Rational Drug Design for Oncotherapy Using Molecular Dynamics Simulations

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Drug Specificity

- A drug has to bind to its target with a suitable affinity
- The drug must stimulate or inhibit the activity of the target
- However, a good fit and high affinity may not always be the best scenario. Modification of key interactions may also be sufficient for increased drug activity

Lead compounds from natural sources

- Finding new and effective drugs is a difficult process
- Many are discovered by serendipity
- The systematic screening of potential drugs involves thousands of compounds
- Computational searches offer speed-up and cost cuts

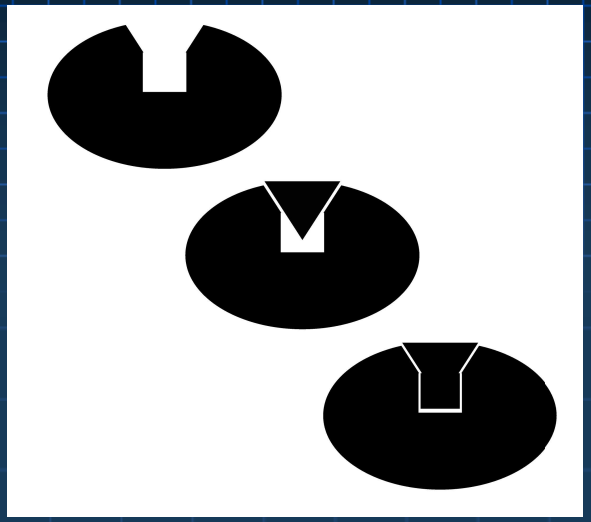
How can computers help design better cancer drugs?

- **Enables us to visualize proteins in 3+1 dimensions**
- **Manipulation of these systems** *in* silico including diffusion calculations
- **Docking fits drug molecules into binding sites and determines the energy of multiple conformations**
- **Statistical Mechanics permits molecular motion and solvent effects to be incorporated**
- **Searching through chemical and structural databases to uncover novel correlations**

Quantitative Structure Activity Relationship (QSAR)

- Structure Activity Relationship that is applied with a degree of predictive ability
- Structural and chemical elements, such as size, hydrophobicity and the electrostatics of a drug are quantitatively scored and used to predict the activity of similar compounds

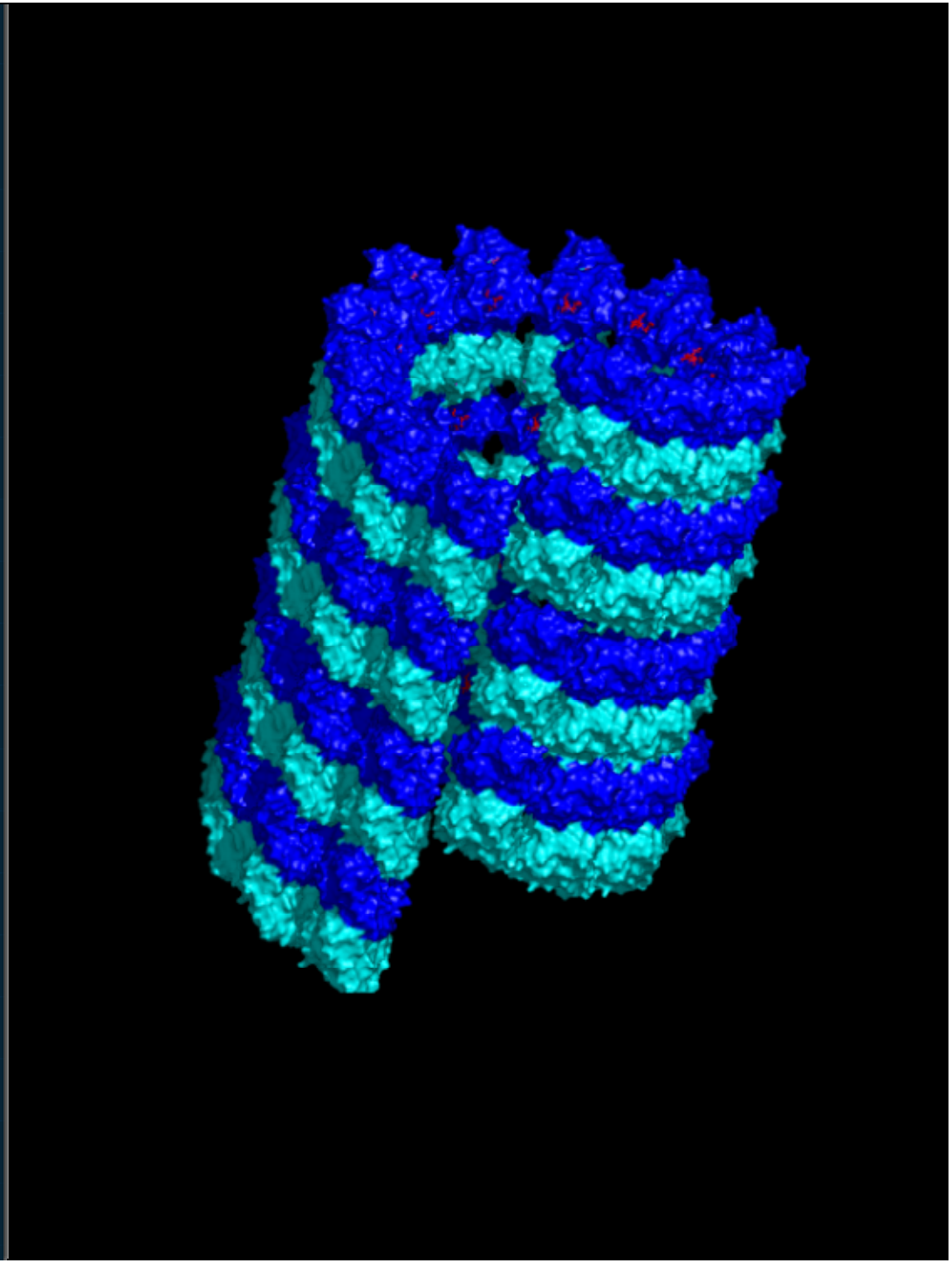
Spatial Fitting



- Visual inspection of binding sites in a target
- Modification of the drug to provide better binding to target
- Involves manual or automated docking of a drug into binding site

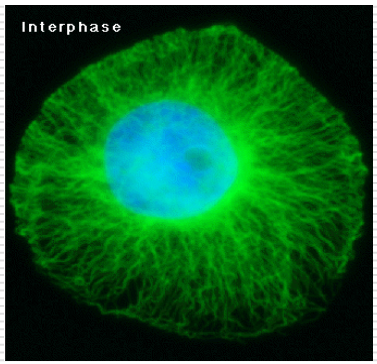
Microtubules

- Microtubules are composed of tubulin
- Assembly and disassembly rates are critical for cell function
- Disruption of these rates with drugs like colchicine and taxol results in cell death

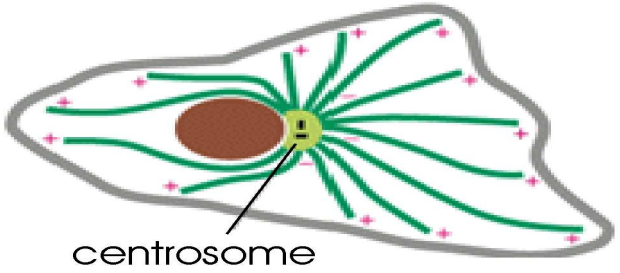


MICROTUBULES PLAY CRITICAL ROLES IN:

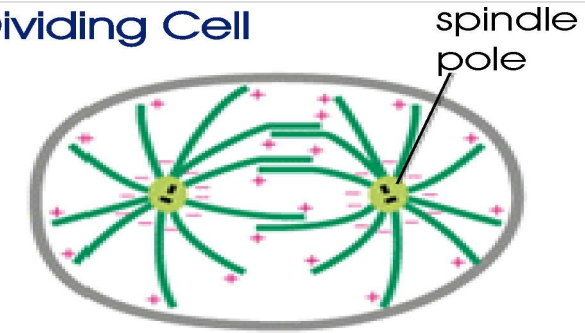
- **Mitosis**
- **Intracellular transport**
- **Intracellular organization**
- **Motility**



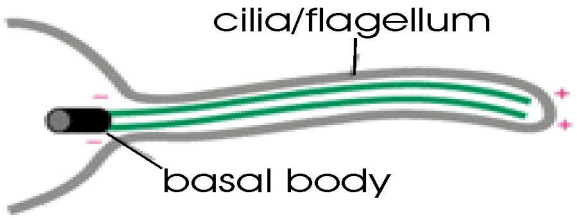
Interphase Cell



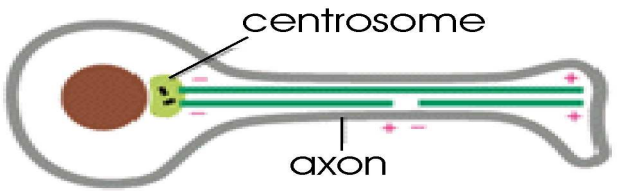
Dividing Cell



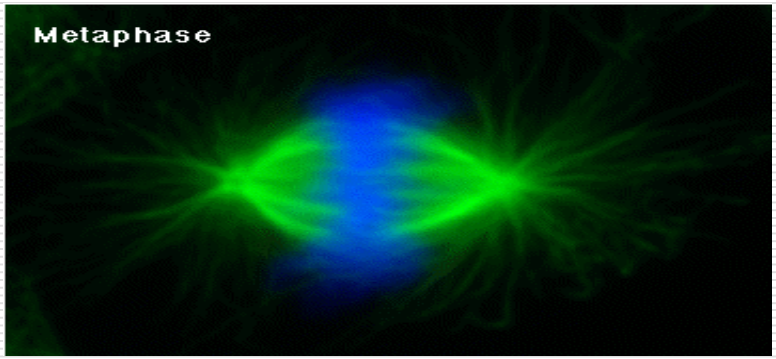
Ciliated Cell

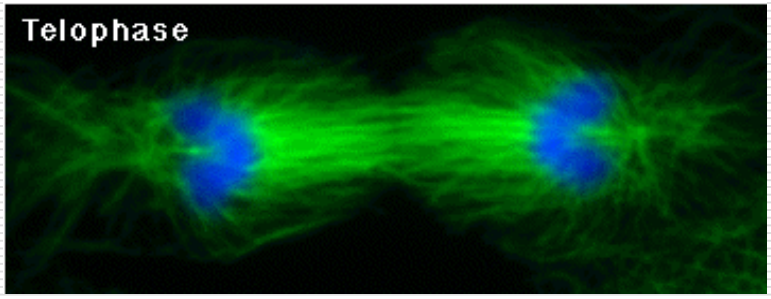


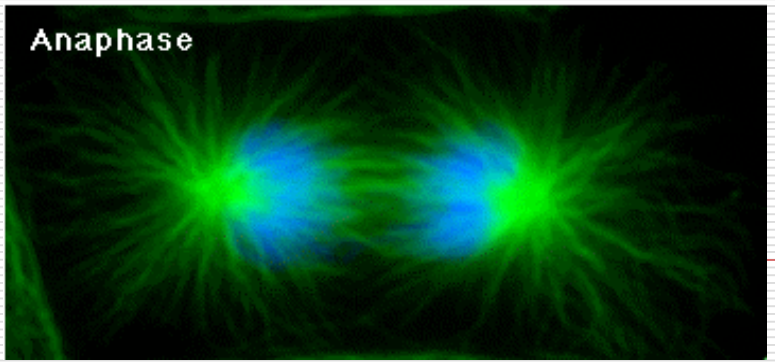
Nerve Cell

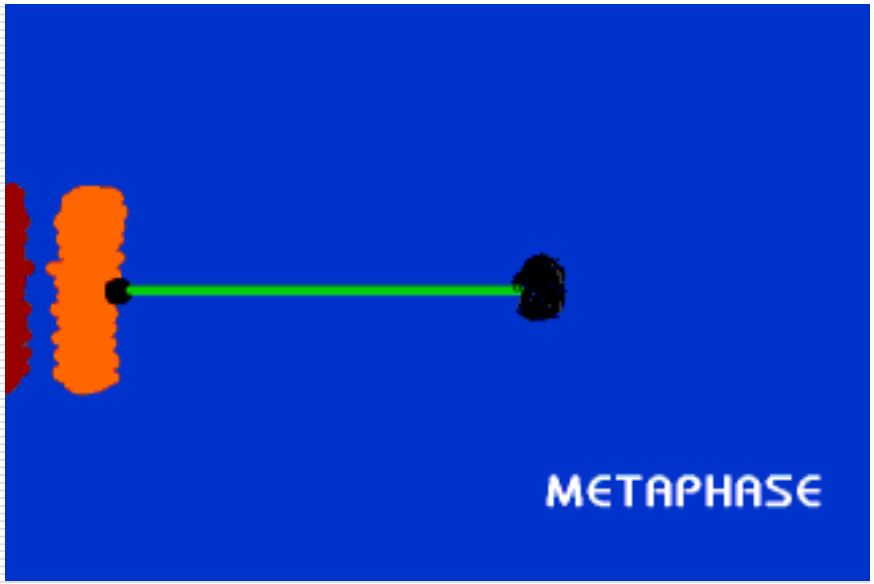


Microtubules in Mitosis

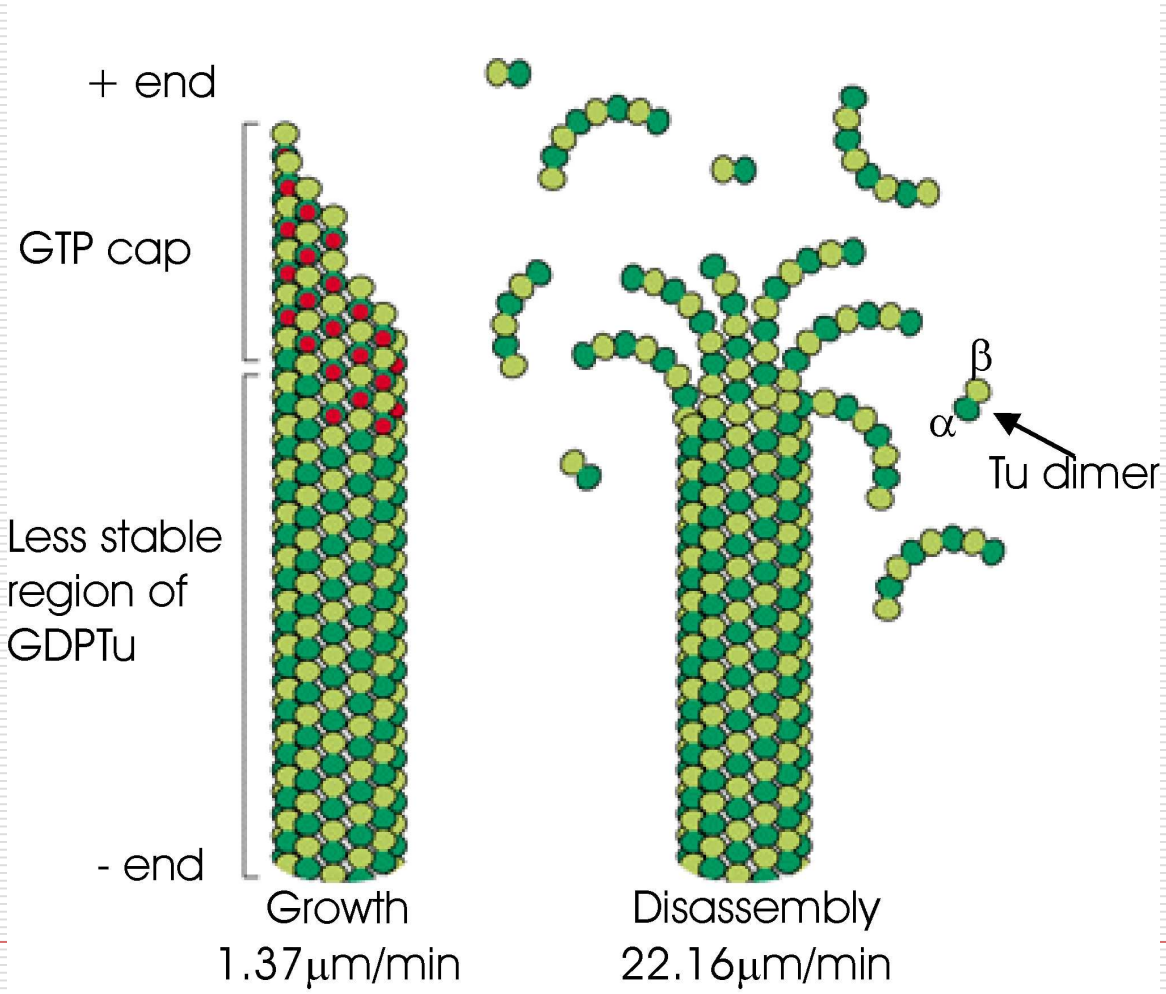






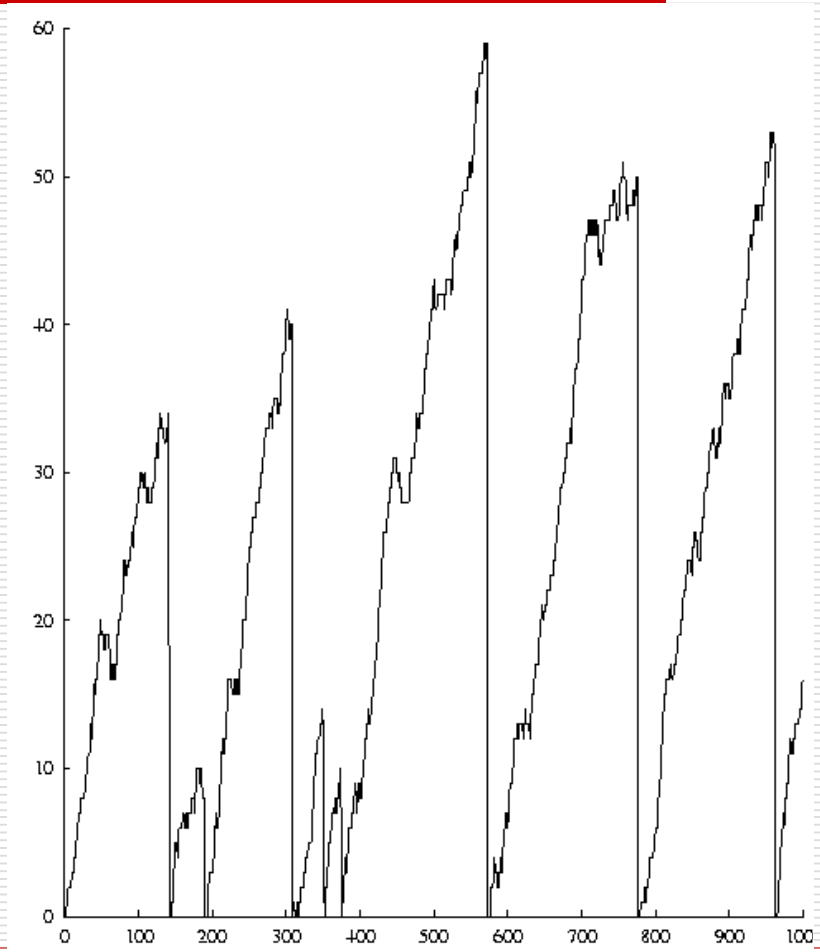


GTP Lateral Cap Model for MT Dynamic Instability



Individual MT life story: dynamic instability

- Catastrophes
- Rescues
- Growth phase
- Shrinking phase



Simulation using a recursive map Shown in the next slide

Modeling of Individual MT Assembly Processes:

States:
- Attachment
- Detachment
- Pause
- Catastrophe

Recursive Map

$$\ell(t+1) = (1 - r_3(1-r_1)(1-r_2))(\ell(t) + r_1 a - r_2 a - a r_1 r_3 + a r_2 r_3)$$

Master Equations

$$\frac{d}{dt}P(n,t) = c_T t_{r1} P(n-1,t) + t_{r2} P(n+1,t) - (c_T t_{r1} + t_{r2} + t_{r3}) P(n,t)$$

for $n > 0$, and

$$\frac{d}{dt}P(0,t) = t_{r2} P(1,t) + t_{r3} \sum_{n=1}^{\infty} P(n,t) - c_T t_{r1} P(0,t)$$

for $n = 0$.

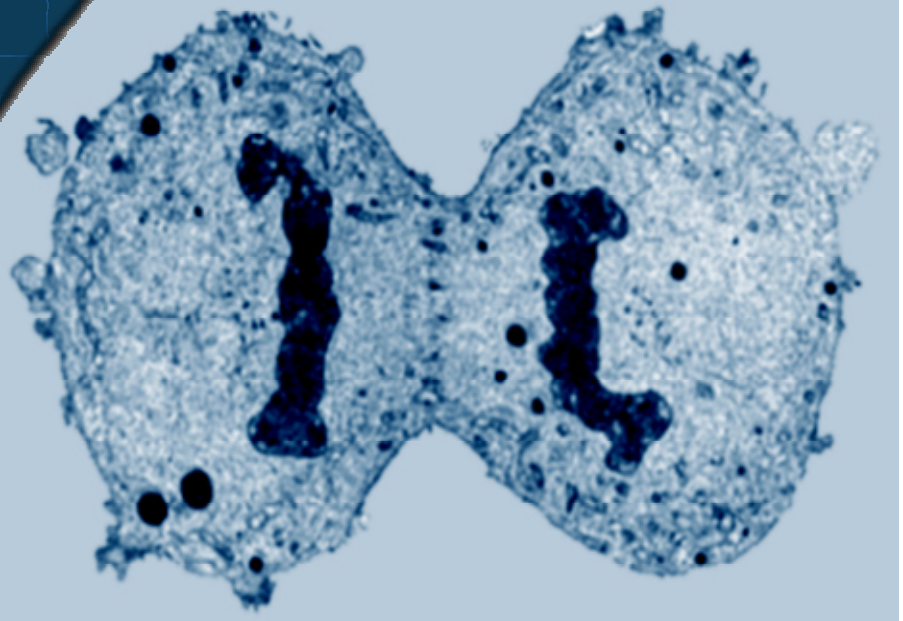
continuum limit

integro-differential equation

$$\frac{\partial P}{\partial t} = \frac{1}{2}(c_T t_{r1} + t_{r2}) \frac{\partial^2 P}{\partial x^2} - (c_T t_{r1} - t_{r2}) \frac{\partial P}{\partial x} - t_{r3} P + t_{r3} \int_x^{\infty} w(x' - x) P(x') dx'$$

Microtubules and Cancer

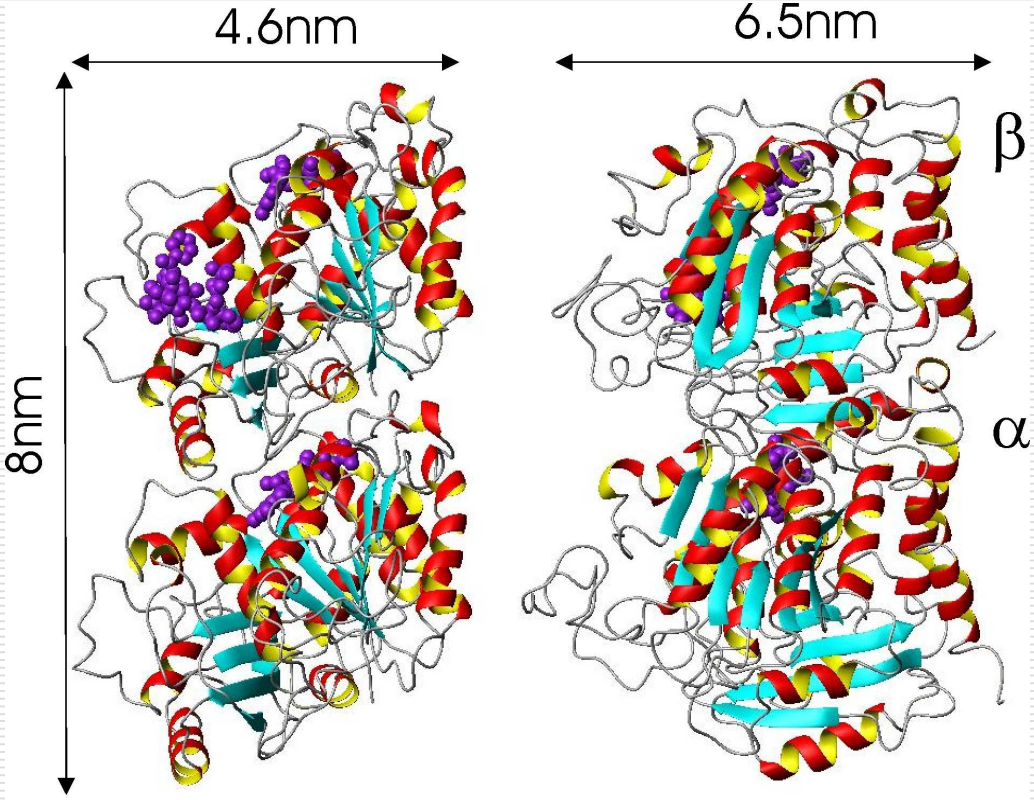
- Cancer is related to a failure of multiple cellular checkpoints that results in uncontrolled division
- we are selectively targeting mitosis during cell division
- a critical component of mitosis are the microtubules



MICROTUBULES AND TUBULIN

- Microns-long cylindrical organelles 25 nm in diameter
- Associated with MAPs and motor proteins
- Formed from 110 kD tubulin α/β heterodimer
- Tubulin dimers bind end to end to form a protofilament; 13 protofilaments arranged side to side to form microtubule
- Both α and β tubulins exist as numerous isotypes each encoded by different genes

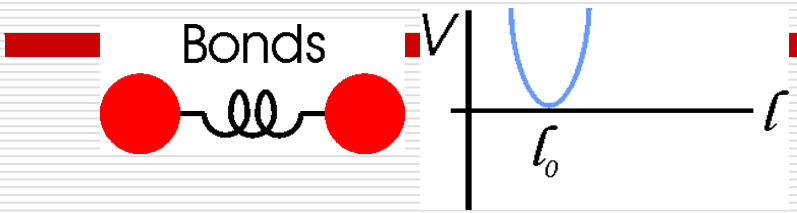
The atomic resolution structure of the tubulin dimer following Downing's electron crystallography (1997)

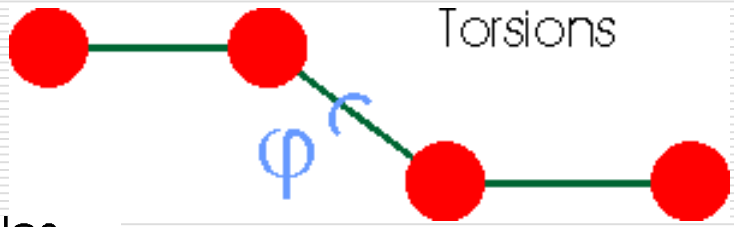


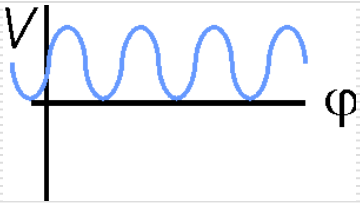
C-terminus
not resolved

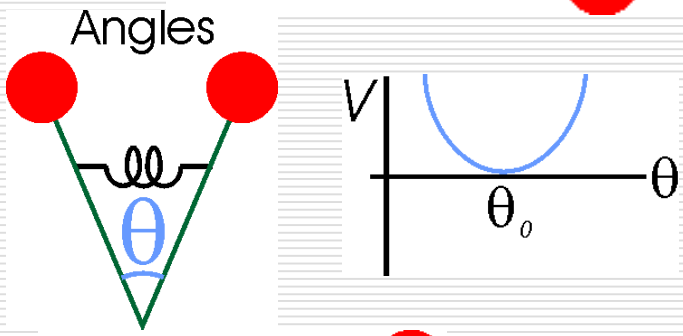
PDB entries for tubulin: 1TUB, 1JFF, 1FFX, 1SAO

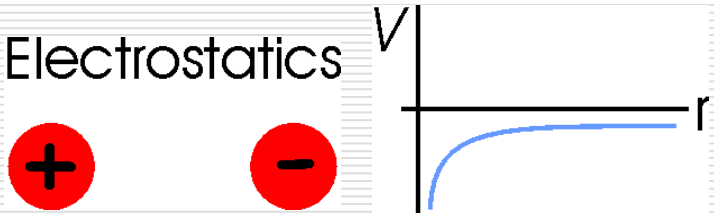
Molecular Dynamics

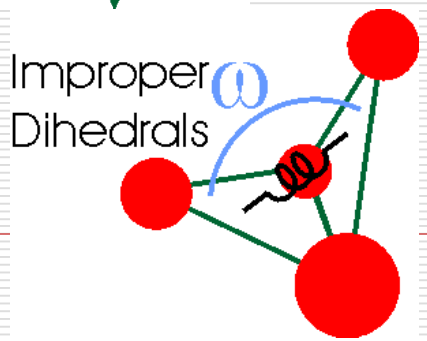


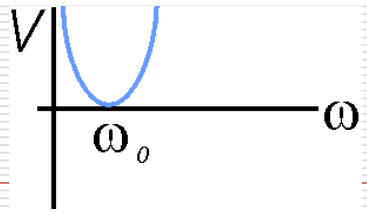


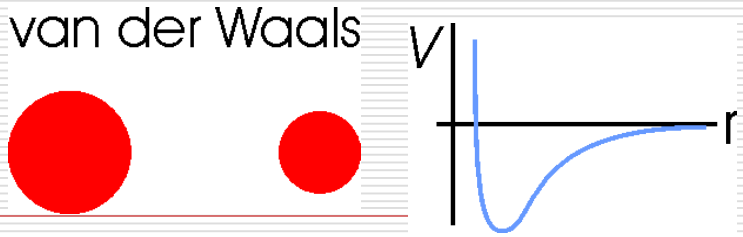




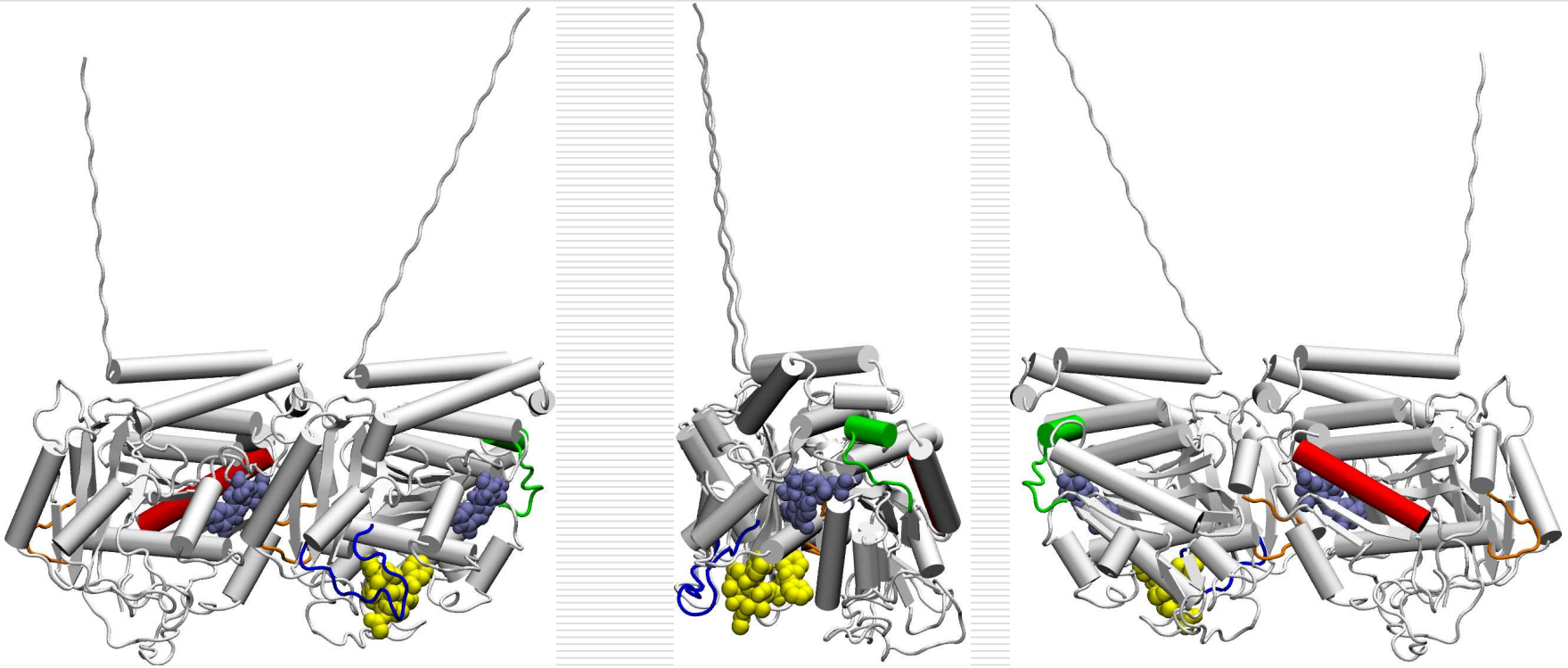








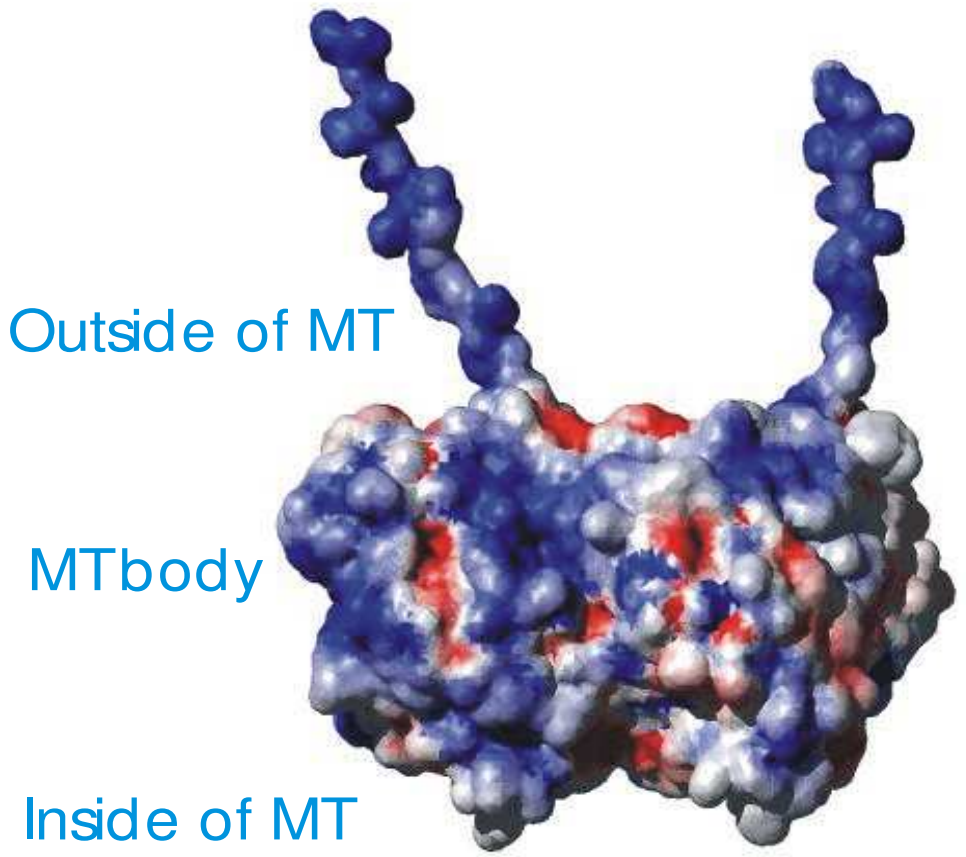
Tubulin Structure: key motifs



GTP/GDP M Loop H3 Helix T3 Loop T7 Loop Taxol

Charge distribution of tubulin dimer including C-termini.

Blue=negative
Red=positive
White=neutral



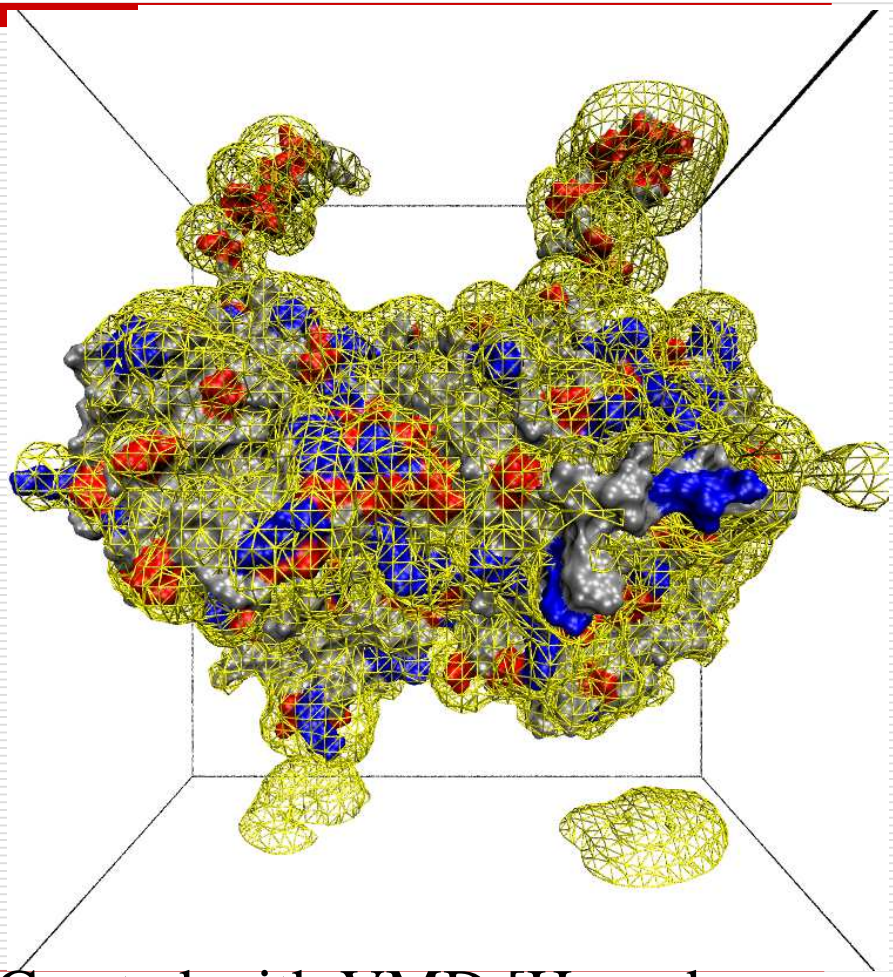
Approx. 25 e per monomer or 50 per dimer, 40% on C-termini

Electric Field around tubulin

- Because each atom has a partial charge we can calculate the electric field of the protein.

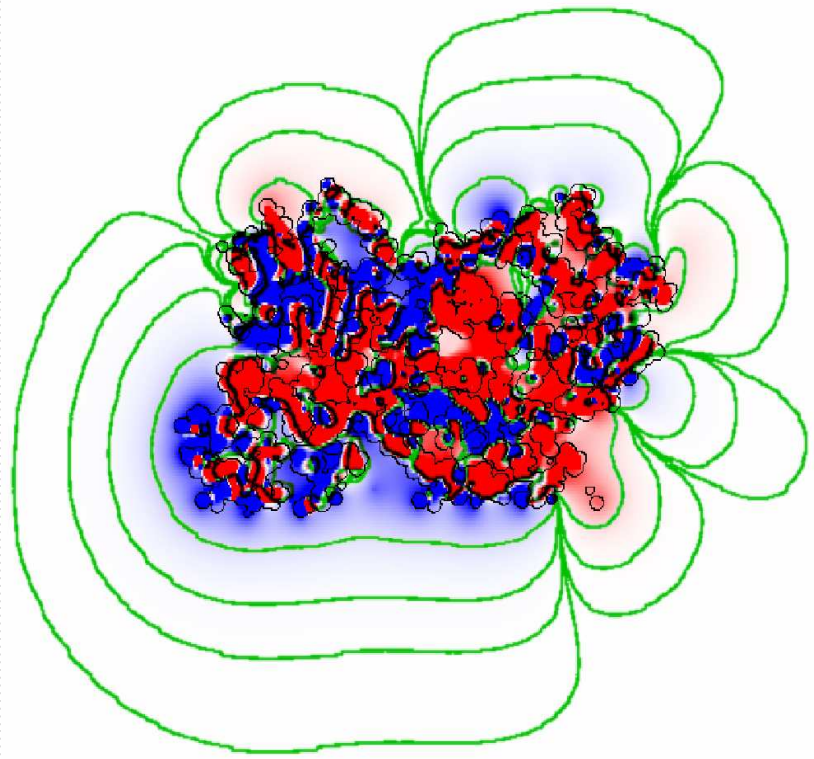
$$\mathbf{E}(\mathbf{x}) = \frac{1}{4\pi\epsilon_0} \sum_{i=1}^{n} \frac{q_i}{r_i^2} \hat{r}_i$$

- Bases (e^+) and acids (e^-) are colored blue and red respectively.
- Iso-surface of the electric field is coloured yellow.



Created with VMD [Humphrey *et al.*, 1996]

Electric Potential

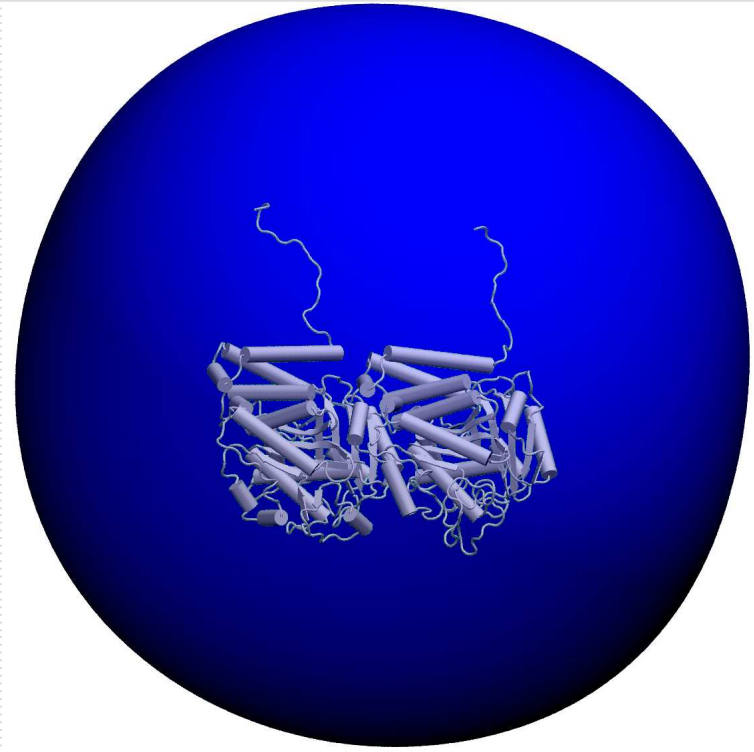


- slice through dimer
- red/blue= negative/positive
- green=isopotential lines

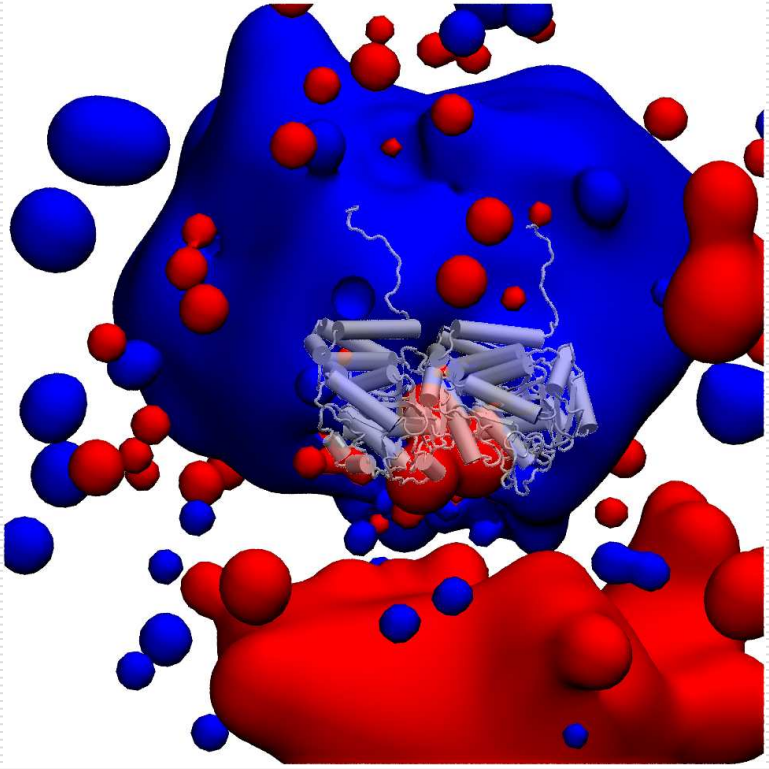
Poisson-Boltzmann equation:

$$\nabla \cdot \left(\epsilon(\vec{x})\nabla\phi(\vec{x})\right) - \kappa(\vec{x})^2 \sinh\left(\phi(\vec{x})\right) = -4\pi\rho(\vec{x})$$

Monopole in vacuum becomes a dipole in solvent

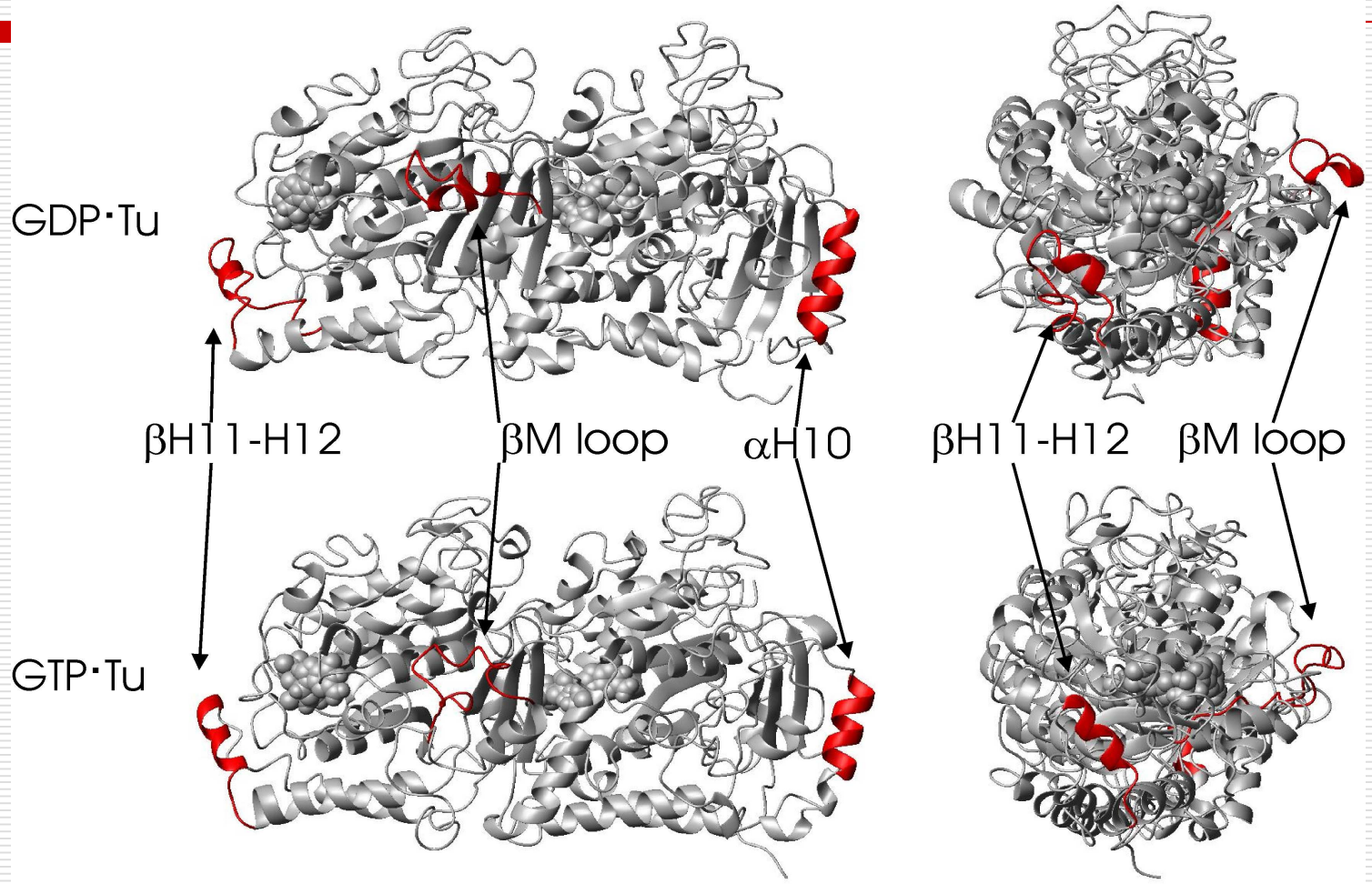


Potential in vacuum

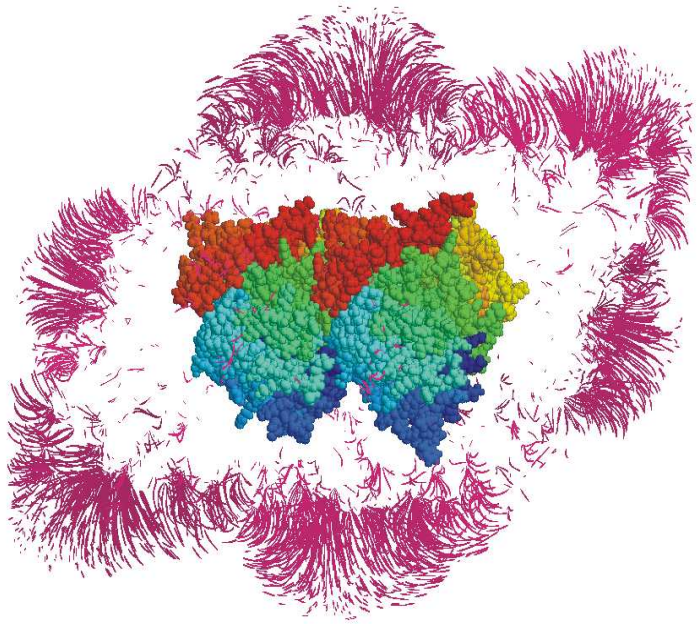


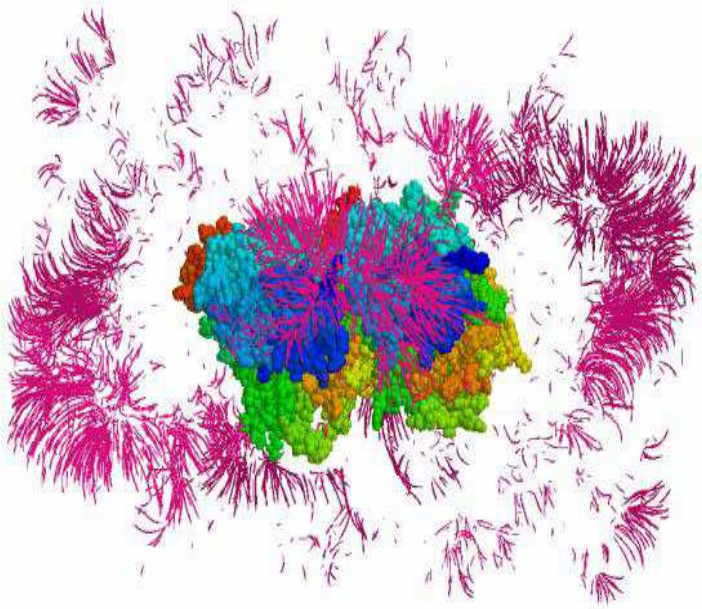
potential in solvent

Conformational effects of GTP hydrolysis in a tubulin dimer



Map of Tubulin Dimer's Sphere of Attraction for a neighbouring dimer





Map of the volume in which two tubulin dimers of identical orientation attract. The magenta "tufts" indicate locations of the centre of mass of a second dimer when it would be attracted by the central dimer and the direction of the force. Darker "tufts" are near the viewer, lighter ones further away.

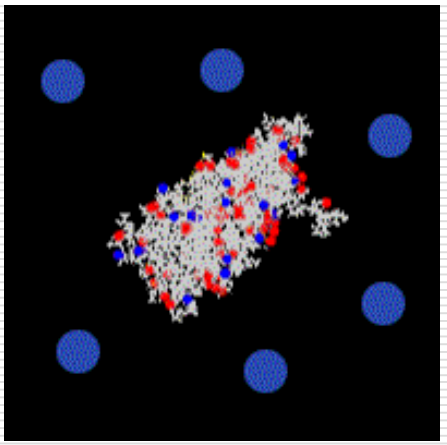
Brownian Dynamics as a tool for MT polymerization simulations:

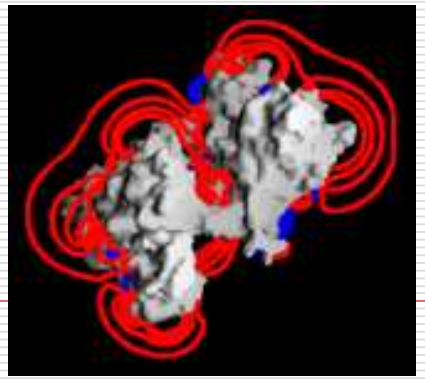
Model protein-protein association using BD methods

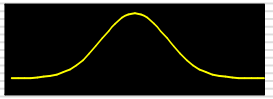
- Electrostatic interactions
- Solvent interactions

$$\Delta R = \frac{D\Delta t}{k_B T} F + S$$

Displacement of Protein



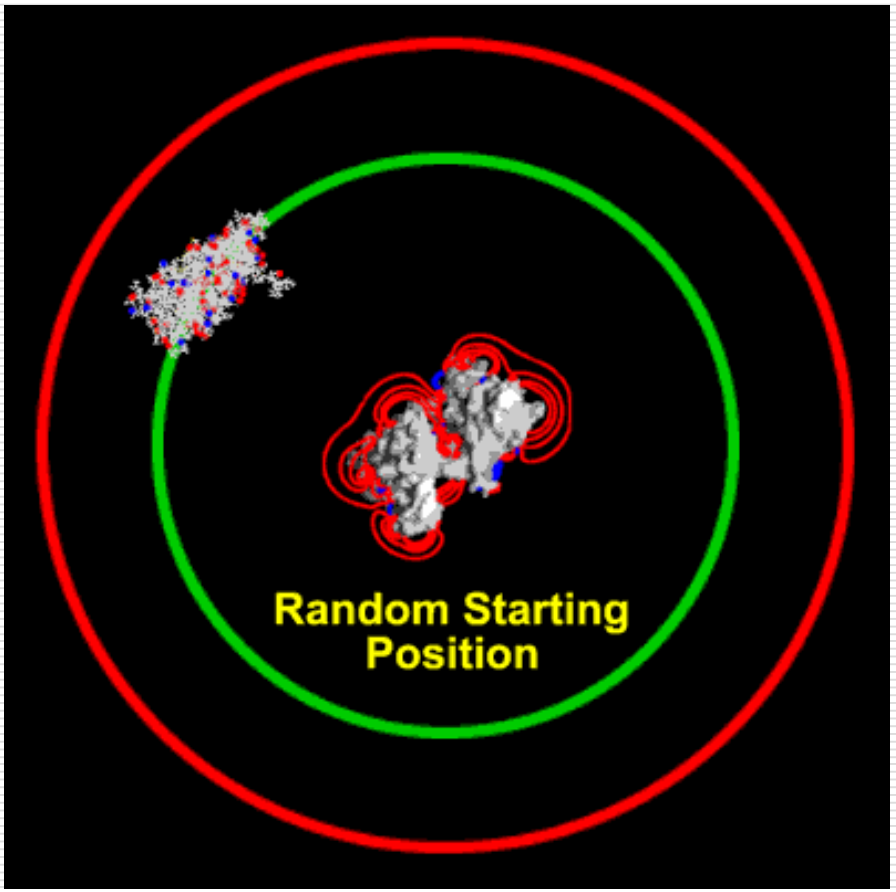




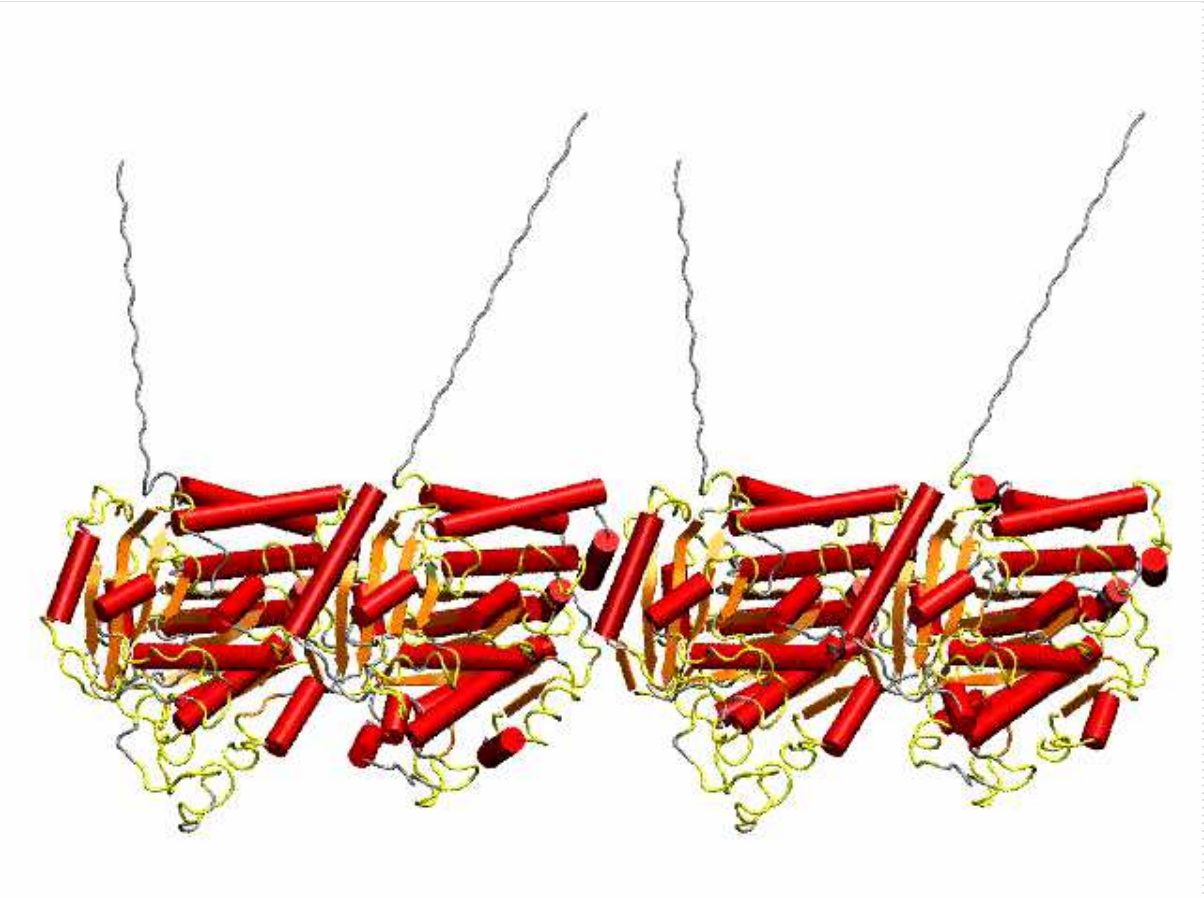
Brownian Dynamics Simulation

- Start at 120 A separation (b)
- End upon escape > 500 A (q) or binding
- From fraction of successful trajectories (β) we can calculate a rate constant:

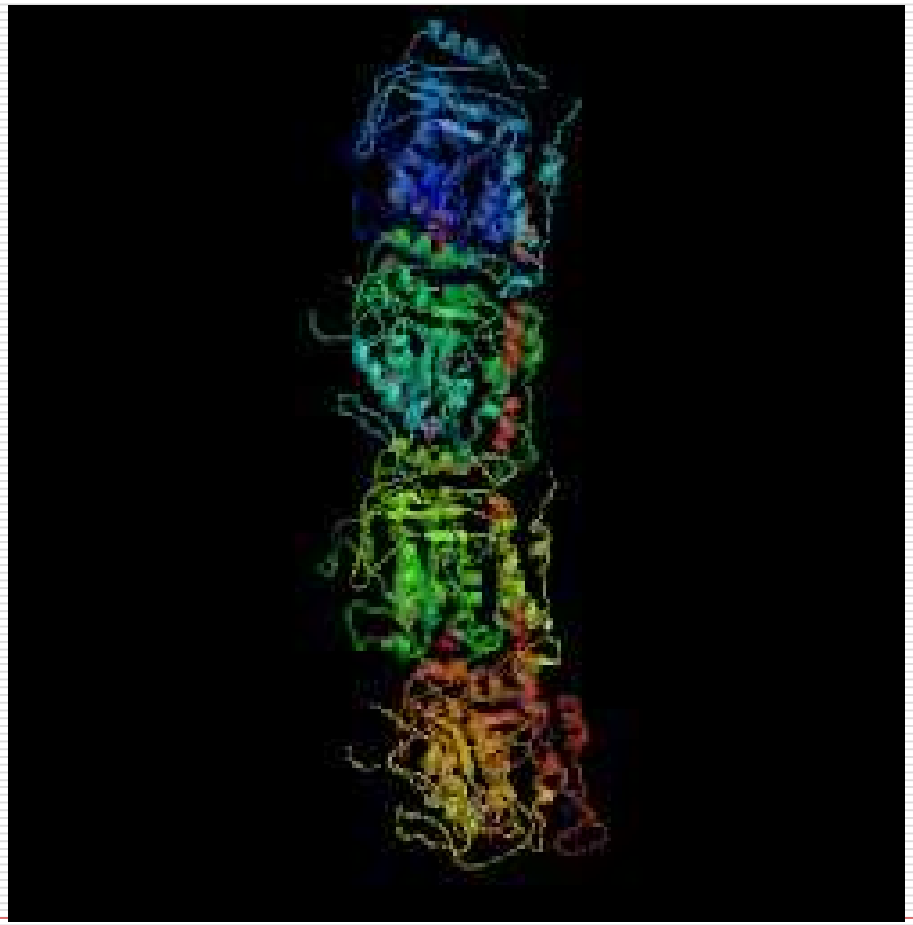
$$k = \frac{k_D(b)\beta}{1 - (1 - \beta)\frac{k_D(b)}{k_D(q)}}$$



Conformational dynamics of protofilament formation



Stathmin-stabilized MT filament



PDB file:
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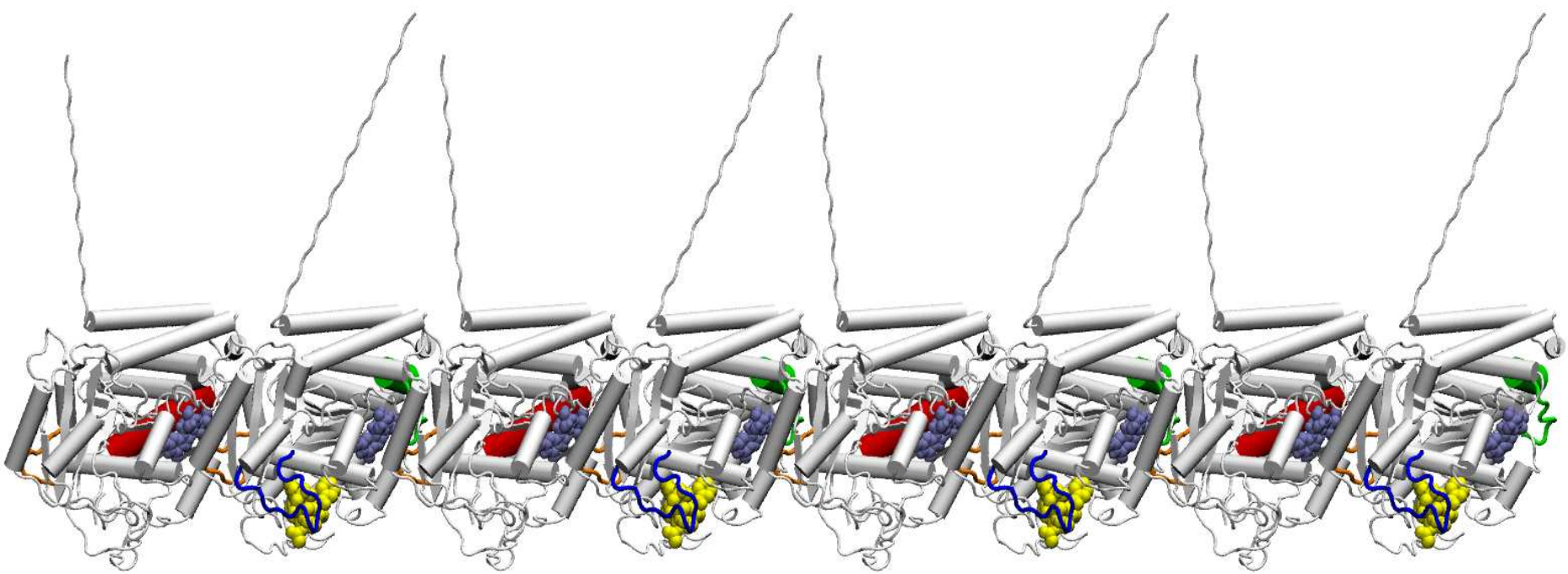
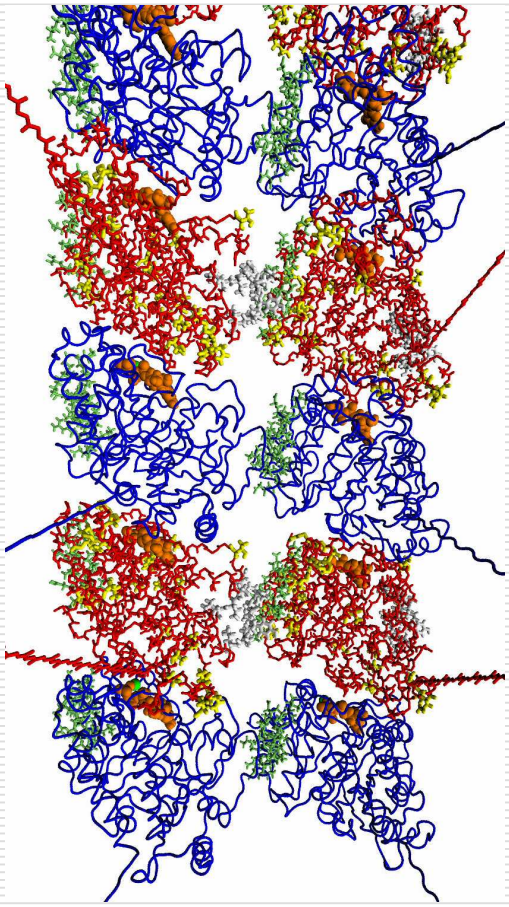
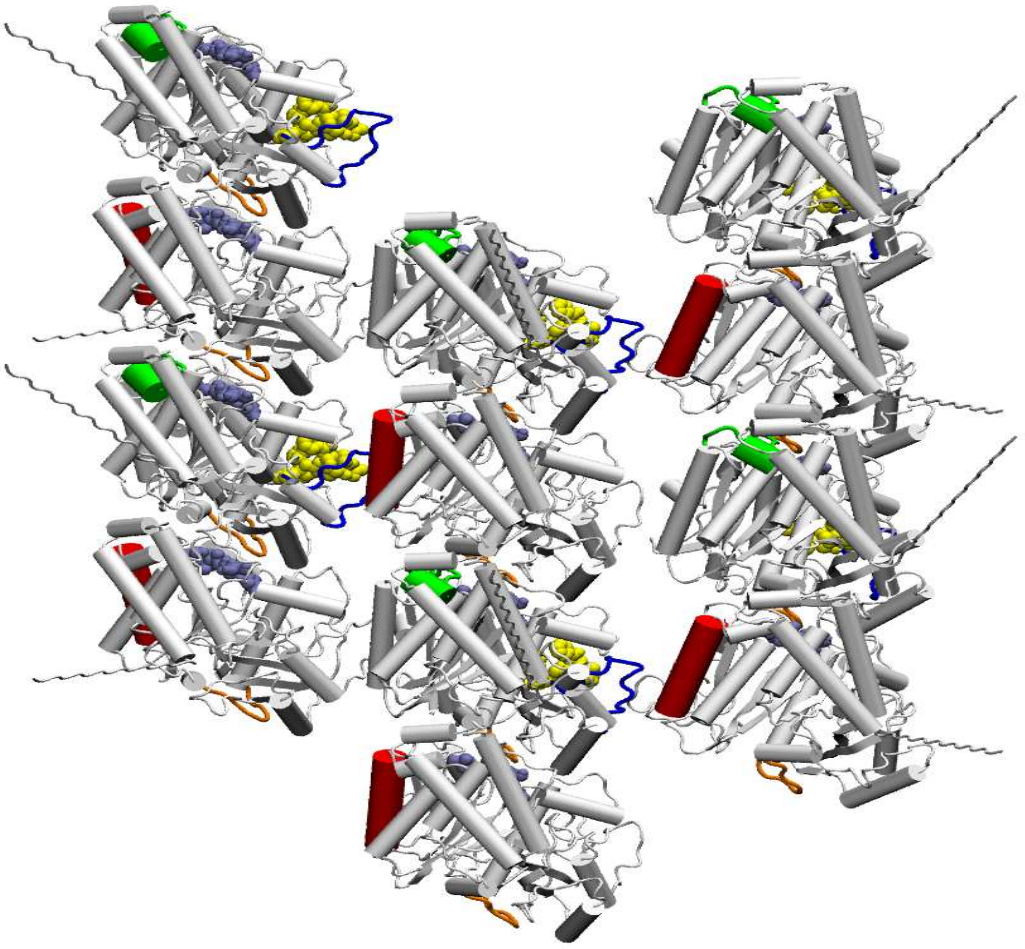
Conclusion: GTP hydrolysis curves protofilaments

Longitudinal Bonds (stronger) in a Protofilament

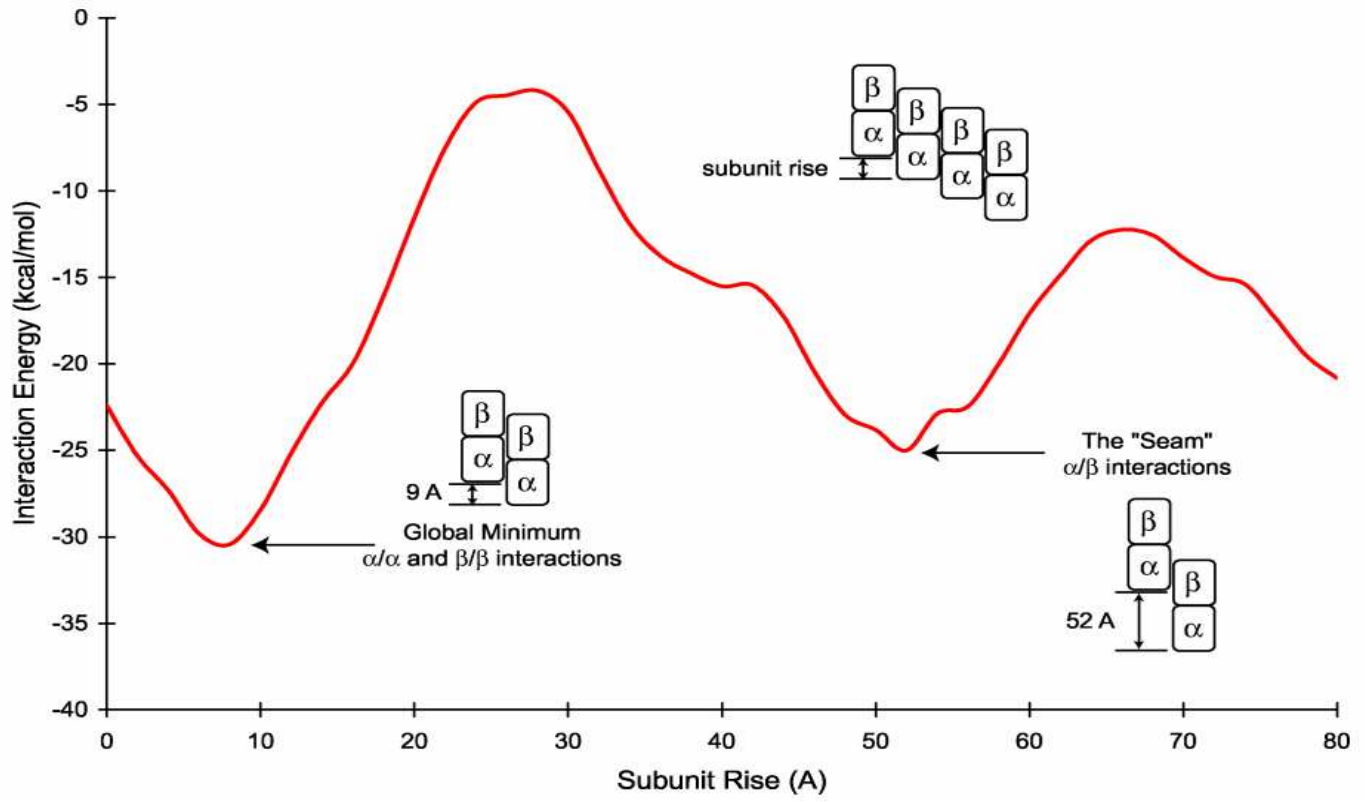


Lateral Bonds: formation of a sheet





Lattice formation: A or B type lattice



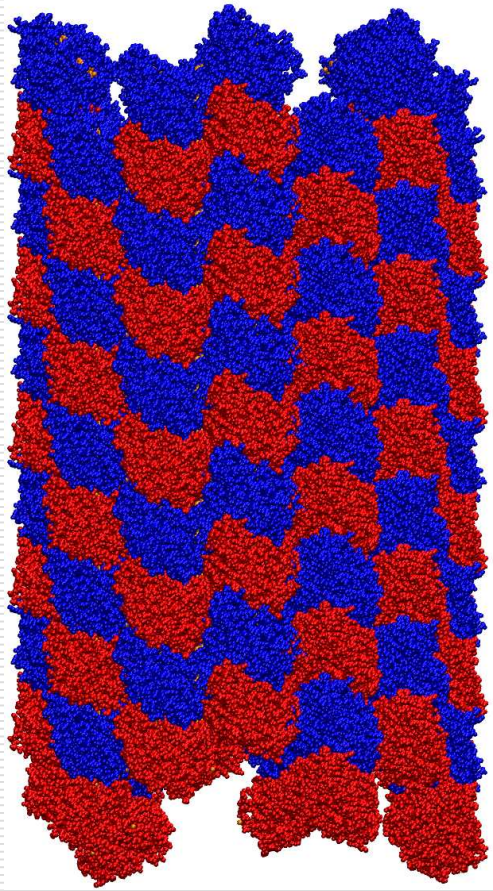
Energy of a lateral tubulin-tubulin interaction: k=4 N/m

Consistent with the elastic properties of MT's (Young's modulus of 10^9 N/m^2)

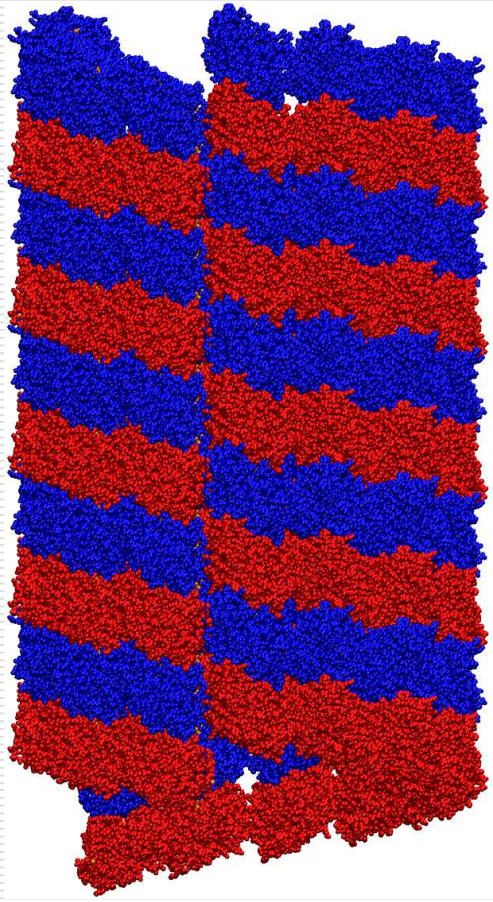
D. Sept

Mircotubule lattices

Blue=α tubulin
Red=b tubulin

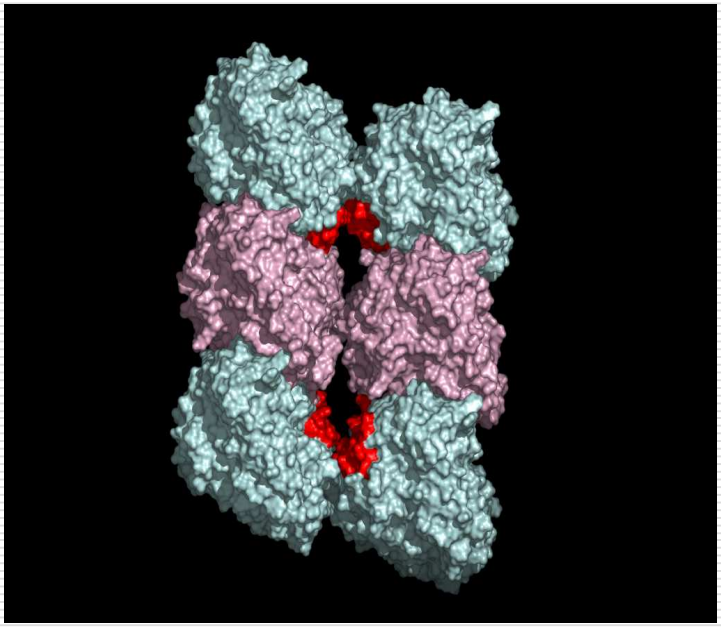


A Lattice

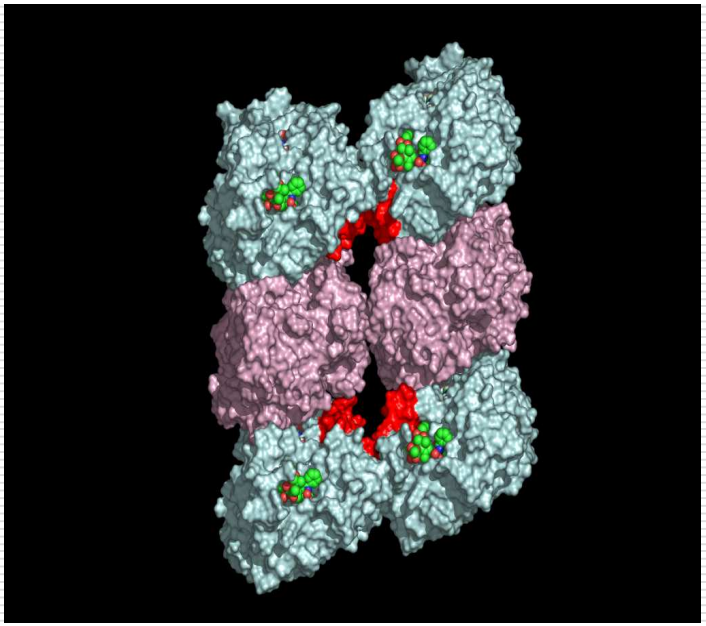


B Lattice

Taxol diffuses through nanometer-size surface pores in a microtubule



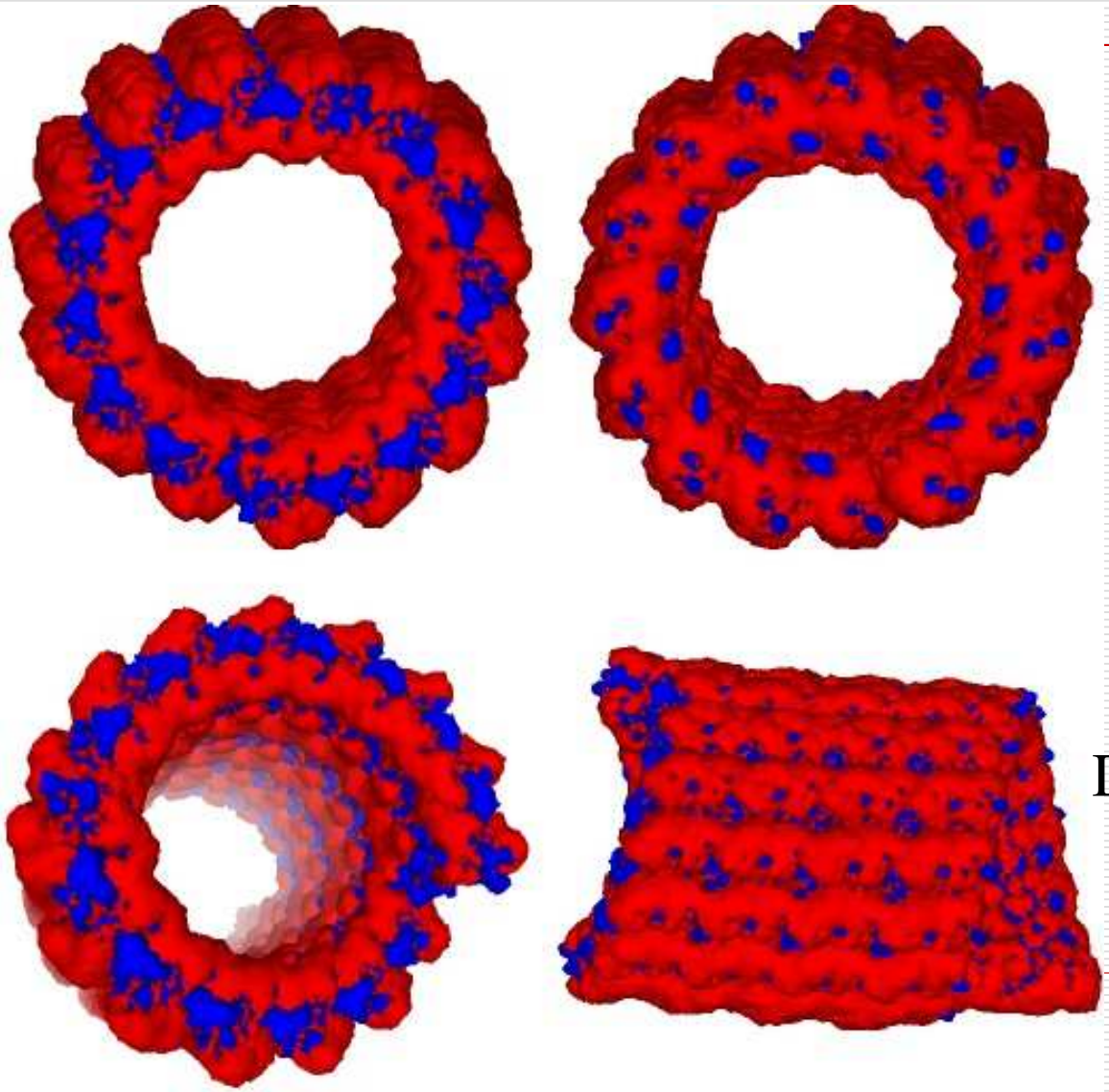
Outside surface



Inside surface

Charge distribution on a microtubule.

-end +end asymmetry



may explain different kinetics

Counter ions
partially
neutralize net charges

D.Sept et al, PNAS 2001

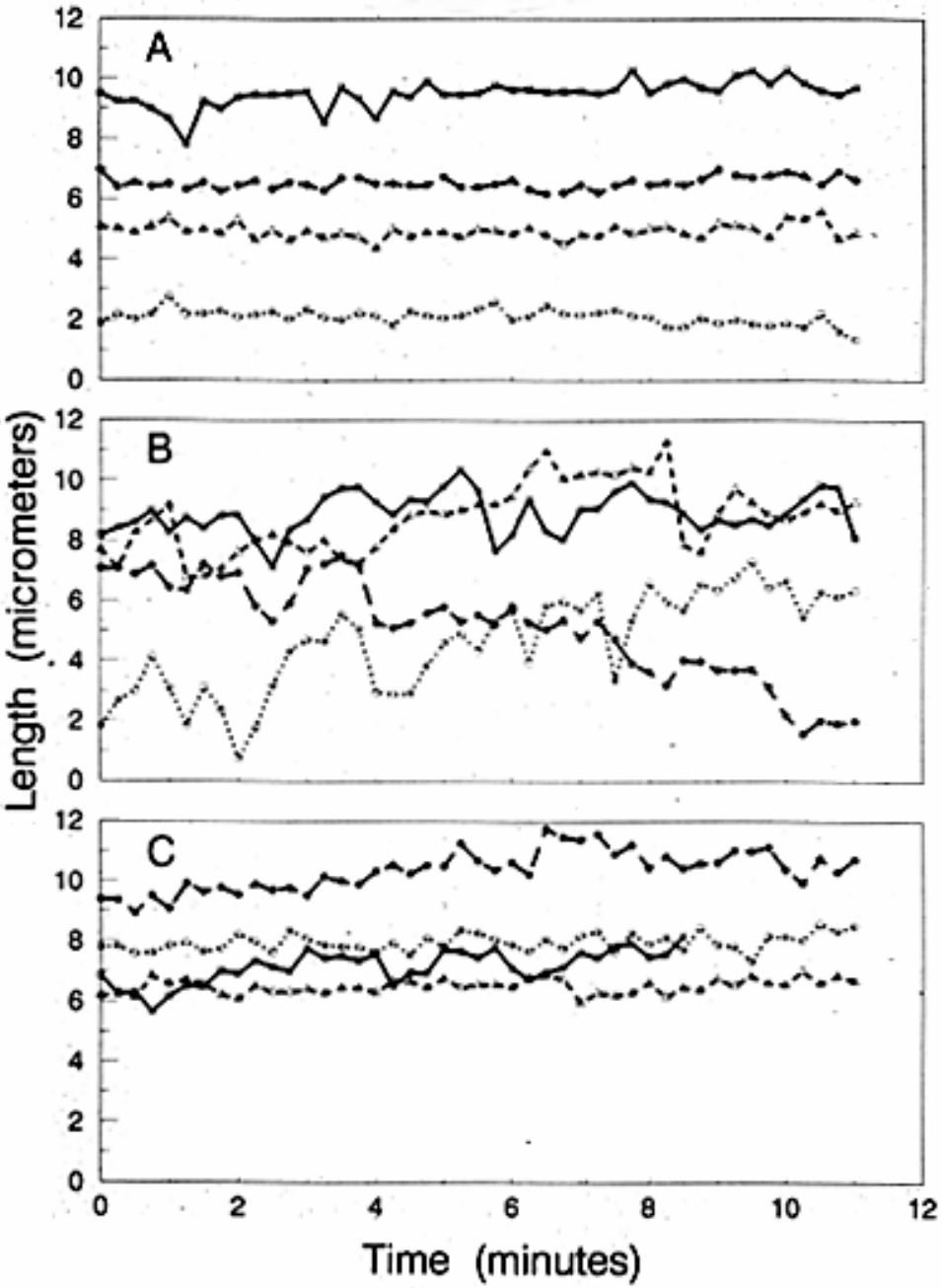
Mammalian β-Tubulin Isotypes

Isotype	Distribution
βI	Most cells
βII	Neurons, glia, inner ear, skeletal muscle, smooth muscle
βIII	Neurons, Sertoli cells, colon
βIVa	Neurons and glia
βIVb	Most tissues
βV	3T3 and CHO cells
βVI	Platelets, marrow, spleen
βVII	Brain

β-Tubulin Isotypes in Mammals

	% Sequence Identity to βI	% Conservation in Evolution
βI	100.0	99.5
βII	94.6	98.9
βIII	90.2	99.6
βIVa	96.0	N/A
βIVb	97.3	96.6
βV	91.4	98.3
βVI	76.7	80.9
βVII	85.5	ND

Dynamics of Isotypically Pure Microtubules



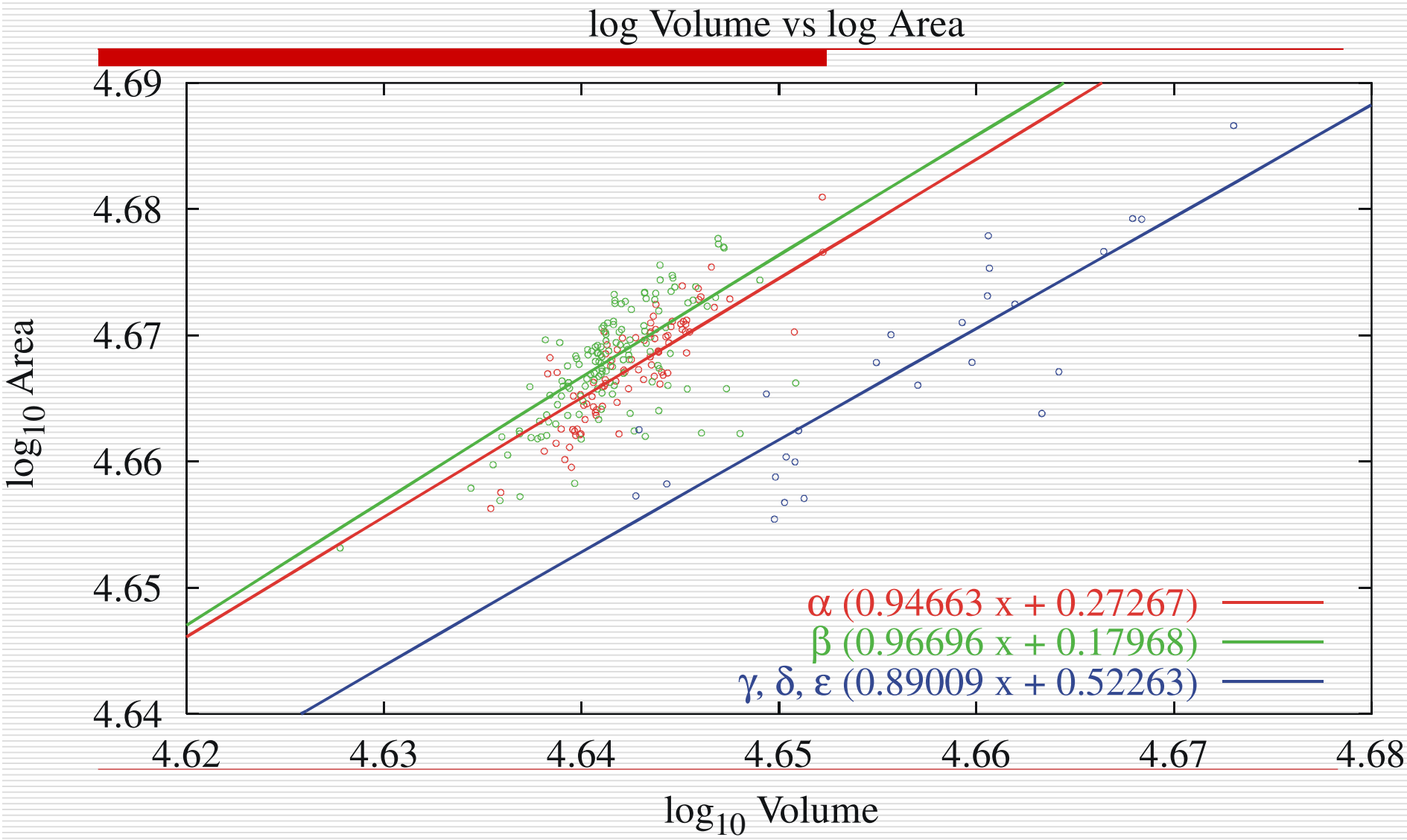
αβII

αβIII

αβIV

Panda et al. (1994) *Proc. Nat. Acad. Sci. USA* **91**, 11358-11362

Slight shape variations



Nature Reviews Cancer **4**, 253 -265 (2004); doi:10.1038/nrc1317 **MICROTUBULES AS A TARGET FOR ANTICANCER DRUGS**

Mary Ann Jordan & **Leslie Wilson:**

- **Mitotic-spindle microtubules are among the most successful targets for anticancer therapy. Microtubule-targeted drugs, including paclitaxel and *Vinca* alkaloids, suppress microtubule dynamics without changing microtubule mass; leading to mitotic block and apoptosis. Some microtubule-targeted drugs can act as vascular-targeting agents, rapidly depolymerizing microtubules of newly formed vasculature to shut down the blood supply to tumours.**
- **Microtubule-active drugs bind to one of three main classes of sites on tubulin, the paclitaxel site, the *Vinca* domain and the colchicine domain. Drugs that bind to the colchicine domain undergo intensive investigation as vascular-targeting agents for cancer therapy.**
- **Development of resistance to microtubule-targeted drugs has several possible causes, some might involve changes in microtubule dynamics resulting from altered expression of tubulin isotypes, tubulin mutations, and altered expression or binding of microtubule-regulatory proteins.**
- **Microtubule-targeted drugs can synergize with one another.**
- **Understanding their modes of action might lead to improved dosing regimens and combinations with other microtubule-targeted drugs, as well as combinations with 'molecularly targeted' drugs.**
- **"Conceivably, if one knew the tubulin isotype and microtubule regulatory protein composition of a specific tumor cell, one could design or choose drugs to selectively target that tumor"**[1]

Taxol, colchicine and vinca bound to a microtubule

LIGAND BINDING SITES ON TUBULIN

1) *Exchangeable GTP* site (β) regulates assembly and dynamic instability.

2) *Non-exchangeable GTP* site (α). Function unknown.

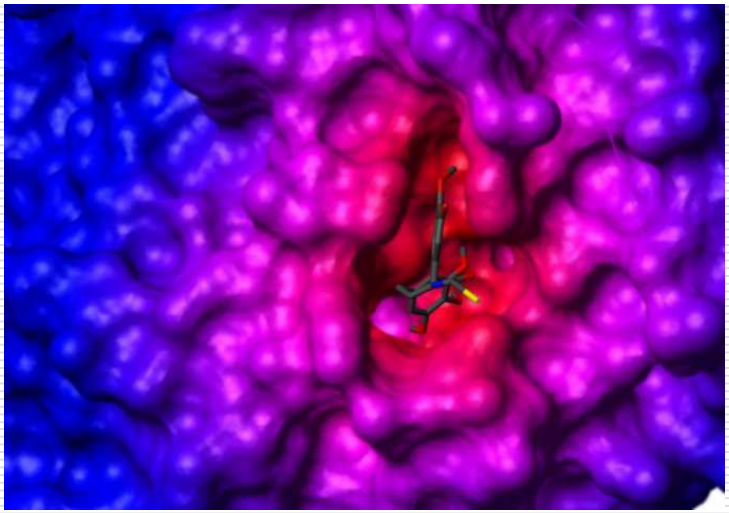
3) *Colchicine* site (α/β interface). Ligands inhibit MT assembly (colchicine, podophyllotoxin, TN16).

4)*Vinca* area (β). Ligands inhibit MT assembly.

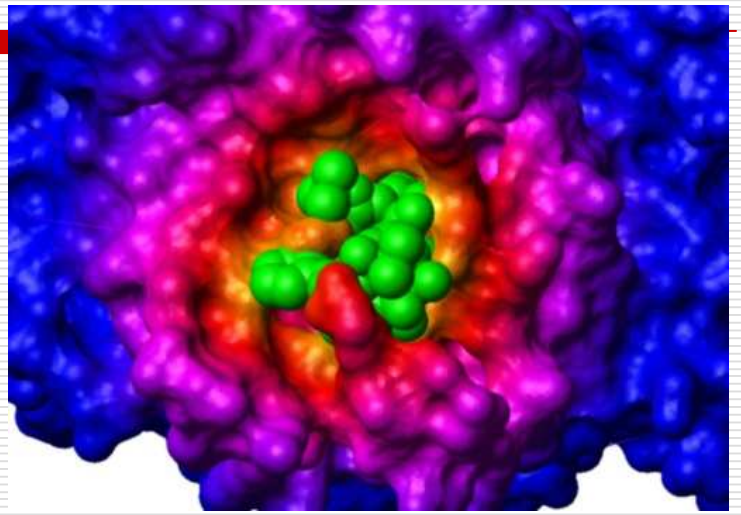
5) *Taxol* site (β). Ligands promote MT assembly, inhibit dynamics.

6) *Other sites: FHIT, Mg++,*

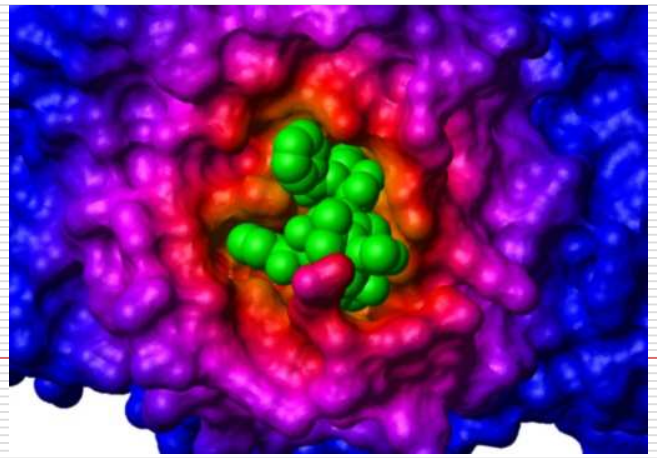
Binding pockets for colchicine and taxol compounds



colchicine

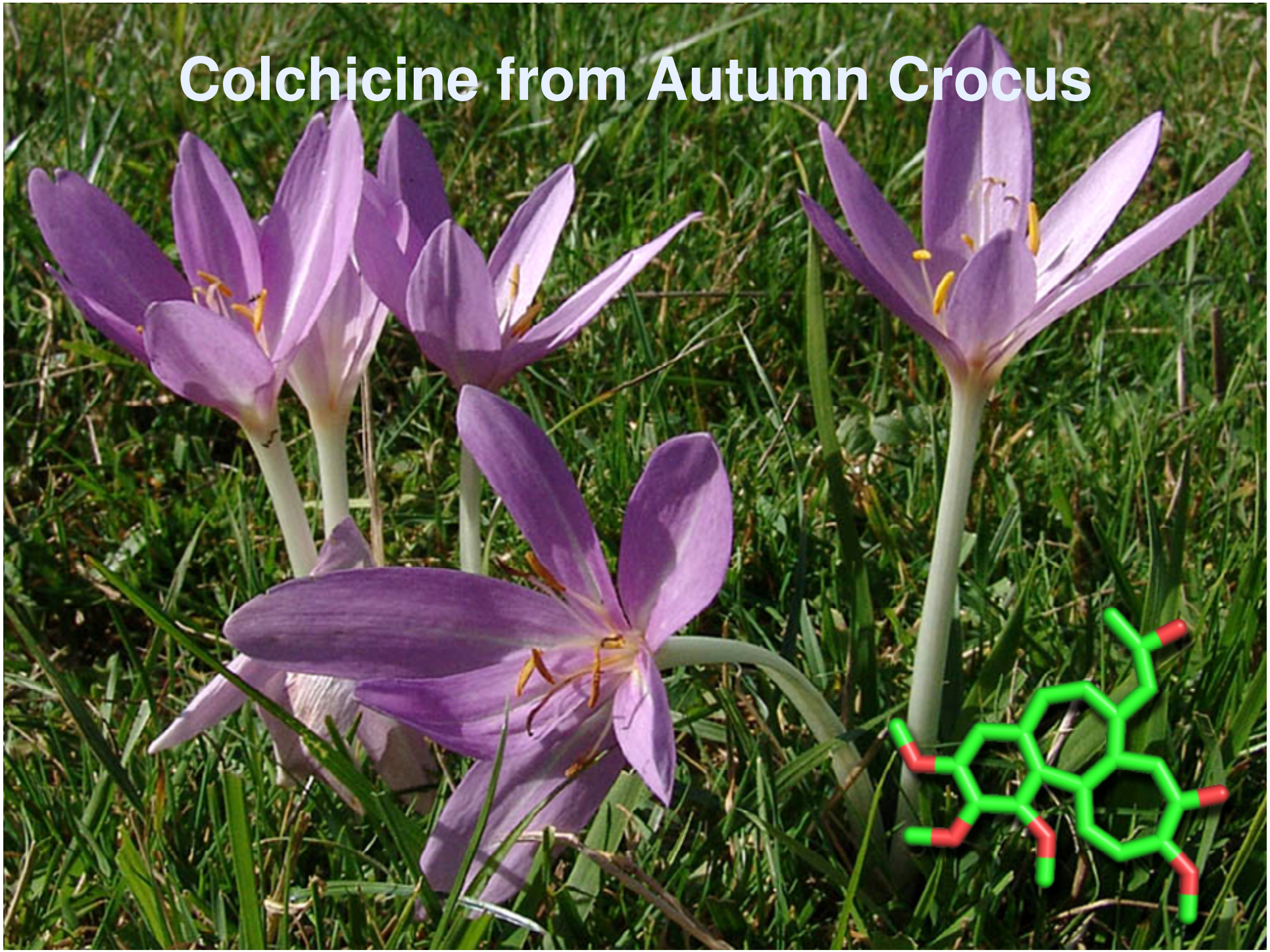


docetaxel

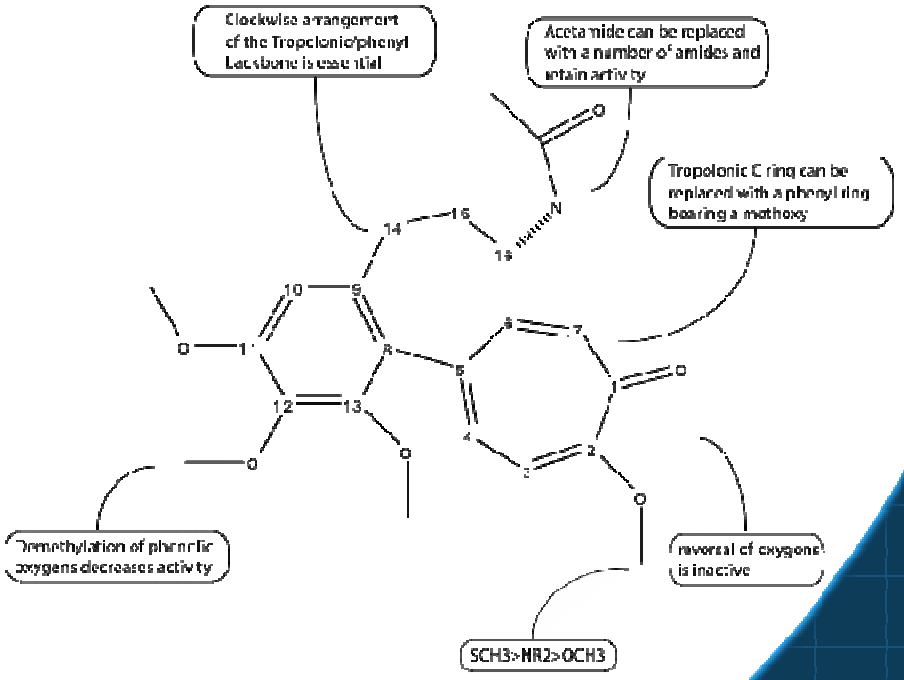


paclitaxel

Colchicine from Autumn Crocus

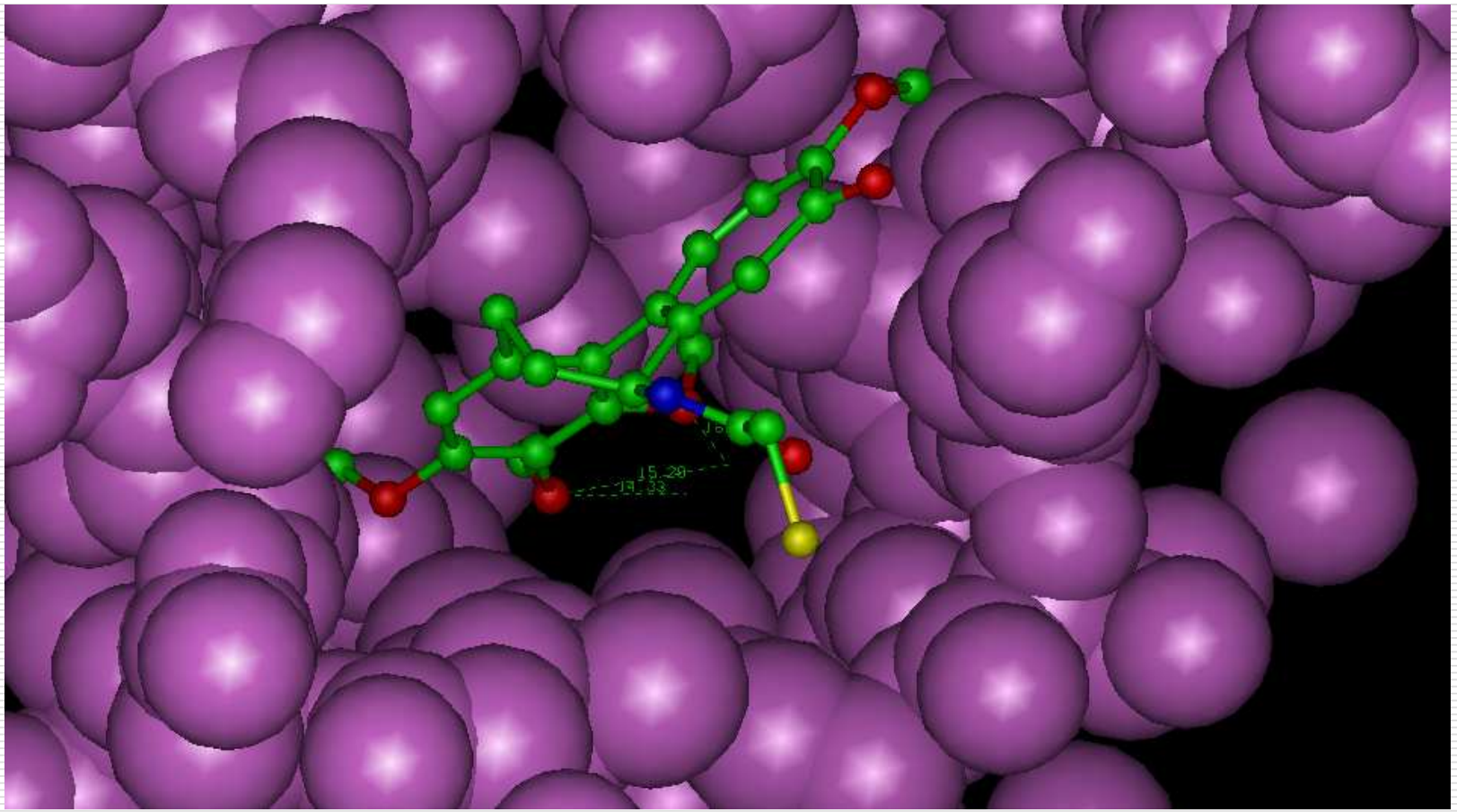


Structure Activity Relationship



- A means by which drugs can be related to their molecular structure
- Assessed by considering a series of modifications to a drug
- The effect upon biological activity is then determined for each modification

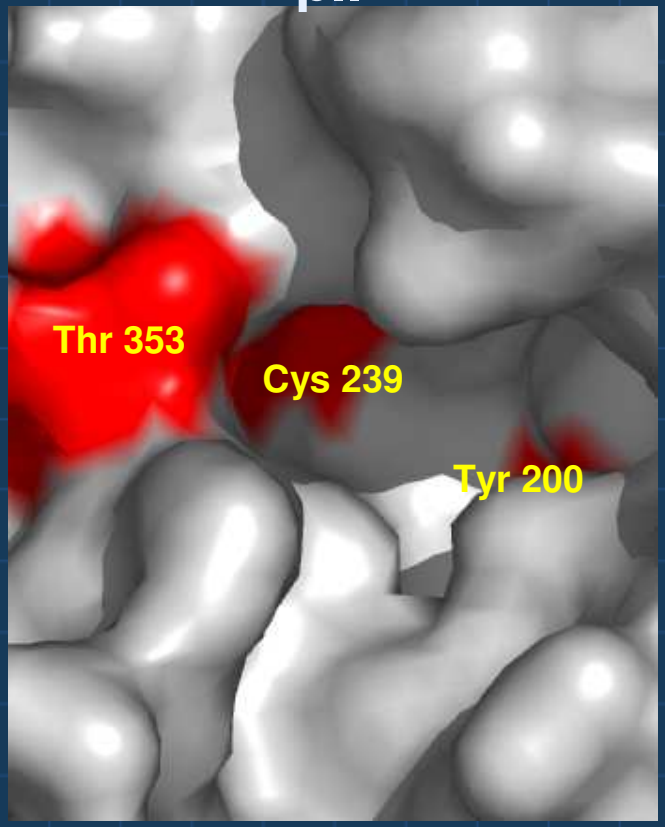
Colchicine binding pocket view from alpha tubulin towards beta



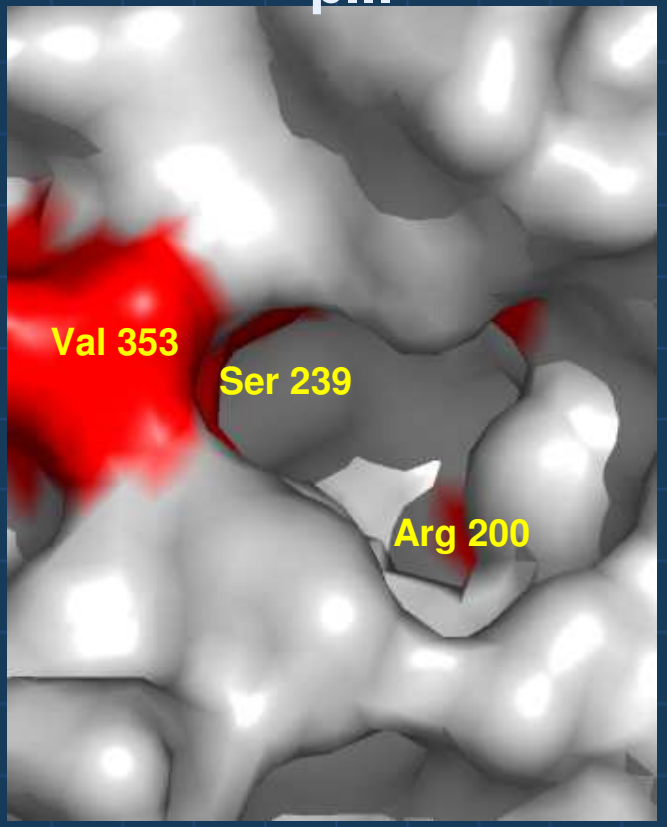
Tubulin residues involved in interactions with Colchicine

The colchicine binding site βII and βIII Tubulin

βII



βIII

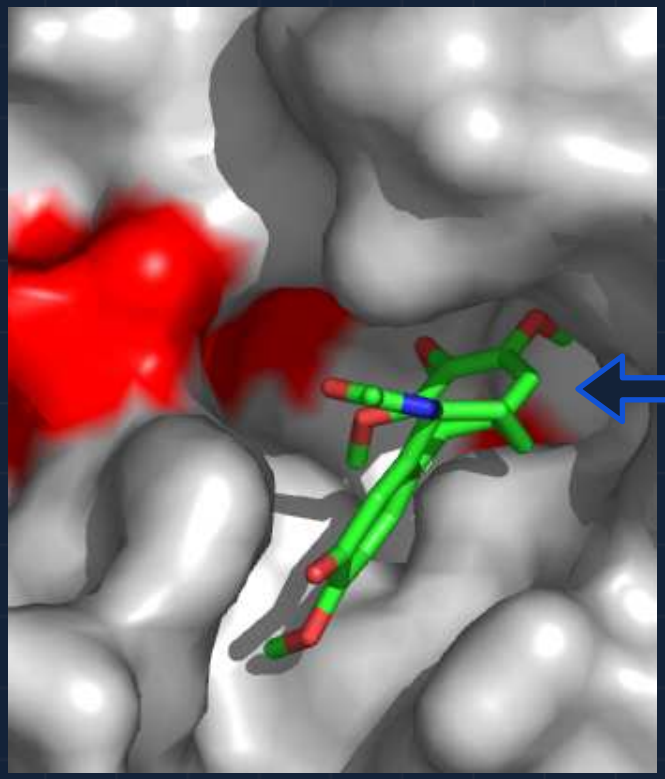


Thr (polar)-> Val (no)
Cys (polar)->Ser (polar)

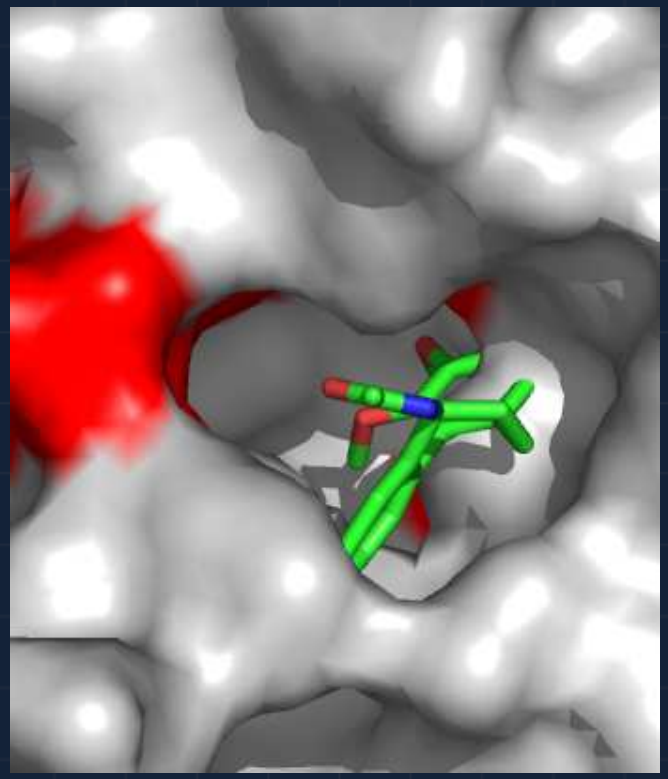
Tyr (polar)->Arg (+charged)

Colchicine bound to βII and βIII Tubulin

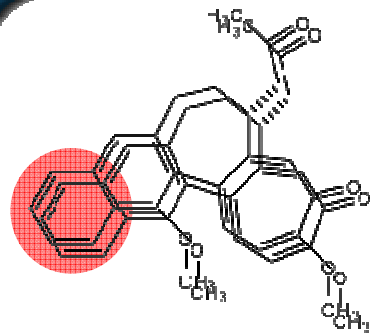
βII



βIII

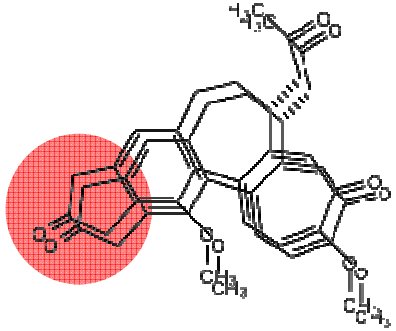


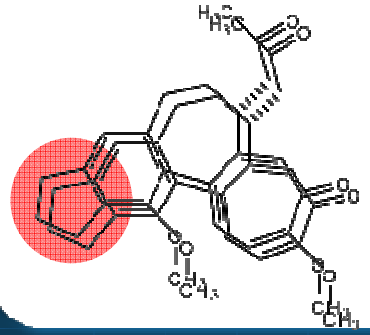
Colchicine derivatives that may differentiate isotypes



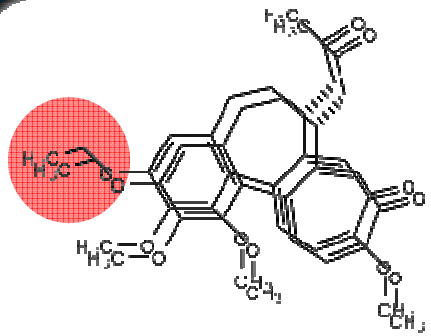
6 membered aromatic ring

5 membered oxygen substituted



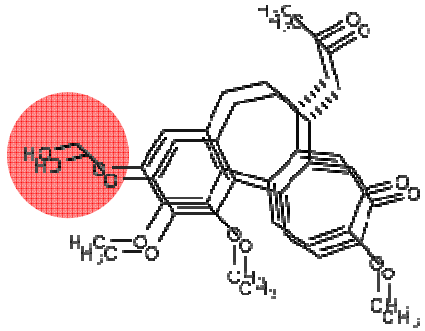


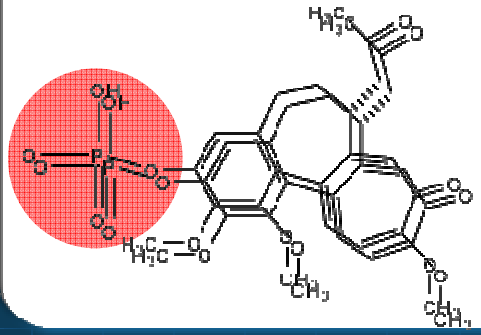
5 membered ring



extended methyl

extended hydroxyl

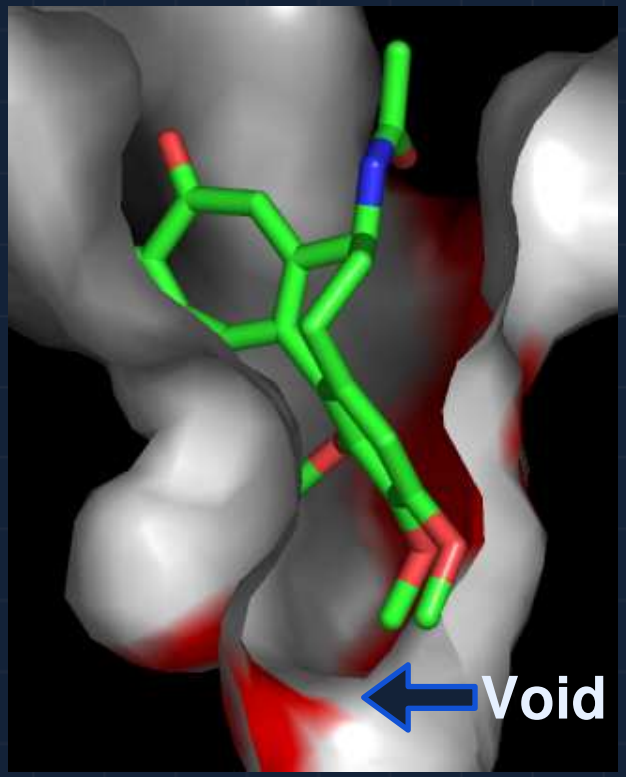




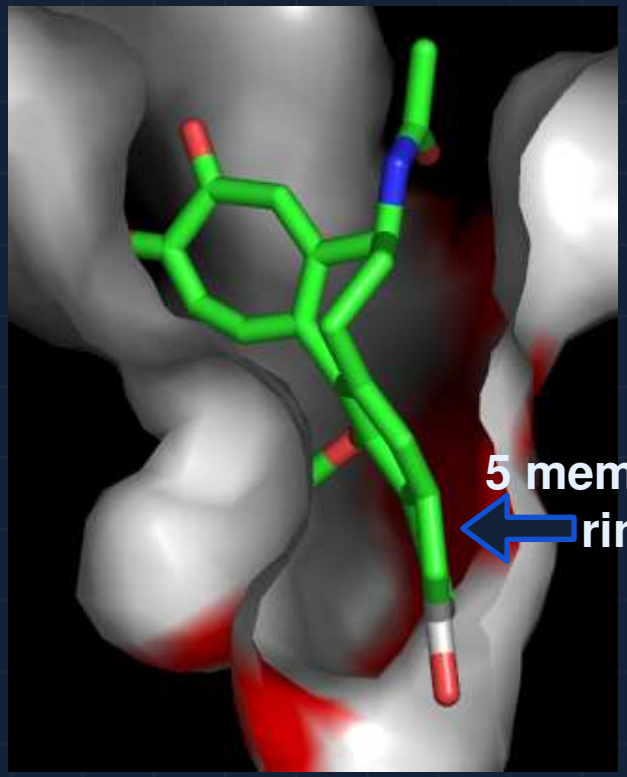
phosphate

Colchicine derivatives bound to Tubulin isotypes

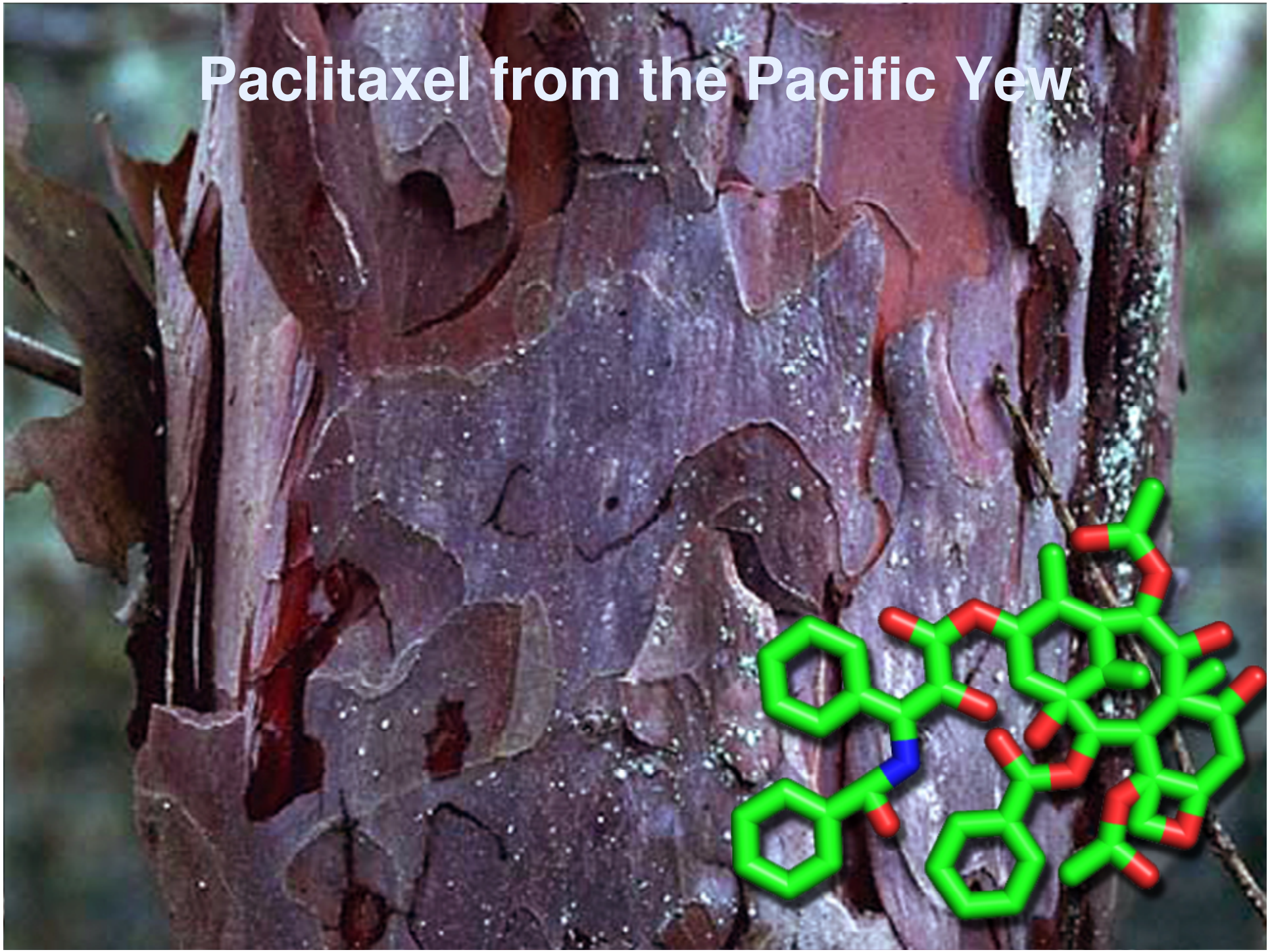
βII



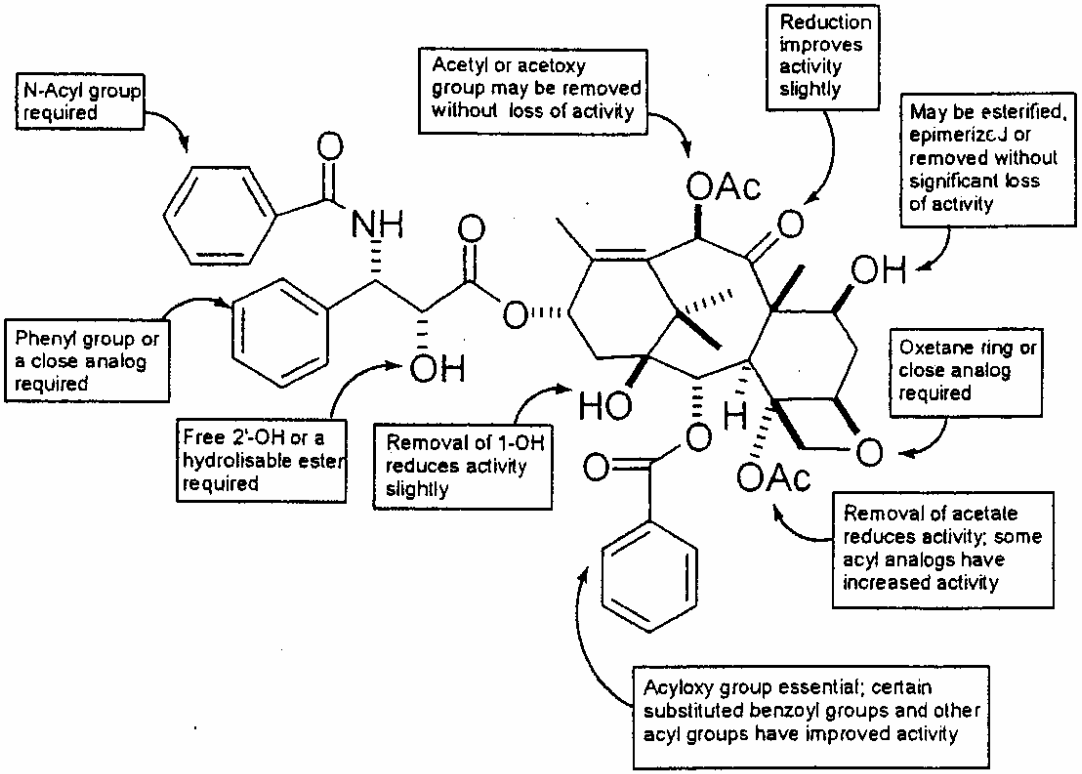
βIII



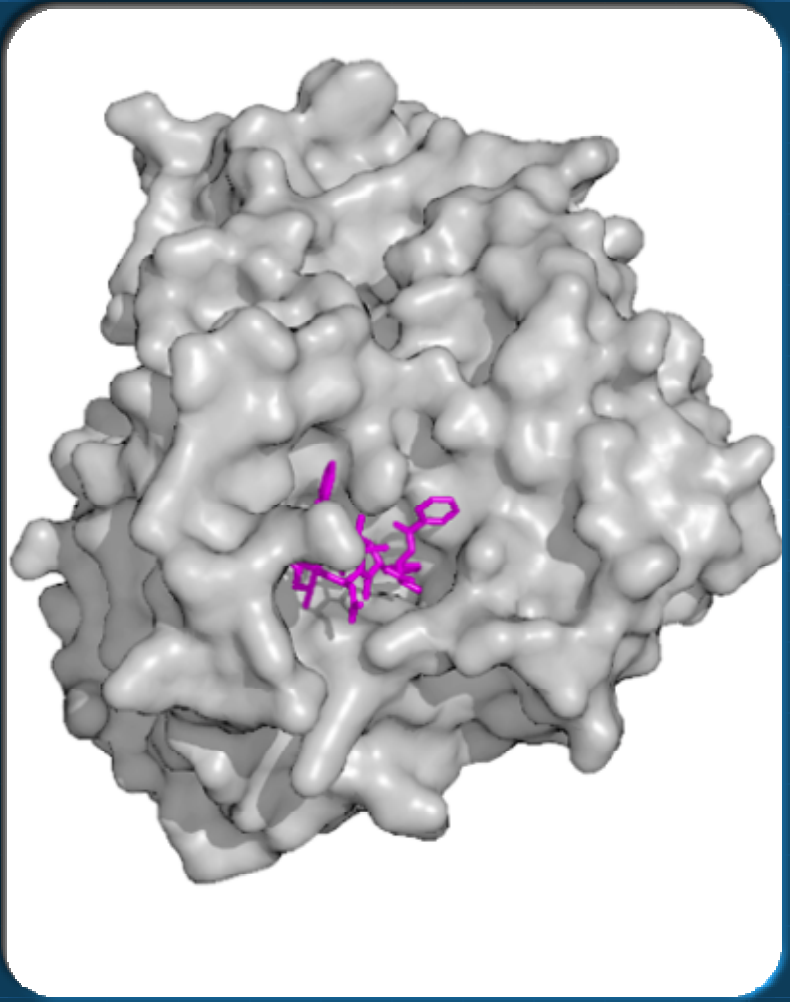
Paclitaxel from the Pacific Yew

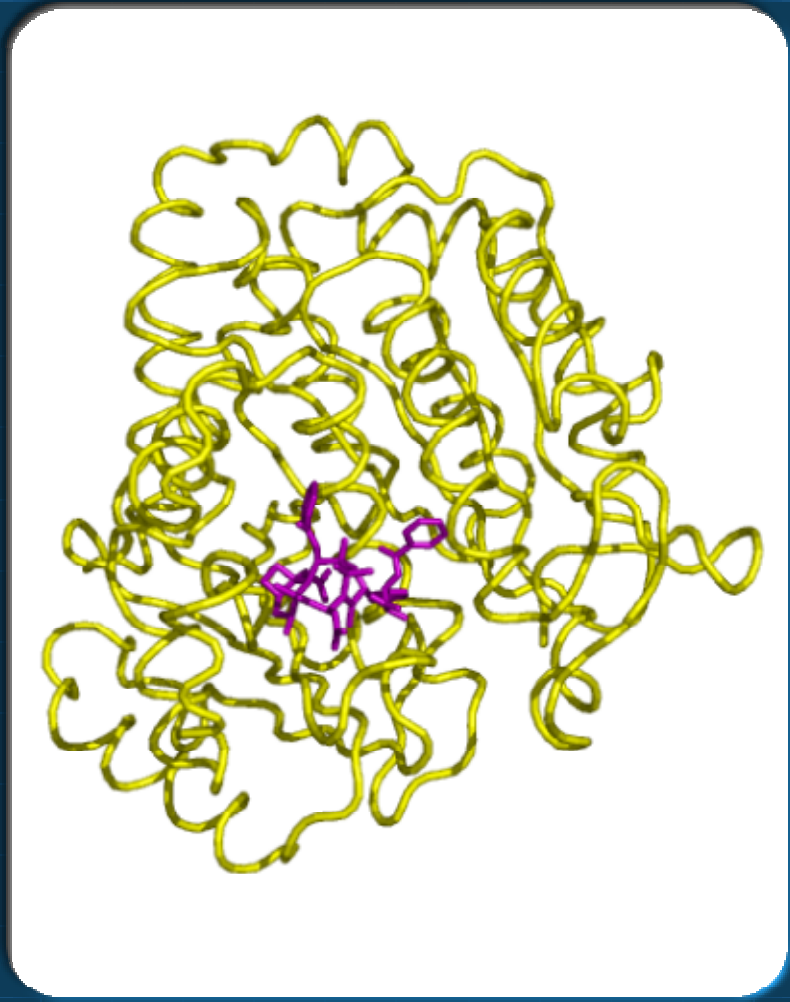


Structure-activity relationship of taxol



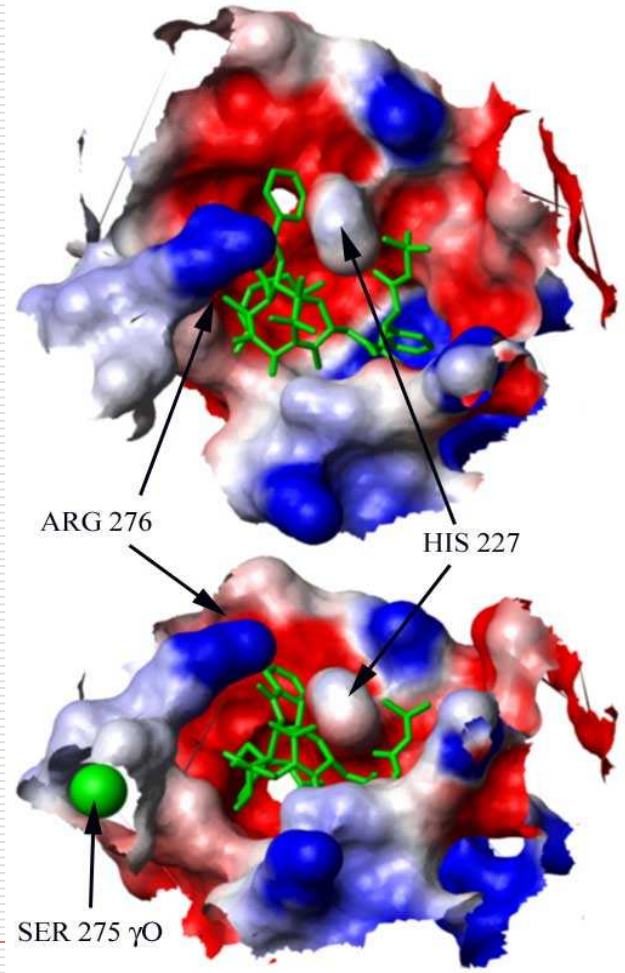
Drug binding example: Taxol bound to Tubulin

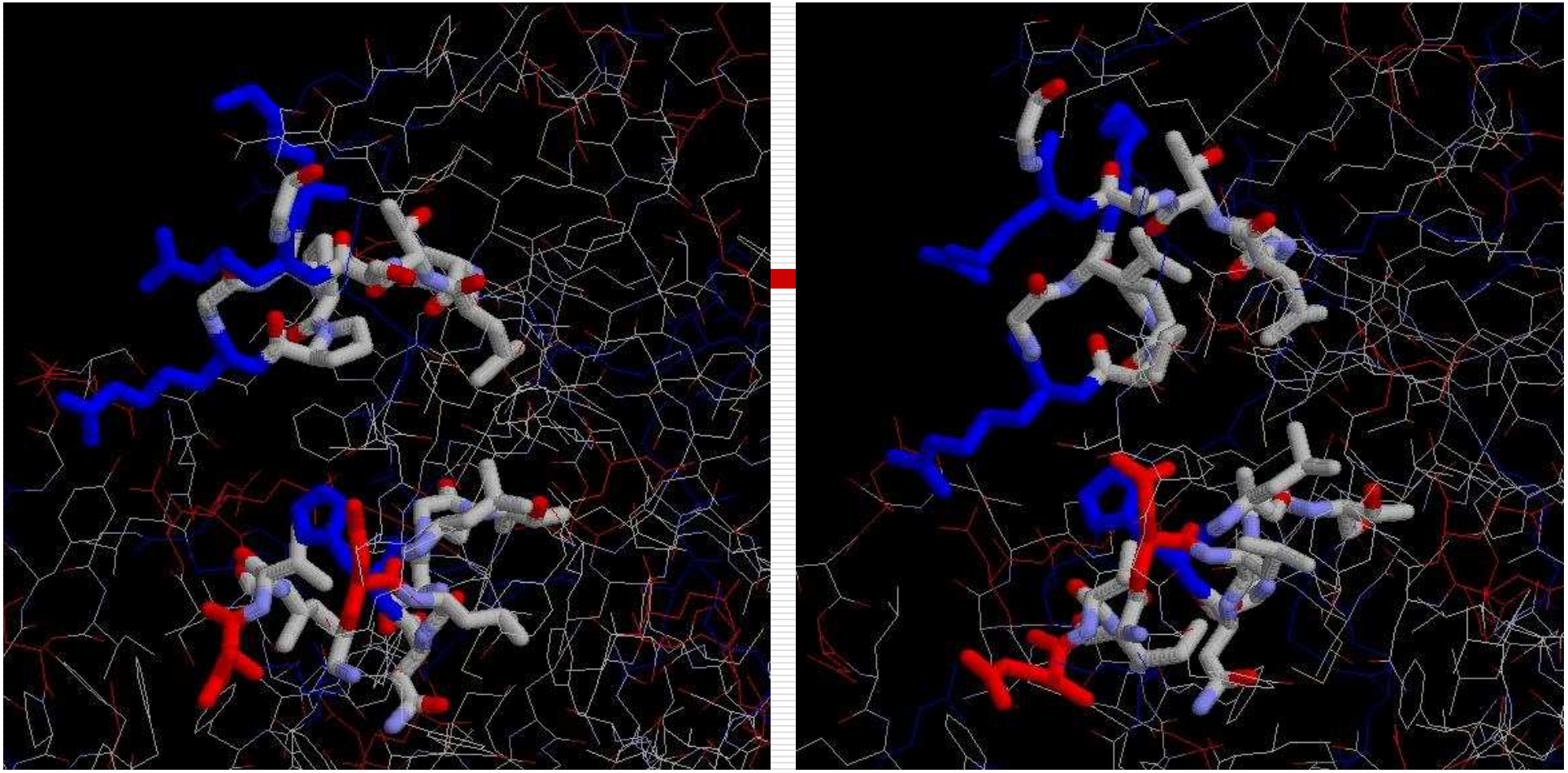




Taxol in its binding site

top view and at a 45 deg angle (bottom).
The white bump in the centre of the pocket is the ring of His 227 viewed edge-on;
the taxoid is wrapped around it.
The oxygen in serine (green in the bottom pic) is about 4 A from taxol which is replaced in TBB4_HUMAN by alanine. That means that OH is striped off.

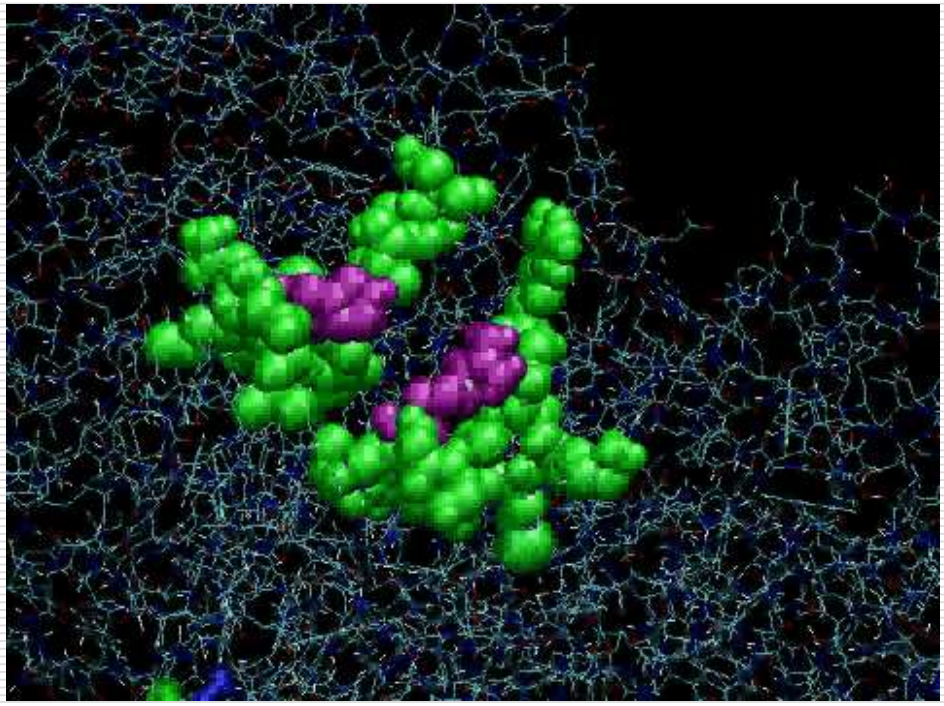




βI βIII

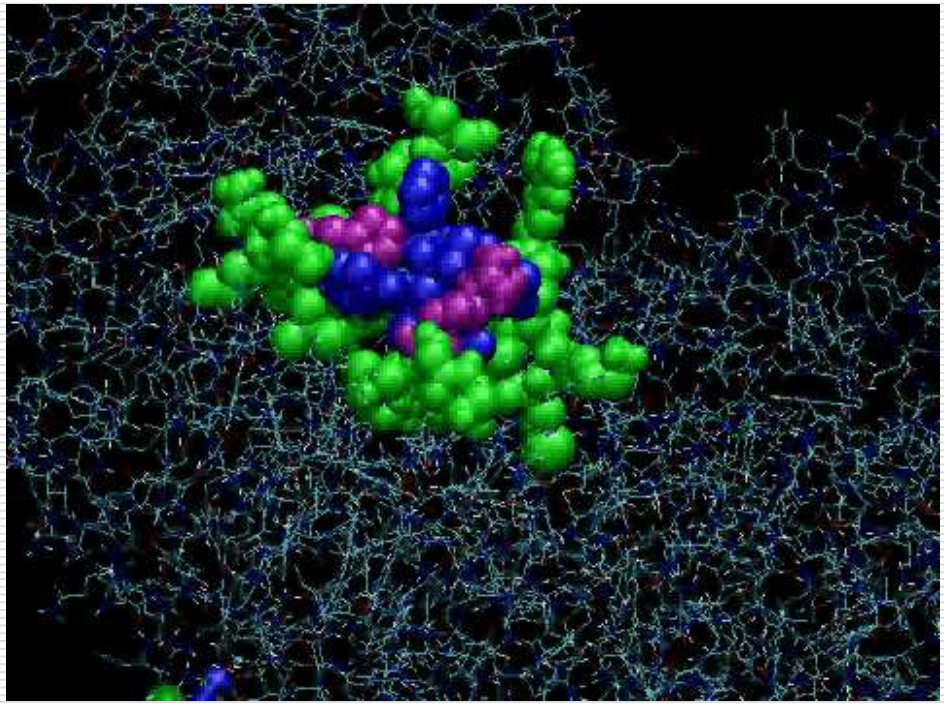
Taxol Binding Site
Acidic residues (red); basic residues (blue)

Taxol binding site in a MT



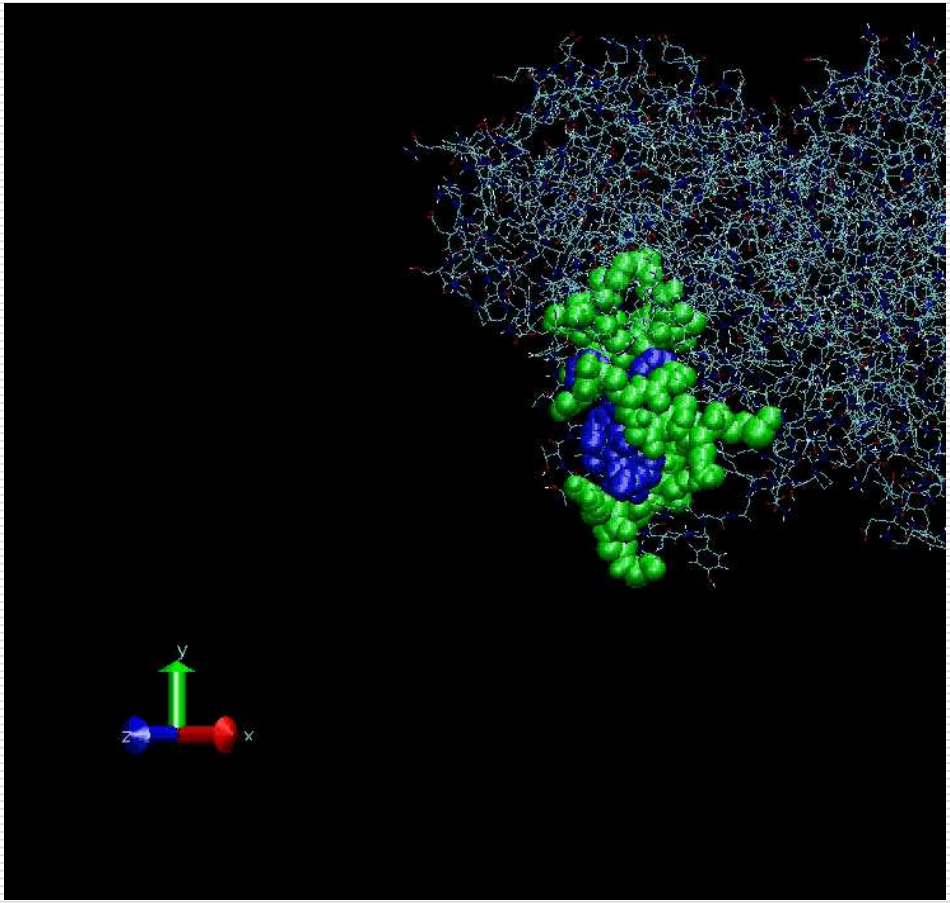
start 300 K, than heat to 500 K for 50 ps and then cool down to 300 K for 240 ps

Taxol bound to a MT



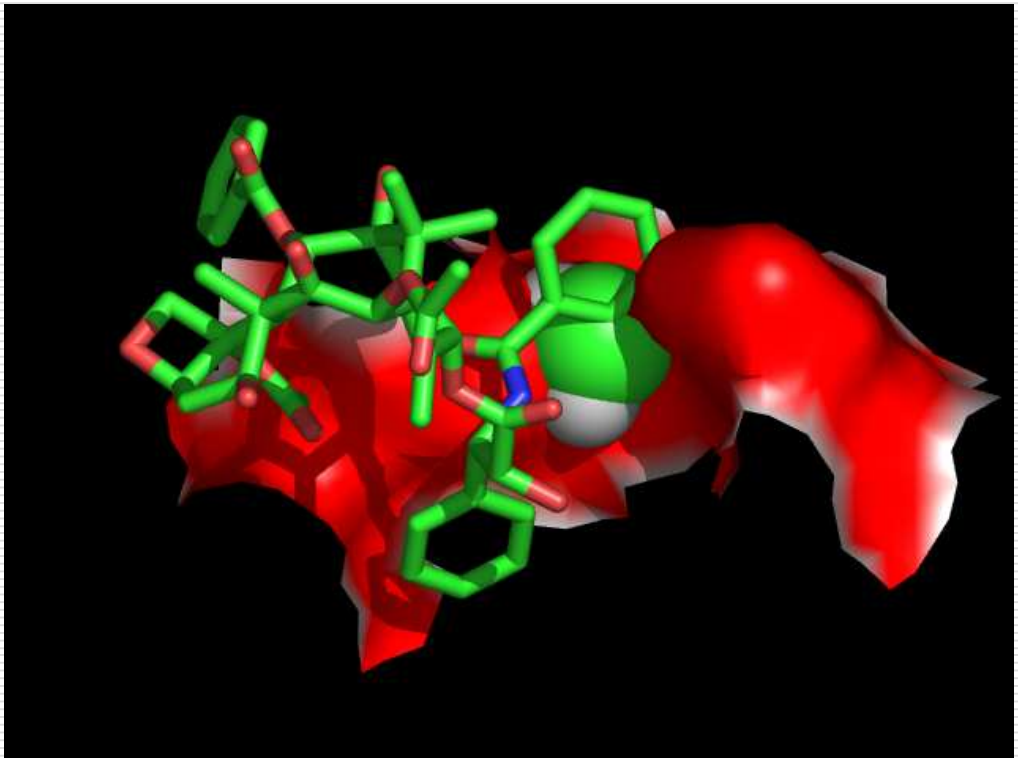
start 300 K, then heat to 500 K for 50 ps then keep at 500 K for 50 ps and finally cool down to 300 K for 240 ps.

Force application to remove taxol in b 1 tubulin

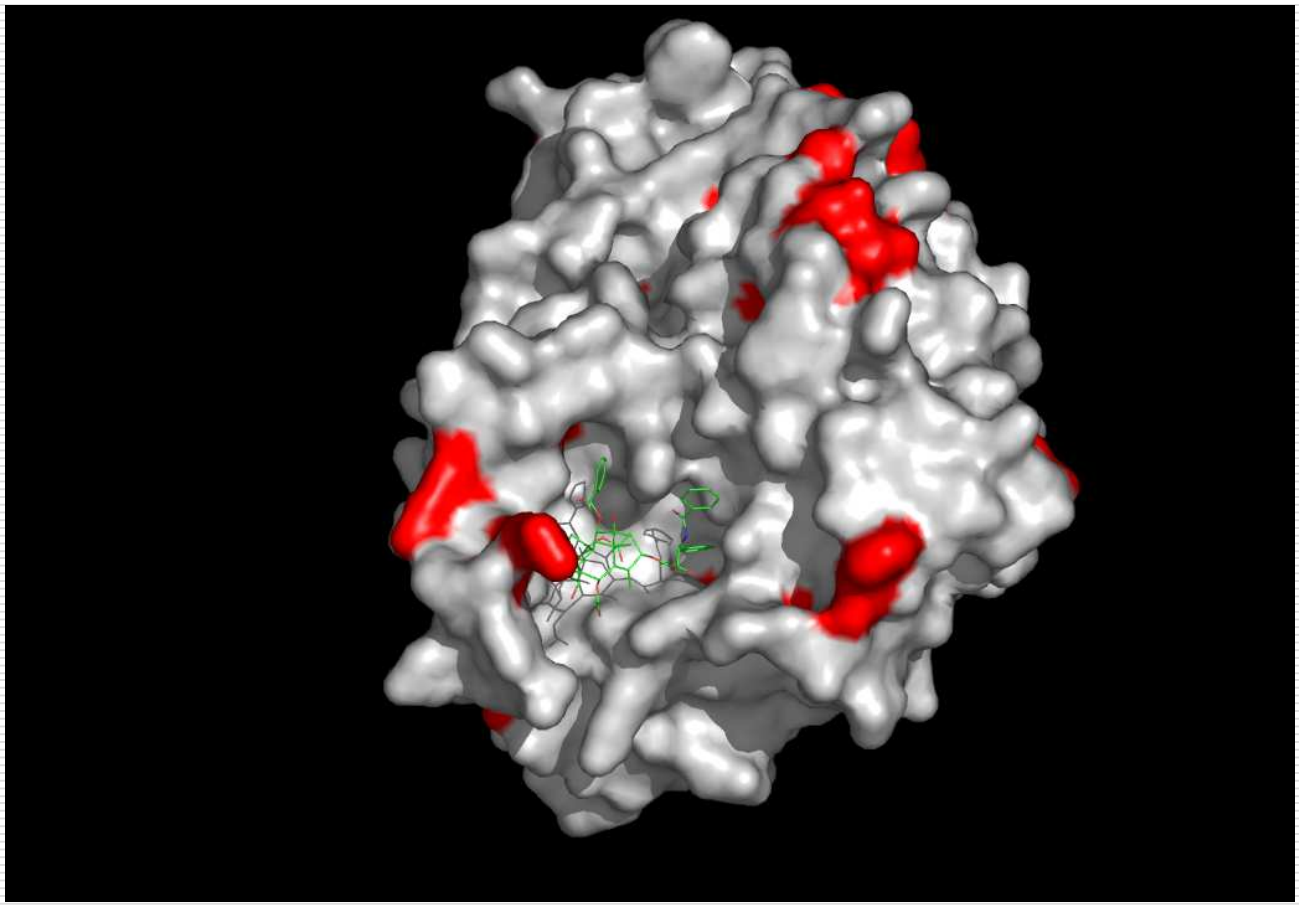


Used to quantify the binding strength for different isotypes of tubulin

New compound to target beta VI: taxol with a methyl group at C-10



Further possibilites: SNP’s



Alberta/Texas/New York collaboration:
OUR GENERAL STRATEGY:

1. Model the isotype-drug binding sites
2. Design isotype-specific drugs
3. Simulate their action by computer models
4. Synthesize the drugs using well-known organic synthetic procedures and try to design drugs less likely to cross the blood-brain barrier.
5. Test the drugs for isotype specificity in vitro using fluorescence assays
6. Test the drugs on normal and transformed cells (e.g., breast and prostate). Effects on cell viability, mitotic arrest, microtubule distribution and tumor colony-forming ability will be measured.
7. Test the drugs in animal models. Mice with tumors will be used. Effectiveness of drugs against tumors will be measured. Side effects will be identified and toxicity determined.
8. Iterate the process.