Long Distance PELDOR Measurements on the Histone Core Particle

Richard Ward,[†] Andrew Bowman,[†] Hassane El-Mkami,[‡] Tom Owen-Hughes,[†] and
David G. Norman*,[†]

*School of Life Sciences, University of Dundee, Dundee DD1 5EH, U.K., and School of Physics and Astronomy,
University of St. Andrews, St. Andrews FE2 4KM, U.K.*

Received October 7, 2008; E-mail: d.g.norman@dundee.ac.uk

The nucleosome core particle is the basic unit of chromatin structure in eukaryotes and consists of 147bp of double-stranded DNA wrapped twice around a core composed of histones H4, H3, H2A, and H2B, assembled into an octameric arrangement. The nucleosome is essential for compaction of the eukaryotic genome but is also a dynamic assembly that is functional with regards to many processes including those of replication, repair, expression, transcription, and recombination. The structure of the histone core[1,2] and the nucleosome[3] have been determined by X-ray crystallography. X-ray crystallography has been very successful in describing the detailed structure of the nucleosome at high resolution, but although great progress has been achieved through crystallography there are many questions still to be answered and alternative ways of studying the structure and dynamics of the nucleosome and related molecules are desirable.

Figure 1. Position of spin labels on the histone octamer. H4, red; H3, green; H2AH2B, gray. Distances measured indicated in blue.

A small number of studies have been reported that have investigated the structure of the nucleosome using electron paramagnetic resonance (EPR)[4] or fluorescence resonance energy transfer (FRET).[5] Previous EPR studies have used CW EPR to investigate nucleosomes labeled in relatively unsophisticated ways owing to the limitations of labeling technology and EPR techniques. FRET has been used to investigate spatial relationships in the nucleosome mainly by looking at FRET intensities of labeled DNA

components.[6] Attempts to label the protein component of the nucleosome has always suffered from the restrictions imposed by the requirement to incorporate two different but complementary fluorophores in a system that is multiply dimeric[7] (PELDOR is most often measured using two chemically identical spin-labels). Advances in EPR technology and site directed spin labeling have allowed us to approach nucleosome structure by the addition of spin-labels to the histone components at specific locations and to use pulsed EPR to measure distance between labels. The dimeric nature of the histones in the core particle results in symmetrical pairs of spin labels, giving extremely good coverage of the overall protein core.

PELDOR (pulsed electron double resonance) is an EPR technique that can provide distance measurement between spin labels.[8−12] Because of the nature of the measurement and the relaxation properties of nitroxide spin labels, the distances available to this technique have often been described as being limited to between approximately 20 and 80 Å. In the case of biological molecules rather than synthetic model compounds, the upper distance limit for the acquisition of high quality experimental data, on a commercially available machine, has so far been about 55 Å[13] (a reasonable definition of quality being that the data should have a large signal-to-noise ratio, show good depth of oscillation, and be acquired over such a time that at least one full oscillation is recorded).

We report results obtained using PELDOR experiments, on site specifically incorporated nitroxide spin-labeled histone H3, included within the core histone octamer (Figure 1).

Among the distances we report are two distances of about 70Å determined from extremely high quality data showing clear and significant dipolar oscillations of up to 8 μs duration. Three other distances are described, all derived from spin labels attached to the H3 histone, of around 60Å, all showing clear and persistent signal oscillation.

Using simple molecular dynamics simulations on spin labeled-models, based on the crystal structure of the nucleosome core octamer (PDB ID 1TZY), we obtain remarkable correspondence between the experimental and predicted distances (Table 1).

Table 1. Experimental and Predicted Distances[a]

mutation site	experimental distance (Å)	distribution width at half-height (Å)	predicted distance (Å)	distribution width at half-height (Å)
H3V46C	60	10	59/64[b]	5.6/4.6[b]
H3R49C	63	9	64	3.5
H3L65C	70	11	74	6
H3Q76C	70	8	70	5.5
H3M90C	59	11	58	4.7

[a] For each labeled octamer is listed an experimental distance obtained by Tikhonov regularization of the PELDOR data (α = 100), the half height distribution width, and a distance and linewidth, predicted by molecular dynamics. [b] Split distribution.

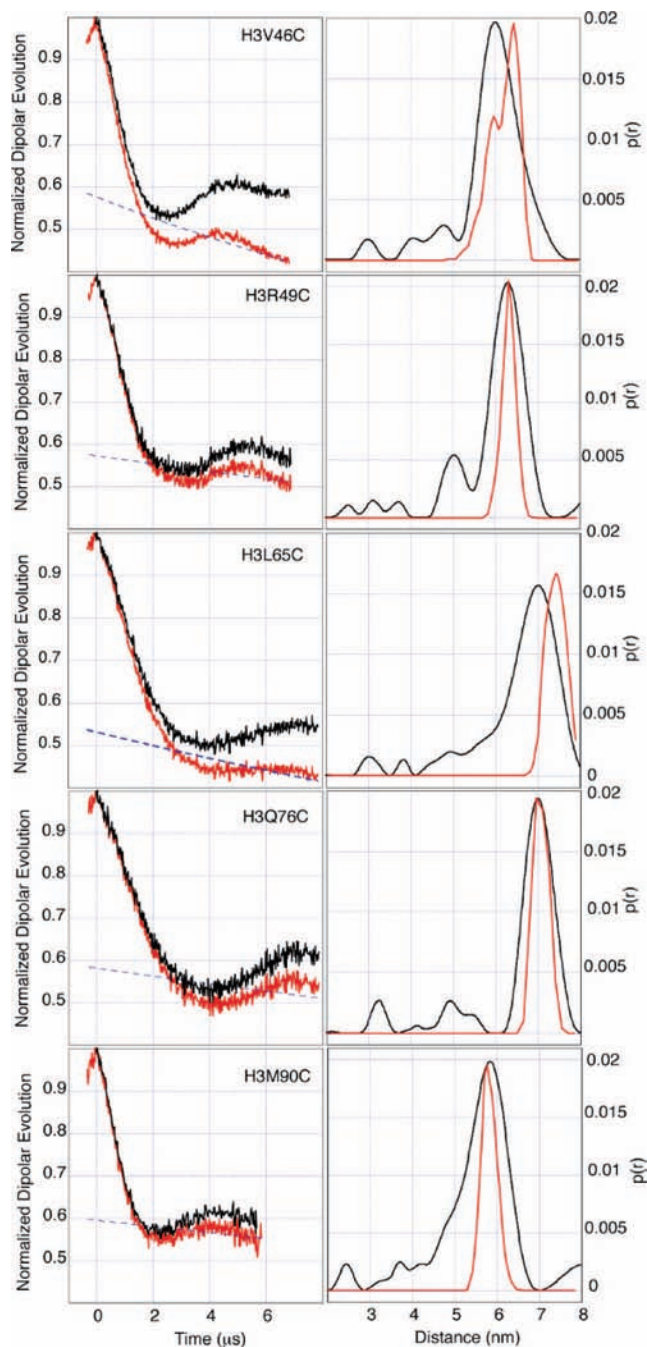[†] University of Dundee.
[‡] University of St Andrews.

Figure 2. Left column, uncorrected PELDOR data (red), background correction (blue), corrected data (black). Right column, distance distributions derived by Tikhonov regularization with regularization parameter 100 (black), dynamics determined distance distribution (red). The splitting of the V46 distribution is an artifact caused by the dynamic simulation of a spin label near the N-terminus of the peptide chain.

The extremely well defined oscillations seen in all the five examples described here, illustrate the quality of PELDOR data that can be achieved even in a complex protein oligomer. The ability

to measure to 8 μs owes much to the use of deuterated solvents[14,15] but also to high labeling efficiency and careful sample preparation (Supporting Information S1). The use of 50% by volume, deuterated glycerol, to ensure that a completely glassy frozen state is achieved, and the optimization of spin-pair density to between 50 and 100 μM are both crucial aspects in obtaining a persistent echo and oscillation. Despite the long detection times the oscillations, shown here, are still truncated, having not decayed to baseline. The truncation of oscillations can lead to inaccurate baseline estimation, giving rise to imprecise distribution widths, inaccurate distances, and the generation of Tikhonov artifacts. To diminish inaccurate baseline calculation and to ensure accurate distribution width determination, at least one full oscillation is required. The largest discrepancy between experimental and calculated distance is seen in L65. This data is at the extreme limit of acceptability in terms of oscillation depth, persistence, and noise level. The distance distribution shown for this mutant is therefore less reliable as demonstrated by comparison to the dynamics derived distance distribution. Although experiment time domains could be extended further, we found that, for these samples, decreased signal-to-noise ratios tended to mitigate any further advantage obtained from a more complete description of the oscillations. To reliably measure to 8 nm one would have to significantly extend the detection time and obtain somewhat greater sensitivity in the PELDOR experiment.

Supporting Information Available: Sequences and mutation sites used in this study; protein purification and sample preparation; EPR data collection, data analysis, molecular dynamics method. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Arents, G.; Burlingame, R. W.; Wang, B. C.; Love, W. E.; Moudrianakis, E. N. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10148–52.
(2) Wood, C. M.; Nicholson, J. M.; Lambert, S. J.; Chantalat, L.; Reynolds, C. D.; Baldwin, J. P. *Acta Crystallogr., Sect. F* **2005**, *61*, 541–5.
(3) Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. *Nature* **1997**, *389*, 251–60.
(4) Chan, D. C.; Piette, L. H. *Biochim. Biophys. Acta* **1980**, *623*, 32–45.
(5) Hoch, D. A.; Stratton, J. J.; Gloss, L. M. *J. Mol. Biol.* **2007**, *371*, 971–88.
(6) Kelbauskas, L.; Sun, J.; Woodbury, N.; Lohr, D. *Biochemistry* **2008**, *47*, 9627–35.
(7) Park, Y. J.; Dyer, P. N.; Tremethick, D. J.; Luger, K. *J. Biol. Chem.* **2004**, *279*, 24274–82.
(8) Milov, A. D.; Salikohov, K. M.; Shirov, M. D. *Fiz. Tverd. Tila* **1981**, *23*, 975–82.
(9) Jeschke, G.; Koch, A.; Jonas, U.; Godt, A. *J. Magn. Reson.* **2002**, *155*, 72–82.
(10) Martin, R. E.; Pannier, M.; Diederich, F.; Gramlich, V.; Hubrich, M.; Spiess, H. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2834–7.
(11) Pannier, M.; Veit, S.; Godt, A.; Jeschke, G.; Spiess, H. W. *J. Magn. Reson.* **2000**, *142*, 331–40.
(12) Schiemann, O.; Prisner, T. F. *Q. Rev. Biophys.* **2007**, *40*, 1–53.
(13) Schiemann, O.; Piton, N.; Mu, Y. G.; Stock, G.; Engels, J. W.; Prisner, T. F. *J. Am. Chem. Soc.* **2004**, *126*, 5722–9.
(14) Jeschke, G.; Bender, A.; Paulsen, H.; Zimmermann, H.; Godt, A. *J. Magn. Reson.* **2004**, *169*, 1–12.
(15) Borbat, P. P.; Davis, J. H.; Butcher, S. E.; Freed, J. H. *J. Am. Chem. Soc.* **2004**, *126*, 7746–7.

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