# The Atomic Structure of the Microtubule Nucleating γ-tubulin Small Complex and its Implications for Regulation

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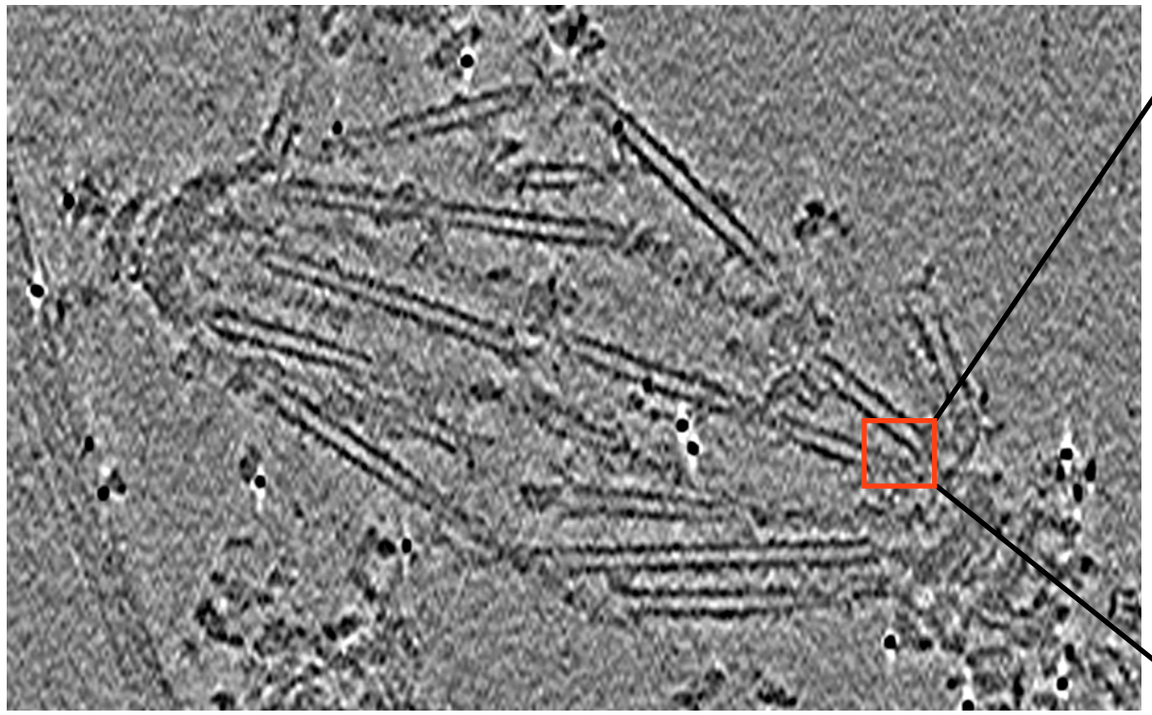
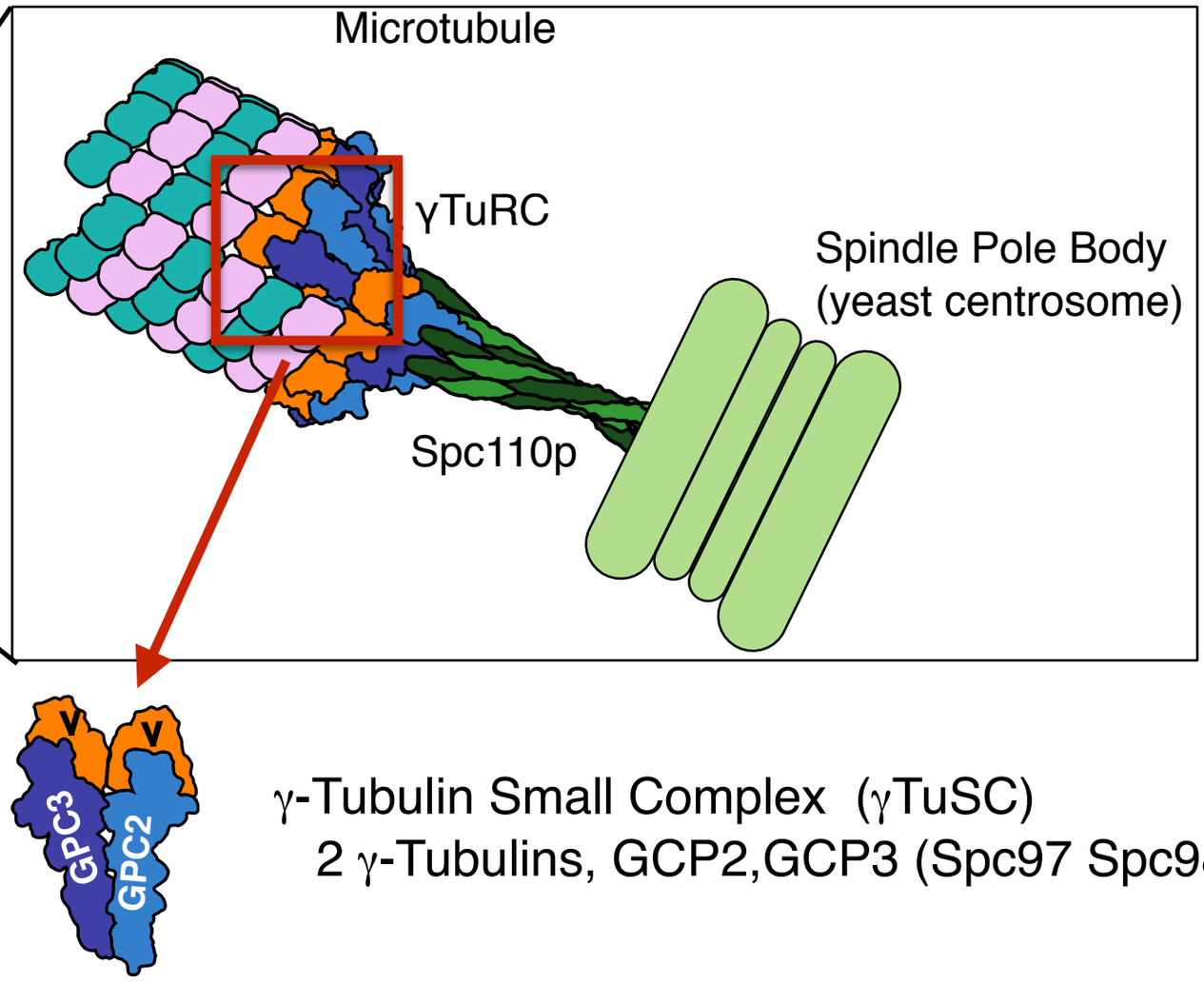
*Agard Lab, UCSF*
*in collaboration with*
*Davis Lab, UW*

*Janet Iwasa*

# *Microtubule nucleation by γ-tubulin Complexes*



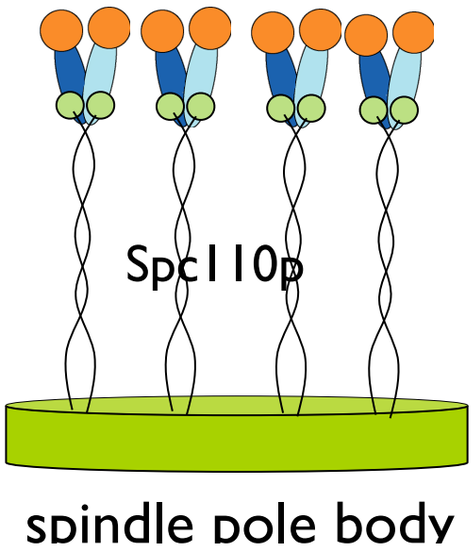
*Yeast Spindle* **Cryo-EM tomogram / Sam Li**

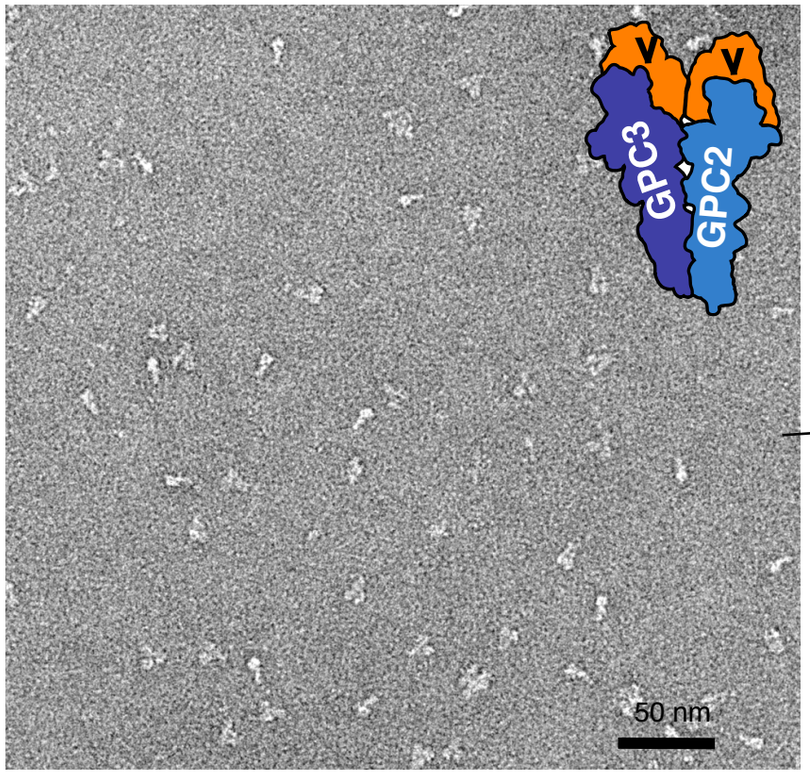



γ-Tubulin Small Complex (γTuSC)
2 γ-Tubulins, GCP2,GCP3 (Spc97 Spc98)

γTuSC/γTuRC structure, assembly, activation

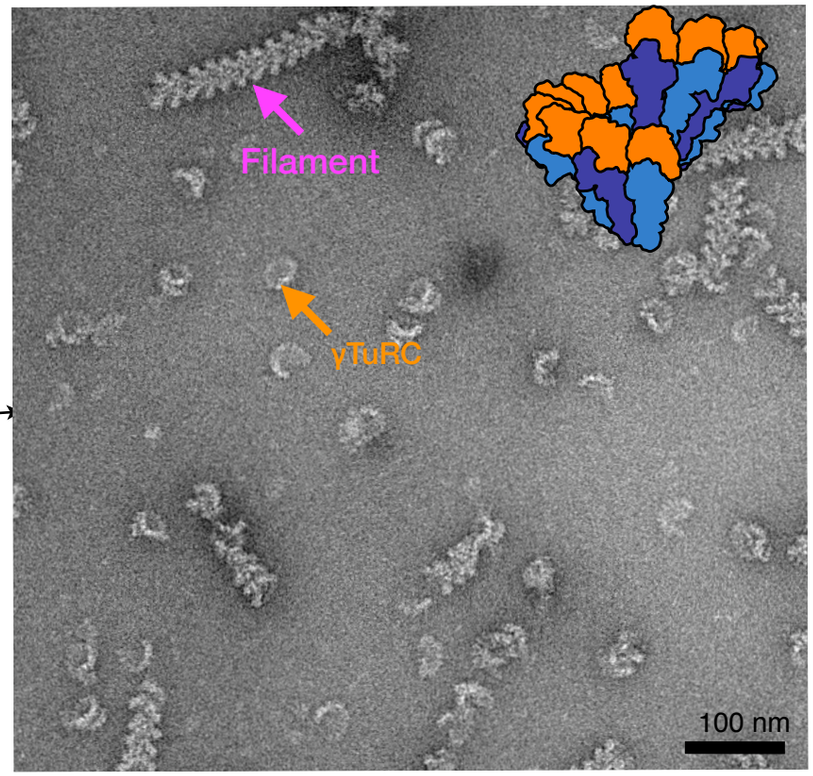
# *Attachment factor Spc110 stabilizes γTuSC assembly*



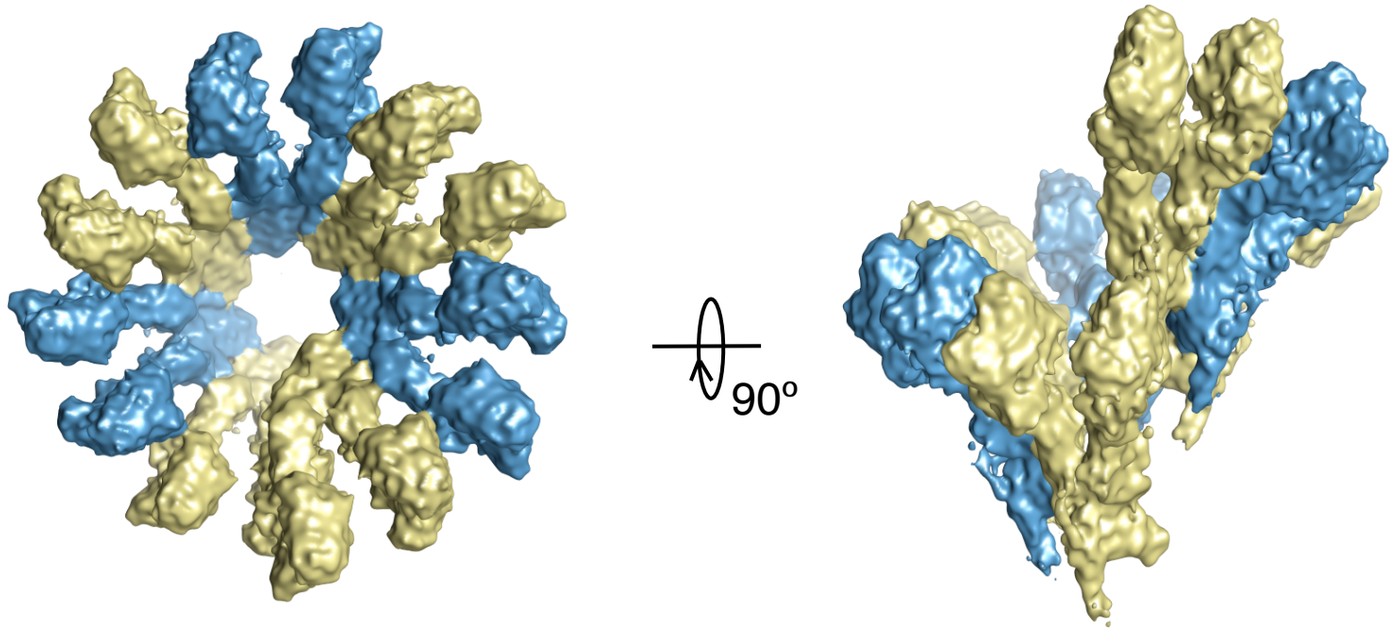




Individual γTuSCs

+ Spc110p$^{1-220}$



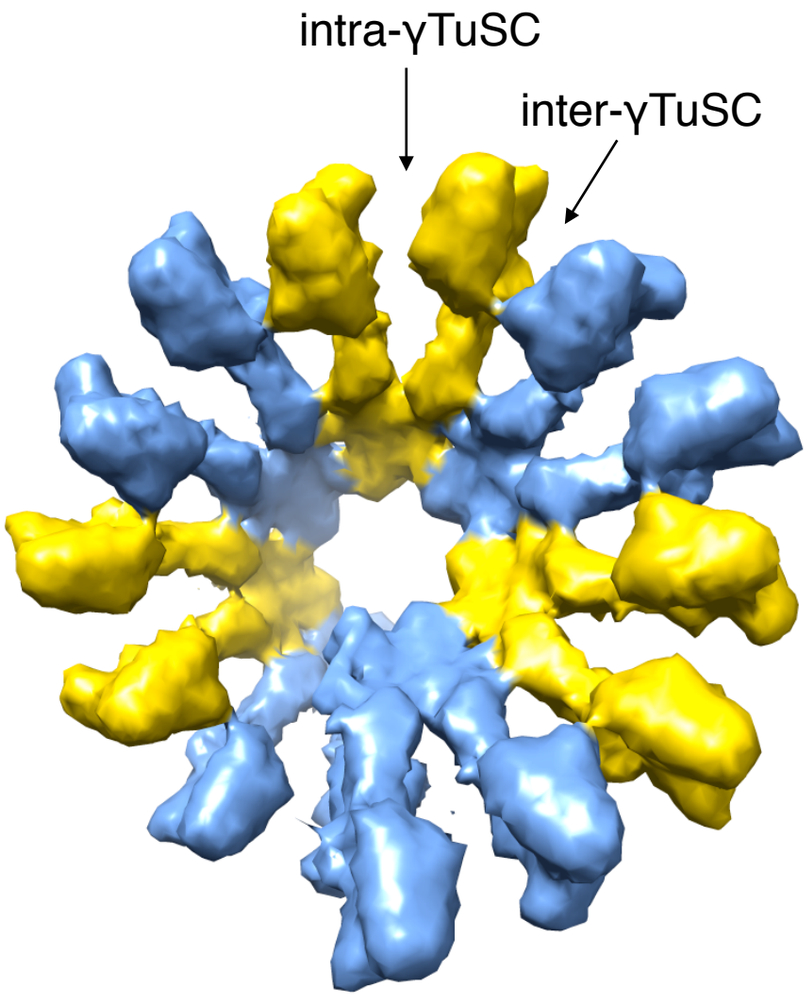

γTuRCs/filaments



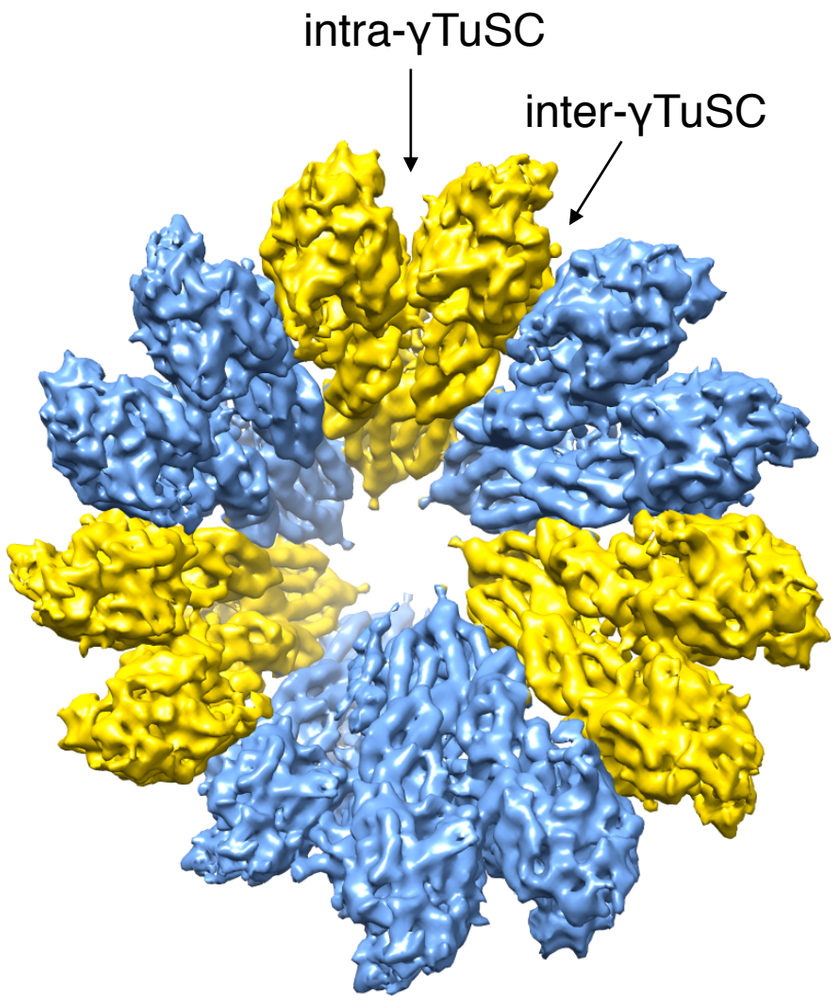

6.5 γTuSCs/turn = 13 γ-tubulins = *in vivo* MT protofilament #

J. Kollman

# *Open - closed transition enhances γ-TuRC MT nucleation*

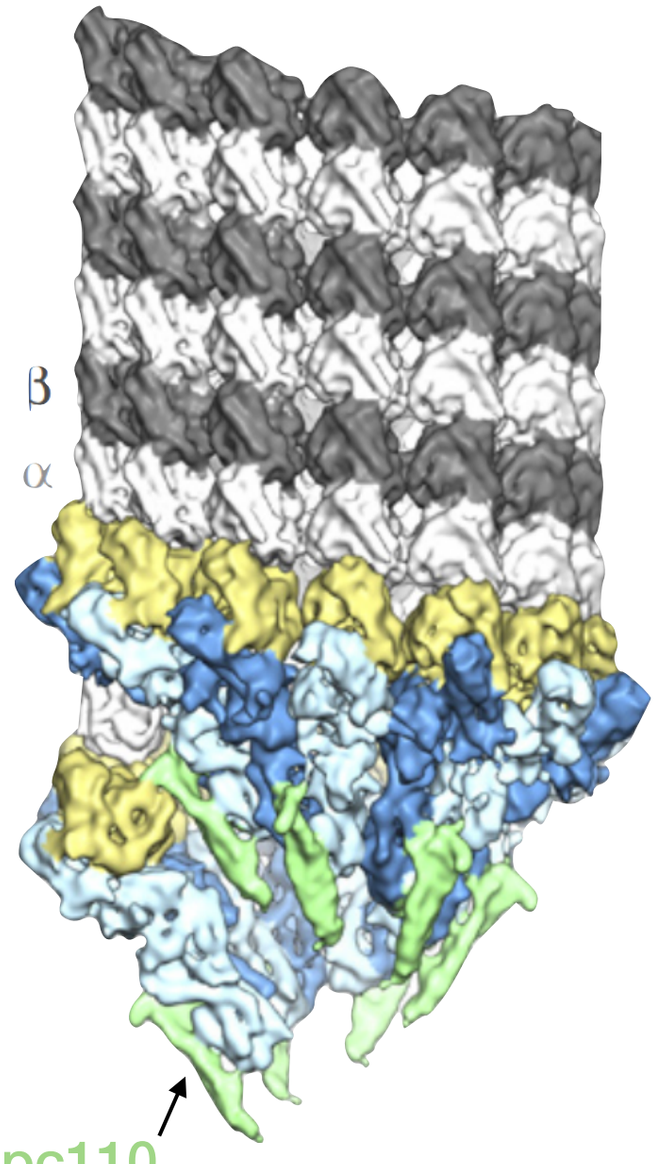



Open state




Closed state
(disulfide stabilized)

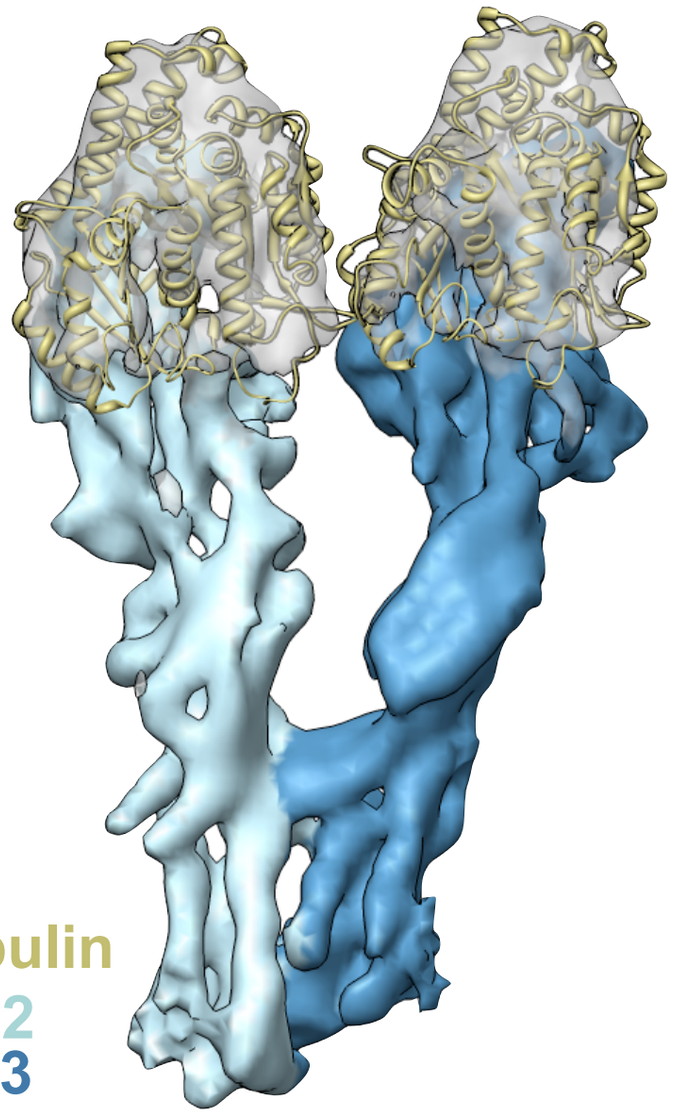
Model:
γTuRC nucleating microtubule




- *closed state better MT nucleator*
- *suggests closure as a regulatory mechanism*

**J. Kollman**

# γTuSC pseudo-atomic model built using 6.5Å oxidized map



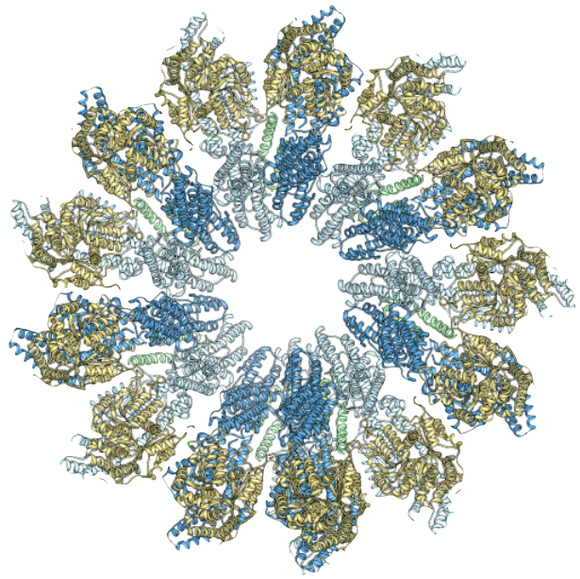

filament interior

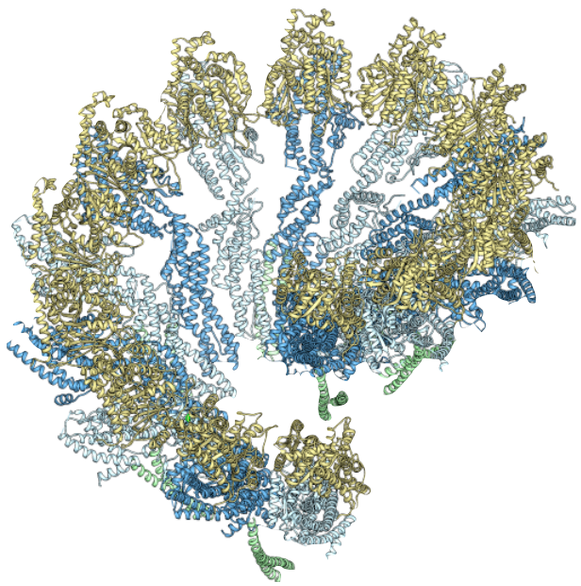
GCP4
crystal structure

C-terminal domain directly binds γ-tubulin

*Merdes, Mourney Guillet, et al. 2011*

less than 20% similarity





**C. Greenberg/A. Sali**
**J. Kollman**

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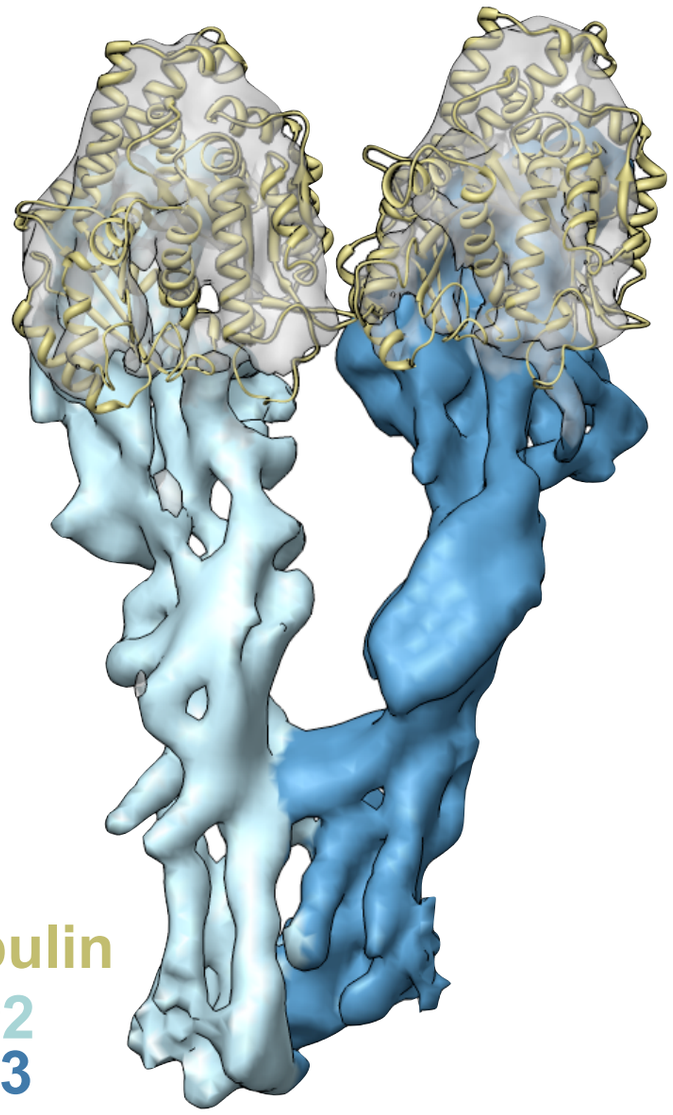


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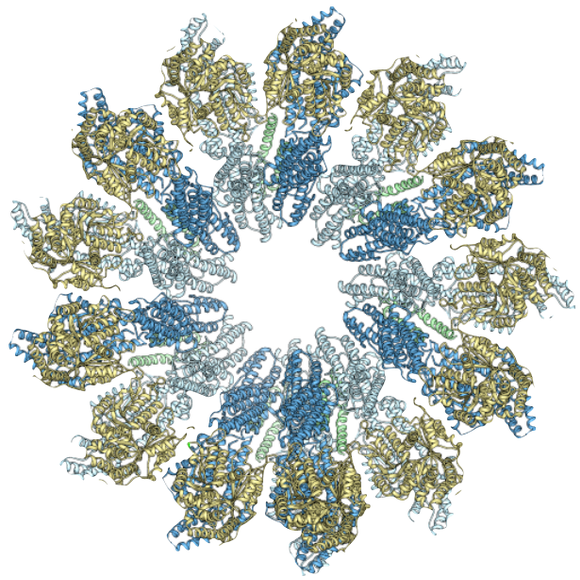
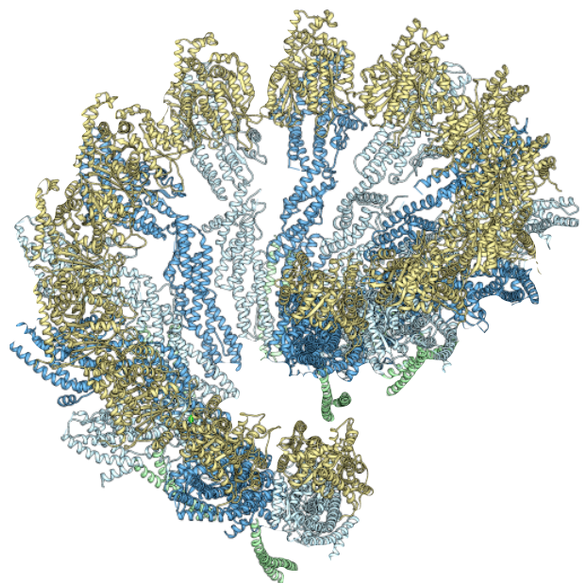
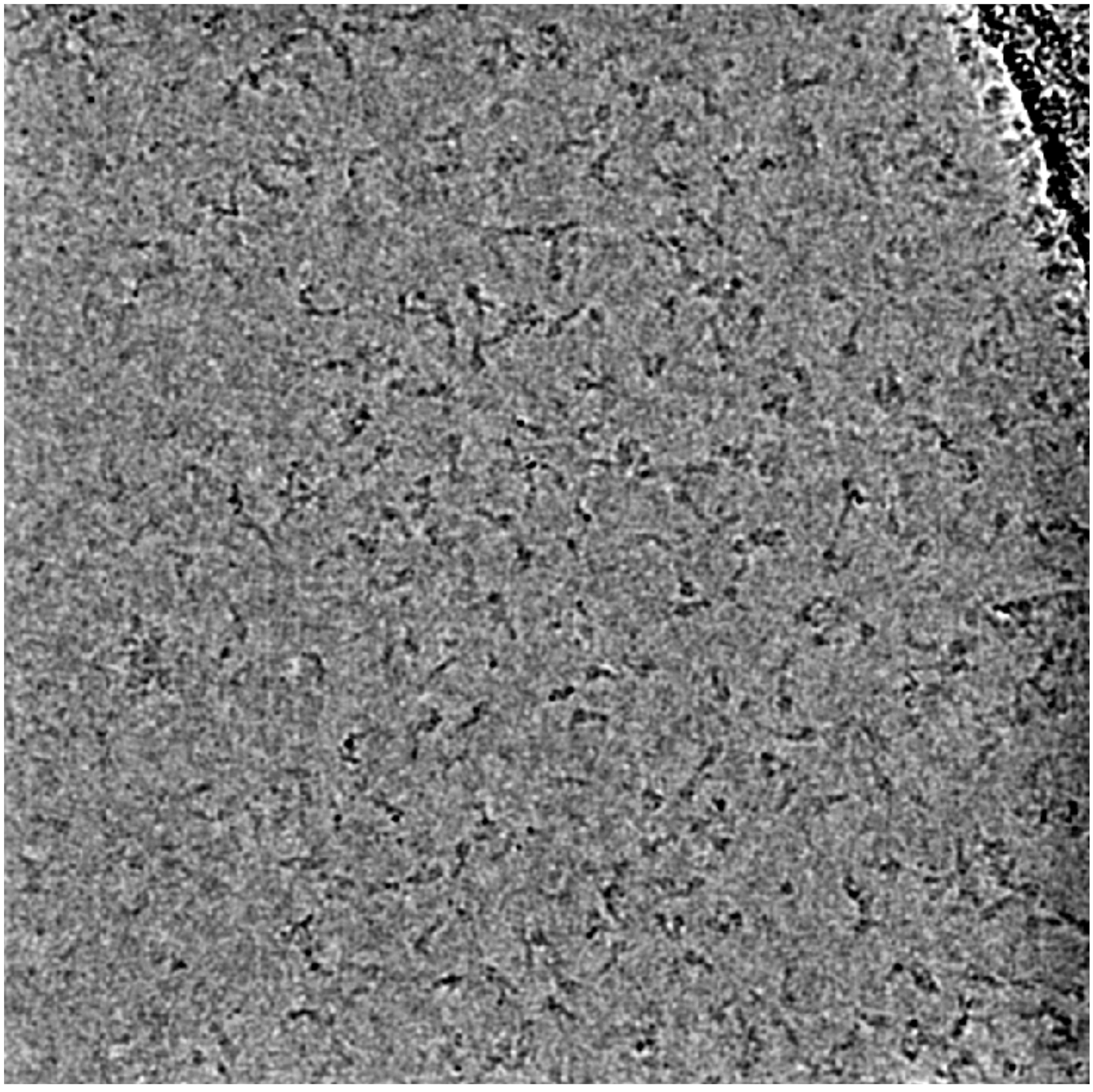




*Missing 234 aa from gcp2, 275 aa from gcp3*
*Built into a ~6.5 Å map*

**C. Greenberg/A. Sali**
**J. Kollman**

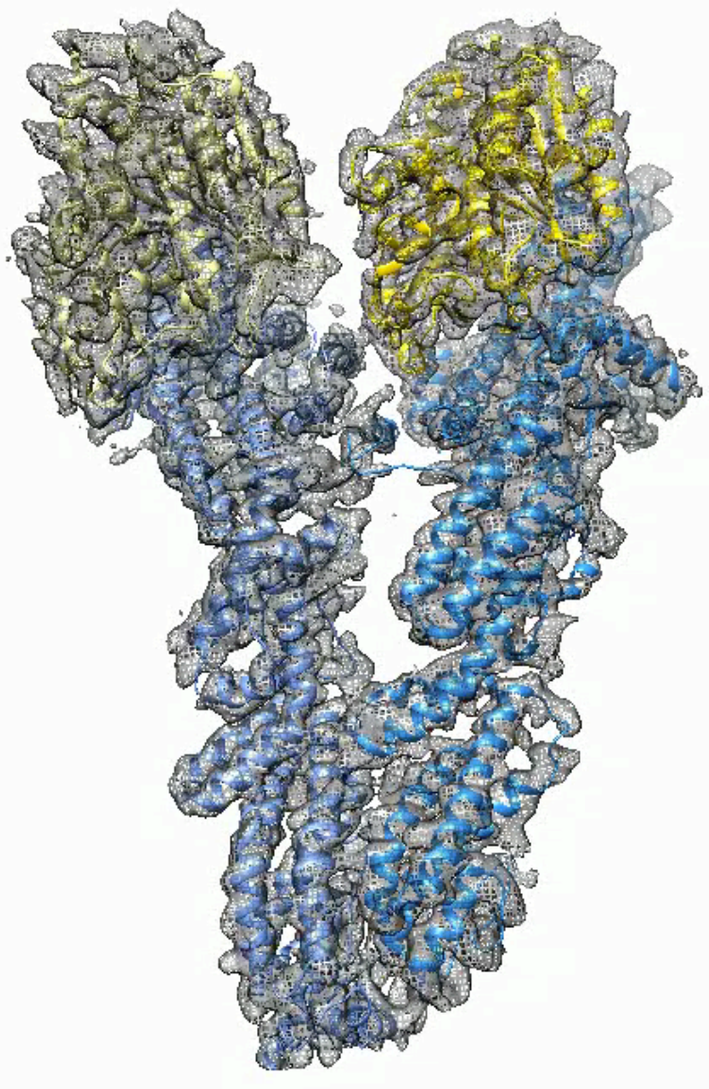
# *The Image Data*

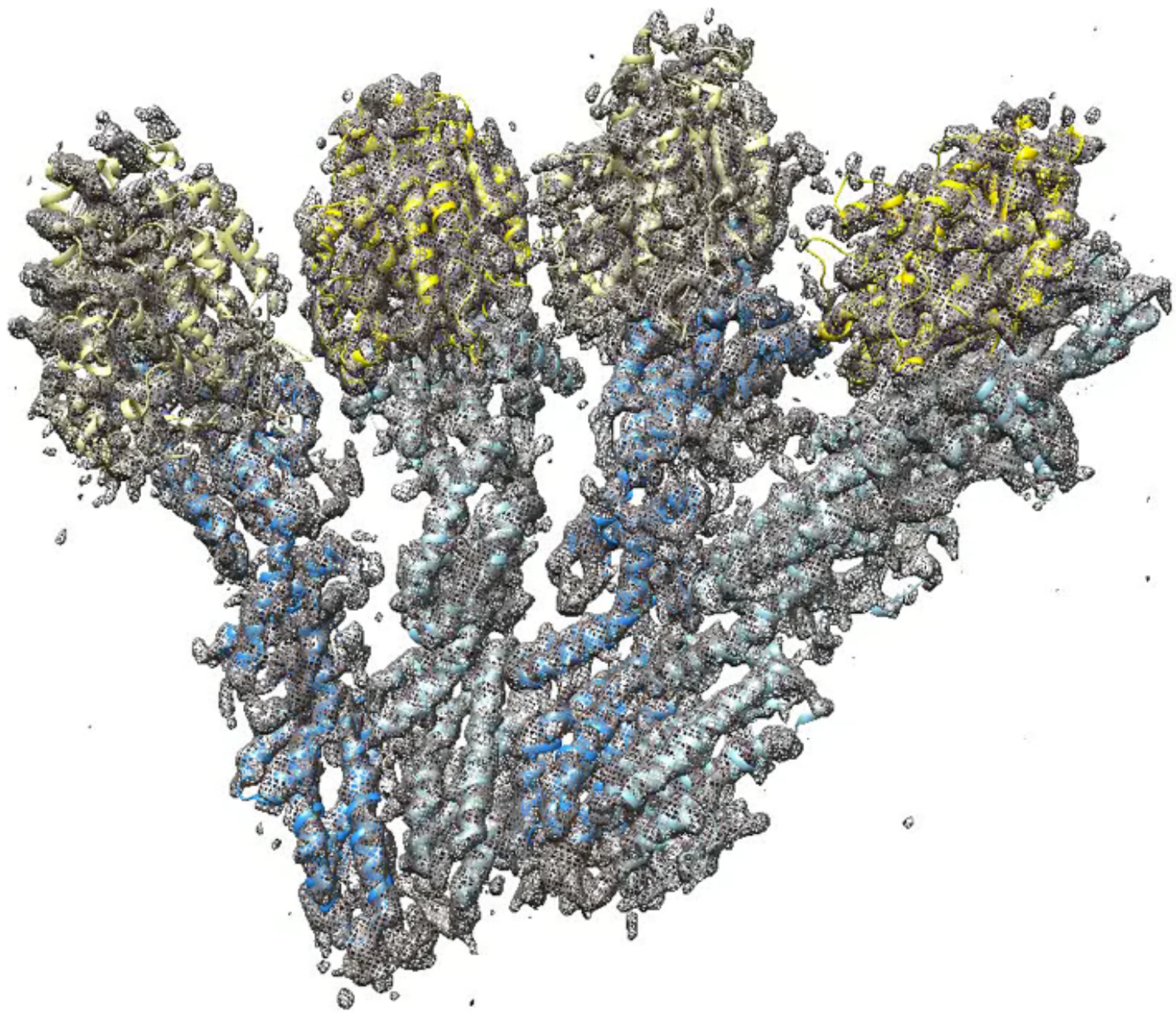


## Polara Data

~80 e-/A2
Dose filtered &
aligned with MotionCorr2
Thon rings 5Å
or better

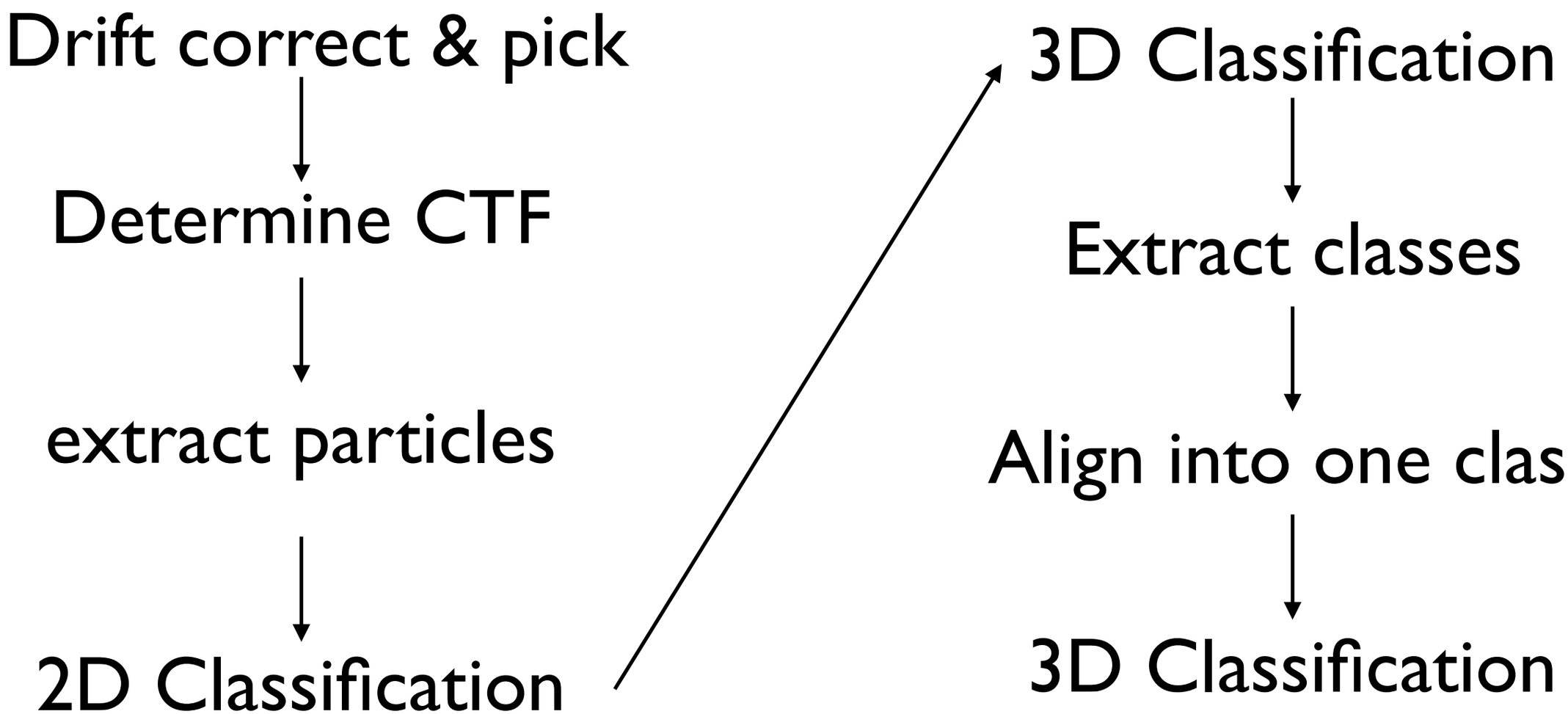
# γTuSC monomer/dimer by single particle cryoEM (3.8Å)





- first true atomic description of γTuSC, numerous inserts, etc
- differences in the interfaces between the γTuSCs vs internal interface
- conformational changes in γ-tubulin upon assembly into γTuSC
- interpretation of phosphorylation sites, mutations

# *Workflow*


Drift correct & pick
Determine CTF
extract particles
2D Classification
3D Classification
Extract classes
Align into one class
3D Classification

# *Improving the Map*

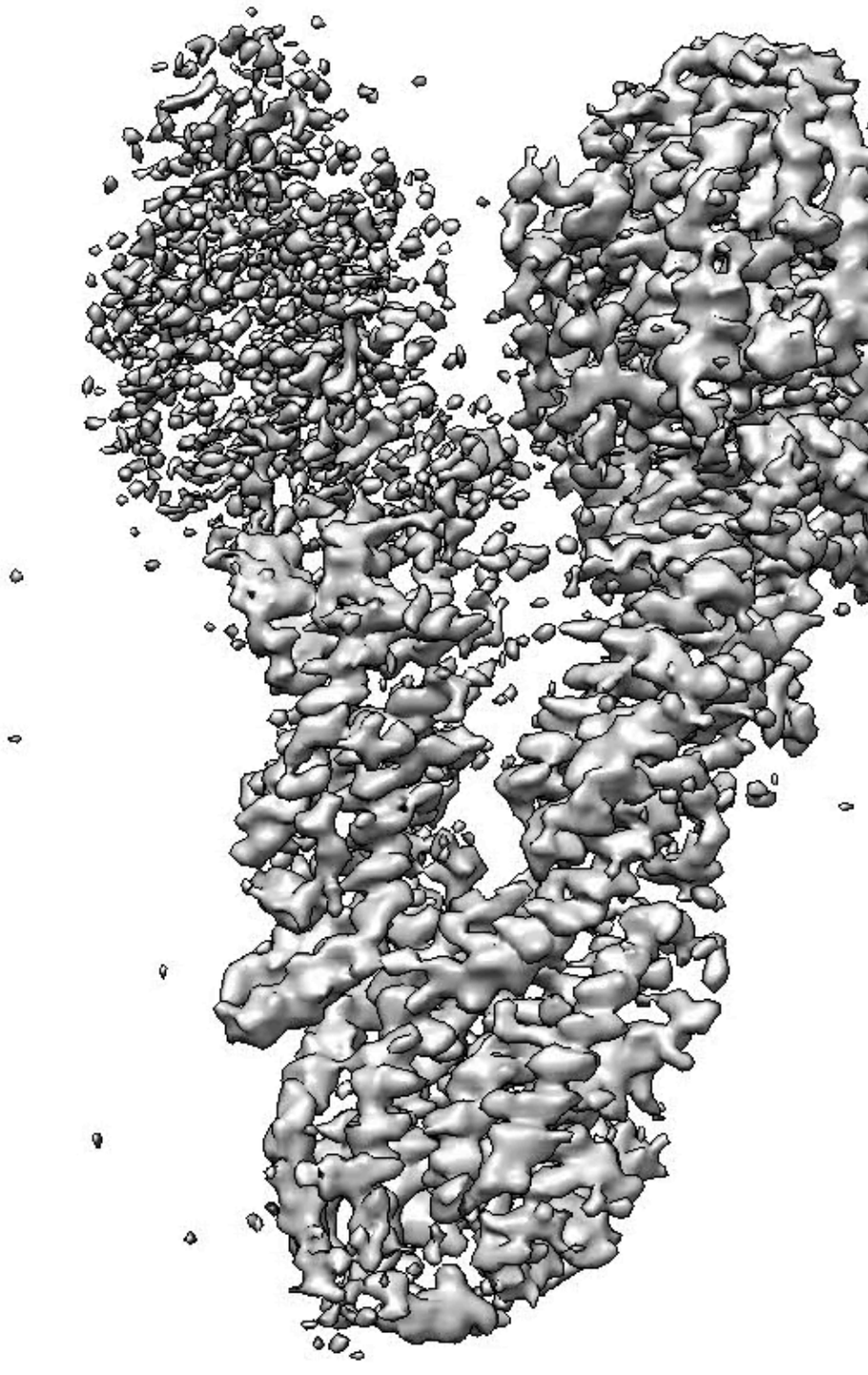
Increase Dataset size (+0.5M particles)

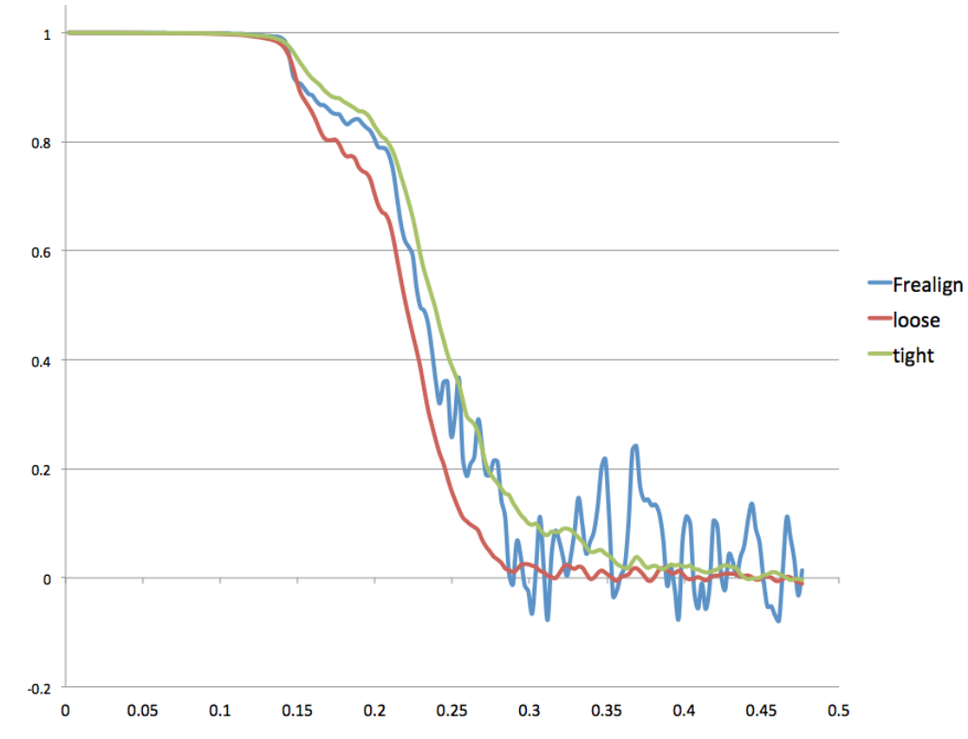
Various Programs (Relion, cryosparc)
  Full workflow, as well as feeding them classification results from Frealign

Focused Classification in Frealign

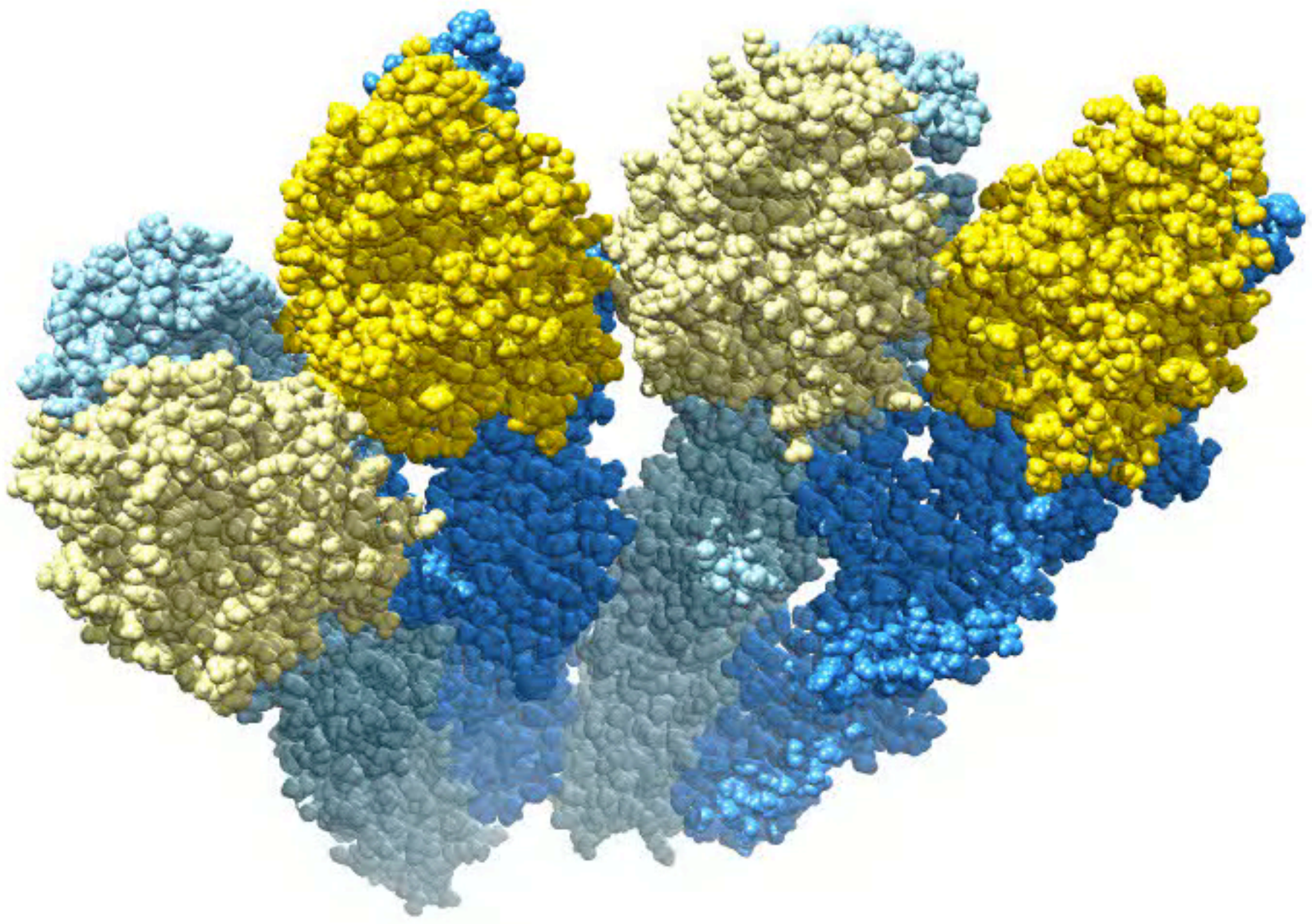
Various Masks
  Half-Tusc, Base only, Base plus one tubulin arm

# *Improving the Map - Frealign, Shaped Masks and Weighting*





# *Assembly driven global conformation changes*



both assembly & allosteric conformational changes required

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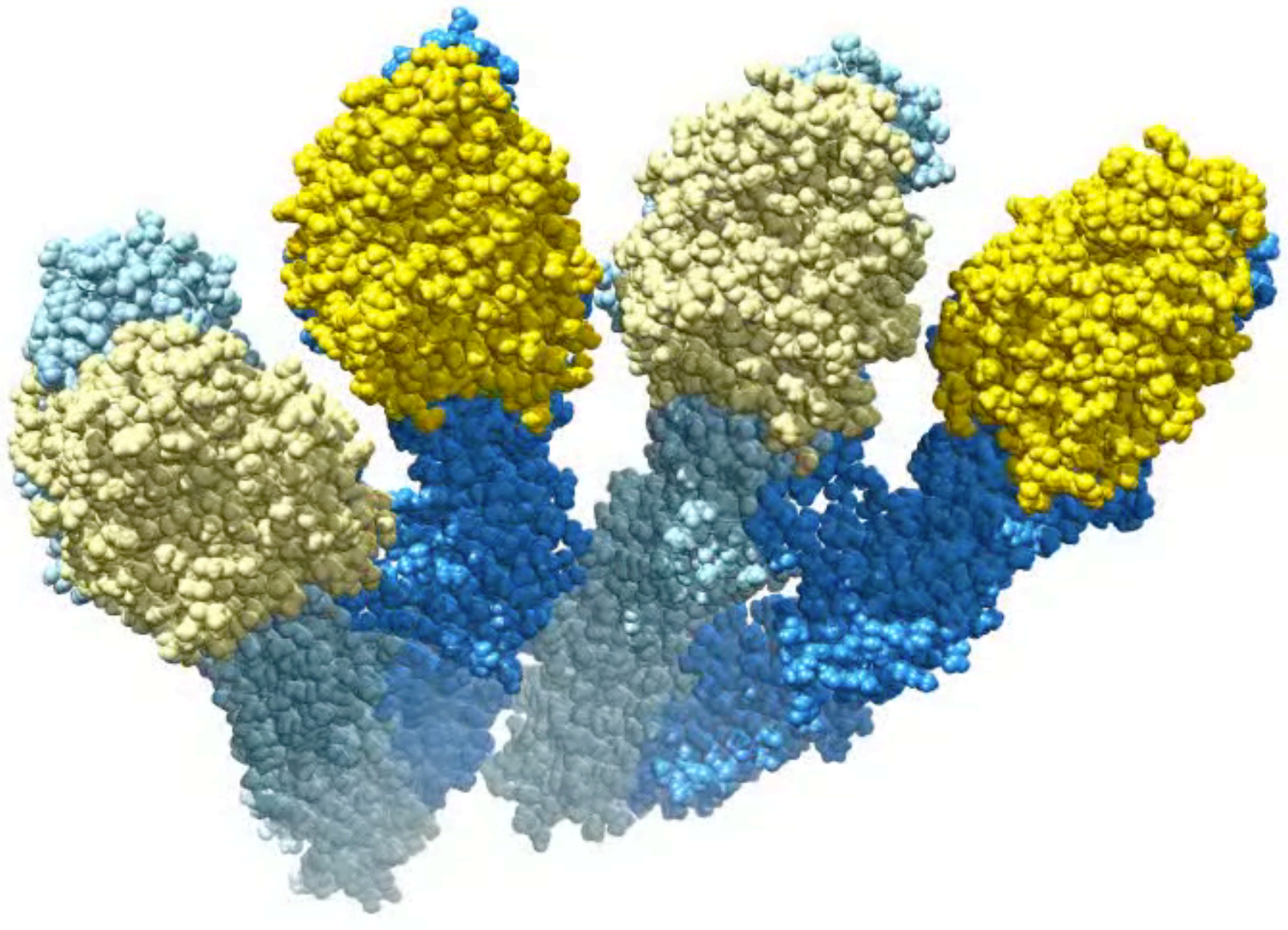
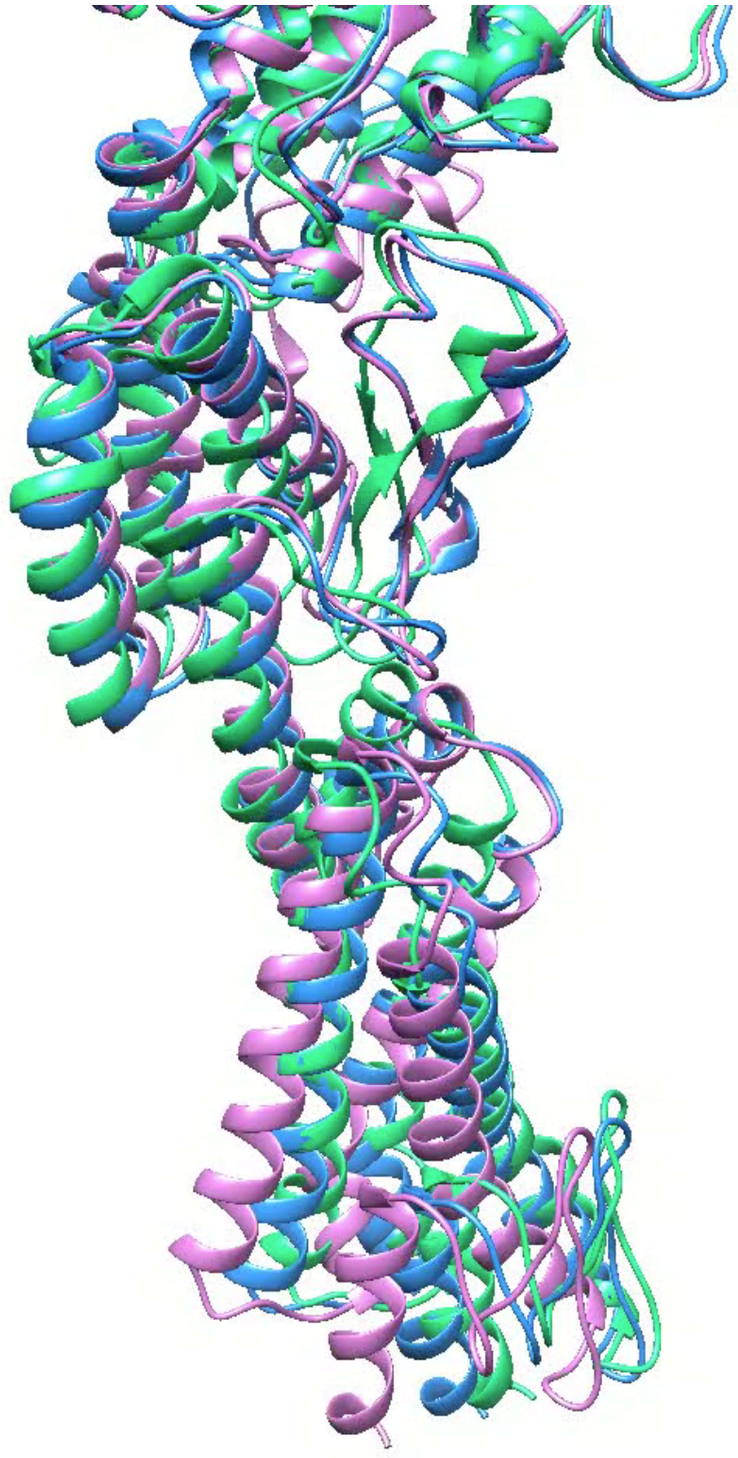


both assembly & allosteric conformational changes required

# *Assembly driven global conformation changes*

Monomer
Closed
Open



GCP3 N-terminus

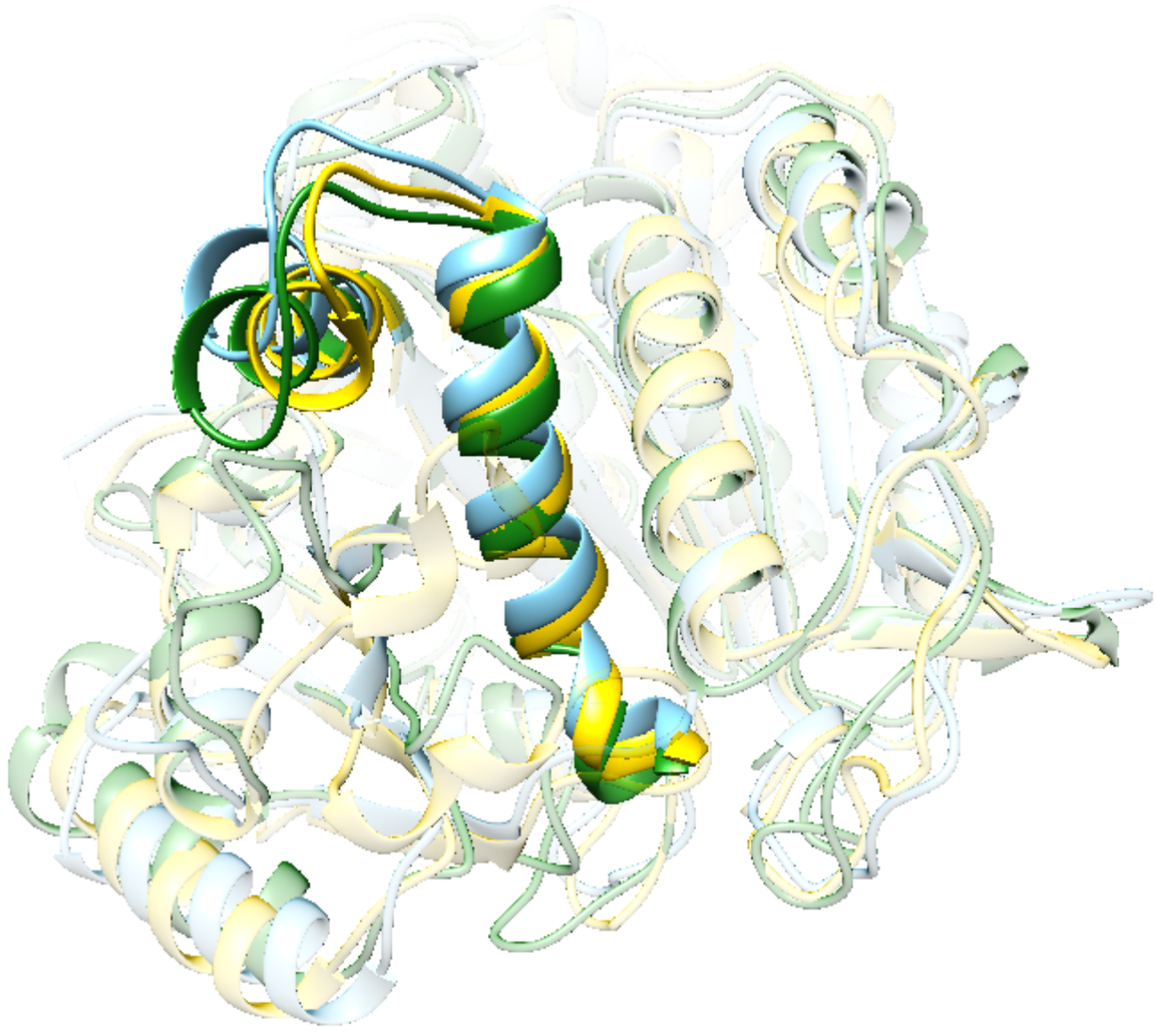
Twist of the conserved GCP domains is the major re-arrangement

# *What is the conformation of γ-tubulin on the γ-TuSC*

98-bound γ

Human
γ xtal (3CB2)

straight β



yeast MT from Nogales & Rice

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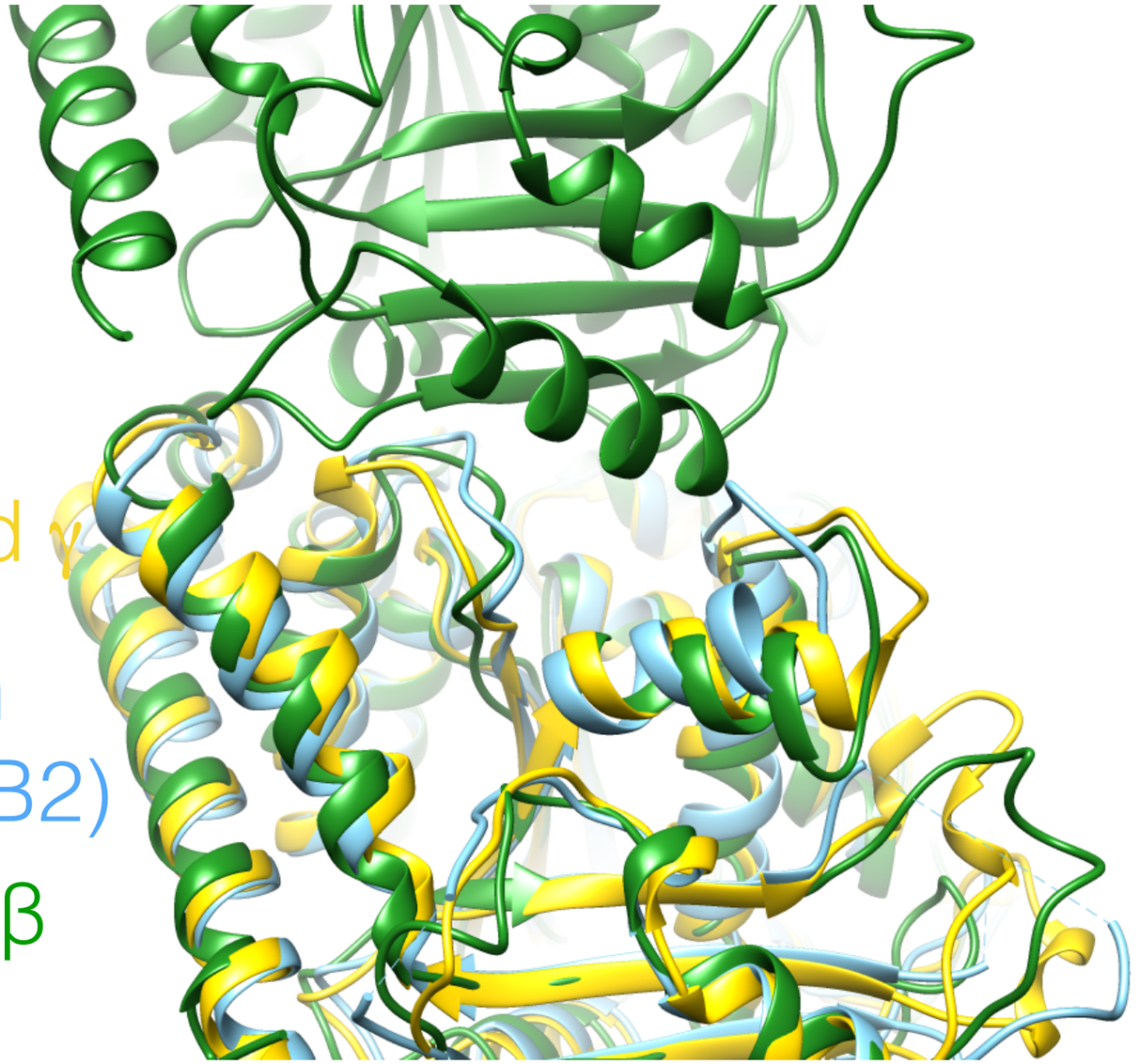

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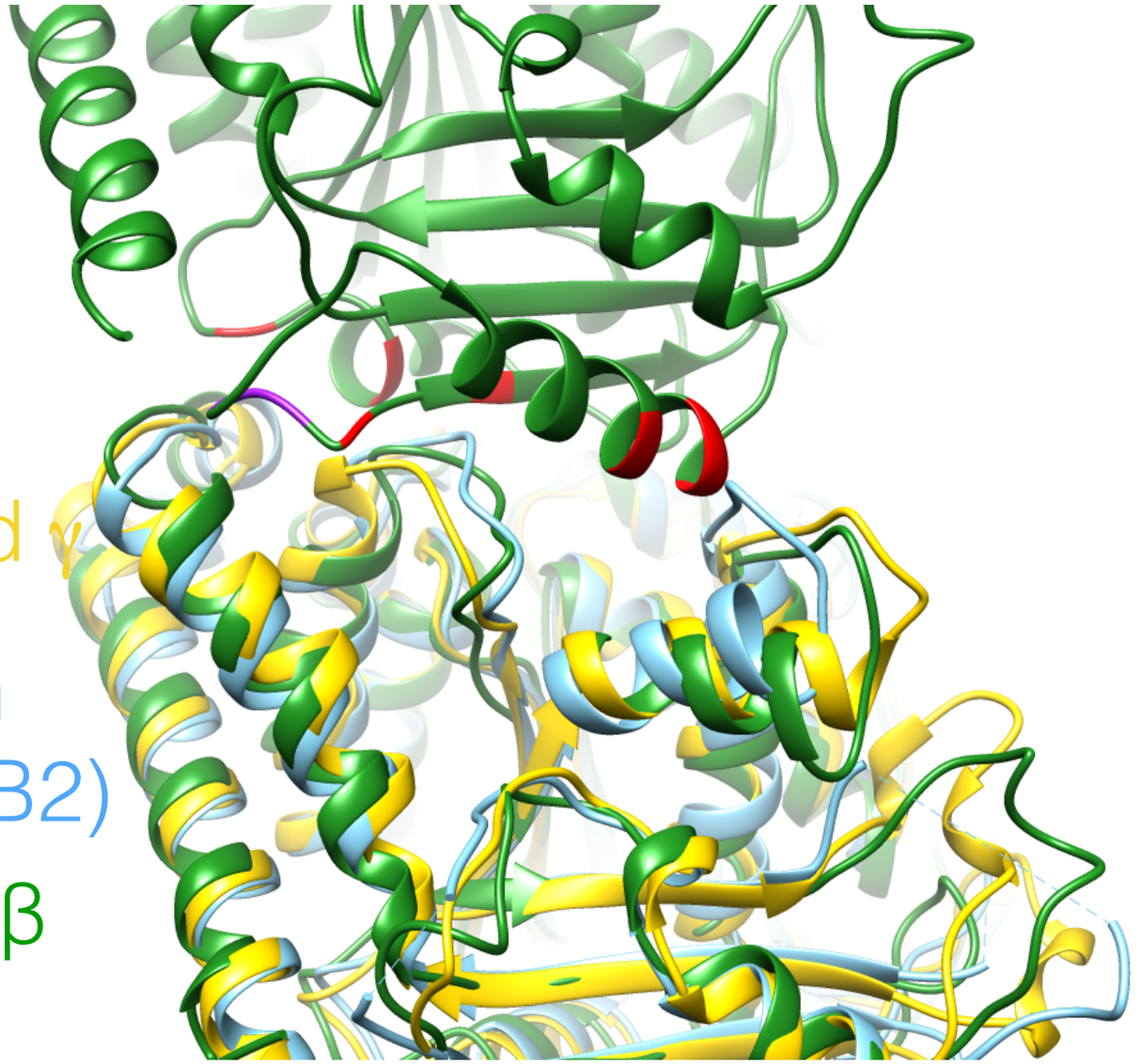
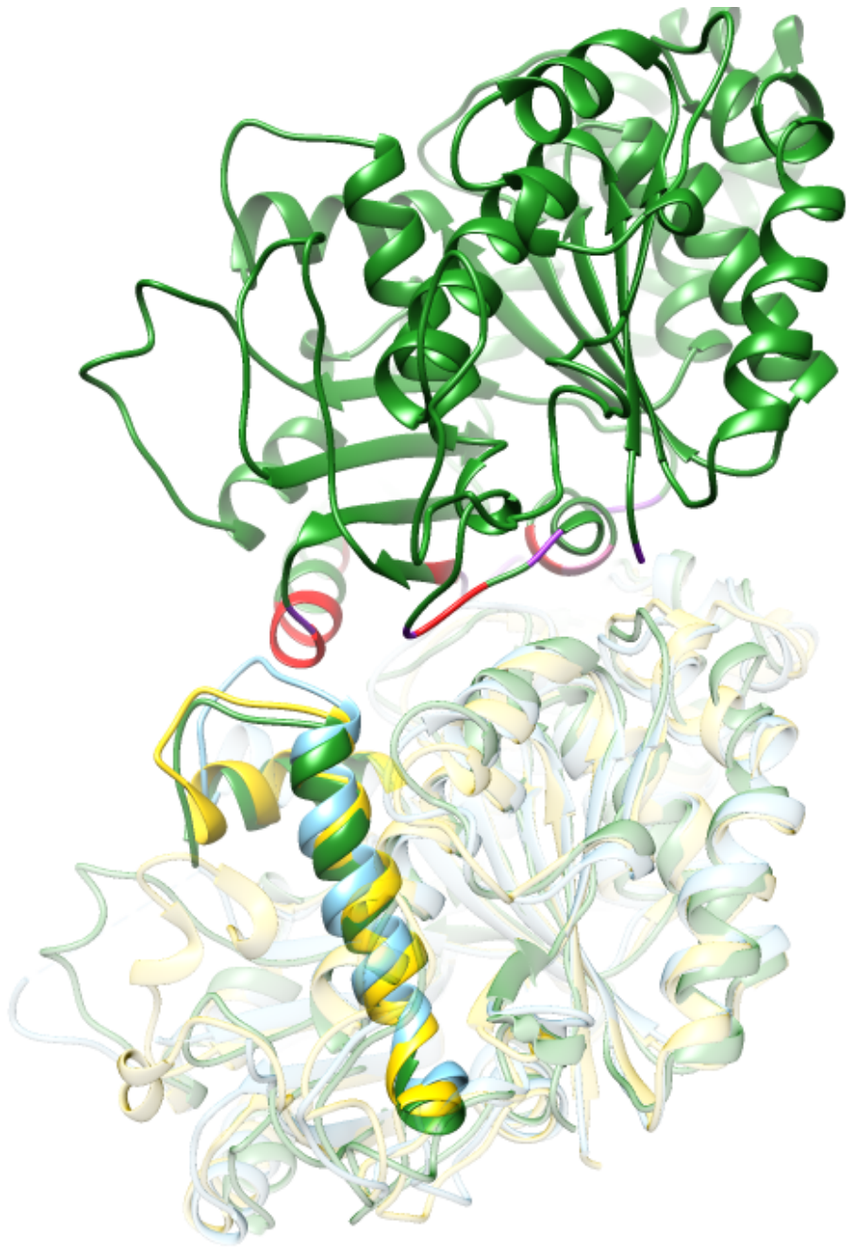




clashes w/human γ-tubulin

clashes with human and yeast γ-tubulin

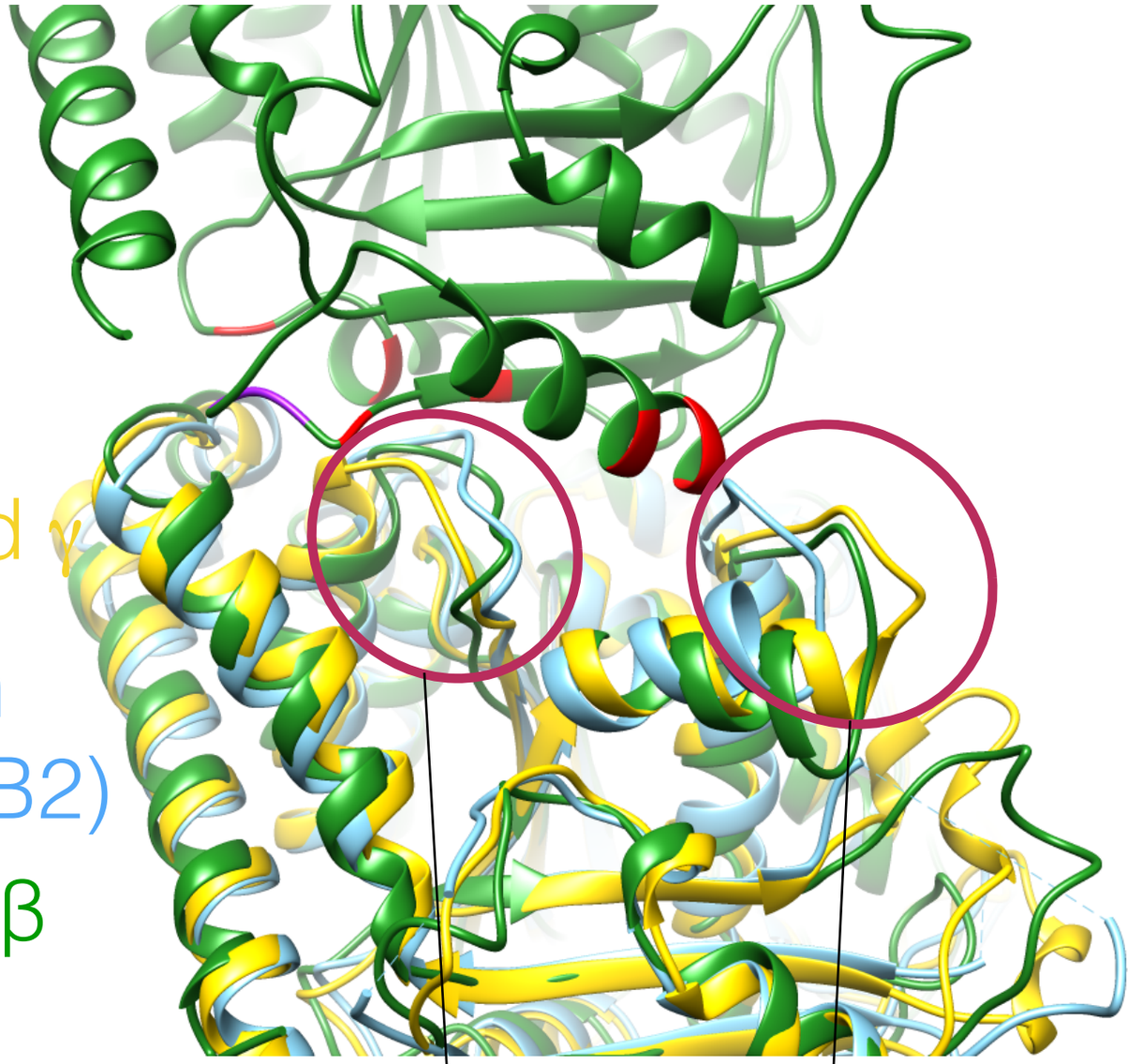
# *What is the conformation of γ-tubulin on the γ-TuSC*

straight α

98-bound γ

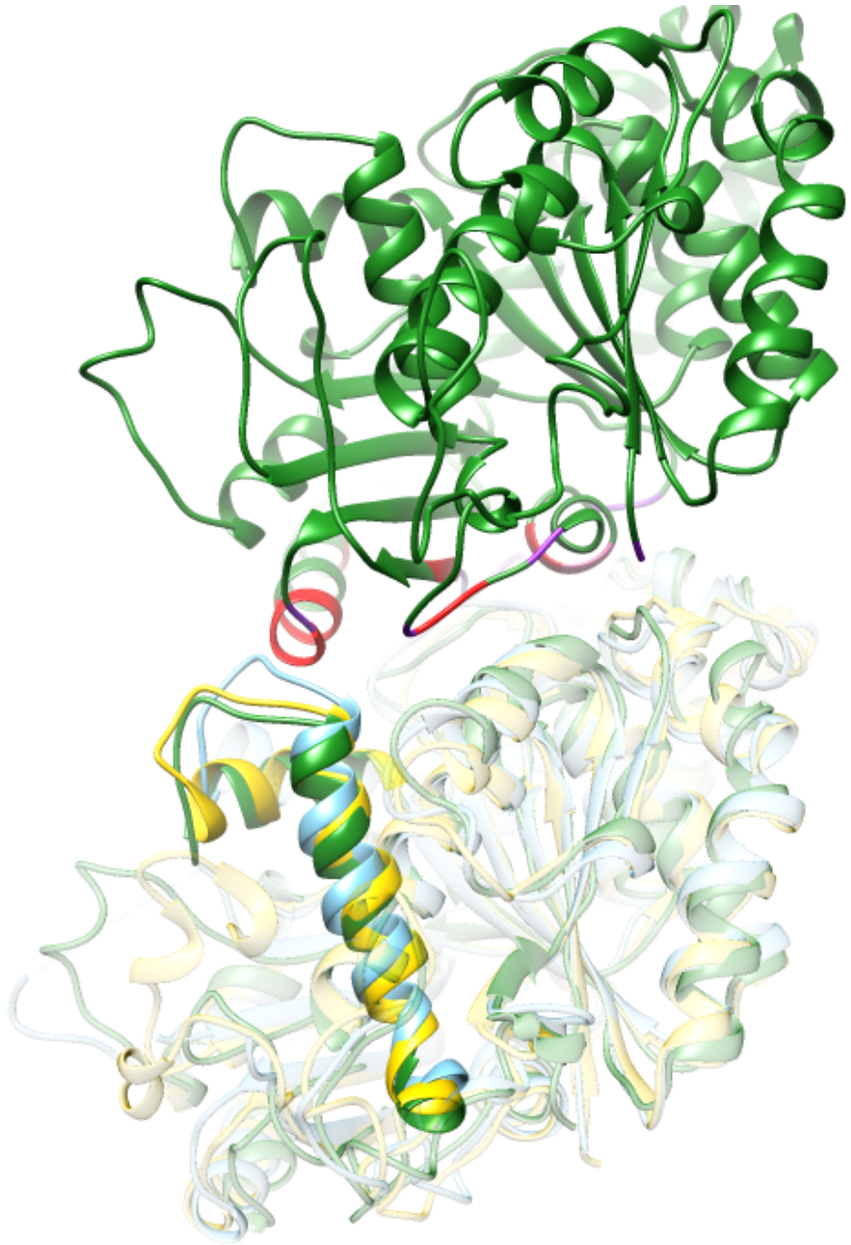
Human
γ xtal (3CB2)

straight β

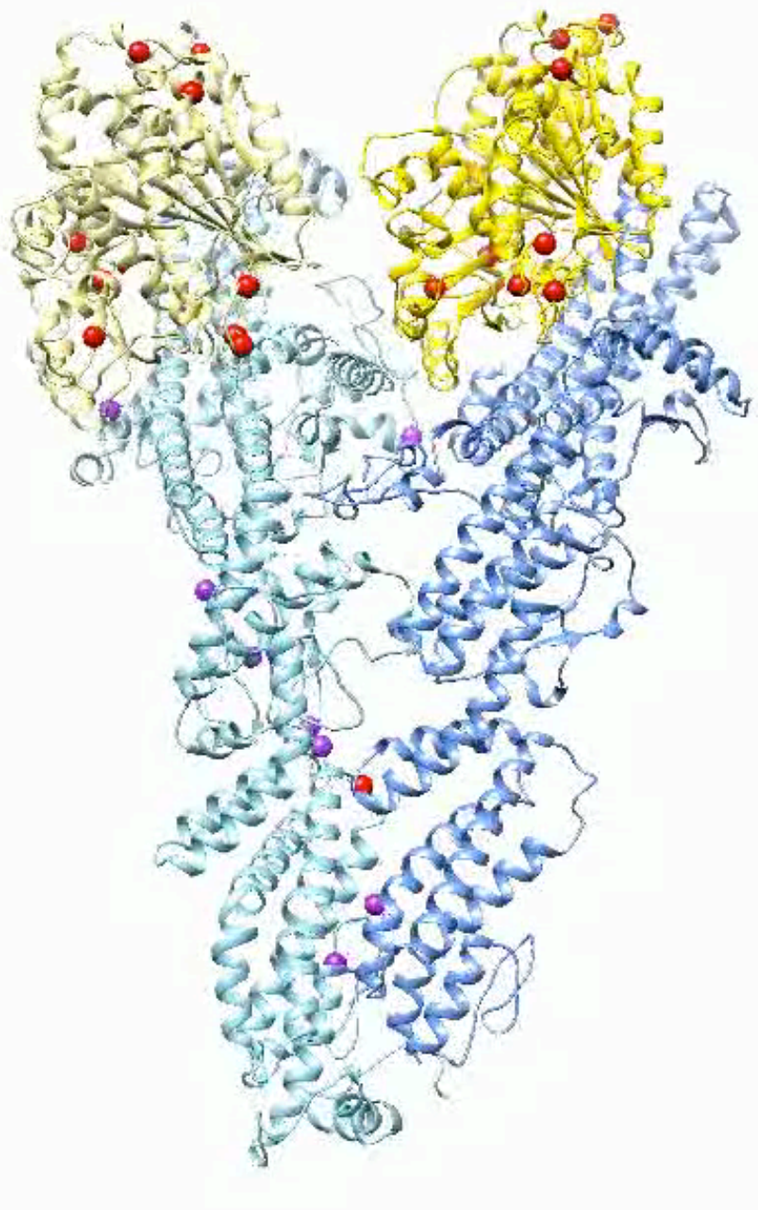


species
specific

conserved
(assembly)



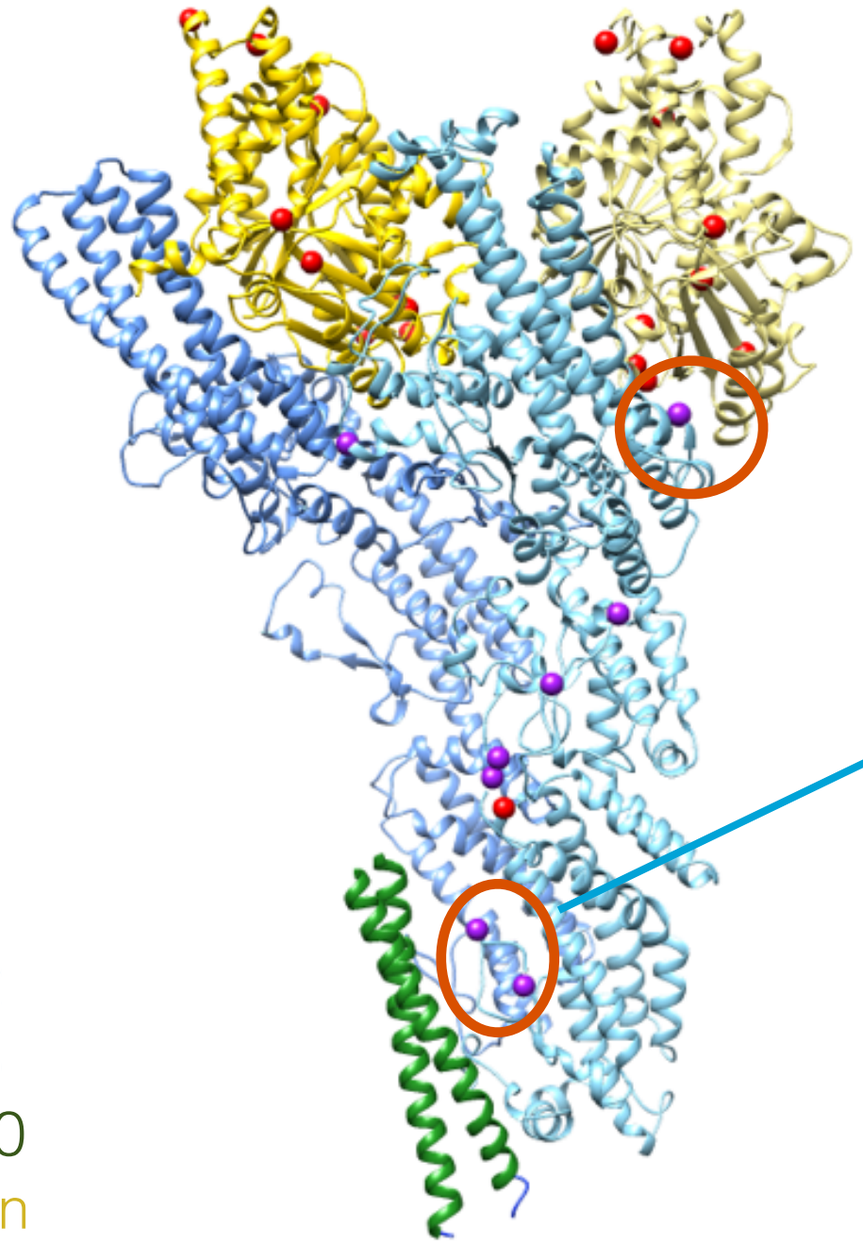
# *GCP2 phospho sites suggests functional roles*



GCP2
GCP3
Spc110
γ-tubulin

new phos

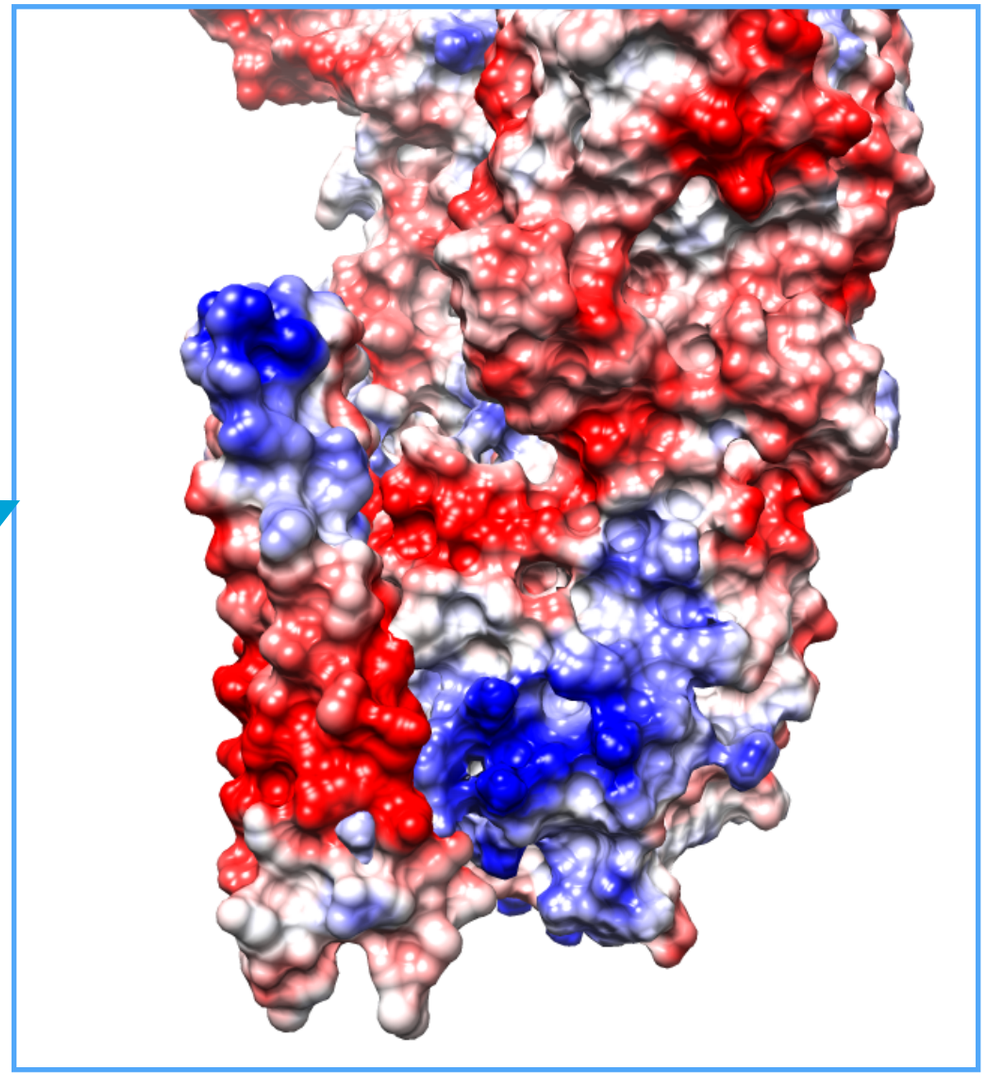
# *GCP2 phospho sites suggests functional roles*



GCP2
GCP3
Spc110
γ-tubulin

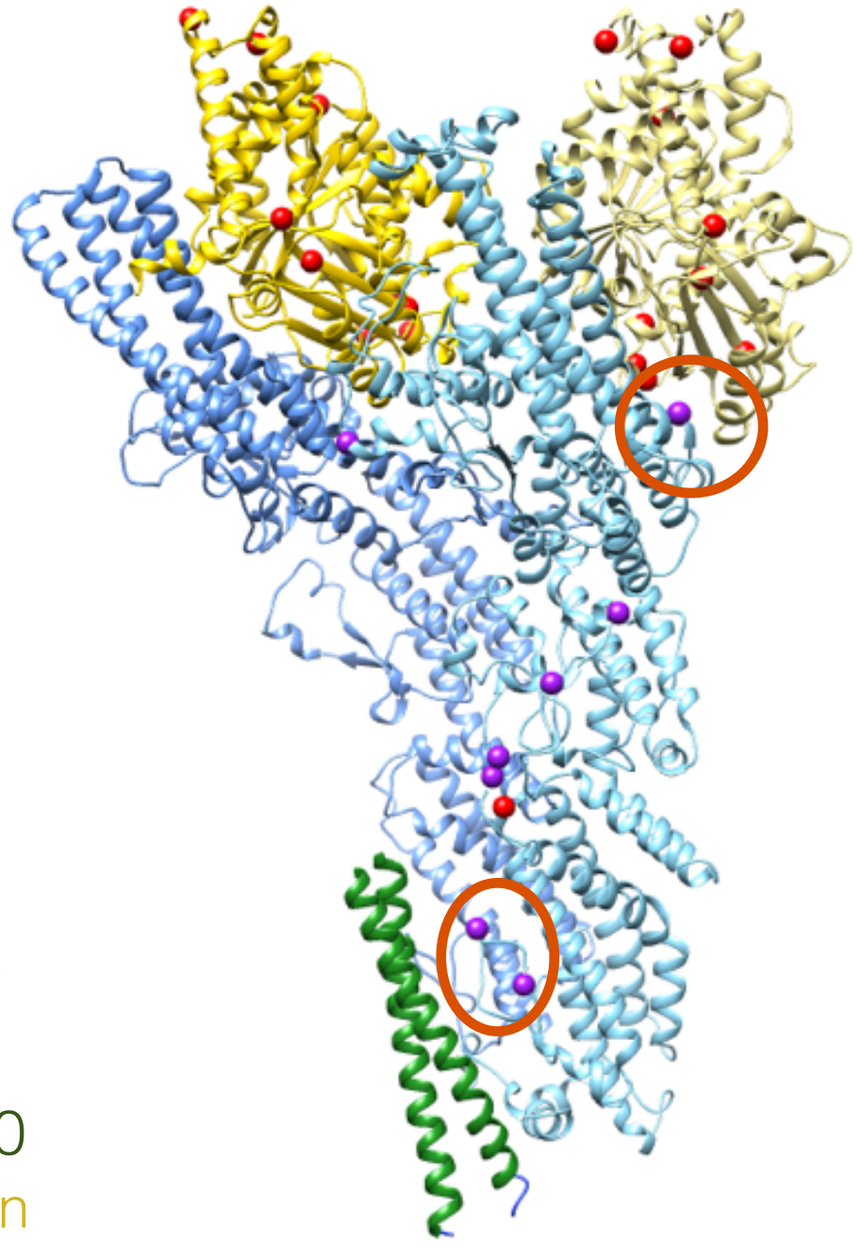
new phos

*Spc110 binding*



*γTuSC recruitment*

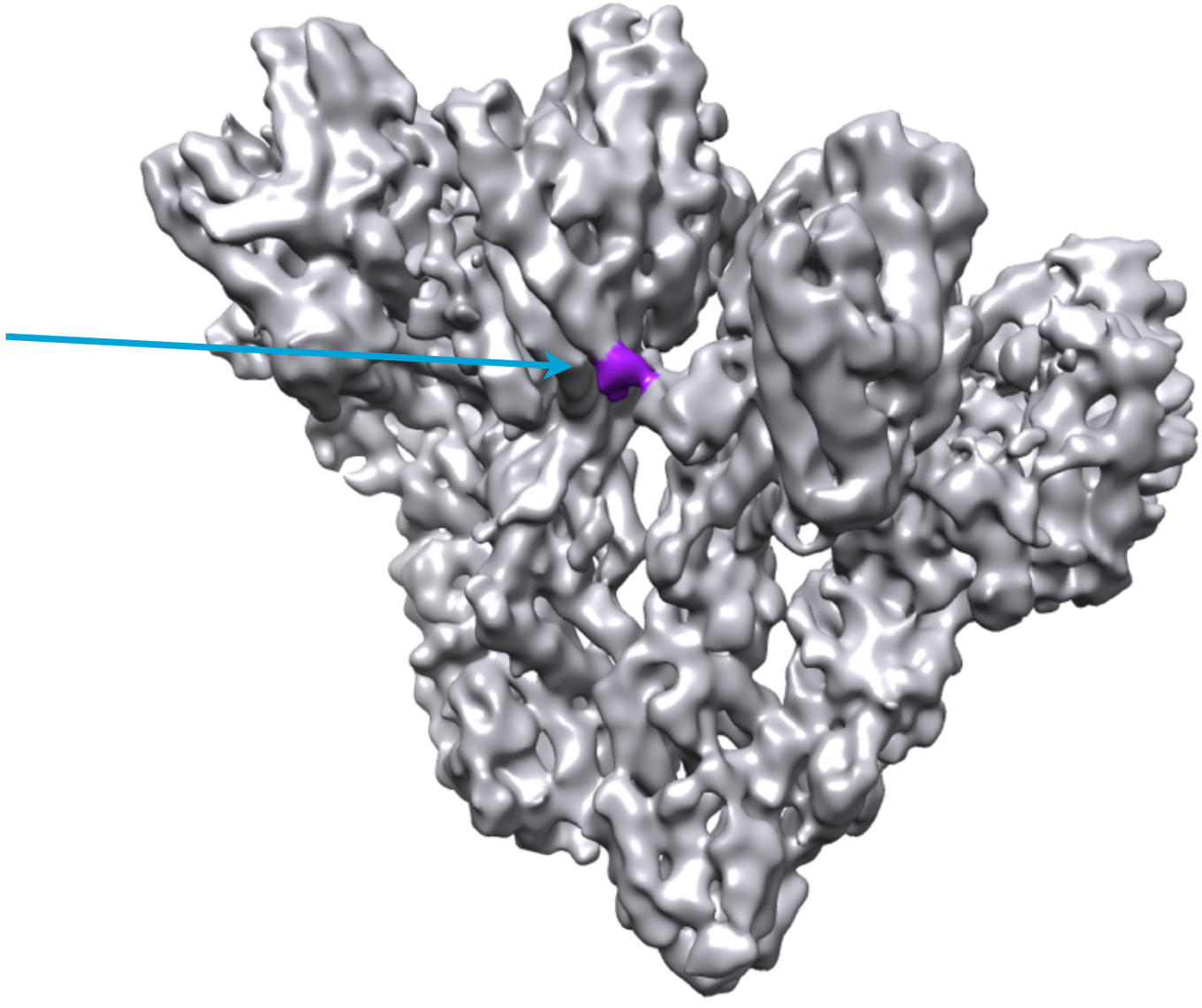
# *GCP2 phospho sites suggests functional roles*



GCP2
GCP3
Spc110
γ-tubulin

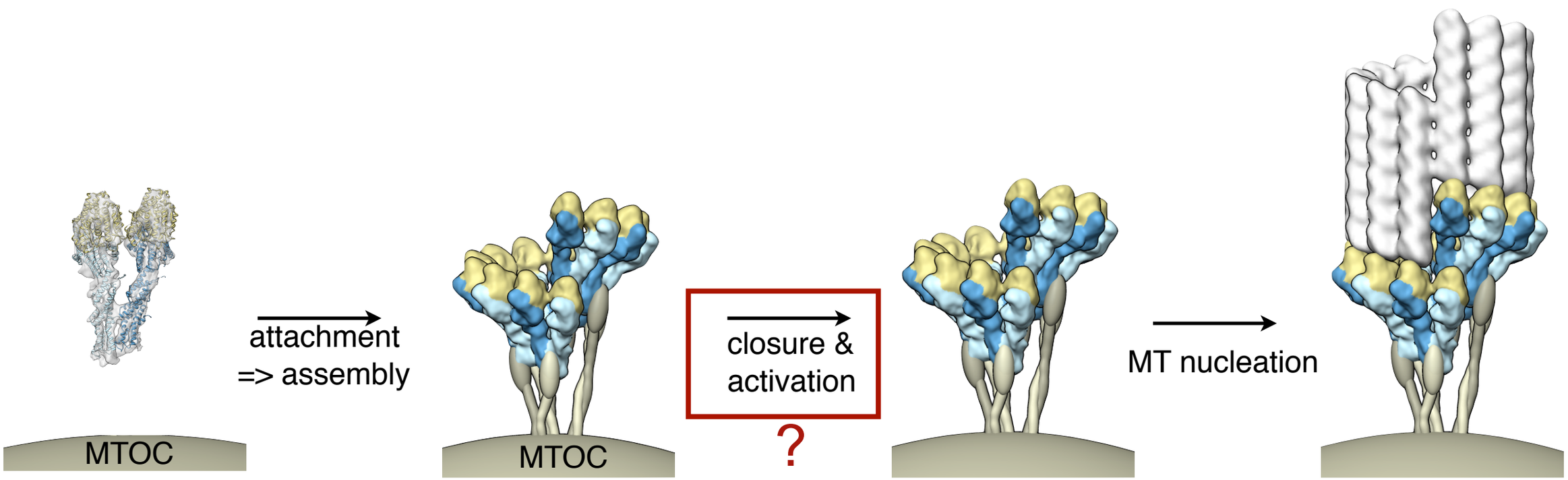
new phos

*Spc110 binding*



*γTuSC assembly*

# *Models for γ-complex mediated attachment and nucleation*




*γ-tubulin "poised" for α-tubulin binding*

*partially active for nucleation*

*fully active for nucleation*

# Acknowledgements


## UCSF

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## Beyond

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King Yabut
Kim Fong
Alex Zelter
Richard Johnson
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