Supporting information for

Single-molecule kinetic analysis of HP1-chromatin binding reveals a dynamic network of histone modification and DNA interactions

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SI Materials and methods

Chemicals

Amino acid derivatives and resins were purchased from Novabiochem, Merck (Hohenbrunn, Germany). Peptide synthesis solvents and reagents were from Acros Organics (Geel, Belgium) (Dimethylformamide, dichloromethane, N,N-diisopropylethylamine and piperidine) or Protein Technologies Inc. (Tucson, AZ, US) (HBTU). All commonly used chemical reagents and solvents were purchased from Sigma-Aldrich Chemical Company (Steinheim, Germany), Fischer Scientific (Fair lawn, NJ, US/Loughborough, UK) or AppliChem (Darmstadt, Germany). Acetonitrile was from Lab-Scan Analytical Sciences (Sowinskiog, Poland). 2, 2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) was purchased from Wako Pure Chemical Industries, Ltf. (Osaka, Japan) and N-Vinylacetamide from (Tokyo Chemical Industry). 76 x 26 mm slides were purchased from Thermo Scientific (Rockford, IL, US) and 24 x 40 mm coverslips from Marienfeld-Superior (Lauda-Königshofen, Germany). (3-Aminopropyl)triethoxysilane (APTES) from Acros Organics, methoxypoly(ethylene glycol) succinimidyl carbonate (mPEG) and biotinylated methoxypoly(ethylene glycol) succinimidyl carbonate (mPEG-Biotin) from Laysan Bio Inc (USA) and Secure Seal adhesive sheets from Grace biolabs (USA). Chemically competent DH5α, Rosetta(DE3), BL21(DE3) and BL21(DE3)plysS were from Novagen (Darmstadt, Germany) and used to generate stocks of competent cells. Casein Kinase 2 (CK2), T4 DNA ligase, restriction enzymes, Phusion DNA polymerase, DNA ladders, DNA loading dyes and dNTPs were purchased from New England Biolabs (Ipswich, MA, US) distributed through BioConcept (Allschwil, Switzerland). Primers were ordered from and synthesized by Integrated DNA technologies (Leuwen, Belgium). Gene sequencing was performed at GATC Biotech (Constance, Germany). Single-point mutations were done using a QuickChange II XL site-directed mutagenesis kit from Agilent (Basel, Switzerland). Atto488 maleimide, Atto532 iodoacetamide and Atto647N NHS ester were purchased from ATTO-TEC Gmbh (Siegen, Germany). Materials for hand-casting of agarose and SDS-PAGE gels (agarose, TEMED, APS, acrylamide), analysis of SDS-PAGE gels (Precision Plus ProteinTM All blue standards and Precision Plus ProteinTM dual color standards) and pre-cast Criterion 5% TBE gels were purchased from BioRad (Hercules, CA, US). QiaQuick spin column for PCR purification, gel extraction
and nucleotide removal as well as QiaPrep spin columns for miniprep plasmid purification were from Qiagen (Hilden, Germany). Slide-a-lyzer dialysis cassettes and MINI dialysis buttons were from Thermo Scientific (Rockford, IL, US). Amicon Ultracentrifugal concentration units were from Merck Millipore (Tullagreen, Ireland). Spectra/por dialysis tubing was from Spectrum laboratories Inc (Rancho Dominguez, CA, US).

**Instrumentation**

Bacterial cells for recombinant protein expression were grown in an HT inforS AG incubator, recovered with an Avanti J-20 XPI centrifuge from Beckman Coulter and sonicated using a Vibra-cell VCX 750 Sonics & Materials sonicator. Manual peptide synthesis and reactions on solid-phase were carried out in reaction vessels from Peptides International and automated peptide synthesis done on a Tribute instrument from Peptides International Inc. Size exclusion chromatography and ion exchange purification was performed on an AKTA Pure FPLC system from GE Healthcare. Size-exclusion were done using a S200 10/300GL or S75 10/300 column from GE Healthcare. Cation exchange and anion exchange purification was done using HiTrap SP HP (5mL) and HiTrap Q FF (1mL) traps from GE Healthcare. Analytical reversed-phase HPLC (RP-HPLC) was performed on an Agilent 1260 series instrument with an Agilent Zorbax C18 column (5µm, 4.6 x 150 mm), employing 0.1% TFA in water (RP-HPLC solvent A), and 90% acetonitrile, 0.1% TFA in water (RP-HPLC solvent B), as the mobile phases. Typical analytical gradients were 0-70% solvent B over 30 min at a flow rate of 1 mL/min. Preparative scale purifications were conducted on an Agilent 1260 preparative HPLC system. A Zorbax C18 preparative column (7 µm, 21.2 x 250 mm) or a semi-preparative column (5 µm, 9.4 x 250 mm) was employed at a flow rate of 20 mL/min or 4 mL/min, respectively. ESI-MS analysis was conducted on a Shimadzu MS2020 single quadrupole instrument connected to a Nexera UHPLC system. Absorbance spectra were recorded with an Agilent 8453 UV-Vis spectrophotometer. SDS-PAGE, native PAGE and agarose gels were imaged using a ChemiDoc MP imaging system from BioRad. Titrations using microscale thermophoresis were done on a MonoLith NT.115 instrument equipped with blue/green or green/red filters from NanoTemper technologies. Live-cell confocal microscopy was done on a LSM700 inverted microscope from Zeiss. For single-molecule TIRF microscopy a Nikon Ti-E inverted fluorescence microscope, controlled by NIS-elements, equipped with a CFI Apo TIRF 100x Oil immersion objective (NA 1.49) was used. Scanning force microscopy was performed with a Bruker FastScan AFM and NCHV AFM cantilevers (Bruker).

**Mathematical modeling.** The kinetic model considers all elementary steps of protein - chromatin interactions, up to third order reactions (Fig. S10a). The protein (HP1) exists in a monomeric state (states denoted by ,) it has the ability to form dimers (HP1:HP1, states denoted by ) it can remain
unbound (index 0) to bind to DNA (HP1:DNA, indicated by index 1) as well as specifically to its cognate histone post-translational modification (PTM), H3K9me3 (HP1:H3K9, indicated by index 2). In our model, we assume that HP1 monomers can bind either to the DNA (DNA binding) or to H3K9me3 (PTM binding). In a dimer, we allow the individual protomers to bind independently to either the DNA or to the histone PTM. Thus, dimers can bind in a monovalent mode as: HP1:HP1:DNA \((Y^0)\) HP1:HP1:H3K9 \((Y^0v)\) or in a bivalent mode as: H3K9:HP1:HP1:DNA (H3K9:2HP1:DNA) \((Y^i2)\), DNA:HP1:HP1:DNA (2HP1:2DNA) \((Y^i2)\) or H3K9:HP1:HP1:H3K9 (2HP1:2H3K9) \((Y^i2)\). We further consider migrations of chromatin-bound proteins between binding sites, in addition to direct binding. Such migrations occur for example if DNA-bound proteins slide along the DNA and then bind to a modified histone tail. In order to avoid biasing the model due to its structure, all possible migration reactions up the third order are considered. Thus, dimers can migrate from monovalent bound states to bivalent bound states as well as from DNA bound states to PTM bound states and vice-versa. To reduce parameters, we introduce thermodynamic constrains (Table S4). Additionally, we assume that the free energy difference of binding to a single site is the same for monomers and dimers: \(K_{00|01} \equiv K_{01|0} \) and \(K_{00|02} \equiv K_{00|2} \). The complete mathematical model accounts also for incomplete fluorescent labeling of HP1. Thus, every monomer can exist in a labeled and an unlabeled form. We used the rule based modeling tool BioNetGen to generate an extended reaction network from the reactions we defined above (Figure S10)(1). The ordinary differential equations (ODEs) of the reaction system were solved using the CVODE solver from the SUNDIALS toolbox for MATLAB (2).

**Parameter Estimation.** We estimated the model parameters using gamultiobj in MATLAB, a genetic multi-objective optimization algorithm based on the NSGA-II algorithm (3). To train the algorithm, we used the results of the single-molecule TIRF experiments with HP1\(\alpha\) at 1 nM concentration and with HP1\(\beta\) at 5 nM concentration (Table 1). For the model calibration, we assigned each experiment with an objective function that compares the dissociation times \(\tau_1 \) and \(\tau_2 \) and the stochastic equivalent relative exponential fraction \(A_2 = \frac{A_{2,\text{exp}}/\tau_2}{A_{1,\text{det}}/\tau_1 + A_{2,\text{det}}/\tau_2} \) as well as the equilibrium value of adsorbed protein, \(N_0 = [\text{HP1}_{\text{labeled}}]VN_a \) with the experimental results. The objective for a single experiment is calculated as:

\[
R = \sqrt{\left(\frac{N_{0,\text{exp}} - N_{0,\text{sim}}}{DN_{0,\text{exp}}}\right)^2 + \left(\frac{\tau_{1,\text{exp}} - \tau_{1,\text{sim}}}{\Delta \tau_{1,\text{exp}}}\right)^2 + \left(\frac{\tau_{2,\text{exp}} - \tau_{2,\text{sim}}}{\Delta \tau_{2,\text{exp}}}\right)^2 + \left(\frac{A_{2,\text{exp}} - A_{2,\text{sim}}}{\Delta A_{2,\text{exp}}}\right)^2}
\]

To simplify the parameter estimation, we used dimensionless quantities to describe our system. The only independent units of our system are concentration and time. Thus we scale our system using a characteristic concentration \(c_{\text{ref}} = [\text{HP1}]_{\text{tot}} \) and a characteristic time \(t_{\text{ref}} = t_{\text{ref}} [\text{HP1}]_{\text{tot}} \). Here we chose the characteristic concentration to be \([\text{HP1}]_{\text{tot}} \) the overall concentration of protein. The
characteristic time is derived from a reference value for the dimerization rate \( r_{D,\text{ref}} \) assumed to be \( 10^6 \text{s}^{-1}\text{M}^{-1} \) and the total concentration of protein. The dimensionless variables are listed in Table S1 and a description of the dimensionless parameter can be found in Table S2 and Table S3. From the parameter optimization, we obtained 168 parameter sets with different parameter combinations (Fig. S11). For the investigations presented in this work, we used the median of these populations as parameters for the model (Tables S2 - S3).

**Stochastic model.** We verified our deterministic parameter estimation using stochastic simulations, directly simulating the single molecule binding experiments. To translate the deterministic model into a stochastic simulation we transferred the rate equations into propensities as described by Gillespie (4). The stochastic model was then simulated using StochPy (5). To evaluate the stochastic simulations, bright and dark times, i.e. the durations a labeled protein is bound to a chromatin array and the times the array remains free, were determined (Fig. 4b). To this end, we simulated a single chromatin array as 24 H3K9 and 120 DNA binding sites. Subsequently, we compared the simulations to the experiments using cumulative histograms of the bright and dark times. Since the experimental procedure cannot resolve events shorter than 0.05 s these events were removed by a low-pass filter in our simulations (Fig. S10b). Comparing the complete lifetime histograms in \( \log(t) \) space we clearly can see that the model captures events that are beyond the resolution of the experimental measurement (Fig. S10c vs. S10d). From the simulated cumulative lifetime histograms, observable rate (time) constants are then retrieved (Fig. 4c-f), dependent on the input parameters. To vary the overall binding affinity of H3K9me3 and DNA interactions we scaled the free energy of the a single DNA or H3K9me3 binding site. Thus, the binding constants of bivalent bound species are scaled by the a square of the scaling factor for the respective monovalent bound species.

**Sensitivity analysis.** To determine parameter sensitivity, we performed variance-based sensitivity analysis (VBSA or Sobol’ Method) (6) using the SAFE tool box in Matlab (7) (Fig. S12). VBSA computes individual and total sensitivities scores, quantifying the impact of a parameter on the model output from the contribution if (i) only the parameter in question is varied or (ii) the parameter is varied in combination with all other parameters. We defined a set of output parameters (bound HP1 concentration, the two apparent dissociation time constants \( \tau_{\text{off},1} \) and \( \tau_{\text{off},2} \) and the percentage of the amplitude of the slow dissociation process). The analysis was performed for all the model parameters (Tables S2-3), varying forward and reverse rate constants for each reaction. For each output parameters, the 5 most significant parameters (parameters with the largest total scores) were determined. For these parameter we extracted the ten-fold change of the model in- and output with respect to the reference model (Tables S2-3) using linear regression (Fig. S13). This yields the most probable global effect effect on the on the model for a given set of parameters. The indicies show
whether correlation, anti-correlation or no-correlation is expected between the respective model output and a parameter (Fig. 5g).

Supporting references

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**Supplementary tables:**

| Symbols | Description | Expression |
|---------|-------------|------------|
| $X_0$   | Unbound monomeric HP1 concentration | $[\text{HP1}]_{\text{tot}}$ |
| $Y_{00}$ | Unbound dimeric HP1 concentration | $[\text{HP1}]_{\text{tot}}$ |
| $D$     | Concentration of free DNA binding sites | $[\text{DNA}]_{\text{tot}}$ |
| $S$     | Concentration of free PTM binding sites (H3K9) | $[\text{H3K9}]_{\text{tot}}$ |
| $X_1$   | Monomeric, DNA bound HP1 concentration | $[\text{HP1:DNA}]_{\text{tot}}$ |
| $Y_{01}$ | Dimeric, monovalent DNA bound HP1 concentration | $[2\text{HP1:DNA}]_{\text{tot}}$ |
| $Y_{11}$ | Dimeric, bivalent 2xDNA bound HP1 concentration | $[2\text{HP1:2DNA}]_{\text{tot}}$ |
| $Y_{12}$ | Dimeric, DNA and PTM bound HP1 concentration | $[\text{H3K9:2HP1}]_{\text{tot}}$ |
| $X_2$   | Monomeric, PTM (H3K9) bound HP1 concentration | $[\text{HP1}]_{\text{tot}}$ |
| $Y_{02}$ | Dimeric, monovalent, PTM (H3K9) bound HP1 concentration | $[\text{H3K9:2HP1}]_{\text{tot}}$ |
| $Y_{22}$ | Dimeric, bivalent PTM (2H3K9) bound HP1 concentration | $[2\text{H3K9:2HP1}]_{\text{tot}}$ |

**Table S1:** A list of all dimensionless model variables given a short description and expression in terms of the reference concentrations

| Symbols | Description | Unit | median | lower quartile | upper quartile |
|---------|-------------|------|--------|----------------|----------------|
| $K_D$   | Dimerization | µM   | 0.36   | 0.30           | 1.92           |
| $K_{1,2,12}$ | 1D mixed Dimerization | µM   | 62.15  | 42.68          | 70.42          |
| $K_{0,1}$ | Monomer DNA bound | µM   | 6.19   | 4.54           | 8.12           |
| $K_{00,01}$ | Dimer monovalent DNA bound | µM   | 6.19   | 4.54           | 8.12           |
| $K_{00,11}$ | Dimer bivalent DNA bound | µM   | $2.20 \times 10^{-4}$ | $1.79 \times 10^{-4}$ | $5.81 \times 10^{-4}$ |
| $K_{0,2}$ | Monomer PTM bound | µM   | 8.35   | 1.74           | 11.76          |
| $K_{00,02}$ | Dimer monovalent PTM bound | µM   | 8.35   | 1.74           | 11.76          |
| $K_{00,12}$ | Dimer bivalent DNA-PTM bound | µM   | $9.10 \times 10^{-5}$ | $2.75 \times 10^{-5}$ | $3.03 \times 10^{-4}$ |
| $K_{00,22}$ | Dimer bivalent DNA-PTM bound | µM   | $9.3 \times 10^{-5}$ | $8.11 \times 10^{-5}$ | $3.72 \times 10^{-3}$ |

**Table S2:** A list of all dimensionless equilibrium constants, their initial assumed values as well as their values after the optimization procedure, given relative to the reference concentration $[\text{HP1}]_{\text{tot}}$
| Symbols | Description of the forward rate equation | unit | median | lower quartile | upper quartile |
|---------|------------------------------------------|------|--------|----------------|---------------|
| $k_{d}$ | Dimerization                             | 1/Ms | 1.12 x 10^7 | 5.88 x 10^6 | 2.16 x 10^7 |
| $k_{d1}$ | Monomer DNA binding                      | 1/Ms | 5.75 x 10^5 | 4.91 x 10^5 | 7.93 x 10^5 |
| $k_{d2}$ | Monomer PTM binding                      | 1/Ms | 9.56 x 10^6 | 3.12 x 10^6 | 1.32 x 10^6 |
| $k_{d01}$ | Dimerization of free and DNA bound protein | 1/Ms | 1.32 x 10^5 | 2.29 x 10^4 | 2.58 x 10^4 |
| $k_{d02}$ | Dimerization of free and PTM bound protein | 1/Ms | 7.93 x 10^4 | 4.72 x 10^4 | 1.23 x 10^4 |
| $k_{d001}$ | Monovalent, DNA binding of a dimer       | 1/Ms | 2.01 x 10^7 | 1.23 x 10^7 | 7.51 x 10^7 |
| $k_{d002}$ | Monovalent, PTM binding of a dimer       | 1/Ms | 1.33 x 10^7 | 7.68 x 10^6 | 1.72 x 10^7 |
| $k_{d012}$ | Bivalent, DNA binding of a dimer         | 1/M^2s | 2.55 x 10^14 | 1.38 x 10^14 | 5.22 x 10^15 |
| $k_{d022}$ | Bivalent, PTM binding of a dimer         | 1/M^2s | 2.07 x 10^13 | 1.03 x 10^13 | 4.04 x 10^14 |
| $k_{d112}$ | Bivalent, PTM-DNA binding of a dimer     | 1/M^2s | 8.37 x 10^13 | 3.34 x 10^13 | 2.33 x 10^14 |
| $k_{d111}$ | Dimerization of two DNA bound monomers | 1/Ms | 1.39 x 10^10 | 5.69 x 10^9 | 4.27 x 10^10 |
| $k_{d222}$ | Dimerization of two PTM bound monomers | 1/Ms | 1.49 x 10^11 | 7.13 x 10^10 | 3.32 x 10^11 |
| $k_{d122}$ | Dimerization of a PTM and a DNA bound monomer | 1/Ms | 6.74 x 10^9 | 3.37 x 10^9 | 1.97 x 10^10 |
| $k_{d011}$ | Migration from monovalent DNA binding to bivalent DNA binding | 1/Ms | 8.75 x 10^10 | 2.59 x 10^10 | 1.22 x 10^11 |
| $k_{d221}$ | Migration from monovalent PTM binding to bivalent DNA binding | 1/Ms | 2.41 x 10^8 | 3.24 x 10^7 | 3.57 x 10^8 |
| $k_{d212}$ | Migration from monovalent DNA binding to bivalent, PTM-DNA binding | 1/Ms | 6.08 x 10^9 | 4.28 x 10^8 | 7.17 x 10^9 |
| $k_{d022}$ | Migration from monovalent, PTM binding to bivalent, PTM-DNA binding | 1/Ms | 6.74 x 10^7 | 2.65 x 10^7 | 1.75 x 10^8 |
| $k_{d112}$ | Migration from bivalent, DNA binding to bivalent, PTM-DNA binding | 1/Ms | 5.72 x 10^8 | 5.12 x 10^8 | 9.49 x 10^8 |
| $k_{d222}$ | Migration from bivalent PTM binding to bivalent, PTM-DNA binding | 1/Ms | 3.67 x 10^7 | 6.48 x 10^6 | 1.46 x 10^10 |
| $k_{d111}$ | Migration from bivalent DNA binding to bivalent, PTM binding | 1/M^2s | 3.17 x 10^18 | 1.02 x 10^18 | 3.70 x 10^19 |
| $k_{d012}$ | Migration from monovalent DNA binding to monovalent, PTM binding | 1/Ms | 7.75 x 10^8 | 3.61 x 10^7 | 1.33 x 10^11 |
| $k_{d112}$ | Migration of a monomer from DNA binding to PTM binding | 1/Ms | 4.08 x 10^8 | 1.75 x 10^7 | 2.60 x 10^9 |
| $k_{d012}$ | Migration from monovalent DNA binding to bivalent, PTM binding | 1/M^2s | 1.02 x 10^19 | 1.10 x 10^17 | 2.67 x 10^19 |
| $k_{d011}$ | Migration from monovalent, PTM binding to bivalent, DNA binding | 1/M^2s | 3.37 x 10^19 | 1.22 x 10^19 | 4.43 x 10^19 |

**Table S3:** A list of all dimensionless forward rates. The first half of the table denotes the reaction rates for the three-dimensional reactions and the second half of the table denotes the reaction rates for the migration rates.
\[
\begin{array}{c|c|c}
K_{0,1|01} &= K_D \frac{K_{00|01}}{K_{0|1}} & K_{01|02} &= K_D \frac{K_{00|02}}{K_{00|01}} & K_{1,1|11} &= K_D \frac{K_{00|01}}{K_{0|1}} \\
K_{0,2|02} &= K_D \frac{K_{00|02}}{K_{0|2}} & K_{01|11} &= K_D \frac{K_{00|11}}{K_{00|01}} & K_{2,2|22} &= K_D \frac{K_{00|02}}{K_{0|2}} \\
K_{1|2} &= \frac{K_{0|2}}{K_{0|1}} & K_{02|22} &= \frac{K_{00|22}}{K_{00|02}} & K_{22|12} &= \frac{K_{00|12}}{K_{00|02}K_{00|22}} \\
K_{11|12} &= \frac{K_{00|12}}{K_{00|01}K_{00|11}} & K_{11|22} &= \frac{K_{00|02}K_{00|22}}{K_{00|01}K_{00|11}} & K_{01|22} &= \frac{K_{00|02}K_{00|22}}{K_{00|01}} \\
K_{02|12} &= \frac{K_{00|12}}{K_{00|02}} & K_{01|12} &= \frac{K_{00|12}}{K_{00|01}} & K_{02|11} &= \frac{K_{00|01}K_{00|11}}{K_{00|02}}
\end{array}
\]

Table S4: Thermodynamic constraints on the equilibrium parameters.
Supplementary Figures:

Figure S1. 177 and 197 bp array DNA labeling. a) Production of fluorescently labeled and biotinylated 177a DNA. In the fluorescence overlay, red: GelRed, green: ATTO647N b) Absorption spectrum of 177a fluorescently labeled array DNA. c) Production of fluorescently labeled and biotinylated 197a DNA. In the fluorescence overlay, red: GelRed, green: ATTO647N d) Absorption spectrum of 197a fluorescently labeled array DNA.
Figure S2. Synthesis of H3K9me3 and H4K16ac. a) RP-HPLC and ESI-MS analysis of the ligation product H3K9me3 H3(A15C)K9me3 after desulfurization (calculated mass: 15251 Da, observed mass: 15248 Da). b) RP-HPLC and ESI-MS analysis of H4K16C (calculated mass: 11211 Da, observed mass: 11211 Da). c) RP-HPLC and ESI-MS analysis of the H4K5ac (calculated mass: 11296 Da, observed mass: 11293 Da).
Figure S3. Production of octamers. a) Octamers were purified by Size exclusion chromatography, black curve: H3K9me3 octamers, red curve: wild-type octamers and blue curve: H3K9me3/H4K16ac octamers. b) SDS-PAGE analysis of the three different refolded octamers pooled and concentrated after size exclusion chromatography.
**Figure S4. Chromatin reconstitution.** a) Agarose gel analysis of reconstituted H3K9me3 or H3K9me3/H4K16ac labeled chromatin arrays with 12x 177a or 197 bp DNA. Green: Atto647N, red: GelRed. b) Native PAGE analysis of Scal digested H3K9me3 or H3K9me3/H4K16ac labeled chromatin arrays with 12x 177a or 197 bp DNA. Green: Atto647N, red: GelRed. c) Agarose gel analysis of reconstituted chromatin with 0-2 eq. of H1-A532. d) Native PAGE analysis of Scal digested chromatin with 0-2 eq. H1.1. Green: Atto647N, red: GelRed.
Figure S5. Expression, purification and labeling of HP1α, HP1β and HP1γ. a) Sequence alignment of the HP1 subtypes HP1α, HP1β and HP1γ. b) Scheme of fluorescent labeling of HP1α, HP1β and HP1γ using split-intein mediated EPL. c) SDS-PAGE analysis before and after labeling of HP1α. d) Purification of HP1α-A532 by size exclusion chromatography. e) RP-HPLC of HP1α-A532. f) MS of HP1α-A532 (HP1α: calculated mass: 23200 Da, observed mass: 23202 Da). g) SDS-PAGE analysis before and after labeling of HP1β. h) Purification of HP1α-A532 by size exclusion chromatography. i) RP-HPLC of HP1β-A532. j) MS of HP1β-A532 (HP1β: calculated mass: 22390 Da, observed mass: 22394 Da). k) SDS-PAGE analysis before and after labeling of HP1γ. l) Purification of HP1γ-A532 by size exclusion chromatography. m) RP-HPLC of HP1γ-A532. n) MS of HP1γ-A532 (HP1γ: calculated mass: 21786 Da, observed mass: 21789 Da).
Figure S6. HP1α binding to mononucleosomes. a) 5% TBE polyacrylamide gel analysis of mononucleosomes detected by GelRed staining and emission of Atto647N. A minimal band of free DNA indicates proper mononucleosome formation. b) Time constants of the fast (τoff,1) and c) slow (τoff,2) dissociation process of HP1α from mononucleosomes. The slow time constant (τoff,2) was poorly defined as long binding events were rarely observed. Numbers indicate % amplitude. Errors bars: standard deviation (SD), n > 3 replicates. d) Agarose gel analysis of reconstituted wild-type octamers and H1 labeled arrays with 12x 197a DNA. e) Representative trace of a smTIRF experiment with HP1α and 12x 197a chromatin containing wild-type octamers and H1.
Figure S7. Histone H1.1 expression and H1.1, H2A fluorescent labeling. a) Size exclusion chromatography purification of Histone H1.1. b) Size exclusion chromatography purification of A532 labeled Histone H1.1. c) RP-HPLC of purified labeled Histone H1.1. d) ESI-MS of purified labeled Histone H1.1 (calculated mass: 22582 Da, observed mass: 22587 Da). e) RP-HPLC of ATTO 488 – labeled H2A (at position 110). f) ESI-MS of purified labeled Histone H2A (calculated mass: 14664 Da, observed mass: 14666 Da). g) Analysis of H1 content of chromatin fibers by step-wise bleaching analysis, demonstrating 8.2 H1/array for a177 chromatin and 11.3 H1/array for a197 chromatin.
Figure S8. Microscale Thermophoresis measurements. a) HP1α binding to a H3K9me3(1-14) peptide, fitting with a quadratic binding equation with a one-site binding model gives $K_D = 11.6 \mu M$. b) HP1β binding to a H3K9me3(1-14) peptide, fitting with a quadratic binding equation with a one-site binding model gives $K_D = 1.4 \mu M$. c) HP1γ binding to a H3K9me3(1-14) peptide, fitting with a quadratic binding equation with a one-site binding model gives $K_D = 4.1 \mu M$. 
Figure S9. Gel shifts with hinge mutant HP1α. a) Size exclusion chromatography purification of HP1α (hinge). b) RP-HPLC of purified HP1α (hinge). c) ESI-MS of purified HP1α (hinge) (calculated mass: 22136 Da, observed mass: 22137. d) EMSA for HP1α and HP1α (hinge) at the indicated concentrations, analyzed by polyacrylamide gel electrophoresis and imaged by fluorescence detection of Cy5 labeled DNA.
Figure S10. Full mathematical model of HP1 chromatin interactions. a) All reactions between the indicated states (for a list see Supporting Table 1) are reversible and governed by equilibrium (Supporting Table 2) and rate constants (Supporting Table 3). b) Ideal low pass filter for fast events. The post-processing is applied to mimic the low pass filtering effect of the microscope that integrates the intensity over a time $\tau \approx 0.05$ s. Events with a duration below this threshold are not taken into account. c) Histogram of the events with in log space with event cutoff (left) and d) without event cutoff (right). e-f) Comparison of the stochastic simulations of e) HP1$\alpha$ wt. with f) a simulation where the dimerization is inhibited i.e. the equilibrium constant of the dimer was increased by a factor of $10^3$. 
Figure S11. Boxplots of the populations obtained for each parameter by optimizing with the GA (168 distinct parameter sets). The horizontal lines denote the bounds for the parameter optimization.
Figure S12. Individual and global effects on the different model outputs. Plotted are the sensitivity indices as calculated by Sobol’s method (6) for the parameters with the largest global effect on the total bound concentration of HP1 (HP1 bound), the fast $\tau_{\text{off},1}$ and slow $\tau_{\text{off},2}$ dissociation times as well as the relative amplitude of the slow phase $A_2$. The individual sensitivity indices account for the sensitivity of the model output with respect to change only in a specific parameter, whereas the total sensitivity indices also accounts for the interaction effects of this parameters with all the other parameters.
Figure S13. Fold change of the model outputs in GSA. The tenfold change of the model outputs (HP1 bound, τ_{off,1}, τ_{off,2}, A2) is plotted vs the tenfold change of the most important model parameters. Data is given as blue dots. The slope of the red lines indicates the mean fold-change of the output parameters with respect to a fold-change in the input parameter. This correspond to the values depicted in Figure 5g.
**Figure S14. Expression of HP1α mutants in cells.** Confocal fluorescence images of NIH 3T3 cells transfected with mEos3.2-HP1α, mEos3.2-HP1α(AAAA) or mEos3.2-HP1α(hinge mutant) and overlayed with Hoechst staining. Scale bar, 5 μm.