2015 Summer School on Polymers in Biology
June 21 – July 3, 2015 at KIAS

Myung Chul Choi
Dept of Bio and Brain Engineering, KAIST

Nanoscale Self-Assemblies in Biological Molecules:

Structures and Interactions of Microtubules and Microtubule-Associated-Molecules

Korea Advanced Institute of Science and Technology (KAIST)

Dept of Bio and Brain Engineering at KAIST

Established in 2002

- With private donation of a Korean venture businessman Moon Soul Chung and counter-match from the Korean government.

Major Areas

- Biomedical Informatics
- Systems Biology
- Nano-Bio Technology
- Bioimaging
- Neural/Brain Engineering

Statistics

- 21 Faculty Members
- ~160 Graduate Students
- ~100 Undergraduate Students

MC Choi lab: Nanoscale Self-Assemblies in Biological Molecules

Model bio-membrane

• Mechanical Properties and Diffusivity Study • FCS


D Jeong


S Lee


H Cho

Microtubules and Tau

• Structures and Interaction Study using SAXS/SANS and cryo-EM


J Lee


J Lee

Optical trapping of liposomes

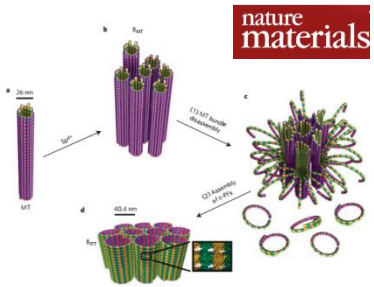
• Trapping energy and 2nd virial coefficient


Dr. S Park

Soft Matter Physics Simulation

• Interactions of Tau and Microtubules


Dr. B Kim

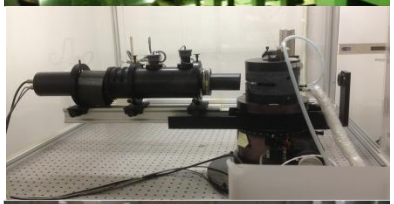


Facilities

- Optical Tweezer
- Fluorescence Correlation Spectroscopy (FCS)
- SAXS BL 4C in PAL
- 40M SANS in HANARO, KAERI
- TEM and Cryo-TEM at NanoFab Center
- Optical Microscopy
- Tau Purification System
- Langmuir Balance
- Laser Light Scattering System
- Pendant Drop Method

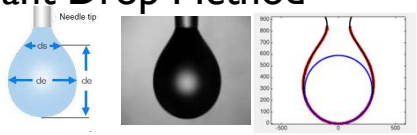
Synchrotron X-ray
at PAL

DLS

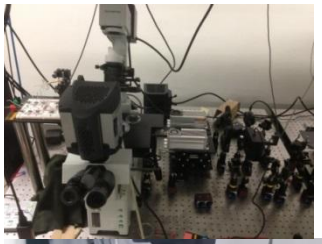


40M SANS
at HANARO, KAERI

Pendant Drop Method

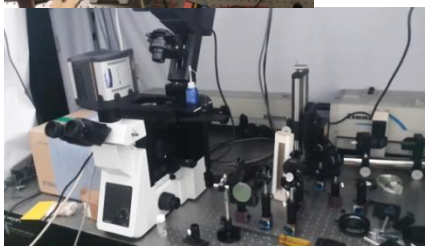


Cryo-TEM



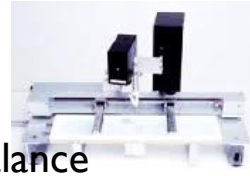
Optical Tweezer

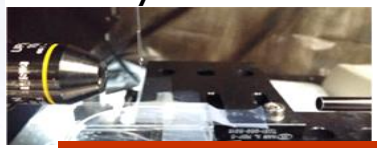
Tau purification system

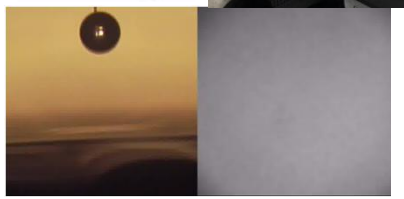


FCS system

FRAM system







Langmuir balance

Collaboration Groups

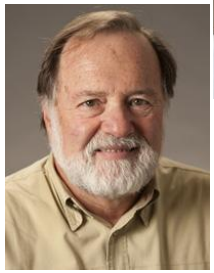
- Cyrus R. Safinya at Physics, Materials, MCDB of UCSB
- Stu C. Feinstein at MCDB and Neuroscience Center of UCSB
- Les Wilson at MCDB of UCSB
- Mahn Won Kim at Physics of KAIST/GIST
- Fyl Pincus at Physics of UCSB.
- Daniel Needleman at Harvard
- Uri Raviv at Hebrew University, Israel
- Daniel Ou-Yang at Lehigh University
- Changbong Hyeon at KIAS.

Mahn Won Kim

Cyrus Safinya

Stu Feinstein

Les Wilson



Fyl Pincus

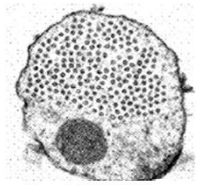
Daniel Ou-Yang

Changbong Hyeon

Nanoscale Self-Assemblies in Biological Molecules:

Structures and Interactions of Microtubules and Microtubule-Associated-Molecules

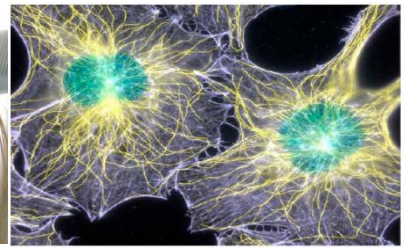
Length Scale



Axon

Squid giant axon ~800 μm

Mammalian axon ~2 μm



10-40μm

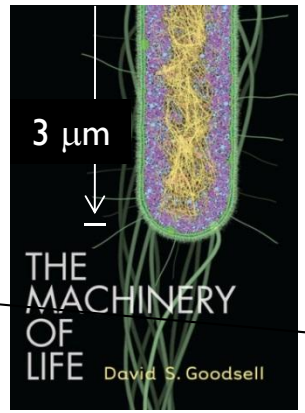
Red Blood Cell

8 μm

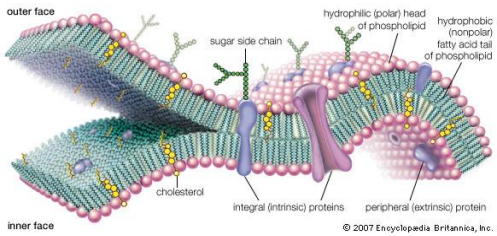
8nm

35nm

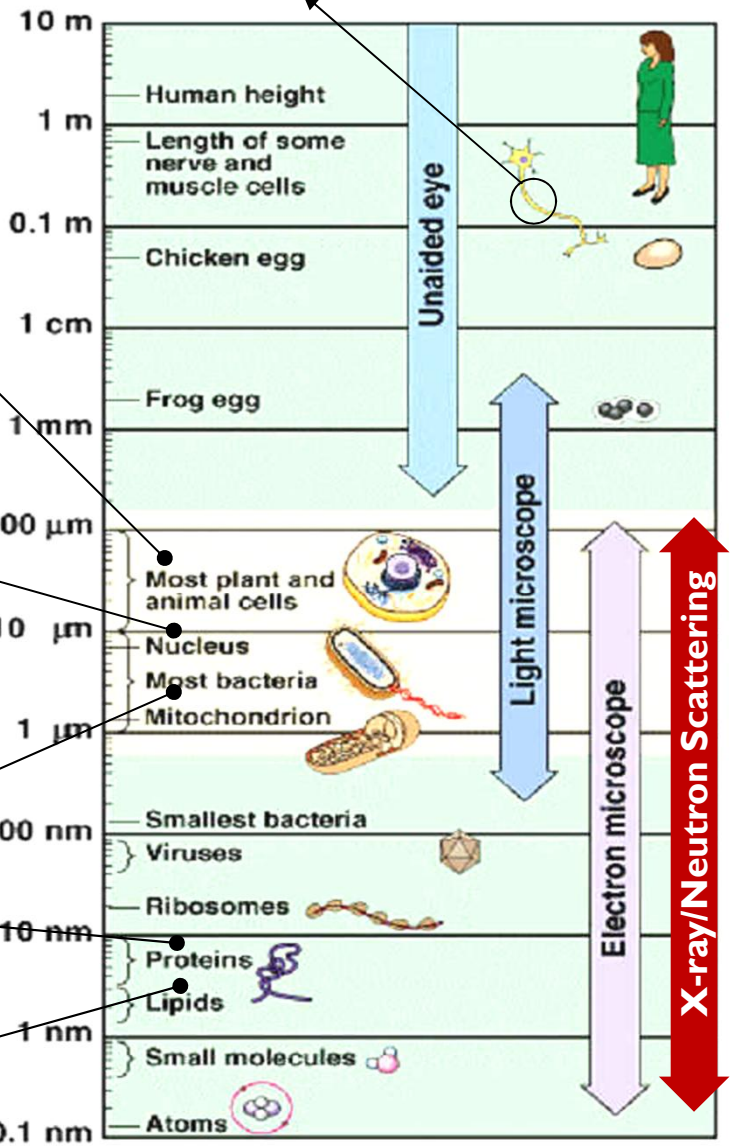
1 μm



E. coli

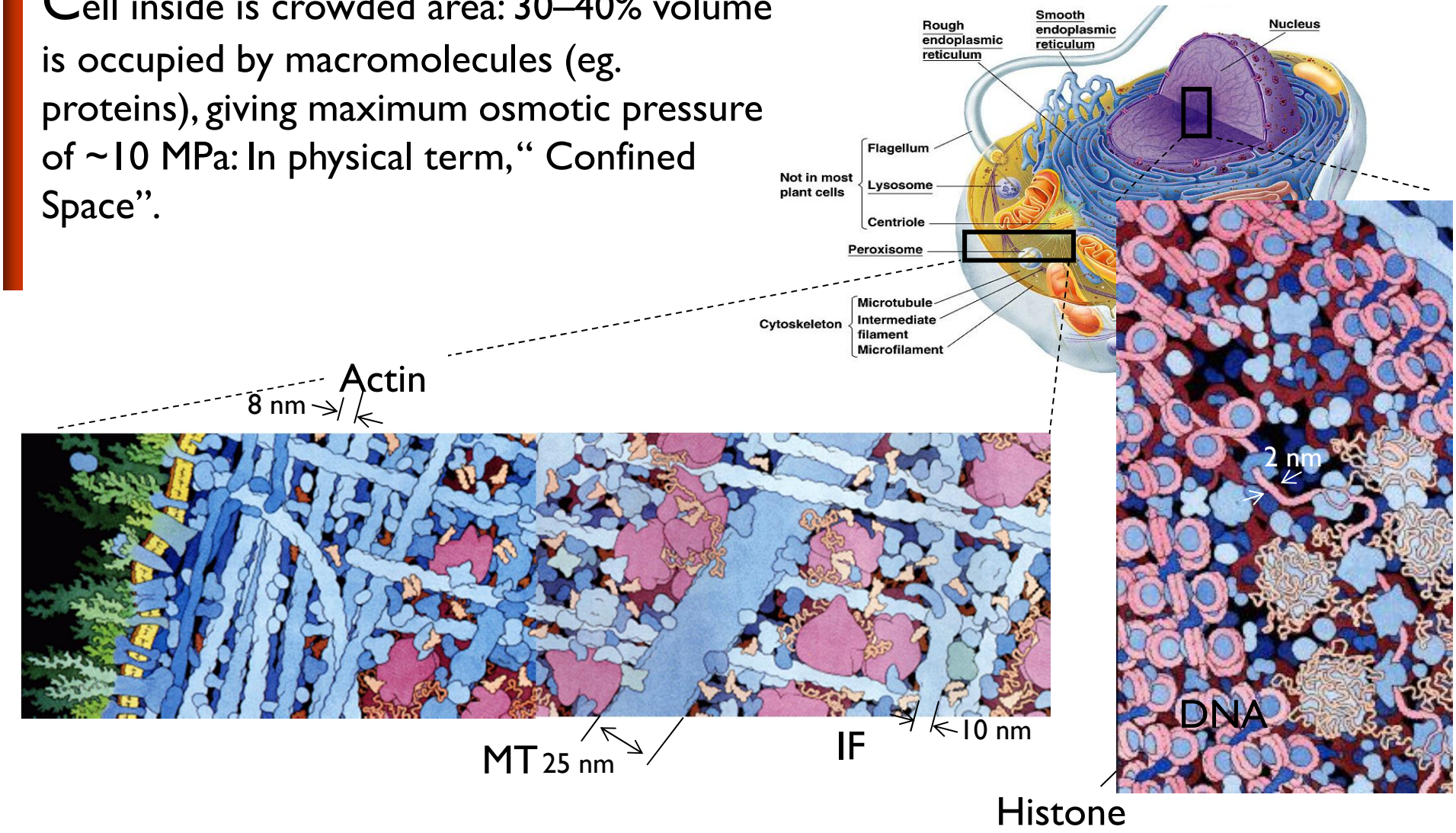


Lipid membrane



Inside Picture of Cell: Molecular Crowding

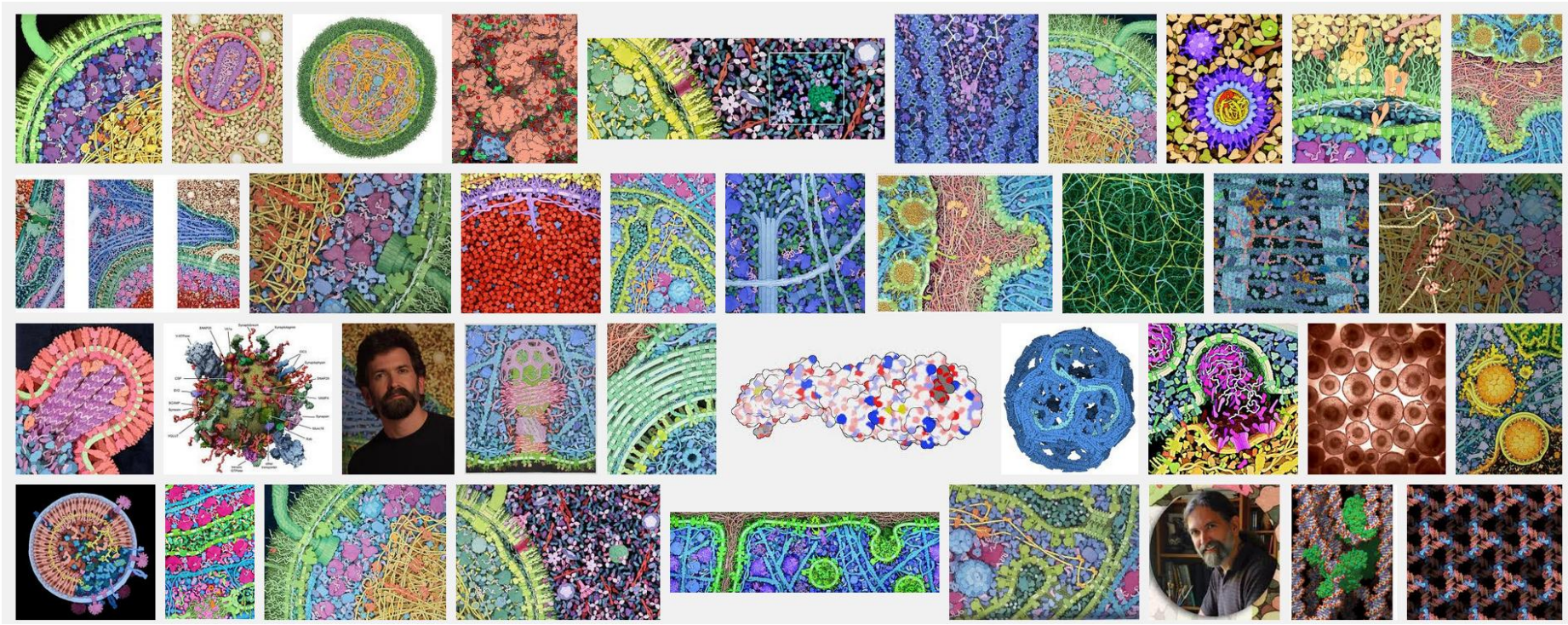
Cell inside is crowded area: 30–40% volume is occupied by macromolecules (eg. proteins), giving maximum osmotic pressure of ~10 MPa: In physical term, " Confined Space".



R. J. Ellis, Curr. Opn. Struct. Biol. (2001); Fulton Cell (1982); Cell movements: from molecules to motility, D. Bray, 2001; The machinery of life, D. Goodsell

Molecular Art

David S. Goodsell in the Scripps Research Institute

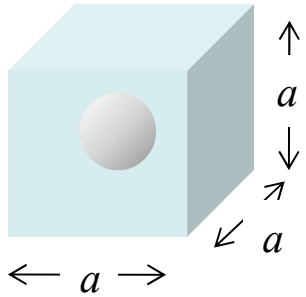


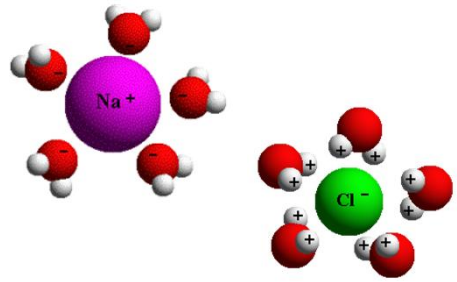
http://mgl.scripps.edu/people/goodsell

Salt Concentration Inside Cell

Monovalent salt concentration inside cell is ~0.1 M, which means 10^{20} ions in cubic cm, which corresponds to one ion in _ nm × _ nm × _ nm box.

a = 2.5 nm



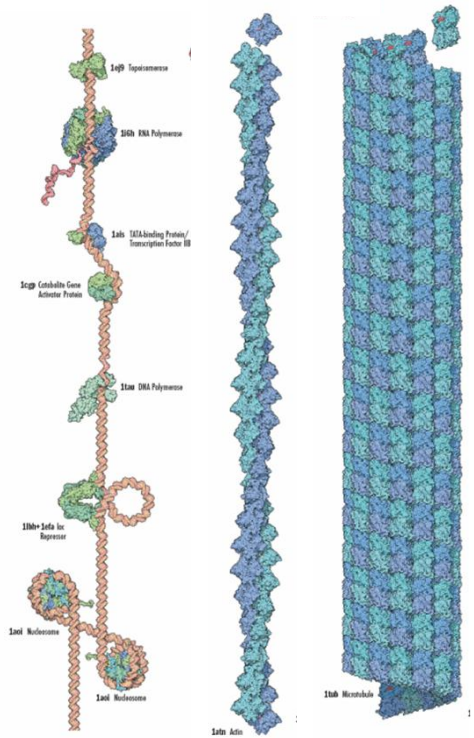


Four Major Filamentous Elements: DNA, MTs, Actin, IFs

All negatively charged with the charge density σ_{MT} (~1 e/nm^2) ≈ σ_{DNA} > σ_{Actin}

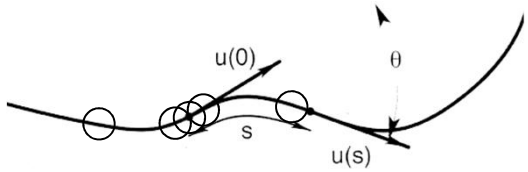
Persistence length is

	Diameter	Persistence length
MTs	25 nm	~ mm
IF	10 nm	0.3 – 1 μm
Actin	8 nm	4 – 10 μm
DNA	2 nm	50 nm



DNA Actin MT

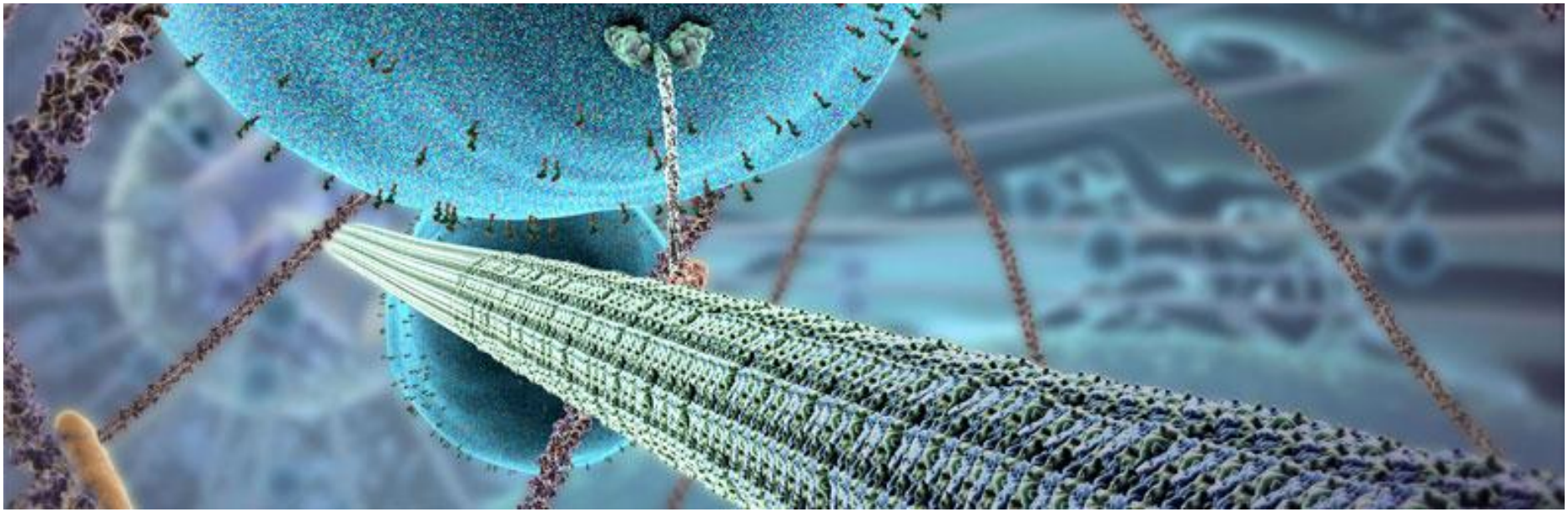
- The persistence length is a basic mechanical property quantifying the stiffness of a polymer. L_P is defined as the length over which correlations in the direction of the tangent are lost.



$$< C(s) >=< \vec{u}(0) \cdot \vec{u}(s) >$$
$$=< \cos[\theta(s) - \theta(0)] >$$
$$\lim_{s\to\infty} < \vec{u}(0) \cdot \vec{u}(s) > \approx \exp(-s / 2L_p)$$

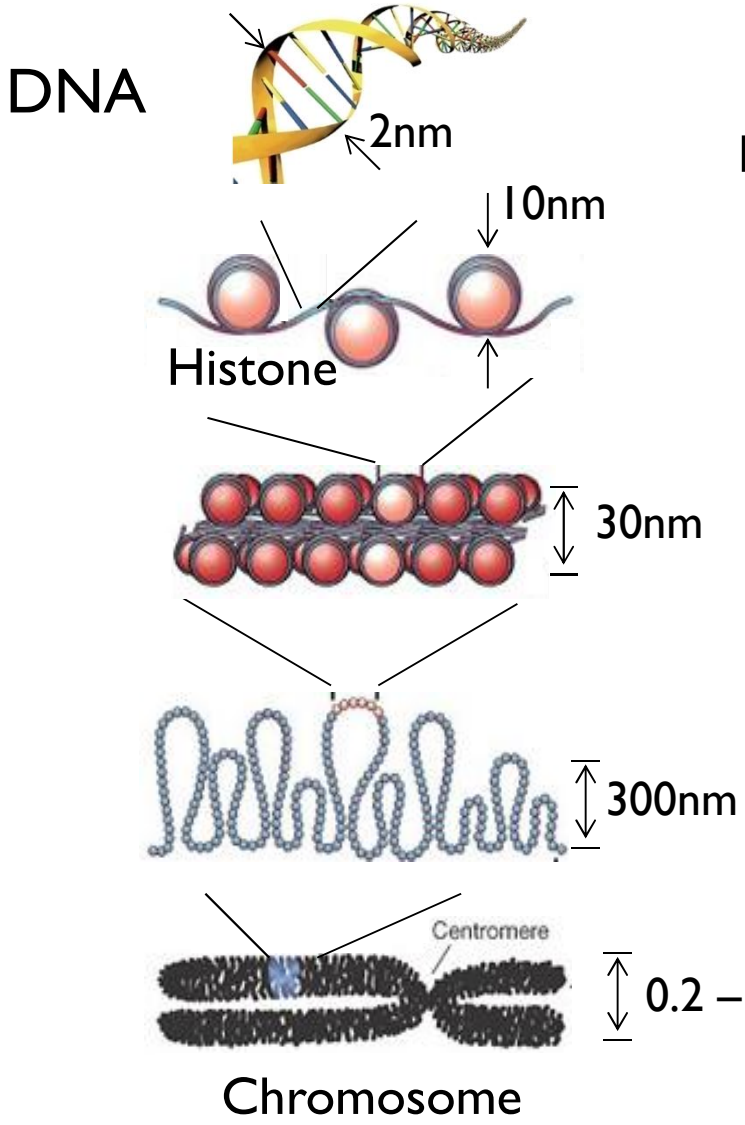
The machinery of life, D. Goodsell

Inner Life of a Cell

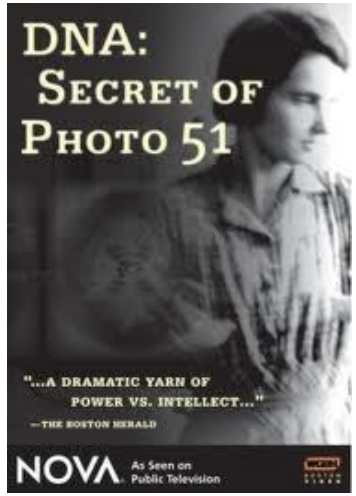


Siggraph Award Winning Animation: http://multimedia.mcb.harvard.edu/media.html

Hierarchical Assembly: DNA



DNA: Secret of Photo 51 (NOVA)
You Tube -55 min



1962

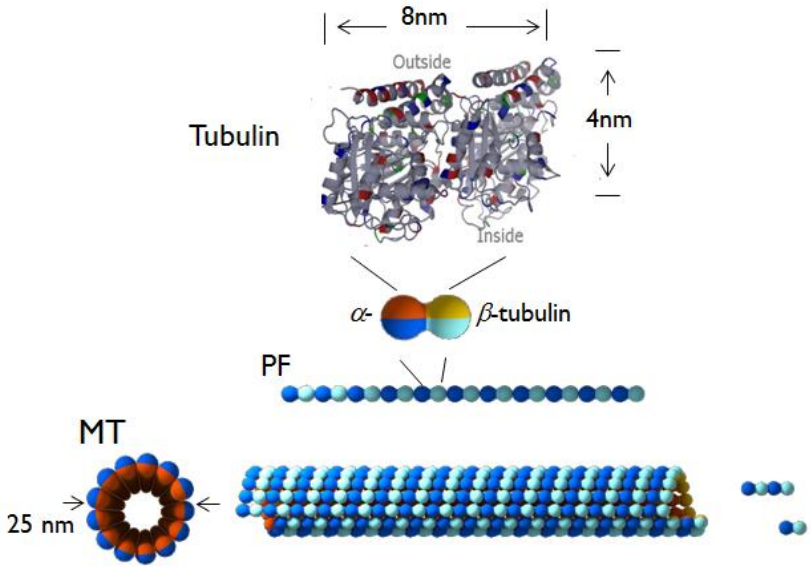
H. Lodish, "Molecular Cell Biology"; raven.zoology.washington.edu

Hierarchical Assembly: Microtubule (MTs)

MTs are 25nm protein nanotube, comprised of globular dimeric $\alpha\beta$ tubulin subunits aligned end-to-end to form linear protofilaments, which interact laterally to form the hollow cylinder.

Charge density of tubulin dimer σ = -0.8 e/nm^2. Most of charge is located on the C-terminal tails of both α- and β- tubulin (the average number of residues N_{CTT} = 19.5).

The electron density of tubulin relative to water $\Delta\rho_{tubulin}$ = 0.07817 e/Å. (ρ_{water} = 0.333 electron/Å^3)



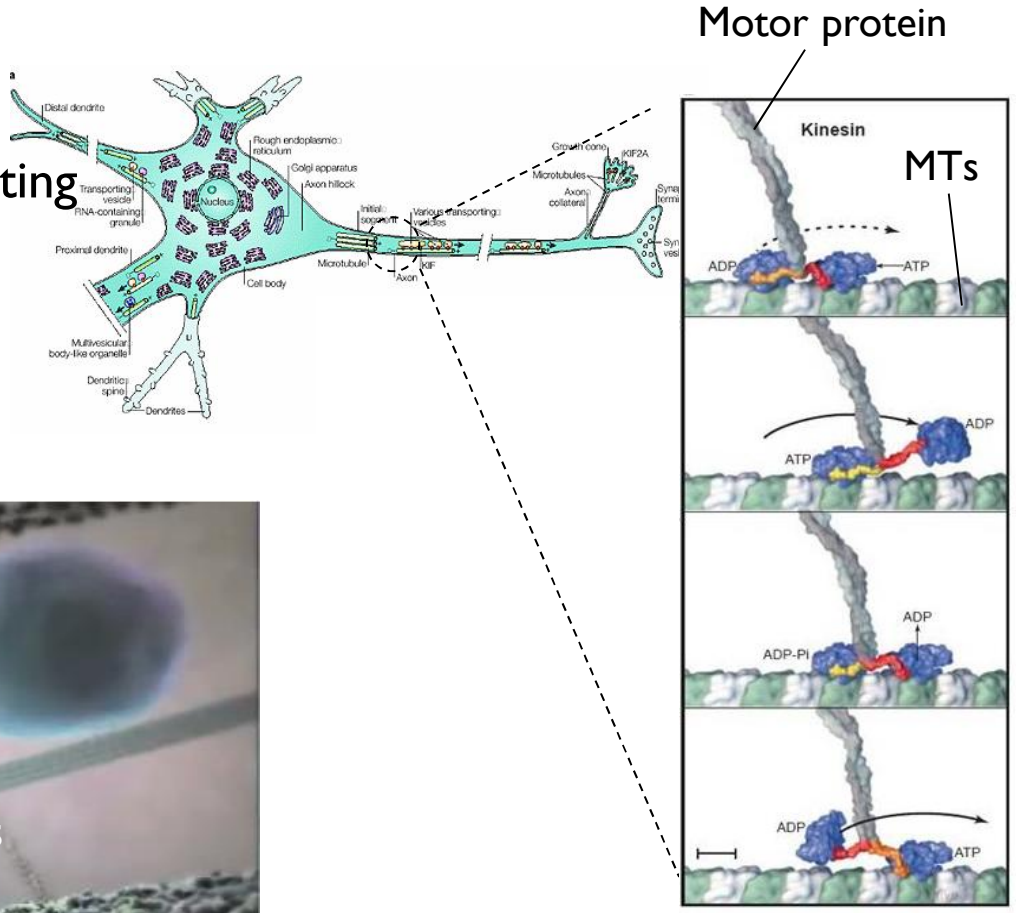
H. Lodish, "Molecular Cell Biology"; raven.zoology.washington.edu

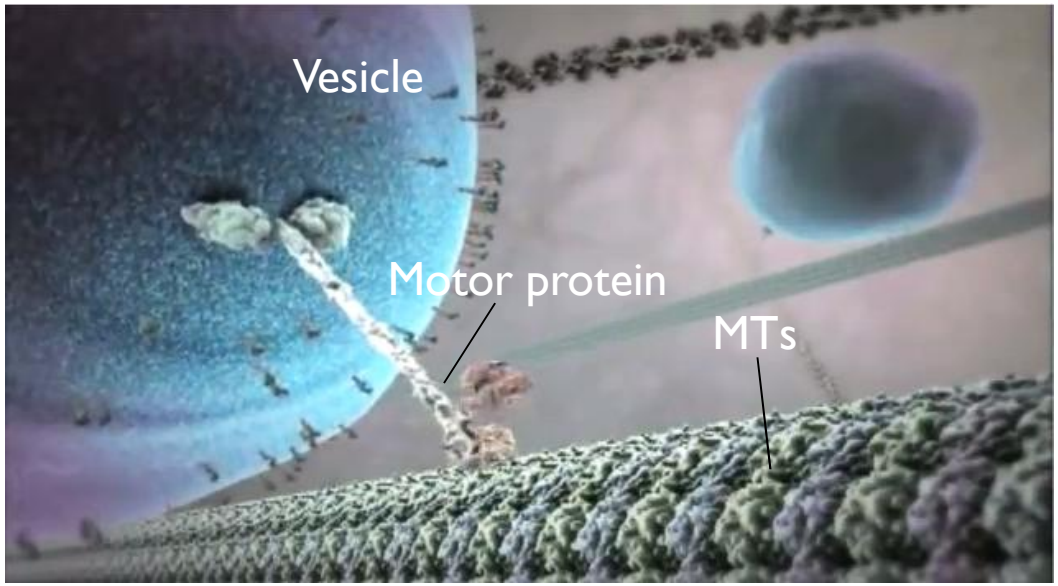
Microtubules

- involved in (i)Cell division, (ii)Intracellular trafficking, and (iii)Maintaining cell shape

Intracellular Trafficking

: Highway for motor protein transporting cellular component





N. Hirokawa (2010) Neuron; Alberts et al Molecular Biology of Cell

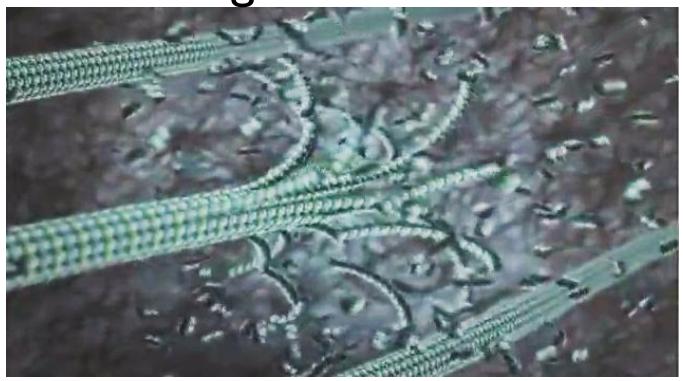
Microtubules

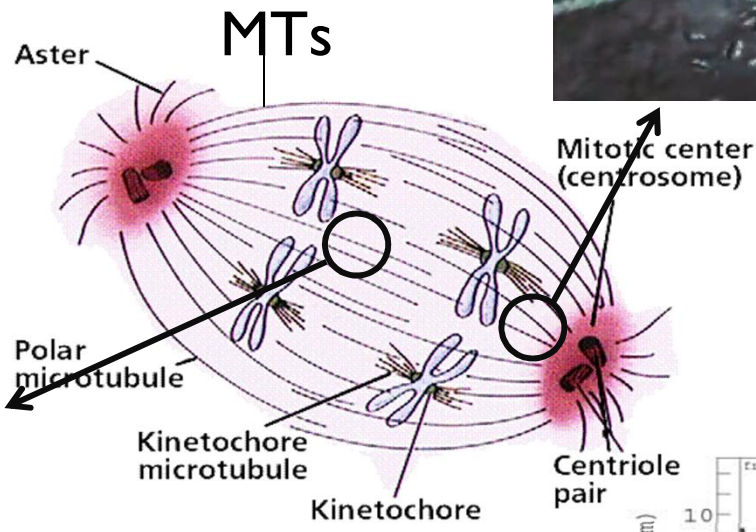
- involved in (i)Cell division, (ii)Intracellular trafficking, and (iii)Maintaining cell shape

Cell Division

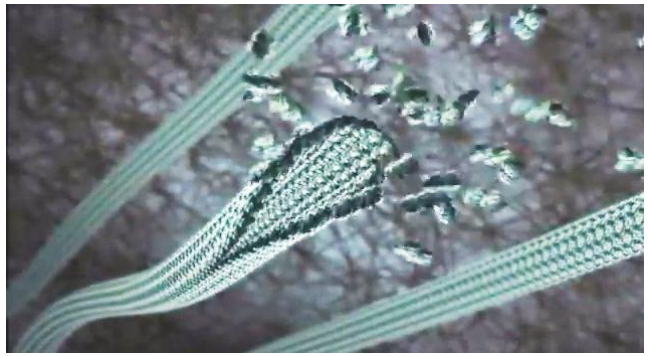
: Search/capture/align and segregate the duplicated chromosomes.

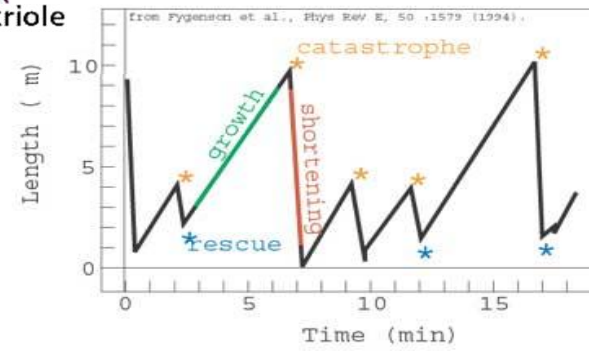
shortening





growth



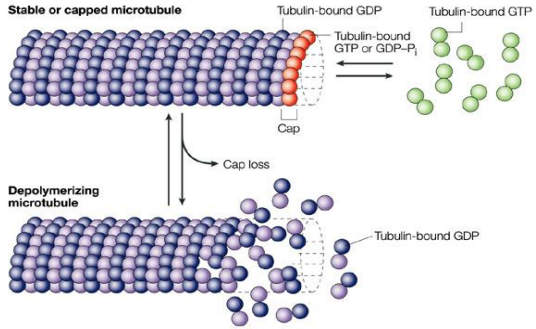


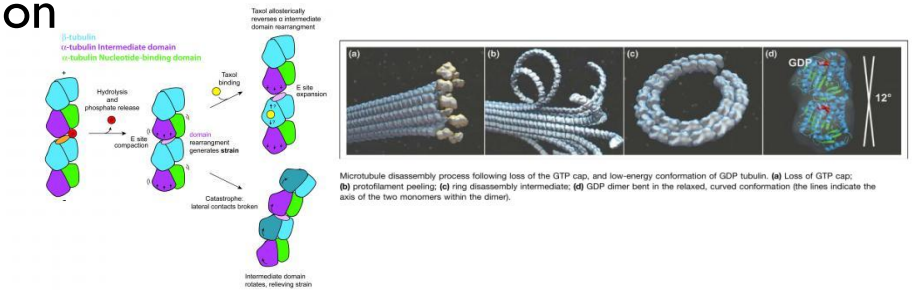
Inner life of cell ; Alberts et al Molecular Biology of Cell; D. Fygenson et al., Phys Rev E (1994)

Dynamic Instability of MTs

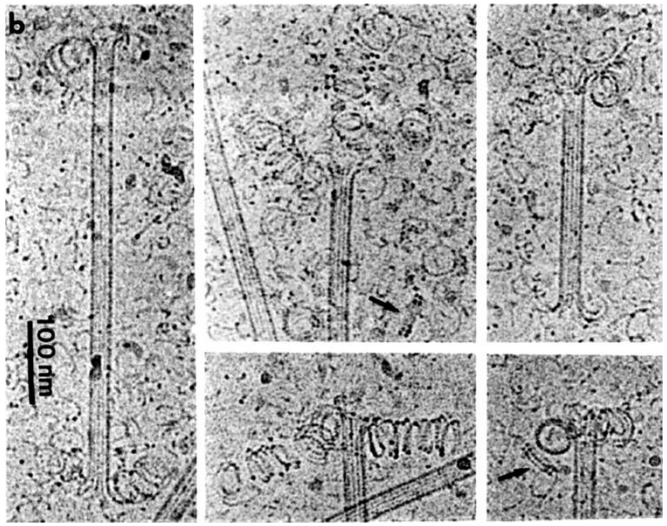
Tubulin-bound GTP is hydrolysed to tubulin–GDP and phosphate P_i. GTP hydrolysis and the release of phosphate induces conformational changes in the tubulin that destabilize MTs, resulting in depolymerization of MTs.

Ultimately, the P_i dissociates from the microtubule, leaving MT core consisting of tubulin with stoichiometrically bound GDP. MT end containing tubulin-bound GTP or GDP–P_i is stable, or 'capped', against depolymerization. Hydrolysis of tubulin-bound GTP and the subsequent release of P_i induces conformational changes in the tubulin molecules that destabilize the MT, resulting in catastrophe and shortening of MT.





Microtubule disassembly process following loss of the GTP cap, and low-energy conformation of GDP tubulin. **(a)** Loss of GTP cap; **(b)** protofilament peeling; **(c)** ring disassembly intermediate; **(d)** GDP dimer bent in the relaxed, curved conformation (the lines indicate the axis of the two monomers within the dimer).

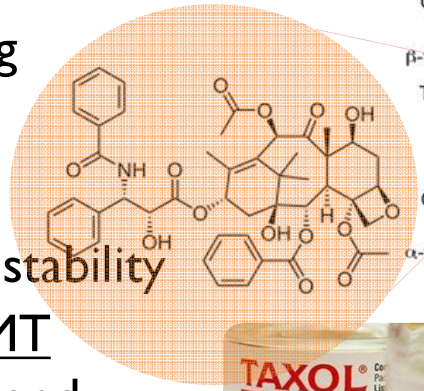


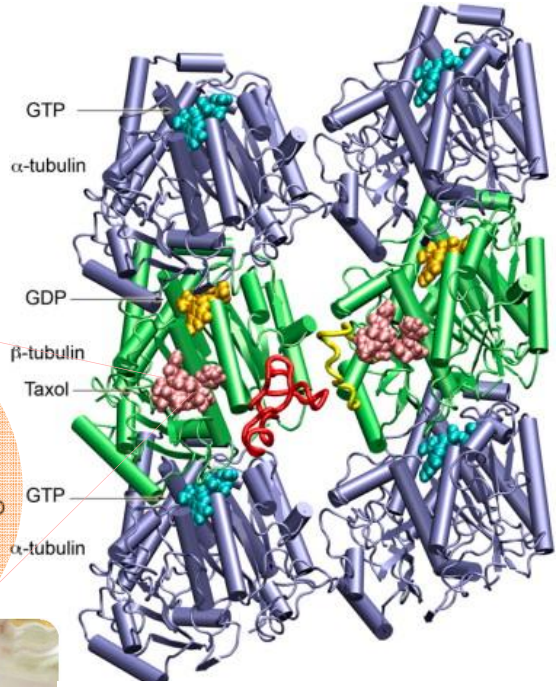
E. Nogales, Current Opinion in Cell Biology 18 179 (2006)

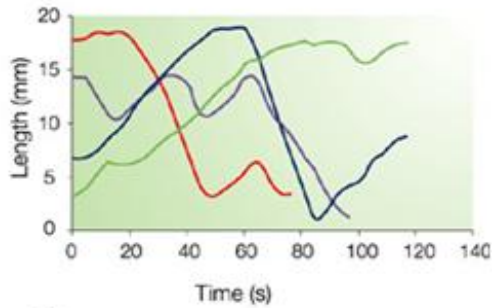
Taxol, Anticancer drug

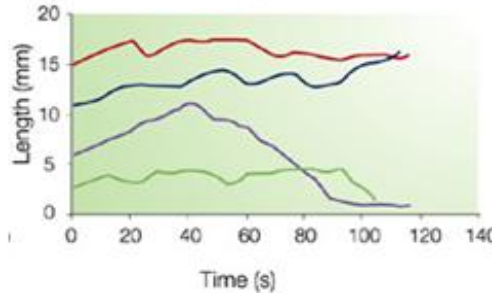
Taxol is a cancer chemotherapeutic agent used for treatment of many types of cancer, including ovary, breast, and lung carcinomas.

Major taxol effects on MT dynamic instability are to reduce the rate and extent of MT shortening. This inhibits the assembly and functioning of the mitotic spindle, thereby preventing or slowing cell cycle progression at the metaphase/anaphase and eventually inducing cell death.





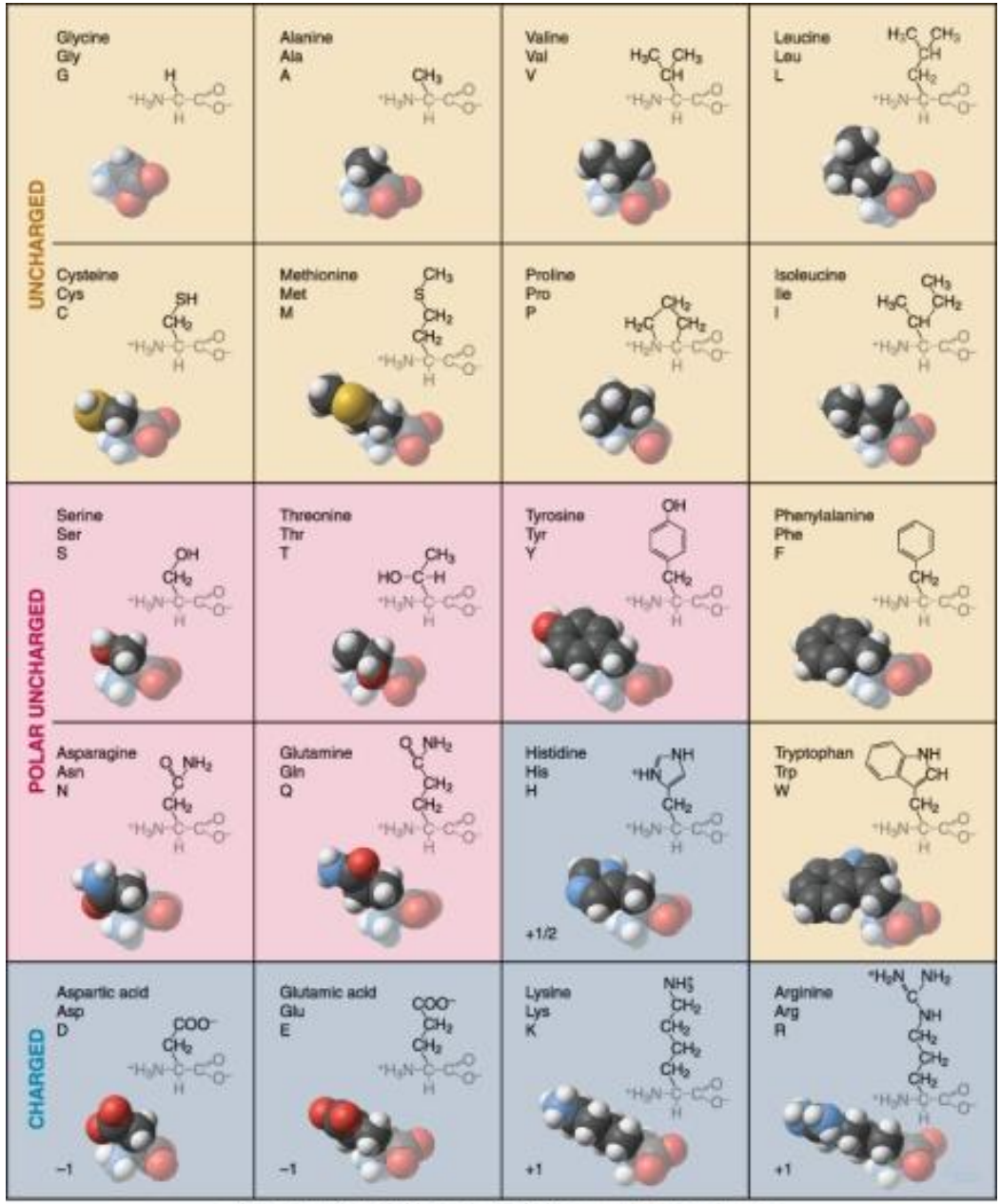




A. Mitra, et al, Biophys J 95 3252 (2008)

20 amino Acids

- Hydrophobic or hydrophilic
- Charged (+/-) or uncharged



Protein Structure

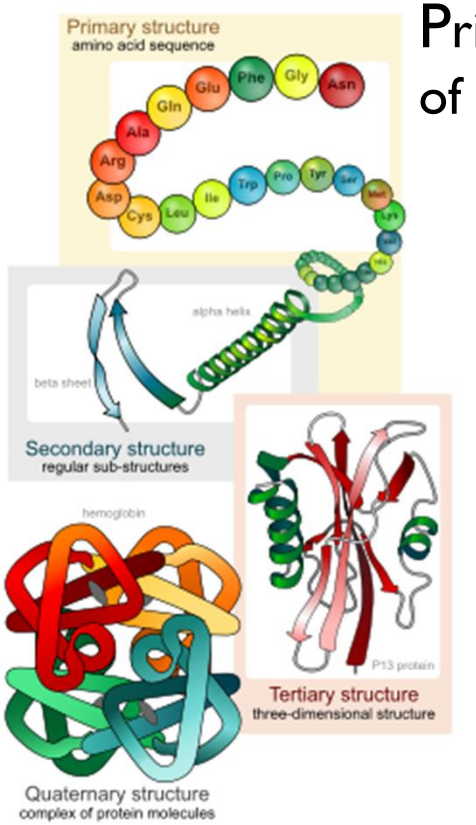
- The strengths of hydrogen bonds lie between 1 – 20 k_BT, which is stronger than van der Waals bond ~ 0.4 k_BT, but still weaker than covalent bonds ~ 200 k_BT.

F—H...:F (63 k_BT)
O—H...:N (12 k_BT)
O—H...:O (9 k_BT)
N—H...:N (5 k_BT)
N—H...:O (3 k_BT)

Primary Structure is the sequence of a chain of amino acids.

C–C (102 k_BT)
C=C (244 k_BT)
C≡C (333 k_BT)
C–H (165 k_BT)
O=O (199 k_BT)
F–F (62 k_BT)

Secondary Structure occurs when the sequence of amino acids are linked by hydrogen bonds



Tertiary Structure occurs when certain attractions are present between alpha helices and pleated sheets

Quaternary Structure is a protein consisting of more than one amino acid chain

Energy k_BT

k_BT is the fundamental unit of energy at the molecular scale. Let's try to get a sense for how much energy this really is:

$$1\ k_BT = 0.6\ \text{kcal/mole} = 4\ \text{pN}\cdot\text{nm}\quad \text{(at RT)}$$

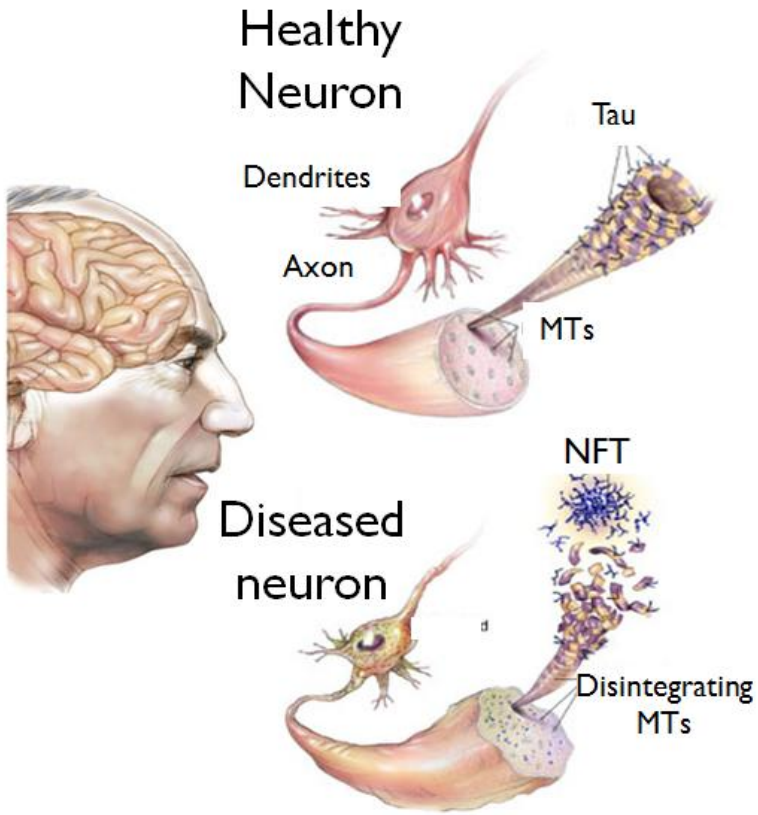
Tau, a Hallmark of Alzheimer's Disease

- Tau regulate tubulin assembly, MT dynamics and stability.

Malfunctions of tau cause neuronal cell death and dementia (e.g. Alzheimer's and Parkinsons' diseases).

In Alzheimer's disease, tau is phosphorylated and detached from MTs, aggregating into NFT, consequently, MTs disintegrate, collapsing neuron's transport system.

This result in malfunctions in communication between neurons and later the death of the cells.



NIH progress report on Alzheimer's disease 2004-2005

Microtubule-associated-protein (MAP) tau

Unfolded protein: $N_{amino\ acid}$= 352 – 441

Polyampholyte (+/-)

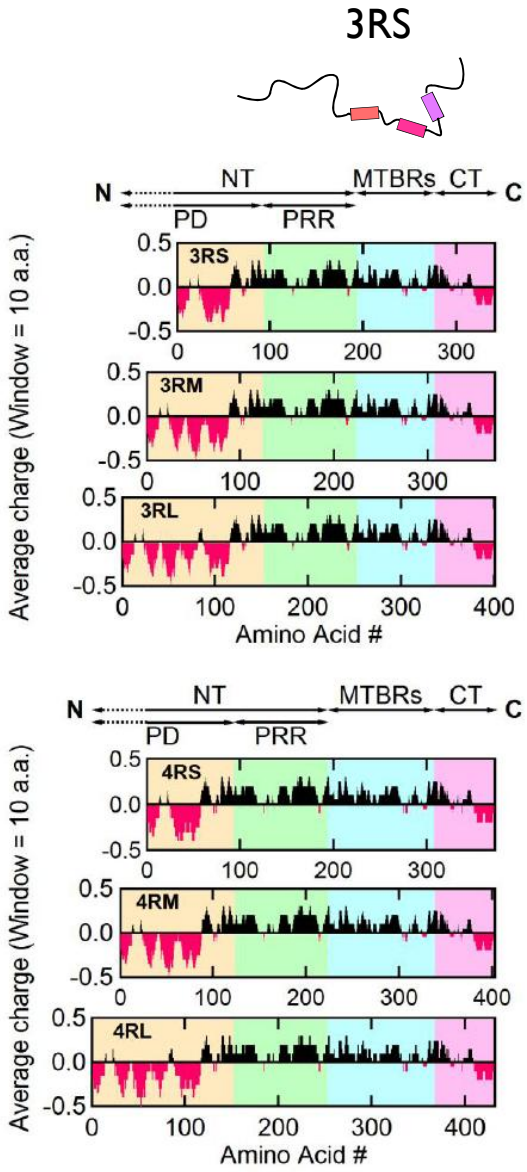
Six isoforms in central nervous system (CNS)

Net charge of tau: $\Sigma\sigma$ = 5 – 20

Average Mass density = 1.41 g/cc

Electron density ρ_{tau} = 0.462 e/Å

Tau	$N_{amino\ acid}$	Σ charge total
3RS	352	16.0
3RM	381	8.0
3RL	410	5.5
4RS	383	19.5
4RM	412	11.5
4RL	441	9.0



- The radius of gyration $R_g = 1.927 \cdot N^{0.6}$ = 41.2 Å.
- $\Delta\rho_{tubulin}$ = 0.07817 electron/Å^3
- $\Delta\rho_{tau}$ = 0.0009 electron/Å^3

Alzheimer's Disease (AD): the most Common Dementia

AD is an age-related and irreversible brain disorder that develops over a period of years.

People experience memory loss, behavior and personality changes, a decline in other cognitive abilities (such as thinking, decision making, and language skills).

Currently 29.4 millions of people in worldwide have the disease. By 2050, 120 million will have the disease if current population trends continue and no preventive treatments become available.

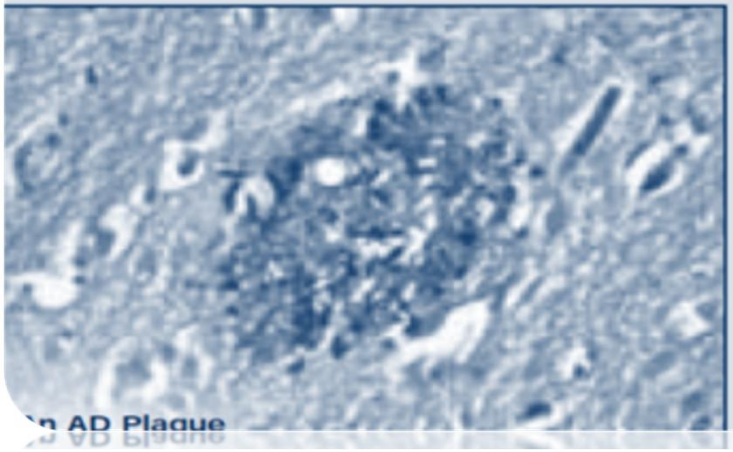
http://en.wikinoticia.com; NIH progress report on Alzheimer's disease

Hallmark of Alzheimer's Disease

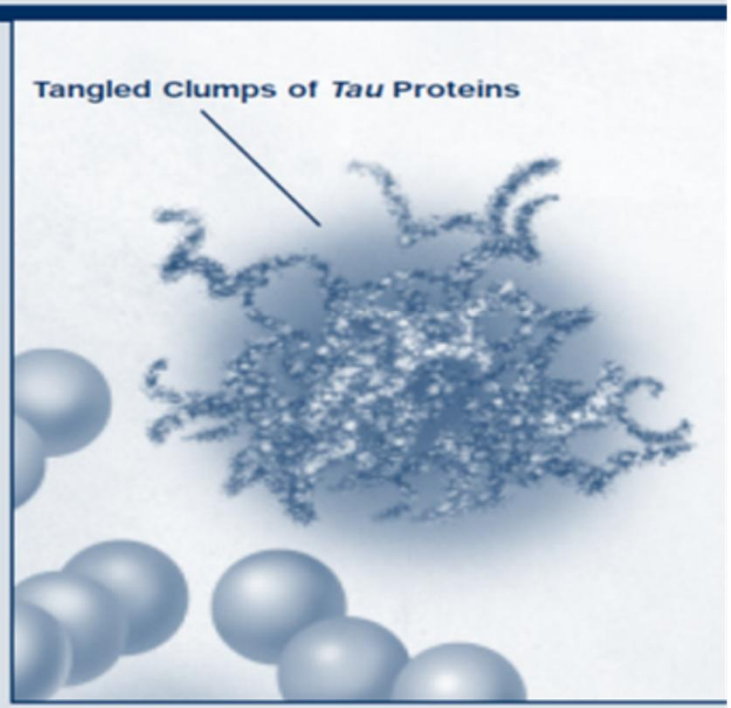
Beta-amyloid and Neurofibrillary Tangles: the Hallmarks of AD

The brains of people with AD have an abundance of two abnormal structures – beta-amyloid plaques and neurofibrillary tangles. This is especially true in certain regions of the brain that are important in memory.

- Plaques are dense, mostly insoluble deposits of protein and cellular material outside and around the neurons. They are made partly of a protein called beta-amyloid, which is a fragment snipped from a larger protein called amyloid precursor protein (APP). We don't yet know whether plaques themselves cause AD or are a by-product of the disease process.



n AD Plaque



- Tangles are insoluble clumps of twisted fibers that build up inside neurons. These fibers are made of a protein called *tau*, which helps to stabilize the neuron's internal support structure. In AD, *tau* is changed chemically, causing it to pair with other threads of *tau* and become tangled up. This may result in malfunctions in communications between neurons and later in the death of the cells.

NIH progress report on Alzheimer's disease