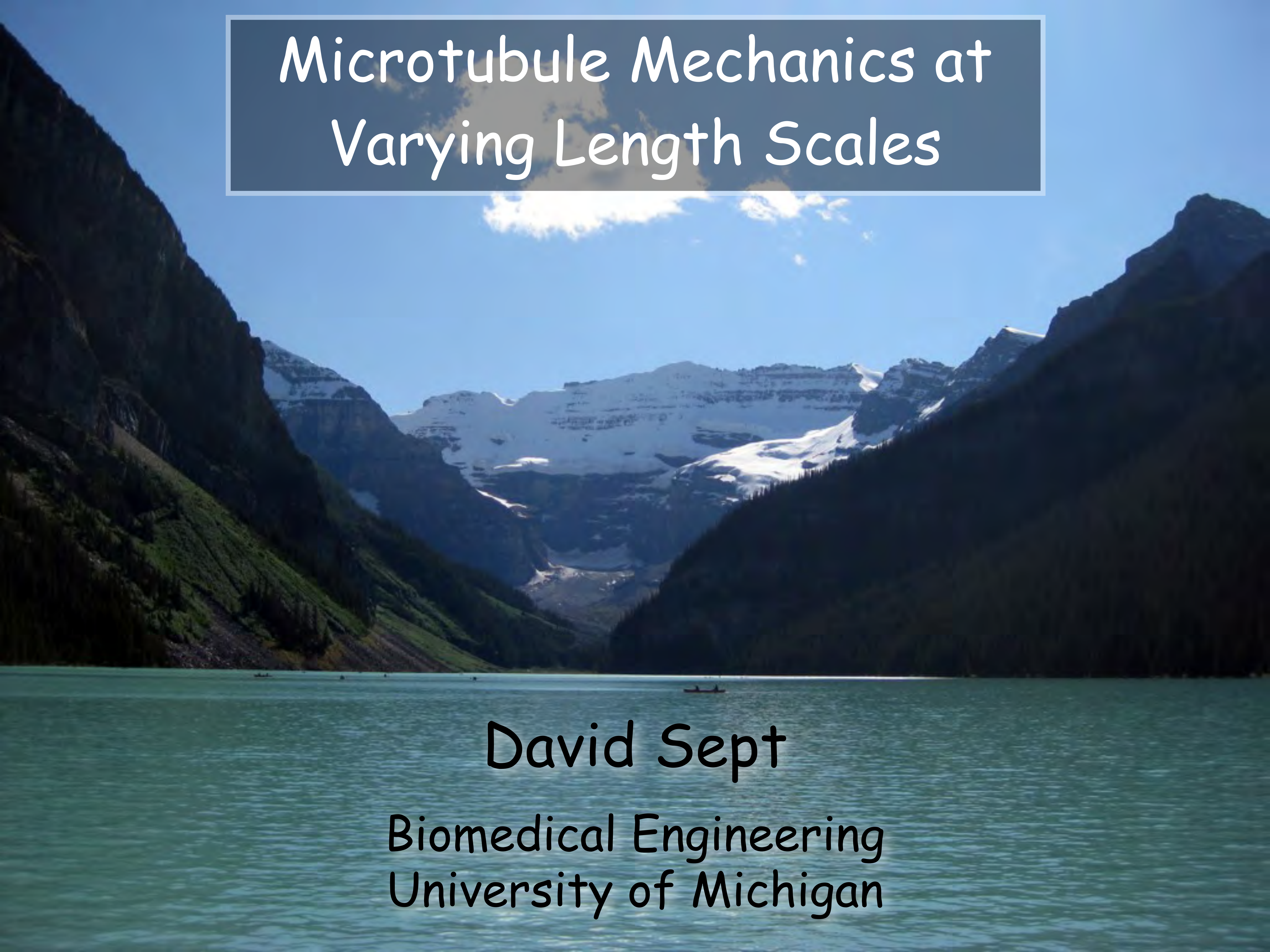
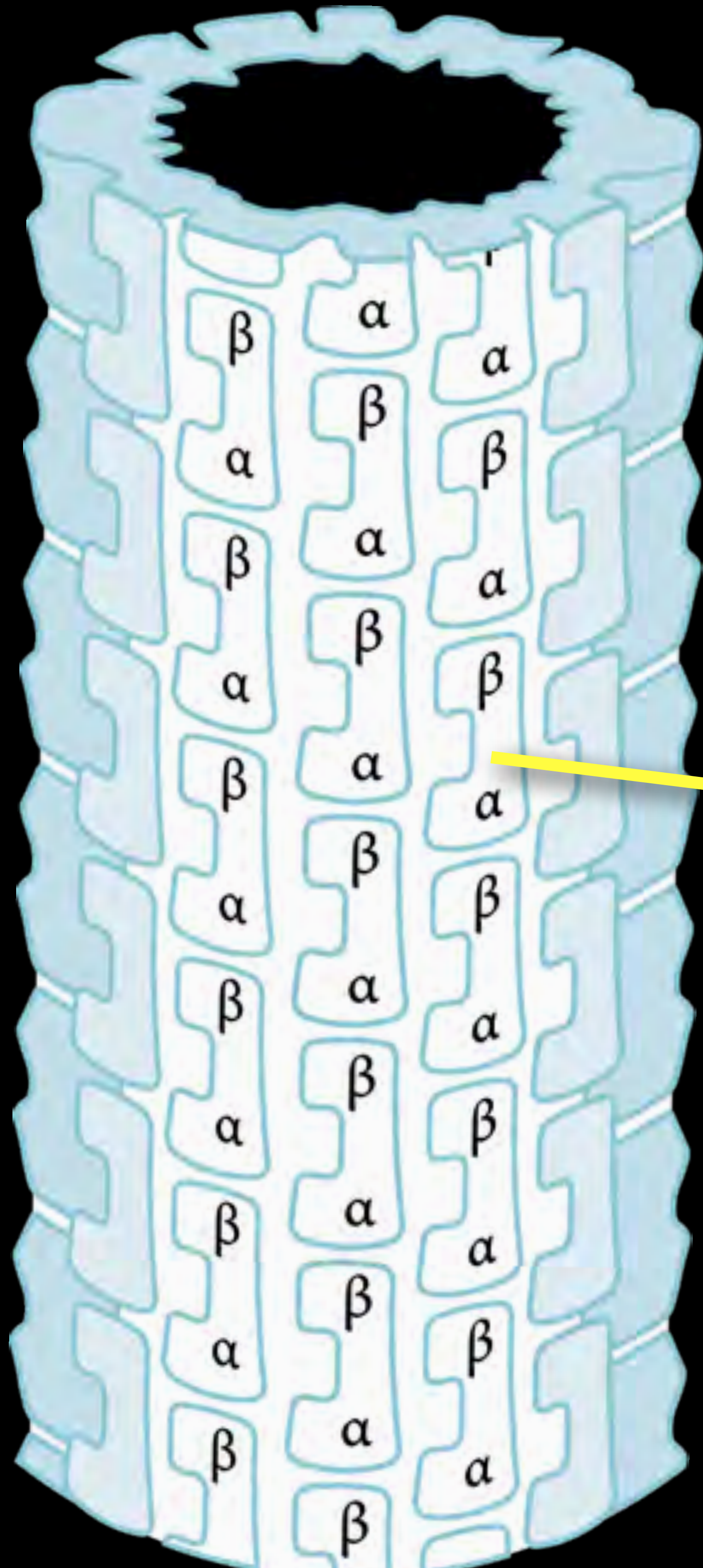


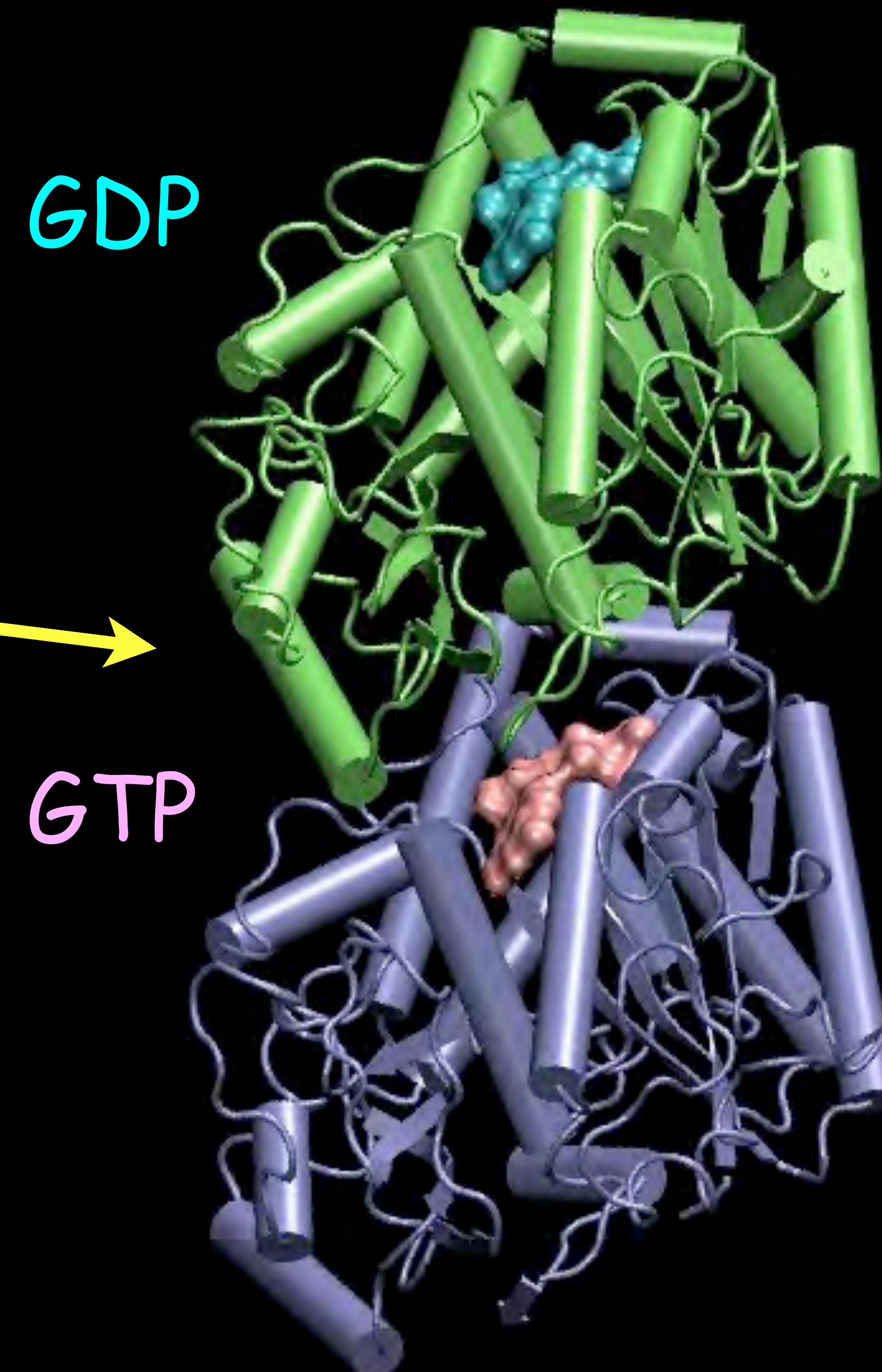
Microtubule Mechanics at Varying Length Scales

David Sept

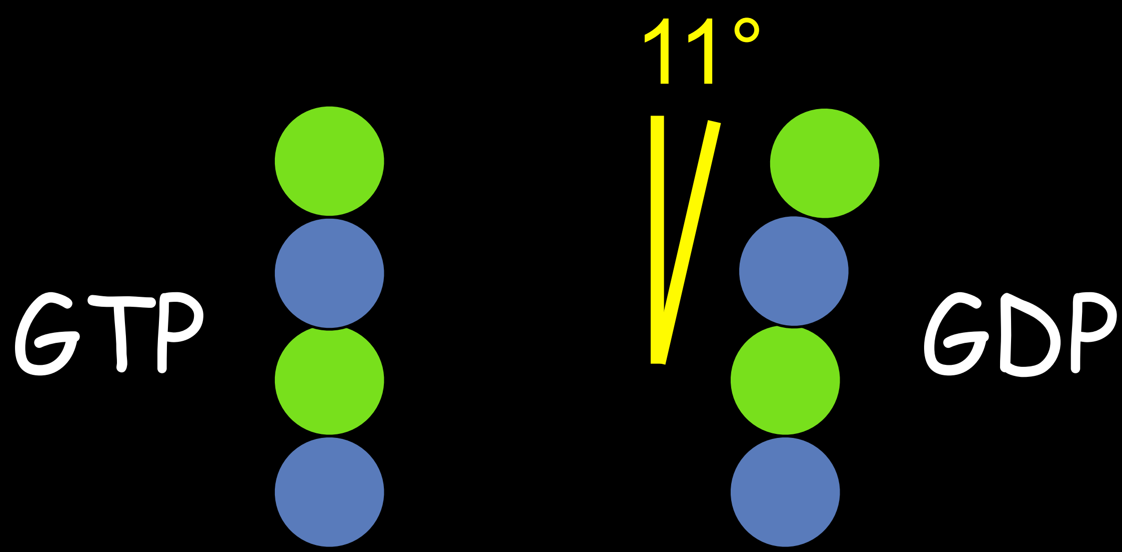
Biomedical Engineering
University of Michigan

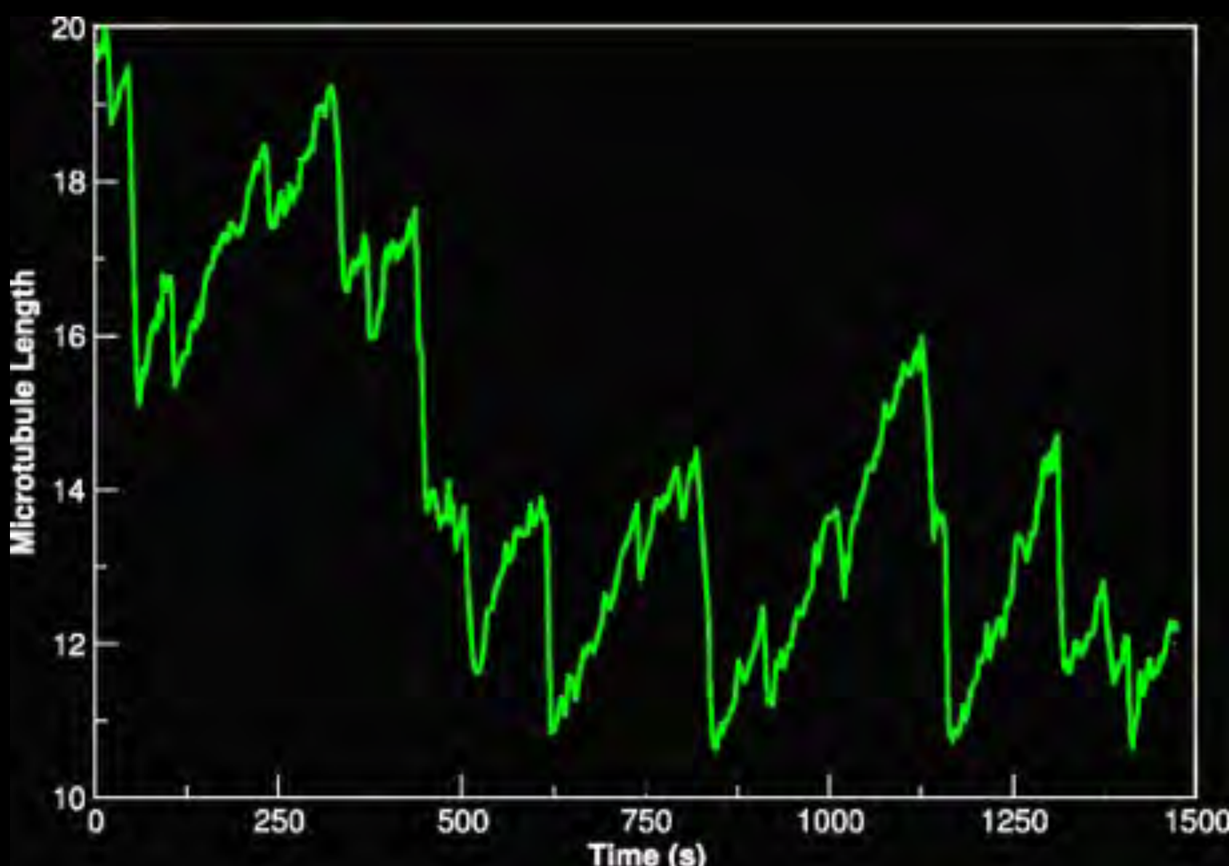
MT Structure

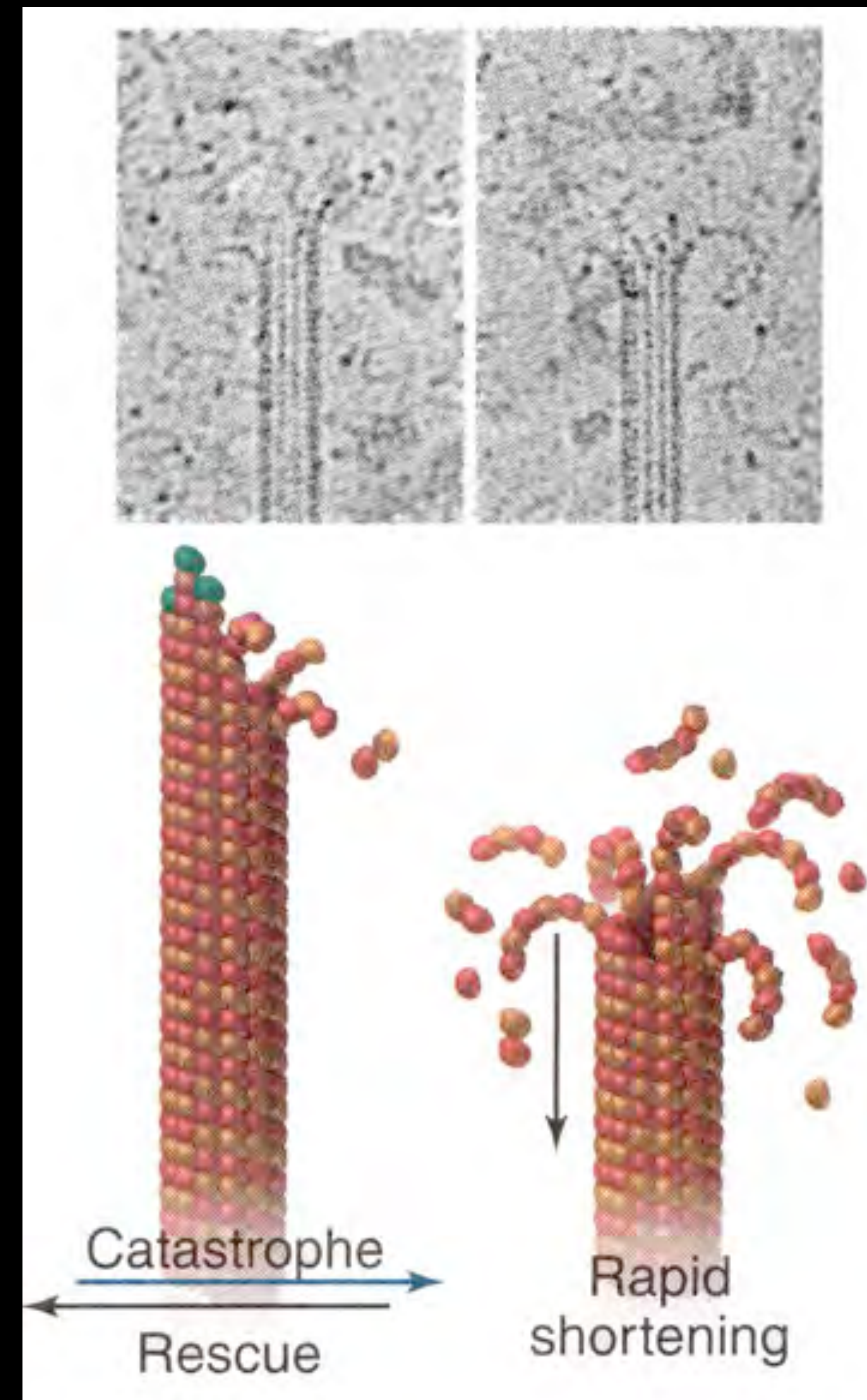




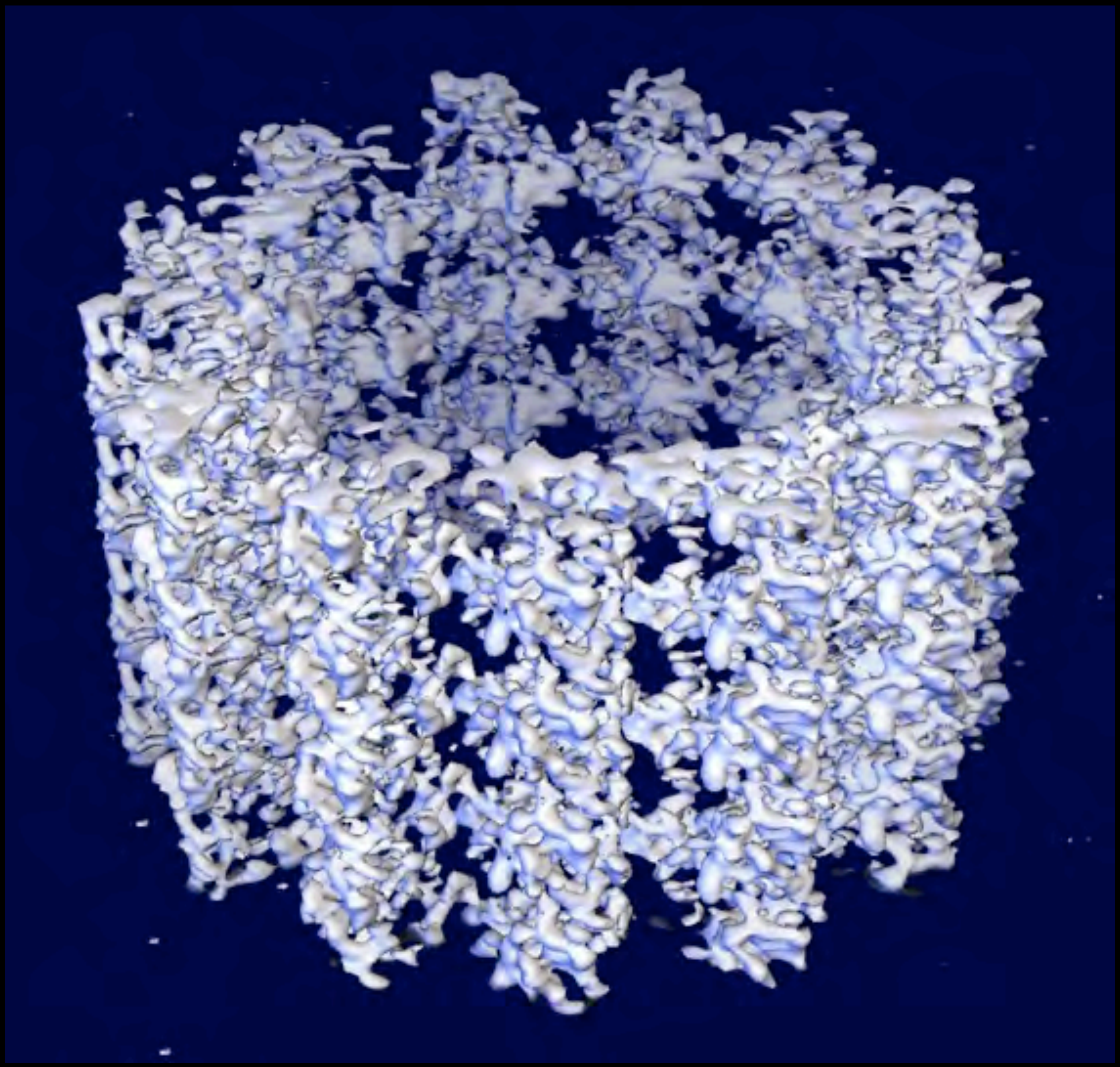
Nucleotide Effects

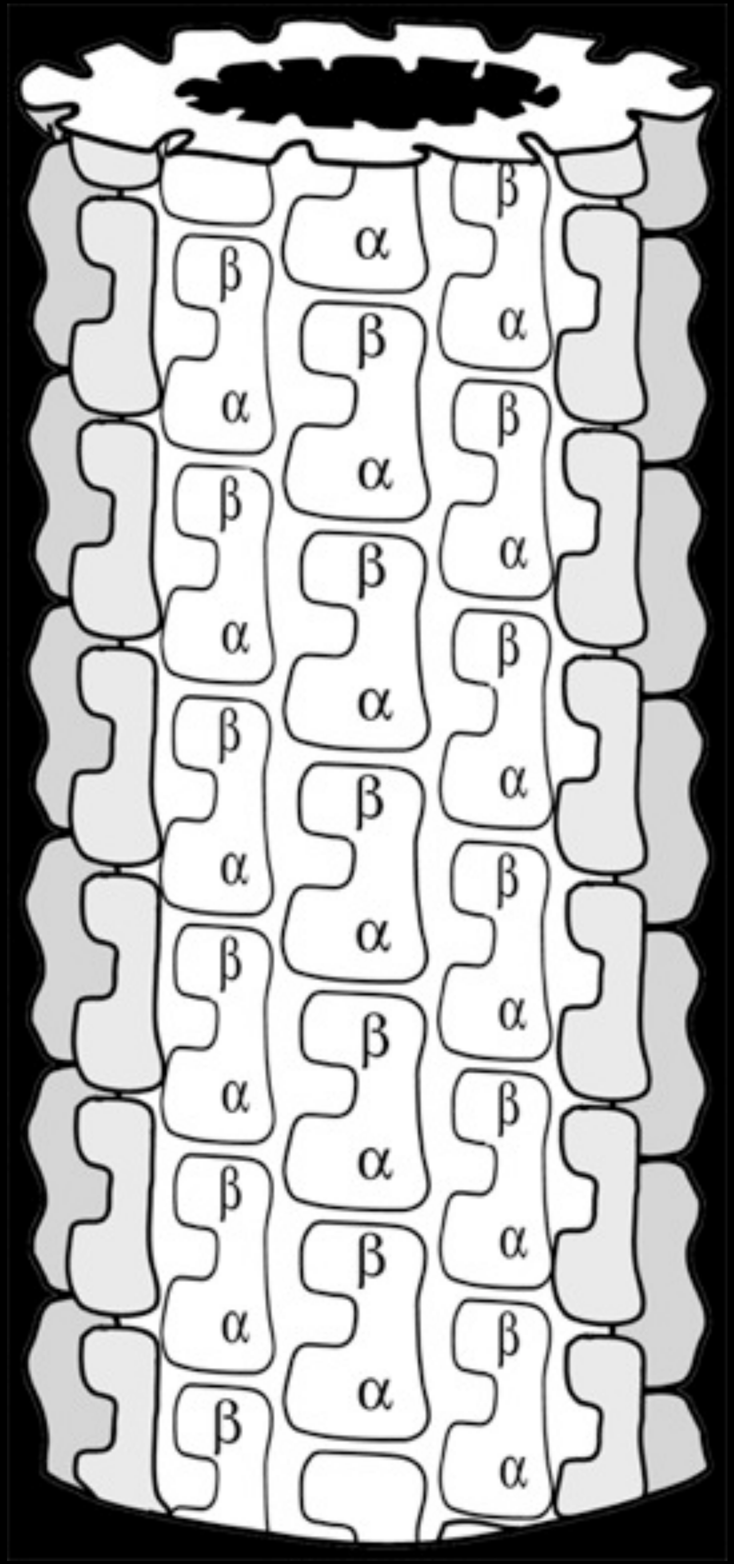




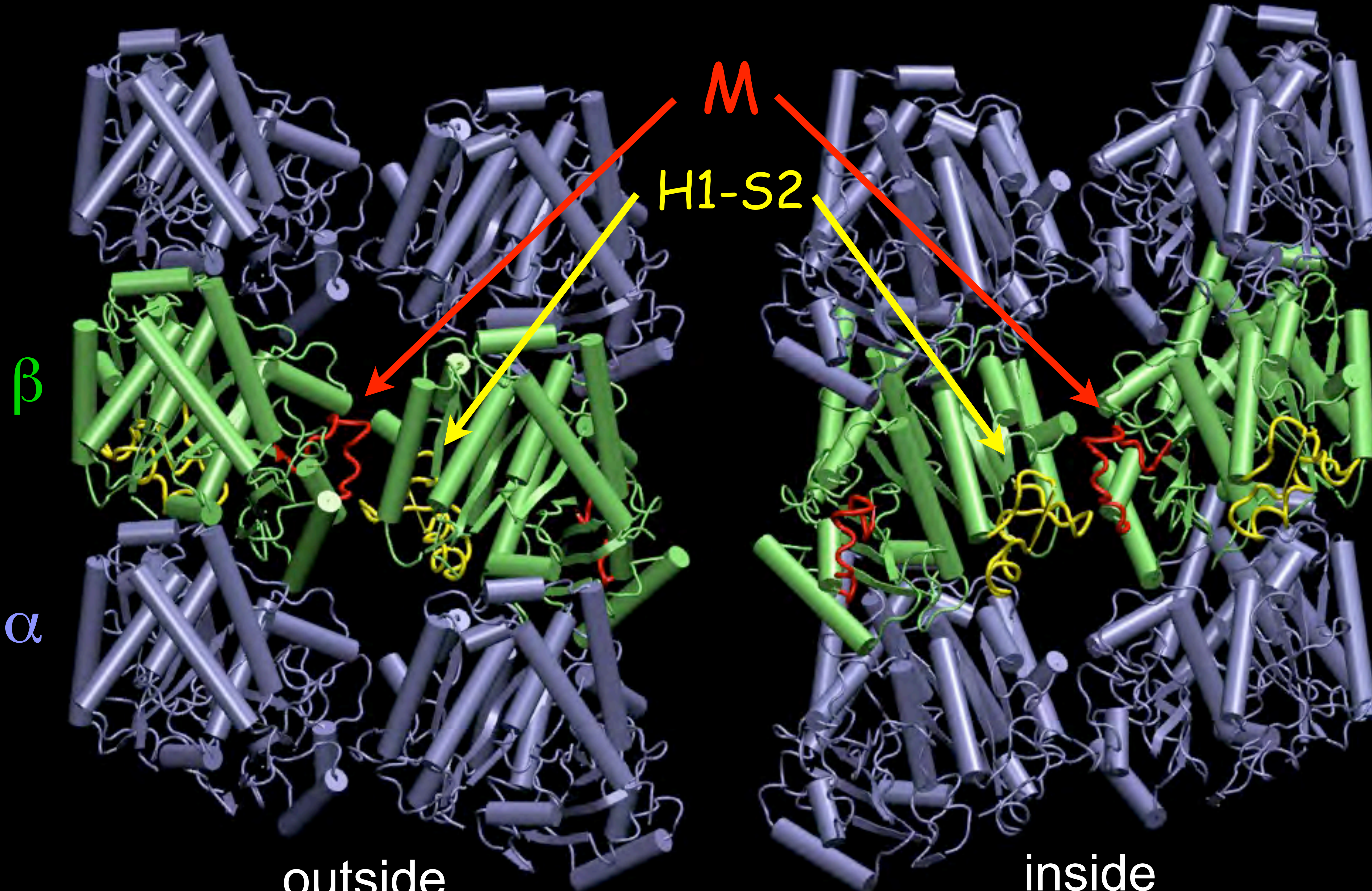


Structure of the Microtubule



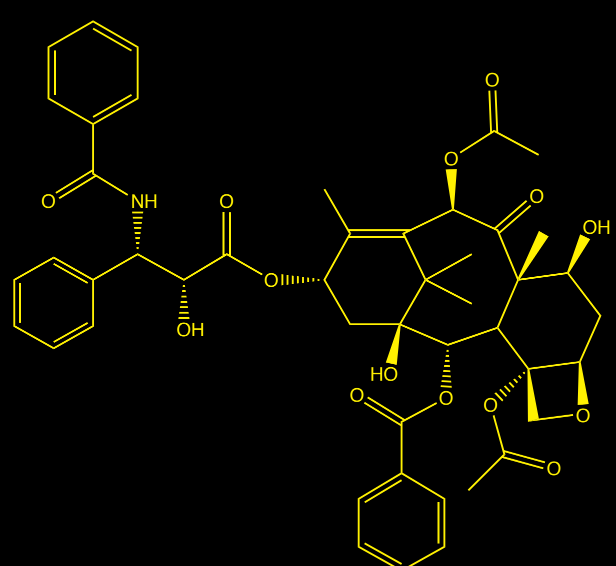


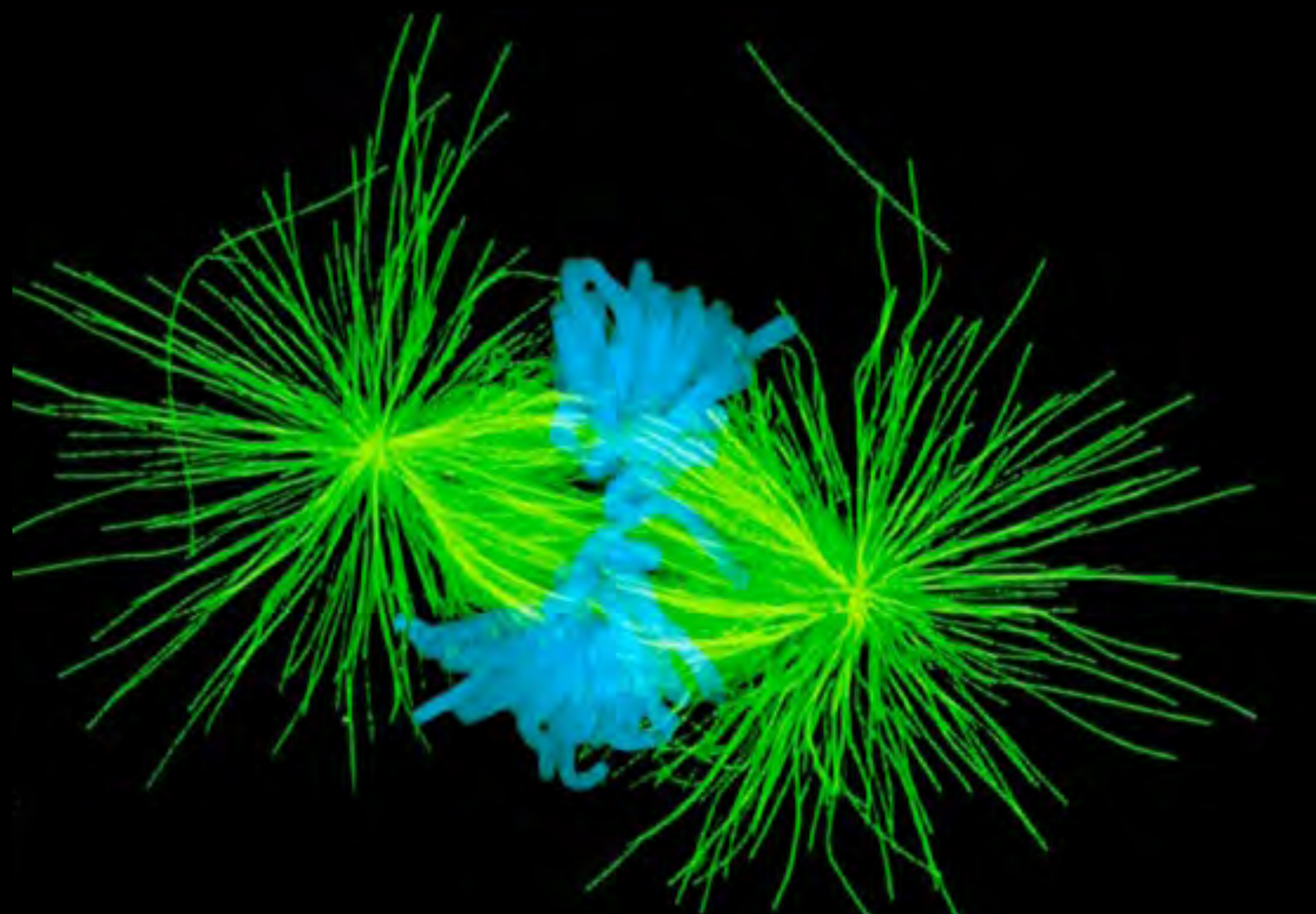
Structure of the Microtubule



Taxol and Mitosis

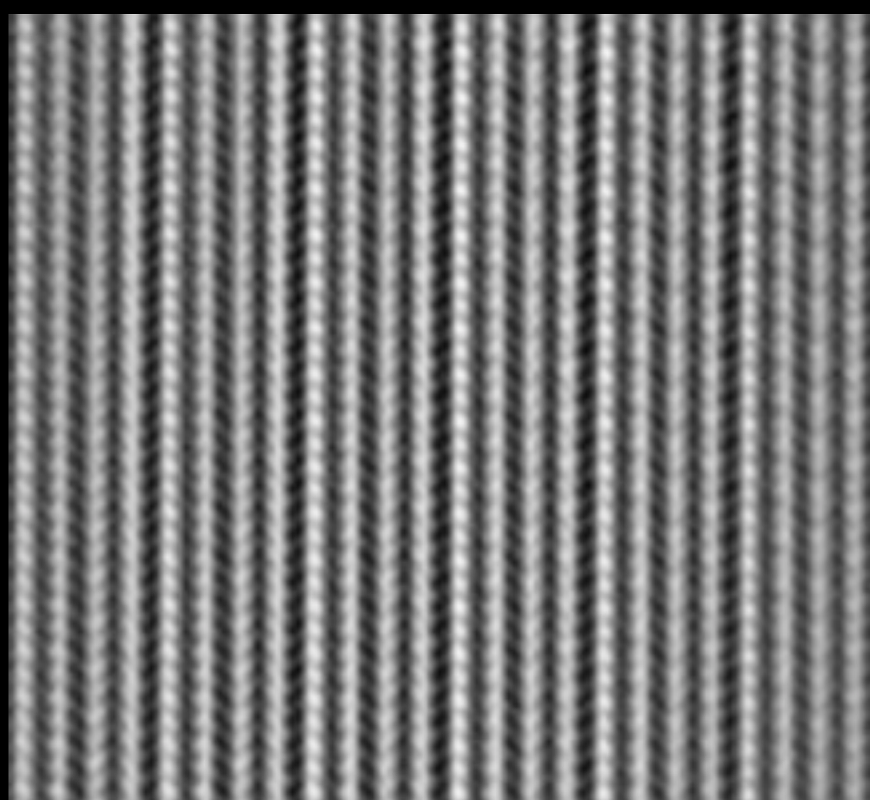
Taxol hyperstabilizes microtubules, prevents their disassembly, and makes the ends blunt.



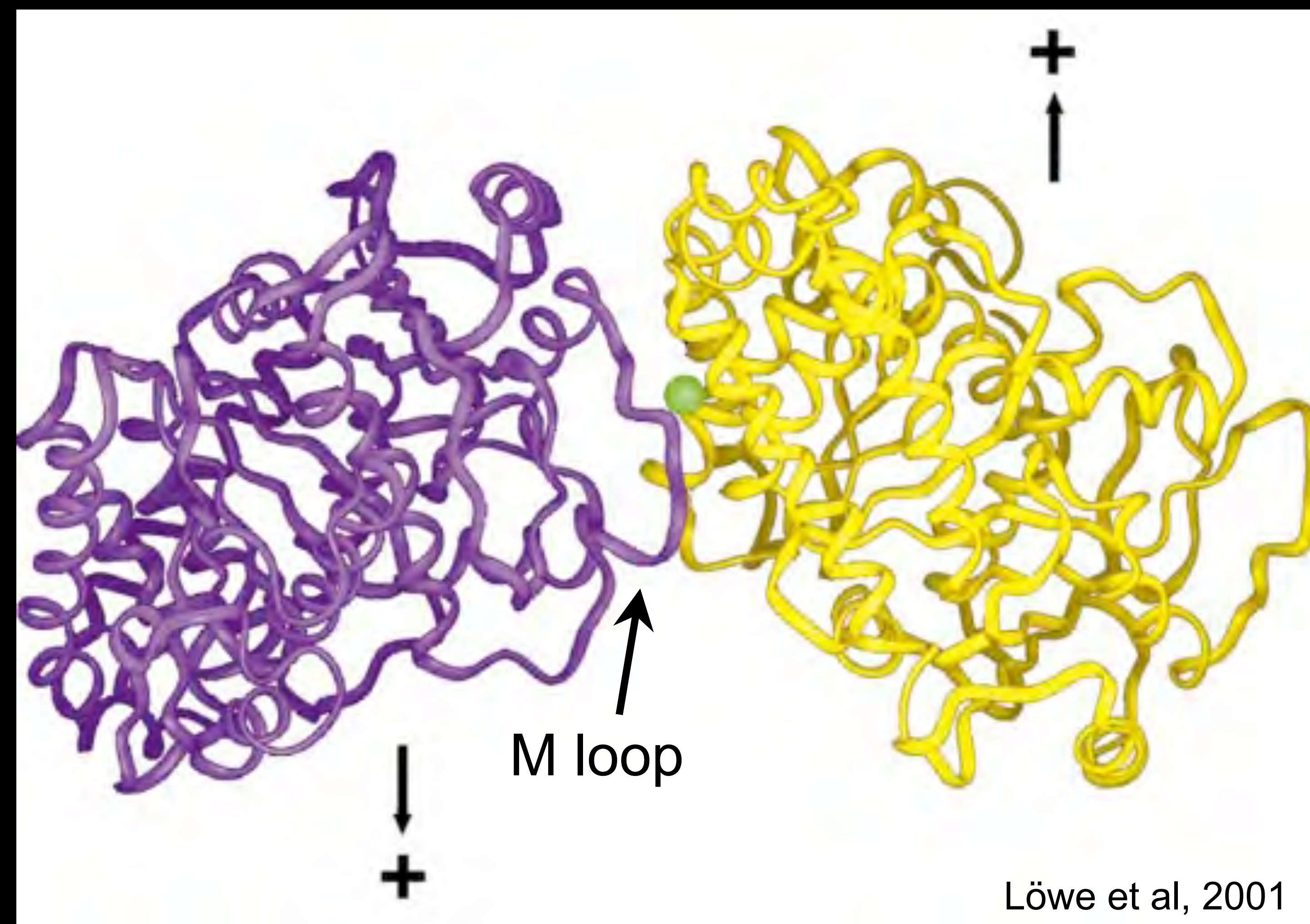


jcs.biologist.org

How Taxol® stabilises microtubule structure
Linda A Amos and Jan Löwe



Löwe and Amos, 1998



Löwe et al, 2001

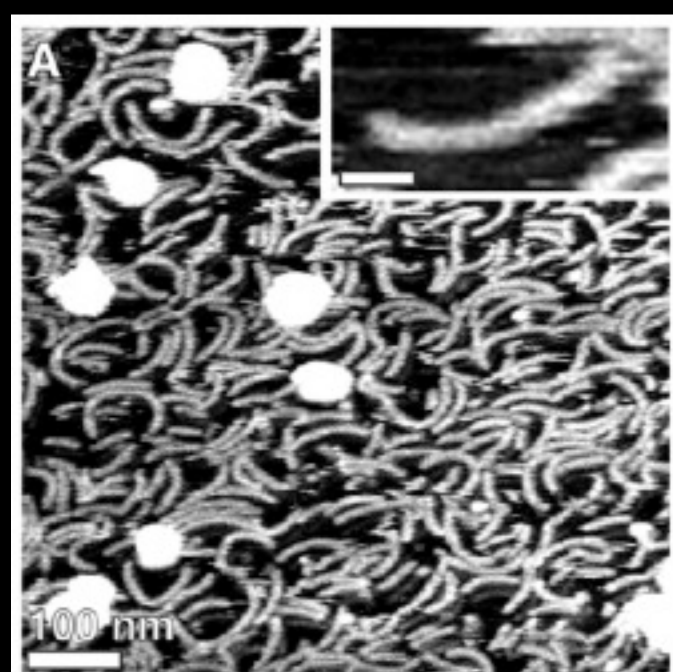
Zn sheets do not have M-loop H1-S2 loop interactions, but Taxol is still required to stabilize them.

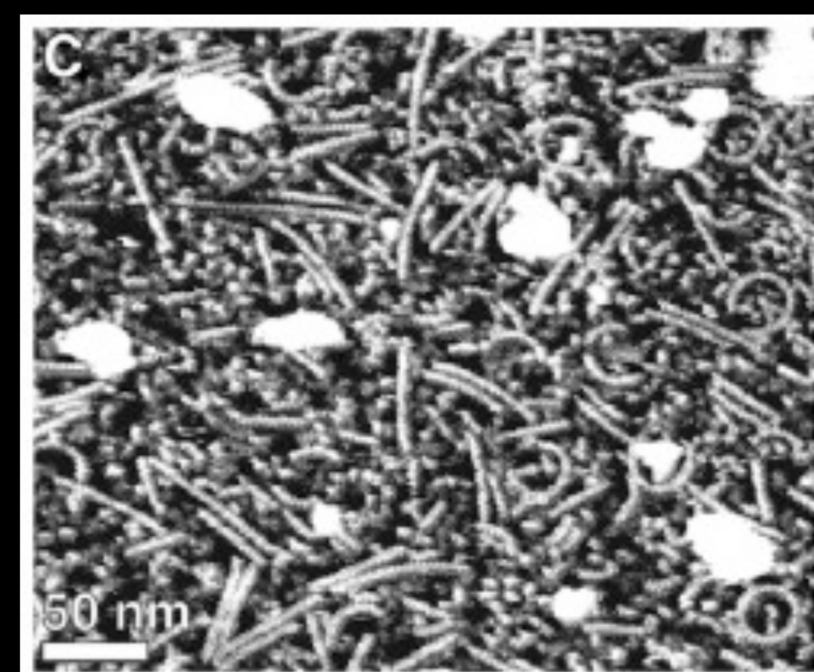
Current Biology 17, 1765–1770, October 23, 2007 DOI 10.1016/j.cub.2007.08.063

Report

Straight GDP-Tubulin Protofilaments Form in the Presence of Taxol

Céline Elie-Caille,[1,2] Fedor Severin,[1] Jonne Helenius,[1]
Jonathon Howard,[3] Daniel J. Muller,[1,*]
and A.A. Hyman[3,*]





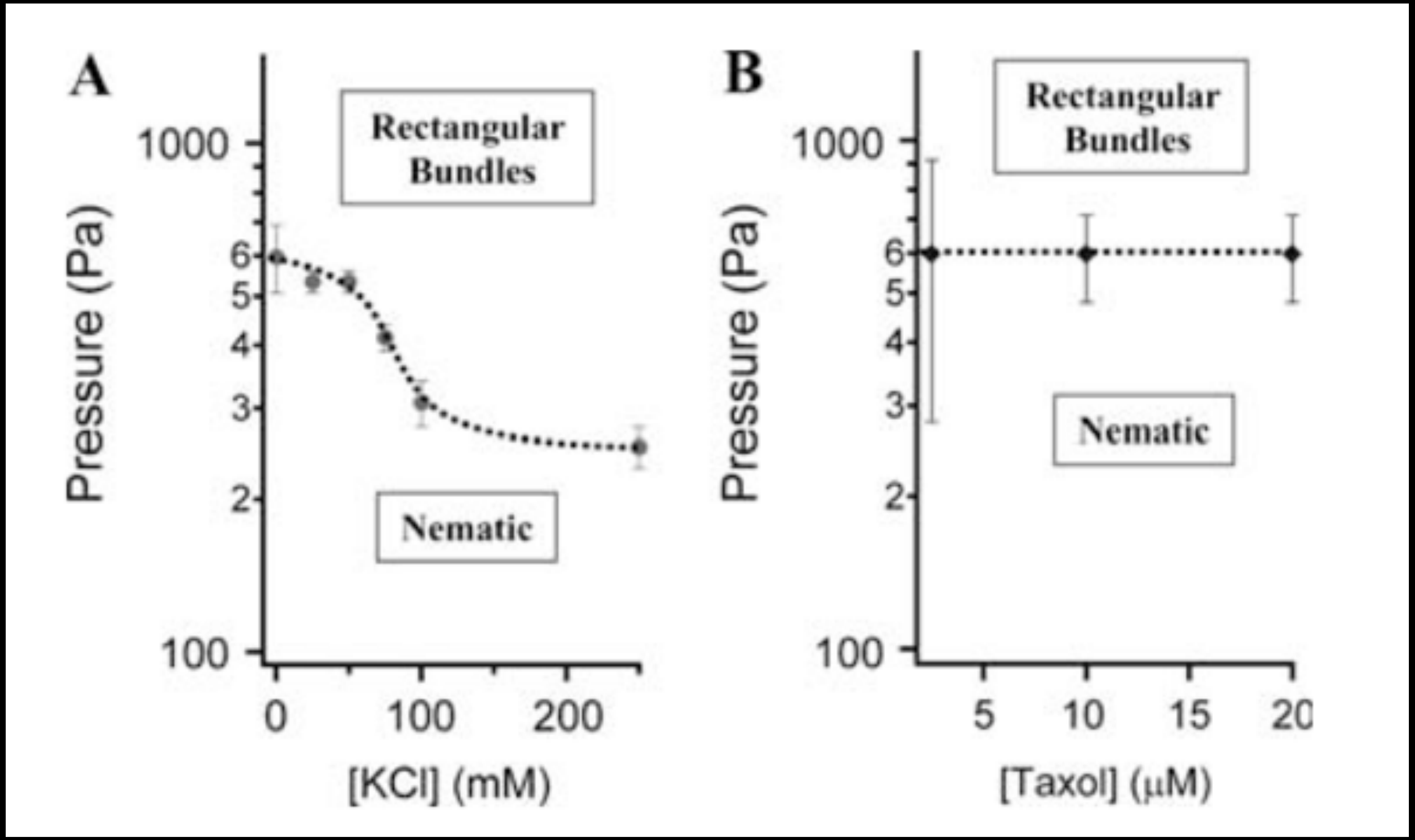
GMPCPP

GTP-Taxol

3410 Biophysical Journal Volume 89 November 2005 3410–3423

Radial Compression of Microtubules and the Mechanism of Action of Taxol and Associated Proteins

Daniel J. Needleman,*† Miguel A. Ojeda-Lopez,*† Uri Raviv,*† Kai Ewert,*† Herbert P. Miller,† Leslie Wilson,† and Cyrus R. Safinya*†
*Materials Department, Physics Department, and †Molecular, Cellular, and Developmental Biology Department, University of California, Santa Barbara, California 93106

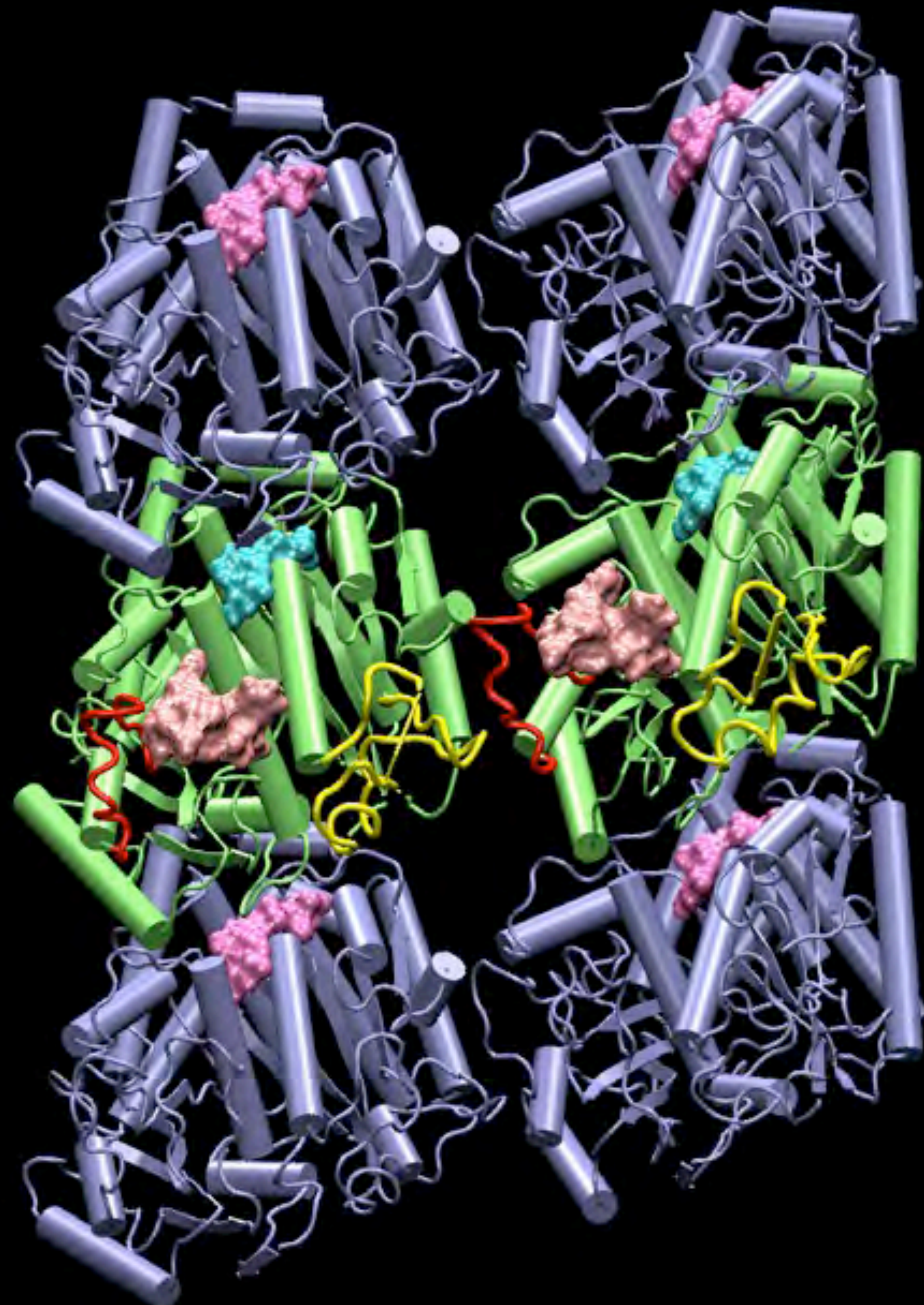


System for MD Simulations

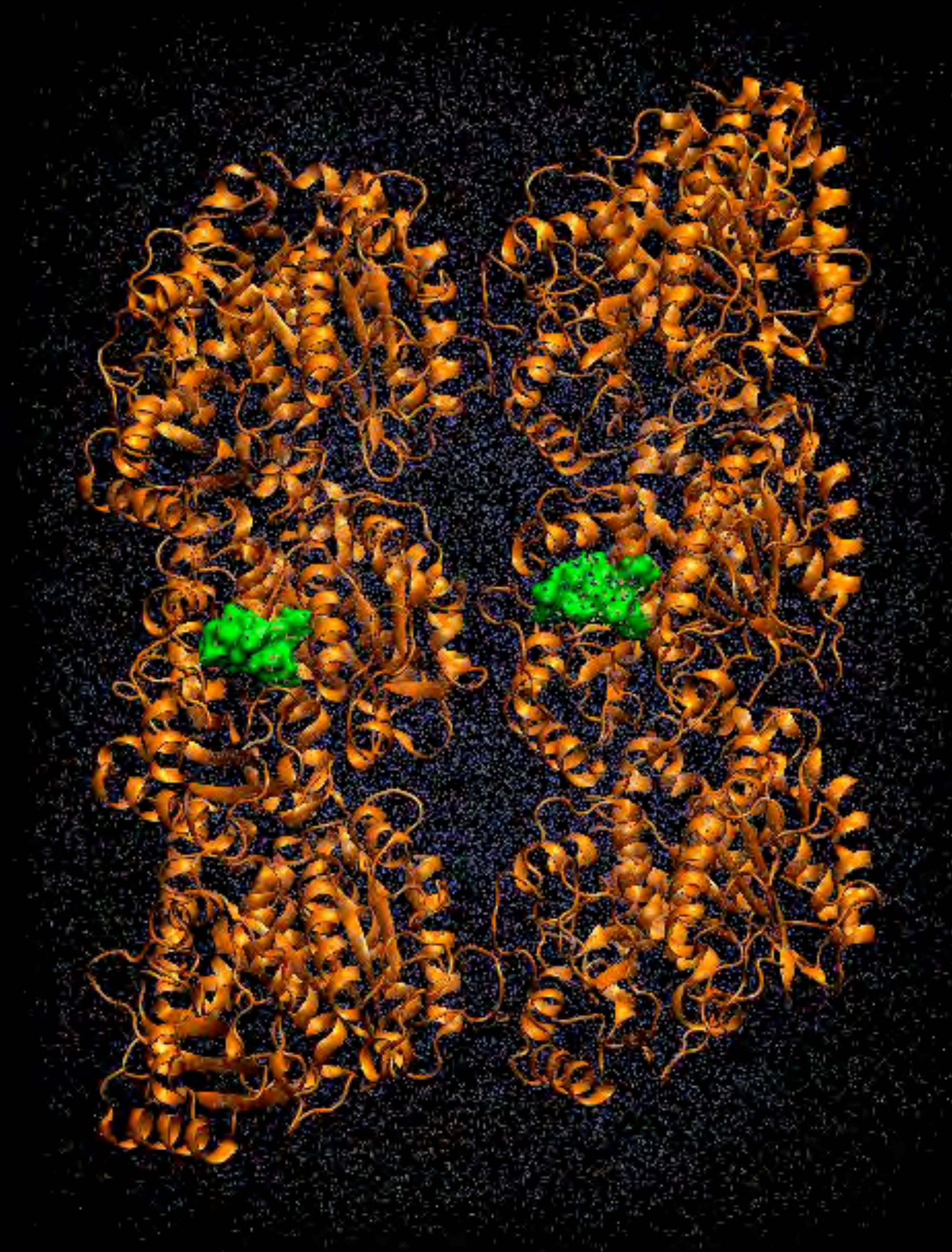
- Used 8Å microtubule model from Ken Downing as template

- Created a system with more than 250,000 atoms (protein, ions and water)

- Performed apo and taxol-bound simulations on 1024 processors on SDSC BlueGene

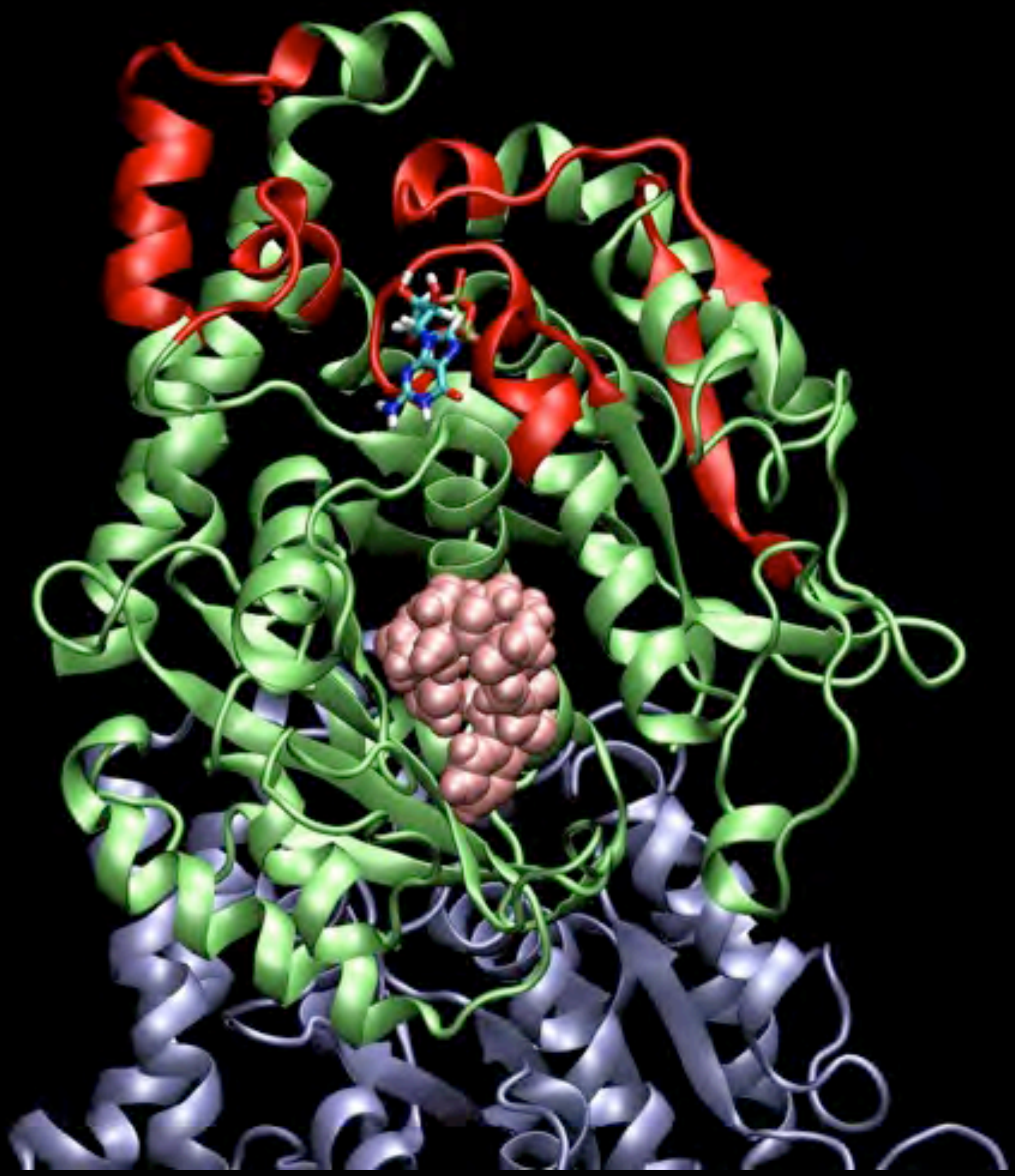


apo



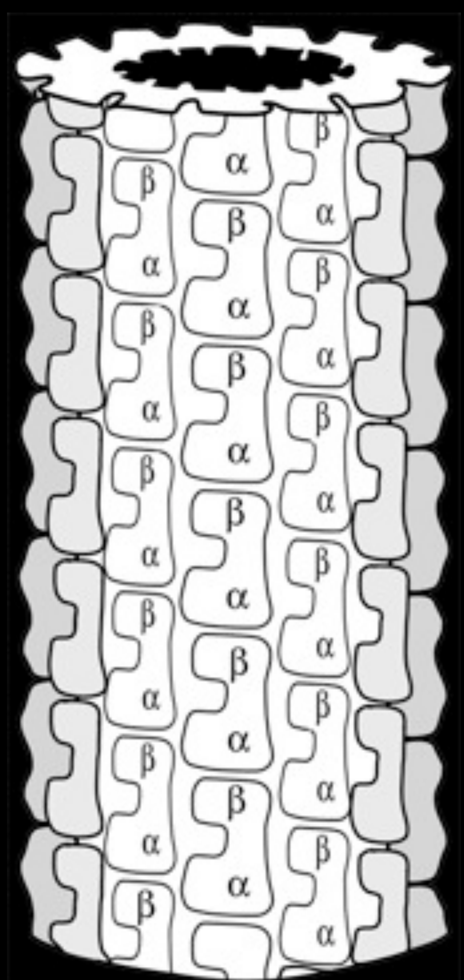
Taxol bound

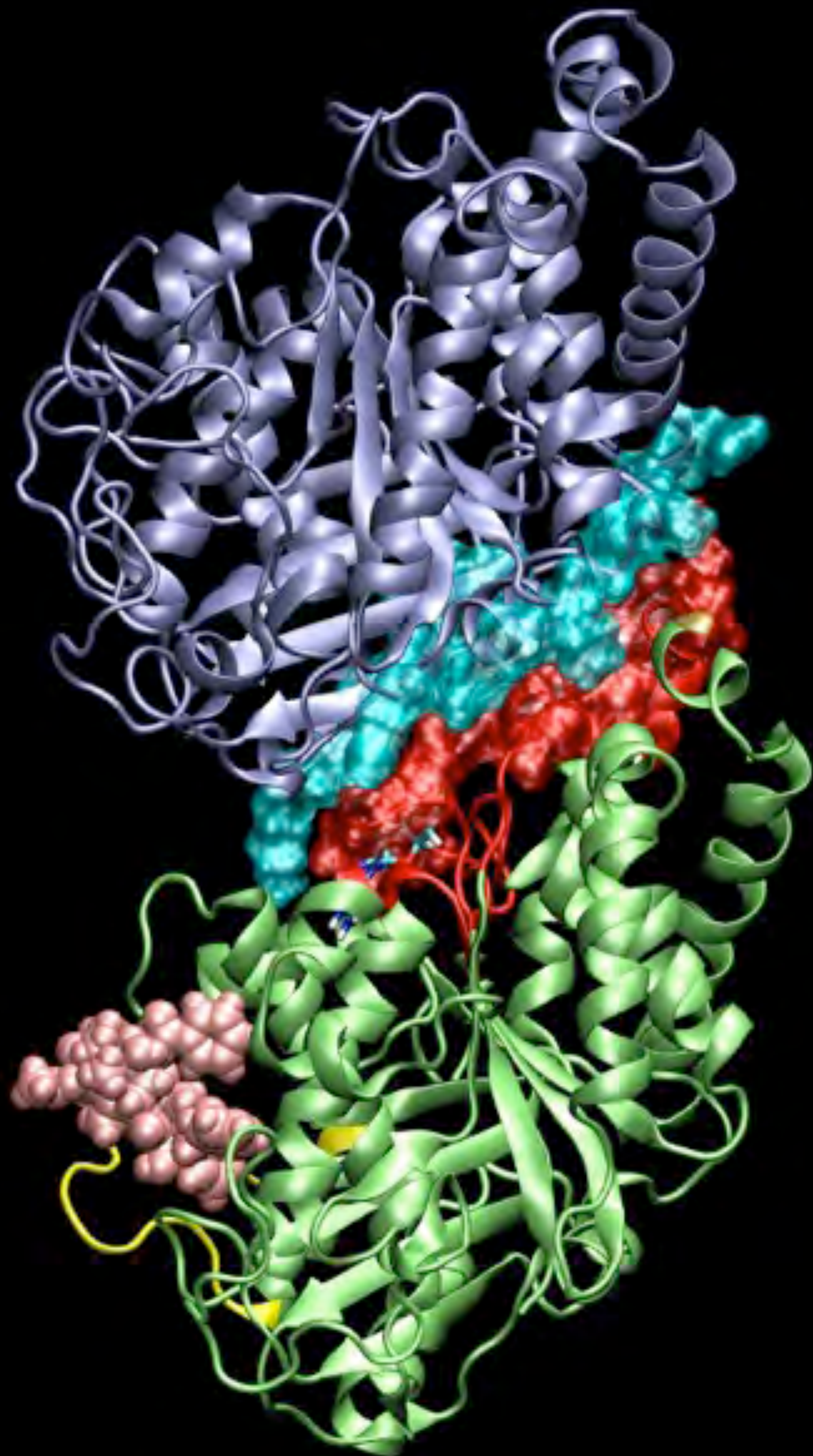
Allosteric Effects in β-Tubulin



- The T1 - T5 loops as well as a portion of H11 all show enhanced flexibility when Taxol binds
- The increase in flexibility should allow the protein to absorb the induced strain resulting from hydrolysis or phosphate loss

Allosteric Effects in β-Tubulin





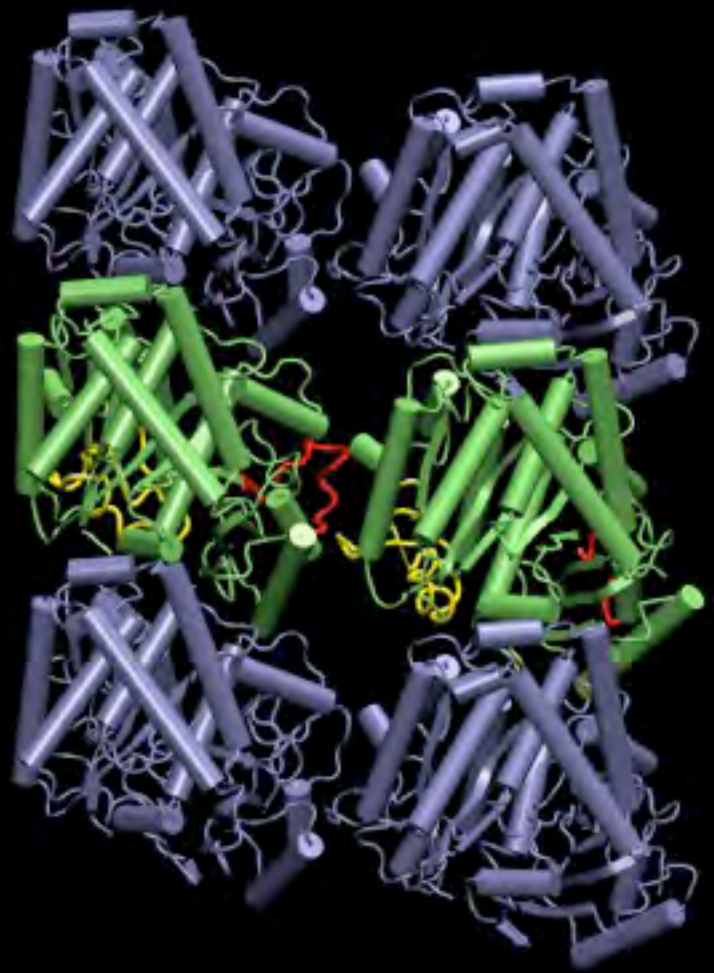
- These same loops also form the interface with the next dimer in the protofilament
- Since this interface is more flexible, the protofilament and hence the entire polymer should appear more flexible.

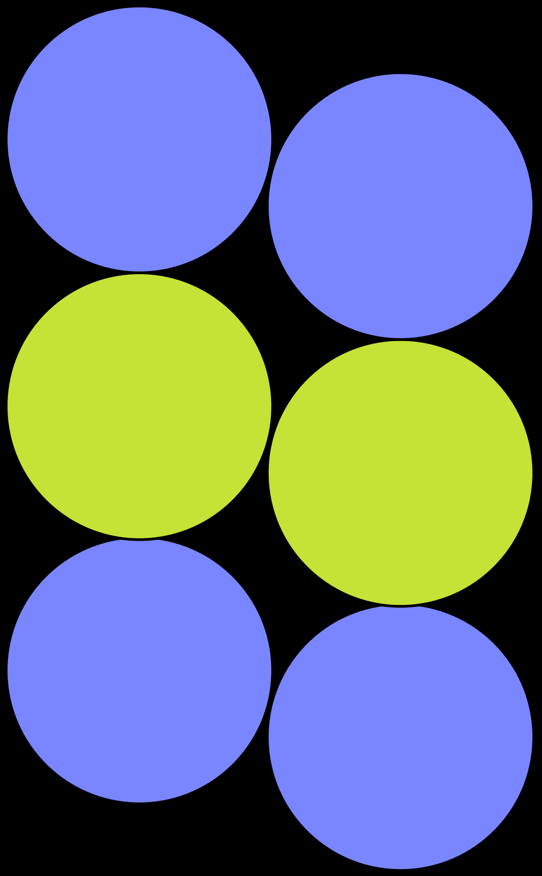
Atomistic Simulations → Mechanics

- We see an increase in flexibility, but we want to relate this to mechanical properties of the protofilaments and the whole microtubule

- Using continuum mechanics descriptions, we can relate dynamics in the simulated structure to quantities such as the Young's modulus, persistence length, etc.

Coarse Graining the System

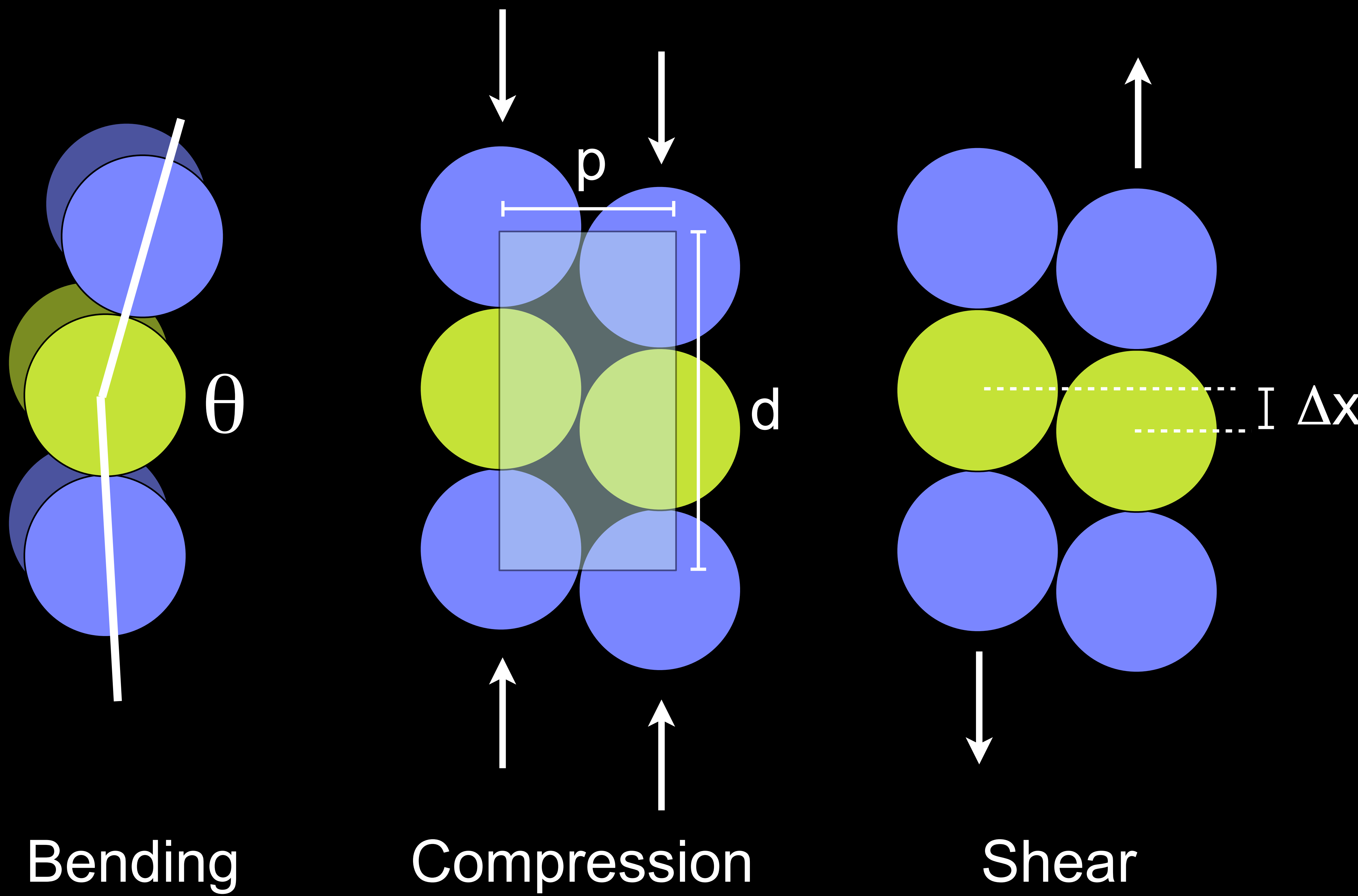




We start with our all-atom simulations ...

and treat the center of each monomer as a particle.

Mechanical Modes

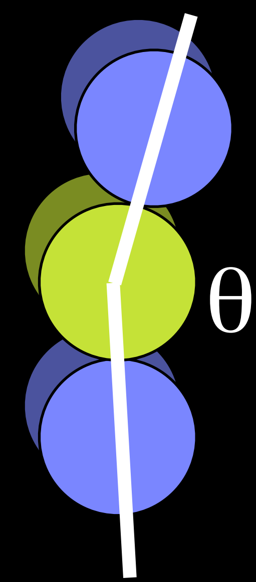


Bending Rigidity

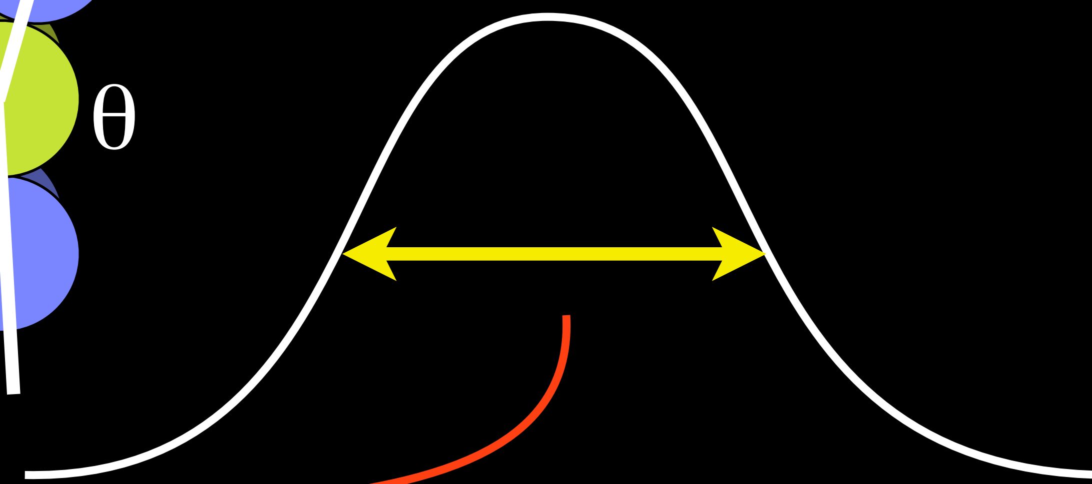
We are not applying an external force to the system (typically calculate a force-displacement curve), but instead we are looking at equilibrium fluctuations

$$\langle E_{bend} \rangle = \frac{kT}{2} = \frac{2p}{d} \kappa \langle \Delta\theta^2 \rangle$$

Bending Rigidity



We measure this

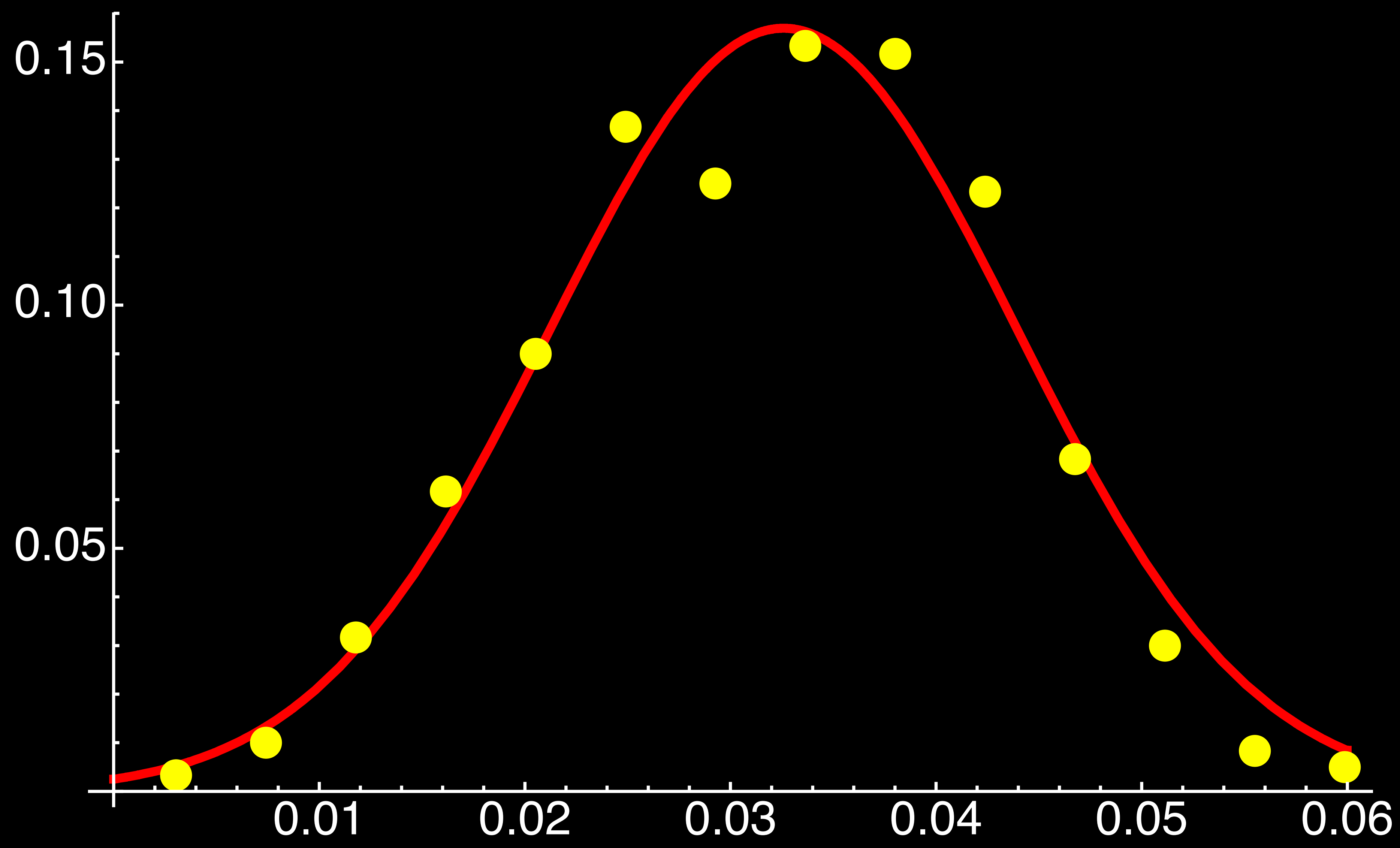


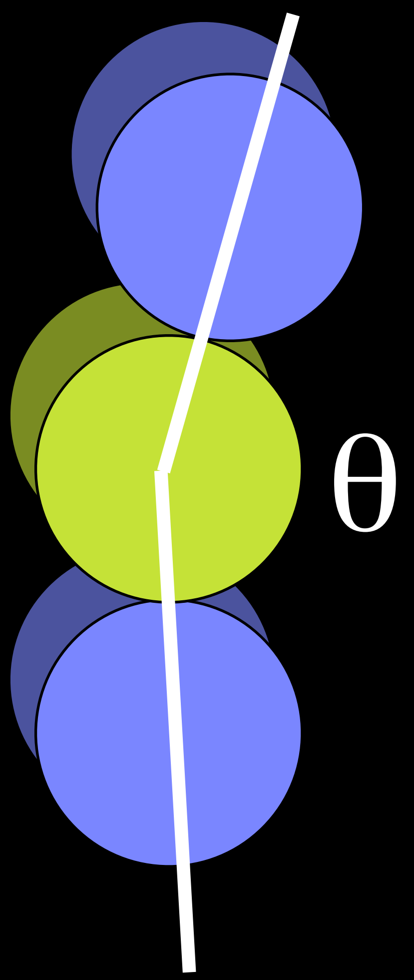
We know these

$$\langle E_{bend} \rangle = \frac{kT}{2} = \frac{2p}{d}\kappa\langle \Delta\theta^2 \rangle$$

To determine the bending rigidity

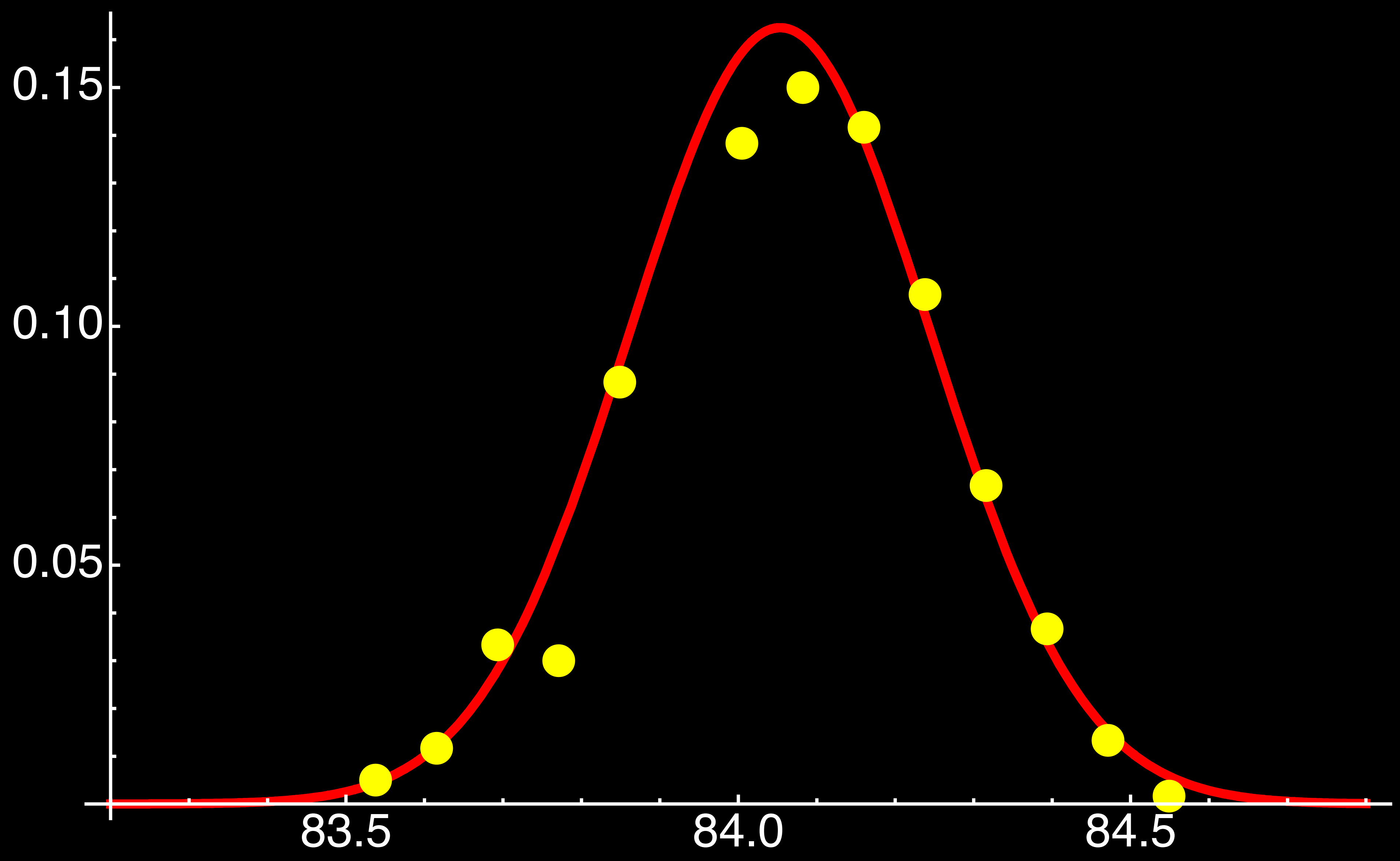
Bending Rigidity

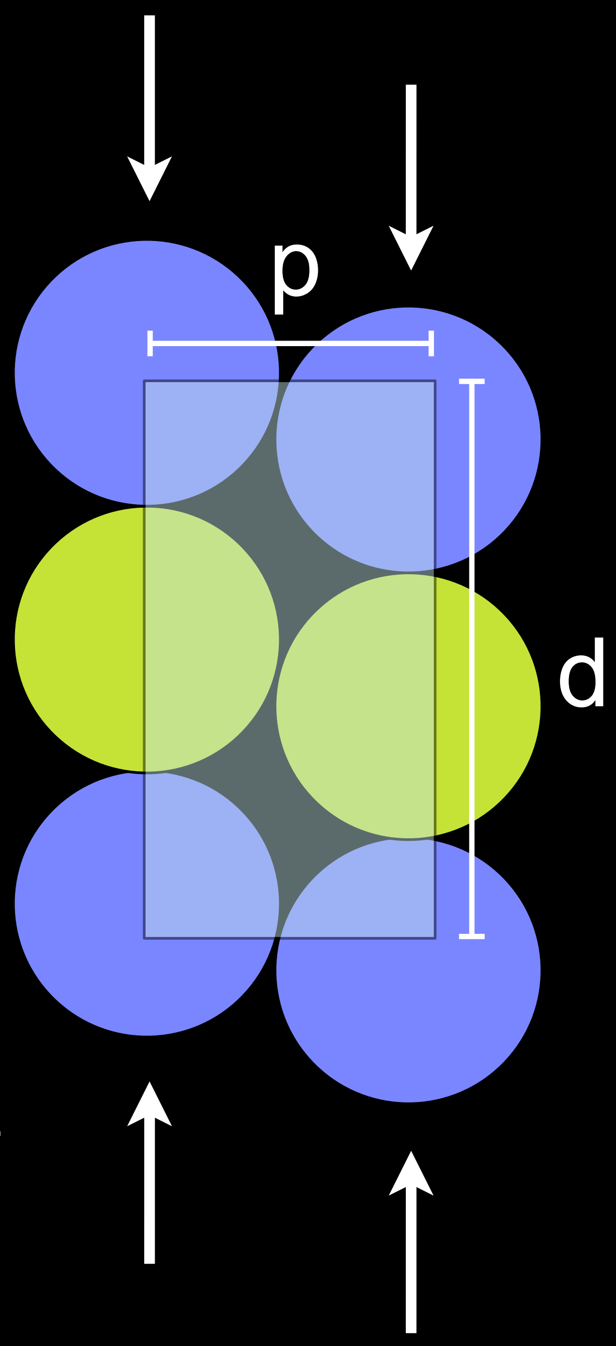




$$\langle E_{bend} \rangle = \frac{kT}{2} = \frac{2p}{d} \kappa \langle \Delta\theta^2 \rangle$$

Compression Modulus





$$\langle E_{comp} \rangle = \frac{kT}{2} = \frac{p}{d} E^{(2D)} \langle \Delta d^2 \rangle$$

Apo Material Numbers

Bending Rigidity

$$\langle \Delta\theta^2 \rangle = 0.000129$$
$$\kappa = 1.24 \times 10^4 \, pN \cdot nm$$

2D Young's Modulus

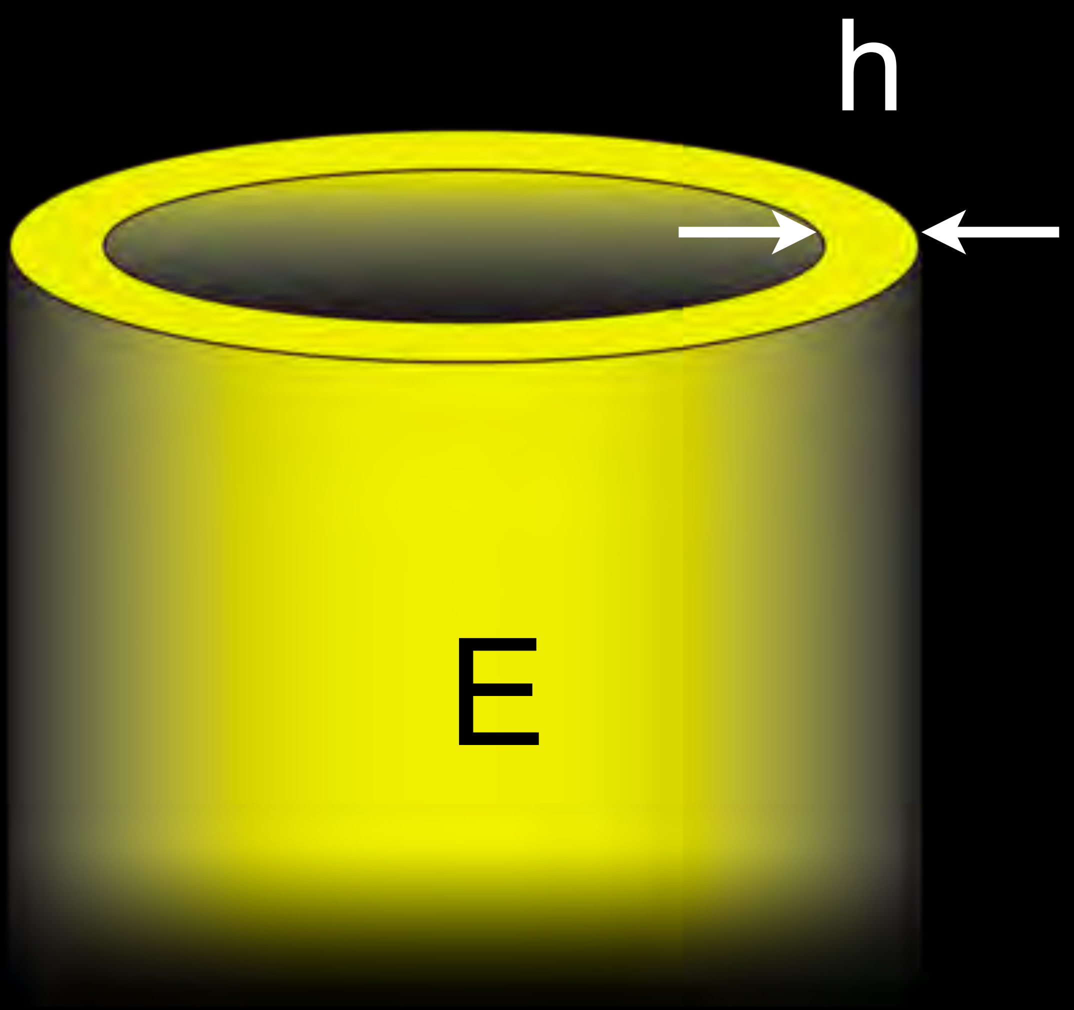
$$\langle \Delta d^2 \rangle = 0.000369 \, nm^2$$
$$\mathrm{E}^{(2D)} = 8.73 \times 10^4 \, pN/nm$$

Continuum MT Mechanics

- We want to treat the microtubule as an isotropic elastic shell, which implies:

$$\kappa = \frac{1}{12(1-\nu^2)} E h^3$$

$$E^{(2d)} = Eh$$



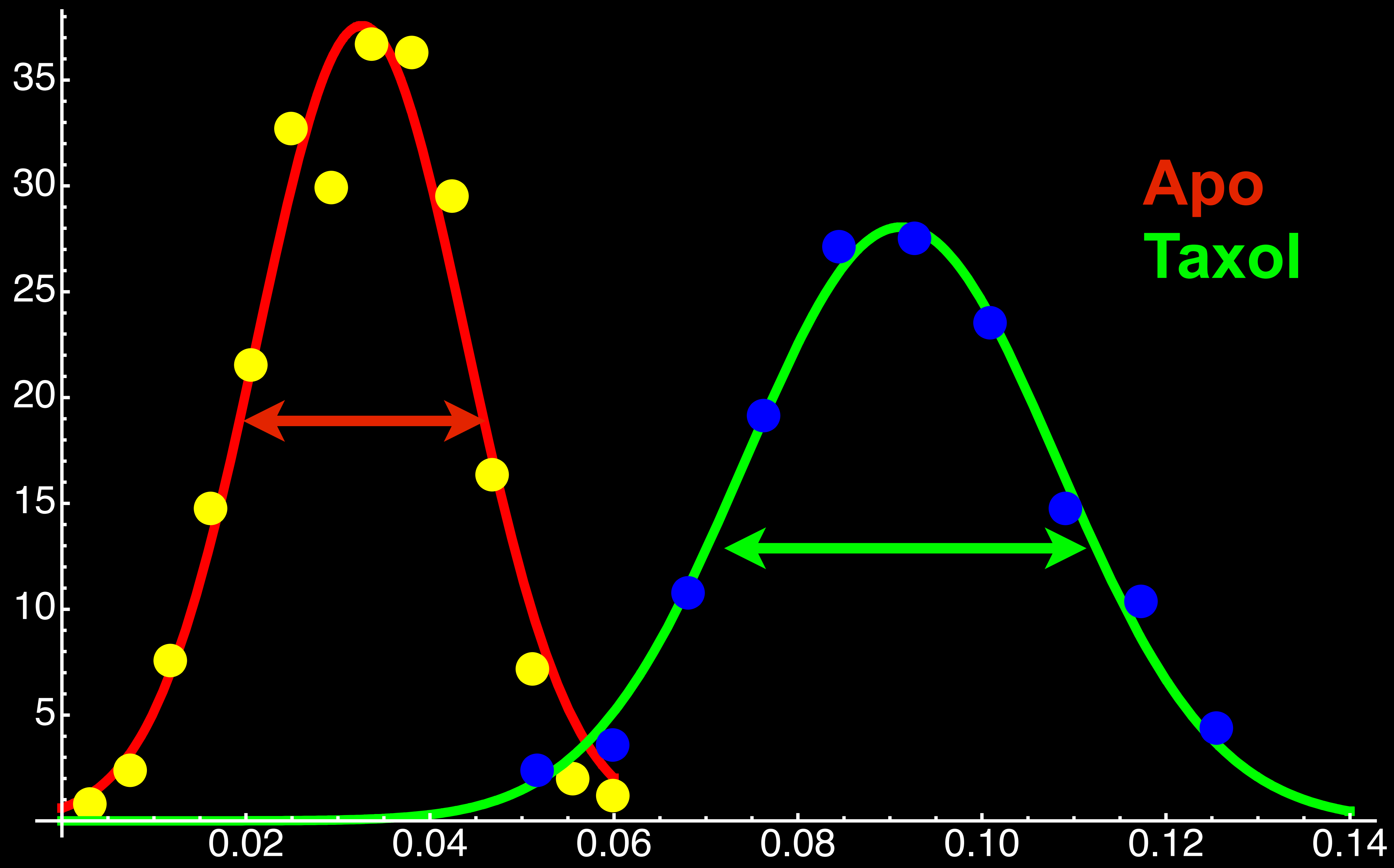
Material Properties

- Using these formulae we find

Simulation	Experiment
$E = 2.2$ GPa	$E = 0.3 - 3$ GPa
$h = 4.0$ nm	$h = 4.0$ nm
$l_p = 6.9$ mm	$l_p = 1 - 10$ mm

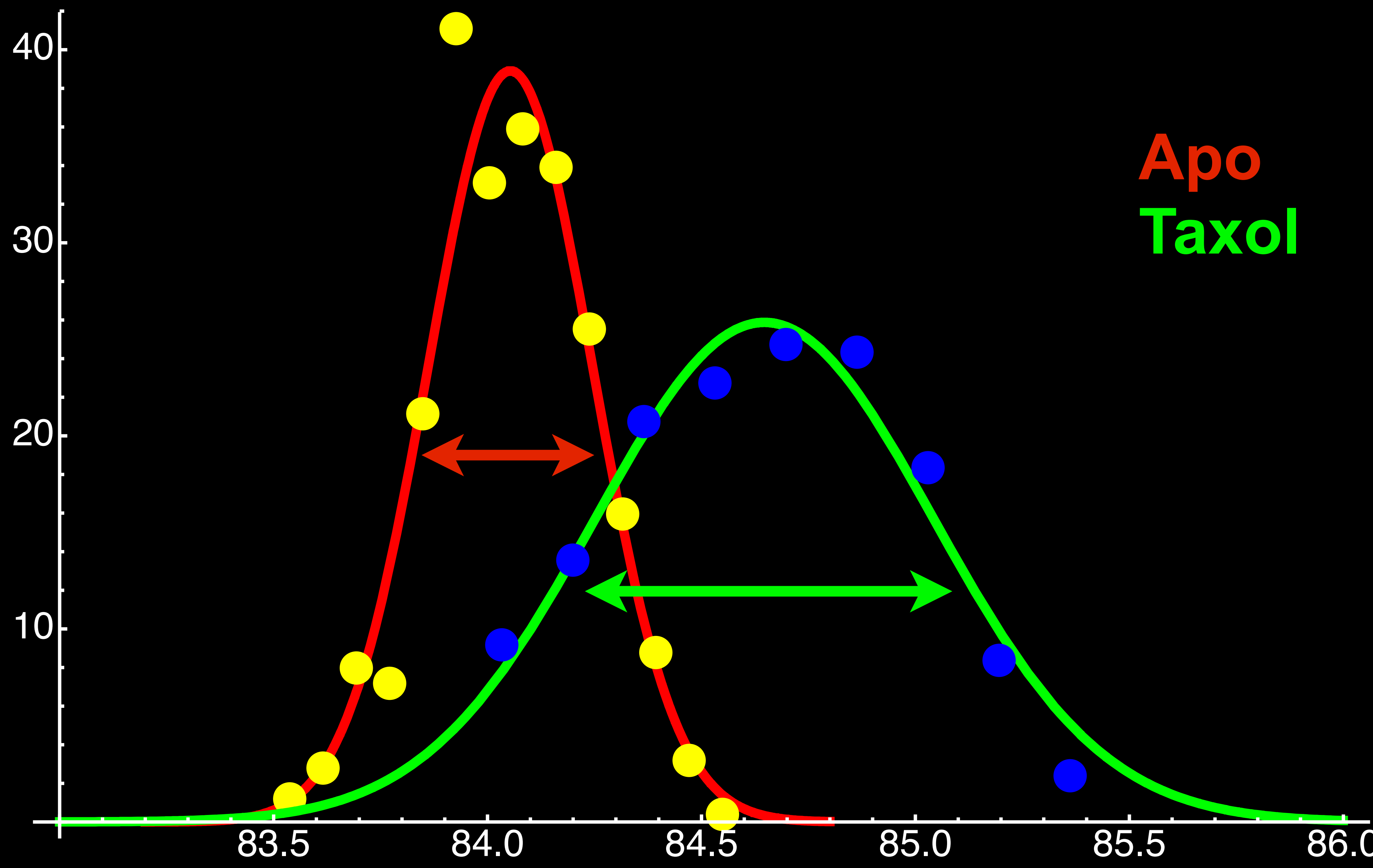
Hawkins et al. J Biomech., 2010

Apo vs. Taxol Bending



Taxol stabilized MTs are more flexible

Apo vs. Taxol Contour



Taxol stabilized MTs are more compressible

Effect of Taxol

Apo	Taxol
$E = 2.2$ GPa	$E = 0.38$ GPa
$h = 4.0$ nm	$h = 5.4$ nm
$l_p = 6.9$ mm	$l_p = 1.2$ mm

Taxol decreases the Young's modulus and the persistence length by a factor of about 5-6.

Biophysical Journal Volume 90 March 2006 1687–1696 1687

Flexural Rigidity of Individual Microtubules Measured by a Buckling Force with Optical Traps

Mahito Kikumoto, Masashi Kurachi, Valer Tosa, and Hideo Tashiro
Photodynamics Research Center, The Institute of Physical and Chemical Research (RIKEN), Miyagi, Japan

The Journal of Biological Chemistry Vol. 269, No. 18, Issue of May 6, pp. 13353–13360, 1994
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Analysis of Microtubule Rigidity Using Hydrodynamic Flow and Thermal Fluctuations*

(Received for publication, November 18, 1993, and in revised form, January 28, 1994)

Pascal Venier, Anthony C. Maggs‡, Marie-France Carlier, and Dominique Pantaloni
From the Laboratoire d'Enzymologie, CNRS, 91198 Gif-sur-Yvette Cedex, France and the ‡Groupe de Physico-Chimie Théorique, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 10 rue Vauquelin, F 75231 Paris Cedex 05, France

✔

Taxol-induced Flexibility of Microtubules and Its Reversal by MAP-2 and Tau*

(Received for publication, January 21, 1993)

Rick B. Dye, Stephen P. Fink, and Robley C. Williams, Jr.‡
From the Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

Flexural rigidity of microtubules measured with the use of optical tweezers

Harald Felgner[1,2,*], Rainer Frank[1] and Manfred Schliwa[1]
[1]Institut für Zellbiologie, Ludwig-Maximilians-Universität, Schillerstrasse 42, D-80336 München, Germany
[2]Lehrstuhl für Biophysik E22, Technische Universität München, Garching, Germany
*Author for correspondence (e-mail: felgner@physik.tu-muenchen.de)

Microtubule curvatures under perpendicular electric forces reveal a low persistence length

M. G. L. van den Heuvel, M. P. de Graaff, and C. Dekker*
Kavli Institute of Nanoscience, Section Molecular Biophysics, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands

Domains of Neuronal Microtubule-associated Proteins and Flexural Rigidity of Microtubules

Harald Felgner,* Rainer Frank,* Jacek Biernat,‡ Eva-Maria Mandelkow,‡ Eckhard Mandelkow,‡ Beat Ludin,§ Andrew Matus,§ and Manfred Schliwa*
*Adolf-Butenandt-Institut, Zellbiologie, 80336 München, Germany; ‡Max-Planck-Unit for Structural Molecular Biology, 22607 Hamburg, Germany; and §Friedrich-Miescher Institute, CH-4002 Basel, Switzerland

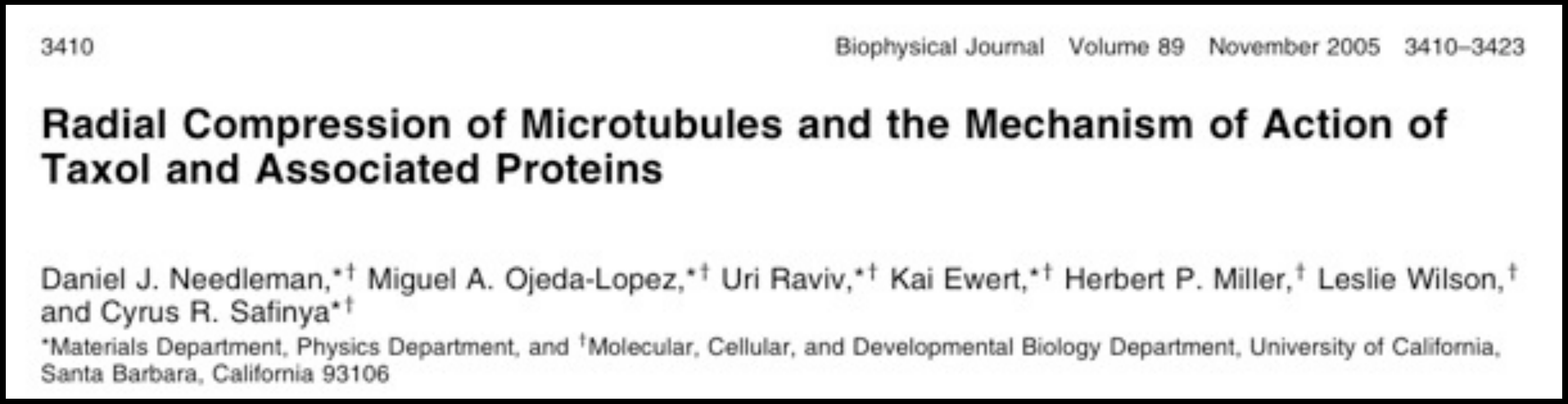
9 out of 10 biophysicists agree
Taxol stabilized MTs are more flexible

✘

Rigidity of Microtubules Is Increased by Stabilizing Agents

Brian Mickey and Jonathon Howard
Department of Physiology and Biophysics, University of Washington, Seattle, Washington 98195-7290

Shear Modulus

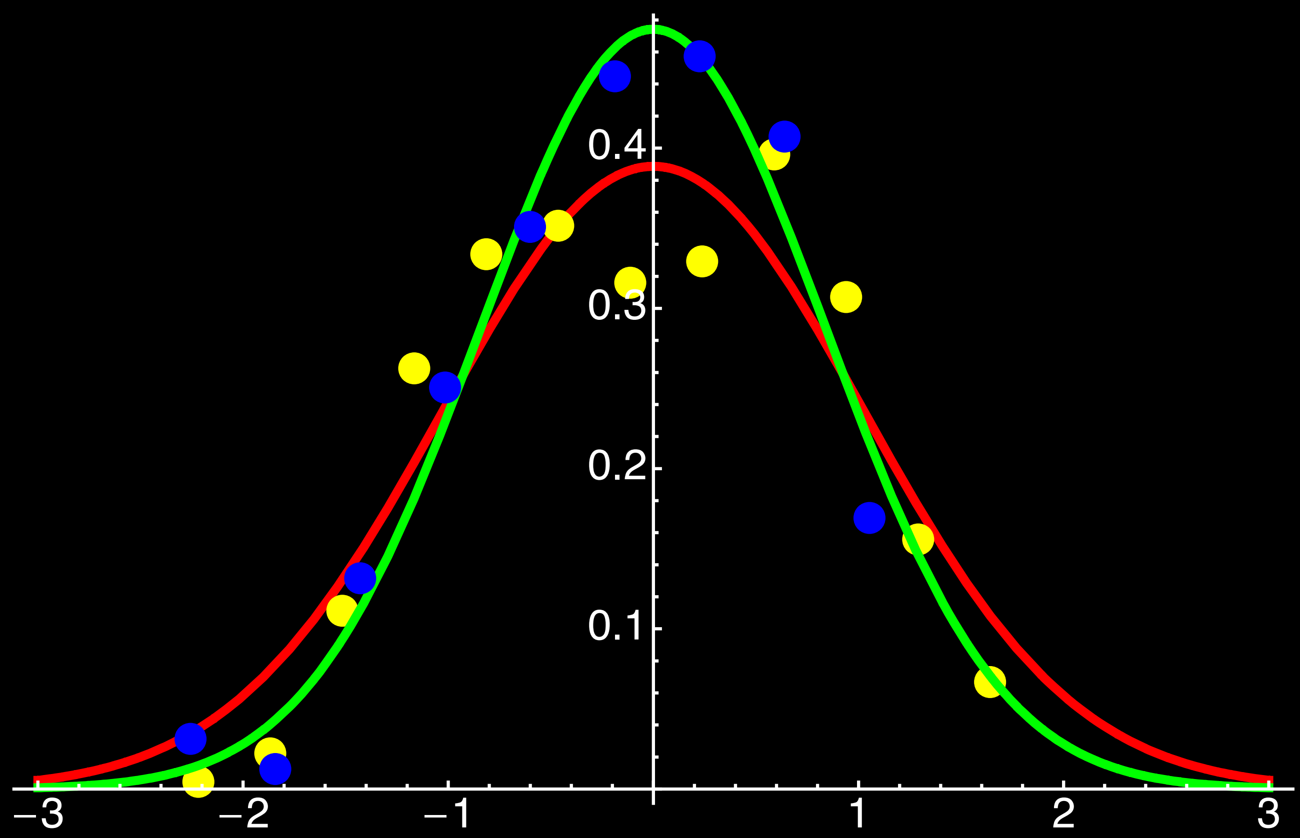


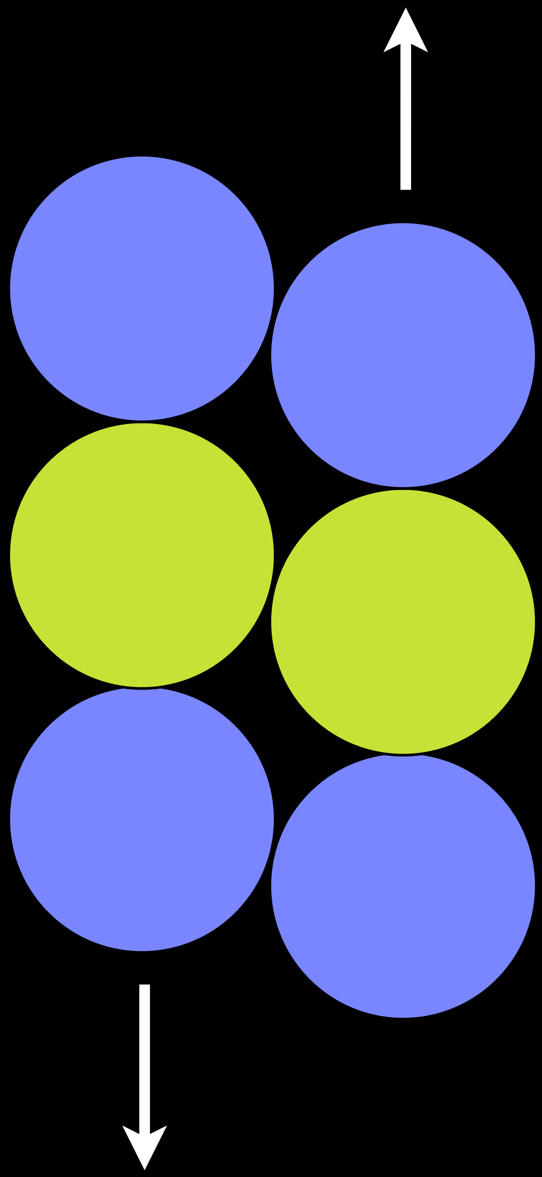
3410 Biophysical Journal Volume 89 November 2005 3410–3423

Radial Compression of Microtubules and the Mechanism of Action of Taxol and Associated Proteins

Daniel J. Needleman,*† Miguel A. Ojeda-Lopez,*† Uri Raviv,*† Kai Ewert,*† Herbert P. Miller,† Leslie Wilson,† and Cyrus R. Safinya*†

*Materials Department, Physics Department, and †Molecular, Cellular, and Developmental Biology Department, University of California, Santa Barbara, California 93106





No difference in shear modulus

$$\langle E_{shear} \rangle = \frac{kT}{2} = \frac{3d}{4p} G^{(2D)} \langle \Delta x^2 \rangle$$

Anisotropy

Thermal fluctuations of grafted microtubules provide evidence of a length-dependent persistence length

Francesco Pampaloni*†, Gianluca Lattanzi†‡, Alexandr Jonáš§, Thomas Surrey*, Erwin Frey¶‖, and Ernst-Ludwig Florin§‖

*Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany; ‡Department of Medical Biochemistry, Biology, and Physics, Innovative Technologies for Signal Detection and Processing Center and Instituto Nazionale Fisica Nucleare, Università di Bari, Piazza Giulio Cesare 11, 70124 Bari, Italy; §Center for Nonlinear Dynamics, University of Texas, Austin, TX 78712; and ¶Arnold Sommerfeld Center for Theoretical Physics and Center for Nano Science, Department of Physics, Ludwig-Maximilians-Universität München, Theresienstrasse 37, D-80333 Munich, Germany

- If we calculate G assuming isotropic elasticity

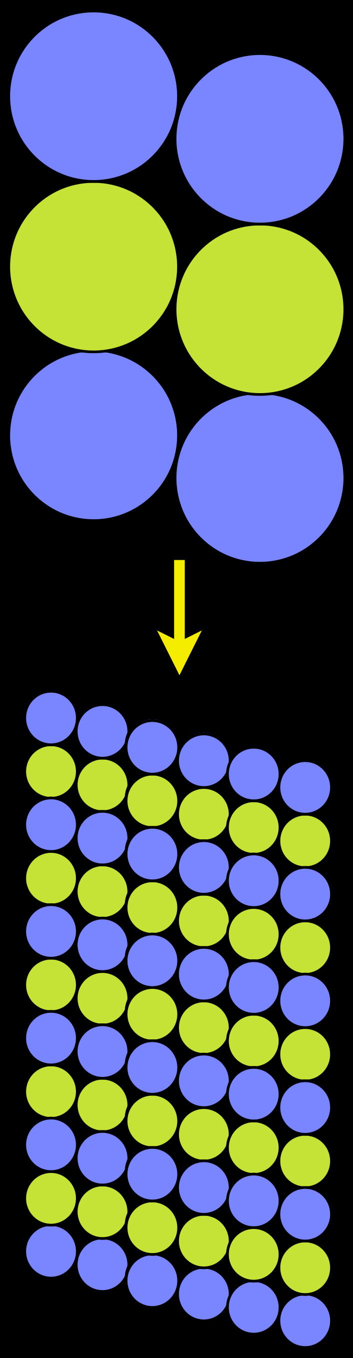
$$E = 2G(1+\nu)$$

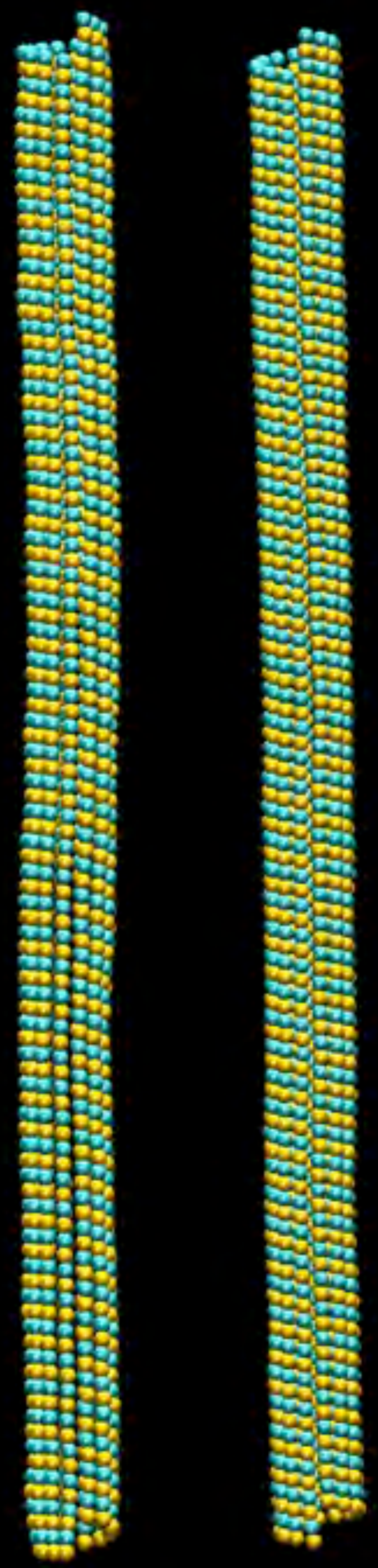
we get a shear modulus of 840 MPa

- We find a shear modulus of 47 MPa - some anisotropy, but not extreme

Increasing Length and Time

- We want to address questions that go beyond our simple model and make more ties to experiment
- In order to increase the size of our system and access longer time scales, we need make some further simplifications to our description and treatment





BD Simulations

- Our molecular dynamics simulations solve a Langevin equation
- If move the the overdamped regime, the acceleration term averages to zero and we can use Brownian dynamics

$$\dot{X} = -\frac{D}{k_B T}\nabla U(X) + \sqrt{2D}S(t)$$

- Our time step increases from 2 fs to 5-10 ps and we use "simple" springs

Anisotropic Mechanics

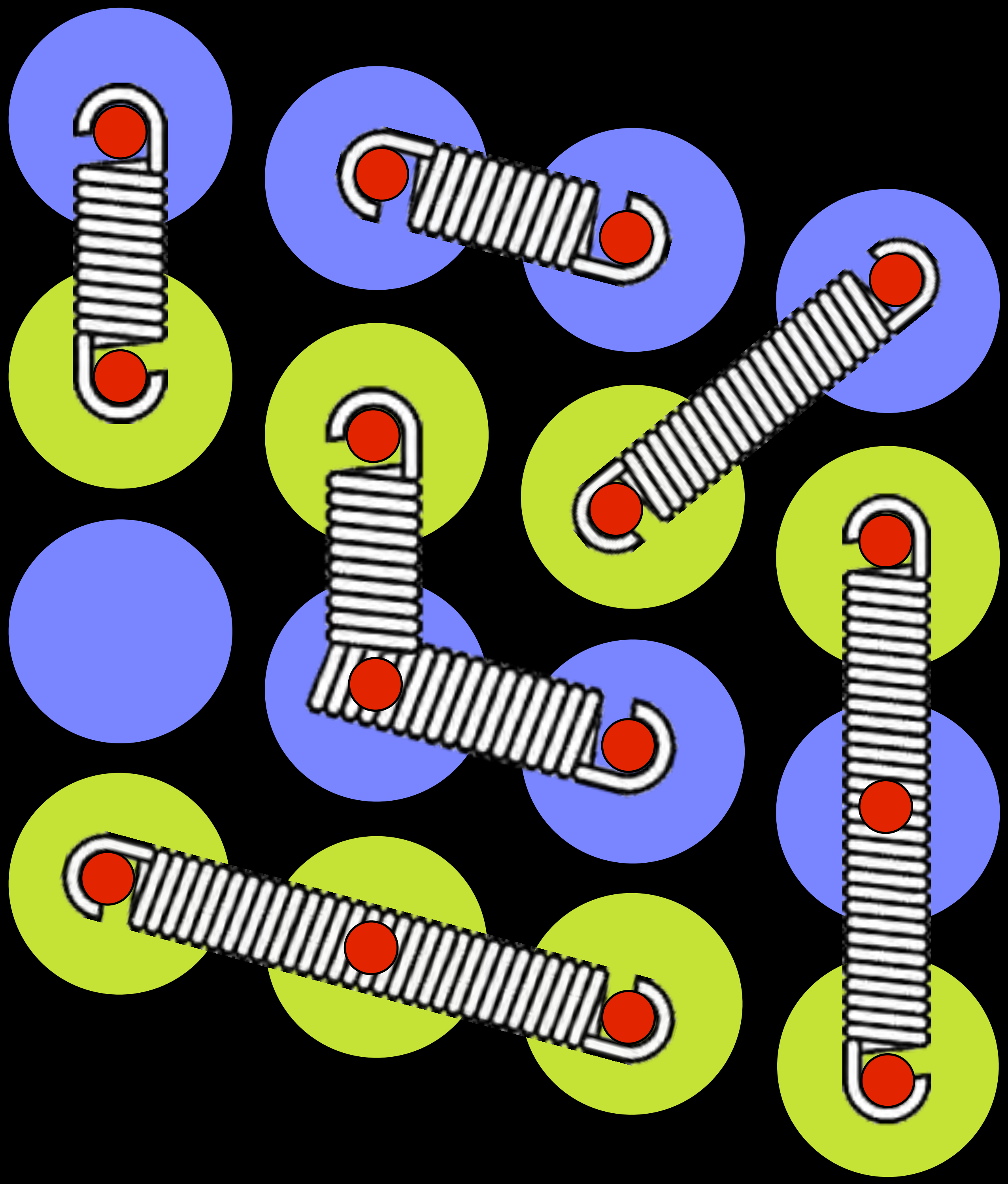
$$\kappa_x = \frac{E_x h^3}{12(1-\nu_x\nu_\theta)}$$

$$\kappa_\theta = \frac{E_\theta h^3}{12(1-\nu_x\nu_\theta)}$$

$$K_{x\theta} = G_{x\theta} h$$

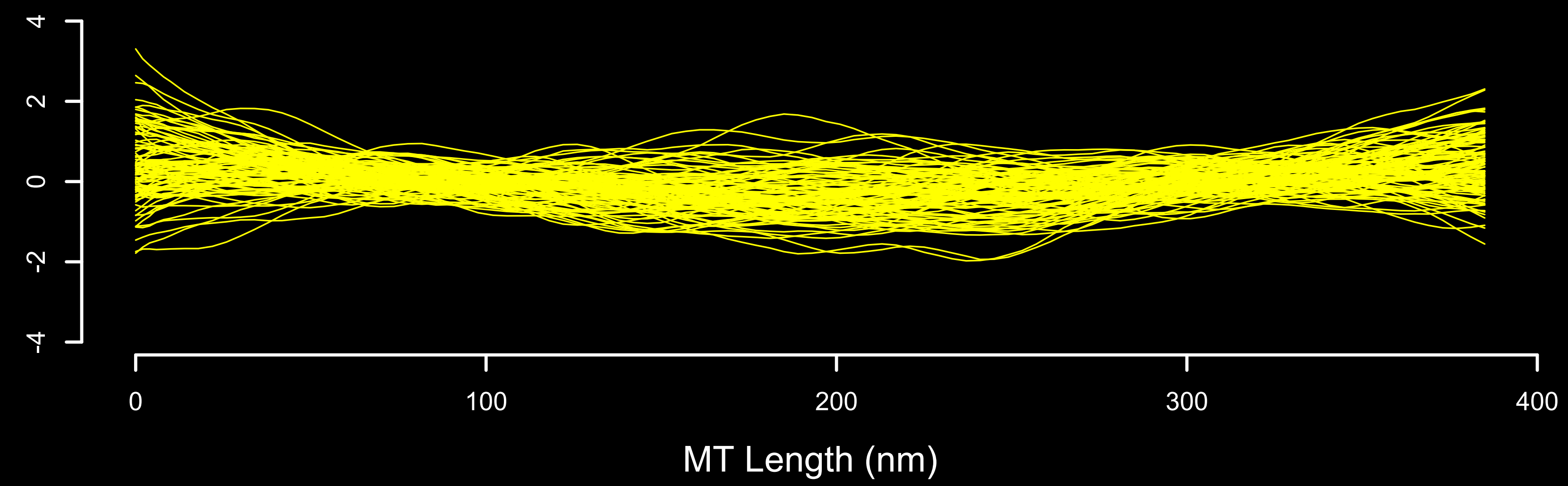
$$K_\theta = \frac{E_\theta h}{1-\nu_x\nu_\theta}$$

$$K_x = \frac{E_x h}{1-\nu_x\nu_\theta}$$



Fourier Analysis

- We decompose the contour traces of the MT into Fourier modes and look at the variance of the modes

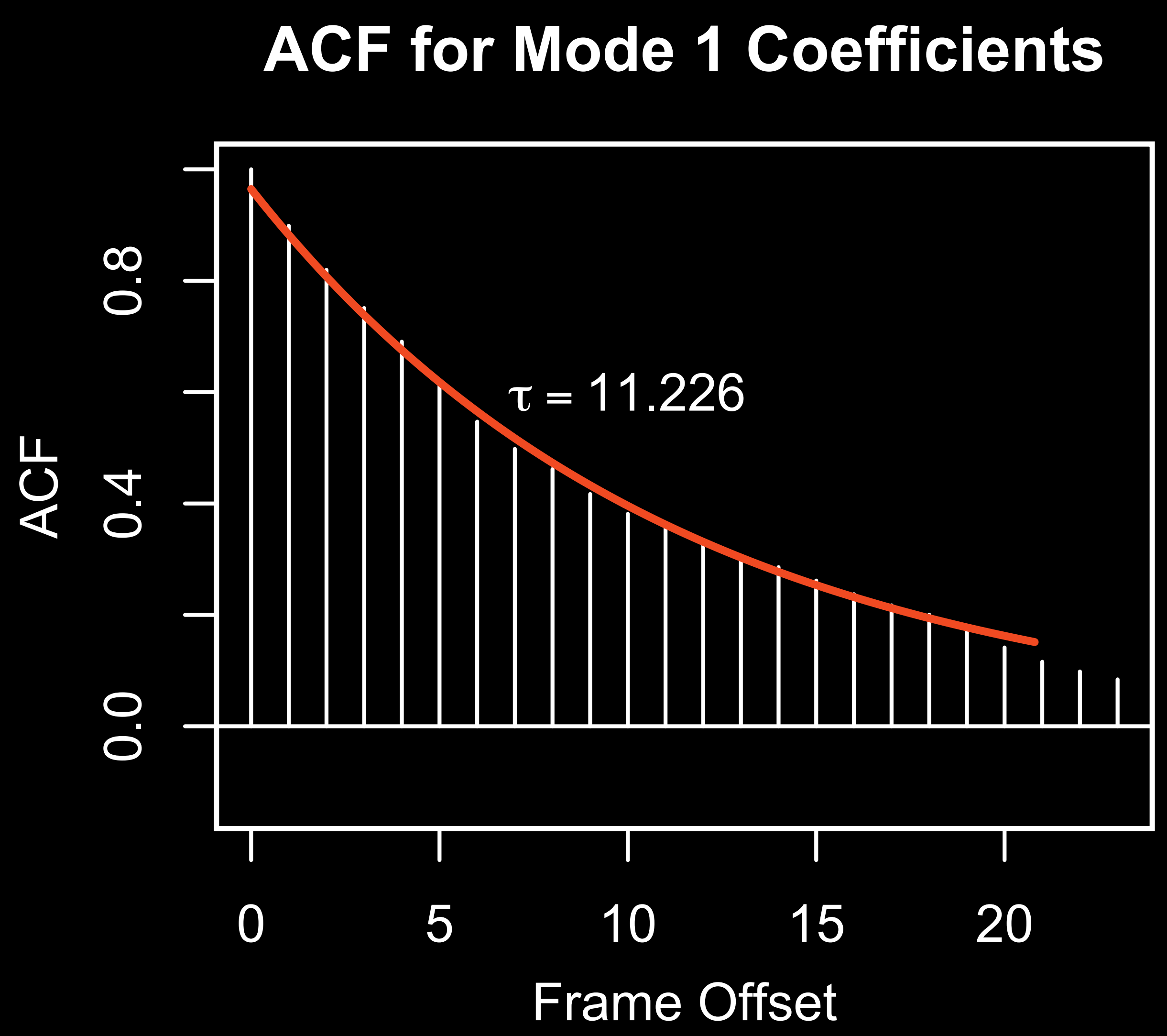


$$\theta(s) = \sqrt{\frac{2}{L}} \sum a_n \cos\left(\frac{n\pi s}{L}\right)$$

$$var(a_n) = \frac{k_B T}{EI}\left(\frac{L}{n\pi}\right)^2$$

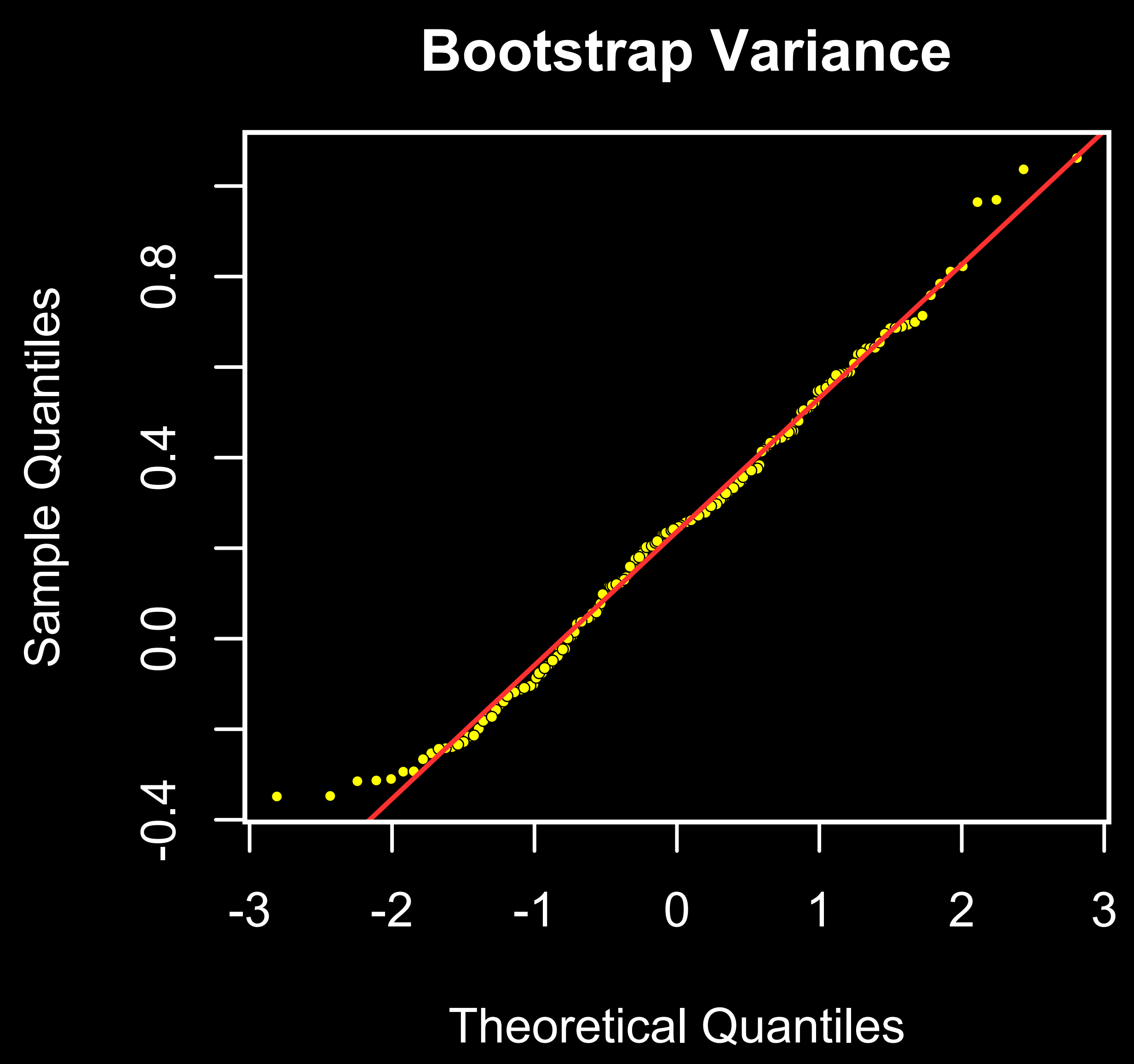
$$\ell_p = \frac{EI}{k_B T}$$

Using the Bootstrap



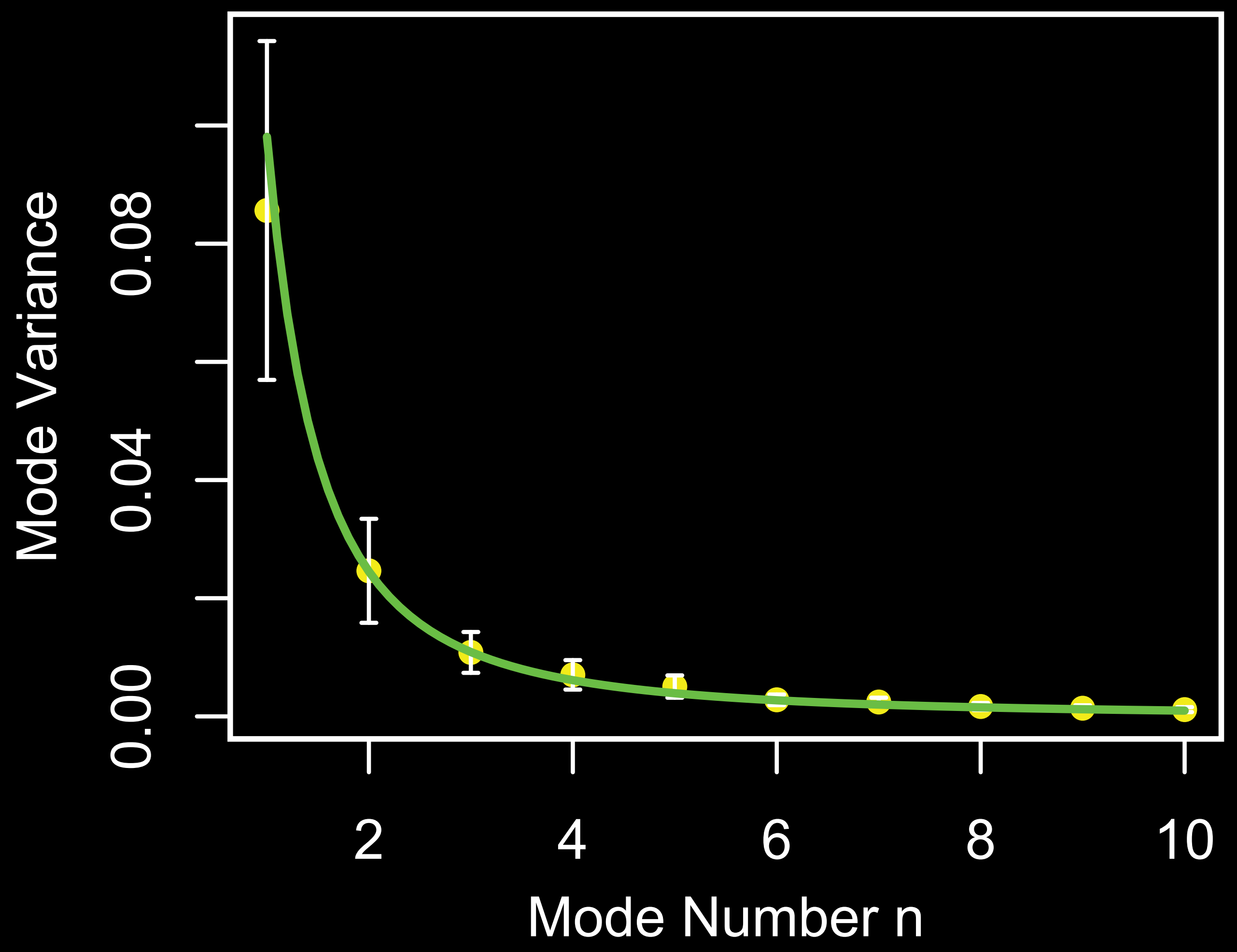
We can determine the correlation time for mode 1

Knowing the number of independent points, we apply the bootstrap



Variance vs. Mode Number

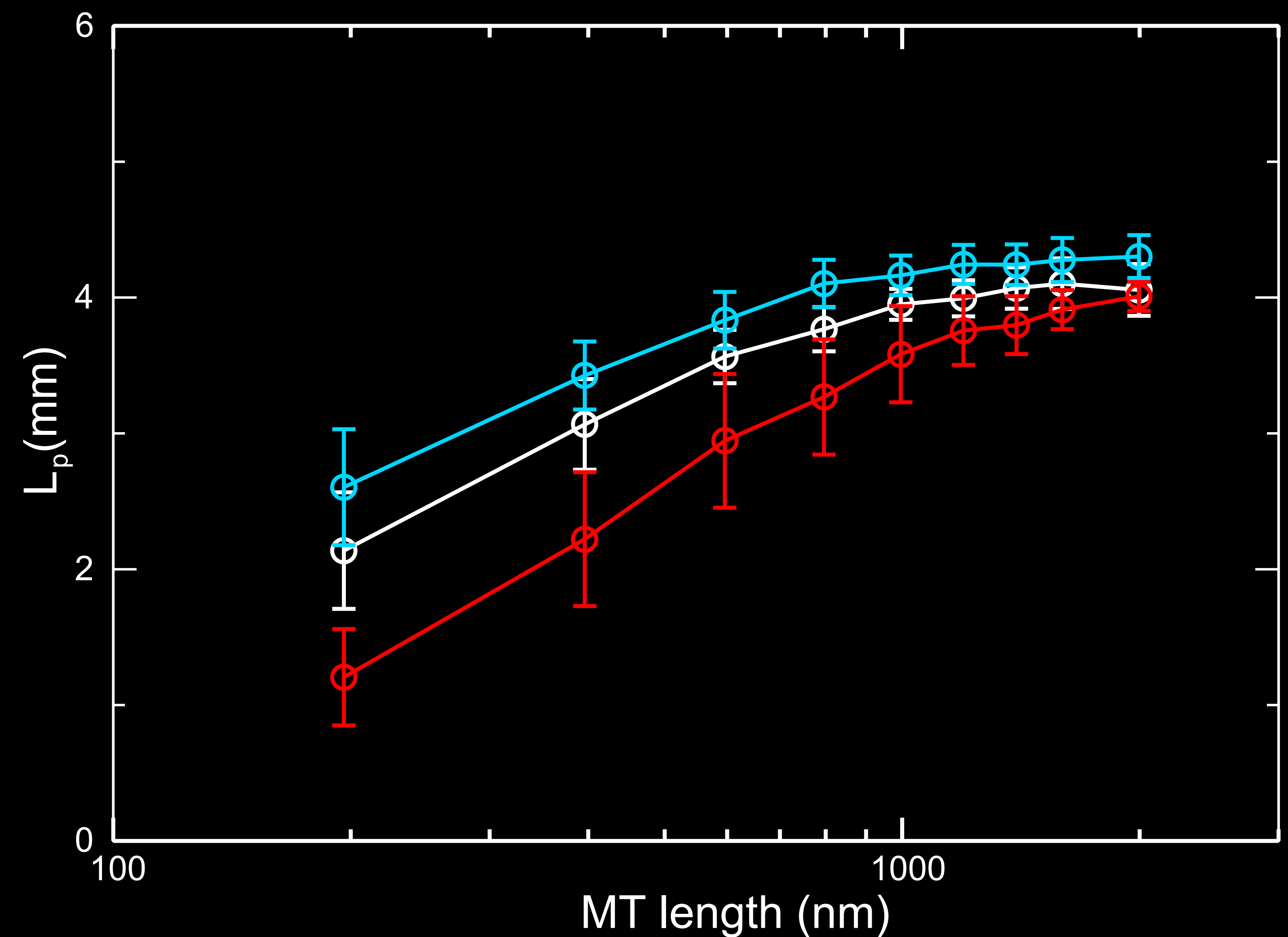
- One key difference in our system is that we have no noise
- With the variances for each mode, we can perform a least-squares fit and get the persistence length with its standard error



$$var(a_n) = \frac{1}{\ell_p}\left(\frac{L}{n\pi}\right)^2$$

MT Persistence Length

- We are still in the process of assessing the influence of each term (E, G, etc.) on the observed mechanics, but we do see a length dependent persistence length emerge

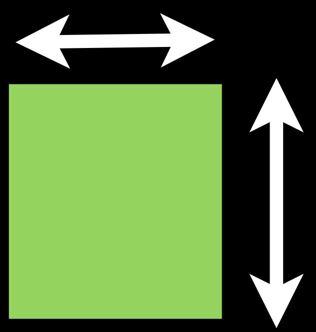


$G_{x\theta} = 40\ \mathrm{MPa}$

$G_{x\theta} = 20\ \mathrm{MPa}$

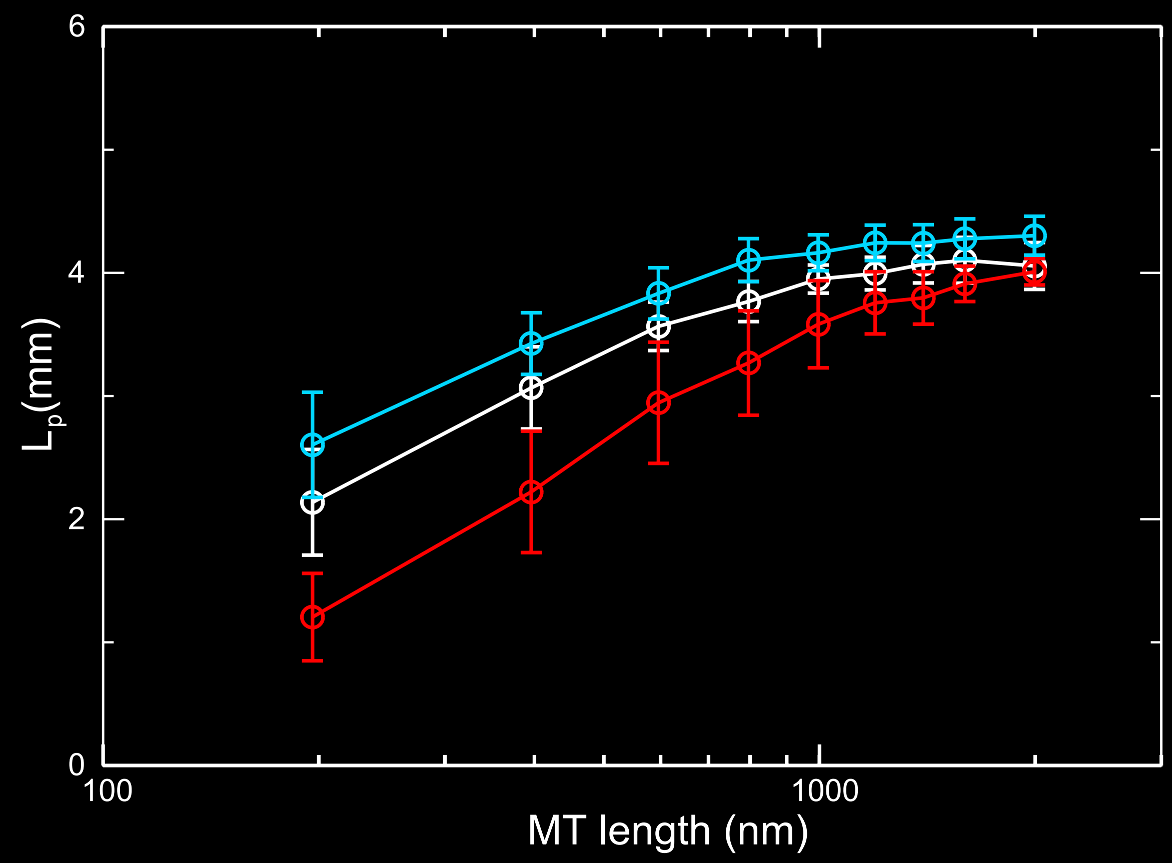
$G_{x\theta} = 4\ \mathrm{MPa}$

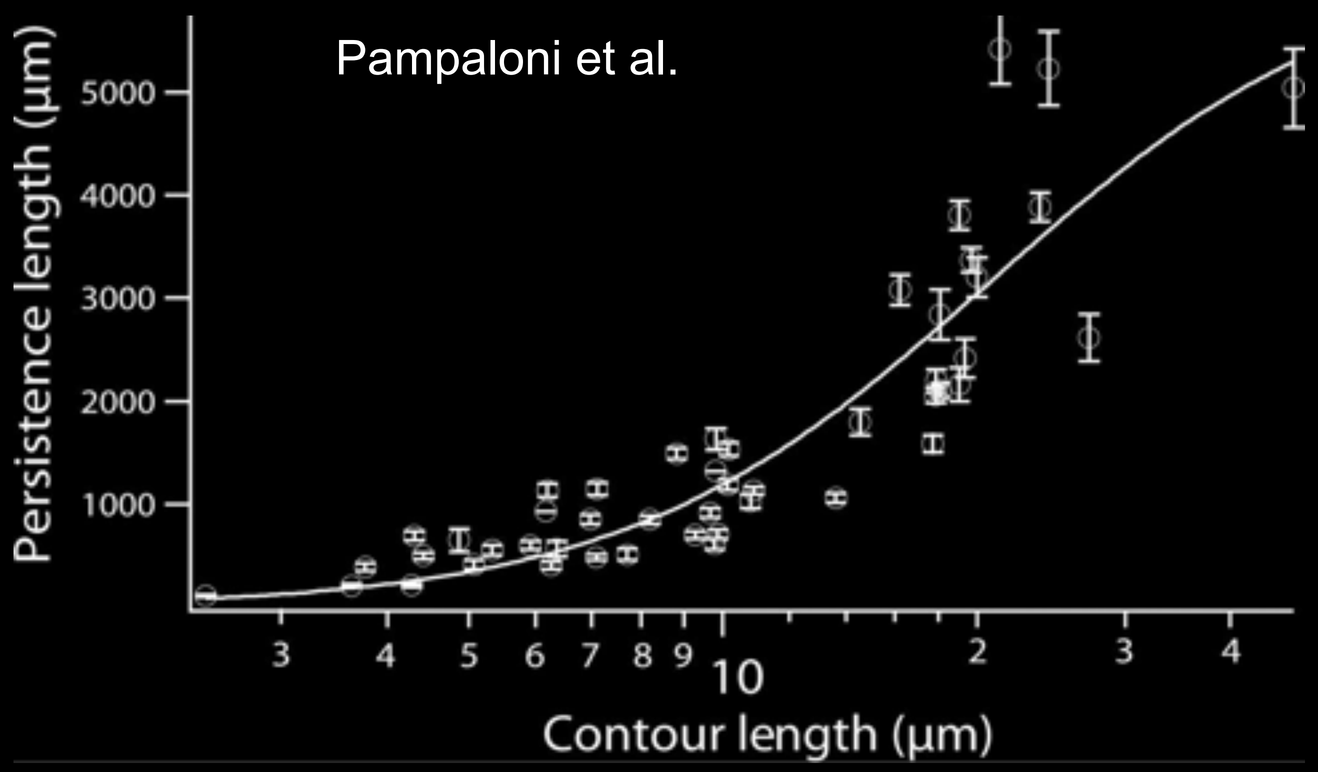
$E_\theta = 750\ \mathrm{MPa}$



$E_x = 1000\ \mathrm{MPa}$

Comparison with Experiment

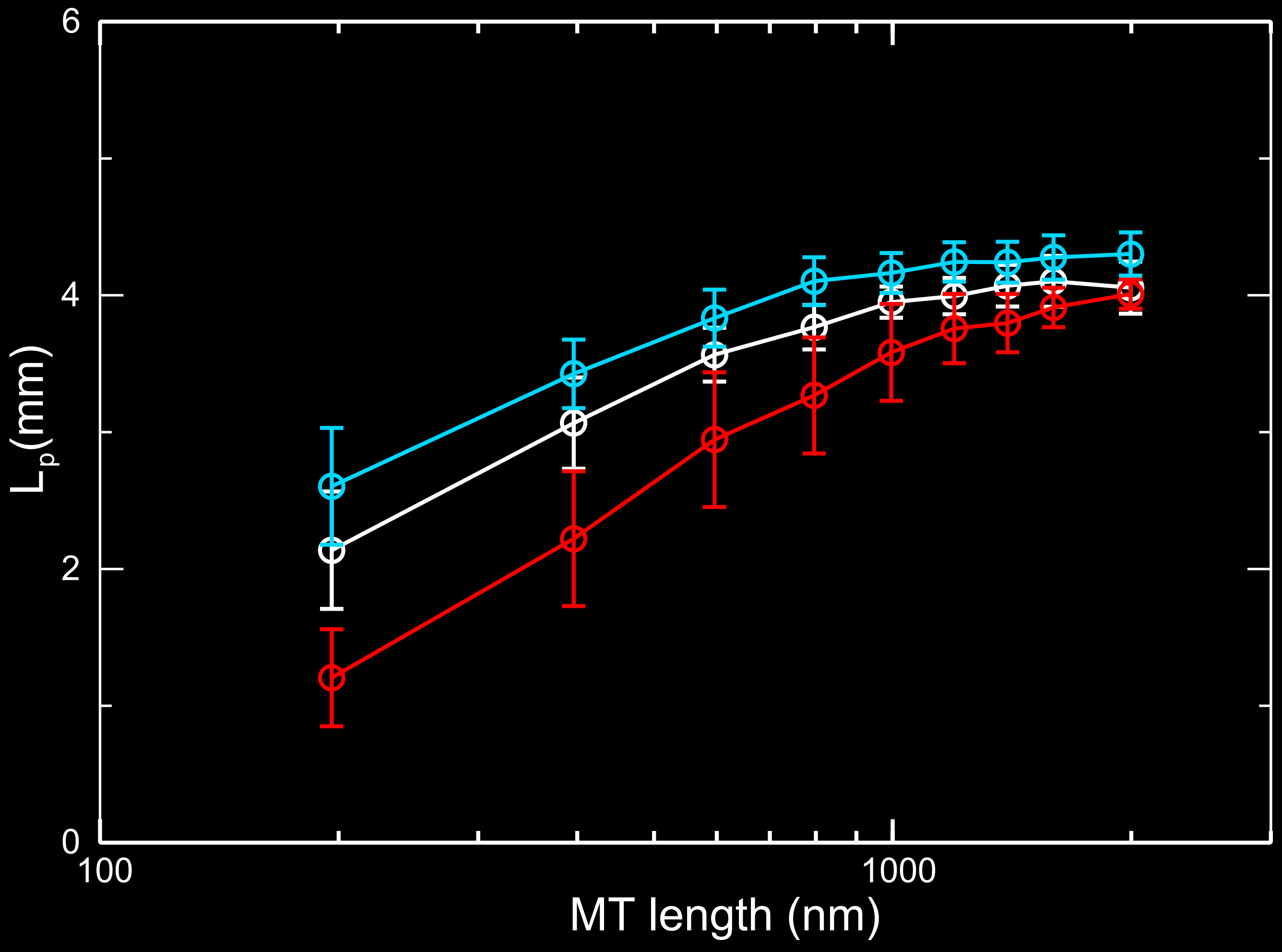




- We are still at relatively short lengths (2 μm), but are moving up to more realistic lengths of 20 μm

Structure vs. Mechanics

- We made our system intrinsically anisotropic through our choice of constants
- Interestingly, even if we make the mechanics isotropic, the anisotropic MT structure gives similar (better?) results



$$E = 250\ \mathrm{MPa}$$

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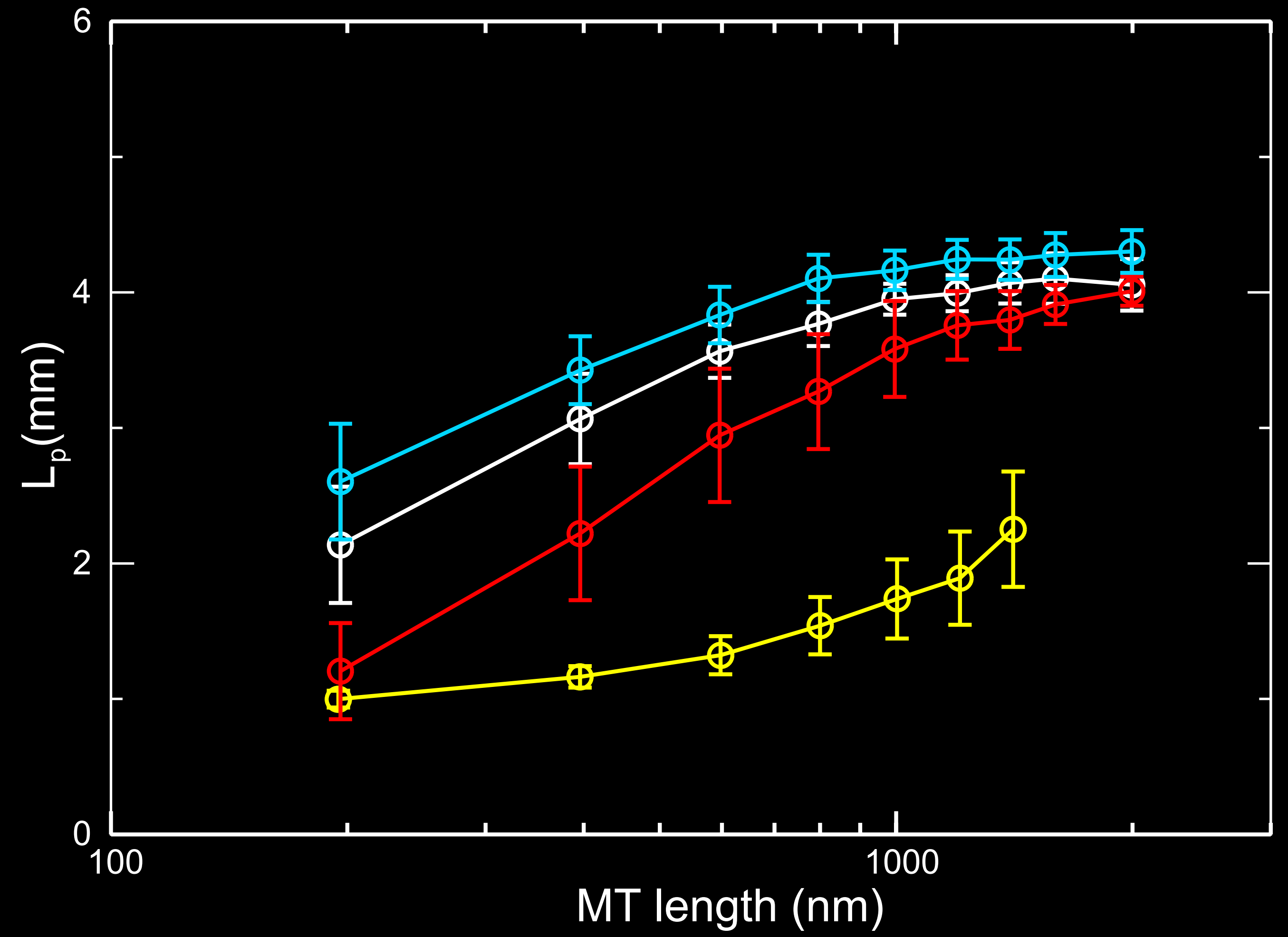


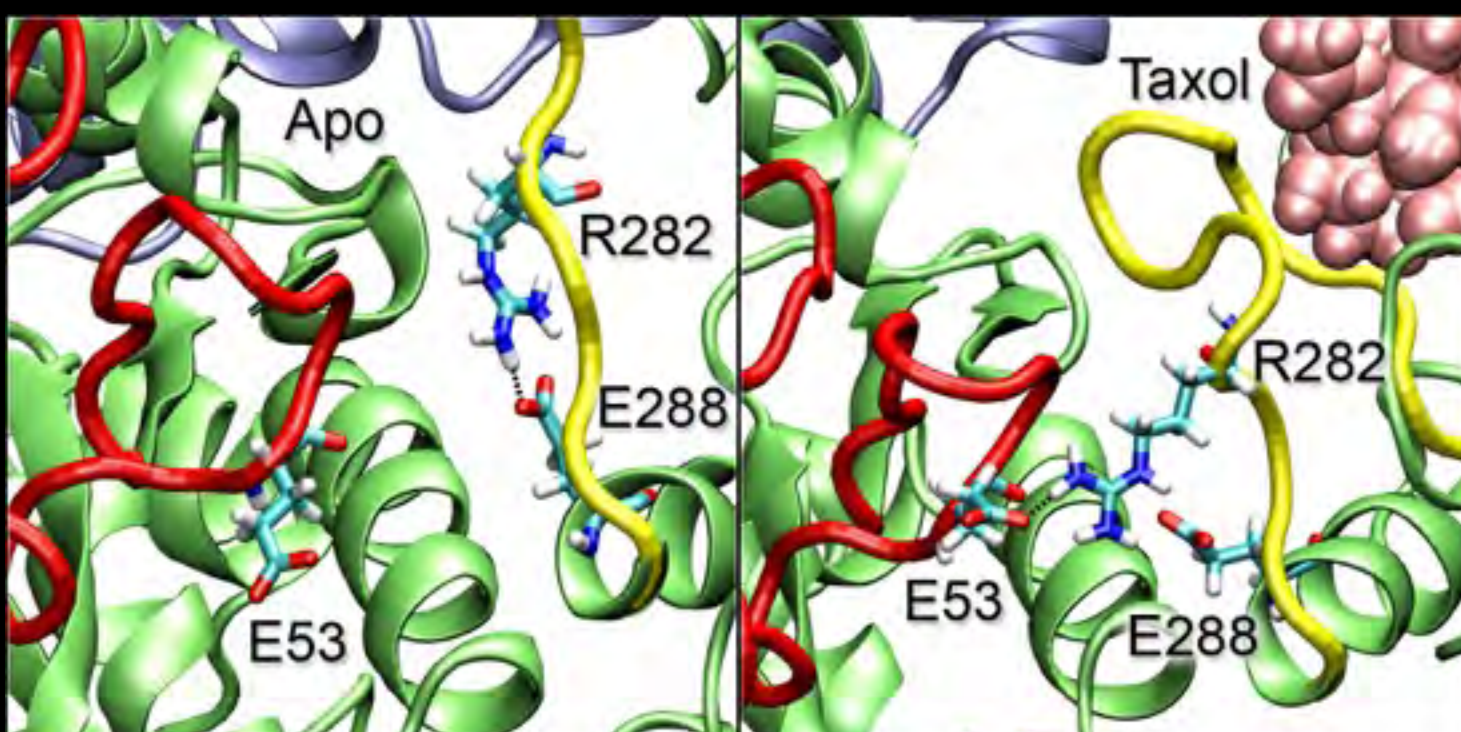
$$E = 250 \text{ MPa}$$

Tubulin Isoform Abundances

Source	β I	β II	β III	β IV
Bovine Brain	3%	58%	25%	13%
Breast Cancer	39.1%	0%	2.5%	58.4%
Ovarian Cancer	97%	0%	0%	3%
Lung Cancer	63.2%	1.5%	5%	30.3%

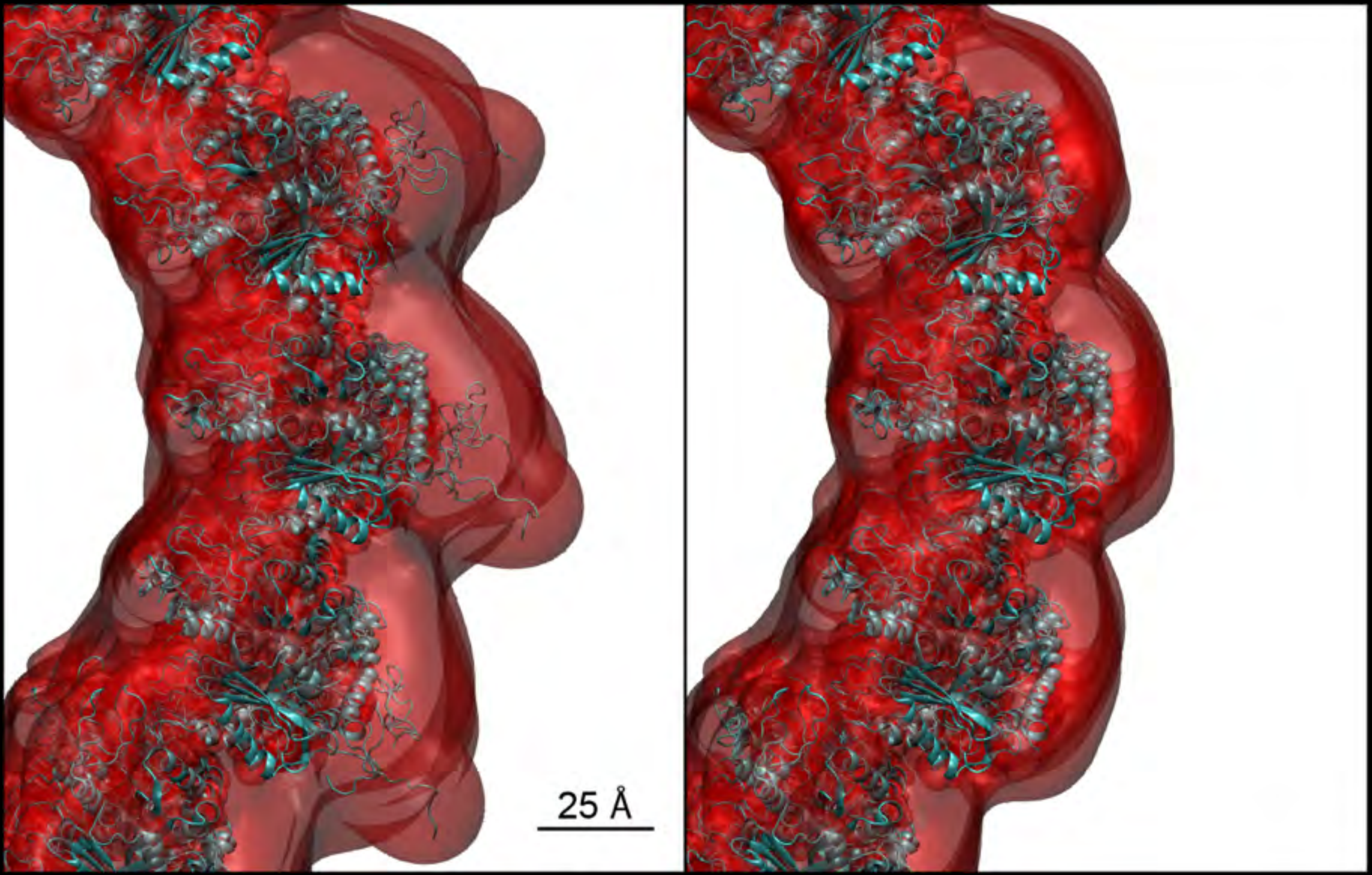
H1-S2 Loop Substitutions

- The various isoforms are largely conserved, but there are substitutions in a few keys regions, including the primary contact points between protofilaments



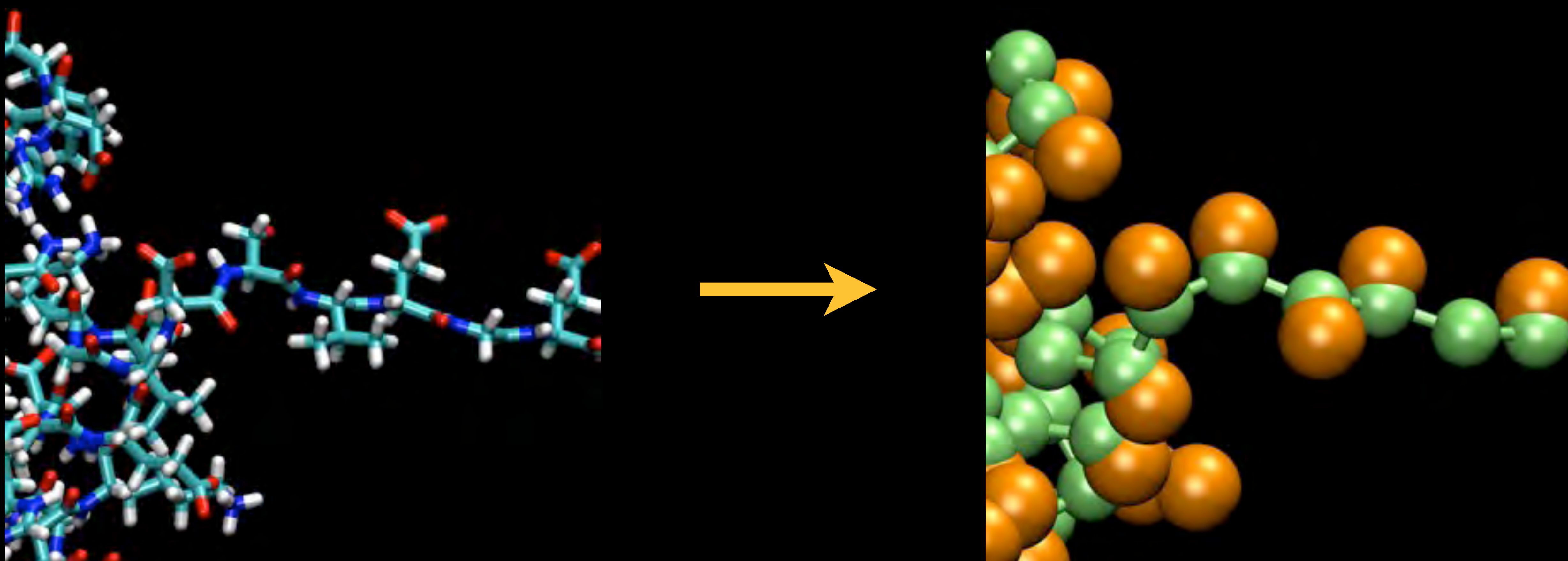
β1 G**T**Y**H**GDSDLQLDR
β2 G**S**Y**H**GDSDLQLER
β3 G**N**Y**V**GDSDLQLER
β4 G**T**Y**H**GDSDLQLER
. *******:*

Importance of the C-Termini



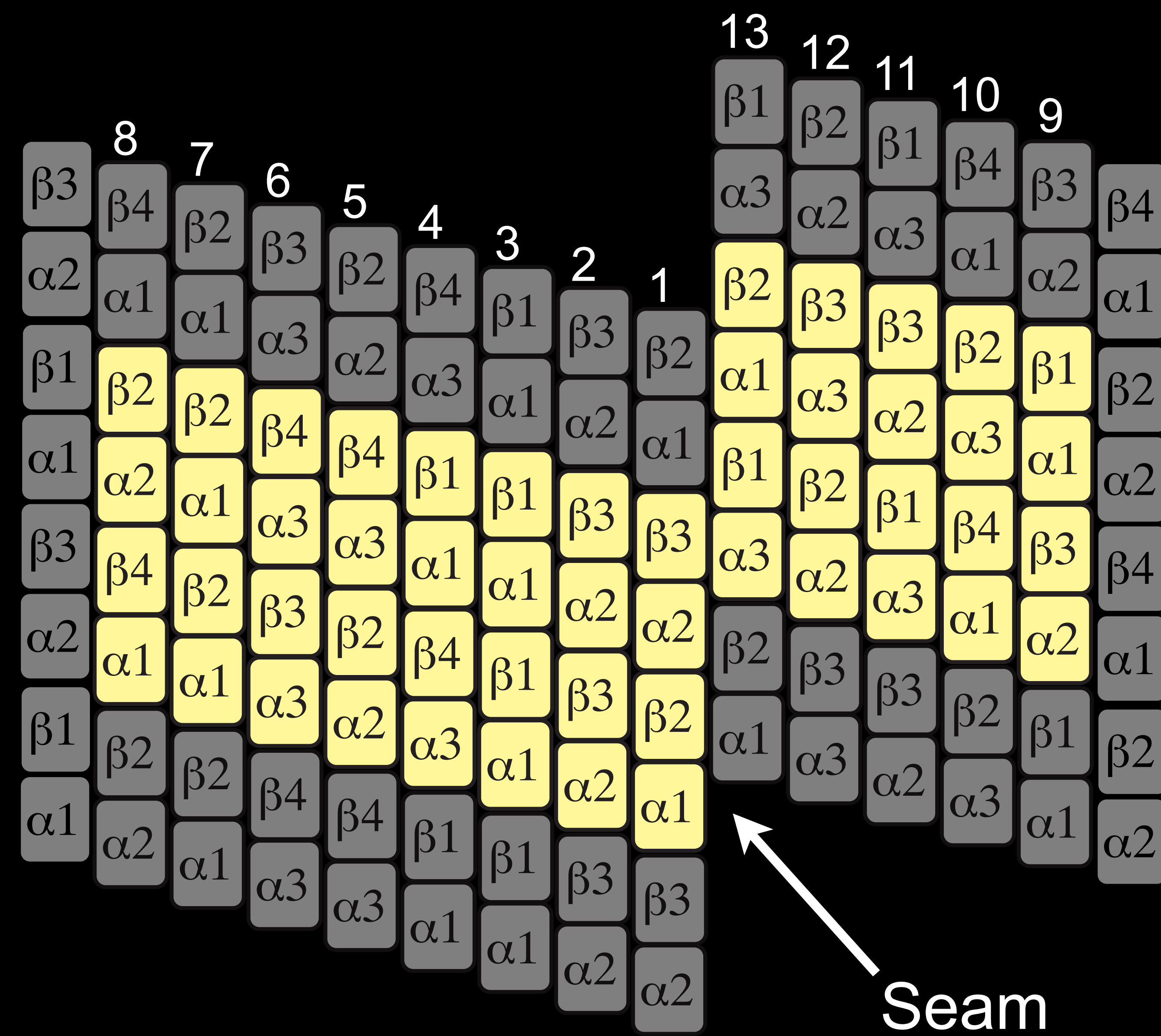
Coarse-Grained MD

- In order to capture atomic level effects, we use the MARTINI coarse graining procedure which reduces the number of atoms



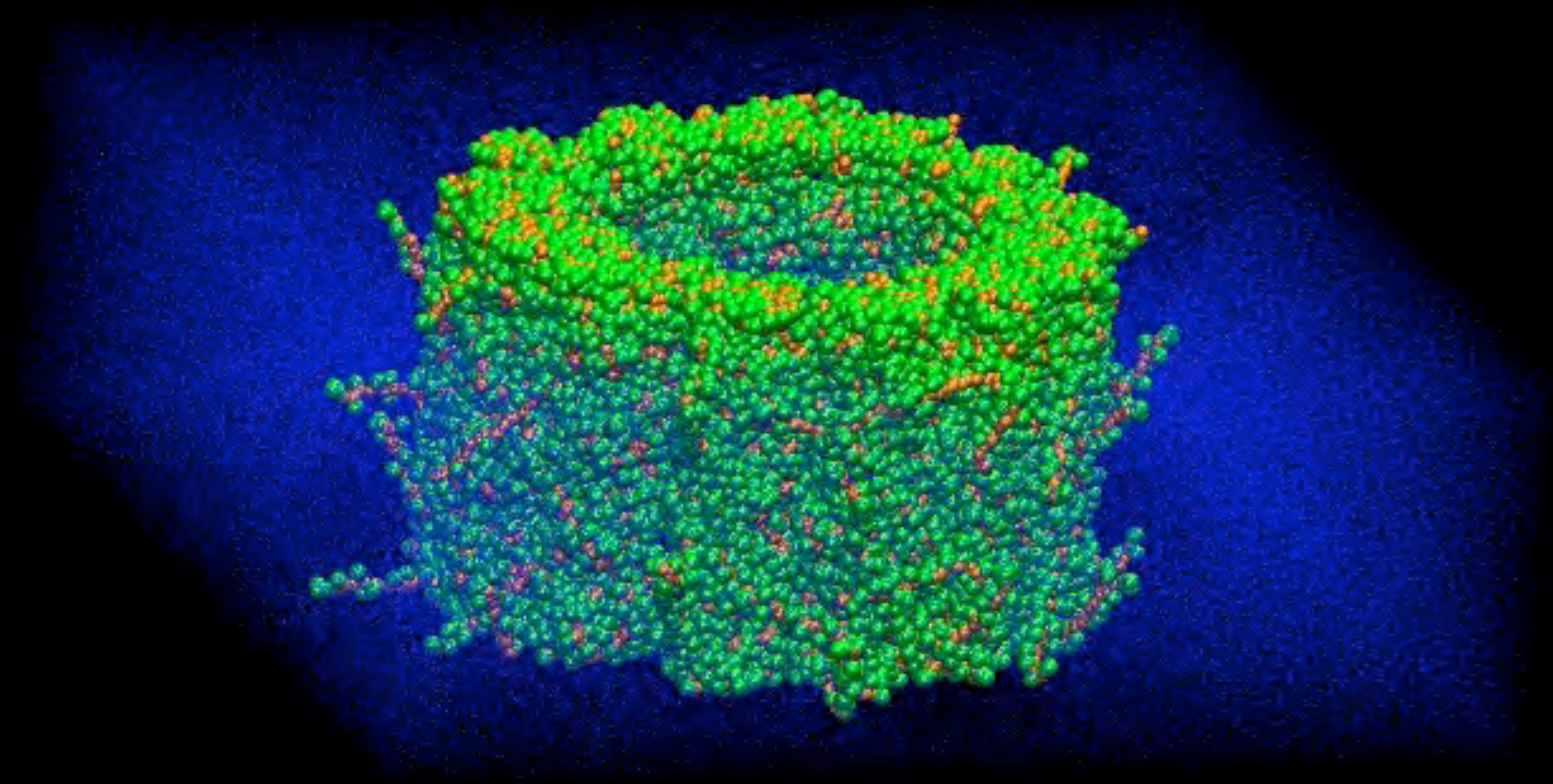
System Set-Up

- We want to make the system large enough to observe the dynamics and interactions of the tails, and see the interactions of the various isoforms



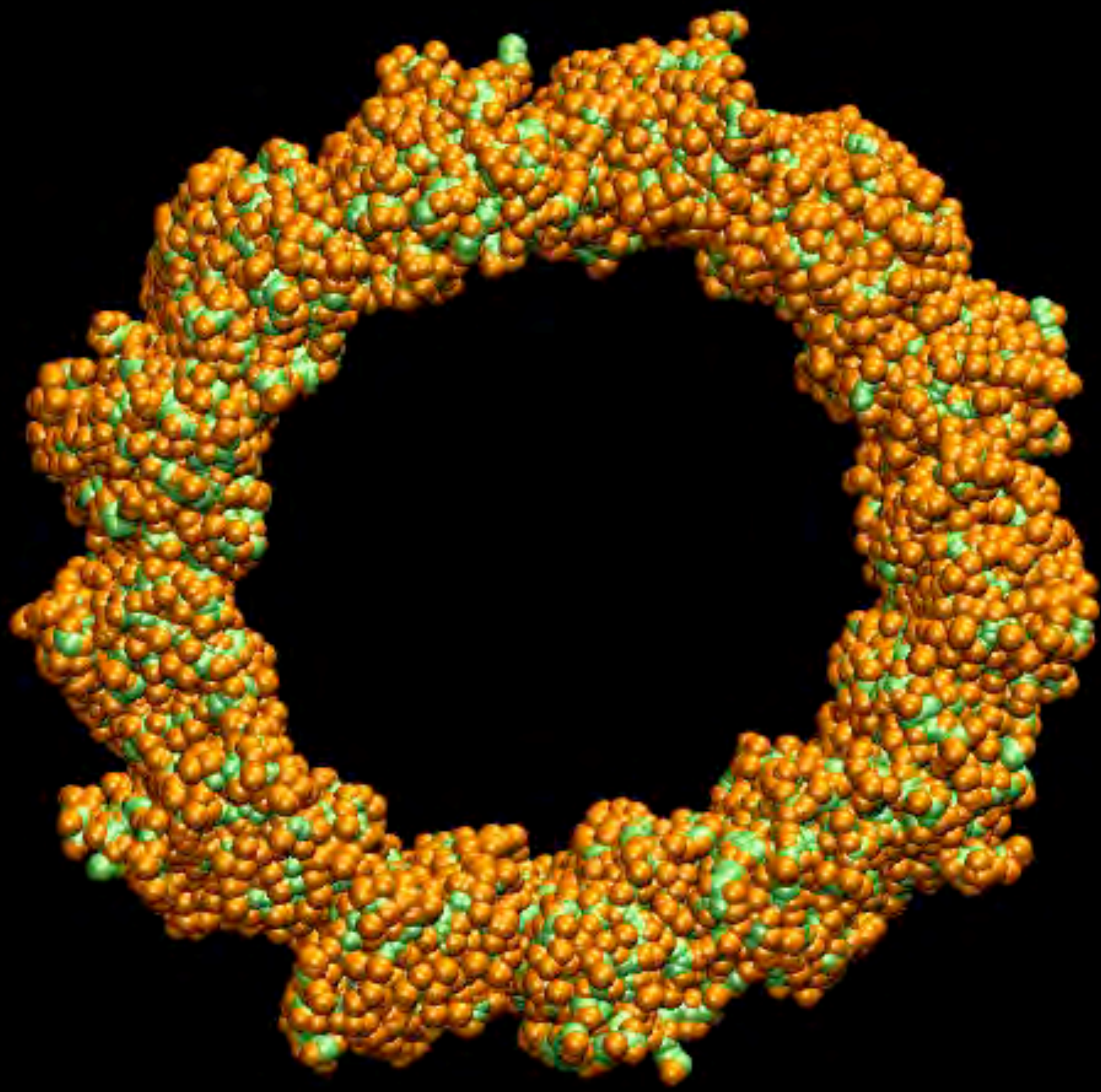
Periodicity = Infinity

- Because our system is periodic in the z-direction, it appears as an infinite polymer
- After adding water and ions, we are ready for simulation



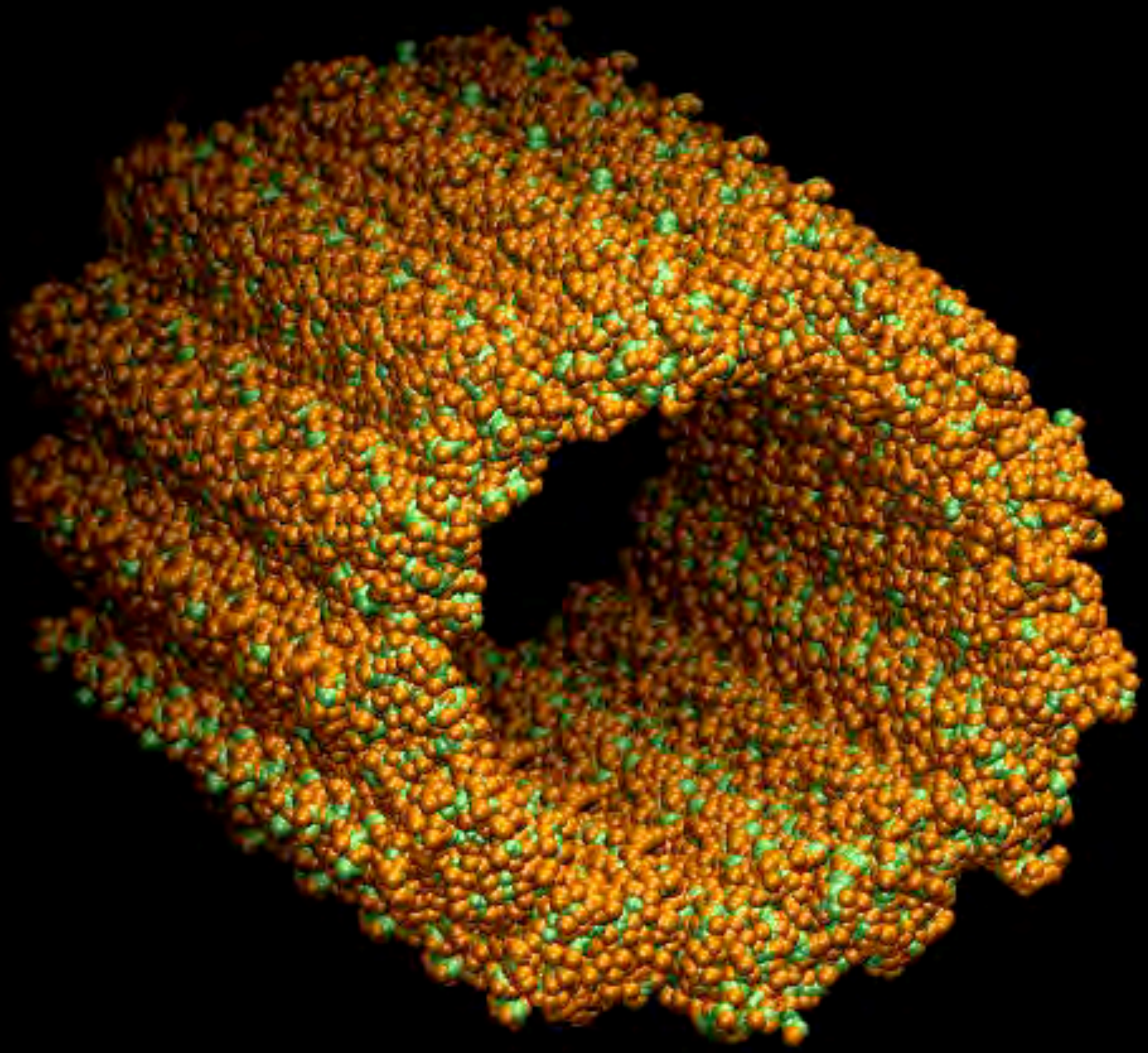
Coarse-Grained MD

- Using the MARTINI description, we can still access time scales in the μs range
- We can get a much better idea of radial compression and shear since we are using a quasi-atomic description



Dynamics to Mechanics

- Since our system is periodic along the MT length and that length is relatively short, we will need to ascertain quantities like E_x from looking at structural fluctuations



Summary

Taxol Increase Microtubule Flexibility

Counteracts the conformational change due to hydrolysis or phosphate loss

Anisotropic Effects Come From Mechanics and Structure

Each contribution still needs to be assessed

CG Models can be Parameterized from MD Simulations

Frank Gu, Hoon Sim - Michigan
Fred MacKintosh - Vrije Universiteit Amsterdam
Arpita Mitra - Corning Inc.
Tav Hawkins, Jenny Ross - UM Amherst

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Aspen Center for Physics

Mitra and Sept, Biophys J. 95 (2008) 3252
Sept and MacKintosh, Phys. Rev. Lett 104 (2010) 18101