Catching DNA with hoops-biophysical approaches to clarify the mechanism of SMC proteins

Eeftens, Jorine; Dekker, Cees

DOI
10.1038/nsmb.3507

Publication date
2017

Document Version
Accepted author manuscript

Published in
Nature Structural and Molecular Biology

Citation (APA)
Eeftens, J., & Dekker, C. (2017). Catching DNA with hoops-biophysical approaches to clarify the mechanism of SMC proteins. *Nature Structural and Molecular Biology*, *24*(12), 1012-1020. https://doi.org/10.1038/nsmb.3507

Catching DNA with hoops – biophysical approaches to clarify the mechanism of SMC proteins

Jorine Eeftens & Cees Dekker

Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, Netherlands

Abstract

Structural Maintenance of Chromosome (SMC) complexes are vital regulators of chromosome architecture, and essential in all domains of life from bacteria to humans. For decades, the field has been debating how these SMC protein complexes are able to mechanistically use their intricate ring-like structure to structurally organize DNA. Single-molecule biophysical techniques might be key to resolve the molecular mechanism of SMC proteins. This review provides an overview of insights obtained so far with such biophysical methods.

Genomes of different organisms vary greatly in size, from a million to hundred billion base pairs, but all share the challenge that they need to be squeezed into a micron-size cell that is many orders of magnitude smaller than the length of the DNA. The spatial organization of the genome within cells is an intriguing scientific question of strong current interest. Structural Maintenance of Chromosome (SMC) protein complexes are the key players in the spatiotemporal organization and maintenance of DNA from bacteria to humans. They are essential for many chromosomal processes such as compaction, chromosome segregation, DNA repair, and gene regulation[1–3].

SMC protein complexes have a unique structural organization, which is characterized by a ring shape consisting of three proteins along its circumference: two SMC proteins complemented by a kleisin subunit (Figure 1a). The main part of the SMC subunits involves a ~50nm long antiparallel coiled coil, connecting a hinge domain on one end with ATPase heads on the other end. The SMC heads are ABC transporter ATPases, characterized by WalkerA and WalkerB motifs. All SMC rings associate with different subunits and co-factors to form functional complexes (Fig.1bc)[4].

The ring-like structure is very well conserved, and thus of vital importance for the function of SMC proteins. Prokaryotes only have a single type of SMC complex. The well-characterized BsSMC in *Bacillus subtilis*, for example, contains a homodimer of SMC proteins and the kleisin protein ScpA, whereas sub-families of γ-proteobacteria (e.g. *E. Coli*) have an SMC complex called MukBEF (Fig. 1b). The structure of MukBEF slightly deviates from the other SMC complexes, as the MukF kleisin domain forms dimers, thus allowing the formation of multimers of SMC complexes[5]. Deletion or mutation of Smc or MukBEF leads to severe chromosomal defects, including disruption of nucleoid structure and failure to segregate sister chromatids [6–9].

In eukaryotes, the SMC complex has evolved to three types of protein complexes that are all essential, but that have different, partially overlapping functions: cohesin, condensin, and Smc5/6 (Fig. 1c). Cohesin is responsible for faithful chromosome segregation during cell division, as it holds sister

chromatids together while they align under the tension of the mitotic spindle (Fig. 2a)[10]. Most of the cohesin is removed from the chromosome arms in prophase, but some stays bound at centromeres until the onset of anaphase, when its kleisin Scc1 is cleaved by separase to release the cohesion[10,11]. In addition, cohesin plays an important role in gene expression (Fig. 2b, recently reviewed in Ref.[12]). Condensin is the main factor in mitotic chromosome assembly (Fig. 2a). Most eukaryotes have two condensin complexes, Condensin I and Condensin II, that work together to ensure proper DNA compaction and segregation[13]. Like cohesin, condensin also has non-mitotic chromosome functions, such as gene regulation, dosage compensation, DNA damage response, and DNA repair[3,14,15]. Finally, the Smc5/6 complex is the least well-understood SMC complex. It is needed for double-strand break repair, while it also has a role in chromosome segregation [16–19].

SMC proteins in chromosome organization

The spatial organization of the genome is a topic of intense current study[20]. Genome mapping studies have provided ample evidence for topological domains and loop formation. How exactly such loops are established and stabilized is still unclear, but SMC proteins are the main candidates for directing these processes. A topological embrace of DNA, where the SMC complex encompasses one or more DNA molecules, is thought to be the basis for the function of the ring-shaped SMC complexes, and this unique principle has been the starting point for many studies on the molecular mechanism[21–24].

The classic, most simple model for chromosome organization by SMC complexes is that random DNA-DNA crosslinks are formed which can be established by trapping DNA inside the SMC ring [25,26]. Using condensin, DNA compaction could be achieved by grabbing two DNA strands and connecting them together inside the condensin ring (Fig. 2c). Linking could be realized by a single SMC ring, or by two mutually interacting SMC rings. The same principle can be applied to cohesin in the context of loop formation and sister-chromatid cohesion. A stochastic non-specific linking does not explain how chromosomes arrange into elongated loop structures, instead of an entangled

random-blob spatial arrangement and mutually cross-linked sister chromatids. To test if crosslinking suffices to compact DNA into chromosomes, a computer-simulation study modeled chromosome compaction as stochastic pairwise bonding between condensin molecules that connect distant DNA sites[27]. This pairwise interaction model condensed the DNA accurately and matched the Hi-C data, indicating that this simple model can go a long way to explain basic features of DNA compaction.

Recently, an alternative model, the so-called loop-extrusion model, gained a lot of attention (Fig. 2d) [28–30]. In this model, an SMC protein binds DNA, initiates formation of a loop, and translocates DNA through its ring to form an extending DNA loop [31,32]. Such a principle could be employed by condensin to compact DNA into mitotic chromosomes, or by cohesin to establish loop formation in TADs. For example, cohesin might halt and anchor the loop when it encounters two CTCF sites. Alipour and Marko first simulated a 1D model with condensin as a loop-extruding enzyme machine that employs two DNA-binding sites per protein[29]. The assumption was that each binding site moves along the DNA, away from the other binding site, in an ATP-hydrolysis-dependent manner. This drives the extrusion of a loop. The authors found that under certain association and dissociation conditions, two possible outcomes could result: either formation of loops of variable size with gaps in between, or a stack of proteins anchoring a single loop. This model was recently applied on a larger scale in two independent studies [31,33]. While these modeled general “extrusion factors”, it was speculated that these factors in fact could be cohesin molecules. Sanborn et al assumed that each SMC extruder would stop extruding upon recognition of a CTCF motif of the correct directionality [33]. This led to formation of stable loops in a manner that was consistent with the experimental Hi-C data that were reported in the same paper. A second study by Fudenberg et al. came to the same conclusion [31]. Yet another large-scale study took parameters from experimental studies to model DNA compaction with condensin as the loop-extruding factor [34]. Depending on parameters, these simulations showed either loops separated by gaps, or tightly stacked loop-arrays (the latter consistent with Hi-C data). The authors showed that one condensin per 10-30kb could lead to loop sizes consistent with Hi-C data.

Loop extrusion by condensin was also shown to be able to compact chromatin into the dense structure characteristic for sister chromatids[35].

Although the random-crosslinking and loop-extrusion models are exemplary for the two major classes of models, many more variations have been proposed throughout the years, including clustering-, translocation-, and supercoiling-based models [36–40]. Many questions remain to be answered for unraveling the mechanism of the SMC protein complexes. At a mechanistic level, we are still in the dark on how exactly SMC complexes interact with DNA. For example: what conformational changes occur within SMC complexes? What is the role of ATP binding and hydrolysis? What is the dynamics of loading and unloading? Is loop extrusion really the all-explaining mechanism? Where are SMC complexes loaded, what drives their processivity, and how do they know when to stop? How does cohesin recognize CTCF orientation? And if all these questions can be addressed: is this mechanism the same for all SMC proteins? How do cohesin, condensin, and Smc5/6 differ? How are these eukaryotic SMC proteins different compared to their prokaryotic counterparts? To what extent is the molecular mechanism different between organisms? Despite numerous cell-biology and biochemical studies in the past decades, many questions thus remain and there is a need for new approaches. As the nature of the most important questions is mechanistic, we feel that single-molecule biophysical techniques are particularly fit to address these issues[41].

Excitingly, in the last five years, a lot of progress has been made on purification of several SMC complexes, enabling researchers to do more *in vitro* type of work[42]. While virtually impossible to deduce from bulk experiments, the mechanical properties of proteins can be probed with various biophysical techniques at the level of individual molecules. The mechanical properties of SMC complexes are of particular interest from a biophysical perspective. SMC rings must withstand external forces in the cell throughout various stages of the cell cycle, such as segregation, and thus must be strong and stable in their association with DNA[43]. Forces can be applied and probed with methods such as magnetic tweezers (Fig.3c), optical

tweezers, and atomic force microscopes. The two most common techniques used to visualize SMC complexes at the single-molecule scale are transmission electron microscopy (TEM, Fig.3a) and atomic force microscopy (AFM, Fig.3b). Visualization of protein-DNA interactions is also possible with optical techniques such as DNA flow stretching (Fig.3d) [44] and DNA curtains (Fig.3e)[45] – techniques that rely on visualizing a stretched DNA molecule with fluorescence microscopy. With Fluorescent Resonance Energy Transfer (FRET), the interaction between two molecules, or two sites within the same molecule, can be investigated (Fig.3f) [46]. Some of the possibilities and limitations of these techniques are summarized in Table 1. Below, we review results obtained with these single-molecule techniques on SMC protein complexes.

Single-molecule imaging of SMC complexes

Their large, multi-subunit architecture make SMC complexes difficult to purify, and structural information is hard to obtain[47]. Throughout the years, parts of SMC subunits have been crystalized, though crystal structures of full SMC complexes are not available (for a recent review on crystallography, see Ref.[47]). Accordingly, most of the information that we have on global SMC architecture is from real-space imaging techniques such as atomic force microscopy and electron microscopy.

TEM can yield high quality images using low-wavelength electrons (Fig. 3a). An electron source emits electrons that are focused into a thin beam that hits the sample (stained with for example heavy metals for increased contrast), where some electrons are scattered, while most travel through, creating a "shadow image" of the sample. Potential artifacts can occur in the sample preparation by transferring proteins from solution to air to vacuum, a challenge which recently has largely been overcome by cryo-EM[48] (which, to our knowledge, has not been applied to SMC complexes yet).

In AFM, a sharp tip at the end of a cantilever scans the surface of the sample of interest, oscillating near its resonance frequency (Fig. 3b). The oscillation of the tip is changed as the tip interacts with the sample, and the deflection is

detected by a photo diode. This information is then translated into a topological image with nanometer resolution. Conventional AFM can be used to take high-resolution static snapshots of molecules on a surface. Due to recent technical advances, it is now also possible to observe the motion of single molecules in real-time with high-speed AFM, that can acquire images at a video rate of 20 images per second[49,50]. In AFM, there is no need for labeling or staining the sample, but a fundamental limitation of both EM and AFM is that proteins need to be bound to a surface for visualization. We note that some caution is needed when interpreting images from most EM and AFM reports, as these techniques dry the molecules, which possibly can kinetically trap them into a nonphysiological conformation.

Despite these potential caveats, imaging techniques have provided a number of valuable insights 1) on the structure of SMC subunits, 2) the shape and dynamics of full SMC complexes, and 3) their interaction with DNA. Specifically, researchers have tried to classify the shape of the SMC dimers and complexes using the letter system depicted in Figure 4a. This is of interest because the shape may relate directly to the SMC function: interaction between the heads will close the loop, interaction between heads and hinge may indicate an intermediate for loading, stiff rods could indicate that the SMC dimers are clamped onto DNA, etc. So far, the imaging efforts have yielded widely scattered results for different species of SMC complexes and varying conditions.

The first images of SMC proteins appeared in the early nineties, when bacterial MukB dimers were visualized with low-angle rotary-shadowing EM[51]. This was the first publication that reported the globular structures (heads and hinge) separated by coiled-coil segments, establishing a key step in determining the structure of SMC proteins. Several years later, higher-resolution EM imaging of MukB and BsSmc dimers revealed another crucial characteristic of SMC proteins: the antiparallel arrangement of the coiled coils, that brings the C- and N-terminals together at the head[52]. EM studies also showed that MukE and MukF bind to the MukB heads[53]. MukB dimers and BsSMC dimers were mostly observed in I-shaped and V-shaped

conformations[51–56], and occasionally in Y- or O-shapes (Fig. 4b) [57]. Similar I- and V-shaped conformations were later found for the full BsSmc-ScpAB complex [58,59].

One of the unanswered questions for SMC proteins is: do they mutually interact and cooperate? Interestingly, MukBEF complexes were shown form either fiber-form multimers or rosette shapes[53]. Similar rosette structures were also observed in liquid AFM for BsSMC[55], while multimers were observed with dry AFM[57]. After incubation with plasmid DNA, MukB complexes were shown to form large networks that appeared to consist of many catenated plasmids [60]. SMC clusters, however, appear to be much less prominent for eukaryotic SMCs. A live-cell imaging study used PALM (Photo Activated Localization Microscopy) super-resolution microscopy to probe the architecture of MukBEF complexes *in vivo*[61]. Despite their different molecular weights, all subunits displayed the same diffusion coefficient indicating that they were moving as a whole. Single-molecule fluorescent-particle tracking estimated a stoichiometry of 4:4:2 molecules for MukB:E:F, and functional units that consisted of 8-10 such MukBEF complexes.

Importantly, the first EM studies on eukaryotic SMC complexes confirmed that cohesin and condensin share the same head-coiled-coil-hinge structure[62]. Another important finding was that the anti-parallel coiled coils of cohesin Smc1/Smc3 dimers are intramolecular, thus folding back on themselves, and not two SMC proteins that are mutually coiled together along their entire length[63]. By imaging individual Smc1 or Smc3 proteins, it was shown that each of them forms an elongated structure with a globular structure on both sides of the coiled-coil, revealing that cohesin thus consists of one Smc1 arm and one Smc3 arm that mutually connect at the hinge.

Cohesin and condensin (both dimers and holocomplexes) were imaged for a variety of species. Budding yeast Smc1/Smc3 dimers in the presence of ATP were reported to be in both V- and O-shapes (Fig. 4b), but mutants deficient in ATP binding showed less head engagement, suggesting that ATP binding influences the interaction between the cohesin heads[64,65]. An AFM study in

liquid reported I-shaped cohesin dimers, and the authors suggested that both coiled coils were in fact mutually intertwined in this I-shape[66]. Interestingly, deacetylated cohesin showed a higher occurrence of V- and Y-shaped Smc1/Smc3 dimers, suggesting that modifications such as acetylation influence the orientation, possibly also for condensin [62]. The majority of full cohesin complexes (human and yeast) was found to be in a V-, O- or Y-shape (Fig, 4b) [63,65,67]. In some cases, kinks in the coiled coils were observed. Several groups have attempted to visualize the interaction of condensin with DNA. With electron spectroscopic imaging[68], *Xenopus* condensin was visualized to interact with plasmid DNA in an ATP-hydrolysis dependent matter [39]. Remarkably, the DNA appeared to be wrapped around the heads, which occurred only in the presence of ATP. This led to the proposal of a model in which condensin, creates supercoils by wrapping DNA around the ATPase heads [39].

It is likely that different conformations exist for the same SMC complex, depending on the function and stage in the cell cycle, and that these conformational changes are dynamic. Condensin Smc2/Smc4 dimers imaged with high-speed AFM in liquid at physiological conditions indeed showed complexes that switched between various conformations over time[69]. The dimers were observed to switch between V-, O-, B- and P-shape, while I-shaped condensin dimers were not detected. While the existence of the head-hinge interaction has been predicted, this is the only report on B- and P-shapes so far [70,71]. Furthermore, this study revealed that the coiled-coils are flexible, with a persistence length of only ~4nm [69]. This indicates that condensin has the structural flexibility to change conformation and engage in chromatin embrace. Cohesin was also imaged with high-speed AFM, showing that the coiled coils were flexible and that the molecules change their configuration within imaging time, but no quantification was given [66].

Out of all SMC complexes, the architecture and function of Smc5/6 is the least well studied. Remarkably, to our knowledge, there has not been *any* imaging or single-molecule study of the Smc5/6 complex. Visualization of this complex and its arrangement of subunits would greatly aid our understanding of its

structure, but the bottleneck will be the purification of a clean and complete complex[19,23].

In conclusion, the abundance of imaging studies has not resulted in the determination of a uniform conformation of SMC complexes, as the results are found to vary between groups, species, imaging techniques, and sample preparation methods. In fact, these studies have shown that these flexible complexes can adopt many different conformations.

Force spectroscopy with magnetic tweezers

The reorganization of DNA by SMC proteins can be studied in real time using single-molecule tweezers. Magnetic tweezers are exceptionally suitable to apply a force clamp on a molecule, monitor changes in DNA length upon protein binding, as well as to study DNA supercoiling induced by SMC complexes [72]. In magnetic tweezers, a DNA molecule is tethered between a surface and a magnetic bead (Fig. 3c). An external magnet is used to manipulate the bead, and thus the molecule. Rotation or vertical movement of the magnets can, respectively, apply torque and force to the molecule. Note that in this technique, the read-out is the z-position of the bead, which can be very precise, allowing a very accurate measurement of the DNA end-to-end length. A limitation of conventional magnetic tweezer techniques is that the proteins acting on DNA cannot be visualized.

Magnetic tweezers have been used to monitor the end-to-end distance of a DNA molecule as it gets shortened by compacting SMCs (Fig. 5a). A pioneering study with condensin holocomplex extracted from mitotic *Xenopus leavis* cells showed compaction and decompaction in large steps (±70nm) upon addition of ATP. Compaction was not observed in the absence of ATP, and only very weak compaction was seen when condensin from interphase cells was used[73]. Although no compaction was observed in the absence of ATP, condensin did interact with DNA in an ATP-independent fashion. Applying forces >10pN reversed compaction. Similar results were found in a recent magnetic tweezers study on the *S. cerevisiae* complex (Fig. 5b)[74] that revealed how the rate of compaction depends on protein concentration, ATP

concentration, and applied force. Compaction was found to be reversible with high salt, but condensin remained bound, indicating topological loading. Interestingly, although previously reported in biochemical studies [37–39], both magnetic tweezers studies on eukaryotic condensin failed to detect a putative supercoiling activity for condensin.

The *E.coli* MukB dimer similarly showed compaction of DNA against low forces in a stepwise manner, with steps of ~70nm [75]. Addition of the subunits MukE and MukF decreased the rate of compaction. The authors argued that MukB formed clusters that could resist forces up to 10pN. ATP had no effect on compaction rate but shortened the time before initiation of compaction. Two DNA molecules were attached to one magnetic bead, probing the ability of MukB to form a bridge between two DNA molecules (Fig. 5c,d)[76]. Interestingly, the probability that an SMC complex would form a bridge was increased in the presence of ATP, and decreased for an ATPase mutant.

Surprisingly, budding yeast's Smc1/3 dimer (i.e. not the full complex but merely the cohesin dimer) was reported to compact DNA in an stepwise manner (130nm steps) as well [67]. This compaction was not dependent on ATP and compaction still occurred when a headless variant (i.e. without the ATPase heads) was used, but not when the hinge was replaced.

We note that all the step sizes reported so far in these SMC-induced DNA condensation studies are strikingly large (70-200 nm). While a detailed step analysis in recent study called for caution in interpreting steps in magnetic tweezers experiments under these low forces [74], it is clear that the steps observed for SMC proteins are much larger than for common DNA-translocating motor proteins such as helicases, translocases or polymerases which typically move in 1-bp increments[77–80]. In fact, these large steps are similar to or even larger than the size of the SMC complexes themselves, which measure maximum 70nm along their longest axis[62]. A similar size suggests conformational changes at the scale of the full SMC complex itself, while even larger steps are puzzling, yet consistently found in different studies. Note that protein aggregation can also lead to a reduced end-to-end

distance of a DNA molecule in magnetic tweezers, calling for caution in interpretation results. Such very large steps may involve the concerted action of multiple SMC complexes, or bursts of fast sequential steps of a single SMC complex – clearly a direction of further future research.

Fluorescent imaging techniques

The interaction between SMC complexes and DNA can be visualized with fluorescent imaging techniques. Typically, the DNA and the protein of interest are fluorescently labeled. In a flow-stretching experiment, a linear DNA molecule is stretched out along a PEGylated glass slide, and SMC complexes may bind to spots on the DNA (Fig. 3d). With the DNA curtain technique, DNA is attached to freely diffusing lipids that, upon applying a flow, diffuse towards micro-fabricated barriers, where “curtains” are formed (Fig. 3e). An advantage of DNA curtains is that many DNA molecules can be visualized in parallel, making it easier to build statistics in these single-molecule experiments. The drawbacks of both techniques are the limited optical resolution (typically >300nm), and the fact that conformational changes (such as compaction) in the DNA are difficult to observe when the DNA is fixated at both ends.

Using single-molecule imaging on flow stretched DNA, fluorescently labeled individual BsSMC complexes were shown to have two types of behavior when bound to DNA: static binding and one-dimensional Brownian diffusion (Fig. 6a) [40]. At higher concentrations, clusters of BsSMC were able to compact the DNA against the flow on a single tethered curtain (Fig. 6b). The presence of ATP had only a marginal influence on the compaction rate, while the presence of non-SMC subunits ScpA and ScpB reduced clustering on DNA. Interestingly, a headless mutant also showed local bending of the DNA. The authors suggested that the ATPase domains are required for cooperative clustering, while single BsSMC dimers might bend the DNA, thereby locally compacting the DNA.

Two studies on cohesin showed a similar diffusive behavior for motion along the DNA. A DNA curtain study on *S.pombe* cohesin found a diffusion constant of 3.8±0.2μm^2/s at 500mM salt [81], which is similar to that found for human

cohesin on flow-stretched DNA (1.7±0.1μm^2/s) [82]. These values correspond well to an *in vivo* estimate for the diffusion of cohesin (3.0±0.2μm^2/s) [83]. Both studies found that ATP or a cohesin loading complex were not necessary for cohesin loading and diffusion. Cohesin remained associated with DNA at high salt concentrations, consistent with biochemical experiments and highly suggestive of a topological-embrace model[84].

Both studies also aimed to probe cohesin's ability to diffuse past obstacles of various sizes. DNA-bound obstacles with a size up to ~10nm could be passed without problems, but complexes >20nm could not be overcome. Cohesin was found to occasionally pause upon encountering a nucleosome, but it could diffuse over it (Fig. 6c). Interestingly, the majority of cohesin failed to pass the transcriptional regulator CTCF, which serves as a boundary element *in vivo* [82]. Both the bacterial DNA translocase FtsK and the T7 RNAP could push the cohesin ring along the DNA. Although the eukaryotic cohesin would not encounter these bacterial complexes *in vivo*, it does indicate that cohesin can in principle be displaced by polymerases.

A third study probed the dynamics of *Xenopus* cohesin on flow-stretched DNA[85]. In contrast to the results reviewed above, these authors claim that cohesin diffusion is dependent on ATP as well as on the cohesion-loading complex Scc-Scc4. The movement they observed was consistent with random diffusion rather than active linear translocation. The presence of Wapl-Pds5 (required for cohesin removal in prophase) was found to reduce cohesin's diffusional motion, an effect that was antagonized by acetylation of cohesin.

Recently, a DNA curtain study showed that the *S. cerevisiae* condensin complex is a mechanochemical molecular motor that translocates on DNA (Fig. 6d) [86]. The translocation was ATP dependent, persisted for very long distances (>10kb), and showed an average velocity of ~60 basepairs per second. Strikingly, condensin was also able to co-translocate a second DNA molecule along the DNA curtains. These findings show that condensin has a DNA-translocating motor domain, which is an essential ingredient for DNA compaction in a mechanism such as loop extrusion. Although loop extrusion

is mostly mentioned in the context of cohesin, eukaryotic condensin is so far the only SMC protein for which motor activity is reported.

Single-molecule FRET techniques were also used to study the dynamics of SMC complexes. The spatial proximity of two fluorescently labeled sites (with separated excitation and emission spectra) can be determined with FRET. This principle relies on the energy transfer by excitation of one fluorophore (donor) to the nearby second fluorophore (acceptor). The efficiency of this transfer is strongly dependent on to the distance between the donor and acceptor, making this technique a very sensitive tool to study inter- and intramolecular interactions, for distances of up to ~10 nm. Incorporation of the suitable fluorescent tags into the proteins of interest at the position of choice can, however, be challenging.

The association of cohesin's head domains was probed with FRET in live cells of budding yeast[87]. A high FRET value was found constitutively throughout the cell cycle, indicating that the ATPase heads are in close proximity of each other at most times. No interactions between the hinge and the heads were detected, indicating that if this interaction exists *in vivo*, it is very transient. No associations between among different cohesin complexes could be detected in this *in vivo* assay. The proximity of the coiled-coils of both MukB and BsSMC was also probed *in vitro* with FRET [54]. A truncated form of BsSMC showed a high FRET efficiency, whereas a MukB fragment showed low FRET, consistent with an I-shape and V-shape respectively.

Perspective

The molecular mechanism of SMC complexes, and their function in directing the chromosomal architecture, is one of the hottest topics in cell biology today. Many open questions remain, and biophysical techniques appear to be key to answering them. Even though crystallography will continue to yield more (partial) protein structures, the flexible and open conformations of the full complexes intrinsically will escape notice. Single-molecule AFM or EM imaging, which circumvents this limitation, has already provided new insights in the structure of SMC proteins, and we can expect many more results from

emerging improved imaging techniques such as high-speed AFM and cryo-EM in the upcoming years. Looking at dynamics with high-speed AFM and FRET will be key to resolve the large conformational changes that supposedly are associated with the function of SMCs.

In vitro single-molecule experiments can provide detailed information on the molecular structure and mechanism, but it remains important to consider how their results can be extrapolated to the *in vivo* environment of the cell. *In vitro* studies with partial complexes in the absence of ATP are tricky to interpret, as partial and ATPase-deficient complexes are not often viable *in vivo*. *In vivo*, SMC complexes are regulated by many co-factors and modifications, depending on the stage in the cell cycle. As the field is progressing in understanding these factors and preparing purified proteins of increasing quality and added co-factors, they will become available for single-molecule experiments [85]. Alternatively, one can perform single-molecule experiments on proteins directly from cell extracts, which may retain their modifications and co-factors. *In vitro* single-molecule experiments can also move up in complexity by studying minimal forms of chromatin instead of naked DNA, which seems well possible since reconstitution of chromosomes requires a surprisingly low amount of factors[88].

It will be of interest to consider the differences between prokaryotic and eukaryotic SMC complexes, as they might employ different mechanisms. For example, the prokaryotic BsSMC was reported to need recruitment factors to become active [89,90]. Such factors were not reported for eukaryotic complexes, and indeed, all *in vitro* single-molecule studies on eukaryotic condensin reported so far showed compaction activity in the absence of a loading factor. This apparent difference between eukaryotic and prokaryotic condensin is unexpected, because from an evolutionary perspective one would expect the eukaryotic SMC to exhibit a higher complexity with additional co-factors.

The differences and similarities of the various eukaryotic SMC complexes have so far largely been unresolved. For example, motor activity has only been identified for eukaryotic condensin, and not for bacterial SMC or for

cohesin. It will be interesting to see if this is an intrinsic difference or related to purification details or functional co-factors. A very recent study combining Hi-C and computer simulations surprisingly found that cohesin, and not condensin, was responsible for chromosome compaction in budding yeast [91]. It may be the case that cohesin and condensin share very similar mechanisms. Or on the contrary, it may be that the same homologous complex, say condensin, functions differently in different organisms. This remains to be resolved in the forthcoming years.

Looking forward, as the field advances in protein purification and *in vitro* loading and more biologists are getting acquainted with biophysical tools, we can expect many more single-molecule studies on SMC proteins in the upcoming years. Critically evaluating differences between species and different SMC complexes with classical assays such as magnetic tweezers and DNA flow stretching is of interest. Such experiments should also be conducted in crowded environments, involving different co-factors known to interact with SMC proteins, as this better mimics *in vivo* conditions.

Almost all experiments discussed in this review probe a single quantity, for example the DNA extension with magnetic tweezers. Progress can also be expected from hybrid techniques that combine multiple single-molecule methods, for example magnetic tweezers that are combined with fluorescence imaging. Such a combination would bring the ability to monitor changes in DNA length or linking number while simultaneously following the action of fluorescently labeled SMC proteins. Similarly, the combination of FRET measurements on flow-stretched DNA could provide information on the local conformational changes within molecules while they perform their function on DNA.

Studying SMC proteins is essential for understanding the organization of the genome in all organisms. New developments in imaging and single-molecule techniques can be expected to significantly advance our understanding in the forthcoming years.

Acknowledgements

We thank Christian Haering, Shveta Bisht, Allard Katan, Jekyung Ryu, Sergii Pud, Yoones Kabiri, and Alexandre Japaridze for discussions. This work was funded by the ERC Advanced Grant SynDiv (ERC-ADG-2014 669598) and the Netherlands Organization for Scientific Research (NWO/OCW) (as part of the Frontiers of Nanoscience program).

Table 1: Possibilities and limitations of single-molecule techniques

	Possibilities	**Limitations**
Single-molecule imaging techniques		
TEM	Near atomic level imaging, i.e., very high resolution	Surface technique, imaging In vacuum, static snapshots, potential artifacts in sample preparation and contrast enhancement
AFM	High resolution (nm-scale) imaging of molecules in air or in liquid, no need for labeling	Surface technique, static snapshots
High-speed AFM	Observing dynamics with nm-scale resolution in liquid. Acquisition of videos at a rate of up to ~20 images per second	Surface technique
Force spectroscopy		
Magnetic tweezers	Controlled application of force and torque, accurate measurement of DNA end-to-end distance	No visualization of proteins acting on DNA
Fluorescent imaging techniques		
DNA flow-stretching	Visualization of fluorescently labeled proteins on stretched, immobilized DNA	Limited optical resolution
DNA curtains	Visualization of many DNA molecules in parallel, i.e., high throughput	Limited optical resolution
FRET	Sensitive measurements of local dynamics of spots within proteins by monitoring the proximity of two fluorescently labeled sites	Incorporation of fluorescent tags at position of interest can be challenging, limited size range (up to ~10nm)

Figure captions

Figure 1: Architecture of SMC complexes

a. General architecture of SMC complexes. SMC complexes consist of two SMC proteins that mutually connect at the hinge. In prokaryotes, this is a homodimer, whereas eukaryote complexes contain a heterodimer. The other ends of the SMC proteins, the "heads", exhibit ATPase activity. A kleisin subunit completes the ring.

b. Overview of prokaryotic SMC complexes.

c. Overview of eukaryotic SMC complexes. The names for the human proteins are listed.

Figure 2: Main mechanisms of SMC complexes

a. Schematic depicting some of the main biological functions of cohesin and condensin. Condensin compacts the DNA into mitotic chromosomes, while cohesin holds sister chromatids together at metaphase.

b. Cohesin acts as a boundary element for topological associating domains that are encoded in CTCF-binding sites.

c. The random crosslinking model. An SMC complex links DNA together by trapping two DNA strands inside its ring. This looping can be achieved by a single SMC complex or by two interacting SMC complexes.

d. The loop extrusion model. DNA gets trapped inside one or two SMC complexes upon which a DNA loop further extrudes.

Note that molecules in the cartoons in this figure are not to scale.

Figure 3: Schematics of common single-molecule techniques

a. Transmission electron microscopy (TEM).

b. Atomic Force microscopy (AFM).

c. Magnetic tweezers.

d. DNA flow-stretching.

e. DNA curtains.

f. Fluorescent resonance energy transfer.

Figure 4: Images of SMC complexes.

a. Classification of shape observations with a letter system.

b. Images of various complexes, showing differences and similarities between species and techniques. Best-quality images are selected. MukB and BsSMC: EM images adapted from [52]. Smc1/3: EM images from [63], dry AFM images from [64]. Smc2/4 dimers: stills from high-speed AFM movies obtained from [69]. The letter in each panel indicates the letter-shape identified, as tabulated in panel a.

Figure 5: SMC-mediated DNA compaction in magnetic tweezers

a. Basic principle of the magnetic tweezers assay to monitor DNA compaction: A DNA molecule is stretched between a magnetic bead and a surface. Upon addition of condensin, the DNA is compacted and the end-to-end distance of the DNA decreases. Adapted from [74].

b. DNA compaction by the *S cerevisiae* condensin complex, in the presence of ATP. Different shades of gray represent different DNA molecules in the same experiment. Adapted from [74].

c. Schematic representation of the time sequence in the magnetic-tweezers DNA-bridging experiment. DNA bridges were introduced by rotating beads that have two attached DNA molecules, in the presence of MukB. Subsequently, the bead was untwisted to zero rotations to attempt to remove bridges.

d. DNA extension (red) decreases as the magnets make one turn (blue), and recovers to the initial extension in the absence of protein (double arrow). In the presence of MukB, a delay in this recovery is observed (arrows, t_{life}) which was attributed to a MukB-induced bridge that was released after some time t_{life}.

Figure 6: SMC motion on flow-stretched DNA

a. Individual BsSMC complexes slide on DNA, switching between static binding at one spot and random one-dimensional diffusion along the DNA. Adapted from [40].

b. At high concentrations, BsSmc complexes cluster and compact DNA. Kymograph shows the quantum-dot-labeled end of a DNA molecule (see inset) that is compacted. Adapted from [40].

c. Obstacles (in this case nucleosomes) restrict the mobility of cohesin. Cohesin is seen to transiently pause at the nucleosome, but it is able to diffuse past it. Adapted from [81].

d. Kymograph showing motor action of condensin as complexes bind and slide along DNA in an ATP-dependent linear motion over very long length scales (>10 μm). Adapted from [86].

References

1. Uhlmann, F. SMC complexes: from DNA to chromosomes. *Nat. Rev. Mol. Cell Biol.* **17,** 399–412 (2016).
   Excellent recent review analysing the currect understanding of the mechanism of SMC complexes.
2. Nasmyth, K. & Haering, C. H. Cohesin: its roles and mechanisms. *Annu. Rev. Genet.* **43,** 525–58 (2009).
3. Hirano, T. Condensins: Universal organizers of chromosomes with diverse functions. *Genes Dev.* **26,** 1659–1678 (2012).
4. Haering, C. H. & Gruber, S. SnapShot: SMC Protein Complexes Part I. *Cell* **164,** 326–326.e1 (2016).
5. Nolivos, S. & Sherratt, D. The bacterial chromosome: architecture and action of bacterial SMC and SMC-like complexes. *FEMS Microbiol. Rev.* **38,** 380–392 (2014).
6. Britton, R. A., Lin, D. C. & Grossman, A. D. Characterization of a prokaryotic SMC protein involved in chromosome partitioning. *Genes Dev.* **12,** 1254–9 (1998).
7. Wang, Q., Mordukhova, E. A., Edwards, A. L. & Rybenkov, V. V. Chromosome condensation in the absence of the non-SMC subunits of MukBEF. *J. Bacteriol.* **188,** 4431–41 (2006).
8. Gruber, S. *et al.* Interlinked sister chromosomes arise in the absence of condensin during fast replication in B. subtilis. *Curr. Biol.* **24,** 293–8 (2014).
9. Danilova, O., Reyes-Lamothe, R., Pinskaya, M., Sherratt, D. & Possoz, C. MukB colocalizes with the oriC region and is required for organization of the two Escherichia coli chromosome arms into separate cell halves. *Mol. Microbiol.* **65,** 1485–1492 (2007).
10. Nasmyth, K., Uhlmann, F. & Lottspeich, F. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature* **400,** 37–42 (1999).
11. Waizenegger, I. C., Hauf, S., Meinke, A. & Peters, J. M. Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. *Cell* **103,** 399–410

(2000).

12. Merkenschlager, M. & Nora, E. P. CTCF and Cohesin in Genome Folding and Transcriptional Gene Regulation. *Annu. Rev. Genomics Hum. Genet.* **17,** 17–43 (2016).
13. Ono, T. *et al.* Differential Contributions of Condensin I and Condensin II to Mitotic Chromosome Architecture in Vertebrate Cells. *Cell* **115,** 109–121 (2003).
14. Frosi, Y. & Haering, C. H. Control of chromosome interactions by condensin complexes. *Curr. Opin. Cell Biol.* **34,** 94–100 (2015).
15. Meyer, B. J. Targeting X chromosomes for repression. *Curr. Opin. Genet. Dev.* **20,** 179–189 (2010).
16. Lehmann, A. R. *et al.* The rad18 gene of Schizosaccharomyces pombe defines a new subgroup of the SMC superfamily involved in DNA repair. *Mol. Cell. Biol.* **15,** 7067–80 (1995).
17. De Piccoli, G. *et al.* Smc5–Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. *Nat. Cell Biol.* **8,** 1032–1034 (2006).
18. Betts Lindroos, H. *et al.* Chromosomal Association of the Smc5/6 Complex Reveals that It Functions in Differently Regulated Pathways. *Mol. Cell* **22,** 755–767 (2006).
19. Jeppsson, K. *et al.* The Chromosomal Association of the Smc5/6 Complex Depends on Cohesion and Predicts the Level of Sister Chromatid Entanglement. *PLoS Genet.* **10,** e1004680 (2014).
20. Dekker, J. & Mirny, L. The 3D Genome as Moderator of Chromosomal Communication. *Cell* **164,** 1110–1121 (2016).
21. Haering, C. H., Farcas, A.-M., Arumugam, P., Metson, J. & Nasmyth, K. The cohesin ring concatenates sister DNA molecules. *Nature* **454,** 297–301 (2008).
22. Cuylen, S., Metz, J. & Haering, C. H. Condensin structures chromosomal DNA through topological links. *Nat. Struct. Mol. Biol.* **18,** 894–901 (2011).
23. Kanno, T., Berta, D. G. & Sjögren, C. The Smc5/6 Complex Is an ATP-Dependent Intermolecular DNA Linker. *Cell Rep.* **12,** 1471–1482 (2015).

24. Wilhelm, L. *et al.* SMC condensin entraps chromosomal DNA by an ATP hydrolysis dependent loading mechanism in *Bacillus subtilis*. *Elife* **4,** 11202–11212 (2015).
25. Cuylen, S., Metz, J., Hruby, A. & Haering, C. H. Entrapment of chromosomes by condensin rings prevents their breakage during cytokinesis. *Dev. Cell* **27,** 469–78 (2013).
26. Thadani, R., Uhlmann, F. & Heeger, S. Condensin, Chromatin Crossbarring and Chromosome Condensation. *Curr. Biol.* **22,** R1012–R1021 (2012).
27. Cheng, T. M. K. *et al.* A simple biophysical model emulates budding yeast chromosome condensation. *Elife* **4,** e05565 (2015).
28. Nasmyth, K. Disseminating the Genome: Joining, Resolving, and Separating Sister Chromatids During Mitosis and Meiosis. *Annu. Rev. Genet.* **35,** 673–745 (2001).
29. Alipour, E. & Marko, J. F. Self-organization of domain structures by DNA-loop-extruding enzymes. *Nucleic Acids Res.* **40,** 11202–11212 (2012).
30. Dolgin, E. DNA's secret weapon against knots and tangles. *Nature* **544,** 284–286 (2017).
31. Fudenberg, G. *et al.* Formation of Chromosomal Domains by Loop Extrusion. *Cell Rep.* **15,** 2038–2049 (2016).
32. Wang, X., Brandão, H. B., Le, T. B. K., Laub, M. T. & Rudner, D. Z. Bacillus subtilis SMC complexes juxtapose chromosome arms as they travel from origin to terminus. *Science (80-.).* **527,** 524–527 (2017).
33. Sanborn, A. L. *et al.* Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *Proc. Natl. Acad. Sci. U. S. A.* **112,** E6456-65 (2015).
34. Goloborodko, A., Marko, J. F. & Mirny, L. A. Chromosome Compaction by Active Loop Extrusion. *Biophys. J.* **110,** 2162–2168 (2016).
35. Goloborodko, A., Imakaev, M. V, Marko, J. F. & Mirny, L. Compaction and segregation of sister chromatids via active loop extrusion. *Elife* **5,** (2016).
36. Diebold-Durand, M.-L. *et al.* Structure of Full-Length SMC and Rearrangements Required for Chromosome Organization. *Mol. Cell* **67,**

334–347.e5 (2017).

37. Kimura, K. & Hirano, T. ATP-Dependent Positive Supercoiling of DNA by 13S Condensin: A Biochemical Implication for Chromosome Condensation. *Cell* **90,** 625–634 (1997).
38. Kimura, K., Rybenkov, V. V, Crisona, N. J., Hirano, T. & Cozzarelli, N. R. 13S Condensin Actively Reconfigures DNA by Introducing Global Positive Writhe. *Cell* **98,** 239–248 (1999).
39. Bazett-Jones, D. P., Kimura, K. & Hirano, T. Efficient Supercoiling of DNA by a Single Condensin Complex as Revealed by Electron Spectroscopic Imaging. *Mol. Cell* **9,** 1183–1190 (2002).
40. Kim, H. & Loparo, J. J. Multistep assembly of DNA condensation clusters by SMC. *Nat. Commun.* **7,** 10200 (2016).
41. Ha, T. Single-molecule methods leap ahead. *Nat. Methods* **11,** 1015–1018 (2014).
42. Murayama, Y. & Uhlmann, F. Biochemical reconstitution of topological DNA binding by the cohesin ring. *Nature* **505,** 367–71 (2014).
43. Tanaka, T., Fuchs, J., Loidl, J. & Nasmyth, K. Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. *Nat. Cell Biol.* **2,** 492–9 (2000).
44. Ganji, M., Kim, S. H., van der Torre, J., Abbondanzieri, E. & Dekker, C. Intercalation-Based Single-Molecule Fluorescence Assay To Study DNA Supercoil Dynamics. *Nano Lett.* **16,** 4699–4707 (2016).
45. Greene, E. C., Wind, S., Fazio, T., Gorman, J. & Visnapuu, M.-L. in *Methods in Enzymology* **472,** 293–315 (2010).
46. Juette, M. F. *et al.* The bright future of single-molecule fluorescence imaging. *Curr. Opin. Chem. Biol.* **20,** 103–111 (2014).
47. Gligoris, T. & Löwe, J. Structural Insights into Ring Formation of Cohesin and Related Smc Complexes. *Trends Cell Biol.* **26,** 680–693 (2016).
48. Bai, X., McMullan, G. & Scheres, S. H. . How cryo-EM is revolutionizing structural biology. *Trends Biochem. Sci.* **40,** 49–57 (2015).
49. Ando, T. *et al.* A high-speed atomic force microscope for studying biological macromolecules. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 12468–72 (2001).

50. Katan, A. J. & Dekker, C. High-speed AFM reveals the dynamics of single biomolecules at the nanometer scale. *Cell* **147,** 979–82 (2011).
51. Niki, H. *et al.* E.coli MukB protein involved in chromosome partition forms a homodimer with a rod-and-hinge structure having DNA binding and ATP/GTP binding activities. *EMBO J.* **11,** 5101–9 (1992).
The first electron micrographs of SMC proteins (MukB) showing the globular ATPase domains and coiled-coils.
52. Melby, T. E. The Symmetrical Structure of Structural Maintenance of Chromosomes (SMC) and MukB Proteins: Long, Antiparallel Coiled Coils, Folded at a Flexible Hinge. *J. Cell Biol.* **142,** 1595–1604 (1998).
53. Matoba, K., Yamazoe, M., Mayanagi, K., Morikawa, K. & Hiraga, S. Comparison of MukB homodimer versus MukBEF complex molecular architectures by electron microscopy reveals a higher-order multimerization. *Biochem. Biophys. Res. Commun.* **333,** 694–702 (2005).
54. Soh, Y. *et al.* Molecular Basis for SMC Rod Formation and Its Dissolution upon DNA Binding. *Mol. Cell* **3,** 1–14 (2015).
55. Mascarenhas, J. *et al.* Dynamic assembly, localization and proteolysis of the Bacillus subtilis SMC complex. *BMC Cell Biol.* **6,** 28 (2005).
56. Hirano, M., Anderson, D. E., Erickson, H. P. & Hirano, T. Bimodal activation of SMC ATPase by intra- and inter-molecular interactions. *EMBO J.* **20,** 3238–50 (2001).
57. Fuentes-Perez, M. E., Gwynn, E. J., Dillingham, M. S. & Moreno-Herrero, F. Using DNA as a fiducial marker to study SMC complex interactions with the atomic force microscope. *Biophys. J.* **102,** 839–48 (2012).
58. Kamada, K., Miyata, M. & Hirano, T. Molecular Basis of SMC ATPase Activation: Role of Internal Structural Changes of the Regulatory Subcomplex ScpAB. *Structure* **21,** 581–594 (2013).
59. Kamada, K., Su'etsugu, M., Takada, H., Miyata, M. & Hirano, T. Overall Shapes of the SMC-ScpAB Complex Are Determined by Balance between Constraint and Relaxation of Its Structural Parts. *Structure* **25,** 603–616.e4 (2017).
60. Bahng, S., Hayama, R. & Marians, K. J. MukB-mediated catenation of

DNA is ATP and MukEF independent. *J. Biol. Chem.* **291,** 23999–24008 (2016).

61. Badrinarayanan, A., Reyes-Lamothe, R., Uphoff, S., Leake, M. C. & Sherratt, D. J. In Vivo Architecture and Action of Bacterial Structural Maintenance of Chromosome Proteins. *Science (80-.).* **338,** (2012).
62. Anderson, D. E., Losada, A., Erickson, H. P. & Hirano, T. Condensin and cohesin display different arm conformations with characteristic hinge angles. *J. Cell Biol.* **156,** 419–24 (2002).
First images of eukaryotic SMC complexes.
63. Haering, C. H., Löwe, J., Hochwagen, A. & Nasmyth, K. Molecular Architecture of SMC Proteins and the Yeast Cohesin Complex. *Mol. Cell* **9,** 773–788 (2002).
64. Elbatsh, A. M. O. *et al.* Cohesin Releases DNA through Asymmetric ATPase-Driven Ring Opening. *Mol. Cell* **61,** 575–588 (2016).
65. Huis in 't Veld, P. J. *et al.* Characterization of a DNA exit gate in the human cohesin ring. *Science* **346,** 968–72 (2014).
66. Kulemzina, I. *et al.* A Reversible Association between Smc Coiled Coils Is Regulated by Lysine Acetylation and Is Required for Cohesin Association with the DNA. *Mol. Cell* **63,** 1044–1054 (2016).
67. Sun, M., Nishino, T. & Marko, J. F. The SMC1-SMC3 cohesin heterodimer structures DNA through supercoiling-dependent loop formation. *Nucleic Acids Res.* **41,** 6149–60 (2013).
68. Bazett-Jones, D. P. & Hendzel, M. J. Electron Spectroscopic Imaging of Chromatin. *Methods* **17,** 188–200 (1999).
69. Eeftens, J. M. *et al. Condensin Smc2-Smc4 Dimers Are Flexible and Dynamic*. *Cell Reports* **14,** (2016).
High-speed AFM study showing that condensin dimers dynamically adopt different conformations.
70. Gruber, S. *et al.* Evidence that loading of cohesin onto chromosomes involves opening of its SMC hinge. *Cell* **127,** 523–37 (2006).
71. Nasmyth, K. Cohesin: a catenase with separate entry and exit gates? *Nature Cell Biology* **13,** 1170–1177 (2011).
72. De Vlaminck, I. & Dekker, C. Recent advances in magnetic tweezers. *Annu. Rev. Biophys.* **41,** 453–72 (2012).

73. Strick, T. R., Kawaguchi, T. & Hirano, T. Real-time detection of single-molecule DNA compaction by condensin I. *Curr. Biol.* **14,** 874–80 (2004).
Pioneering magnetic tweezers study showing that condensin can compact DNA.
74. Eeftens, J. M., Bisht, S., Kerssemakers, J., Haering, C. & Dekker, C. Real-time detection of condensin-driven DNA compaction reveals a multistep binding mechanism. *bioRxiv* (2017). at <http://biorxiv.org/content/early/2017/06/15/149138>
75. Cui, Y., Petrushenko, Z. M. & Rybenkov, V. V. MukB acts as a macromolecular clamp in DNA condensation. *Nat. Struct. Mol. Biol.* **15,** 411–8 (2008).
76. Petrushenko, Z. M., Cui, Y., She, W. & Rybenkov, V. V. Mechanics of DNA bridging by bacterial condensin MukBEF in vitro and in singulo. *EMBO J.* **29,** 1126–35 (2010).
77. Pyle, A. M. Translocation and Unwinding Mechanisms of RNA and DNA Helicases. *Annu. Rev. Biophys.* **37,** 317–336 (2008).
78. Singleton, M. R., Dillingham, M. S. & Wigley, D. B. Structure and Mechanism of Helicases and Nucleic Acid Translocases. *Annu. Rev. Biochem.* **76,** 23–50 (2007).
79. Yang, W. Lessons Learned from UvrD Helicase: Mechanism for Directional Movement. *Annu. Rev. Biophys.* **39,** 367–385 (2010).
80. Seidel, R., Bloom, J. G., Dekker, C. & Szczelkun, M. D. Motor step size and ATP coupling efficiency of the dsDNA translocase EcoR124I. *EMBO J.* **27,** 1388–1398 (2008).
81. Stigler, J., Çamdere, G. Ö., Koshland, D. E. & Greene, E. C. Single-Molecule Imaging Reveals a Collapsed Conformational State for DNA-Bound Cohesin. *Cell Rep.* **15,** 988–998 (2016).
One of three studies that showed diffusion of cohesin on stretche DNA, and their ability to move across roadblocks.
82. Davidson, I. F. *et al.* Rapid movement and transcriptional re-localization of human cohesin on DNA. *EMBO J.* **35,** e201695402 (2016).
83. Ladurner, R. *et al.* Cohesin's ATPase Activity Couples Cohesin Loading

onto DNA with Smc3 Acetylation. *Curr. Biol.* **24,** 2228–2237 (2014).

84. Murayama, Y. & Uhlmann, F. Chromosome segregation: how to open cohesin without cutting the ring? *EMBO J.* **32,** 614–6 (2013).
85. Kanke, M., Tahara, E., Huis In 't Veld, P. J. & Nishiyama, T. Cohesin acetylation and Wapl-Pds5 oppositely regulate translocation of cohesin along DNA. *EMBO J.* **35,** 2686–2698 (2016).
86. Terakawa, T. *et al.* The condensin complex is a mechanochemical motor that translocates along DNA. 1–26 (2017). doi:10.1101/137711 *Science, accepted*
87. Mc Intyre, J. *et al.* In vivo analysis of cohesin architecture using FRET in the budding yeast Saccharomyces cerevisiae. *EMBO J.* **26,** 3783–3793 (2007).
88. Shintomi, K., Takahashi, T. S. & Hirano, T. Reconstitution of mitotic chromatids with a minimum set of purified factors. *Nat. Cell Biol.* **17,** 1014–1023 (2015).
89. Gruber, S. *et al.* Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in B. subtilis. *Cell* **137,** 685–96 (2009).
90. Sullivan, N. L. *et al.* Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. *Cell* **137,** 697–707 (2009).
91. Schalbetter, S. A. *et al.* SMC complexes differentially compact mitotic chromosomes according to genomic context. *Nat Cell Biol* **advance on,** (2017).

Figure 1

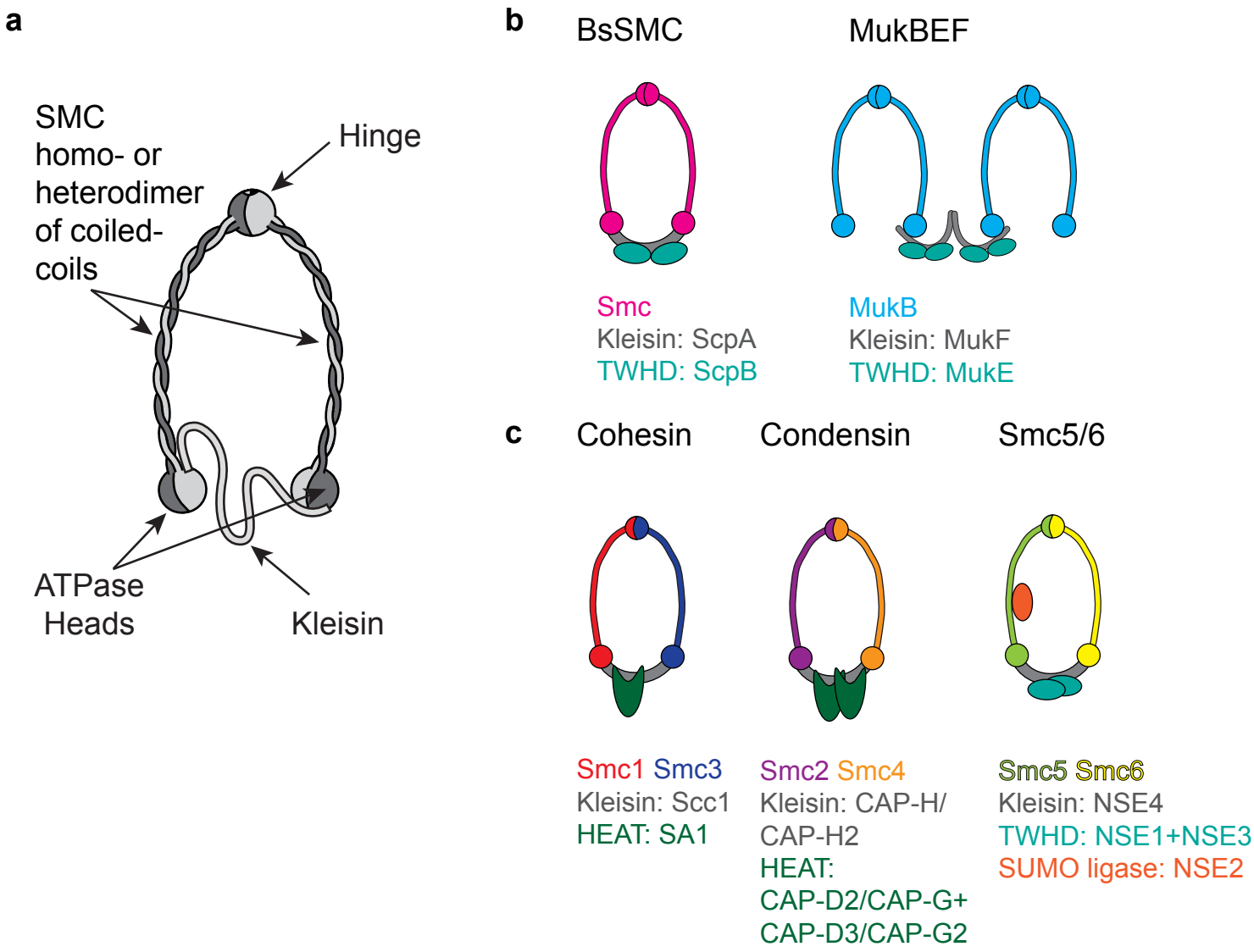

a
SMC
homo- or
heterodimer
of coiled-
coils
Hinge
ATPase
Heads
Kleisin
b
BsSMC
MukBEF
Smc
Kleisin: ScpA
TWHD: ScpB
MukB
Kleisin: MukF
TWHD: MukE
c
Cohesin
Condensin
Smc5/6
Smc1 Smc3
Kleisin: Scc1
HEAT: SA1
Smc2 Smc4
Kleisin: CAP-H/
CAP-H2
HEAT:
CAP-D2/CAP-G+
CAP-D3/CAP-G2
Smc5 Smc6
Kleisin: NSE4
TWHD: NSE1+NSE3
SUMO ligase: NSE2

Figure 2

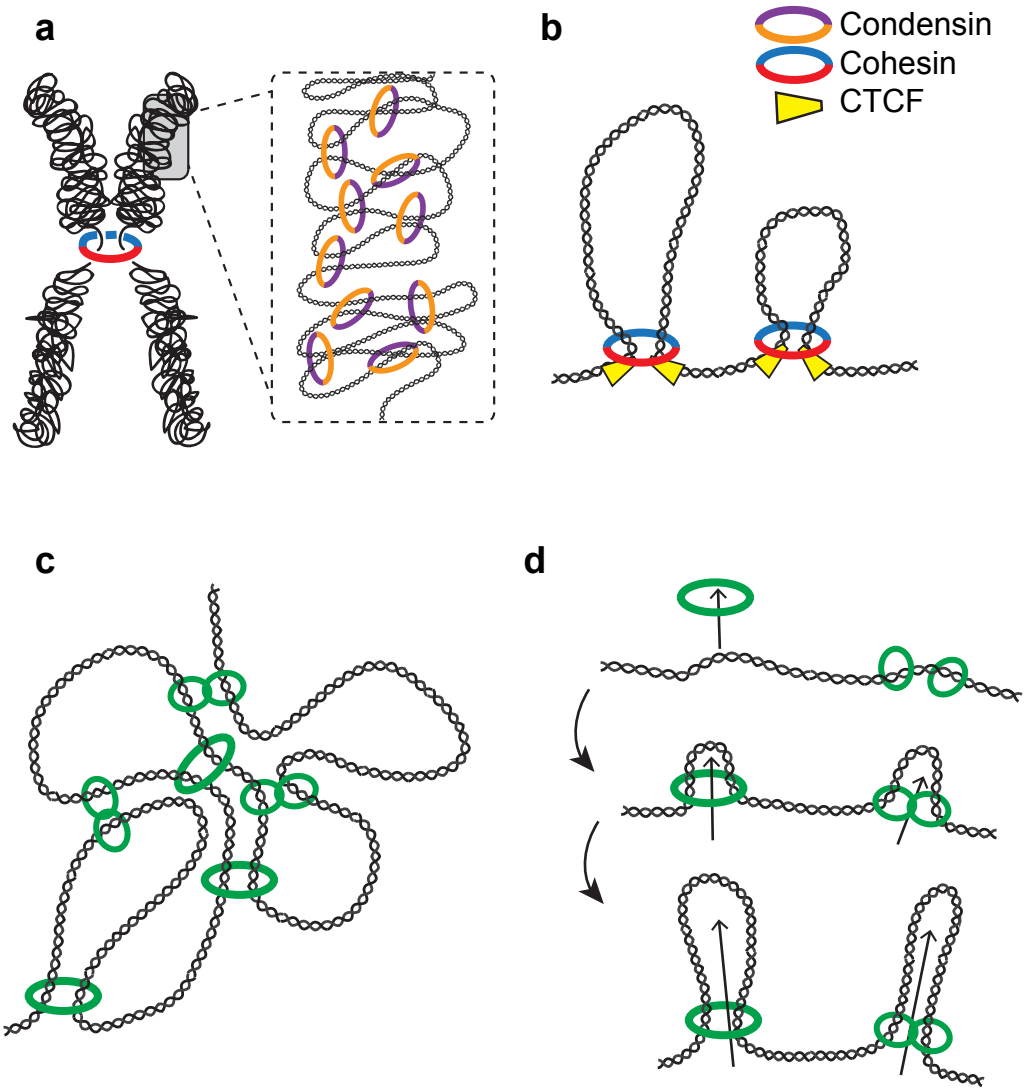


Figure 3

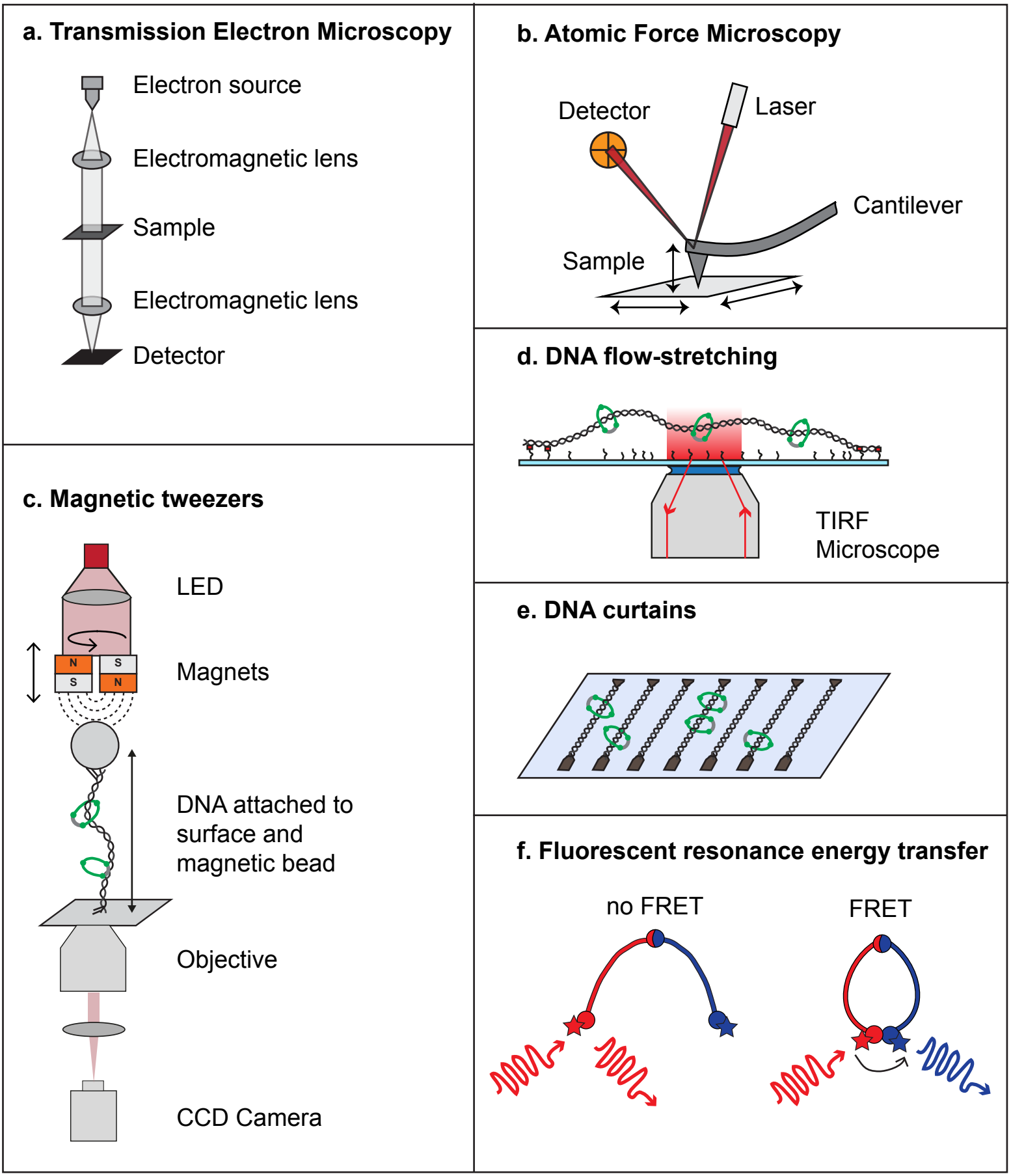

a. Transmission Electron Microscopy
Electron source
Electromagnetic lens
Sample
Electromagnetic lens
Detector
b. Atomic Force Microscopy
Detector
Laser
Cantilever
Sample
c. Magnetic tweezers
LED
N
S
S
N
Magnets
DNA attached to surface and magnetic bead
Objective
CCD Camera
d. DNA flow-stretching
TIRF Microscope
e. DNA curtains
f. Fluorescent resonance energy transfer
no FRET
FRET

Figure 4

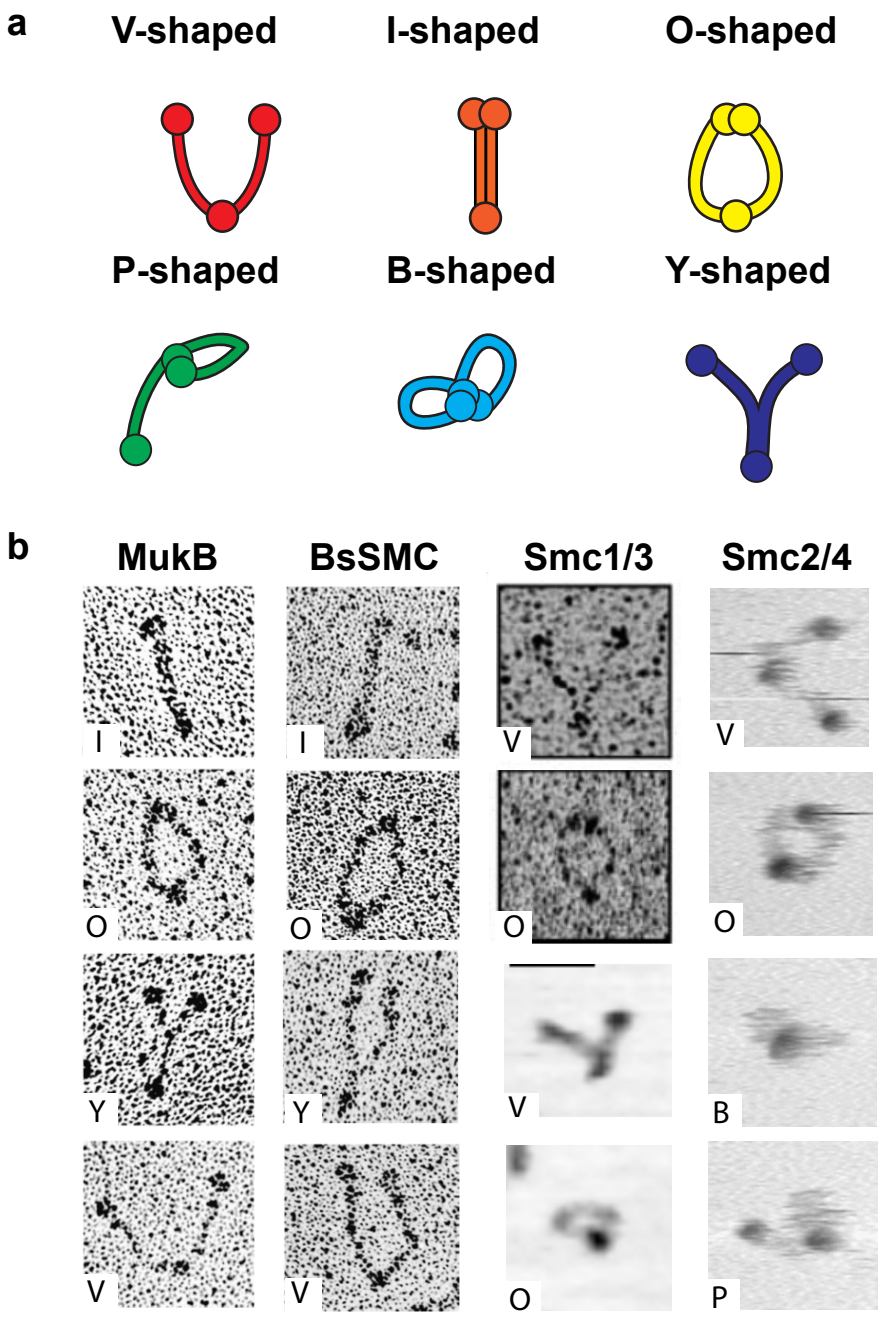

a
V-shaped
I-shaped
O-shaped
P-shaped
B-shaped
Y-shaped
b
MukB
BsSMC
Smc1/3
Smc2/4
I
I
V
V
O
O
O
O
Y
Y
V
B
V
V
O
P

Figure 5

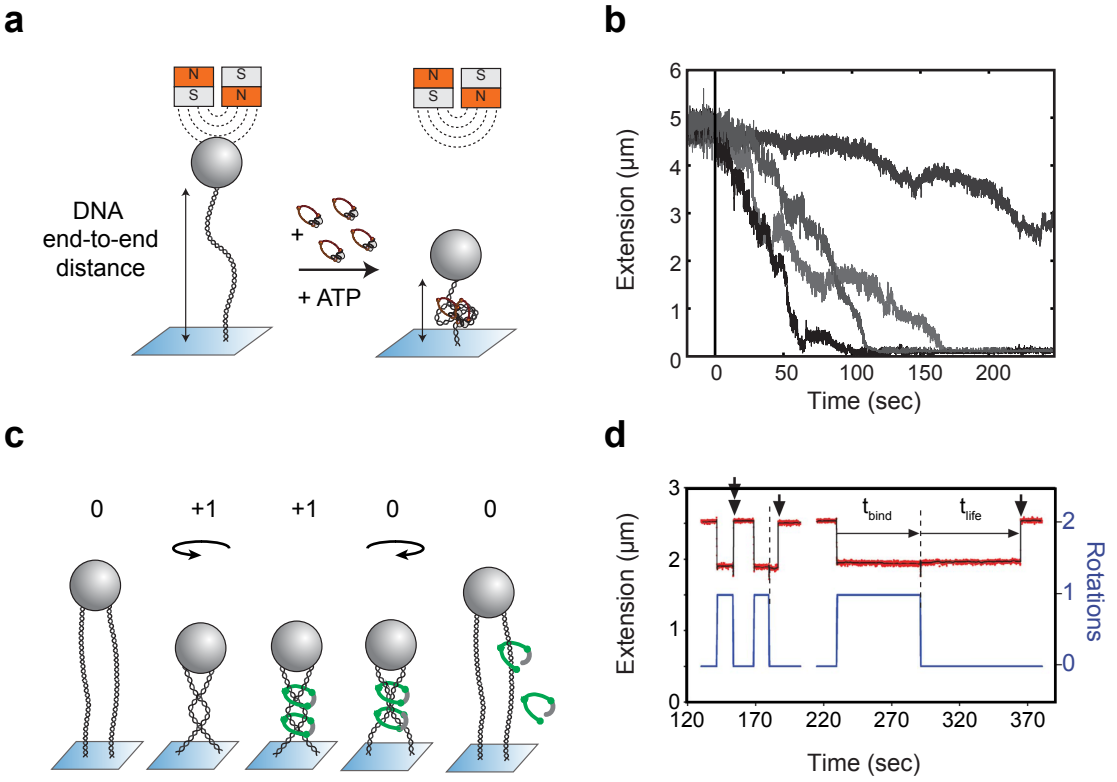
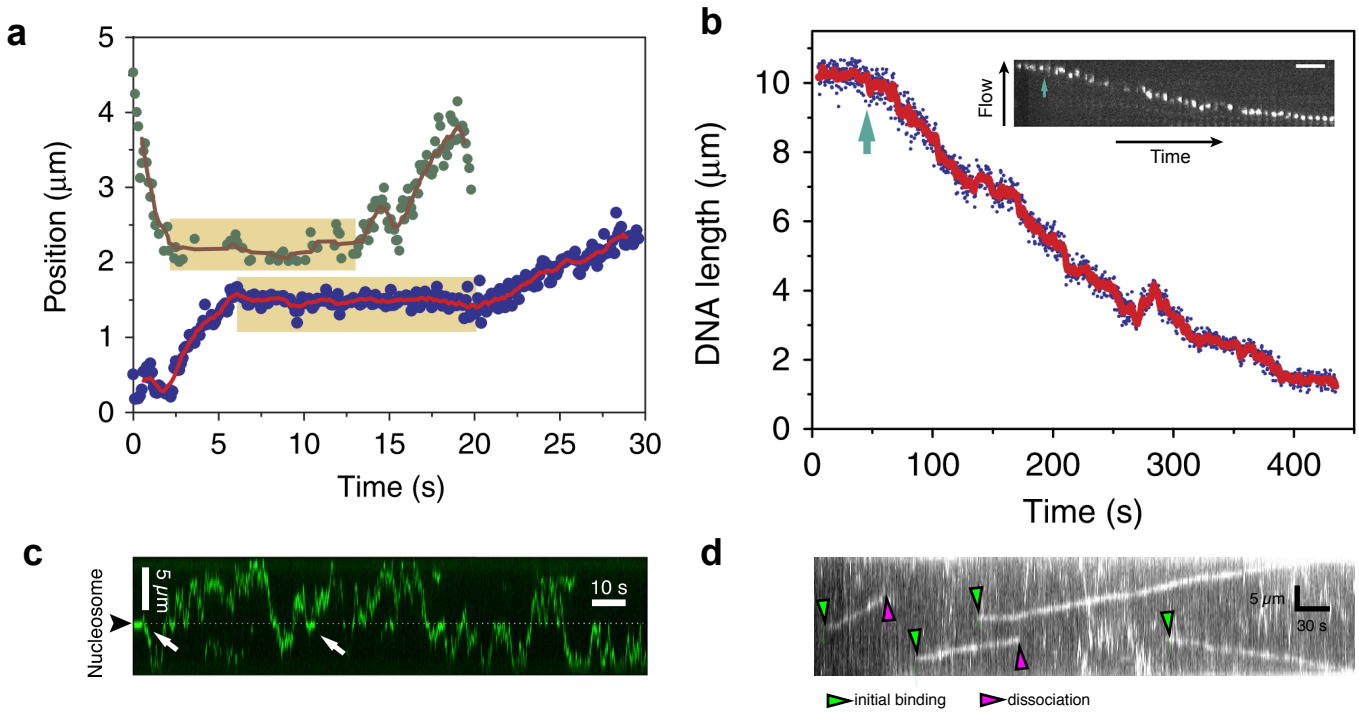

a
N
S
S
N
DNA
end-to-end
distance
+
+ ATP
b
Extension (μm)
Time (sec)
c
0
+1
+1
0
0
d
Extension (μm)
Rotations
t_{bind}
t_{life}
Time (sec)

Figure 6


a
Position (µm)
Time (s)
b
DNA length (µm)
Time (s)
Flow
Time
c
Nucleosome
5 µm
10 s
d
5 µm
30 s
initial binding
dissociation