An emerging mechanistic paradigm for self-organization and functional properties of biological materials: the power of weak binding

SIAM Mathematical Aspects of Materials Science

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Historically, I have benefitted from support from AFOSR and ARO for "engineering nanocomposites through mathematics & computation", focusing now at the energy-water nexus.
A topic for another day.

Collaborators and Contributors to Today's Lecture

- Jay Newby, UNC, moving to U. Alberta
- Paula Vasquez, Qi Wang, U. So. Carolina
- Jia Zhao, Utah State
- Tim Wessler, Caitlin Hult, now at U. Michigan
- Xuezheng Cao, Ben Walker, Yunyan He, David Adalsteinsson, Peter Mucha, Boyce Griffith, Bill Shi, Josh Lawrimore, Kerry Bloom, Sam Lai, David Hill, Kelsey Gasior, Amy Gladfelter, UNC Mathematics, Biology, Pharmacy & Physics
- Dane Taylor, U @ Buffalo
- Alex Chen, General Electric & Simi Wang, Amazon
- Scott McKinley, Melanie Jensen, Tulane
- Feifei Xu, Google
- Bob Guy, Becca Thomases, Greg Miller, UC-Davis

Overview

- In several collaborations with molecular/cellular biologists and pharmaco-engineers, ***the role of transient molecular anchors that crosslink larger species has emerged as essential.*** One cannot directly observe the binding and unbinding of the anchors; rather one observes mobility of the effector species.
- ***Paradigm: weak, short-lived binding kinetics of many anchors enhances, even optimizes, diverse functionalities. 5 such functionalities we study are:***

Spatial and dynamic self-organization of the genome in all living cells

Immobilization of pathogens (active and passive) in mucus barriers

Tuning the rheological and self-healing properties of mucus

Cellular cargo transport by molecular motors along microtubules

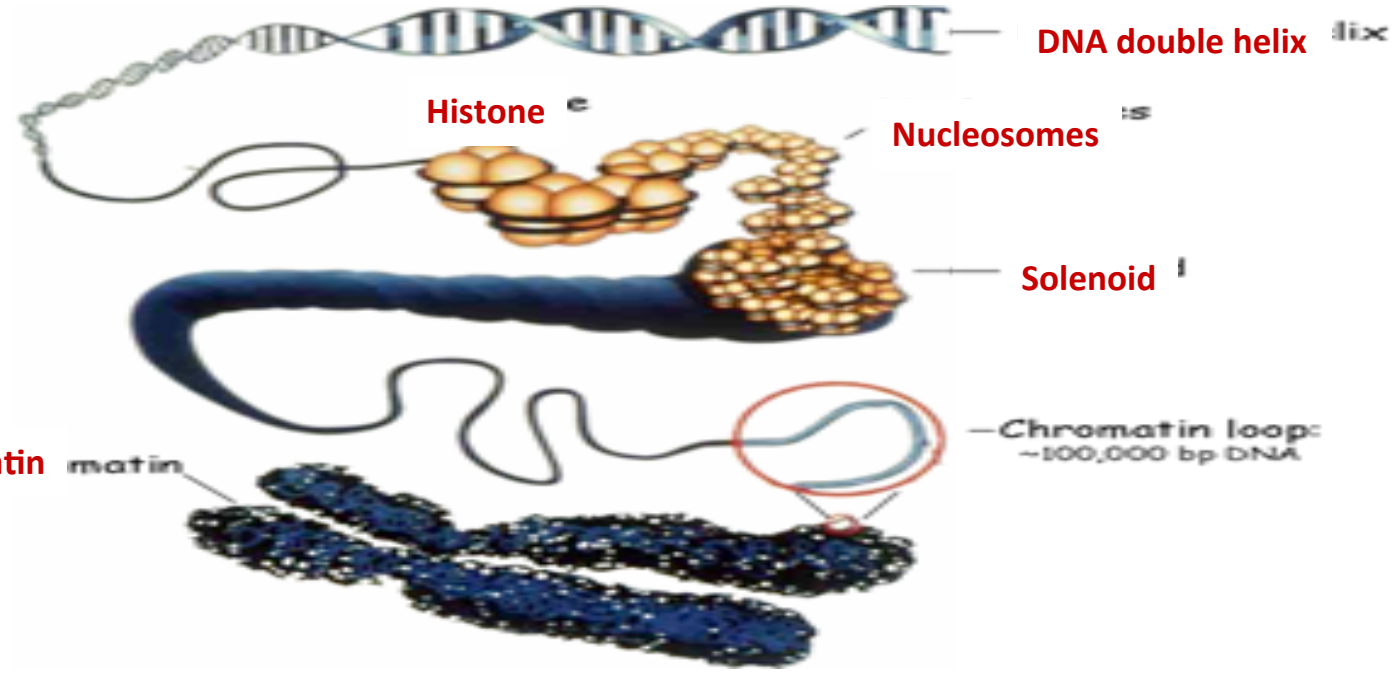
Sequestration of cytoplasmic membrane-less condensates

Specific biological systems discussed in this lecture (we'll be lucky to get to 3rd system)

- **In the nuclei of eukaryote cells, genes within chromosomal DNA are the effectors, "structural maintenance of chromosome" (SMC) proteins, condensin and cohesin, are the anchors.**
- **In mucus barriers of every organ (lung, intestinal and reproductive tracts), pathogens (viruses or bacteria) or particulates are the effectors, antibodies are the anchors**
- **Mucus – binding sites in the highly entangled polymeric gel are crosslinked by families of protein anchors**

Post genomics era: epigenomics of chromosomal DNA

How do chromosomes organize in living cells to facilitate cellular processes? What DNA and DNA-associated protein modifications occur "on top of the genome" to facilitate gene activity? (e.g., manufacture of ribosomal RNA, transcription, DNA repair)



/www.med.unc.edu/~bstrahl/research.html

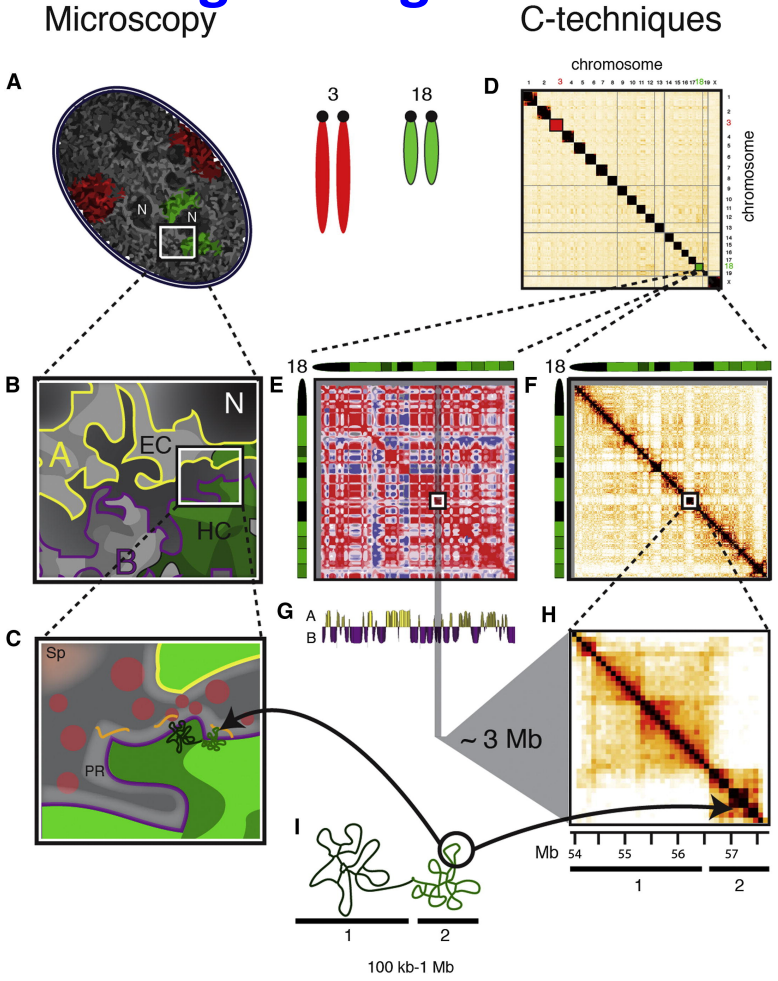
Chromosome Conformation Capture 3C – Hi C: Formaldehyde cross-linked chromosomes, population averages over 1000s of cells have produced many inferences about gene organization

Chromosomes occupy territories – *how?*

Genes preferentially associate – *how?*

Structure-within-structure, loops-within-loops, fractal-like conformations of chromosomes – *how?*

Hi-C implicitly assumes Ergodicity: an equivalence between the dynamics of a living cell and a population average. Are minutes sufficient for the time average of living genome conformations to sample the landscape?

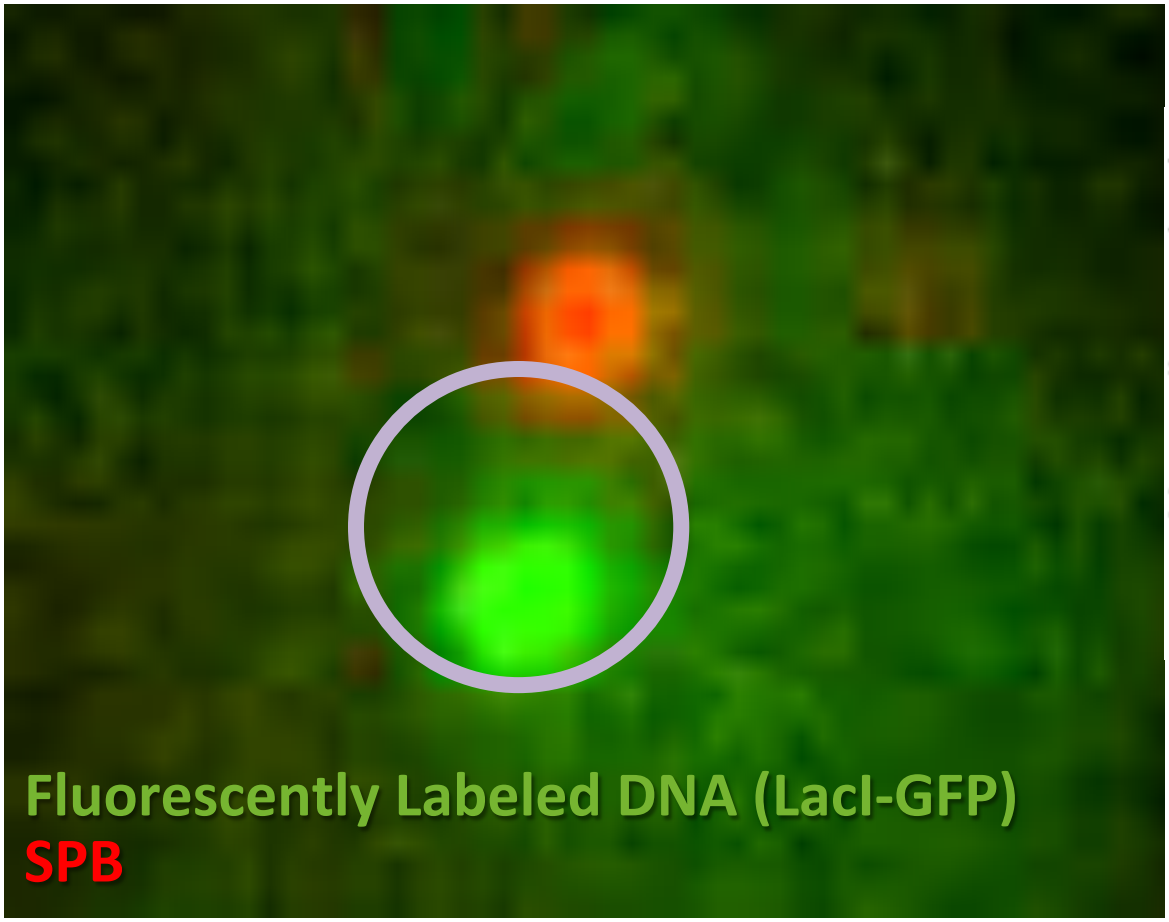


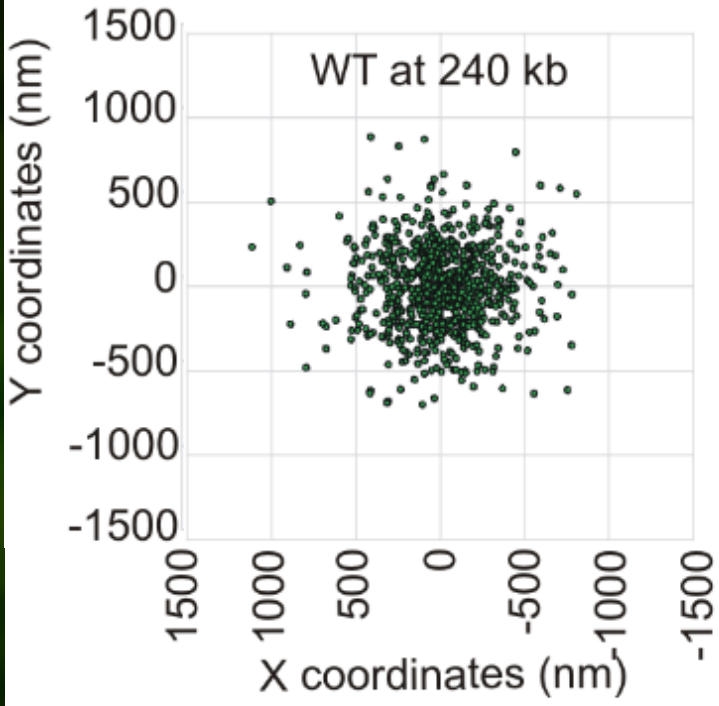
Molecular Cell 2013 49, 773-782DOI: (10.1016/j.molcel.2013.02.011)

The Hierarchy of the 3D Genome, Gibcus and Dekker

Look up Dekker, Mirny, and many other contributors

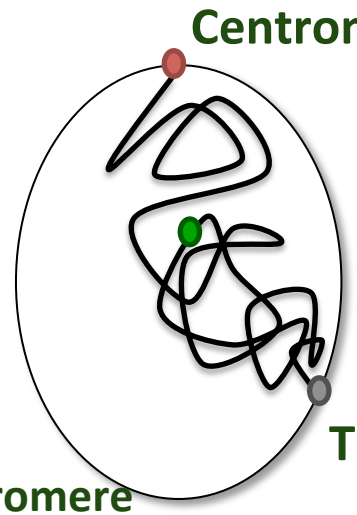
Bloom lab at UNC explores 5-10k base pair domain fluctuations on chromosomes in Live Yeast Cells. Explore interphase today, mitosis another day, transitions between cycles in future

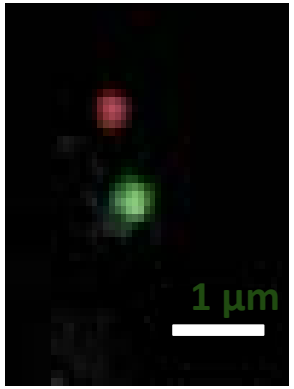




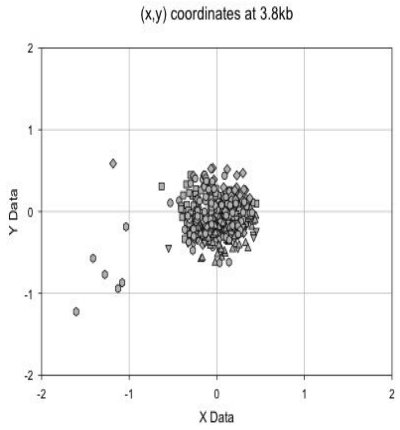
"DNA spot" fluctuation data relative to tether sites: MSD of fluorescently tagged 5-10k base pair spots has been the "industry standard"

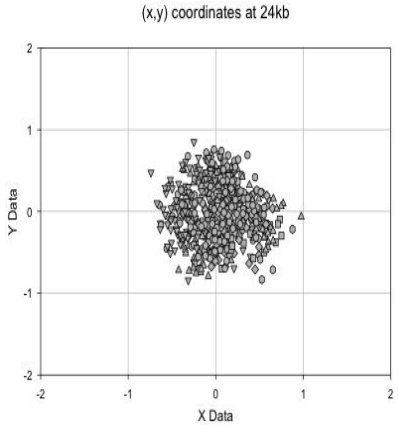
LacI-GFP bound to lacO integrated into the genome at specific sites

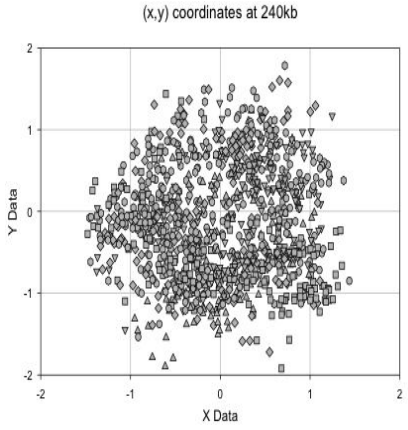




Farther from centromere



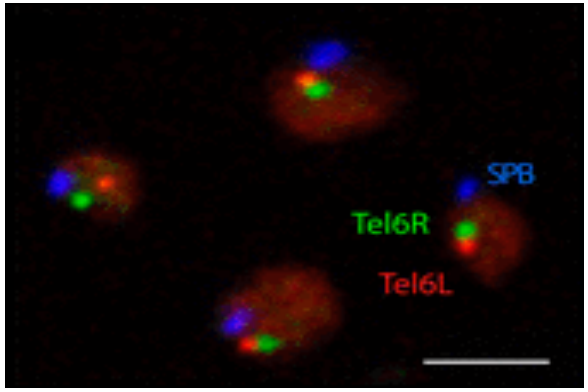


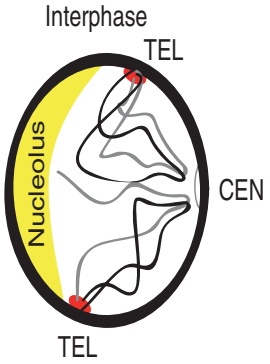


Mean squared displacement (MSD) and step size distributions per lagtime

$$\left\langle \Delta r^2(\tau) \right\rangle \qquad \Delta r^2(\tau) = \left[x(t+\tau) - x(t) \right]^2 + \left[y(t+\tau) - y(t) \right]^2$$

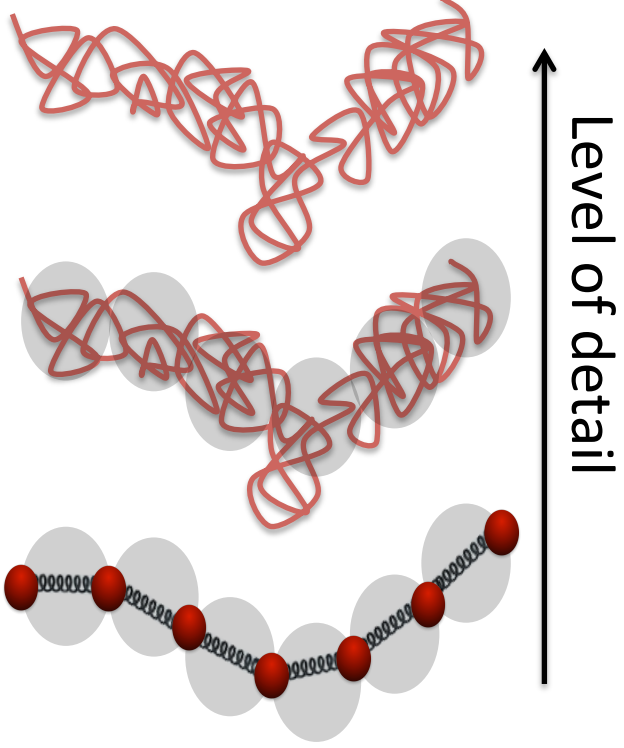
Coarse graining to a representation of interphase chromosomes as *entropic, geometrically confined, tethered, "bead-spring" polymers*



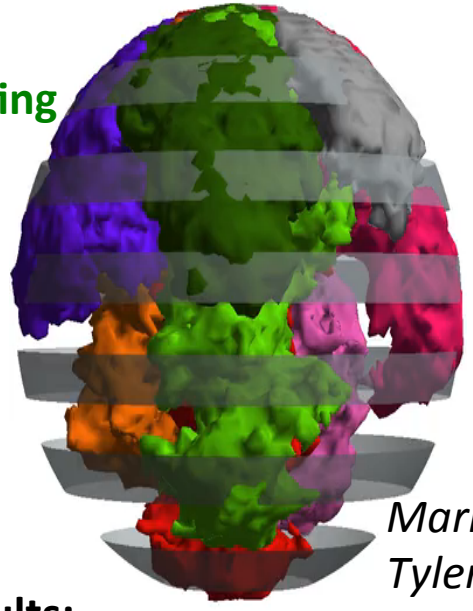


Taddei and Gasser. Genetics (2012)

Coarse-grained representation of chromosomes



Common centromere w/ 32 "arms" emanating to 7 telomeres
Inherent degeneracy!



Foreshadowing of our dynamic 16 chromosome, all nucleus, 3D model results: Chromosome territories

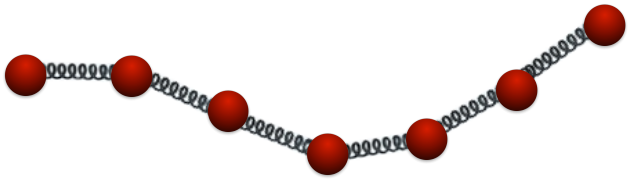
David Adalsteinsson, UNC-CH

Marko & Siggia, 1997, Mol. Biol. Cell
Tyler, Vasquez..., Forest, Bloom, 2013, Molecular Cell
Vasquez et al., Nucleic Acids Research, 2016
Hult et al., Nucleic Acids Research, 2017

Many groups around the world are in this game.

Model Chromatin as an Effective Rouse-like Bead-Spring Polymer Chain

Choose 5k bp resolution to be able to compute all chromosomes in a 3D nucleus w/ interphase tethering in a couple days on our supercomputers.



Equation of motion bead *i*

$$\zeta \frac{d\mathbf{X}_i}{dt} = \mathbf{F}_i^B(t) + \mathbf{F}_i^S(t) + \mathbf{F}_i^{EV}(t) + \mathbf{F}_i^W(t)$$

- **Brownian motion**

$$\mathbf{F}_i^B(t) = \sqrt{2k_BT\zeta}\, W_i$$

- **Excluded volume**

$$\mathbf{F}_i^{EV}(t) = \frac{zk_s}{2d^5}\left(\sum_{j=0, j\neq i}^{N} \mathbf{r}_{ij} \exp\left[-k_s \mathbf{r}_{ij}^2 / 2k_BTd^2 \right] \right)$$

- **Bead-Bead spring force**

$$\mathbf{F}_i^S(t) = -k_s\left(2\mathbf{X}_i - \mathbf{X}_{i+1} - \mathbf{X}_{i-1}\right)$$

- Interaction with nucleus wall $\mathbf{F}_i^W(t)$

Geometric confinement

We use a **worm-like chain** in all simulations to follow whereby the force ramps up dramatically when the polymer coils have been pulled out. Results are very similar if we use different nonlinear springs (FENE)

~2800 dimensional (# beads) nonlinear & nonlocal geometrically constrained stochastic dynamical system

Double-Tethered Rouse Chain: Exactly Solvable Limit (linear springs, no excluded volume, no sphere confinement, single chain) --- code validation

$$\zeta \frac{d\mathbf{X}_i}{dt} = \mathbf{F}_i^B(t) + \mathbf{F}_i^S(t) \qquad \zeta d\mathbf{X}_i = -k_0^s\left(2\mathbf{X}_i - \mathbf{X}_{i+1} - \mathbf{X}_{i-1}\right)dt + \sqrt{2k_BT\zeta}\,d\mathbf{W}_i$$

Change of variables[1] $\mathbf{R}_i = \mathbf{X}_i - \frac{i}{N}\mathbf{X}_N, \quad \mathbf{R}_0 = 0, \mathbf{R}_N = 0$

$$\zeta d\mathbf{R}_i = -k_0^s A_{i,j}\mathbf{R}_j dt + \sqrt{2k_BT\zeta}\,d\mathbf{W}_i$$

$$\mathbf{A} = \begin{pmatrix} 2 & -1 & 0 & \cdots & 0 & 0 \\ -1 & 2 & -1 & \cdots & 0 & 0 \\ 0 & -1 & 2 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & 2 & -1 \\ 0 & 0 & 0 & \cdots & -1 & 2 \end{pmatrix}$$

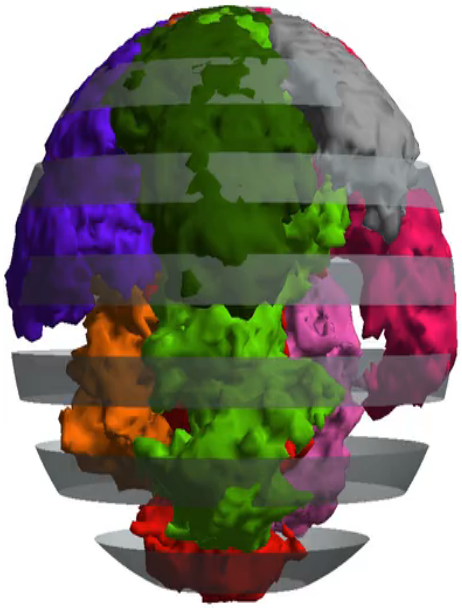
Normal mode decomposition[1] $\mathbf{R}_i = 2\sum_{p=1}^{N-1}\mathbf{Y}_p \sin\left(\frac{\pi i p}{N}\right)$

$$\zeta_p d\mathbf{Y}_p = -k_p\mathbf{Y}_p dt + \sqrt{2k_BT\zeta_p}\,d\mathbf{W}_p \qquad \left\langle \mathbf{Y}_p \cdot \mathbf{Y}_p \right\rangle = \frac{k_BT}{k_p}$$

$$\left\langle \left(\mathbf{R}_j - \mathbf{R}_0\right)^2 \right\rangle = \frac{b^2}{3N} j(N-j) = \frac{k_BT}{k_{s,j}} \Rightarrow \qquad k_{s,j} = \frac{3k_BT}{b^2}\left(\frac{N}{j(N-j)}\right) = k_s^0\left(\frac{N}{j(N-j)}\right)$$

Alexei Likhtman (http://www.personal.reading.ac.uk/~sms06al2/short_course/rouse.pdf)

32 chromosome arm "point clouds" = territories swept out by beads per arm



Entropic fluctuations, excluded volume, tethering & confinement suffice to yield: territories, "TADs", segregation

(contrary to heterochromatin hypothesis −heterogeneity of chromosomes is not necessary)

Ref: Nucleic Acids Research (2016)
Vasquez, Hult, Adalsteinsson, Lawrimore, Yeh, Forest, Bloom

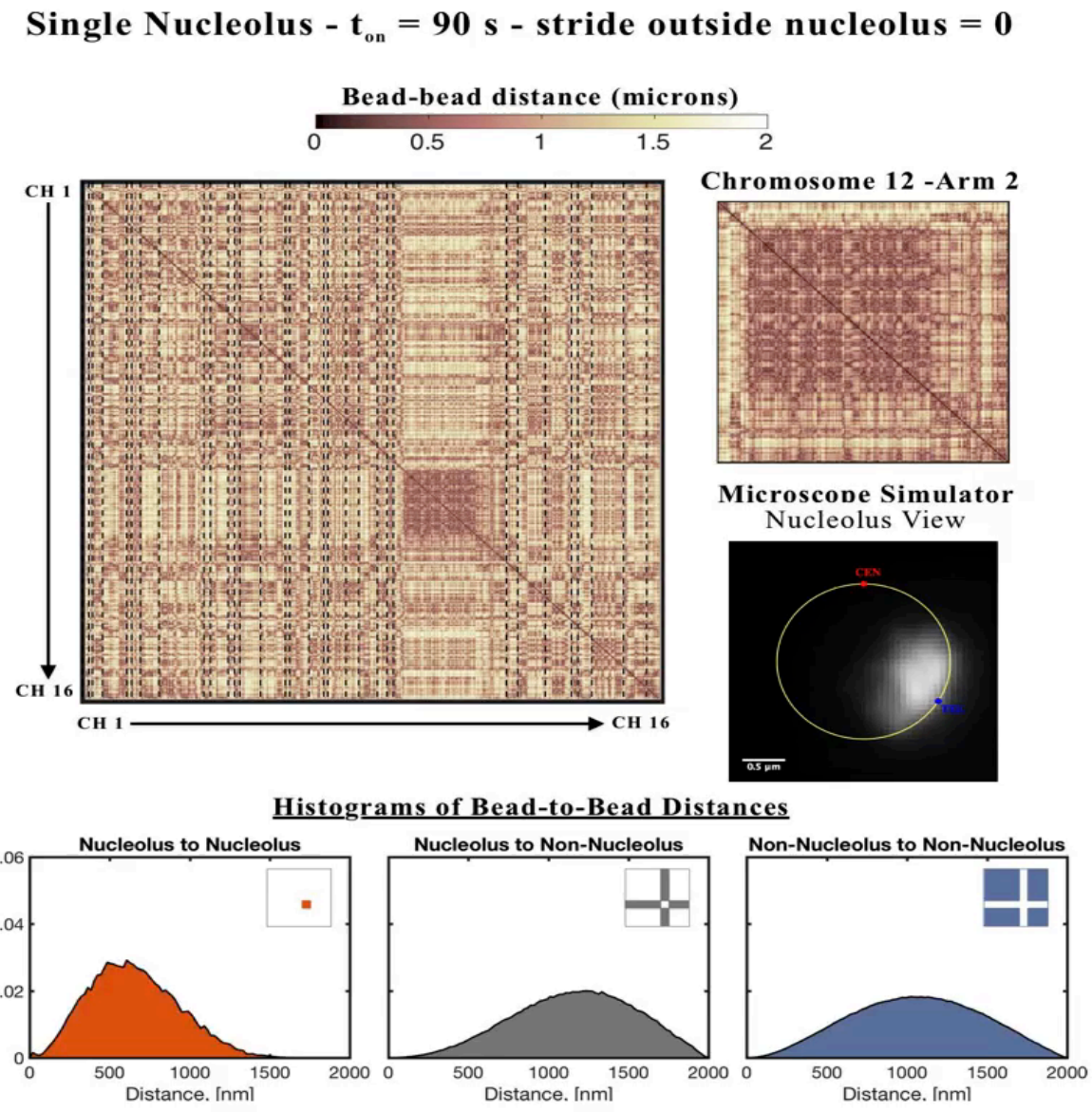
Now add "intelligent design": explore consequences of structural maintenance of chromosome (SMC) proteins that act "on top of the genome" by transient binding kinetics

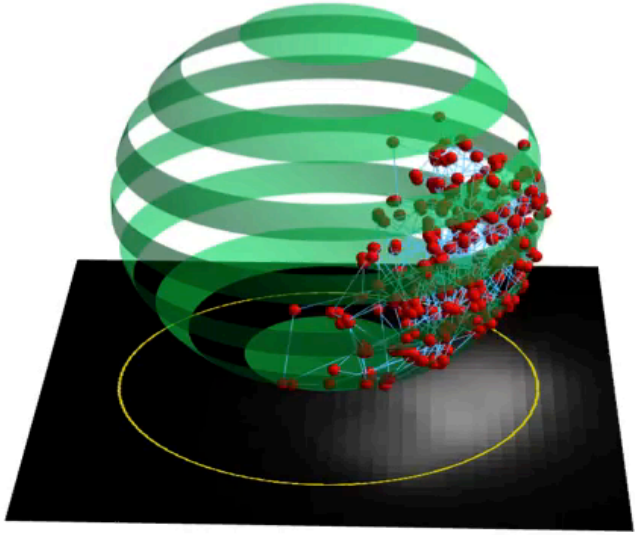
Two mechanisms are implicated: Crosslinking of genes (5-10 kbp domains) explored here to generate structure, morphology and gene communities; and, motor-like loop extrusion that acts below the lengthscale of our model, which we embed in our model of mitosis

Ref: Nucleic Acids Research, Hult, Vasquez, Lawrimore, Adalsteinsson, Forest, Bloom 2017

Nucleolus -- a **repeat** Ribosomal **DNA** (rDNA) **sequence** that is a manufacturing center for proteins. **Movie below is for weak, short-lived binding-unbinding kinetics of condensin SMC proteins within the nucleolus, ~ 365 beads on Chromosome XII**

Nucleolus with strong, long-lived binding of SMC proteins





Upshot: binding kinetics of SMC proteins *(condensins, cohesins)* weak (fast) vs. strong (slow) binding kinetics yields behavior consistent with experimental observations:

***greater compaction of the ~360 bead nucleolus with 20-30 gene communities that persist for minutes with slow exchange of genes**

***seclusion of the nucleolus in a crescent-shaped territory at nuclear wall**

***robust structure-within-structure (loops-within-loops)**

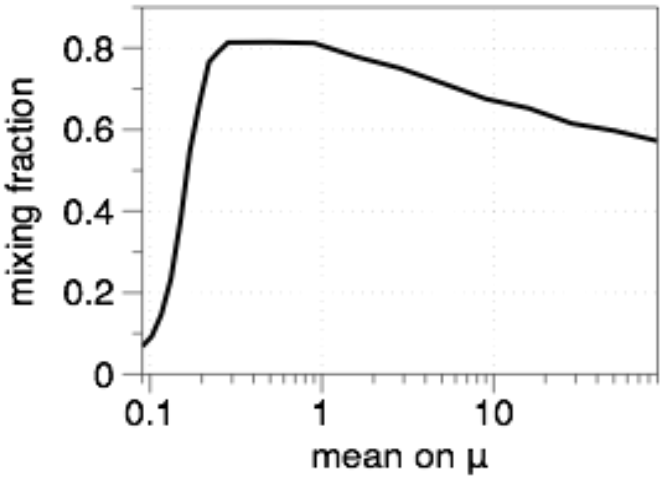
***persistent multiscale structure, upon population averaging goes away**

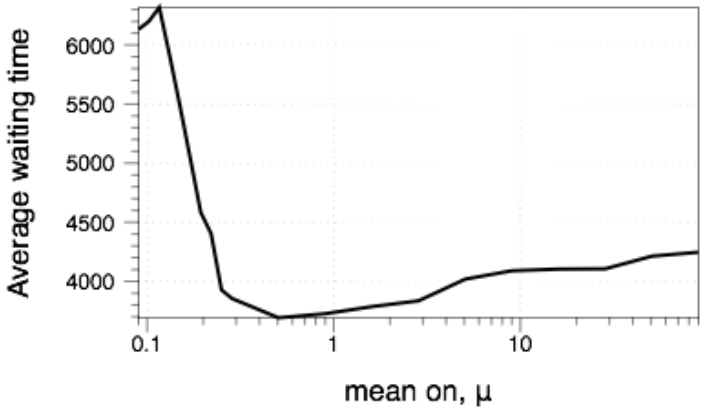
***we put "active" beads (5 kbp domains) on different chromosomes, expt'ly & in the model, with same results!**

Functional Effects of Crosslinking Kinetics on Behavior

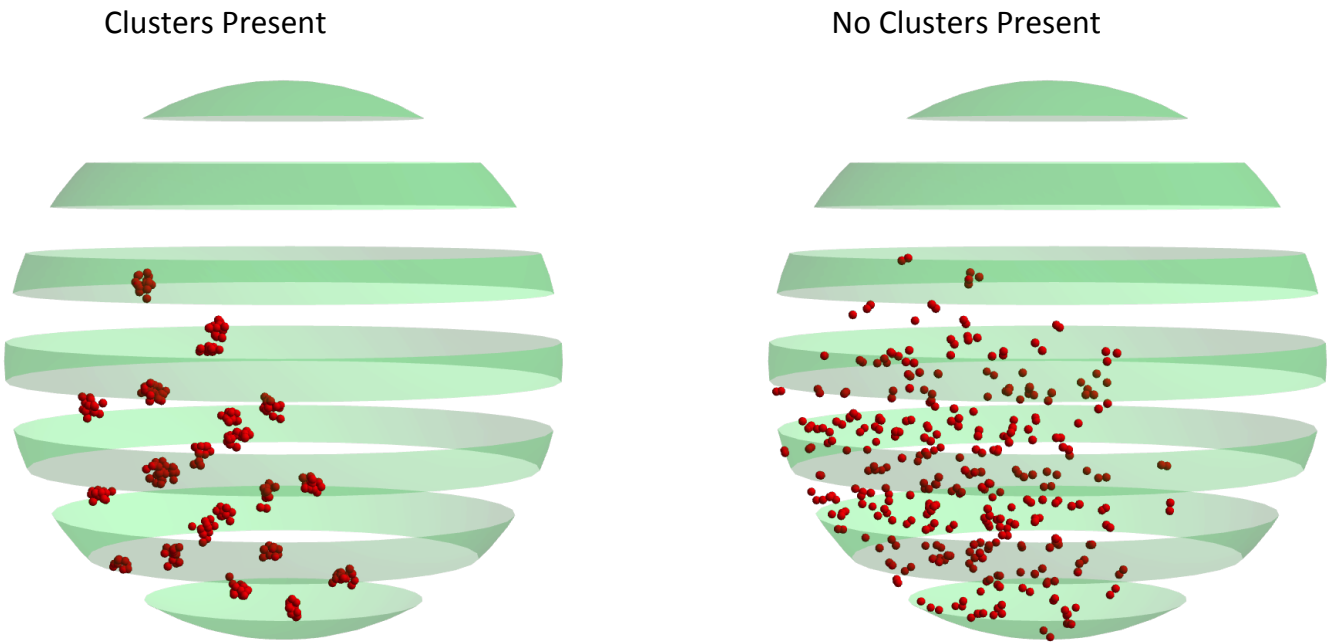
Vary the mean of the waiting time distribution for bonds to break (Walker, Taylor, Hult, gf, to be submitted)

- **Fast crosslinking produces loops within loops and *persistent* gene clusters**
- **When kinetics are slightly slowed down, "sweet spot" maximizes gene-gene interactions**
  - **Top: *Fraction of all pairs of active genes that get within the distance required to bind over 30 minutes***
  - **Bottom: *Average waiting time between successive interactions of all pairs of beads in the nucleolus***





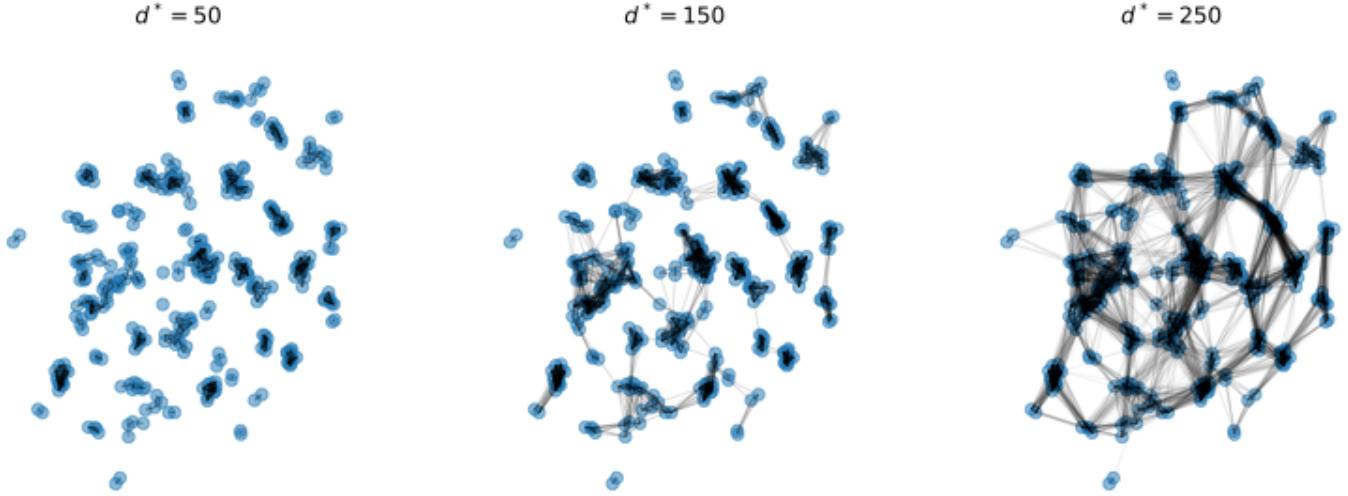
- **We want agnostic metrics to detect**
  - *Is **gene clustering** present? (beyond 2-pt correlations)*
  - ***Which beads/genes are in which clusters**? (Labeling)*



Network Community Detection

Walker, Taylor, Hult, gf, to be submitted

- Translate bead positions into a network - connections between beads whose distance is below threshold d^*. As threshold increases, more edges are added to the network.
- Time series leads to multilayer network



Advantages of network community detection algorithmic diagnostics (B. Walker, D. Taylor)

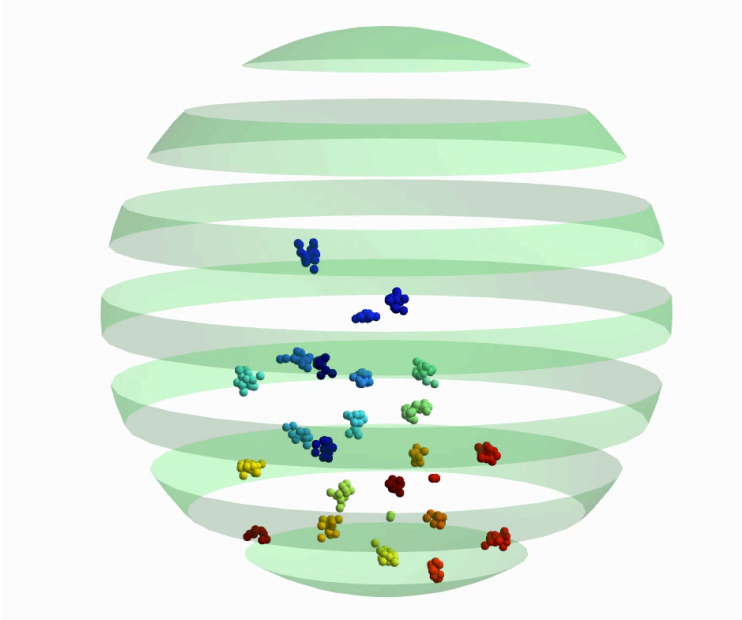
- Automation - applied to many datasets at once
- Objectivity - output is computed according to known and consistent rules versus the eyeball norm based on statistics of bead-bead / gene-gene distances
- Rigorous parameter analysis to Assess robustness
- Algorithm of choice: Louvain modularity optimization

Louvain Modularity Optimization

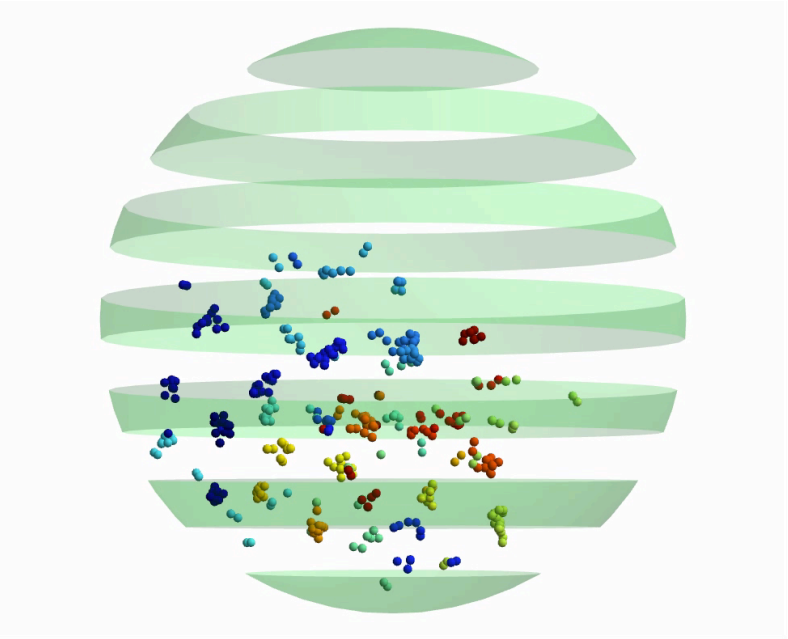
- **Modularity**: cost function for community partition that rewards high edge weight within communities, implicitly penalizes low edge weight between communities
- **Multilayer (dynamic) modification**: add a penalty if a bead is not in the same community in successive layers
- **Louvain optimization**: Each bead starts in its own community, proceed to iterate over beads and *change community label to the one that maximizes modularity*. Then recursively iterate until convergence.

Labeling of clusters over time from network community detection -- then visualize results

Fast Crosslinking, i.e., very weak bonds



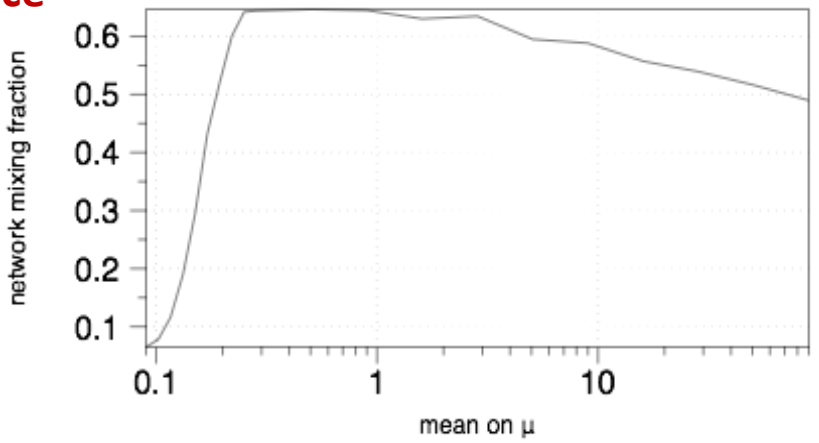
Intermediate Crosslinking, i.e., weak bonds

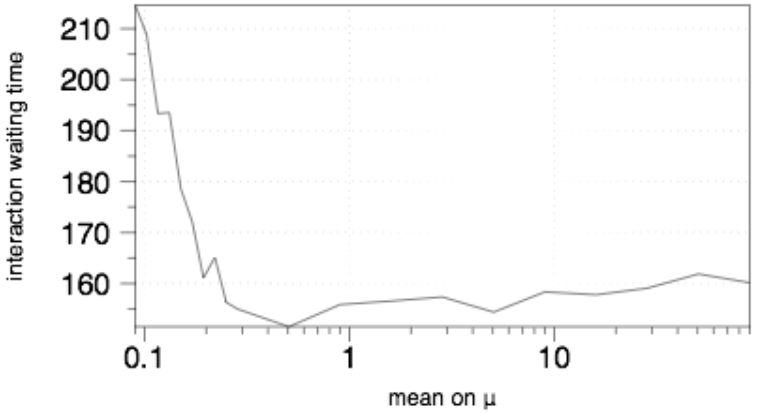


Recover previous "sweet spot" in gene communication with community detection metrics rather than distances

Fraction of bead pairs that reside in the same community at least once

- Community detection version of distance 2-pt statistics produces qualitatively similar results
- Reinforces that community detection finds clusters in alignment with our expectations
- **Future: explore community networks underlying transcription, repair, ….**





Instead of "when beads return to within a set distance"
Impose "when beads return to common community"

Viruses, epitopes, and antibodies (Ab)

The accepted dogma in immunology: Ab (~10 nm) populate the epitopes that a virus (~100 nm) uses to dock to and infect cells, thereby neutralizing viruses

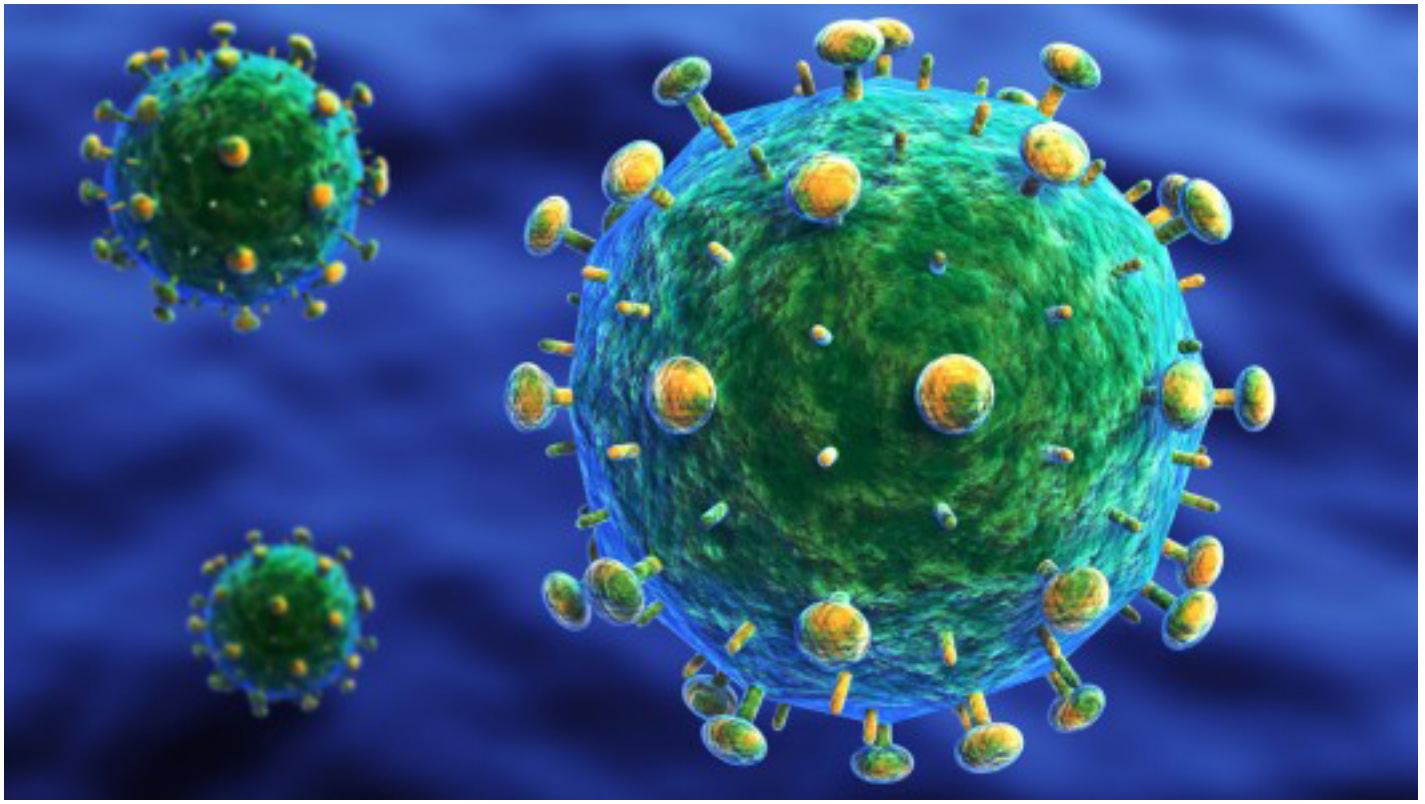


Image: Shutterstock

There are more Ab in mucus than in blood or the lymphatic system! Intriguing fact worth understanding why.

How do antibodies (Ab) *really* protect against viruses and bacteria in mucosal barriers?

Modeling these diffusion-reaction-advection systems based on data from the lab of Sam Lai

Jay Newby, Scott McKinley & Melanie Jensen (Tulane), Alex Chen (GE), Tim Wessler (U. Mi), Feifei Xu (Google), Simi Wang (Amazon), Peter Mucha, Bill Shi (UNC), Forest, plus members of the Lai lab, series of papers last 5-6 years

What hints were out there to suggest Ab do something other than cover epitopes?

- **Remarkable precedent with HIV clinical trial reported in the NE J. of Med, 2009, Vol 361, No. 23**
- Thai RV144 trial gave evidence a vaccine (ALVAC and AIDSVAX) may block HIV transmission.
- Provided no protection against progression for already infected individuals (no effect on T-cell count)

Some facts to guide our thinking:

- Virus diffusivity is weakly diminished in the presence of Ab, therefore deemed insignificant with respect to virus mobility (**Ab-virus weak binding**)
- Ab diffusivity in mucus versus buffer is very weakly diminished, therefore deemed insignificant (**Ab-mucus very weak binding**)
- **Nobody considered the tandem mucus-Ab-virus effect - many weak bonds can be very strong, a rather non-intuitive concept**

Our joint experimental, theoretical, computational work =>

Ab dramatically boost mucus diffusive barrier properties of viruses and bacteria by crosslinking 1, 2, 3,... many (N>>1) viral epitopes to the mucin mesh --- a Velcro effect

Furthermore, tandem effect of:

very weak Ab-mucus binding affinity, i.e., very short-lived AM complex timescale τ_{AM}

weak Ab-virus affinity, i.e., short-lived anchor-particulate AP complex timescale τ_{AP}

with a couple other conditions, especially N>>1, is *optimal*

Nature Communications 2017, Newby et al.

Theoretical framework and assumptions that give **optimal trapping potency**

- **Three players: anchors A (antibodies); particulates P (viruses); matrix constituents M (the mucin mesh)**
- **Transient complexes & mean timescales:**

anchor-matrix (AM) timescale, τ_{AM}

anchor-particulate (AP) timescale, τ_{AP}

timescale of virus diffusivity: $\tau_v = L^2/(2D_v)$

We show: optimal trapping arises if $\tau_{AM} << \tau_{AP} << \tau_v$ plus other conditions
numerically by stochastic simulations,
theoretically by stochastic averaging,
experimentally in the Lai lab, where anchors are **antibodies**, particulates are **viruses**, and the matrix is **mucus**
Nature Communications, 2017, Newby, Schiller, Wessler, Forest, Lai

Kinetics of complexes

$$\mathrm{M}+\mathrm{A} \underset{a_{\mathrm{off}}}{\overset{a_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{MA}, \quad \mathrm{MA}+\mathrm{P} \underset{k_{\mathrm{off}}}{\overset{k'_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{MAP}, \quad \mathrm{A}+\mathrm{P} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{AP}, \quad \mathrm{M}+\mathrm{AP} \underset{a_{\mathrm{off}}}{\overset{\frac{D_{\mathrm{P}}}{D_{\mathrm{A}}}a_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{MAP}.$$

Binding rates via Smoluchowski encounter relation

$$k_{\mathrm{on}} = (D_{\mathrm{P}} + D_{\mathrm{A}})\varphi[\mathrm{A}]R_0, \quad k'_{\mathrm{on}} = (D_{\mathrm{P}} + D_{\mathrm{M}})(1-\varphi)[\mathrm{A}]R_0,$$

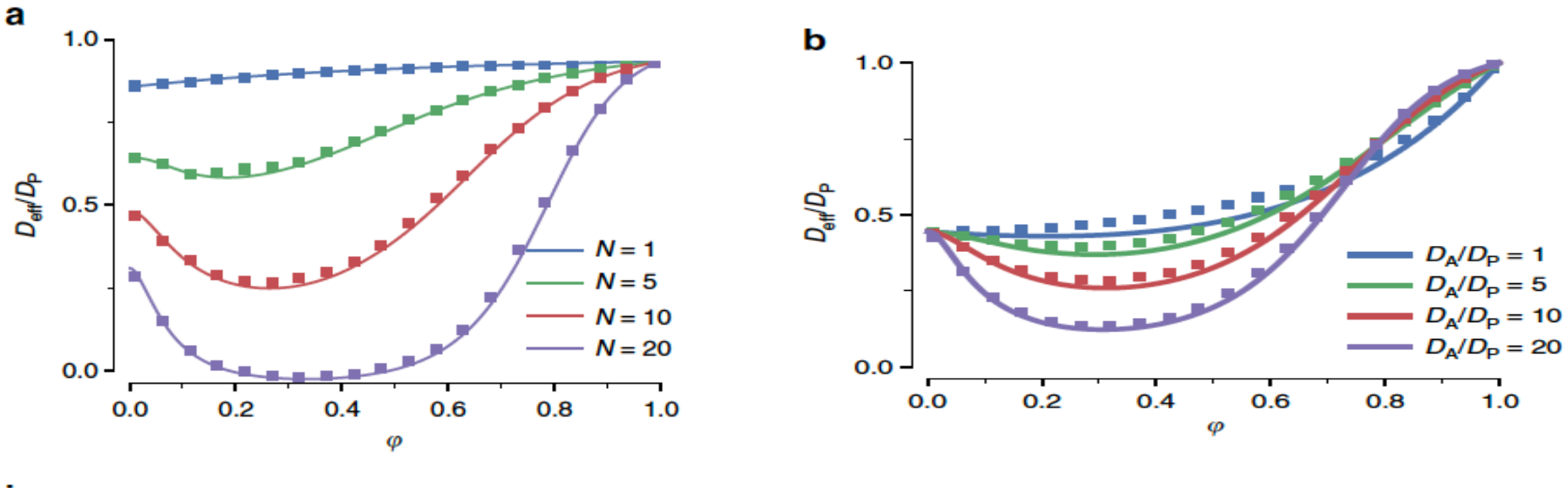
Fraction φ of free A at steady state

$$\varphi = \frac{a_{\mathrm{off}}}{a_{\mathrm{on}} + a_{\mathrm{off}}}$$

Note that $\varphi = 0$ and $\varphi = 1$ represent extremes where all anchors and no anchors are bound to the matrix, respectively.

Key Results Effective diffusivity of nanoparticulates vs. free fraction of anchors.

Monte-Carlo simulations varied values of **a** N, the maximum number of binding sites on the nanoparticle, and **b** D_A/D_P, the ratio of the anchor diffusivity to the nanoparticle diffusivity. Parameter values used were $\tau_{AP}/\tau_{AM} = 20$, $N = 15$, and $D_A/D_P = 20$



- Stochastic averaging result: **optimal trapping arises if**: **N>>1, D_A>>D_P** , and **very weak anchor-matrix interactions** $\varphi \sim 0.2\text{–}0.4$, where $\varphi = \frac{a_{off}}{a_{on} + a_{off}}$
- **plus a separation of the key timescales** in the Chapman-Kolmogorov equation $\tau_{AM} << \tau_{AP} << \tau_v$

Stochastic quasi steady state approximation has been applied to similar models of molecular motor transport

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3rd system: Transient Polymer Networks (TPNs)

A Molecular Dynamics Model of Mucus

Results to follow are not validated by experimental data *yet*

Ex vivo experiments underway in Ronit Freeman lab

Families of short-lived to longer-lived anchors collectively tune the viscous and elastic properties of highly entangled, transiently crosslinked biopolymer networks.

TPNs are self-healing due to reversibility, on timescales dictated by anchor / crosslink kinetics. *In nonlinear stress regimes, mucus self heals over a cascade of timescales, from entanglement dynamics that is modulated & modified by transient anchor return to equilibrium.*

In materials science: Vitromers in materials science literature (Leibler, CNRS; transient biopolymers for regenerative medicine (Freeman, Stupp)

Goal: a model that recapitulates linear and nonlinear rheology, is not over-parametrized, amenable to learning from micro and macro rheological data, & compatible with fluid-structure simulation tools

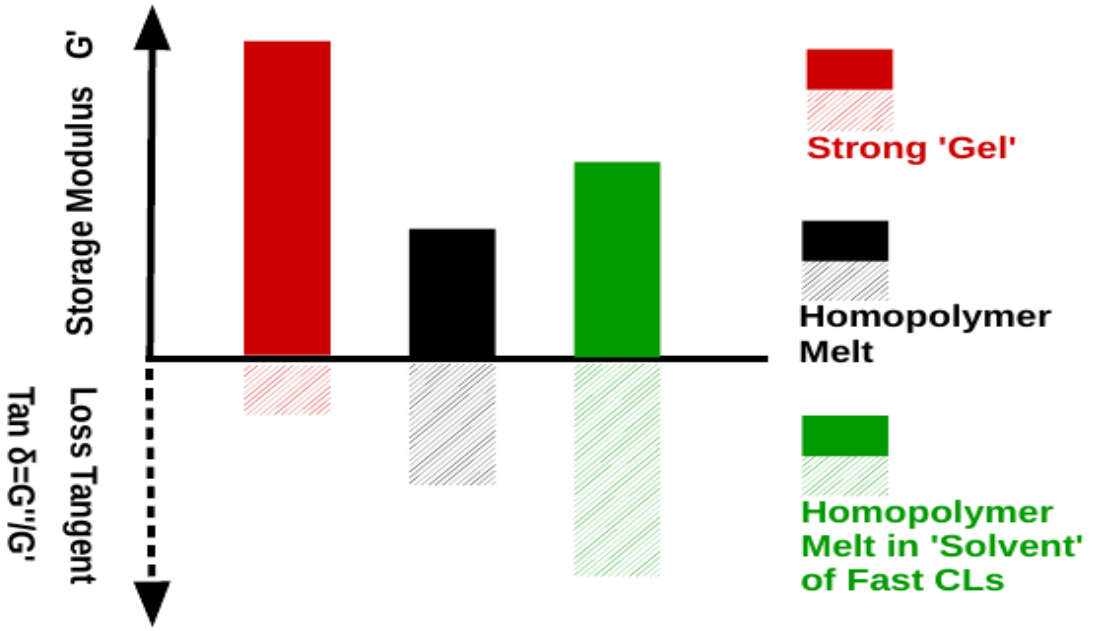
Transient Crosslinking Tunes the Mechanical and Rheological Properties of Highly Entangled Polymer Networks
MD model of mucus – similar to chromosomes + SMC proteins

(1) Determine the **lifetime and number concentration of crosslinkers (Cls) as dictated by binding-unbinding kinetics**
(2) Determine the **effects of crosslinking kinetics on mechanical and rheological properties**

Key Results:
Baseline is a Rouse-like highly entangled homopolymer melt

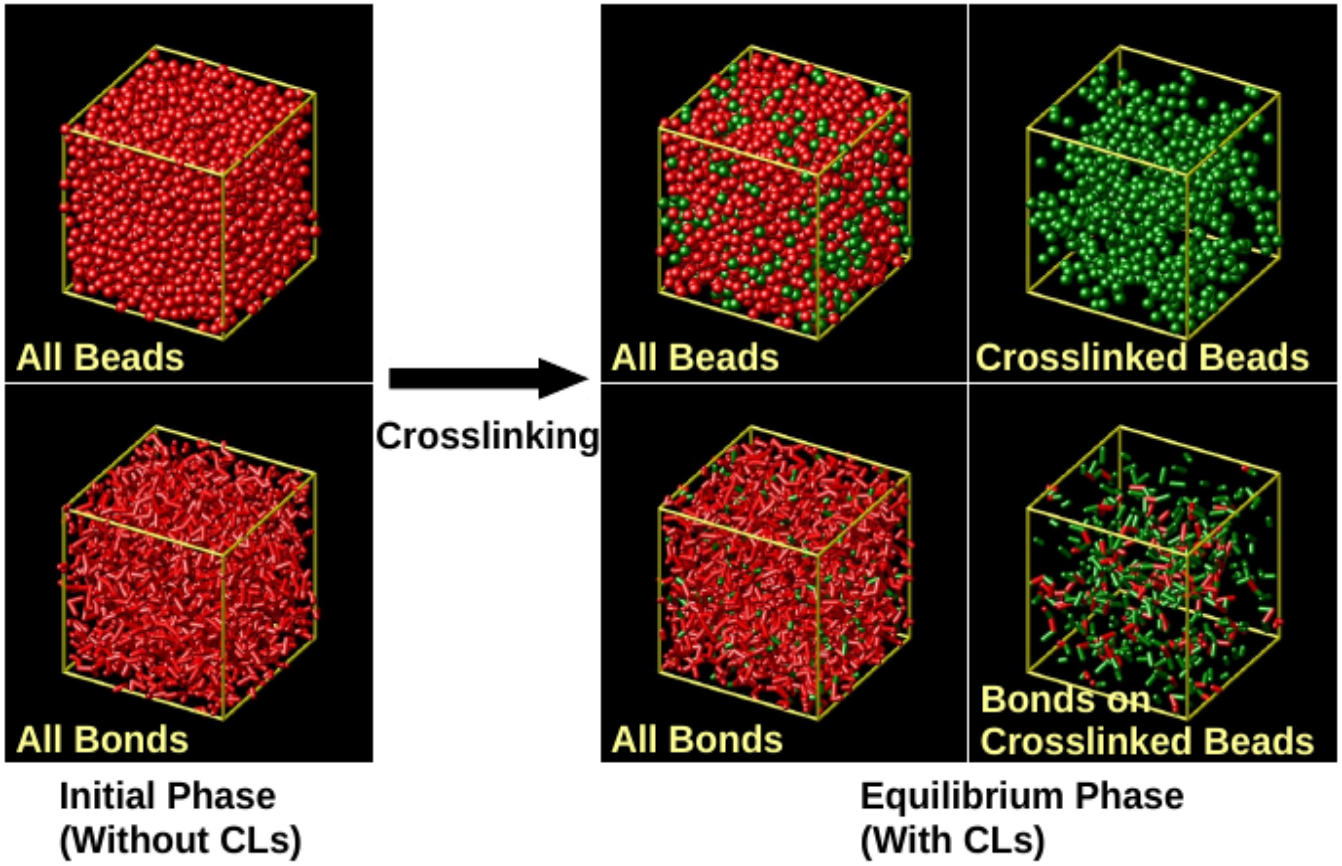
Add long-lived anchors ➔ "stronger & solid-like" G' enhanced, G' > G"

Add weak, short-lived anchors ➔ "stronger & liquid-like" G' enhanced but G" enhanced even more, G">G'



MD Simulations for Transient Crosslinking

Snapshots prior to (left) and after (right) crosslinking. Permanent and transient bonds are shown in red and green rods respectively



Langevin dynamics of Rouse-like bead-spring polymer chains (for mucins instead of chromosomes) is adopted

Calculation of stress relaxation, storage and loss moduli

Stress is defined via the virial theorem and measured directly from the simulations

$$\sigma_{ij}(t) = \frac{1}{V}\left(\Sigma_{k=1}^{NM} m_k v_k^{(i)} v_k^{(j)} + \frac{1}{2}\Sigma_{k=1}^{NM}\Sigma_{l=1}^{NM} F_{kl}^{(i)} r_{kl}^{(j)}\right)$$

In order to reduce the noise, stress is pre-averaged over time

$$\overline{\sigma}_{ij}(t) = \frac{1}{t_{avg}}\Sigma_{\Delta t=-t_{avg}/2+1}^{t_{avg}/2}\sigma_{ij}(t+\Delta t)$$

The stress relaxation modulus G(t) is computed from the stress

$$G_{ij}(t) = \frac{V}{k_B T}\langle\overline{\sigma}_{ij}(t)\overline{\sigma}_{ij}(0)\rangle$$

$$G(t) = \frac{1}{3}(G_{xy}(t)+G_{xz}(t)+G_{yz}(t))$$

Storage and loss moduli are defined as sine and cosine transforms of G(t)

$$G'(\omega) = \omega\int_0^\infty G(t)sin(\omega t)dt$$

$$G''(\omega) = \omega\int_0^\infty G(t)cos(\omega t)dt$$

Relaxation function behavior for a Rouse-like homopolymer melt

the baseline prior to addition of transient anchors

$$G_{HPM}(t) = \begin{cases} \frac{k_B T}{L_b^3} * C_b & t < \tau_0 \\ \frac{k_B T}{L_b^3} * C_b * \left(\frac{t}{\tau_0}\right)^{-0.5} & \tau_0 < t < \tau_e^{HPM} \\ G_e & \tau_e^{HPM} < t < \tau_{ter}^{HPM} \\ G_e * exp(-\frac{t}{\tau_{ter}^{HPM}}) & t > \tau_{ter}^{HPM} \end{cases}$$

τ_0 monomer relaxation time

τ_e^{HPM} end of Rouse relaxation

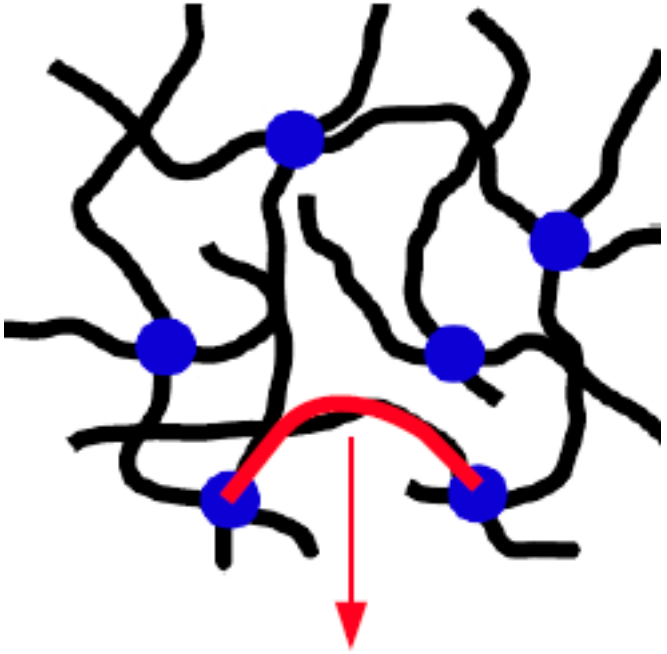
τ_{ter}^{HPM} terminal time of reptation due to entanglements, after which there is an abrupt relaxation and loss of memory

Introduce transient crosslinks, first assuming all domains can participate in the anchoring kinetics

Transient crosslinking introduces
*new lengthscales (distribution of contour lengths of the strands created by CLs)
*new timescales (relaxation times of the induced strands) which are "activated" if the crosslinks persist long enough

Permanent gels (long-lived crosslinks) introduce a mean strand contour length & relaxation time, easier to analyze

Depending on the kinetic timescales relative to the entangled polymer timescales, stochastic homogenization results are possible. For now, rely on MD simulations as in all other systems.



Contour length a strand between two Cls S^{CL}_{strand}

The corresponding relaxation time of the strand τ^{CL}_{strand}

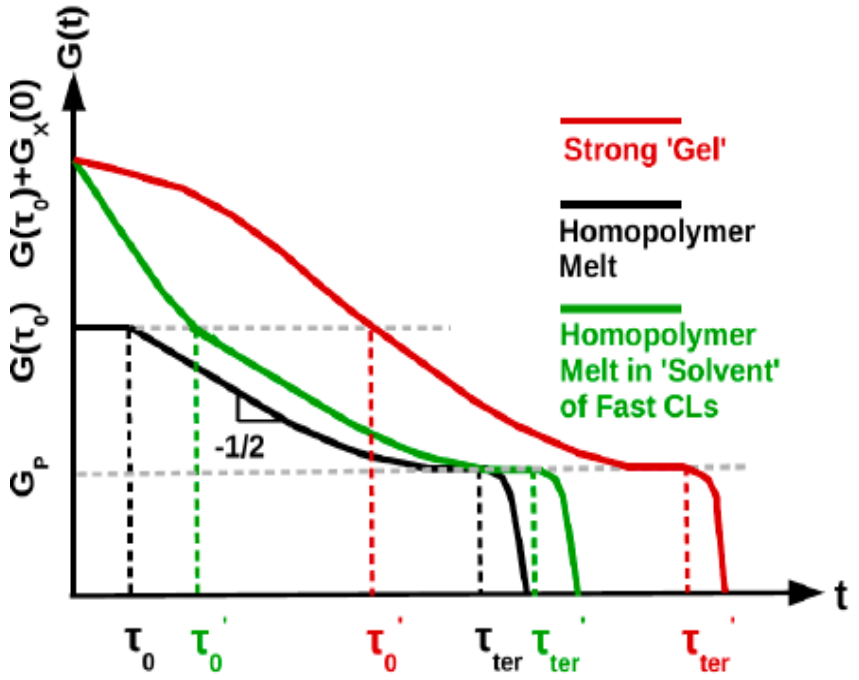
Results relative to the Rouse-like entangled polymer melts:

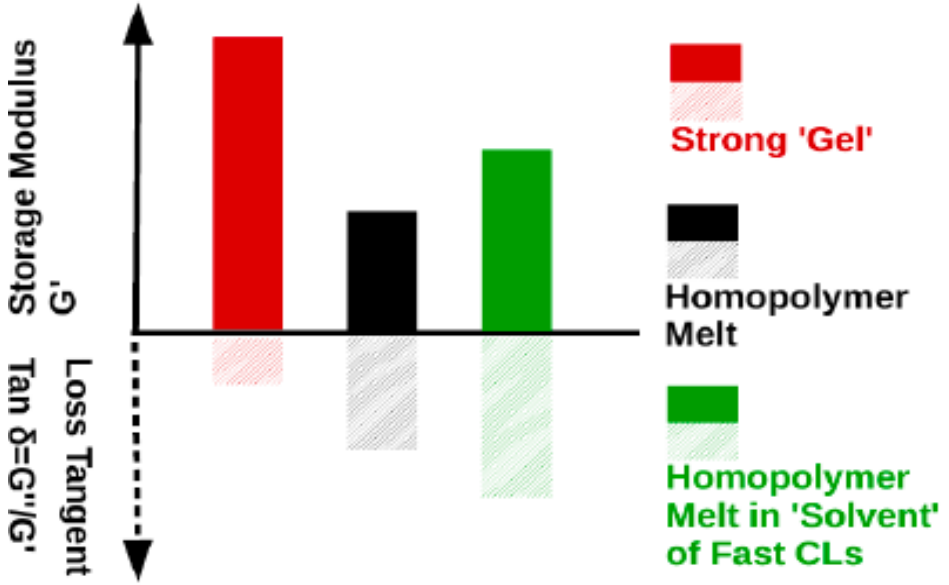
long-lived strong anchor family ➔ more elastic and more viscous, but enhances elasticity more than viscosity **gel-like behavior**

short-lived weak anchor family ➔ higher elasticity but even higher viscosity **sol-like behavior**

N.B. Pathological human lung mucus is highly viscous & elastic & gel-like

Healthy human lung mucus is highly viscous & elastic & sol-like





Effects of transient crosslinks on the relaxation function:
renormalized entangled polymer $G_{HPM}(t)$ ➔ $G_{Tran}(t)$
Can think of this as a collected coordinate description

$$G_{Tran}(t) = \begin{cases} G_{HPM}(t) + G_X(t) & t < \tau_{rel}^{CL} \\ G_{HPM}^{Eff}(t) = G_{HPM}(t/a_{shift}) & t > \tau_{rel}^{CL} \end{cases}$$

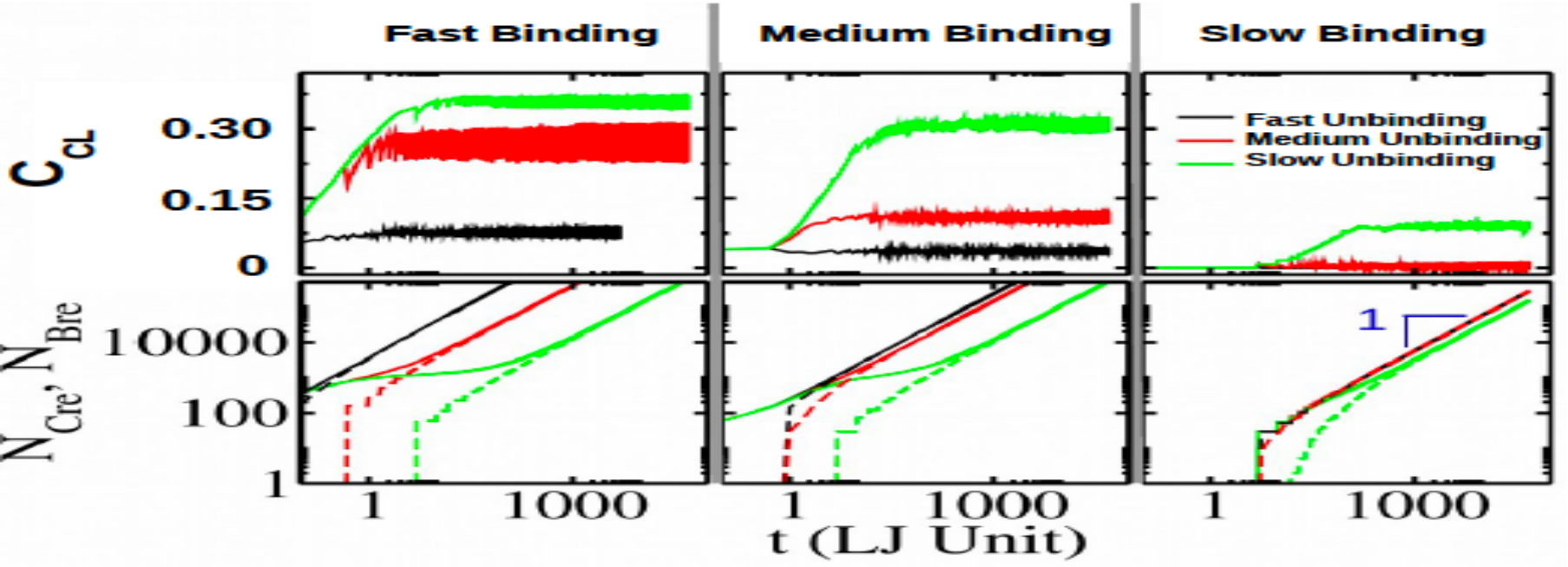
τ_{rel}^{CL} a timescale below which there is an additive transient crosslink relaxation mode

τ_{strand}^{CL} a relaxation timescale of the strands created by crosslinks

$a_{shift} = \frac{\tau_{rel}^{CL}}{\tau_{strand}^{CL}}$ rescaling factor due to crosslinks, value relative to 1
Is dictated by anchor binding-unbinding kinetics

$$G_X(t) = C_{CLs} * \nu k_B T * exp(-\frac{t}{\tau_{ref}^{CL}})$$

Phase Diagram of Transiently Crosslinked Entangled Polymers
Track time to recover quasi-equilibrium (heal after rupture) & # of transient bonds (mean + fluctuations) vs (binding, unbinding times)



Primary outcomes versus timescales of binding and unbinding:

- ***High affinity, short-lived anchors self-heal fast, low % mean crosslinks***
- ***Recall these anchors create strength (elasticity), maintain "sol" state***
- Current direction: allow families of anchors with variable affinities to different domains distributed according to known heterogeneous structure of mucus, and use sufficient experimental micro/macro rheology data to learn kinetics.

Thank you for your attention, to the organizers for the honor of the invitation, and to our sponsors for their support.

To anyone interested in the biological contexts where transient, especially weak anchors, convey diverse functionality, we are a collaborative family ☺

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