分子モーターから観た生命科学

分子モーターはどのように動くか

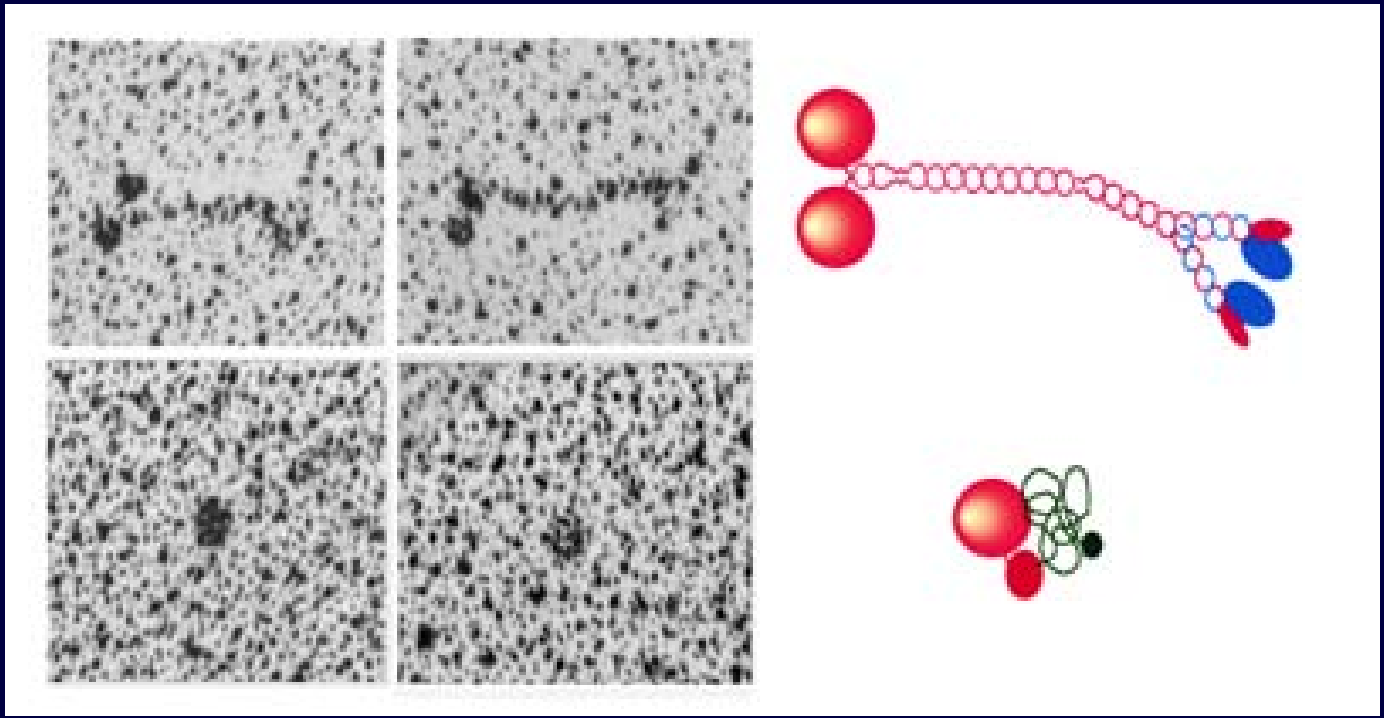
2006年11月27日

東京大学医学研究科　廣川信隆

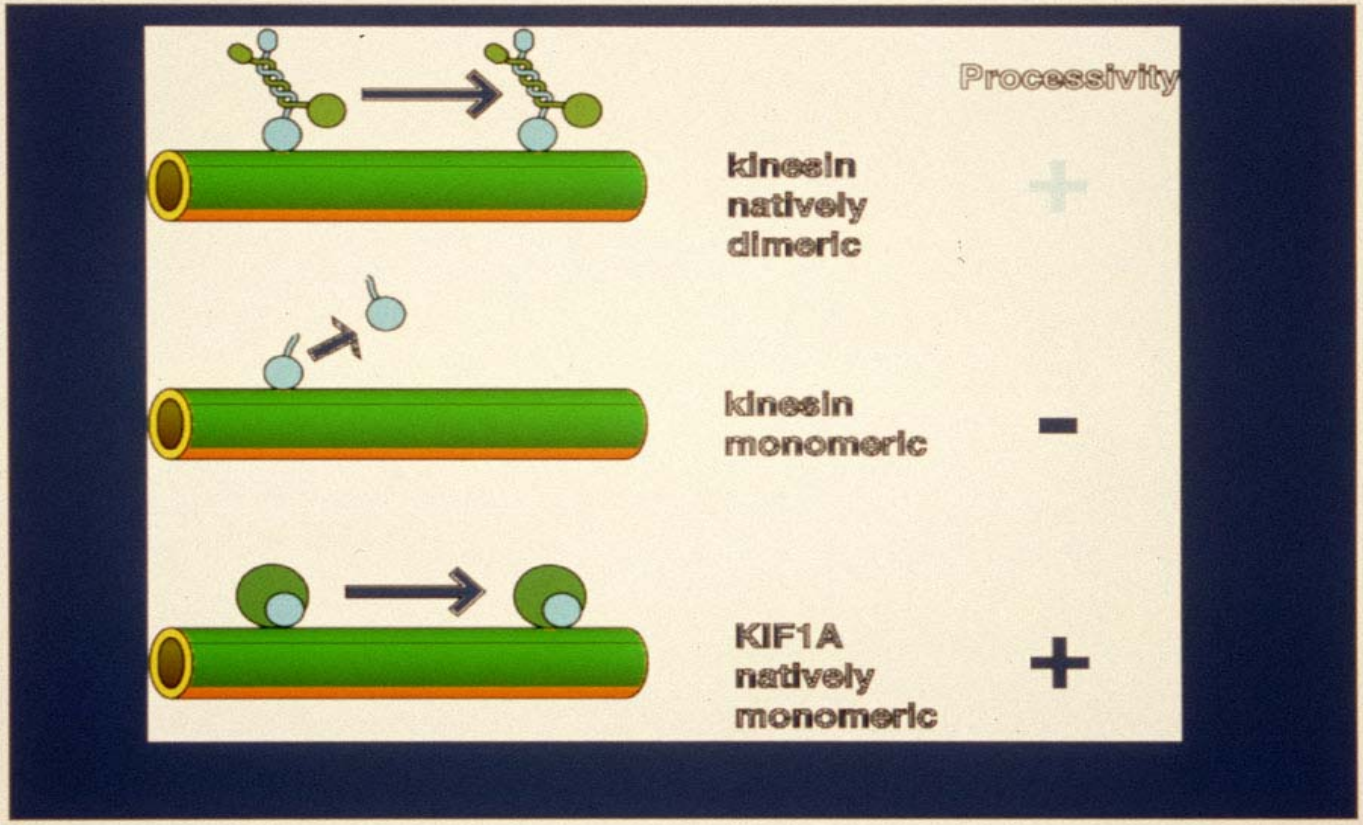
Low-angle, rotary shadowing electronmicrograph

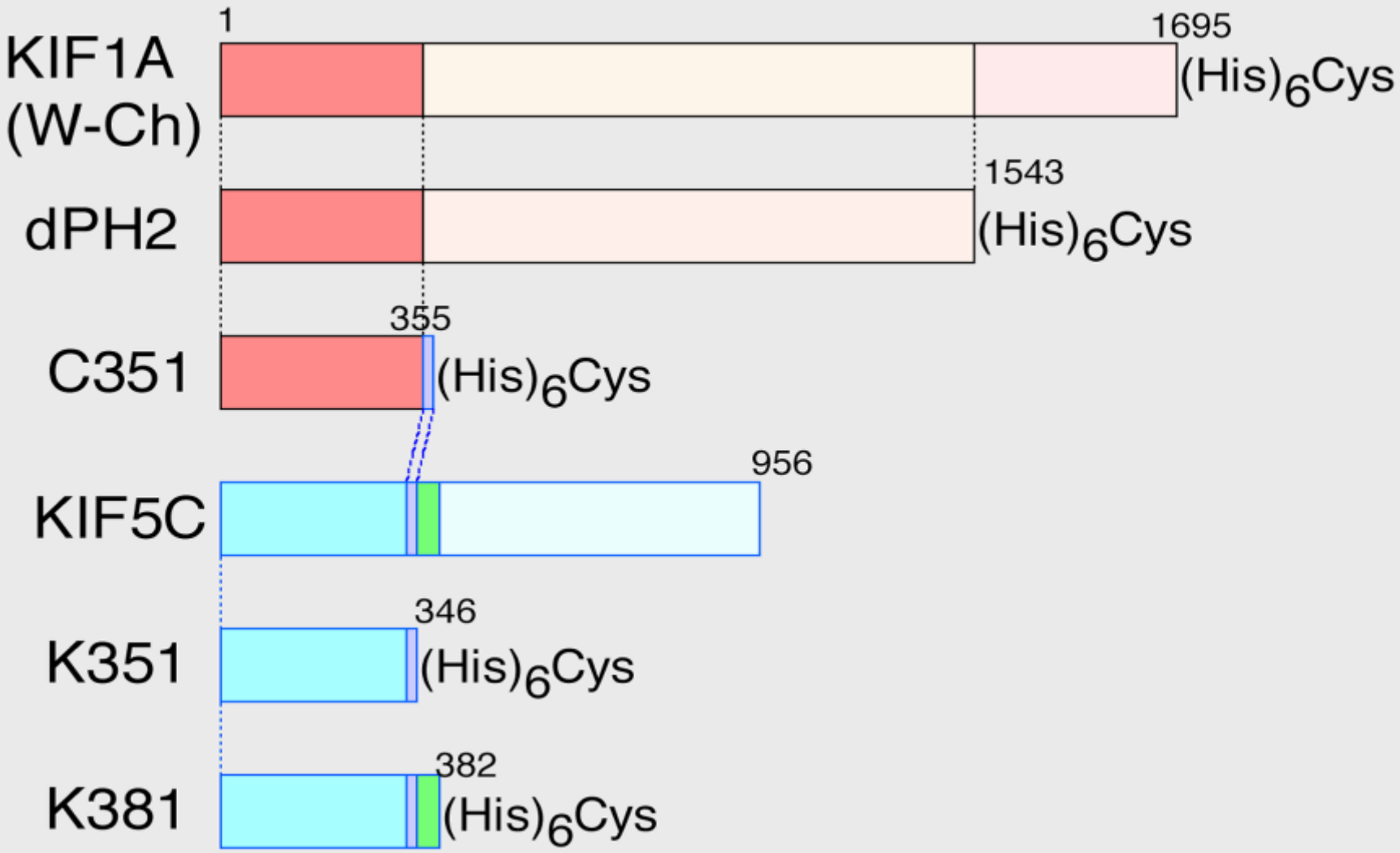
Kinesin

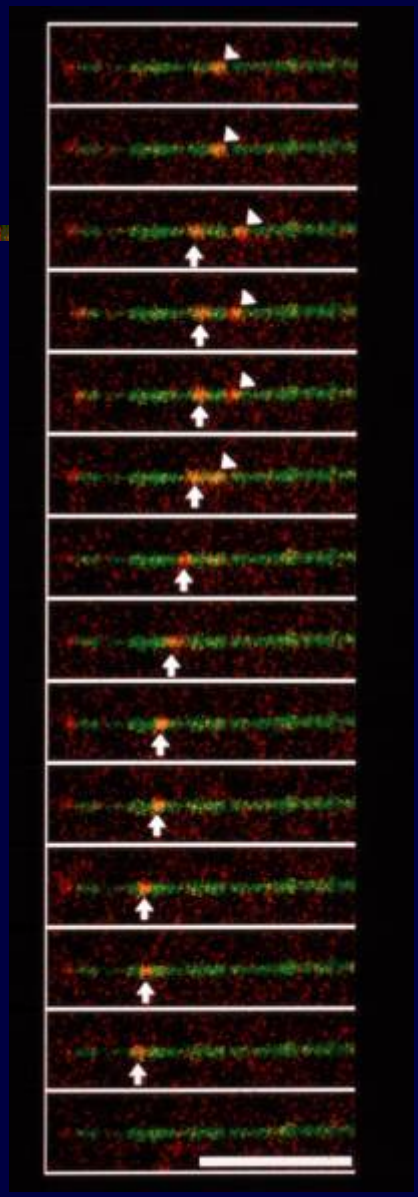
KIF1A



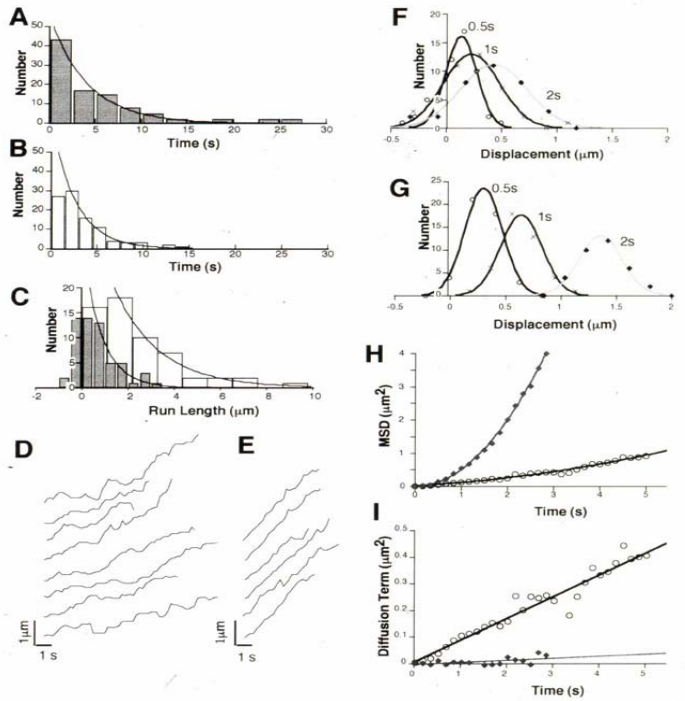
Question

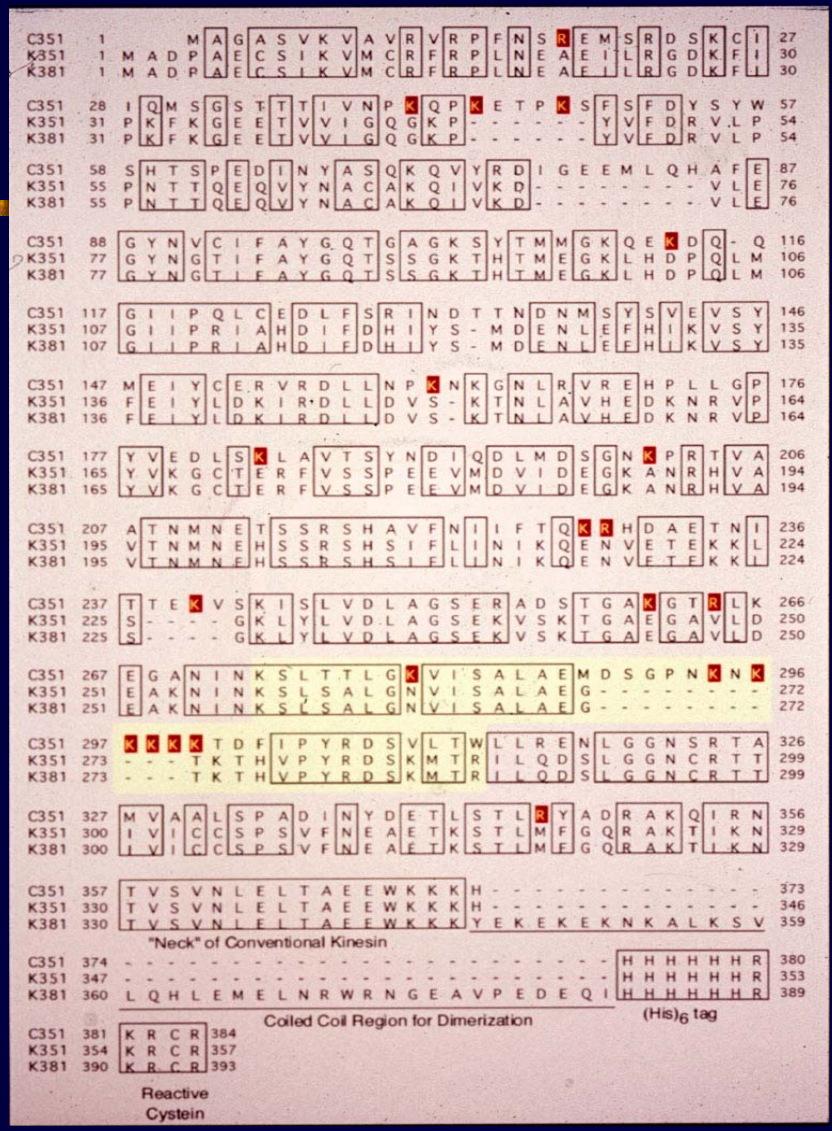

Processivity
kinesin
natively
dimeric
+
kinesin
monomeric
-
KIF1A
natively
monomeric
+

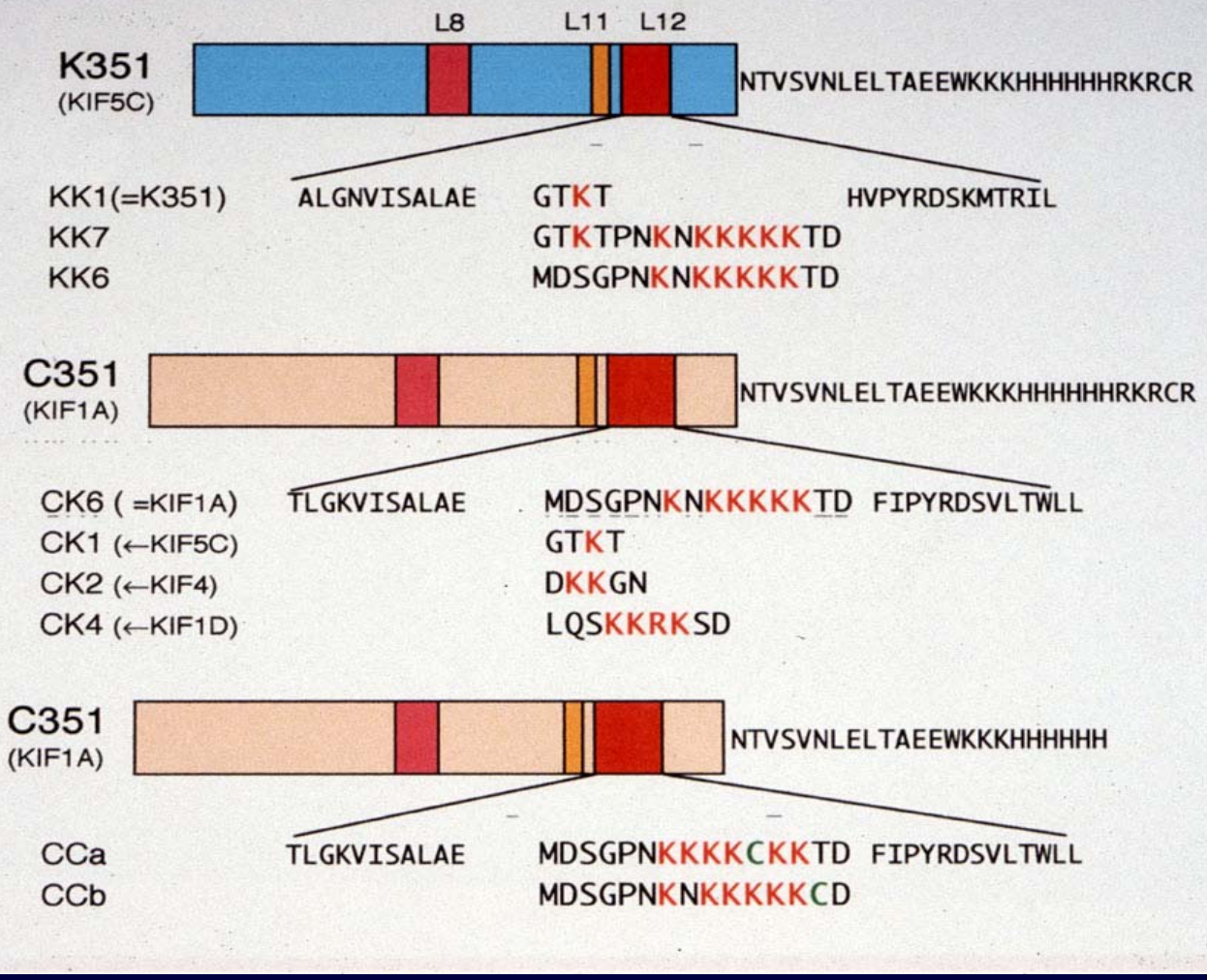

KIF1A
(W-Ch)
1
1695
(His)6Cys
dPH2
1543
(His)6Cys
C351
355
(His)6Cys
KIF5C
956
K351
346
(His)6Cys
K381
382
(His)6Cys

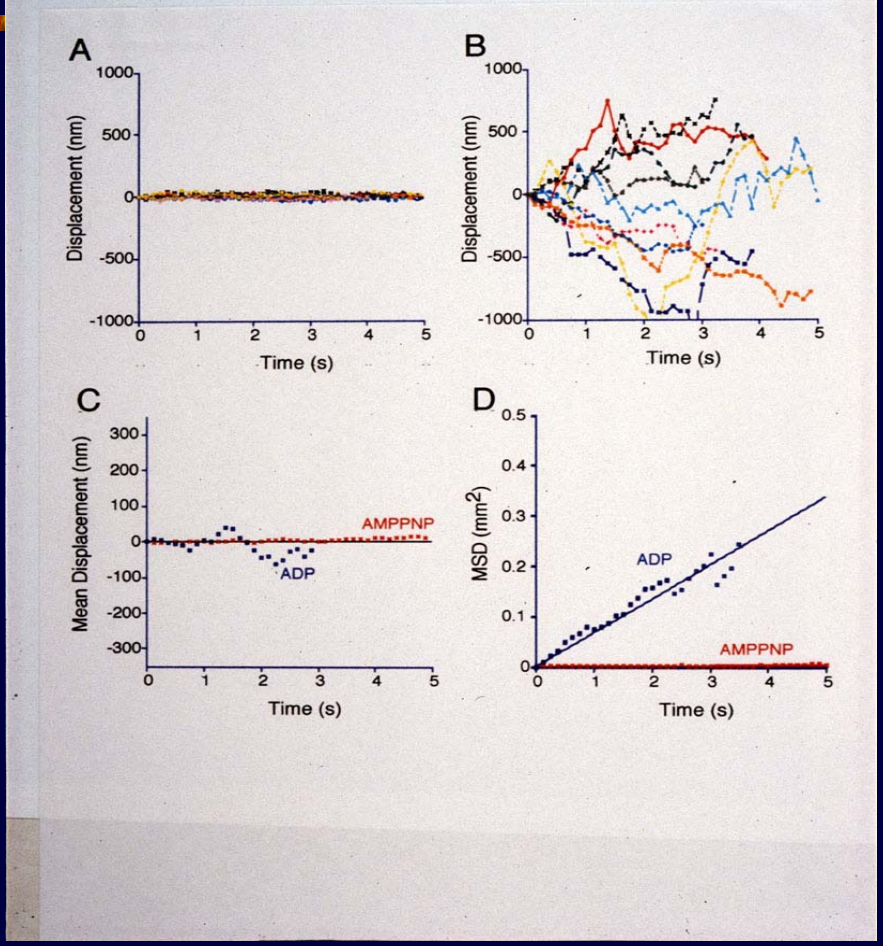


Okada et al. Science 283:1152－, 1999

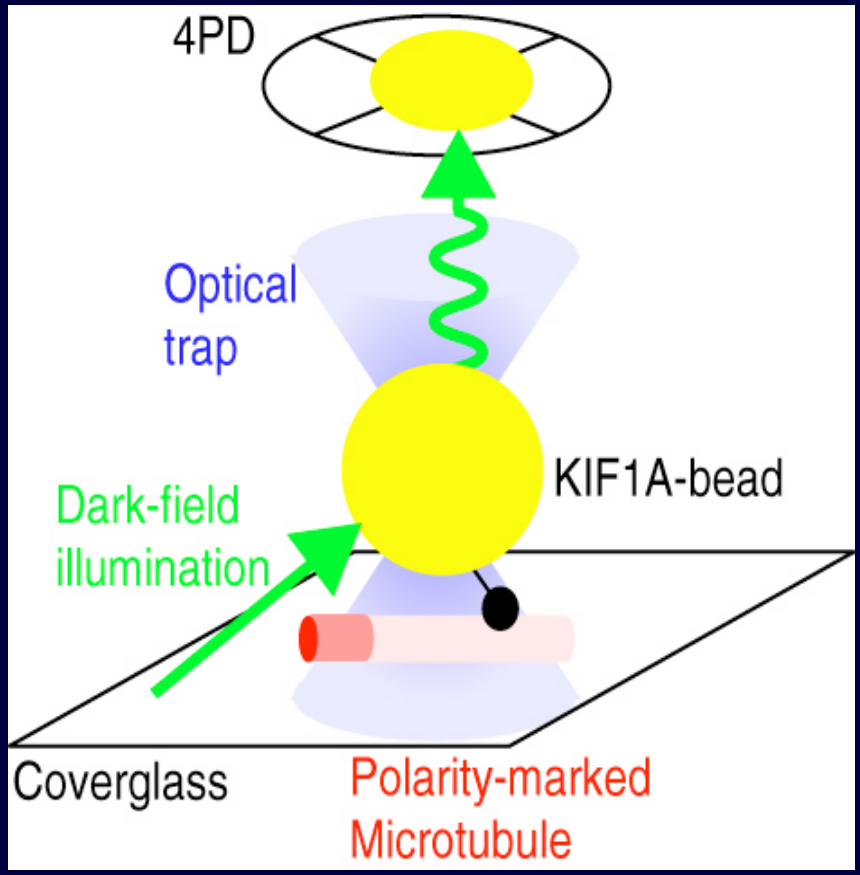

A
Number
Time (s)
B
Number
Time (s)
C
Number
Run Length (μm)
D
1 μm
1 s
E
1 μm
1 s
F
0.5s
1s
2s
Number
Displacement (μm)
G
0.5s
1s
2s
Number
Displacement (μm)
H
MSD (μm²)
Time (s)
I
Diffusion Term (μm²)
Time (s)


"Neck" of Conventional Kinesin
Coiled Coil Region for Dimerization
(His)6 tag
Reactive Cystein


L8
L11
L12
K351
(KIF5C)
NTVSVNLELTAEEWKKKHHHHHHRKRCR
KK1(=K351) ALGNVISALAE GTKT HVPYRDSKMTRIL
KK7 GTKTPNKNKKKKKTD
KK6 MDSGPNKNKKKKKTD
C351
(KIF1A)
NTVSVNLELTAEEWKKKHHHHHHRKRCR
CK6 (=KIF1A) TLGKVISALAE MDSGPNKNKKKKKTD FIPYRDSVLTWLL
CK1 (←KIF5C) GTKT
CK2 (←KIF4) DKKGN
CK4 (←KIF1D) LQSKKRKSD
C351
(KIF1A)
NTVSVNLELTAEEWKKKHHHHHH
CCa TLGKVISALAE MDSGPNKKKKCKKTD FIPYRDSVLTWLL
CCb MDSGPNKNKKKKKCD


A
Displacement (nm)
1000
500
0
-500
-1000
0 1 2 3 4 5
Time (s)
B
Displacement (nm)
1000
500
0
-500
-1000
0 1 2 3 4 5
Time (s)
C
Mean Displacement (nm)
300
200
100
0
-100
-200
-300
AMPPNP
ADP
0 1 2 3 4 5
Time (s)
D
MSD (mm2)
0.5
0.4
0.3
0.2
0.1
0
ADP
AMPPNP
0 1 2 3 4 5
Time (s)

Optical Trapping Nanometry



Spatial resolution < 0.2 nm

Temporal resolution < 1 ms

0.2μm beads

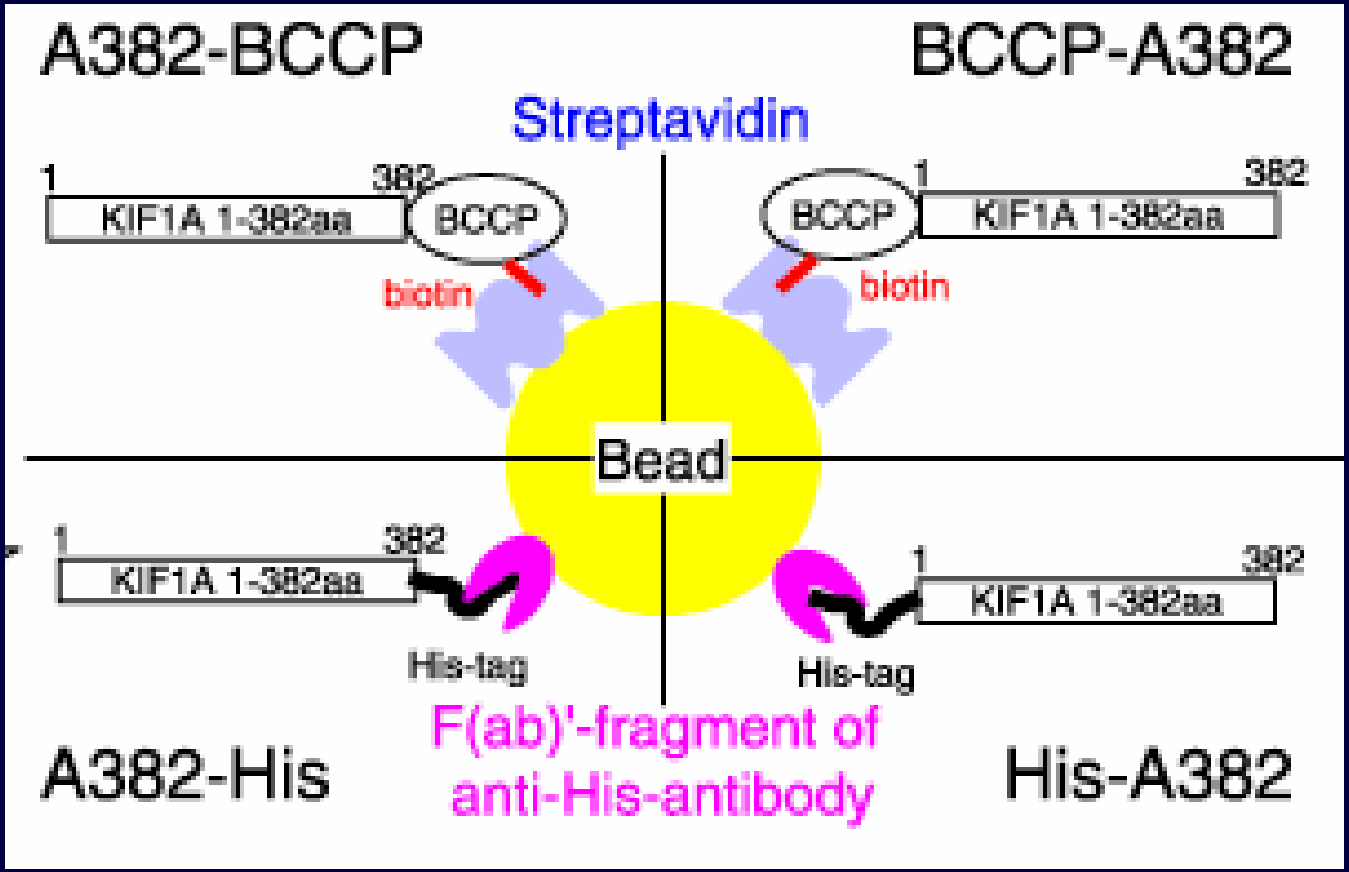
⇒Small viscous drag

⇒ High temporal resolution

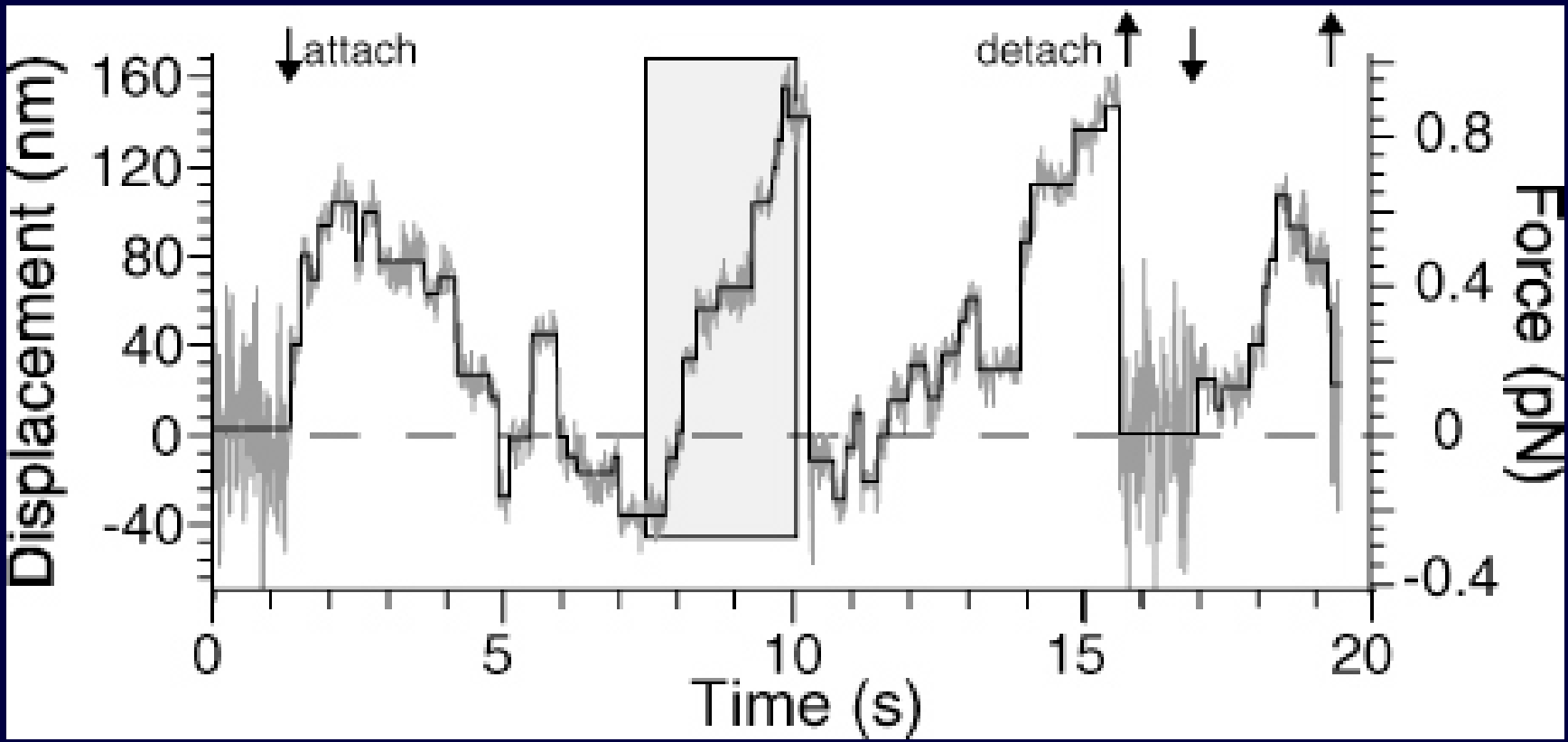
(Response time ≦ 0.25 ms)

Okada, Higuchi, and Hirokawa Nature 424:574－2003

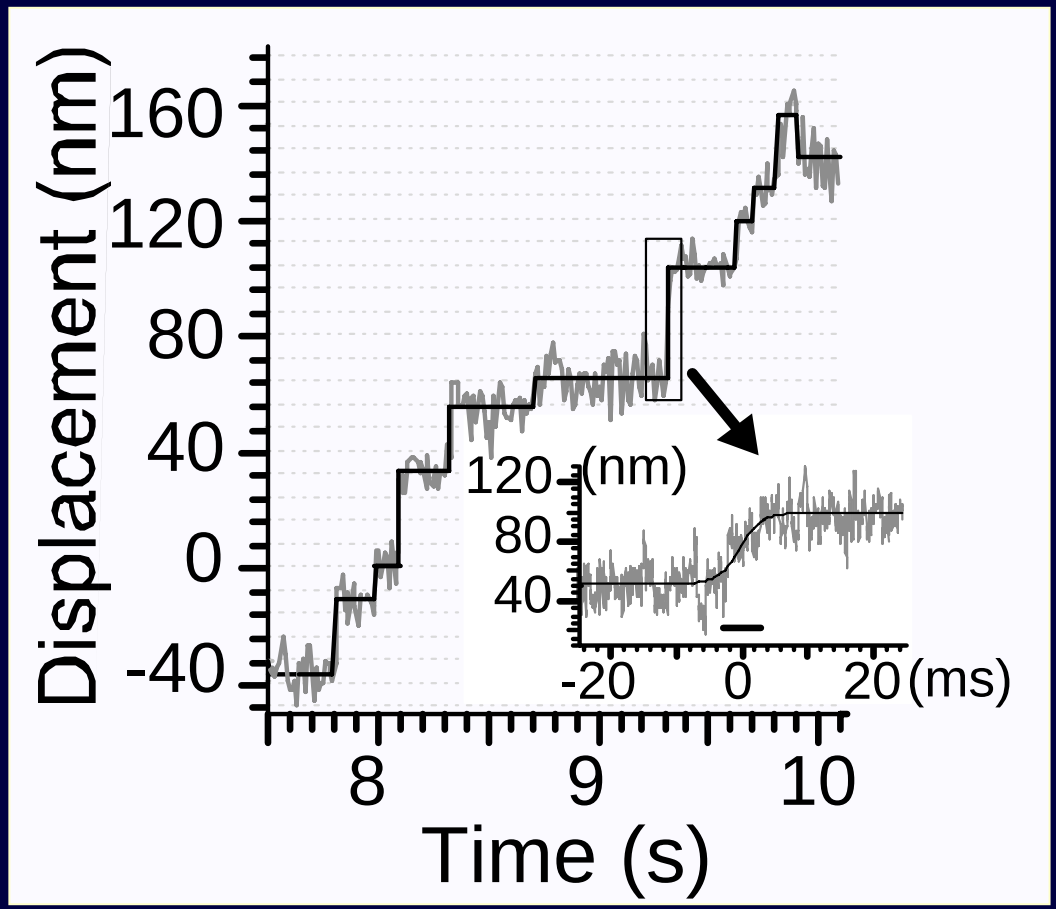
Immobilization of KIF1A to Bead

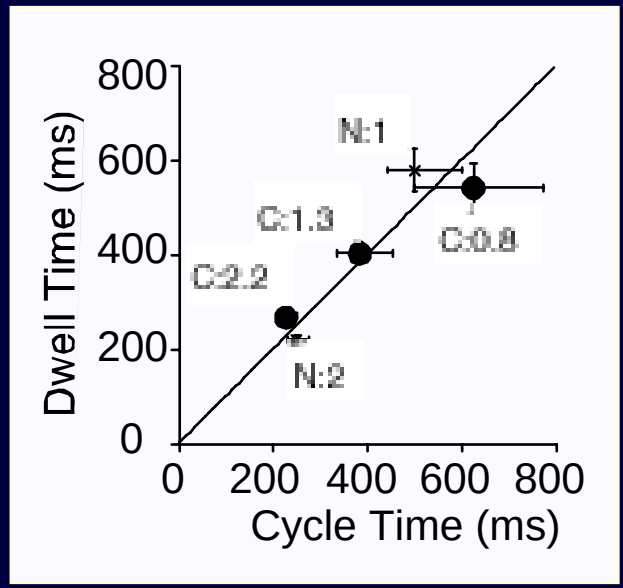


Bidirectional and Step-wise Movement of a Single KIF1A Monomer

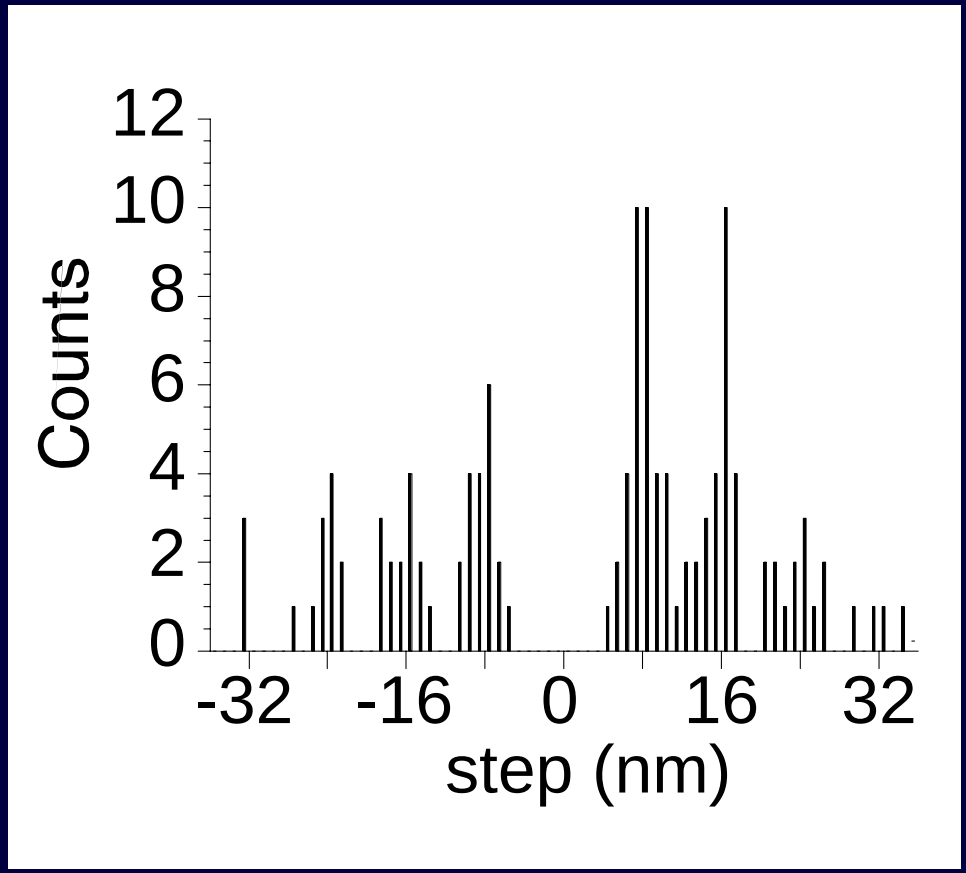


One ATP Hydrolysis Triggers One Stepping Movement.

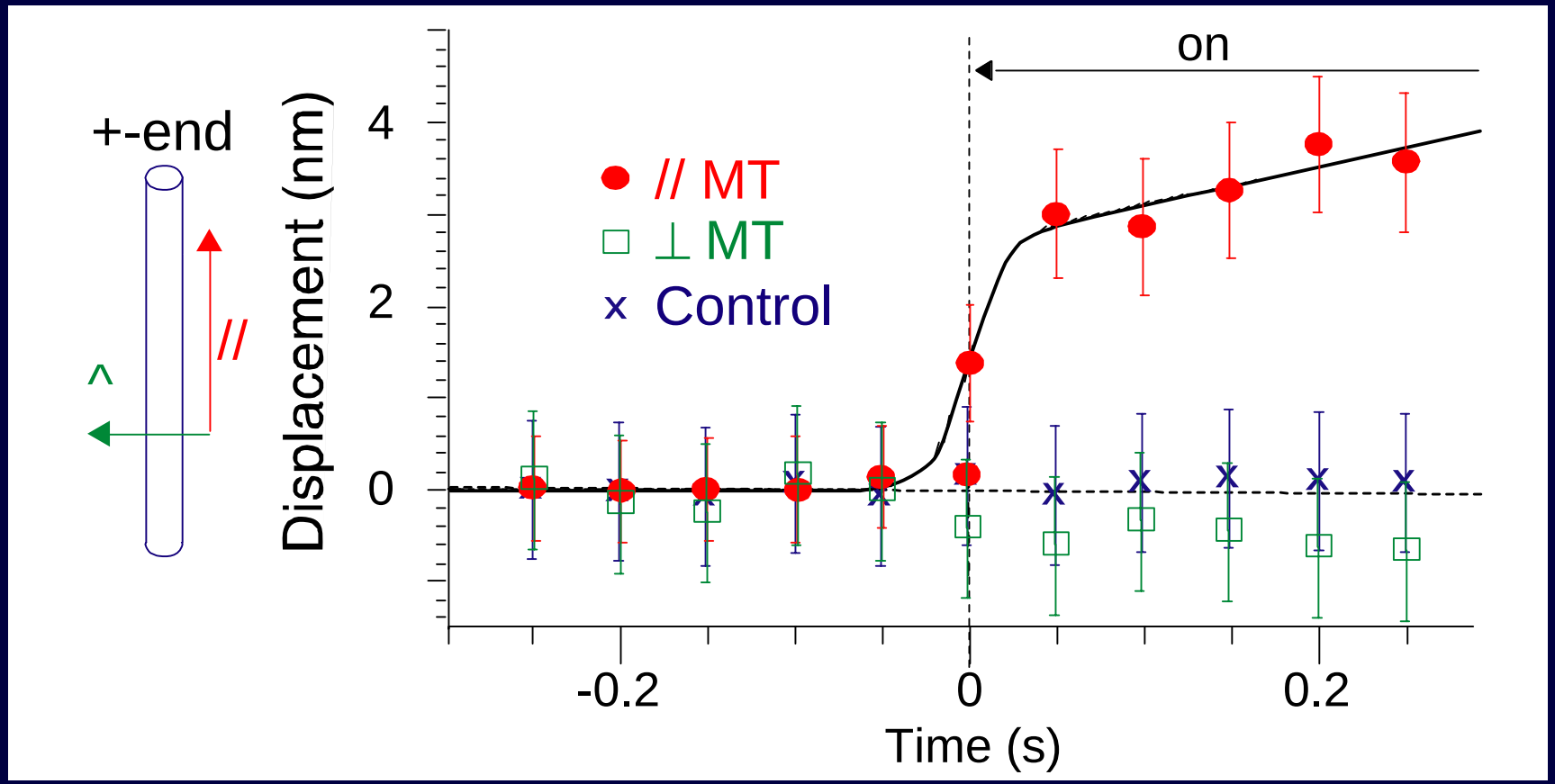




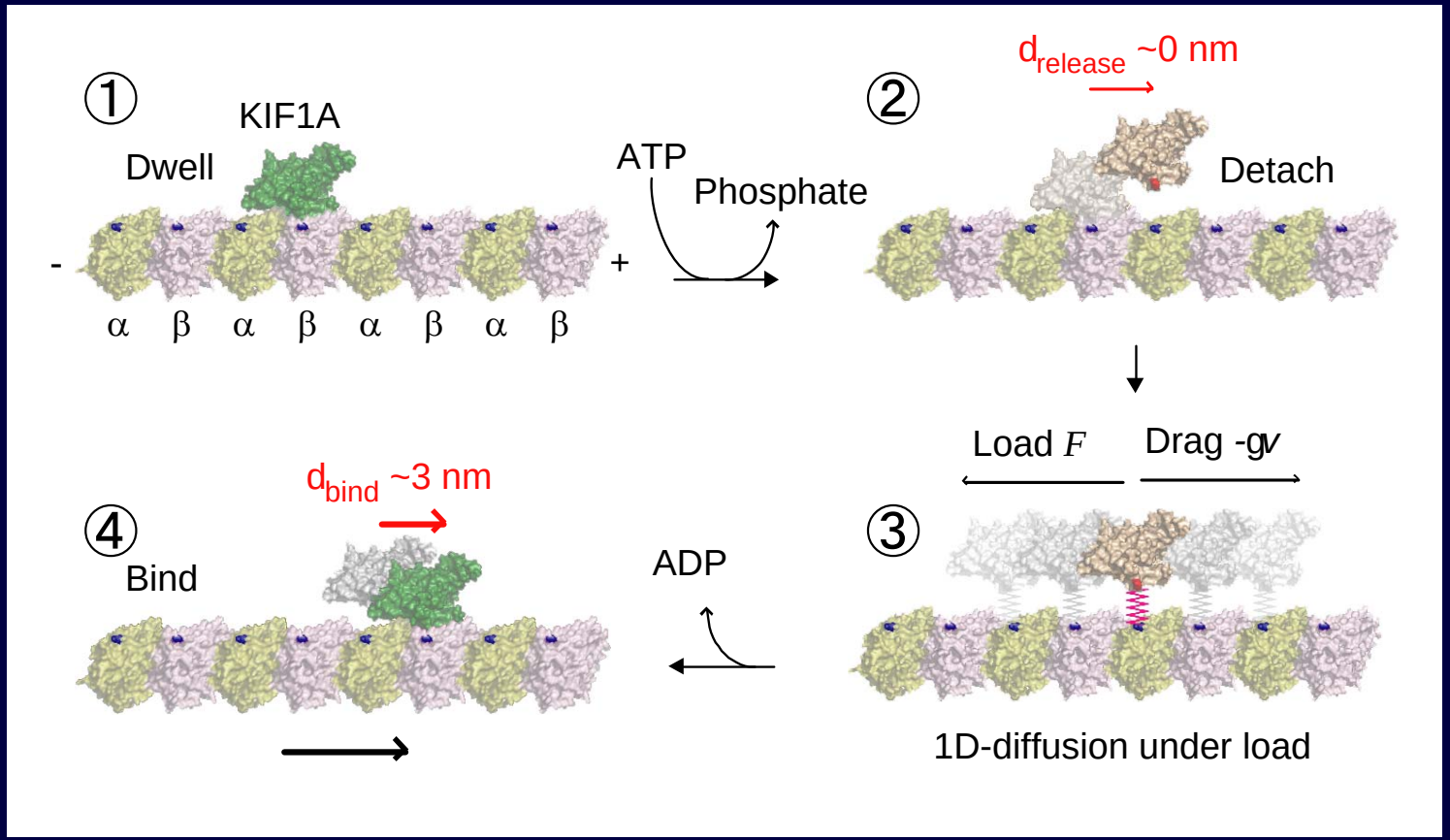
Step size of KIF1A is 8 × *n* nm, and varies stochastically.

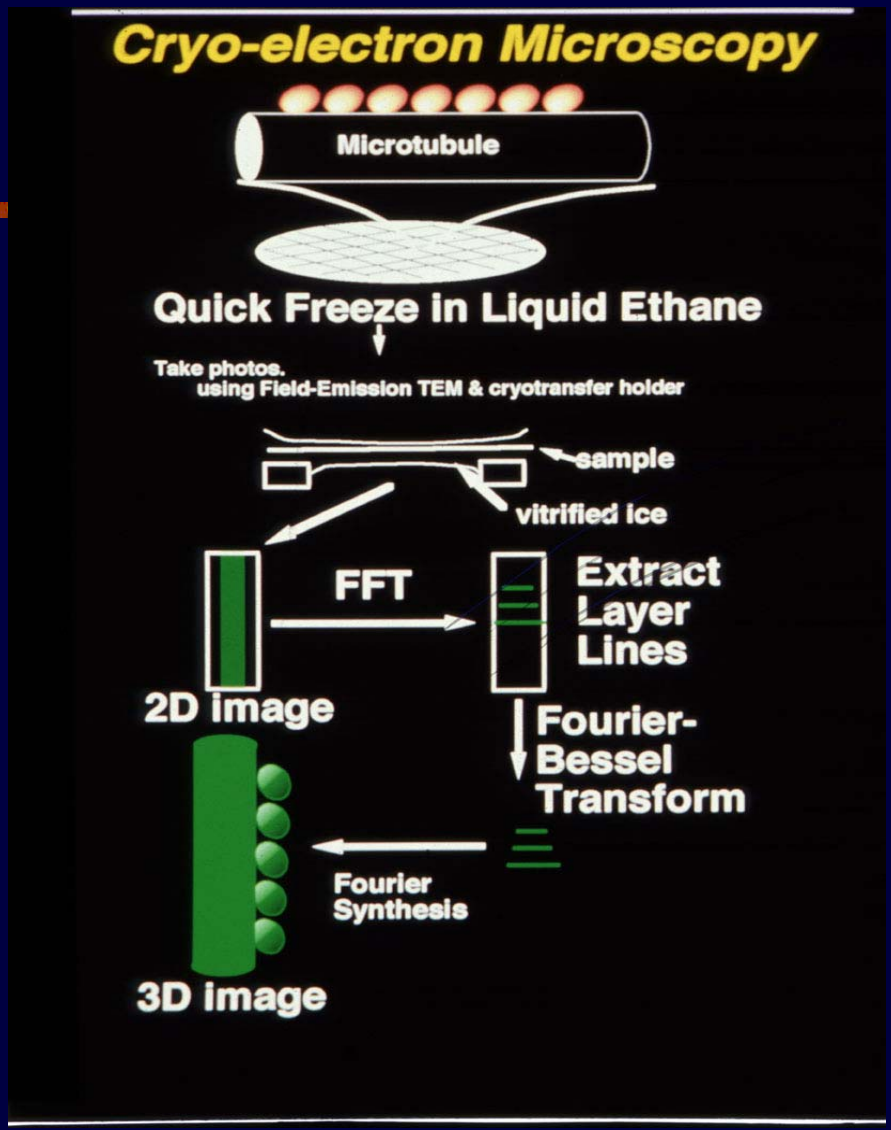


Plus-end biased binding of KIF1A to the microtubule: ensemble average



Flush Ratchet Model

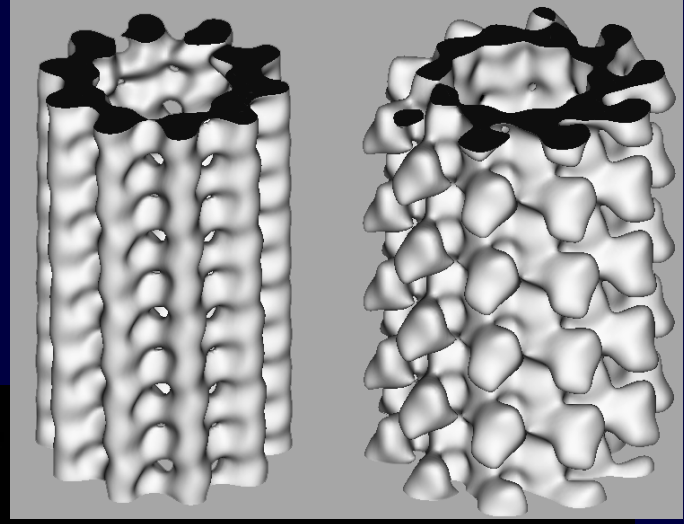



Cryo-electron Microscopy
Microtubule
Quick Freeze in Liquid Ethane
Take photos.
using Field-Emission TEM & cryotransfer holder
sample
vitrified ice
FFT
Extract
Layer
Lines
2D image
Fourier-
Bessel
Transform
Fourier
Synthesis
3D image

Microtubule Structure

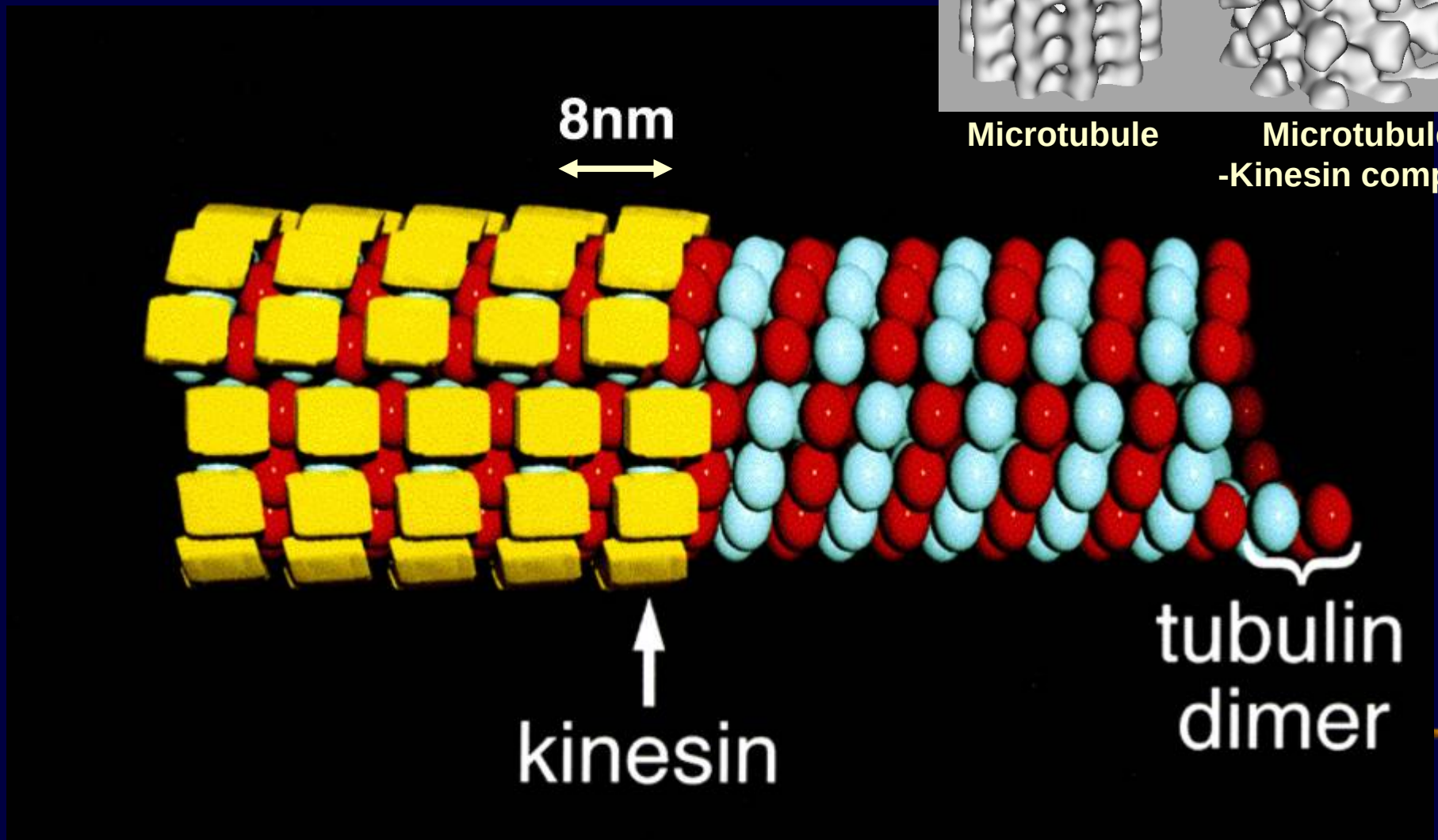
Kikkawa et al.

JCB, 1994: Nature, 1995



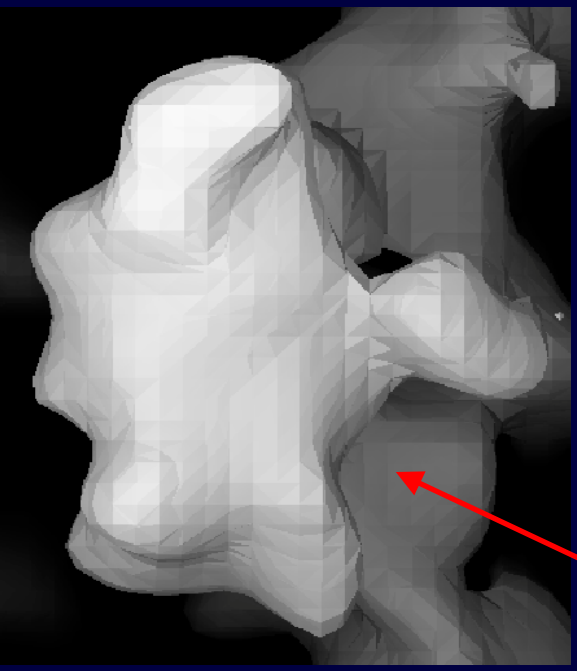
Microtubule

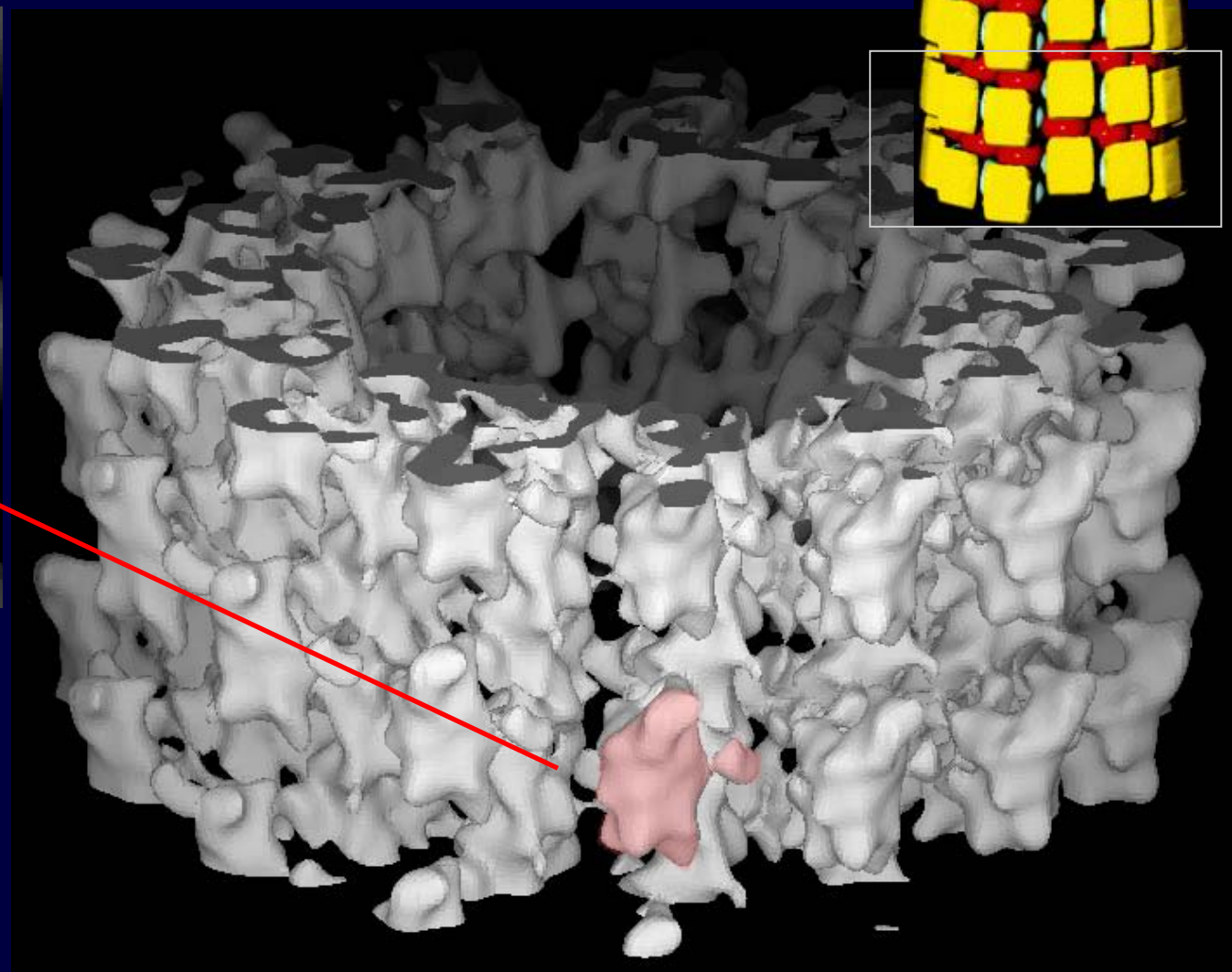
Microtubule
-Kinesin complex

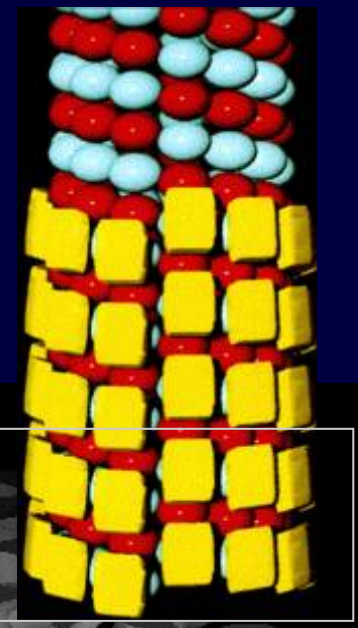


KIF1A-Microtubule Complex: cryo EM（15Å resolution）

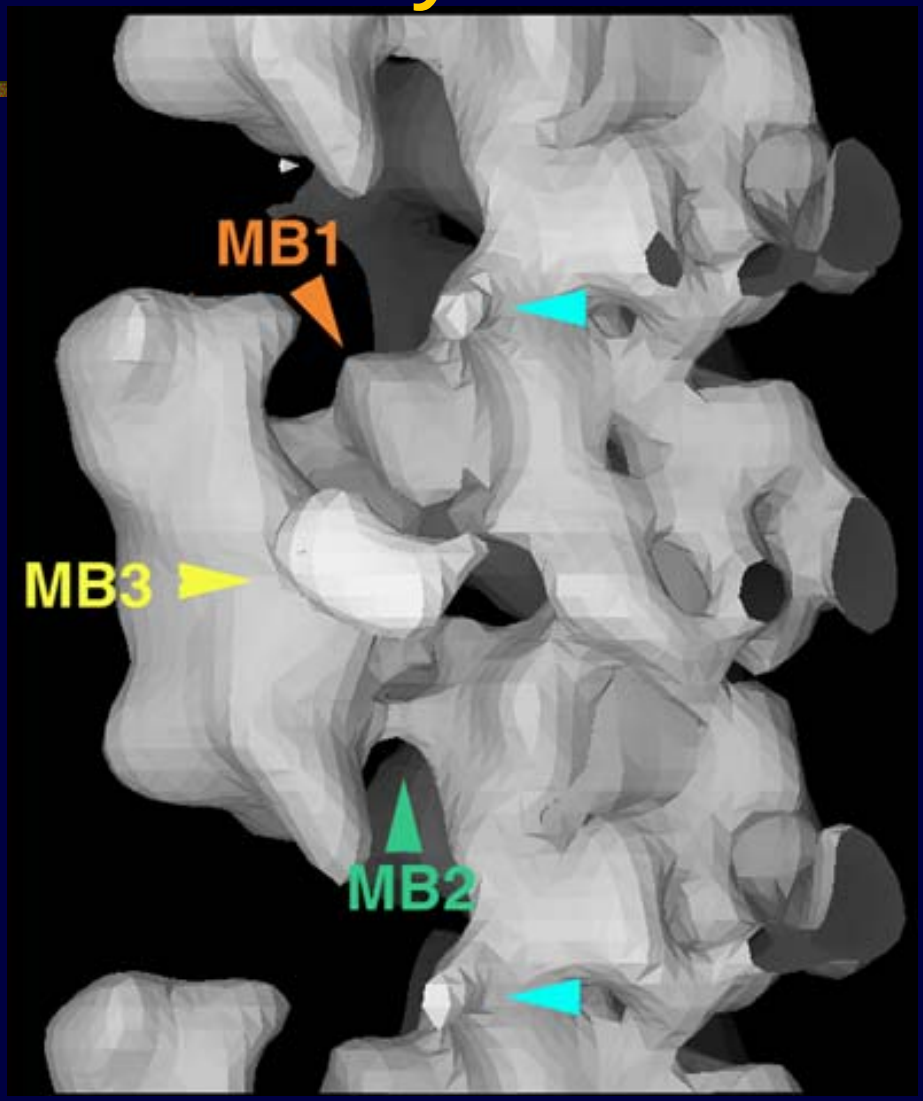
Kikkawa et al. **Cell** 100: 241-, 2000

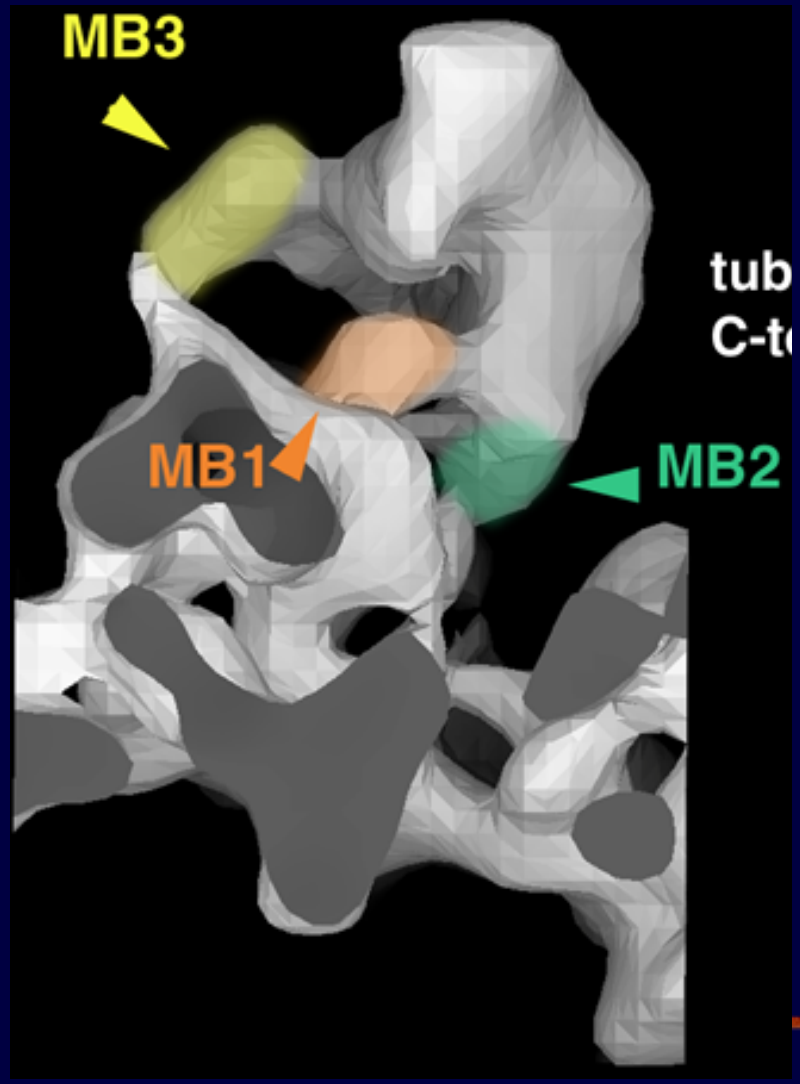


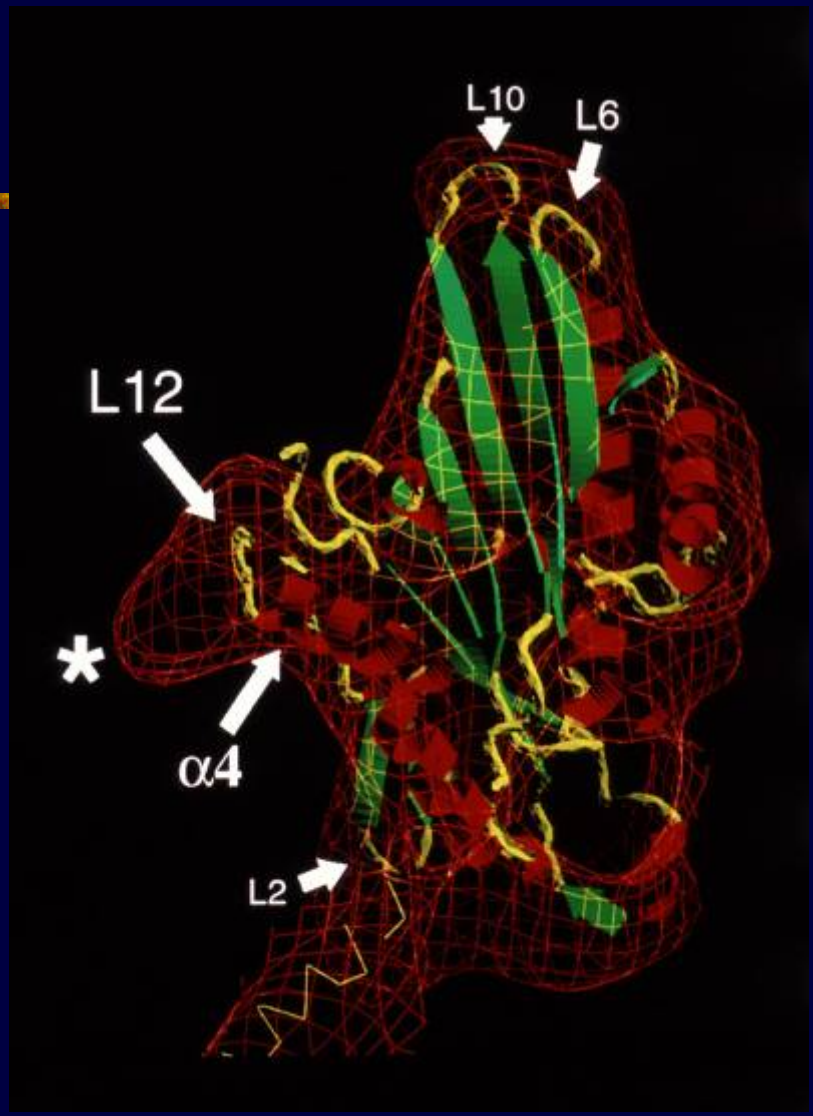


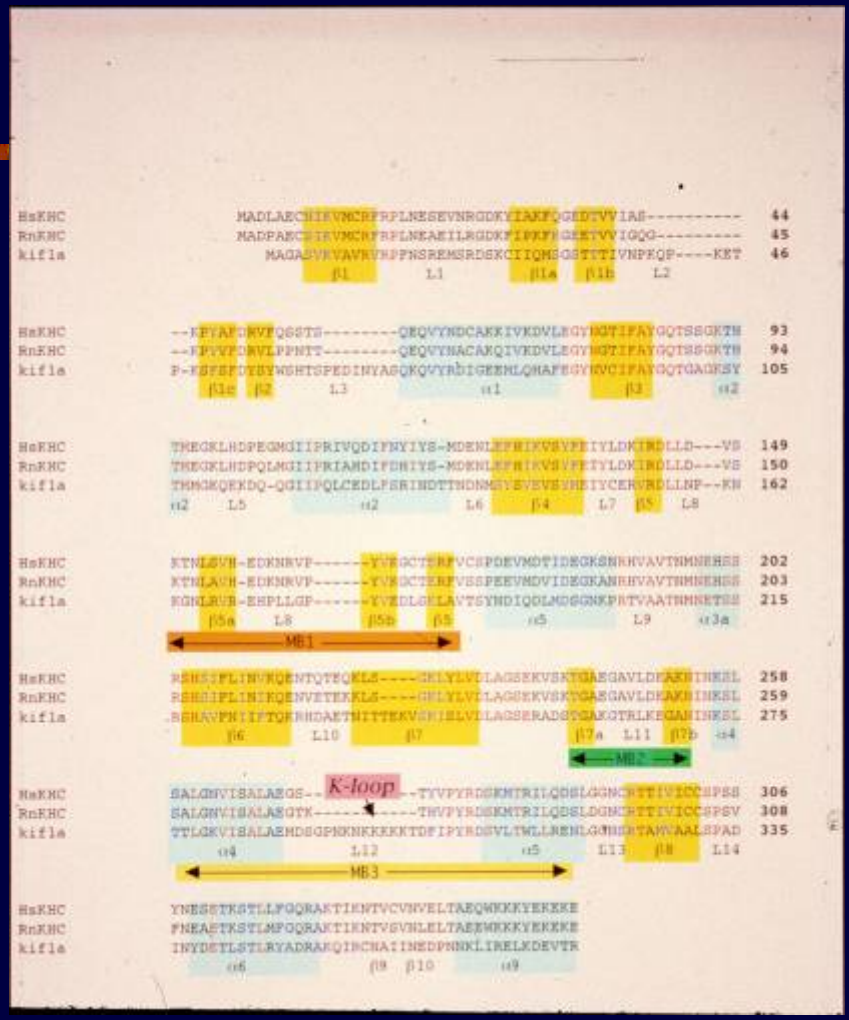


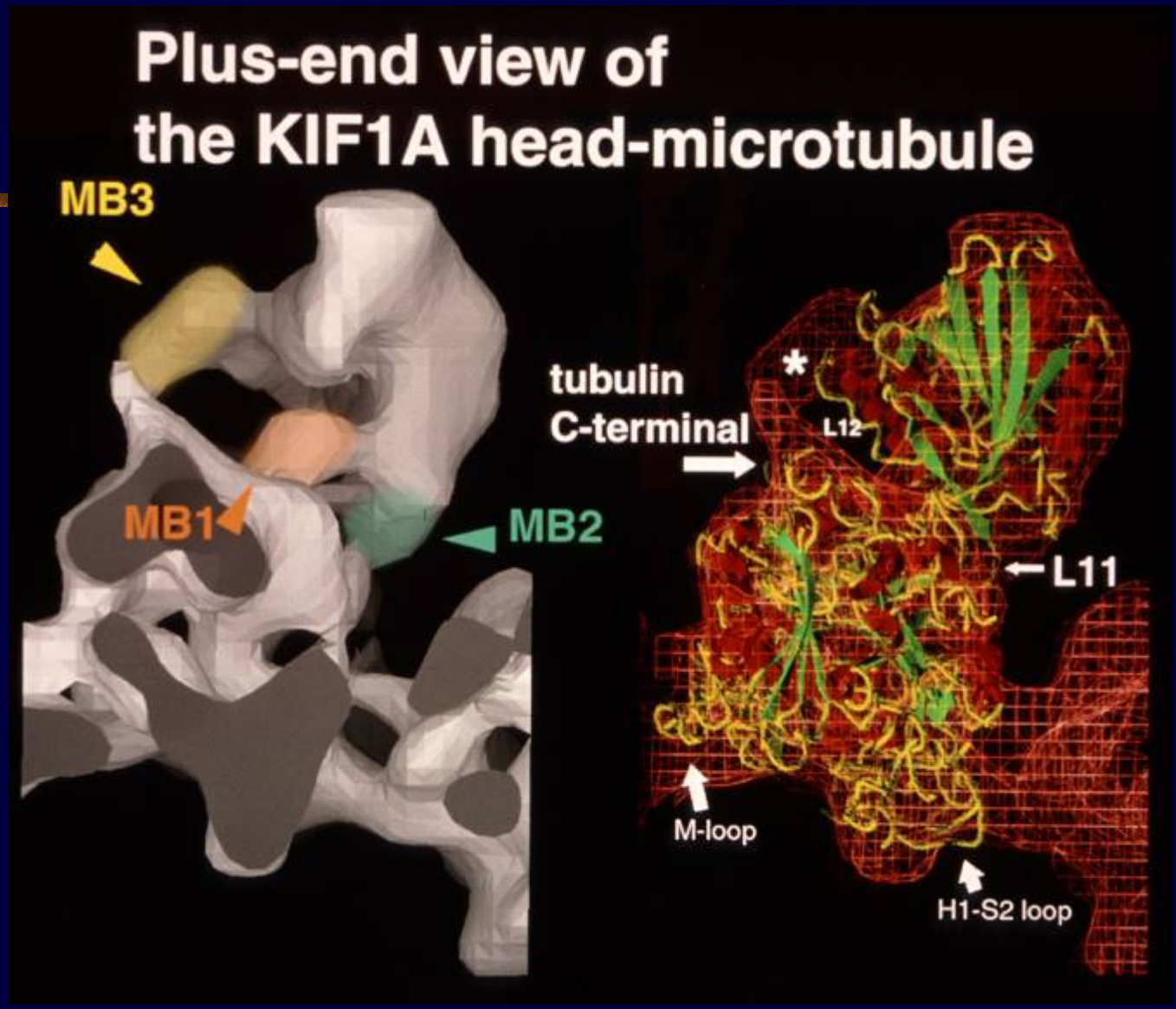
KIF1A-Microtubule Complex: cryo EM（15Å resolution）



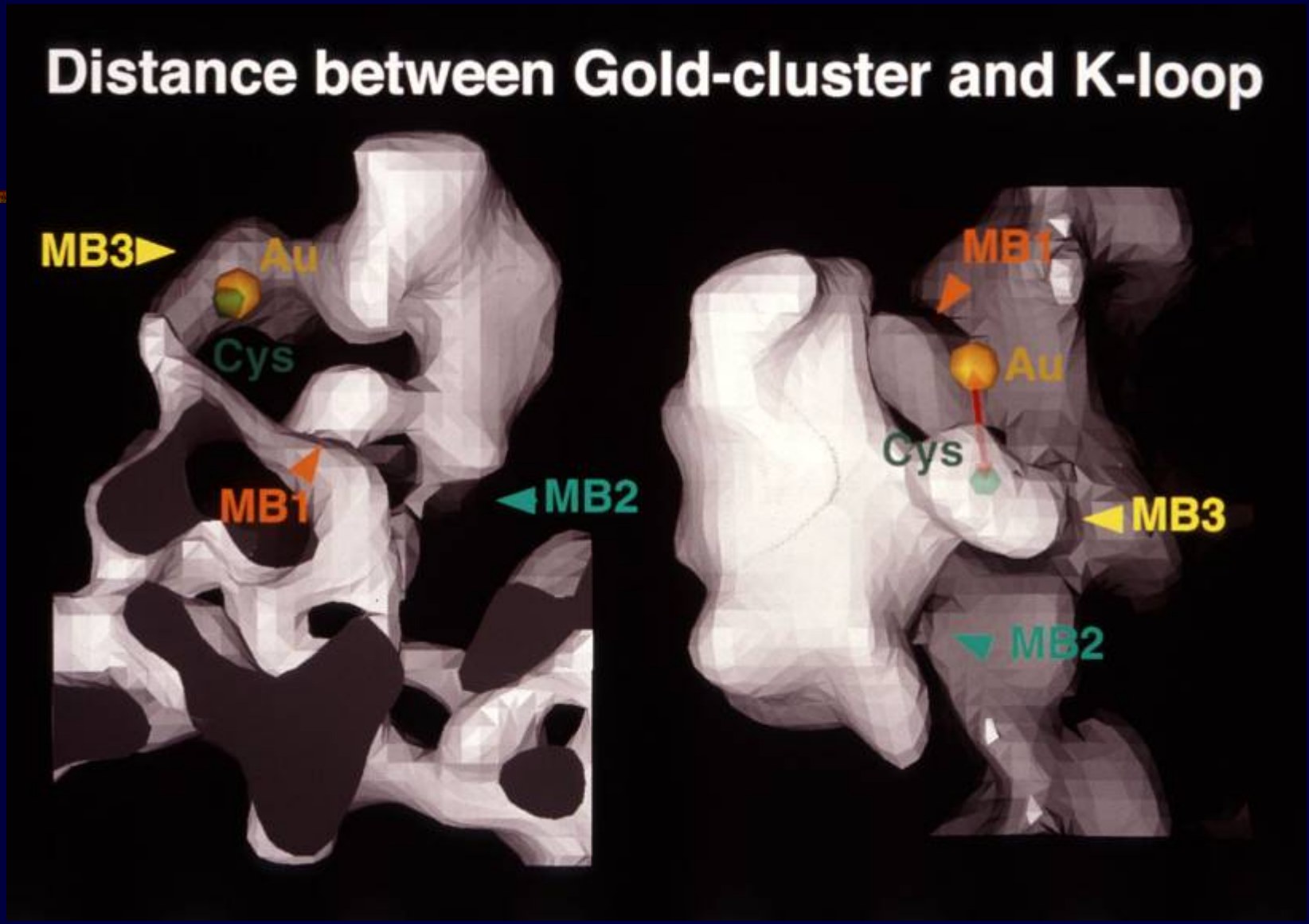



L10
L6
L12
*
α4
L2

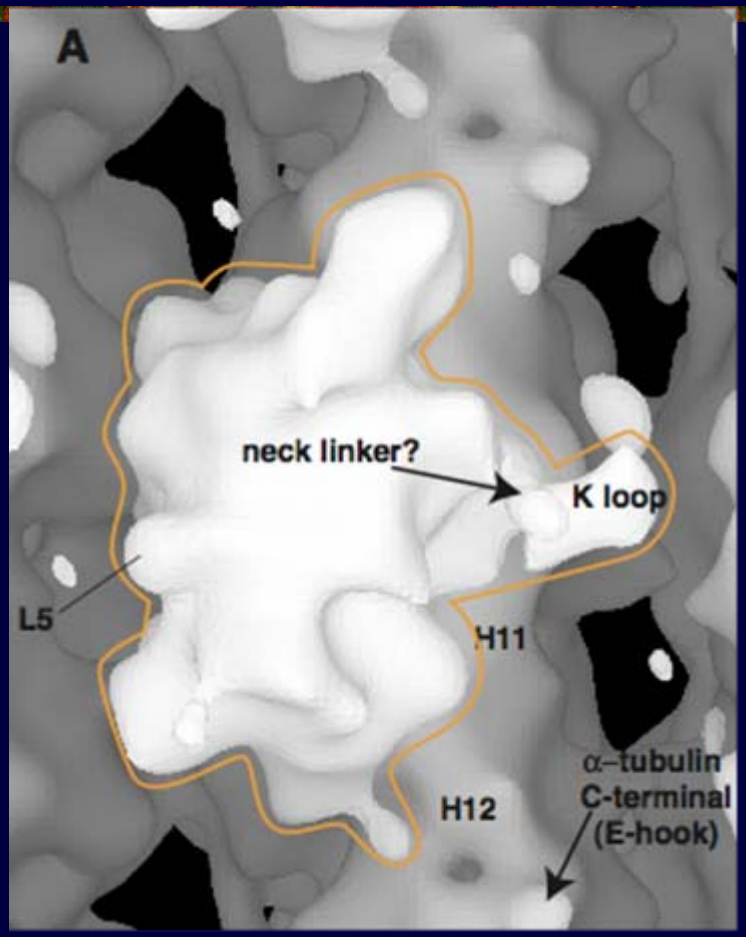

K-loop
ME1
ME2
ME3

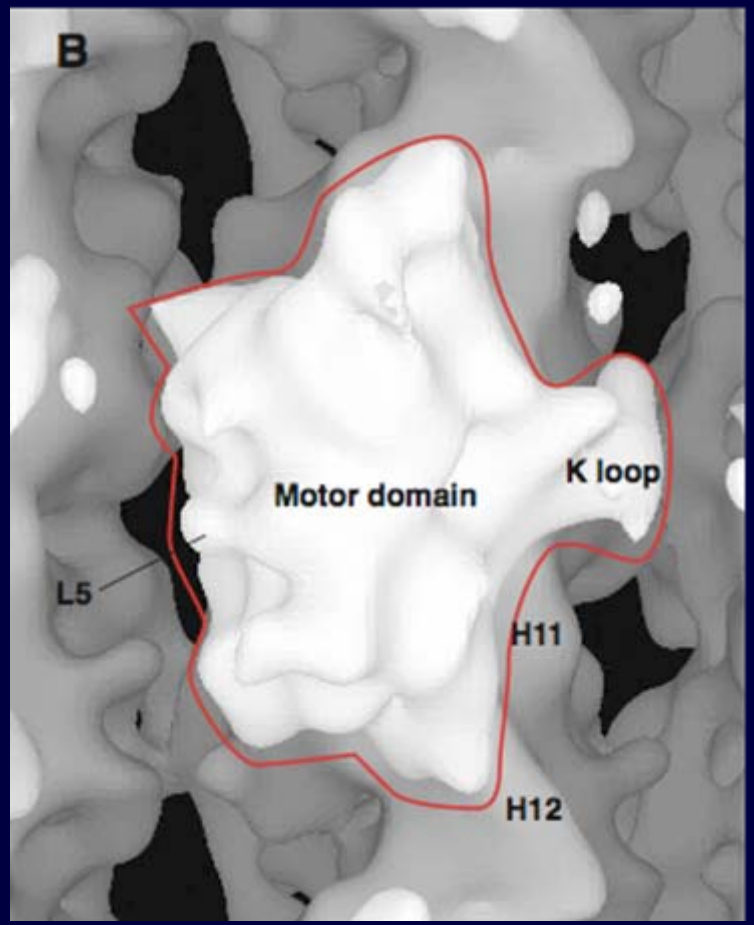

Plus-end view of
the KIF1A head-microtubule
MB3
MB1
MB2
tubulin
C-terminal
*
L12
L11
M-loop
H1-S2 loop

Distance between Gold-cluster and K-loop



View from outside

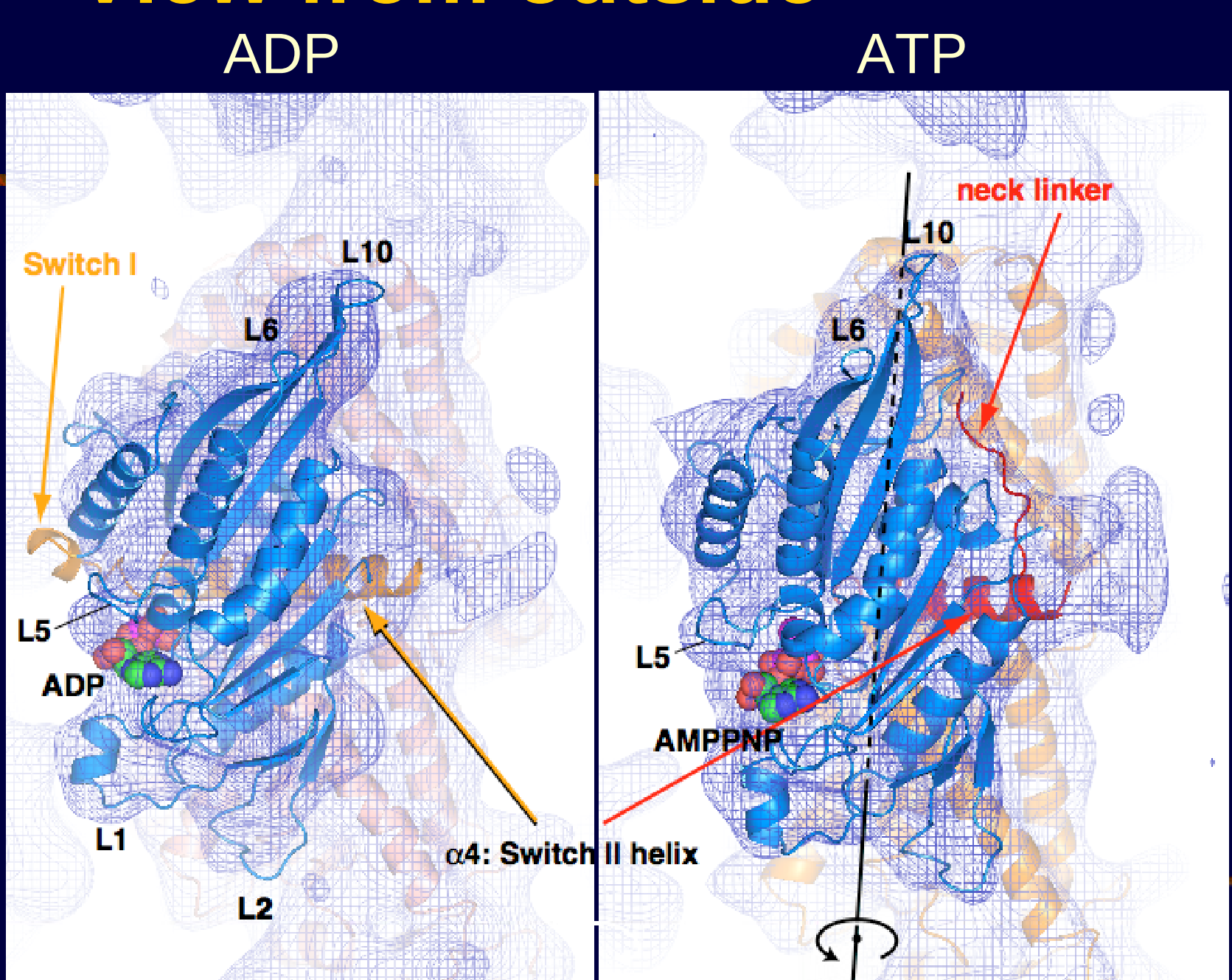




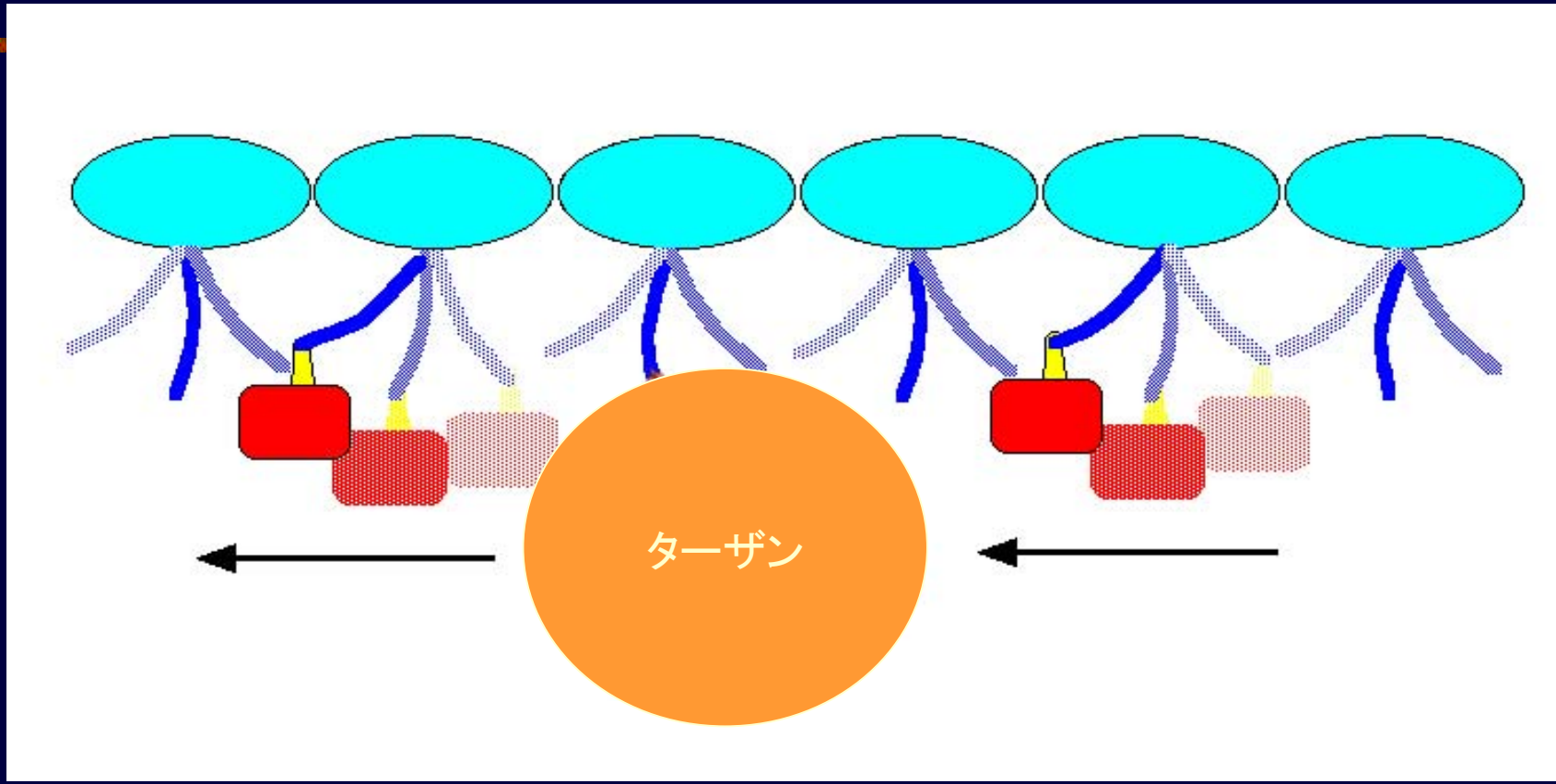
ADP

ATP(AMPPNP)

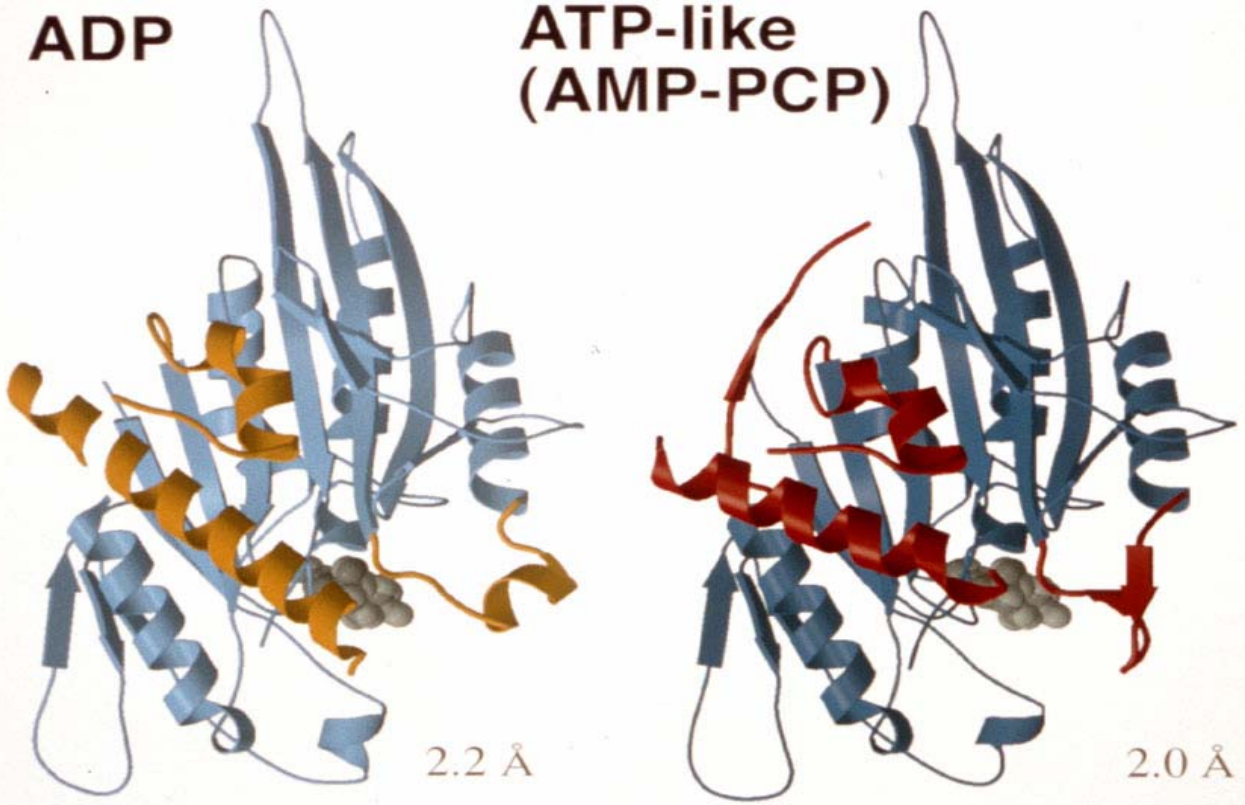
View from outside



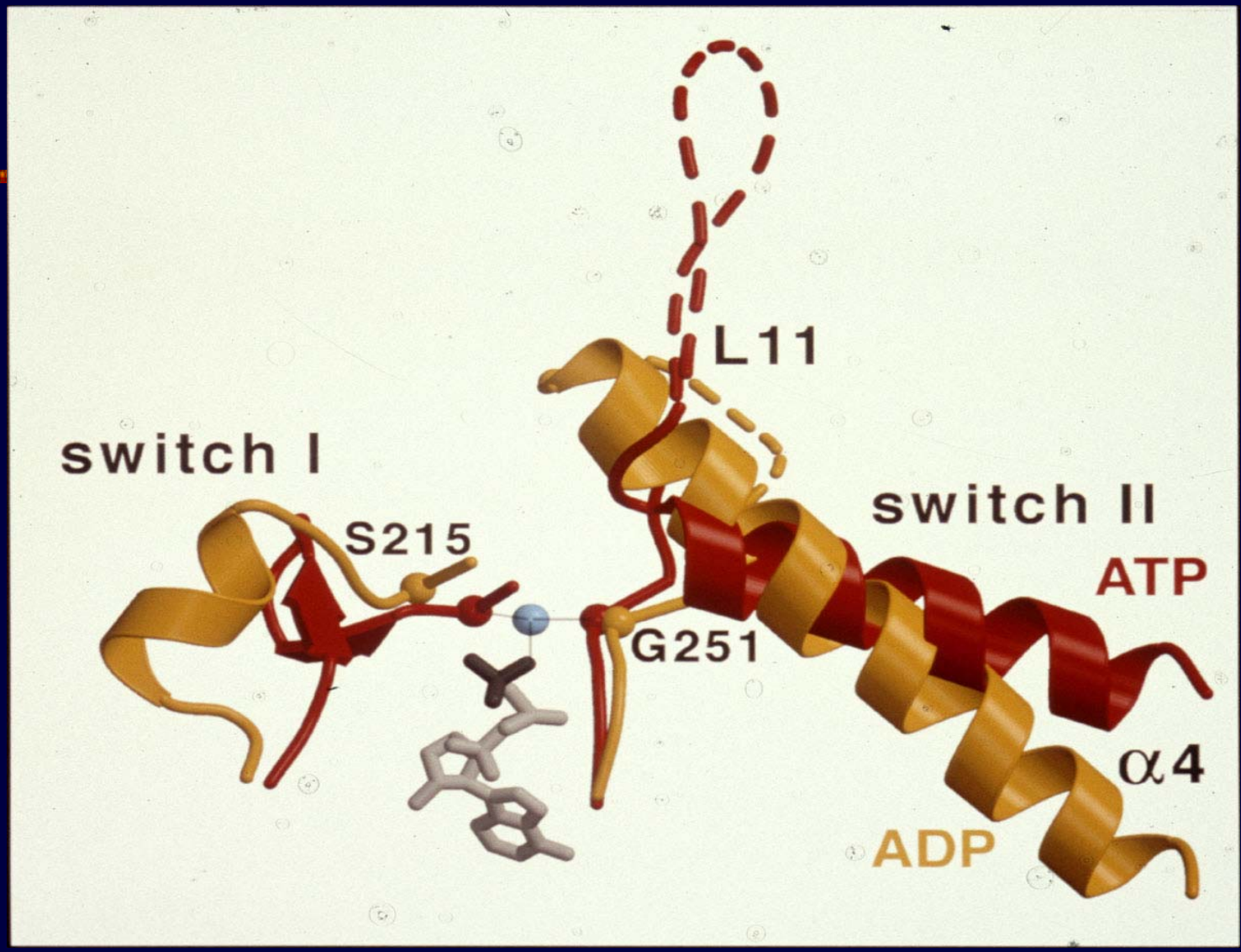
Tarzan Model



“Drag force” = diffusion might reflect the fluctuation of the flexible tether between KIF1A and tubulin (K-loop & E-hook).



k Kikkawa et al. Nature 411: 439- ,2001
j

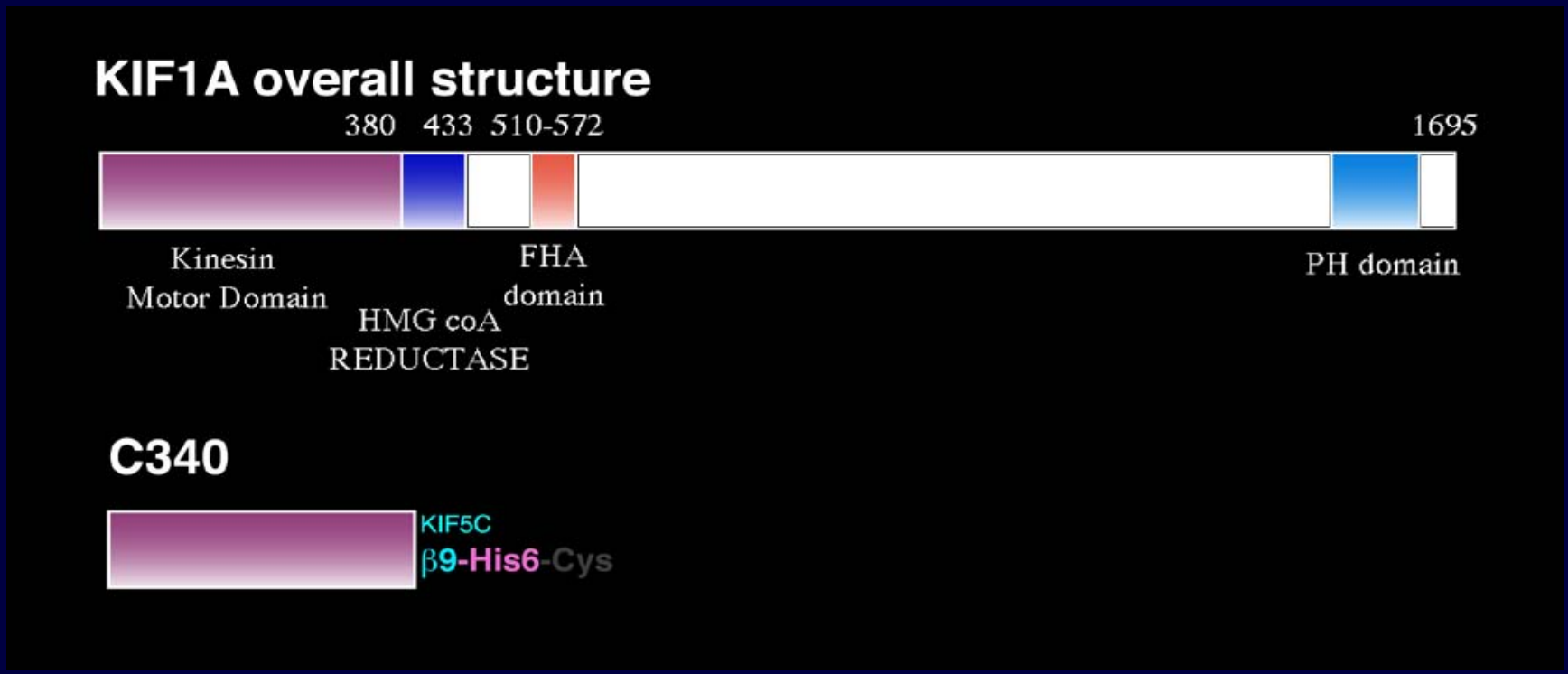

L11
switch I
switch II
S215
ATP
G251
α4
ADP

Structural Biological Analysis of kinesin motors

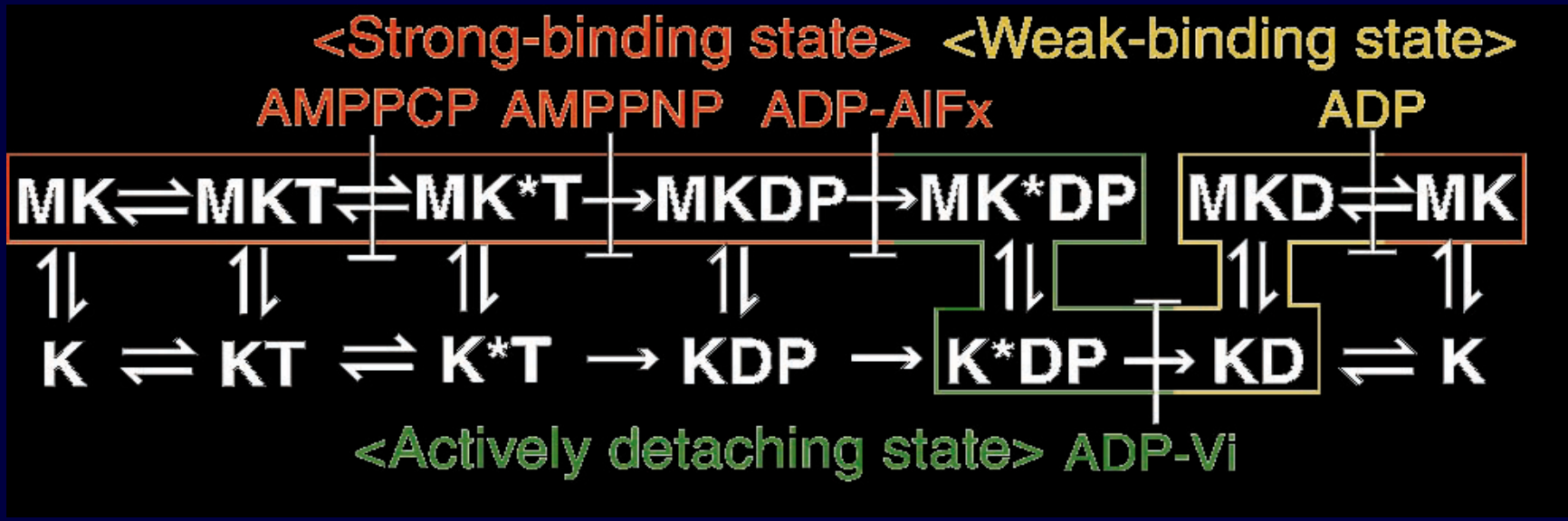
KIF1A alternately uses two loops to bind microtubules

Nitta,R. et al. Science 305:678- , 2004

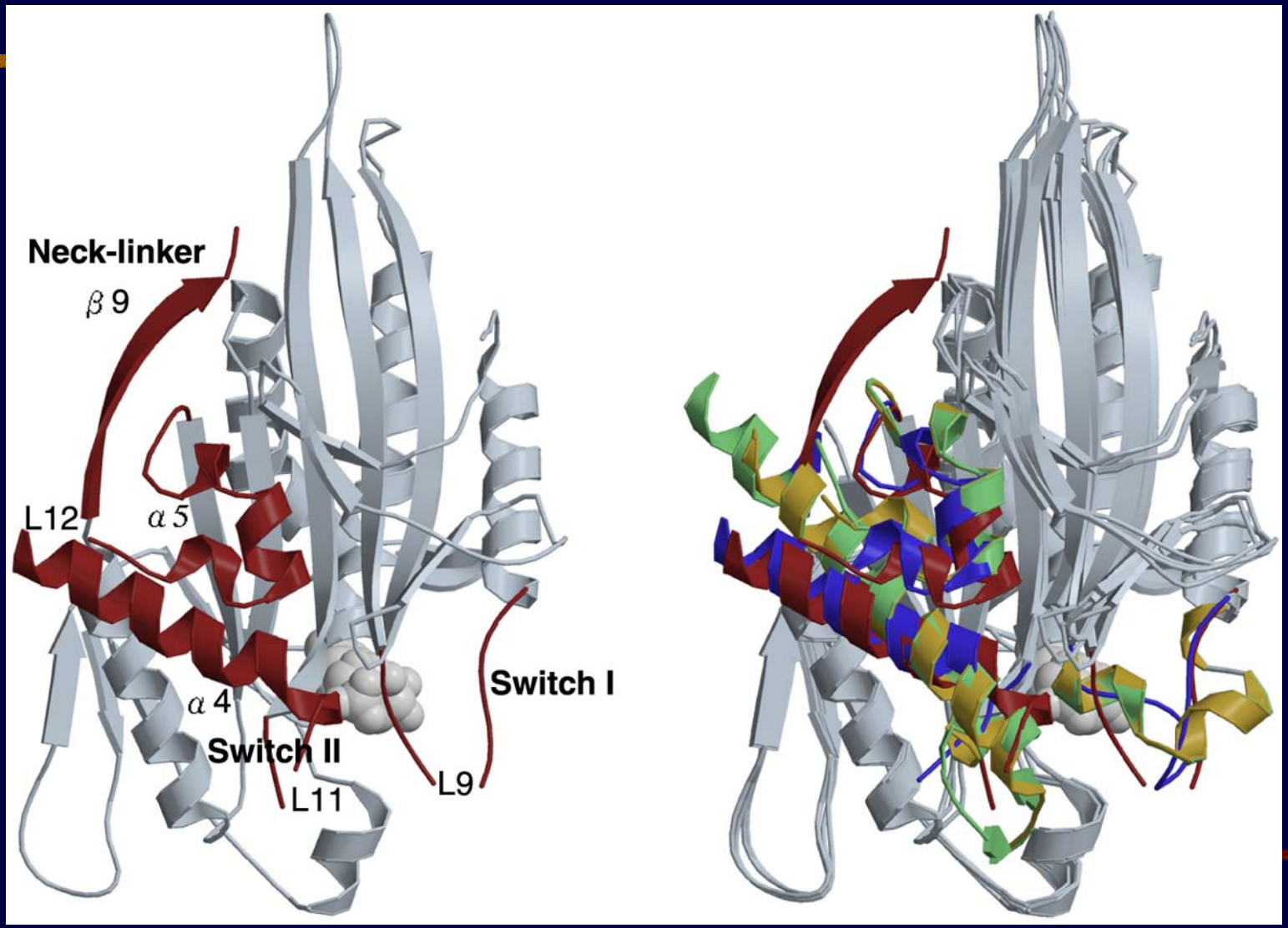
Construction of KIF1A Motor Domain



Chemo-mechanical cycle of kinesin motors



Overall architecture of KIF1A



ATP
(AMPPNP)

↓

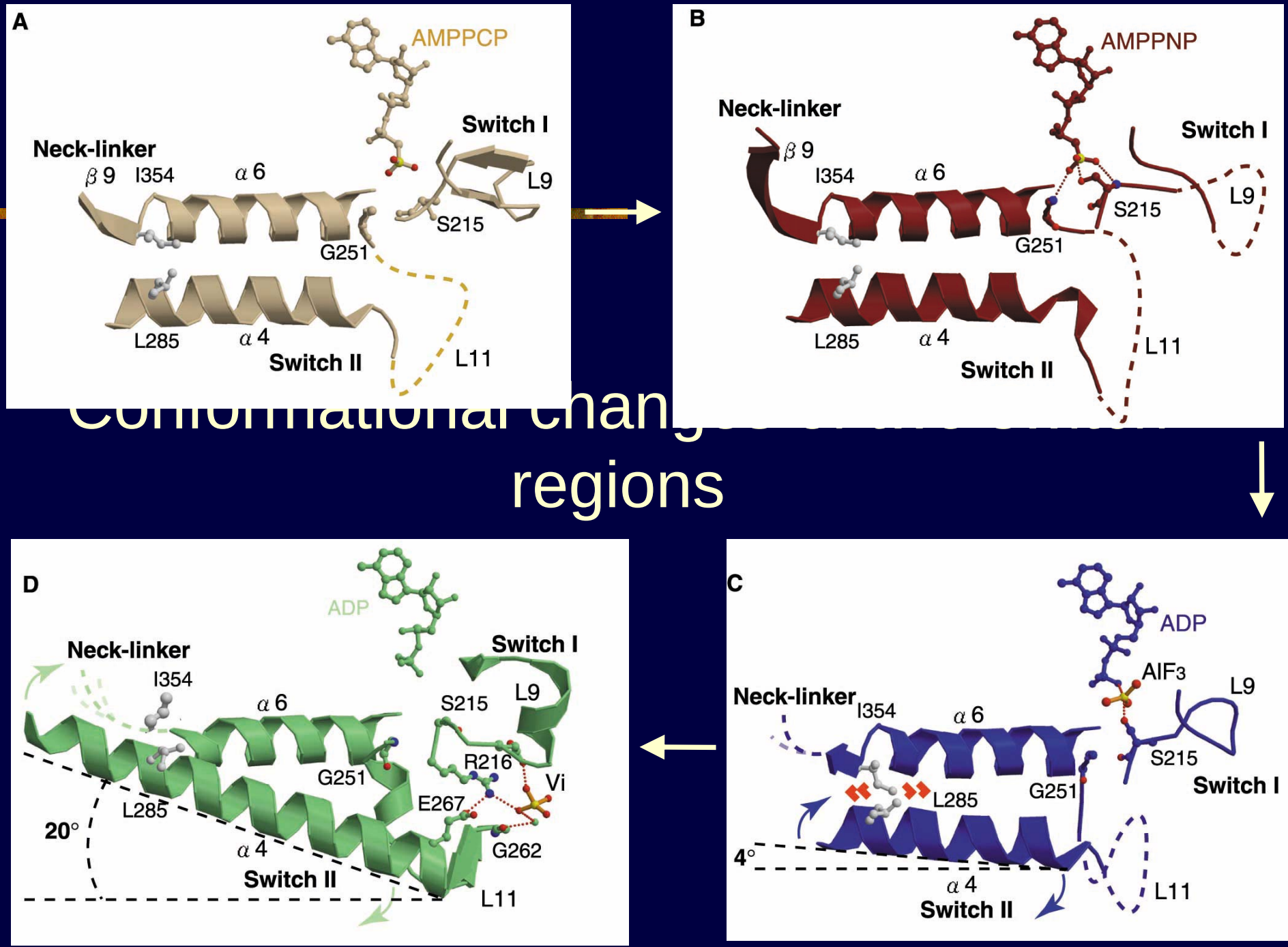
ADP-Pi (1)
(ADP-AlF$_3$)

↓

ADP-Pi (2)
(ADP-VO$_4$)

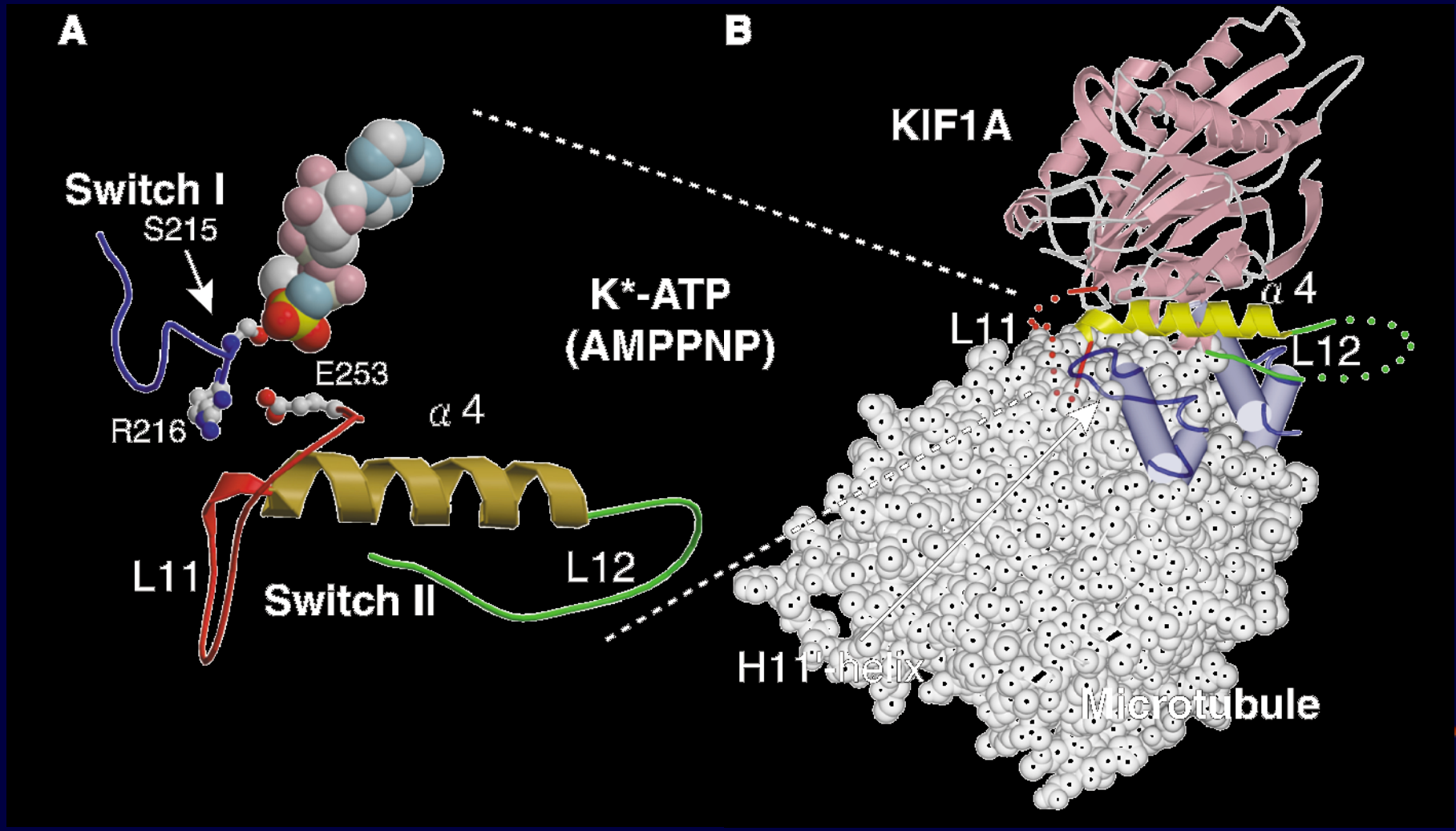
↓

ADP

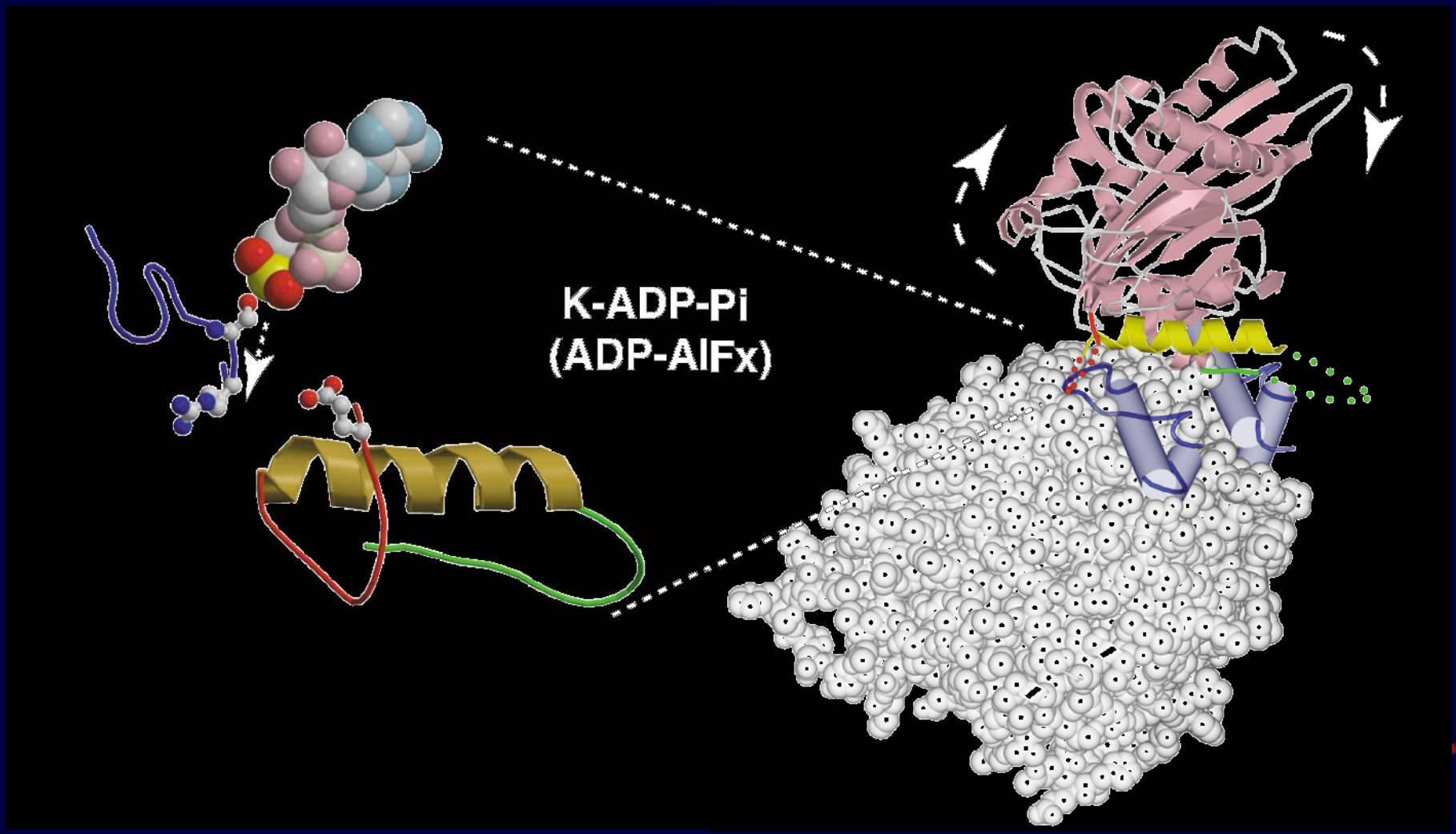


Conformational changes in the switch regions

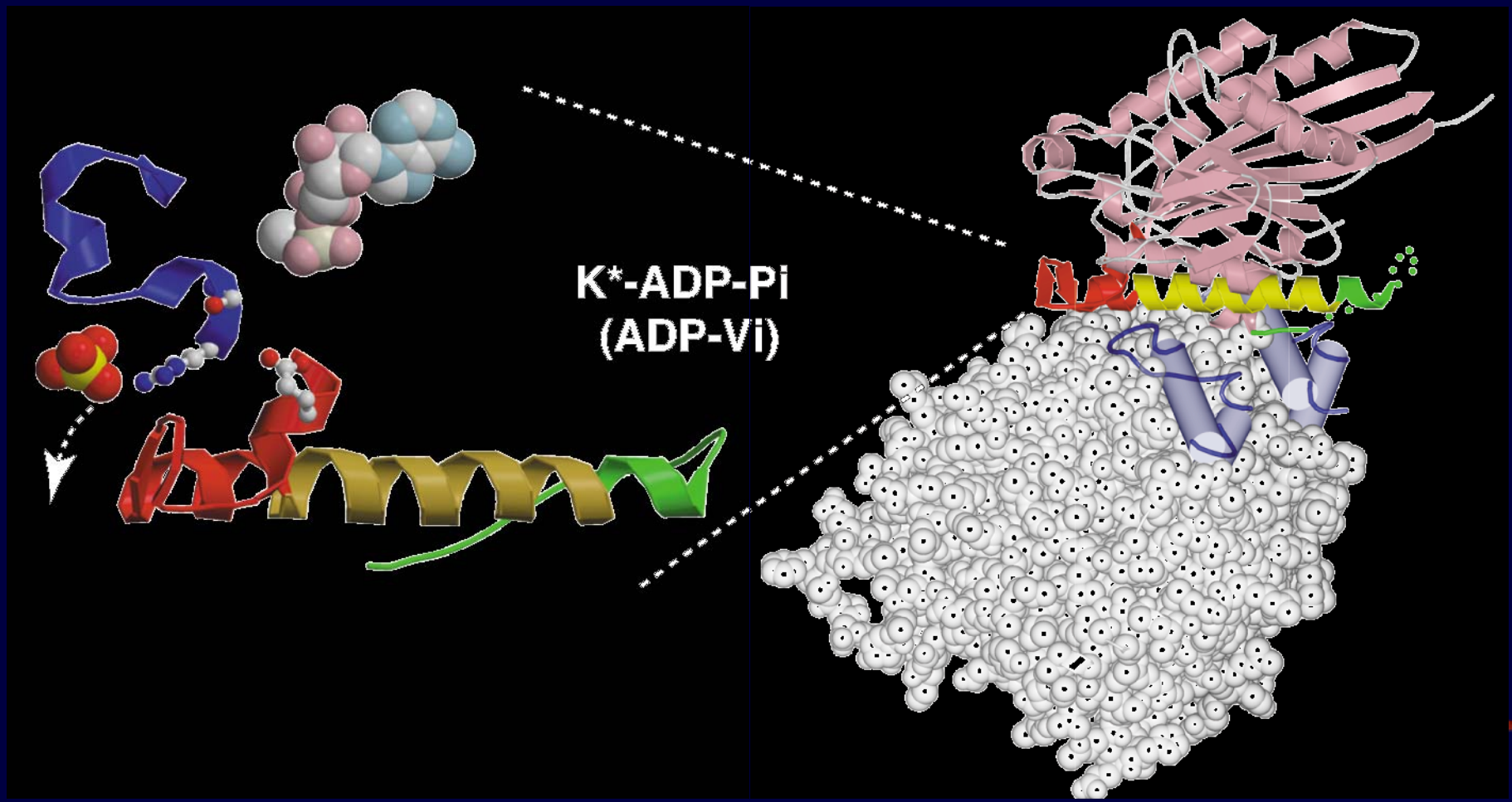
KIF1A*=ATP- Microtubule



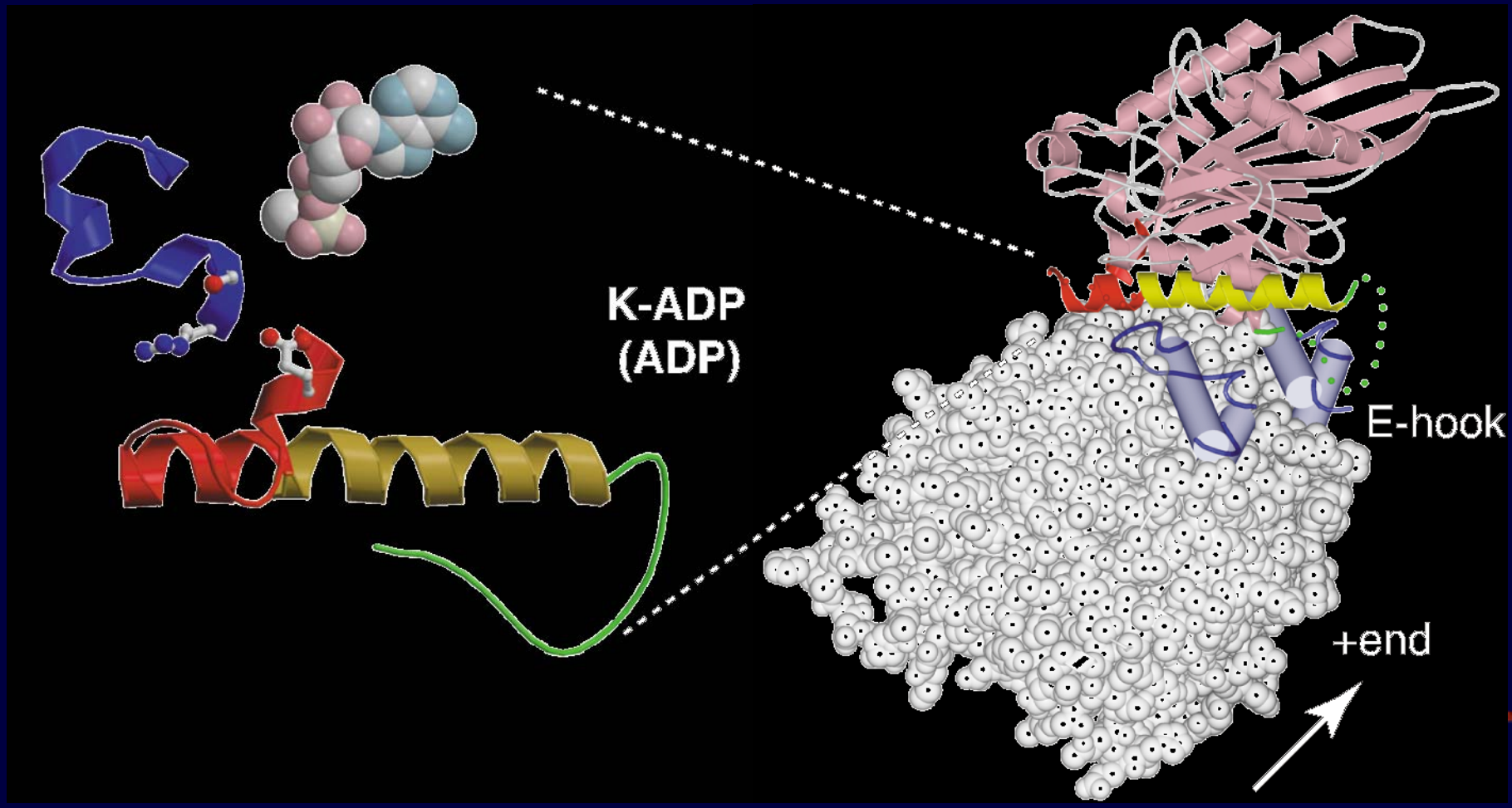
KIF1A=ADP-Pi -Microtubule



KIF1A*=ADP-Pi -Microtubule



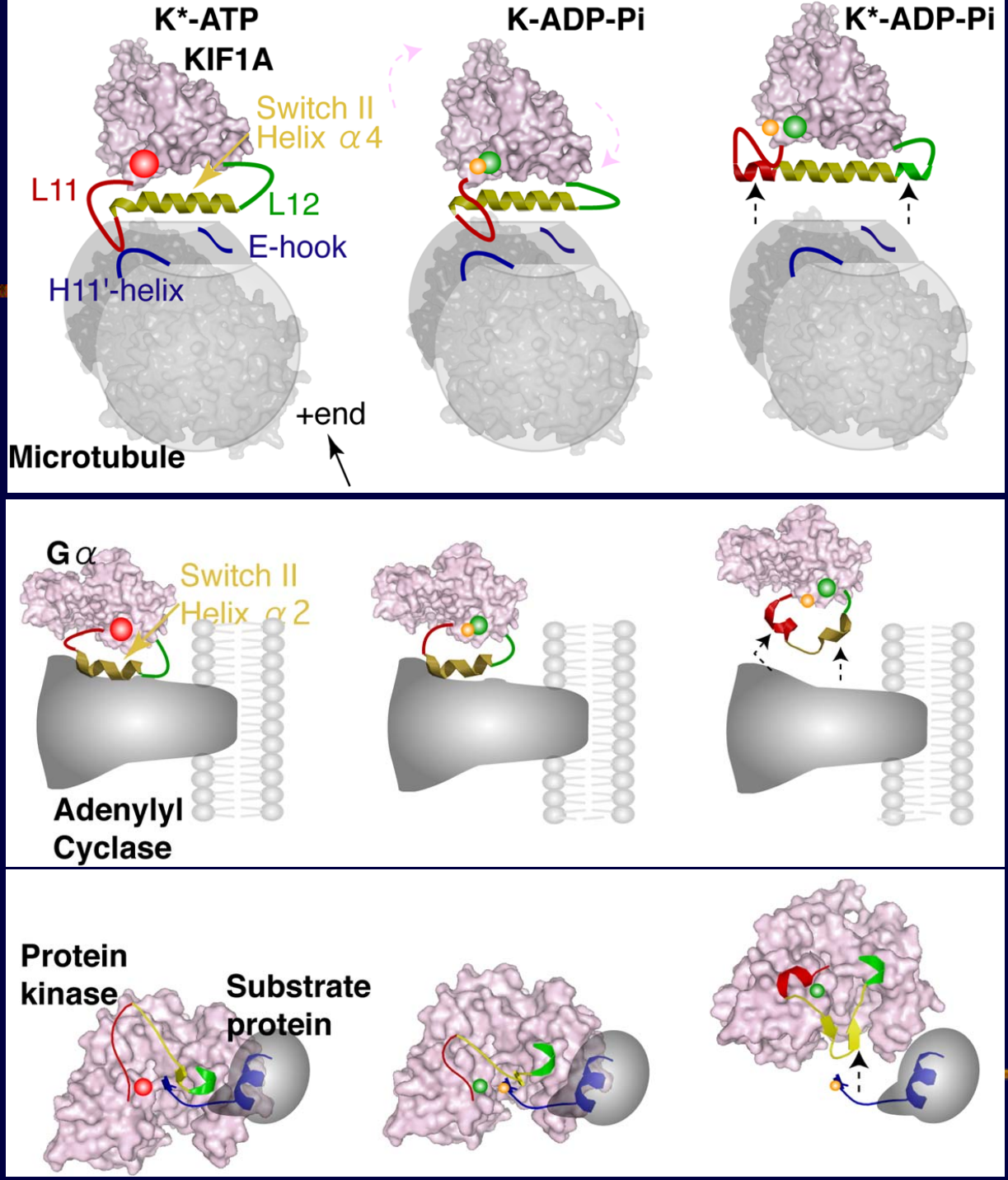
KIF1A=ADP -Microtubule



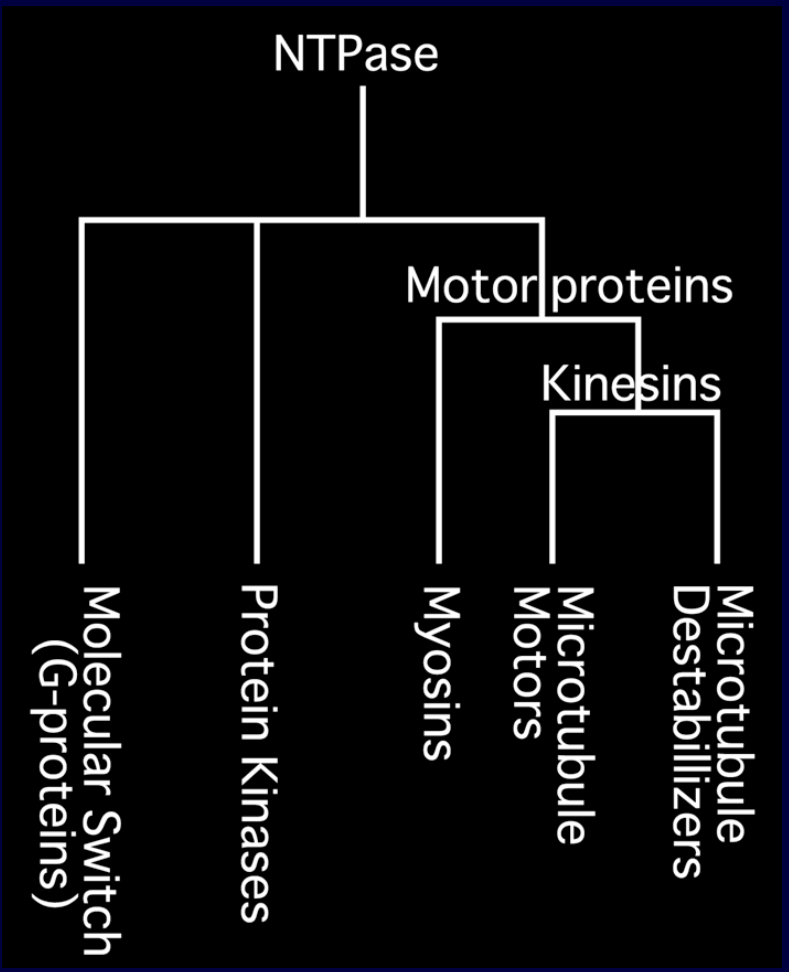
Equilibrium Binding Constants of KIF1A mutant

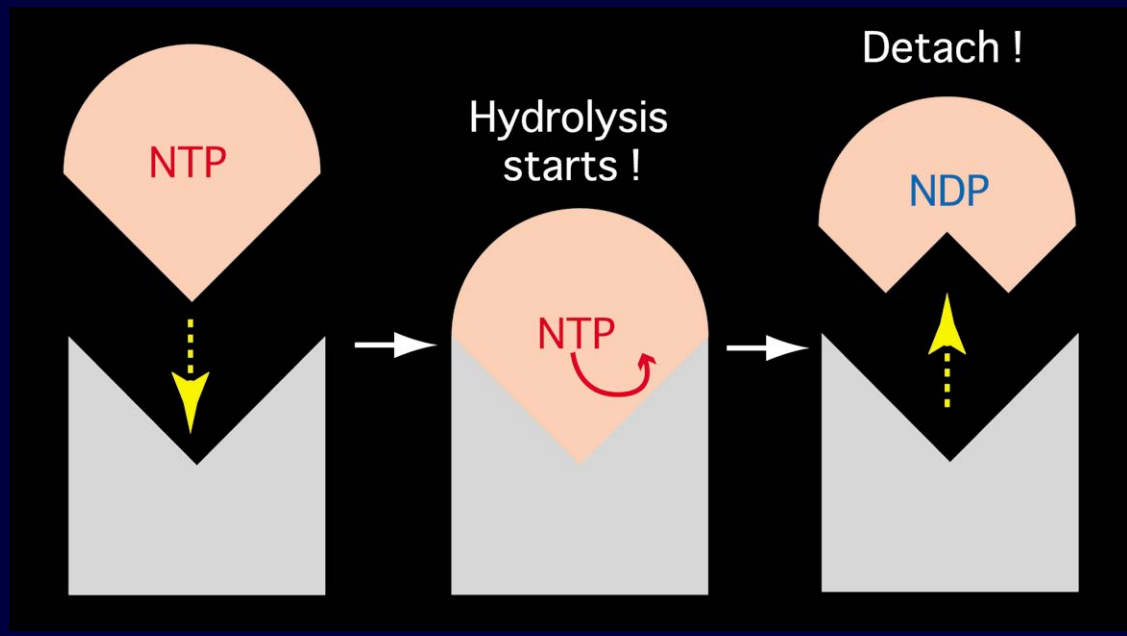
Nucleotide	construct			
	wild type	L12	L11	L8
AMPPNP	4.2±1.3	6.0±1.4	20.2±4.0	25.0±6.0
ADP	6.8±2.5	23.5±8.4	12.3±4.0	26.5±5.0
ATP	10.8±1.8	40.5±11.8	nd	nd
ADP-AlFx	5.9±1.5	7.1±1.7	nd	nd
ADP-Vi	21.4±4.3	167±66	nd	nd

Design principle of Walker-type NTPases



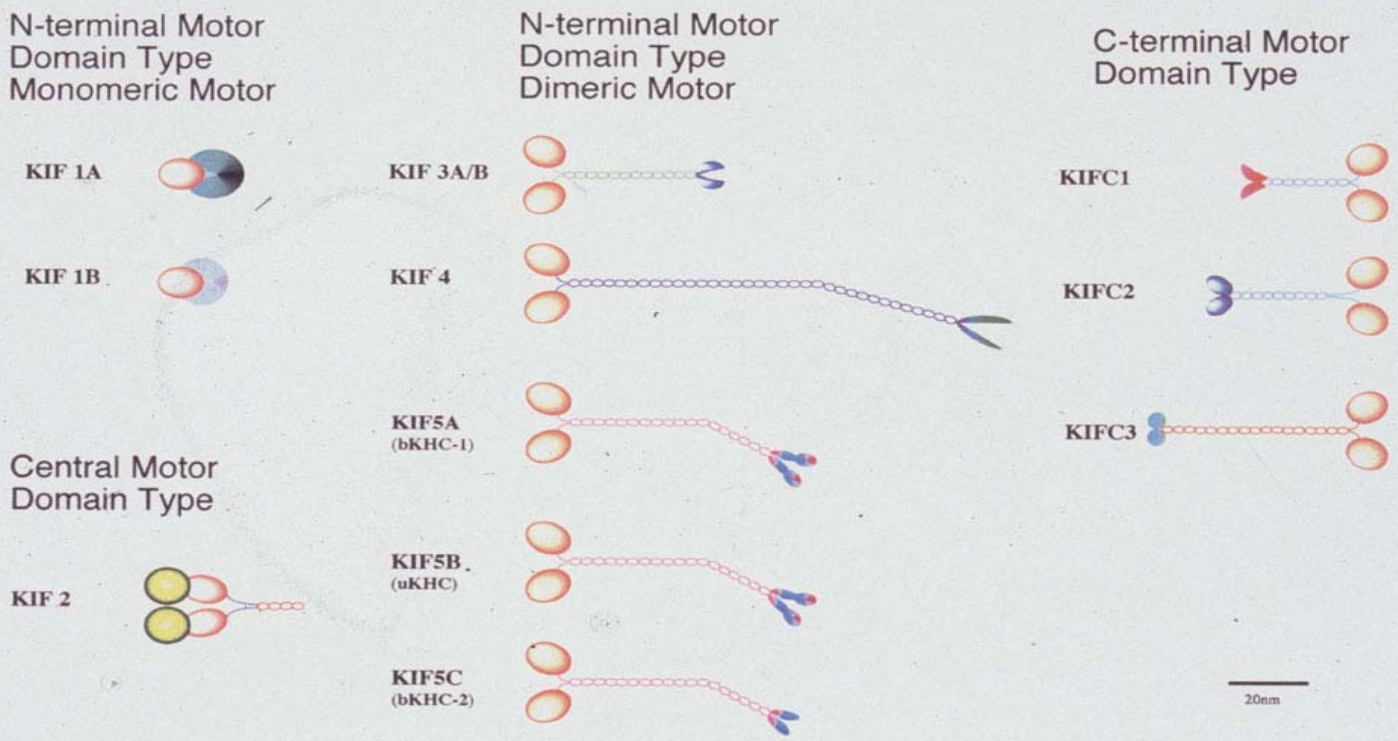
Design principle of Walker-type NTPases

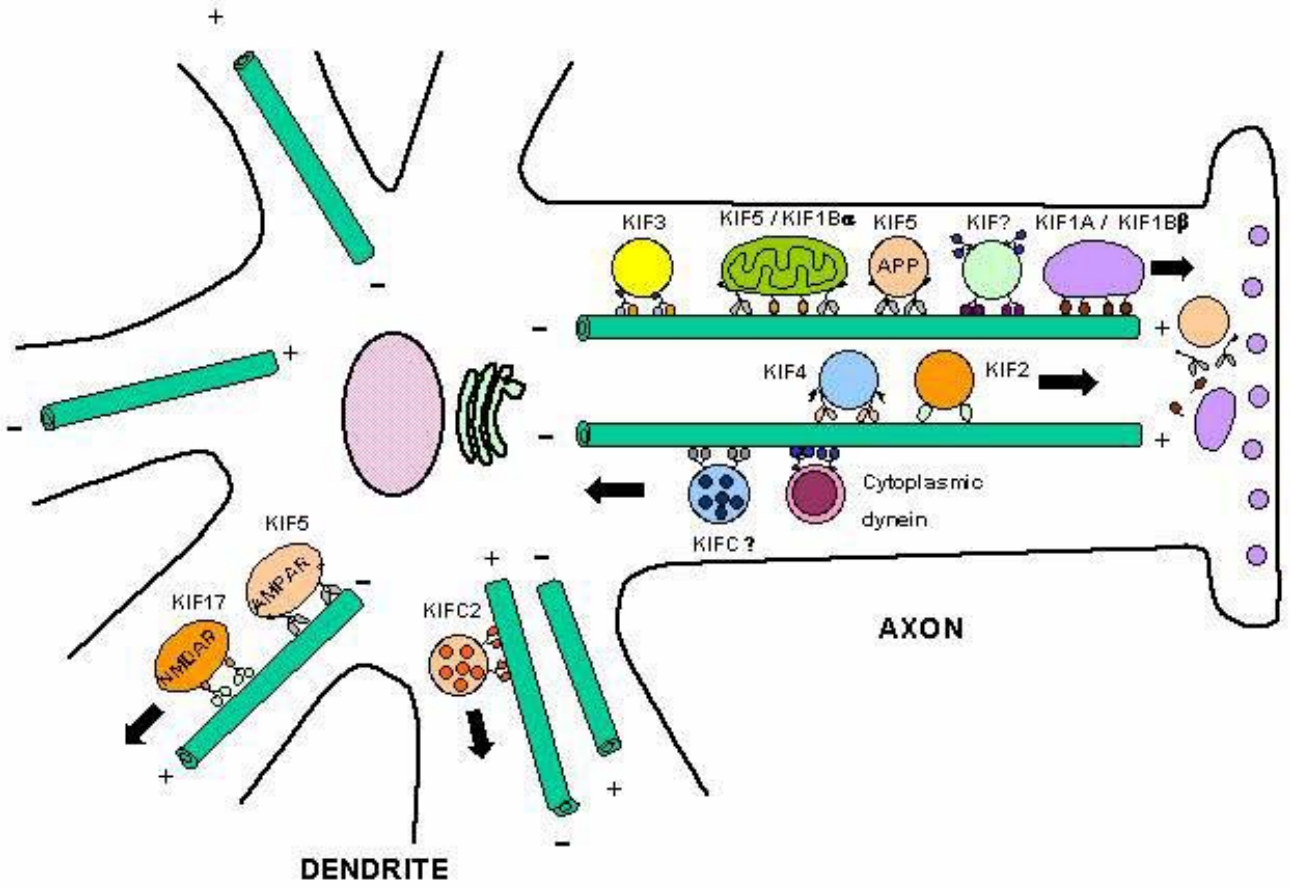




Model for the processive movement of the monomeric kinesin KIF1A

Kinesin SuperFamily Proteins in Murine CNS




KIF3
KIF5 / KIF1Bα
KIF5
APP
KIF?
KIF1A / KIF1Bβ
KIF4
KIF2
Cytoplasmic
dynein
KIFC ?
KIF5
AMPAR
KIF17
NMDAR
KIFC2
AXON
DENDRITE

Univ. Tokyo

Takao NAKATA
Yoshimitsu KANAI
Yasuko NODA
Sumio TERADA
Yosuke TAKEI
Sen TAKEDA
Yasushi OKADA
Yosuke TANAKA
Masahiko KAWAGISHI
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