Dynamic and structural properties of interphase chromatin mapped in vivo with fluorescence correlation spectroscopy and quantitative modelling

Malte Wachsmuth[1], Tobias A. Knoch[2], Karsten Rippe[3]

[1]Cell Biology & Biophysics Unit, EMBL, Heidelberg, Germany;
[2]Biophysical Genomics, Erasmus MC, Rotterdam, The Netherlands; [3]DKFZ/BioQuant, Heidelberg, Germany

EMBL 40 YEARS

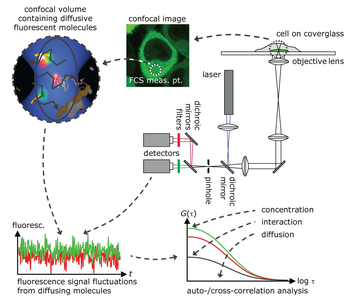
Abstract

The three-dimensional organization of chromosomes of eukaryotic interphase cells is emerging as an important parameter for the regulation of storage, replication and expression of the genome. While a range of techniques like electron microscopy (EM), fluorescence in situ hybridization(FISH), or chromosome conformation capture techniques (3C, 4C, 5C, HiC, or the novel targeted chromatin capture, T2C) have been very successfully used to study chromosomal architecture, many details of structural and especially of dynamic properties remain unresolved.

Here, we present a novel approach to dissect intramolecular polymer dynamics from fluorescence intensity fluctuations measured with fluorescence correlation spectroscopy (FCS) to investigate the higher order chromatin dynamics in living cells. Using fluorescently tagged linker histone H1 and core histones H2A and H2B as tracer molecules, we find distinct chromatin relaxation times of ~160 ms for open and ~90 ms for dense chromatin areas, corresponding to radii of gyration of 240 and 300 nm for the topologically independent chromatin units. According to their genomic content of ~1 Mb, these units correspond to the distinct topologically associating domains (TADs) found recently by 3C-derived techniques and to sub-chromosomal domains seen earlier by FISH and in vivo chromatin labelling. We have also obtained light-sheet microscopy-based FCS maps of chromatin domain dynamics.

Based on these results, we have developed a quantitative analytical and numerical model of chromatin dynamics that provides access to mass density, persistence length and topological information of chromatin. It allows to extract these parameters from dynamics and 5C/HiC results, to predict chromatin conformation and distance data, and to identify complex looping as crucial for domain formation. Data and model suggest the existence of several connected loops of ~100 kb each per domain. Especially in combination with the recently developed highly selective T2C method (see abstract T.A. Knoch & M. Wachsmuth, "Determination of the three-dimensional organization of chromatin by modelling-supported targeted chromosomal interaction capture (T2C)"), which provides very good signal-to-noise ratio at high genomic resolution, we present a comprehensive systematic approach for the understanding of chromatin dynamics and its relation to structure as well as for insight into the impact on gene regulation.

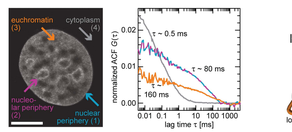
Confocal fluorescence correlation spectroscopy (FCS)

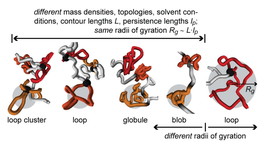


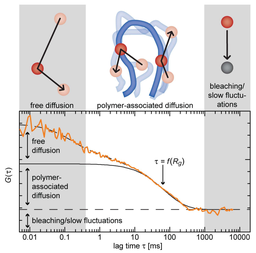
Yields:
- diffusion coefficients
- concentrations
- fractions of bound and free molecules
- dissociation constants

Limitations:
- photobleaching
- time-consuming
- tedious data processing

Distinct chromatin relaxation modes reveal indynamically and topologically independent domains



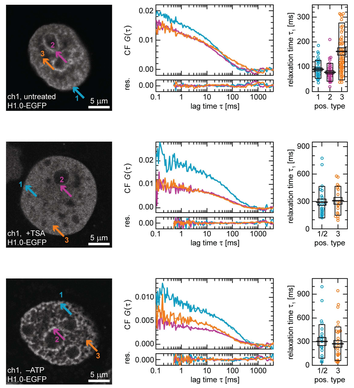




Observation:
- distinct chromatin relaxation modes when studying core and linker histones in living cell nuclei in interphase

Interpretation:
- chromatin-associated diffusion
- reveals chromatin dynamics on intermediate scales
- evidence for topologically independent units
- could be previously observed topologically associating domains (TADs)
- relaxation times depend on radius of gyration, defined by persistence length, contour length, mass density of nucleosome chain

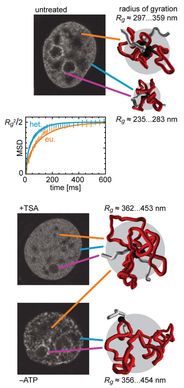
Polymer models: 1 Mb content and 240-300 nm size of domains



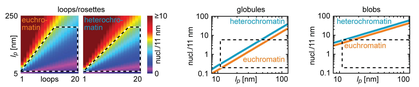
Untreated cells:
- faster relaxation/ more confined in heterochromatin
- genomic content 1 Mb

TSA treatment:
- homogenized chromatin
- increased radii and genomic content

ATP depletion:
- more aggregated, clumpy chromatin
- increased radii and genomic content

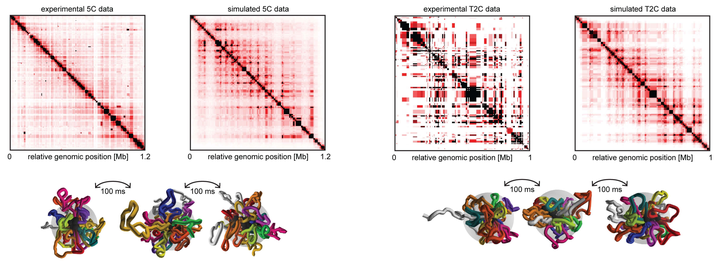


Physical parameter space for different conformations



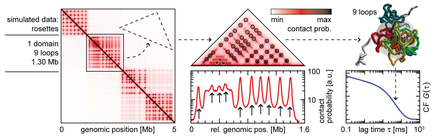
Persistence length, mass density and loop number:
- loops/loop cluster compatible
- globule compatible
- blobs unlikely

Analysis and simulations of contact probability maps

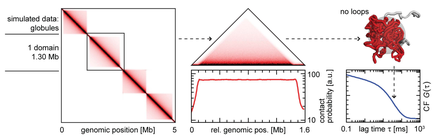


Loop-/loop cluster-based analysis and simulation: good agreement

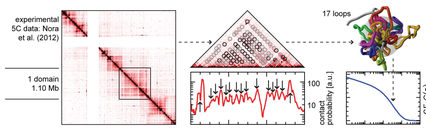
Rosettes or globules or ...?



Simulated rosettes: T2C/5C data – conformation – relaxation

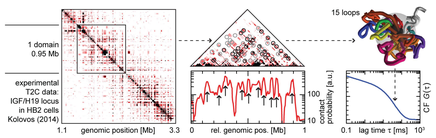


Simulated globule: T2C/5C data – conformation – relaxation



5C experimental data: 5C data – conformation – relaxation

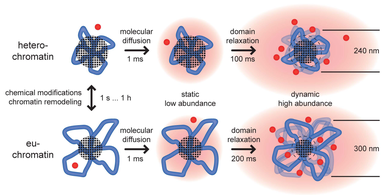
Nora et al., Nature 485, 2012



T2C experimental data: T2C data – conformation – relaxation

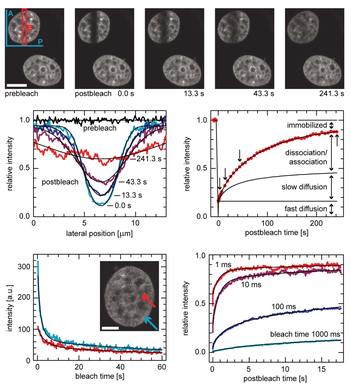
Kolovos et al., Epigenetics & Chromatin 7, 2014

Differential regulation of chromatin accessibility

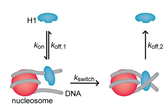


Acessibility:
- concentration-
- and size-dependent

H1.0 binding is slower than the observed relaxations

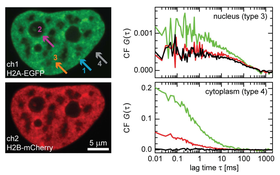


Fluorescence recovery after/ continuous photobleaching:
- three-step binding kinetics
- 1st step: mean dwell time ~1 s
- 2nd step: switching to longer-lived state
- 3rd step: mean dwell time ~120 s



Interpretation:
overall, association/ dissociation is far slower than the observed relaxations

Cross-correlations of core histones: no photophysics



FCCS of H2A-EGFP and H2B-mCherry:
- strong cross-correlation in the nucleus
- no cross-correlation in the cytoplasm
- photophysics as source of fluctuations can be ruled out

Summary

- The observation of distinct chromatin relaxations indicates the existence of dynamically and topologically independent domains
- Chromatin fiber dynamics provides additional contraints for domain architecture
- Relaxation data restrict compatible models to loops/loop clusters or globules
- Additional consideration of T2C and 5C data suggests loop/rosette formation
- Chromatin accessibility depends on size and concentration of tracer molecules

Dynamic and Structural *in vivo* Properties of Interphase Chromatin Mapped with Fluorescence Correlation Spectroscopy (FCS) and Quantitative Modelling

Wachsmuth, M. & **Knoch, T. A.**

The American Society for Cell Biology/International Federation of Cell Biology Meeting 2014, Pennsylvania Convention Center, Philadelphia, Pennsylvania, USA,
6th – 10th December, 2014.

Abstract

The three-dimensional organization of chromosomes of eukaryotic interphase cells is emerging as an important parameter for the regulation of genes as well as the storage, replication and expression of genetic information in general. Whereas a couple of techniques like electrone microscopy (EM), fluorescentce *in situ* hybridization, or chromosome capture techniques as 3C, 4C, 5C, HiC, or the novel T2C have been used for researching genomic architecture, its dynamics has remained underexplored. Here, we present a novel approach to dissect intramolecular polymer dynamics from fluorescence intensity fluctuations measured with fluorescence correlation spectroscopy (FCS) to investigate the higher order chromatin dynamics in living cells. Using fluorescently tagged linker histone H1 and core histones H2A and H2B as tracer molecules, we found distinct chromatin relaxation times of ~160 ms for open and ~90 ms for dense chromatin areas, corresponding to radii of gyration of 240 and 300 nm for the topologically independent chromatin units. According to their genomic content of ~1 Mb, these domains correspond to subchromosomal domains, already seen by FISH, and histone GFP labelling beforehand and correspond to so called distinct topologically associating domains (TADs). This is an impressive advancements in the field, since details on the dynamic and structural properties of subchromosomal domains/TADs in living cells are scarce.
Based on these results, quantitative analytical and numerical modelling provided access to mass density, persistence length and topological information of chromatin. It allowed to extract these parameters from 3C/5C/HiC/T2C results, to predict chromatin conformation and distance data, and to identify complex looping as crucial for domain formation. Especially, in combination with T2C, a recently developed selective high-throughput high-resolution chromosomal interaction capture technique (see abstract T.A. Knoch & M. Wachsmuth, Determination of the three-dimensional organization of chromatin by modelling-supported selective chromosomal interaction capture (T2C)), which provides very good signal-to-noise ratio, we present a comprehensive systematic approach for the understanding of chromatin dynamics and structure as well as for insight into their impact on gene regulation. As an outlook, we show light-sheet microscopy-based FCS maps of chromatin domain dynamics.

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

Keywords:

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome statistical mechanics, genomic uncertainty principle, multilism genotype-phenotype, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin quasi fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, polymer model, analytic mathematical model, Brownian Dynamics, Monte Carlo, fluorescence *in situ* hybridization (FISH), targeted chromatin capture (T2C) confocal laser scanning microscopy, fluorescence correlation spectroscopy, spatial precision distance microscopy, super-resolution microscopy, two dimensional fluorescence correlations spectroscopy (2D-FCS) auto-fluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, *in vivo* labelling, information browser, visual data base access, holistic viewing system, integrative data management, extreme visualization, three-dimensional virtual environment, virtual paper tool.

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.