HIV integrase compacts viral DNA into biphasic condensates

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ABSTRACT

The human immunodeficiency virus (HIV) infects non-dividing cells and its genome must be compacted to enter the cell nucleus. Here, we show that the viral enzyme integrase (IN) compacts HIV DNA mimetics *in vitro*. Under physiological conditions, IN-compacted genomes are consistent in size with those found for pre-integration complexes in infected cells. Compaction occurs in two stages: first IN tetramers bridge DNA strands and assemble into "rosette" structures that consist of a nucleo-protein core and extruding bare DNA. In a second stage, the extruding DNA loops condense onto the rosette core to form a disordered and viscoelastic outer layer. Notably, the core complex is susceptible towards IN inhibitors, whereas the diffuse outer layer is not. Together, our data suggest that IN has a structural role in viral DNA compaction and raise the possibility to develop inhibitors that target IN-DNA interactions in disordered condensates.

INTRODUCTION

To establish infection, the human immunodeficiency virus (HIV) traverses multiple cellular micro-environments of human host cells, using only a limited arsenal of viral proteins. HIV infects non-dividing cells, and viral particles must enter the cell nucleus to perform integration, thereby irreversibly establishing a provirus in the human genome. While the molecular structures and mechanisms of reverse transcription[1,2] and integration[3–7] are reasonably well understood, the composition and structural assembly of the large nucleoprotein complexes wherein they occur, *i.e.* the reverse transcription complex and the pre-integration complex (PIC) respectively, remain obscure.

Recent work has dramatically impacted our view on the intracellular nature of the virus particle: in contrast to previous belief, HIV transport towards the nucleus occurs within the confines of a (largely) intact viral capsid.[8,9] The new insights suggest that reverse transcription takes place in the confines of the capsid, which in turn requires constraining the increasing mechanical stresses due to the conversion of single-stranded HIV RNA into a much stiffer double-stranded copy-DNA (cDNA) of the HIV genome. Together, the observations suggest the need for a molecular mechanism to compact the DNA within the capsid.

In this context, it was found that the estimated copy-number of the viral protein integrase (IN) in PICs[10] is ~150-250, at least one order of magnitude larger than the number of IN (4-16 copies[4,11]) required for intasome assembly and catalysis of integration. This raises the question of whether and how such a large excess of IN is beneficial during the replication cycle. While IN serves a functional role during the later stages of HIV replication, in particular during viral assembly and maturation through binding to viral RNA,[12] IN mutants defective for non-specific DNA binding are impaired for nuclear import[13] and reverse transcription,[14] suggesting a critical role of IN within the cytoplasmic viral capsid also during the early stages of infection.

Here, we provide evidence for an additional functional role of IN by showing that IN can effectively compact viral DNA mimetics under physiologically relevant conditions. Dimensions of the resulting condensates agree favorably with those of PICs purified from[15] or within the infected cell.[16] Our data, obtained by single molecule force microscopy and spectroscopy techniques, and complemented with coarse-grained polymer simulations, suggest a bridging-induced attraction mechanism[17–19] that features an on-pathway “rosette” intermediate preceding full collapse. The fully collapsed condensates retain the mechanically robust rosette core and a diffuse viscoelastic outer layer, reminiscent of current models of eukaryotic genome folding[20] – albeit at a length scale orders of magnitude shorter. Importantly, we demonstrate a differential susceptibility towards allosteric IN inhibitors for the rosette folding intermediate *versus* the fully collapsed condensate.

RESULTS

IN binds viral DNA ends with moderate selectivity

Binding of IN to viral DNA ends is a prerequisite for the catalysis of the integration reaction. Here, we use atomic force microscopy (AFM) imaging to quantify the selectivity of recombinant IN for the specific ends in short viral cDNA mimics (Fig. 1a), as a function of the level of 3' processing. From AFM images, we determine the position of the bound IN complexes with respect to the nearest DNA end in IN-DNA nucleoprotein complexes (Fig. 1 and Supplementary Fig. S1), which enables us to estimate the selectivity of end-binding[21] $S = \frac{P_{end}}{P_{background}}$. We first investigated binding of IN to a 491 bp cDNA mimic with blunt ends in buffer containing 1 mM Mg^{2+}. The distribution of binding events along the internal DNA is essentially homogenous (Supplementary Fig. S1), implying the absence of high affinity internal binding sites. Assuming[22] a footprint of 16 bp, there are 476 potential binding sites in the cDNA mimic, and we estimate a selectivity $S = 64 \pm 13$ (from N=159 molecules; errors were estimated from counting statistics using standard error propagation; Fig. 1g). Next, we studied binding in buffer wherein Mg^{2+} is replaced by Ca^{2+} to impede catalysis of 3' processing[23] and we find a reduced selectivity $S = 19 \pm 7$ ($N = 122$; Fig. 1c). Last, we quantify binding to HIV DNA mimetics with 3' pre-processed ends (see Methods), which results in an increased selectivity, $S = 256 \pm 15$ ($N = 129$). Thus, the selectivity of binding to the specific blunt HIV ends increases by more than 10-fold by catalysis of 3' processing. Nevertheless, binding selectivity overall is only moderate; the probability of binding to a random position in genomic length viral DNA (~8-9 kbp) is still 1-2 orders of magnitude larger than binding to the specific ends.

Unspecific IN binding infers DNA bending and looping

As most IN-DNA complexes bind to internal sequences, *i.e.*, non-sequence specifically, we next investigate whether non-specific binding alters DNA structure. We quantify IN-induced DNA bending (Fig. 1e,h) and find that the bend angle distribution is much broader and shifted to larger bend angles at positions where IN is bound, as compared to the distribution of bare DNA bends. At higher ionic strength (250 mM NaCl, 5 mM $MgCl_2$), the fraction of complexes with large bend angles (> 50º bending; Fig. 1h) increases with respect to complexes with smaller bend angles, suggesting the presence of (at least) two binding modes: a first mode that is salt-sensitive and minimally invasive, and a second binding mode that is less sensitive to ionic strength and infers strong DNA bending.

Beyond bending, a small fraction (~5% of all internally bound complexes) forms synapses, i.e., intramolecular DNA loops mediated by IN (Fig. 1f). Using a longer viral DNA mimetic (1000 bp) and otherwise identical conditions (100 nM IN), the fraction of looped DNA increases to 30 ± 5% of all internally bound nucleoprotein complexes ($N = 93$). This increase is consistent with the expectation that looping is facilitated by the longer DNA length, since ~500 bp corresponds to only ~4 times the bending persistence length of DNA.

AFM volumetric analysis suggests that IN in the absence of DNA is monomeric and mostly dimeric, whereas in the DNA-bound state it takes on higher oligomers, in particular tetramers (Fig. 1i and Supplementary Fig. S2). Together, our results demonstrate that IN binding to random positions along the DNA is prevalent, induces higher order IN oligomerization, and can alter DNA conformation by bending and looping.

Two-stage compaction of genomic length viral DNA

To investigate how IN binding affects DNA of lengths similar to the HIV genome, we titrated linearized mini-HIV DNA[7] of different lengths (3.4 kbp, 4.8 kbp, 9.1 kbp) with IN and quantify the resulting complexes using AFM (Fig. 2a and Supplementary Fig. S3 to S5). To directly relate the conformations of adsorbed complexes to their solution state, we performed AFM imaging under kinetic trapping conditions, where the resulting images are close to a 3D to 2D projection.[24–26]

We first used a 4.8 kbp mini-HIV DNA[7] for titration with IN in the concentration range [IN] = 0-2 μM. At IN concentrations of < 500 nM, we observe localized IN binding and IN-induced looping. Increasing the concentration to [IN] = 500 - 1200 nM results in the formation of large nucleoprotein complexes featuring a central nucleoprotein core surrounded by bare DNA loops. Because of their appearance, we refer to these complexes as "rosettes". At even higher IN concentrations (> 1200 nM), the DNA is further compacted, and the nucleoprotein complexes are predominantly in globular conformations wherein individual DNA strands are no longer visible. On occasion, these intramolecular complexes self-associate to form large conglomerates comprising multiple DNA copies (Supplementary Fig. S4f, S5d,e,f).

We quantified DNA compaction upon titration of mini-HIV DNA with IN by automated analysis of the height-weighted 2D radius of gyration $R_g = \sqrt{\frac{\sum_{i=1}^{n} h_i \cdot s_i^2}{\sum_{i=1}^{n} h_i}}$ where s_i is the distance of pixel i with respect to the molecular center of mass, and h_i is the height value in pixel i. Consistent with the qualitative observations of DNA-IN complex geometries, the R_g data reveal that IN significantly compacts DNA in two successive, highly cooperative transitions: a first transition occurs at [IN] ~ 400 nM and reduces R_g by approximately 40% (from 75 nm at [IN] ≤ 250 nM, to 45 nm for [IN] = 500-1200 nM). A second transition is observed at [IN] ~ 1400 nM, and further reduces R_g ~ two-fold (Fig. 2 and Supplementary Fig. S6).

Condensation of nucleic acids with proteins and the formation of nucleoprotein complexes are in general highly dependent on environmental parameters. We mapped out the dependence of IN-induced DNA condensation on environmental conditions and observed that compaction is independent of incubation temperature (we tested 37 °C and room temperature, i.e. 22.5 °C; Supplementary Fig. S7) and incubation time (Supplementary Fig. S8), but strongly dependent on ionic strength[27] (Supplementary Fig. S9 and S10). We constructed a phase diagram of condensate formation as a function of integrase and salt concentration (Fig. 2c and Supplementary Fig. S11). The data show that both rosette formation and full collapse are disfavored by high salt concentration but occur under physiologically relevant conditions. As expected from the modest end-binding

selectivity (Fig. 1g), we find no effect of the specific miniHIV terminal sequences on compaction efficiency (Supplementary Fig. S12).

Having characterized the compaction behavior of a 4.8 kbp HIV DNA mimic, we next explore how DNA length alters IN-induced condensation. We find that the degree of compaction is independent of the DNA contour length and reduces R_g approximately 3-fold at full compaction compared to bare DNA without IN present (Supplementary Fig. S13). Nevertheless, the critical concentrations for the compaction transitions increases for shorter DNA lengths (Fig. 2c and Supplementary Fig. S6). Most notably, full compaction of the 3.4 kbp HIV mimetic occurs in a single transition (i.e., the rosette state is no longer observed), in contrast to the biphasic behavior observed for 4.8 kbp and 9.1 kbp DNA. The absence of a rosette state with DNA loops for the 3.4 kbp construct is in line with a previously identified DNA length threshold for bridging induced looping.[19] Last, the radius of gyration of the fully compacted state for 9.1 kbp DNA (approximately the length of the viral genome) is ~ 32 nm, close to the Stokes' radius found for purified pre-integration complexes[15] (28 nm) and in agreement with the molecular dimensions of pre-integration complexes in infected cells.[16] Taken together, our quantitative AFM analysis indicates that genomic length viral DNA is compacted via two distinct transitions into condensates with dimensions that are in quantitative agreement with those found in infected cells.

Simulations suggest bridging-mediated attraction mechanism with a critical role for IN-IN interactions

To obtain a mechanistic understanding of how local interactions lead to DNA compaction, we turn to Monte Carlo (MC) simulations (Fig. 3; Supplementary Methods). We model DNA as a chain of beads, each representing 12 bp (equal to the approximate binding site size of IN) joined by springs. A bending potential between subsequent beads is added to reproduce the experimentally determined bending stiffness of DNA. IN tetramers are similarly modeled as beads of the same size as the DNA beads, with an isotropic binding interaction with the DNA beads. We capture the tetrameric binding nature of the active IN complex by introducing a maximum valence of 4: each IN bead can bind to at most 4 DNA beads (Fig. 3a) (see Supplementary Information). We determine the interaction strength of IN binding to a single DNA segment to be $\varepsilon = 5\ k_B T$ (where k_B is Boltzmann's constant and T the absolute temperature) by direct quantitative comparison[21] to experimental data (Fig. 3h). For the IN-IN interaction we consider two cases: (i) no IN-IN interaction (beyond excluded volume interactions) and (ii) IN-IN interactions (i.e. IN-IN attractions in addition to the excluded volume interactions). We perform extensive simulations at different IN concentration for both cases and analyze the DNA-IN conformations as a function of time. During these simulations, the IN concentration is controlled via a grand canonical ensemble (see Supplementary Information) to ensure that the concentration of free IN remains constant. To identify different states of compaction and the pathways between them, we develop a classification pipeline using principal component analysis of 15 different structural parameters and a Gaussian mixture model (Fig. 3b-g and Supplementary Information). A key result is that we observe distinct pathways in the presence and absence of IN-IN attractions: MC simulations that only include IN-DNA binding[17], but no IN-IN attraction, exhibit DNA compaction, but fail to reproduce the two-

phase compaction and the experimentally observed rosette conformations. In contrast, if IN-IN attraction is included[18] (Fig. 3i), we observe transitions and structures that are in good agreement with the experimental observations (Fig. 2). In summary, our simulations demonstrate that a simple model that includes both IN-DNA and attractive IN tetramer-tetramer interactions can semi-quantitatively reproduce the observed DNA compaction.

IN-DNA condensates feature a rigid core surrounded by a soft coat

To further characterize the IN-DNA condensates and to probe their mechanical properties that likely play a role in the interior of the capsid and/or during nuclear entry, we use AFM force-volume mapping[28,29]. In this modality, a full force-distance curve (i.e., an approach and retract cycle) is recorded in every pixel of the image (Fig. 4a). Localized interaction forces on the tip are recorded as it moves towards and away from the sample surface (Fig. 4b) and can be directly correlated to the sample topography. We analyze the approach force curves to extract the indentation stiffness k, the retract curves to quantify elastic deformation energy $E_{Elast.}$, and the hysteresis between both curves to obtain a measure of plastic deformation $E_{Plast.}$ (Fig. 4a, inset and Supplementary Fig. S14). From the elastic and plastic contributions to the deformation energy, we can quantify the dimensionless viscoelasticity index[30] $\eta = 1 - \frac{E_{Elast.}}{E_{Elast.}+E_{Plast.}}$, where $\eta = 0$ for a perfectly elastic deformation and $\eta = 1$ for a completely viscous deformation, i.e. where the bonds broken during indentation are not reformed during the stress release.

We first perform force-volume mapping on complexes in the rosette state (Fig. 4c). The approach and retract curves are uniform across the nucleoprotein core and exhibit little hysteresis. The fitted values of k, $E_{Elast.}$, and $E_{Plast.}$ from at least 100 approach and retract curves are pooled and averaged per complex. Averaging over different rosette complexes, we find a mean stiffness $\langle k \rangle = 31 \pm 7$ pN/nm (mean ± SD from $N = 5$ complexes) and a mean viscoelasticity index $\langle \eta \rangle = 0.34 \pm 0.07$ (Fig. 4e,f).

In a second set of experiments, we use force-volume mapping on fully collapsed condensates (Fig. 4d; $N = 5$). In contrast to force curves recorded on rosettes, we consistently observe substantial hysteresis between the approach and retract curves. Furthermore, we find the overall slopes of the force curves to vary depending on their radial position: at constant peak forces, larger indentations are observed near the edges of the condensates. Indentation curves do not show breakthrough events, and because one would expect decreased indentations due to finite AFM tip size effects near steep topographic features we interpret the spatially resolved features in the condensates to result from complex material properties.[31,32] The spatially resolved stiffness map is used to identify a "core" and outer layer or "coat". We pool force curves in the core and find $\langle k \rangle = (53 \pm 8)$ pN/nm (error is SD) and a mean viscoelasticity index $\langle \eta \rangle = 0.48 \pm 0.09$ over the different condensates (Fig. 4e,f). An analogous treatment for force curves recorded on the condensate coats gives $\langle k \rangle = (29 \pm 2)$ pN/nm (error is SD), significantly less than the stiffness of the rosette, and a mean viscoelasticity index $\langle \eta \rangle = 0.55 \pm 0.08$.

We conclude that the fully collapsed condensates feature a relatively rigid, stiff core and more viscous, softer outer layer. The observed stiffness of the fully compacted condensates are on the same order of magnitude, but still significantly lower (by at least ~5-fold) than the reported stiffnesses of full virus particles[33], consistent with the possibility that they could remain in the capsid during transport to and into the nucleus. The pronounced viscoelasticity and hysteresis between approach and retract curves suggests that intrinsic dynamics and re-arrangement of bonds in the complex are much slower than the time scale of the force application and release by AFM (~5 ms).

Force spectroscopy of IN-DNA reveals heterogeneous dynamics of compacted loops

To investigate the dynamics and stability of IN-DNA condensates, we performed magnetic tweezers (MT) force spectroscopy experiments. In our assay, we tethered long linear DNA molecules (21 kbp) between the flow cell surface and superparamagnetic beads (Fig. 5a). Using external magnets, we can apply precisely calibrated stretching forces to the tethered molecules.[34] To investigate the forces involved in IN-DNA condensation, we first introduced IN (2 μM) in the flow cell to interact with tethered DNA molecules under very low stretching forces (F < 0.01 pN) for 10 min, to allow for condensate formation, and then increase the applied forces to mechanically probe the stability and structure of the condensate (Fig 5b). In a first set of experiments, we gradually increase the force from 0.1 to 5 pN with plateaus of constant force of 60 min (0.1 to 0.9 pN) or 30 min (1 to 5 pN), respectively (Fig 5b and Supplementary Fig. S15), while tracking the extension of the DNA tether. In the presence of IN, the DNA is shortened, compared to control experiments in the absence of IN and to the prediction of the WLC model (red line in Fig 5b), in particular in the force range 0.5 – 1 pN (Fig. 5b, inset). At very low forces (< 0.5 pN), we observe slow increases and decreases in DNA extension at constant force (Fig. 5c and Supplementary Fig. S15c-f), suggesting that the breakage and re-formation of interactions is in equilibrium under these conditions, pointing towards a dynamic and reversible nature of the interactions involved in loop compaction. Notably, the largest deviations from bare DNA behavior are observed in the same force range in force-extension experiments (Fig 5b, inset). The observed reversibility of compaction in the MT is consistent with the observation that rosette formation and compaction are reversible in AFM imaging experiments upon dilution.

In a second set of experiments, we again incubated tethered DNA molecules at a very low force (F < 0.01 pN) in presence of 2 μM IN for 5 min, to allow for the formation of IN-DNA condensates. Then, we apply a force-jump to a constant stretching force of 1 pN. We observe that the extension only increases very gradually – in contrast to control experiments with bare DNA, where the DNA extension increases essentially instantaneously (≤1 s) to the full extension of DNA at the given force (Supplementary Fig. S16). During force-induced extension in the presence of 2 μM IN, we observe extension increments or “bursts”, interspersed by long (>min) pauses or “dwells” where extension remains constant (Fig 5d, top panel). We quantify the extension time traces at constant force by determining i) the dwell times between extension events and for each extension event, ii)

the total increase in tether length for the event, and iii) the extension velocity (Fig 5d, bottom panel and Supplementary Fig. S17 to S19).

The increase in length per extension event is in reasonable agreement with the loop sizes found in AFM measurements with short DNA (Fig. 1 and Fig. 5e), suggesting that individual extension events correspond to the release of compacted loops. The extension velocities are much smaller (by at least two orders of magnitude) than what would be expected for the release of free DNA loops[35,36] and show a broad velocity distribution (Fig. 5f), suggesting that IN-compacted DNA loops are released under forces of ~1 pN, but that the release is slowed down by protein interactions that cause an effective friction and exhibit heterogeneous dynamics, consistent with the viscous nature of the soft coat region observed by AFM probing. The distribution of dwell times between extension events has a mean lifetime of (24 ± 3) s and does not follow a single exponential, but rather a power law with scaling exponent −1.0 ± 0.1 (Fig. 5g), suggesting heterogeneous interactions and dynamic disorder.[37]

The finding that extension plateaus (characterized by long dwell times ~1 min) separate bursts of extension increments (which occur on the ~s timescale), suggests that force is first exerted on mechanically stable and long-lived DNA-protein bonds that shield the hidden length in compacted loops from opening up. Once these long-lived bonds break, compacted loops are released and open under the applied force, but still experience friction originating from protein-protein and protein-DNA interactions. Together, our MT data further confirm the biphasic nature of fully compacted condensates, and indicate heterogeneous interactions with high levels of dynamic disorder.

Allosteric IN inhibitors interact differentially with rosettes versus collapsed condensates

Our experimental data suggest that interactions in the core are mediated by IN tetramers and higher order assemblies thereof (Fig. 1i). As IN tetramers are the preferred target for allosteric integrase inhibitors,[38] we tested the susceptibility of condensates in the rosette and fully compacted states towards the small molecule, quinoline-derivative inhibitor CX014442, an integrase inhibitor originally developed to inhibit association with the coactivator LEDGF/p75 (LEDGIN; this class of inhibitors has also been referred to as ALLINI for *allosteric integrase inhibitor*).[39] In the following, we will refer to CX014442 from now on as the "inhibitor" for simplicity.

In a first set of experiments, we introduced the inhibitor at 1 and 2 μM, at fixed [IN] = 1 μM, i.e. under condition where the rosette is formed, and studied the resulting complexes via AFM imaging (Supplementary Fig. S20). At equimolar [inhibitor]:[IN] ratio, the typical single nucleoprotein clusters of the rosette conformations appear partly disintegrated (Fig. 6a). We observe an increase in the radius of gyration of the entire molecule, albeit statistically not significant (p = 0.35; Fig. 6b). In contrast, the radius of gyration of the core decreases significantly (p = 0.024; Fig. 6c and Supplementary Fig. S21). At 2:1 [inhibitor]:[IN] ratio, the rosette core is disintegrated completely: individual IN complexes are still bound along the DNA contour but do not exhibit bridging interactions. Consistently, we find significant changes in cluster size and integrity (via $R_{g,core}$; $p = 10^{-25}$), as well as significant changes in compaction of the condensate (via $R_{g,mol}$; $p = 2 \cdot 10^{-14}$).

In a second set of experiments, we introduced the inhibitor at 1.5 and 3 μM, at a fixed [IN] = 1.5 μM, i.e. to fully compacted condensates (Fig. 6d). Strikingly, independent of the inhibitor concentration, the nucleoprotein assemblies retain their fully compacted conformations akin to those found in the absence of inhibitor and we observe no significant changes in cluster size (Fig. 6e,f). We note that the influence of the inhibitor on rosette or fully compacted conformations does not depend on order of addition, indicating that steric effects on inhibitor binding cannot explain our observations (Supplementary Fig. S21).

In general, LEDGINs have multiple mechanisms of action during HIV replication. By interference with the binding of LEDGF/p75 they redirect integration into less optimal sites. LEDGINs also allosterically interfere with the enzymatic activity of integrase and induce aberrant multimerization[40]. Aberrant multimerization of IN during virus assembly results in defective virus particles. LEDGINs do affect IN-IN interactions and, in particular induce the loss of IN into inactive aggregates at high IN concentrations.[12,41] Consistent with this view, we observe protein aggregates by AFM imaging (Supplementary Fig. S20). However, the fact that at 1.5 μM IN no visible changes on fully compacted nucleoprotein complexes are observed in the presence of inhibitor strongly suggests that the major effect on IN-induced condensation is not due to the loss of IN in inactive aggregates. In addition, the differential effects of the inhibitor on rosette formation and the fully collapsed state indicate that the protein-protein and/or protein-DNA interactions involved are different for the different compaction stages.

DISCUSSION

Here we use single molecule experiments in combination with Monte Carlo simulations to analyze nucleoprotein condensates formed between viral cDNA mimics and recombinantly expressed HIV IN. IN induces (flexible) DNA bends and mediates the formation of DNA loops. Furthermore, DNA-bound IN complexes were found to be larger than unbound complexes, suggesting that DNA binding induces conformational changes leading to higher order oligomerization (Fig. 7). At concentrations 500-1000 nM, IN induces formation of large DNA complexes with multiple DNA loops extending from the center that we refer to as rosettes. At higher IN concentrations 1000-1500 nM, a second compaction transition occurs in which the extruding loops of the rosette structure collapse onto the core of the condensate and full compaction is achieved. The resulting fully collapsed condensates drastically (~30-fold) reduce the volume occupied by the viral genome. In addition, our data indicate that in the fully collapsed condensate, a rigid central core is surrounded by a diffuse outer layer with relaxation times on the ~min timescale. It is interesting to note that this architecture resembles a polymer brush, the model that similarly describes the folding of eukaryotic chromosomes[42] that are orders of magnitude larger and also plays key roles in the organization of prokaryotic genomes.[43] Importantly, we experimentally demonstrate that the center of the rosette and the outer layer of the fully compacted IN-DNA complexes interact differently with the inhibitor CX014442: while the center of the rosette dissolves in an inhibitor concentration-dependent manner, the outer layer of the fully compacted state is not susceptible to the inhibitor in

the concentration range probed, irrespective of the order of addition of the inhibitor (Supplementary Fig. S21). Intriguingly, this drug-susceptibility (Fig. 6) is in stark contrast to the mechanical (Fig. 4 and 5) and thermodynamic stability (Fig. 2).

Provided that our experiments give a very reductionist view of HIV DNA genome compaction, it is important to evaluate their potential relevance with regards to viral condensates in the infected cell. In this regard, the size of IN-DNA condensates in vitro compare very favorably to sizes found by super-resolution microscopy in vivo and to those of PICs purified from infected cells.[16] In addition, in vivo IN plays a crucial role during capsid uncoating[44], and IN mutants defective for non-specific DNA binding are impaired for nuclear import[13] and reverse transcription.[14] Thus, it appears that IN plays a critical role within the cytoplasmic viral capsid during the early stages of infection. Maybe, these effects involve an architectural role for IN as a DNA compaction agent – similar to the established role of IN during folding of the RNA genome in nascent virus particles.[12] Nevertheless, we stress that our data do not rule out the involvement of other DNA interacting viral proteins that have been shown to induce DNA compaction in vitro, such as the nucleocapsid[45,46] and VpR proteins.[47]

Finally, we are convinced that the highly quantitative approaches presented here will be instrumental in future efforts to unravel the molecular mechanisms governing retroviral nucleoprotein condensation, e.g. to further establish the role of IN and other DNA-binding viral proteins in compaction of viral DNA. We especially anticipate breakthrough insights into PIC architecture by combining bottom-up (i.e. reconstitution) and top-down (i.e. purification) approaches. Similarly, it would be intriguing to use similar methods to probe the effects of IN on HIV RNA compaction, which has an established biological role during virus maturation.[12] Last, we expect our experimental platform to be valuable for the in-depth mechanistic investigation of strand transfer and allosteric inhibitors of IN on DNA compaction, and potentially uncover the mode of action of future inhibitors targeting disordered phases of IN and its condensates.

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FIGURES

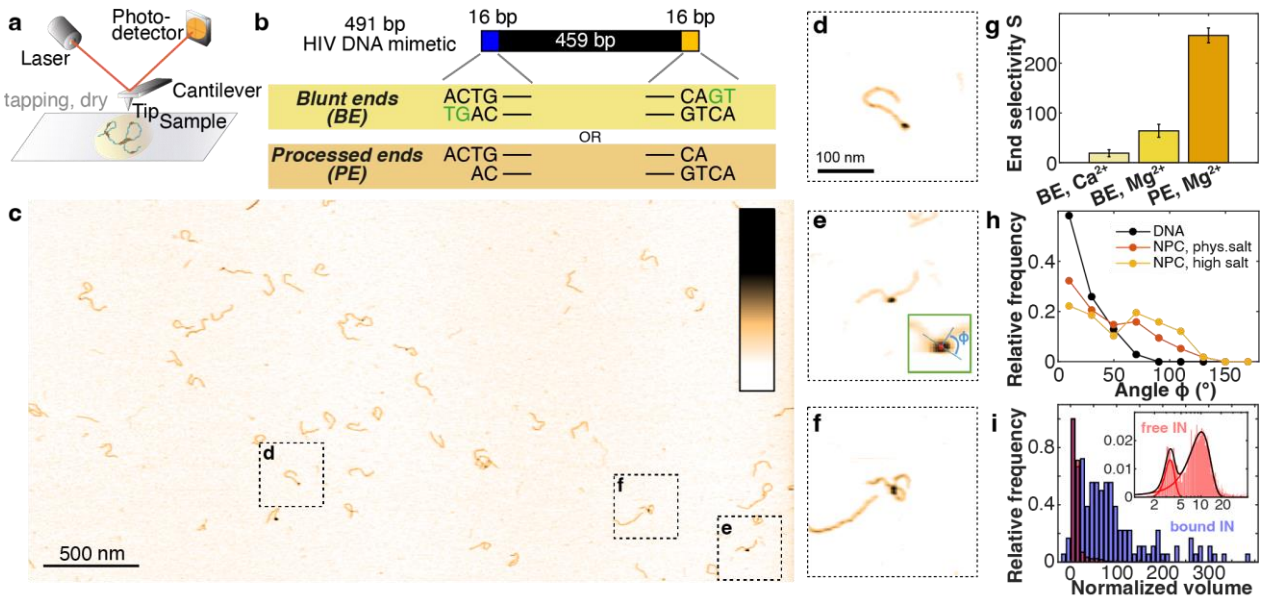

Figure 1. IN specific and aspecific binding to HIV DNA mimetics. a. Schematic representation of the experimental set up for AFM imaging experiments. **b.** Schematic of the 491 bp DNA used to mimic the HIV cDNA, both as a blunt ended (BE) or as a 3'-preprocessed substrate (PE). **c.** Overview AFM topograph of the 491 bp DNA in the presence of 100 nM IN. Color range is 1.5 nm. **d.** HIV DNA mimetic with end-bound IN. **e.** HIV DNA mimetic with integrase bound at internal sites and definition of bend angle (inset). **f.** HIV DNA mimetic with integrase-mediated DNA looping. **g**. Selectivity for end-binding over binding to internal sites in the mimetic as a function of 3' processing and buffer conditions. From left to right: blunt DNA ends, in the presence of Ca^{2+}; blunt DNA ends, in the presence of Mg^{2+}; processed DNA ends, in the presence of Mg^{2+}. The IN binding site size is assumed to be 16 bp.[22] Error bars are based on counting statistics. **h.** Bend angle distribution measured at 10 nm length scale for bare DNA and IN-DNA nucleoprotein complexes (NPCs) deposited from near-physiological salt buffer (50 mM NaCl, 1 mM $MgCl_2$, 10 mM Tris-HCl pH = 7.4; N = 232) or high salt buffer (250 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris-HCl pH = 7.4; N = 109). **i.** Histogram of the normalized volume of IN free in solution (red) and IN bound to DNA (blue). In free solution, we find a small fraction of IN monomers and a dominant fraction of IN dimers (inset). For IN bound to DNA, we find the resulting protein volume distribution to be significantly broader and shifted to larger volumes as compared to the DNA-unbound IN population.

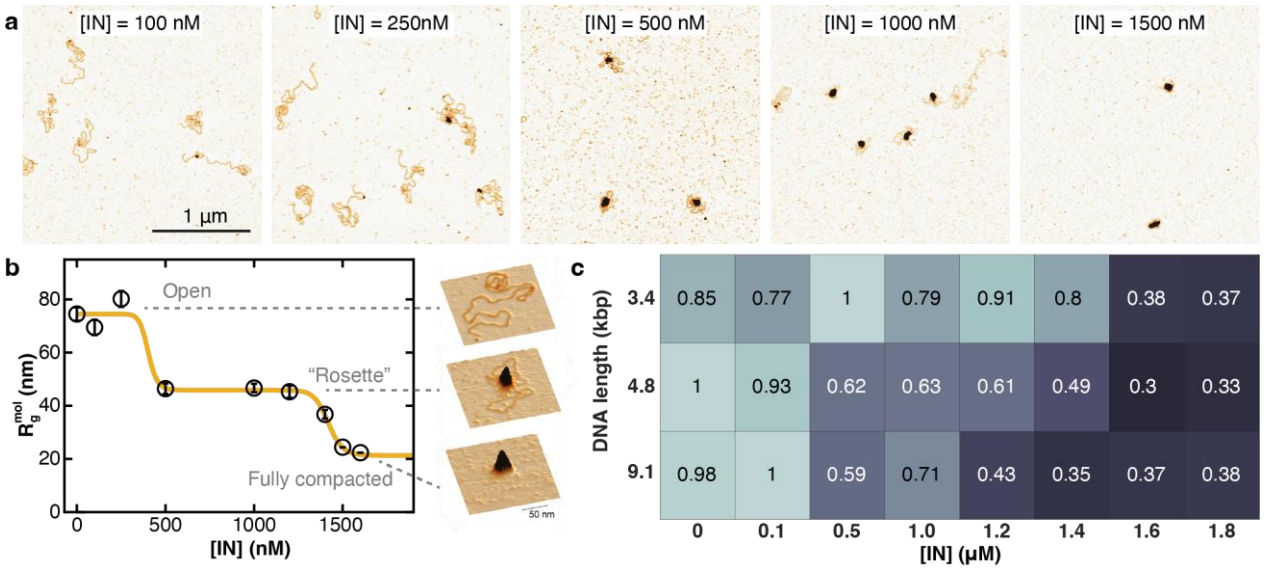

Figure 2. IN compacts long viral DNA mimetics into biphasic condensates. a. AFM topographs of 4.8 kbp DNA in the presence of (from left to right) increasing IN concentrations. **b.** Radius of gyration of the nucleoprotein complex for 4.8 kbp DNA at 60 mM ionic strength as a function of [IN]. The data reveal two distinct compaction transition and are fitted by a double-Hill-fit: the first transition from open state to rosette state occurs at ~ 500 nM, the second transition from rosette state to fully compacted state at ~ 1100 nM. Representative AFM images are shown as insets on the right. Symbols and error bars are the mean and s.e.m. from typically ≥ 100 molecules per condition. **c.** Phase diagram depicting the normalized radius of gyration of the nucleoprotein complexes formed as a function of [IN] and DNA length, normalized to the largest R_g observed for each DNA length. With increasing DNA length, the compaction transitions shift to lower IN concentration. Intriguingly, at DNA length > 3.4 kbp a biphasic compaction transition is observed, whereas at 3.4 kbp the transition occurs in one step.

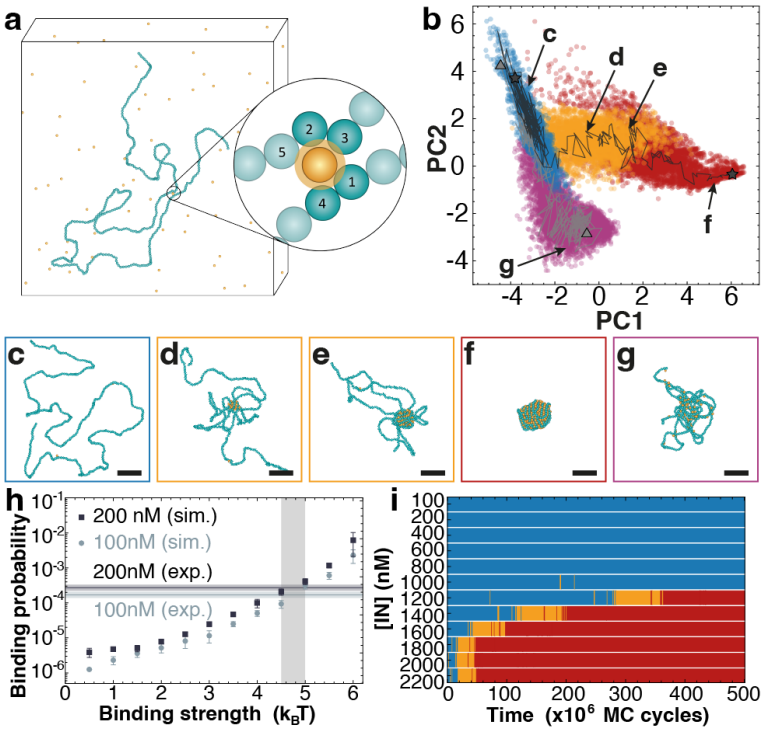

Figure 3. Monte Carlo (MC) simulations show that IN protein-protein interactions are required for biphasic compaction. a. Schematic representation of the setup for MC simulations. DNA beads (4 nm) are colored in light blue and IN beads (same size) are colored in yellow. The box size is not to scale. **b.** Principal component analysis separates the large dataset of DNA-IN complexes into several categories of DNA-IN complexes (depicted as different colors, which were assigned using a Gaussian mixture model). The grey trajectories indicate two typical simulation paths of 4.8 kbp DNA (408 beads). Without IN-IN attraction, the left path is taken (triangles at start and end point), here no rosette intermediates are formed. Only if IN-IN attractions are added (right path; stars at start and end point), rosette formation (yellow) as well as full compaction are possible (red). **c-g.** Some typical conformations along the pathways in panel b. The scale bar indicates 40 nm. Free IN are not depicted. **h.** IN-DNA binding probability (mean ± SD from 5 independent simulations) as a function of binding strength (in k_BT) from MC simulations. The horizontal lines indicate the experimentally determined binding probability for 100 nM and 200 nM IN from AFM images. The crossing area of experiment and simulations allows to estimate the IN-DNA binding energy to be 4.5-5 k_BT. **i.** Time evolution of the state of compaction (same color as in panel b) of a 4.8 kbp DNA during a MC simulation with IN-IN attractions for varying IN concentrations, well in line with the experimental data (Fig. 2). Each simulation was selected as the most typical one from 12 simulations per IN concentration.

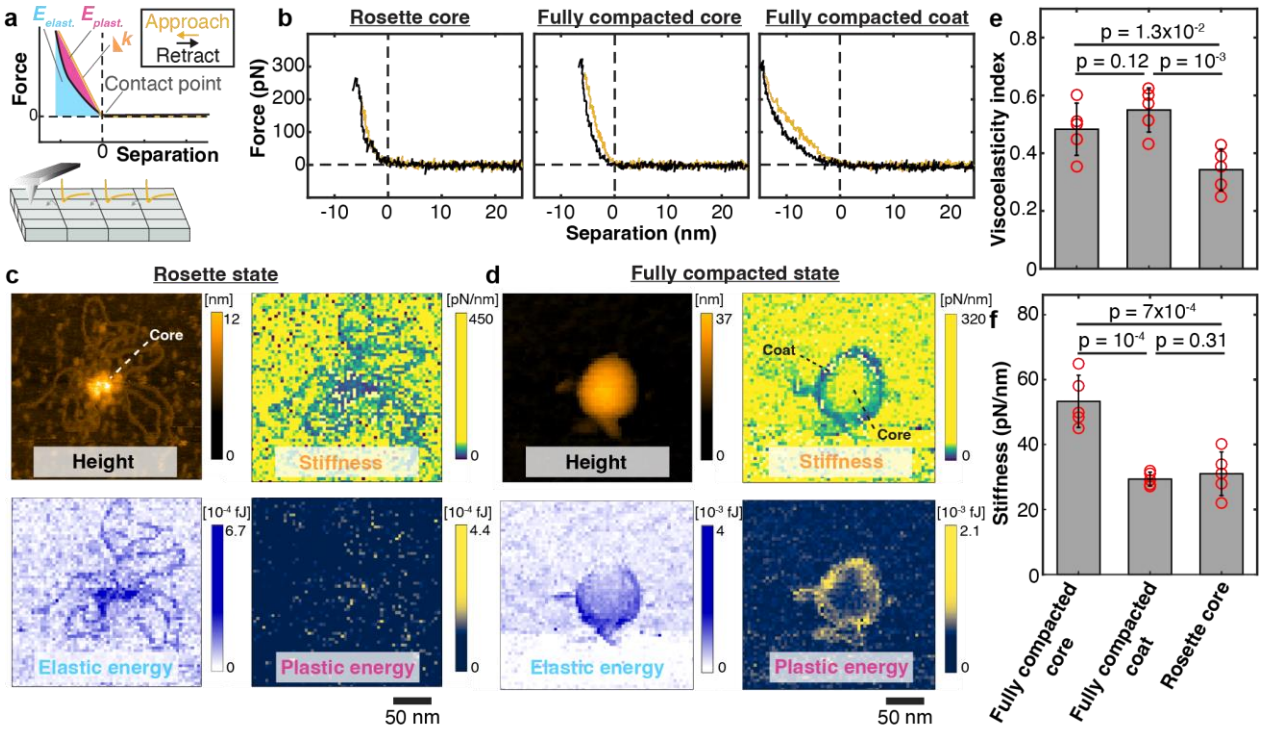

Figure 4. AFM force-volume based multiparametric imaging of IN-DNA condensates. a. Schematic depiction of AFM-based force-volume mapping, and derived parameters *k* (indentation stiffness), $E_{\text{plast.}}$ (plastic deformation energy), and $E_{\text{elast.}}$ (elastic deformation energy). **b.** Example force distance curves (yellow: approach; black: retract). **c.** Multiparametric imaging of a condensate in the rosette state: zero-force height (top left), stiffness (top right), elastic energy (bottom left), plastic energy (bottom right). **d.** Multiparametric imaging of a DNA-IN condensate in the fully compacted state, indicating a soft "coat" surrounding a rigid "core". **e.** Viscoelasticity index (mean ± SD) of the core and coat of fully compacted state, and of core of rosette state. *p*-values are based on 2-sample Kolmogorov-Smirnov test. **f.** Indentation stiffness (mean ± SD) of the core and coat of fully compacted state, and of core of rosette state. *p*-values are based on 2-sample Kolmogorov-Smirnov test.

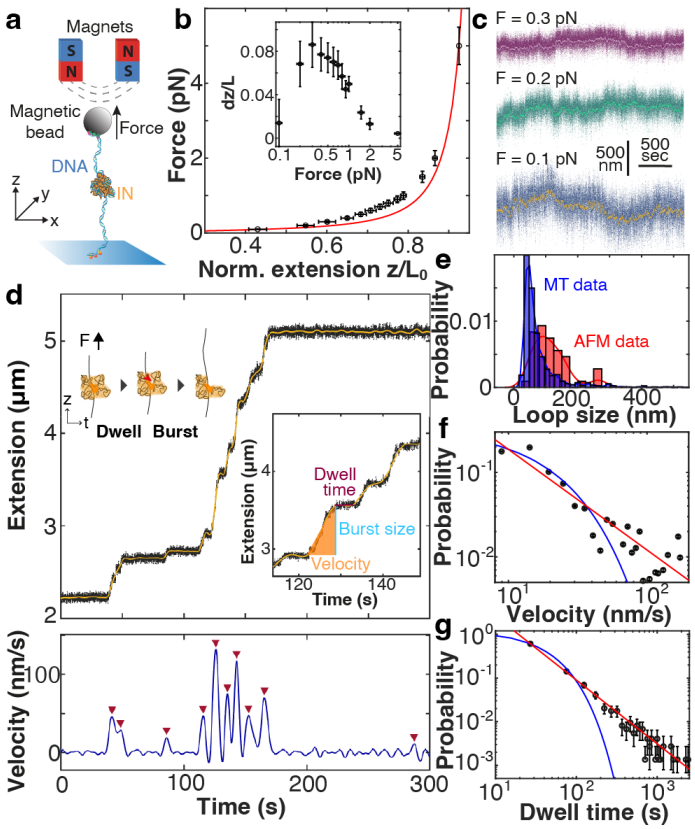

Figure 5. Single-molecule force spectroscopy quantifies dynamics condensate unfolding. a. Schematic depiction of the MT force spectroscopy measurements. 21 kbp DNA is tethered between a flow cell surface and magnetic beads and (partially) compacted by IN. External magnets enable the application of precisely calibrated stretching forces. **b.** Force-extension relation at forces ≤5 pN. Extension z is normalized to L_0, the extension of bare DNA at F = 5 pN. Red line is a worm-like chain (WLC) fit of bare DNA. The inset shows the deviation of the experimental data (mean ± SD) in the presence of IN from bare DNA WLC behavior; the maximum deviation occurs at ~ 0.3 pN. **c.** Extension-time traces of IN-DNA condensates at constant forces ≤0.3 pN exhibiting dynamic compaction and extension. **d.** Top: Extension-time trace following a force jump from 0.01 to 1 pN. Raw data (68 Hz) are depicted in black, yellow line is smoothed data (Butterworth filter, see Methods and SI for filter parameters). Insets depict a schematic of the events governing dwells (corresponding to extension plateaus), and extension bursts following dissociation of IN-DNA bridge in the core of the complex (red arrow). Bottom: Velocity-time trace, as obtained by differentiation of the filtered extension trace. Red marks indicate extension bursts detected by thresholding. **e.** Burst size distribution (blue bars; N= 777 events from 41 independent DNA tethers). For comparison, the loop size distribution obtained by AFM imaging are shown (red bars). A two sample t-test suggest no significant difference between both distributions (2-sample t-test; p = 0.5) **f.** Weighted bursts velocity distribution fitted with exponential decay (blue line; fitted decay constant 12 ± 3 nm/s) and power law (red line; scaling exponent –1.9 ± 0.3) for 1 nm/s < v < 100 nm/s. **g.** Distribution of dwell times between extension bursts and fits using an exponential decay (blue line; decay time of 24 ± 3 s) and power law (red line; scaling exponent –1.0 ± 0.1). Errors reflect 95% confidence intervals on the fit parameter.

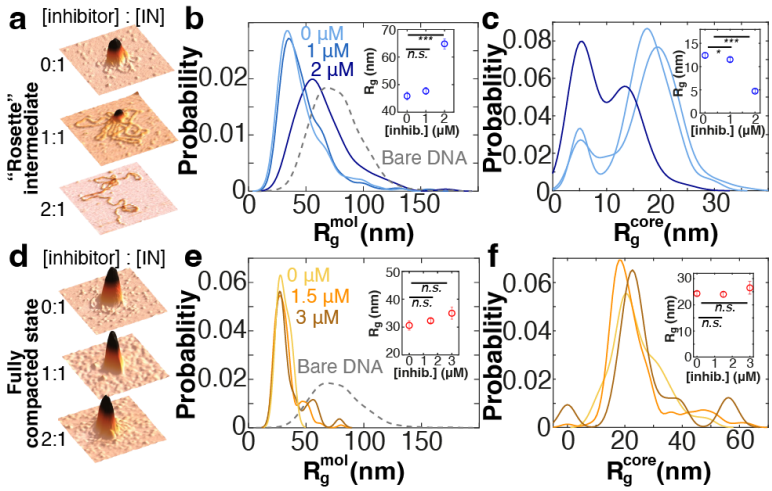

Figure 6. Allosteric inhibitors affect compaction behavior of long viral DNA mimetics. a. AFM topographs of the 4.8 kbp DNA in the presence of 1000 nM IN (rosette state) and no, 1000 nM, 2000 nM inhibitor. The inhibitor was added after complex formation. **b.** R_g-distributions for the entire molecule at 1000 nM IN and no, 1000 nM, 2000 nM inhibitors. As a reference, the distribution for bare DNA is shown as well. With increasing inhibitor concentration, less compaction is observed. **c.** Same conditions and color coding as in panel b only the R_g-distributions for the core are shown. **d.** AFM topographs of the 4.8 kbp DNA in the presence of 1500 nM IN (fully compacted state) and no, 1500 nM, 3000 nM inhibitor. The inhibitor was added after complex formation. **e.** R_g-distributions at 1500 nM IN and no, 1500 nM, 3000 nM inhibitors. As a reference, the distribution for bare DNA is shown as well. **f.** Same conditions and color coding as in panel e only the R_g-distributions for the core are shown. Even at high inhibitor concentrations, the fully compacted DNA-IN complexes do not disassemble under influence of the inhibitor. The insets in panels b, c, e, and f shows the means ± SEM of the respective distributions. Significance values are based on 2-sample Kolmogorov-Smirnov test: * indicates $p < 0.05$; *** indicate $p < 0.001$, *n.s.* indicates not significant.

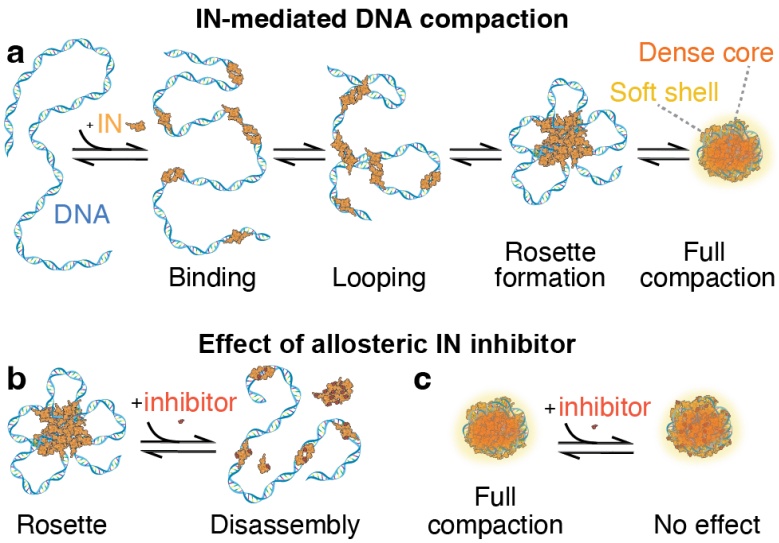

Figure 7. Model for IN-mediated DNA compaction. a. Schematic model of DNA compaction by IN. At increasing IN concentrations, the protein binds to the DNA and can form DNA loops. At intermediate concentration, loop-extruding complexes, so-called rosettes, form. At high IN concentrations, full compaction occurs. **b.** The allosteric IN inhibitor CX014442 can de-compact rosettes. **c.** The allosteric IN inhibitor CX014442 cannot de-compact fully compacted DNA-IN condensates. We observe that all compaction and de-compaction processes are reversible.