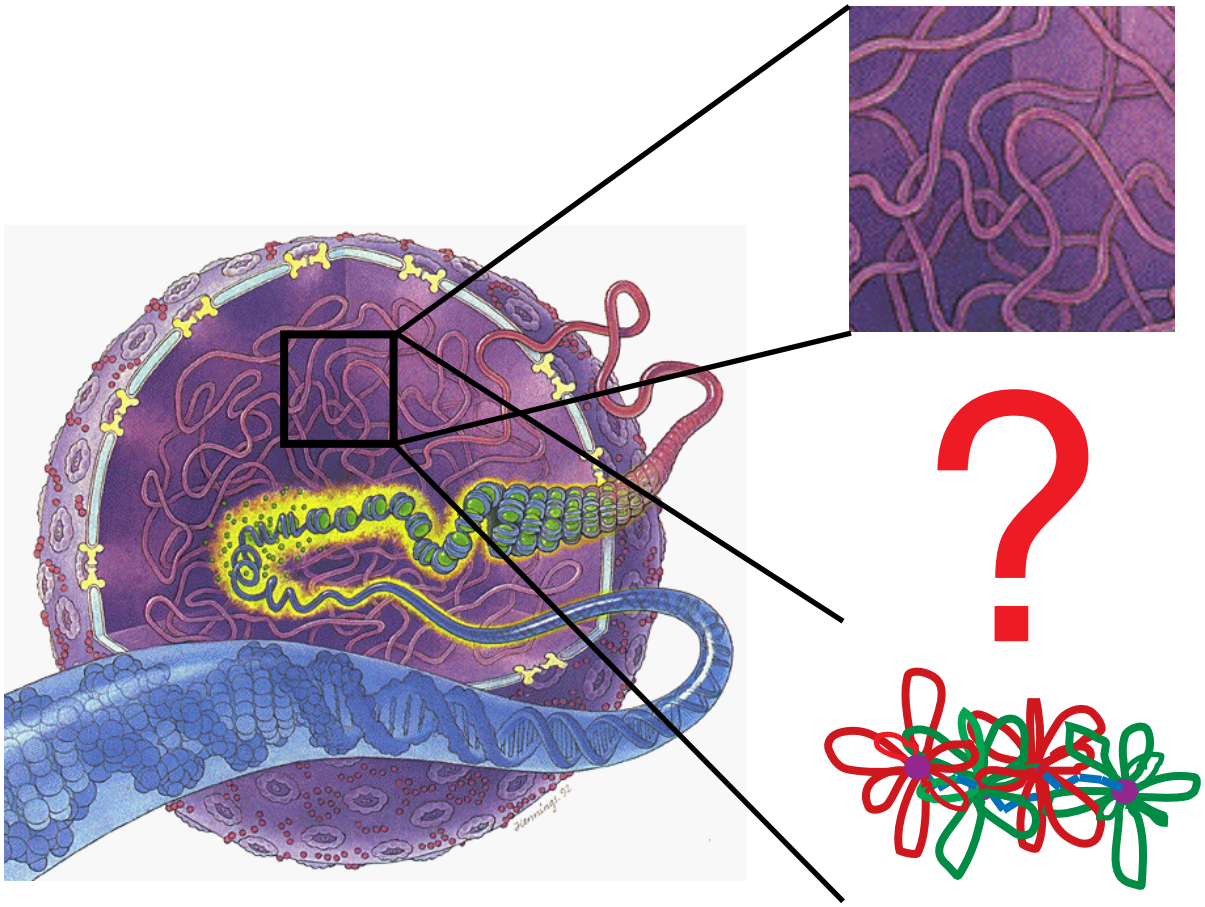
Three-Dimensional Organization of

Chromosome Territories and the Human Cell Nucleus



Tobias A. Knoch, Christian Münkel, Jörg Langowski

Biophysics of Macromolecules

German Cancer Research Center (DKFZ)

Heidelberg - Germany

The dynamic and hierarchical organization of cell nuclei span between 10 and 13 orders of magnitude concerning length and time scales.

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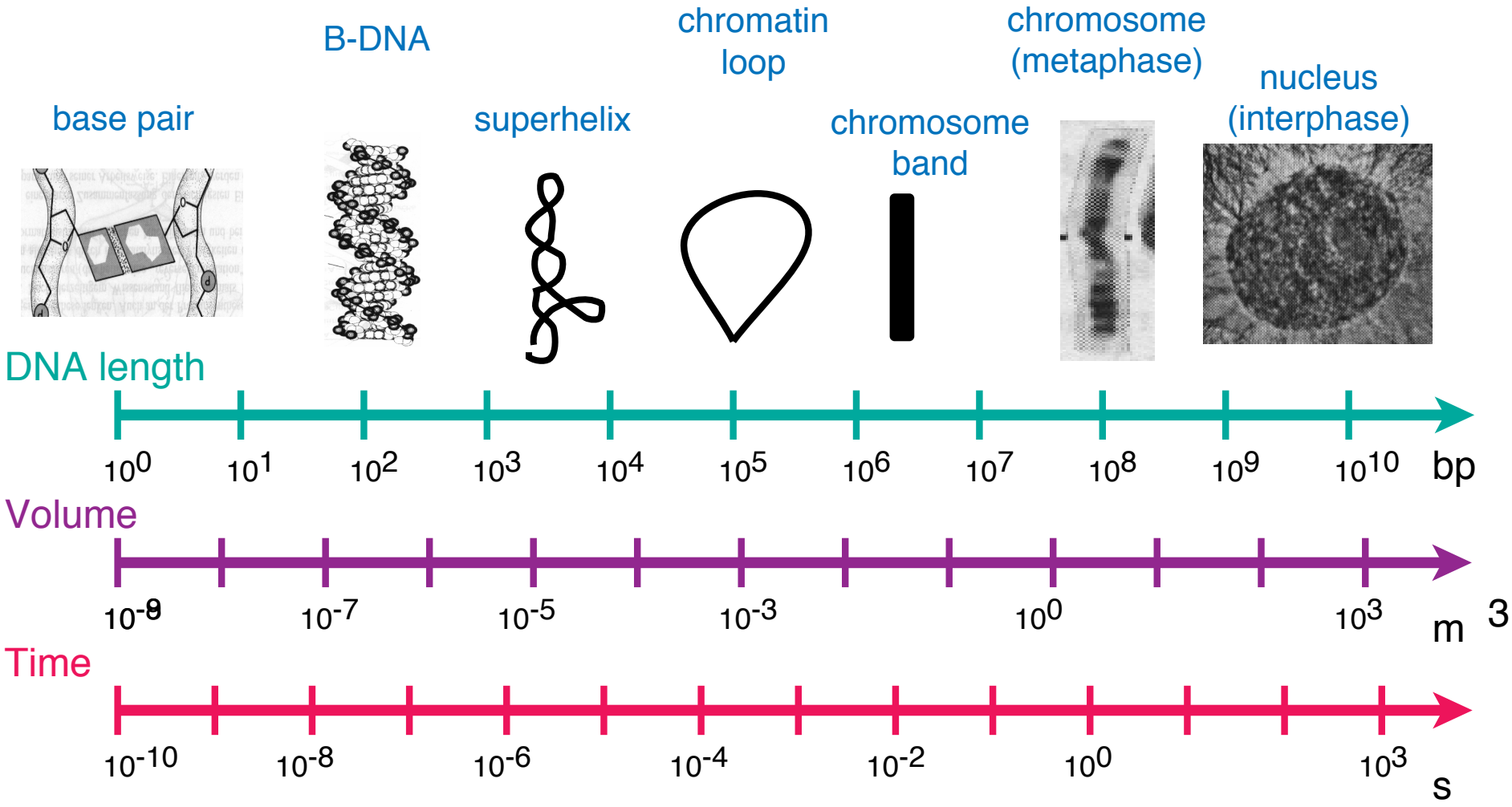
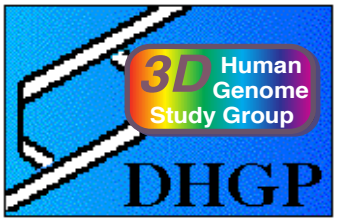


Overview



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Experiment

Genomic Region
(Chromosome or Gene)

↓

fluorescence in-situ
hybridization (FISH)

↓

3D confocal scanning
microscopy

Simulation

Multi-Loop-
Subcompartment
and
Random Walk/
Giant Loop
model

↓

polymer model
for simulation of the
chromatin fiber

↘ ↙

Conclusions for the human cell nucleus

chromosome-, chromosome-arm and subcompartment overlap

3D-distances between genomic markers as function of their genomic separation

behaviour of marker ensembles and dynamics of structural features

fractal properties of chromosomes

decondensation of chromosomes from metaphase into interphase and chromosome stretching

conclusions from simulating whole cell nuclei

Fluorescence in-situ Hybridization

FISH

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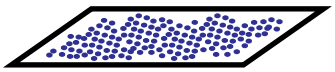
Cell - Preparation

cells on coverslip grown to confluent layer

fixation of cells on coverslip (formaldehyde) and permeabilisation



DNA double strand

TACGTTAACGGTAGCATT
ATGCAATTGCCATCGTAA

Probe - Preparation

finding of genomic site for marking and cloning of this sequence

AACGG
TTGCC

labeling of the DNA probe (Nick translation or PCR) with

Digoxigenin (indirect)

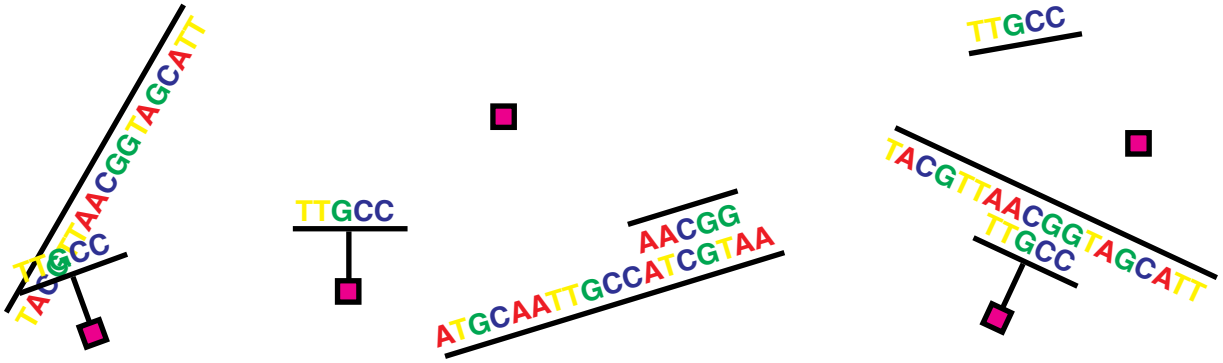
AACGG
TTGCC

Fluorophor (direct)

AACGG
TTGCC

Hybridization

probe is put on coverslip and melting of the double strands at 70C



amplification with fluorescent labeled antibodies

TACGTTAACGGTAGCATT
TTGCC

Chromosomes form distinct territories in interphase and genomic markers lie within the territories and are clearly separable.

Left: Territory painting by FISH of chromosome 15; by chance the two territories neighbour each other.

Right: Genomic markers YAC48 and YAC60, genomic separation 1 Mbp.

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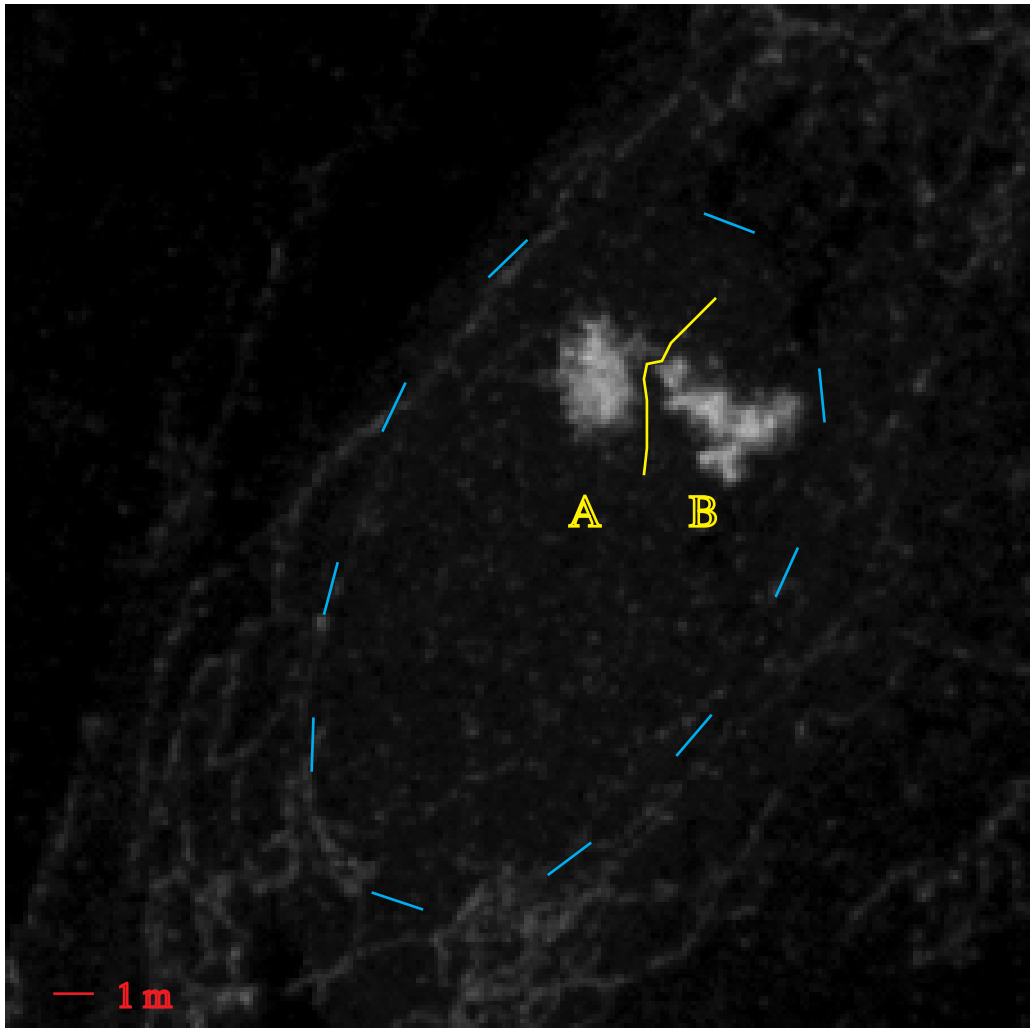

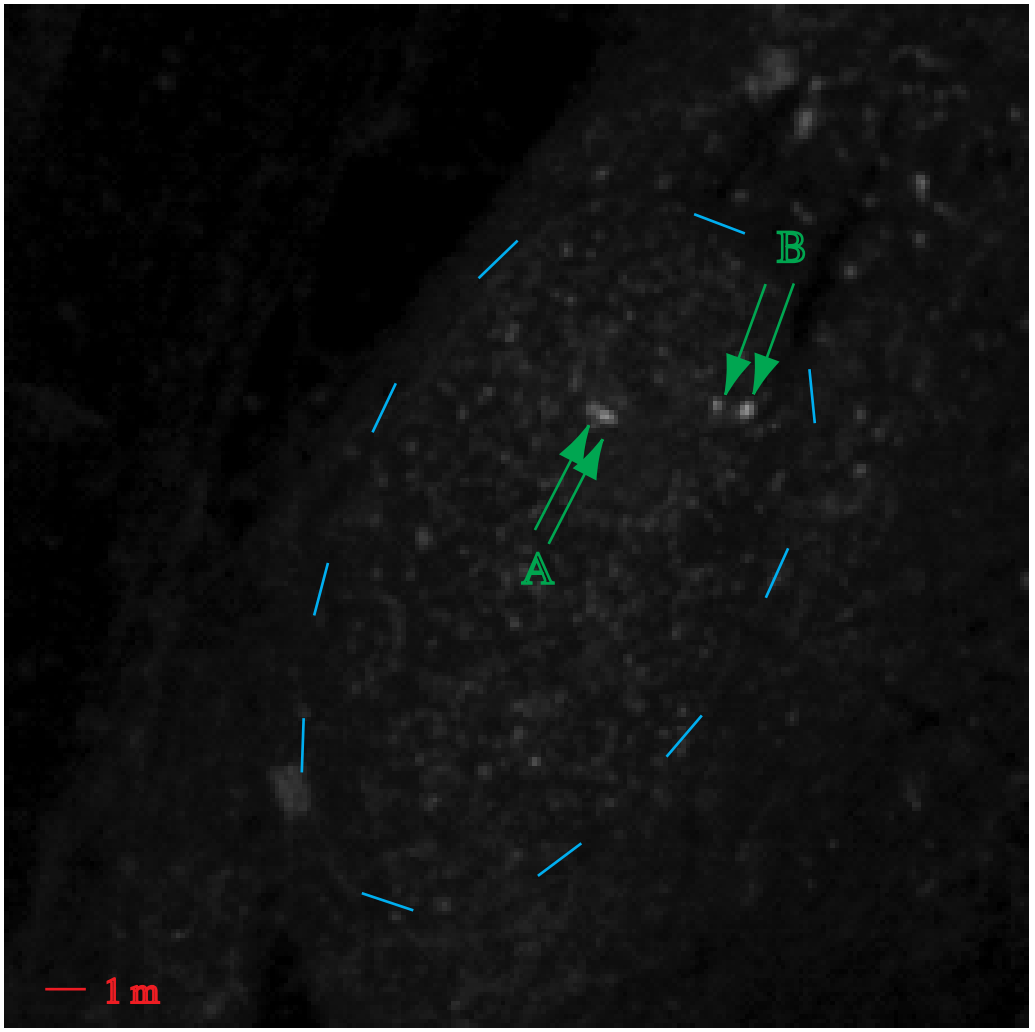

Ideogram of chromosome 15 with
Prader - Willi Region and Angelmann Region.
The size and genomic distance of the clones
are sufficiently small and well characterized to measure
the fine structure and organization of chromosome territories.

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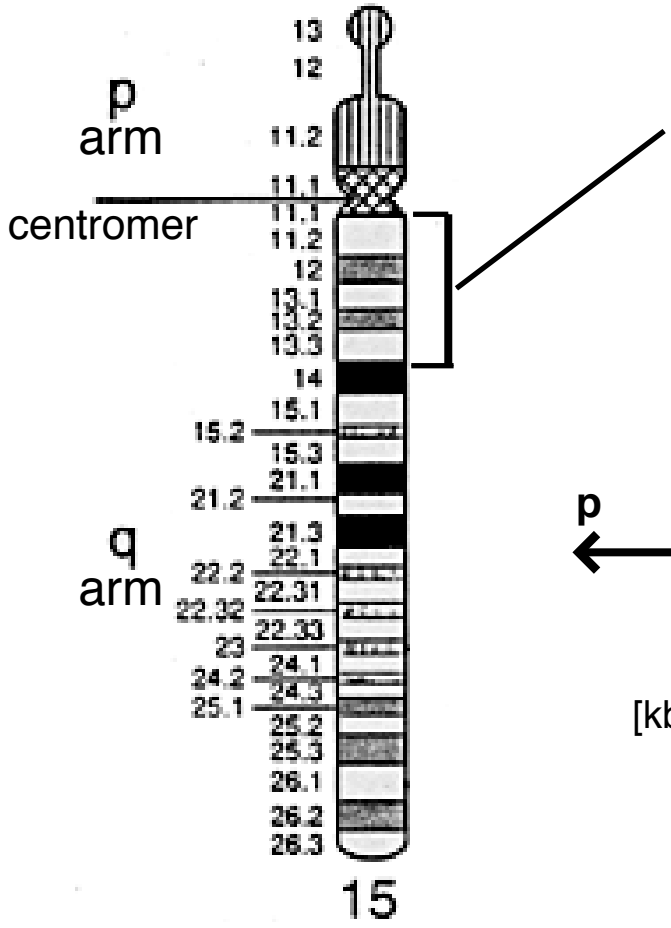
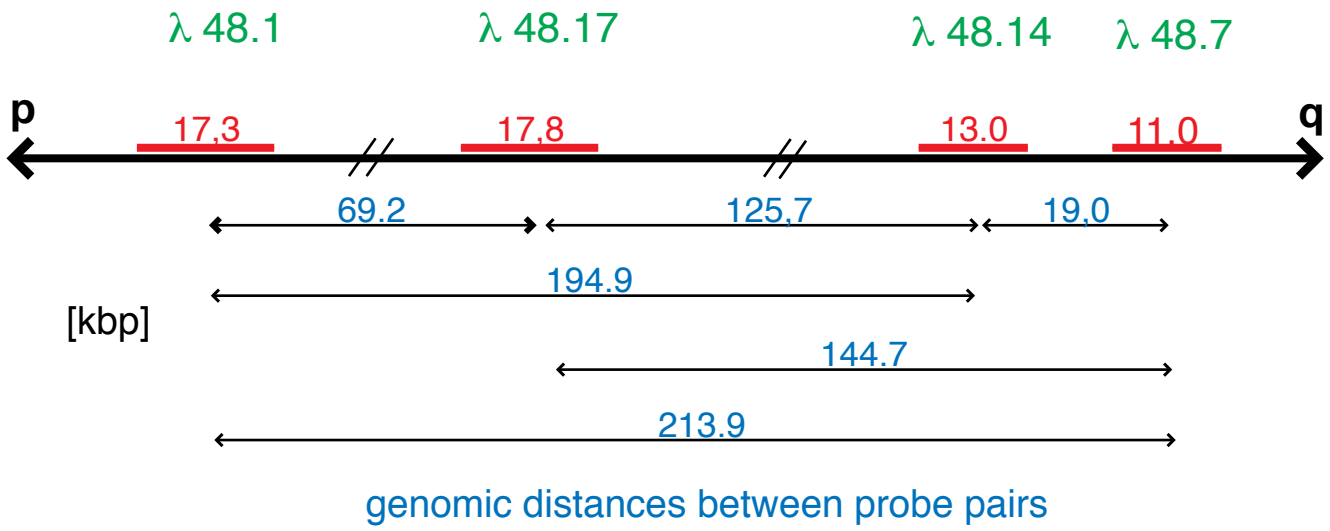


Prader-Willi Region (paternal chromosome) **YAC 48**
Angelmann Region (maternal chromosome) **YAC 60**

↕ 1Mbp

clones and their genomic size



genomic distances between probe pairs

Dual colour FISH of genomic markers leads to measurements of 3D-distances which are below the resolution of the microscope. Critical signals could also be excluded with higher confidence.

Genomic marker λ48.1 in red and marker λ48.14 in green, genomic separation 195 kbp.

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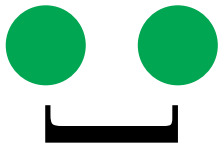
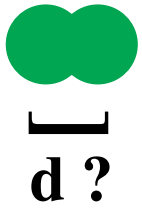
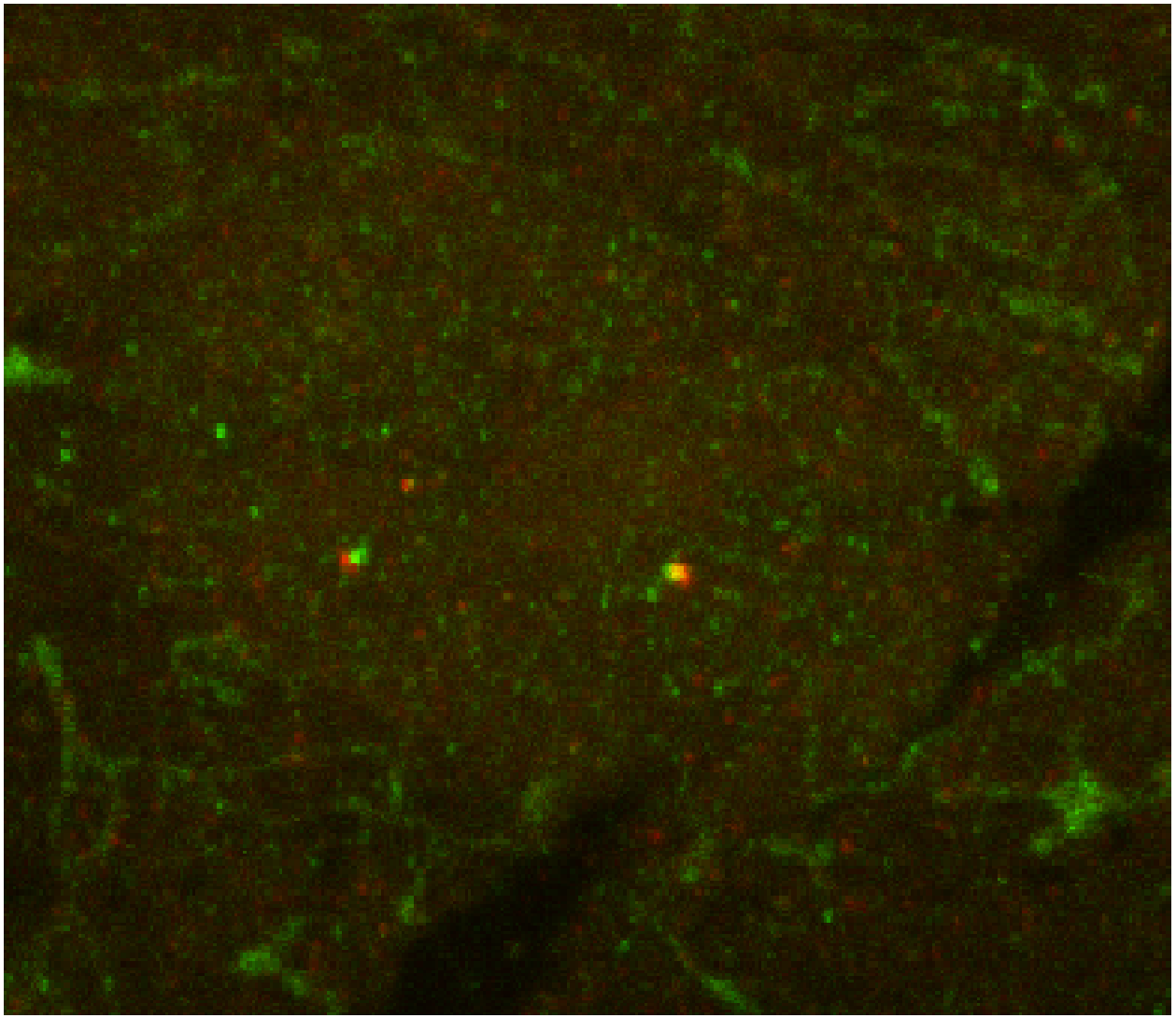
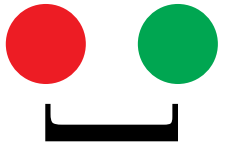
one colour

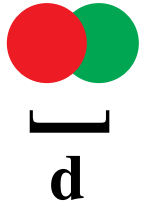






dual colour





Statistical analysis of the spatial distances between the PWS-Region (YAC48) and AS-Region (YAC60) with a genomic distance of 1Mbp = 10m chromatin fiber.

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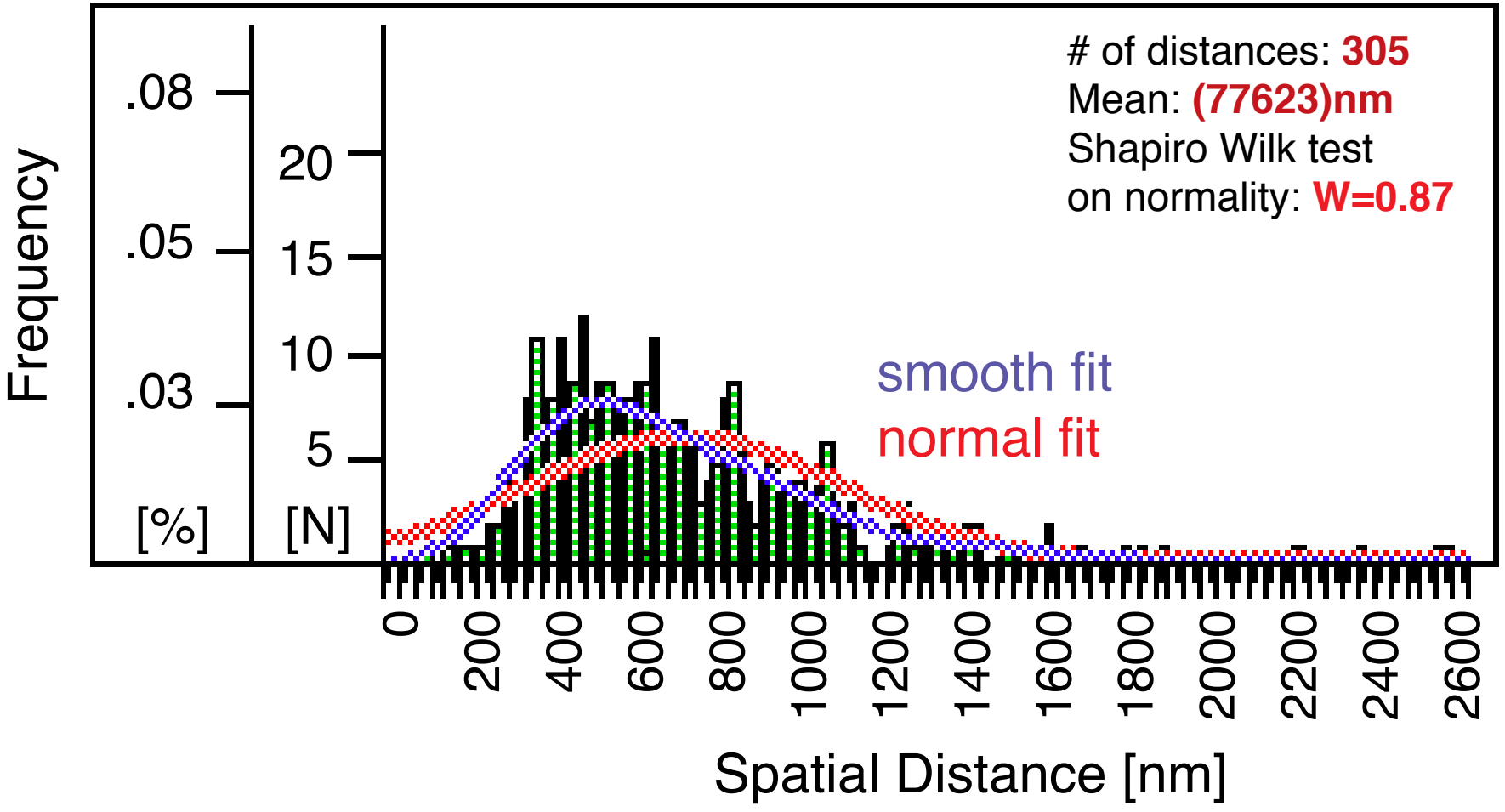
Distance Distribution



The Multi-Loop-Subcompartment (MLS) and the Random Walk / Giant Loop (RW/GL) Model. Rosettes in the MLS-Model correspond to the size of chromosomal interphase band domains.

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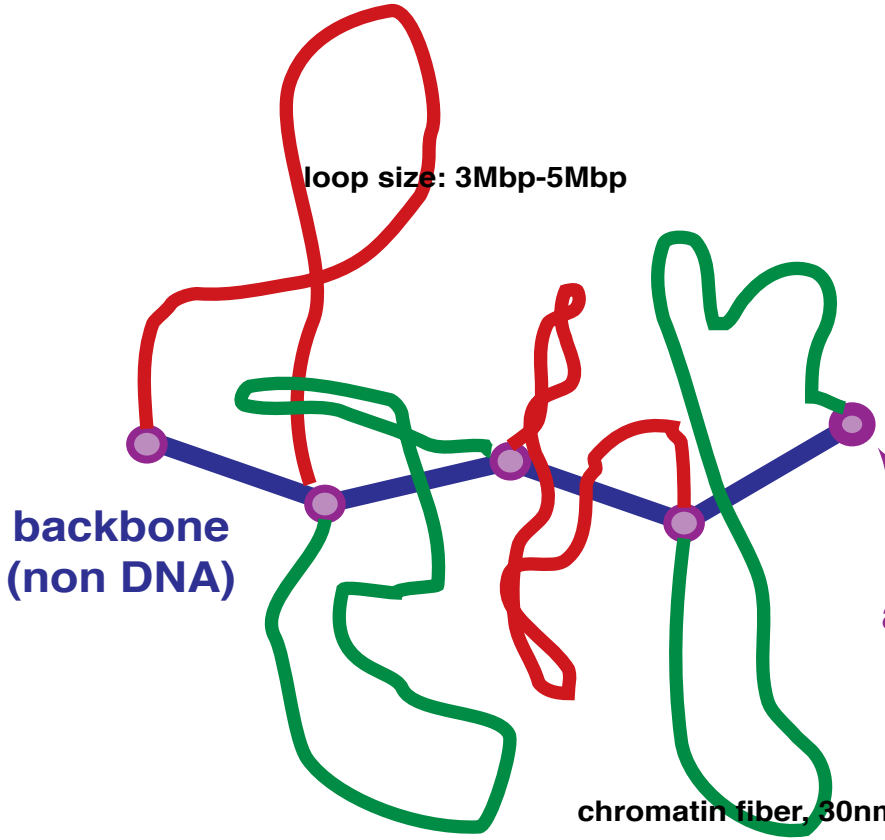
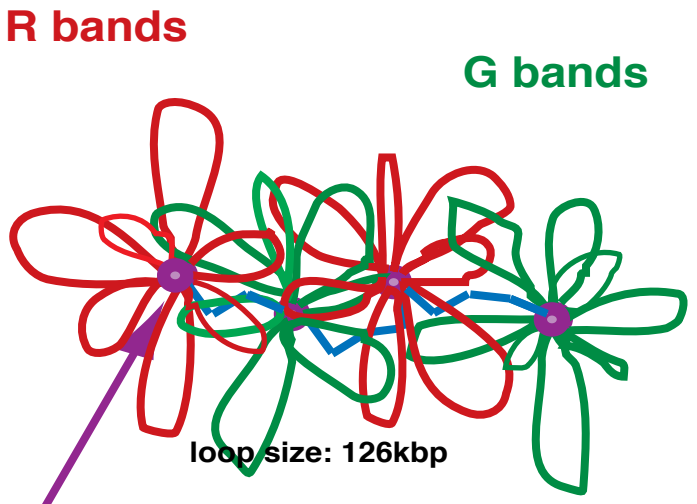
Random Walk / Giant Loop model (RW/GL) Sachs et al. (1995)



Multi-Loop-Subcompartment model (MLS) Münkel et al. (1997)



Linker consists of DNA (126 kbp) (in contrast to backbone)

Polymer Chain and Potentails

The chromosome fiber is simulated assuming a polymer chain and harmonic potentials. No hydrodynamic interaction is used due to hydrodynamic shielding.

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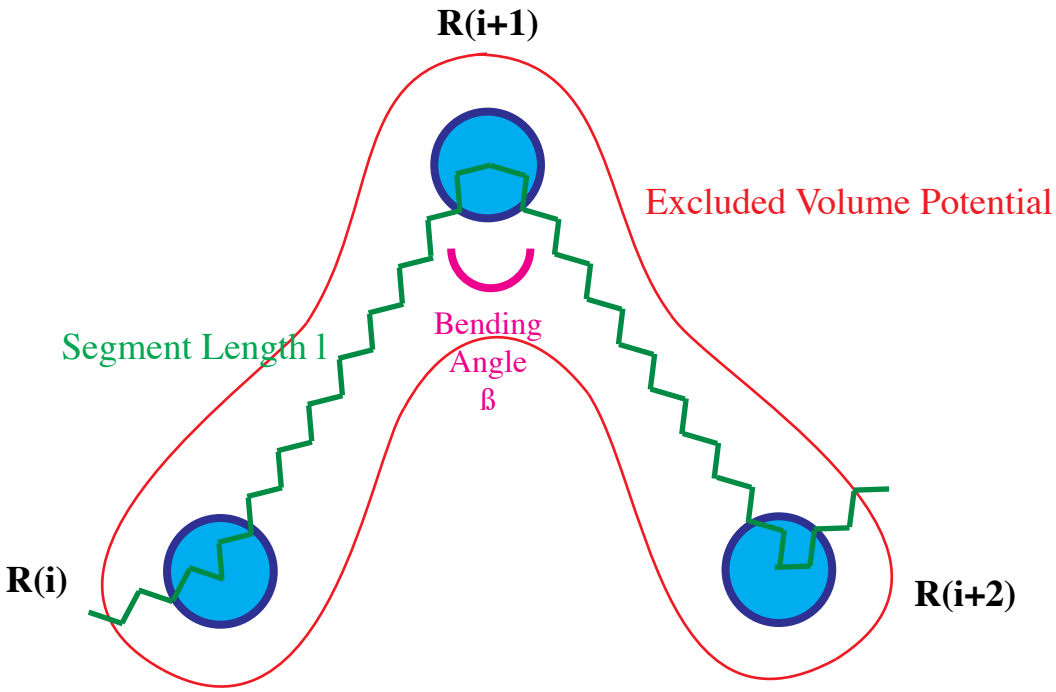

Stretching Potential

$$U_s(l) = \frac{k_B T}{2}^2 (l - l_0)$$

Bending Potential

$$U_b(ß) = \frac{k_B T}{2}^2 ß^2$$

Excluded Volume Potential

$$U_{ev}(r) = U_{ev}^0 k_B T \left(1 + \frac{r^4 - 2r_c^2 r^2}{r_c^4}\right)$$

k_B : Bolzmann constant
T : Temperature, 310 K
: stretching elasticity, = 0.1
: bending elasticity
r_c : minimum distance of segments
L_k : Kuhn length, 300 nm, $L_k = b_0/2$ [2]

VirtNucSim

The programme code is written in C++ and uses Message Passing Interface (MPI) for parallelization and scales well at least up to 64 processors depending on compilation.

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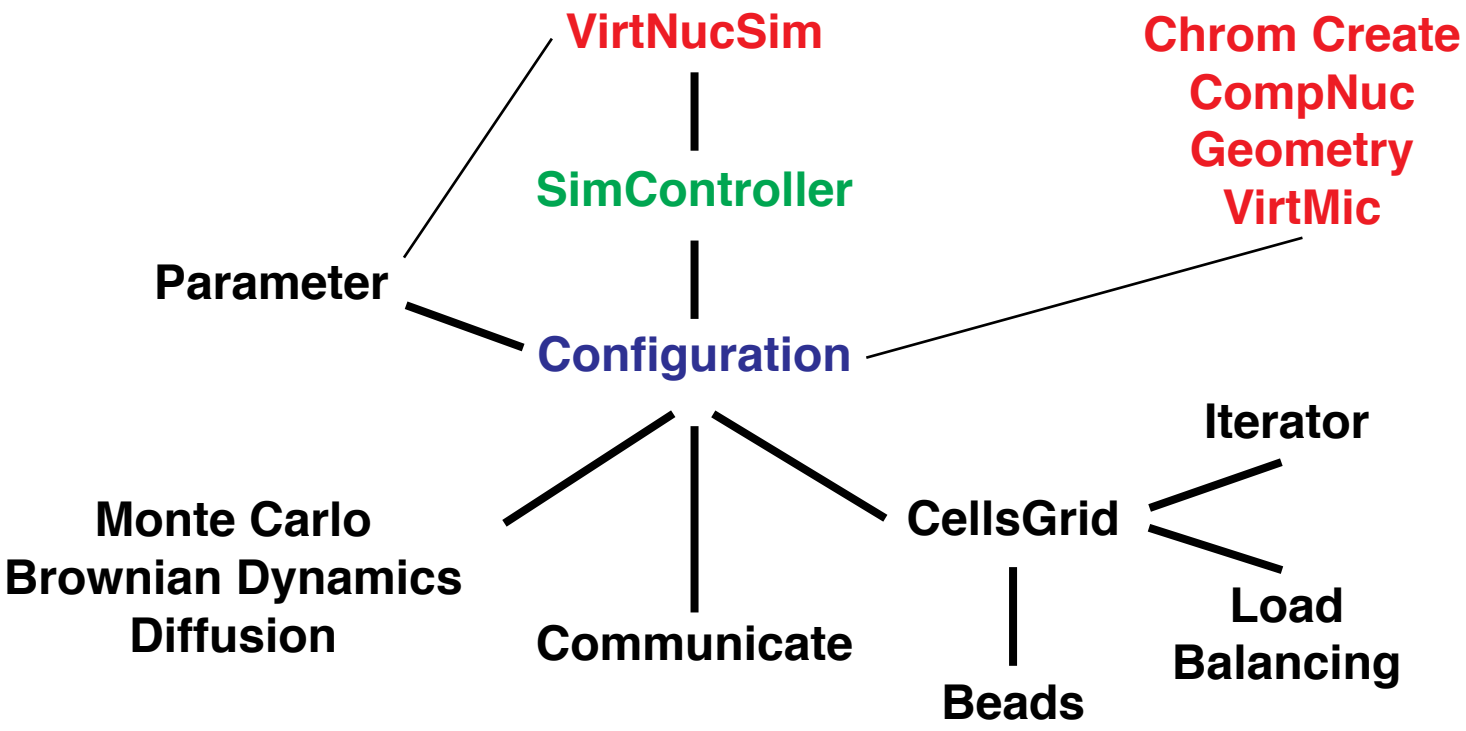

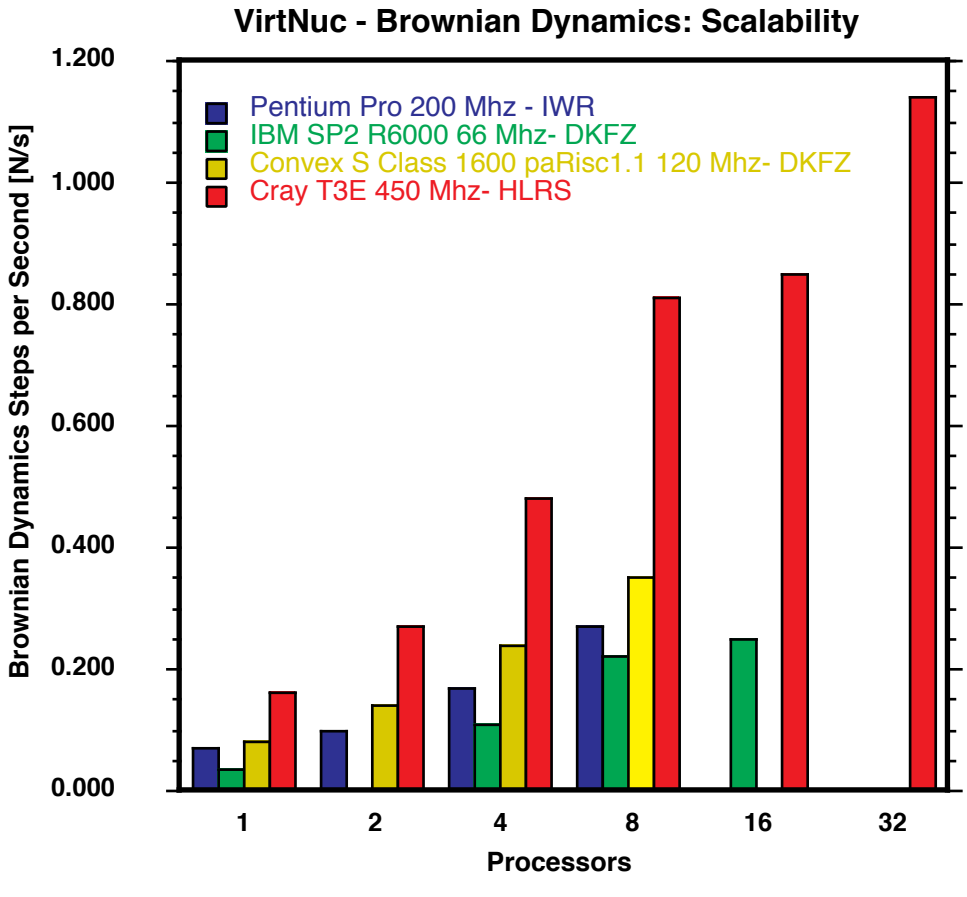

Linked-Cell Algorithm and Dynamic Load Balancing

A linked-cell algorithm reduces the computation time for the pairwise Excluded Volume interaction using all beads within one cell and half of its 26 neighbour cells.
Dynamic Load Balancing reduces the computation time by projecting the 3D cell grid *dynamically* on the 2D processor grid (spherical nucleus time reduction: 1/3).
To avoid communication overhangs asynchronious, buffered communication is used.

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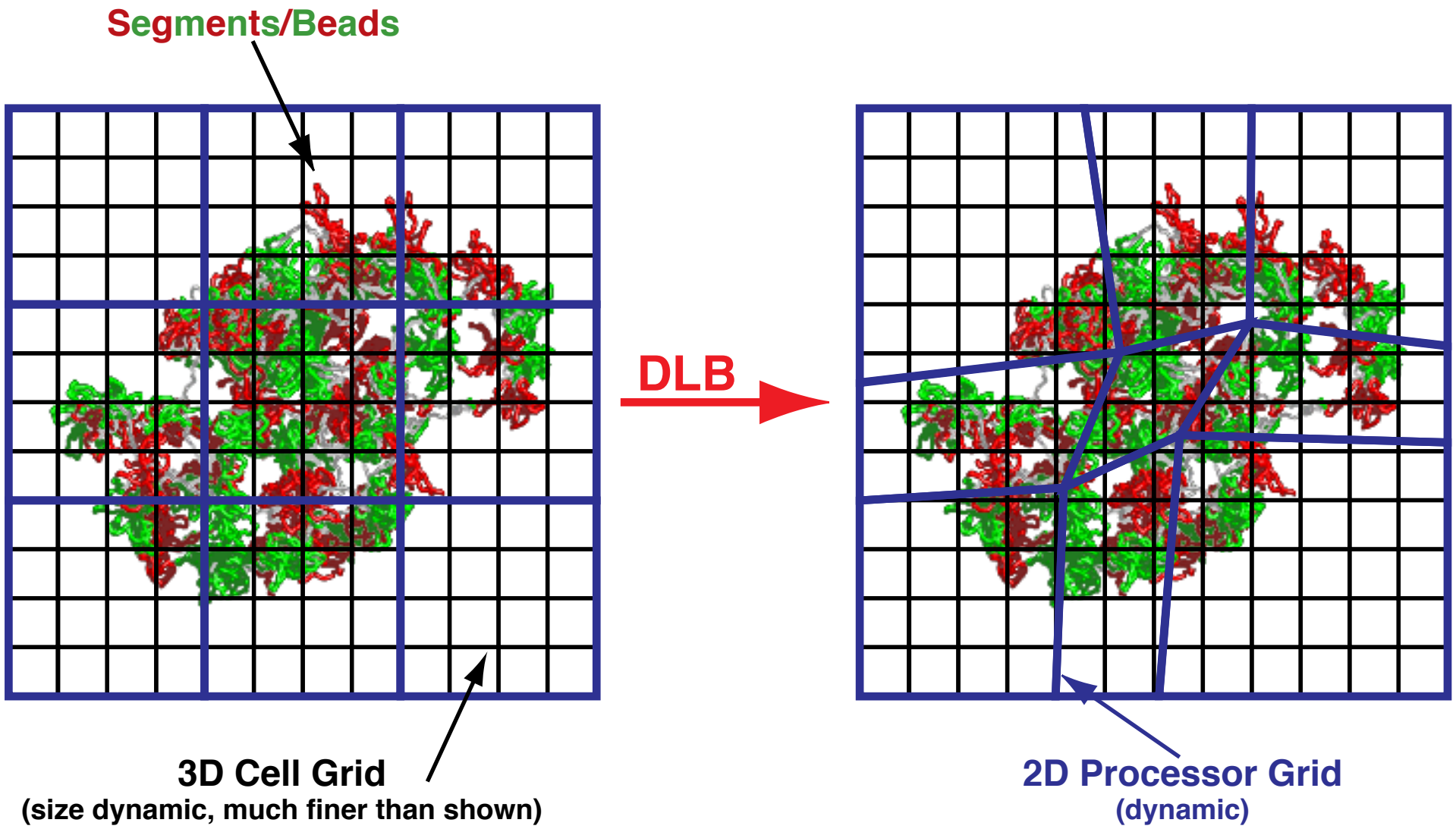

Random-Walk/Giant-Loop model versus Multi-Loop-Subcompartment model. Simulation results of chromosome 15.

The chromosome is simulated assuming a flexible polymer chain, starting with ~ 3500 segments of 300nm = 31kbp and relaxing with ~ 21,000 segments 50nm = 5.2kbp. The starting configuration has the approximate form and size of a metaphase chromosome.

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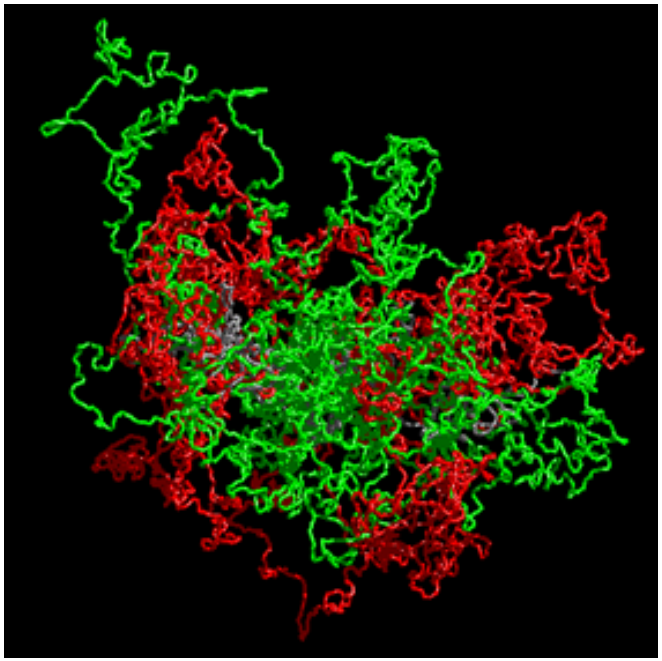
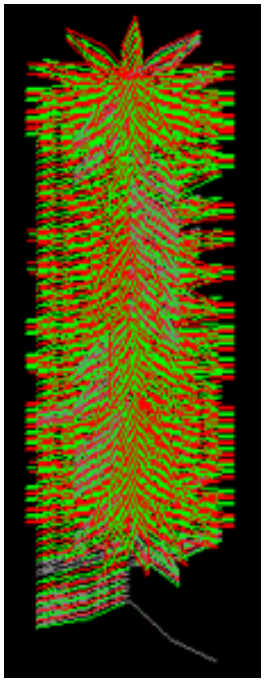
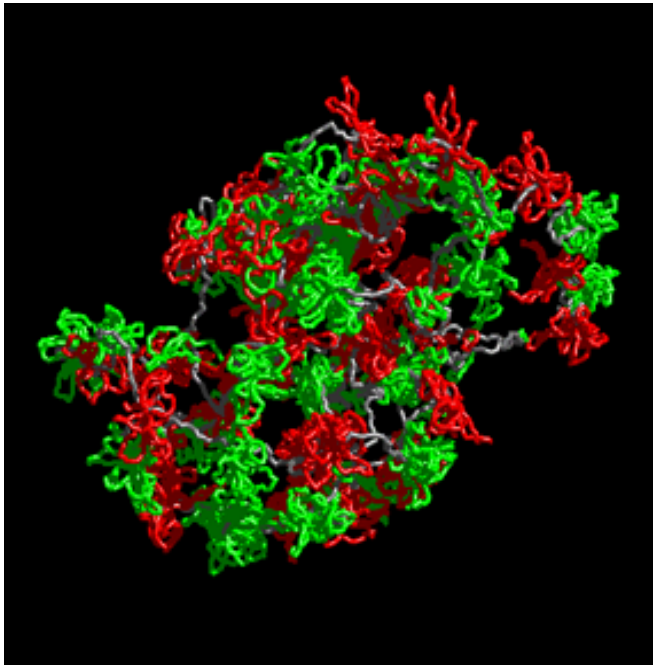
Ray traced image of the Random-Walk/Giant-Loop model, loop size 5Mbp, after ~80.000 Monte-Carlo and 1000 relaxing Brownian-Dynamics steps. Large loops intermingle freely thus forming no distinct features like in MLS model.





Wire frame image of the starting configuration.

Ray traced image of the Multi-Loop-Subcompartment model, loop size 126kbp, linker size 126 kbp, after~50.000 Monte-Carlo and 1000 relaxing Brownian-Dynamics steps. Here rosettes form subcompartments as separated organizational and dynamic entities.



Chromosome arms and bands do not overlap.
The MLS-model predicts this behavior.

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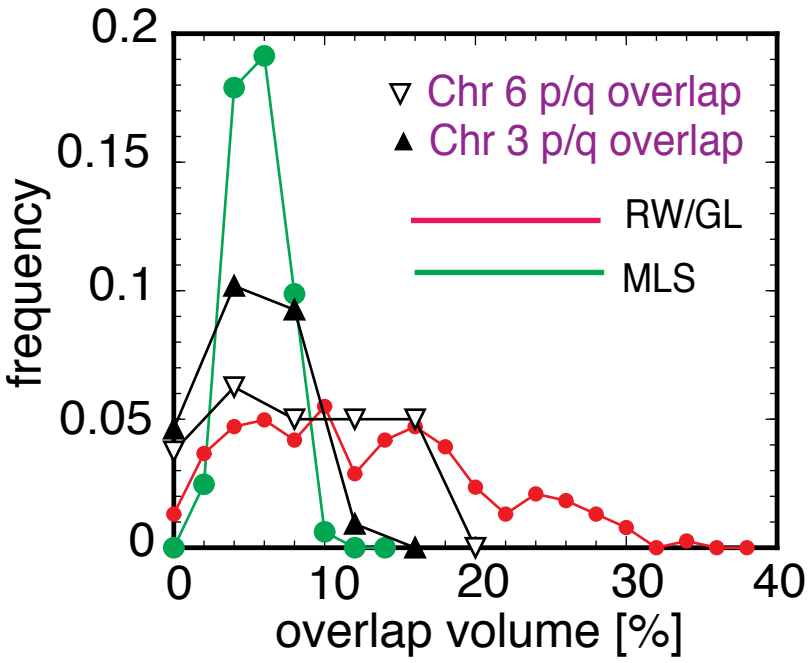
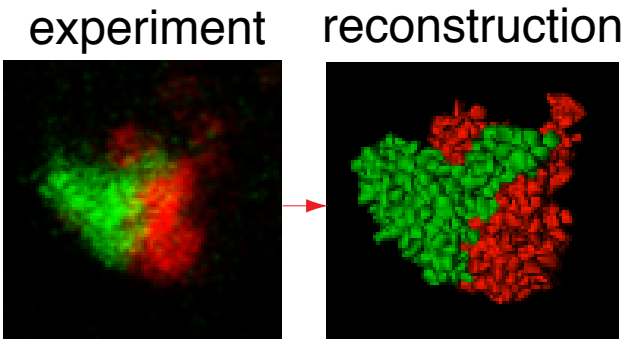
Arm Overlap

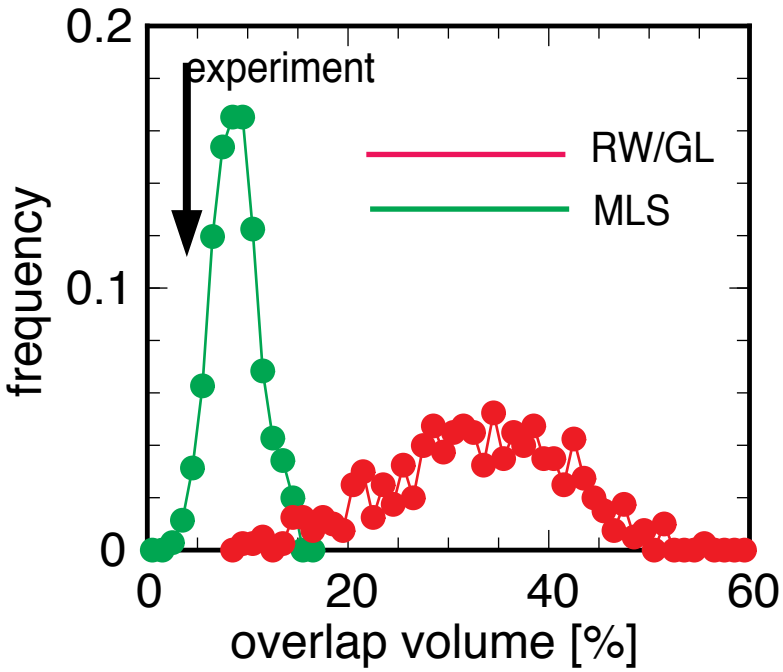


Confocal images of interphase p- and q- arms of human chromosome 3

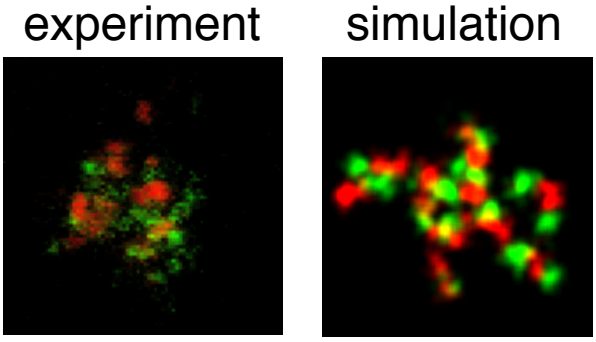


S. Dietzel R. Eils

Subcompartment Overlap



Confocal images of interphase R- and G- bands of human chromosome 15



D. Zink

Comparison of the RW/GL- and MLS model with experimentally determined interphase distances.

Best agreement between simulations and experiments is reached for a MLS-model with a loop size of 126kbp and a linker length of 126kbp.

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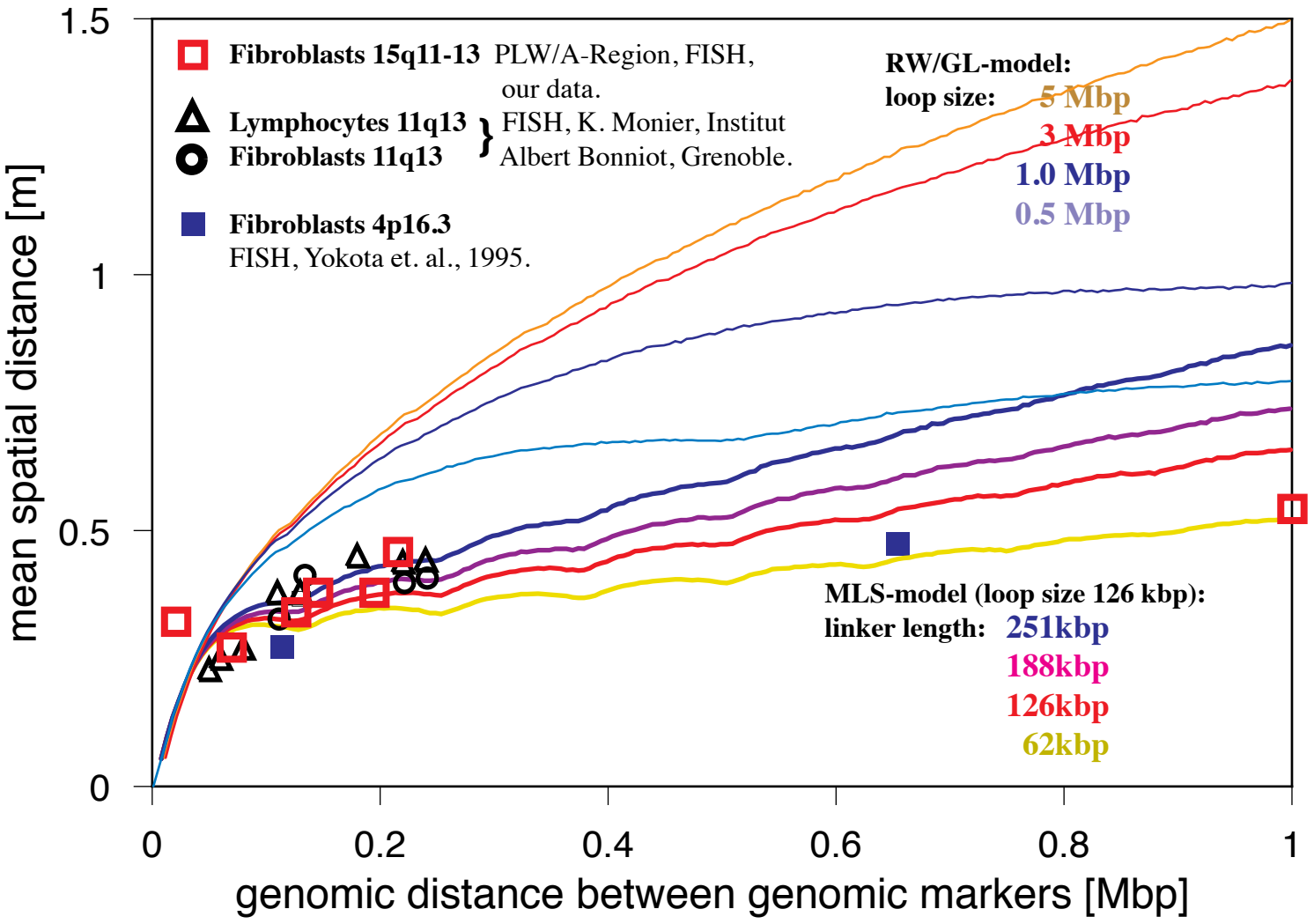

Shift of a marker ensemble through a rosette in the MLS-model in respect to loop bases.
This leads to different sets of 3D-distances for every ensemble position.
Due to the symmetry of the MLS-rosettes periodicities are found.

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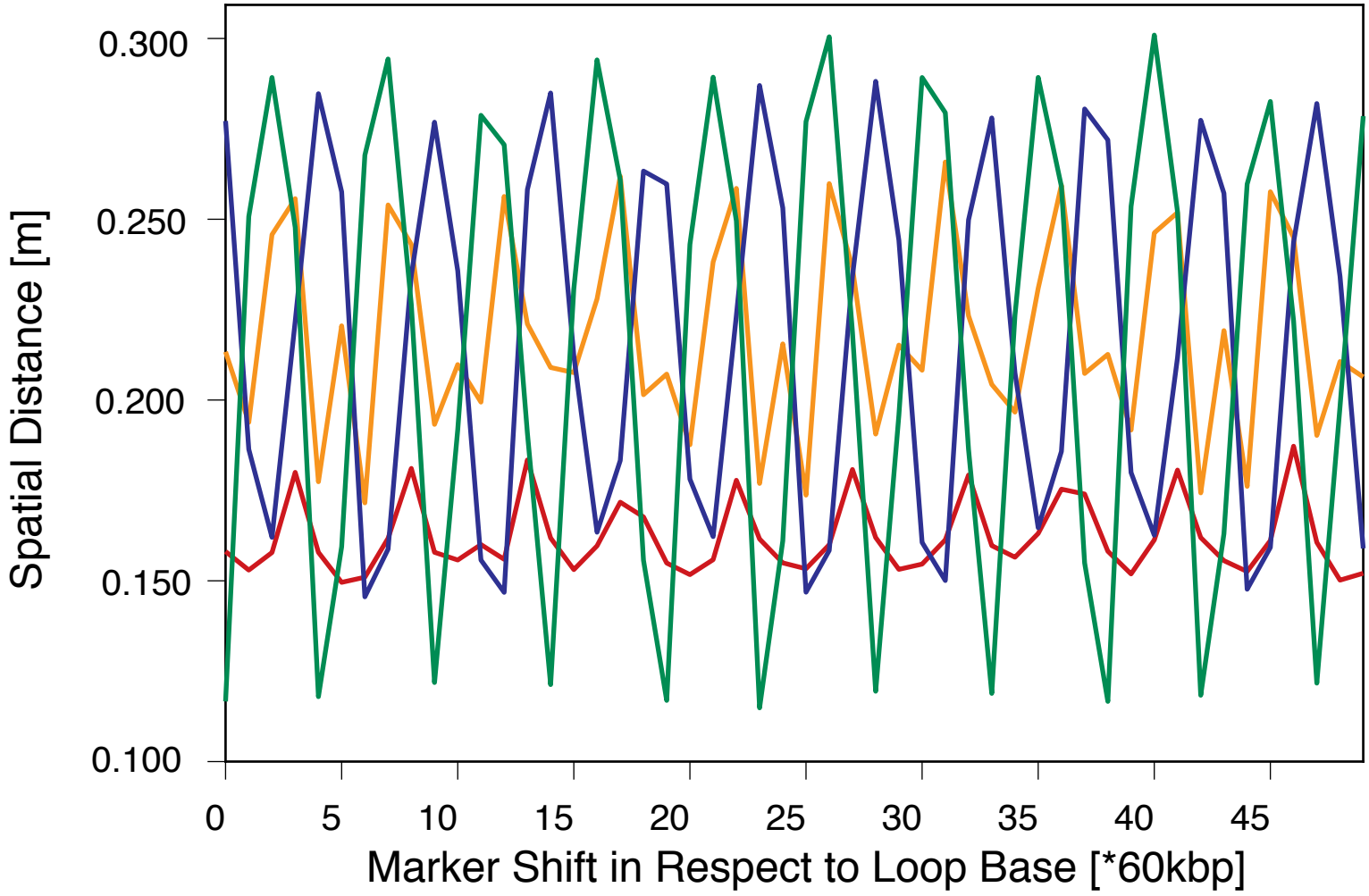

In agreement with porous network research fractal analysis show multifractal behaviour in simulations of chromosome 15. Different fractal dimensions mean different process-dynamics in these spaces. Therefore chromosomal territories show a higher degree of determinism than previously assumed.

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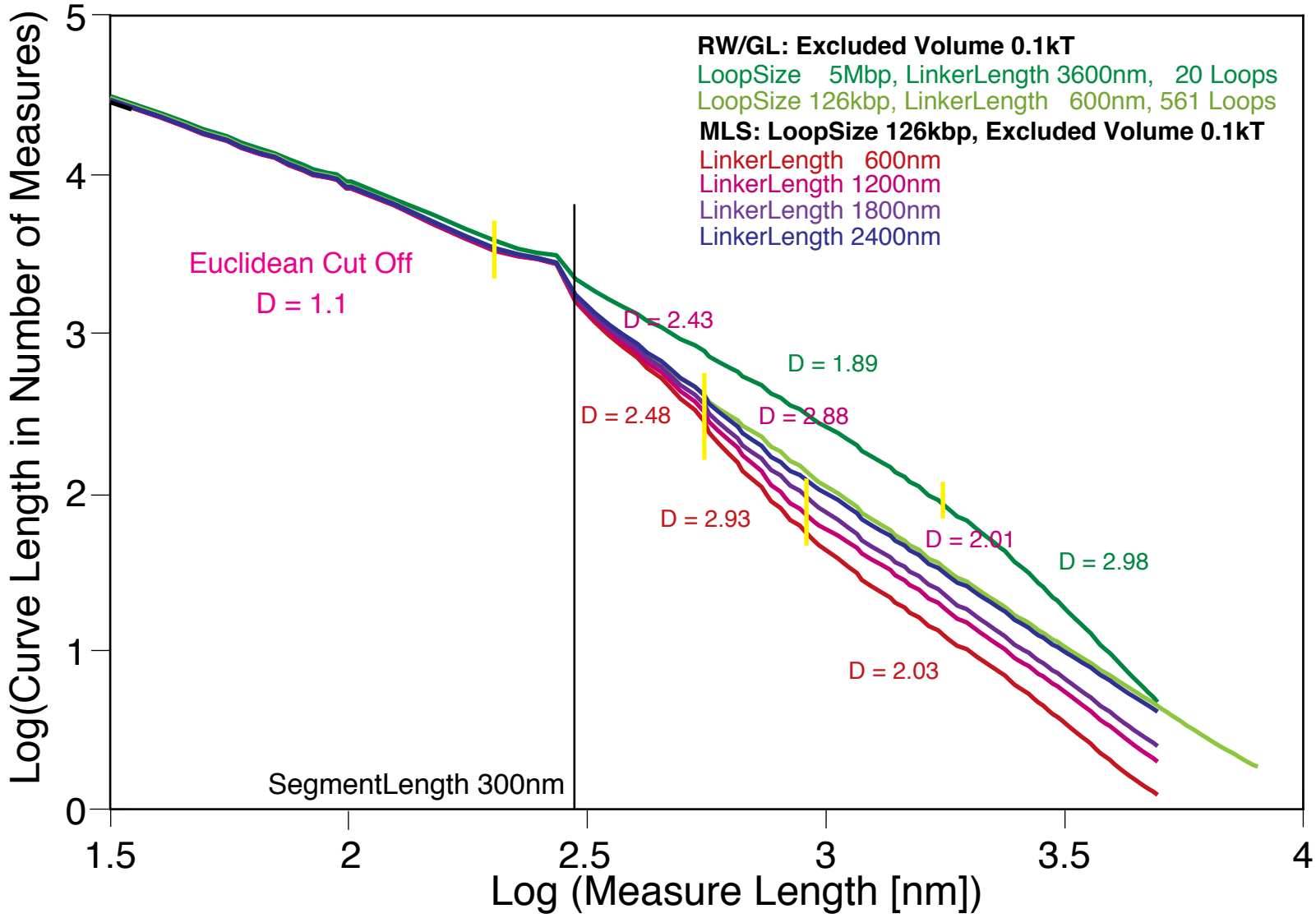

Creation of a ´Virtual Human Cell Nucleus´ with all 46 chromosomes using the MLS-model.

a) 46 metaphase configurations are randomly placed in a spherical potential and dencondensed into interphase by Brownian Dynamic or Monte Carlo methods.

b) 46 chains of spheres (number of spheres ~ chromosome size) are randomly placed in a spherical potential and relaxed with Simulated Annealing. Then the fine structure is added to receive the same resolution as in a).

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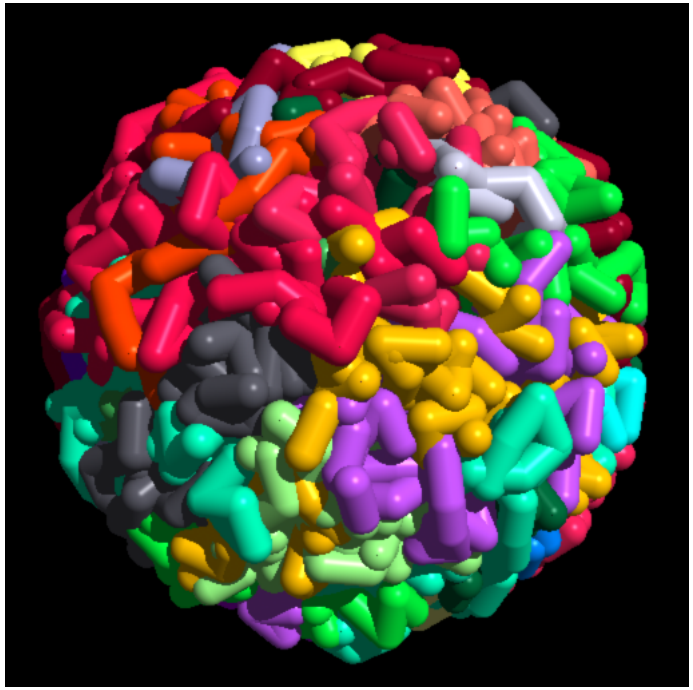
0 ms

10 ms



50 ms

´Virtual Human Cell Nucleus´

Simulation of all 46 chromosomes using the MLS-model.

The nucleus is simulated assuming a flexible polymer chain, modelling the 46 chromatin fibers with a total 1,248,794 segments of 50 nm = 5.2 kbp. Pictures are shown after a 0.5 ms Brownian Dynamics simulation, one step taking 10s.

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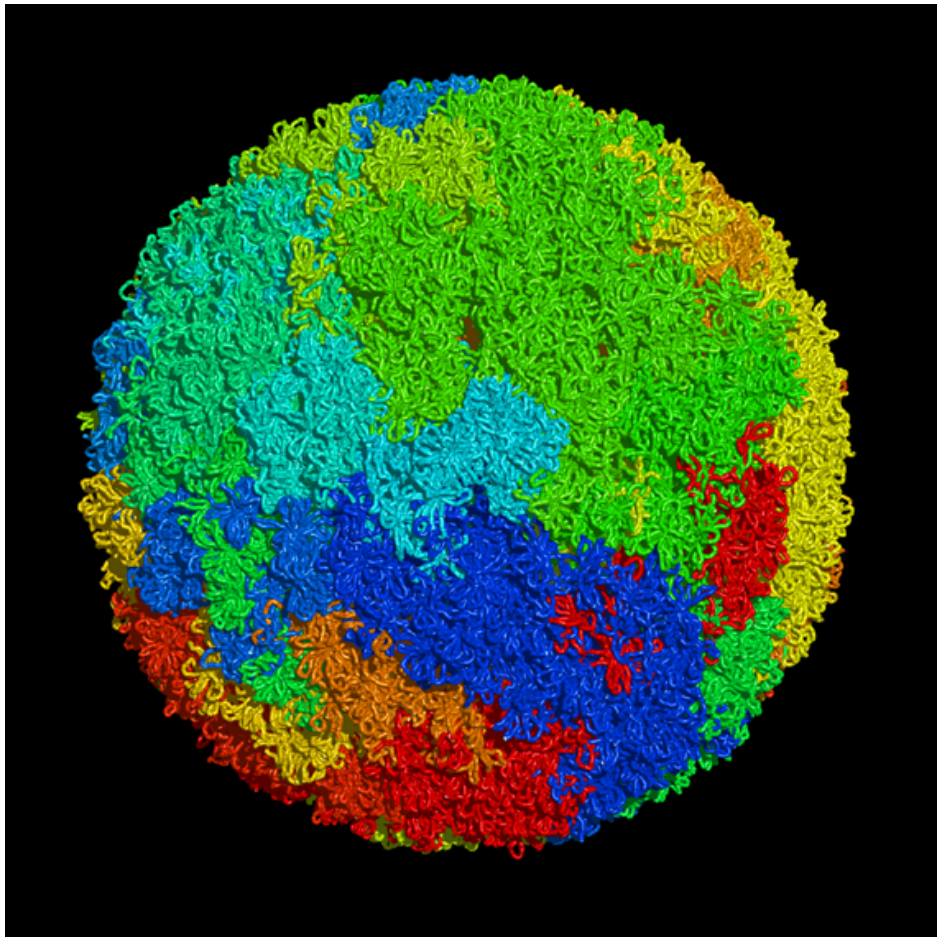
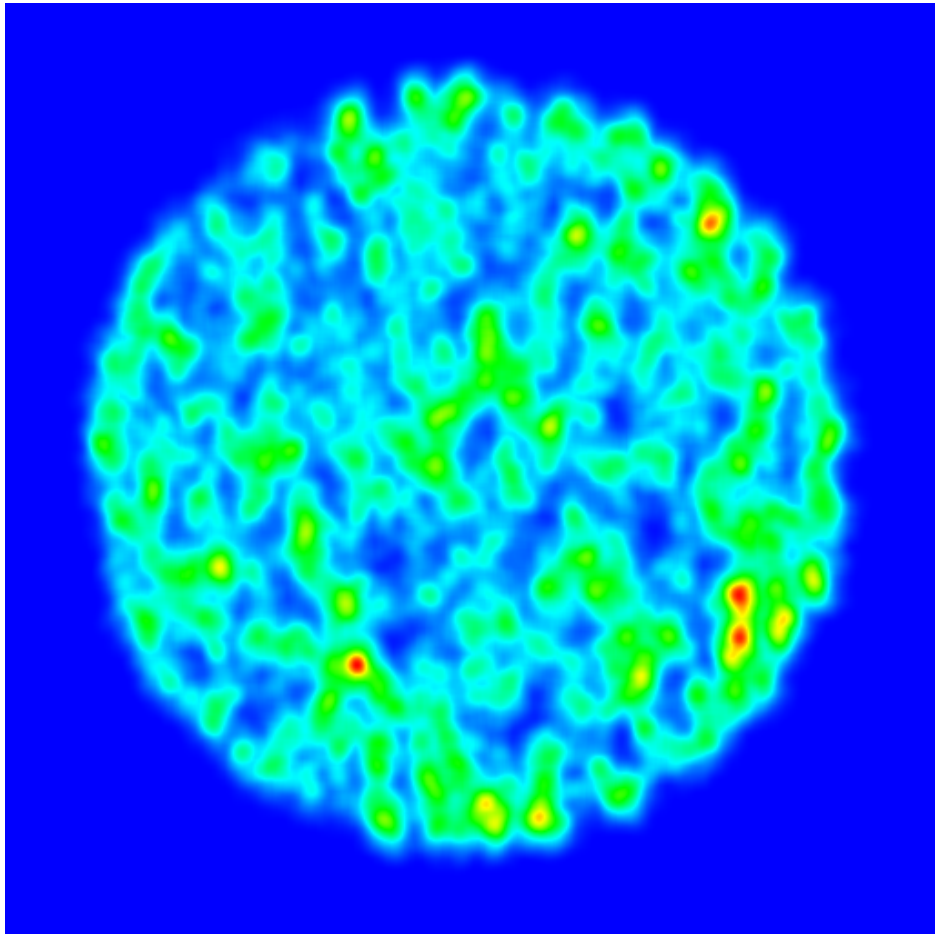


3-D rendering



simulated confocal section

The MLS-model leads to low overlap of chromosome-arms and subcompartments in contrast to the RWGL-model. This is also seen in experiments.

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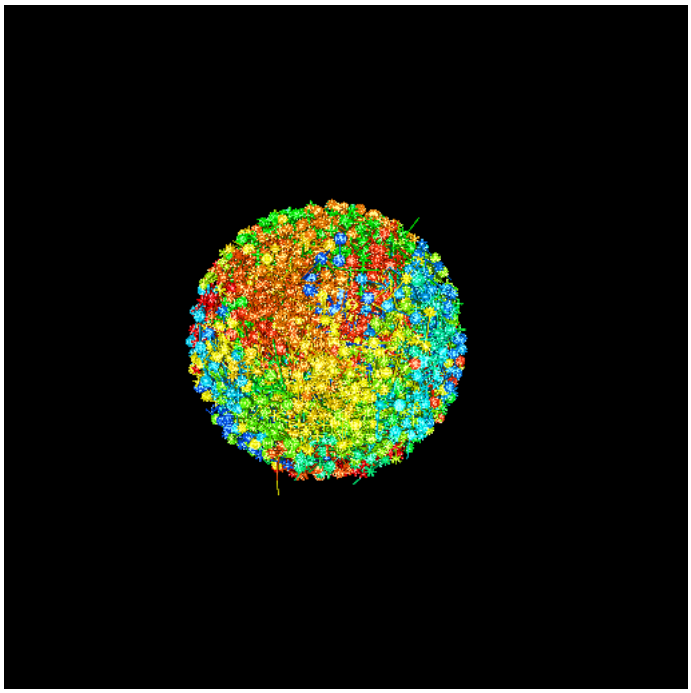
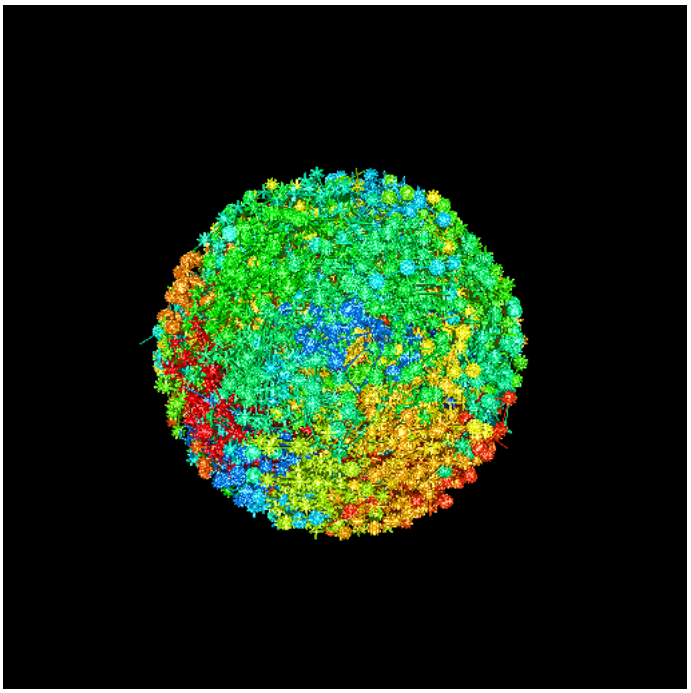
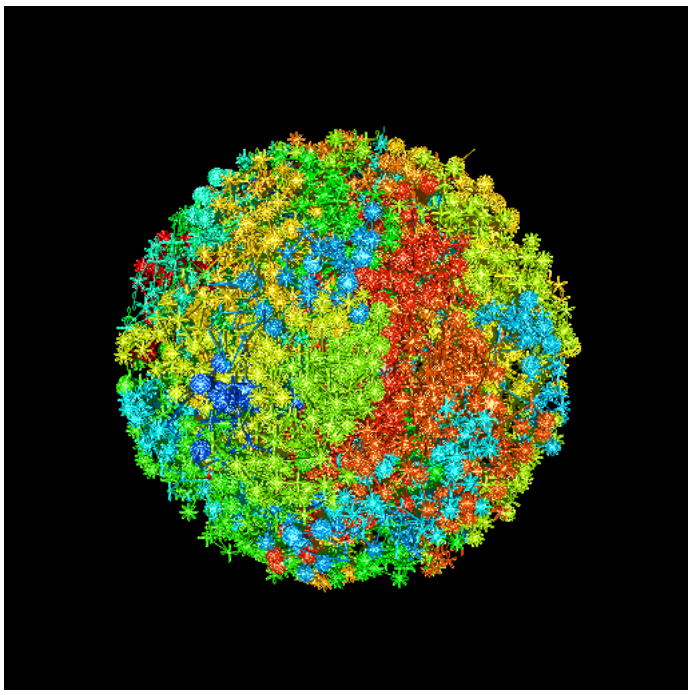


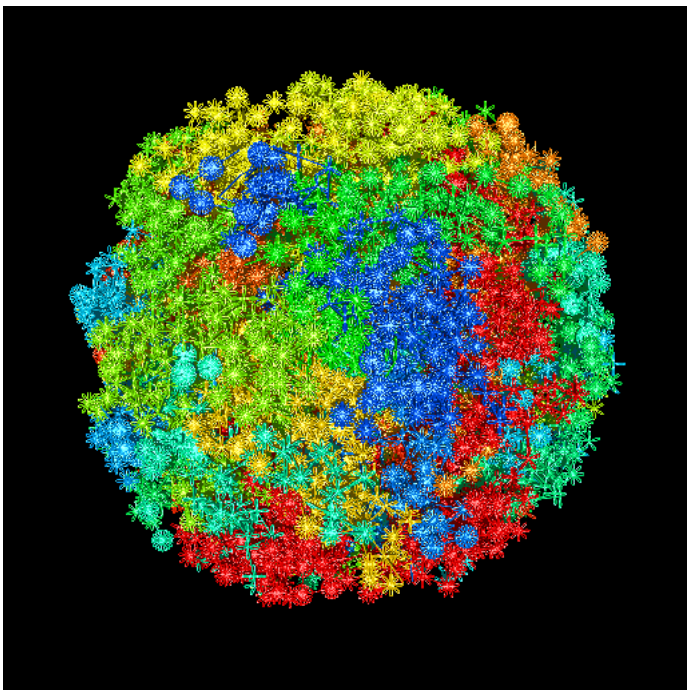
nucleus
6 m diametre



nucleus
8 m diametre



nucleus
10 m diametre



nucleus
12 m diametre

Mapping of Histone H2B-GFP and H1-GFP distribution in vivo.

The Histone-GFP reflects the distribution of chromatin in interphase.
The structure visible in the images is similar to those found in simulations.

Left: HeLa cells stably transfected with H2B-GFP (K. Sullivan, Scripps Institute). Confocal in vivo section of a cell nucleus and a mitosis.
Right: Cos7 cell stabaly transfected with H1-GFP (A. Alonso, DKFZ). Confocal in vivo section.

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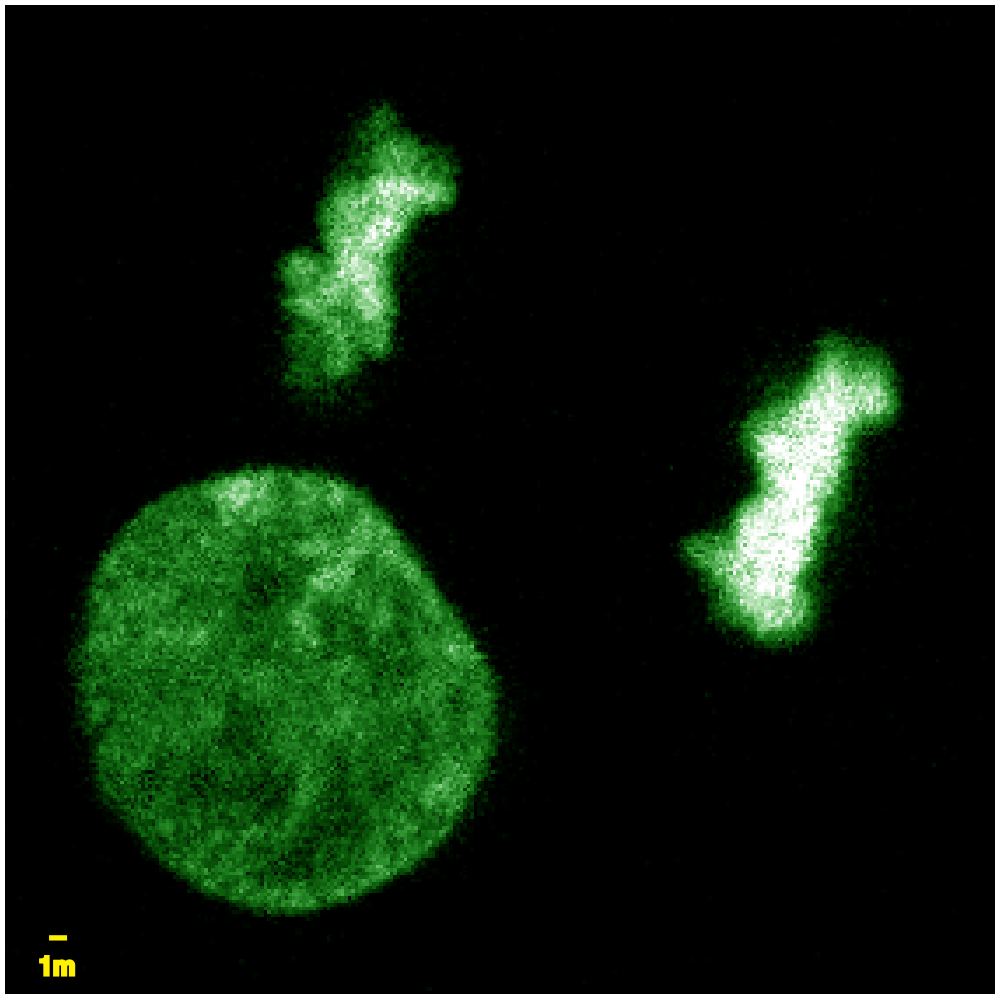

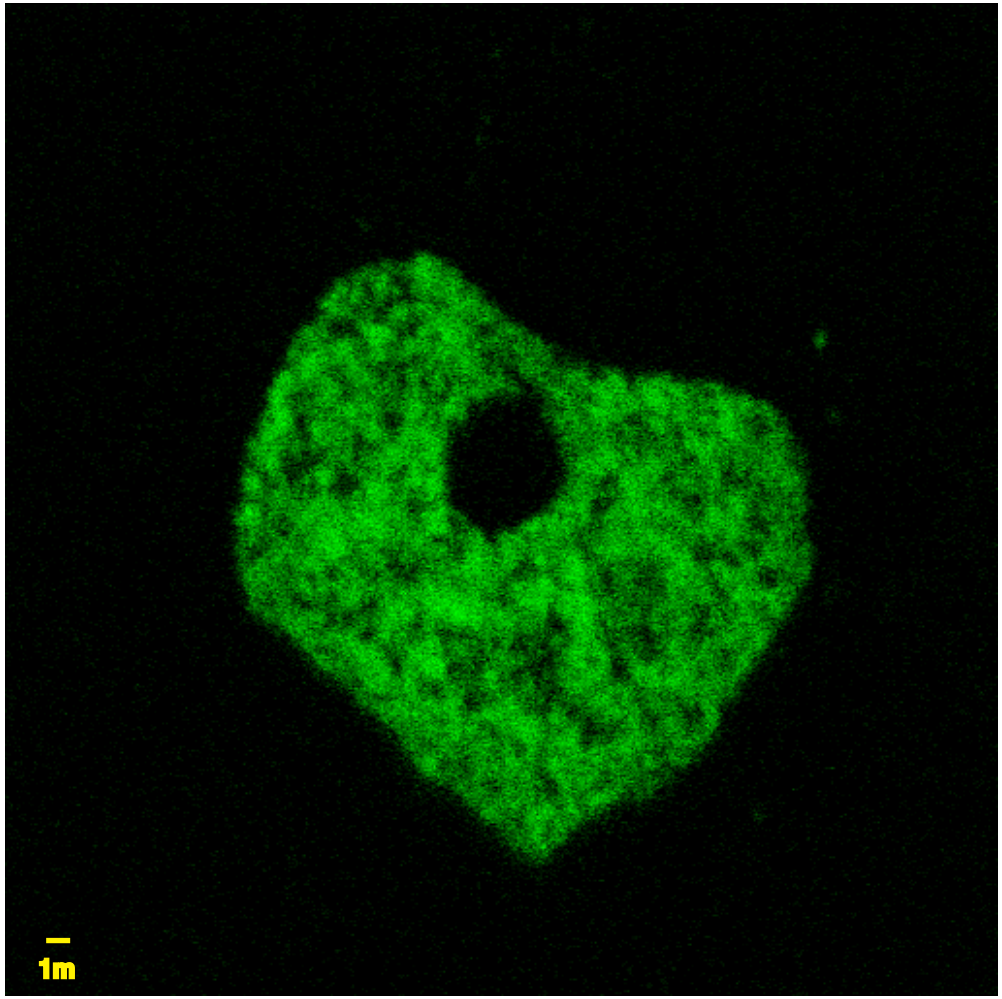

DNA fragmentation by irradiation with carbon ions.

Irradiation with carbon ions results in DNA double strand breackage. The length of the fragments follow distributions depending o the spatial arrangement of the 30 nm chromatin fiber in the nucleus. Together with P. Quicken, GSF, Munich.

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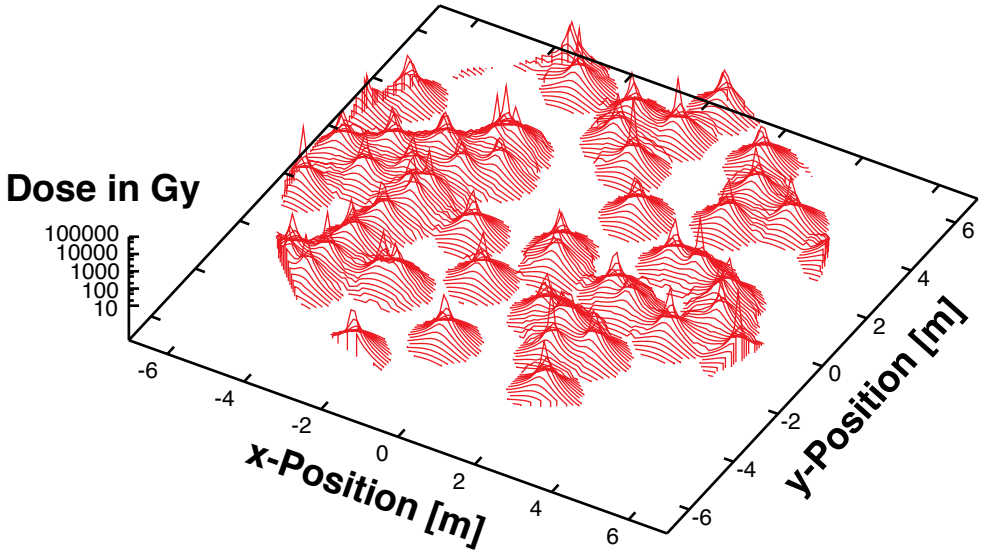
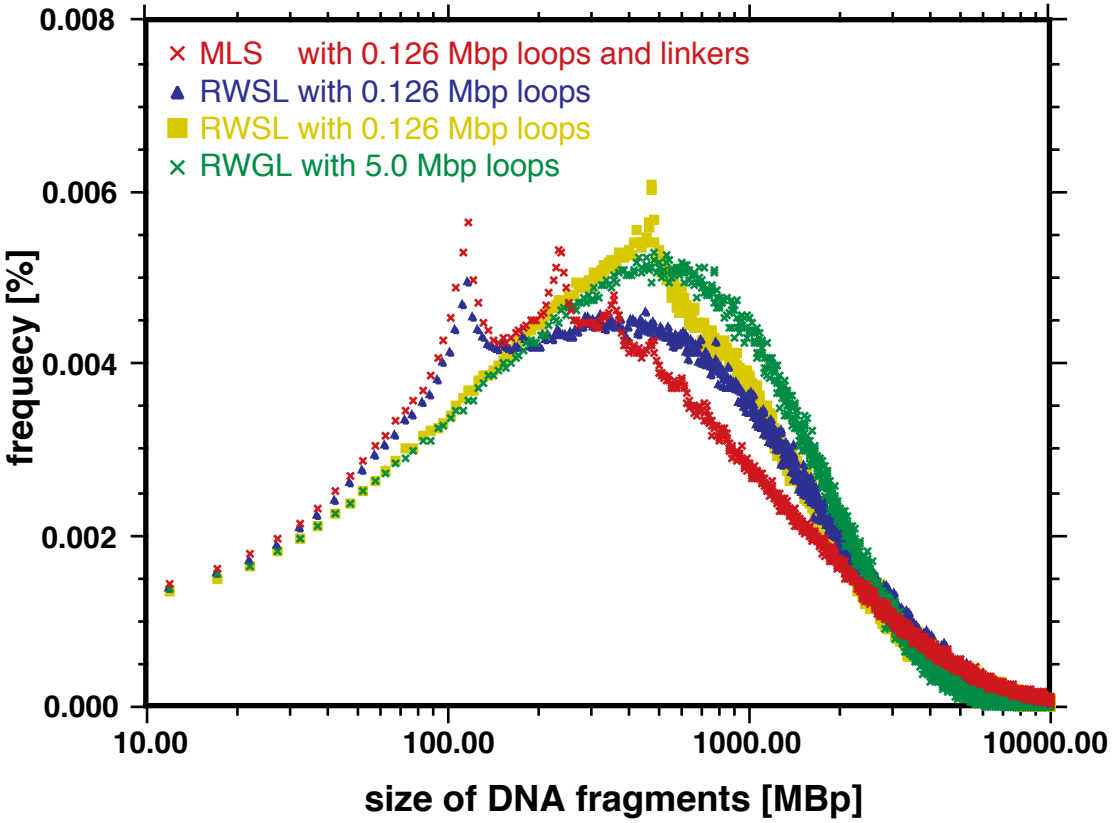
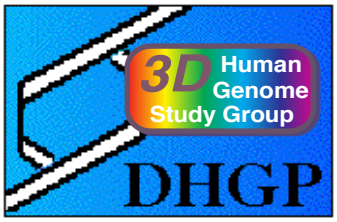
Radiation dose distribution in a cell nucleus irradiated with carbon ions



Comparison between experimental and simulated fragment distributions after carbon irradiation.



People



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Computer Modelling of Chromosome Territories

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German Cancer Research Centre (DKFZ), Heidelberg, Germany, February, 1999.

Abstract

Despite the successful linear sequencing of the human genome its three-dimensional structure is widely unknown. However, the regulation of genes - their transcription and replication - has been shown to be closely connected to the three-dimensional organization of the genome and the cell nucleus. On the bases of polymer physics we have recently developed detailed and quantitative structural models for the folding of the 30 nm chromatin fiber within the human interphase cell nucleus. A quantitative test of several plausible theories resulted in a best agreement between computer simulations of chromosomes, cell nuclei and experiments for the so-called Multi-Loop-Subcompartment (MLS) model. Results concern the following properties: overlap of chromosome territories, -arms, -bands, 3D spatial distances between genomic markers as function of their genomic separation in base pairs, fractal analysis of simulations, mass distribution of chromatin in cell nuclei and the fragmentation distribution of cellular DNA after irradiation with carbon ions. Thus in an analogy to the Bauhaus principle that „form follows function“, analyzing in which form DNA is organized might help us to understand genomic function.

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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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