

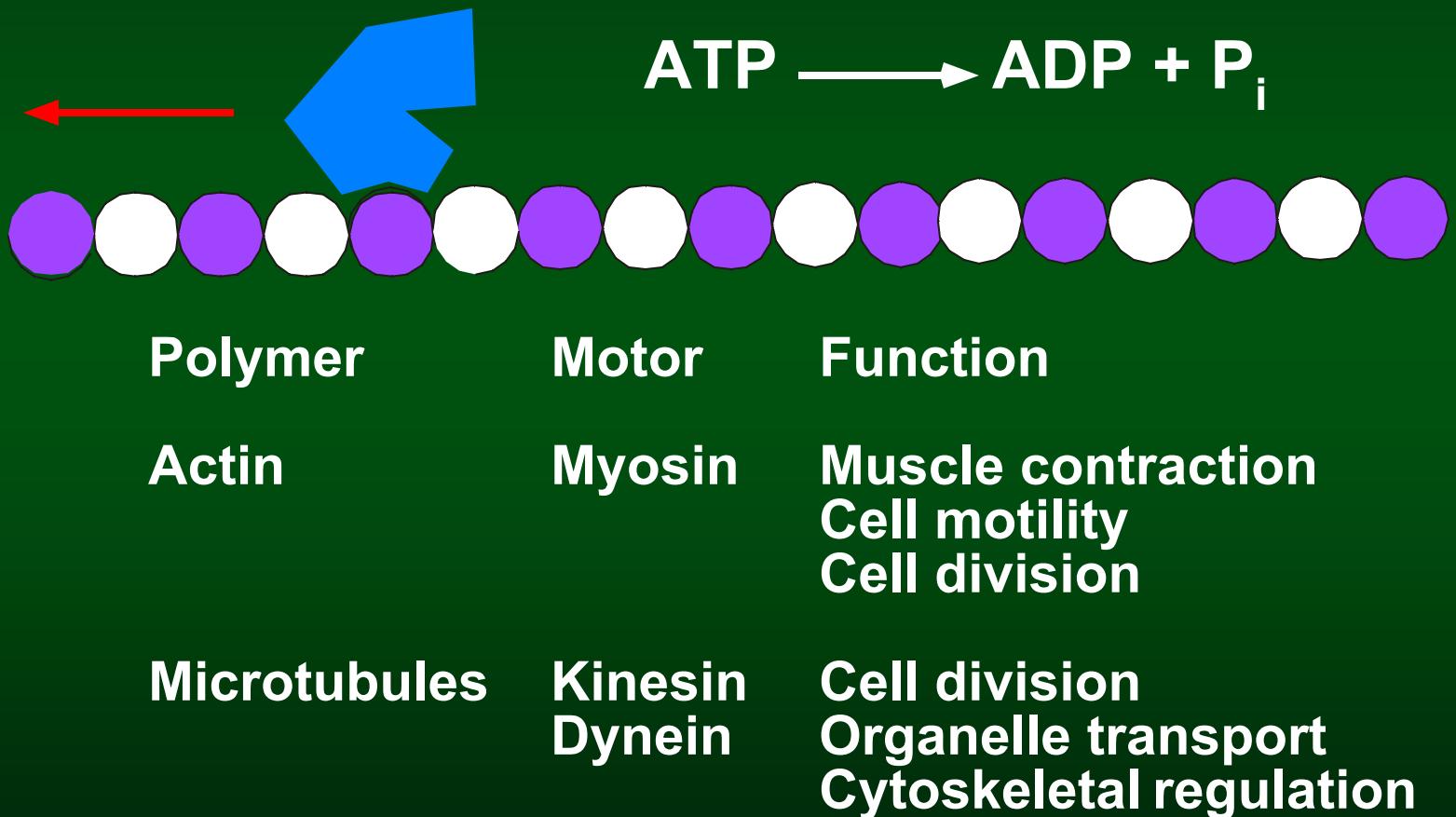
The missing link between motor proteins and transcription factors

F. Jon Kull

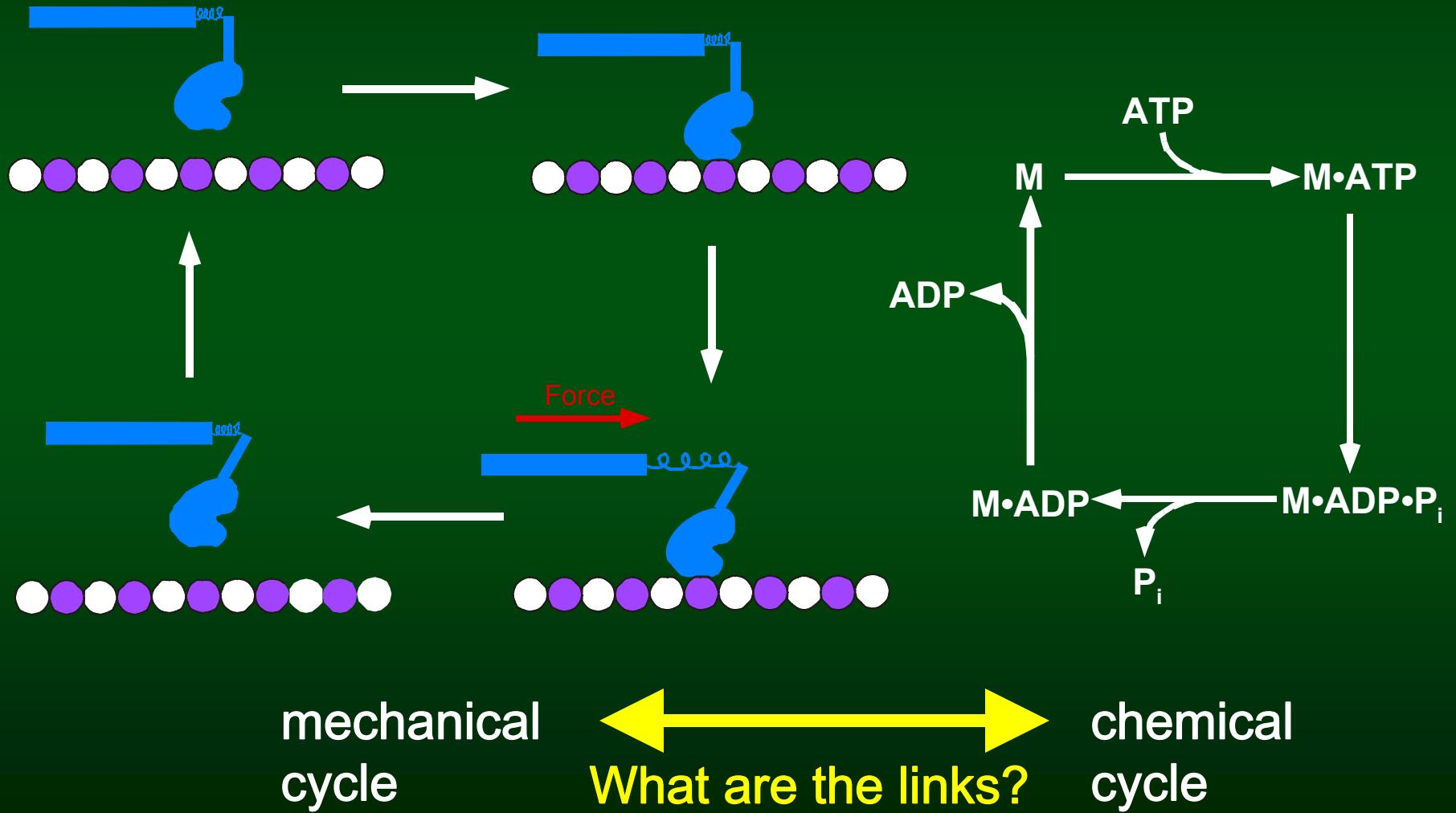
Department of Chemistry
Dartmouth College



Molecular Motors



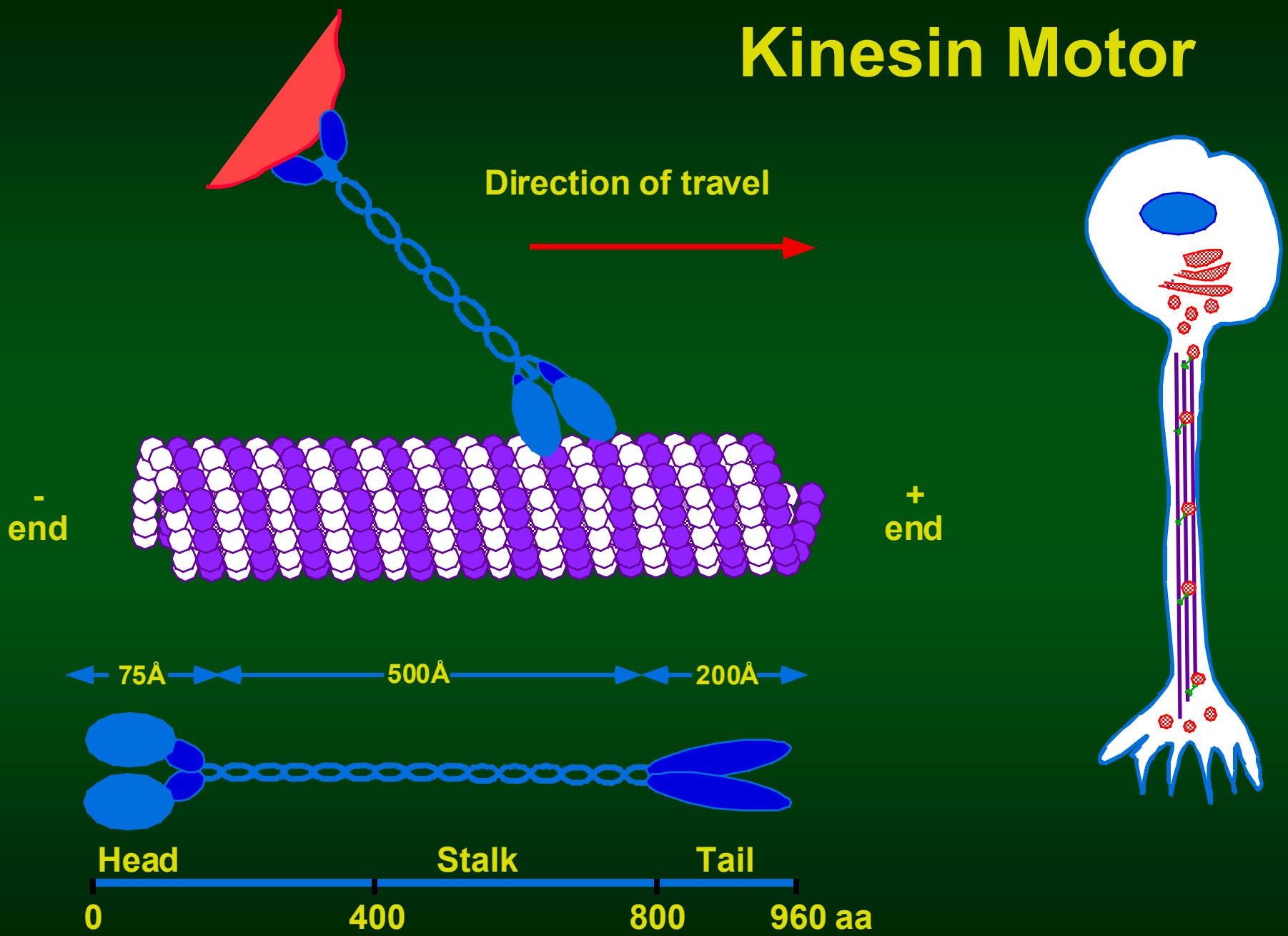
A simple ATP-fueled molecular motor



Molecular Motor Components

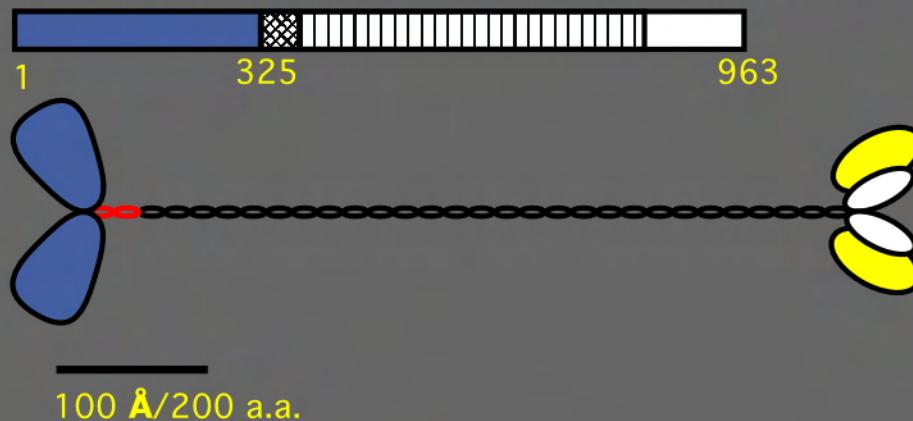
- Bind nucleotide
- Sense γ -phosphate
- Initial conformational response
- Alter protein surface (motor-track interactions)
- Force generating conformational change
- Inter- and intra-motor communication/
synchronization

Kinesin Motor



Kinesin Superfamily Members

N-terminal - conventional kinesin

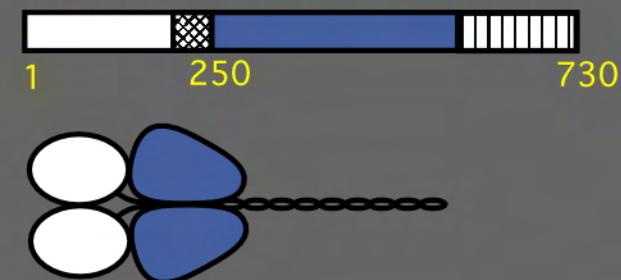


- 8 nm step
- 100 steps/association
- 5-7 pN/step
- 1 step/ATP
- 60% efficient
- ATPase stimulated 1000-2000x by MT's

C-terminal - Ncd



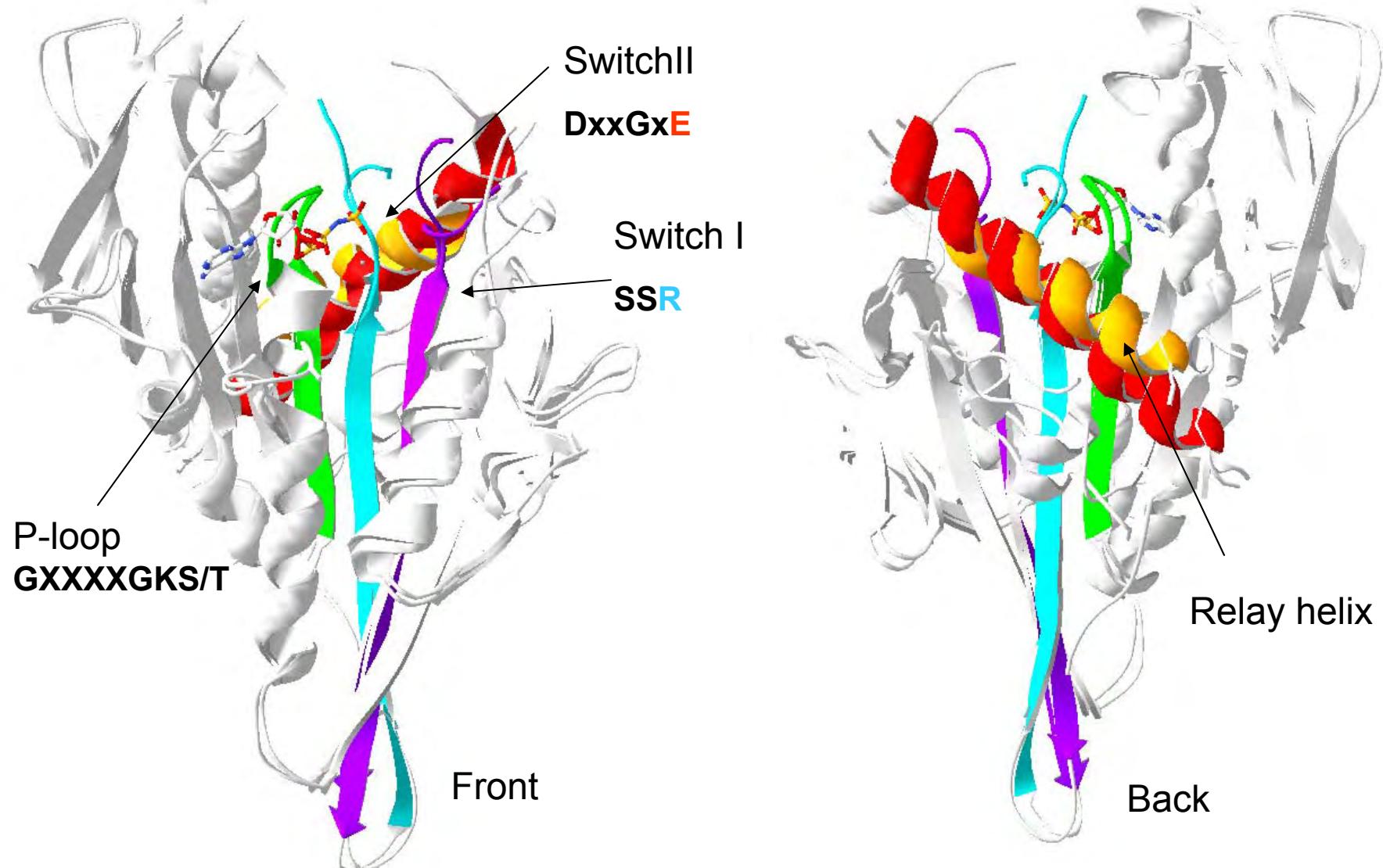
Internal - XKCM1



Directionality in kinesin motors

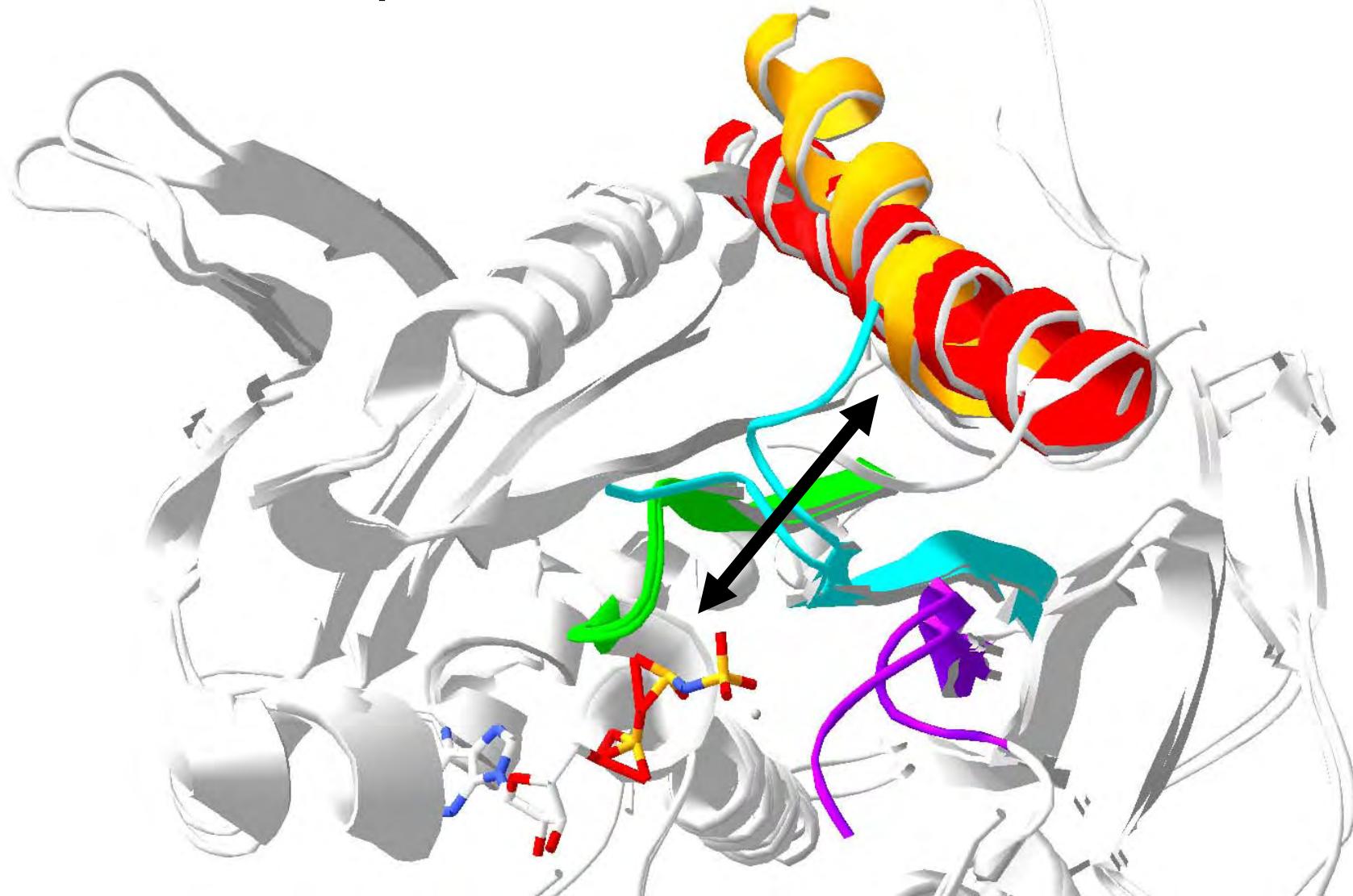
Aligned Kif1a ADP and ANP-PNP structures

Nitta, R., Kikkawa, M., Okada, Y., and Hirokawa, N. (2004). KIF1A alternately uses two loops to bind microtubules. *Science* 305, 678-683.



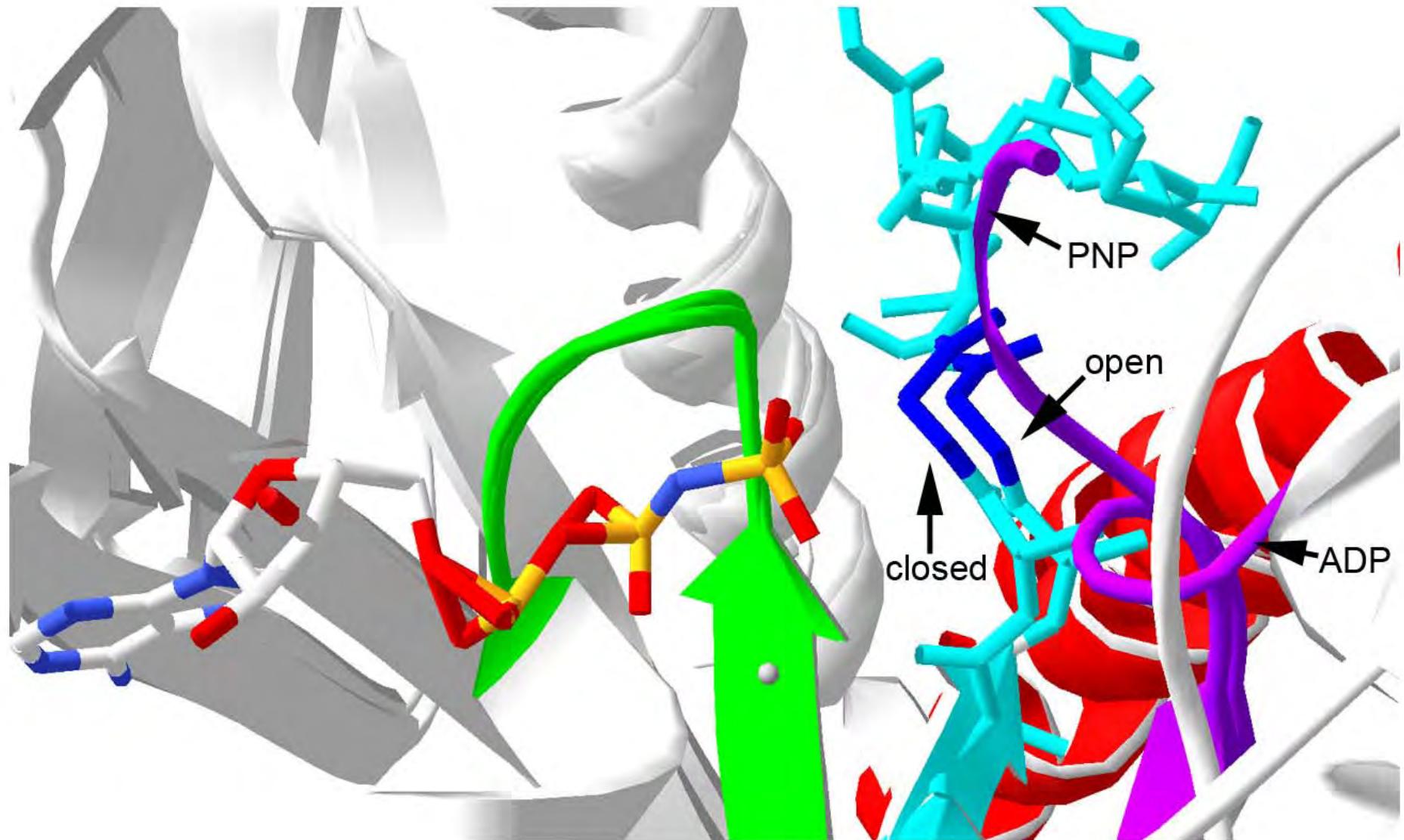
View from the top

Microtubule-binding face

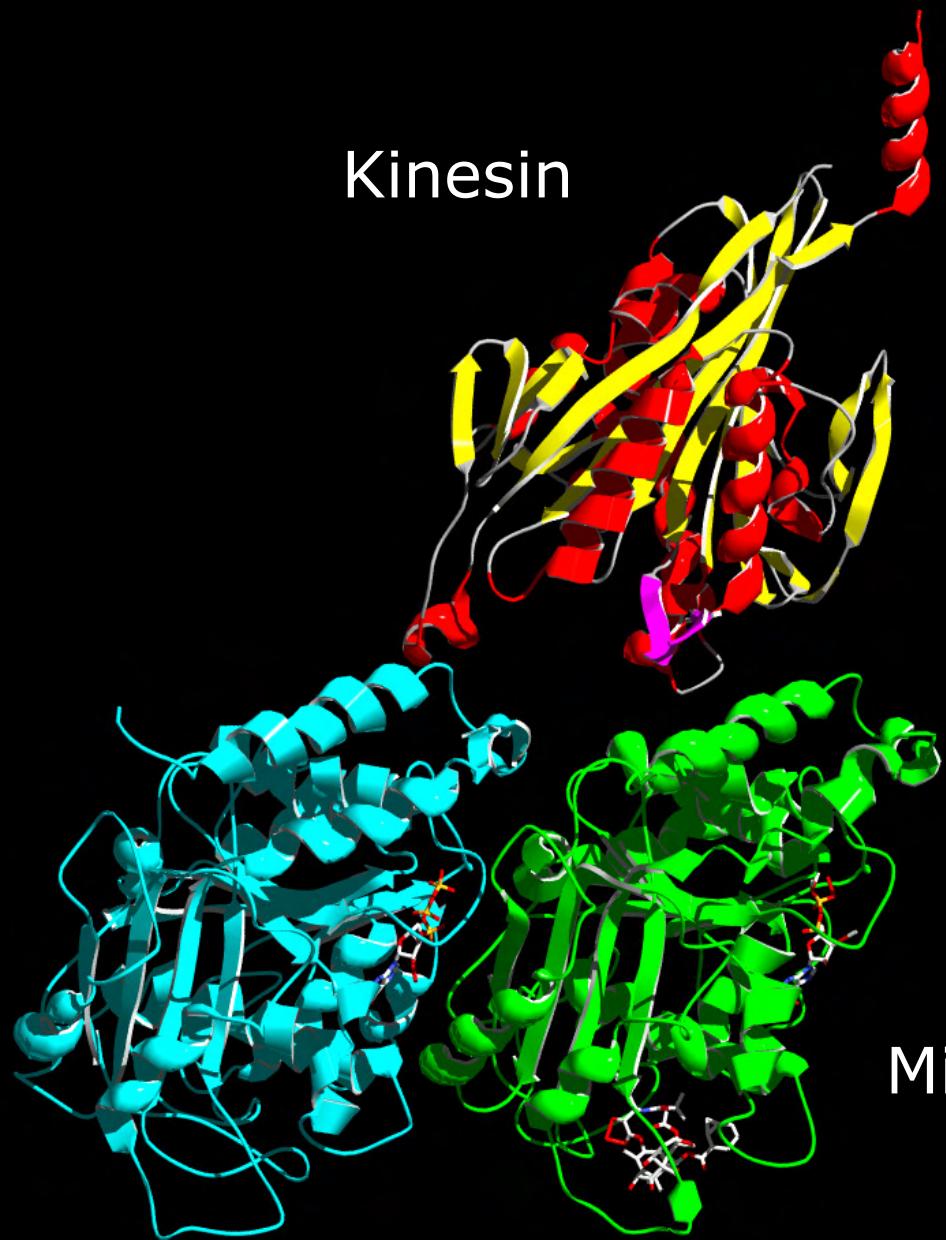


Nucleotide-binding face

Kif1a switch II region



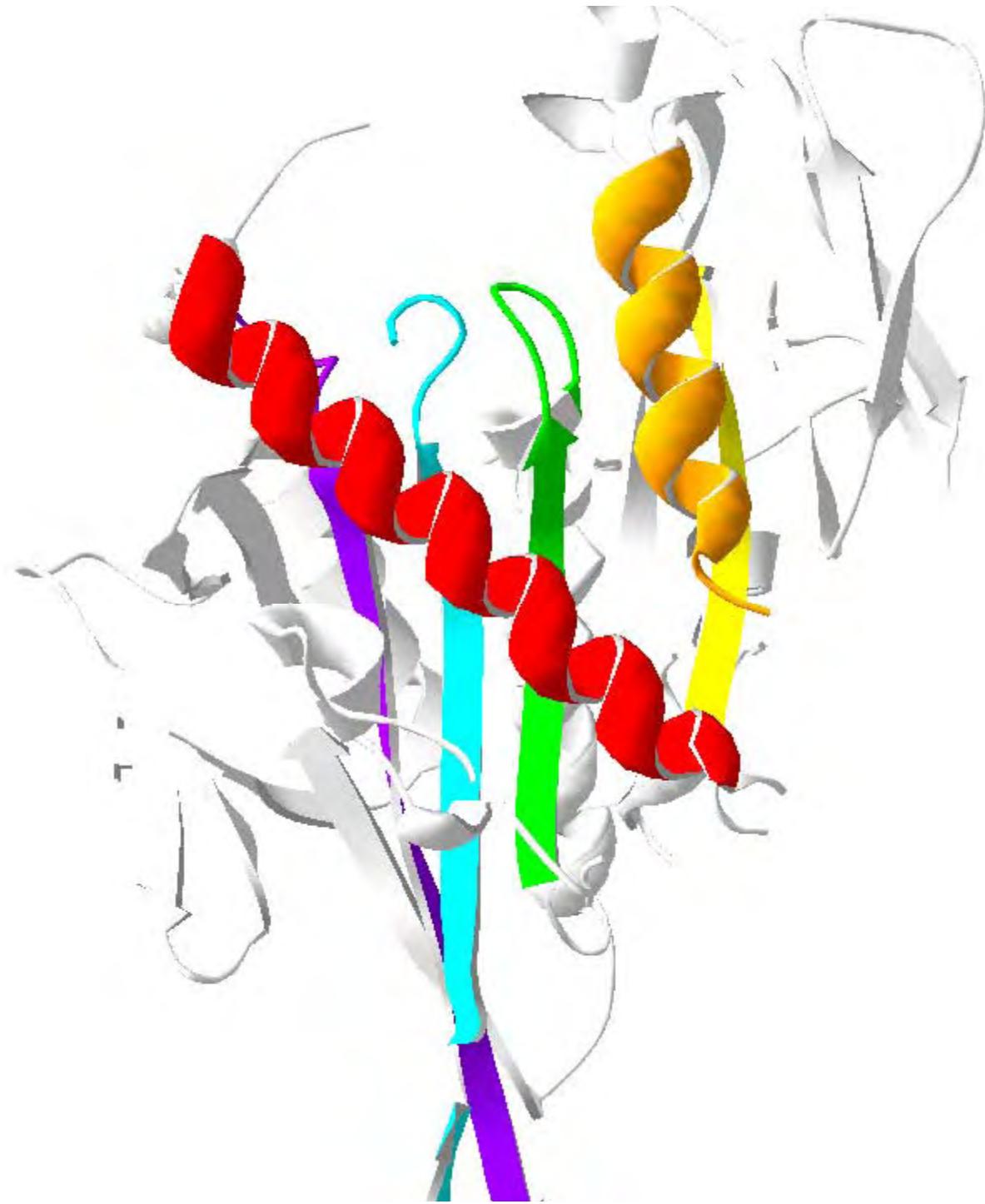
When bound to MT, loop 11 forms a link between Switch II and the relay helix.



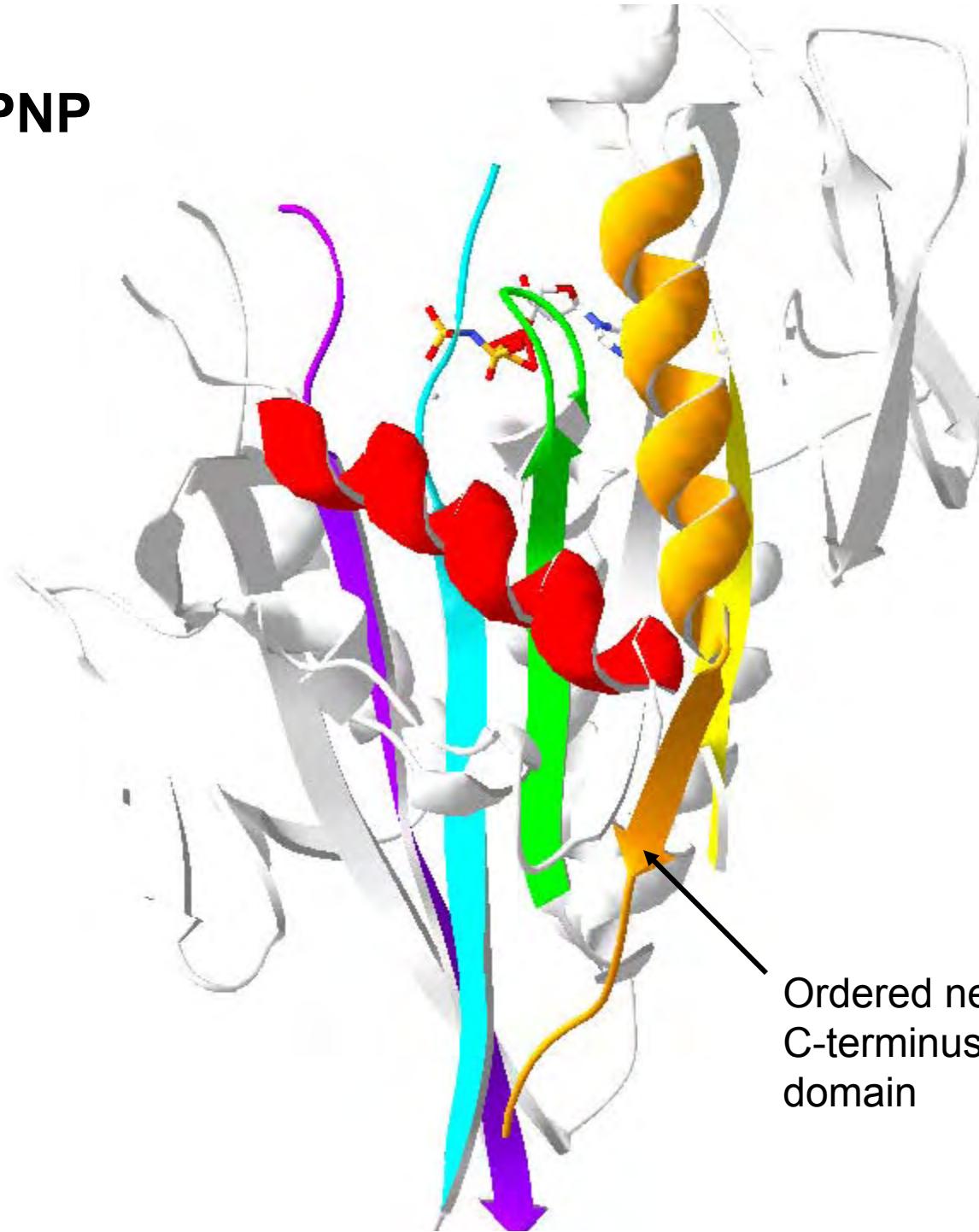
Microtubule



ADP

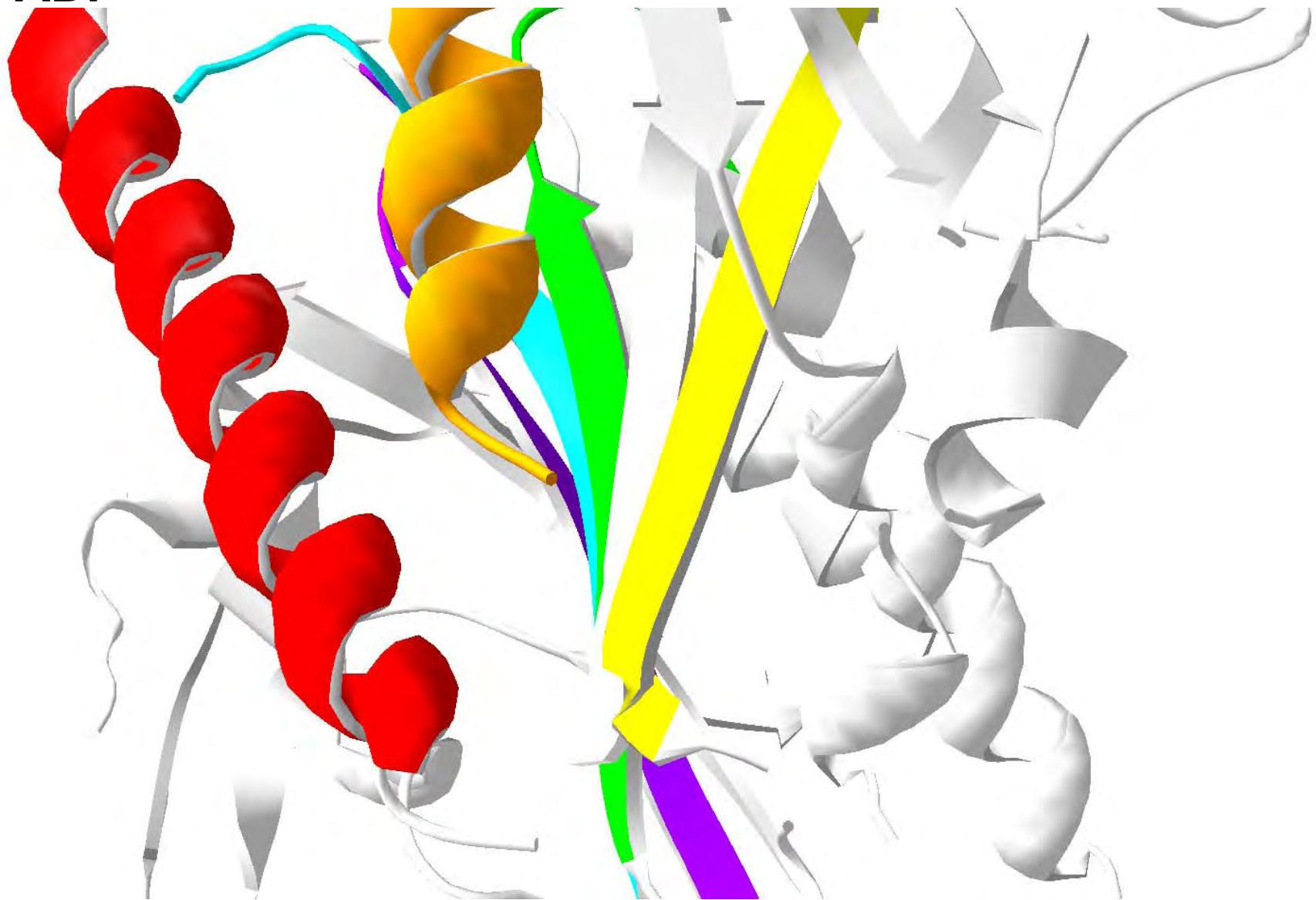


AMP-PNP

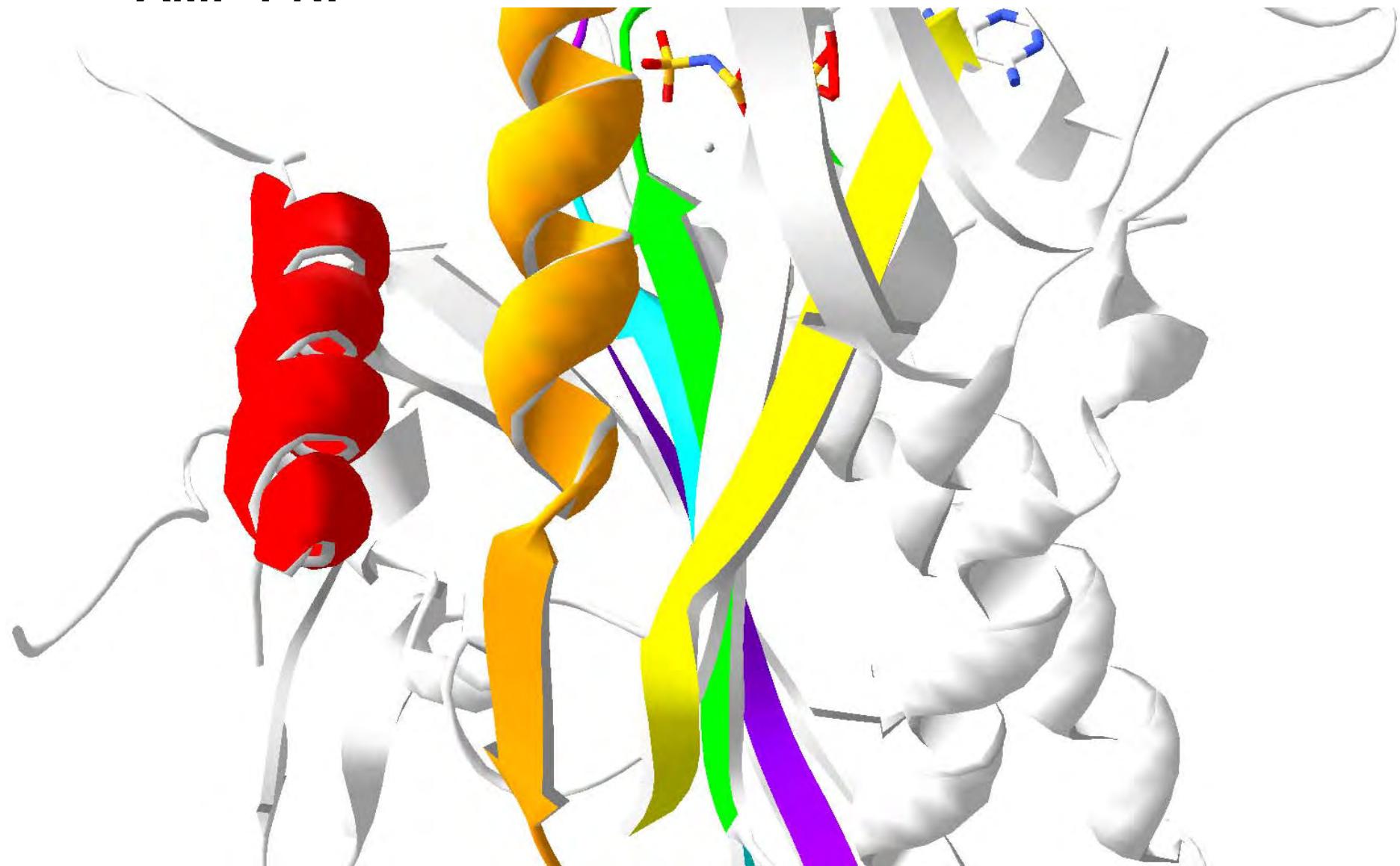


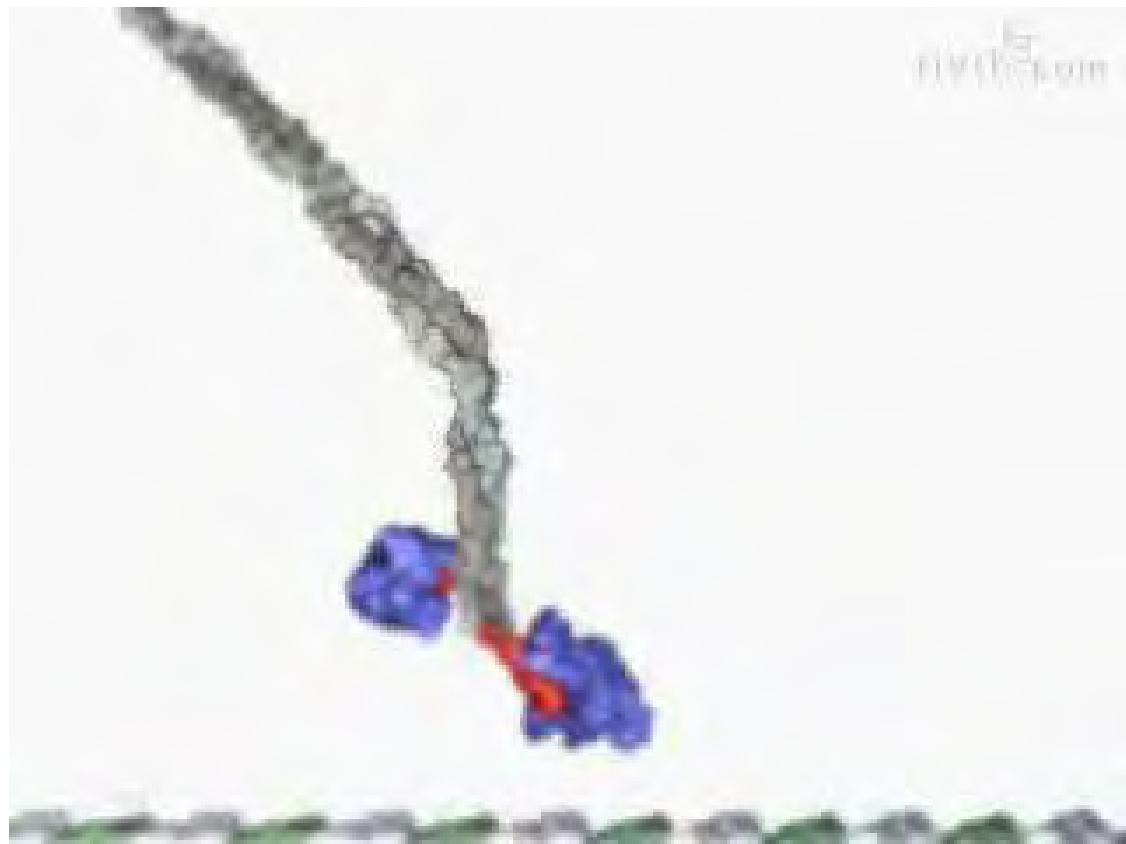
Ordered neck linker at
C-terminus of motor
domain

ADP



AMP-PNP

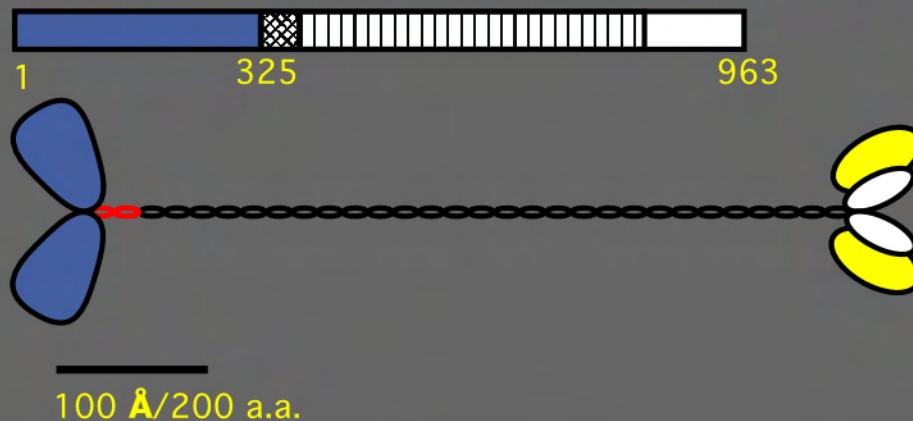




Vale RD, Milligan RA. (2000) The way things move: looking under the hood of molecular motor proteins. *Science*. Apr 7;288(5463):88-95.

Kinesin Superfamily Members

N-terminal - conventional kinesin

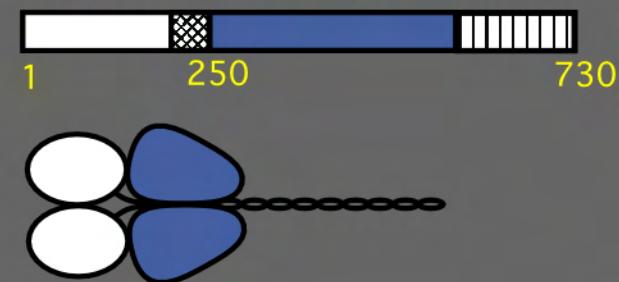


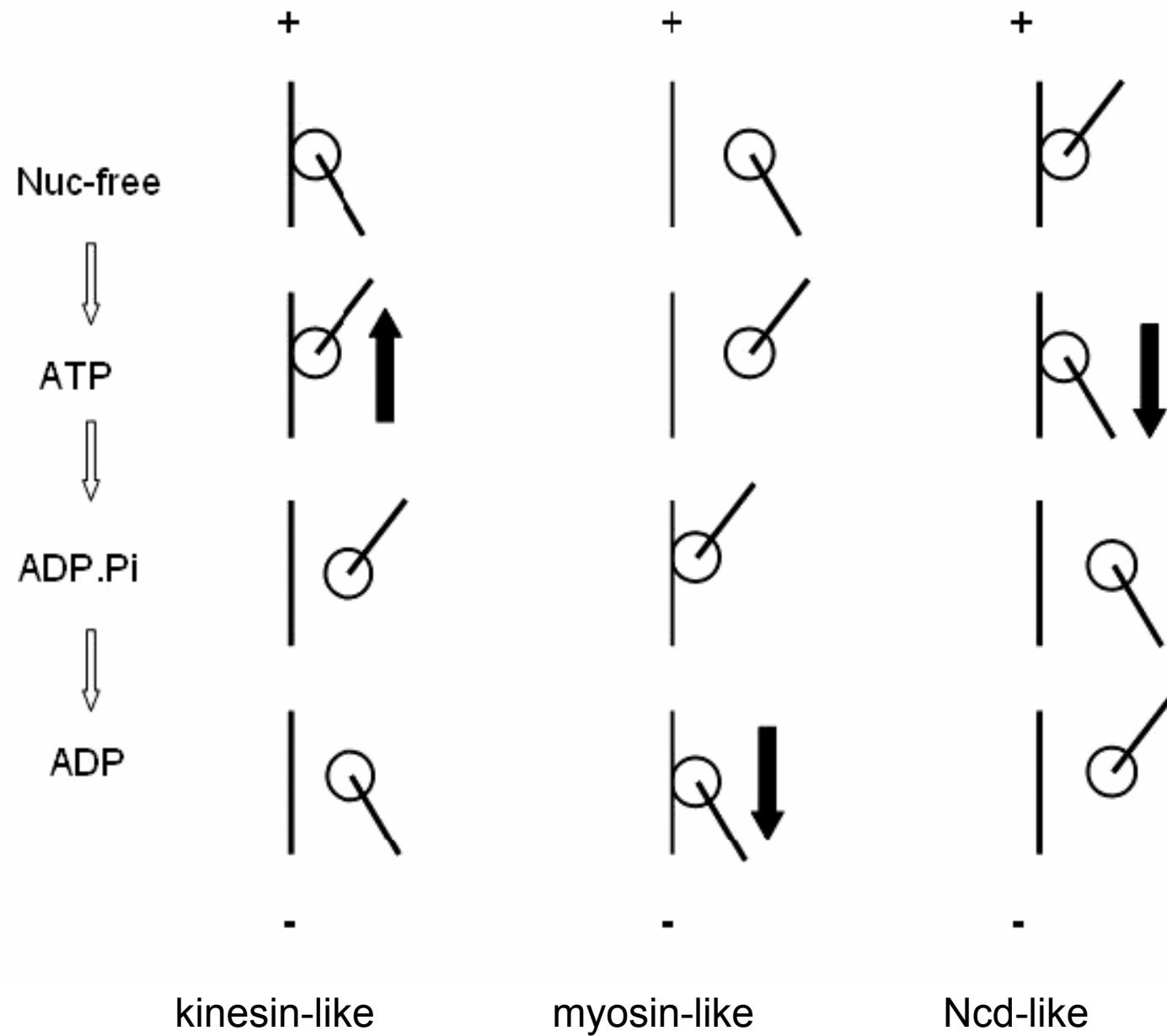
- 8 nm step
- 100 steps/association
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- 1 step/ATP
- 60% efficient
- ATPase stimulated 1000-2000x by MT's

C-terminal - Ncd



Internal - XKCM1



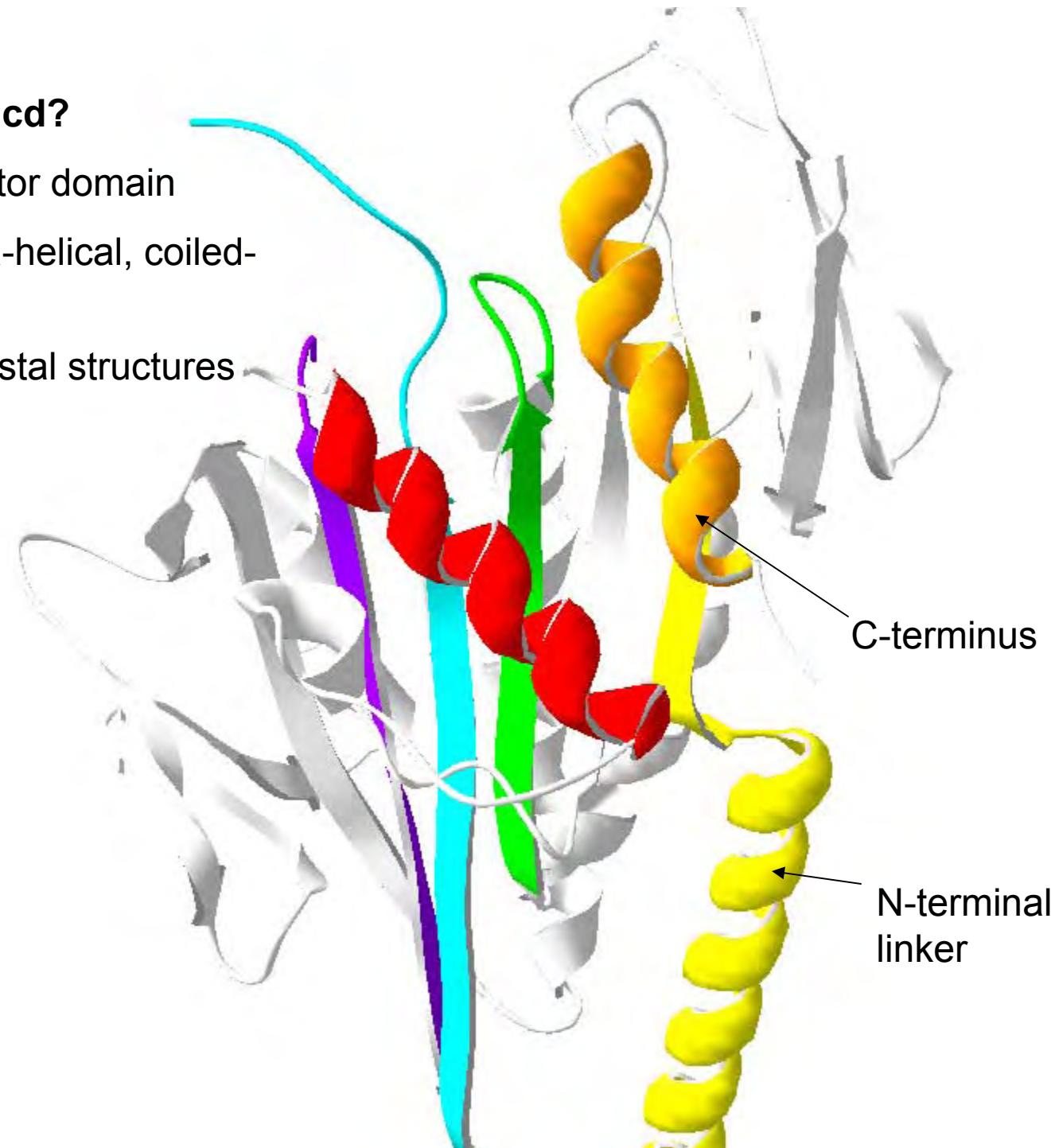


What about Ncd?

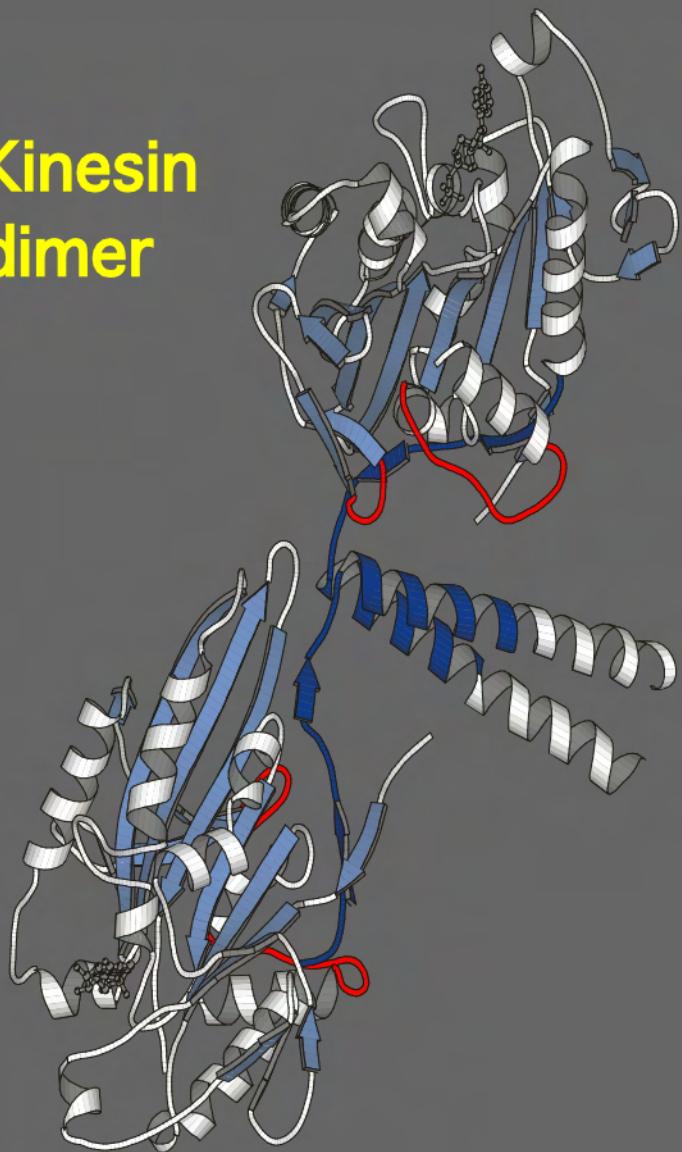
C-terminal motor domain

Linker is alpha-helical, coiled-coil dimer.

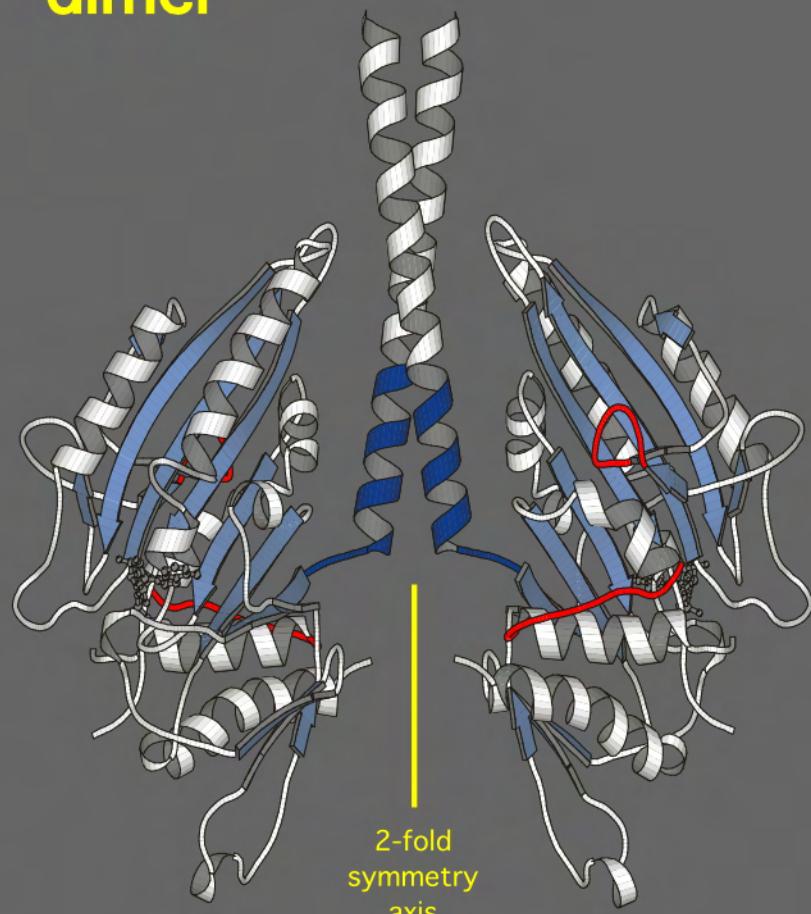
Ordered in crystal structures



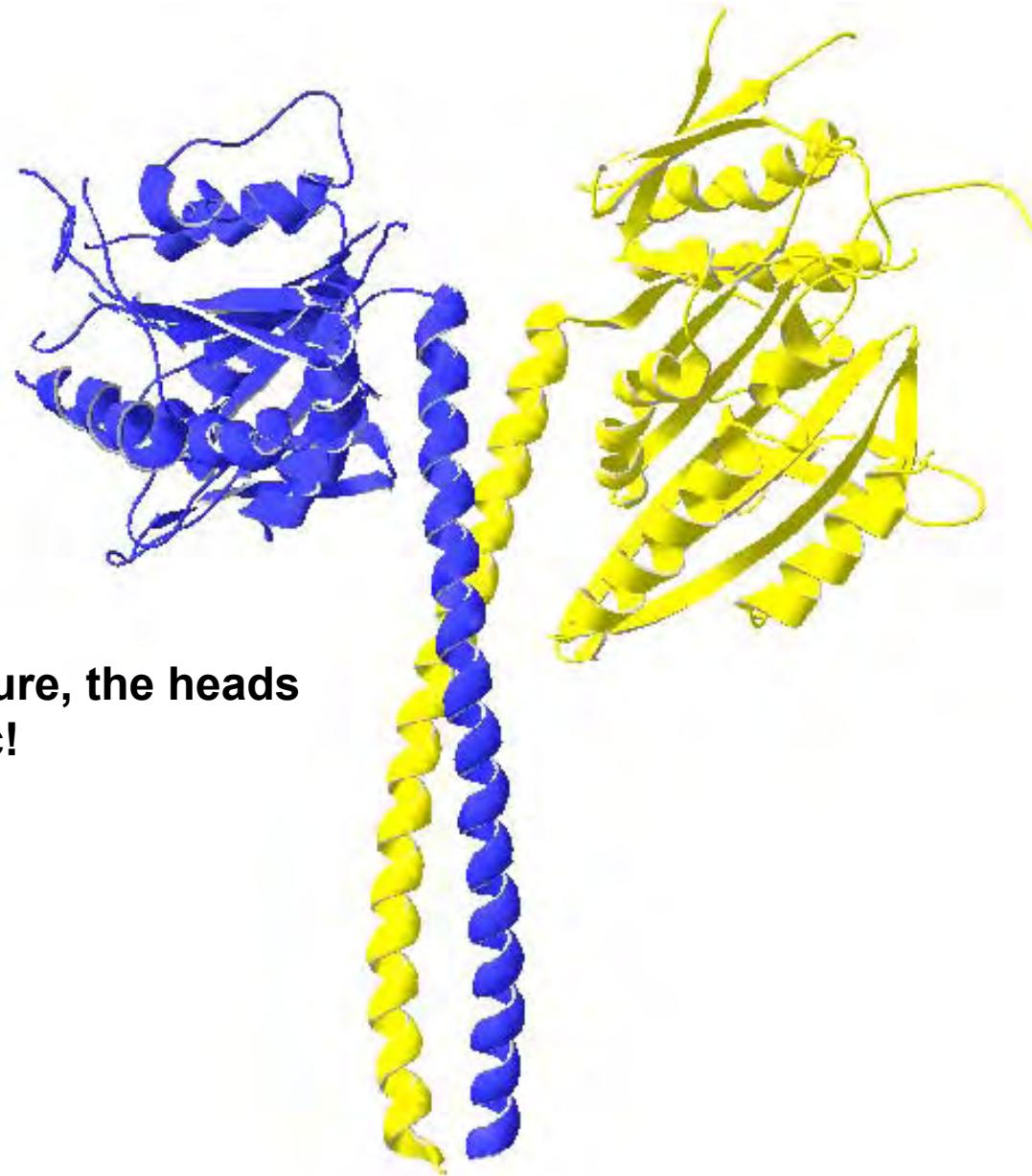
Kinesin
dimer



Ncd
dimer



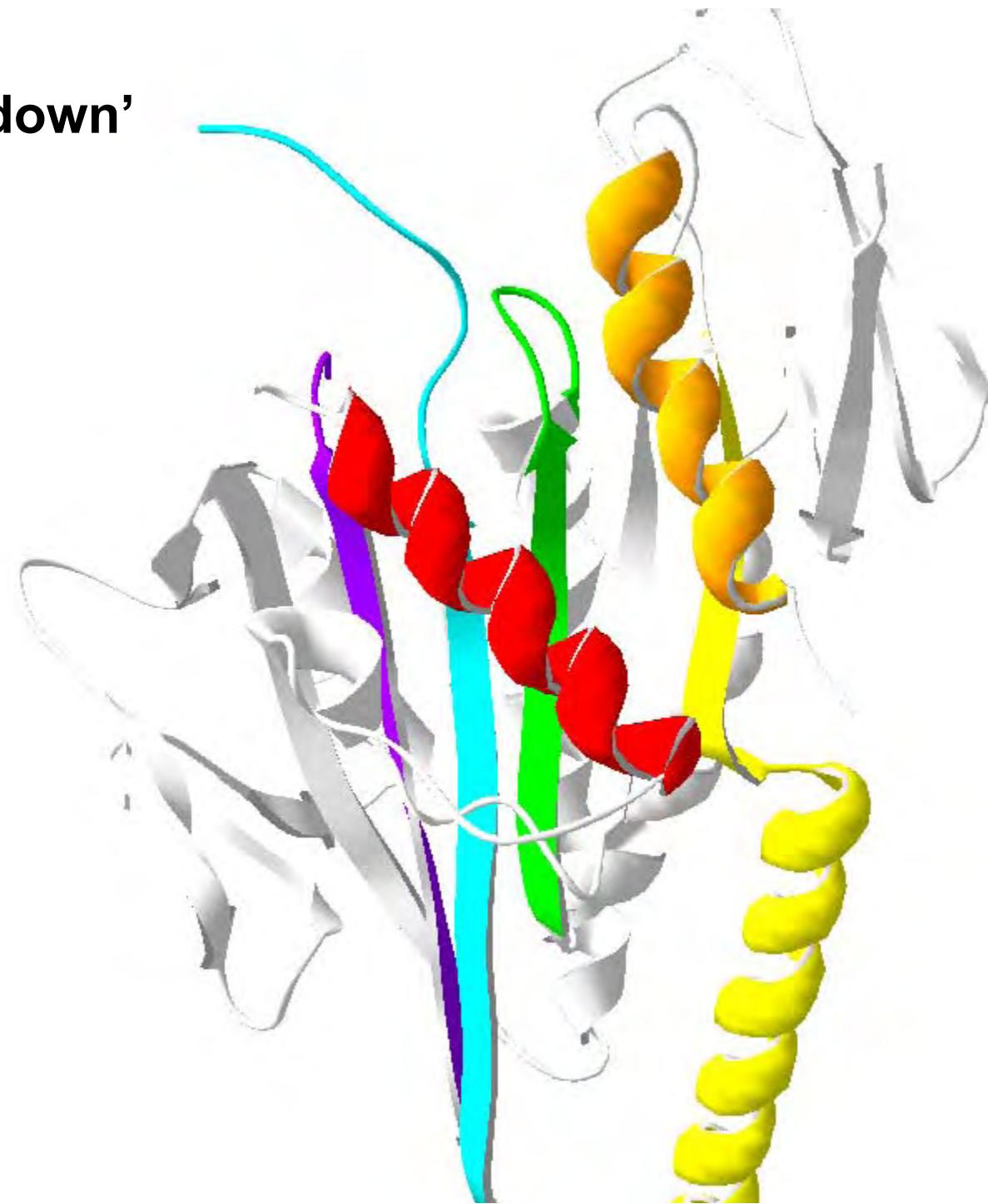
2-fold
symmetry
axis



**In a new structure, the heads
are asymmetric!**

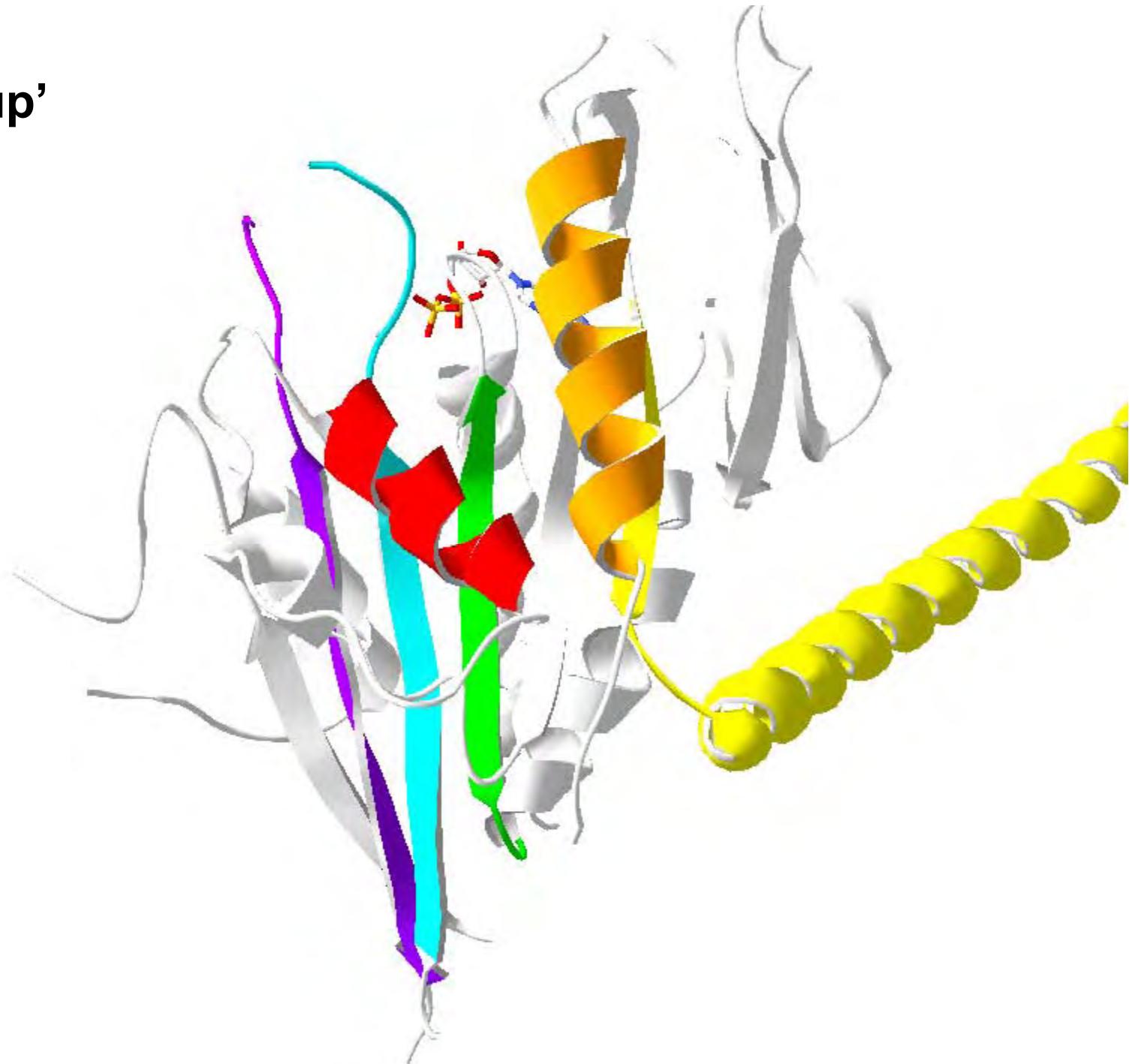
Ncd 'down'

ADP



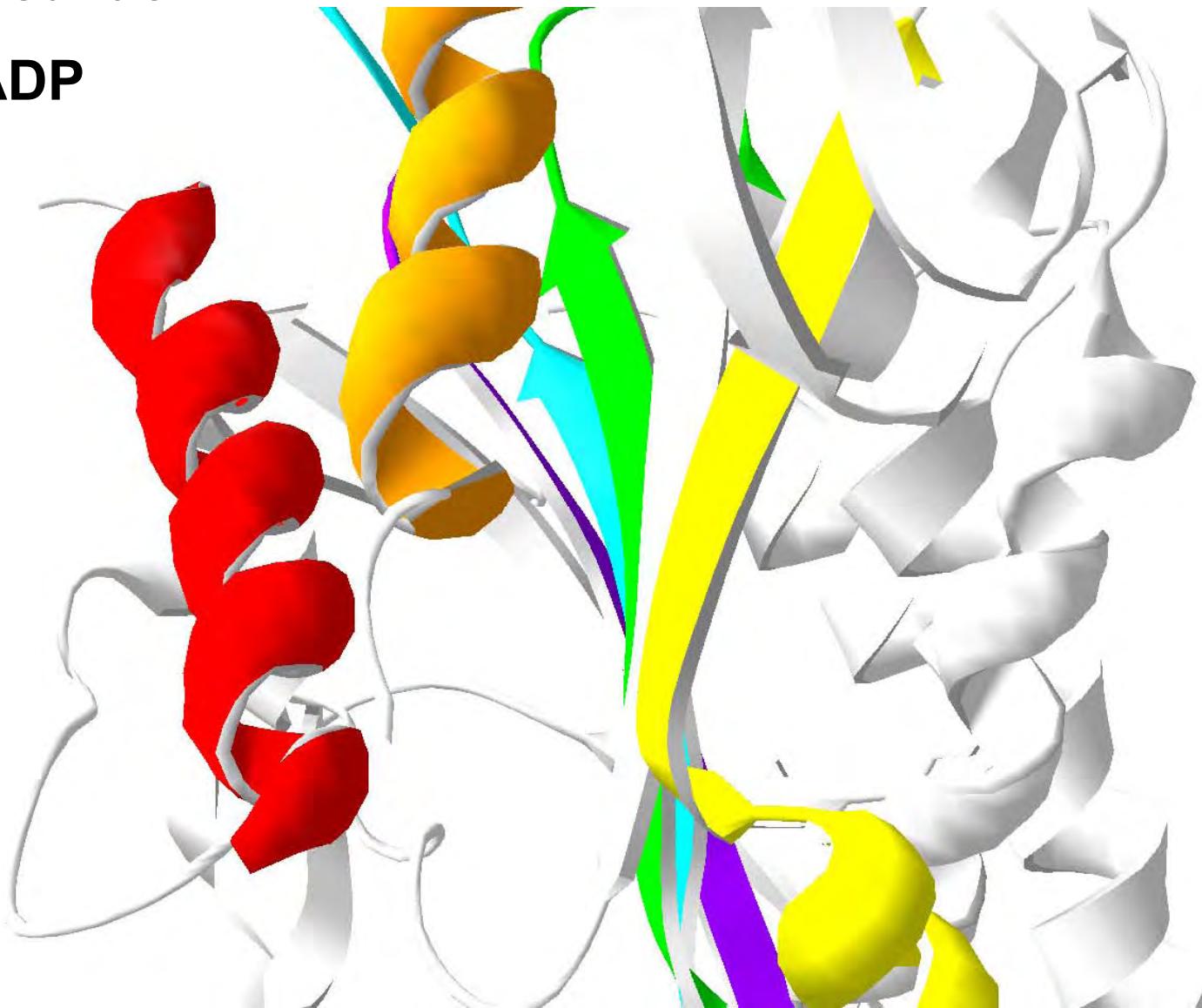
Ncd 'up'

ATP?



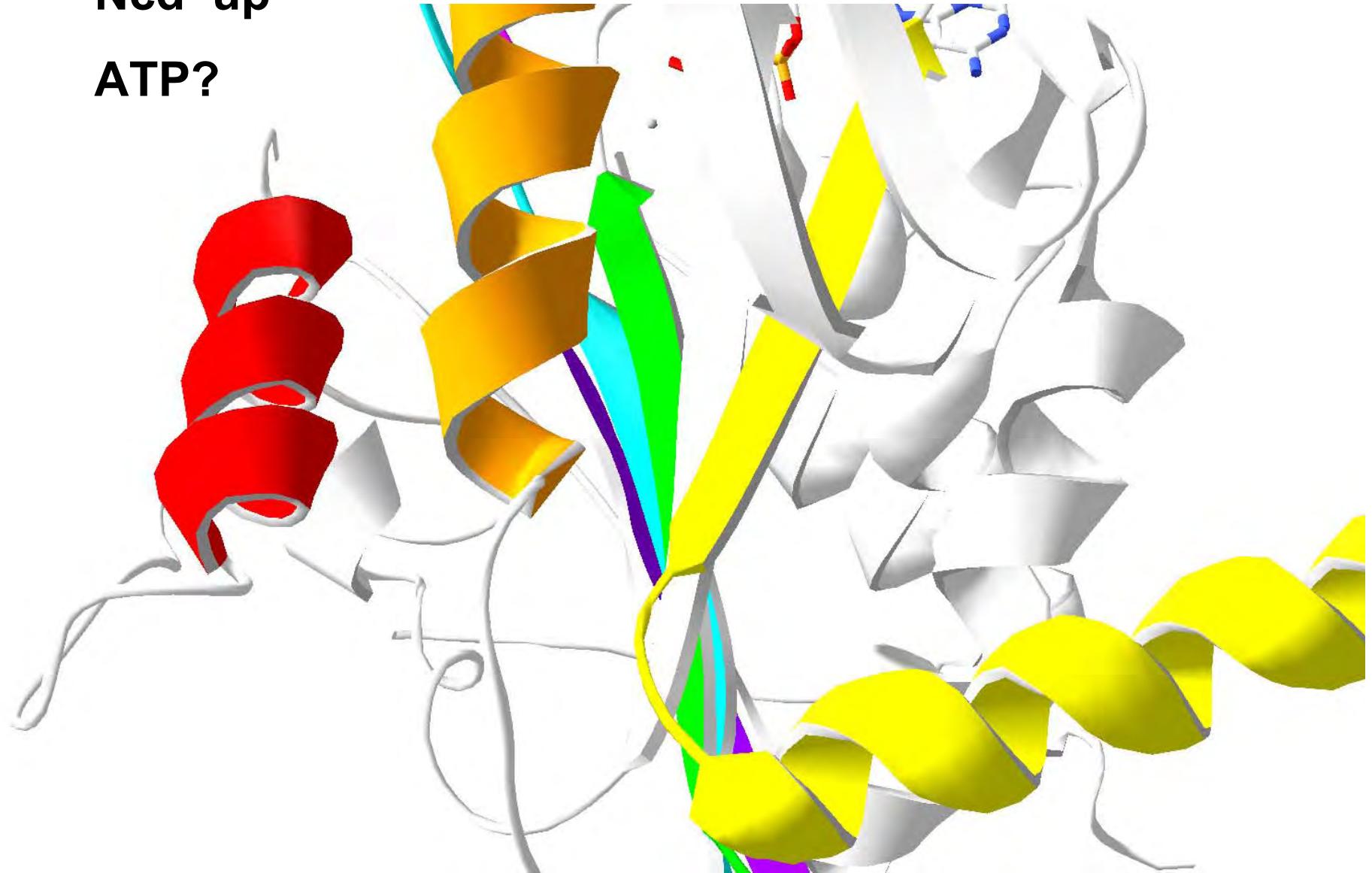
Ncd 'down'

ADP

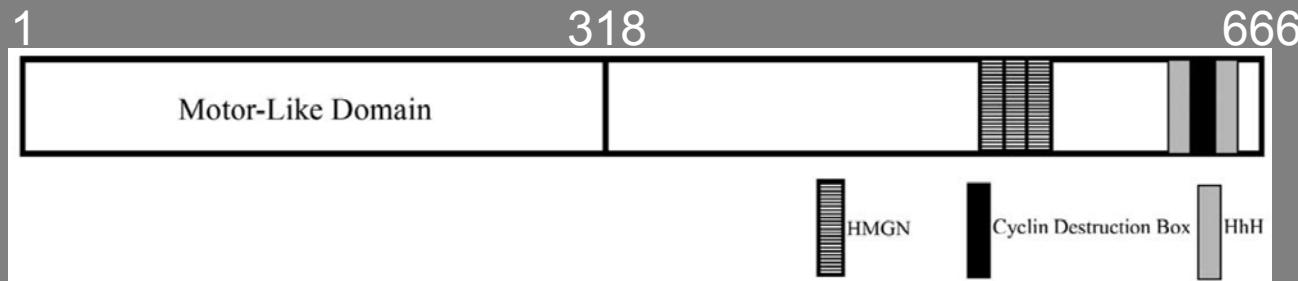


Ncd 'up'

ATP?



Nod: Kinesin-10 Protein in *Drosophila*

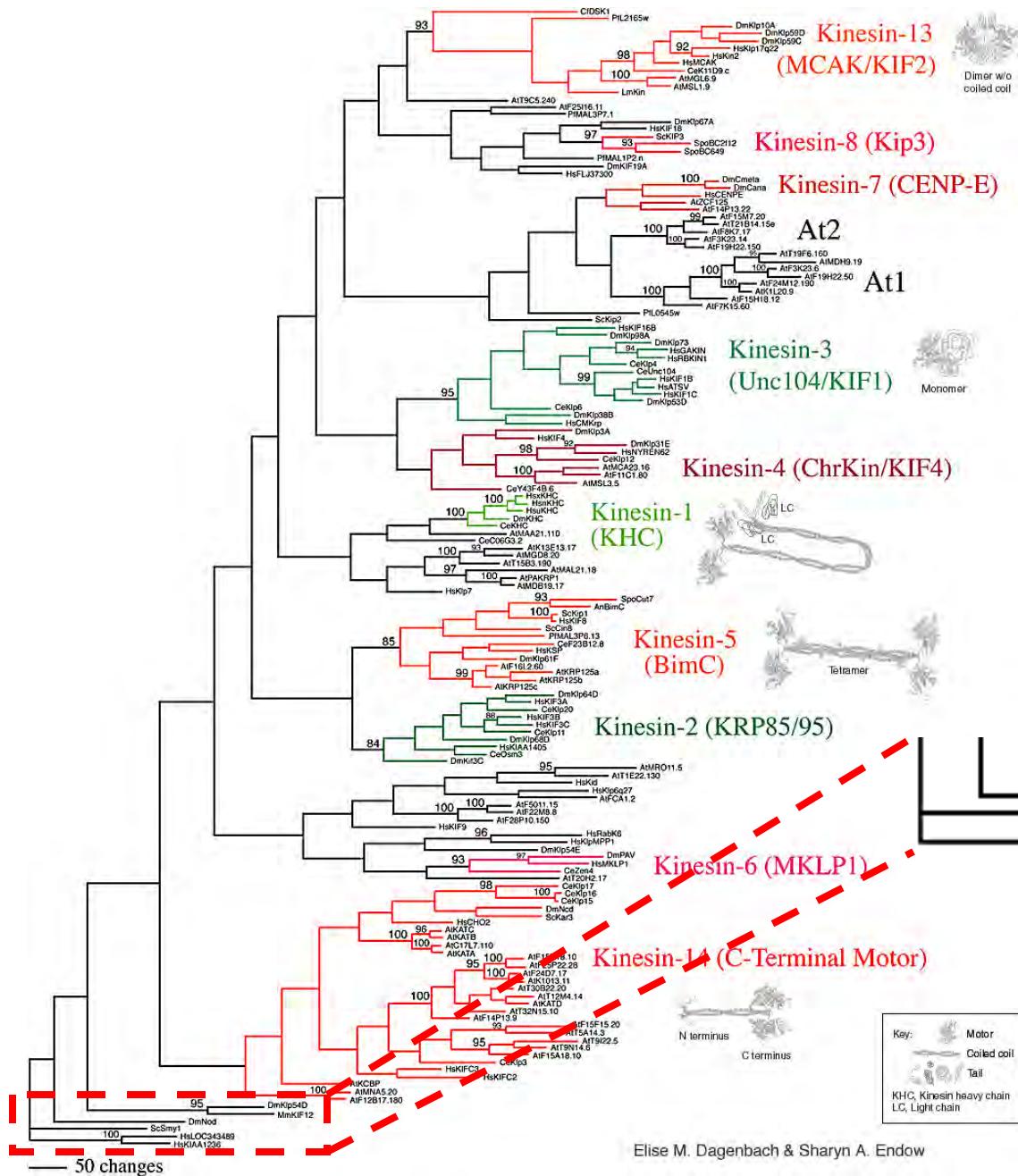


- 666 aa - localizes along the arms of meiotic chromosomes
- Plays critical role in segregating non-exchanging (achiasmate) chromosomes during female meiosis
- Preferentially binds plus-ends of microtubules
- Modulates microtubule polymerization dynamics
 - Stabilizes microtubule polymers *in vitro*
 - ATP hydrolysis NOT required
- Non-motile (no microtubule gliding *in vitro*)

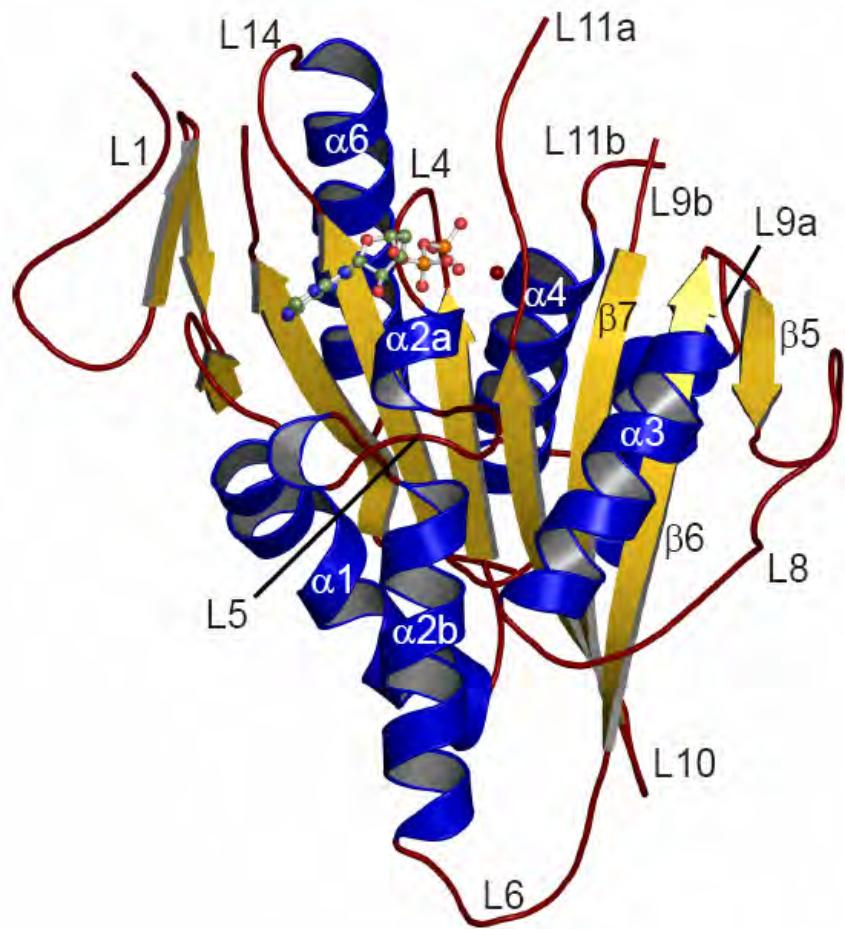
Is NOD really a kinesin ‘motor’?

Kinesin Superfamily

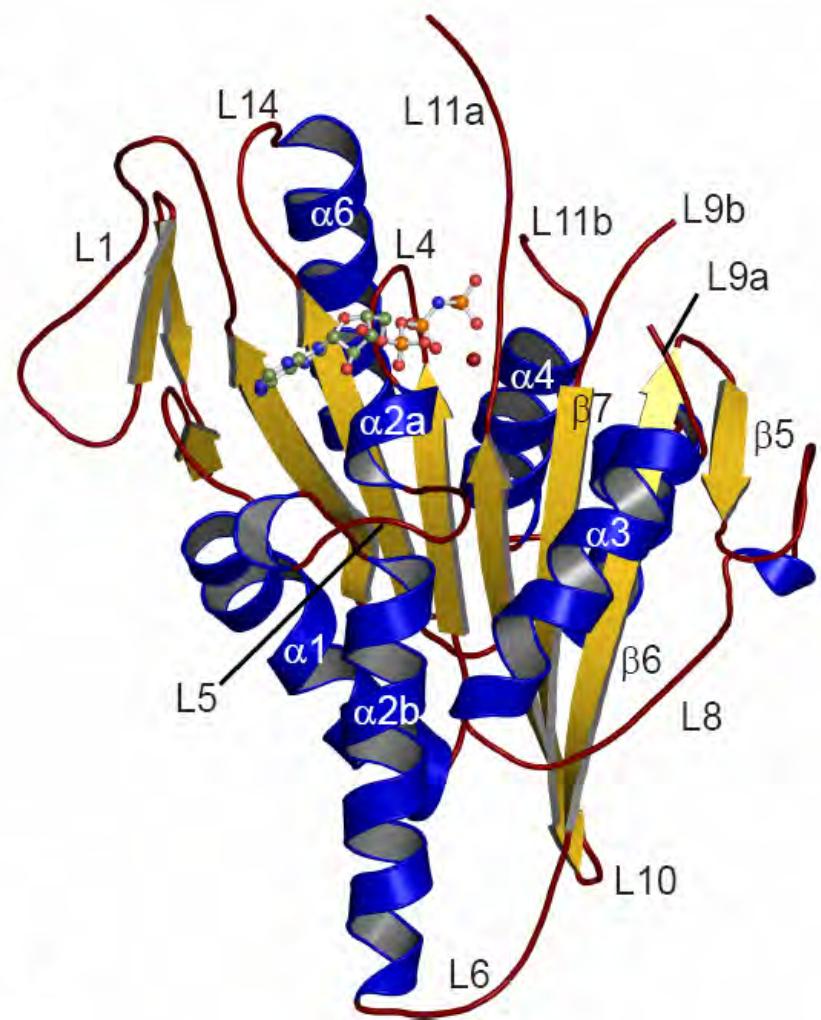
- 14 families
 - 7 families have motor domains that have been crystallized and solved



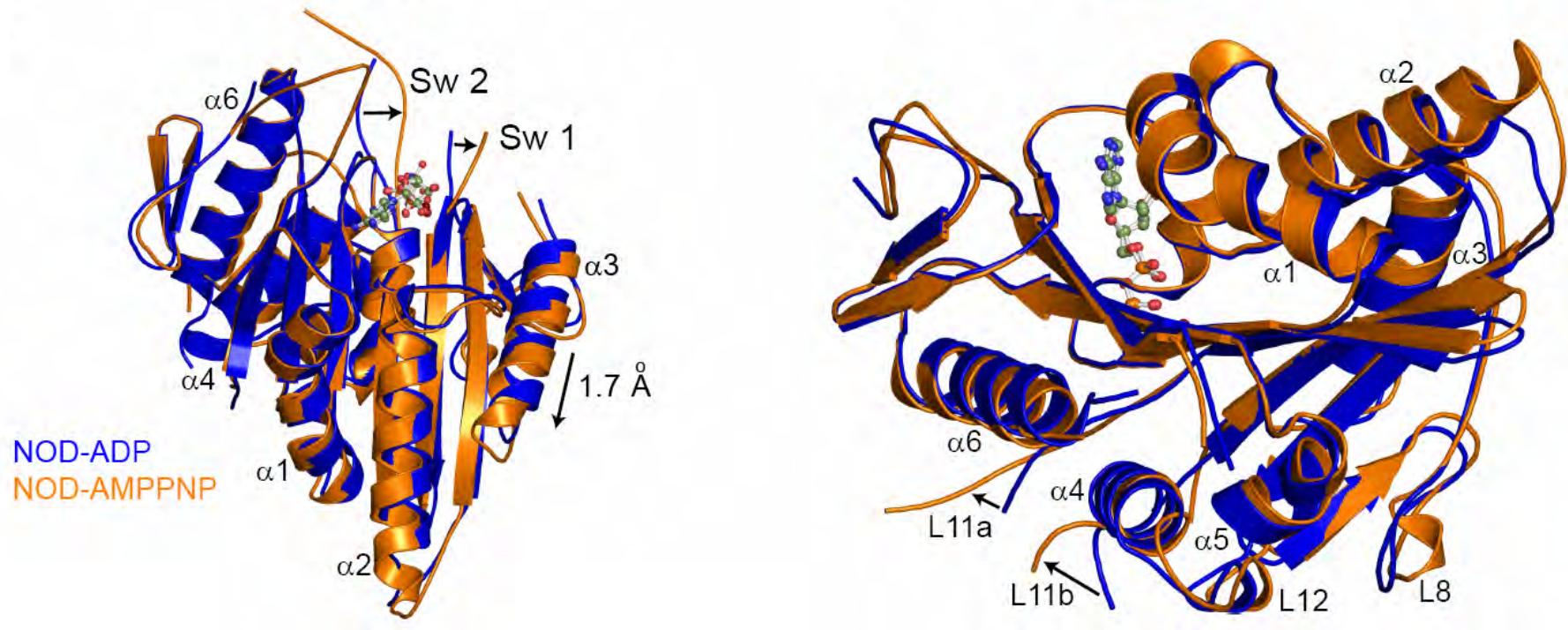
NOD motor domain



ADP (1.9 Å)

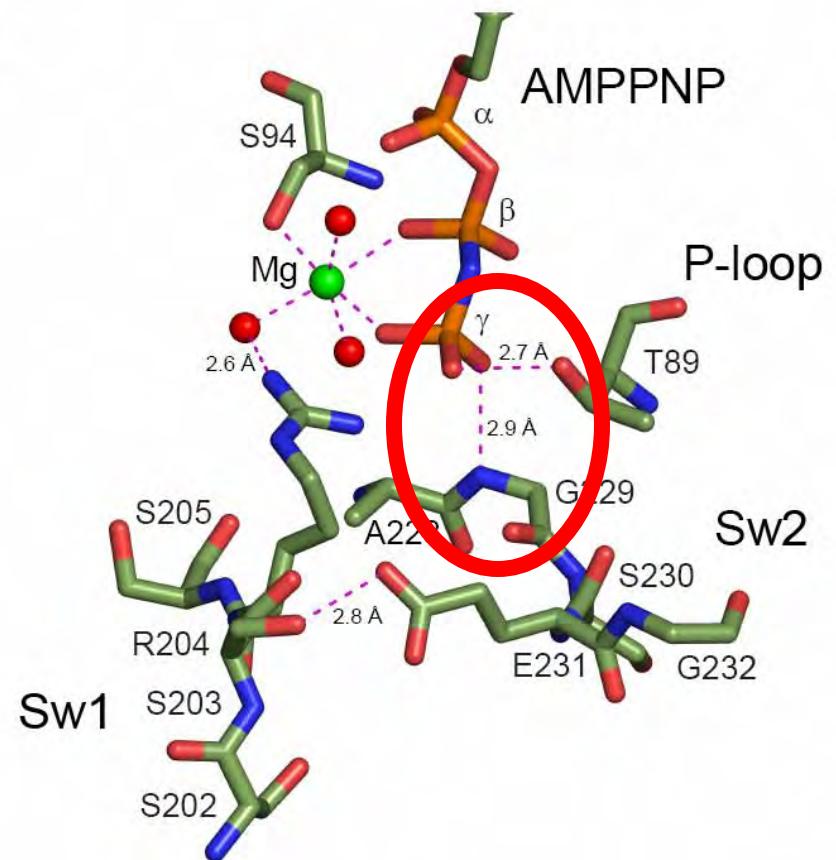
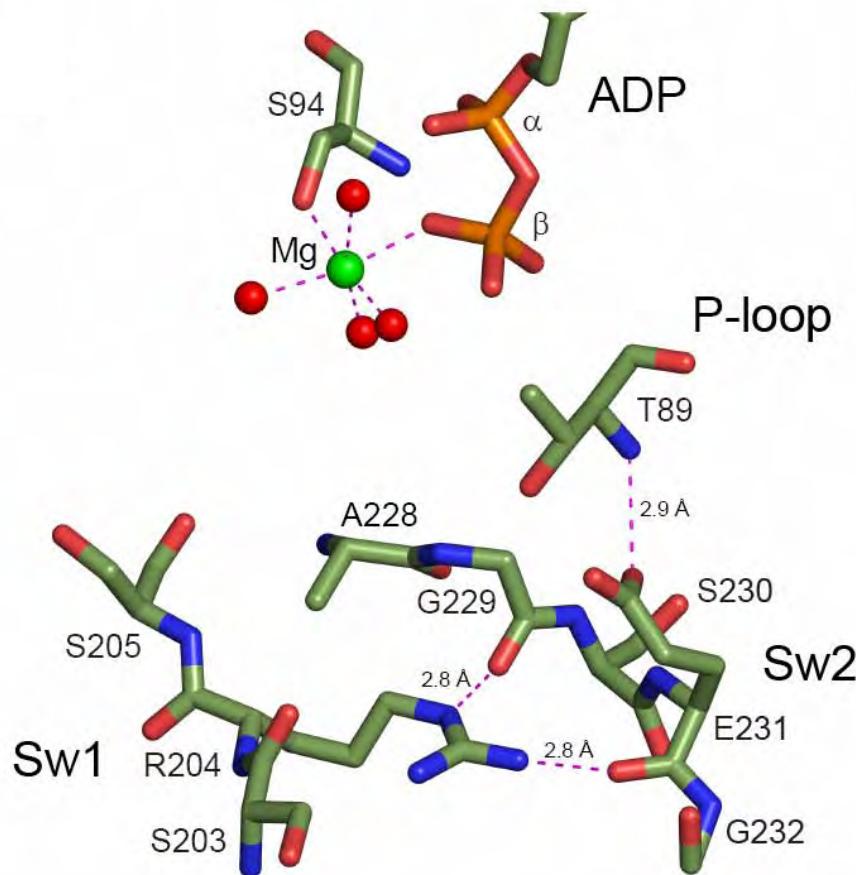


AMP-PNP (2.5 Å)



No big differences or surprises...

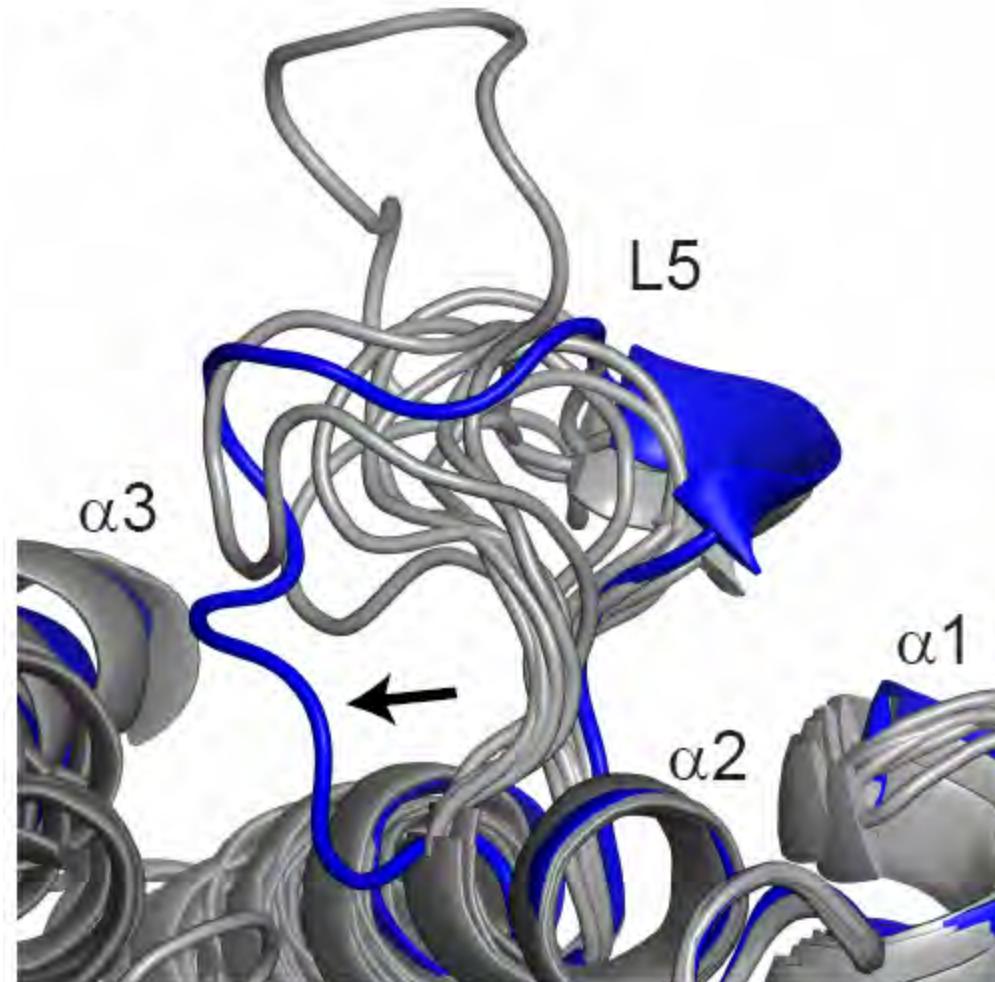
Except...



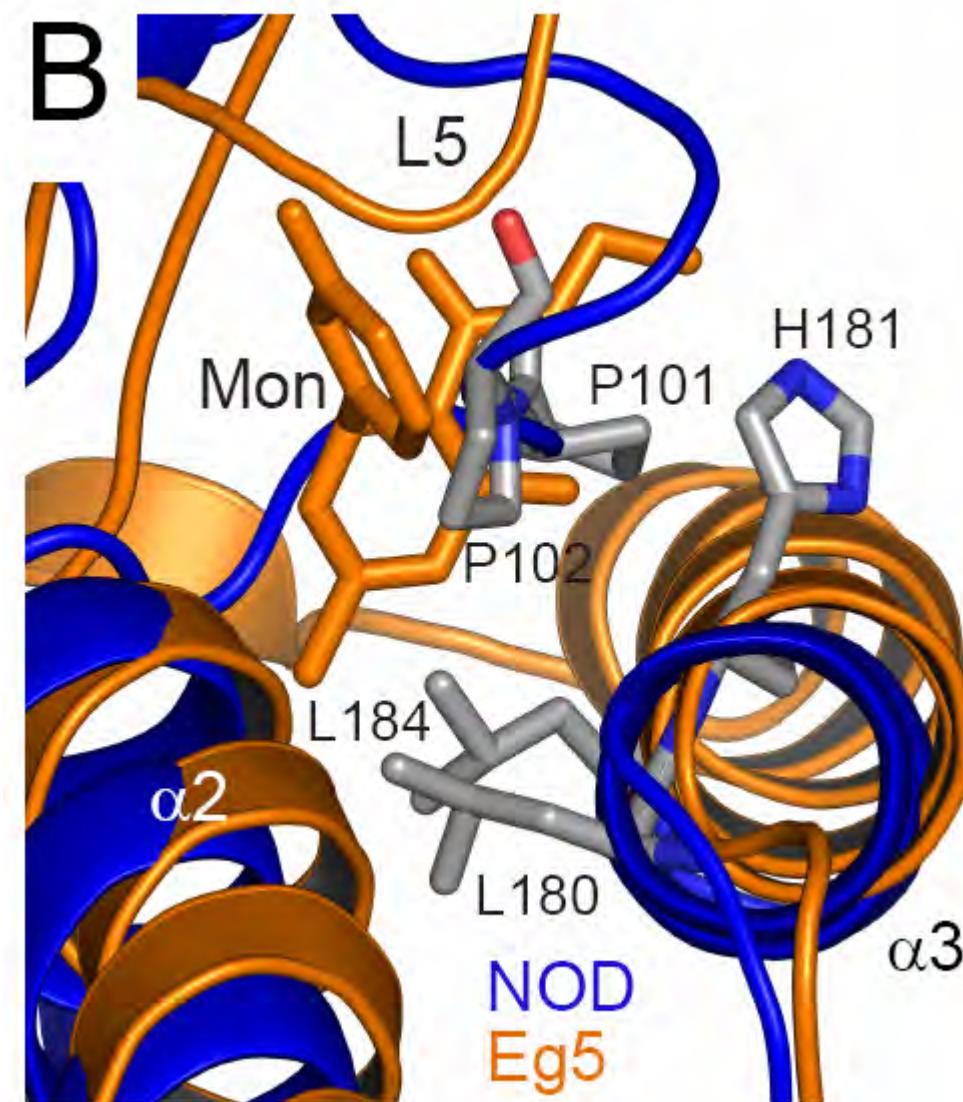
Fully closed Switch 2

Catalytically competent off of MTs?

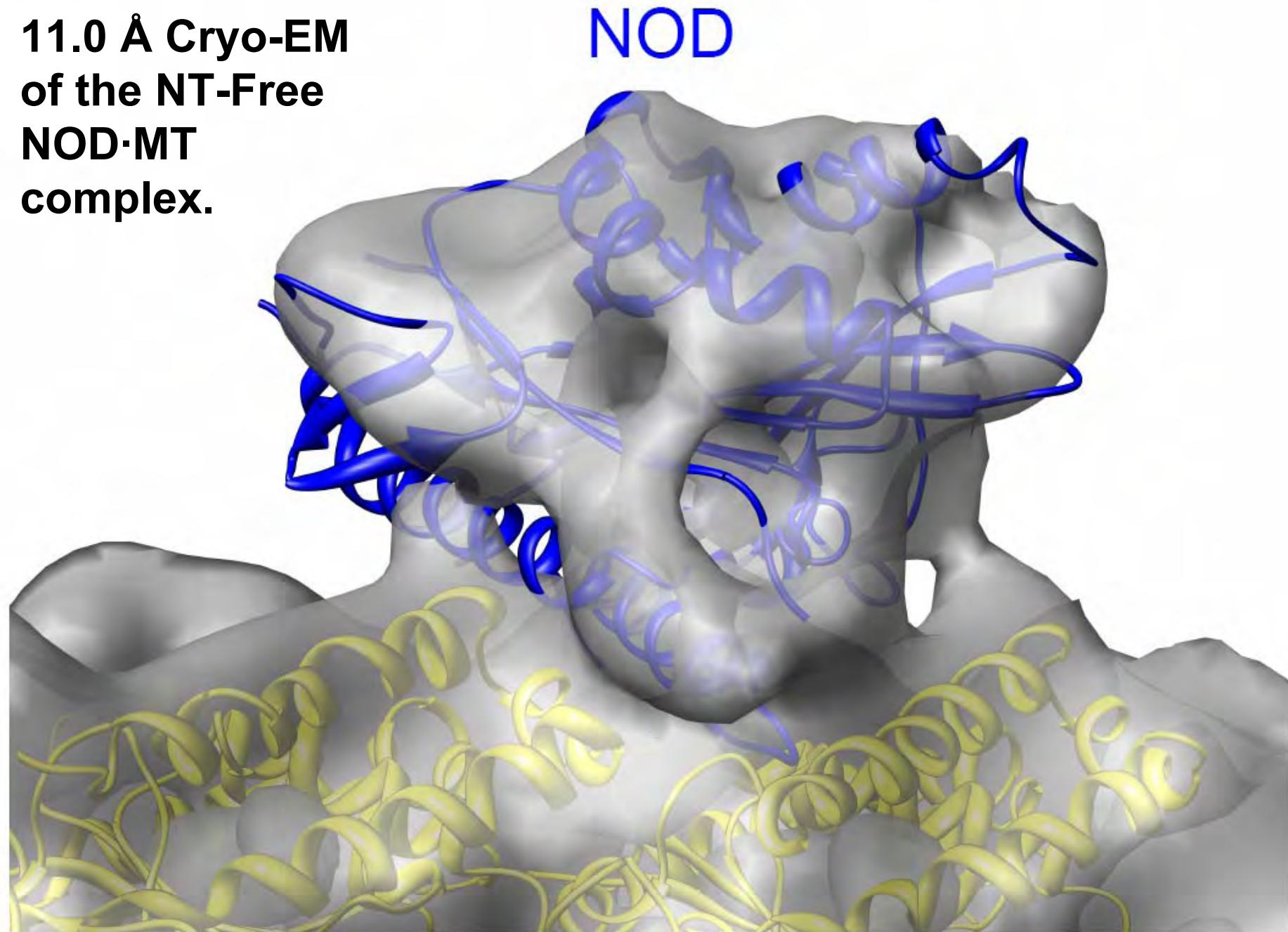
What is the role of Loop 5 in kinesins?



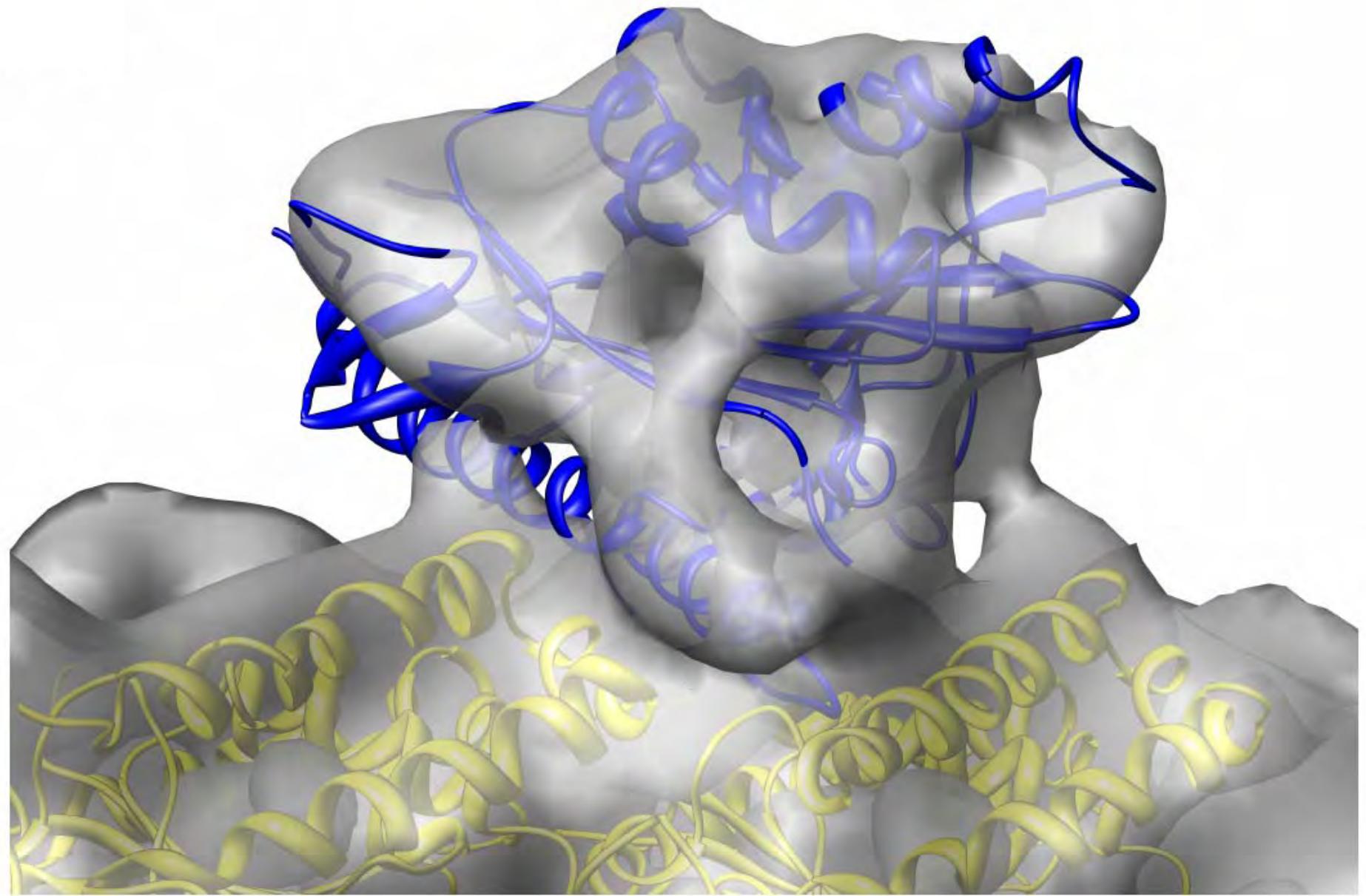
**Monastrol, a potent inhibitor of the Kinesin-5,
Eg5, binds near this region...**



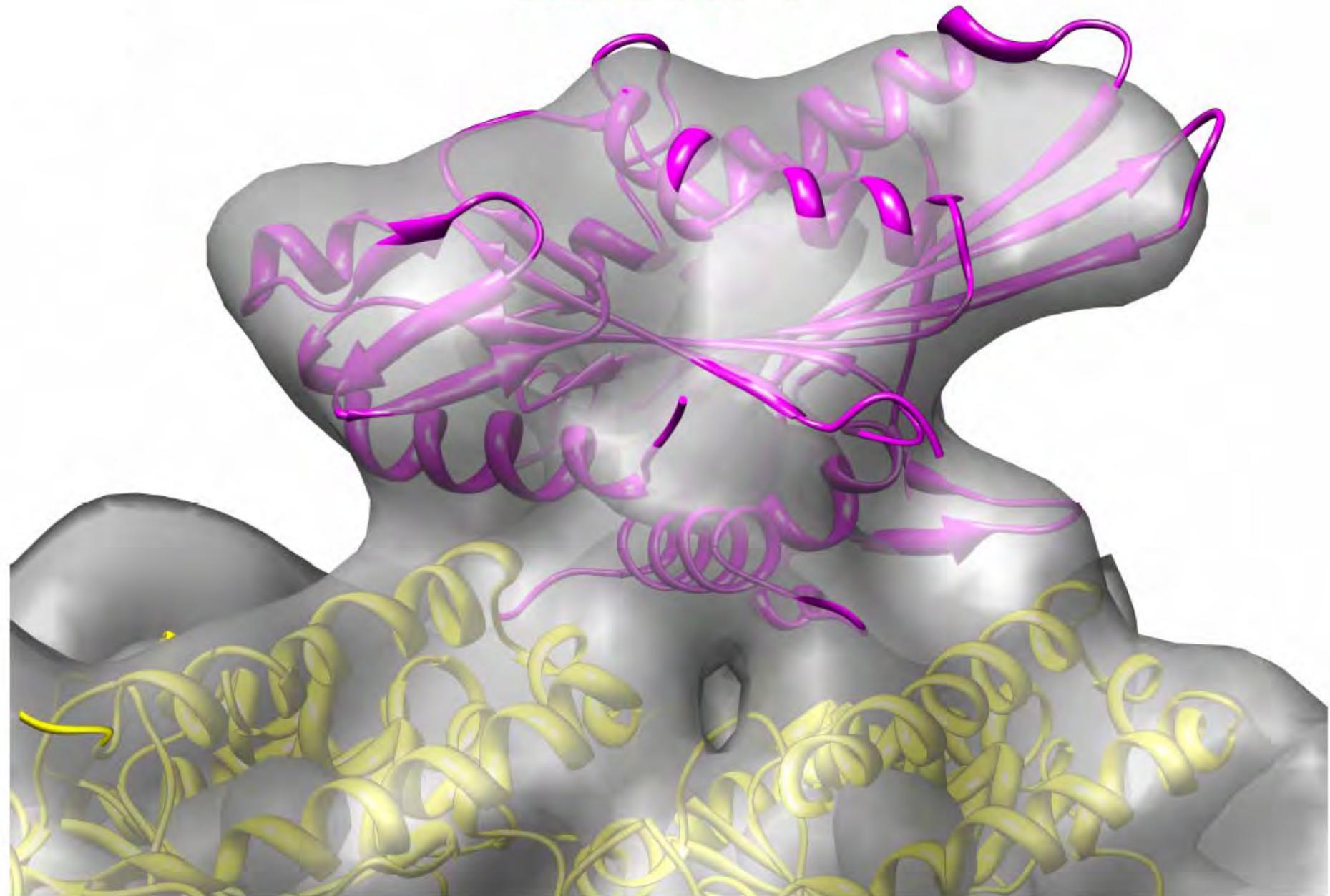
**11.0 Å Cryo-EM
of the NT-Free
NOD-MT
complex.**

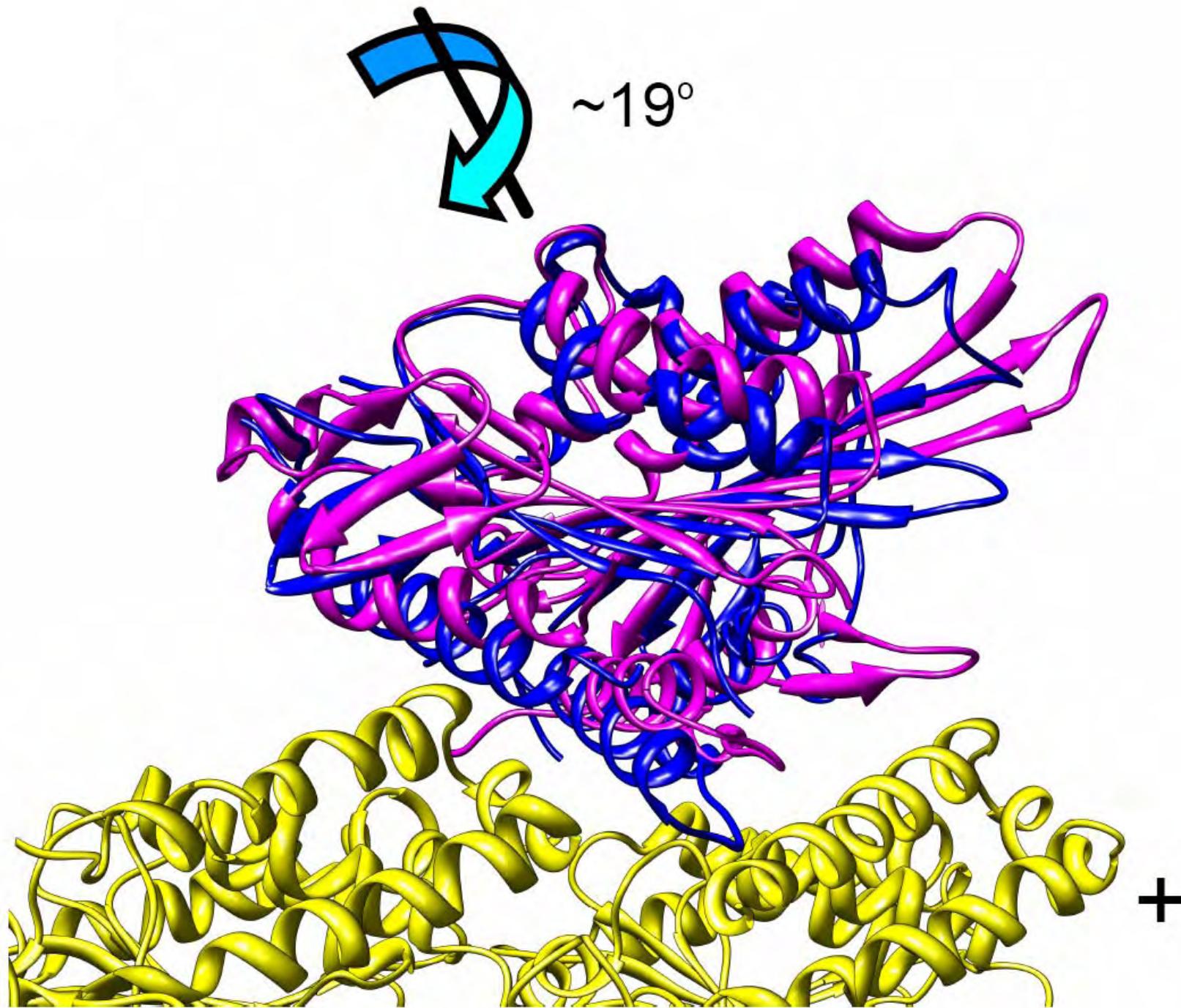


NOD

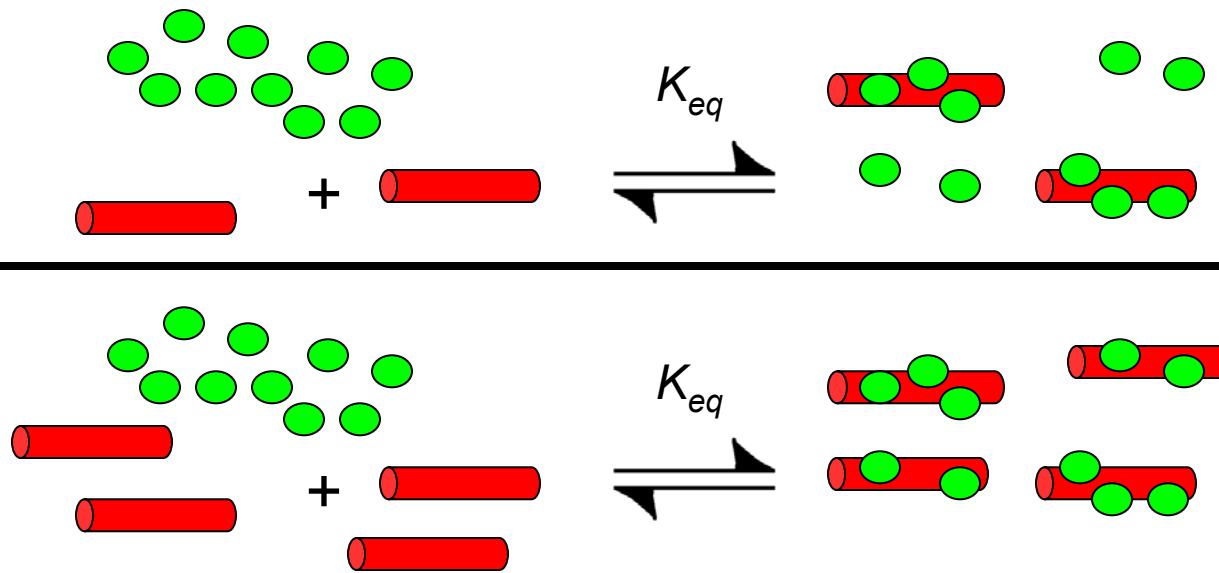


Kinesin-1



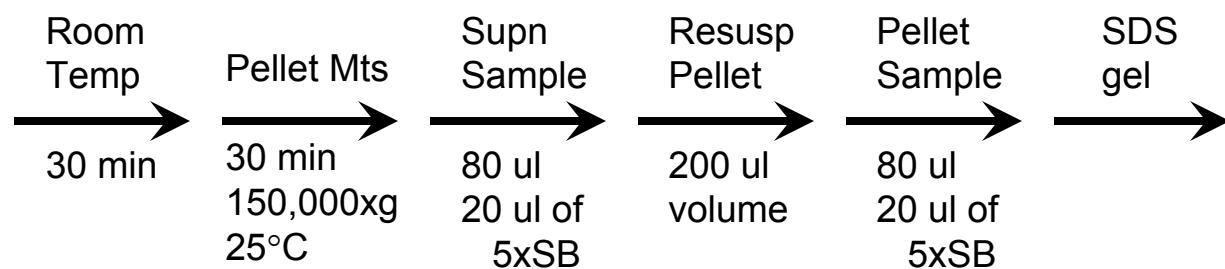


MT Equilibrium Binding Expts (MT-Nod Cosedimentation Expts)



Expt Conditions:

2 μ M Nod
0-50 μ M Mts
 \pm 1 mM MgAXP
(ATP, ADP, ANP)
 \pm 1 U Alk. Phosph.
200 ul total volume

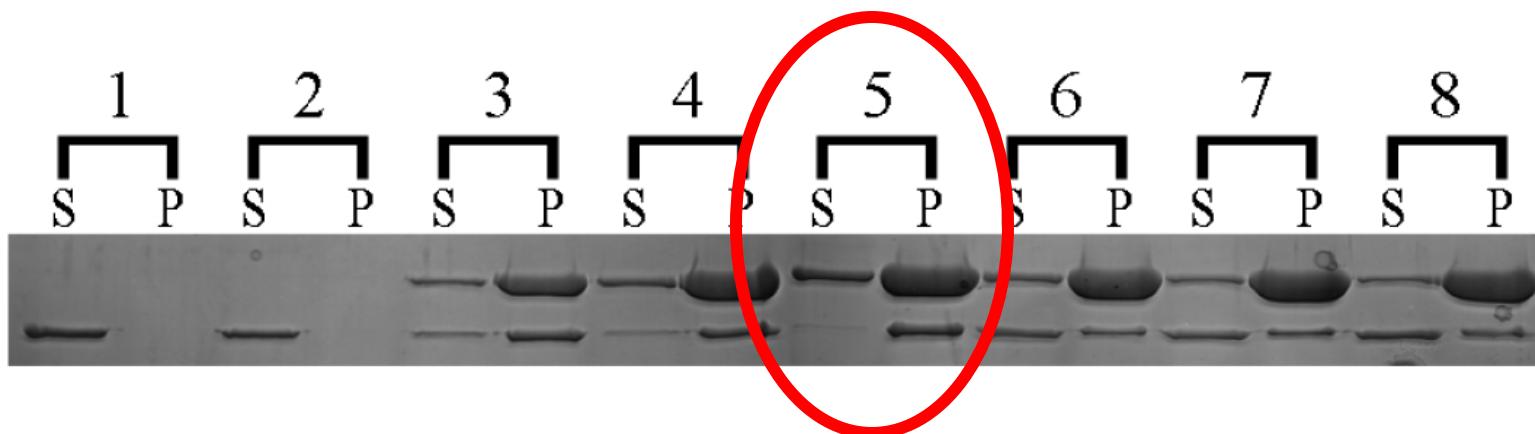


MT Equilibrium Binding Expts (MT-Nod Cosedimentation Expts)

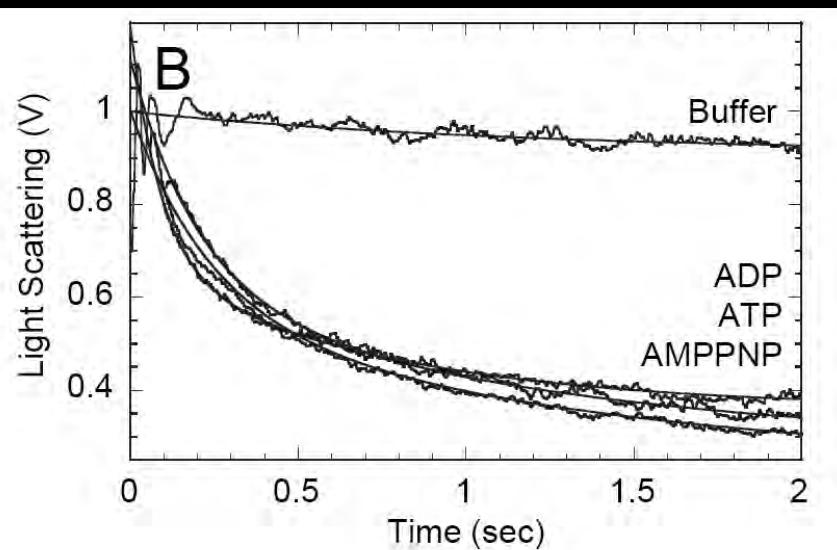
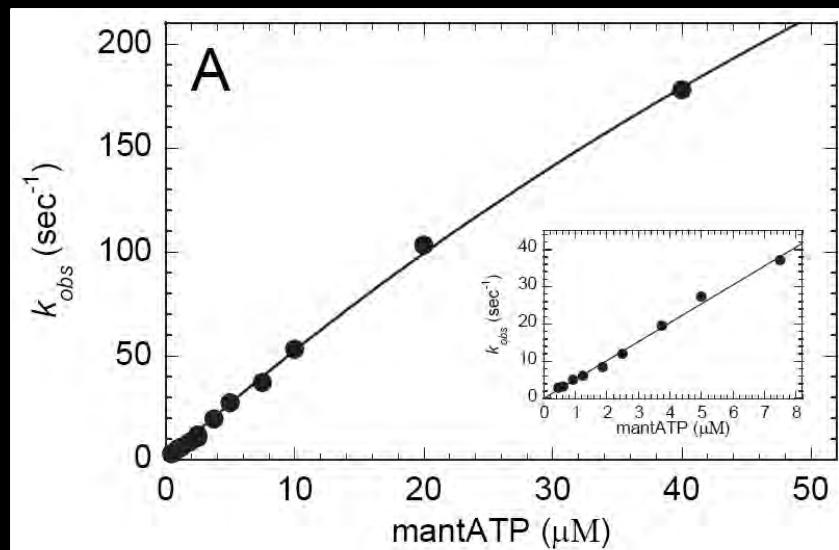
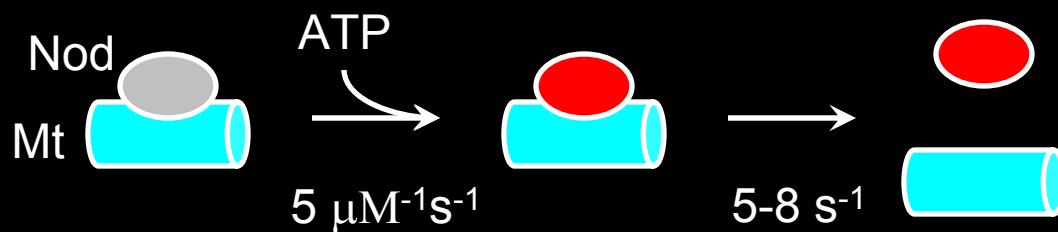
A

Rxn Conditions

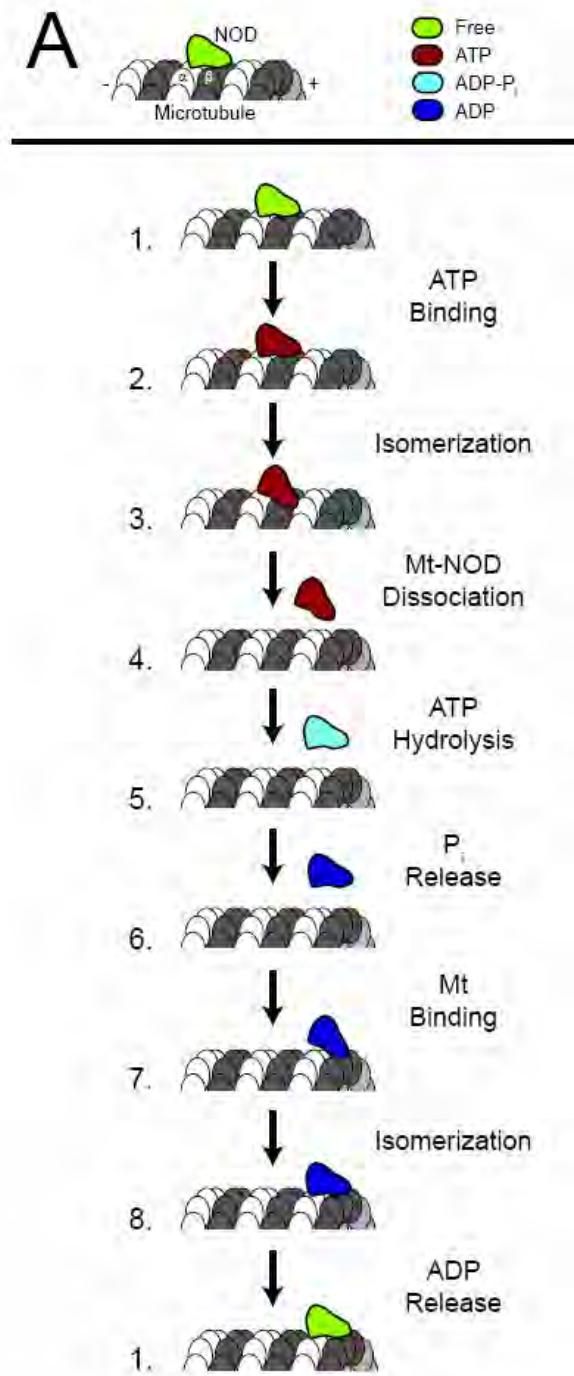
- | Rxn | Conditions |
|-----|--|
| 1 | 2 μ M Nod |
| 2 | 2 μ M Nod + 1 U Alk. Phos. |
| 3 | 2 μ M Nod + 2 μ M Mts |
| 4 | 2 μ M Nod + 4 μ M Mts |
| 5 | 2 μ M Nod + 4 μ M Mts + 1 U Alk Phos. |
| 6 | 2 μ M Nod + 4 μ M Mts + 1 mM MgATP |
| 7 | 2 μ M Nod + 4 μ M Mts + 1 mM MgAMPPNP. |
| 8 | 2 μ M Nod + 4 μ M Mts + 1 mM MgADP |



Rapid ATP Binding Promotes Nod Detachment from Mt



Hydrolysis occurs OFF of filament



Summary of sedimentation and kinetics: a model for the NOD ATPase cycle

NOD binds ATP tightly

Dissociates from the MT

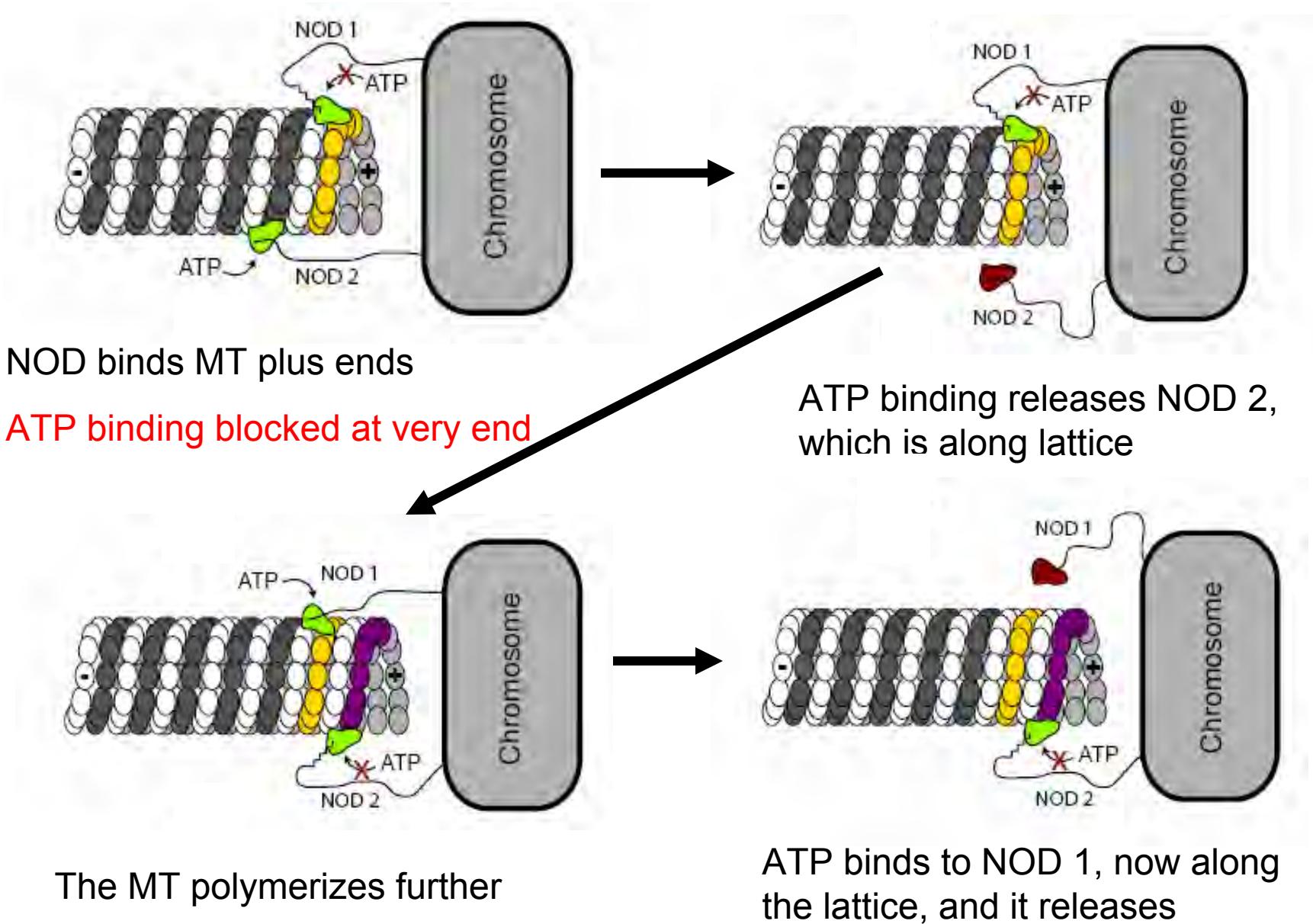
Slowly hydrolyzes ATP

Rapid phosphate release

Rebinds MT

Isomerization and ADP release

How can this move chromosomes?

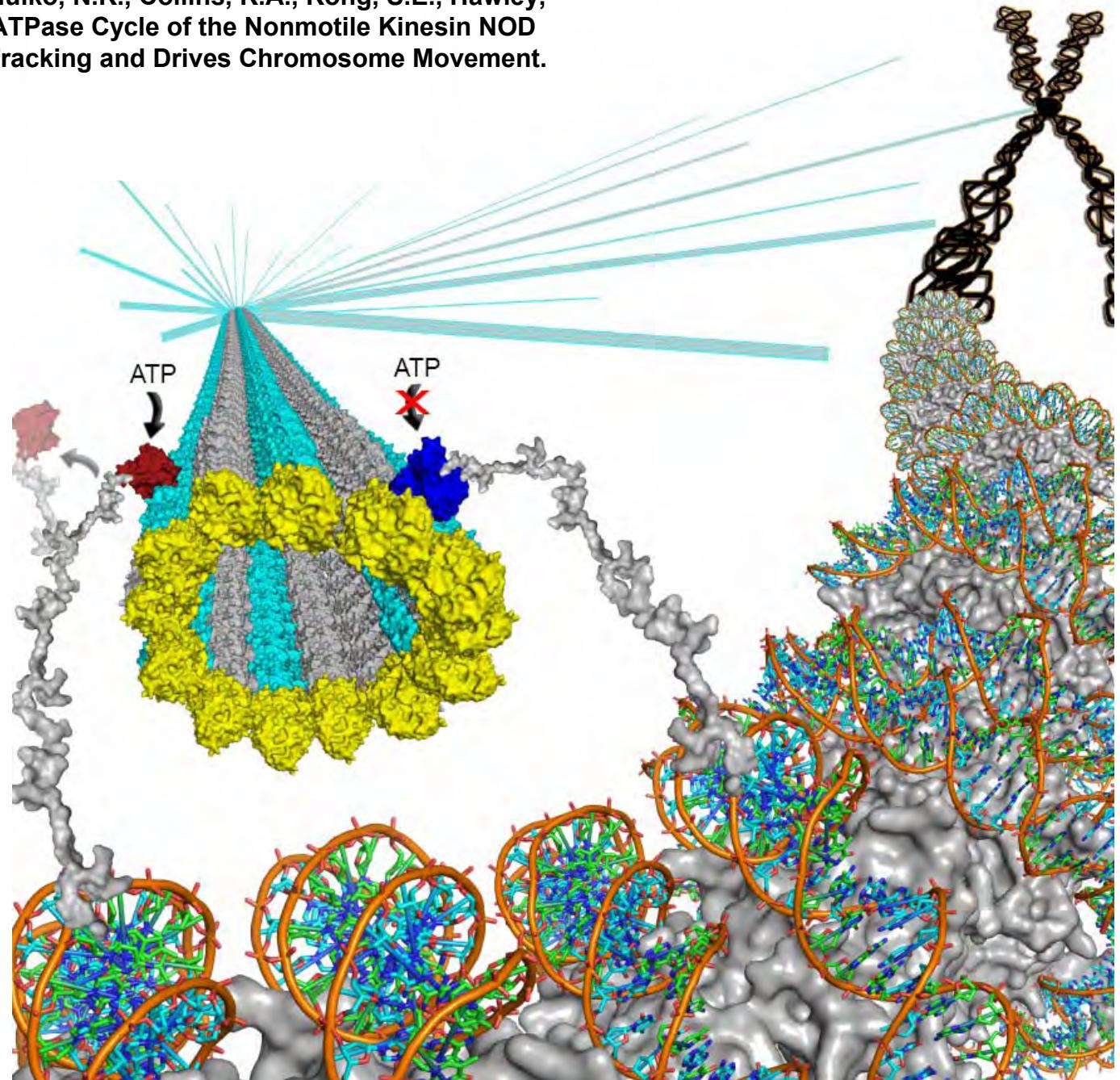


Cochran, J.C., Sindelar, C.V., Mulko, N.K., Collins, K.A., Kong, S.E., Hawley, R.S., and Kull, F.J. (2009) The ATPase Cycle of the Nonmotile Kinesin NOD Allows Microtubule Plus-End Tracking and Drives Chromosome Movement. *Cell*. Jan 9;136(1):110-22.

Dartmouth College
Jared Cochran,
Postdoctoral fellow
Natasha K. Mulko '07

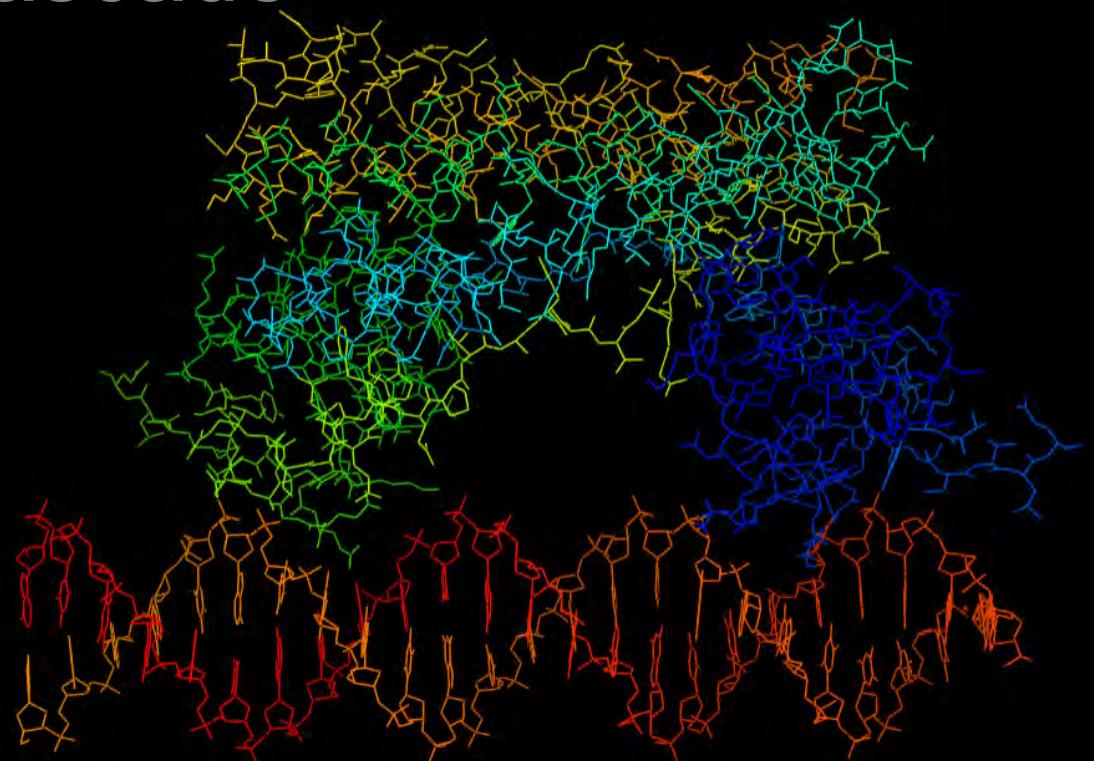
Stowers Institute for
Medical Research
R. Scott Hawley
Kimberly A. Collins
Stephanie E. Kong

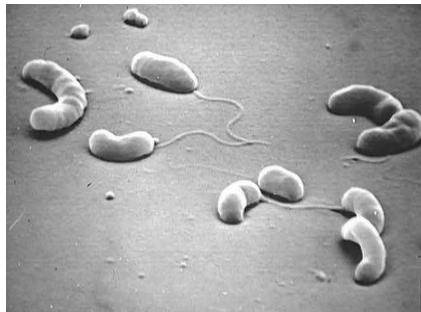
Lawrence Berkeley
National Lab
Charles V. Sindelar



Structural studies towards understanding the interactions of the *Vibrio* *cholerae* virulence gene activation cascade

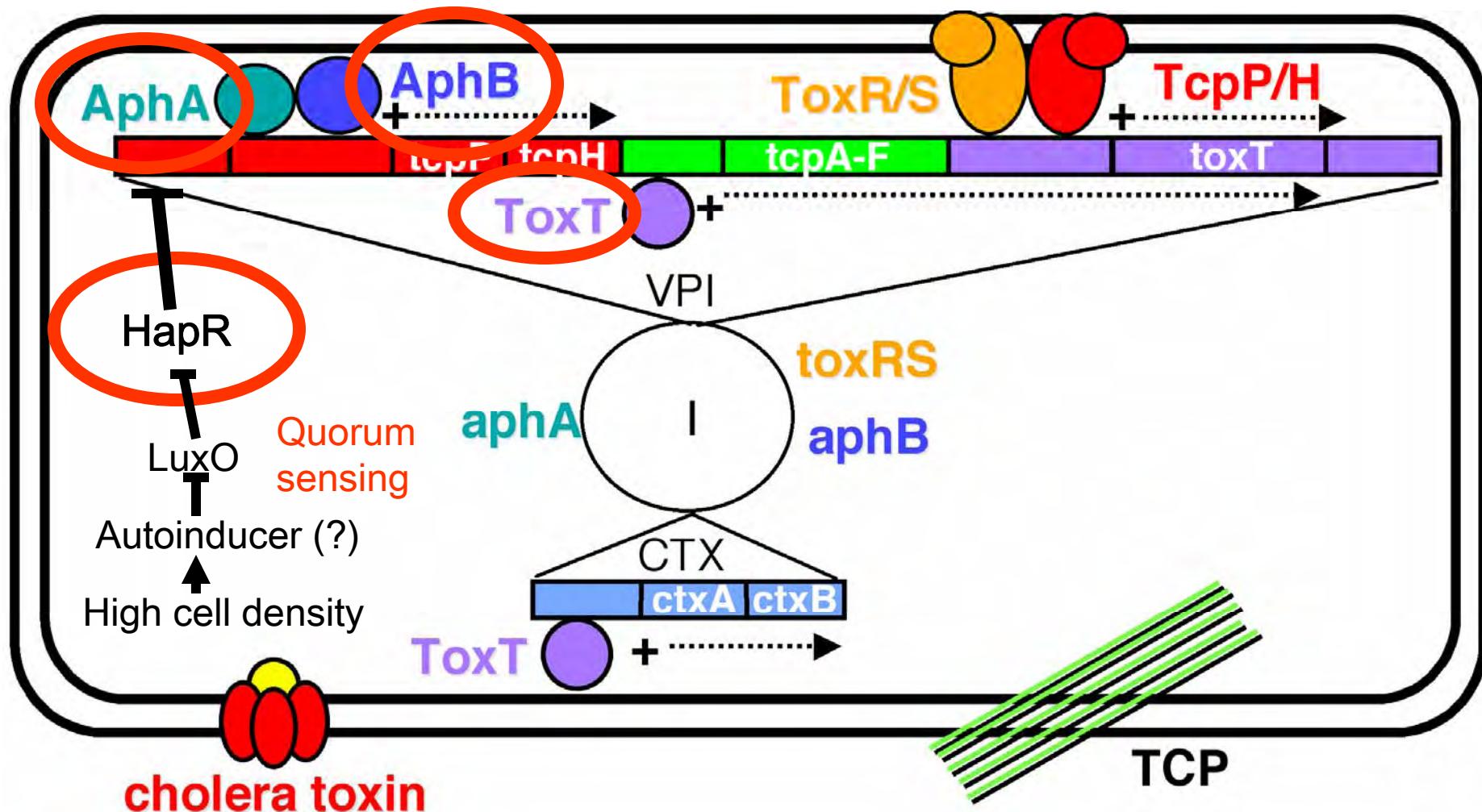
Jon Kull
Rukman De Silva
Karen Skorupski
Ron Taylor





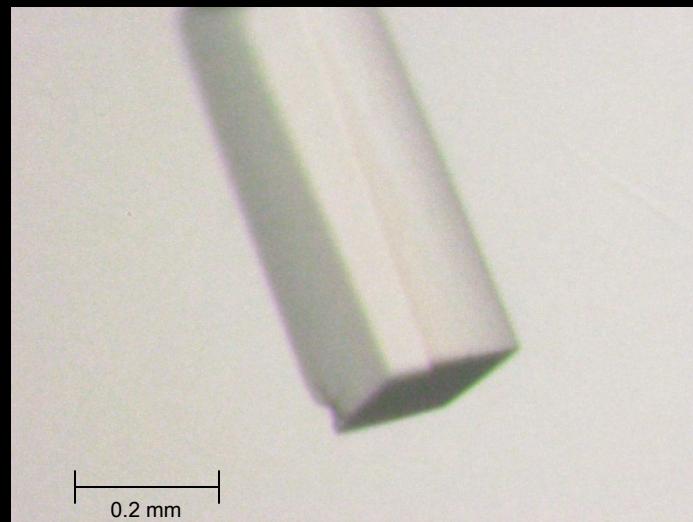
Pathogenic strains of *V. cholerae* require:

- Vibrio pathogenicity island (VPI)
- Lysogenic CTX phage



AphA crystallization

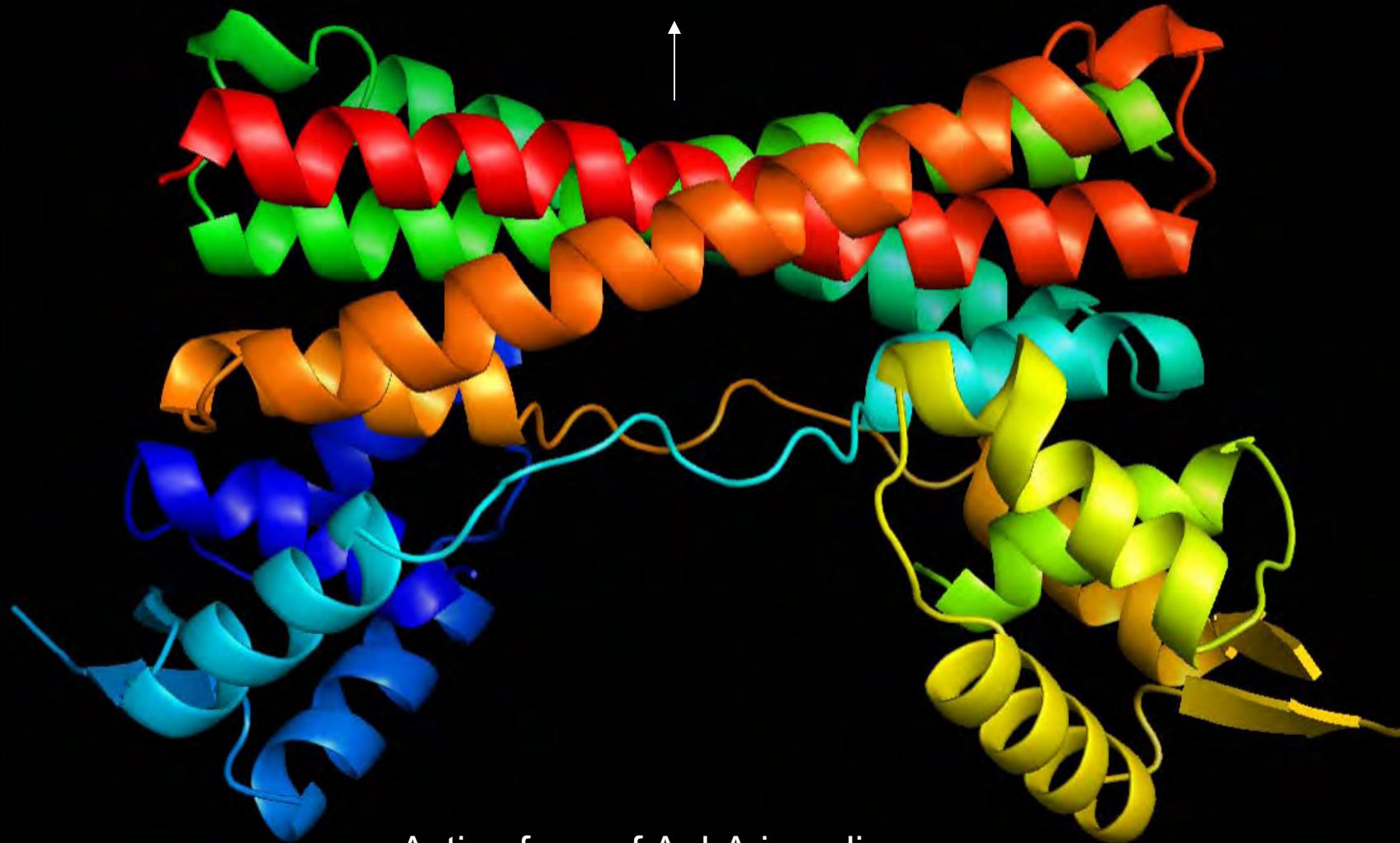
- Native AphA protein crystals were obtained at room temperature
- AphA crystals was soaked in CH_3HgCl to obtain derivatives for SIRAS (single isomorphous replacement anomalous scattering) phasing



Statistics

Crystal	Native (in-house)	Anomalous Hg derivative	Native (NSLS X6A)
Space group	C222 ₁	C222 ₁	C222 ₁
Unit cell dimensions	a=76.9 b=94.3 c=59.2 $\alpha=\beta=\gamma=90^\circ$	a=77.0 b=93.3 c=59.7 $\alpha=\beta=\gamma=90^\circ$	a=77.5 b=94.6 c=59.6 $\alpha=\beta=\gamma=90^\circ$
Resolution range (highest resolution shell)	20-3.0 (4.0-3.0)	20-2.6 (2.8-2.6)	20-2.2 (2.4-2.2)
Measured reflections	48,899 (20,241)	48,182 (9,013)	80,059 (17,400)
Unique reflections	4,543 (2,574)	12,614 (2,406)	11,425 (2,546)
Completeness [%]	99.8 (100.0)	98.5 (95.0)	99.6 (99.2)
R _{sym} ^a [%]	11.0 (13.0)	8.1 (26.1)	6.3 (30.0)
Phasing			
Resolution Range [Å]	15.0-3.0		
Phasing power isomorphous/anomalous	1.2/1.2		
Overall figure of merit	0.45		

Overall AphA structure



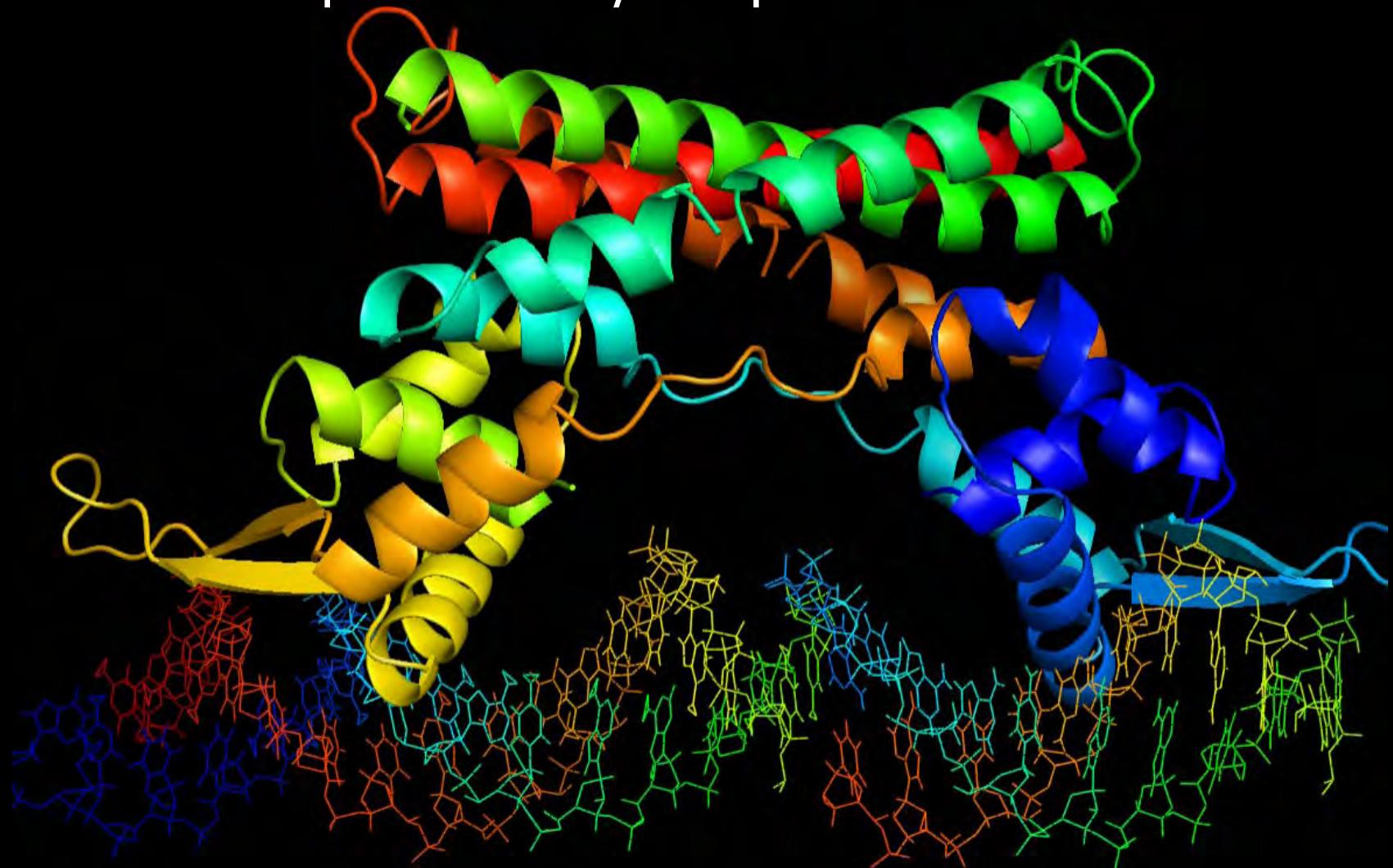
Interactions of AphA-*tcpPH* promoter

- AphA recognized its binding site in the *tcpPH* promoter with partial dyad symmetry (between -101 and -71)
- AphA facilitates the binding of AphB to *tcpPH* promoter



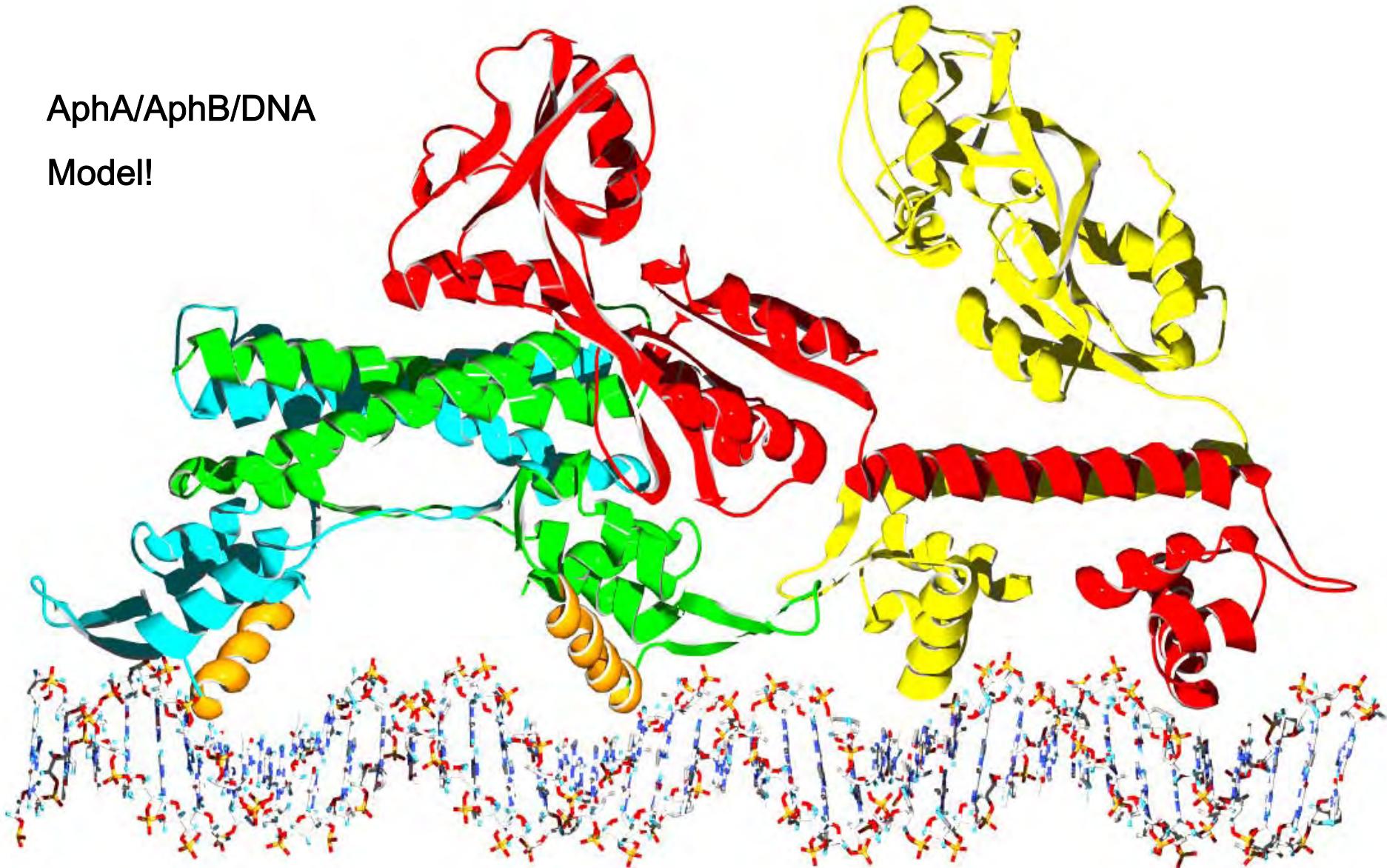
Kovacikova & Skorupski ,Mol Microbiol 44(2), 533-547 (2002).

Model of AphA and *tcpPH* promoter interaction



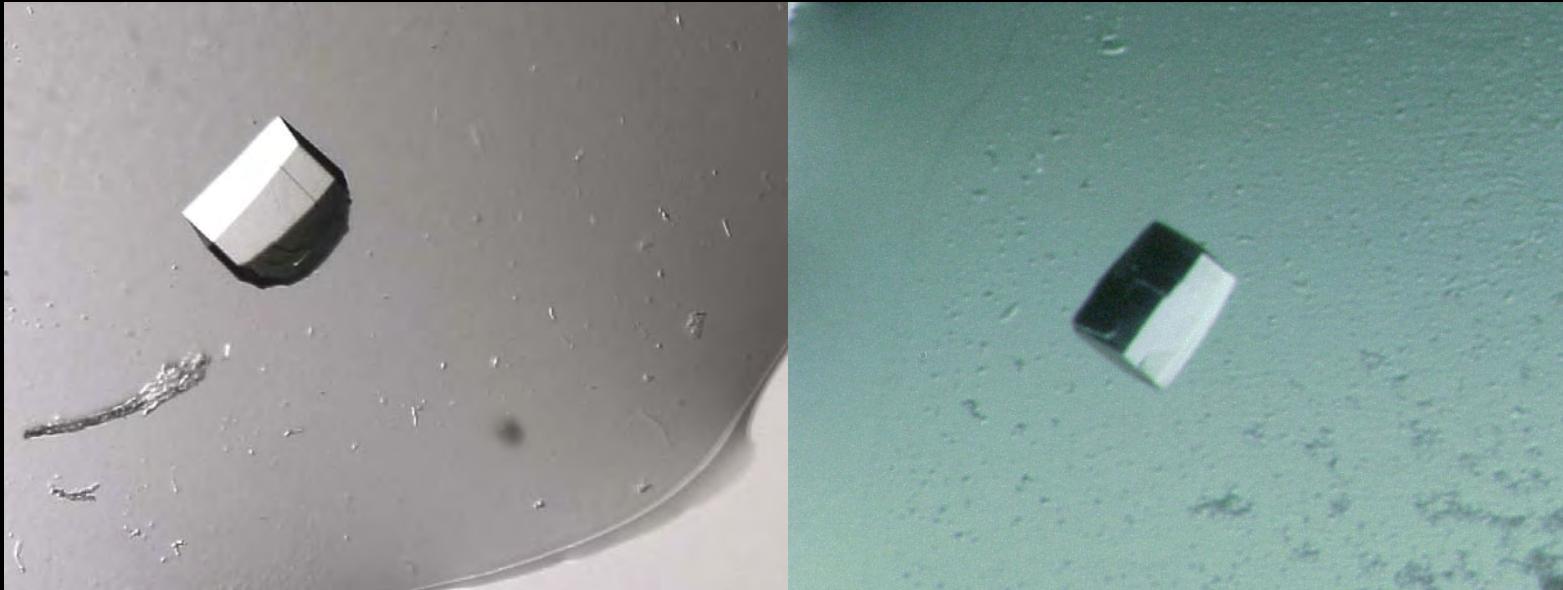
AphA subunits are too far apart ($\sim 6\text{-}8 \text{ \AA}$) to fit symmetrically into the DNA binding site

AphA/AphB/DNA
Model!



De Silva, R.S., Kovacikova, G., Lin, W., Taylor, R.K., Skorupski, K., and Kull, F.J. (2005) Crystal Structure of the Virulence Gene Activator AphA from *Vibrio cholerae* Reveals It Is a Novel Member of the Winged Helix Transcription Factor Superfamily. *J. Biol. Chem.*, 280 (14):13779-13783.

HapR Crystallization

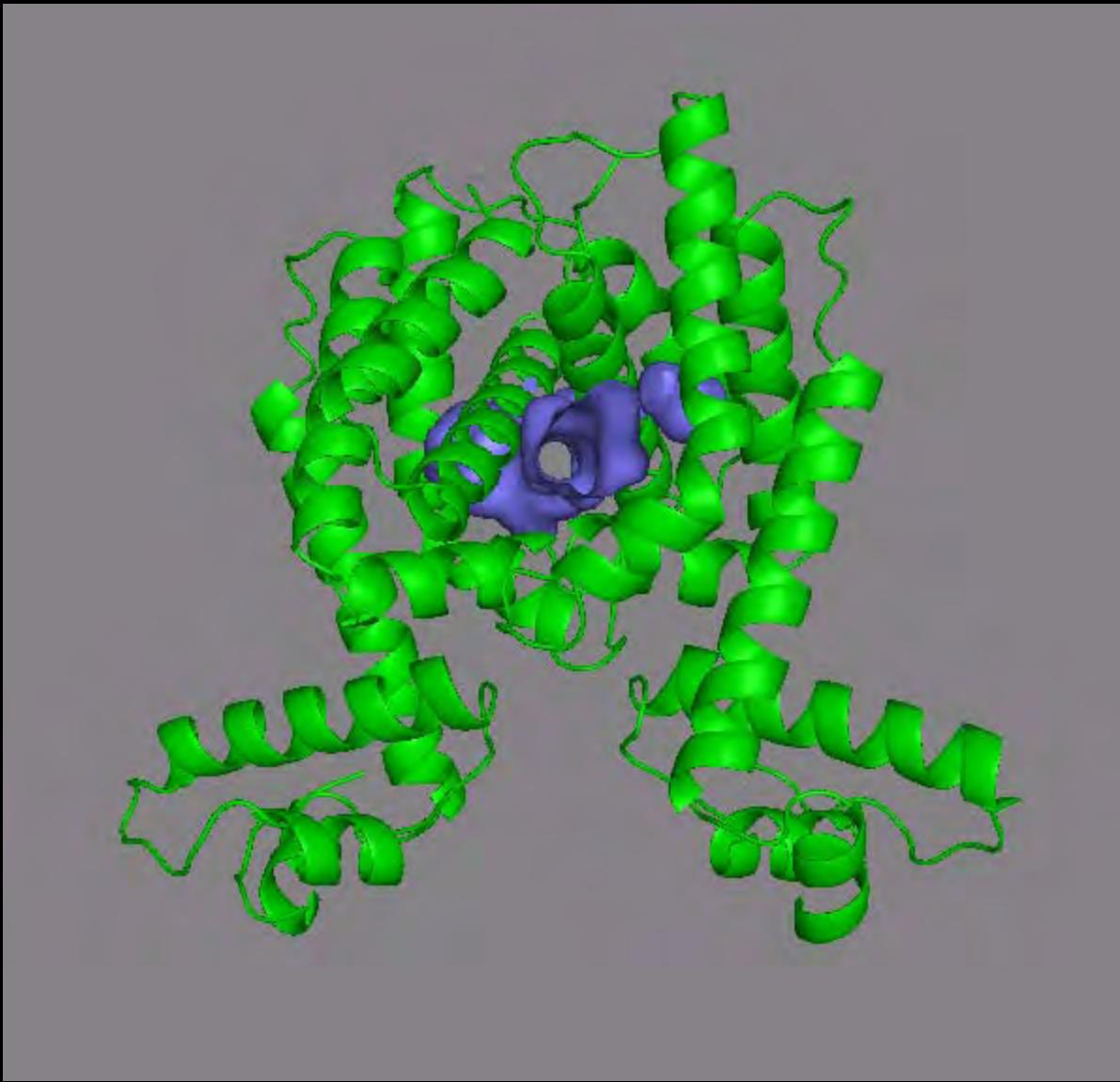


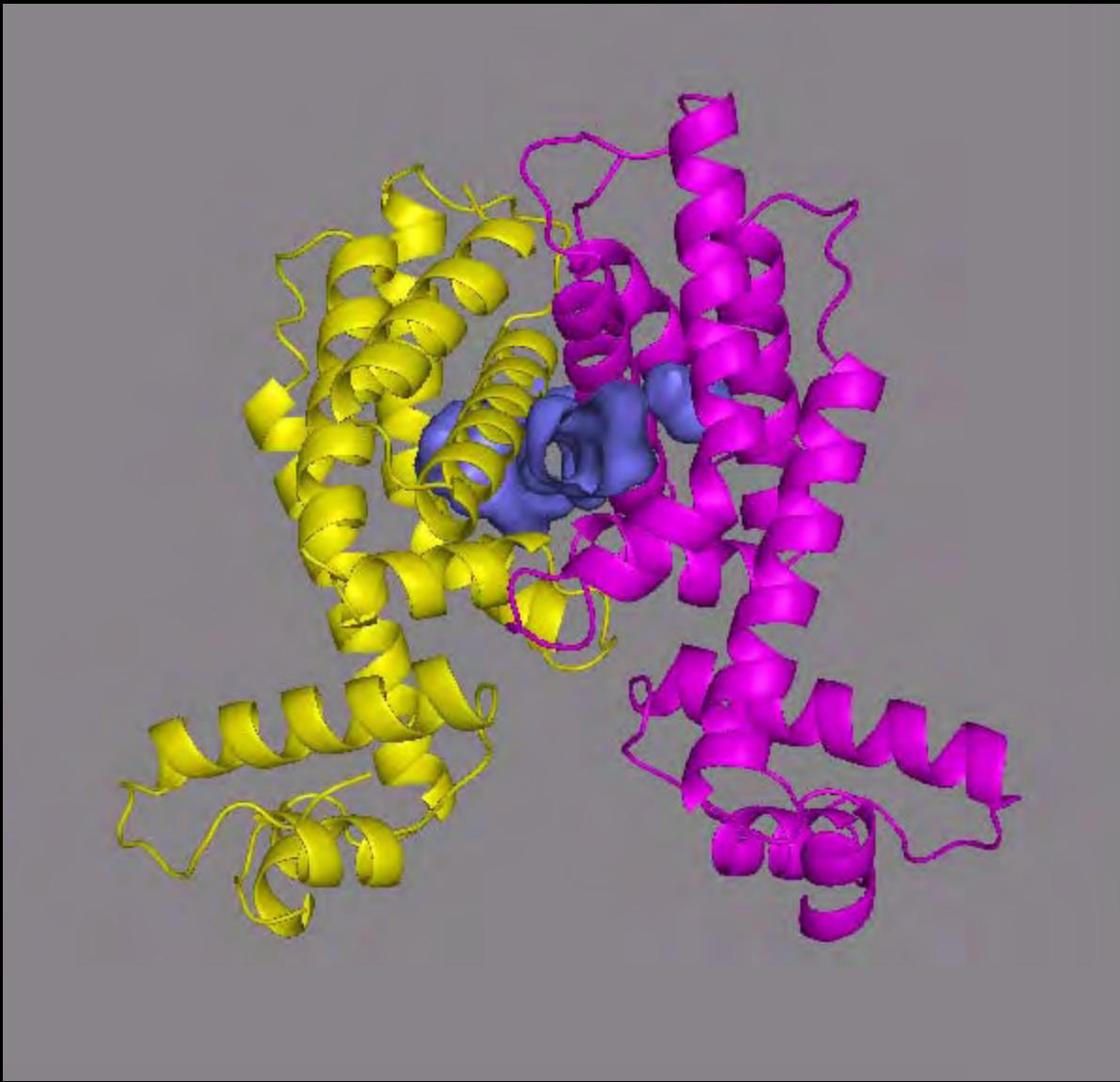
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION									
RESOLUTION LIMIT	NUMBER OF OBSERVED REFLECTIONS	NUMBER OF UNIQUE REFLECTIONS	NUMBER OF POSSIBLE REFLECTIONS	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED I/SIGMA	R-meas	Rmrgd-F
8.00	1468	725	897	80.8%	3.3%	3.2%	1356	24.64	4.4%
6.00	2498	1211	1230	98.5%	2.9%	3.2%	2322	24.76	3.9%
5.00	3137	1529	1544	99.0%	3.1%	3.2%	2891	24.36	4.2%
4.00	7116	3470	3505	99.0%	2.8%	3.2%	6525	23.78	3.8%
3.50	7021	3450	3516	98.1%	3.6%	3.5%	6398	20.97	4.9%
3.00	12762	6206	6314	98.3%	5.0%	5.0%	11713	14.86	6.7%
2.60	18441	9016	9121	98.8%	9.7%	9.2%	16862	8.76	13.1%
2.40	14227	6983	7058	98.9%	15.8%	15.5%	12986	5.79	21.3%
2.30	9178	4505	4555	98.9%	21.9%	21.5%	8384	4.29	29.3%
2.20	10507	5265	5356	98.3%	30.9%	29.8%	9451	3.17	41.7%
total	86355	42360	43096	98.3%	4.7%	4.8%	78888	11.51	6.3%
									10.4%

Data statistics for native crystals of HapR. The space group was identified as P2₁2₁2₁, with unit cell dimensions are a=43.786, b=85.085, c=110.385, $\alpha=\beta=\gamma=90^\circ$.

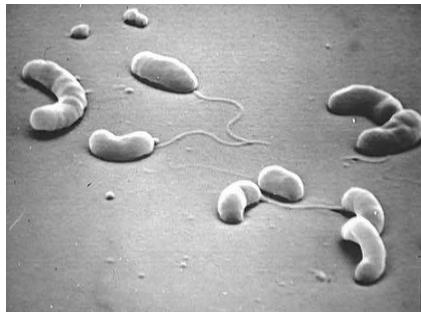
HapR structure solution

- MeHgCl₃ derivative
- MAD data collected at NSLS Beamline X6A
- Structure solved with RE/SOLVE
- Fold similar to that in: TetR, QacR, EthR, YcdC, YsiA, YfiR, CprB, YbiH, and YxaF



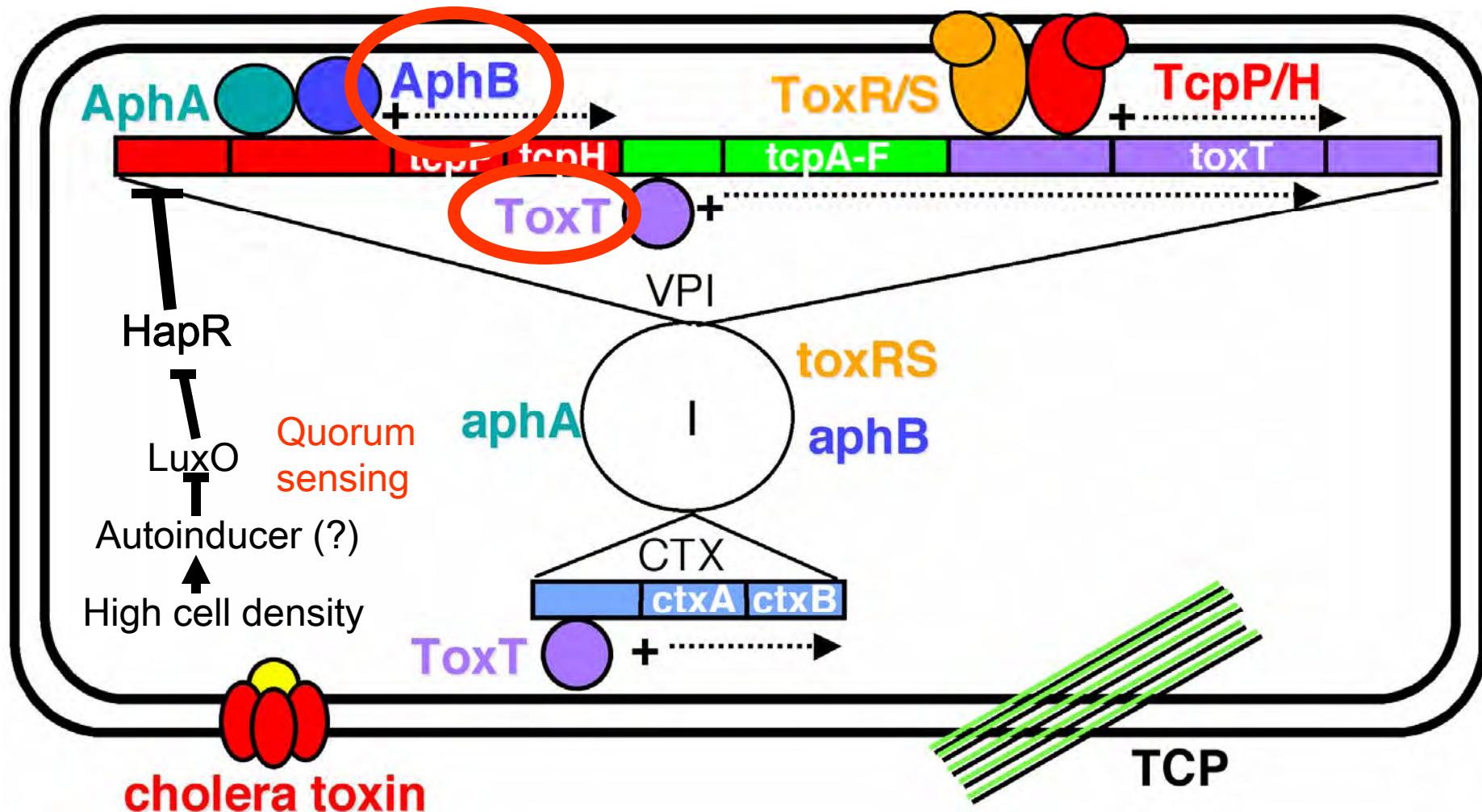


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Pathogenic strains of *V. cholerae* require:

- Vibrio pathogenicity island (VPI)
- Lysogenic CTX phage



So what is the missing link? X6A!



- Great staff – special thanks to Vivian Stojanoff and Jean Jakoncic
- Great beamline – data quality is super, equipment works, beam is up
- Easy scheduling!



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