Supplementary 1

1. Modelling of the 30nm fibre

A nucleosome in an array rotates if it slides away from (or towards) its preceding neighbour and thus, the length of the linker DNA changes. This rotation in turn alters the trajectory of the linker DNA (exiting the nucleosome) that follows. Therefore, within the array, the path of each linker is governed by the length of the preceding one. The overall topology of the array is then determined by the pattern of its nucleosome spacing. A nucleosome rotates 360° when sliding over a distance of 10.5 bp (helical repeat of relaxed DNA) along the DNA molecule. This means nucleosomes positioned at every 10.5 bp (or n x 10.5 bp) in an array will have parallel orientations, such that in a zigzag arrangement two opposing nucleosome stacks lie in the same plane (Figure 1a). However, the observation of n x 10 bp linkers *in vivo* suggests that this geometry is less likely. For example two nucleosome cores separated by 3 x 10 bp DNA rotate relative to each other by -51° (left-handed), as a nucleosome travelling the length of 3 x 10 bp will have a 51° deficit from making three complete turns (3 x 360°) which would be the case if the distance were to be 3 x 10.5 bp. Consequently, an array with this spacing will have a different geometry in which two opposing stacks coil into a left-handed double helix (Figure 1b). The helical pitch is determined by the amount of rotation amongst successive nucleosomes (~ 140° in Figure 1b). This geometry is equivalent to the proposed crossed-linker model. On the other hand, binding of linker histone alters the relative orientation of the stacks since it imparts a positive twist in the linker DNA (see main text). In the above example, the -51° deficit would be reduced —making it less negative and tending towards 0° . In this situation, the compensating writhe converts the crossed linker form to a helical ribbon (Figure 2). With longer linkers this effect is offset by increasing negative twist between successive nucleosomes, in which case the writhe element is small so that the overall geometry remains largely crossed-linker. The transition from a helical ribbon towards a crossed-linker alters the inclination of the linker DNA, in view of this the two models are essentially ‘same’structures. In all cases, the pitch of the helical stack is determined by the amount of rotation amongst successive nucleosomes (~140° in Figure

1b).

We emphasise here that helicity is intrinsic in arrays of n x 10 bp linker lengths (or any linker lengths ≠ n x 10.5 bp). In other words, structural consequences are predetermined by the chromatin remodelling machinery that specifies nucleosome spacing. Linking number is unlikely to change during fibre compaction, and consequently the ends of an array do not rotate during local folding/unfolding, an effect which would otherwise stress adjacent chromatin structures. Importantly a constant linking number drives topological compensation, for example, the writhe brought about by linker histone (see above) and therefore facilitates folding. Furthermore linker histone orders the trajectory of linker DNA by maintaining its entry/exit angle (to/from a nucleosome). Both uniformity in linker length and linker trajectory are essential for folding. Relaxing either constraint breaks down compaction (and regularity) and the fibre unfolds.

In principle a nucleosome array may assume a crossed-linker conformation (see above) or its topological equivalence, a helical ribbon (Figure 2). However, it is likely that the end point of folding is subject to additional constraints in vivo, for example minimising the helical pitch to optimise nucleosome stacking. The topology of the array can be modelled assuming a constant distance between stacked nucleosomes and that linker DNAs remain straight. The helical pitch, and hence packing density and diameter, is determined by linker length, which affects the relative rotations of nucleosomes (see above). We derived:

$$diameter_{\text{crossed-linker}} = 2 \cdot \cos\beta \cdot a \cdot \tan\lambda / \sin(2 \cdot \lambda) + 11 \quad (1)$$

$$density_{\text{crossed-linker}} = \left(2 + 360^\circ/(4 \cdot \lambda)\right) \cdot 11 \Big/ \left(\left(360^\circ/(4 \cdot \lambda)\right) \cdot \sin\theta \cdot a \cdot \sin\beta\right) \quad (2)$$

$$diameter_{\text{helical ribbon}} = \left(\cos\delta \cdot \left(360^\circ/(8 \cdot \lambda)\right) \cdot b + \sin\delta \cdot (\cos\beta \cdot a + 11)\right) \Big/ \pi + 11 \quad (3)$$

$$density_{\text{helical ribbon}} = 2 \cdot \left(2 + 360^\circ/(4 \cdot \lambda)\right) \cdot 11 \Big/ \left(2 \cdot \sin\delta \cdot \left(360^\circ/(8 \cdot \lambda)\right) \cdot b\right) \quad (4)$$

where a = linker length, b = distance between nucleosome i and i+2 (a, b measure the nucleosome centre to centre distance), 2α = relative rotation between nucleosome i and

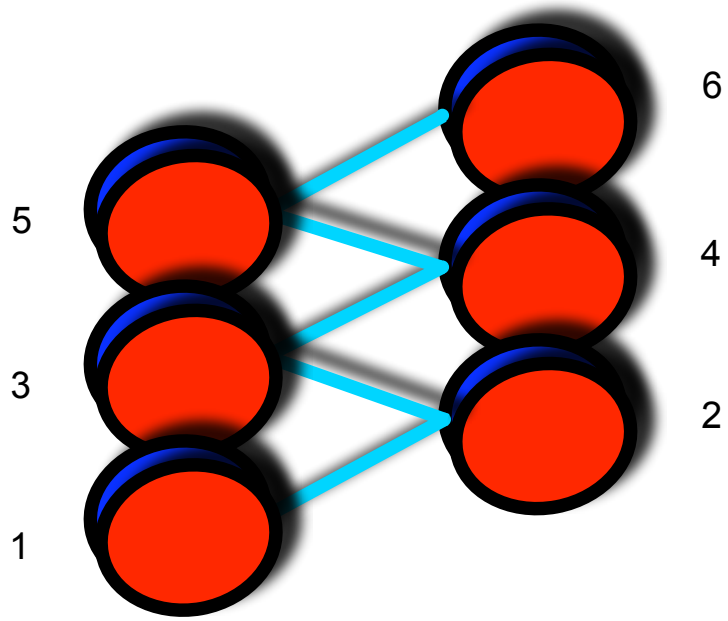
i+1, $\beta = \arcsin\left(b/2/a\right)$, $\lambda = \arcsin\left(\sin\alpha \cdot \sin\beta\right)$, $\theta = \arccos\left(\cot\beta \cdot \tan\lambda\right)$, $\delta = \arctan\left(\left(\cos\beta \cdot a + 11\right)\Big/\left(\left(360^\circ/\left(8 \cdot \lambda\right)/2\right) \cdot b\right)\right)$.

We asked whether the folding pathway we consider here gives rise to the degrees of compaction that are consistent with the fibre dimensions observed in EM studies (12), that is to say whether equations (1)-(4) predict the trend of fibre diameter and density in response to the change in linker length. We applied the principles of global curve fit, in which b was set as the global parameter, while a and 2α varied concomitantly with an increment of 3.4 nm and 17° respectively. We found that experimental data points are well described by (1)-(4) together with $a = 15.8 + n \times 3.4$ nm, $b = 4.4$ nm, $2\alpha = 44.6^\circ + n \times 17^\circ$, where n = 0, 1, 2, 3, 4, 5, 6 corresponding to 177, 187, 197, 207, 217, 227, 237 bp repeats. The predictions improve further where we allowed 2α to increase by variable increments. In this situation, $a = 17.2 + n \times 3.4$ nm, $b = 3.9$ nm, $2\alpha = 57.6^\circ + n \times 17^\circ \left(n \le 3\right)$ and $2\alpha = 57.6^\circ + n \times 17^\circ - \left(n - 4\right) \times 12.6^\circ \left(n \ge 4\right)$.

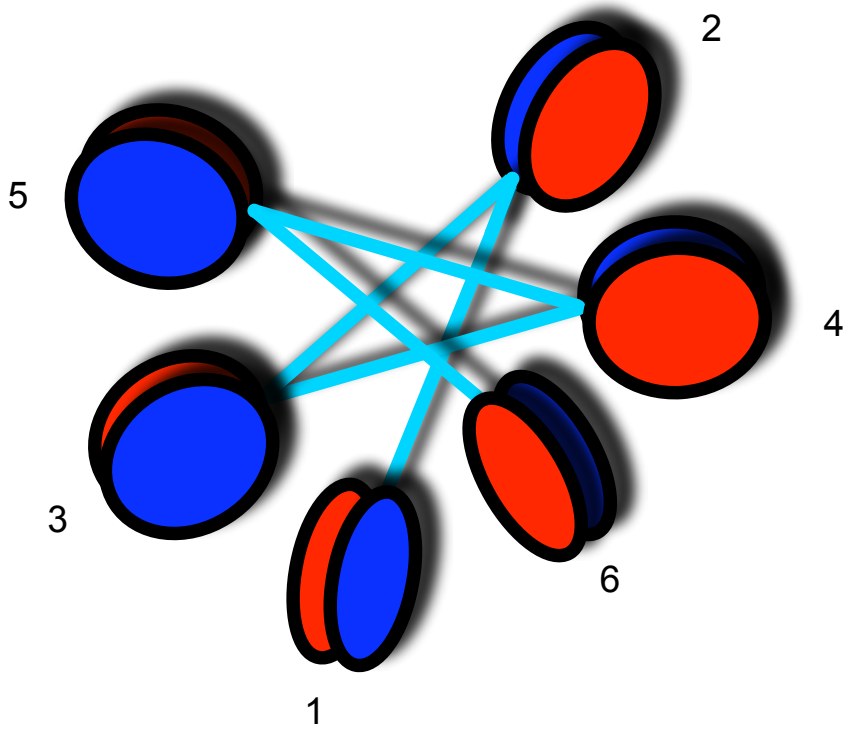
Legends to Figures

Figure 1. Different topologies of nucleosome arrays. Individual nucleosomes are drawn as units of red and blue discs to mark the relative orientations of successive nucleosomes. Note that the linker DNA enters the red side of the nucleosome and exits from the blue side. a) linker DNA = n x 10.5 bp. b) linker DNA = n x 10 bp.

Figure 2. The topological conversion between twist and writhe. The blue and red columns represent the opposing nucleosome stacks, where dotted lines approximate the orientations of linkers. Equilibrating from left to right converts a crossed-linker conformation to a helical ribbon. The linking number remains constant.



a. linker length = n x 10.5 bp



b. linker length = n x 10 bp