Supporting information

Long Distance PELDOR Measurements on the Histone Core Particle

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Sequences and mutation sites used in this study

H4

MSGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVFLENVIRDAVTYTEH
AKRKTVTAMDVVYALKRQGRTLYGFGG

H3C110A

MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRL
VREIAQDFKTDLRFQSSAVMALQEASEAYLVALFEDTNLAAIHAKRVTIMPKDIQLARRIRGERA

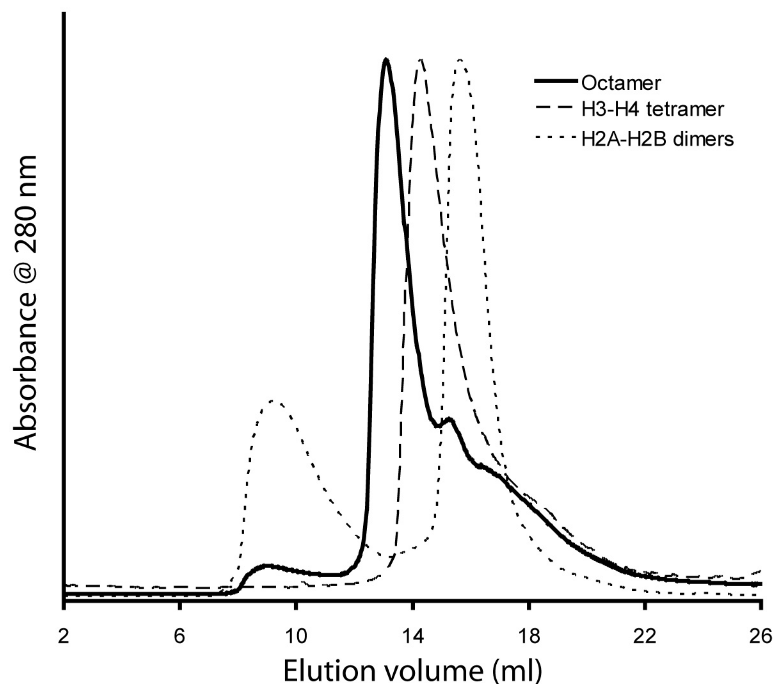
Protein purification and sample preparation

Site directed mutagenesis was used to introduce cysteine residues at strategic locations on histone H3C110A, aided by the crystal structure of the histone octamer (1AOI.pdb) using standard cloning procedures. Recombinant *Xenopus* histones were purified from *E. coli* (strain BL21(DE3) pLysS, Stratagene) as described previously [1], dialysed into H_2O and stored as lyophilates. The core histones were dissolved in unfolding buffer (7M gunadinium chloride, 20 mM sodium acetate pH 5.2, 1 mM EDTA, 5 mM dithiothreitol) and mixed in equimolar amounts, typically in the region of ~200 nmoles (octamer), to obtain a final concentration of ~100 μM (octamer). Refolding proceeded through dialysis into refolding buffer (2M sodium chloride, 10 mM tris-hydrogen chloride pH 7.5, 1 mM EDTA, 5 mM β-mercaptoethanol), with any precipitated material removed by centrifugation before concentration (Amicon® Ultra - 4, Millipore) and size exclusion chromatography (SEC). Prior to SEC 10 mM of dithiothriatol was added to the refolded octamer and left on ice for ten minutes to ensure complete reduction of labelling sites. SEC was carried out on an AKTApurifier FPLC system (Amersham Pharmacia Biotech) using a 10/300 GL column prepacked with Superdex™ 200 resin (Amersham Pharmacia Biotech) at a flow rate of 0.4 ml/min. Histone octamers eluted at 13 ml, with residual H3-H4 tetramer and H2A-H2B dimers present at 14.3 and 15.6 ml, respectively (supplementary figure 1). Histone octamer fractions were pooled and labelled with a 10-fold molar excess of MTSSL and incubated overnight at 4 ºC in the dark. Unreacted MTSSL was dialysed away in two changes of 1L of refolding buffer, without reducing agent, before the sample buffer was exchanged to deuterated refolding buffer by three rounds of concentration and dilution in a centrifugal concentrator (Amicon® Ultra - 4, Millipore). Finally, the sample was concentrated to 180-330 μM, excess H2A-H2B dimer added (see below), diluted 1:1 with D8-glycerol, resulting in a final concentration of 61-98 μM octamer, and stored at -20 ºC until distance extraction. We noticed that upon dilution of the octamer with glycerol the equilibrium of the histone complex was shifted more towards a dimer-tetramer state than an octamer state, i.e. upon decrease in ionic strength the dimers dissociated from the tetramer[2]. However, we found that we could drive the equilibrium back to the octamer state by having excess dimers in solution. Dimers were prepared in the same way as for the octamer, except without the labelling steps, and were used in an equimolar excess.

Estimated concentrations of histone octamer PELDOR samples

V46C	82 μM
R49C	64 μM
L65C	98 μM
Q76C	65 μM
M90C	61 μM

Figure S1. Size exclusion chromatography of H3M90C histone octamer (solid line), H3 M90C histone tetramer (dashed line) and wtH2A-H2B dimers (dotted line). Histone octamer was isolated from aggregates (peak at 8.5 ml) that occur during refolding, producing a major elution peak at 13 ml and two minor peaks at 14.3 and 15.6 ml. The minor peaks are likely to be dissociated H3-H4 tetramer and H2A-H2B dimer, respectively, as they eluted in the same volume as H3-H4 and H2A-H2B that were refolded separately. Elution of all three complexes was in refolding buffer (see text) with the absorbance at 280 nm normalised to the H3-H4 tetramer.



EPR Data Collection

PELDOR experiments were executed using a Bruker ELEXSYS E580 spectrometer operating at X-band with a dielectric ring resonator and a Bruker 400U second microwave source unit. All measurements were made at 50 K with an overcoupled resonator giving a Q factor of approximately 100. The video bandwidth was set to 20MHz. The four pulse, dead-time free, PELDOR sequence, was used, with the pump pulse frequency positioned at the center of the nitroxide spectrum; the frequency of the observer pulses was increased by 80 MHz. The observer sequence used a 32 ns π– pulse; the pump π- pulse was typically 28 ns. The experiment repetition time was 4 ms, the video bandwidth 20 MHz, and the number of scans accumulated was typically 440-632 with 309-409 time points per scan and 50 shots at each time point.

PELDOR data analysis

PELDOR data was analysed as follows using the DeerAnalysis 2006 software package [3] The dipolar coupling evolution data was corrected for background echo decay using a homogeneous three-dimensional spin distribution. The starting time for the background fit was optimised to give the best fit Pake pattern in the fourier transformed data and the lowest root mean square deviation background fit.

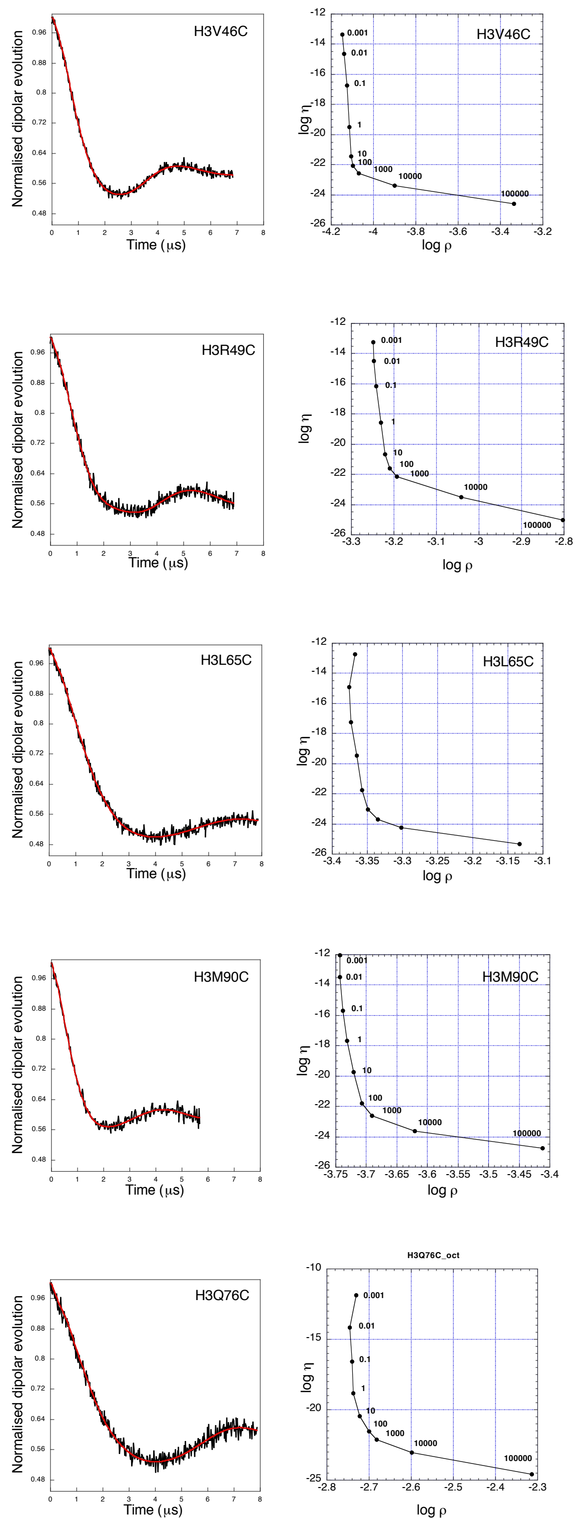
The Pake pattern can allow distance determination using equation S2,

$$f_{Dip}(r,\theta) = \frac{\mu_B^2 g_A\, g_B \mu_0}{2\pi h} . \frac{1}{r_{AB}^3}(3\, cos^2 - 1) \quad (S2)$$

The angle θ is between the spin-spin vector r and the direction of the applied magnetic field, μ_B is the Bohr magneton, μ_0 is the permeability of free space, g_A and g_B are the g values for the two spin labels A and B, and r is the spin-spin distance, assuming the exchange coupling constant can be neglected. If a resolved perpendicular turning point feature is observed in the spectrum a mean distance can be inferred

The background corrected data was analysed using Tikhonov regularisation. The resultant distributions are shown in the main communication figure 2 and the fit to background corrected experimental data is shown as a red line in figure S3 collumn 1. The alpha term, a measure of the peak widths, used was judged by reference to a calculated L-curve (figure S3 column 2). The L curve is a plot of the alpha term against quality of fit, measured by mean square deviation between the experimental data and the simulation. The most appropriate alpha term to be used is at the corner of the L curve, since this provides the best compromise between smoothness (artifact suppression) and fit to the experimental data.

Figure S3. Graphs showing background corrected dipolar evolutions (black) with superimposed fit from Tikhonov regularisation using an α value of 100 (red). (column 1). Tikhonov L curves (column 2).



Molecular dynamics

Molecular dynamics was carried out using XPLOR-NIH [4]. Coordinates were taken from PDB code 1TZY and mutated within PyMol[5] to replace required positions with Cysteine. Parameter and topology files for MTSSL were created using PRODG [6]. Unknown coordinates were generated at random positions and minimized to give initial structures on the remaining fixed protein structure. Non-Cα backbone positions were restrained by an harmonic function to initial positions and the unrestrained atoms were allowed to move under molecular dynamics at a temperature of 400 deg K. Structures were taken at regular intervals and the distance between MTSL Nitrogen atoms were calculated, collected in groups (40 bins between 2 and 8 nm) and displayed as interpolated lines.

(1) Luger, K.; Rechsteiner, T. J.; Flaus, A. J.; Waye, M. M.; Richmond, T. J. *J Mol Biol* **1997**, *272*, 301-11.
(2) Banks, D. D.; Gloss, L. M. *Biochemistry* **2003**, *42*, 6827-39.
(3) Jeschke, G.; Chechik, V.; Ionita, P.; Godt, A.; Zimmermann, H.; Banham, J.; Timmel, C. R.; Hilger, D.; Jung, H. *Applied Magnetic Resonance* **2006**, *30*, 473-498.
(4) Schwieters, C. D.; Kuszewski, J. J.; Tjandra, N.; Clore, G. M. *J Magn Reson* **2003**, *160*, 65-73.
(5) DeLano, W. L.; DeLano Scientific, Palo Alto, CA, USA.: 2002.
(6) Schuttelkopf, A. W.; van Aalten, D. M. *Acta Crystallogr D Biol Crystallogr* **2004**, *60*, 1355-63.