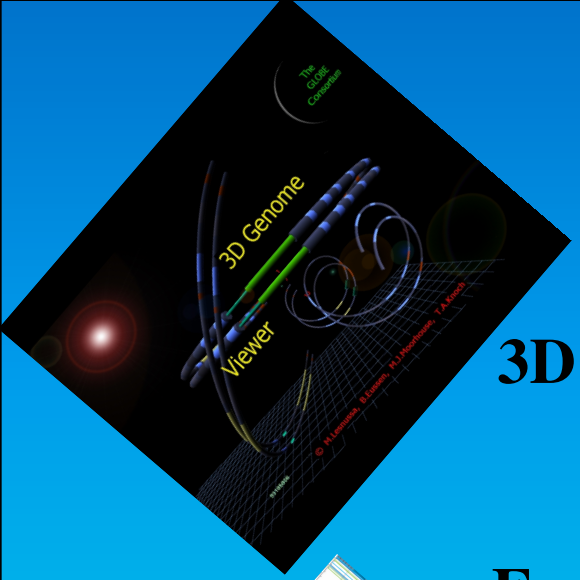
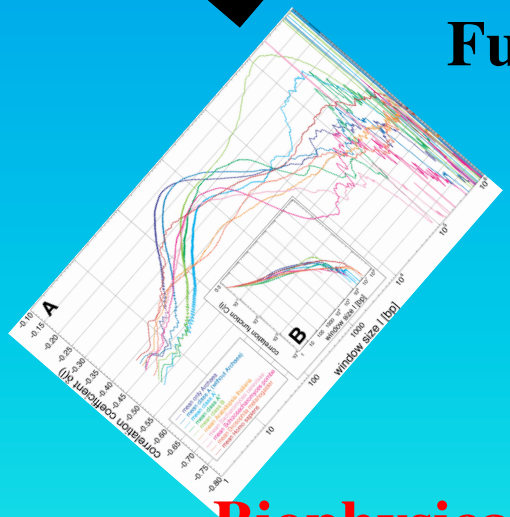
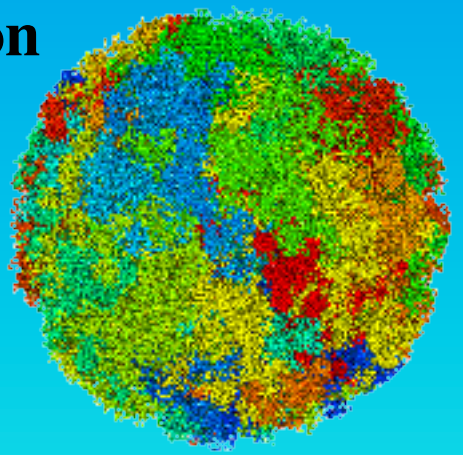


The Detailed 3D Multi-Loop Aggregate/Rosette Chromatin Architecture and Functional Dynamic Organization of the Human and Mouse Genomes





Tobias A. Knoch

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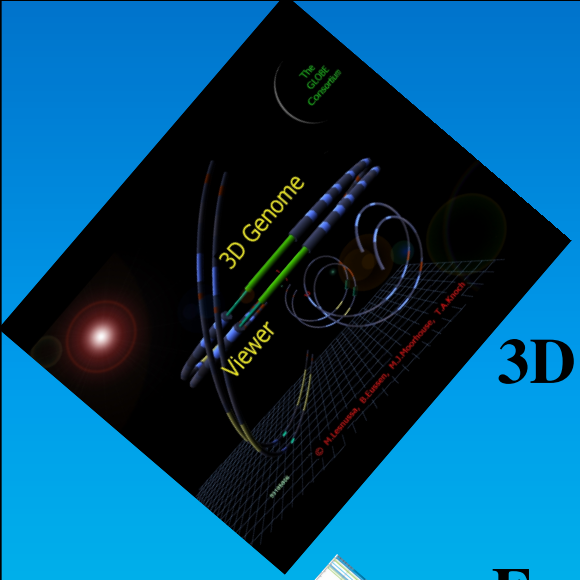
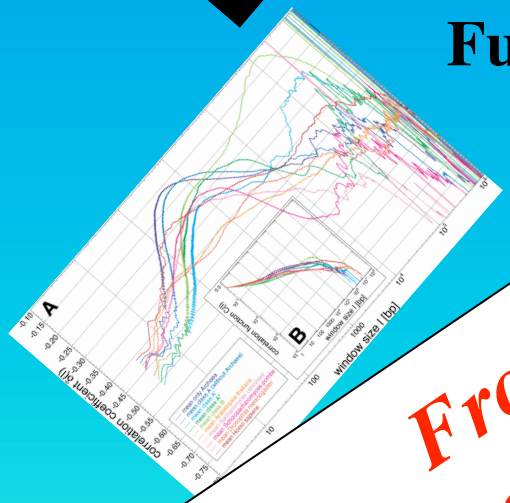
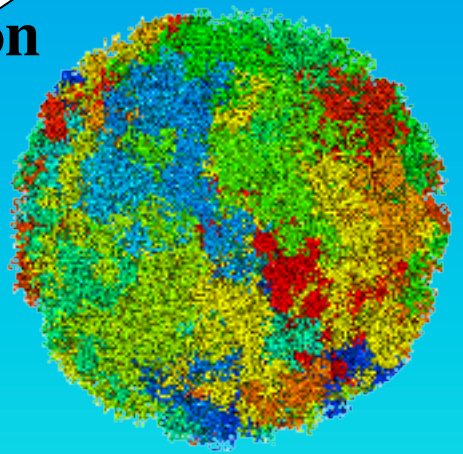


The Detailed 3D Multi-Loop Aggregate/R… Chromatin Archite… and Functional Dyn… …tion

Hum… …Genomes





From Sequence to Morphology: Towards a Holistic Understanding of Genomes!

…bias A. Knoch

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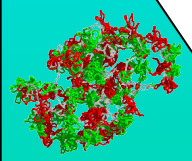
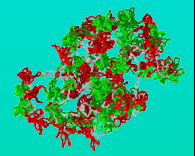
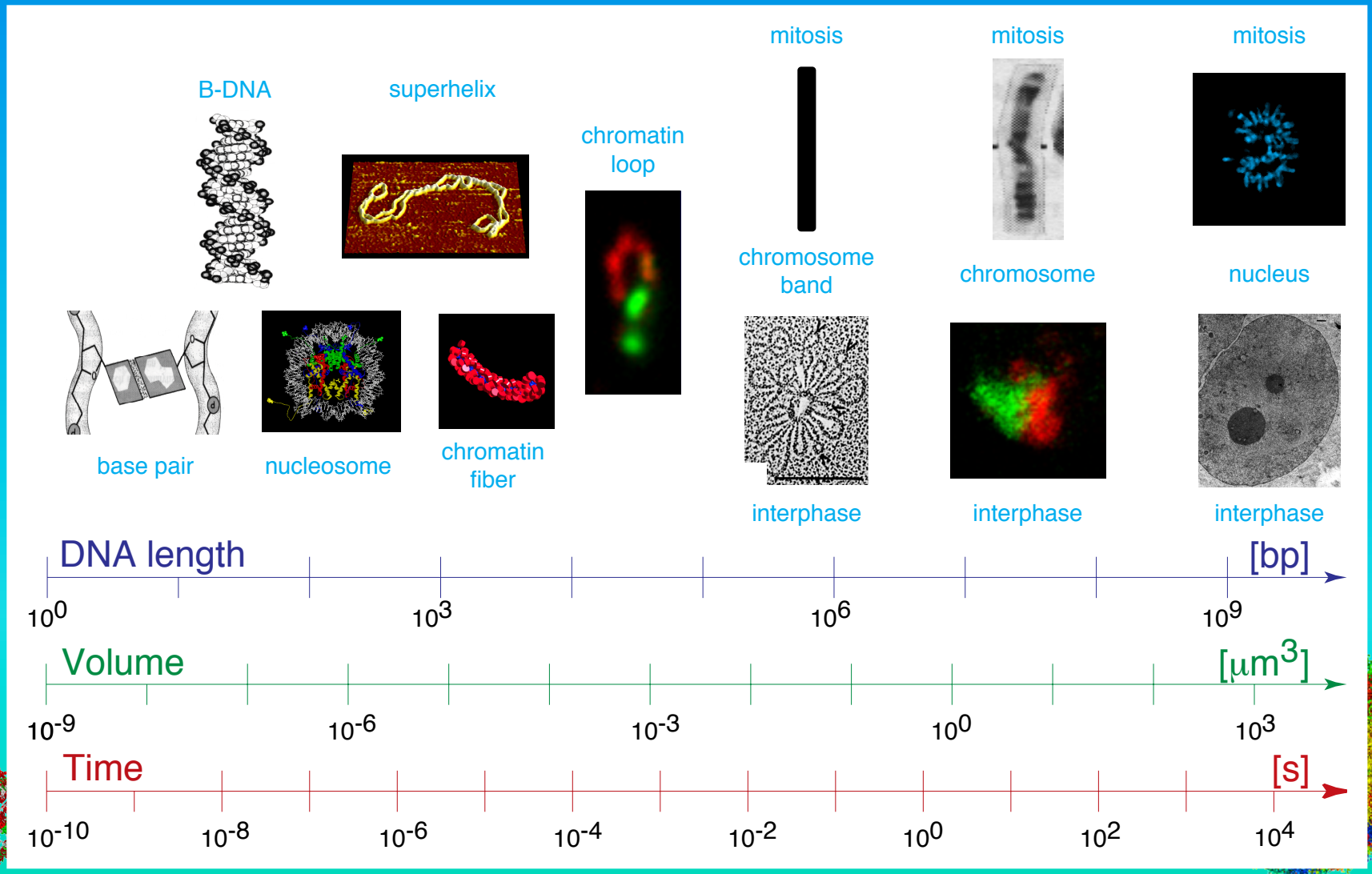




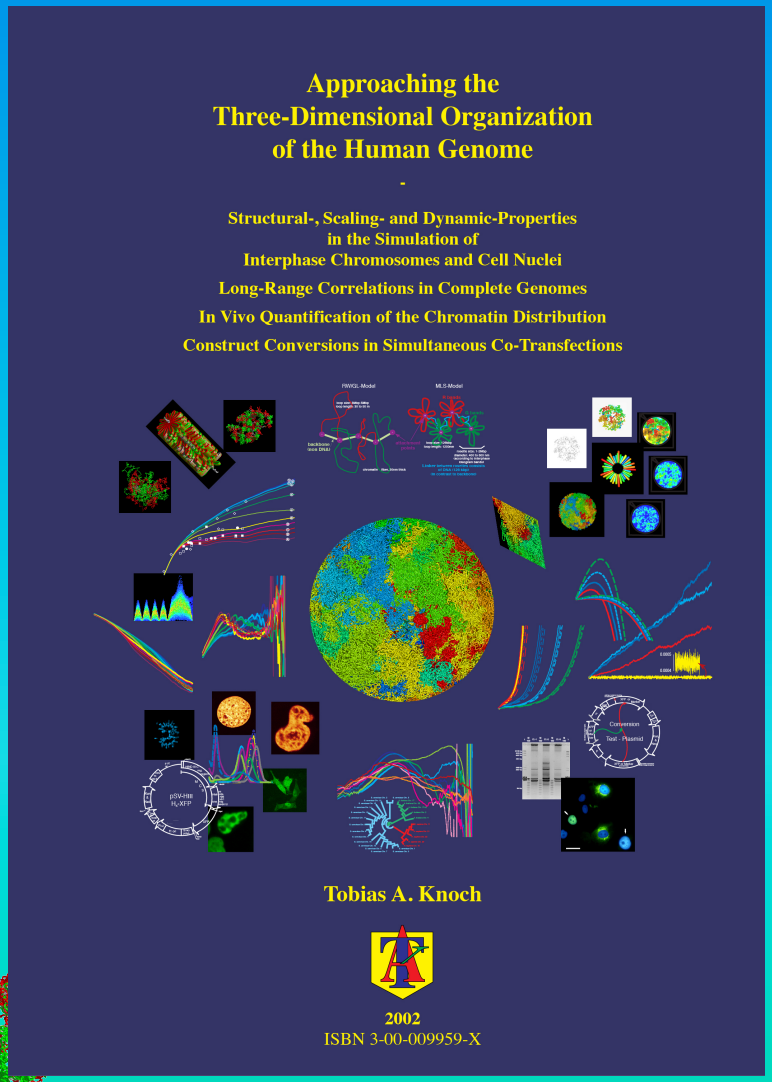
Dynamic and Hierarchical Genome Organization

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?



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!



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:

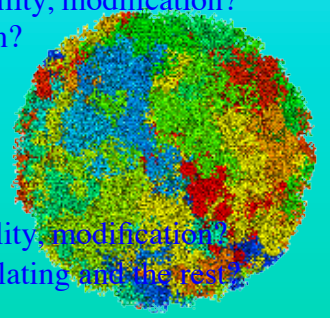
-> Chromatin fiber organization: prevalence, variation, dynamics?
-> Chromatin code: coding, regulation, modification?

Nucleosome:

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

DNA Sequence:

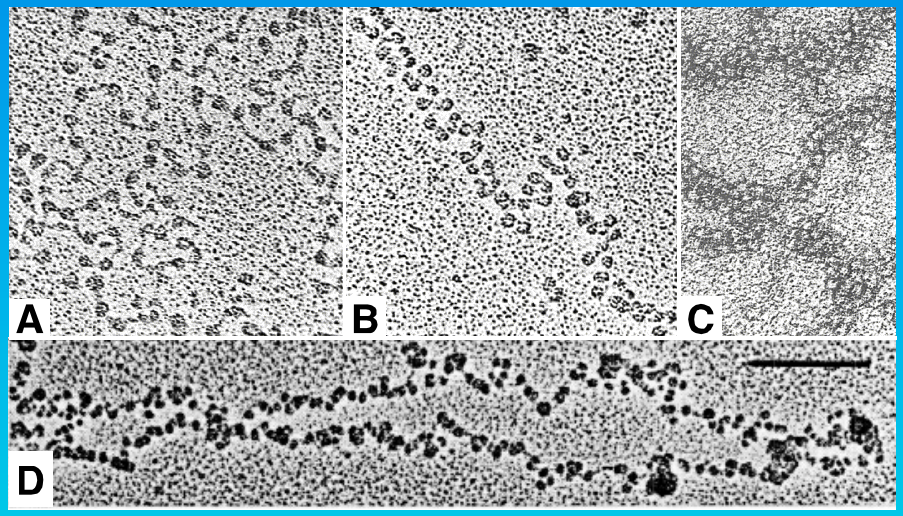
-> DNA local structure: bending, melting, stability, modification?
-> General sequence organization: coding, regulating and the rest?



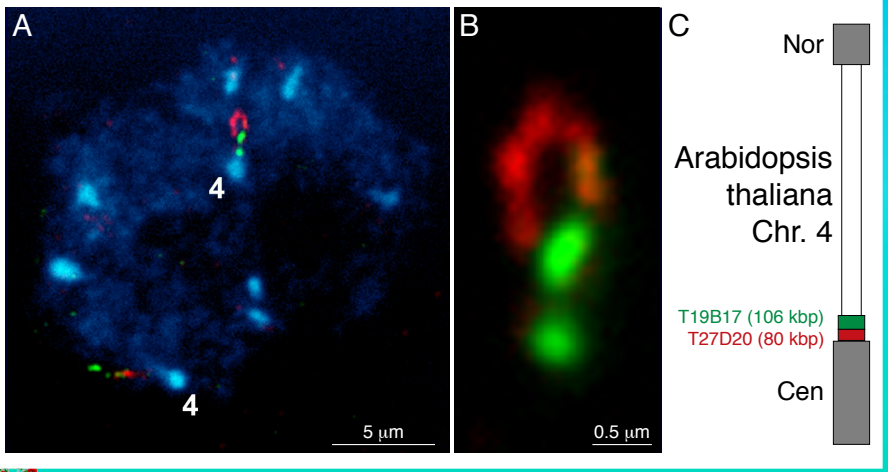
Chromatin Conformation and Higher-Order Topologies

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.

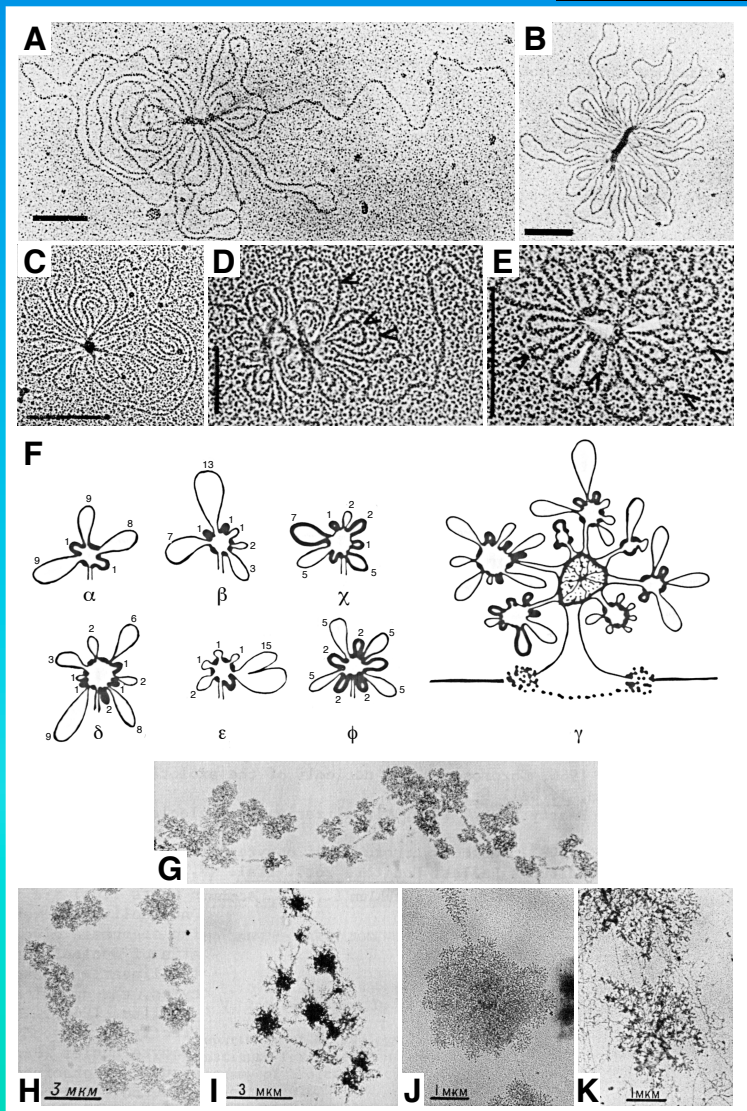
A-C: Voet & Voet D: Reznik *et al.*



Courtesy P. Fransz, Amsterdam



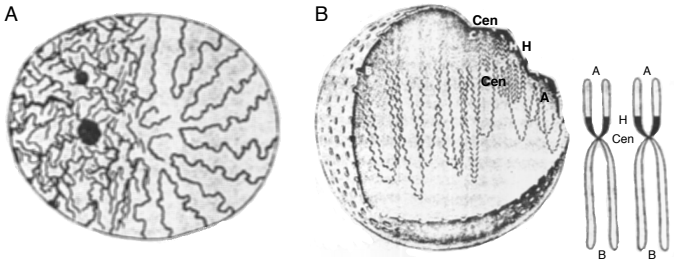
A, B: Avramova *et al.*; C: Salganik *et al.*; D-G:Frznik *et al.*; G-K: Tsvetkov & Parvenov

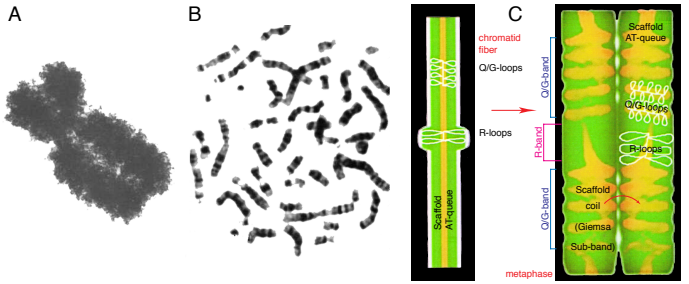


Integral Models of Cell Nuclear Organization I

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.

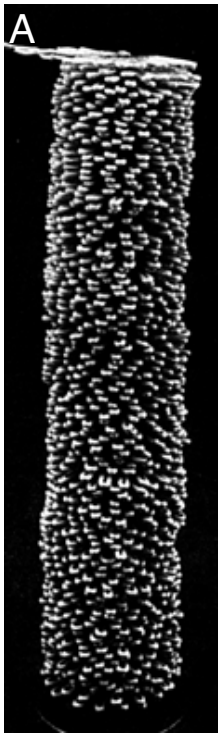
Rabl & Boveri

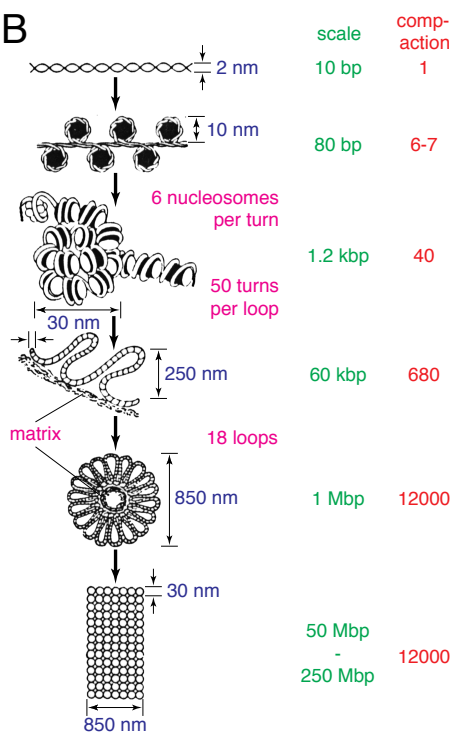




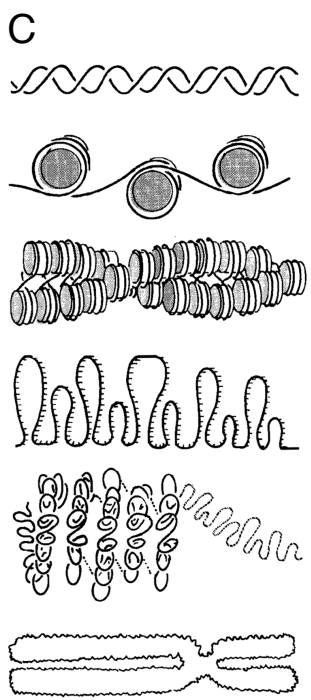
A: Bloom & Fawcett
B: Alberts *et al.*
C: Paulson & Laemmli

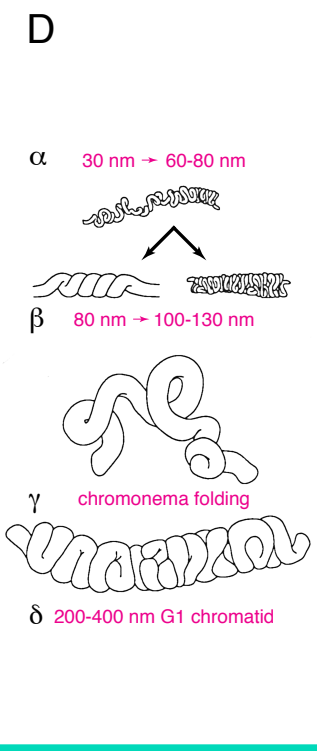
A, B: Pienta & Coffey; C: Alberts *et al.*; D: Belmont & Bruce

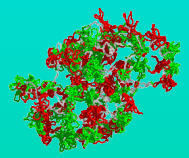


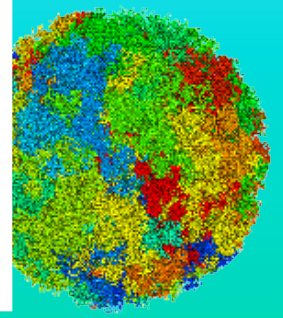


scale	comp-action
10 bp	1
80 bp	6-7
1.2 kbp	40
60 kbp	680
1 Mbp	12000
50 Mbp - 250 Mbp	12000



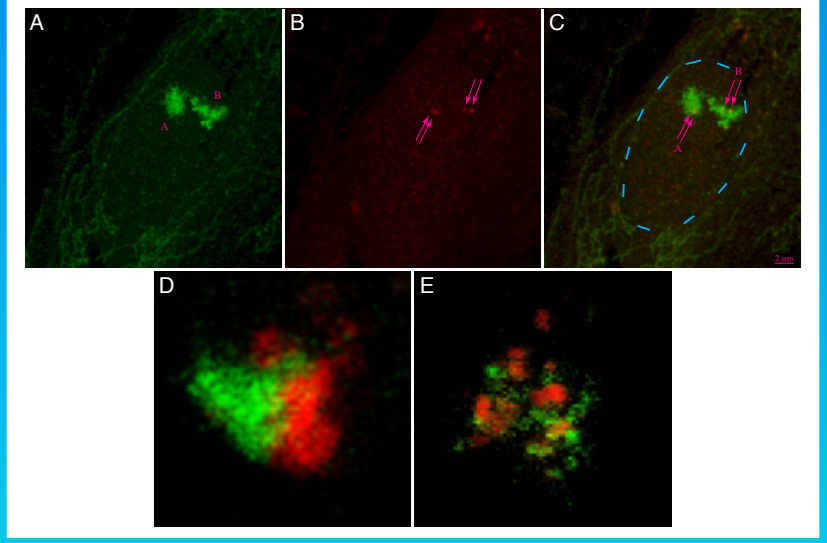




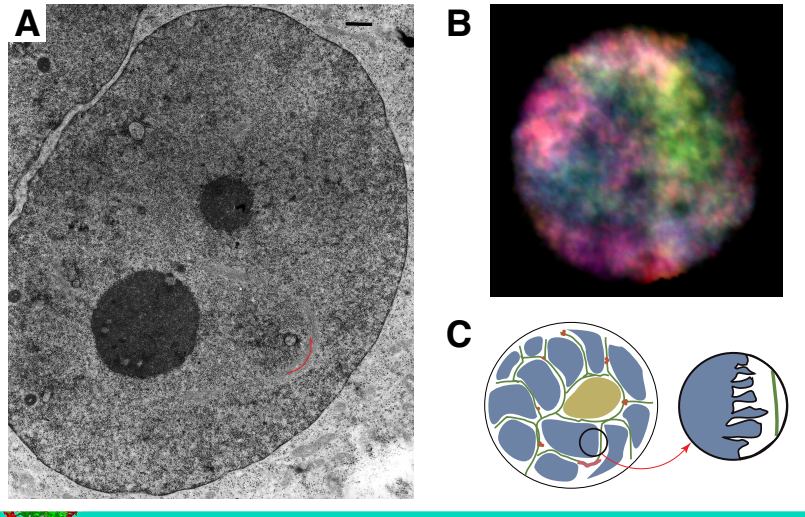


Integral Models of Cell Nuclear Organization II

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…

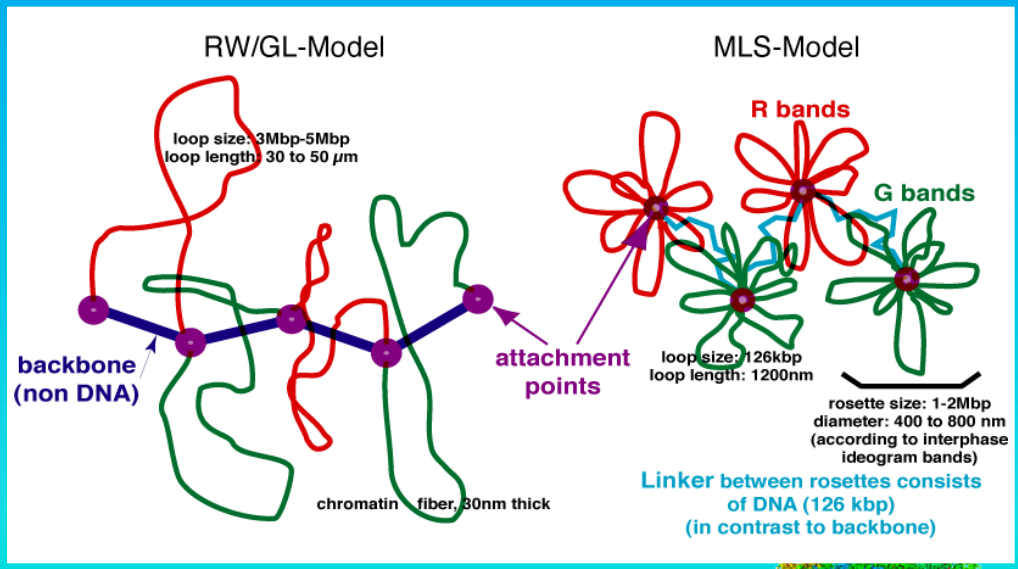


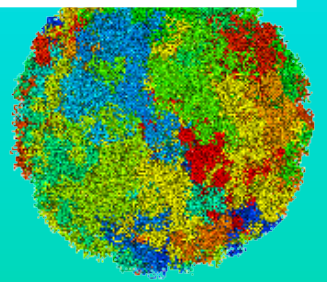
D: courtesy S. Dietzel; E: courtesy D. Zink



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

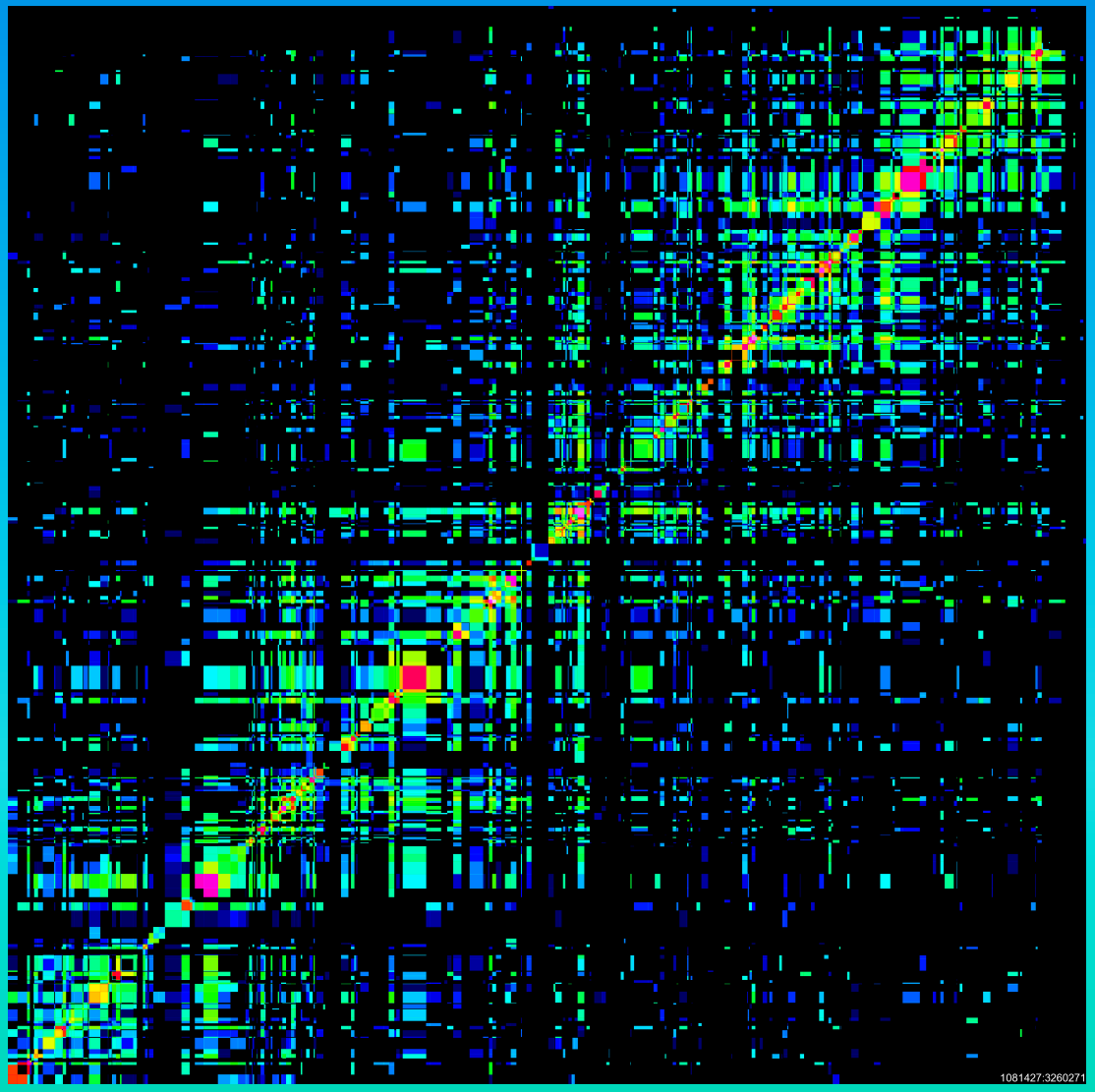
Random-Walk/Giant-Loop Multi-Loop-Subcompatment Model

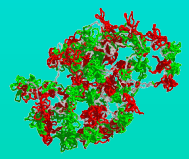


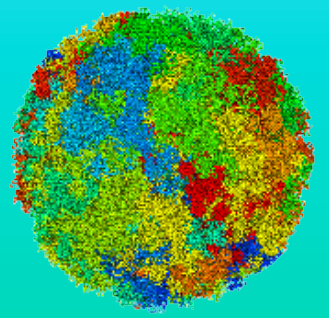


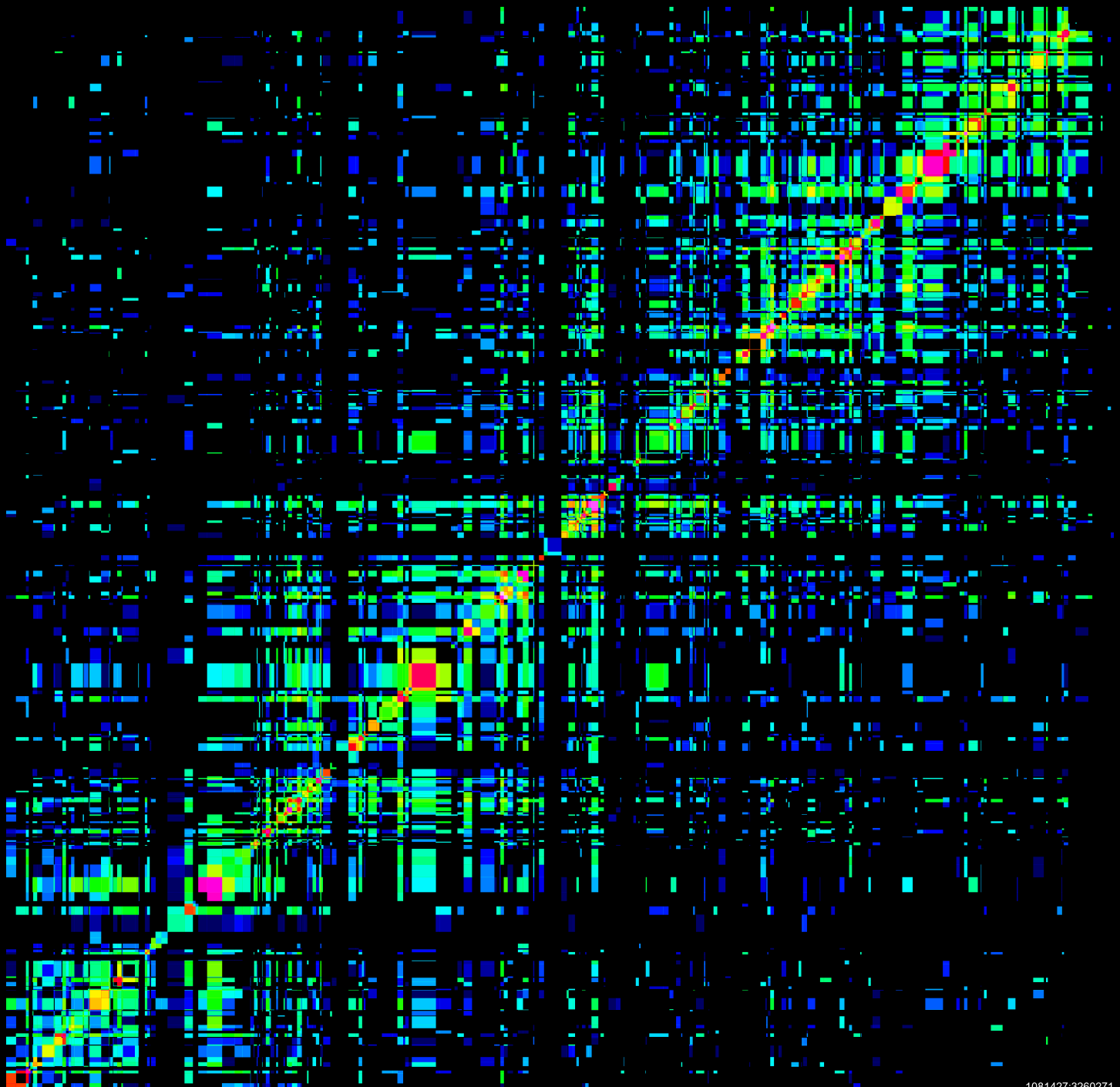
Selective Chromosome Interaction Capture (T2C)

T2C is a novel selective high-resolution high-throughput chromosome interaction capture, in which the relation between, region size, resolution, interaction frequency range, and sequencing depth can be designed towards the goal of the experiment. T2C reaches the limit of the “genomic” uncertainty principle and statistical mechanics.







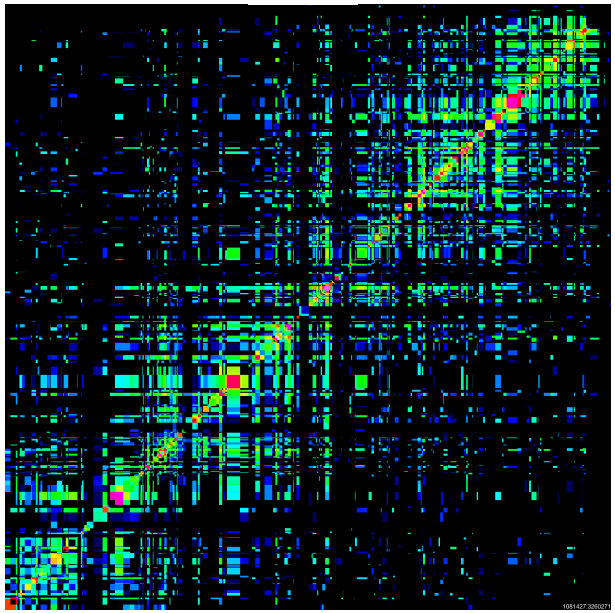


We Reached the Level of the Genomic Uncertainty Principle!
The Conformation of Chromatin and the 3D Architecture
can be derived with Tremendous Resolution !

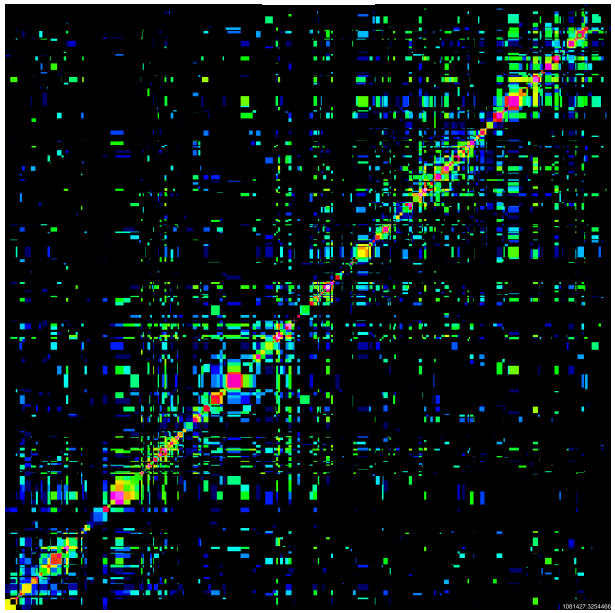
Stable Consensus Architecture of Genomes

Due to the high signal-to-noise ratio of T2C reaching 5-6 orders of magnitude interaction maps reveal definitely an extremely high degree of similarity between different species, cell types, or functional states, thus functional differences are variation of a stable theme persisting through the cell cycle

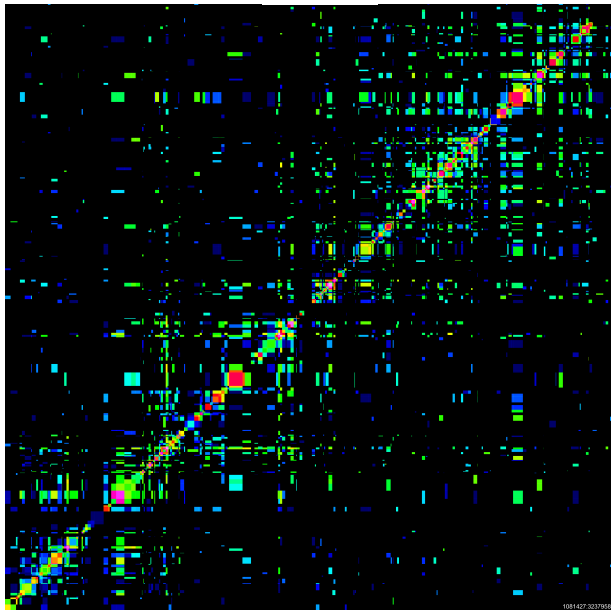
HB2

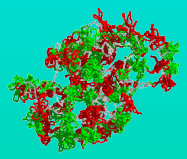


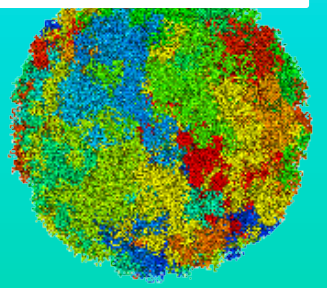
TEV



HRV







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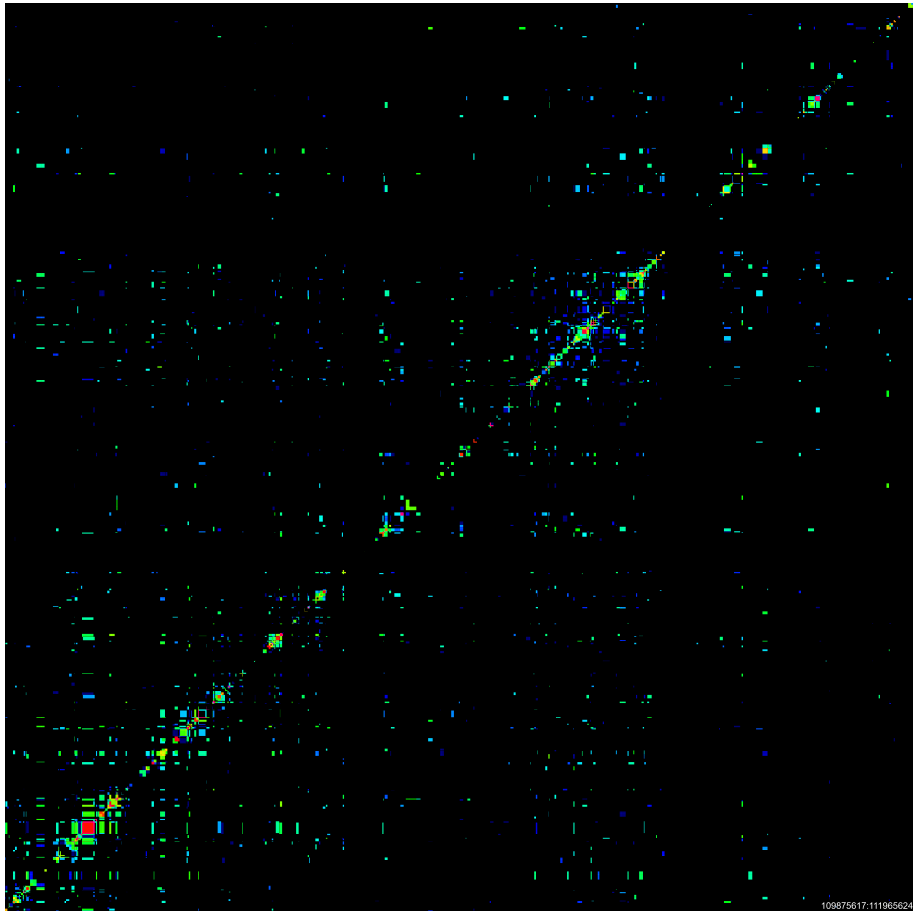
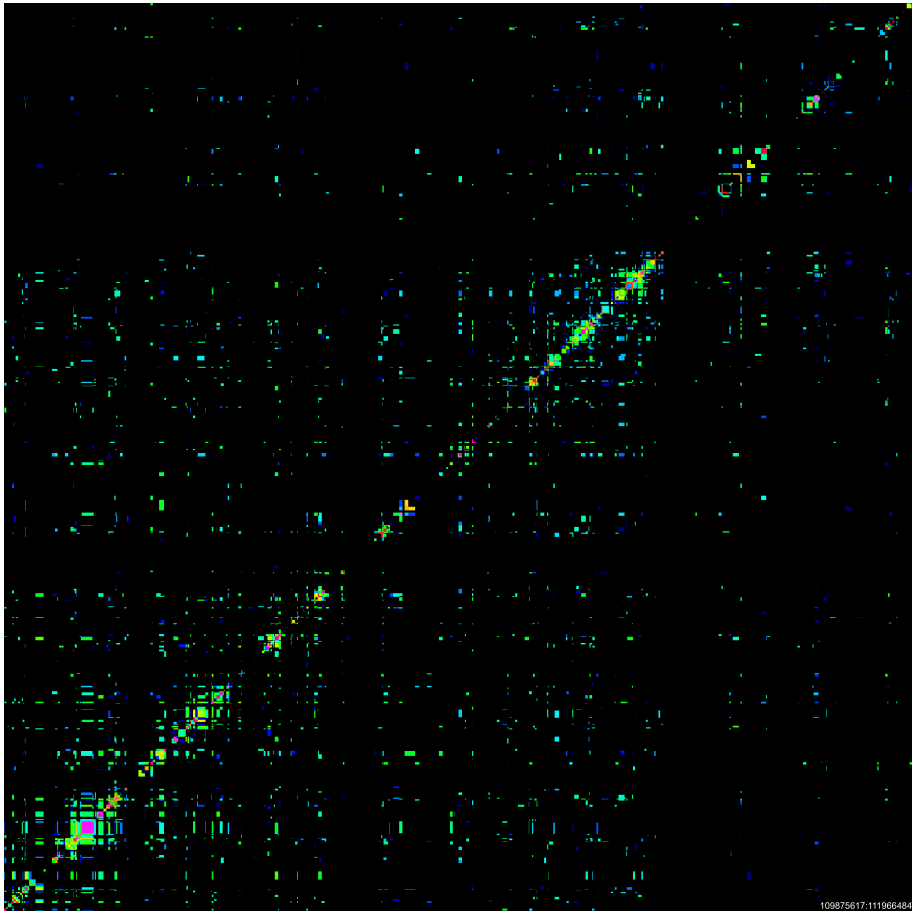
fetal brain



fetal liver

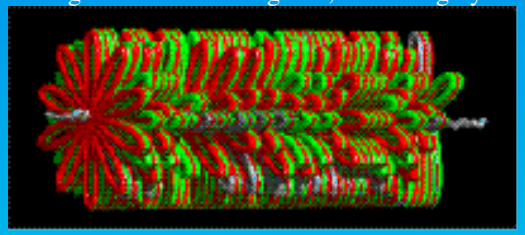


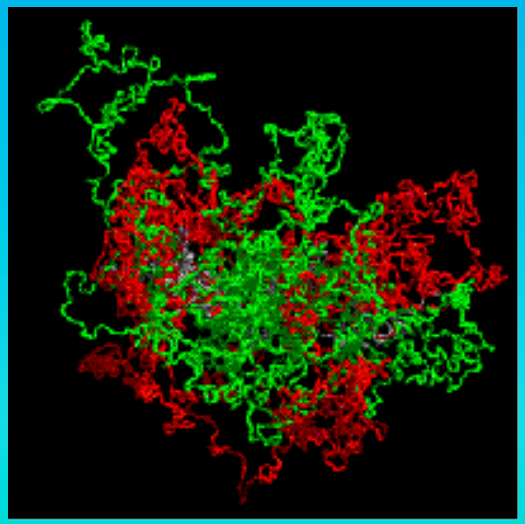
Simulation of Single Chromosomes

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.

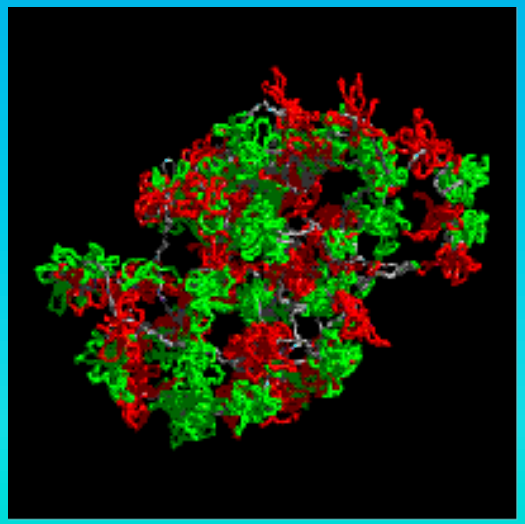
All models form chromosome territories with big voids and different chromatin morphologies. Experimental territory and subcompartment diameters agree best with an MLS model with 80 to 120 kbp loops and linkers.

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

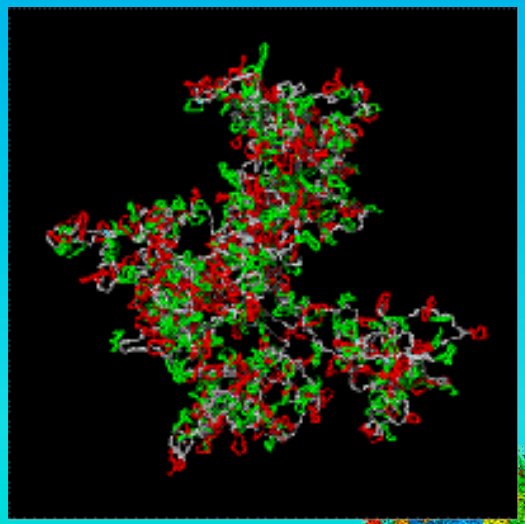




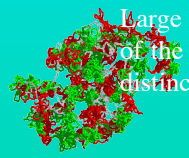
RW/GL model, loop size 5 Mbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromsome territory, thus forming no distinct features like in MLS model.

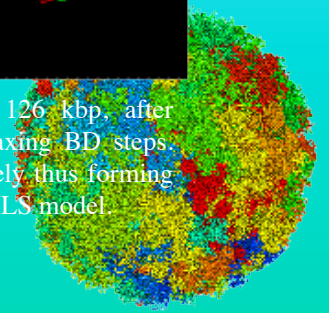


MLS model, loop size 126kbp, linker size 126 kbp, after ~50.000 MC and 1000 relaxing BD steps. Here rosettes form subcompartments as separated organizational and dynamic entities.



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





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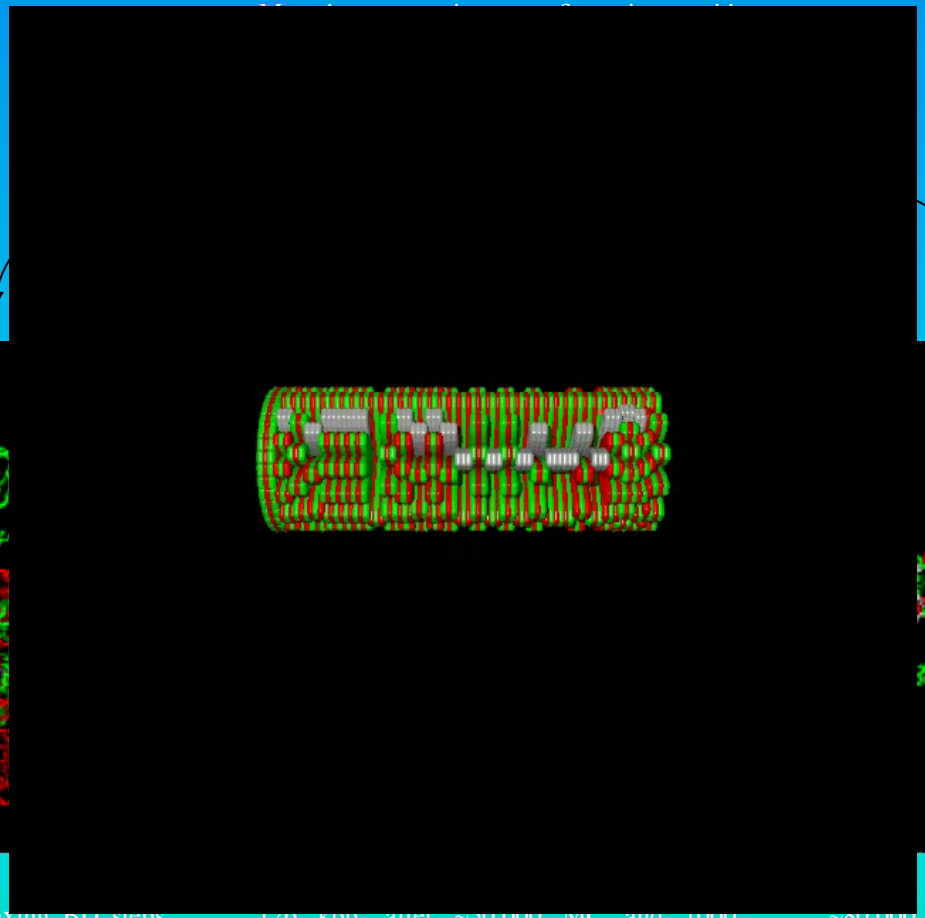
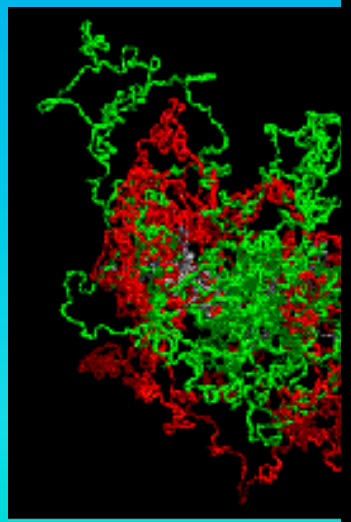
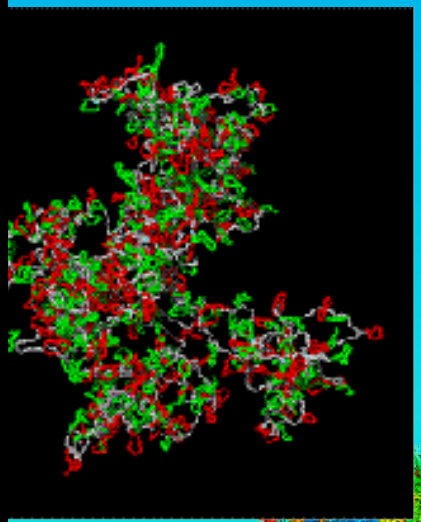
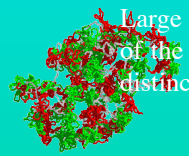


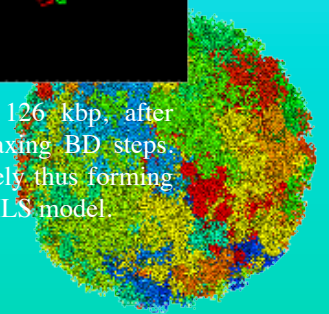


RW/GL model, loop size ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromsome territory, thus forming no distinct features like in MLS model.



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

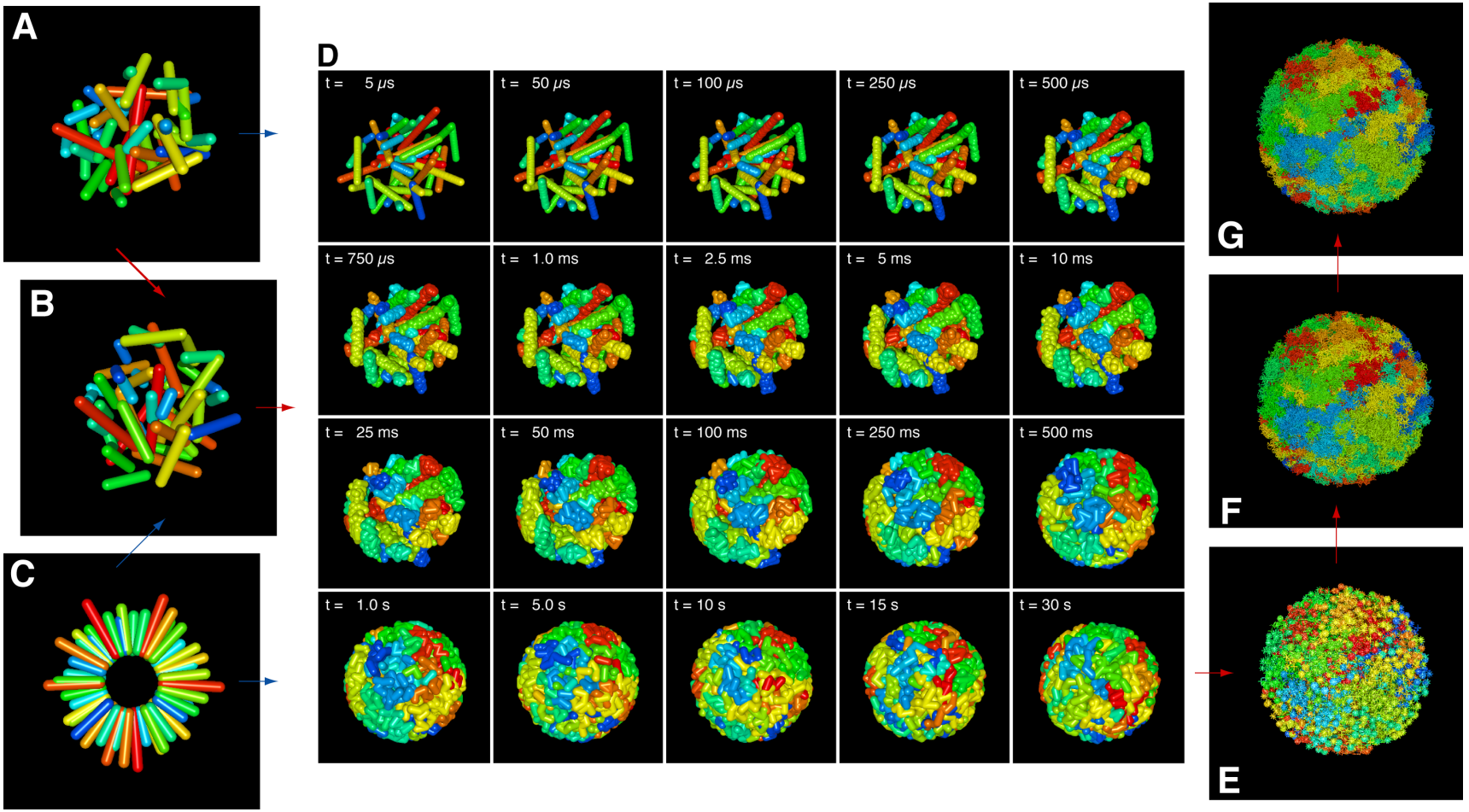




Simulation of Whole Nuclei with all 46 Chromosomes

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

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



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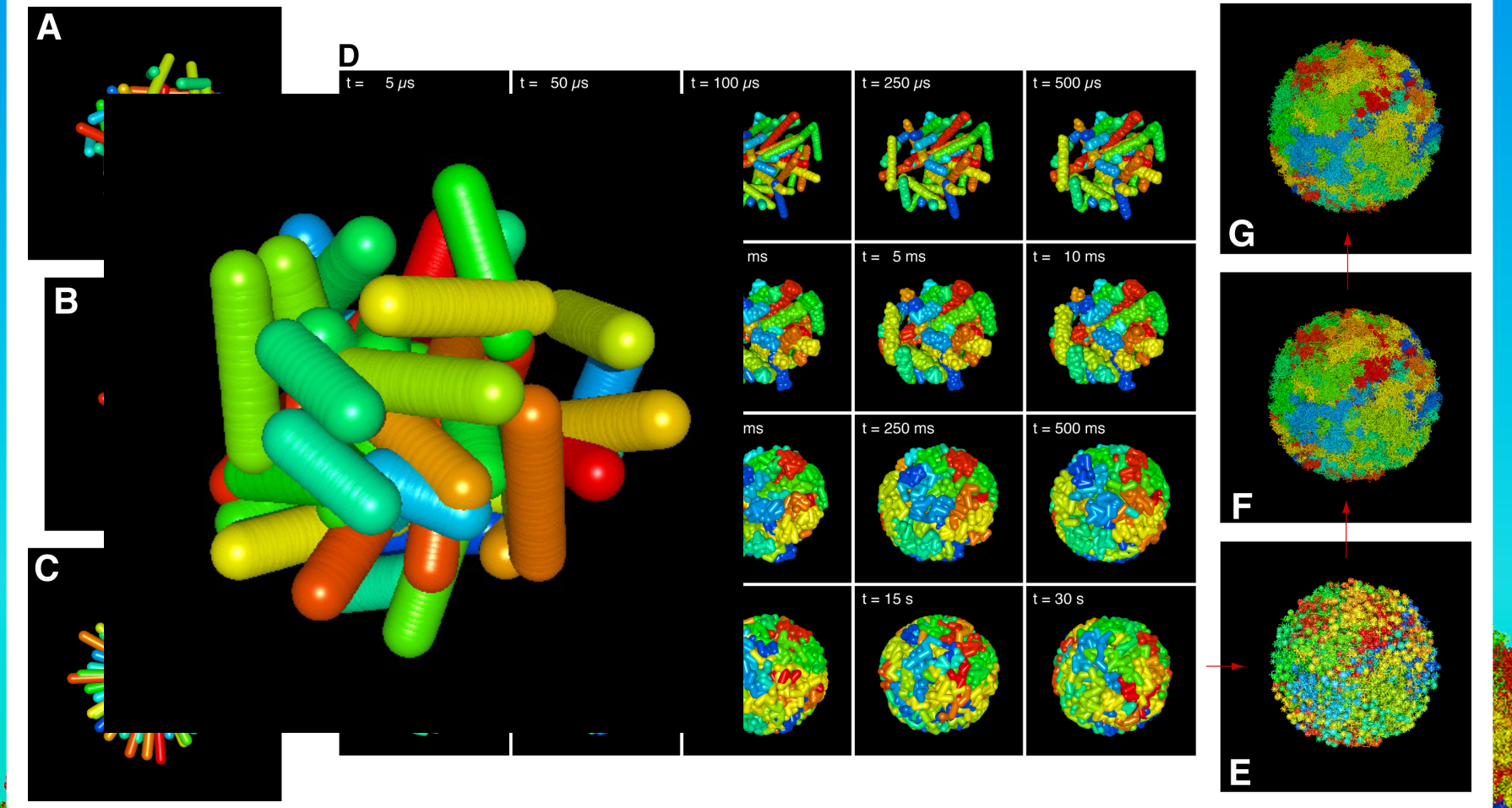

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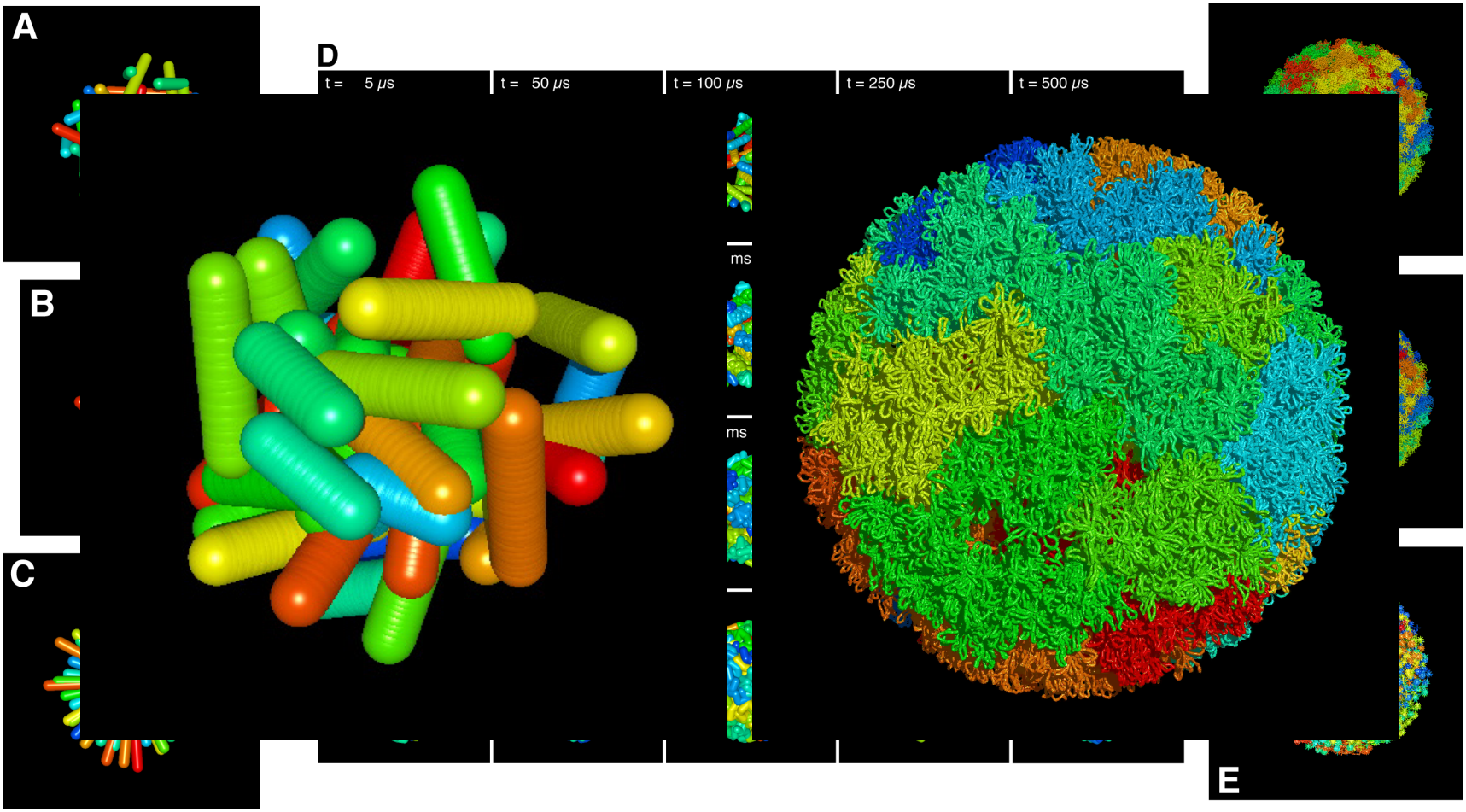

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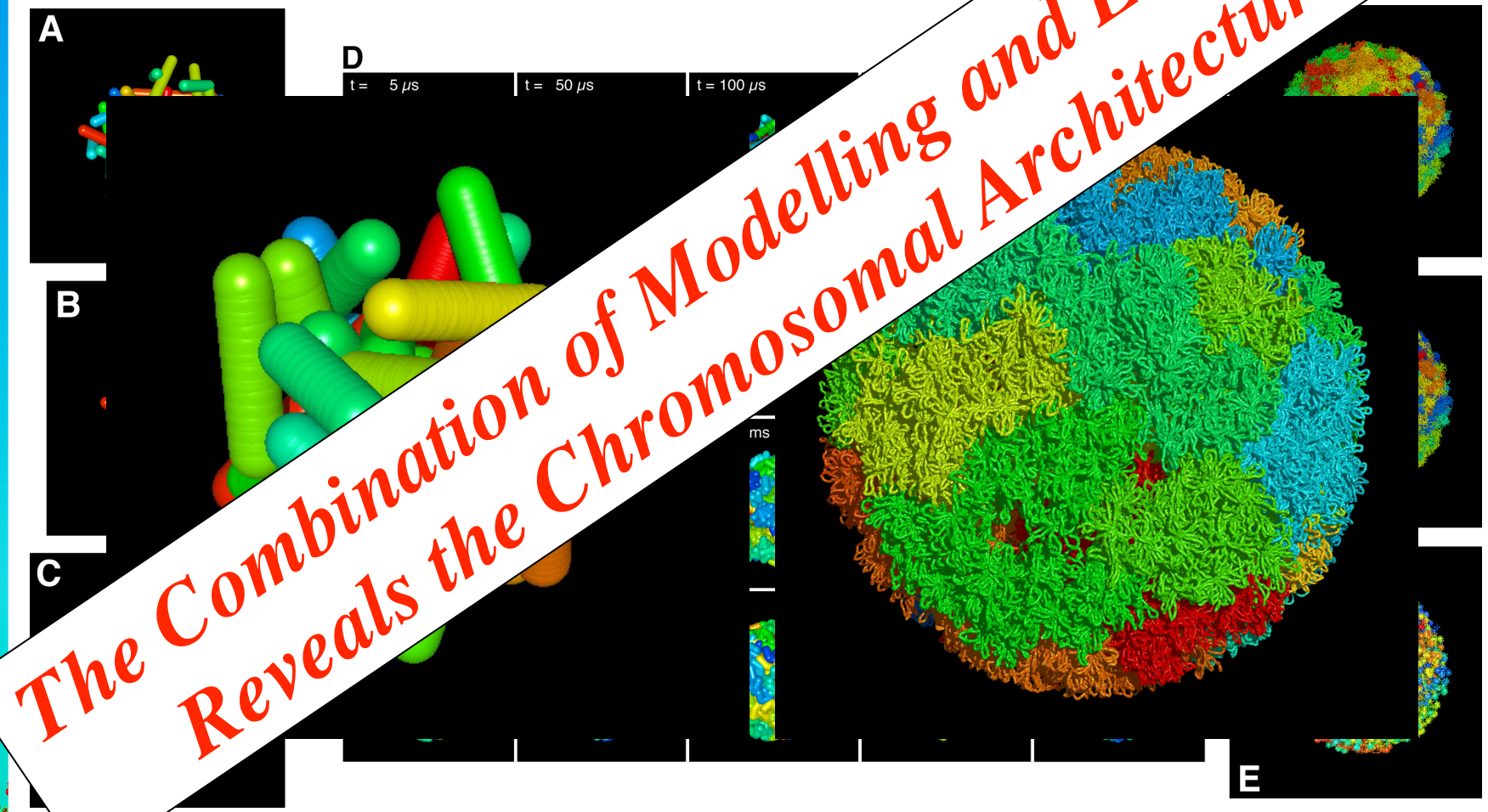
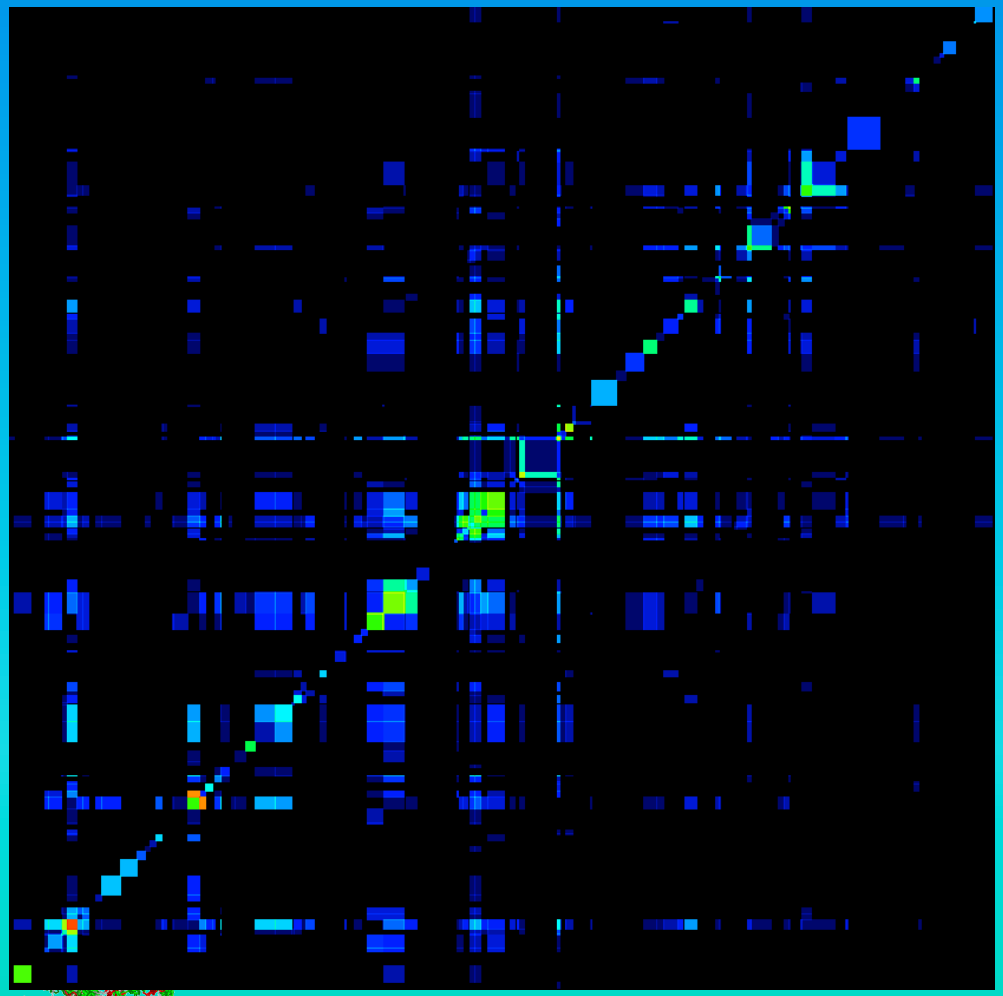
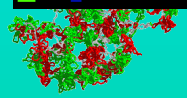


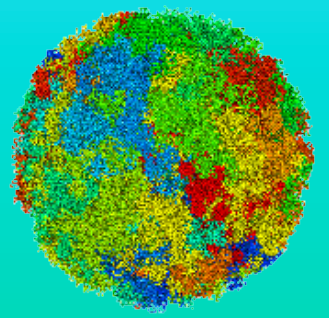
The Combination of Modelling and Experiment Reveals the Chromosomal Architecture !

Fine Structure of Loop Aggregates/Rosettes

Depending on the resolution, the loops within a domain an their arrangement in loop aggregates/rosettes can be shown as well as the details of how the loops are organized at their base as well as their aggregated rosette core: parallel loop fibres exist at the loop base with ~6kbp and these form the core.







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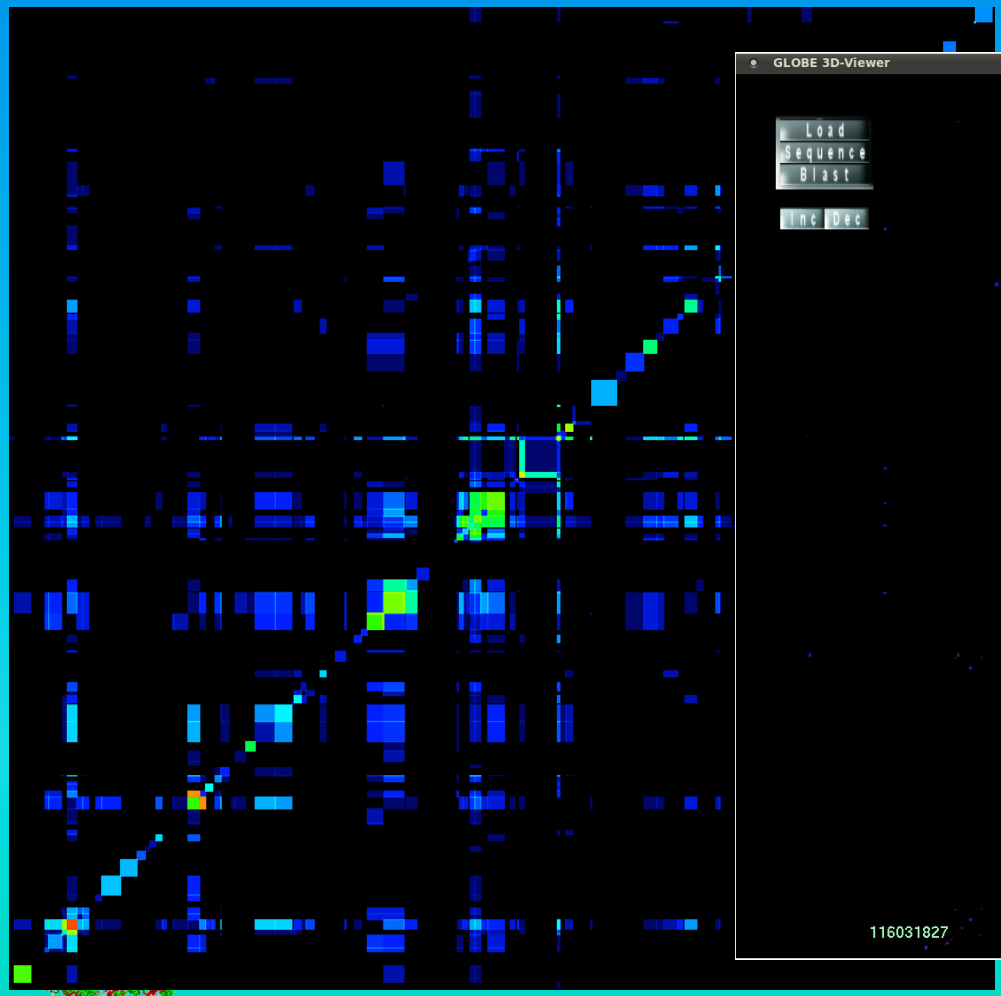

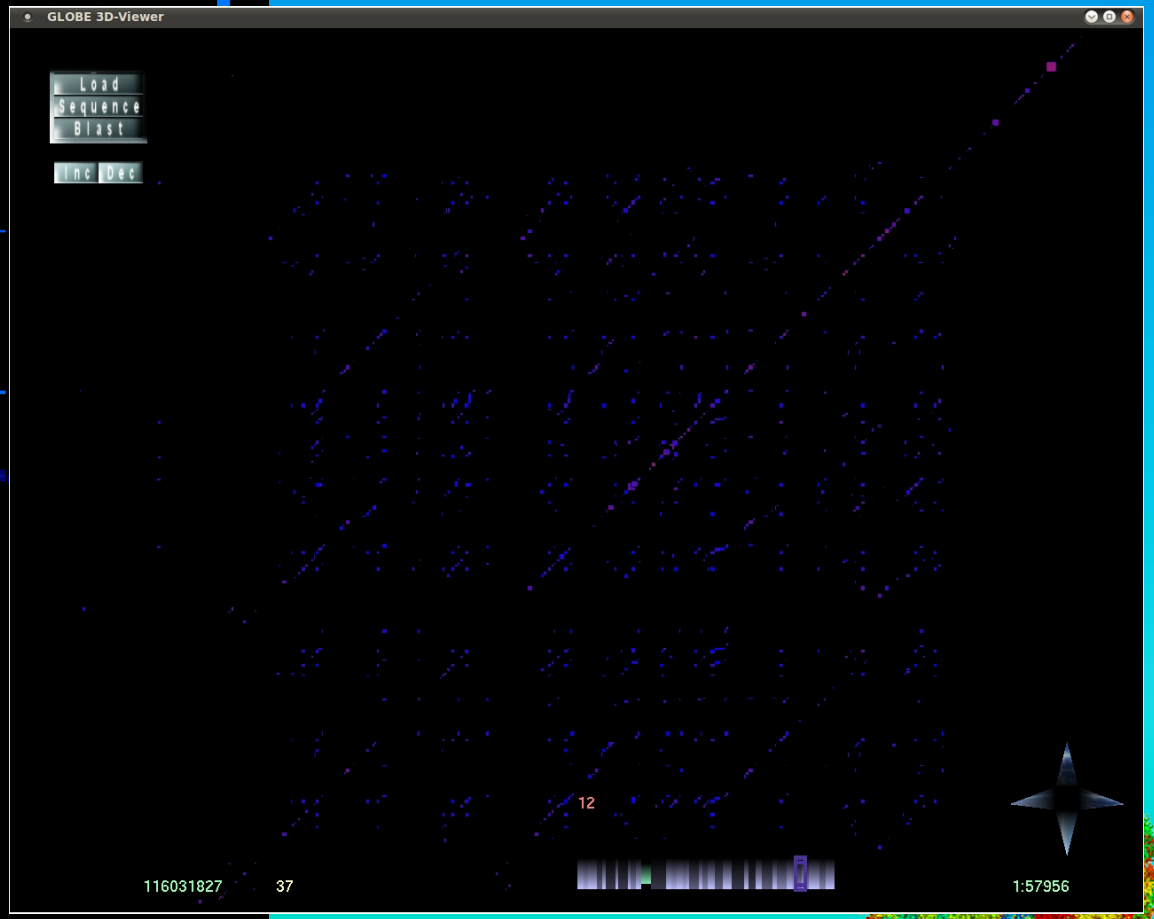

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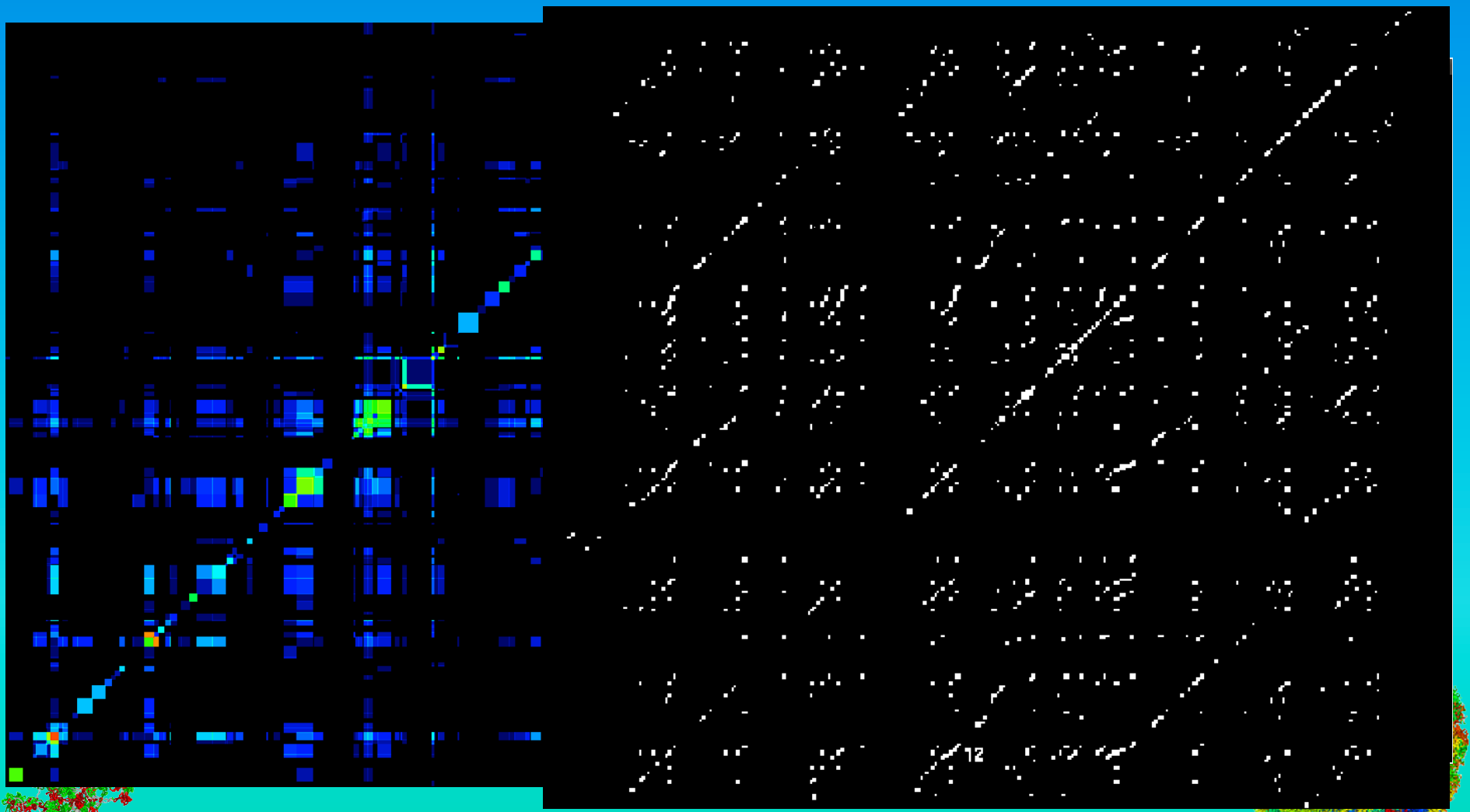

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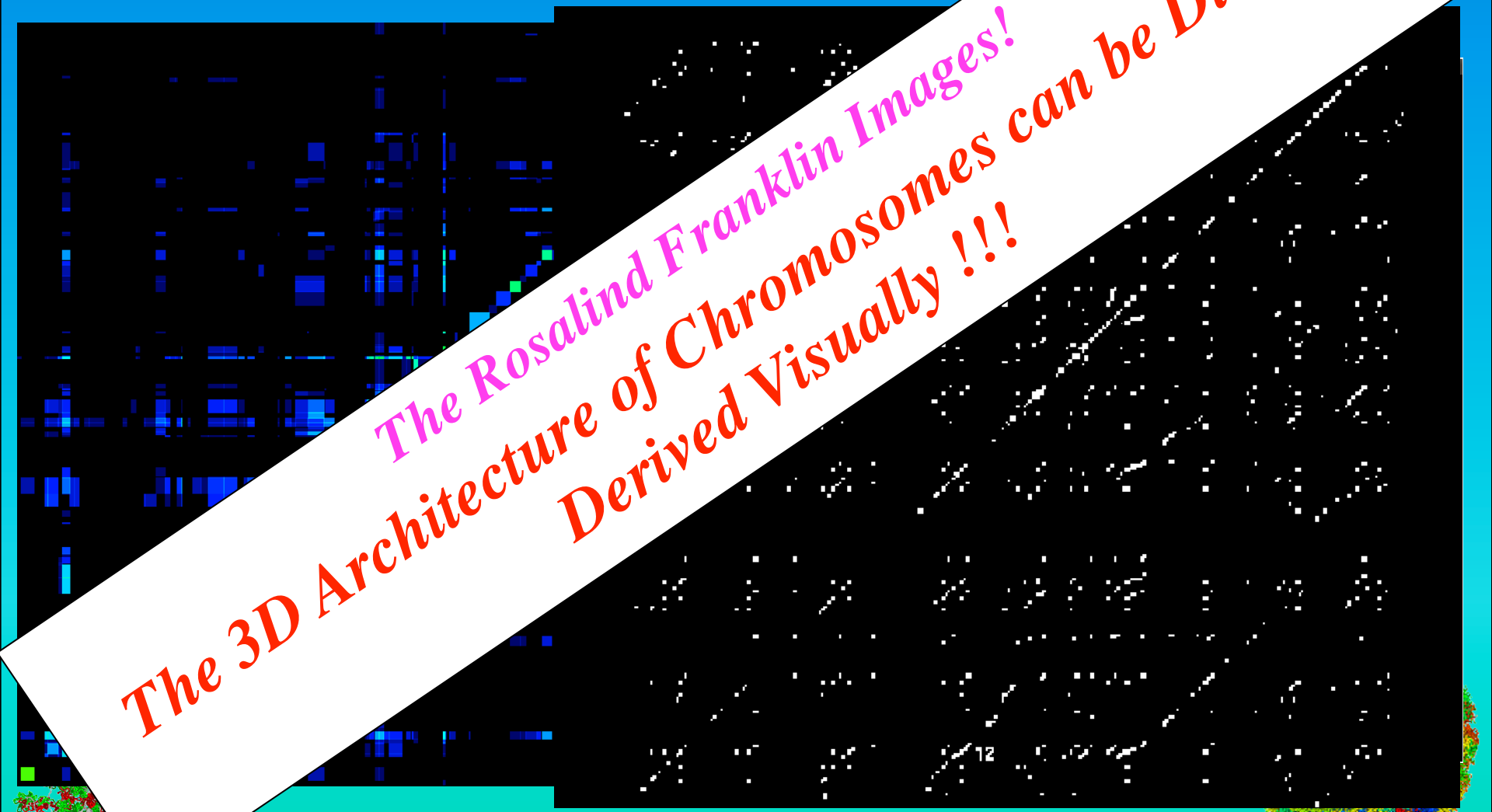
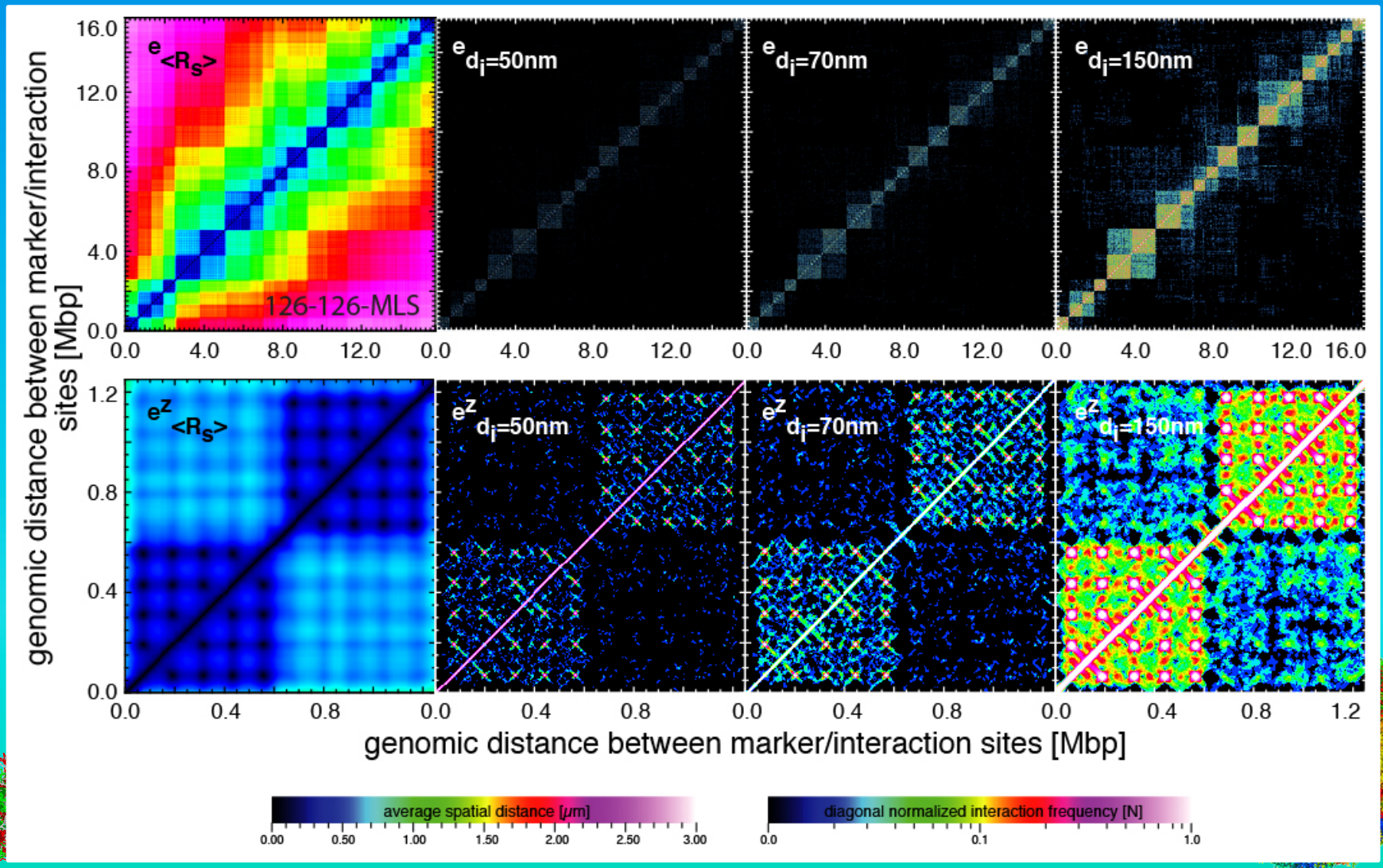


The Rosalind Franklin Images!

The 3D Architecture of Chromosomes can be Directly Derived Visually !!!

Simulated Interaction Maps

Simulated spatial distance maps as well as simulated interaction maps result in the representation of every parameter variation, and also exhibit the fine-structure describing the loop base as well as rosette core. Thus from the quasi-fibre to the entire chromosome the architecture can be understood in detail.



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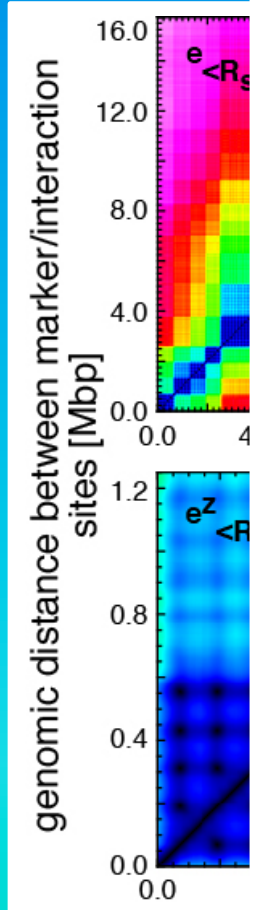
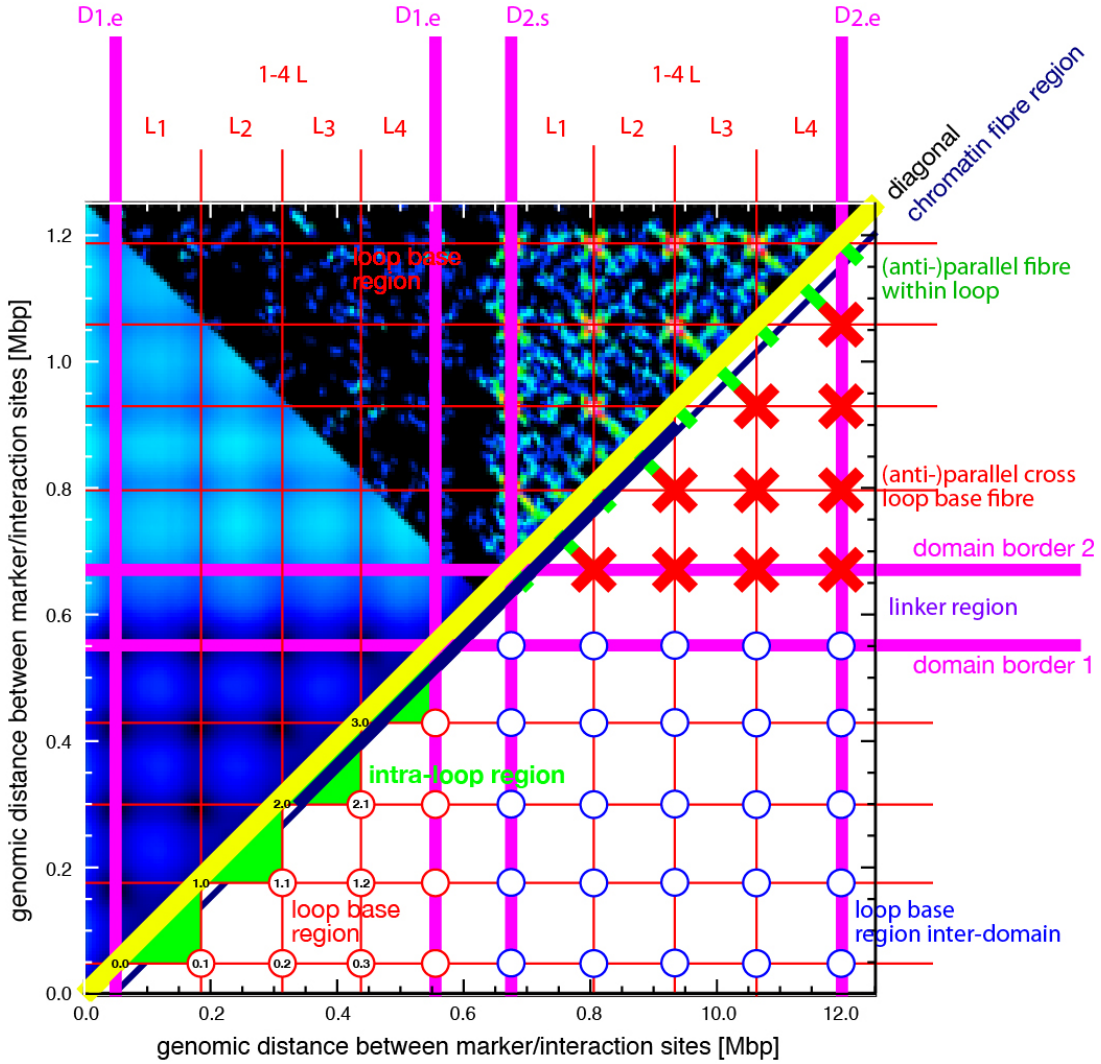
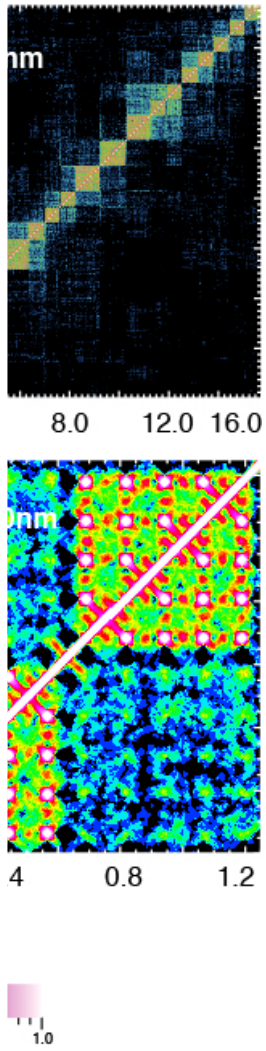
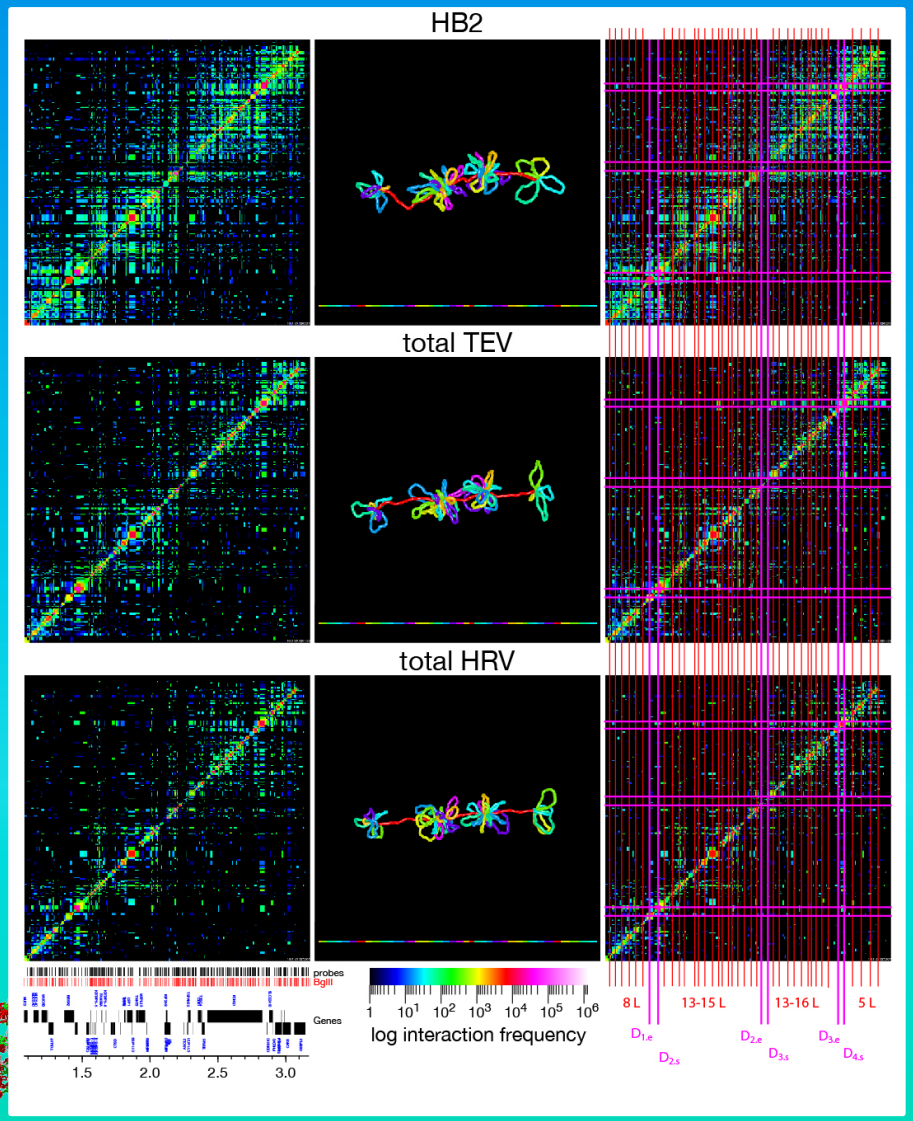


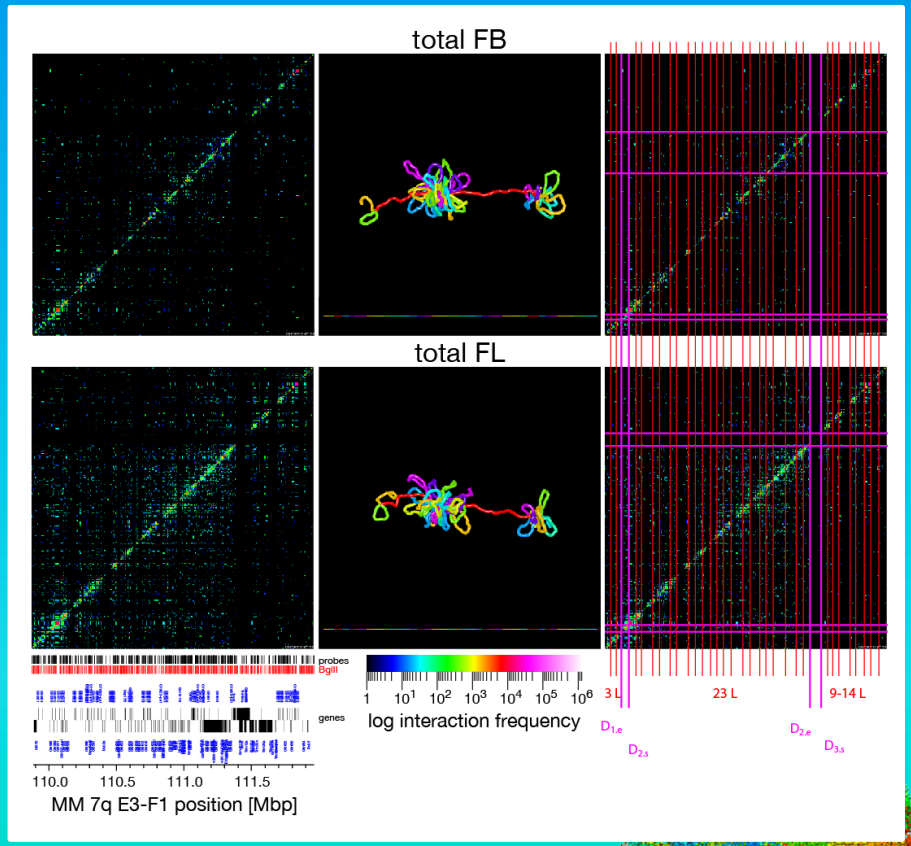




Variation of a Consensus Architecture Scheme

The difference between different cell types, functional states or even species is minor despite depending on the region. From this, the chromatin fibre conformation, loop position, and their association into loop aggregates/ rosettes can be derived, simulated by polymer models and finally visualized.





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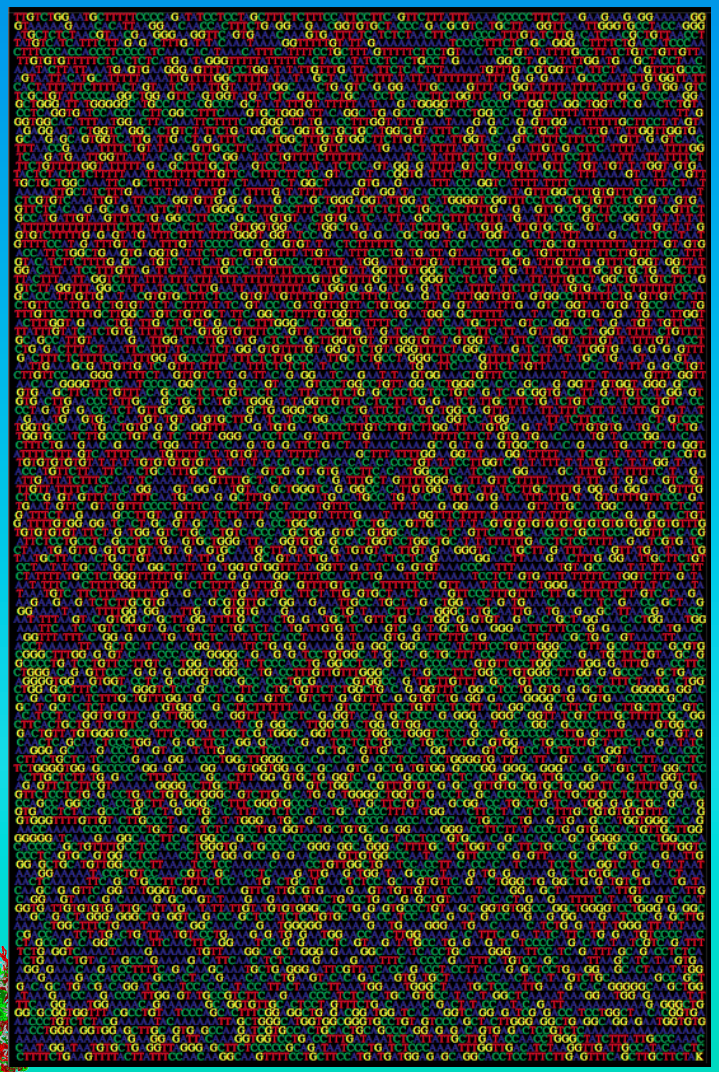
DNA Sequence Organization

Determination of the concentration fluctuation function $C(l)$ and its local slope the correlation coefficient $\delta(l)$ 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.



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

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

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

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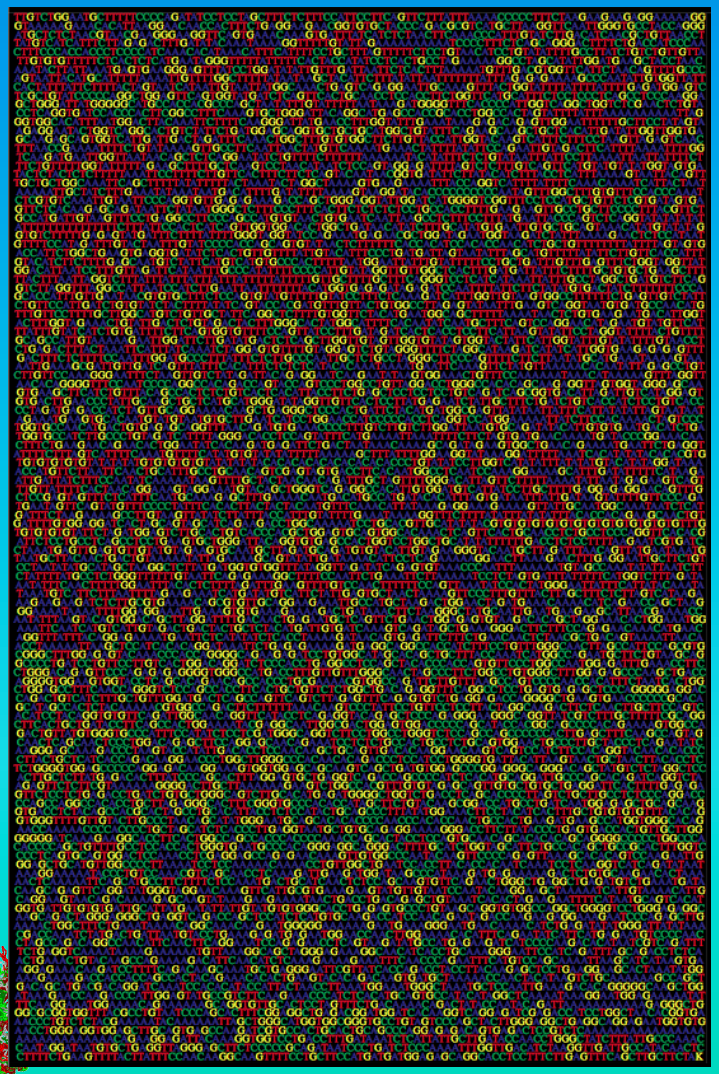

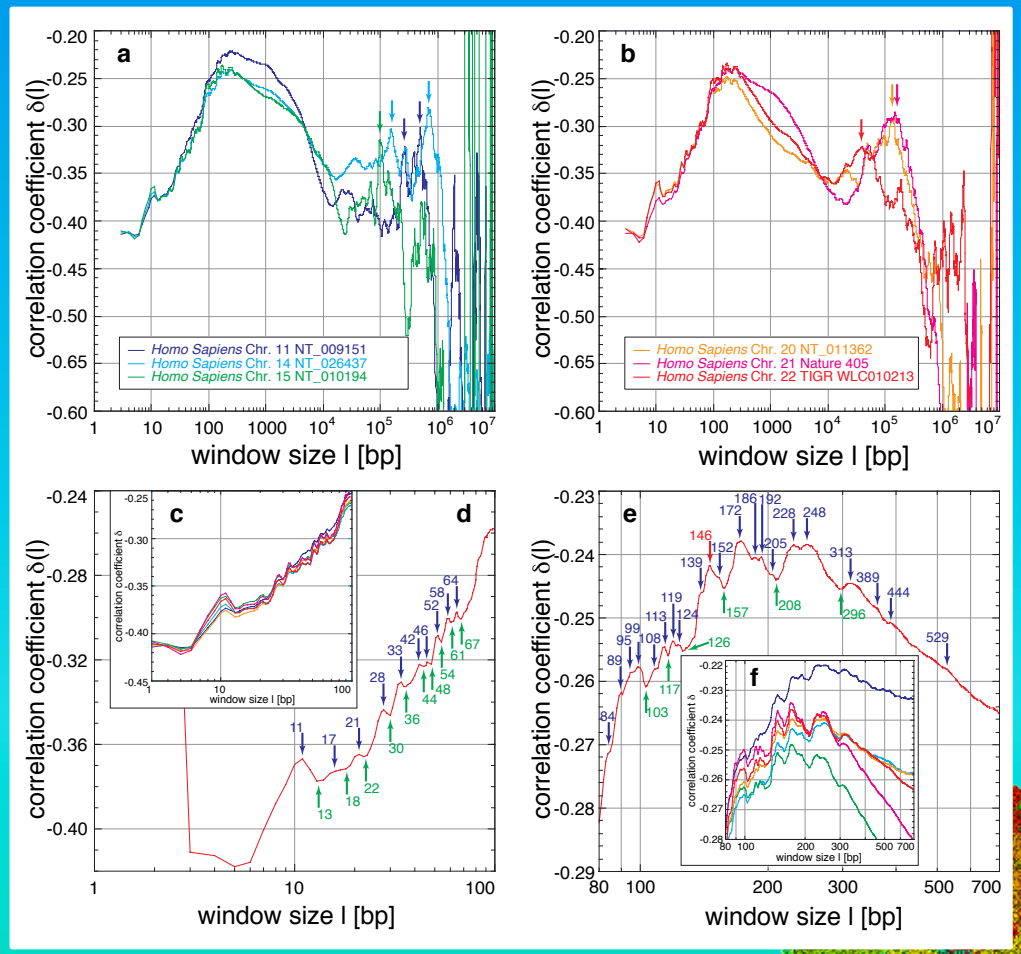

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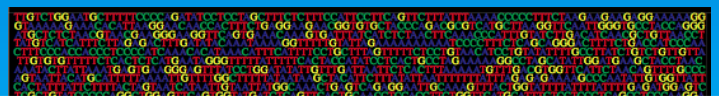

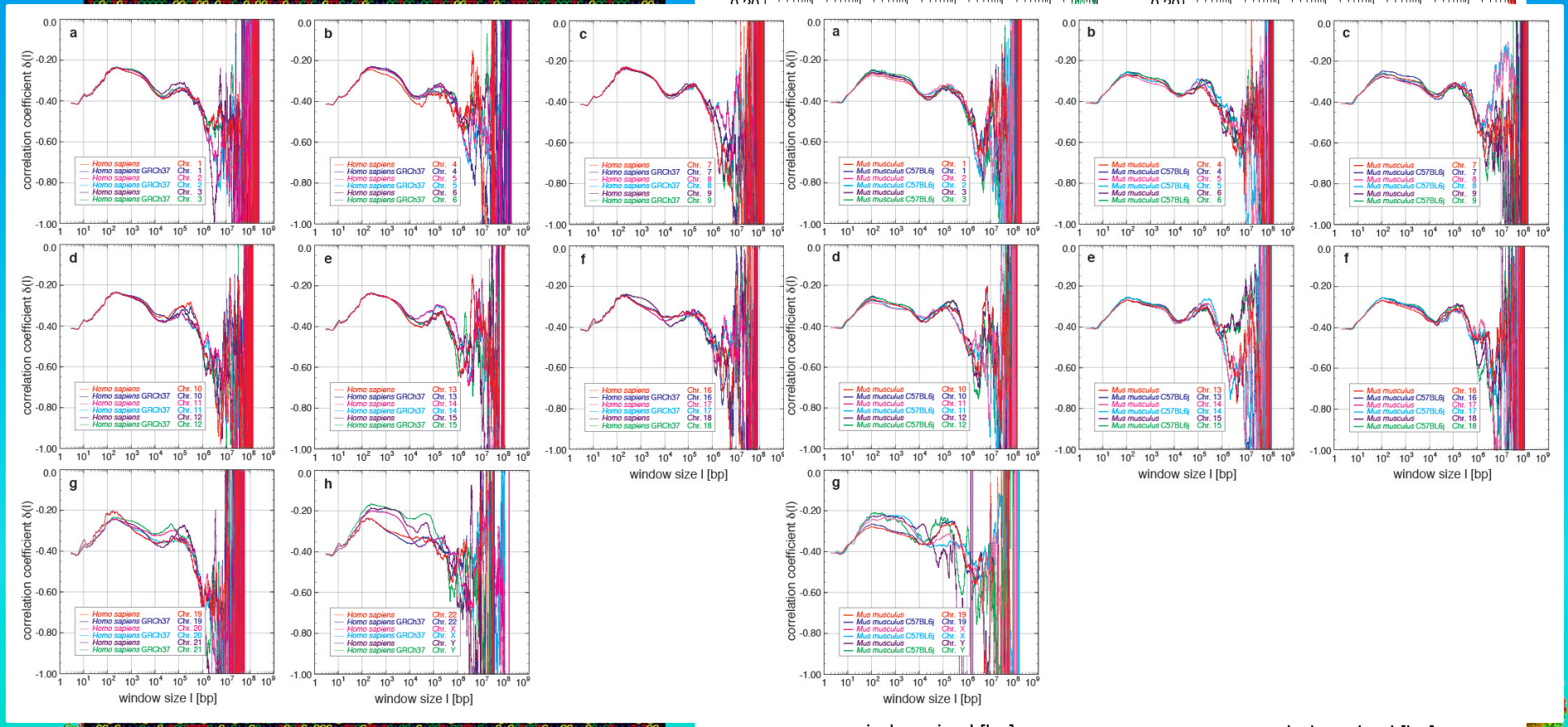

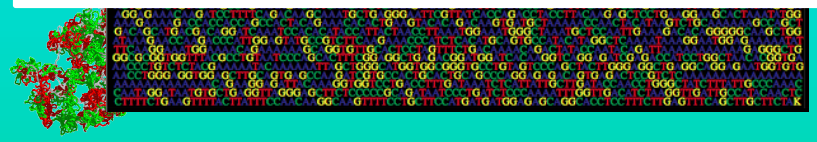

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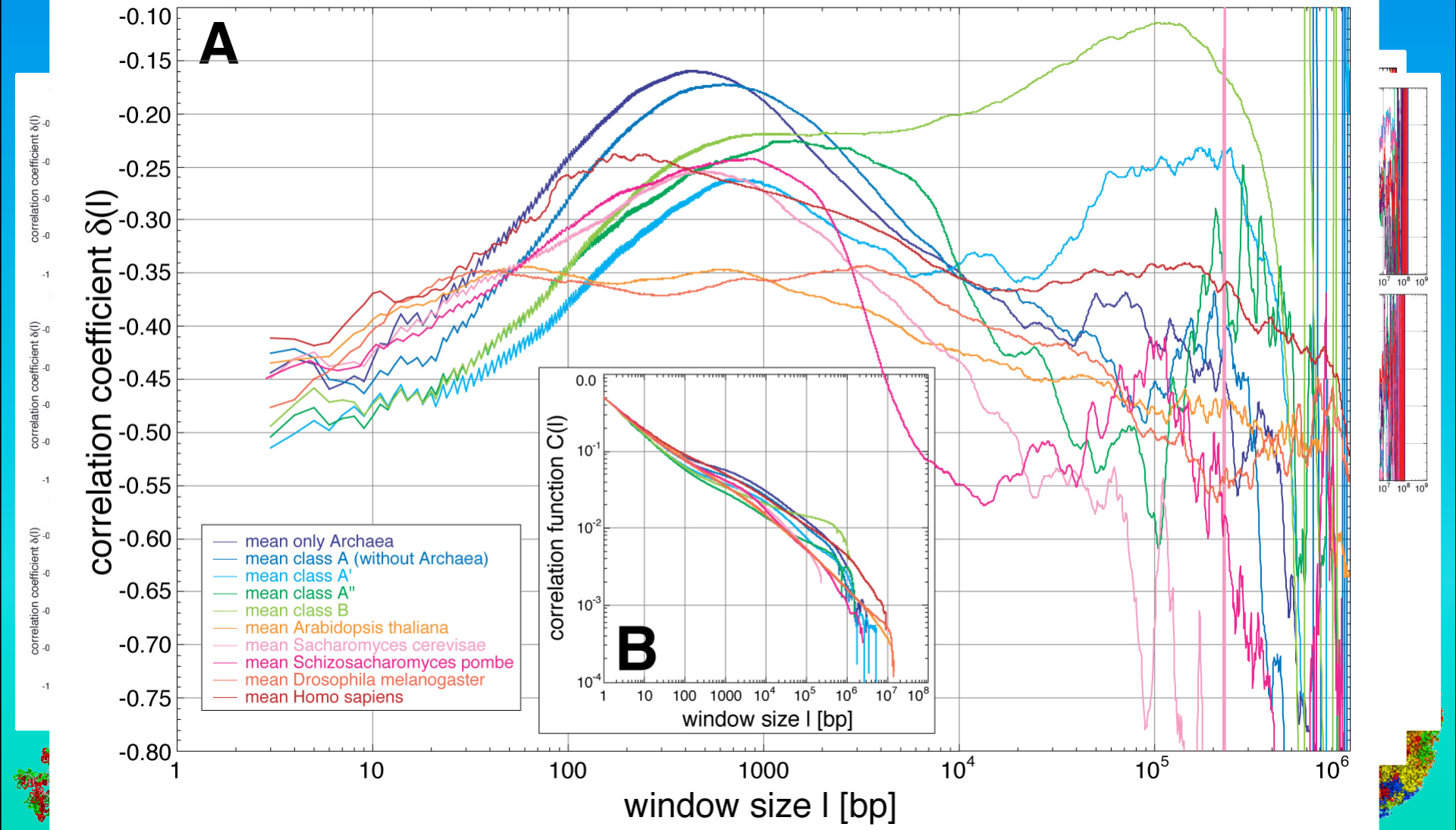

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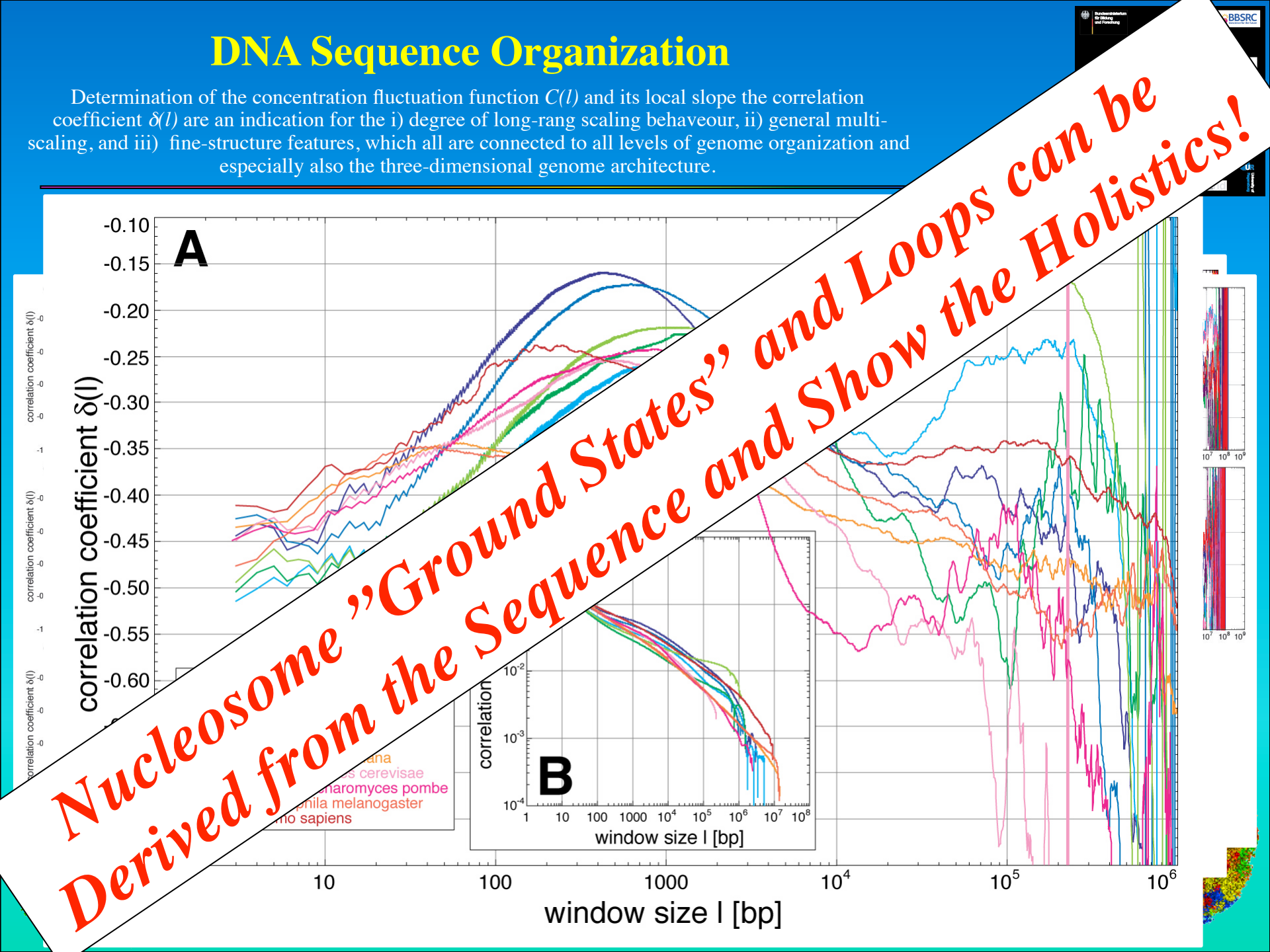
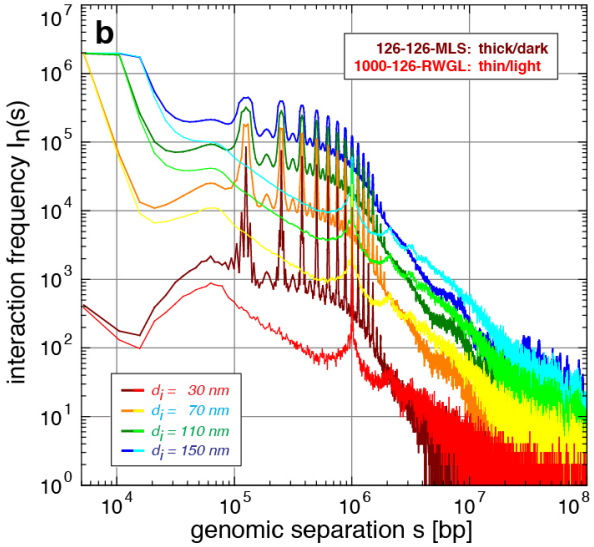
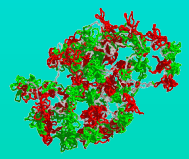


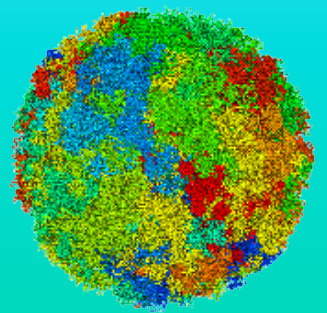
Nucleosome "Ground States" and Loops can be Derived from the Sequence and Show the Holistics!

Scaling Analysis

Scaling analysis show again the entire bandwidth of architectural effects in an aggregated manner. Beyond, they show the scale bridging of the structures and the evolutionary holistic entanglement between the 3D architecture and the DNA sequence organization itself.







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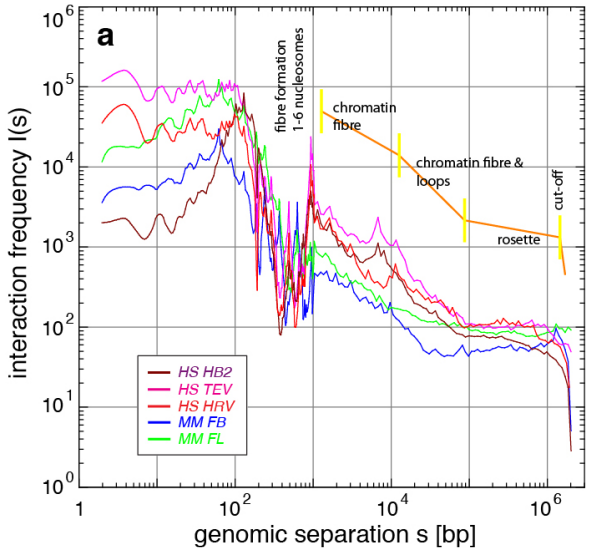

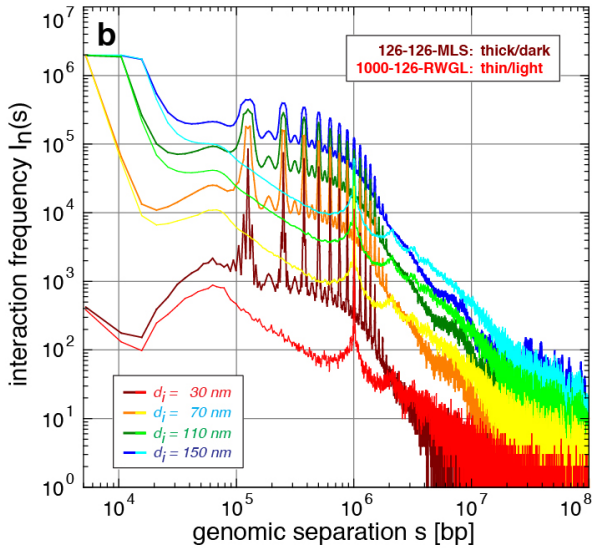

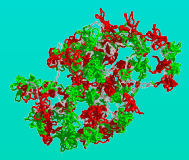

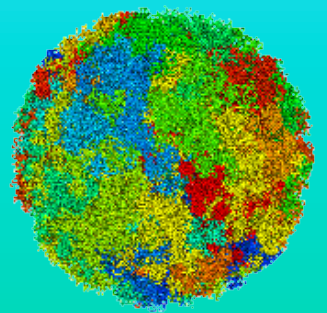

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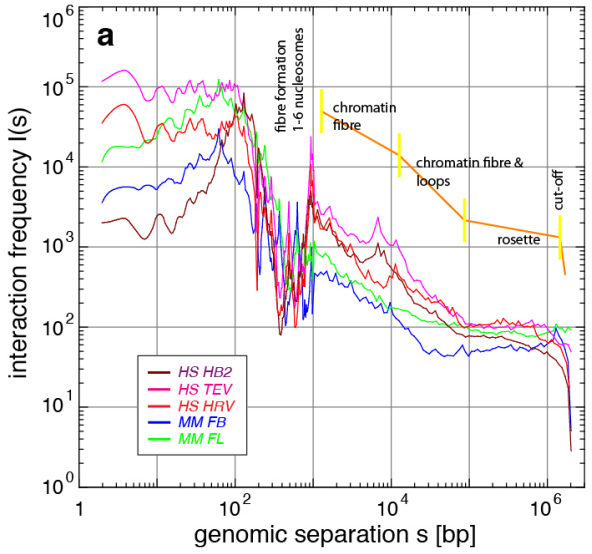

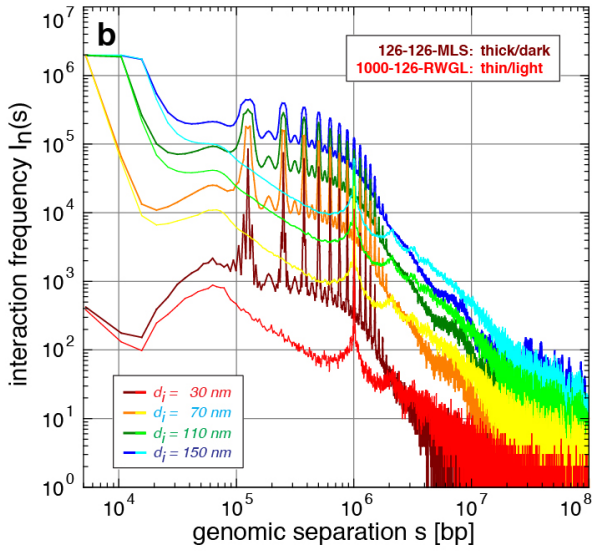

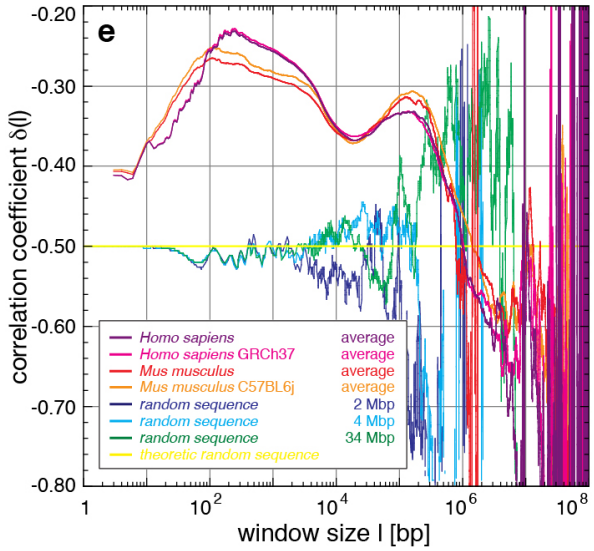

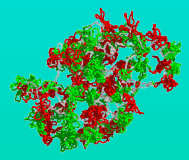

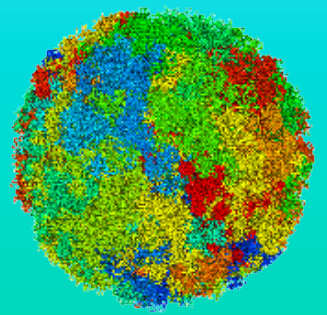

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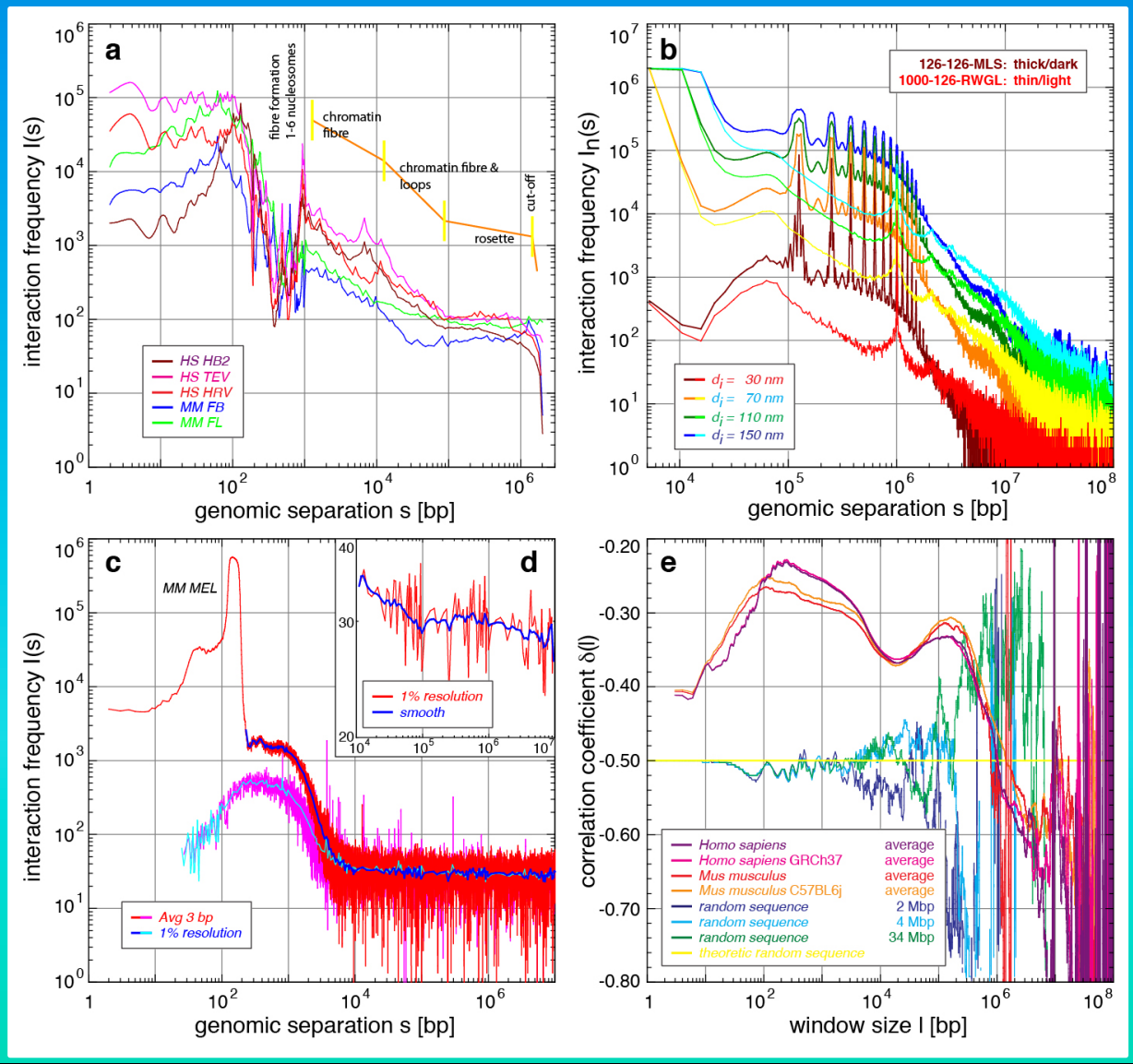

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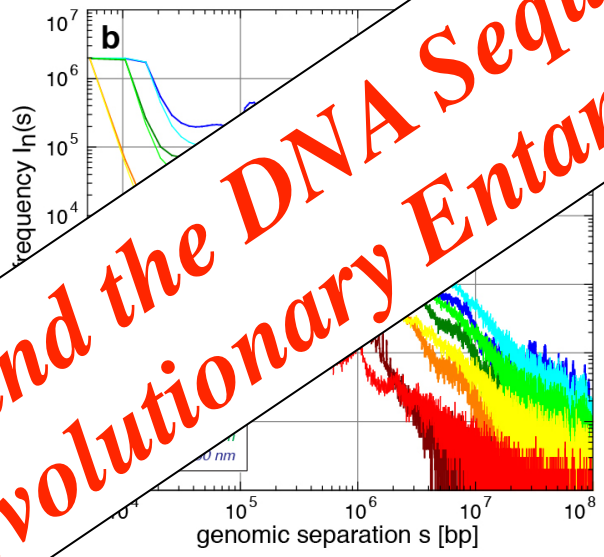
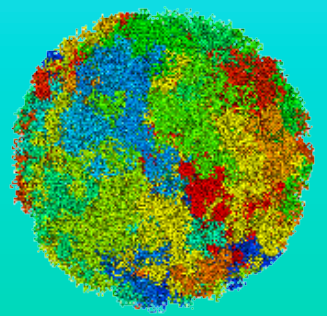
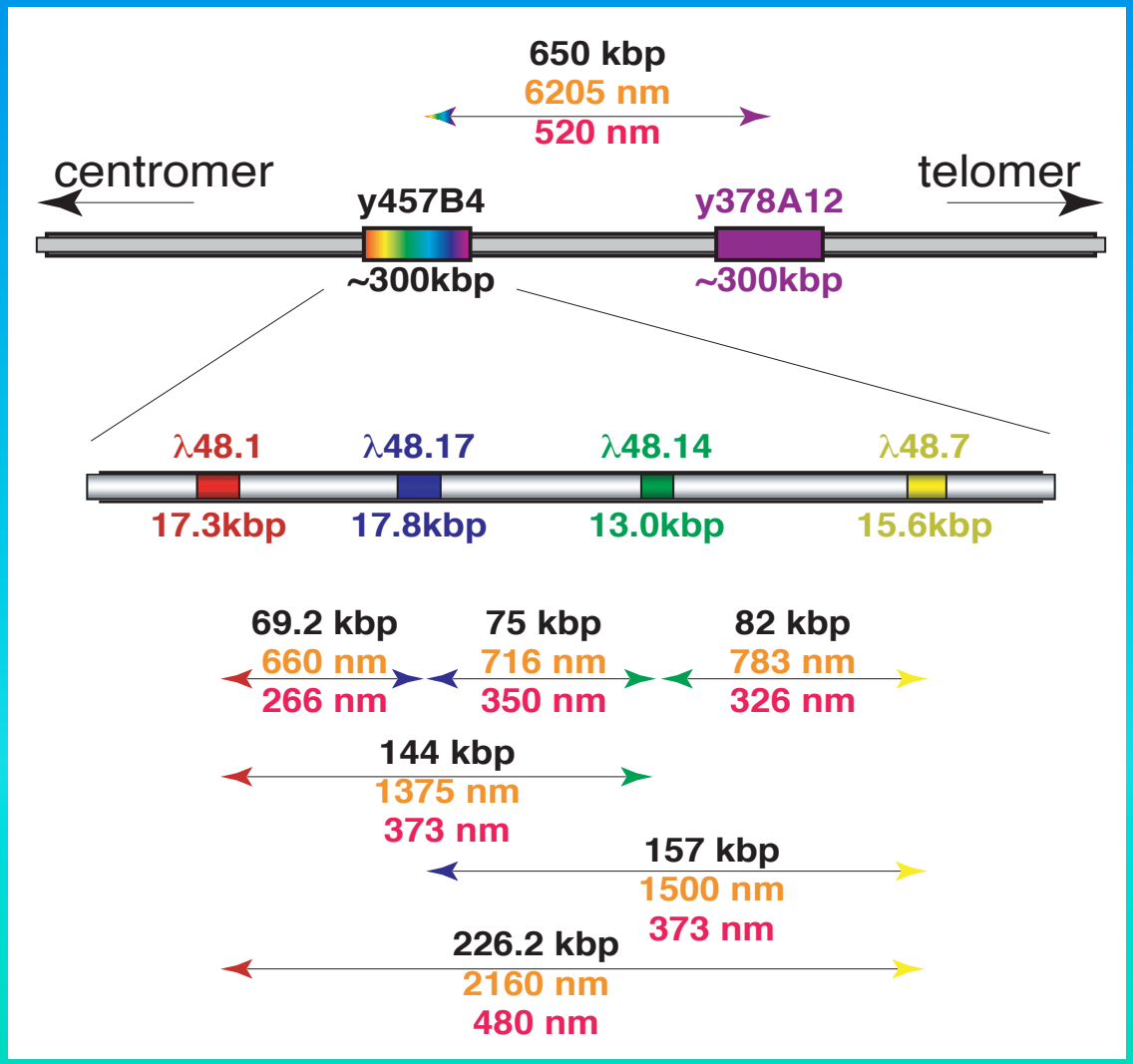


The 3D Architecture and the DNA Sequence Organization are Evolutionary Entangled!



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.



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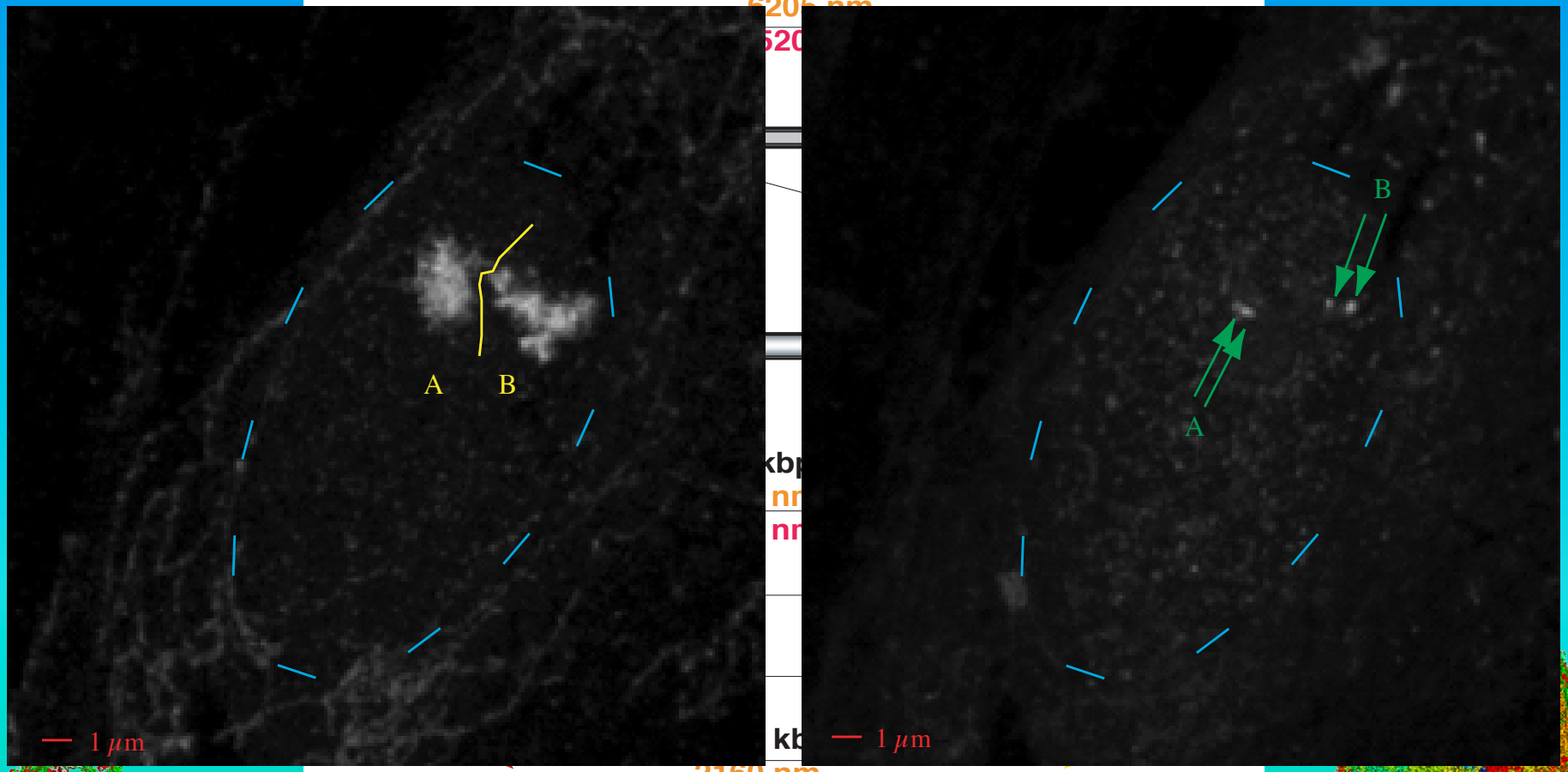

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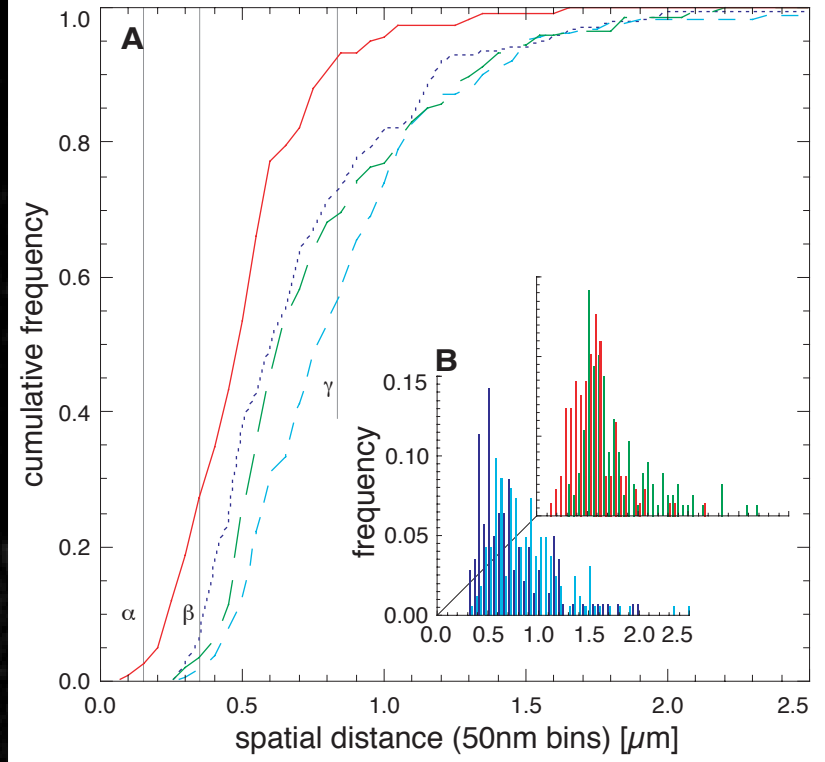
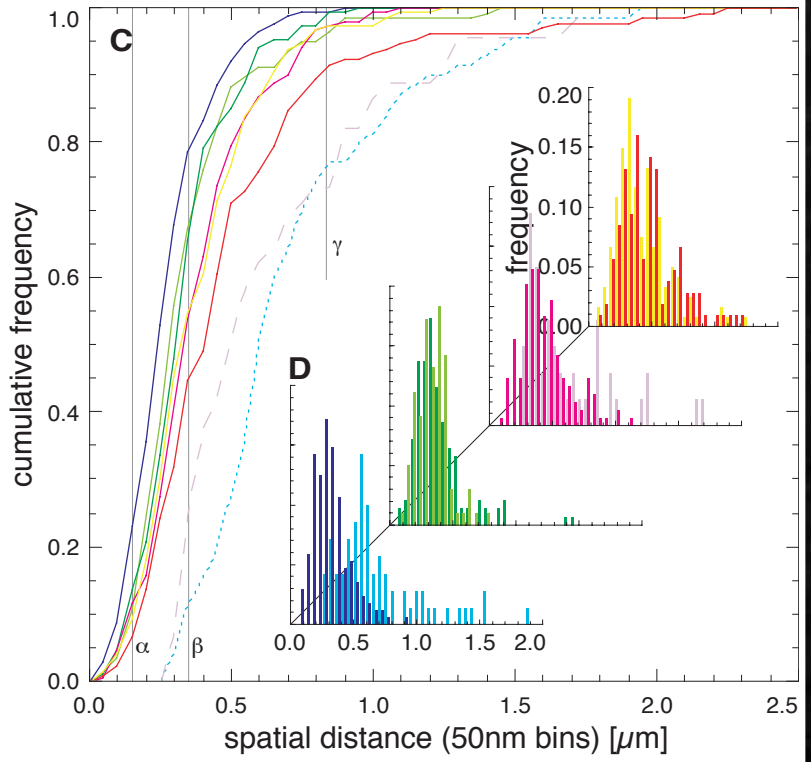
650 kbp





1 μm

1 μm

2160 nm

480 nm

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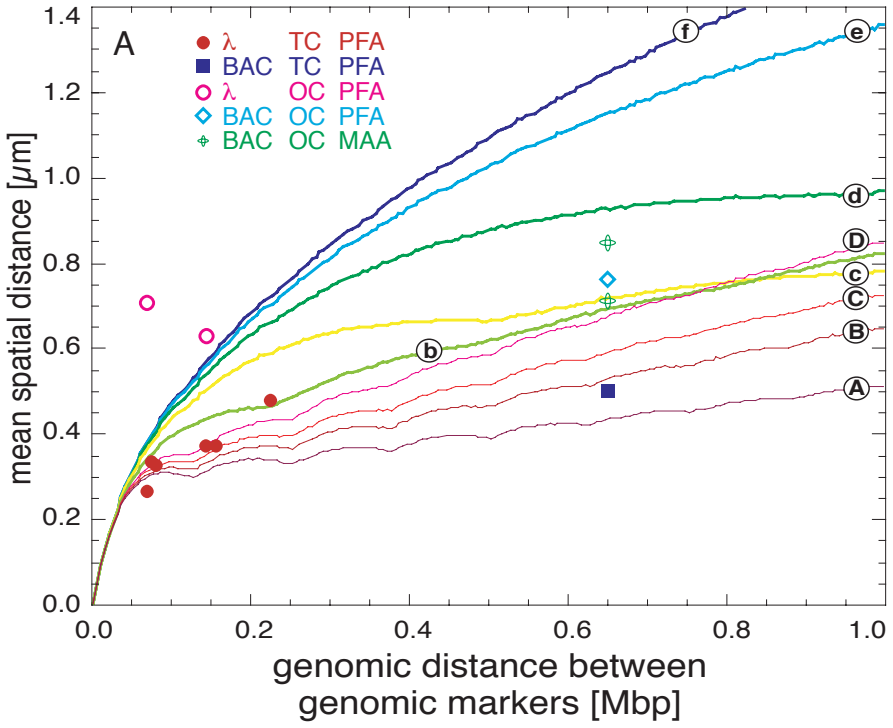

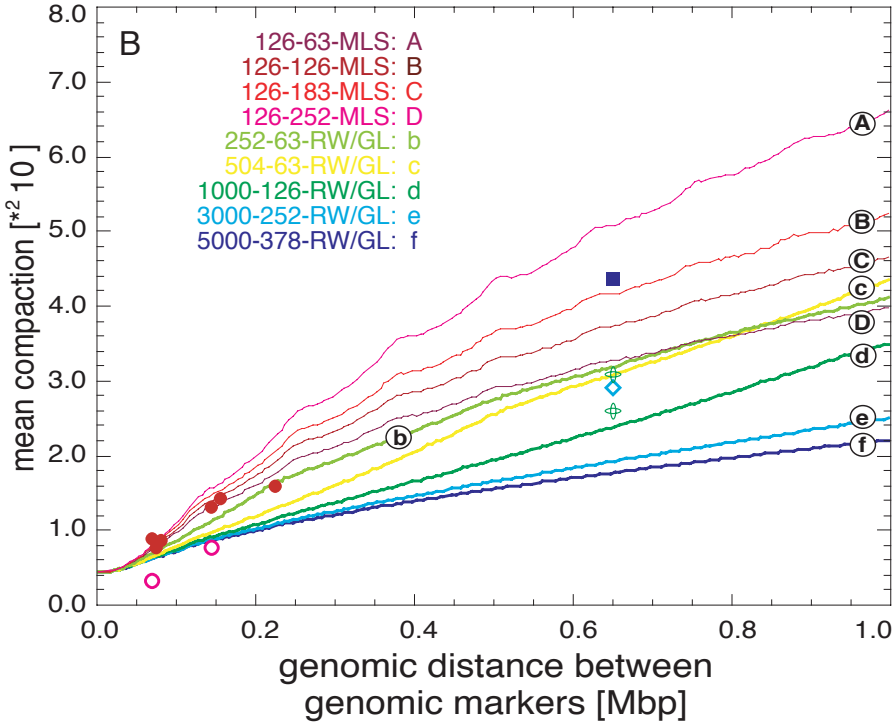

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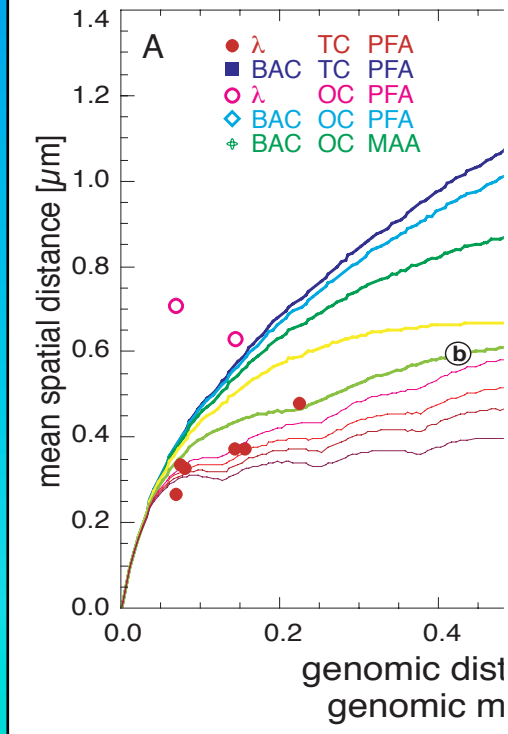
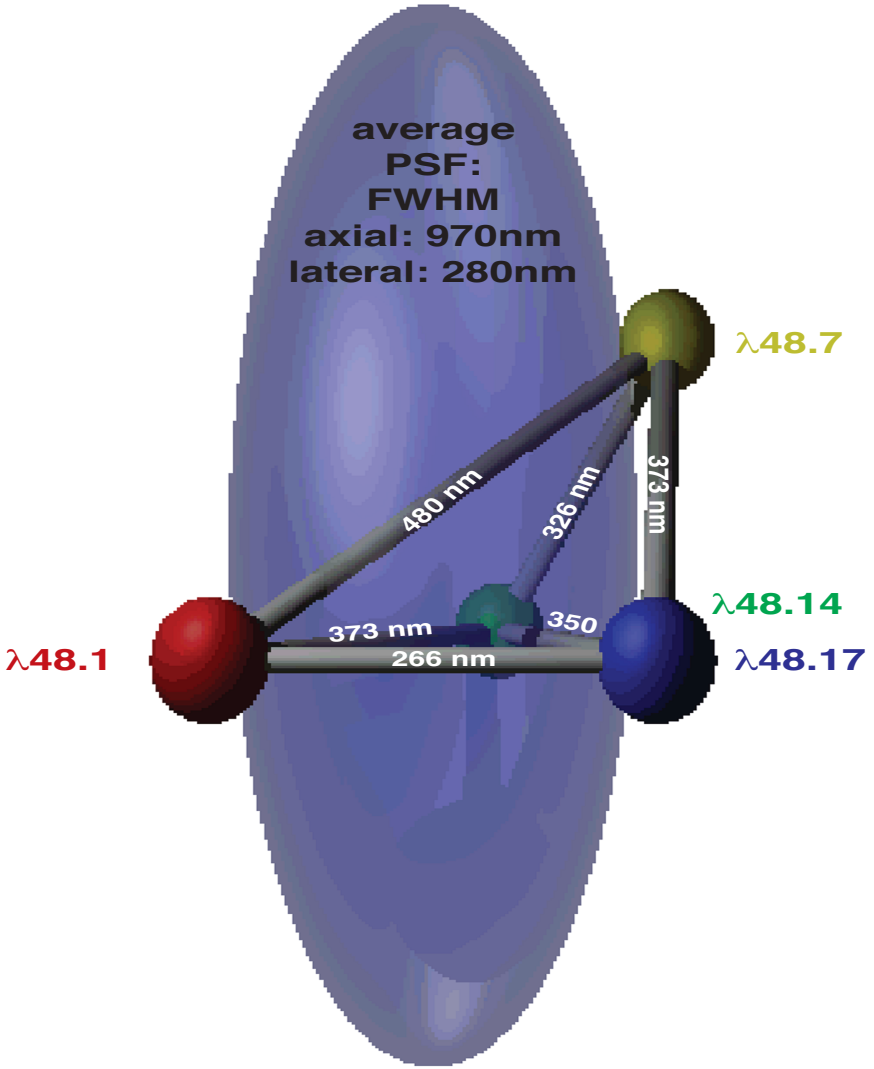
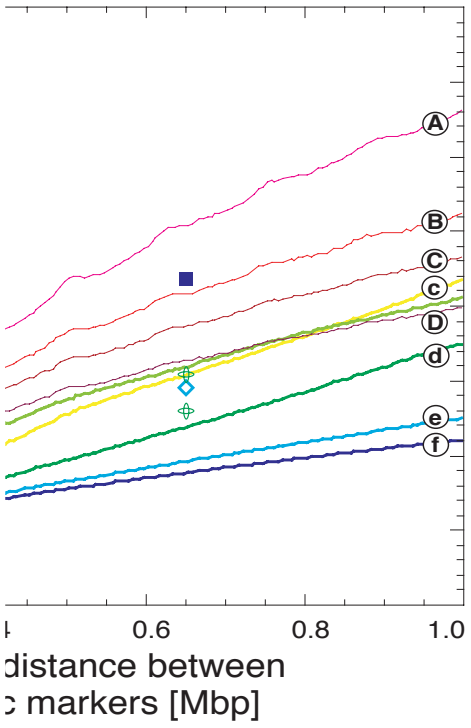
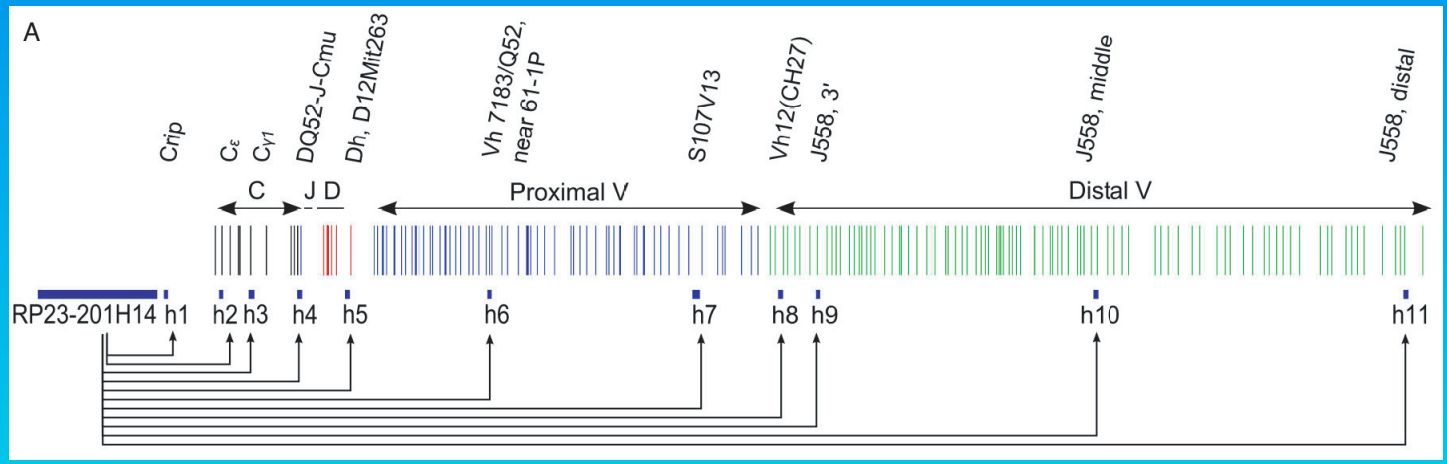


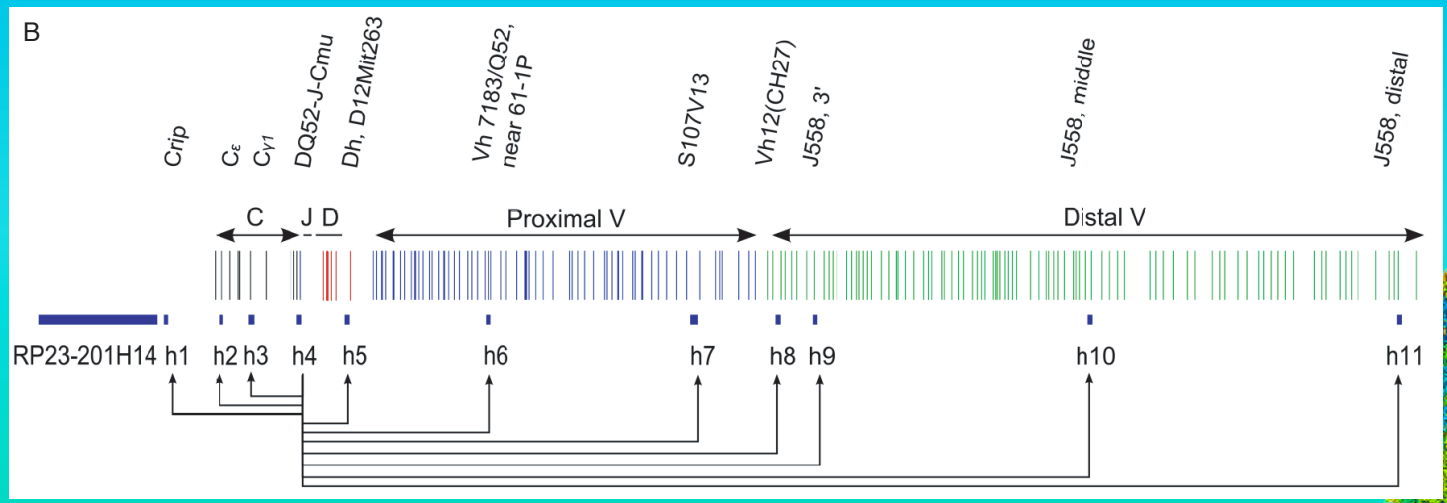




3D Archticture & Function of the IgH Locus

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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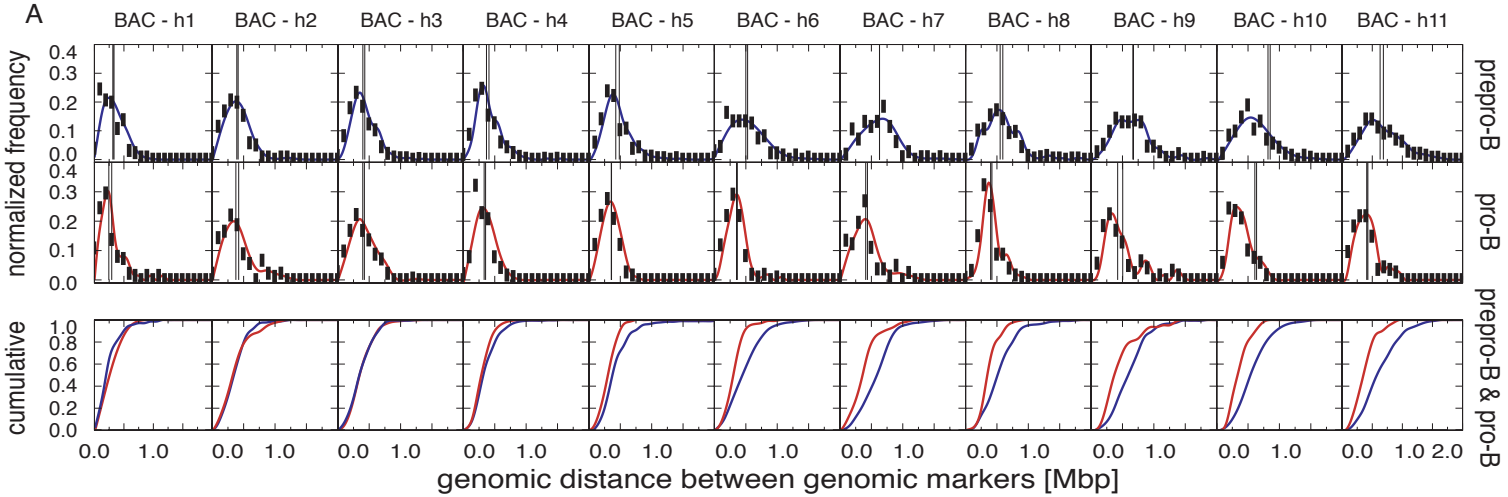

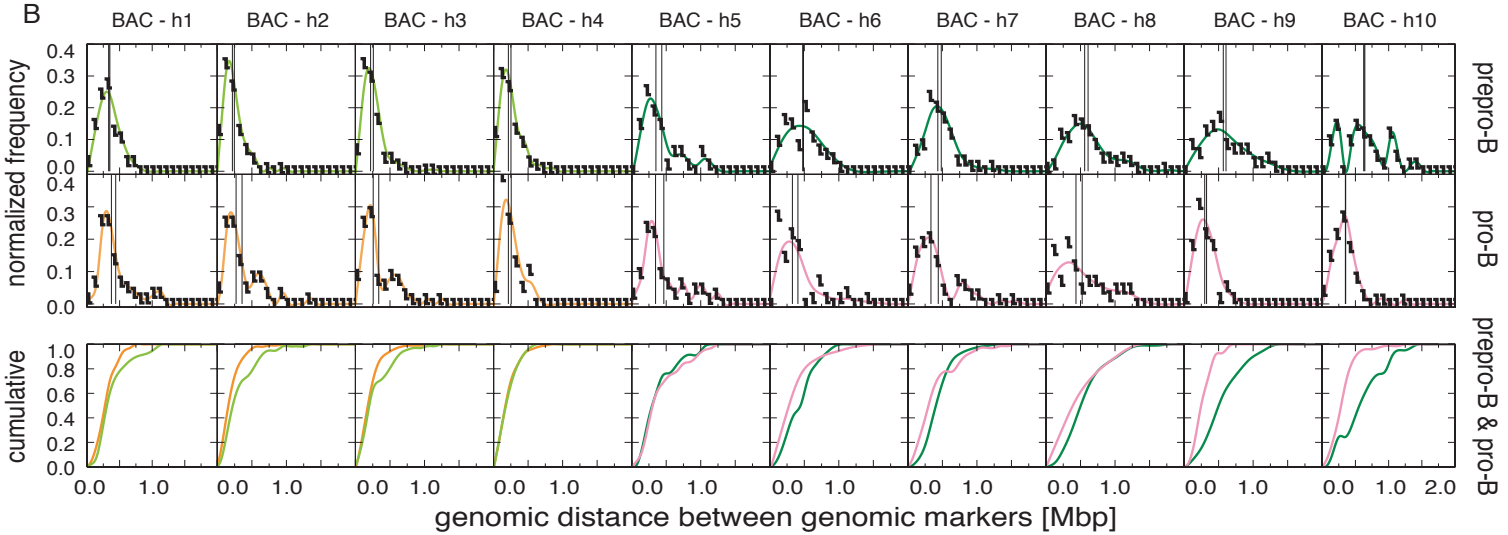

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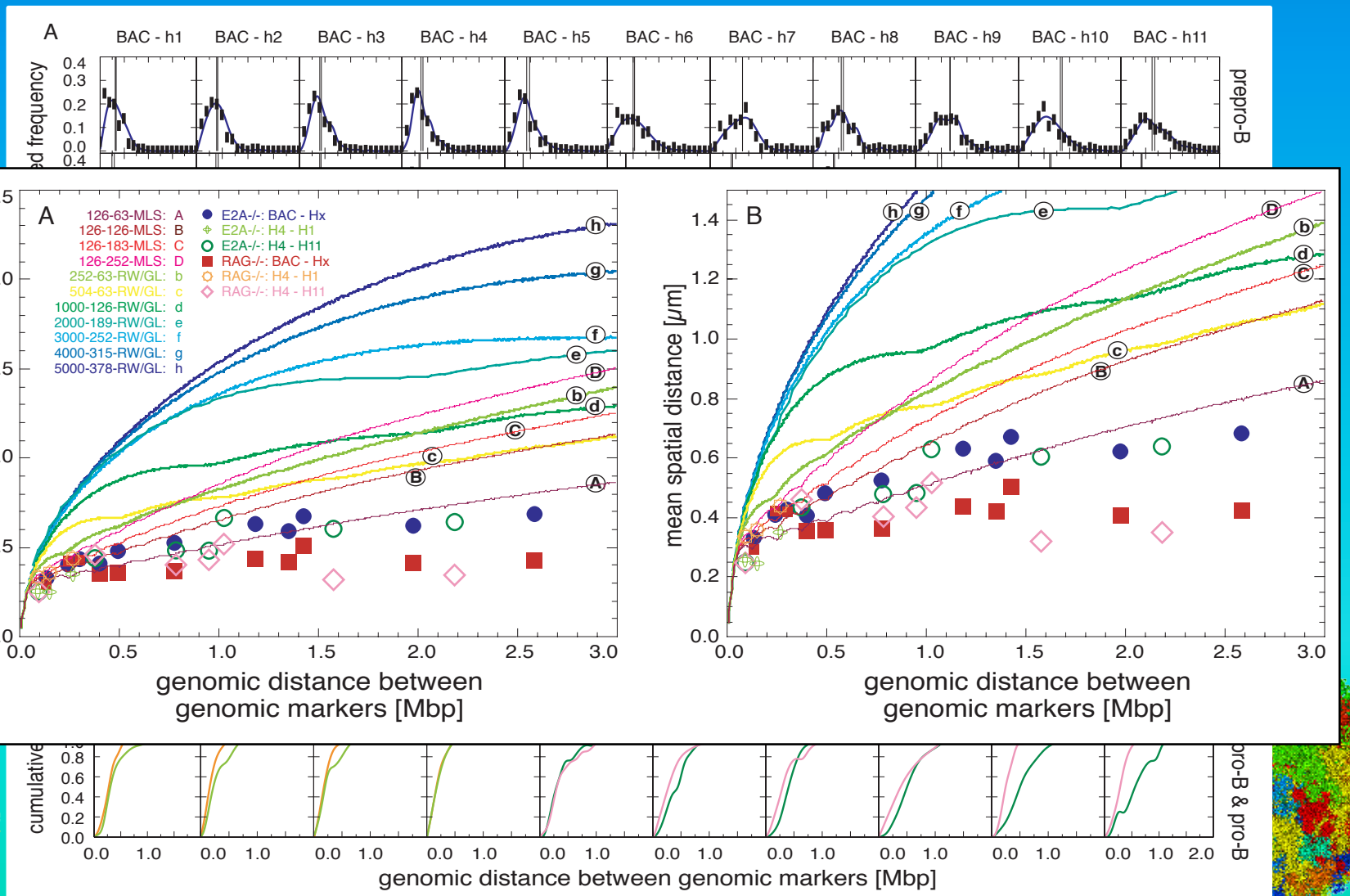

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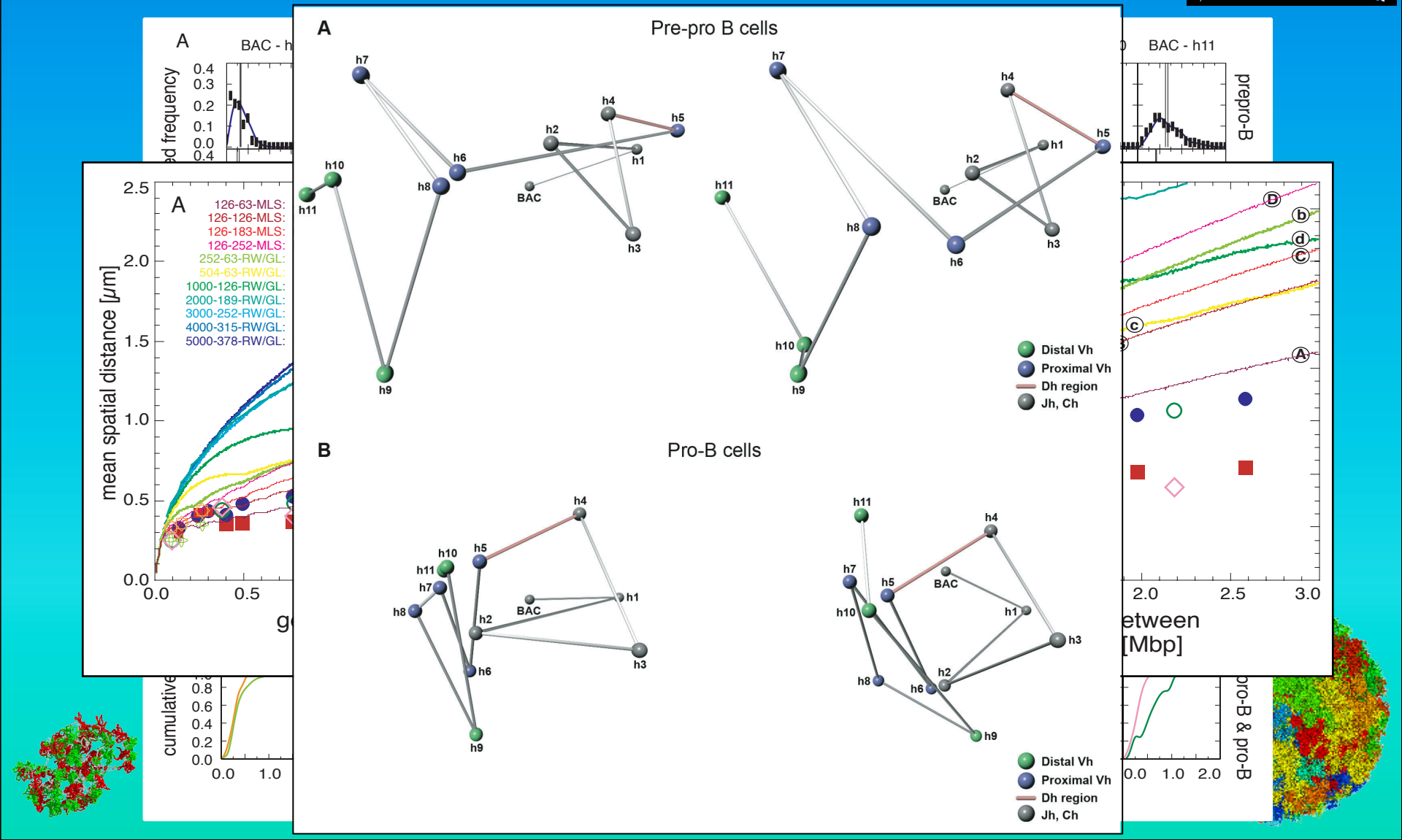
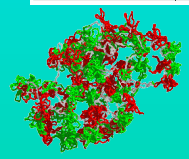


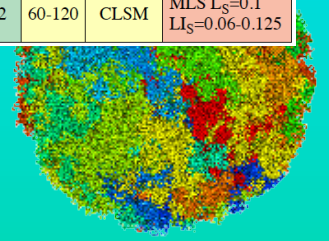
“Synoptic” 3D Architecture of Various Loci

A history “synoptic” comparison of the spatial distance mapping from their original background and aim, FISH methodological protocols, via microscopic imaging and restoration analysis procedures, to their interpretation, reveals that with time Multi-Loop Subcompartment models are fovoured.

Study	Location	Preparation of Cells			FISH			Microscopy		Fit to model
		Cell cycle	KCl [nM]	Fix-ative	Melt-ing	Label	Col-ours	# of nuclei	Image acquisi-tion	
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	Dystro-phin	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]	Fix-ative	Melt-ing	Label	Col-ours	# of nuclei	Image aquisi-tion	
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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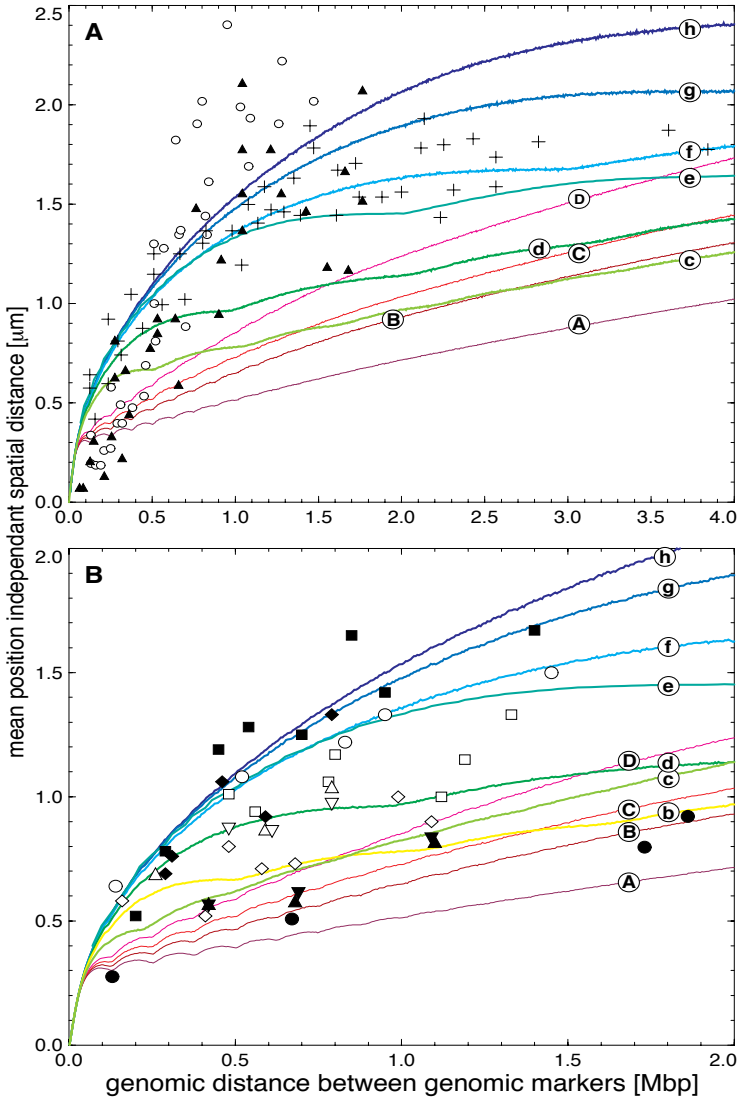
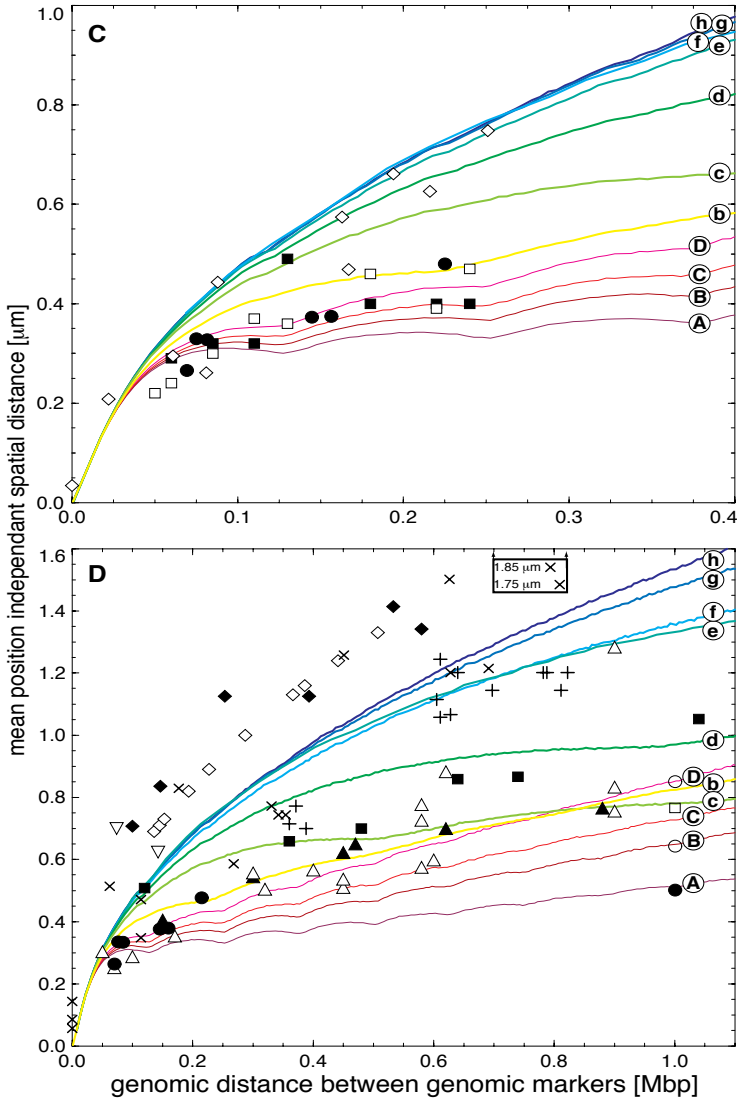
Study	to model
Fig. 3B, Tr ’89	L 2.0-3.0
Fig. 3B, Lawrence’	L 4.0-5.0
Fig. 3A, Tr ’91	L 1.0-2.0
Fig. 3B, Tr ’91	L 0.5-0.9
Fig. 3, v.d. ’92 or Fig. Trask ’93	L 1.0-5.0j
Fig. 5B, Tr ’93	L 0.25 L_S=0.126 0.200
Fig. 5, Senger ’93	L 1.0
Fig. 5, Senger ’93	L 0.25 L_S=0.126 0.200
Fig. 1, Warrington	L 1.0
Tab. 1, Warrington	L_S=0.126 180
Fig. 2B, Yo ’95	L_S=0.1 0.18-0.24
Fig. 3B, Yo ’95	L_S=0.1 0.06-0.125

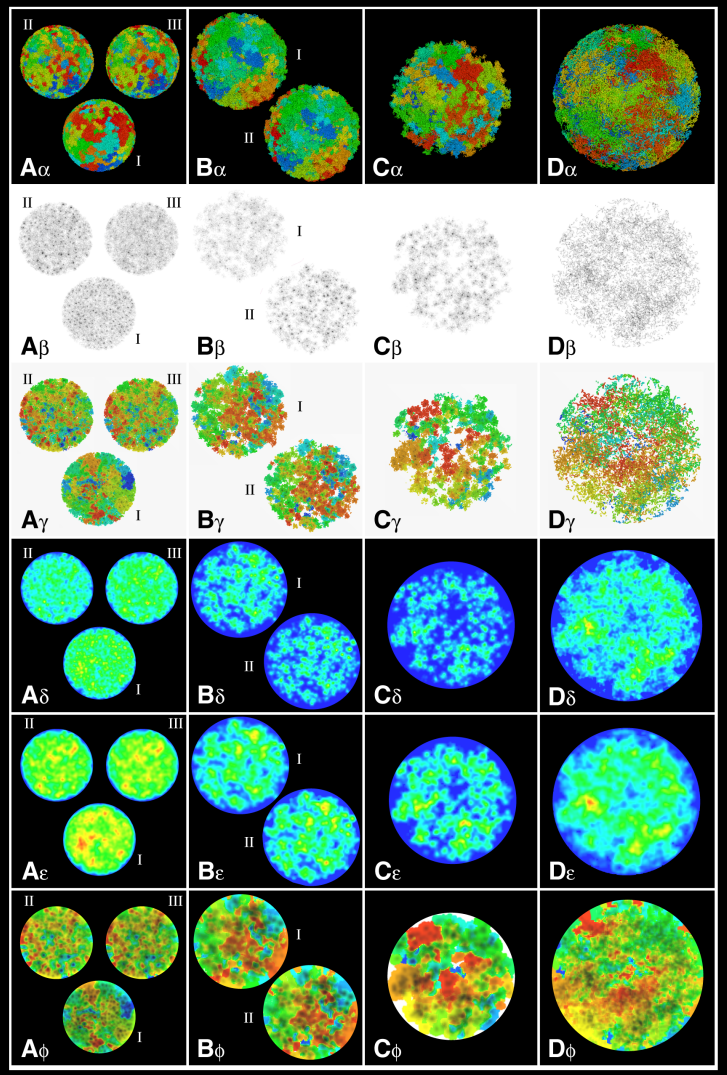




From Fiber Topology to Nuclear Morphology

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.

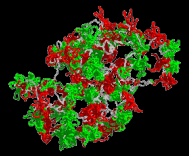


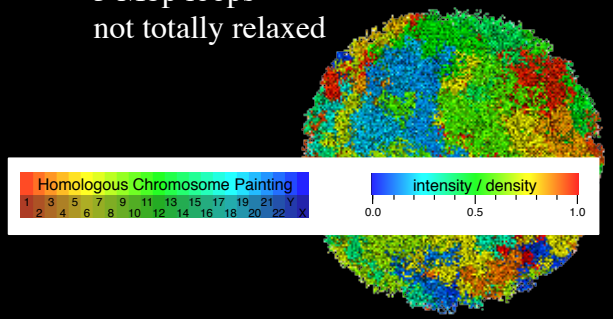
A: MLS in 6 μm nucleus
I: 63 kbp loops, 63 kbp linkers
II: 63 kbp loops, 252 kbp linkers
III: 126 kbp loops, 252 kbp linkers

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

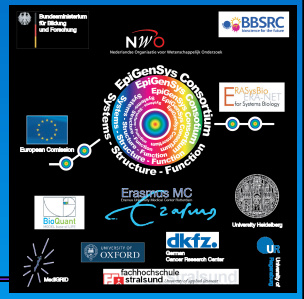




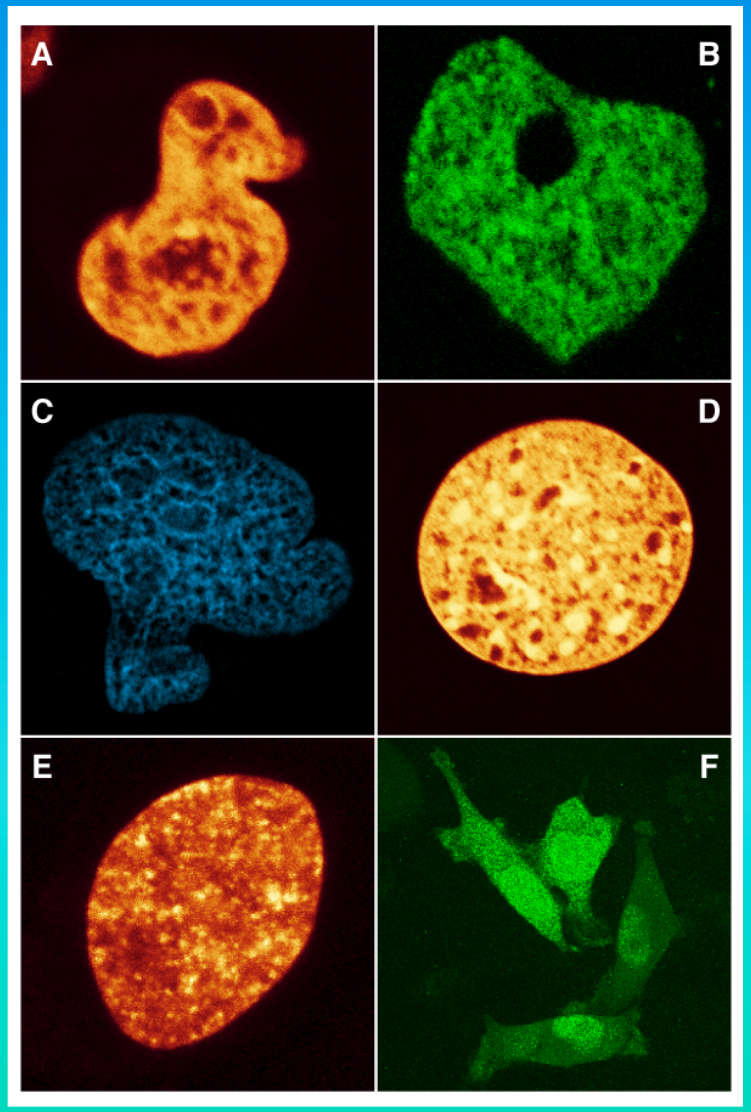
In vivo Morphology & Chromatin Distribution

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

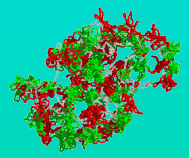


Cos7, H1.0-GFP

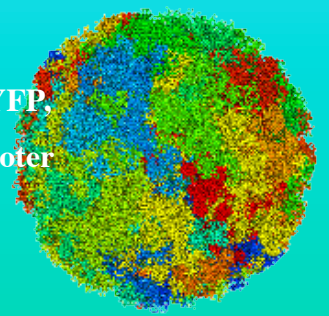
LCLC 103H, H2A-CFP

ID13, H2A-YFP

HeLa, mH2A1.2-YFP

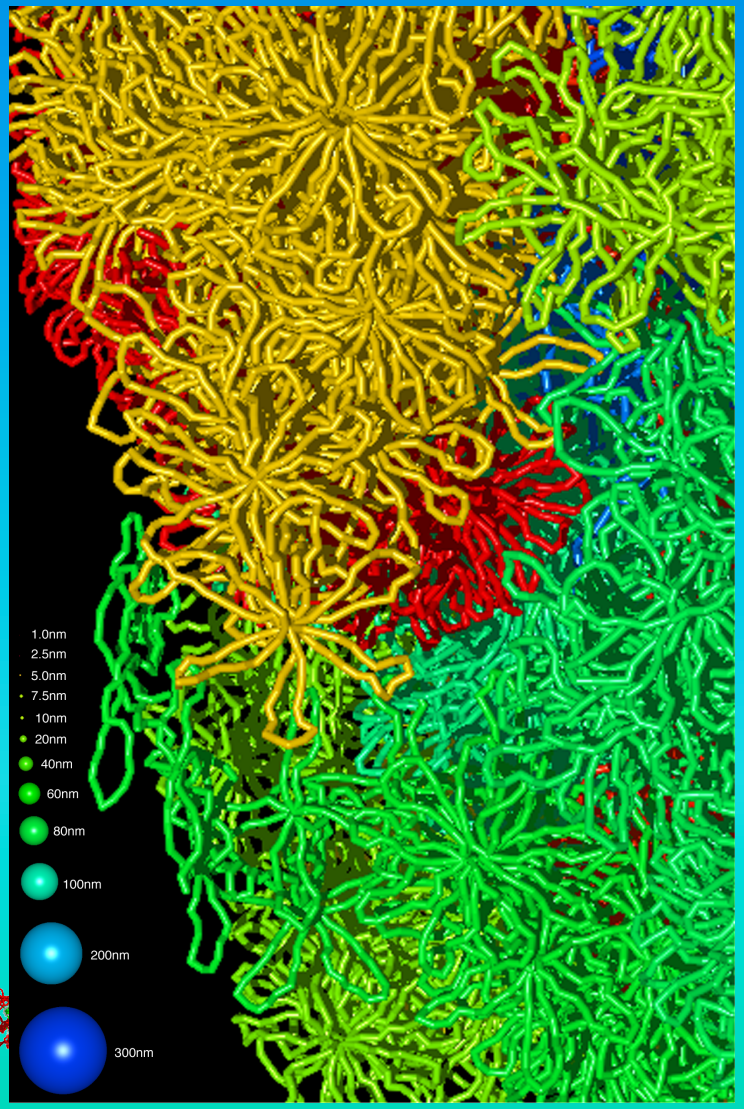


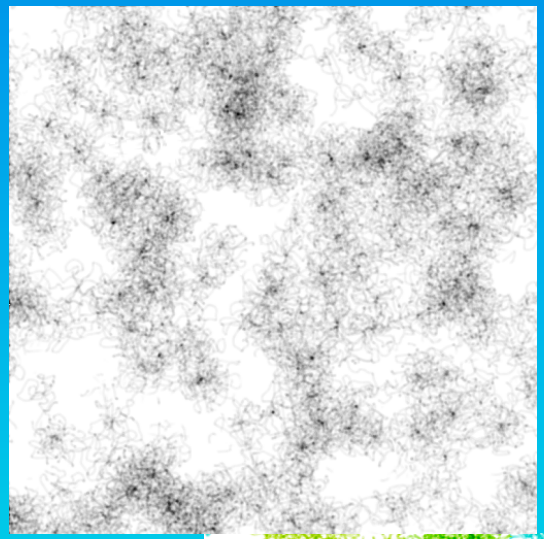
HeLa, H2A-YFP,
natural promoter

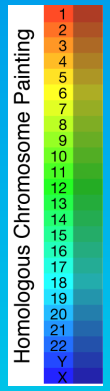


Fine Morphology of Nuclei

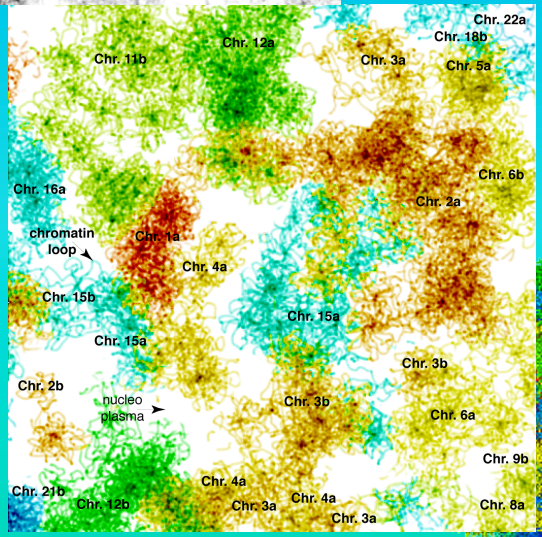
High resolution rendering and simulated electron microscopy including territory painting reveal not only again the model details but also that any location in the nucleus is accessible to biological molecules <15 nm in diameter and that even the Extended Interchromosomal Domain hypothesis is oversimplified.

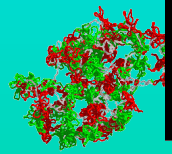


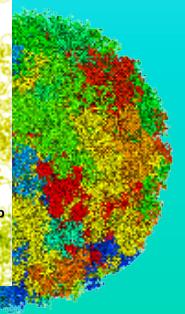




MLS models model with 126 kbp loops and linkers in a 10 μm nucleus.

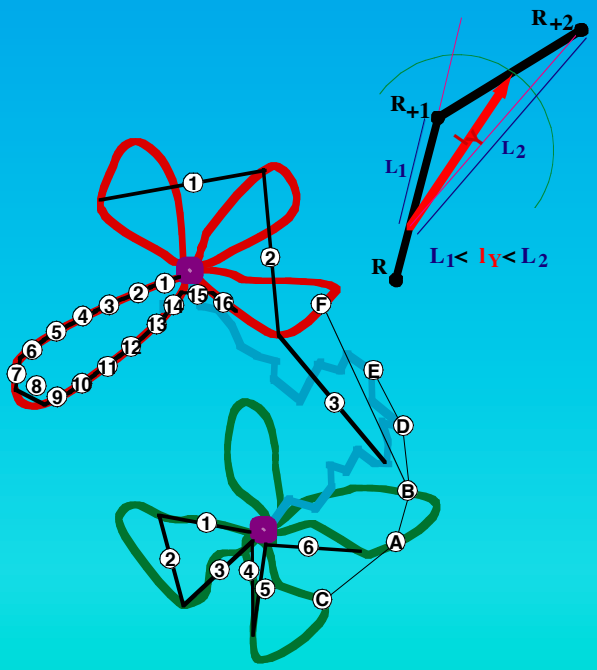


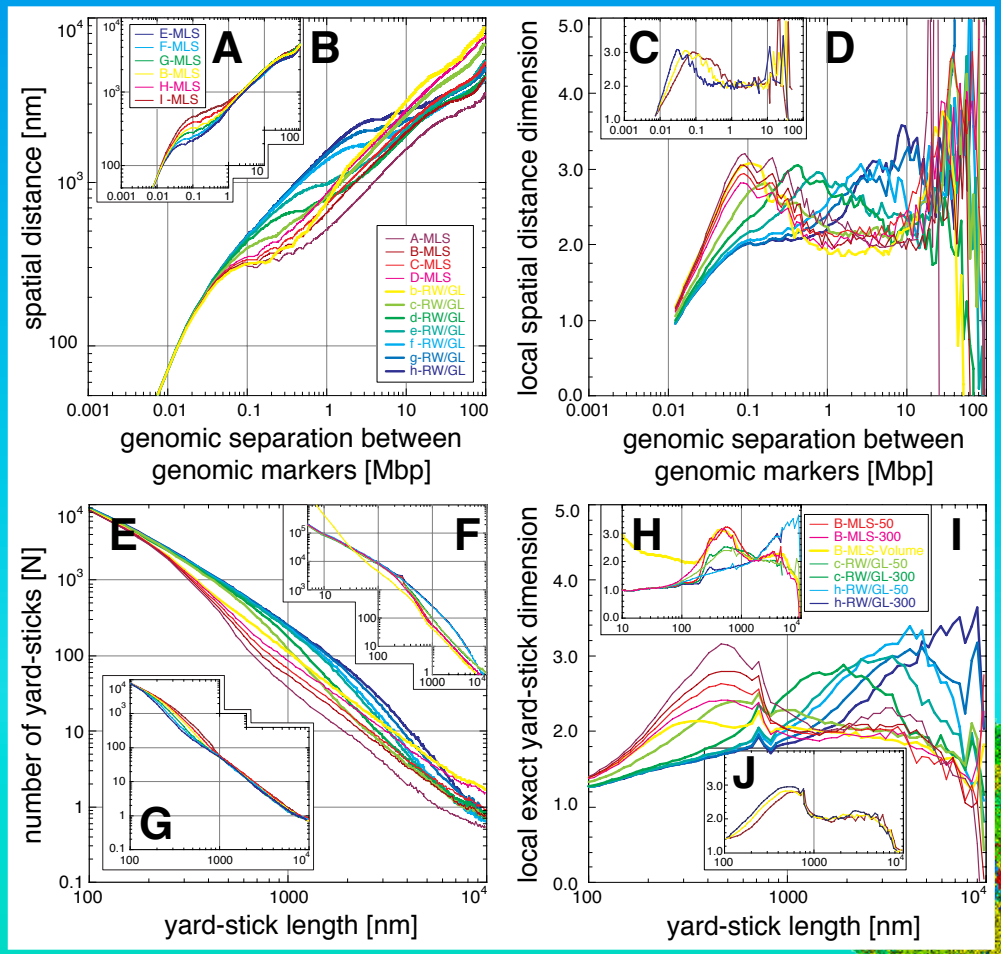




Scaling of the Chromatin Fiber Topology

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.

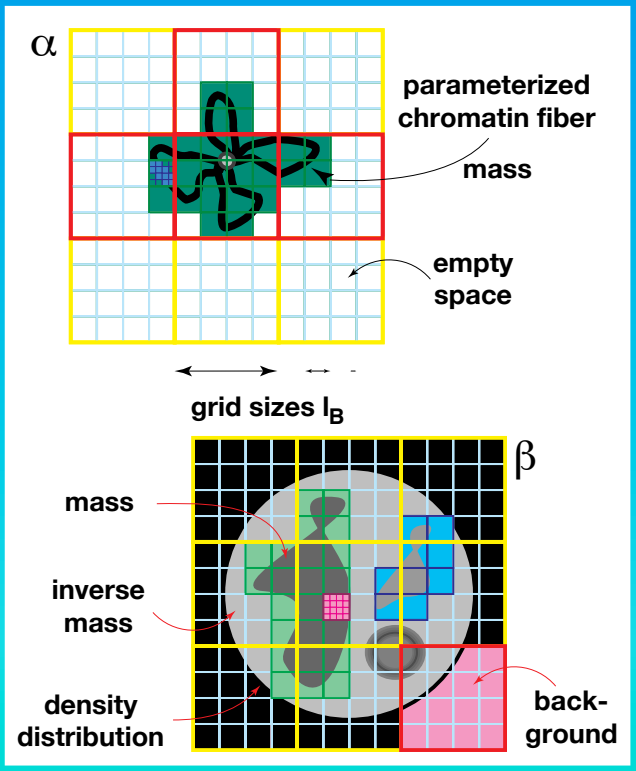




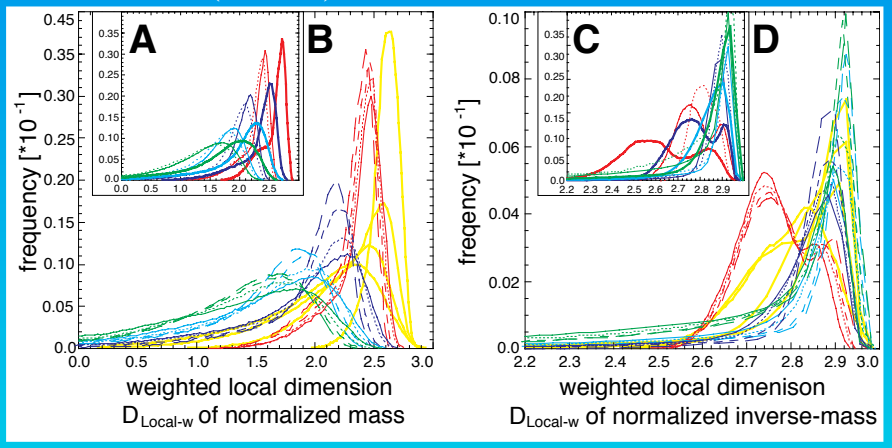
Scaling of the Chromatin Morphology & Distribution

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

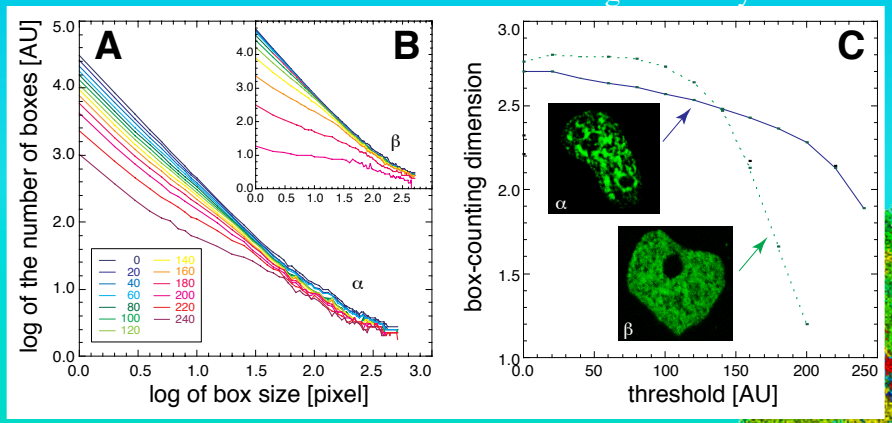
Consequently, the chromatin morphology is causally and quantitatively connected to the fiber topology.



(inverse-) mass dimension distribution

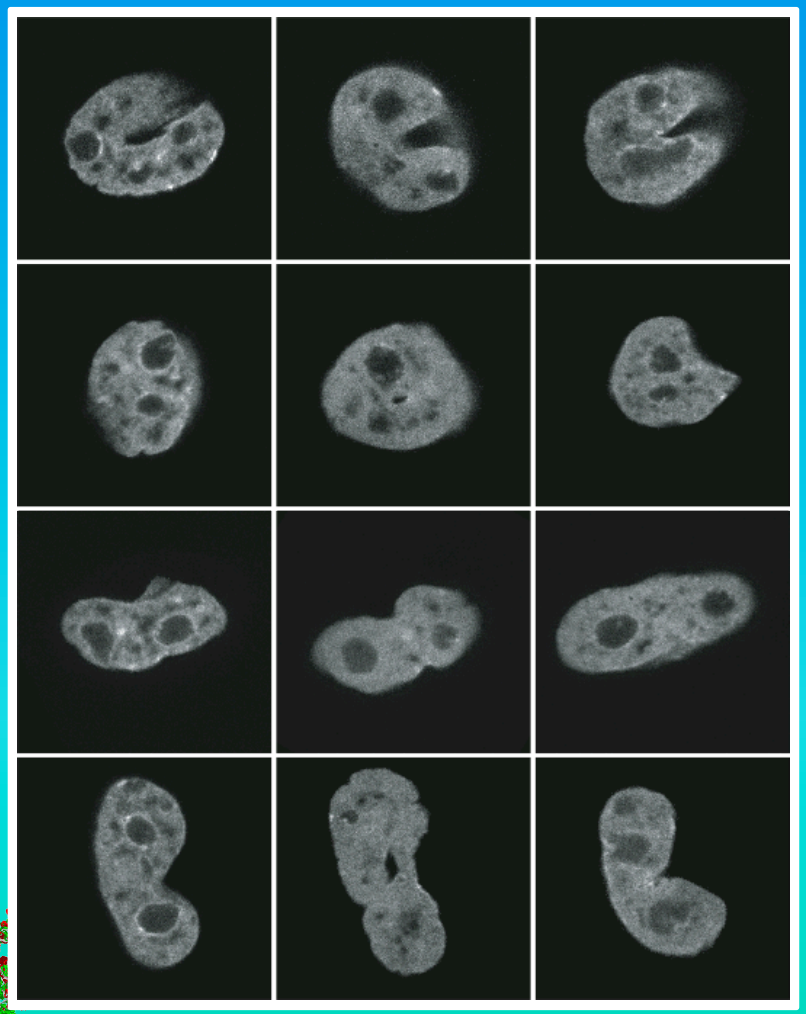


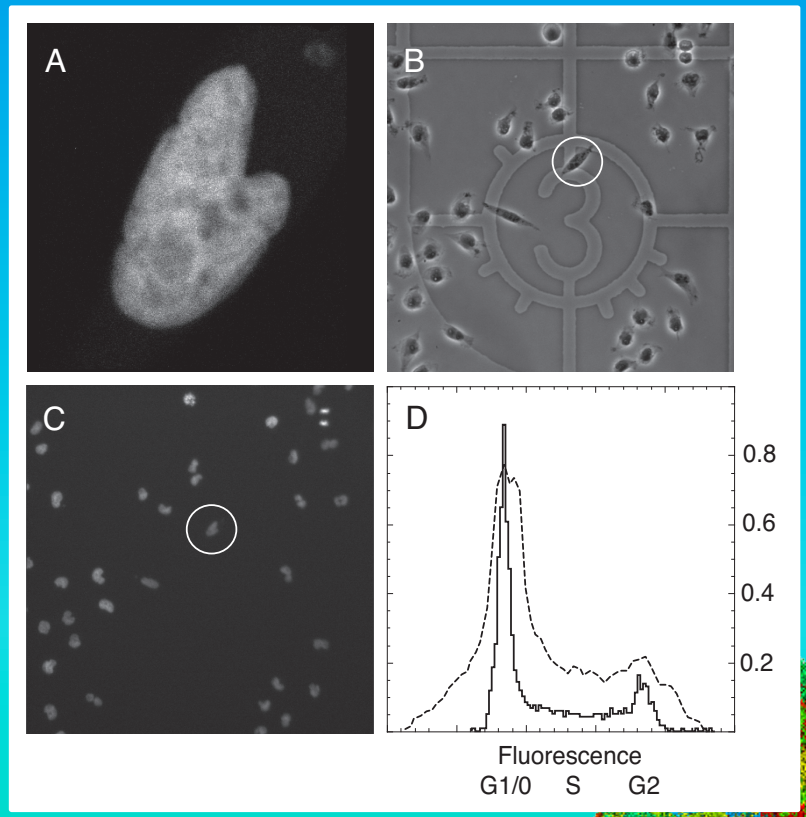
mass dimension as function of image intensity



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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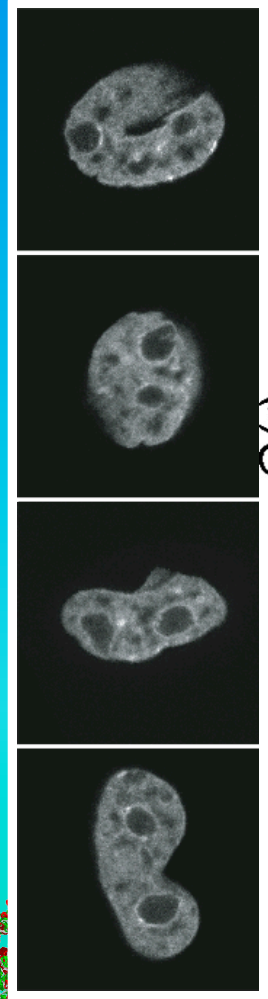
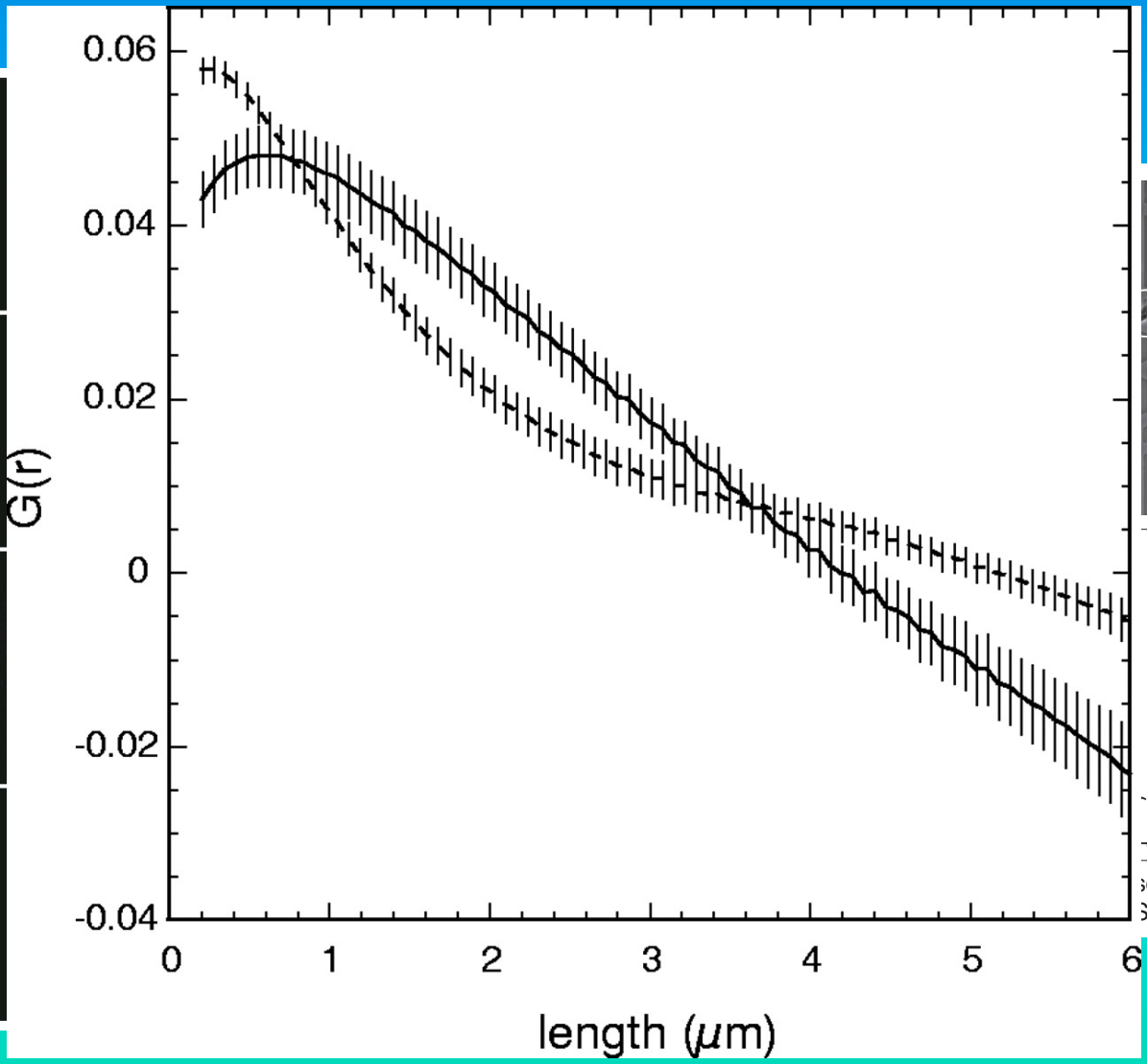
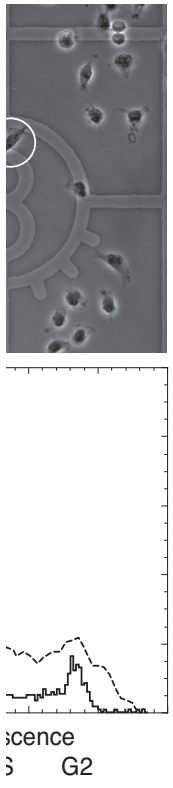
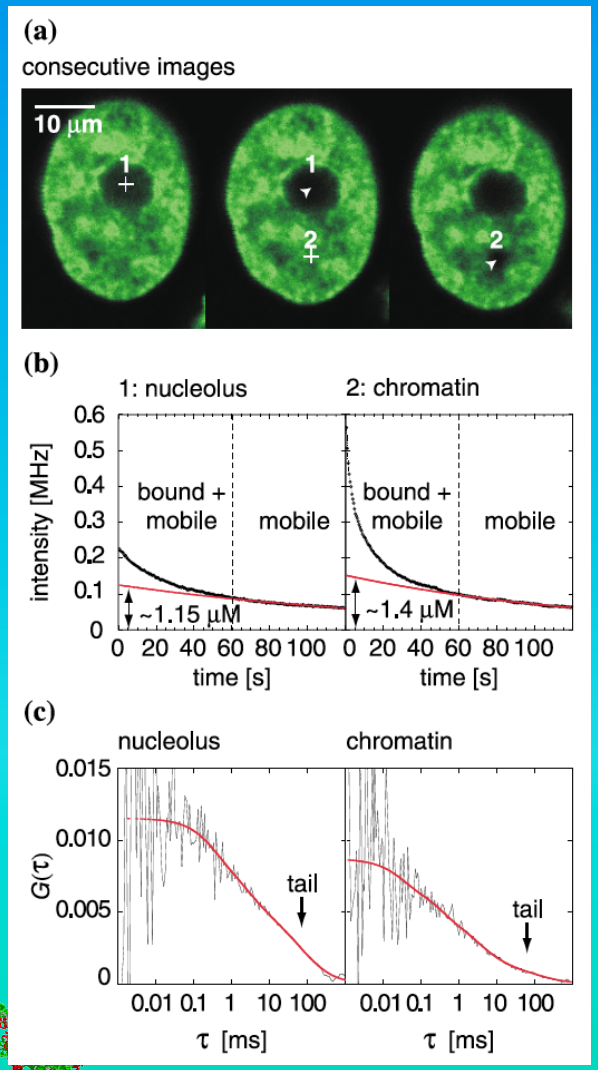


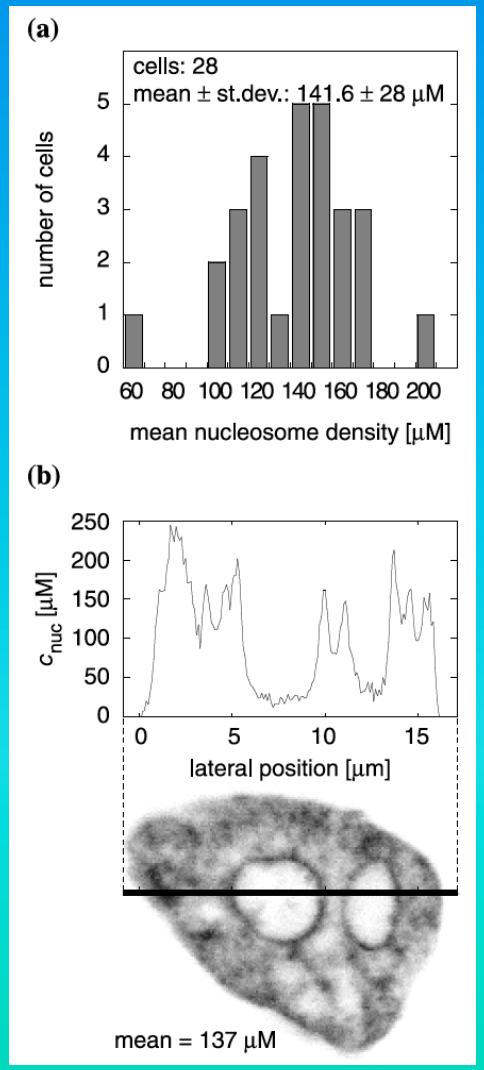


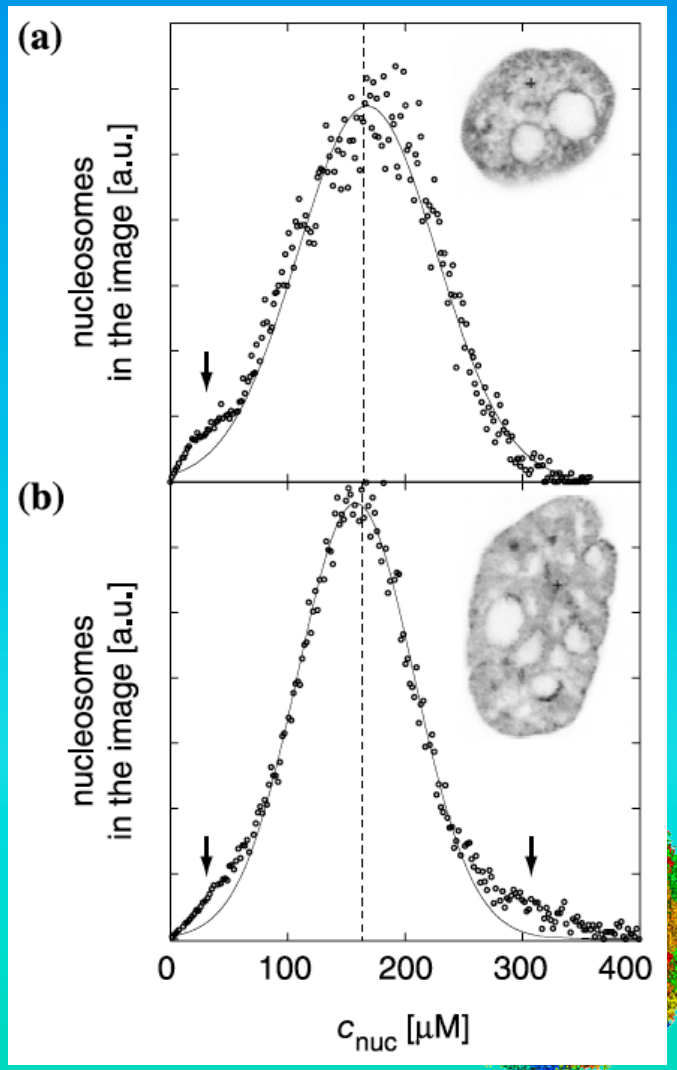


Counting Nucleosomes *In Vivo*

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.







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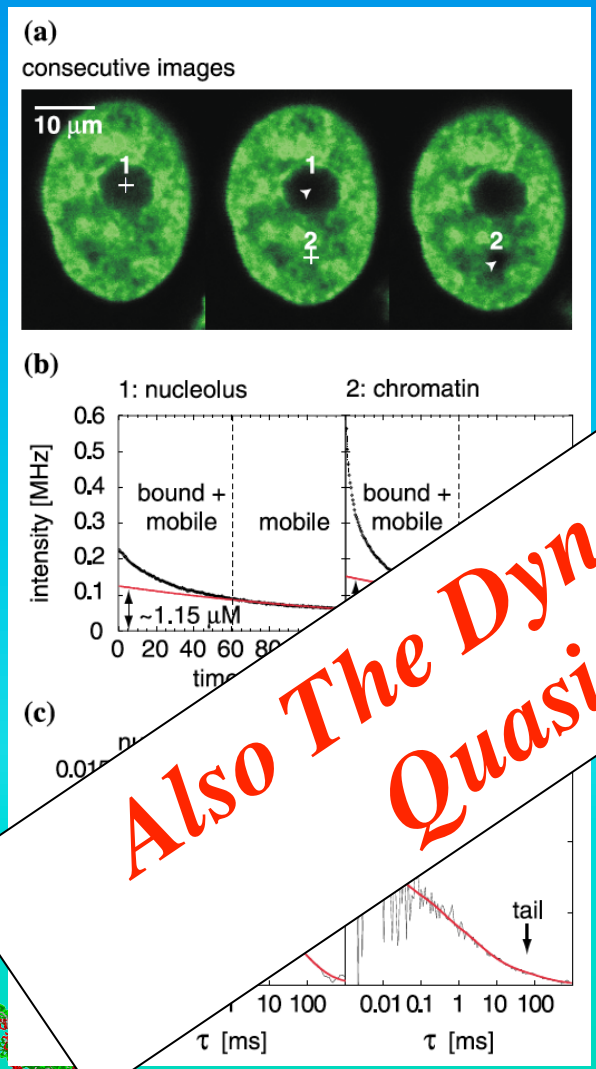
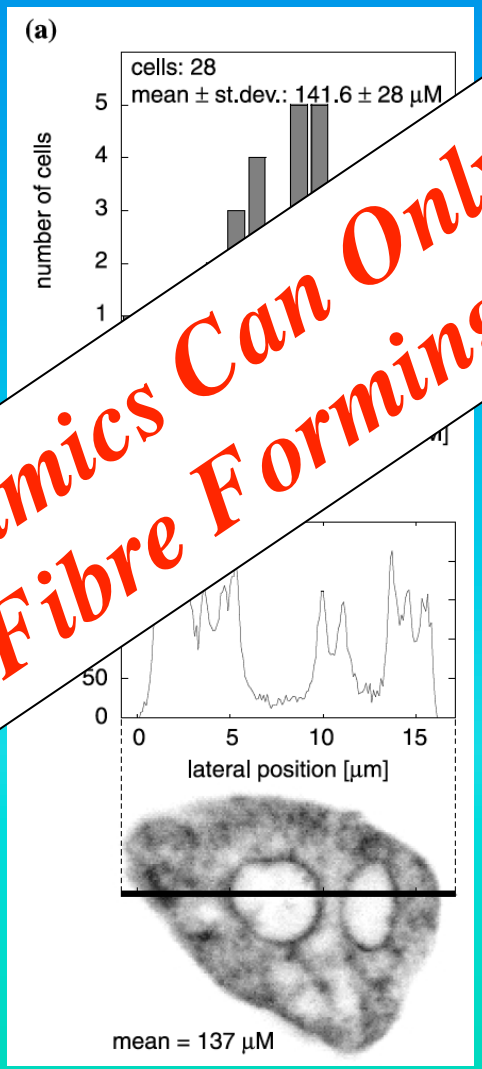
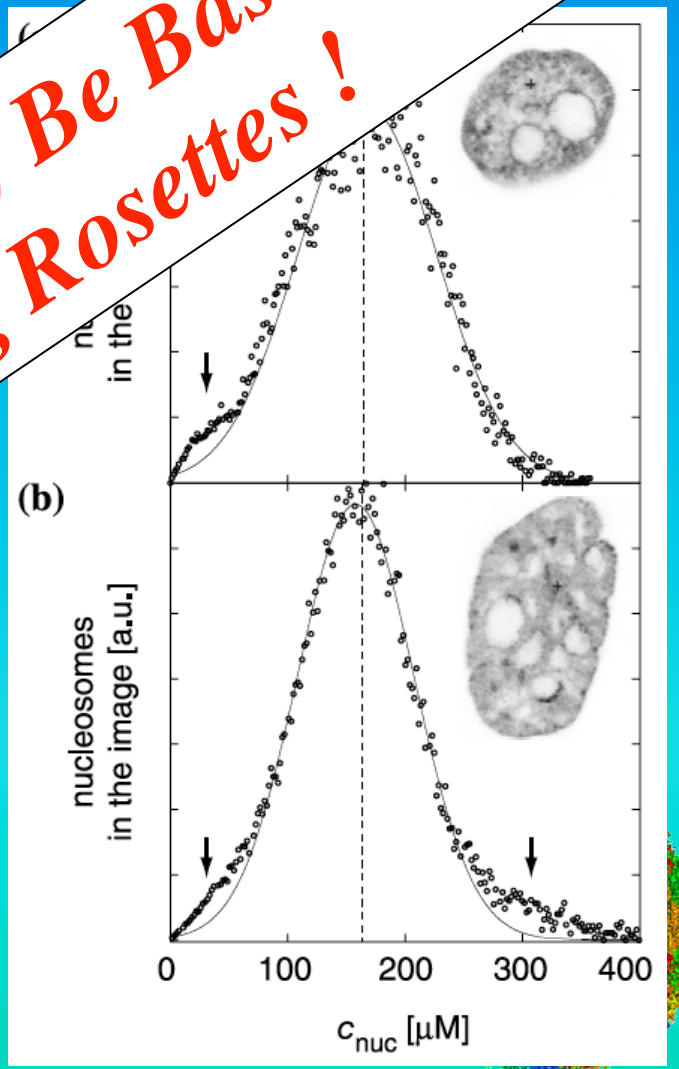






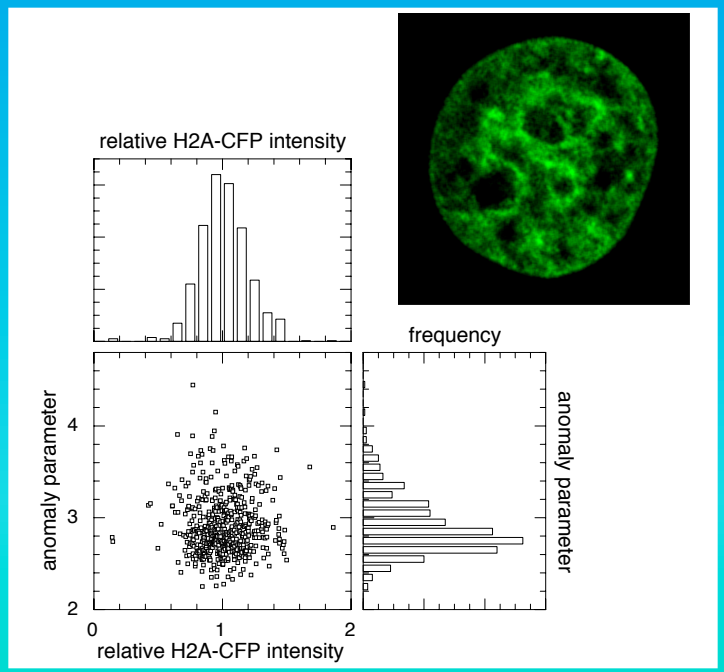
Also The Dynamics Can Only Be Based On Quasi-Fibre Forming Rosettes !

Diffusion of Particles in the Nucleus

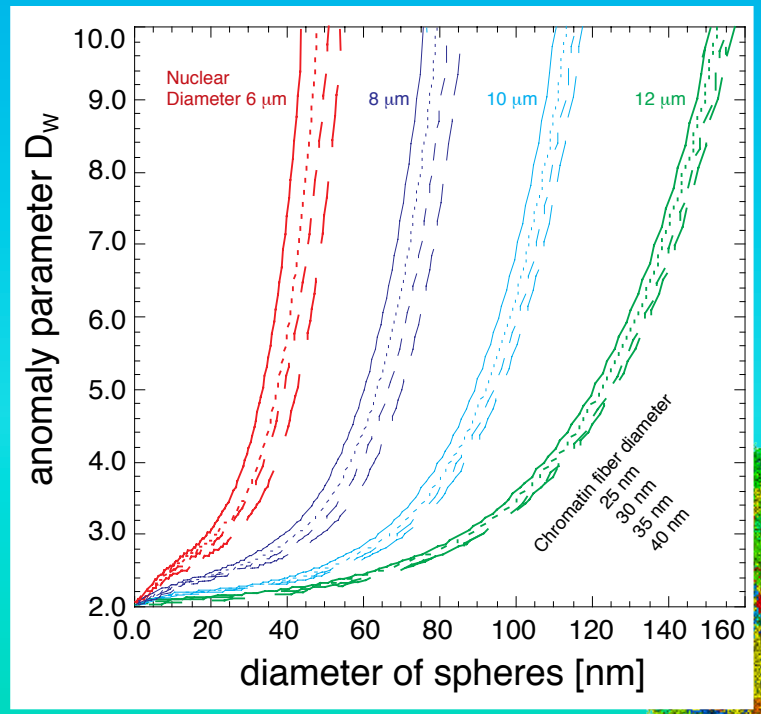
Due to the volume and spatial relation ships in the nucleus typical particles reach almost any location in the nucleus by moderately obstructed diffusion: a 10 nm particle moves 1 to 2 μm within 10 ms.

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)

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

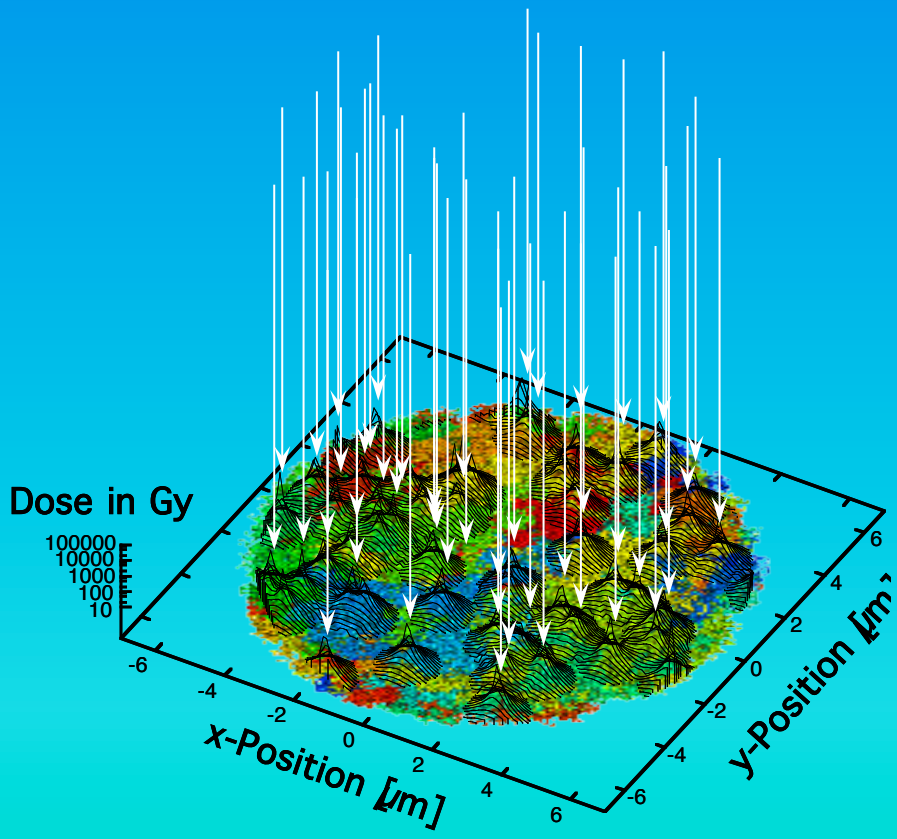


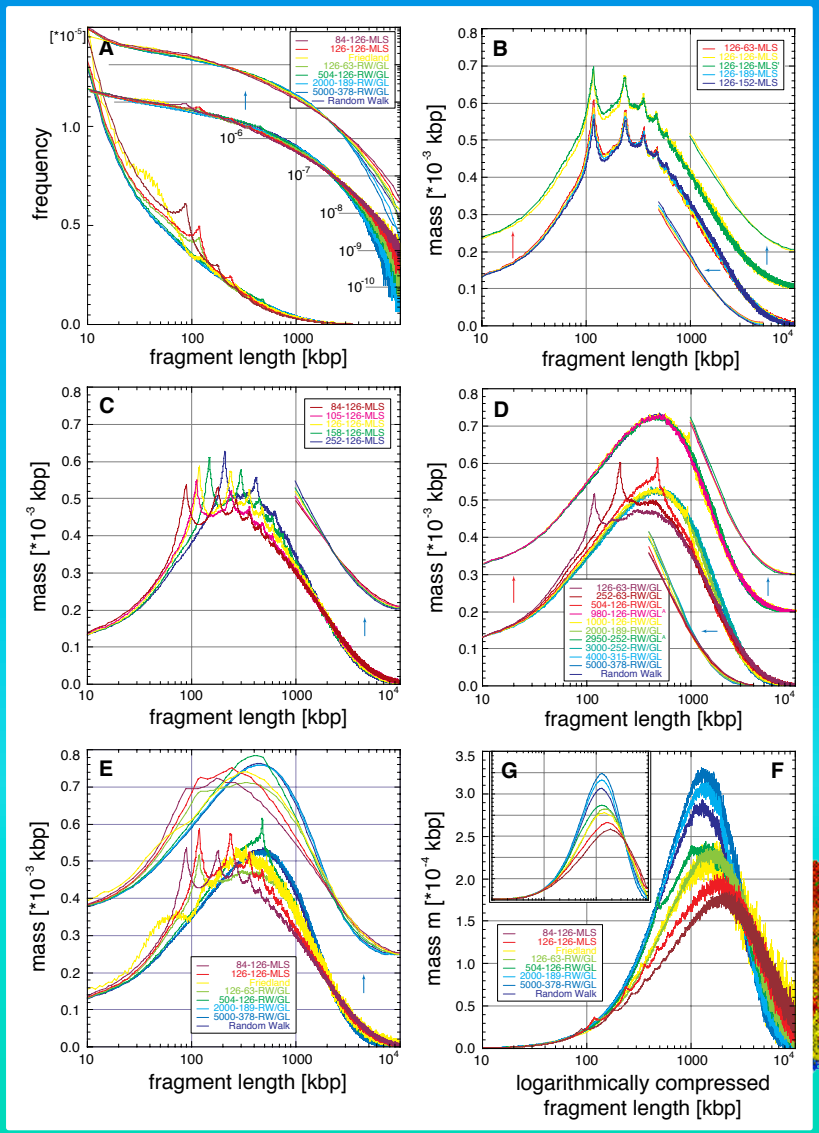
Nuclear diameter [μm]	Nuclear Volume [μm^3]	Mean Nucleosome Concentration [μM]	Chromatin Volume Fraction [%]	Mean Isotropic Mesh Spacing [nm]
6	115	251	20.1	41
8	268	107	8.6	64
10	523	55	4.4	90
12	904	32	2.6	117



DNA Fragment Distribution after Ione-Irradiation

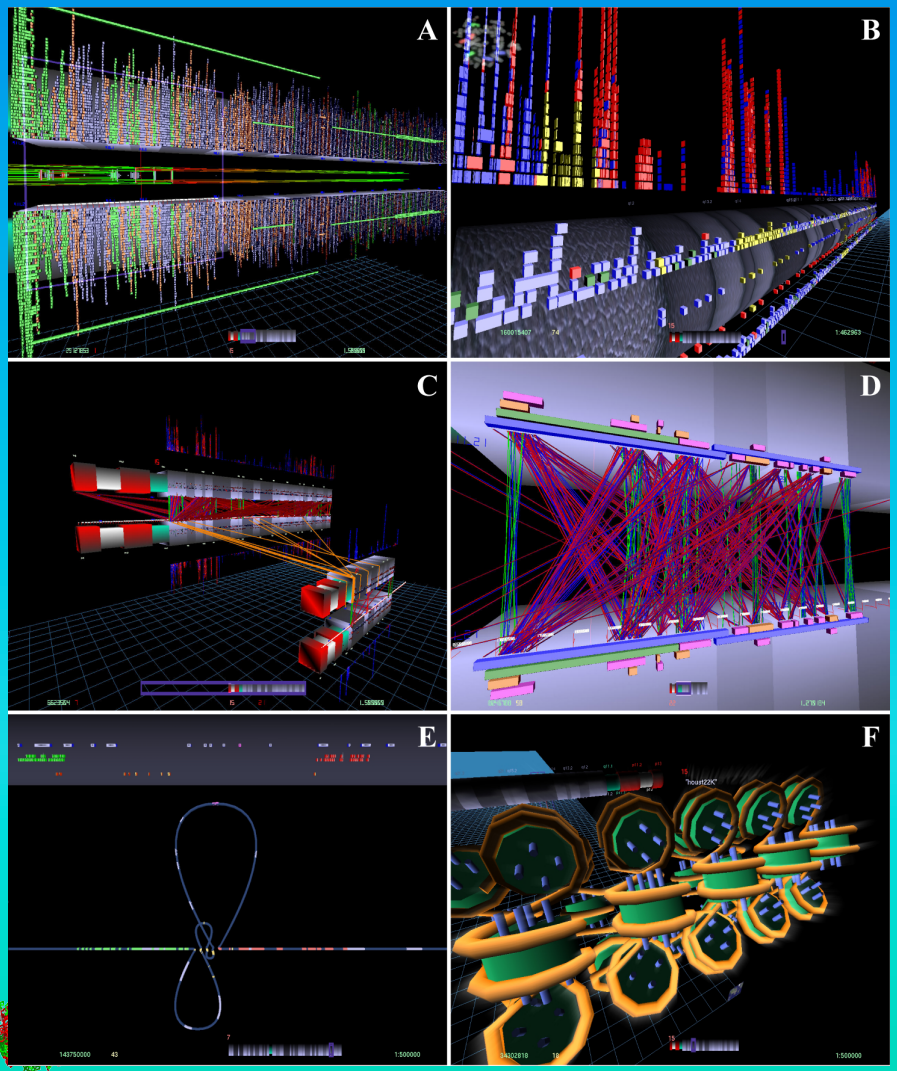
The length distribution of DNA fragments after irradiation with e. g. C or Ca with an inhomogeneous spatial double strand breackage probability depends on the detailed folding topology of the chromatin fiber and the RW/GL and MLS models differ largely.

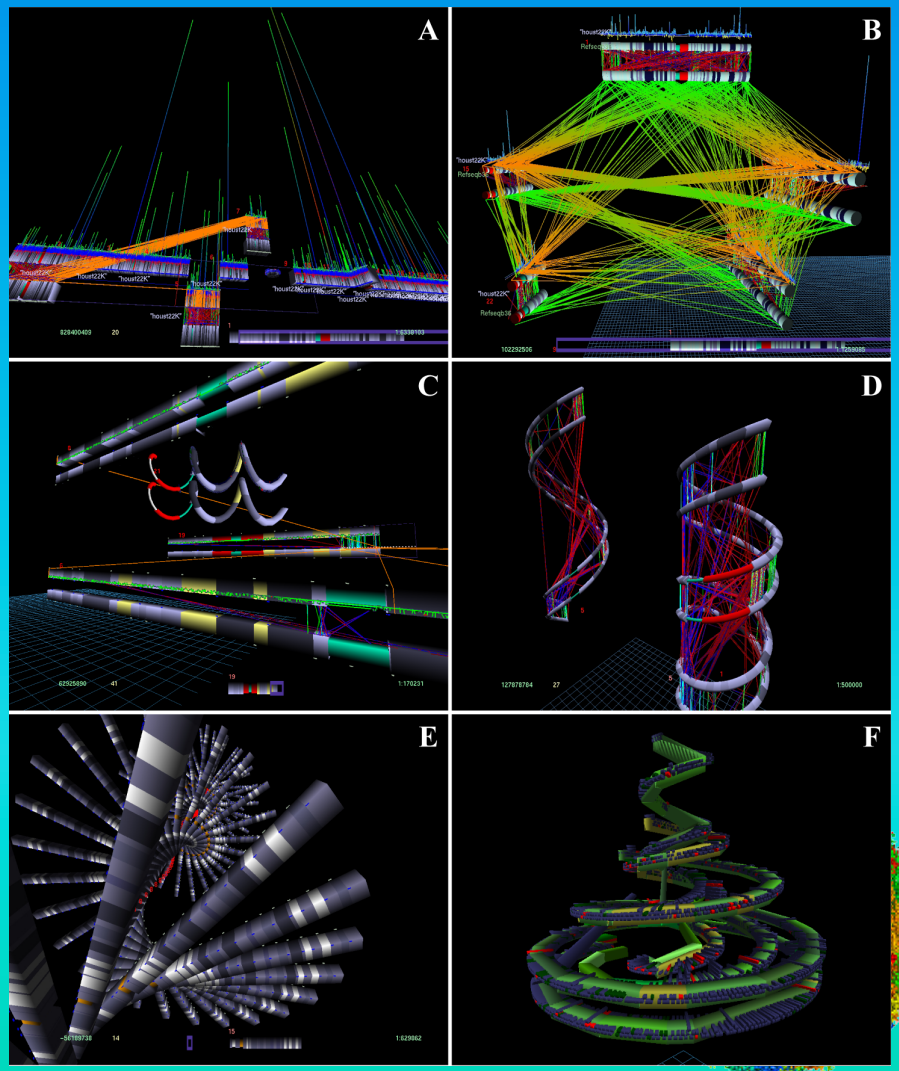




Systems Biological Result Integration via the GLOBE 3D Genome Platform

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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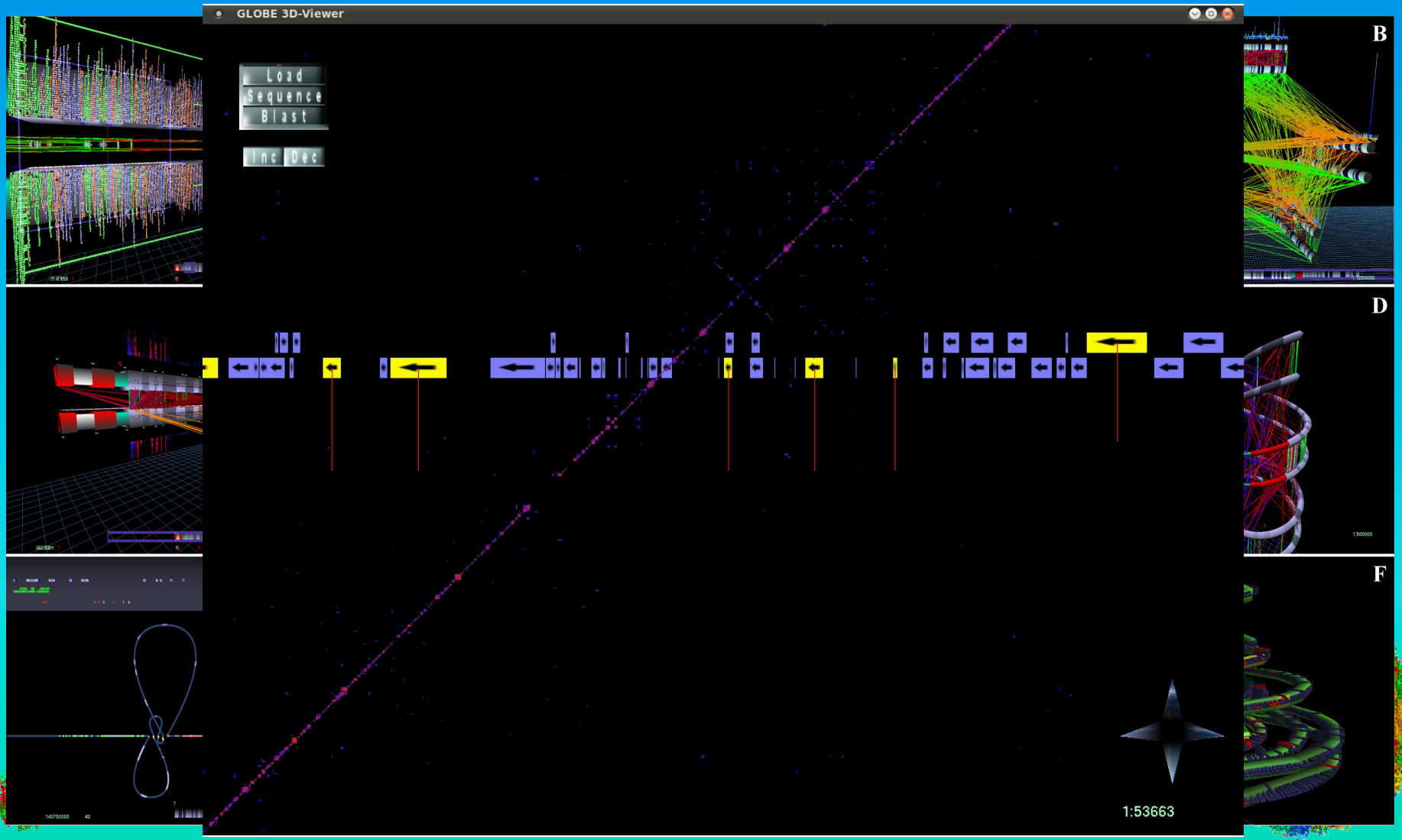

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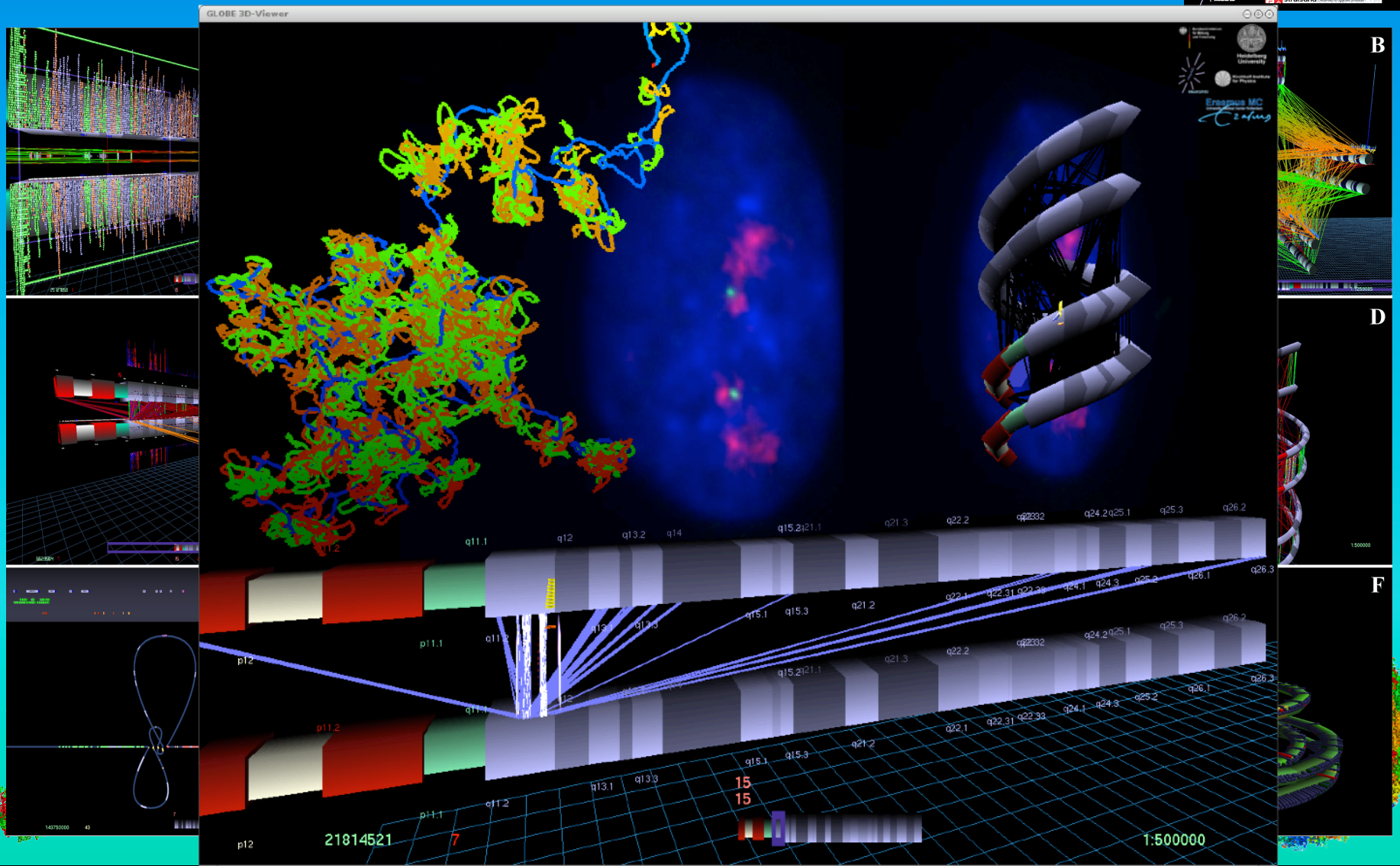

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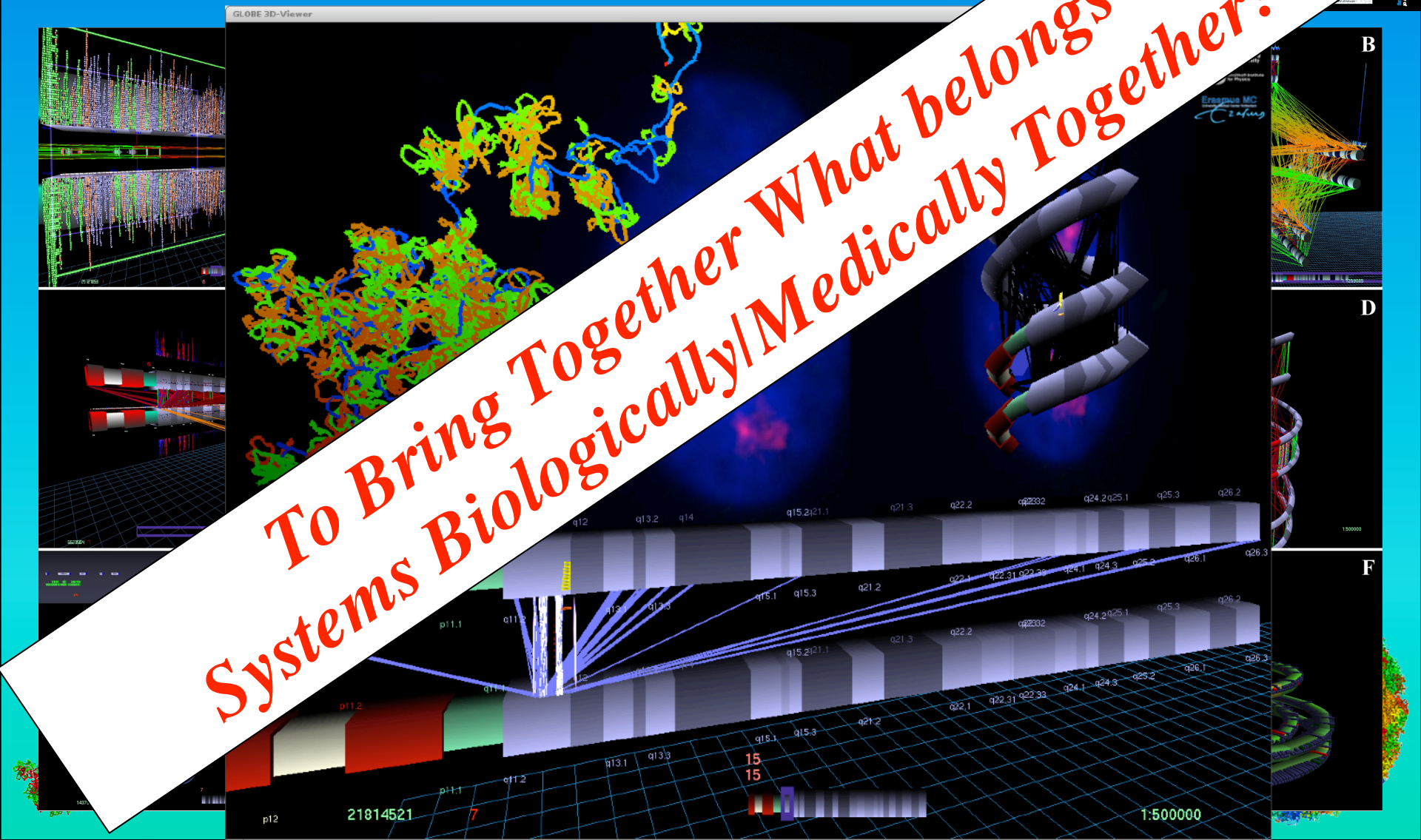


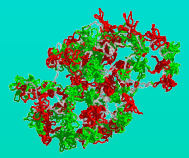
To Bring Together What belongs Systems Biologically/Medically Together!

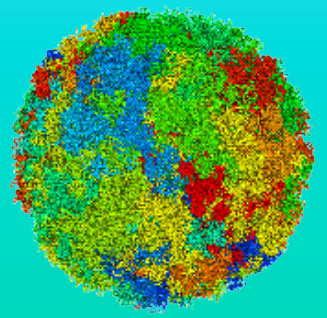
Conclusion

The compacted chromatin quasi-fibre, folds into loop-aggregates connected by a linker !

Every structural level of nuclear organization including its dynamics is connected and represented in all the other levels in a holistic systems genomics manner.

- The 3D genomes architecture consists of chromatin quasi-fibres (5±1 nuc. / 11 nm, L_p of 80-120 nm), forming stable loop aggregates/rosettes (~40-100 kbp loops, ~60 kbp linkers).
- The dynamics of genomes follows the 3D genome architecture in detail and determines in an inseparable entanglement with the architecture genome function.
- From the single base pair to the entire cell nucleus, all genomic levels represent all other levels and by modification a code is present and used to store genetic information.
- Genomes have a consensus organization with only small variation from the basic theme on each compaction level of the genome and these small variations determine genome function.
- Genome organization and function cannot be determined or understood from a single organizational level but only in a holistic systems genomics manner integrating all parts of the system.
- **The genome behaves on the basis of a genomic statistical mechanics with a genomic uncertainty principle attached !**





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Frank G. Grosveld

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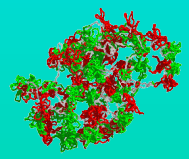

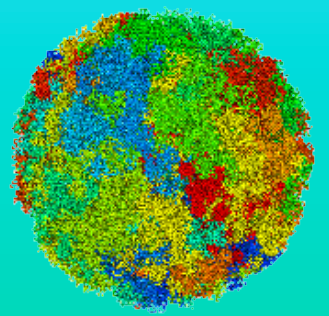

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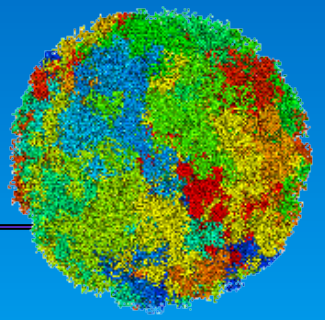

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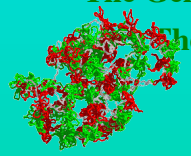
The European Commission



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The Real Fun
Is Yet To Come!

nter and BioQuant & German Cancer Research Center

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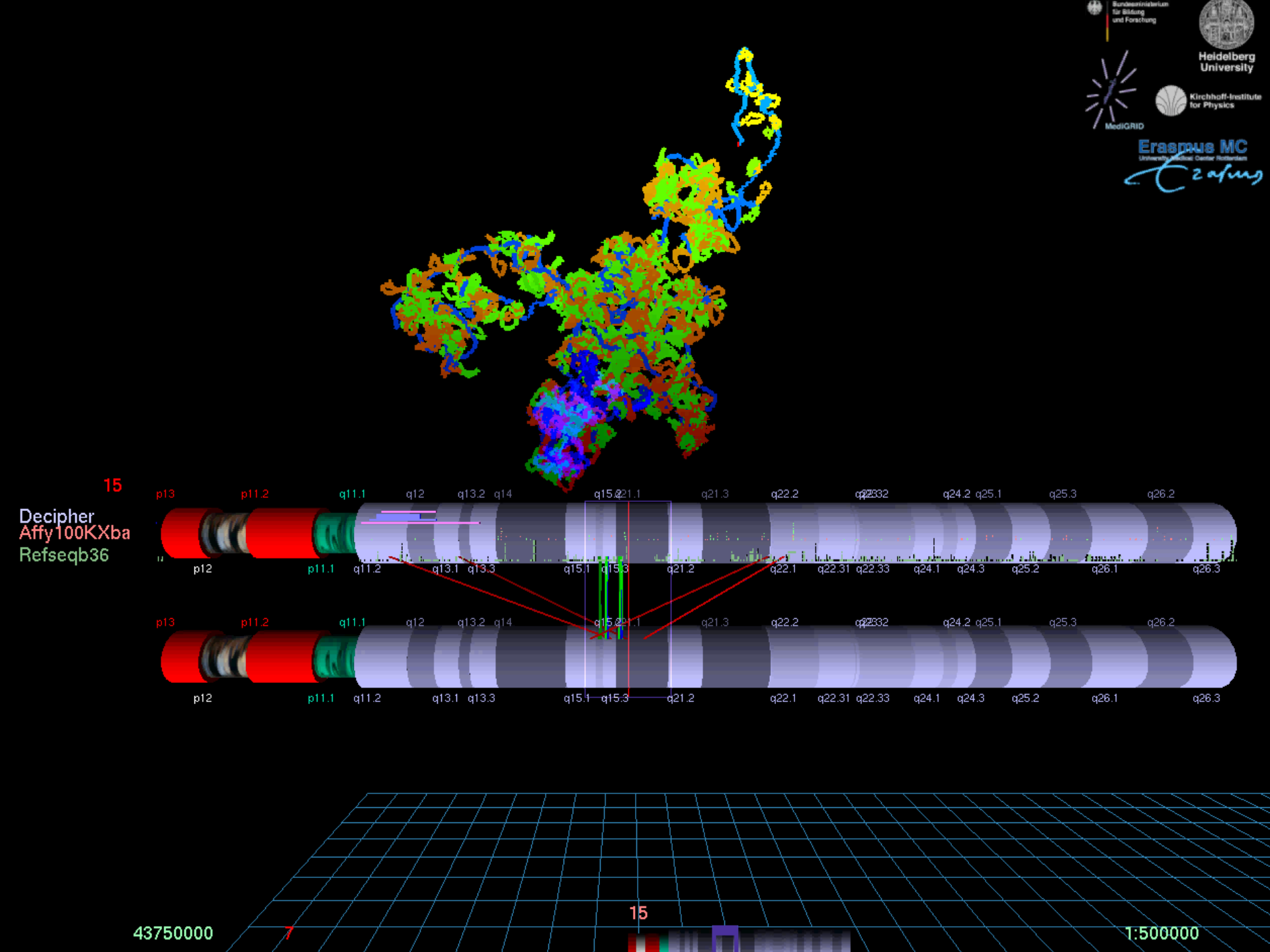
The European Commission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MediGRID
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The
Detailed 3D Multi-Loop Aggregate/Rosette Chromatin Architecture
and
Functional Dynamic Organization
of the
Human and Mouse Genome

Knoch, T. A.,

Wachsmuth, M., Kepper, N., Lesnussa, M., Abuseiris, A., Kolovos, P., Zuin, J., van den Werken, H. J. G., Wendt, K. S. & Grosveld, F. G.

Chromatin and Epigenetics: From Omics to Single Cells 2014, Institut de Genetique et de Biologie Moleculaire et Cellulair (IGBMC), Strasbourg, France, 14th - 15th October, 2014.

Abstract

The dynamic three-dimensional chromatin architecture of genomes and the obvious co-evolutionary connection to its function – the storage and expression of genetic information – is still, after ~170 years of concentrated research, one of the central issues of our time. With a systems genomics combination of T2C and FCS together with analytical and simulated polymer models as well as scaling analysis of the architecture and the DNA sequence itself, we determined the architecture and dynamics with with molecular resolution from the single base to the mega base pair level! T2C is a novel superior selective high-throughput high-resolution chromosomal interaction capture of all physical genomic interactions (T2C) which has great advantages since it allows to reach an optimal combination of resolution, interaction frequency range, and multi-plexability, at unprecidented signal-to-noise ratio of multiple orders of magnitude. Actually, with T2C the ultimate limits at the level of the "genomic uncertainty principle" and "genomic statistical mechanics", is reached. In parallel, with a novel FCS approach it is possible to determine the relaxation of local concentration fluctuations of the chromatin fibre and derive based on an analytical polymer model the relation between average persistence length, mass density and chromatin architecture. Whereas T2C measures architectural parameters, the FCS approach allows to measure *in* vivo the dynamics of the architecture. As common ground polymer simulations using Monte Carlo and Brownian Dynamics approaches as well as scaling analysis of the architecture as well as the DNA sequence organization itself round-off the experimental techniques in detail. In combination all four approaches reach agreement concerning the 3D architecture and dynamics of genomes in detail. With this we investigated several loci in detail of different species, cell type, and functional states from *some to the mega-base pair level and thus spanning 6 orders of magnitude (!)*, and finally determined the three-dimensional organization and dynamics for the first time in a consisten system genomics manner from the four mentioned angles. Beyond, the results are also in agreement with the heuristics of the research of the last 170 years. Consequently, we have not only finally determined the architecture and dynamics of the mouse and human genome with base pair/molecular resolution, but this also opens now the detailed architectural and dynamic sequencing of genomes in a systems genomic manner which is of fundamental importance from fundamental genome understanding to diagnosis and treatment.

Corresponding author email contact: TA.Knoch@taknoch.org

Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome statistical mechanics, genomic uncertainty principle, multilism genotype-phenotype, 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 quasi 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, polymer model, analytic mathematical model, Brownian Dynamics, Monte Carlo, fluorescence *in situ* hybridization (FISH), targeted chromatin capture (T2C) confocal laser scanning microscopy, fluorescence correlation spectroscopy, spatial precision distance microscopy, super-resolution microscopy, two dimensional fluorescence correlations spectroscopy (2D-FCS) 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, human ecology, e-human grid ecology, society, social systems, e-social challenge, inverse tragedy of the commons, grid phenomenon, micro-sociality, macro-sociality, autopoietic tragedy of social sub-systems, micro subsystems, macro subsystems, micro operationality, macro operationality, grid psychology micro riskmanagement, macro riskmanagement.

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