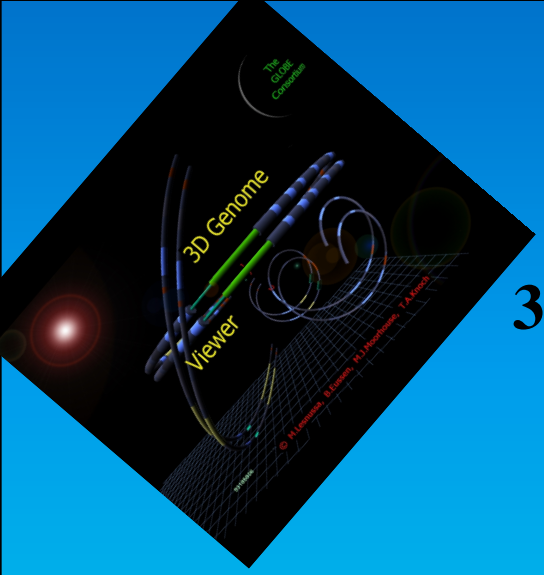
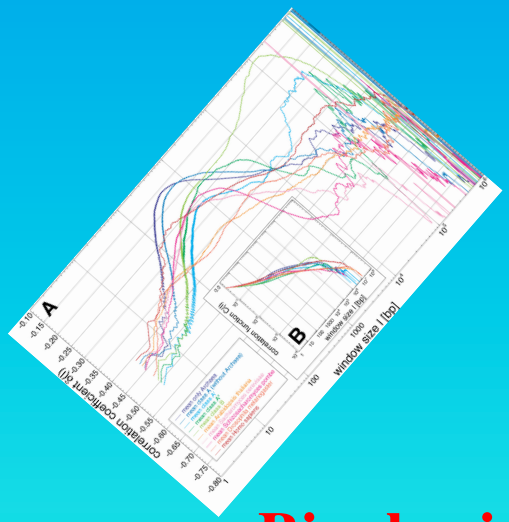
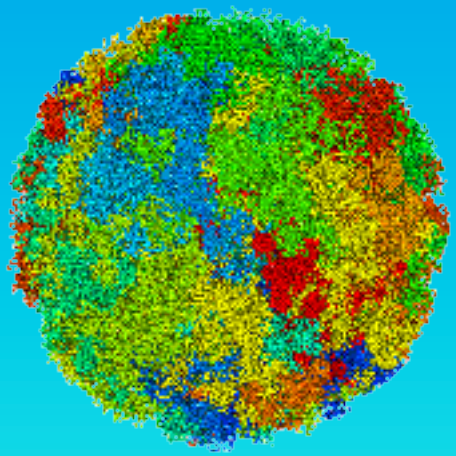


Decoding the 3D Multi-Loop Aggregate/Rosette Chromatin Architecture, Dynamics, and Functional Epigenetics of Genomes





Tobias A. Knoch

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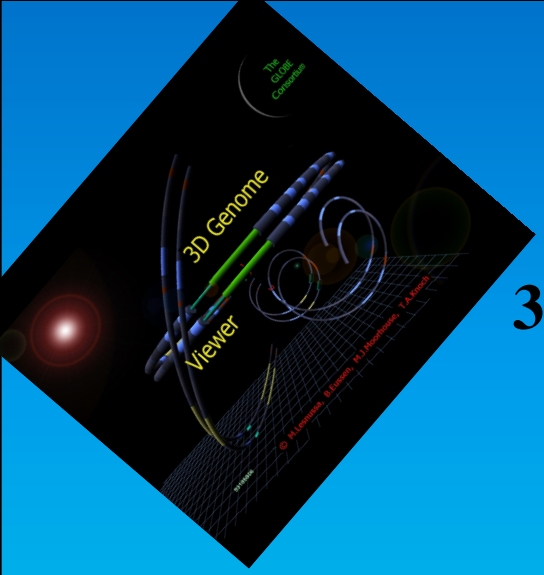
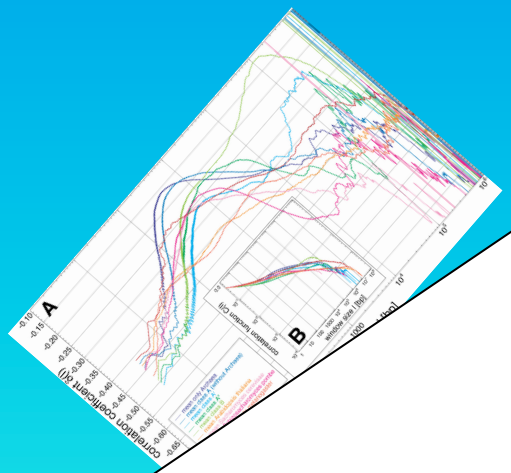
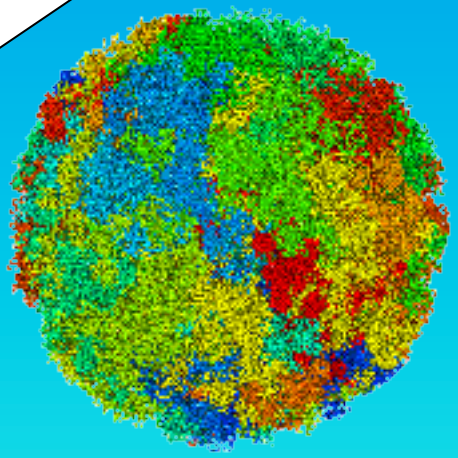
2nd Annual Epigenetics Discovery Congress, Heathrow Marriott Hotel, London, United Kingdom, 8th – 9th September, 2016.



Decoding the 3D Multi-Loop Aggregate/B Chromatin Architecture, F Funct

Results and Perspectives of a Finally Solved Challenge!





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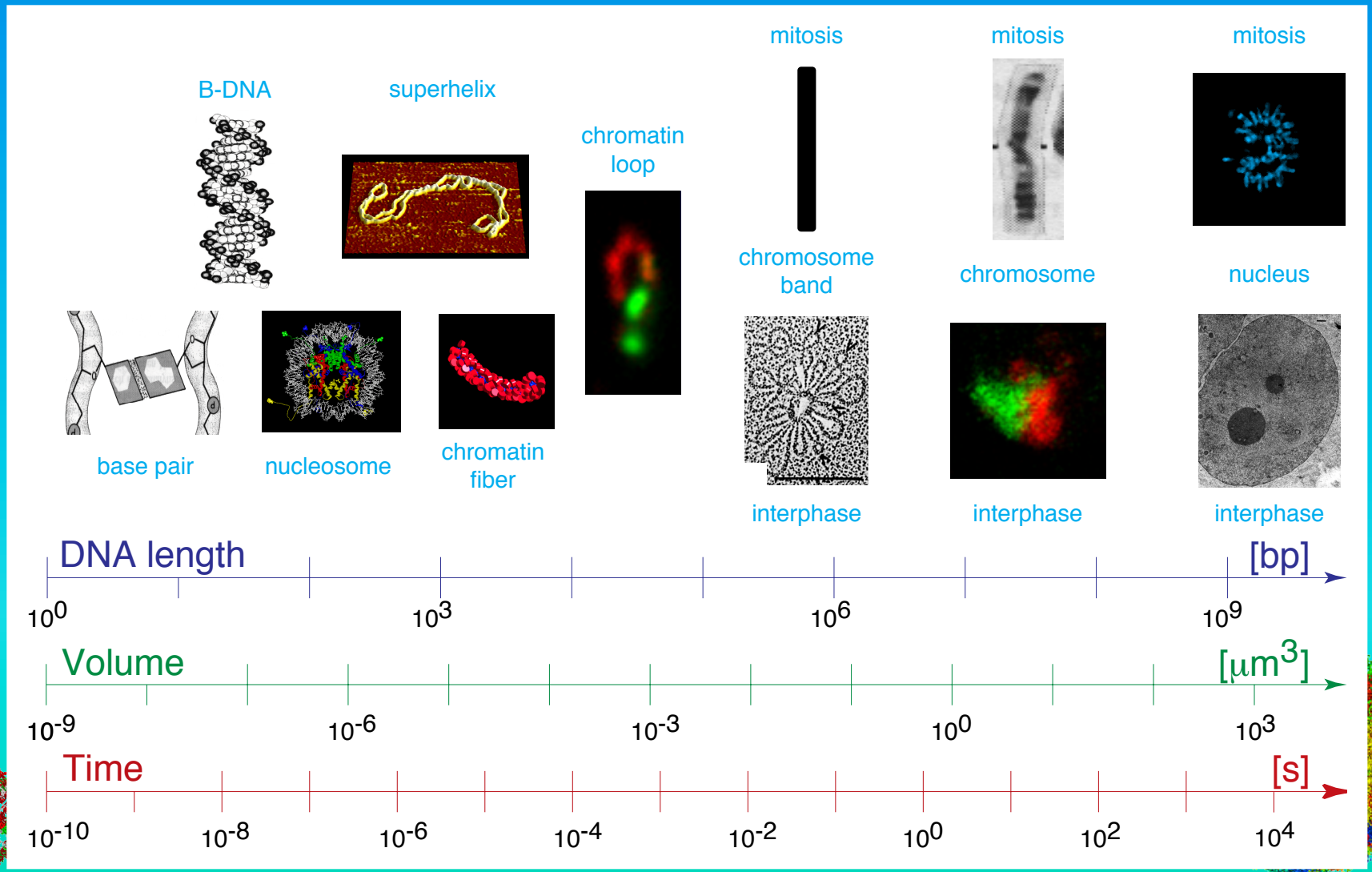
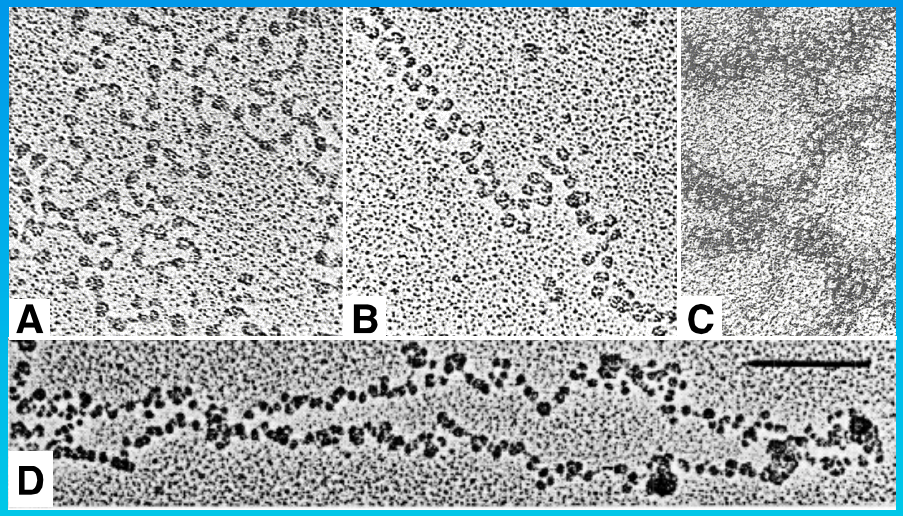
Dynamic and Hierarchical Genome Organization

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?

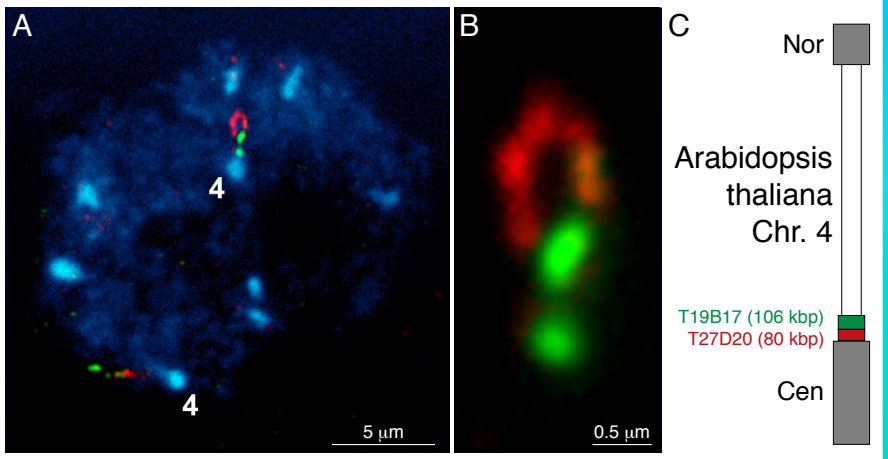


Chromatin Conformation and Higher-Order Topologies

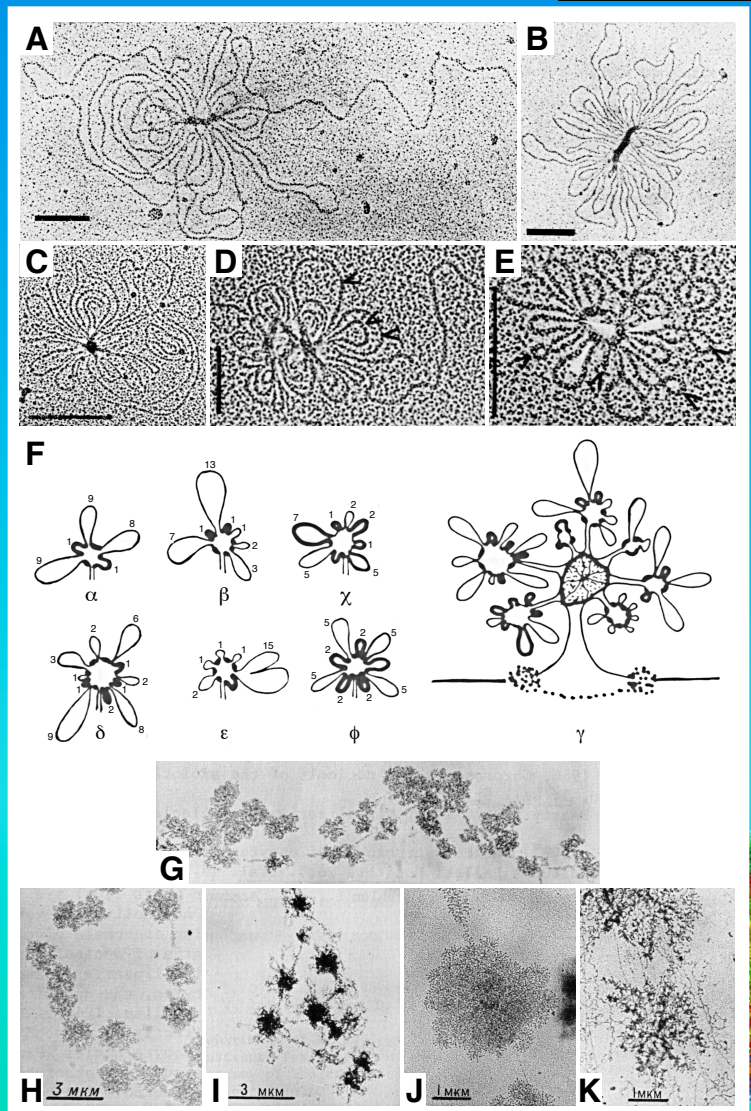
It becomes increasingly clearer, that the chromatin conformation is a random organization of nucleosomes, which depending on external or modification conditions has different condensation degrees, with a prevalence for the 30nm fiber with ~6nucleosomes per 11nm. This seems to make loops which further 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

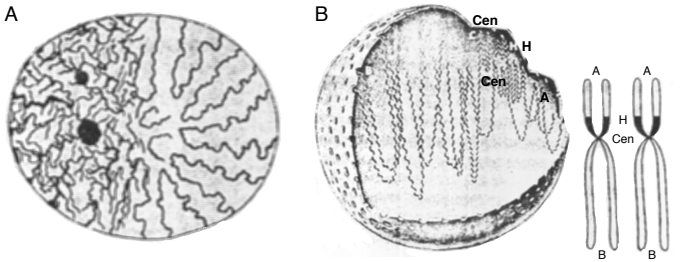


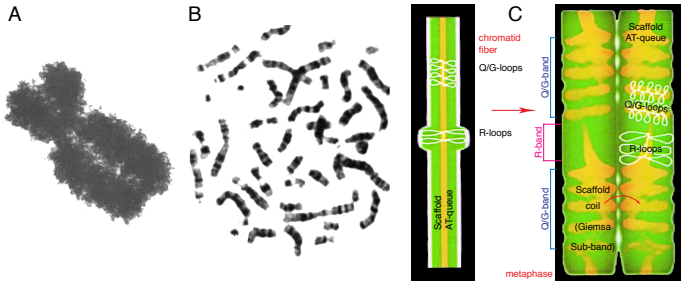
A, B: Avramova *et al.*; C: Salganik *et al.*; D-G:Frznik *et al.*; G-K: Tsvetkov & Parvenov.

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.

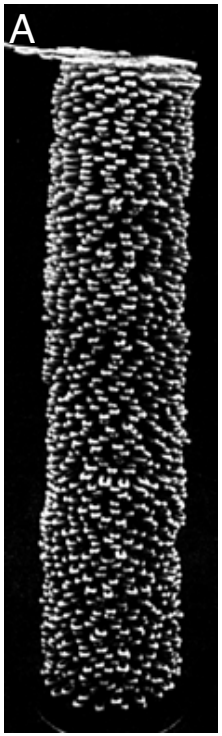
Rabl & Boveri

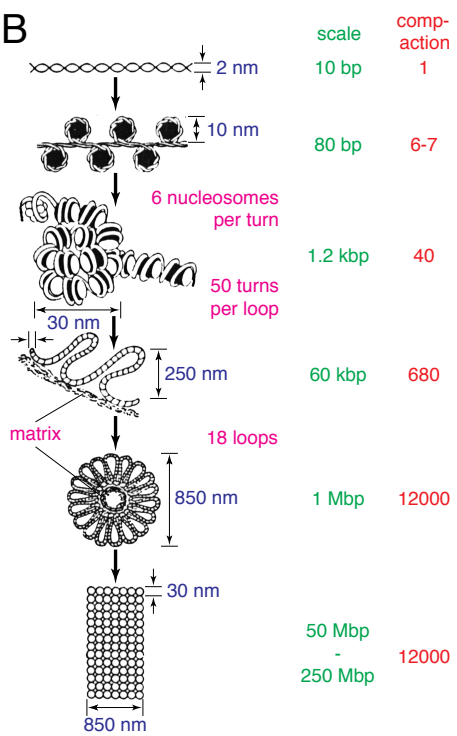


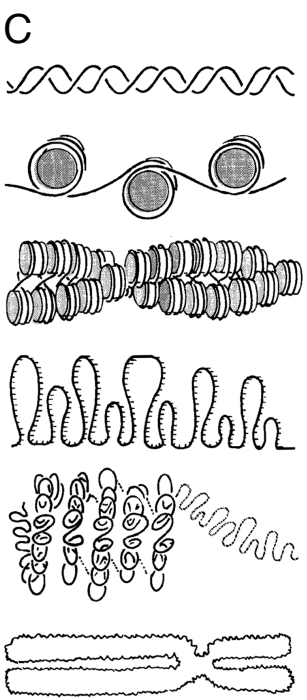


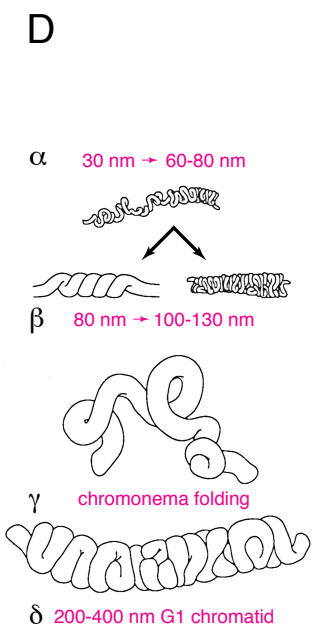
A: Bloom & Fawcett
B: Alberts *et al.*
C: Paulson & Laemmli

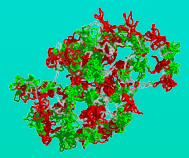
A, B: Pienta & Coffey; C: Alberts *et al.*; D: Belmont & Bruce

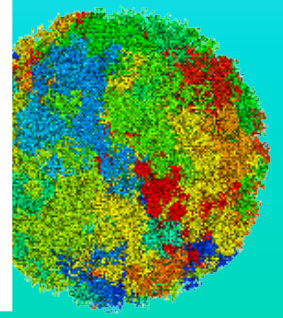






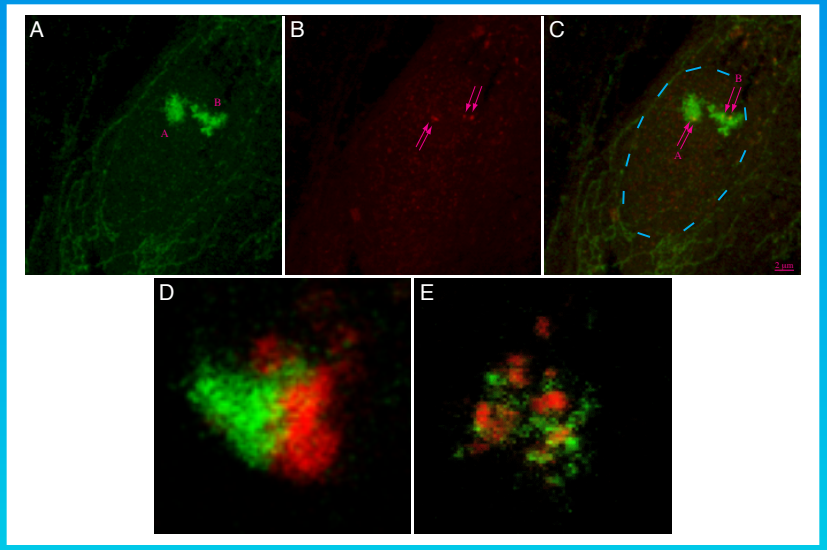




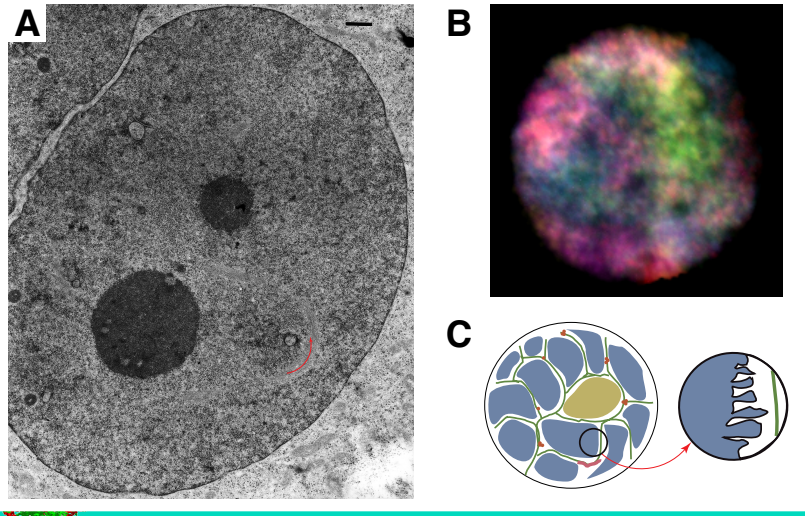


Integral Models of Cell Nuclear Organization

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…

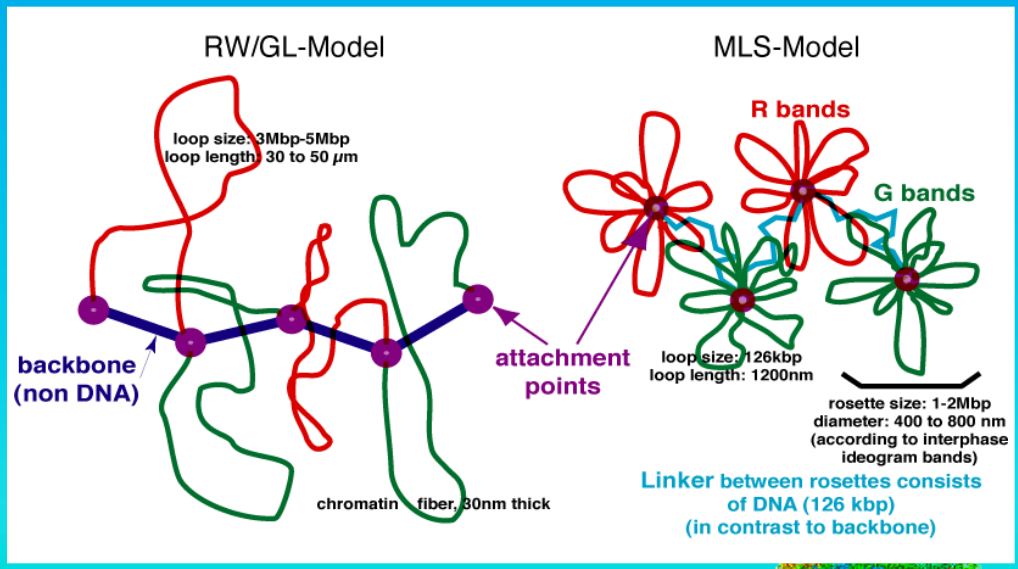


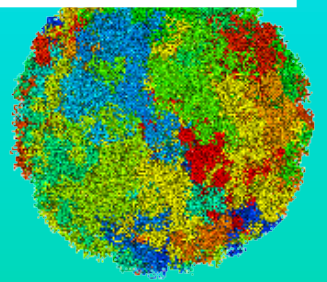
D: courtesy S. Dietzel; E: courtesy D. Zink



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

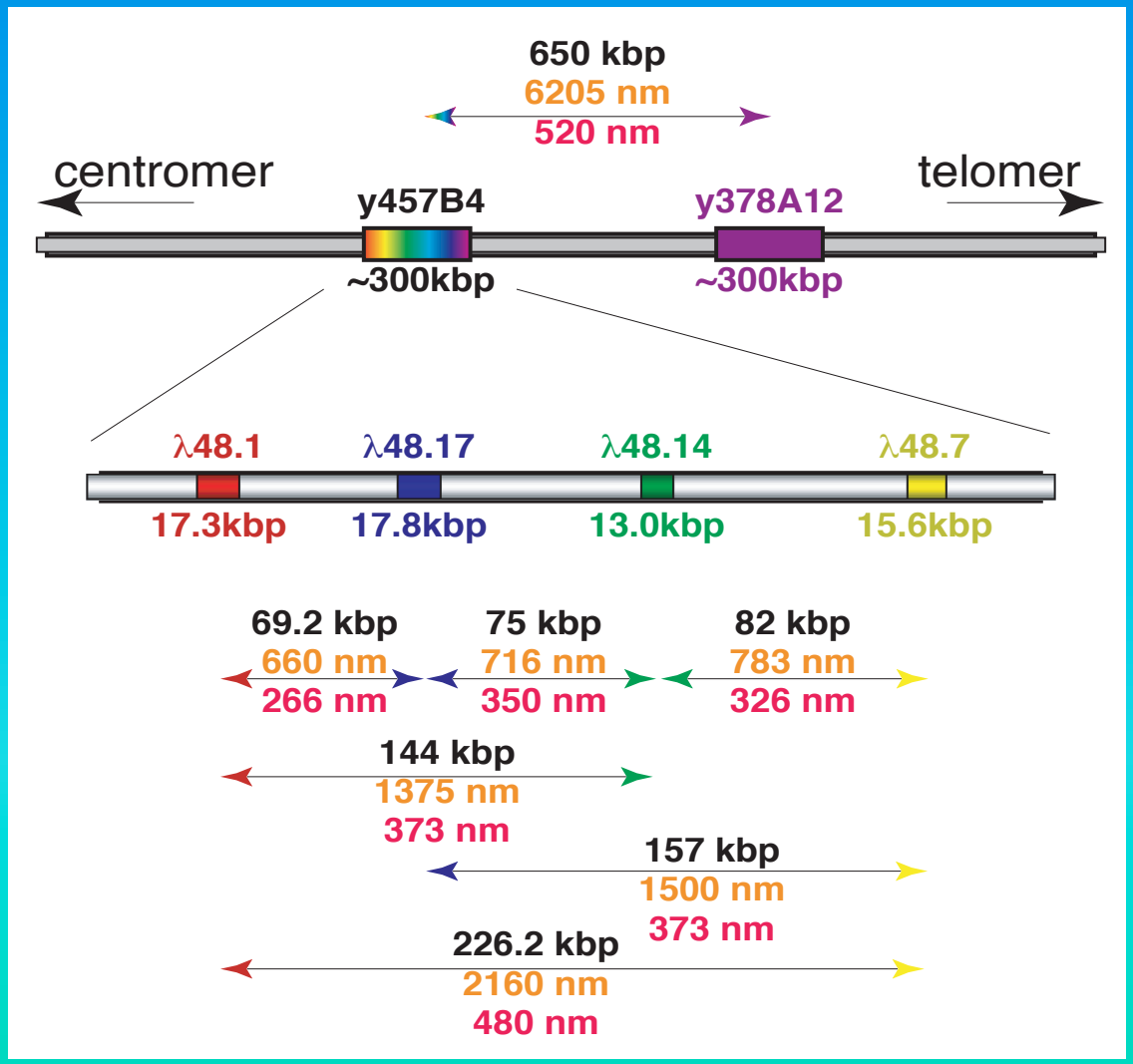
Random-Walk/Giant-Loop Multi-Loop-Subcompatment Model





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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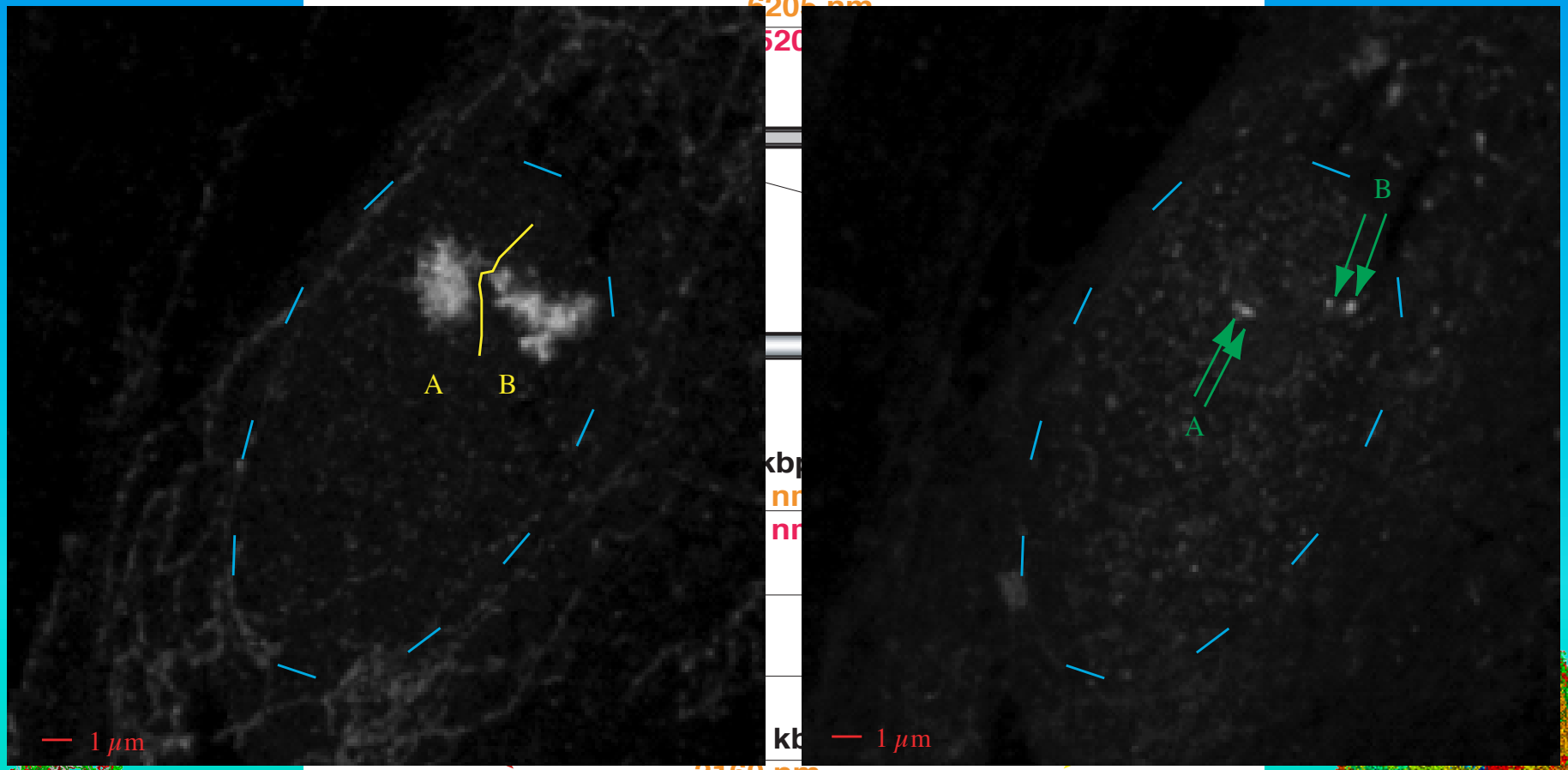

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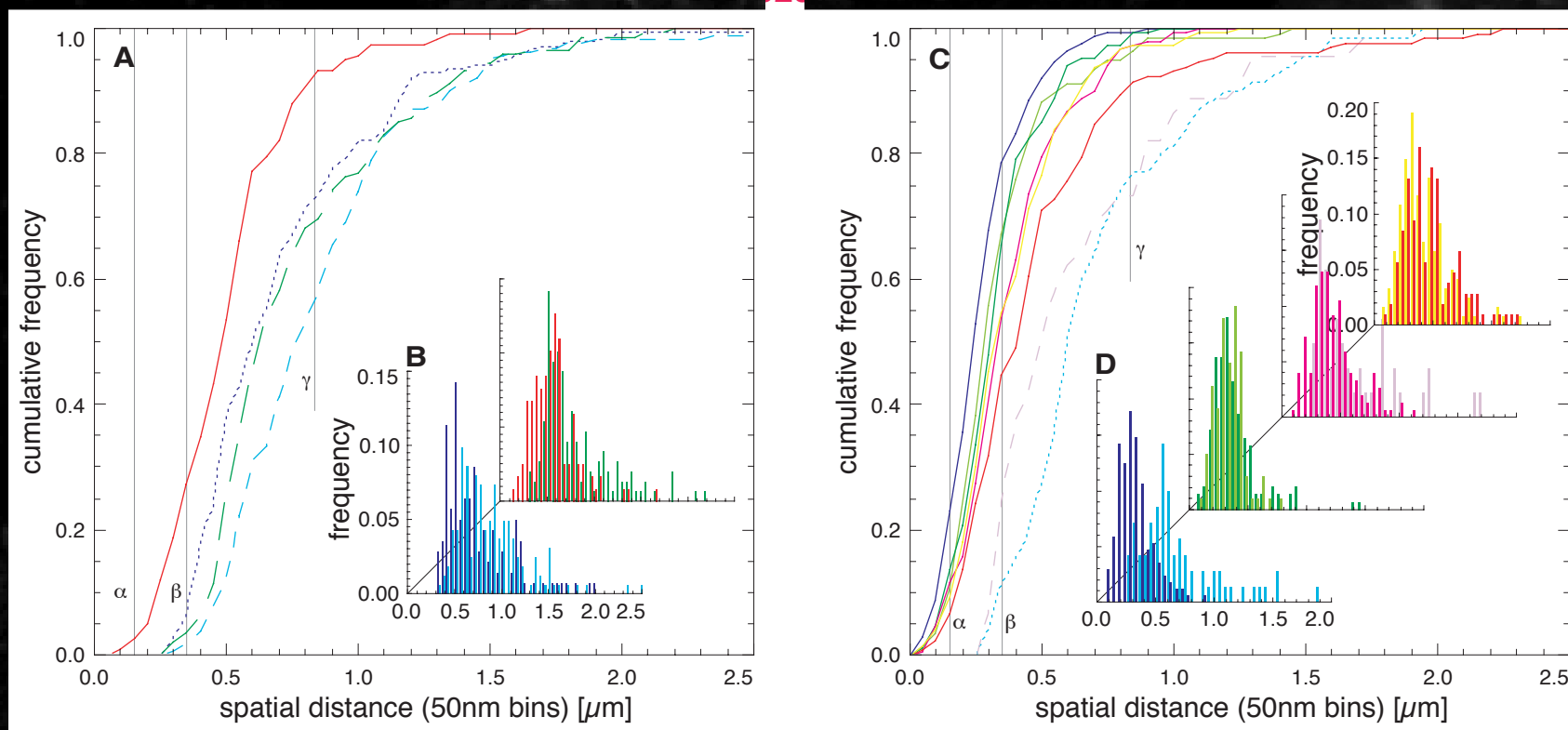
650 kbp

6205 nm

520



1 μm

kb

1 μm

2160 nm

480 nm

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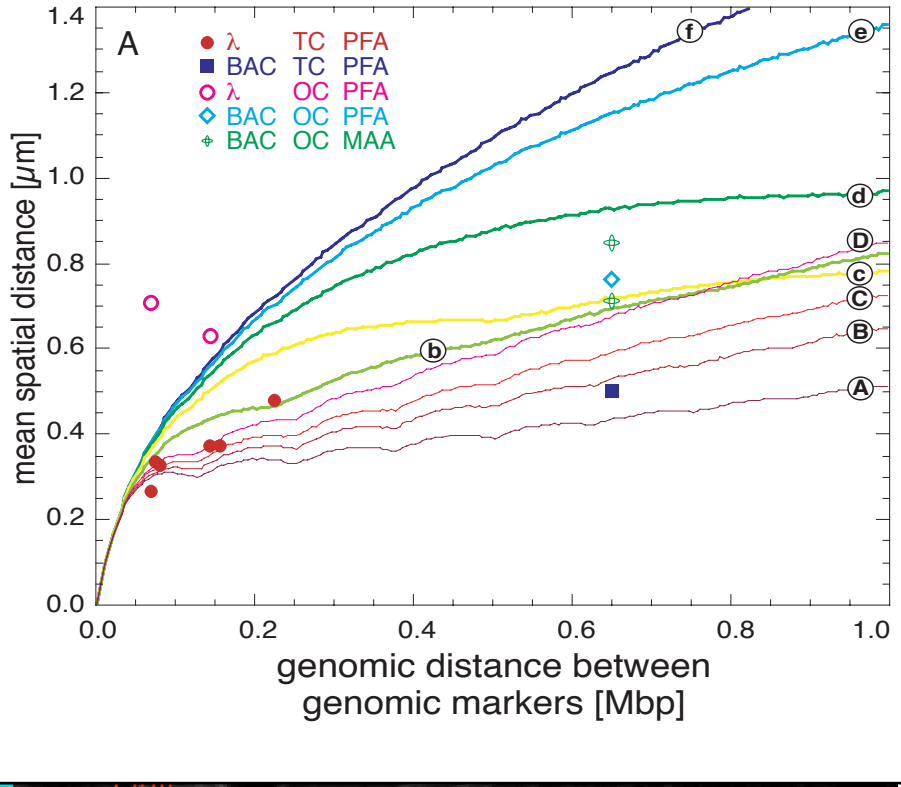

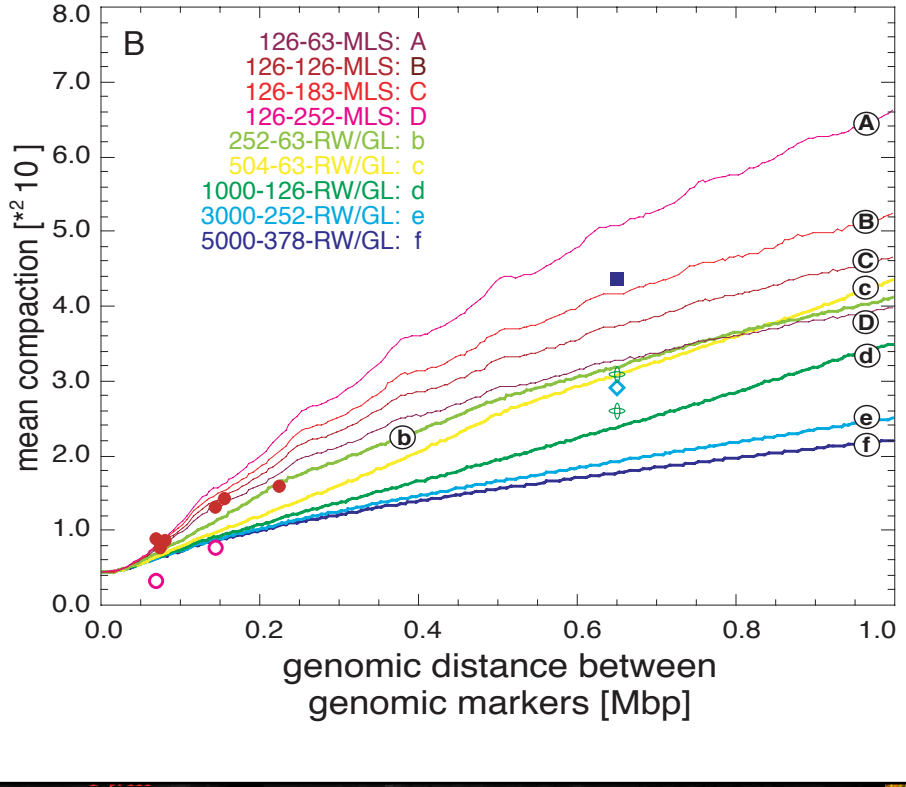

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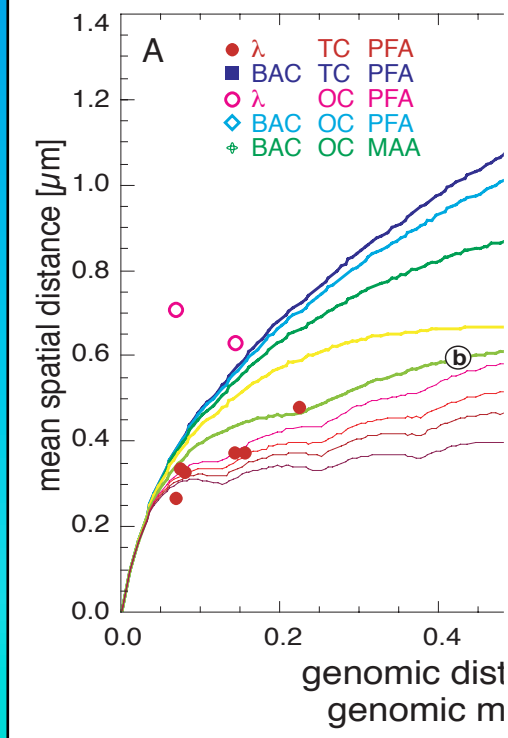
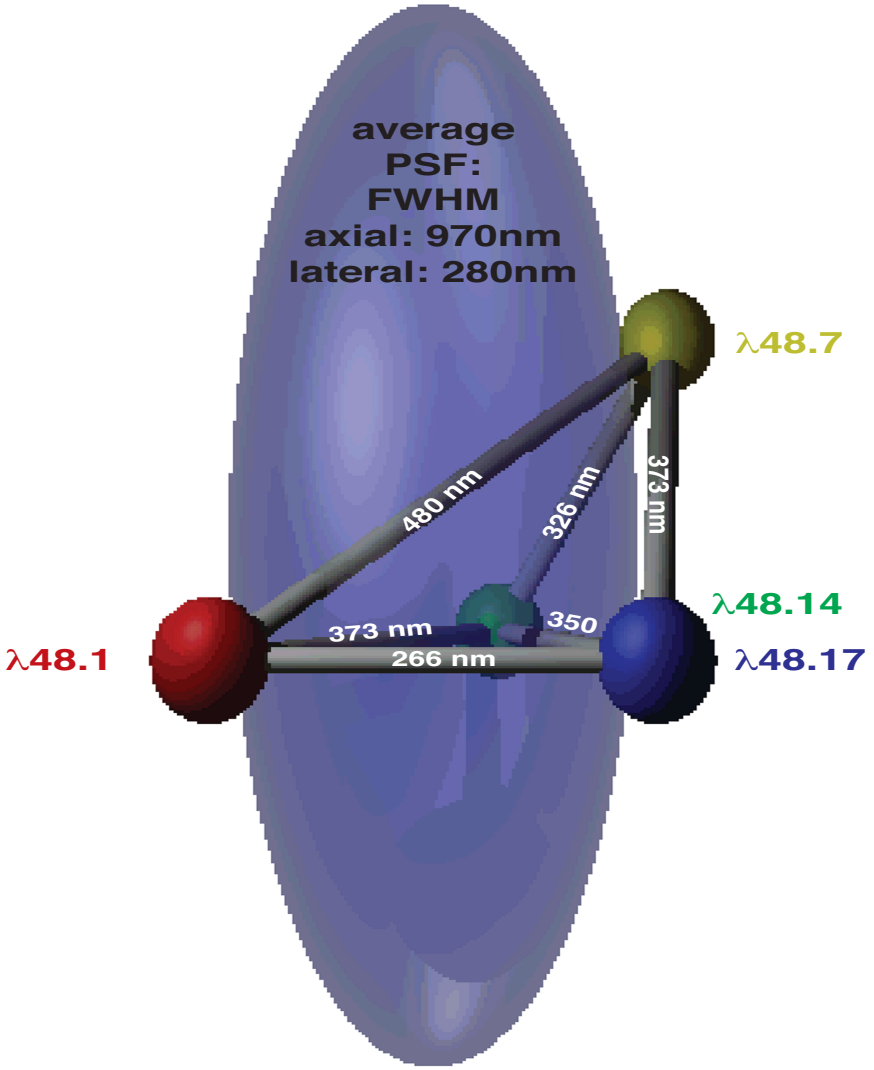
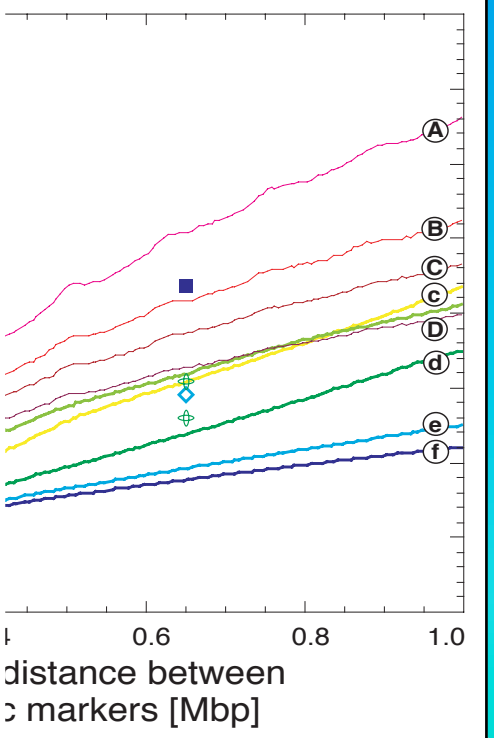
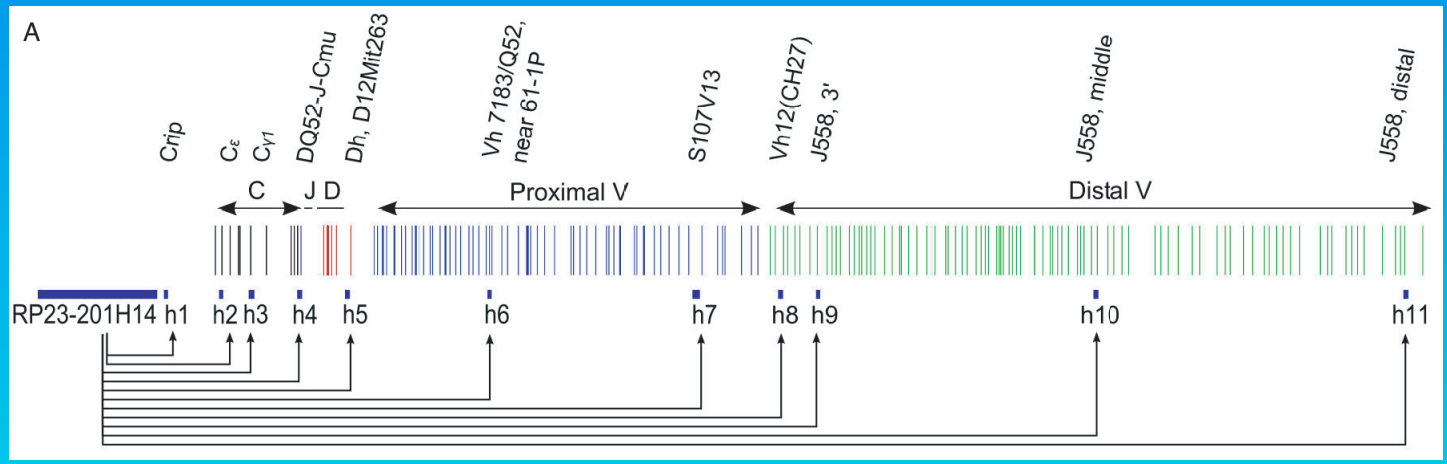


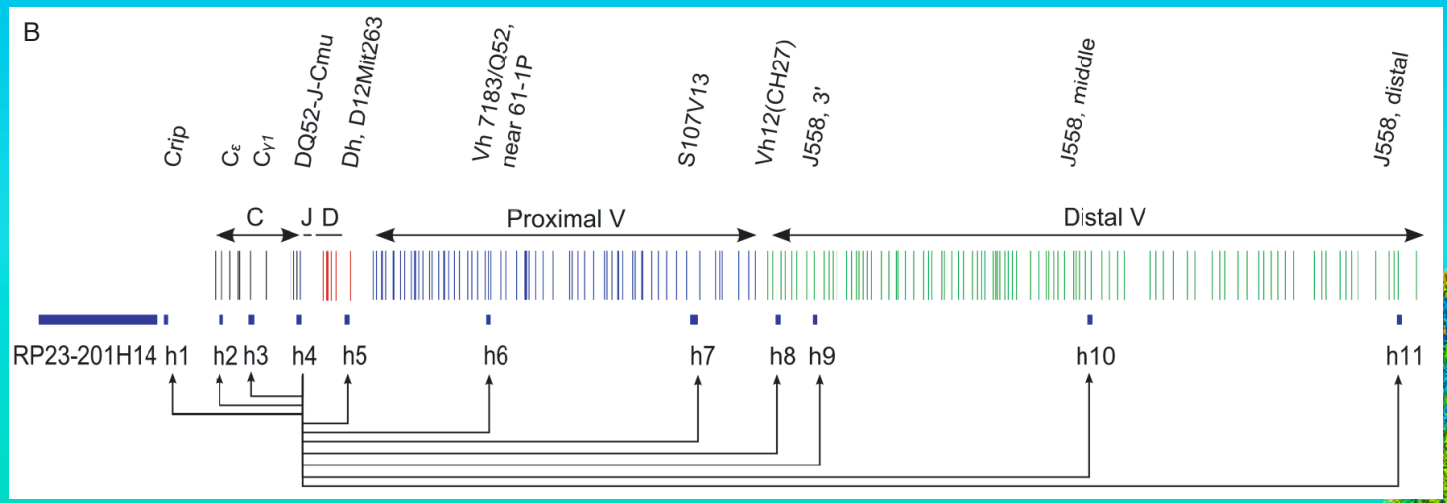




3D Archticture & Function of the IgH Locus

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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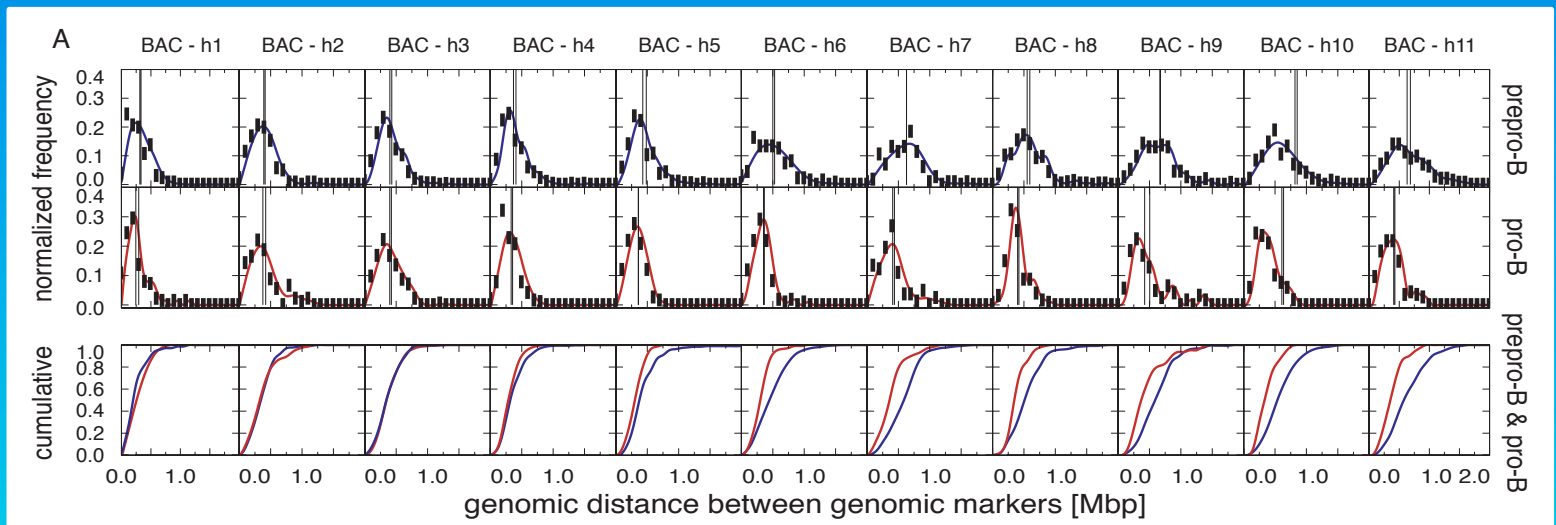

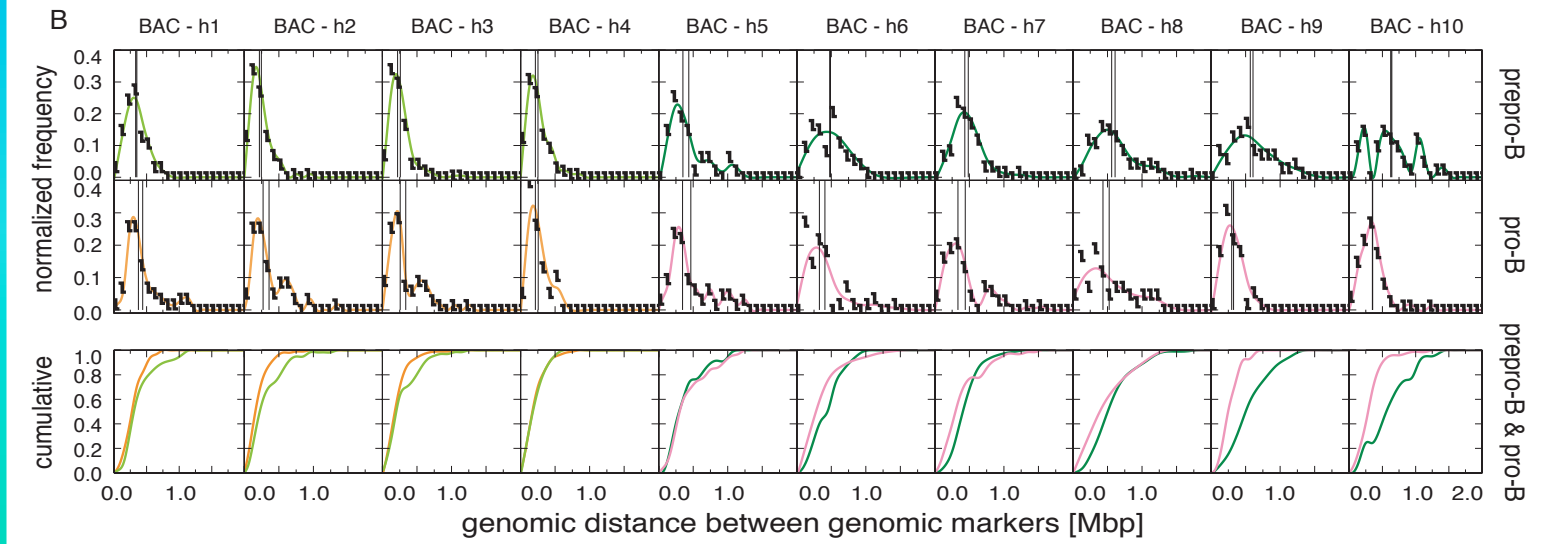

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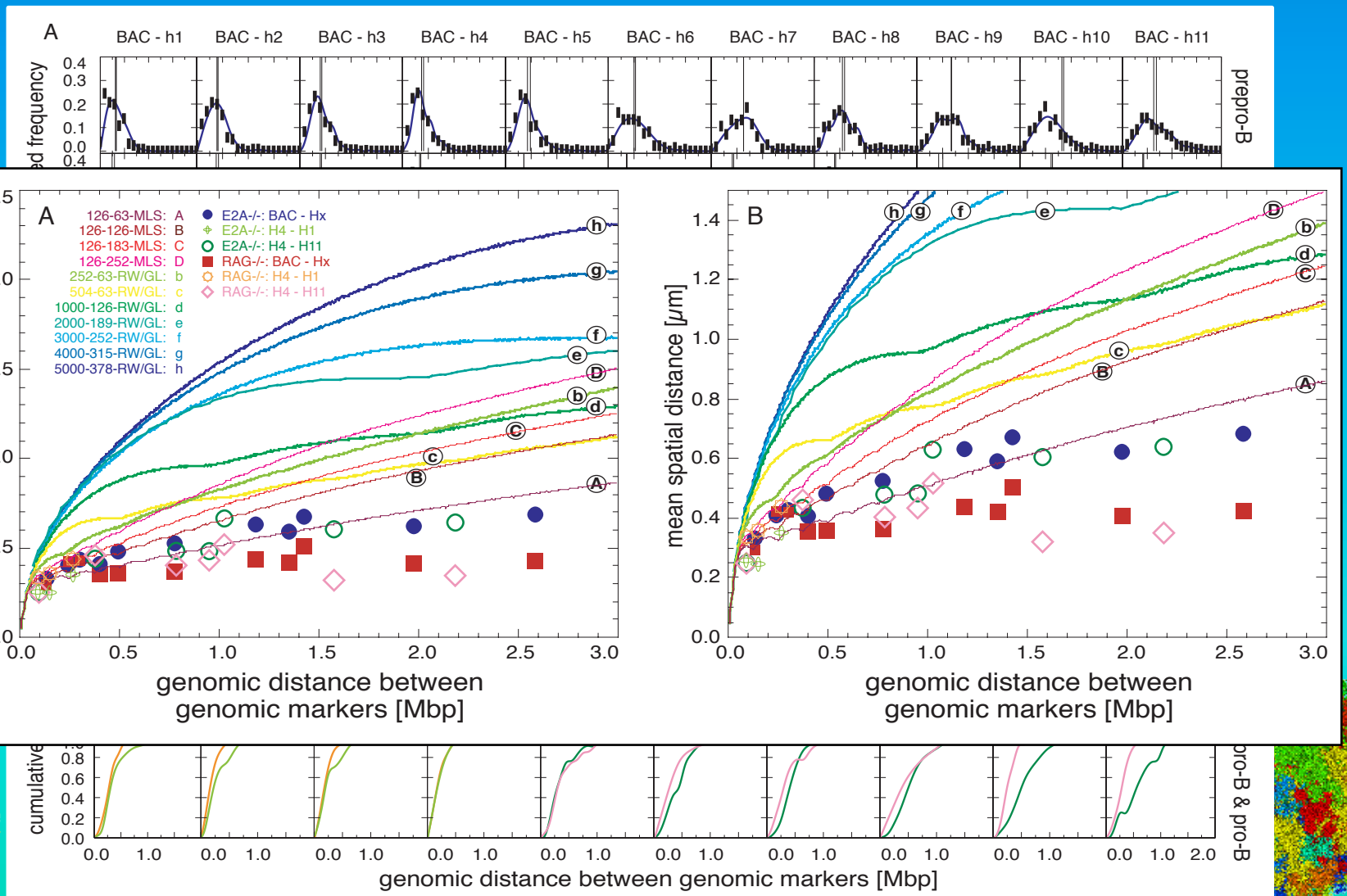

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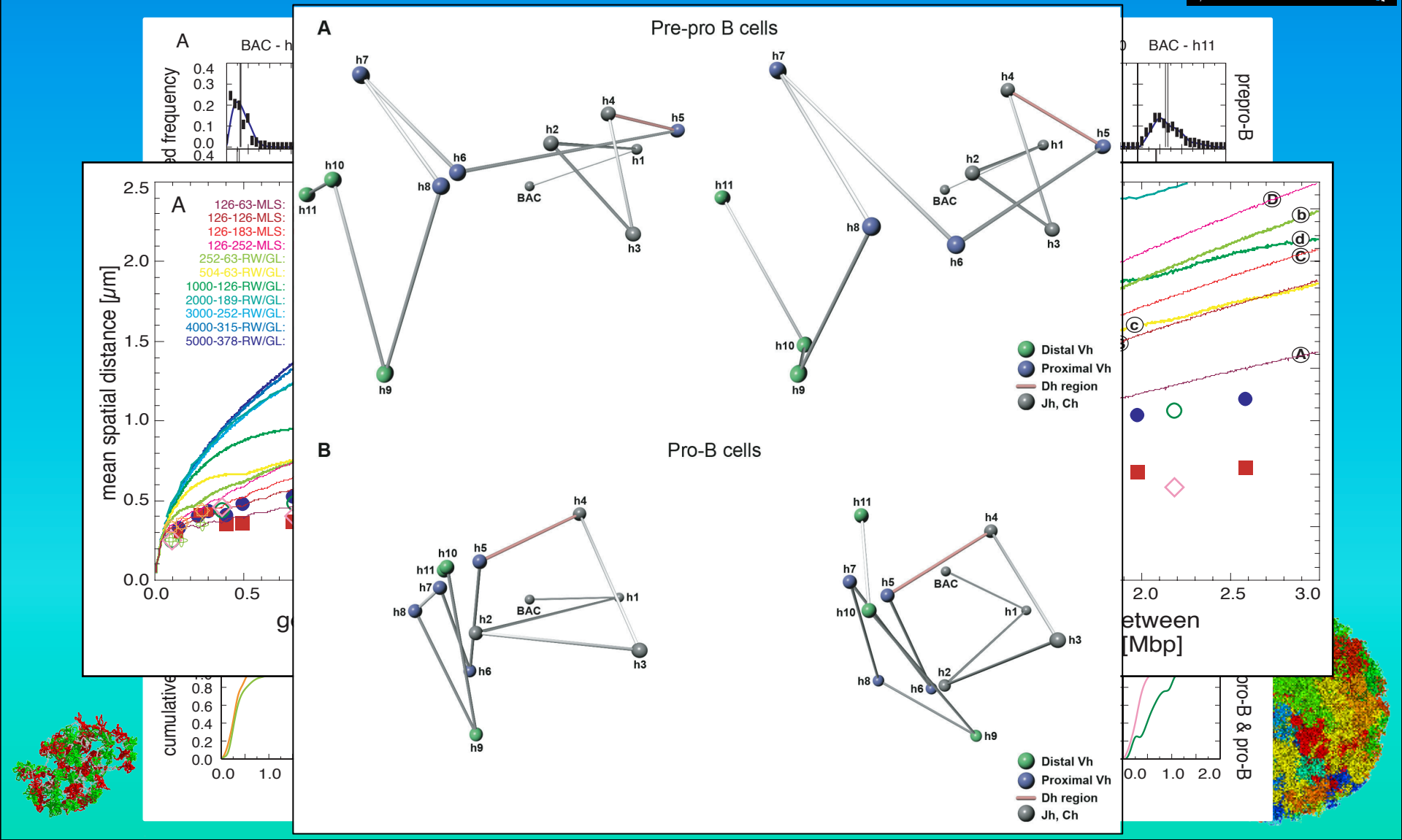
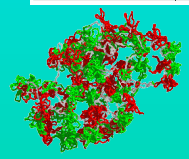


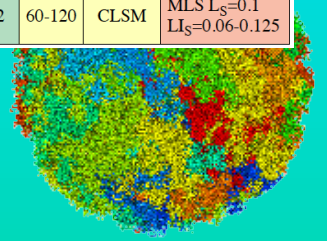
“Synoptic” 3D Architecture of Various Loci

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

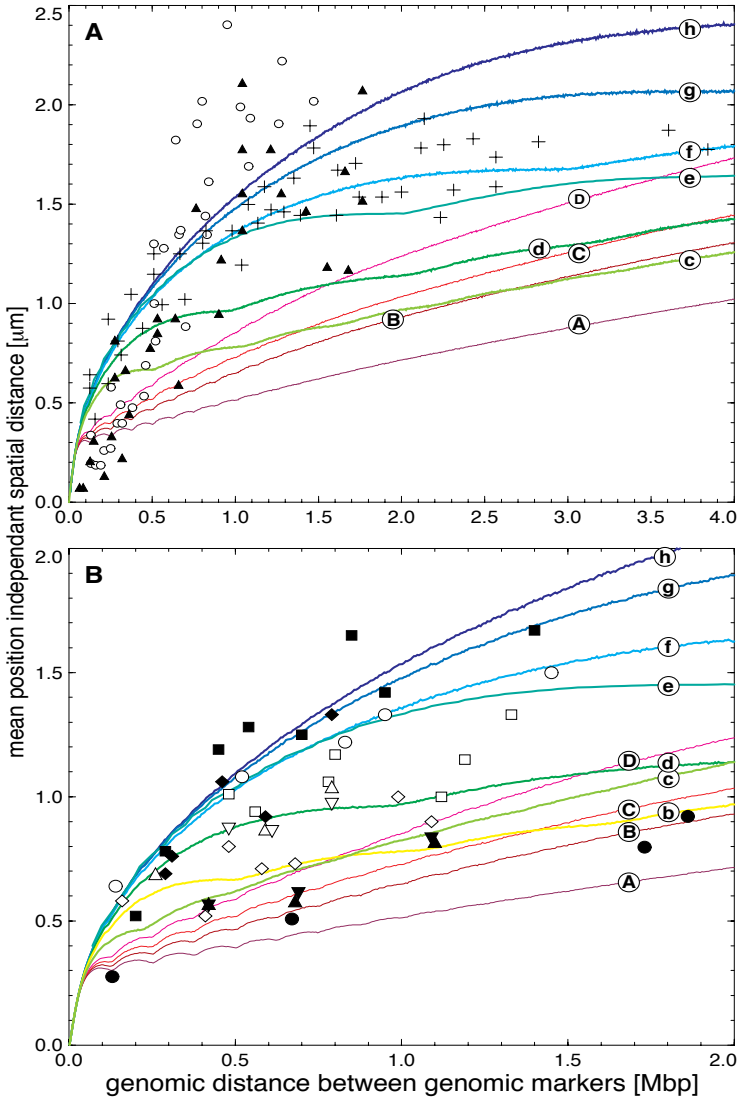


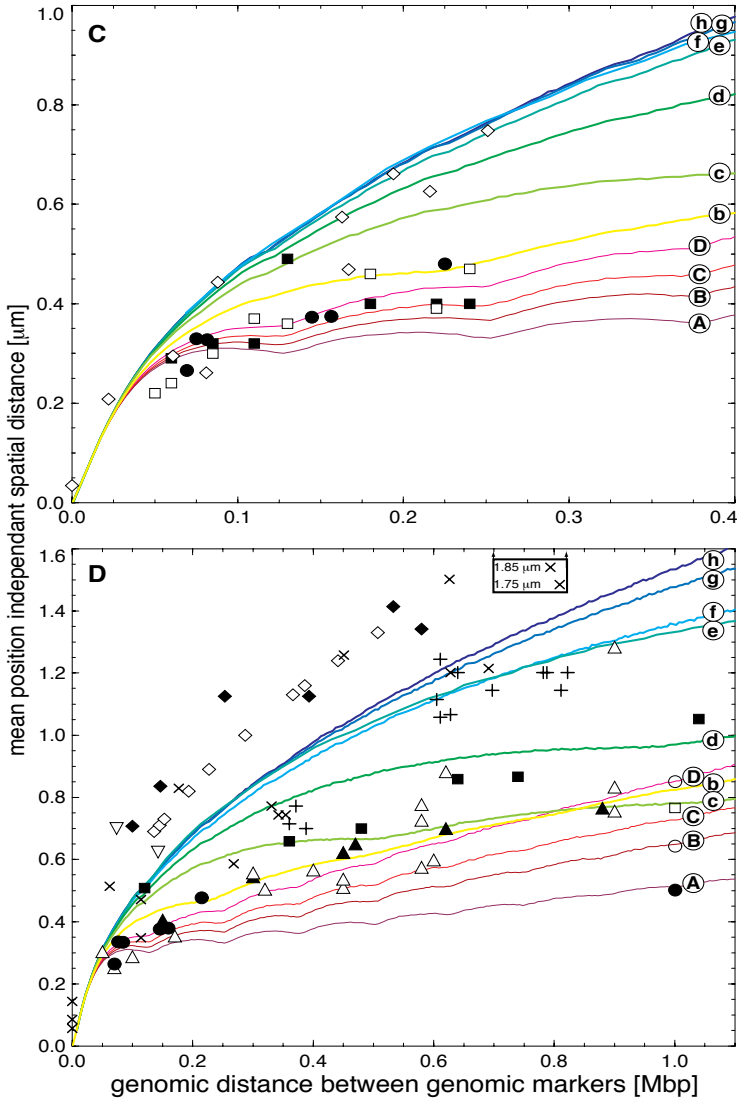


“Synoptic” 3D Architecture of Various Loci

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 favoured.

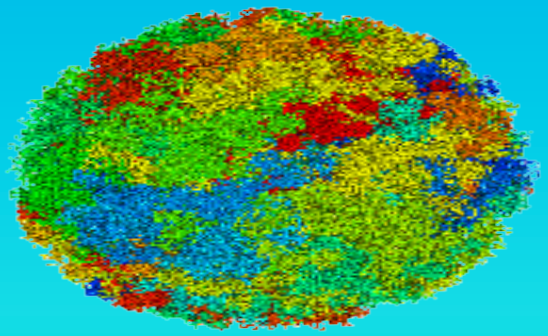
Study	to model
Fig. 3B, Tr ’89	L 2.0-3.0
Fig. 3B, Lawrence’	L 4.0-5.0
Fig. 3A, Tr ’91	L 1.0-2.0
Fig. 3B, Tr ’91	L 0.5-0.9
Fig. 3, v.d. ’92 or Fig. Trask ’93	L 1.0-5.0j
Fig. 5B, Tr ’93	L 0.25 L_S=0.126 0.200
Fig. 5, Senger ’93	L 1.0
Fig. 5, Senger ’93	L 0.25 L_S=0.126 0.200
Fig. 1, Warrington	L 1.0
Tab. 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

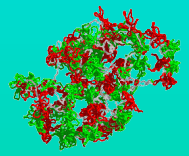


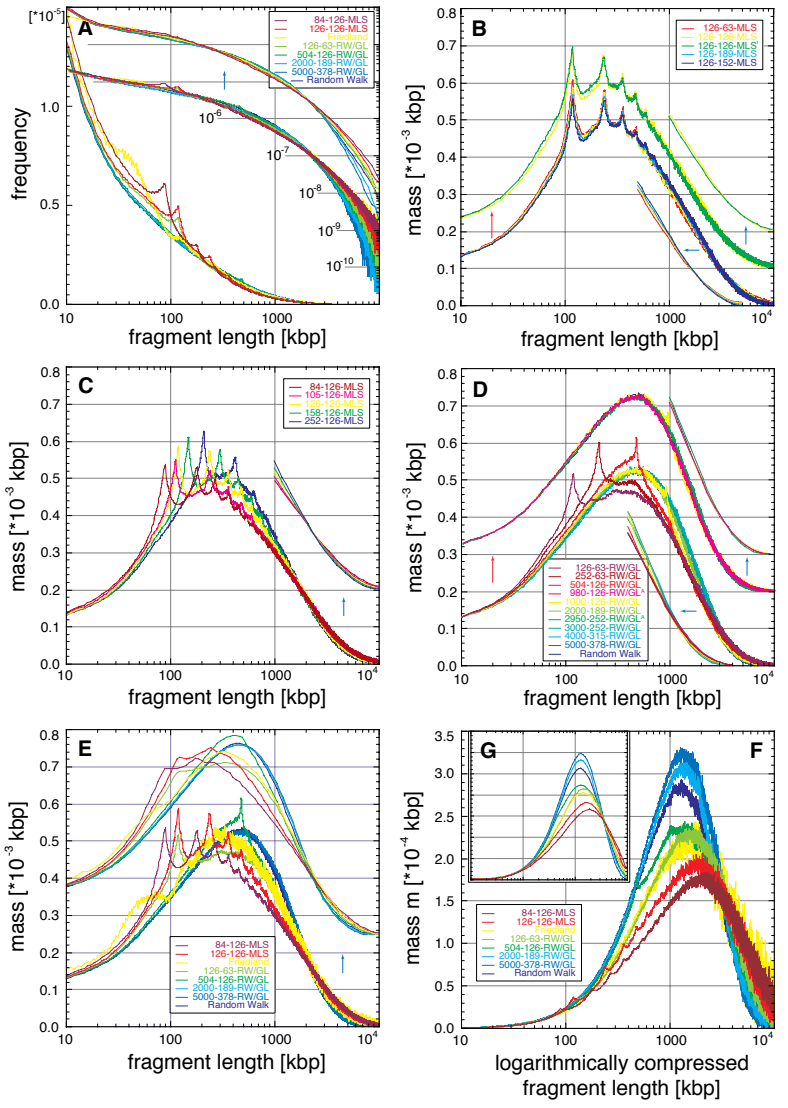


DNA Fragment Distribution after Ione-Irradiation

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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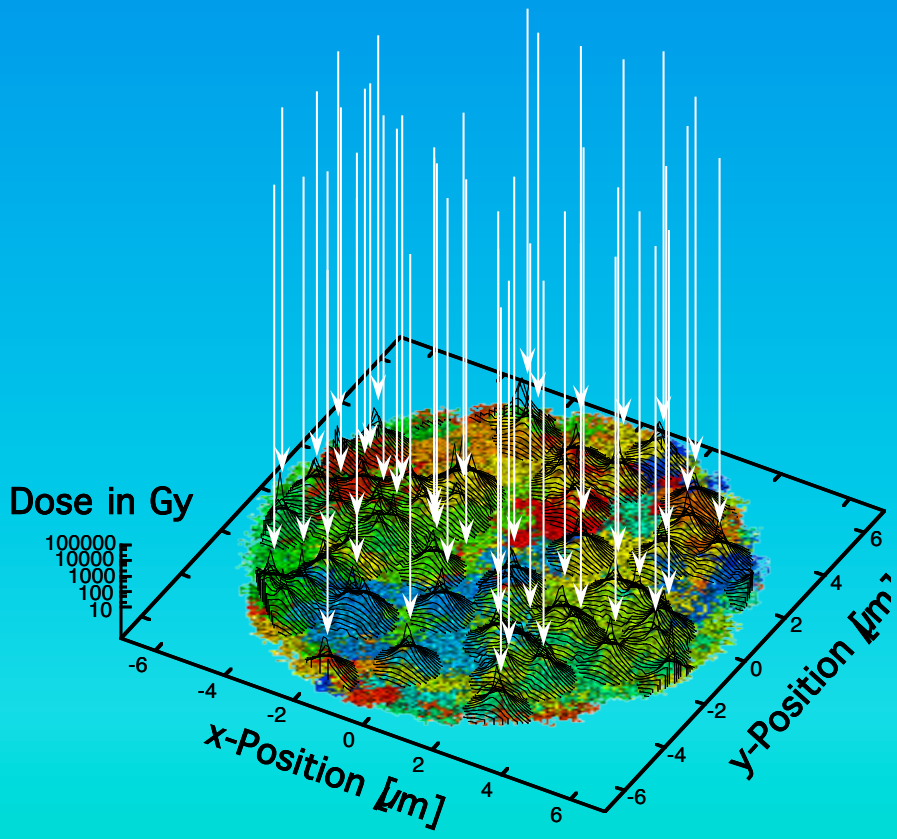
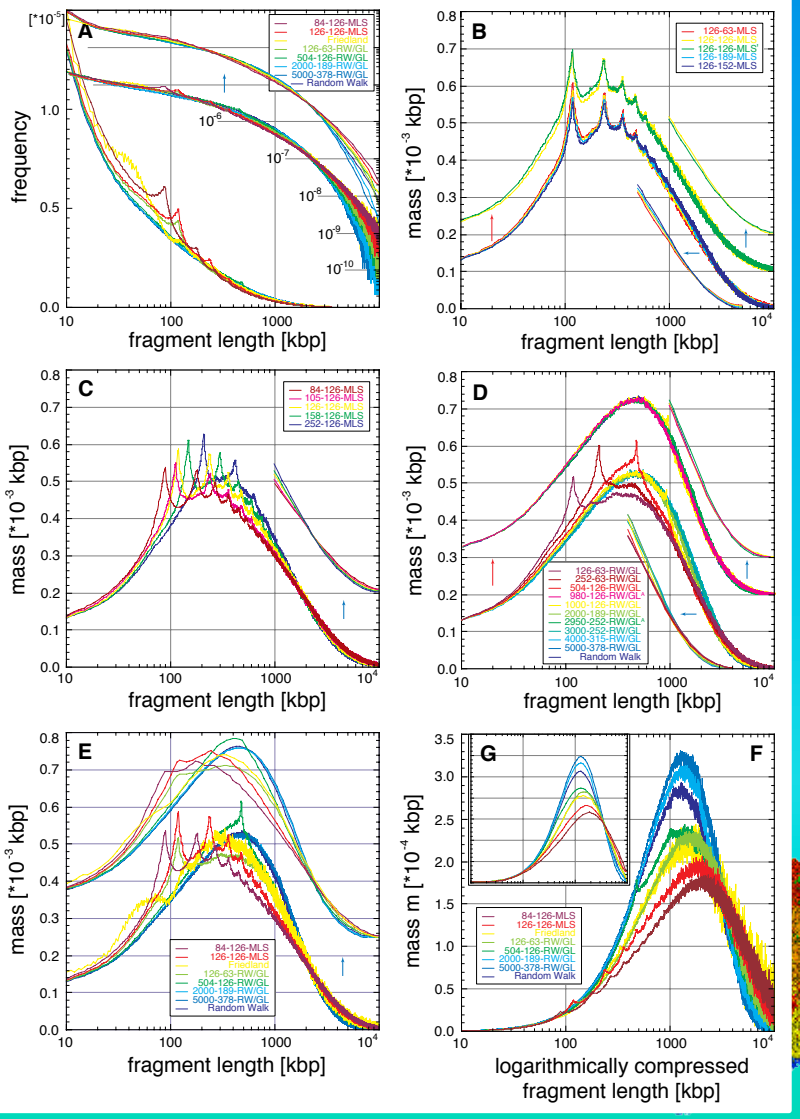
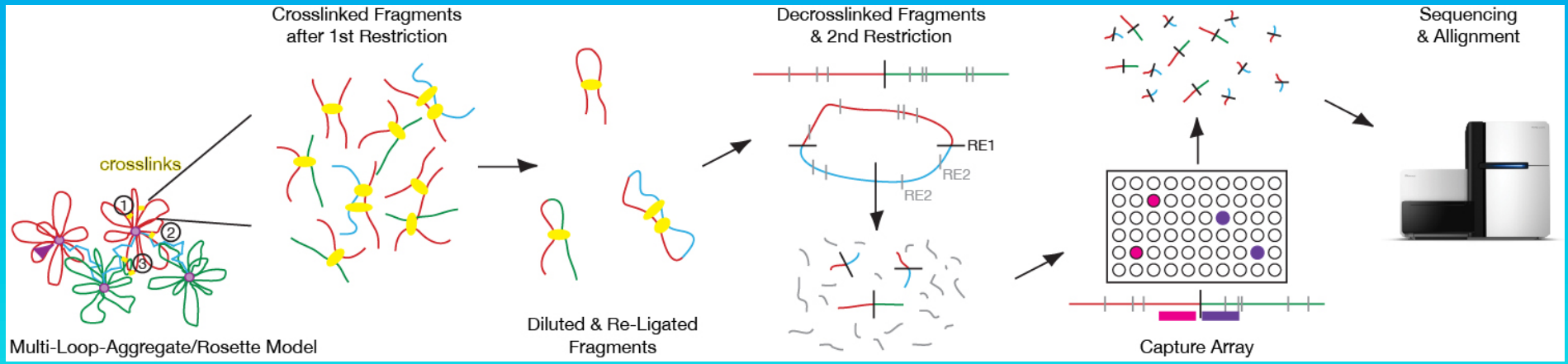




Selective Chromosome Interaction Capture (T2C)

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.



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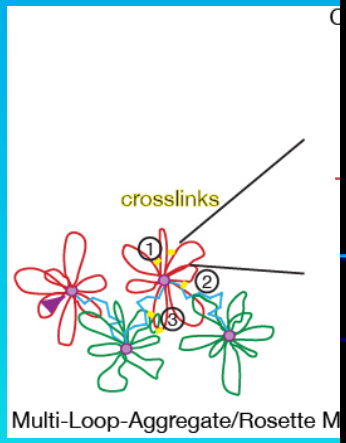
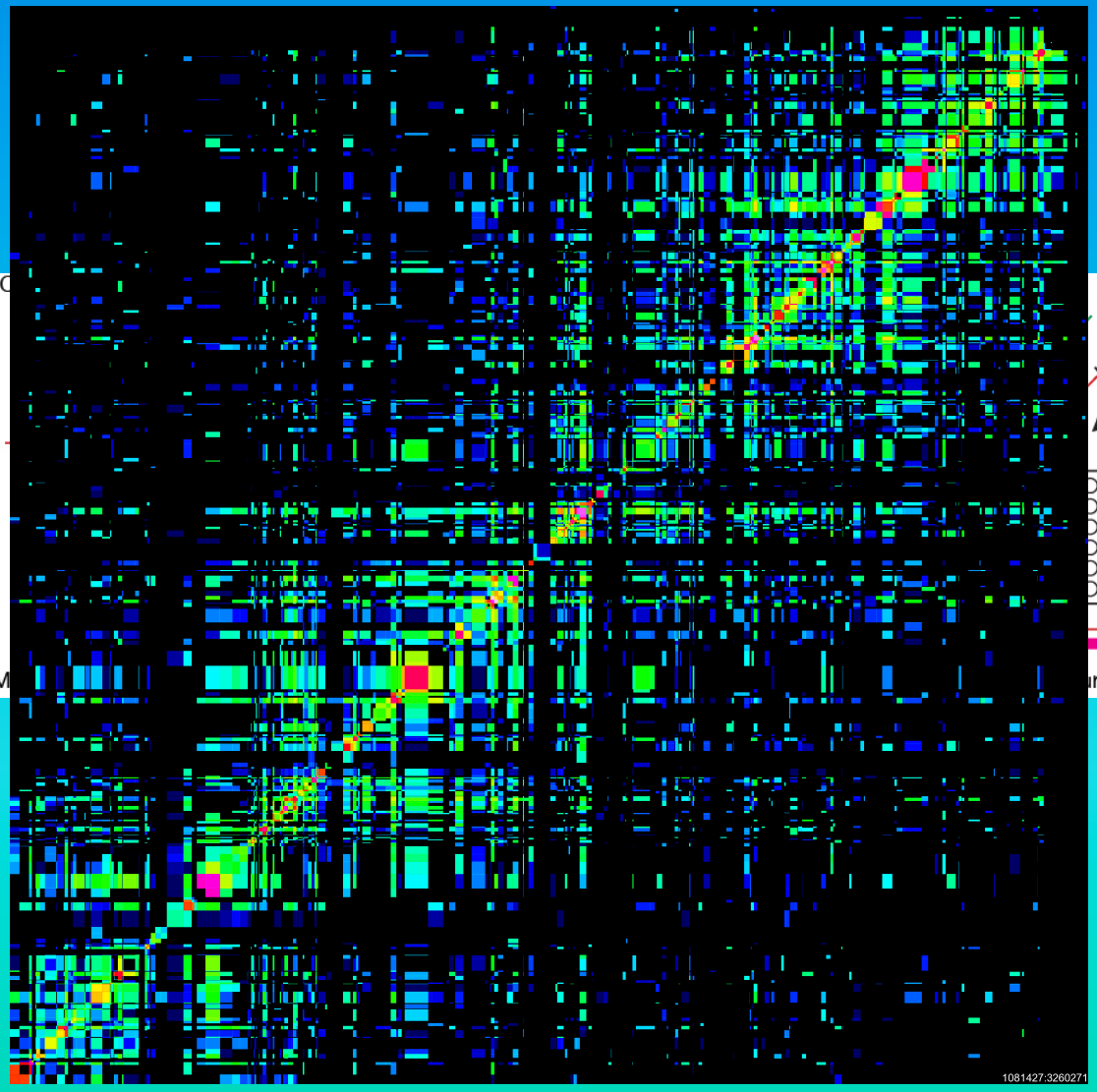
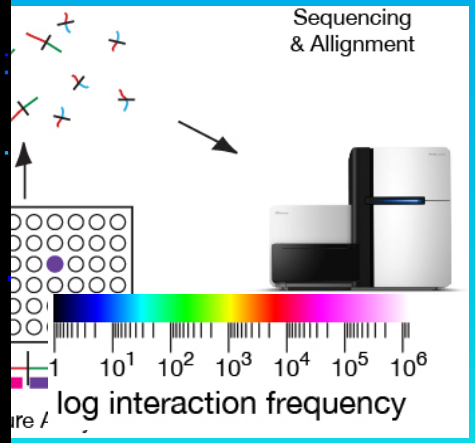
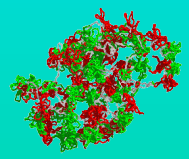
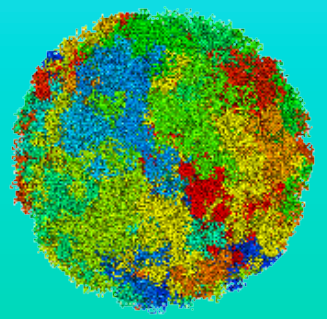


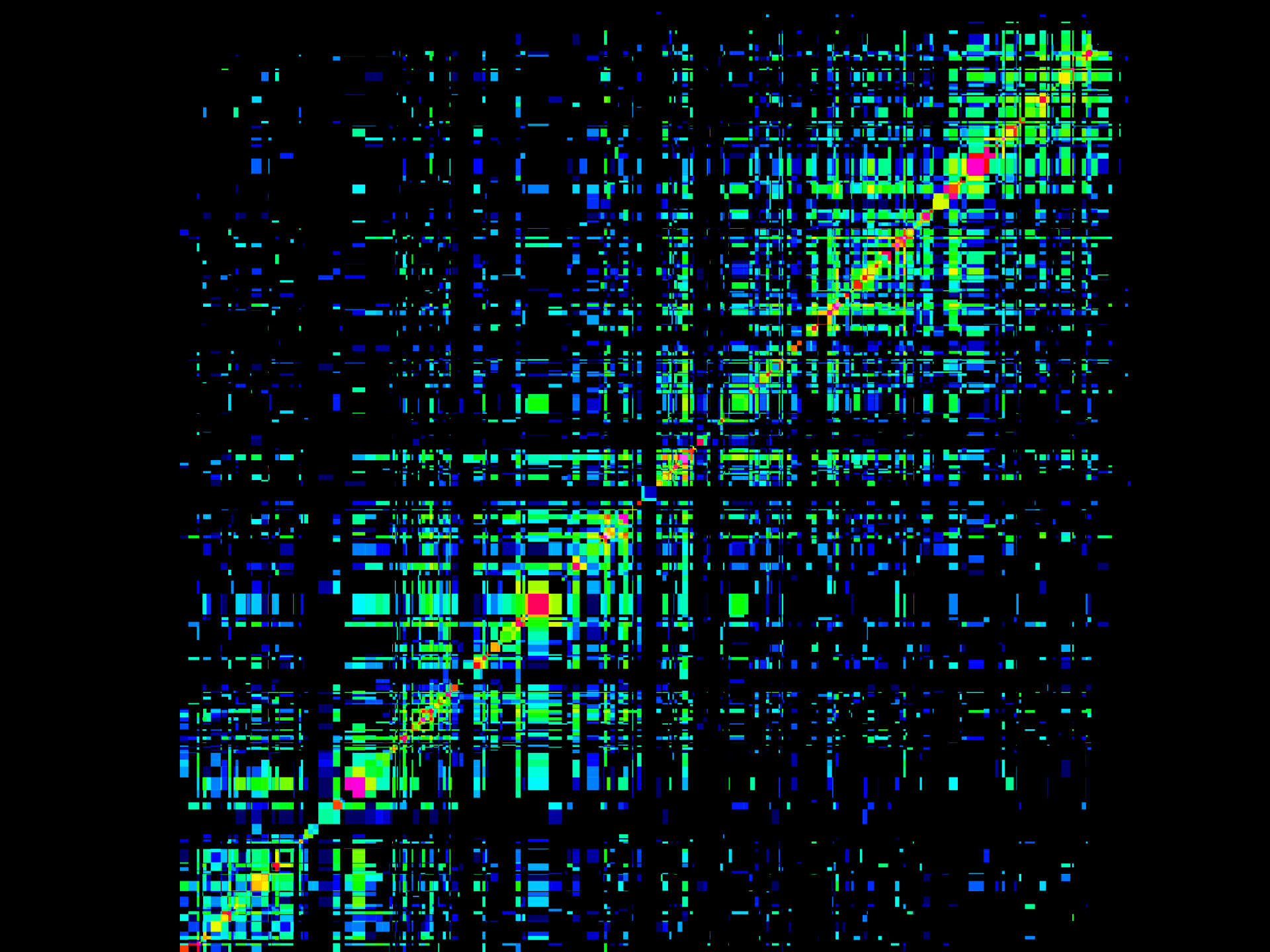




HS IGF locus
~2.1 Mbp



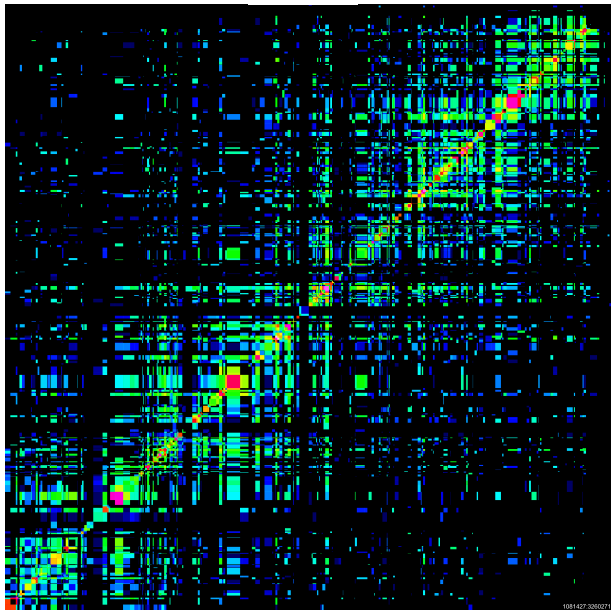




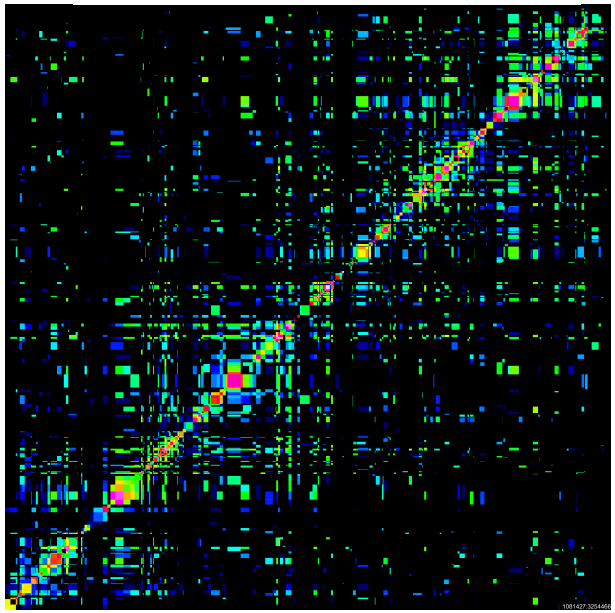
Stable Consensus Architecture of Genomes

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

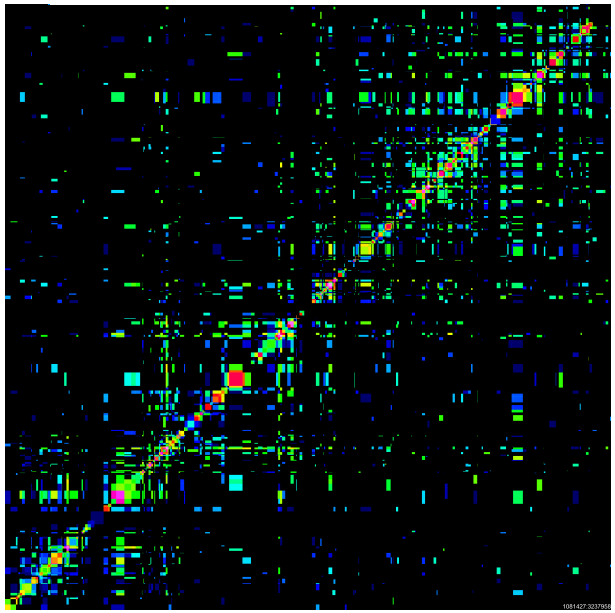
HB2

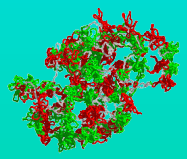


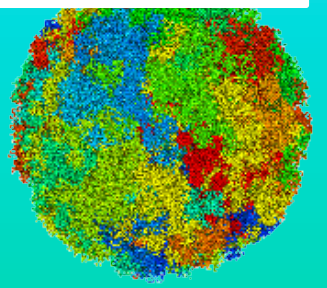
TEV-HEK293T cohesin intact



HRV-HEK293T cohesin cleaved







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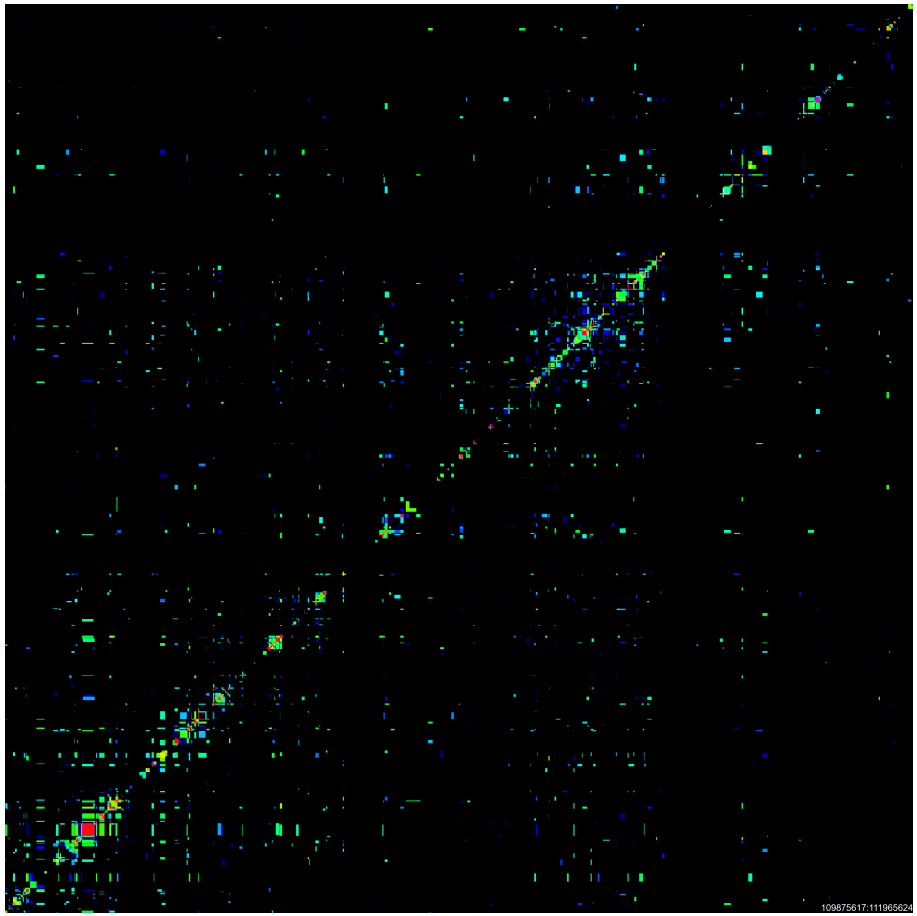
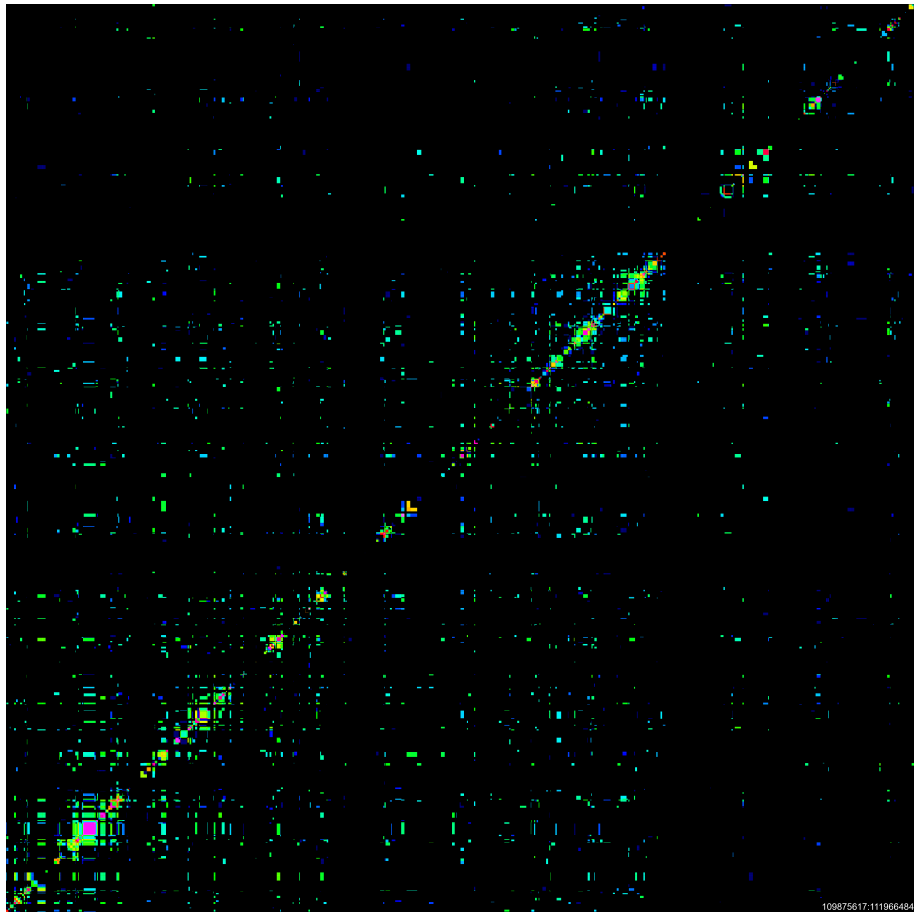
Fetal Brain (inactive β-Globin)



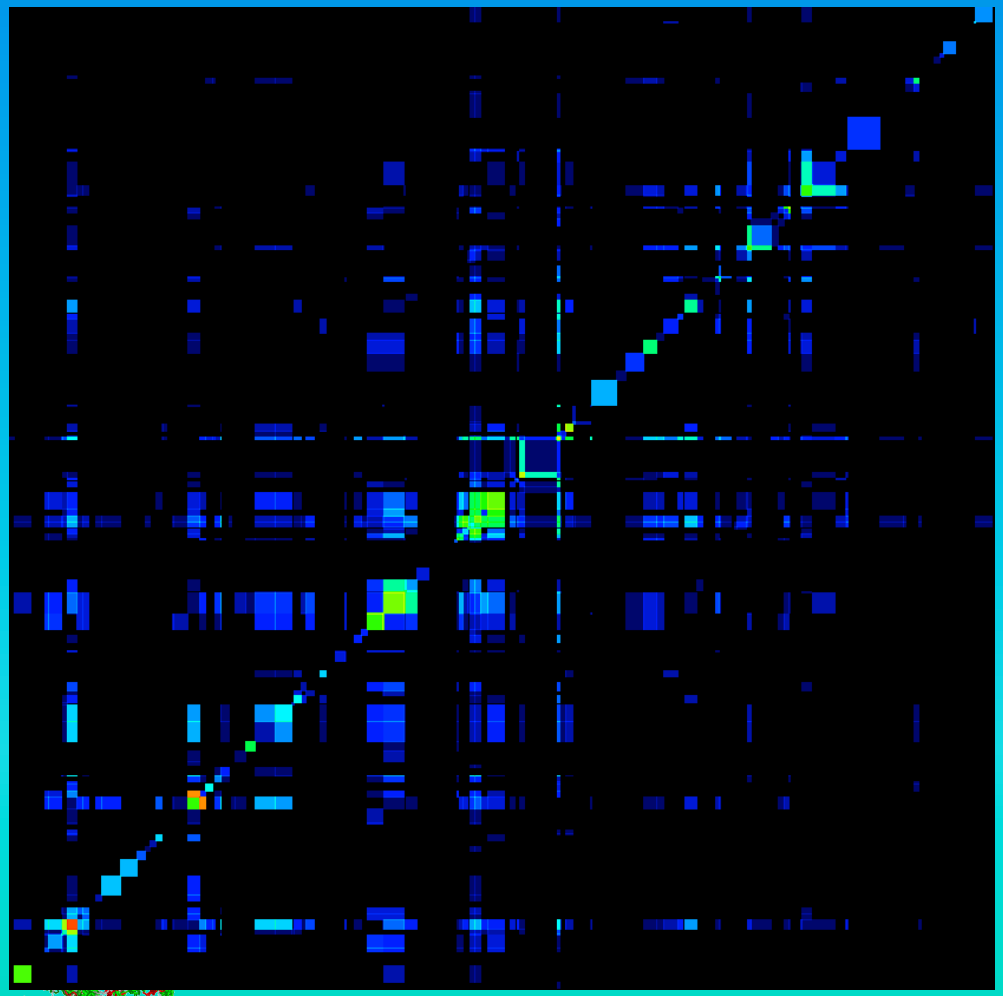
Fetal Liver (active β-Globin)

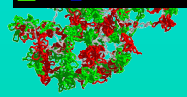


MM β-Globin 2.1 Mbp

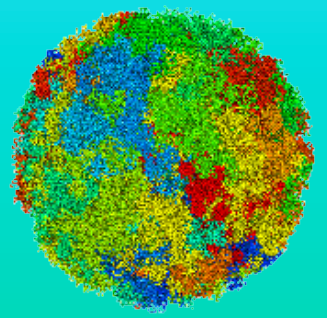
Fine Structure of Loop Aggregates/Rosettes

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.





~ 400 kbp



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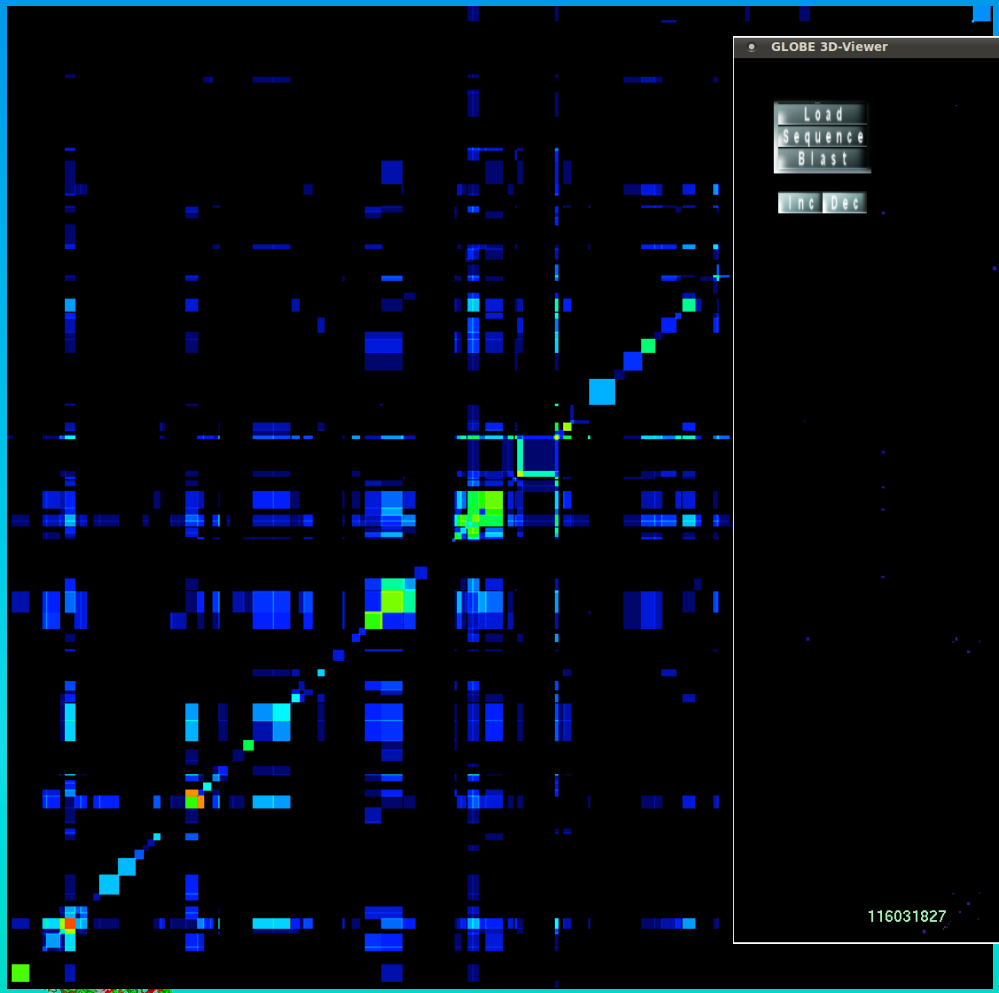

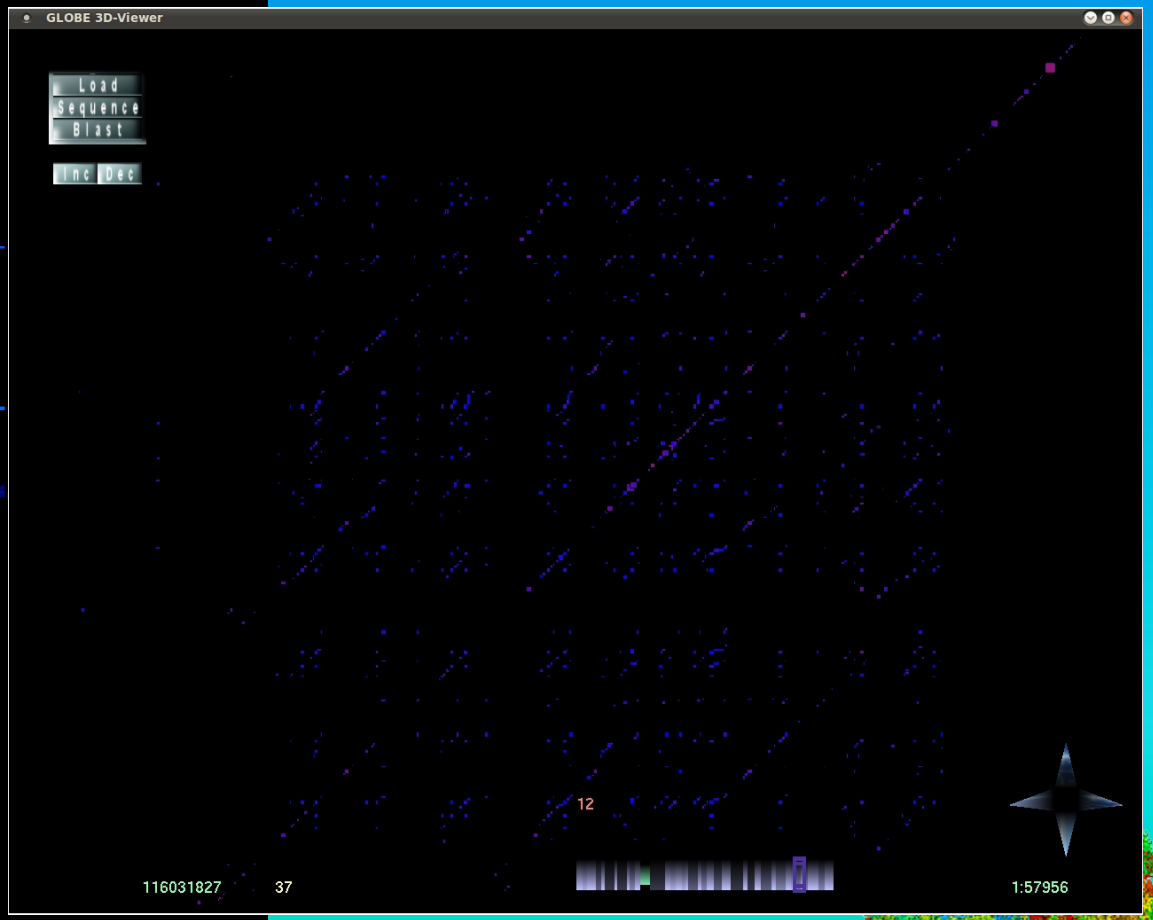

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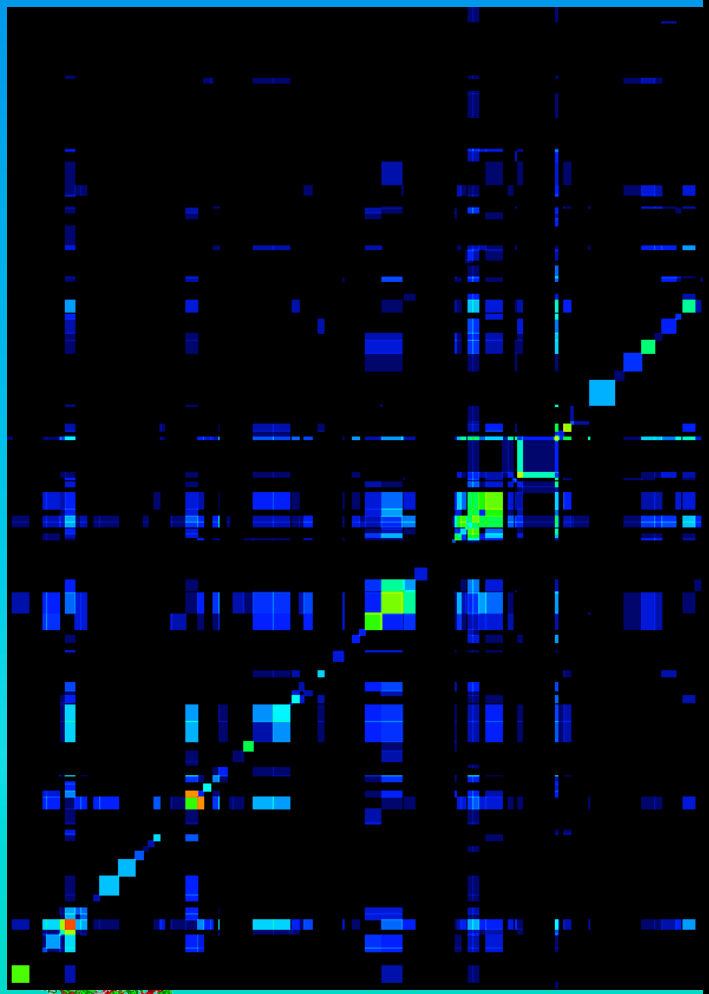

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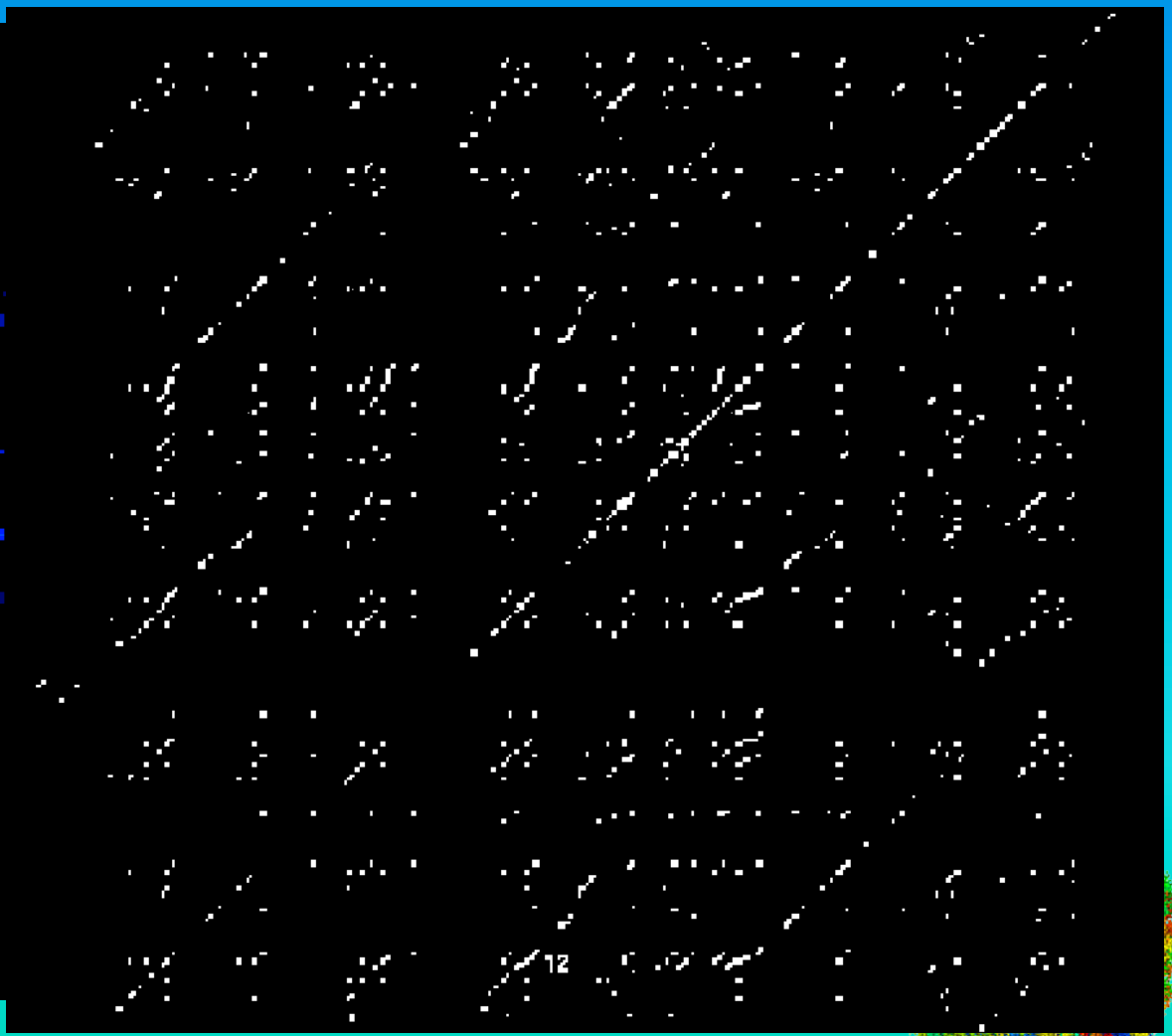

~ 380 kbp

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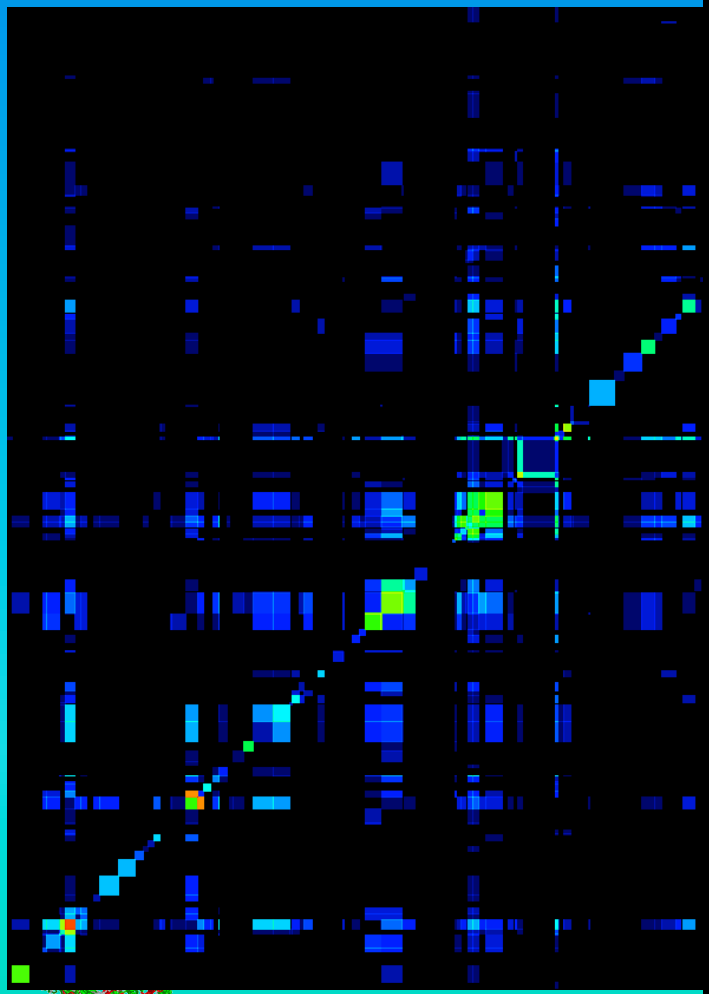

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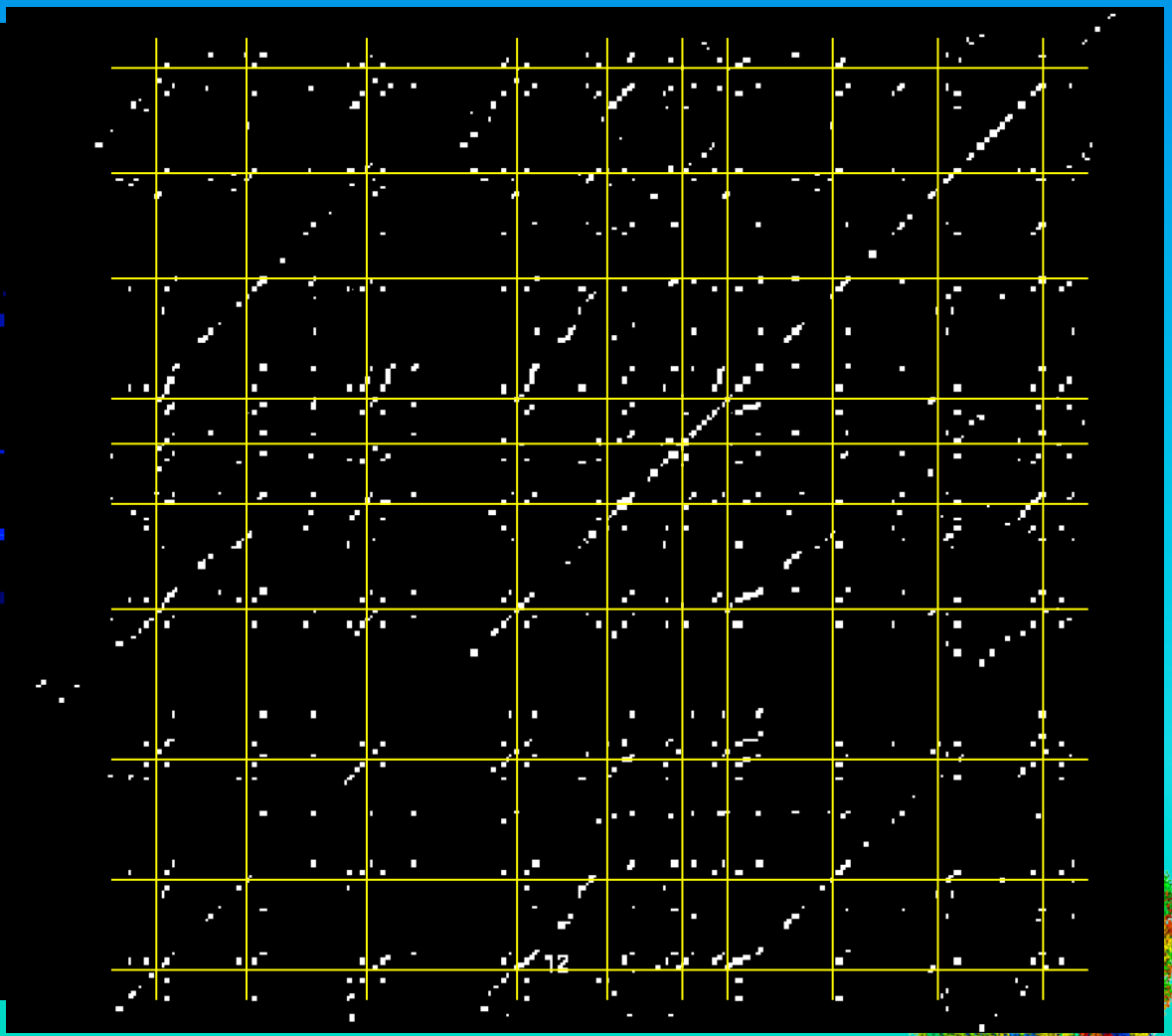

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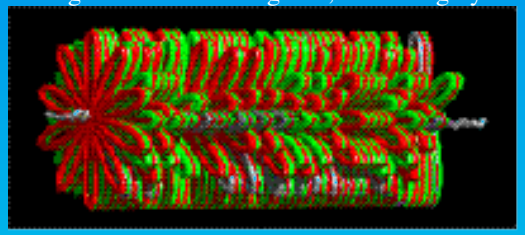
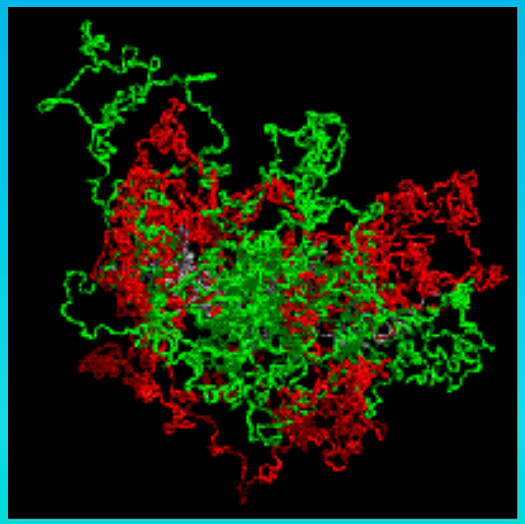
Simulation of Single Chromosomes

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.

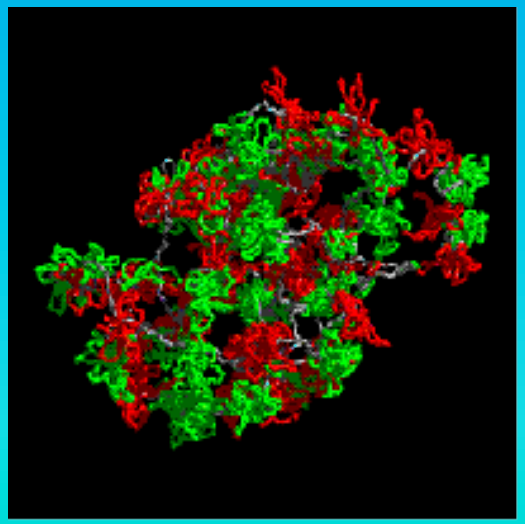
All models form chromosome territories with big voids and different chromatin morphologies. Experimental territory and subcompartment diameters agree best with an MLS model with 80 to 120 kbp loops and linkers.

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

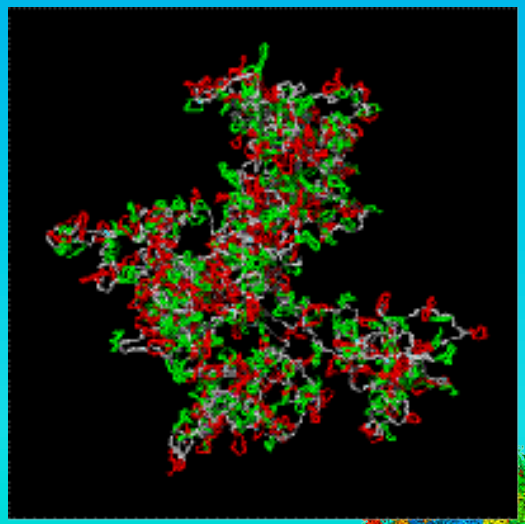




RW/GL model, loop size 5 Mbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromsome territory, thus forming no distinct features like in MLS model.



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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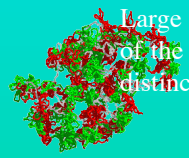

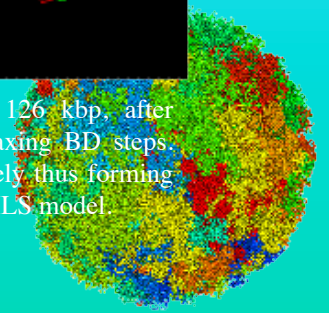

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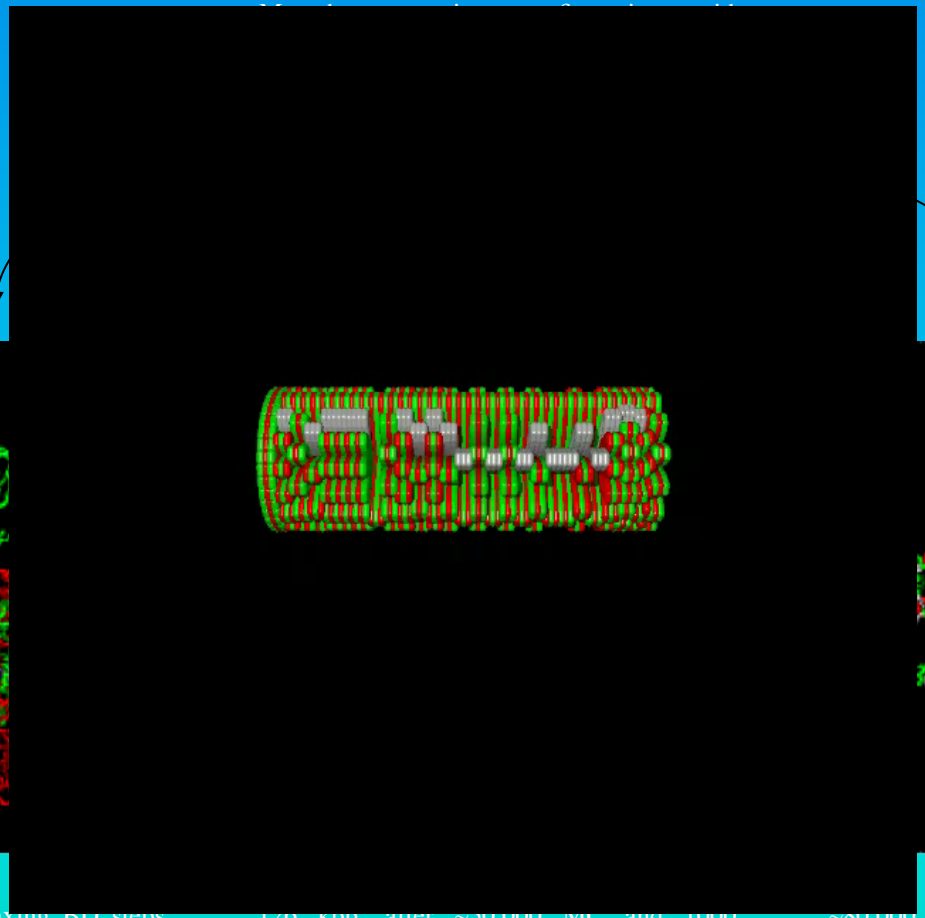

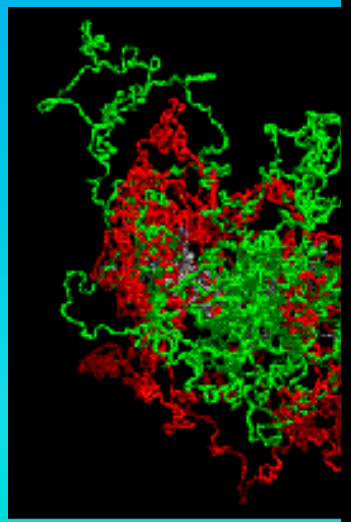

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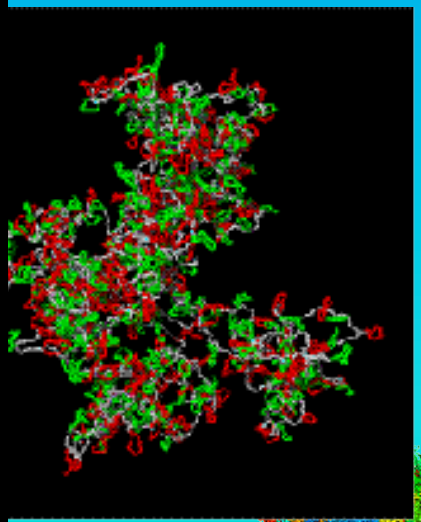

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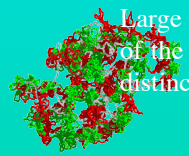
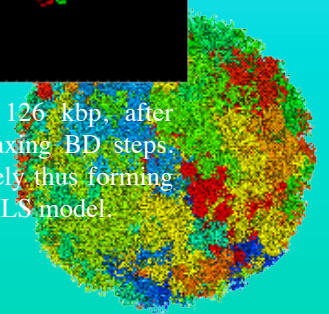
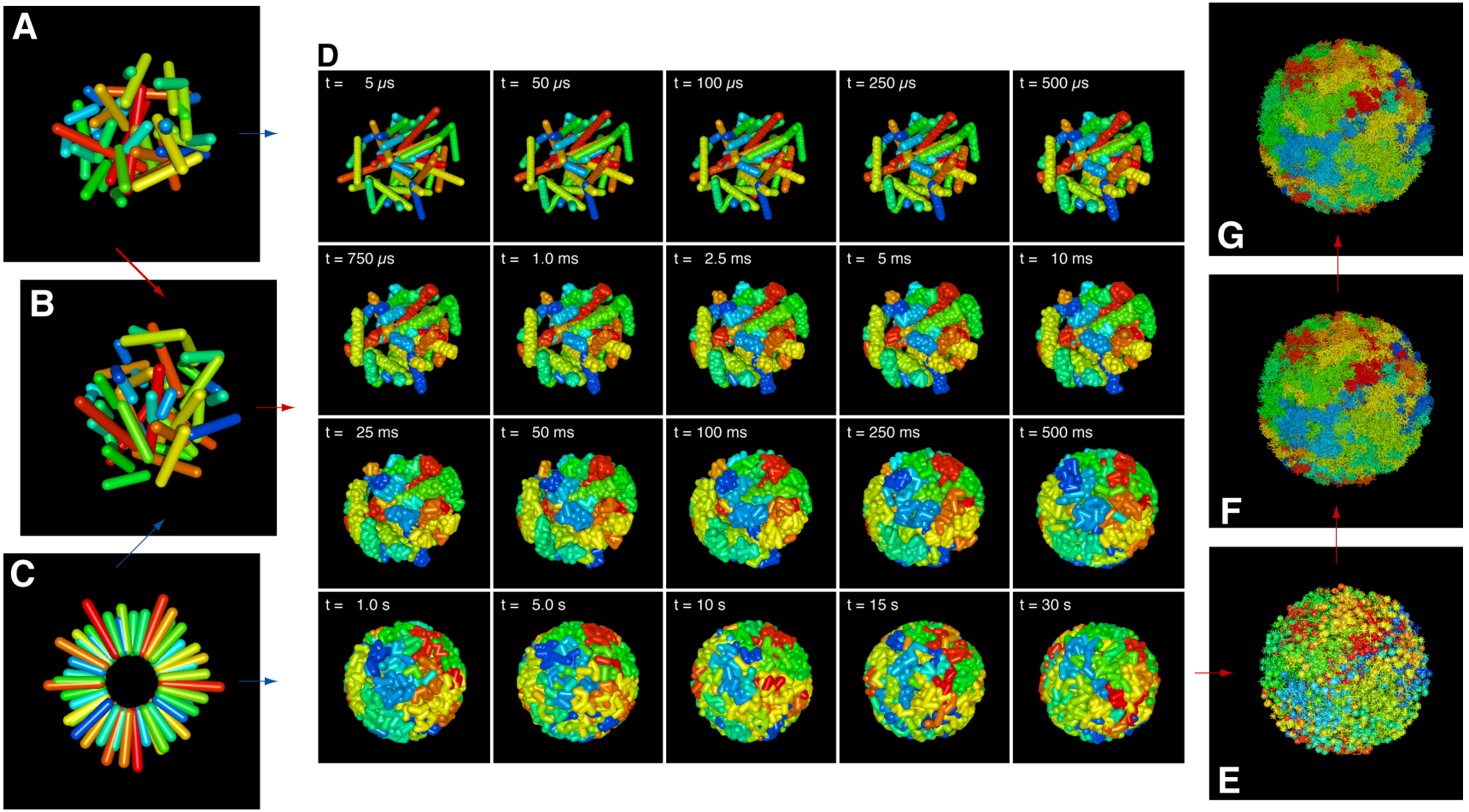




Simulation of Whole Nuclei with all 46 Chromosomes

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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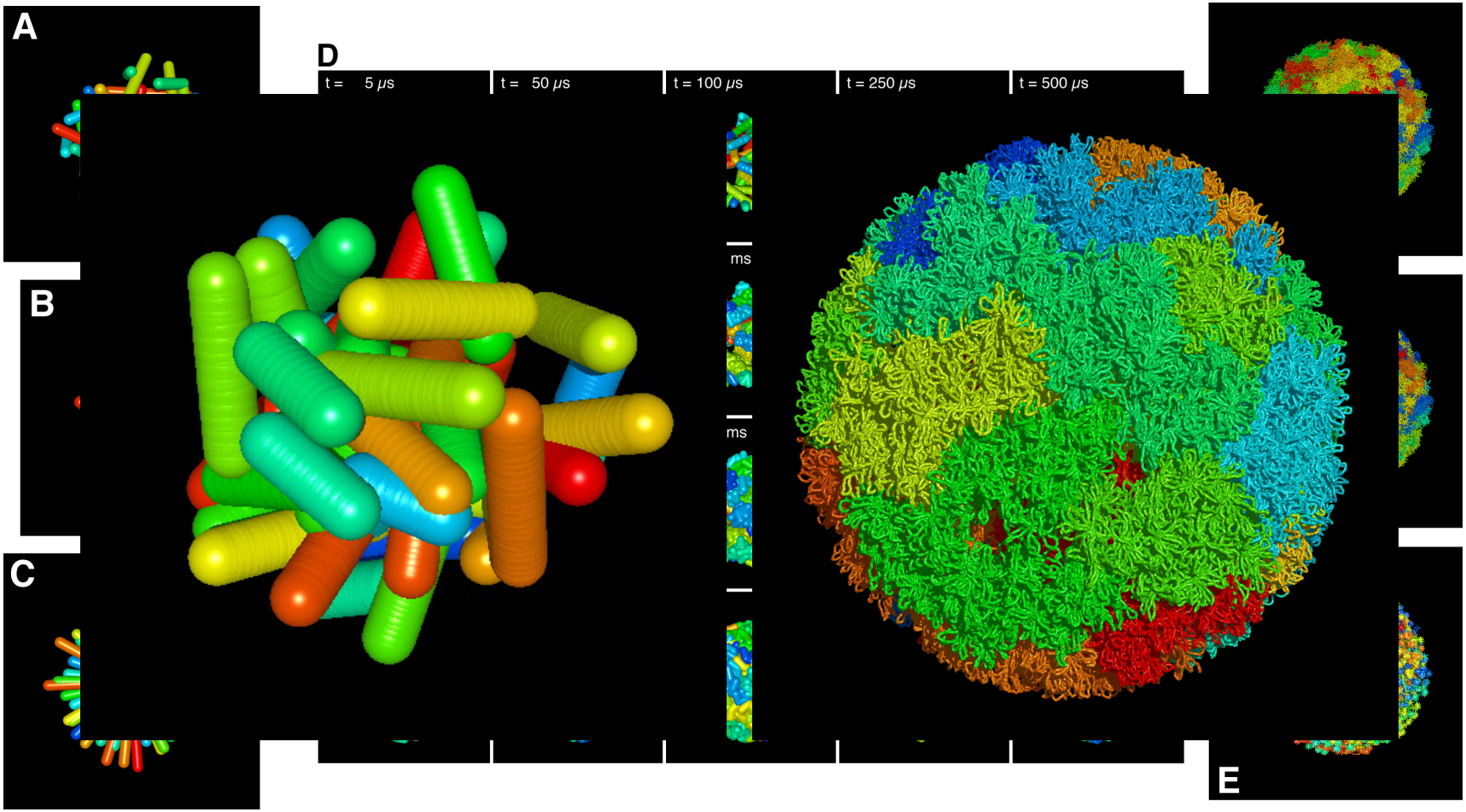
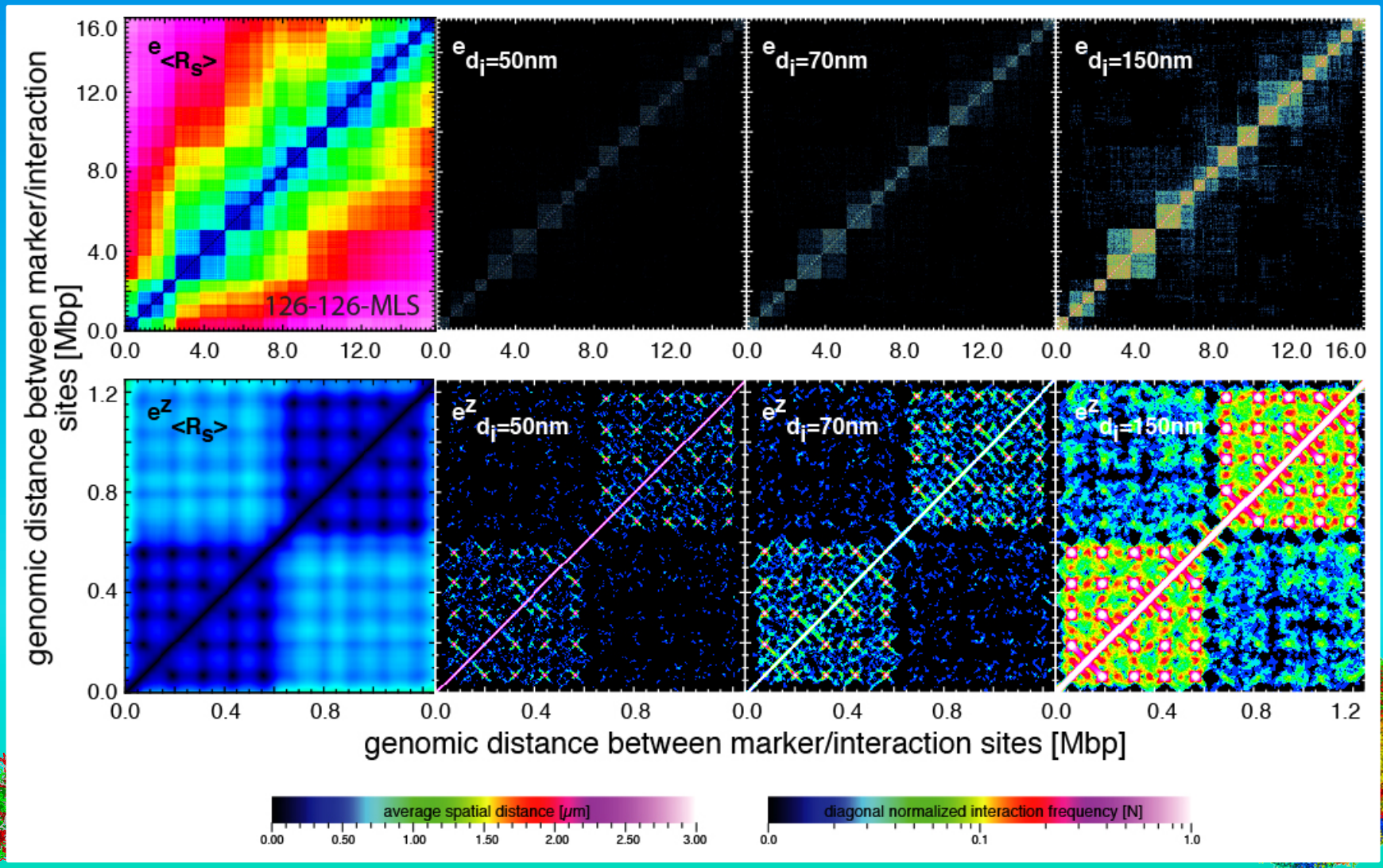


Simulated Interaction Maps

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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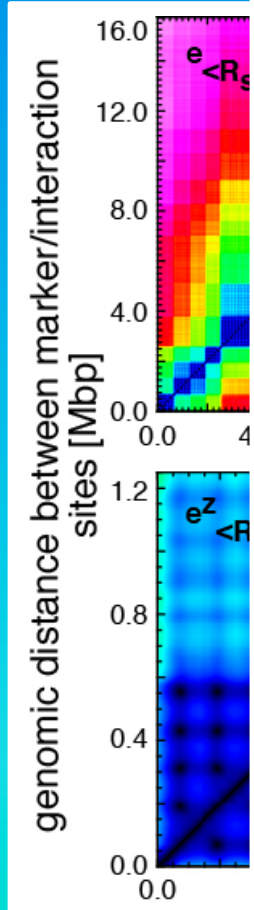
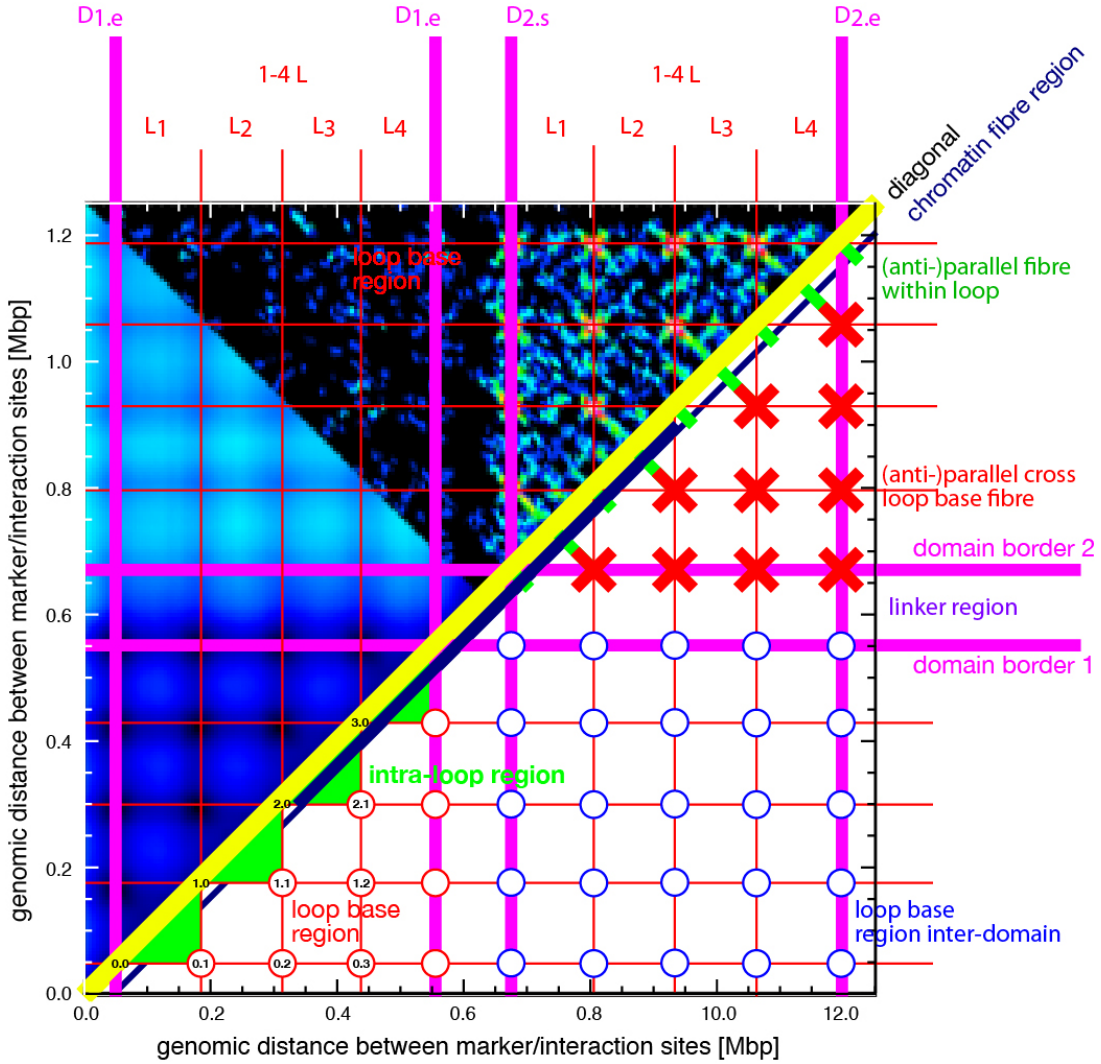
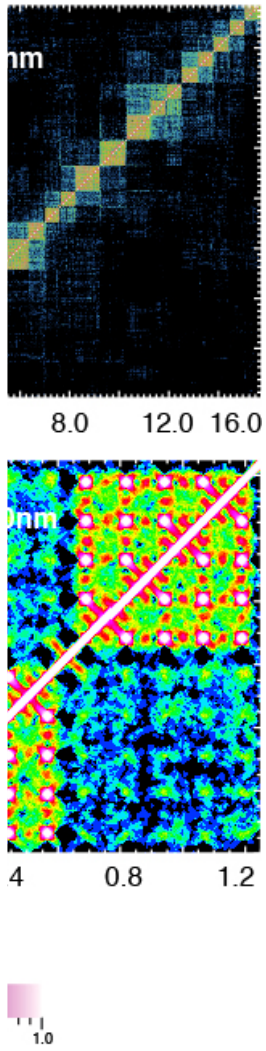
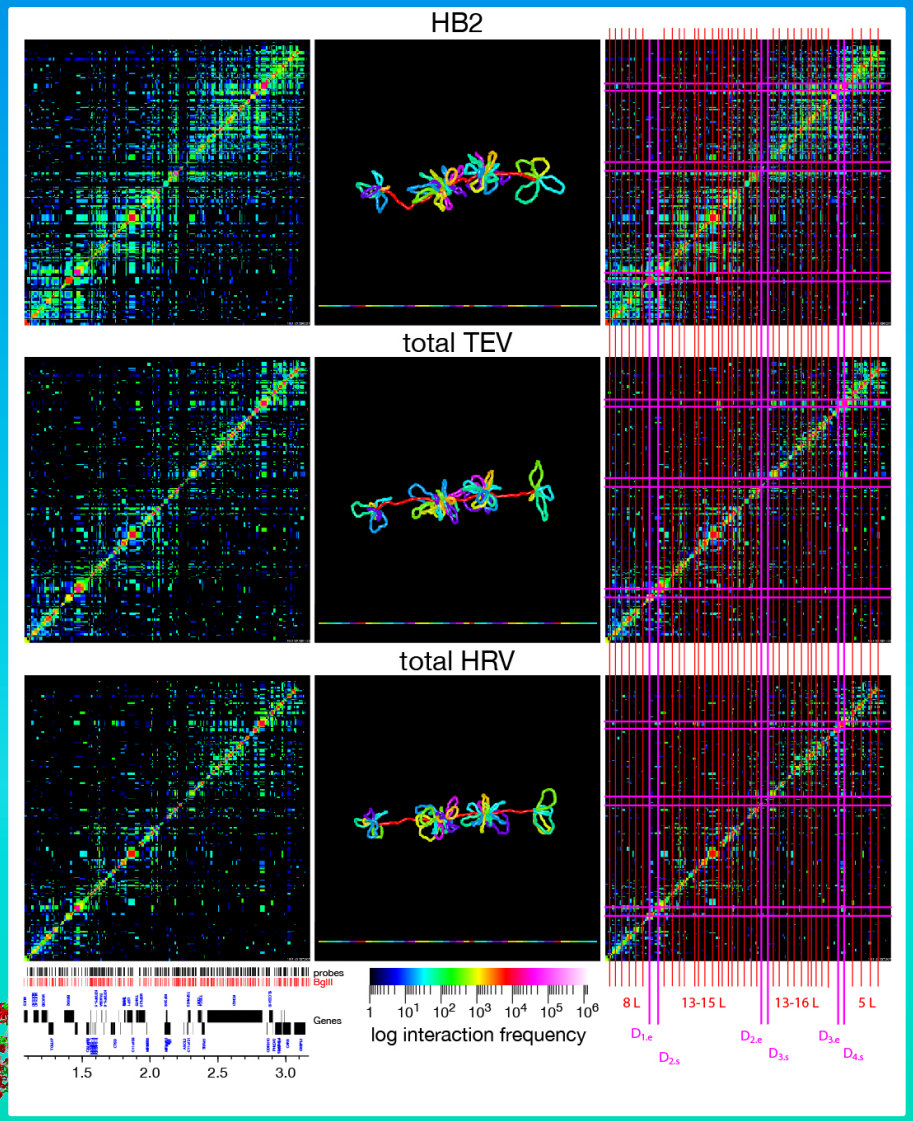


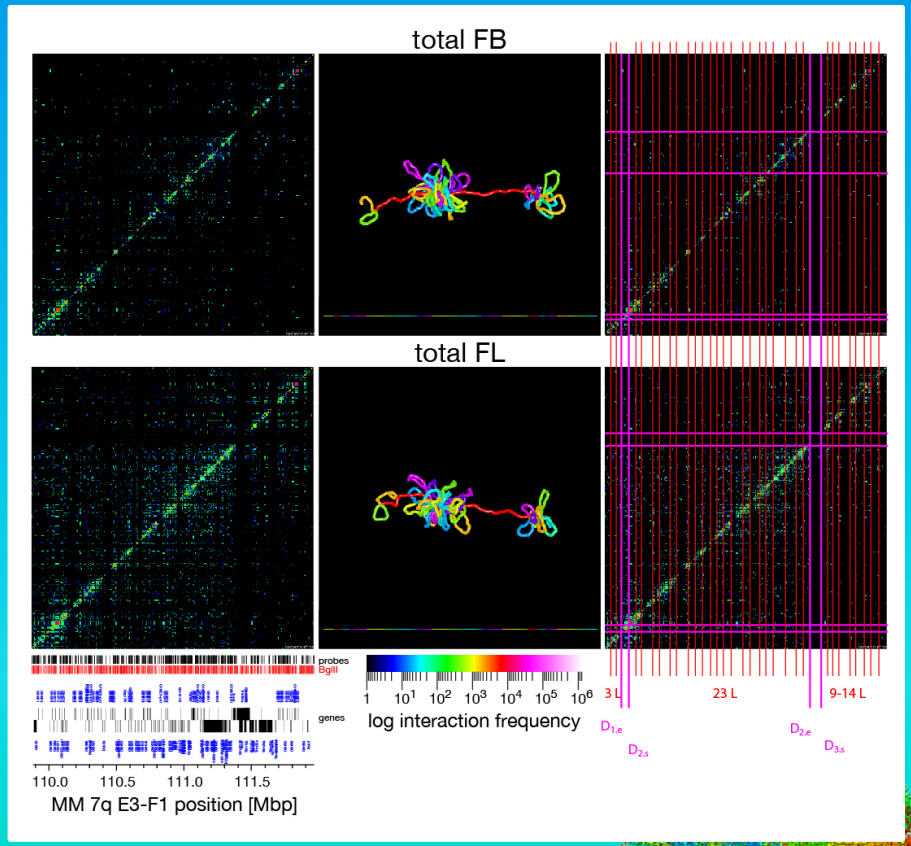




Variation of a Consensus Architecture Scheme

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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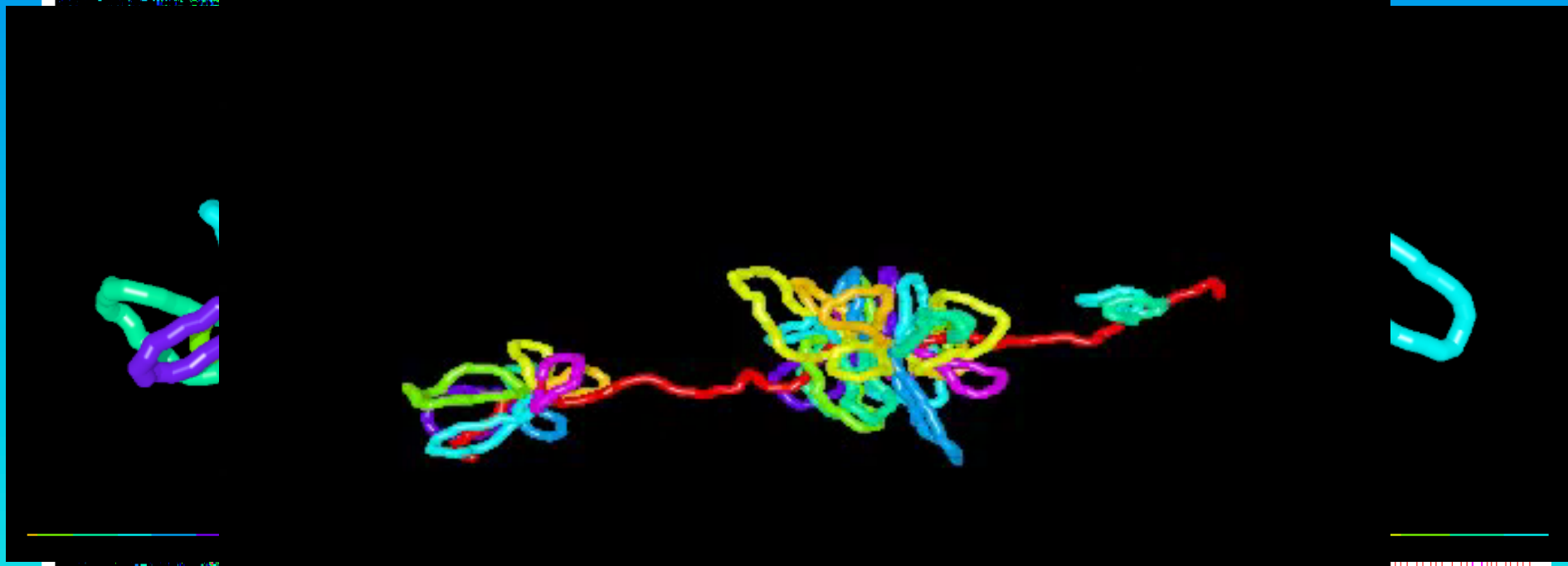
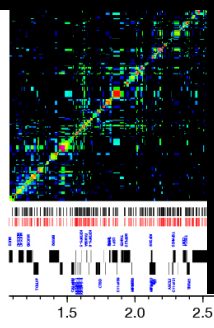
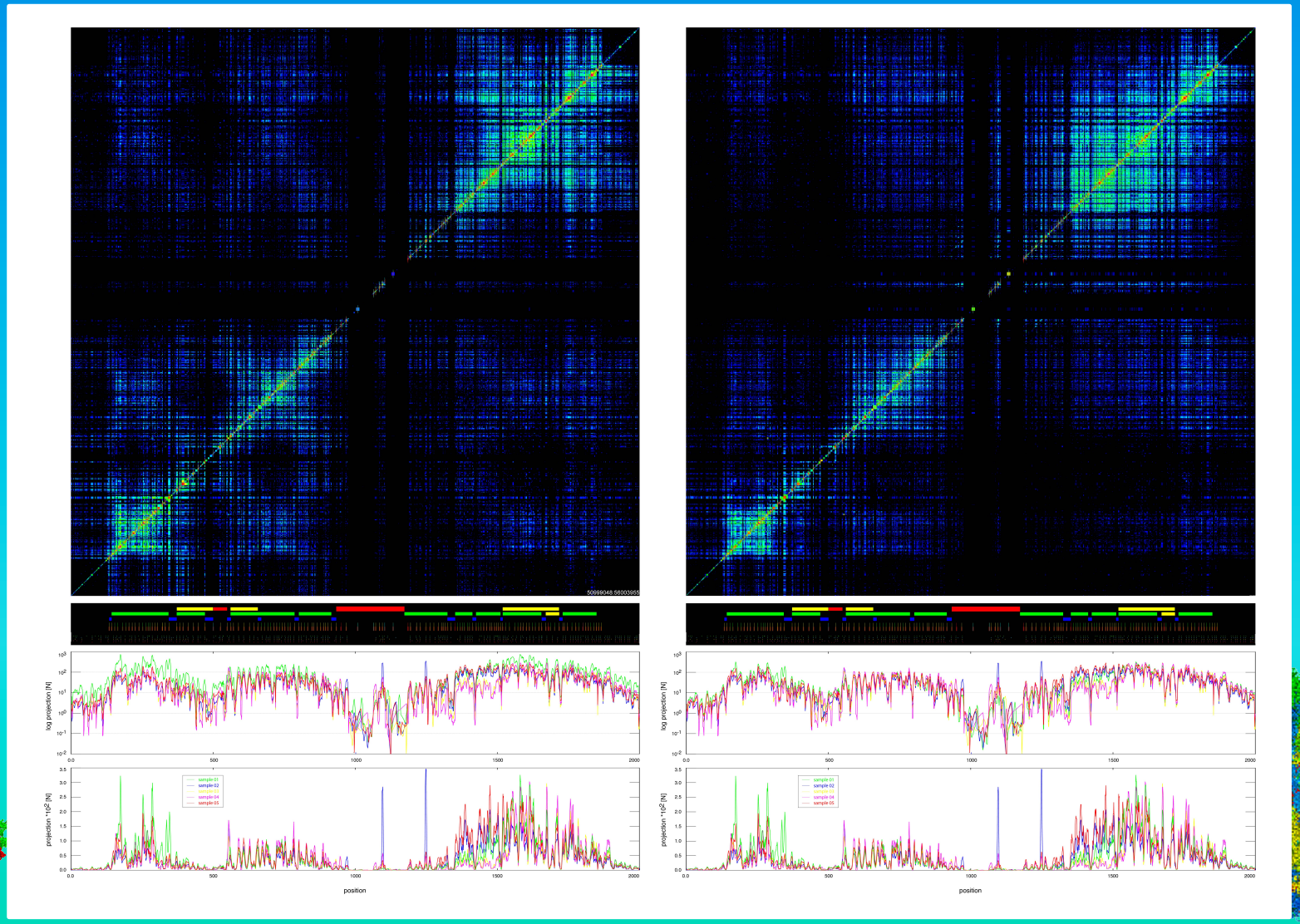




Clinical Epigentic Functional 3D Architecture Context

Although the ground state architecture remaining the same, depending on the local functional status of genetic regions, the quasi-fibre compaction, loop and their arrangement in loop aggregates/rosettes allow for different functional interaction dynamics and thus regulation of a genetic regions represented by characteristic interaction map changes. If the variation is beyond the evolutionary bandwidth the system turns into malfunctional or disease states.



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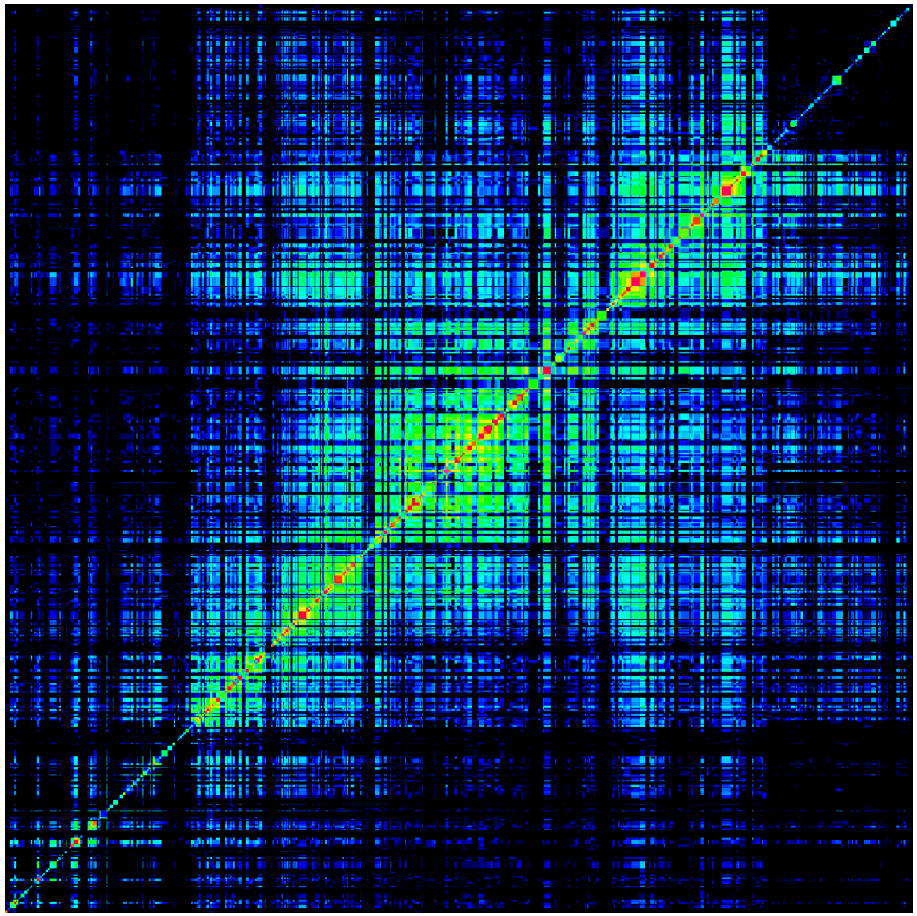
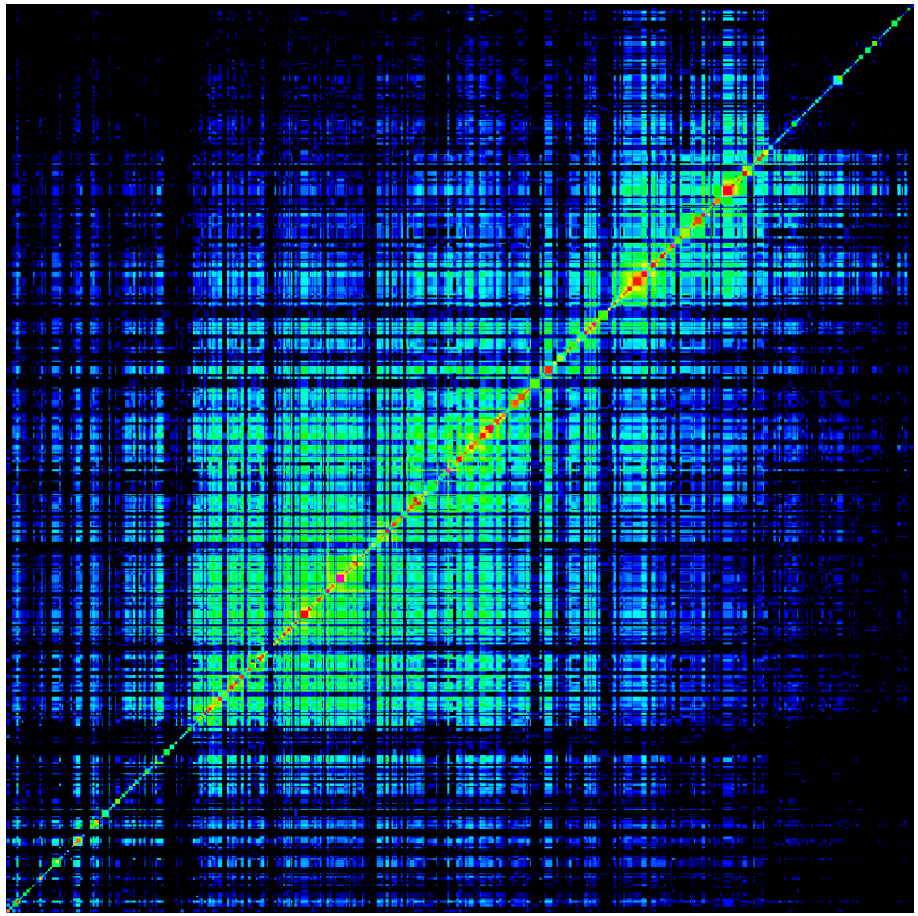
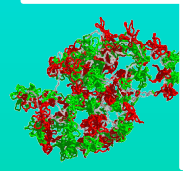
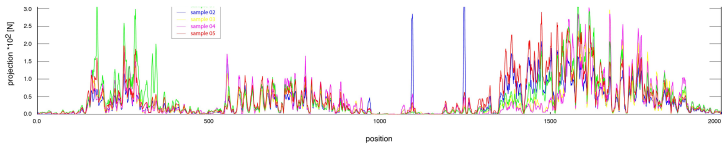
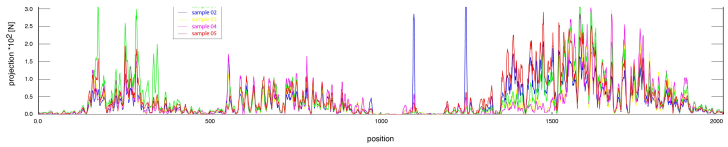
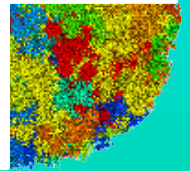






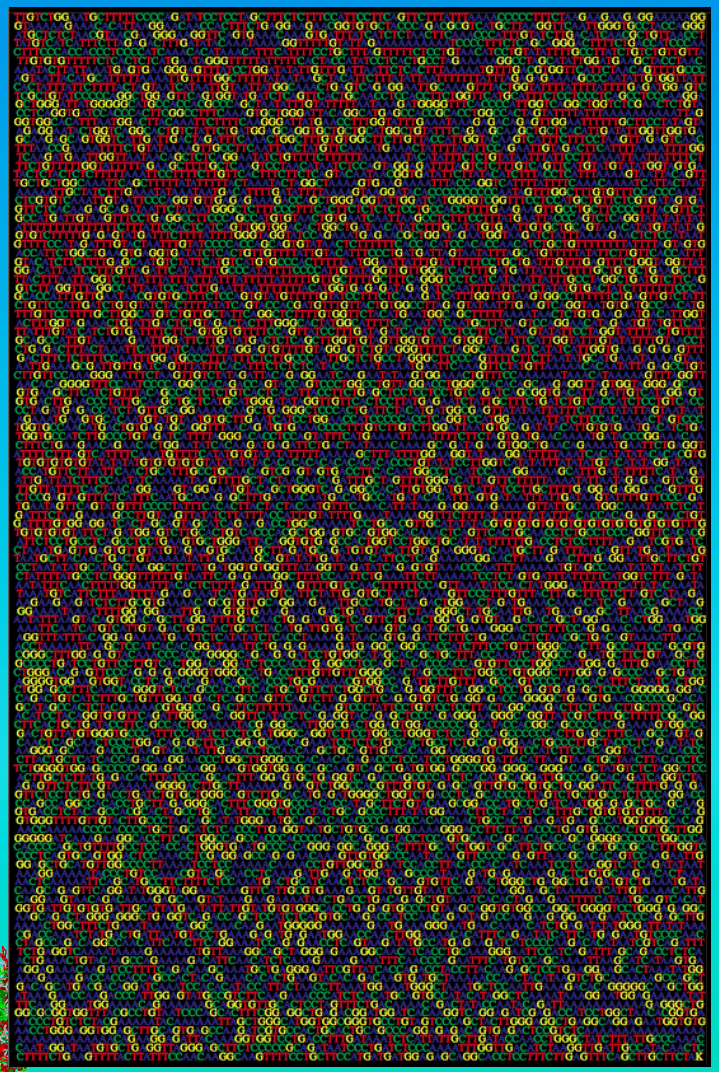


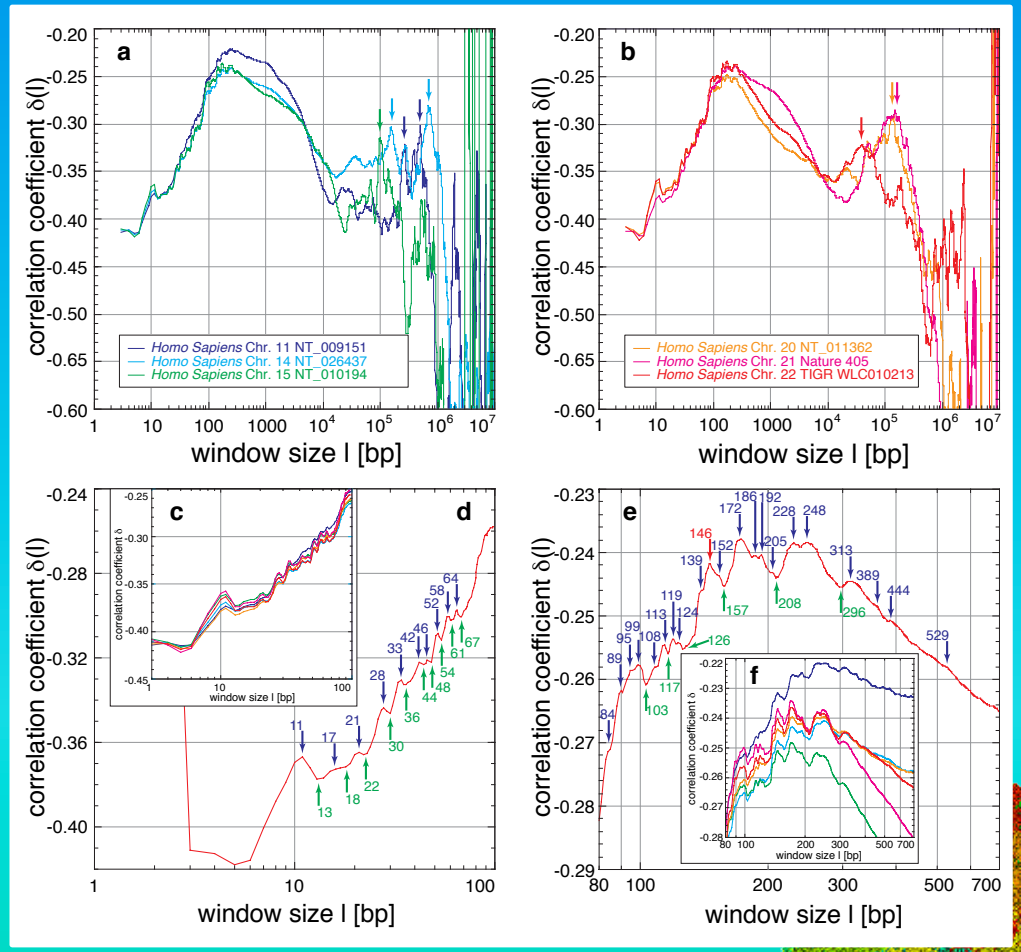




DNA Sequence Organization

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.





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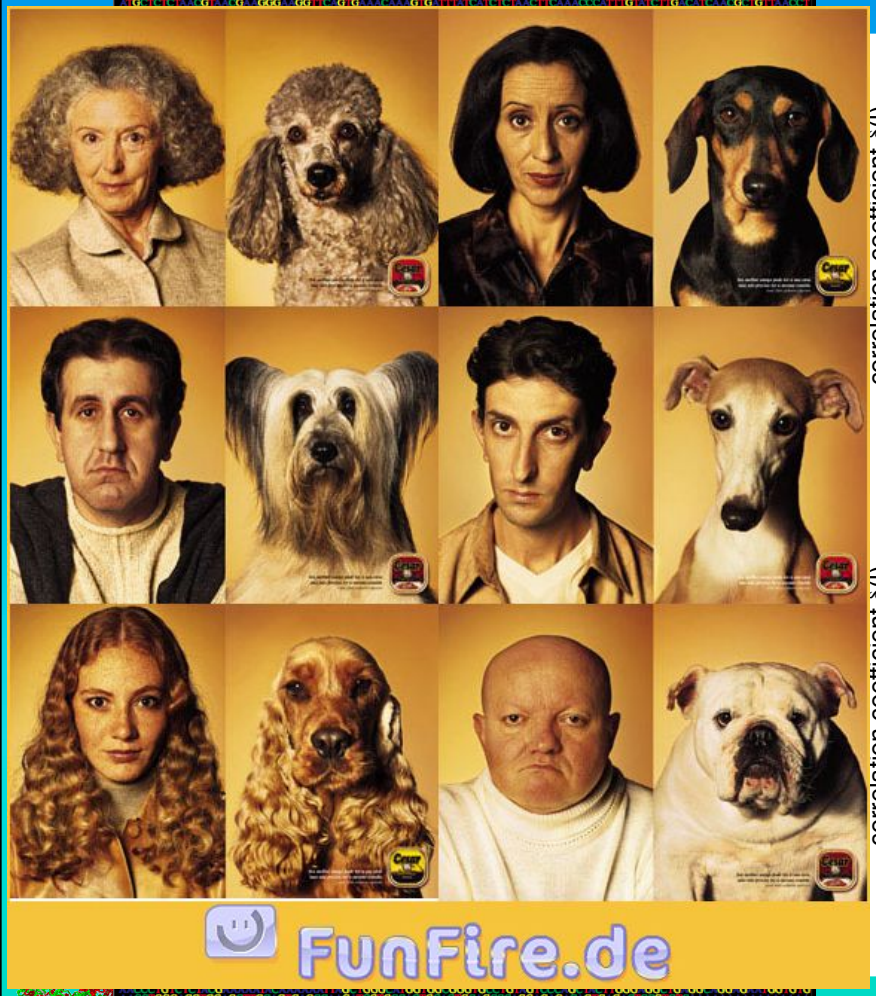

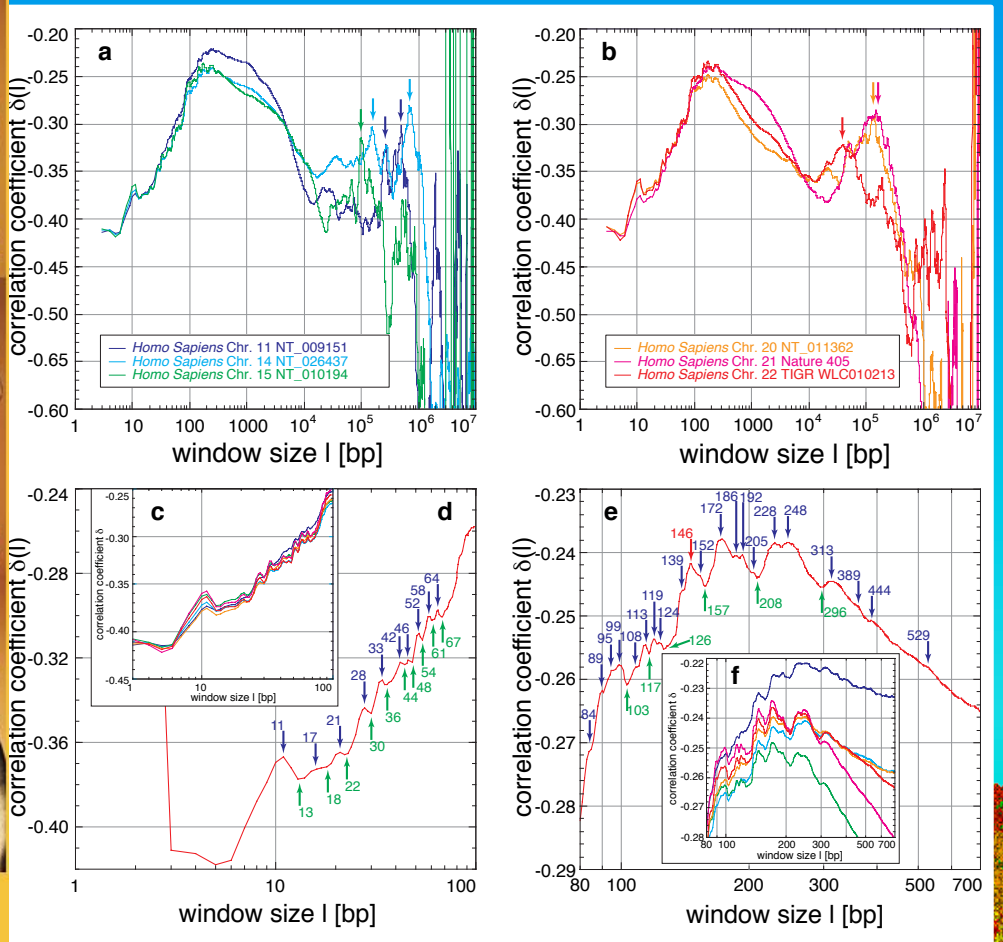

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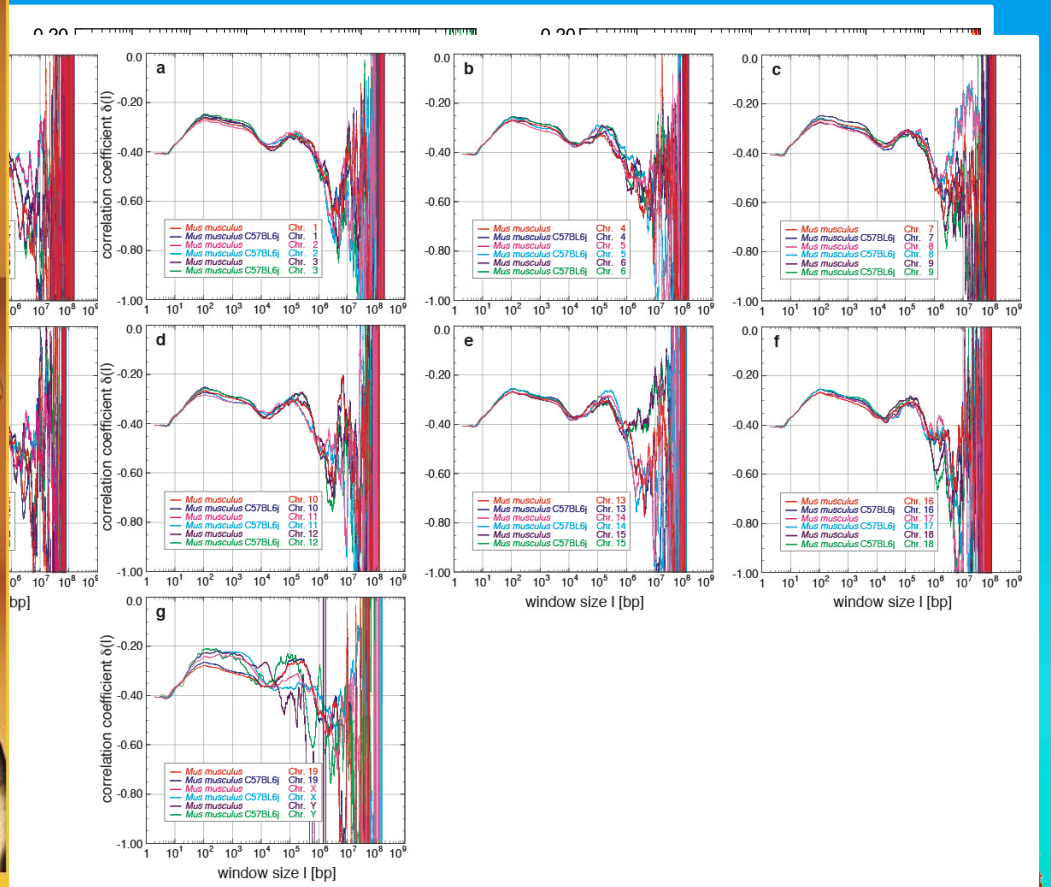

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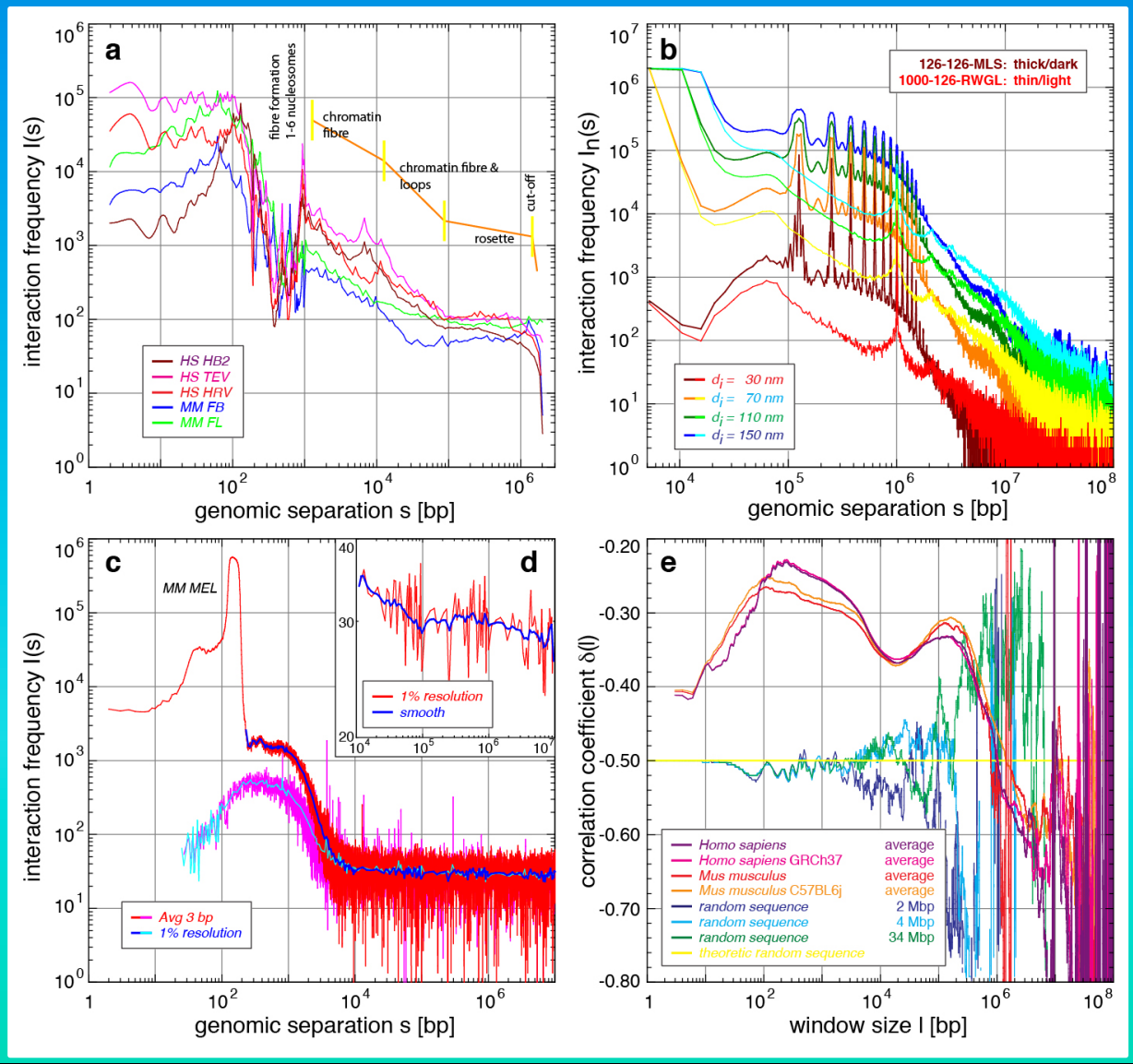
Scaling Analysis

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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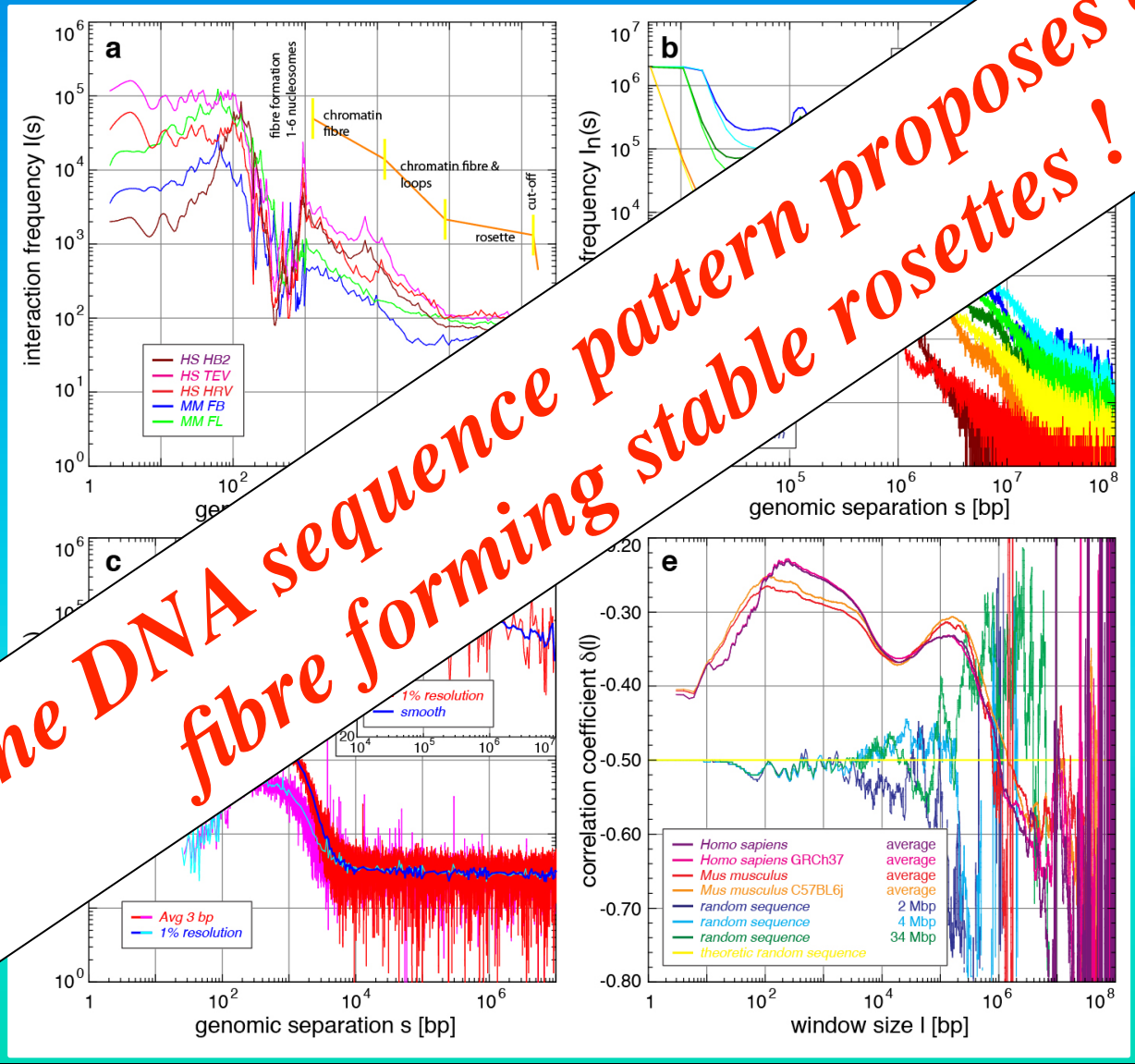
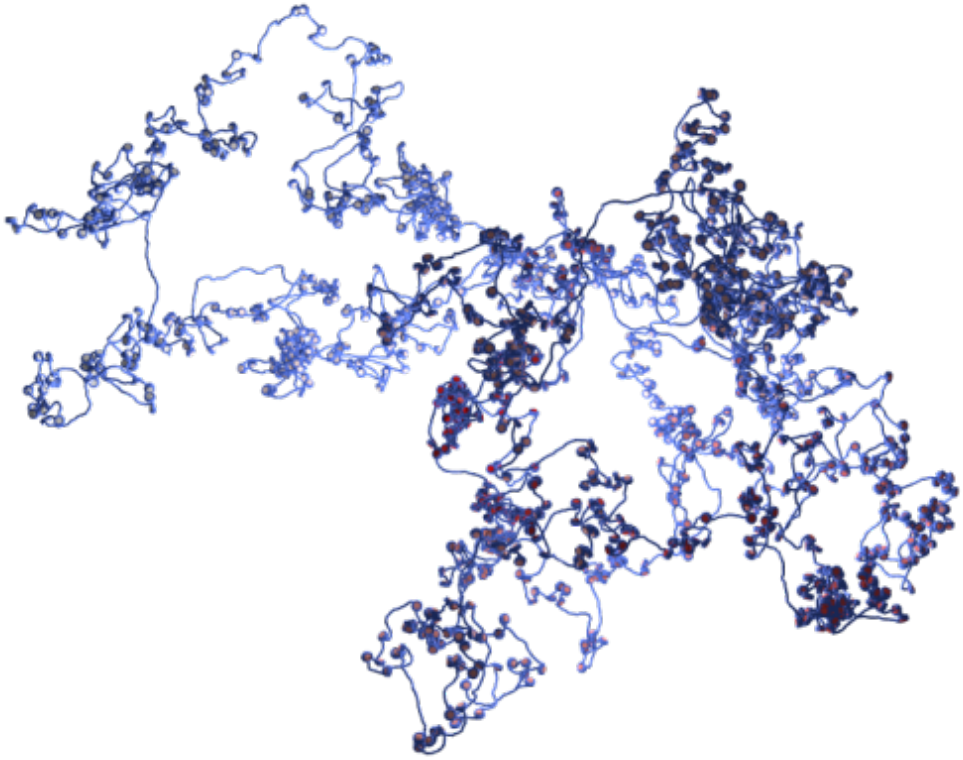


Also the DNA sequence pattern proposes a quasi-fibre forming stable rosettes !

Simulation of Chromatin Quasi-Fibres

The position of nucleosomes influence greatly the structure of chromatin fibers done on super-computers. Here a dedicated workflow is applied, with which overlapping nucleosome populations can be analyzed and the best positioning of nucleosomes by Monte Carlo simulated annealing can be achieved. For an actual locus in a spherical confinement then a 3D independent nucleosome fiber conformation can be simulated.

Monte Carlo Simulation of SAMD4A Mouse ES Cells



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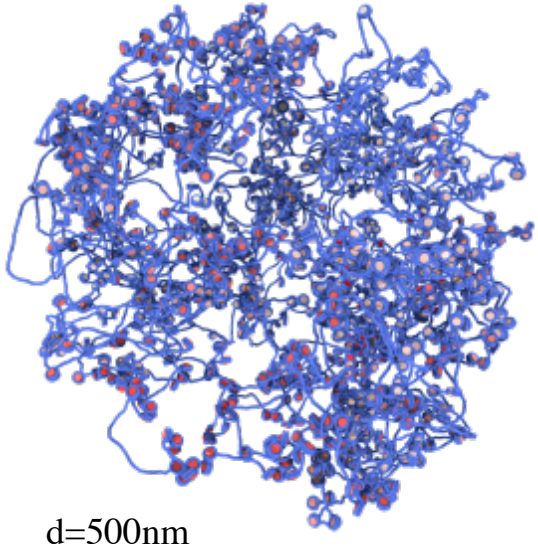

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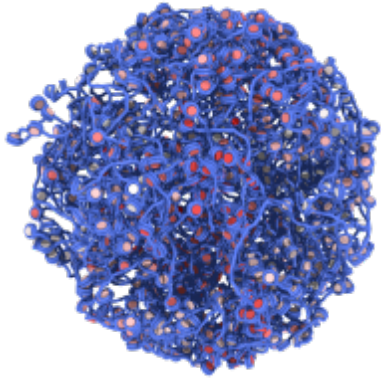
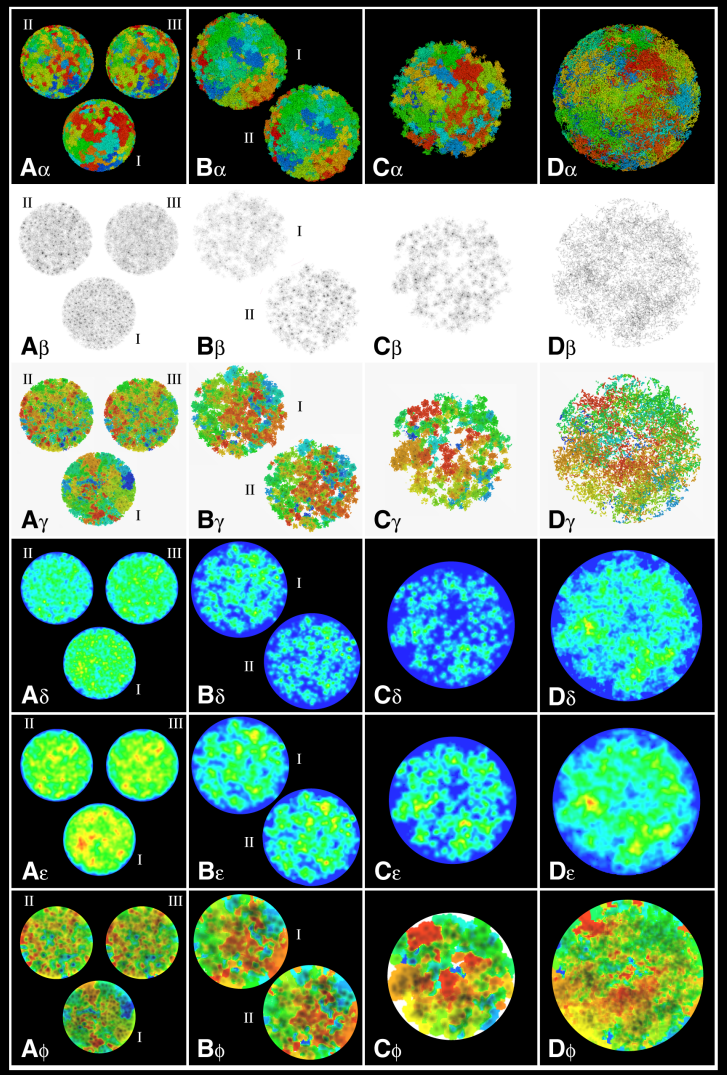


d=200nm

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.

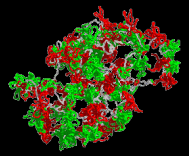


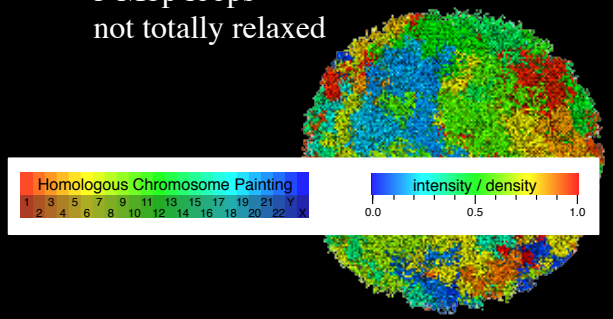
A: MLS in 6 μm nucleus
I: 63 kbp loops, 63 kbp linkers
II: 63 kbp loops, 252 kbp linkers
III: 126 kbp loops, 252 kbp linkers

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

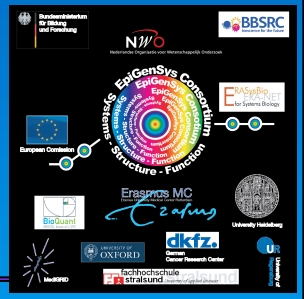




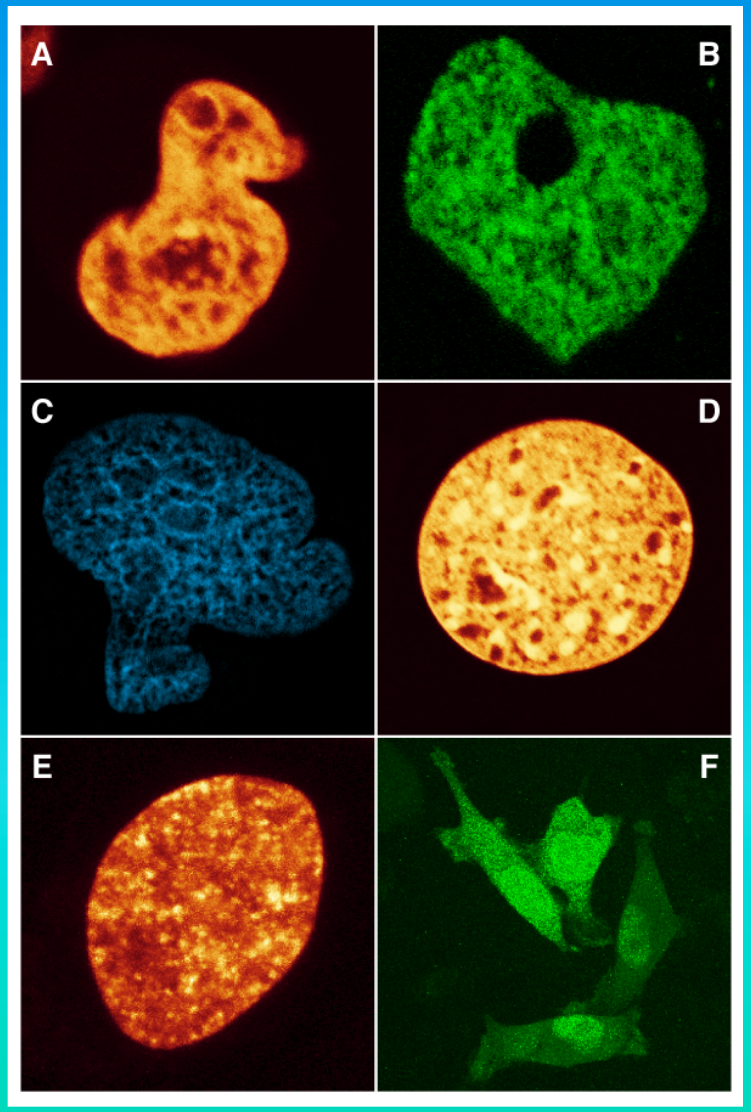
In vivo Morphology & Chromatin Distribution

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

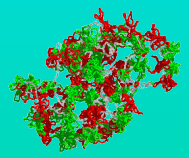


Cos7, H1.0-GFP

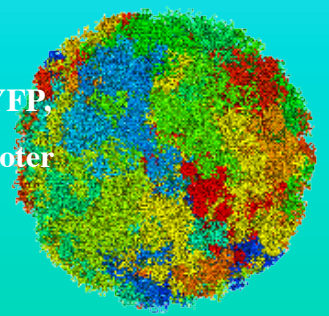
LCLC 103H, H2A-CFP

ID13, H2A-YFP

HeLa, mH2A1.2-YFP

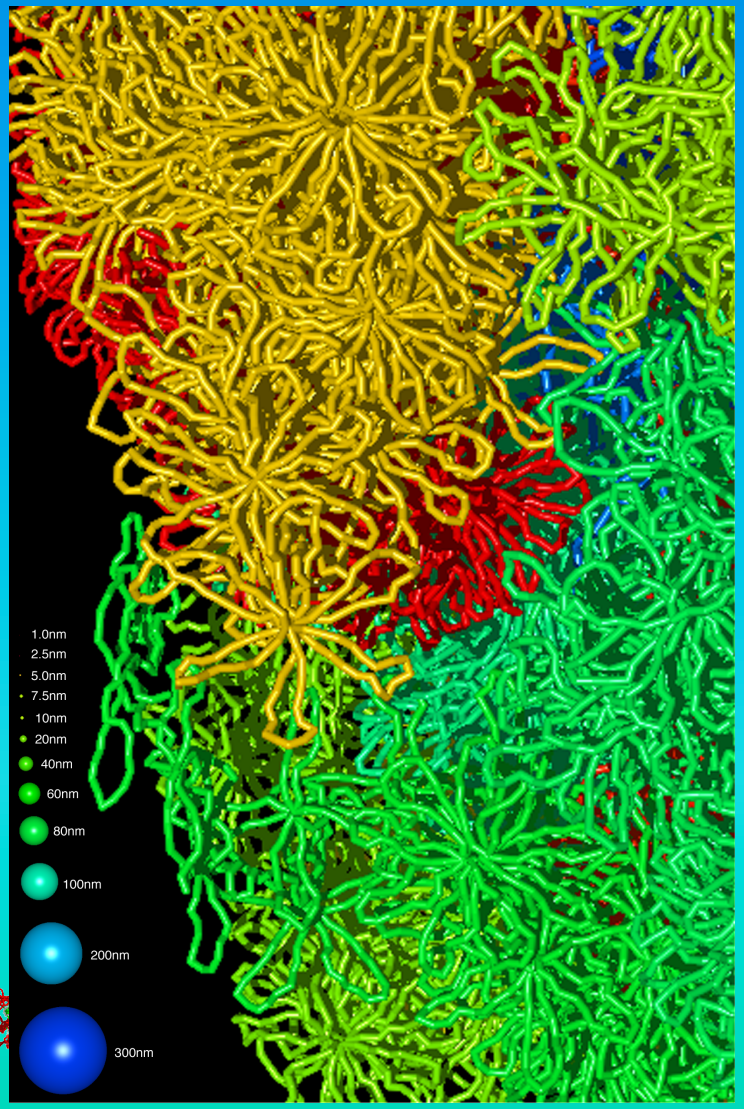


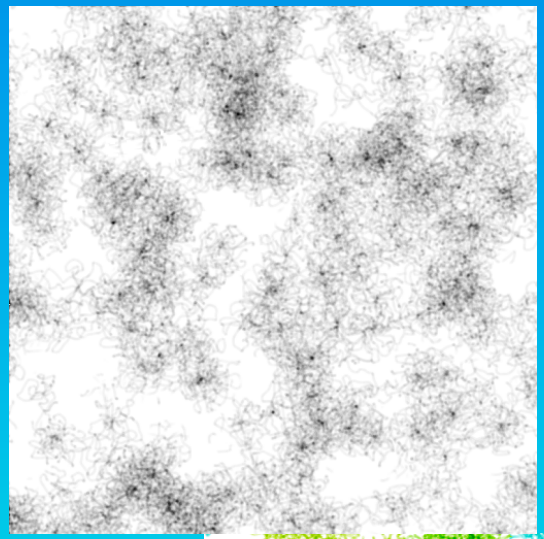
HeLa, H2A-YFP,
natural promoter

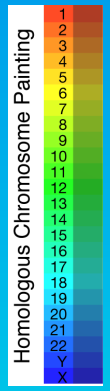


Fine Morphology of Nuclei

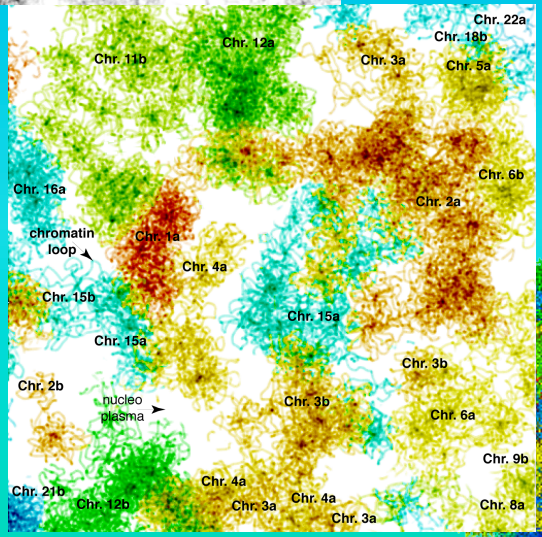
High resolution rendering and simulated electron microscopy including territory painting reveal not only again the model details but also that any location in the nucleus is accessible to biological molecules <15 nm in diameter and that even the Extended Interchromosomal Domain hypothesis is oversimplified.

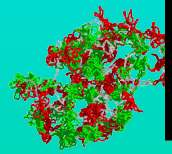


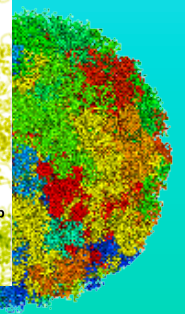




MLS models model with 126 kbp loops and linkers in a 10 μm nucleus.

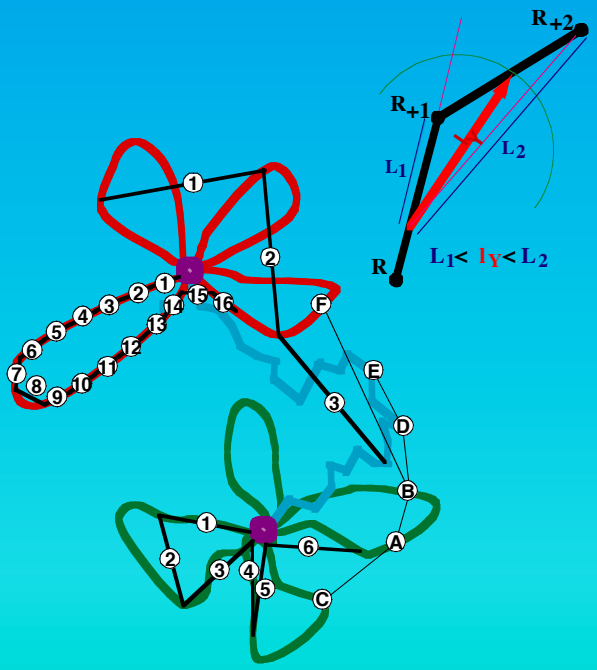


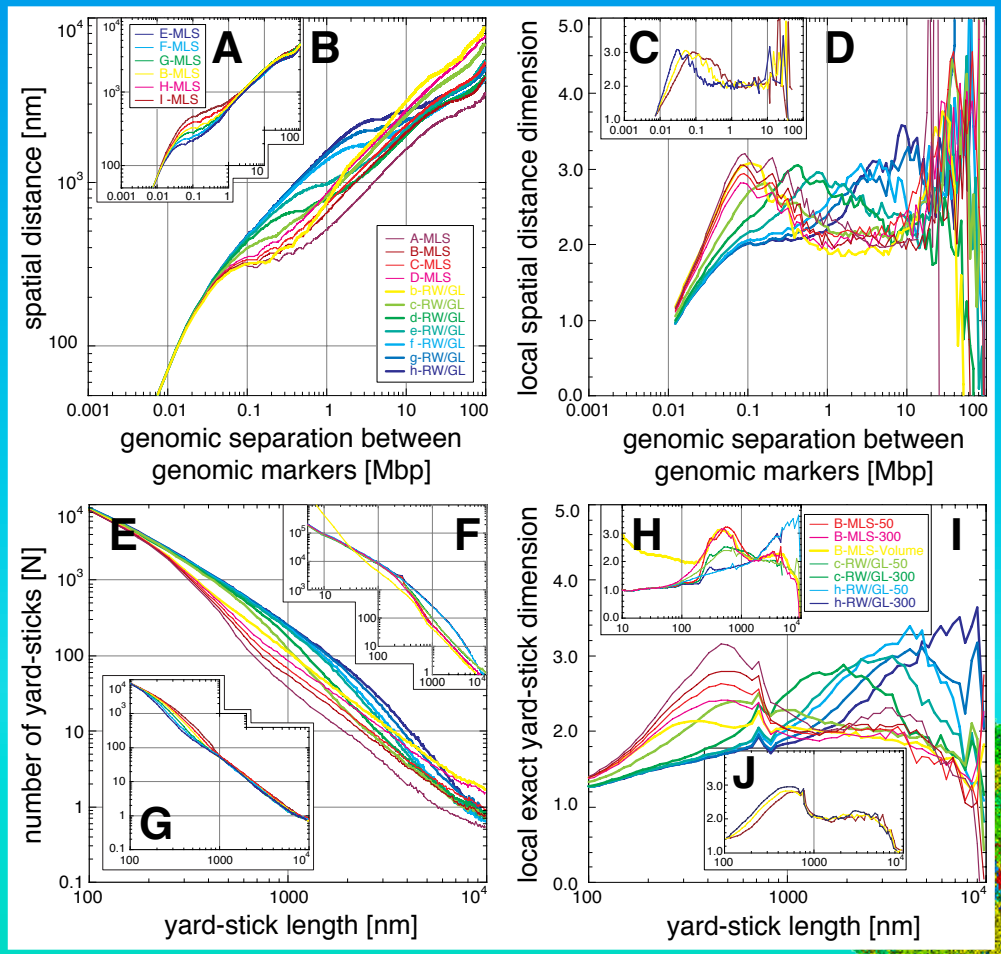


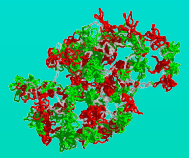


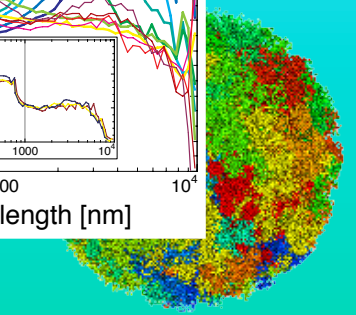
Scaling of the Chromatin Fiber Topology

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.





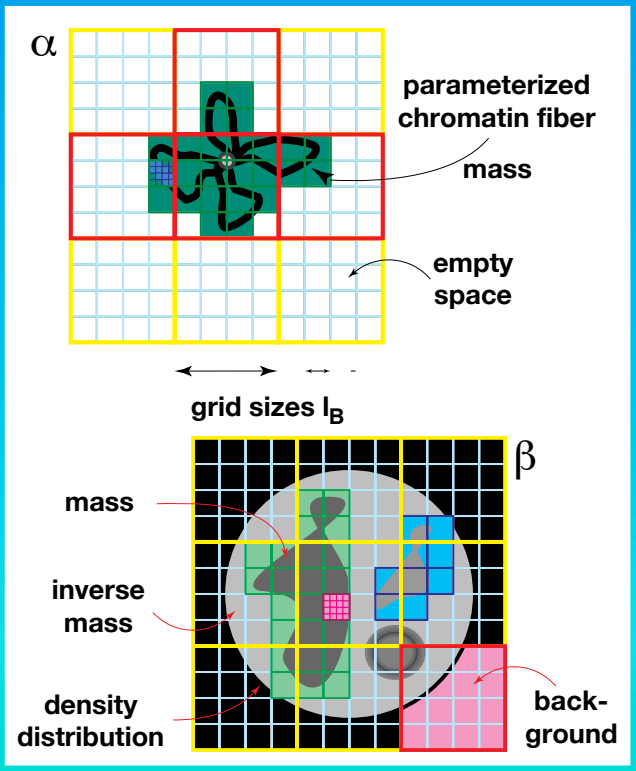




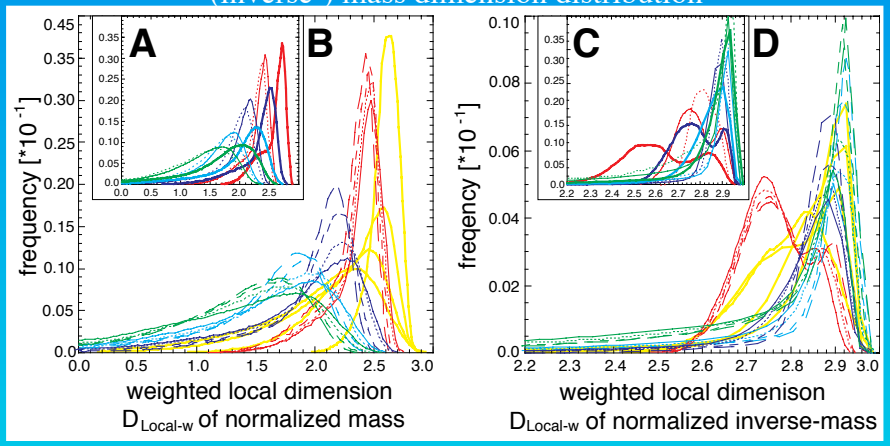
Scaling of the Chromatin Morphology & Distribution

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*.

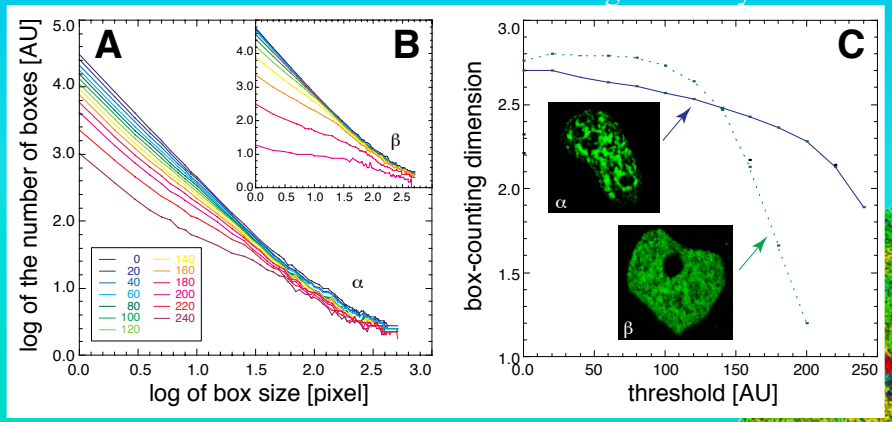
Consequently, the chromatin morphology is causally and quantitatively connected to the fiber topology.



(inverse-) mass dimension distribution

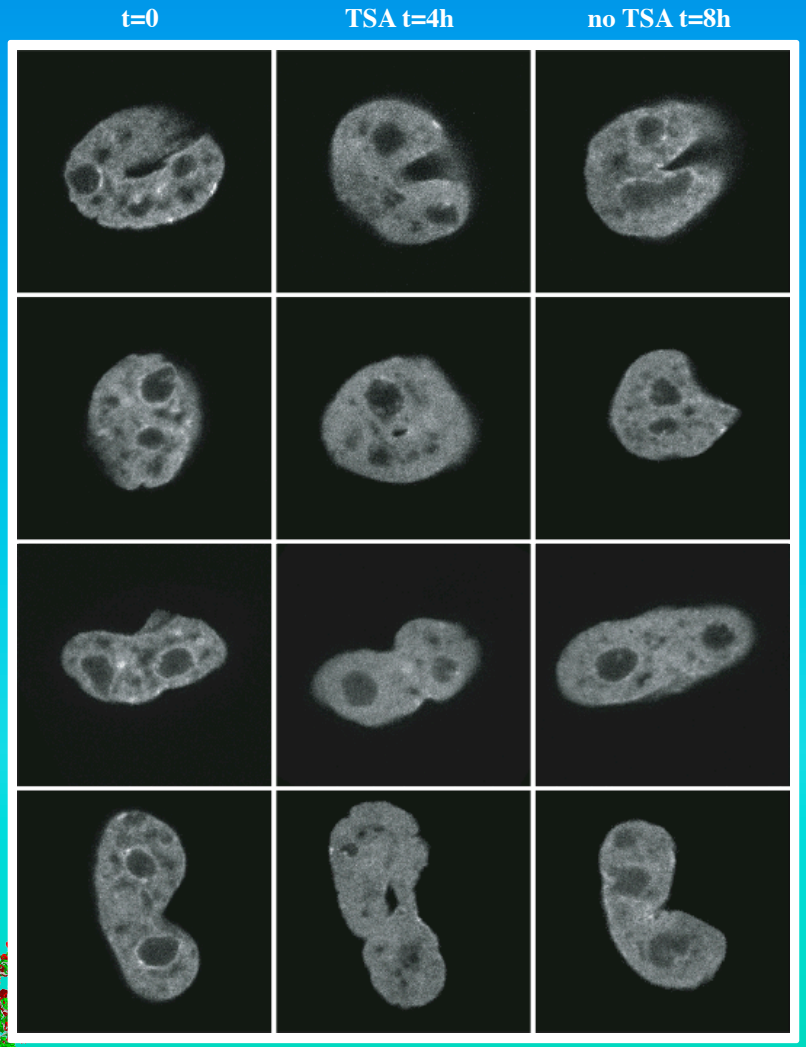


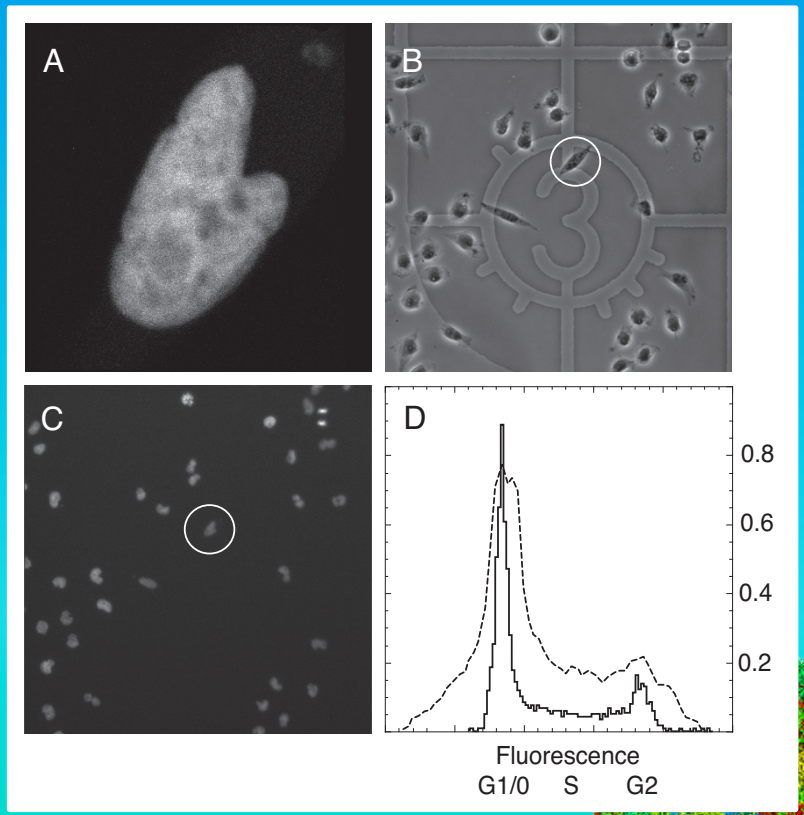
mass dimension as function of image intensity



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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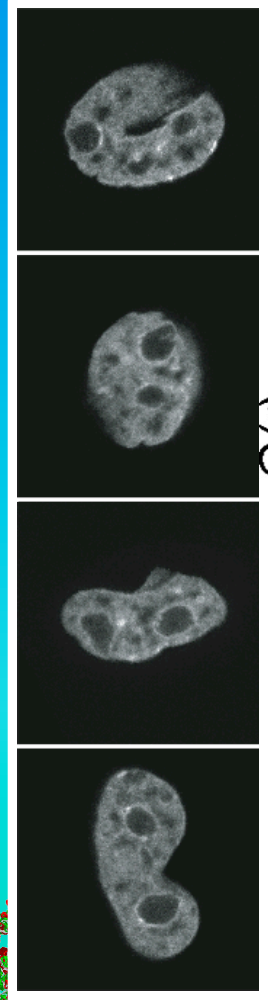
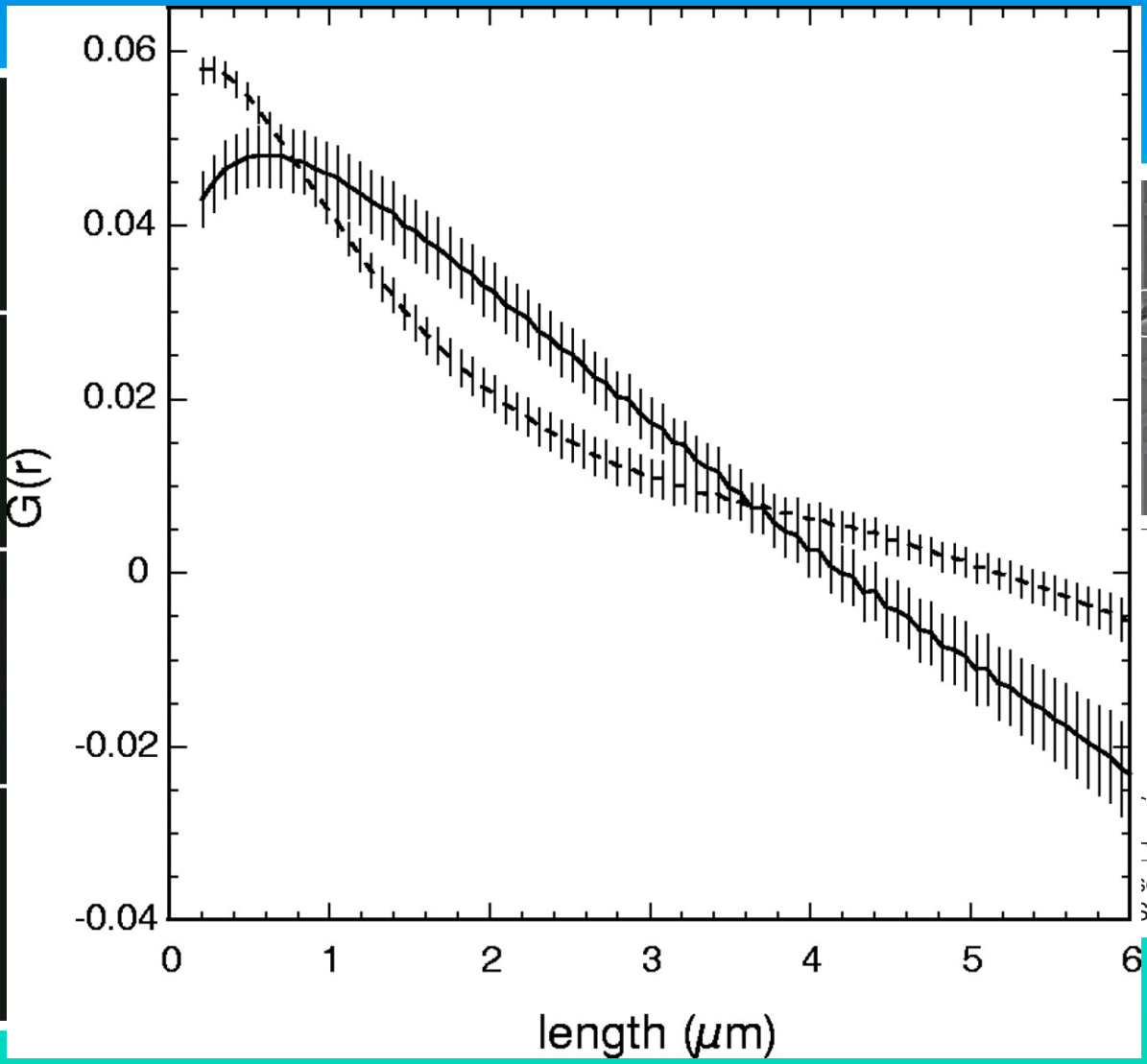
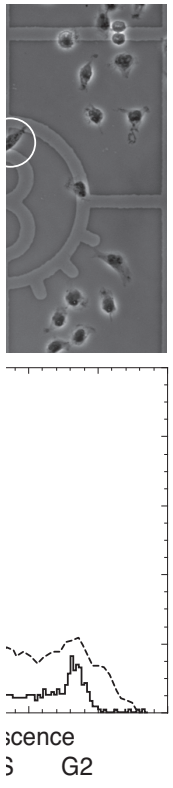




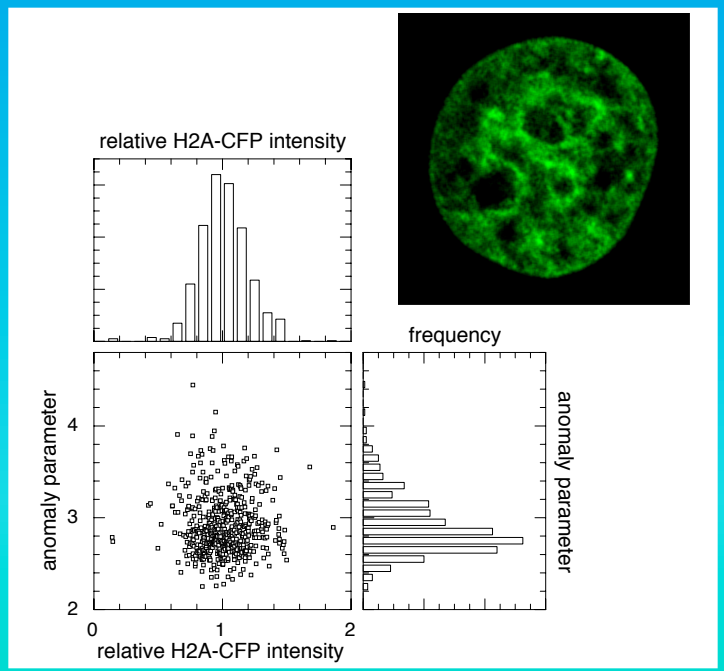


Diffusion of Particles in the Nucleus

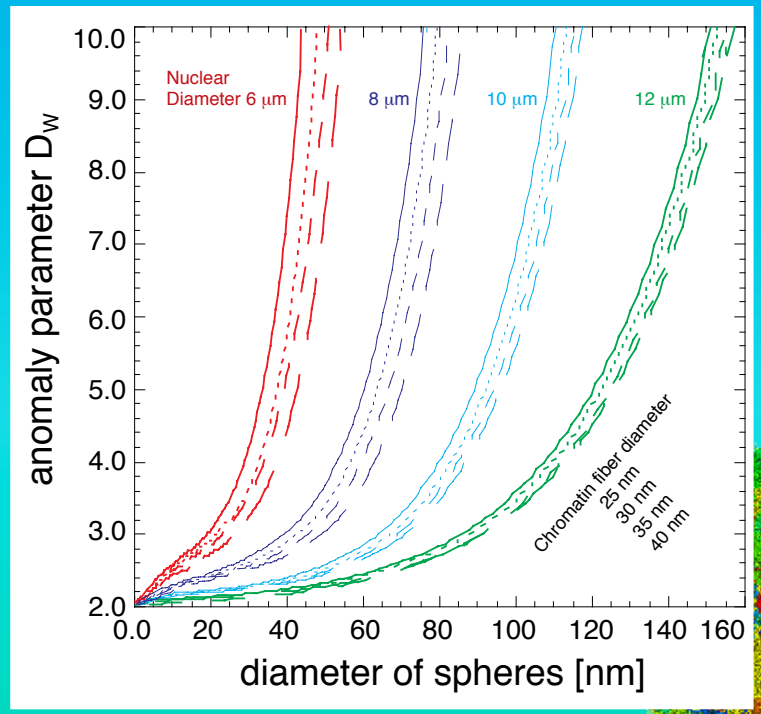
Due to the volume and spatial relation ships in the nucleus typical particles reach almost any location in the nucleus by moderately obstructed diffusion: a 10 nm particle moves 1 to 2 μm within 10 ms.

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)

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

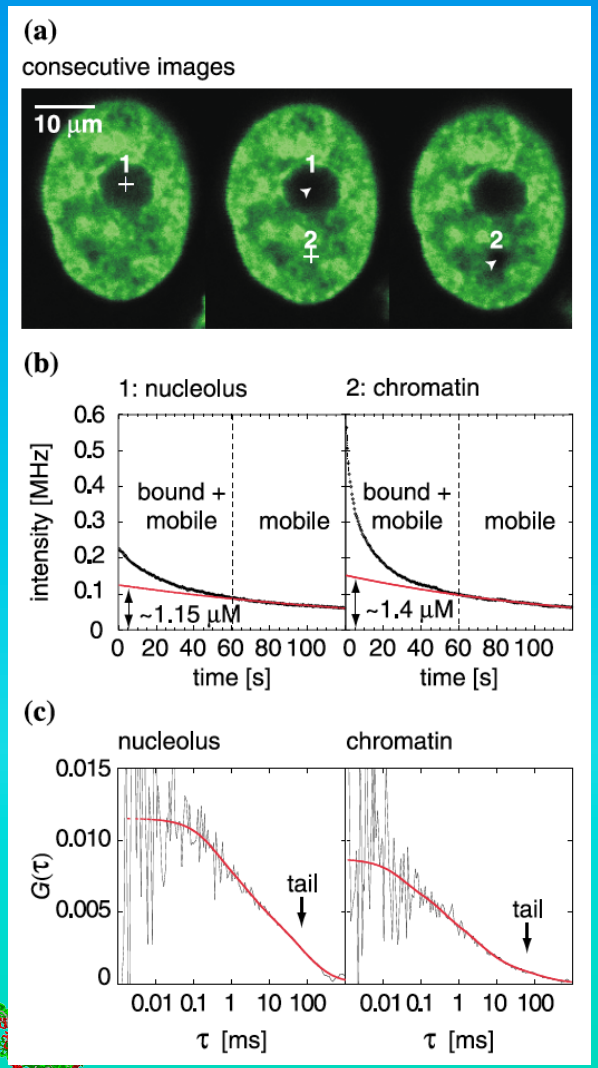


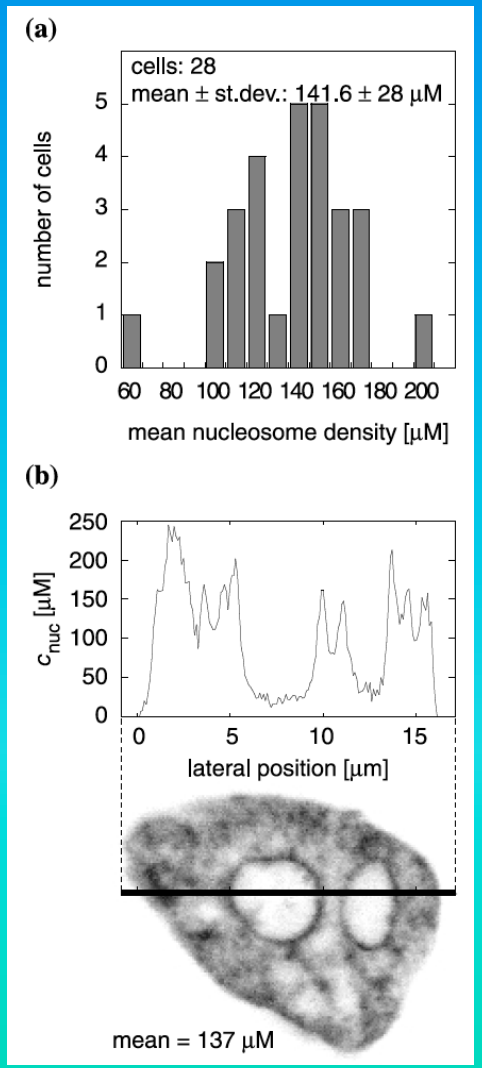
Nuclear diameter [μm]	Nuclear Volume [μm^3]	Mean Nucleosome Concentration [μM]	Chromatin Volume Fraction [%]	Mean Isotropic Mesh Spacing [nm]
6	115	251	20.1	41
8	268	107	8.6	64
10	523	55	4.4	90
12	904	32	2.6	117

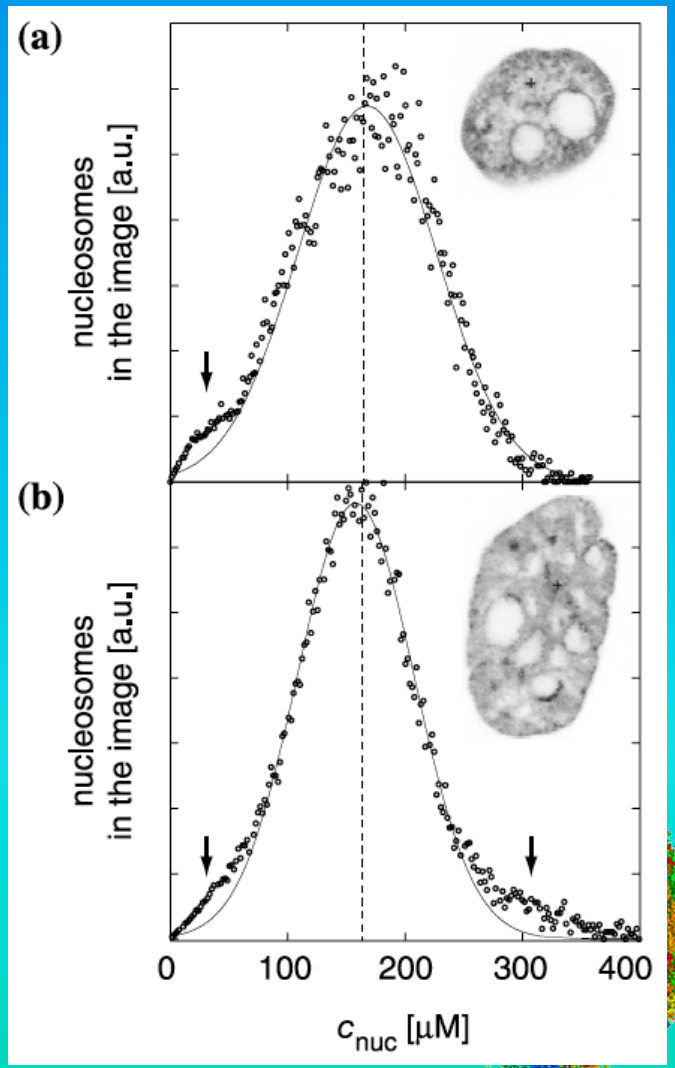


In Vivo Nucleosome Concentrations and 3D architecture

Counting nucleosomes in living cells with a combination of fluorescence correlation spectroscopy (FCS) and confocal laser scanning microscopy (CLSM) reveals the association of nucleosomes and their kinetics as well as again the typical expected distribution of a multi-loop aggregate/rosette.

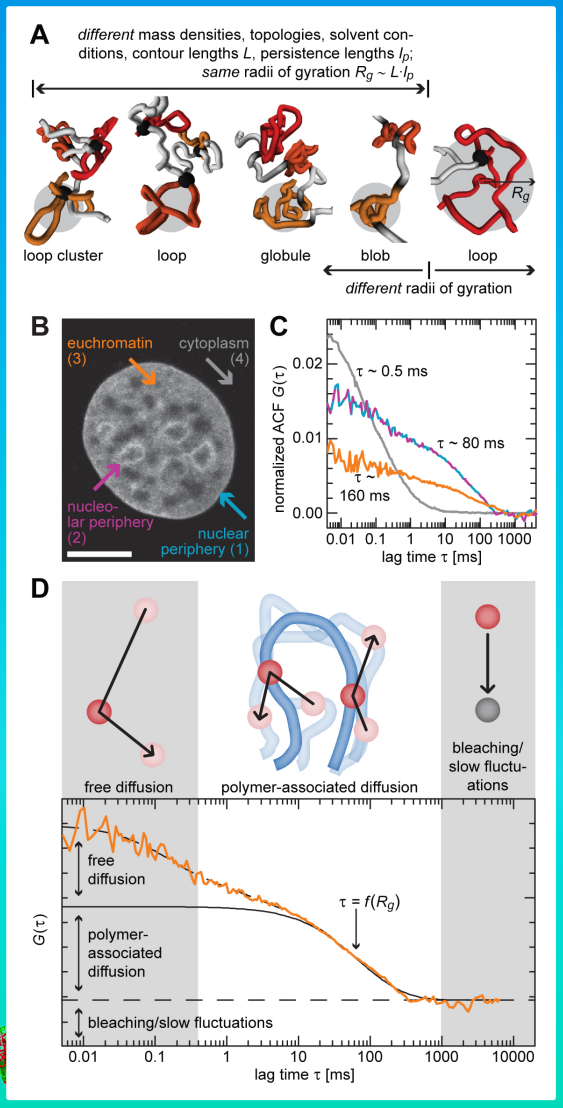


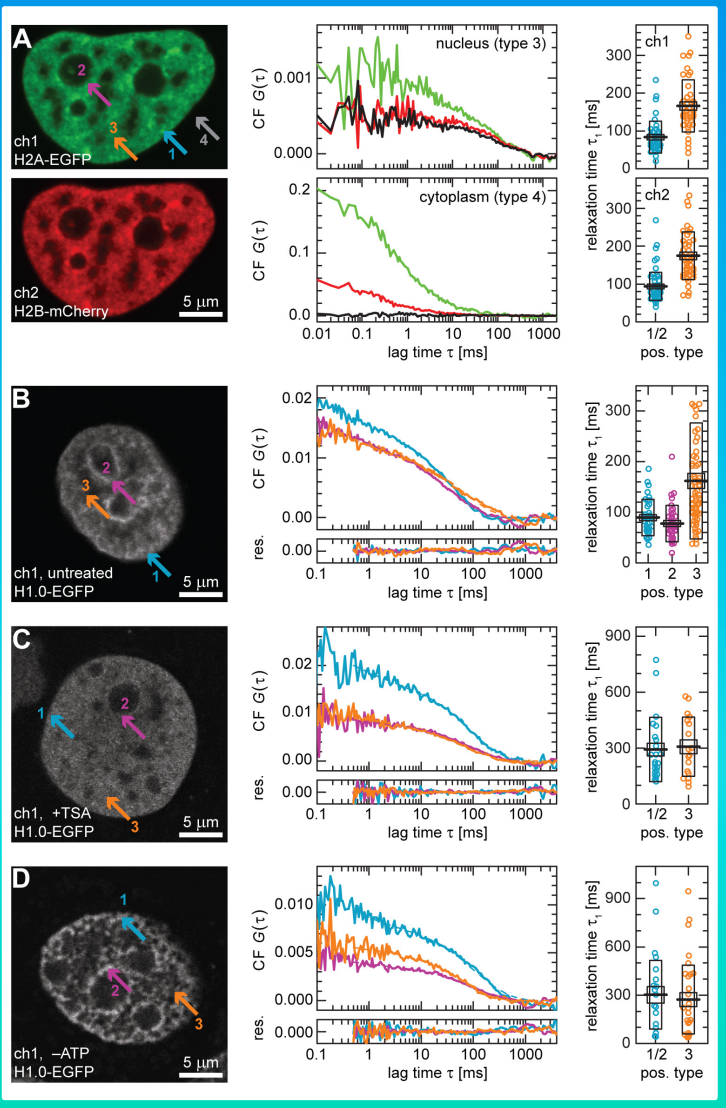


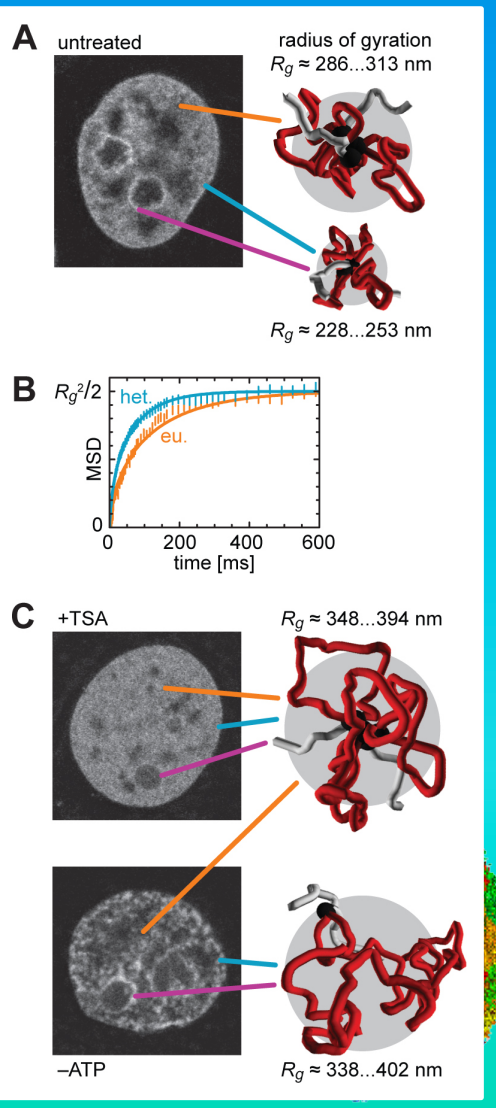


In Vivo Dynamics and 3D architecture

Fluorescence correlation spectroscopy (FCS) also reveals the dynamics of nucleosomes bound to DNA, i.e. FCS measures the movement of the chromatin quasi-fibre and its constraining architecture. This shows again a differentially compacted quasi-fibre folded into multi-loop aggregates/rosettes with functional differences as to e.g. hetero- and euchromatin or induced disturbances chromatin fiber (de-)condensation (+TSA, -ATP)







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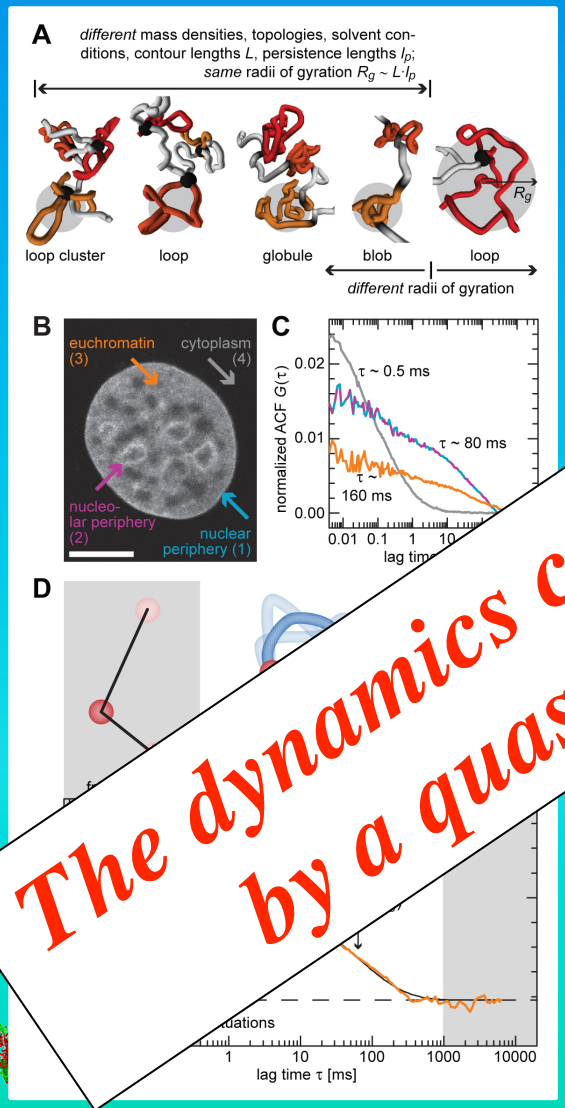

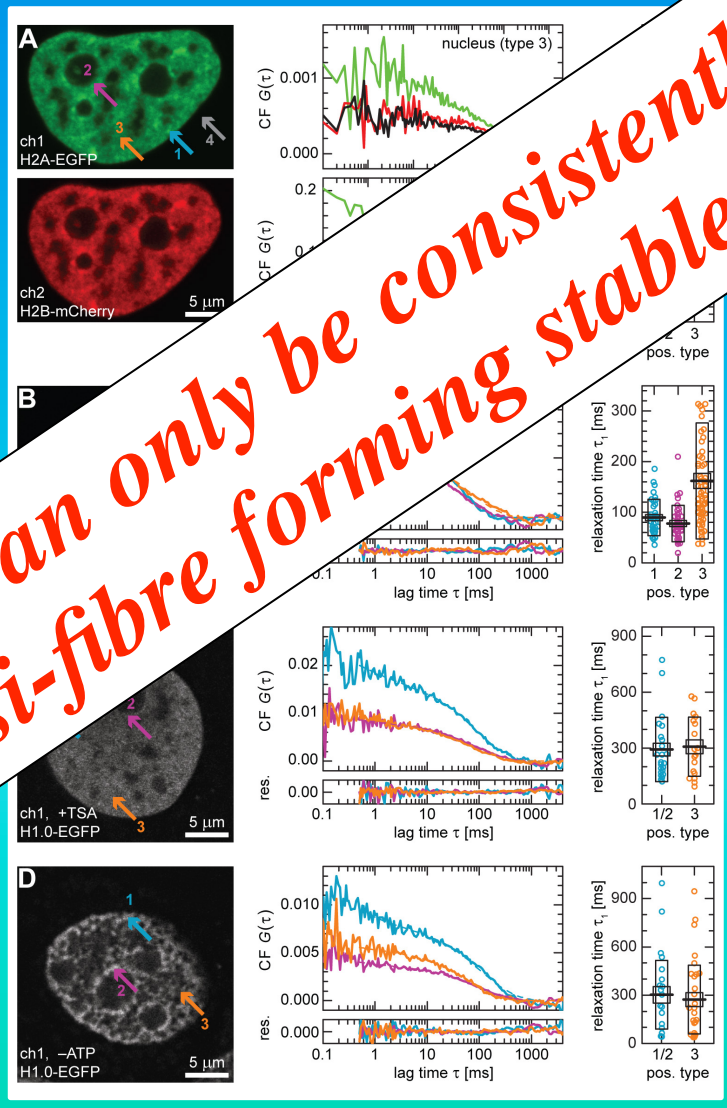

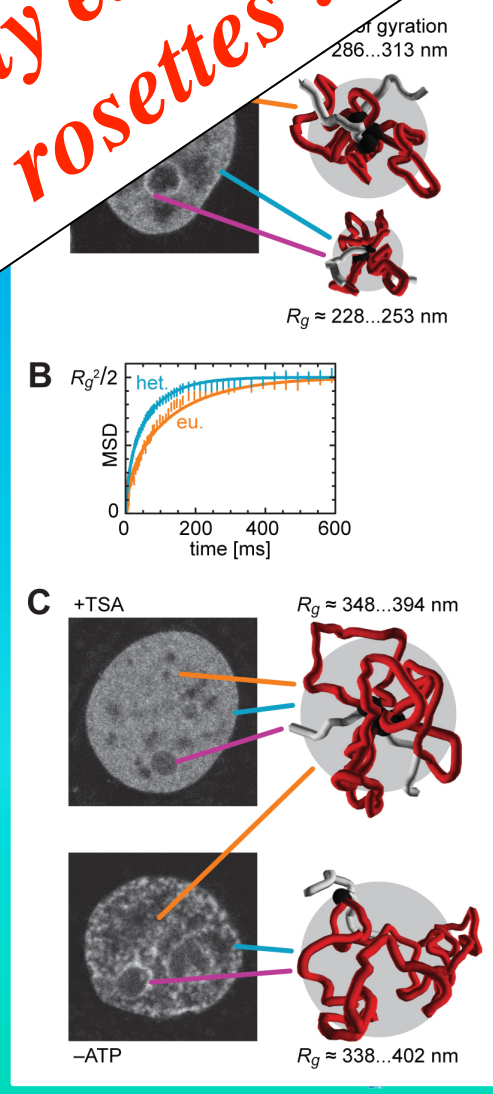

The dynamics can only be consistently explained by a quasi-fibre forming stable rosettes !

Conclusion

The compacted chromatin quasi-fibre, folds into stable 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.

Parameter/Observable			Simulation Single Chromosomes						Simulation Whole Nuclei							General Comparison Experiment vs. Simulation	
Architectural Level	Observable Class	Spec. Feature	Applic. & Quality	Topological Parameter Dependence				Modell	Applic. & Quality	Topological Parameter Dependence					Modell	Comp. Degree	Modell
				L_S/R_S	LI_S	C_{VOL}	EV			L_S/R_S	LI_S	NUC_{VOL}	EV	$Chrom_S$			
Morphology: Chromatin Fibre to Nucleus	Morphology	Rendering	+++	+++	+++	+++	+++	MLS	+++	+++	+++	+++	+++	-	MLS	+	MLS
		EM	ND; but similar to the simulation of whole nulcei.						+	+	+	+++	+	-	~MLS	+	~ MLS +
		FISH-EM							++	++	++	+++	++	+++	~MLS	+	~ MLS +
		CLSM							+	+	++	+++	+	-	MLS	++	MLS ++
		FISH-CLSM							++	++	+++	+++	++	+++	MLS	+++	MLS +++
Nucleus	Radial Distribution Nuclei	Mass							+++	+	+	+++	++	+++	≈MLS	+	≈ MLS
		Density							+++	+	+	+++	++	+++	≈MLS	+	≈ MLS
	CLSM-Image Distribution	Intensity							+++	+	+	+++	++	-	MLS	+++	MLS +++
		Mass							+++	+	+	+++	++	-	MLS	+++	MLS +++
	Chromosome Position	Distance Mass Centre							+++	+	++	+++	++	+++	~MLS	+	~ MLS +
Chromosome	Radial Distribution Chromosomes	Mass	+++	++	+++	+++	++	MLS	+++	++	+++	++	+	+++	MLS	+	MLS +
		Density	+++	++	+++	+++	++	MLS	+++	++	+++	++	+	+++	MLS	+	MLS +
	Territory Shape	Roundness	ND; but similar to the simulation of whole nulcei.						++	+	+++	++	+	+++	MLS	++	MLS ++
	Territory Distance	Nearest							+++	+	++	+++	+	+++	MLS	++	MLS ++
		Arbitrary							+++	+	++	+++	+	+++	MLS	++	MLS ++
	Territory CLSM/Interaction	Volume CLSM							+++	+	++	+++	++	+++	MLS	+++	MLS +++
		Overlap CLSM							+++	+	++	+++	++	+++	MLS	+++	MLS +++
Subchromosomal Domain	SD Radial Distribution	Mass	+++	+++	+	+	+	MLS	+++	+++	+	+	~+	-	MLS	+++	MLS +++
		Density	+++	+++	+	+	+	MLS	+++	+++	+	+	~+	-	MLS	+++	MLS +++
	SD Distance	Genetically Adjacent	+++	++	+++	+	+	MLS	+++	++	+++	+	~+	-	MLS	+++	MLS +++
		Spatial Arbitrary	+++	+	+	++	++	MLS	+++	+	+	+++	~+	-	MLS	+++	MLS +++
		Spatial Nearest	+++	++/+	+++/+	++	++	MLS	+++	++/+	+++/+	+/+++	~+	-	MLS	+++	MLS +++
	SD CLSM/Interaction	Volume CLSM	ND; but similar to the simulation of whole nulcei.						+++	+++	+	+	~+	-	MLS	+++	MLS +++
		Overlap CLSM							+++	+++	+	+++	~+	-	MLS	+++	MLS +++
Chromatin Loop/Fibre	Spatial Distance	Position Independent	+++	+++	+++	-/+++	++	MLS	+++	+++	+++	-/+++	+	-/+++	MLS	+++	MLS +++
		Position Dependent	++++	+++	+++	-/+++	++	MLS	++++	+++	+++	-/+++	+	-/+++	MLS	+++	MLS +++
		Marker Ensemble	++++	+++	+++	-/+++	++	MLS	++++	+++	+++	-/+++	+	-/+++	MLS	+++	MLS +++

Evolutionary Architecture Perspective

Only a compacted chromatin quasi-fibre, folded into stable loop-aggregates connected by a linker allows to guaranty the functional informational requirements of genomes:

i) storage stability/flexibility, ii) readout, and iii) replication !

- **Storage stability/flexibility:**

  The packaging ratio/scale into a quasi-fibre and stable loops forming rosettes is optimal for the physical stability of genomes, while it is flexible enough to allow functional differences as well as react to entropic and other damages.

- **Readout:**

  The dynamics this architecture allows expression/regulation by self-organization into (in-)active units already in proximity, and guaranties at the same time accessibility to and from the information for factors as well transcripts.

- **Replication:**

  The 2D knot-free topology as well as the packaging ratio/scale into a quasi-fibre and stable loops forming rosettes, allows concatenation free replication with low error/damage rate due to the easy block-wise proximity organization as well as the easy physical (de-)condensation during cell division.

Form follows function and function follows form!

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Genomes are an Evolutionary Holistic Form-Function Entanglement !

Form follows function and function follows form!

Clinical Epigenetic Context

Only a compacted chromatin quasi-fibre, folded into stable loop-aggregates connected by a linker allows to guaranty the functional informational requirements of genomes:

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- **Clinical Epigentic Context of Storage Stability/Flexibility:**

  Epigenetic alteration of the packaging ratio/scale into a quasi-fibre and stable loops forming rosettes alters the optimum ground state of the physical stability of genomes. While this ground state is flexible enough to allow for functional differences as well as react to entropic and other damages, functional alterations beyond this flexibility turns into malfunction and disease.

- **Clinical Epigenetic Context of the Readout:**

  Epigenetic variation of the dynamics of this architecture alters the expression/regulation preset by self-organization into (in-)active units already in proximity, and thus changes at the same time the guaranteed accessibility to and from the information for factors as well transcripts. Beyond, the evolutionary limits this also leads to malfunction and disease.

- **Clinical Epigenetic Context of Replication:**

  Epigenetic changes influence the 2D knot-free topology as well as the packaging ratio/scale into a quasi-fibre and stable loops forming rosettes, and thus changes the degree of concatenation free replication with low error/damage rate due to the easy block-wise proximity organization as well as the easy physical (de-)condensation during cell division. Again, beyond the limits, this results in malfunction and disease.

Form Follows Function and Function Follows Form

ONLY within the Limits of Evolutionary Preset Multilism between Genotype and Phenotype!

Clinical Epigenetic Context

Only a compacted chromatin quasi-fibre, folded into stable loop-aggregates connected by a linker allows to guaranty the functional informational requirements of genomes:

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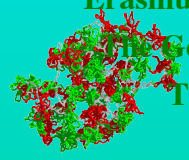

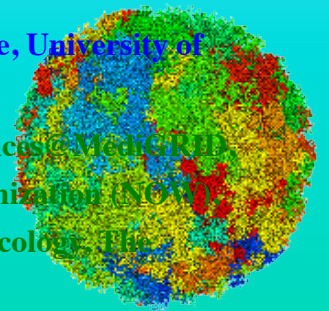

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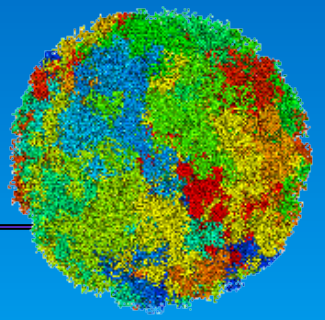

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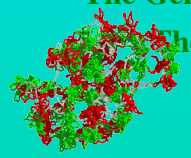

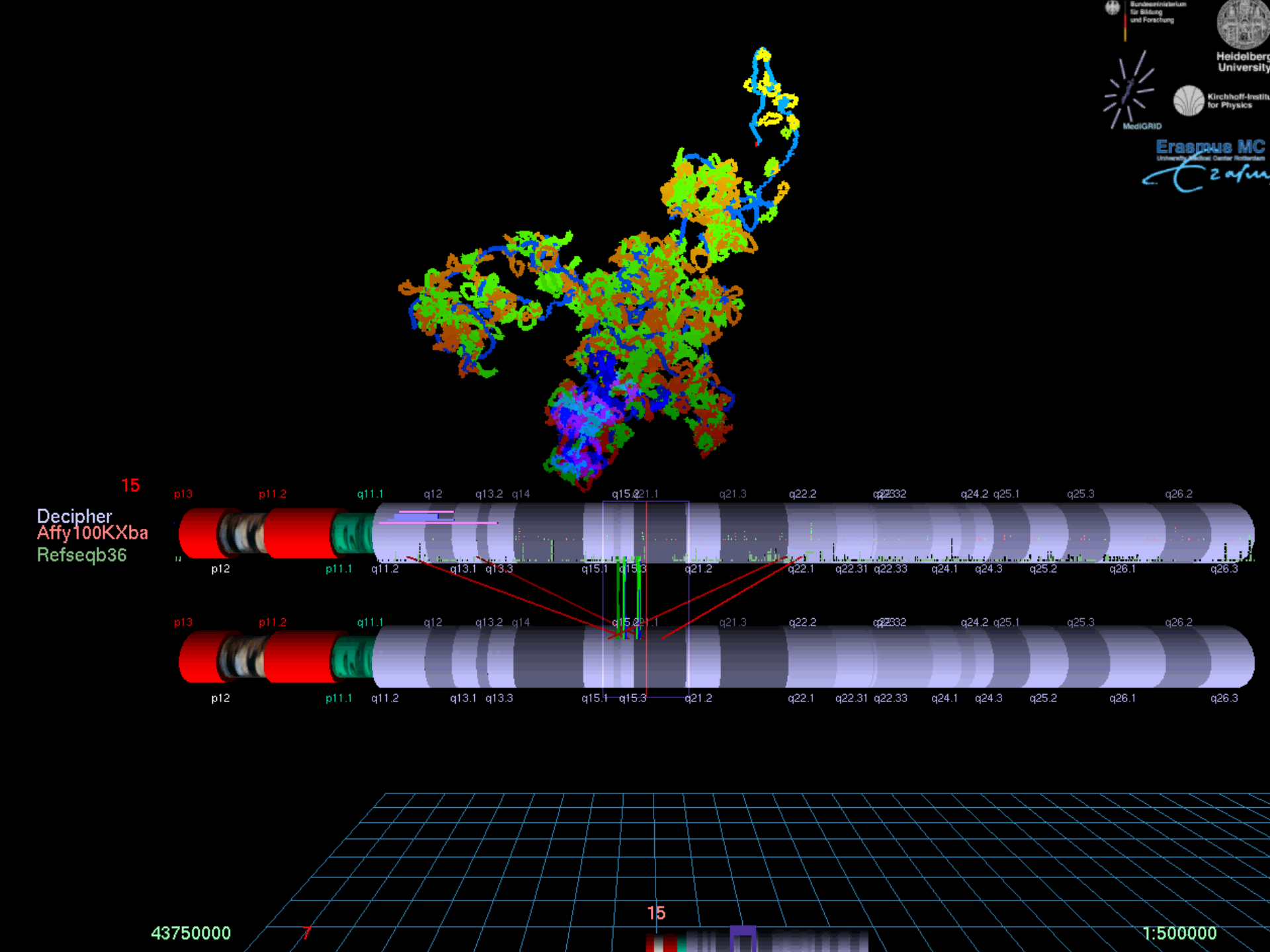
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15
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Refseqb36
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7
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Decoding
the
3D Multi-Loop Aggregate/Rosette Chromatin Architecture, Dynamics,
and
Functional Epigenetics of Genomes

Knoch, T. A.

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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 debated after ~170 years. With a systems genomics approach combining a novel selective high-throughput chromosomal interaction capture (T2C) with quantitative polymer simulations and scaling analysis of architecture and DNA sequence, we determined and cross-proved the final architecture of genomes with unprecedented molecular resolution and dynamic range from single base pairs to entire chromosomes: for a variety of genetic loci of different species, cell type, cell cycle, functional states and system distortion a chromatin quasi-fibre exists with 5±1 nucleosome per 11 nm, which folds into stable(!) 40-100 kbp loops forming stable(!) aggregates/rosettes which are connected by a ~50 kbp chromatin linker. Modifications on all these organizational levels are variations of the aforementioned scheme. Beyond, functional variations on various levels are reflected also on others. Spatial isotropy breaking is also found. Polymer simulations using Monte Carlo and Brownian dynamics approaches confirm this and predict and explain additional experimental findings. Beyond, a novel fluorescence correlation spectroscopy (FCS) approach combined with analytical polymer models measures the architectural dynamics *in vivo* in the entire genome and agrees with the before mentioned conclusion using completely independent means. System distortions are reflected in the corresponding variations as well. Beyond, we find a fine-structured multi-scaling behaviour of both the architecture and the DNA sequence, showing for the first time directly the tight entanglement between architecture and sequence. All this agrees with the outcome of a synopsis e.g. with previous spatial distance measurement studies, *in vivo* morphology of entire cell nuclei, or electron microscopy of chromosome spreading studies, as well as the heuristics of the field in the last 170 years. This now complete architecture and dynamics of these genomes has fundamental consequences for the entire system of the storage and expression of genetic information, for its investigation in general as well as for clinical epigenetics: E.g. this architecture, its dynamics, and accessibility balance stability and flexibility ensuring genome integrity and variation enabling gene expression/regulation by self-organization of (in)active units already in proximity. Thus, both the T2C and FCS approaches open the door to "architectural and dynamic sequencing" of genomes at a resolution where a genome mechanics with corresponding uncertainty principles applies. Consequently, this will lead now to a detailed understanding of genomes with fundamental new insights and huge novel perspectives for diagnosis, treatment and genome engineering efforts in the future.

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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, 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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