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**Modeling chromatin fibre folding  
for human embryonic stem cells**

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# Modeling chromatin fibre folding for human embryonic stem cells

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## ABSTRACT

In this study we analyze the chromatin state of human pluripotent stem cells by geometric modelling of fibre conformation. The model takes into account local structure of chromatin organized into euchromatin, permissive for gene activation, and heterochromatin, transcriptionally silenced. Euchromatin modelled using linear DNA while heterochromatin by means of a solenoid structure in which DNA winds onto six nucleosome spools per turn. Two geometric models are presented and are compared in terms of geometric quantities. The models are tested using in vivo data generated from chromatin immunoprecipitation human from embryonic stem cells. This study provides insight into and tools for identifying the relationships between chromosome geometry and epigenomic processes associated with chromatin remodeling, cellular reprogramming and maintenance of cellular pluripotency.

**Keywords:** chromatin structure, pluripotency, epigenomics, geometric modelling, euchromatin, heterochromatin.

## 1. Introduction

The human genome is estimated to contain approximately 30,000 unique genes. Though every gene exists within every cell in the human body, only a small percentage of these genes is active in any given cell. What promotes the transcription of cell-specific genes and determines the cell identity? The chromatin structure and its ability for remodeling into different states.

The chromatin state recently emerged as one of the governing factors for self-renewal and pluripotency of embryonic stem (ES) cells. Recent data show that stem cell chromatin is distinct from that of somatic or differentiated cells in several different structural and functional aspects such as global chromatin arrangement and condensation and compaction and that undifferentiated ES cells are characterized by hyperdynamic plasticity of chromatin proteins, supporting an open conformation model of chromatin in undifferentiated stem cells.

The chromatin state of a cell is defined through the establishment and the maintenance of localized open and closed states of the chromatin structure, determined by epigenomic interactors.

In fact mutations in epigenomic regulators have the potential to alter the chromatin structure, leading to mis-regulation of gene expression and contributes to cancer or other diseases.

Therefore understanding chromatin remodeling is of fundamental significance in understanding cancer and for regenerative medicine.

The focus of our research is on identifying and characterizing the chromatin state of Pluripotent Stem Cells (PSCs). PSCs are characterized by extensive self-renewal and multi-lineage differentiation potential. PSCs generate all functional tissues of the body during development and adult stem cells (SCs) that allow for regeneration of these tissues following injury or degenerative processes. In this study we present a summary of an extensive analysis on the chromatin state using in vivo data generated from chromatin immunoprecipitation of human embryonic stem cells with Oct4, Sox2 and Nanog transcription factors from [1]. The investigation is carried out using geometrical models of the chromatin fibre conformation.

## 2. Measures and energetics of filament coiling

In this section we show how to investigate several geometric features of the DNA filament, such as writhing, inflexional configuration, torsion and twist localization, in relation to properties of physical interest, such as elastic deformation energy and filament compaction [2,3].

For this purpose we refer to an inextensible, smooth, simple closed curve  $C$  in the three-dimensional space  $\mathbb{R}^3$ , thought of as the central axis of a closed, double-stranded DNA filament. Each point on  $C$  is labeled by the position vector  $\mathbf{X}=\mathbf{X}(\xi)$ , where  $\xi\in[0,L_{fin}]$ , its curvature by  $c(\xi)$ , torsion  $\tau(\xi)$  and  $\hat{\mathbf{t}} = \frac{\mathbf{X}'(\xi)}{\|\mathbf{X}'(\xi)\|}$  the unit tangent to  $C$  at  $\xi$  and  $l(\xi)$  the length function.

Coiling is measured by the *normalized total curvature* of  $C$  that is given

$$\mathcal{K} := \frac{1}{l(\xi)} \int_0^{L_{fin}} c(\xi) \|\mathbf{X}'(\xi)\| d\xi .$$

The folding process conserves topology. For a thin filament this means conservation of the linking number  $Lk$  [4,5], given by

$$Lk := Wr + Tw ,$$

where  $Wr$  represents the *writhing number* [6] and  $Tw$  represents the total twist of the filament fibers. These are assumed to be wound uniformly around the axis  $C$ .

Denoting by  $\Omega = \Omega(\xi)$  the angular twist rate of the fibers, we have that the *normalized total twist* is given by

$$Tw := \frac{1}{l(\xi)} \left( \frac{1}{2\pi} \int_0^{L_{fin}} \Omega(\xi) \|\mathbf{X}'(\xi)\| d\xi \right) ,$$

which is related to the geometry of the filament axis through the decomposition

$$Tw := \frac{1}{l(\xi)} \left( \frac{1}{2\pi} \int_0^{L_{fin}} \tau(\xi) \|\mathbf{X}'(\xi)\| d\xi + \frac{1}{2\pi} [\Theta]_{\mathcal{F}} \right) ,$$

where the first term in the r.h.s. is the normalized total torsion  $\mathcal{T}$  and the second term is the normalized intrinsic twist  $\mathcal{N}$  of the fibers *around*  $C$ .

In terms of deformation energy, the process of folding and coiling of DNA filaments due to the relaxation of high twist is characterized by a transfer of torsional energy to bending energy. To analyze the energetics of folding we consider the DNA filament modeled by a thin, inextensible rod, of uniform circular cross-section of area  $A = \pi a^2$  centered on  $C$ .

The elastic characteristics are specified by the bending rigidity  $K_b$  and the torsional rigidity  $K_t$  of the filament. In general  $\chi \in [1,1.5]$ , with  $\chi = \frac{K_b}{K_t}$ . The stress-strain relation leads to the conventional quadratic form of the deformation energy, given by

$$E = E_b + E_t = \frac{1}{l(\xi)} \int_0^{L_{fin}} \left[ K_b (c(\xi))^2 + K_t (\Omega(\xi))^2 \right] \|\mathbf{X}'(\xi)\| d\xi ,$$

where we assumed a zero natural twist rate of the filament fibers. The deformation energy  $E$  is thus given by the sum of the bending energy  $E_b$  due to curvature effects, and the torsional energy  $E_t$ , due to torsion and intrinsic twist.

## 3. Geometric models of the 30nm chromatin fiber

In eukaryotes, the DNA is organized in a chromatin fiber, a complex made of DNA wrapped around a core histone octamer, which consists of histones of 2 copies each of H2A, H2B, H3, H4. The chromatin building block is the nucleosome. In the core particle, 147 base pairs (*bp*) are wrapped in 1.7 left-handed superhelical turns around the histone octamer. Each nucleosome core is connected by a *linker* DNA to make repetitive motifs so the fiber forms a “beads-on-a-string” like model [7]. More than 30 years ago, Finch and Klug first proposed that the nucleosome is folded into 30-nm chromatin fibres [8].

The detailed structure of the chromatin fiber has remained controversial for over three decades [9,10], with evidence supporting both zigzag (“two-start”) [11,12,13] and solenoid (“one-start”) [8] models coming from various sources, including X-ray crystallography and electron microscopy. In

the solenoid model, consecutive nucleosomes are located next to each other in the fibre, folding into a simple one-star helix. Subsequently, a second model of the two-star helix was proposed on the basis of microscopic observations of isolated nucleosomes, where a nucleosome in the fibre is bound to the second neighbor but not the first.

Over several years, chromatin models have been designed in increasing level of detail, each of which is suitable for certain biological problems and applications. The first-generation “macroscopic” models treated the nucleosome and the wound DNA according to general mechanical and electrostatic properties, with the histone tails approximated as rigid bodies and linker histones neglected [14,15,16,17,18]. It captured the fundamental monovalent-salt dependent mechanics of chromatin and the thermal fluctuations of the nucleosome and linker DNA.

Chromatin has finally been crystallized, providing some measurements on the way DNA is wrapped around an octamer. We will restrict our study on the basis of geometry without introducing any kind of interaction. We will use the general assumption that the local structure of the chromatin is periodical such that each pattern has exactly the same environment. We assume that there are six nucleosomes per turn in the solenoid with each nucleosome radius of  $r = 5.5 \text{ nm}$ , and a wrapping angle of the DNA molecule around a nucleosome given by  $\theta = 3.5 \pi$ , shaped into a helix with a pitch of  $P = 2.8 \text{ nm}$  and with a nucleosome wrapping length:  $\Lambda = \theta \sqrt{r^2 + \frac{P^2}{4\pi^2}} \approx 5.5 \text{ nm} \approx 147 \text{ bp}$ . The connection between two nucleosomes is described by a linker-DNA length of  $b = 50 \text{ bp}$ . The quantity  $L = \Lambda + b = 197 \text{ bp}$  is referred to as the *repeat length* and a value of  $200 \text{ bp}$  is often considered to be realistic [19,20].

We introduce now two geometric models for a single solenoid turn: the *six helices model* and the *torus unknot model*.

### 3.1 Six helices model for a solenoid turn (Heterochromatin)

Assigning a number  $i = 0, \dots, 5$  to the  $i$ -nucleosomes which belong to a single solenoid turn, the following parametric equation  $\mathbf{X}_i(\xi)$  represents the DNA filament wrapped around the corresponding nucleosome  $i = 0, \dots, 5$  (see Figure 1(left side)), where the parameter  $i(2\pi) \leq \xi \leq (i+1)2\pi$  parameterizes the points of the curve along its length:

$$\mathbf{X}_i: \begin{cases} x = \frac{\pi(P - 2r \sin(\frac{1}{6}(2\pi i + \pi)) \cos(\theta(\xi - 4\pi i))) + P(-4\pi i + \xi - \pi) \cos(\frac{1}{6}(2\pi i + \pi)) + 2\pi R \cos(\frac{1}{3}\pi(i-1))}{2\pi} \cdot \frac{\Lambda}{l(\xi)} \\ y = \frac{P(-4\pi i + \xi - \pi) \sin(\frac{1}{6}(2\pi i + \pi)) + 2\pi r \cos(\frac{1}{6}(2\pi i + \pi)) \cos(\theta(\xi - 4\pi i)) + 2\pi R \sin(\frac{1}{3}\pi(i-1))}{2\pi} \cdot \frac{\Lambda}{l(\xi)} \\ z = r \sin(\theta(\xi - 4\pi i)) \cdot \frac{\Lambda}{l(\xi)} \end{cases}$$

and where, in order to ensure inextensibility, each component has been normalized by the length function

$$l(\xi) = \int_0^{L_{fin}} \left[ \left( \frac{\partial x}{\partial \xi} \right)^2 + \left( \frac{\partial y}{\partial \xi} \right)^2 + \left( \frac{\partial z}{\partial \xi} \right)^2 \right]^{\frac{1}{2}} d\xi.$$

This re-scaling ensures that the total length is kept fixed at  $\Lambda = 147 \text{ bp}$ . (for further details see [1]). Parameters  $P$ ,  $\theta$ , and  $r$  are defined as above and  $R = 9.5 \text{ nm}$  such that  $R + r = 15 \text{ nm}$ .

Connections  $\mathbf{Y}_i(\xi)$ , between two successive nucleosomes (DNA linkers) are approximated by a cubic Hermite spline interpolation [21], a particular class of a third-degree spline interpolation with each polynomial of the spline in *Hermite form*, which consists of two control points and two control tangents for each polynomial. For a detailed description of the mathematical model in Hermite form adopted to describe DNA linkers we refer to [1]. A single turn of the six helices model composed

by the 6 helices  $\mathbf{X}_0, \mathbf{X}_1, \mathbf{X}_2, \mathbf{X}_3, \mathbf{X}_4, \mathbf{X}_5$ , the connections between two successive nucleosomes  $\mathbf{Y}_0, \mathbf{Y}_1, \mathbf{Y}_2, \mathbf{Y}_3, \mathbf{Y}_4$  and the connection  $\mathbf{Y}_5$  with the next solenoid layer is displayed in Figure 1 (left side).

### 3.2 Torus unknot model for a solenoid turn (Heterochromatin)

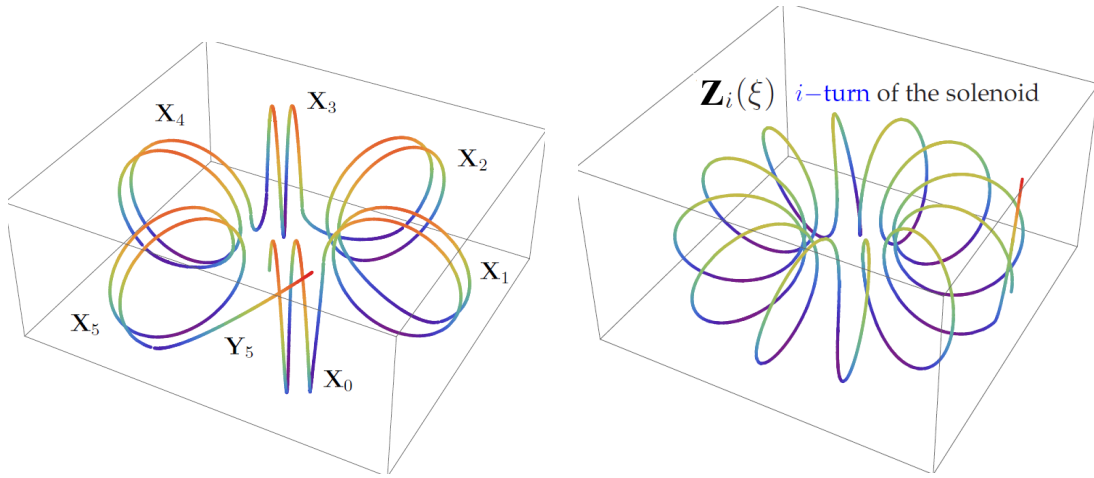
We propose now another model for a single solenoid layer. Let  $\mathbf{Z}_i(\xi)$  represents an open *Fourier torus unknot* [22], where  $i(2\pi) \leq \xi \leq (i+1)2\pi - h$ ,  $i = 0, \dots, N-1$ , is a parameter along the curve,  $N$  the total number of solenoid turns and  $h$  a small value in order to leave the curve open and to be connected with the next solenoid turn.

$$\mathbf{Z}_i(\xi): \begin{cases} x = \cos(\xi) \left( R + r \cos \left[ \left( \frac{Q}{P} \right) \xi \right] \right) (6L - b) / l(\xi) \\ y = \sin(\xi) \left( R + r \cos \left[ \left( \frac{Q}{P} \right) \xi \right] \right) (6L - b) / l(\xi) \\ z = r \sin \left[ \left( \frac{Q}{P} \right) \xi \right] (6L - b) / l(\xi) \end{cases}$$

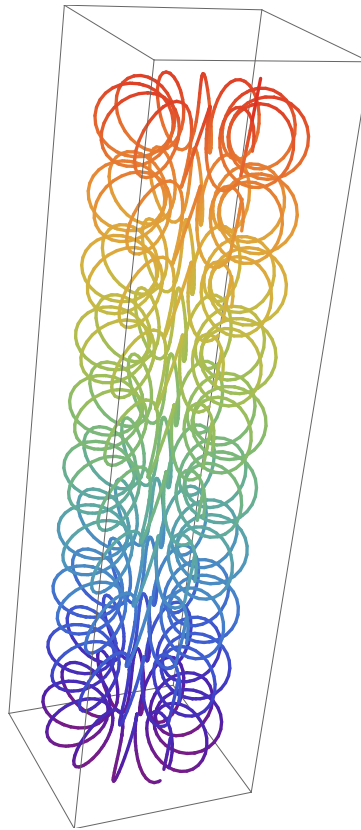
The DNA filament winds around a torus  $P$ -times ( $P=1$ ) in the longitudinal direction and  $Q$ -times ( $Q=12$ ) in the meridian direction so to have 2 turns per nucleosome (see Figure 1 right side).

In order to ensure inextensibility, each component has been normalized by the length function  $l(\xi)$  where its length is fixed at  $6L - b = 1132 bp$  and  $r$  and  $R$  chosen as above.

The  $i$ -turn  $\mathbf{Z}_i(\xi)$  of the solenoid finally is connected with the  $(i+1)$ -turn  $\mathbf{Z}_{i+1}(\xi)$  by means of cubic hermite spline functions of length  $b$ . An example of the final solenoid structure composed by 10 turns is shown in Figure 2.



**Figure 1:** (On the left) Side view of a single turn of the six helices model composed by the 6 helices with a parametric equation  $\mathbf{X}_0, \mathbf{X}_1, \mathbf{X}_2, \mathbf{X}_3, \mathbf{X}_4, \mathbf{X}_5$ , the connections between two successive nucleosomes and the connection  $\mathbf{Y}_5$  with the next solenoid layer. (On the right) Side view of a single turn of the *torus unknot model* composed of 12 turns in the meridian direction and one in the longitudinal direction. The connection with the next solenoid layer is also shown.



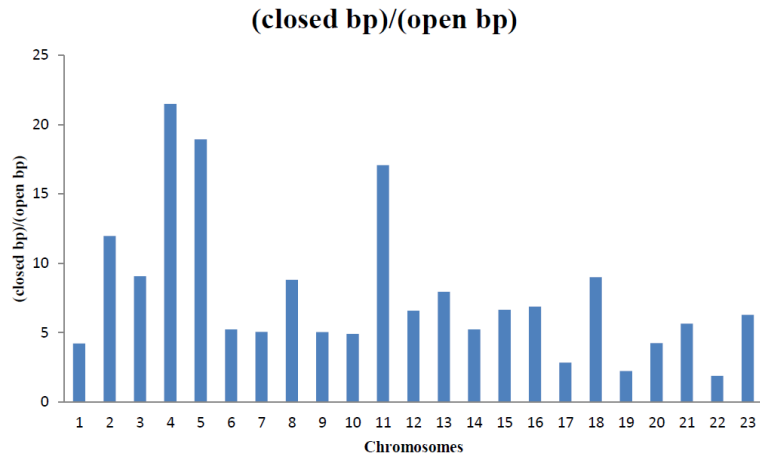
**Figure 2:** Example of the solenoid model (10 turns).

#### **4. Numerical results (ChIP Data Analysis)**

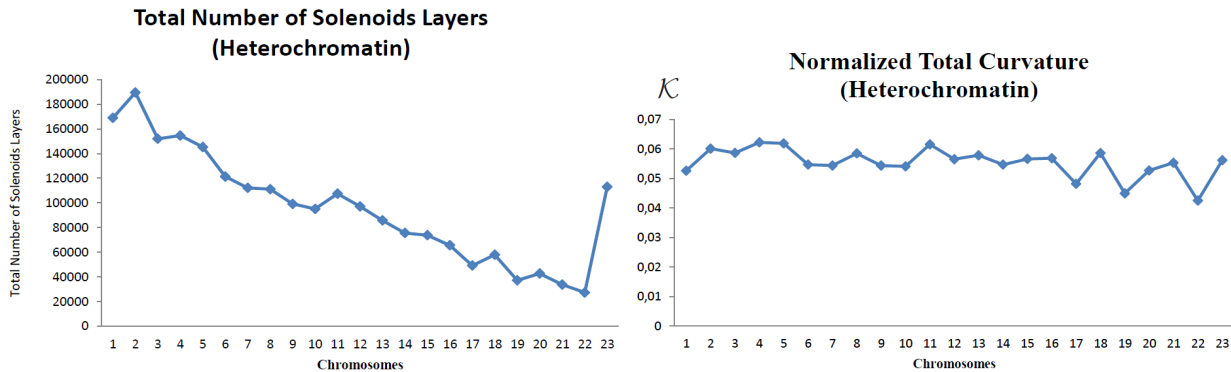
To study the chromatin state of PSCs we utilize extensive ChIP (Chromatin Immunoprecipitation) data generated from native pluripotent human embryonic stem cells (ESCs) [23]. ChIP experiments were performed with antibodies specifically against Oct4, Sox2 and Nanog, transcription factors (TFs) important for maintaining the pluripotential state. ChIP analysis was used to identify binding sites for Oct4, Sox2 and Nanog transcription factors on all chromosomes. Recently it was demonstrated that Oct4 alone, or Oct4, Sox2 and Klf4 in combination were sufficient to generate iPSCs (induced pluripotent stem cells) from various types of cells of the human body. This suggests that iPSCs are generated by, local and/or global chromatin structural changes that result in chromatin remodeling induced by these transcription factors binding to particular regions of the chromosomes. Chip analysis generates local and global pictures of the chromosome structure of iPSCs. Structural changes can be investigated at different time points during cellular reprogramming since changes are maintained for analysis by a chemical cross-linking step utilized with ChIP. Following crosslinking, chromatin is broken and immunoprecipitation is performed resulting in the purification of protein–DNA complexes. DNA sequencing or microarray analysis identify on the chromosomes where the protein binds on a genome-wide scale.

We have developed an algorithm allowing us to model the chromatin structure and therefore state of human pluripotent cells, based on the observation that Oct4, Sox2 and Nanog are sufficient to initiate a cascade of histone protein modifications that result in an epigenomically induced permanent change of cell state, a process called cell transdifferentiation. Regions where Oct4, Sox2 and Nanog bind we conjecture are sites in which the chromosome are more responsive to, and therefore in a more open state for transcription. We analyzed approximately 3000 chromosome regions bound by Oct4, Nanog and Sox2 proteins (see supplementary material). Results are plotted in Figures 3-4. Figure 3 represents the ratio between base pair regions that are open or close over all

the 23 chromosome pairs of PSCs. Figure (left side) shows the total number of solenoid layers, computed according to the models presented in Section 3, reflecting the total length of the corresponding chromosomes. Finally Figure 4(right side) show the normalized total curvature over all the 23 chromosome pairs computed according to the torus unknot model. From the graphics we can deduce that in PSCs, the chromatin state is not uniformly similar, with Oct4, Sox2 and Nanog promoting a more decondensed-like state of chromatin in ch. 22. Variability in the decondenstations states of the different chromosome pairs, provides an distinctive image of the chromatin conformation state of human embryonic pluripotent cells. For further details see [1].



**Figure 3.** Ratio between closed and open base pairs according to Oct4, Sox2 and Nanog binding regions over all the 23 chromosome pairs of human PSCs.



**Figure 4:** (left side) Total number of solenoid layers, and (right side) normalized total curvature over all the 23 chromosome pairs of PSCs.

	$\mathcal{K}$ per turn	$E_b$ per turn	$bp$ per turn
Six helices model	76.97	7.61	1182
Torus model	75.43	5	1182

**Table 1:** Normalized total curvature, normalized bending energy and total number of base pairs per turn, respectively according to the *six helices model* (see Section 3.1) and *torus model* (see Section 3.2).

## 5. Conclusions

Induced pluripotent cells hold great promise for regenerative medicine, like embryonic stem cells since they have the potential to generate all cells of the human body and therefore, can be utilized for cell or tissue replacement therapies. The iPSCs are generated by chromatin remodeling of non-pluripotent cells, an epigenomic process that is still not well characterized. The chromatin state of iPSCs is determined by local and global chromatin structural changes that are generated by transcription factor binding to particular regions of the chromosomes. The binding of the transcription factors initiates chromatin structural remodeling induced by epigenomic factors that have the capacity to modify histone proteins. Two geometric models for chromatin fibre conformation are presented and compared in terms of geometric quantities. The models are tested on ChIP Data generated from human pluripotent embryonic stem cells (PSCs) [23]. These models provide insight into the chromatin structural state of human pluripotent stem cells in terms of geometric spatial information.

We have found that in PSCs the chromatin state is not uniform for all the chromosomes, with Oct4, Sox2 and Nanog activity promoting more decondensation of chromatin structure in certain chromosomes than in others, in particular on chromosomes 19 and 22 (open state). These differences in chromosome geometry provide a deeper insight into processes associated with cellular reprogramming.

Future bio-mathematical-informatics modeling of chromosome structural states of particular cell types, is an important contribution for identifying epigenomic agents and mechanisms in regenerative medicine and disease models such as cancer and ailments associated with particular life style conditions, such as cardiovascular disease and diabetes [24,25].

Ongoing work in our laboratory with ChIP analysis, using antibodies against specific components of the transcriptional machinery will allow us to define at higher resolution the structural chromatin state of specific cell types and the various transitional chromatin states during cellular reprogramming and disease.

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