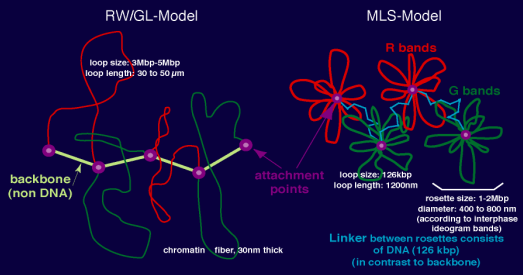
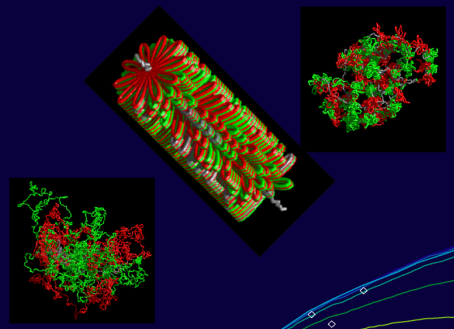
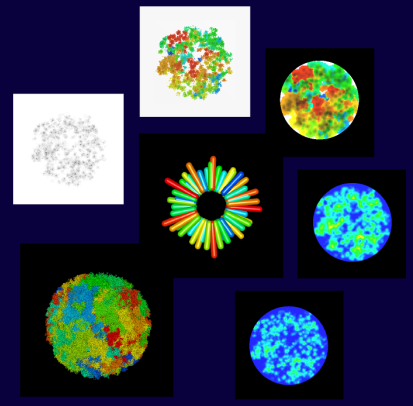
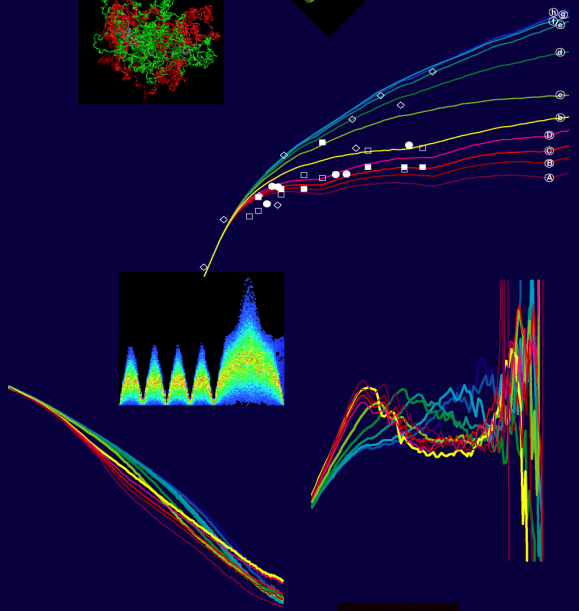
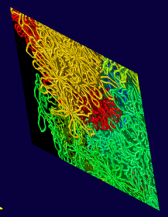
Structural-, Scaling and Dynamic-Properties in the Simulation of Interphase Chromosomes and Cell Nuclei



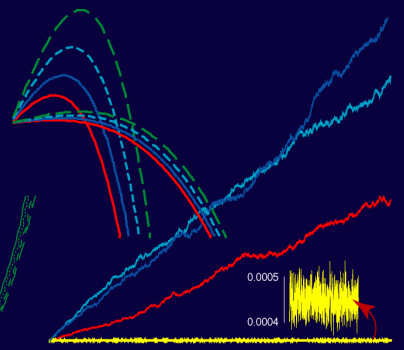


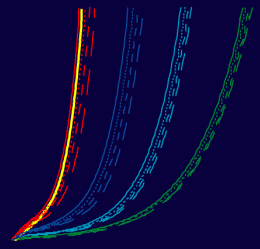




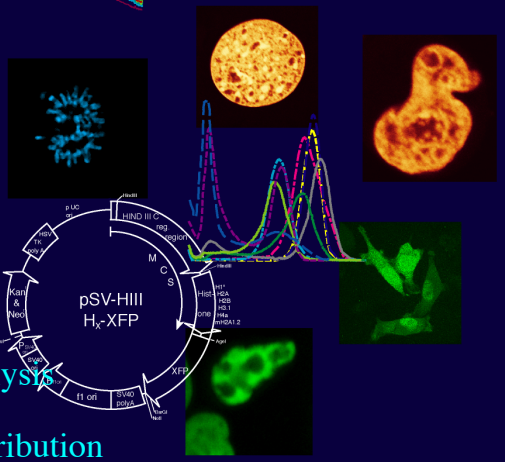


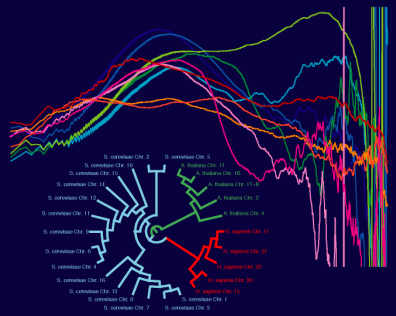
Approaching the Three-Dimensional Organization of the Human Genome

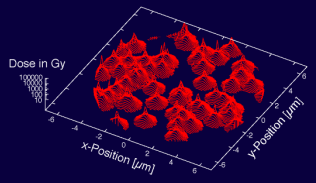


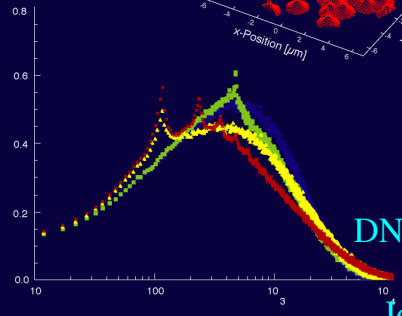


Tobias A. Knoch









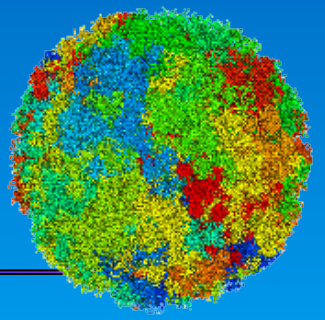
In Vivo Analysis of the Chromatin Distribution

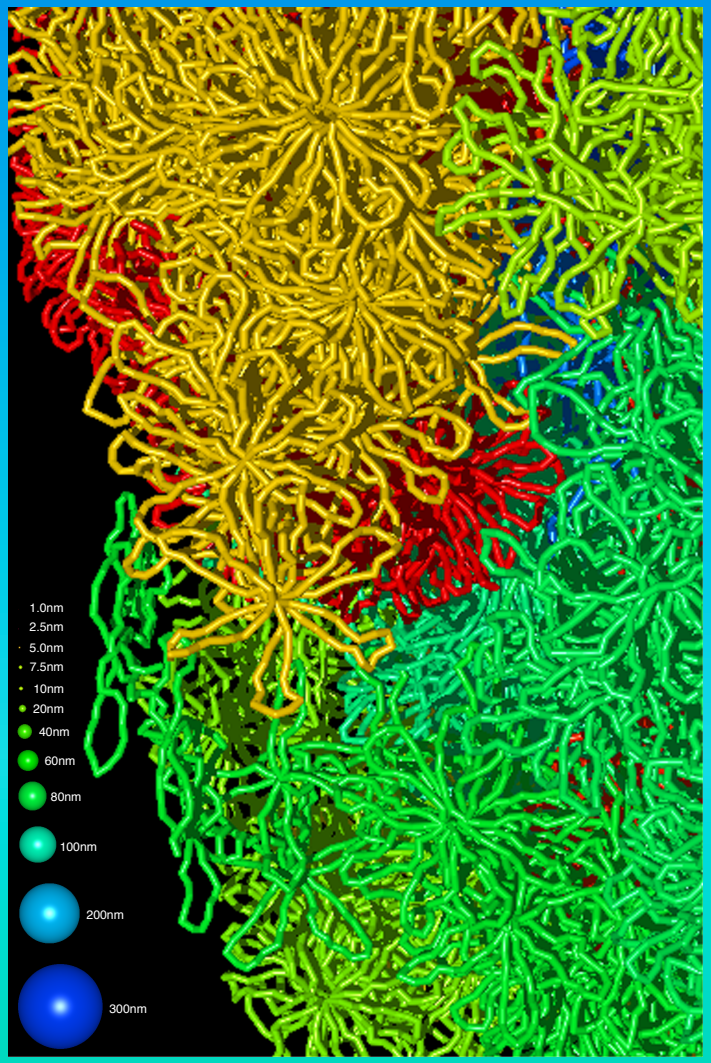
DNA Fragmentation by Ion - Irradiation

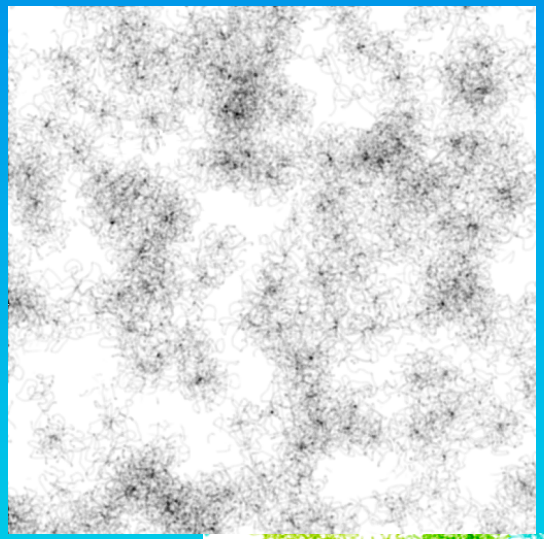
Long-Range Correlations in Completely Sequenced Genomes

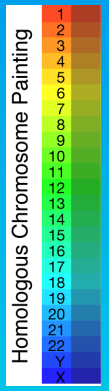
Fine Morphology of Nuclei

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.

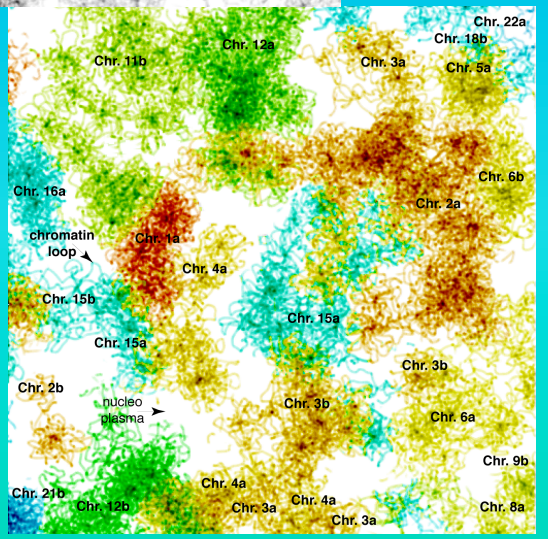


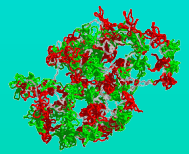




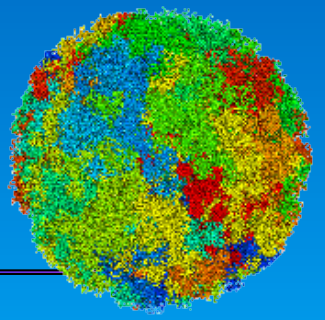


MLS models model with 126 kbp loops and linkers in a 10 μm nucleus.





Acknowledgements



Biophysics of Macromolecules DKFZ

Thomas Weidemann

Gabriele Müller

Waldemar Waldeck

Jörg Langowski

Molecular Biophysics KIP

Katalin Fejes-Toth

Malte Wachsmuth

Karsten Rippe

Biomedical Structure Analysis DKFZ

Felix Bestvater

Eberhard Spiess

The Cremer Labs

Joachim Rauch

Irina Solovei

Michael Hausmann

Christoph Cremer

Thomas Cremer

Molecular Genetics DKFZ

Karsten Richter

Peter Lichter

University Tübingen

Markus Göker

LMU Munich

Peter Quicken

Anna Friedl

Supercomputin Center Karlsruhe

Rudolph Lohner

Scripps Research Institute

Karin Monier

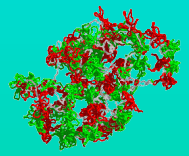
Kevin Sullivan

Others from the DKFZ:
Monika Stöhr, Michael Stöhr, Andreas Hunziker, Angel Alonso

High-Performance Computing Center Stuttgart, University of Stuttgart; Supercomputing Center Karlsruhe, University of Karlsruhe; Computing Center, Deutsches Krebsforschungszentrum Heidelberg (DKFZ)

Bundesministerium für Forschung und Technology (BMFT) 01 KW 9602/2
(3D-Human Genome Study Group Heidelberg, German Human Genome Projekt)

Deutsches Krebsforschungszentrum (DKFZ)



Approaching the Three-Dimensional Organization and Dynamics

of the

Human Genome

Knoch, T. A.

Short Poster Lecture, 5. Nachwuchswissenschaftler-Tagung, Deutsche Gesellschaft für Zellbiologie, Jena, Germany, 25th - 27th October, 2003.

Abstract

Genomes are one of the major foundations of life due to their role in information storage, process regulation and evolution. However, the sequential and three-dimensional structure of the human genome in the cell nucleus as well as its interplay with and embedding into the cell and organism only arise scarcely. To achieve a deeper unterstanding of the human genome the three-dimensional organization of the human cell nucleus, the structural-, scaling- and dynamic properties of interphase chromosomes and cell nuclei were simulated and combined with the analysis of long-range correlations in completely sequenced genomes as well as the chromatin distribution *in vivo*. Using Monte Carlo and Brownian Dynamics methods, the 30 nm chromatin fiber was simulated according to the Multi-Loop-Subcompartment (MLS) model, in which ~100 kbp loops form rosettes, connected by a linker, and the Random-Walk/Giant-Loop (RW/GL) topology, in which 1-5 Mbp loops are attached to a flexible backbone. Both the MLS and the RW/GL model form chromosome territories but only the MLS rosettes result in distinct subcompartments visible with light microscopy and low overlap of chromosomes, -arms and subcompartments. The MLS morphology, the size of subcompartments and chromatin density distribution of simulated confocal (CLSM) images agree with the expression of fusionproteins from the histones H1, H2A, H2B, H3, H4 and mH2A1.2 with the autofluorescent proteins CFP, GFP, YFP, DsRed-1 and DsRed-2 which also revealed different interphase morphologies for different cell lines. Even small changes of the model parameters induced significant rearrangements of the chromatin morphology. Thus, pathological diagnoses, are closely related to structural changes on the chromatin level. The position of interphase chromosomes depends on their metaphase location, and suggests a possible origin of current experimental findings. The scaling behaviour of the chromatin fiber topology and morphology of CLSM stacks revealed fine-structured multi-scaling behaviour in agreement with the model prediction and correlations in the DNA sequence. Review and comparison of experimental to simulated spatial distance measurements between genomic markers as function of their genomic separation also favour an MLS model with loop and linker sizes of 63 to 126 kbp. Simulated and experimental DNA fragment distribution after ion-irradiation revealed also best agreement with such an MLS. Correlation analyses of completely sequenced Archaea, Bacteria and Eukarya chromosomes revealed fine-structured positive long-range correlation due to codon, nucleosomal or block organization of the genomes, allowing classification as well as tree construction. This shows a complex sequential organization of genomes closely connected to their three-dimensional organization. Visual inspection of the morphology reveals also big spaces between the chromatin fiber allowing high accessibility to nearly every spatial location, due to the chromatin occupancy <30% and a mean mesh spacing of 29 to 82 nm for nuclei of 6 to 12 µm diameter. This agrees with a simulated displacement of 10 nm sized particles of ~1 to 2 µm takes place within 10 ms, i. e. a moderately obstructed diffusion of biological molecules in agreement with experiments. Thus, the local, global and dynamic characteristics of cell nuclei are not only tightly inter-connected, but also are integrated holisticly to fulfill the overall function of the genome.

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

Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, autofluorescent proteins, CFP, GFP, YFP, DsRed, fusionprotein, in vivo labelling.

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.

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.

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.