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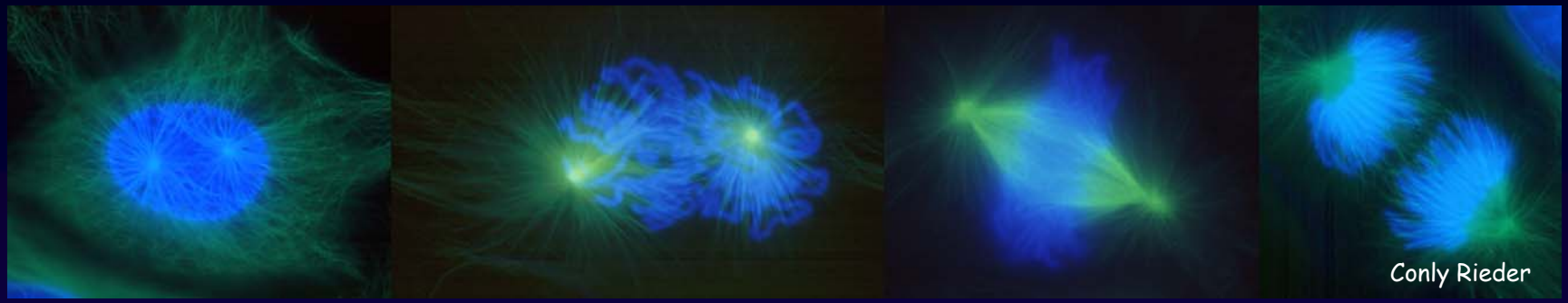
Structural Basis of Microtubule Dynamic Instability

Eva Nogales

Howard Hughes Medical Institute
UC Berkeley
Lawrence Berkeley Natl. Lab.

The Dynamic Nature of Microtubules



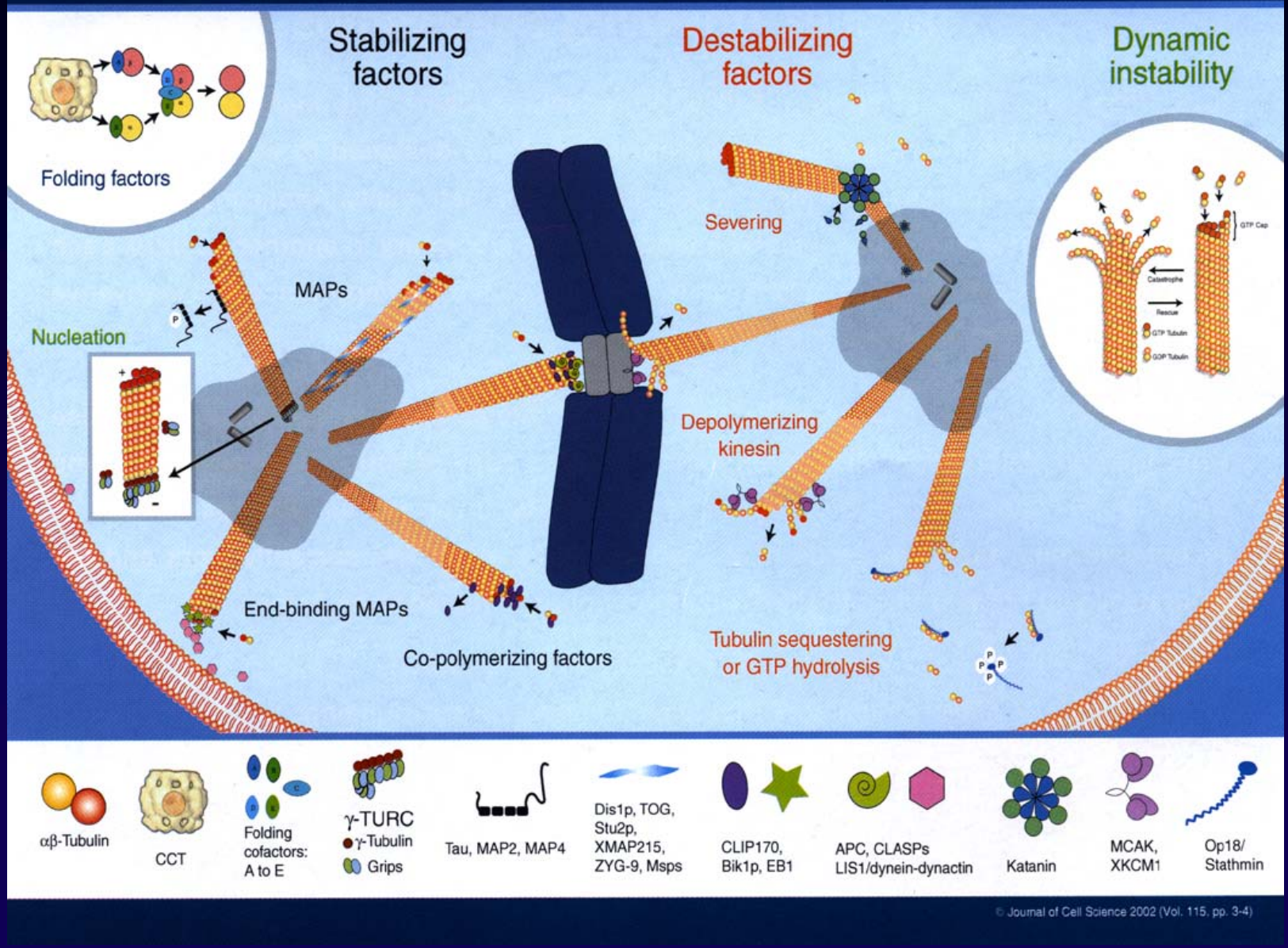
QuickTime™ and a Photo - JPEG decompressor are needed to see this picture.

Gary Borisy

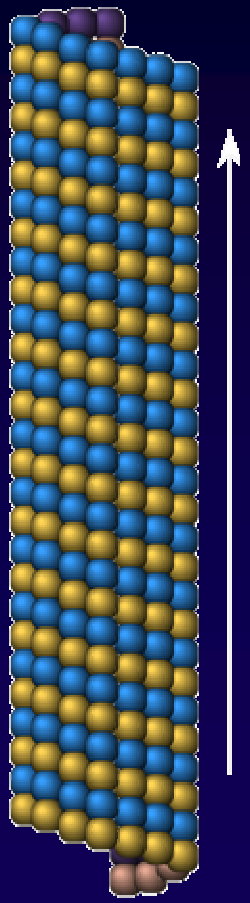
Journal of Cell Science

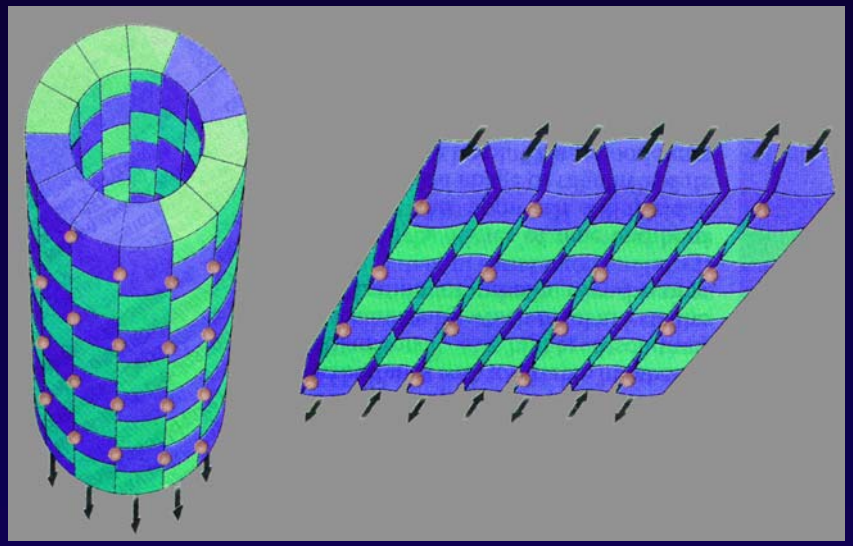
Microtubule Dynamics

Rebecca Heald and Eva Nogales

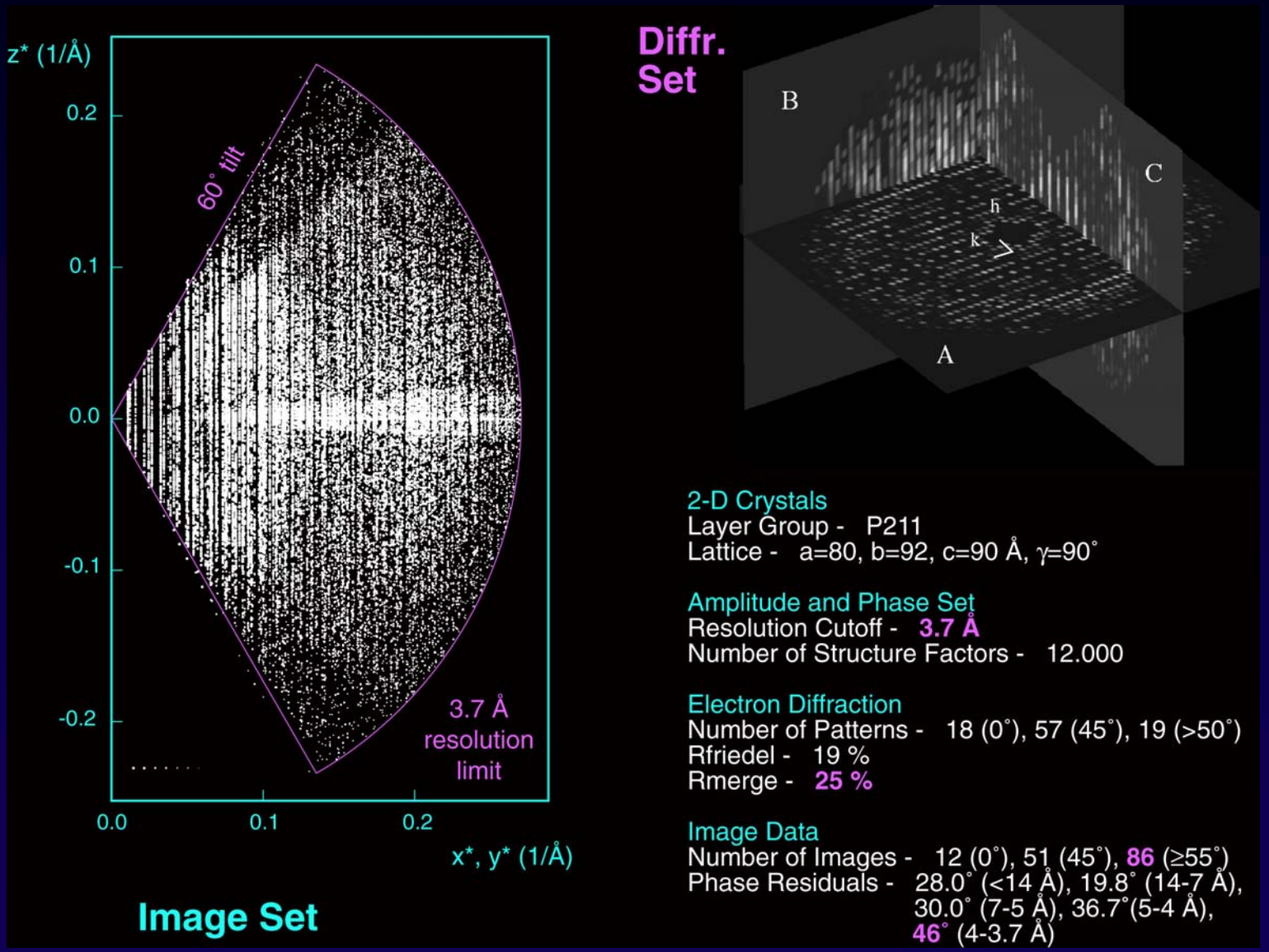


Microtubule Structure & Zinc-Sheets

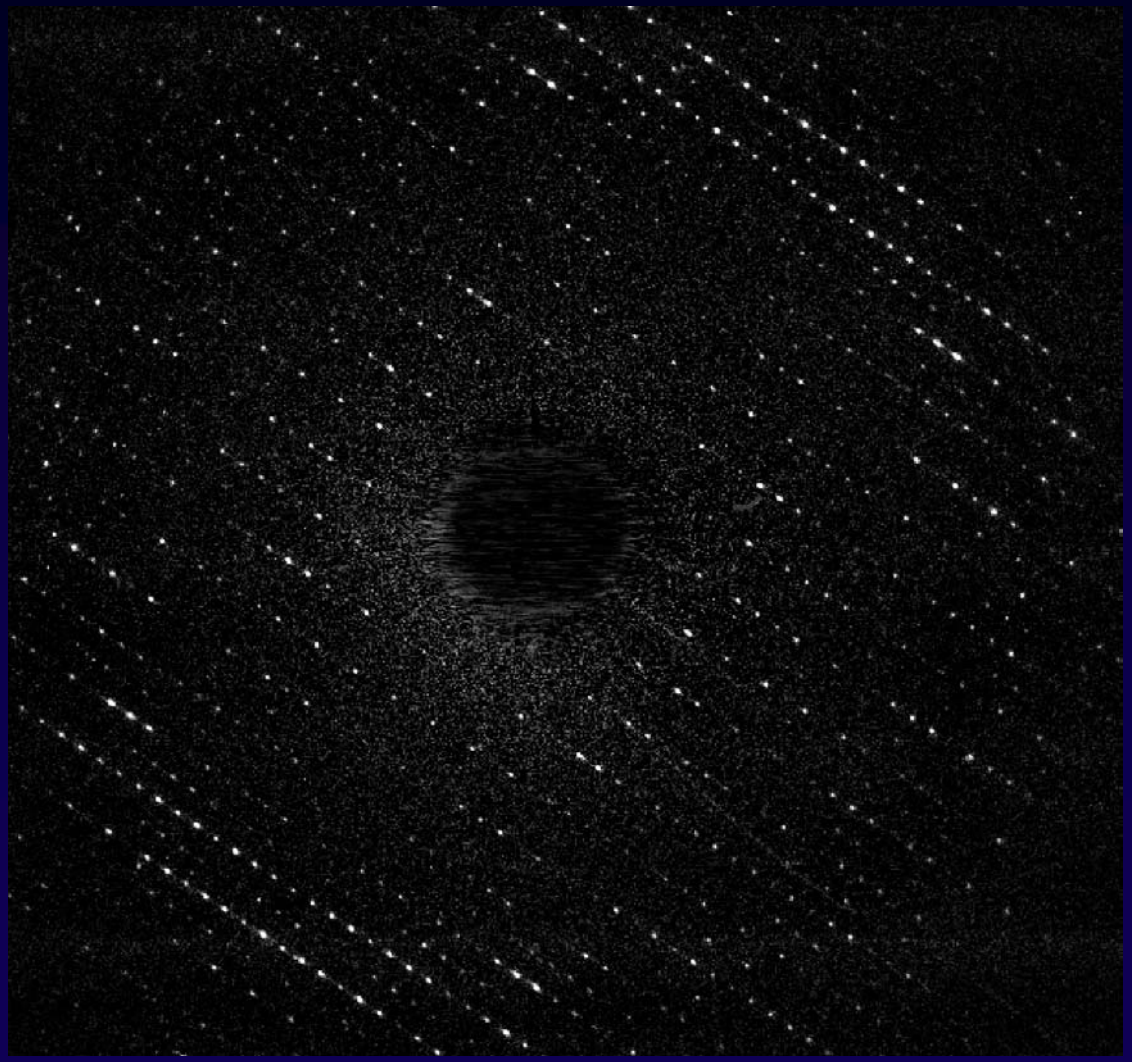


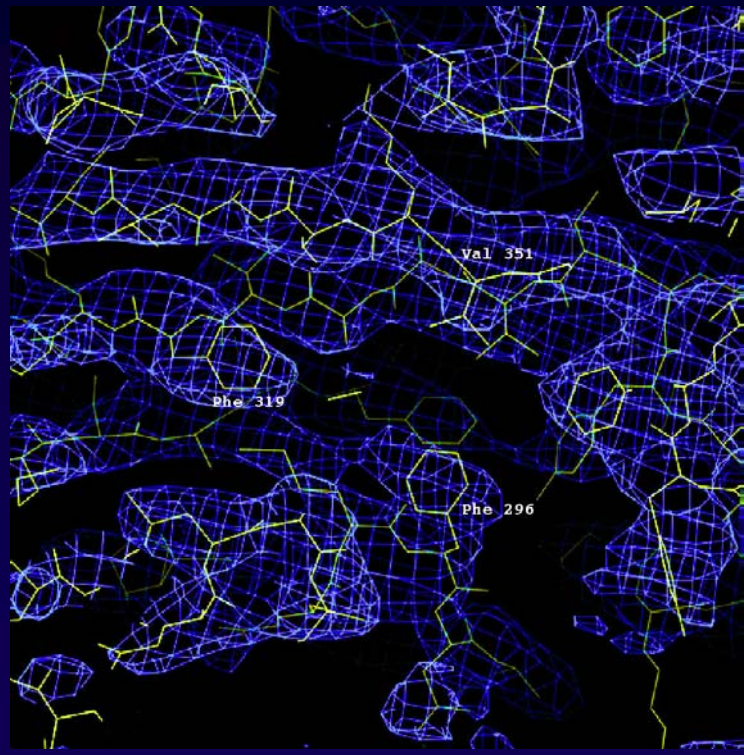


Electron Crystallography

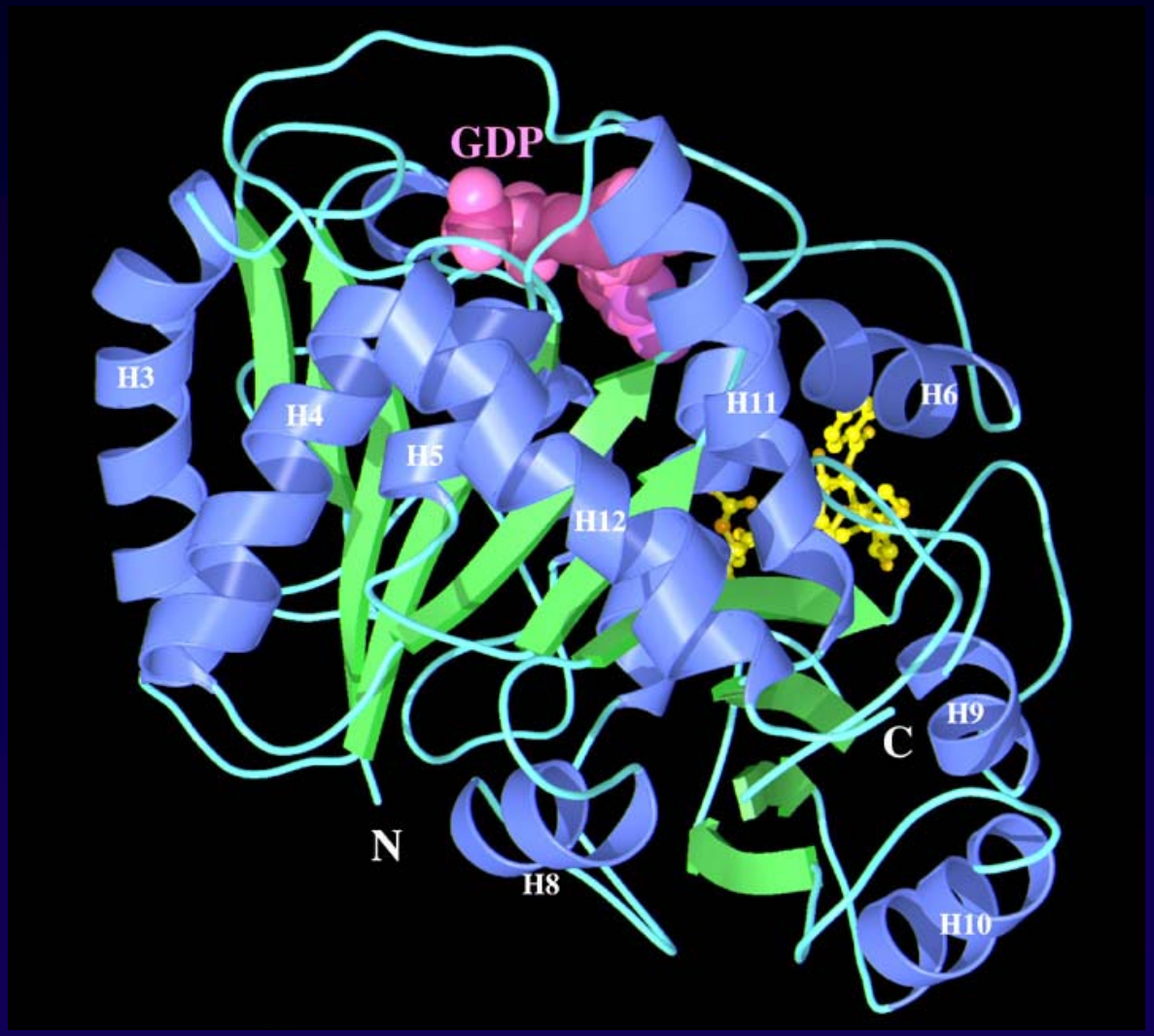


Raw Data and Density Map

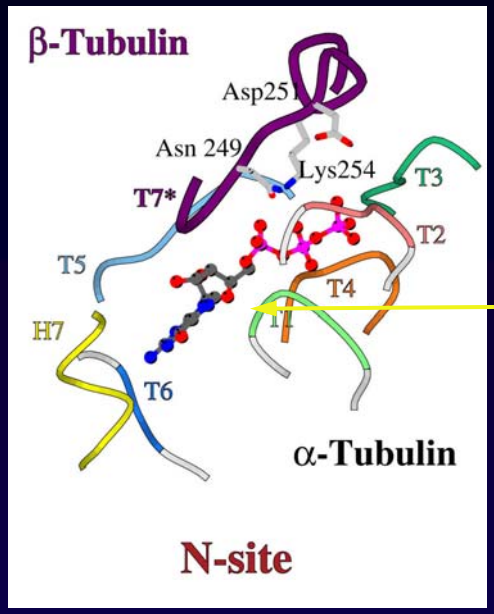




Tubulin Structure



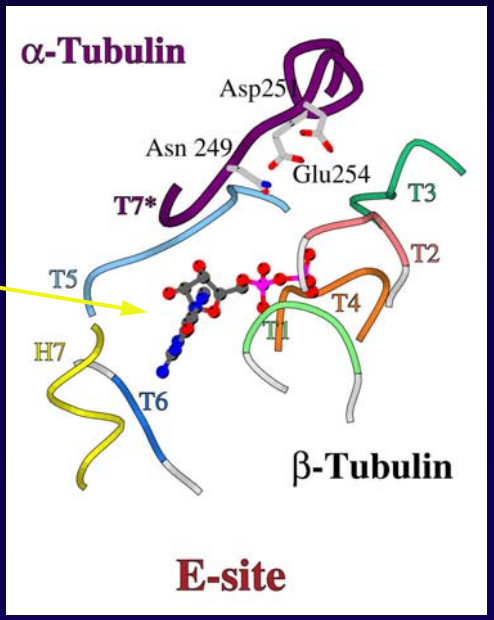
Protofilament assembly and GTP Hydrolysis



Intradimer

Polymerization Interface

α-Glu 254

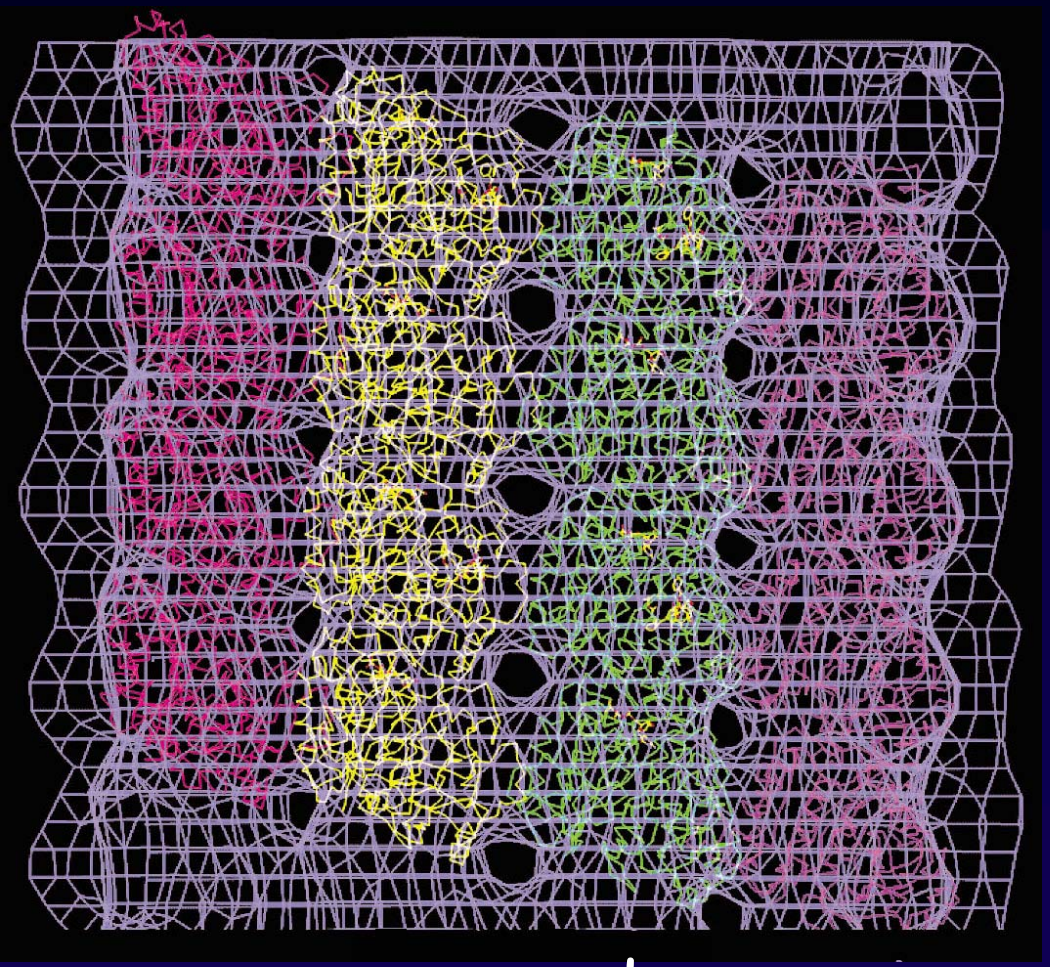


Interdimer

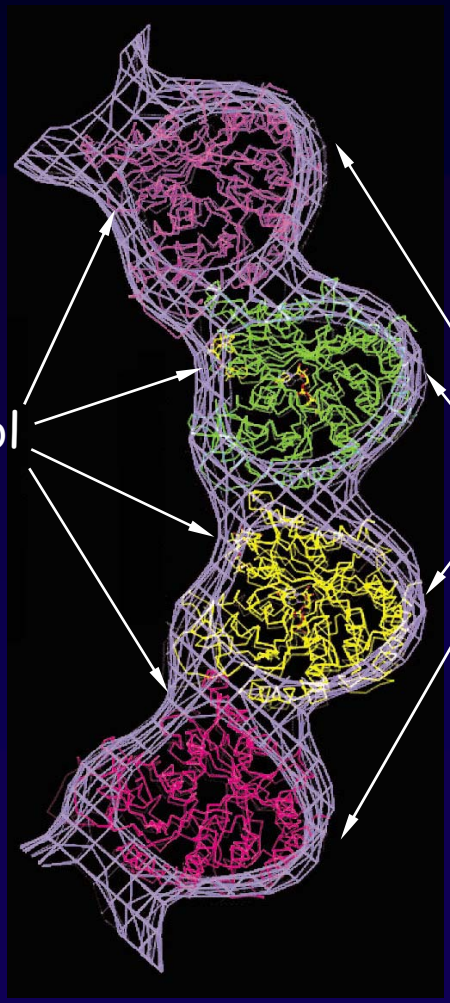
Nogales et al. (1998)
Nat. Struc. Biol. 5, 451-458

Microtubule Structure

+ end

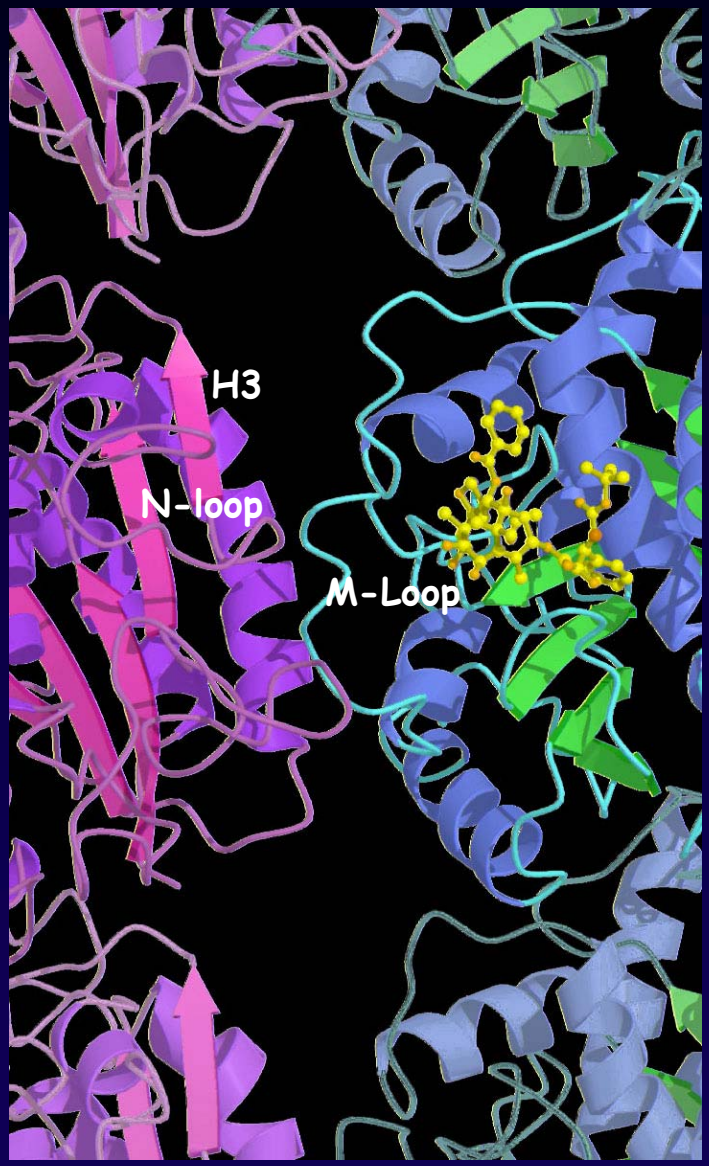


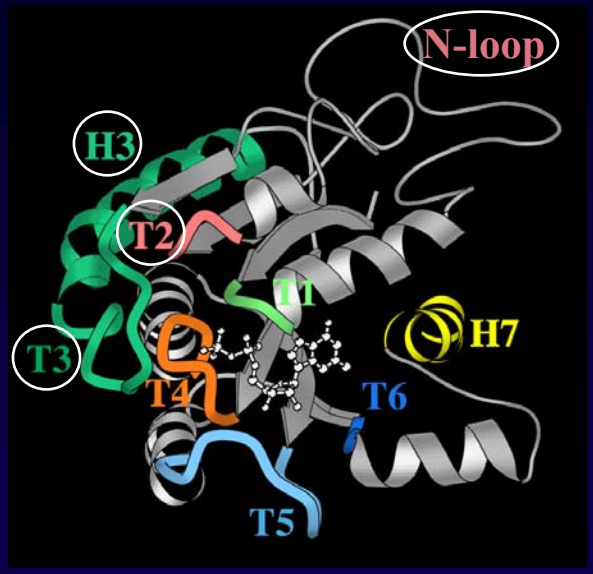
− end



Nogales et al. (1999)
Cell 96, 79-88

GTP Hydrolysis and Microtubule Depolymerization





γ-phosphate bound by T2 and T3

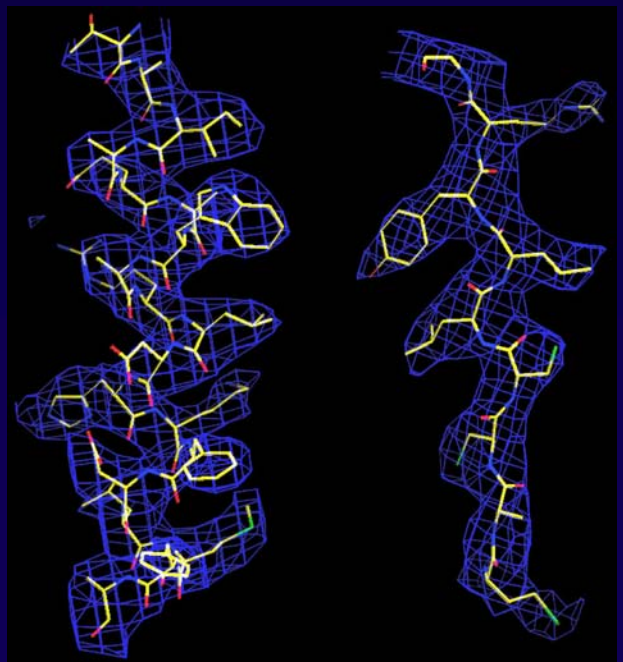
N-loop and H3 equivalent of Switch I and Switch II regions

Input Data & Refinement

Original Data:
149 images
94 diffraction patterns
Additional Data:
114 diffraction patterns
Huilin Li & Ken Downing (LBNL)

•Maximum likelihood - Phases in target function
• Constrained temperature factor refinement:
• overall anisotropic temperature factor
Jan Löwe (MRC)

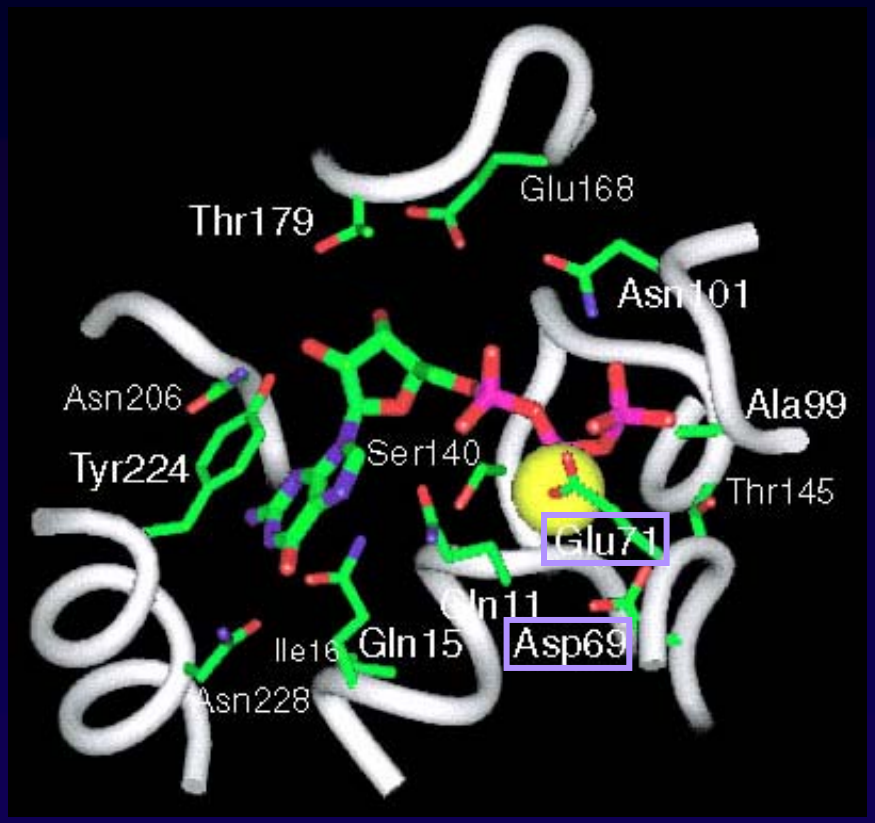
QuickTime™ and a Sorenson Video 3 decompressor are needed to see this picture.

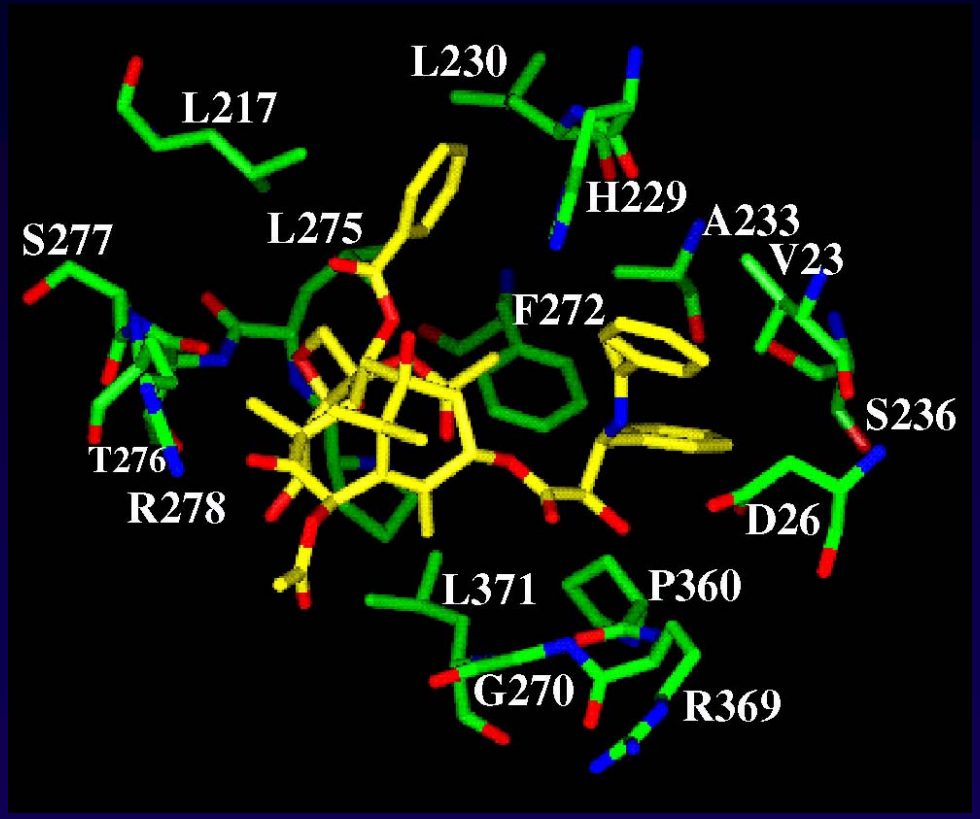


Final Resolution: 3.5 Å

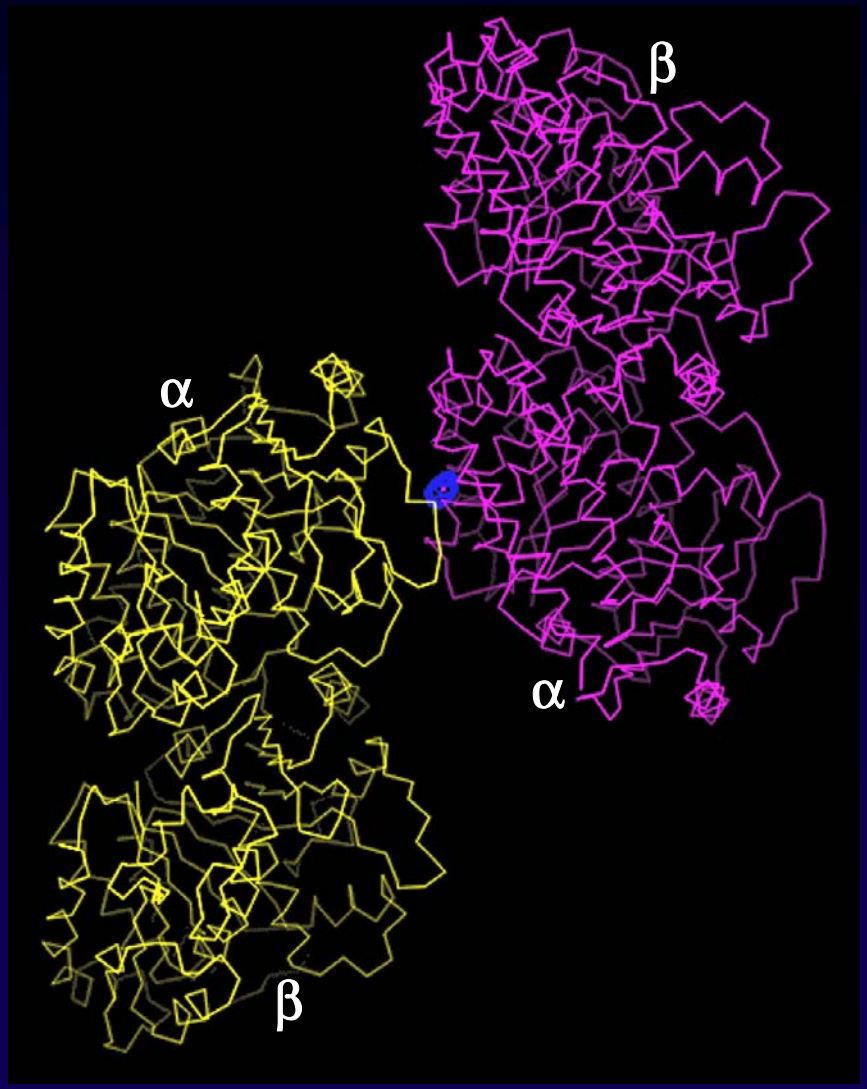
Rfactor - 23.2
Free Rfactor - 29.7

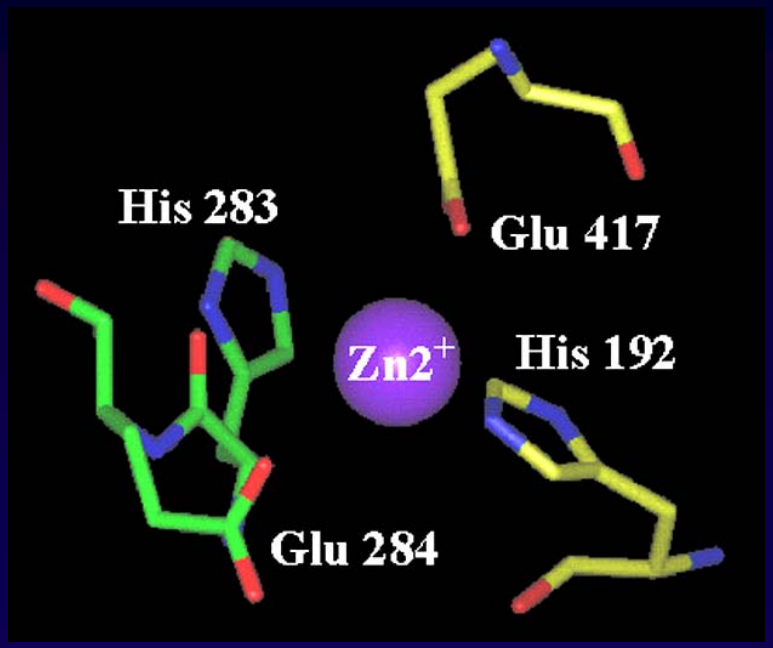
GTP and Taxol Sites





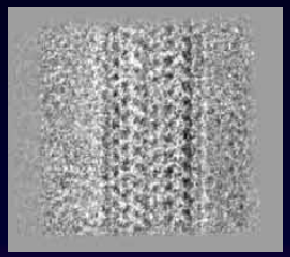
Zinc and Sheet Formation

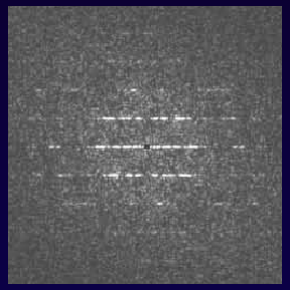


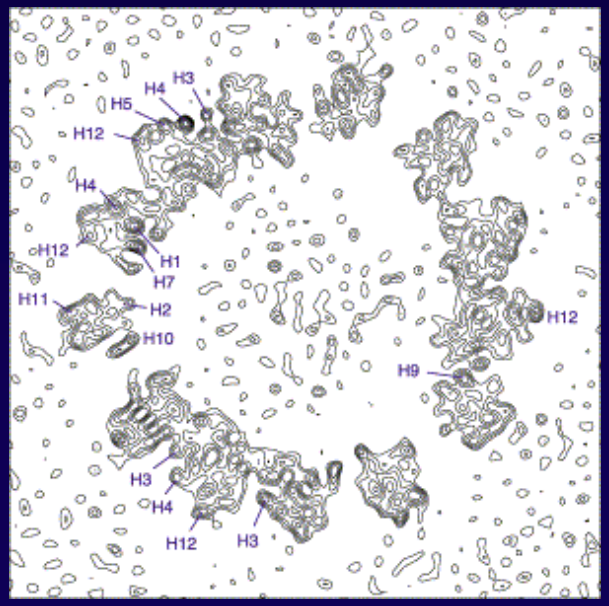


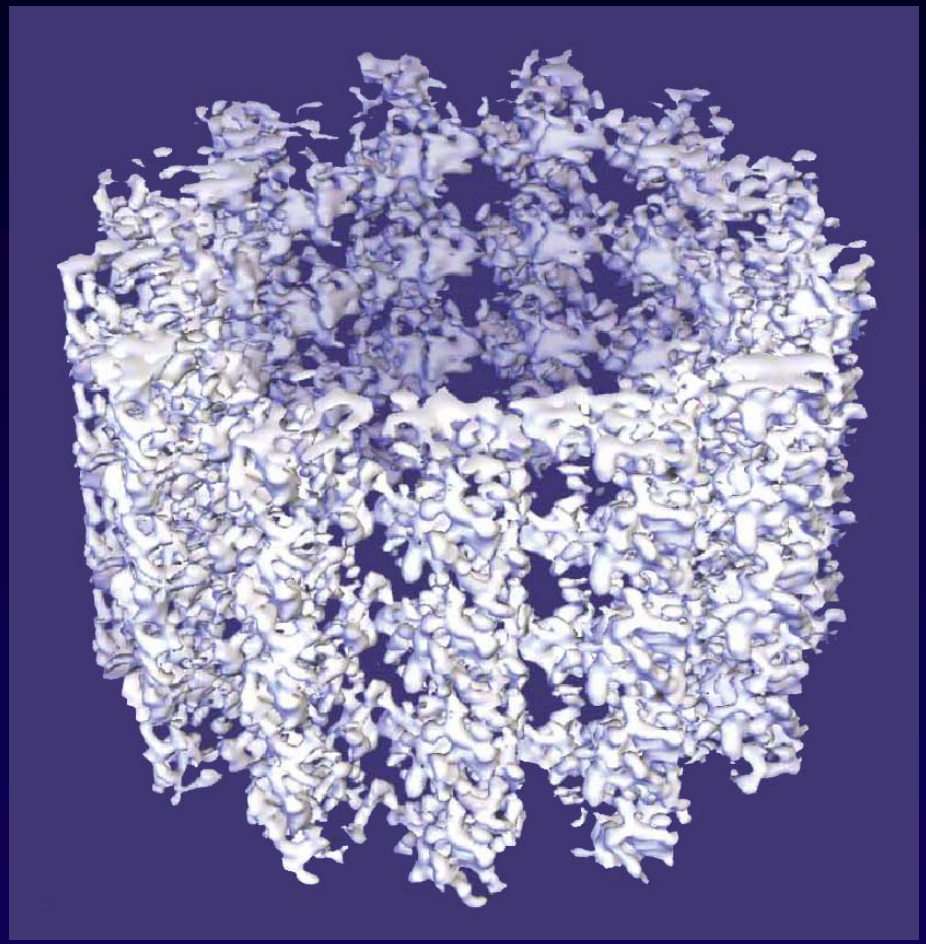
High Resolution Microtubule Structure

Single Particle Approach



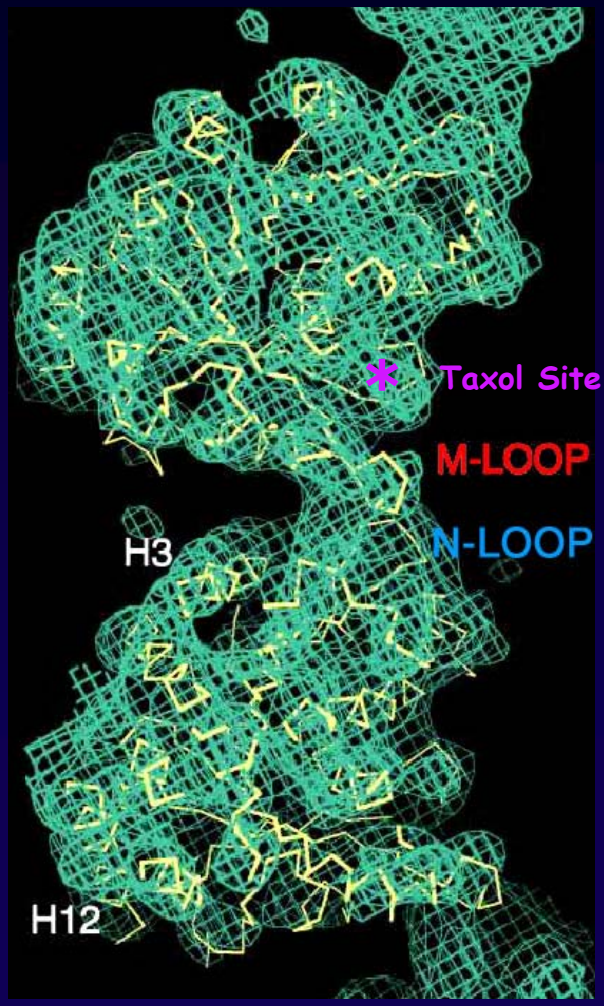


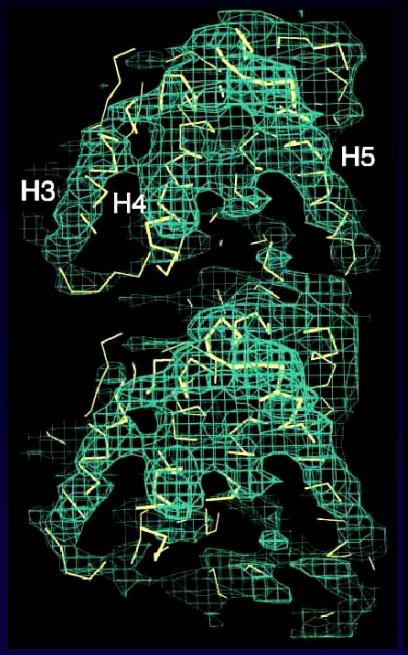


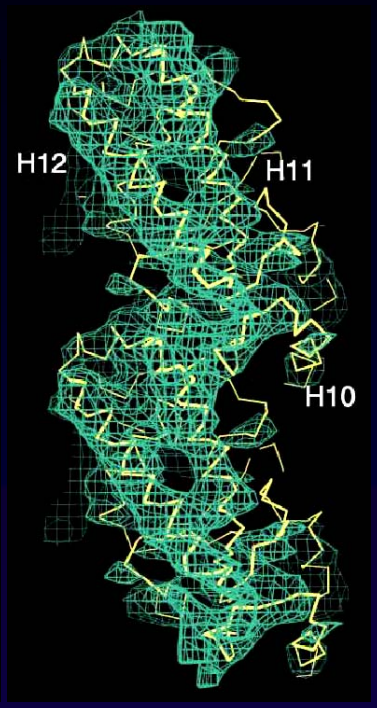


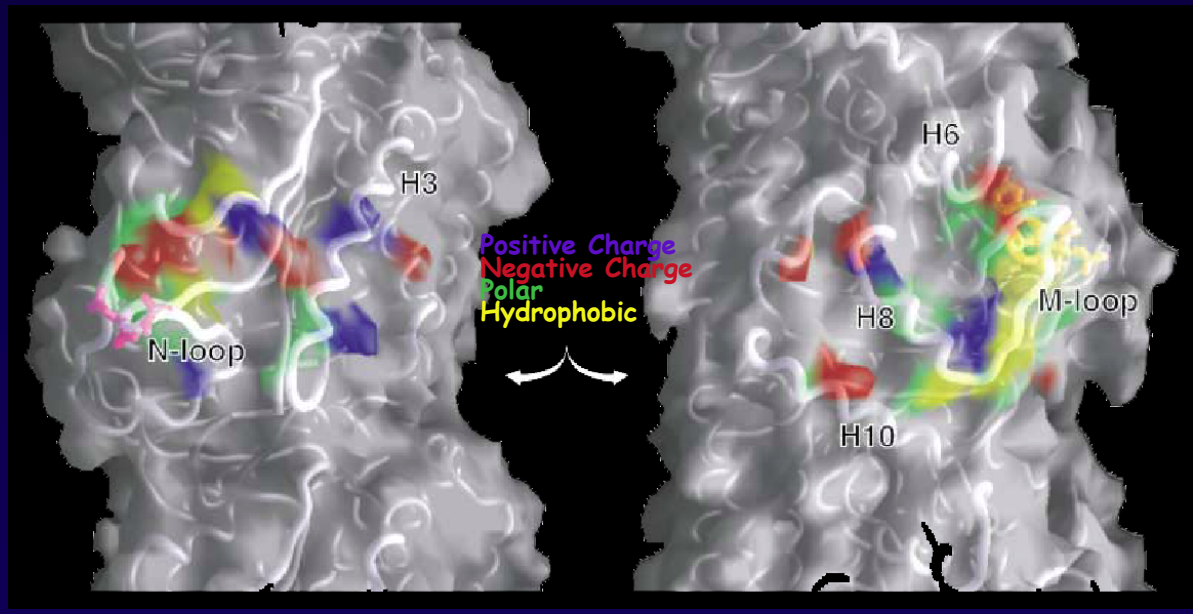
Li et al. (2002)
Structure 10, 1317-1328

Lateral Contacts

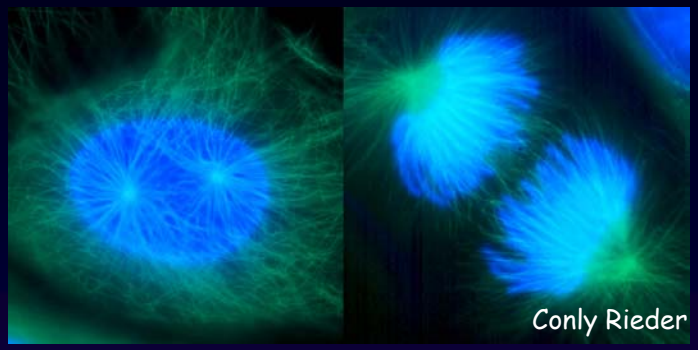






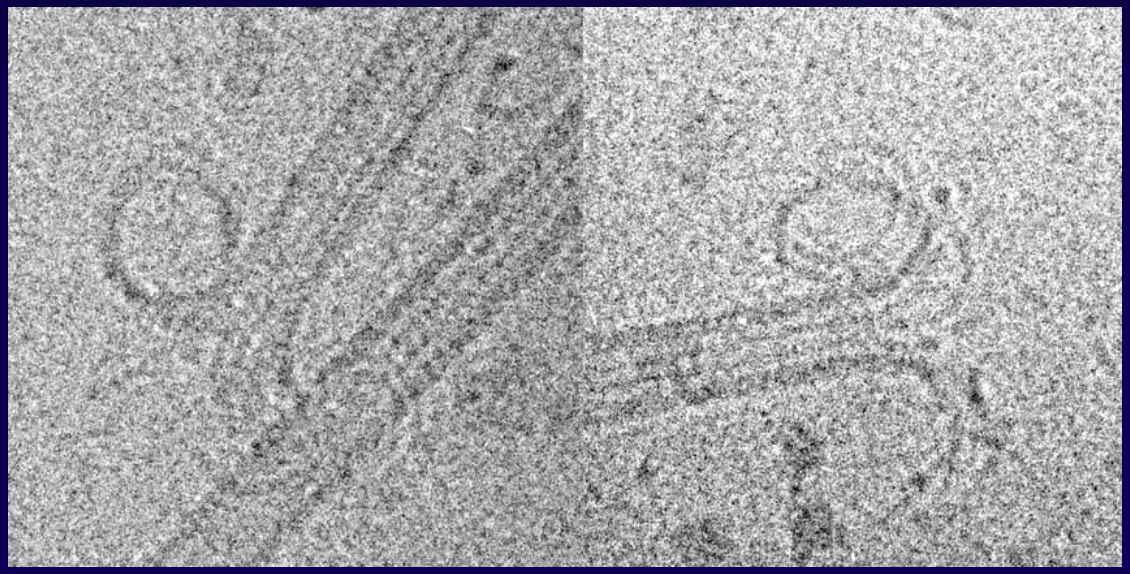


The Dynamic Nature of Microtubules



GTP and Tubulin Structure

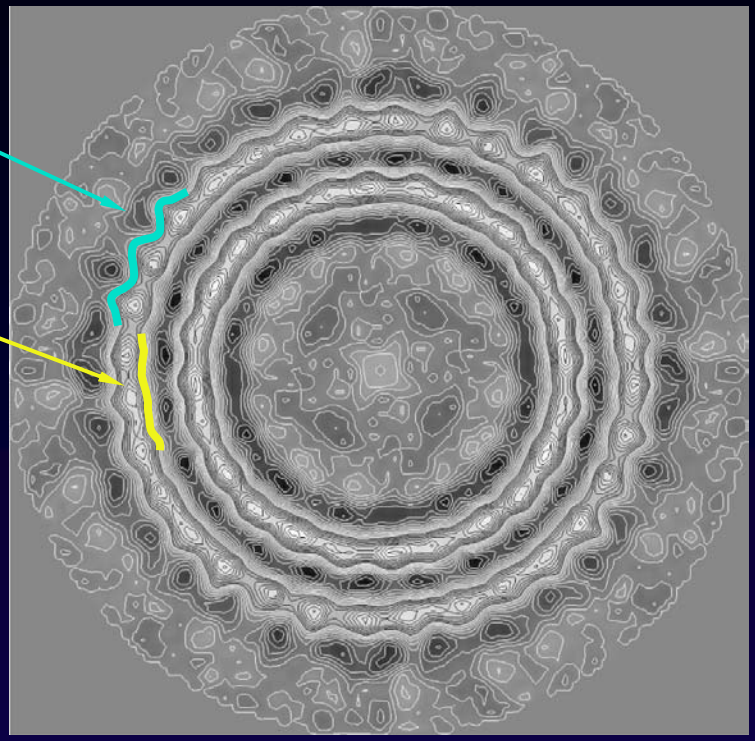
1 - GTP-bound tubulin required for self-assembly
2 - Hydrolysis upon polymerization
3 - Metastable Structure:
"GTP cap"
Conformational Strain
4 - Endwise Peeling (relaxed conformation)

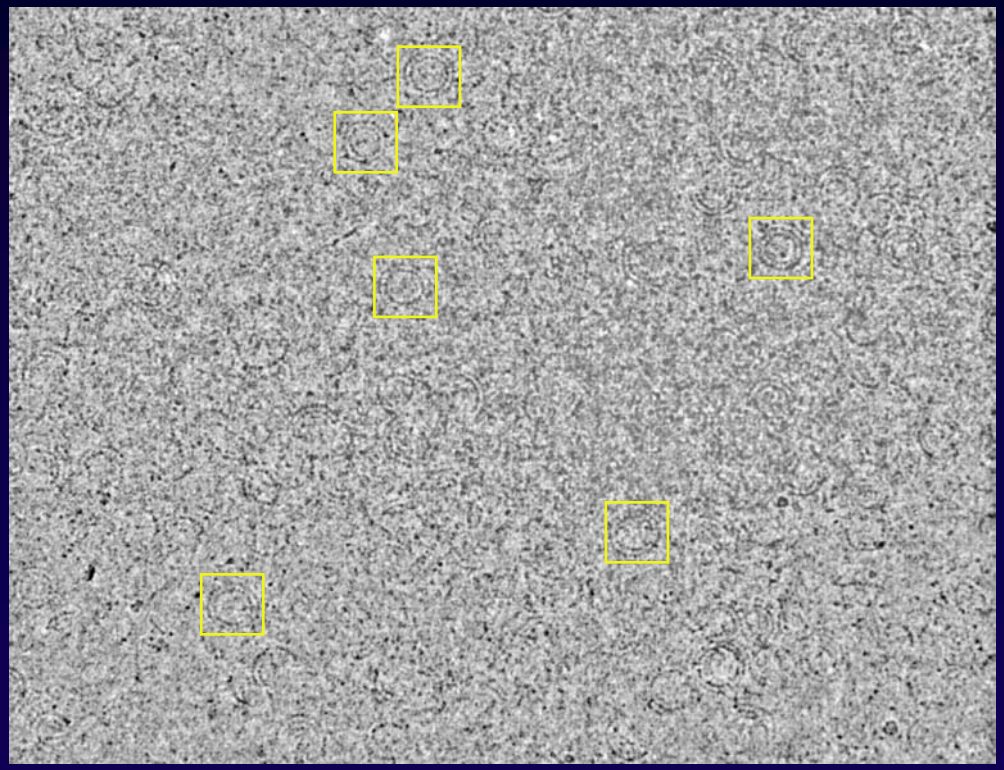


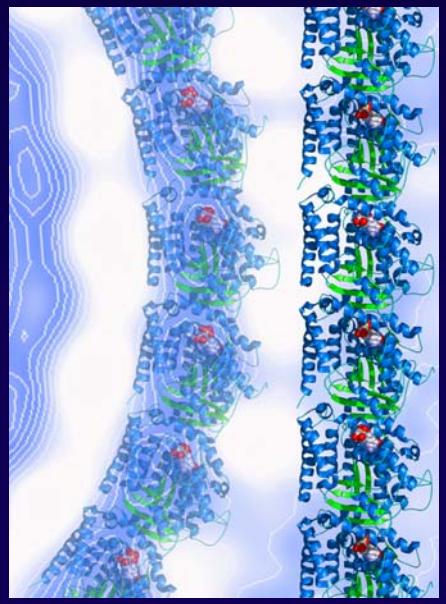
GDP-Tubulin Rings

"Bumpy" side
(MT inside surface)

"Flat" side
(MT outside surface)

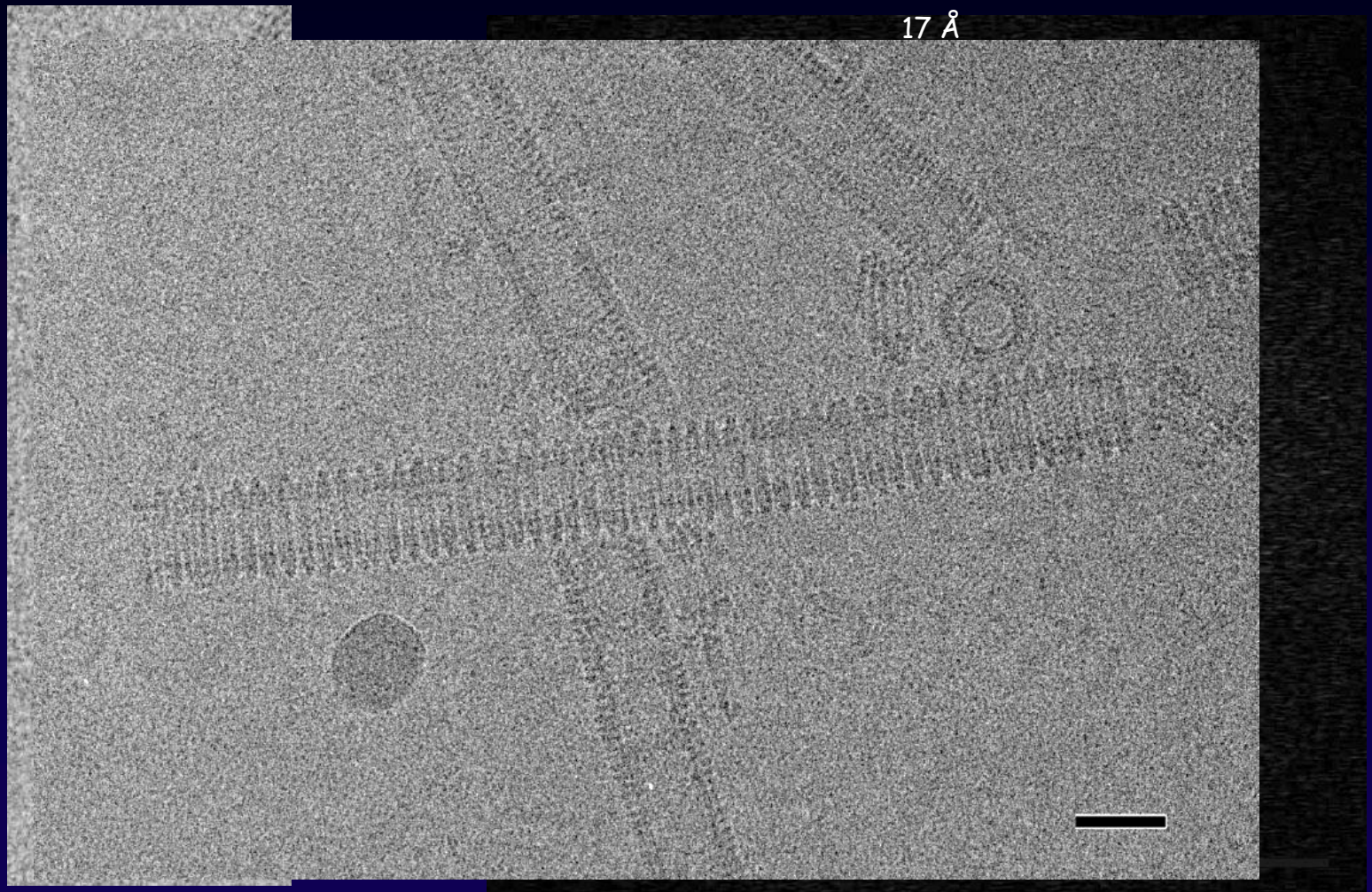






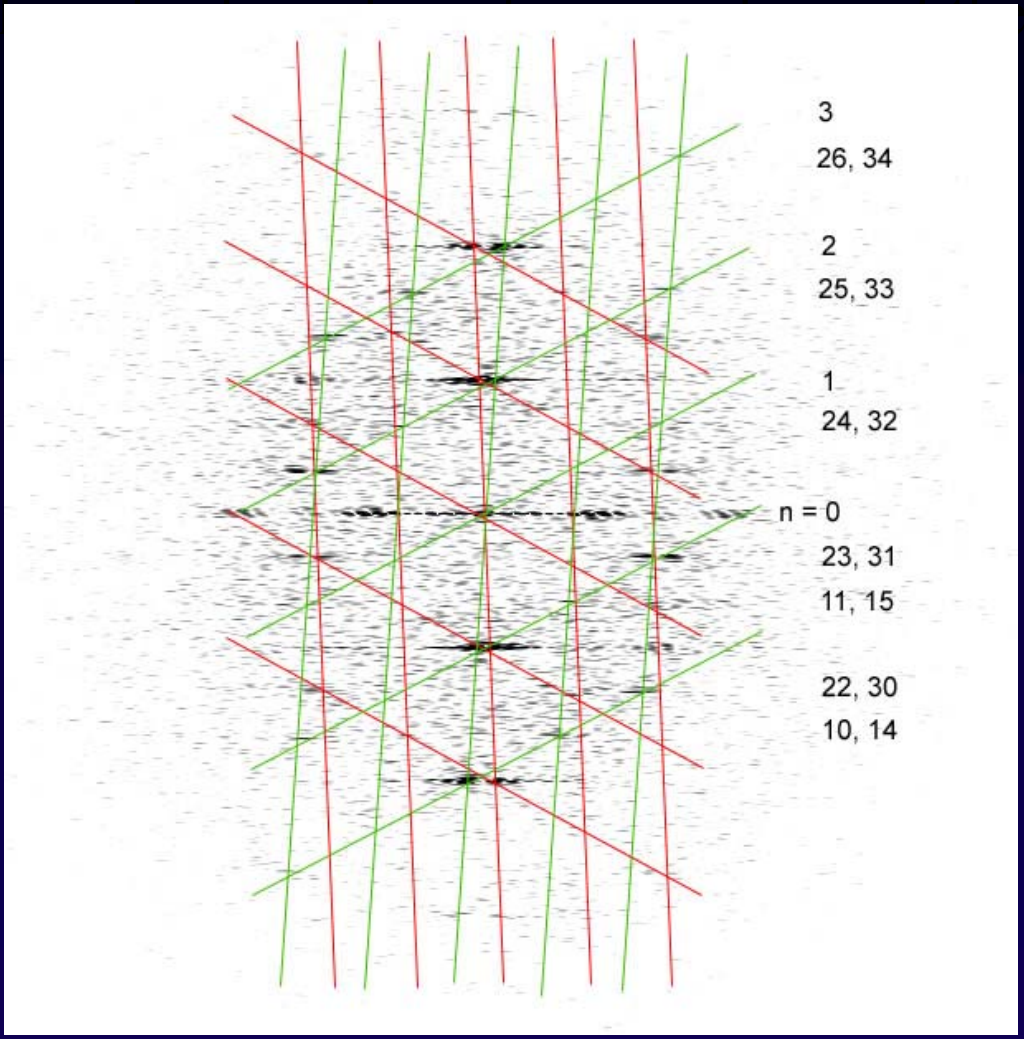
Nicholson et al. (1999)
Cell Biochem. Biophys. 31, 175-183

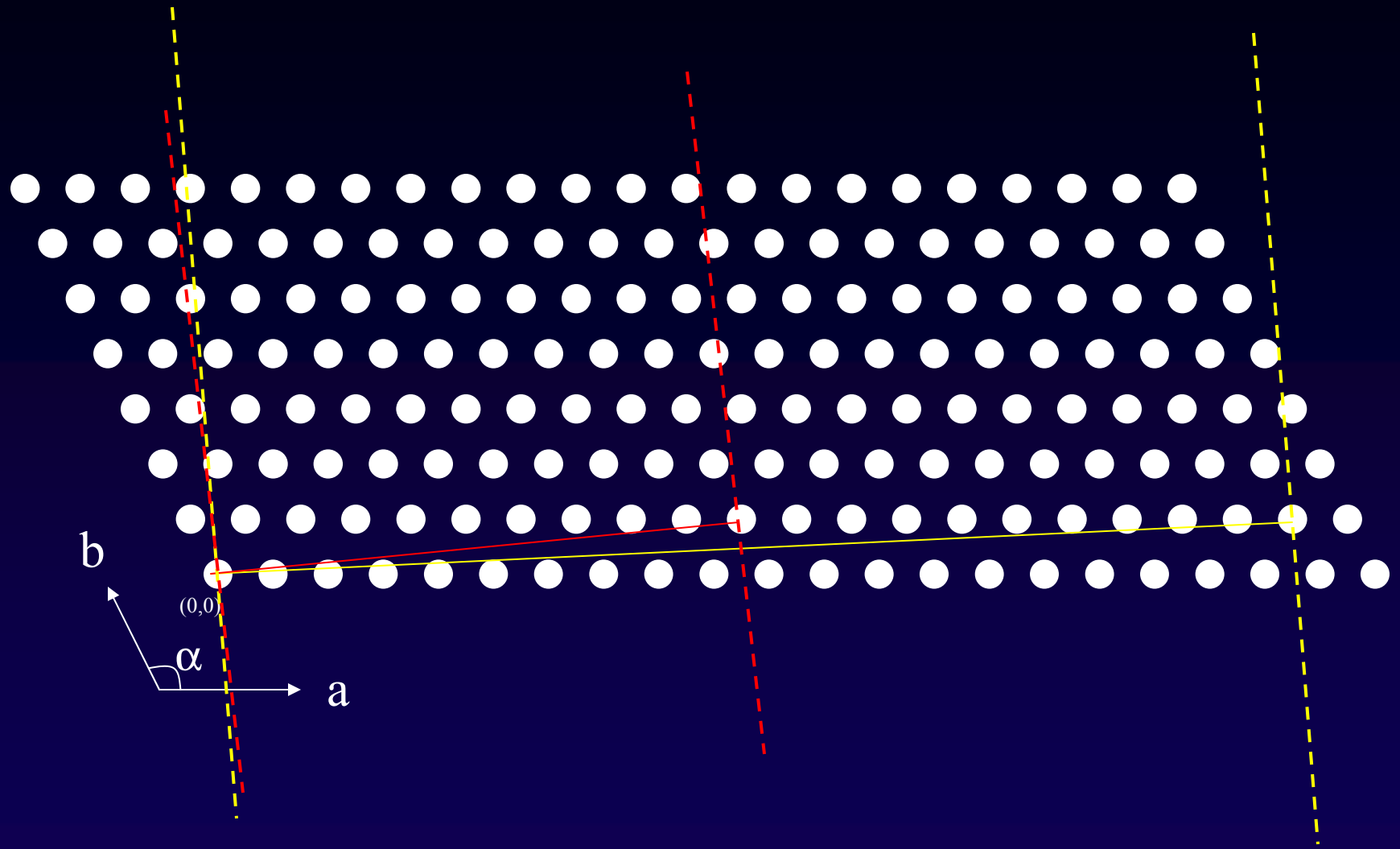
GDP-Tubulin Helical Crystals



Nogales et al. (2003)
Curr. Opi. Struc. Biol. **13**, 256-261.

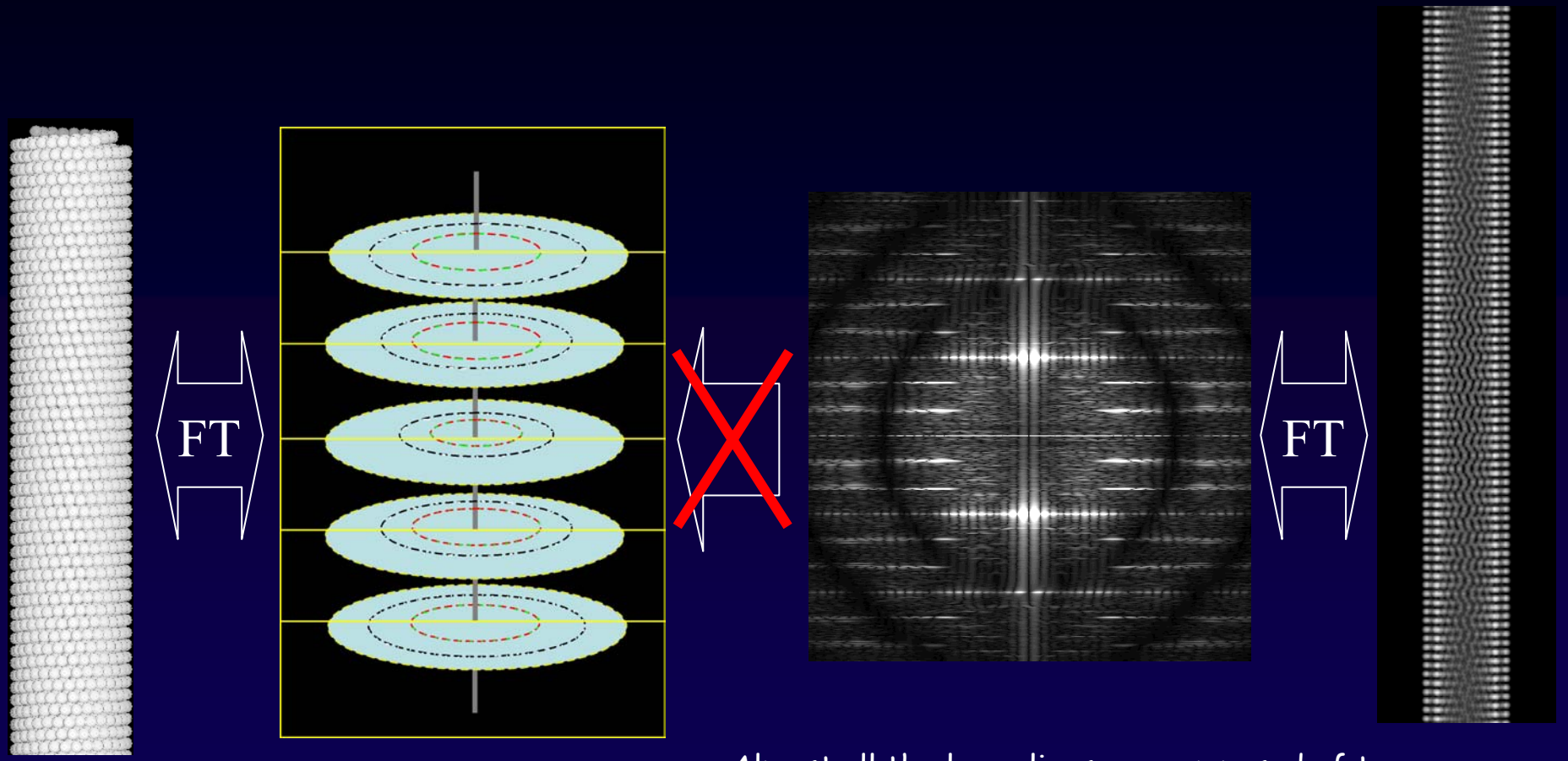
Loo tubes





The two layers of the helical crystals come from the same plane lattice where, a=47 Å, b=61 Å, α=123.8.

Traditional Helical Reconstruction

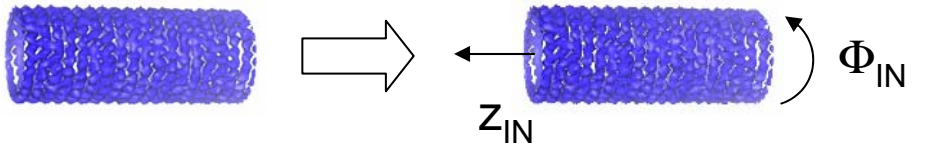


Almost all the layer lines are composed of two Bessel orders, except those with N= 1, 2, 3 …

$$F(R,\Phi,Z) = G_{N_{in}}(R,Z)\exp\{iN_{in}(\Phi+\pi/2)\} + G_{N_{out}}(R,Z)\exp\{iN_{out}(\Phi+\pi/2)\}$$

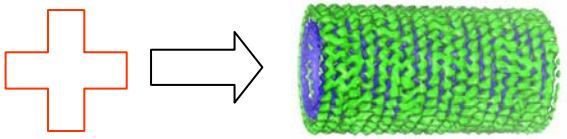
Theory of the reconstruction algorithm

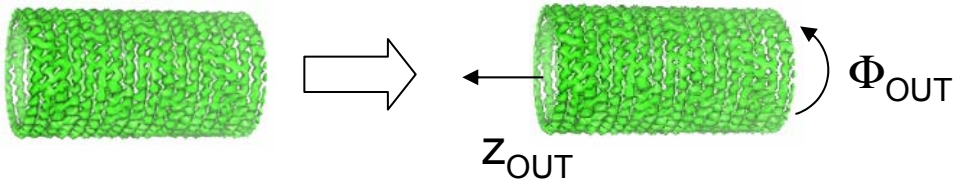
The double-layered tube is a summation of two tubes with independent orientations, which in Fourier-Bessel space is mathematically described by the following equation relationships on each layer line.



$G_{IN}(R,Z)$

$G_{IN}(R,Z)\exp\{i[N_{IN}\Phi_{IN}-2\pi Zz_{IN}]\}$





$G_{OUT}(R,Z)$

$G_{OUT}(R,Z)\exp\{i[N_{OUT}\Phi_{OUT}-2\pi Zz_{OUT}]\}$

$$F(R,Z)=G_{IN}(R,Z)\exp\{i[N_{IN}\Phi_{IN}-2\pi Zz_{IN}]\}$$
$$+G_{OUT}(R,Z)\exp\{i[N_{OUT}\Phi_{OUT}-2\pi Zz_{OUT}]\}$$

In the above equations, N is the Bessel order of the certain layer line of helix. For the reconstruction of the double-layered tubes, what we actually need is the G_{IN} and G_{OUT}. Once they are calculated, the reconstruction thus can be done.

The Fourier Transform of a given projection image of a tube is given by:

$$F_{obs}(R,Z) = G_{IN}(R,Z)\exp\{i[N_{IN}\Phi_{IN} - 2\pi Z z_{IN}]\}$$
$$+ G_{OUT}(R,Z)\exp\{i[N_{OUT}\Phi_{OUT} - 2\pi Z z_{OUT}]\}$$

If we collect projection images of M tubes, we"ll have M equations such as:

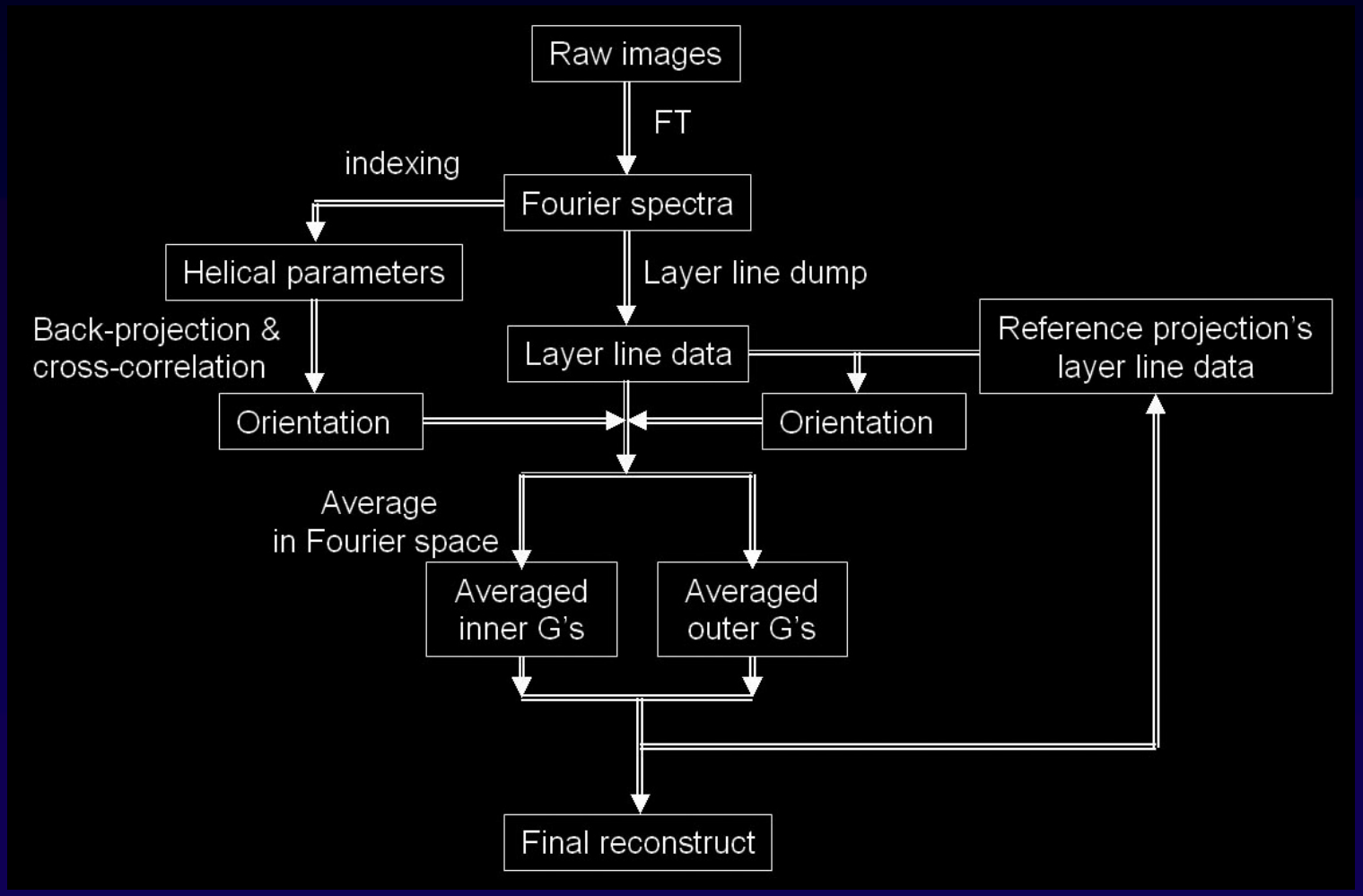
$$F_{obs,k}(R,Z) = G_{IN}(R,Z)\exp\{i[N_{IN}\Phi_{IN,k} - 2\pi Z z_{IN,k}]\}$$
$$+ G_{OUT}(R,Z)\exp\{i[N_{OUT}\Phi_{OUT,k} - 2\pi Z z_{OUT,k}]\}, k = 1,2...M$$

If the orientations of the inner and outer helices of each image could be determined (all the Φ_{IN}, z_{IN}, Φ_{OUT}, z_{OUT}), the G_{IN} and G_{OUT} could then be calculated by solution of the above equations (Crowther et al., J. Mol. Biol. 1985, method I).

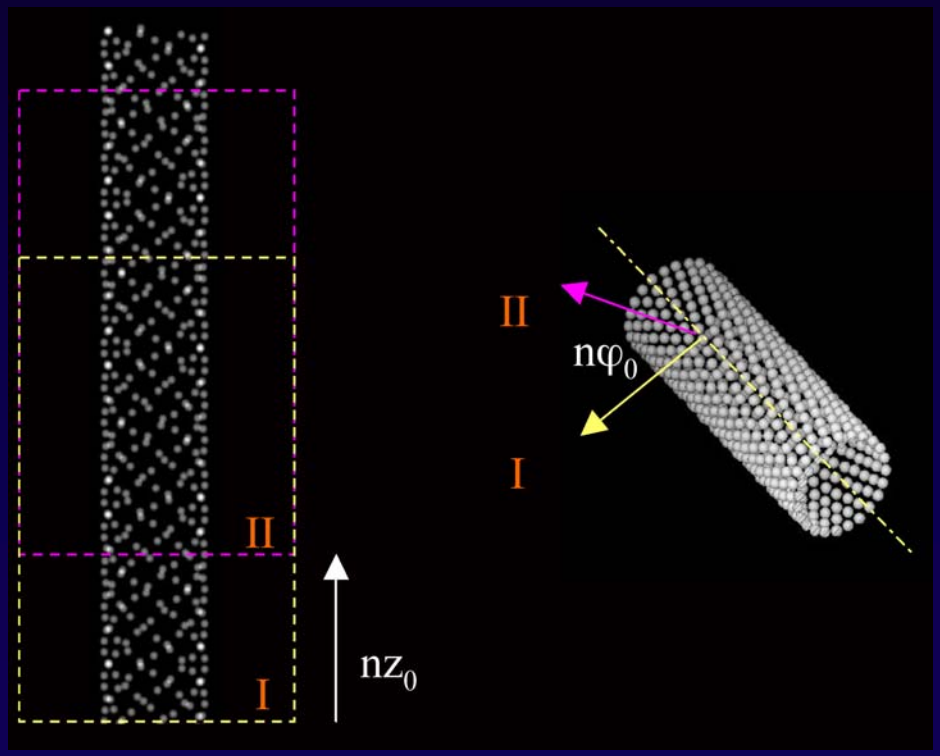
An alternative way to obtain G_{IN} and G_{OUT} is averaging the Fourier terms of certain layer lines from many different images after assign the Bessel orders and orientations of the goal helix (Miyazawa et al., J. Mol. Biol. 1999, method II). For instance, apply the Bessel orders and orientations of the inner helix to all the observed Fourier terms and average them:

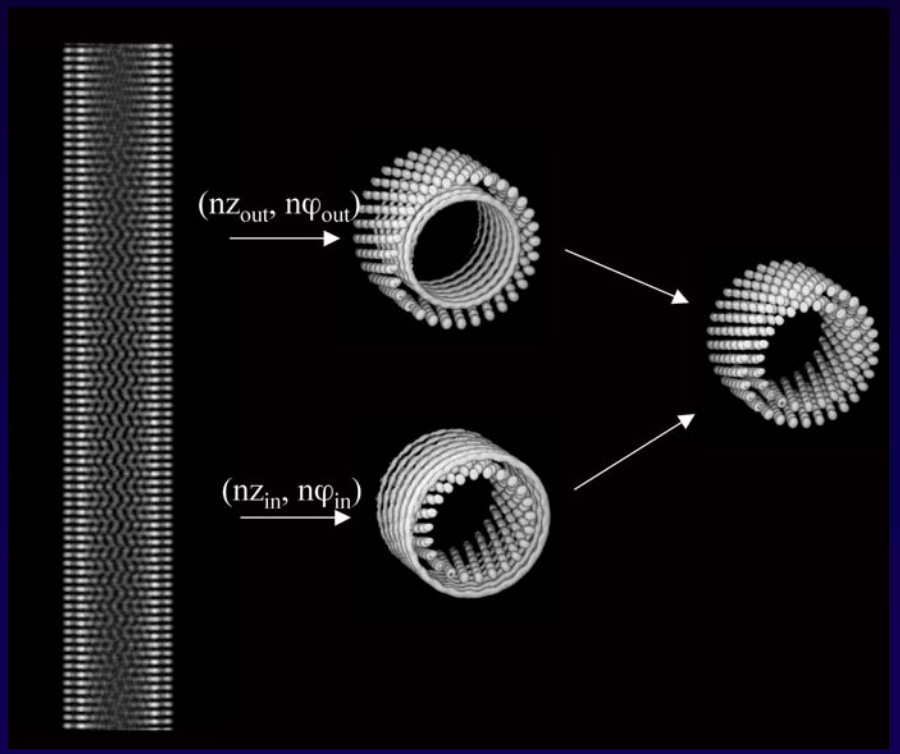
$$\overline{F} = \frac{1}{M}\sum_{k=1}^{M} F_{obs,k}\exp\{i[2\pi Z z_{IN,k} - N_{IN}\Phi_{IN,k}]\} = G_{IN}(R,Z)$$
$$+ G_{OUT}(R,Z)\frac{1}{M}\sum_{k=1}^{M}\exp\{i[(N_{OUT}\Phi_{OUT,k} - 2\pi Z z_{OUT,k}) - (N_{IN}\Phi_{IN,k} - 2\pi Z z_{IN,k})]\}$$

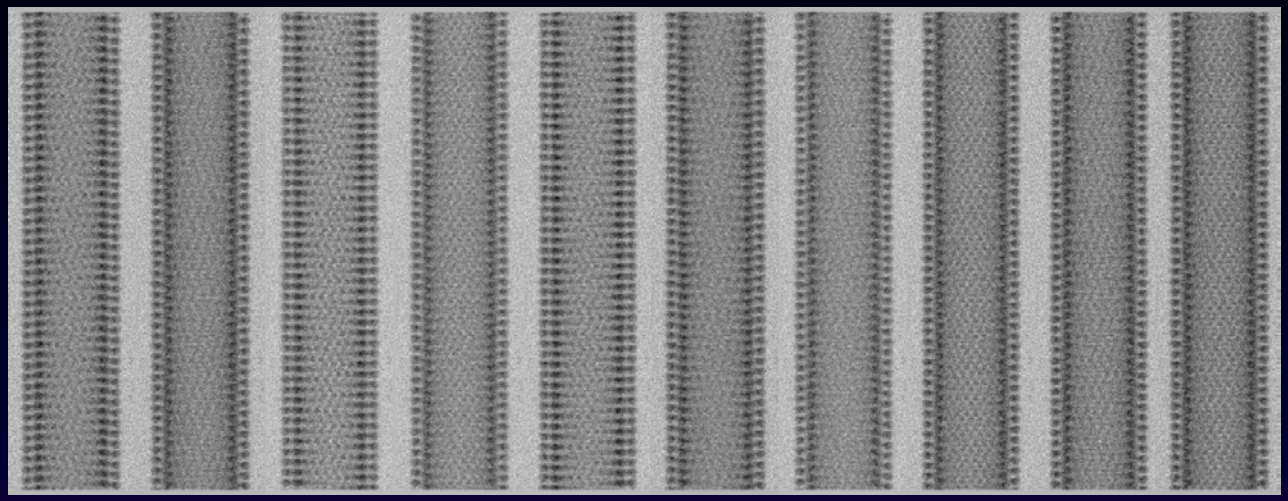
Iterative Algorithm

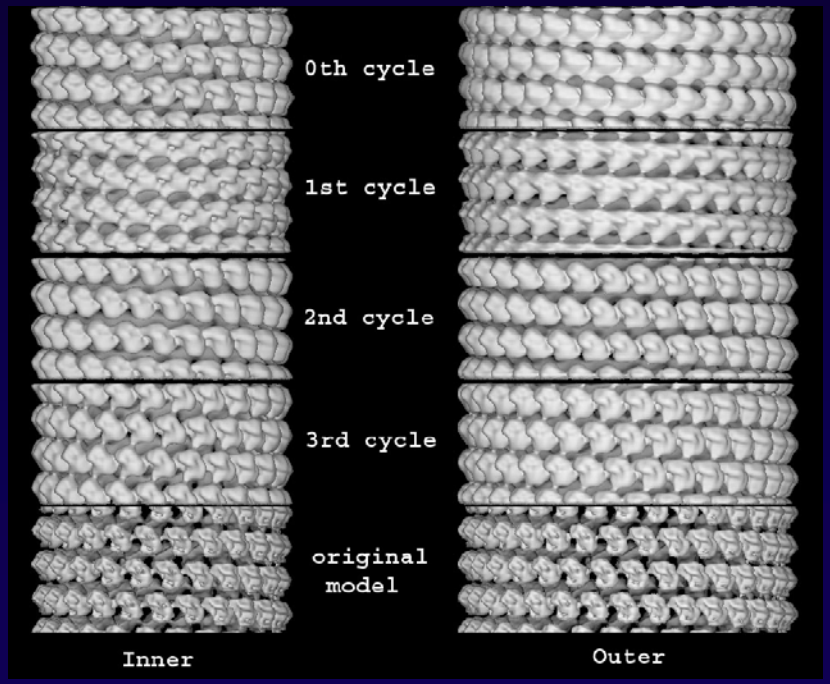


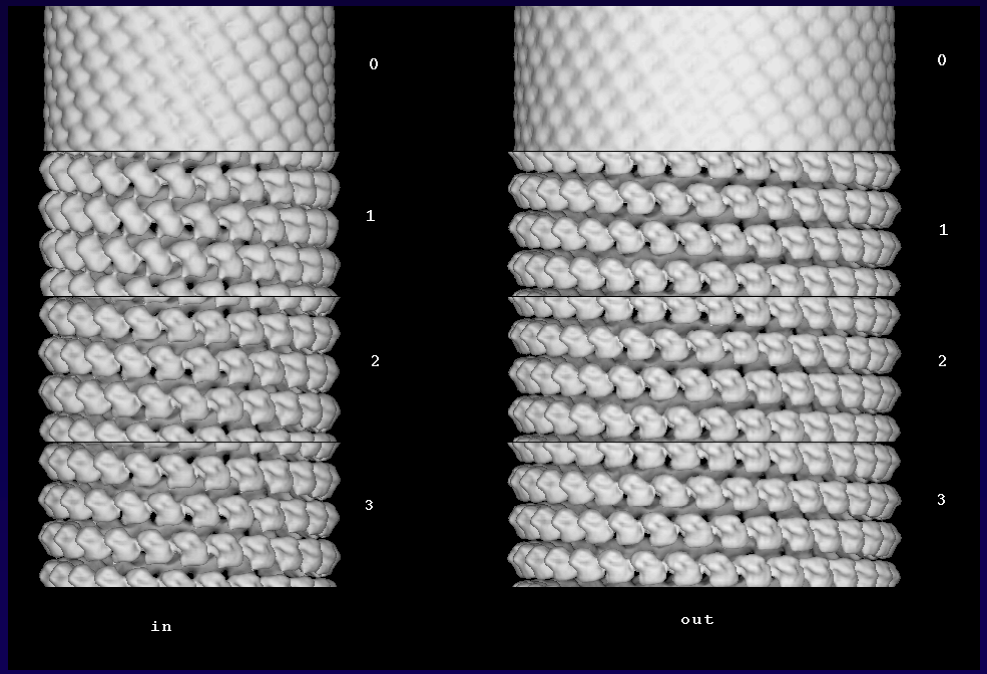
Backprojection for Inner and Outer Layers








0th cycle
1st cycle
2nd cycle
3rd cycle
original model
Inner
Outer

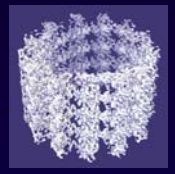

0
1
2
3
in
0
1
2
3
out

Acknowledgements

Jan Löwe (MRC)
Ken Downing, Huilin Li (LBNL)

Milligan's Lab (Scripps)

Ken Downing, Huilin Li (LBNL)
David DeRosier (Brandeis Univ.)



William V. Nicholson, Minou Le

Hong-Wei Wang