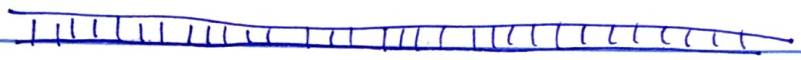
Lecture 1 - Part 1

Life of Tubulin

Like all life, the life of tubulin starts of as another, different Biopolymer: DNA.

You, no doubt, have discussed DNA
You should know what it is
how it coils + folds
how it stretches

Know how it bounds to polymerize out of A, T, G, C



Sequence for tubulin codes the RNA that codes the polypeptide (Central Dogma)

Sequences of Tubulin DNA – Use projector to show

		Sequences	exons/introns	
Yeast: (Budding)	α	TUB1, TUB3	X	
	β	TUB2	X	
	γ	TUB4	X	
Human:	α	TUBA 1, 2, 3, 8	numerous	
	β	TUBB a, b, c, d, e (1, 2, 3, ...)	numerous	pseudogenes
	γ	TUBG 1, 2	numerous	

Methods in Cell Biology

Projector: ① Yeast Tubulin sequences.
② Human Tubulin isoforms tab

Types of tubulin:

alpha - part of α-β dimer that forms filaments.

beta - same ↗

gamma - part of γ-TUB ring complex that nucleates filaments in cell.

isotypes of tubulin - different sequences

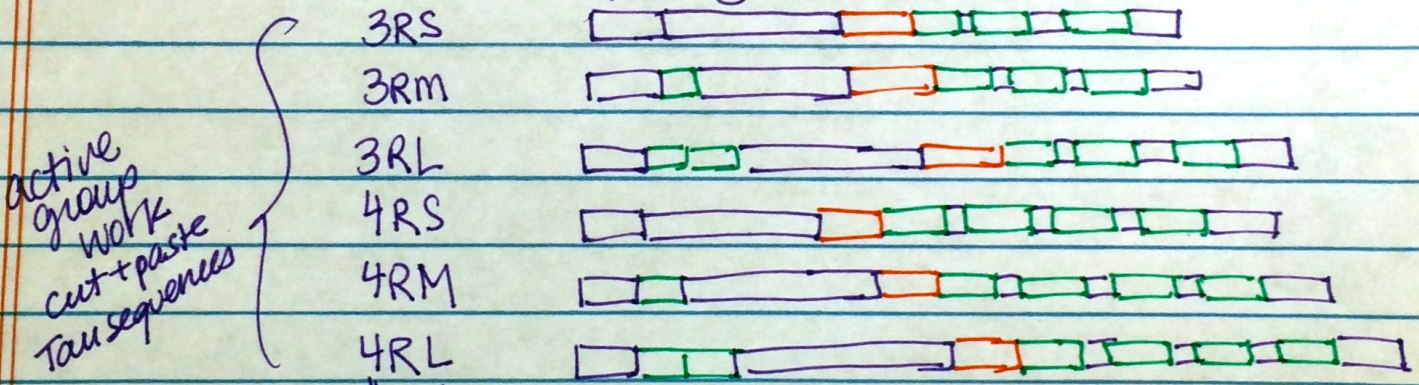
vs.

isoforms - one sequence, different splice variants

⊛ An aside and a reminder about Tau.

Tau has 6 isoforms

~~Comp~~ Come from splicing DNA → RNA



# of MT binding repeats in Carboxy terminus

3 vs 4

short / medium / long } # of repeats in amino terminus

*There is also a PNS/Spinal version that is even longer!

Tubulin isotypes: Table

DNA → RNA splicing out unwanted regions

define: exons: segment of DNA that does code for protein

introns: segment of DNA that does not code for protein

RNA - single chain, flexible, self-interacting + foldable.

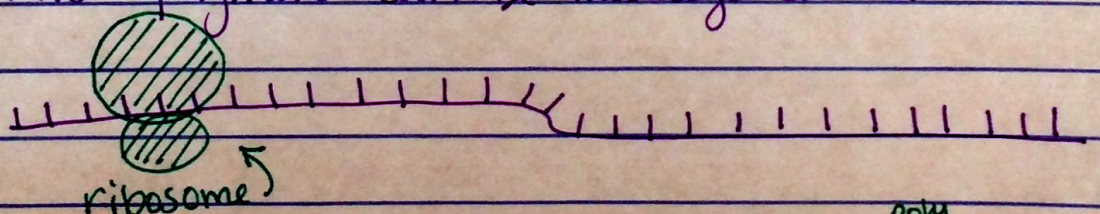
Folds have functions

Riboswitch: regulatory segment of mRNA that binds to a small molecule that can result in production of proteins encoded by the mRNA

mRNA: messanger RNA, carries sequence to be encoded into protein

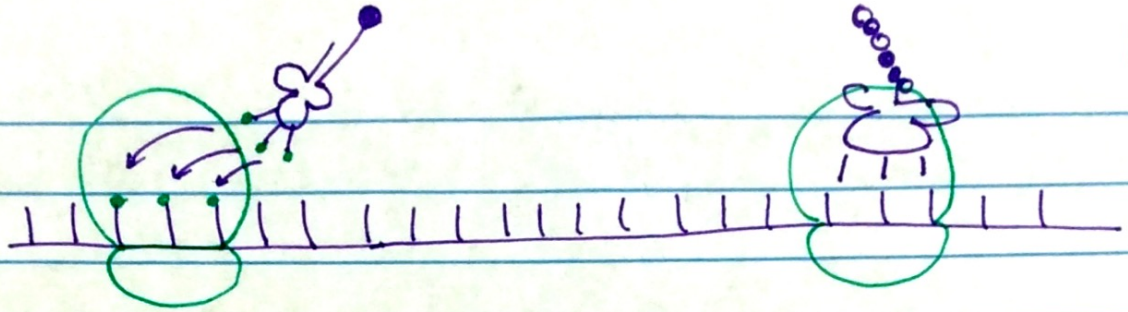
tRNA: transfer RNA, decodes / binds to mRNA and brings amino acid for polymerization.

Polymers can be machines when folded.
-AND- polymers can be message carriers
} OR both



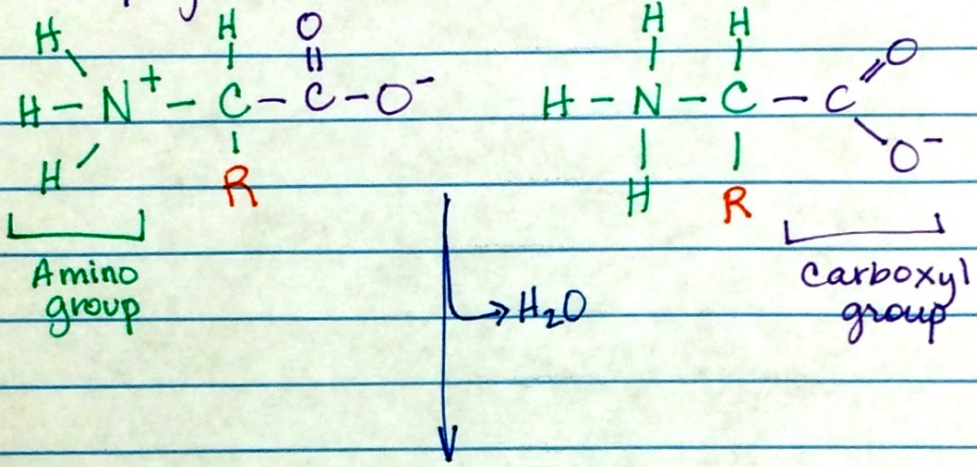
↳ ribosome is a machine made of polypeptides + RNA polymers

Machine job: to make protein from mRNA sequence.



creates the polypeptide chain

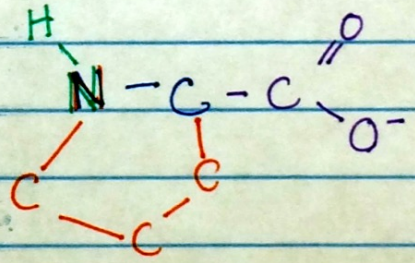
Peptide polymerization



Repeat...

Most polypeptides are freely jointed chains
($L_p \sim 1$ amino acid) (no persistence/no memory)

EXCEPT: Proline



Amino terminus also part of the R-group.

proteins with stretches of prolines are kinky + not freely jointed

Reminder back to Tau protein:

N-terminus

C-terminus

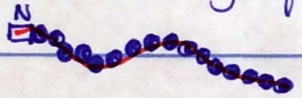
proline rich domain has "structure" - more than freely jointed

BACK TO TUBULIN SYNTHESIS:

OK, so now the tubulin peptide sequence is coming out.

What does it do?

Does it stay freely jointed?



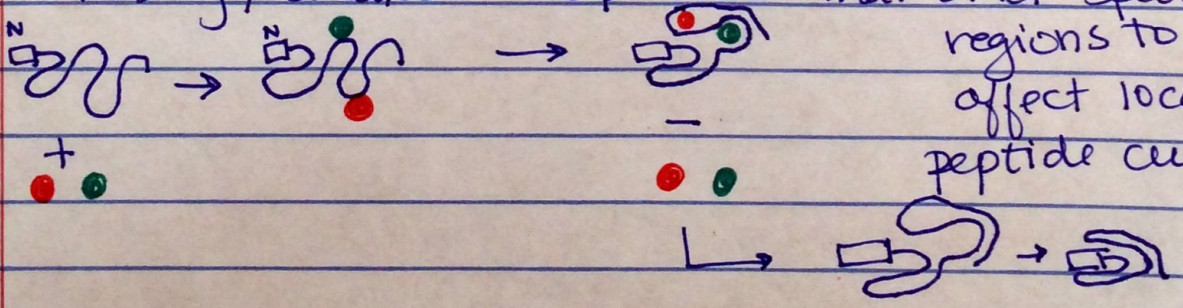
or does it begin folding?

Likely to begin folding thru electrostatic + hydrophobic R-group interactions

TUBULIN is a machine. specifically a GTPase.

folded structure requires chaperones

Mostly, smaller chaperones that bind specific regions to affect local peptide curvature



Folded Structure of tubulin

Project on screen

Note: N-terminus, bound in structure
C-terminus, hanging off.
α, β dimer.
α, β similarities
GTP-bind site.

Compare structures of 2 yeast tubulin isotypes

Project on screen

Compare TUB1 and TUB3
sequences + structures.

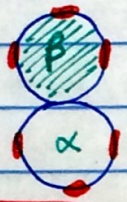
-BREAK-

Ended last time: we now have correctly folded + dimerized α, β tubulin

Let's build some things!

pop beads

Tubulin dimers are like sticky bricks



Red regions can stick to each other

Can make a lot of different structures

Projection of pictures

Microtubules, "necklaces", double rings

GDP Rings, Zn sheets

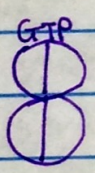
Many of structures are curved

b/c dimer has intrinsic curvature depending on GTP hydrolysis state

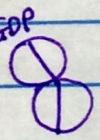
GDP/GTP ← E-site (Exchangeable) can have GTP, hydrolysis to GDP

GTP ← N-site (non-exchangeable) always has GTP

Project Dimer structure.



vs.



12°

GDP rings

radius of curvature:

if double ring, angles are 11° and 15°

11° ← outer

15° → inner

curvature change is one function of GTP hydrolysis.

Goal: To build a MT

Need to understand: ① end state structure
② subunits + their interactions

Part 1 - structure

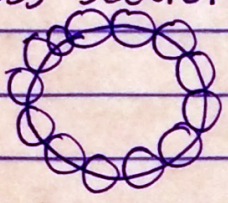
Structure typically found in cells

13 Protofilaments

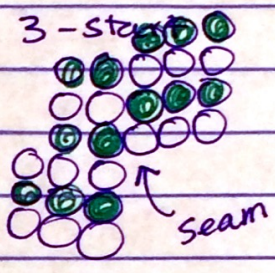
3- Start Helix

Look at projector

Cross-section



13 Protofilaments.



LOOK AT 3-D Printed model pass around.

Helix is very clear. in model

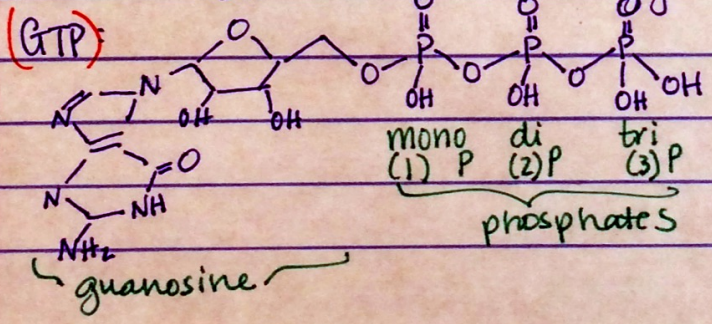
Activity - Pop Beads to make lattice
Spread out on desk.

Homework - assemble MT structure
from Beads or other stuff

Part 2 - dimer-dimer interactions

Start w/ GTP (or GMPCPP) dimers in straight conformation

Side note about nucleotide energy sources: ATP, GTP, CTP, TTP



GTP OR GMPCPP Tubulin 8 straight

in good salt / buffer PIPES-K pH to 6.8

free acid → Base to pH

with Mg^{2+} ions → enzymes NEED Magnesium to function

without Ca^{2+} ions → Ca^{2+} ions cause MTs to fall apart

↳ use EGTA to sequester + "Chelate" Ca^{2+} ions

Competes w/ Mg^{2+}? Same charge, wrong size

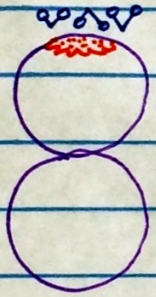
High concentration > 1 μM

+ 37°C

↳ polymerization driven by ENTROPY

(*) Microtubule Polymerization: Non-covalent, entropy driven (*)

8 sticky patches ~ electrostatic + hydrophobic

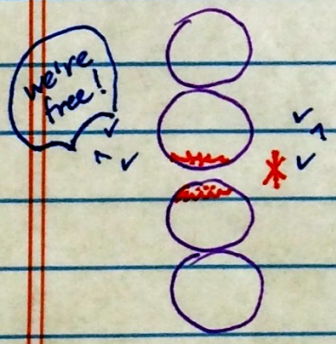


← water molecules low affinity w/ greasy (oil, hydrophobic) patch. Water has to be there - no vaccuums

so, spreads out (water "lattice")

When two grease patches match together, water molecules are released

S_{water} ↑



So, process is driven by Entropy of water.

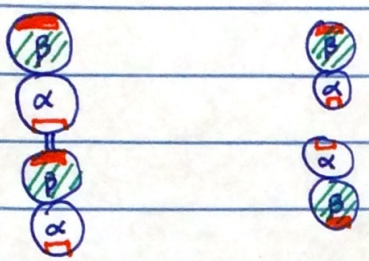
Specific dimer-dimer binding

{and subsequent long-lasting polymerization}

SPECIFIED by electrostatic interactions + Steric interactions

Lock + key biochemical binding.

Due to specific binding, dimers bind w/ specific orientation



YES

NO

1D lattice protofilament

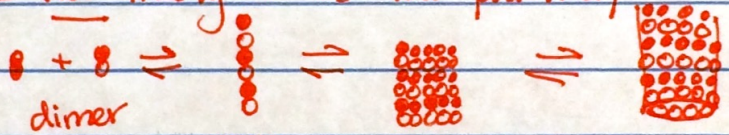
But MT is not a 1D lattice.

On THURSDAY, we will discuss models of MT assembly. 2D nature needed to recapitulate dynamics.

To start polymerization rxn, always need NUCLEATION

The 1D lattice is not a stable nucleation state!

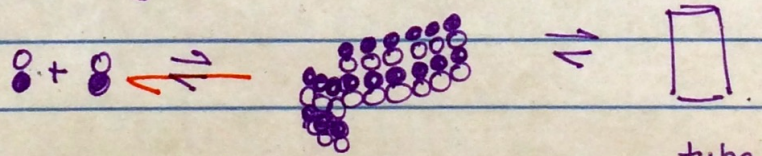
This is not likely to be the pathway:



No

How do we know? We never see protofilament or sheet when making MTs.

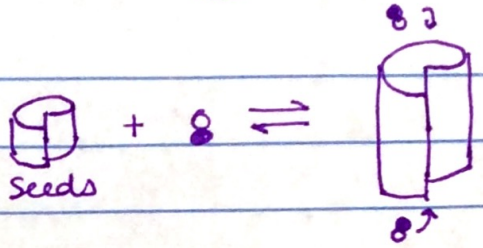
Likely pathway



Seed of $13 \times 2 = 26$ Dimers

nucleation site of 26 dimers

Once the seeds are established can elongate off the seeds to grow MTs.



Dimers bind to both ends.

Plus End – topped w/ β tubulin exposed

Minus End – topped w/ α tubulin exposed

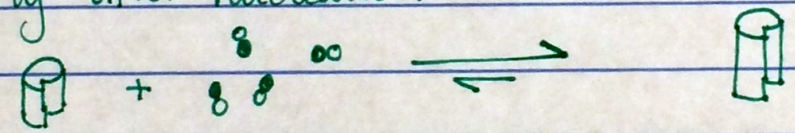
Two ends are chemically + structurally distinct

Dimers add to plus end faster (higher affinity) than minus end (lower affinity)

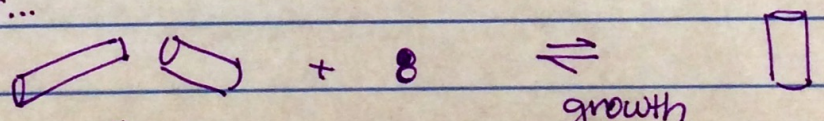
✱ This is weird. This is open. Why is plus-end different from minus-end?

minus-end regulation is modern / fashionable topic currently.

Early after nucleation



Later...



✱ At EQUILIBRIUM, # dimers adding to filaments / sec equals # dimers dissociating from filaments / sec

Dynamic Instability ⟸

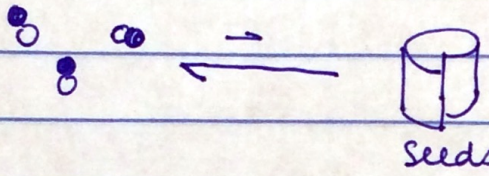
~~if using GMPCPP slowly hydro~~

GTP hydrolysis → dimer curvature

↓

destabilization of structure

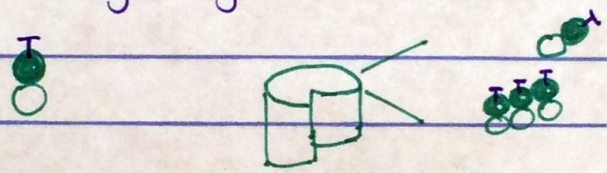
Affects nucleation

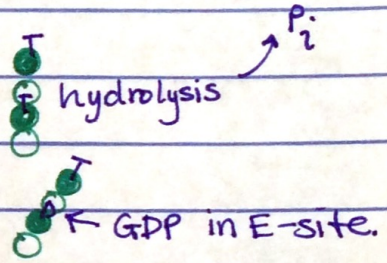


seeds are not stable
Very few form.

Affects equilibrium structure. → dynamic instability

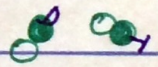
When does hydrolysis occur?



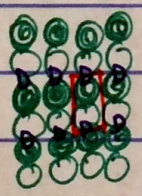


This is why protofilaments are unstable (ways to stabilize them exist: Ca^{2+}, binding proteins)

↳ release of dimer



Inside the MT body, all GDP (mostly) (it is probabilistic if hydrolysis occurs)



Dimer in middle is held in place by lattice - lateral and ~~tog~~ longitudinal binding partners.

It wants to bend back, but is mechanically constrained to be straight. ⊛ Entire filament wants to fall apart, but held together by neighbors + GTP-cap

dimers at top are most vulnerable to loss, but they have GTP, so

① They are straight

② They have tighter neighbor-dimer interactions

These dimers at top w/ GTP constitute the GTP-cap.

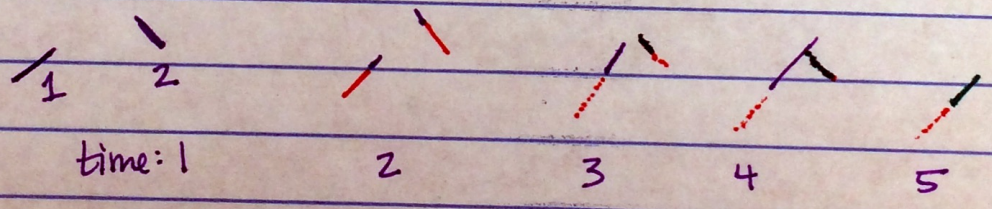
The ~~random~~ probabilistic hydrolysis of GTP coupled with the shape of distribution of dimers at end

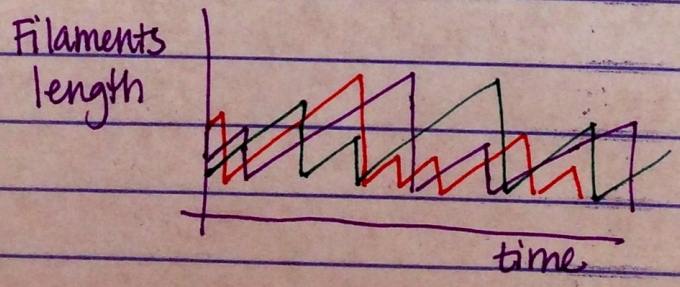
+ GTP hydrolysis causing bending - mechanical strain

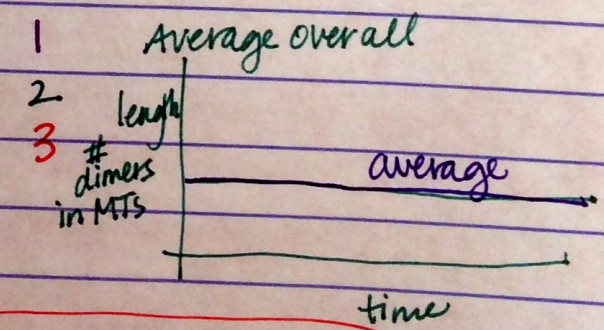
Leads to dynamic instability

At EQUILIBRIUM, total # dimers associating / sec = total # dimers dissociating / sec

but not all from ~~the same~~ all the filaments



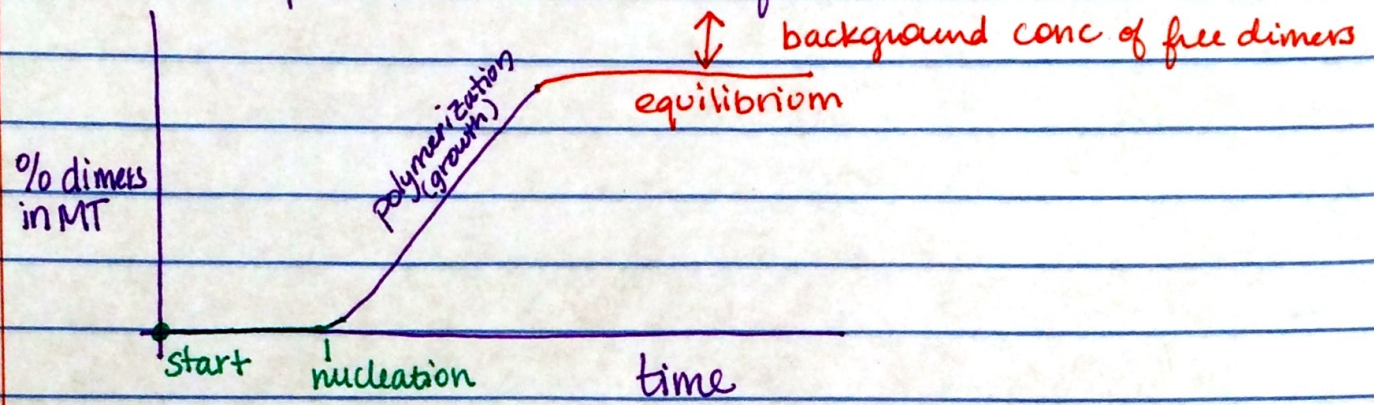




Projector - dynamic instability movie - Megan

Problems:

We can plot a bulk data of Dimer state over time



At t=0, 0 dimers are polymerized

1st step = nucleation, only very few dimers make up nucleation states (seeds) seeds are unstable

2nd step = growth. once enough seeds formed can get growth. Dynamic instability still exists

3rd step - equilibrium. MTs undergoing dynamic instability. Background level of dimers is constant. Flux of dimers into MTs = Flux out of MTs

Take home problems:

① How will plot change, if ~~ed~~ use GMPCPP instead of GTP to stabilize structures against hydrolysis-based changes.

② How will the plot change, if we add nucleation seeds at t=0?