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Rapport sur les contributions

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ID de Contribution: **1** Type: **Non spécifié**

Multiple-scale polymer model of bacterial chromosomes

Supercoiled DNA often adopts tree-like double-folded branching configurations. In this context, we proposed a framework to generate expected bacterial chromosome structures at multiple scales. We built a coarse-grained model of bacterial DNA, which is known to adopt tree-like plectonemic structures due to negative DNA supercoiling. To this end, we extended our previous model of elastic polymer chains on an FCC lattice for tightly double-folded ring polymers [1] to include the possibility of generating long branches, with the average length becoming the parameter of the model. Considering DNA concentration and cylindrical confinement similar to the in vivo situation, we adjusted this average length parameter to reproduce as well as possible contact properties between chromosomal loci as obtained from high- throughput chromosome conformation capture methods (Hi-C). Finally, we obtained various coarse-grained models that are consistent with each other and that allow capturing contact properties of various bacteria, from 2 kb to 1 Mb scale. In other words, we are able to rationalize from first principles contact properties between bacterial chromosomal loci as measured from Hi-C methods.

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ID de Contribution: **2** Type: **Non spécifié**

A Dynamic Single-Molecule Approach to Directly Visualize the Molecular Mechanisms of DNA-Binding Proteins.

Studying and validating the molecular mechanism of DNA processes often requires direct functional evidence. Here we present the C-Trap: a dynamic single-molecule microscope that allows researchers to directly visualize the dynamics and assembly of biological complexes under different conditions. All at the single-molecule level, providing direct proof of the biological mechanisms being studied.

Fully understanding the mechanism underlying DNA repair, transcription, editing, or organization requires a multidisciplinary approach. Structural techniques such as cryo-EM reveal unprecedented detail into the structure of the proteins, but they are static representations and do not provide direct functional evidence. On the other hand, bulk biochemical assays provide insights into the function, but the outcomes are averaged in time and over many molecules, hindering information regarding the exact effect of different actors in the molecular mechanism. By adding dynamic single-molecule information one gains direct proof of how each component works together in the molecular machinery.

Here, we present our efforts to further enable discoveries in the field of DNA-protein interactions and DNA organization using the combination of optical tweezers with correlative confocal fluorescence microscopy. We present several examples in which our technologies enhanced the understanding of the DNA repair mechanisms, chromatin structure and DNA editing tools. Furthermore, we show that advances in hybrid single- molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new venues in the field of DNA-protein interactions.

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ID de Contribution: **3** Type: **Non spécifié**

Cell cycle dependent oscillations of gene expression in E. coli

A long-standing hypothesis sees DNA replication control in *E. coli* as a central cell cycle oscillator at whose core is the DnaA protein. The consensus is that the activity of the DnaA protein, which is dependent on its nucleotide bound state, is an effector of initiation of DNA replication and a sensor of cell size. However, while several processes are known to regulate the change in DnaA activity, the oscillations in DnaA production and DnaA activity have never been observed at the single cell level, and their correlation with cell volume has yet to be established. Here, we measured the volume-specific production rate of a reporter protein under control of the *dnaA*P2 promoter in single cells. By a careful dissection of the effects of DnaA-ATP- and SeqA-dependent regulation of dnaAP2 promoter activity two distinct cell cycle oscillators emerge. The first one, driven by both DnaA activity and SeqA repression, is strongly coupled to cell cycle and cell size, and its minima show the same behaviour as initiation events, following each other by a constant added size. The second, a reporter of DnaA activity, is still coupled with cell size but not to the time of cell division and the added size between its minima is dependent on the cell size. These findings suggest that while DnaA activity and gene dosage perform volume sensing, SeqA repression primes the DnaA oscillator to follow initiation of DNA replication, thus setting the cell size of initiation of the next replication round in the daughter cells.

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ID de Contribution: **4** Type: **Non spécifié**

STRESSING CHROMATIN, FROM THE CELL TO THE NUCLEOSOME SCALE

The physical impact of the nucleus on cellular function becomes evident during migration in 3-D environments. With its large volume and relative rigidity, governed by the nuclear envelope proteins and chromatin organization, the nucleus acts as physical barrier, particularly relevant to immune cells and invading cancer cells. These must move through tissue pores and clefts often smaller than the size of the nucleus, which induce substantial compressive and shearing stresses, and may also lead to the temporary rupture of the nuclear envelope. Recent studies amply demonstrated that extreme nuclear deformations during confined migration can lead to DNA damage and increased genomic instability in cancer cells.

Here we shed a first light on the molecular processes of stress transfer and relaxation down the scale of the individual chromatin units, the nucleosomes. In this talk, we will briefly outline our innovative experimental techniques aimed at measuring and biophysical signatures of cancer cells, notably single-cell MEMS nano-tweezers that provide high sensitivity to examine different biophysical properties (size, stiffness, viscosity, etc.), and high-throughput MEMS devices oriented at clinical applications. Then, by using molecular dynamics simulations of force-induced poly-nucleosome deformation under ideally controlled conditions, we show that external forces acting on the nucleosome core particle transmit a mechanical stress, which is mainly translated as elastic energy stored in the elastic and plastic response of DNA. The ability of the double-stranded DNA helix to absorb and release this stress, most notably in the form of bending and twisting deformations, may constitute a platform to elicit or repress the interaction with remodeler proteins, by controlling their access to active histone domains.

The concerted actions of mechanical deformation and remodeler enzymes open the way for a new framework, to understand the microscopic control of chromatin organization by mechanical forces, and the attending modifications of gene expression and transcription factor activity.

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ID de Contribution: **5** Type: **Non spécifié**

Why chromosomal translocations are cell type-specific?

Most cancer-related chromosomal translocations appear to be cell type-specific. It is currently unknown why different chromosomal translocations occur in different cells. This can be either due to the occurrence of particular translocations in specific cell types or adaptive survival advantage conferred by translocations only in specific cells. We experimentally addressed this question by double-strand break (DSB) induction at *MYC, IGH, AML, ETO* loci in the same cell to generate chromosomal translocations in different cell lineages. Our results show that any translocation can potentially arise in any cell type. We have analyzed different factors that could affect the frequency of the translocations and only the spatial proximity between gene loci after the DSB induction correlated with the resulting translocation frequency, supporting the "breakage-first"model. Furthermore, upon long term culture of cells with the generated chromosomal translocations, only oncogenic MYC-IGH and AML-ETO translocations persisted over a 60-day period. Overall, the results suggest that chromosomal translocation can be generated after DSB induction in any type of cell, but as to whether the cell with the translocation would persist in a cell population depends on the cell type-specific selective survival advantage that the chromosomal translocation confers to the cell.

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ID de Contribution: **6** Type: **Non spécifié**

Poster: Targeting the 3D genomic and epigenetic changes in Mantle Cell Lymphoma

Recurrent chromosomal translocations found in most lymphomas frequently lead to overexpression of a certain oncogene, but in many cases, the expression of the oncogene alone does not suffice to produce a malignant phenotype. This is the case in mantle cell lymphoma (MCL), an aggressive B-cell non-Hodgkin lymphoma associated with the t(11;14)(q13;q32) translocation that results in the overexpression of cyclin D1 (CCND1), a potent cell-cycle regulator. Nevertheless, not all MCLs overexpress CCND1, and the CCND1 overexpression alone does not lead to malignancies in animal models. Thus, the development of MCL should be triggered by additional factors, which may guide the development of new therapies once discovered.
A chromosomal translocation can trigger large-scale changes in the 3D genome organization, as well as the transcriptional and epigenetic changes in the translocated loci. Here we demonstrated that the regions in the vicinity of the translocation breakpoint on derivative chromosomes 11 and 14 are relocated closer to the nuclear center in MCL cells. This was accompanied by the upregulation of gene expression in these regions, as well as the perturbation of the enhancer landscape of MCL cells. Several novel enhancers and superenhancers predicted to regulate the genes overexpressed in MCL were discovered, suggesting the potential utility of the enhancer-modifying substances for MCL treatment. We tested two substances with such properties, Abemaciclib and Minnelide, in MCL cell lines and the B cells from the venous blood of MCL patients. Both substances effectively reduced the viability of the malignant cells. These results provide valuable preclinical data and novel insights into the MCL pathogenesis.

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ID de Contribution: **7** Type: **Non spécifié**

Reconstitution in vitro of chromatin patterning

Mammalian cells package their genomic DNA into a complex with proteins called chromatin. The assembly and compaction of chromatin are regulated by multiple molecular cues, including DNA modifications, histone post-translational modifications, and histone variants. The spatial partitioning of chromatin into active and inactive domains has also been proposed to be regulated by LLPS. The interplay between histone modifications and spatial chromatin partitioning is currently unclear. To address this issue, I develop a reconstituted in vitro system to study chromatin partitioning and the deposition of histone methylation by single-molecule microscopy. To this end, we reconstitute chromatin fragments using fluorescently labeled histones, and we methylate them with the purified histone methyltransferase DIM5. I am currently studying the kinetics of this methylation and the spreading of methylated marks at the single-molecule level using differently-sized nucleosomes arrays. My aim is to reconstitute compacted and methylated chromatin domains in vitro, in an assay where domain formation and compaction can be directly observed by fluorescence microscopy in real-time. To this end, I tether biotinylated chromatin fibers to a fluid lipid bilayer, where they can laterally diffuse and associate with each other. I will use this system to tackle the following questions: What is the critical number of chromatin fibers required for domain formation? What are their mechanical properties? What is the interplay between histone methylation and domain formation?

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ID de Contribution: **8** Type: **Non spécifié**

Poster: Exploring chromatin dynamics in Streptomyces, antibiotic-producing bacteria

Streptomyces are gram-positive, multicellular, filamentous aerobic bacteria characterized by a large linear chromosome divided into a central region harboring cores genes and two extremities enriched in genomic islands (GIs) such as the specialized metabolite biosynthetic gene clusters (SMBGCs) and prophage. Nevertheless, the majority of GIs remain transcriptionally silent over growth under laboratory conditions. Interestingly, a stress condition (HT medium) was recently found to be associated with awakening of a prophage. Characterizing *Streptomyces* physiology and chromosome conformation in this growth condition and identifying the cellular machineries involved in the regulation of prophage expression are hot topics to explore. First, we have identified a variant of HT medium named BM (Bacteriophage production Medium) in which the phage production is optimal and we demonstrated that the production of this phage mediates multicellular bacteria dispersal in response to metabolic stress. Moreover, we studied the effect of the prophage on chromosome conformation and the results have shown that phage dormancy is associated with a specific DNA contact pattern, named condensate, and this motif disappears when the phage is produced. In addition, by performing the Chromatin ImmunoPrecipitation followed by sequencing (ChIP-seq), we found out that Lsr2A, which is a nucleoid associated protein (NAP) that acts as xenogeneic silencer, targets the prophage and this reflects the involvement of this NAP in the prophage modulation. The project is ongoing and the effect of prophage on chromosome conformation and gene expression as well as the role of other NAPs in the chromatin dynamics of *Streptomyces* over growth are currently being investigated.

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ID de Contribution: **9** Type: **Non spécifié**

Genome stability: Interplay between Double Strand Break (DSB), chromatin mobility and SMC complexes.

Chromosome organization has recently emerged as essential for maintaining genome integrity. In the budding yeast, chromosomes follow a Rabl configuration where centromeres are clustered close to the nuclear envelope near the Microtubule Organization Center. The attachment of microtubules to kinetochores, multiprotein complexes associated with centromeres, ensures the faithful segregation of chromosomes. Remarkably, DSB only near centromeres, leads to an increase in global chromatin mobility that favors repair by homologous recombination, pointing to specific features of pericentromeres.

We investigate pericentromeric specificity by analyzing chromosome maintenance proteins required for chromosomes spatial folding in response to DSBs, focusing on the Smc5/6 complex. This complex is enriched at pericentromeres and recruited to DSBs. Moreover, the Smc5/6 complex is able to interact with microtubules through lysines K624, K631 and K667 of the Smc5 protein. Here we question the role of the microtubule-Smc5 interaction in genome integrity, by analyzing chromatin organization and DSB repair in a mutant where the two lysines (K624 and K631), present in the characteristic hinge of Smc5, are mutated into glutamic acid (smc5-2KE).

The 2KE mutations lead to declustering of centromeres and increased global chromatin mobility, specifically at pericentromeres, all correlated with a decreased binding to microtubules in vitro. This suggests an effect of microtubules attachment to kinetochore on pericentromeric chromatin organization. Moreover, we examined a potential link between DSB repair and Smc5-microtubule interaction. The smc5-2KE mutant showed a strong decrease in Non-homologous end joining (NHEJ) and HR repair suggesting a role of microtubules stabilization through the Smc5/6 complex in DNA damage repair.

Overall, our results show that the Smc5/6 complex controls the binding of microtubules to kinetochores via two lysines in Smc5, thereby limiting global chromatin mobility. Furthermore, our findings suggest that in addition to global mobility, the Smc5-microtubule interaction is required to promote DSB repair.

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ID de Contribution: **10** Type: **Non spécifié**

TANGO II: an integrated framework for 3D bioimage analysis of cell nuclei

The study of nuclear architecture has become an important field of cellular biology. Extracting quantitative data from large sets of 3D fluorescence images helps understanding how the genome is functionaly organized within different nuclear compartments. We present here Tango II, a new image analysis tool for studying nuclear architecture from fluorescence microscopy images. It is based on the Bacmman plugin in ImageJ for processing and extracting quantitative data from 3D stacks. Tango II intuitive interface enables users to define compartments, perform segmentation, and analyze multiple nuclei in microscopy fields. It integrates the most common quantitative measurements, including colocalization and shell analysis. Statistical processing and analysis of subpopulations is facilitated by a Python library that enables interactions between statistical analysis and image datasets. We illustrate here the capabilies of Tango II by investigating the impact of the cell cycle on heterochromatin distribution.

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ID de Contribution: **11** Type: **Non spécifié**

Poster: Salmonella chromatin reorganization upon SPI-1 expression

Salmonella enterica serovar Typhimurium (*S.* Typhimurium) is a facultative intracellular bacterium that is a major cause of foodborne gastroenteritis in humans. It has the ability to infect numerous cell types, thanks to a broad arsenal of virulence genes. The majority of the genes encoding the most important virulence factors of *S.* Typhimurium are located within the highly conserved *Salmonella* pathogenicity islands (SPIs). There are 17 SPIs; among them SPI-1 encodes for a Type 3 Secretion System and several effector proteins needed to trigger the invasion of epithelial cells. SPIs expression is tightly regulated by a complex regulatory network in which nucleoid-associated proteins (NAPs) are among the key regulators. Besides acting as transcriptional regulators, NAPs also play an important role in bacterial chromosome architecture. Thus, we propose that the expression of SPIs in response to environmental stresses (e.g., infection) might promote changes in *S.* Typhimurium chromosome conformation that could be driven by the differential binding of NAPs. By using HiC, we studied for the first time the 3D organization of *S.* Typhimurium chromosome in conditions mimicking infection and promoting the bistable expression of SPI-1. Our results show that, irrespective of SPI-1 expression, the chromosome of *Salmonella* is partitioned into two structurally distinct entities: the *terminus* (*ter*) and the non-*ter* regions. In the non-*ter* region, long-range DNA contacts can extend up to ~1Mb and in the *ter* region, contacts are constrained up to ~600Kb by the MatP protein. Furthermore, expression of SPI-1 revealed a local reorganization of the chromatin at the SPI-1 locus. Remarkably, by applying HiC to sorted SPI-1 ON subpopulations, we unveiled transcriptionally induced virulence domains (VIDs) within SPI-1 locus. The contribution of NAPs to the chromatin remodeling observed is been currently investigated. Altogether, our results revealed for the first time the global 3D organization of the *Salmonella* genome in conditions mimicking epithelial cells invasion and the specific folding of SPI-1 chromatin upon virulence expression.

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ID de Contribution: **12** Type: **Non spécifié**

Poster: Polymer modelling to investigate yeast's 3D chromosome organization in S phase and Mitosis

In Eukaryotes, DNA is tightly packed into a polymer-like structure called chromatin. Chromatin self-organization remains an elusive phenomenon whose impact is extensive in several cells' fundamental processes.
In our project, we focus on the specific problem of how chromatin folding couples with DNA replication dynamics. Taking into account chromosomes' conformation properties is in fact crucial to fully characterize the one dimensional process of eukaryotic genomes' replication.
While the molecular actors and mechanisms involved in DNA replication on one side and in genome folding on the other start to be well characterized independently, the interplay of the two remains elusive. For this reason, we used polymer modelling to fill this gap by implementing a polymer class capable of replicating itself starting from several origins of replication.
The developed computational framework allowed us to simultaneously describe 3D chromatin folding and 1D replication dynamics and address specific biological problems.
In particular, we adapted the model to describe *Saccharomyces cerevisiae*'s chromosomes during S phase, focusing on the hypothesized co-localization of sister forks (*Kitamura et al.* , *Saner et al.*). From our simulated Hi-C maps, we predicted two different scenarios for contact enrichment around the origin of replication: entropic compaction due to bubbles' topology and active extrusion of newly replicated sister chromatid (chromatin fountains).
We also adapted our simulations to describe mitotic chromosomes to exploit the SisterC data by *Oomen et al.* . We showed that even with a minimal set of parameter, it is possible to recover some structural properties of yeast mitotic chromosome, such as cohesin loops mediated compaction (*Costantino et al.*) and loose alignment between the two sister chromatids.

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ID de Contribution: **13** Type: **Non spécifié**

Bacterial chromosome organization by condensins: functional analysis of MukBEF in E. coli

Abstract : Chromosome condensation and segregation are two essential processes for the transmission of genetic material in all living organisms. Indeed, chromosomes are composed of a DNA molecule and several families of proteins that perform various functions related to DNA. Ubiquitous SMC complexes in the living world are among these proteins that constitute the chromosome, and their study is of great importance in understanding the mechanisms of condensation, segregation, and their possible links.
The MukBEF SMC complex is the condensin of the bacterium *Escherichia coli.* Although MukBEF is essential under fast growth conditions, its deletion is tolerated under slow growth, and is characterized by the production of anucleate cells reflecting a defect in chromosome segregation. The chromosome conformation capture technique (3C-seq/HiC) has revealed that MukBEF promotes long-distance contacts throughout the chromosome, except in the Ter domain where it is inhibited by the MatP/*matS* system. During my thesis, I focused on characterizing the activity of MukBEF on the chromosome. I was able to demonstrate that the loading of MukBEF, unlike other bacterial condensins that have been studied, occurs without site specificity.Additionally, I identified that this loading is more efficient on newly replicated DNA. I also investigated the characterization of the inhibition mechanism of MukBEF by the MatP/*matS* system in the Ter domain. MatP is a protein that specifically binds to 28 *matS* sites distributed in the Ter domain. My results suggest a mechanism of inhibition that involves unloading MukBEF from the chromosome near the borders of the Ter domain. Furthermore, I demonstrated that 3 matS sites are sufficient to inhibit MukBEF, but the addition of additional sites strengthens the effectiveness of this inhibition. Moreover, the analysis of the distribution of *matS* sites in several bacteria suggests that increasing the number of *matS* sites and their density near the dif site aims to prevent MukBEF loading near the *dif* site.

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ID de Contribution: **14** Type: **Non spécifié**

Modeling the coupling between epigenome regulation and 3D chromatin organization

The relation between the 3D chromosome organization and the epigenome (DNA methylation, histone modifications, etc.) has been long established. Several studies have demonstrated that epigenomic-driven interactions (mediated by architectural proteins like HP1 or PRC1) between loci sharing the same chromatin content are key drivers of the 4D Genome leading to (micro)phase separation and compartmentalization of active and repressive regions. However, on contrary, how genome folding impacts epigenome is still unclear. To explore the coupling between epigenome regulation and 3D chromatin organization, we introduce the "Living Painter"model incorporating 3D polymer dynamics and diffusing histone modifying enzymes (HMEs) along with architectural proteins (APs). The model elucidates how the concentration and kinetics of HMEs and APs impact the spreading and maintenance of epigenetic marks and at the same time as organize the 3D genome. We show that a limited number of enzymes does facilitate the formation of confined, stable chromatin state domains. Interestingly, favoring nucleation of APs at specific genomic recruitment sites (RSs) over their self-association creates multiple meta-stable nanodroplets localized around RSs, while favoring self-association of APs drives the system towards fully phase separated droplets englobing all RSs. These contrasting regimes provide mechanistic insights about how multiple nucleation sites can create local clustering of chromatin regulators.

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ID de Contribution: **15** Type: **Non spécifié**

Quantitative insights into topoisomerase activity during gene transcription

While the feedback between transcription and DNA supercoiling is well understood theoretically and *in vitro*, it remains to be quantified *in vivo*. In this talk, I will present our work where we fill this gap by realizing, on a plasmid in *Escherichia coli*, the conceptual "twin transcriptional-loop model"[1] that is the basis of theoretical and *in vitro* studies. In particular, we measured how gene expression varies with promoters and distances to the topological barriers. We find that gene expression depends on the distance to the upstream barrier but not to the downstream barrier, with a promoter-dependent intensity. I will then present a first-principle biophysical model of DNA transcription that is able to quantitatively rationalize these findings. This model integrates binding, initiation and elongation of RNA polymerases parametrized with available *in vitro* measurements, as well as the action of topoisomerases for which parameters are constrained by our experimental results. By comparing it with the data, it supports that TopoI and gyrase must both act specifically, respectively upstream and downstream the gene, and predicts TopoI to be less active than gyrase. It also highlights antagonistic effects of TopoI, which both facilitates elongation and tends to repress initiation [2].

[1] Liu, L.F., Wang, J.C., 1987. Supercoiling of the DNA template during transcription. PNAS. 84, 7024–7027.

[2] Boulas, I., Rimsky, S., Espeli, O., Junier, I., Rivoire, O., 2022. Assessing in vivo the impact of gene context on transcription through DNA supercoiling. bioRxiv.

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ID de Contribution: **16** Type: **Non spécifié**

DNA supercoiling quantiatively regulates bacterial transcription by untwisting the promoter spacer

DNA supercoiling acts as a global transcriptional regulator in bacteria, but the promoter sequence or structural determinants controlling its effect remain unclear. It was previously proposed to modulate the torsional angle between the −10 and −35 hexamers, and thereby regulate the formation of the closed-complex depending on the length of the 'spacer'between them. We develop a thermodynamic model of this notion based on DNA elasticity, providing quantitative and parameter-free predictions of the relative activation of promoters containing a short
versus long spacer when the DNA supercoiling level is varied. The model is tested through an analysis of in vitro and in vivo expression assays of mutant promoters with variable spacer lengths, confirming its accuracy for spacers ranging from 15 to 19 nucleotides, except those of 16 nucleotides where other regulatory mechanisms likely overcome the effect of this specific step. An analysis at the whole-genome scale in Escherichia coli then demonstrates a significant effect of the spacer length on the genomic expression after transient or inheritable superhelical variations, validating the model's predictions. Altogether, this study shows an example of mechanical constraints associated to promoter binding by RNA Polymerase underpinning a basal and global regulatory mechanism.

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ID de Contribution: **17** Type: **Non spécifié**

Poster: Revealing DNA's topology : A spectral energy approach to loop detection

Loop formation is a crucial aspect of understanding chromatin architecture and its functional role. However, detecting loops remains challenging due to cell population variability and conformation randomness. In this study, we propose a novel approach utilising spectral analysis to identify and quantify loops in experimental conformations obtained through super-resolution imaging. Applying our method to published data by Bintu et al. (2018), we successfully provide a comprehensive and statistically validated description of the global chromosomal architecture, affirming the effectiveness of our approach.

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