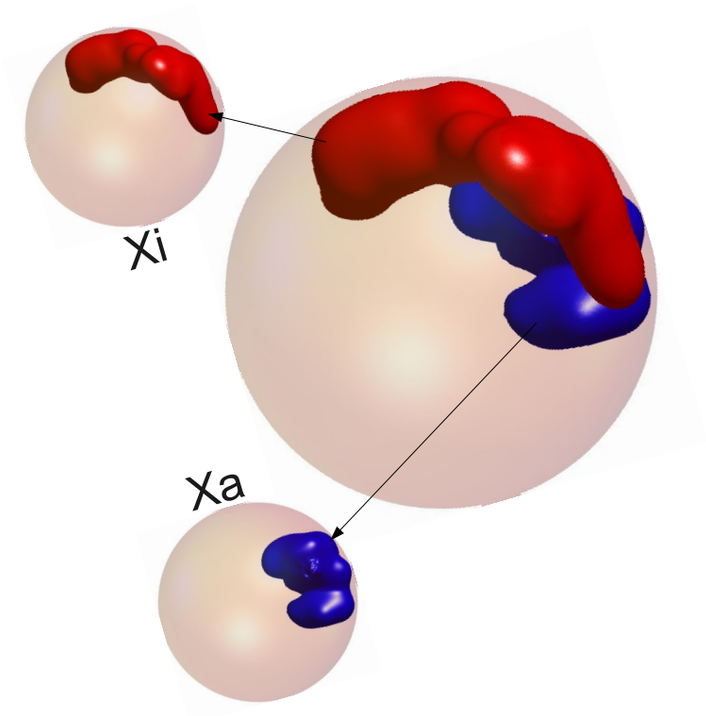
Active Matter and Nuclear Physics



Gautam I. Menon

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Outline

Non-equilibrium physics

Self-propelled objects

Living matter as active matter

Nuclear architecture

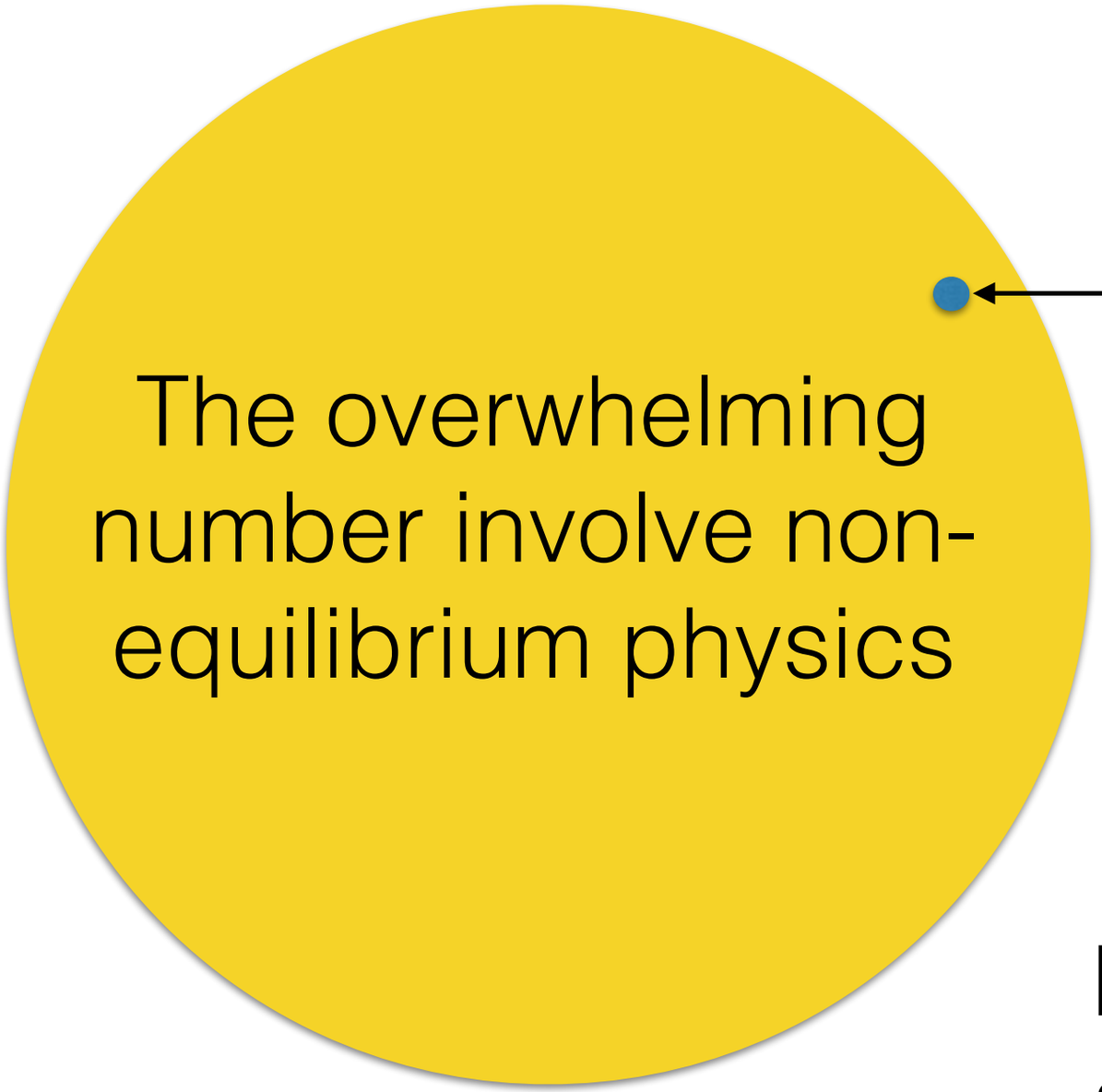
Chromosome positioning

Applying active matter ideas

Results & Predictions

Conclusions

In the space of all interesting problems in condensed matter physics (largely)



Those involving equilibrium physics are a truly tiny fraction

Many ways in which a system can be non-equilibrium

Non-equilibrium

Driving at <u>macroscopic</u> scales, bulk or boundary, a route to non equilibrium

http://en.wikipedia.org/wiki/Kelvin–Helmholtz_instability
http://en.wikipedia.org/wiki/File:Kelvin-Helmholtz_Instability.ogv

Indian traffic

https://www.youtube.com/watch?v=_Yj36G9ukDc

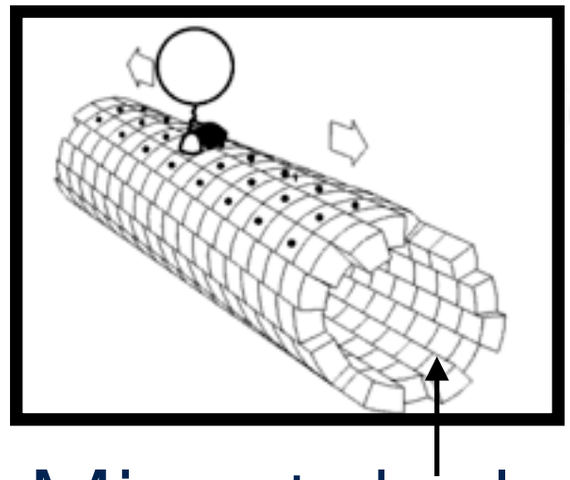
Molecular Motor

Single molecule, mechano-chemical cycle.

Energy-rich molecule, ATP, broken down to ADP (Hydrolysis)

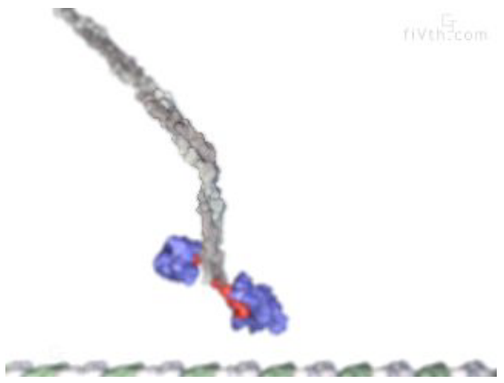
Cell makes lots of ATP. Concentrations are not in equilibrium

Kinesin



http://valelab.ucsf.edu/moviepages/movies.html
http://python.rice.edu/~kolomeisky/kinesin_walking.gif

Examples of “self-propelled particles”

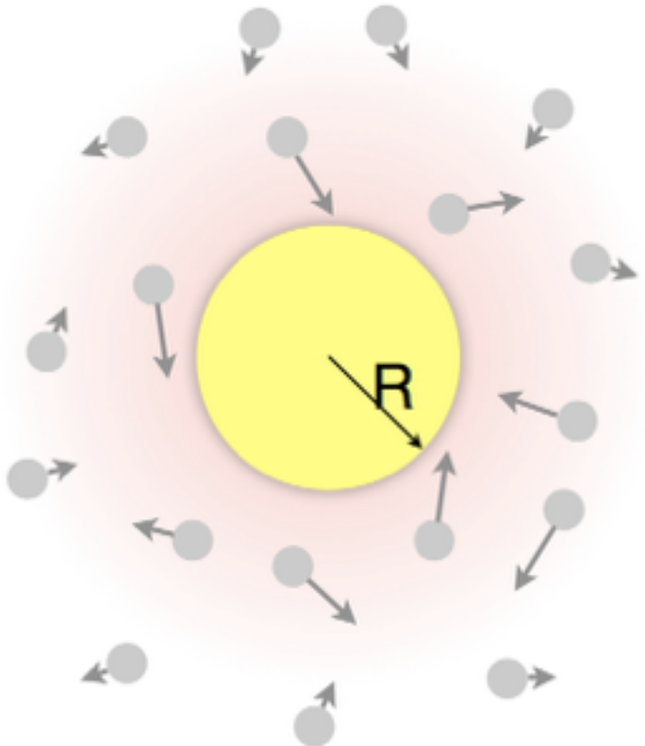


Thinking about self-propelled particles and how to describe them (individually, collectively) was the initial motivation for the study of <u>active matter</u>

But what is “passive” matter?

http://www.uni-leipzig.de/~physik/tl_files/mona/images/Photothermal%20Detection/HBM.png

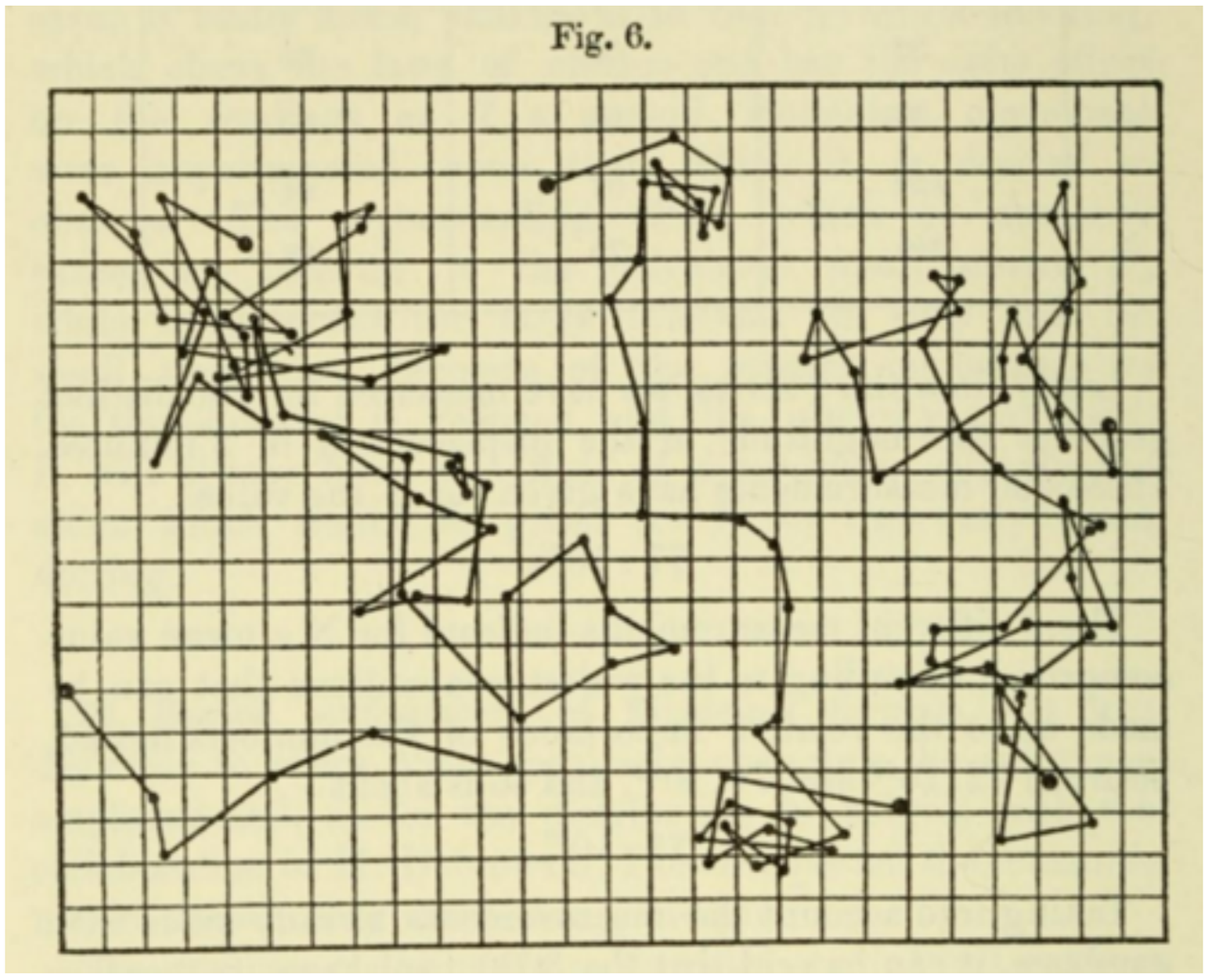
“Passive” Matter



Trajectory of a micron sized particle in a fluid

Random, Brownian motion

Particle buffeted about by other particles which we cannot see, but whose presence we infer



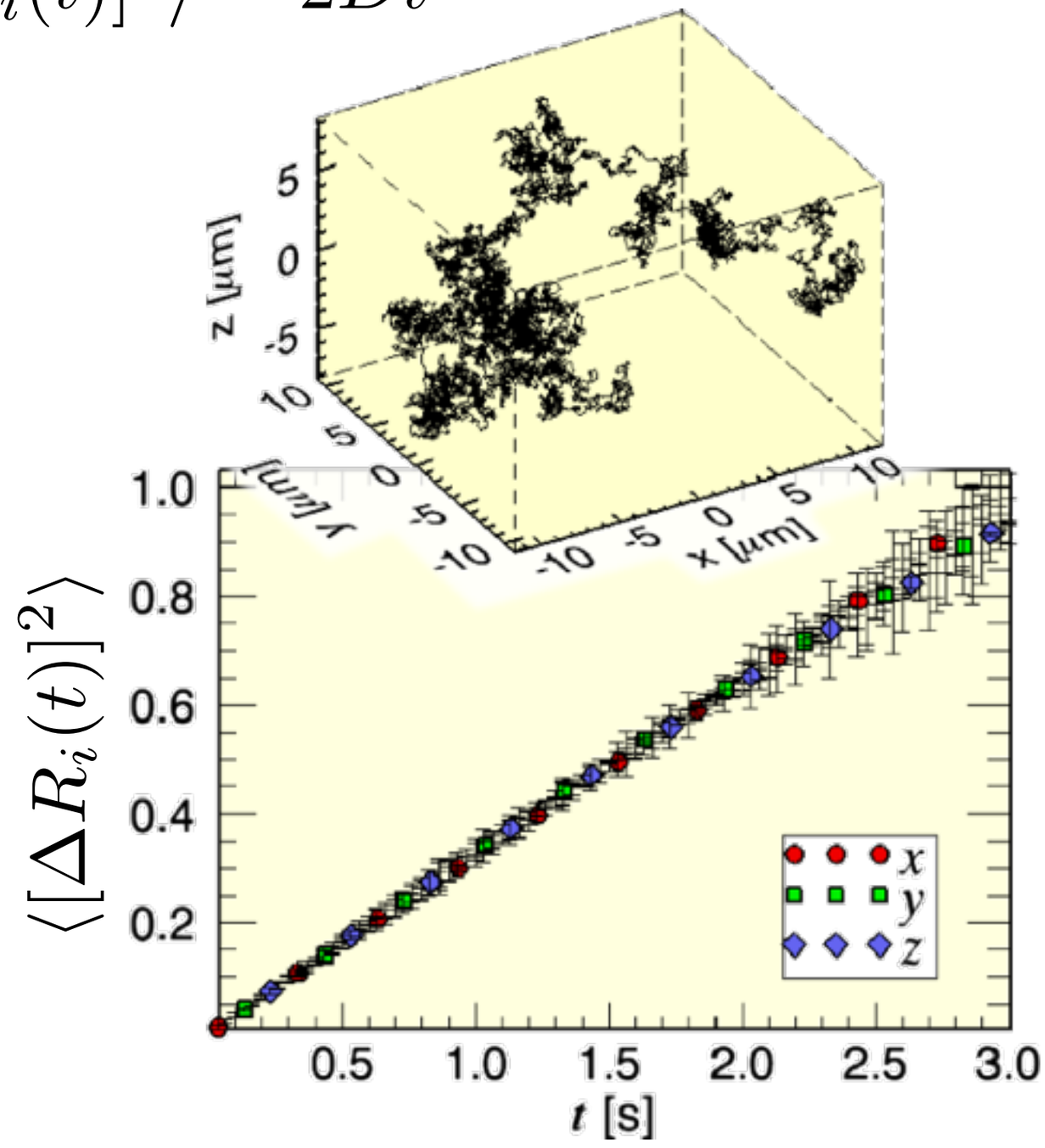
http://4.bp.blogspot.com/-ORcSEZZOqKU/UJAy64PFKjI/AAAAAAAABbo/P6tRH_u4Vq4/s1600/perrin-1909.png

http://physics.nyu.edu/grierlab/biofilm8c/x2.png

$$\langle [R_i(t) - R_i(0)]^2 \rangle = \langle [\Delta R_i(t)]^2 \rangle = 2Dt$$

Diffusion

Experiment: Three-dimensional trajectory of a polystyrene bead (radius 0.75 microns) freely moving in a fluid.



D = 0.1695 ± 0.0001 μ m²/s

In three dimensions

$$\langle |\vec{R}|^2 \rangle = 6Dt$$

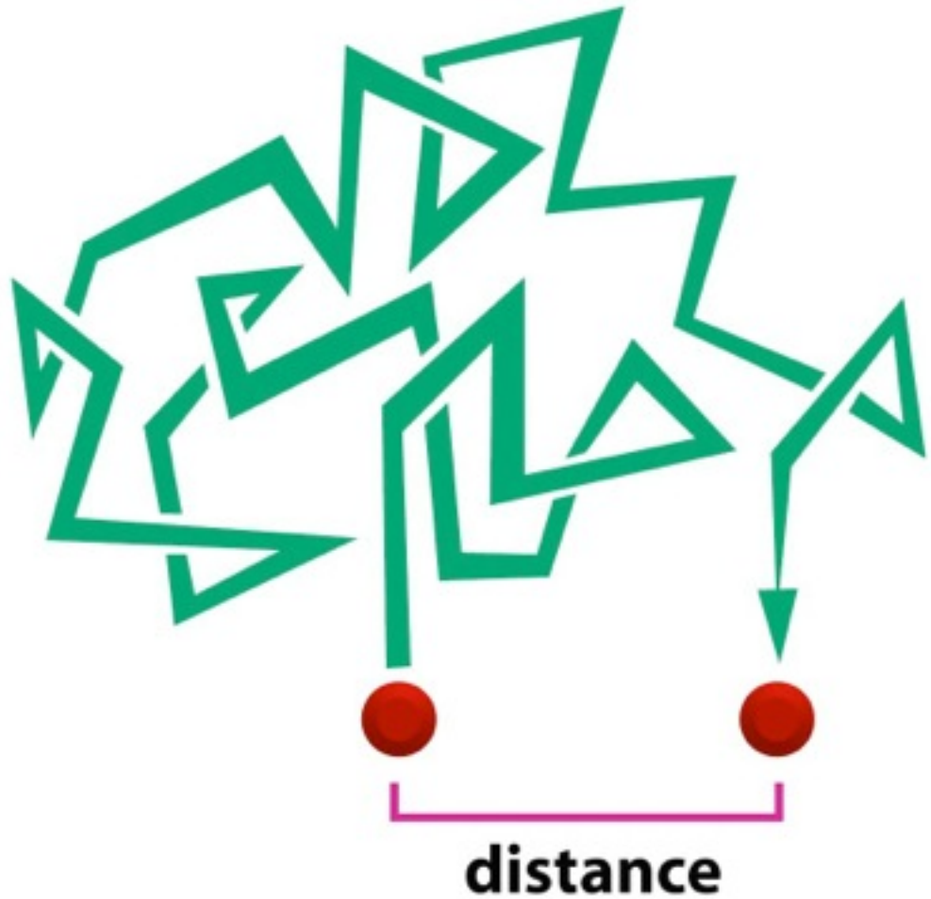


Figure 2-48 Molecular Biology of the Cell 5/e (© Garland Science 2008)

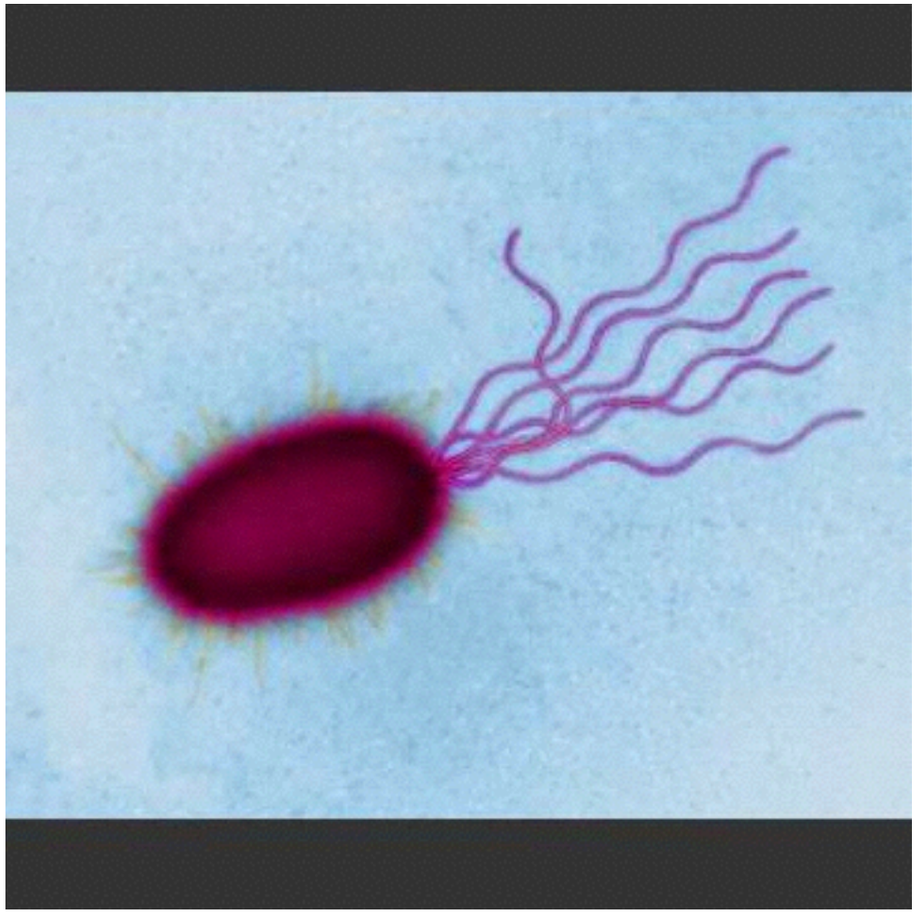
What does the diffusion constant depend on?

Stokes-Einstein

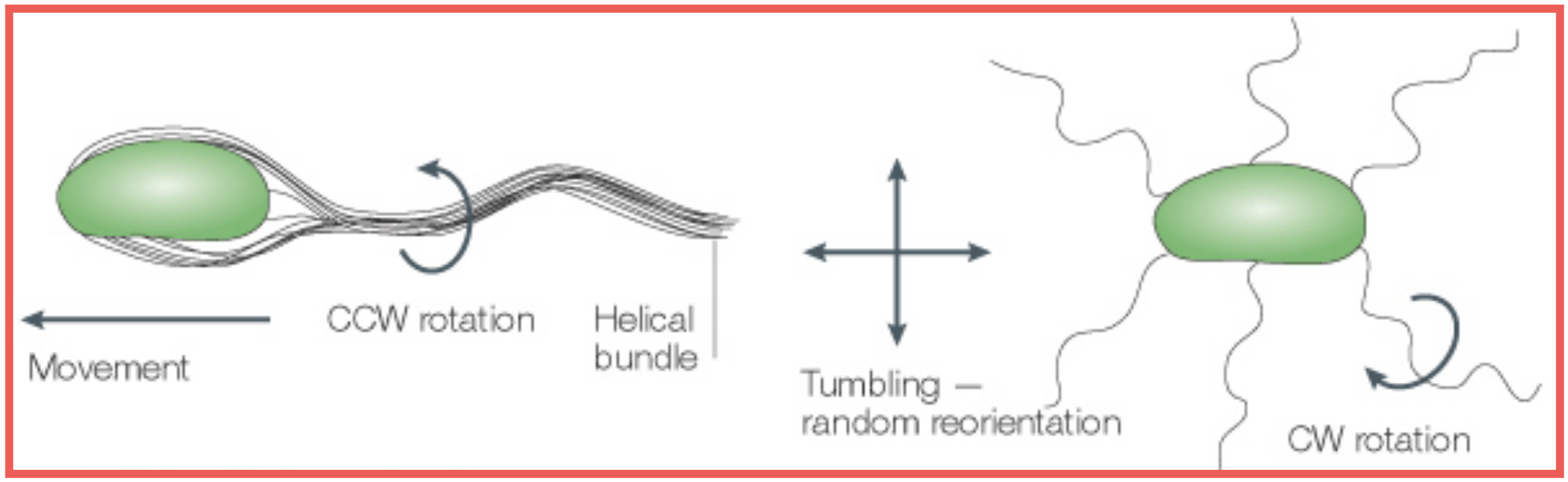
It's given by $$D = \frac{k_B T}{6\pi\eta a}$$

A fluctuation-dissipation relation

What about the motion of a similarly sized, live bacterium, *E. coli*?

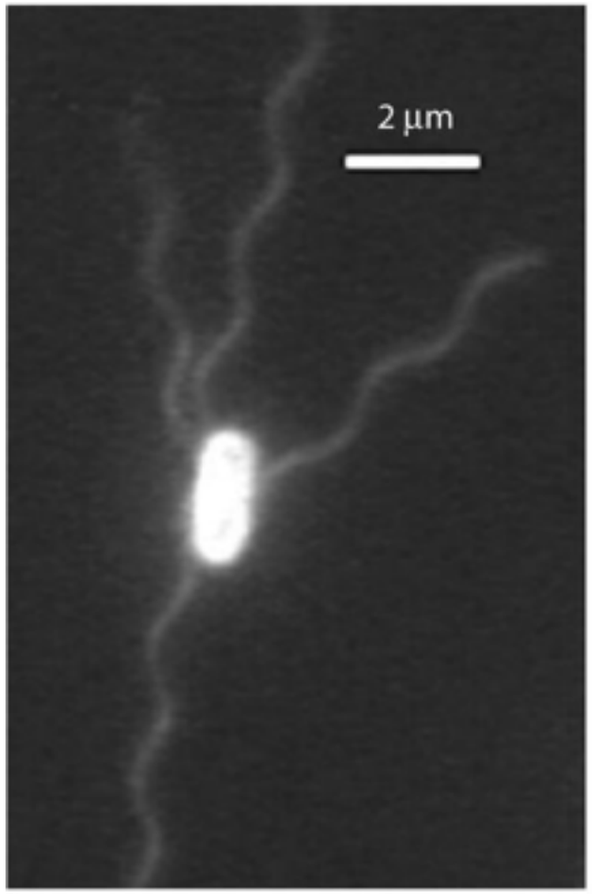


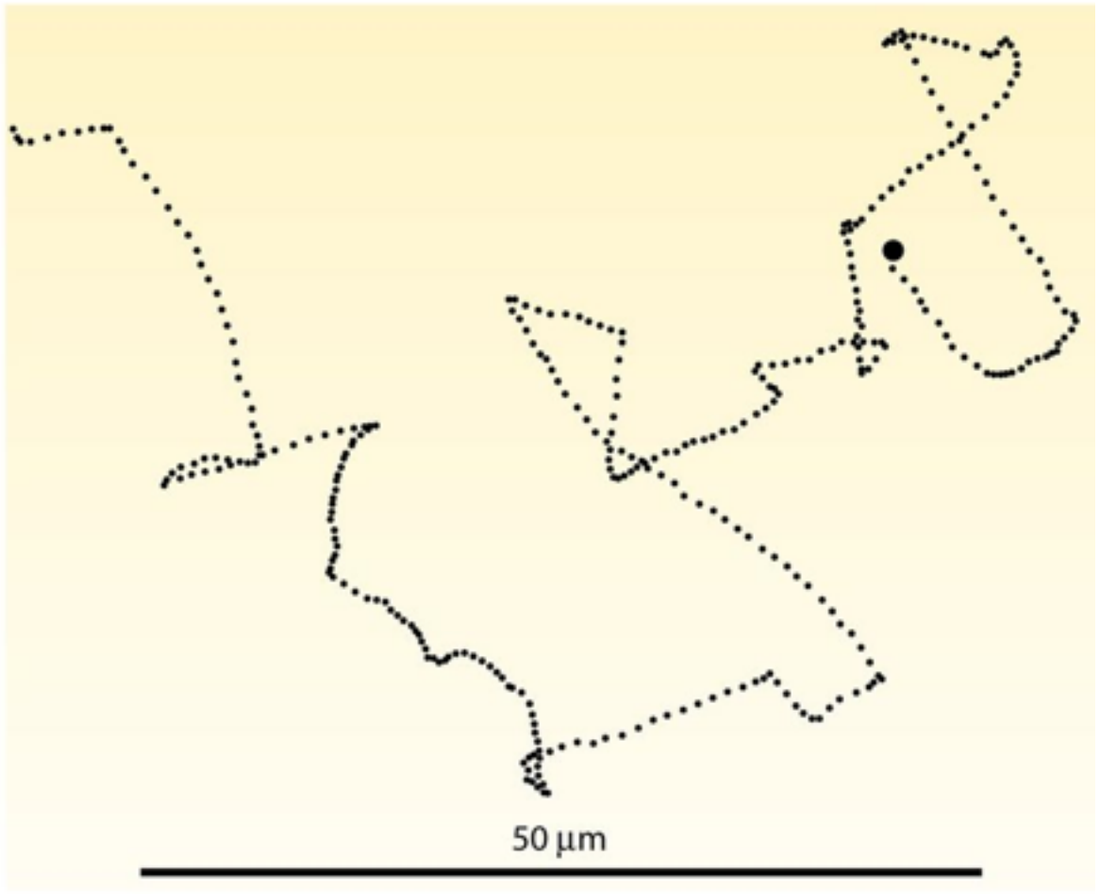
http://ccdb.wishartlab.com/CCDB/GP2118.jpg



http://www.ebi.ac.uk/biomodels/ModelMonth/2009-09/figure1.gif

http://mmbr.asm.org/content/76/4/792/F9.large.jpg



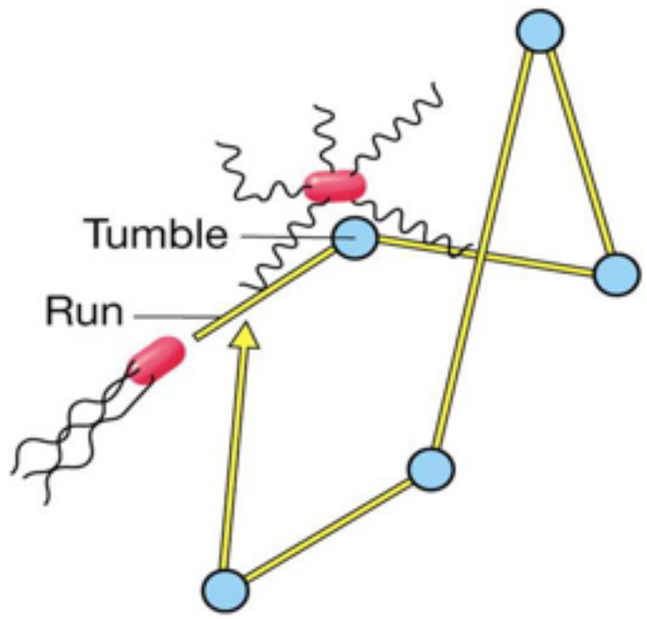


When looked at over a long time, the trajectories look "Brownian", but are they?

Remember, in thermal equilibrium

$$\langle [\mathbf{R}(t) - \mathbf{R}(0)]^2 \rangle = 2Ddt$$

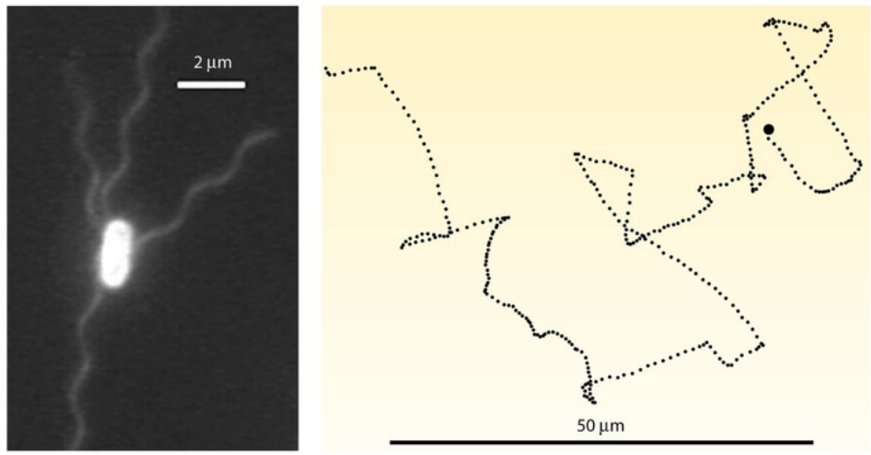
$$D = \frac{k_B T}{6\pi\eta a}$$



http://202.204.115.67/jpkch/jpkch/2008/wswx/chapter%203/65.jpg

http://mmbr.asm.org/content/76/4/792/F9.large.jpg

The bacterium, *E. coli* moves along its axis at fixed velocity (runs). Micron sized so susceptible to noise



"directed motion" "noise"

$$\partial_t \vec{r} = v_0 \hat{u} + \vec{\eta}$$

Angle diffuses with diffusion constant D_R

But axis also changes (2d) - an angle θ

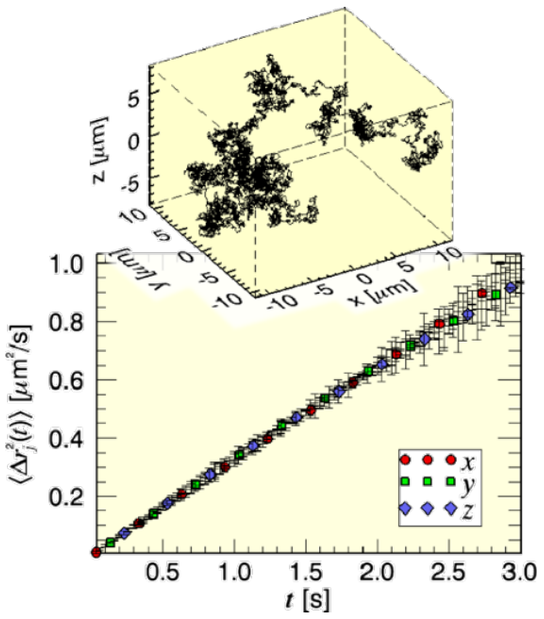
$$\partial_t \theta = \eta^R$$

The final result for the particle displacement is

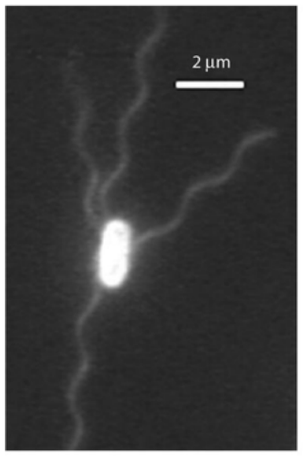
$$\langle [\vec{r}(t) - \vec{r}(0)]^2 \rangle = 4(D + \frac{v_0^2}{4D_R})t = 4D_{eff}t$$

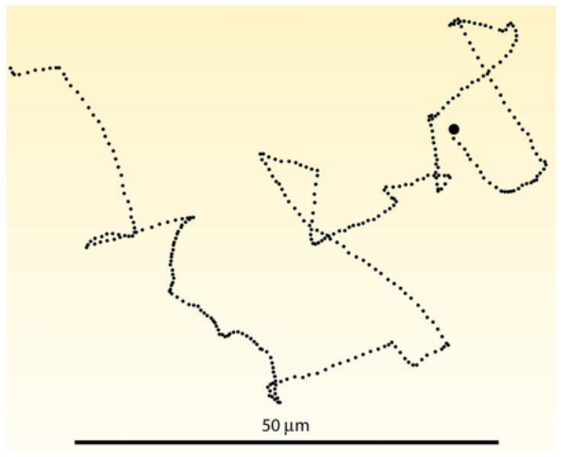
D_{eff} can be much, much bigger than D!

Aparna Baskaran, Cristina Marchetti, M. E. Cates



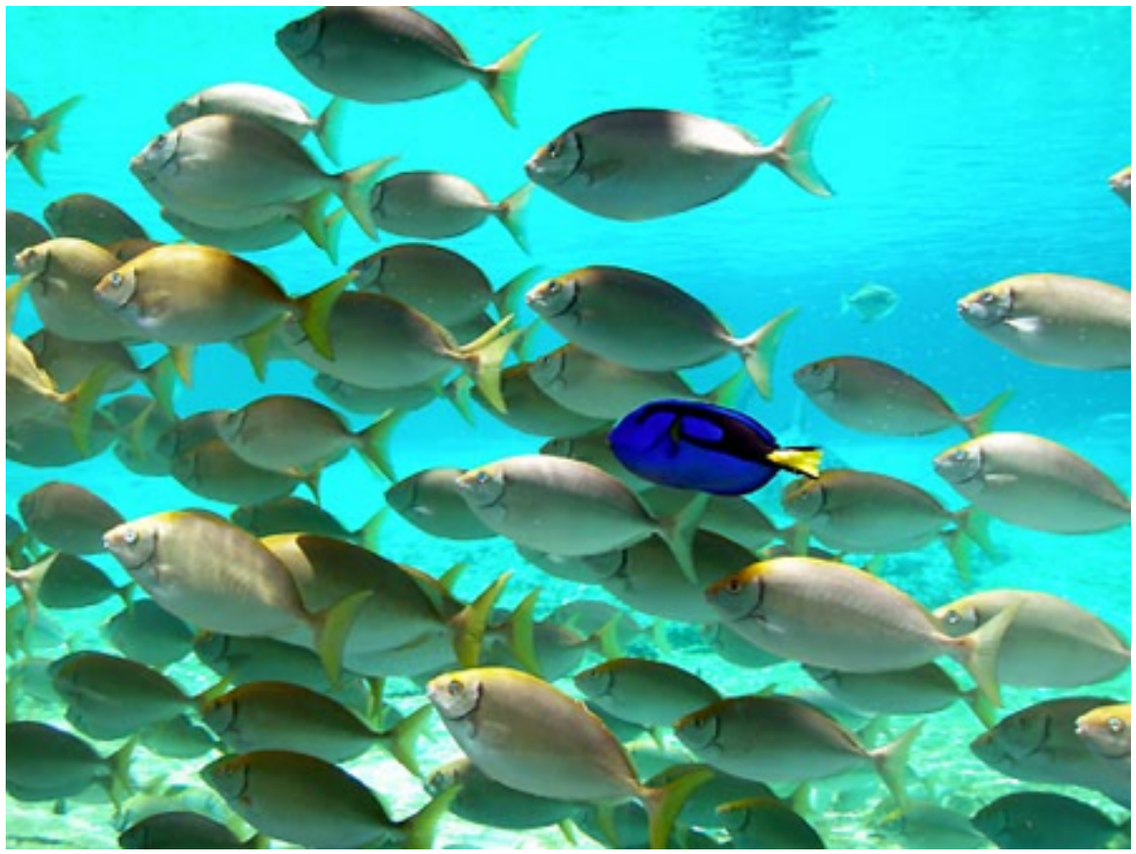
versus





The “passive” colloid and the “living” bacterium are very different. The bacterium appears as if it is diffusing, at long times, but D is not what equilibrium physics predicts. An FDT violation.

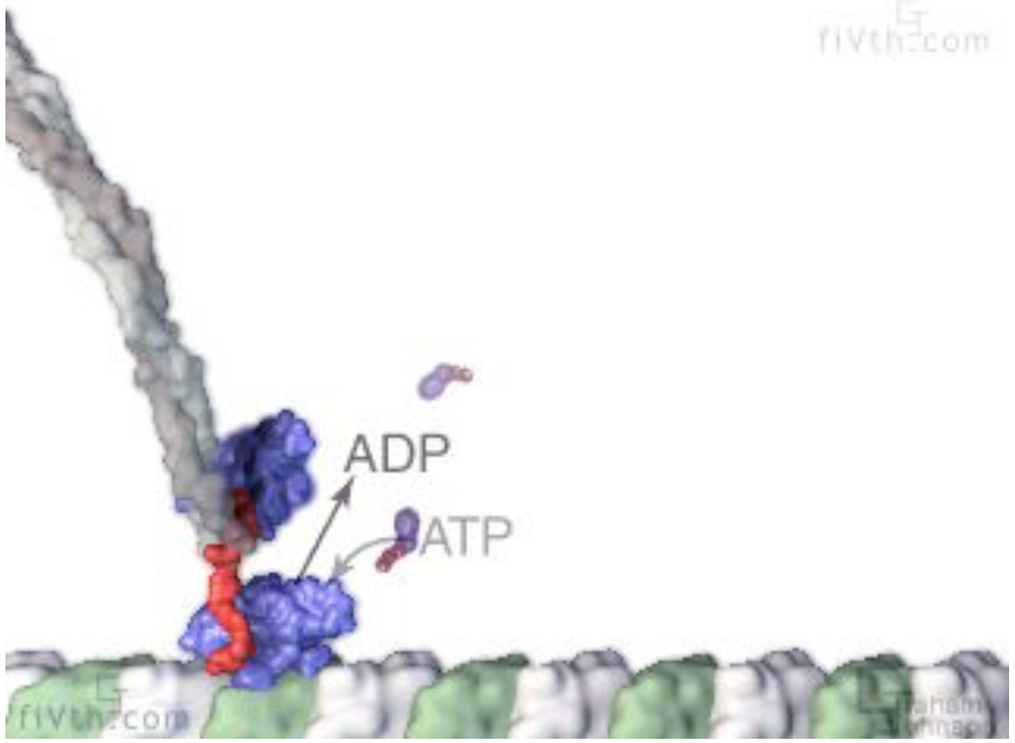
The bacterium is “self-propelled” and thus out of equilibrium!



Idea motivating active matter studies: Drive the system out of equilibrium by adding energy on a microscopic scale, as for the self-propelled particle

Viewed from a very general perspective: This is how all cells function!

http://1.bp.blogspot.com/_eK29PUa7DGw/TJ1QjCgpeeI/AAAAAAAAABY/BBrqqMAhR8w/s1600/fish.jpg



http://valelab.ucsf.edu/moviepages/movies.html

Energy input at the nanoscale in biological systems.

ATP hydrolysis releases energy powering cellular processes

Summary (so far)

Living matter
Driven at a microscopic scale.

http://manbir-online.com/grafics/ageing.jpg

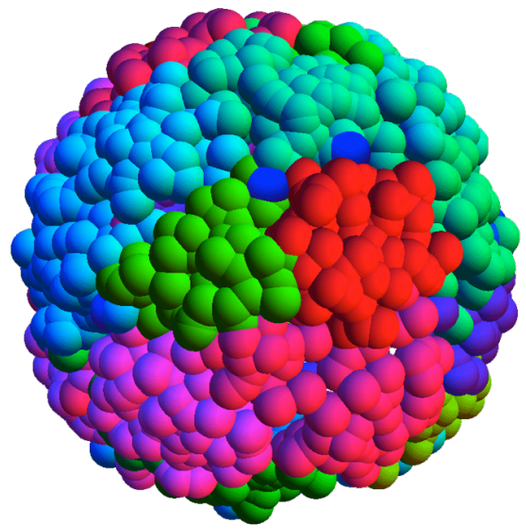
Such systems are active systems

Active matter ideas provide a powerful way of describing non-equilibrium aspects of biological systems

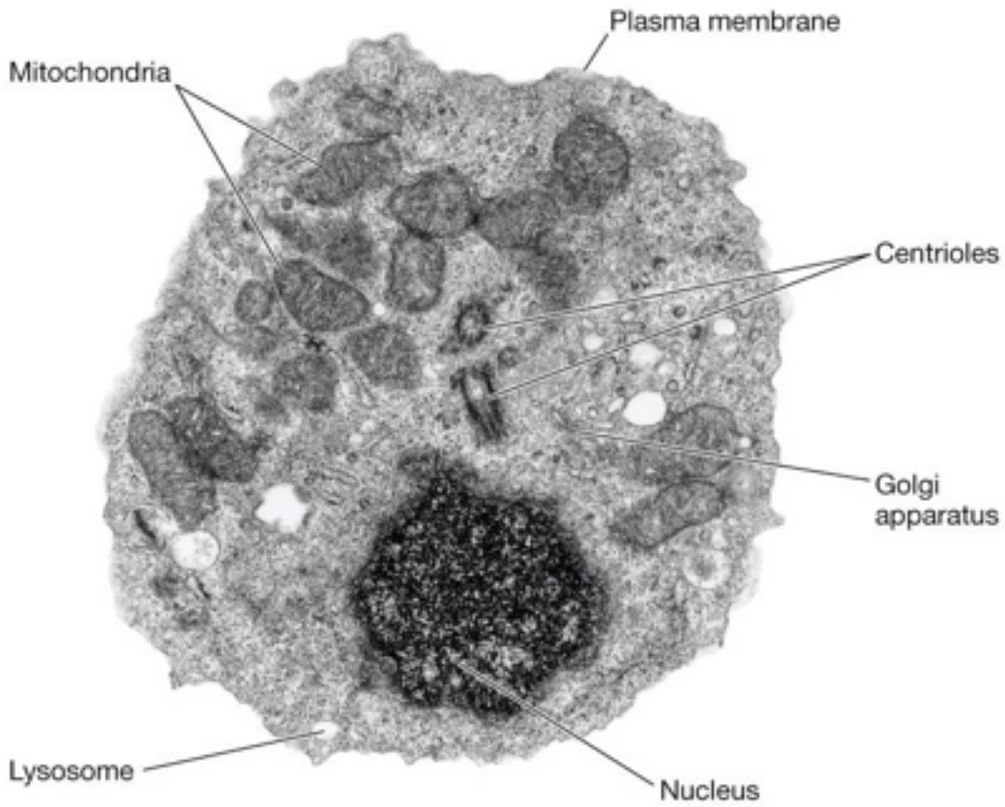
Active Gel Physics, Prost, Julicher and Joanny Nature Physics (2015);
Hydrodynamics of Soft Active Matter, Marchetti at al. Rev. Mod. Phys. (2103);
The Mechanics and Statistics of Active Matter, Ramaswamy, Ann. Rev. Cond. Matt. Phys. (2010).

Used to model: bacterial swimming, cell motility, developmental processes ..

Our work: Use active matter ideas to understand the large-scale architecture of the cell nucleus



About 10^{13} cells in the human body and ~ 200 cell types



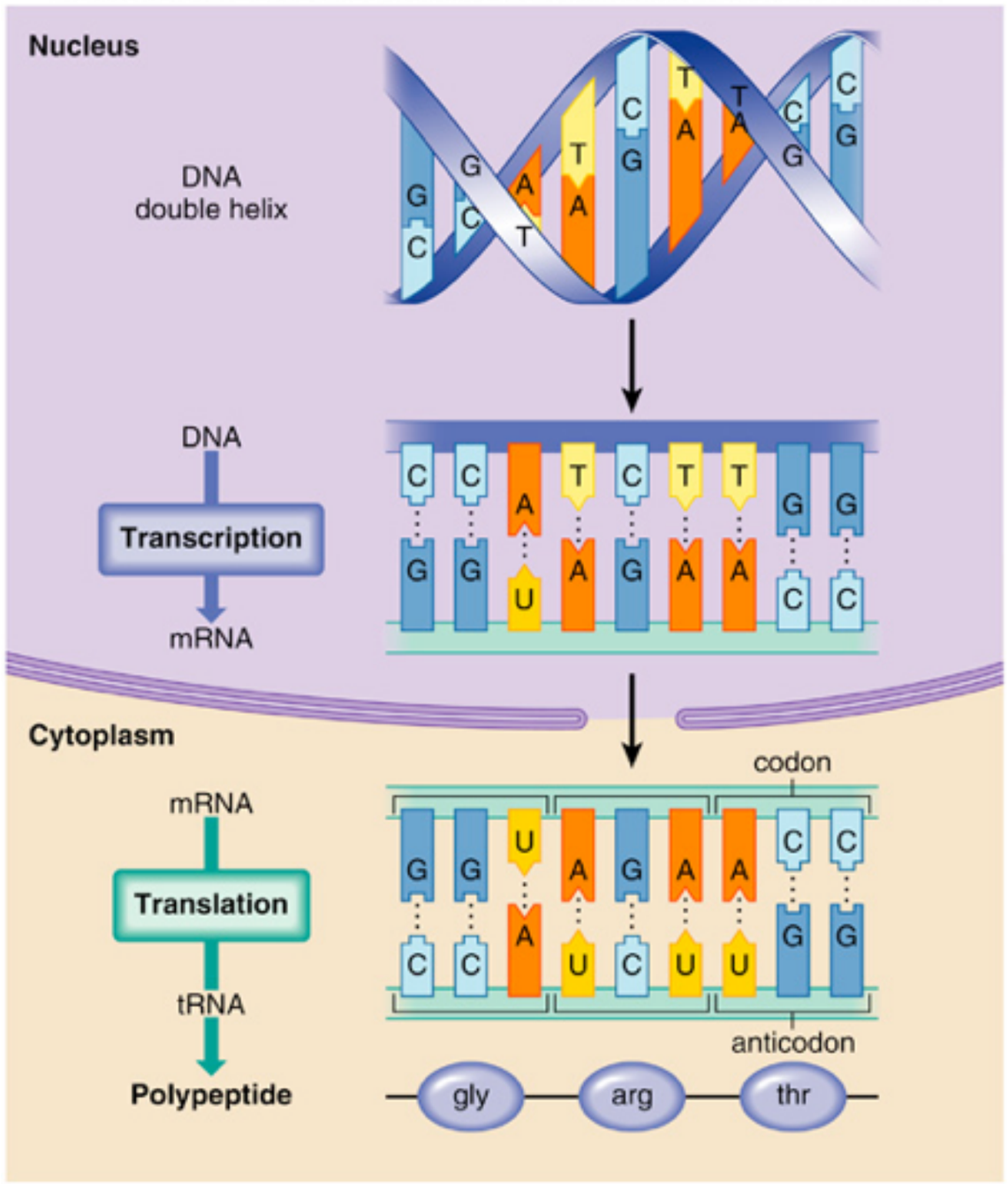
http://www.oncoursesystems.com/

23 pairs of chromosomes in the nucleus of each (somatic) cell

Each chromosome is a single long DNA molecule

Total DNA length of about 2m in each nucleus, packed into about a 5-10 micron region

DNA as information carrier



Transcription

Sequence in coding DNA

Translation

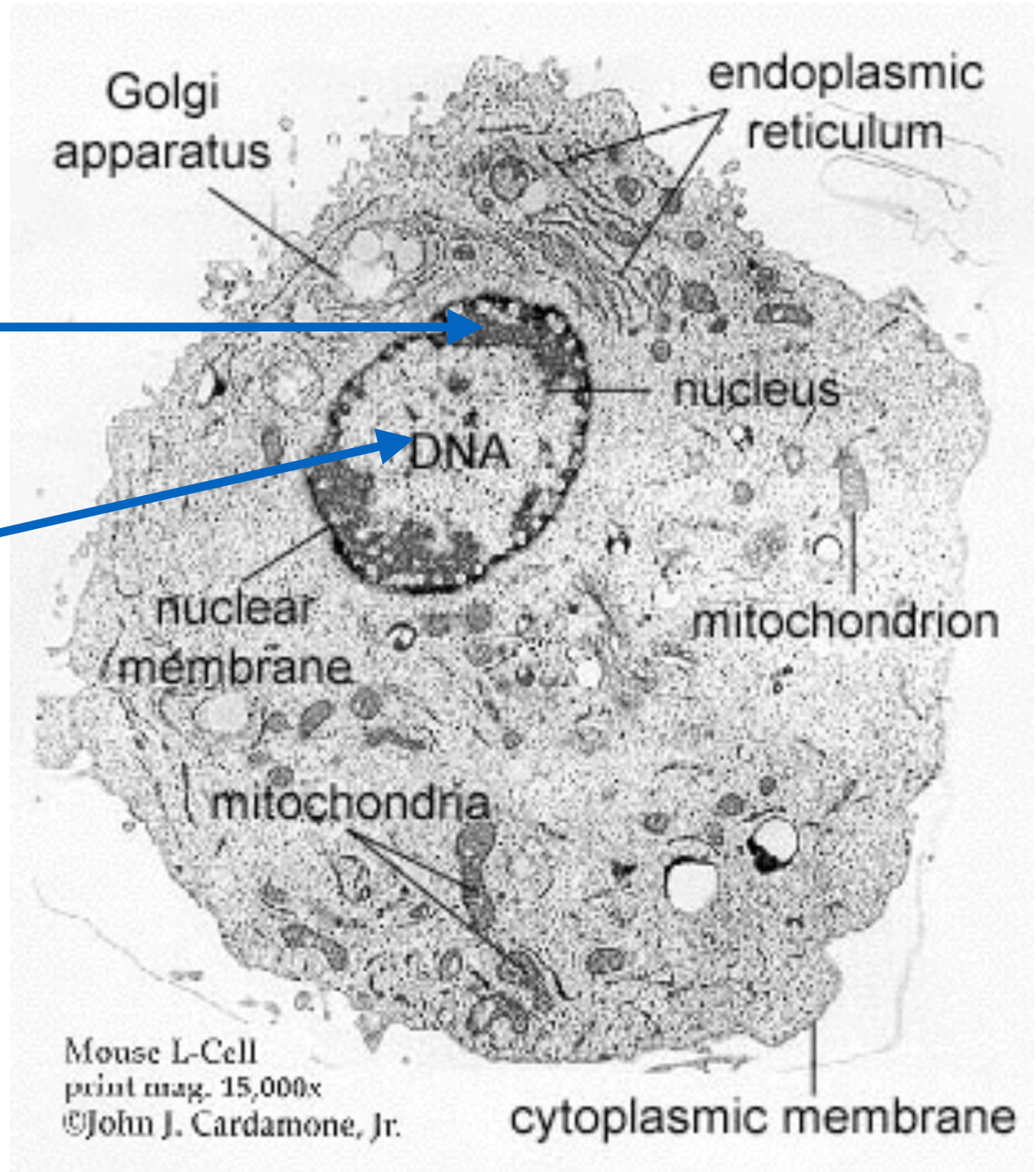
Proteins

.. and as physical molecule

Nucleus in animal cells

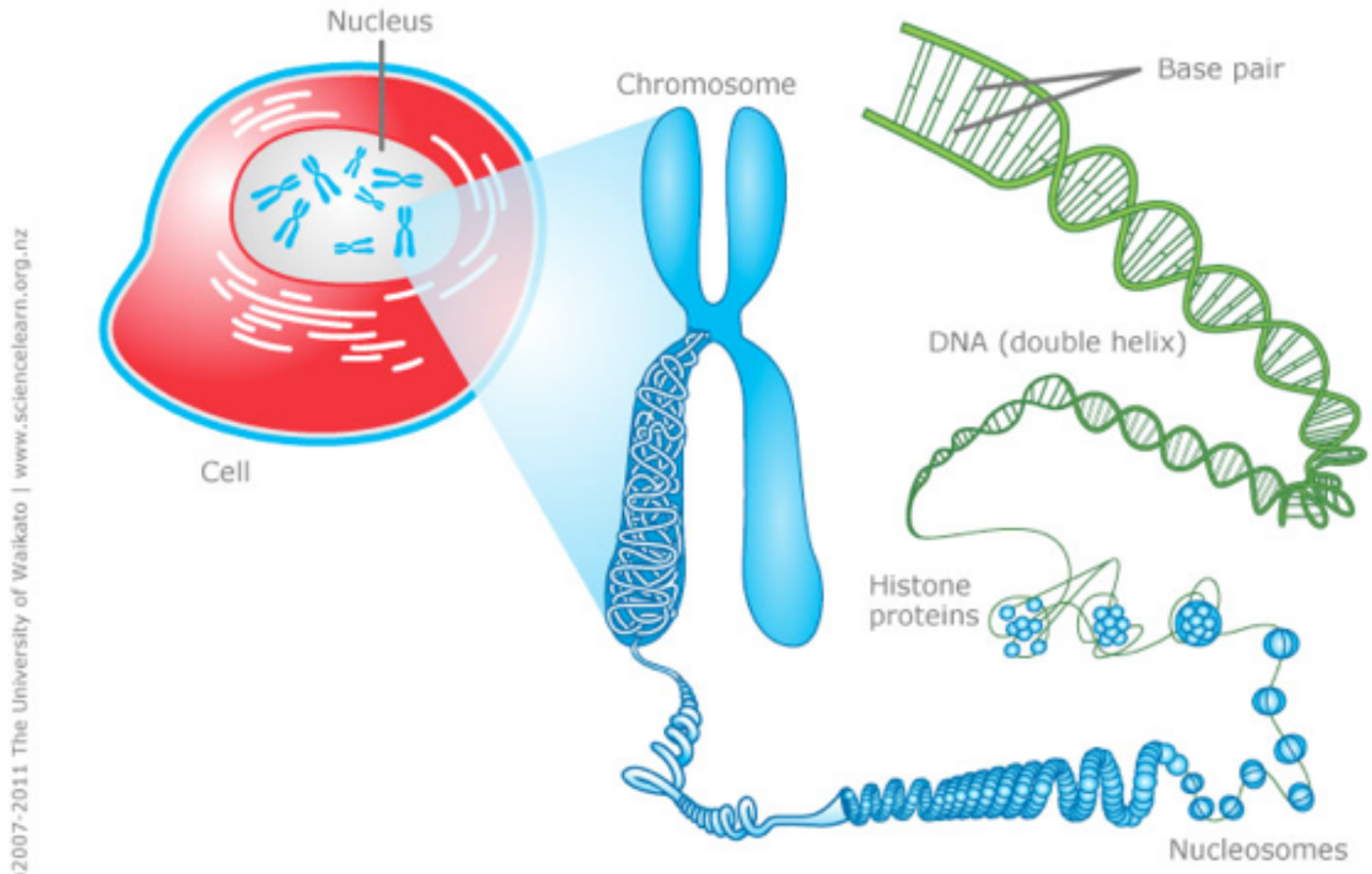
Tightly packed DNA, gene-poor, **HETEROCHROMATIN**

Loosely packed DNA, gene-rich, **EUCHROMATIN**



http://student.ccbcmd.edu/~gkaiser/biotutorials/eustruct/u1fig2.html

Total DNA length of about 2m in each nucleus, packed into about a 5-10 micron region



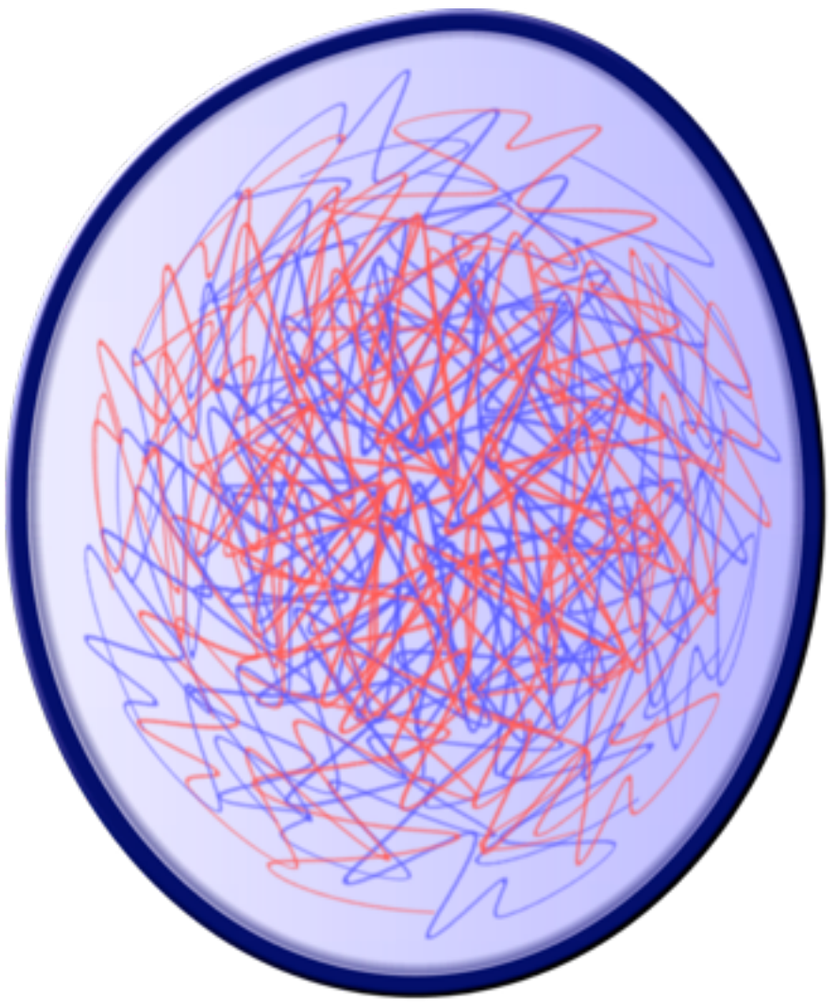
How is DNA compacted within the nucleus?

DNA compacted by **histones**

DNA + histones: **chromatin**

Elementary unit: **nucleosome**

What we used to think …



http://www.edupic.net

During the 1970s and 1980s, most researchers seemed content with the assumption that the nucleus is filled with intermingling chromatin fibers and loops like a dish of spaghetti, an assumption widely reflected by textbooks of cell biology .....

T. Cremer and M. Cremer, Cold Spring Harb. Perspect. Biol. (2010)

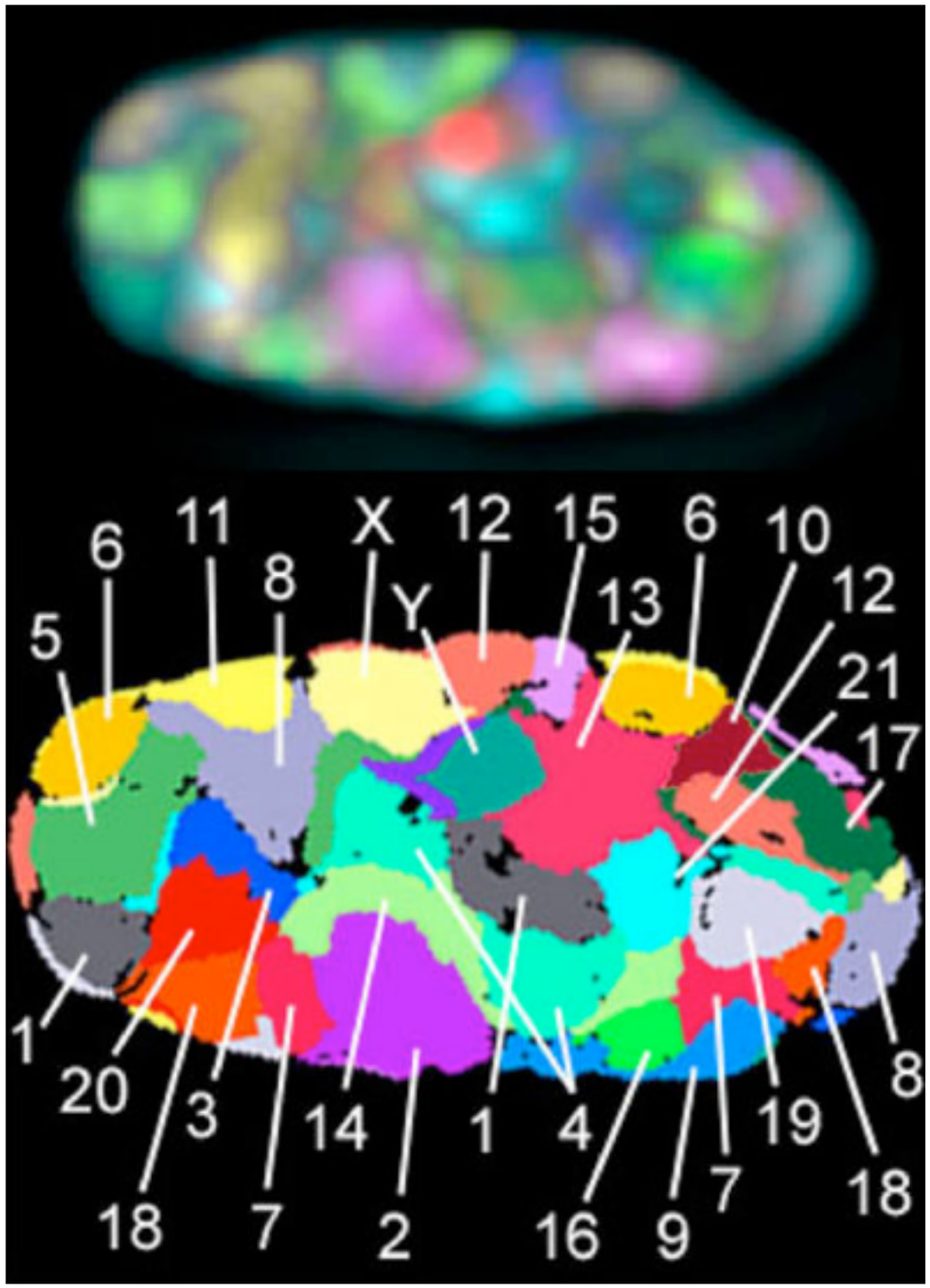
What we know about the large-scale architecture of interphase (between cell divisions) chromatin

Individual chromosomes

- Are territorial
- Have nonrandom arrangements

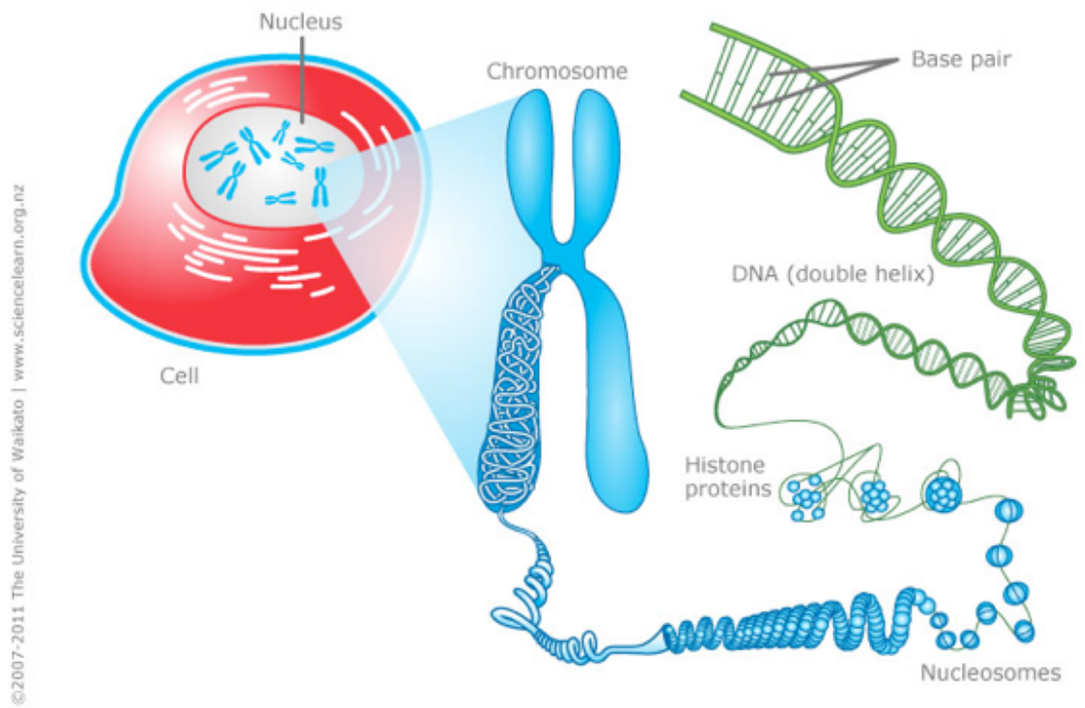
Rabl 1885, Boveri 1908, Stack 1977,
90's: Cremer, Bickmore, Misteli, ..

Bolzer et al, PLoS Biol 3(5) : e157 (2005)



FISH: Each chromosome coloured a different colour by a fluorescent label

Reminder



Chromosome: Long DNA molecule, 23 pairs in nucleus

Histones: Special proteins that pack DNA tightly

Chromatin: DNA + histones + “things binding to DNA”

Nucleosomes: Basic unit of DNA + histones

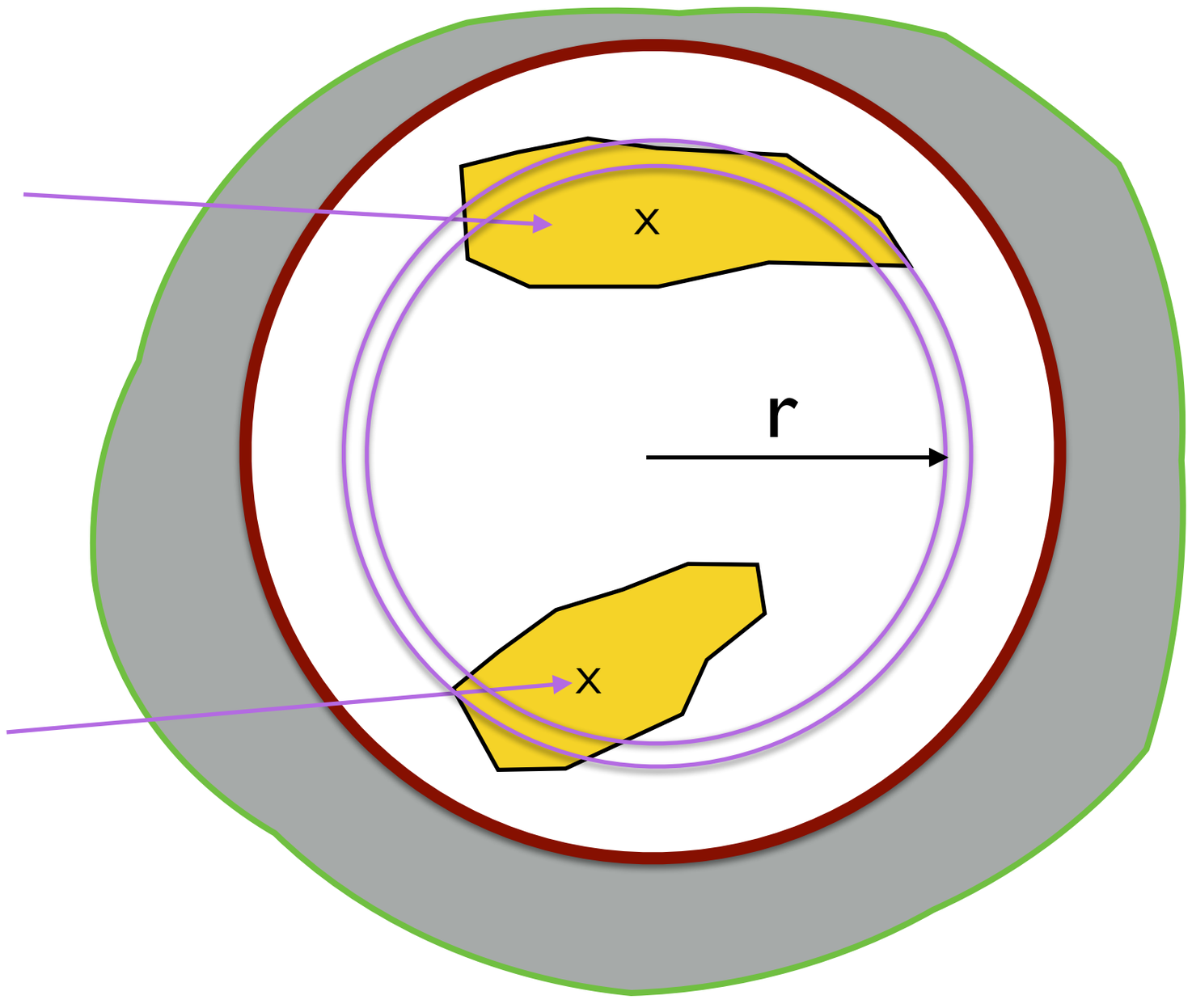
ATP: Energy storage. Hydrolysis releases energy

Chromatin at large scales: What is measured?

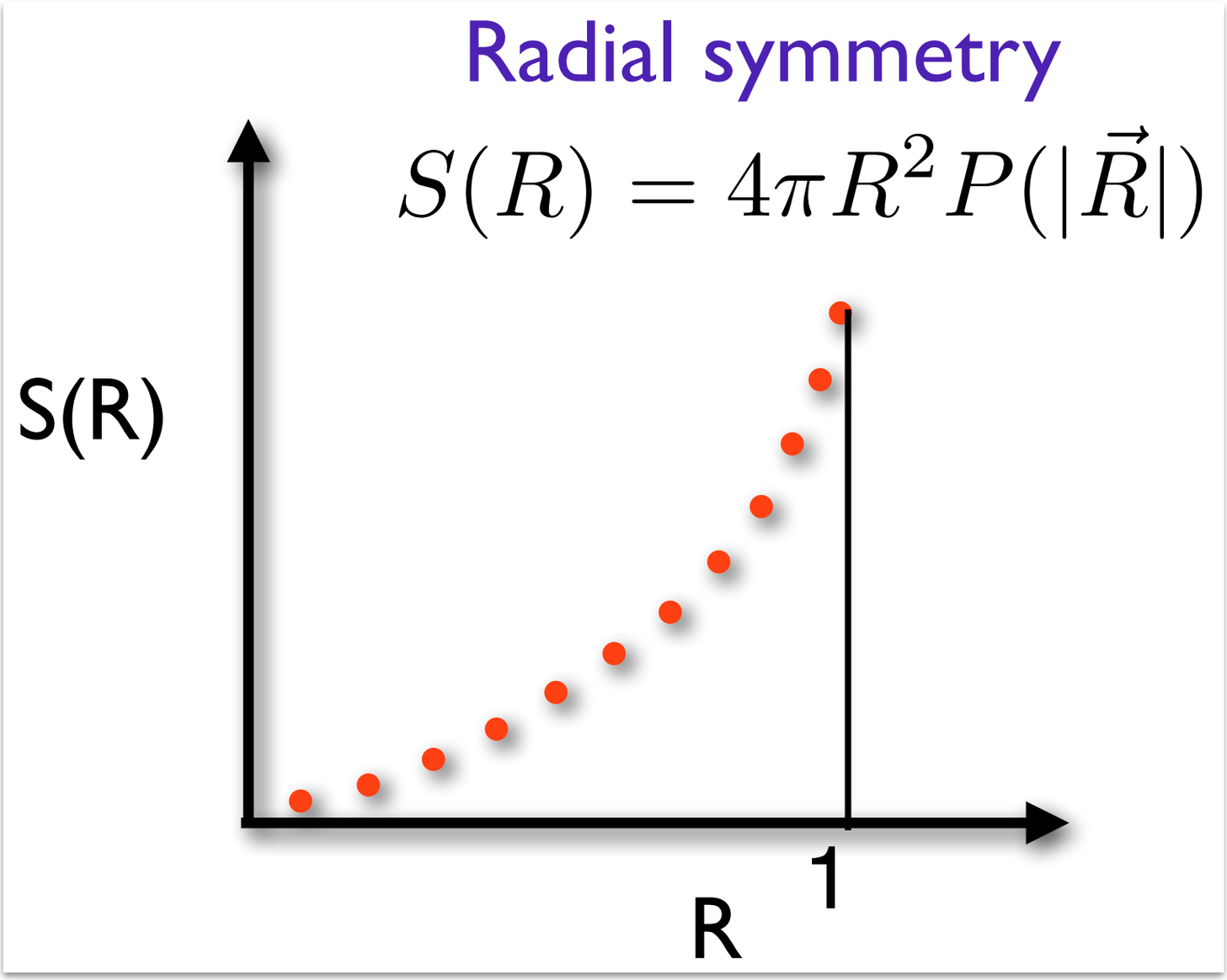
3-d radial densities

DNA density associated with a chromosome

Centre of mass distribution of a chromosome's DNA



Diagnostic for non-random placement



If each chromosome distributed uniformly, then S(R) quadratic, maximum at edge of nucleus

Nuclear architecture is not random

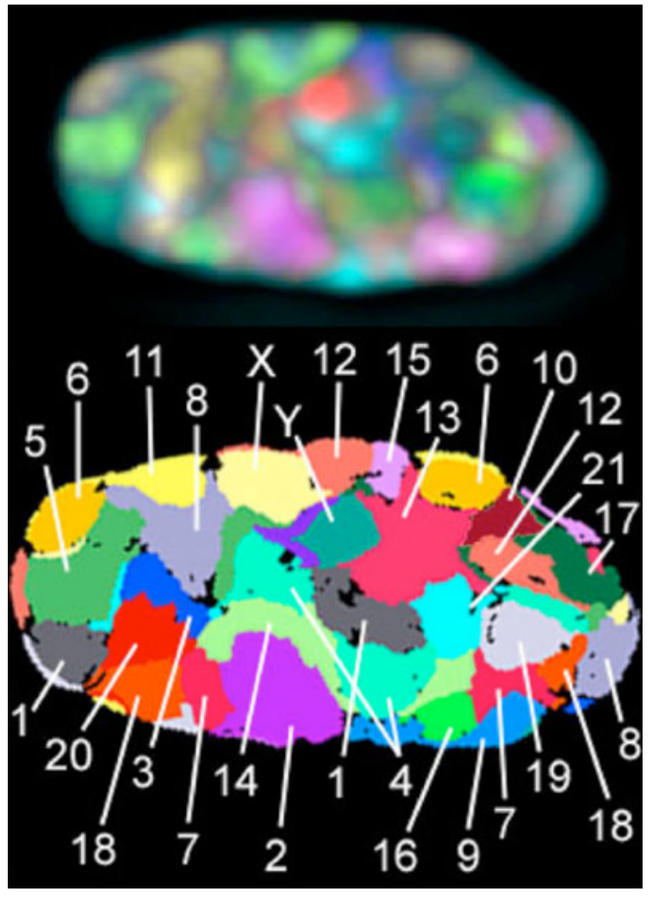
1. Chromosomes are territorial (don't intermingle in the bulk)

2. Gene-rich, actively transcribed DNA (euchromatin) more centrally located than gene-poor regions of DNA (heterochromatin)

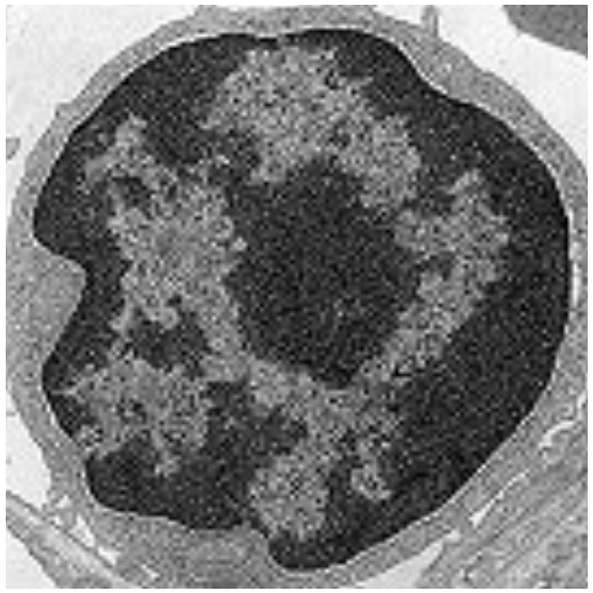
3. Individual chromosomes often radially positioned by gene density. More gene-dense chromosomes associated to nuclear interior

4. Radial positioning by chromosome size also seen in some cell types

Bolzer et al, PLoS Biol 3(5) e157 (2005)



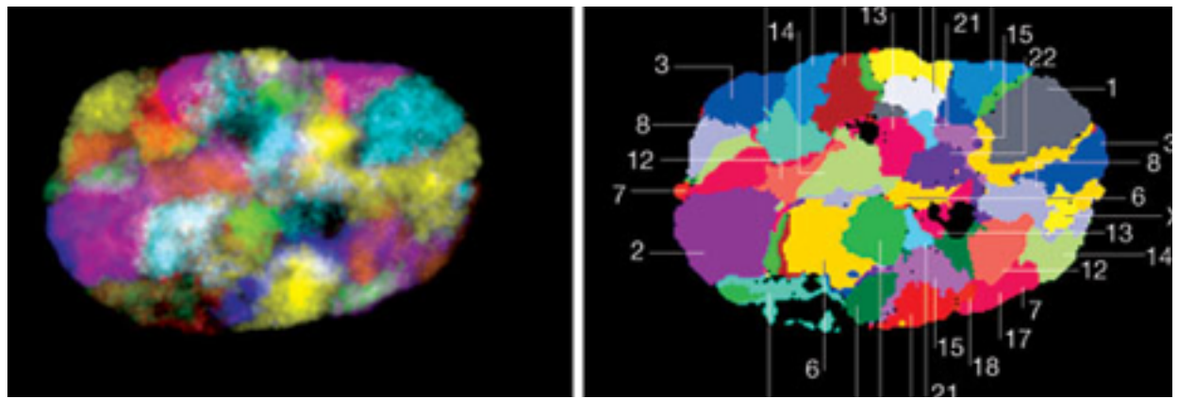
H. Jastrow (Mainz): Anatomy for the Internet (EM)



Peripheral gene-poor chromatin/Interior gene-rich chromatin

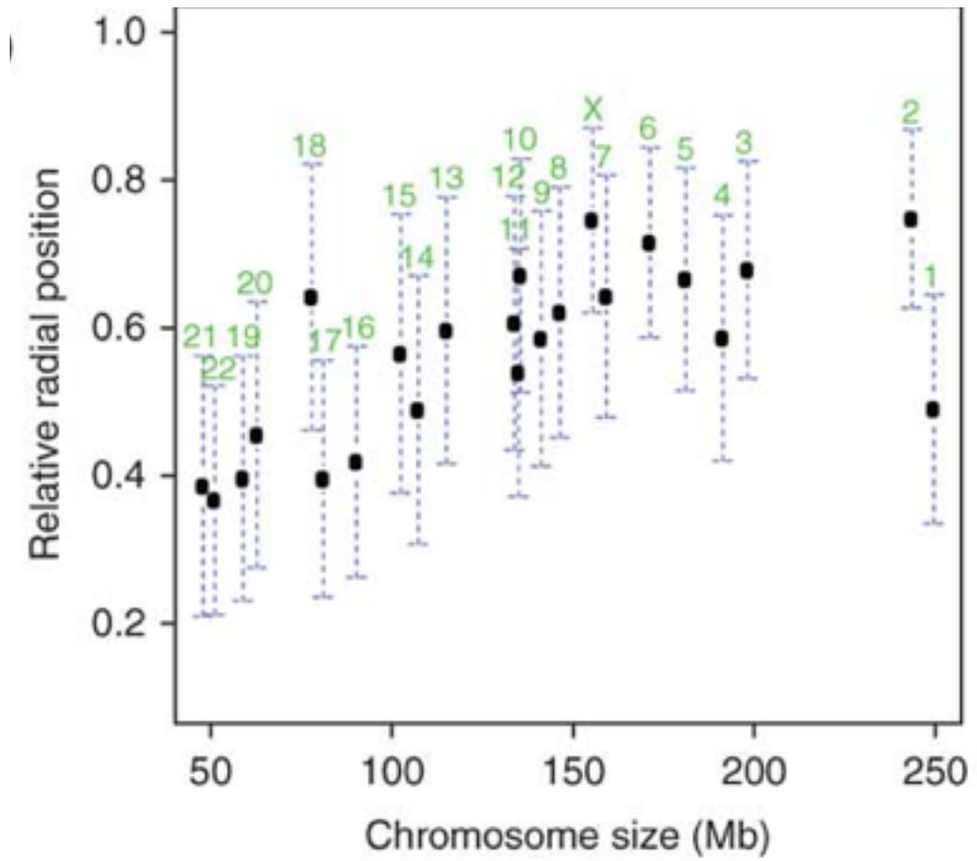
2

Speicher & Carter, Nat. Rev. Genet. 6(10) 782-92(2005)



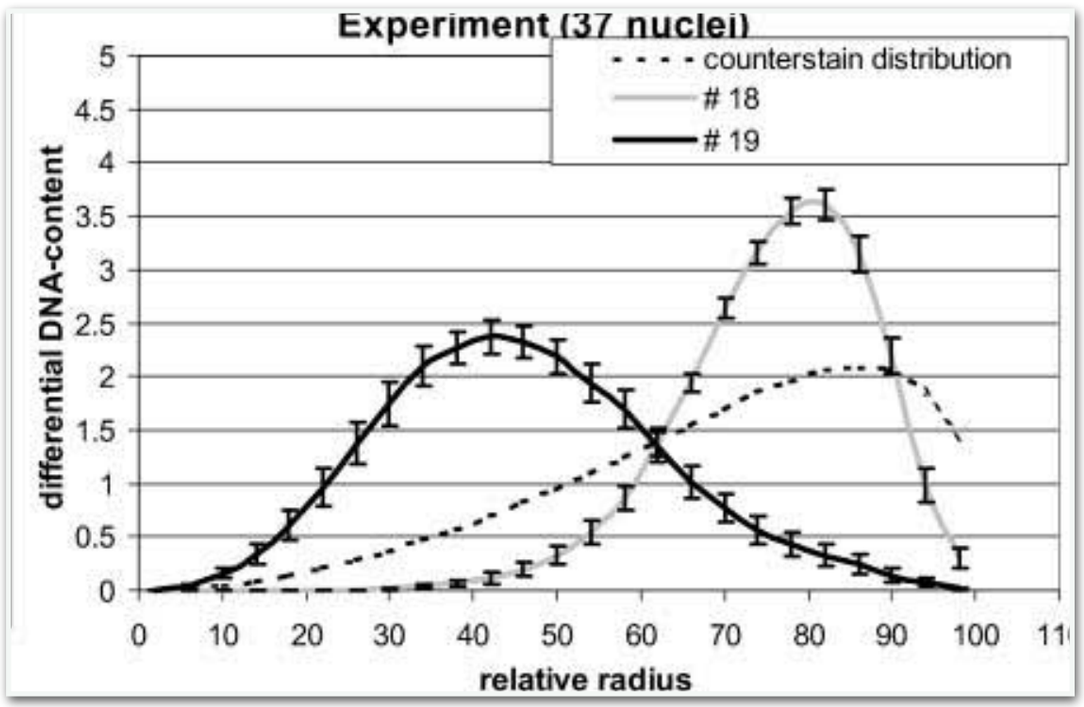
1 Chromosome territories

Experimental Data



Kalhor et al, Nat Biotech 30, 90–98 (2012)

3 Chromosome positioning by gene density



Kreth et al Biophys J 86(5): 2803-12 (2004)

4 Chromosome positioning by size

Hypothesis

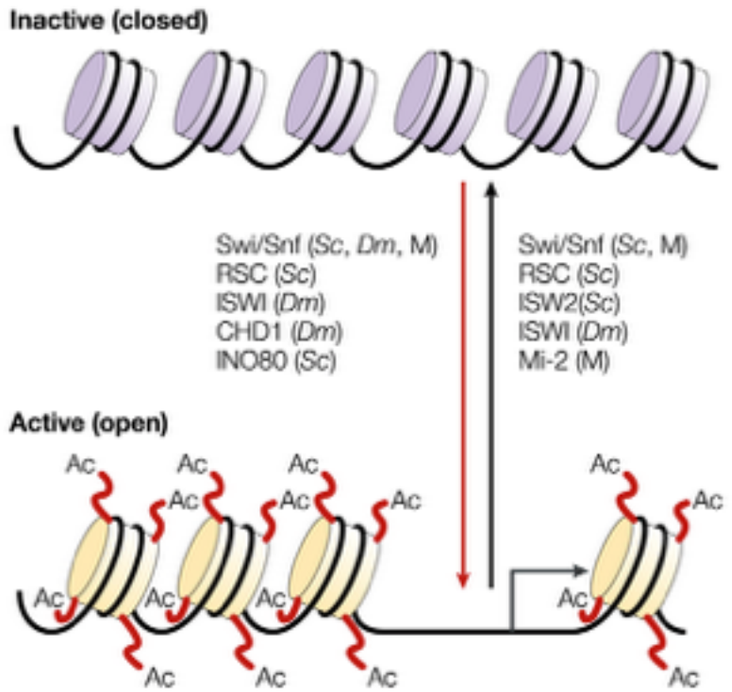
Activity, inhomogeneous across gene-rich and gene-poor regions, is a central determinant of large-scale nuclear architecture

N. Ganai, S. Sengupta and G.I. Menon, *Nucl. Acids Res.*, 42(7) 4145-59.(2014)

Where does activity come from?

Chromatin in living cells has many energy consuming (active) processes which act on it, exerting forces locally

Transcription, chromatin remodelling, DNA repair ..



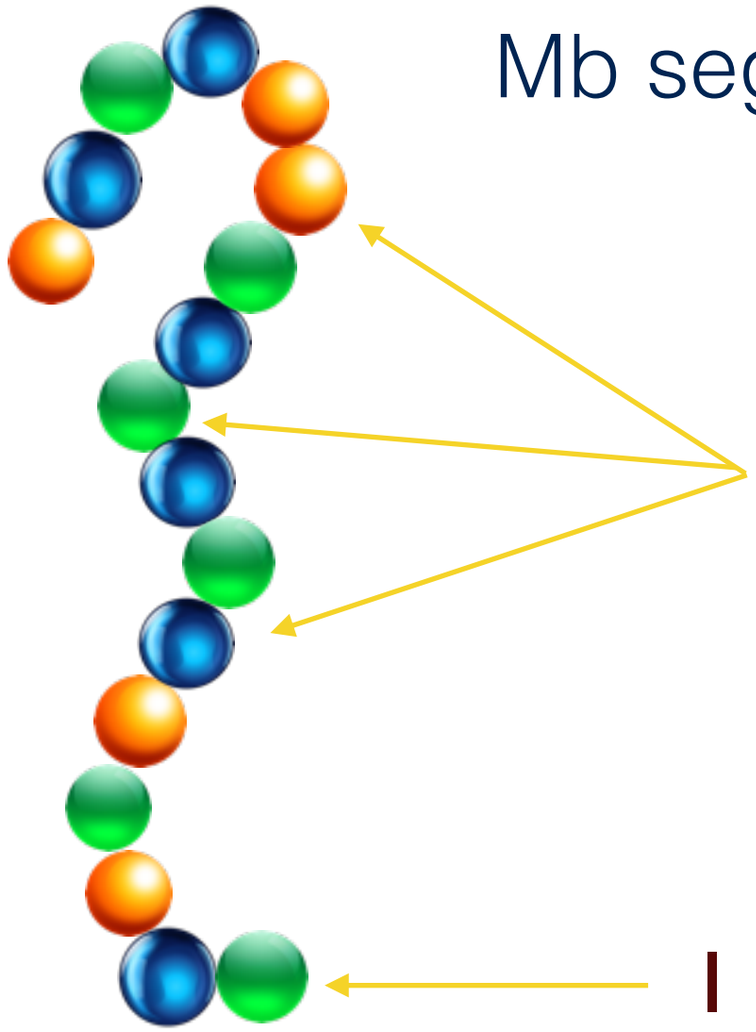
Nature Reviews | Molecular Cell Biology

Tsukiyama, Nat. Rev. Mol. Cell Biol. 3, 422-429 (2002)

All theoretical work so far on nuclear architecture ignores such effects

A model for activity of chromatin

Model each chromosome as a set of 1 Mb segments (monomers)

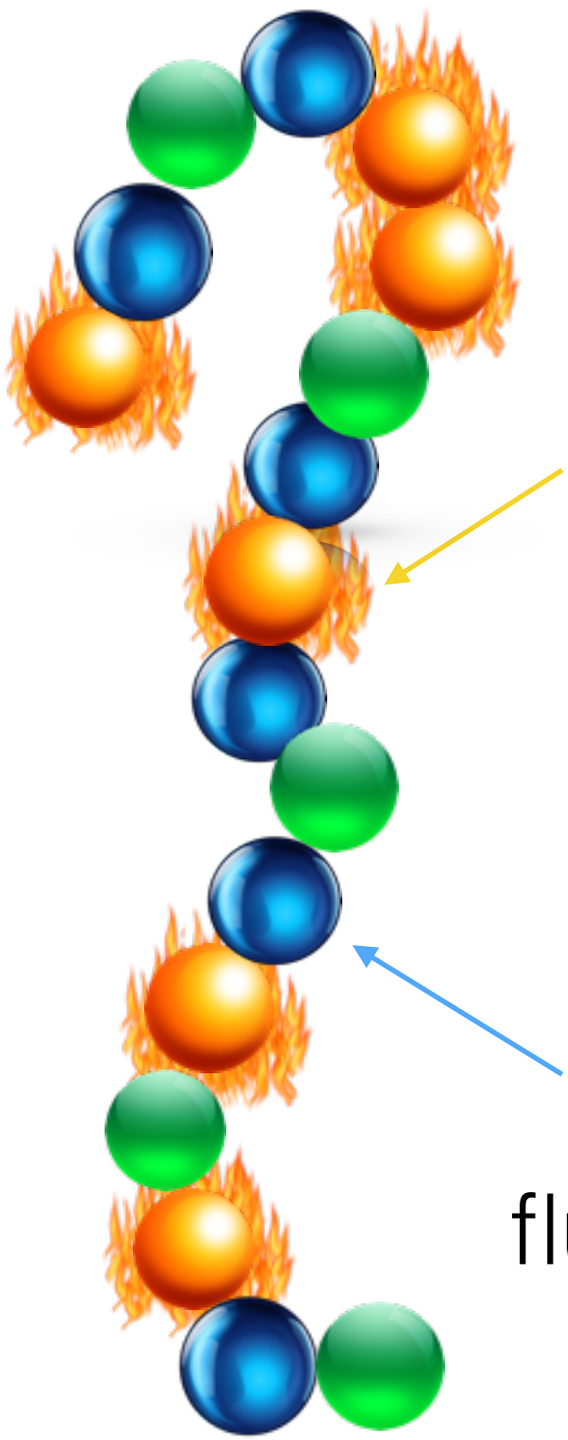


Different gene densities in these monomers

1 Mb monomer

Such 1 Mb segments are known to be "building blocks" of chromosome territories

The "activity" of each monomer should be set by (transcription levels of) the genes it contains



<u>More active</u>: Larger fluctuations. High effective temperature

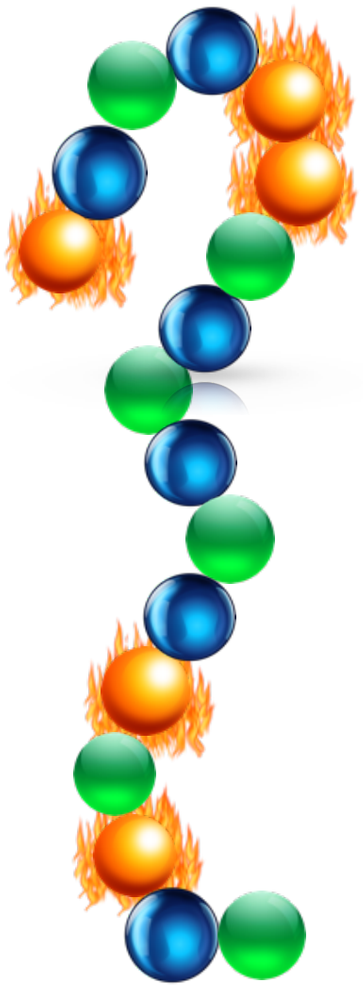
<u>Less active</u>: Smaller fluctuations. Low effective temperature (= physiological)

The “activity” of each 1 Mb monomer comes from driving at 10-20 bp scale. Large scale separation

Each such event consumes energy and exerts forces. Once averaged,translates physically to random forces on monomers

Coarse-grained, equivalent to a temperature, but not the same across monomers Models physical effects of activity most simply

Simplest model ignores cell type specificity

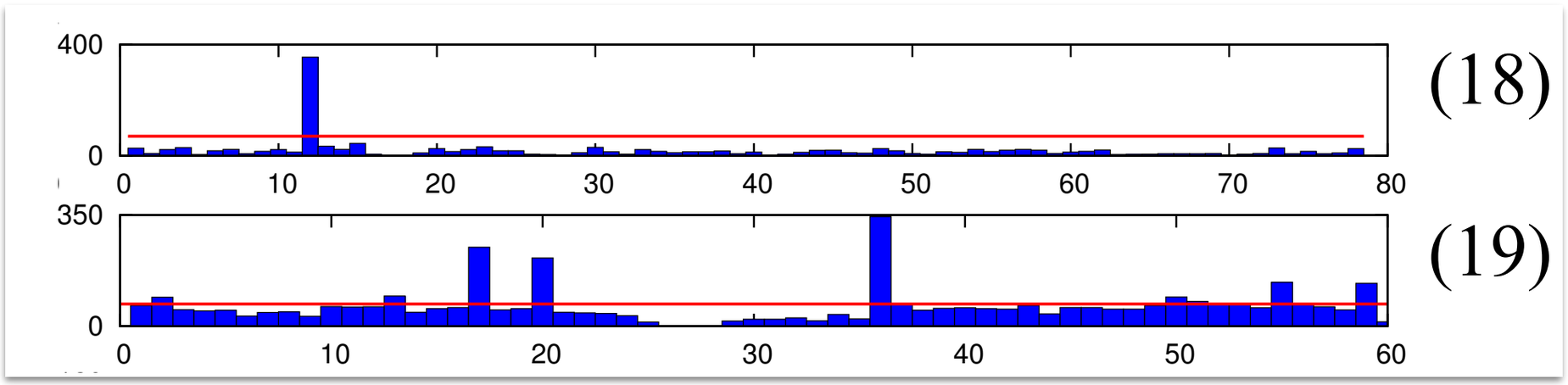


1. Compute gene density across each 1 Mb segment on each chromosome

2. Consider the top 5% of monomers by gene density.

3. Make them "active"; see a larger effective temperature (T = 20; passive: T = 1)

4. Each chromosome has a unique pattern (a fingerprint) of active/passive monomers



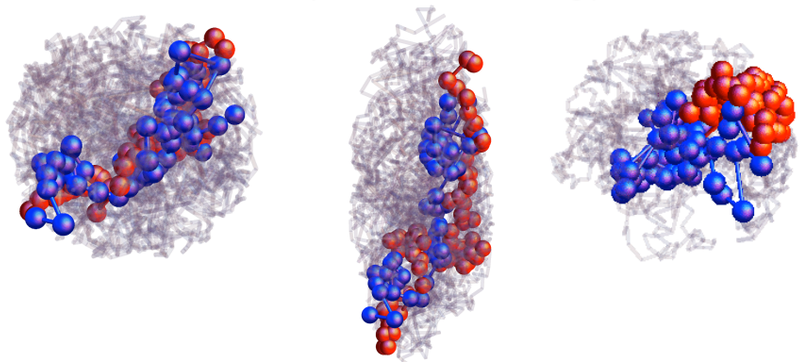
Brownian dynamics, 3-d: 6098 monomers, Gene density: GENECARDS database
Self-repelling monomers, spring potential for bonds, Gaussian core potential
Varied nuclear shape: spherical, ellipsoidal + passive & active confinement
Fraction of permanent loops Random loop model: Bohn et al [PRE 76, 051805 (2007)]

$$\zeta \frac{d\mathbf{r}_i}{dt} = \mathbf{F}_i + \eta_i$$

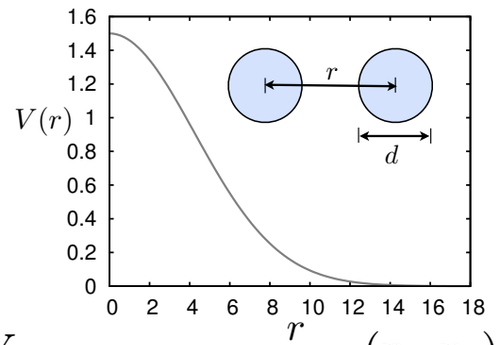
$$\langle \eta_i^\alpha(t)\eta_j^\alpha(t')\rangle = 2k_BT_i\zeta\delta_{ij}\delta(t-t').$$

We generalise the model of [Kreth et al, Biophys J 86(5): 2803-12 (2004)] to include activity, active and passive confinement and varied nuclear shapes

A. B. C.

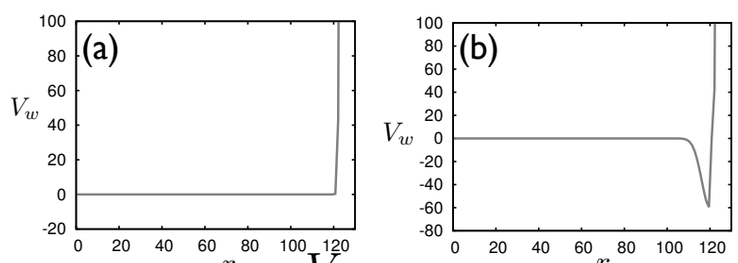


$$V_{neighbour\ monomers}(\mathbf{r}_i,\mathbf{r}_{i+1}) = \frac{1}{2}k(|\mathbf{r}_i-\mathbf{r}_{i+1}|)^2$$



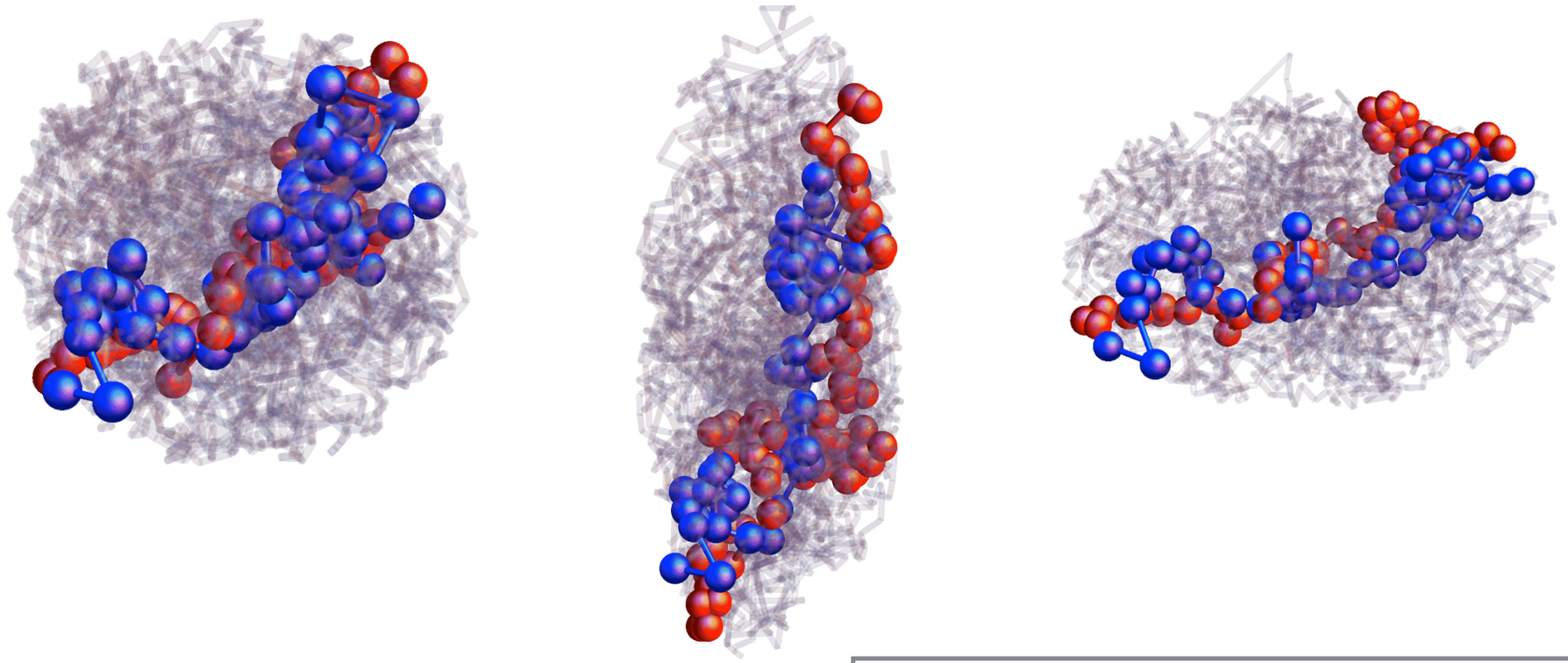
$$d \simeq 500nm,$$
$$R_0 \simeq 6.7\mu m$$
$$\ell_0 \simeq 600nm$$
$$V_0 \simeq 1.5k_BT_{eq}$$

$$V_{monomer-monomer}(\mathbf{r}_i,\mathbf{r}_j) = V_0\exp\left(-|\mathbf{r}_i-\mathbf{r}_j|^2/\sigma^2\right)$$



$$V_{wall}(\mathbf{r}_i) = \frac{V_{conf}}{a^5}(|\mathbf{r}_i|-R_0)^5, \qquad |\mathbf{r_i}| > R_0$$

Our model simulates individual chromosomes within a nucleus

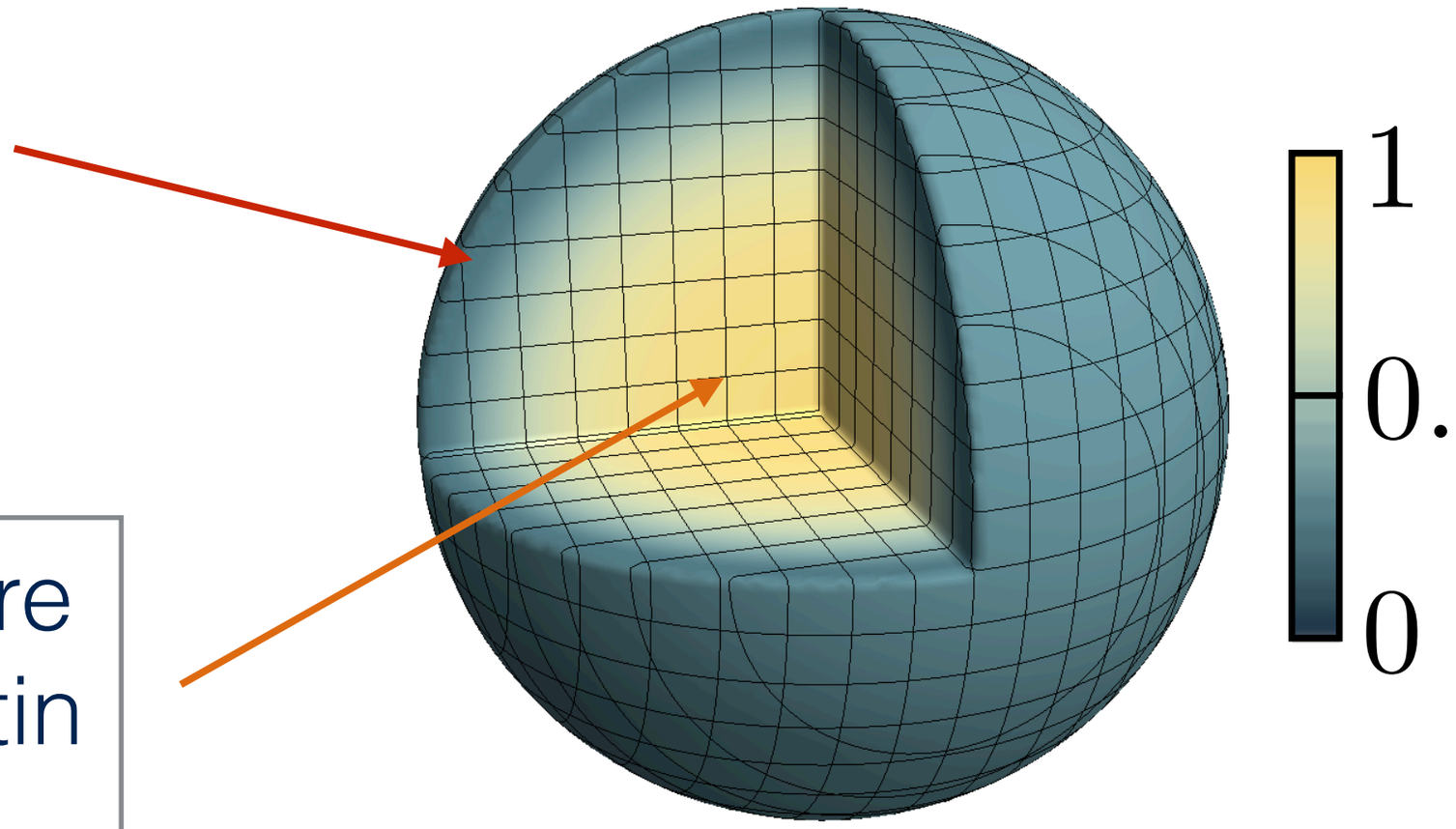


Chromosome 18/19

Theoretical Predictions

Gene-poor, less active chromatin (heterochromatin) near periphery

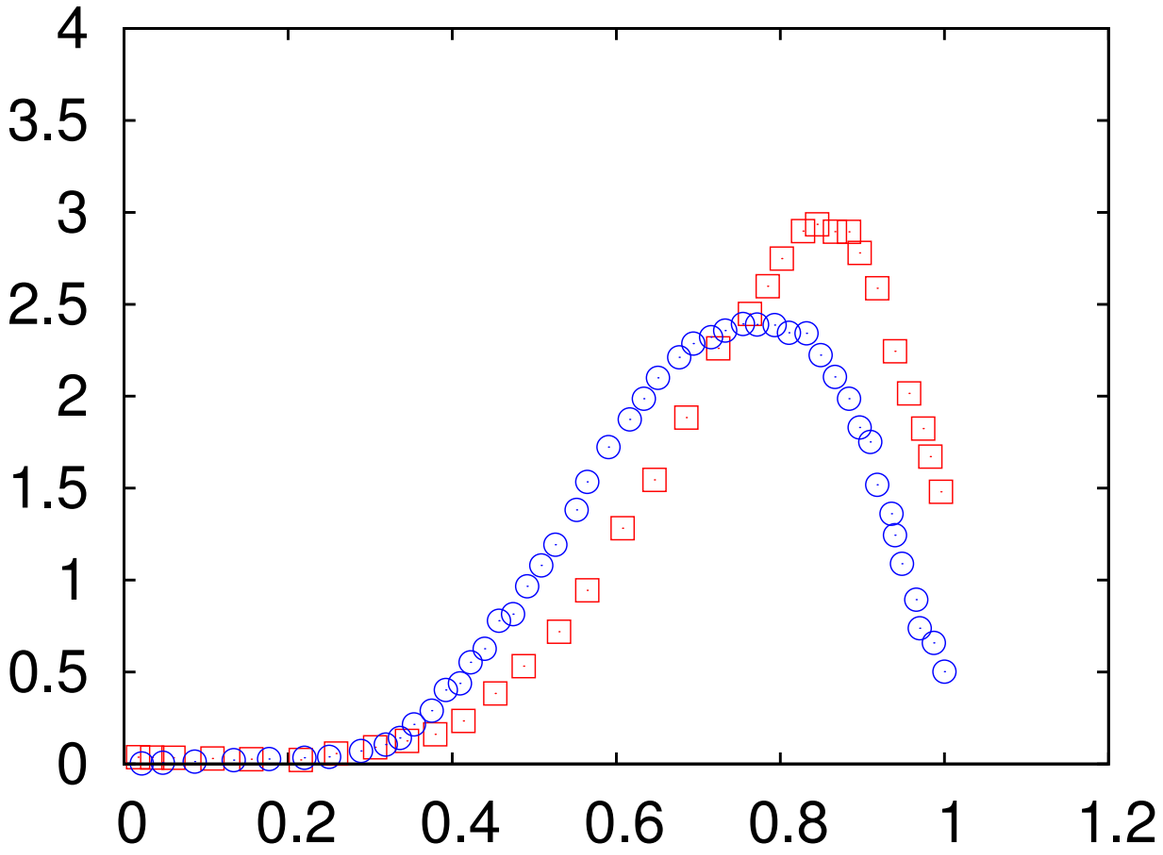
Gene-rich, more active chromatin (euchromatin) more central



Shading indicates local gene density, low = dark, high = bright

Theoretical Predictions

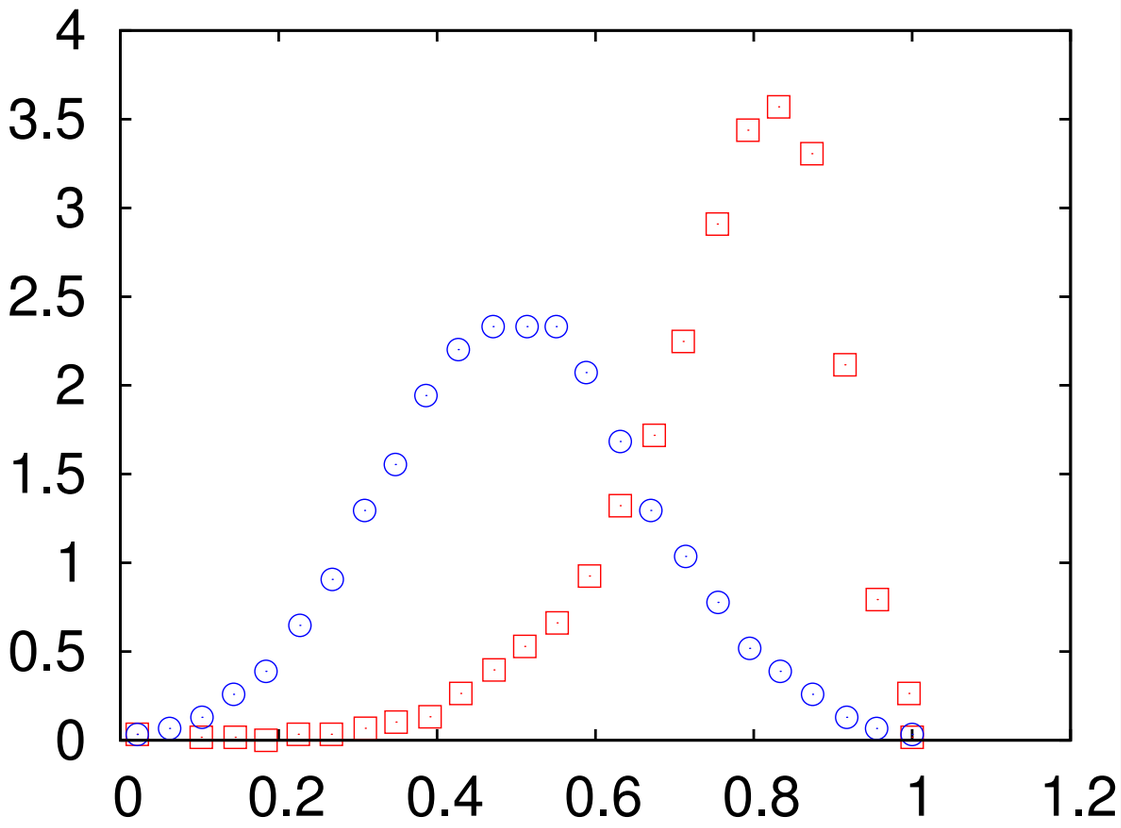
Expts: Cremer et al, Chrom. Res. 2001, Weierich et al Chrom Res. 2003, Kreth et al Biophys J 2005



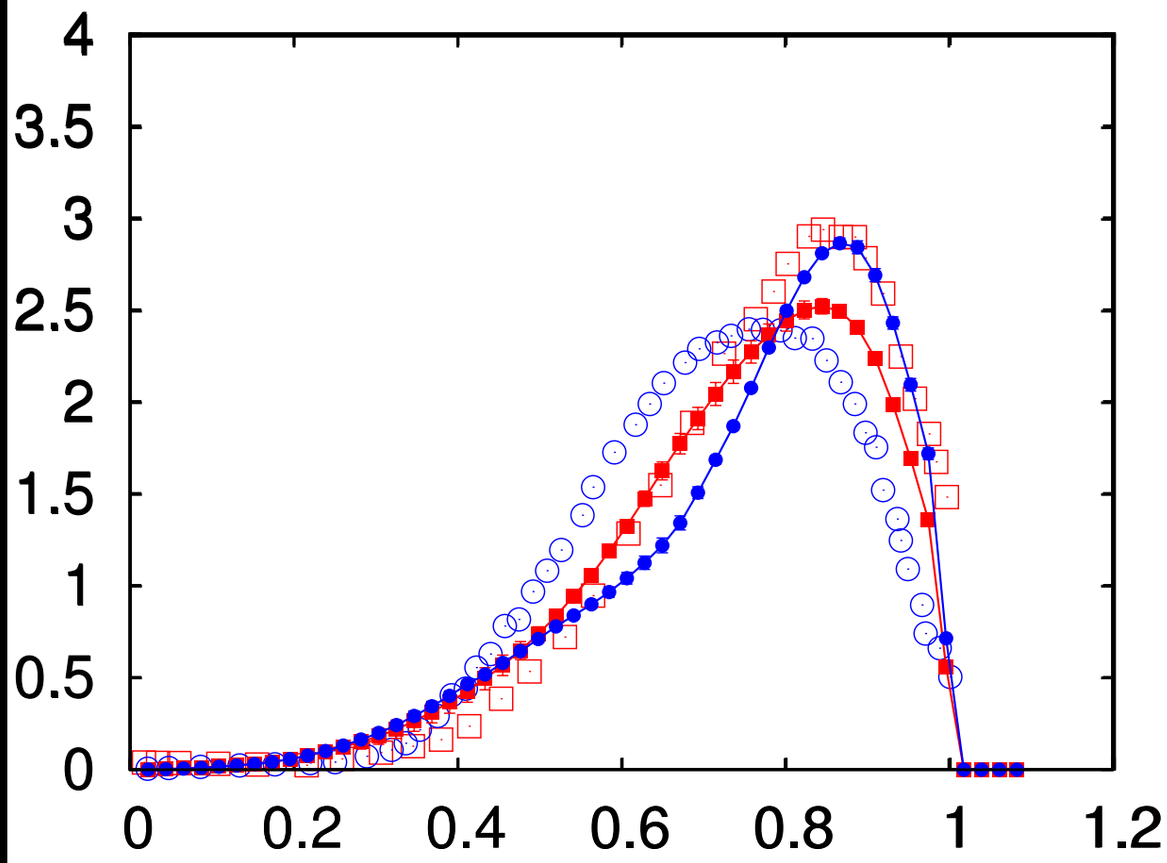
Chromosome 12/20

Experimental data

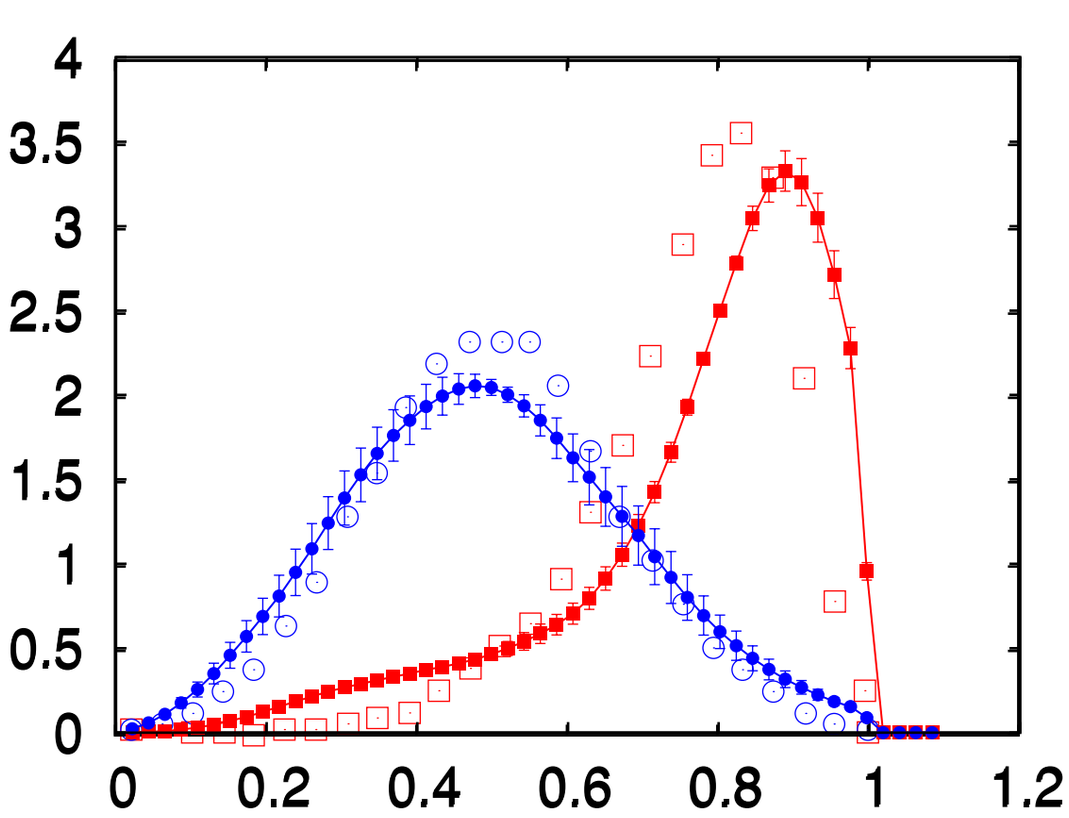
Chromosome 18/19



Theoretical Predictions



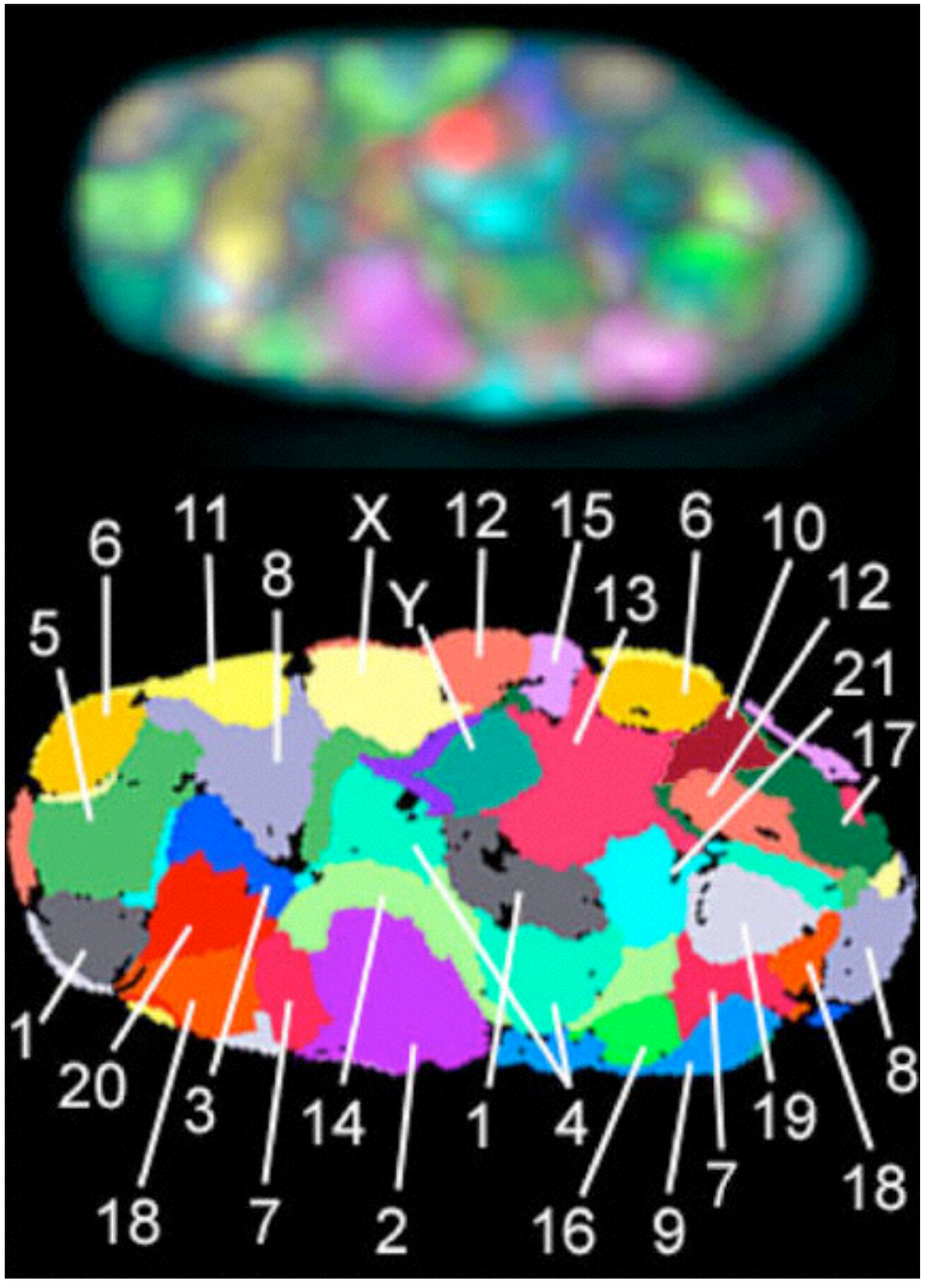
Chromosome 12/20



Chromosome 18/19

Symbols: Experimental data
Lines: Simulations

Chromosome Territories: Rabl 1885, Boveri 1908, Stack 1977 +

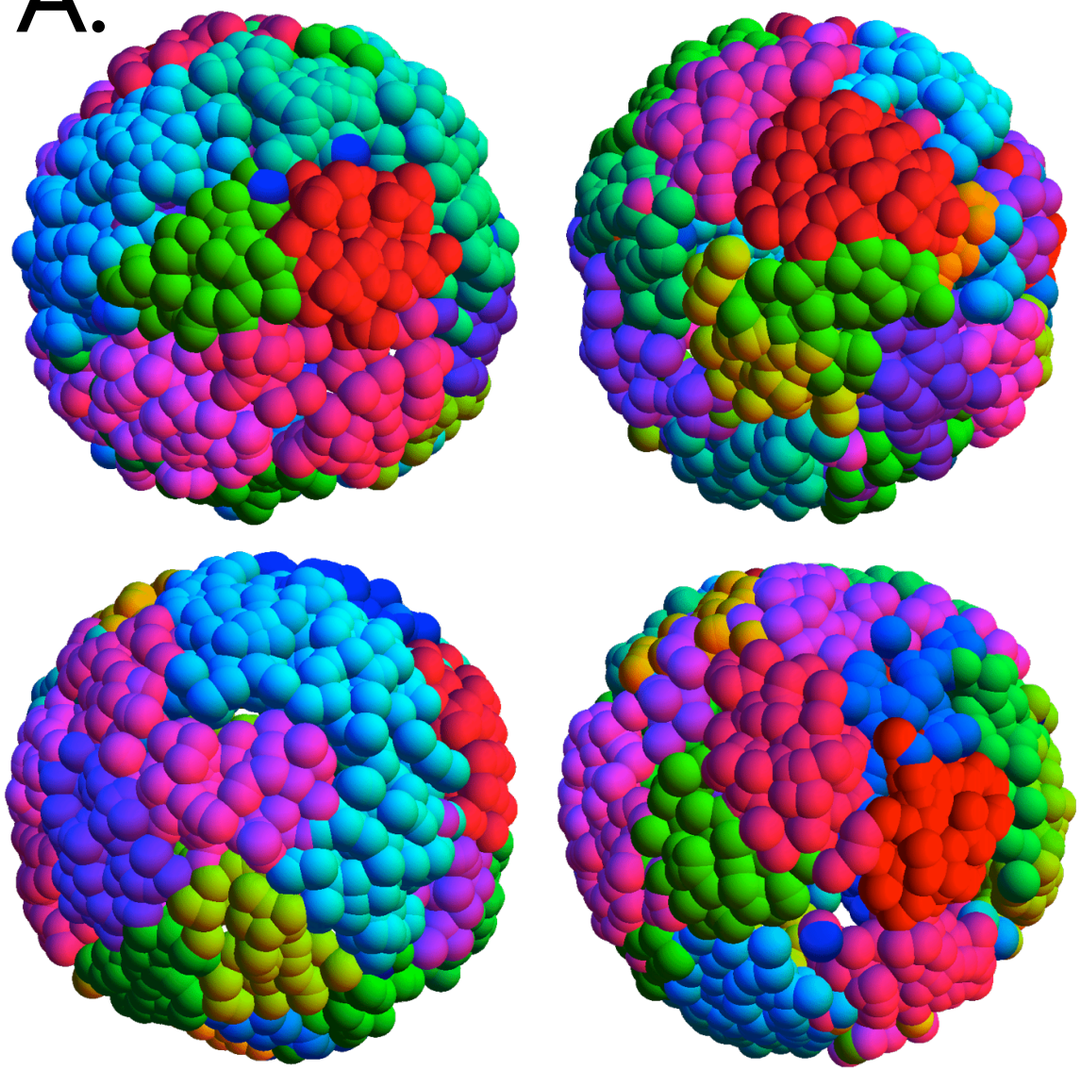


3-d FISH images

Bolzer et al, PloS Biology (2005)

Theoretical Predictions

A.

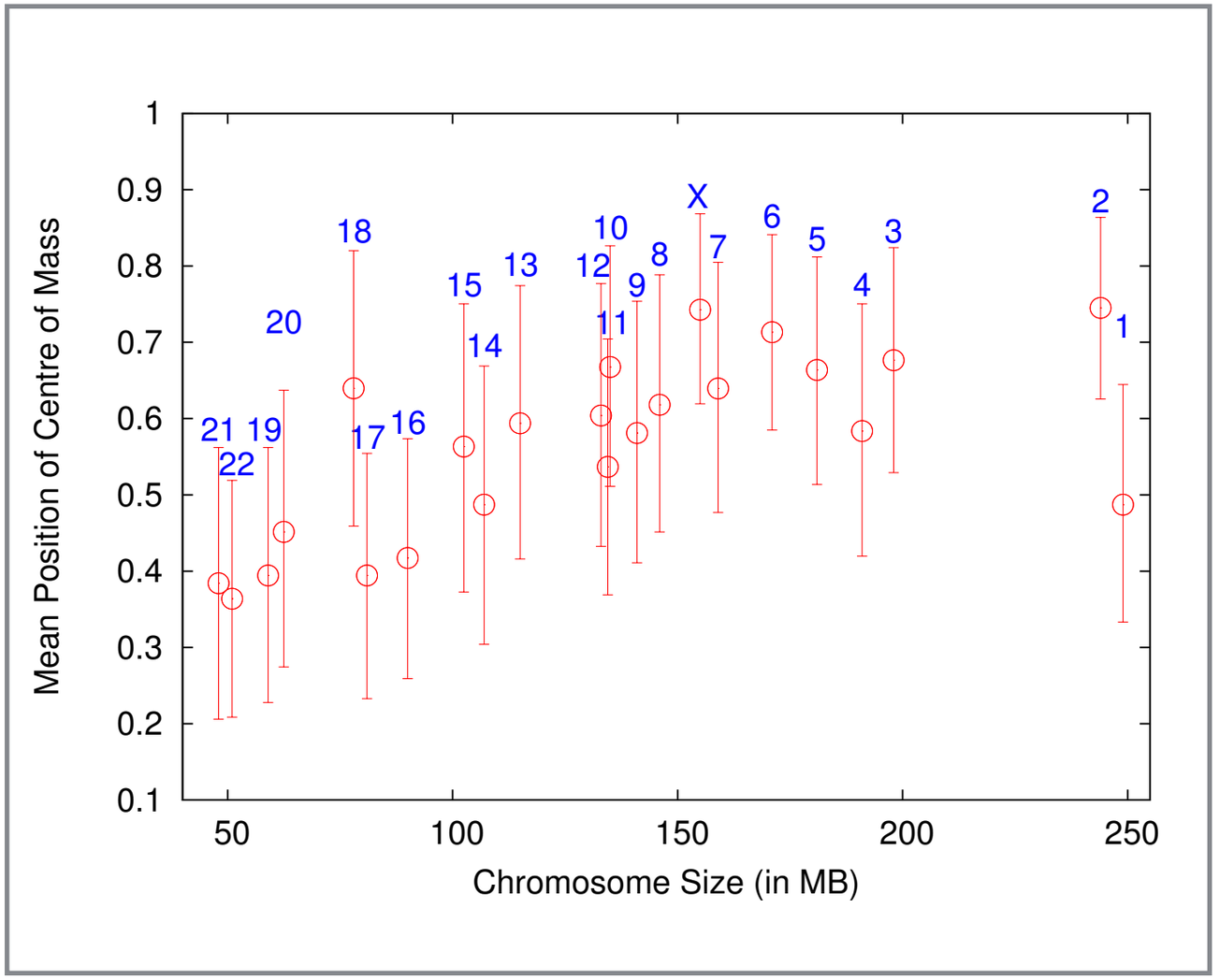


Simulations starting from many different initial conditions. Each chromosome a different colour

Chromosome territoriality follows naturally from our model

Chromosome territoriality is robust

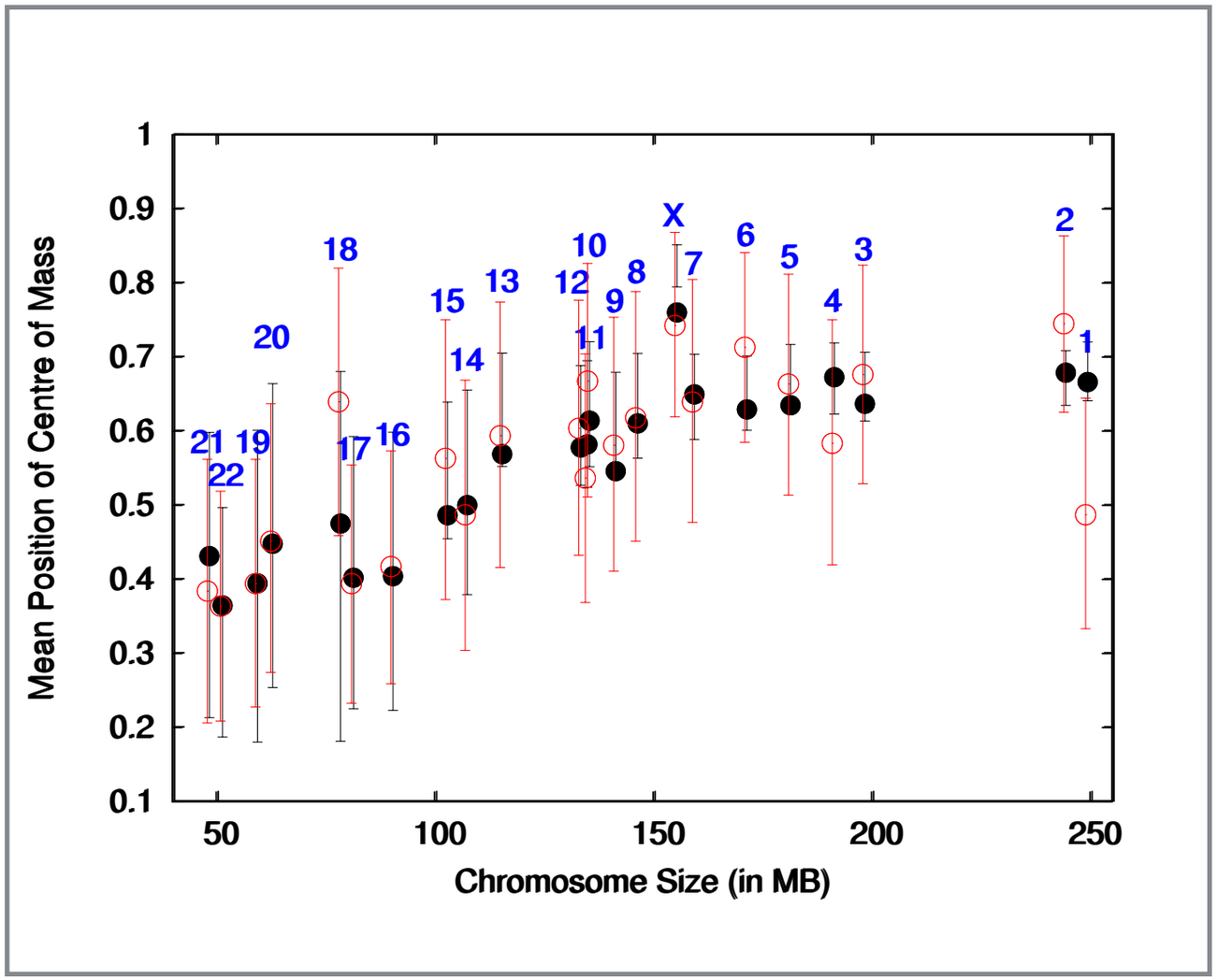
Centre of Mass Distribution



Kalhor et al, Nat Biotech (2012)

Open circles: Experiment

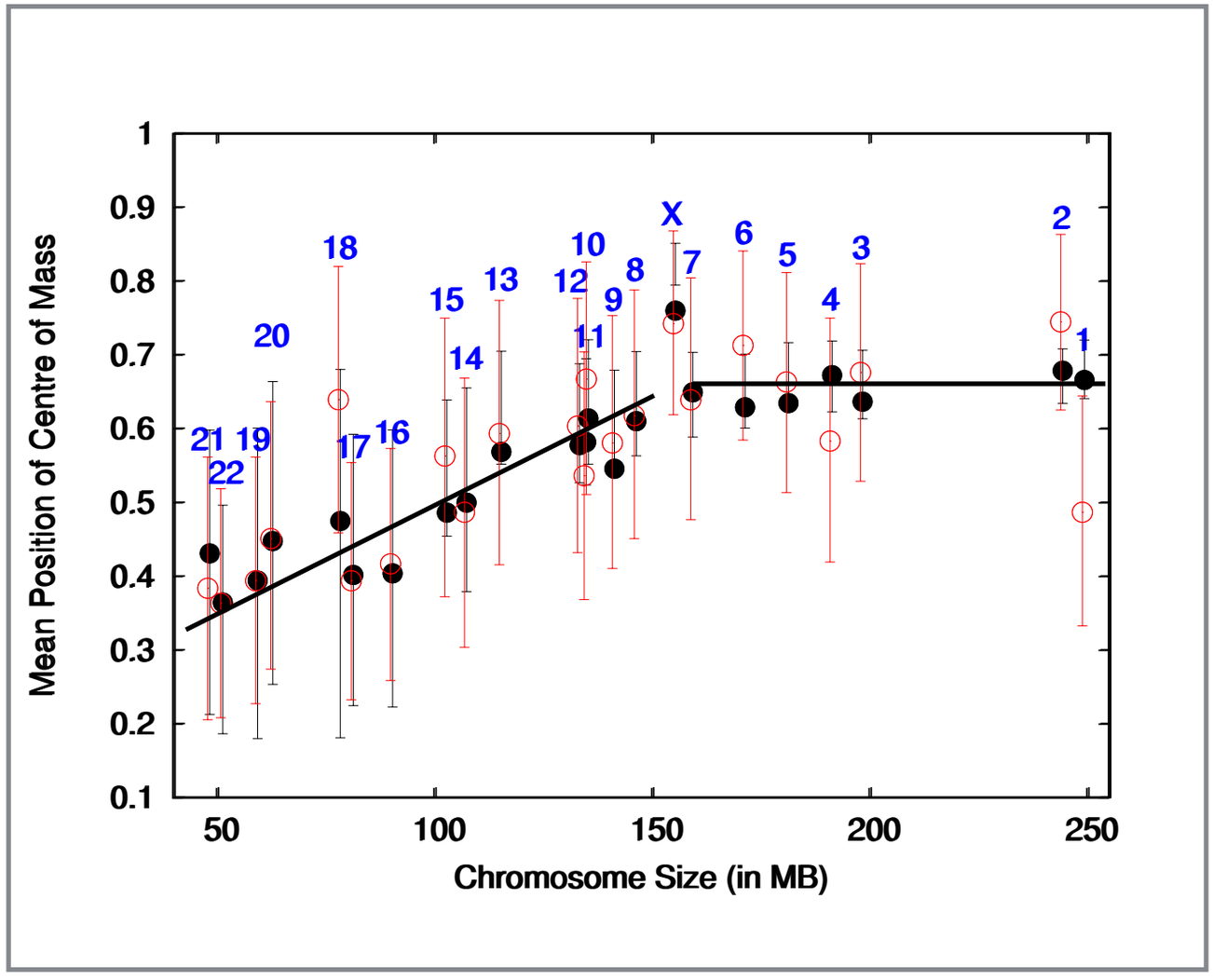
Theoretical Predictions



Open circles: Experiment

Closed circles: Theory

Theoretical Predictions



Captures systematics of the experiments. Reasonable qualitative and quantitative agreement

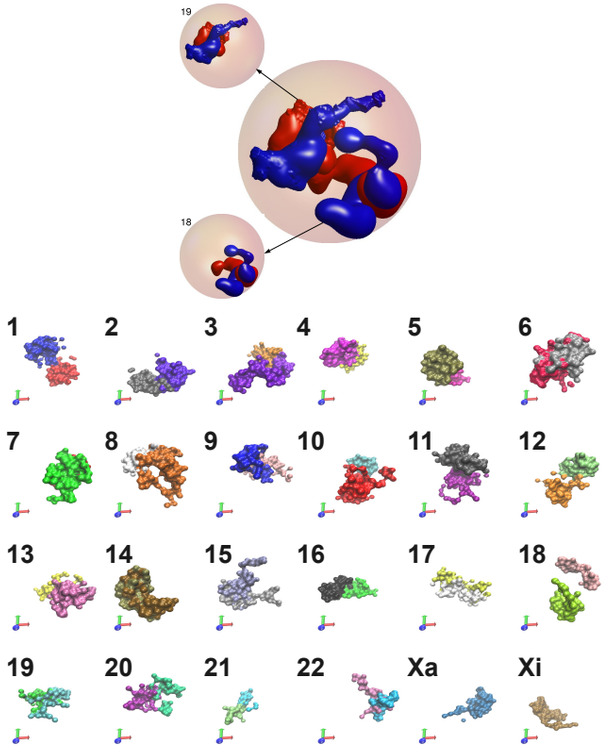
A statistical mechanics and biophysics of large-scale nuclear architecture?

Simplest model (surprisingly good)

- No cell-type specificity
- Number of genes, averaged over 1 Mb region, proxy for activity.
- Random loop model enforces compactness at large scales

More refined model (additional biological input)

- Transcriptome data (RNA-seq) for cell-type specific transcription levels - infer activity from expression
- Hi-C measurements - infer looping of individual chromosomes



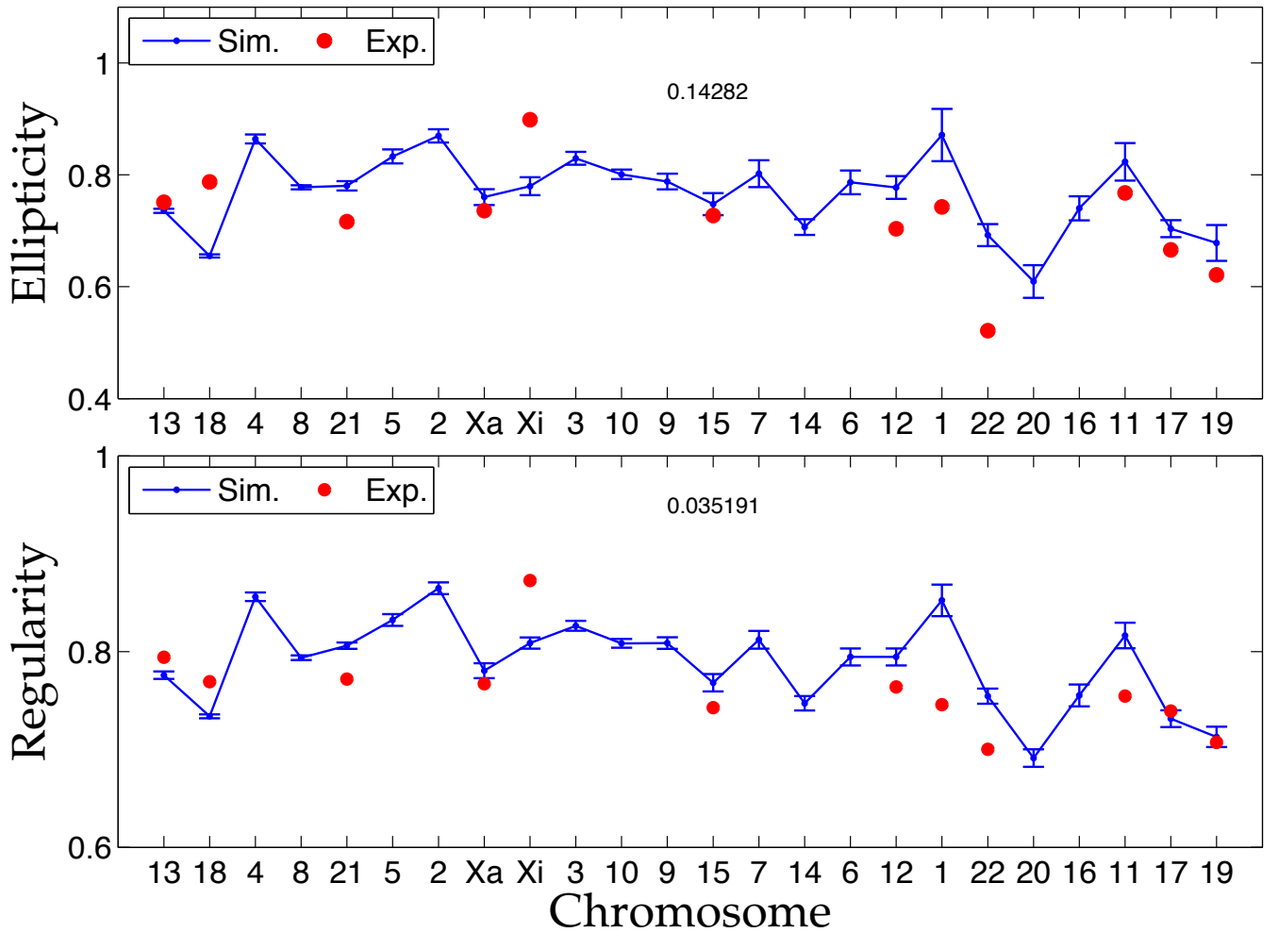
- Configurational Snapshot: Chromosomes 18 and 19 (2 homologs each)
- Territories for all chromosomes. 2d projections to compare to available data (2d FISH)
- Structural measures: Regularity from surface to volume ratio, ellipticity measures anisotropy

- More active chromosomes are rougher and less spherical in character
- Qualitative agreement with experimental data

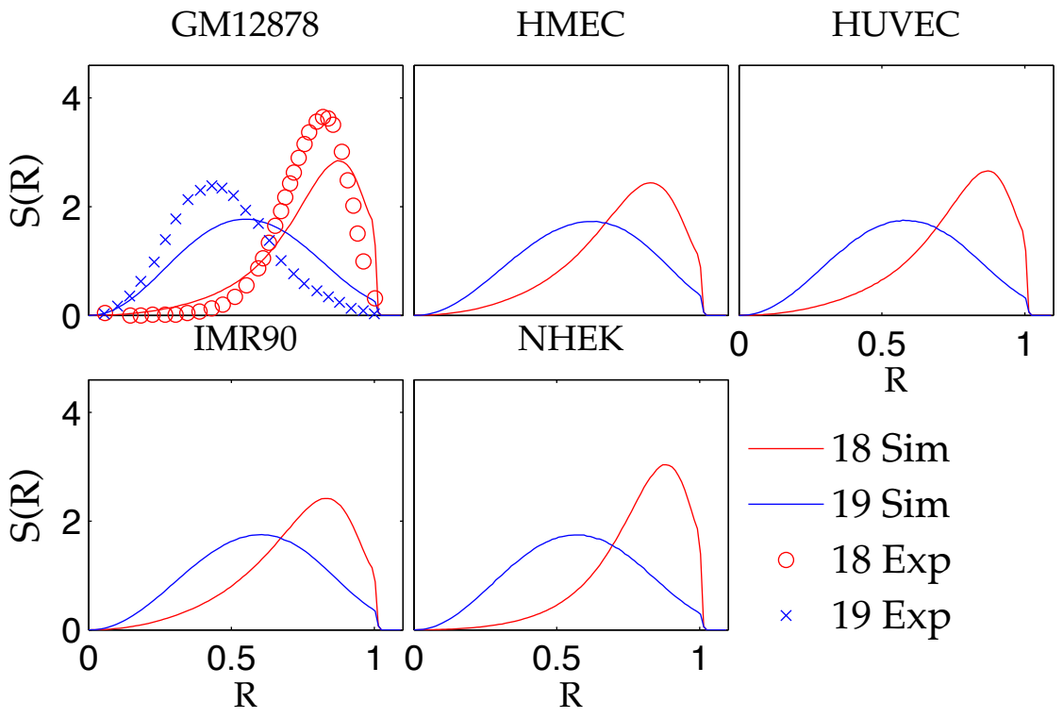
Data: Sehgal at al, *Chromosoma* (2014)

WI-38 and MRC5 normal human fibroblast cells

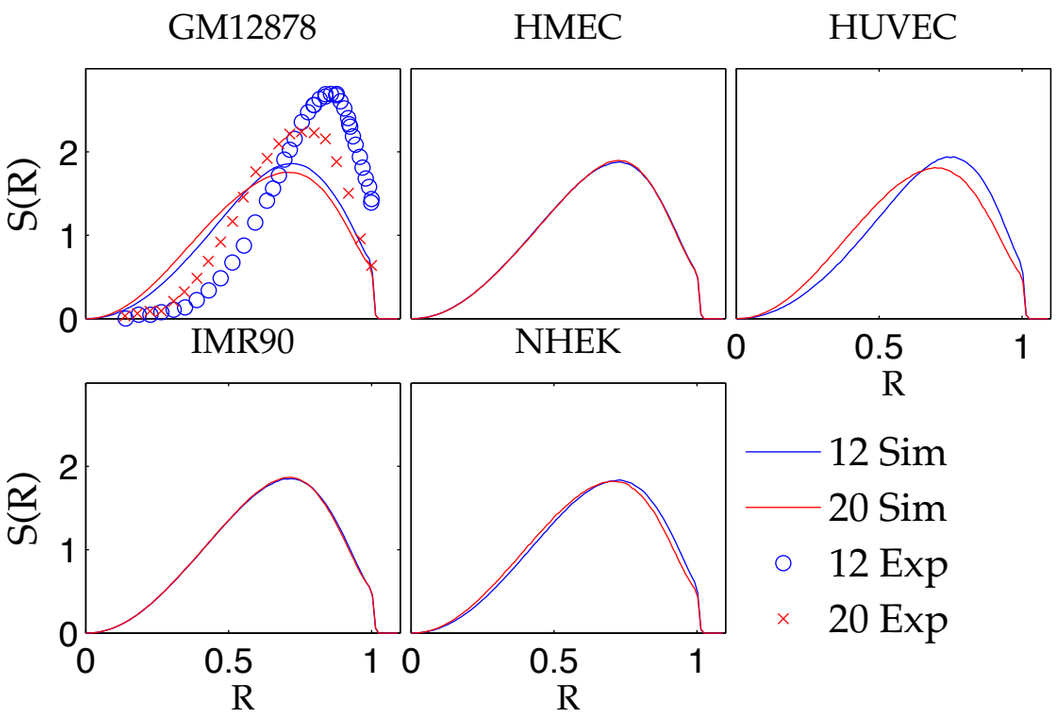
Simulations vs experimental data



DNA density distribution: 18/19

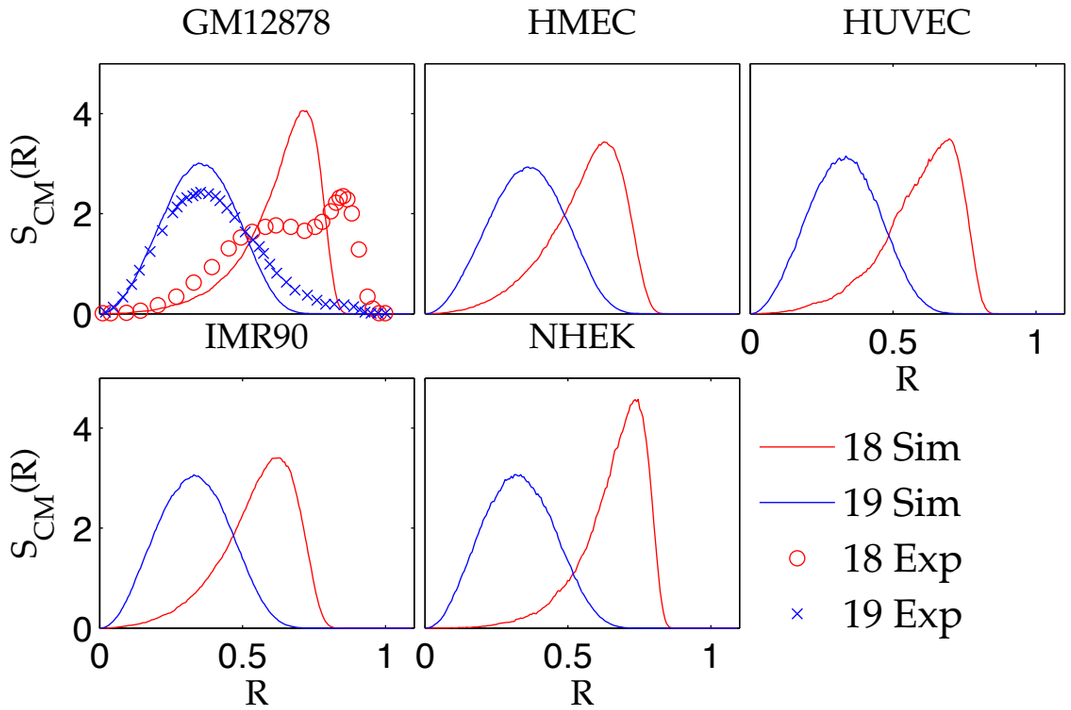


DNA density distribution: 12/20



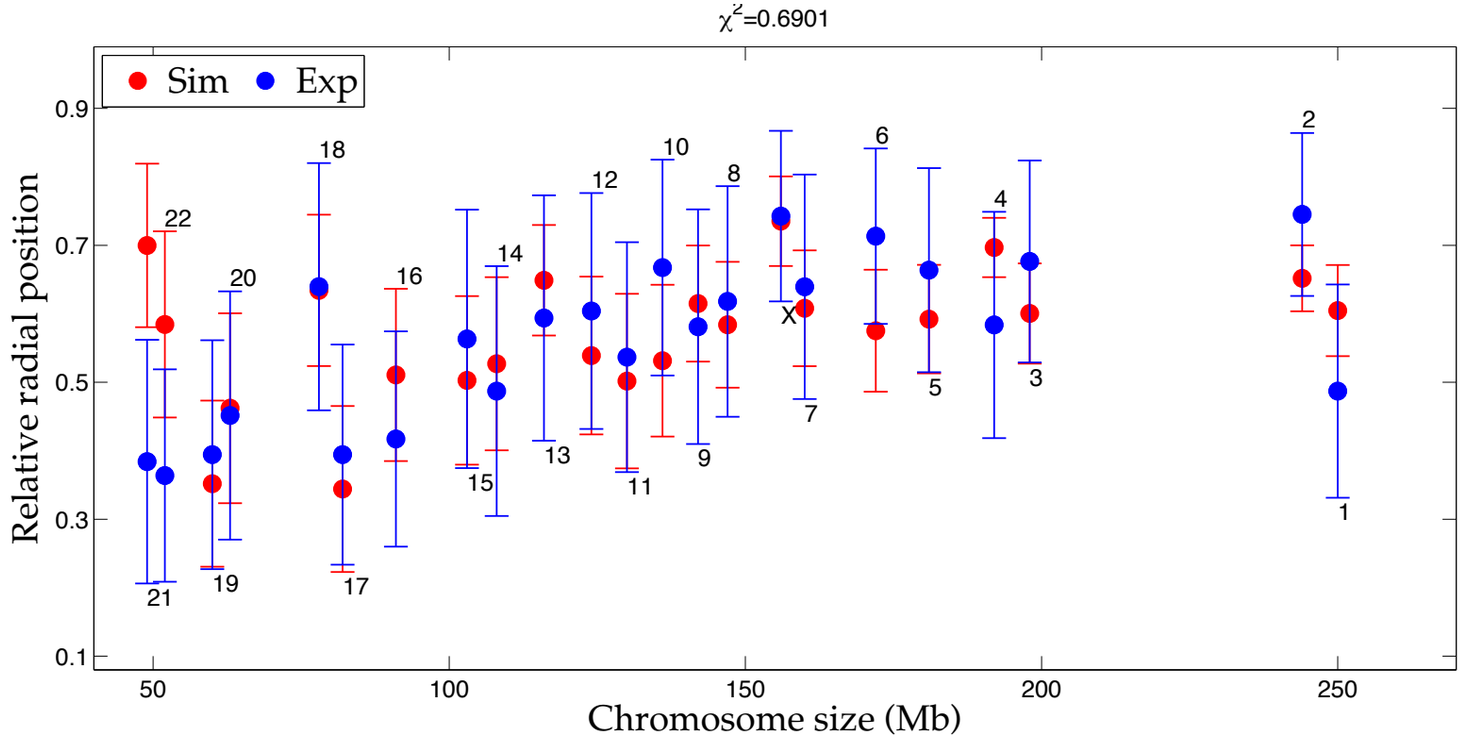
Data: Kalhor et al, Nat Biotech 30, 90–98 (2012)

Chromosome centre-of-mass distribution: 18/19



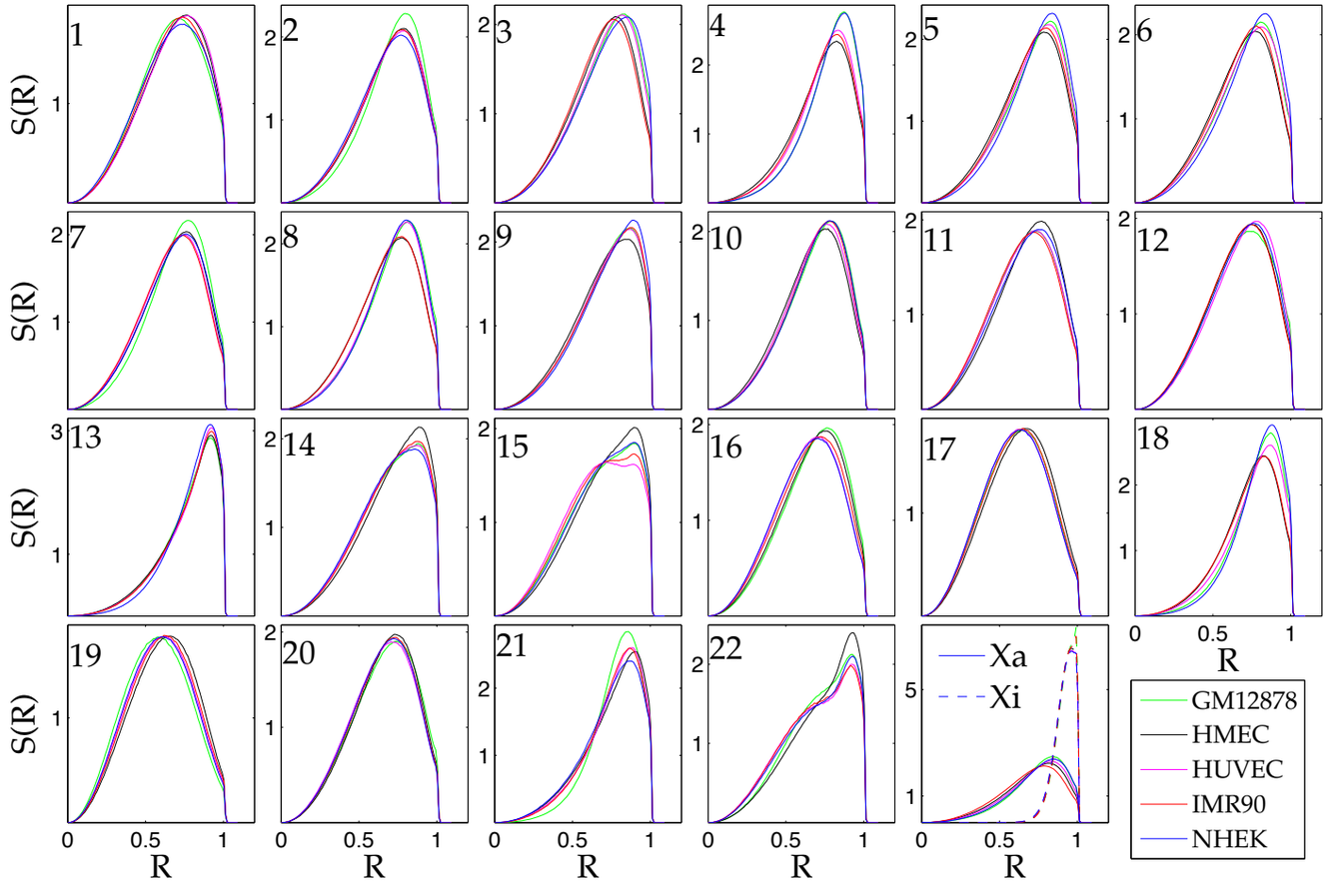
- Reasonable agreement with experiment
- Stronger cell-type dependence in centre-of-mass distributions.

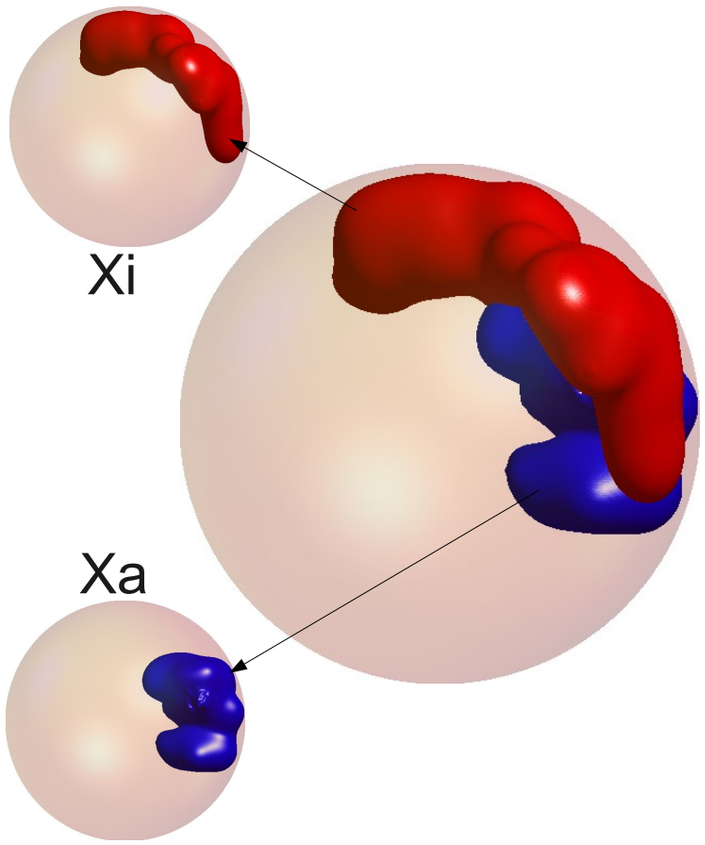
Data: Kalhor et al, Nat Biotech 30, 90–98 (2012)

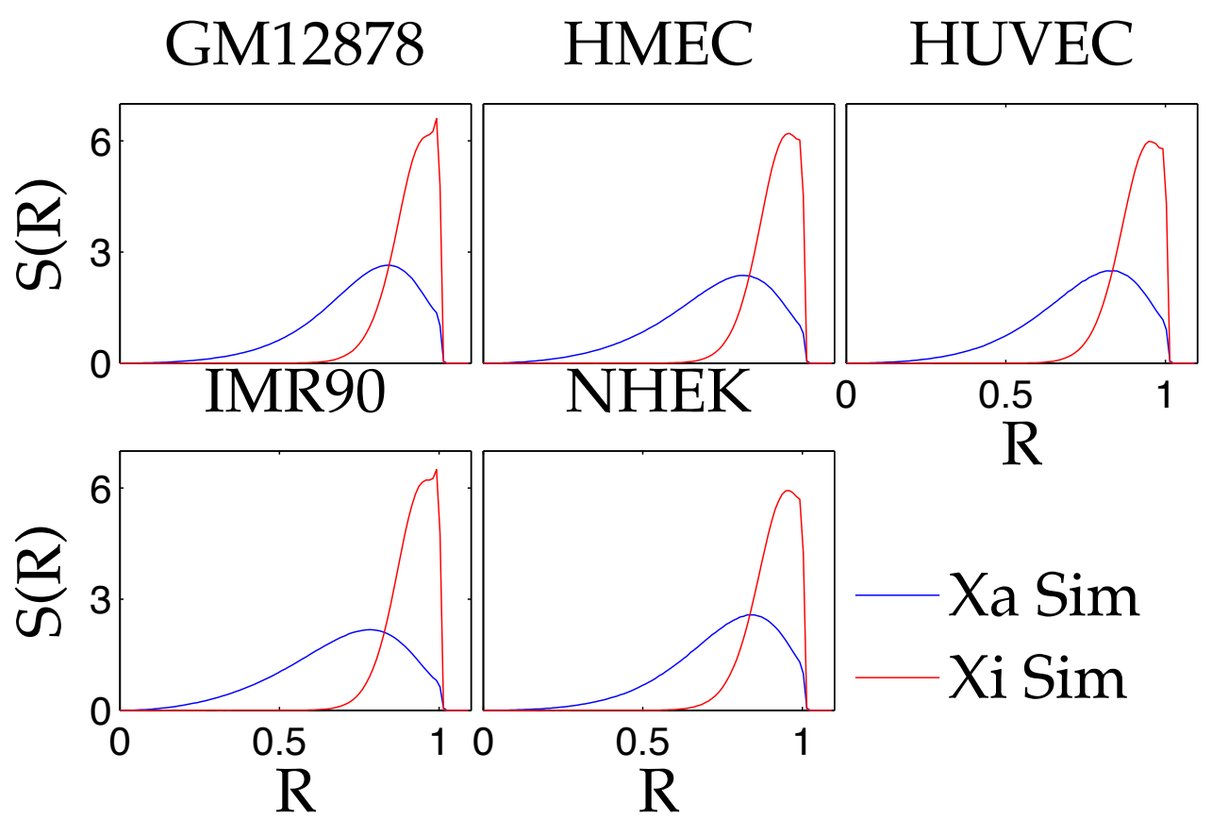


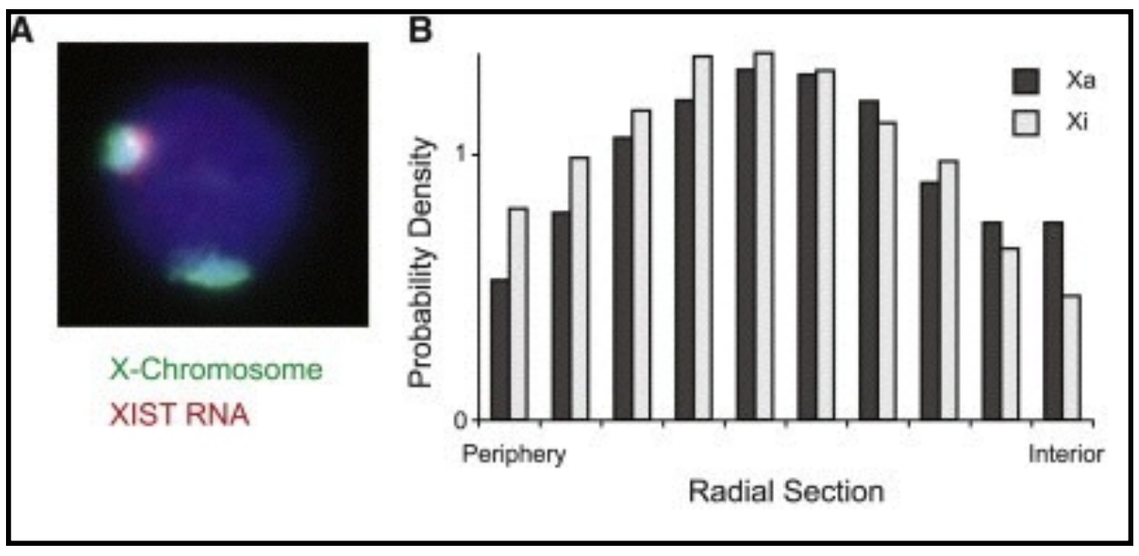
- Chromosome centre-of-mass positions for all chromosomes
- Fair agreement for larger chromosomes

- DNA density distribution S(R) for all 5 cell types and all chromosomes
- Largely the same across cell types, cell-type differences larger in centre-of-mass distribution



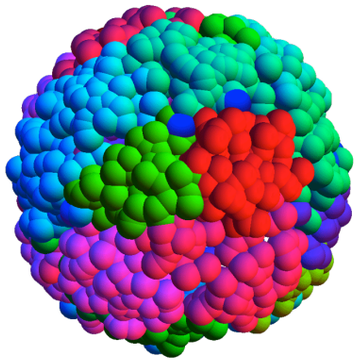




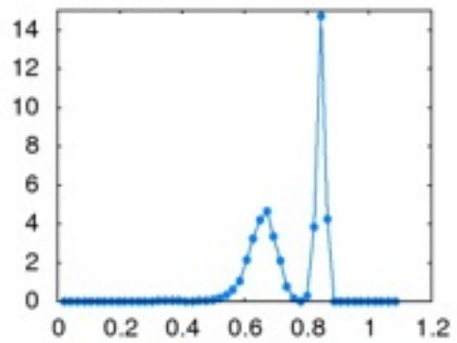


Data: Naughton et al, *Mol Cell* 40(3):397-409 (2010)
Female SAT03 cells

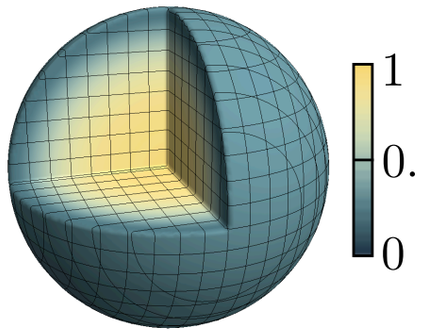
- X-chromosomes in female cells (XX) asymmetrically distributed, with the inactive X chromosome located more peripherally than the active X
- We recover this very non-trivial feature



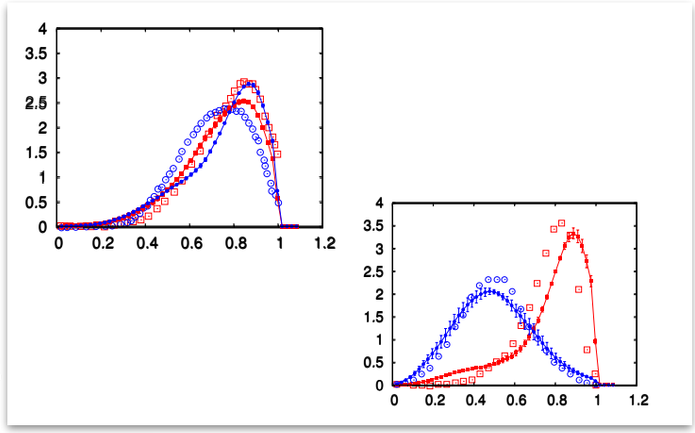
1. Chromosome territories



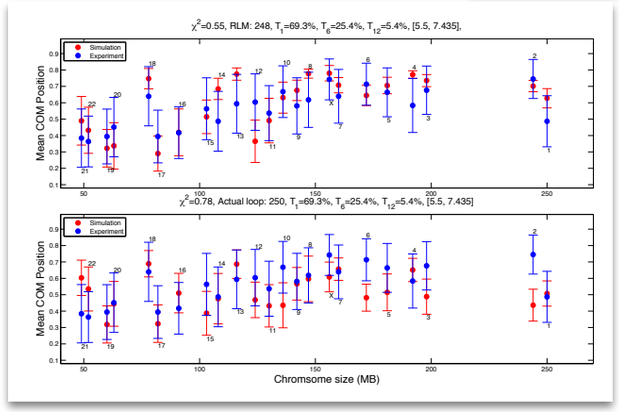
2. Heterochromatin & euchromatin distribution



Visual Summary

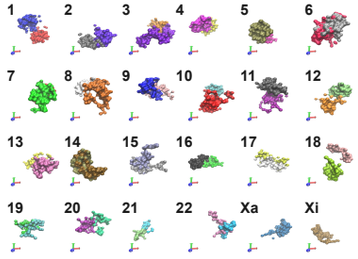


4. Chromosome COM distributions

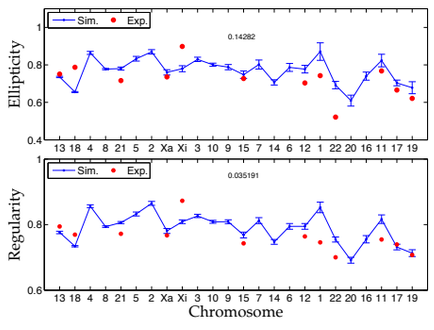


3. S(R) for individual chromosomes (18/19 & 12/20)

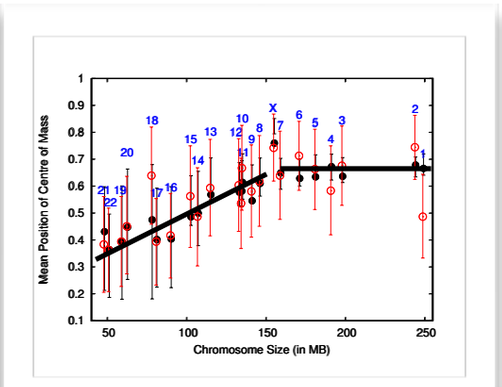
8. X-chromosome positioning



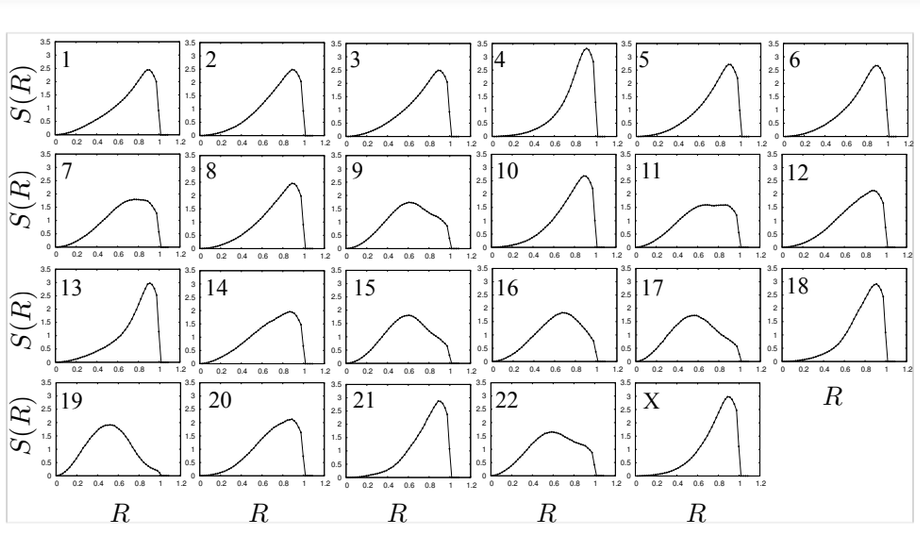
These results follow once we specify the pattern of activity on each chromosome and how they loop



5. Shape statistics of chromosome territories



6. Positioning by chromosome size



7. Radial DNA distributions for all chromosomes

K. Meaburn and T. Misteli, Nature 445, 379-381(2007)

Conclusions

" .. the position of each chromosome is largely determined by the activity of all its genes; that is, the number and pattern of active and silent genes on a given chromosome .. "

1. A proposal for the physics of large-scale nuclear architecture which emphasizes inhomogenous activity, conceptually rooted in very modern statistical mechanics and biophysical ideas.

2. Positioning by gene density and/or size and chromosome territory formation both originate in the physical phenomenon of "activity-based segregation". An activity-based positioning code?

3. A predictive approach to nuclear architecture (inhomogeneous activity, looping + specific interactions with the nuclear envelope)

Summary

In thermal equilibrium
and out of it

Self-propelled objects
and active matter

Living matter as
active matter

Nuclear architecture

Chromosome positioning

Application of active
matter ideas

Predictions

Conclusions

Active matter and the architecture of the cell nucleus

In what way(s) does living matter differ from non-living matter?

"Nothing" in physical biology makes sense except in the light of non-equilibrium (active) processes

Cf. T. Dobzhansky's classic essay: "Nothing in Biology Makes Sense Except in the Light of Evolution"

Search for unifying principles through active matter, applications to physical biology via such examples

Importance of “coarse-graining”, the fundamental modelling step.

Complexity of what is measured. Only indirect probes and noisy data. Statistical analysis and relevant models crucial

Value of simple models

Ganai, Sengupta and Menon, Nucl. Acids Res. 42(7) 4145 (2014)
Agrawal, Ganai, Sengupta, Menon, J. Stat. Mech (2017)
Agrawal, Ganai, Sengupta, Menon, in preparation
Agrawal, Menon, in preparation

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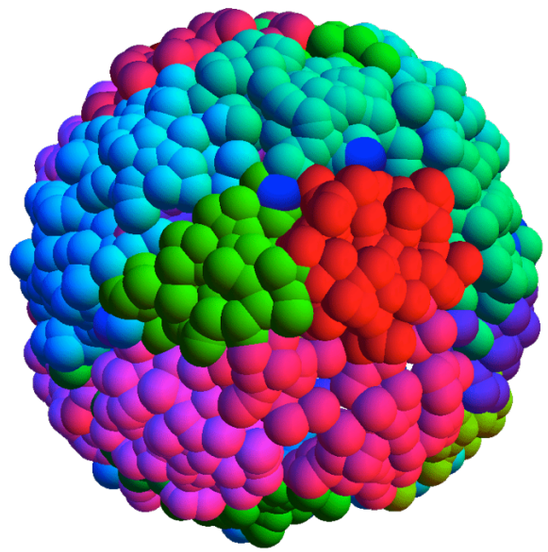

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DAE-SRC Fellowship

PRISM, IMSc

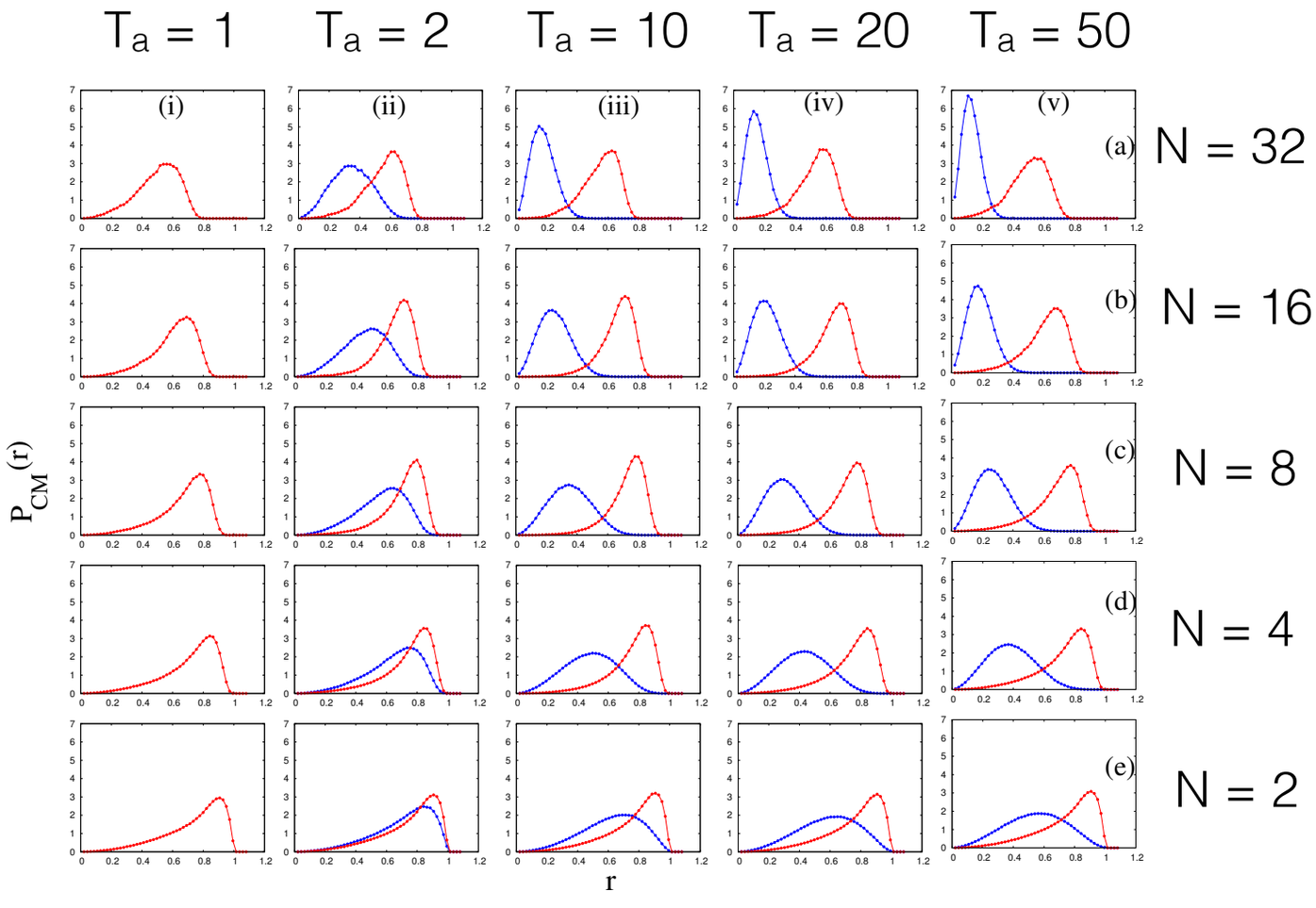
Activity-based segregation?

Phase-separation generic to self-propelled systems, e.g. motility-induced phase separation (Cates, Tailleur, Baskaran, Marchetti + ..) Also [Weber et al, PRL (2016)] in binary mixtures of particles with different diffusivities.

Confinement important here, also polymer character

Hot particles exclude the cold ones, drive them to the boundary. Relation to “reverse osmosis” in active systems. [Lion & Allen, EPL (2014)]

Simple model of active-inactive polymer mixture, no polydispersity



See similar segregation

Brownian dynamics, 3-d, spherical confinement: 512 monomers subdivided into polymers of N monomers each, 50% active and 50% inactive