

3D models of fungal chromosomes to enhance visual integration of omics data

Thibault Poinsignon, Mélina Gallopin, Pierre Grognet, Fabienne Malagnac,
Gaëlle Lelandais* and Pierre Poulain*

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Abstract


The functions of eukaryotic chromosomes and their spatial architecture in the nucleus are reciprocally dependent. Hi-C experiments are routinely used to study chromosome 3D organization by probing chromatin interactions. Standard representation of the data has relied on contact maps that show the frequency of interactions between parts of the genome. In parallel, it has become easier to build 3D models of the entire genome based on the same Hi-C data, and thus benefit from the methodology and visualization tools developed for structural biology. 3D modeling of entire genomes leverages the understanding of their spatial organization. However, this opportunity for original and insightful modeling is underexploited. In this paper, we show how seeing the spatial organization of chromosomes can bring new perspectives to omics data integration. We assembled state-of-the-art tools into a workflow that goes from Hi-C raw data to fully annotated 3D models and we re-analysed public omics datasets available for three fungal species. Besides the well-described properties of the spatial organization of their chromosomes (Rabi conformation, hypercoiling and chromosome territories), our results highlighted (i) In *Saccharomyces cerevisiae*, the backbones of the cohesin anchor regions, which were aligned all along the chromosomes, (ii) In *Schizosaccharomyces pombe*, the oscillations of the coiling of chromosome arms throughout the cell cycle and (iii) In *Neurospora crassa*, the massive relocalization of histone marks in mutants of heterochromatin regulators. 3D modeling of the chromosomes brings new opportunities for visual integration of omics data. This holistic perspective supports intuition and lays the foundation for building new concepts.

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3D models of fungal chromosomes to enhance visual integration of omics data

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Abstract

The functions of eukaryotic chromosomes and their spatial architecture in the nucleus are reciprocally dependent. Hi-C experiments are routinely used to study chromosome 3D organization by probing chromatin interactions. Standard representation of the data has relied on contact maps that show the frequency of interactions between parts of the genome. In parallel, it has become easier to build 3D models of the entire genome based on the same Hi-C data, and thus benefit from the methodology and visualization tools developed for structural biology. 3D modeling of entire genomes leverages the understanding of their spatial organization. However, this opportunity for original and insightful modeling is underexploited. In this paper, we show how seeing the spatial organization of chromosomes can bring new perspectives to omics data integration. We assembled state-of-the-art tools into a workflow that goes from Hi-C raw data to fully annotated 3D models and we re-analysed public omics datasets available for three fungal species. Besides the well-described properties of the spatial organization of their chromosomes (Rabi conformation, hypercoiling and chromosome territories), our results highlighted (i) In *Saccharomyces cerevisiae*, the backbones of the cohesin anchor regions, which were aligned all along the chromosomes, (ii) In *Schizosaccharomyces pombe*, the oscillations of the coiling of chromosome arms throughout the cell cycle and (iii) In *Neurospora crassa*, the massive relocalization of histone marks in mutants of heterochromatin regulators. 3D modeling of the chromosomes brings new opportunities for visual integration of omics data. This holistic perspective supports intuition and lays the foundation for building new concepts.

Introduction

What if it were possible to see all the details of chromosomes inside the nucleus of a cell? In eukaryotic cells, the nucleus is a dynamic organelle which is highly organized and characterized by extensive compartmentalization of structural components in its three-dimensional space (see (1–4) for examples of reviews). In such a crowded environment, the arrangement of chromosomes is constrained and requires the formation of multiple chromatin domains to limit gene positions to preferred locations within the nuclear space (5). The spatial organization of chromosomes is of great interest, helping molecular biologists represent the objects they work with, and understand their interactions. Even if immense progress has been made in cell imaging, biological molecules (e.g. DNA, RNA, proteins) are too small to be individualized with optical microscopes (6,7) and consequently, the interior of the cell (and the interior of a nucleus even more) remains largely invisible to the human eye. Alternative solutions are based on molecular-scale techniques like X-ray crystallography (8), Nuclear Magnetic Resonance (NMR) microscopy (9) or electron microscopy (10). By analyzing atom arrangements in molecules, these techniques produce informative views of macromolecular complexity (11,12), but they require complex technical skills and expensive equipment. Furthermore, it is important to keep in mind that in the end, these images are still artificial representations of reality. In other words, they are 'models'.

The use of models is widespread in biology. From model organisms to model systems, their interest is to understand a phenomenon in a simplified context, in order to, later, generalize to more complex situations. In cell biology for instance, structural models are used to represent cell components (membranes, nucleus, cytoplasm, etc.), to understand their organization, and to describe their constituent molecules (13). The work of David Goodell provides an emblematic example (14). His drawings representing cellular compartments and their molecular actors are so striking because of the unexpected density of molecules and the complexity of their organization. Goodell's illustrations have been featured as 'Molecule of the Month' on the Protein Data Bank website for over twenty years and the scientific journal Nature chose one of his paintings to make the cover of a special issue on COVID-19 (August 20, 2020 issue). Models make it possible to represent and summarize, in an intuitive but still scientifically rigorous way, the massive knowledge of cell molecular structures. Creating them thus represents a stimulating challenge, at the crossroads of multiple disciplines (biology, physics, computer science, art) (15).

Modeling chromosomes is challenging because they belong to the mesoscale, i.e. a length-scale that is larger than

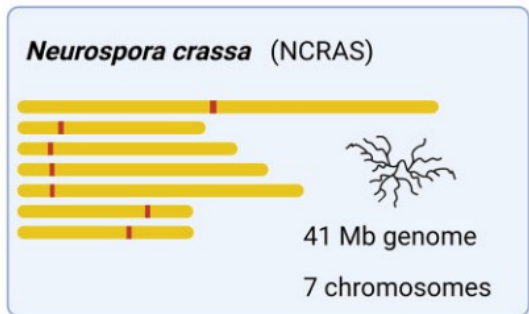
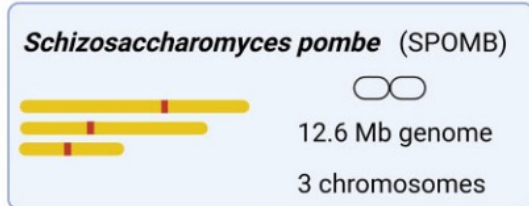
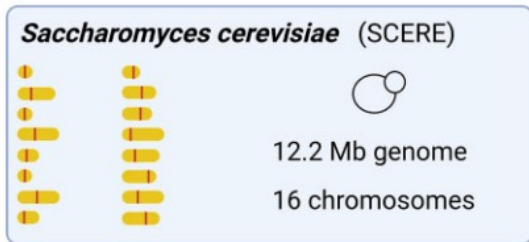
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<https://academic.oup.com/nargab/article/5/4/lqad104/7458894>

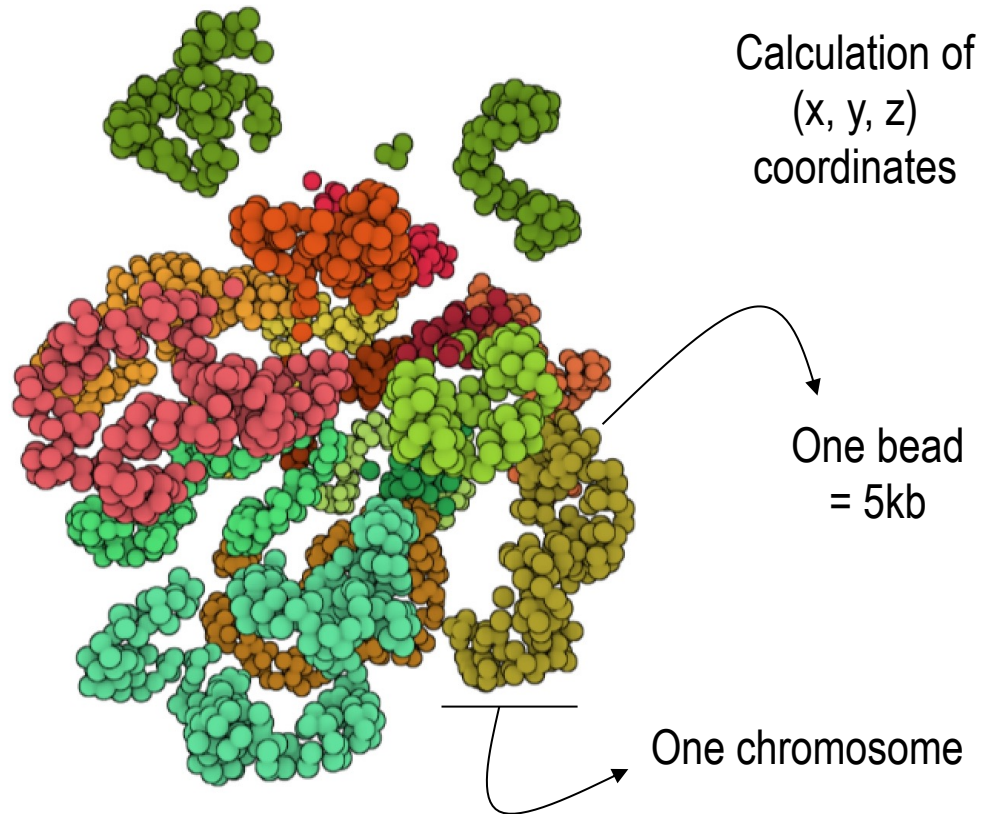
<https://doi.org/10.1093/nargab/lqad104>

3D fungal chromosome models from public HiC data

➤ Three different species:



➤ Example of SCERE genome:
(HiC data from public database)



Poinsignon et al. (2023)

Methods to reconstruct 3D models of genome organization

- In the last decade, **several strategies** have been presented in the literature (reviewed in Oluwadare et al., 2019).
- In this work, we chose to use **the Pastis-NB method**, which is a probability-based method, recently published (Varoquaux et al., 2023).
- We explored the **potential of using 3D models** as complementary information to Hi-C contact maps, to better understand the spatial organization of fungal chromosomes and to improve **the visual integration** of omics data.

Three examples are presented in the article

Detailed view of
chromosome organization

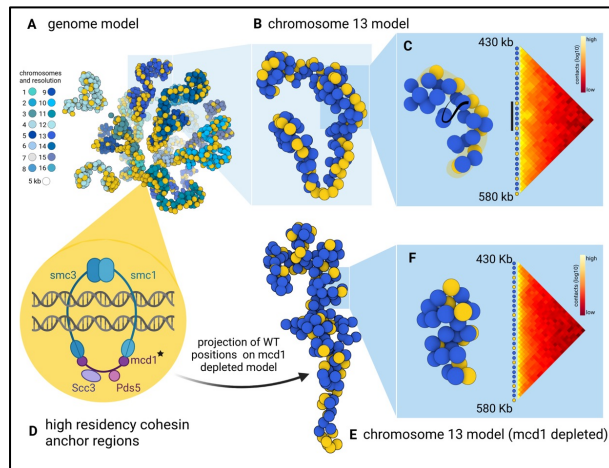
Poinsignon et al. (2023)

General view of
chromosome organization

➤ SCERE

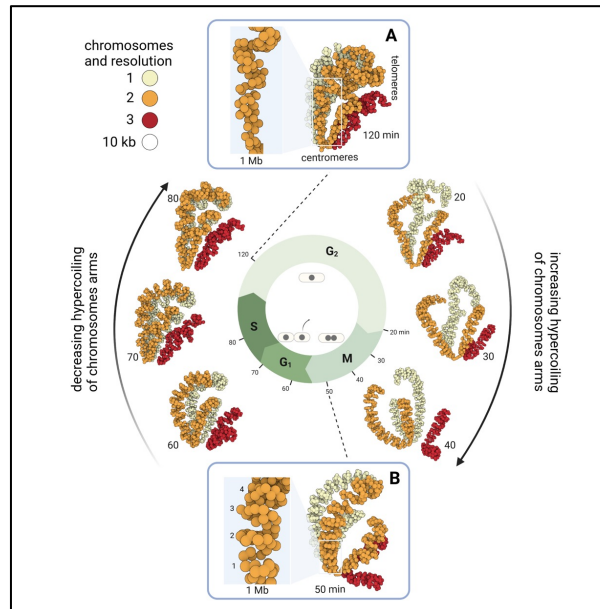
➤ SPOMBE

➤ NCRASSA



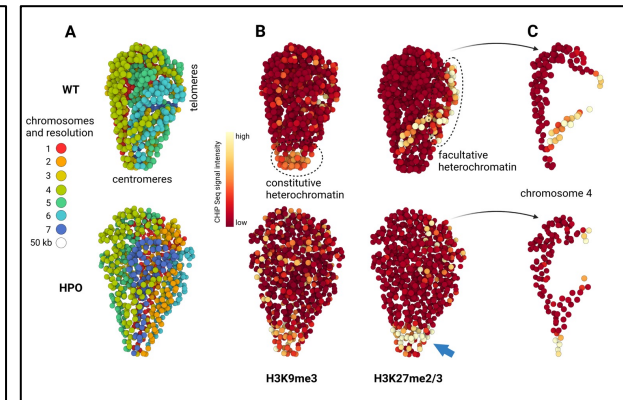
Example #1: Spatial alignment of cohesin binding sites along chromosomes in *S. cerevisiae*.

HiC (WT and mutant) + ChIPseq



Example #2: Oscillations in coiling of chromosome arms during the cell cycle in *S. pombe*.

HiC (WT only, at several time points)



Example #3: Massive relocalisation of histone marks in *N. crassa* heterochromatin regulator mutants.

HiC (WT and mutant) + ChIPseq

Re-analysis of a large omics dataset

➤ More than 40 FASTQ files were collected:

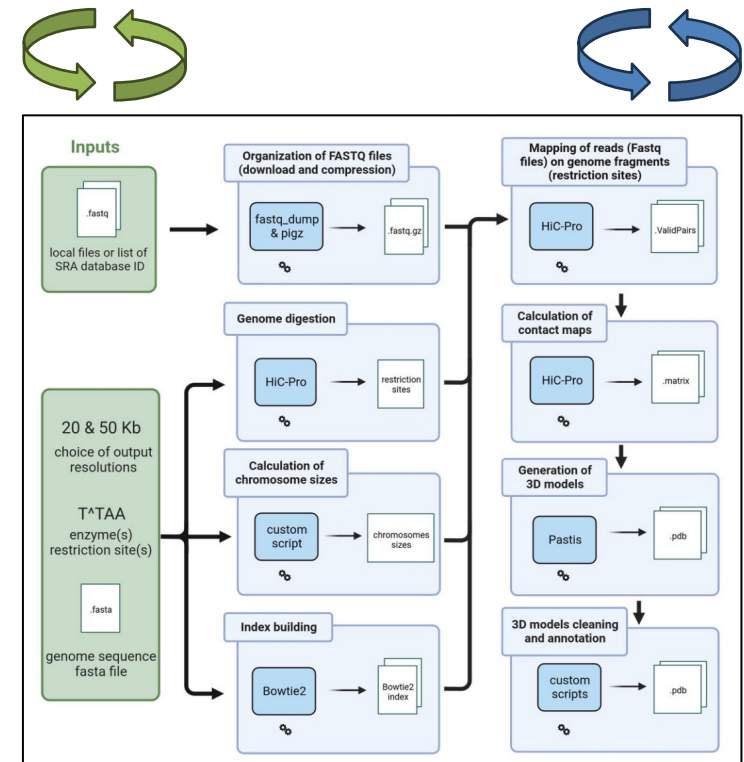
Table 1. Main characteristics of raw data used in this study to create 3D models of fungal chromosomes

Model	Fungal species (genome version)	Strain	Hi-C data sources	SRA IDs	Final number of valid read pairs	Number of beads in the final model	Figure (model resolution in bp)
1	<i>N. crassa</i> (nc14)	WT	Rodriguez <i>et al.</i> 2022 (31)	SRR14362684	107 636 304	3976	2 (10 000)
2		WT	Galazka <i>et al.</i> 2016 (30)	SRR14362685 SRR16761088 SRR16761089 SRR16761090 SRR16761091 SRR16761092 SRR2105869	5 030 121	800	5 (50 000)
3		hpo		SRR2105870 SRR2105871 SRR2105872 SRR2105876 SRR2105877 SRR2105878 SRR5149251	2 922 526	808	
4	<i>S. pombe</i> (ASM294v2.19)	WT	Tanizawa <i>et al.</i> 2017 (27)	SRR5149252 SRR5149253 SRR5149254 SRR5149255 SRR5149256 SRR5149257 SRR5149258 SRR5149259 SRR5149260 SRR5149261 SRR5149263 SRR5149264 SRR5149265 SRR5149267 SRR5149268 SRR5149269 SRR5149270 SRR5149271 SRR5149272 SRR5149273 SRR5149274 SRR5149275 SRR5149276 SRR5149277 SRR5149278 SRR5149279 SRR5149280 SRR5149281 SRR5149282 SRR5149283 SRR5149284 SRR5149285 SRR5149286 SRR5149287 SRR5149288 SRR5149289 SRR5149290 SRR5149291 SRR5149292 SRR5149293 SRR5149294 SRR5149295 SRR5149296 SRR5149297 SRR5149298 SRR5149299 SRR5149300 SRR5149301 SRR5149302 SRR5149303 SRR5149304 SRR5149305 SRR5149306 SRR5149307 SRR5149308 SRR5149309 SRR5149310 SRR5149311 SRR5149312 SRR5149313 SRR5149314 SRR5149315 SRR5149316 SRR5149317 SRR5149318 SRR5149319 SRR5149320 SRR5149321 SRR5149322 SRR5149323 SRR5149324 SRR5149325 SRR5149326 SRR5149327 SRR5149328 SRR5149329 SRR5149330 SRR5149331 SRR5149332 SRR5149333 SRR5149334 SRR5149335 SRR5149336 SRR5149337 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13		Mccl1 depleted		SRR11893085 SRR11893086 SRR11893087	14 180 445	2323	

The sources of Hi-C data (original articles and SRA identifiers) are given. All datasets were obtained on wild type strains, with the exception of models #3 and #13 which correspond to the *N. crassa* hpo mutant (used in Figure 5) and *S. cerevisiae* mccl1 depleted (used in Figure 3). The number of valid pairs of reads gives an estimation of the overall quality of the data and indicates the density of the contact frequencies measured in the experiment. The number of beads in the 3D model is determined by the chosen resolution during Hi-C data analysis and the length of the reference genome.

Poinsignon et al. (2023)

➤ Standardized bioinformatics protocols for the creation of 3D models:



Workflow 3D genome builder (3DGB)



The workflow 3DGB

- It automatically performs the **critical bioinformatics steps** required to:
 - (i) compute Hi-C contact frequencies,
 - (ii) infer associated 3D models of the chromatin organization,
 - (iii) annotate and control the quality of the 3D models.
- It also adds **further processing** of the 3D model output as PDB files, suitable for **advanced visualization** with molecular viewer software (Mol*, PyMol, etc.).
- It is **available to other scientists** who wish to add 3D models to their analyses of Hi-C data.
 - It requires only **four basic inputs** to be specified (FASTQ files, a FASTA file, restriction sites, targeted resolutions).

A link is available from the Integrative Bioinformatics platform of the I2BC

bioi2.i2bc.paris-saclay.fr/tools/dna-rna-genomes/

Select Language

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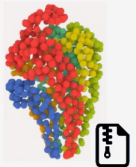
Tools & DBs

DNA, RNA & Genomes

The tools and databases developed at the I2BC are categorised by subject. The following are tools & databases to do with genomics data.

3DGB


DNA, RNA & Genomes, Software



A workflow to build 3D models of genomes from raw HiC data and visually explore them

ARNold


DNA, RNA & Genomes, Webservice



Identify Rho-independent transcription terminators in your sequences

CRISPR-Cas++

DNA, RNA & Genomes, Webservice



CRISPR database and tools to detect and view CRISPRs and cas genes in sequences

BADGET 2.0

DNA, RNA & Genomes, Webservice

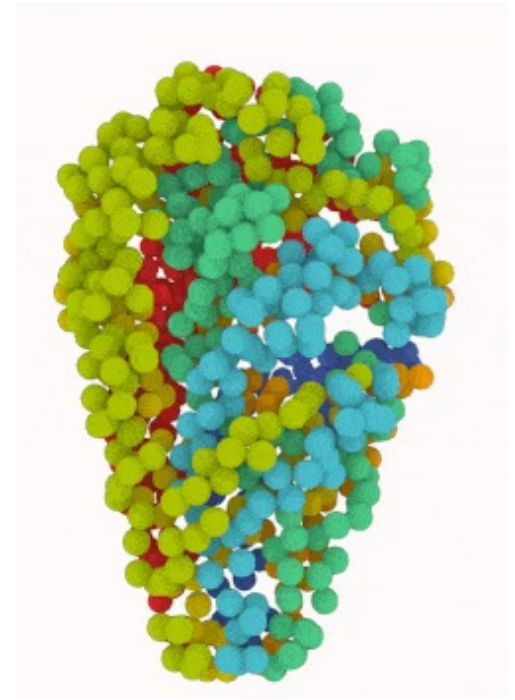
BAGET 2.0

Dynamically explore over 54000 replicons from 25000+ prokaryotic genomes

DETR'PROK

DNA, RNA & Genomes, Software

<https://bioi2.i2bc.paris-saclay.fr/tools/dna-rna-genomes/>



Contact : gaelle.lelandais@universite-paris-saclay.fr

THANK YOU FOR YOUR ATTENTION