Structural Plasticity in Human Heterochromatin Protein 1β

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Introduction

Proteins of the Heterochromatin Protein 1 (HP1) family are important regulators of chromatin structure and function in almost all eukaryotes. The human genome encodes three HP1 isoforms, hHP1α, hHP1β and hHP1γ with distinct sub-nuclear localization and potential activity [1]. HP1α and HP1β are mainly found at heterochromatin sites where they mediate chromatin condensation and gene silencing. HP1γ has also been found in euchromatin domains where it seems to be involved in the expression of active genes [2]. The pivotal role of HP1 proteins in genome regulation and their putative connection to the development of cancer [3] have motivated increasing efforts to understand the molecular basis of their biological activity.

HP1β is of particular interest as it is the only isoform essential for viability in mammals [4]. This protein has a multi-domain organization common to the whole HP1 family. A long weakly conserved hinge region links two globular modules, the chromo-domain (CD) and the chromoshadow domain (CSD) at the N- and C-terminal sides, respectively. Two additional highly charged regions constitute the N- and C-terminal tails. CD is a “histone post-translational modification (PTM) reader”. A binding pocket characterized by a conserved aromatic cage selectively discriminates the methylation states of Lys9 in histone H3 [5–7]. The trimethylated form (H3me3K9) of this chemical modification is one of the most studied epigenetic marks associated with gene silencing [8]. The NMR structure of CD [9] has revealed a globular shape made of a three-stranded anti-parallel β-sheet packed against a C-terminal α-helix. In the complex, the histone H3 peptide acquires an extended conformation and forms an intermolecular β-sandwich with CD [3]. The CSD has a similar fold with the key difference of two α-helices at the C-terminus that constitute an interface for HP1 dimerization [10]. The CSD dimerization interface provides an additional binding platform for diverse protein partners containing a common PXVXL motif [11]. For HP1α, it was shown that the C-terminal tail cooperates with CSD to discriminate the binding among different partners [12]. HP1, via the CSD, can bind proteins from different biological pathways such as transcriptional repression (KAP1) [10,13], chromatin assembly (CAF1) [14], nucleosome remodeling (ATRX) [15], nuclear lamina organization (LBR) [16] and DNA replication (ORC) [17]. The ability of CD and CSD to recruit protein partners from diverse biological networks makes HP1 a powerful molecular connector of different cellular pathways.

The hinge region contains a nuclear localization sequence and has the most variable amino acid sequence among human HP1 isoforms and HP1 from different species. It has been reported to be highly accessible to proteases [9] and it was suggested to be unstructured [10]. Chemical modifications on the linker, especially phosphorylation [18], influence HP1 localization, interaction and function in Drosophila melanogaster. Beyond the basic CD-CSD
connection function, the hinge region seems therefore of functional relevance in tuning HP1 activity. Moreover, for some HP1 isoforms, it can bind DNA [19,20]. Recently we showed that interactions of the hinge region and the N-terminal tail with DNA mediate the weak association of hHP1b to unmodified nucleosomes, thus providing an alternative mechanism of chromatin binding besides the specific recognition of methylated histone H3 by the CD [21]. Similarly, the N-terminal tail is weakly conserved and contains residues available for post-translational modifications that can modulate HP1 binding to chromatin [22].

The bifunctional and dimeric nature of HP1 appears to be the key for its biological function. While structures have been deposited for the isolated CD and CSD, no detailed information is available for the full-length protein. In particular, little information about the conformational propensities and dynamics of the hinge region and the long N- and C-terminal tails is available. We therefore used NMR spectroscopy to investigate the structural and dynamical properties of both the non-globular and folded domains in full-length human HP1b. Our study reveals both inter-domain motions and internal dynamics in CD and CSD that can promote complex formation with different binding partners.

Results and Discussion

hHP1b Populates an Extended Ensemble

To obtain insight into the global conformation of the 184-residue full-length hHP1b in solution we investigated its hydrodynamic behaviour. Dynamic light scattering measurements resulted in a well-defined monodisperse peak with a hydrodynamic radius of 4.4±0.1 nm (figure 1A). The value is in agreement with the results from pulse field gradient NMR (figure 1B) and indicates that the protein does not assume a compact state in solution.

SAXS experiments were then performed to obtain information about the size and shape of the conformational ensemble populated by hHP1b in solution. For hHP1b at 1.0–5.0 mg/mL concentrations, we obtained a MW of 40±4 kDa and an excluded volume of the particle of 85±5 nm³. The data confirmed that the protein is present in a dimeric state within the range of tested concentrations. Values of the radius of gyration (Rg) and maximal size of the particle (D max) (4.7±0.2 nm and 15.5±0.5 nm, respectively) as well as the long tail of the distance distribution function p(r) (figure 1C, inset) pointed to a relatively elongated shape of hHP1b. To take into account the dynamic nature of hHP1b, SAXS data were also subjected to the ensemble optimization method, which is particularly suited for flexible multi-domain proteins as it accounts for multiple configurations of disordered linkers [23]. Comparison of the Rg distribution derived from the optimized ensembles with that obtained from a pool of randomly generated models is shown in figure 1D. The Rg distribution of the selected ensemble is nearly as broad as the one from the initial random pool, with the maximum shifted towards longer distances, indicating that the hinge region is highly flexible with a preference for more extended conformations.

CD and CSD do not Form Stable Inter-domain Contacts in Full-length hHP1b

The 1H-15N TROSY-HSQC spectrum of 15N-perdeuterated hHP1b (figure 2A) highlights its multi-domain nature. Besides the two structurally related CD (21–71) and CSD (110–170), the remainder of the protein, which accounts for more than one third of the sequence, has a non-globular character with a high percentage of charged residues. The non-globular nature of the hinge region and the N- and C-terminal tails lead to severe signal...
overlap between 8 and 8.5 ppm in the 1H dimension and a large dynamic range of peak intensities. In particular, the clustering of lysine and glutamate residues in the sequence complicates the sequence-specific resonance assignment by conventional triple-resonance techniques, thus precluding the structural characterization of the full-length protein so far. By using automated projection spectroscopy (APSY) experiments on hHP1β (2–185), in combination with the use of protein fragments, we were able to assign most of the hHP1β backbone signals [21].

Analysis of the average 1H-15N chemical shift differences between CD in hHP1β (2–185) and the isolated CD (residues 2–79) (figure 2B, upper panel), and between CSD in hHP1β (2–185) and the isolated CSD (residues 107–176) (figure 2B, lower panel), suggested that the structures of both domains are retained in the full-length protein. Moreover, the lack of chemical shift changes except at the termini of the domains excludes significant inter-domain contacts between CD and CSD, in line with the conclusions reported by Brasher et al. [10]. The mutual independence of the two domains was further supported by 1H-15N residual dipolar coupling (RDC) analysis. Alignment tensors calculated from experimental RDCs and 3D structures had different magnitudes: 9.67 Hz for CD and 29.54 Hz for CSD.

Structural Propensities in the Non-globular Domains

The conformational properties of the hinge region and of the N- and C-terminal tails were addressed by analysis of NMR secondary chemical shifts, that are highly sensitive probes of local conformation [24]. For most residues in the tails and the hinge region the absolute values of Cα secondary chemical shifts were below 0.3 ppm (figure 2C and, for the combined secondary chemical shifts see figure S1), supporting their intrinsically disordered nature. In the hinge region a weak helical tendency expands the α-helix of the CD beyond residue 70 up to 73. In addition, a continuous stretch of small negative Cα secondary shifts in proximity to residue 20 points to a propensity for extended conformation in the N-terminal tail. The extended conformation in this region might be favored by the high density of charged residues and may be functionally relevant for formation of the β-sheet sandwich between strand β1 of the CD and the induced β-strand in methylated histone H3. In line with this hypothesis, β1 is N-terminally extended in the dmHP1CD-methylated histone H3 complex [3].

Modular Dynamics of hHP1β

To obtain insight into the dynamic properties of hHP1β, we performed 15N spin relaxation measurements [25]. In the globular CD and CSD the average R1 (R2) relaxation rate was 1.24±0.11 s⁻¹ (1.48±0.17 s⁻¹) and 0.71±0.11 s⁻¹ (37.01±5.54 s⁻¹), respectively (figures 3A–B). In addition, average
The average uncertainty threshold is estimated as 0.1 ppm for the non-
globular parts, 0.2 ppm for CD and 0.3 ppm for CSD due to different
relaxation properties of these regions. In CD and CSD the presence of
segments of continuous positive and negative secondary Cα shifts
identifies, respectively, the helix and β-sheet elements in agreement
with the secondary structure, schematically shown at the top, as
defined in IAP0 [9] and 1D21 [10] PDB files and definition by DSSP [58].
Blue (extended) and green (helical) stripes highlight the additionally
identified secondary structure propensities.

doi:10.1371/journal.pone.0060887.g002

hetNOE values of 0.70±0.04 for CD and 0.64±0.13 for CSD
(figure 3C) indicated that the protein backbone is very rigid in the
two domains. From the $R_2/R_1$ ratios average $\tau_c$ values of
10.68±1.45 ns and 23.47±2.41 ns were calculated for the CD
and CSD, respectively. Residue-specific $\tau_c$ values in the C-
terminal α-helical region of CD were consistently higher than the
average $\tau_c$ of the domain (figure 3E), indicating that the global
motion of CD is anisotropic. Also CSD shows features of anisotropic
global motion: the product of $R_1$ and $R_2$ ($R_1R_2$)
efficiently removes the anisotropy that causes a large distribution
of $R_2/R_1$ [26] (figure S2A).

To characterize the anisotropy of the global motion of CD and
CSD within full-length hHP1β, we determined the rotational
diffusion tensor of the two domains using the program ROTTIF
[27] (Table 1). The best fit for CD was obtained with the axially-
symmetric diffusion model ($Q = 0.35$), resulting in average values for
$\tau_c$ and anisotropy of 10.19±1.18 ns and 2.02±0.38, respectively.
The orientation of the diffusion tensor of CD is illustrated in figure 3F. The C-terminal helix of CD is aligned
nearly parallel to the z-axis of the diffusion tensor, providing a rationale for its large $\tau_c$ values. The CSD data were best fit using
the fully-anisotropic diffusion model ($Q = 0.37$) (Table 1 and
figure 3F) with average $\tau_c$ of 24.57±4.95 ns. The anisotropy and
rhombicity of the diffusion tensor were 3.55±1.41 and 0.37±0.13,
respectively.

Next we compared the rotational correlation times for the two
global domains in full-length hHP1β to values predicted for the
isolated domains using the program HYDROPRO [28]. HY-
DROPRO estimated the $\tau_c$ values of the isolated CD and the
isolated dimeric CSD as 4 ns and 10 ns, respectively. Thus,
rotational correlation times in the isolated domains are more than
a factor of two smaller than in full-length hHP1β. The strong
increase in $\tau_c$ for the two globular domains in the full protein
points to the presence of motional coupling. With the apparent
lack of any persistent structure in the intervening hinge region or
any stable contact between the two domains, this motional
coupling seems to be mainly contributed by hydrodynamic
interaction: in the spatial proximity of the other domains, tumbling
of each domain is slowed down in comparison with the isolated
state as a consequence of a stronger resistance to accompanied
solvent displacement. The presence of hydrodynamic coupling has
been demonstrated for several multi-domain proteins, for example
two-domain model protein [29], and appears to be a generic feature of modular proteins with flexible linkers.

According to $^{15}$N spin relaxation rates the backbone outside of
CD and CSD is highly mobile (figure 3). The high mobility of
these regions precludes the analysis of their dynamic properties
through separation of global and internal motions. Therefore, we
analyzed $^{15}$N spin relaxation rates by reduced spectral density
mapping to describe protein NH vector motions at time scales
corresponding to three different frequencies $\omega_0$, $\omega_{68}$ and
0.87$\omega_{68}$ [30] (figure 3D and figure S3). The N- and C-terminal tails as well as
the hinge region showed much smaller $J(0)$ and bigger $J(0.87\omega_{68})$ than the two globular domains, indicating a very slow decay of

their spectral density function characteristic of fast tumbling
molecules with high internal mobility. The $J(0.87\omega_{68})$ profile
demonstrated that the N-terminal tail experiences smaller internal
dynamics when compared to the hinge region, with the C-terminal
tail being the most flexible part among the disordered domains
(figure S3A). A reduced mobility on the pico-to-nanosecond time
scale in the N-terminal tail is further supported by an average
hetNOE value of 0.10±0.00. Interestingly, the N-terminal tail is
the most rigid at its beginning up to residue L14, where the hetNOE
reaches a local minimal value of 0.06, and then starts to rise
afterwards (figure 3C).

Internal Dynamics in the Binding Pockets of CD and CSD

Internal dynamics play a key role for molecular recognition
[31]. Therefore, we investigated the internal motions in CSD and
CD, the two domains that mediate binding of HP1 to a wide
variety of protein partners. In CSD, residues E165 to W170
showed the largest $^{15}$N linewidths (figure 4A). Besides a possible
local effect due to H171, the observed signal broadening points to
slow conformational rearrangements. Importantly, this region is
involved in interactions with different proteins, suggesting that this
conformational plasticity may support interaction with PXVXL
motif and its variants in CSD binding partners.

In CD, which is essential for binding to methylated histone H3
in chromatin, strong signal broadening (figure 4A) and slow
chemical exchange (slower than ~100 μs) (figure 4B) was observed
for H75, close to the hinge region, and K33, which is located in
the short loop between β-strands β1 and β2. The mobility of the
loop comprising K33 was further supported by a prominent
exchange contribution to the $^{15}$N $R_2$ relaxation rate and distinct
temperature sensitivity of its peak intensity (figure S4). Besides
K33, peak intensities of four residue stretches in CD (L27–D28;
E56–C60; L63–I64; Q69–S70) showed pronounced temperature
sensitivity that points to a change in the flexibility of these regions
as a function of temperature (figure S4C).

N57 to C60 of the CD Populate Binding-competent
Conformations Prior to Binding to Methylated Histone
H3

How is the structure of CD and CSD in solution affected by the
observed conformational dynamics? To address this question,
we measured residual dipolar couplings that describe the orientation
of internuclear vectors and are therefore highly sensitive probes of
structure and dynamics [32]. Experimental $^{1}H-^{15}N$ RDCs of CSD
in full-length hHP1β correlated well with values back-calculated
from the X-ray structure of CSD (Pearson’s correlation coefficient
$R = 0.97$ and dipolar coupling quality factor $Q = 0.15$) (figure 5A).
The fit of experimental RDCs to the X-ray structure of the CD
was of much lower quality with $R = 0.89$ and $Q = 0.44$. In
particular, six RDC values deviated significantly. Their removal
improved the quality of the fit to $R = 0.98$ and $Q = 0.18$ (figure 5B).
The six RDC values belong to the two residue stretches V32–K33
and N57–C60, which experience a wide range of internal motions
(figure 4 and figure S4).

Inspection of the 3D structure of CD reveals that N57–C60 is
part of the intervening region between strand β3 and helix α1
(figures 5B and C). In the X-ray structure of the isolated, unbound
CD, a helical turn following the β3-strand has been assigned to
residues E56–L58 (PDB code: 3FU2 [33]). However, NMR
secondary chemical shifts (marked in figure 2G and figure S1A–C)
rather point to a propensity for extended conformation in the
L58–D59 region. Thus, crystallization may have stabilized the β3–
α1 intervening region in a conformation that is weakly represented

Conformational Characterization of hHP1β
Figure 3. Backbone dynamics probed by 15N-relaxation rates. A, B, C. 15N spin-relaxation rates for 15N-perdeuterated hHP1 measured at a proton Larmor frequency of 500 MHz at 298 K. Residues with severe peak overlap or insufficient signal-to-noise ratio were excluded. Rs (A), R2 (B) and steady-state 1H-15N heteronuclear NOE (C) are shown along the protein sequence. The R2 rates were derived from R1 measurements upon correction for the off-resonance tilted field as described in the Methods. D. Graphical analysis of reduced spectral density mapping. The solid line represents the theoretical function of J(νN) versus J(0) assuming a rigid single Lorentzian motion. Experimental values are plotted as points with labels specific for each hHP1 domain. E. The rotational correlation time t_c, determined for each residue from the R2/R1 ratio, is shown as a function of residue number. F. Illustration of the diffusion tensor of CD (left, axially symmetric) and CSD (right, fully anisotropic) in full-length hHP1. The x-, y- and z-axes are shown as half-axes only for the fully anisotropic tensor. Pictures were prepared using MolMol [57].

doi:10.1371/journal.pone.0060887.g003

Conformational Characterization of hHP1
in solution providing a rationale for why N57–C60 coordinates from the X-ray structure are not compatible with experimental RDCs. Upon interaction with methylated histone H3, residues L58–D59 are stabilized in a well-ordered intermolecular β-sandwich, as shown for CD of hHP1α (PDB code: 3F2U [6], displayed in figure 5C in comparison with 3F2U structure), hHP1β (PDB code: 1GUW [7]) and dmHP1 (PDB code: 1KNE [5]). We therefore tested whether our experimental 1H-15N RDCs better fit to those back-calculated from the available crystal structures of bound CD (figure 5D–E and figure S1D). A best fit of structural plasticity of hHP1 promotes its activity in binding and connecting a variety of proteins related to epigenetic events.

**Methods**

**Production of Recombinant hHP1β Proteins**

The following hHP1β sequences (GenBank NM_001127228; UniProt accession number P83916) were cloned into a pET16b expression vector (Novagen) modified with an N-terminal TEV-protease-cleavable His-tag site: full-length (2–185), CD (2–79), CD (19–79) and CSD (107–176). Details of plasmid constructs are available upon request.

hHP1β (2–185) protein for SAXS and DLS experiments and isotope-labeled hHP1β (2–185), CD (2–79), CD (19–79) and CSD (107–176) proteins for NMR measurements were expressed and purified as described previously [21]. An additional size-exclusion chromatography on a Superdex200 column (GE Healthcare) was performed for SAXS and DLS samples.

All samples were finally prepared in 20 mM sodium phosphate (pH 6.5), 50 mM NaCl, 2 mM DTT and 0.02% NaN3 and filtered before the experiments.

The H3K9me3 1–15 peptide was obtained from a synthetic peptide H3[1–15]K9C where a Cys residue replaced the Lys at position 9. This modification allowed the site-specific installation of a tri-methyl lysine analog by Cys-alkylation reaction using (2-bromoethyl)-trimethylammonium bromide (Sigma). Details of the alkylation reaction were described previously [21,42].
Dynamic Light Scattering

Dynamic light scattering measurements were performed on a Wyatt DynaPro Titan instrument (Wyatt Technology, California) at 303 K on a sample containing 0.1 mM hHP1β.

SAXS Experiments

Synchrotron radiation X-ray scattering data were acquired on the EMBL X33 beamline at the DORIS III storage ring, DESY, in Hamburg [43]. Experiments were carried out at 283 K, with protein concentrations of 1.0, 2.0 and 5.0 mg/mL. A pixel detector PILATUS 1 M (DECTRIS, Switzerland) at sample-detector distance 2.7 m and wavelength $\lambda = 0.15$ nm, covering the momentum transfer range $0.12 \leq s < 4.9$ nm$^{-1}$ ($s = 4\pi \sin (\theta)/\lambda$, where $2\theta$ is the scattering angle), was employed. Data were processed with the ATSAS program package [44]. For each measurement, four 30 sec exposures were compared to check for radiation damage. No radiation effects were observed. The data were averaged after normalization to the intensity of the incident beam. The signal of the buffer was subtracted and the difference data were extrapolated to zero solute concentration by standard procedures.

Data analysis was performed with the program PRIMUS [45]. The forward scattering $I(0)$ and the radius of gyration $R_g$ were obtained using the Guinier approximation, assuming that at very small angles ($s < 1.3/R_g$) the intensity is represented as $I(s) = I(0) \exp \left(- (1/3) (R_g s)^2 \right)$. These parameters were also computed from the entire scattering patterns with the program GNOM [46], giving the distance distribution function $p(r)$ and the maximum particle dimension $D_{max}$. The MW was obtained comparing the forward scattering to that from reference solutions of bovine serum albumin (66 kDa). Possible flexibility of hHP1β was assessed by the ensemble optimization method (EOM) [23], which allows for coexistence of different conformations contributing to the experimental scattering pattern. These conformers were selected by a genetic algorithm from a pool containing $10^5$ randomly generated models. Genetic algorithms were employed to find the subsets of the conformers that fit the experimental data best. The obtained subsets were analyzed to yield the $R_g$ distributions in the optimal ensembles.

NMR Experiments

NMR spectra for sequence-specific backbone resonance assignment of full-length hHP1β were acquired at 303 K on 600, 700 and 800 MHz Bruker spectrometers equipped with triple resonance cryogenic probes. A combination of standard TROSY-based 3D [47] and APSY 5D, 6D and 7D experiments [48] was used. $^1$H-$^{15}$N assignments were transferred to the CD (2–79), CD (19–79) and CSD (107–176) and verified by additional standard TROSY-based 3D experiments when required.

Secondary chemical shifts were calculated based on the random coil chemical shifts predicted by the Neighbor Corrected Structural Propensity Calculator [49] and corrected for the $^2$H isotope shift. The 4,4-dimethyl-4-silapentane-1-sulfonic acid (0.0 ppm) was used for chemical shift referencing. Consensus chemical shift index (CSI) values were obtained using the RCI webserver [50].

Pulse field gradient stimulated-echo diffusion experiments were performed at 303 K on a sample containing 0.12 mM of hHP1β. $Q$ was defined according to [51]. Diffusion gradient length (little delta) and diffusion delay (big delta) were set to 3 ms and 200 ms, respectively. Gradient calibration was achieved by measuring the diffusion of residual HDO in 99.8% D$_2$O at 298 K.

Figure 4. Internal dynamics. A. $^{15}$N linewidths of perdeuterated hHP1β as a function of residue number, measured from a TROSY-HSQC recorded at 700 MHz and 303 K. B. $R_{ex}$ values of CD in full-length hHP1β as a function of residue number. C. Comparison of steady-state $^1$H-$^{15}$N heteronuclear NOE values of CD in full-length hHP1β in the free state (black circles) with those of CD in full-length hHP1β in complex with the H3K9me3 peptide (1–15) at a molar ratio of 1:4 (white circles). Both measurements were performed at 298 K, 600 MHz proton Larmor frequency, 5 s recycle delay, on a 0.3 mM $^{15}$N-perdeuterated hHP1β sample.

doi:10.1371/journal.pone.0060887.g004
15N relaxation experiments were performed at 600 MHz, 298 K, using 0.7 mM 15N-perdeuterated hHP1b sample. 15N longitudinal relaxation rates (R1) were measured using relaxation delays of 8, 30, 60, 100, 180, 320, 500, 800 and 1200 ms. 15N transverse relaxation rates ("total R2") were measured using relaxation delays of 8, 16, 24, 34, 52, 86, 120, 180 and 240 ms. 15N longitudinal relaxation rates in the rotating frame (R1r) were measured in a near-resonance mode with relaxation delays of 20, 40, 60, 80, 100, 120, 140 and 180 ms and a spin-lock field strength of 2.5 kHz. R1, R2 and R1r relaxation rates were determined from the best single exponential fit to the experimental intensity data. Effective R2 rates were derived from the relation: R2 = (R1p - R1 cos^2 θ)/sin^2 θ, where θ = tan^-1 (v1/Ω), v1 is 15N spin-lock field strength (in Hz) and Ω is the resonance offset from the spin-lock carrier (Hz). Rnx values were determined as the difference between the "total R2" rates and the effective R2 rates derived from R1r values. Steady-state 1H-15N heteronuclear nuclear Overhauser enhancement (NOE) was measured with a total recycle delay of 10 s. NOE values were calculated by the ratio of the peak intensities between saturated and reference spectra. With the reduced spectral density mapping, the relaxation rates R1 and effective R2 and hetNOE values were transformed to spectral densities at zero frequency J(0), at 15N frequency J(ωN) and at the effective 1H frequency J(0.87ωH) [30]. The theoretical relation between J(ω) and J(0) was obtained with the assumption of a single Lorentzian motion, as J(ω) = J(0)/(1 + 6.25ω^2J(0)^2).

Figure 5. Analysis of CD and CSD structures by RDCs. A. Correlation between experimental 1H-15N RDCs and values back-calculated from the 3D structure of CSD of hHP1b (PDB code: chain A, 2FMM [59]) using singular value decomposition method. The analysis comprised 36 residues, spanning R111–F163, that are marked in green on the model structure. B. Correlation between experimental 1H-15N RDCs and values predicted from the 3D structure of CD of hHP1b (PDB code: 3F2U). The 45 residues analyzed, spanning Y21–Q69, are marked in green on the model structure. Outliers are marked in orange. In A and B, residues affected by overlap or with insufficient signal-to-noise ratio were excluded from the analysis. C. Alignment between free CD of hHP1b (PDB code: 3F2U) and bound CD of hHP1b (PDB code: 3FDT). The β3-α1 intervening region that includes the N57–C60 residues (outliers in the RDCs analysis) is shown in red for free CD and in blue for bound CD. The histone peptide of 3FDT PDB is coloured in light blue. D. Correlations between experimental 1H-15N RDCs and values predicted from the 3D structure of free CD of hHP1b (PDB code: 3F2U) (red) or from bound CD of hHP1b (PDB code: 3FDT) (blue). Residues showing a remarkably different correlation among the compared structures are highlighted as filled circles. E. Comparison of the fitting quality parameters, Q and R, from analysis of experimental RDCs using different X-ray structures of CD.

doi:10.1371/journal.pone.0060887.g005
For rotational diffusion analysis the program ROTDIFF7 [27] and the crystal structures of the isolated CD (PDB code: 3F2U) and CSD (PDB code: chain A, 2FMM) were used. For each of the two domains, only residues within the main secondary structural elements were included in the analysis. Three diffusion models, isotropic, axially-symmetric and fully-anisotropic were utilized for calculation of rotational diffusion tensors, and the F-test was applied to evaluate if the improvement of the fit by the more complex model was statistically significant. 500 Monte-Carlo calculations were performed for uncertainty analysis. The R$_2$ used in this analysis was the exchange-free R$_2$ derived from the $^{15}\text{N}$-$^{1}\text{H}$ dipole-dipole/$^{15}\text{N}$ chemical shift anisotropy cross-correlated relaxation rates ($\eta_0$) according to $R_2^0 = \kappa_1 \eta_0 + 1.3\tau$, where $\tau = (\text{NOE} - 1)R_1^0/\gamma_B^2$. The results with the R$_{1p}$-based effective R$_2$ were consistent with these results, but had larger uncertainty range for the CSD domain. R$_{1p}$-based relaxation dispersion experiments for K33 were performed on a sample containing 0.48 mM of $^{15}\text{N}$-labelled CD(19–79) at 303 K and a proton Larmor frequency of 600 MHz. R$_{1p}$ rates were obtained at $^{15}\text{N}$ spin-lock field strengths of 200, 600, 1000 and 1500 Hz centered on the $^{15}\text{N}$ resonance of K33, using six relaxation time delays between 5 and 190 ms.

One bond $^{1}$H-$^{15}$N coupling constants of protein backbone were obtained using a TROSY-HSQC interleaved experiment recorded on a 900 MHz NMR spectrometer equipped with a cryoprobe. The sample contained 0.2 mM of $^{15}\text{N}$-perdeuterated hHP1B. The temperature was 293 K. Partial alignment was achieved using a dilute liquid crystalline phase of 5% C12E5/ hexanol (Sigma) [54] resulting in 16 Hz of quadrupolar splitting. RDC data were analyzed using the PALES software [55]. Alignment tensors calculated from experimental RDCs and 3D structures (PDB codes: 3F2U and 2FMM, chain A) had magnitudes of 9.67 Hz for CD and 29.54 Hz for CSD with rhombicities of 0.09 and 0.12, respectively.

NMR data were processed with NMRPipe [56] and analyzed with Sparky (T. D. Goddard and D. G. Kneller, University of California, San Francisco). Origin® and Wolfram Mathematica® were used for mathematical and graphical analyses. CD and CSD PDBs were visualized with MolMol [57] and PyMOL (The PyMOL Molecular Graphics System, Version 1.1r1, LLC).

Supporting Information

Figure S1 Secondary chemical shifts and RDCs analysis. A, B. Combined Cz+CO (A) and Cz-Cβ (B) secondary chemical shifts as a function of residue number. The average uncertainty threshold is estimated 0.1 ppm for the non-globular parts, 0.2 ppm for CD and 0.3 ppm for CSD. Blue (extended) and green (helical) stripes highlight the additionally identified secondary structure propensities. The Cz-Cβ secondary chemical shifts have the advantage that they are not affected by any possible imperfection in $^{13}$C chemical shift referencing. C. Consensus chemical shift index (CSI) values for CD from RCI analysis. D. Correlations between experimental $^{1}$H-$^{15}$N RDCs and values predicted from the atomic coordinates of free CD of hHP1B (PDB code: 3F2U) (red) or from bound CD of hHP1B (PDB code: 5T2D) (green). Residues showing a remarkably different correlation among the compared structures are highlighted as filled circles. (TIF)

Figure S2 Analysis of $^{15}$N spin-relaxation data. A. The anisotropy of global motion can be detected in the plot of R$_2$/R$_1$ versus $^{15}$N chemical shift referencing. The condition is met for CSD where the large distribution of R$_2$/R$_1$ values along constant R$_2$/R$_1$ denotes an anisotropic rotational diffusion. B. Transverse cross-correlated relaxation rates ($\eta_0$) of hHP1B as a function of residue number. (TIF)

Figure S3 Reduced spectral density mapping. A, B, C. hHP1B $^{15}$N spin-relaxation rates were analysed by reduced spectral density mapping. Spectral densities at the effective proton frequency, J[0.87ω_ν] (A), at the $^{15}$N frequency J[ω_ν] (B) and at zero frequency J(0) (C) are shown as a function of residue number. (TIF)

Figure S4 The slow motion of K33. A. Relaxation dispersion profile of K33 in $^{15}$N-labelled CD (19–79). Residue L40 serves as control. B. Selected regions of the $^{1}$H-$^{15}$N TROSY-HSQC spectrum of hHP1B at 283 K (blue), 290 K (light blue), 298 K (green), 303 K (yellow), and 310 K (red). Spectra at different temperatures are displayed with equal counter level. K33 shows strong signal broadening at increasing temperature. C. Change of $^{1}$H-$^{15}$N signal intensity with increasing temperature. The signal intensities at 290 K (blue), 298 K (green), 303 K (yellow), and 310 K (red), relative to the intensity at 283 K, are shown. (TIF)

Acknowledgments

We thank Peter Konarev and Dmitriy Svergun of the European Molecular Biology Laboratory Outstation, c/o DESY, in Hamburg, Germany, for the access to the EMBL X33 beamline and support in analysis and interpretation of SAXS data.

Author Contributions

Supervised the project: MZ. Conceived and designed the experiments: FM MZ. Performed the experiments: FM NR-G SN WF. Analyzed the data: FM NR-G. Wrote the paper: FM MZ.

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