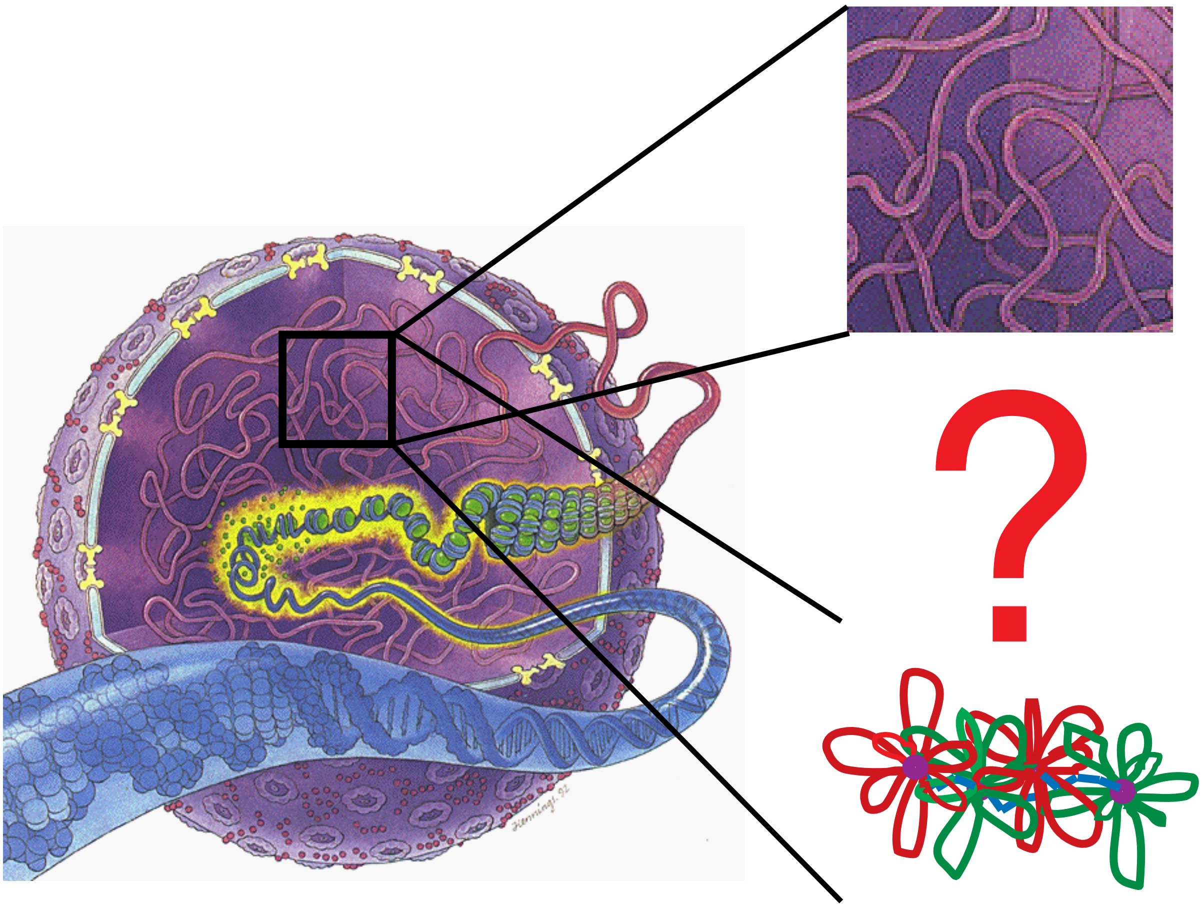
Three-Dimensional Organization of Chromosome Territories and the Human Cell Nucleus



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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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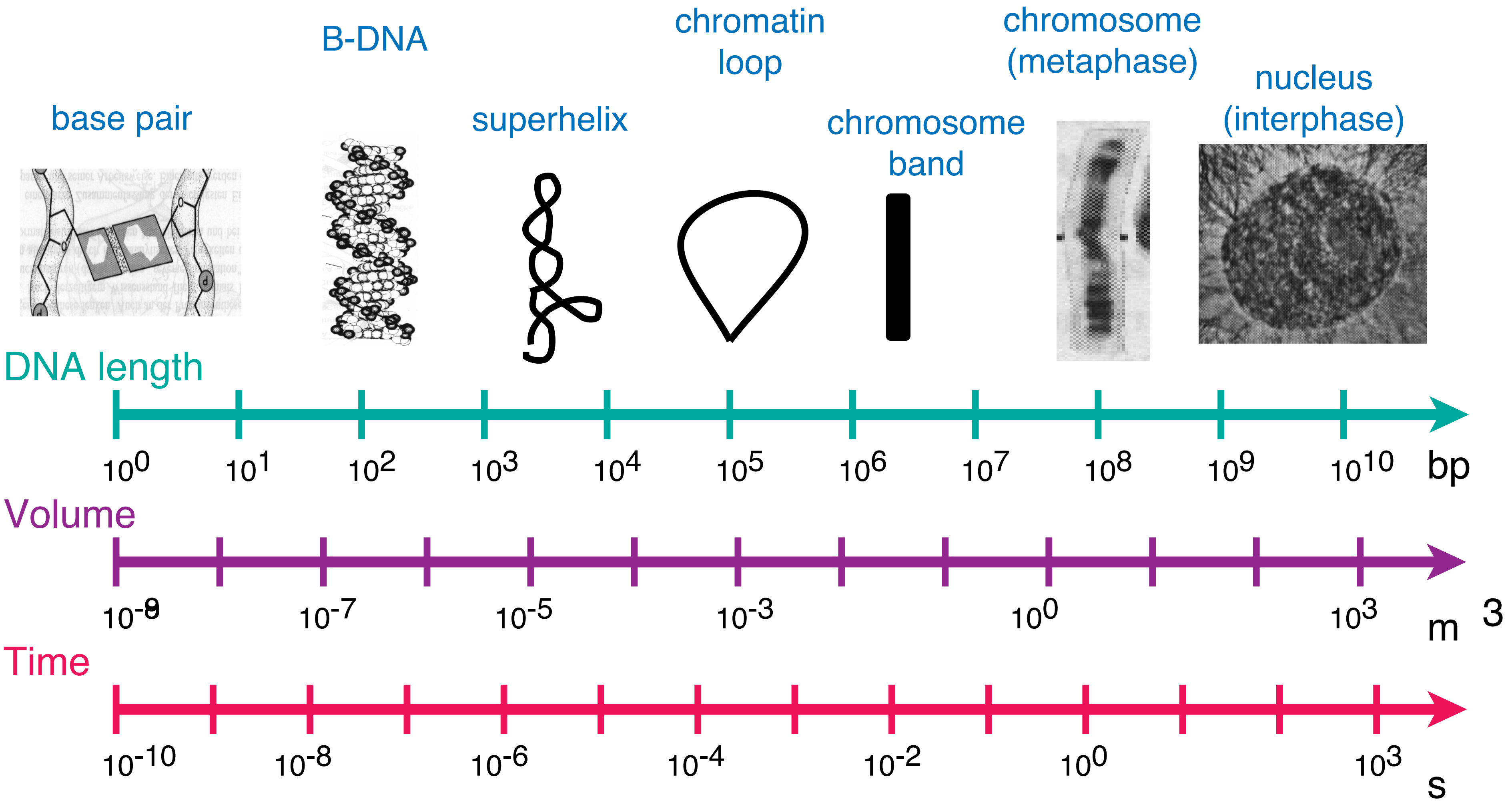

base pair

B-DNA

superhelix

chromatin loop

chromosome band

chromosome (metaphase)

nucleus (interphase)

DNA length

10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7 10^8 10^9 10^{10} bp

Volume

10^{-9} 10^{-7} 10^{-5} 10^{-3} 10^0 10^3 m^3

Time

10^{-10} 10^{-8} 10^{-6} 10^{-4} 10^{-2} 10^0 10^3 s

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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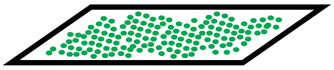
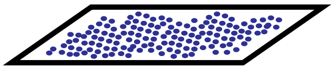
Cell - Preparation

cells on coverslip grown
to confluent layer



fixation of cells on coverslip
(formaldehyde) and
permeabilisation



DNA double strand

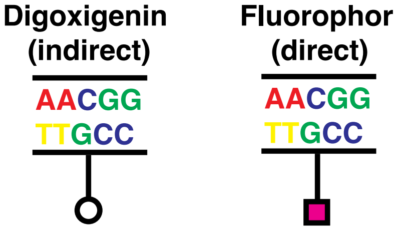
TACGTTAACGGTAGCATT
ATGCAATTGCCATCGTAA

Probe - Preparation

finding of genomic site for marking
and
cloning of this sequence

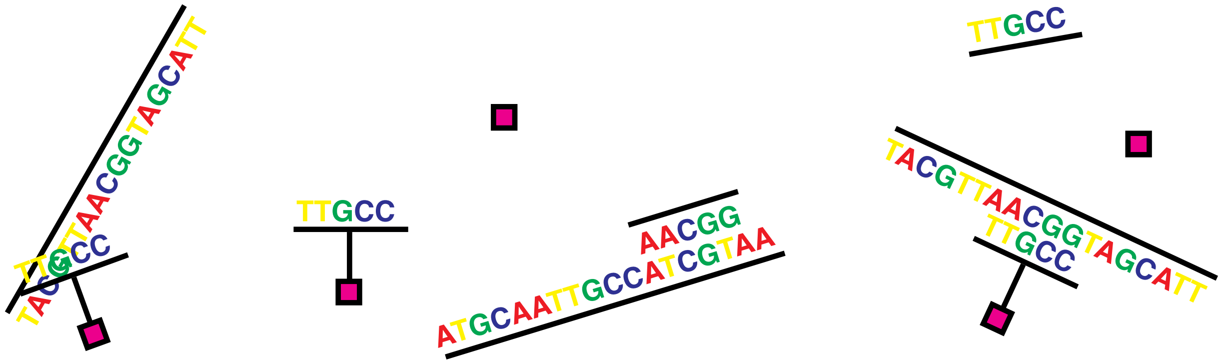
AACGG
TTGCC

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

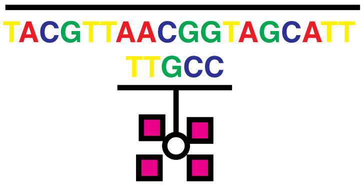


Hybridization

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



amplification with fluorescent labeled antibodies



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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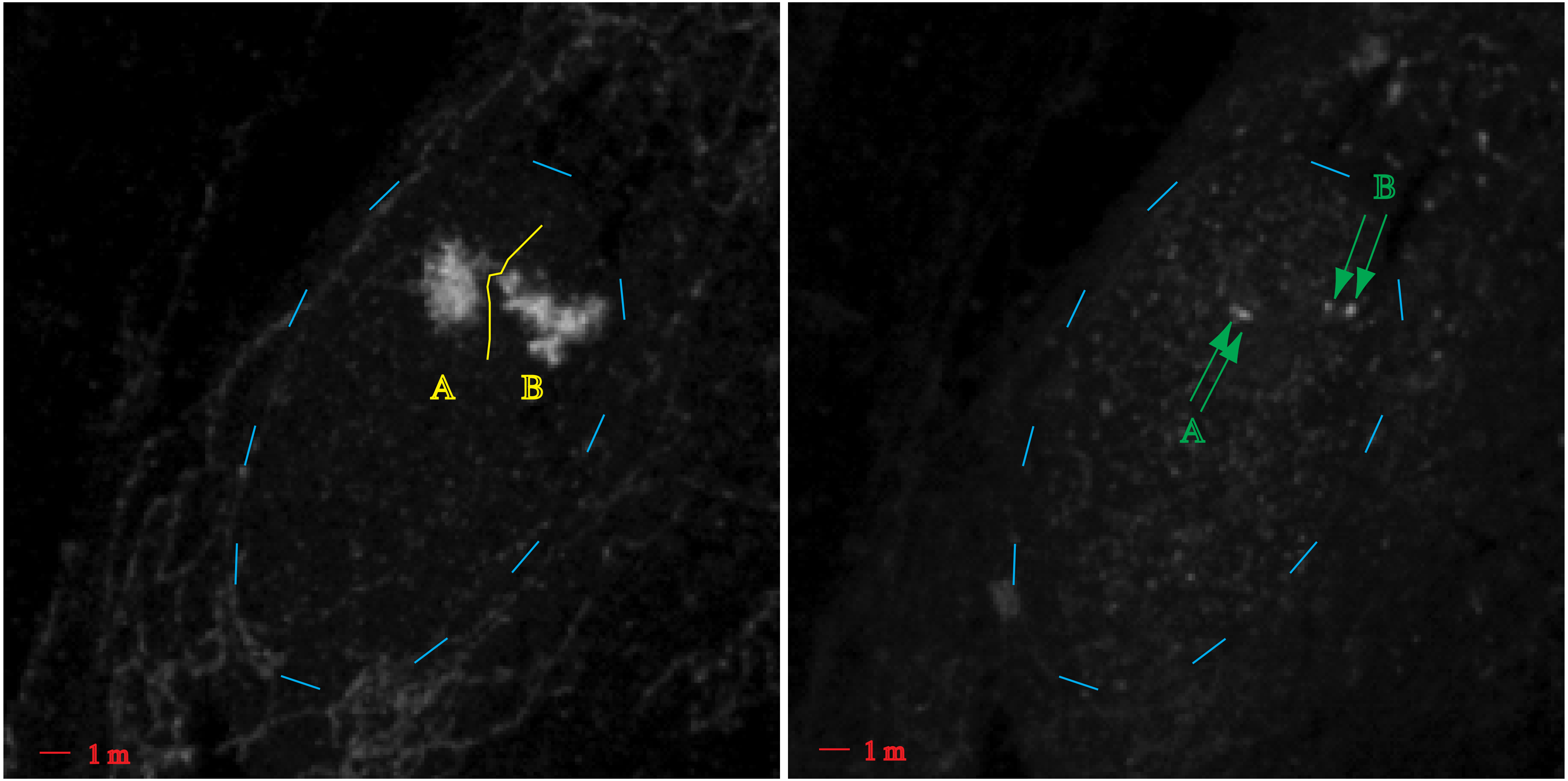

Ideogram of chromosome 15 with Prader - Willi Region and Angelmann Region. The size and genomic distance of the clones are sufficiently small and well characterized to measure the fine structure and organization of chromosome territories.

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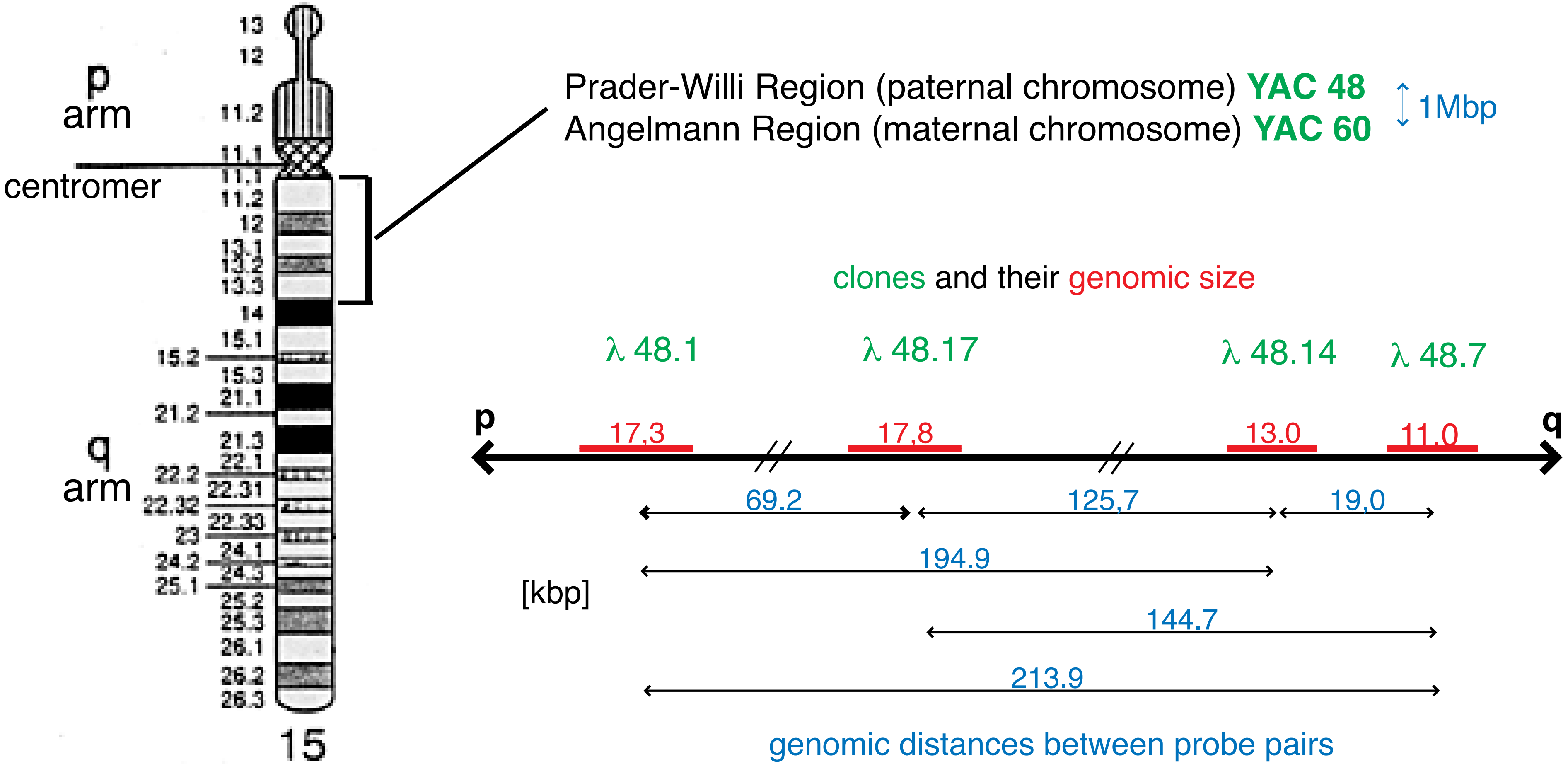

Prader-Willi Region (paternal chromosome) **YAC 48**
Angelmann Region (maternal chromosome) **YAC 60**

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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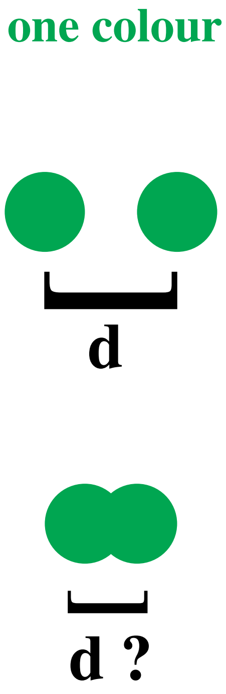
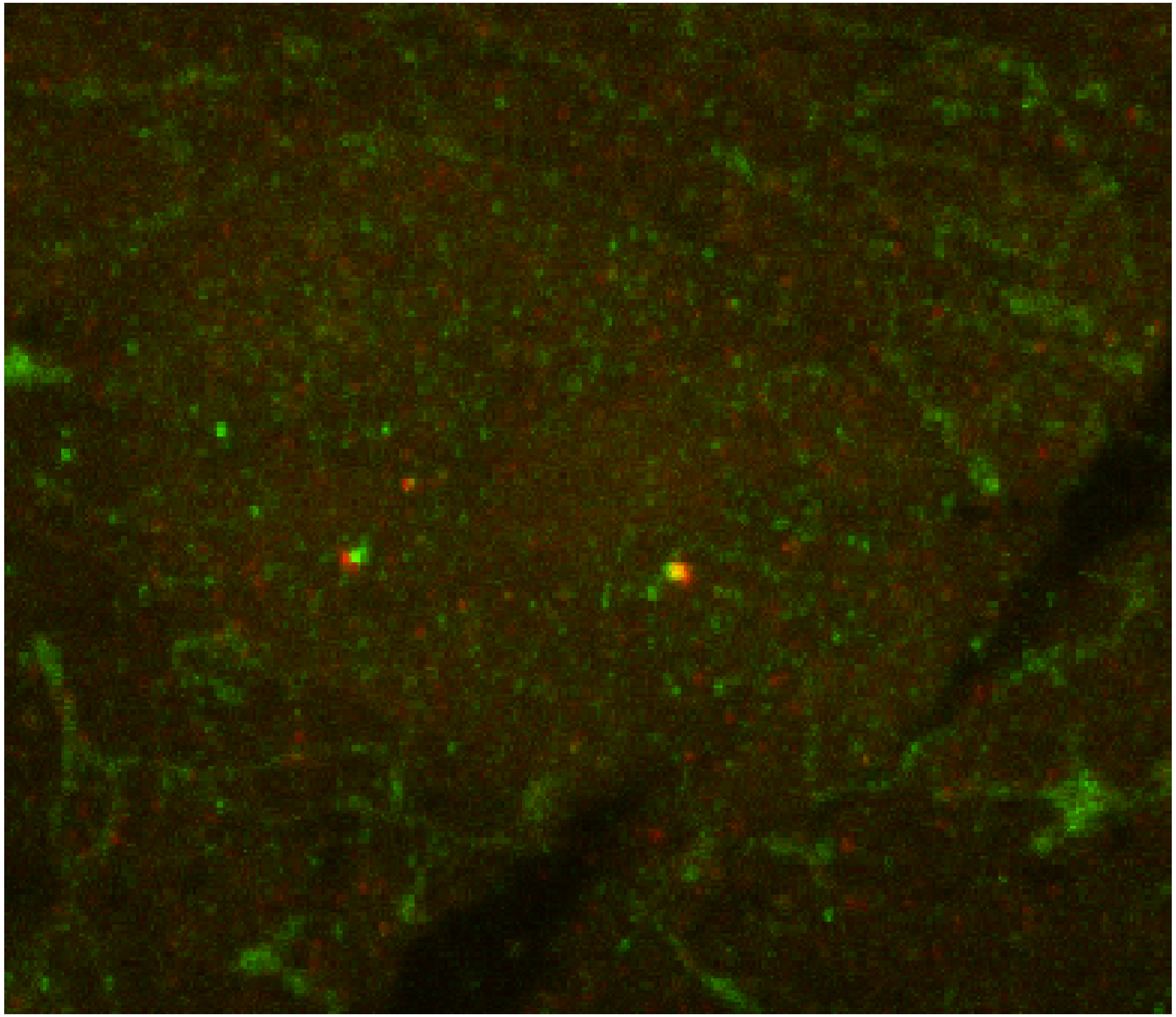
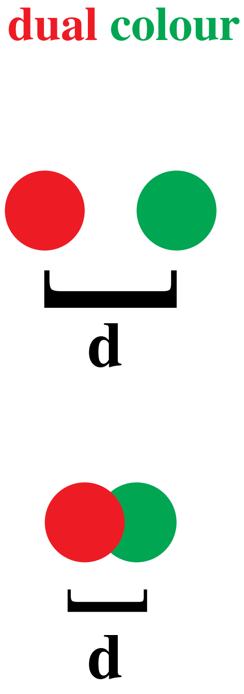


one colour

d

d ?





dual colour

d

d

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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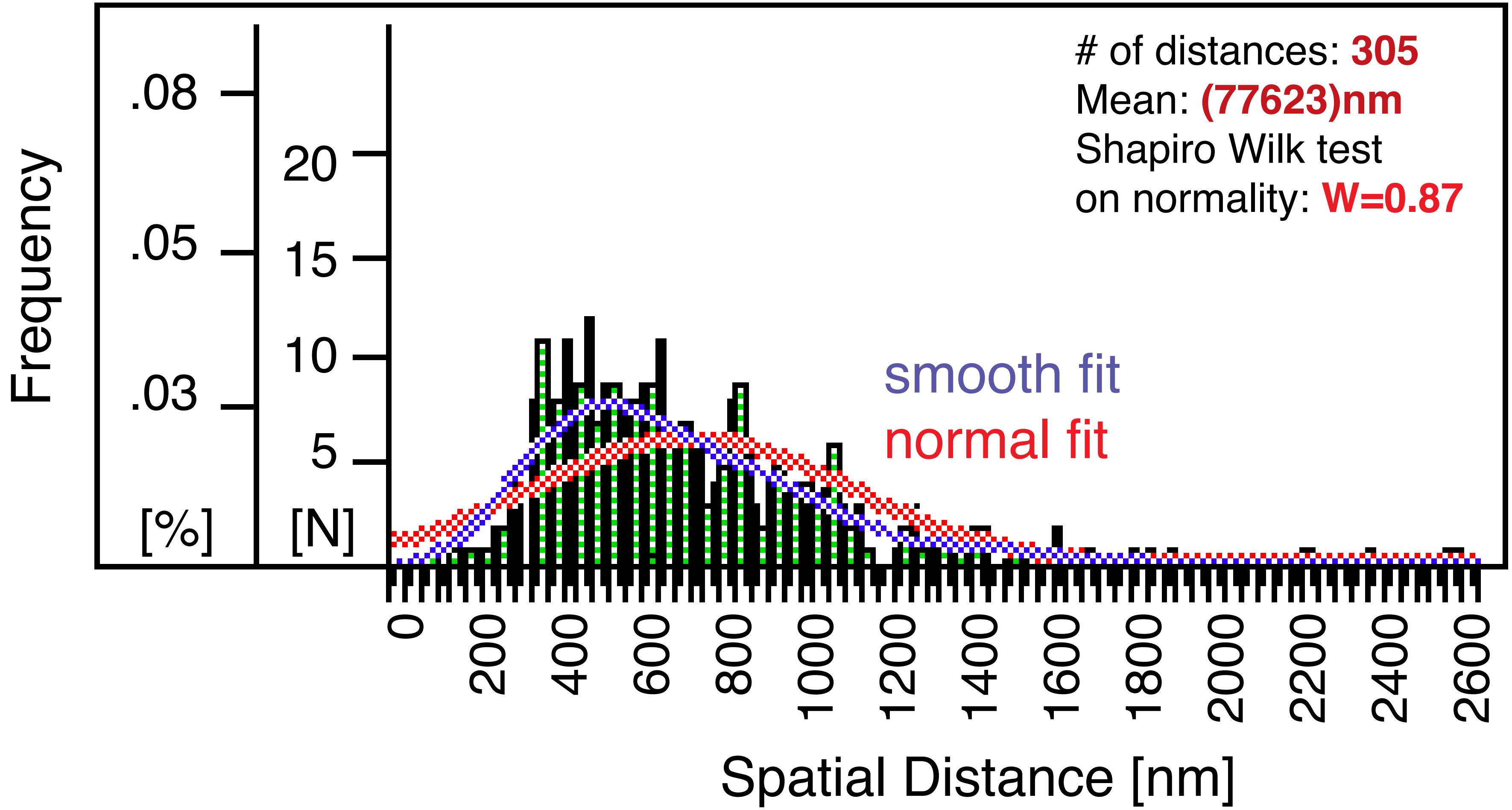
Distance Distribution



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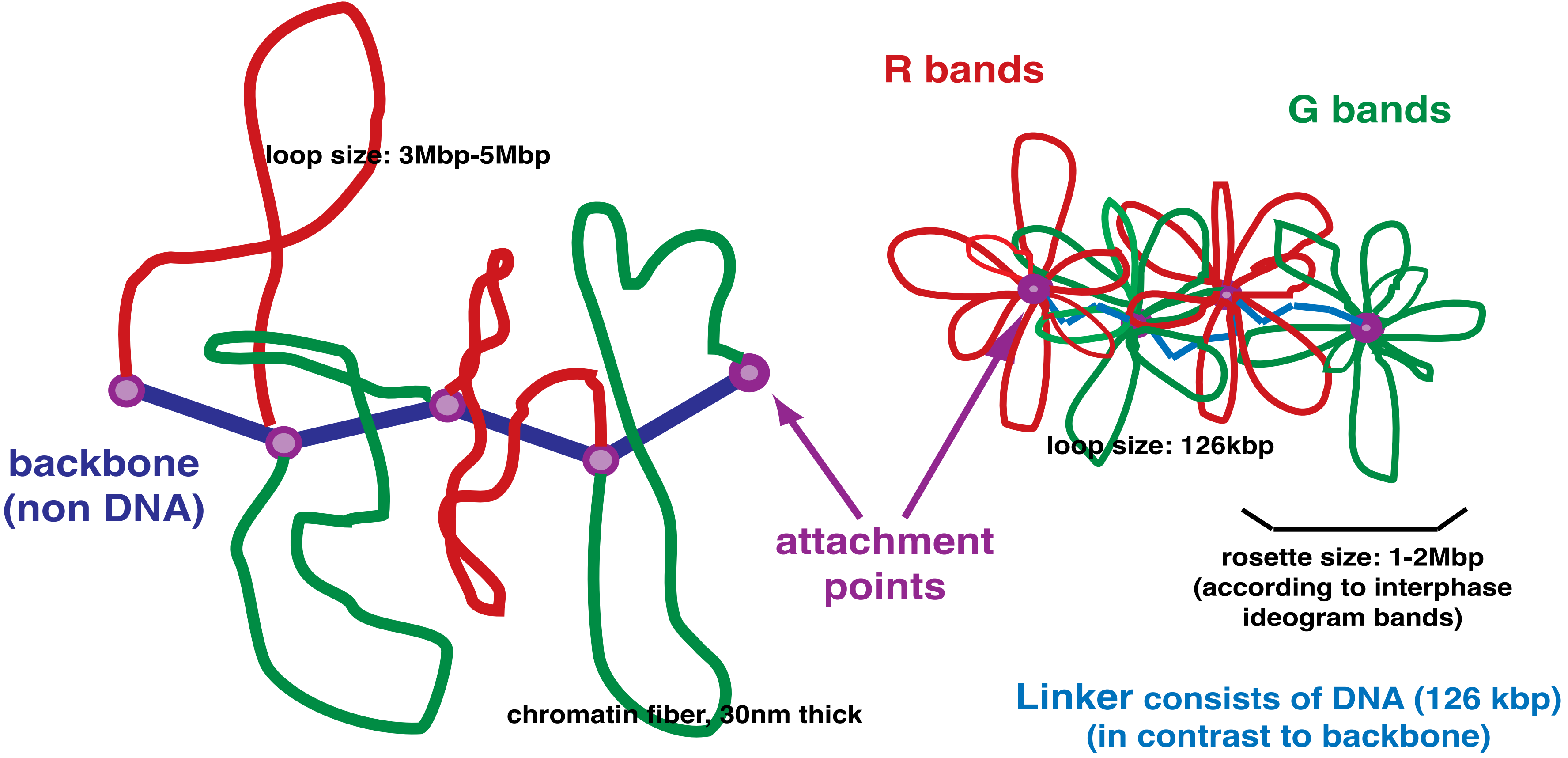
Random Walk / Giant Loop model
(RW/GL)
Sachs et al. (1995)

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



R bands

G bands

loop size: 3Mbp-5Mbp

backbone
(non DNA)

attachment
points

loop size: 126kbp

rosette size: 1-2Mbp
(according to interphase
ideogram bands)

chromatin fiber, 30nm thick

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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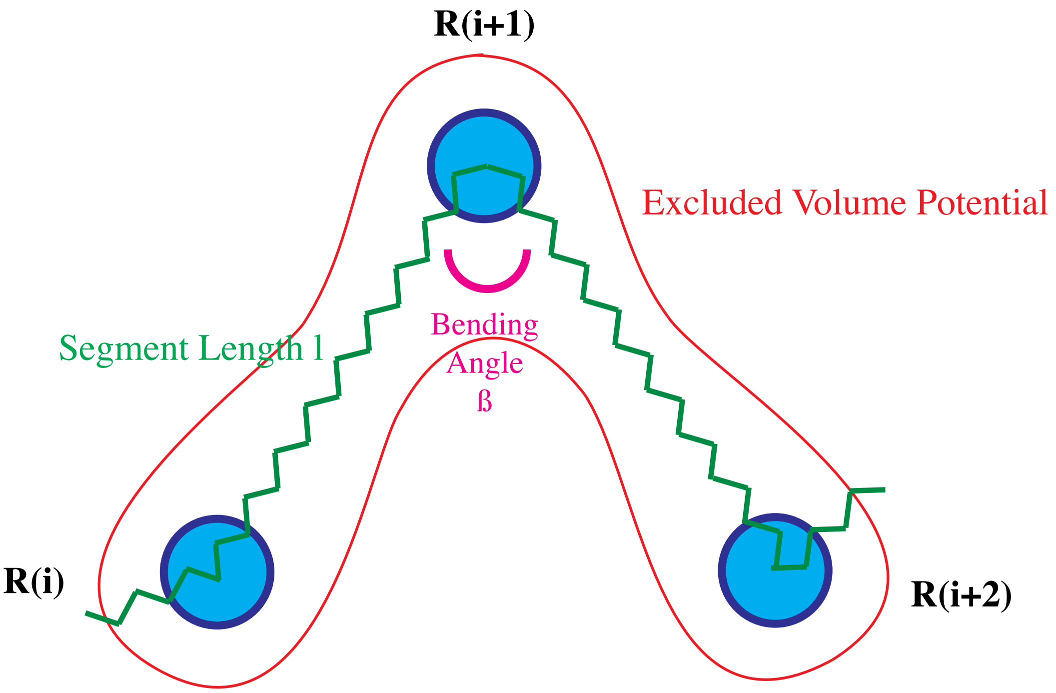

Stretching Potential

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

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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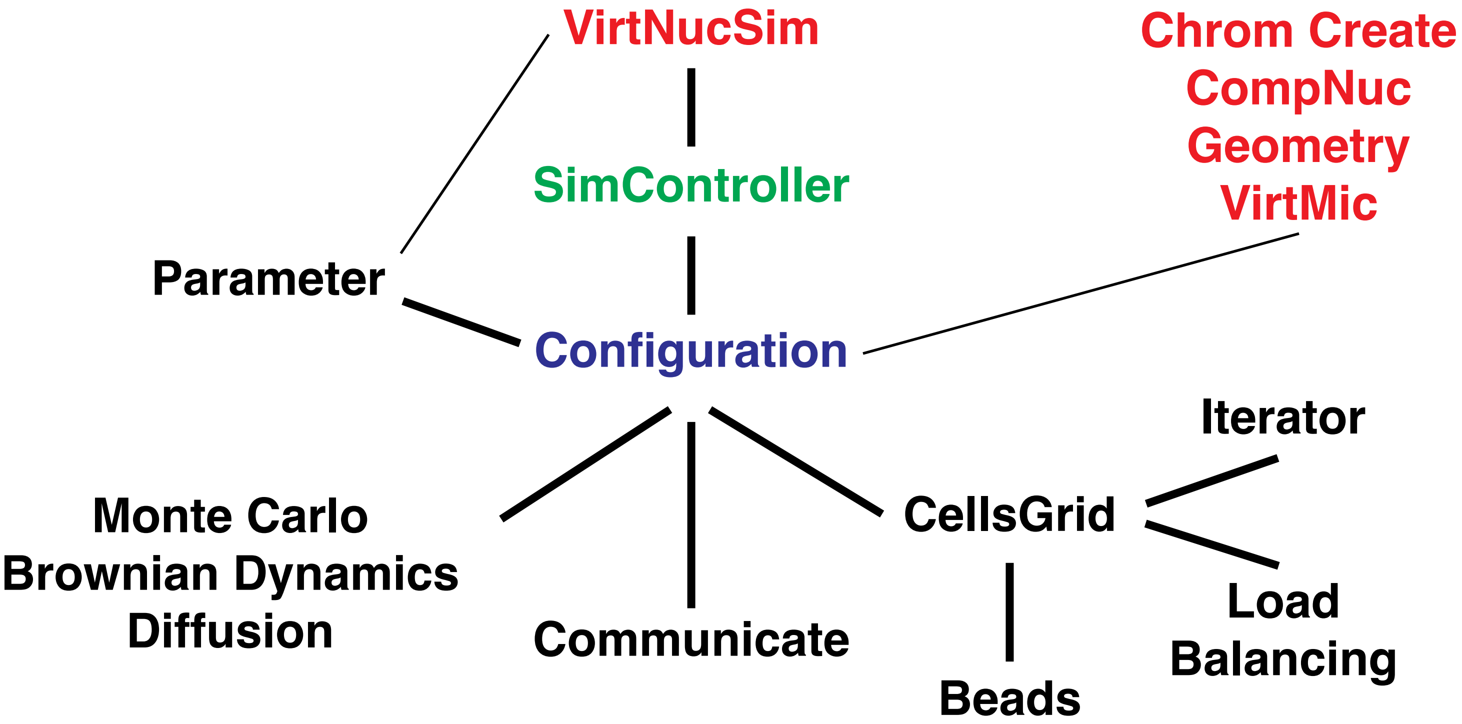


VirtNucSim

SimController

Chrom Create
CompNuc
Geometry
VirtMic

Parameter

Configuration

Iterator

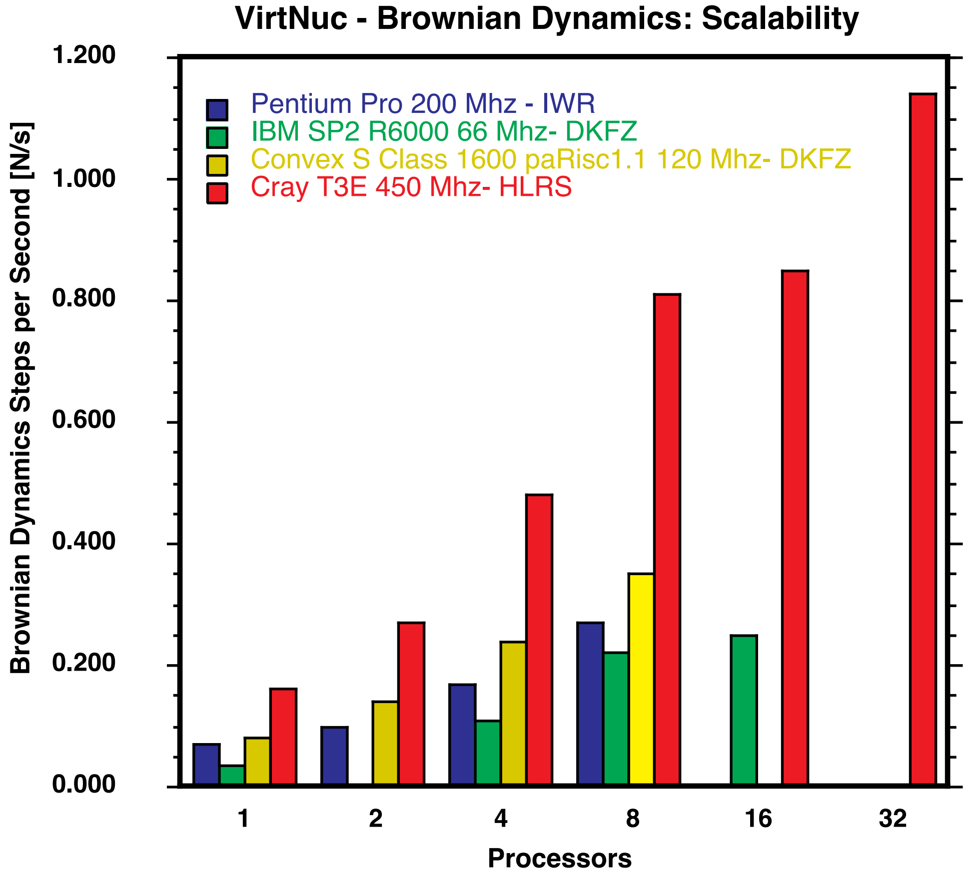
Monte Carlo
Brownian Dynamics
Diffusion

Communicate

CellsGrid

Load Balancing

Beads



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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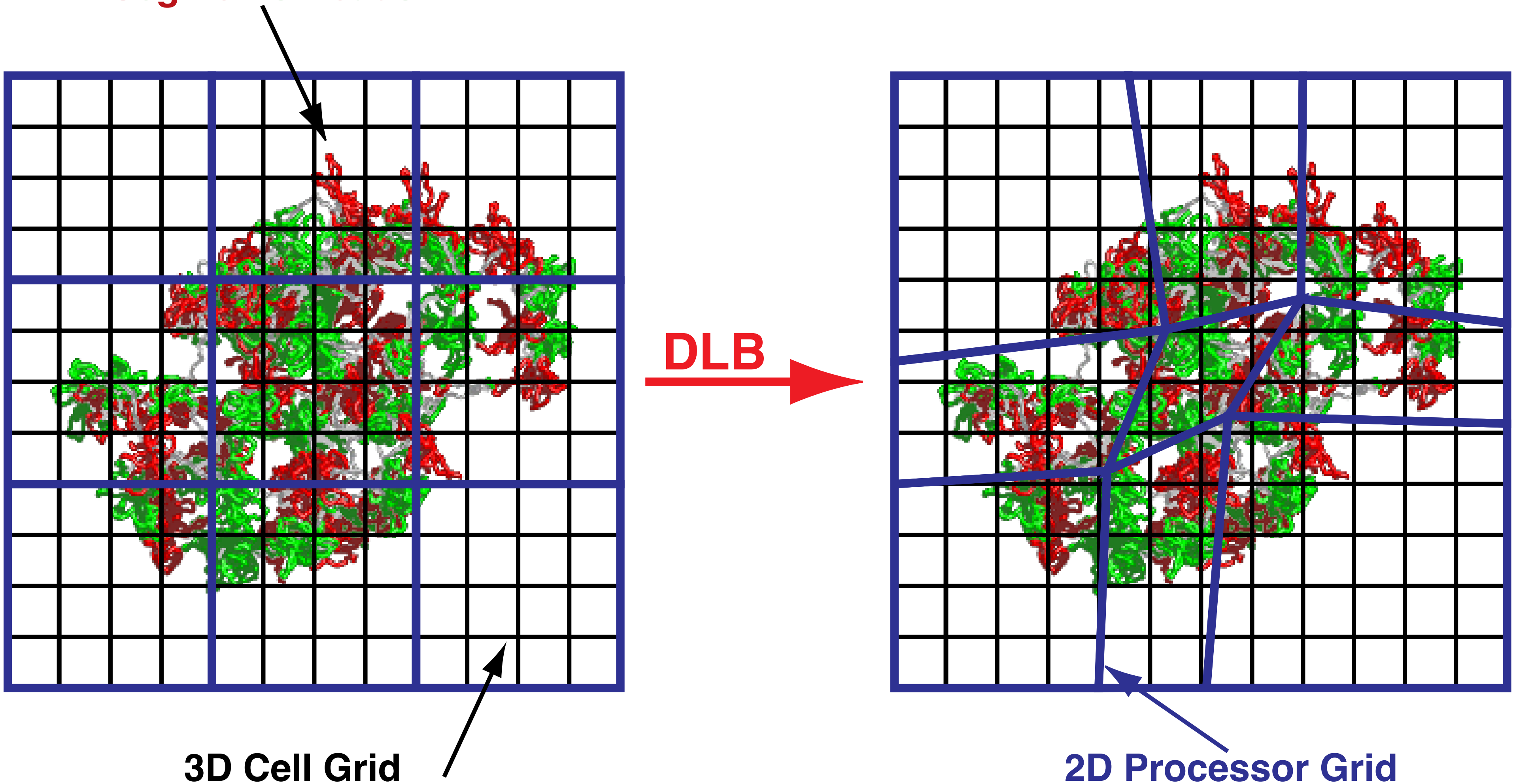

Segments/Beads

DLB

3D Cell Grid
(size dynamic, much finer than shown)

2D Processor Grid
(dynamic)

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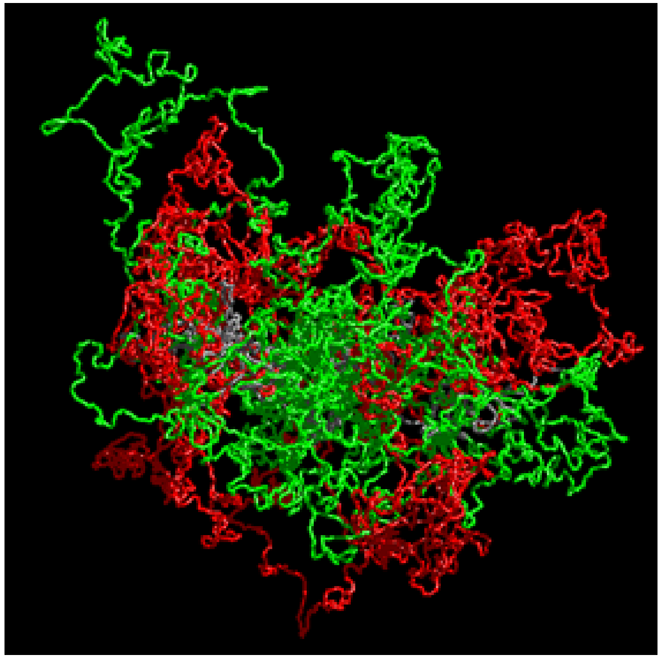
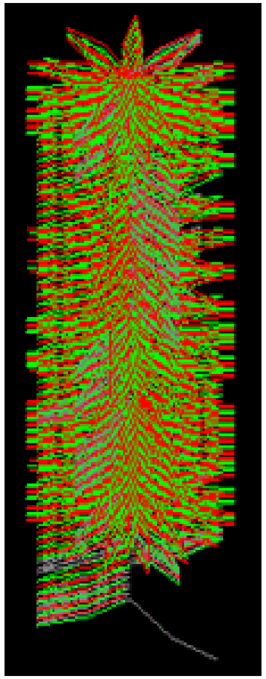
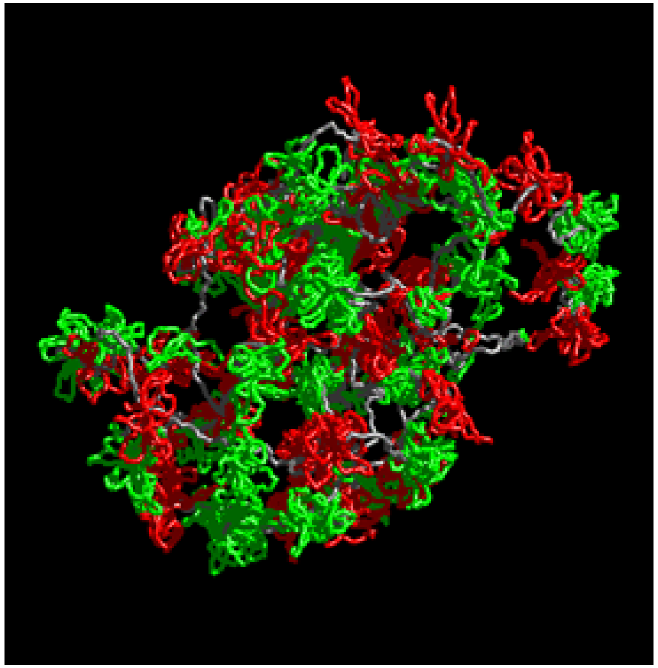
Ray traced image of the Random-Walk/Giant-Loop model, loop size 5Mbp, after ~80.000 Monte-Carlo and 1000 relaxing Brownian-Dynamics steps. Large loops intermingle freely thus forming no distinct features like in MLS model.





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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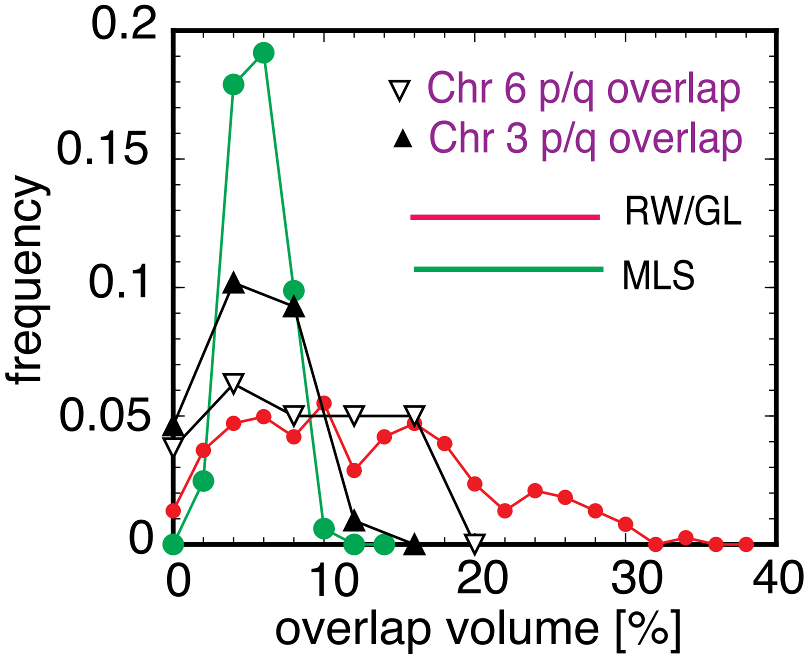
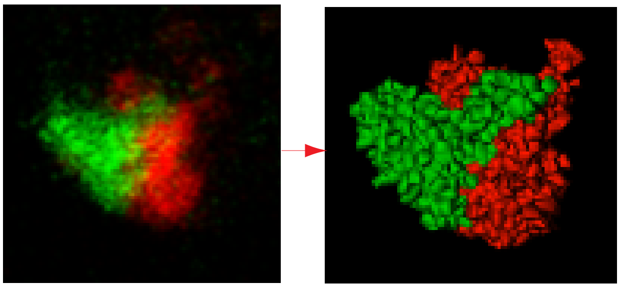
Arm Overlap



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



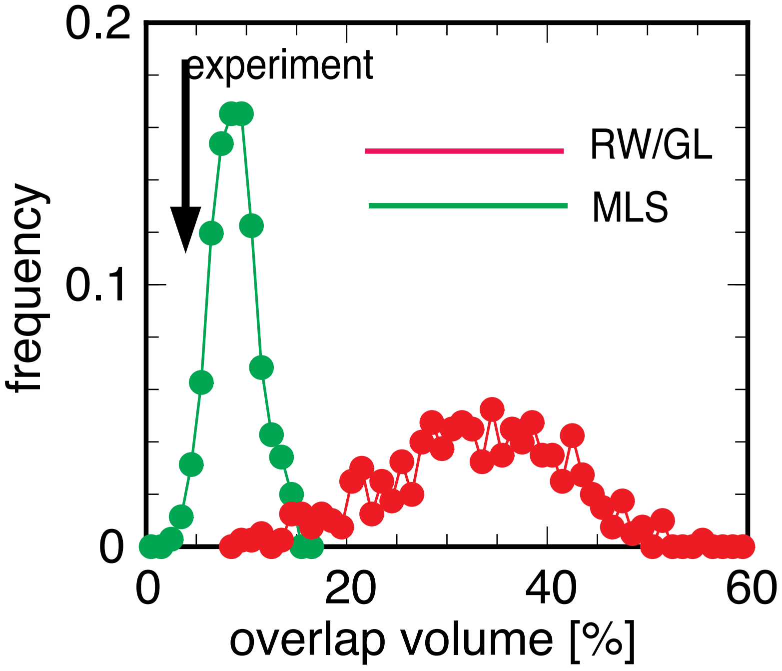
experiment

reconstruction

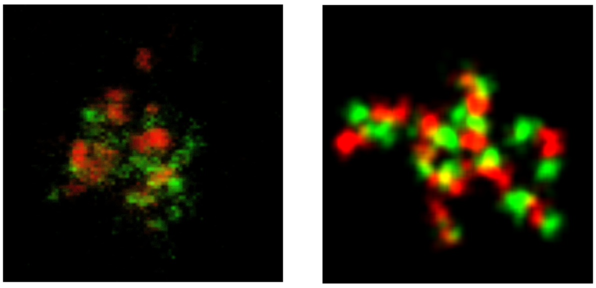
S. Dietzel

R. Eils

Subcompartment Overlap



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



experiment

simulation

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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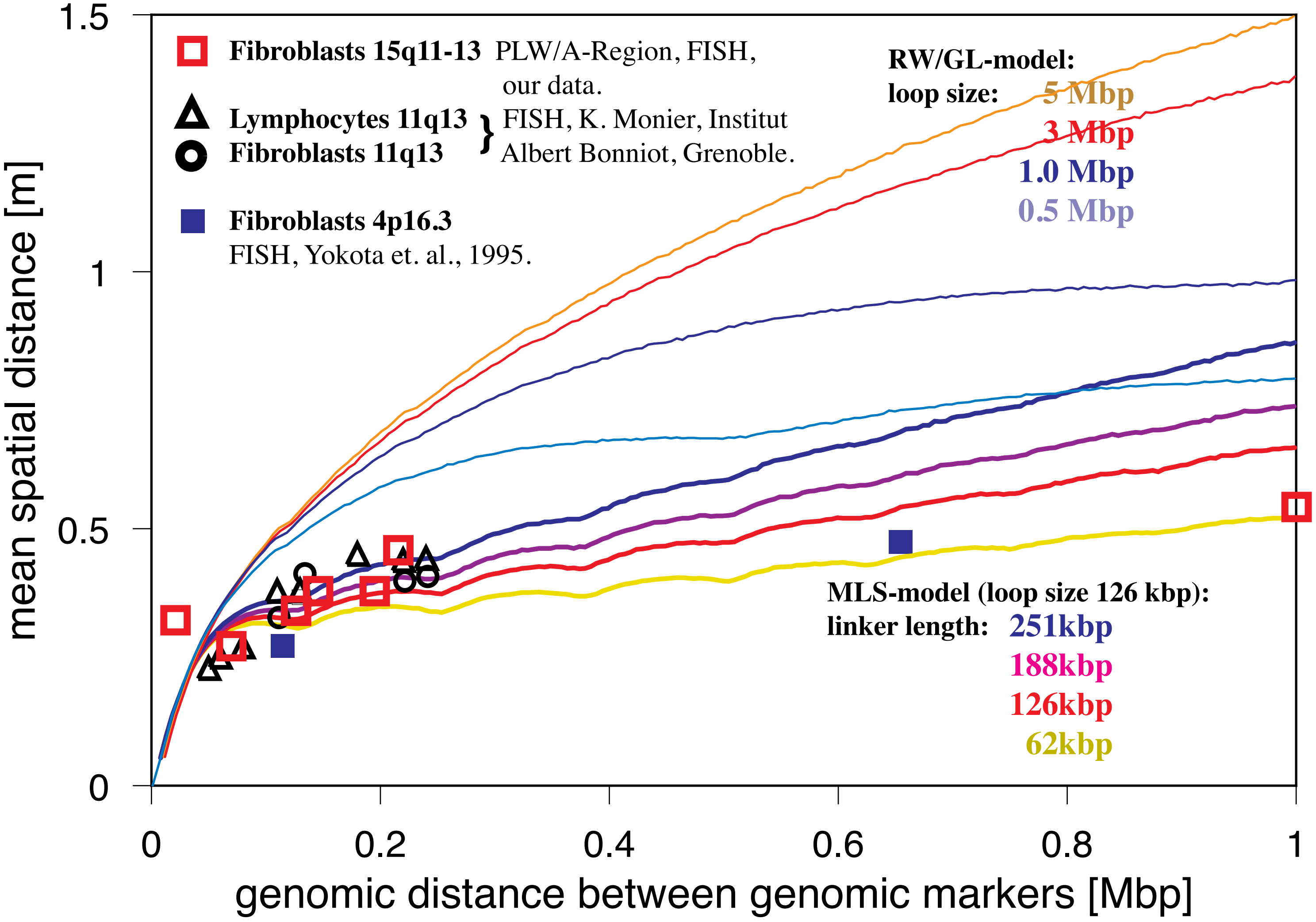

Shift of a marker ensemble through a rosette in the MLS-model in respect to loop bases.
This leads to different sets of 3D-distances for every ensemble position.
Due to the symmetry of the MLS-rosettes periodicities are found.

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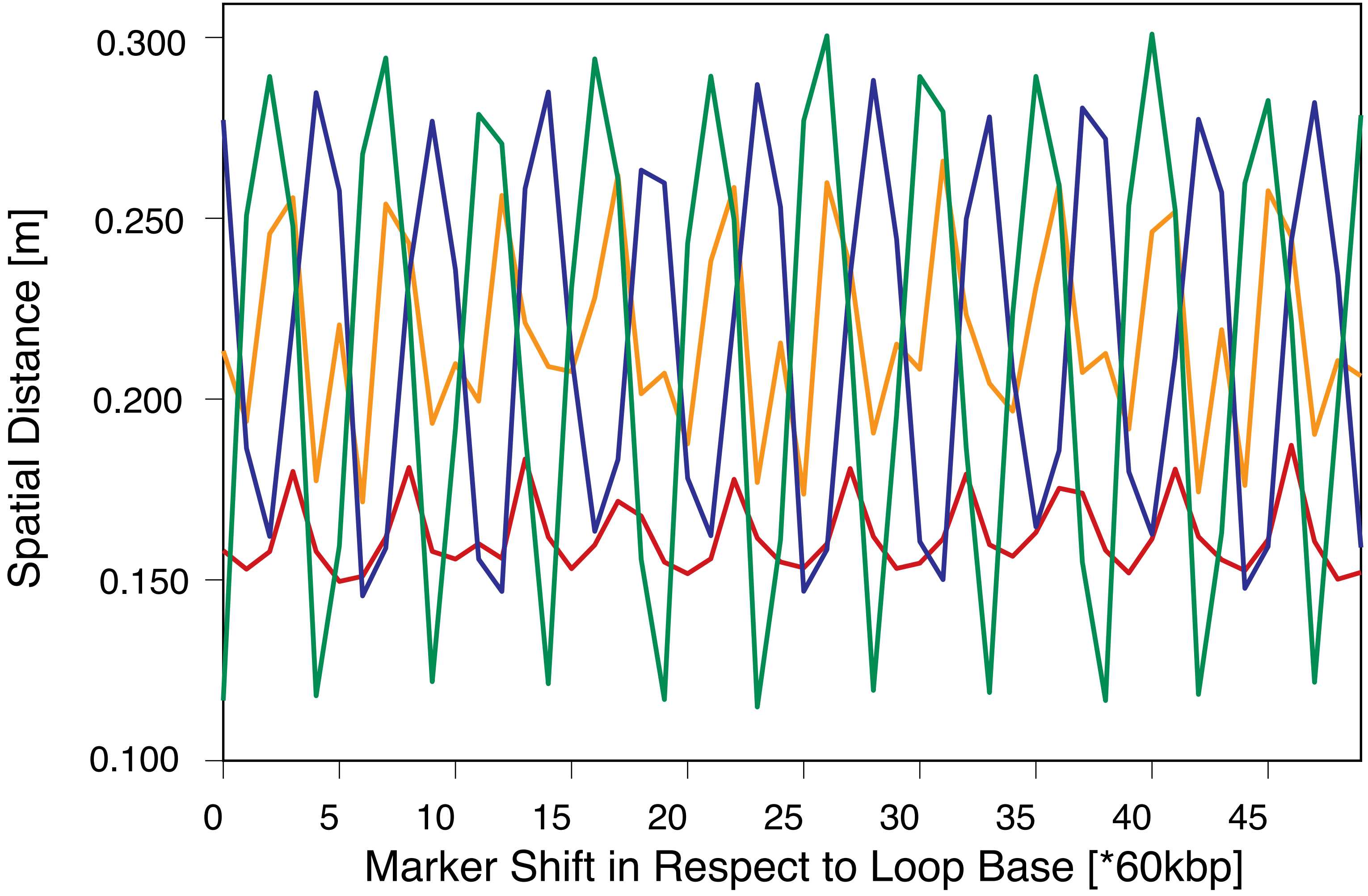

Genomic Marker Distance: 🟥 31kbp 🟧 145kbp 🟦 171kbp 🟩 215kbp

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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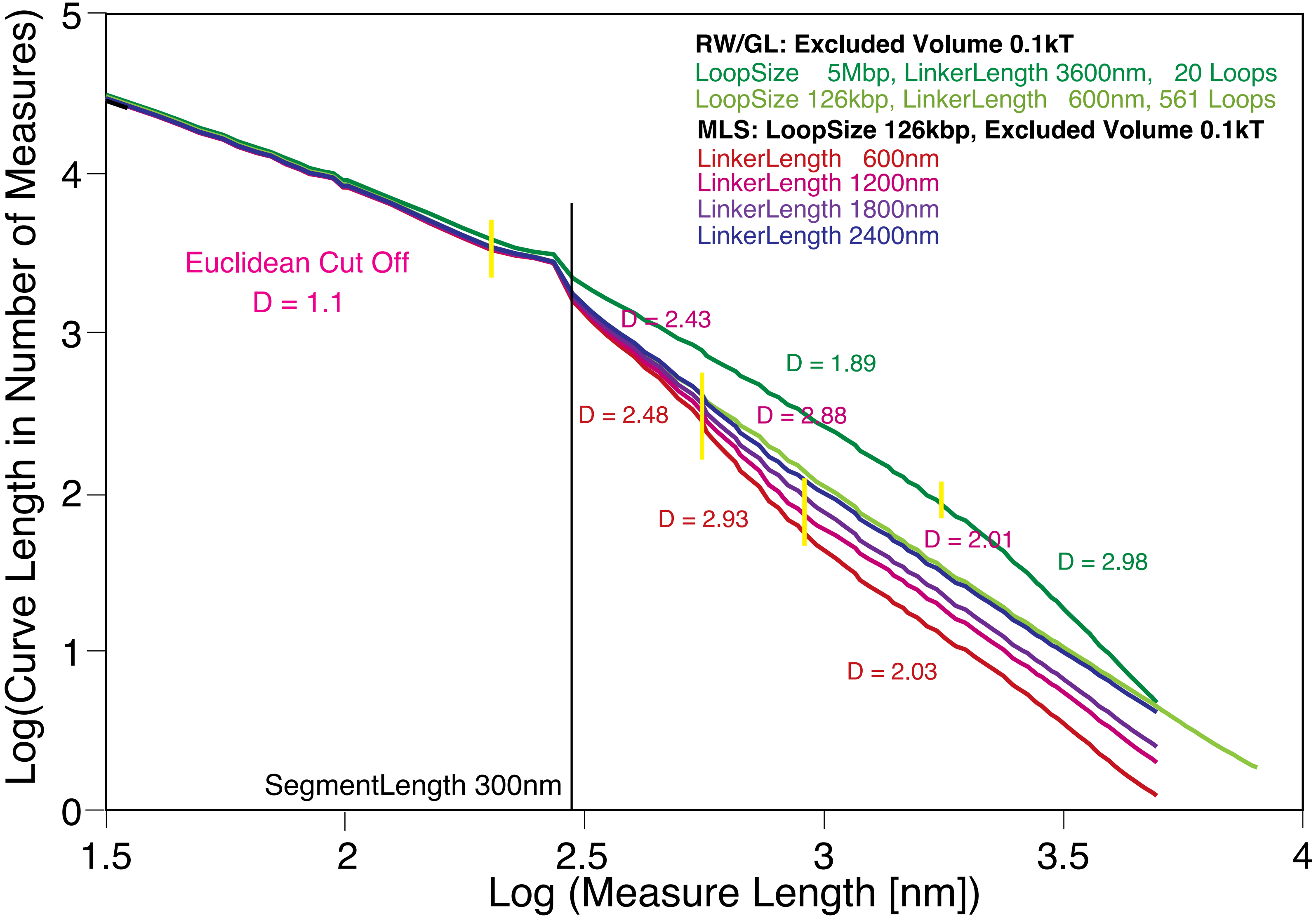

Creation of a ´Virtual Human Cell Nucleus´ with all 46 chromosomes using the MLS-model.

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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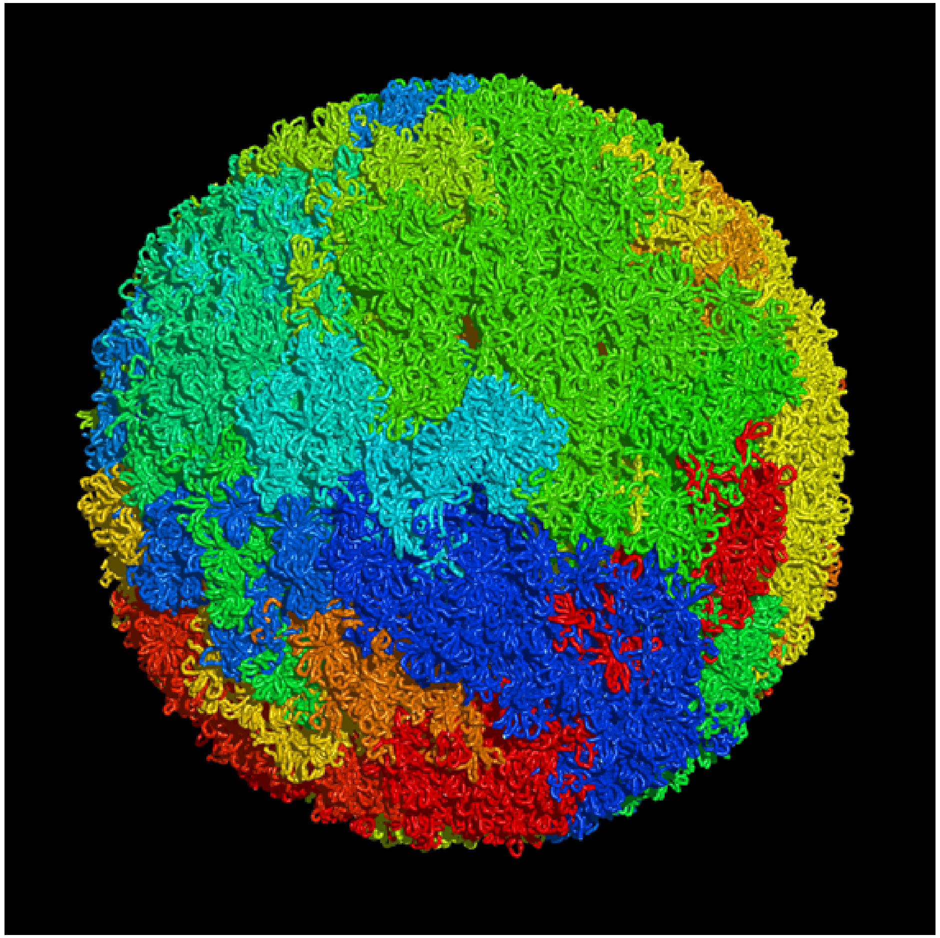
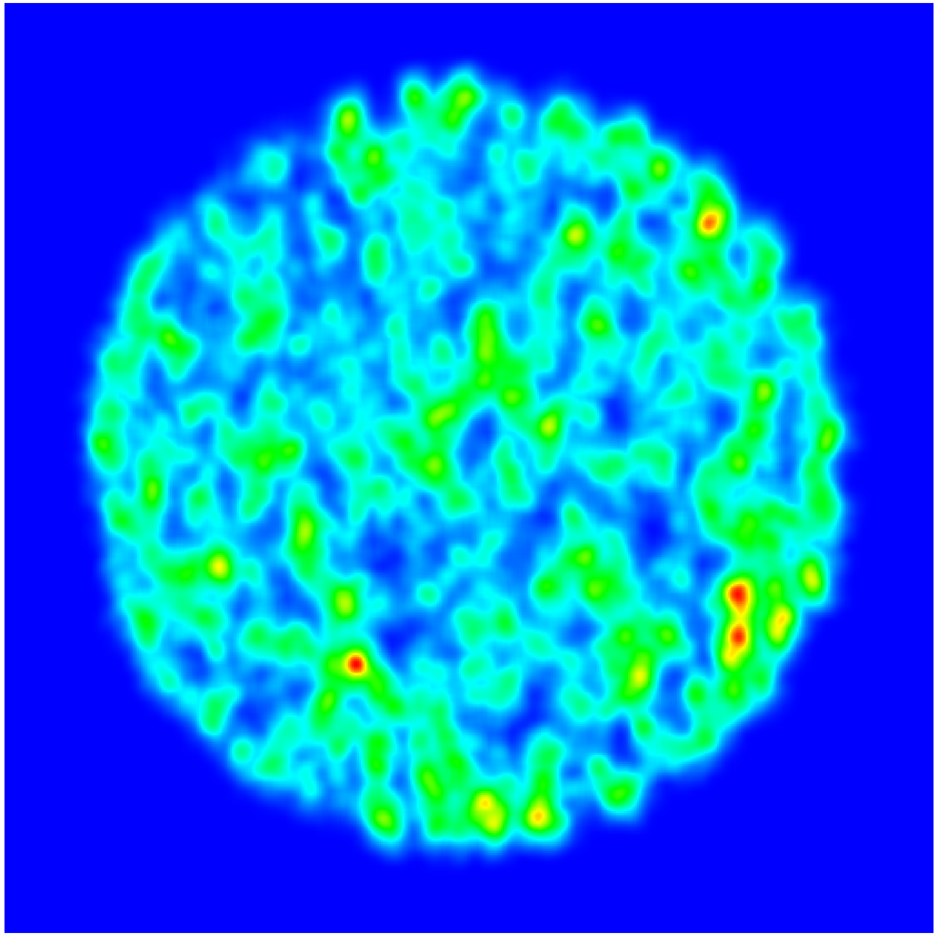


3-D rendering



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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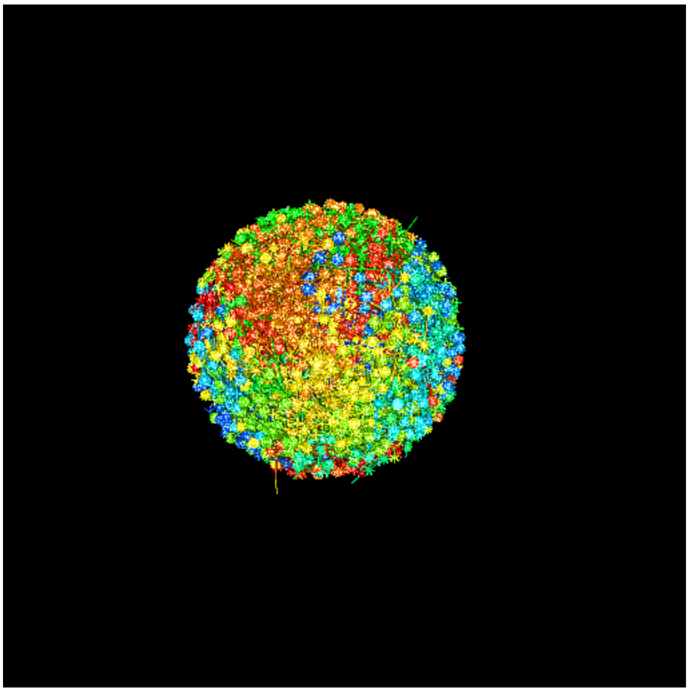
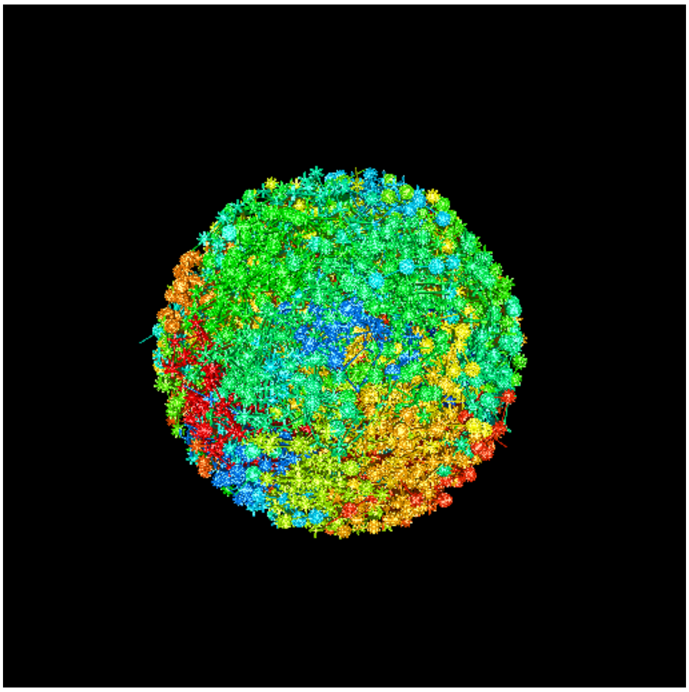
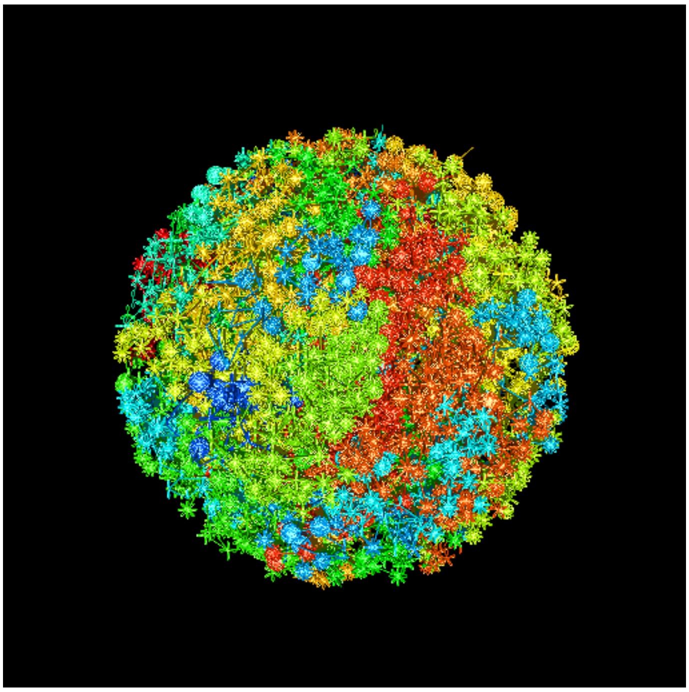


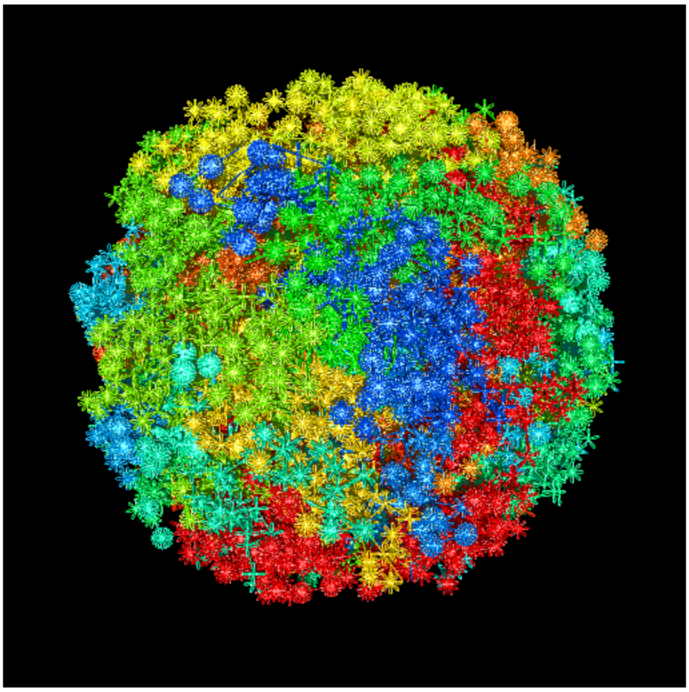
nucleus
6 m diametre



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.

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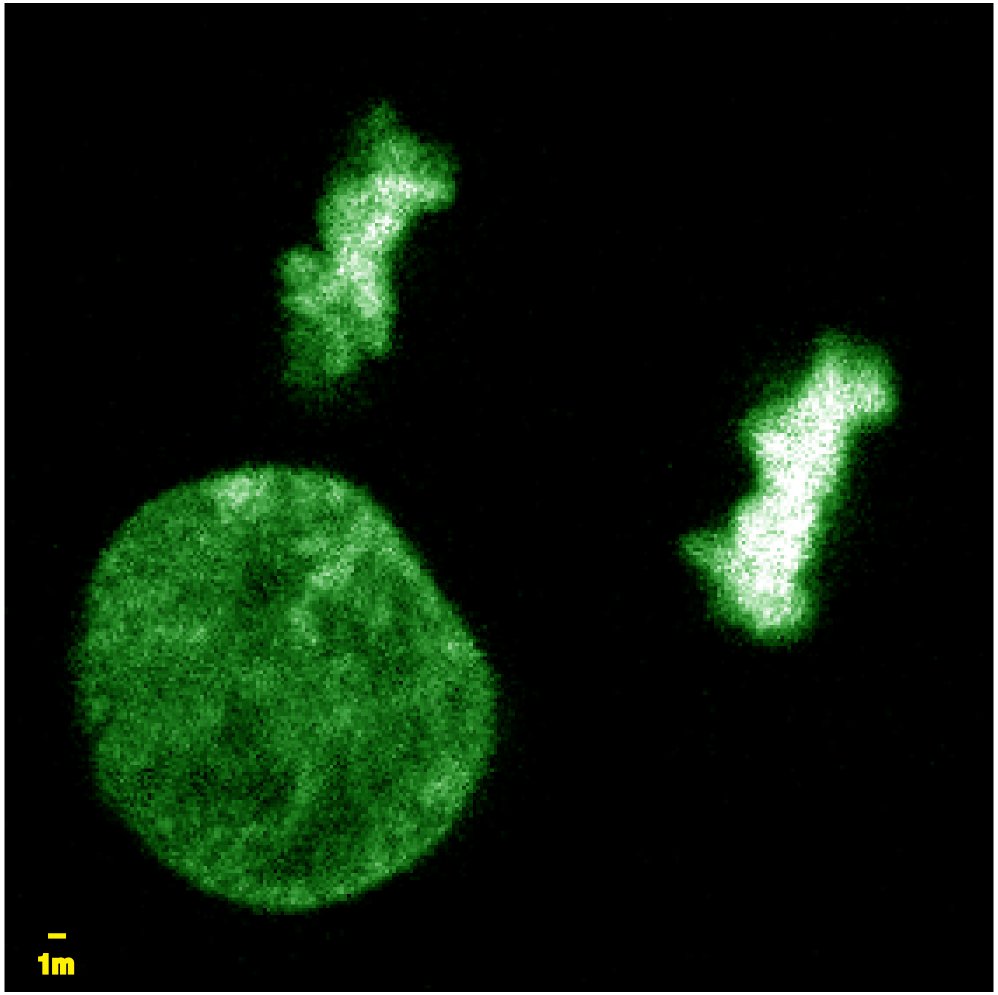
Left: HeLa cells stably transfected with H2B-GFP (K. Sullivan, Scripps Institute).
Confocal in vivo section of a cell nucleus and a mitosis.
Right: Cos7 cell stabaly transfected with H1-GFP (A. Alonso, DKFZ). Confocal in vivo section.



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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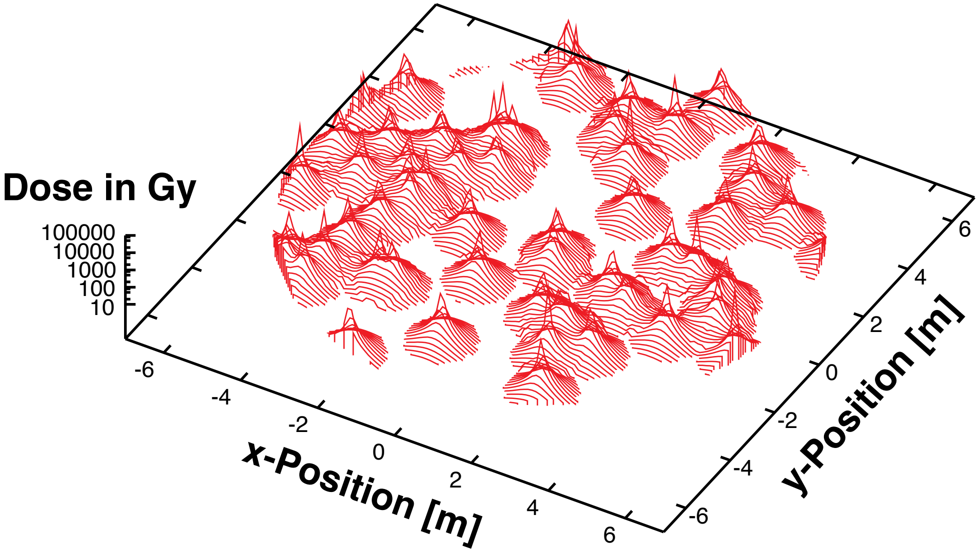
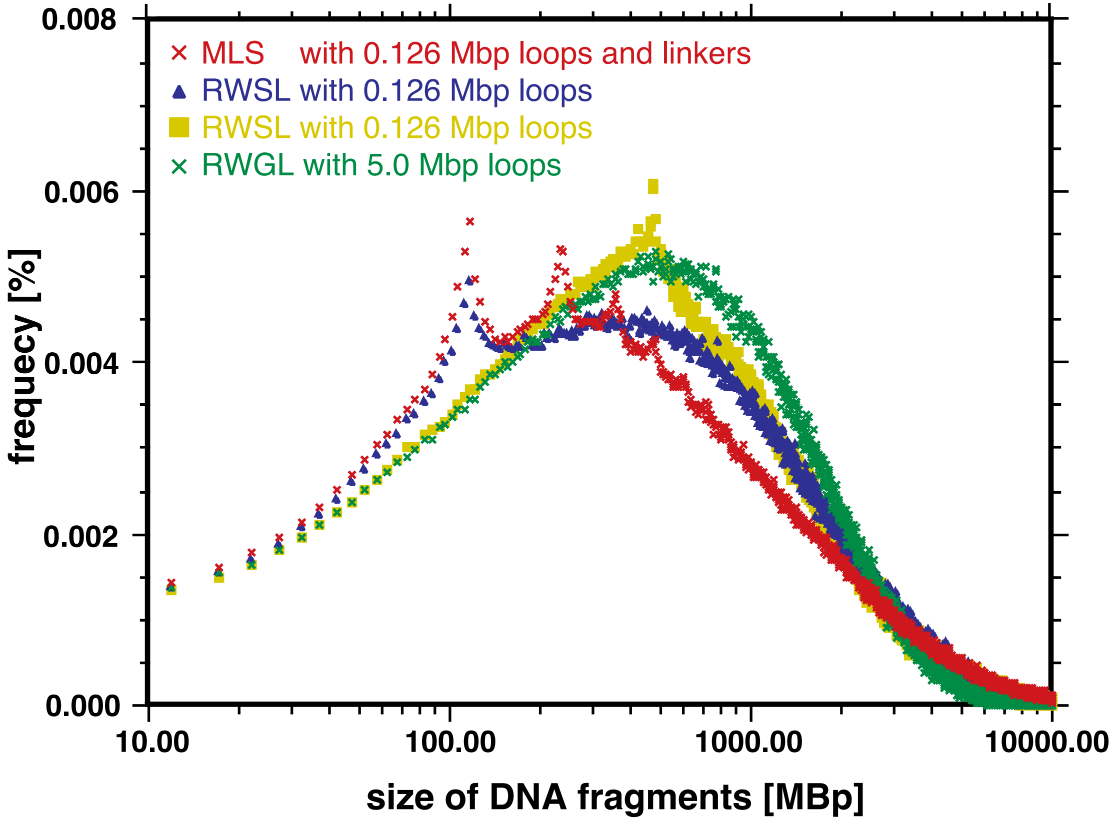
Radiation dose distribution
in a cell nucleus
irradiated with carbon ions



Comparison between experimental and simulated fragment distributions after carbon irradiation.



People

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IBM-SP2, Computing Centre, Karlsruhe
Silicon Graphics-Graphic-Lab, Institute for Scientific Computing (IWR), Heidelberg

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Approaches leading to the Three-Dimensional Organization of the Human Interphase Nucleus:
Simulations, FISH, Chromatin Labelling *in vivo*, Fractal Analysis, Carbon Ion Irradiation

Knoch, T. A.

LabFair 2000, Merck-Eurolab, Technology Centre Jülich, Jülich, Germany, 18th - 19th October 2000.

Abstract

Despite the successful linear sequencing of the human genome its three-dimensional structure is widely unknown, although it is important for gene regulation and replication. For a long time the interphase nucleus has been viewed as a 'spaghetti soup' of DNA without much internal structure, except during cell division. Only recently has it become apparent that chromosomes occupy distinct 'territories' also in interphase. Two models for the detailed folding of the 30 nm chromatin fiber within these territories are under debate: In the Random-Walk/Giant-Loop-model big loops of 3 to 5 Mbp are attached to a non-DNA backbone. In the Multi-Loop-Subcompartment (MLS) model loops of around 120 kbp are forming rosettes, which are also interconnected by the chromatin fiber. Here we show with a comparison between simulations and experiments an interdisciplinary approach leading to a determination of the three-dimensional organization of the human genome:

For the predictions of experiments various models of human interphase chromosomes and the whole cell nucleus were simulated with Monte Carlo and Brownian Dynamics methods. Only the MLS-model leads to the formation of non-overlapping chromosome territories and distinct functional and dynamic subcompartments in agreement with experiments. Fluorescernce in situ hybridization is used for the specific marking of chromosome arms and pairs of small chromosomal DNA regions. The labeling is visualized with confocal laser scanning microscopy followed by image reconstruction procedures. Chromosome arms show only small overlap and globular substructures as predicted by the MLS-model. The spatial distances between pairs of genomic markers as function of their genomic separation result in a MLS-model with loop and linker sizes around 126 kbp. With the development of GFP-fusion-proteins it is possible to study the chromatin distribution and dynamics resulting from cell cycle, treatment by chemicals or radiation in vivo. The chromatin distributions are similar to those found in the simulation of whole cell nuclei of the MLS-model. Fractal analysis is especially suited to quantify the unordered and non-euklidean chromatin distribution of the nucleus. The dynamic behaveour of the chromatin structure and the diffusion of particles in the nucleus are also closely connected to the fractal dimension. Fractal analysis of the simulations reveal the multi-fractality of chromosomes. First fractal analysis of chromatin distributions in vivo result in significant differences for different morphologies and might favour a MLS-model-like chromatin distribution. Simulations of fragment distributions based on double strand breakage after carbon-ion irradiation differ in different models. Here again a comparison with experiments favours a MLS-model.

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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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