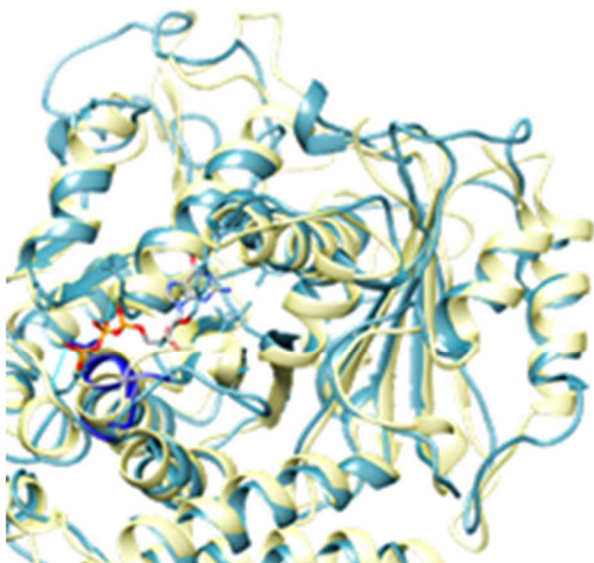


## **Microtubules lab: «evolution of tubulins and microtubules»**



2020



SMTB

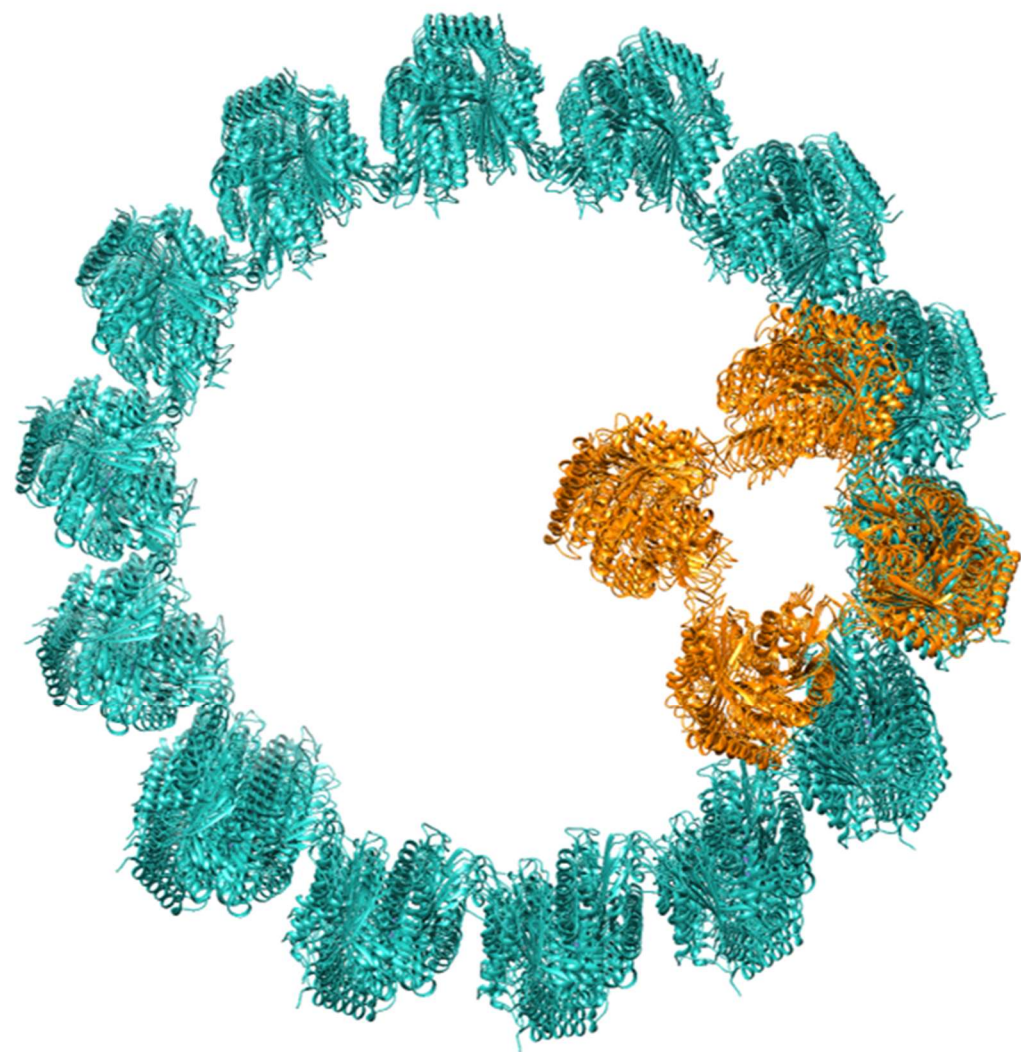


**Project leader:** Nikita Gudimchuk, Alena Korshunova

**Faculty team members:** Anastasia Masaltseva, Lyuba Makarova,, Iuliia Lopanskaia, Varvara Dreval

**Students:** Alexey Kulikov, Anvar Normurodov, Yaroslav Demenchuk, Anna Kalygina, Youssef Hossam, Dasha Semyonova, Vera Turtapkina, Julia Trofimova



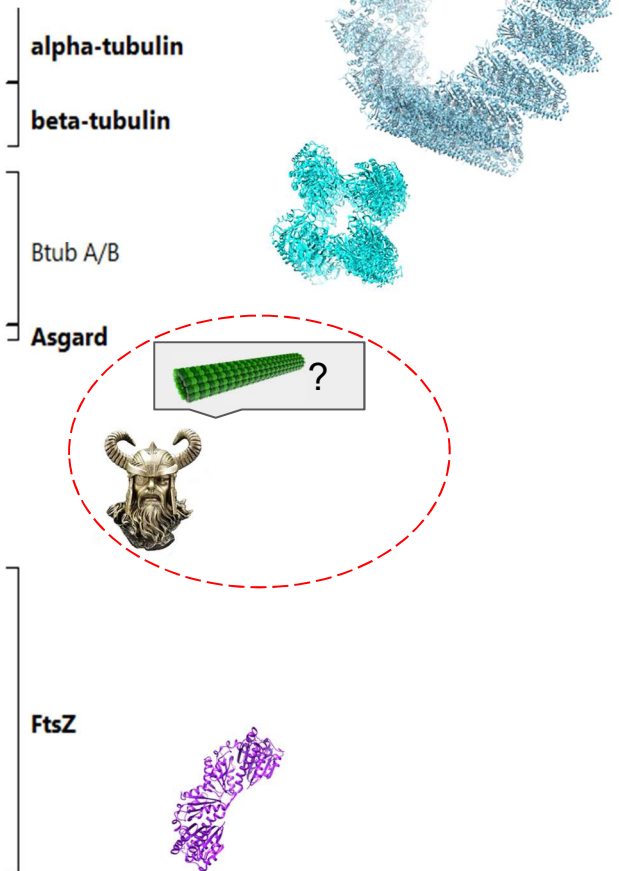
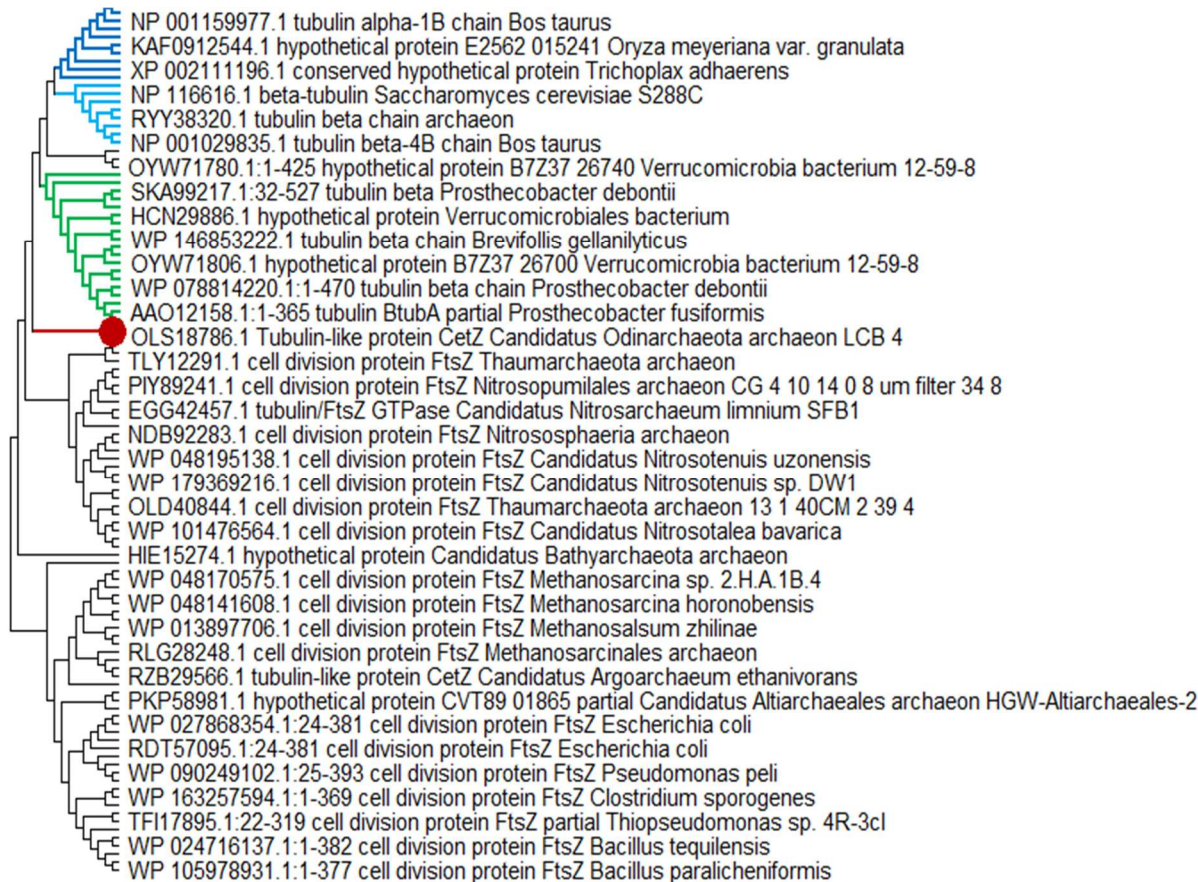


# Outline

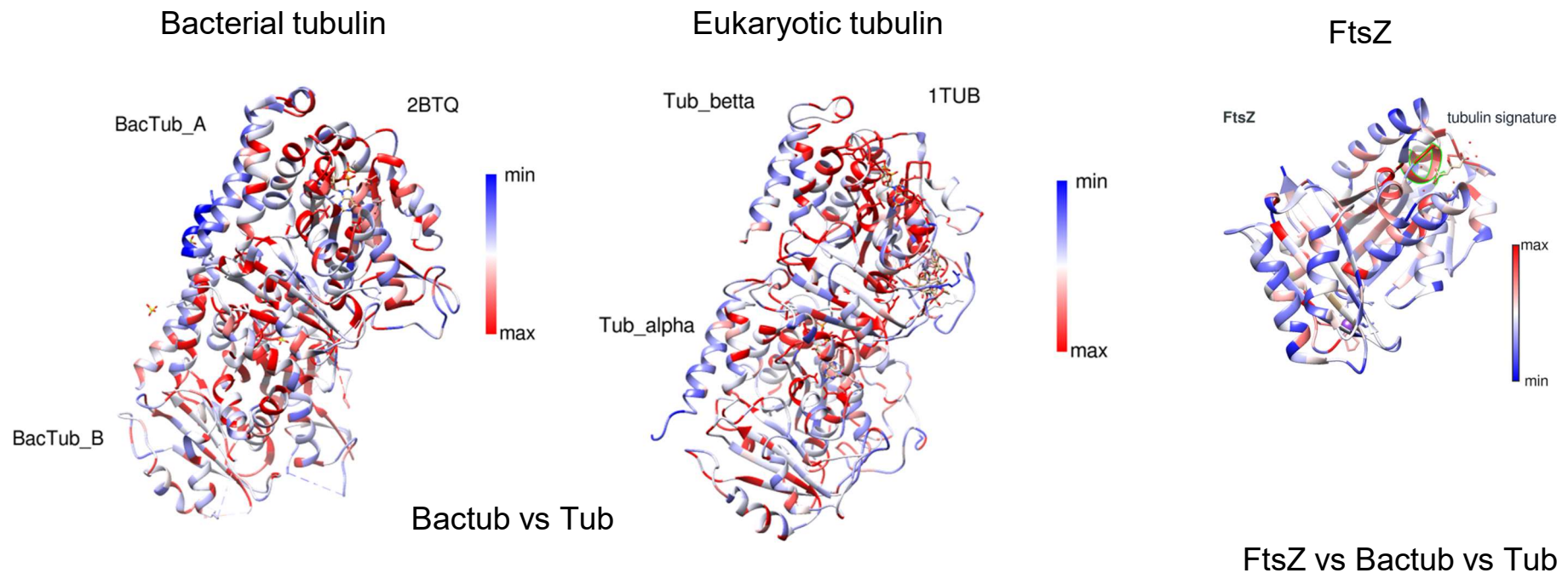
- 1) Part 1: Tubulin evolution
- 1) Part 2: Intra- and inter-dimer interfaces of eukaryotic and bacterial tubulins
- 1) Part 3: Sequence conservation and structure of bacterial tubulin tails

# Part 1: Tubulin evolution

## Tubulin-like proteins Phylogenetic Tree: is the **Odin-tubulin** a missing link from filaments to MT?

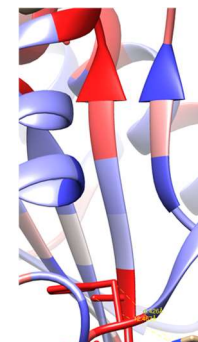
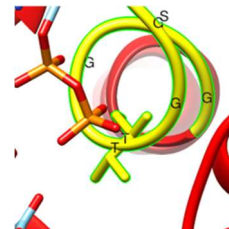
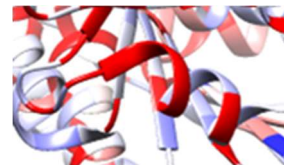


## Amino acids conserved region comparison reveals the bona fide tubulin specific sites



## The “dimerization site” and “MT harpoon” region - hypothetical signs of MT formation?

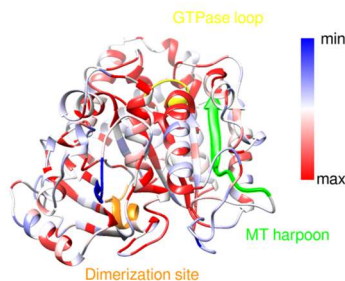
	dimerization site	GTPase loop	MT harpoon	
CAJ14012.3_bacterial_tubulin_A_Prostheco	LRELLTINLVQPQSLHFLM	IIIVLHATGGGGSGFGALLI	YVPRRAVMVDLEFSVID	bacteria
HCN78743.1_hypothetical_protein_Verrucomicrobia	LRELLTINLVQPQSLHFLM	VIIVLHAVGGGGSGGLGALLVI	FVPRRAVMVDLEFSVIQ	
NNE90788.1_tubulin_beta_chain_Verrucomicrobiale	IREFVITINLVKPKGLHFLI	IFIIHSLGGGTGSGLGCLMI	YVPRITVMIDLEFGVID	
OAI56928.1_hypothetical_protein_AYO49_03290_Ver	LRELLTINLVQPQSLHFLM	IIITLHSLGGGTGSGLGARIM	YVPRRAVMCDLEFSVIE	
OAI56929.1:1-416_hypothetical_protein_AYO49_032	LGEEFLTNLVFPFGCHFLT	FLITLHSLGGGTGSGGLGALLII	YVPRRAVLIDLEFGVIA	
SKA99217.1:32-527_tubulin_beta_Prostheco	LRELLTINLVQPQSLHFLM	IIIVLHAIIGGGSGSGFGSLLI	YVPRRAVMVDLEFSVIN	eukaryotes
WP_133797138.1:1-468_tubulin_beta_chain_Prosthe	LRELLTINLVQPQSLHFLM	IIIVLHAIIGGGSGSGFGTLLI	YVPRRAIMVDLEFSVID	
WP_133797138.1_tubulin_beta_chain_Prostheco	LRELLTINLVQPQSLHFLM	IIIVLHAIIGGGSGSGFGTLLI	YVPRRAIMVDLEFSVID	
NP_001029835.1_tubulin_beta-4B_chain_Bos_taurus	LRKLAVNMVFPFRLHFFM	FQLTHSLGGGTGSGMGTLLI	YVPRRAVLVDLEFGTMD	
NP_116093.1_tubulin_alpha-1C_chain_isoform_c_Ho	LTEFQITINLVYPRIHFPL	FLVFHSTGGGTGSGFTSLLM	HVPRRAVFVDLEFTVID	
NP_476772.1_alpha-Tubulin_at_84B_Drosophila_mel	LTEFQITINLVYPRIHFPL	IIIVHSTGGGTGSGFGSLLI	HVPRRAVFVDLEFTVVD	FtsZ
WP_146853222.1_tubulin_beta_chain_Brevifollis_g	LRELLTINLVQPQSLHFLM	YVVPFAMGGGTGSGFSSAFI	FVPRRAVMVDLEFSVIE	
WP_007550732.1_MULTISPECIES: cell division prot	MSNYRTWLATK.KPKFAI	FLITFHSFGGGTSGSGFTSLLM	LIPRALLVDLEFRAN	
RDT57095.1:24-381_cell_division_protein_FtsZ_Es	FADVRTIVMS...EMGYAM	VFTIAGMGGGTGTGAAPVVA	...FVNTDAQALR	
WP_027868354.1:24-381_cell_division_protein_Fts	FADVRTIVMS...EMGYAM	VFTIAGMGGGTGTGAAPVVA	...FVNTDAQALR	
HII79815.1_cell_division_protein_FtsZ_Methanosa	LGDFKTVMSG..GAGLAT	...GVGANNRNIGKQVEQN.	...LAFNTAVNDLK	
TBR06802.1_cell_division_protein_FtsZ_Candidatu	MSNYRTWLATK.RPKFAI	...GAARNWAEGRGRFKNE.	LIPRALLVDLEFRAN	
WP_010476989.1_cell_division_protein_FtsZ_Therm	ASDLKFVVKAF.GS.FAT	GGKGVNANFVLGREAMKRD.	FES..LAINTSRGDLE	
WP_101476564.1_cell_division_protein_FtsZ_Candi	MSNYRTWLATK.RPKFAI	...GAARNWAEGRTRFIKE.	LIPRALLVDLEFRAN	
WP_137710828.1_cell_division_protein_FtsZ_Halor	PADITRTLDLG.GVITVG	...GVGGDFDVGAQVAMAD.	TNGNVLAFHTESQPF	
WP_154809763.1_cell_division_protein_FtsZ_Metha	LGDFKTVMSG..GAGLAT	...GVGANNRNVGKHFVEDN.	...LAINTAVNDLK	
WP_163257594.1:1-369_cell_division_protein_FtsZ	FADVRTIMV...DKGLAH	..LGAGANPEIGKKAAEES.	...LAINTDKALM	



# Can “Odin tubulin” make microtubules?

	dimerization site	GTPase loop	MT harpoon
OLS18786.1 Tubulin-like protein CetZ_Candidatus	LMEMAHNLVLPETKFII	AFIIASASGGTGSFSPILI	FHPRALFIDLEFLAVE
RZB29566.1 tubulin-like protein CetZ_Candidatus	LGDFKTLTLAS..GARLAT	FLVIHTLGGGTGSGFGSLIT	...MAINTALNDLK
AOZ55973.1 beta-tubulin uncultured archaeon	LMEMAHNLVLPETKFII	FLVIHTLGGGTGSGFGSLIT	FHPRALFIDLEFLAVE
NJK78101.1 cell_division_protein_FtsZ_Nanoarcha	MSNYRTWLATK.KPKFAI	YVVPFAMGGGTGSGFSSAFI	LIPRALLVLDLDFRAAN
RYG61971.1 tubulin_beta_chain_archaeon	LRKLAVNMVFPFRLHFFM	FQLTHSGGGGTGSGMGTLLI	YVPRAILMDLDFGTMD
CAJ14012.3 bacterial_tubulin_A_Prosthecobacter	LRELLTNLVQPSSLHFLM	IIIVLHATGGGGSGFGALLI	YVPRAVMVDLEFSVID
HCN78743.1 hypothetical_protein_Verrucomicrobia	LRELLTNLVQPSSLHFLM	VIIVLHAVGGGGSGGLGALVI	FVPRAVMVDLEFSVIQ
NNE90788.1 tubulin_beta_chain_Verrucomicrobiale	IREFVTNLVFKPGLHFLI	IFIHSLGGGTGSGLGLCMI	YVPRTVMIDLEFGVID
OAI56928.1 hypothetical_protein_AYO49_03290_Ver	LRELLTNLVQPSSLHFLM	IIILHSLGGGTGSGLGARIM	YVPRVMCDLEFSVIE
OAI56929.1:1-416 hypothetical_protein_AYO49_032	LGDFLTNLVFPFGCHFLT	FLTHSGGGGGSGGLGALLI	YVPRVLIDLEFGVIA
SKA99217.1:32-527 tubulin_beta_Prosthecobacter	LRELLTNLVQPSSLHFLM	IIIVLHAIIGGGSGSGFGSLI	YVPRSMVDLEFSVIN
WP_133797138.1:1-468 tubulin_beta_chain_Prosthe	LRELLTNLVQPSSLHFLM	IIIVLHAIIGGGSGSGLGTLLI	YVPRIMVDLEFSVID
WP_133797138.1 tubulin_beta_chain_Prosthecobact	LRELLTNLVQPSSLHFLM	IIIVLHAIIGGGSGSGLGTLLI	YVPRIMVDLEFSVID
NP_001029835.1 tubulin_beta-4B_chain_Bos_taurus	LRKLAVNMVFPFRLHFFM	FQLTHSGGGGTGSGMGTLLI	YVPRVLVDLEFGTMD
NP_116093.1 tubulin_alpha-1C_chain_isoform_c_Ho	LTEFQTNLVFPRIHFPL	FLVFHSGGGGTGSGFTSLLM	HVPRVFDLEFTVID
NP_476772.1 alpha-Tubulin_at_84B_Drosophila_mel	LTEFQTNLVFPRIHFPL	IIIVHSTGGGTGSGFGSLI	HVPRVFDLEFTVID
WP_146853222.1 tubulin_beta_chain_Brevifollis_g	LRELLTNLVQPSSLHFLM	YVVPFAMGGGTGSGFSSAFI	FVPRVFDLEFSVIE
WP_007550732.1 MULTISPECIES: cell_division_prot	MSNYRTWLATK.KPKFAI	FLIFHSGGGGTGSGFTSLLM	LIPRALLVLDLDFRAAN
RDT57095.1:24-381 cell_division_protein_FtsZ_Es	FADVRTVMS...EMGYAM	VFIAGMSGGGGTGTGAAPVVA	...FAVNTDAQALR
WP_027868354.1:24-381 cell_division_protein_Fts	FADVRTVMS...EMGYAM	VFIAGMSGGGGTGTGAAPVVA	...FAVNTDAQALR
HII79815.1 cell_division_protein_FtsZ_Methanosa	LGDFKTVMSG..GAGLAT	...GVGANRNIQKVFEDN	...LAFNTAVNDLK
TBR06802.1 cell_division_protein_FtsZ_Candidatu	MSNYRTWLATK.RPKFAI	...GAARNWAEGRSRFKNE	LIPRALLVLDLDFRAAN
WP_010476989.1 cell_division_protein_FtsZ_Therm	ASDLKFVVKAF.GS.FAT	GGKGVNANFVLGREAMKRD	FES...LAINTSRGDLE
WP_101476564.1 cell_division_protein_FtsZ_Candi	MSNYRTWLATK.RPKFAI	...GAARNWAEGRTRFIKE	LIPRALLVLDLDFRAAN
WP_137710828.1 cell_division_protein_FtsZ_Halor	PADITRTLDTLG.GVTTVG	...GVGGDFDVGAQVAMAD	TNGNVLAFTESQPFPR
WP_154809763.1 cell_division_protein_FtsZ_Metha	LGDFKTVMSG..GAGLAT	...GVGANRNVGKHVFEDN	...LAINTAVNDLK
WP_163257594.1:1-369 cell_division_protein_FtsZ	FADVRTIMV...DKGLAH	...LGAGANPEIGKKAAEES	...LAINTDKQALM

Hypothetical OdinTub

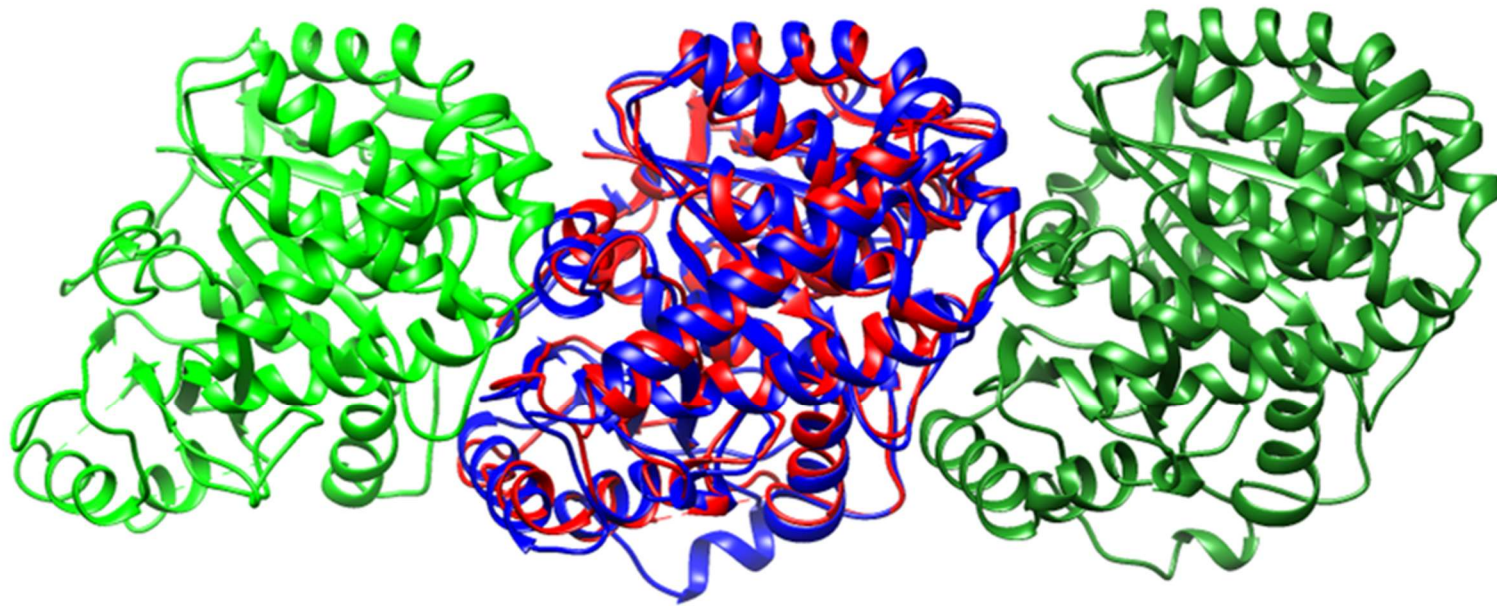


Maybe..  
Why not?



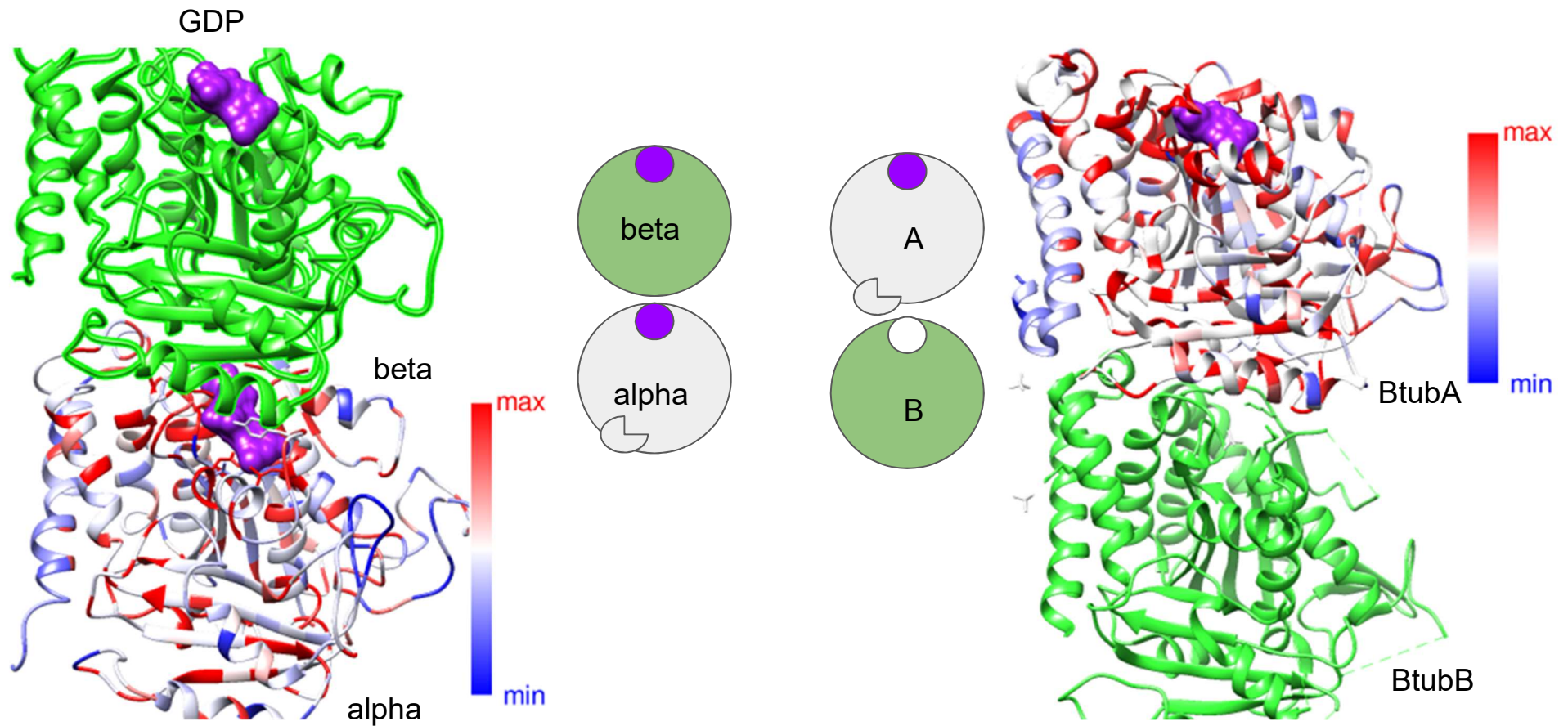
Part 2:  
Intra- and inter-dimer  
interfaces of eukaryotic and  
bacterial tubulins

Structural alignment of bacterial and eukaryotic tubulin dimers reveals different arrangement

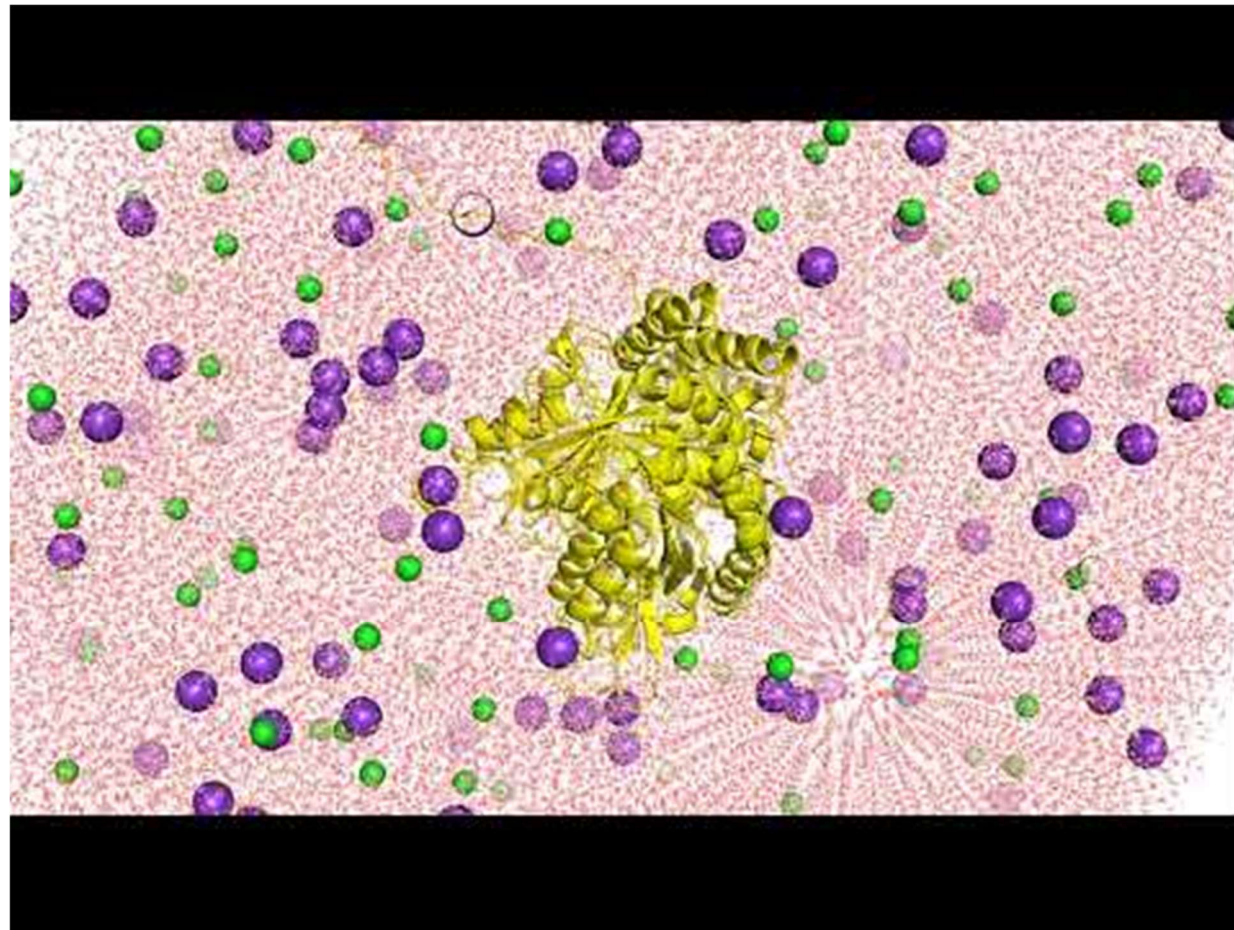


What is going on?

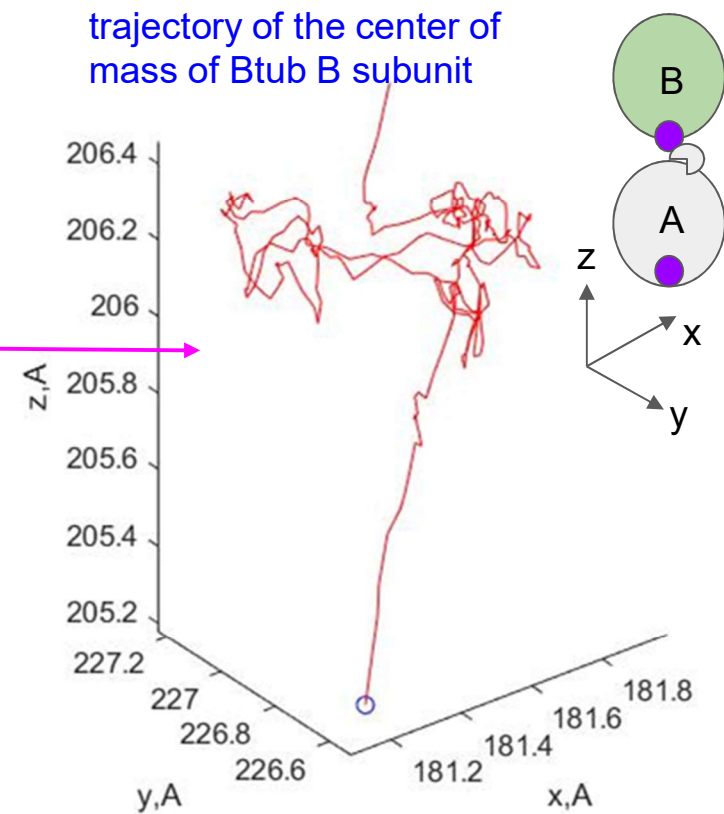
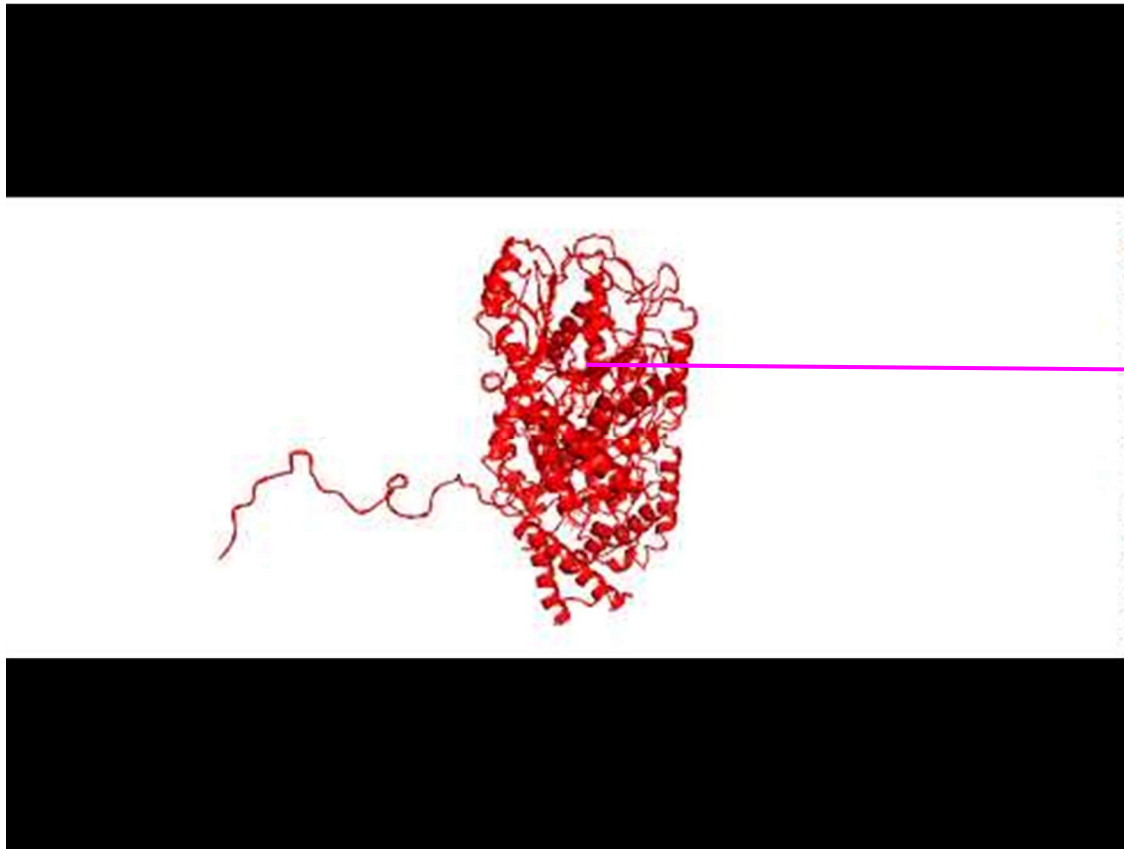
# Comparison of $\alpha\beta$ tubulin and BtubAB dimers



# Simulation box

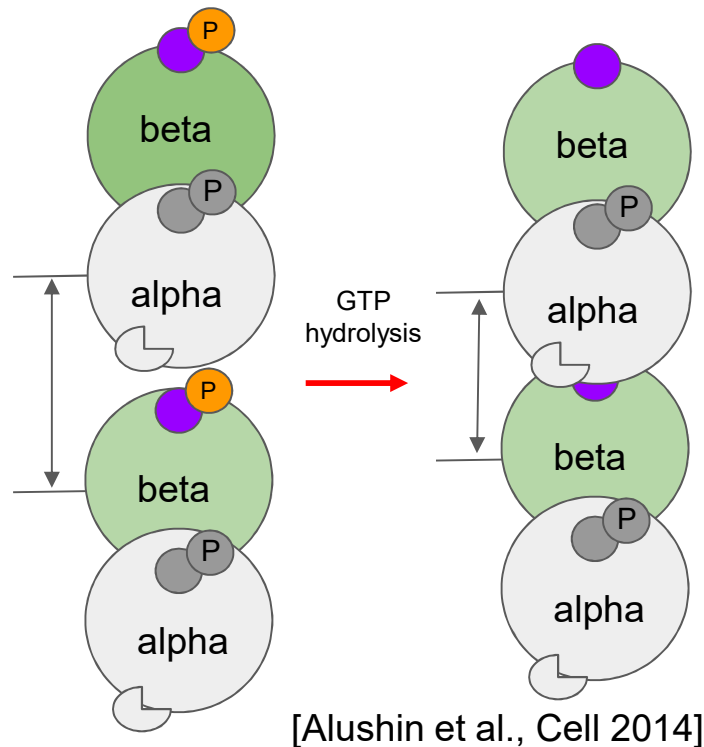


**28-nanoseconds-long simulation of bacterial tubulin predicts dimer elongation  
(‘extension of intr-dimer interface) but no bending**

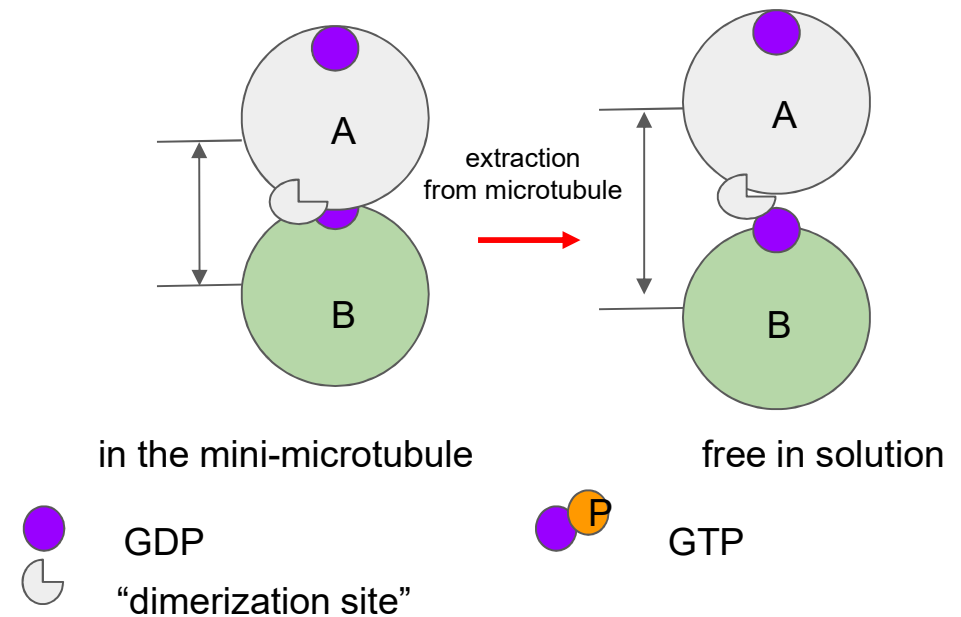


Eukaryotic **inter**-dimer interface and bacterial **intra**-dimer interfaces have similar 'compaction-extension' conformational change

**Eukaryotes:** Inter-dimer interface becomes compact after GTP hydrolysis

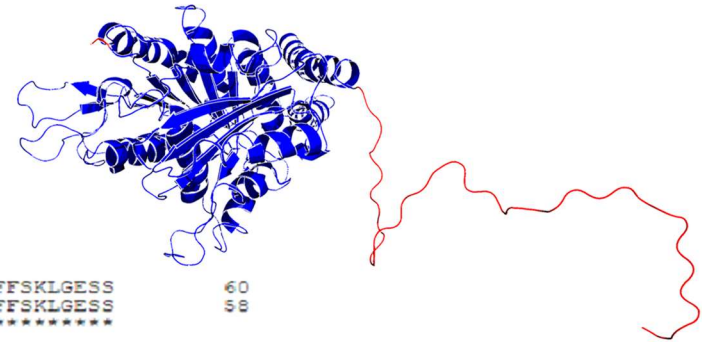


**Bacteria:** Intra-dimer interface becomes compact when incorporated into mini-microtubule



# Part 3: Sequence conservation and structure of bacterial tubulin tails

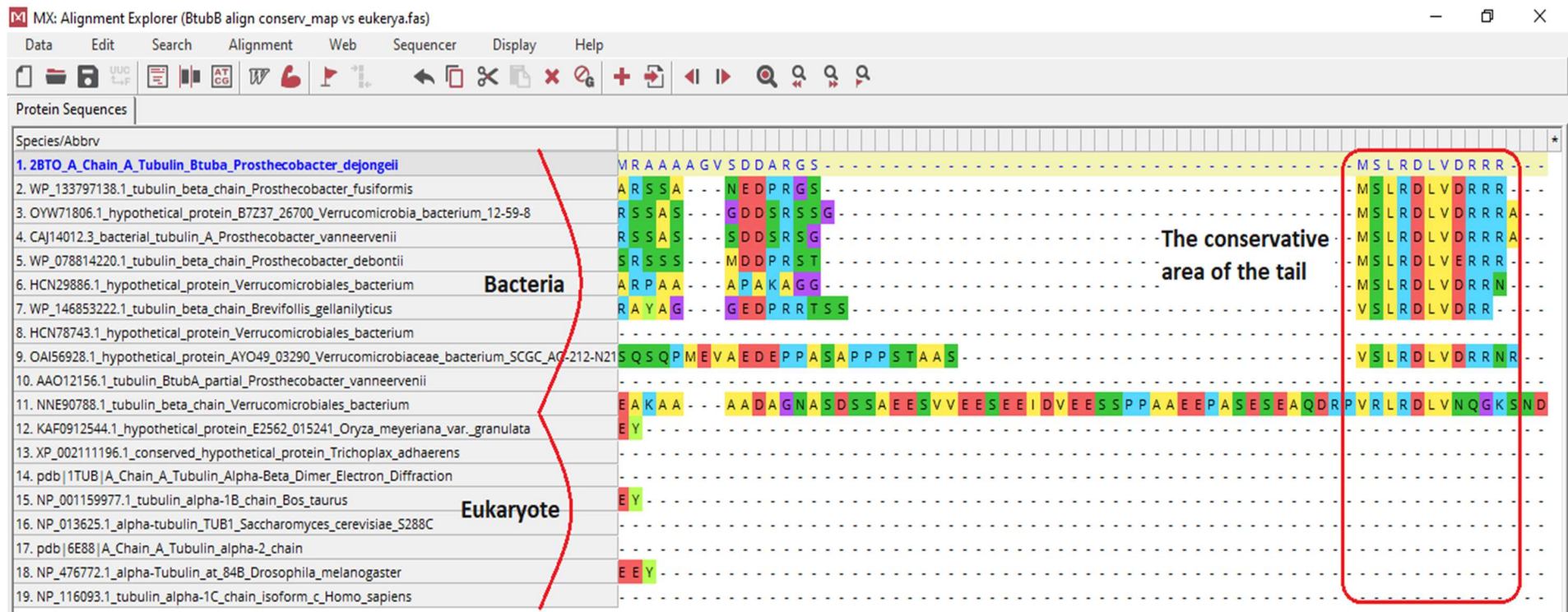
# Alignment of btubA amino seq revealed a 38-amino acids tail



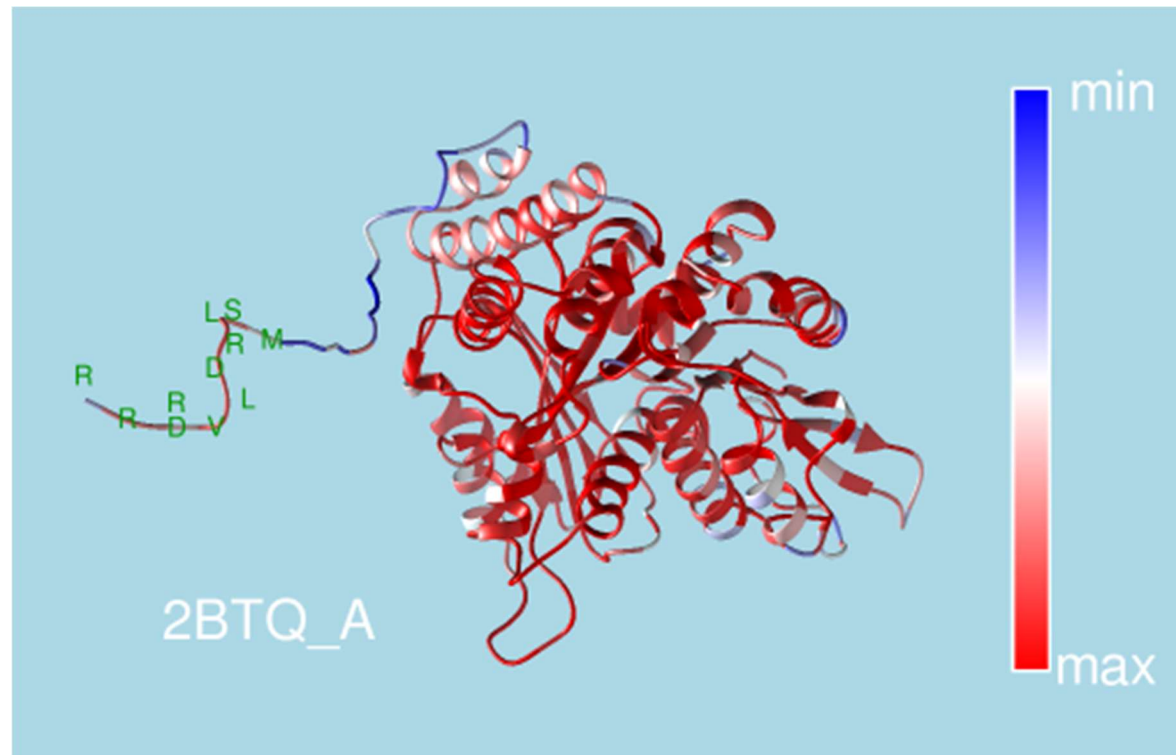
Q8GCC5 Q8GCC5_9BACT	1	MKVNNTIVVSIGQAGNQIARAFWKIVCLEHGIDPLTGQTAPGVAPRGNWSSFFSKLGESS	60
Sc09-pdb-bundle1A_ATOM	1	--VNNTIVVSIGQAGNQIARAFWKIVCLEHGIDPLTGQTAPGVAPRGNWSSFFSKLGESS	58
		*****	
Q8GCC5 Q8GCC5_9BACT	61	SGSYVPRAIMVDLEPSVIDNVKATSGSLFNPANLISRTEGAGGNFAVGYLGA GREVLPEV	120
Sc09-pdb-bundle1A_ATOM	59	SGSYVPRAIMVDLEPSVIDNVKATSGSLFNPANLISRTEGAGGNFAVGYLGA GREVLPEV	118
		*****	
Q8GCC5 Q8GCC5_9BACT	121	MSRLDYEIDKCDNVGGIIVLHAIGGGTSGSGFGALLIESLKEYGEIPVLSCAVLPS PQVS	180
Sc09-pdb-bundle1A_ATOM	119	MSRLDYEIDKCDNVGGIIVLHAIGGGTSGSGFGALLIESLKEYGEIPVLSCAVLPS PQVS	178
		*****	
Q8GCC5 Q8GCC5_9BACT	181	SVVTEPYNTIVFALNTLRRSADACLIFDNEALFDLAHRKWNIESPTVDDLNL LITEALAGI	240
Sc09-pdb-bundle1A_ATOM	179	SVVTEPYNTIVFALNTLRRSADACLIFDNEALFDLAHRKWNIESPTVDDLNL LITEALAGI	238
		*****	
Q8GCC5 Q8GCC5_9BACT	241	TASMRFSGFLTVEITIRELLTNLVPQPSLHFLMCAFAPLTPPDRSKFEELGIEEMI KSLF	300
Sc09-pdb-bundle1A_ATOM	239	TASMRFSGFLTVEITIRELLTNLVPQPSLHFLMCAFAPLTPPDRSKFEELGIEEMI KSLF	298
		*****	
Q8GCC5 Q8GCC5_9BACT	301	DNGSVFAACSPMEGRFLSTAVLYRGIMEDKPLADAALAAMREKLPLTYWIPTAFKIGYVE	360
Sc09-pdb-bundle1A_ATOM	299	DNGSVFAACSPMEGRFLSTAVLYRGIMEDKPLADAALAAMREKLPLTYWIPTAFKIGYVE	358
		*****	
Q8GCC5 Q8GCC5_9BACT	361	QPGISHRKSMVLLANNTETIARVLDRIHNFDKLWQKAFANWYLN EGMSEEQINVL RASA	420
Sc09-pdb-bundle1A_ATOM	359	QPGISHRKSMVLLANNTETIARVLDRIHNFDKLWQKAFANWYLN EGMSEEQINVL RASA	418
		*****	
Q8GCC5 Q8GCC5_9BACT	421	QELVQSYQVAEESGAKAKVQDSAGDTGMRAAAAGVSDDARGSM SLRDLVDRRR	473
Sc09-pdb-bundle1A_ATOM	419	QELVQSYQVAEESGA-----	433
		*****	

long tail of BtubA

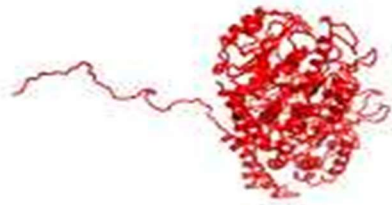
Bacterial tubulin A has a long tail with a conservative sequence at the C-terminal



Bacterial tubulin A has a long tail with a conservative sequence at the C-terminal

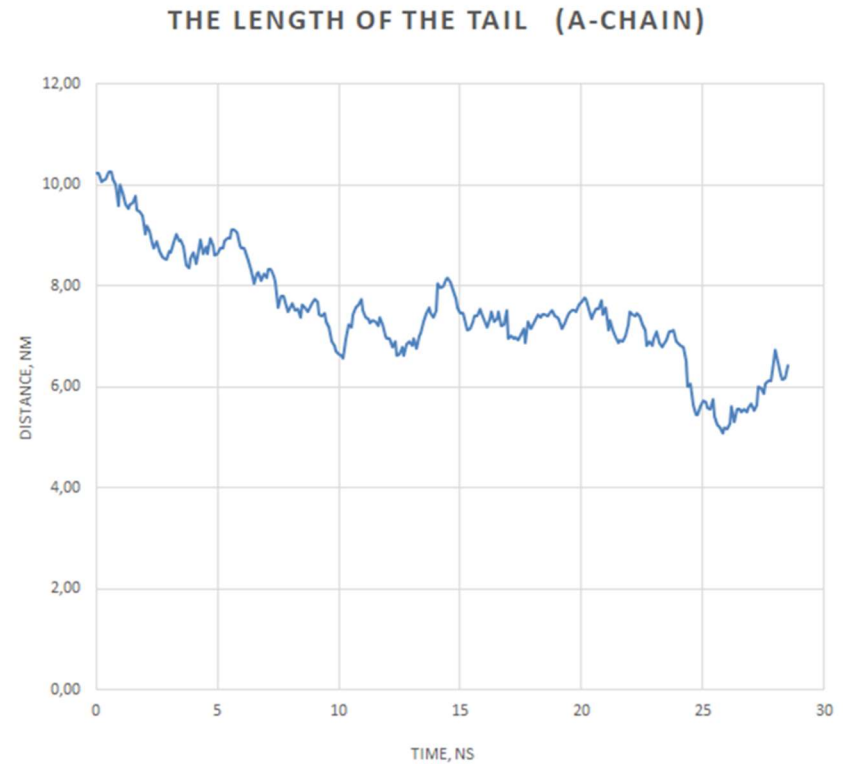
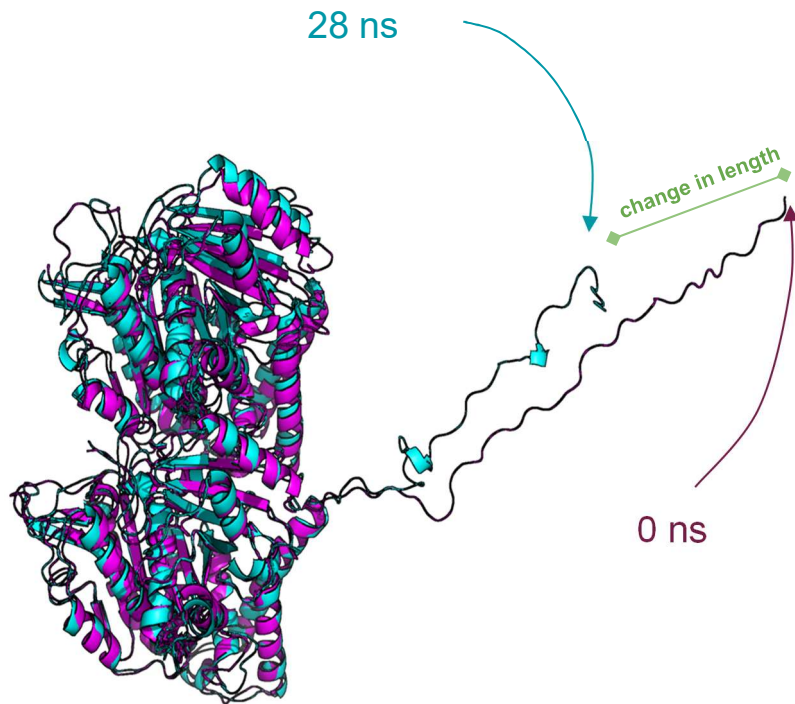


## **Molecular dynamics of BtubAB: it moves!!!**



**The movie in PyMOL, illustrating 28 ns of the conformational changes in protein structure, was obtained by GROMACS. The tail belongs to the A-chain of bacterial tubilin**

## Tubulin tail becomes progressively more compact during the simulation process



The graph made using coordinates of two atoms: the last  $\alpha$ -carbon (ALA-435) in non-tail region and penultimate one (ARG-473) of the tail

# Conclusions

- 1) We found two regions, which are similar between tubulins that can form microtubules and different to FtsZ, which cannot form microtubules.
- 2) We identified CetZ from Odinarchaeota as a potential common ancestor of btubAB and eukaryotic tubulins. We predict that it might form mini-microtubules
- 3) Bacteria have swapped places of A and B monomer in a dimer, compared to eukaryotic tubulin. This is confirmed by bioinformatics and molecular dynamics analysis of properties of bacterial tubulin dimers.
- 4) Bacterial tubulin A has a long tail with a conservative sequence at the C-terminal, which adopts a compacted configuration in molecular dynamics simulations.