Relating Nucleotide-dependent Conformational Changes in Free Tubulin Dimer to Microtubule Dynamic Instability

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MMSM2009, JNCASR, Dec. 17-20, 09

in femto - nanosecond timescale

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soft matter

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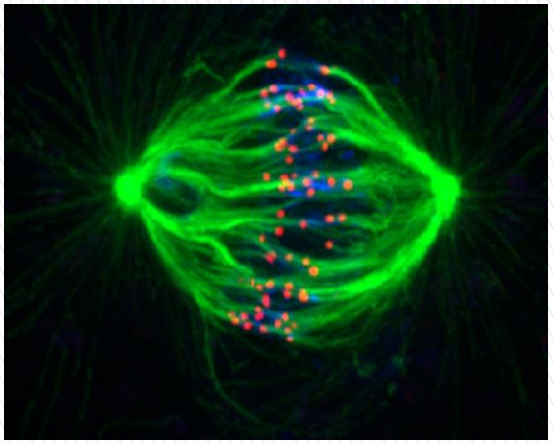
Overview

- Why to study?
- What is microtubule?
- What is dynamic instability?
- How is tubulin dimer related to?
- A theorist's simple approach to the complex problem!!!
- Suggestion welcome.

Why are Microtubules important?

Microtubules are involved in many fundamental biological processes:



- segregating the chromosomes correctly during cell division
- most effective drug target to cancer therapy
- organize cytoplasm by positioning the organelles
- understanding microtubule rigidity/flexibility may bring new nano materials.

STRUCTURE OF MICROTUBULE

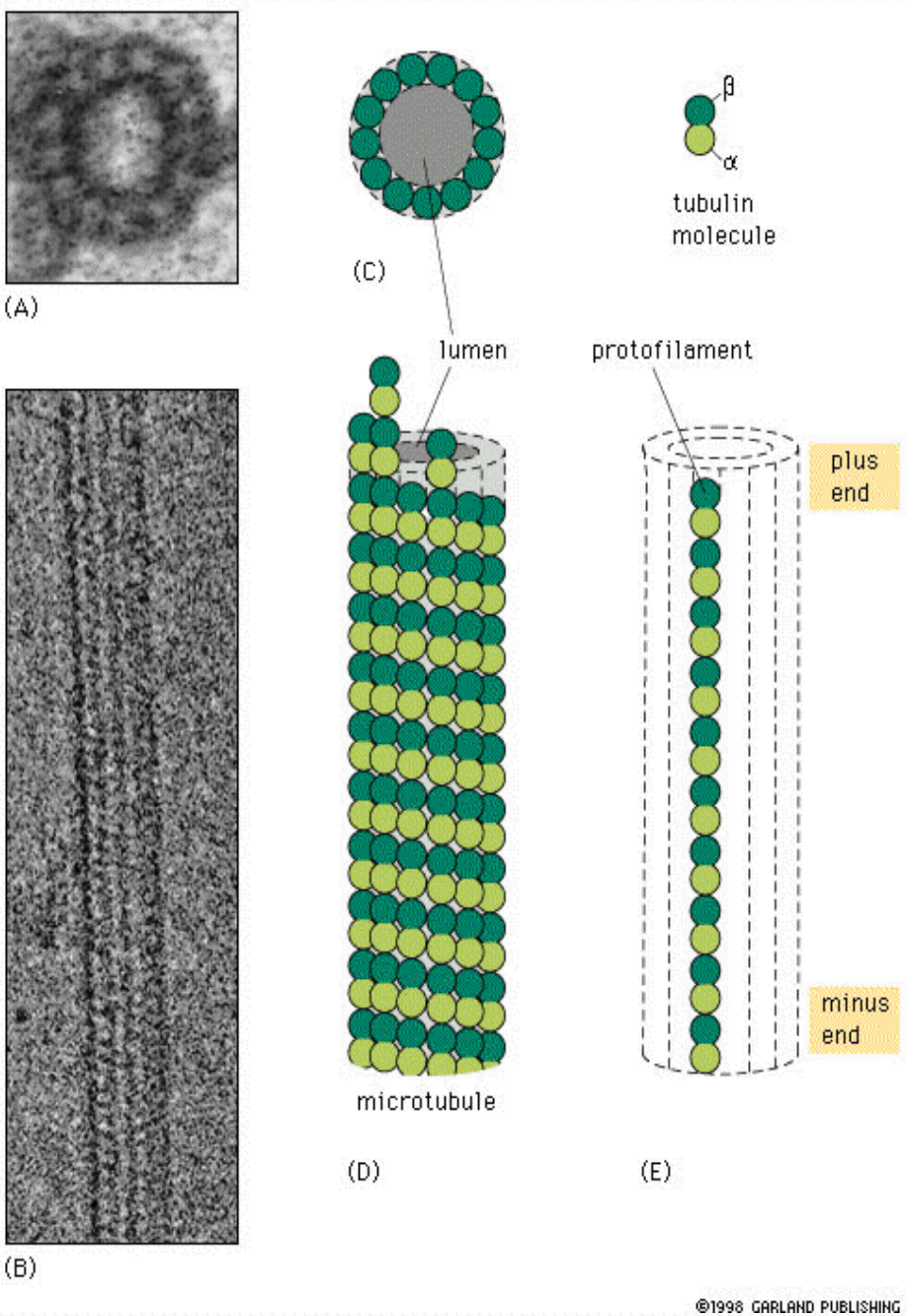
13 parallel protofilaments

25 nm in diameter; 200 nm - 25μm in length

Built by the assembly of **α- and β- tubulin dimers**

Alpha tubulin exposed at one end **(- end)** & beta tubulin exposed at the other end **(+ end)**

Grows by adding individual tubulin dimers



Microtubule Assembly/Disassembly

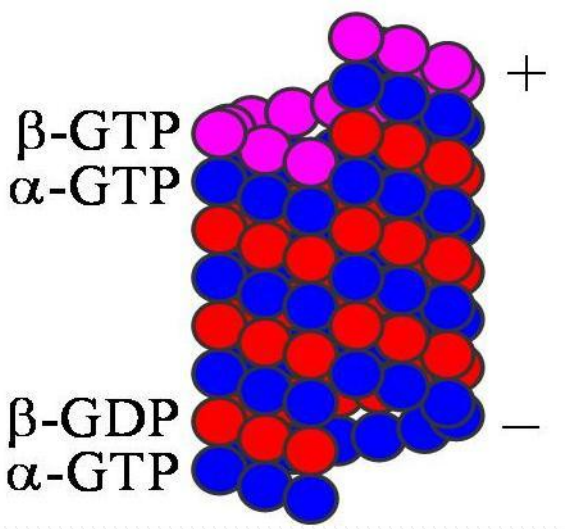
Tubulin dimers can add or dissociate at either end of a microtubule

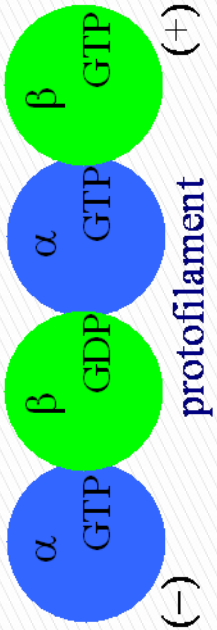
Greater tendency for subunits to add at **plus end**, where **β-tubulin** is exposed.

Contains two non-identical nucleotide binding sites – non-exchangale GTP site at α and GTP ↔ GDP exchangeable site at β

GTP must be bound to both α & β subunits for a tubulin heterodimer to assemble

Subunit addition promotes **hydrolysis of GTP** bound to β-tubulin.

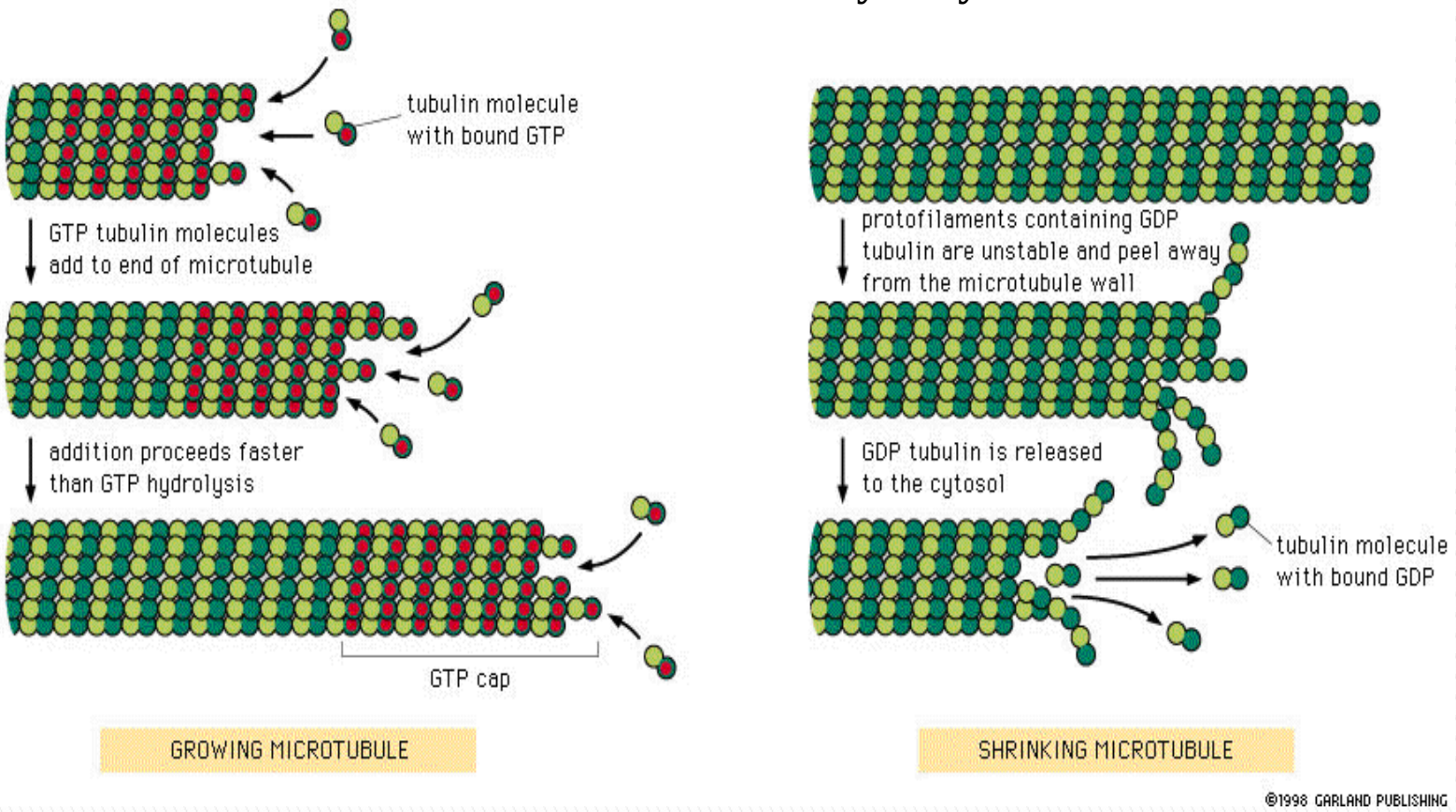




Microtubule Dynamic instability

Only GTP-tubulins assemble to microtubules

Subunit addition promotes hydrolysis

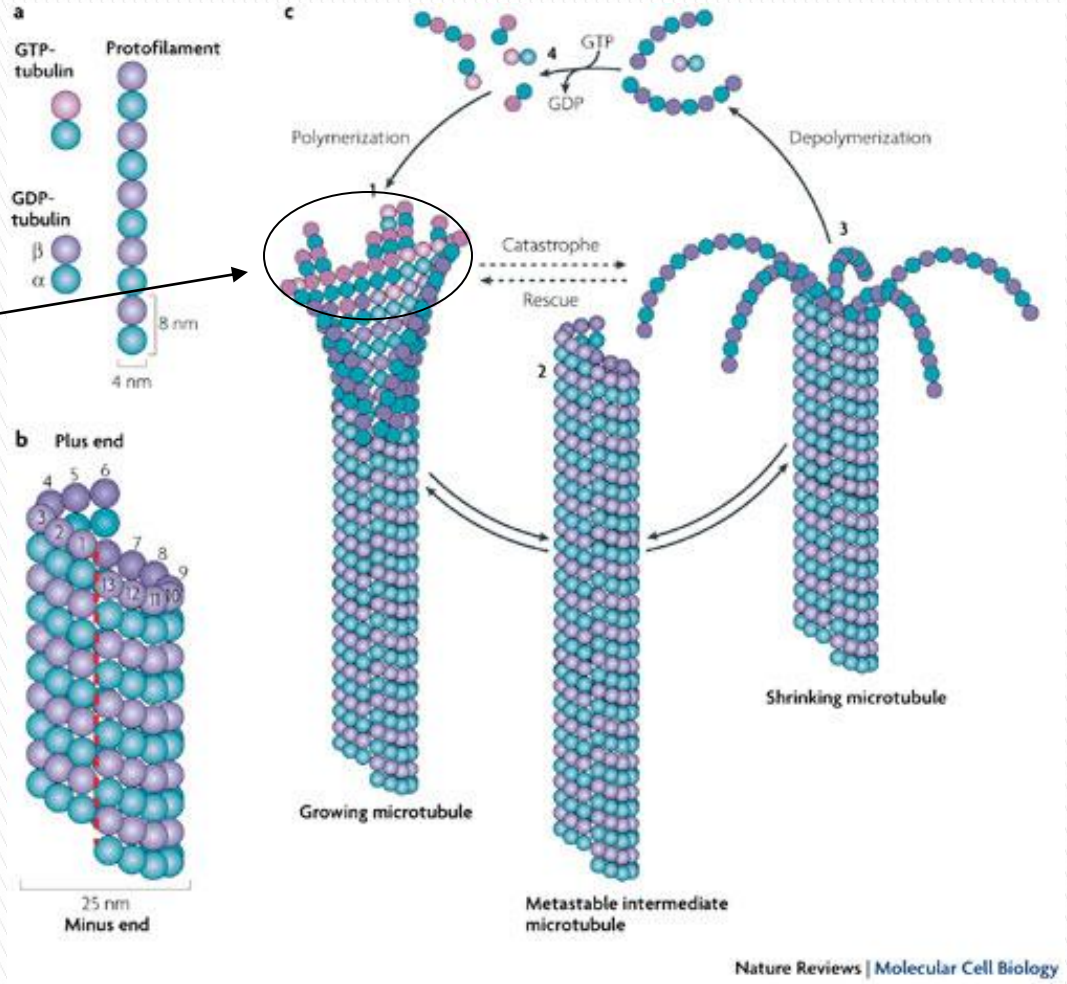


GDP Tubulin

GTP Tubulin

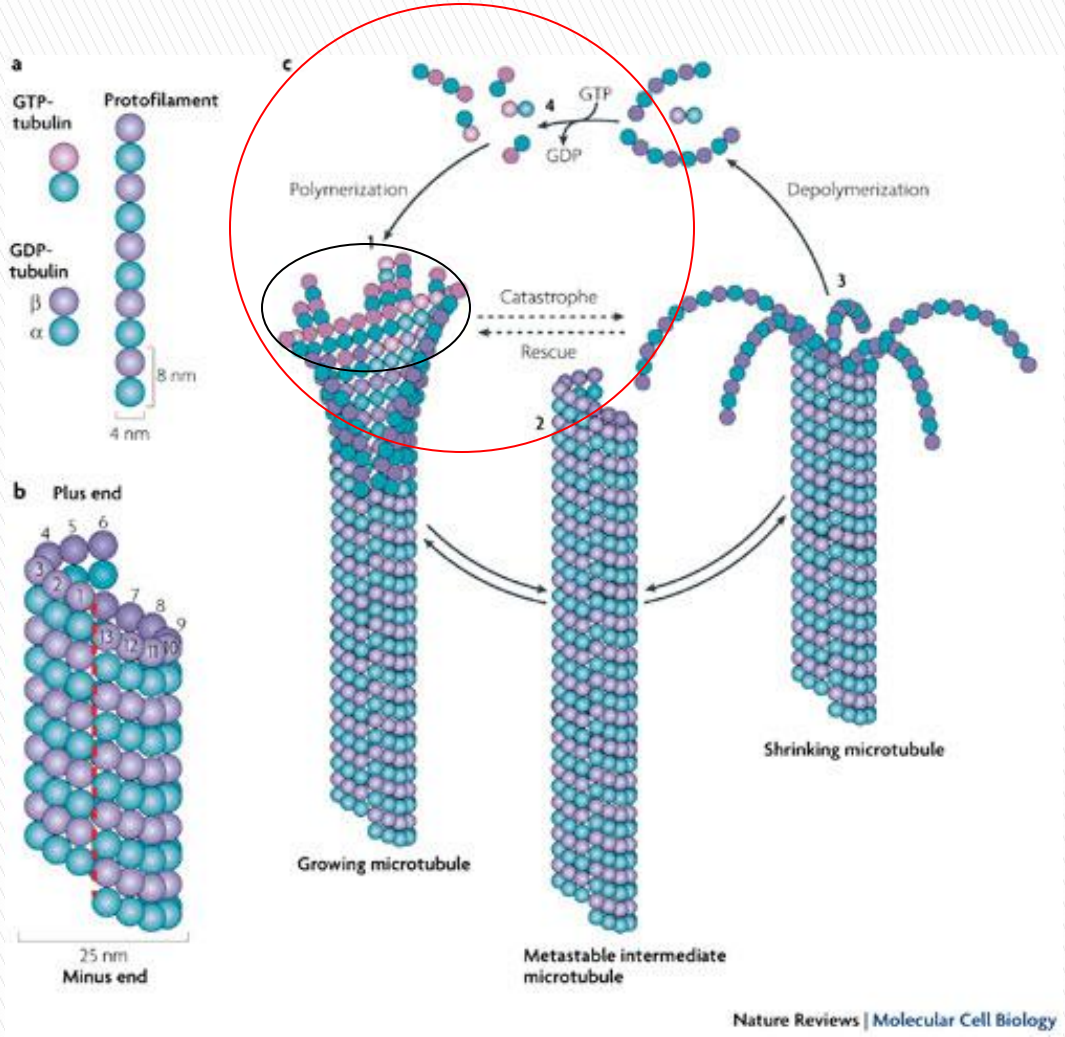
Microtubule Dynamic instability

GTP Cap



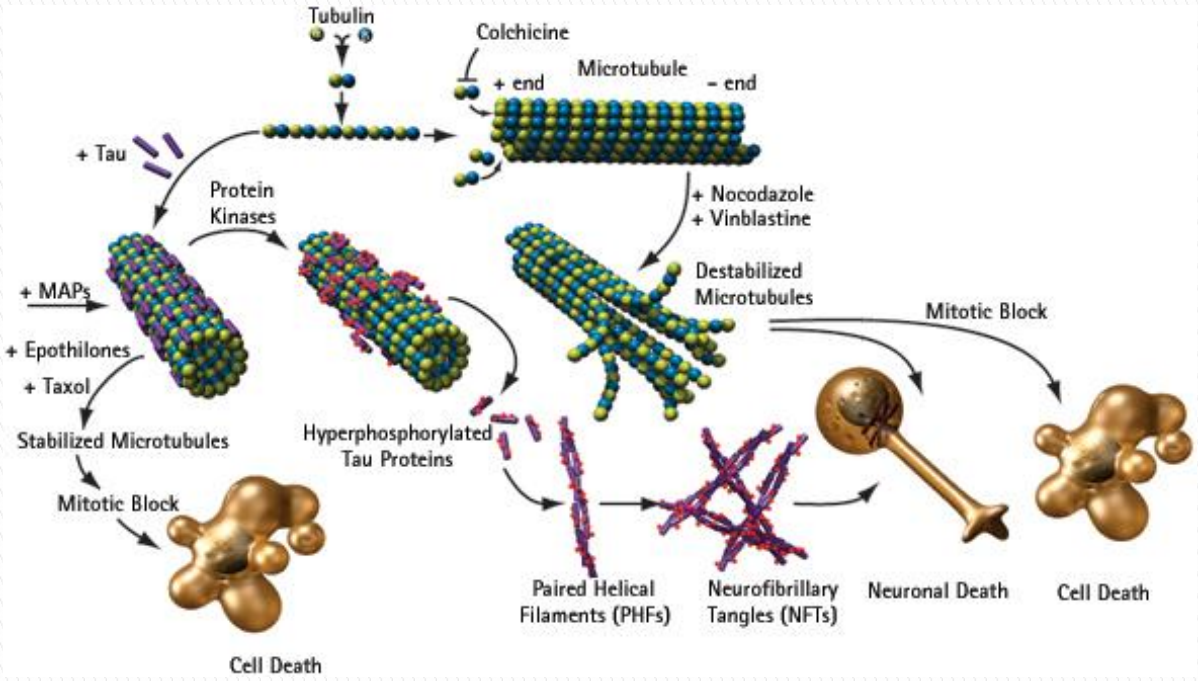
Akhmanova, A. and Steinmetz, M.O.Nat. Rev. Mol. Cell Biol. 9, 309 - 322 (2008)

Microtubule Dynamic instability



Akhmanova, A. and Steinmetz, M.O.Nat. Rev. Mol. Cell Biol. 9, 309 - 322 (2008)

Tubulin Pathway



Taxol is a mitotic inhibitor used in cancer therapy

It interferes with the normal function of microtubule by blocking the GDP-tubulin disassembly

Intrinsic GTPase activity of Tubulin dimer

- Only GTP-tubulin dimer can assemble to form protofilaments
- GDP-tubulin dimer can't bind effectively rather peels away from microtubule wall
- However, Taxol bound GDP-tubulin can keep the protofilament stable
- GTP-tubulin: α-GTP-β-GTP
  GDP-tubulin: α-GTP-β-GDP

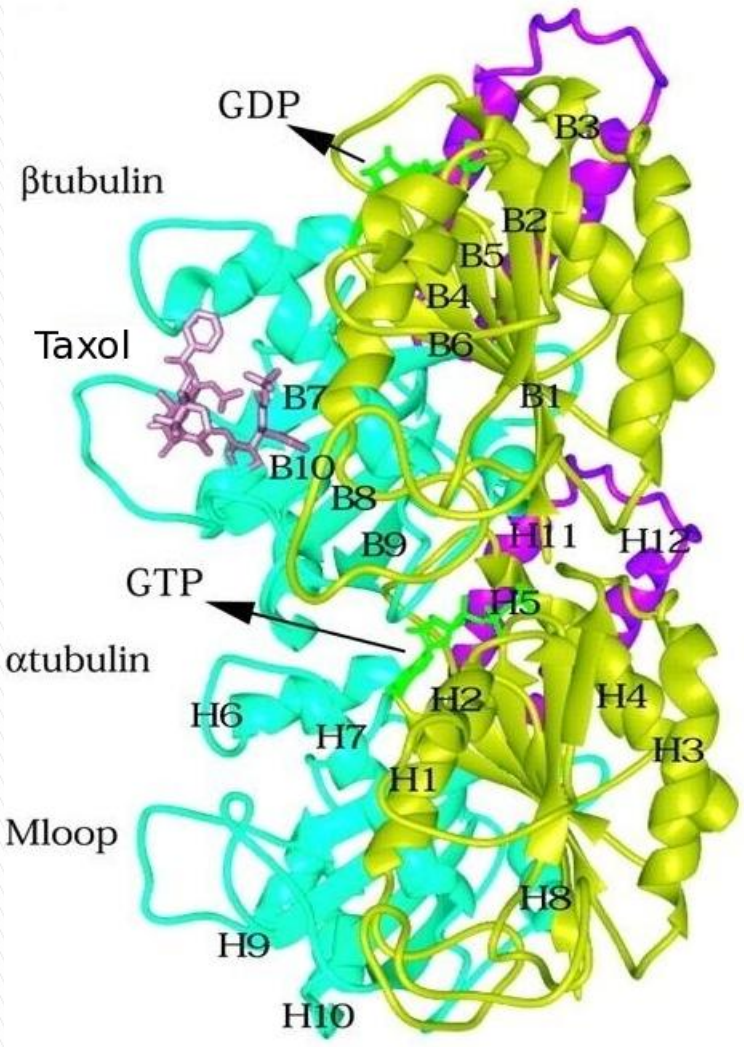
Literature Review

- Nogales, CMLS 1999, “The complex dynamic behavior of microtubules, though can be modulated by other cellular factors, is primarily due to the tubulin subunit architecture and its intrinsic GTPase activity”.

Problems and our Approach to tackle them

- Structural information of free tubulins would help in understanding the function
- Crystalizing free GTP- and GDP-tubulins are difficult
- Modeling these structures would be important
- Can we correlate the structures with microtubule dynamic instability?
- We perform all atom MD simulations and Protein-Protein docking studies to gain insight

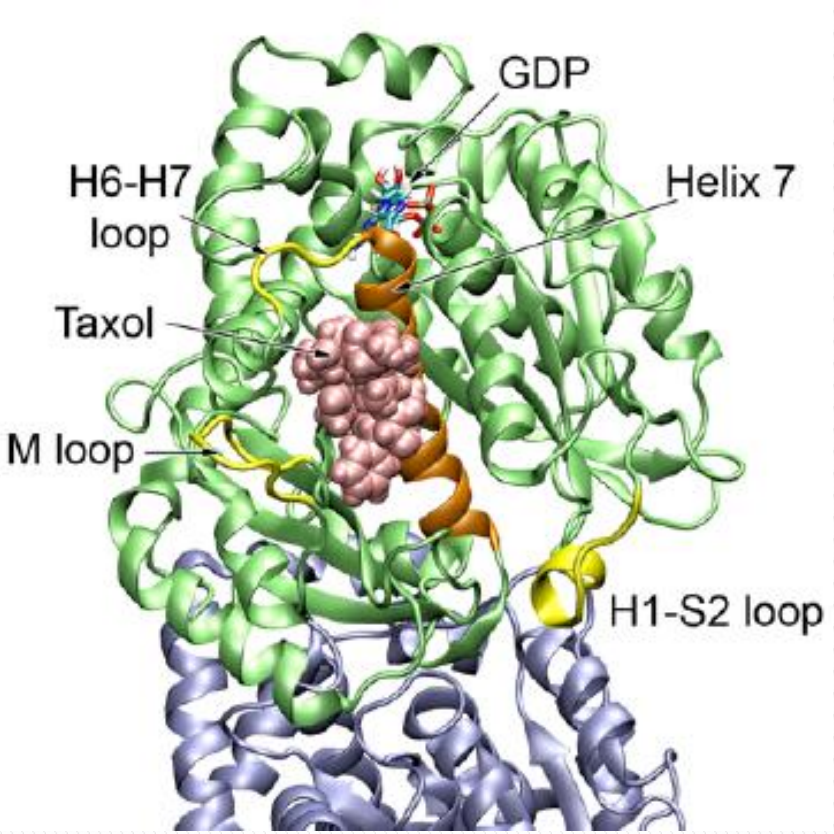
Our Starting Model to Explore the Tubulin Subunit Architecture



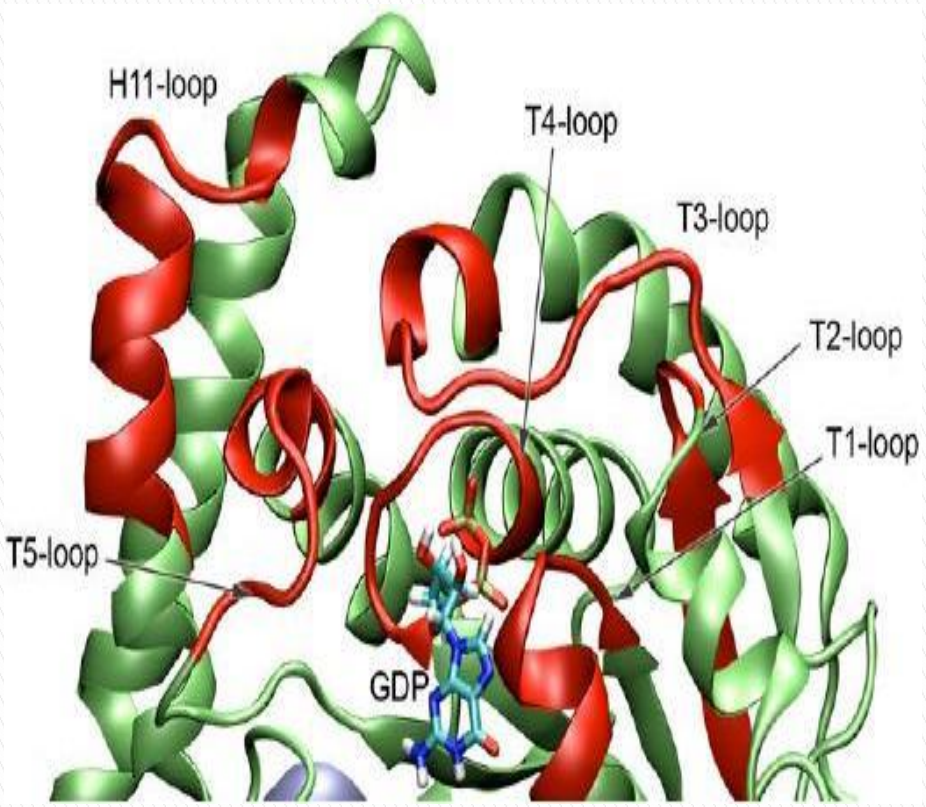
- Taxol-bound Tubulin X-ray structure from Zn^{+2} induced β-sheet
- α-GTP-β-GDP Tubulin heterodimer bound to Taxol
- 3.5 Å resolution
- Each monomer consists of 3 domains
  - N terminal: S1 – S6, H1 – H7, T1 – T7
  - Intermediate: S7 – S10, H8 – H10, M loop
  - C terminal: H11, H12

Lowe et al. J. Mol. Biol. 2001

Helices and Loops that are directly involved in interactions



Taxol binding Domain

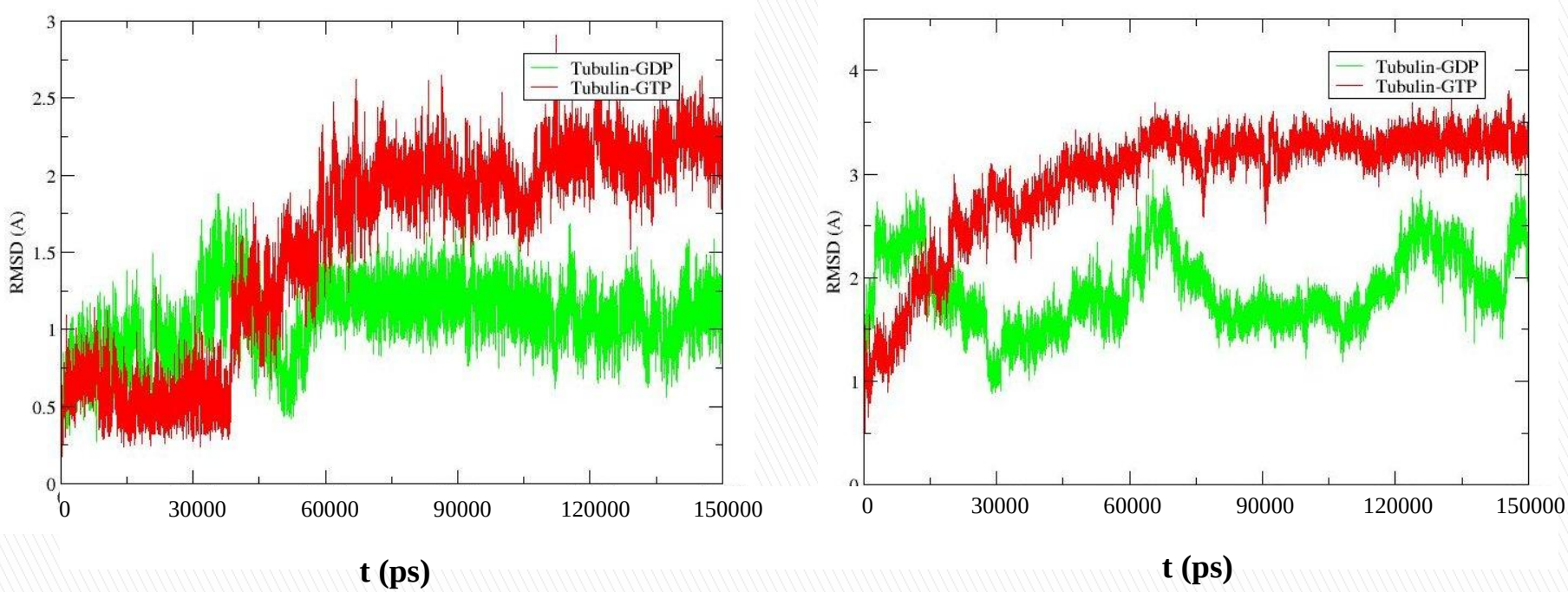


Nucleotide binding Domain

Simulation details

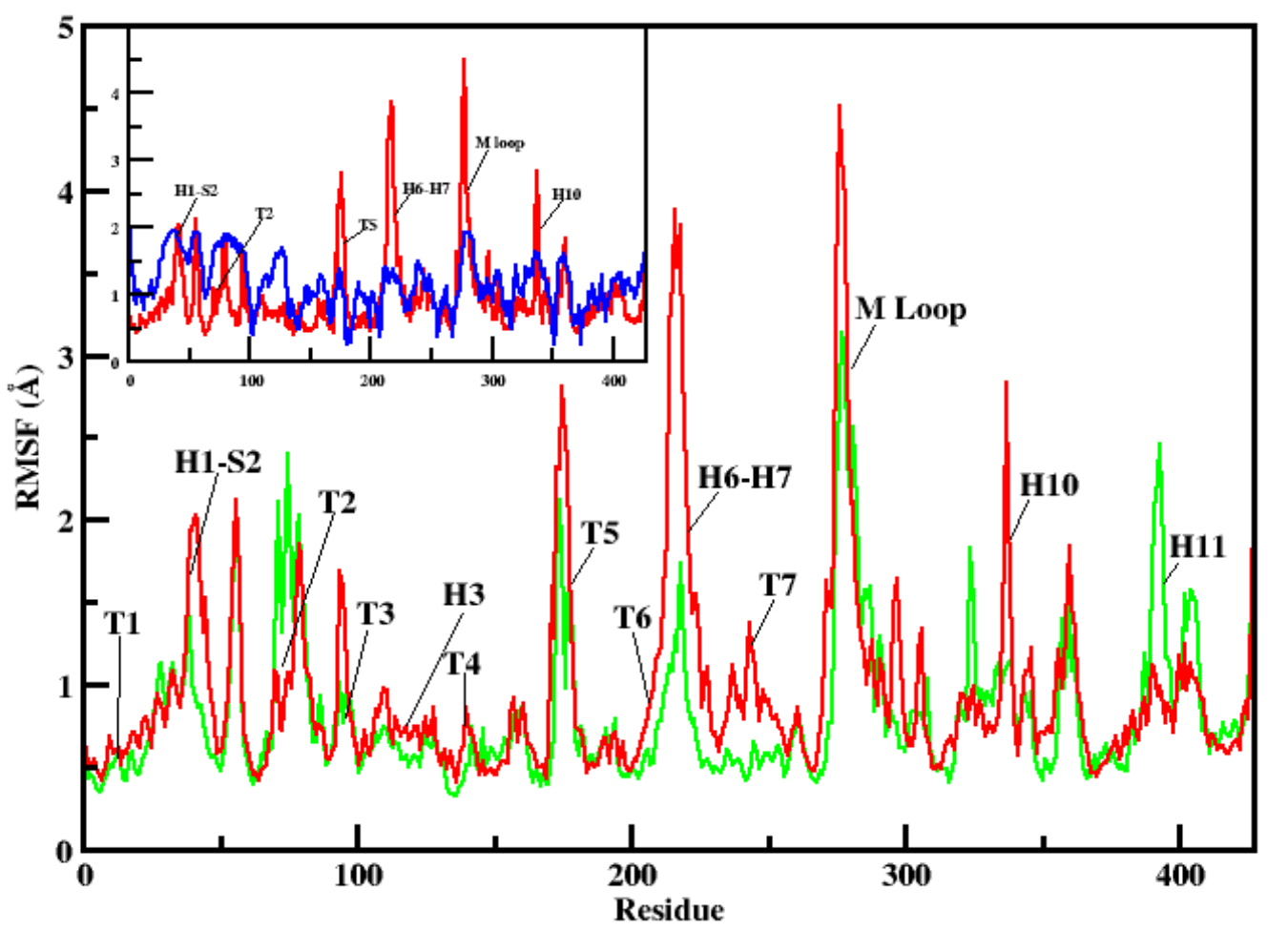
- We performed two independent MD simulations of free tubulin dimers bound, respectively, to GDP and GTP at the E site
- Control simulation of Taxol bound to GDP-tubulin
- Amber force field on 16 processors of an Infiniband Xeon E5472 linux cluster
- 150 ns each

Evolution of the Systems



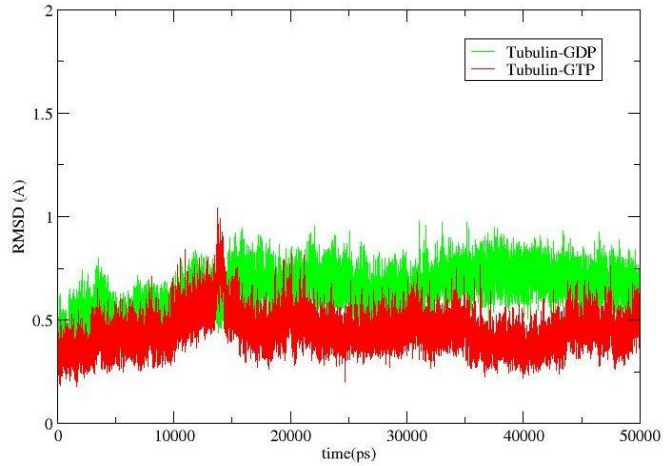
RMSD analyses of (a) full tubulin and (b) β-tubulin alone.

B factor analysis of β-tubulin

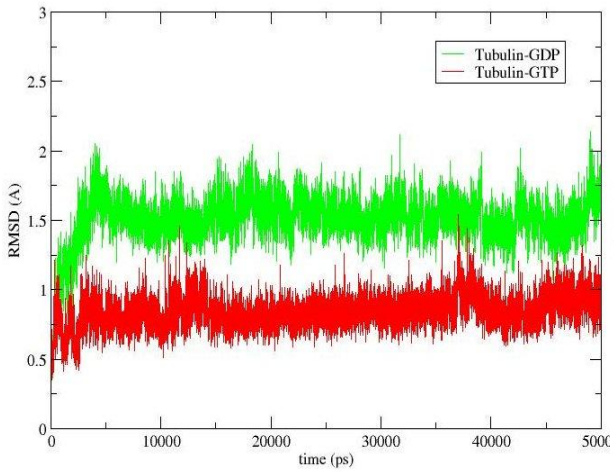


GDP and GTP bound form show differential flexibility in the Taxol binding site **(allosteric effect?)**. Color scheme: GDP (green), GTP (red), Crystal (blue).

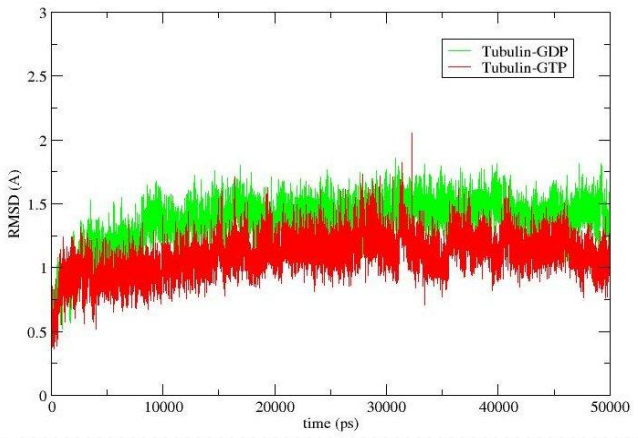
Allosteric effect in place



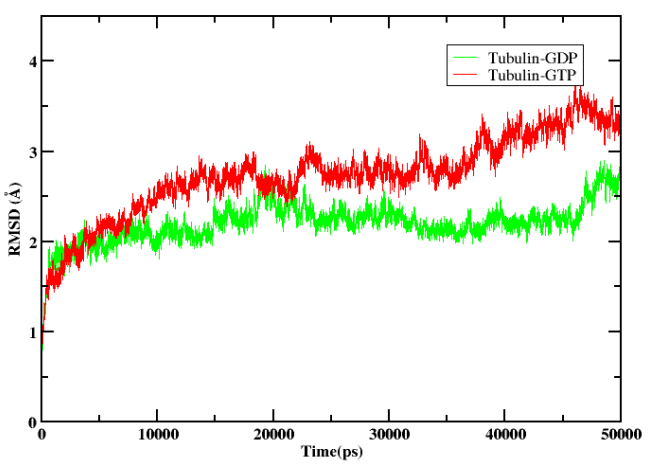
T1 loop



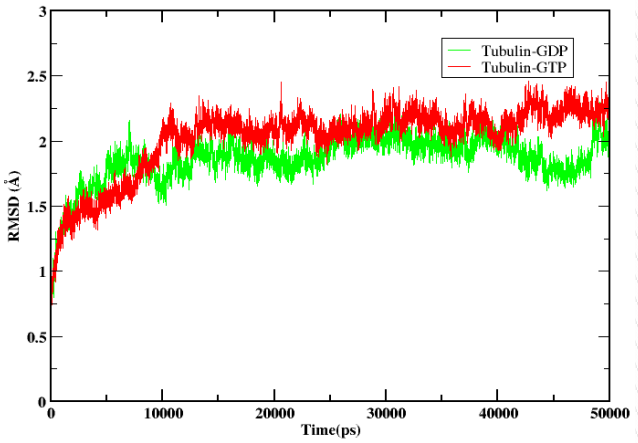
T2 loop



T3 loop

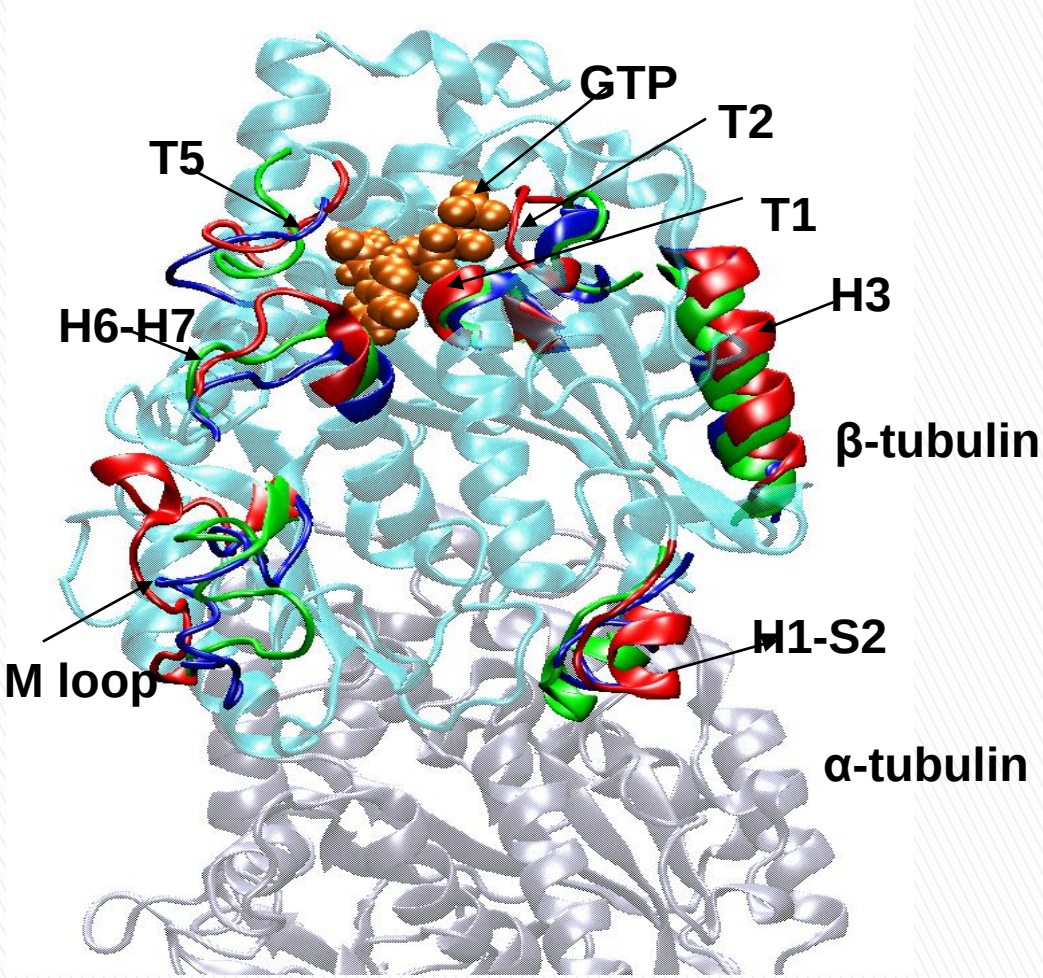


M loop



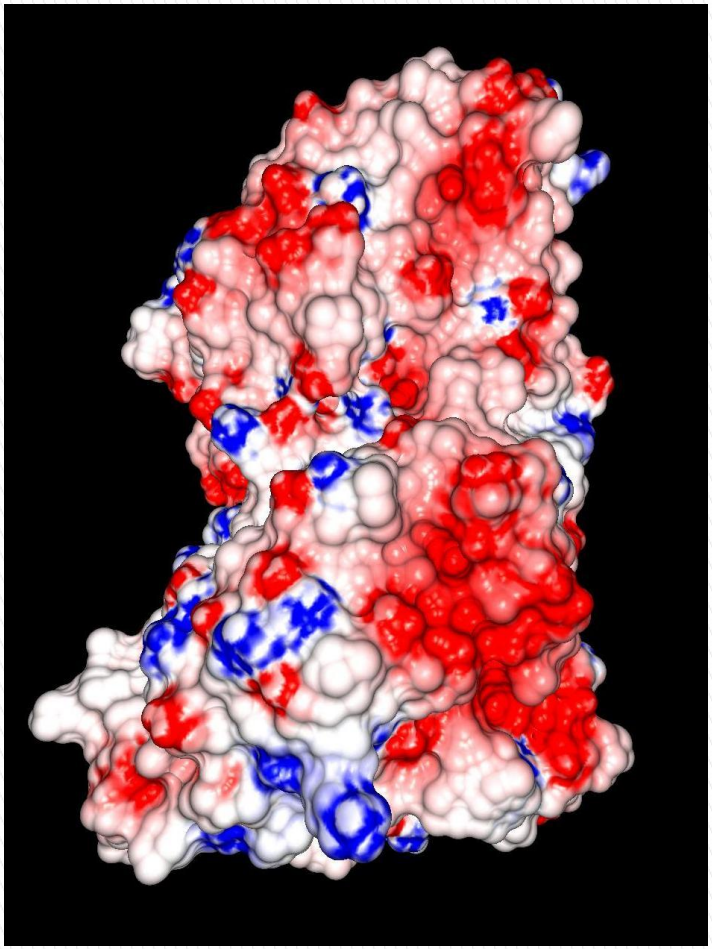
H1-S2 loop

Structural Comparison Between free GTP- and GDP-tubulins

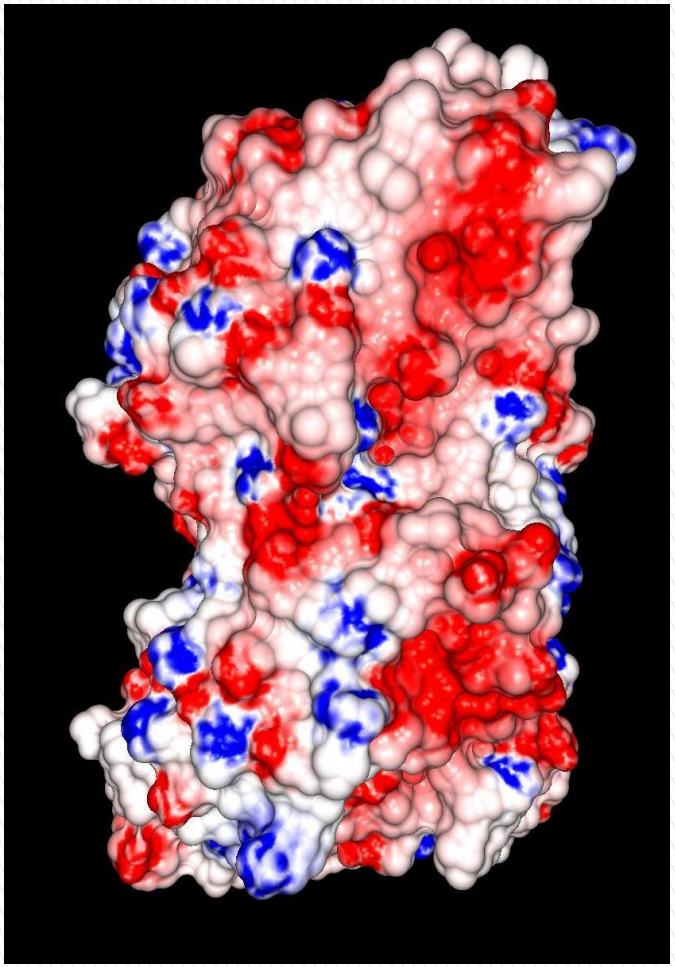


- Nucleotide state confers significant conformational changes in tubulin dimer
- M loop, H6-H7 loop, H3 helix, H1-S2 loop expose greatly in GTP-bound form
- Color scheme: GDP (green), GTP (red), Crystal (blue)

Electrosatic Surface of Tubulin

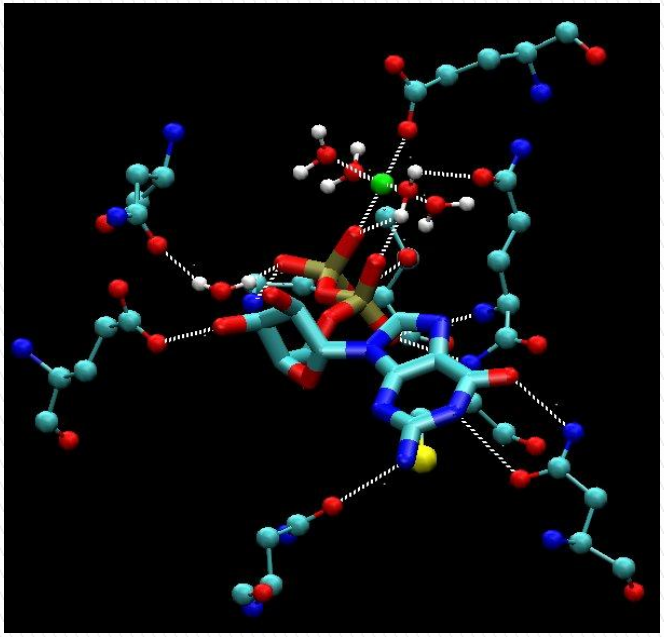


Tubulin-GDP

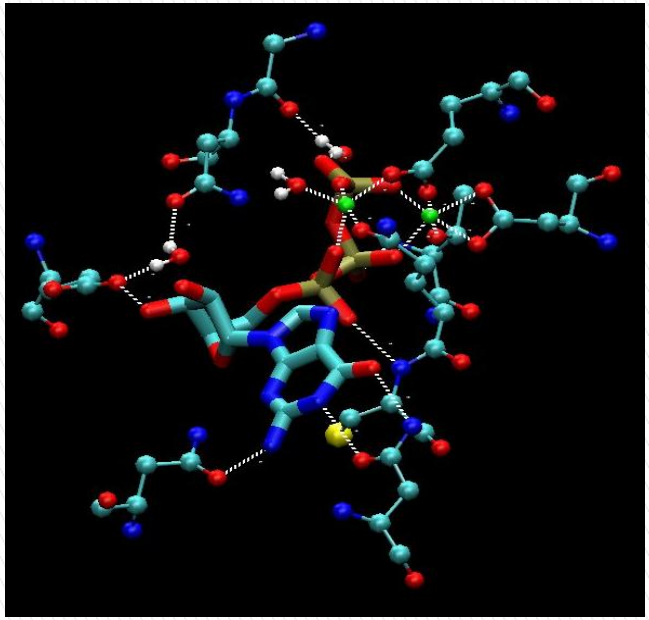


Tubulin-GTP

Mg^{2+} dependence of guanine nucleotide



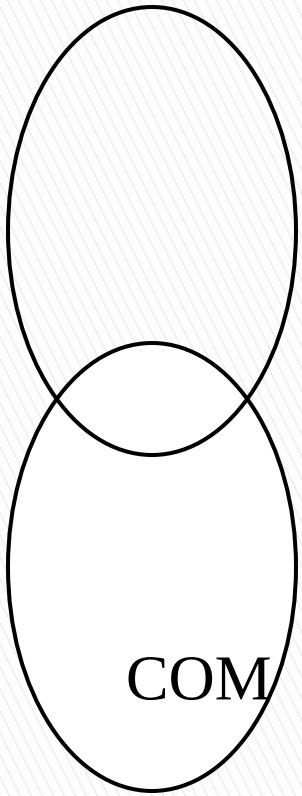
GDP-tubulin

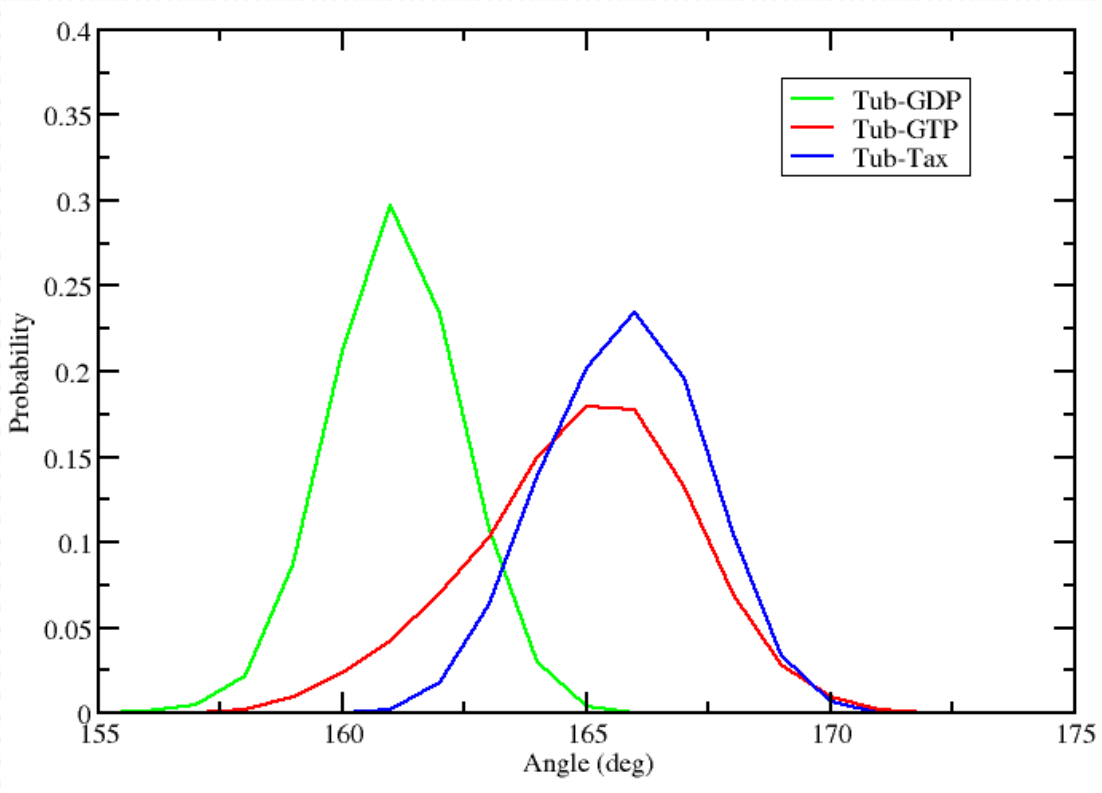


GTP-tubulin

- Spontaneous appearance of Mg^{+2} ions into the nucleotide binding pocket of β-tubulin – as seen in other GTPase X-ray structures
- The role of water in hydrolysis is implied by the occurrence of 2 Mg^{+2} and 2 water in GTP-bound form
- Mg^{+2} is hexacoordinated

Angular Distribution of Tubulin dimers





→ Free GTP-tubulin and Taxol-bound GDP-tubulin are 5 – 7 degrees straighter than free GDP-tubulin

Looking Back to Literature

- Nogales et al. Nature 2005, We hypothesize that GTP-tubulins are straight but GDP-tubulins are kinked, and hence the later is energetically unfavorable on microtubule wall.

- Elie-Caille et al. Curr. Biol. 2007, “Straight GDP-tubulin protofilaments form in the presence of taxol”.

Model Structures of Free Tubulin Dimers seem to be good

- Model structures of free GTP- and GDP-tubulin dimers are ready
- Let's try correlating that to microtubule dynamic instability

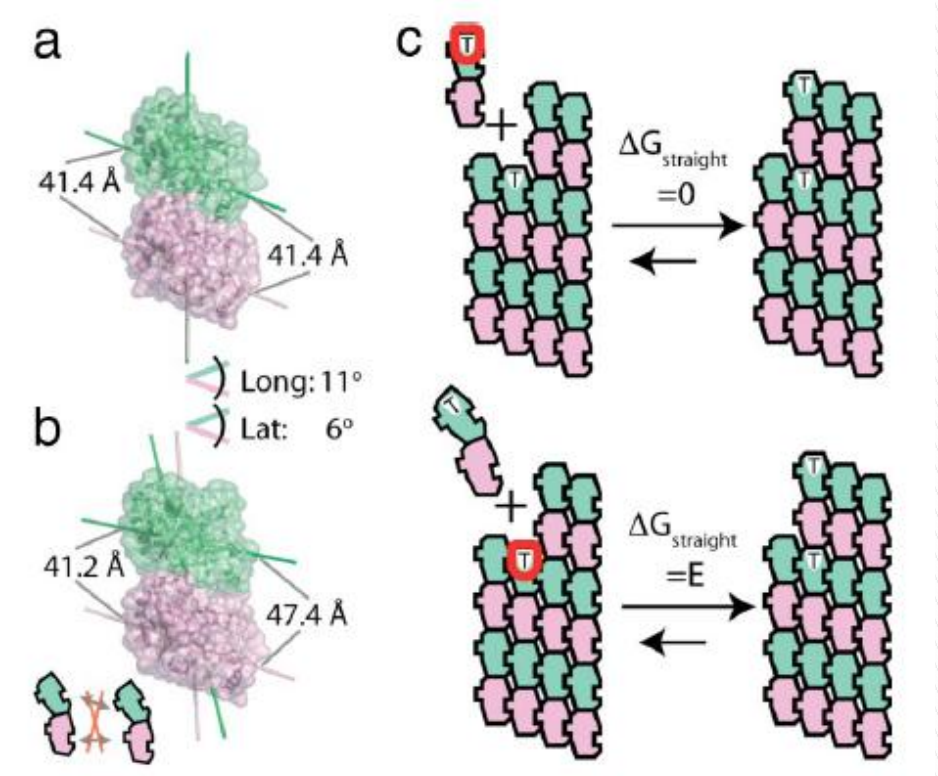
Conflicting models of Tubulin assembly in protofilament

Allosteric model

J. Cell. Biol. 1991, PNAS 1998, Nature 2004

Lattice model

PNAS 2008



Protein-protein docking to check the mode of tubulin assembly

- Two dimers are docked laterally, both for GDP- and GTP-tubulins
- Two dimers are docked longitudinally, both for GDP- and GTP-tubulins
- Strength of interactions is estimated by SASA
- Docking performed through HADDOCK

HADDOCK: High Ambiguity Driven protein-protein Docking

- Protein-Protein docking approach based upon available experimental data

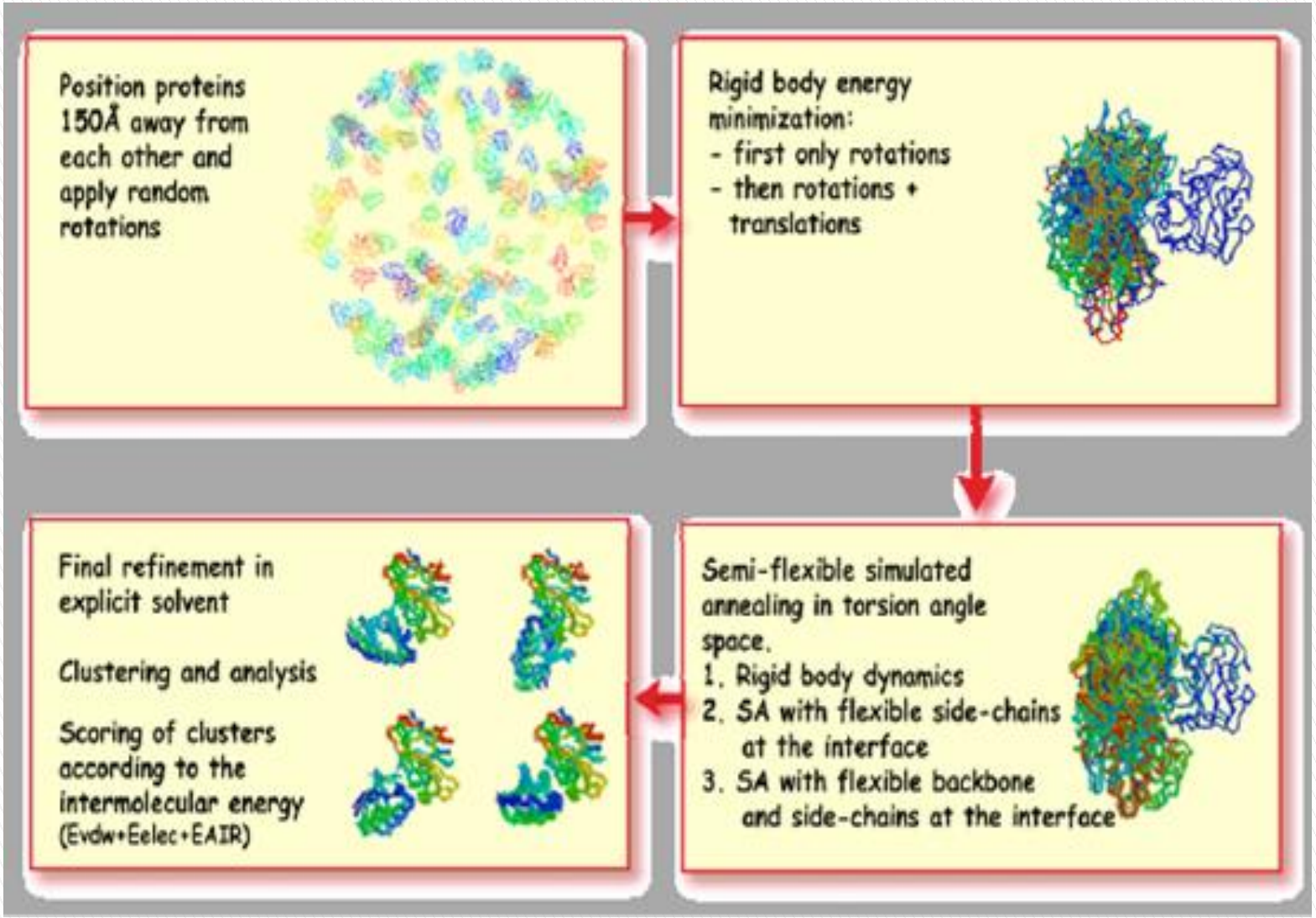
Data directed docking

- Include data directly in the docking by adding an additional energy term or limiting the search space

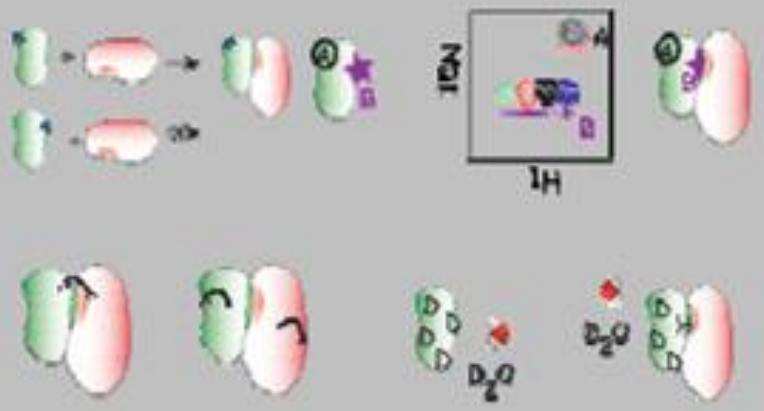
Requisites

- 3D structures of the two partners must be known
- No large conformational change must take place upon complex formation
- Information available to map the interaction interface of the both partners, e.g.
  - Chemical shift perturbation
  - Mutagenesis data
  - Any type of other data

Haddock docking protocol



Combine biochemical or biophysical data and semi-flexible docking to model biomolecular complexes



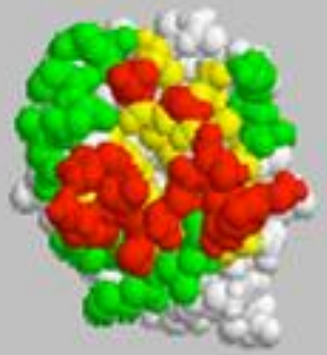
Interface definition:

- **Active residues:**

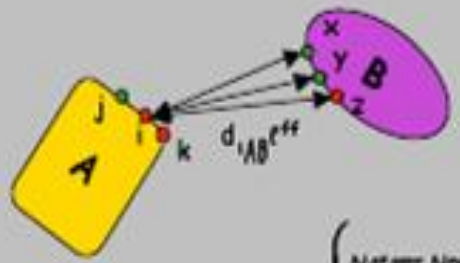
involved in the interaction (e.g. from NMR data, mutagenesis, ...) and high solvent accessibility

- **Passive residues:**

all solvent accessible neighbors



Ambiguous interaction restraints:



$$d_{I,AB}^{eff} = \left(\sum_{m_I=1}^{N_{atoms\,I}} \sum_{k=1}^{N_{res\,B}} \sum_{n_k=1}^{N_{atoms}} \frac{1}{d_{m n_k}^{r}} \right)^{\frac{1}{r}}$$

Scoring

- The structures are ranked after each docking stage.
- Rigid-body docking:
  - $Score = 1.0 * E_{elec} + 1.0 * E_{vdW} - 0.05 * BSA + 1.0 * E_{desolv} + 1.0 * E_{AIR}$
- Semi-flexible and water refinement:
  - $Score = 1.0 * E_{elec} + 1.0 * E_{vdW} + 1.0 * E_{desolv} + 1.0 * E_{AIR}$

- E_{elec} = electrostatic energy
- E_{vdW} = Van der Waals energy
- BSA = Buried Surface Area
- E_{desol} = desolvation energy[1]
- E_{AIR} = Ambiguous Interaction Restraint energy

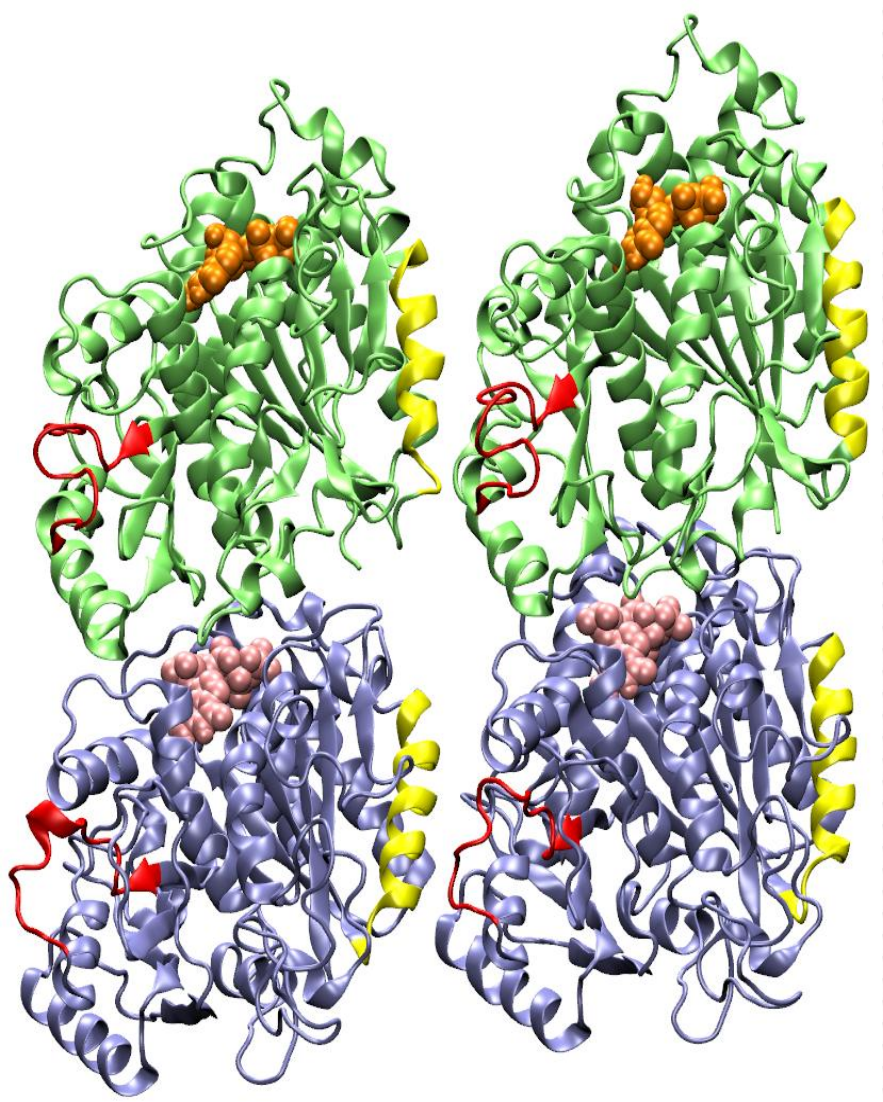
(8.5Å cutoff, OPLS / DNA-RNA param)

Ref. Bonvin et al. JACS 2003, 125, 1731

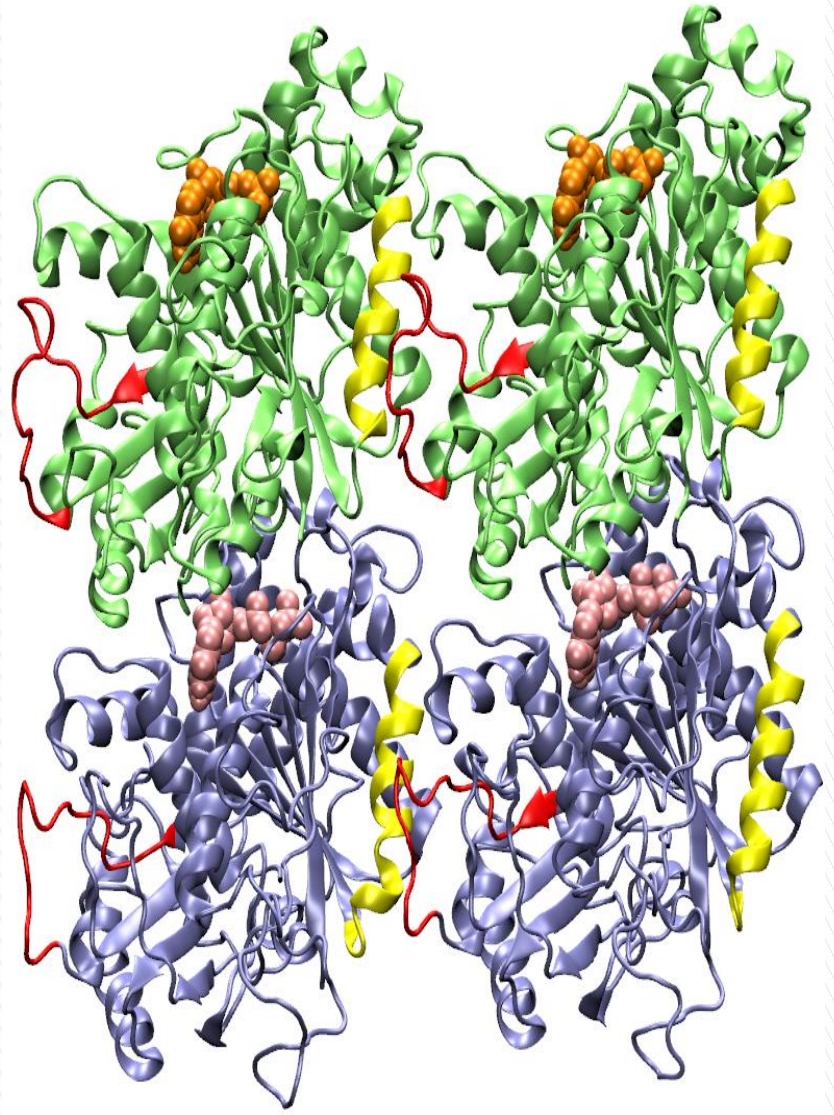
Lateral and Longitudinal Docking Protocol

- Two tubulin dimers were docked to obtain a model of the tubulin complex formed due to lateral or longitudinal interactions
- Active and passive residues include M, H1-S2, H6-H7 loops, H3 helix and adjacent residues
- 1000 structures were sampled in the first stage of **rigid body docking**
- Out of these 1000 structures, 500 were then selected for the second step which is a **semi-flexible refinement**
- Then 200 out of the 500 were selected for the final step of **explicit solvent refinement.**
- The selection in each step was done based on the HADDOCK scoring function

Structures of Tubulin-Tubulin Complex via Lateral Interactions

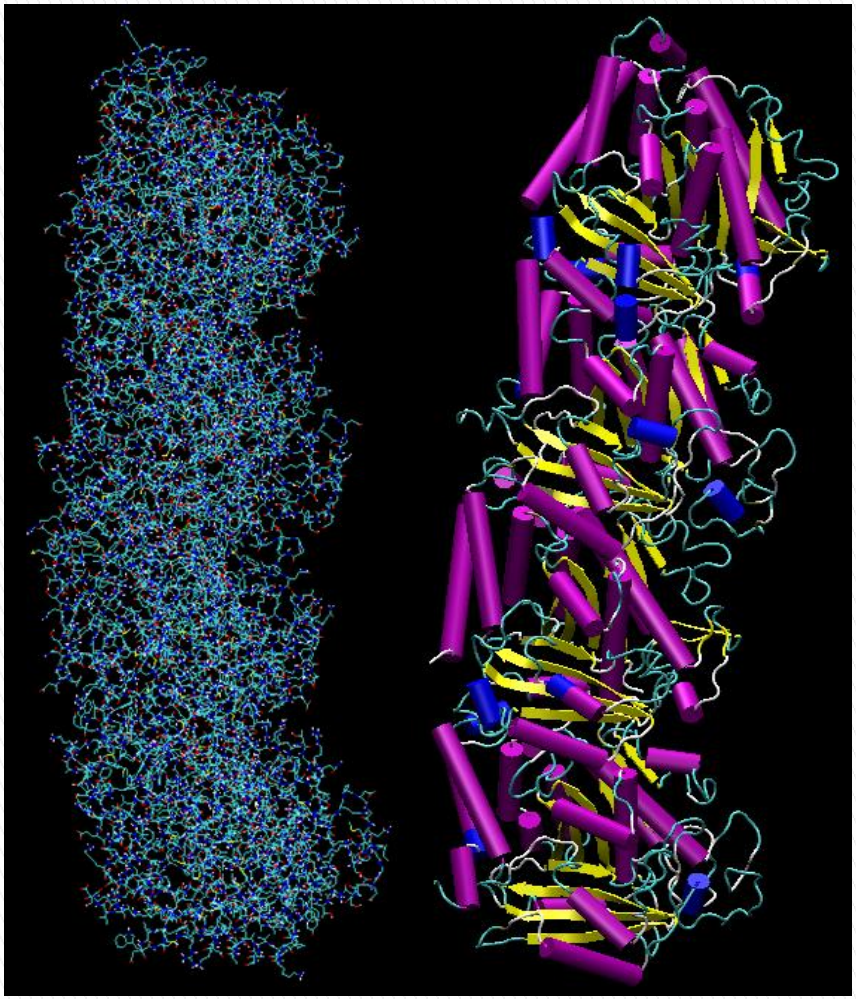


Tubulin-GDP state
Week sidewise interactions

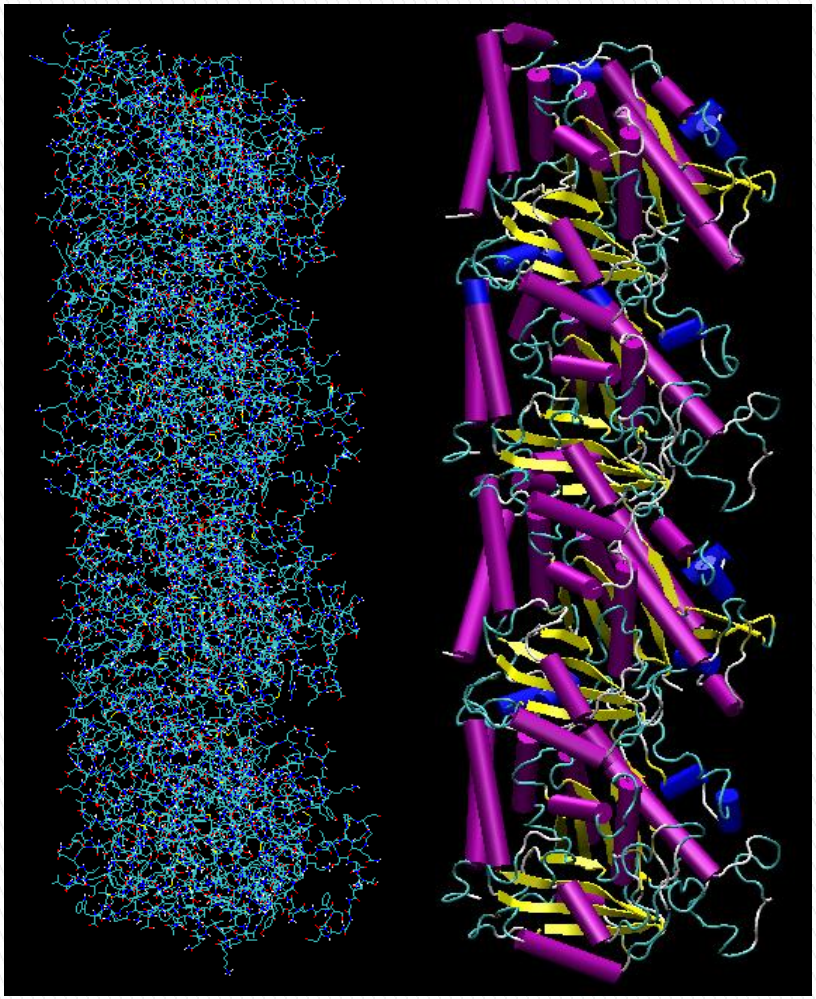


Tubulin-GTP state
Strong sidewise interactions

Structures of Tubulin-Tubulin Complex via Longitudinal Interactions



GDP state
⇒Bent protofilament might peel away



GTP state
⇒Straight protofilament can increase inter-protofilament interactions

A Quantitative Comparison of Straight vs. Kinked Tubulin Structures

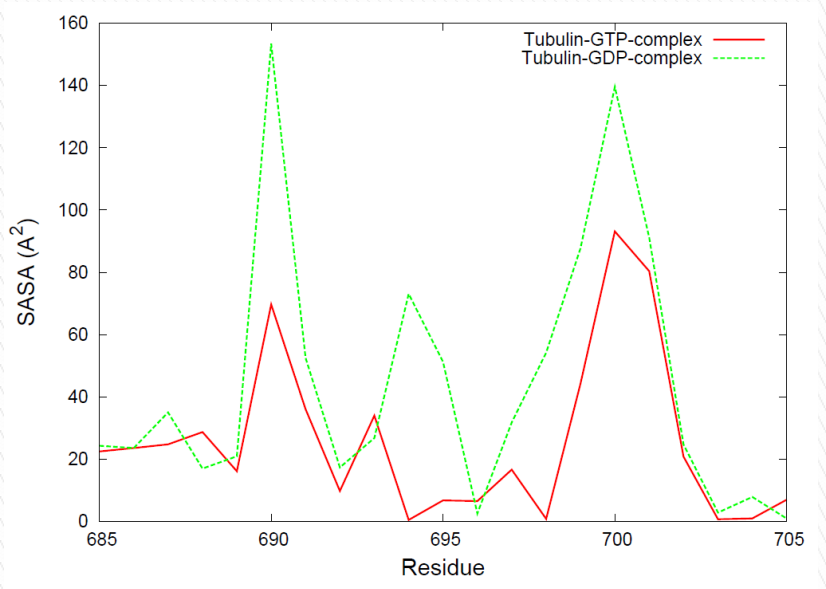
Intradimer Angle

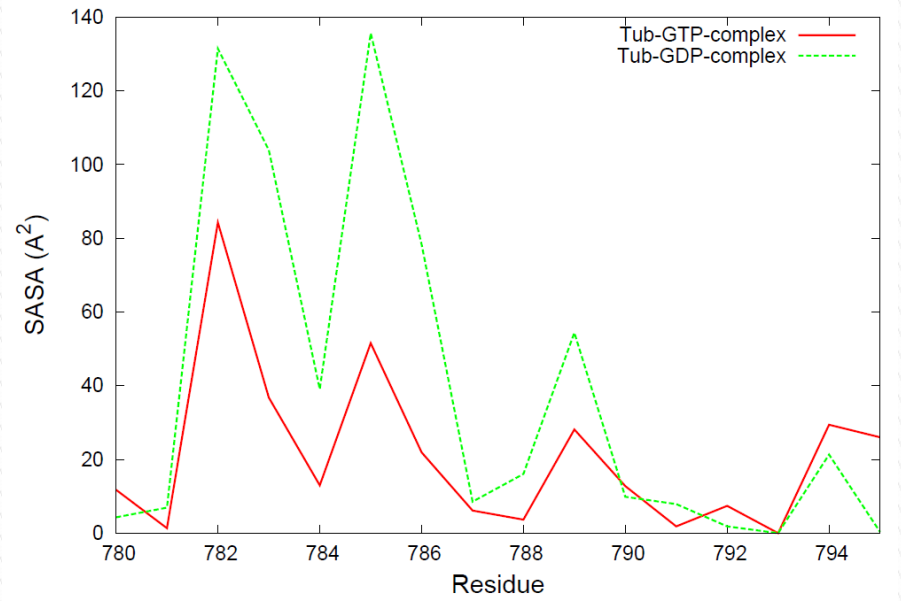
Lateral docking	Angle (deg)	Angle (deg)
Tubulin-GDP	150.55	150.30
Tubulin-GTP	166.57	166.15
Longitudinal docking		
Tubulin-GDP	154.74	155.18
Tubulin-GTP	165.95	165.29

Interdimer Angle

Longitudinal docking	Angle (deg)
Tubulin-GDP	165.38
Tubulin-GTP	178.52

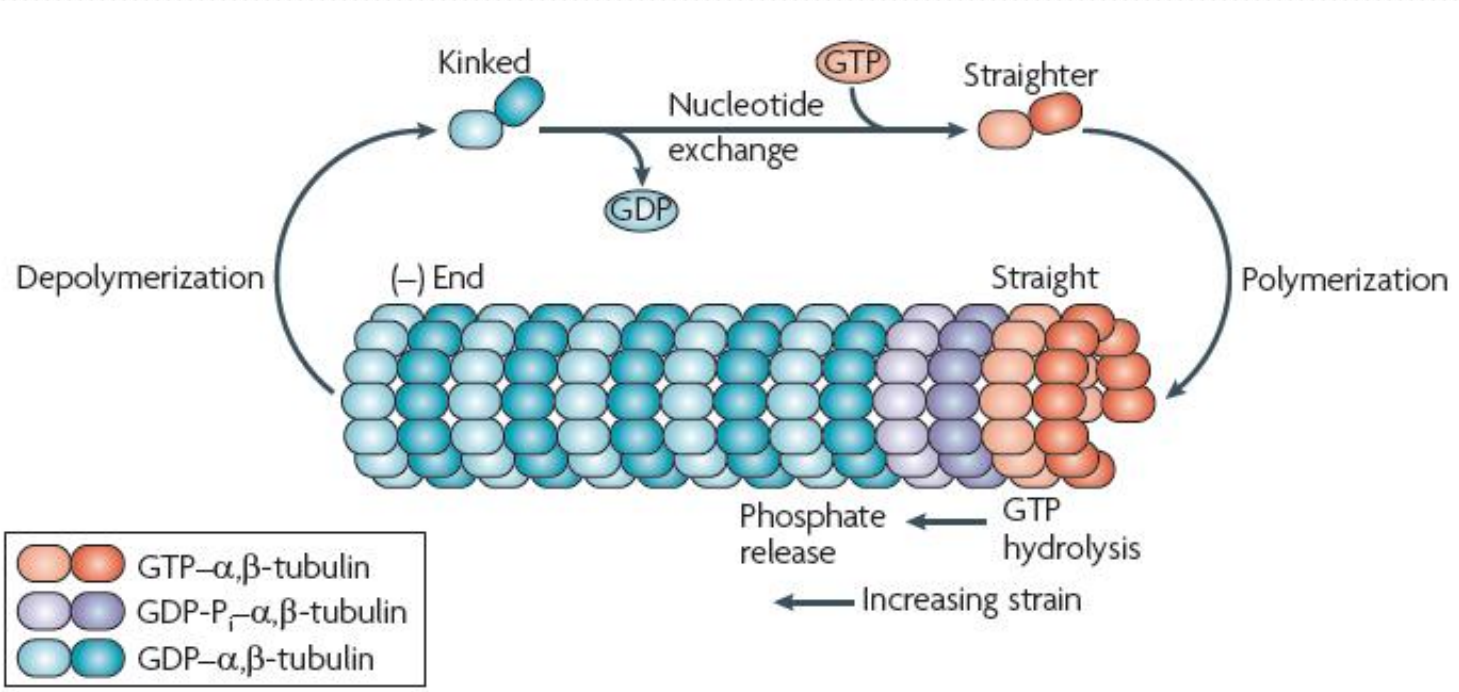
Solvent Accessibility at inter-dimer interfaces





Solvent accessibility at inter-dimer interface of GDP-tubulin is more during lateral docking

Summary


Kinked
GTP
Straighter
Nucleotide
exchange
GDP
Depolymerization
(–) End
Straight
Polymerization
Phosphate
release
GTP
hydrolysis
Increasing strain
GTP–α,β-tubulin
GDP-P$_i$–α,β-tubulin
GDP–α,β-tubulin

Conclusion

- We examine the intrinsic conformational changes of free tubulin dimer in its different nucleotide states
- Results suggest that the nucleotide state has a direct influence on lateral and longitudinal interactions among tubulin dimers by modifying the geometry and chemical properties of the interacting surfaces
- M, H3, H6-H7 loops in free GTP-tubulin are observed to experience high flexibility, which could be a requirement for its efficient reactivity
- MD in conjunction with Protein-Protein docking provide insight on microtubule dynamic instability
- Model suggests an Allosteric Model of tubulin assembly in protofilament

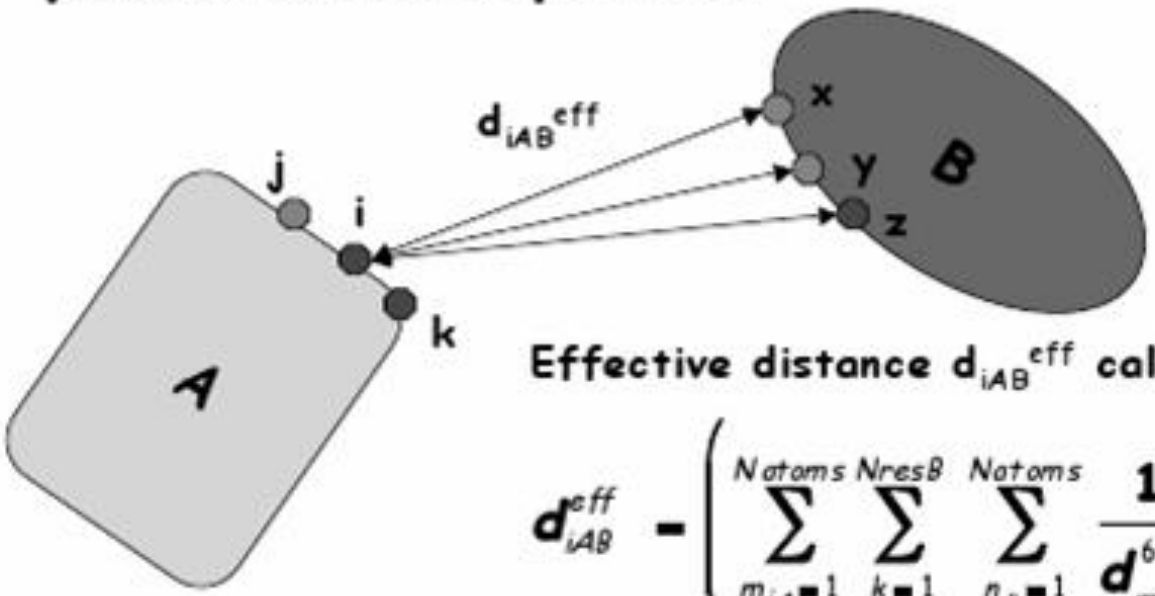
Acknowledgements

- N. Kathiresan
- T. P. Karthigeyan
- Jagan Mohan

- DST, DBT, SERC FAST, IITM

Ambiguous Interaction Restraints (AIRs)

- **AIR defined as an ambiguous distance restraint with a maximum value of 2-3Å between any atom of an active residue i of protein A and any atom of all active and passive residues of protein B**



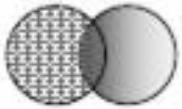
Effective distance d_{iAB}^{eff} calculated as

$$d_{iAB}^{eff} = \left(\sum_{m_{iA}=1}^{Natoms} \sum_{k=1}^{NresB} \sum_{n_k=1}^{Natoms} \frac{1}{d_{mn_k}^{6}} \right)^{-\frac{1}{6}}$$

Dealing with flexibility in HADDOCK

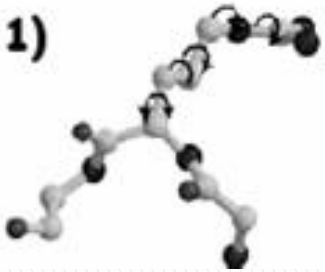
- Docking from ensembles of starting structures (e.g. from MD)

- "Soft" docking by scaling down intermolecular interaction



- Explicit flexibility introduced step-wise:
  1) First side-chains at interface
  2) Then both side-chains and backbone at interface

1)



2)