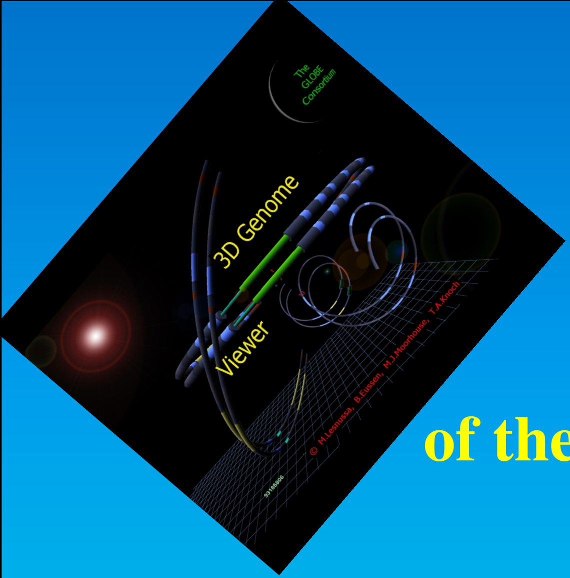

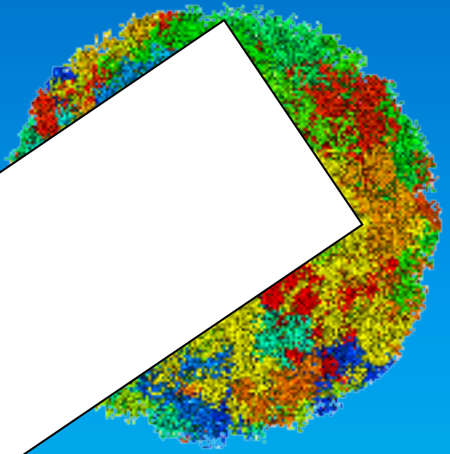

The Three-Dimensional
Architecture and Dynamic
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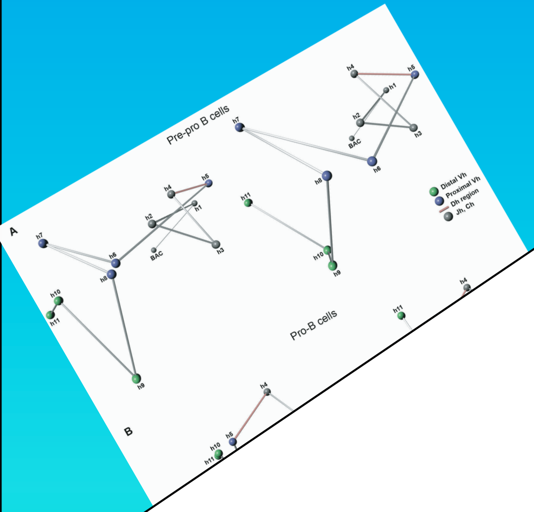
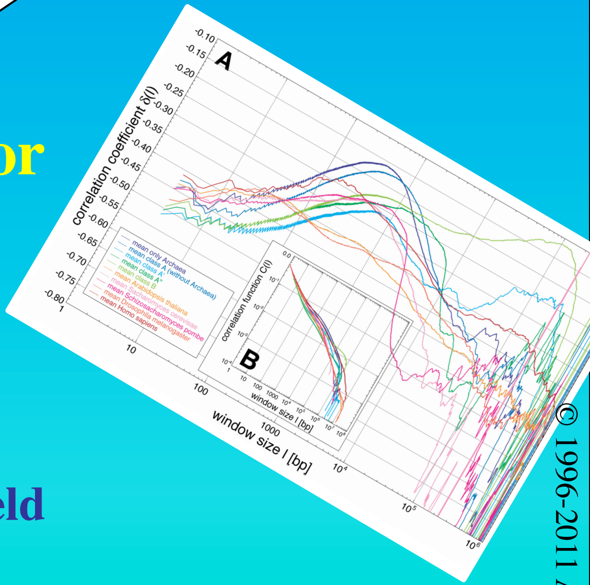


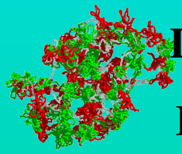


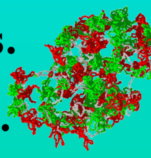
Tobias A. Knoch

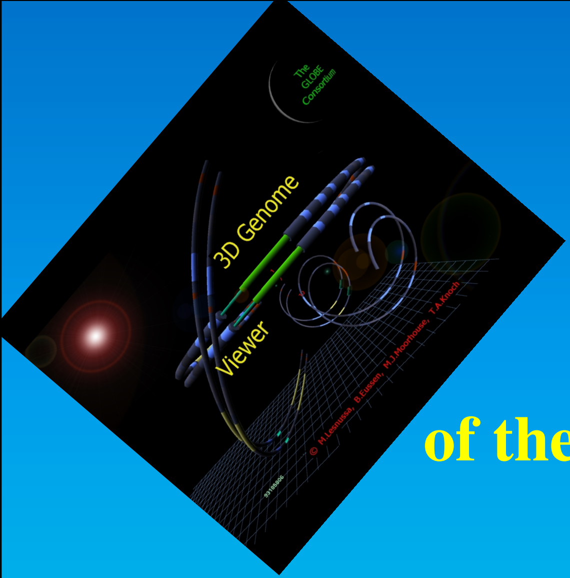
With

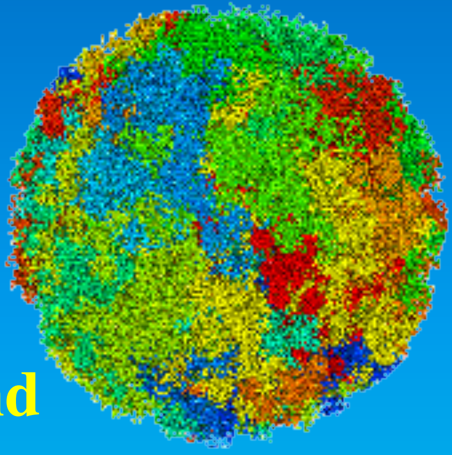
ornelis C. Murre & Frank G. Grosveld

Biophysical Genomics & Erasmus Computing Grid
Dep Cell Biology & Genetics, Erasmus Medical Center, Rotterdam, The Netherlands.
BioQuant Center & German Cancer Research Center (DKFZ), Heidelberg, Germany.





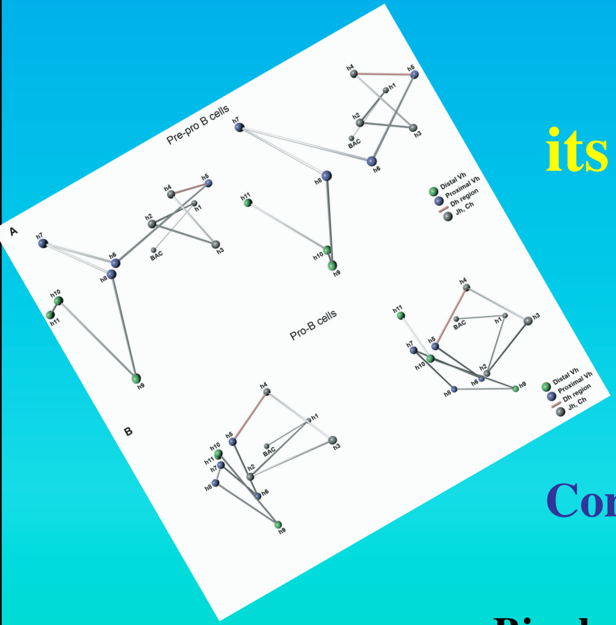


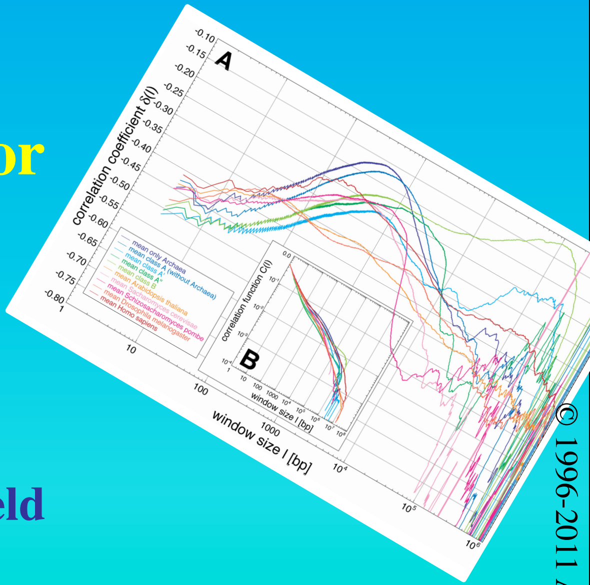


The Three-Dimensional Architecture and Dynamids of the Immunoglobin Heavy-Chain and other Loci

-

its Functional Implications for Genome Organization





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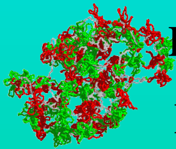
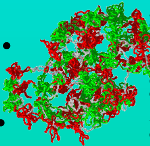
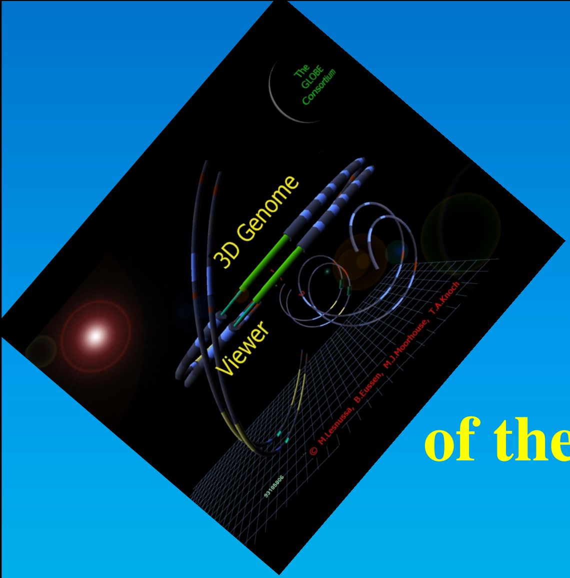


Dept. Cell Biology & Genetics, Erasmus Medical Center, Rotterdam, The Netherlands.

BioQuant Center & German Cancer Research Center (DKFZ), Heidelberg, Germany.





The Three-Dimensional
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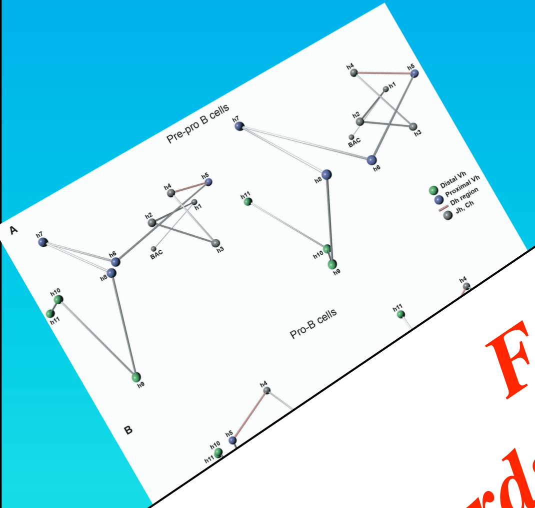
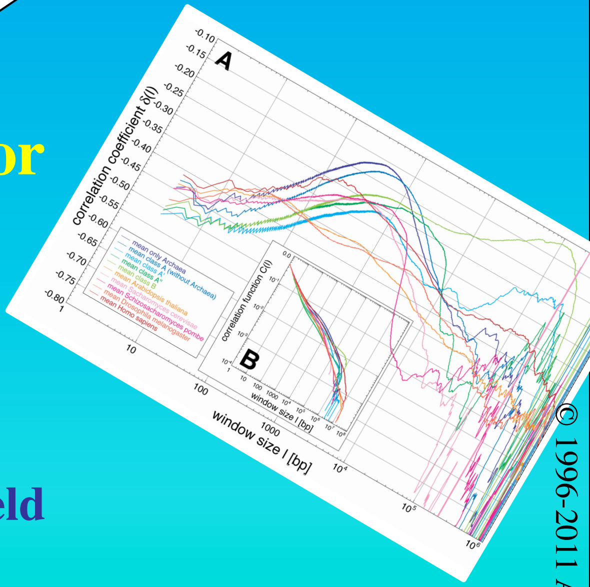
From Sequence to Morphology:
Towards a Holistic Understanding of Genomes!





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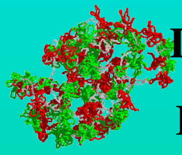
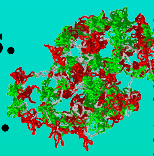
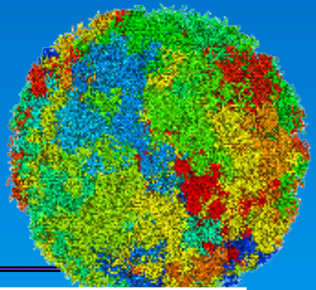
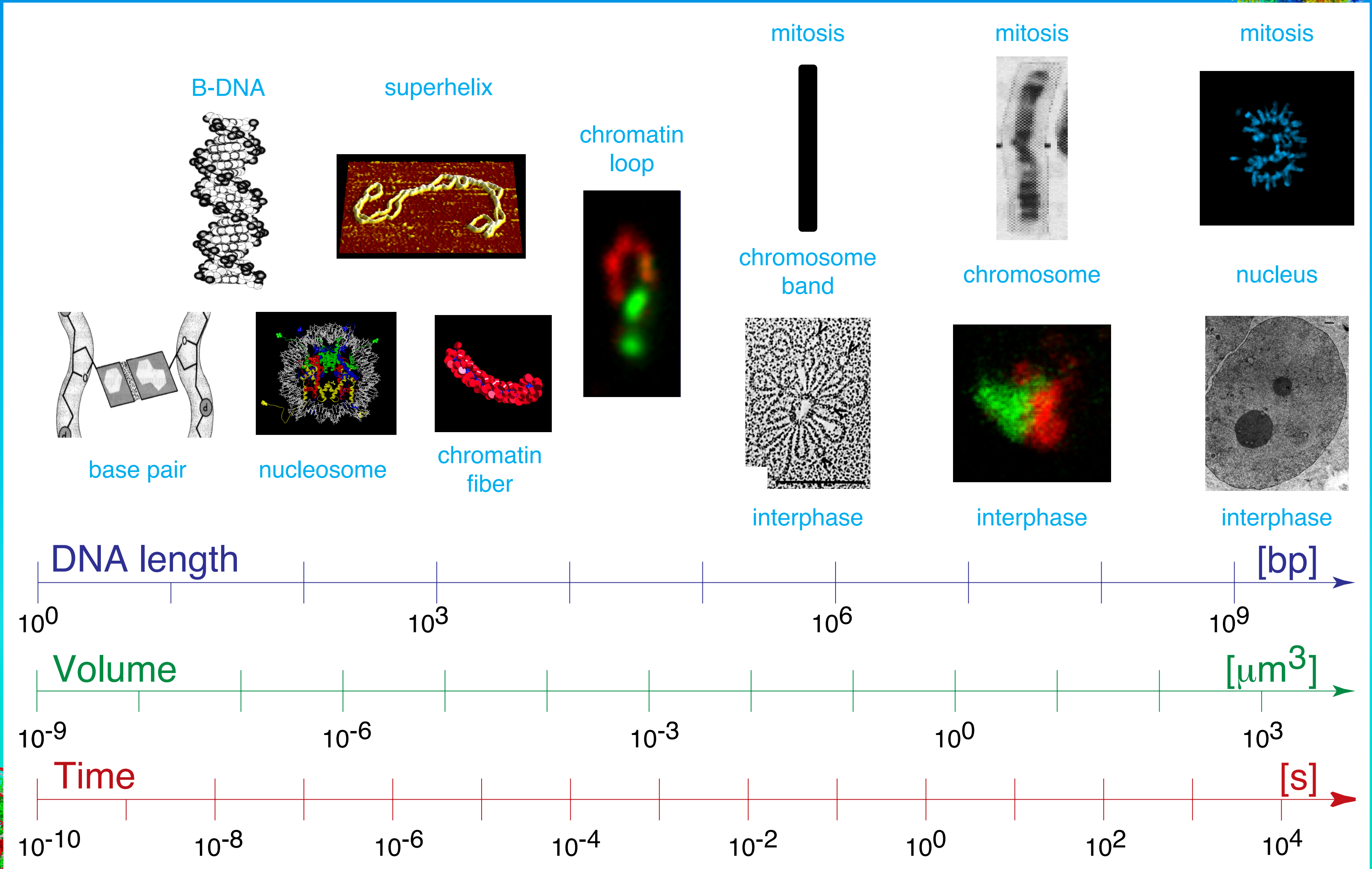
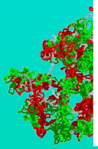



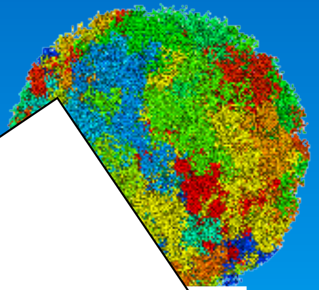


Dynamic and Hierarchical Genome Organization

The different organization levels of genomes bridge several orders of magnitude concerning space and time. How all of these organization levels connect to processes like gene regulation, replication, embryogeneses, or cancer development is still unclear?







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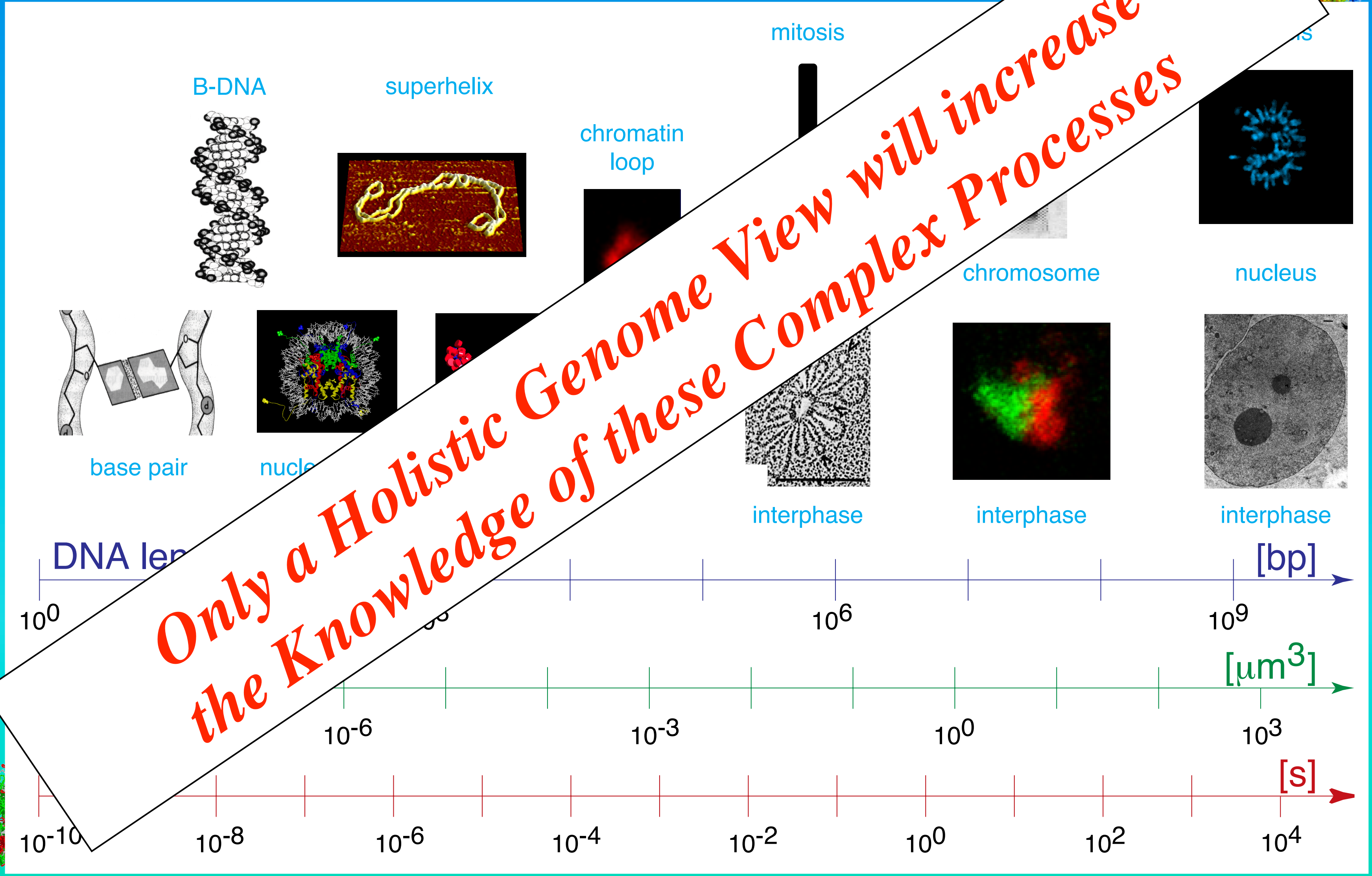
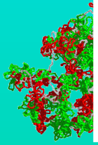
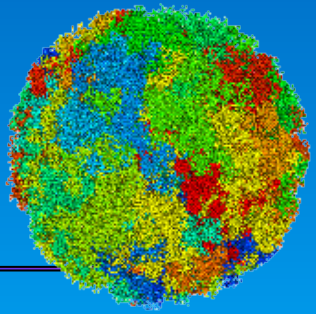


Only a Holistic Genome View will increase the Knowledge of these Complex Processes

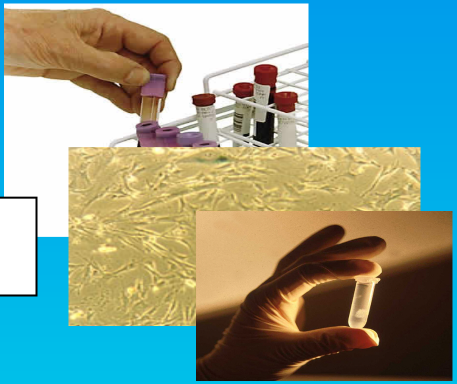


The Complexity of Cytogenetic Diagnostics

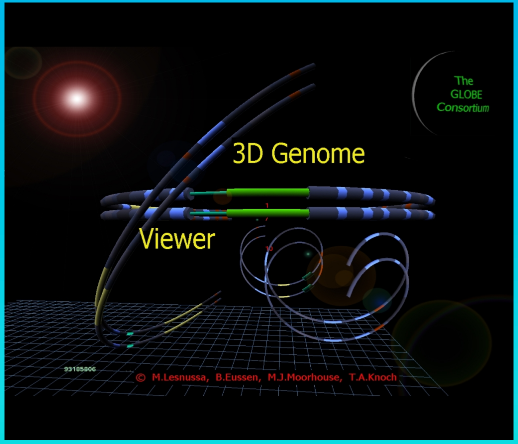
The process of cytogenetic analysis requires proper patient and sample analysis
as well asa comprehensive evaluation of the results.

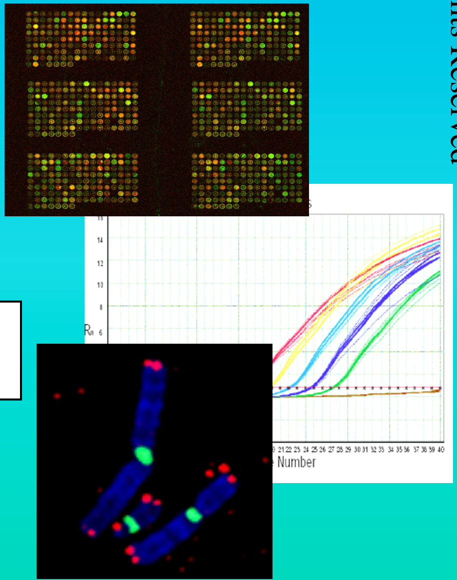


Patient



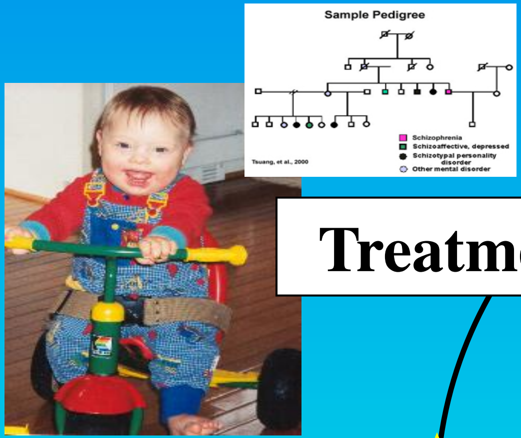
Sample





Analysis

Diagnosis



Treatment

The Complexity of Cytogenetic Diagnostics

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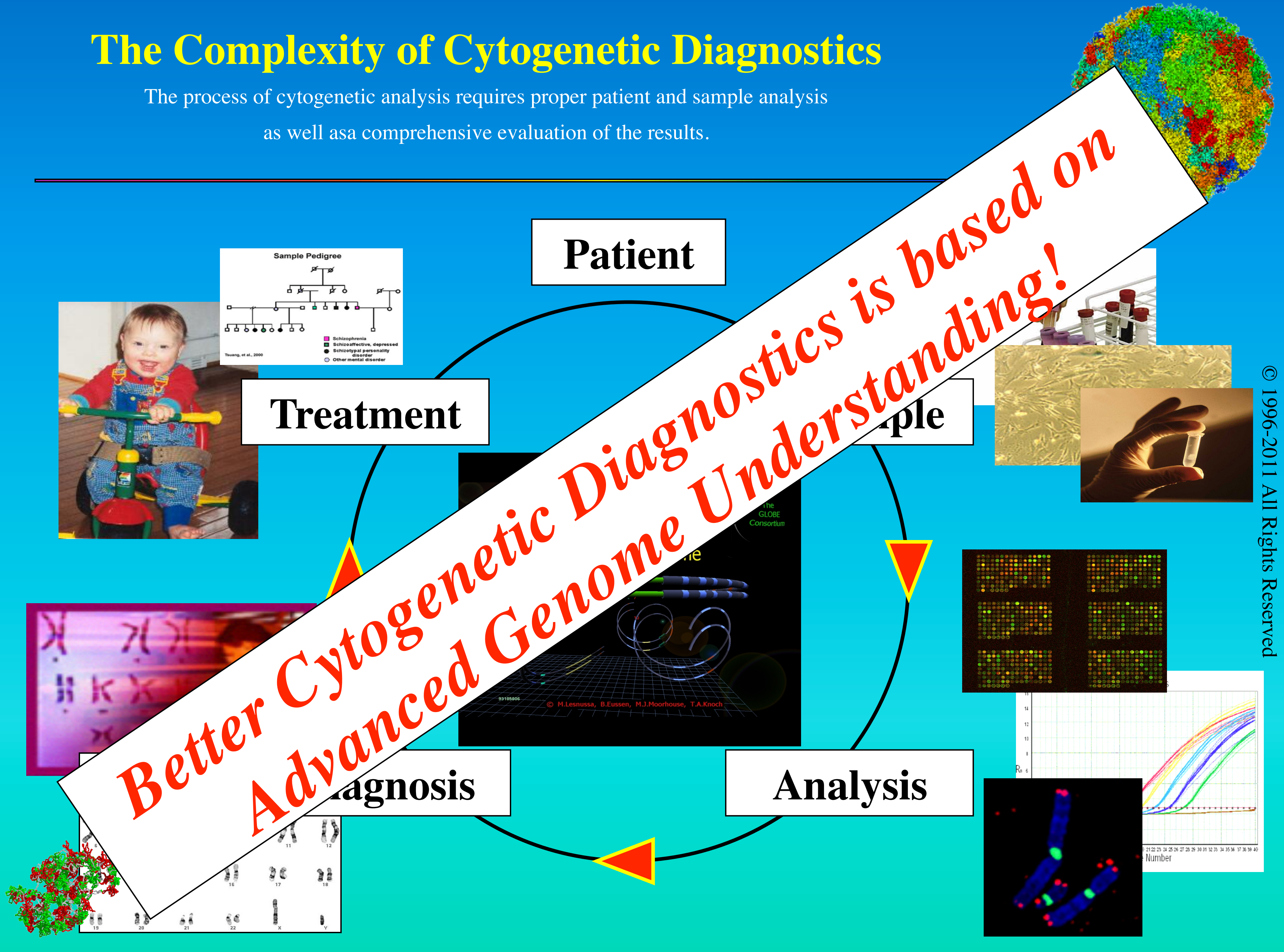

Patient

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Analysis

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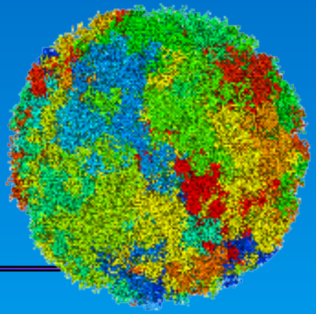
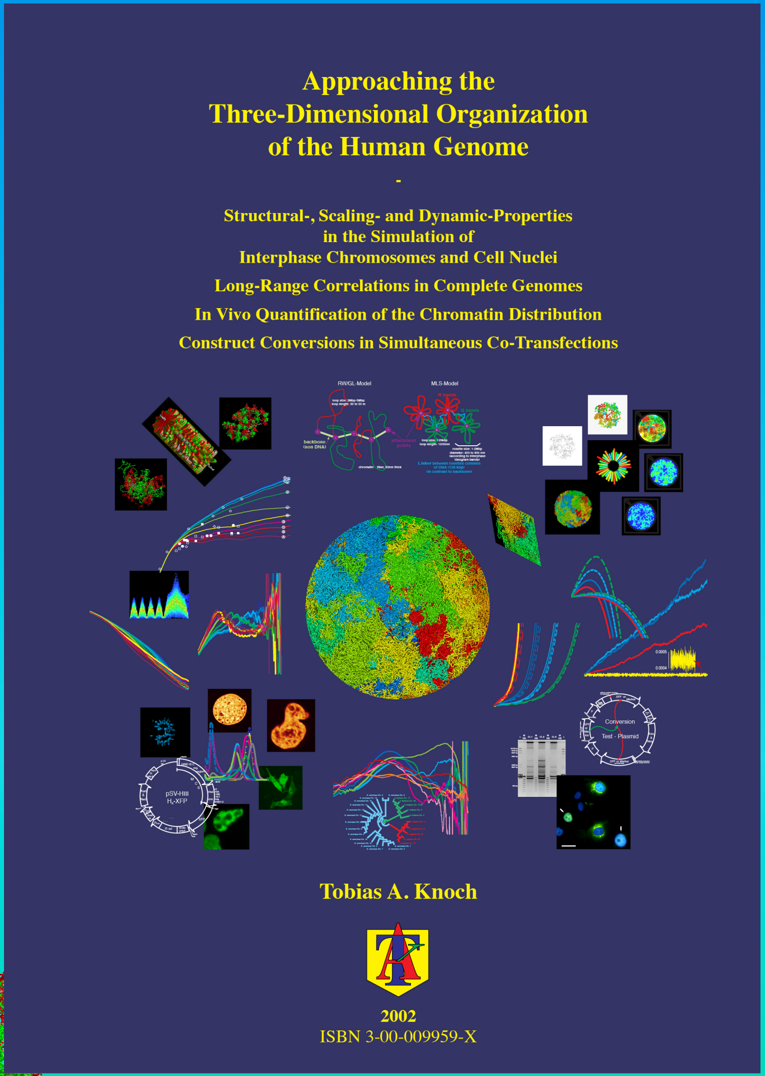
Treatment

Better Cytogenetic Diagnostics is based on Advanced Genome Understanding!

WE KNOW THAT WE HARDLY KNOW ANYTHING

From the sequence to the morphology we just have begun to elucidate the organization of genomes and we also just have begun to understand that it is not only the detailed knowledge about one organizational level but beyond the holistic entity of the whole cell nucleus or genome which makes genomes function!





Approaching the
Three-Dimensional Organization
of the Human Genome
-

Structural-, Scaling- and Dynamic-Properties
in the Simulation of
Interphase Chromosomes and Cell Nuclei

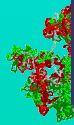
Long-Range Correlations in Complete Genomes

In Vivo Quantification of the Chromatin Distribution

Construct Conversions in Simultaneous Co-Transfections

Tobias A. Knoch

2002
ISBN 3-00-009959-X



Nucleus:

-> Nuclear organization: chromosome arrangement, morphology?
-> Nuclear Code: information content, regulation, variability?

Chromosome:

-> Chromosome organization: loops, loop aggregates, extension?
-> Chromosome code: information content, regulation, variability?

Chromatin:

-> Chromatin fiber organization: prevalence, variation, dynamics?
-> Chromatin code: coding, regulation, modification?

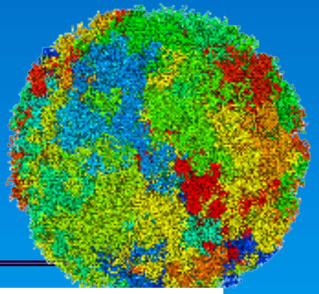
Nucleosome:

-> Nucleosome organization: tail position, mobility, modification?
-> Histon code: coding, regulation, modification?

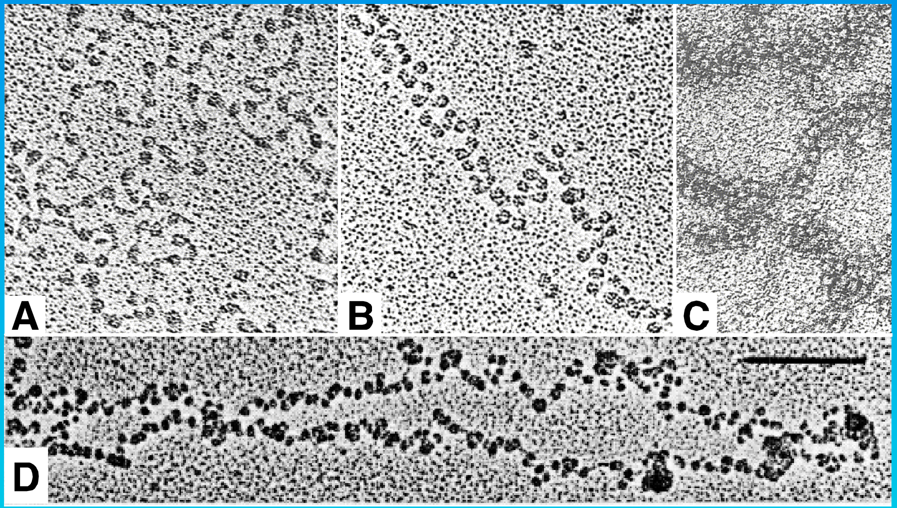
DNA Sequence:

-> DNA local structure: bending, melting, stability, modification?
-> General sequence organization: coding, regulating and the rest?

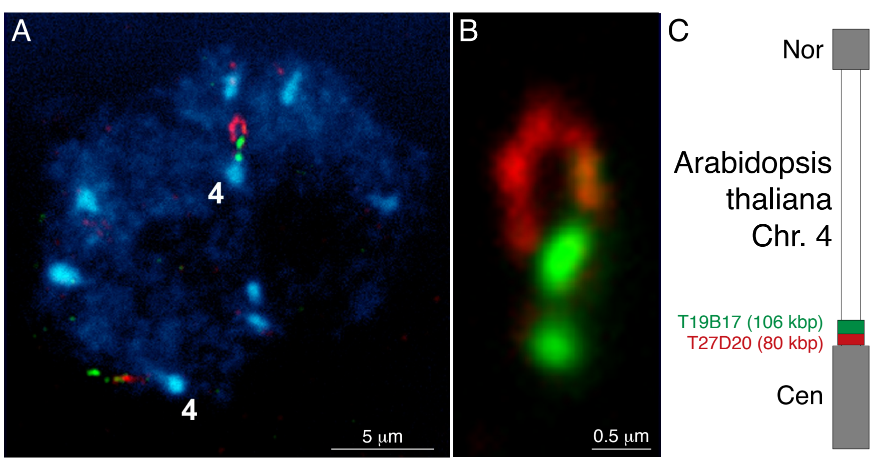
Chromatin Conformation and Higher-Order Topologies



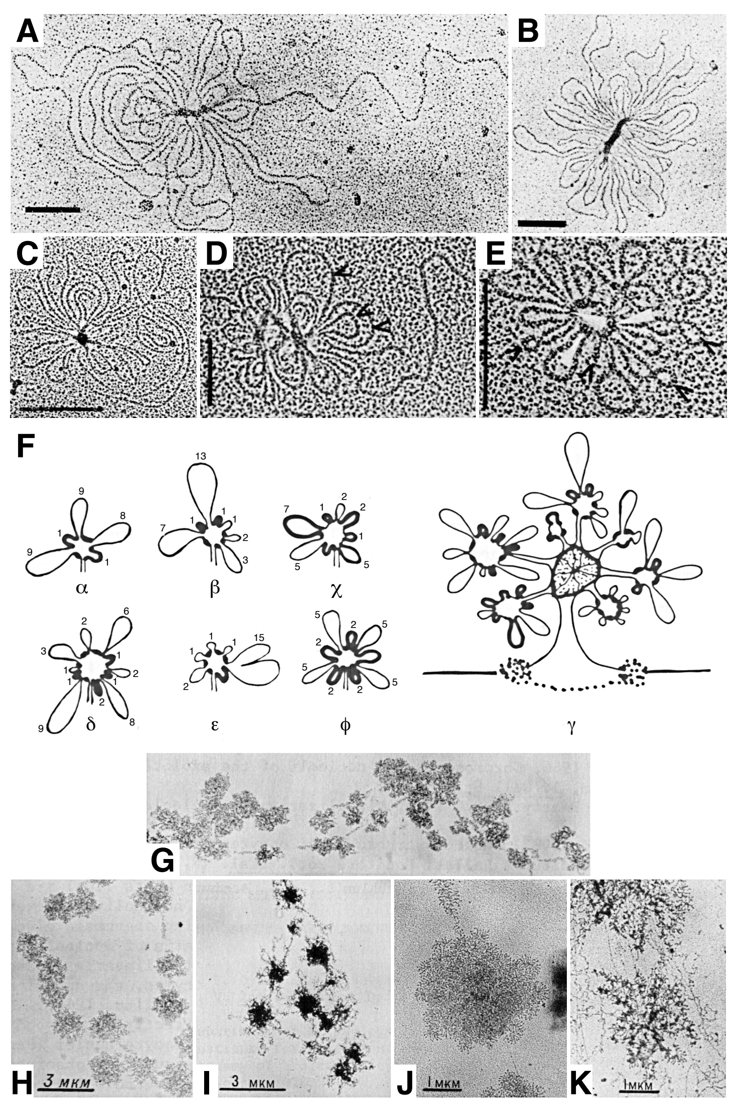
It becomes increasingly clearer, that the chromatin conformation is a random organization of nucleosomes, which depending on external or modification conditions has different condensation degrees, with a prevalence for the 30nm fiber with ∼6nucleosomes per 11nm. This seems to make loops which further cluster to form aggregates more or less rosette-like which then constitute the chromosome.



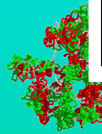
A-C: Voet &Vo;t D: Reznik *et al.*



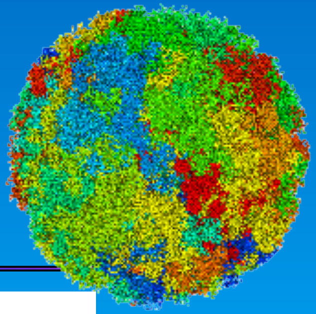
Courtesy P. Fransz, Amsterdam



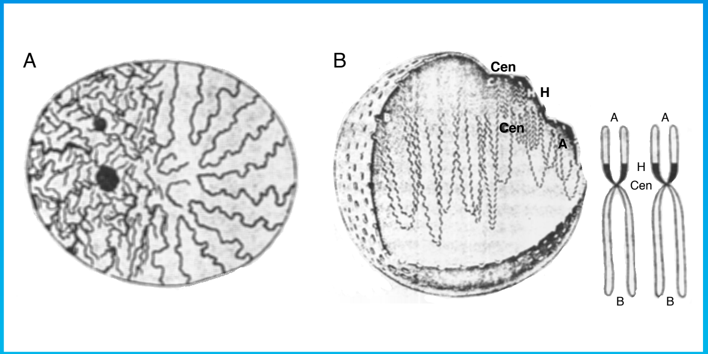
A, B: Avramova *et al.*; C: Salganik *et al.*; D-G:Frznik *et al.*; G-K: Tsvetkov & Parvenov.



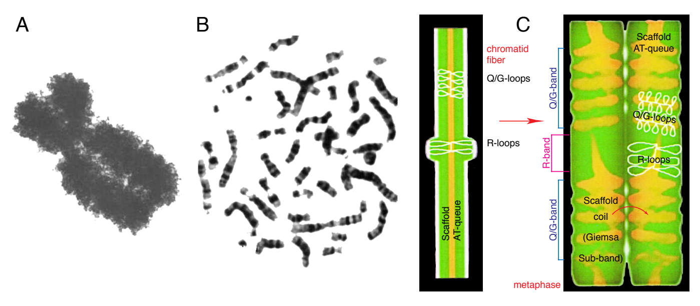
Integral Models of Cell Nuclear Organization



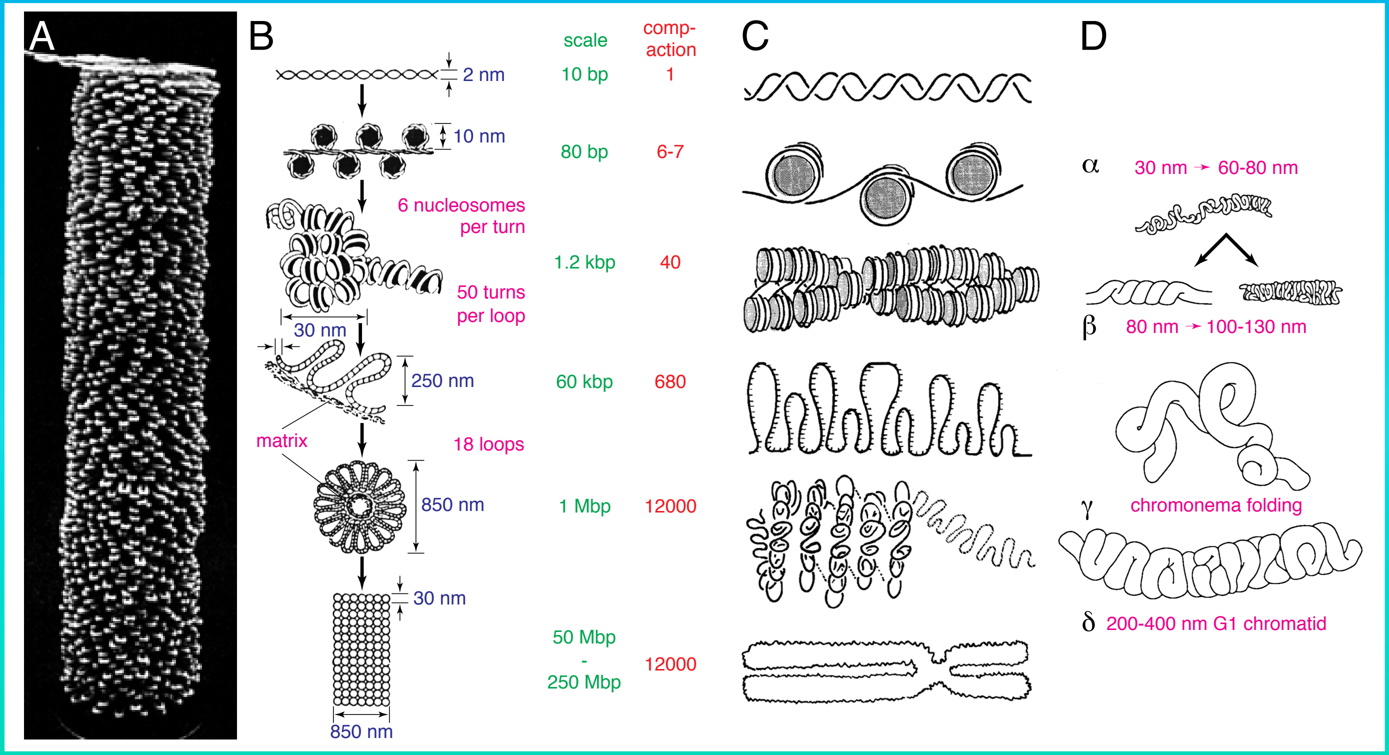
Already Rabl and Boveri were aware of the obvious fact that the organization of genomes has to be consistent from the sequence level to the morphology of the whole cell nucleus. Although they might be different in detail their common seem is recursive folding and clustering thereof with variation/ modification and dynamics accounting for different nuclear states and function.



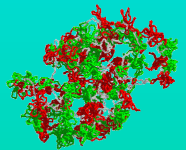
Rabl & Boveri



A: Bloom & Fawcett
B: Alberts *et al.*
C: Paulson & Laemmli

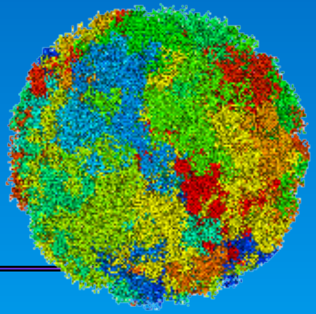


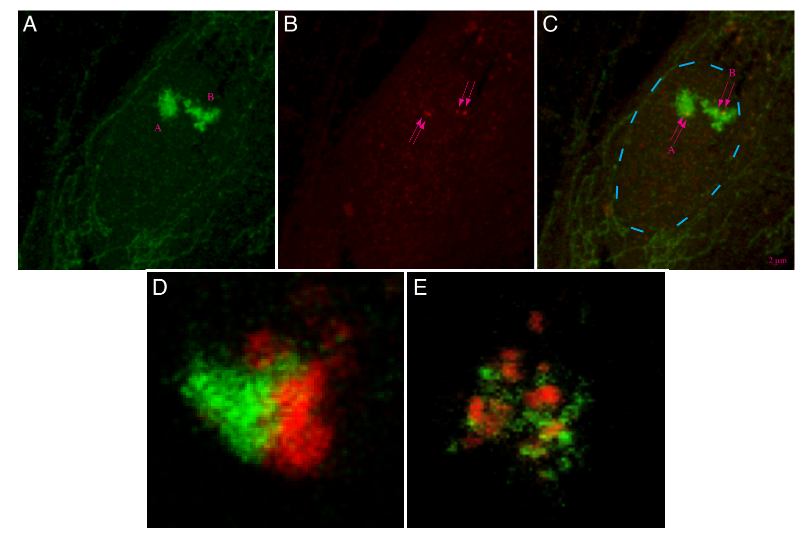
A, B: Pienta & Coffey; C: Alberts *et al.*; D: Belmont & Bruce



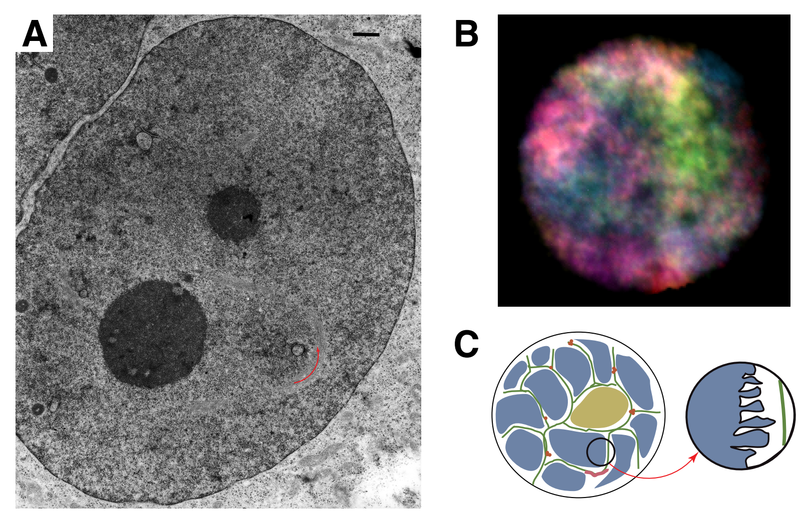
Integral Models of Cell Nuclear Organization

The biggest advantage of integral models is the again obvious and simple fact, that they allow the validation from the consistency of different levels of organization from the other levels. Thus, e.g. the so called Interchromosmal Domain Model can be ruled out by simple volumenous thought…

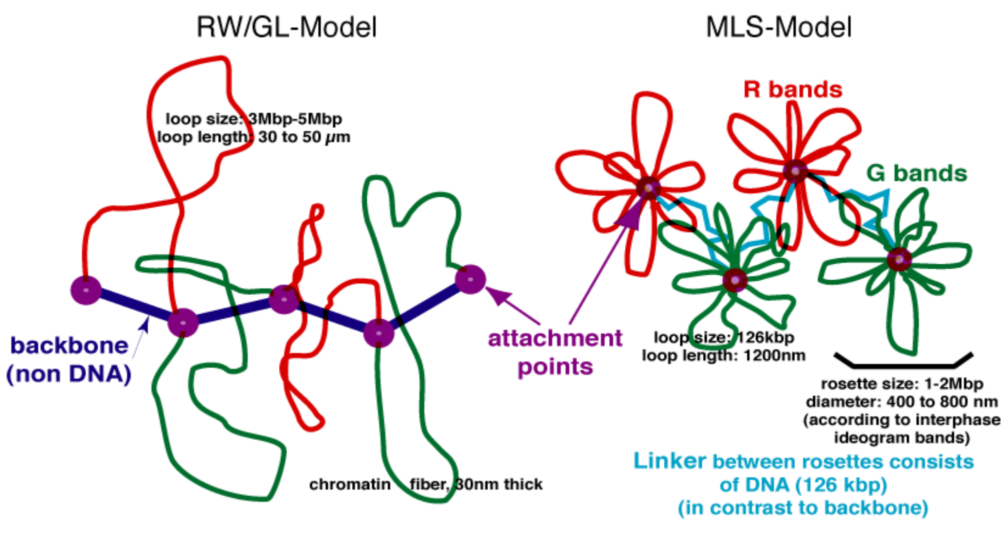




D: courtesy S. Dietzel; E: courtesy D. Zink

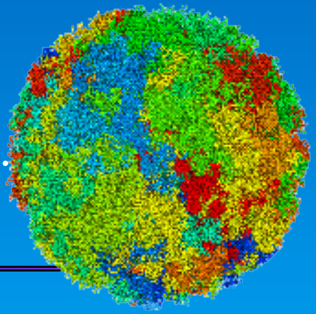


Random-Walk/Giant-Loop Multi-Loop-Subcompatment Model



A: courtesy K. Richter; B: courtesy K. Greulich-Bode

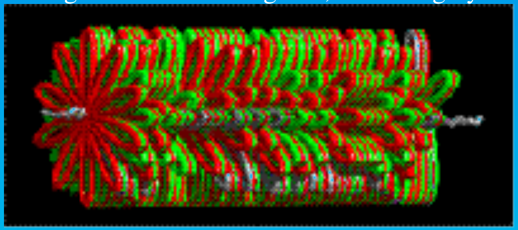
Simulation of Single Chromosomes

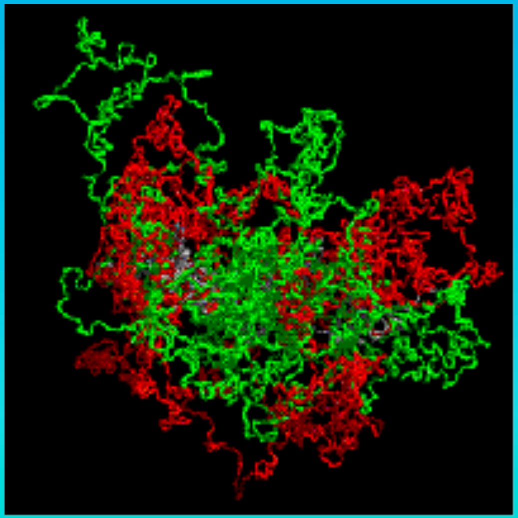


The 30 nm chromatin fiber is modeled as a polymer chain with stretching, bending, and excluded volume interactions. Monte Carlo and Brownian Dynamic methods lead to thermodynamical equilibrium configurations.

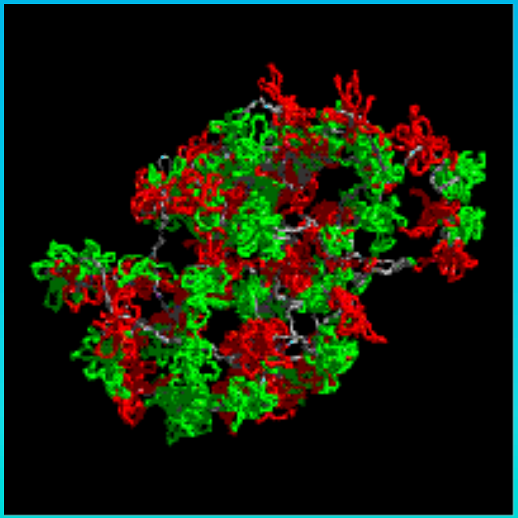
All models form chromosome territories with big voids and different chromatin morphologies. Experimental territory and subcompartment diameters agree best with an MLS model with 80 to 120 kbp loops and linkers.

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

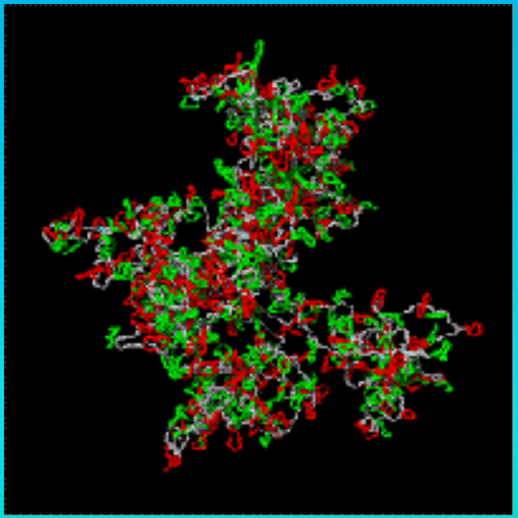




RW/GL model, loop size 5 Mbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromsome territory, thus forming no distinct features like in MLS model.



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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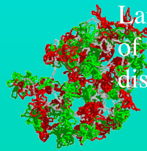

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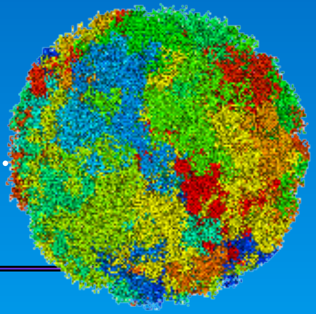

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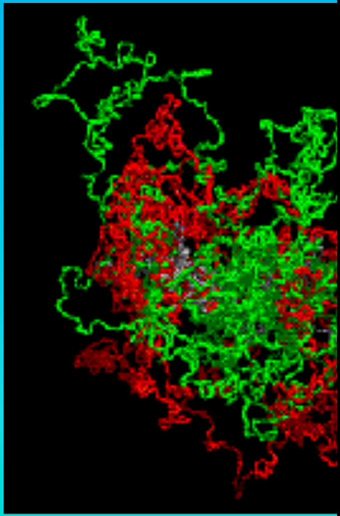

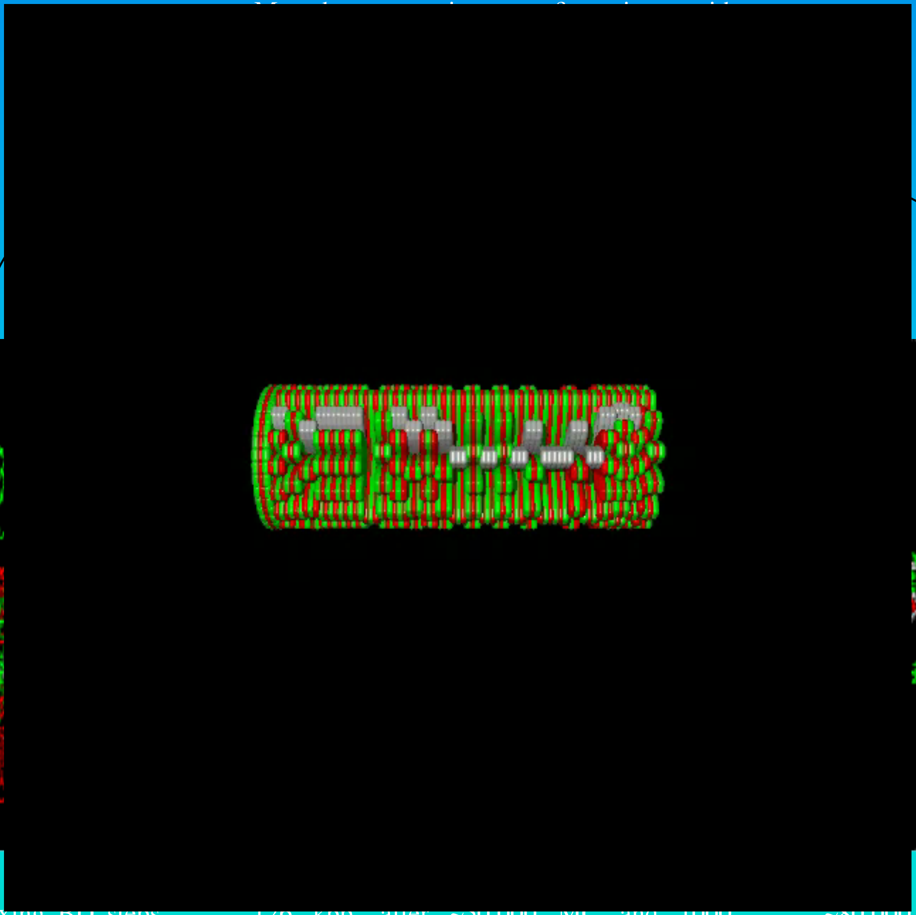

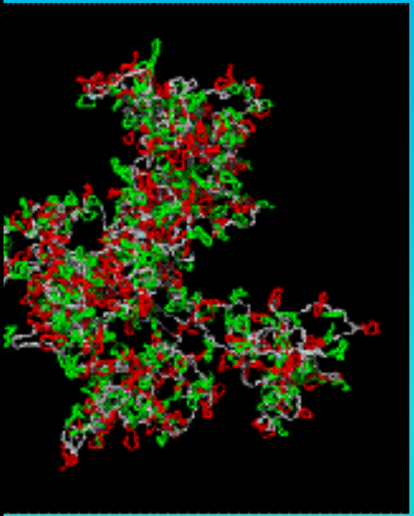

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126 kbp, after ~50.000 MC and 1000 relaxing BD steps. Here rosettes form subcompartments as separated organizational and dynamic entities.

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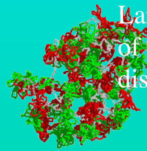
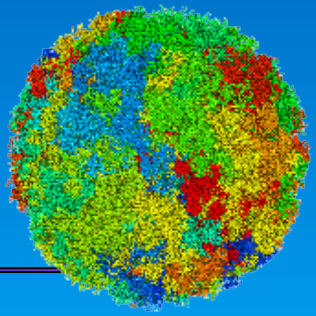
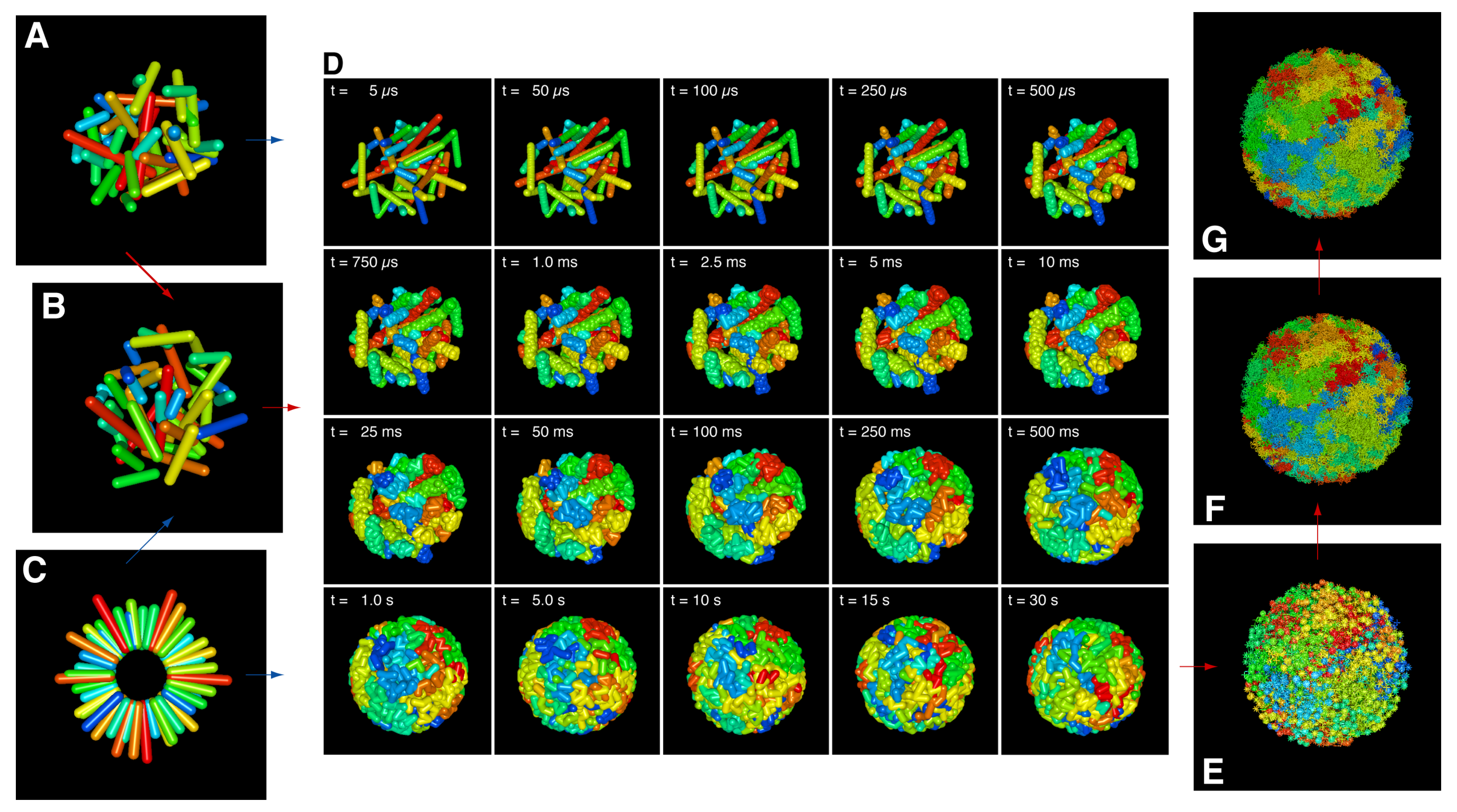


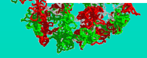
Simulation of Whole Nuclei with all 46 Chromosomes



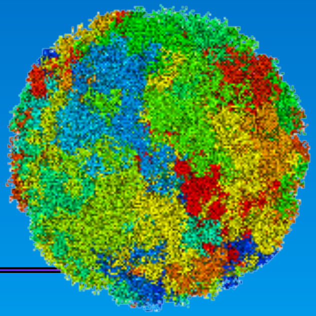
Starting with some metaphase arrangement of cylindrical chromosomes, interphase nuclei with a 30 nm fiber resolution and at thermodynamical equilibrium are created in 4 steps using simulated annealing and Brownian Dynamics methods with stretching, bending, excluded volume and a spherical boundary interactions.

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



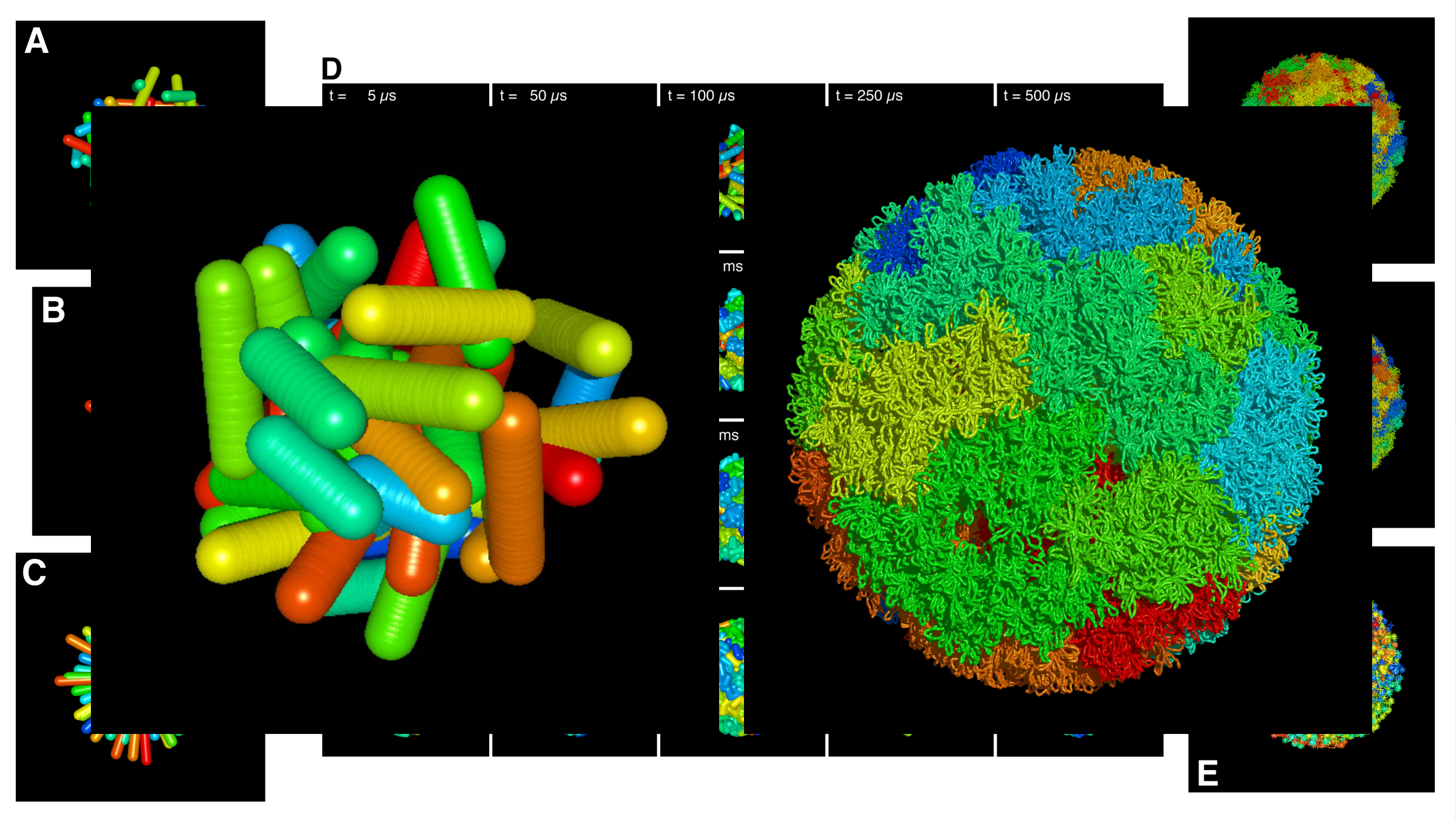


Simulation of Whole Nuclei with all 46 Chromosomes



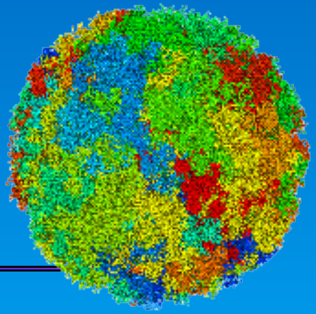
Starting with some metaphase arrangement of cylindrical chromosomes, interphase nuclei with a 30 nm fiber resolution and at thermodynamical equilibrium are created in 4 steps using simulated annealing and Brownian Dynamics methods with stretching, bending, excluded volume and a spherical boundary interactions.

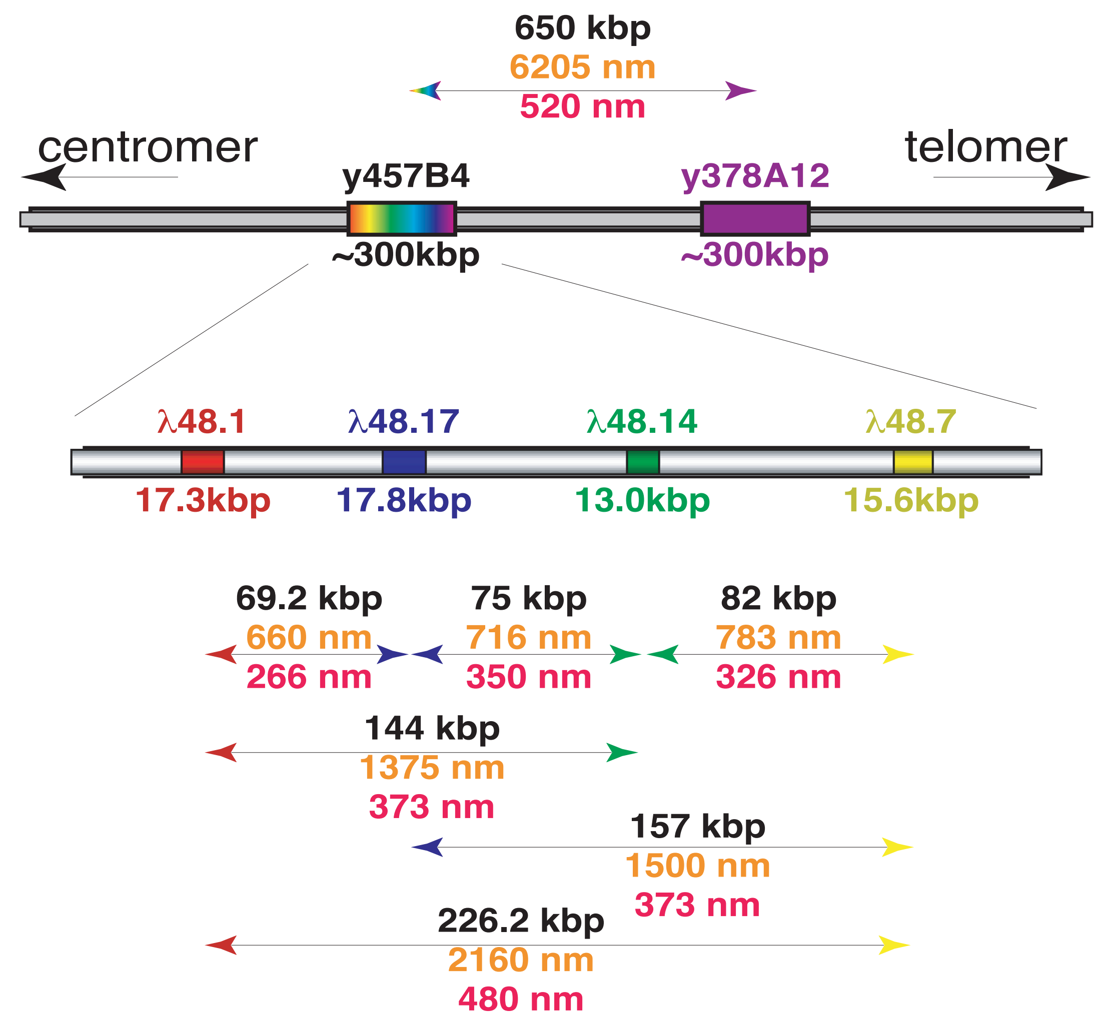
The chromosome territory position depends on their metaphase position and is reasonably stable.



3D Architecture of the Prader-Willi Region

Fluorescence *in situ* hybridization with various protocols of small probes within the Prader-Willi region combined with spectral precision distance confocal laser scanning microscopy and comparison with large-scale computer simulations shows a Multi-Loop Subcompartiment organization of the Prader-Willi region.





650 kbp
6205 nm
520 nm

centromer
telomer

y457B4
~300kbp

y378A12
~300kbp

λ48.1
17.3kbp

λ48.17
17.8kbp

λ48.14
13.0kbp

λ48.7
15.6kbp

69.2 kbp
660 nm
266 nm

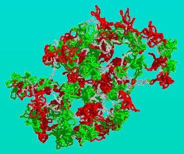
75 kbp
716 nm
350 nm

82 kbp
783 nm
326 nm

144 kbp
1375 nm
373 nm

157 kbp
1500 nm
373 nm

226.2 kbp
2160 nm
480 nm



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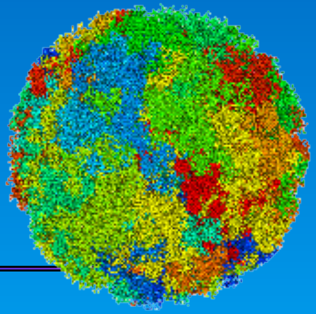

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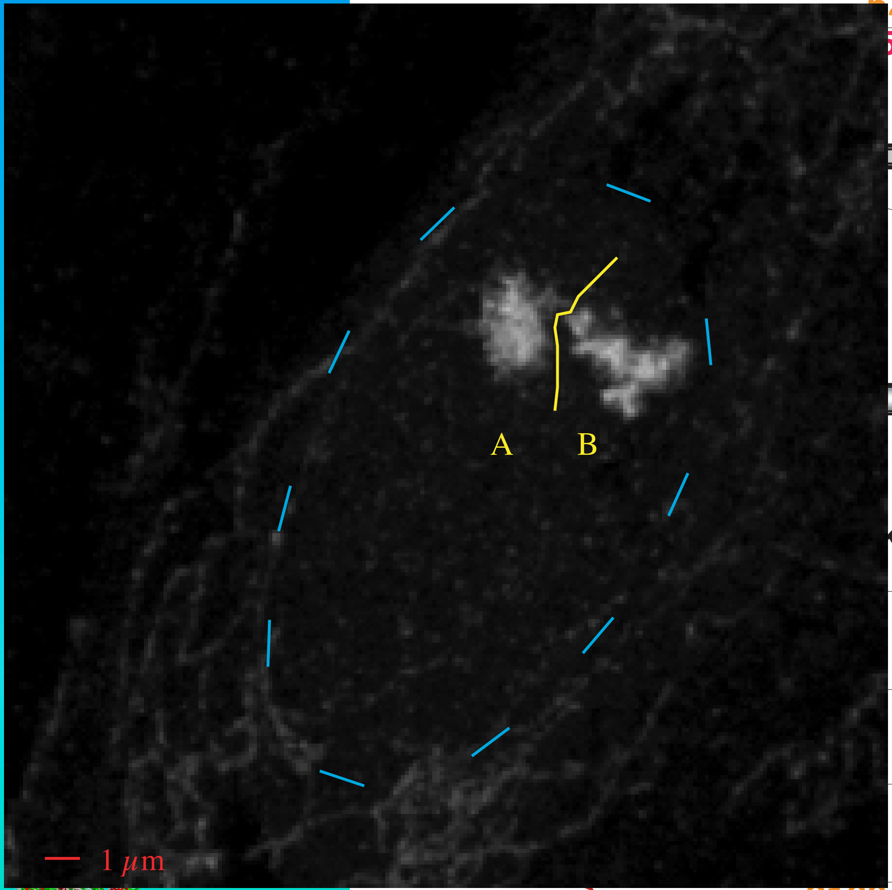
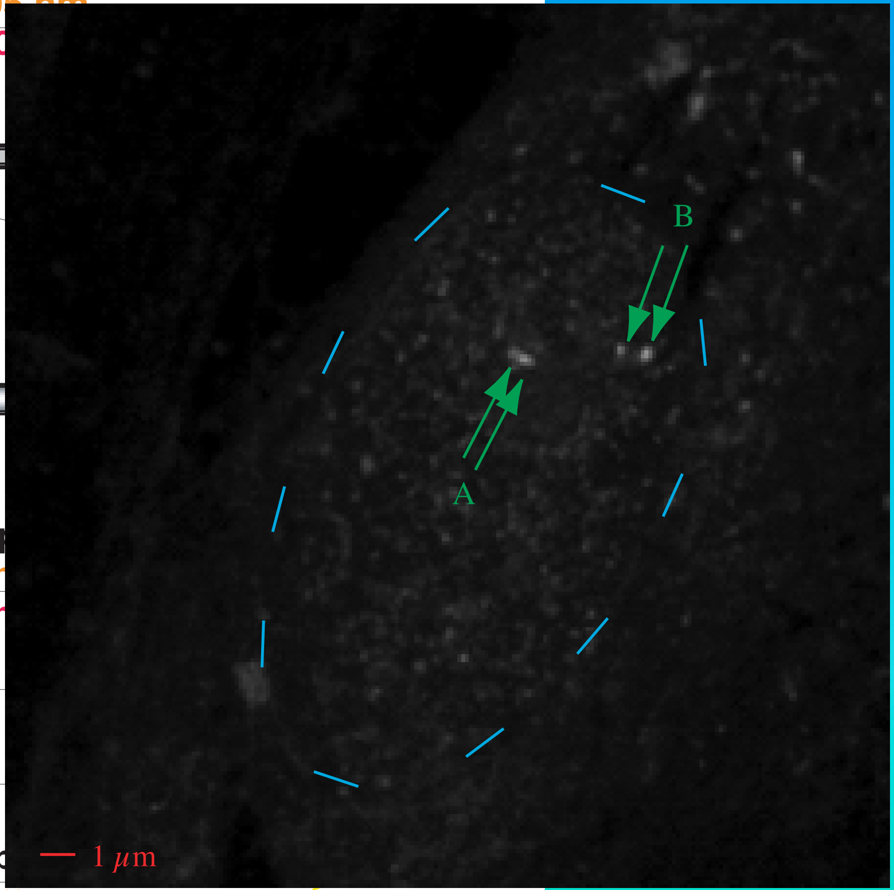




kbp

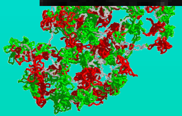
nm

nm

kb

2160 nm

480 nm



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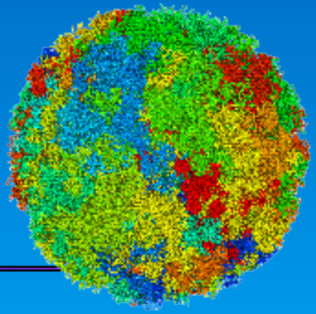
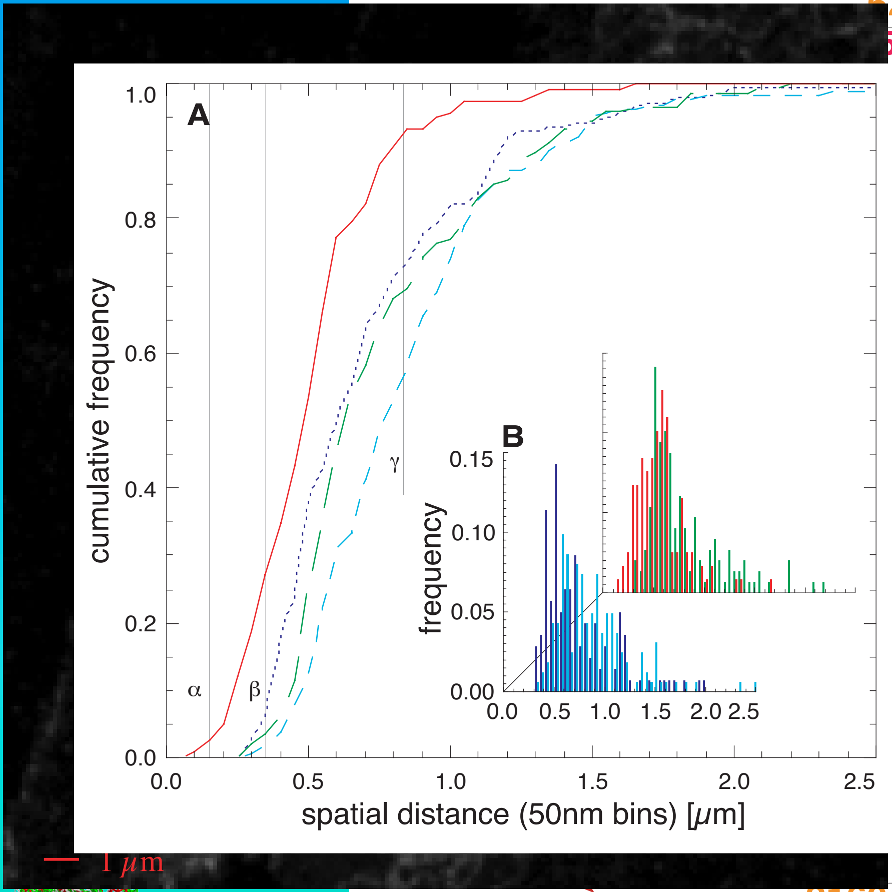
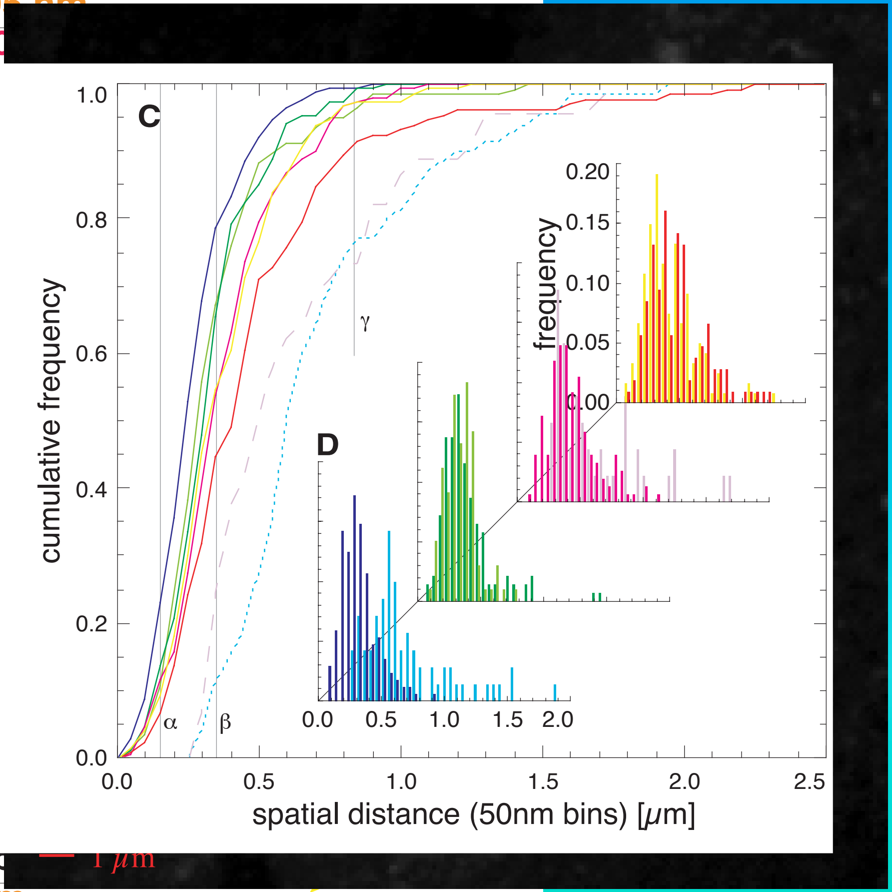


650 kbp

6205 nm

520

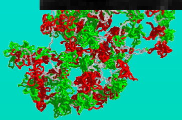




kb

2160 nm

480 nm



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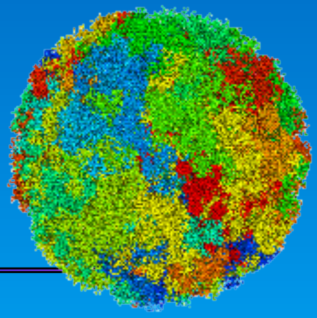

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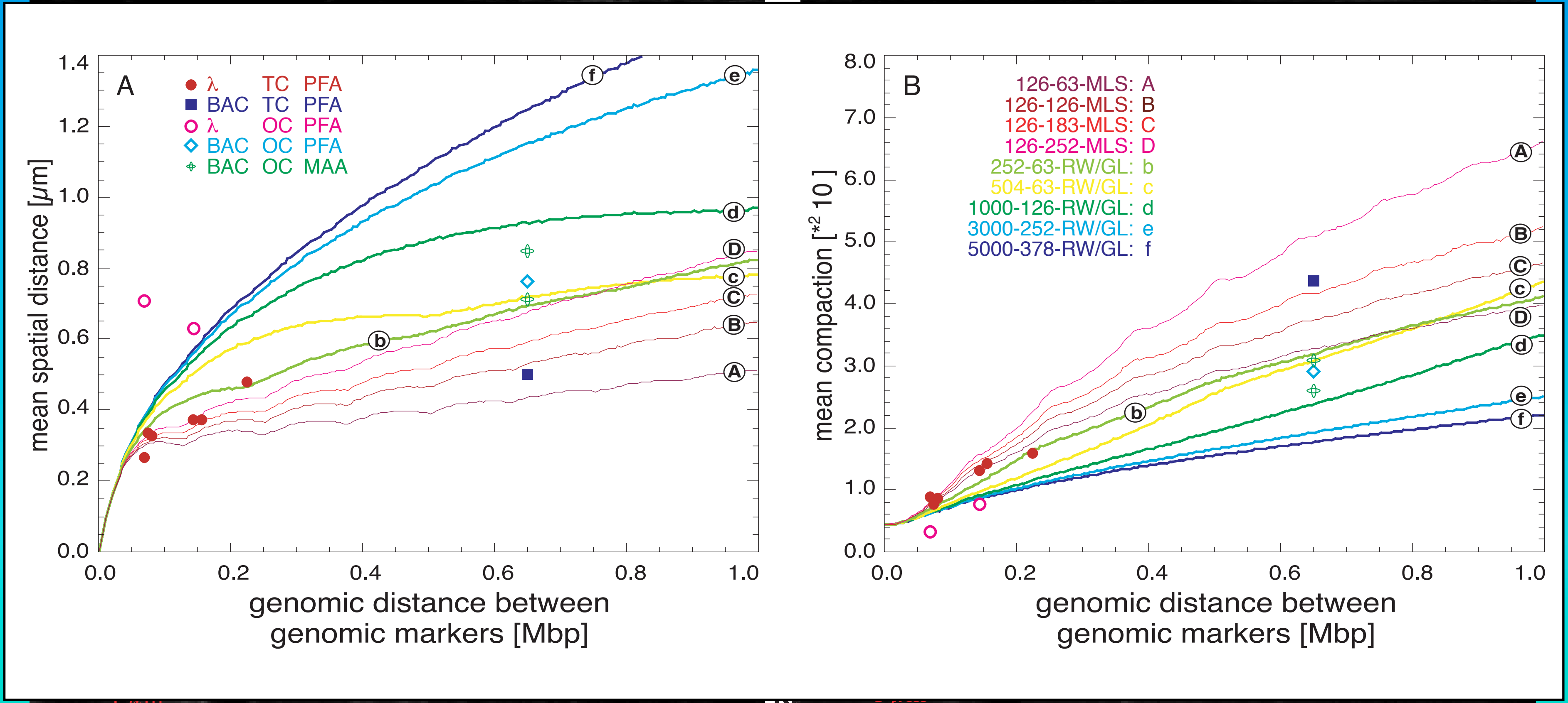

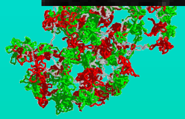

2160 nm

480 nm

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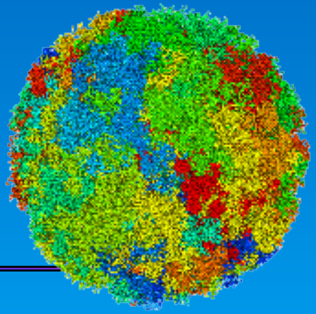
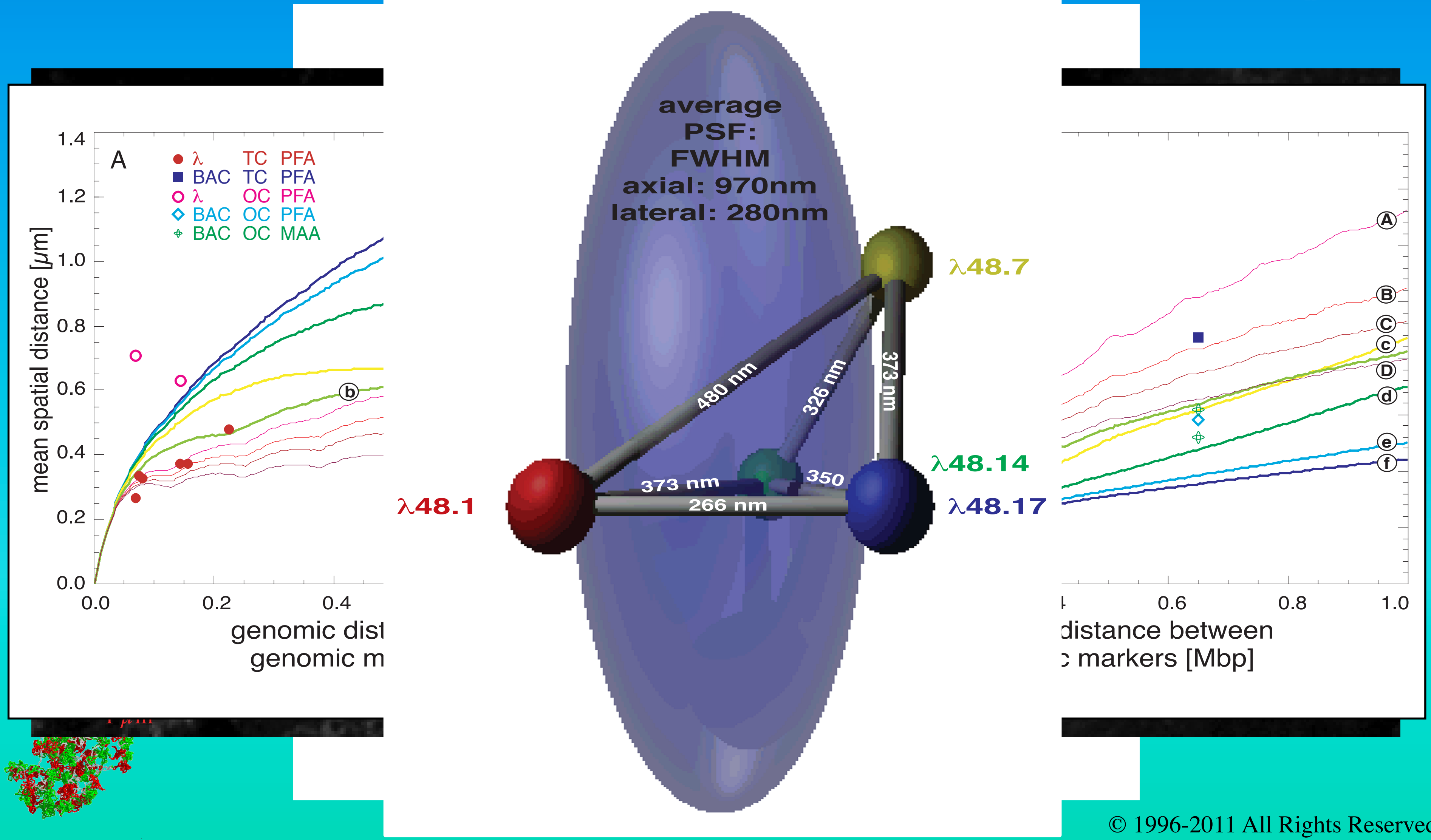
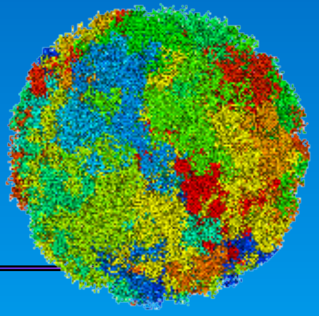
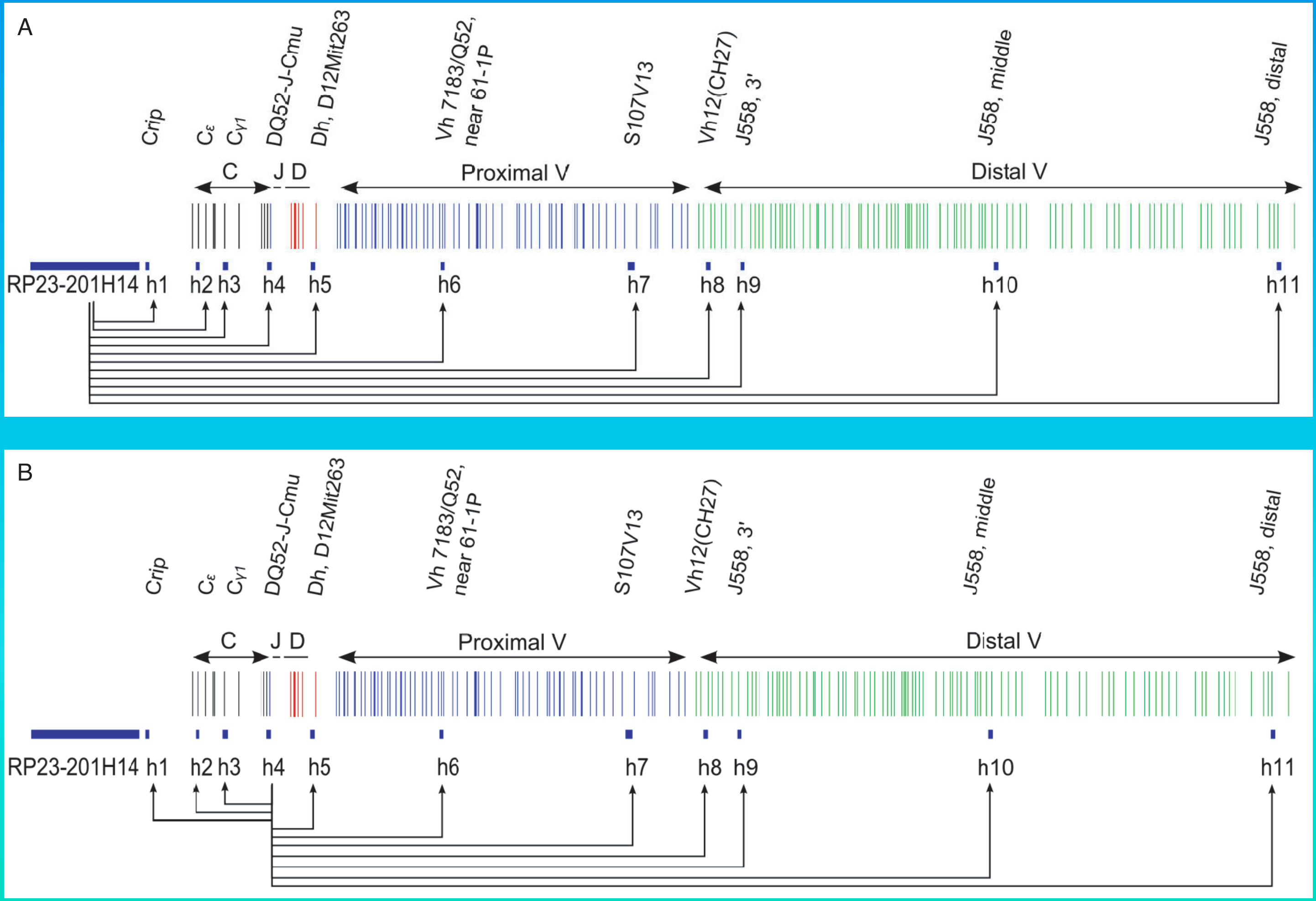


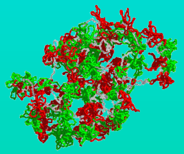


3D Archtiture & Function of the IgH Locus

Fluorescence *in situ* hybridization of the IgH locus combined with spectral precision distance epifluorescence microscopy, analytical trilateration and comparison with computer simulations shows again a Multi-Loop Subcompartiment organization of the IgH locus with functional relevant distances.







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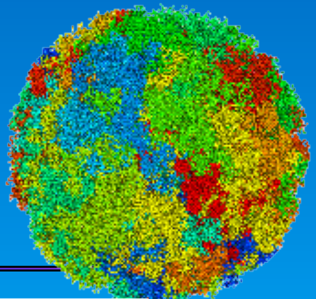

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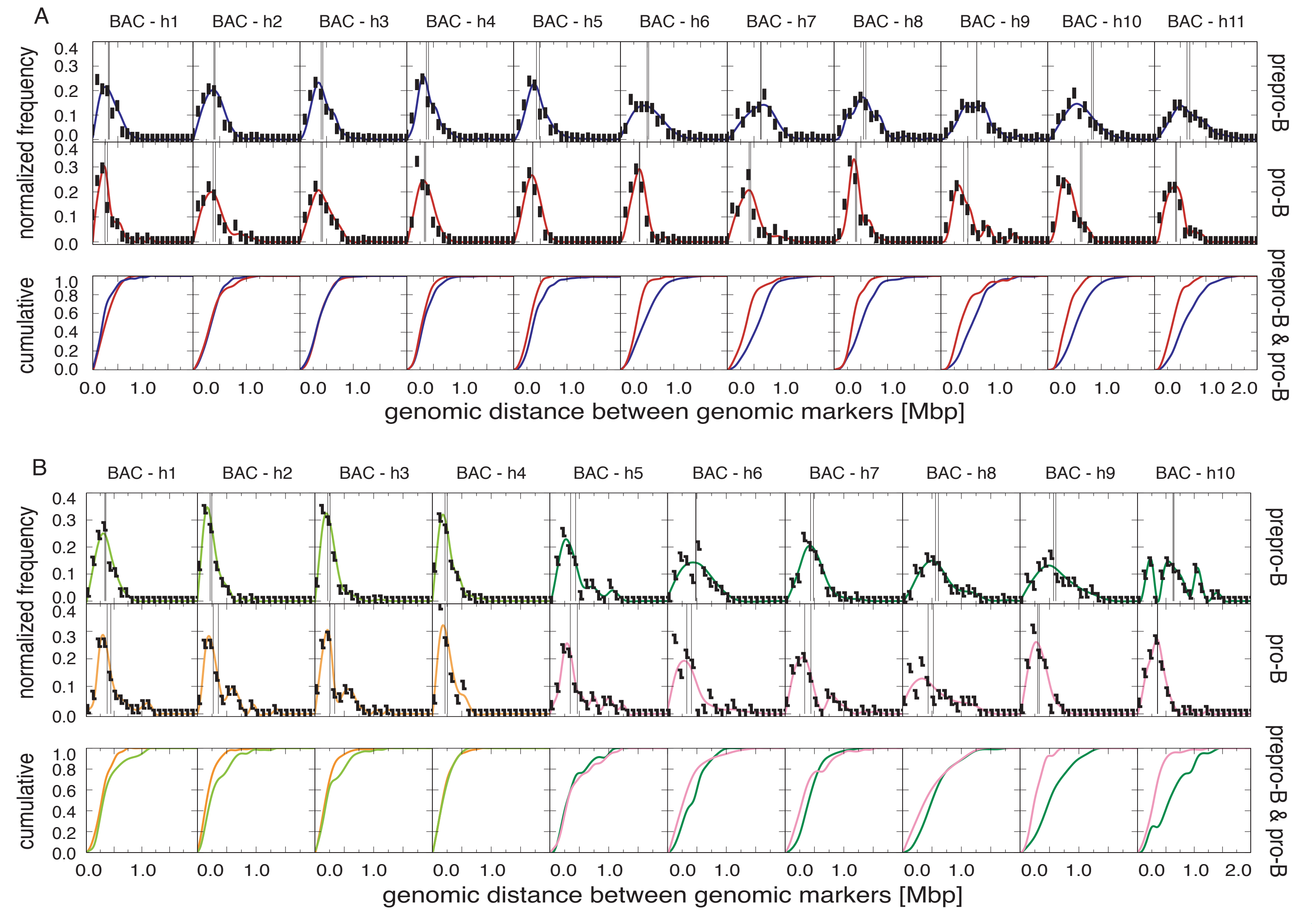

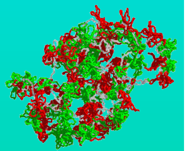

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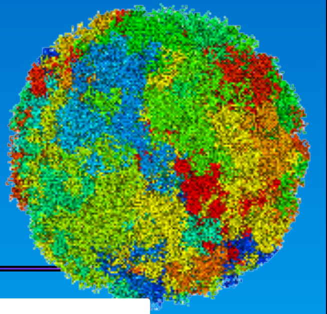

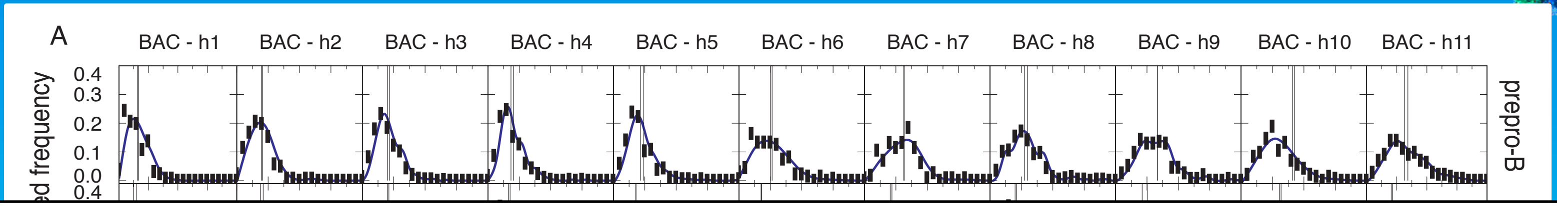

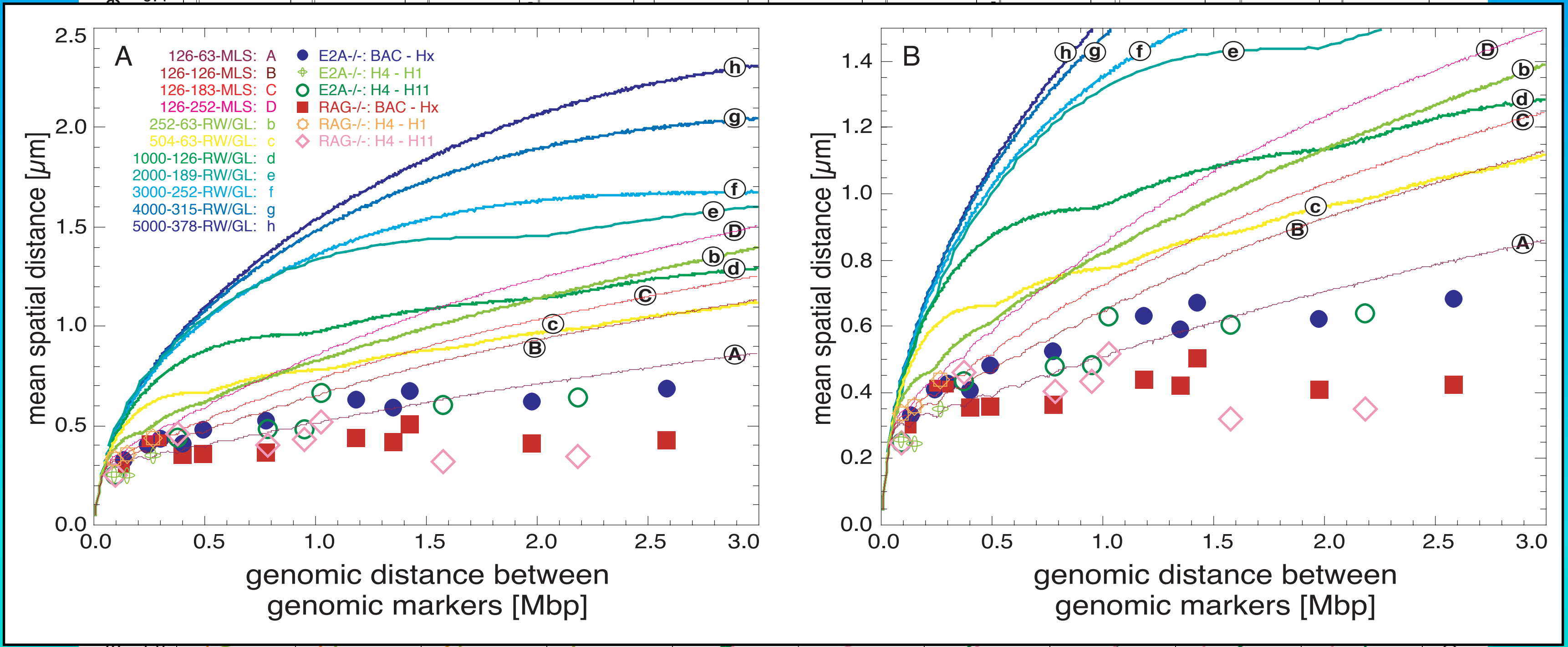

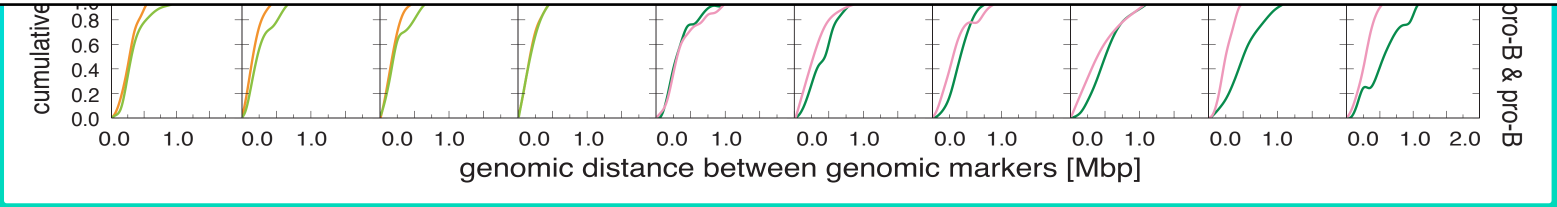

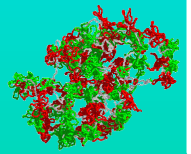

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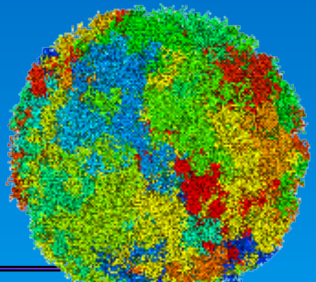

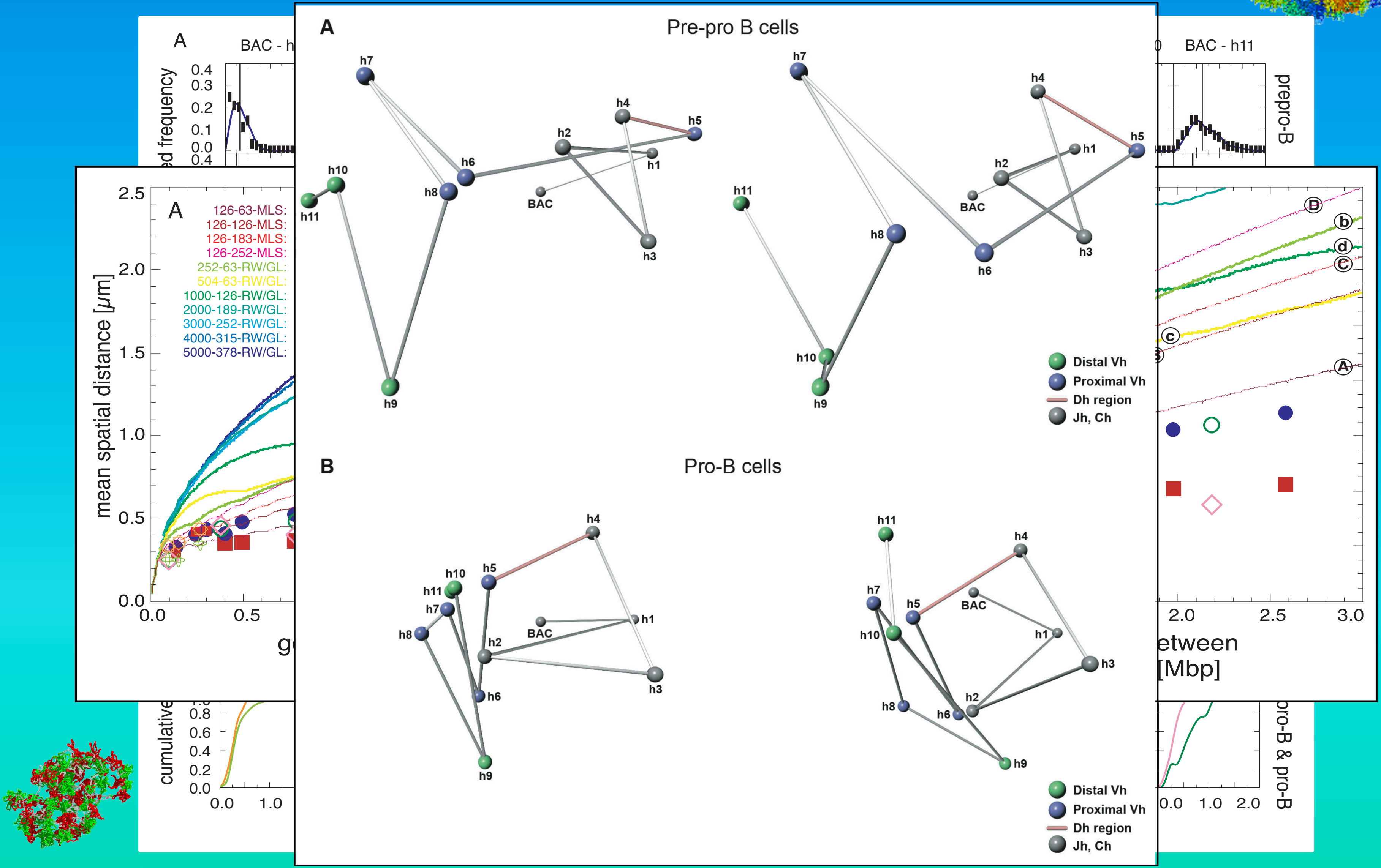

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“Synoptic” 3D Architecture of Various Loci

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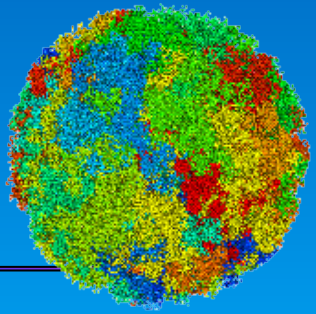
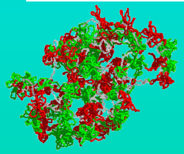


Study	Location	Preparation of Cells			FISH			Microscopy		Fit to model
		Cell cycle	KCl [nM]	Fixative	Melting	Label	Colours	# of nuclei	Image acquisition	
Fig. 3B, Trask ’89	DHFR	UA41 G1-cf	75 dropped	MAA 3:1	FM 50 %	Biotin	1	20-37	photo, wall	RWGL 0.08-J RWGL 1.0
Fig. 3B, Lawrence’90	Dystrophin	WI38F G1	75 dropped	MAA 3:1	FM 50 %	Biotin	1	20-60	photo, wall	RWGL 0.5-1
Fig. 3A, Trask ’91	Xq28	F G1-cf	75 dropped	MAA 3:1	FM 50 %	Biotin	1	30-60	photo, wall	RWGL 0.7 J RWGL 2.0->5.0
Fig. 3B, Trask ’91	Xq28	F G1-cf	75 dropped	MAA 3:1	FM 50 %	Biotin Dig	2	30-60	photo, wall	RWGL 1.0-3.0
Fig. 3, v.d. Engh ’92 or Fig. 5A, Trask ’93	4p16.3	F G1-cf	75 dropped	MAA 3:1	FM 50 %	Biotin Dig	2	?	photo, d-board	L_S <=0.1 for GS < 0.5 < RWGL >5.0
Fig. 5B, Trask ’93	6p21	F G1-cf	75	MAA 3:1	FM 50-70 %	Biotin Dig	2	?	photo, d-board	L_S <=0.1 for GS < 1.0 < RWGL 1.0-5.0
Fig. 5, Senger ’93	MHC 6p21.31	HFF G1-cf	?	?	FM 50 %	Biotin	1	> 30	photo, wall	MLS L_S&LI_S= 0.12-0.25 RWGL 0.1-0.5
Fig. 5, Senger ’93	MHC 6p21.31	HFF G1-cf	?	?	FM 50 %	Biotin Dig	2	> 30	photo, wall	MLS L_S=0.1 LI_S=0.18 RWGL 0.1-0.5
Fig. 1, Warrington ’94	4p16.3	F G1-cf	75	MAA 3:1	FM 50 %	Biotin Dig	2	?	?	RWGL > 5.0
Tab. 1, Warrington ’94	5q31-33	L	?	?	?	?	?	?	CLSM BioRad	RWGL > 5.0
Fig. 2B, Yokota ’95	4p16.3	F G1-cf	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	40-360	photo, d-board	RWGL 2.0-4.0
Fig. 3B, Yokota ’95	4p16.3	F G1-cf	-	PFA 4 %	FM 70 %	Biotin Dig	2	40-350	photo, d-board	MLS L_S&LI_S= 0.1-0.125

Study	Location	Preparation of Cells			FISH			Microscopy		Fit to model
		Cell cycle	KCl [nM]	Fixative	Melting	Label	Colours	# of nuclei	Image aquisition	
Fig. 2A, Yokota ’97	4p16.3 R-band	F G1-cf	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 2.0-3.0
Fig. 2B, Yokota ’97	6p21.3 R-band	F G1-o	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 4.0-5.0
Fig. 2C, Yokota ’97	21q22.2 G-band	F G1-o	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 1.0-2.0
Fig. 2D, Yokota ’97	Xp21.3 G-band	F G1-o	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 0.5-0.9
Fig. 2D, Yokota ’97	Xq28 R-band	F G1-of	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 1.0-5.0j
Fig. 2A, Yokota ’97	Xp21.3 G-band	F	-	PFA 4 %	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 0.25 MLS L_S=0.126 LI_S=0.200
Fig. 2A, Yokota ’97	Xq28 R-band	F	-	PFA 4 %	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 1.0
Fig. 4B, Yokota ’97	Xp21.3 G-band	HeLa	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 0.25 MLS L_S=0.126 LI_S=0.200
Fig. 4B, Yokota ’97	Xq28 R-band	HeLa	40 dropped	MAA 3:1	FM 70 %	Biotin Dig	2	37-178	photo, d-board	RWGL 1.0
Monier ’97	11q13	F	-	PFA 4%	FM 70 %	Biotin Dig	1	22-69	CLSM	MLS L_S=0.126 LI_S=180
Monier ’97	11q13	L	-	PFA 4%	FM 70 %	Biotin Dig	1	22-69	CLSM	MLS L_S=0.1 LI_S=0.18-0.24
Knoch ’98/ Rauch ’99	15q11-21	F	-	PFA 4%	FM 70 %	Biotin Dig	1 & 2	60-120	CLSM	MLS L_S=0.1 LI_S=0.06-0.125



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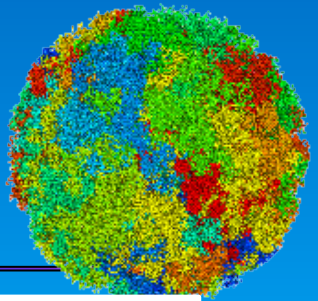
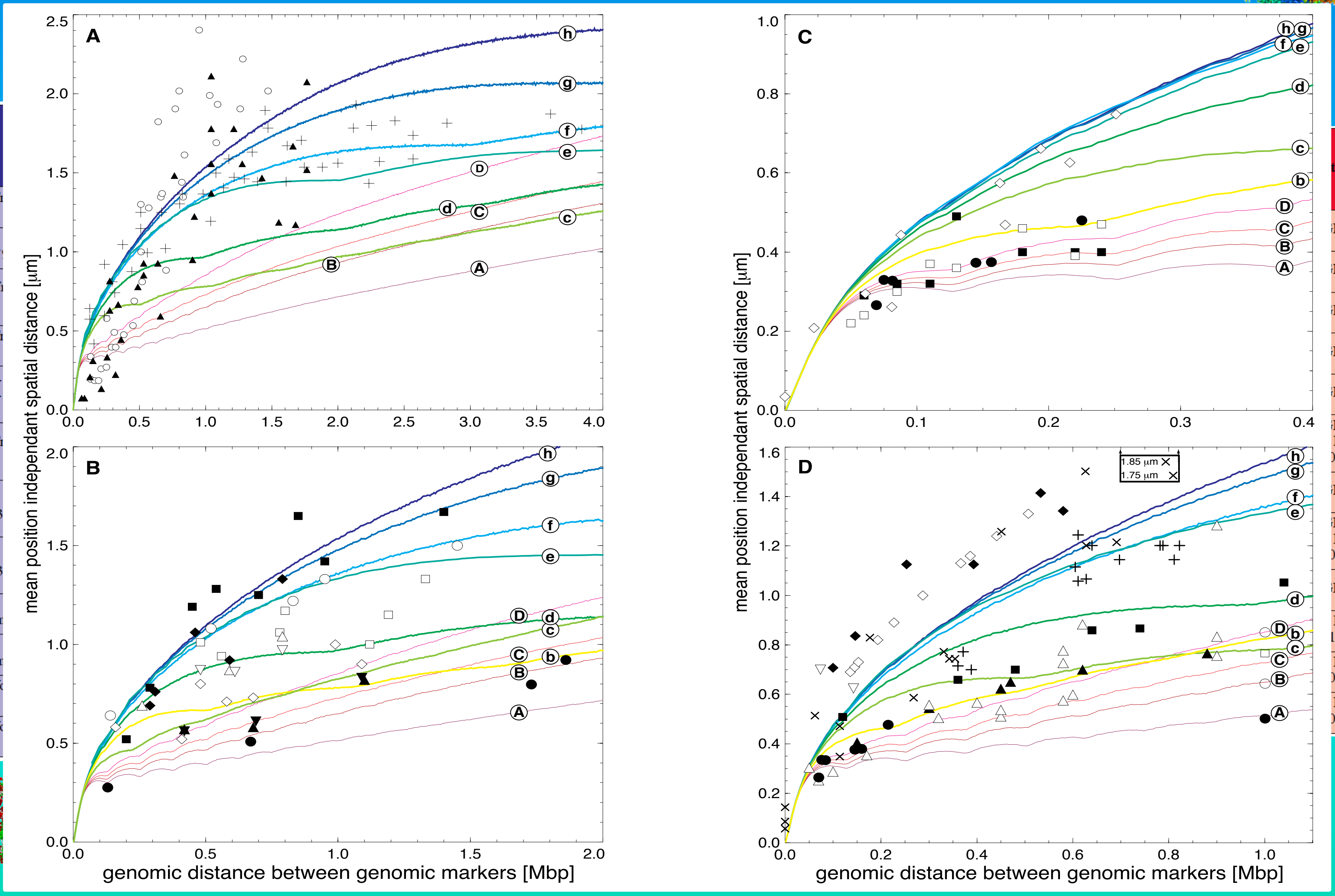
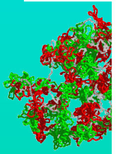


Study
Fig. 3B, Tr ’89
Fig. 3B, Lawrence’
Fig. 3A, Tr ’91
Fig. 3B, Tr ’91
Fig. 3, v.d. ’92 or Fig. Trask ’93
Fig. 5B, Tr ’93
Fig. 5, Senger ’93
Fig. 5, Senger ’93
Fig. 1, Warrington
Tab. 1, Warrington
Fig. 2B, Yo ’95
Fig. 3B, Yo ’95



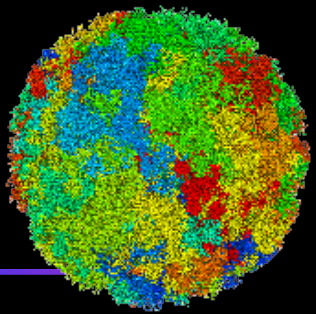
to model
L 2.0-3.0
L 4.0-5.0
L 1.0-2.0
L 0.5-0.9
L 1.0-5.0j
L 0.25 L_S=0.126 .200
L 1.0
L 0.25 L_S=0.126 .200
L 1.0
L_S=0.126 180
L_S=0.1 .18-0.24
L_S=0.1 .06-0.125

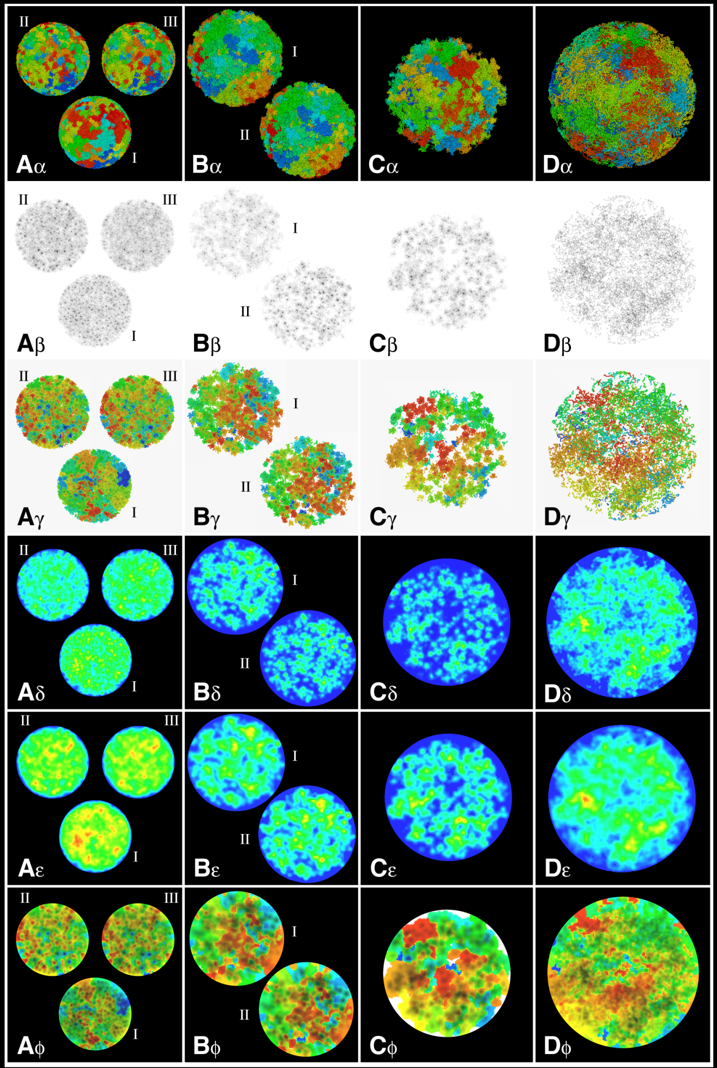


From Fiber Topology to Nuclear Morphology

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

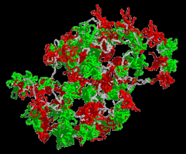
confocal microscopy
territory painting

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

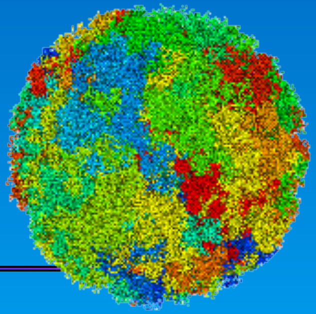
B: MLS in 8 μm nucleus
- I: 126 kbp loops, 126 kbp linkers
- II: 84 kbp loops, 126 kbp linkers

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

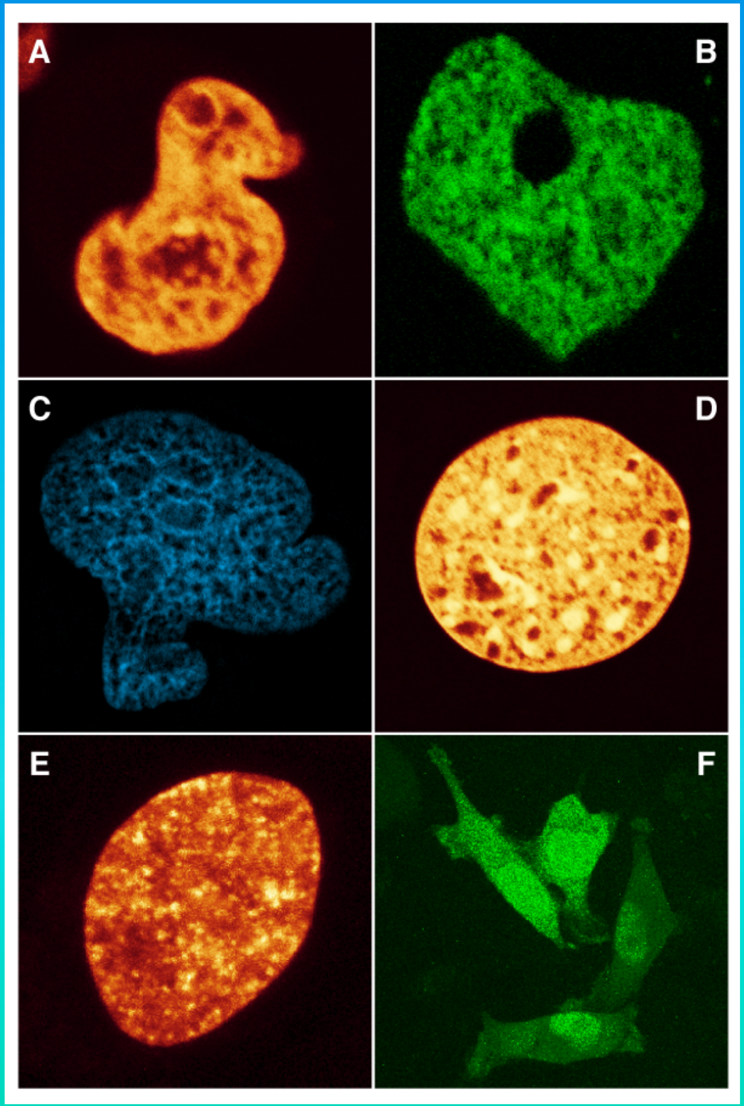


In vivo Morphology & Chromatin Distribution



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

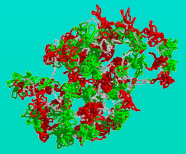
LCLC 103H, H2A-CFP

ID13, H2A-YFP

HeLa, mH2A1.2-YFP

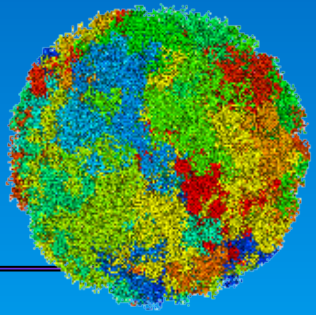
HeLa, H2A-YFP,

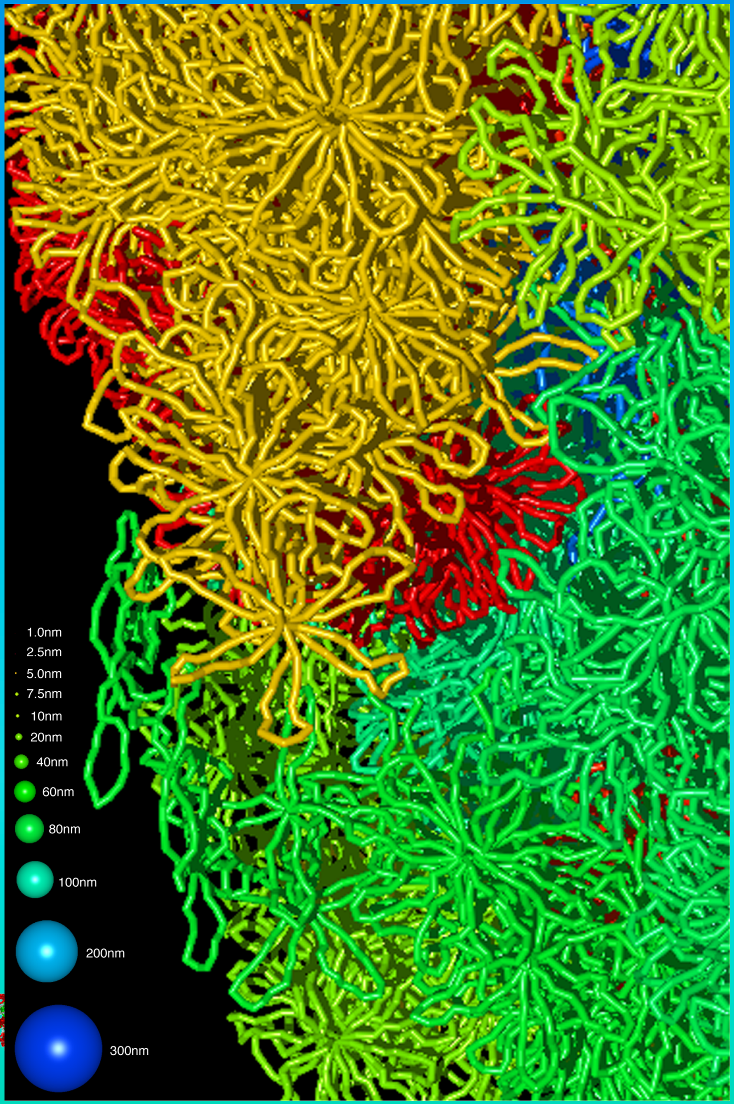
natural promoter

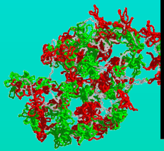


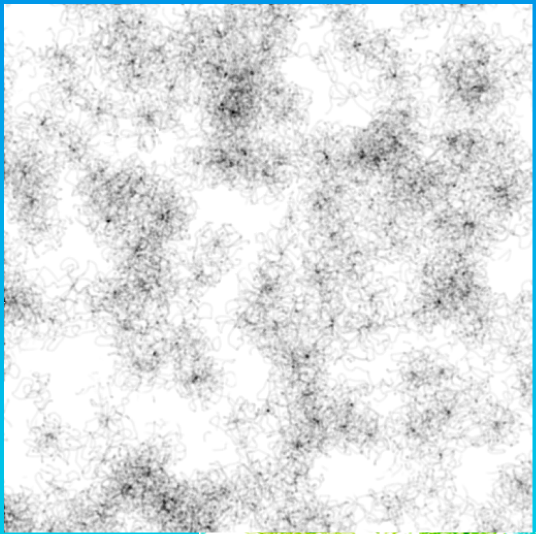
Fine Morphology of Nuclei

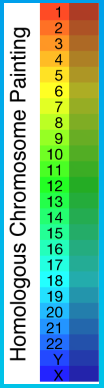
High resolution rendering and simulated electron microscopy including territory painting reveal not only again the model details but also that any location in the nucleus is accessible to biological molecules <15 nm in diameter and that even the Extended Interchromosomal Domain hypothesis is oversimplified.



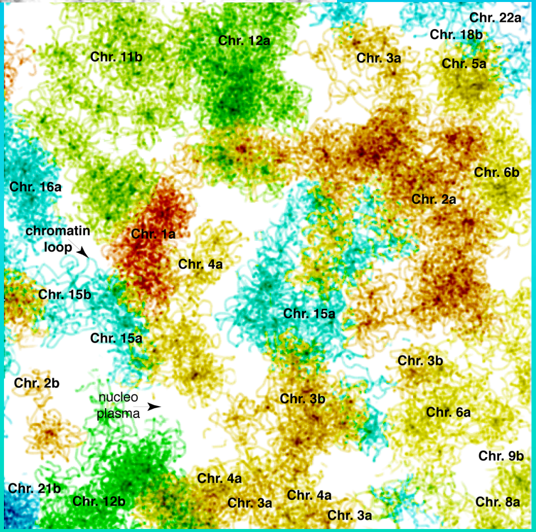






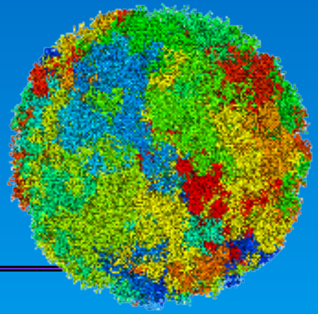


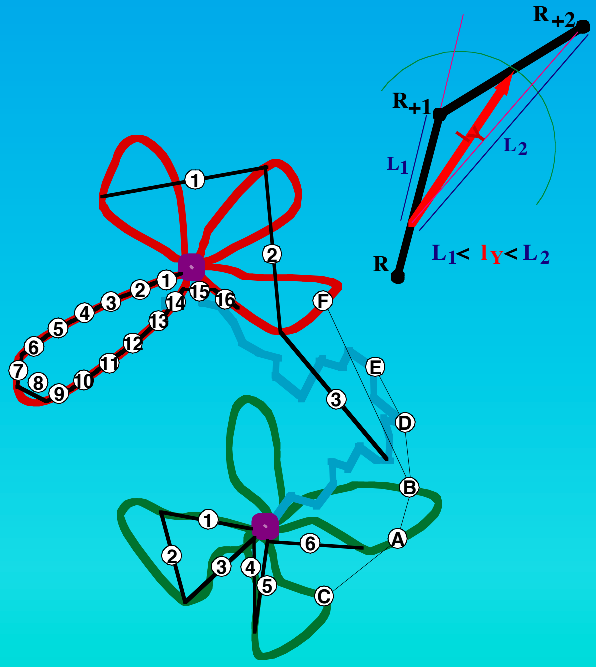
MLS models model with 126 kbp loops and linkers in a 10 μm nucleus.

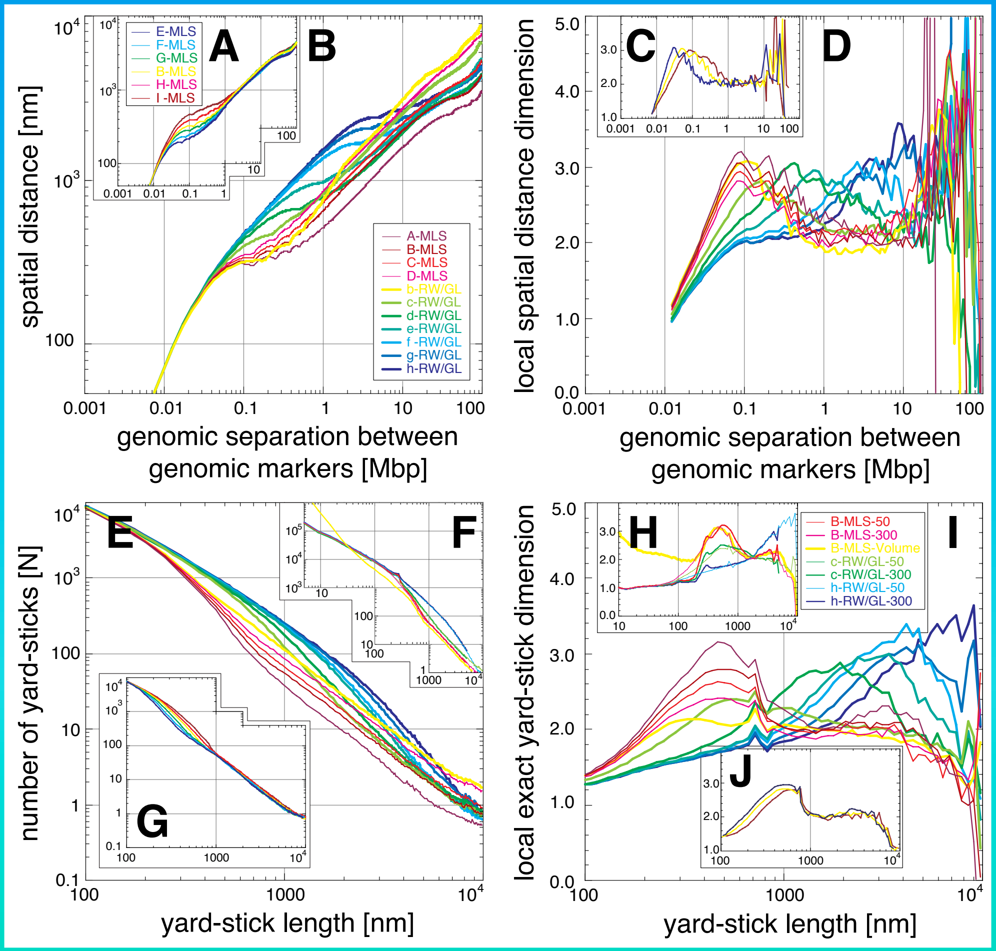


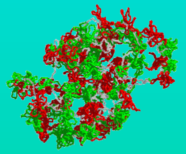
Scaling of the Chromatin Fiber Topology

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 froming 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.

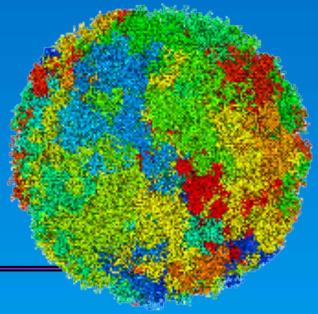






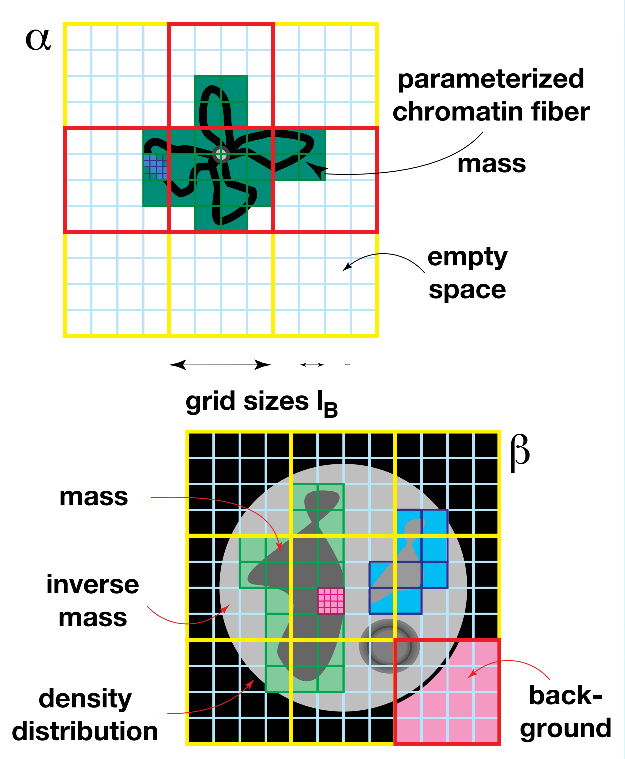


Scaling of the Chromatin Morphology & Distribution



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.



α

parameterized chromatin fiber

mass

empty space

grid sizes l_B

β

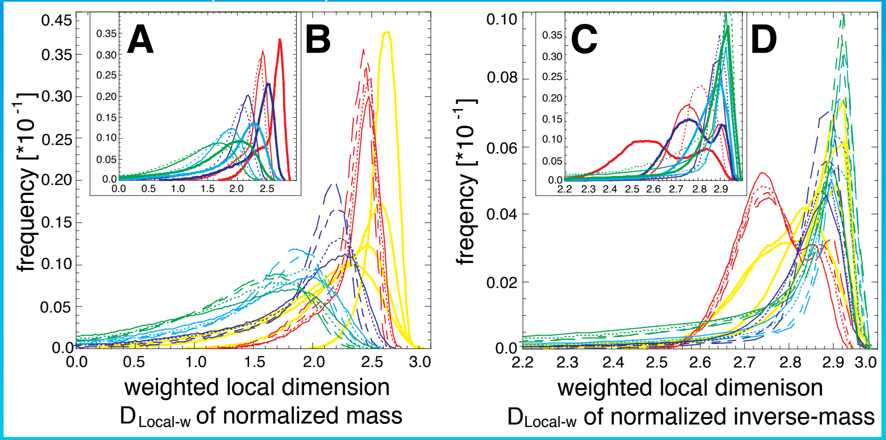
mass

inverse mass

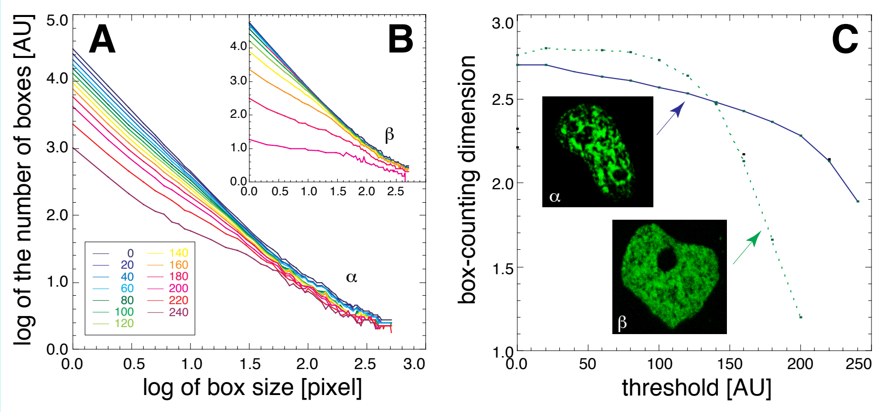
density distribution

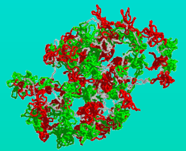
back-ground

(inverse-) mass dimension distribution

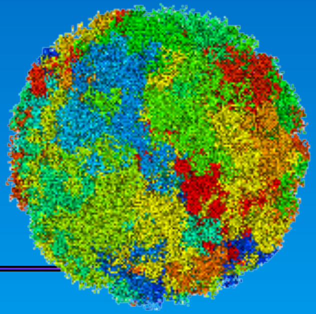


mass dimension as function of image intensity

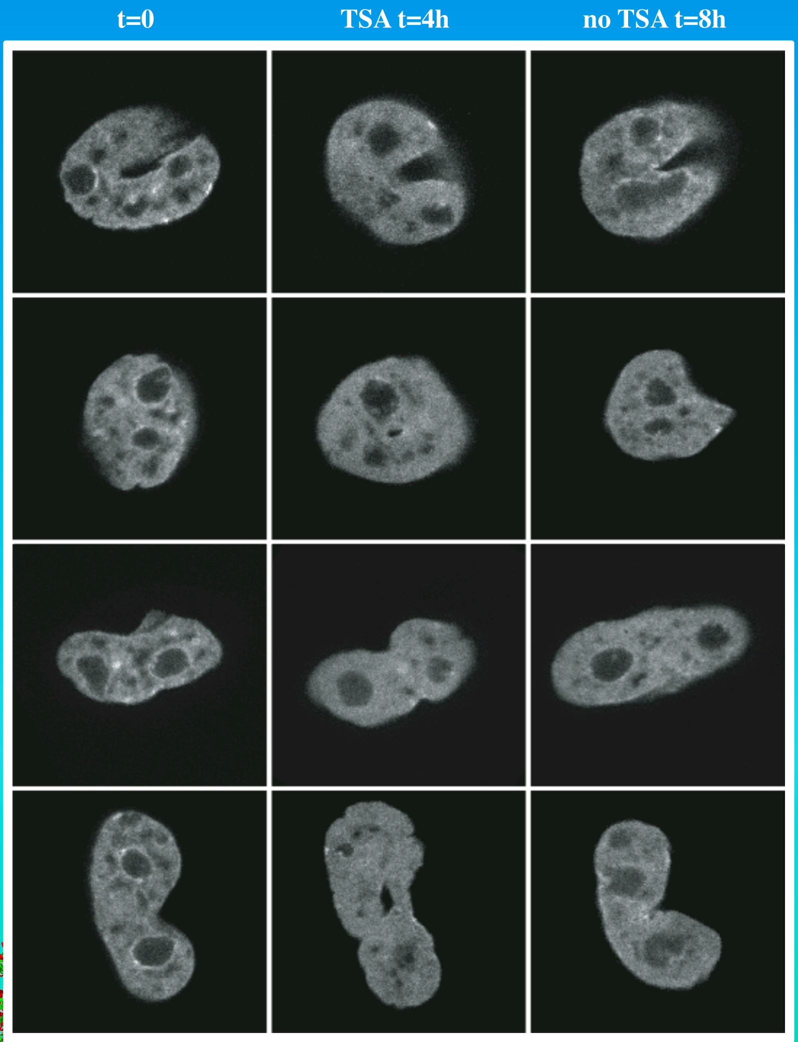


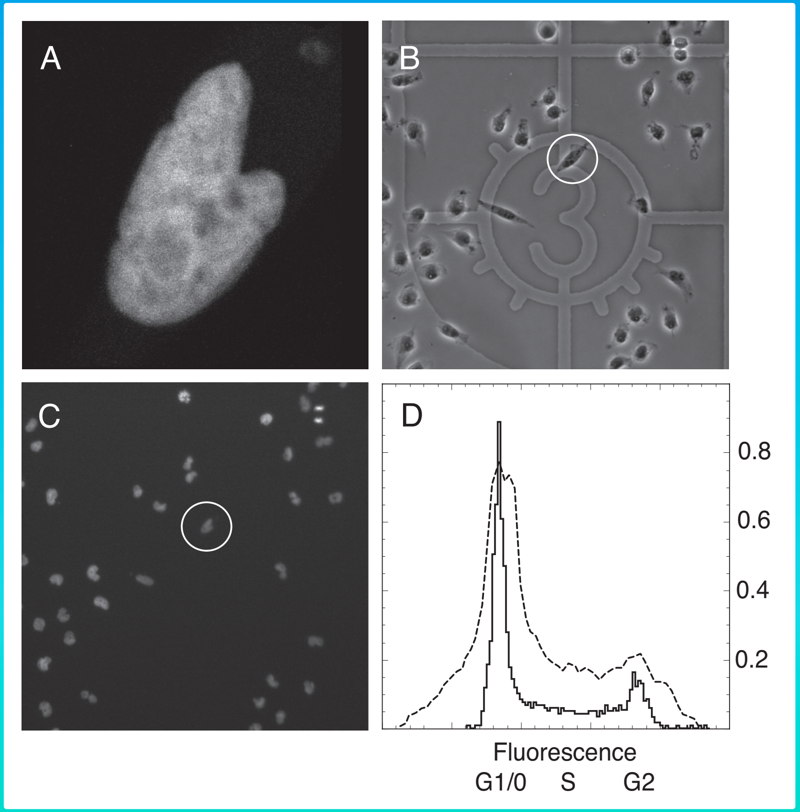


Quantified TSA induced Morpholoy Changes



Trichostatin A induced histone acetylation can be quantified by *in vivo* H2A-GFP confocal images and image correlation spectroscopy (iFCS), which is a scaling analysis, and reveals the opening of chromatin, and thus reorganization changes on scales from 0.2 to ~1µm, consistent with MLS models.





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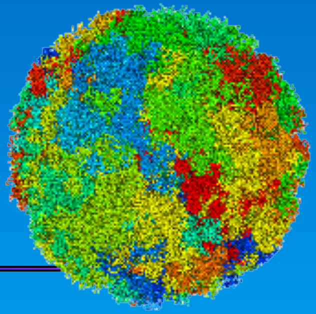
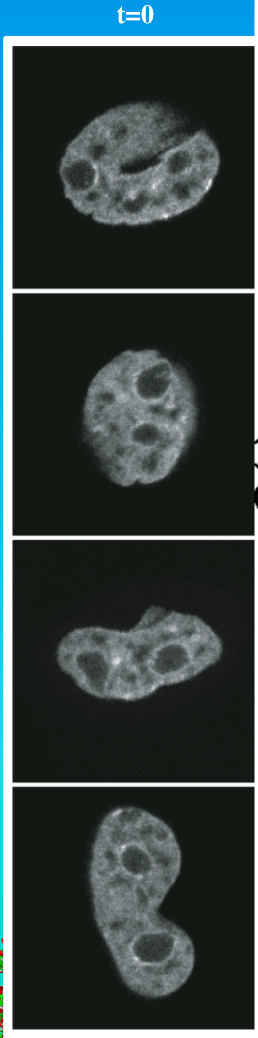
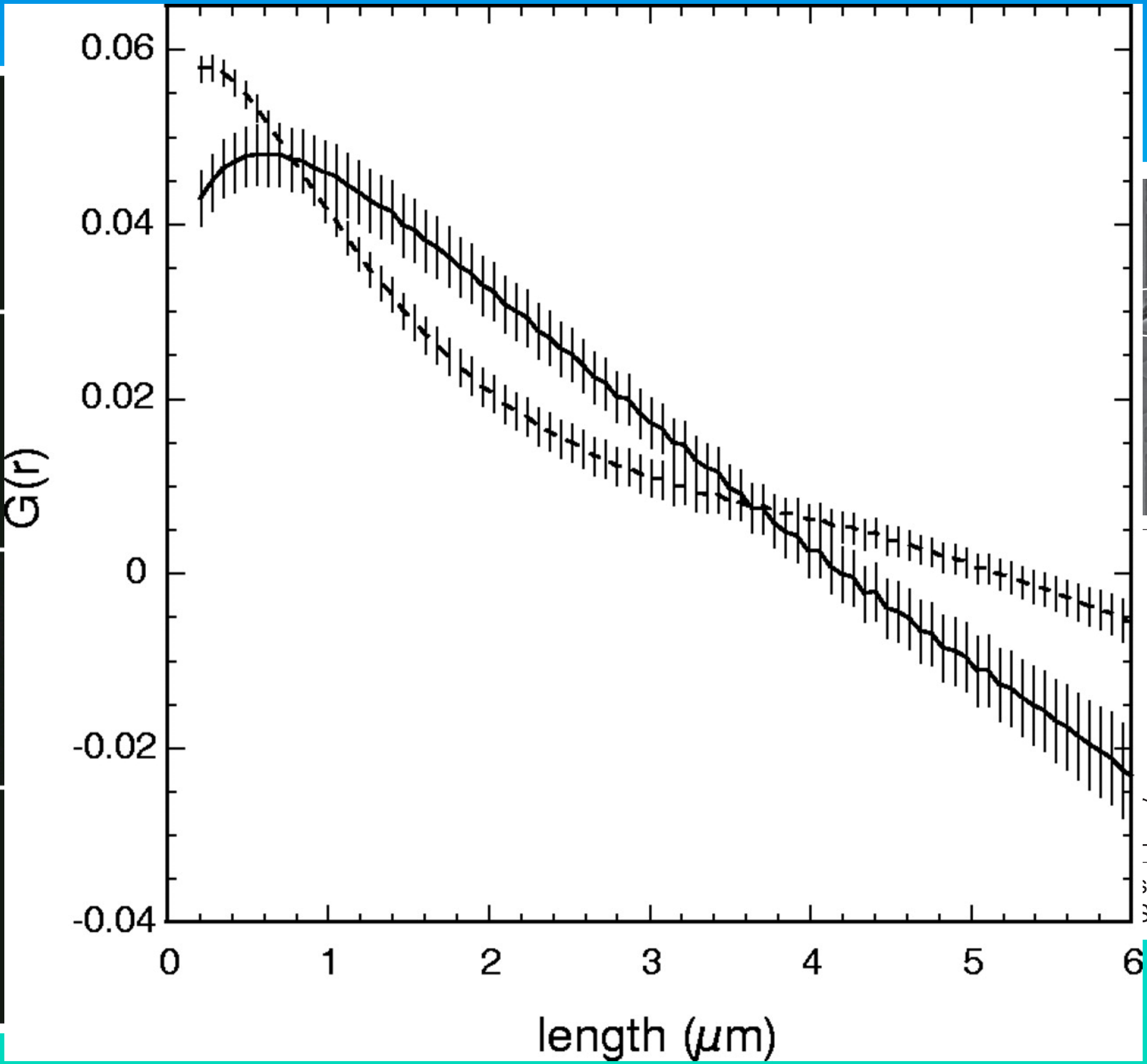
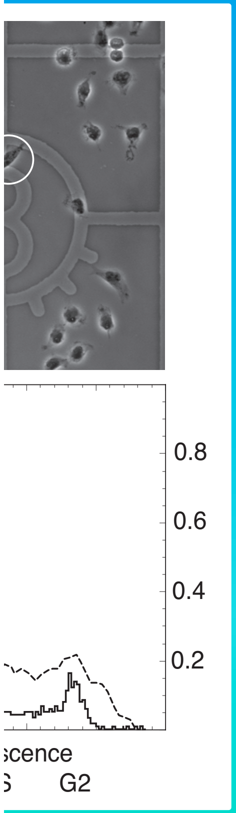
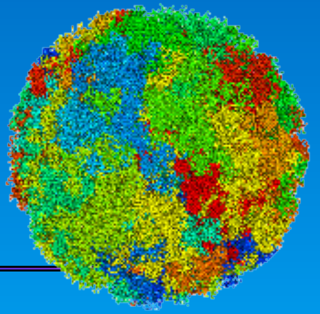




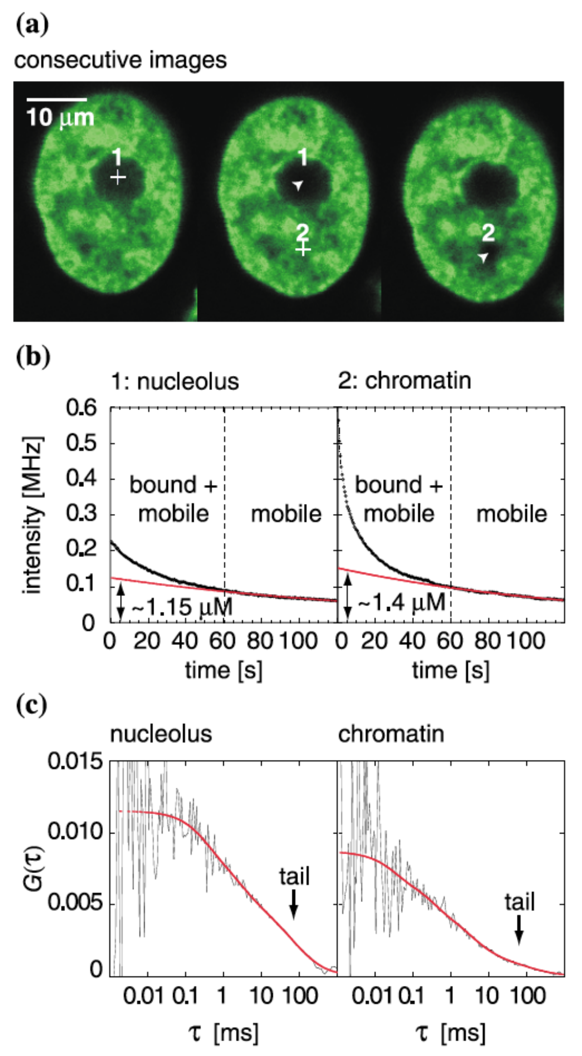


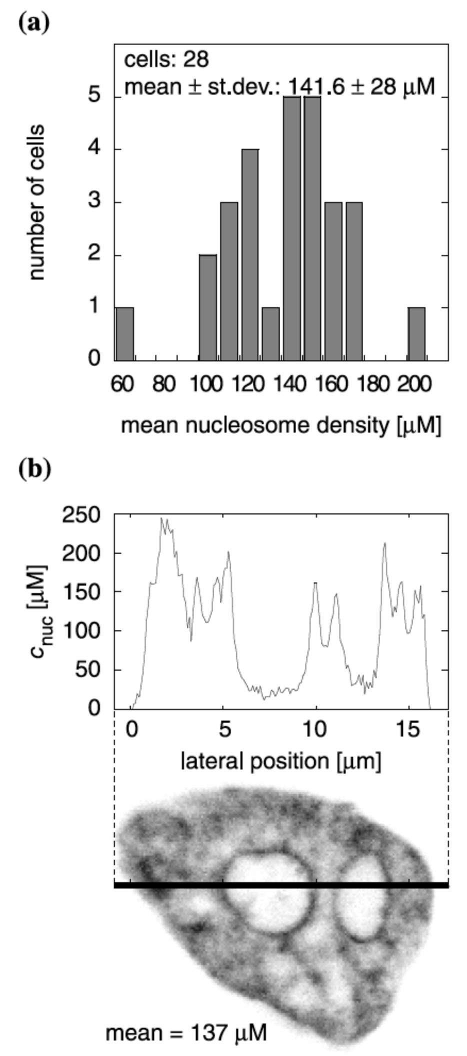


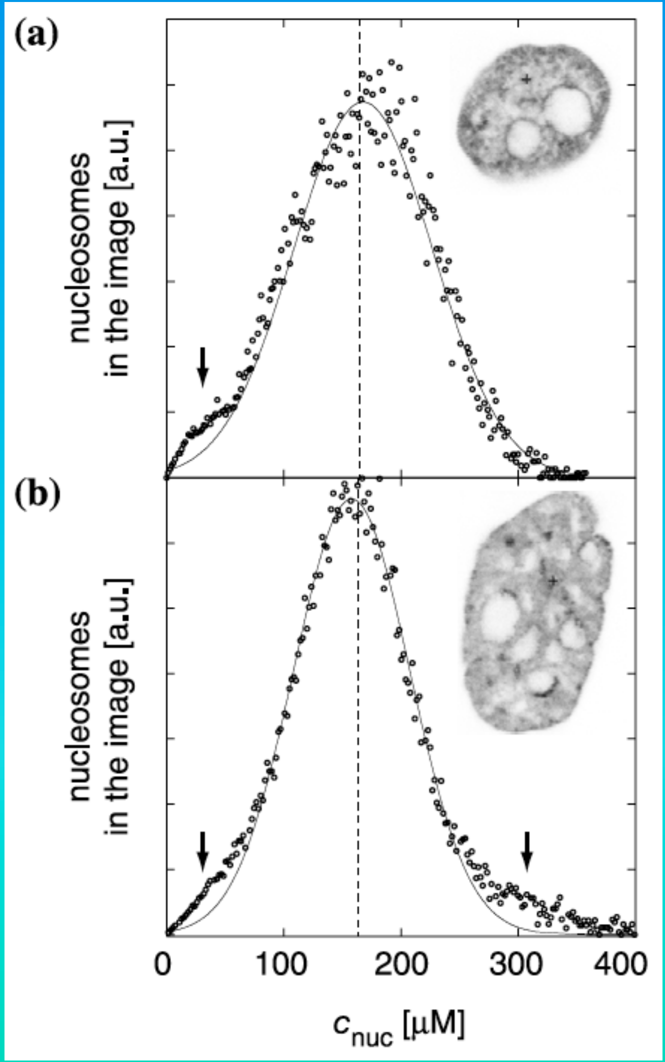
Counting Nucleosomes *In Vivo*



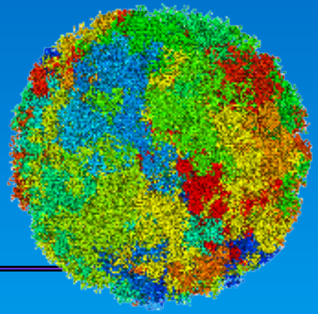
Counting nucleosomes in living cells with a combination of fluorescence correlation spectroscopy and confocal laser scanning imaging reveals not only the free unbound histone component but also the concentration in absolute numbers of bound histones. Thus, the absolute concentration distribution of histones can be determined and reveals again the typical expected distribution of aggregated chromatin loops.







Diffusion of Particles in the Nucleus

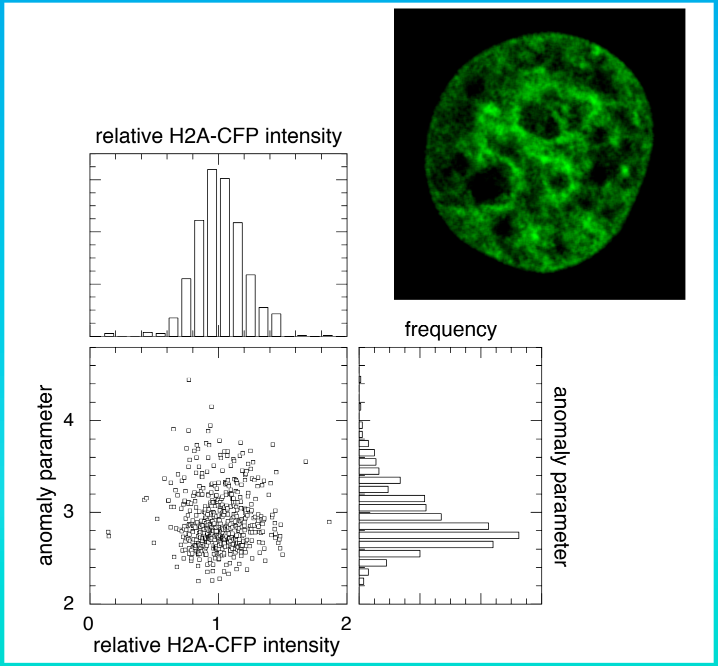


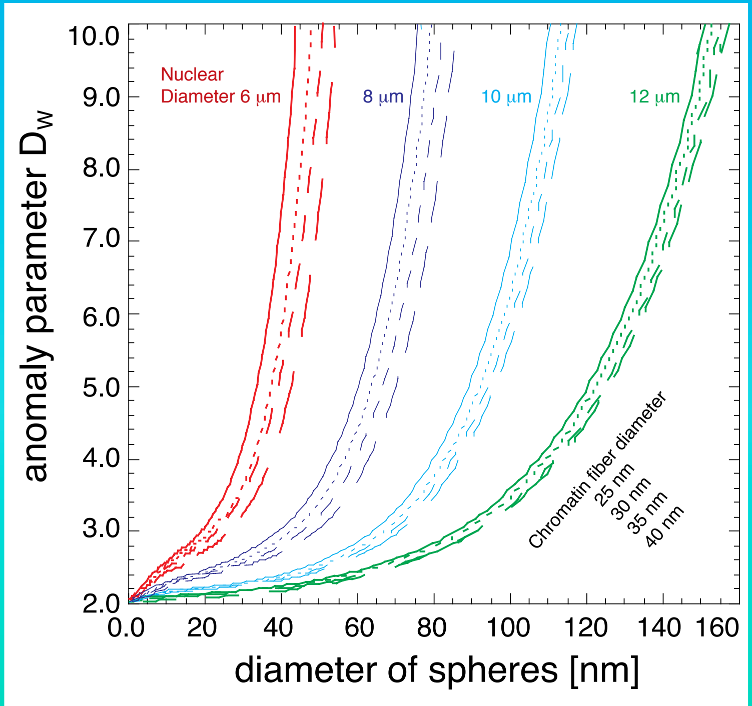
Due to the volume and spatial relation ships in the nucleus typical particles reach almost any location in the nucleus by moderately obstructed diffusion: a 10 nm particle moves 1 to 2 μm within 10 ms.

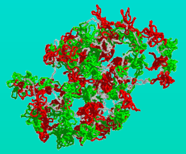
The structural influence on the obstruction degree is random for Alexa 568 as function of the chromatin distribution visualized by H2A CFP in vivo and measured by fluorescence correlation spectroscopy (FCS)

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

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

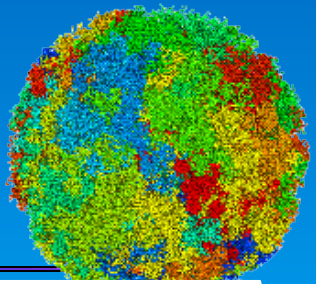


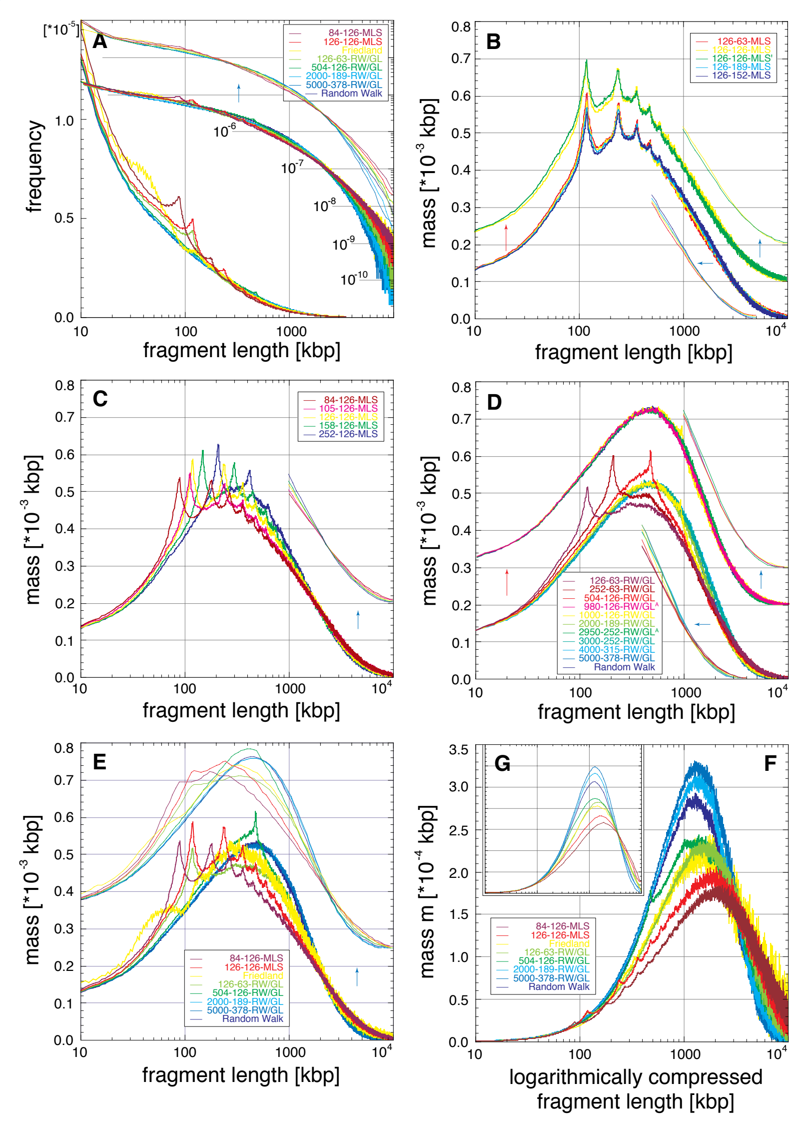


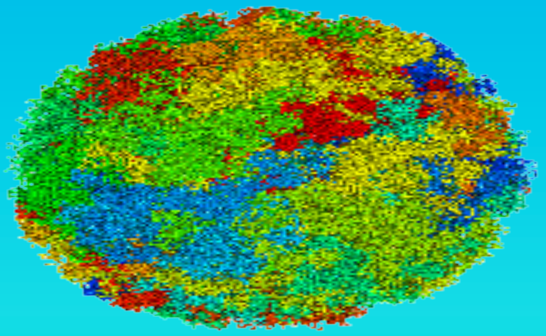


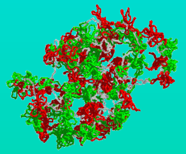
DNA Fragment Distribution after Ione-Irradiation

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.









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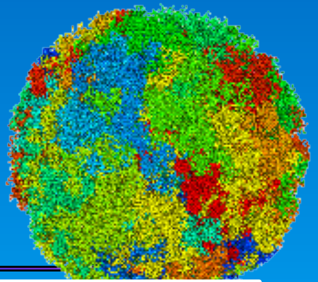

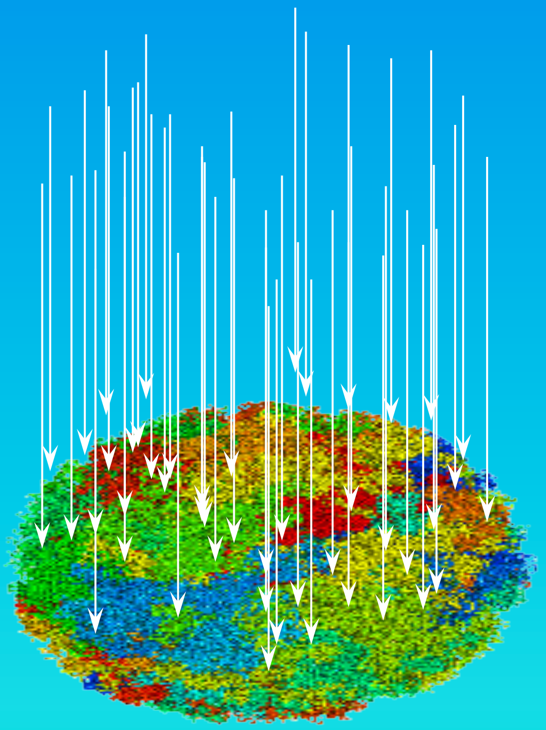

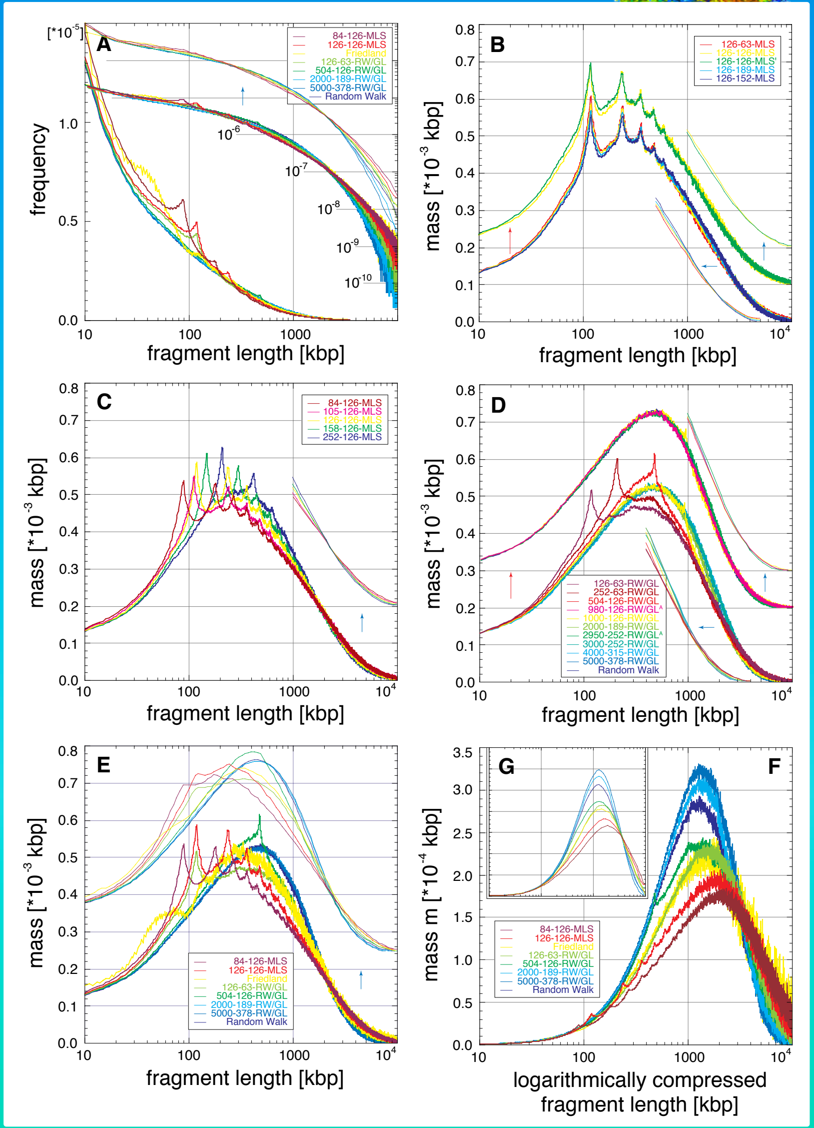

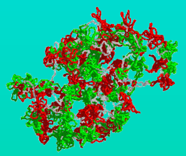

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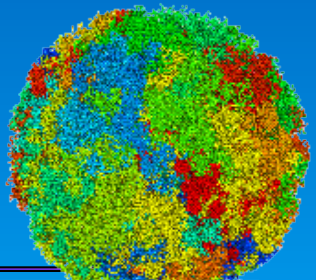

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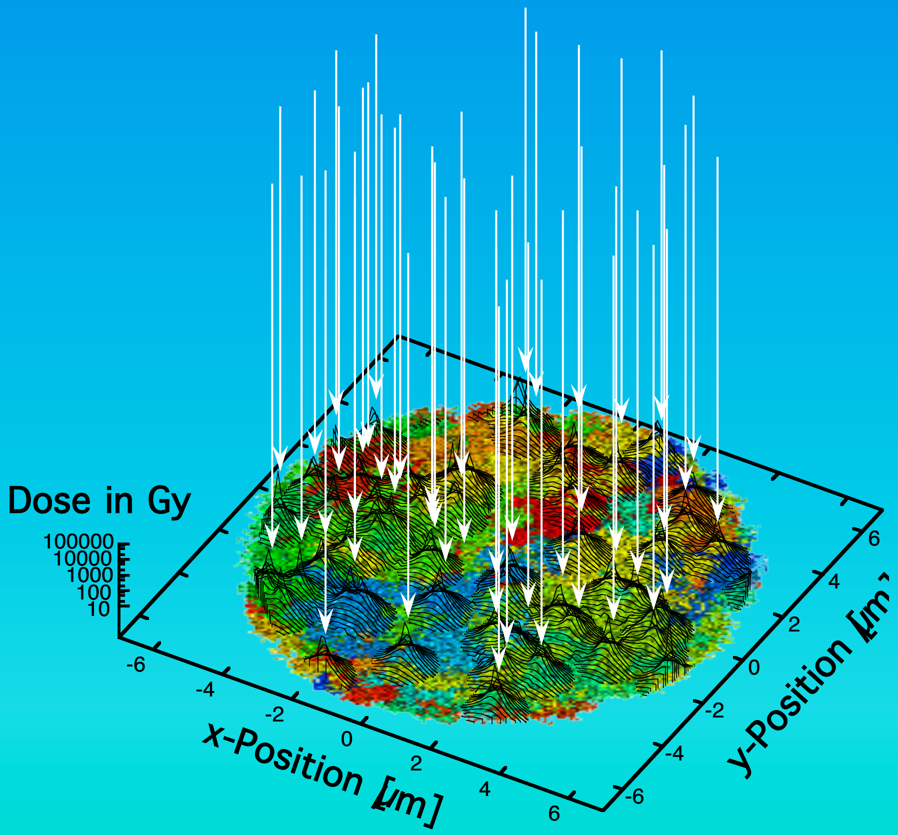
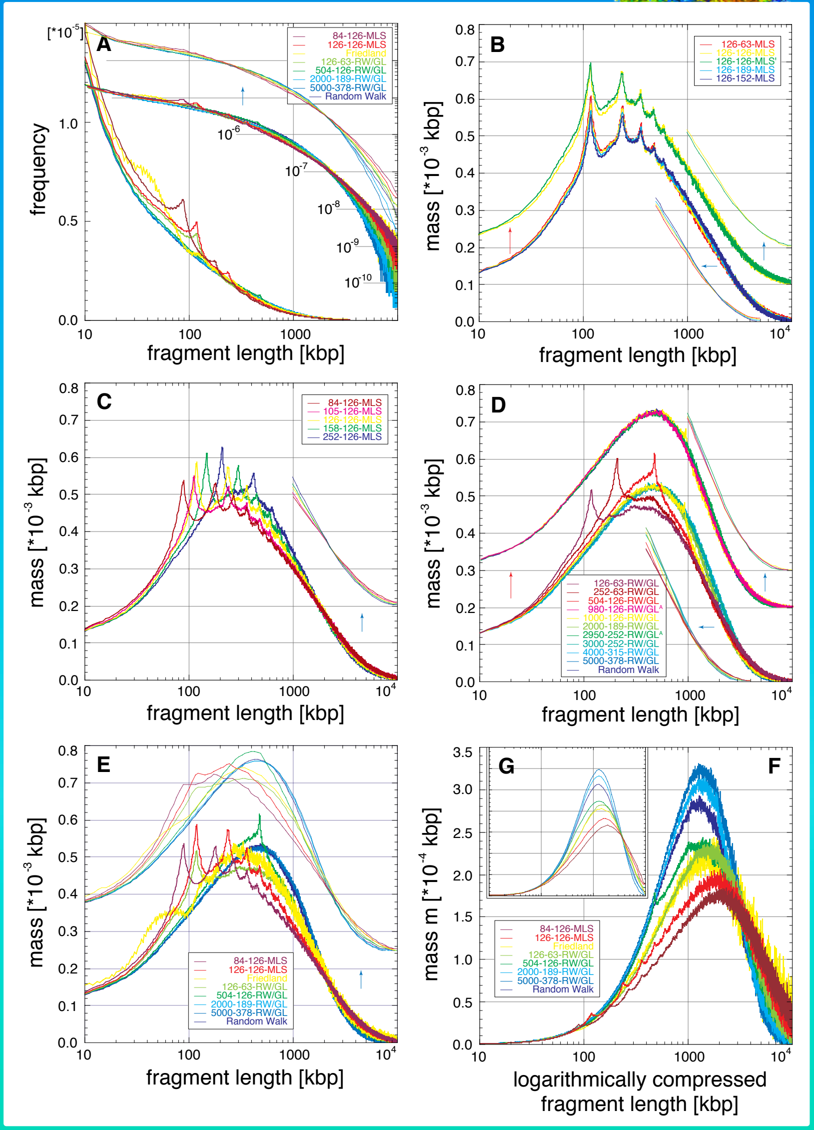
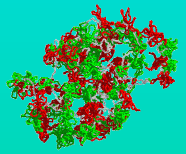
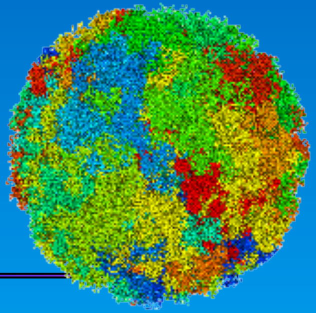


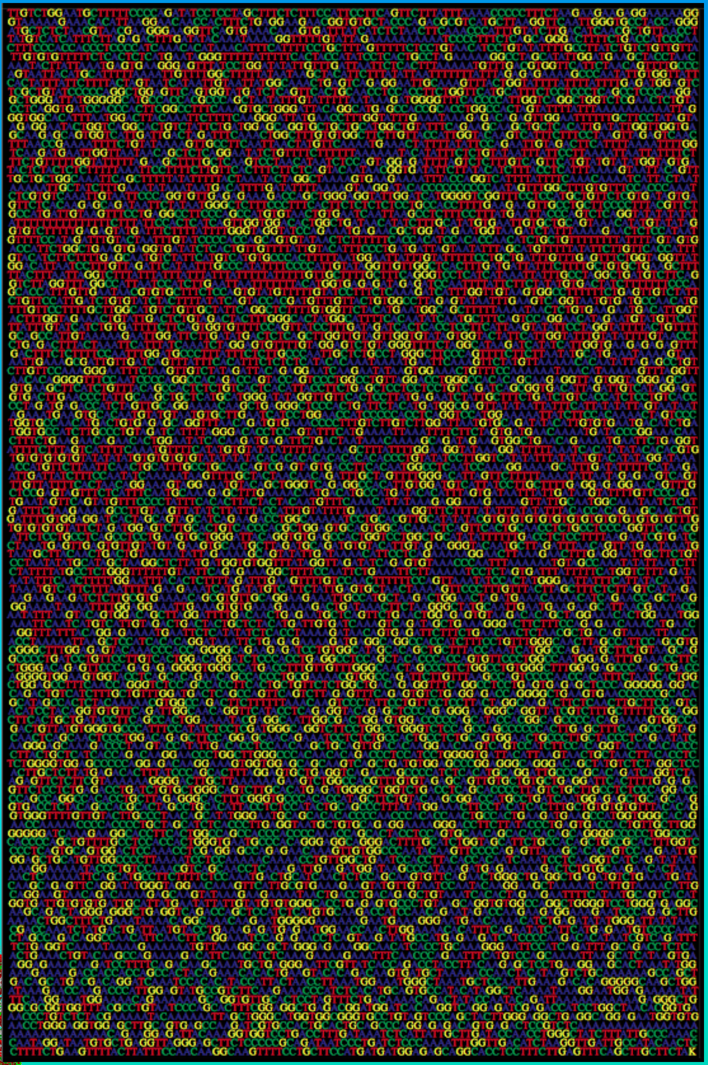


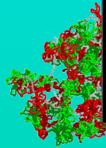


DNA Sequence Organization



Determination of the concentration fluctuation function *C(l)* and its local slope the correlation coefficient *δ(l)* are an indication for the i) degree of long-rang scaling behaveour, ii) general multi-scaling, and iii) fine-structure features, which all are connected to all levels of genome organization and especially also the three-dimensional genome architecture.



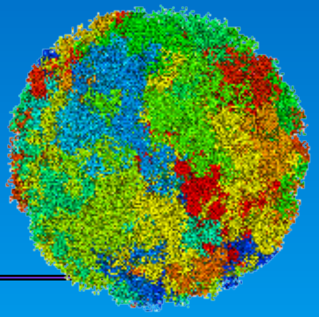


$$C(l) = \sqrt{\langle (c_l - \bar{c}_L)^2 \rangle_s}$$

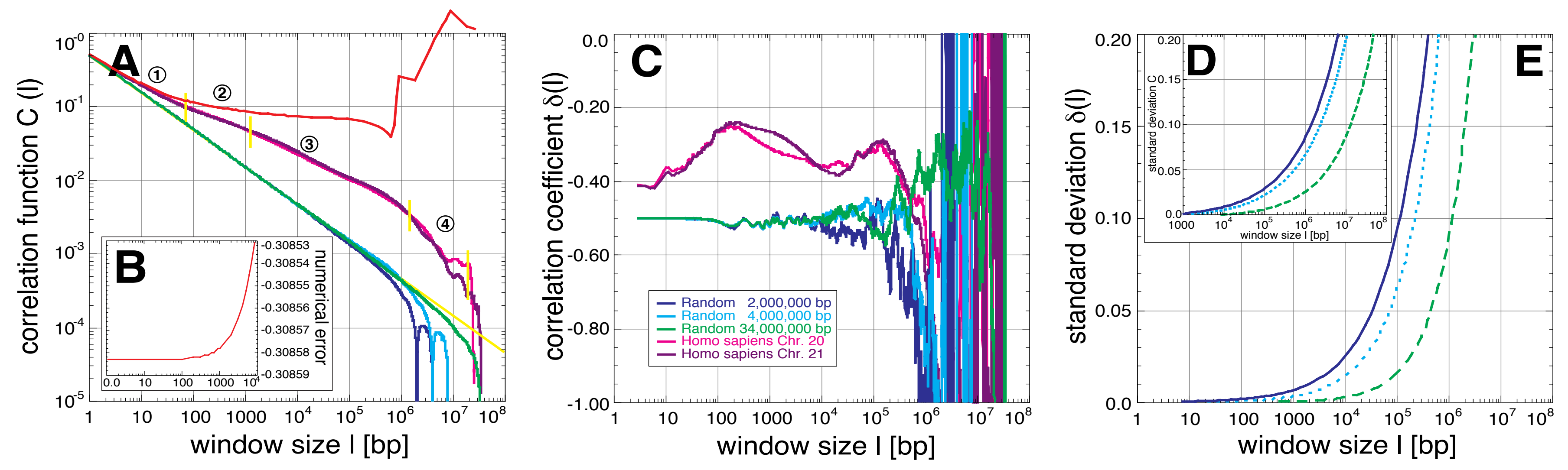
$$C(l) = \sqrt{\frac{1}{L-l+1}\sum_{s=1}^{L-l}\left(\frac{1}{l}\sum_{k=1}^{l} n - \frac{1}{L}\sum_{k=1}^{L} N\right)^2}$$

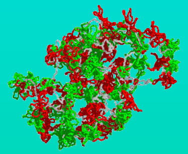
$$C(l) = \frac{1}{Ll}\sqrt{\frac{1}{L-l}\sum_{s=1}^{L-l}\left[\left(\sum_{k=1}^{l} Ln\right) - \left(\sum_{k=1}^{L} lN\right)\right]^2}$$

Long-Range Correlations in DNA Sequences



Determination of the concentration fluctuation function *C(l)* and its local slope the correlation coefficient *δ(l)* show that genomes show characteristic long-range correlations up to 10^8 bp, i.e. in principle over their complete length. Beyond, the show a specific multi-scaling behaveour, as well as a characteristic fine-structure. All correlates with the 3D-architecture of genomes.





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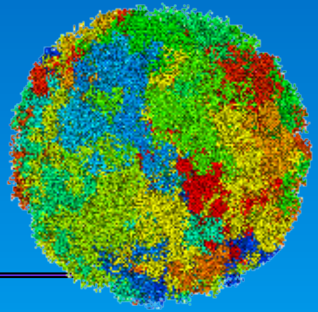
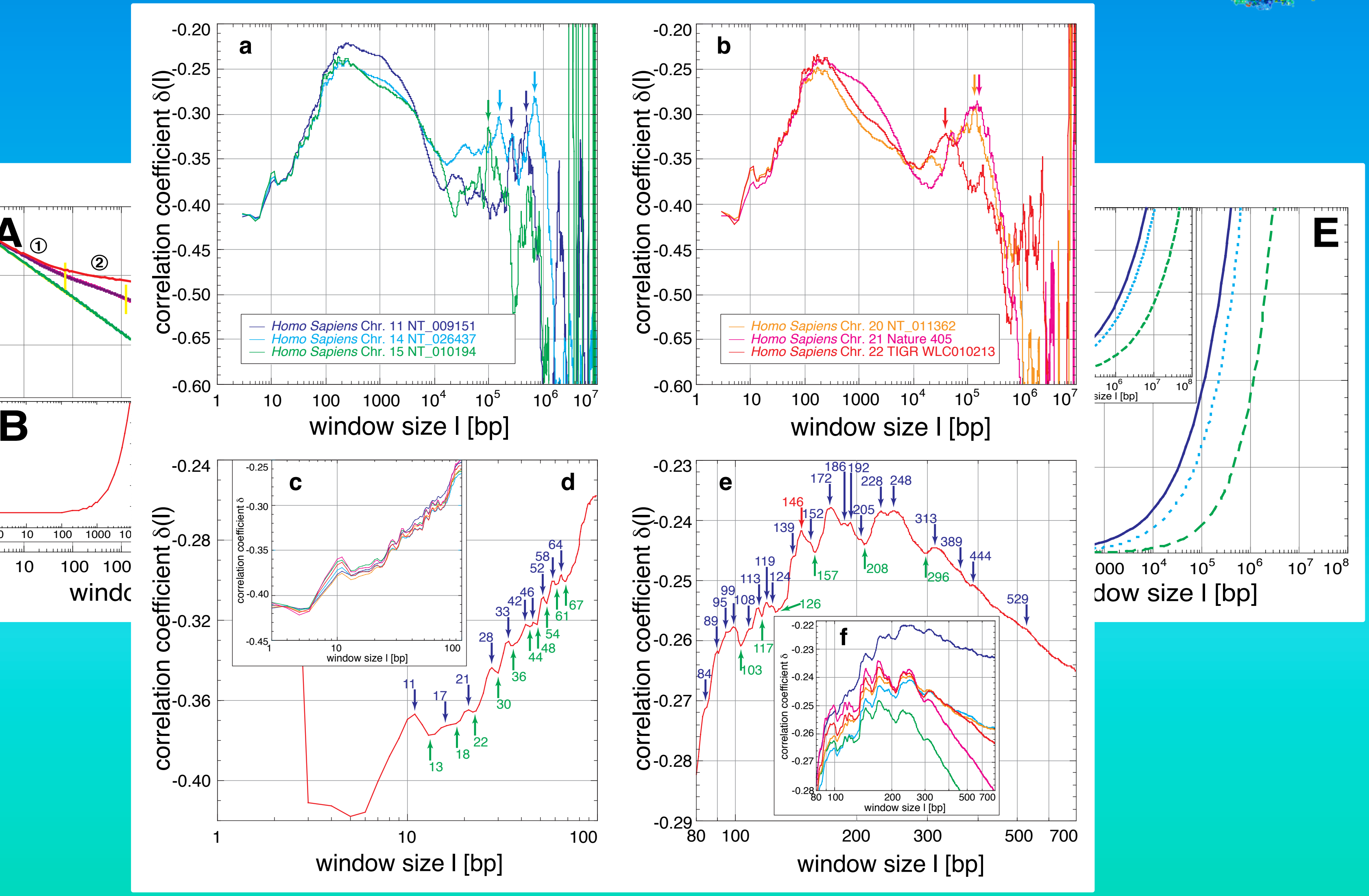
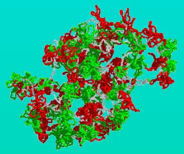
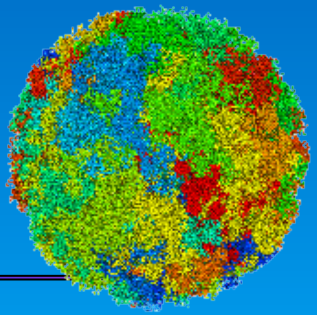


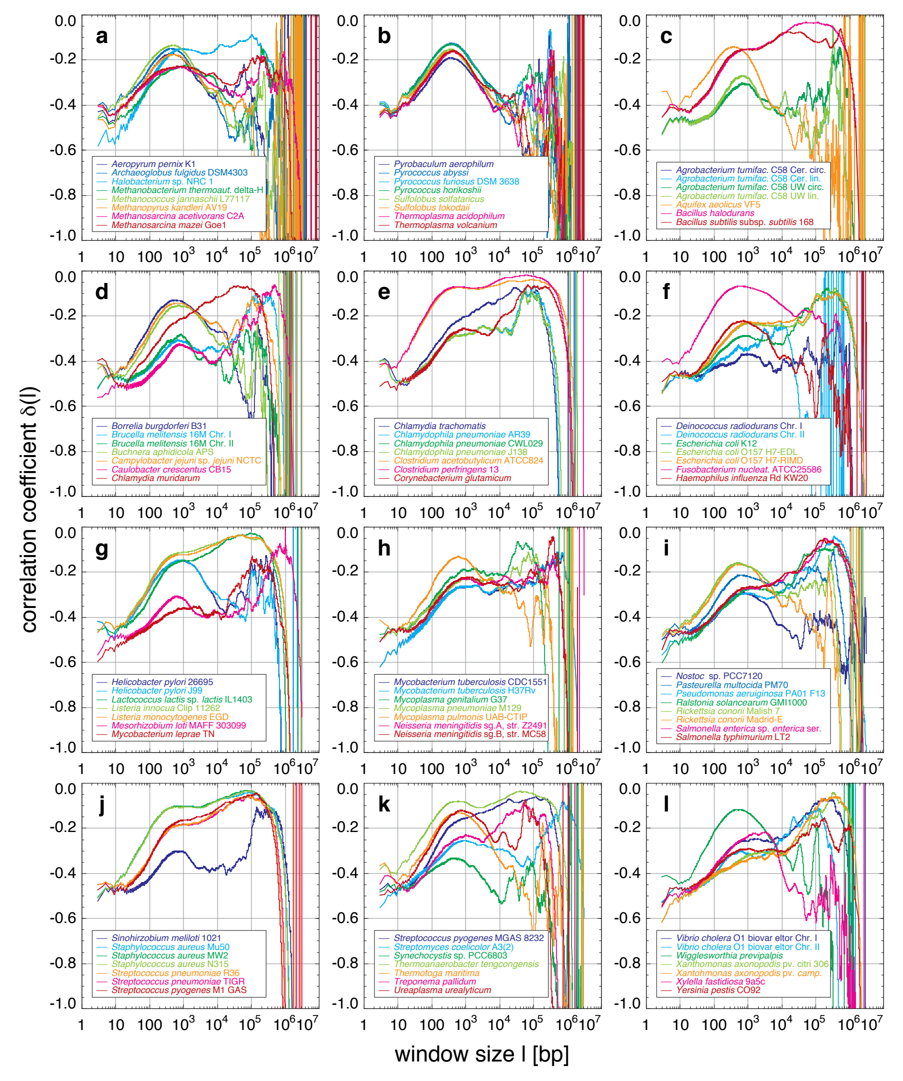




Sequence Organization and Phylogeny

The concentration fluctuation function *C(l)* and its local slope the correlation coefficient *δ(l)* characteristic long-range fine-structured multi-scaling behaveour, which is specy specific. Tree construction thus leads to characteristic groups, which are similar to those suggested by classic phylogenetic trees. Thus, the sequence organization represents evolutionary lines.





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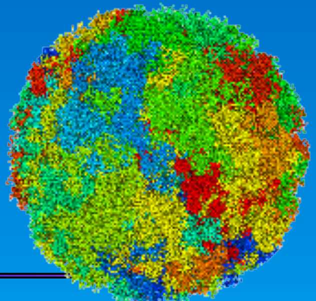
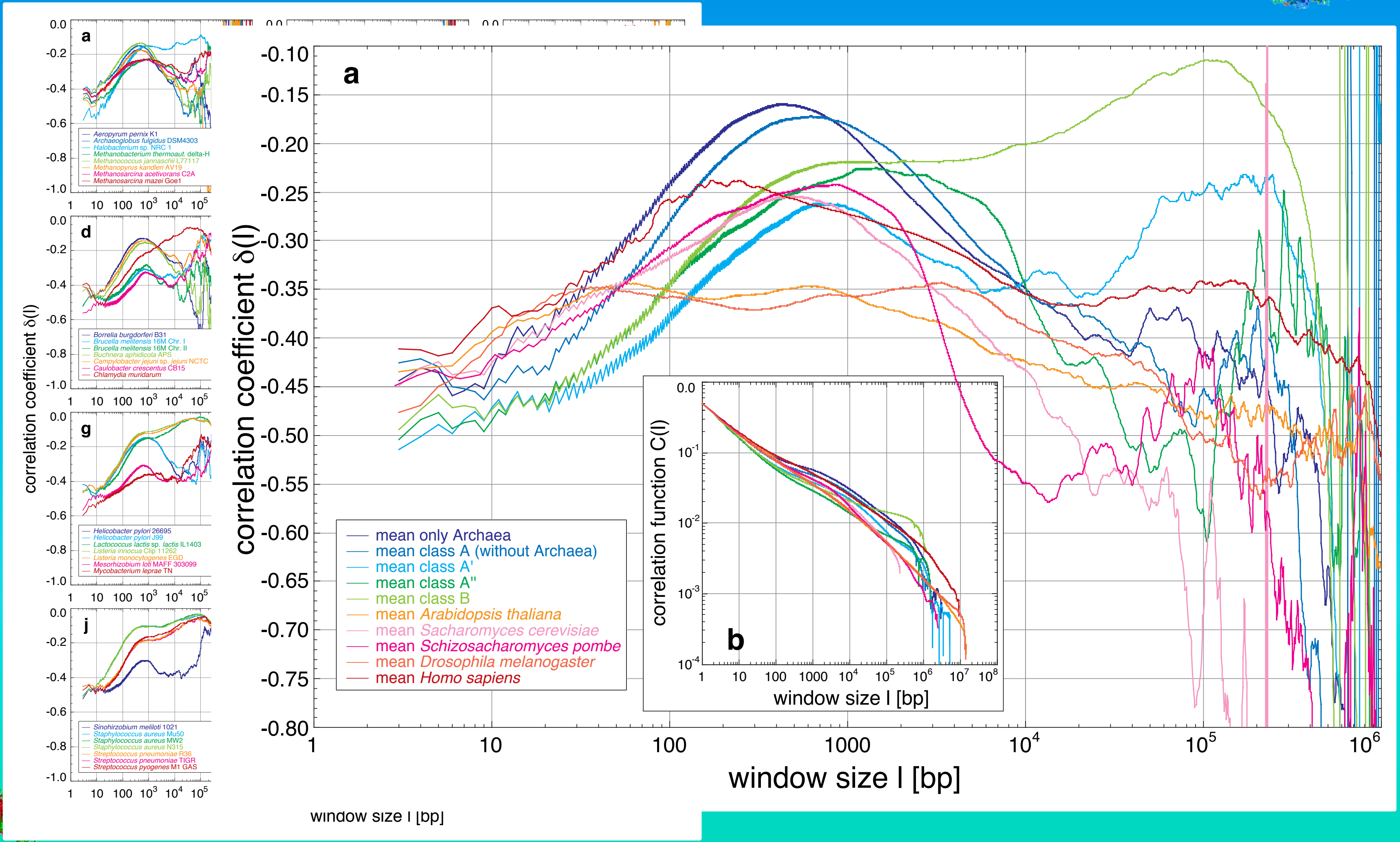
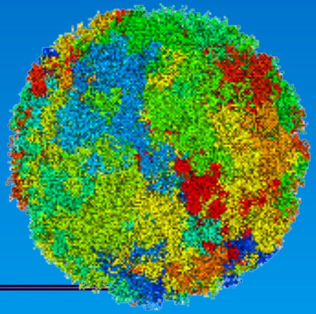
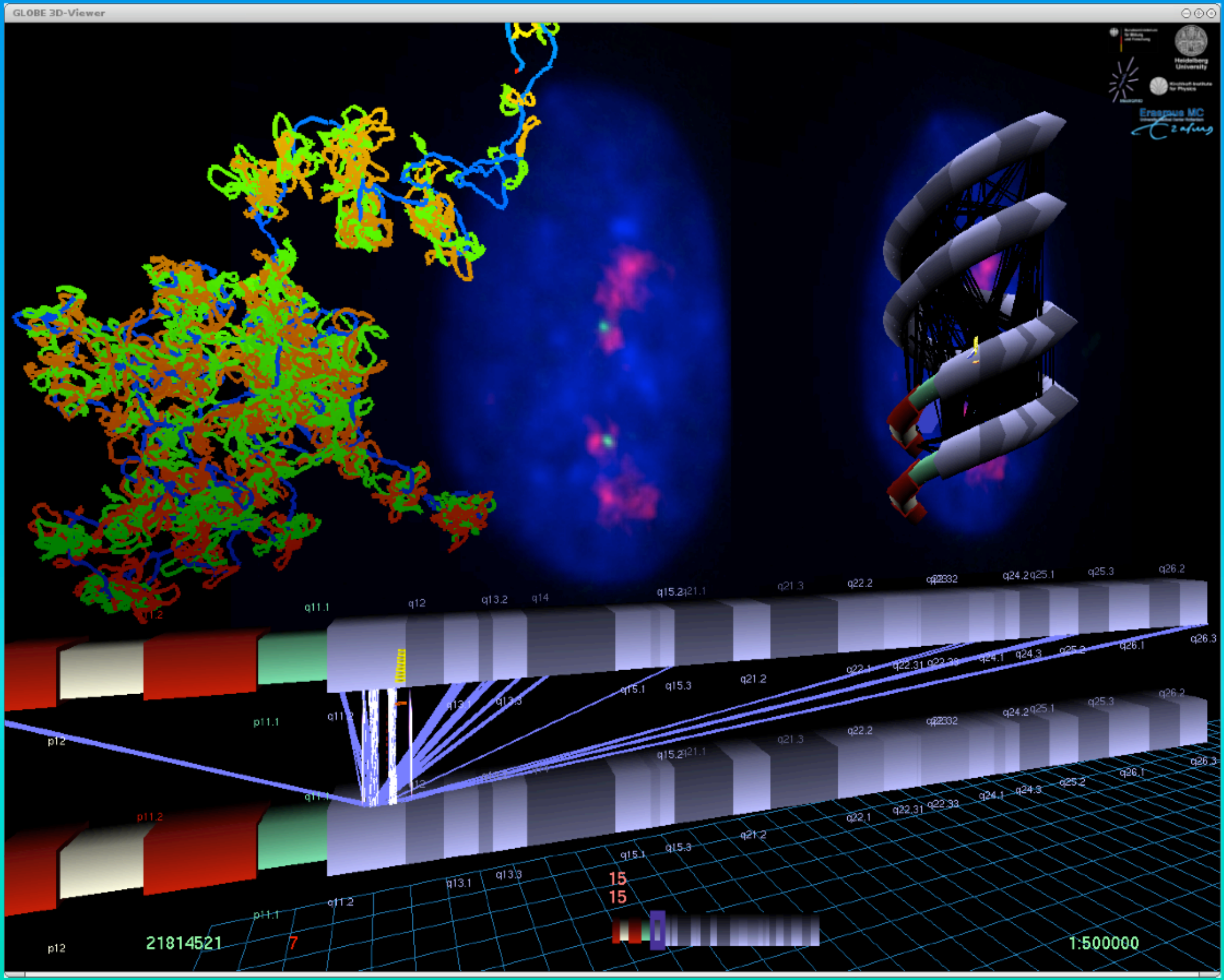


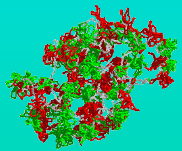


Systems Biological Result Integration via the GLOBE 3D Genome Platform

All results will be integrated using our GLOBE 3D Genome Platform, established for analysis, manipulation and understanding of multi-dimensional complex genome wide data. Thus in reiterative cycles between experiments and simulations a systems biological/medical genome model will be achieved.







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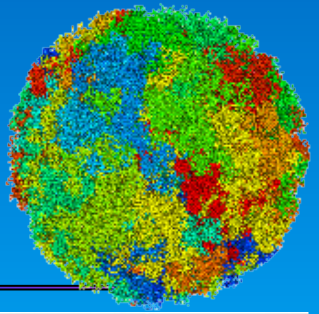

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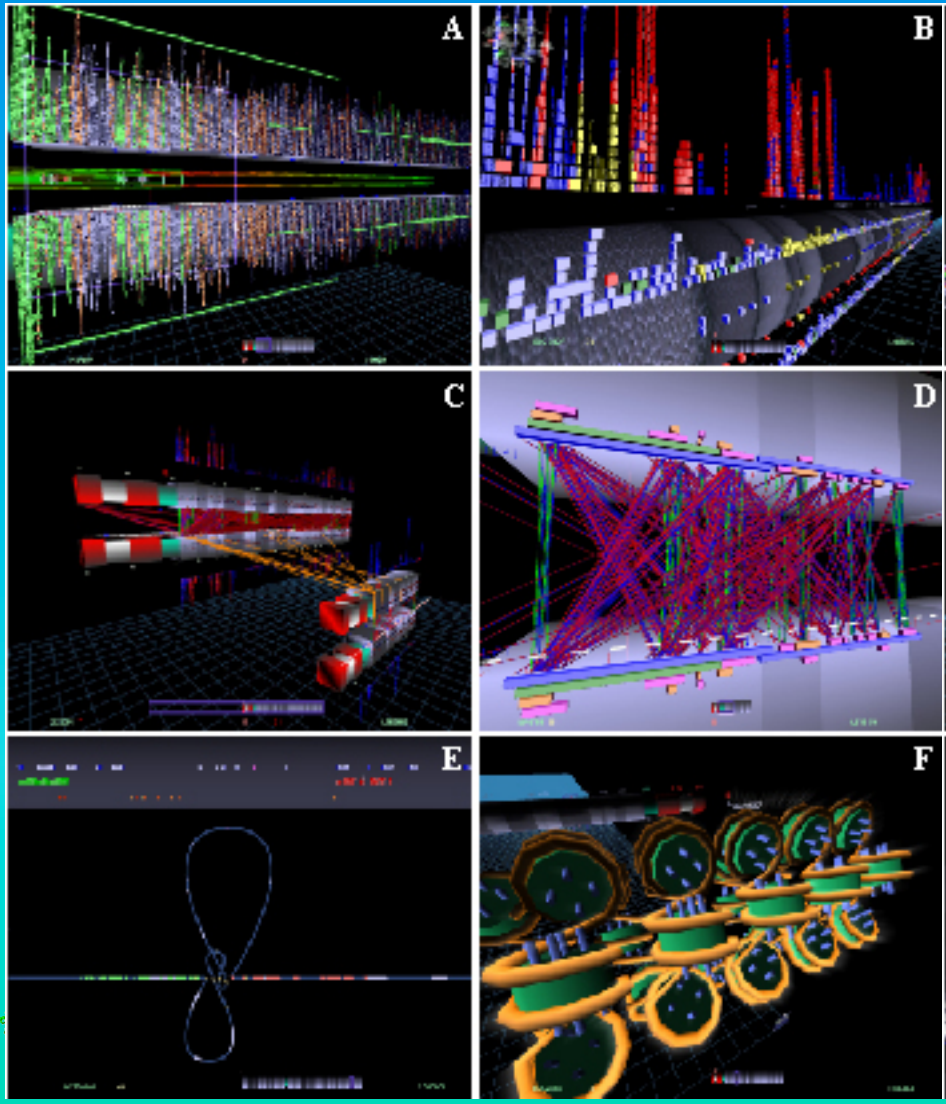
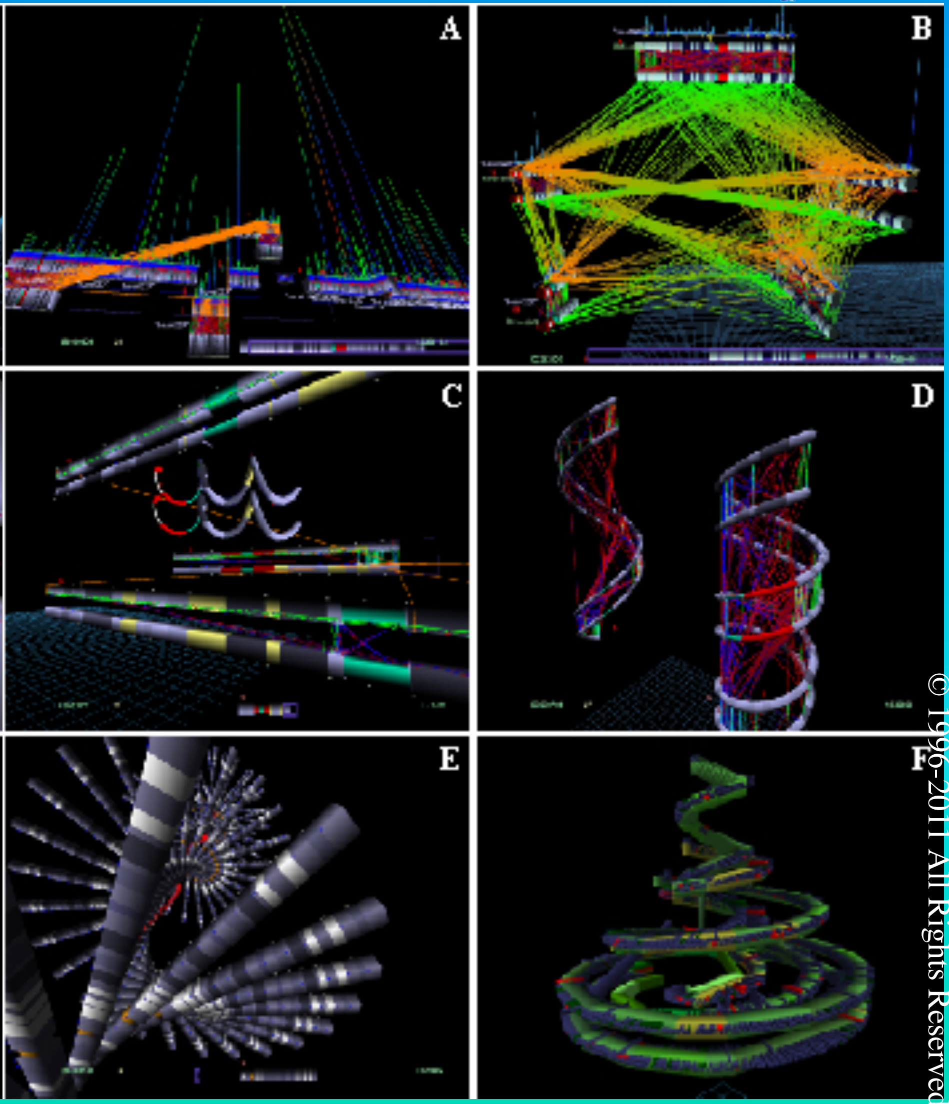
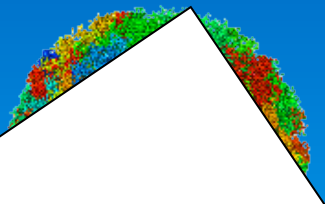




Systems Biological Result Integration via the GLOBE 3D Genome Platform



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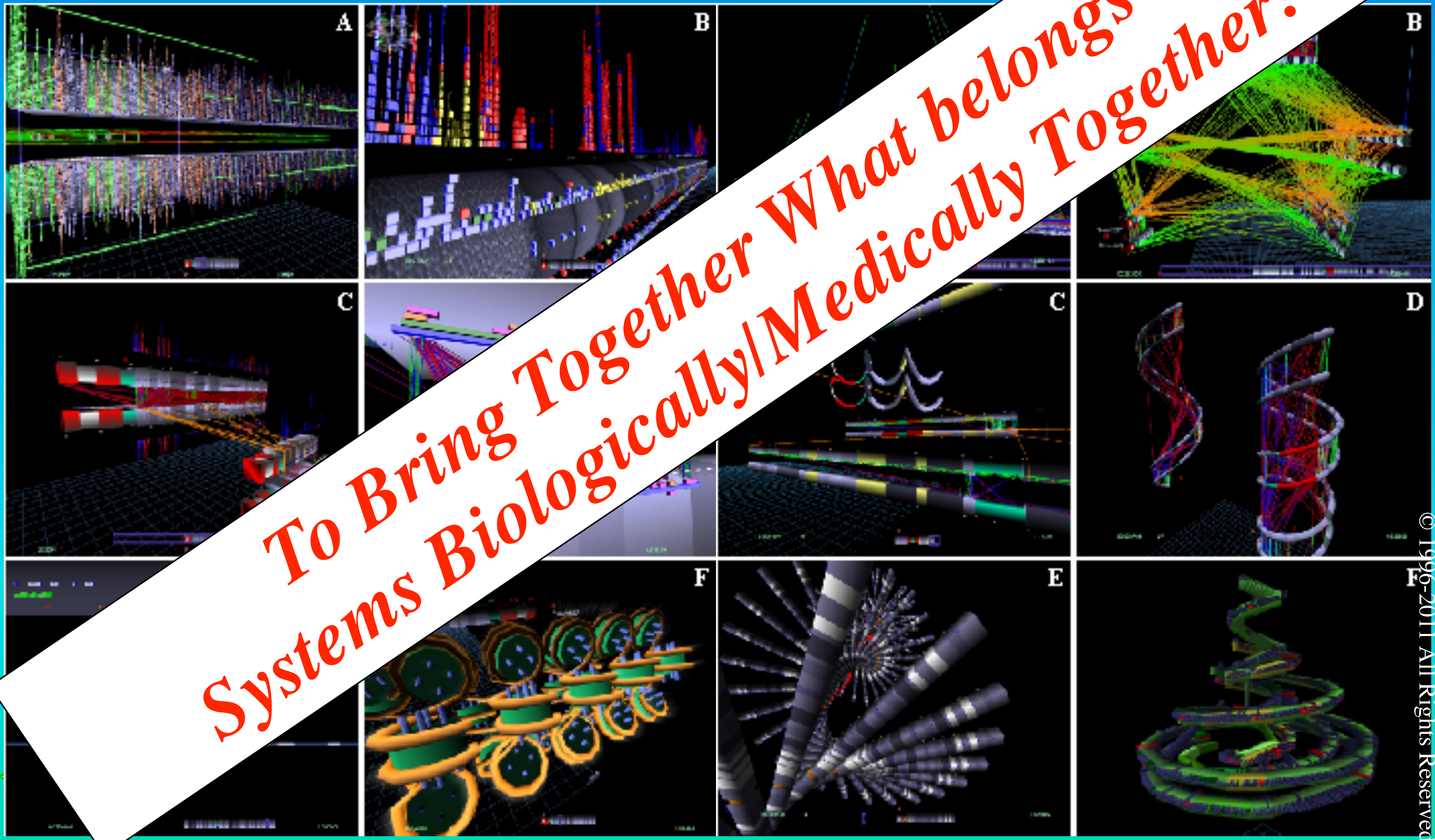
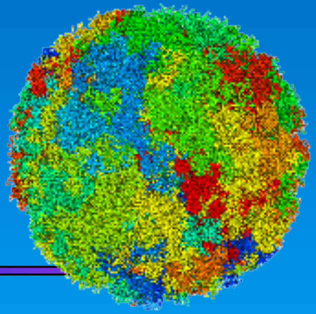


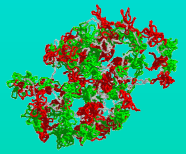
To Bring Together What belongs Systems Biologically/Medically Together!

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 behaveour 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 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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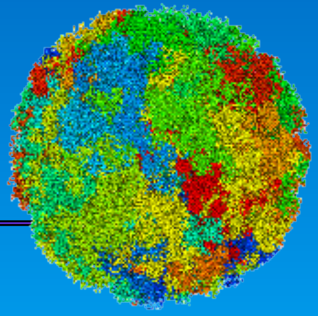

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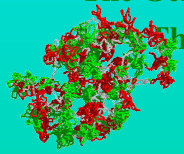
Erasmus Medical Center, Hogeschool Rotterdam, The Fraunhofer Society, The German MediGRID and Services@MediGRID, The German D-Grid Initiatives, The German Ministry for Science and Technology, The Dutch Science Organization (NOW), The European EGEE Initiative, The European EDGES Consortium, The German Society for Human Ecology, The International Society for Human Ecology,

The European Commission



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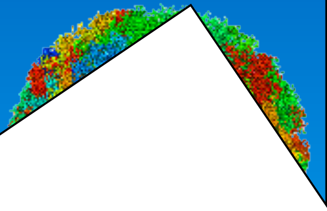

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The Real Fun
Is Yet To Come!

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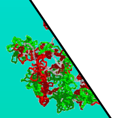

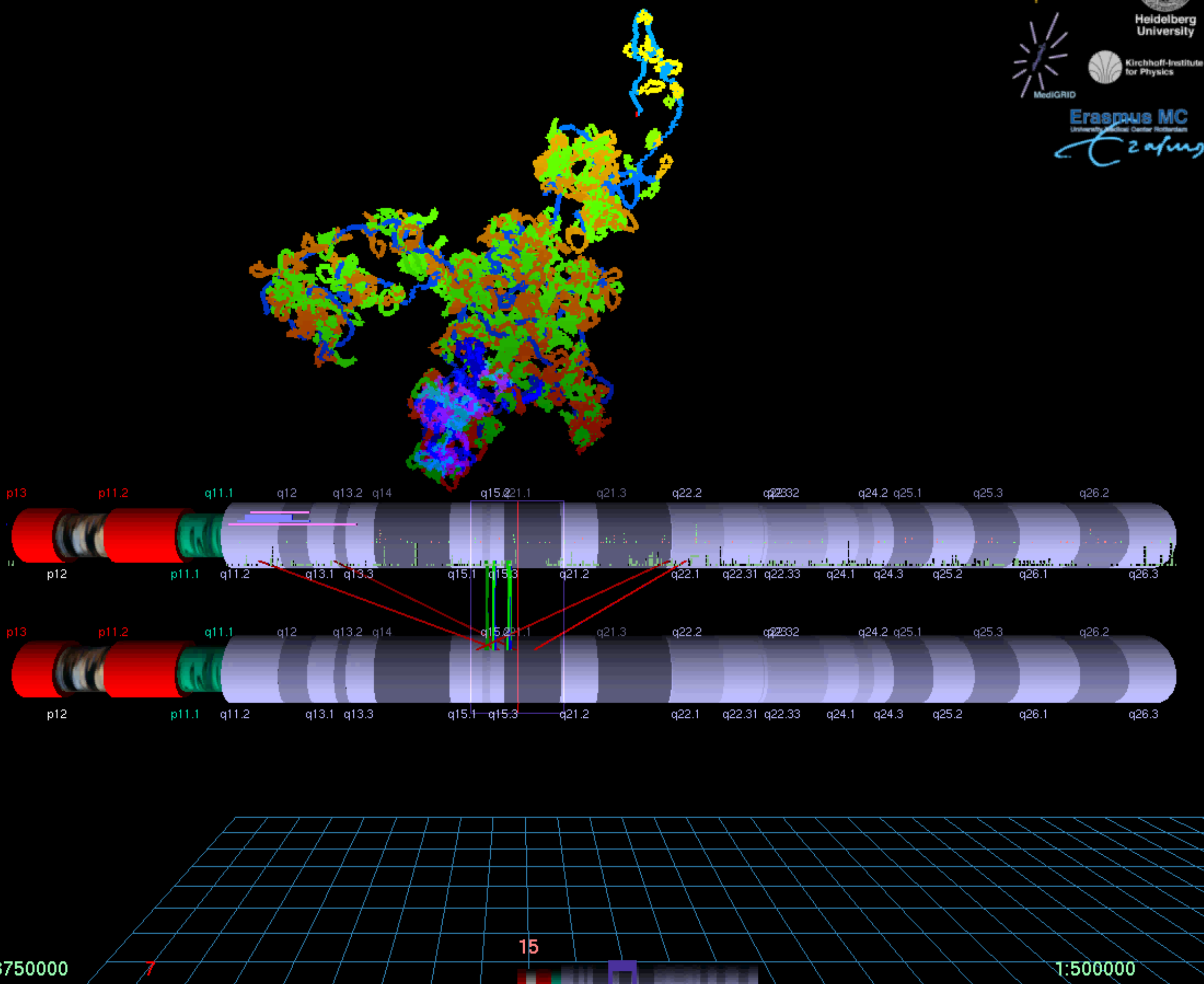
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The 3D Architecture and Dynamics
of the
Immunoglobin Heavy-Chain and other Loci
and its
Functional Implications for Genome Organization

Knoch, T. A., Murre, C. & Grosveld, F. G.

The 22nd Wilhelm Bernhard Workshop, Hotel Riga, Riga, Latvia,
25th - 30th August, 2011.

Abstract

The general 3D architecture of the immunoglobin heavy-chain (Igh) and other loci was determined by a novel interdisciplinary combination of high-resolution FISH and high-resolution epifluorescence spectral distance microscopy with analytical analysis, computer simulations, as well as trilateration (*Cell 133, 265-279, 2008*). The Igh locus is organized into distinct regions that contain multiple variable (V_H), diversity (D_H), joining (J_H) and constant (C_H) coding elements. Determination of distance distributions between genomic markers across the entire locus showed that the Igh locus is organized into compartments consisting of small loops separated by linkers with in detail dynamic functional relevance: V_H, D_H, J_H, and C_H elements showed striking conformational changes involving V_H and D_H-J_H elements during early B cell development, culminating in a merger and juxtaposition of the entire repertoire of V_H regions to the D_H elements in pro-B cells allowing long-range genomic interactions with relatively high frequency. This is in agreement with our recent study of the Prader-Willi/Angelmann region using a similar approach (*Differentiation 76, 66-82, 2008*) and in agreement with the Multi-Loop-Subcompartment (MLS) model of chromosome organization predicting 60-150 kbp loop aggregates separated by a similar linker (Knoch, ISBN 3-00-009959-X, 2002). With a new technology we are also able to proof, that this holds for other loci as well. Synopsis with previous spatial distance measurement studies and combination with sequence correlation analysis of the DNA sequence, fine-structure multi-scaling analysis of the chromatin fiber topology or *in vivo* morphology of entire cell nuclei, electron microscopy of chromosome spreading studies and even the diffusion behaviour within the cell nucleus, are all suggesting such an MLS architecture. This framework reveals a consistent picture of genome organization joining structural and dynamical aspects ranging from the DNA sequence to the entire nuclear morphology level with functional aspects of gene location and regulation. Many previously contradictory viewpoints are resolved by this framework as well. Consequently, the determination of the general 3D architecture of the Igh and other loci has beyond its major functional relevance, huge implications for the understanding of the entire genome understanding in a holistic system-biological manner.

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Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome statistical mechanics, genomic uncertainty principle, 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, chromatin cross-linking, chromosome conformation capture (3C), selective high-resolution high-throughput chromosome interaction capture (T2C), confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling, information browser, visual data base access, holistic viewing system, integrative data management, extreme visualization, three-dimensional virtual environment, virtual paper tool.

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