



# Release of linker histone from the nucleosome driven by polyelectrolyte competition with a disordered protein

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**Highly charged intrinsically disordered proteins are essential regulators of chromatin structure and transcriptional activity. Here we identify a surprising mechanism of molecular competition that relies on the pronounced dynamical disorder present in these polyelectrolytes and their complexes. The highly positively charged human linker histone H1.0 (H1) binds to nucleosomes with ultrahigh affinity, implying residence times incompatible with efficient biological regulation. However, we show that the disordered regions of H1 retain their large-amplitude dynamics when bound to the nucleosome, which enables the highly negatively charged and disordered histone chaperone prothymosin  $\alpha$  to efficiently invade the H1-nucleosome complex and displace H1 via a competitive substitution mechanism, vastly accelerating H1 dissociation. By integrating experiments and simulations, we establish a molecular model that rationalizes the remarkable kinetics of this process structurally and dynamically. Given the abundance of polyelectrolyte sequences in the nuclear proteome, this mechanism is likely to be widespread in cellular regulation.**

A large fraction of the human genome codes for proteins that contain extensive disordered regions or even lack any well-defined three-dimensional structure<sup>1</sup>. These intrinsically disordered proteins (IDPs) are known to play important roles in the cell nucleus, especially in the formation of nuclear organelles via liquid–liquid phase separation, chromatin condensation and transcriptional regulation, via mediating key interactions with other proteins or nucleic acids<sup>2,3</sup>. DNA- and RNA-binding proteins often contain disordered regions highly enriched in positively charged residues<sup>4,5</sup>, which are expected to facilitate electrostatic interactions with their cellular targets<sup>5</sup>. The affinities can be remarkably high, even if no structure is formed upon binding<sup>6,7</sup>. Such polyelectrolyte interactions have long been known in the fields of polymer chemistry and soft matter physics<sup>8,9</sup>, but their importance in biology only recently started to be recognized<sup>6,7,10–12</sup> and is thus largely unexplored.

A ubiquitous group of nuclear proteins with disordered polycationic regions are the histones, which are responsible for packaging DNA into chromatin, predominantly via their strong electrostatic interactions with the polyanionic nucleic acids<sup>13</sup>. Among these, the linker histones are particularly notable<sup>14</sup>; they are largely disordered and highly positively charged, with two disordered regions flanking a small folded globular domain. By binding to the linker DNA on the nucleosome (Fig. 1a), linker histones contribute to chromatin condensation and transcriptional regulation<sup>14–17</sup>. The extent of disorder in the complex between the nucleosome and linker histones suggests that it may have important roles for function<sup>16</sup>. For

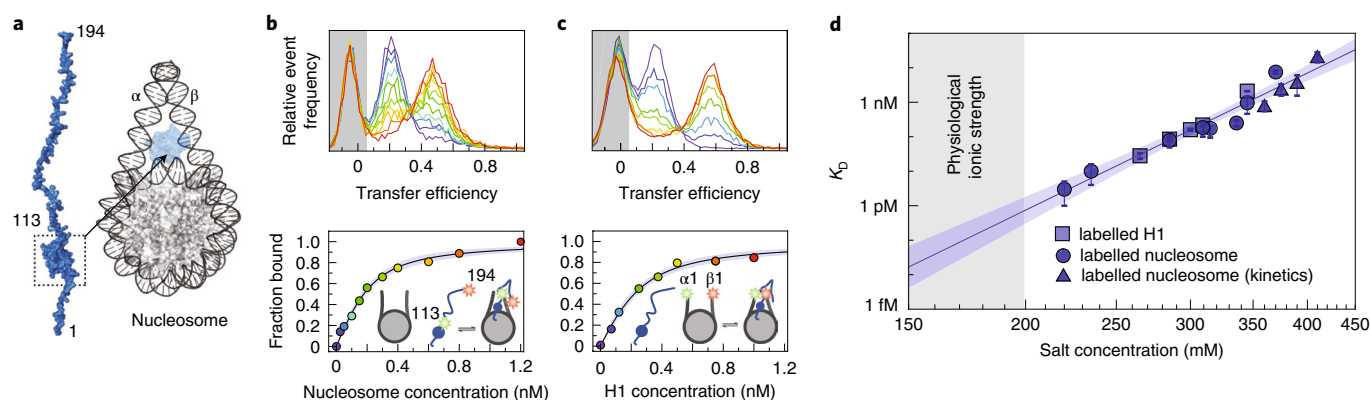
example, it was recently demonstrated that the linker histone H1 can promote the formation of liquid–liquid phase separation of reconstituted chromatin arrays under physiological conditions<sup>18</sup>.

Here, we show how the disordered tail regions of H1 facilitate a remarkable mechanism of molecular chaperoning that keeps the H1–nucleosome complex in a rapid association–dissociation equilibrium despite the extremely high affinity of the interaction. By integrating single-molecule experiments and simulations, we establish a molecular model of the H1–nucleosome complex, in which the H1 tails remain highly disordered and dynamic even when bound. We demonstrate that the highly negatively charged and disordered human protein prothymosin  $\alpha$  (ProT $\alpha$ ), a well-known histone chaperone<sup>19–22</sup> that forms a high-affinity disordered complex with linker histone H1 (ref. 6), can efficiently displace H1 from the nucleosome and accelerate its dissociation. The results can be explained by a mechanism in which ProT $\alpha$  initially invades the linker-histone/nucleosome complex and forms interactions with H1 mediated by the extensive disorder in both proteins, resulting in facilitated H1 dissociation. The underlying process of competitive substitution<sup>23</sup> via a transient ternary complex may be widespread in IDP-mediated nuclear interactions.

## Results

**Probing H1–nucleosome interactions.** To understand the interactions between nucleosomes, H1 and ProT $\alpha$ , we probed both the conformational distributions of these highly disordered molecules as well as the equilibria and kinetics of their complex formation and

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**Fig. 1 | H1 binds nucleosomes tightly but reversibly.** **a**, Structural representation of histone H1 and the nucleosome–H1 complex, with the position of the H1 globular domain on the nucleosome indicated (PDB no. 5NLO)<sup>29</sup>. H1, blue; DNA, dark grey; core histones, light grey. The entry and exit linker DNA are denoted  $\alpha$  and  $\beta$ , respectively. **b,c**, Binding of H1 to the nucleosome. Single-molecule transfer efficiency histograms and binding isotherms (see symbols in binding isotherms below for the colour code of concentrations) of freely diffusing H1 fluorescently labelled at positions 113 and 194 (**b**) or nucleosomes labelled at positions  $\alpha 1$  and  $\beta 1$  (**c**) titrated with the corresponding unlabelled binding partner (10 mM tris(hydroxymethyl)aminomethane (Tris), 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4, 300 mM KCl). See cartoon insets for illustration of labelling positions. Transfer efficiency range dominated by signal from molecules lacking an active acceptor dye shaded in grey. **d**, Equilibrium dissociation constant ( $K_D$ ) as a function of salt concentration, from binding isotherms using labelled H1 (squares) or labelled nucleosomes (circles), and from rate coefficients ( $K_D = k_{off}/k_{on}$ ) measured with surface-immobilized labelled nucleosomes (triangles). The data were fit and extrapolated to the physiological ionic-strength range (grey-shaded area) with the Lohman–Record model<sup>24</sup> (solid line with shaded 95% confidence band). Error bars indicate uncertainties estimated from dilution errors (see Methods for details).

dissociation. We first investigated the binding of the human linker histone H1.0 (referred to herein as H1) to nucleosomes with confocal single-molecule spectroscopy combined with Förster resonance energy transfer (FRET). We attached a donor and an acceptor fluorophore at positions 113 and 194 in H1, spanning its disordered C-terminal region (Fig. 1a), and monitored the binding to nucleosomes freely diffusing in solution.

Unbound H1 shows a low mean FRET efficiency,  $\langle E \rangle$ , as expected for a highly expanded configuration due to intramolecular repulsion between the positive charges in H1 (refs. 6,24,25). Upon addition of unlabelled reconstituted nucleosomes based on the 197-base-pair (bp) 601 Widom sequence<sup>26</sup> (Fig. 1a), a population with higher  $\langle E \rangle$  is formed (Fig. 1b), indicating a compaction of H1 on binding, in line with previous results<sup>27</sup>. From the bound fraction as a function of nucleosome concentration, we obtained the equilibrium dissociation constant ( $K_D$ ), which is highly dependent on salt concentration: across the range where reliable measurements were feasible (220–410 mM),  $K_D$  increases from  $\sim 3$  pM to  $\sim 10$  nM (Fig. 1d and Supplementary Table 1). Measurements at lower salt concentrations are complicated by the high and non-specific affinity of H1 to surfaces<sup>6</sup>, but extrapolation suggests subpicomolar  $K_D$  values in the physiological ionic-strength range below 200 mM—even slightly tighter than estimated previously<sup>28</sup>. To control for the possible influence of dye labelling on the interaction, we also performed single-molecule measurements with the fluorophores attached to the termini of the nucleosomal linker DNA arms (positions  $\alpha 1$  and  $\beta 1$ ; Fig. 1a) and titrated with unlabelled H1 (Fig. 1c). The  $\langle E \rangle$  value increases upon binding, in line with the expected closure of the linker DNA arms in the H1-bound nucleosome<sup>29–31</sup>. The resulting  $K_D$  values agree with the measurements using labelled H1 over a wide range of salt concentrations (Fig. 1d), attesting to the robustness of the approach and to a lack of strong perturbations by the dyes.

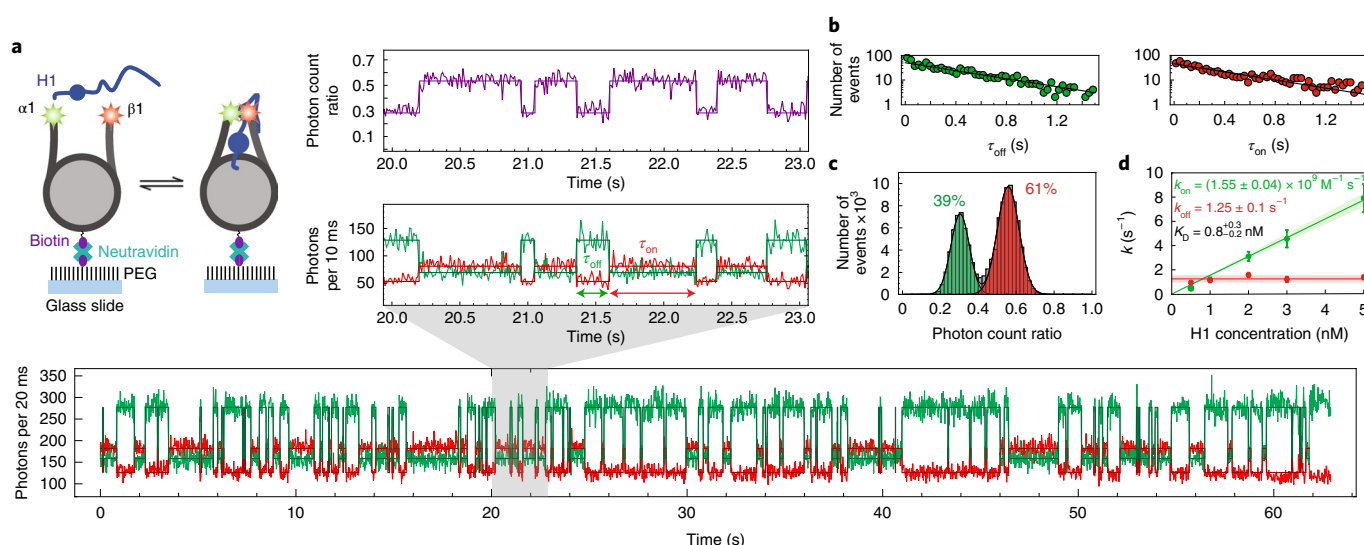
The pronounced salt concentration dependence of  $K_D$  (Fig. 1d) reflects the important role of electrostatic interactions in the binding process. Although a substantial fraction of the DNA charge in the nucleosome is compensated by the histone octamer, the resulting polyanion–polycation complex retains a large negative net charge, especially at the dyad and on the linker DNA, which favours the

binding of additional positively charged proteins such as H1 (ref. 13). The interaction of H1 with the nucleosome can be described in terms of a counterion exchange reaction, in which H1 binding results in a release of inorganic counterions—under our experimental conditions mainly chloride ions associated with H1 and potassium ions associated with the DNA<sup>32,33</sup>. The entropy gain from this counterion release is a major thermodynamic driving force for such protein–DNA interactions, and the dependence of the corresponding  $K_D$  on salt concentration has been shown for a variety of systems to be well approximated by the relation

$$\log K_D = \log [K_D(1 \text{ M})] - \Delta n \log c_M,$$

where  $c_M$  is the salt concentration,  $\Delta n$  is the number of released counterions, and the  $K_D$  at 1 M salt reports on non-electrostatic contributions to binding<sup>32</sup>. Applied to the H1–nucleosome interaction (Fig. 1d), we obtain a release of  $\Delta n = 13 \pm 1$  inorganic ions, and the  $K_D$  extrapolated to 1 M salt is  $\geq 1$  mM, indicating a weak non-electrostatic contribution to binding, possibly mediated by the interactions of the H1 globular domain with the DNA at the dyad<sup>29,34</sup>.

**Association kinetics of H1 on immobilized nucleosomes.** For probing the kinetic mechanism of H1–nucleosome interactions, we immobilized labelled nucleosomes via a biotin–streptavidin tether on polyethylene-glycol-passivated cover slides and recorded fluorescence time traces from single nucleosomes with confocal single-photon counting for up to several minutes (Fig. 2a, Supplementary Fig. 1). Nucleosomal unwrapping events were identified by persistent changes in transfer efficiency<sup>35,36</sup> and excluded from the analysis (Supplementary Fig. 1c). In the presence of low concentrations of unlabelled H1, the time traces exhibit the characteristic anti-correlated changes in donor and acceptor fluorescence expected from the association and dissociation of individual H1 molecules, with high  $\langle E \rangle$  in the bound state and low  $\langle E \rangle$  in the unbound state (Fig. 2a). To quantify the kinetics, thousands of such H1 association and dissociation events were analysed by likelihood maximization based on a two-state Markov model<sup>37</sup> (Fig. 2, see Methods for details), which describes the data well (Fig. 2a).



**Fig. 2 | H1 binds nucleosomes with diffusion-limited association rates. a**, Example of a single-molecule fluorescence time trace of 2 nM unlabelled H1 binding to surface-immobilized labelled nucleosomes (see cartoon; PEG, polyethylene glycol) at 360 mM salt (acceptor signal,  $N_A$ , red; donor signal,  $N_D$ , green). An expanded segment is shown above, along with the photon count ratio ( $N_A/(N_A + N_D)$ , purple) and appropriately scaled state trajectories (dark green, red, purple) based on Viterbi analysis with a two-state model. **b**, Dwell-time distributions for the unbound (green) and bound (red) states with exponential fits (solid lines). **c**, Photon count ratio histograms from 46 fluorescence time traces at 360 mM salt and 2 nM H1, showing the unbound (green) and bound (red) populations. **d**, Observed association rate (green) and dissociation rate coefficient (red) as a function of H1 concentration at 360 mM salt concentration and fits assuming a one-to-one binding model (solid lines) with resulting values. Error bars show one standard deviation estimated from ten bootstrapping trials; shaded bands for all fits represent 95% confidence intervals.

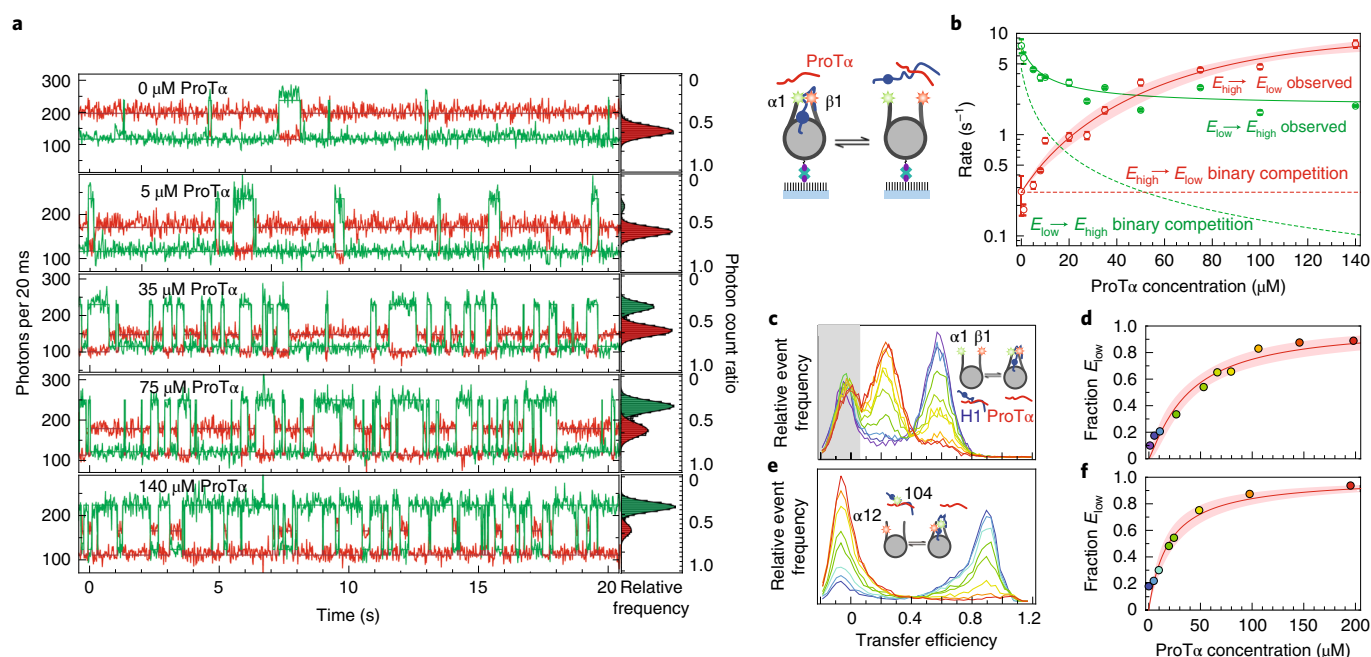
The resulting association ( $k_{on}$ ) and dissociation ( $k_{off}$ ) rate coefficients yielded values of  $K_D$  in accord with the free-diffusion experiments (Fig. 1d and Supplementary Table 1), indicating that surface immobilization does not interfere with H1 binding. Association is extremely rapid, with rate coefficients at the diffusion limit,  $>10^9 \text{ M}^{-1} \text{ s}^{-1}$  (Supplementary Table 1), which indicates binding without a substantial activation barrier. The increase in affinity with decreasing salt concentration (Fig. 1d) is dominated by a decrease in  $k_{off}$ , whereas  $k_{on}$  is much less salt dependent (Supplementary Fig. 2 and Supplementary Table 1). The similarity of the salt concentration dependences of  $k_{off}$  ( $12 \pm 1$  ions bound upon dissociation; Supplementary Fig. 3a) and  $K_D$  ( $13 \pm 1$  ions bound upon dissociation; Fig. 1d) further supports a binding mechanism that is downhill in free energy.

These results illustrate a classic conundrum regarding H1–nucleosome interactions<sup>28,38,39</sup>: at physiological ionic strengths, dissociation of H1 from the nucleosome *in vitro* is much too slow to be compatible with efficient cellular regulation of transcription and chromatin condensation<sup>40</sup>. At 165 mM salt, for instance, extrapolating our results yields an average dwell time in the bound state of  $3^{+2}_{-1}$  hours (Supplementary Fig. 3a), similar to previous results *in vitro*<sup>41</sup> but much longer than the timescale of about a minute observed in cells<sup>38,39</sup>. Clearly, other cellular factors must be involved in regulating the interaction of H1 with chromatin.

**ProTα efficiently displaces H1 from the nucleosome.** A well-known linker histone chaperone is ProTα<sup>19–22</sup>, which binds to H1 with high affinity<sup>6,12,42,43</sup> and is thus expected to compete with the nucleosome for H1 binding. Indeed, if the experiments probing H1–nucleosome binding are performed in the presence of ProTα, the kinetics of the system change markedly (Fig. 3a). For example, at 340 mM salt and 3 nM H1 in the absence of ProTα, H1 remains nucleosome-bound for  $\sim 4$  s on average, and association occurs rapidly after dissociation, leading to an unbound population of only  $\sim 2\%$ , just enough to detect occasional dissociation events in fluorescence time traces. At lower salt concentrations, the H1–nucleosome

affinity is too high to detect spontaneous dissociation. Upon addition of ProTα, the frequency of transitions to low  $\langle E \rangle$  increases (Fig. 3a), with a change in rate from  $0.3 \text{ s}^{-1}$  to  $8 \text{ s}^{-1}$  when the ProTα concentration is increased from 0 to 140  $\mu\text{M}$  (Fig. 3b). This rate enhancement occurs for a wide range of salt concentrations, and it exceeds two orders of magnitude at near-physiological salt concentrations (Supplementary Fig. 3a). Intra- and intermolecular single-molecule FRET experiments on freely diffusing molecules confirm the accelerated and complete dissociation of H1 from the nucleosome by ProTα (Fig. 3c–f and Supplementary Fig. 3). Thermodynamically, this reaction can be described as a competition between two polyanions, ProTα and DNA, for the polycation H1; the larger number of counterions released upon binding of H1 to ProTα ( $18 \pm 1$ )<sup>6,43</sup> compared to the nucleosome ( $13 \pm 1$ ; Fig. 1d) contribute entropically to the release of H1. Notably, the tens-of-micromolar ProTα concentrations we require for enhanced H1 dissociation are in the range reported for intracellular ProTα<sup>44</sup>, which is thus likely to contribute to the high mobility of H1 in cells<sup>38,39</sup>. What is the mechanism underlying this rate enhancement by ProTα?

The observed increase in rate (Fig. 3b) is not compatible with the simplest competition mechanism, where H1 forms exclusively binary complexes, either with the nucleosome or with ProTα. In this case, H1 would first have to dissociate from the nucleosome before binding to ProTα. In other words, dissociation of H1 from the nucleosome would be a unimolecular reaction with a rate independent of ProTα concentration (red dashed line in Fig. 3b), in contrast to what we observe. Moreover, the observed rate of transitions to high  $\langle E \rangle$  changes much less than expected for the association rate in this mechanism, where the presence of ProTα should sequester free H1 to an extent that H1 association would decrease to a virtually undetectable level (green dashed line in Fig. 3b). These deviations from the simplest competition mechanism are a typical indication for the formation of ternary complexes, a paradigm that has recently emerged for gene regulation and usually involves multivalent interactions<sup>45–49</sup>. Such ternary complex formation immediately suggests itself for complexes of IDPs such as H1 and ProTα, whose interactions can



**Fig. 3 | ProTα facilitates H1 dissociation from the nucleosome.** **a**, Fluorescence time traces monitoring H1 binding to surface-immobilized nucleosomes (cartoon) at different ProTα concentrations and corresponding photon count ratio histograms (see Fig. 2 for details). **b**, Kinetic analysis of time traces, with observed transition rates from low to high FRET efficiency ( $E_{\text{low}} \rightarrow E_{\text{high}}$ , green symbols) and from high to low FRET efficiency ( $E_{\text{high}} \rightarrow E_{\text{low}}$ , red symbols) as a function of ProTα concentration, described with different kinetic models (dashed line, competition with binary interactions only; solid line, competition including ternary-complex formation; see Methods for details). Error bars show one standard deviation estimated from ten bootstrapping trials. **c–f**, Transfer efficiency histograms (**c** and **e**) from free-diffusion single-molecule FRET experiments in the presence of different ProTα concentrations (see symbols in binding isotherms for the colour code, and cartoon insets for illustration of components and labelling positions) and corresponding fractional populations (**d** and **f**). Intramolecular FRET of double-labelled nucleosomes in the presence of 1 nM unlabelled H1, at increasing concentration of unlabelled ProTα (shown in the cartoon inset; **c**) and the corresponding fraction of low-FRET population (symbols in **d**) fit with a binding isotherm (**d**). Intermolecular FRET between 5 nM acceptor-labelled nucleosomes and 100 pM donor-labelled H1, with increasing concentration of unlabelled ProTα (shown in the cartoon inset; **e**) and the corresponding fraction of low-FRET population (symbols in **f**) fit with a binding isotherm (**f**). Note that in **c**, the peak at FRET efficiency  $E \approx 0$  (grey shading) originates from molecules lacking an active acceptor fluorophore, resulting in an amplitude independent of ProTα concentration; in **e**, we explicitly use the increase in the peak at  $E \approx 0$  and concomitant decrease of the peak at  $E \approx 0.9$  to monitor donor-labelled H1 dissociation. Data in all panels were collected at 340 mM salt; shaded bands for fits represent 95% confidence intervals.

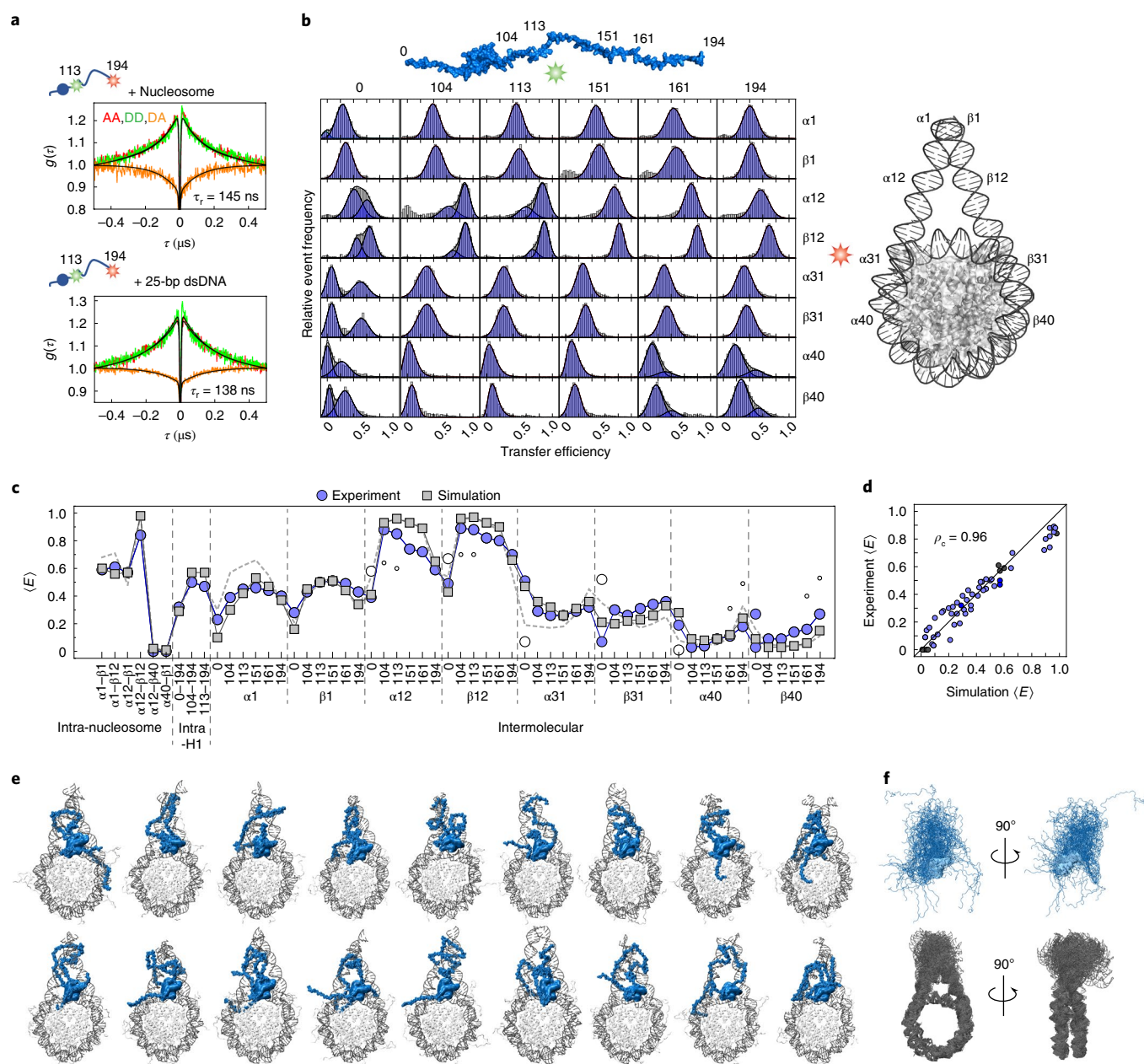
be considered an extreme case of multivalency<sup>10,45,49</sup>: if the positively charged tails of H1 remain largely disordered and dynamic while bound to the nucleosome, as suggested by recent experimental work on the C-terminal region of H1 bound to double-stranded DNA<sup>7</sup> and by simulations<sup>30,50</sup>, the negatively charged ProTα would still be able to invade the complex via local association–dissociation events and bind to H1 while both proteins remain disordered<sup>6</sup>. To obtain more insight into the underlying molecular mechanism of ProTα action, we thus first sought a structural description of H1 on the nucleosome.

**H1 is highly disordered and dynamic on the nucleosome.** The globular domain of H1 is known to localize to the dyad axis and interact with the nucleosomal core DNA and both linkers<sup>29,31</sup>. However, the conformational distributions of the disordered regions of H1 on the nucleosome have been difficult to elucidate because of their dynamics and lack of persistent structure<sup>4,7,29,51</sup>. Since fluctuations in distance cause fluctuations in the fluorescence intensity of donor and acceptor<sup>52</sup>, we can monitor molecular motion in single-molecule FRET experiments with nanosecond fluorescence correlation spectroscopy (nsFCS<sup>53</sup>; Fig. 4a and Supplementary Fig. 4). Indeed, H1 shows pronounced long-range chain dynamics (Fig. 4a) on the 100-ns timescale characteristic of disordered proteins<sup>52</sup>, both in isolation<sup>6</sup>, bound to the nucleosome (Fig. 4a), and bound to 25-bp double-stranded DNA, in agreement with recent NMR results<sup>7</sup> and with the insensitivity of the intermolecular FRET efficiency between H1 and 25-bp double-stranded DNA to

label position (Supplementary Fig. 5a,b). Distance fluctuations on this timescale can be observed throughout the C-terminal region of H1, as well as between the ends of the nucleosomal linker DNA (Supplementary Fig. 4b,c), highlighting the exceedingly dynamic nature of the complex. The presence of broad distance distributions and rapid molecular motion resulting from disorder is further supported by fluorescence lifetime analysis (Supplementary Fig. 6). These results suggest a picture in which local segments of the H1 tails continuously disengage and reengage in non-specific electrostatic interactions with the DNA, leading to rapid local and global reconfiguration dynamics.

We thus require a molecular model that takes this dynamical disorder into account, allows a description of H1 in terms of a structurally diverse conformational ensemble and can be optimized based on experimental data. In view of the large system size, we employed a coarse-grained simulation model that captures both the known structure of the nucleosome and the pronounced dynamics of the linker DNA and the disordered regions of H1. We used a ‘top-down’ coarse-grained model based on matching experimental observations, rather than a ‘bottom-up’ coarse-grained model matched to all-atom simulations<sup>30</sup>. We combined a structure-based model<sup>54,55</sup> for the nucleosomal core particle and the globular domain of H1 and its position on the dyad based on the crystal structure (Protein Data Bank (PDB) no. 5NL0)<sup>29</sup> with a polymer-like representation of the histone tails. The interaction between disordered regions of histones and DNA was encoded in terms of non-specific



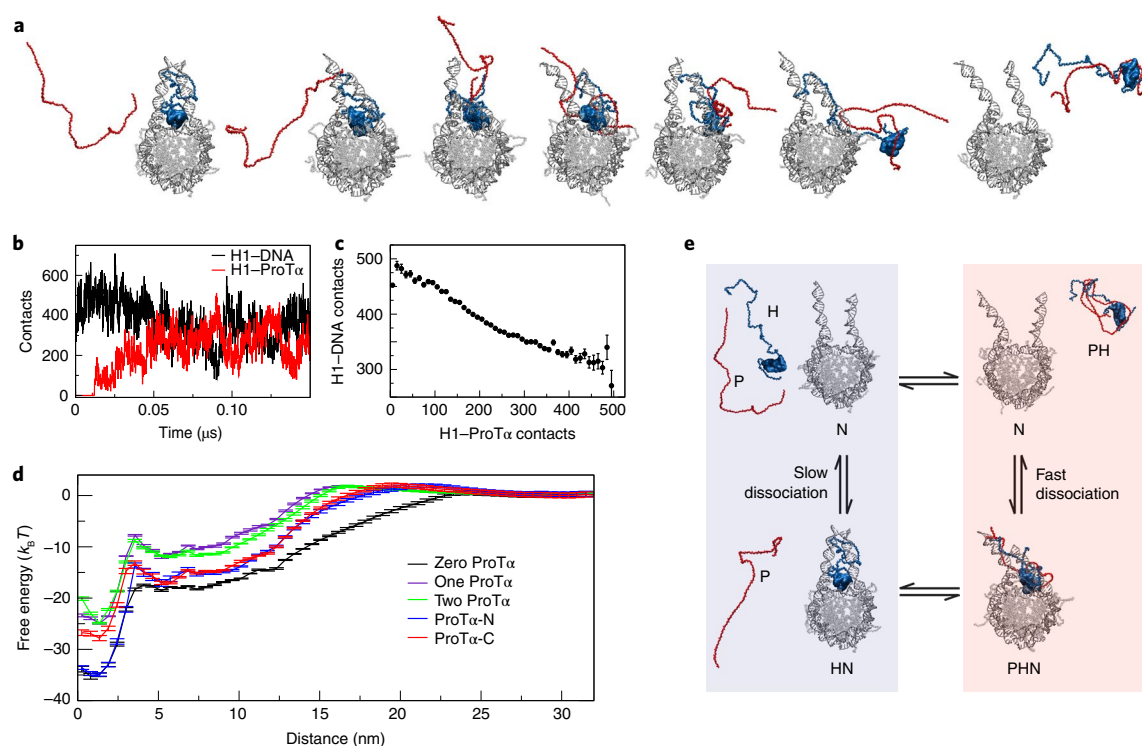


**Fig. 4 | H1 remains disordered and dynamic on the nucleosome. a**, nsFCS of double-labelled H1 bound to the nucleosome (top) and double-stranded 25-bp DNA (bottom), with donor-donor (DD, green), acceptor-acceptor (AA, red) and donor-acceptor (DA, orange) correlations,  $g(\tau)$ , with fits (see Methods for details;  $\tau_r$ , chain reconfiguration times;  $\tau$ , lag time). **b**, Intermolecular single-molecule FRET experiments with dyes at different positions along the H1 (donor dye, green star) and nucleosome sequences (acceptor dye, red star). Labelling positions are indicated as numbers along the structural representations. Subpopulations are fit with Gaussian peak functions (black lines). **c**, Comparison between average FRET efficiencies,  $\langle E \rangle$ , from experimental subpopulations and simulation for 57 different labelling pairs (**b** and Supplementary Fig. 5). Filled blue circles, main experimental FRET population; empty circles, minor experimental FRET population (size of symbol indicates population size); grey squares,  $\langle E \rangle$  from simulations using the H1 globular domain orientation as in structure 5NLO (ref. 29); dashed line,  $\langle E \rangle$  from simulations using the crystal structure but with the H1 globular domain rotated by 180° on the dyad. **d**, Correlation between experimental and simulated  $\langle E \rangle$ , with concordance correlation coefficient,  $\rho_c$ . **e**, Representative snapshots of the H1-nucleosome complex from simulations (see Methods for details, and Supplementary Video 1). **f**, Overlay of 50 conformations of H1 (top) and nucleosome (bottom) in the complex. All measurements and simulations performed at 165 mM salt concentration.

short-range and electrostatic interactions<sup>56</sup>, including a screening term to account for the experimental ionic strength (see Methods for details). To attain a realistic description of the disordered and dynamic parts of the H1-nucleosome complex, we adjusted the strength of the short-range protein-DNA interactions via a single parameter to maximize the agreement between the measured FRET efficiencies and those computed from the simulation ensemble.

Although this model does not explicitly represent counterions and all details of chemical structure<sup>57</sup>, its previous success in explaining the interaction of H1 with ProTα in terms of both overall structure and affinity<sup>6</sup> indicates that it can be used to obtain insight into the overall properties of the system.

To test and optimize the model of H1 on the nucleosome, we obtained FRET data reporting on a total of 57 intra- and inter-



**Fig. 5 | Mechanism of H1 chaperoning on the nucleosome by ProT $\alpha$ .** **a**, Structural model based on simulation snapshots depicting the association of ProT $\alpha$  (red) to the H1-nucleosome complex followed by the dissociation of ProT $\alpha$ -H1 from the nucleosome (Supplementary Video 1). **b,c**, Formation of H1-ProT $\alpha$  contacts and correlated breaking of H1-DNA contacts as ProT $\alpha$  invades the H1-DNA complex. Error bars in **c** are standard errors of the mean number of H1-DNA contacts for each bin in H1-ProT $\alpha$  contacts, with the bin width being ten contacts. **d**, Potentials of mean force for unbinding of H1 from the nucleosome (distance is between the globular domain and the DNA residues it binds in the natively bound state), in the presence or absence of ProT $\alpha$  or its fragments (legend). ProT $\alpha$ -N/ProT $\alpha$ -C, N/C-terminal halves of ProT $\alpha$ . **e**, Kinetic scheme depicting the relevant molecular states. H1 (H) binds to the nucleosome (N) rapidly (forming HN), leading to compaction of the C-terminal tail of H1 and a closing of the linker DNA. H1 dissociates extremely slowly from the nucleosome by itself, but binding of ProT $\alpha$  (P) to H1 on the nucleosome results in a ternary complex (PHN) that facilitates dissociation of H1 from the nucleosome in the ProT $\alpha$ -H1 complex (PH).

molecular distances from single-molecule FRET experiments. We created constructs with labelling sites on the nucleosomal DNA, both in positions corresponding to the linker DNA and in the core particle, and along the H1 sequence (Fig. 4b, Supplementary Fig. 5c,d and Supplementary Table 2). Intermolecular FRET experiments using alternating excitation of donor and acceptor indicate 1:1 H1-nucleosome complexes under our experimental conditions (Supplementary Fig. 6), in accord with previous results<sup>28</sup>. Most of the resulting transfer efficiency histograms exhibit a single peak, as expected from the rapid conformational averaging within the complex observed by nsFCS (Fig. 4c)<sup>53</sup>. The similarity of the transfer efficiencies involving positions of the labels on the entry and exit linker DNA that are rotationally symmetric with respect to the dyad axis attests to the robustness of the results.

The conformational ensembles resulting from the optimized simulation model show good agreement with the measured FRET efficiencies and other experimental data. In the H1-bound state, the characteristic pattern of transfer efficiencies for different labelling positions along the H1 and DNA sequences are described remarkably well by this simple model (Fig. 4c and Supplementary Fig. 7), and even the absolute efficiency values show excellent overall agreement (concordance correlation coefficient, 0.96; Fig. 4d). The simulations also reproduce the increase in FRET efficiency between the labelled linker DNA arms observed experimentally upon binding of H1 (Fig. 4c and Supplementary Fig. 5c,d), in accord with electron cryo-microscopy results showing a compaction of the nucleosome and crossing of the linker DNA arms when H1 is bound<sup>29</sup>.

In some cases, reproducible broadening or double peaks were observed in the transfer efficiency histograms (Fig. 4b), especially if the labels were located close to the exit and entry points of the nucleosomal DNA and to the globular domain of H1. We hypothesized that they are caused by the different orientations of the H1 globular domain on the dyad that were previously identified by crosslinking experiments and are expected to interconvert slowly<sup>14,15,29</sup>. Indeed, when the simulations were performed with the globular domain of H1 rotated by 180° on the dyad compared to the crystal structure<sup>29</sup>, the FRET efficiencies of several of the secondary populations could be reproduced (Fig. 4c). Explicitly including a representation of the fluorophores in the simulations yielded very similar results (Supplementary Fig. 7). Overall, these findings indicate that this coarse-grained, charge-dominated description of the disordered regions of H1 on the nucleosome captures the essential conformational and dynamic properties of the ensemble and further supports the notion that H1 retains its disorder when bound to the nucleosome<sup>7,29,58</sup>. Figure 4e,f and Supplementary Video 1 illustrate the broad range of conformations that are populated in the H1-nucleosome complex.

**Disorder underlies the mechanism of histone chaperoning.** The disorder and the large-amplitude conformational fluctuations of the positively charged regions of H1 on the nucleosome suggest a simple mechanism for its displacement by the negatively charged chaperone ProT $\alpha$  via the multivalent interactions possible in such a complex (Fig. 5a): initial contacts between ProT $\alpha$  and H1 are formed in a fly-casting-like process<sup>59</sup>, during which the number of

contacts between the two proteins progressively increases owing to the favourable electrostatic interactions. As a result, ProT $\alpha$  competes with the DNA for the disordered regions of H1 (Fig. 5b) and reduces the number of H1–DNA contacts (Fig. 5c) and thus the interaction strength of H1 with the nucleosome, leading to an opening of the linker DNA arms (Fig. 5a and Supplementary Video 2).

Unlike the essentially barrierless competitive substitution in polyelectrolyte complexes that occurs when the invading chain is longer and more charged than the one it is displacing<sup>23</sup>, spontaneous dissociation of H1 was too slow to be observed on the timescale of the simulations, likely because of the much smaller charge on ProT $\alpha$  compared with the nucleosome. We therefore performed umbrella sampling simulations to obtain potentials of mean force for the interaction of H1 with the nucleosome in the absence and presence of ProT $\alpha$  (Fig. 5d); these show barrierless binding of H1 in all cases, consistent with the diffusion-limited association (Fig. 2d and Supplementary Table 1) and the similarity in salt concentration dependences of  $k_{\text{off}}$  and  $K_{\text{D}}$  (Supplementary Fig. 3) observed experimentally. The resulting binding free energy is reduced from  $-38k_{\text{B}}T$  to  $-28k_{\text{B}}T$  by ProT $\alpha$ , where  $k_{\text{B}}$  is the Boltzmann constant and  $T$  is temperature. Since the difference in  $K_{\text{D}}$  is expected to be dominated by  $k_{\text{off}}$ , this change corresponds to a pronounced increase in dissociation rate, as observed experimentally (Fig. 3 and Supplementary Fig. 3). Simulations using the N- and C-terminal halves of ProT $\alpha$  individually suggest that the C-terminal part of the protein makes a larger contribution to H1 dissociation, as expected from the higher charge density in this region<sup>6,25</sup>. Furthermore, addition of a second ProT $\alpha$  does not appreciably alter the potential of mean force (Fig. 5d), supporting our use of a kinetic model in which binding of a single ProT $\alpha$  molecule explains the experimentally observed increase in dissociation rate (Fig. 3b).

## Discussion

Our results suggest that disordered proteins can facilitate a remarkable mechanism of molecular competition: highly disordered, electrostatically driven biomolecular complexes aid the formation of transient ternary interactions, where a competing binding partner invades an existing complex and accelerates dissociation. This mechanism of ‘competitive substitution’ has been known in the field of synthetic polyelectrolytes<sup>23</sup>, but its role in biology has largely remained unexplored. Given the great abundance of disorder in nuclear proteins, including transcriptional regulators, polymerases and other RNA- and DNA-binding factors<sup>4</sup>, competitive substitution is likely to be widespread among biomolecular interactions in the nucleus and may play an important role in cellular regulation.

Several lines of evidence indicate that ProT $\alpha$  is also an important effector *in vivo*. First, ProT $\alpha$  has been shown to increase H1 mobility in cells<sup>20</sup>; second, the tens-of-micromolar ProT $\alpha$  concentrations reported in cells<sup>44</sup> correspond to the range of concentrations where we observe strong effects on H1–nucleosome affinity; and finally, the facilitated H1 dissociation we observe is a large effect and robust to salt concentration (Supplementary Fig. 3). Correspondingly, variations in ProT $\alpha$  concentration, for example during the cell cycle<sup>60</sup>, may be involved in regulating H1 association with chromatin. We note that from our experimental data, we cannot exclude the possibility that multiple molecules of ProT $\alpha$  participate in the detachment of H1 from the nucleosome. However, the difference between the potentials of mean force from simulations with one and two ProT $\alpha$  molecules is negligible (Fig. 5d), suggesting no additional effect on the dissociation rate. Recent results demonstrated the formation of H1–ProT $\alpha$ <sub>2</sub> trimers in the presence of a large excess of ProT $\alpha$  at high micromolar concentrations<sup>43</sup>, which may contribute to shifting the equilibrium, even if the trimers form only after dissociation of the H1–ProT $\alpha$  complex from the nucleosome.

Another observation is noteworthy: the affinity of H1 to free DNA of the length of our linker DNA or the entire nucleosomal DNA

is about two orders of magnitude lower (Supplementary Fig. 5) than the affinity of H1 to intact nucleosomes (Fig. 1), probably at least in part owing to the spatial arrangement of the DNA in the nucleosome and the more specific interactions of the globular domain at the dyad<sup>29,34,61</sup>. As a result, ProT $\alpha$  is expected to efficiently prevent non-specific binding of H1 to DNA and ensure its targeting to the nucleosomal dyad. However, a multitude of contributions are likely to modulate the interaction of H1 with chromatin, such as other histone chaperones<sup>19</sup>, DNA-interacting machinery<sup>4</sup> or local variations in ion concentrations. Proteins that compete with H1 for binding to the nucleosome, ranging from transcription factors to the high-mobility group chromatin remodelers, will also contribute to enhanced mobility of H1 *in vivo*<sup>62</sup>. In addition, post-translational modifications<sup>13,63</sup> are expected to have an important effect on the interaction of H1 with the nucleosome and ProT $\alpha$  by fine-tuning their affinities.

Even though the existence of charged disordered regions in proteins has been known for decades, we are only now starting to elucidate the physical basis underlying their functions. Established concepts regarding the physical chemistry of polyelectrolyte interactions and the competition with inorganic ions will be essential for a detailed thermodynamic understanding of the affinities of charged IDPs and their strong sensitivity to other organic and biomolecular ions present in the cell<sup>13,32,33,64</sup>. The physical properties of the charged disordered regions of H1 and the core histones are also likely to be crucial for processes involving liquid–liquid phase separation in chromatin<sup>10,11,18,65</sup>. Phase separation is often driven by weak, multivalent interactions between disordered proteins and nucleic acids. The highly charged C-terminal tail of H1 has already been established to promote phase separation in chromatin, with the disordered regions allowing for multivalent interactions between nucleosomes<sup>18</sup>. The highly disordered nature of H1 on the nucleosome that emerges from our results therefore provides a plausible basis for these interactions to occur. Phase separation may be regulated further by chaperones such as ProT $\alpha$  or by post-translational modifications, for example phosphorylation, which has been shown to reduce the formation of H1–DNA condensates<sup>7</sup>.

Finally, it is worth emphasizing that our results suggest that two long-standing questions in the chromatin field, namely the nature of the structural ensemble of H1 on the nucleosome<sup>10</sup> and the discrepancy between the residence times of H1 *in vivo*<sup>39</sup> and *in vitro*<sup>40</sup>, are closely connected: it may be precisely the large degree of structural disorder and long-range dynamics in the tails of nucleosome-bound H1 that enable chaperones such as ProT $\alpha$  to invade the H1–nucleosome complex via electrostatic interactions and in this way accelerate H1 dissociation by competitive substitution instead of passively scavenging H1 once dissociated. Related processes involving charged disordered proteins may affect many aspects of chromatin assembly and dynamics, and cellular regulation in general<sup>11,30,65</sup>.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41557-021-00839-3>.

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## Methods

**Protein preparation and labelling.** Recombinant wild-type human histone H1.0 (H1; New England Biolabs, catalog no. M2501S) was used for experiments with fluorescently labelled nucleosomes. ProTx was prepared as previously described<sup>25</sup>. Variants of H1 for fluorescent labelling were produced by bacterial expression using a modified version of the pRSET vector<sup>66</sup> containing the human H1F0 gene (UniProt P07305), a hexahistidine tag and a thrombin cleavage site<sup>6</sup>. All protein variants were confirmed to have the correct molecular weight by mass spectrometry. Cysteine mutations were introduced for labelling the protein with fluorescent dyes using site-directed mutagenesis. H1 variants were expressed, purified and fluorescently labelled as previously described<sup>6</sup>. Briefly, all H1 variants were expressed in *Escherichia coli* C41 cells in terrific broth medium at 37 °C, using isopropylthiogalactopyranoside (IPTG) to induce expression. The cells were pelleted after three hours of growth, resuspended in denaturing buffer containing 6 M guanidinium chloride (GdmCl) and had the soluble fraction applied to a Ni-IDA resin (Agarose Bead Technologies). After washing, the protein was eluted using 250–500 mM imidazole, then dialysed against phosphate-buffered saline (PBS), followed by thrombin (Serva) cleavage to remove the hexahistidine tag. The uncleaved protein and cleaved hexahistidine tag were removed from the eluted mixture with a HisTrap HP 5 ml column (GE Healthcare) in PBS with 25 mM imidazole. The final purification step was performed by anion exchange chromatography using a Mono S column (GE Healthcare). Before fluorescent labelling, samples were reduced with dithiothreitol (DTT) and purified by reversed-phase high-performance liquid chromatography (RP-HPLC) using a Reprosil-Gold C4 column. H1-containing fractions were resuspended and labelled in denaturing buffer (6 M GdmCl, 50 mM sodium phosphate, pH 7.0), before a final RP-HPLC purification step. Lyophilized proteins were resuspended in 8 M GdmCl, frozen in liquid nitrogen and stored at –80 °C. All experiments were performed in TEK buffer (10 mM Tris, 0.1 mM EDTA, pH 7.4) at different salt concentrations adjusted by addition of KCl. Protein sequences and labelling positions are shown in Supplementary Table 2. The dye pairs Alexa Fluor 488/Alexa Fluor 594 (Förster radius  $R_0$  = 5.4 nm) and Cy3B/CF660R ( $R_0$  = 6.0 nm) were used for experiments with freely diffusing molecules and for surface-immobilized nucleosomes, respectively.

**Preparation of fluorescently labelled oligonucleotides.** A ~10  $\mu$ l solution of 5–10 nmol oligonucleotide (thymine modified with a C6-amino linker for the reaction with the succinimidyl ester of the fluorescent dye; Integrated DNA Technologies; Supplementary Table 2) was diluted with 50  $\mu$ l labelling buffer (0.1 M sodium bicarbonate, pH 8.3). For analytical purposes, a 1  $\mu$ l sample was diluted with 50  $\mu$ l of RP-HPLC solvent A (95% 0.1 M triethylammonium acetate, 5% acetonitrile), followed by RP-HPLC using a Reprosil-Pur 200 5  $\mu$ m, 250  $\times$  4 mm C18 column (Dr. Maisch) using a gradient of 0–100% RP-HPLC solvent B (100% acetonitrile) in 50 minutes. Then 50–100  $\mu$ g fluorescent dye succinimidyl ester (Cy3B/CF660R) dissolved in dimethylsulfoxide was sonicated for 10 minutes and added to the oligonucleotide solution in labelling buffer and the reaction incubated at room temperature for at least two hours. After ethanol precipitation to remove excess dye, the pelleted oligonucleotide was redissolved in 100  $\mu$ l, 95% A, 5% B RP-HPLC solvent. Labelled oligonucleotides were purified using the same column and gradient as above, lyophilized and resuspended in double-distilled water (ddH<sub>2</sub>O) to a final concentration of 2.5  $\mu$ M and stored at –20 °C. The correct molecular weights of the labelled oligonucleotides were confirmed by mass spectrometry.

**Preparation of core-histone octamer.** Human wild-type core histones (H2A, H2B, H3, H4) were prepared as previously described<sup>67</sup>. Each core-histone protein was expressed in BL21 DE3 pLysS cells using a pET3a plasmid carrying the corresponding gene. Cells were grown at 37 °C in LB media including 100  $\mu$ g ml<sup>–1</sup> ampicillin and 35  $\mu$ g ml<sup>–1</sup> chloramphenicol until an optical density of 0.6 at a wavelength of 600 nm (OD<sub>600</sub>) was reached, at which point protein expression was induced by addition of IPTG to a final concentration of 0.5 mM. The cells were harvested 3 hours after induction, the cell pellets resuspended in lysis buffer (20 mM Tris, 1 mM EDTA, 200 mM NaCl, 1 mM 2-mercaptoethanol (2-ME), one cOmplete Protease Inhibitor Cocktail tablet (Roche) per 50 ml, pH 7.5), followed by cell lysis by freeze–thawing and sonication. The inclusion body pellet was washed three times with the histone lysis buffer (twice with and once without 1% Triton X-100). The histones were resolubilized in histone resolubilization buffer (6 M GdmCl, 20 mM Tris, 1 mM EDTA, 1 mM 2-ME, pH 7.5) and then dialysed against urea buffer (7 M urea, 10 mM Tris, 1 mM EDTA, 0.1 M NaCl, 5 mM 2-ME, pH 7.5). The proteins were then purified by cation exchange using a 5 ml HiTrap SP HP column (GE Healthcare) and the collected fractions analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. The final purification step was performed by preparative RP-HPLC on a Zorbax 300SB 7  $\mu$ m 21.2  $\times$  250 mm column using a gradient of 30–70% solvent B (A, water with 0.1% trifluoroacetic acid (TFA); B, 9.9% water, 90% acetonitrile with 0.1% TFA). The collected fractions were characterized by analytical RP-HPLC and electrospray ionization mass spectrometry, lyophilized and stored at –20 °C.

To refold the core-histone octamer, 0.4–1.5 mg of each of the purified lyophilized human histones were dissolved in 6 M GdmCl, 10 mM Tris and 5 mM

DTT, pH 7.5, and the protein concentrations quantified by UV absorbance. Equimolar amounts of H3 and H4 were mixed with 1.05 equivalents of H2A and H2B to a final concentration of 1 mg ml<sup>–1</sup>, and octamers were refolded by dialysing against 2 M NaCl, 10 mM Tris, 1 mM EDTA and 5 mM DTT, pH 7.5. The refolded octamers were then purified by gel filtration on a Superdex S200 10/300GL column (GE Healthcare) and the collected fractions analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. The octamer-containing fractions were pooled and concentrated to ~50  $\mu$ M; glycerol was added to a final concentration of 50% (v/v) and the samples stored at –20 °C.

### PCR amplification of 197-bp DNA containing the 601 Widom sequence.

DNA for nucleosome reconstitution was generated by PCR amplification of a pJ201 plasmid template containing the 147-bp Widom sequence<sup>68</sup>, either with unlabelled oligonucleotides (Integrated DNA Technologies) or fluorescently labelled oligonucleotides (as described in the Preparation of fluorescently labelled oligonucleotides section). DNA for nucleosomes for surface immobilization was generated by using oligonucleotides with a modified thymidine containing a C6-amino linker and a biotin moiety (Integrated DNA Technologies). The oligonucleotides were designed so that the Widom sequence was extended by a linker DNA of 25-bp length on either side. PCR was typically performed in 10  $\times$  50  $\mu$ l volume by mixing in PCR tubes Phusion HF buffer ( $\times$ 1, New England Biolabs), plasmid template (0.02 ng  $\mu$ l<sup>–1</sup>), 0.25  $\mu$ M forward primer, 0.25  $\mu$ M reverse primer and deoxynucleotide triphosphates (dNTPs, 0.2 mM each) with ddH<sub>2</sub>O and 1.0 units of Phusion high-fidelity DNA polymerase (New England Biolabs). Thermocycles included 30 s for initial denaturation at 94 °C, followed by 30 cycles of 20 s for denaturation at 94 °C, 10 s for annealing at 66 °C and 15 s for extension at 72 °C. The completed PCR reactions were pooled and ethanol-precipitated prior to purification using a DNA Clean and Concentrator Kit (DCC-25, Zymo Research). The concentration of the labelled 197-bp PCR products was determined by UV absorbance. DNA sequences of the 197-nucleotide  $\alpha$  and  $\beta$  strands containing the 601 Widom sequence, primer sequences and positions of nucleotides that were modified for labelling or biotinylation are listed in Supplementary Table 2.

**Nucleosome reconstitution.** Nucleosomes were reconstituted<sup>69</sup> using 10 pmol of purified DNA containing the 147-bp 601 Widom sequence<sup>68</sup> flanked by 25-bp linkers. The DNA was mixed with 0.9–1.5 molar equivalents of recombinant core-histone octamer at a final concentration of 2 M NaCl on ice. The 30  $\mu$ l reaction was then transferred to a Slide-A-Lyzer MINI dialysis device (Thermo Fisher Scientific) and dialysed against a linear gradient of buffer with decreasing salt concentration, starting from 10 mM Tris, 0.1 mM EDTA and 2 M KCl, pH 7.5 to 10 mM Tris, 0.1 mM EDTA and 10 mM KCl, pH 7.5, over ~20 hours. The gradient was created by slowly removing buffer from the dialysis container with a constant flow rate using a peristaltic pump and, simultaneously, supplying fresh buffer with 10 mM KCl using the same flow rate and thus keeping the volume constant. The reactions were then transferred to microcentrifuge tubes and centrifuged for 5 min at 21,000g and 4 °C to remove aggregates; the supernatant was transferred to a new tube. After determining the volumes and concentrations of the samples via absorbance at 260 nm, 0.2–0.5 pmol of the reaction products were loaded on a 6% agarose gel (Invitrogen) and run for 90 min at 90 V with  $\times$ 0.25 Tris–borate as running buffer. The gels were stained with GelRed (Biotium) for 30 minutes and visualized under UV light. Only nucleosome preparations containing less than 5% of free DNA in the sample were used for measurements.

**Surface immobilization of nucleosomes.** Quartz coverslips coated with polyethylene glycol and biotin (MicroSurfaces) were sonicated in Tween-20 (Thermo Fisher Scientific) and extensively washed with ddH<sub>2</sub>O before binding to silicone hybridization chambers (SecureSeal hybridization chambers, Grace BioLabs), forming chambers with a sample volume of 150  $\mu$ l. The chambers were washed several times with 50 mM sodium phosphate buffer containing 0.01% Tween-20 and then incubated with a 1  $\mu$ M neutravidin solution (Vector Labs) for 10 minutes. After three washing steps with TEK buffer including 0.01% Tween-20, the chambers were filled with a 10 to 20  $\mu$ M solution of biotinylated and fluorescently labelled nucleosomes in TEK buffer with 0.1 mg ml<sup>–1</sup> bovine serum albumin (BSA) and 0.01% Tween-20 for 5–10 minutes, followed by another two washing steps in TEK buffer. The agreement between data taken on surface-immobilized and freely diffusing molecules with and without BSA, respectively (Fig. 1d), indicates that despite its negative charge, BSA does not interfere with the H1–nucleosome interactions. The transfer efficiency and binding affinity of the H1–nucleosome complex were also tested explicitly using freely diffusing molecules in the presence and absence of BSA, and were found to be unaffected.

**Single-molecule fluorescence spectroscopy.** Single-molecule experiments on freely diffusing molecules were conducted at 22 °C using either a custom-built confocal instrument or a MicroTime 200 (PicoQuant), including a HydraHarp 400 time-correlated single-photon counting module (PicoQuant). The donor dye was excited using a 485 nm diode laser at 100  $\mu$ W power (measured at the back aperture of the objective), either in continuous-wave mode or with pulsed interleaved excitation<sup>70</sup> to enable alternating excitation of donor and acceptor dyes.

For acceptor excitation, the light from a supercontinuum laser (NKT Photonics) operating at 20 MHz repetition rate was passed through a z582/15 band-pass filter (Chroma) and adjusted to an average power of 35  $\mu$ W at the back aperture of the objective. Excitation and emission light was focused and collected, respectively, using a high-numerical-aperture microscope objective (Olympus UPlanApo  $\times 60/1.20$  W). Emitted fluorescence was focused onto a 100  $\mu$ m pinhole and separated into four detection channels by polarization and donor and acceptor emission wavelengths. Single-photon avalanche diodes were used for detection (SPCM-AQR-15, PerkinElmer, or  $\tau$ -SPADs, PicoQuant). All experiments involving freely diffusing molecules were performed in  $\mu$ -Slide sample chambers (Ibidi) at 22 °C in TEK buffer with varying KCl concentrations of 150–400 mM (the salt concentrations quoted throughout the manuscript include the 8 mM ionic strength of 10 mM Tris at pH 7.4); 140 mM 2-ME and 0.01% (v/v) Tween-20 were added for photoprotection and for minimizing surface adhesion, respectively.

Single-molecule experiments with surface-immobilized nucleosomes were conducted on a custom-built confocal instrument with a 532 nm continuous-wave laser (LaserBoxx LBX-532-50-COL-PP, Oxixus). The objective (UPlanApo  $\times 60/1.20$  W, Olympus) was mounted on a piezo stage (P-733.2 and PIFOC, Physik Instrumente) for scanning. Fluorescence emission was collected and split into two channels with a dichroic mirror (T635LPXR, Chroma). Donor emission was filtered with an ET585-65m band-pass filter (Chroma) and detected with a  $\tau$ -SPAD (PicoQuant); acceptor emission was filtered with a LP647RU long-pass filter (Chroma) and detected with a SPCM-AQRH-14 single-photon avalanche diode (PerkinElmer). After surface immobilization of nucleosomes, experiments were performed by filling the reaction chamber with degassed TEK buffer containing H1 and/or ProTx $\alpha$ , under an argon atmosphere to exclude atmospheric oxygen. The buffer contained KCl to adjust the salt concentration, 0.1 mg ml $^{-1}$  of BSA to reduce non-specific surface interactions and D $_2$ O instead of H $_2$ O to increase the quantum yield of the dyes<sup>71</sup>. An oxygen scavenging system containing 2.5 mM protocatechuic acid and 1 mM protocatechuate-3,4-dioxygenase was used to improve photostability<sup>72</sup>, and 1 mM methyl viologen and 1 mM ascorbic acid<sup>73</sup> were used for triplet quenching and radical scavenging. A surface area of 20  $\times$  20  $\mu$ m was scanned (78 nm per pixel) at a laser power of 2  $\mu$ W to locate single immobilized nucleosomes. The axial position and the objective correction collar were adjusted to maximize the fluorescence intensity detected.

Data for FRET efficiency histograms from freely diffusing molecules were collected on samples containing 50–100 pM double-labelled H1 or nucleosomes; intermolecular FRET efficiencies were measured on samples with up to 500 pM of acceptor-labelled nucleosomes to ensure saturation of binding, and up to 5 nM in time-resolved FRET experiments at elevated salt concentrations (Supplementary Fig. 3). FRET efficiencies were calculated according to  $E = \hat{N}_A/(\hat{N}_A + \hat{N}_D)$ , where  $\hat{N}_D$  and  $\hat{N}_A$  are the number of donor and acceptor photons in a fluorescence burst, respectively, after correction for background, direct acceptor excitation, channel crosstalk, and differences in dye quantum yields and photon detection efficiencies<sup>74</sup>. Aggregates, identified as occasional fluorescence bursts with photon counts greater than three standard deviations from the mean signal binned at 1 s, were removed before data analysis. For experiments on surface-immobilized nucleosomes, we determined photon count ratios and summed for all traces recorded at the same salt concentration and H1 concentration, which report accurately on the ratio of bound and unbound populations (even though the photon count ratio may differ from the corrected absolute transfer efficiencies).

**nsFCS.** Data for nsFCS<sup>52,75</sup> were collected using continuous-wave excitation at 485 nm and an  $\sim$ 100 pM sample of either donor-labelled H1 with acceptor-labelled nucleosomes, or double-labelled H1 with an excess of unlabelled dsDNA or nucleosomes. Donor and acceptor fluorescence photons from the bound subpopulation were used for the correlations at 1 ns binning time. Photons were recorded with two detectors each for donor and acceptor and cross-correlated between detectors to avoid the effects of detector dead times and after-pulsing on the correlation functions. Autocorrelation curves of acceptor and donor channels and cross-correlation curves between acceptor and donor channels were fit and analysed as described previously<sup>6</sup>. Briefly, the correlation curves were fit over a lag time interval from  $-2.5 \mu$ s to  $+2.5 \mu$ s using

$$g_{ij}(\tau) = a \left( 1 - c_{ab} e^{-\frac{|\tau|}{\tau_{ab}}} \right) \left( 1 + c_{cd} e^{-\frac{|\tau|}{\tau_{cd}}} \right) \left( 1 + c_{\tau} e^{-\frac{|\tau|}{\tau_{\tau}}} \right),$$

where  $i$  and  $j$  indicate donor (D) or acceptor (A) fluorescence emission; the amplitude  $a$  depends on the effective mean number of molecules in the confocal volume and on the background signal;  $c_{ab}$ ,  $\tau_{ab}$ ,  $c_{cd}$  and  $\tau_{cd}$  are the amplitudes and time constants of photon antibunching (ab) and chain dynamics (cd), respectively;  $c_{\tau}$  and  $\tau_{\tau}$  refer to the triplet blinking component occurring on the microsecond timescale. Fluctuations in the inter-dye distance result in specific features in the correlation functions: donor and acceptor autocorrelations have a positive amplitude ( $c_{cd} > 0$ ), whereas the donor–acceptor cross-correlation has a negative amplitude ( $c_{cd} < 0$ ), but all correlations have identical decay times<sup>6</sup>. All three correlation curves were thus fit globally with the same value of  $\tau_{cd}$  applied to all curves, but allowing for different values of  $c_{ab}$ ,  $c_{cd}$ ,  $\tau_{ab}$ ,  $\tau_{\tau}$  and  $c_{\tau}$  as free fit parameters.  $\tau_{cd}$  can be converted to the reconfiguration time of the chain,  $\tau_r$ , by modelling chain

dynamics as a diffusive process in the potential of mean force derived from the sampled inter-dye distance distribution  $P(r)$ <sup>75,76</sup> based on the SAW- $\nu$  model<sup>77,78</sup>. The correlation functions are displayed throughout the paper with a normalization to 1 at their respective values at 0.5  $\mu$ s to facilitate direct comparison.

**Fluorescence lifetime and fluorescence anisotropy.** Fluorescence lifetimes were estimated from the mean donor ( $t_D$ ) and acceptor ( $t_A$ ) detection times after the excitation pulse. The average lifetimes were then combined with the transfer efficiencies in two-dimensional plots (Supplementary Fig. 6) where  $\tau_D^0/\tau_D^0 = t_D/t_D^0$  and  $\tau_A^0/\tau_A^0 = (t_A - \tau_A^0)/\tau_D^0$  were calculated for each burst. Here,  $\tau_D^0$  and  $\tau_A^0$  are the intrinsic donor and acceptor lifetimes, respectively. For a static inter-dye distance, the normalized mean fluorescence lifetimes are equal to  $1 - E$  (diagonal lines in Supplementary Fig. 6), whereas systems rapidly sampling a broad distance distribution deviate from the diagonal<sup>79,80</sup>.

Fluorescence anisotropies for donor and acceptor dyes were determined for all intra- and intermolecular labelling pairs from the single-molecule measurements and yielded values of 0.09–0.14 for the donor dye and 0.16–0.22 for the acceptor dye (upon direct excitation), which indicates sufficiently rapid reorientational dynamics of the dyes for approximating the orientational factor in Förster theory by 2/3 (ref. <sup>81</sup>).

**Binding affinities.** Transfer efficiency histograms were recorded for either double-labelled H1 or nucleosomes with increasing concentrations of the unlabelled binding partner until no further change in transfer efficiency was observed. The histograms were fit with two or more Gaussian peak functions to quantify the relative areas of the bound and unbound subpopulations and the resulting fraction of bound species ( $\theta$ ). The binding isotherm ( $\theta$  versus ligand concentration) was then fit to quantify the dissociation constant ( $K_D$ ) using

$$\theta = \frac{c_{X,tot} + K_D + c_{Y,tot} + \sqrt{(c_{X,tot} + K_D + c_{Y,tot})^2 - 4c_{X,tot}c_{Y,tot}}}{2c_{Y,tot}},$$

where  $c_{X,tot}$  and  $c_{Y,tot}$  are the total concentrations of H1 or nucleosome, depending on which molecule is kept at constant concentration ( $c_{X,tot}$  variable,  $c_{Y,tot}$  constant). Errors on  $K_D$  values are based on pipetting error estimates, propagated through corresponding dilution steps at each salt concentration<sup>6</sup>. The number of counterions released upon complex formation can be estimated from the salt concentration dependence of the  $K_D$  according to the model of Record et al.<sup>32</sup>.

#### Analysis of fluorescence time traces from surface-immobilized molecules.

Single-molecule fluorescence time traces were first inspected visually, and any recordings showing pronounced changes in count rate, that is, those indicative of nucleosome unwrapping (Supplementary Fig. 1), were excluded from further analysis. These traces could furthermore be identified by a markedly increased off-rate of H1 that results in a much lower affinity than expected from the experiments using freely diffusing molecules. The trajectories of immobilized nucleosomes binding to H1 free in solution (Fig. 2a) exhibited transitions between two states: the H1-bound nucleosome with high FRET efficiency and the unbound nucleosome with low FRET efficiency, in accordance with the free-diffusion experiments (Fig. 1b,c). We therefore analysed the system in terms of a two-state model described by a rate matrix,  $\mathbf{K}$ , with transitions between unbound (N) and bound (HN) nucleosome:

$$\mathbf{K} = \begin{pmatrix} -k_{on}^{HN} c_H & k_{off}^{HN} \\ k_{on}^{HN} c_H & -k_{off}^{HN} \end{pmatrix},$$

where  $c_H$  is the concentration of H1 in solution, and  $k_{on}^{HN}$  and  $k_{off}^{HN}$  are the association and dissociation rate coefficients, respectively. The photon detection rates for donor and acceptor are estimated by applying maximum likelihood analysis to the binned time traces<sup>82</sup>, where the likelihood ( $L_m$ ) for time trace  $m$  with bin size  $\Delta$ , number of bins ( $T_m$ ) and ( $N_{D,i}$ ,  $N_{A,i}$ ) photons detected in time bin  $i$  is calculated according to

$$L_m = 1^T \left[ \prod_{i=1}^{T_m} \mathbf{F}_i e^{\mathbf{K}\Delta} \right] \mathbf{p}_{eq},$$

The population vector  $\mathbf{p}_{eq}$  describes the equilibrium distribution of states, for which  $\mathbf{K}\mathbf{p}_{eq} = 0$  and  $1^T \mathbf{p}_{eq} = 1$ , where  $1^T = (1 \ 1 \ \dots)$  is the transposed vector of ones.  $\mathbf{F}_i$  is a diagonal matrix with elements

$$(\mathbf{F}_i)_{ii} = \frac{(n_{D,i}\Delta)^{N_{D,i}}}{N_{D,i}!} e^{-n_{D,i}\Delta} \times \frac{(n_{A,i}\Delta)^{N_{A,i}}}{N_{A,i}!} e^{-n_{A,i}\Delta},$$

assuming Poisson statistics for the number of photons per bin;  $n_{D,i}$  and  $n_{A,i}$  are the mean photon detection rates of the  $i$ th state in the donor and acceptor channels, respectively. The photon detection rates and transition rate coefficients are then found by maximizing the sum of the logarithm of the likelihoods,  $\sum_m \ln(L_m)$ . Using the Viterbi algorithm<sup>83,84</sup>, we also identified the most likely state trajectories using the photon detection rates and transition rate coefficients determined from the maximum likelihood analysis, and obtained the distributions of dwell times,

which yielded rate coefficients in good agreement with those obtained with the maximum likelihood analysis when fit with single-exponential decays.

For describing the kinetics in the presence of ProTα, we assume that the nucleosome can additionally be in complex with both H1 and ProTα, forming the ternary complex PHN. Accordingly, we describe the measurements (Fig. 3b) with a three-state model (including N, HN and PHN) based on the rate matrix

$$\mathbf{K}(c_P) = \begin{pmatrix} -k_{\text{on}}^{\text{HN}} c_{\text{H}} - k_{\text{on}}^{\text{PH-N}} c_{\text{PH}} & k_{\text{off}}^{\text{HN}} & k_{\text{off}}^{\text{PH-N}} \\ k_{\text{on}}^{\text{HN}} c_{\text{H}} & -k_{\text{off}}^{\text{HN}} - k_{\text{on}}^{\text{P-HN}} c_{\text{P}} & k_{\text{off}}^{\text{P-HN}} \\ k_{\text{on}}^{\text{PH-N}} c_{\text{PH}} & k_{\text{on}}^{\text{P-HN}} c_{\text{P}} & -k_{\text{off}}^{\text{P-HN}} - k_{\text{off}}^{\text{PH-N}} \end{pmatrix},$$

where  $c_{\text{PH}}$  and  $c_{\text{P}}$  are the concentrations of PH and P in solution, respectively. Even though a unique determination of all four additional rate coefficients besides the independently known  $k_{\text{off}}^{\text{HN}}$  and  $k_{\text{off}}^{\text{PH-N}}$  is not feasible based on the available data, the dependence of the observed transition rate coefficients between low- and high-FRET states on ProTα concentration can be described well with this model (solid lines in Fig. 3b) according to

$$k_{\text{off}}^{\text{obs}} = \frac{K_{\text{D},1} K_{\text{D},3} k_{\text{on}}^{\text{HN}} c_{\text{H}} (k_{\text{on}}^{\text{PH-N}} + k_{\text{on}}^{\text{P-HN}} c_{\text{P}})}{(c_{\text{H}} c_{\text{P}} + (c_{\text{P}} + K_{\text{D},1}) K_{\text{D},3}) k_{\text{off}}^{\text{HN}}}$$

and

$$k_{\text{on}}^{\text{obs}} = k_{\text{off}}^{\text{HN}} + c_{\text{P}} k_{\text{on}}^{\text{P-HN}},$$

showing that the formation of the ternary complex PHN can explain the observed kinetic behaviour, in contrast to a model without PHN (dashed

lines in Fig. 3b). In the ternary complex model,  $K_{\text{D},1} = \frac{k_{\text{off}}^{\text{PH}}}{k_{\text{on}}^{\text{PH}}}$  ( $\text{PH} \rightleftharpoons \text{P} + \text{H}$ ),

$K_{\text{D},2} = \frac{k_{\text{off}}^{\text{HN}}}{k_{\text{on}}^{\text{HN}}}$  ( $\text{HN} \rightleftharpoons \text{H} + \text{N}$ ),  $K_{\text{D},3} = \frac{k_{\text{off}}^{\text{PH-N}}}{k_{\text{on}}^{\text{PH-N}}}$  ( $\text{PHN} \rightleftharpoons \text{PH} + \text{N}$ ) and

$K_{\text{D},4} = \frac{k_{\text{off}}^{\text{P-HN}}}{k_{\text{on}}^{\text{P-HN}}}$  ( $\text{PHN} \rightleftharpoons \text{P} + \text{HN}$ ). For deriving the equations of the observed transition rate coefficients, we assumed detailed balance, which implies

$K_{\text{D},1} K_{\text{D},3} = K_{\text{D},2} K_{\text{D},4}$ . We calculated  $k_{\text{off}}^{\text{obs}}$  as the reciprocal mean dwell time in the low-FRET states N and PHN, and  $k_{\text{on}}^{\text{obs}}$  as the reciprocal mean dwell time in the

high-FRET state, that is HN.  $K_{\text{D},3}$  and  $k_{\text{on}}^{\text{P-HN}}$  are the only fit parameters, since  $K_{\text{D},1}$  is known from Borgia et al.<sup>6</sup>. Interestingly, the simulations suggest that N and PHN exhibit similar inter-dye distances, and the experimental data (Fig. 3b) can indeed only be described if we assume that HN exhibits high FRET efficiency and that both N and PHN exhibit low FRET efficiency, suggesting that ProTα binding leads to the opening of the linker DNA in the H1–nucleosome complex. Intermolecular FRET experiments (Supplementary Fig. 3) demonstrate, however, that H1 dissociation from the nucleosome in the presence of ProTα occurs on a similar timescale as the transitions from high to low  $\langle E \rangle$  (Fig. 3), indicating that nucleosome opening is coupled to rapid dissociation of H1 with ProTα.

**Simulations. Protein model.** Each residue of ProTα and the core and linker histones was represented as a bead (C-beads) mapped on the Cα atom of the X-ray crystal structure (PDB no. 5NL0). The potential energy had the following functional form<sup>6</sup>:

$$V_{\text{Protein}} = \frac{1}{2} \sum_i k_b (d_i - d_i^0)^2 + \frac{1}{2} \sum_{i=1}^{N-1} k_\theta (\theta_i - \theta_i^0)^2 + \sum_{i=1}^{N-2} \sum_{m=1}^4 k_{i,m} (1 + \cos(n\phi_i - \delta_{i,m})) + \sum_{i,j} \frac{q_i q_j}{4\pi\epsilon_0 d_{ij}} e^{-\frac{d_{ij}}{\lambda_D}} + \sum_{(ij) \in \text{Native}} \epsilon_{ij} \left[ 13 \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - 18 \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{10} + 4 \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right] + \sum_{(ij) \notin \text{Native}} 4\epsilon_{\text{pp}} \left[ \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right]$$

The first and second terms describe bond lengths ( $d_i$ ) and angles ( $\theta_i$ ), respectively, using harmonic potentials with force constants  $k_b$ ,  $k_\theta$  and equilibrium values for bond lengths ( $d_i^0$ ) and angles ( $\theta_i^0$ ) for all  $N$  bonds assigned according to the distances and angles between Cα atoms in the all-atom structure, as previously described<sup>85</sup>; values for unstructured regions were taken from an extended conformation. The third term defines the torsions (dihedrals) between four beads linked by three bonds. The dihedral parameters (force constants  $k_{i,m}$  and phase shifts  $\delta_{i,m}$ ) were obtained from a knowledge-based potential based on the PDB<sup>85</sup>. The fourth term describes electrostatic interactions between charged residues using a screened Coulomb potential. Aspartate and glutamate residues were assigned a charge of −1, lysine and arginine a charge of +1 and histidine a charge of +0.5 to account for the  $pK_a$  of histidine of ~6.5. A charge of 0 was assigned to all the other beads. In the Coulomb term,  $q_i$  is the charge of residue  $i$ ;  $\epsilon_0$  is the permittivity of free space; and  $\epsilon_d$  is the relative dielectric constant of water, which was set to a value of 80. The Debye screening length,  $\lambda_D$ , is given by

$$\lambda_D = \left( \frac{\epsilon_0 \epsilon_d k_B T}{2 N_A e^2 I} \right),$$

where  $N_A$  is Avogadro's constant,  $e$  is the elementary charge,  $I$  denotes the ionic strength,  $k_B$  is the Boltzmann constant and  $T$  is temperature.

The fifth and sixth terms define short-range attractive interactions between beads  $i$  and  $j$  separated by distance  $d_{ij}$ . These interactions are applied differently to disordered and folded regions of the proteins. For the folded regions, native interactions are enforced using a 12–10–6 pair potential<sup>85</sup>. The values of  $\epsilon_{ij}$  were calculated based on the globular domain of H1 and core histones according to the native-centric model of Karanicolas and Brooks<sup>85</sup>. The coefficient  $\sigma_{ij}$  was set according to the Cα distances in the crystal structure. For disordered regions and interactions between non-native residue pairs and the folded regions (that is, those not in contact in the native state), the  $\epsilon_{\text{pp}}$  term defining the strength of the interaction between beads was tuned to the value of 0.16  $k_B T$  (~0.40 kJ mol<sup>−1</sup>), the value that was shown to produce the best agreement with the experimentally determined FRET efficiencies (Fig. 4) for the 1:1 ProTα/H1 complex<sup>6</sup>, while the value of  $\sigma_{ij} = (\sigma_i + \sigma_j)/2$  was determined based on  $\sigma_i$  specific to each residue type<sup>86</sup>.

**DNA model.** The coarse-grained model of the DNA comprised three beads representing the phosphate (P), ribose (R) and base (B) moieties of a nucleotide and were mapped to the P, C4' and N1 atoms from the all-atom DNA structure, respectively. Phosphate beads were assigned a charge of −1. The functional form of the potential for the DNA was

$$V_{\text{DNA}} = \frac{1}{2} \sum_{i < j} k_b (d_i - d_j^0)^2 + \frac{1}{2} \sum_{i < j-1} k_\theta (\theta_i - \theta_j^0)^2 + \sum_{i < j} \frac{q_i q_j}{4\pi\epsilon_0 d_{ij}} e^{-\frac{d_{ij}}{\lambda_D}} + \sum_{(ij) \in \text{Stack}} 4\epsilon_{\text{Stack}} \left[ \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right] + \sum_{(ij) \in \text{Pair}} 4\epsilon_{\text{Pair}} \left[ \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right] + \sum_{(ij) \notin \text{Pair, Stack}} 4\epsilon_{\text{ns}} \left[ \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right]$$

The first two terms describe bonds and angles between beads, with parameters chosen to reproduce the equilibrium structure of B-DNA. A screened Coulomb potential was used for electrostatic interactions as described above. Non-bonded Lennard-Jones potentials were used to reproduce stacking and pairing between bases. The corresponding values of  $\epsilon_{\text{Stack}}$  and  $\epsilon_{\text{Pair}}$  were set to 3.0  $k_B T$  (~7.5 kJ mol<sup>−1</sup>) and 3.5  $k_B T$  (~8.8 kJ mol<sup>−1</sup>), respectively, consistent with previously estimated free energy values for base stacking<sup>87</sup>. Interactions between DNA beads not involved in stacking or native base pairing were described by a very weakly attractive potential of  $\epsilon_{\text{ns}} = 0.04 k_B T$  (~0.10 kJ mol<sup>−1</sup>). This is the same model used previously to describe a DNA hairpin<sup>86</sup>; only  $\epsilon_{\text{Pair}}$  was optimized to obtain the persistence length (~50 nm) characteristic of double-helical DNA<sup>87</sup>.

**Protein–DNA interaction potential.** The potential energy for the interactions between DNA and protein beads is composed of a screened Coulomb potential, which describes the interaction between charged beads, and short-range potentials:

$$V_{\text{Protein-DNA}} = \sum_{i < j} \frac{q_i q_j}{4\pi\epsilon_0 \epsilon_d d_{ij}} e^{-\frac{d_{ij}}{\lambda_D}} + \sum_{((ij) \in \text{Native})} \epsilon_{ij} \left[ 13 \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - 18 \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{10} + 4 \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right] + \sum_{(ij) \notin \text{Native}} 4\epsilon_{\text{pd}} \left[ \left( \frac{\sigma_{ij}}{d_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{d_{ij}} \right)^6 \right]$$

In this case, pairs of beads interacting via native interactions were identified based on the crystal structure of the nucleosome (PDB no. 5NL0). Native interactions were defined between protein beads and ribose or nucleobase beads if any heavy atom of an amino acid falls within 0.5 nm from any heavy atom of that ribose or nucleobase. The  $\epsilon_{ij}$  for these interactions was set to 2  $k_B T$  (~5 kJ mol<sup>−1</sup>) to ensure these interactions remained formed. For the interactions between the H1 globular domain and the DNA dyad<sup>88,89</sup>, we reduced the native  $\epsilon_{ij}$  between protein and DNA to allow for globular domain fluctuations and identified the interaction strength that yielded the best agreement between experimental and computed FRET efficiencies, yielding a value of 1  $k_B T$  (~2.5 kJ mol<sup>−1</sup>). For non-native interactions between the disordered regions of the proteins (histone tails and ProTα) and the DNA,  $\epsilon_{\text{pd}}$  was set to 0.06  $k_B T$  (~0.15 kJ mol<sup>−1</sup>) and  $\sigma_{ij}$  to 0.6 nm. The values of  $\epsilon_{ij}$  and  $\epsilon_{\text{pd}}$  were chosen to optimize the agreement between simulated and experimentally obtained FRET efficiencies. Note that the optimal strength of non-native interactions,  $\epsilon_{\text{pd}}$ , is the same as that obtained for a non-specific complex between a protein chaperone and DNA hairpin<sup>86</sup>; only the native contact



parameters representing specific interactions needed to be tuned for this system. For simulations with the H1 globular domain rotated by 180° on the nucleosomal dyad, a coordinate transformation was performed and steric clashes relaxed by energy minimization. Native interactions between the rotated globular domain and the DNA were retrieved from the rotationally symmetric list of contacts between the globular domain and the DNA in its native conformation. A summary of all parameter values defining bonded and non-bonded interactions in the models is provided in Supplementary Table 3. In summary, the similarity of the parameters to those used in previous work indicates their transferability. The parameters that were tuned in this work were (1) the DNA base-pairing energy  $\epsilon_{\text{pair}}$  and (2) the strength of the specific, native protein–DNA interactions  $\epsilon_{ij}$ .

**Replica-exchange Langevin dynamics simulations of the nucleosome–H1 complex.** Langevin dynamics simulations were performed using a modified version of GROMACS version 5.4.1 (refs. <sup>90,91</sup>). The nucleosome was placed at the centre of a box of 30 nm × 40 nm × 30 nm and simulated with and without H1 bound using periodic boundary conditions and charge screening equivalent to 165 mM salt. After energy minimization, 33 replicas were simulated in a temperature range between 298.15 and 648.23 K. Exchange attempts between conformers of neighbouring replicas in temperature space were made every 5 ps and accepted according to the Metropolis criterion. Each replica was run for 1 μs, using a time step of 10 fs and a friction coefficient set at 0.2 ps<sup>−1</sup>. Particle velocities for each replica were randomly seeded according to a Boltzmann distribution. Interaction energies were calculated in direct space using a cut-off of 2.5 nm, with neighbour searching performed every 0.1 ps.

To test the influence of the FRET dyes on the simulation results, we performed a set of simulations in which the dyes were represented explicitly. Each dye and linker was described by five beads, reflecting the approximate equivalence to a five-amino-acid chain<sup>92</sup>, linked by bonds with a length of 0.38 nm and bond angles of 110° (the dihedral term was omitted). The interaction between the dyes and the protein or DNA was characterized by a weakly attractive short-range interaction energy, with  $\epsilon = 0.001 \text{ kJ mol}^{-1}$  and  $\sigma = 0.6 \text{ nm}$ . The fourth and the fifth beads carried a charge of −1 each to reflect the total charge of −2 of the Alexa fluorophores used in the measurements. Mean FRET efficiencies were calculated from simulations according to the inter-bead distance distributions,  $P(r)$ , of the fluorescently labelled residues:

$$\langle E \rangle = \int E(r) P(r) dr$$

and the Förster equation:

$$E(r) = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6},$$

using the Förster radius,  $R_0$ , of 5.4 nm of the Alexa Fluor 488/594 dye pair. The agreement between  $E$  from experiments and simulations was quantified by the concordance correlation coefficient<sup>93</sup>,  $\rho_c$ , between the two datasets:

$$\rho_c = \frac{2\rho\sigma_{\text{exp}}\sigma_{\text{sim}}}{\sigma_{\text{exp}}^2 + \sigma_{\text{sim}}^2 + (\mu_{\text{exp}} - \mu_{\text{sim}})^2},$$

where  $\sigma_{\text{exp}}$  and  $\sigma_{\text{sim}}$  indicate the standard deviations,  $\mu_{\text{exp}}$  and  $\mu_{\text{sim}}$  the mean values and  $\rho$  the linear (Pearson) correlation coefficient between the values from experiments and simulations, respectively. The concordance correlation coefficient is a stricter measure than the linear correlation coefficient:  $\rho_c$  is in general smaller than  $\rho$  and is equal to it only if  $\sigma_{\text{exp}} = \sigma_{\text{sim}}$  and  $\mu_{\text{exp}} = \mu_{\text{sim}}$ .

**Langevin dynamics simulations of the nucleosome–H1 complex in the presence of ProTα.** Langevin dynamics simulations of the nucleosome were performed at a concentration of ProTα of ~10 μM, by placing one molecule of ProTα and the nucleosome bound to H1 in a cubic box of 55 nm × 55 nm × 55 nm. Six independent simulations of the association of ProTα with the H1–nucleosome complex were run at 298.15 K for ~1.4 μs each with the same H1–nucleosome parameters optimized to match the equilibrium FRET data using a modified version of Gromacs 2019 (refs. <sup>90,91</sup>), and the same simulation parameters as above. The number of contacts between H1 and either ProTα or the nucleosomal DNA was determined as the number of pairs of beads, one from each molecule, that were within 1.0 nm of each other.

To sample the dissociation of H1 from the nucleosome (with or without ProTα), umbrella sampling was used (implemented using the Plumed plug-in, version 2.5.3 (ref. <sup>94</sup>)), with the bias applied to the distance between the centre of mass of the globular domain (residues 23–97) of H1 and the centre of mass of the linker DNA residues contacting the globular domain in the native complex. For distances between 0 and 8 nm, 24 umbrella windows with equally spaced centres and a force constant of 100 kJ mol<sup>−1</sup> nm<sup>−2</sup> were used, and from 8 to 32 nm, a further 24 equally spaced windows with a force constant of 10 kJ mol<sup>−1</sup> nm<sup>−2</sup> were used, for a total of 48 windows. In addition to the bias applied between H1 and

the nucleosome, a weak harmonic bias with force constant 1 kJ mol<sup>−1</sup> nm<sup>−2</sup> was applied between H1 and the centre of mass of any ProTα present to ensure that ProTα was consistently bound in all umbrella windows. Replica exchange moves between adjacent umbrellas were attempted every 500 steps<sup>95</sup>. Potentials of mean force (PMFs) were reconstructed from the equilibrated portion of the trajectories using the weighted histogram analysis method<sup>96</sup>, and the Jacobian contribution  $-2k_B T \ln r$  was subtracted. This procedure was performed without ProTα present; with either one or two full-length ProTα molecules; and with the N-terminal (ProTα-N, residues 1–56) and C-terminal (ProTα-C, residues 57–112) halves of ProTα; and PMFs were determined in each case. The dissociation constants  $K_D$  were determined by integration of the PMFs via<sup>64,97</sup>

$$\frac{1}{K_D} = 4\pi N_A \int_0^b \exp[-F(r)/k_B T] r^2 dr,$$

where  $N_A$  is Avogadro's number,  $F(r)$  is the PMF shifted so that  $\lim_{r \rightarrow \infty} F(r) = 0$  and

$b$  is the radius defining the maximum extent of the bound state. This approach yielded dissociation constants for H1 from the nucleosome without ProTα, with one ProTα, with two ProTα, with ProTα-N and with ProTα-C, respectively, of  $4.1 \times 10^{-17} \text{ M}$ ,  $9.4 \times 10^{-13} \text{ M}$ ,  $1.3 \times 10^{-12} \text{ M}$ ,  $3.3 \times 10^{-17} \text{ M}$  and  $5.5 \times 10^{-14} \text{ M}$ .

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

Data supporting the findings of this study are available within the paper and its Supplementary Information. Source data are provided with this paper.

## Code availability

A custom WSTP add-on for Mathematica (Wolfram Research) used for the analysis of single-molecule fluorescence data is available upon request and at <https://schuler.bioc.uzh.ch/programs>. A modified version of GROMACS was used for coarse-grained simulations, which is available at <https://github.com/bestlab/gromacs-2019.4.git>.

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## Author contributions

P.O.H., D.M., R.B.B. and B.S. designed the research; P.O.H. and S.K. prepared the reconstituted nucleosomes; P.O.H., M.B.B., A.B., A.S., S.K. and B.F. prepared the fluorescently labelled and/or unlabelled proteins; P.O.H. and A.S. performed the single-molecule experiments; P.O.H., A.S., D.N. and B.S. analysed the single-molecule data; D.M. and R.B.B. performed and analysed the simulations; R.B.B., B.F. and B.S. supervised the research; and P.O.H. and B.S. wrote the paper with help from all authors.

## Competing interests

The authors declare no competing interests.

## Additional information

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**Correspondence and requests for materials** should be addressed to Pétur O. Heidarsson, Robert B. Best or Benjamin Schuler.

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- |                 |  |
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| Data collection | SymPhoTime 64 (PicoQuant) was used for single-molecule data collection. A modified version of GROMACS was used for coarse-grained simulations, which is available at <a href="https://github.com/bestlab/gromacs-2019.4.git">https://github.com/bestlab/gromacs-2019.4.git</a> . |
| Data analysis   | A custom WSTP add-on for Mathematica (Wolfram Research, v.11, v.12) was used for the analysis of single-molecule fluorescence data and is available upon request and at <a href="https://schuler.bioc.uzh.ch/programs">https://schuler.bioc.uzh.ch/programs</a> .                |

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Data exclusions	Freely diffusing bursts arising from molecules with only a single fluorophore and with < 50 photons were excluded from analysis. Surface immobilized time traces were inspected to ensure that no substantial brightness variations were occurring (e.g., drift of the molecule's position, long-lived dark states, nucleosomal unwrapping events, or background fluctuations). Single-step photobleaching indicated that only one molecule was present in the confocal volume. For the simulations, the first half of each trajectory was discarded as equilibration.
Replication	All main results were successfully reproduced using different samples (protein, DNA, or nucleosome preparations), and measured on different days in fresh buffers.
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**Supplementary information**

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**Release of linker histone from the nucleosome driven by polyelectrolyte competition with a disordered protein**

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# Supplementary Information

## Release of linker histone from the nucleosome driven by polyelectrolyte competition with a disordered protein

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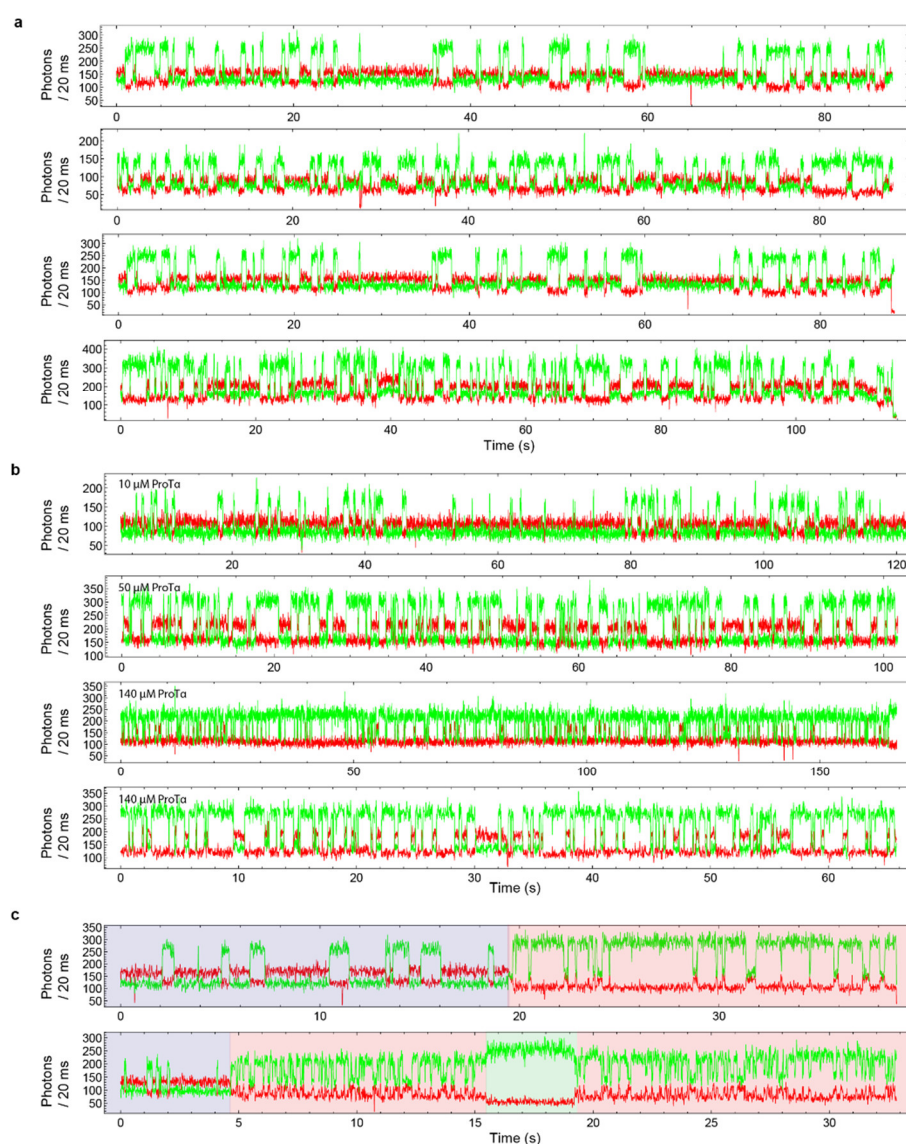
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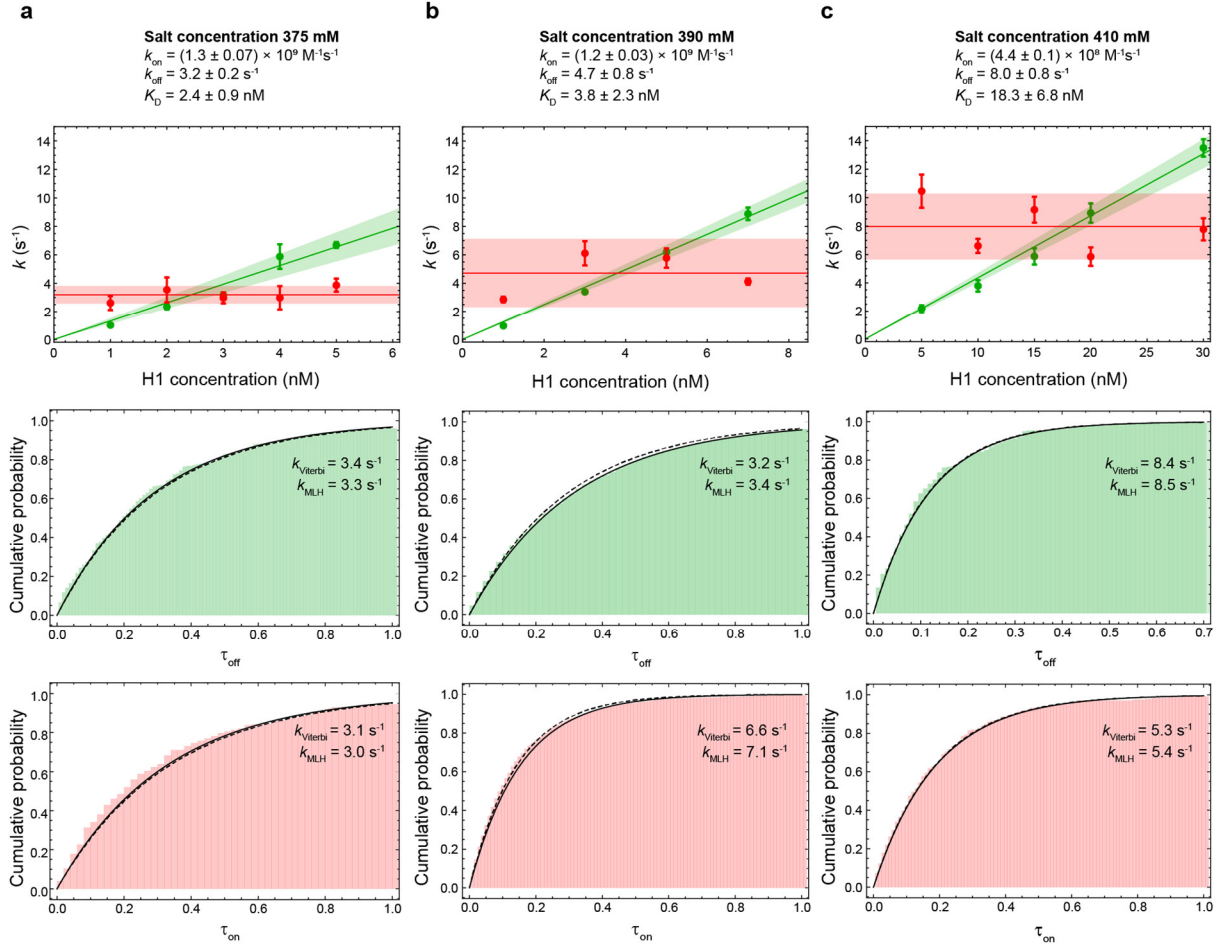
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## Supplementary Figures

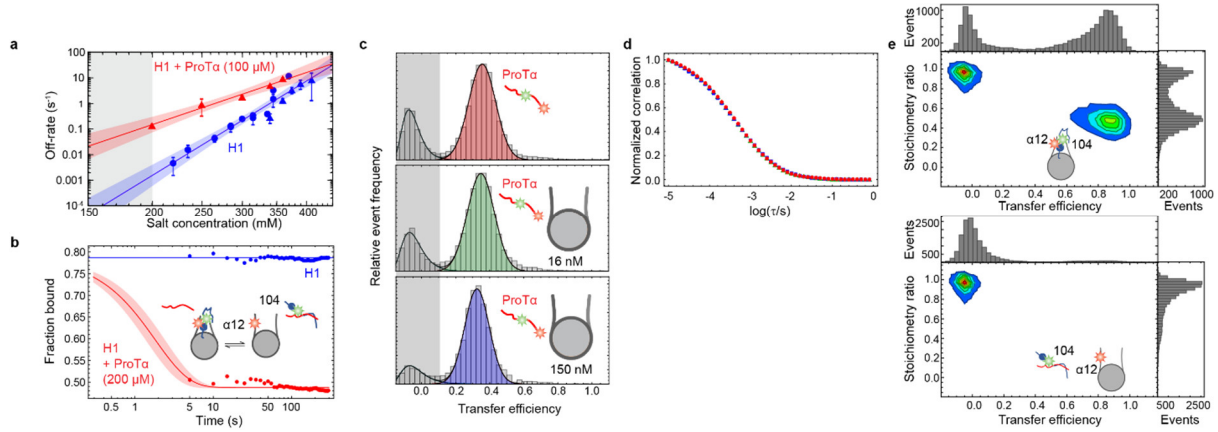


**Fig. S1. Fluorescence time traces of surface-immobilized nucleosomes.**

**a**, Representative time traces of unlabeled H1 binding to double-labeled nucleosomes, at 360 mM salt concentration and 2 nM H1 in the absence of ProTa. **b**, Representative time traces of H1 binding to nucleosomes in the presence of 10, 50, or 140  $\mu\text{M}$  ProTa, at 340 mM salt concentration and 3 nM H1. **c**, Representative time traces showing unwrapping of nucleosomes. Unwrapping occurs after ~20 s in the upper trace (red-shaded range). Such partially wrapped states, which have previously been observed, may consist of one or both linker DNA arms partially detached from the nucleosome core<sup>1, 2</sup>; corresponding segments or the entire traces were excluded from the analysis (see Materials and Methods). Binding events to these partially wrapped states are still observed, consistent with reports that H1 retains high affinity to nucleosomes that contain only one linker DNA arm<sup>3</sup>. The lower trace shows a further unwrapping event (15.5-19 s, green-shaded range) that displays no anti-correlated changes in donor and acceptor signal before returning to a binding-competent partially unwrapped state.

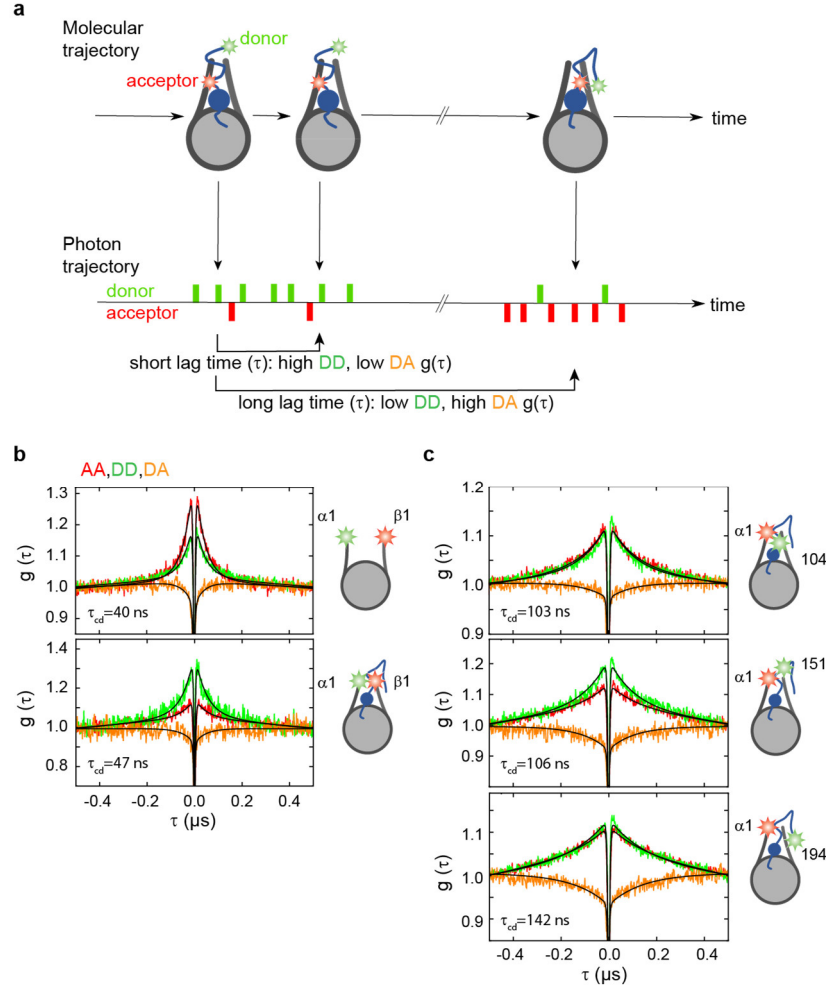


**Fig. S2. H1-nucleosome binding kinetics at different salt concentrations.** Association (green) and dissociation rates (red) of the H1-nucleosome complex as a function of H1 concentration, at (a) 375 mM, (b) 390 mM, and (c) 410 mM salt concentrations, and corresponding examples of cumulative dwell-time probability distributions, at 2 nM, 3 nM, and 20 nM H1, respectively. The rate coefficients and equilibrium dissociation constants resulting from the fits (lines) are shown in each panel. Error bars show one standard deviation estimated from ten bootstrapping trials; shaded areas indicate 95% confidence intervals. The dwell times determined with the Viterbi algorithm are displayed as cumulative probability distributions and fits with a single-exponential function to yield the transition rates (solid lines). A single-exponential function based on the transition rates determined by MLH analysis is plotted for comparison (dashed lines) to illustrate the agreement with the rates determined by Viterbi analysis.

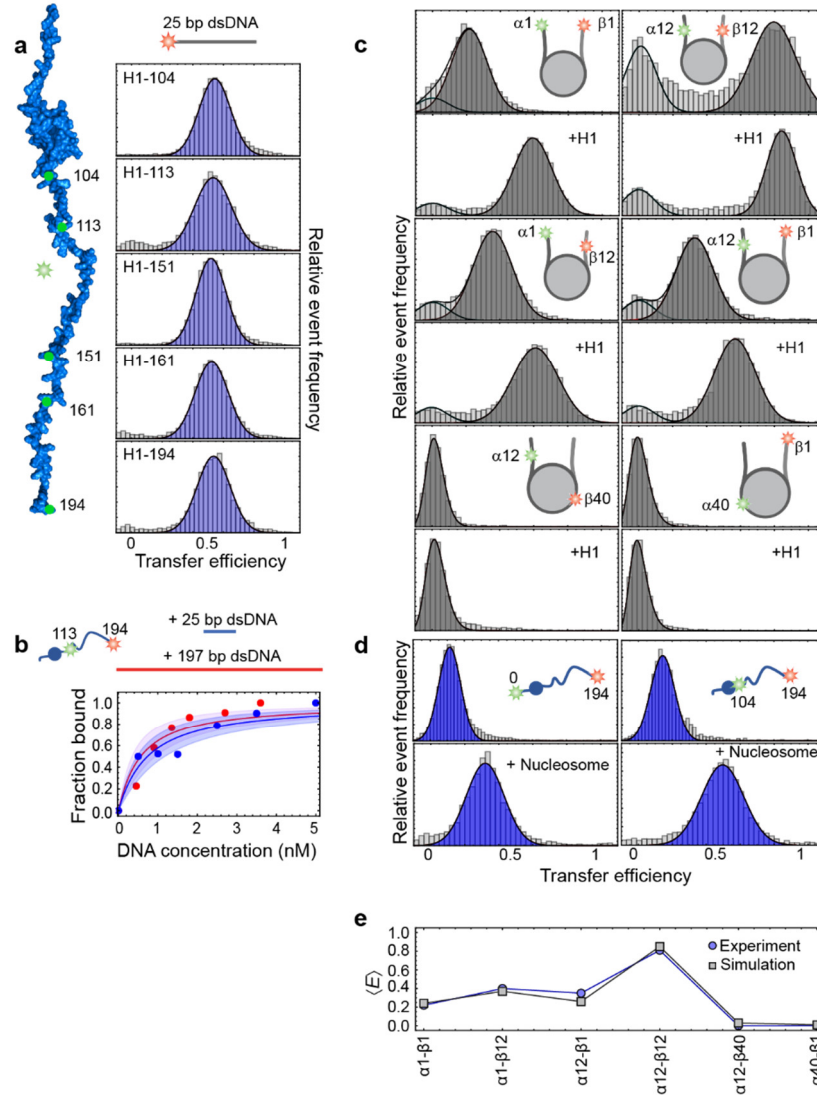


**Fig. S3. ProT $\alpha$  accelerates H1 dissociation from the nucleosome.** **a**, Observed off-rates of H1 from nucleosomes in the absence of ProT $\alpha$  (blue triangles: surface experiments, blue circles: from  $K_D$  and diffusion-limited on-rate coefficient) and transition rate from high to low FRET efficiency ( $E_{\text{high}} \rightarrow E_{\text{low}}$ , red triangles) in the presence of 100  $\mu\text{M}$  ProT $\alpha$ , as a function of salt concentration with fits to the Lohman-Record model<sup>4</sup> for extrapolation into the physiological salt concentration range (gray shading) and for estimating the changes in the numbers of monovalent counter ions bound. The results for the dissociation reaction  $\text{NH} \rightarrow \text{N} + \text{H}$  ( $12 \pm 1$  ions getting bound) are similar to the salt dependence of  $K_D$  for  $\text{NH} \rightleftharpoons \text{N} + \text{H}$  ( $13 \pm 1$  ions getting bound, Fig. 1d), providing further evidence that the binding reaction is downhill in free energy, as suggested by the diffusion-limited association rates (Table S1) and the simulations (Fig. 5d). **b**, Fraction of donor-labeled H1 (labeled with Alexa Fluor 488 in position 104) bound to acceptor-labeled nucleosome (labeled with Alexa Fluor 594 at position  $\alpha 12$ ), based on transfer efficiency histograms from time-resolved intermolecular FRET experiments on freely-diffusing molecules, in the absence (blue) and presence (red) of 200  $\mu\text{M}$  ProT $\alpha$ , at 200 mM salt concentration. A transfer efficiency histogram was constructed every 5 s after mixing of ProT $\alpha$  (dead time 5 s). A fit of the data shows that ProT $\alpha$  accelerates dissociation of H1 from the nucleosome (fraction bound is reduced from  $\sim 0.8$  to  $\sim 0.5$ ), resulting in a dissociation rate of at least  $0.6 \pm 0.1 \text{ s}^{-1}$ , much faster than the value of  $\sim 10^{-3} \text{ s}^{-1}$  expected in the absence of ProT $\alpha$  (panel A). **c**, Transfer efficiency histograms of fluorescently labeled ProT $\alpha$  (Alexa Fluor 488 and Alexa Fluor 594 in positions 56 and 110), either in isolation (top), in the presence of 16 nM (middle), or 150 nM (bottom) unlabeled nucleosomes, at 165 mM salt concentration. The differences in average transfer efficiency between the histograms are negligible; in the bottom histogram, the apparent transfer efficiency is slightly decreased by the considerably higher background fluorescence at this high nucleosome concentration. For comparison, when bound to H1, the transfer efficiency of this ProT $\alpha$  variant increases from 0.36 to 0.54.<sup>5</sup> **d**, Donor-acceptor cross-correlation curves were used to compare the translational diffusion of ProT $\alpha$  in isolation and in the presence of nucleosomes, at 165 mM salt concentration (same color scheme as in panel b). Binding of ProT $\alpha$  to the much larger nucleosome particles would be expected to substantially increase its diffusion time. The measured diffusion time of ProT $\alpha$  in the presence of nucleosomes is, however, indistinguishable from its diffusion time in isolation. The data in panels c and d indicate that, without H1 present, a direct interaction between ProT $\alpha$  and nucleosomes does not take place at these protein concentrations. **e**, Stoichiometry ratio vs. transfer efficiency histograms from pulsed interleaved excitation of donor-labeled H1 (Alexa Fluor 488 at position 104) and acceptor-labeled nucleosome (Alexa Fluor 594 at position  $\alpha 12$ ), in the absence (upper) and presence (lower) of 106  $\mu\text{M}$  ProT $\alpha$ . In the absence of ProT $\alpha$ , a donor-only peak ( $S \approx 1$ ,  $E \approx 0$ ) and a FRET peak ( $S \approx 0.5$ ,  $E \approx 0.9$ ) are visible. Upon addition of ProT $\alpha$ , the FRET peak disappears, and the donor-only peak increases in amplitude, indicating that the donor-labeled H1 has fully dissociated from the acceptor-labeled nucleosome.

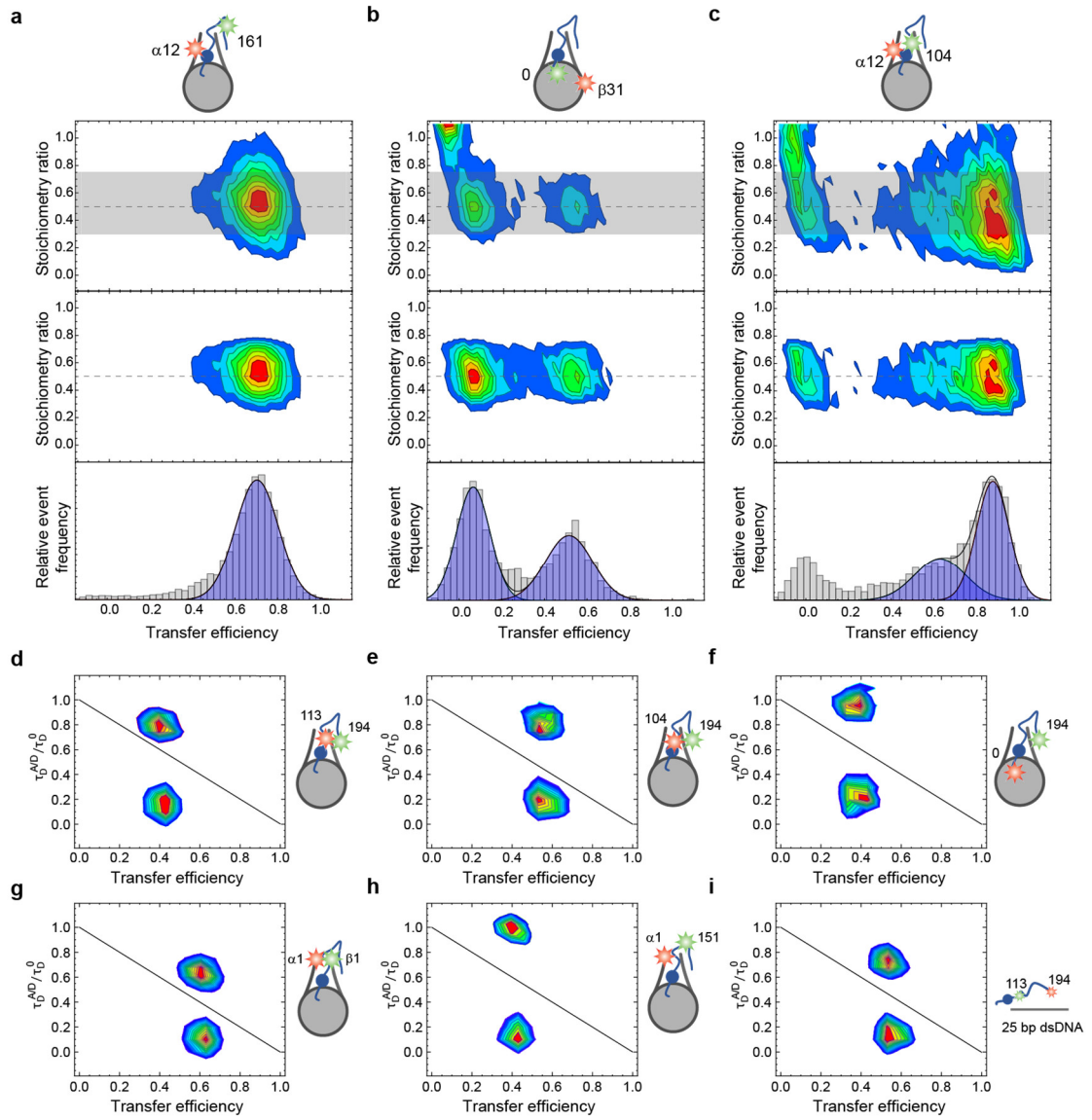




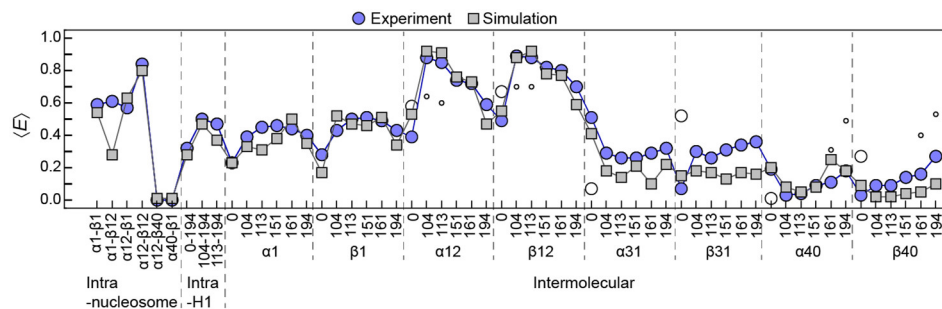
**Fig. S4. Rapid dynamics in the H1-nucleosome complex.** **a**, Schematic illustration of nanosecond fluorescence correlation spectroscopy (nsFCS)<sup>6</sup>, which uses photon arrival times to report on fluctuations in distance between two fluorophores. The effect of the delay between photons on the donor-donor (DD) and donor-acceptor (DA) correlations is illustrated (acceptor-acceptor (AA) is analogous to DD). **b**, nsFCS of nucleosomes labeled at the  $\alpha 1$  and  $\beta 1$  positions, without (upper) and with (lower) unlabeled H1 bound; with DD (green), AA (red), and DA (orange) correlations. Fits of the correlation decays indicate rapid chain dynamics between the two dyes on a sub-microsecond timescale (Materials and Methods). **c**, nsFCS with intermolecular FRET between H1 donor-labeled at positions 104, 151, and 194, and nucleosomes acceptor-labeled at position  $\alpha 1$  shows that rapid dynamics of H1 relative to the linker DNA are present throughout the C-terminal region of H1 in complex with the nucleosome.



**Fig. S5. Interactions and dynamics of H1, nucleosomes, and DNA.** **a**, Transfer efficiency histograms from intermolecular FRET experiments between donor-labeled H1 and acceptor-labeled 25-bp dsDNA with a sequence corresponding to the  $\alpha$ -linker used in the nucleosome constructs (see Table S2). **b**, Binding isotherms for binding of double-labeled H1 to dsDNA, either the  $\alpha$ -linker 25-bp sequence (blue) or the entire Widom sequence flanked by the linker sequences (197 bp, red), at 245 mM salt concentration. The affinity of H1 for 25 bp and 197 bp DNA ( $\sim 0.7 \pm 0.1$  nM and  $0.5 \pm 0.2$  nM, respectively) is almost two orders of magnitude lower than for the nucleosome at the same salt concentration ( $\sim 11^{+18}_{-7}$  pM, Fig. 1d). **c**, Intramolecular transfer efficiency histograms of nucleosomes with FRET dyes at different positions on the  $\alpha$  and  $\beta$  strands, in the presence and absence of saturating concentrations of unlabeled H1. Changes in transfer efficiency report on changes in linker DNA arrangement upon H1 binding. For variants  $\alpha 1/\beta 1$ ,  $\alpha 1/\beta 12$ ,  $\alpha 12/\beta 12$ , and  $\alpha 12/\beta 1$ , the peaks at  $E \approx 0$  (light gray) are incompatible with a correctly formed nucleosome and were thus excluded from the analysis. **d**, Intramolecular single-molecule FRET experiments with dyes at different positions along the H1 sequence and unlabeled nucleosomes. Labeling positions are indicated as numbers and arrows along the structural or cartoon representations of H1. **e**, Comparison between average intra-nucleosome FRET efficiencies from experiment (circles) and simulation (squares), in the absence of H1. The agreement attests to the quality of the simulation model for describing the nucleosome structure. All data in the figure, except panel b, were collected at 165 mM salt concentration.



**Fig. S6. Representative examples of stoichiometry measurements and fluorescence lifetime analysis of H1-nucleosome and H1-DNA complexes.** **a-c**, 2D-histograms of stoichiometry ratio vs. transfer efficiency from intermolecular FRET between donor-labeled H1 and acceptor-labeled nucleosome using pulsed interleaved excitation. The labeling positions are indicated in the schematics in the graphs. The gray-shaded areas indicate the ranges of stoichiometry ratios used for filtering out donor-only bursts. A 1:1 complex results in a stoichiometry ratio of 0.5 (dashed horizontal line). Most of the transfer efficiency histograms in Figure 4 contain a single population, as in **(a)**. In some cases, such as in **(b)**, a clear second population is observed that is well separated from the donor-only population. In other cases, i.e. in **(c)**, an additional residual population at a transfer efficiency close to zero can remain even after filtering due to a large signal from molecules lacking an active acceptor dye. **(d-i)** Fluorescence lifetime analysis. Plots showing the fluorescence lifetimes of Alexa Fluor 488 ( $\tau_D^D$ ) and Alexa Fluor 594 ( $\tau_D^A$ ) normalized by the intrinsic donor lifetime ( $\tau_D^0$ ) as a function of transfer efficiency. If fluctuations in transfer efficiency occur on a timescale between the donor fluorescence lifetime and the burst duration ( $\sim 4$  ns–1 ms), the normalized donor and acceptor lifetimes cluster above and below the static FRET line (solid diagonal line)<sup>7,8</sup>, respectively. The substantial deviations from the diagonal indicate a broad distribution of distances in all samples, as previously observed for intrinsically disordered proteins<sup>9,10</sup>. **(d)** H1<sup>113-194</sup> + unlabeled nucleosome; **(e)** H1<sup>104-194</sup> + unlabeled nucleosome; **(f)** H1<sup>0-194</sup> + unlabeled nucleosome; **(g)** Nucleosome <sup>$\alpha 1-\beta 1$</sup>  + unlabeled H1; **(h)** H1<sup>151</sup> + nucleosome <sup>$\alpha 1$</sup> ; **(i)** H1<sup>113-194</sup> + 25 bp dsDNA.



**Fig. S7. Influence of fluorophores on simulation results.** Comparison between FRET efficiencies from experiment and simulation using an explicit representation of dyes. Experimental FRET efficiency values (purple and empty circles) are identical to the ones shown in Figure 4c. FRET efficiency values from simulation (gray squares) are computed from simulations where a representation of the dyes was included in the corresponding labeling positions (see Materials and Methods).

**Table S1. Binding parameters for H1-nucleosome interactions.**

H1-nucleosome complex					H1-nucleosome complex + 100 $\mu$ M ProT $\alpha$		
Salt concentration (mM)	$K_D^{Diff}$ (nM)	$K_D^{Surf}$ (nM)	$k_{on}$ ( $M^{-1}s^{-1}$ )	$k_{off}$ ( $s^{-1}$ )	Salt concentration (mM)	$K_D^{Diff}$ (nM)	$K_D^{Surf}$ (nM)
360	$1.7 \pm 0.1$	$0.9 \pm 0.3$	$(1.5 \pm 0.04) \cdot 10^9$	$1.3 \pm 0.1$	200	$0.05 \pm 0.02$	$0.35 \pm 0.03$
375	$3 \pm 0.2$	$2.4 \pm 0.9$	$(1.3 \pm 0.07) \cdot 10^9$	$3.2 \pm 0.2$	250	$0.16 \pm 0.02$	$0.76 \pm 0.05$
390	$5 \pm 0.3$	$3.8 \pm 2.3$	$(1.2 \pm 0.03) \cdot 10^9$	$4.7 \pm 0.8$	300	$0.95 \pm 0.2$	$2.45 \pm 0.07$
410	$9.7 \pm 0.6$	$18.3 \pm 6.8$	$(4.4 \pm 0.01) \cdot 10^8$	$8.0 \pm 0.8$	340	$3.15 \pm 0.5$	$8.48 \pm 0.06$
					360	$10.7 \pm 0.9$	$24.4 \pm 1.1$

Equilibrium and rate constants for nucleosome-H1 interactions from single-molecule experiments at different salt concentrations, in the absence and presence of 100  $\mu$ M ProT $\alpha$ . The equilibrium dissociation constants are either from the ratio of the rate coefficients based on the surface experiments ( $K_D^{Surf}$ ) or from the binding isotherms measured on freely diffusing molecules ( $K_D^{Diff}$ ). The agreement between the two methods confirms that surface immobilization does not interfere with the interactions between nucleosomes, H1, and ProT $\alpha$ . Rate constants were determined via maximum-likelihood analysis of fluorescence time traces collected with surface-immobilized nucleosomes in the presence of 0.5-30 nM of unlabeled H1. Errors on rate constants and  $K_D^{Surf}$  are based on ten bootstrapping trials from global likelihood maximization. For experiments on freely diffusing H1-nucleosome complex with 100  $\mu$ M ProT $\alpha$ , H1 was titrated into a solution containing nucleosome until binding was saturated. Errors are based on pipetting error estimates<sup>5</sup>.



**Table S2. Sequences of H1, ProTα, and nucleosome DNA, and transfer efficiencies of all labeling pairs.**

H1.0	<p><b>0</b></p> <p>CTENSTSAAPAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRSIQYIKSHYKVGENADSOIKLSIKRLVT</p> <p>104 113 151</p> <p>TGVLKQTKGVGASGSFRLAKSDEPKSVAFKKTKEIKKVATPKKASKPKKAASKAPTKKPKATPVKKAKKKLAATP</p> <p>161 194</p> <p>KKAKKPKTVKAKPVKASKPKKAKPVKPAKSSAKRAGKKKGPR</p>
ProTα	<p><b>1</b></p> <p>GPMSDAAVDTSEITTKDLKEKKEVEEAENGRDAPANGNANEENGEQADNEVDEEEEEEGEEEEEEEGDGE</p> <p>109</p> <p>EEDGDEEEAESATGKRAAEDDEDDVDTKKQKTDEDD</p>
'601'-Widom sequence, α-strand	<p><b>α1 α12 α31 α40</b></p> <p>5'-TCCATGACC CTATACGCGG CCGCCCTGGA GAATCCCGT GCCGAGGCCG CTCAATTGGT CGTAGACAGC</p> <p>1 2 3</p> <p>TCTAGCACCG CTAAACGCA CGTACGCGCT GTCCCCGCG TTTAACC GC CAAGGGGATT ACTCCCTAGT</p> <p>CTCCAGGCAC GTGTCAGATA TATACATCT GTAGATCATG ACGACAAGTT ATGTACG-3'</p>
'601'-Widom sequence, β-strand	<p><b>β1 β12 β31 β40 Biotin</b></p> <p>5'-TCTAGTACTG CTGTTCAATA CATGCACAGG ATGTATATAT CTGACACGTG CCTGGAGACT AGGGAGTAAT</p> <p>4 5</p> <p>CCCCTTGGCG GTTAAACGC GGGGGACAGC GCGTACGTGC GTTTAAGCGG TGCTAGAGCT GTCTACGACC</p> <p>AATTGAGCGG CCTCGGCACC GGGATTCTCC AGGGCGGCCG CGTATAGGGT CCATGGA-3'</p>

Intermolecular	Nucleosome position							
H1 position	α1	β1	α12	β12	α31	β31	α40	β40
<b>0</b>	0.23	0.28	0.39,0.58	0.49,0.67	0.07,0.51	0.07,0.52	0.01,0.19	0.03,0.27
<b>104</b>	0.39	0.43	0.64,0.88	0.70,0.89	0.29	0.30	0.03	0.09
<b>113</b>	0.45	0.50	0.6,0.85	0.70,0.88	0.26	0.26	0.04	0.09
<b>151</b>	0.46	0.51	0.74	0.82	0.26	0.31	0.09	0.14
<b>161</b>	0.44	0.49	0.72	0.80	0.29	0.34	0.11,0.31	0.16,0.40
<b>194</b>	0.40	0.43	0.59	0.70	0.32	0.36	0.18,0.49	0.27,0.53

Intra-nucleosome	β-linker				
α-linker	β1	β12	β40	Intra-H1	
<b>α1</b>	0.59	0.61	ND	<b>0-194</b>	0.32
<b>α12</b>	0.57	0.84	0.00	<b>104-194</b>	0.50
<b>α40</b>	0.00	ND	ND	<b>113-194</b>	0.47

*Upper table:* Sequences of H1 and ProTα constructs and the 601-Widom sequence<sup>11</sup> used for nucleosome reconstitution. Residues and nucleotides in red indicate fluorophore labeling sites. H1 residues in yellow remain after proteolytic cleavage of the hexahistidine tag with thrombin. The underlined segment of H1 indicates the globular domain<sup>5</sup>. The nucleotide indicated in green was modified with a biotin linker for surface immobilization of the nucleosomes. Underlined nucleotides constitute the 147-bp 601-Widom sequence, which forms the core nucleosome particle; the remaining nucleotides correspond to the linker DNA<sup>12</sup>. For DNA labeling, thymine modified with a C6-aminolinker was incorporated for the reaction with the succinimidyl ester of the fluorescent dye. Purple labels (1-5) denote the 3'-end of oligonucleotide primers used to PCR-amplify the corresponding nucleosomal DNA, either with or without previous fluorescence labeling (1: unlabeled and α1-labeled nucleosomes, 2: α-12/α31-labeled nucleosomes, 3: α-40-labeled nucleosome, 4: β1/β12/β31/β-40-labeled nucleosomes, 5: biotinylated nucleosomes). In all cases, the 5'-end nucleotide of the respective PCR primer is the one shown at the 5'-end of the Widom sequence.

*Lower table:* Transfer efficiencies of all labeling pairs measured in this study. For intermolecular and intra-nucleosome FRET efficiencies, the donor and acceptor dye positions are indicated in green and red, respectively. In cases where two peaks were observed, both values are given. The accuracy of the transfer efficiency values is approximately ±0.04,<sup>13</sup> the precision approximately ±0.02.

**Table S3. Bonded and non-bonded interactions between protein and DNA beads used for simulations.**

Bonds	$r_0$ (nm)	$k_b$ (kJ·mol <sup>-1</sup> ·nm <sup>-2</sup> )
$C_i - C_{i+1}$	see text <sup>75</sup>	316,310
$B_i - R_i$	0.586	300,000
$P_i - R_i$	0.390	300,000
$P_i - R_{i+1}$	0.390	300,000
Angles	$\theta_i$ (degrees)	$k_\theta$ (kJ·mol <sup>-1</sup> ·rad <sup>-2</sup> )
$C_i - C_{i+1} - C_{i+2}$	see text <sup>75</sup>	632.62
$B_i - R_i - P_i$	100.8	1000.0
$P_i - R_{i+1} - B_{i+1}$	91.1	1000.0
$R_i - P_i - R_{i+1}$	95.2	1000.0
Torsions	$\phi_i$ (degrees)	$\delta_\phi$ (kJ·mol <sup>-1</sup> ·rad <sup>-2</sup> )
$C_i - C_{i+1} - C_{i+2} - C_{i+3}$	See Methods	See Methods

Pair type	$\sigma$ (nm)	$\epsilon$ (kJ·mol <sup>-1</sup> )
<b>Protein-Protein</b>		
$C_i - C_j$ (non-native)	see text <sup>75</sup>	0.40
$C_i - C_j$ (native)	see text <sup>75</sup>	see text <sup>75</sup>
<b>DNA-DNA</b>		
$B_i - B_{i+1}$ (i.e. stacking)	0.360	8.786
$B_i - B_j$ (i.e. pairing)	0.360	7.531
$P_i - P_j$	0.600	0.100
$R_i - R_j$	0.600	0.100
$B_i - R_j$	0.600	0.100
$B_i - P_j$	0.600	0.100
$R_i - P_j$	0.600	0.100
<b>DNA-Protein (non-native)</b>		
$B_i - C_j$	0.600	0.151
$P_i - C_j$	0.600	0.151
$R_i - C_j$	0.600	0.151
<b>DNA-Protein (native)</b>		
$P_i / R_i / B_i / - C_j$ (DNA-core histones)	see text	5.115
$P_i / R_i / B_i / - C_j$ (DNA-H1 globular domain)	see text	2.574

List of bonded and non-bonded parameters describing the interaction between protein and DNA beads. Details for some parameters are given in the text.

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