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Structural and dynamic studies of chromatin by solid-state NMR spectroscopy

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Abstract

Chromatin is a complex of DNA with histone proteins organized into nucleosomes that regulates genome accessibility and controls transcription, replication and repair by dynamically switching between open and compact states as a function of different parameters including histone post-translational modifications and interactions with chromatin modulators. Continuing advances in structural biology techniques including X-ray crystallography, cryo-electron microscopy and nuclear magnetic resonance (NMR) spectroscopy have facilitated studies of chromatin systems, in spite of challenges posed by their large size and dynamic nature, yielding important functional and mechanistic insights. In this review we highlight recent applications of magic angle spinning solid-state NMR – an emerging technique that is uniquely-suited toward providing atomistic information for rigid and flexible regions within biomacromolecular assemblies – to detailed characterization of structure, conformational dynamics and interactions for histone core and tail domains in condensed nucleosomes and oligonucleosome arrays mimicking chromatin at high densities characteristic of the cellular environment.

Keywords: gene regulation; nucleosome; histone; MAS solid-state NMR; dynamic nuclear polarization

Introduction

Eukaryotic DNA is organized into chromatin, a highly conserved complex that controls essential genome functions. The basic unit of chromatin is the nucleosome (Figure 1), which contains ~147 bp of DNA wrapped nearly twice around a histone octamer complex containing two copies each of histones H2A, H2B, H3 and H4 [1,2]. Human genomic DNA is wrapped into ~30 million nucleosomes, which are connected by linker DNA varying between ~20-70 base pairs (bp) in length [3,4] and compact into dynamic higher order structures [4–8] to regulate genome accessibility and control transcription, replication and repair [6–10].

Chromatin regulates gene expression by converting between open active euchromatin and compact repressive heterochromatin, with dynamic switching between chromatin states required for the expression of a gene to change [4–8]. Chromatin function is modulated by numerous covalent histone post-translational modifications (PTMs), including acetylation and methylation of lysine residues and phosphorylation of serines and threonines [11,12]. The majority of PTM sites are located on the positively charged N-terminal tail domains [13–15], which extend out of the nucleosome and range from approximately 15 to 35 amino acid (aa) residues in length for the different histone proteins [2]. Histone tail PTMs control chromatin compaction and act to recruit histone reader proteins that recognize specific PTMs [6,13–18] or combinations of PTMs constituting the "histone code" [13,19,20]. Additionally, multiple PTMs in the globular histone domains within the nucleosome core, which modulate DNA unwrapping and breathing, nucleosome stability and transcription factor binding to nucleosomal DNA, have been identified [20–24].

Atomic resolution structures of the relatively rigid domains for *in vitro*-assembled nucleosomes, oligonucleosome arrays and their numerous complexes with chromatin modulators

have been determined by X-ray crystallography and, more recently, cryo-electron microscopy (cryo-EM) [25–28], yielding important insights into the function and mechanisms of chromatin. These structural biology techniques, however, are not able to visualize the histone N-terminal tails due to missing density or to directly probe biomacromolecular motions occurring on timescales of microseconds to seconds [29], which appear to play key roles in chromatin function [7,8,25,30,31]. In contrast, nuclear magnetic resonance (NMR) spectroscopy – in solution and the solid state – is ideally suited toward atomic level analysis of protein conformation, conformational dynamics over a wide range of timescales, and interactions for both structured and conformationally flexible domains [29,32–36].

In the context of chromatin, a multitude of solution NMR studies targeting histone N-terminal tails in mononucleosomes have been performed [37,38]; overall, data emerging from these experiments, complemented by results of atomistic microsecond timescale molecular dynamics (MD) simulations [39], indicate that histone tail domains within nucleosomes are conformationally dynamic yet engage in transient, fuzzy complex type interactions [40] with nucleosomal DNA [37,38]. While considerably more challenging due to the relatively large size of the nucleosome particle (~200 kDa), solution NMR approaches based on site-specific incorporation of $^{13}CH_3$ methyl groups on an otherwise highly deuterated background [41] can also be employed and these methods have enabled characterization of the structure, conformational dynamics and interactions for the globular histone core as well as nucleosomal DNA [25,38,42]. On the other hand, high-resolution structural and dynamic studies of condensates of nucleosomes and nucleosome arrays with effective sizes in the multi-megadalton regime under conditions that correspond to typical concentrations of chromatin in cells (~100-200 mg/mL) [43] fall outside the realm of solution NMR spectroscopy. Instead, such studies may

be pursued by magic-angle spinning (MAS) solid-state NMR, which is subject to few limitations related to sample solubility, molecular size or degree of order, as demonstrated in recent years for a broad range of large biomacromolecular complexes and assemblies [35,36] including chromatin [42,44]. By utilizing multidimensional radiofrequency pulse sequences that rely on through-space dipolar coupling or through-bond J-coupling based magnetization transfers under MAS [35,36], this technology facilitates, in principle, the selective observation of NMR signals corresponding to all backbone and side-chain atoms located in relatively rigid and highly conformationally flexible protein domains, respectively, thereby enabling comprehensive investigation of local structure and dynamics at specific protein sites.

In this review we highlight recent studies of condensed nucleosomes and nucleosome arrays mimicking chromatin at physiological concentrations by multidimensional MAS solid-state NMR techniques, including studies aimed at the detailed analysis of structure, conformational dynamics and interactions for the nucleosome core and histone tail domains as a function of different chromatin parameters as well as emerging applications to the characterization of complexes with chromatin-binding proteins.

Chromatin samples for solid-state NMR

The preparation of *in vitro* samples of nucleosomes and nucleosome arrays suitable for analysis by MAS solid-state NMR has been discussed at length in comprehensive recent reviews [42,44] and is only briefly summarized here. These samples are generally prepared by reconstituting appropriately isotope enriched (e.g., ^{2}H,^{13}C,^{15}N) recombinant histone proteins with purified DNA, generated by synthetic or recombinant methods, corresponding to a high-affinity nucleosome positioning sequence (NPS) such as Widom 601 [45] with possible linker DNA

overhangs (e.g., ~10-20 bp) or to multiple tandem NPS repeats, typically on the order of 10 to 20, separated by linker DNA (Figure 2).

For nucleosome arrays distinct chromatin conformational states can be generated in the presence of varying concentrations of cations corresponding to different levels of DNA charge neutralization, with divalent cations such as Mg^{2+} added as $MgCl_2$ (or other multivalent cations) being particularly effective in this regard [46]. Namely, in the absence of Mg^{2+} extended "beads-on-a-string" fibers that model open chromatin are obtained, which can be readily sedimented by ultracentrifugation [47], especially for arrays larger than ~10 nucleosome units, and transferred to a MAS solid-state NMR sample holder (rotor) for subsequent spectroscopic analysis [48]; low Mg^{2+} concentrations (~1-2 mM) generally result in folded fibers, while Mg^{2+} concentrations significantly higher than 2 mM yield large aggregates of fibers displaying an extensive network of interactions between nucleosome subunits located within different fibers [46] (Figure 2). Interestingly, in specific buffers containing combinations of Mg^{2+} and K^+ ions nucleosome arrays have been found to form liquid-liquid phase separated condensates [46,49–51], which are also amenable to characterization by MAS solid-state NMR methods. To generate condensates of mononucleosomes for solid-state NMR studies, both low (2 mM) Mg^{2+} concentration combined with ultracentrifugation over an extended period [52,53] and precipitation using higher (20 mM) Mg^{2+} concentration [54] have been used, with the former approach resulting in sediments devoid of regular packing or long-range ordering of nucleosomes expected for high Mg^{2+} concentrations [53].

Structural and dynamic studies of histone tails by solid-state NMR

As noted above, NMR studies of mononuclosomes in solution indicate that histone N-terminal tail domains form dynamic fuzzy complexes with nucleosomal DNA [37,38]. While early studies of histone H3 tails within condensed nucleosome arrays based on backbone amide hydrogen/deuterium exchange detected by solution NMR suggested that histone tails may become immobilized and structured within the chromatin environment [55], direct J-based MAS solid-state NMR experiments targeting H3 and H4 tails in nucleosome arrays as a function of degree of condensation, achieved by varying the Mg^{2+} concentration in the 0-5 mM range, revealed that these domains actually retain significant conformational flexibility even in compact chromatin states [48].

These initial MAS solid-state NMR studies were performed at a moderate magnetic field strength and relied on ^{13}C and ^{15}N detection, offering somewhat limited spectral resolution and sensitivity and precluding residue-specific analysis. With increased availability of high and ultrahigh field magnets and MAS probes optimized for ^{1}H detection, however, high-quality 2D and 3D correlation MAS solid-state NMR spectra of histone tails in condensed nucleosomes and nucleosome arrays can nowadays be routinely recorded (Figure 2), which permits quantitative site-resolved measurements of NMR chemical shifts and spin relaxation rates enabling more comprehensive analyses of histone tail conformation, dynamics and interactions in chromatin. In one recent MAS solid-state NMR study of H3 tails in nucleosome arrays with DNA linkers of different length ranging from 15 to 60 bp, backbone ^{15}N spin relaxation rates were determined and used to evaluate residue-specific rotational correlation times [56]. Comparison of these data with results of analogous solution NMR measurements for 147 bp nucleosomes and nucleosomes containing two flanking 20 bp linker DNA overhangs [56,57] revealed considerable attenuation

of H3 tail dynamics in the arrays irrespective of the specific linker DNA length relative to nucleosomes with or without DNA linkers, consistent with transient electrostatic interactions of H3 tail residues with linker DNA segments amplified by the structured chromatin environment [56,57]. In another recent study, MAS solid-state NMR was used to investigate nucleosome arrays reconstituted with H4 containing mono- or tri-methylated lysine 20 concluding that H4K20 mono-methylation results in an altered H4 tail conformation and enhanced tail dynamics relative to unmethylated and trimethylated tails [58]. Remarkably, this finding appears to correlate with chromatin function given that these two PTMs modulate chromatin structure in distinct ways, with H4K20 mono-methylation associated with increased chromatin accessibility and gene expression [58] and H4K20 tri-methylation with a more compact chromatin structure [59].

The dynamic nature of histone tails is likely to be an important functional feature and suggests that these domains remain accessible to various chromatin modulators [25,37,38,42,44,60], and the ability to directly visualize these domains by MAS solid-state NMR methods in systems modeling condensed chromatin is, in principle, expected to enable their conformations, dynamics and interactions in complexes with chromatin-binding proteins to be evaluated in detail. A recent study demonstrated the feasibility of this approach for a histone reader, PHD2 domain of CHD4, that weakly binds unmodified H3 tails, using co-sediments of nucleosomes containing ^{2}H,^{13}C,^{15}N-enriched H3 with unlabeled PHD2 [53]. Reduced cross-peak intensities were noted for several H3 residues located near the N-terminus in J-based experiments, consistent with PHD2 interacting with the H3 tail. At the same time, no new resonances in dipolar-based experiments that would be consistent with the structuring and rigidification of the H3 tail upon PHD2 binding were detected, suggesting that the PHD2-H3 tail

complex exhibits considerable dynamics in the compact chromatin environment [53]. Another recent study focused on dissecting the interactions between phosphorylated heterochromatin protein 1α (pHP1α), which binds H3K9 tri-methylated tails, and condensed nucleosomes and nucleosome arrays under conditions of liquid-liquid phase separation [61]. Interestingly, J-based MAS solid-state NMR experiments indicated a reduction in conformational dynamics of not only the H3 tails, as may be anticipated due to pHP1α binding, but also of the H4 tails which are not believed to engage in specific interactions with pHP1α. Though the exact nature of interactions responsible for the observed decrease in H4 tail flexibility remains unclear, such large-scale changes in histone tail dynamics upon protein binding may have significant functional consequences [61].

Structural and dynamic studies of the nucleosome core by solid-state NMR

Dipolar-based MAS solid-state NMR techniques enable the selective detection and structural and dynamic characterization of relatively rigid domains of biomacromolecular assemblies [35]. In the context of chromatin systems, such techniques enable high resolution and sensitivity 2D and 3D solid-state NMR spectra to be recorded that, in principle, can report on all histone globular core residues in condensed nucleosomes or nucleosome arrays as illustrated in Figure 2 for histone H3 in 16-mer Widom 601 DNA arrays with 60 bp DNA linkers [62].

The initial MAS solid-state NMR study of the histone core in sedimented nucleosomes by van Ingen and co-workers [52] used ^{1}H-detected methods and focused primarily on characterizing the conformation and dynamics of histone H2A as well as nucleosome interactions with a peptide derived from the viral LANA protein that has been previously established to specifically recognize the H2A/H2B acidic patch [25]. Notably, in addition to

establishing sequential backbone ^{1}H, ^{13}C and ^{15}N resonance assignments for the majority of H2A core residues and confirming that histone tails in sedimented nucleosomes are conformationally flexible akin to those in condensed nucleosome arrays, the study was able to successfully confirm the LANA binding site by monitoring perturbations in the H2A chemical shifts and to model the conformation of the bound peptide on the nucleosome surface that was consistent with the crystal structure. In a series of elegant studies along similar lines, Nordenskiöld and co-workers [54,63–65] established the nearly complete sequential resonance assignments and investigated the backbone dynamics of histones H2B, H3 and H4 in the nucleosome core for both condensed nucleosomes and nucleosome arrays using ^{13}C-detected MAS solid-state NMR experiments. Dynamics on the ns-μs timescale were determined using quantitative residue-specific measurements of $^{1}H^{N}$-^{15}N and $^{1}H\alpha$-$^{13}C\alpha$ dipolar order parameters, while the presence of μs-ms timescale motions was assessed by inspecting the cross-peak intensities in 3D CANCO chemical shift correlation spectra. By mapping the histone core residue dynamics on the nucleosome structure, as illustrated in Figure 3 for H3 and H4 μs-ms motions in Widom 601 DNA nucleosomes [64], the authors proposed the existence of dynamic networks in nucleosomes that include many residues engaged in contacts with nucleosomal DNA and may be important for regulating nucleosome stability and DNA accessibility [54,64,65]. Remarkably, comparison of the dynamic profiles for nucleosomes reconstituted with Widom 601 DNA and telomeric DNA revealed that the latter display enhanced μs-ms motions for the H3 and H4 core residues (and more conformationally flexible H3 and H4 N-terminal tails), consistent with the reduced stability of telomeric nucleosomes [64]. In another recent study, ^{1}H-detected MAS solid-state NMR methods were used to evaluate the impact of DNA linker length in the 15-60 bp range on H3 core domain structure and interactions in nucleosome arrays [62]. While the histone core did not

appear to undergo any large-scale conformational changes multiple residues at the DNA-H3 interface showed pronounced chemical shift and linewidth differences for arrays with 15 bp DNA linkers relative to those with 60 bp linkers (Figure 4), consistent with alternate backbone and side-chain conformations likely resulting from structural strain and increased heterogeneity in nucleosome packing for arrays with short DNA linkers.

The ability to detect signals for most histone core backbone and side-chain sites in dipolar-based MAS solid-state NMR spectra permits intermolecular interactions in chromatin to be investigated, as demonstrated in the early study highlighted above of histone H2A in sedimented nucleosomes free and in complex with the LANA peptide fragment [52]. Several additional studies in this direction have been recently reported by Debelouchina and co-workers [61,66,67] as expanded upon below. In one study, low temperature dynamic nuclear polarization (DNP) MAS solid-state NMR, which enables direct observation of both rigid and flexible protein domains in dipolar-based experiments with concomitant significant (order of magnitude or larger) enhancements in spectral sensitivity [68,69], was applied to nucleosome arrays reconstituted with isotope labeled H3 and/or H4 [66]. While selective detection of intermolecular ^{15}N-^{13}C histone-histone interactions was found to be complicated by the naturally abundant ^{13}C atoms present in these samples, the study showed that multidimensional chemical-shift correlation spectra for the nucleosome core residues display sufficient resolution to enable detection of potentially functionally relevant DNA-histone contacts [66] despite the inherent line broadening associated with low temperature DNP solid-state NMR spectra of many biological systems [69]. In another study, carried out for condensed nucleosomes and nucleosome arrays in complex with pHP1α under conditions of liquid-liquid phase separation [61], in addition to assessing the influence of pHP1α binding on the conformational dynamics of H3 and H4 histone

tails as discussed above the impact of pHP1α binding on the histone core domain conformation and dynamics was evaluated by comparing cross-peak positions and intensities for the H3 and H4 core residues in 2D ^{13}C-^{13}C chemical shift correlation spectra recorded in the presence and absence of pHP1α. Interestingly, the binding of pHP1α was found to not cause any major rearrangements of the nucleosome core, in significant contrast to the fission yeast HP1 protein Swi6 [70] and suggestive of different modes of interaction for pHP1α and Swi6 with nucleosomes [61]. Finally, a recent study probed the association and interactions of the microtubule-associated protein tau with nucleosome arrays under conditions of liquid-liquid phase separation [67]. The study used 2D J- and dipolar-based MAS solid-state NMR methods to assess with site-specific resolution any potential structural and dynamic perturbations of the histone H4 core and tail domains upon tau binding, concluding that in the chromatin context tau interacts primarily with nucleosomal DNA rather than histones without major impact on the conformation and dynamics of the nucleosome core and histone tails.

Concluding remarks and future outlook

As illustrated by the recent studies highlighted in this review, multidimensional MAS solid-state NMR spectroscopy is uniquely positioned to furnish atomic level structural and dynamic information for histone core and tail domains that are comprised of residues exhibiting different degrees of conformational flexibility within *in vitro*-assembled samples of nucleosomes and nucleosome arrays at high densities corresponding to cellular concentrations of chromatin. Such studies promise to contribute fundamental insights into the molecular mechanisms of chromatin compaction and gene regulation and are synergistic with investigations of chromatin systems by complementary experimental and computational techniques [25–28,37–39].

Consequently, applications of MAS solid-state NMR to condensed nucleosomes and nucleosome arrays as a function of different parameters including DNA sequence, PTMs and complexes with chromatin modulators are anticipated to continue and to grow considerably in the coming years.

While to date most applications of solid-state NMR to chromatin systems have focused on monitoring the histone protein signals, studies that directly probe the chromatin-binding partners and nucleosomal DNA are beginning to emerge or can be readily envisioned [71–74]. Notably, all of the above applications are expected to significantly benefit from recent and ongoing developments in NMR instrumentation, including ultrahigh field (>25 T) magnets and ultrafast (>100 kHz) MAS probes, and associated methodologies including ^{1}H-detection [75], dynamic nuclear polarization [66,68,69,72] and paramagnetic NMR [76–79], combined with continuing advances in atomistic MD simulations [39] and chemical biology tools [44,80] tailored toward chromatin systems.

Declaration of interest

The authors declare no conflict of interest.

Data availability

This is a review article.

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- •• of outstanding interest

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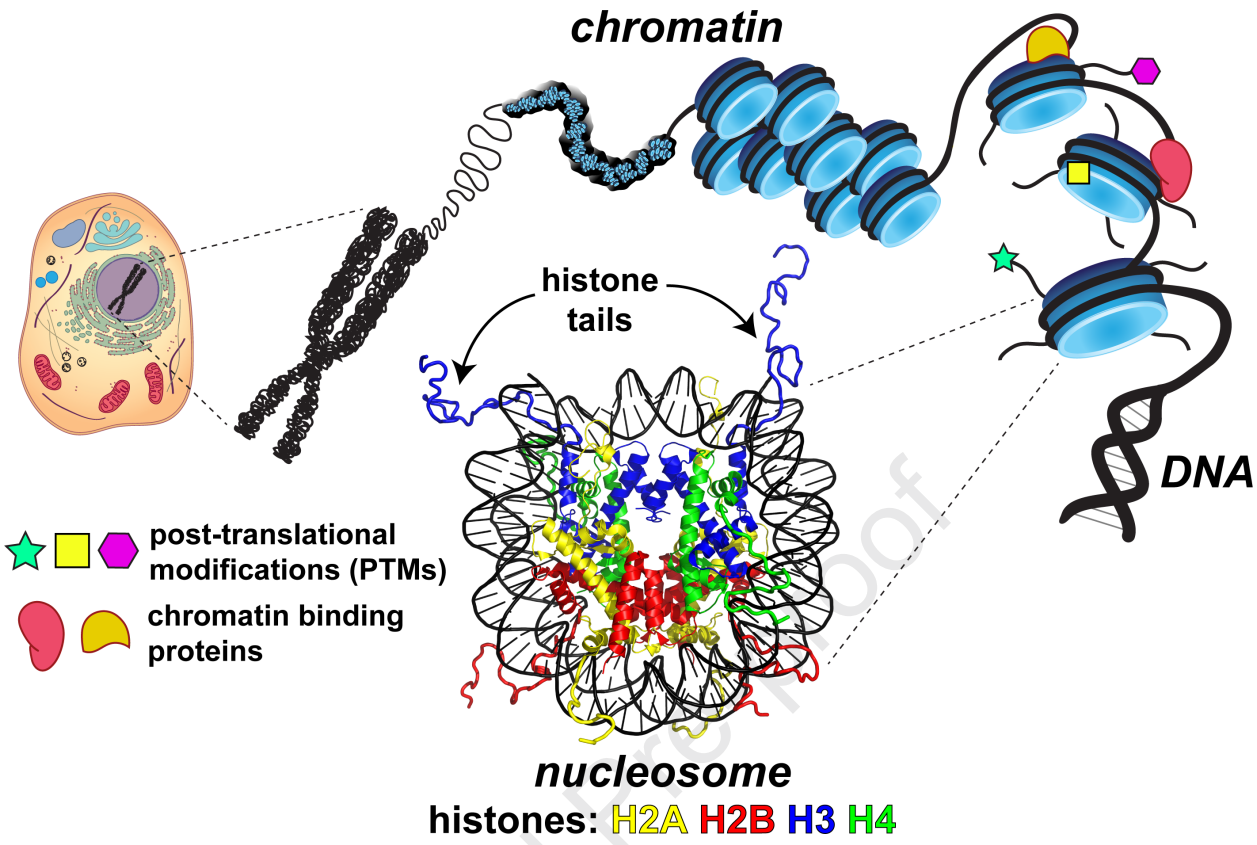
Figure Captions

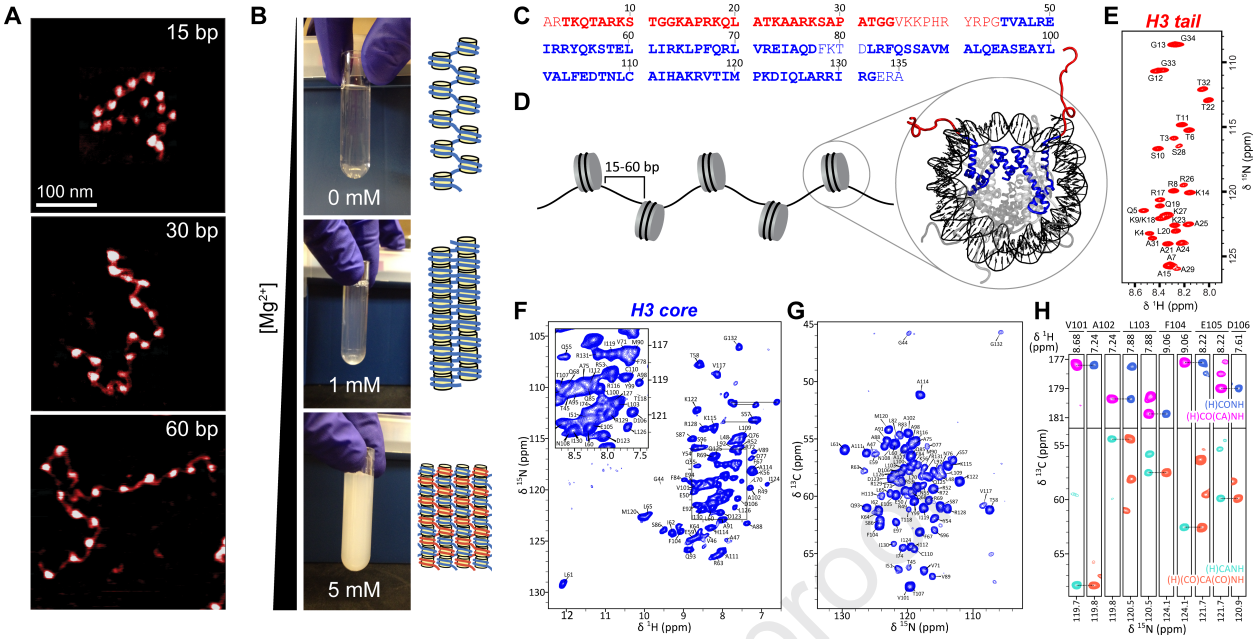
Figure 1. DNA packaging into nucleosomes and chromatin, schematically showing locations of post-translational modifications of histone core and tail residues and interactions with chromatin binding proteins. The crystal structure of the nucleosome (PDB entry 1KX5) is also shown with nucleosomal DNA colored in black and histones H2A, H2B, H3 and H4 colored in yellow, red, blue and green, respectively.

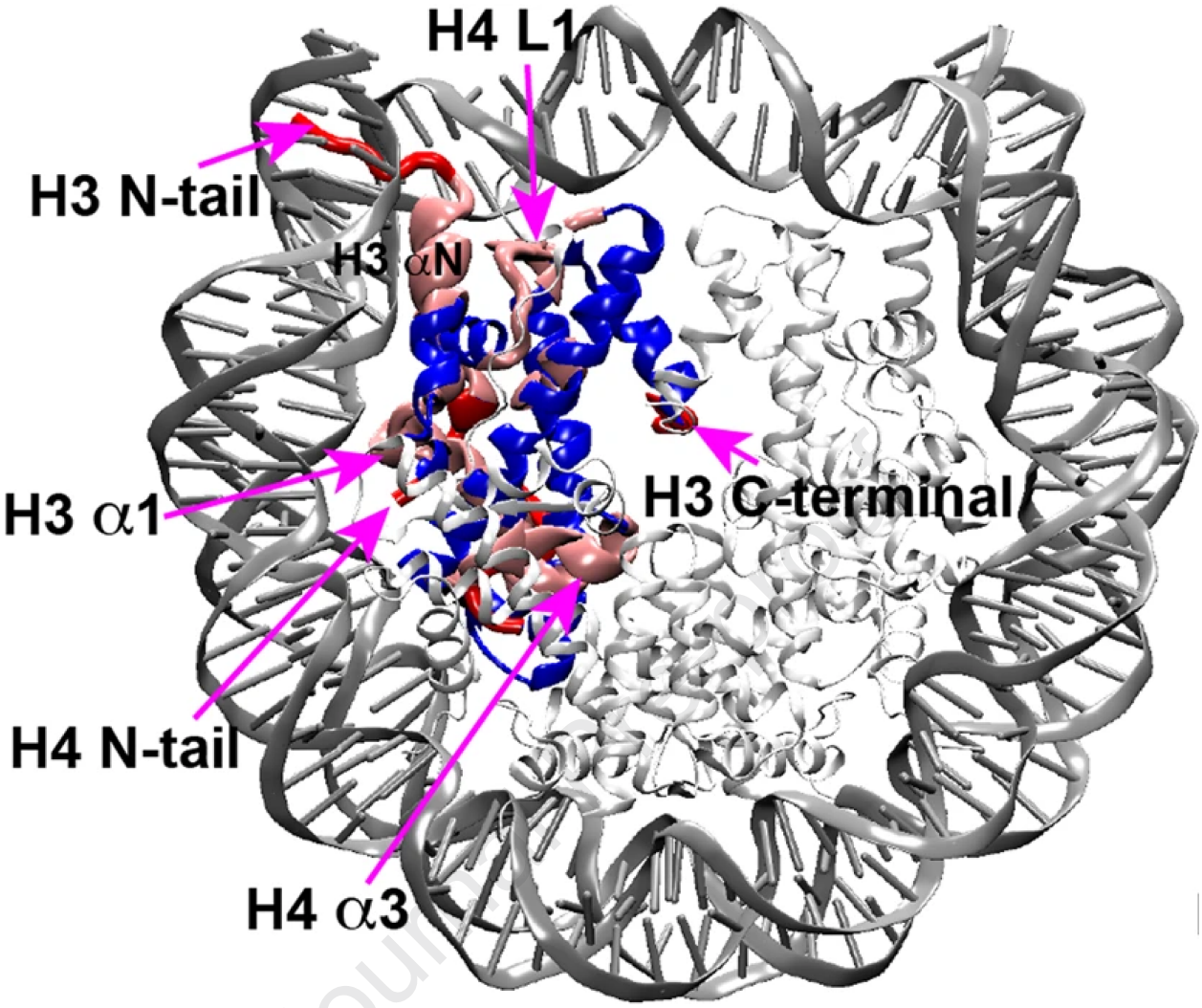
Figure 2. MAS solid-state NMR studies of histone tail and core domains in nucleosome arrays. (A) Representative atomic force microscopy images of sucrose gradient purified 16-mer nucleosome arrays with 15, 30 and 60 bp DNA linkers. (B) Nucleosome arrays incubated with 0, 1 and 5 mM $MgCl_2$, corresponding to open, folded and aggregated chromatin. (C) H3 amino acid sequence (*Xenopus laevis*), with residues structured and unstructured in nucleosome crystals (PDB entry 1KX5; panel D) shown in blue and red font, respectively. Dynamically disordered (bold red) and relatively rigid (bold blue) residues observed in J-based (E) and dipolar-based (F-H) MAS solid-state NMR spectra. (D) Schematic representation of nucleosome arrays. ^{15}N-^{1}H NMR spectra of H3 tail (E) and core (F) domains in 16-mer nucleosome arrays with 60 bp DNA linkers reconstituted with ^{2}H,^{13}C,^{15}N-H3 back-exchanged with H_2O. (G) ^{13}C-^{15}N projection of a 3D (H)CANH spectrum and (H) small regions of ^{1}H-detected 3D spectra for H3 core residues used to establish sequential resonance assignments. Spectra were recorded at 800 MHz, 60 kHz MAS rate and sample temperature of ~25 ºC. Figure adapted from Refs. [48], [56] and [62].

Figure 3. Histone H3 and H4 residues exhibiting significant backbone motions on the μs-ms timescale in the nucleosome core. The most dynamic residues associated with negligible and low cross-peak intensities in 3D CANCO chemical shift correlation spectra are colored in red and pink, respectively. The remaining H3 and H4 residues are colored in blue and histones H2A/H2B and nucleosomal DNA are colored in light grey and dark grey, respectively. Figure adapted from Ref. [64].

Figure 4. Backbone ^{1}H, ^{15}N and ^{13}C chemical shift perturbations (CSPs) for histone H3 core residues in 16-mer Widom 601 DNA nucleosome arrays with 60 vs. 15 bp DNA linkers (A), mapped on the nucleosome structure (B). The 0.1-0.15 ppm and >0.15 ppm thresholds correspond to ~2.5-5σ and >5σ from the average CSP. Figure adapted from Ref. [62].






H4 L1
H3 N-tail
H3 αN
H3 α1
H3 C-terminal
H4 N-tail
H4 α3