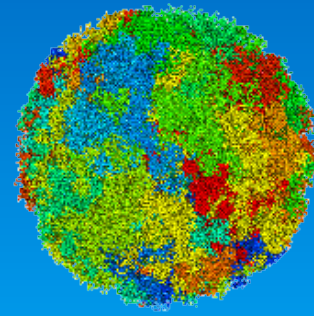


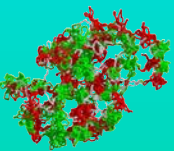
Towards a Holistic Understanding of the Human Genome by Determination and Integration of its Three-Dimensional and Sequential Organization



**Structural-, Scaling and Dynamic-Properties
in the Simulation of
Interphase Chromosomes and Cell Nuclei
Long-Range Correlations in Complete Sequenced Genomes**

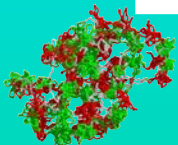
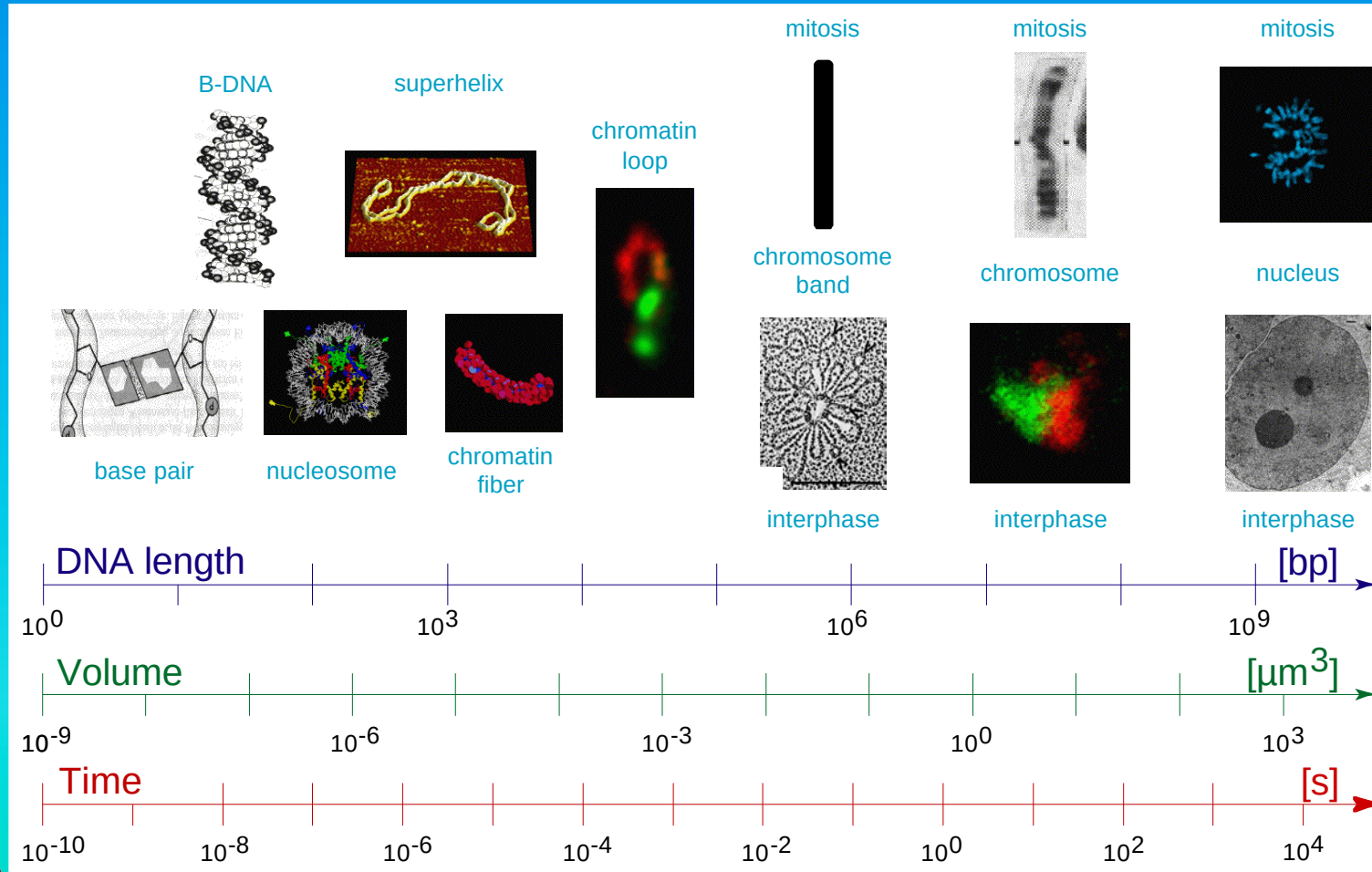
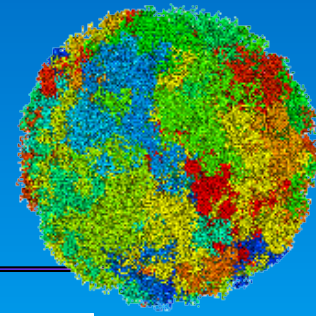
**by
Tobias A. Knoch**

**Kirchhoff Institut for Physics, Ruperto-Carola University
&
Deutsches Krebsforschungszentrum (DKFZ)
Heidelberg
Germany**



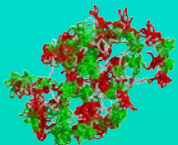
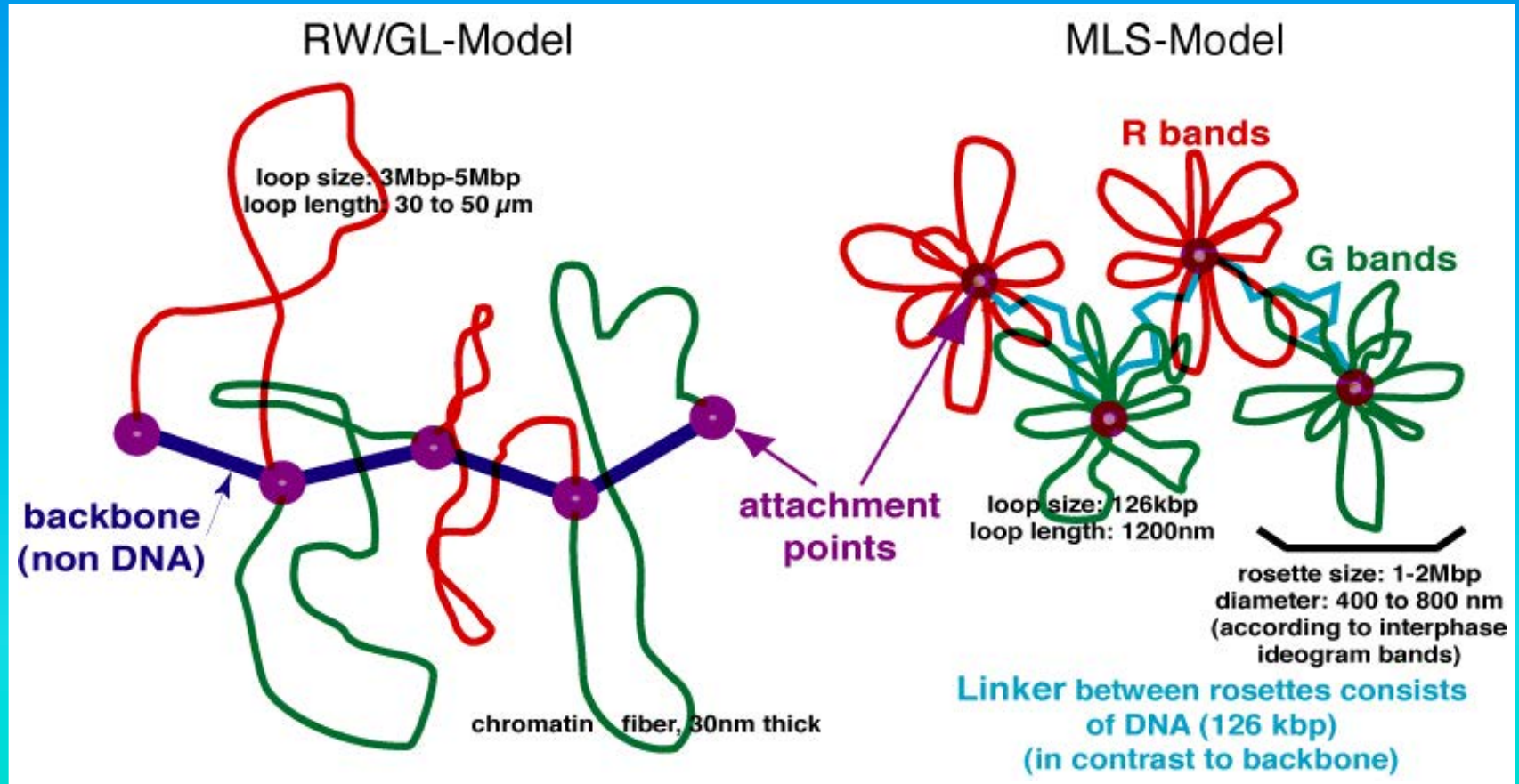
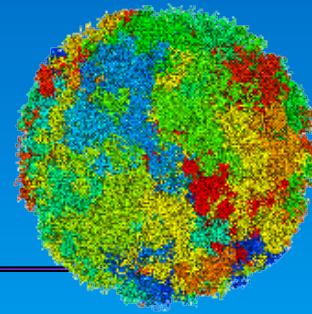
Dynamic and Hierarchical Genome Organization

10 and 13 orders of magnitude concerning length and time scales are bridged.
Are and how are all of these organization levels connected to fulfill their obvious functions, e. g. gene regulation or replication, since they are optimized by evolution ?



Simulated Interphase Chromosome Models

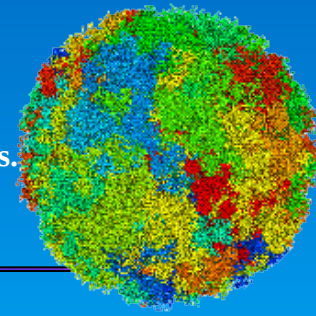
Random-Walk/Giant-Loop (RW/GL) and Multi-Loop-Subcompartment (MLS) Model



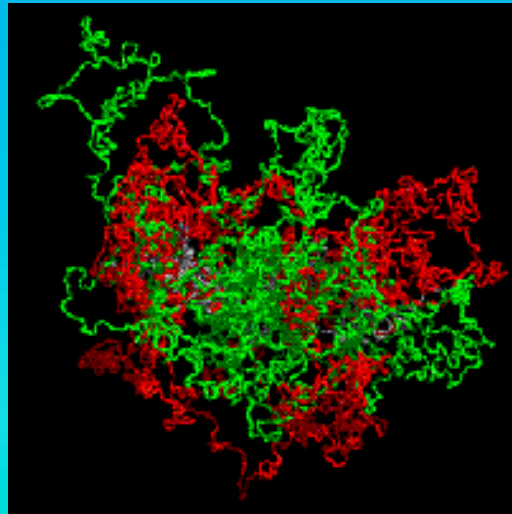
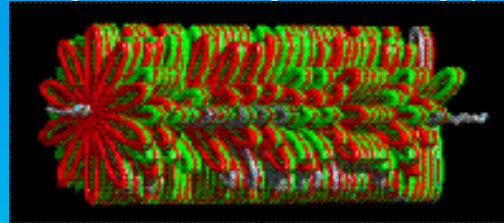
Simulation of Single Chromosomes

The 30 nm chromatin fiber is modeled as a polymer chain with stretching, bending, and excluded volume interactions. Monte Carlo and Brownian Dynamic methods lead to thermodynamical equilibrium configurations.

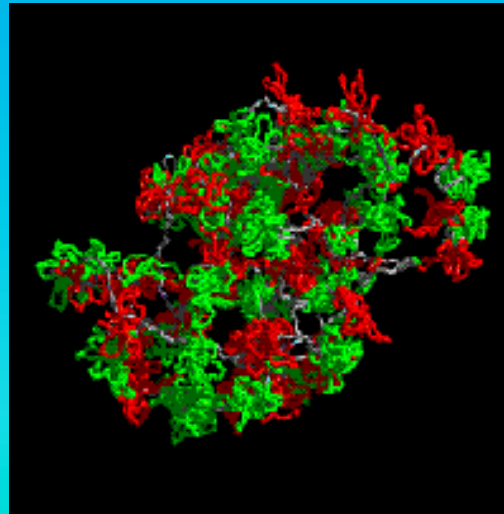
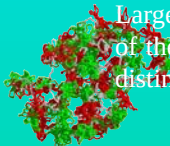
All models form chromosome territories with big voids and different chromatin morphologies. Experimental territory and subcompartment diameters agree best with an MLS model with 80 to 120 kbp loops and linkers.



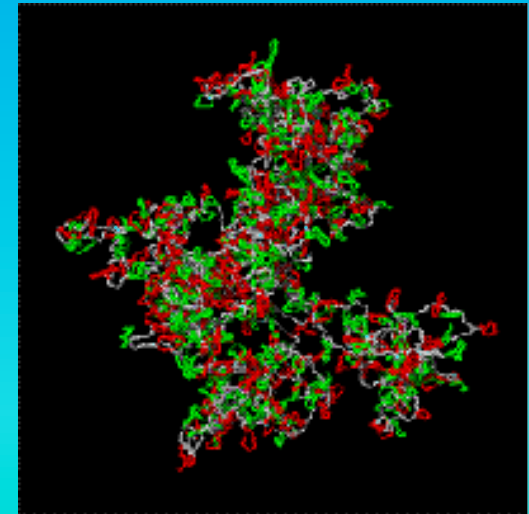
Metaphase starting configuration with ideogram bands in red/green, linker in grey.



RW/GL model, loop size 5 Mbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromosome territory, thus forming no distinct features like in MLS model.



MLS model, loop size 126kbp, linker size 126 kbp, after ~50.000 MC and 1000 relaxing BD steps. Here rosettes form subcompartments as separated organizational and dynamic entities.

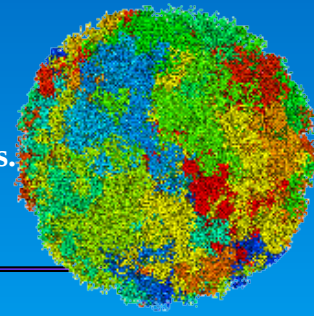


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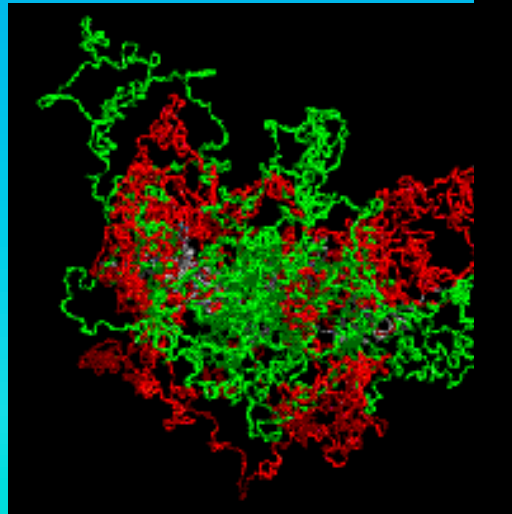
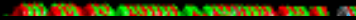
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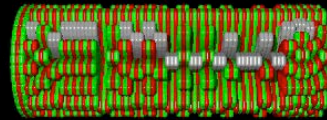
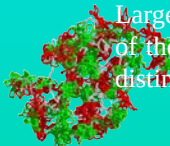
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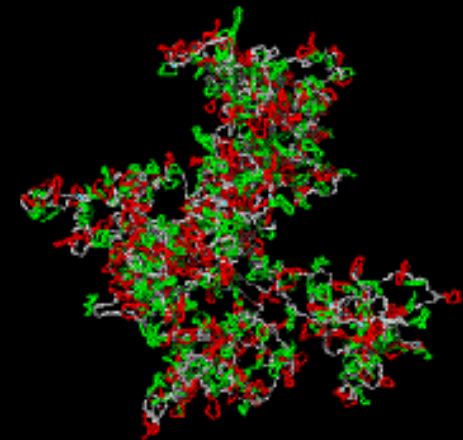
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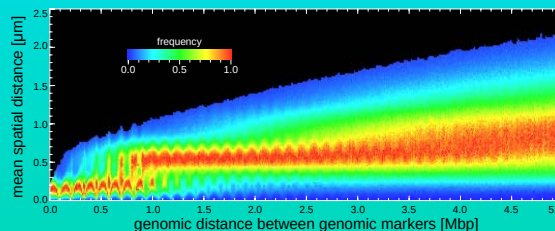
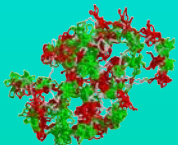
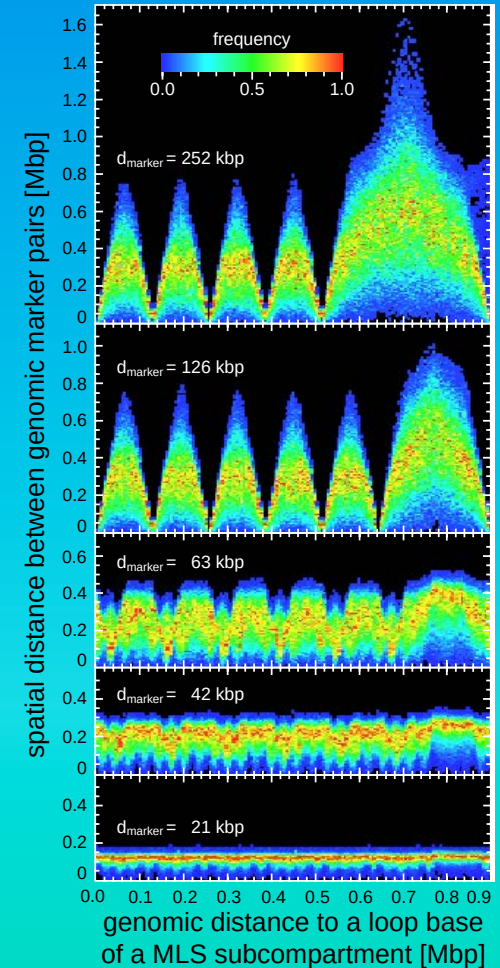
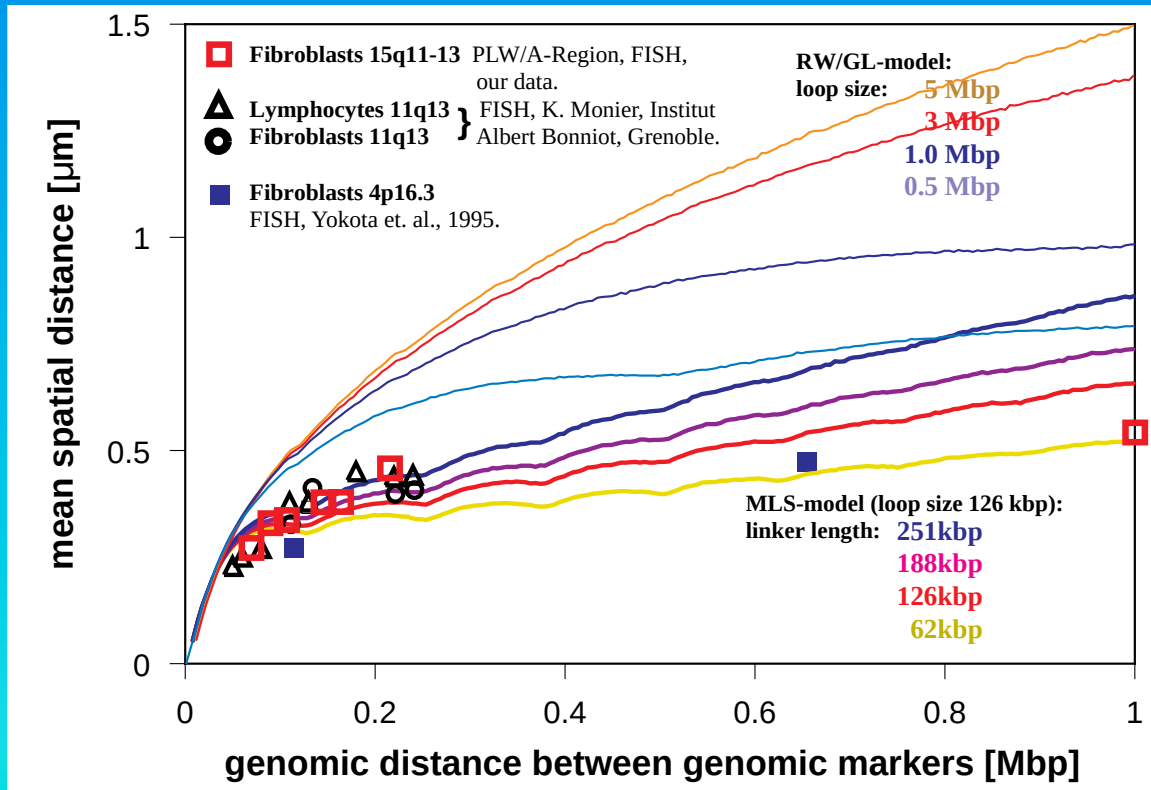
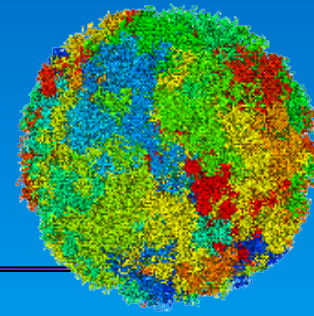


RW/GL model, loop size 126 kbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely thus forming no distinct features like in MLS model.

Spatial Distances between Genetic Markers

Simulated spatial distances between random genetic markers as function of their genetic separation leads to best agreement in a comparison to experiments for an MLS model with 80 to 120 kbp loops and linkers.

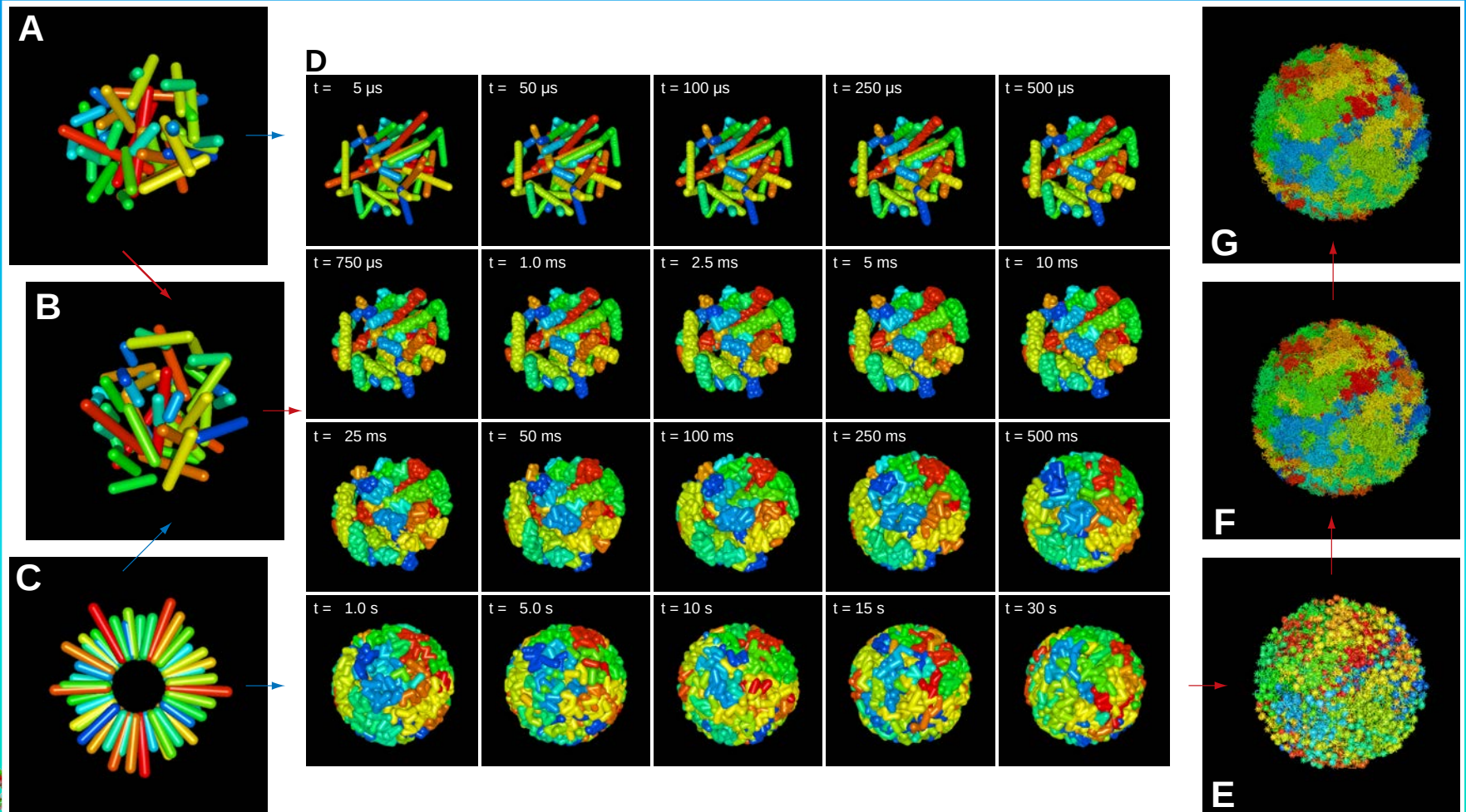
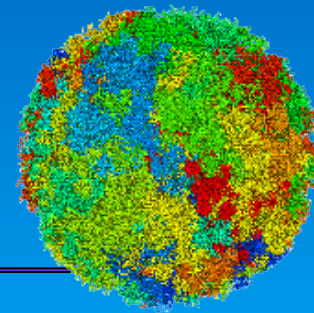
The spatial distance distributions are also model characteristic and show in a set of markers as function of their relative position to the chromatin fiber topology characteristic variation, strongly connected.



Simulation of Whole Nuclei with all 46 Chromosomes

Starting with some metaphase arrangement of cylindrical chromosomes, interphase nuclei with a 30 nm fiber resolution and at thermodynamical equilibrium are created in 4 steps using simulated annealing and Brownian Dynamics methods with stretching, bending, excluded volume and a spherical boundary interactions.

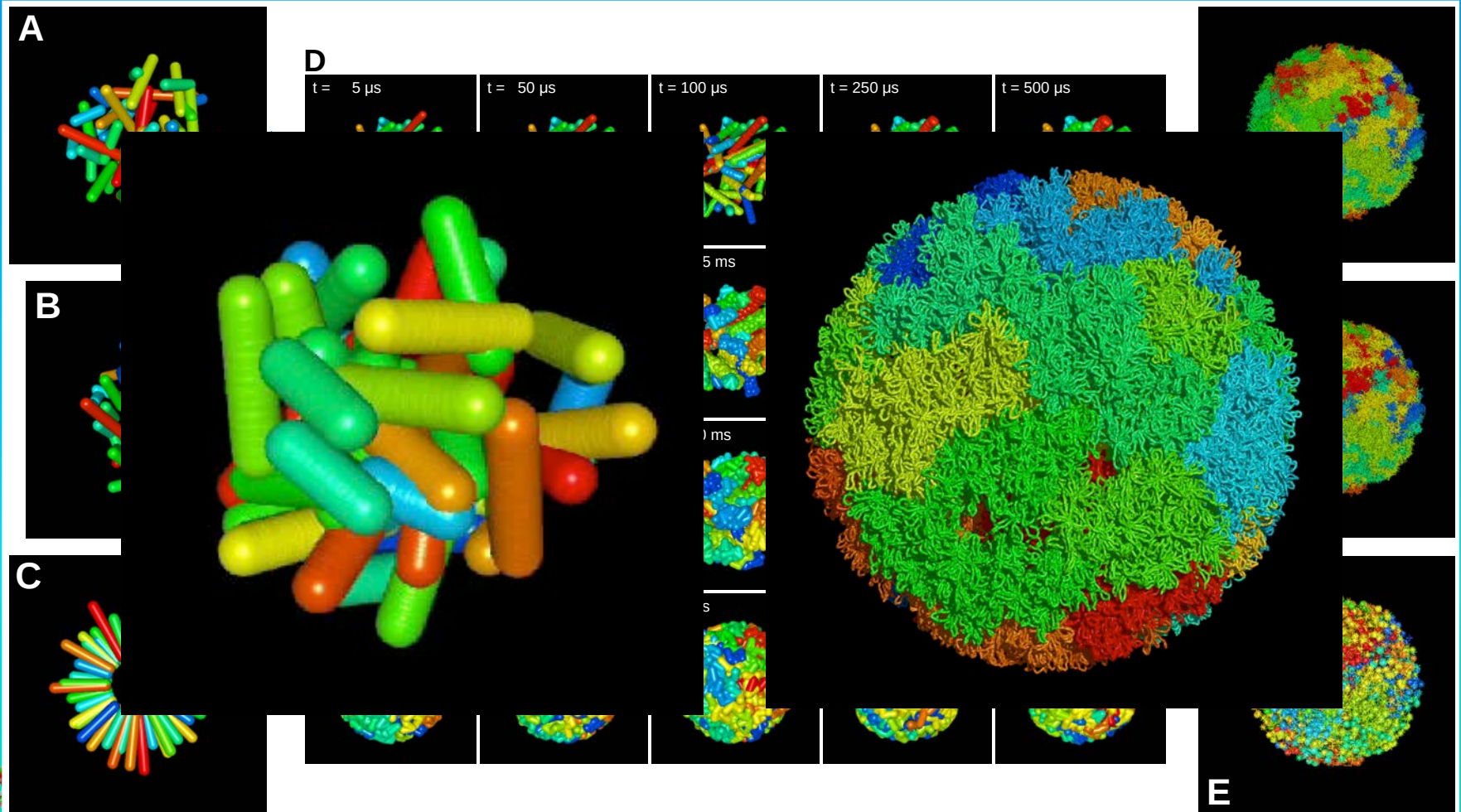
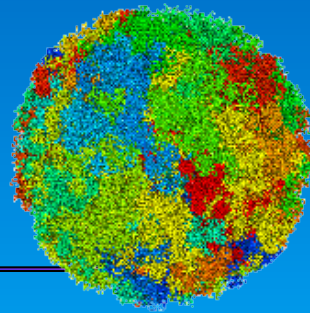
The chromosome territory position depends on their metaphase position and is reasonably stable.



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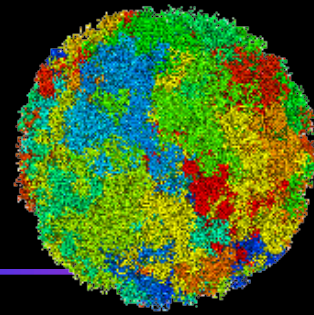
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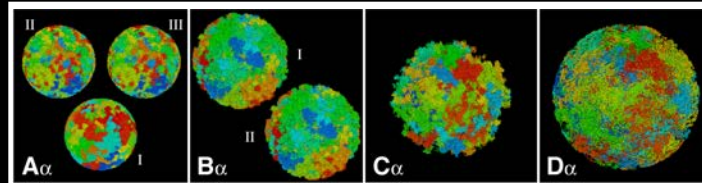
From Fiber Topology to Nuclear Morphology

Chromosome territories form in the RW/GL and the MLS model. However, only the MLS model leads distinct subcompartments and low chromosome and subcompartment overlap. Best agreement is reached for an MLS model with 80 to 120 kbp loops and linkers in nuclei with 8 to 10 μm diameter.

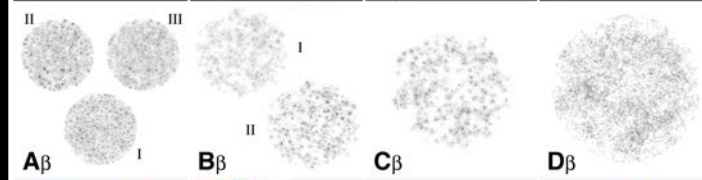
The simulated nuclear morphology reflects the chromosome fiber topology of different models in detail.



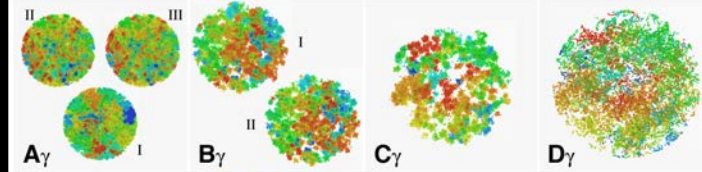
rendering



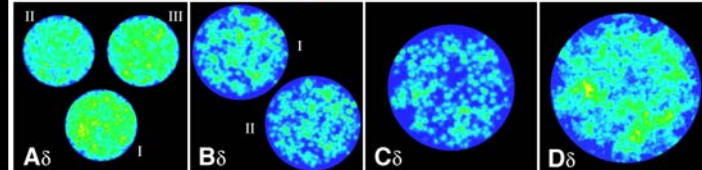
electron microscopy



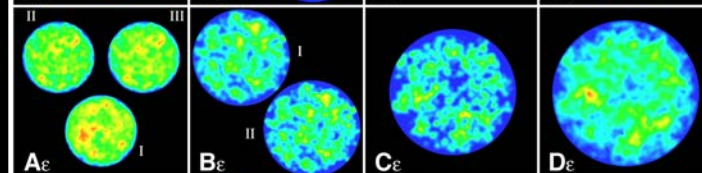
electron microscopy territory painting



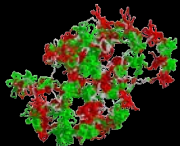
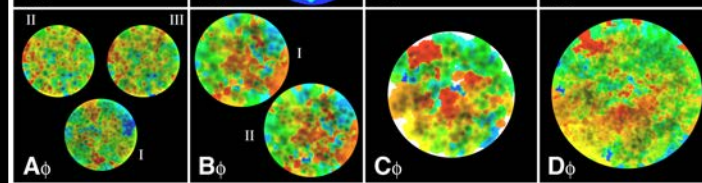
confocal microscopy
100x objective, theoretic resolution



confocal microscopy
63x objective, real resolution



confocal microscopy
territory painting

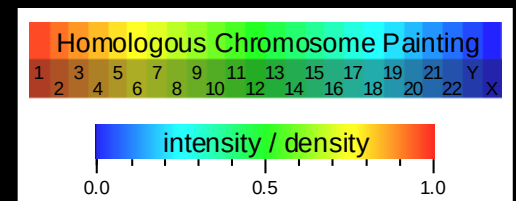


A: MLS in 6 μm nucleus
I: 63 kbp loops, 63 kbp linkers
II: 63 kbp loops, 252 kbp linkers
III: 126 kbp loops, 252 kbp linkers

B: MLS in 8 μm nucleus
I: 126 kbp loops, 126 kbp linkers
II: 84 kbp loops, 126 kbp linkers

C: MLS in 10 μm nucleus
126 kbp loops, 126 kbp linker,
not totally relaxed

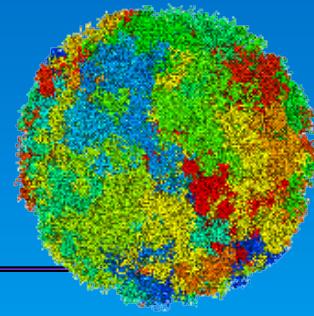
D: RW/GL in 12 μm nucleus
5 Mbp loops
not totally relaxed



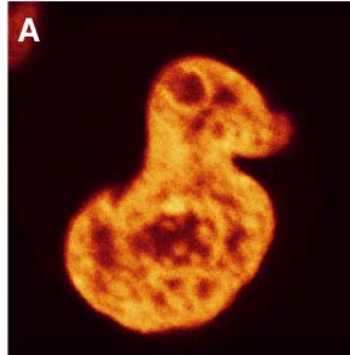
In vivo Morphology & Chromatin Distribution

The stable expression of fusions between histones and autofluorescent proteins and the integration into nucleosomes allows the minimal invasive investigation of the structure and dynamics of chromatin.

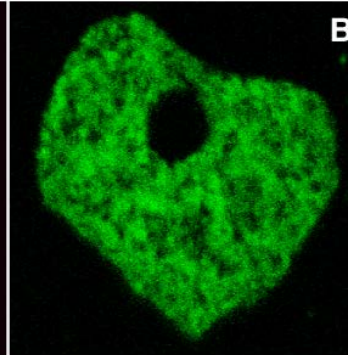
The clustered morphology in detail favour an MLS like chromatin topology.



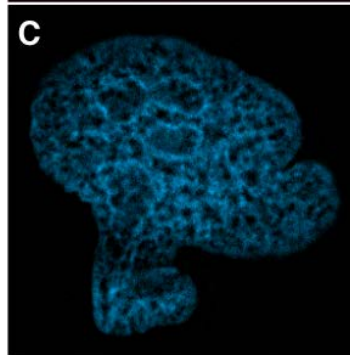
HeLa, H2A-YFP



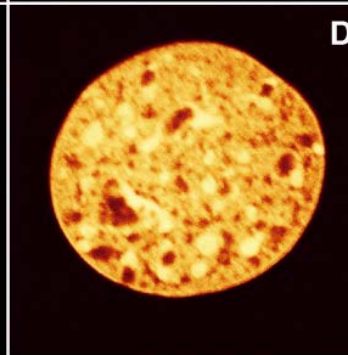
Cos7, H1.0-GFP



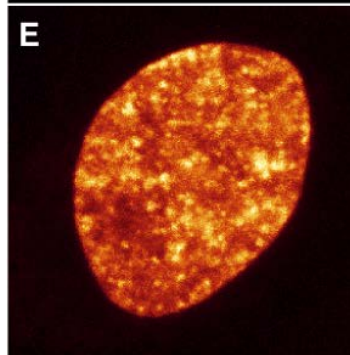
LCLC 103H, H2A-CFP



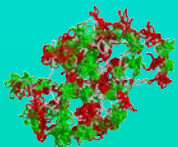
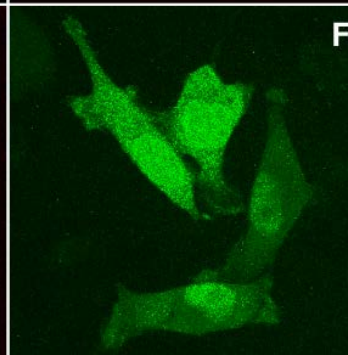
ID13, H2A-YFP



HeLa, mH2A1.2-YFP

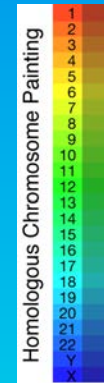
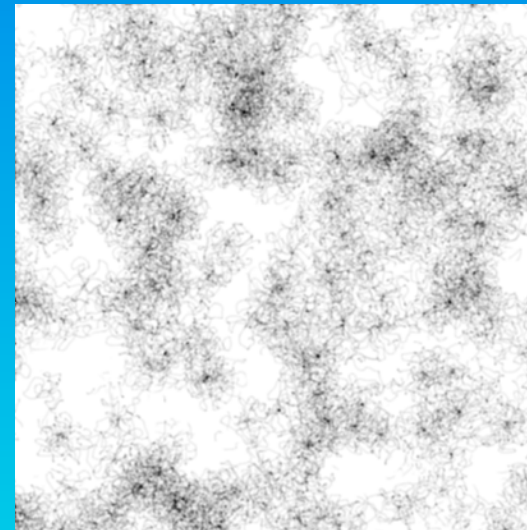
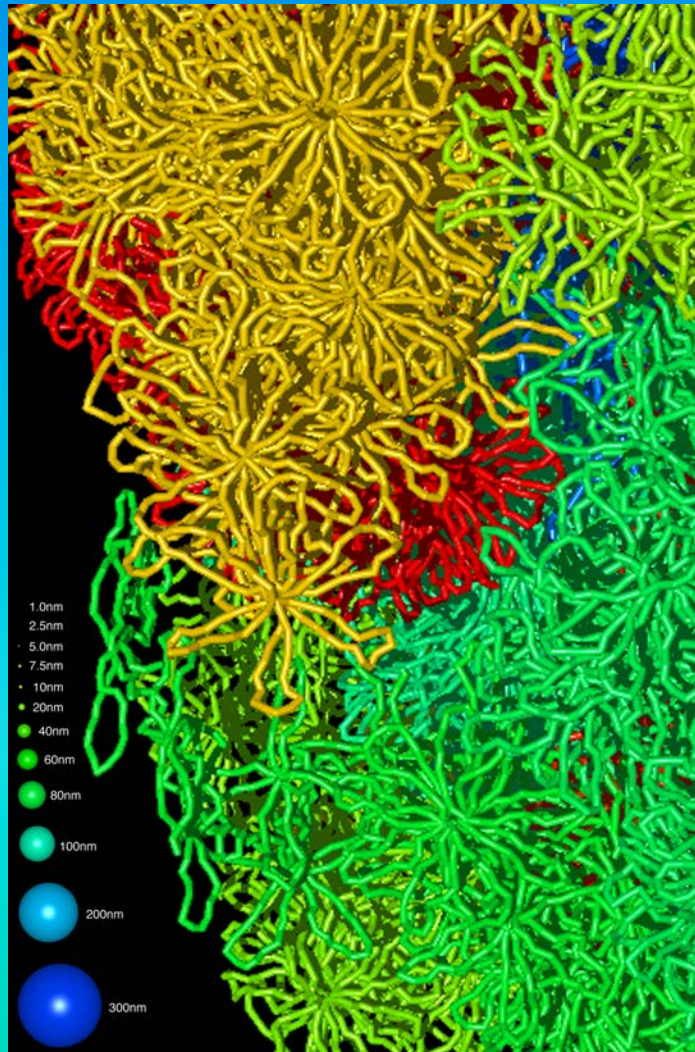
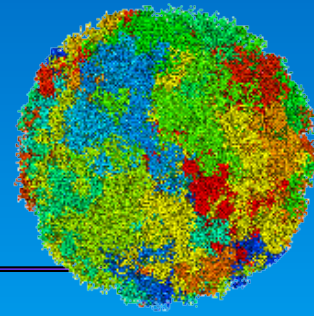


HeLa, H2A-YFP,
natural promoter

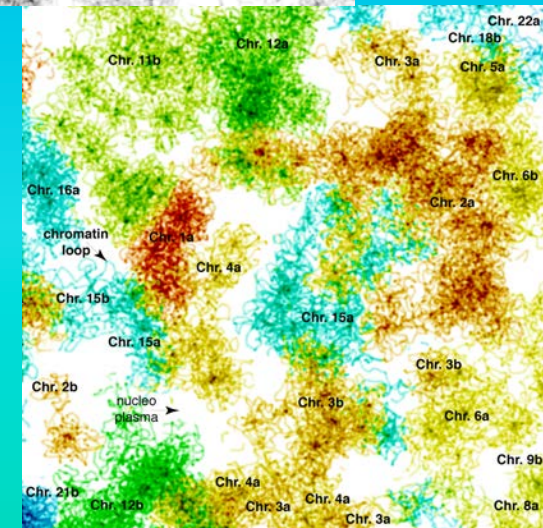


Fine Morphology of Nuclei

High resolution rendering and simulated electron microscopy including territory painting reveal not only again the model details but also that any location in the nucleus is accessible to biological molecules <15 nm in diameter and that even the Extended Interchromosomal Domain hypothesis is oversimplified.

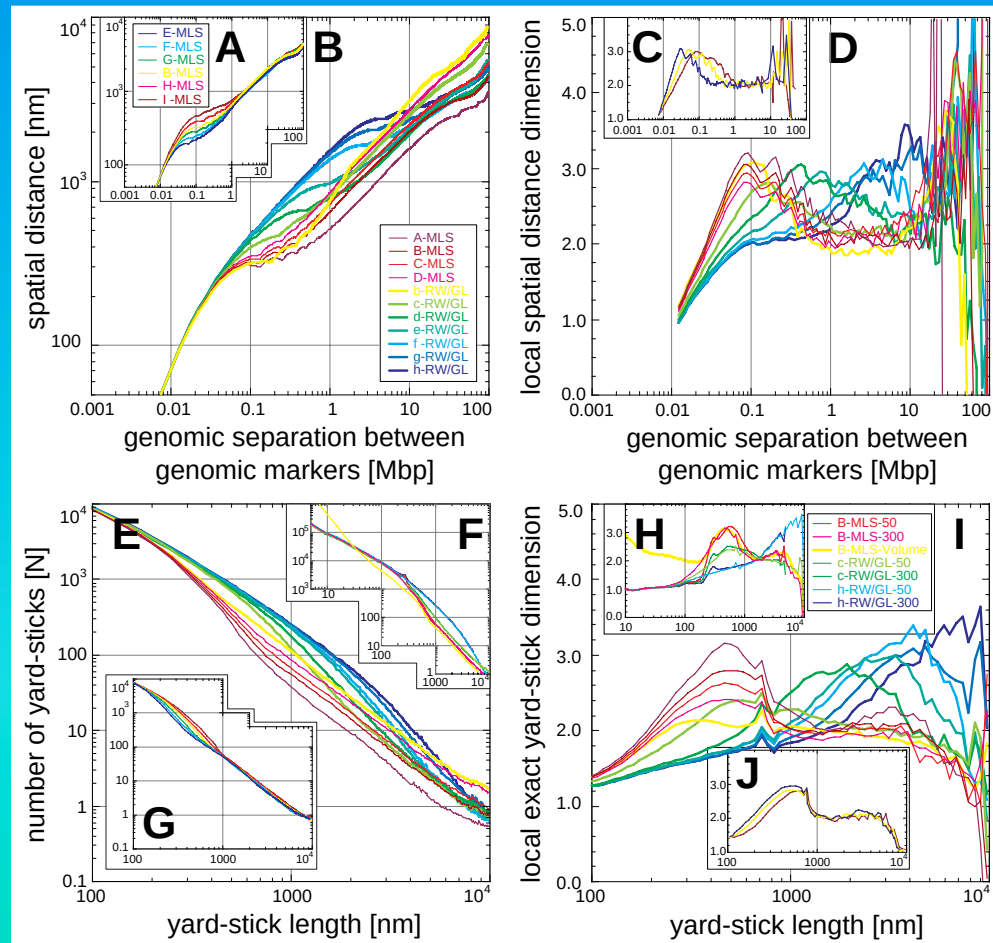
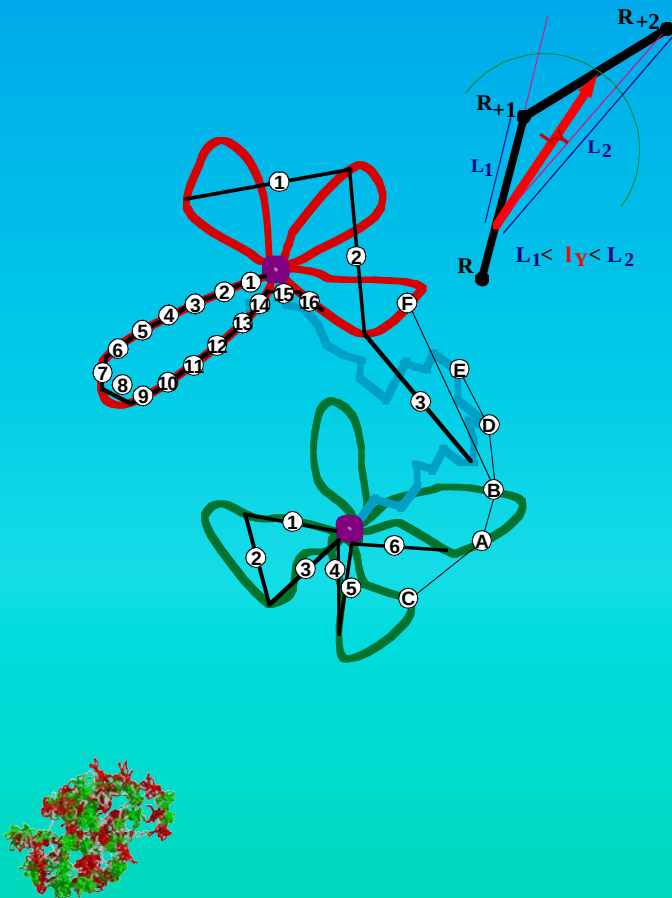
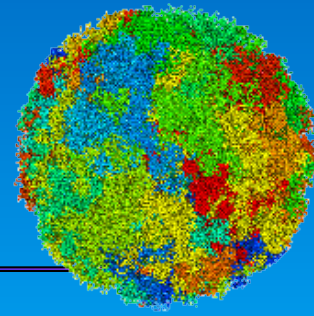


MLS
models
model with
126 kbp
loops and
linkers in a
10 μ m
nucleus.



Scaling of the Chromatin Fiber Topology

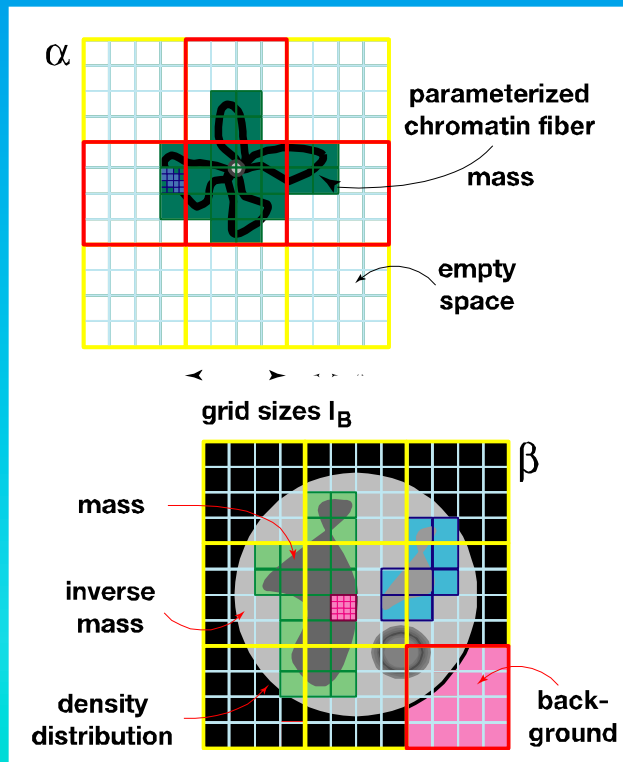
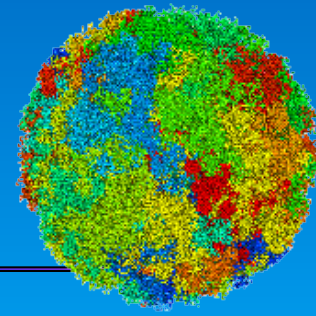
The spatial-distance and exact yard-stick dimension distinguish between the simulated models in detail. The MLS model shows a globular and fine-structured multi scaling behaviour due to the loops forming rosettes. This agrees with DNA fragmentation by Carbon ion irradiation and the appearance of fine-structured multi-scaling long-range correlations found in the sequential organization of genomes.



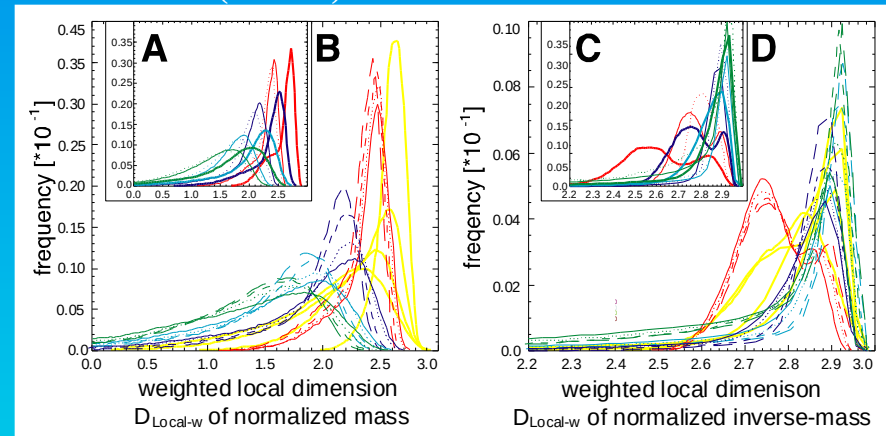
Scaling of the Chromatin Morphology & Distribution

The local (inverse-) mass dimension distribution distinguishes between the models in detail and show also a multi-scaling behaviour with globular feature for the MLS model like the scaling of the fiber topology. With the mass dimension as function of intensity separates very well between different nuclei *in vivo*.

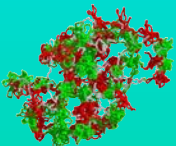
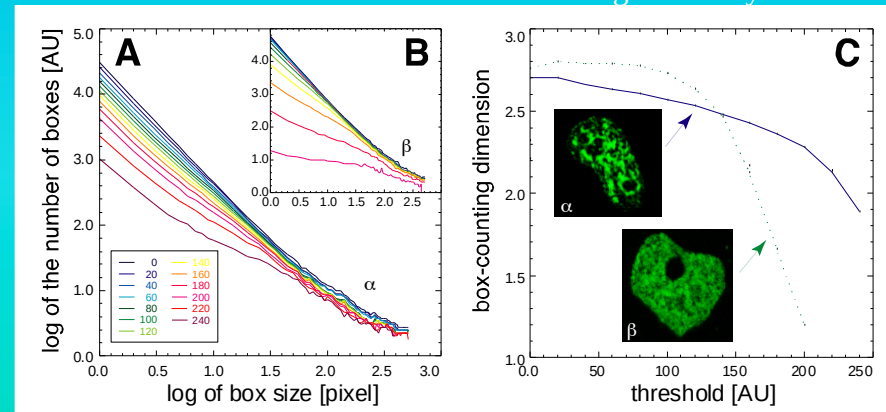
Consequently, the chromatin morphology is causally and quantitatively connected to the fiber topology.



(inverse-) mass dimension distribution



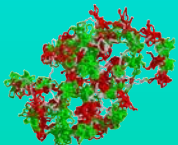
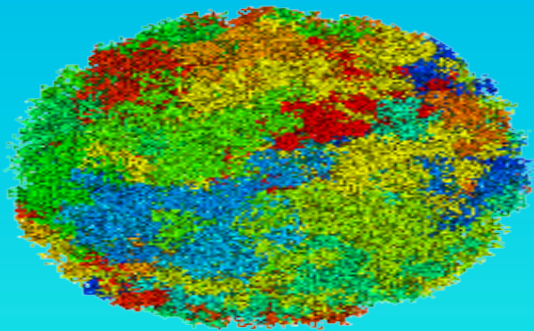
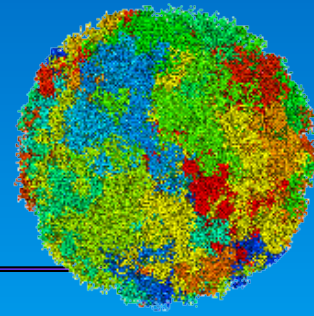
mass dimension as function of image intensity



DNA Fragment Distribution after Ione-Irradiation

The length distribution of DNA fragments after irradiation with e. g. C or Ca with an inhomogeneous spatial double strand breackage probability depends on the detailed folding topology of the chromatin fiber and the RW/GL and MLS models differ largely.

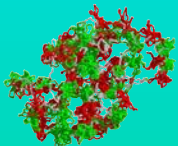
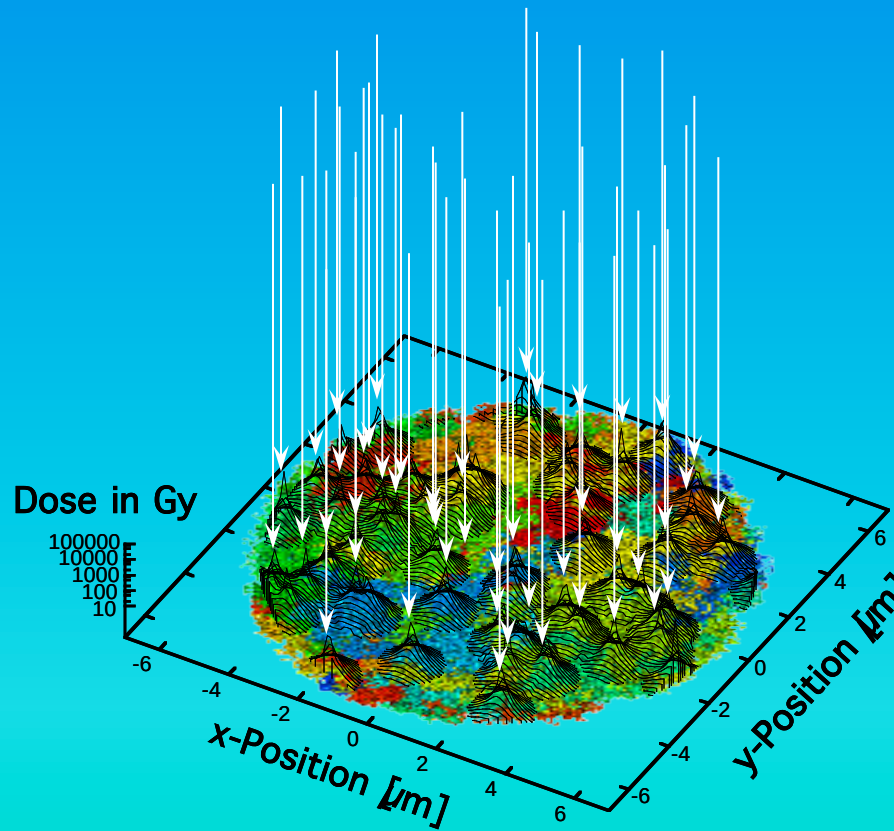
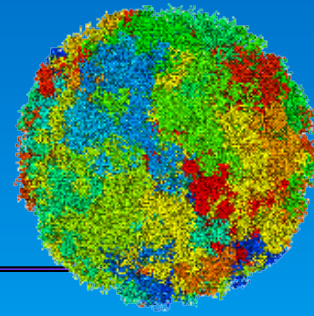
Experiments always agree best with the MLS model independent of the irradiation conditions.



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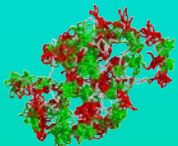
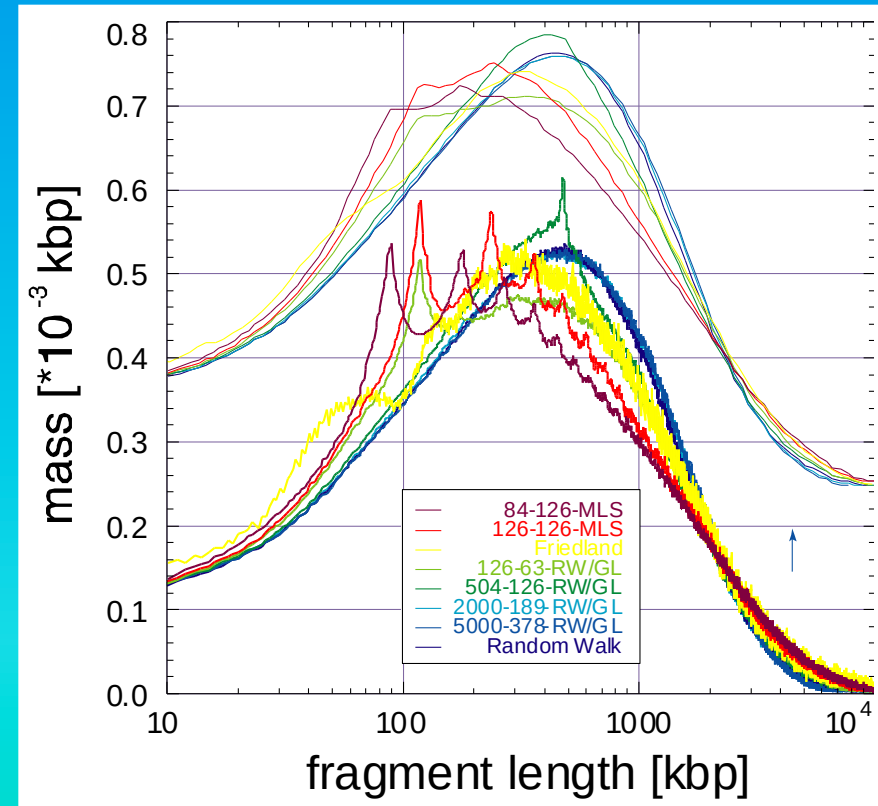
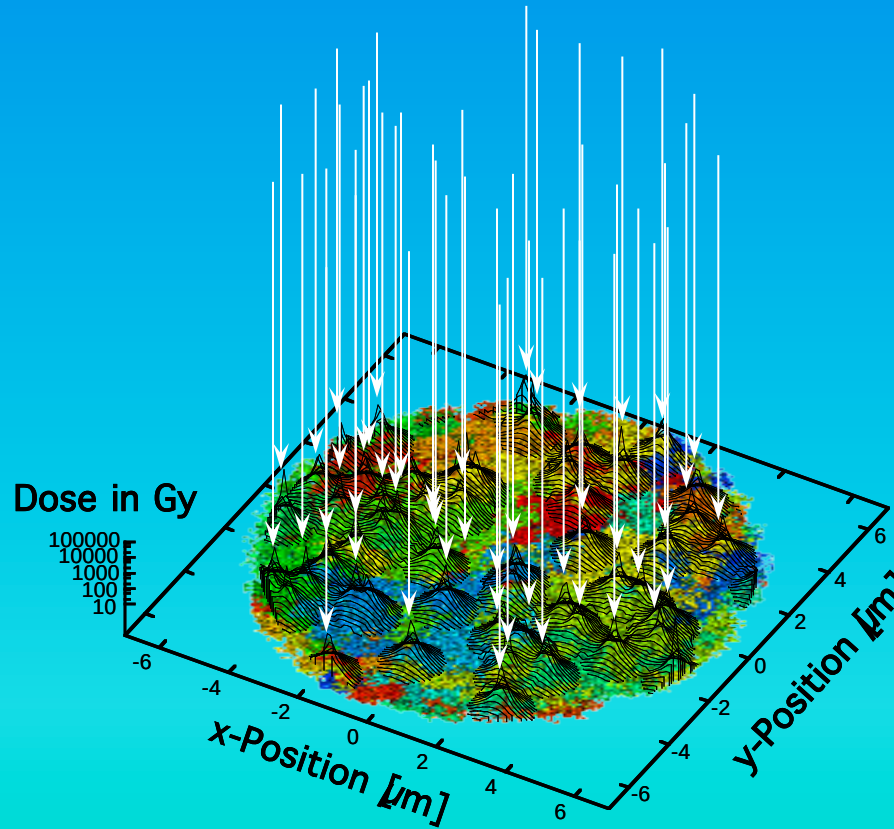
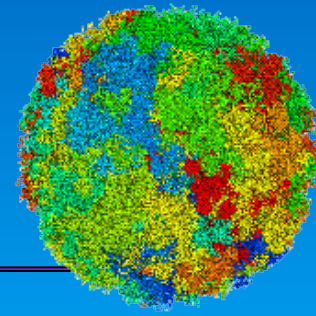
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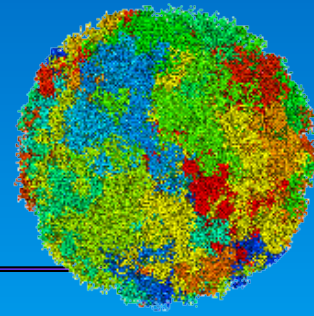
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Diffusion of Particles in the Nucleus

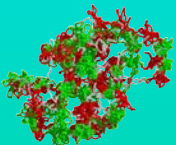
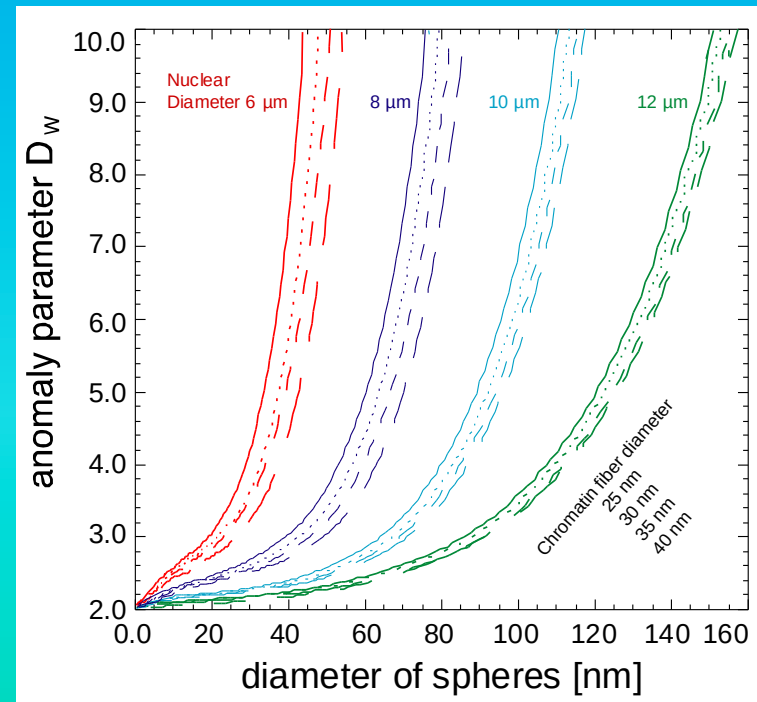
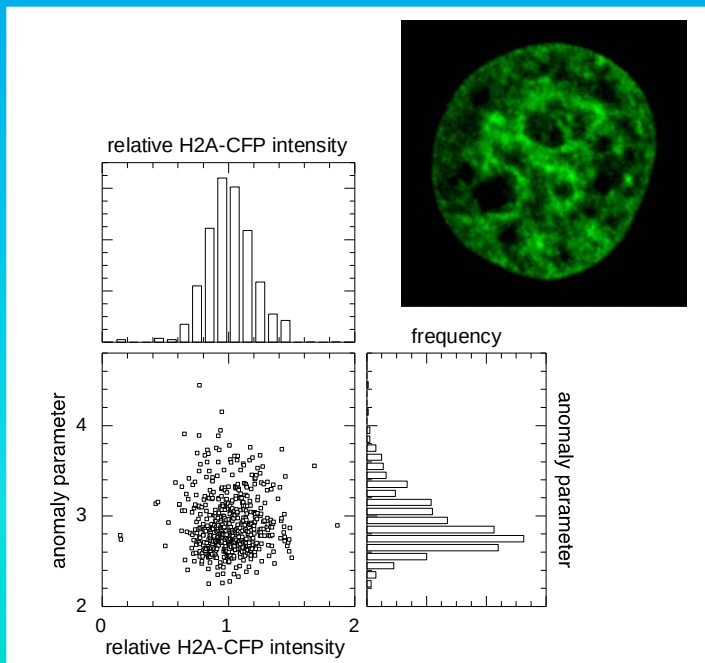
Due to the volume and spatial relations in the nucleus typical particles reach almost any location in the nucleus by moderately obstructed diffusion: a 10 nm particle moves 1 to 2 μm within 10 ms.

The structural influence on the obstruction degree is random for Alexa 568 as function of the chromatin distribution visualized by H2A CFP in vivo and measured by fluorescence correlation spectroscopy (FCS)



$$\langle r^2 \rangle \propto t^{2/D_w}$$

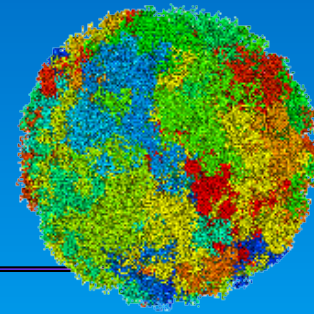
Nuclear diameter [μm]	Nuclear Volume [μm^3]	Mean Nucleosome Concentration [μM]	Chromatin Volume Fraction [%]	Mean Isotropic Mesh Spacing [nm]
6	115	251	20.1	41
8	268	107	8.6	64
10	523	55	4.4	90
12	904	32	2.6	117



Sequential Organization of Genomes

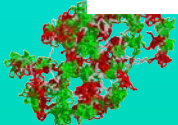
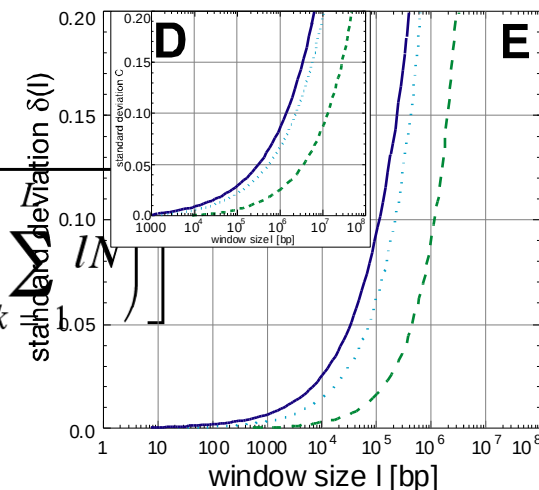
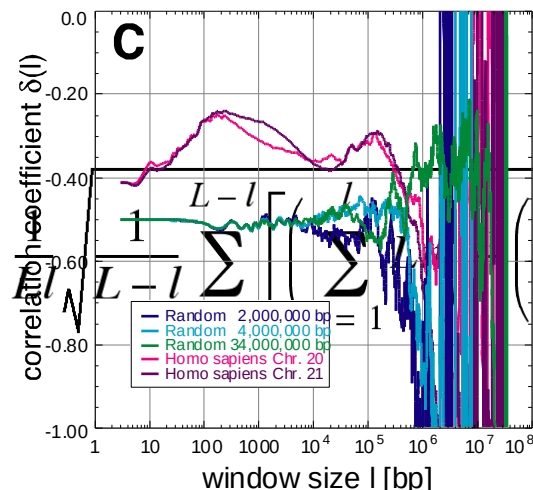
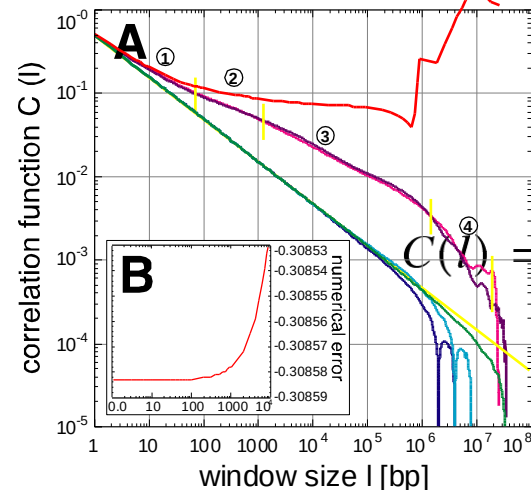
Determination of the concentration fluctuation function $C(l)$ and its local slope the correlation coefficient $\delta(l)$ reveal multi-scaling long-range correlation up to 10^6 to 10^7 bp in *Homo sapiens* which clearly deviate from random sequences with high significance (decreasing the nearer to the cut-off).

On large scales this might only be due to a strong and definite three-dimensional genome organization.

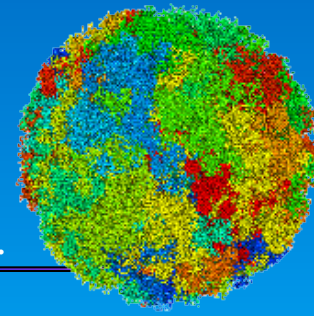


$$C(l) = \sqrt{\langle (c_l - \bar{c}_L)^2 \rangle_s} \quad \text{numerically unstable}$$

$$C(l) = \sqrt{\frac{1}{L-l+1} \sum_{s=1}^{L-l} \left(\frac{1}{l} \sum_{k=1}^l n - \frac{1}{L} \sum_{k=1}^L N \right)^2} \quad \text{numerically stable}$$



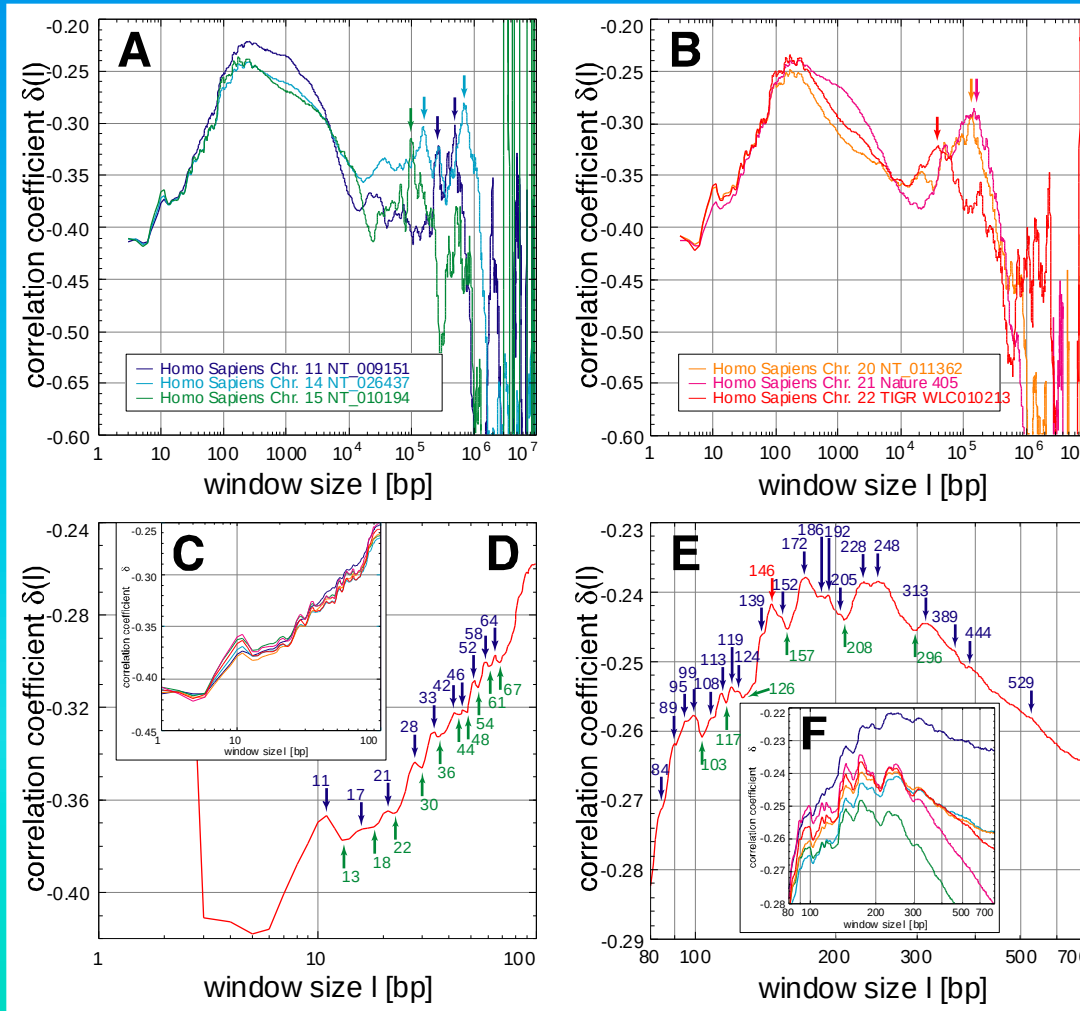
Fine-Structured Multi-Scaling Long-Range Correlations of *Homo sapiens*



The general behaviour is characterized by first maximum of the correlation coefficient $d(l)$ at ~ 250 bp and at 1×10^5 to 3×10^5 bp, both due to a globular block structure of genomes. Due to their fine structure the first is attributable to nucleosomal binding and the latter due to aggregation of chromatin loops as in the MLS model.

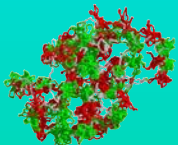
Thus, the sequential organization is closely connected to the three-dimensional organization of genomes.

general behaviour



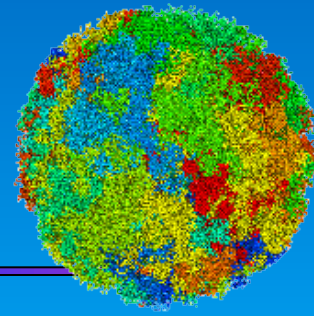
fine structure

the fine structure survives averaging over several human chromosomes.

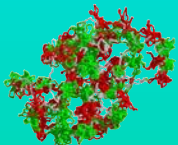


Conclusion

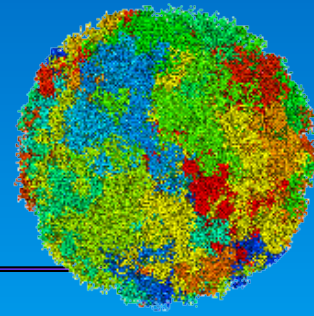
Every structural level of nuclear organization including its dynamics is connected and represented in all the other levels.



- Only the MLS model leads to chromosome territories with subcompartments agreeing qualitatively and quantitatively with experiments.
- Comparison between simulated and experimental spatial distances between genetic markers favours and MLS model with 80 to 120 kbp loops and linkers.
- The nuclear morphology or chromatin distribution is tightly connected to the folding topology of the chromatin fiber.
- Scaling analysis of the chromatin fiber topology and nuclear morphology reveals a fine-structured multi-scaling behaviour and allows a detailed description model changes.
- Most biological particles (molecules, proteins...) could reach almost any location in the nucleus by only moderately obstructed diffusion in agreement with *in vivo* experiments.
- The DNA fragment distribution after ion irradiation reflects the chromatin fiber topology not only in detail but also favours always an MLS model.
- The sequential organization of genomes is characterized by fine-structured multi-scaling long-range correlations, which are specie specific and tightly connected to the three-dimensional organization of genomes. On large-scales again an MLS model is favoured.



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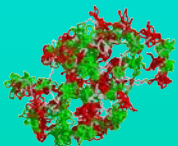
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Towards a Holistic Understanding of the Human Genome by Determination and Integration of its Sequential and Three-Dimensional Organization

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Computational Methods in the Nanoscopy of Supramolecular Biological Complexes,
University of Mannheim, Mannheim, Germany, 2nd October, 2003.

Abstract

Genomes are one of the major foundations of life due to their role in information storage, process regulation and evolution. However, the sequential and three-dimensional structure of the human genome in the cell nucleus as well as its interplay with and embedding into the cell and organism only arise scarcely from the unknown. To achieve a deeper understanding of the human genome the three-dimensional organization of the human cell nucleus, the structural-, scaling- and dynamic properties of interphase chromosomes and cell nuclei were simulated and combined with the analysis of long-range correlations in completely sequenced genomes as well as the chromatin distribution *in vivo*. Using Monte Carlo and Brownian Dynamics methods, the 30 nm chromatin fiber was folded according to the Multi-Loop-Subcompartment (MLS) model, in which ~100 kbp loops form rosettes, connected by a linker, and the Random-Walk/Giant-Loop (RW/GL) topology, in which 1-5 Mbp loops are attached to a flexible backbone. Both the MLS and the RW/GL model form chromosome territories but only the MLS rosettes result in distinct subcompartments visible with light microscopy and low overlap of chromosomes, -arms and subcompartments. This morphology and the size of subcompartments agree with the morphology found by expression of histone auto-fluorescent protein fusions and fluorescence *in situ* hybridization (FISH) experiments. Even small changes of the model parameters induced significant rearrangements of the chromatin morphology. Thus, pathological diagnoses based on this morphology, are closely related to structural changes on the chromatin level. The position of interphase chromosomes depends on their metaphase location, and suggests a possible origin of current experimental findings. The chromatin density distribution of simulated confocal (CLSM) images agrees with the MLS model and with recent experiments. The scaling behaviour of the chromatin fiber topology and morphology of CLSM stacks revealed fine-structured multi-scaling behaviour in agreement with the model prediction and correlations in the DNA sequence. Review and comparison of experimental to simulated spatial distance measurements between genomic markers as function of their genomic separation also favour an MLS model with loop and linker sizes of 63 to 126 kbp. Simulation of the fragment distribution after ion-irradiation revealed also best agreement with the MLS model by comparison with experimental distributions. Correlation analyses of completely sequenced Archaea, Bacteria and Eukarya chromosomes revealed fine-structured positive long-range correlation due to codon, nucleosomal or block organization of the genomes, allowing classification as well as tree construction. This shows a complex sequential organization of genomes closely connected to their three-dimensional organization. Visual inspection of the morphology reveals also big spaces allowing high accessibility to nearly every spatial location, due to the chromatin occupancy <30% and a mean mesh spacing of 29 to 82 nm for nuclei of 6 to 12 μm diameter. The simulation of diffusion agreed with this structural prediction, since the mean displacement for 10 nm sized particles of ~1 to 2 μm takes place within 10 ms. Therefore, the diffusion of biological relevant tracers is only moderately obstructed, with the degree of obstruction ranging from 2.0 to 4.0 again in experimental agreement. Thus, the local, global and dynamic characteristics of cell nuclei are not only tightly inter-connected, but also are integrated holistically to fulfill the overall function of the genome.

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Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, autofluorescent proteins, CFP, GFP, YFP, DsRed, fusionprotein, in vivo labelling.

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