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1 Abstract

2 Structural maintenance of chromosomes (SMC)-kleisin complexes organize chromosomal
3 DNAs in all domains of life, where they have key roles in chromosome segregation, DNA
4 repair and regulation of gene expression. They function through topological entrapment and
5 active translocation of DNA, but the underlying conformational changes are largely unclear.
6 Using structural biology, mass spectrometry and cross-linking, we investigated the
7 architecture of two evolutionarily distant SMC-kleisin complexes: proteobacterial MukBEF
8 and eukaryotic cohesin. We show that both contain a dynamic coiled-coil discontinuity, the
9 elbow, near the middle of their arms that permits a folded conformation. Bending at the
10 elbow brings into proximity the hinge dimerization domain and the head/kleisin module,
11 situated at opposite ends of the arms. Our findings favor SMC activity models that include a
12 large conformational change in the arms, such as a relative movement between DNA binding
13 sites during DNA loading and translocation.

1 **Introduction**

2 All organisms organize and maintain enormous chromosomal DNA molecules whose contour
3 lengths exceed cellular dimensions by orders of magnitude. Hence both regulation of gene
4 expression and accurate chromosome segregation require a high degree of spatial
5 organization. Structural maintenance of chromosomes (SMC)-kleisin complexes are ancient
6 machines that help to organize chromosome superstructure in bacteria, archaea and
7 eukaryotes (Hirano, 2016). They are essential for chromosome segregation in many bacteria
8 and are retained in the set of 438 protein-coding genes from an organism with an artificially
9 reduced genome (Britton et al., 1998; Hutchison et al., 2016; Jensen and Shapiro, 1999; Niki
10 et al., 1991). Similarly, SMC-kleisin complexes are essential for both mitosis and meiosis in
11 eukaryotes (Guacci et al., 1997; Hirano and Mitchison, 1994; Klein et al., 1999; Michaelis et
12 al., 1997; Pebernard et al., 2004; Saka et al., 1994).

13 At the core of SMC-kleisin complexes is a conserved tripartite protein ring, composed of an
14 SMC homo- or hetero-dimer, bridged by a kleisin subunit (Bürmann et al., 2013; Haering et
15 al., 2002; Onn et al., 2007; Woo et al., 2009; Zawadzka et al., 2018). SMC proteins are
16 elongated molecules containing an ABC-type ATPase head and a hinge dimerization domain
17 at opposite ends of an approximately 50 nm long intramolecular and antiparallel coiled-coil
18 arm (Anderson et al., 2002; Diebold-Durand et al., 2017; Haering et al., 2002; Melby et al.,
19 1998; Niki et al., 1992). The kleisin asymmetrically connects the two heads of an SMC dimer
20 and contains additional binding sites for additional KITE or HAWK subunits (Palecek and
21 Gruber, 2015; Wells et al., 2017).

22 A widely conserved and possibly fundamental aspect of SMC-kleisin activity is the ability to
23 entrap chromosomal DNA within their ring structure (Cuylen et al., 2011; Gligoris et al.,
24 2014; Wilhelm et al., 2015). DNA entrapment is the molecular basis for sister chromatid
25 cohesion by the cohesin complex, and might also be used by cohesin, condensin and bacterial
26 Smc-ScpAB to organize DNA into large loops. Loading of DNA into the complex is thought to
27 involve transient opening of a ring interface in cohesin (Gruber et al., 2006; Murayama and
28 Uhlmann, 2015), and is likely mediated by the SMC arms in Smc-ScpAB (Bürmann et al.,
29 2017).

1 The second possibly universal aspect of SMC-kleisin activity is their translocation along DNA.
2 Cohesin and bacterial SMC-kleisin complexes associate with chromosomes in a manner that
3 requires ATP binding and they redistribute or translocate from initial loading sites to
4 adjacent regions dependent on ATP hydrolysis (Badrinarayanan et al., 2012; Hu et al., 2011;
5 Minnen et al., 2016; Wang et al., 2018). Translocation of bacterial Smc-ScpAB coincides with
6 progressive linking of distant chromosomal loci *in vivo*, indicating that the complex might
7 actively extrude DNA loops (Wang et al., 2017). Recently, ATPase dependent DNA
8 translocation and loop extrusion reactions have been reconstituted using purified condensin
9 *in vitro* (Ganji et al., 2018; Terakawa et al., 2017). These findings support the notion that
10 SMC-kleisin complexes are motor proteins that use the ATPase activity of their SMC subunits
11 to track along DNA, and some, by doing so, might actively organize chromosomes by building
12 up loops (Alipour and Marko, 2012; Marsden and Laemmli, 1979; Nasmyth, 2001).

13 To explore how the core activities of SMC-kleisin complexes might be implemented on a
14 structural level, we have investigated the architecture of two representative complexes that
15 are separated by a billion years of evolution: MukBEF from *E. coli* and cohesin from budding
16 yeast. We find that both complexes contain a bendable structural coiled-coil discontinuity in
17 their arms that allows them to interconvert between extended and folded conformations, in
18 the latter bringing the hinge dimerization domain close to the head/kleisin module. Our
19 findings show that SMC proteins have the capacity for a large conformational
20 transformation, and provide the basis for investigating long-distance domain movements
21 during DNA loading and translocation reactions.

1 **Results**

2 **A folded conformation of MukBEF and cohesin**

3 MukBEF is a diverged SMC-kleisin complex that serves as an essential chromosome
4 organization machine in *E. coli* (Badrinarayanan et al., 2012; Liroy et al., 2018; Woo et al.,
5 2009; Yamanaka et al., 1996; Yamazoe et al., 1999). The complex comprises the SMC protein
6 MukB, the kleisin MukF and the KITE protein MukE. We co-overexpressed the MukBEF
7 subunits in *E. coli* and prepared the complex by a multi-step procedure that yielded purified
8 material without extra residues on any of the subunits (**Fig. 1a**). The purified complex eluted
9 as a single peak in size exclusion chromatography (SEC) (**Fig. 1b**) and was analyzed by
10 negative stain electron microscopy (EM) immediately after elution from the column (**Fig.**
11 **1c**). Although subject to heterogeneity, most particles had a characteristic double cherry-like
12 shape, composed of a two-lobed density (the MukB head/MukEF module) from which a stalk
13 emerged (the MukB arms). Surprisingly, many particles possessed a stalk length of about 24
14 nm, roughly half of what is expected for an extended MukB arm consisting of canonical
15 coiled-coil segments. As evident from partially extended particles, this conformation was
16 caused by folding at a kink close to the center of the stalk, consisting of the MukB arms. We
17 propose to refer to this kink as the “elbow”, as it connects the upper and lower parts of the
18 MukB arms (**Fig. 1c**). Fully extended particles were also observed, but were less apparent.
19 Using reference-free 2D image classification we obtained class averages for the
20 conformationally less heterogeneous closed form (**Fig. 1c**). Class averages displayed the
21 MukB head/MukEF module as a bow-tie shaped density with a central bridge and showed a
22 clear signal for the folded arm with the elbow at its vertex. We also observed the presence of
23 the elbow by cryo-electron microscopy imaging (cryo-EM) of a distantly related (~26 %
24 sequence identity) MukBEF complex embedded in vitreous ice, without the use of particle
25 support or contrast agent (**Supplementary Fig. 1**).

26 We noticed the presence of a considerable fraction of what appeared to be broken particles
27 on the negative stain EM grids, possibly caused by the grid preparation procedure. To
28 decrease heterogeneity, we subjected the *E. coli* MukBEF to mild cross-linking with the
29 amine-reactive compound BS3 (bis(sulfosuccinimidyl)suberate). This treatment caused the

1 complex to elute from SEC in two major peaks: one at a retention volume similar to native
2 material, and one eluting earlier, indicative of an increased hydrodynamic radius (**Fig. 1d**).
3 Electron micrographs of material eluting close to native material revealed particles mostly
4 in the folded conformation with significantly reduced heterogeneity (**Fig. 1e**). The faster
5 eluting fraction migrated differently from reconstituted MukBEF doublets (Petrushenko et
6 al., 2006) (**Supplementary Fig. 2a**) and was, interestingly, enriched for singlet particles in
7 an extended conformation (**Fig. 1e**). We readily obtained 2D class averages for both open
8 and closed conformations of BS3 cross-linked MukBEF using this fractionation approach
9 (**Fig. 1f**). In the averages, the MukB elbow is positioned at a clearly resolved near-central
10 position in the arm and allows MukB's hinge to approach the head/MukEF module.
11 Importantly, comparison of SEC profiles from native and cross-linked material suggest that
12 native MukBEF adopts mostly a closed conformation under the conditions used (**Fig. 1d**).

13 It has been noted in previous studies that other SMC arms sometimes contain kinks
14 (Anderson et al., 2002; Haering et al., 2002; Hons et al., 2016; Yoshimura et al., 2002). This
15 led us to address whether eukaryotic cohesin would also be able to adopt a defined folded
16 conformation similar to that of MukBEF. We purified budding yeast cohesin containing Smc1,
17 Smc3, the kleisin Scc1 and the HAWK protein Scc3 produced in insect cells, and as for
18 MukBEF, stabilized the complex by mild cross-linking with BS3 and imaged it by negative
19 stain EM (**Fig. 1g, Supplementary Fig. 2b,c**). The complex appeared in a folded
20 conformation resembling that of MukBEF and reference-free 2D classification revealed well-
21 resolved features in the averages. The head/kleisin/HAWK (Smc1/Smc3 ATPase heads, Scc1,
22 Scc3) module of cohesin is visible as a cherry-shaped density at one end of the complex. It is
23 adjacent to a small constriction that likely represents the conserved head-proximal coiled-
24 coil discontinuity called 'joint' (Diebold-Durand et al., 2017; Gligoris et al., 2014). The
25 cohesin hinge, which is larger than the MukB hinge, is visible as a circular density in direct
26 vicinity of the joint. The cohesin elbow is located at an off-center position within the SMC
27 arms in contrast to MukBEF, but, similar to MukBEF, allows them to bend at an angle close
28 to 180 degrees. We conclude that the ability to bend at an SMC elbow is shared by two very
29 distantly related SMC-kleisin complexes.

1 **Identification of the elbow position in MukBEF and cohesin**

2 To ascertain where the elbow might be located at the sequence level, we used mass-
3 spectrometry to identify the BS3 cross-linked residue pairs in both MukBEF and cohesin. We
4 observed 176 distinct inter-subunit cross-links for MukBEF (**Fig. 2a**) as well as 352
5 additional intra-subunit cross-links, whereas analysis of cohesin identified 241 inter- and
6 503 intra-subunit cross-links (**Supplementary Fig. 2d**). Using spatial information from
7 crystal structures of the MukEF and the MukB head / MukF C-terminal winged-helix domain
8 (cWHD) subcomplexes, respectively, we computed kernel density estimates for the
9 distribution of inter-subunit cross-links (**Fig. 2b**) (Fennell-Fezzie et al., 2005; Woo et al.,
10 2009). The distribution of observed cross-links is congruent with the position of known
11 subunit interfaces, indicating that our cross-linking experiment faithfully reports the
12 structure of the complex. We used the same approach to localize regions at the MukB hinge
13 that cross-linked to head-proximal sites and the MukEF module, respectively (**Fig. 2c**).
14 Cross-links clustered at a coiled-coil region near the hinge (Li et al., 2010), consistent with
15 the idea that the complex folds at an elbow. To pinpoint the elbow region precisely, we next
16 mapped all MukB coiled-coil residues onto a unified sequence coordinate system along the
17 arm (accounting for the antiparallel nature of the SMC arm coiled coils), using available
18 disulfide cross-linking data as a guide (Weitzel et al., 2011) (**Fig. 2d**). We then filtered intra-
19 molecular cross-links in MukB for long-distance residue pairs in this coordinate system and
20 determined the midpoint for each pair. If the coiled-coil arm folds at a defined elbow
21 position, then the midpoints should reveal it, and indeed, midpoints clustered at a central
22 region of the MukB arm (**Fig. 1d**). As a negative control, clustering was not observed in
23 randomly permuted data (**Supplementary Fig. 2e**). Kernel density estimates produced a
24 pronounced peak close to the 180th coiled-coil residue in the arm coordinate system
25 (corresponding to MukB residues 427 and 970 on the N- and C-terminal coiled-coil strands,
26 respectively).

27 We used a similar approach to identify the elbow's position in a cohesin complex comprising
28 Smc1, Smc3, Scc1, Scc3 and the loader protein Scc2. As was the case for MukBEF, kernel
29 density estimates for inter-subunit cross-links are in good agreement with available crystal
30 structures (**Fig. 2e**) (Gligoris et al., 2014; Haering et al., 2004). Consistent with our

1 observations by EM, the arms of Smc1 and Smc3 both showed midpoint clustering of cross-
2 links at a position away from the center, indicating the presence of the elbow close to
3 residues 391/806 in Smc1 (coiled-coil residue 215 in the arm coordinate system) and
4 396/808 in Smc3 (residue 212 in the arm coordinate system), respectively. These findings
5 suggest that cohesin's elbow is shifted towards the hinge, in contrast to MukBEF's center
6 position (**Fig. 2f**), consistent with our imaging data (**Fig. 1c, f, g**). Using the same method, we
7 re-analyzed published cross-link/mass-spectrometry (CLMS) data for human and budding
8 yeast cohesin (Chao et al., 2017; Huis in 't Veld et al., 2014) and obtained similar results
9 (**Supplementary Fig. 2f**). We conclude that although cohesin and MukBEF each contain a
10 defined elbow that enables folding, its relative position within the SMC proteins appears to
11 be different.

12 **Structure of the MukB elbow**

13 To investigate if the MukB arm contains structural features that would allow it to bend at the
14 elbow position, we purified a fusion construct between matching N- and C-terminal
15 fragments containing the elbow as determined above and solved its structure by X-ray
16 crystallography (**Fig. 3a**). Consistent with findings from disulfide cross-linking experiments,
17 the arm contains two coiled-coil discontinuities or "knuckles" in this region (Weitzel et al.,
18 2011). The knuckle that has been previously named K1/2 is at a central position, joining the
19 coiled-coil regions formed by helices $\alpha 1/\alpha 7$ and $\alpha 2/\alpha 6$. Knuckle K1/2 is followed by the
20 K2/3a break formed by helices $\alpha 3$, $\alpha 4$ and $\alpha 5$. Mapping the long-distance cross-link
21 midpoints onto the structure identifies the K1/2 break as the elbow (**Fig. 3b, 2d**). In the
22 crystal, the elbow adopts an extended and gently bent conformation. It contains an "anchor"
23 segment in its $\alpha 1$ helix, which is part the N-terminal coiled-coil region (**Fig. 3c**). This anchor
24 helix connects to $\alpha 2$ via a loop. The C-terminal part of the coiled-coil segment winds around
25 the elbow anchor helix, starting at $\alpha 6$ that connects to $\alpha 7$ via a distorted helical stretch. A
26 conserved Tyr residue (Y416) (**Fig. 3b, c, Supplementary Fig. 3**) is wedged into the $\alpha 6/\alpha 7$
27 connection and might contribute to its distortion by obstruction with the bulky Tyr
28 sidechain. The tip of $\alpha 6$ in the C-terminal coiled-coil region forms a short interface with the
29 anchor helix of the N-terminal coil (**Fig. 3c**). It is conceivable that unzipping of this interface

1 might further destabilize the $\alpha 6/\alpha 7$ connection to allow the elbow to bend and fold. Codon
2 substitutions at the chromosomal *mukB* locus that either changed Leu960, located at the
3 $\alpha 1/\alpha 6$ /anchor helix interface, to Glu (L960E) or changed the central Tyr416 to Asp or Pro
4 (Y416D and Y416P, respectively) caused a *mukB* null phenotype: mutant strains were not
5 viable on rich media at 37 °C, despite MukB proteins being present at wild-type levels
6 (**Supplementary Fig. 3**). These finding suggest that an intact elbow region is critical for
7 MukBEF activity *in vivo*. Although the structural details of the folded conformation remain to
8 be determined, our findings support the notion that bending of MukBEF occurs at a
9 predetermined, structurally defined and essential coiled-coil discontinuity in its SMC arms.

10 **Proximity of cohesin HAWK Pds5 and the hinge *in vivo***

11 A crucial question is whether the coiled coils of SMC-kleisin complexes adopt a folded
12 conformation *in vivo* as well as *in vitro*. We reasoned that if such folding occurred at cohesin's
13 elbow, then proximity of its hinge domain to ATPase head proximal sequences might permit
14 site-specific chemical cross-linking between residues within the hinge and those associated
15 with ATPase heads. To this end, we generated yeast strains in which residues within the
16 Smc1 hinge were substituted by the unnatural amino acid BPA (p-benzoyl L-phenylalanine)
17 (**Fig. 4a**), the sidechain of which can be activated by ultra-violet (UV) light to cross-link to
18 residues in its vicinity. After UV treatment of intact cells, we immunoprecipitated cohesin
19 and analyzed the cross-linking reaction by Western blotting (**Fig. 4b**). An Smc1 mutant with
20 a BPA substitution at Glu593 efficiently cross-linked to Smc3 (**Fig. 4b**) because this residue
21 is positioned directly at the Smc1/Smc3 hinge interface. Strikingly, a BPA substitution
22 mutant of Lys620, located on the coiled-coil distal face of the hinge, efficiently cross-linked
23 to a large protein other than Smc3. This protein was identified as Pds5 (**Fig. 4b**). We verified
24 that BPA was incorporated into Smc1(K620BPA) (**Supplementary Fig. 4**) and that cross-
25 linking between Smc1(K620BPA) and Pds5 was dependent on UV treatment (**Fig. 4c**). Pds5
26 is a HAWK protein that binds Scc1 sequences close to the kleisin's N-terminal domain
27 associated with the coiled coil emerging from the Smc3 ATPase head (Lee et al., 2016; Muir
28 et al., 2016). Importantly, mutation of the Pds5 binding site in Scc1 by substitution of Val137
29 for lysine (V137K) greatly diminished Pds5 recruitment and prevented cross-linking to the
30 Smc1(K620BPA) hinge (**Fig. 4d**) (Chan et al., 2013).

1 At present, we cannot exclude the possibility that cross-linking between Pds5 and the Smc1
2 hinge occurs between two different cohesin complexes or that Pds5 binds close to the hinge
3 in a way that only indirectly depends on Scc1. However, if cross-linking happens within a
4 single cohesin complex and Pds5 contacts the hinge while bound to Scc1, then this would
5 necessitate a folded conformation similar to that observed by EM (**Fig. 1g**). We note that it
6 has previously been observed that a fluorescent tag inserted into the Smc1 hinge of cohesin
7 produces a weak FRET signal when combined with a tag on the head proximal HAWK subunit
8 Pds5 (Mc Intyre et al., 2007), which is consistent with our observations. We conclude that
9 folding of cohesin's coiled coils most probably occurs *in vivo* as well as *in vitro*.

10 **Conservation of the SMC elbow**

11 It has been noted before that coiled-coil prediction profiles for SMC sequences often contain
12 at least two considerable drops in coiled-coil probability within both the N- and C-terminal
13 parts of the arm (Waldman et al., 2015). One of the predicted breaks is located close to the
14 SMC heads, and another one is often found at a more central position within the arm. We
15 wished to confirm and corroborate these findings by extending the analysis to a large set of
16 protein sequences. We predicted coiled-coil probabilities for hundreds of individual full-
17 length sequences using MARCOIL (Delorenzi and Speed, 2002), extracted profiles for N- and
18 C-terminal halves, aligned them on the arm center, and averaged the profiles to remove noise
19 (**Fig. 5**). The aggregate profiles for different classes of SMC proteins clearly indicate the
20 position of the head proximal coiled-coil discontinuity, mapping it to the structurally
21 conserved joint (Diebold-Durand et al., 2017; Gligoris et al., 2014). Importantly for our work,
22 the profiles also predict the presence of a centrally located coiled-coil discontinuity in most,
23 if not all, SMC protein families with high confidence as judged by random resampling (**Fig.**
24 **5**). For MukB, the predicted central position is in excellent agreement with the elbow
25 position estimated here experimentally by cross-linking / mass-spectrometry (minimum
26 coiled-coil probability at residues 432 and 970, maximum cross-link midpoint probability
27 density close to residues 427 and 970). These residues are located directly within the K1/2
28 break present in our crystal structure (**Fig. 3**). Similarly, the predicted elbow positions for
29 Smc1 and Smc3 (residues 374/790 and 397/796, respectively) are close to our experimental
30 estimates (residues 391/806 and 396/808, respectively). In addition, the coiled coils of both

1 bacterial and archaeal Smc proteins (*B. subtilis* and *P. yayanosii*) contain a discontinuity close
2 to the elbow position predicted by our aggregate profiling approach (Diebold-Durand et al.,
3 2017; Waldman et al., 2015) (**Supplementary Fig. 5**). Interestingly, in *B. subtilis* Smc this
4 region is among the few that tolerates peptide insertions (Bürmann et al., 2017). It appears
5 that the prediction method is accurate for the two distantly related SMC-kleisin complexes,
6 which we have investigated here, and hence likely generalizes to other SMC proteins. We
7 conclude that a central coiled-coil discontinuity is present in most if not all classes of SMC
8 proteins, indicating that the ability to bend at a defined elbow is likely a fundamental feature.

1 Discussion

2 Conformational states and their interconversions in SMC-kleisin complexes

3 The first electron microscopic images of isolated SMC proteins were obtained by rotary
4 metal shadowing of mica-adsorbed proteins and revealed a characteristic shape: positioned
5 at the ends of a long coiled-coil arm were identified two globular domains, a hinge
6 dimerization domain and a head ATPase domain (Haering et al., 2002; Melby et al., 1998;
7 Niki et al., 1992). In these early studies, SMC dimers held together by their hinges were
8 largely observed as V-, I- (rod) or O-shaped particles. Furthermore, it was noticed that the
9 SMC arms would sometimes be kinked (Anderson et al., 2002; Haering et al., 2002; Hons et
10 al., 2016). Other studies, employing atomic force microscopy, have suggested that the
11 isolated SMC2-4 heterodimer of condensin may adopt a bent conformation (Yoshimura et al.,
12 2002) or may have highly flexible arms with a short persistence length (Eeftens et al., 2016).
13 The apparent presence of coiled-coil breaks within SMC arms prompted the prediction that
14 "the coiled coil undergoes a dramatic conformational change to allow a direct interaction
15 between the hinge domain and the head domain or a head-proximal portion of the coiled
16 coil" (Waldman et al., 2015).

17 Here, we demonstrate that two substantially diverged SMC-kleisin complexes, namely
18 bacterial MukBEF and eukaryotic cohesin, are able to adopt a well-defined folded
19 conformation that brings their hinge into proximity of their heads. Folding of the complexes
20 occurs at a centrally positioned coiled-coil discontinuity, the 'elbow', that is present in most
21 if not all SMC proteins. The elbow is apparent as a sharp kink also in cryo-EM images of
22 MukBEF particles, without the use of surface immobilization, dehydration, staining or
23 mechanical probing of the sample (**Supplementary Fig. 1**), and it is detectable by in-solution
24 cross-linking and mass spectrometry. Observed contact sites are fully consistent with a
25 folded state and are in excellent agreement with computational predictions for the elbow
26 position and also crystallographic data (**Figs. 2, 3, 5**). Size-exclusion chromatography of
27 MukBEF suggests that a considerable fraction of this complex adopts a folded conformation
28 (**Fig. 1d**). As indicated by SEC and negative stain EM, a smaller fraction adopts an extended
29 ('I' or rod) conformation, which resembles the shape of *B. subtilis* Smc-ScpAB (Diebold-

1 Durand et al., 2017; Soh et al., 2015). Interestingly, treatment of MukBEF with the cross-
2 linker BS3 strongly enriches the extended rod fraction. Hence, we would like to propose that
3 MukBEF switches between folded and extended forms, and that reaction with BS3 artificially
4 triggers this switch.

5 If MukBEF and cohesin are able to alternate between folded and extended states, then it is
6 likely that the interconversion is coupled to their DNA binding and ATP hydrolysis cycle. The
7 SMC arms are firmly anchored in the ATPase heads, which are ideally positioned to drive
8 such a conformational change. A link between the ATPase cycle and arm conformation is
9 supported by site-specific cross-linking experiments with Smc-ScpAB that indicate a
10 conformational change in its coiled coil upon binding of ATP and DNA (Minnen et al., 2016;
11 Soh et al., 2015). This conformational change has been interpreted as a disengagement of the
12 arm/arm interface, converting Smc-ScpAB from a rod-like to ring-like state. If both folded
13 and extended conformations interconvert in MukBEF and cohesin, we suspect that they may
14 do so via an intermediate that accommodates considerable strain in its arms. Such an
15 intermediate might correspond to this "open" or ring-like state (**Fig. 6a**).

16 How could an ATPase driven folding/extension cycle be implemented at the structural level?
17 One conundrum is how an SMC dimer with a central 2-fold symmetry axis is able to adopt a
18 folded state such as those observed here. Making an SMC dimer bend to one side severely
19 breaks this symmetry, as the symmetry dictates that the two arms bend to opposite sides if
20 the same bending angle direction is applied to each SMC coiled coil (**Supplementary Figure**
21 **6**). To bend the 2-fold symmetric dimer at the elbow to one side, there are two options: the
22 monomers might bend into opposite directions within their respective body frames, or they
23 might rotate 180 degrees relative to each other and then bend into the same direction. This
24 insight allows the construction of a simple hypothesis: conformational switching between
25 folded and extended states might be achieved by rotating the arms against each other. This
26 would bring the monomer elbows into an orientation that either is or is not compatible with
27 folding at the elbow, depending on the starting conformation. If such a rotation introduced
28 or removed strain, for example by twisting the heads while keeping the hinges fixed, this
29 could actively promote switching. The reader is encouraged to elaborate on these insights
30 by building and twisting the accompanying paper model (Fig. 6b). Of note, asymmetric

1 binding of SMC proteins by the kleisin appears to be a widely conserved feature and might
2 facilitate the asymmetric twisting (Bürmann et al., 2013; Haering et al., 2002; Zawadzka et
3 al., 2018). A mechanism based on a simple mechanical principle such as this would be robust
4 because it could induce bending at arbitrary positions, only depending on the position of the
5 elbow. However, it requires heads and hinge to have a particular relative angular orientation.
6 Consistent with such a geometric constraint, function of Smc-ScpAB appears to be influenced
7 by the superhelical phase-relationship between the ends of its arms (Bürmann et al., 2017)
8 suggesting that ATPase heads and hinges are attached to the coiled-coil arms in fixed and
9 relevant relative orientations.

10 **Shape transformations for DNA transactions**

11 SMC-kleisin complexes in pro- and eukaryotes appear to share three possibly overlapping
12 activities that are likely central to their biological function: DNA translocation, DNA
13 entrapment and DNA loop extrusion. How these activities are interconnected and how they
14 are biochemically implemented remain exciting and important questions. Understanding the
15 DNA translocation mechanism appears especially relevant, because it is almost certainly
16 required for DNA loop extrusion. Translocation along DNA depends on ATP hydrolysis by
17 SMC proteins (Ganji et al., 2018; Hu et al., 2011; Minnen et al., 2016), and is likely driven by
18 an internal motor activity at least in condensin (Terakawa et al., 2017). The SMC arms likely
19 play a role in the translocation process because mutations in the arms of bacterial Smc-
20 ScpAB largely uncouple ATP hydrolysis from the movement on chromosomes (Bürmann et
21 al., 2017). Moreover, recent findings indicate a dependency of translocation on the SMC hinge
22 in cohesin because mutations in this domain appear to uncouple nucleotide hydrolysis and
23 translocation in a similar fashion (Srinivasan et al., 2018). Apart from DNA translocation,
24 cross-talk between hinge and head module has been suspected to mediate DNA loading of
25 cohesin (Murayama and Uhlmann, 2015; Xu et al., 2018). We envision that a large-scale
26 conformational change in the arms, such as folding at the elbow, is involved in DNA
27 translocation or entrapment by SMC-kleisin complexes.

28 How might a folded conformation of SMC-kleisin complexes take part in the translocation
29 reaction? One intriguing possibility is that switching between extended and folded

1 conformations might change the distance between two DNA binding sites, possibly located
2 at the ends of the SMC-kleisin rod, thereby permitting an inchworm-like movement along
3 the substrate (**Fig. 6c, Supplementary Fig. 7a**). DNA binding activity has been reported for
4 the head domains of MukB, the SMC-like Rad50 and Smc-ScpAB (Lammens et al., 2004; Liu
5 et al., 2016; Löwe et al., 2001; Woo et al., 2009). Moreover, isolated hinge domains of cohesin,
6 condensin, Smc5-6 and Smc-ScpAB are also able to bind DNA (Alt et al., 2017; Chiu et al.,
7 2004; Griese et al., 2010; Soh et al., 2015). The MukB hinge, although lacking a strong
8 interaction with DNA (Ku et al., 2010; Kumar et al., 2017), associates with the DNA binding
9 proteins topoisomerase IV and MatP, suggesting that it might at least come into proximity of
10 the substrate (Nolivos et al., 2016; Vos et al., 2013). Translocation via an inchworm-like
11 mechanism would require at least one of the binding sites to act as a “grapple”, i.e. it must
12 have regulated DNA affinity for capture and release of the substrate (**Supplementary Fig.**
13 **7a**). The second site would act as an “anchor”, keeping the complex attached to DNA while
14 the grapple is released. DNA binding at either site might not be purely enthalpic but may
15 involve entropic (steric or even topological) entrapment of the substrate, similar to the
16 sliding clamp of DNA polymerase (Hedglin et al., 2013). In addition, the DNA binding sites
17 could also be located on different complexes that act in concert, as is clearly a possibility for
18 chromosomal MukBEF (Badrinarayanan et al., 2012). If a distance change in DNA binding
19 sites was responsible for translocation of SMC-kleisin complexes, then loop extrusion might
20 be implemented by addition of a third site that stabilizes the loop (**Supplementary Fig. 7b**).
21 The HAWK subunits Ycg1 and Scc3 of condensin and cohesin, respectively, are part of a DNA
22 binding site that might act as such an anchor (Ganji et al., 2018; Kschonsak et al., 2017; Li et
23 al., 2018).

24 It has been proposed that DNA translocation of SMC-kleisin complexes involves the
25 transition from a rod- to a ring-like conformation, whereby DNA is captured in between the
26 SMC arms to be pushed from a hinge-proximal site towards a head-proximal compartment
27 (Diebold-Durand et al., 2017; Marko et al., 2018). In such a scenario, which is not mutually
28 exclusive with the principles outlined here, the SMC elbow might have a twofold role: First,
29 it might support bending of the relatively rigid arms into a ring, which in MukBEF might be
30 assisted by secondary coil-coil discontinuities (**Fig. 3**) (Li et al., 2010; Weitzel et al., 2011).

1 Second, active transition to a folded conformation might help pushing DNA from one end of
2 the complex to the other (**Fig 6c**). However, at present it is unclear whether DNA
3 translocation, or loop extrusion, requires entrapment within the tripartite ring. Clarifying
4 the nature of the DNA bound states of SMC-kleisin complexes and tracing the path of
5 associated DNA is now of utmost importance.

6 In summary, we show that the evolutionarily distant SMC-kleisin complexes MukBEF and
7 cohesin adopt very similar folded conformations by bending at a central coiled-coil
8 discontinuity, the elbow. We provide evidence that the elbow is a general feature of SMC-
9 kleisin complexes and propose it is involved in a conformational switch that drives DNA
10 transactions of all SMC-kleisin complexes.

1 **Methods**

2 **Purification of MukBEF**

3 Coding sequences for *E. coli* MukF, MukE and MukB (GeneBank IDs: NP_415442.1,
4 NP_415443.2, NP_415444.1) were inserted as a polycistronic expression construct into a
5 pET-28 derived vector using Golden Gate cloning (Engler et al., 2008). MukB was N-
6 terminally fused to budding yeast His6-SUMO. The complex was produced in *E. coli* BL21-
7 Gold(DE3) grown in ZYP-5052 autoinduction medium at 24 °C (Studier, 2005). Purification
8 of the complex was performed at 4 °C. About 15 g cells were resuspended in 90 mL buffer
9 IMAC (50 mM sodium phosphate, 300 mM NaCl, 20 mM imidazole, pH 7.4 @ 4 °C) including
10 RNase A, DNase I and protease inhibitors and lysed in a high-pressure homogenizer at 172
11 MPa. The lysate was briefly sonicated to reduce viscosity, and cleared by centrifugation for
12 30 min at 96,000 x g. The extract was incubated with 25 mL NiNTA agarose (Qiagen) for 30
13 min. The resin was packed into a gravity flow column and washed with 80 mL IMAC buffer
14 followed by 40 mL SENP buffer (10 mM sodium phosphate, 50 mM NaCl, 20 mM imidazole,
15 pH 7.4 @ 4 °C). The resin was resuspended in 25 mL SENP buffer containing 1 mM DTT and
16 1 mg GST-hSENP1 protease and incubated for 1 h to cleave off the His6-SUMO-tag. The flow-
17 through containing the complex was collected and combined with an additional 12.5 mL
18 wash of the column. The eluate was then loaded onto a 20 mL Heparin HP column (GE
19 Healthcare), washed with 2 column volumes (CV) of buffer HA (10 mM sodium phosphate,
20 50 mM NaCl, pH 7.4 @ 4 °C) and eluted with a 20 CV gradient into buffer HB (10 mM sodium
21 phosphate, 1 M NaCl, pH 7.4 @ 4 °C). The complex eluted in two peaks, whereby the low salt
22 peak contained a prominent contaminant. The high salt peak fractions (at about 400 mM
23 NaCl) were pooled and diluted with 4 volumes of buffer (10 mM Tris, 70 mM NaCl, pH 8.0 @
24 4 °C). The solution was loaded onto a 5 mL Q HP column (GE Healthcare). The column was
25 washed with 2 CV of buffer QA (10 mM Tris, 200 mM NaCl, pH 8.0 @ 4 °C) and eluted with a
26 20 CV gradient into buffer QB (10 mM Tris, 1 M NaCl, pH 8.0 @ 4 °C). The complex eluted as
27 a single peak at about 450 mM NaCl. Peak fractions were pooled, concentrated to about 10
28 mg/mL on a Vivaspin 100k ultrafiltration membrane (Sartorius), aliquoted, frozen in liquid
29 nitrogen and stored at -80° C. An aliquot of MukBEF was then thawed and injected into a

1 Superose 6 Increase 3.2/300 column (GE Healthcare) in T200 buffer (10 mM Tris, 200 mM
2 NaCl, pH 8.0 @ 4 °C) to remove aggregates. The monomer fraction appeared stable for
3 several days as judged by SEC but was freshly used for all experiments. MukBEF from
4 *Desulfovermiculus halophilus* (GeneBank IDs: WP_027370798.1, WP_027370797.1, WP_
5 027370796.1) was produced and purified similar to the *E. coli* complex.

6 **Purification of MukB and MukEF and reconstitution of MukBEF complexes**

7 MukB was produced and purified similar to the MukBEF holocomplex. MukEF was produced
8 from a polycistronic expression vector with a His6-SUMO-tag on MukE and purified similar
9 to the holocomplex, but omitting the Heparin step and using Sephacryl S200 as the size-
10 exclusion resin. Complexes were reconstituted similar to the protocols from (Petrushenko
11 et al., 2006) at 2 μM MukB₂ and 4 μM MukE₄F₂ in either 10 mM Tris, 40 mM NaCl, 2 mM
12 MgCl₂, pH 8.0 (singlets, MukBEF^S) or in 10 mM Tris, 200 mM NaCl, pH 8.0 (doublets,
13 MukBEF^D). Reactions were run over Superose 6 Increase in the respective reconstitution
14 buffer and peak fractions were re-injected into Superose 6 Increase in T200 buffer.

15 **Purification of cohesin**

16 Cohesin expression constructs were cloned as described previously (Petela et al., 2018).
17 Briefly, coding sequences for Smc1, Smc3, Scc1, Scc2 and Scc3 from *S. cerevisiae* (Genbank
18 IDs: NP_116647.1, NP_012461.1, NP_010281.1, NP_010466.3, NP_012238.1) were
19 synthesized with codon optimization for insect cell expression (Genscript). Sequences were
20 individually cloned as Smc1, 8xHis-Smc3, Scc1-2xStrepII, 2xStrepII-Scc3 and 2xStrepII-(151-
21 1493)Scc2 into Multibac vectors (Bieniossek et al., 2008), yielding Smc1-pACEbac1, 8xHis-
22 Smc3-pACEbac1, 2xStrepII-ΔN150-Scc2-pACEbac1, 2xStrepII-Scc3-pACEbac1 and Scc1-
23 2xStrepII-pIDC. Tagged constructs contained an HRV 3C protease site (LEVLFQ/GP) in the
24 tag linker. The vectors Smc1-pACEbac1, 8xHis-Smc3-pACEbac1 and Scc1-2xStrepII-pIDC
25 were combined through Gibson assembly and *in vitro* Cre-*lox* recombination yielding a
26 transfer vector for the Smc1-Smc3-Scc1 trimer. The trimer, 2xStrepII-ΔN150-Scc2-
27 pACEbac1 and 2xStrepII-Scc3-pACEbac1 transfer vectors were individually transformed
28 into chemically competent DH10Embacy cells (Vijayachandran et al., 2011). The purified
29 bacmids were transfected into Sf9 cells using Fugene HD reagent (Promega), and the

1 generated P1 viruses were infected into fresh Sf9 cells. The cells were grown in Insect
2 XPRESS protein free medium with L-glutamate (Lonza) at 27 °C for ~72 h, and the harvested
3 cells were frozen in liquid nitrogen.

4 The frozen pellets of Sf9 culture were re-suspended in lysis buffer (20 mM Hepes pH 7.5, 125
5 mM NaCl, 1 mM TCEP, and 10 % (w/v) glycerol) supplemented with DNase, RNase, 1 mM
6 PMSF and EDTA-free protease inhibitor (cOmplete, Roche). Cells were lysed by sonication,
7 and the lysates were clarified by ultracentrifugation at 200,000 x g. The clarified lysates were
8 applied to Strep resin (5 mL StrepTrap, GE Healthcare) and eluted with 2 mM desthiobiotin
9 in lysis buffer. 3C protease was added to the eluents to cleave the affinity tags and the
10 cleavage products were further purified by anion exchange columns (HiTrap Q FF or mini Q
11 (GE healthcare)) with buffers of QA (50 mM Tris, 100 mM NaCl, 1 mM TCEP, and 5 % (w/v)
12 glycerol, pH 8.0) and QB (50 mM Tris, 1 M NaCl, 1 mM TCEP, and 5 % (w/v) glycerol, pH 8.0).
13 The fractions were pooled, concentrated using a Vivaspin 100 kDa ultrafiltration membrane
14 (Sartorius). The purified trimer, NΔ150-Scc2, Scc3 proteins were then frozen in liquid
15 nitrogen and stored at -80 °C until further use.

16 **BS3 cross-linking / SEC**

17 An aliquot of MukBEF Q eluate was thawed and injected into a Superose 6 Increase 3.2/300
18 column in P200 buffer (10 mM sodium phosphate, 200 mM NaCl, pH 7.4 @ 4 °C). The
19 monomer fraction was incubated for 2 h on ice at 0.4 mg/mL with or without 1 mM BS3 and
20 was injected into a Superose 6 Increase 3.2/300 column in T200 buffer (10 mM Tris, 200
21 mM NaCl, pH 8 @ 4 °C). Chromatography was performed at a flow rate of 40 μL/min.

22 **Negative stain electron microscopy**

23 For imaging of native MukBEF, an aliquot of Q eluate was thawed and injected into a
24 Superose 6 Increase 3.2/300 column in T200 buffer. The monomer fraction was reinjected,
25 and the peak fraction applied to freshly glow-discharged EMS Cu Mesh 400 continuous
26 carbon grids. Grids were stained with 2 % uranyl acetate and imaged in a Tecnai Spirit
27 microscope (FEI) using an Orius CCD camera at a pixel size of 3.5 Å and an electron dose of
28 30 e⁻/Å² at 120 kV. For data collection, native MukBEF was applied to Quantifoil CuRh R2/2

1 Mesh 200 grids covered with a homemade continuous carbon film. The grids were stained
2 with 2 % uranyl formate and imaged on a Tecnai F30 Polara microscope (FEI) with a Falcon
3 III detector using a pixel size of 1.72 Å and an electron dose of 30 e-/Å² at 300 kV.

4 BS3 cross-linked MukBEF was prepared as described above and imaged on EMS Cu Mesh
5 400 continuous carbon grids stained with 2 % uranyl formate. Data for SEC peak 1 (extended
6 conformation) were collected on a Tecnai Spirit with an UltraScan CCD camera using a pixel
7 size of 3.95 Å and an electron dose of 30 e-/Å² at 120 kV. Data for SEC peak 2 (folded
8 conformation) were collected on a Tecnai G2 F20 microscope (FEI) with a Falcon II detector
9 using a pixel size of 2.08 Å and an electron dose of 30 e-/Å² at 200 kV.

10 For imaging of cohesin, the purified trimer and Scc3 were mixed at a 1:1.5 molar ratio and
11 injected into a Superose 6 Increase 3.2/300 column in P200 buffer. The tetramer fraction
12 was incubated with 1 mM BS3 for 2 h on ice and injected into a Superose 6 Increase 3.2/300
13 column in T200 buffer. Peak fractions were applied to Quantifoil CuRh R2/2 Mesh 200 grids
14 covered with a homemade continuous carbon film and stained with 2 % uranyl formate. Data
15 were collected on a Tecnai G2 F20 microscope (FEI) with a Falcon II detector using a pixel
16 size of 2.08 Å and an electron dose of 30 e-/Å² at 200 kV.

17 **Cryo-electron microscopy**

18 *D. halophilus* MukBEF at 0.2 mg/mL was applied to glow-discharged Quantifoil CuRh R2/2
19 Mesh 200 grids, blotted on a Vitrobot (FEI) and plunge frozen in liquid ethane. Particles were
20 imaged on a FEI Titan Krios equipped with a Volta phase plate (Danev et al., 2014) and a
21 Falcon III detector operating in linear mode, using a pixel size of 1.07 Å, defocus of -0.6 µm
22 to -0.8 µm and a total electron dose of 100 e-/Å² at 300 kV.

23 **Image processing**

24 The contrast transfer function (CTF) for electron micrographs was estimated with CTFFIND-
25 4.1 (Rohou and Grigorieff, 2015). For movies collected on a Falcon III detector, motion
26 correction was performed with Motioncor2 (Zheng et al., 2017). Particle picking and
27 reference-free 2D classification were performed in RELION2 (Fernandez-Leiro and Scheres,
28 2017).

1 **Cross-linking/mass spectrometry**

2 For cross-linking/mass spectrometry analysis of MukBEF, aliquots of Q eluate were thawed
3 and injected into a Superose 6 Increase 3.2/300 column in buffer XL (20 mM Hepes, 150 mM
4 NaCl, 5 mM MgCl₂, pH 7.8 @ 23 °C). The monomer fractions were pooled and incubated at
5 0.4 mg/mL with 2.5 mM BS3 for 2 h on ice before quenching with 20 mM ammonium
6 bicarbonate for 30 min on ice. The sample was incubated for 2 min at 98 °C in the presence
7 of LDS-PAGE sample buffer (Life Technologies) containing 6 % 2-mercaptoethanol. Reaction
8 products were separated on Criterion TGX 4-20 % SDS-PAGE gels (BioRad).

9 For analysis of cohesin, the purified trimer, NΔ150-Scc2 and Scc3 were mixed at a 1:1.5:1.5
10 ratio and into a Superose 6 Increase 3.2/300 column in buffer (20 mM Hepes, 150 mM NaCl
11 and 1 mM TCEP, pH 7.7). Pentamer fractions were incubated at 2 mg/mL with 5 mM BS3 for
12 2 hr at 4 °C and then the reaction was quenched with 50 mM ammonium bicarbonate for 45
13 min on ice. Reaction products were separated on a Criterion TGX 4-15% SDS-PAGE gel
14 (BioRad).

15 Gel bands corresponding to the cross-linked species were excised and digested with trypsin
16 (Pierce, Germany) (Shevchenko et al., 2006). The resulting tryptic peptides were extracted
17 and desalted using C18 StageTips (Rappsilber et al., 2003).

18 For MukBEF, peptides eluted from StageTips were dried in a Vacuum Concentrator
19 (Eppendorf, Germany) and dissolved in running buffer A prior to strong cation exchange
20 chromatography (100 x 2.1 mm Poly Sulfoethyl A column; Poly LC, Columbia, MD, USA).
21 Mobile phases A consisted of 30 % acetonitrile (v/v), 10 mM KH₂PO₄ at pH 3, and mobile
22 phase B additionally contained 1 M KCl. The separation of the digest used a non-linear
23 gradient (Chen et al., 2010) at a flow rate of 200 µl/min. Five fractions a 2 min in the high-
24 salt range were collected and cleaned by StageTips for subsequent LC-MS/MS analysis. For
25 cohesin, peptides were fractionated on an ÄKTA Pure system (GE Healthcare) using a
26 Superdex Peptide 3.2/300 (GE Healthcare) at a flow rate of 10 µL/min using 30% (v/v)
27 acetonitrile and 0.1% (v/v) trifluoroacetic acid as mobile phase. Five 50 µl fractions were
28 collected and dried.

1 Samples for analysis were resuspended in 0.1% v/v formic acid 1.6% v/v acetonitrile. LC-
2 MS/MS analysis was conducted in duplicate for SEC fractions and triplicate for SCX fractions,
3 performed on an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher
4 Scientific, Germany) coupled on-line with an Ultimate 3000 RSLCnano system (Dionex,
5 Thermo Fisher Scientific, Germany). The sample was separated and ionized by a 50 cm EASY-
6 Spray column (Thermo Fisher Scientific). Mobile phase A consisted of 0.1% (v/v) formic acid
7 and mobile phase B of 80% v/v acetonitrile with 0.1% v/v formic acid. Flow-rate of 0.3
8 μ L/min using gradients optimized for each chromatographic fraction from offline
9 fractionation ranging from 2% mobile phase B to 45% mobile phase B over 90 min, followed
10 by a linear increase to 55% and 95% mobile phase B in 2.5 min, respectively. The MS data
11 was acquired in data-dependent mode using the top-speed setting with a three second cycle
12 time. For every cycle, the full scan mass spectrum was recorded in the Orbitrap at a
13 resolution of 120,000 in the range of 400 to 1,600 m/z. Ions with a precursor charge state
14 between 3+ and 6+ were isolated and fragmented. Fragmentation by Higher-energy
15 collisional dissociation (HCD) employed a decision tree logic with optimized collision
16 energies (Kolbowski et al., 2017). The fragmentation spectra were then recorded in the
17 Orbitrap with a resolution of 30,000. Dynamic exclusion was enabled with single repeat
18 count and 60-second exclusion duration.

19 The fragment spectra peak lists were generated from the raw mass spectrometric data using
20 msConvert (version 3.0.11729) (Chambers et al., 2012) with default settings. A recalibration
21 of the precursor m/z was conducted based on high-confidence (<1% FDR) linear peptide
22 identifications, using an in-house script (Lenz et al., 2018). The recalibrated peak lists were
23 searched against the sequences and the reversed sequences (as decoys) of cross-linked
24 peptides using the Xi software suite (version 1.6.739) (Giese et al., 2016)
25 (<https://github.com/Rappsilber-Laboratory/XiSearch>) for identification. The following
26 parameters were applied for the search: MS1 accuracy = 3 ppm; MS2 accuracy = 10 ppm;
27 enzyme = trypsin (with full tryptic specificity) allowing up to four missed cleavages; cross-
28 linker = BS3 with an assumed reaction specificity for lysine, serine, threonine, tyrosine and
29 protein N termini); fixed modifications = carbamidomethylation on cysteine; variable
30 modifications = oxidation on methionine, hydrolysed / aminolysed BS3 from reaction with

1 ammonia or water on a free cross-linker end. The identified candidates were filtered to 5%
2 FDR on link level using XiFDR (Fischer and Rappsilber, 2017).

3 **Analysis of cross-linked residue pairs**

4 For mapping of contact sites, kernel density estimation was performed on a per-protein basis
5 for the C-alpha coordinates of cross-link residue pairs present in the respective structures.
6 The coordinates were convolved with a three-dimensional Gaussian kernel (bandwidth: 25
7 Å), and the resulting probability density distributions were sampled at all C-alpha
8 coordinates of the respective proteins.

9 For the determination of long-distance cross-link midpoints, we first mapped each residue
10 onto a unified coordinate system along the arm (running from the head at coordinate 0 to
11 the hinge at coordinate 1). Using this approach, residues that are at the same position along
12 the coiled-coil axis but reside on opposite coiled-coil helices map to the same coordinate. For
13 MukB, we used the coiled-coil register established by disulfide cross-linking to build a
14 piecewise linear interpolation function for the coordinate transformation (Weitzel et al.,
15 2011). For each arm segment with a length mismatch between N- and C-terminal parts we
16 used the shorter part as the length of the segment. Residues located in the head were mapped
17 to coordinate 0, residues in the hinge were mapped to 1, and residues located on either the
18 N- or C-terminal arm helix were mapped to the interval (0, 1) according to the disulfide
19 cross-linking data. We used the same approach for cohesin but with single interval
20 interpolation for the N- and C-terminal helices, respectively, due to the mostly unknown
21 coiled-coil register. Finally, coordinates were scaled to an arm length in amino acids (aa)
22 given by the sum of the individual arm segments (MukB: 365 aa, Smc1/3: 323 aa). Cross-link
23 residue pairs with coordinates transformed according to this procedure were filtered for
24 distances of at least 100 aa, and the corresponding midpoints were determined. Kernel
25 density estimation for the distribution of midpoints was performed by convolution with a
26 Gaussian kernel (bandwidth: 10 aa). Cross-link data are available in **Table S1**.

1 **Purification of the MukB elbow fragment**

2 Residues 333-526 of MukB (GenBank ID: NP_415444.1) were fused to residues 893-1053
3 using an SGGG linker. The construct contained a C-terminal GSHHHHHH tag and was inserted
4 into a pET-16 derived vector using Golden Gate cloning (Engler et al., 2008).
5 Selenomethionine (SeMet) labeled protein was produced in *E. coli* BL21-Gold(DE3) grown
6 in autoinduction medium PASM-5052 at 24 °C (Studier, 2005). Purification was performed
7 at 4 °C. About 40 g of cells were resuspended in 200 mL buffer NA (50 mM sodium phosphate,
8 300 mM NaCl, 40 mM imidazole, 1 mM DTT, pH 7.4 @ 4 °C) containing DNase I, RNase A and
9 protease inhibitors. Cells were lysed in a high-pressure homogenizer at 172 MPa, the lysate
10 was briefly sonicated to reduce viscosity, and was cleared by centrifugation at 96,000 x g for
11 30 min. The extract was passed over a 5 mL HisTrap HP column (GE Healthcare), the column
12 was washed in 10 CV NA and eluted with buffer NB (40 mM sodium phosphate, 240 mM NaCl,
13 400 mM imidazole, 1 mM DTT, pH 7.4 @ 4 °C). The eluate was diluted in 2 volumes of buffer
14 (10 mM Tris, 1 mM TCEP, pH 8.0 @ 4 °C) and loaded onto a 5 mL Q HP column (GE
15 Healthcare). The column was washed with 3 CV of buffer QA (10 mM Tris, 100 mM NaCl, 1
16 mM TCEP, pH 8.0 @ 4 °C) and eluted with a 20 CV gradient into buffer QB (10 mM Tris, 1 M
17 NaCl, 1 mM TCEP, pH 8.0 @ 4 °C). Peak fractions were pooled and concentrated in a Vivaspin
18 10k filter (Sartorius) to about 10 mL before injection into a Sephacryl S200 26/60 column
19 (GE Healthcare) in buffer SEC (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP, 1 mM
20 NaN₃, pH 7.4 @ 23 °C). Peak fractions were pooled, concentrated to 21 mg/mL in a Vivaspin
21 10k filter, aliquoted, frozen in liquid nitrogen and stored at -80 °C. The construct had lost its
22 N-terminal methionine as judged by ESI-TOF mass-spectrometry.

23 **Crystallization of the MukB elbow and structure determination**

24 An aliquot of the MukB elbow construct was thawed and exchanged into buffer X (10 mM
25 Mes, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP, 1 mM NaN₃, pH 6.5 @ 23 °C) using a Zeba Spin
26 column (Thermo Scientific). Crystallization conditions were found by screening a set of 1728
27 conditions using an in-house robotic setup (Stock et al., 2005). Crystals grew as thin plates
28 at 19 °C in sitting drops with mother liquor ML1 (22 % PEG 3350, 0.25 M sodium
29 thiocyanate) or mother liquor ML2 (23.5 % PEG 3350, 2 % PEG 4000, 0.375 M sodium

1 thiocyanate). Crystals mounted in nylon loops were dipped into cryoprotectant solution (23
2 % PEG 3350, 0.257 M sodium thiocyanate, 30 % glycerol in buffer X) before freezing in liquid
3 nitrogen. X-ray diffraction data were collected at Diamond Light Source I04-1 at a
4 wavelength of 0.91587 Å. Several crystals were tested, whereby a crystal grown in ML1
5 diffracted to the highest resolution (2.6 Å) but produced weak anomalous signal. A crystal
6 grown in ML2 diffracted to about 3.0 Å but yielded good anomalous signal. The space group
7 of the crystals was determined as $P2_1$ using Pointless (Evans and Murshudov, 2013).
8 Diffraction data were integrated with XDS, scaled and merged with Aimless, and converted
9 to structure factor amplitudes with Ctruncate (Evans and Murshudov, 2013; Kabsch, 2010).
10 Automated structure solution with CRANK2 using data from the ML2 crystal yielded an
11 almost complete initial model (Winn et al., 2011). This was used as a search model for
12 molecular replacement in Phaser AutoMR with the ML1 dataset (Bunkoczi et al., 2013). A
13 random set of 5 % of the reflections was retained for validation, and the model was rebuilt
14 from scratch using Buccaneer (Cowtan, 2006). The model was iteratively refined by manual
15 building in Coot and reciprocal space refinement using REFMAC5 (Emsley and Cowtan,
16 2004; Murshudov et al., 2011). At later stages, manual building was alternated with
17 reciprocal and real space refinement using Phenix.refine (Afonine et al., 2012). Data
18 collection and refinement statistics are listed in **Table S2**.

1 ***E. coli* strain construction and growth**

2 *E. coli* strains are based on MG1655 (DSM 18039). All chromosomal modifications were done
3 by λ -Red recombineering using a temperature sensitive plasmid carrying the λ phage genes
4 *exo*, *bet* and *gam* under control of the heat-labile CI857 repressor (Datta et al., 2006). A *neoR*
5 coding sequence was joined with a terminator sequence by Golden Gate assembly and the
6 product was integrated downstream of the *mukFEB* terminator. An in-frame deletion of
7 *mukB* and a *mukB-HaloTag* allele were constructed similarly, terminated by the *mukFEB*
8 terminator and linked to the *neoR* cassette downstream of the operon. For construction of
9 marker-free strains carrying point mutations in *mukB*, target regions were first replaced by
10 a cassette containing the counter-selection marker *pheS(R251A, A294G)* (Miyazaki, 2018)
11 linked to a *hygR* selection marker. The cassette was then ejected by recombination with a
12 PCR product containing the point mutation and counter-selection on media containing 2.5
13 mM 4-chlorophenylalanine. Strains with a *mukB* null phenotype were grown on LB or TYE
14 at 22 °C or on M9 (lacking thiamine) at 37 °C. Recombineering plasmids were cured in either
15 LB at 37 °C (functional *mukB* alleles) or in M9 (lacking thiamine) at 37 °C (*mukB* null alleles).
16 Strains were single-colony purified and verified by marker analysis, PCR and Sanger
17 sequencing. Strains are listed in **Table S3**. Phenotypic analysis was performed by streaking
18 on TYE and growth at 37 °C for 13 h.

19 ***E. coli* HaloTag labelling**

20 Cells were grown to stationary phase in LB at 22 °C, diluted in LB to OD₆₀₀ = 0.02, and grown
21 to OD₆₀₀ = 0.3-0.4 at 37 °C (non-permissive temperature). Cultures were mixed with 30 %
22 (w/v) ice and harvested by centrifugation. Cells were resuspended in B-PER (Thermo
23 Fisher) containing 1 mM EDTA (pH 7.4), 5 μ M HaloTag-TMR substrate (Promega), Ready-
24 Lyse lysozyme (Epicentre), Benzonase (Sigma), protease inhibitor cocktail (Roche) and 28
25 mM 2-mercaptoethanol. Samples were incubated for 10 min at 37 °C, mixed with LDS sample
26 buffer (Thermo Fisher), incubated at 95 °C for 5 min and resolved by SDS-PAGE. Gels were
27 scanned on a Typhoon imager (GE Healthcare) using a Cy3 filter setup, and subsequently
28 stained with InstantBlue (Expedeon).

29

1 **Yeast strain construction**

2 Smc1-myc9 with its endogenous promoter was cloned into the LEU2 2 μ plasmid YEplac181
3 and the codon for E620 was replaced with the amber codon TAG. The TRP1 2 μ pBH61
4 expressing the *E. coli* nonsense suppressor tRNA/tRNA synthetase system was a gift from
5 Steven Hahn's lab. The endogenous Scc1 and Pds5 were fused to 9xPK and 6xFLAG epitope
6 tags at their C-terminus, respectively. All strains are derived from the W303 background and
7 are listed in **Table S3**.

8 ***In vivo* photo cross-linking**

9 The yeast strains bearing the TAG-substituted Smc1-myc9 plasmid and pBH61 were grown
10 in -Trp -Leu SD medium containing 1 mM BPA. Cells were collected and resuspended in 1
11 ml of ice-cold PBS buffer. The cell suspension was then placed in a Spectrolinker XL-1500a
12 (Spectronics Corp.) and irradiated by at 360 nm for 2x5 min. Extracts were prepared as
13 described previously (Hu et al., 2011) and 5 mg of protein were incubated with 5 μ l of Anti-
14 PK antibody (Bio-Rad) for 2 hours at 4 °C. Next, 50 μ l of Protein G Dynabeads (Life
15 Technology) were added and incubated overnight at 4 °C to immunoprecipitate Scc1. After
16 washing 5x with lysis buffer the beads were boiled in 2x SDS-PAGE buffer. Samples were run
17 on a 3%–8% Tris-acetate gel (Life Technology) for 3.5 hours at 150 V. For Western blot
18 analysis, Anti-Myc (Millipore) and Anti-FLAG (Sigma) antibodies were used to probe for
19 Smc1 and Pds5, respectively.

20 **Coiled-coil predictions and conservation analysis**

21 A set of SMC sequences and their domain delineations was used for coiled-coil prediction
22 analysis (Bürmann et al., 2017). Individual coiled-coil probability profiles were generated
23 with MARCOIL (Delorenzi and Speed, 2002), and both N- and C-terminal arm regions were
24 extracted. N- and C-terminal profiles were separately aligned on their center coordinates,
25 zero padded and averaged. We estimated 95 % confidence intervals for the averaged profiles
26 using the 5 % and 95 % quantiles of 100 identically processed sequence sets generated by
27 random resampling with replacement.

1 For conservation analysis, MukB sequences were aligned using MSAProbs (Liu et al., 2010).
2 Jensen-Shannon divergences were computed for each alignment column according to (Capra
3 and Singh, 2007), but using equal weights at positions with more than 30 % gaps.

4 **Data availability**

5 Crystallographic structure factors and model coordinates will be deposited in the PDB.

6 **Code availability**

7 The Xi software suite is available at <https://github.com/Rappsilber-Laboratory/XiSearch>.
8 Custom code for statistical analysis is available on request.

9 **Author Contributions**

10 Protein purification, F.B. and B.-G.L.; Electron microscopy, F.B. and B.-G.L.; Mass
11 spectrometry and identification of cross-links, L.S., F.O.; CLMS data analysis and
12 bioinformatics, F.B.; Crystallography, F.B. and J.L.; *E. coli* strain construction, F.B.; Yeast strain
13 construction and *in vivo* cross-linking experiments, T.T.; Conception of the paper model, S.Y.;
14 Preparation of the manuscript with input from all authors, F.B.; Supervision of the work, J.R.,
15 B.H., K.N., J.L.

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1 **Figure Legends**

2 **Figure 1**

3 **Folded conformation of MukBEF and cohesin. (a)** Purification of MukBEF. Elution of the
4 MukBEF complex from a Q ion exchange (IEX) column. Peak fractions were separated by
5 SDS-PAGE and stained with Coomassie Blue. **(b)** Size-exclusion chromatography (SEC) of the
6 MukBEF complex, MukB and MukEF. Proteins were separated on Superose 6 Increase. **(c)**
7 Negative stain EM of native MukBEF. A typical field of view is shown in the top left panel.
8 Particle instances for observed conformations are shown in the top right panel. 2D class
9 averages for the folded conformation are shown on the bottom left, using a circular mask of
10 640 Å. **(d)** Cross-linking of MukBEF with BS3. SEC profiles for native and cross-linked
11 material are shown. **(e)** Negative stain EM microscopy of BS3 cross-linked MukBEF. Typical
12 fields of view for particles from SEC peak 1 and SEC peak 2 are shown. **(f)** Negative stain 2D
13 class averages for extended (top) and folded (bottom) conformations, using circular masks
14 of 948 Å and 640 Å, respectively. Data was collected from samples of peak 1 and peak 2 of
15 the SEC shown in (d). **(g)** Negative stain EM of BS3 cross-linked cohesin. A typical field of
16 view is shown on the left. Class averages using a circular mask of 500 Å are shown in the
17 middle panel.

18 **Figure 2**

19 **Elbow positions revealed by cross-linking/mass spectrometry. (a)** Inter-subunit cross-
20 links of MukBEF. Links are colored according to their false discovery rate (FDR). The bottom
21 panel illustrates a likely topology of the complex. **(b)** Kernel density estimates for the
22 position of cross-link sites mapped onto MukBEF subcomplex structures. Cross-link
23 probability densities were mapped onto the *E. coli* MukEF subcomplex (PDB: 3EUH, top) and
24 the partial structure of the *H. ducreyi* MukBEF head module (PDB: 3EUH, bottom). **(c)** Kernel
25 density estimates for long-distance cross-links at the MukB hinge. Probability density for
26 MukB cross-links to MukB sites located at least 500 aa away (left) or to MukEF (right). The
27 bottom panel illustrates an explanation for the observed cross-linking pattern. **(d)**
28 Identification of the MukB elbow region. Long-distance cross-links in a coordinate system
29 along the coiled-coil arm and their midpoints are shown on top. The bottom panel shows the

1 kernel density estimate for the midpoint positions. An inset shows the piecewise
2 interpolation function used to map residue numbers to the arm coordinate system. **(e)**
3 Kernel density estimates for the position of cross-link sites mapped onto cohesin
4 subcomplex structures (PDB: 4UX3, top; 1W1W, bottom). The top panel illustrates the
5 topology of the complex. **(f)** Identification of the cohesin elbow region as in (d).

6 **Figure 3**

7 **Structure of the MukB elbow. (a)** Crystal structure of an *E. coli* MukB arm fragment. The
8 top panel illustrates the design of the fusion construct used for crystallography. The bottom
9 panel shows the refined atomic model obtained from the X-ray diffraction experiment. **(b)**
10 Identification of the elbow. Cross-link midpoint density (see **Fig. 2d**) was mapped onto the
11 structure (left). The right panel shows sequence conservation (Jensen-Shannon divergence)
12 mapped onto the structure (high conservation: purple, low conservation: cyan). **(c)**
13 Structure of the elbow. The C-terminal coiled-coil helix is distorted (kinked) close to the
14 conserved Tyr416 on the N-terminal helix. Residues for visual reference between the views
15 are shown in grey. Residues targeted by mutagenesis (**Supplementary Fig. 3**) are
16 highlighted in black.

17 **Figure 4**

18 **In vivo cross-linking of Pds5 to the Smc1 hinge. (a)** Illustration of the BPA cross-linking
19 experiment (top) and mapping of tested BPA substitutions onto a homology model of the
20 cohesin hinge (bottom). **(b)** Screen for Smc1(BPA) cross-links to Pds5 and Smc3. BPA was
21 incorporated into the indicated Smc1 positions, cells were treated with UV, cohesin was
22 immunoprecipitated via a PK9-tag on Scc1 and products were analyzed by Western blotting.
23 **(c)** UV-dependent cross-linking of Smc(K620BPA) and Pds5. Cells were either treated or not
24 treated with UV and products were analyzed as in (b). **(d)** Cross-linking of Smc(K620BPA)
25 and Pds5 depends on Pds5 binding to Scc1. The left panel shows the position of Scc1 V137
26 in its Pds5 binding site (mapped to the *L. thermotolerans* structure, PDB: 5F00). The right
27 panel shows a cross-linking experiment of Smc1(K620BPA) in the presence of Scc1(V137K)-
28 PK9 as in (b).

1 Figure 5

2 **Conservation of the SMC elbow.** Coiled-coil prediction profiles for a diverse set of SMC
3 protein sequences were generated by MARCOIL. Profiles for N- and C-terminal parts of the
4 arms were separately aligned on their center coordinate and averaged. 95 % confidence
5 intervals (purple shading) were estimated by 100 times random resampling with
6 replacement. The Mms21 binding site of Smc5 is highlighted in green. N, number of
7 sequences used for generating the respective aggregate profiles.

8 Figure 6

9 **Model for DNA translocation by relative movements of DNA binding sites. (a)** Model for
10 conformational switching of SMC-kleisin complexes. Transitioning between extended and
11 folded states might be driven by the ATPase cycle introducing mechanical strain into the SMC
12 arms. **(b)** Paper model: conversion between extended and folded states is achieved by
13 twisting the arms of the model. **(c)** Models for DNA translocation and loop extrusion
14 involving a folded state. (left) “Inchworm translocation” using distance changes between two
15 DNA binding sites, one of which might be a topological entrapment device/ring. Folding at
16 the elbow might cause the distance change. (right) Translocation using the segment-capture
17 mechanism that enlarges a loop held in a bottom chamber by merging with a smaller loop
18 captured in a top chamber. Folding at the elbow might drive DNA from top to bottom.

19 Supplementary Figure 1

20 Cryo-EM of *Desulfovermiculus halophilus* MukBEF. Particles were imaged in unsupported
21 vitreous ice and contrast was enhanced by use of a Volta phase plate. The presence of an
22 elbow is indicated by a sharp central kink in the arm of several particles. Sequence identity
23 between *D. halophilus* and *E. coli* MukBEF complexes is ~26 %.

24 Supplementary Figure 2

25 Cross-linking and mass spectrometry of MukBEF and cohesin. **(a)** SEC profiles of native co-
26 expressed MukBEF (blue), BS3 treated co-expressed MukBEF (orange), singlet MukBEF
27 (MukBEF^S) reconstituted in buffer containing 40 mM NaCl, 2 mM MgCl₂ (red) and doublet

1 MukBEF (MukBEF^D) reconstituted in buffer containing 200 mM NaCl (green). Reconstitution
2 was similar to the protocol established in (Petrushenko et al., 2006). **(b)** SDS-PAGE analysis
3 of a purified cohesin complex containing Smc1, Smc3, Scc1 and Scc3. The gel was stained
4 with Coomassie. **(c)** SEC profiles of the cohesin complex containing Smc1, Smc3, Scc1 and
5 Scc3 before and after treatment with BS3 (see **Fig. 1g**). **(d)** Inter-subunit cross-links of a
6 cohesin complex containing Smc1, Smc3, Scc1, Scc3 and Scc2. As in Fig. 2a. **(e)** Cross-link
7 midpoint analysis for MukB performed as in **Fig. 2d** but using random resampling without
8 replacement before data processing. **(f)** Cross-link midpoint analysis for various cohesin
9 datasets (as in **Fig. 2**). Peak density for human cohesin corresponds to residues 375/813
10 (Smc1) and 379/811 (Smc3), respectively.

11 **Supplementary Figure 3**

12 Mutagenesis of the MukB elbow. **(a)** Sequence alignment of the N-terminal (left) and C-
13 terminal (right) parts of the MukB elbow. The mutated residues are highlighted by triangles.
14 *Eco*, *Escherichia coli*; *Mmo*, *Morganella morganii*; *Tmo*, *Thioflavicoccus mobilis*; *Emo*,
15 *Endozoicomonas montiporae*; *Tau*, *Tolumonas auensis*; *Osp*, *Oceanimonas sp. GK1*; *Btr*,
16 *Bibersteinia trehalosi*; *Hdu*, *Haemophilus ducreyi*. **(b)** Growth of strains containing point
17 mutations at the elbow in the endogenous *mukB* gene. **(c)** Construction of a functional *mukB*-
18 *HaloTag* allele. **(d)** Protein levels of elbow mutants fused to a HaloTag. Extracts were labelled
19 with a HaloTag-TMR substrate and were analyzed by in-gel fluorescence (top) and
20 Coomassie staining (bottom) after SDS-PAGE. WT, wild-type.

21 **Supplementary Figure 4**

22 BPA-dependent expression of Smc1(K620BPA). Strains were grown either in the absence or
23 presence of 1 mM BPA, and extracts were analyzed by Western blotting.

24 **Supplementary Figure 5**

25 The elbow of *P. yayanosii* Smc. The predicted coiled-coil probability from aggregate analysis
26 of 3115 different prokaryotic Smc proteins (see **Fig. 5**) is mapped onto the crystal structure
27 of a central arm fragment from *P. yayanosii* (PDB: 5XG2). Positions of the predicted and
28 chrystrallographically determined discontinuities are shown.

1 **Supplementary Figure 6**

2 Bending of SMC dimers. **(a)** An SMC dimer with C2 symmetry. Monomers and their body-
3 frame coordinate systems are shown in black or blue. The symmetry axis of the dimer is
4 shown in purple. **(b)** Symmetry breaking upon elbow bending. Option 1: monomers bend
5 into opposite directions; Option 2: monomers twist and bend into the same direction.
6 Orientations of the relevant body-frame coordinate axes are shown at the bottom.

7 **Supplementary Figure 7**

8 **Detailed models for DNA and translocation and loop extrusion. (a)** DNA translocation
9 model requiring a regulated grapple DNA binding site and a sliding anchor DNA binding site.
10 DNA binding may or may not involve a DNA entrapping ring that could be used to enhance
11 processivity. **(b)** Loop extrusion using a second anchor site. DNA binding may or may not
12 involve a DNA entrapping ring that could be used to enhance processivity.

1 **Supplementary Tables**2 **Supplementary Table 1**

3 Cross-linking/mass spectrometry are available as a separate table online.

4 **Supplementary Table 2****Crystallography table**

Dataset	MukB elbow SeMet (Crystal 1)	MukB elbow SeMet (Crystal 2)
Construct	(333-526, SGGs, 893-1053)MukB-GSHHHHHH	(333-526, SGGs, 893-1053)MukB-GSHHHHHH
GenBank ID	NP_415444.1	NP_415444.1
Data collection		
Beamline	DLS I04-1	DLS I04-1
Wavelength [Å]	0.91587	0.91587
Data reduction		
Resolution [Å]	40.8-2.6 (2.72-2.60)	42.4-3.0 (3.18-3.00)
Space group	P2 ₁	P2 ₁
Cell dimensions [Å]	81.12, 35.04, 81.71	80.92, 35.01, 84.89
Completeness [%]	99.9 (100)	99.8 (99.8)
Multiplicity	6.5 (6.9)	6.7 (6.8)
Anomalous completeness [%]		99.3 (99.5)
Anomalous multiplicity		3.5 (3.5)
Anomalous correlation		0.437 (0.005)
I / σ	9.7 (2.0)	16.1 (2.2)
R _{pim}	0.041 (0.390)	0.031 (0.312)
CC1/2	0.998 (0.917)	0.999 (0.969)
Phasing		
Scatterer		Se
Number of sites		6
Model refinement		
Modeled residues	(335-526)-SGGS-(893-1047)	
Coverage [%]	95.9	
R / R _{free}	0.243 / 0.297	
Bond length RMSD [Å]	0.002	
Bond angle RMSD [°]	0.43	
MolProbity score	1.14 (100th percentile)	
Ramachandran favored [%]	97.4	
Ramachandran disallowed [%]	0.3	

PDB ID to be released

1 **Supplementary Table 3**

2

Strain ID	Genotype	Figures
<i>E. coli</i> strains		
MG1655	F ⁻ , λ ⁻ , <i>rph-1</i> , <i>fmr+</i>	S3b, S3c
SFB012	MG1655, <i>mukB::neoR</i>	S3c
SFB017	MG1655, <i>mukB-HaloTag(C61V, C262A)::neoR</i>	S3c, S3d
SFB018	MG1655, Δ <i>mukB::neoR</i>	S3b, S3c
SFB022	MG1655, <i>mukB(Y416D)</i>	S3b
SFB025	MG1655, <i>mukB(Y416P)</i>	S3b
SFB026	MG1655, <i>mukB(L960E)</i>	S3b
SFB030	MG1655, <i>mukB(Y416D)-HaloTag(C61V, C262A)::neoR</i>	S3d
SFB031	MG1655, <i>mukB(Y416P)-HaloTag(C61V, C262A)::neoR</i>	S3d
SFB032	MG1655, <i>mukB(L960E)-HaloTag(C61V, C262A)::neoR</i>	S3d
<i>S. cerevisiae</i> strains		
W303	Mat a, <i>ade2-1</i> , <i>trp1-1</i> , <i>can1-100</i> , <i>leu2-3, 112</i> , <i>his3-11, 15</i> , <i>ura3</i> , GAL, psi	-
2017	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH826 (<i>Smc1(D588TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2018	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH827 (<i>Smc1(E562TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2019	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH828 (<i>Smc1(T565TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2020	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH829 (<i>Smc1(K620TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2021	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH830 (<i>Smc1(E591TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2022	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH831 (<i>Smc1(D592TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2023	W303, <i>Smc3-HA6::HIS3</i> , <i>Scs1-PK9::NatMX</i> , pBH832 (<i>Smc1(D593TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2069	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH826 (<i>Smc1(D588TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2070	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH827 (<i>Smc1(E562TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2071	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH828 (<i>Smc1(T565TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2072	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH829 (<i>Smc1(K620TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b, c, S4
2073	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH830 (<i>Smc1(E591TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2074	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH831 (<i>Smc1(D592TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2075	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>Scs1-PK9::NatMX</i> , pBH832 (<i>Smc1(D593TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4b
2221	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>ura3::Scs1-PK9::URA3</i> , pBH829 (<i>Smc1(K620TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4d
2223	W303, <i>Pds5-6xHis-6xFLAG::KanMX</i> , <i>ura3::Scs1(V137K)-PK9::URA3</i> , pBH829 (<i>Smc1(K620TAG)-myc9</i> in YEplac181), pBH61 (BPA crosslink, Trp1)	4d

2357 W303, Pds5-6xHis-6xFLAG::KanMX, Scc1-PK9::NatMX, pBH768 (Smc1-myc9 in YEplac181), 4c, S4
pBH61 (BPA crosslink, Trp1)

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Figure 2

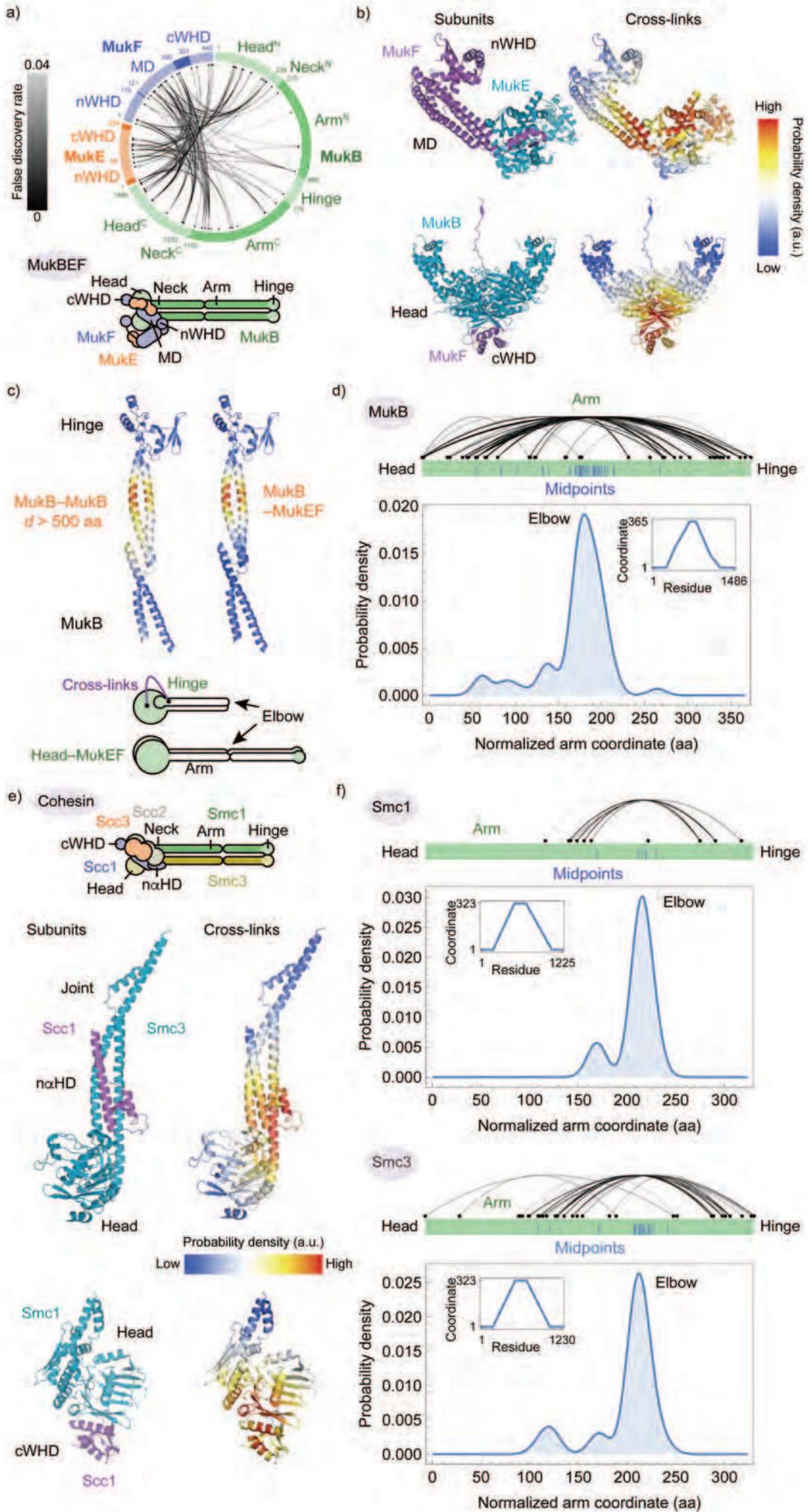


Figure 3

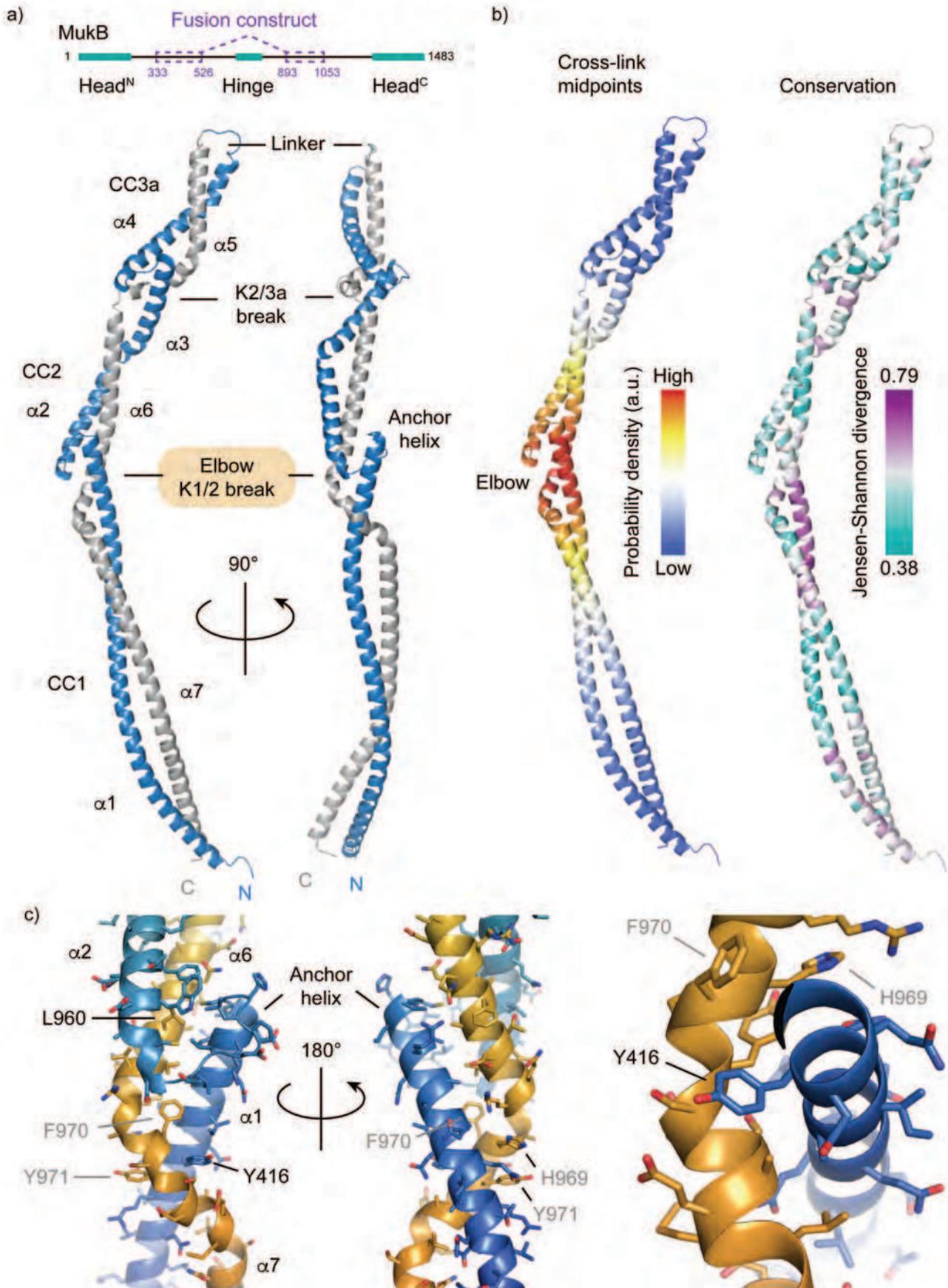


Figure 4

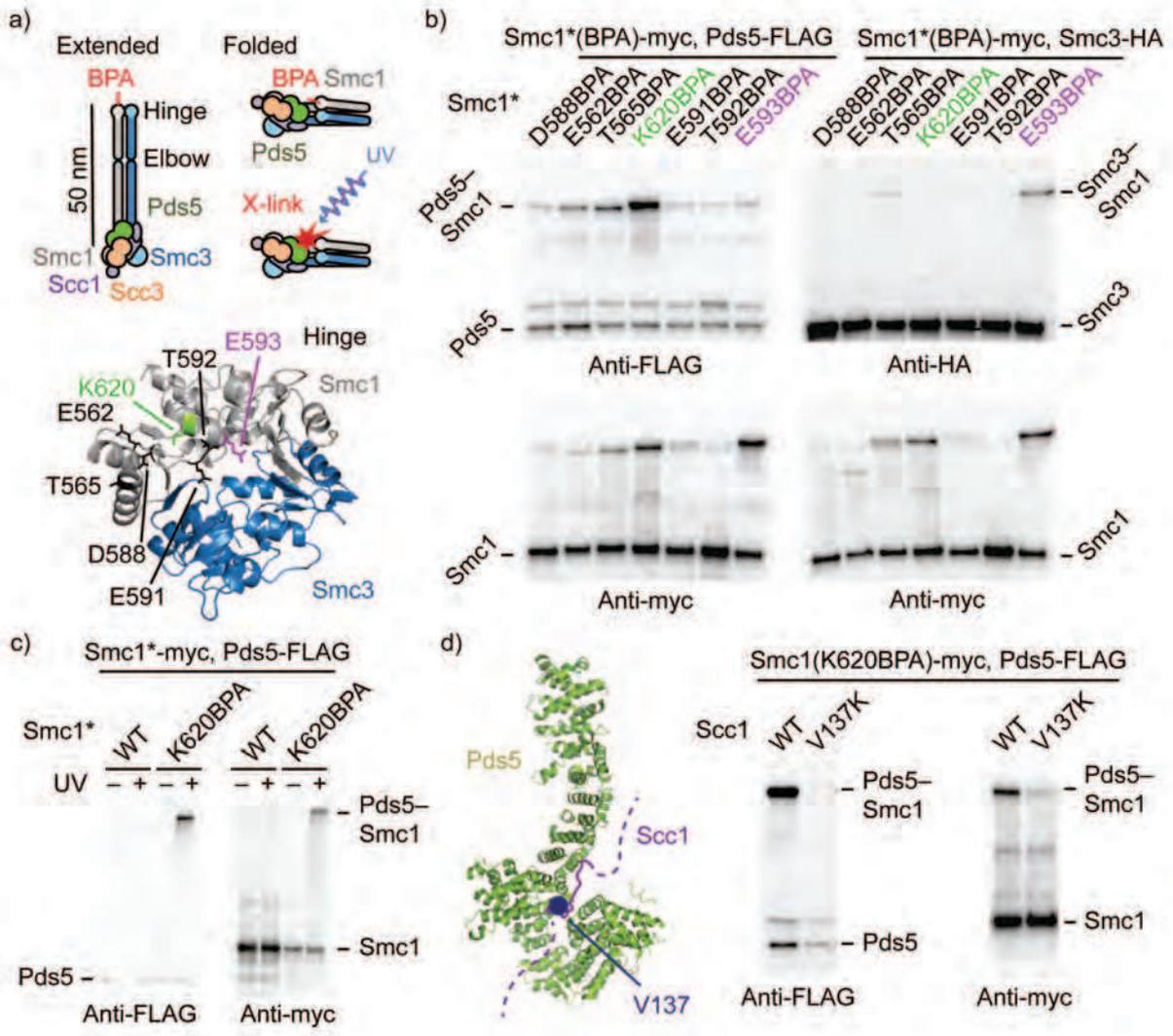


Figure 5

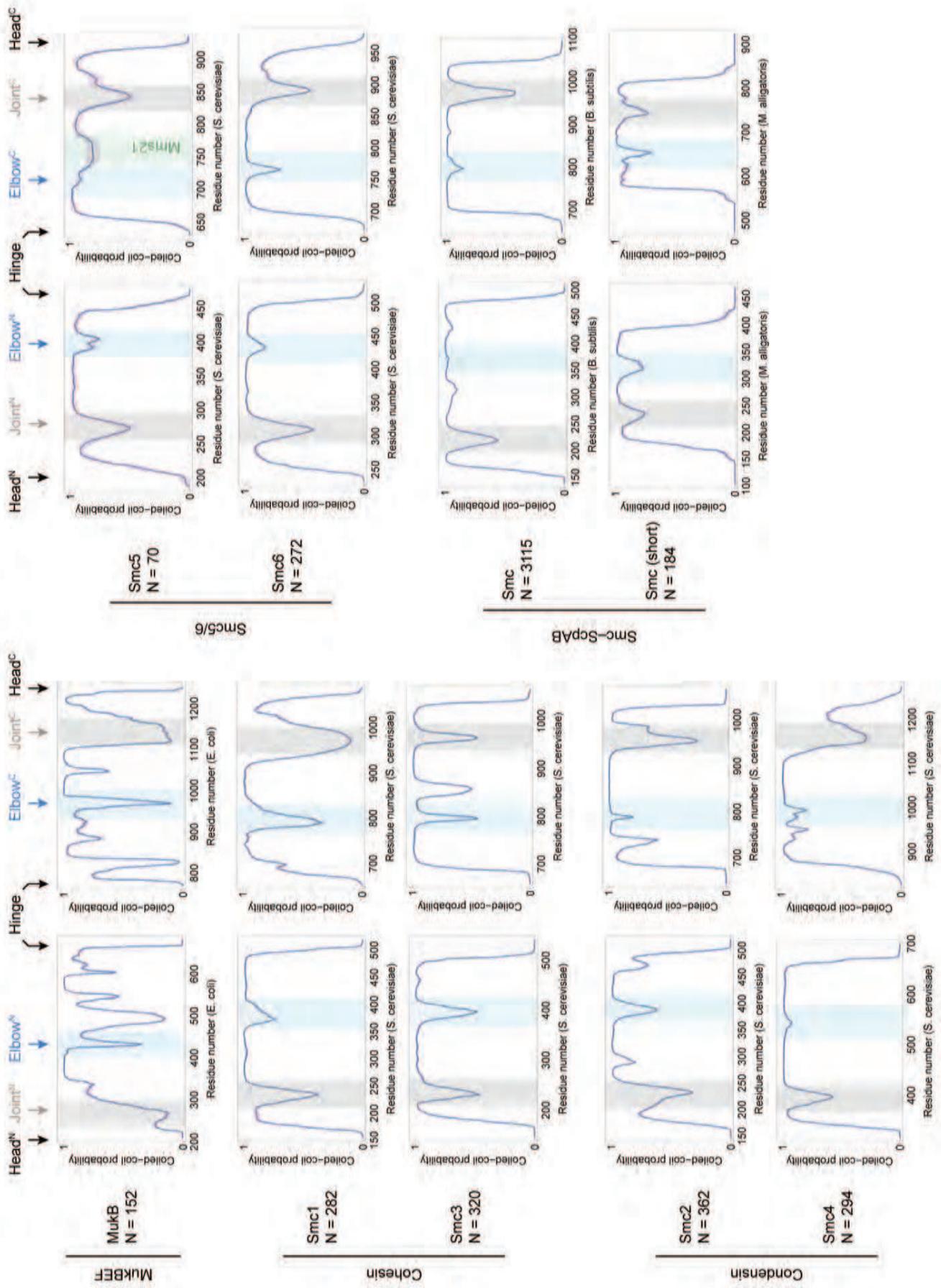
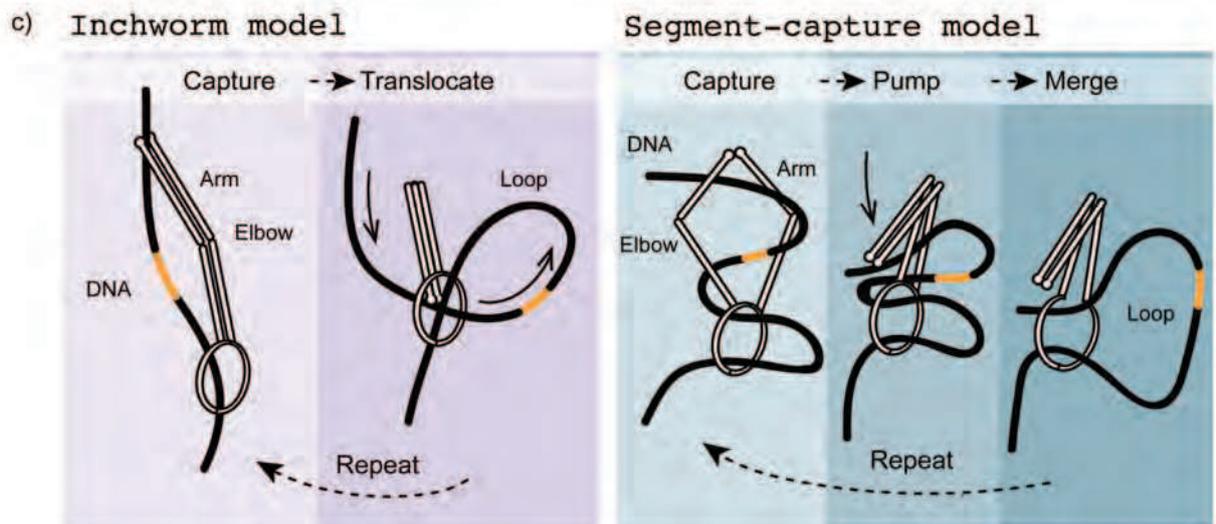
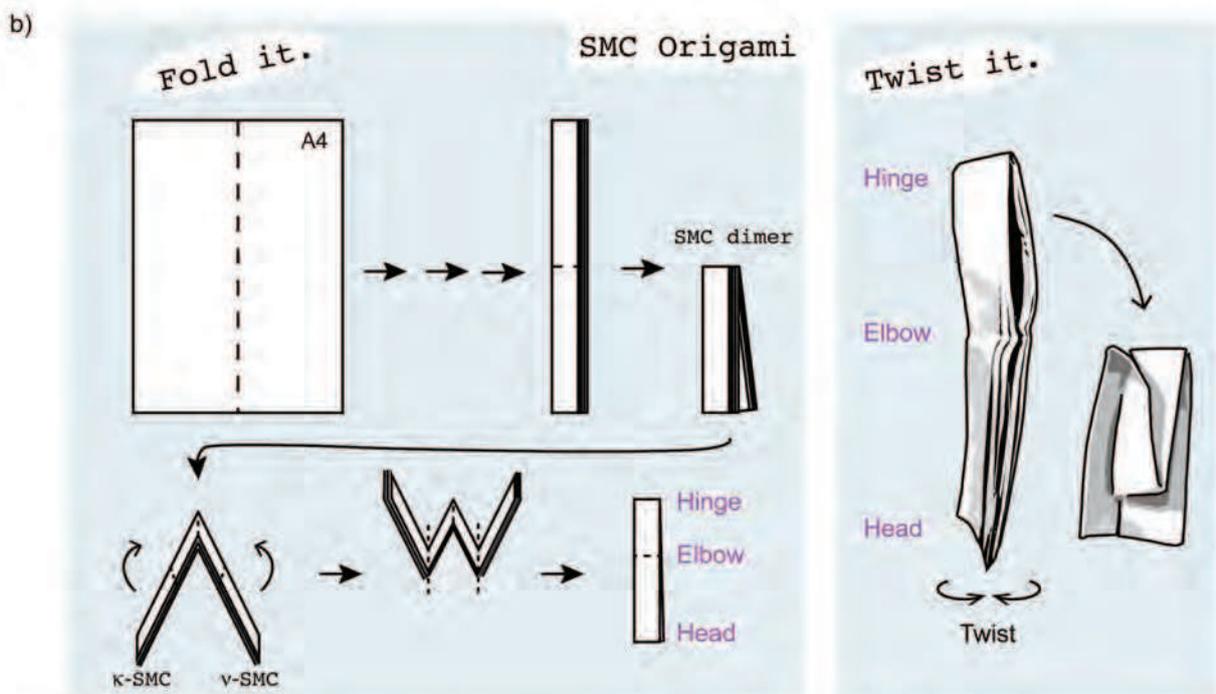
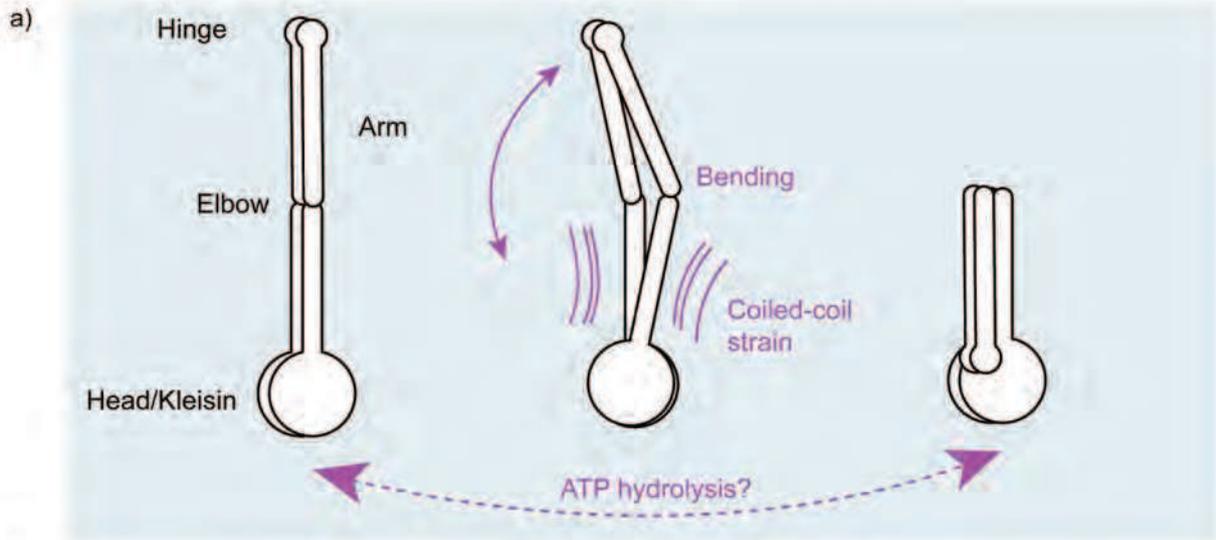


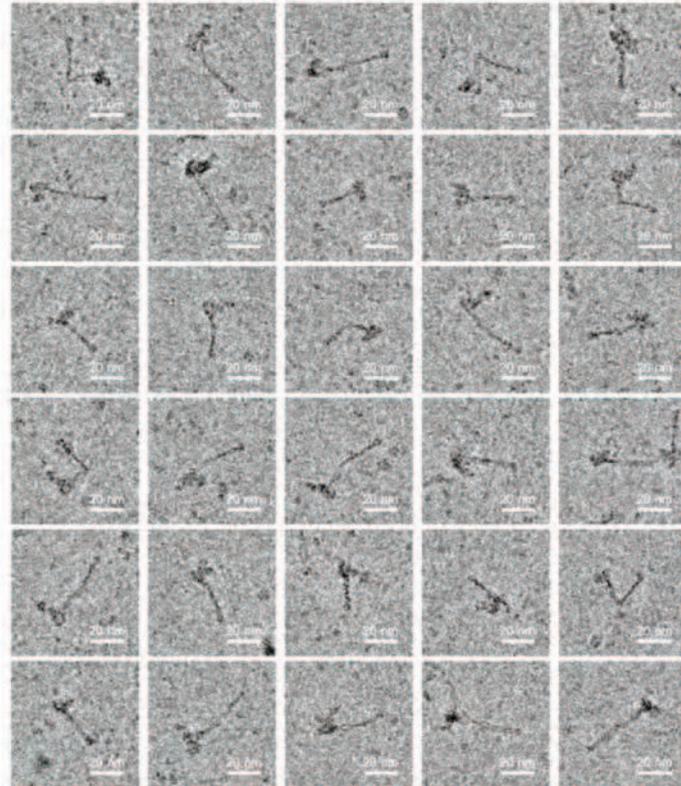
Figure 6



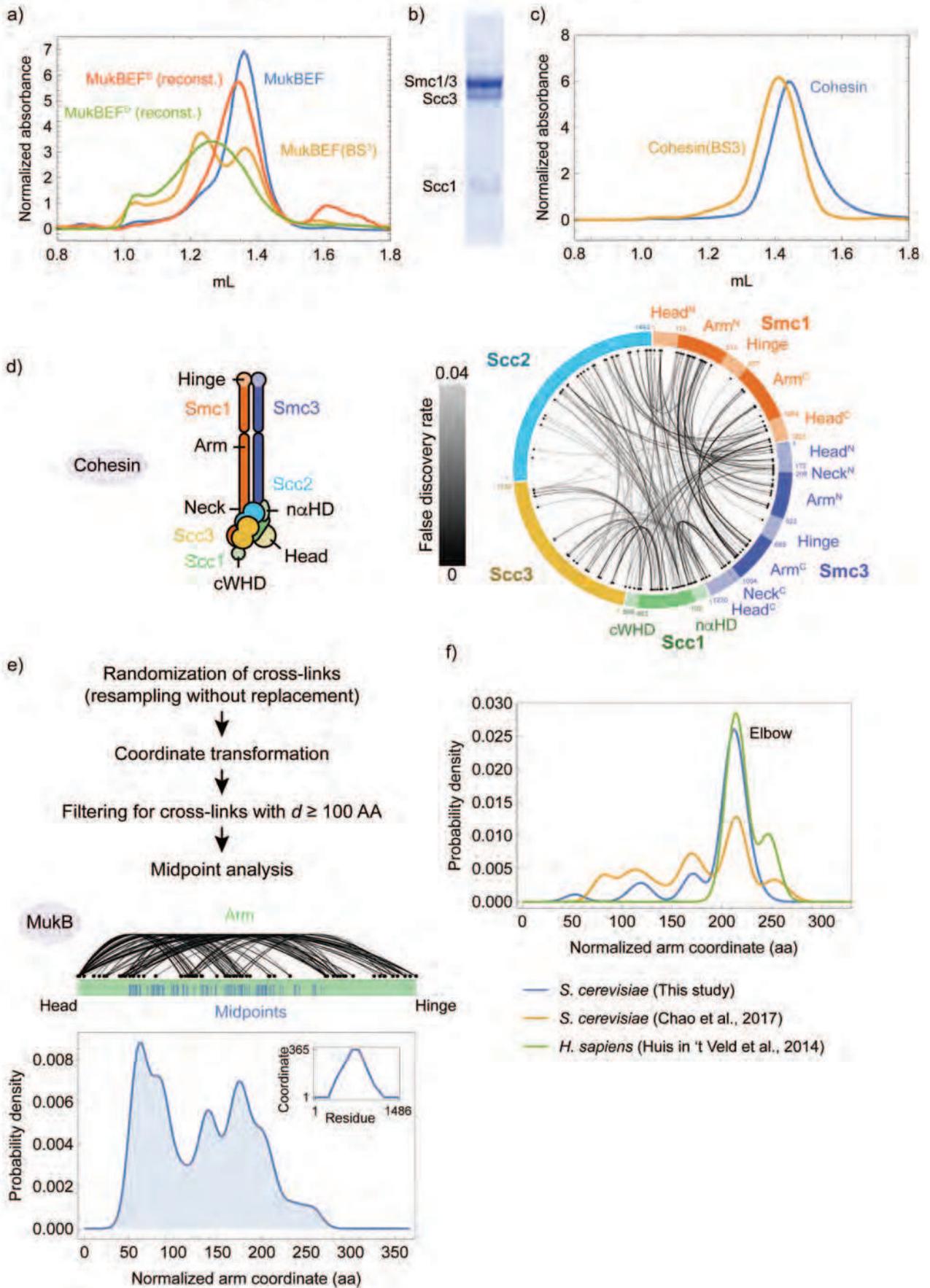
Supplementary Figure 1



Cryo-EM

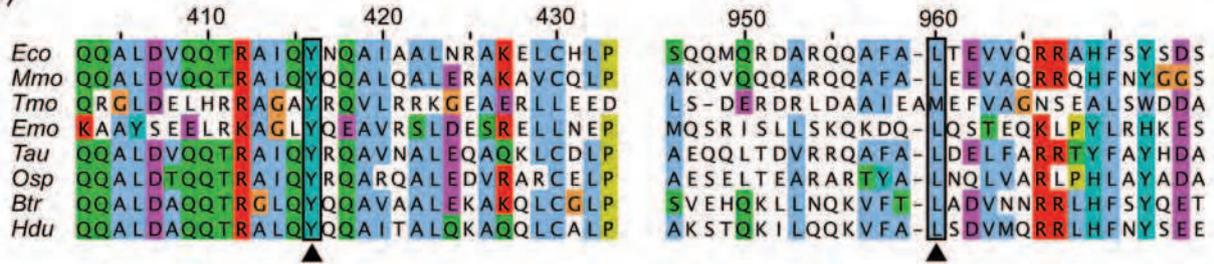


Supplementary Figure 2



Supplementary Figure 3

a)



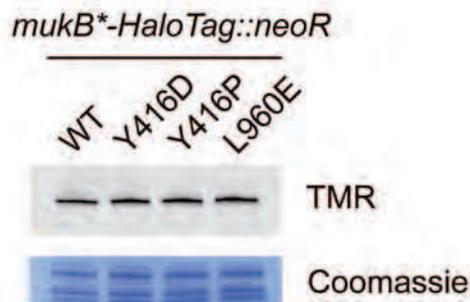
b)



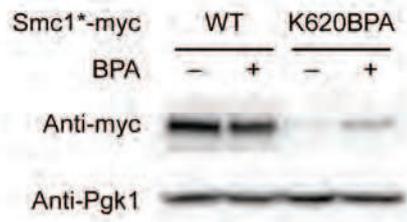
c)



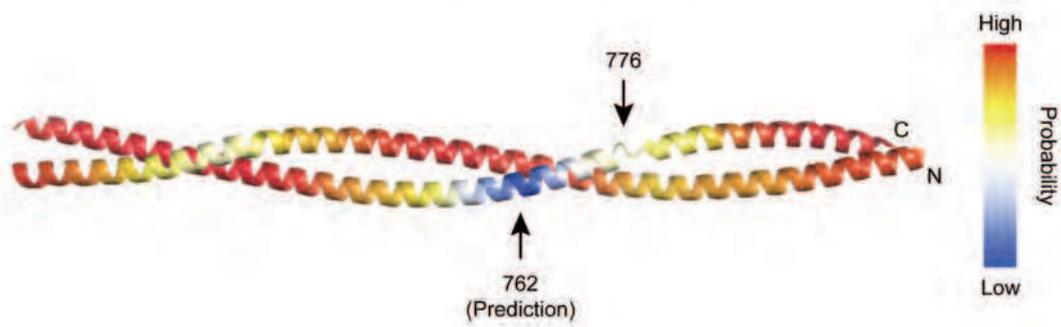
d)



Supplementary Figure 4



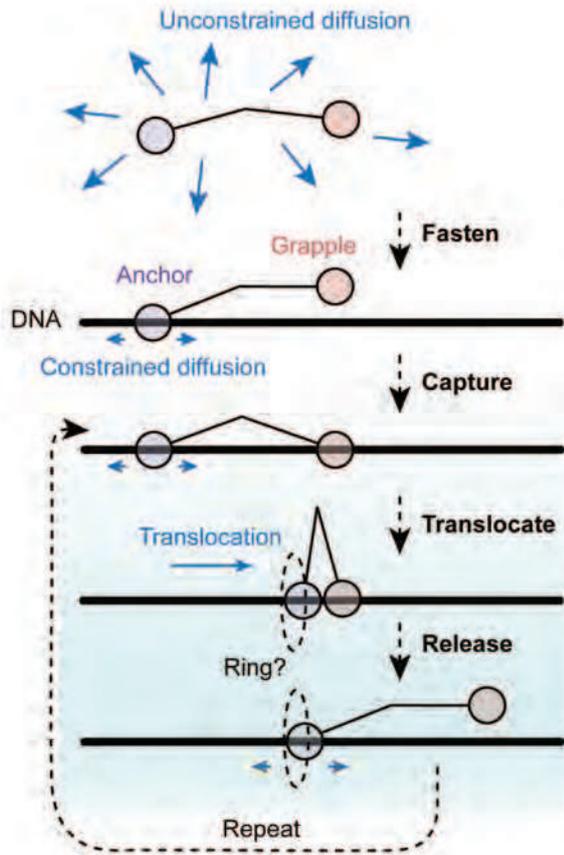
Supplementary Figure 5



Supplementary Figure 7

a)

Translocation



b)

Loop extrusion

