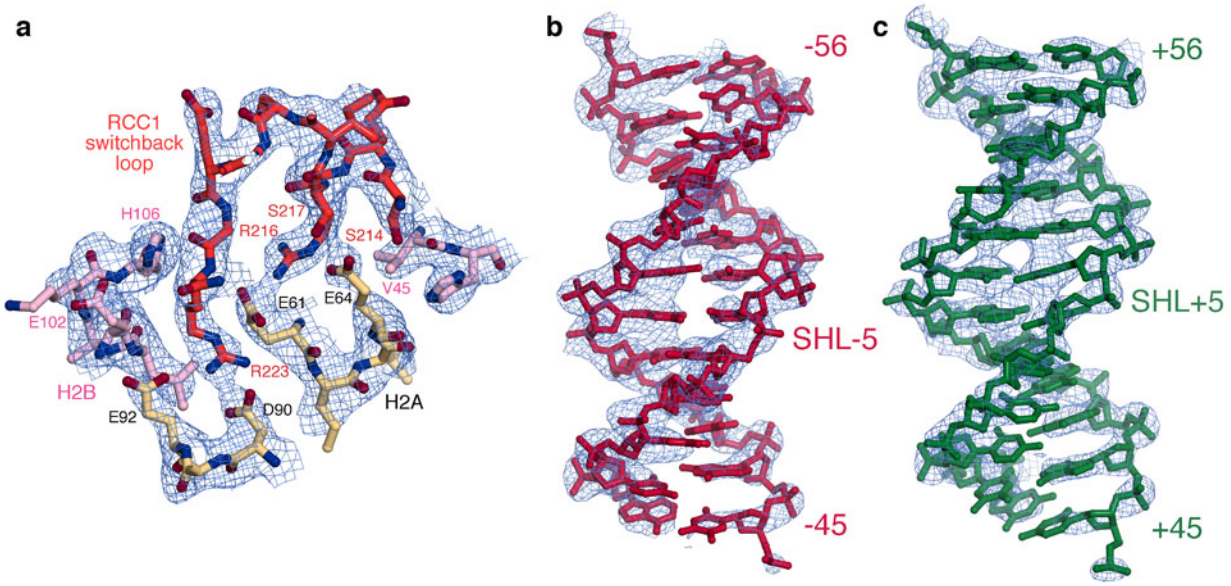
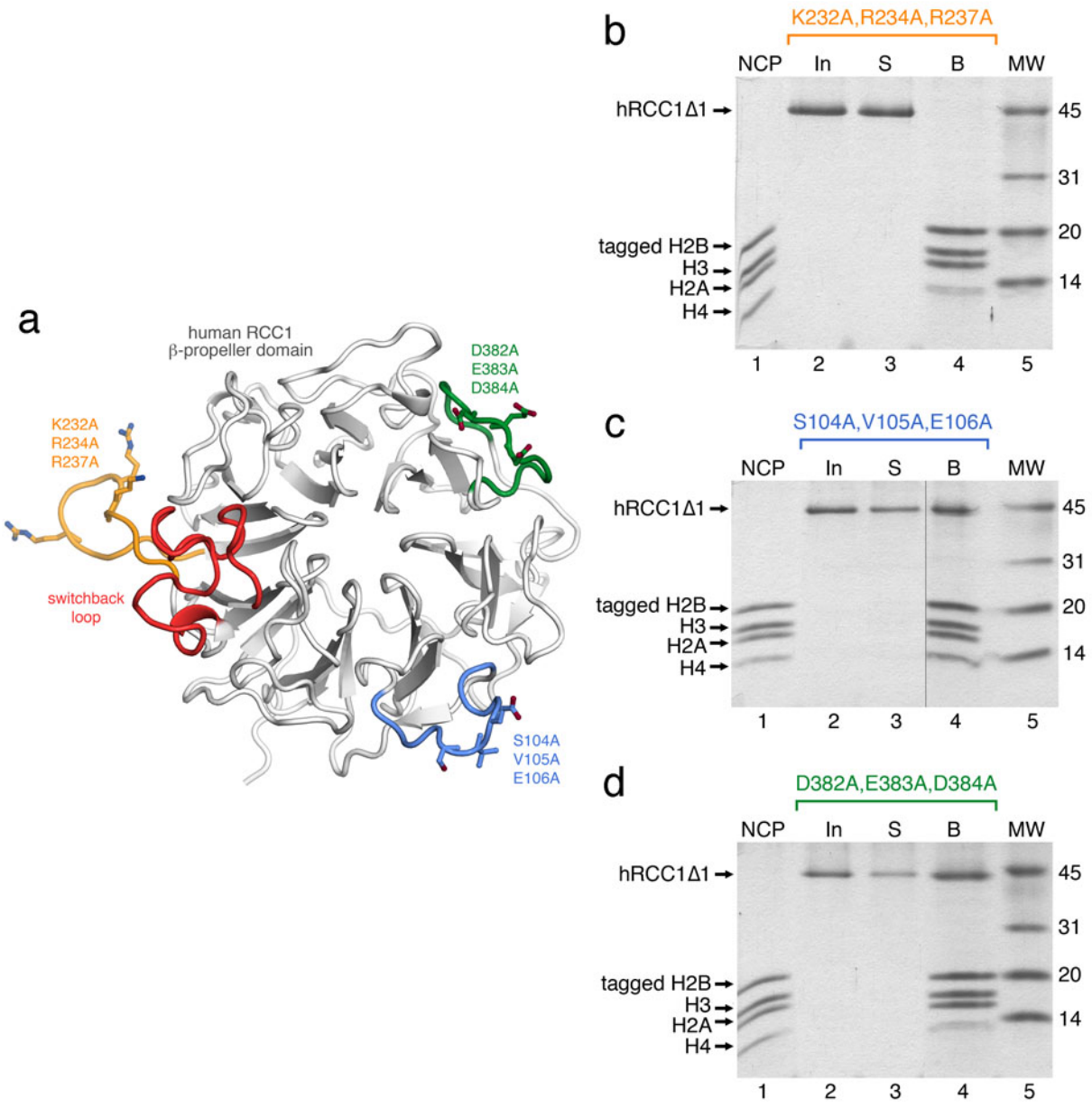
Supplementary Figures

Suppl. Figure 1: RCC1 sequence and sequence alignments. (a) Amino acid sequence of Drosophila RCC1. Same colors are for Figure 1 with sequence of β-wedge that interacts with Ran in purple. β-strands are indicated by horizontal arrows, and the conserved glycine residues towards the ends of strands A and C are highlighted with a grey background. Residues that interact with the nucleosome in the complex are indicated by asterisks. (b) Sequence alignment of nucleosome interacting region for RCC1 homologs. Similar comments as for (a). The conserved switchback loop arginine residue that interacts with the acidic histone dimer patch is highlighted with a pink background, while the basic residues in the predicted human RCC1 DNA-binding loop and analyzed in Supplementary Figure 3 are shown with a grey background.

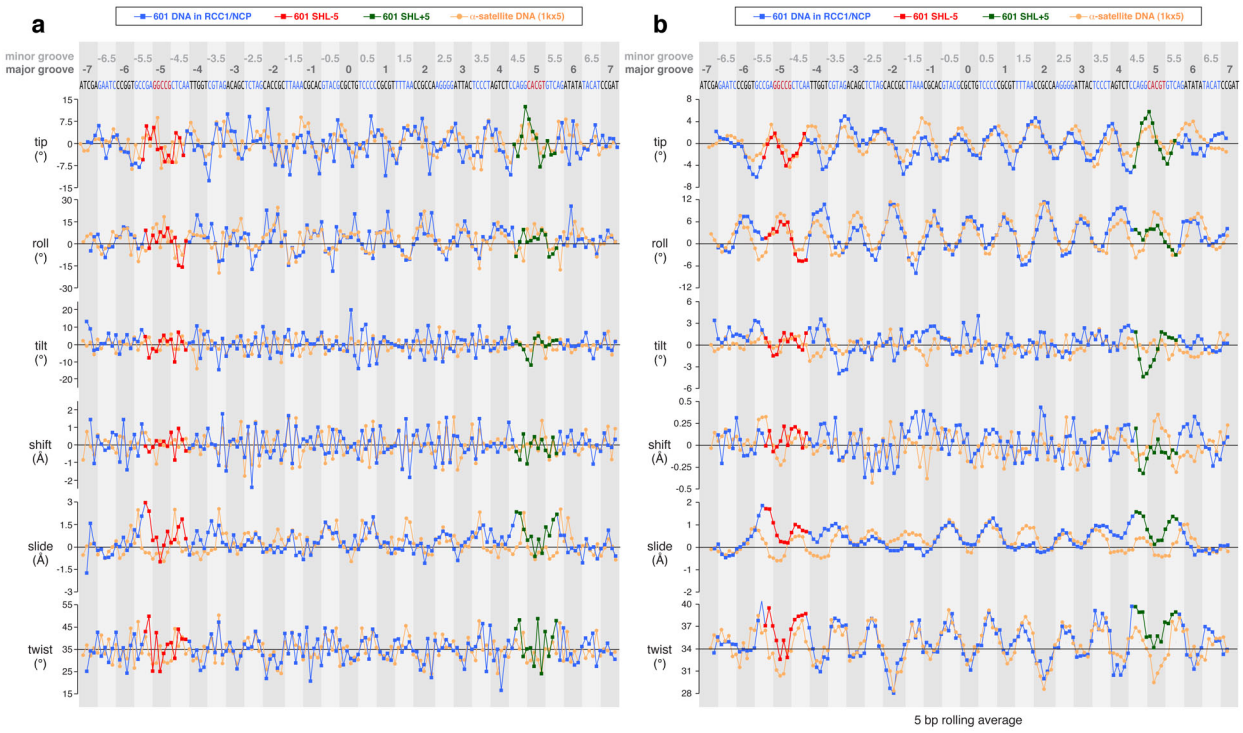


Suppl. Figure 2: Electron density for RCC1-nucleosome core particle structure. (a) Maximum-likelihood $2mF_o$-DF_c difference Fourier electron density map for RCC1-histone interface contoured at 1.5 σ. Same orientation as for Fig. 2a and b. (b) and (c) Simulated annealing omit mF_o-DF_c electron density maps for nucleosomal DNA around SHL-5 and SHL+5 respectively contoured at 5 σ. Same orientations as for Figs. 4 b and c.

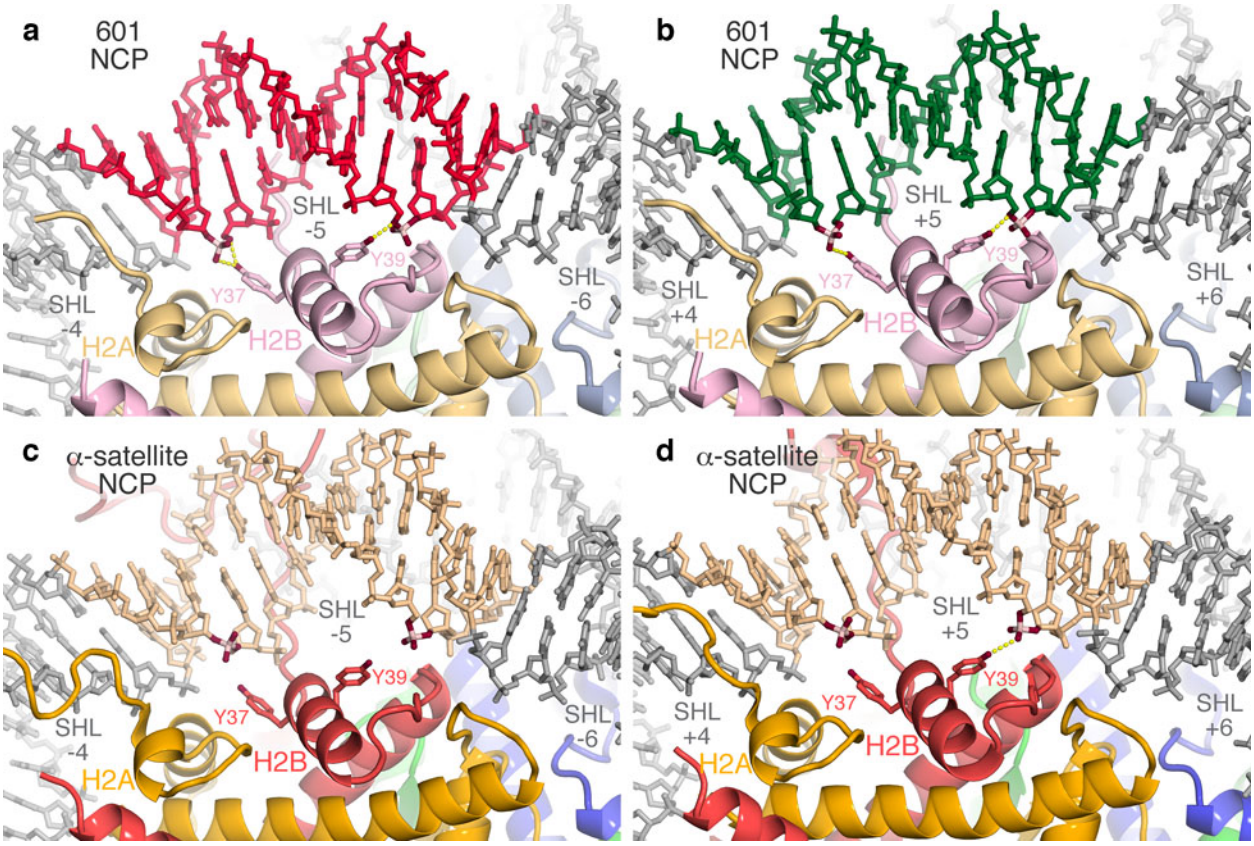


Suppl. Figure 3: Mutations in the predicted DNA-binding loop in human RCC1 abrogate binding to the nucleosome. (a) Sites of mutated residues mapped onto the crystal structure of human RCC1 β-propeller domain (PDB id 1A12, chain B). The switchback loop is shown in red, while the loop region between strands 4D and 5A (putative DNA-binding loop), strands 2C and D, and strands 7C and D are shown in yellow, blue and green respectively. (b) - (d) Immobilized nucleosome pulldown assay results for human RCC1 β-propeller domain [hRCC1Δ1 = hRCC1(21-421)] containing

K232A/R234A/R237A, S104A/V105A/E106A and D382/E383A/D384 mutations respectively. All RCC1 proteins were determined to be monodisperse by dynamic light scattering, indicating that the mutations did not affect the folding of the proteins (polydispersity of RCC1 wild type, K232A/R234A/R237A, S104A/V105A/E106A and D382/E383A/D384 at 13.6%, 6.7%, 15.9% and 13.2% respectively). NCP = input nucleosome core particles, In = input hRCC1Δ1 protein, S = unbound protein in supernatant fraction, B = bound protein eluted from the immobilized nucleosome beads, MW = molecular weight markers (sizes in kDa). In panel c, a control lane removed from the original gel is delineated by the vertical line between lanes 3 and 4.



Suppl. Figure 4: DNA tip base pair parameter and other base pair step parameters calculated using CURVES+ for 601 nucleosome core particle (blue) in the RCC1-nucleosome structure compared to the 147 bp human α-satellite nucleosome core particle (peach). The regions around superhelical locations (SHL) -5 and +5 are shown in red and green respectively. The major and minor groove blocks are indicated by dark and light grey stripes respectively. The Widom 601 sequence used in the RCC1-nucleosome core particle structure is shown near the top (black major groove blocks, blue minor groove blocks, red SHL±5 major groove blocks). (a) Tip base pair parameter and roll, tilt, shift, slide and twist base pair step parameters for each of the nucleotides in the 601 and human α-satellite (1KX5) nucleosome core particles, (b) Same parameters as in (a) except plotted as a 5 bp rolling average (average values for 5 base pairs centered on given base pairs). Similar results are obtained using a 3 bp rolling average.



Suppl. Figure 5: Histone-DNA interactions around superhelical locations (SHL) ±5 in the 601 nucleosome core particle (NCP) (this work) and the 147 bp human α-satellite nucleosome core particle (PDB id 1KX5). Hydrogen bonds are shown in yellow dotted lines. (a) and (b) Interactions in 601 nucleosome core particle for SHL -5 and +5 respectively. Same colors as for Figures 1 and 4. (c) and (d) Interactions in human α-satellite nucleosome core particle for SHL -5 and +5 respectively. Histones H2A, H2B, H3 and H4 shown in yellow, red, blue and green respectively, while the DNA at SHL±5 are shown in peach.

Supplemental Table 1:
Data collection and refinement statistics

Data Collection		
Space group	$P2_1$	
Cell dimensions		
a, b, c (Å)	102.7, 183.0, 107.0	
α, β, γ (°)	90, 101.5, 90	
Resolution (Å)	50 - 2.90	(2.95 - 2.90)
Mosaicity (°)	0.15 - 0.44	
Reflections (total/unique)	329,804/85,040	
I/sigmaI	22.25	(1.67)
Completeness (%)	98.5	(98.0)
Rmerge (%)	5.8	(59.4)
Refinement		
R_{work}/R_{free}	17.5%/21.6%	
Number of atoms	17743	
Protein	11757	
DNA	5986	
Water	0	
B-factors	87.5	
Protein	73.6	
DNA	114.9	
Water	N/A	
R.m.s deviations		
Bond lengths (Å)	0.002	
Bond angles (°)	0.627	
Ramachandran plot[a]		
Most favored (%)	89.4	
Allowed (%)	10.3	
Generously allowed (%)	0.1	
Disallowed (%)	0.1	

[a] Calculated using the program PROCHECK

Supplementary Results

Crystallization and structure determination

We grew crystals of Drosophila RCC1 (Bj1) bound to recombinant Xenopus nucleosome core particle after initial attempts using RCC1 from other species produced poorly ordered or no crystals. By optimizing the post-crystallization dehydration and cryocooling conditions, we were able to improve the diffraction of the Drosophila RCC1/nucleosome core particle crystals from about 5 Å to 2.9 Å. The structure was solved by molecular replacement using the crystal structures of Drosophila RCC1 β-propeller domain (M. Saxena, R. Makde and S. Tan, unpublished data) and the 147 bp α-satellite nucleosome core particle[12].

RCC1 switchback loop interactions with nucleosomal histones

Amino acid side chains dominate the hydrogen bond network between the RCC1 switchback loop and the histone dimer: all hydrogen bonds with RCC1 in the network are made to side chain atoms, and all but one of these hydrogen bonds are made to H2A side chain atoms. The guanidium group of RCC1 Arg223 forms five charged hydrogen bonds with H2A residues Glu92, Asp90 and Glu61. H2A Glu61 uses its other side chain carboxyl group to hydrogen bond with RCC1 Arg216, which in turn hydrogen bonds to H2A Glu64. This positions H2A Glu64 for hydrogen bonding interactions with RCC1 Ser214 and Ser217, which are held in a 3_{10} helix, the only helix in the RCC1 structure. RCC1 Ser217 then makes the only polar interaction mediated by the switchback loop to histone H2B, through the side chain hydroxyl group to the H2B Val45 carboxyl main chain atom. This hydrogen bonding network of RCC1 with the histone dimer acidic patch is facilitated by van der Waals interactions between RCC1 residues and histone H2B residues including Glu102, Leu103, His106 and Val45. The side chains of these H2B residues form the walls of the acidic patch depressions and these walls help position the flexible Arg216 and Arg223 for their extensive interactions with the histone dimer (Fig. 2a and 2b).

All the histone residues involved in RCC1-histone contacts are conserved among yeast, Drosophila, Xenopus and human, and also adopt either similar or could adopt similar conformations in the crystal structures of corresponding nucleosome core particles[11,12,16,17]. Thus, the histone surface recognized by Drosophila RCC1 appears to be conserved. It is curious, however, that the sequence of the RCC1 switchback loop itself is not better conserved among different species (Suppl. Fig. 2b). Despite a lack of high sequence similarity, we anticipate that at least some elements of the interaction will be conserved. For example, although only 5 of the 16 residues in the switchback loop are conserved between Drosophila and human, the conformation of the switchback loop in our Drosophila RCC1/nucleosome complex structure and in the human RCC1 protein alone crystal structure are similar (main chain atoms rmsd of 1.54 and 1.34 Å for Drosophila RCC1 in the nucleosome complex compared to human RCC1 chains B and C respectively in PDB 1A12, human RCC1 chain A has a different conformation from chains B and C in the crystal[4,25]). A possible common recognition element may be Drosophila Arg223 (equivalent to human Arg217) which appears to be generally conserved. We suspect that the equivalent residue in RCC1 from other species will interact with some or all of the triad of H2A residues Glu61, Asp90 and Glu92 in the histone dimer acidic patch.

RCC1 DNA-binding loop van der Waals contacts

The interaction of RCC1's DNA-binding loop with nucleosomal DNA at superhelical location 6 are supplemented by van der Waals contacts with histone H2A. The RCC1 Arg239 side chain that interacts with the DNA phosphate backbone is within 4.0 Å of H2A Thr76 side chain atoms. H2A Thr76 further makes van der Waals interactions with RCC1 Thr238 via side chain contacts. Separately, RCC1 Ile236 is in van der Waals contact with H2A Arg71. These RCC1-histone interactions around the DNA-binding loop all involve H2A side chains and none are made to histone H2B or to main chain atoms.

Comparison of 601 DNA and α-satellite DNA nucleosome structures

The 601 DNA and α-satellite DNA nucleosome share overall similar structures: the rmsd for histone main chain atoms is 0.86 Å for all main chain atoms, 0.49 Å if divergent tail residues are removed; the rmsd for DNA sugar C1' atoms in the central 91 bp is 1.11 Å (all comparisons made to 147 bp human α-satellite nucleosome core particle, PDB id 1KX5).

However, the structures of the 601 DNA and the 147 bp α-satellite DNA are significantly different at SHL±5 due to the unanticipated loss of one base pair for the 601 nucleosome at these positions. Other crystallographic structures of variant α-satellite nucleosome core particle have also observed localized changes around SHL±2 and SHL±5. Richmond and colleagues have noted that DNA in a nucleosome core particle forms alternating 5 bp minor and major groove blocks facing the histones[12,14]. The only exceptions occur at SHL±2 and SHL±5 where the major groove blocks can accommodate either 5 or 6 bp (Fig. 4d). For example, in the canonical 147 bp α-satellite nucleosome core particle, each of these four major groove blocks contains 6 bp[14]. However, several nucleosome core particle crystal structures incorporating variant DNA lengths (145 or 146 bp) or histone species (human vs Xenopus) contain 5 bp blocks at SHL±2 or SHL±5[12,13,16]. We find that the 601 nucleosome also contains 5 and not 6 bp major groove blocks at both SHL-5 and +5, but retains 6 bp blocks at SHL±2. In comparison, the crystal structure of a nucleosome core particle containing 145 bp of α-satellite DNA shows that the major groove at SHL±2 accommodates the two missing bases as compared to the 147 bp structure[13]. It is interesting that the path of the 601 DNA also differs from the 147 bp α-satellite DNA around SHL±2, although without significant changes in the DNA twist or number of bases in the major groove at this position (6 bp in both 601 and 147 bp α-satellite DNA, Fig. 4d).

The stretching of DNA around SHL±5 in the 601 nucleosome is accompanied by changes in the histone-DNA contacts at this location. SHL±5 is flanked by histone-DNA interactions made by histones H2A-H2B α1α1 helices to the minor groove at SHL±4.5 and histones H2B-

H2A L1L2 loops to the minor groove at SHL±5.5. In the 601 nucleosome, the 601 DNA is positioned closer to the histone H2B α1 helix, permitting hydrogen bonding between Tyr37 and Tyr39 with the phosphate backbone at SHL-5 and SHL+5 (Suppl. Fig. 5a, b). In contrast, only Tyr37 hydrogen bonds with the phosphate backbone in the 147 bp α-satellite nucleosome core particle and then only at SHL+5 (Suppl. Fig. 5c, d).

Our structural understanding of nucleosome core particles has been facilitated by crystal structures of 12 or more variant nucleosome core particles. The internal arrangements of nucleosome core particles within these crystals have been essentially the same, with all molecules packing in the same space group ($P2_12_12_1$) and making DNA end to DNA end crystal contacts at both DNA ends[9-13,15-17]. The RCC1/nucleosome core particle complex packs differently (space group $P2_1$), and all crystal contacts are mediated by RCC1. There are no visible direct contacts made between nucleosome core particles in the RCC1/nucleosome core particle crystal. Since our 601 DNA contained 147 bp and the central 145 bp are contained within the nucleosome core particle, the additional one base pair on each end extend beyond the canonical nucleosome core particle. On the downstream end, the terminal base pair is visible due to crystal contacts with RCC1, while the terminal base pair on the upstream end is disordered and no crystal contacts are evident here. We suspect that the 601 nucleosome structure presented here is representative of the structure in solution since relatively few direct crystal packing interactions are made by the 601 nucleosome, but this conclusion will require further validation since one of the two DNA ends does mediate a crystal contact with RCC1.