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Given August 17, 2006 during the GEM4 session at MIT in Cambridge, MA.

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Cytoskeleton dynamics simulation of the red blood cell

Ju Li

Collaborators: Subra Suresh, Ming Dao, George Lykotrafitis, Chwee-Teck Lim

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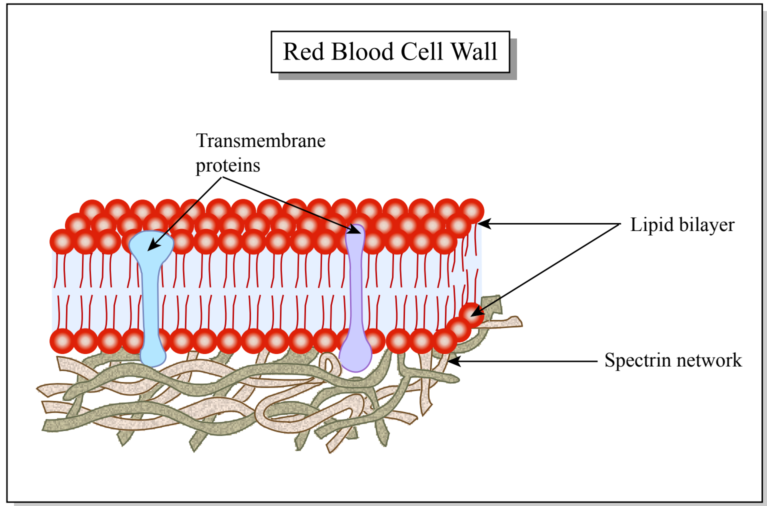
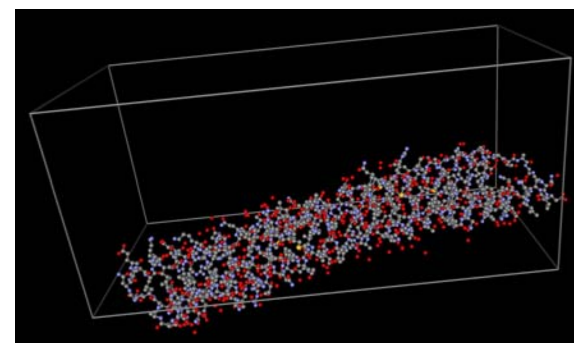


Figure by MIT OCW.

One spectrin tetramer has 39 segments, contour length ~200 nm.
Room-temperature length ~80nm due to thermal fluctuations.

one segment ~5 nm



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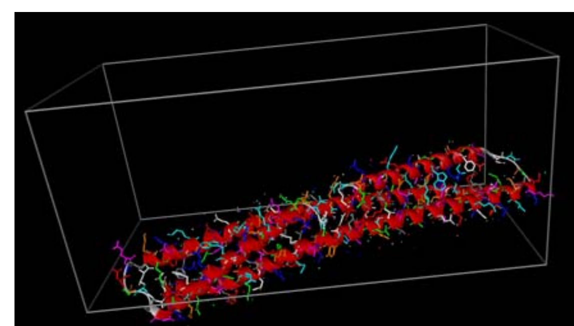

Images removed due to copyright restrictions.
See Fig. 2 in Takeuchi, et al. "Structure of the Erythrocyte Membrane Skeleton as Observed by Atomic Force Microscopy." *Biophys* J 74 (1998): 2171-2183.

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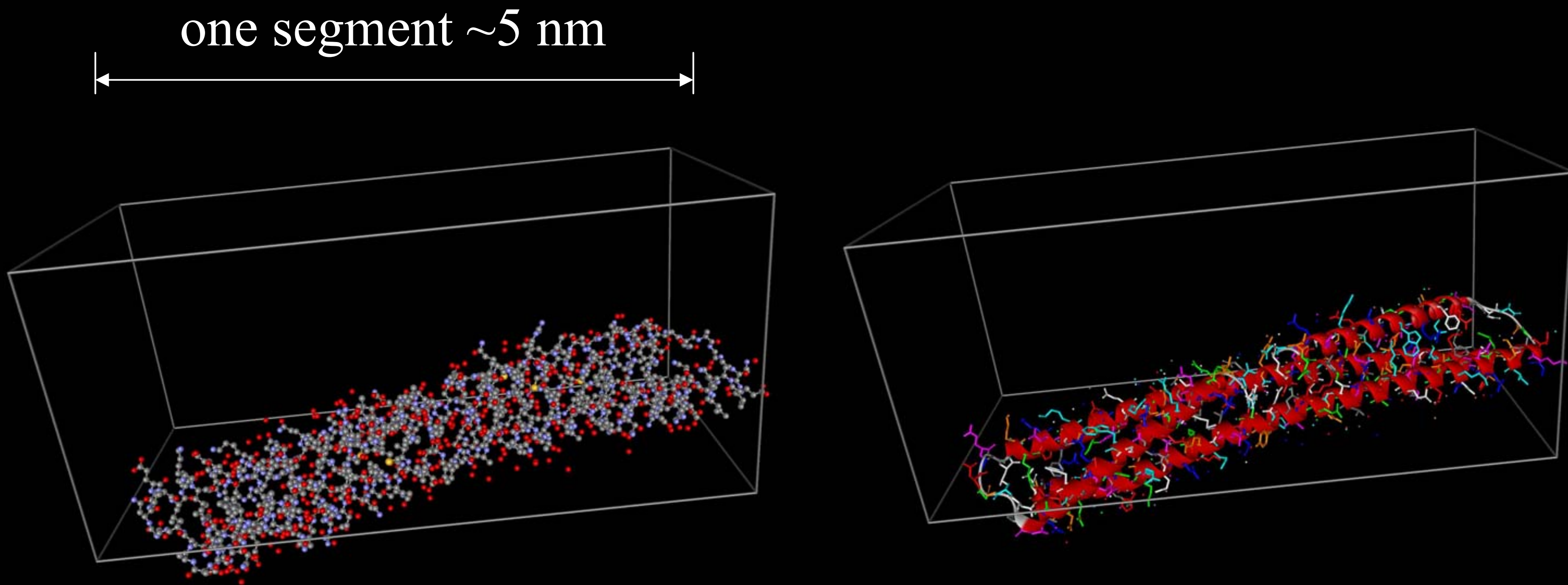
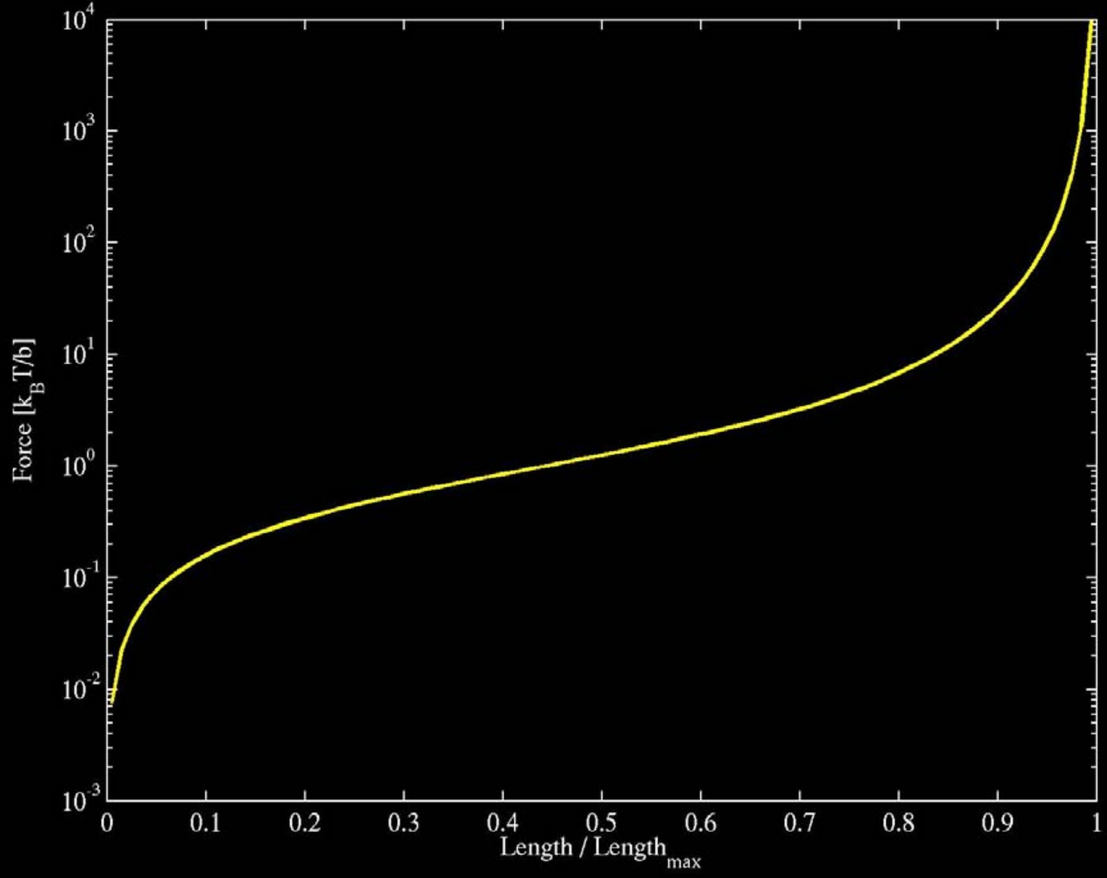
See movies in Dao, M., C. T. Lim, and S. Suresh. "Mechanics of The Human Red Blood Cell Deformed by Optical Tweezers." *J Mech Phys Solids* 51 (2003): 2259-2280.

Spectrin Elasticity



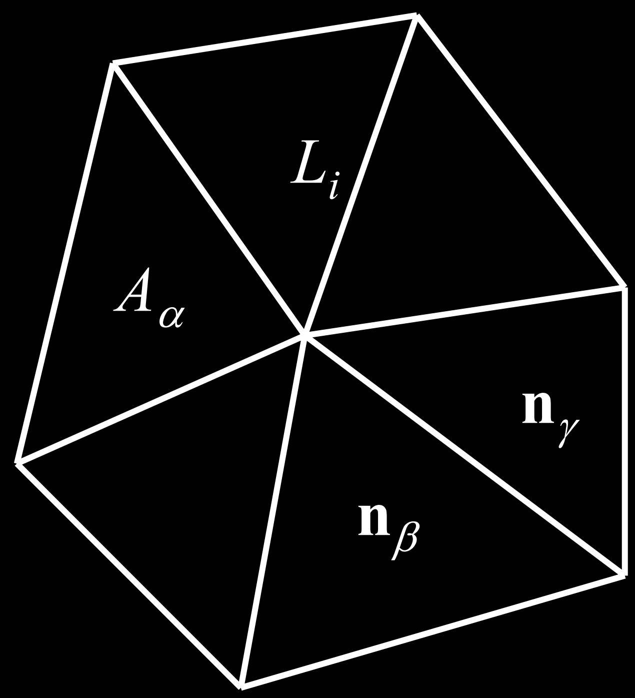
One spectrin tetramer has ~40 segments, contour length ~200 nm.
Room-temperature length ~80nm due to thermal fluctuations.

Worm-like Chain Coarse-Grained Free Energy



$$V_{\mathrm{WLC}}(L) = \frac{k_{\mathrm{B}} T L_{\max}}{4b} \cdot \frac{3x^2 - 2x^3}{1-x}, \quad x \equiv \frac{L}{L_{\max}}$$

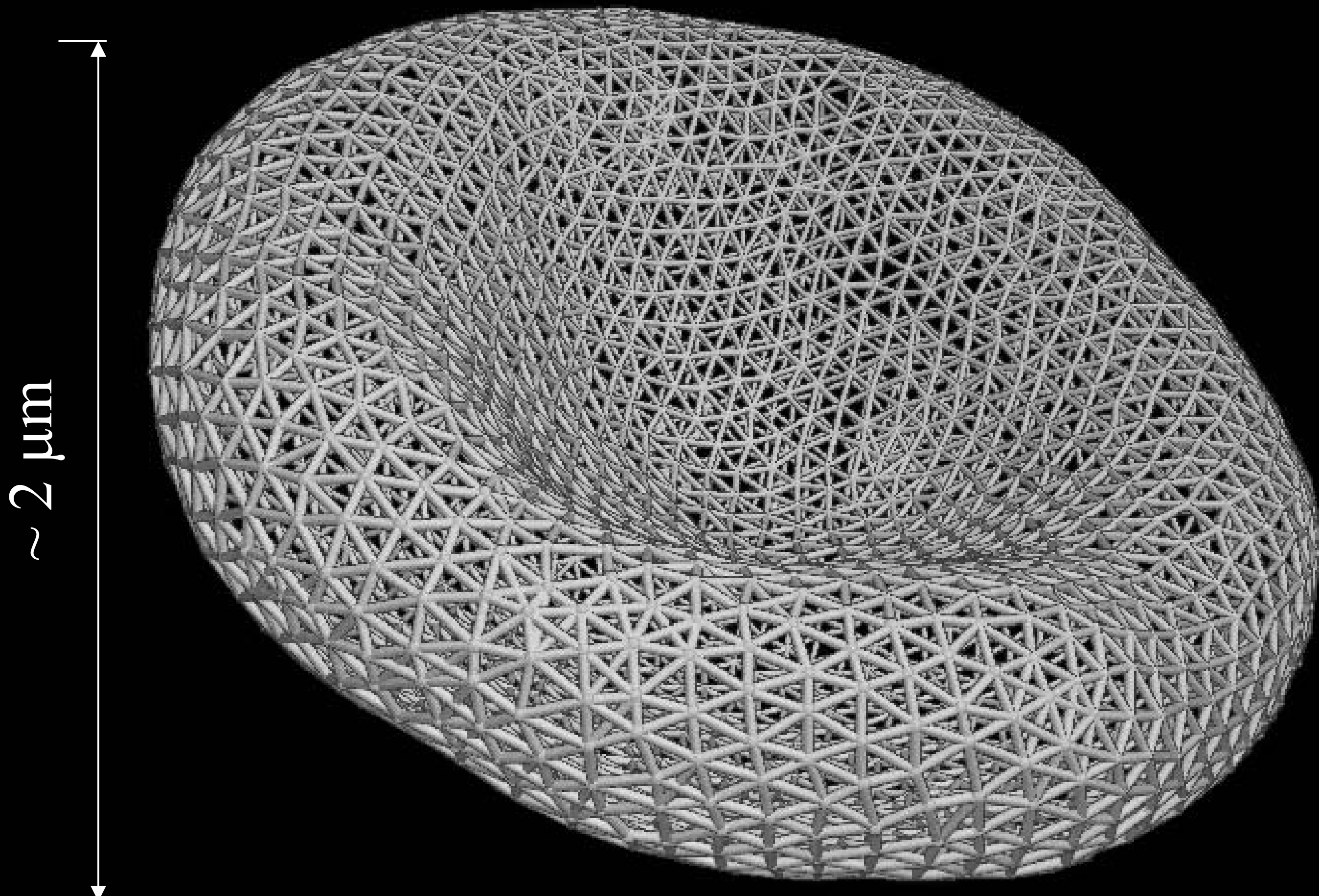
Spectrin-Net Level, Whole Red Blood Cell model (Discher, Boal, Boey, 1998)



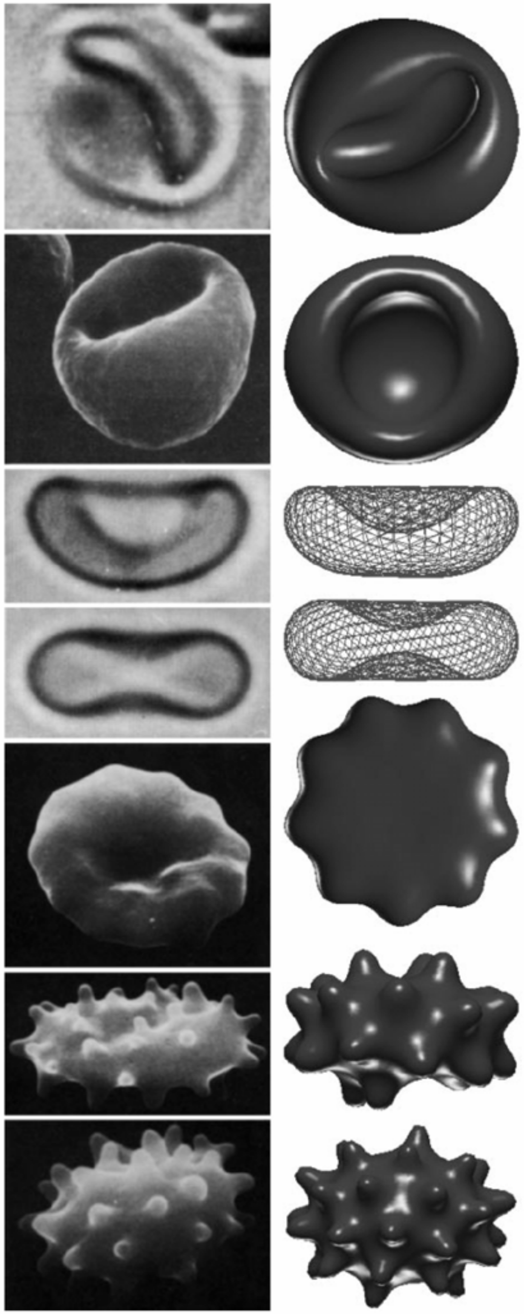
$$V_{\text{total}} = \sum_{i \in \text{spectrin link}} V_{\text{WLC}}(L_i) + \sum_{\alpha \in \text{triangle}} \frac{C}{A_\alpha} + \sum_{\beta,\gamma \in \text{triangle}} K_{\text{bend}} (1 - \mathbf{n}_\beta \cdot \mathbf{n}_\gamma)$$

+ total volume constraint + total area constraint

Small Cell Simulation
(“volume quench” to get discocyte shape)



2562 vertices



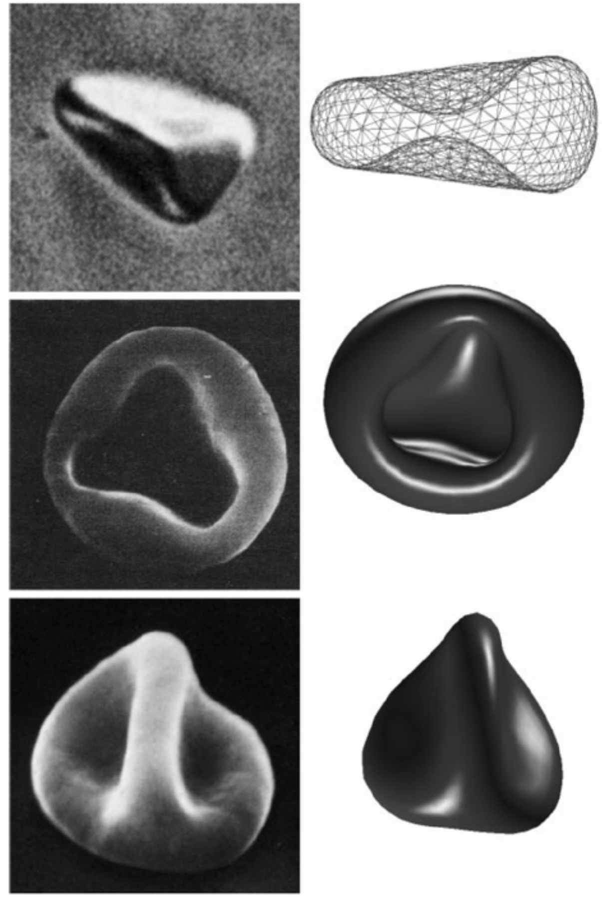


Fig. 2. A sample of observed non-main-sequence shapes, including (top to bottom) nonaxisymmetric discocyte, stomatocyte with triangular mouth, and knizocyte. (*Left*) Laboratory images reproduced with permission from refs. 27 (Copyright 1981, Biophysical Society), 32 (Copyright 1980, Academic Press), and 2 (Copyright 1973, Springer). (*Right*) Minimum-energy shapes calculated from our model with values of v_0 and $\overline{\Delta a_0}$ of 0.989 and 0.215%, 0.950 and −0.858%, and 1.000 and 1.144% (from top to bottom) with all other parameters remaining fixed.

required to make them conform. The shape–free–energy functional that incorporates these two effects is

$$F_{\mathrm{ADE}}[S] = \frac{\kappa_b}{2}\oint_S d\mathcal{A}(2H - C_0)^2 + \frac{\bar{\kappa}}{2}\frac{\pi}{AD^2}(\Delta A - \Delta A_0)^2, \quad [1]$$

where D is the membrane thickness,‖ A is the membrane area, κ_b

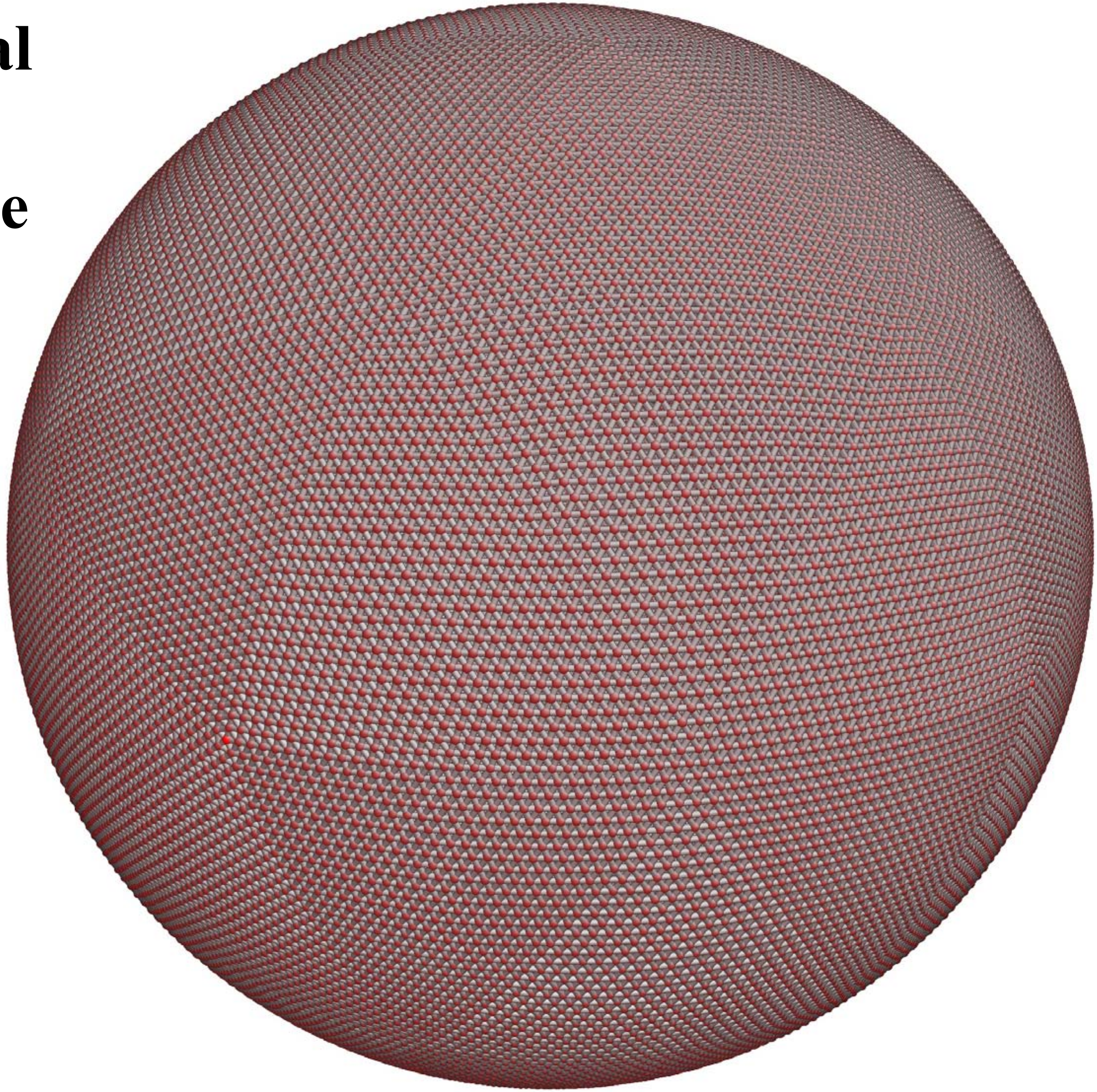
Lim, Wortis, Mukhopadhyay, *PNAS* **99** (2002) 16766

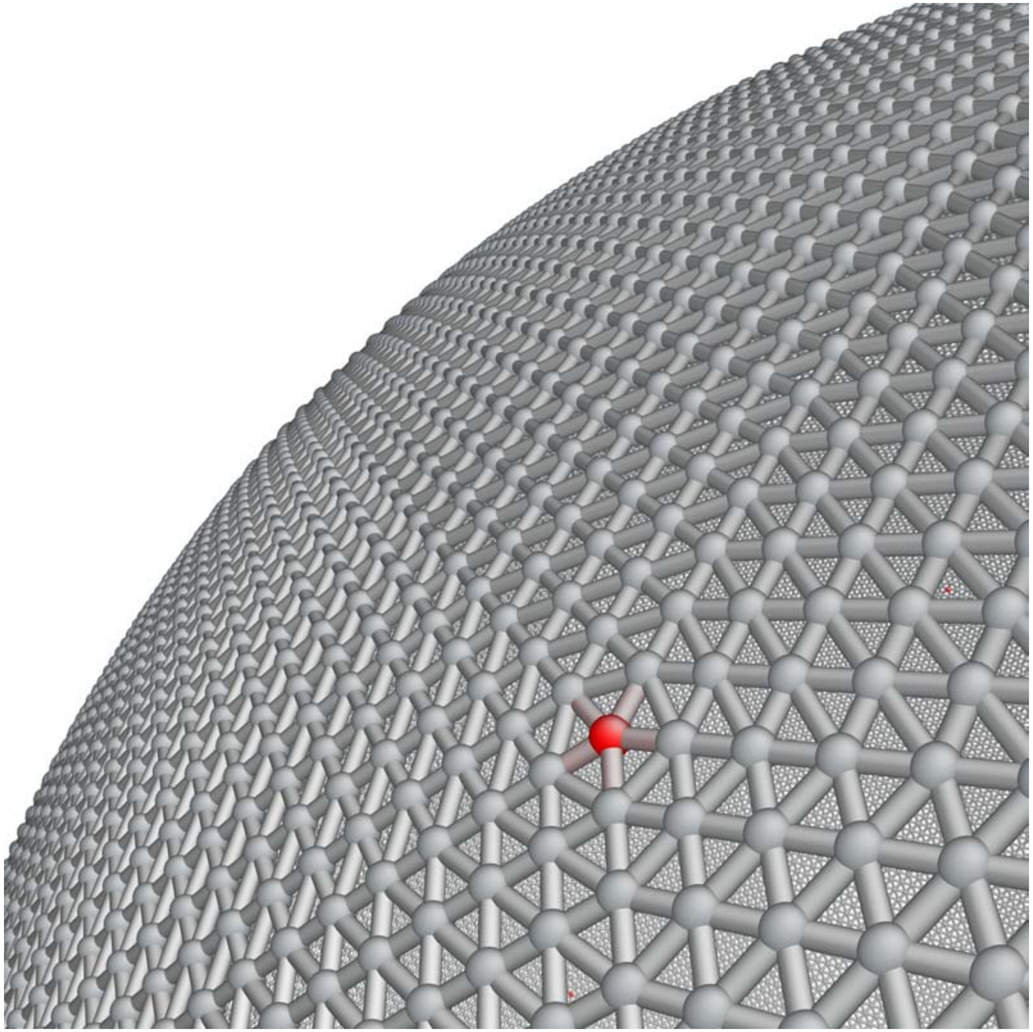
Stomatocyte - discocyte - echinocyte Sequence

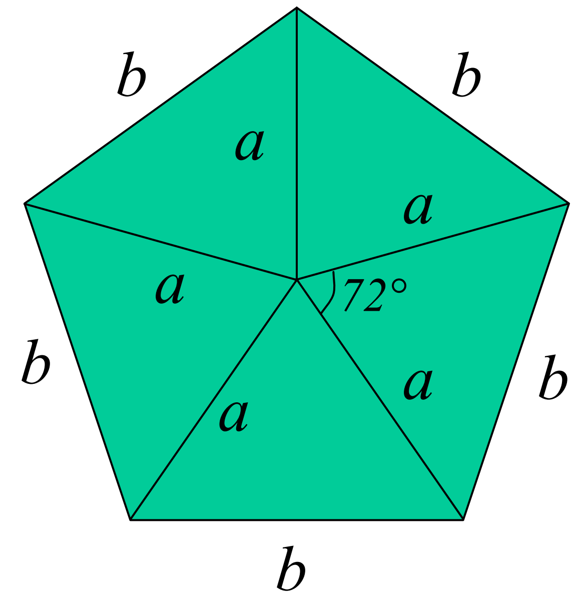
spontaneous curvature parameter

Source: Lim, Gerald, Michael Wortis, and Ranjan Mukhopadhyay. "Stomatocyte–discocyte–echinocyte Sequence of the Human Red Blood Cell: Evidence for the Bilayer– Couple Hypothesis from Membrane Mechanics." *PNAS* 99 (2002): 16766-16769.

Icosahedral network on a sphere



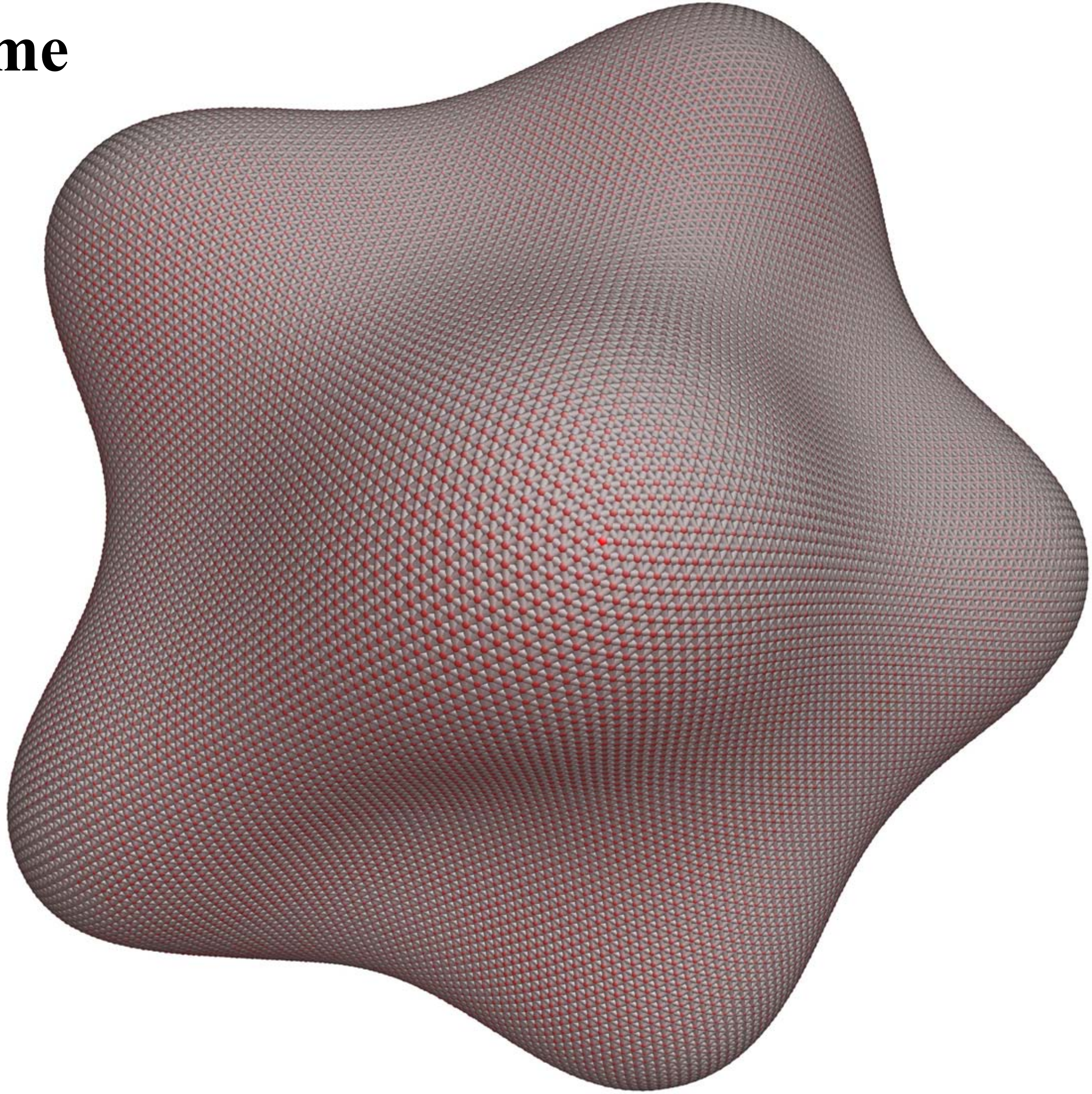




Geometrically Necessary Disinclinations

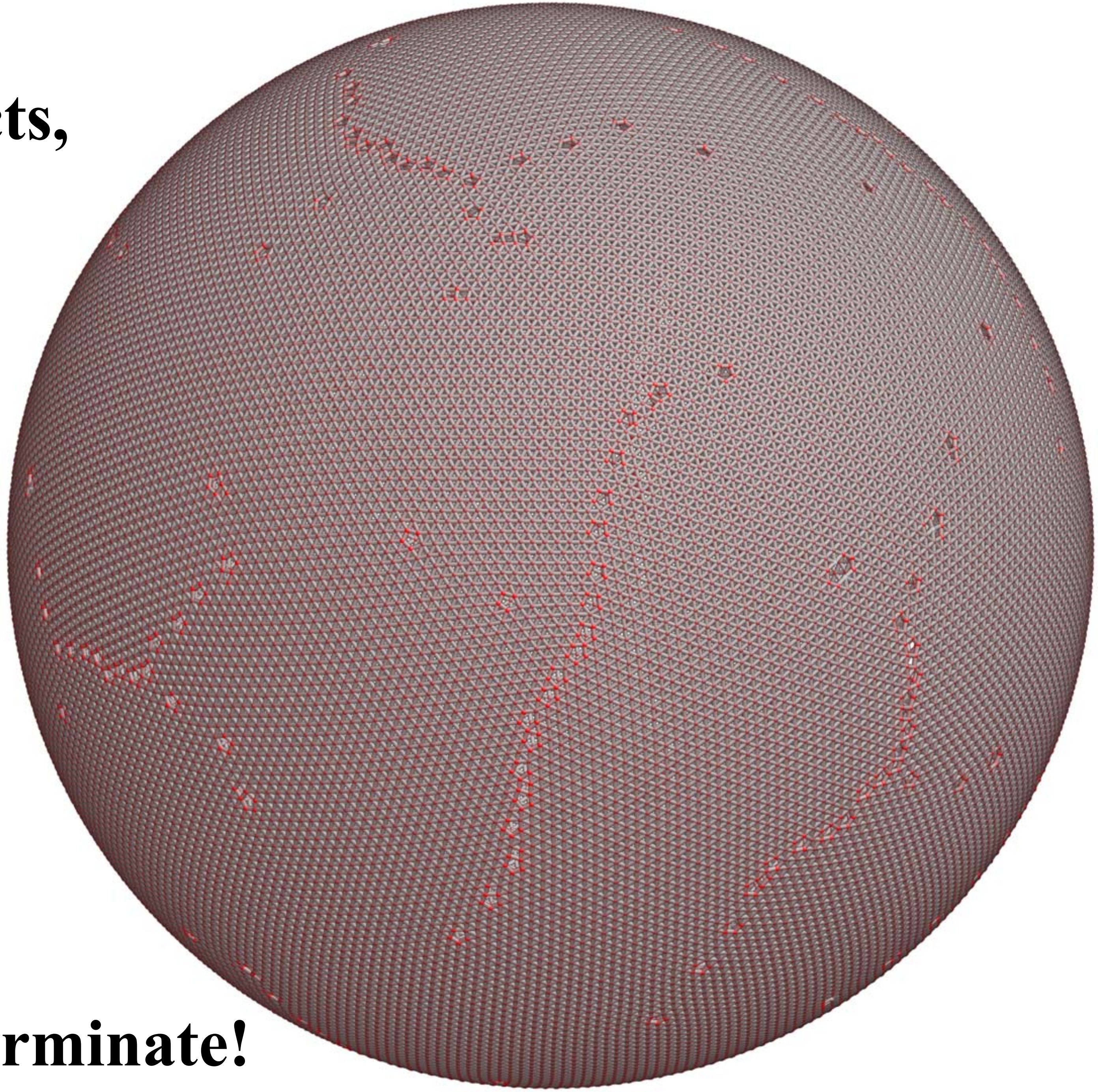
If each carries disinclination charge 60°, need 12

100% volume



60% volume

To rid of the shape artifacts, melt and quench the network



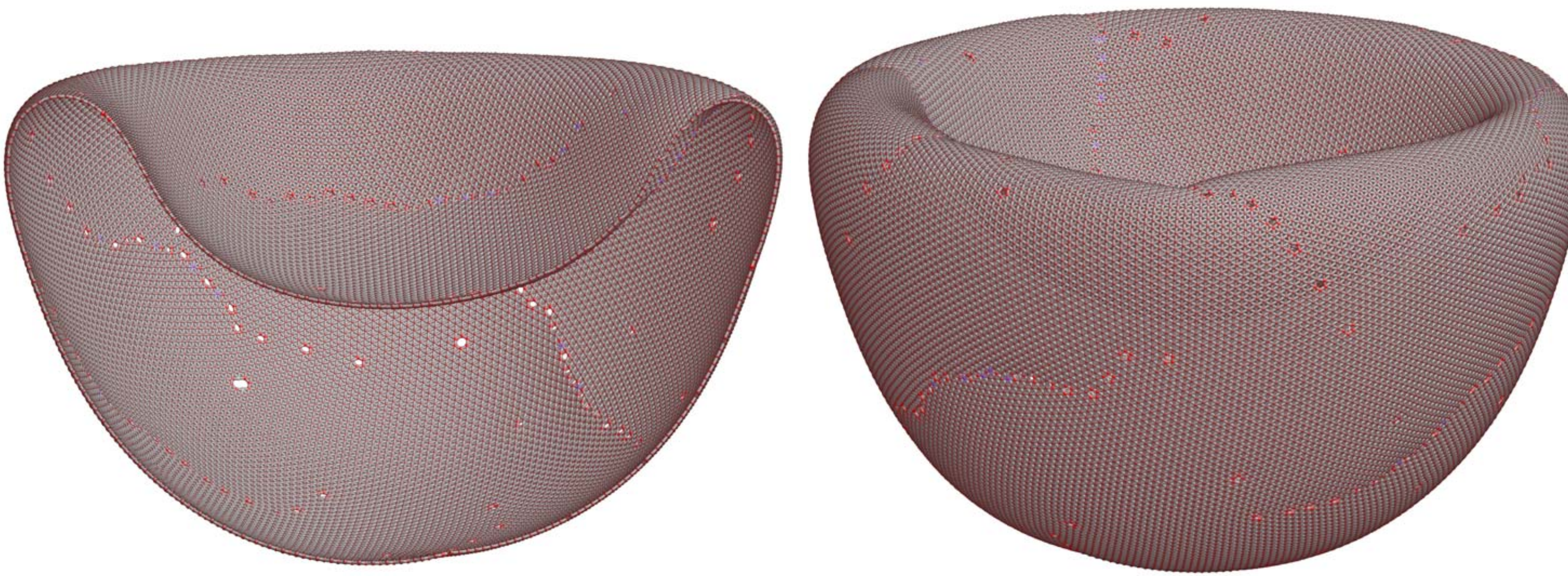
GBs freely terminate!

Images removed due to copyright restrictions.
See Fig. 1 in Bausch, et al. "Grain Boundary Scars and Spherical Crystallography." *Science* 299 (2003): 1716-1718.

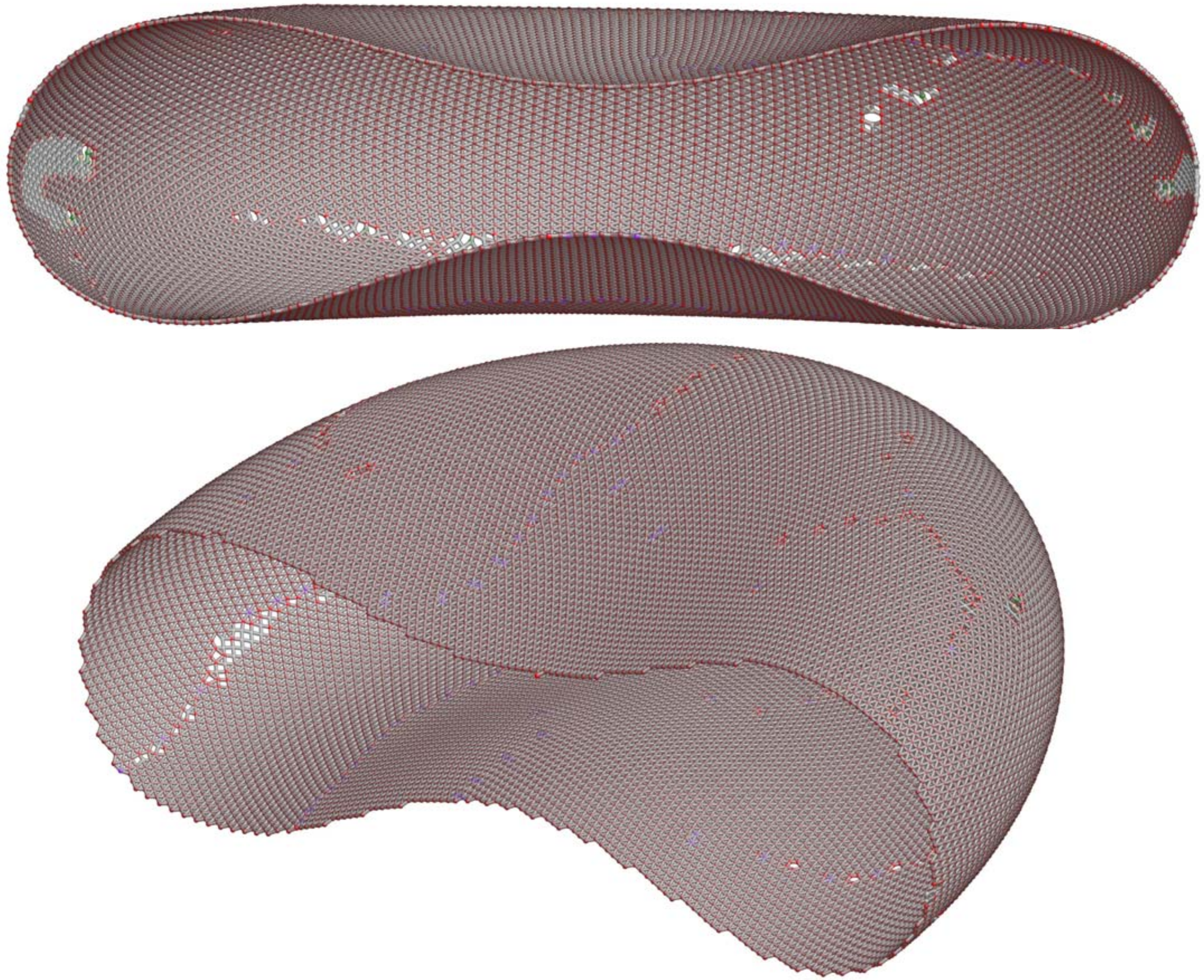
These GBs should be widespread in nature: large viral protein capsids, giant spherical fullerenes, spherical bacterial surface layers, siliceous skeletons of spherical radiolaria (aulosphaera), etc.
Sites for chemical reactions, initiation points for bacterial cell division, influence the mechanical response.

Material reference state

for the in-plane shear energy E_{shear}



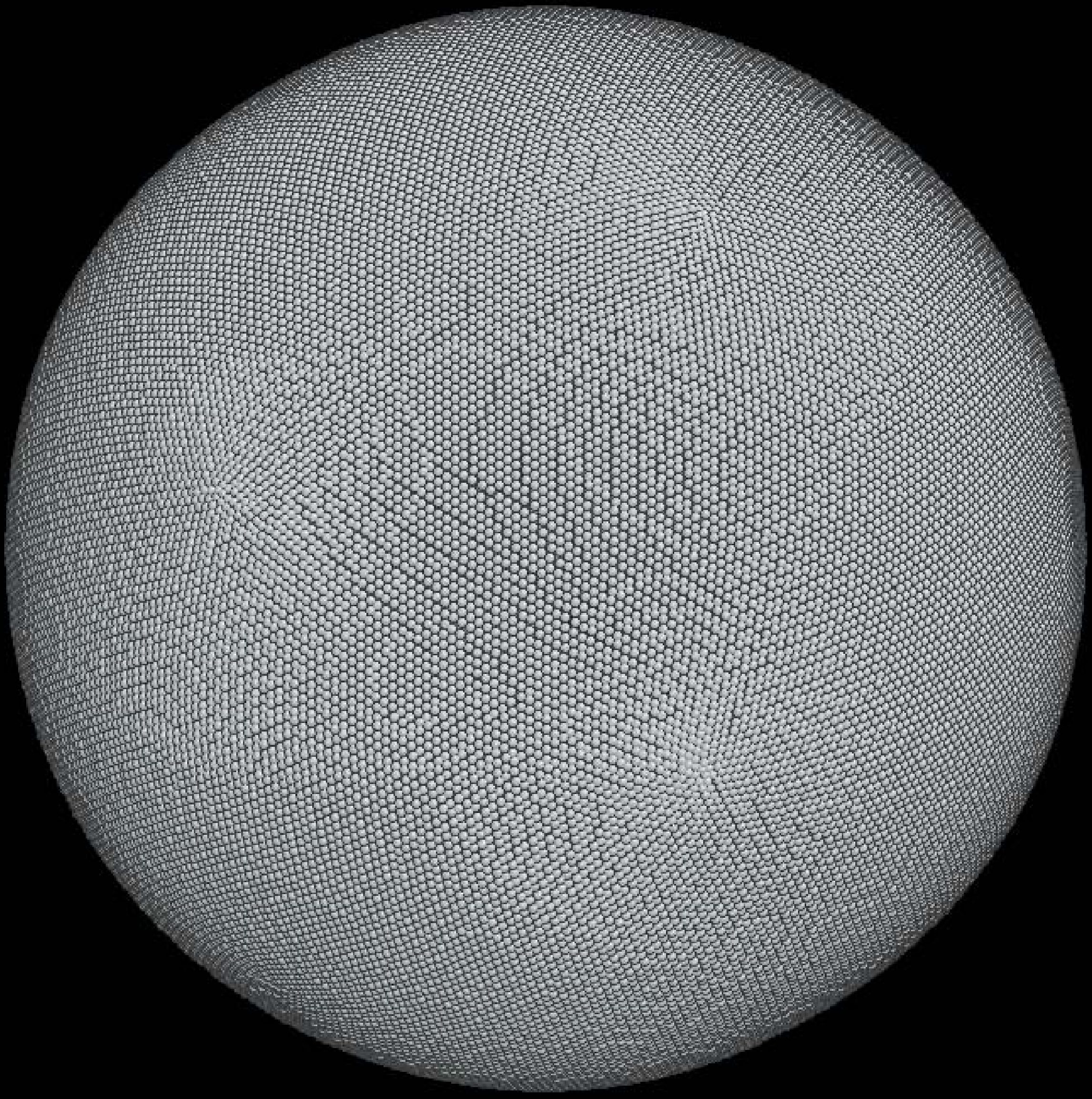
60% volume: **spherical state** as stress-free reference.

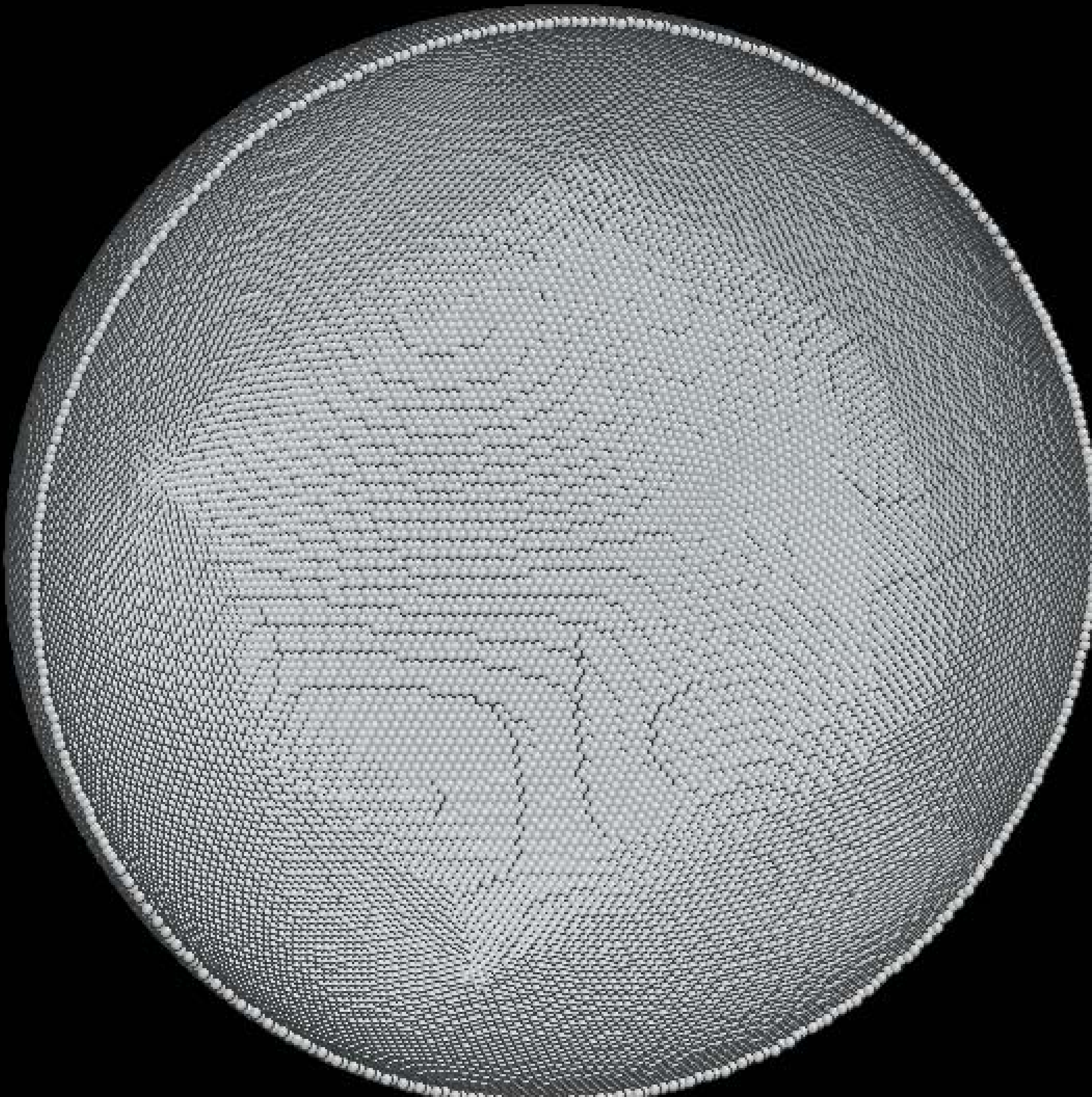


W/ experimental range of parameters and sphere as stress-free reference state, the biconcave shape is only metastable at 60% volume.

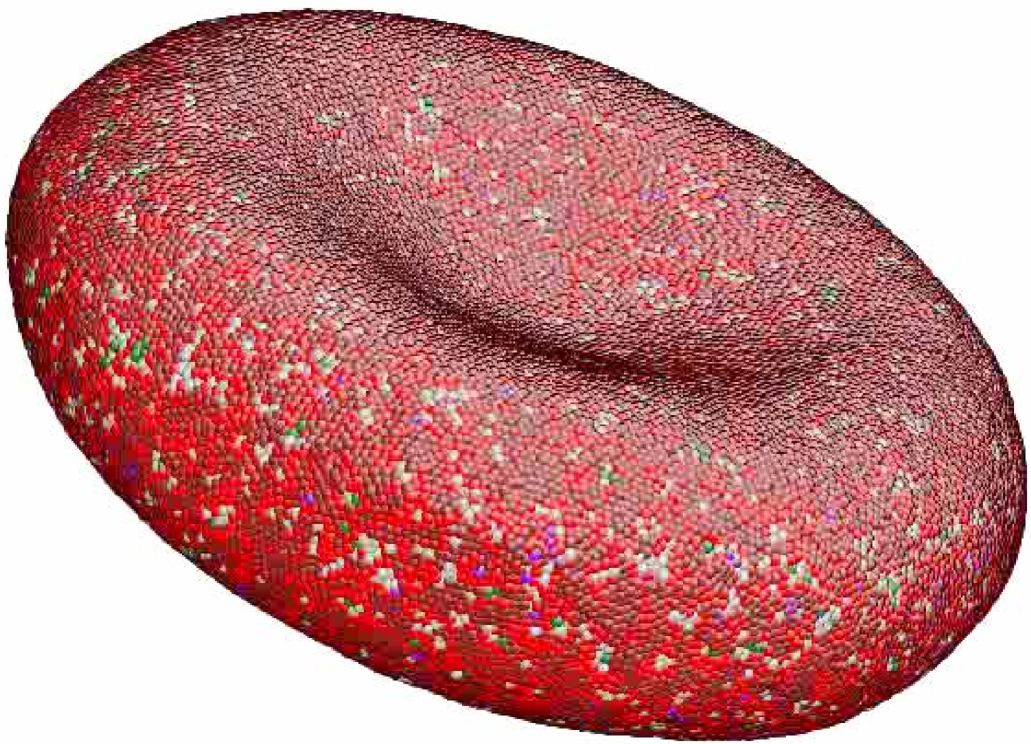
With bending energy E_{bend} only

Canham (1970)
Helfrich (1973)

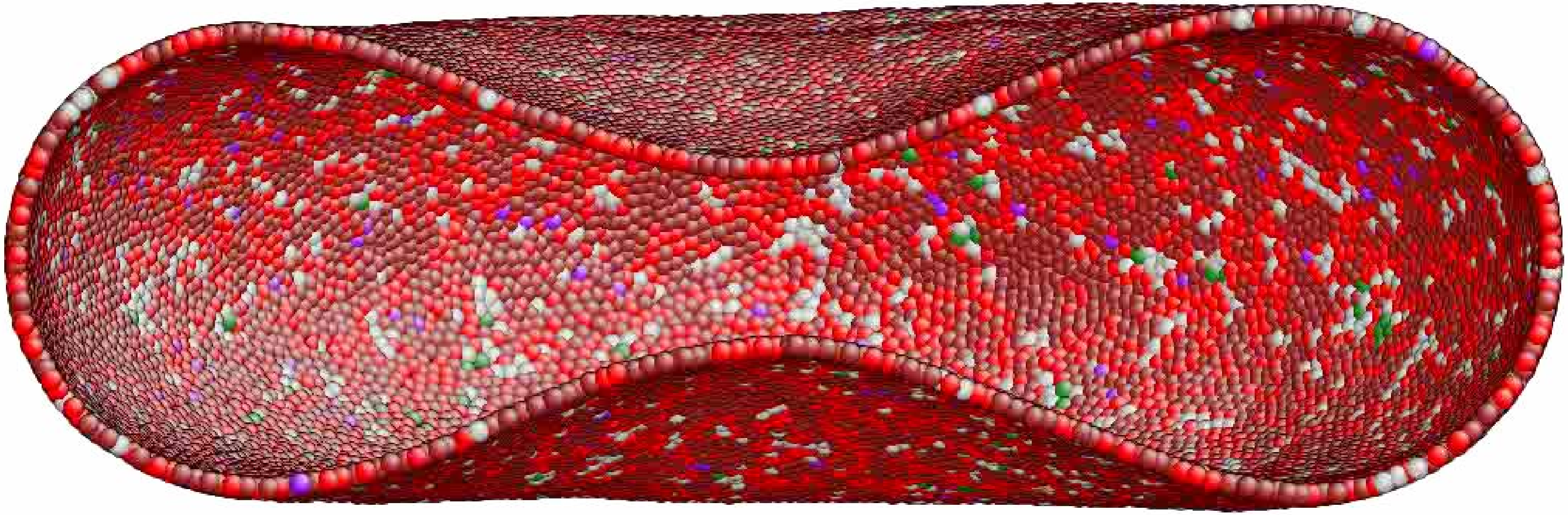


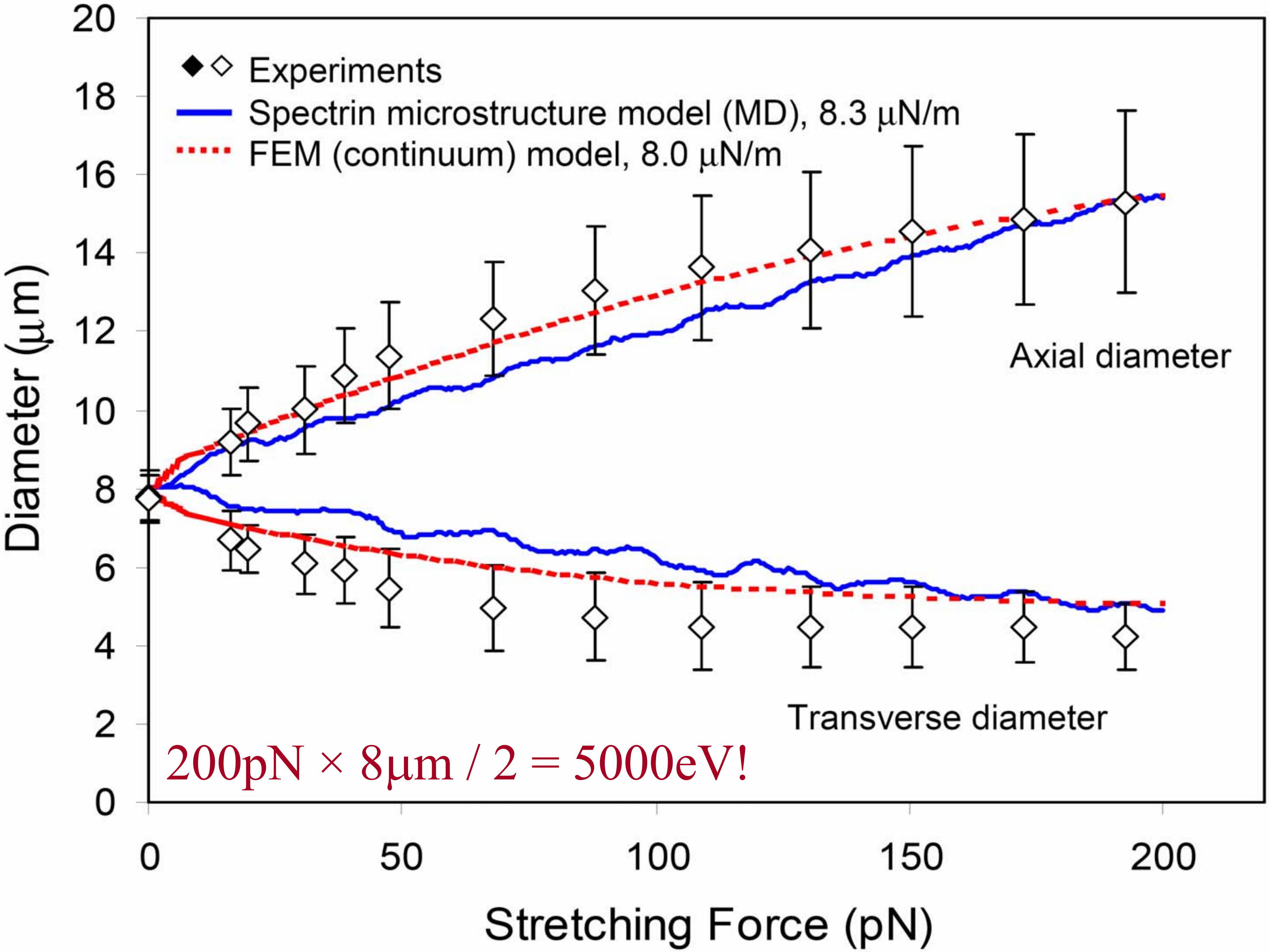


Optical Tweezers Stretching Simulation



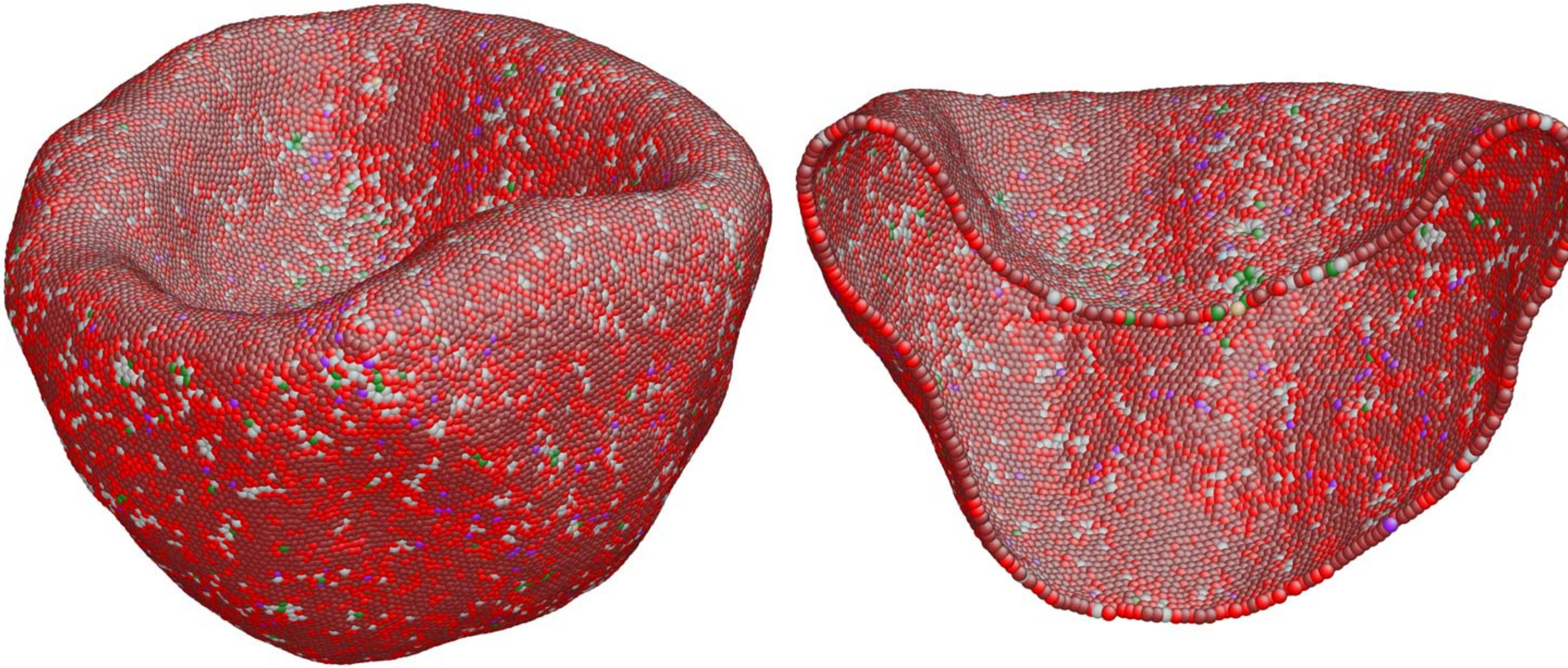
Cross Sectional View





Experiments
Spectrin microstructure model (MD), 8.3 μN/m
FEM (continuum) model, 8.0 μN/m
Axial diameter
Transverse diameter
200pN × 8μm / 2 = 5000eV!
Diameter (μm)
Stretching Force (pN)
0
2
4
6
8
10
12
14
16
18
20
0
50
100
150
200

Why is biconcave the stable equilibrium shape?



$E_{\mathrm{bend}} \sim 8\pi\kappa$: $\kappa \sim 2\times10^{-19}$ J $\rightarrow$ $E_{\mathrm{bend}} \sim 30$ eV

$E_{\mathrm{shear}} \sim \mu\varepsilon^2 A$: $\mu \sim 8\mu$N/m, $\varepsilon \sim 0.1$, $A \sim 140\mu\mathrm{m}^2$
$\rightarrow$ $E_{\mathrm{shear}} \sim 70$ eV

Material Concept Hypothesis

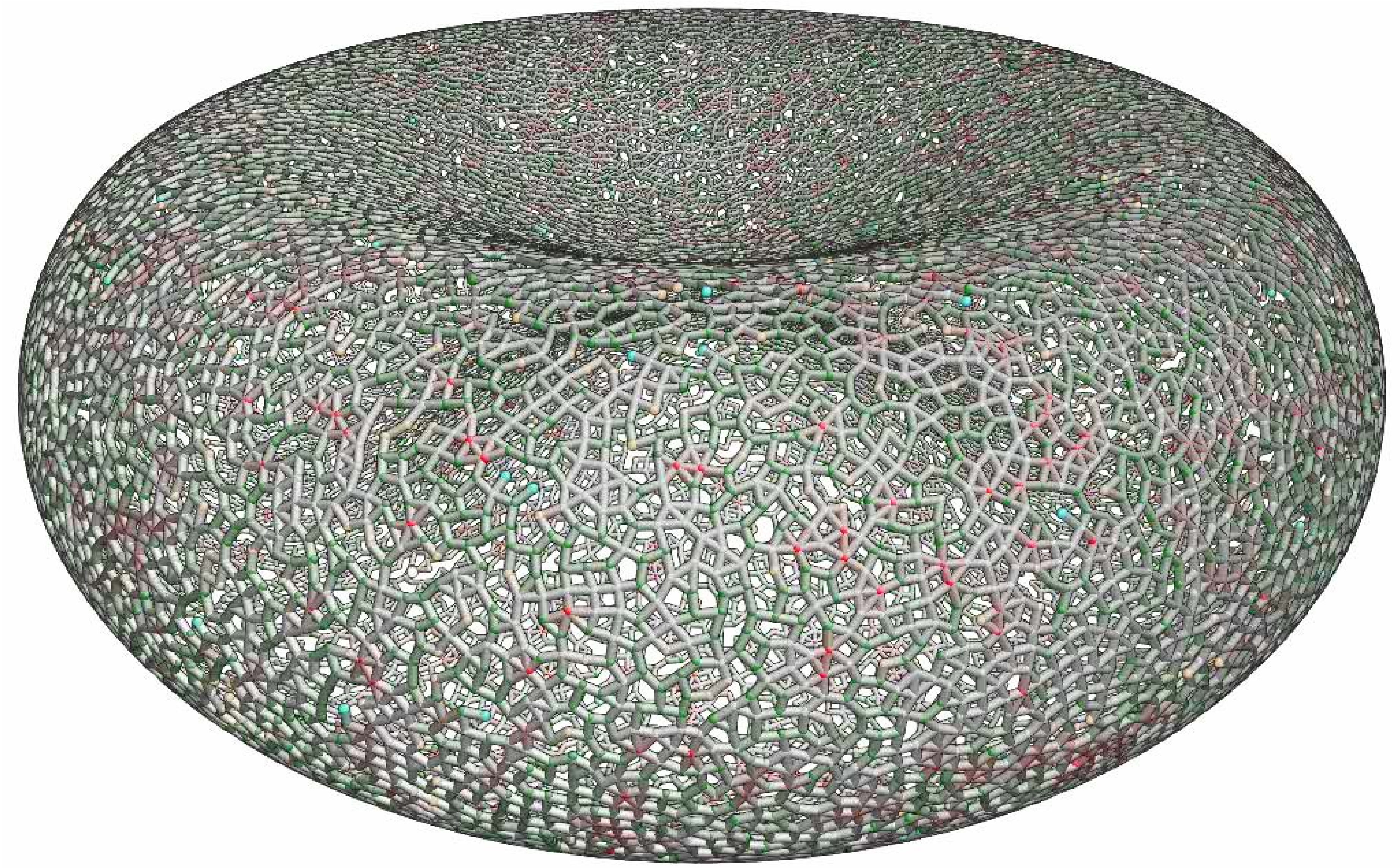
Li, Dao, Lim & Suresh, *Biophys. J.* **88** (2005) 3707.

- In an ideal limit, for any RBC shape, the cytoskeleton will always undergo remodeling in topological connectivity at a slow rate to relax its in-plane *shear* elastic energy to *zero*.

  "liquefaction", "slow-flowing glass"

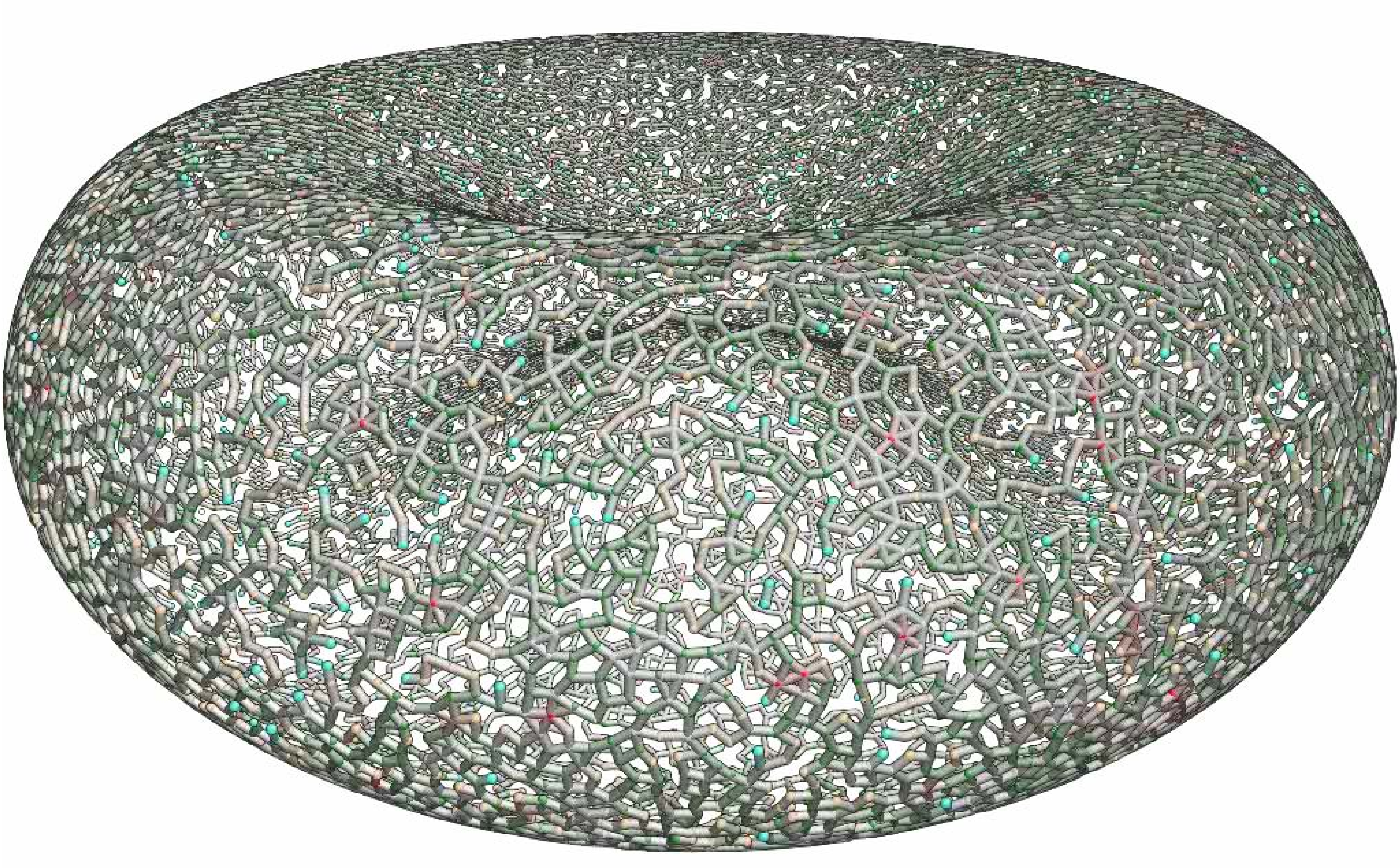
- At the timescale of optical tweezers stretching, the above relaxation is not significant, so large shear energy can be injected temporarily.

Stillinger-Weber liquid on curved surface:



no shear energy can survive long!

RBC cytoskeleton at reduced spectrin density



very large holes start to percolate ...

Extreme Statistics of Cytoskeletal Defects in RBC

	actin#	spectrin#	largest polygon hole
normal	28673	81718	6
degree-4.5	26880	57523	8
degree-4	24372	48012	11
degree-3.5	21504	37416	22
degree-3	18637	26837	35

But this is basically from a "geometrical" simulation
no biophysical basis, yet.

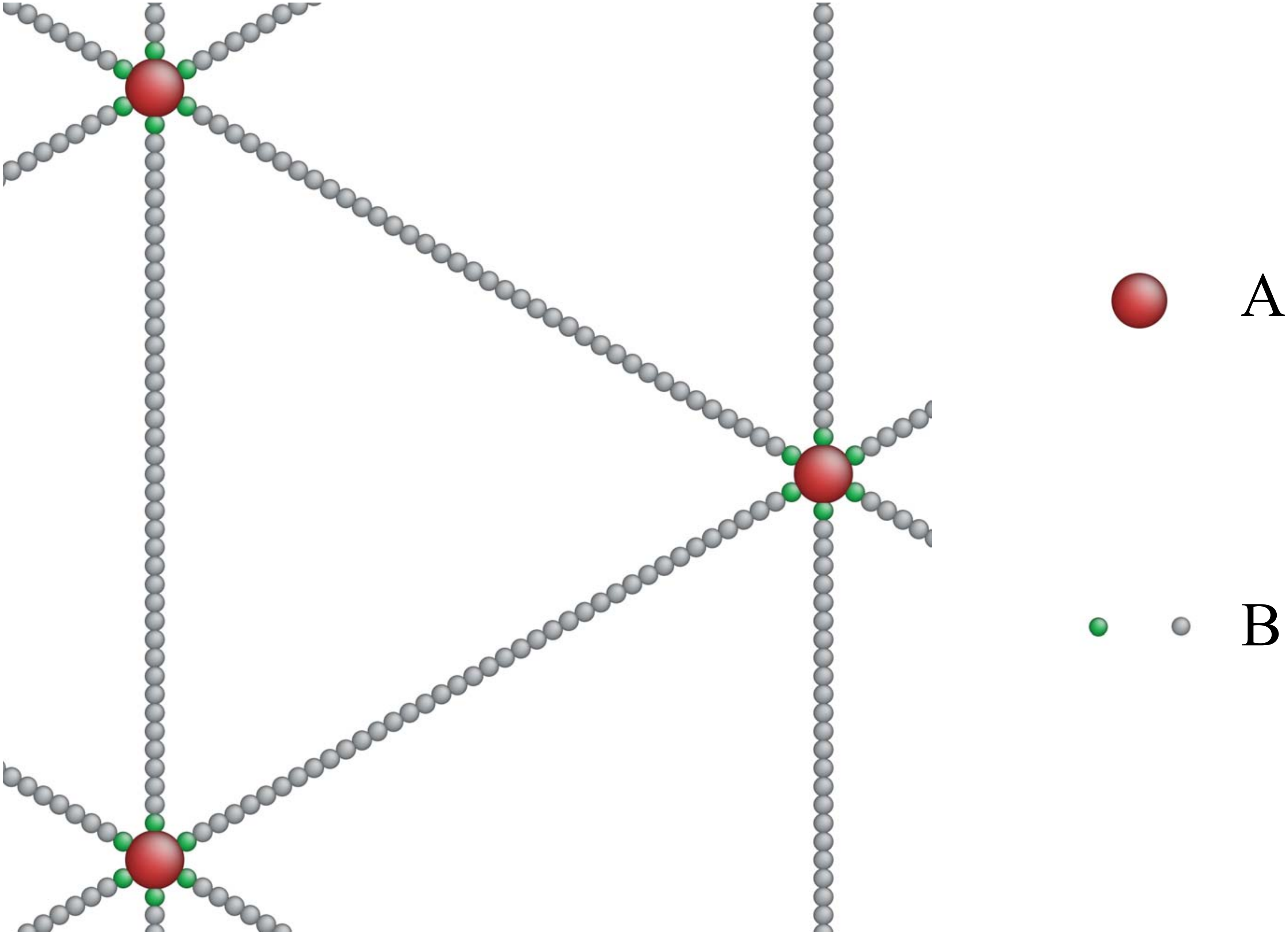
Intermediate Summary

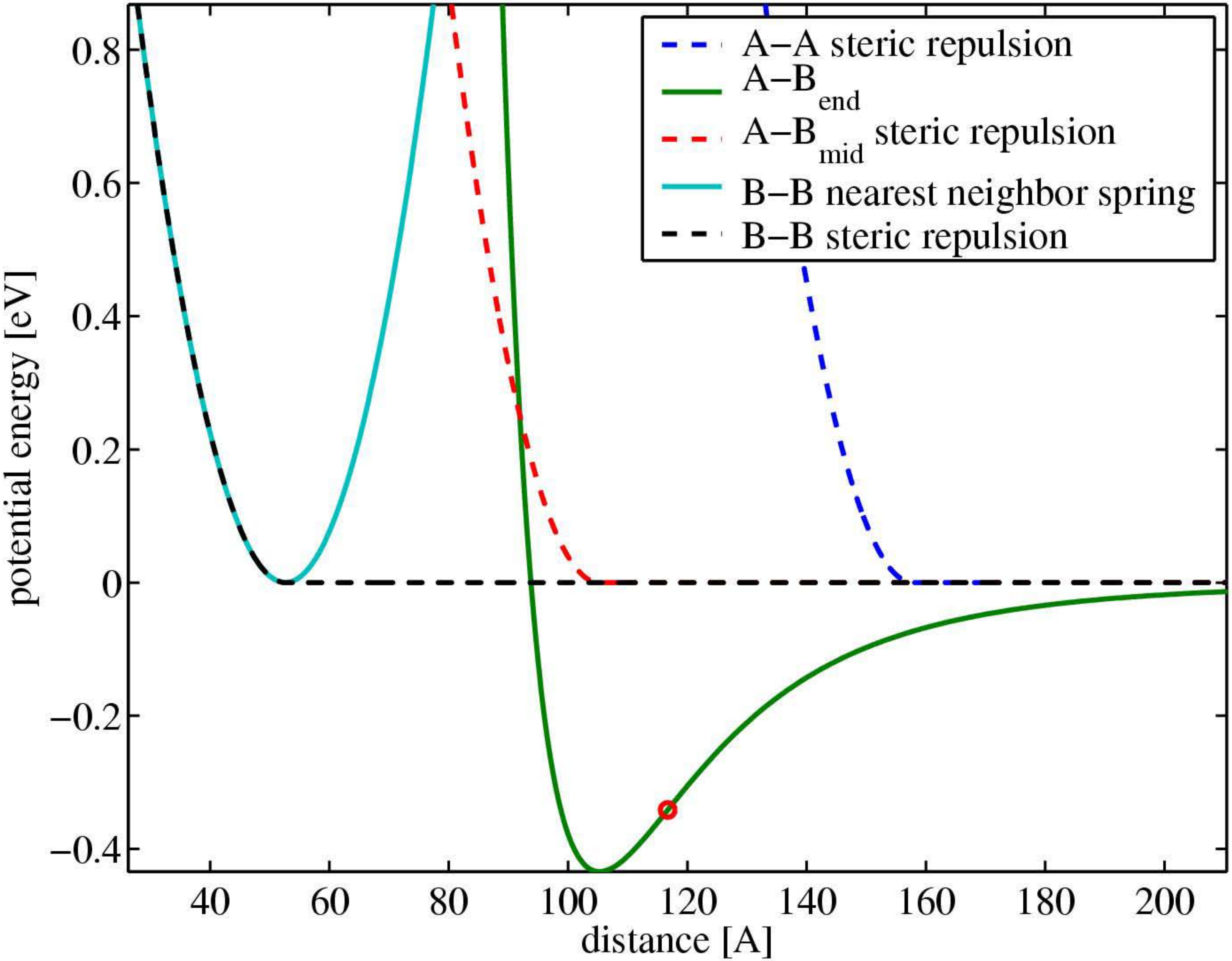
- Spectrin-level and continuum FEM analyses indicate our optical tweezers experiments give approximately the same in-plane shear modulus as micropipette aspiration experiments: $\mu = 5$ to 10×10^{-6} N/m.

- Stabilization of biconcave equilibrium shape strongly suggests the cytoskeleton undergoes slow but constant remodeling topologically to always relax the in-plane shear elastic energy to zero.

- Connection to single-molecule stretching experiments (“intermolecular potential development”).

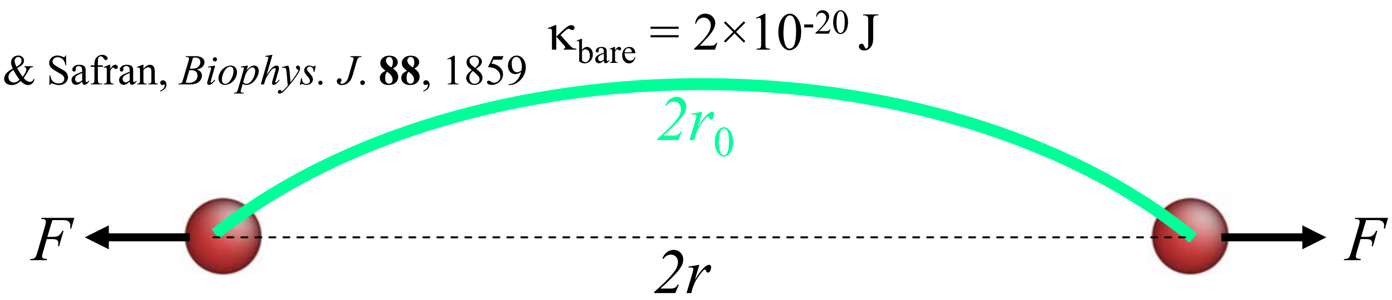
CGMD model with *breakable* actin-spectrin junction



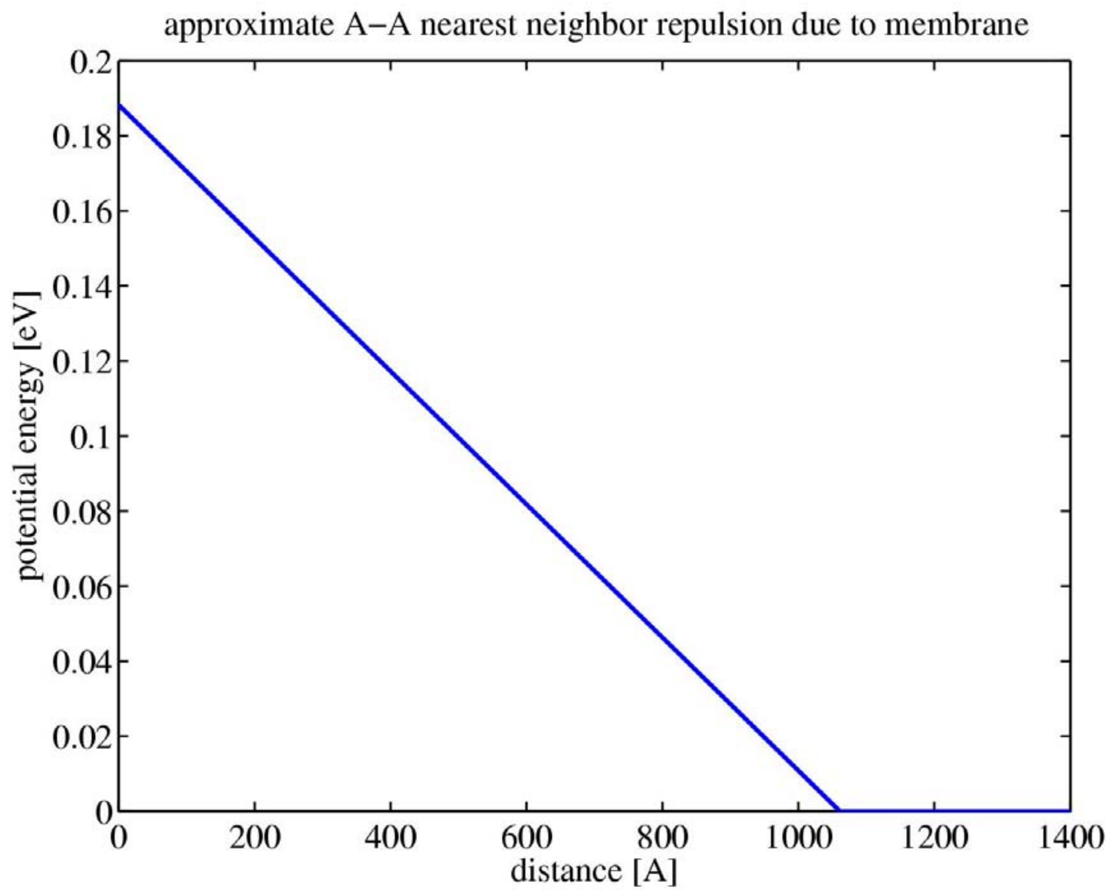


A−A steric repulsion
A−B$_{end}$
A−B$_{mid}$ steric repulsion
B−B nearest neighbor spring
B−B steric repulsion
potential energy [eV]
distance [A]
0.8
0.6
0.4
0.2
0
−0.2
−0.4
40
60
80
100
120
140
160
180
200

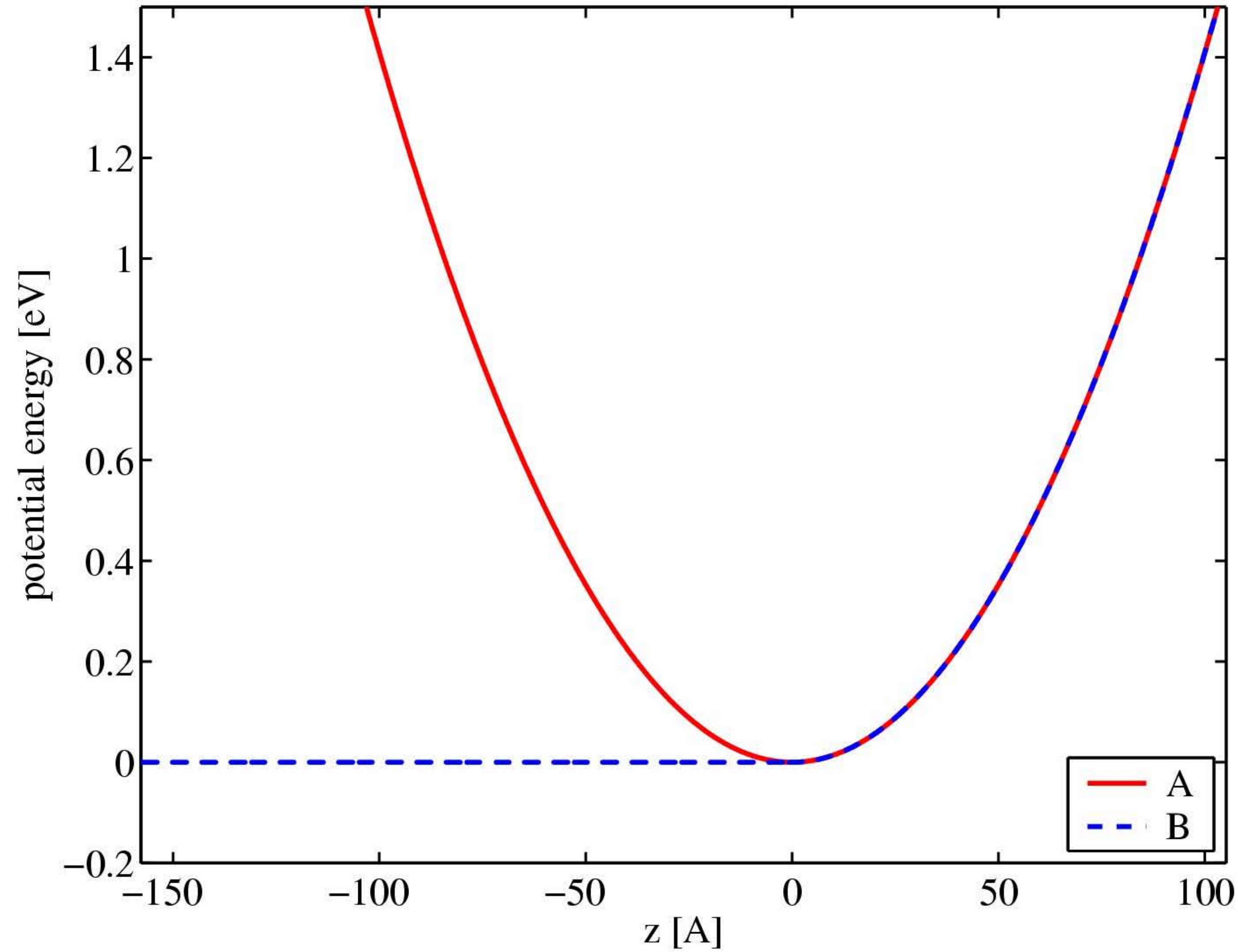
Reference: Gov & Safran, *Biophys. J.* **88**, 1859

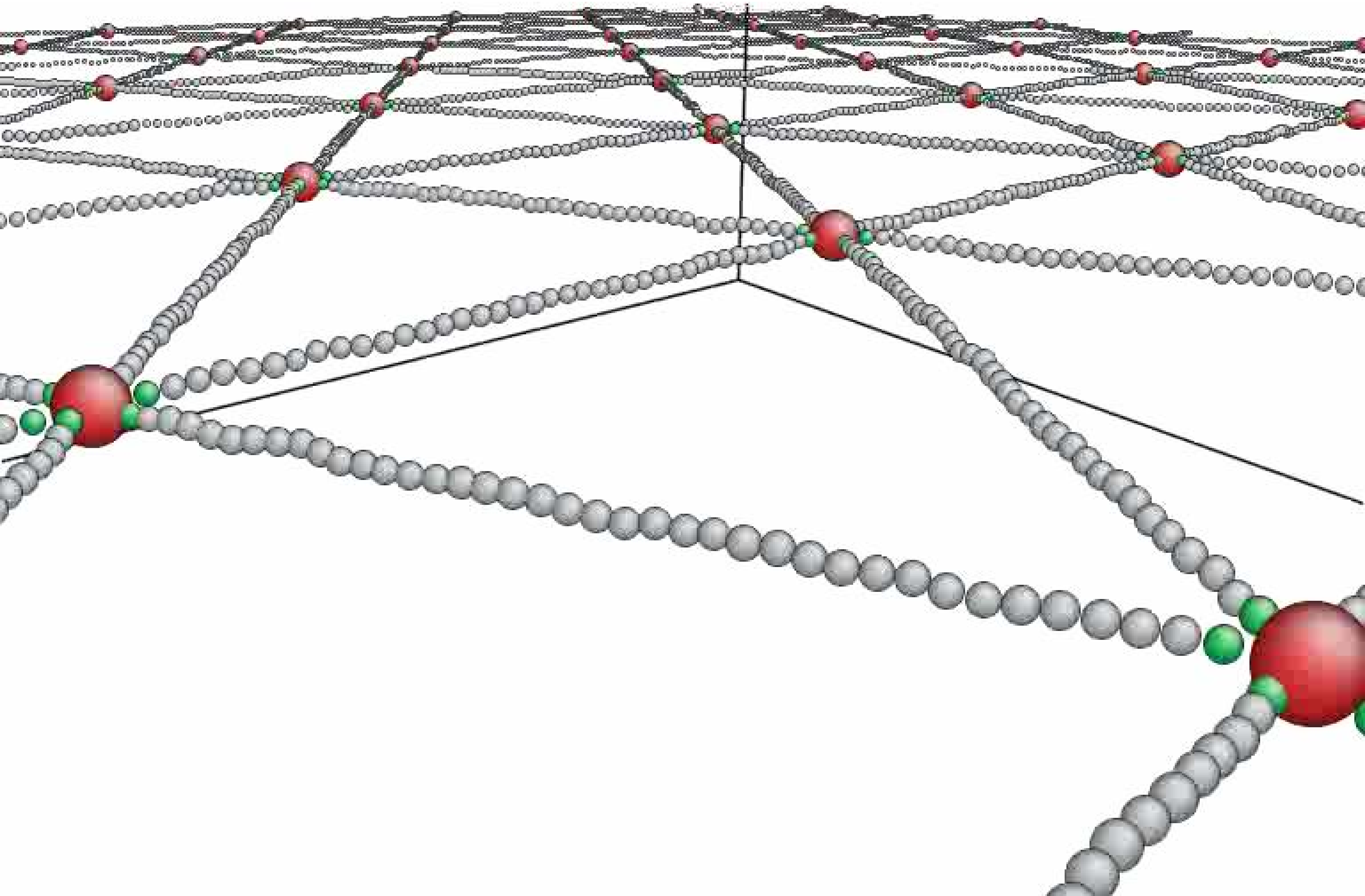


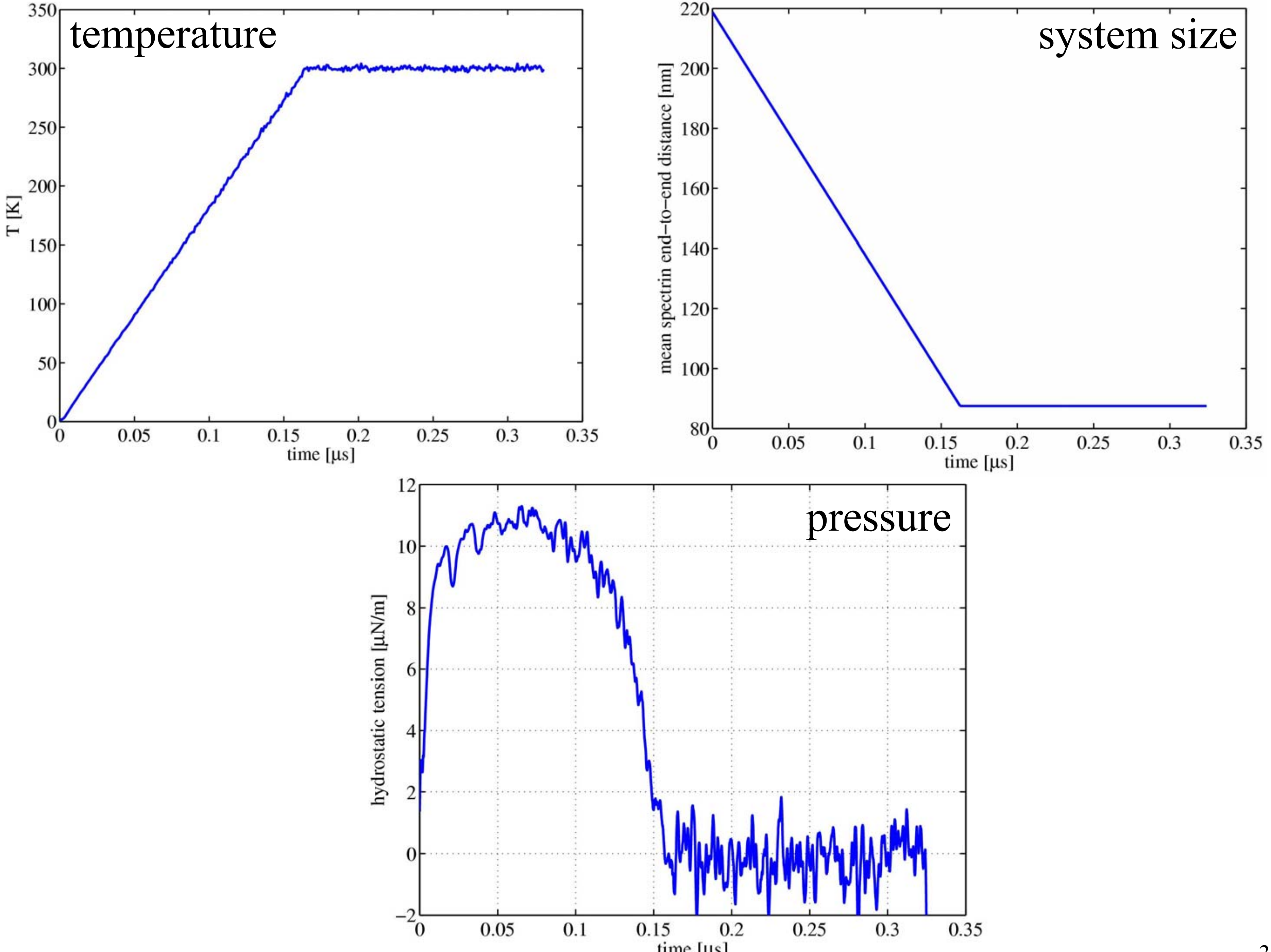
$$F = \alpha \frac{4\pi\kappa_{bare}}{3r_0}, \qquad \alpha \text{ chosen to be } 0.36$$



We also put soft ($0.1k_{\mathrm{BB}}$) confinement potential on A and B in z to mimic interaction with the membrane without actually simulating the membrane.

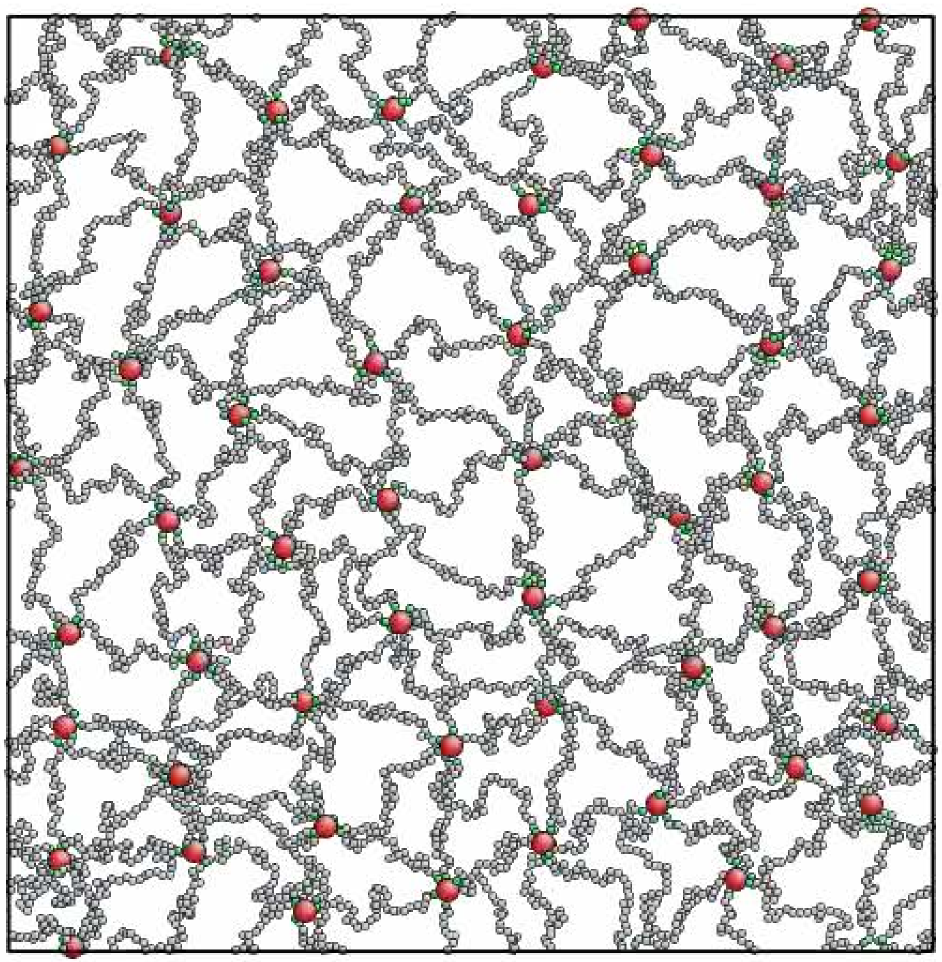




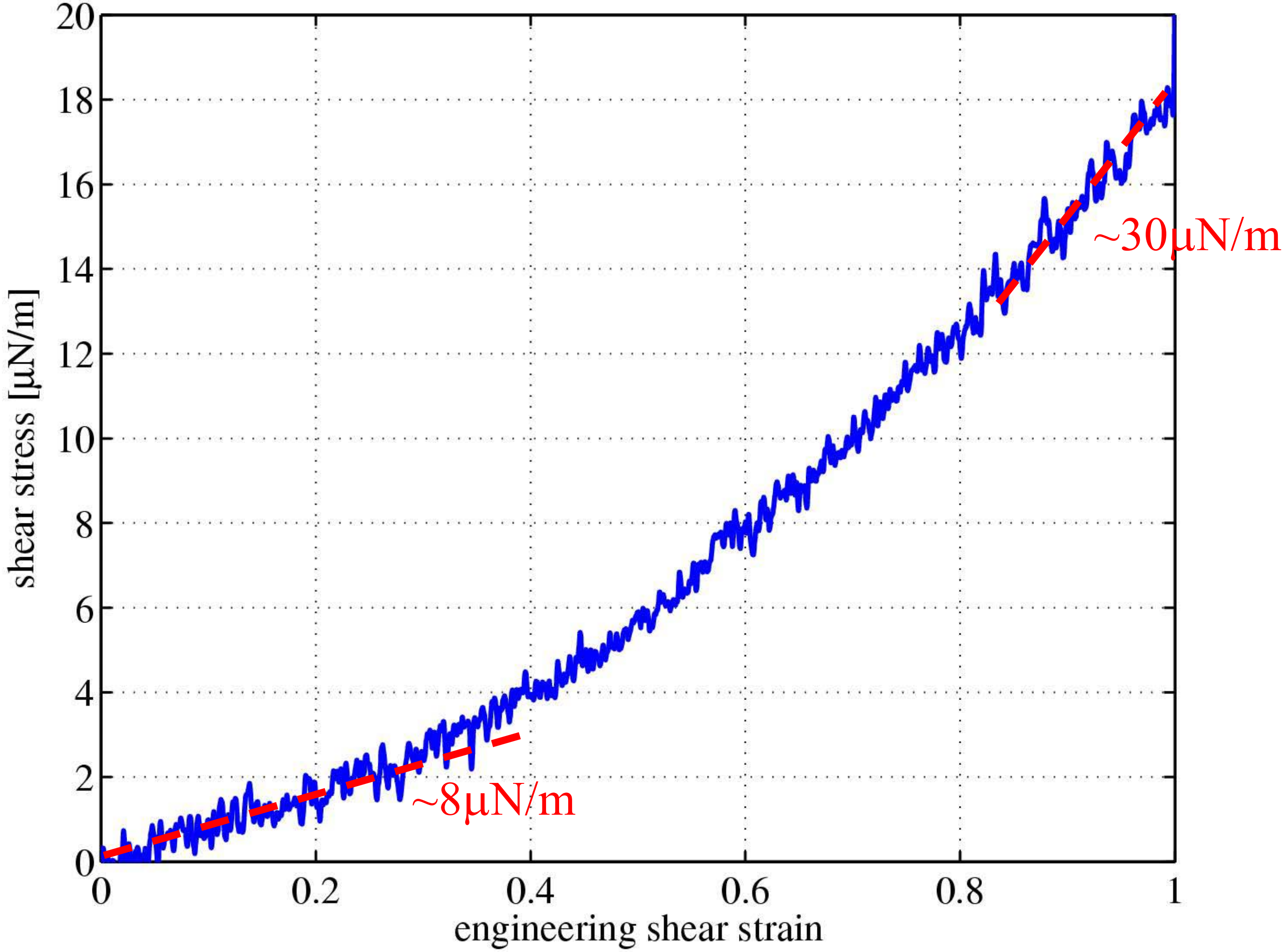


temperature
T [K]
time [μs]
system size
mean spectrin end-to-end distance [nm]
time [μs]
pressure
hydrostatic tension [μN/m]
time [μs]

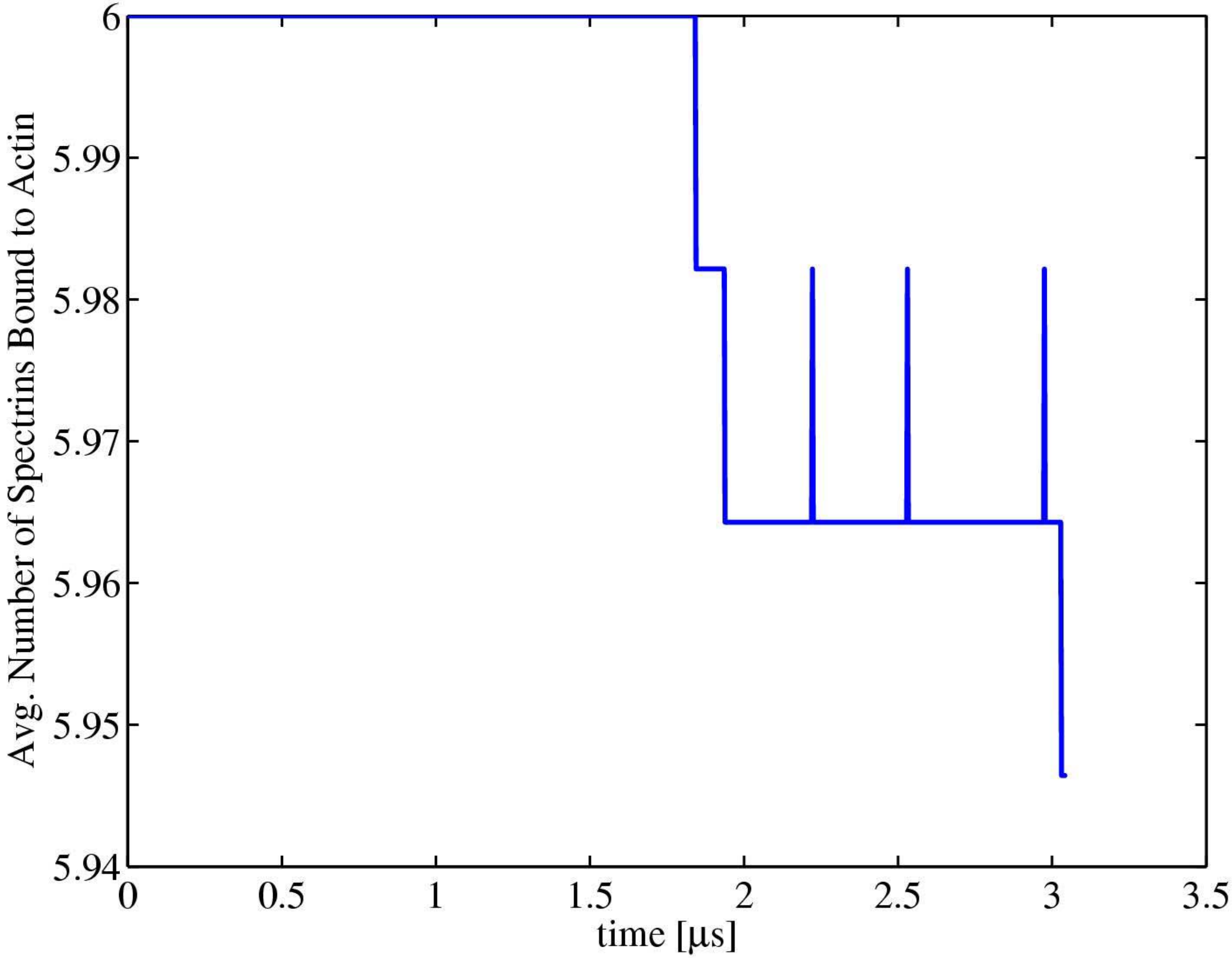
Pure shear deformation at 300K and strain rate 3×10^5/s



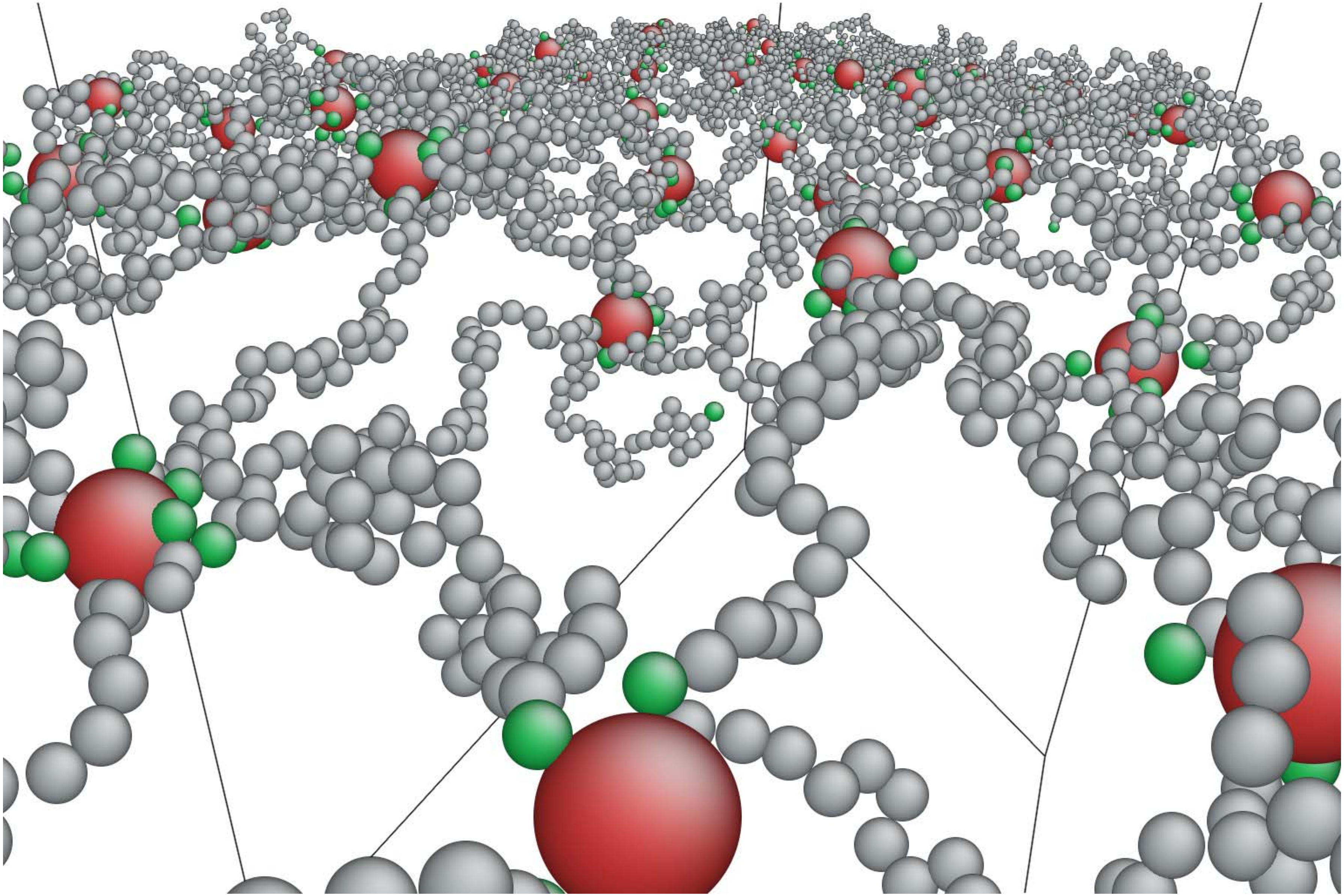
Stress-strain curve at 300K and no ATP

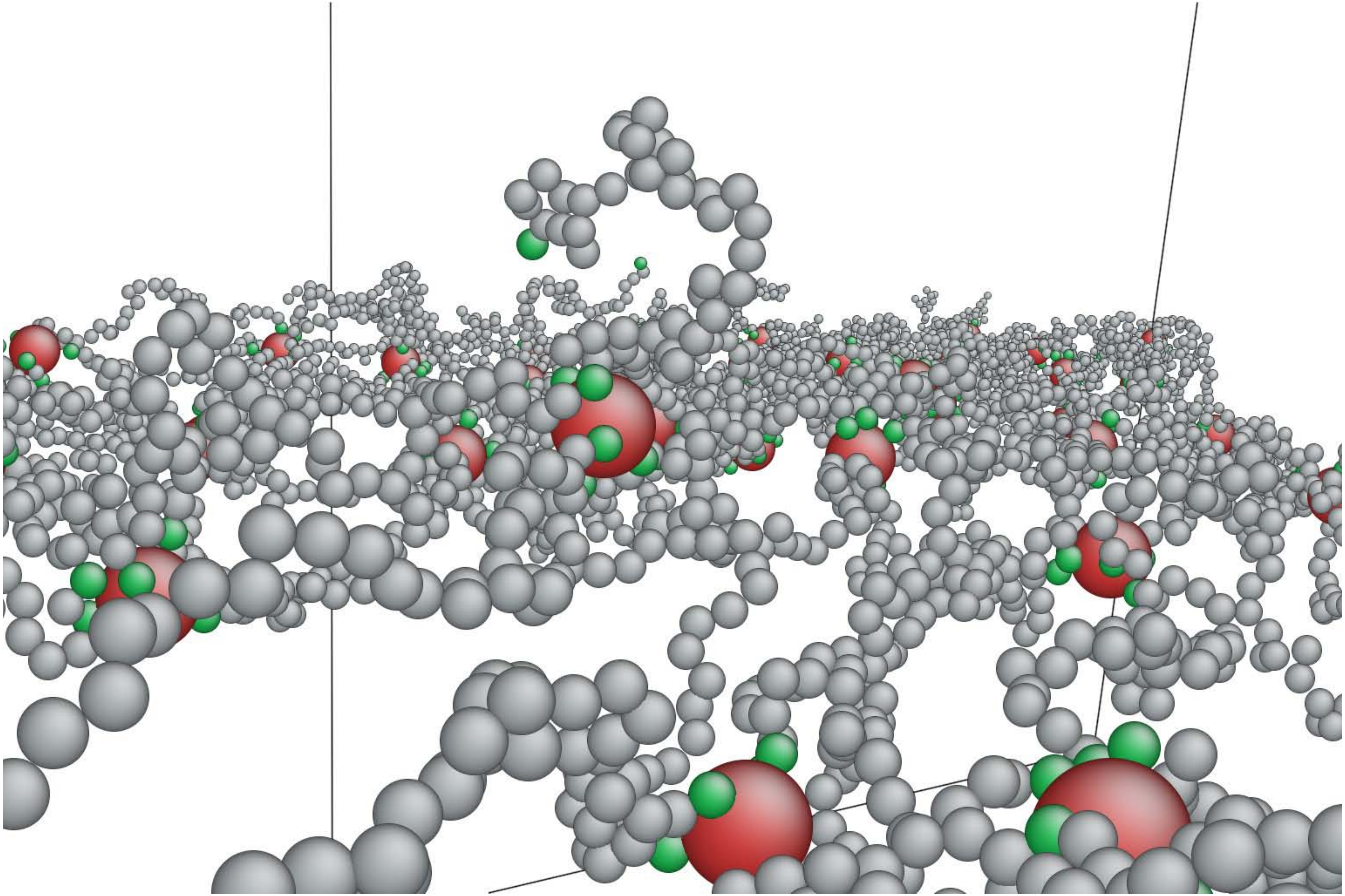


Defect statistics at 300K with no ATP



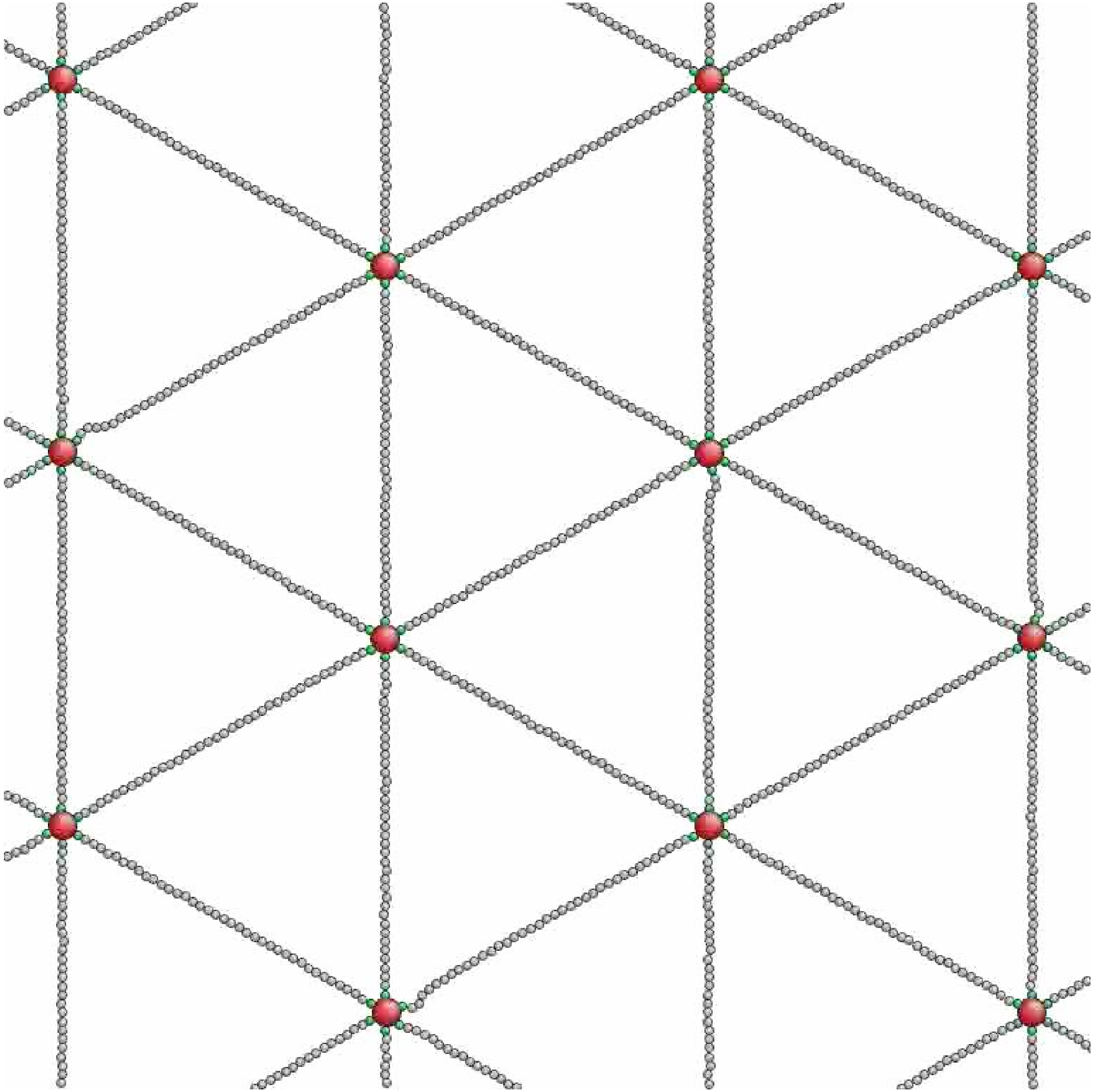
A broken link 5-fold defect



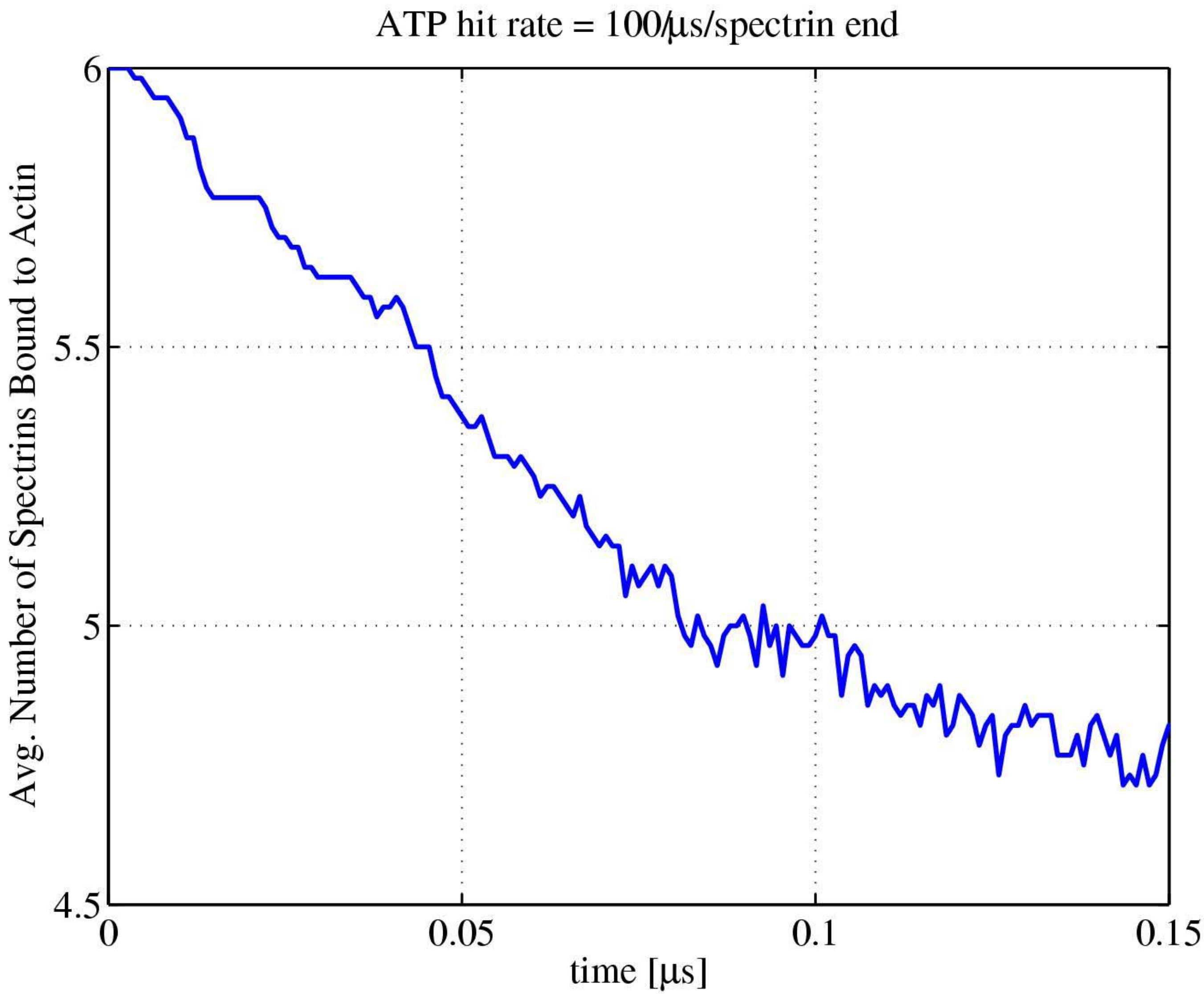


Corrugation due to buckling: elevated / depressed in height

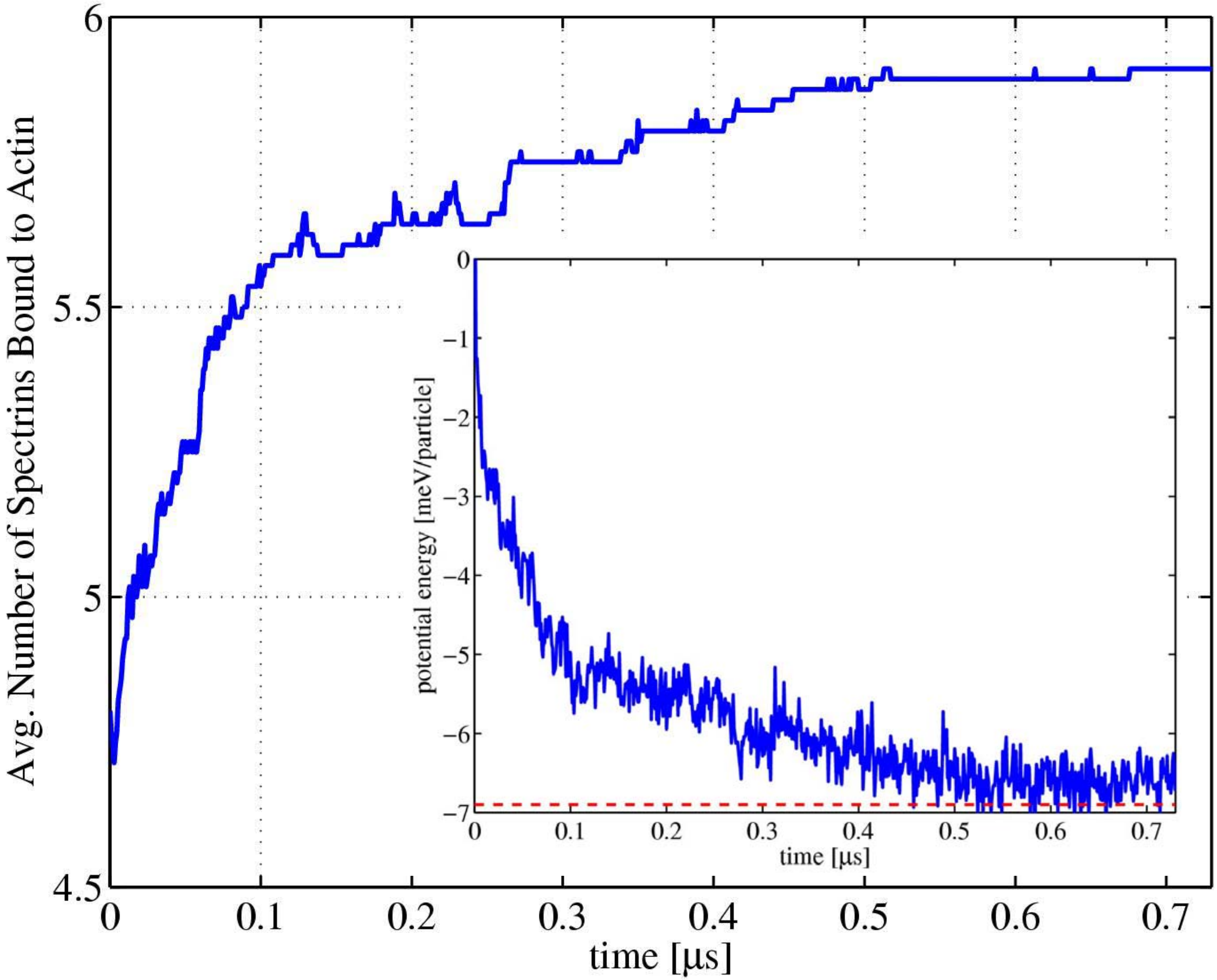
Now add ATP (0.5eV random kinetic energy to green ball): hit rate = 100/μs per spectrin end



Defect statistics at 300K, ATP hit rate 100/μs

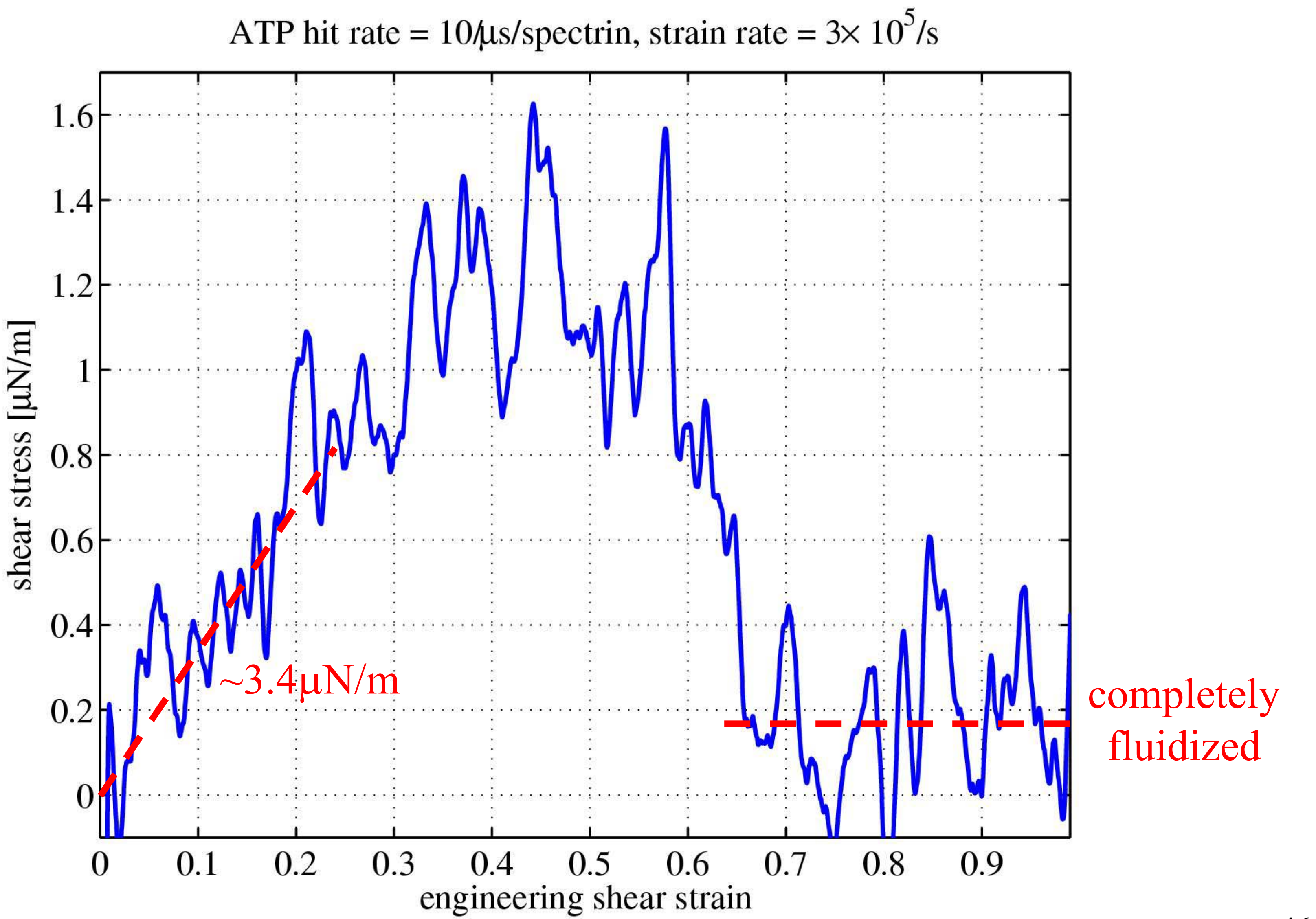


Now turn off ATP hits, “anneal” at 300K…

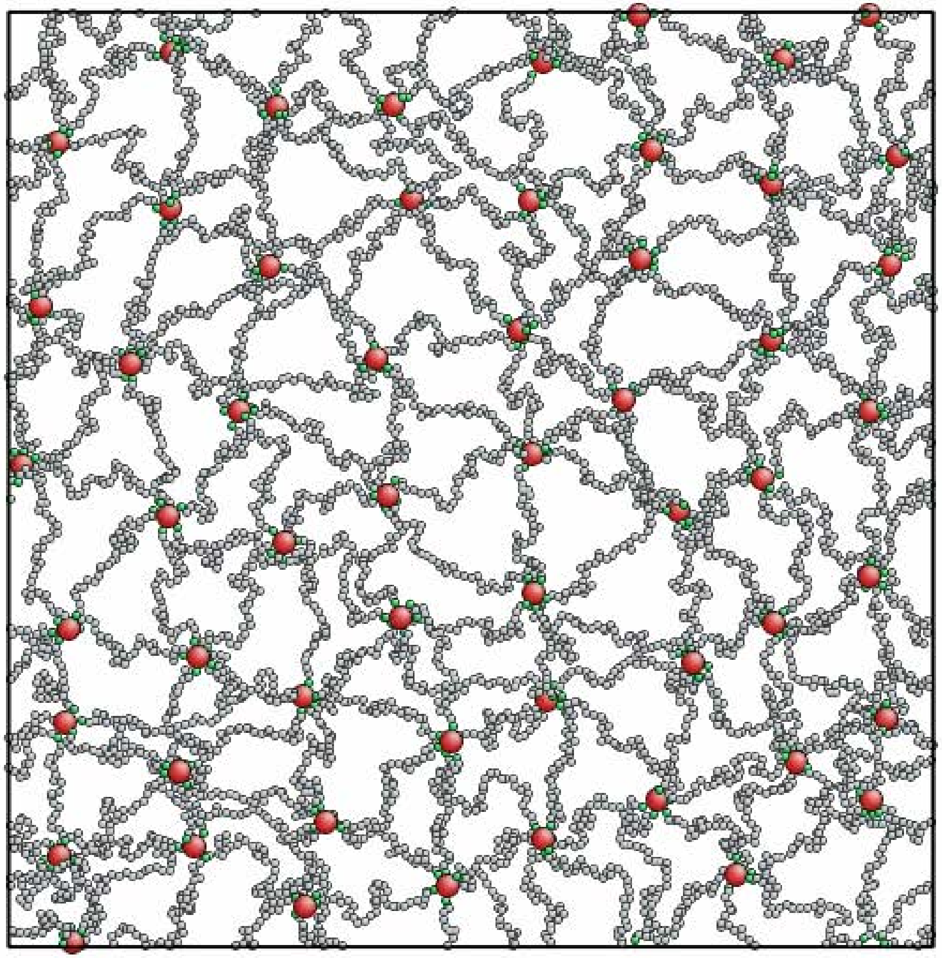


Miraculously, the system recovers, within CGMD simulation timescale.

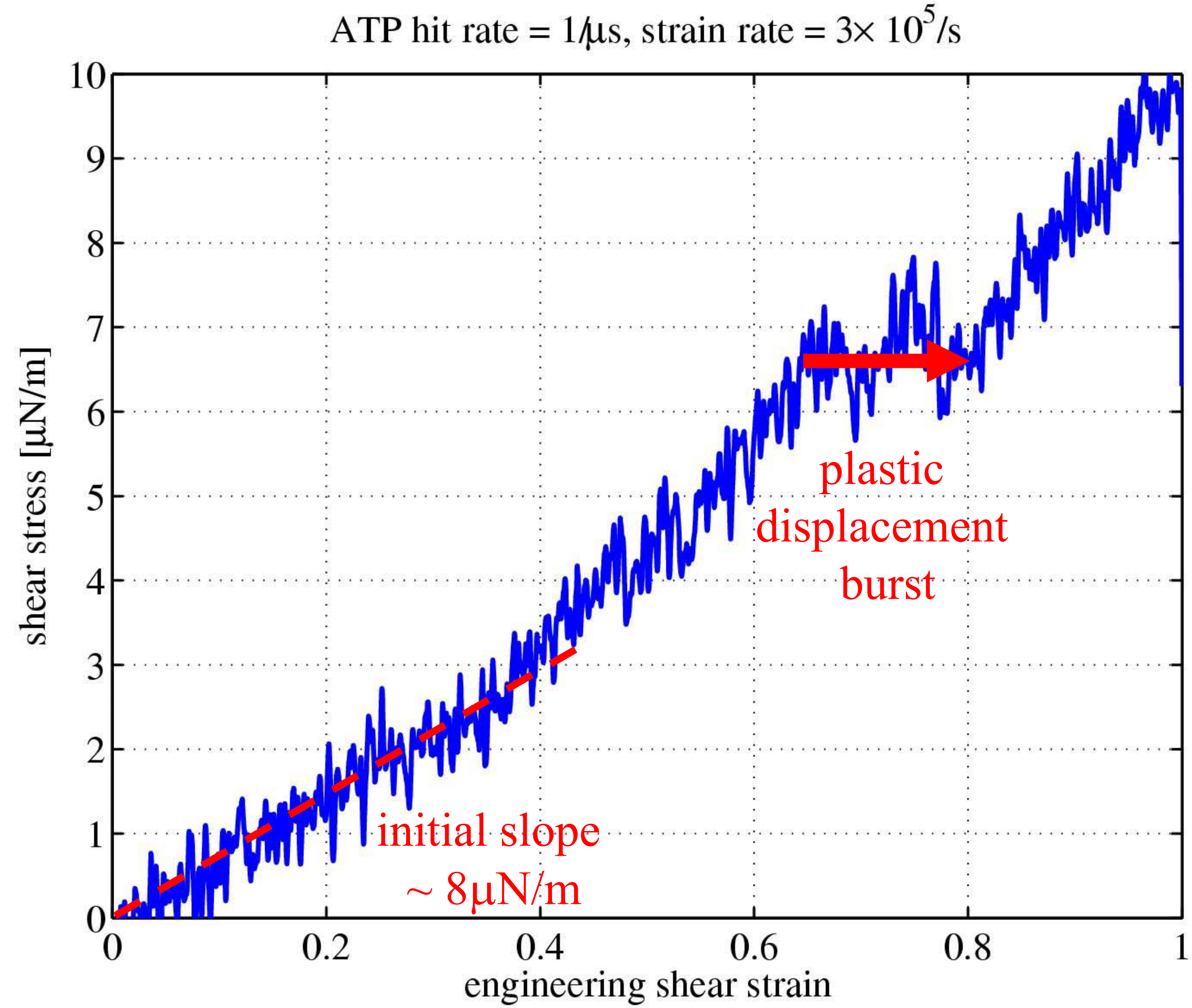
A more reasonable ATP hit rate: 10/μs. Simultaneously, also shear deform.



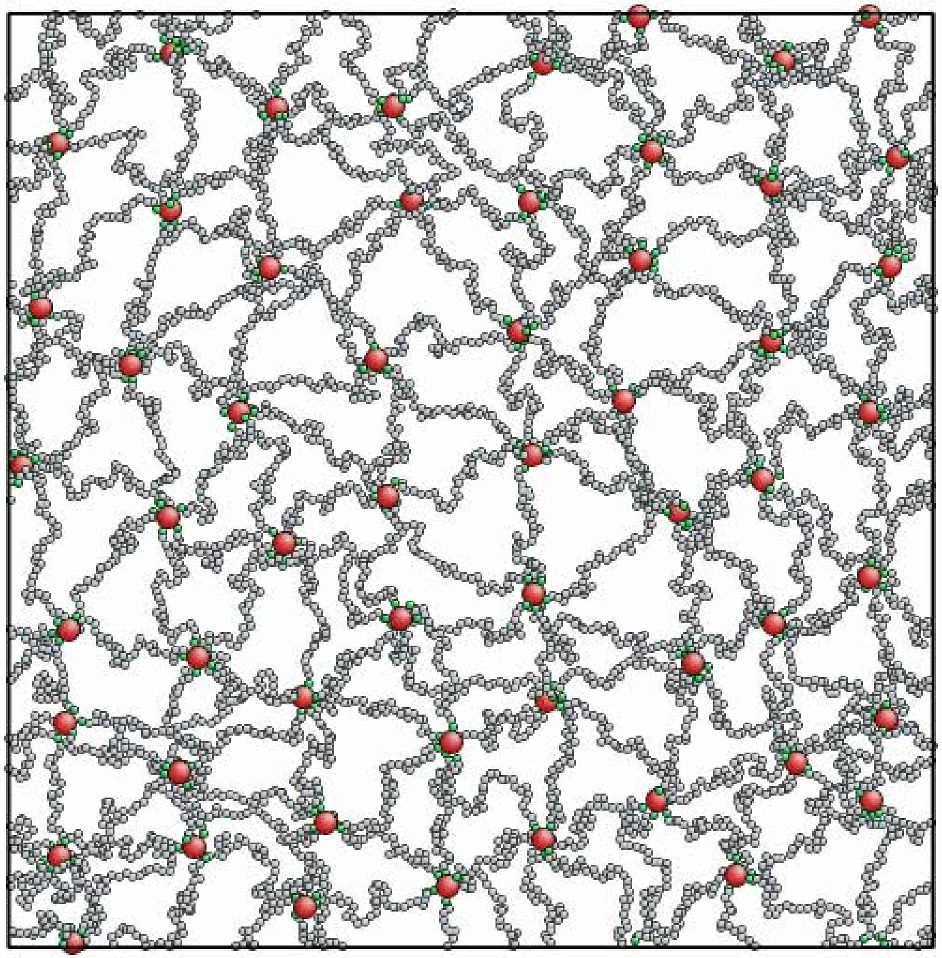
ATP hit rate = 10/μs



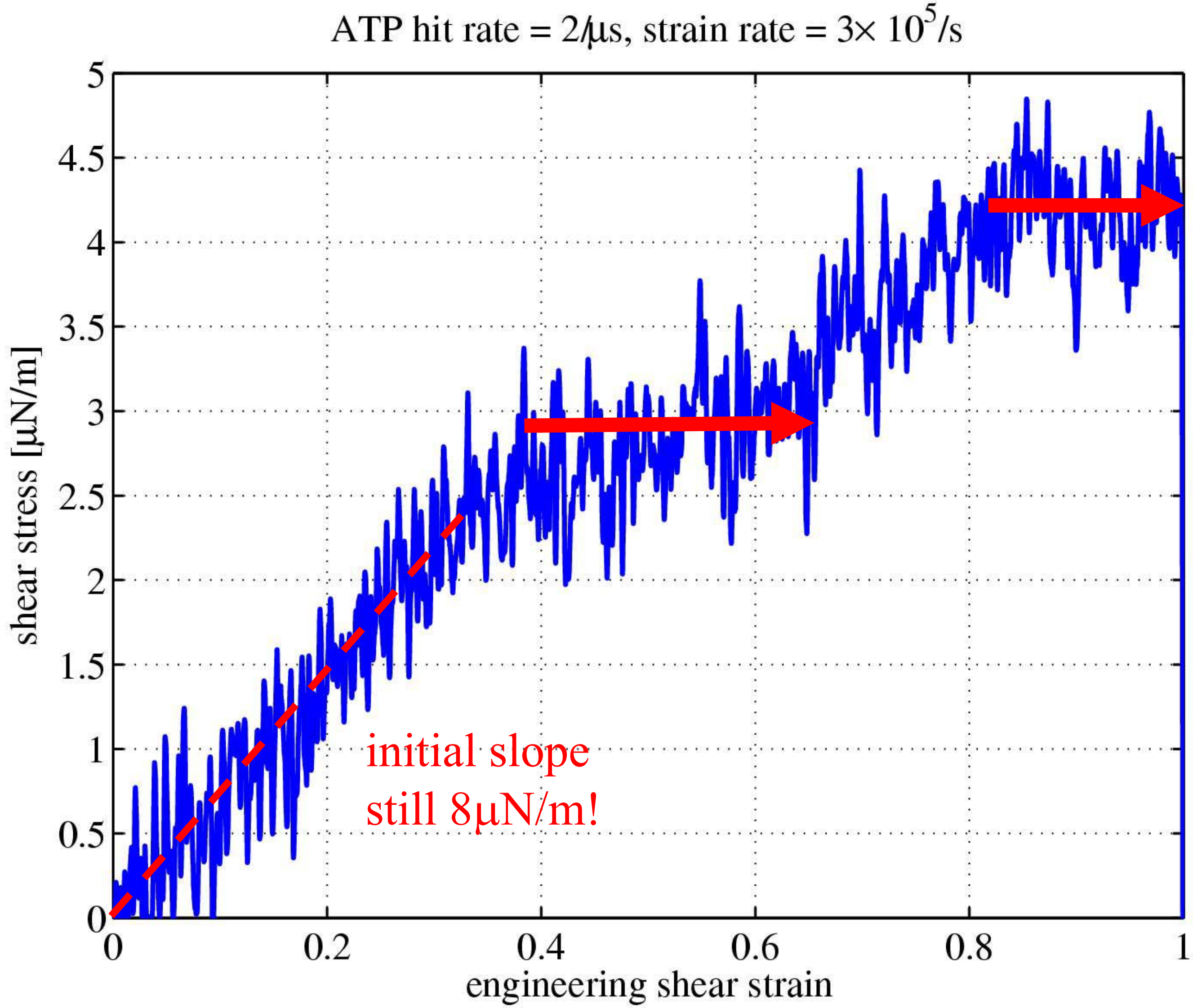
ATP hit rate = 1/μs:



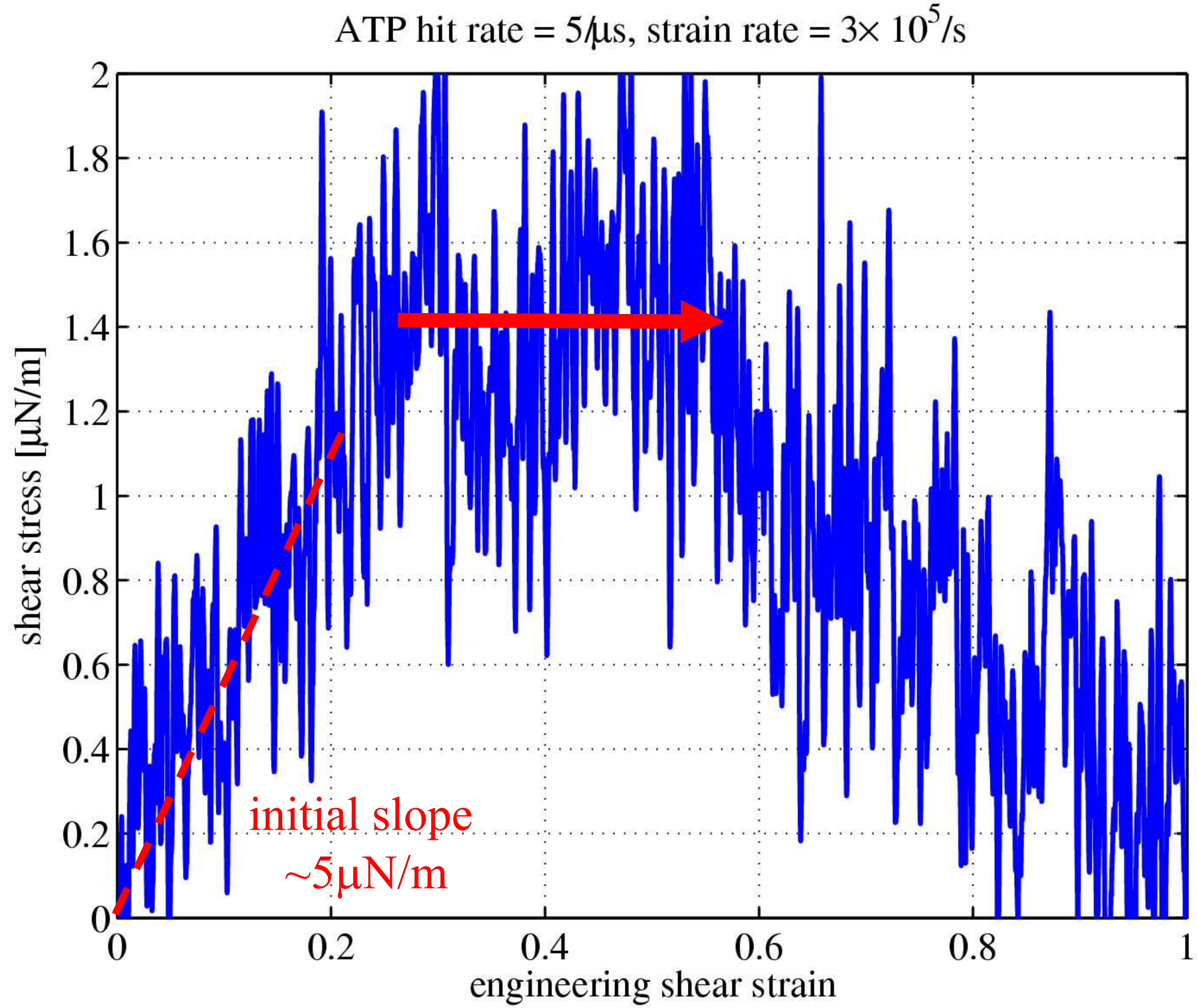
ATP hit rate = 1/μs



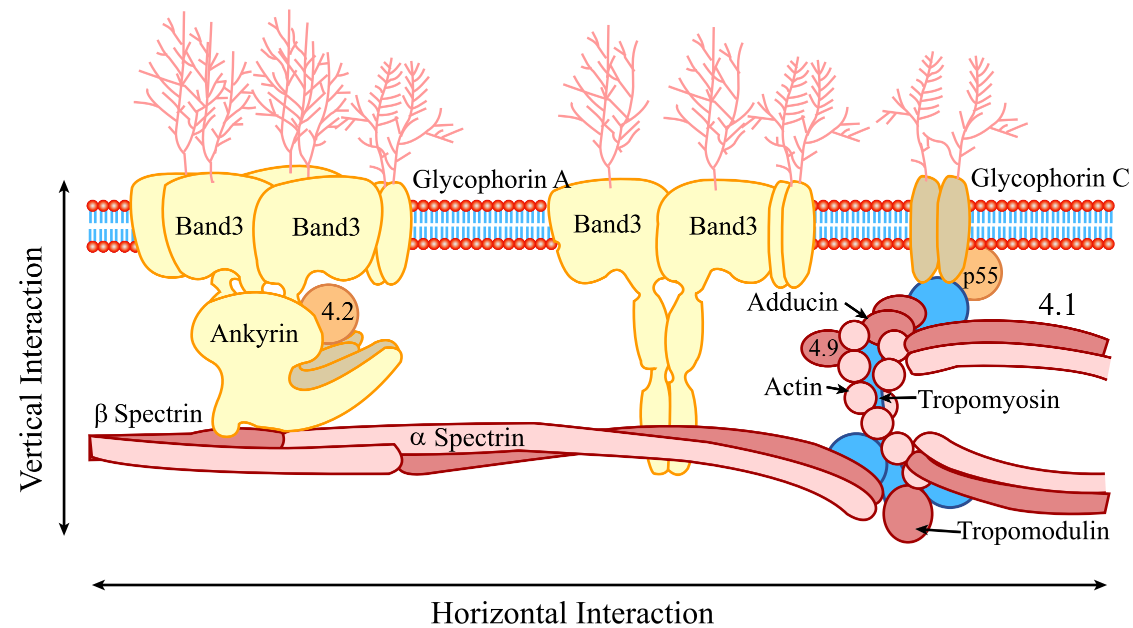
ATP hit rate = 2/μs: two plastic displacements… also longer



ATP hit rate = 5/μs:
large-strain resistance collapses, manifest global yield



Schematic Model of the RBC Membrane

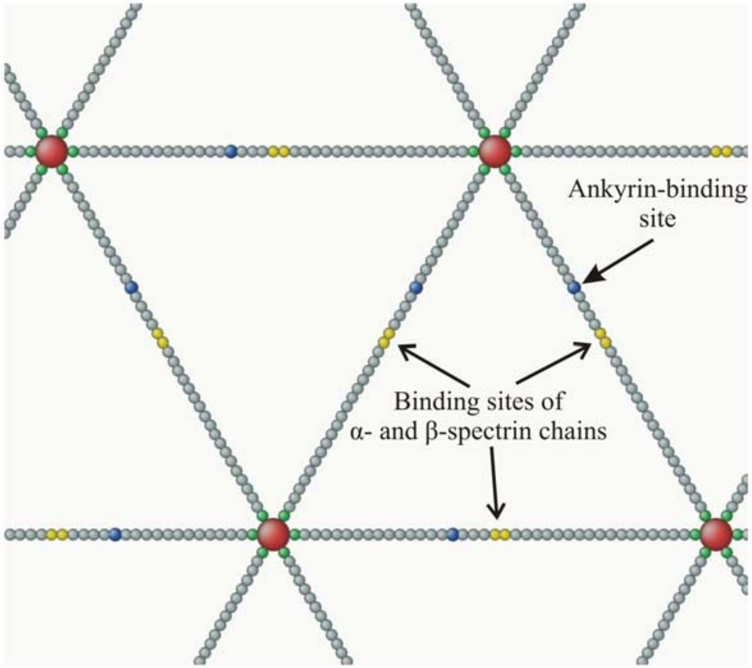


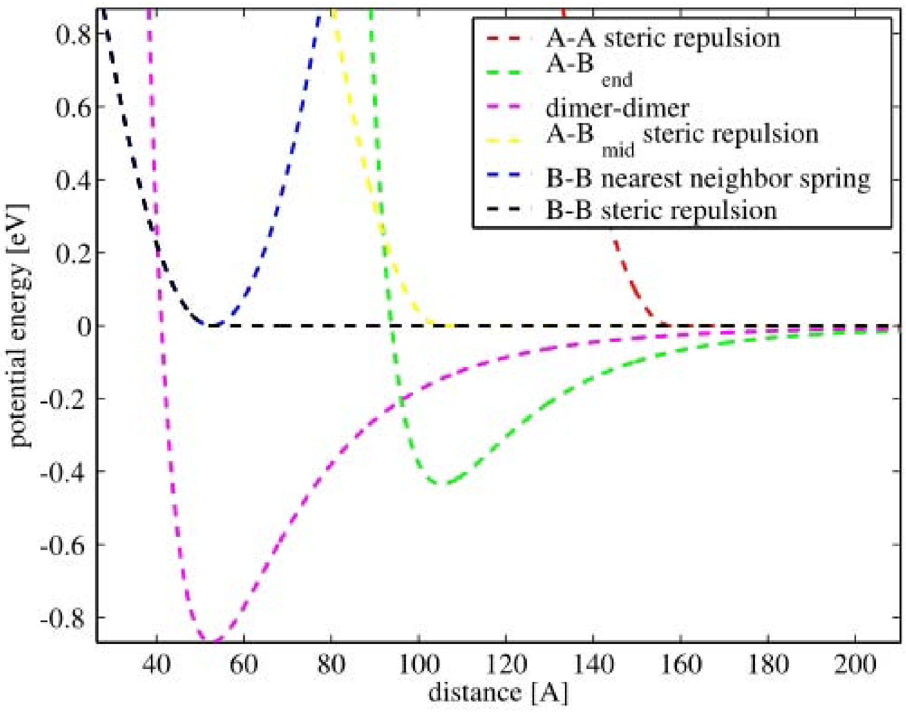
Schematic model of the red cell membrane, with the vertical and horizontal interaction of its components indicated. Estimated frequencies of mutations in different membrane proteins in HS and HE/HPP are as follows. Vertical interaction: hereditary spherocytosis: band 3,~20%; protein 4.2, ~5%; ankyrin , ~45%; spectrin, ~30%. Horizontal interaction: hereditary elliptocytosis /hereditary pyropoikilocytosis; β spectrin, ~5%; α spectrin, ~80%; protein 4.1,~15%. The relative position of the various proteins is correct, but the proteins and lipids are not drawn to scale.

Figure by MIT OCW. After Tse and Lux, 1999.

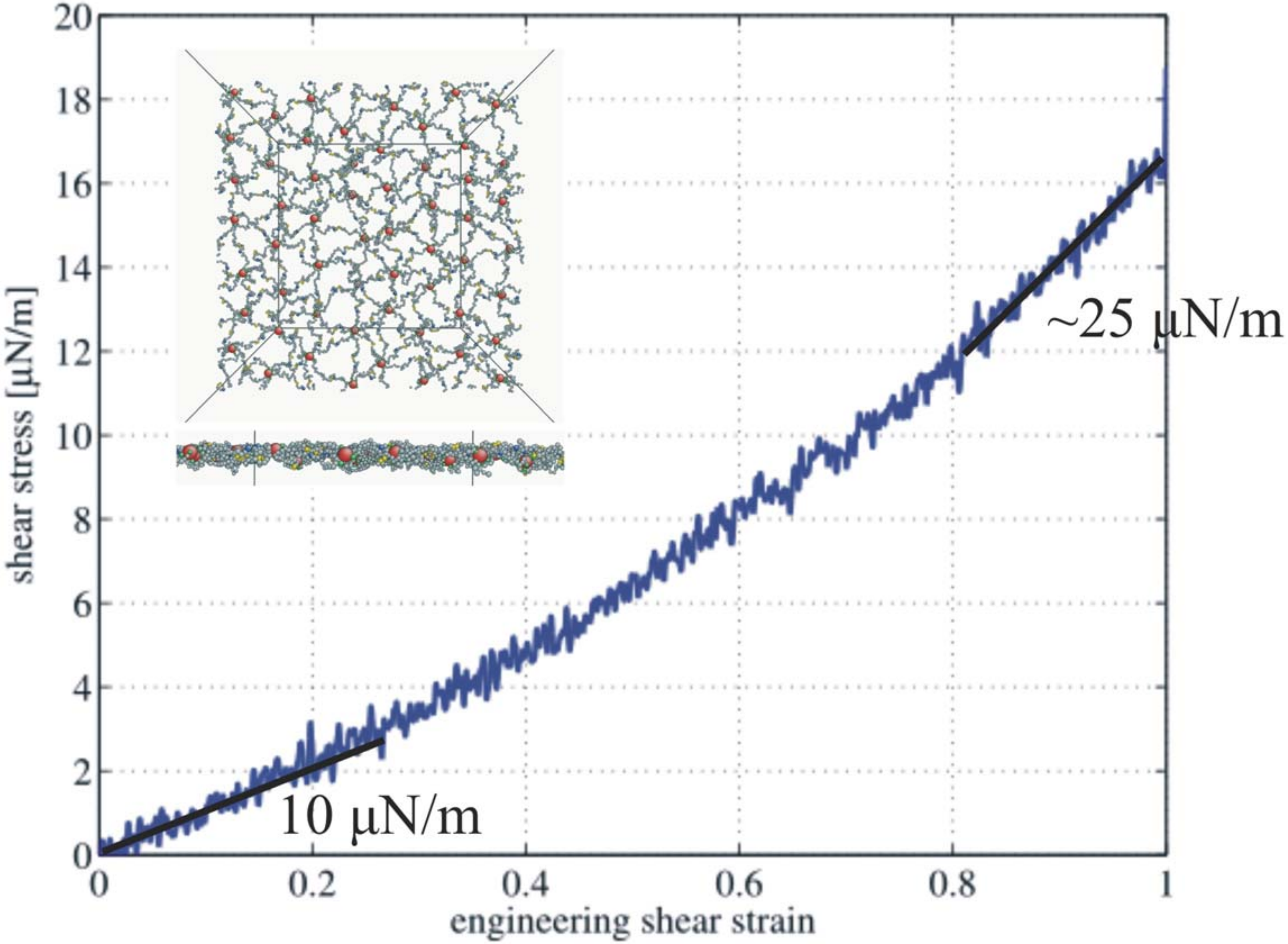
See figures in Discher, D. E., N. Mohandas, and E. A. Evans. "Molecular Maps of Red Cell Deformations: Hidden Elasticity and in Situ Connectivity." *Science* 266 (1994): 1032-1038.

Coarse Grain Molecular Dynamic Modeling

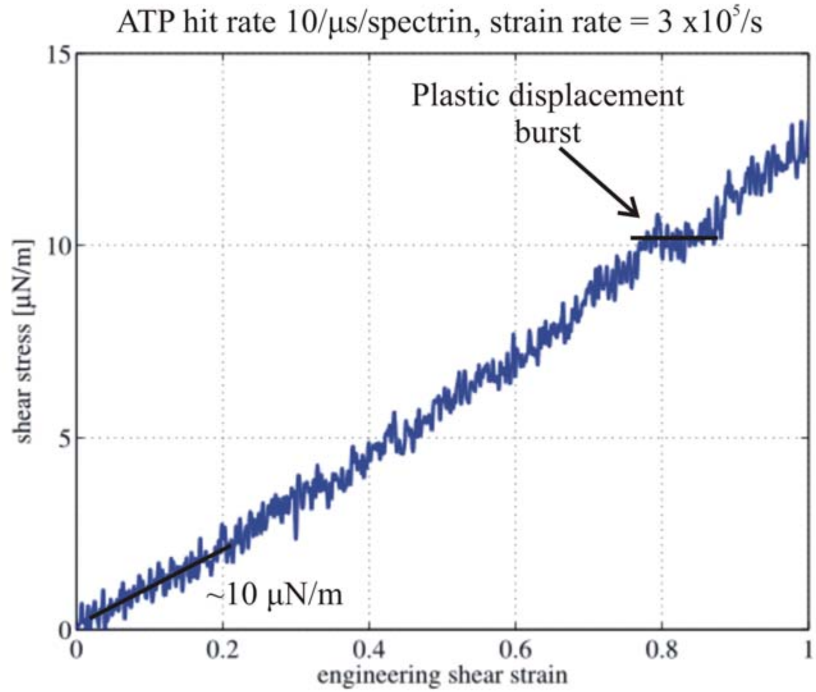


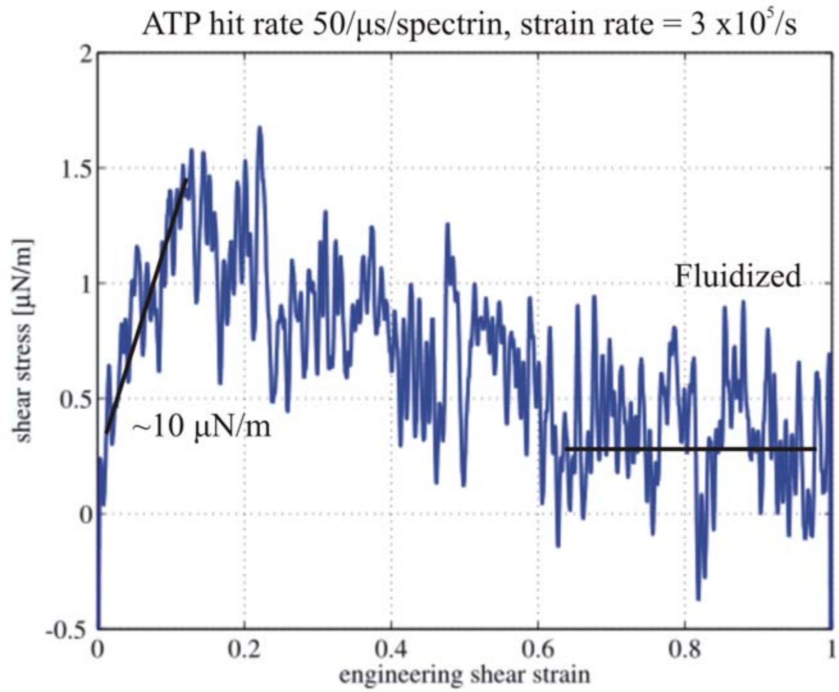


Shear Deformation



Shear Deformation and Promoted Dimer – Dimer Dissociation





Summary

- A minimal CGMD model with *breakable* actin-spectrin junction has been developed, with physically reasonable parameters and behavior.
- ATP hydrolysis is modeled as stochastic kinetic energy transfer. As ATP hit rate rises, we see initiation of plastic displacement excursions, followed by macroscopic yield, and eventually, complete fluidization.
- Practical timescale of CGMD able to simulate recovery.