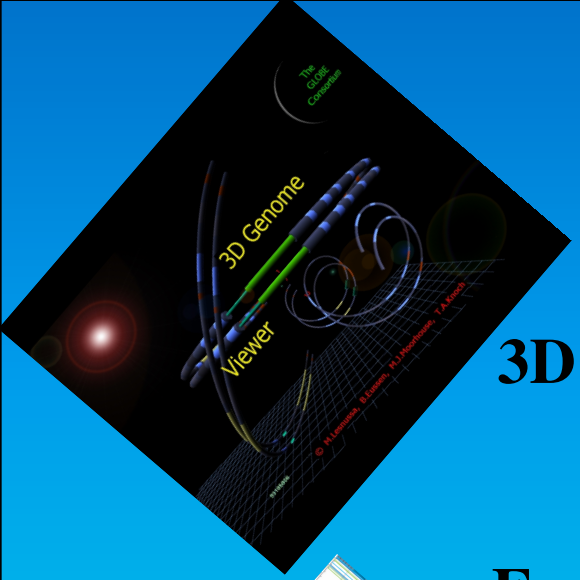
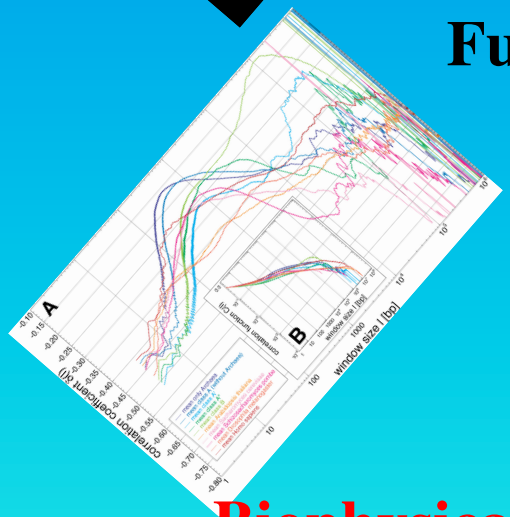
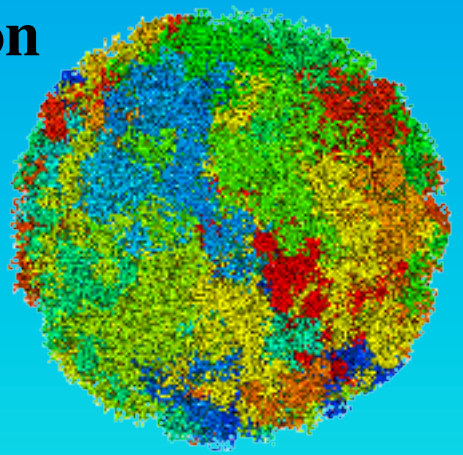


The Detailed 3D Multi-Loop Aggregate/Rosette Chromatin Architecture and Functional Dynamic Organization of the Human and Mouse Genomes





Tobias A. Knoch

Biophysical Genomics & Erasmus Computing Grid

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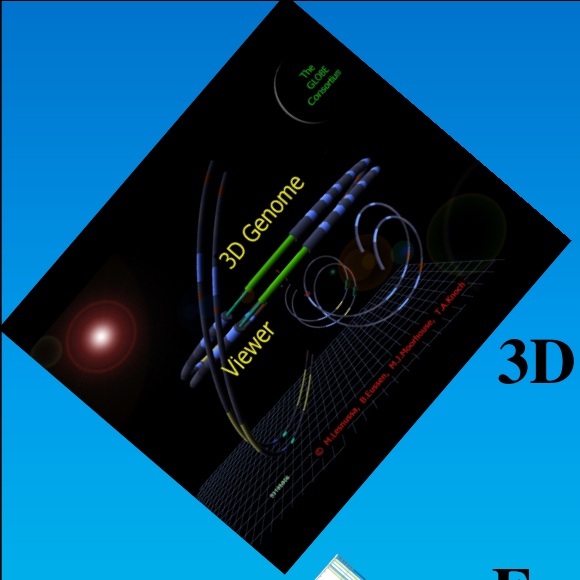
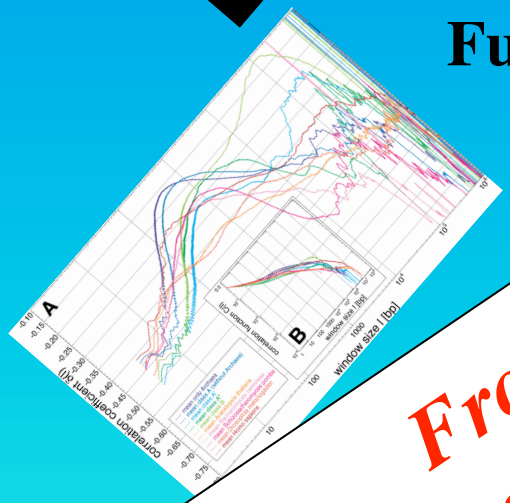
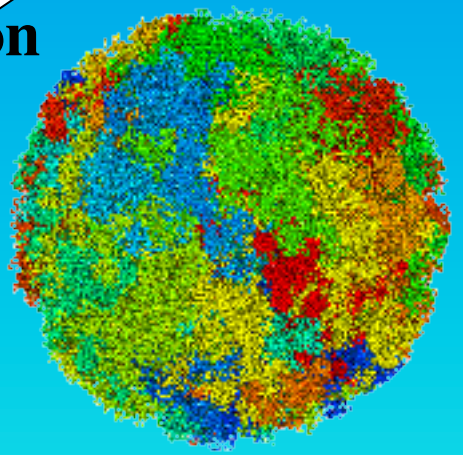
TA.Knoch@taknoch.org



The Detailed 3D Multi-Loop Aggregate/P… Chromatin Archit… and Functional Dyn… …tion

Hum… …Genomes





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

…bias A. Knoch

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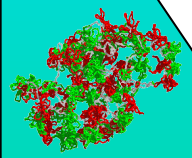
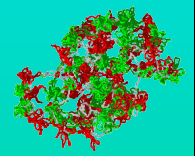
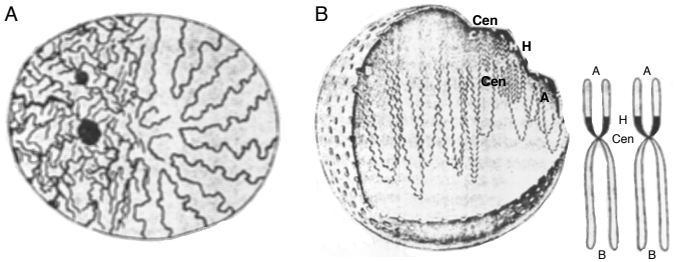


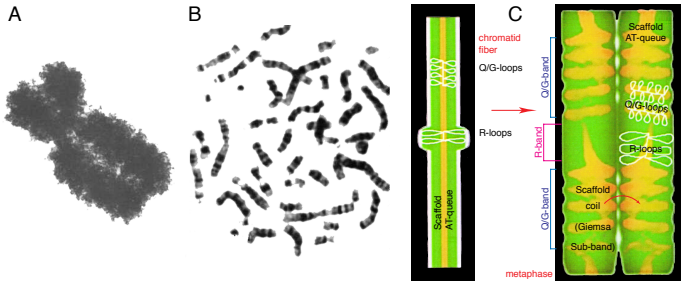


Integral Models of Cell Nuclear Organization I

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.

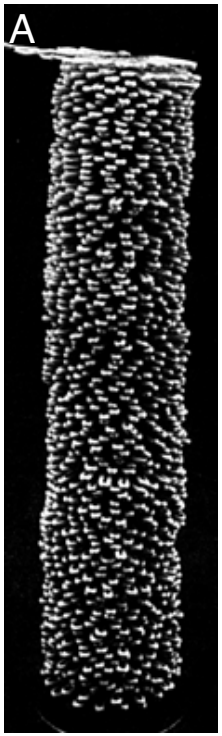
Rabl & Boveri

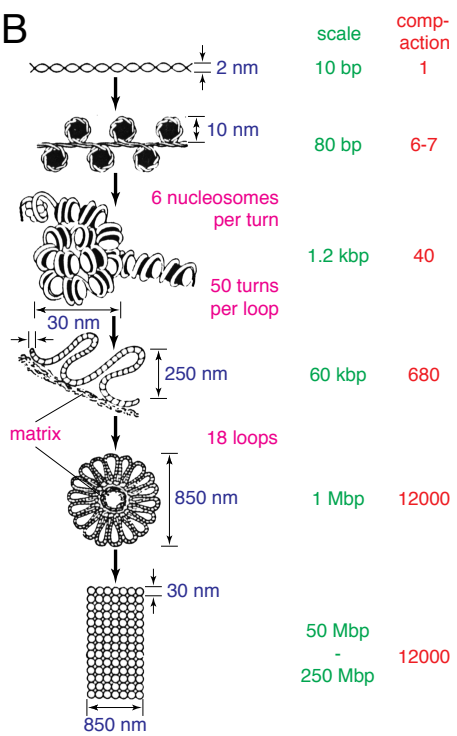




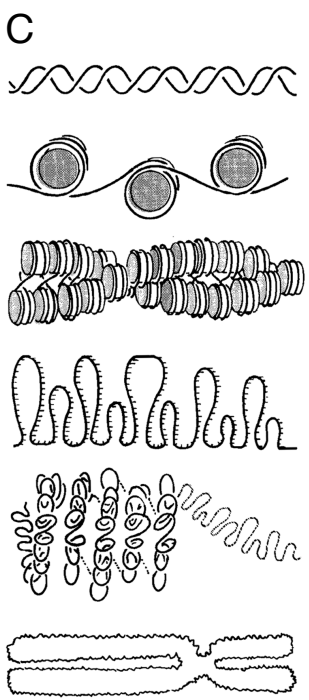
A: Bloom & Fawcett
B: Alberts *et al.*
C: Paulson & Laemmli

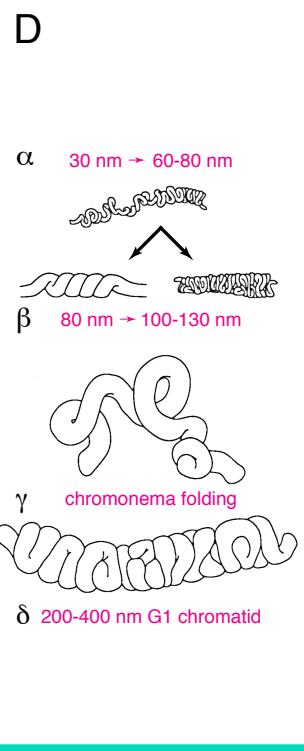
A, B: Pienta & Coffey; C: Alberts *et al.*; D: Belmont & Bruce

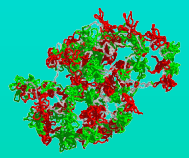


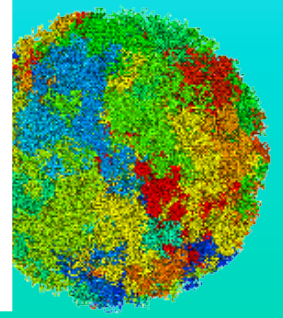


scale	comp-action
10 bp	1
80 bp	6-7
1.2 kbp	40
60 kbp	680
1 Mbp	12000
50 Mbp - 250 Mbp	12000



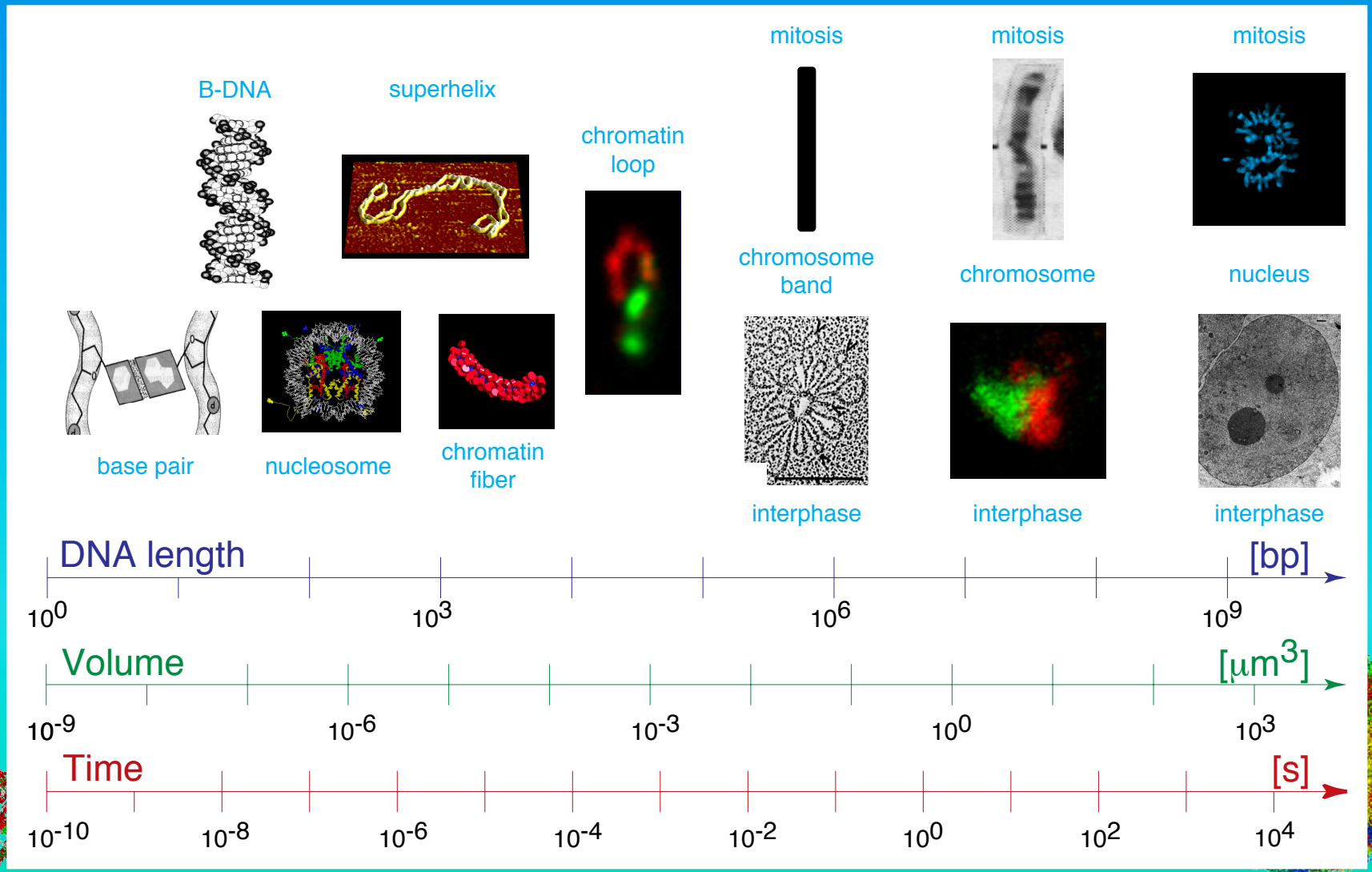






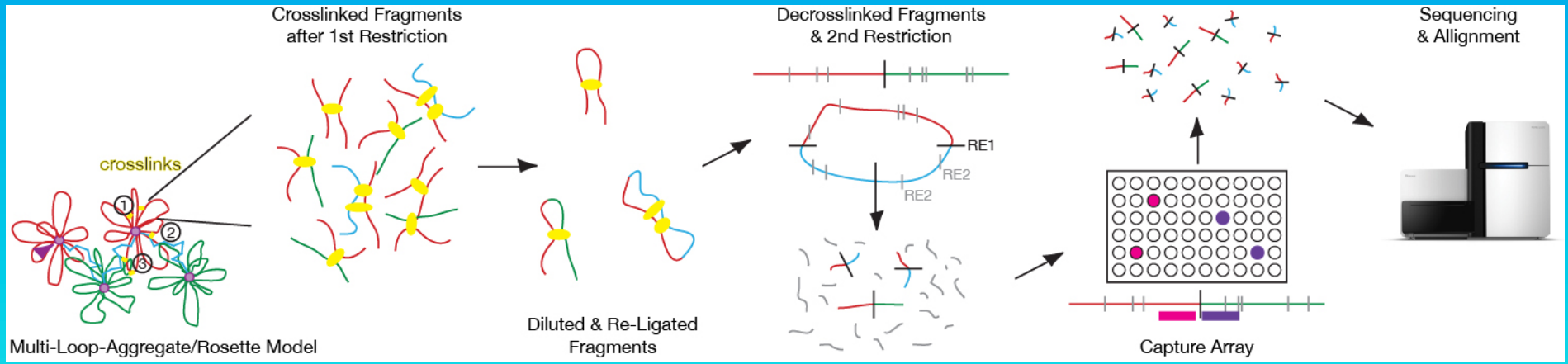
Dynamic and Hierarchical Genome Organization

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?



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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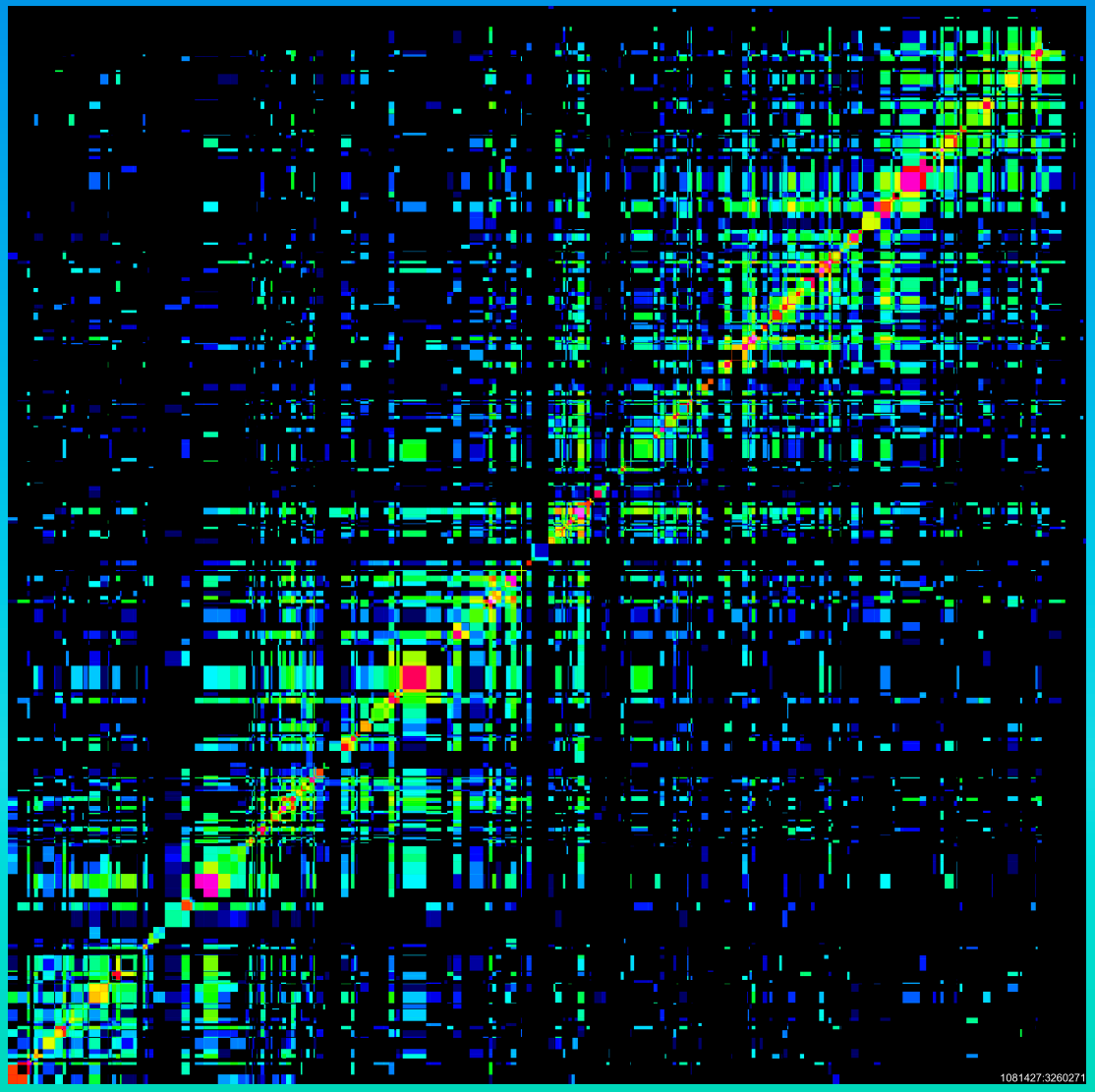
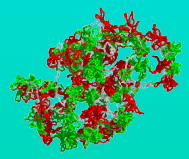
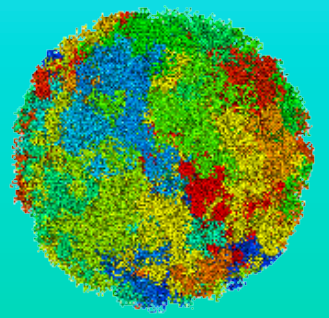
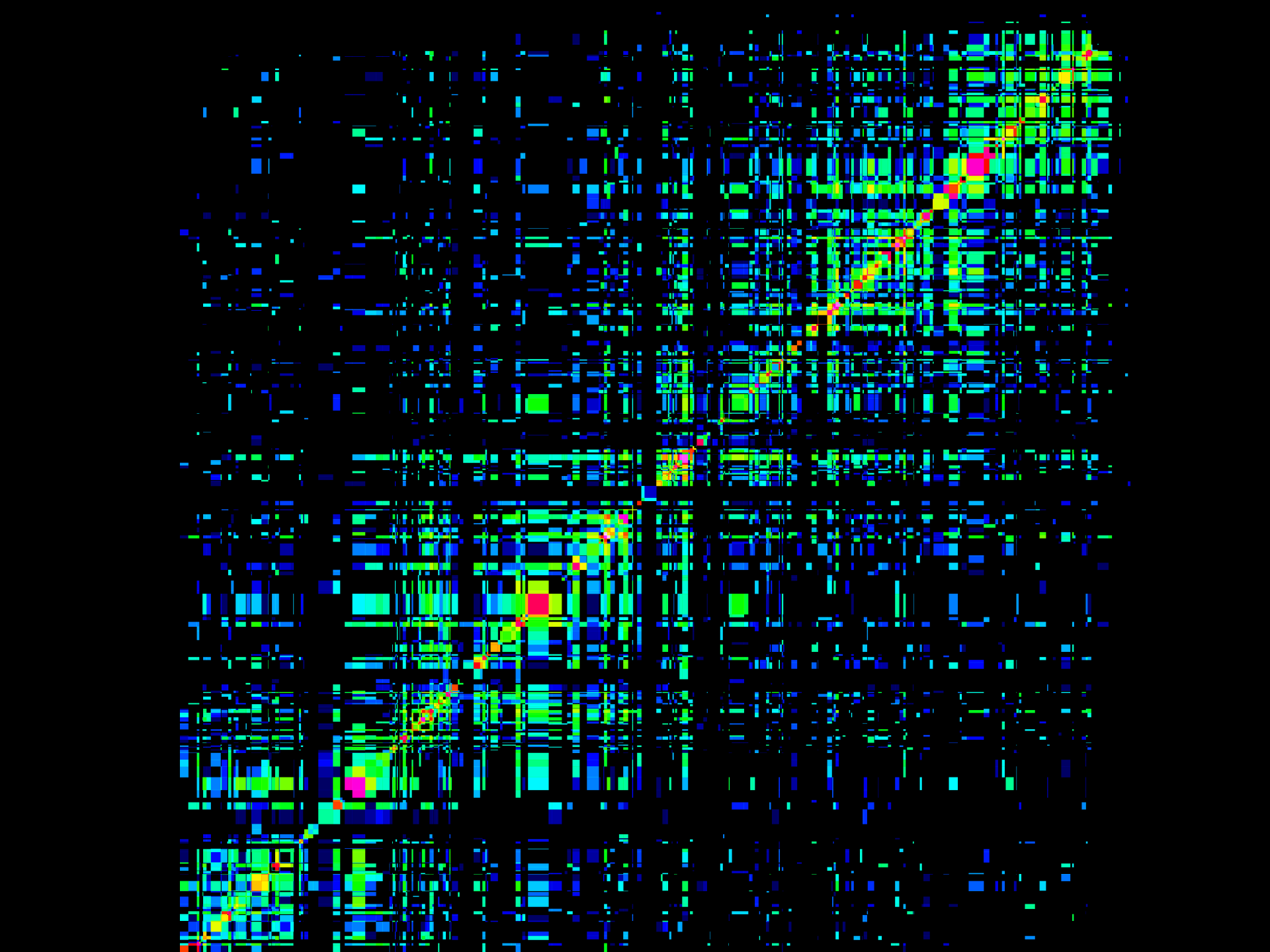
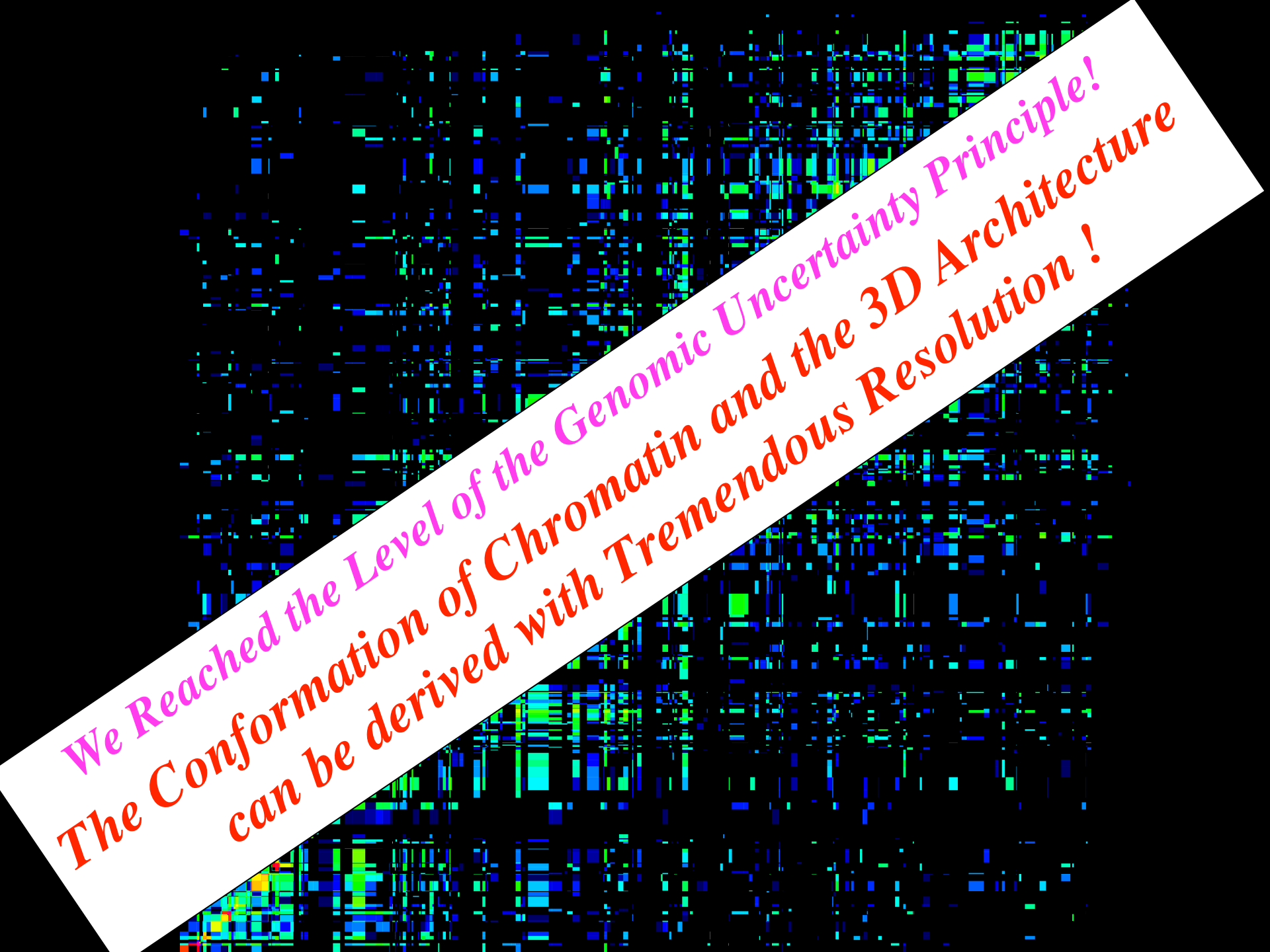


HS IGF locus
~2.1 Mbp





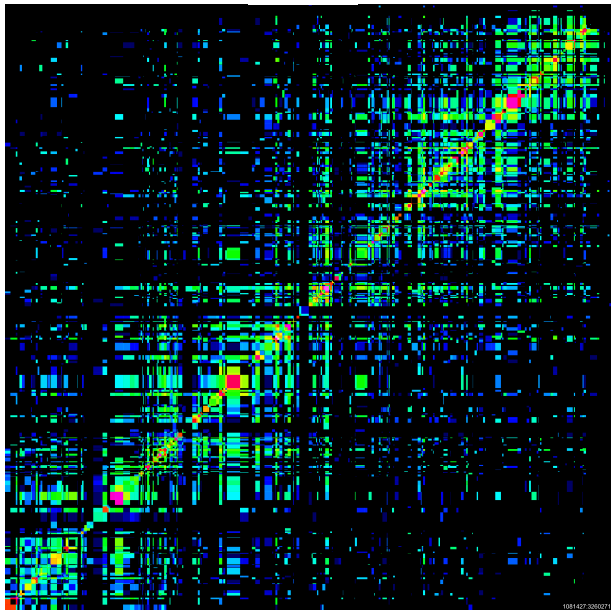



We Reached the Level of the Genomic Uncertainty Principle!
The Conformation of Chromatin and the 3D Architecture
can be derived with Tremendous Resolution !

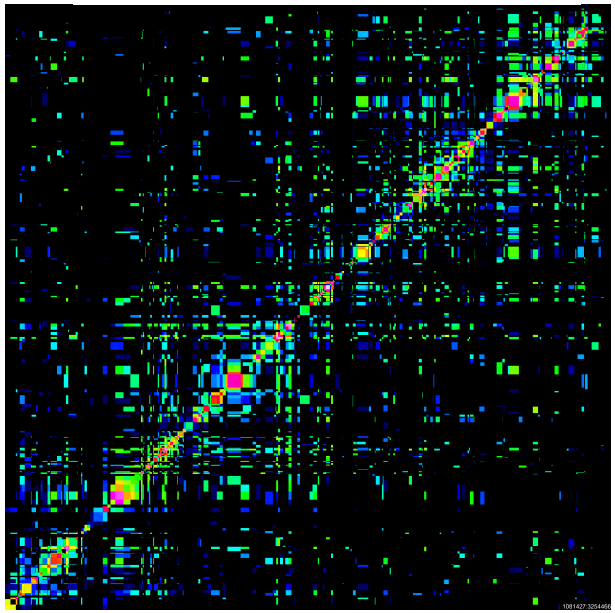
Stable Consensus Architecture of Genomes

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

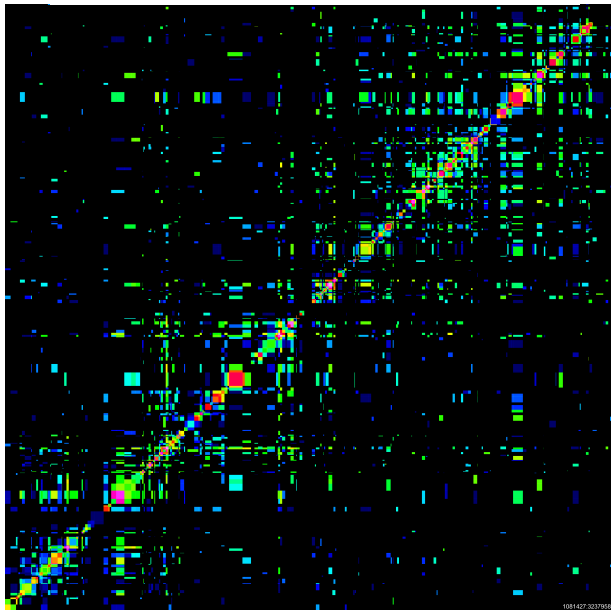
HB2

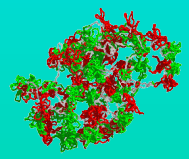


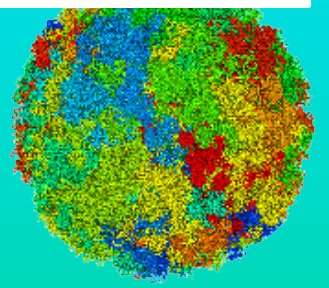
TEV-HEK293T cohesin intact



HRV-HEK293T cohesin cleaved







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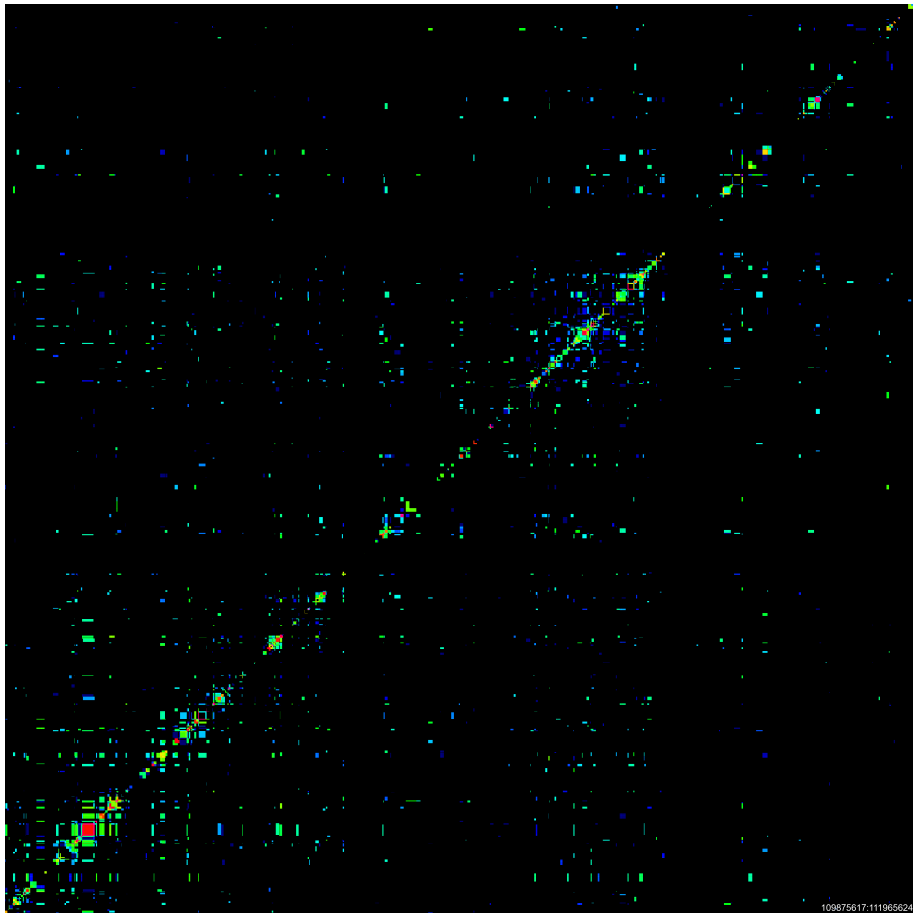
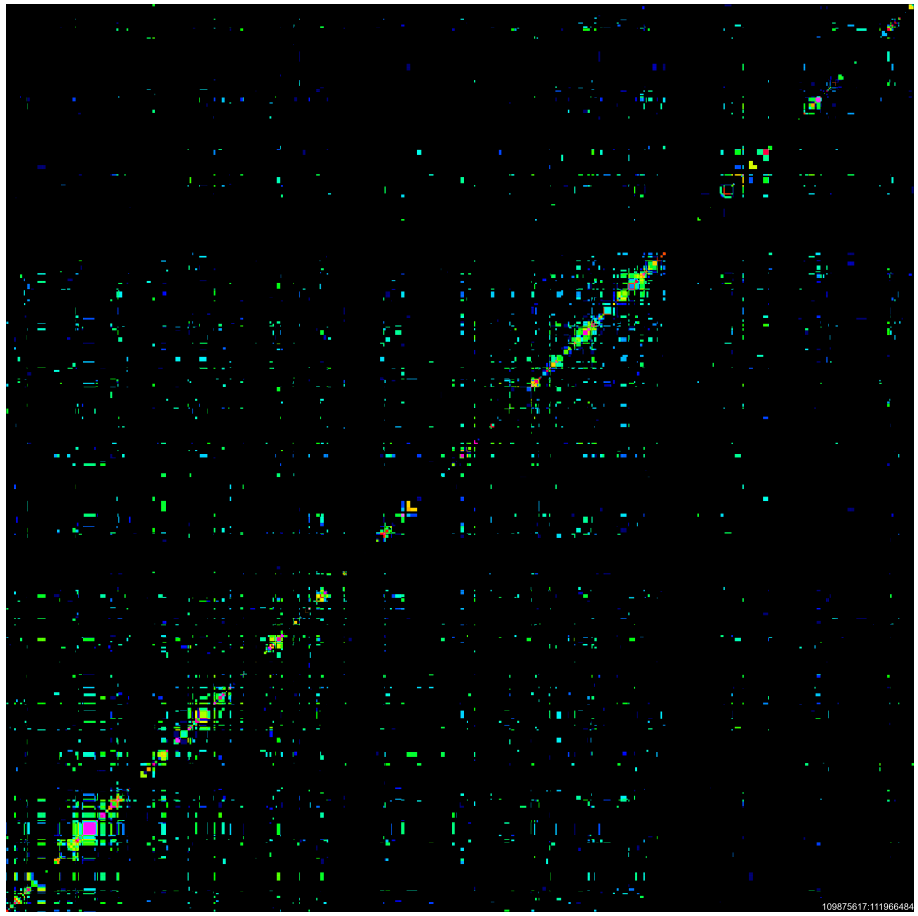
Fetal Brain (inactive β-Globin)



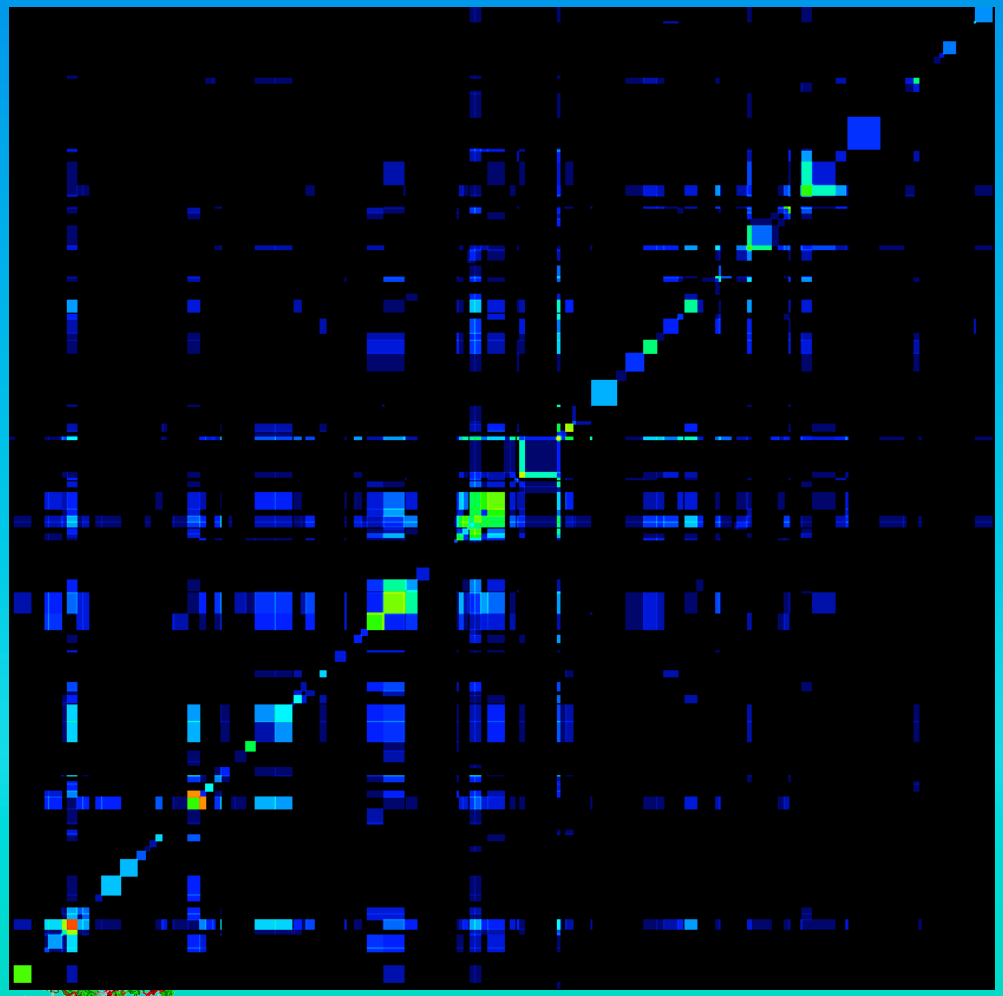
Fetal Liver (active β-Globin)

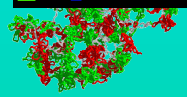


MM β-Globin 2.1 Mbp

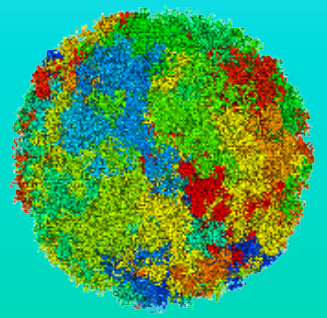
Fine Structure of Loop Aggregates/Rosettes

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.





~ 800 kbp



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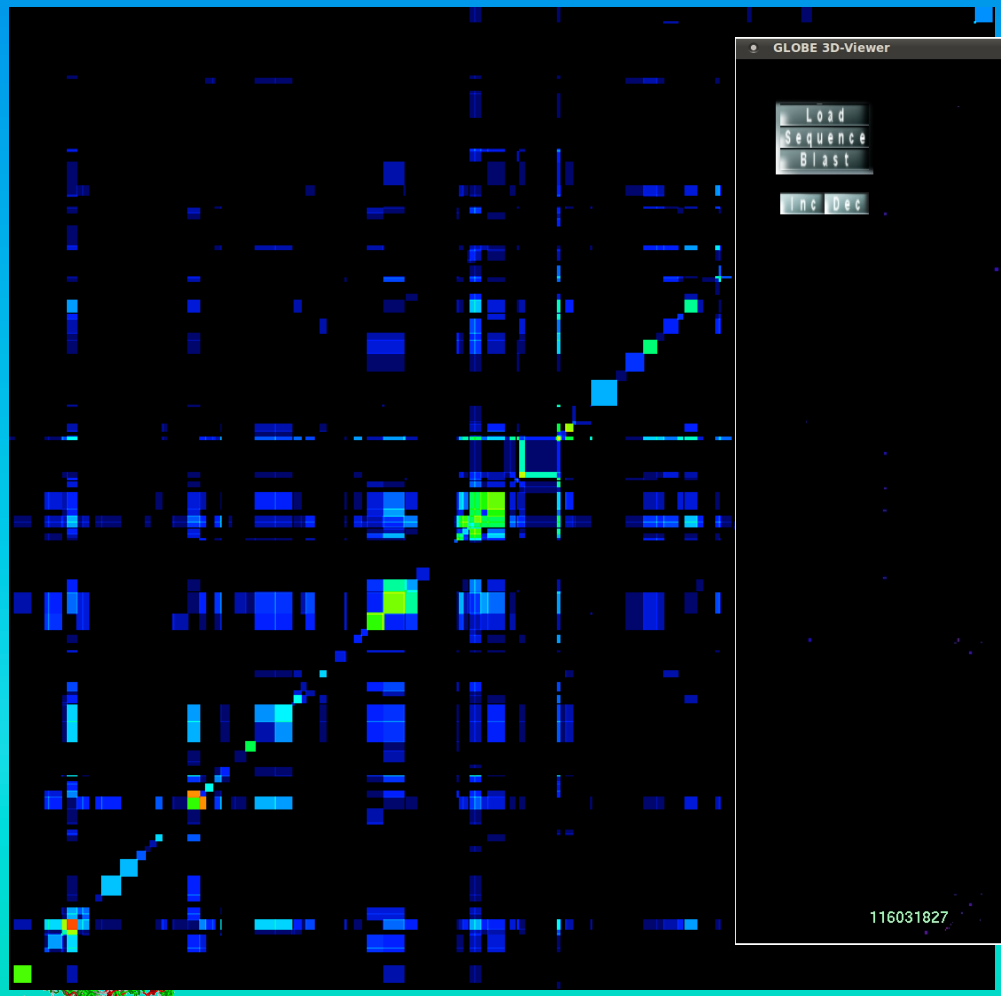

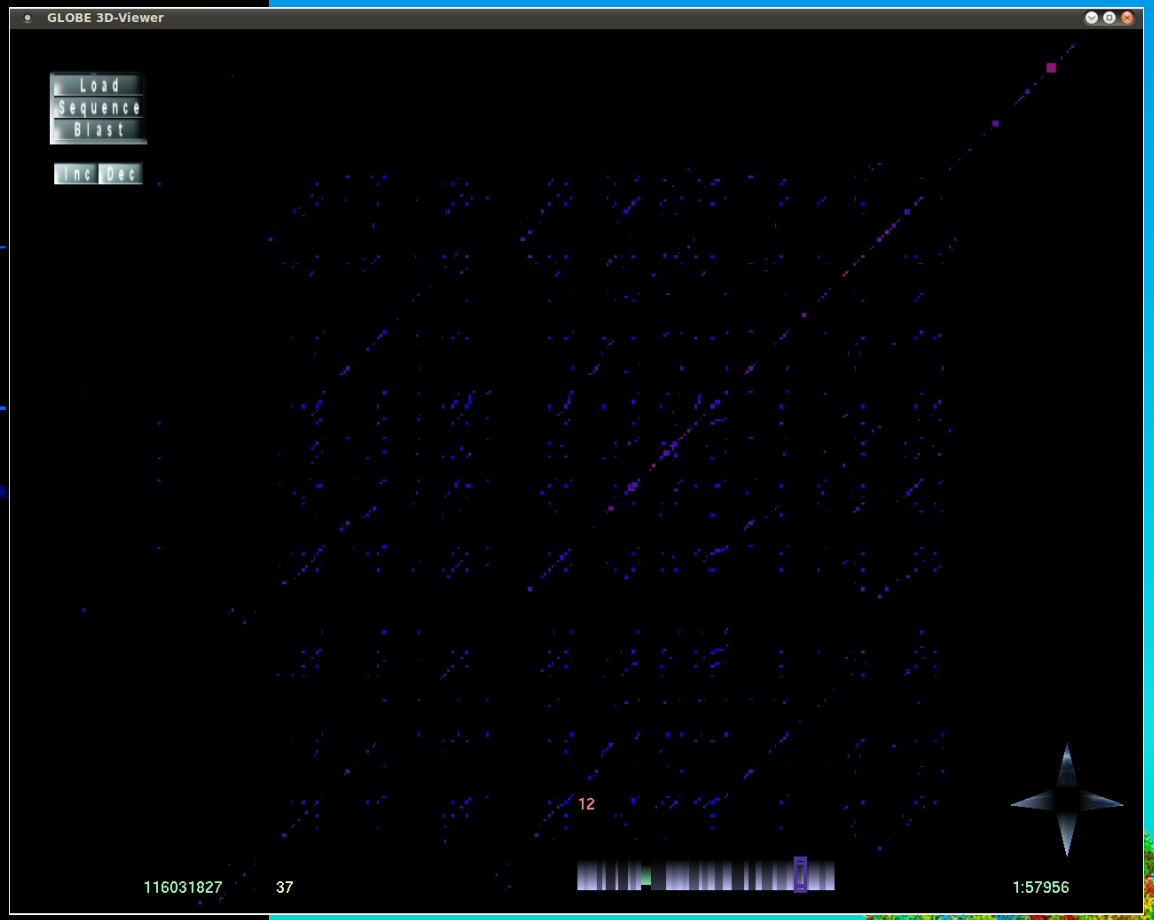

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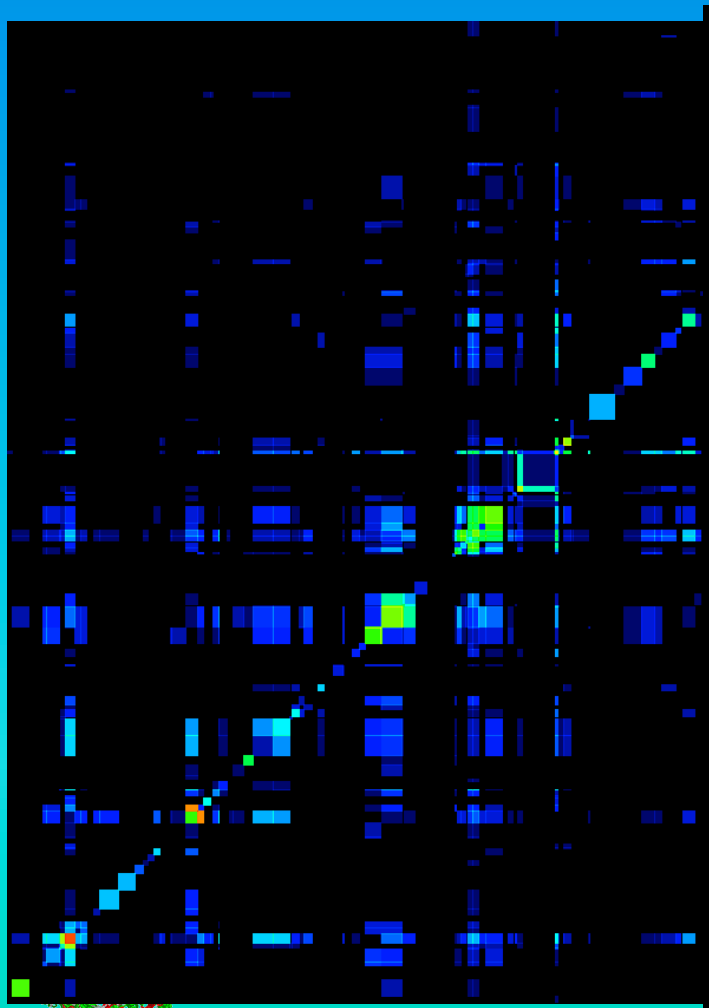

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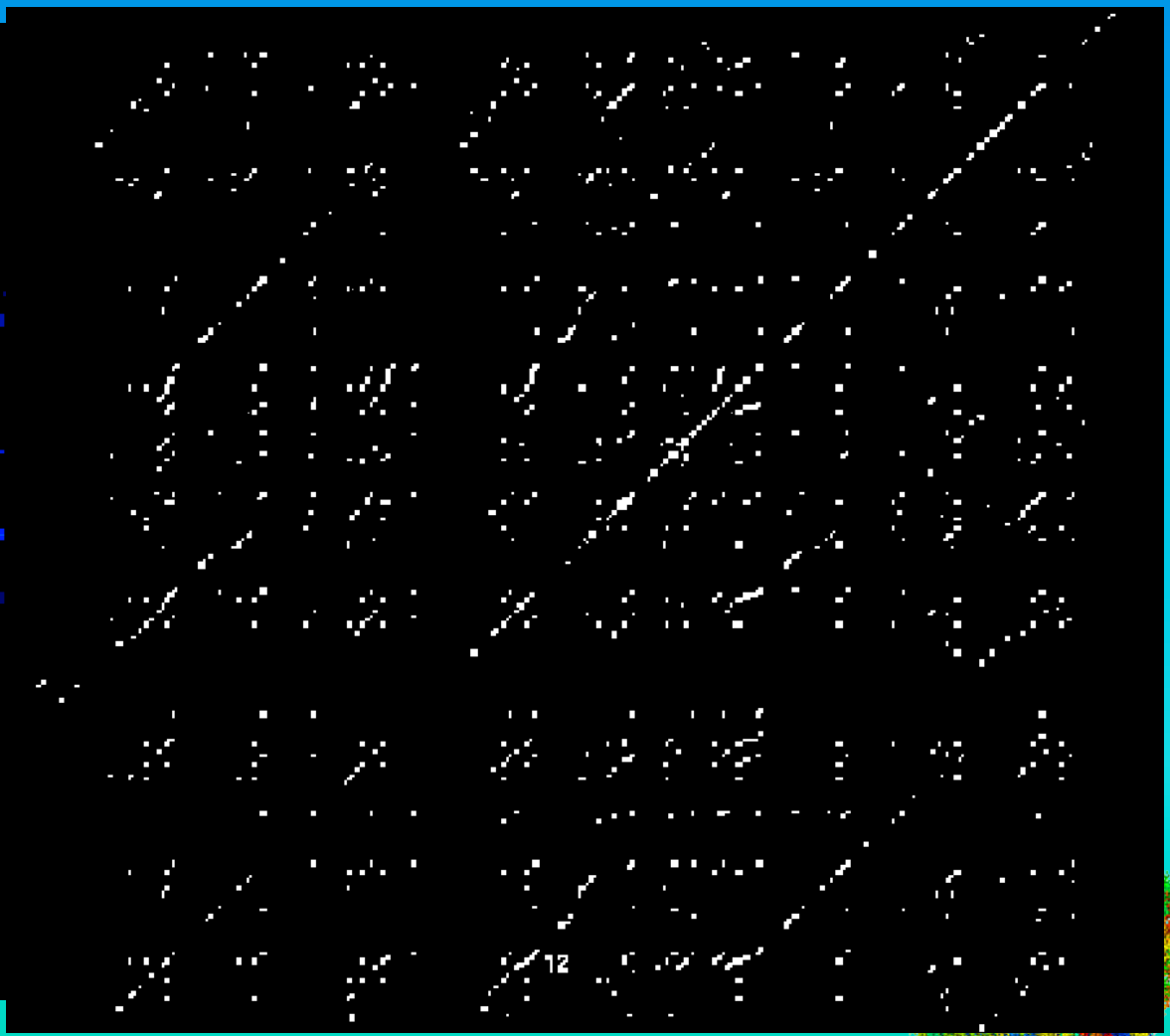

~ 380 kbp

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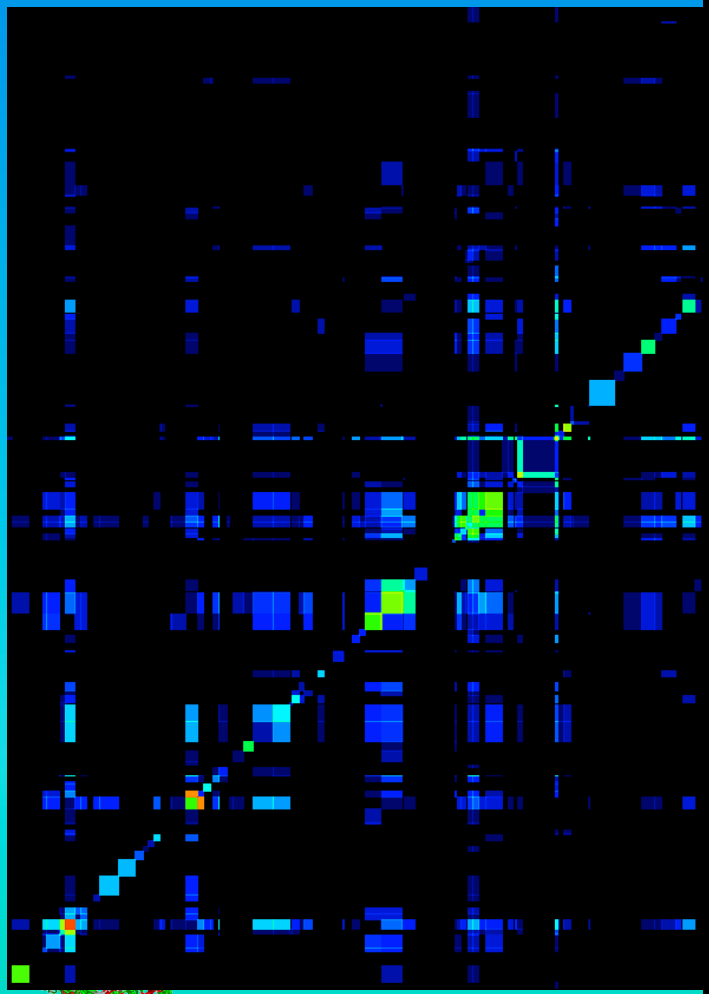
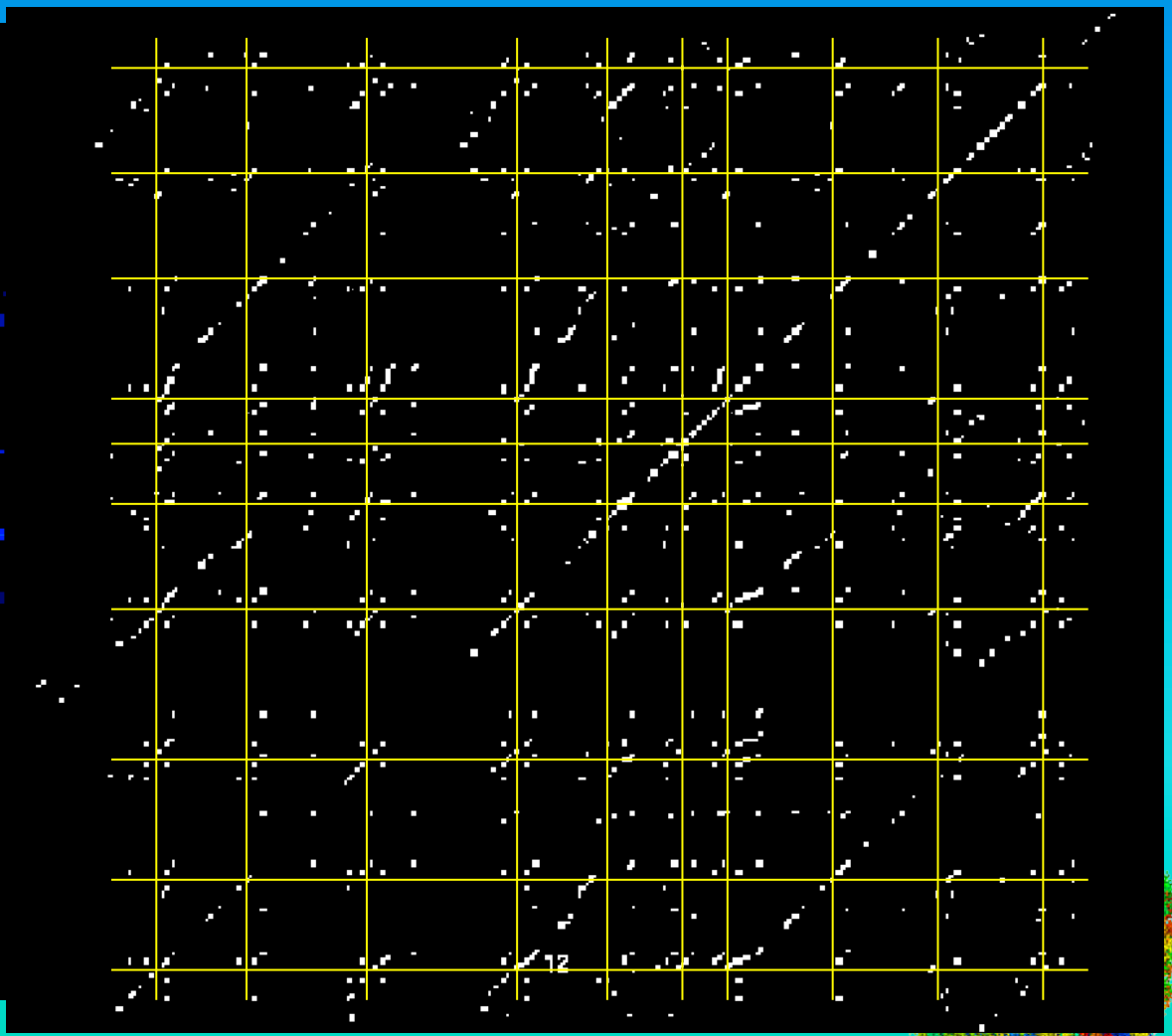


~ 800 kbp



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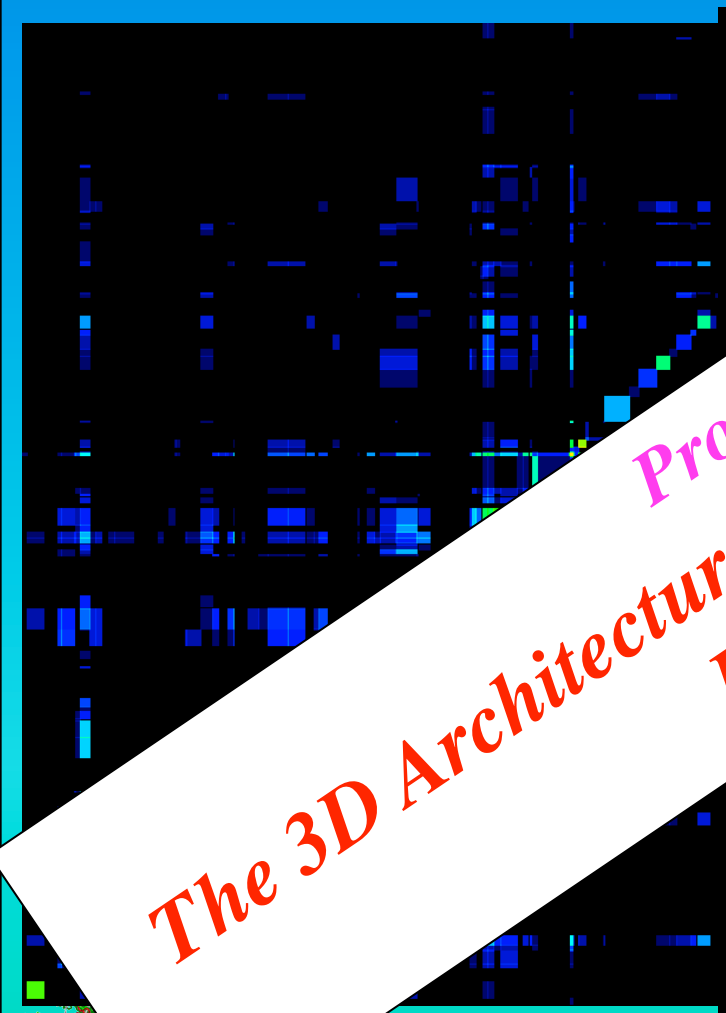

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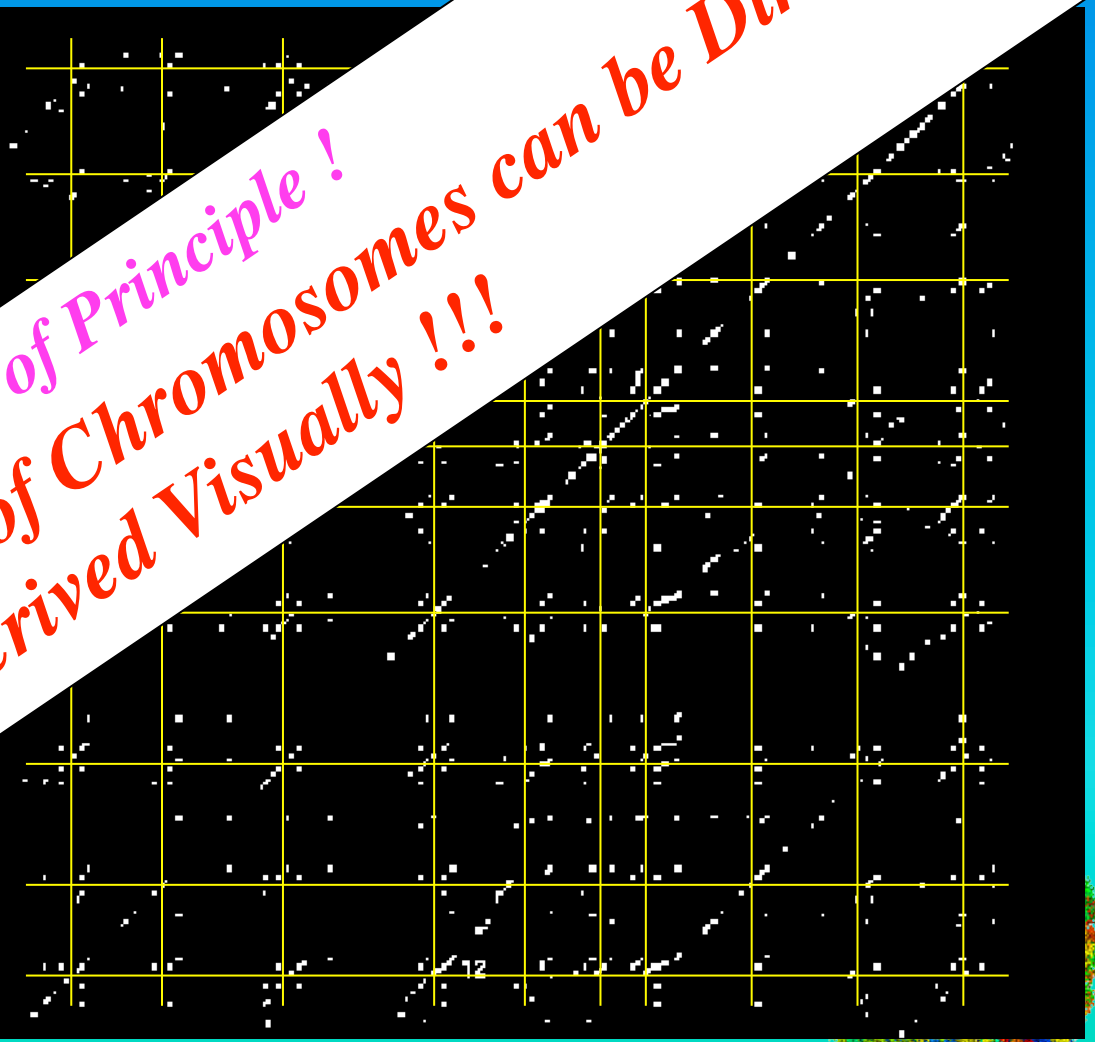


~ 380 kbp

Proof of Principle !

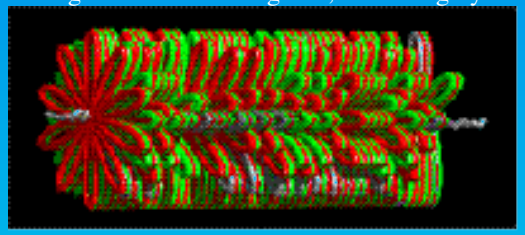
The 3D Architecture of Chromosomes can be Directly Derived Visually !!!

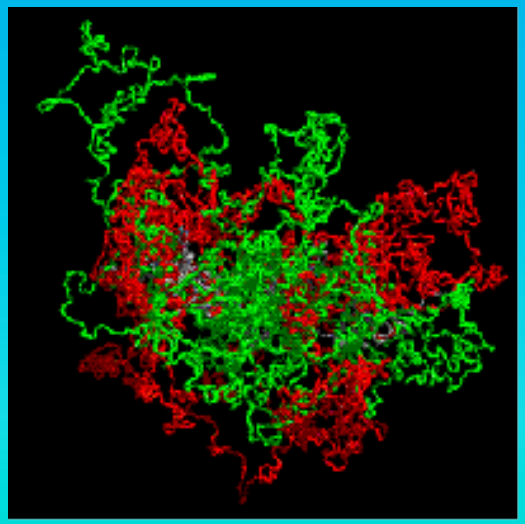
Simulation of Single Chromosomes

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.

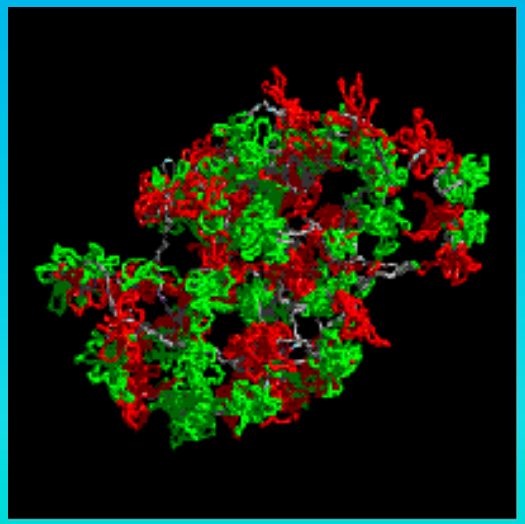
All models form chromosome territories with big voids and different chromatin morphologies. Experimental territory and subcompartment diameters agree best with an MLS model with 80 to 120 kbp loops and linkers.

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

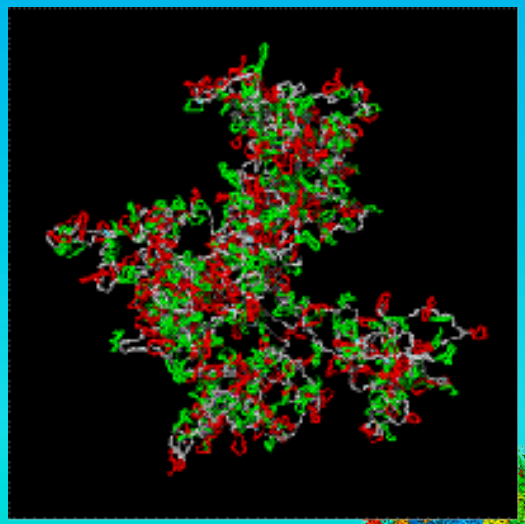




RW/GL model, loop size 5 Mbp, after ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromsome territory, thus forming no distinct features like in MLS model.



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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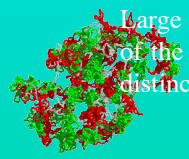

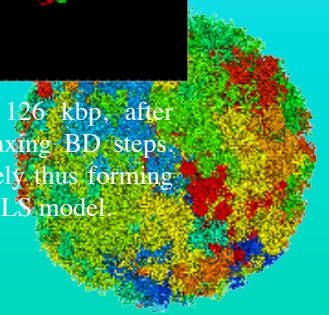

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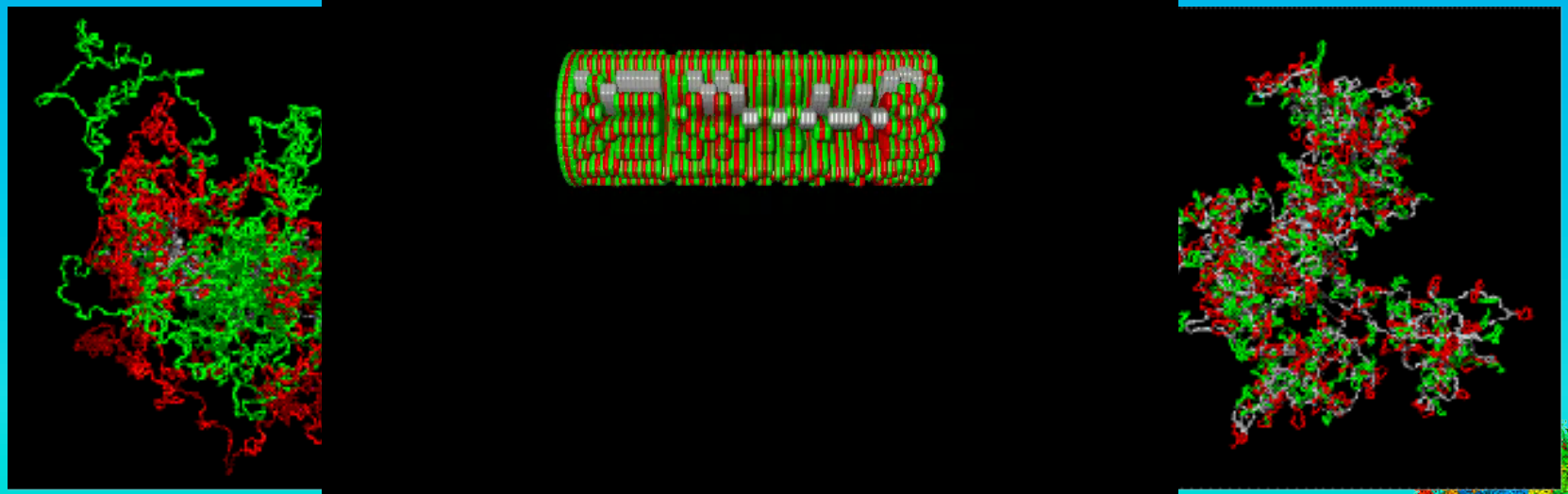
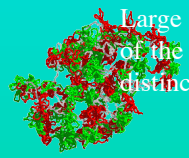
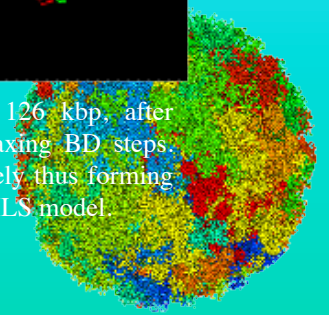


RW/GL model, loop size ~80.000 MC and 1000 relaxing BD steps. Large loops intermingle freely and reach out of the chromsome territory, thus forming no distinct features like in MLS model.

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

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.

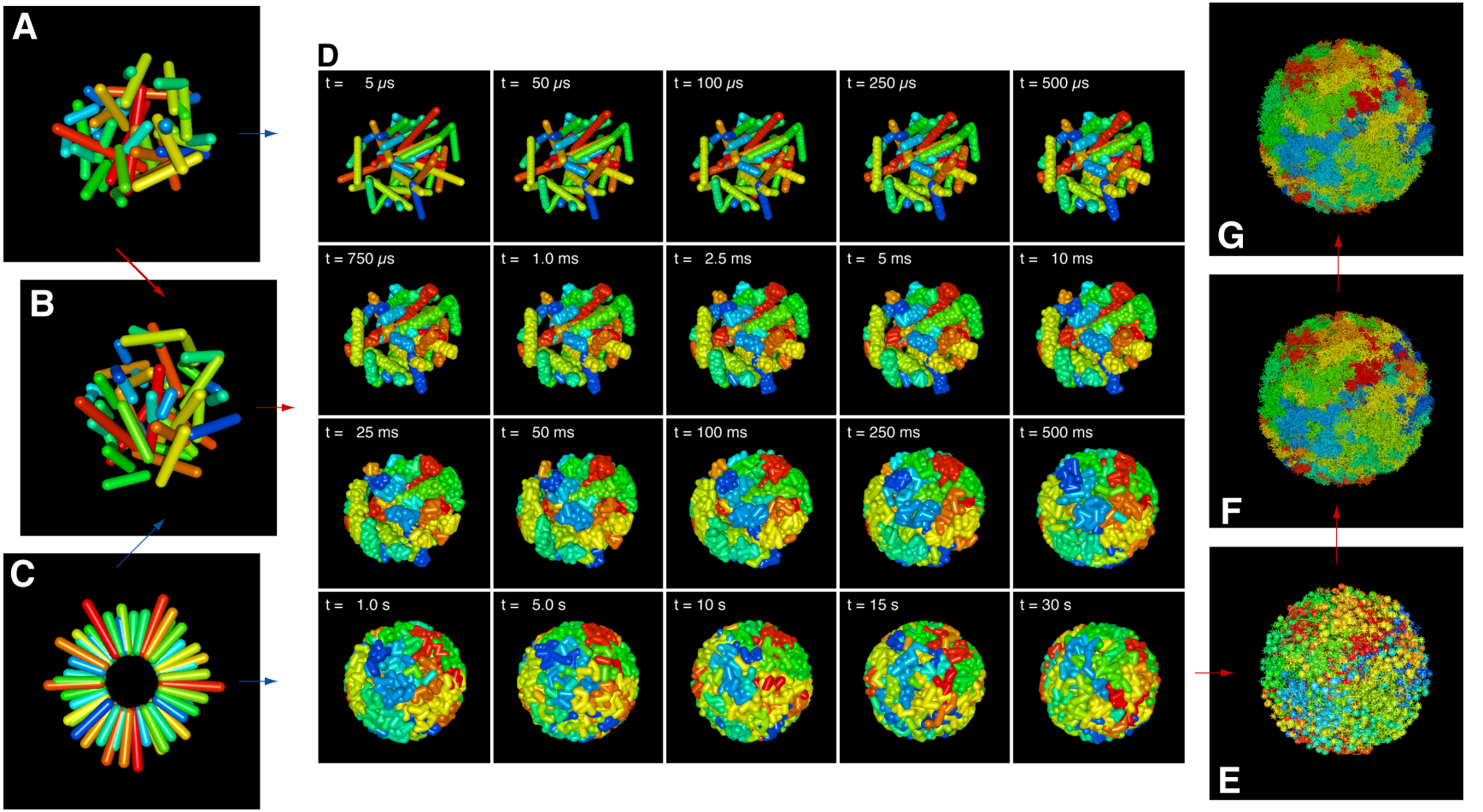




Simulation of Whole Nuclei with all 46 Chromosomes

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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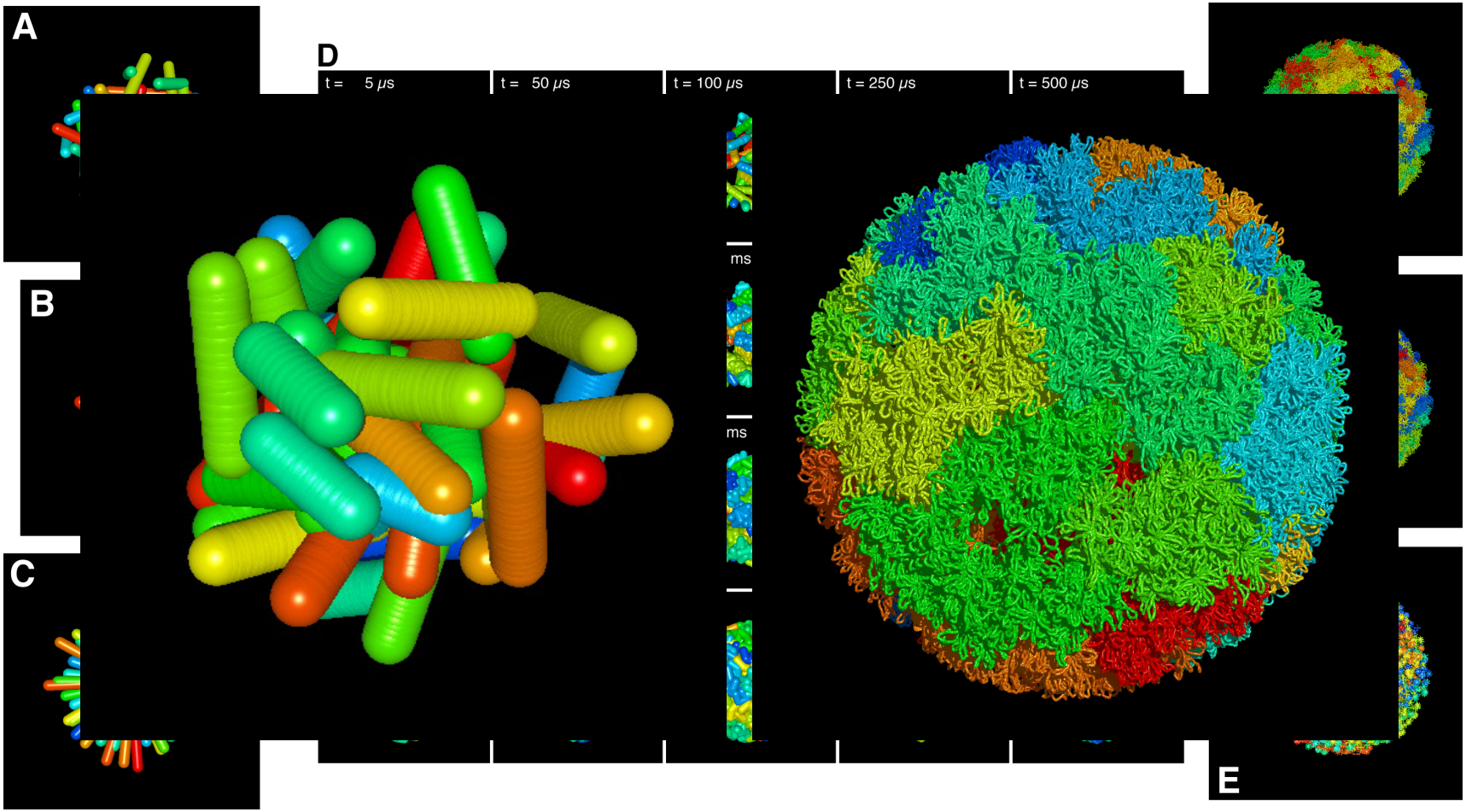

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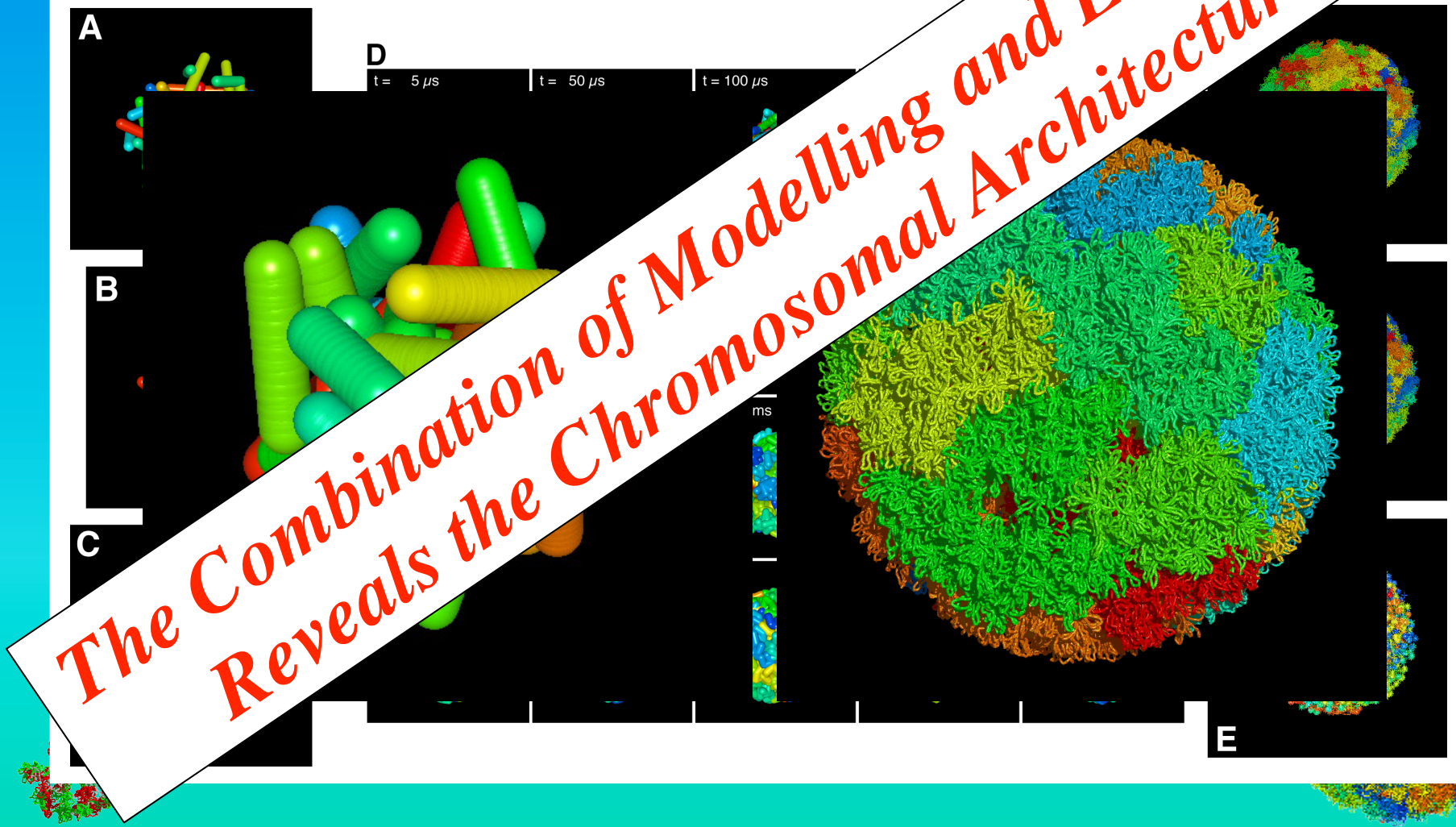
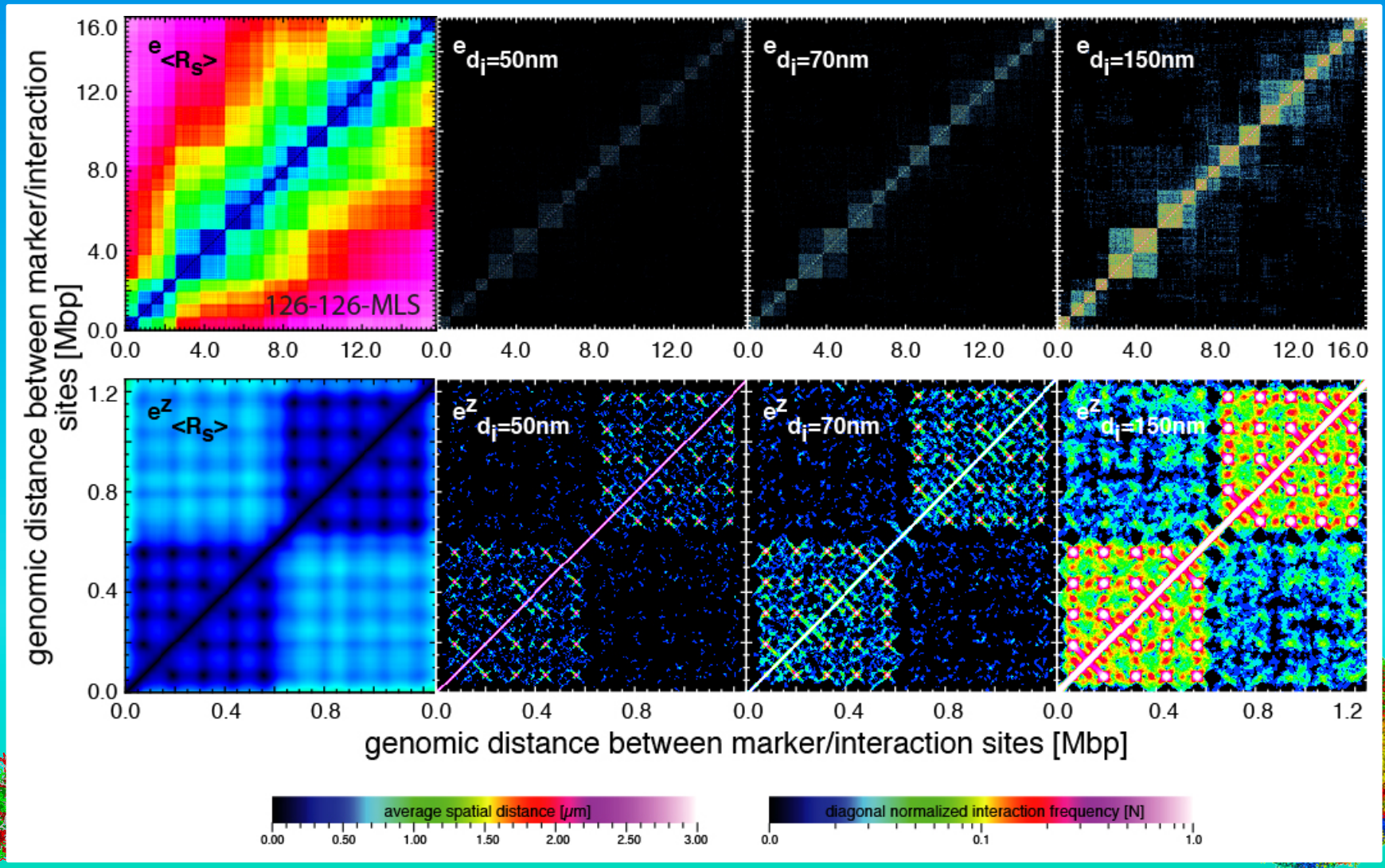


The Combination of Modelling and Experiment Reveals the Chromosomal Architecture !

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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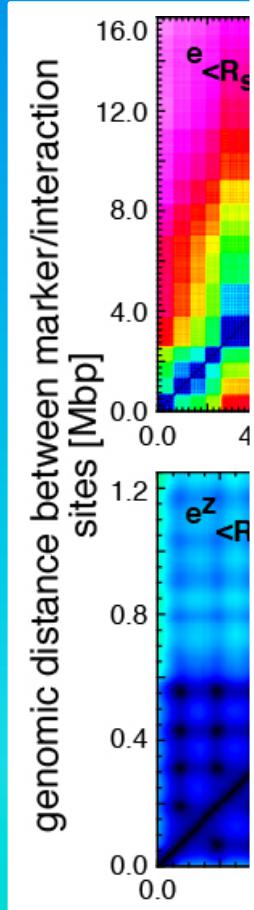
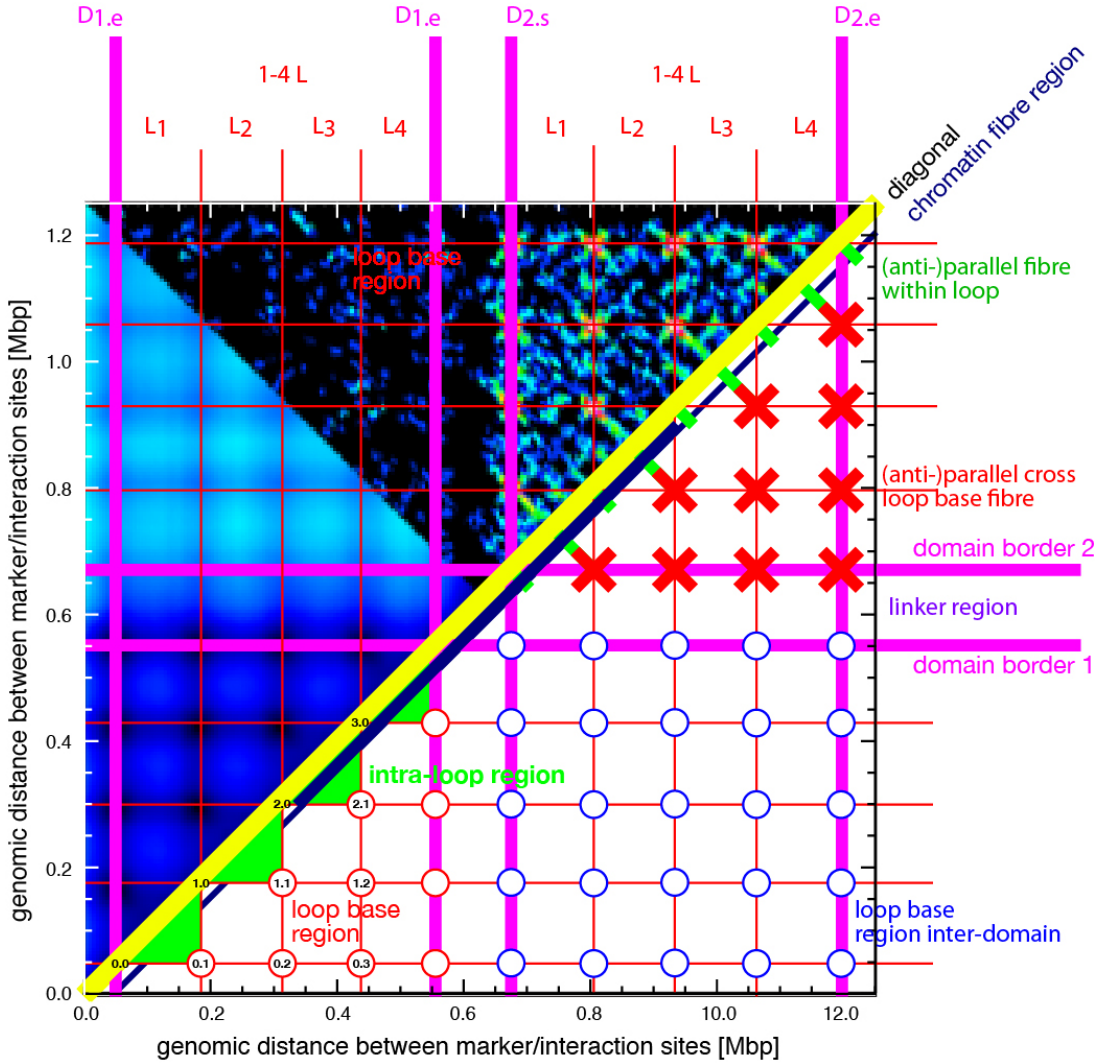
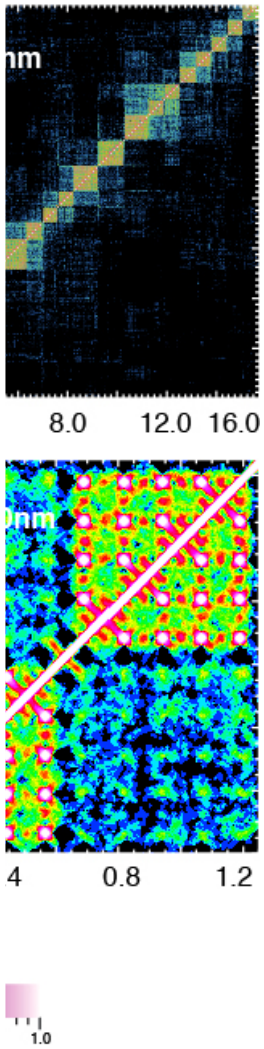
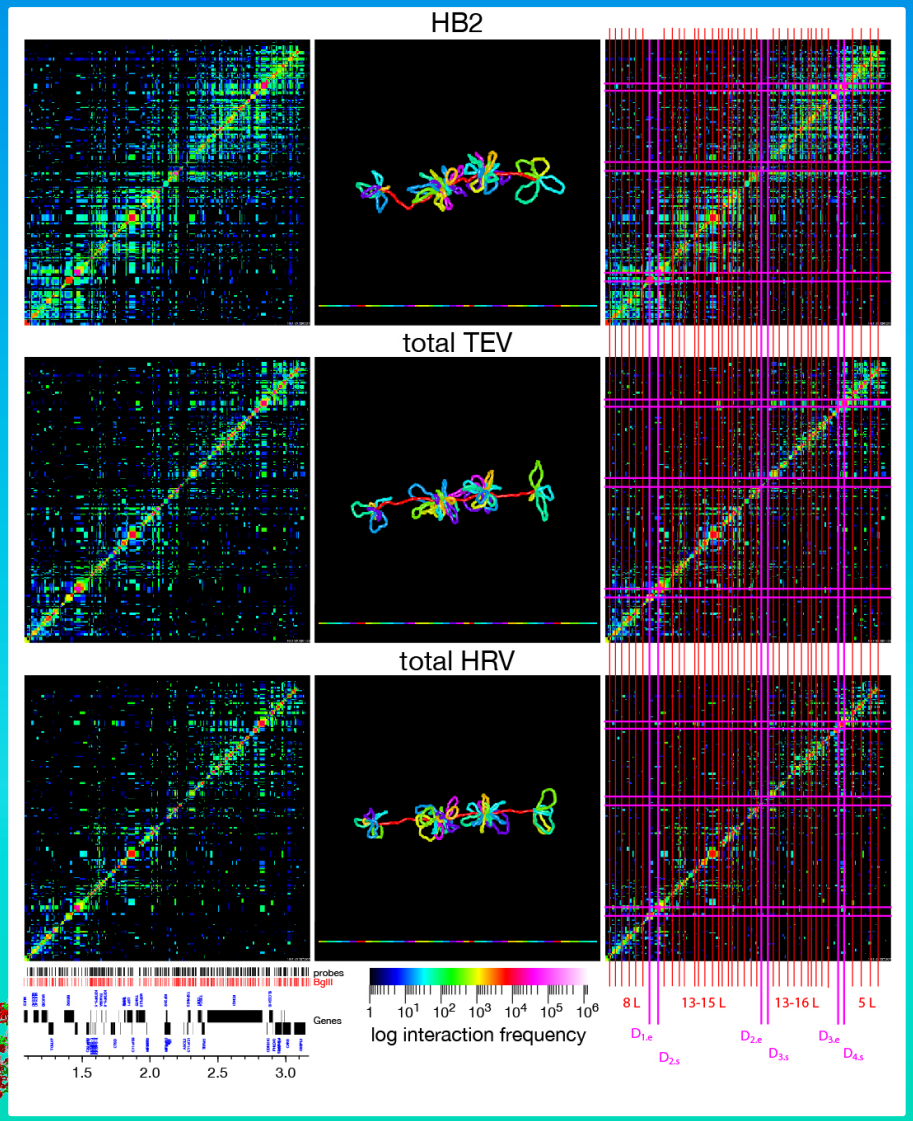


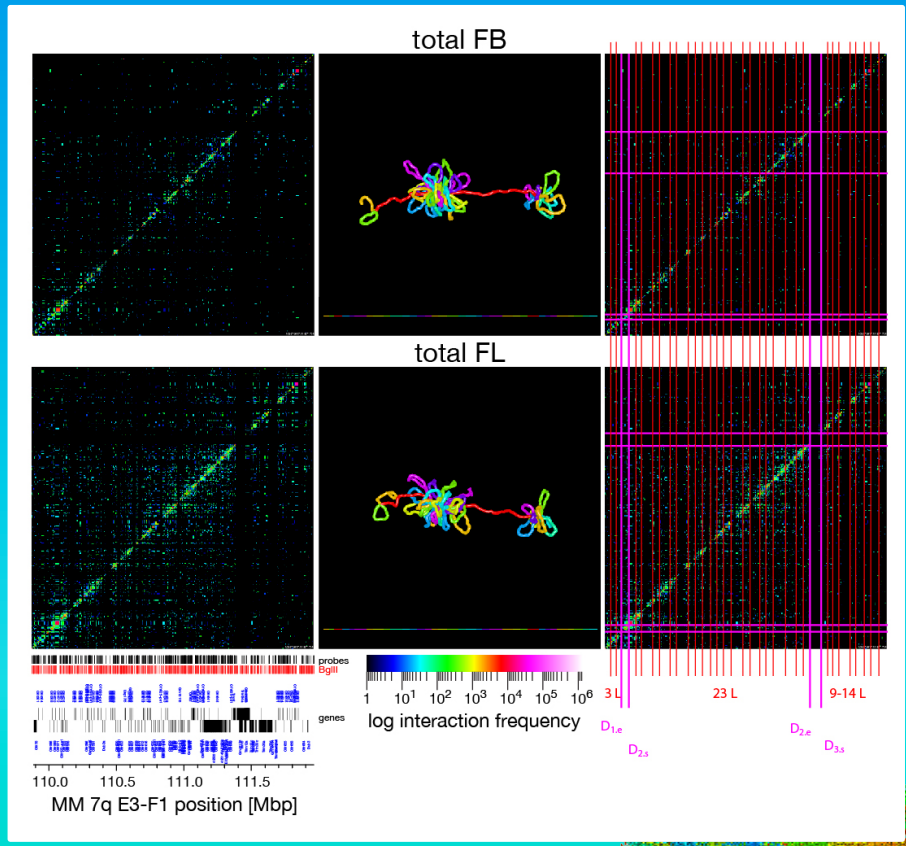




Variation of a Consensus Architecture Scheme

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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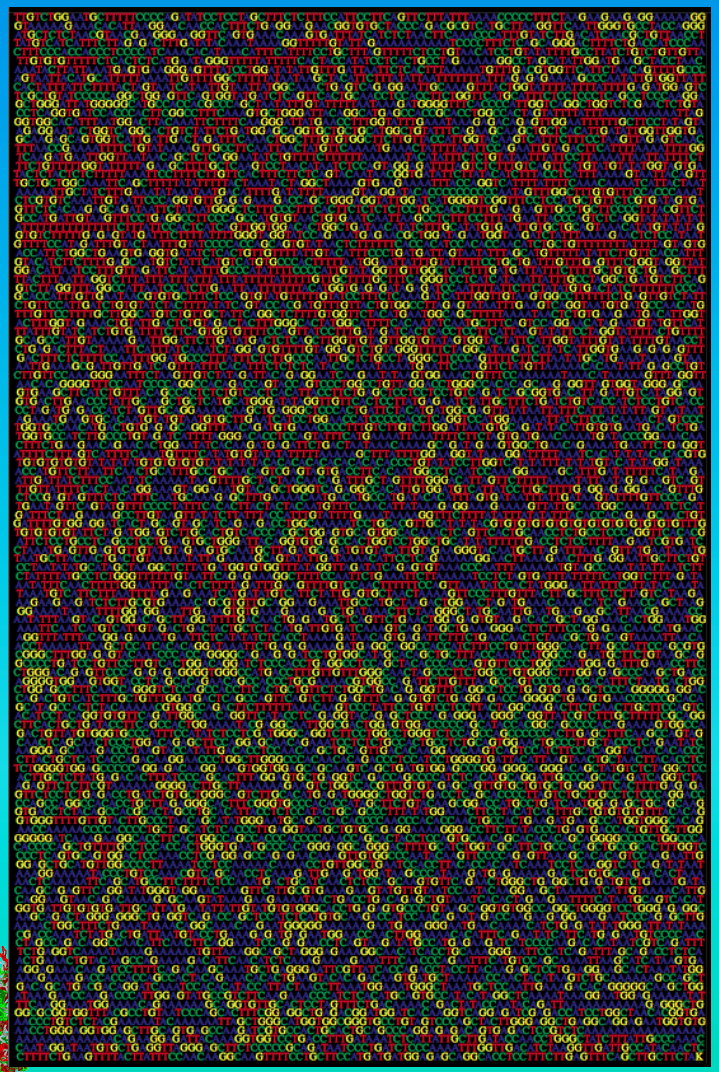
DNA Sequence Organization

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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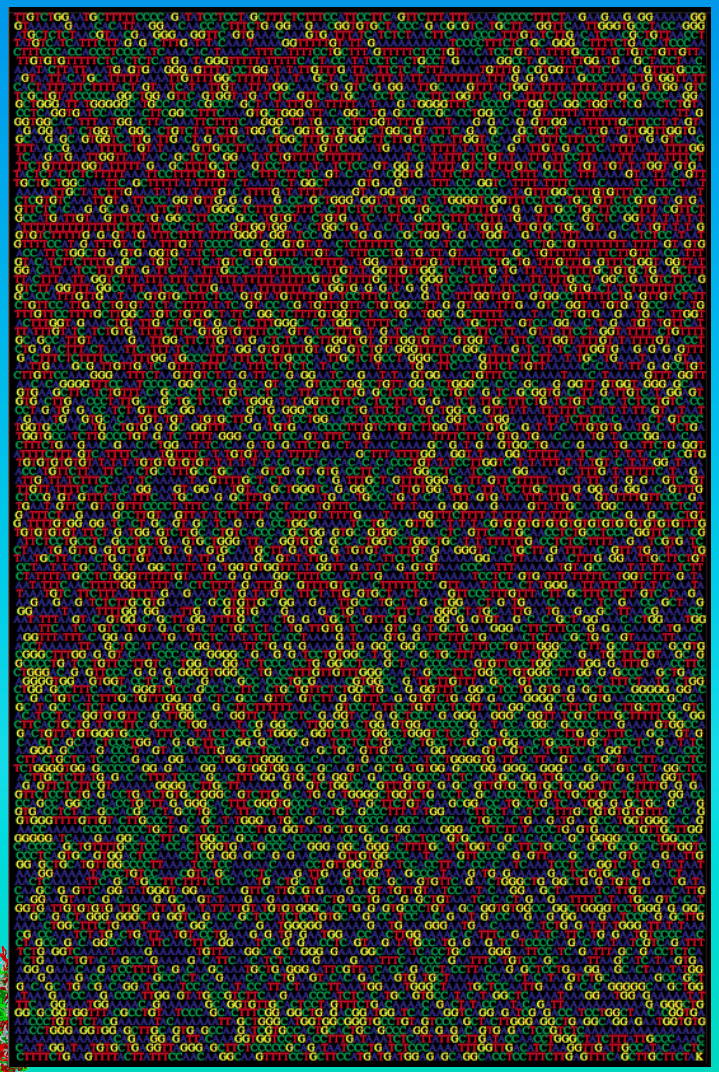

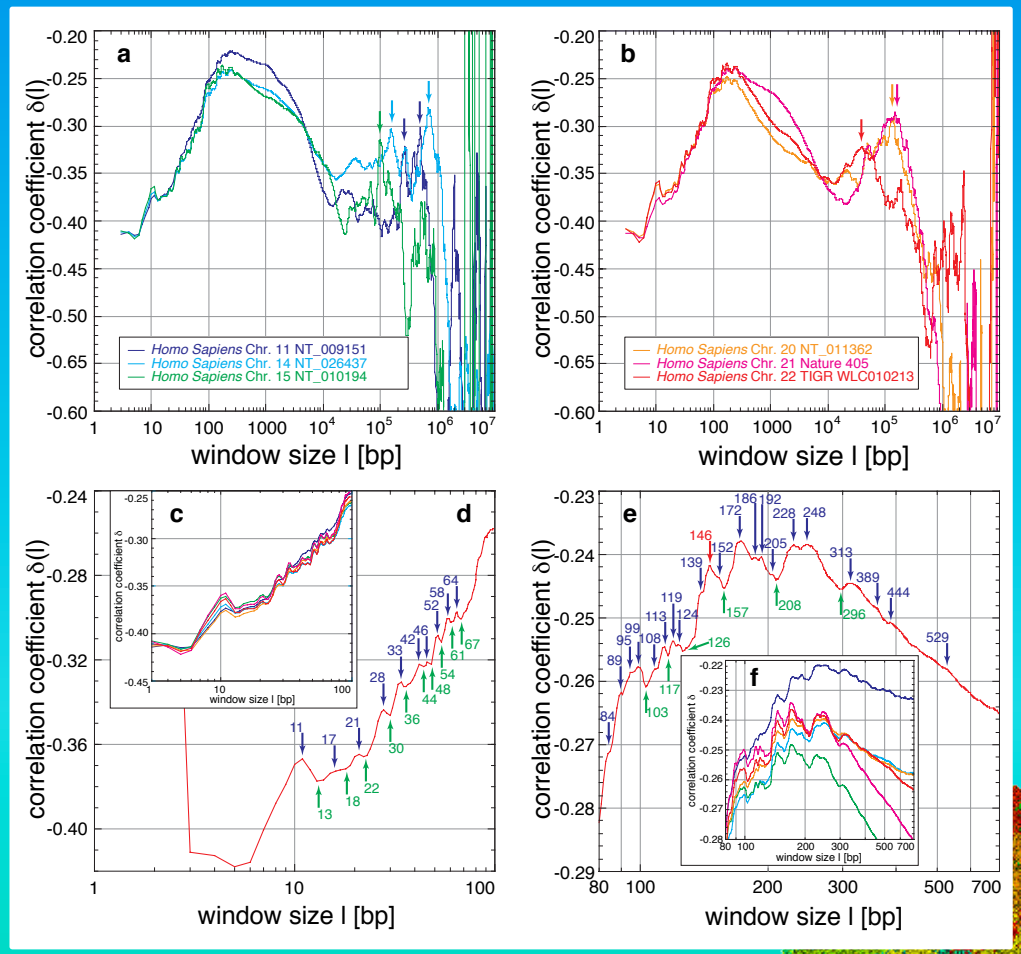

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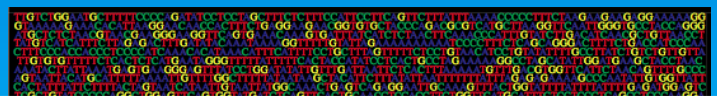

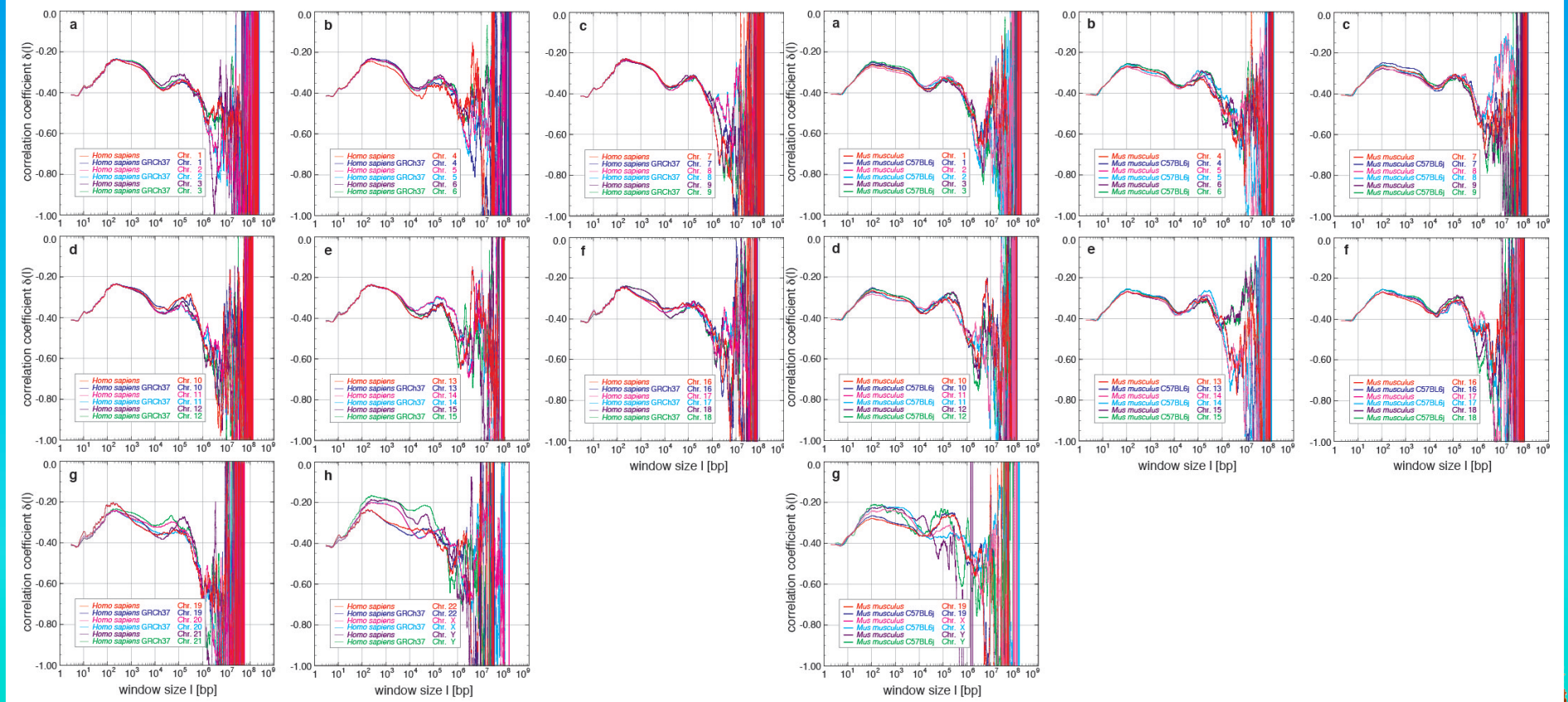

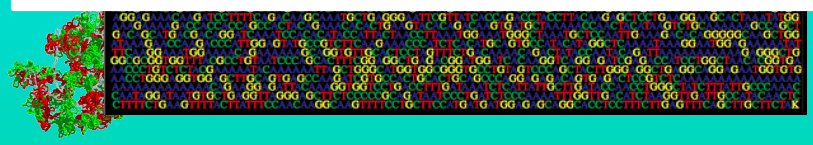

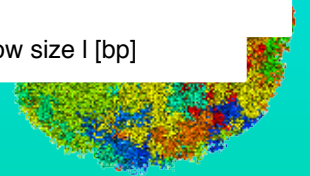

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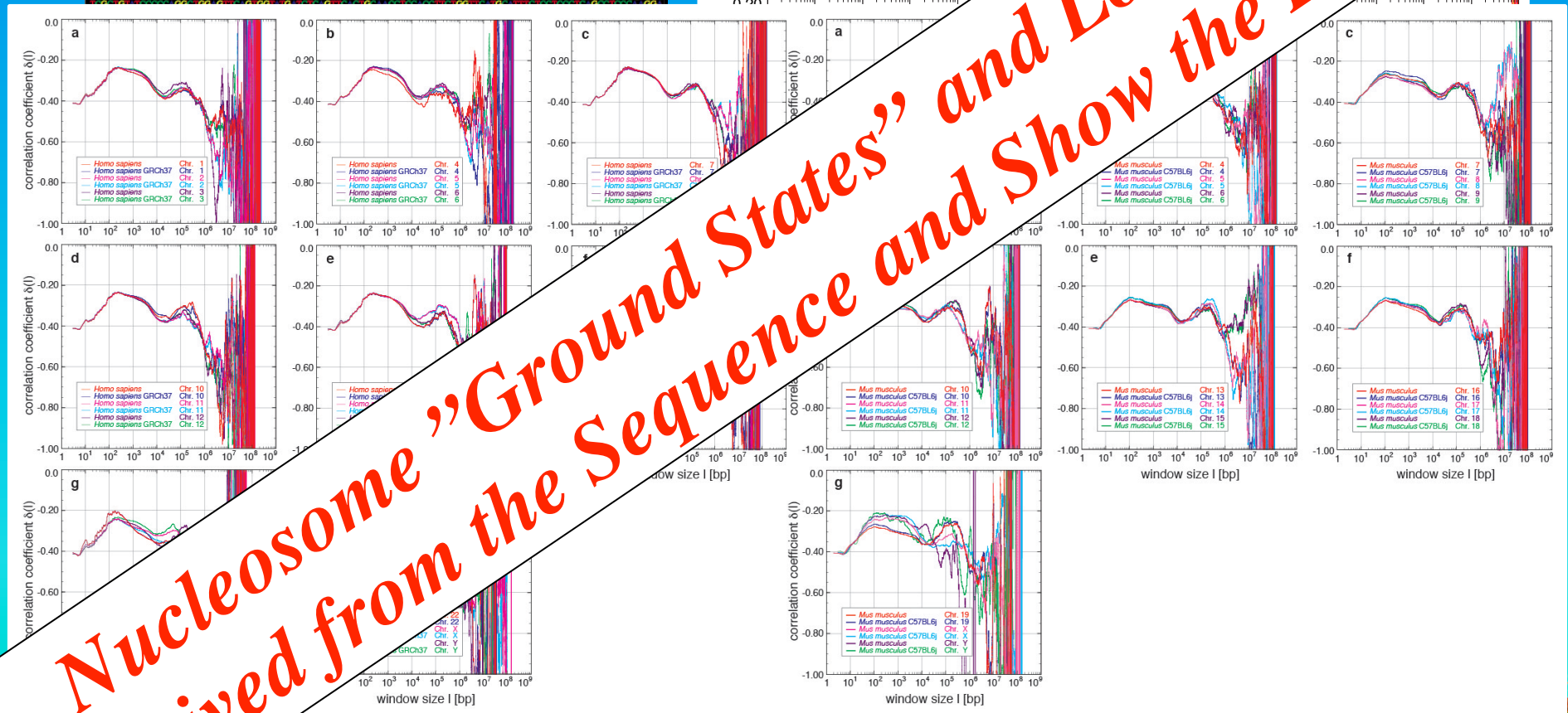
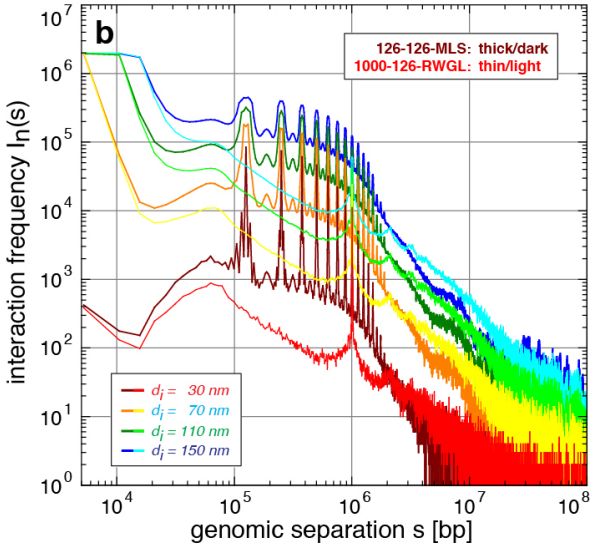
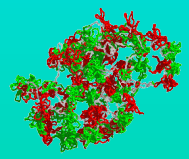


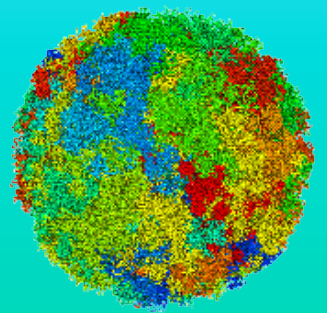
Nucleosome "Ground States" and Loops can be Derived from the Sequence and Show the Holistics!

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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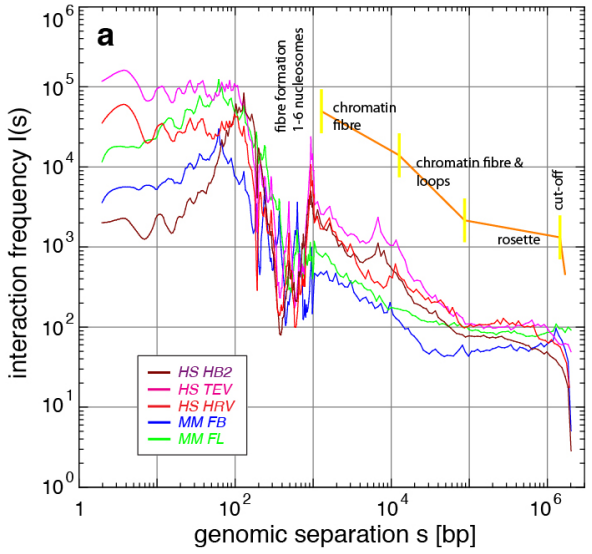

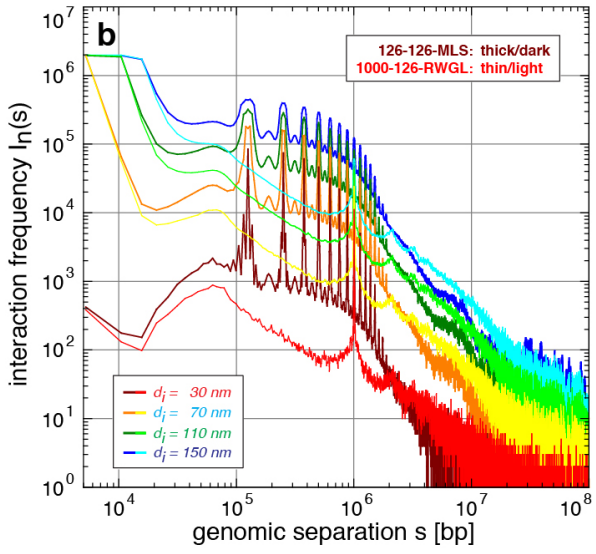

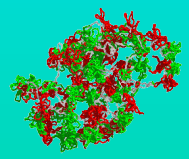

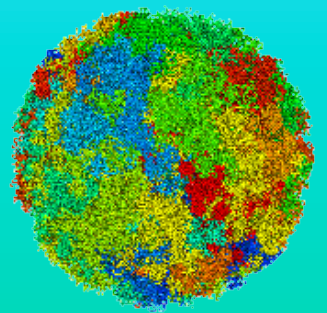

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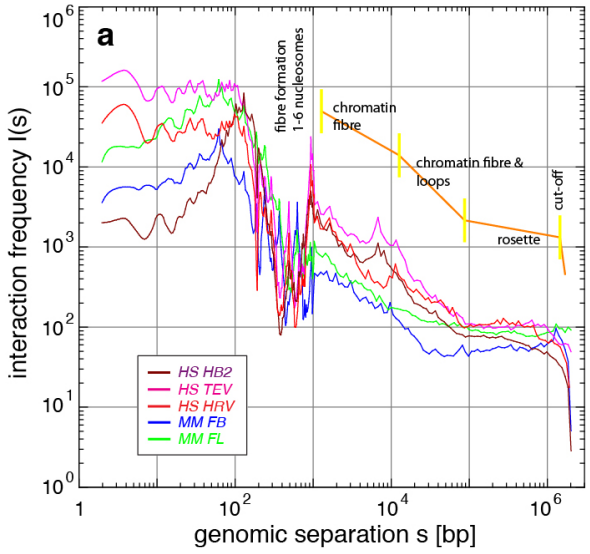

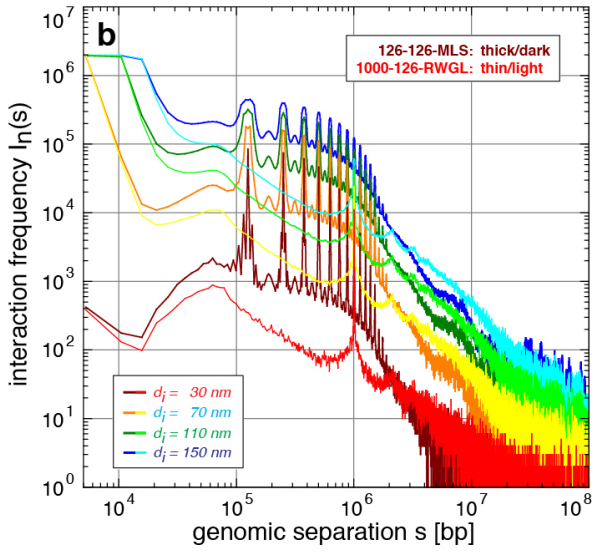

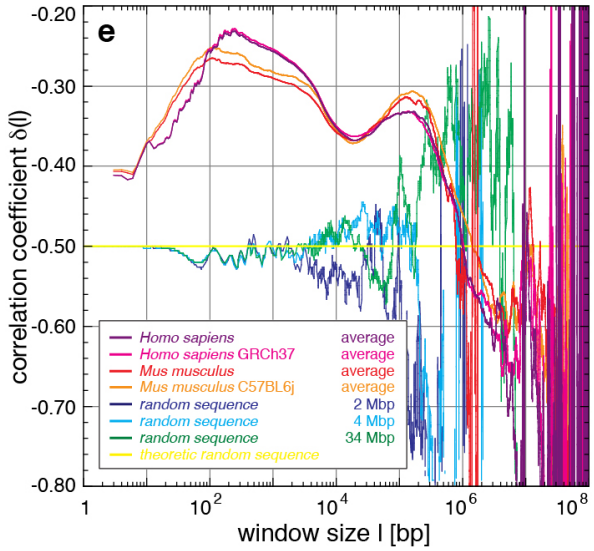

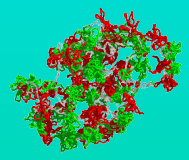

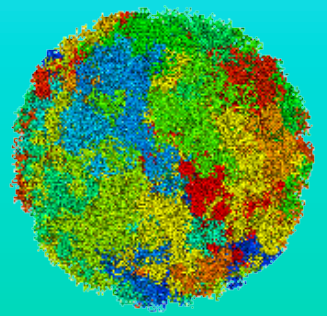

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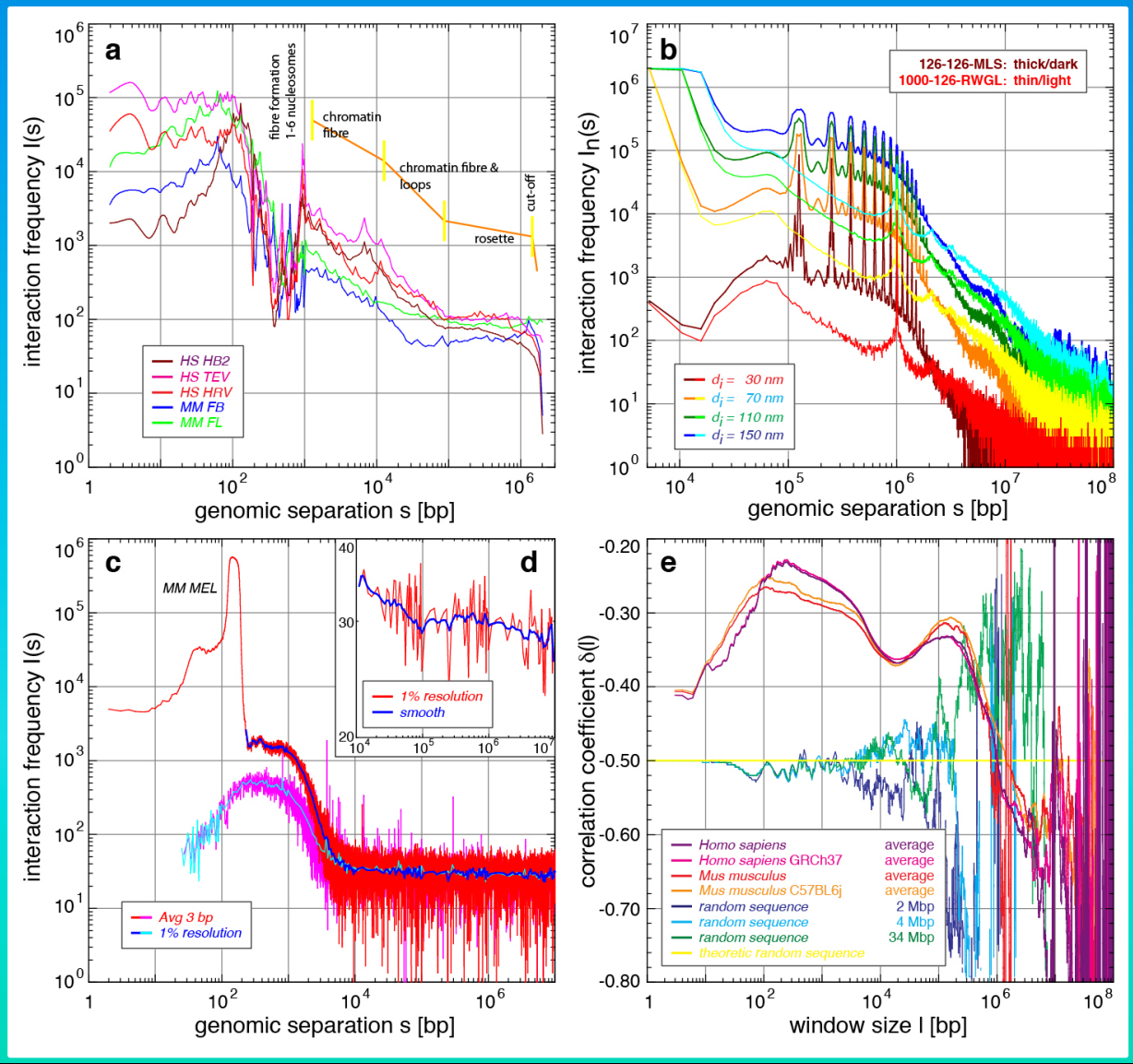

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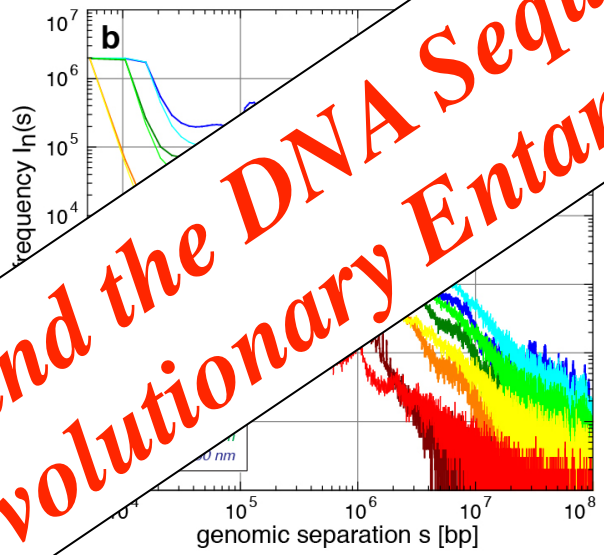
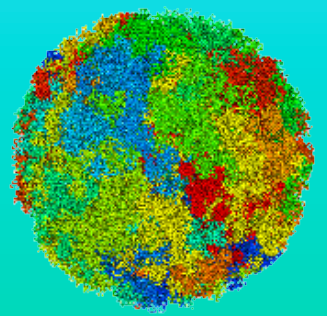


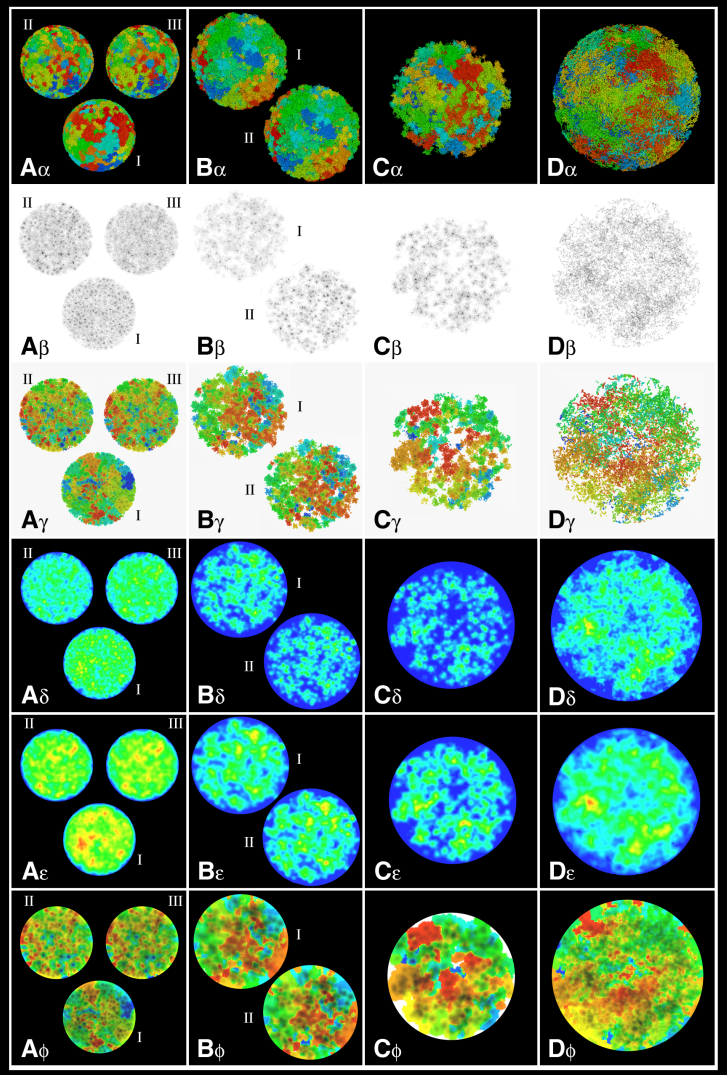
The 3D Architecture and the DNA Sequence Organization are Evolutionary Entangled!



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.

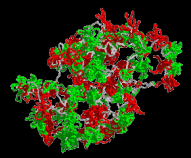


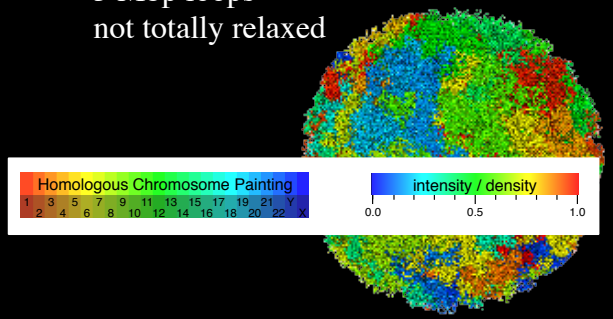
A: MLS in 6 μm nucleus
I: 63 kbp loops, 63 kbp linkers
II: 63 kbp loops, 252 kbp linkers
III: 126 kbp loops, 252 kbp linkers

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

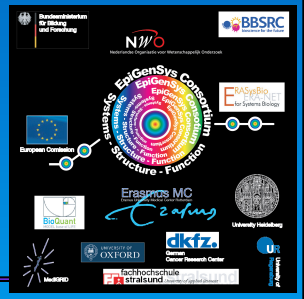




In vivo Morphology & Chromatin Distribution

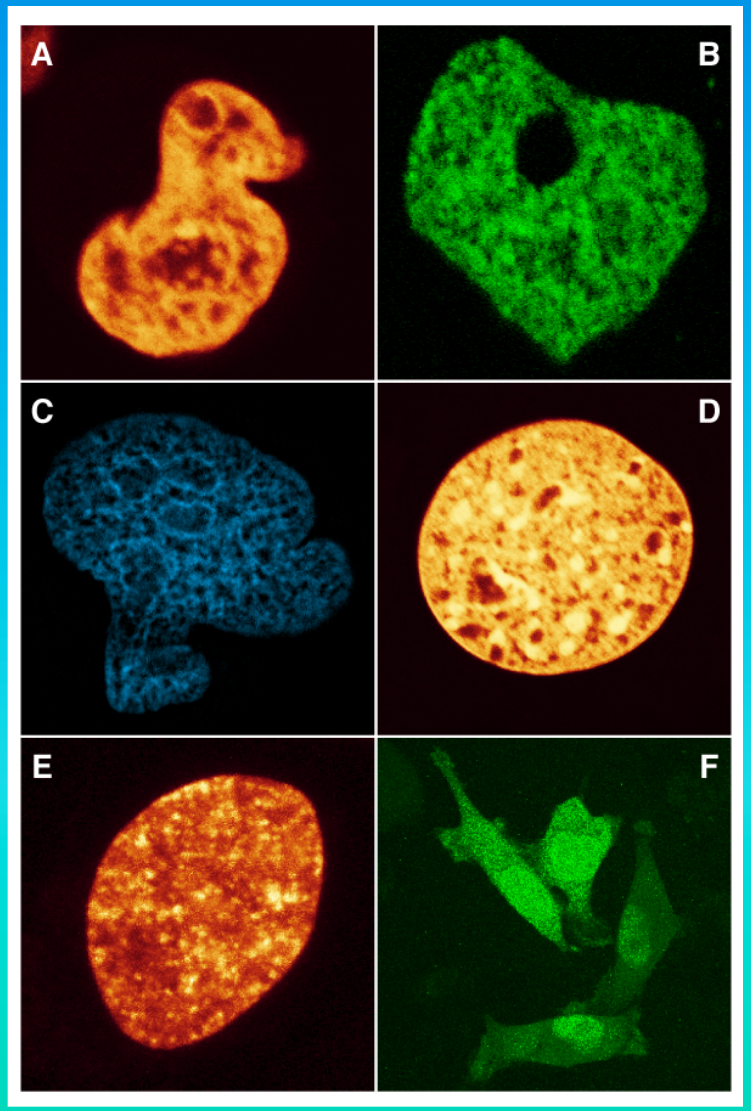
The stable expression of fusions between histones and autofluorescent proteins and the integration into nucleosomes allows the minimal invasive investigation of the structure and dynamics of chromatin.

The clustered morphology in detail favour an MLS like chromatin topology.



HeLa, H2A-YFP

Cos7, H1.0-GFP

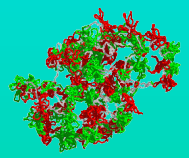


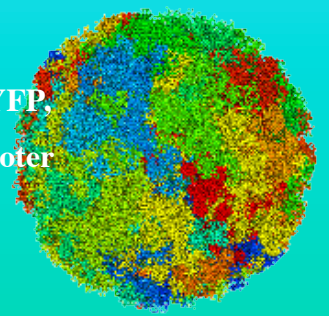
LCLC 103H, H2A-CFP

ID13, H2A-YFP

HeLa, mH2A1.2-YFP

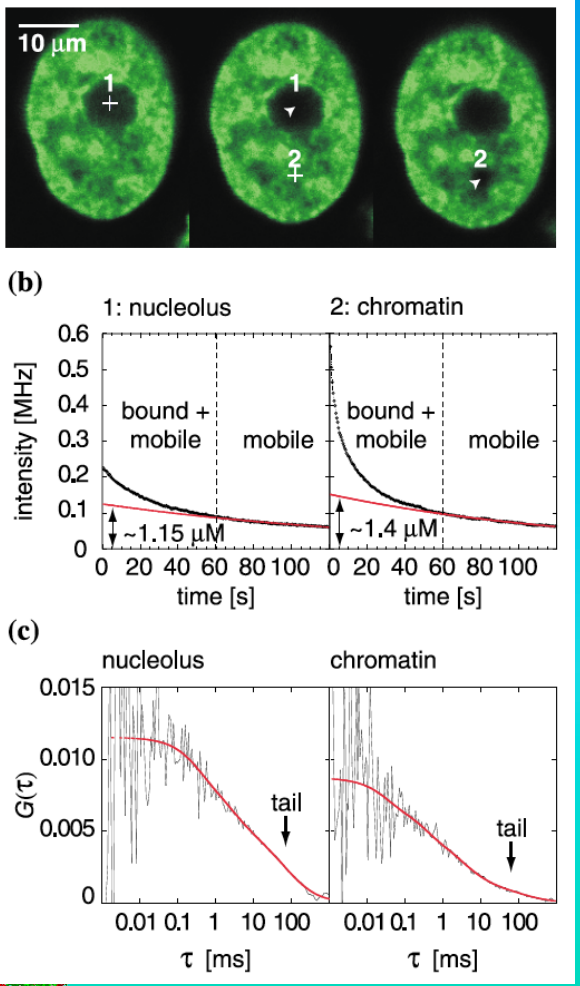
HeLa, H2A-YFP, natural promoter

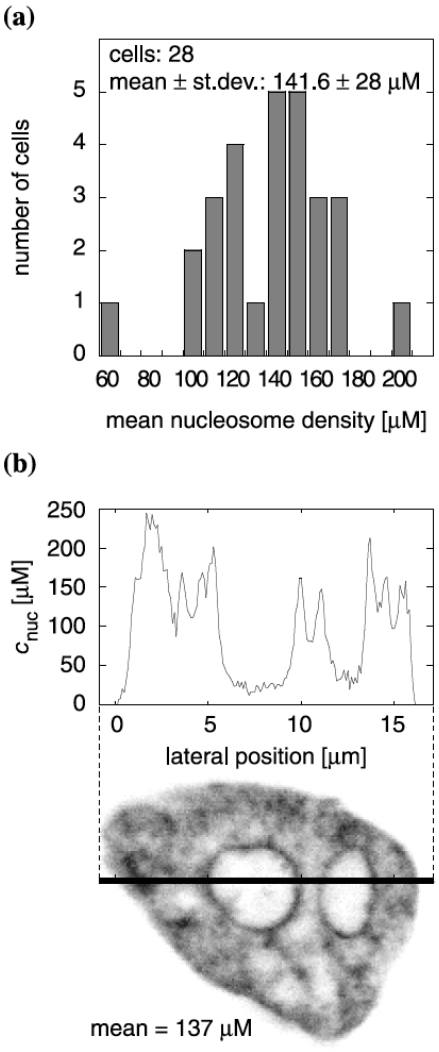


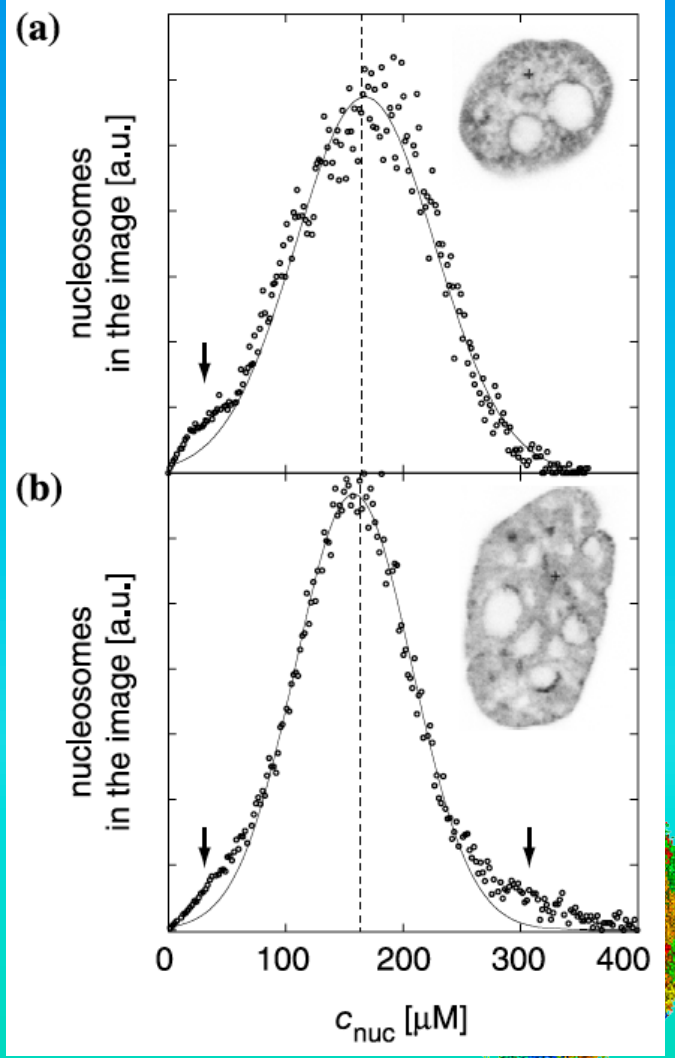


Counting Nucleosomes *In Vivo*

Counting nucleosomes in living cells with a combination of fluorescence correlation spectroscopy (FCS) and confocal laser scanning microscopy (CLSM) 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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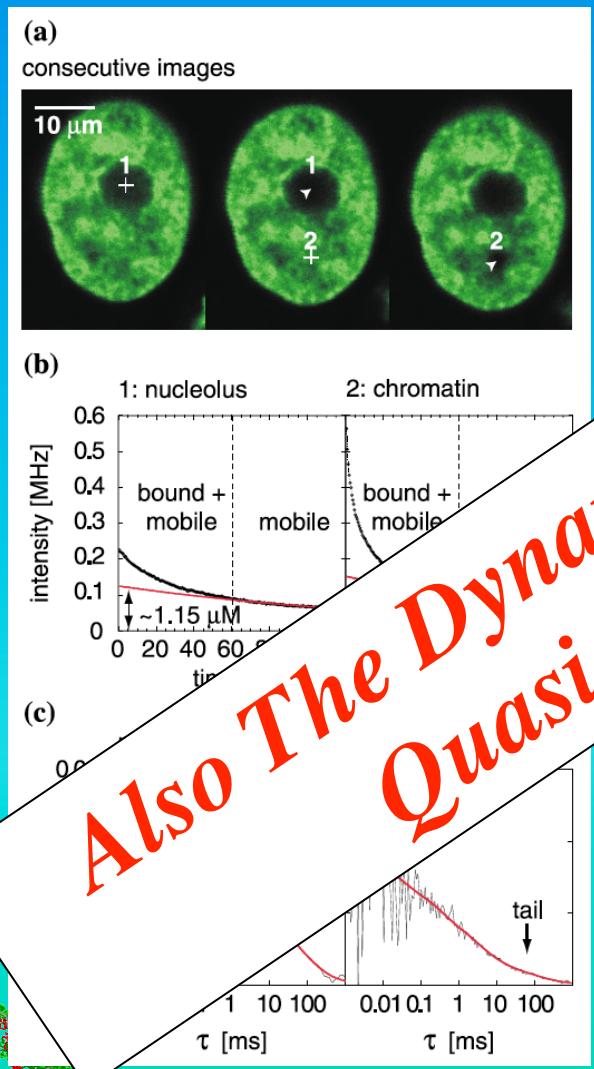
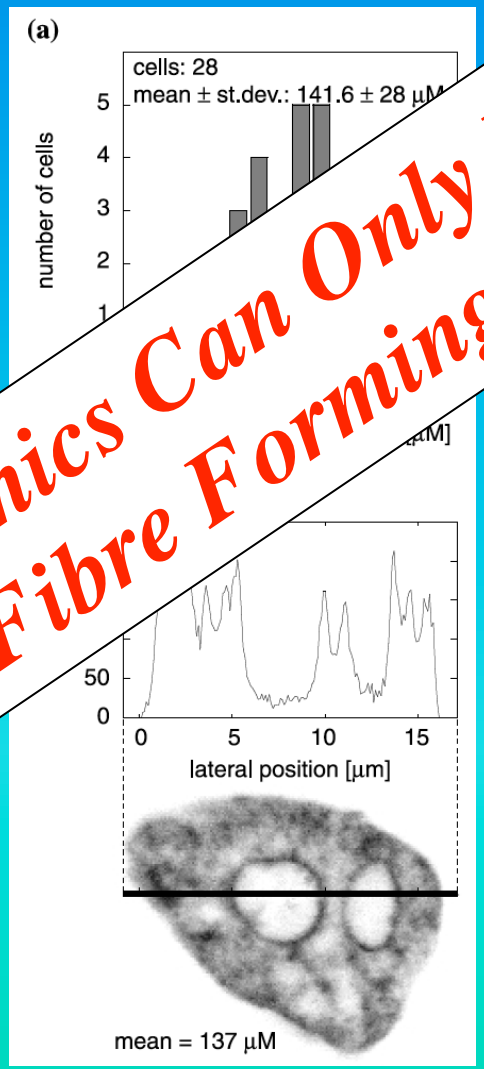
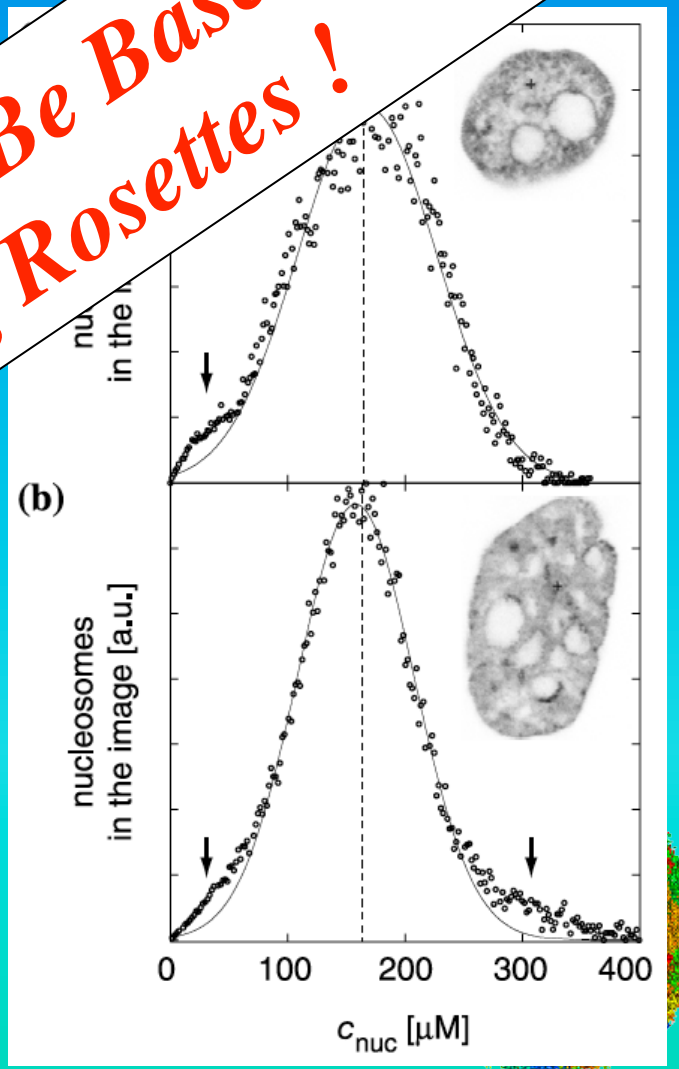






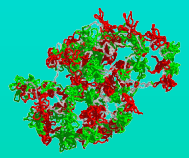
Also The Dynamics Can Only Be Based On A Quasi-Fibre Forming Rosettes !

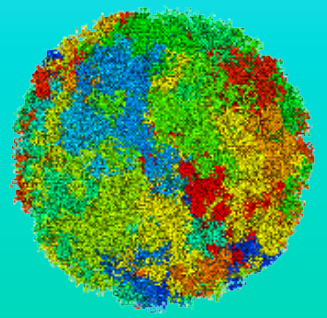
Conclusion

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 !**





Acknowledgements

Thanks go to all the lab local lab members, those people who supported this work in the last decades, the institutions providing their infrastructure, and the national and international computing infrastructures.

Special thanks go to the reviewers, the EraSysBio+ initiative and the national and EU funding bodies.

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

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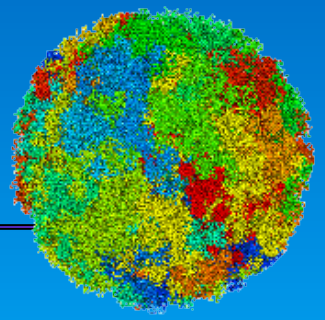

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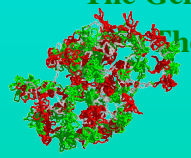

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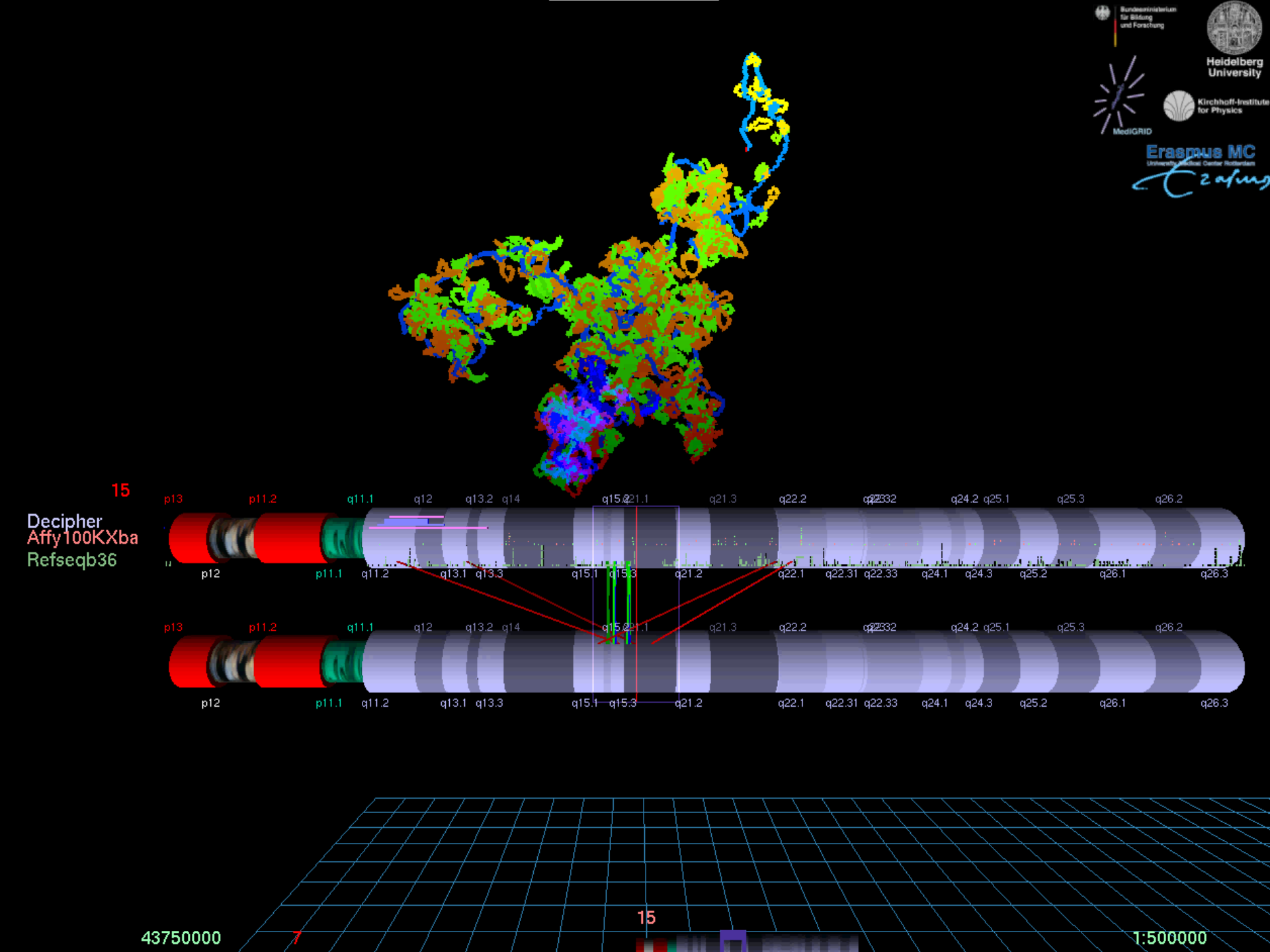
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The Detailed Three-Dimensional Organization of the Human and Mouse Genome

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

12th Dutch Chromatin Meeting, University Library De Uithof Utrecht, Utrecht, The Netherlands, 30th 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 one of the central issues of our time. With a systems genomics combination of a novel superior selective high-throughput high-resolution chromosomal interaction capture (T2C), a novel FCS, polymer models, architectural and DNA sequence scaling analysis, we determined the 3D architecture and dynamics with *molecular resolution from some to the mega-base pair level spanning 6 orders of magnitude (!)*: for several genetic loci, different species, cell type and states we find a chromatin quasi-fibre, folding into loops forming aggregates/rosettes, connected by chromatin linkers. Whereas T2C measures architectural parameters, the FCS approach allows to measure *in* vivo the dynamics of the architecture. Beyond, we find the same fine-structured multi-scaling behaveour in the architecture and the DNA sequence, thus both are tightly evolutionarily entangled. Hence, we determined the three-dimensional organization and dynamics for the first time in a consistent system genomics manner from several angles which are all in agreement as well as additionally also with the heuristics of the research of the last 170 years. This is of fundamental importance for genome understanding, diagnosis, and treatment.

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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.

Literature References

Knoch, T. A. Dreidimensionale Organisation von Chromosomen-Domänen in Simulation und Experiment. (Three-dimensional organization of chromosome domains in simulation and experiment.) *Diploma Thesis*, Faculty for Physics and Astronomy, Ruperto-Carola University, Heidelberg, Germany, 1998, and TAK Press, Tobias A. Knoch, Mannheim, Germany, ISBN 3-00-010685-5 and ISBN 978-3-00-010685-9 (soft cover, 2rd ed.), ISBN 3-00-035857-9 and ISBN 978-3-00-0358857-0 (hard cover, 2rd ed.), ISBN 3-00-035858-7, and ISBN 978-3-00-035858-6 (DVD, 2rd ed.), 1998.

Knoch, T. A., Münkel, C. & Langowski, J. Three-dimensional organization of chromosome territories and the human cell nucleus - about the structure of a self replicating nano fabrication site. *Foresight Institute - Article Archive*, Foresight Institute, Palo Alto, *CA, USA,* http://www. foresight.org, 1- 6, 1998.

Knoch, T. A., Münkel, C. & Langowski, J. Three-Dimensional Organization of Chromosome Territories and the Human Interphase Nucleus. *High Performance Scientific Supercomputing*, editor Wilfried Juling, Scientific Supercomputing Center (SSC) Karlsruhe, University of Karlsruhe (TH), 27- 29, 1999.

Knoch, T. A., Münkel, C. & Langowski, J. Three-dimensional organization of chromosome territories in the human interphase nucleus. *High Performance Computing in Science and Engineering 1999*, editors Krause, E. & Jäger, W., High-Performance Computing Center (HLRS) Stuttgart, University of Stuttgart, Springer Berlin-Heidelberg-New York, ISBN 3-540-66504-8, 229-238, 2000.

Bestvater, F., **Knoch, T. A.**, Langowski, J. & Spiess, E. GFP-Walking: Artificial construct conversions caused by simultaneous cotransfection. *BioTechniques* 32(4), 844-854, 2002.

Gil-Parado, S., Fernández-Montalván, A., Assfalg-Machleidt, I., Popp, O., Bestvater, F., Holloschi, A., **Knoch, T. A.**, Auerswald, E. A., Welsh, K., Reed, J. C., Fritz, H., Fuentes-Prior, P., Spiess, E., Salvesen, G. & Machleidt, W. Ionomycin-activated calpain triggers apoptosis: A probable role for Bcl-2 family members. *J. Biol. Chem.* 277(30), 27217-27226, 2002.

Knoch, T. A. (editor), Backes, M., Baumgärtner, V., Eysel, G., Fehrenbach, H., Göker, M., Hampl, J., Hampl, U., Hartmann, D., Hitzelberger, H., Nambena, J., Rehberg, U., Schmidt, S., Weber, A., & Weidemann, T. Humanökologische Perspectiven Wechsel - Festschrift zu Ehren des 70. Geburtstags von Prof. Dr. Kurt Egger. Human Ecology Working Group, Ruperto-Carola University of Heidelberg, Heidelberg, Germany, 2002.

Knoch, T. A. Approaching the three-dimensional organization of the human genome: structural-, scaling- and dynamic properties in the simulation of interphase chromosomes and cell nuclei, long- range correlations in complete genomes, *in vivo* quantification of the chromatin distribution, construct conversions in simultaneous co-transfections. *Dissertation*, Ruperto-Carola University, Heidelberg, Germany, and TAK†Press, Tobias A. Knoch, Mannheim, Germany, ISBN 3-00-009959-X and ISBN 978-3-00-009959-5 (soft cover, 3rd ed.), ISBN 3-00-009960-3 and ISBN 978-3-00-009960-1 (hard cover, 3rd ed.), ISBN 3-00-035856-9 and ISBN 978-3-00-010685-9 (DVD, 3rd ed.) 2002.

Westphal, G., van den Berg-Stein, S., Braun, K., **Knoch, T. A.**, Dümmerling, M., Langowski, J., Debus, J. & Friedrich, E. Detection of the NGF receptors TrkaA and p75NTR and effect of NGF on the growth characteristics of human tumor cell lines. *J. Exp. Clin. Canc. Res.* 21(2), 255-267, 2002.

Westphal, G., Niederberger, E., Blum, C., Wollman, Y., **Knoch, T. A.**, Dümmerling, M., Rebel, W., Debus, J. & Friedrich, E. Erythropoietin Receptor in Human Tumor Cells: Expression and Aspects Regarding Functionality. *Tumori* 88(2), 150-159, 2002.

Gil-Parado, S., Popp, O., **Knoch, T. A.**, Zahler, S., Bestvater, F., Felgenträger, M., Holoshi, A., Fernández-Montalván, A., Auerswald, E. A., Fritz, H., Fuentes-Prior, P., Machleidt, W. & Spiess, E. Subcellular localization and subunit interactions of over-expressed human μ-calpain. *J. Biol. Chem. 278(18),* 16336-15346, 2003.

Knoch, T. A. Towards a holistic understanding of the human genome by determination and integration of its sequential and three-dimensional organization. *High Performance Computing in Science and Engineering 2003*, editors Krause, E., Jäger, W. & Resch, M., High-Performance Computing Center (HLRS) Stuttgart, University of Stuttgart, Springer Berlin-Heidelberg-New York, ISBN 3- 540-40850-9, 421-440, 2003.

Wachsmuth, M., Weidemann, T., Müller, G., Urs W. Hoffmann-Rohrer, **Knoch, T. A.**, Waldeck, W. & Langowski, J. Analyzing intracellular binding and diffusion with continuous fluorescence photobleaching. *Biophys. J.* 84(5), 3353-3363, 2003.

Weidemann, T., Wachsmuth, M., **Knoch, T. A.**, Müller, G., Waldeck, W. & Langowski, J. Counting nucleosomes in living cells with a combination of fluorescence correlation spectroscopy and confocal imaging. *J. Mol. Biol.* 334(2), 229-240, 2003.

Fejes Tóth, K., **Knoch**, **T. A.**, Wachsmuth, M., Frank-Stöhr, M., Stöhr, M., Bacher, C. P., Müller, G. & Rippe, K. Trichostatin A induced histone acetylation causes decondensation of interphase chromatin. *J. Cell Science* 177, 4277-4287, 2004.

Ermler, S., Krunic, D., **Knoch, T. A.**, Moshir, S., Mai, S., Greulich-Bode, K. M. & Boukamp, P. Cell cycle-dependent 3D distribution of telomeres and telomere repeat-binding factor 2 (TRF2) in HaCaT and HaCaT-myc cells. *Europ. J. Cell Biol.* 83(11-12), 681-690, 2004.

Kost, C., Gama de Oliveira, E., **Knoch, T. A.** & Wirth, R. Spatio-temporal permanence and plasticity of foraging trails in young and mature leaf-cutting ant colonies (*Atta spp.*). *J. Trop. Ecol.* 21(6), 677- 688, 2005.

Winnefeld, M., Grewenig, A., Schnölzer, M., Spring, H., **Knoch, T. A.**, Gan, E. C., Rommelaere, J. & Cziepluch, C. Human SGT interacts with BAG-6/Bat-3/Scythe and cells with reduced levels of either protein display persistence of few misaligned chromosomes and mitotic arrest. *Exp. Cell Res.* 312, 2500-2514, 2006.

Sax, U., Weisbecker, A., Falkner, J., Viezens, F., Yassene, M., Hartung, M., Bart, J., Krefting, D., **Knoch, T. A.** & Semler, S. Grid-basierte Services für die elektronische Patientenakte der Zukunft. *E- HEALTH-COM - Magazin für Gesundheitstelematik und Telemedizin*, 4(2), 61-63, 2007.

de Zeeuw, L. V., **Knoch, T. A.,** van den Berg, J. & Grosveld, F. G. Erasmus Computing Grid - Het bouwen van een 20 TeraFLOP virtuelle supercomputer. *NIOC proceedings 2007 - het perspective of lange termijn*. editor Frederik, H. NIOC, Amsterdam, The Netherlands, 52-59, 2007.

Rauch, J., **Knoch, T. A.** , Solovei, I., Teller, K. Stein, S., Buiting, K., Horsthemke, B., Langowski, J., Cremer, T., Hausmann, M. & Cremer, C. Lightoptical precision measurements of the Prader- Willi/Angelman Syndrome imprinting locus in human cell nuclei indicate maximum condensation changes in the few hundred nanometer range. *Differentiation* 76(1), 66-82, 2008.

Sax, U., Weisbecker, A., Falkner, J., Viezens, F., Mohammed, Y., Hartung, M., Bart, J., Krefting, D., **Knoch, T. A.** & Semler, S. C. Auf dem Weg zur individualisierten Medizin - Grid-basierte Services für die EPA der Zukunft. *Telemedizinführer Deutschland 2008*, editor Jäckel, A. Deutsches Medizinforum, Minerva KG, Darmstadt, ISBN 3-937948-06-6, ISBN-13 9783937948065, 47-51, 2008.

Drägestein, K. A., van Capellen, W. A., van Haren, J. Tsibidis, G. D., Akhmanova, A., **Knoch, T. A.**, Grosveld, F. G. & Galjart, N. Dynamic behavior of GFP-CLIP-170 reveals fast protein turnover on microtubule plus ends. *J. Cell Biol. 180(4)*, 729-737, 2008.

Jhunjhunwala, S., van Zelm, M. C., Peak, M. M., Cutchin, S., Riblet, R., van Dongen, J. J. M., Grosveld, F. G., **Knoch, T. A.**$^{+}$ & Murre, C.$^{+}$ The 3D-structure of the Immunoglobulin Heavy Chain Locus: implications for long-range genomic interactions. *Cell 133(2)*, 265-279, 2008.

Krefting, D., Bart, J., Beronov, K., Dzhimova, O., Falkner, J., Hartung, M., Hoheisel, A., **Knoch, T. A.**, Lingner, T., Mohammed, Y., Peter, K., Rahm, E., Sax, U., Sommerfeld, D., Steinke, T., Tolxdorff, T., Vossberg, M., Viezens, F. & Weisbecker, A. MediGRID - Towards a user friendly secured grid infrastructure. *Future Generation Computer Systems 25(3)*, 326-336, 2008.

Knoch, T. A., Lesnussa, M., Kepper, F. N., Eussen, H. B., & Grosveld, F. G. The GLOBE 3D Genome Platform - Towards a novel system-biological paper tool to integrate the huge complexity of genome organization and function. *Stud. Health. Technol. Inform. 147*, 105-116, 2009.

Knoch, T. A., Baumgärtner, V., de Zeeuw, L. V., Grosveld, F. G., & Egger, K. e-Human Grid Ecology: Understanding and approaching the Inverse Tragedy of the Commons in the e-Grid Society. *Stud. Health. Technol. Inform. 147*, 269-276, 2009.

Dickmann, F., Kaspar, M., Löhnardt, B., **Knoch, T. A.,** & Sax, U. Perspectives of MediGRID. *Stud. Health. Technol. Inform. 147*, 173-182, 2009.

Knoch, T. A., Göcker, M., Lohner, R., Abuseiris, A. & Grosveld, F. G. Fine-structured multi-scaling long-range correlations in completely sequenced genomes - features, origin and classification. *Eur. Biophys. J. 38(6)*, 757-779, 2009.

Dickmann, F., Kaspar, M., Löhnhardt, B., Kepper, N., Viezens, F., Hertel, F., Lesnussa, M., Mohammed, Y., Thiel, A., Steinke, T., Bernarding, J., Krefting, D., **Knoch, T. A.** & Sax, U. Visualization in health-grid environments: a novel service and business approach. *LNCS 5745*, 150-159, 2009.

Dickmann, F., Kaspar, M., Löhnhardt, B., Kepper, N., Viezens, F., Hertel, F., Lesnussa, M., Mohammed, Y., Thiel, A., Steinke, T., Bernarding, J., Krefting, D., **Knoch, T. A.** & Sax, U. Visualization in health-grid environments: a novel service and business approach. *Grid economics and business models - GECON 2009 Proceedings, 6th international workshop, Delft, The Netherlands.* editors Altmann, J., Buyya, R. & Rana, O. F., GECON 2009, LNCS 5745, Springer-Verlag Berlin Heidelberg, ISBN 978-3-642-03863-1, 150-159, 2009.

Estrada, K.[*], Abuseiris, A.[*], Grosveld, F. G., Uitterlinden, A. G., **Knoch, T. A.**[+] & Rivadeneira, F.[+] GRIMP: A web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. *Bioinformatics 25(20), 2750-2752*, 2009.

de Wit, T., Dekker, S., Maas, A., Breedveld, G., **Knoch, T. A.,** Langeveld, A., Szumska, D., Craig, R., Bhattacharya, S., Grosveld, F. G.[+] & Drabek, D. Tagged mutagenesis of efficient minos based germ line transposition. *Mol. Cell Biol 30(1)*, 66-77, 2010.

Kepper, N., Schmitt, E., Lesnussa, M., Weiland, Y., Eussen, H. B., Grosveld, F. G., Hausmann, M. & **Knoch T. A.,** Visualization, Analysis, and Design of COMBO-FISH Probes in the Grid-Based GLOBE 3D Genome Platform. *Stud. Health Technol. Inform. 159*, 171-180, 2010.

Kepper, N., Ettig, R., Dickmann, F., Stehr, R., Grosveld, F. G., Wedemann, G. & **Knoch, T. A.** Parallel high-performance grid computing: capabilities and opportunities of a novel demanding service and business class allowing highest resource efficiency. *Stud. Health Technol. Inform. 159*, 264-271, 2010.

Skrowny, D., Dickmann, F., Löhnhardt, B., **Knoch, T. A.** & Sax, U. Development of an information platform for new grid users in the biomedical field. *Stud. Health Technol. Inform. 159*, 277-282, 2010.

Knoch, T. A., Baumgärtner, V., Grosveld, F. G. & Egger, K. Approaching the internalization challenge of grid technologies into e-Society by e-Human "Grid" Ecology. *Economics of Grids, Clouds, Systems, and Services – GECON 2010 Proceedings*, 7th International Workshop, Ischia, Italy, editors Altman, J., & Rana, O. F., Lecture Notes in Computer Science (LNCS) 6296, Springer Berlin Heidelberg New York, ISSN 0302-9743, ISBN-10 3-642-15680-0, ISBN-13 978-3-642-15680-9, 116-128, 2010.

Dickmann, F., Brodhun, M., Falkner, J., **Knoch, T. A.** & Sax, U. Technology transfer of dynamic IT outsourcing requires security measures in SLAs. *Economics of Grids, Clouds, Systems, and Services – GECON 2010 Proceedings*, 7th International Workshop, Ischia, Italy, editors Altman, J., & Rana, O. F., Lecture Notes in Computer Science (LNCS) 6296, Springer Berlin Heidelberg New York, ISSN 0302-9743, ISBN-10 3-642-15680-0, ISBN-13 978-3-642-15680-9, 1-115, 2010.

Knoch, T. A. Sustained Renewability: approached by systems theory and human ecology. *Renewable Energy 2*, editors M. Nayeripour & M. Keshti, Intech, ISBN 978-953-307-573-0, 21-48, 2011.

Kolovos, P., **Knoch, T. A.**, F. G. Grosveld, P. R. Cook, & Papantonis, A. Enhancers and silencers: an integrated and simple model for their function. *Epigenetics and Chromatin 5(1)*, 1-8, 2012.

Dickmann, F., Falkner, J., Gunia, W., Hampe, J., Hausmann, M., Herrmann, A., Kepper, N., **Knoch, T. A.**, Lauterbach, S., Lippert, J., Peter, K., Schmitt, E., Schwardmann, U., Solodenko, J., Sommerfeld, D., Steinke, T., Weisbecker, A. & Sax, U. Solutions for Biomedical Grid Computing - Case Studies from the D-Grid Project Services@MediGRID. *JOCS 3(5)*, 280-297, 2012.

Estrada, K., Abuseiris, A., Grosveld, F. G., Uitterlinden, A. G., **Knoch, T. A.** & Rivadeneira, F. GRIMP: A web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. *Dissection of the complex genetic architecture of human stature and osteoporosis.* cummulative

dissertation, editor Estrada K., Erasmus Medical Center, Erasmus University Rotterdam, Rotterdam, The Netherlands, ISBN 978-94-6169-246-7, 25-30, 1st June 2012.

van de Corput, M. P. C., de Boer, E., **Knoch, T. A.**, van Cappellen, W. A., Quintanilla, A., Ferrand, L., & Grosveld, F. G. Super-resolution imaging reveals 3D folding dynamics of the β-globin locus upon gene activation. *J. Cell Sci. 125 (Pt 19)*, 4630-4639, 2012.

da Silva, P. S. D., Delgado Bieber, A. G., Leal, I. R., **Knoch, T. A.**, Tabarelli, M., Leal, I. R., & Wirth, R. Foraging in highly dynamic environments: leaf-cutting ants adjust foraging trail networks to pioneer plant availability. *Entomologia Experimentalis et Applicata 147*, 110-119. 2013.

Zuin, J., Dixon, J. R., van der Reijden, M. I. J. A., Ye, Z., Kolovos, P., Brouwer, R. W. W., van de Corput, M. P. C., van de Werken, H. J. G., **Knoch, T. A.**, van IJcken, W. F. J., Grosveld, F. G., Ren, B. & Wendt, K. S. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. *PNAS 111(3)*, 9906-1001, 2014.

Kolovos, P., Kepper, N., van den Werken, H. J. G., Lesnussa, M., Zuin, J., Brouwer, R. W. W., Kockx, C. E. M., van IJcken, W. F. J., Grosveld, F. G. & **Knoch, T. A.** Targeted Chromatin Capture (T2C): A novel high resolution high throughput method to detect genomic interactions and regulatory elements. *Epigenetics & Chromatin 7:10*, 1-17, 2014.

Diermeier, S., Kolovos, P., Heizinger, L., Schwartz, U., Georgomanolis, T., Zirkel, A., Wedemann, G., Grosveld, F. G., **Knoch, T. A.**, Merkl, R., Cook, P. R., Längst, G. & Papantonis, A. TNFα signalling primes chromatin for NF-kB binding and induces rapid and widespread nucleosome repostioning. *Genome Biology 15(12)*, 536-548, 2014.

Knoch, T. A., Wachsmuth, M., Kepper, N., Lesnussa, M., Abuseiris, A., A. M. Ali Imam, Kolovos, P., Zuin, J., Kockx, C. E. M., Brouwer, R. W. W., van de Werken, H. J. G., van IJcken, W. F. J., Wendt, K. S. & Grosveld, F. G. The detailed 3D multi-loop aggregate/rosette chromatin architecture and functional dynamic organization of the human and mouse genomes. *bioXriv preprint*, 16.07.2016.

Kolovos, P., Georgomanolis, T., Koeferle, A., Larkin, J. D., Brant, J., Nikolić, M., Gusmao, E. G., Zirkel, A., **Knoch, T. A.**, van IJcken, W. F. J., Cook, P. R., Costa, I. G., Grosveld, F. G. & Papantonis, A. Binding of nuclear kappa-B to non-canonical consensus sites reveals its multimodal role during the early inflammatory response. *Genome Research* 26(11), 1478-1489, 2016.

Wachsmuth, M., **Knoch, T. A.** & Rippe, K. Dynamic properties of independent chromatin domains measured by correlation spectroscopy in living cells. *Epigenetics & Chromatin 9:57*, 1-20, 2016.

Knoch, T. A., Wachsmuth, M., Kepper, N., Lesnussa, M., Abuseiris, A., A. M. Ali Imam, Kolovos, P., Zuin, J., Kockx, C. E. M., Brouwer, R. W. W., van de Werken, H. J. G., van IJcken, W. F. J., Wendt, K. S. & Grosveld, F. G. The detailed 3D multi-loop aggregate/rosette chromatin architecture and functional dynamic organization of the human and mouse genomes. *Epigenetics & Chromatin 9:58*, 1-22, 2016.