

Life Science Seen from Molecular Motor

How molecular motor acts

2006.11.27.

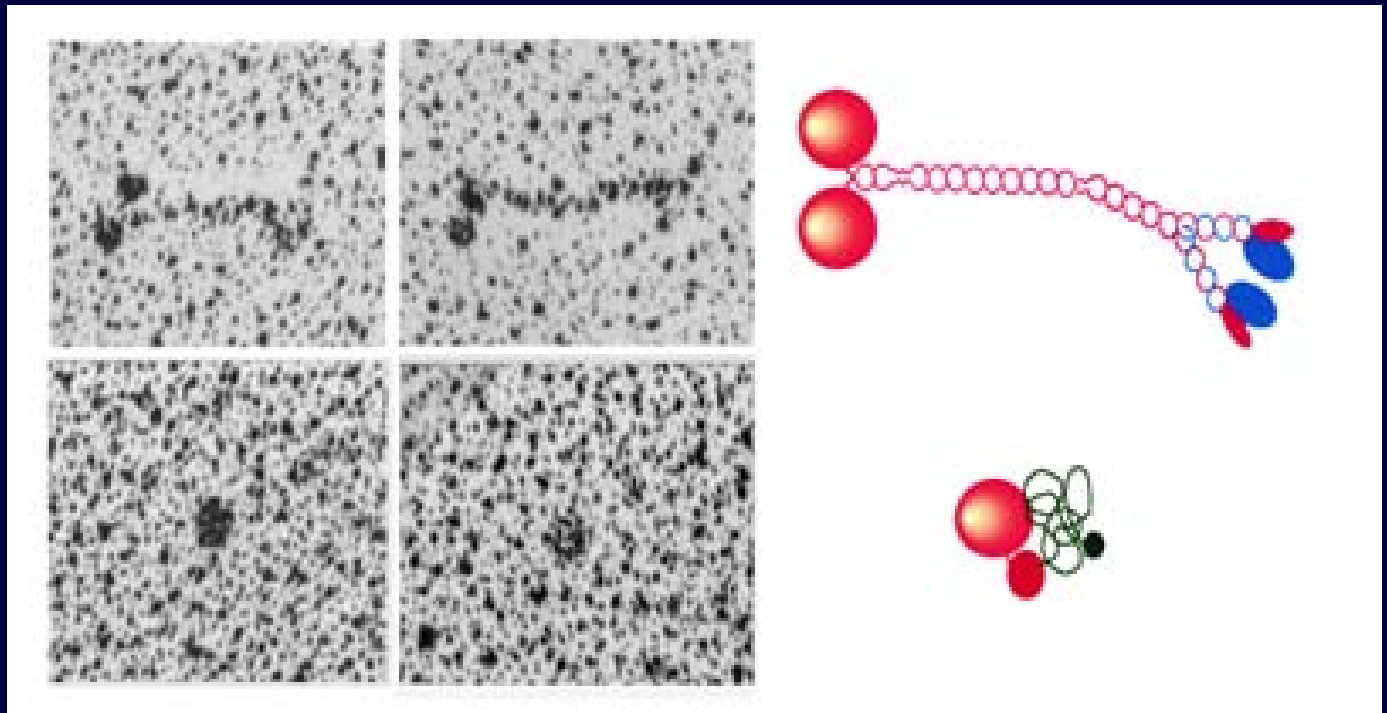
graduate school of medicine, the University of Tokyo

Nobutaka Hirokawa

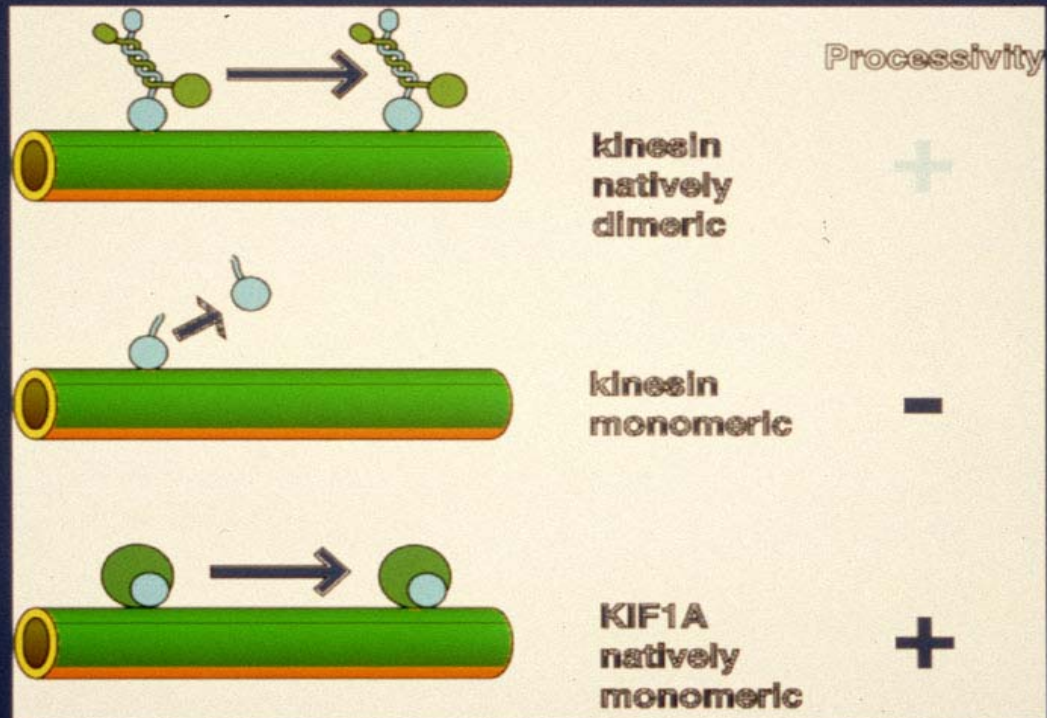
Low-angle, rotary shadowing electronmicrograph

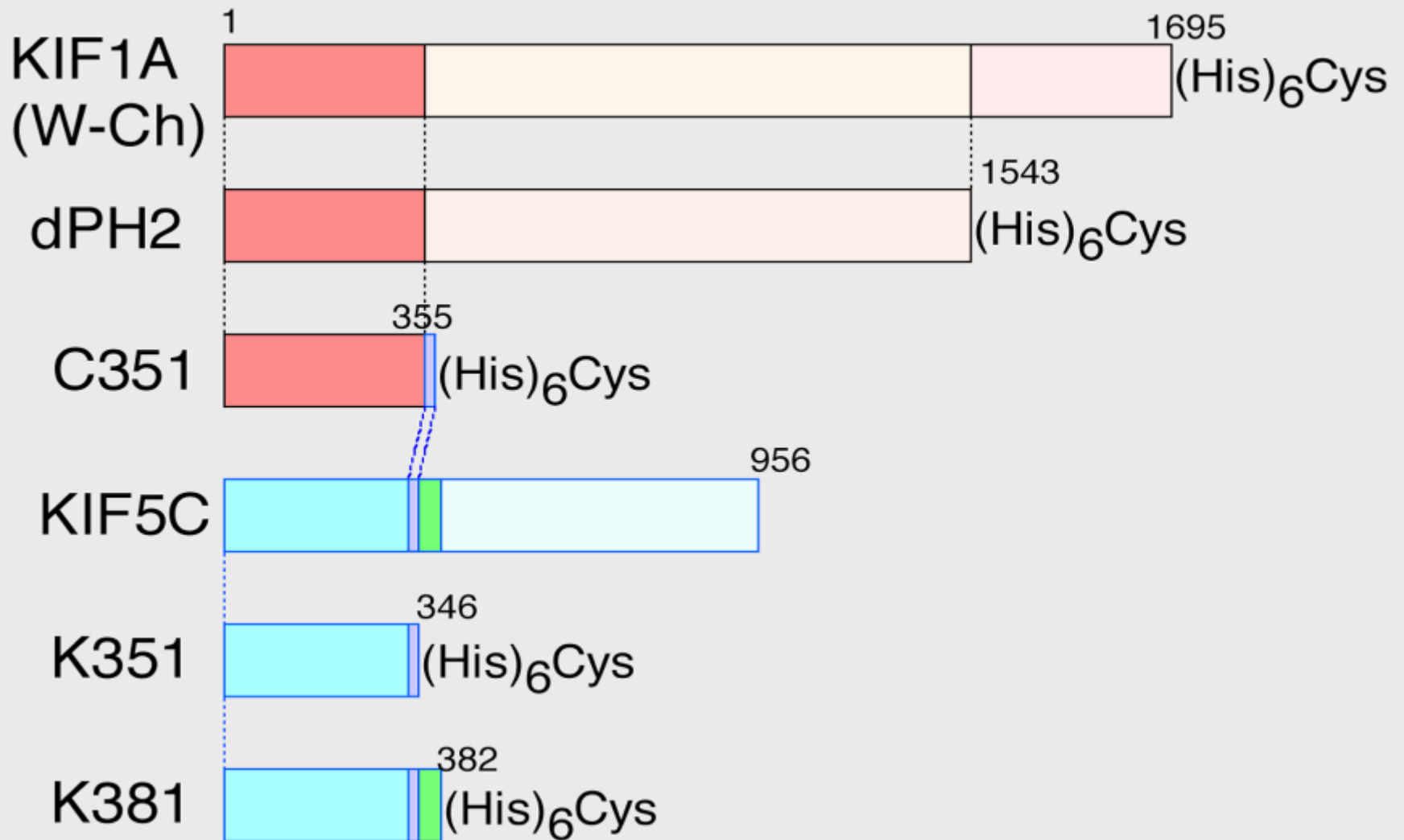
Kinesin

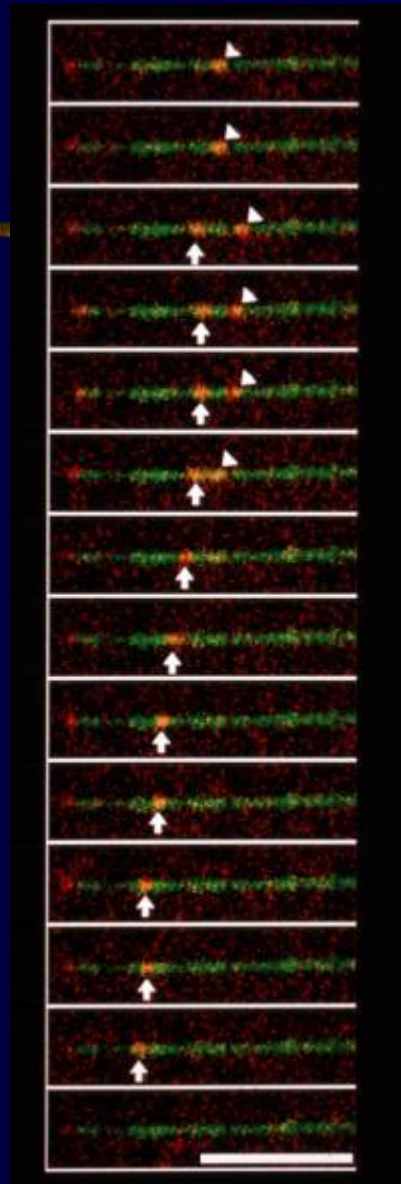
KIF1A



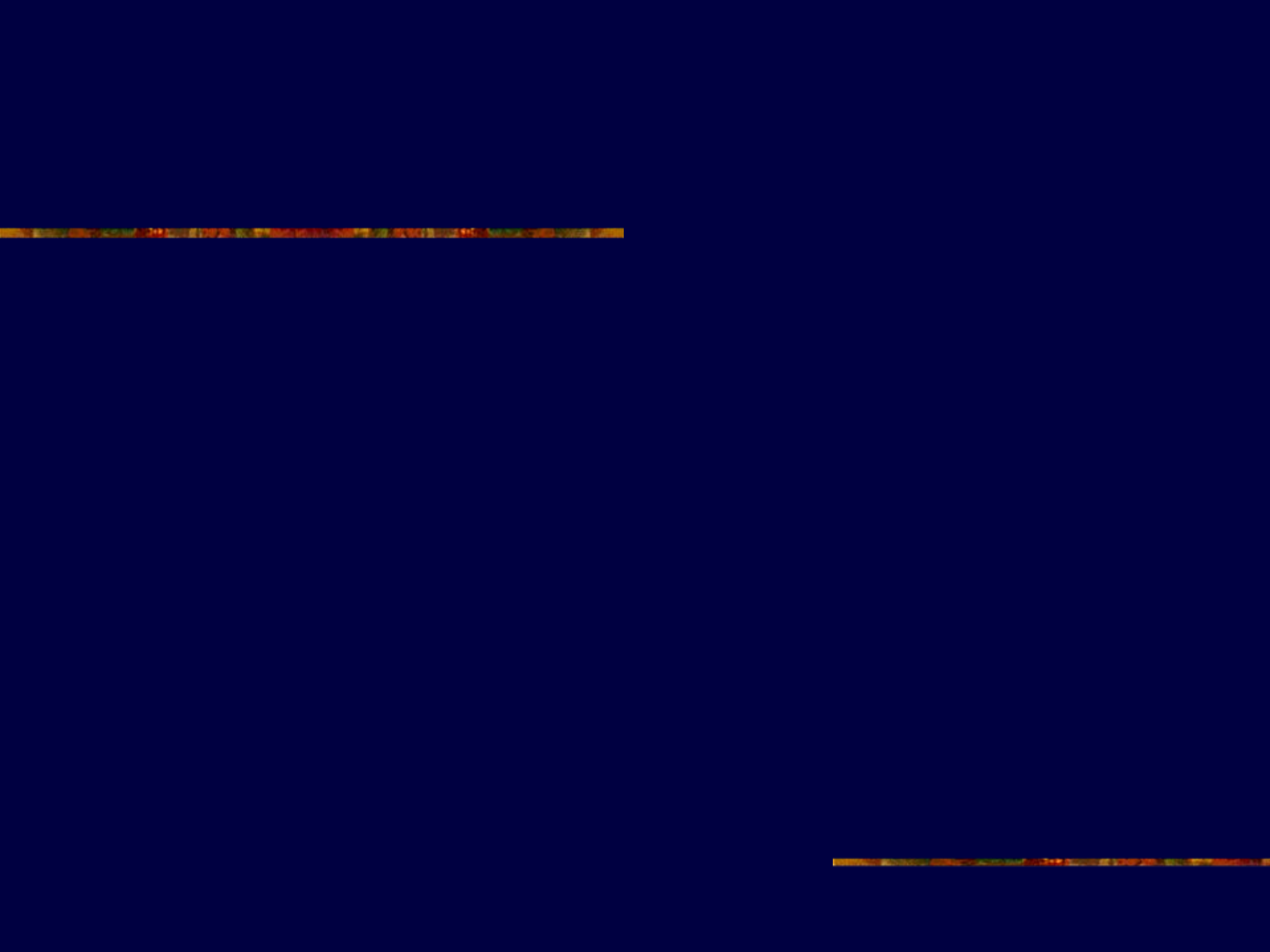
Question

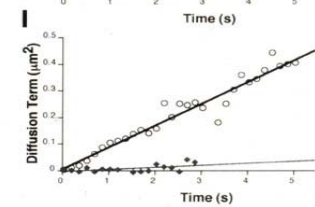
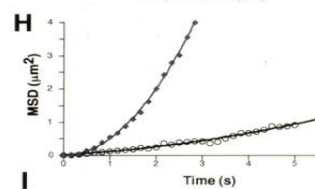
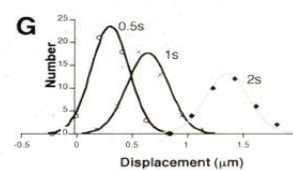
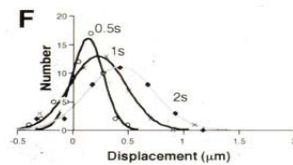
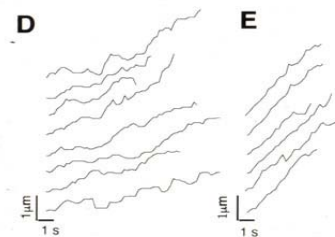
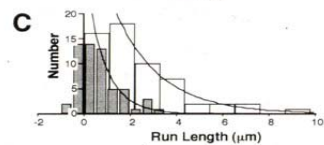
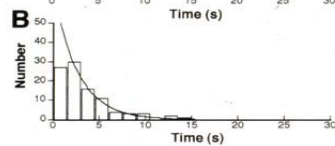
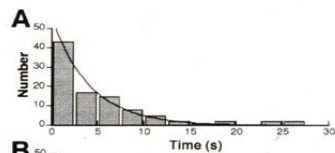




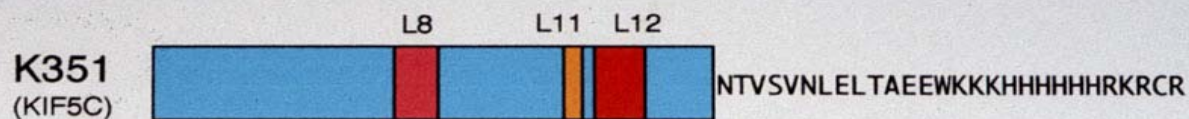


Okada et al. Science 283:1152—, 1999





C351	1	M	A	G	S	V	K	V	S	V	A	V	R	V	R	P	F	N	S	R	D	S	R	S	K	C	I	27				
K351	1	M	A	D	P	A	E	C	S	I	K	V	M	C	R	F	R	P	L	N	E	A	E	I	L	R	G	30				
K381	1	M	A	D	P	A	E	C	S	I	K	V	M	C	R	F	R	P	L	N	E	A	E	I	L	R	G	30				
C351	28	I	Q	M	S	G	E	T	T	T	I	V	N	P	K	Q	P	K	E	T	P	K	S	F	S	F	D	57				
K351	31	P	K	F	K	G	E	E	T	V	V	I	G	Q	G	K	P	-	-	-	-	-	-	Y	V	F	D	54				
K381	31	P	K	F	K	G	E	E	T	V	V	I	G	Q	G	K	P	-	-	-	-	-	-	Y	V	F	D	54				
C351	58	S	H	T	S	P	E	D	I	N	Y	A	S	Q	K	Q	V	Y	R	D	I	G	E	E	M	L	Q	87				
K351	55	P	N	T	T	Q	E	Q	V	Y	N	A	C	A	K	Q	I	V	K	D	-	-	-	-	-	-	V	L	76			
K381	55	P	N	T	T	Q	E	Q	V	Y	N	A	C	A	K	Q	I	V	K	D	-	-	-	-	-	-	V	L	76			
C351	88	G	Y	N	V	C	I	F	A	Y	G	Q	T	G	A	G	K	S	Y	T	M	M	G	K	Q	E	K	116				
K351	77	G	Y	N	G	T	I	F	A	Y	G	Q	T	S	S	G	K	T	H	T	M	E	G	K	L	H	D	106				
K381	77	G	Y	N	G	T	I	F	A	Y	G	Q	T	S	S	G	K	T	H	T	M	E	G	K	L	H	D	106				
C351	117	G	I	P	Q	L	E	D	I	F	S	R	I	N	D	T	N	D	N	M	S	Y	S	V	I	E	V	135				
K351	107	G	I	P	R	I	A	H	D	I	F	D	H	I	Y	S	-	M	D	E	N	L	E	F	H	I	K	146				
K381	107	G	I	P	R	I	A	H	D	I	F	D	H	I	Y	S	-	M	D	E	N	L	E	F	H	I	K	146				
C351	147	M	E	I	Y	L	C	E	R	V	R	D	L	L	N	P	K	N	K	G	N	L	R	V	R	E	H	176				
K351	136	F	E	I	Y	L	D	K	I	R	D	L	L	D	V	S	-	K	T	N	L	A	V	H	E	D	K	164				
K381	136	F	E	I	Y	L	D	K	I	R	D	L	L	D	V	S	-	K	T	N	L	A	V	H	E	D	K	164				
C351	177	Y	V	E	D	L	S	K	L	A	V	T	S	Y	N	D	I	Q	D	L	M	D	S	G	N	K	P	206				
K351	165	Y	V	K	G	C	T	E	R	F	V	S	S	P	E	E	V	M	D	V	I	D	E	G	K	A	N	194				
K381	165	Y	V	K	G	C	T	E	R	F	V	S	S	P	E	E	V	M	D	V	I	D	E	G	K	A	N	194				
C351	207	A	T	N	M	N	E	T	S	S	R	S	H	A	V	F	N	I	I	F	T	Q	K	R	H	D	A	236				
K351	195	V	T	N	M	N	E	H	S	S	R	S	H	S	I	F	L	N	I	N	I	K	Q	E	N	V	E	224				
K381	195	V	T	N	M	N	E	H	S	S	R	S	H	S	I	F	L	N	I	N	I	K	Q	E	N	V	E	224				
C351	237	T	T	E	K	V	S	K	I	S	L	V	D	L	A	G	S	E	R	A	D	S	T	G	A	K	G	266				
K351	225	S	-	-	-	G	K	L	Y	L	V	D	L	A	G	S	E	K	V	S	K	T	G	A	E	G	A	250				
K381	225	S	-	-	-	G	K	L	Y	L	V	D	L	A	G	S	E	K	V	S	K	T	G	A	E	G	A	250				
C351	267	E	G	A	N	I	N	K	S	L	T	T	L	G	K	V	I	S	A	L	A	E	G	M	D	S	G	292				
K351	251	E	A	K	N	I	N	K	S	L	T	T	L	G	N	V	I	S	A	L	A	E	G	-	-	-	-	276				
K381	251	E	A	K	N	I	N	K	S	L	T	T	L	G	N	V	I	S	A	L	A	E	G	-	-	-	-	272				
C351	297	K	K	K	K	T	D	F	I	P	Y	R	D	S	V	L	T	W	L	L	R	E	N	L	G	G	N	326				
K351	273	-	-	-	-	T	K	T	H	V	P	Y	R	D	S	K	M	T	R	I	L	Q	D	S	L	G	G	N	299			
K381	273	-	-	-	-	T	K	T	H	V	P	Y	R	D	S	K	M	T	R	I	L	Q	D	S	L	G	G	N	299			
C351	327	M	V	A	A	L	S	P	A	D	I	N	Y	D	E	T	L	S	T	L	M	Y	A	D	R	A	K	356				
K351	300	I	V	I	C	C	S	P	S	V	F	N	E	A	E	T	K	S	T	L	M	F	G	Q	R	A	K	329				
K381	300	I	V	I	C	C	S	P	S	V	F	N	E	A	E	T	K	S	T	L	M	F	G	Q	R	A	K	329				
C351	357	T	V	S	V	N	L	E	L	T	A	E	E	W	K	K	K	H	-	-	-	-	-	-	-	-	-	373				
K351	330	T	V	S	V	N	L	E	L	T	A	E	E	W	K	K	K	H	-	-	-	-	-	-	-	-	-	346				
K381	330	T	V	S	V	N	L	E	L	T	A	E	E	W	K	K	K	H	-	-	-	-	-	-	-	-	-	359				
		Neck of Conventional Kinesin																														
C351	374	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	H	H	H	380	
K351	347	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	H	H	H	353	
K381	360	L	Q	H	E	M	E	L	N	R	W	R	N	G	E	A	V	P	E	D	E	Q	I	-	-	H	H	H	H	H	H	389
		Coiled Coil Region for Dimerization																														
C351	381	K	R	C	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	384	
K351	354	K	R	C	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	357	
K381	390	K	R	C	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	393	
		Reactive Cystein																														



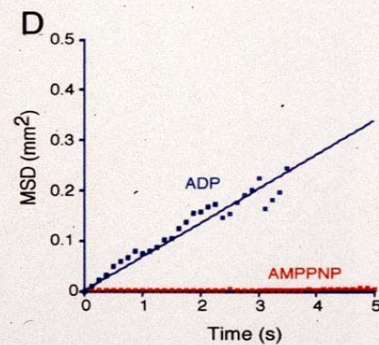
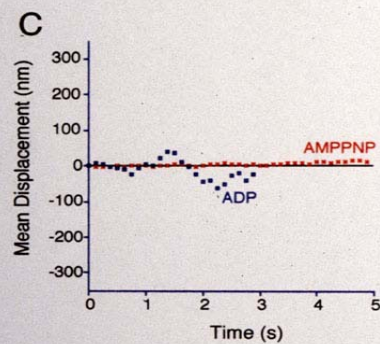
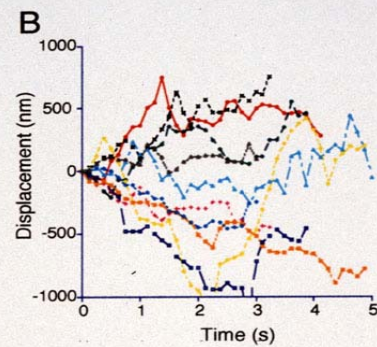
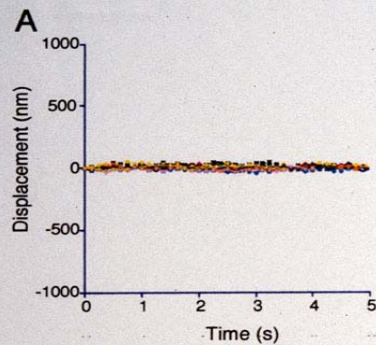
KK1(=K351) ALGNVISALAE GTKT HVPYRDSKMTRIL
 KK7 GTKTPNKNKKKKKTD
 KK6 MDSGPNKNKKKKKTD



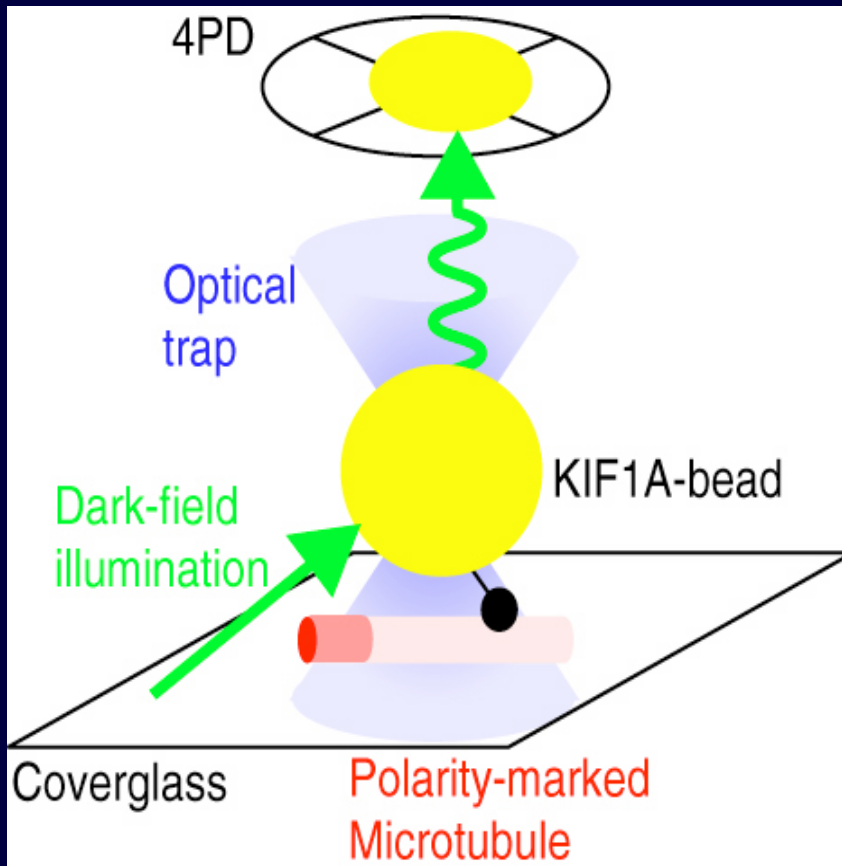
CK6 (=KIF1A) TLGKVISALAE MDSGPNKNKKKKKTD FIPYRDSVLTWLL
 CK1 (←KIF5C) GTKT
 CK2 (←KIF4) DKKGN
 CK4 (←KIF1D) LQSKKRKSD



CCa TLGKVISALAE MDSGPNKKKKCKKTD FIPYRDSVLTWLL
 CCb MDSGPNKNKKKKKCD



Optical Trapping Nanometry



Spatial resolution $< 0.2 \text{ nm}$
Temporal resolution $< 1 \text{ ms}$

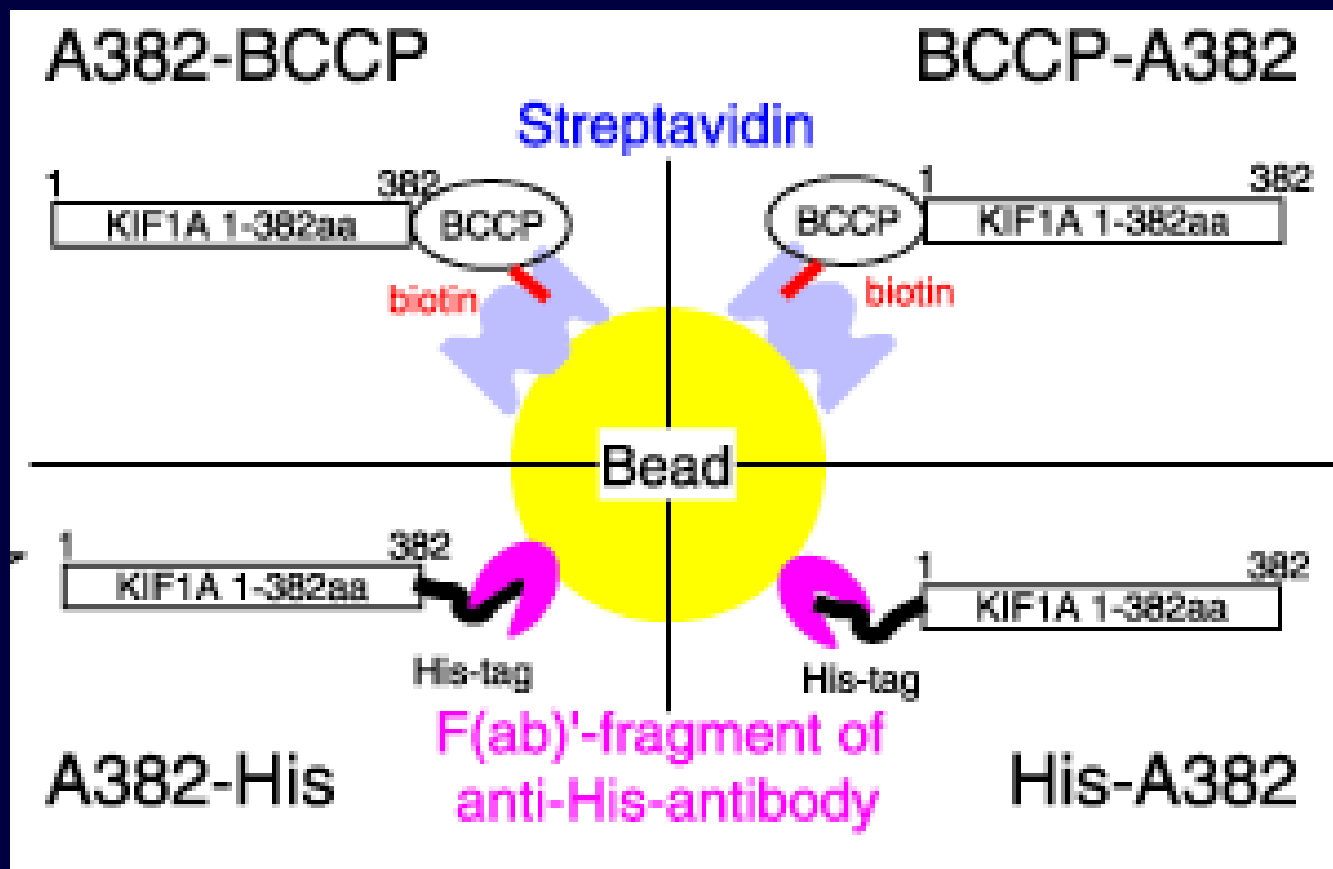
$0.2 \mu\text{m}$ beads

$\Rightarrow$ Small viscous drag

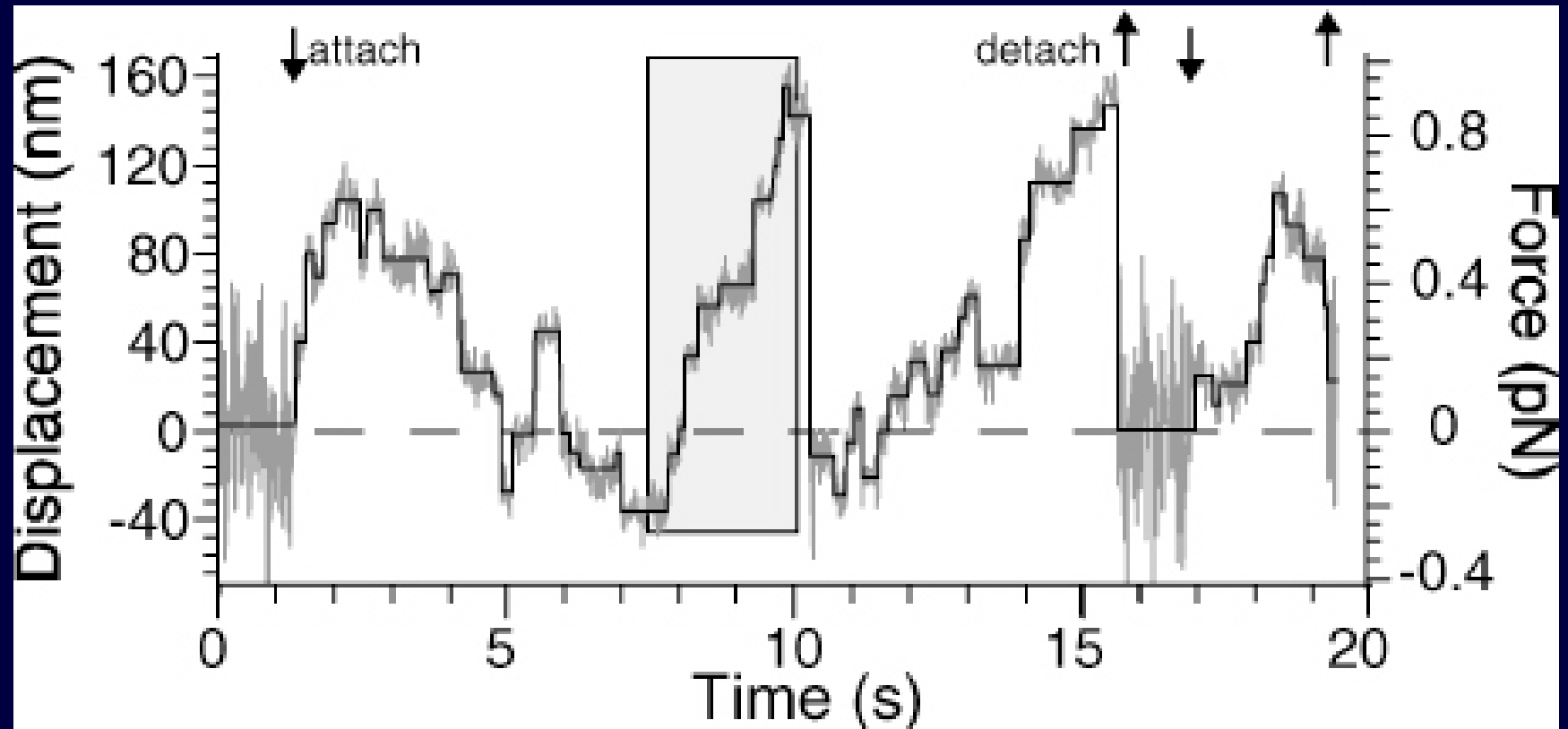
$\Rightarrow$ High temporal
resolution

(Response time $\leq 0.25 \text{ ms}$)

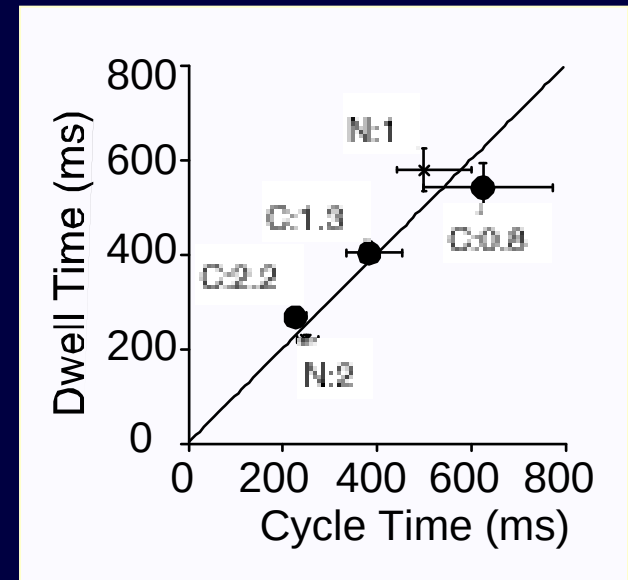
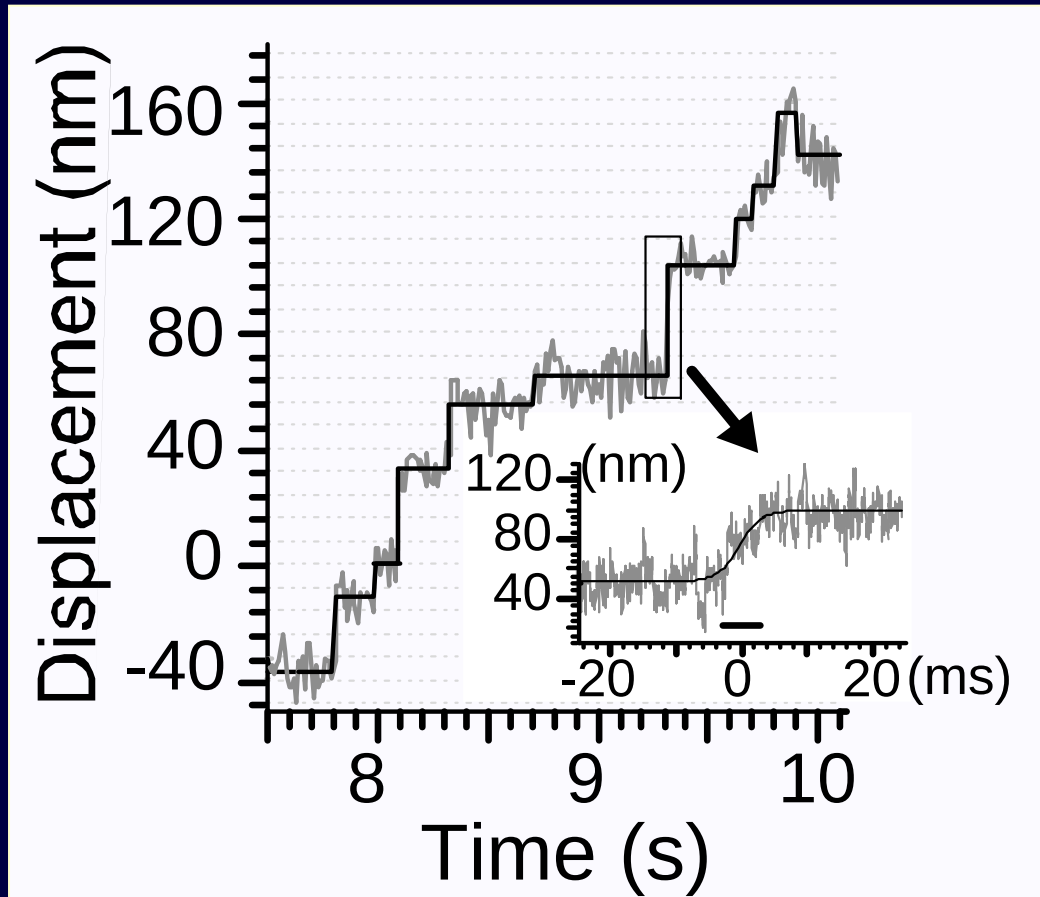
Immobilization of KIF1A to Bead



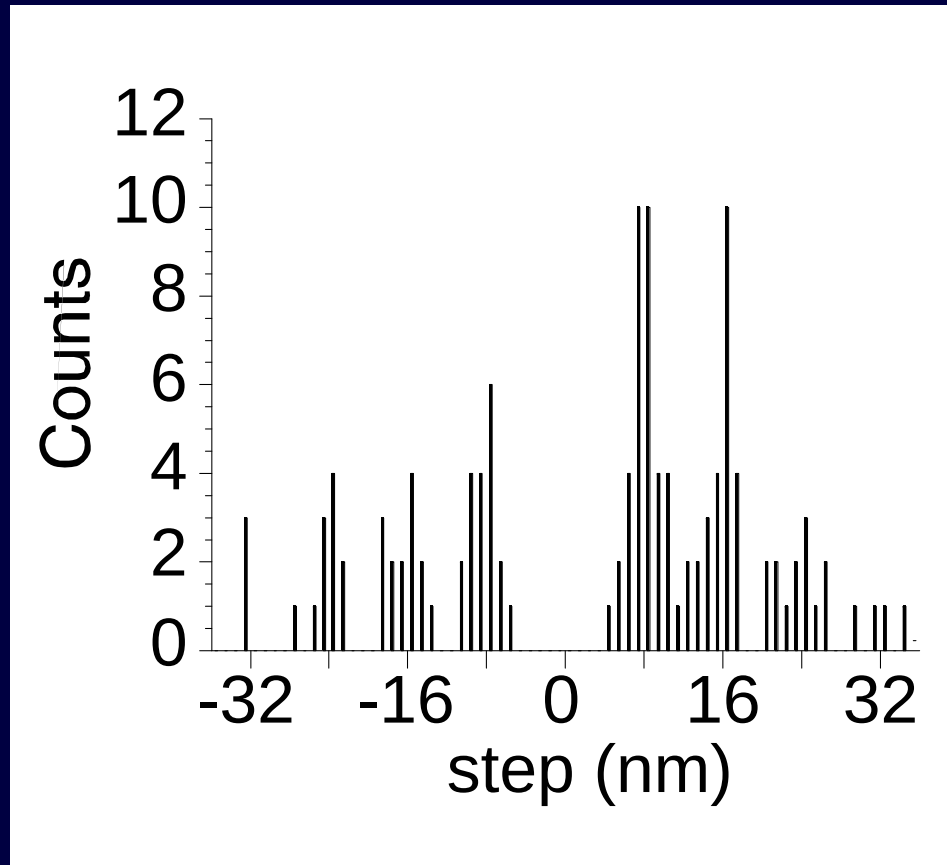
Bidirectional and Step-wise Movement of a Single KIF1A Monomer



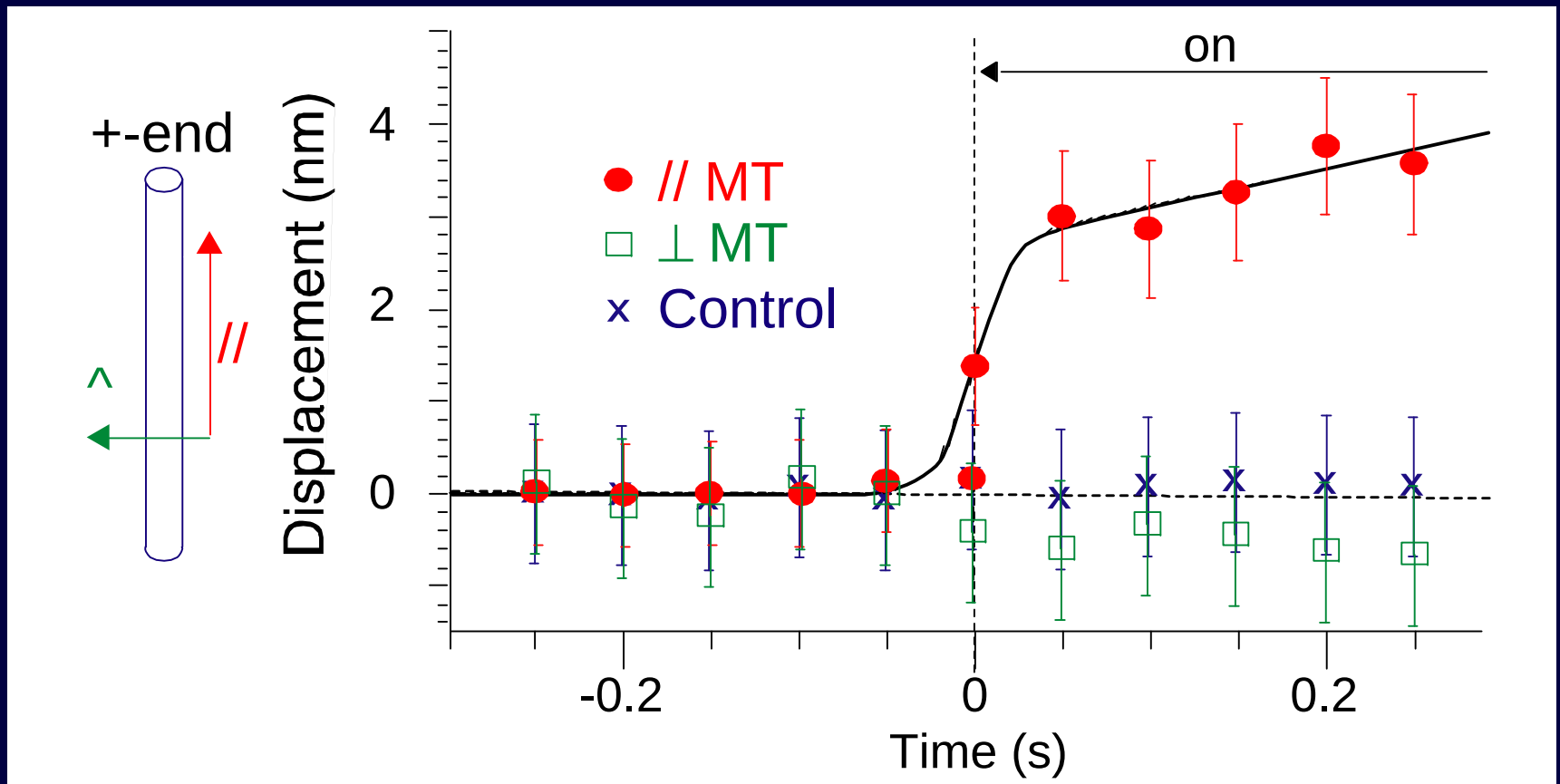
One ATP Hydrolysis Triggers One Stepping Movement.



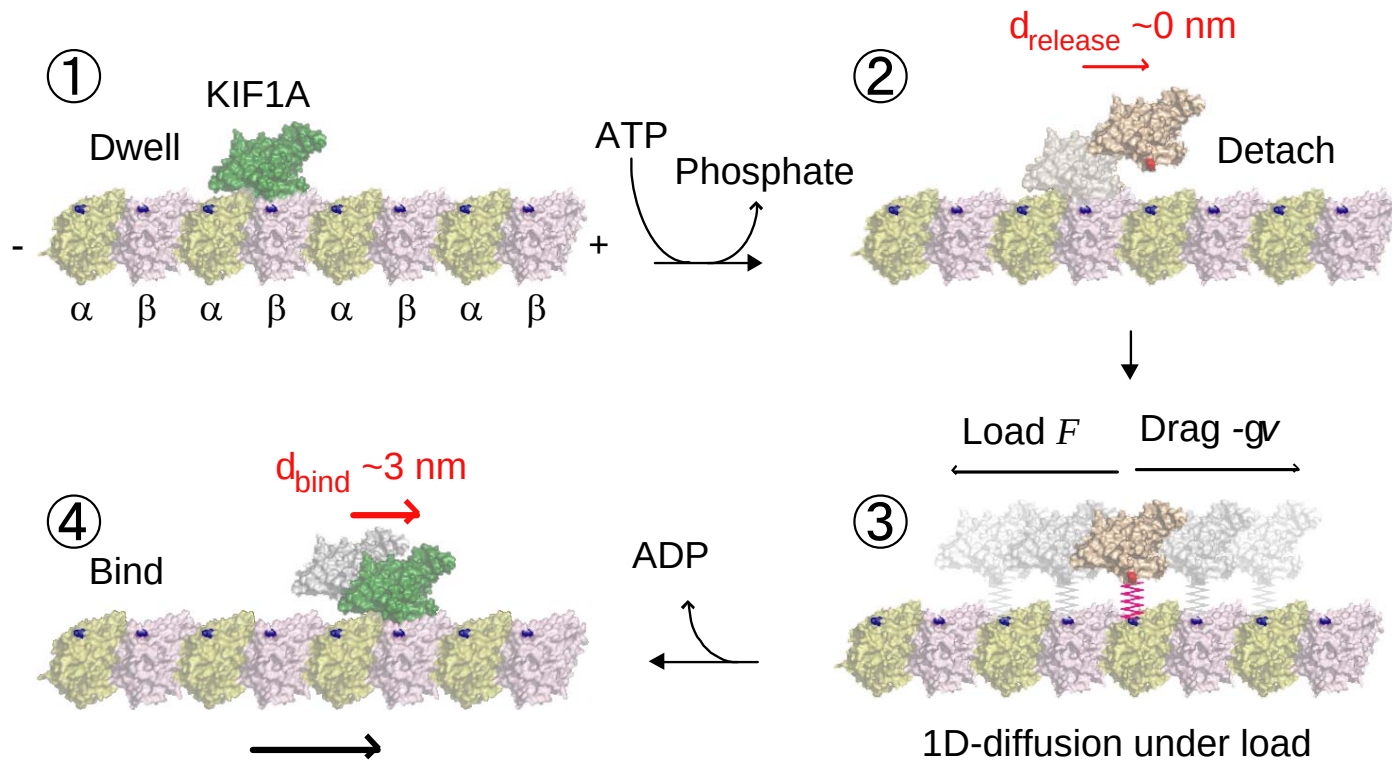
Step size of KIF1A is $8 \times n$ nm,
and varies stochastically.



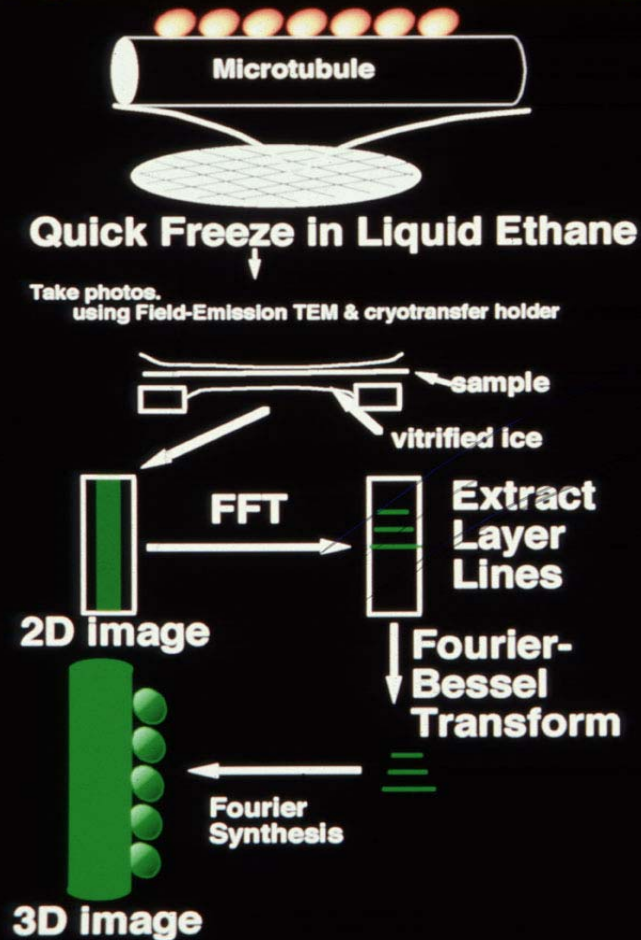
Plus-end biased binding of KIF1A to the microtubule: ensemble average



Flush Ratchet Model



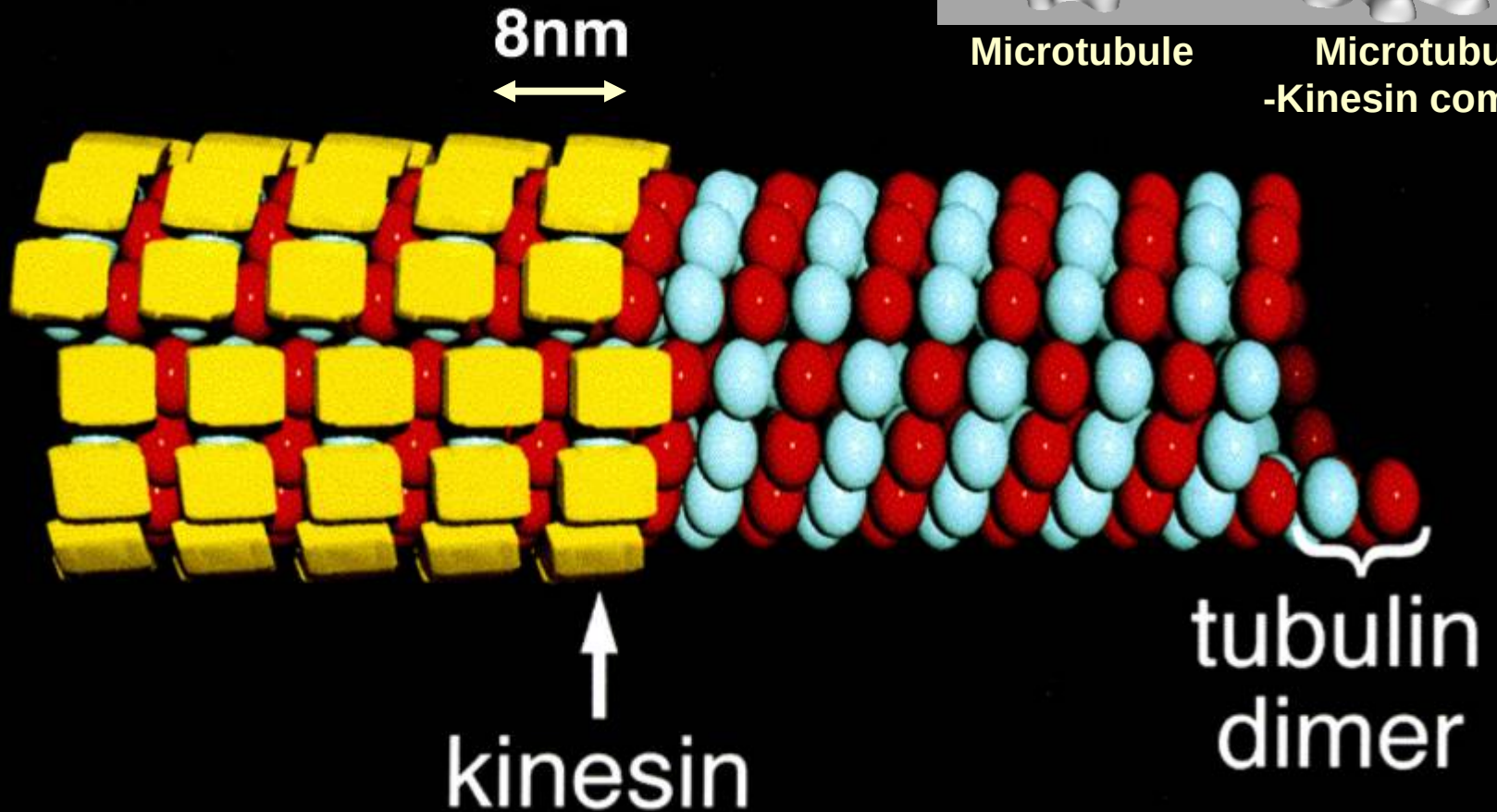
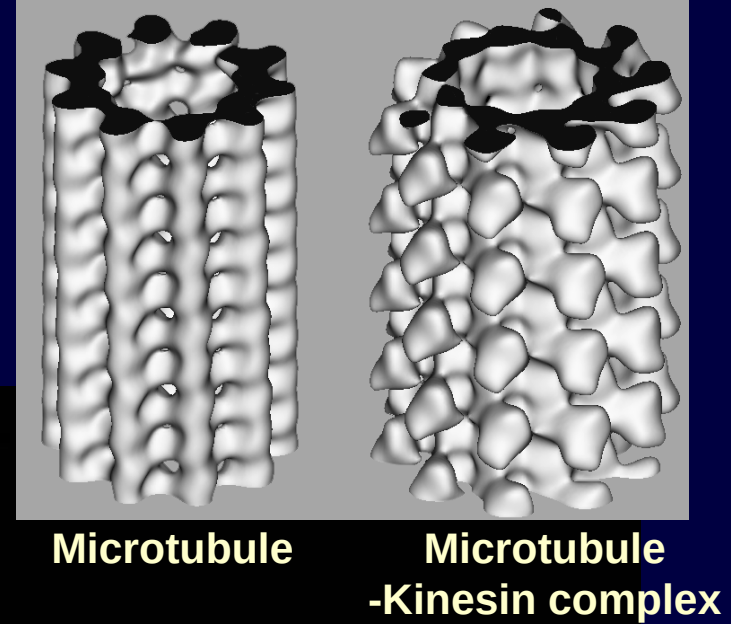
Cryo-electron Microscopy



Microtubule Structure

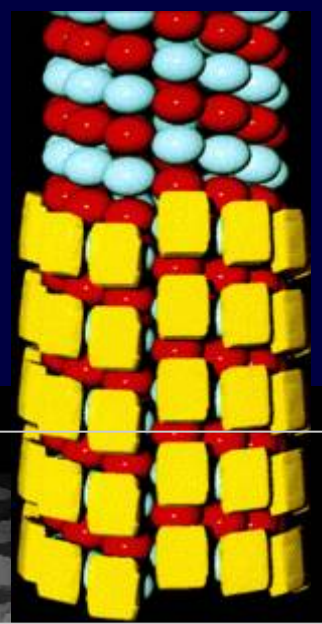
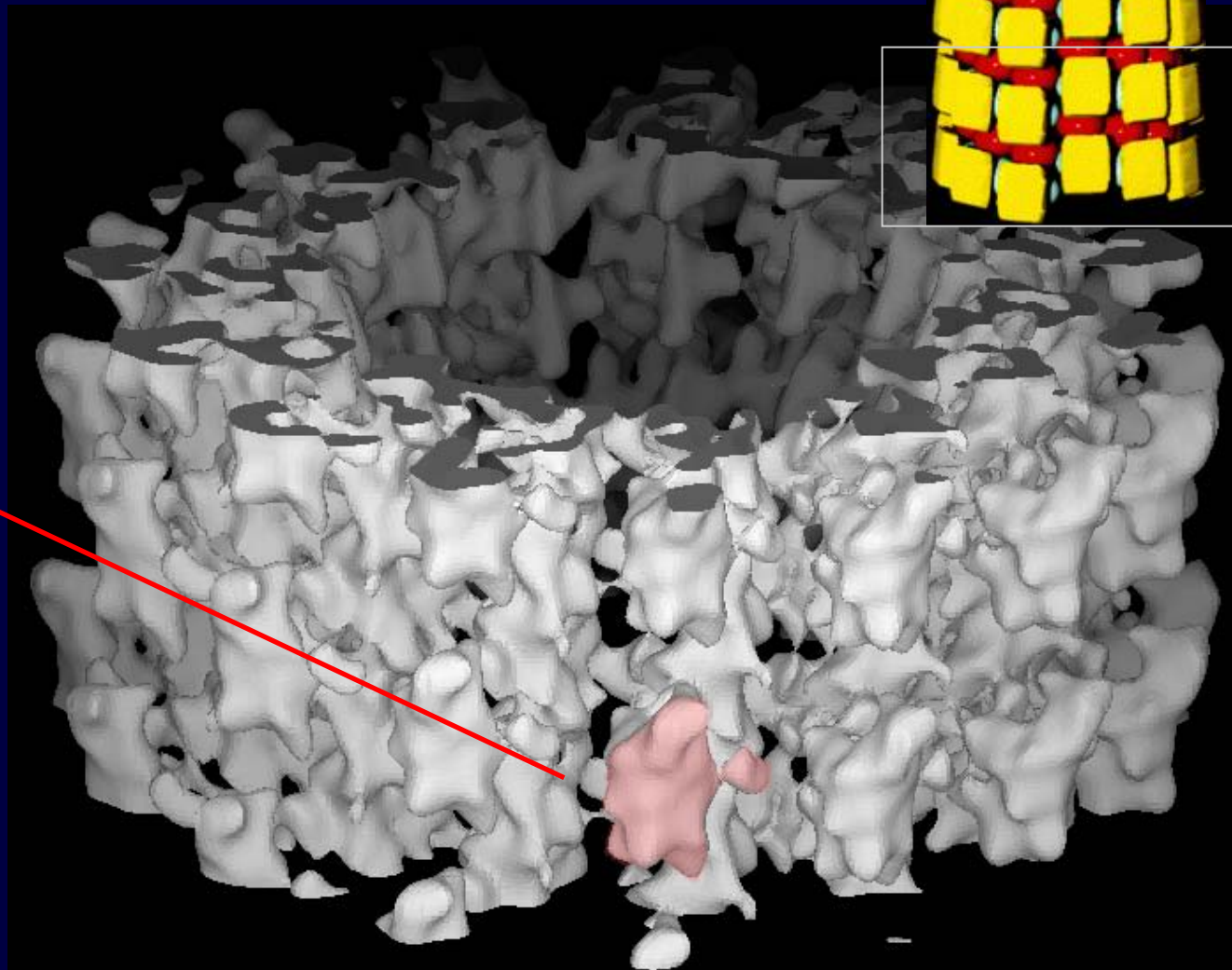
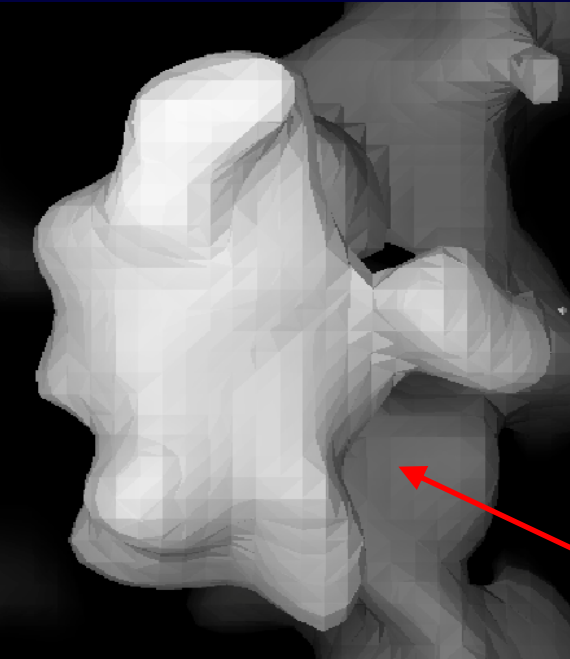
Kikkawa et al.

JCB, 1994; Nature, 1995

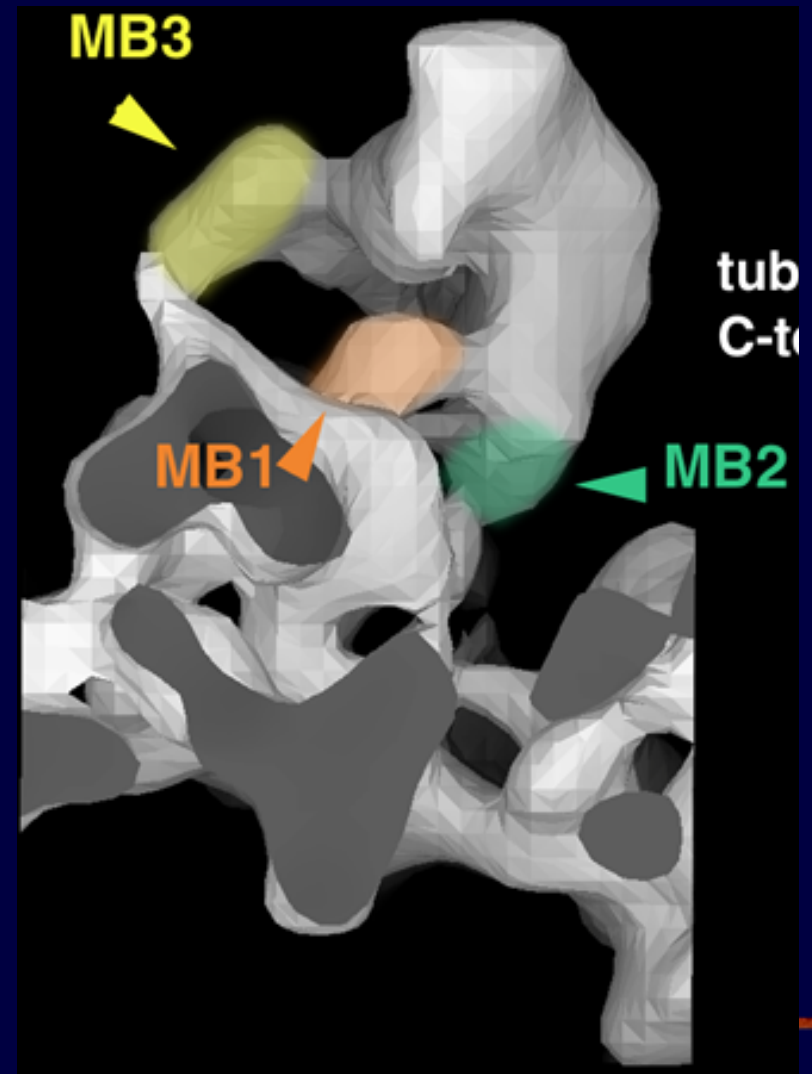
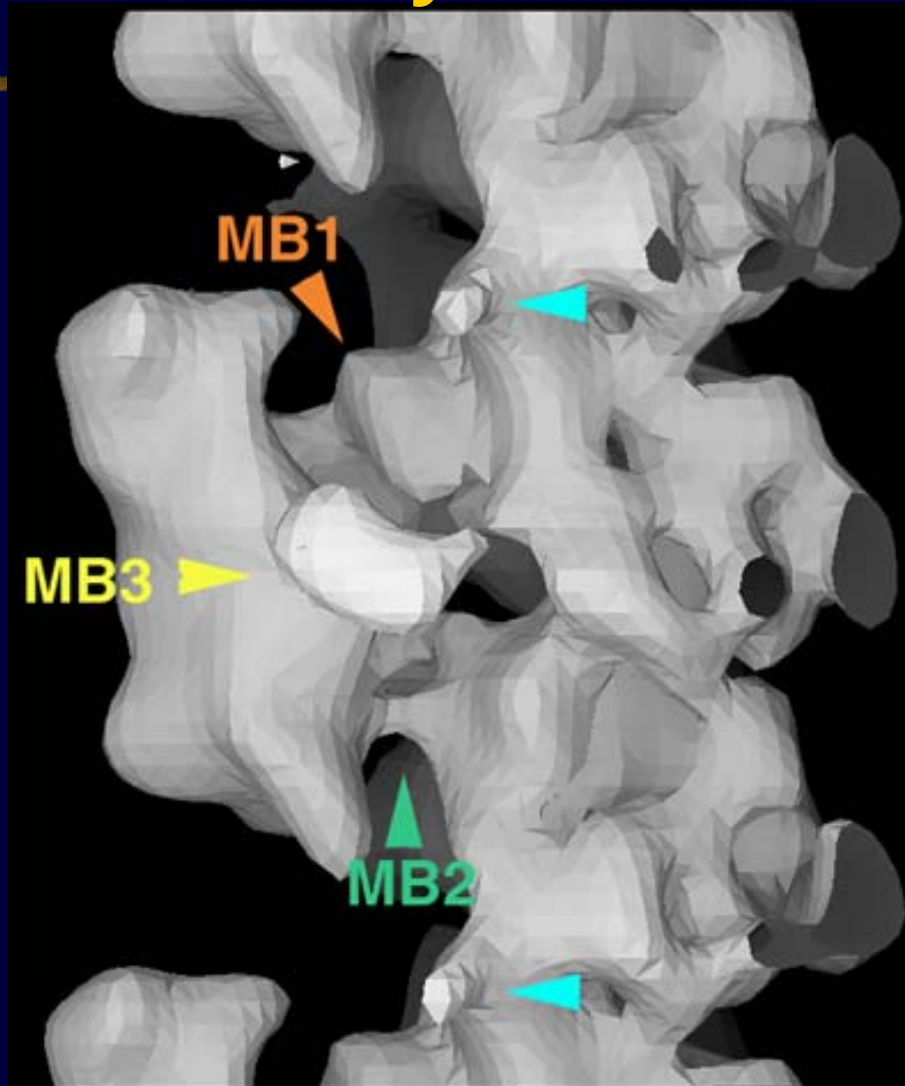


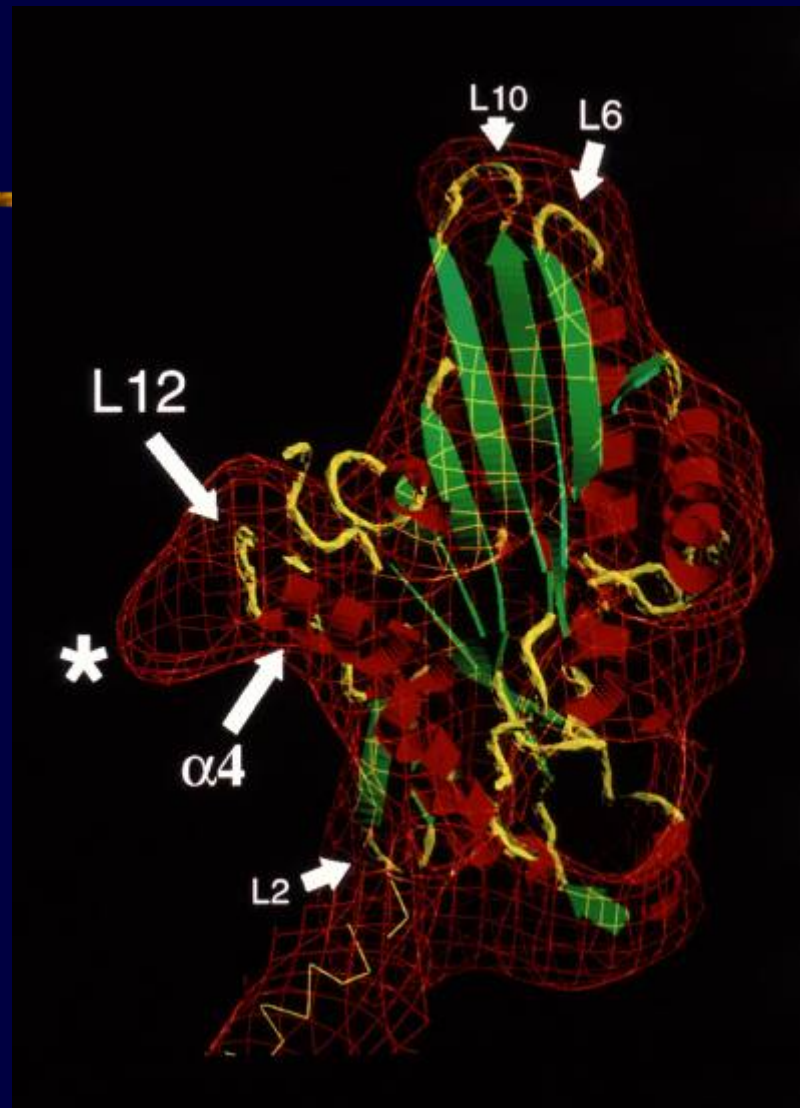
KIF1A-Microtubule Complex: cryo EM (15Å resolution)

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



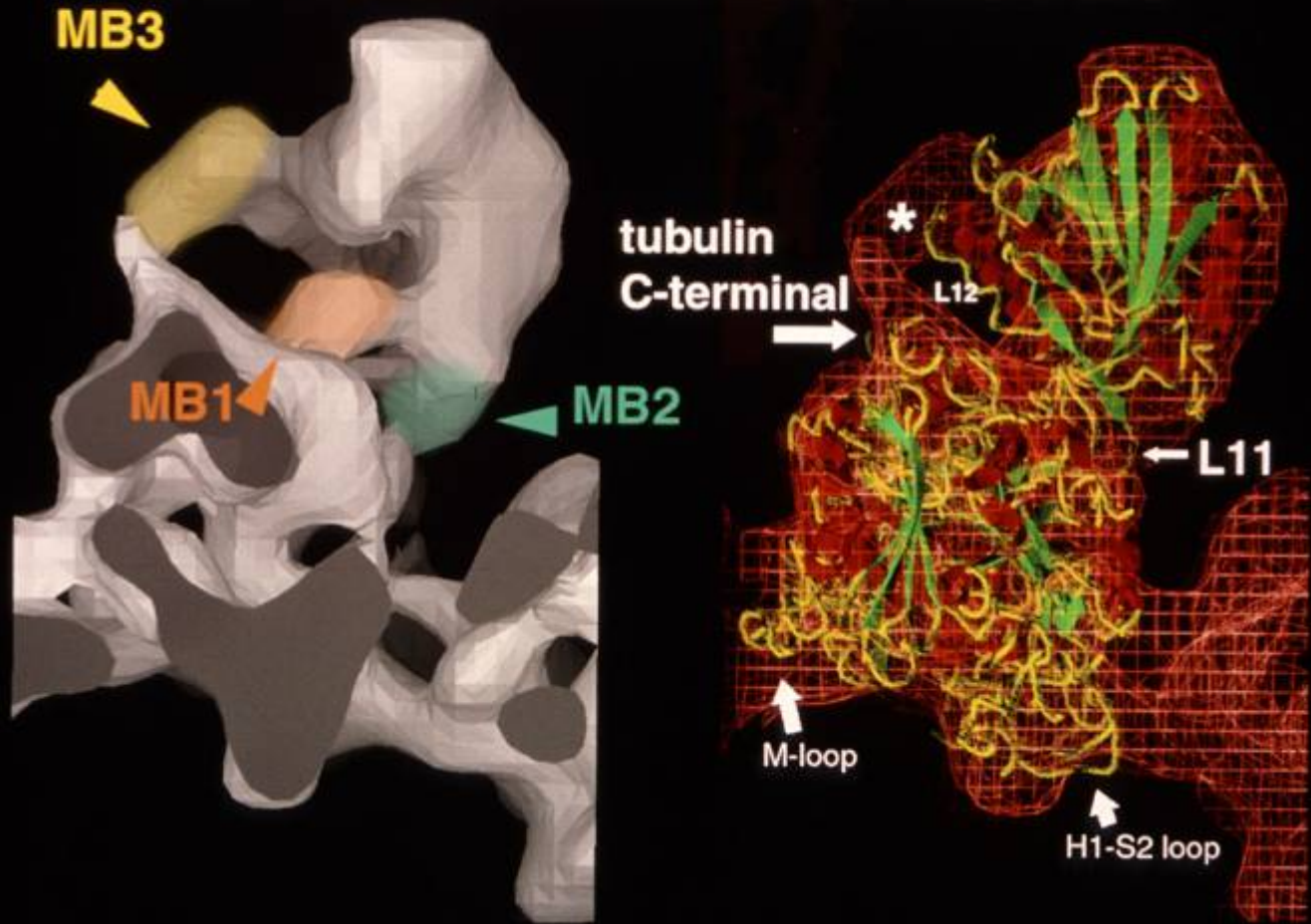
KIF1A-Microtubule Complex: cryo EM (15Å resolution)



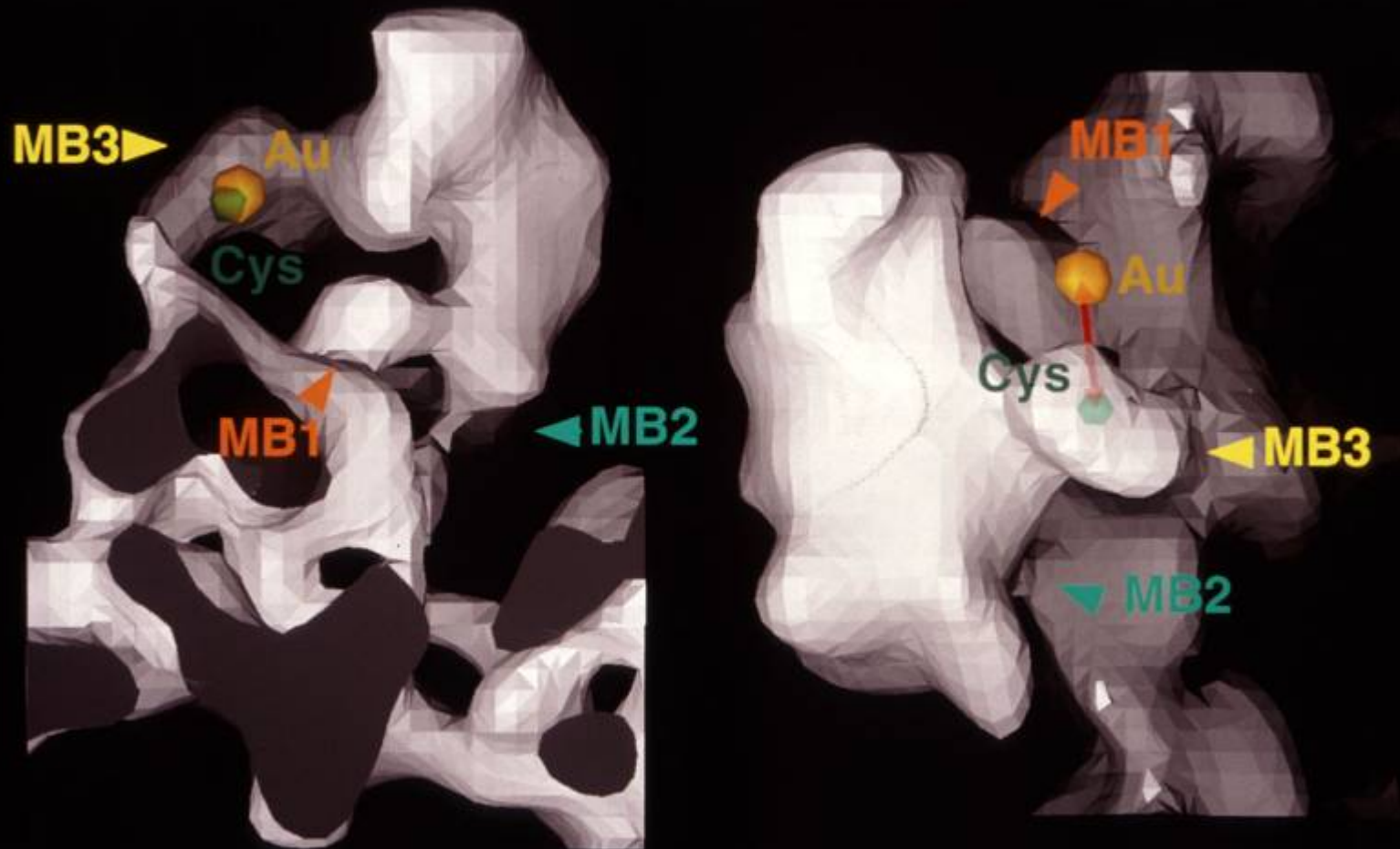


	MsKNC	44
	MADLAECHEICVNCVTRPFLNESEVNRGDKYIAKFGQEDVYIAR	44
RnRNC	MADPAECITVCMKTRPFLNEAEILRGDKYIPKFGQETVYIQGQ	45
kif1a	MADAGYKVAIVVPPNSREMSRDKCIYQMGQTTIVNFGQP---KET	46
MsKNC	--KFIAPQKVFQSGSTG-----QEQVYNDCAKIKVKDLVGLGYIGTIFAYQQTSSGKTH	93
RnRNC	--KPIYVQKVLPPHIT-----QEQVTNACAKIQVEDVGLGYIGTIFAYQQTSSGKTH	94
kif1a	P-KSTSGEYVWHTISFEDIHVAKQKQVYRDIIGENLGNAPGSGVIVCIPATQGTGAQDSY	105
MsKNC	THREGKLDHPGQMGIIPRIVQDIPNYYIS-MDENLEPRIFVSYEPIYLDKINLDD--VS	149
RnRNC	THREGKLDQPLNGIIPRIAMIDFIRHYI-MDKHLPRIFVSYEPIYLDKINLDD--VS	150
kif1a	THMGKQKQKQ-QGIIYQLCDLPSRINOTTHNNMYSVEVSHETCYERDGLNLP--KN	162
MsKNC	KTNLGVH-EDNRNV-----YVSGCTIRFVCPDPEVMQDIEGKSNRNVAVTMMNHEES	202
RnRNC	KTNLASH-EDNRNV-----YVSGCTIRFVSPPEVMQDIEGKSNRNVAVTMMNHEES	203
kif1a	EGNLRVH-EHPLLP-----YVDELGLAVTSYNDIQQLMDGGGKPRVVAATHNHEES	215
MsKNC	REHSIFLINIRQENTQTECKLS---IELYLVLAGGKVKSKTGASGAVLDIAKINHEL	258
RnRNC	REHSIFLINIRQENVETEKLS---IELYLVLAGGKVKSKTGASGAVLDIAKINHEL	259
kif1a	REHAYVPIITQKHDAETHTTEKVRVIELVLAGGERADGTGAGSTRLEKAGINHEL	275
MsKNC	SALGNVISALAGS--K-loop--TVVPYRDSKMTRIIQDGLDGNHPTTVICCPFS	306
RnRNC	SALGNVISALAGSK--TVVPYRDSKMTRIIQDGLDGNHPTTVICCPFS	308
kif1a	TLGQVISALAGSDGPNKKKKKKTPIPYRDSVLTWLRENHGNHPTANVAALSPAD	335
MsKNC	YNESSTRGTLFQGRAKTINHTVCMVELTAQWKKKYEKKE	
RnRNC	FNEATKSTLMPQGRAKTINHTVGMVELTAQWKKKYEKKE	
kif1a	INHYDELSTLYADRAKQINCHA:INSDPNKKLINELDEVTR	

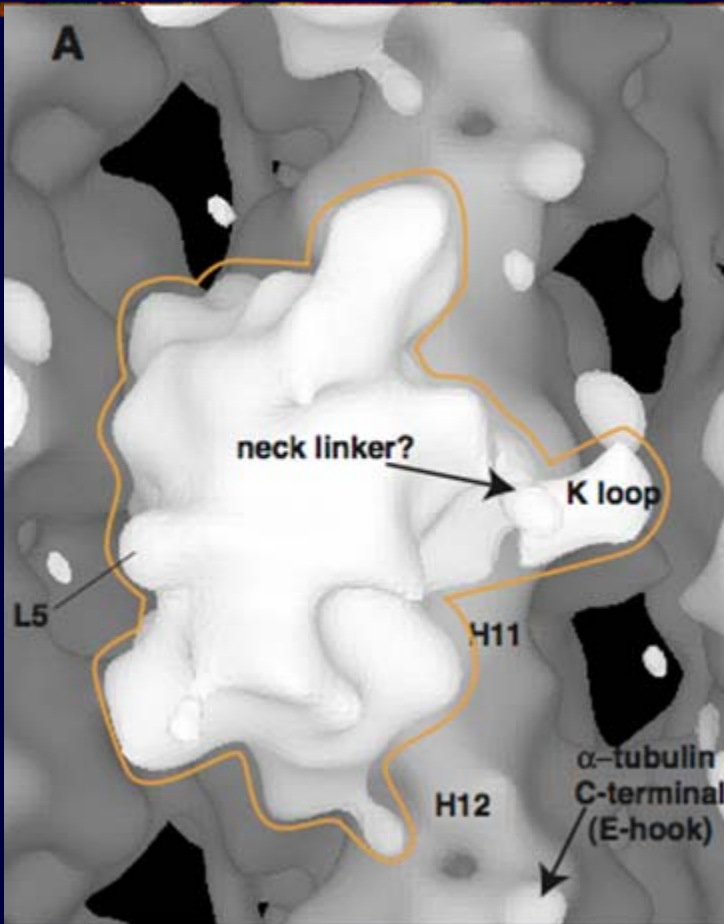
Plus-end view of the KIF1A head-microtubule



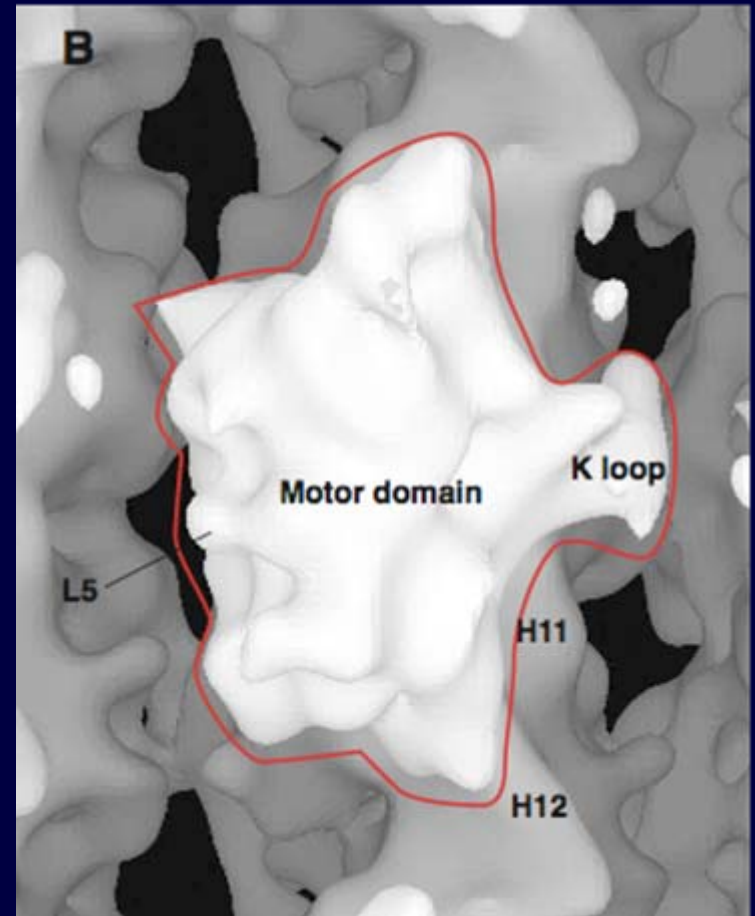
Distance between Gold-cluster and K-loop



View from outside



ADP

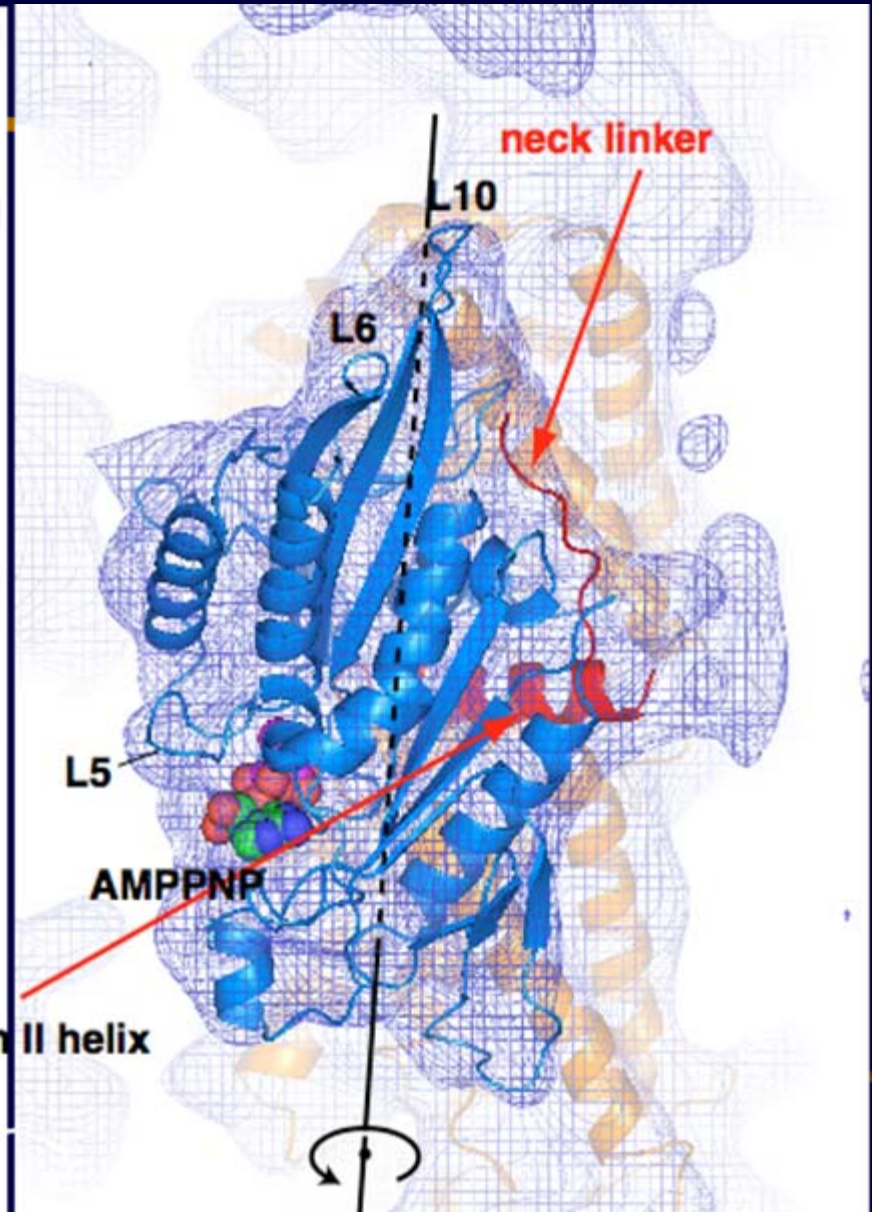
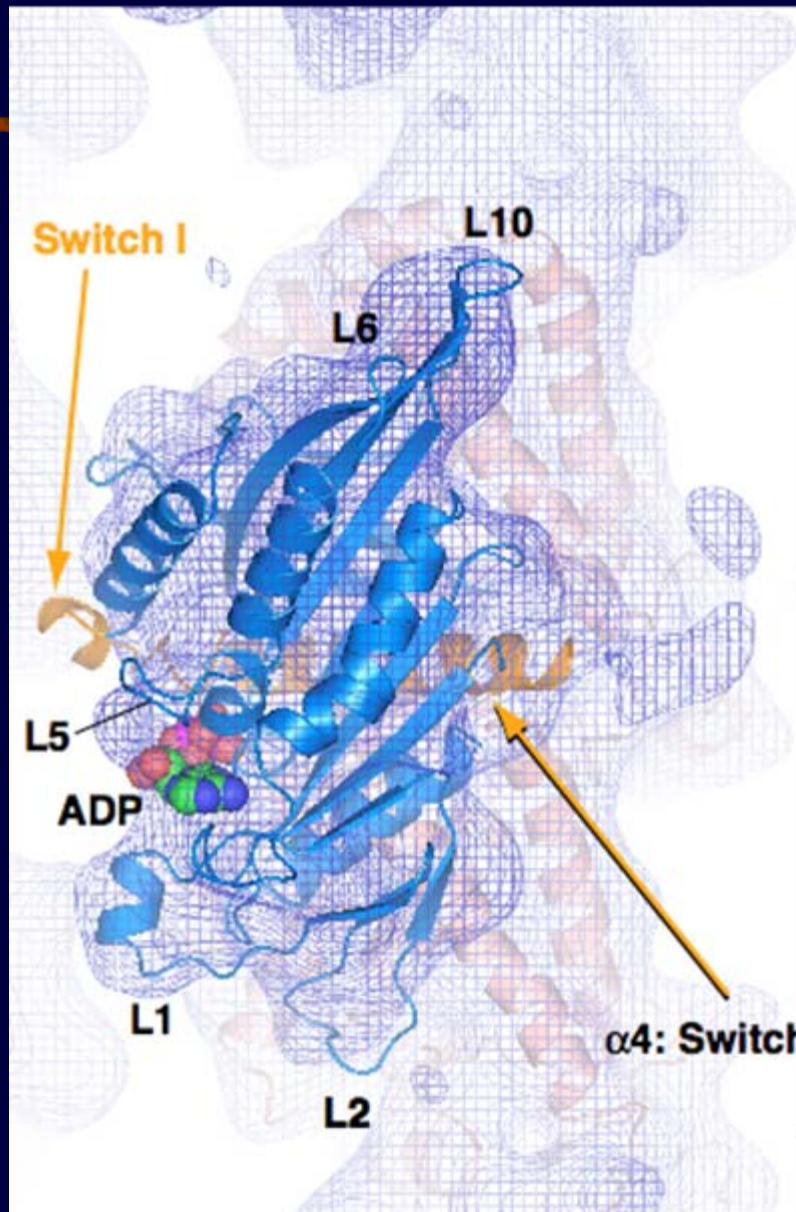


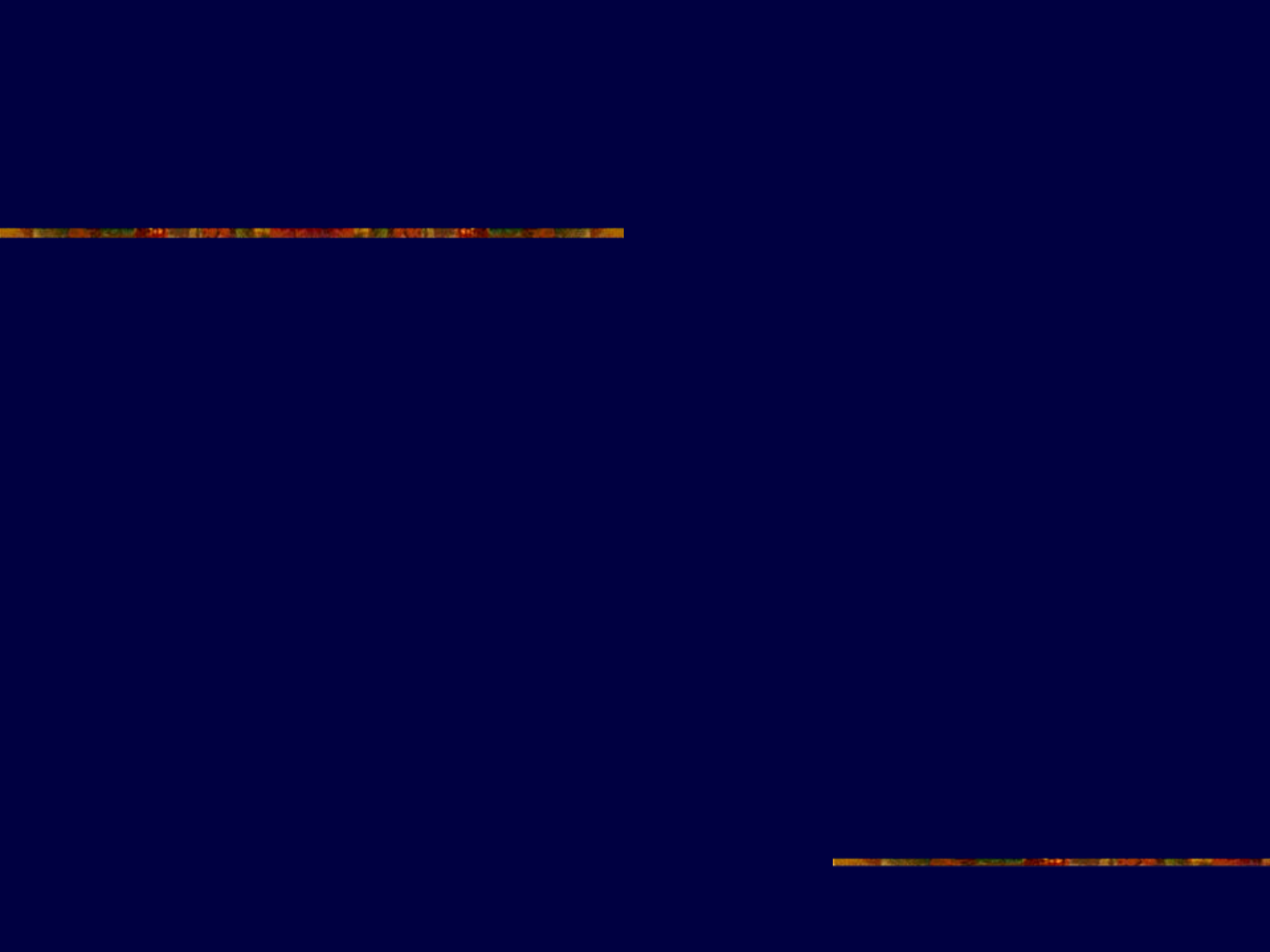
ATP(AMPPNP)

View from outside

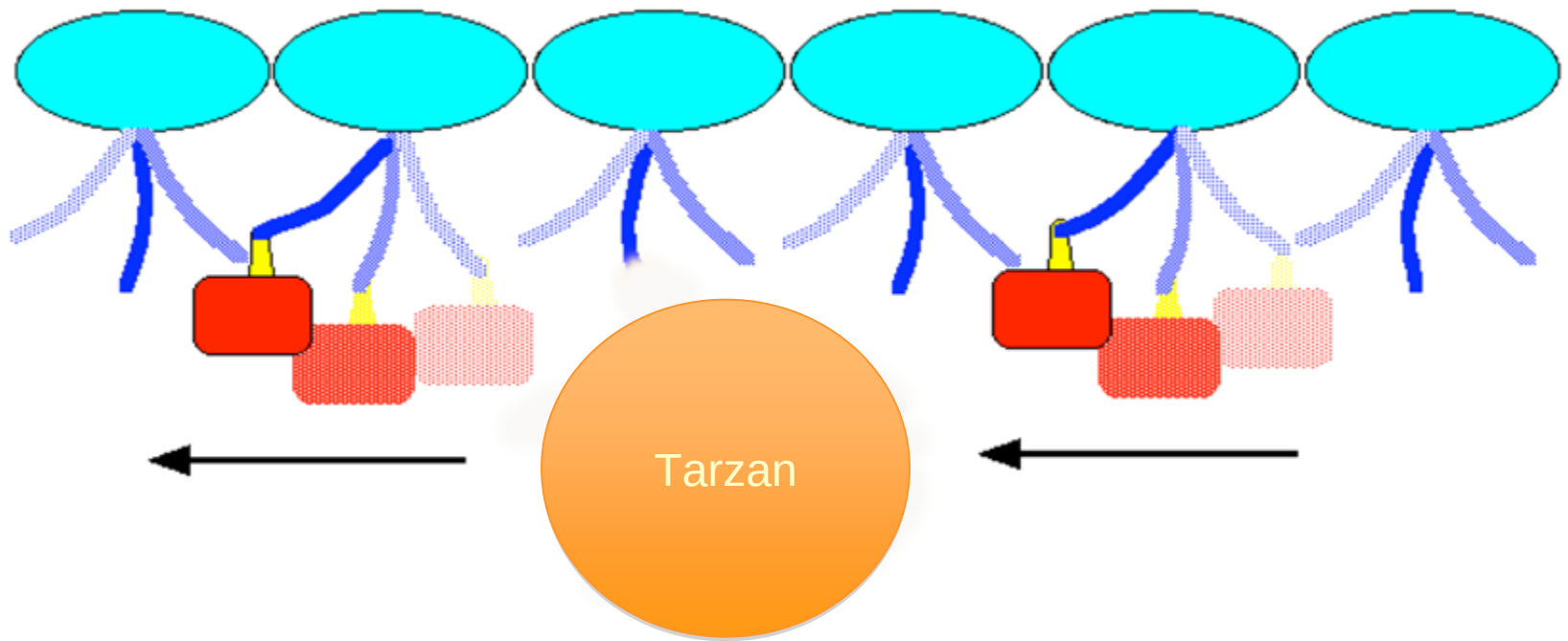
ADP

ATP

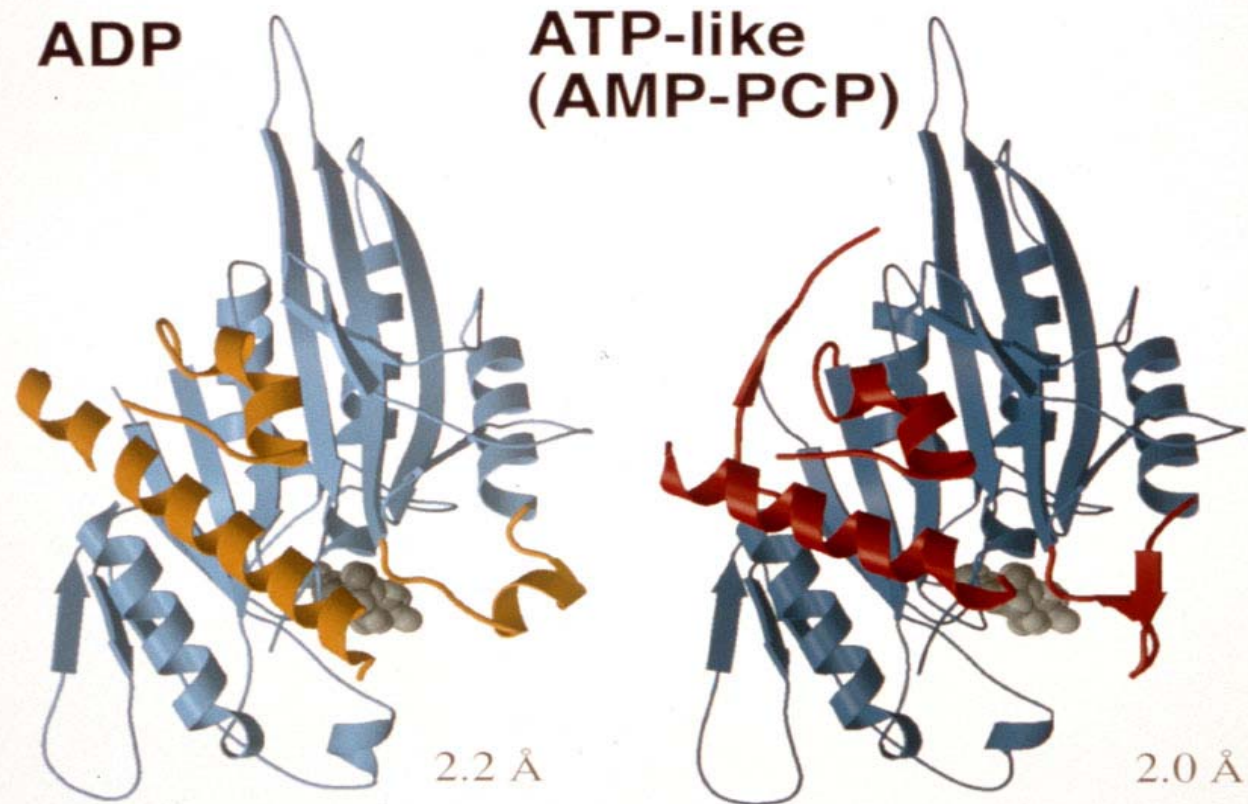


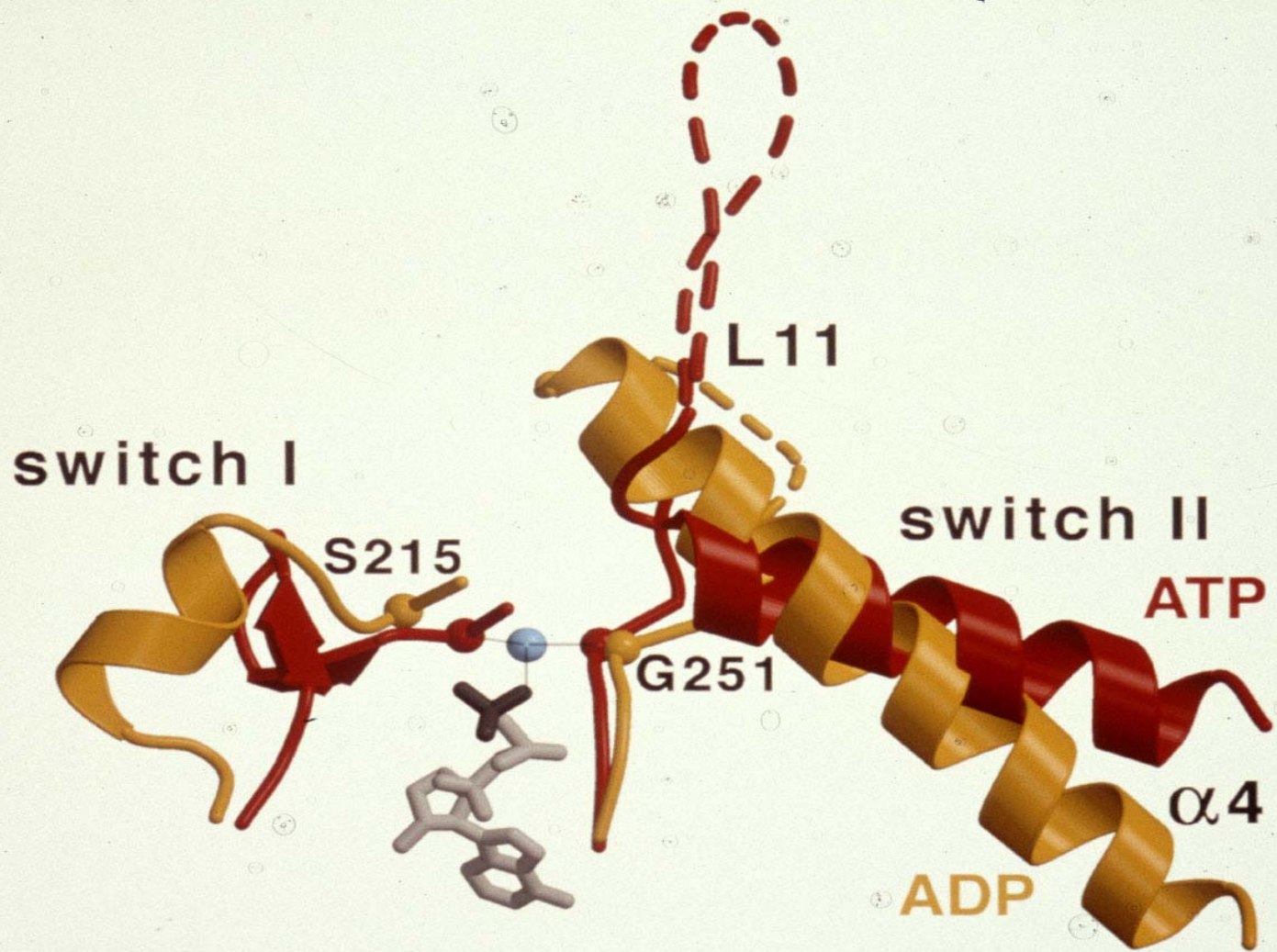


Tarzan Model



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







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

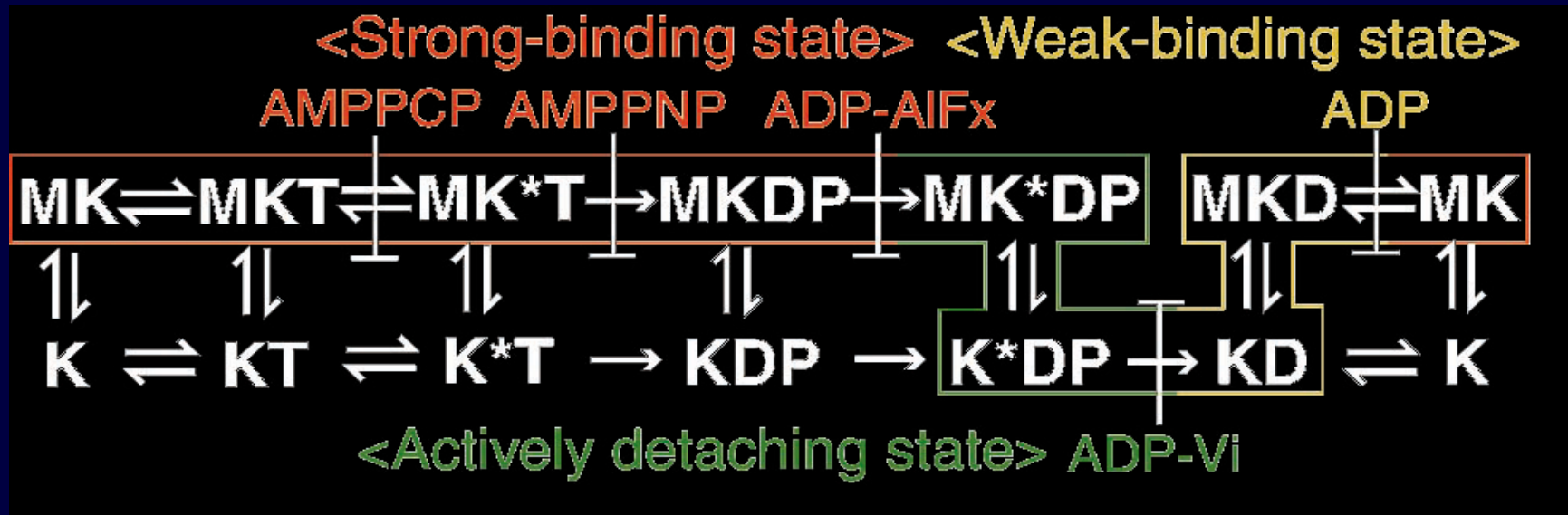
KIF1A overall structure



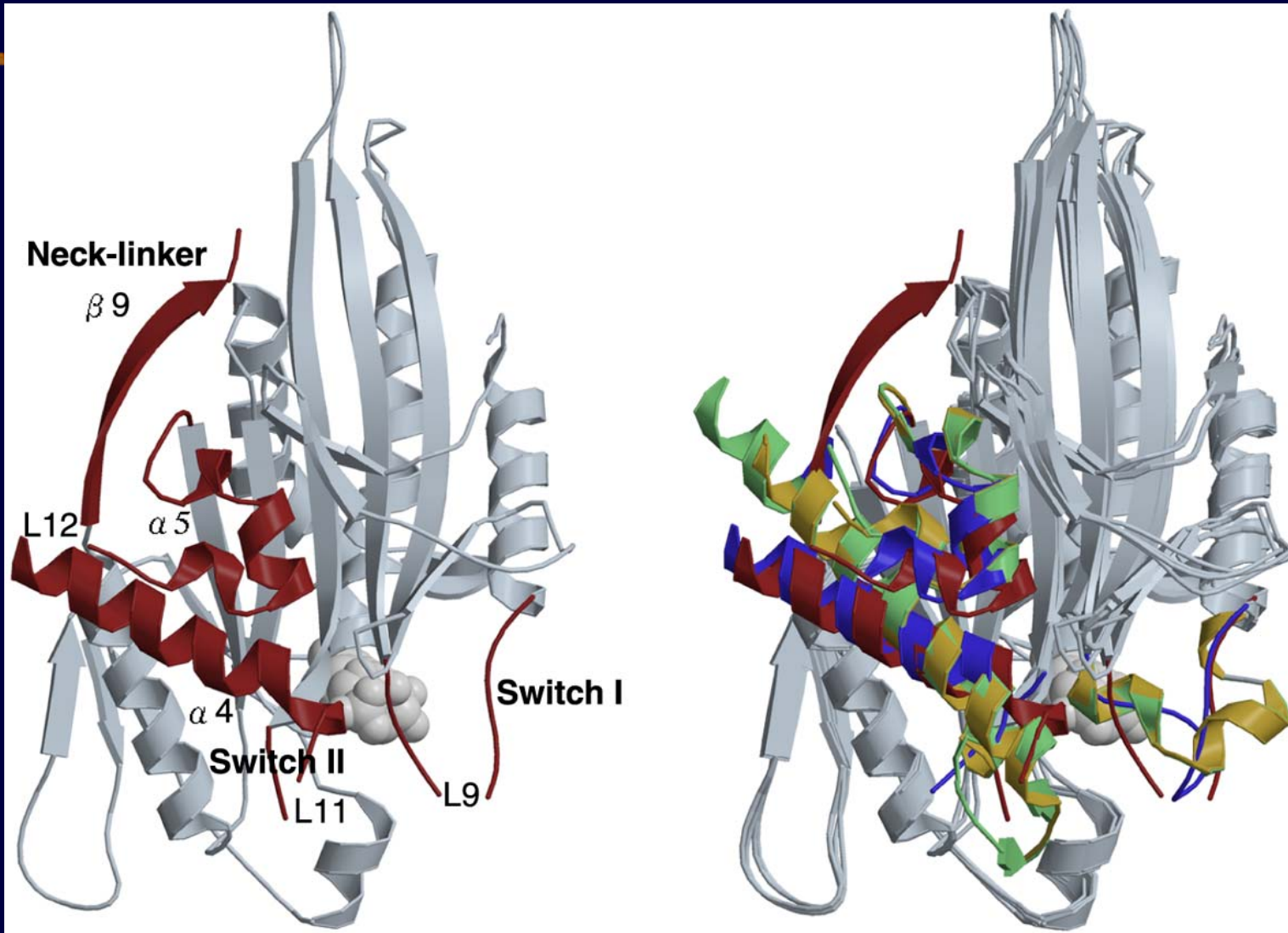
C340



Chemo-mechanical cycle of kinesin motors



Overall architecture of KIF1A

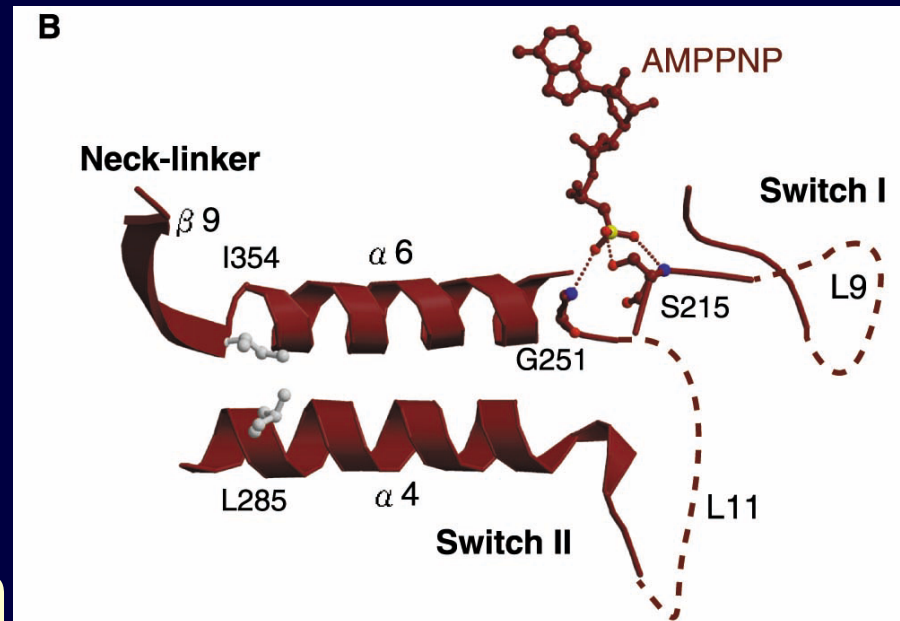
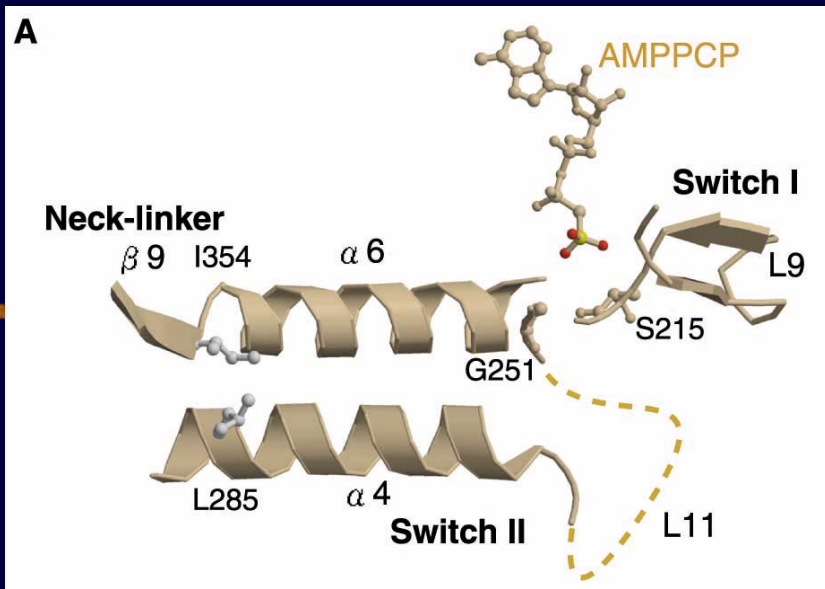


ATP
(AMPPNP)

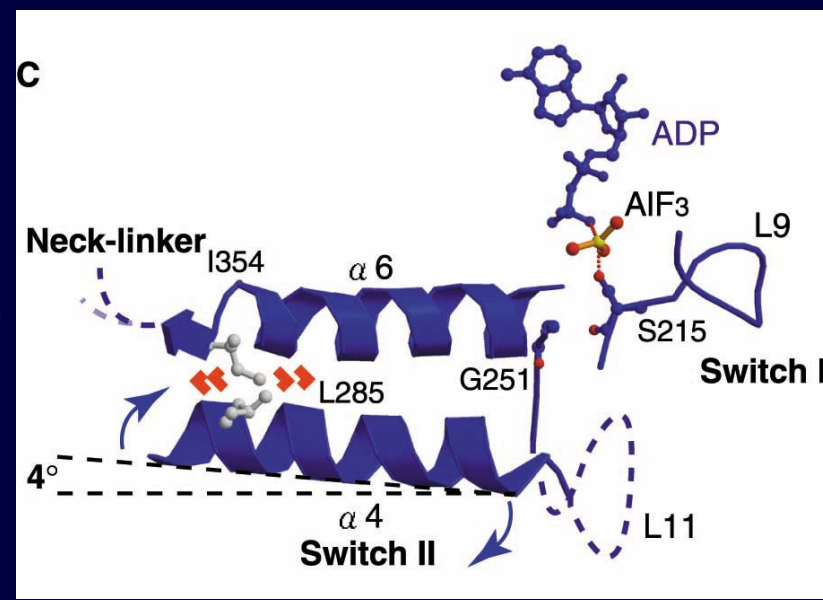
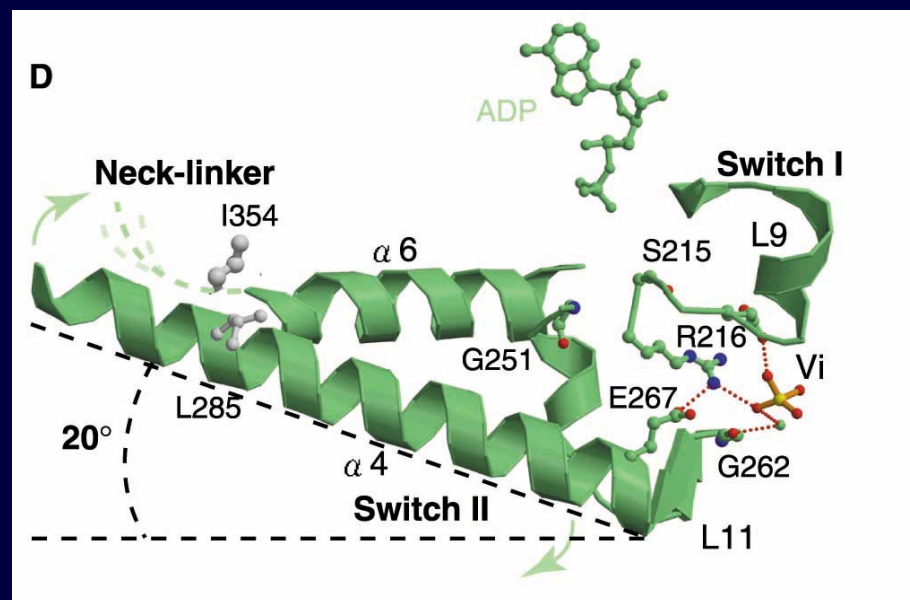
↓
ADP-Pi (1)
(ADP-AlF₃)

↓
ADP-Pi (2)
(ADP-VO₄)

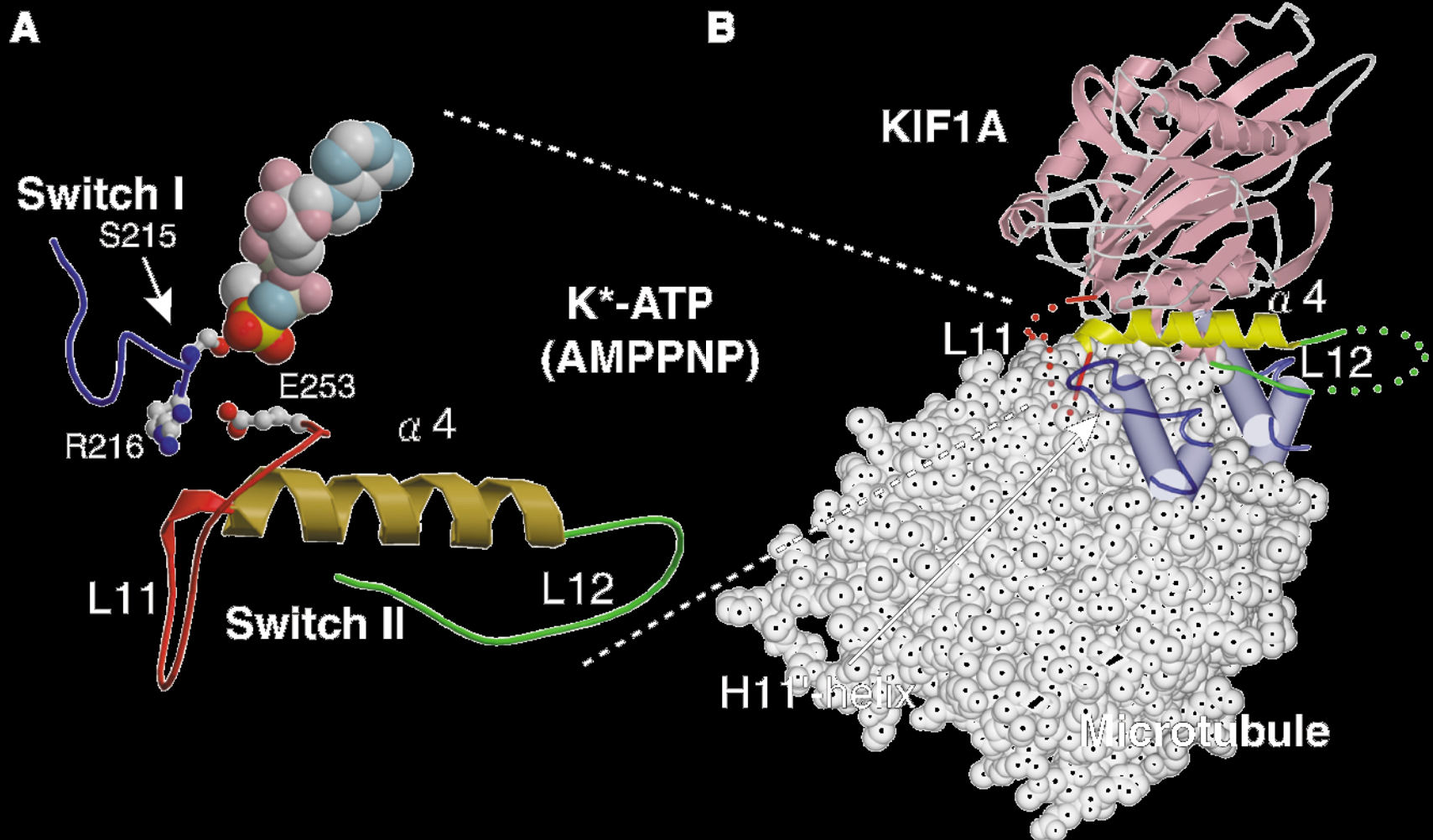
↓
ADP



Conformational change
regions



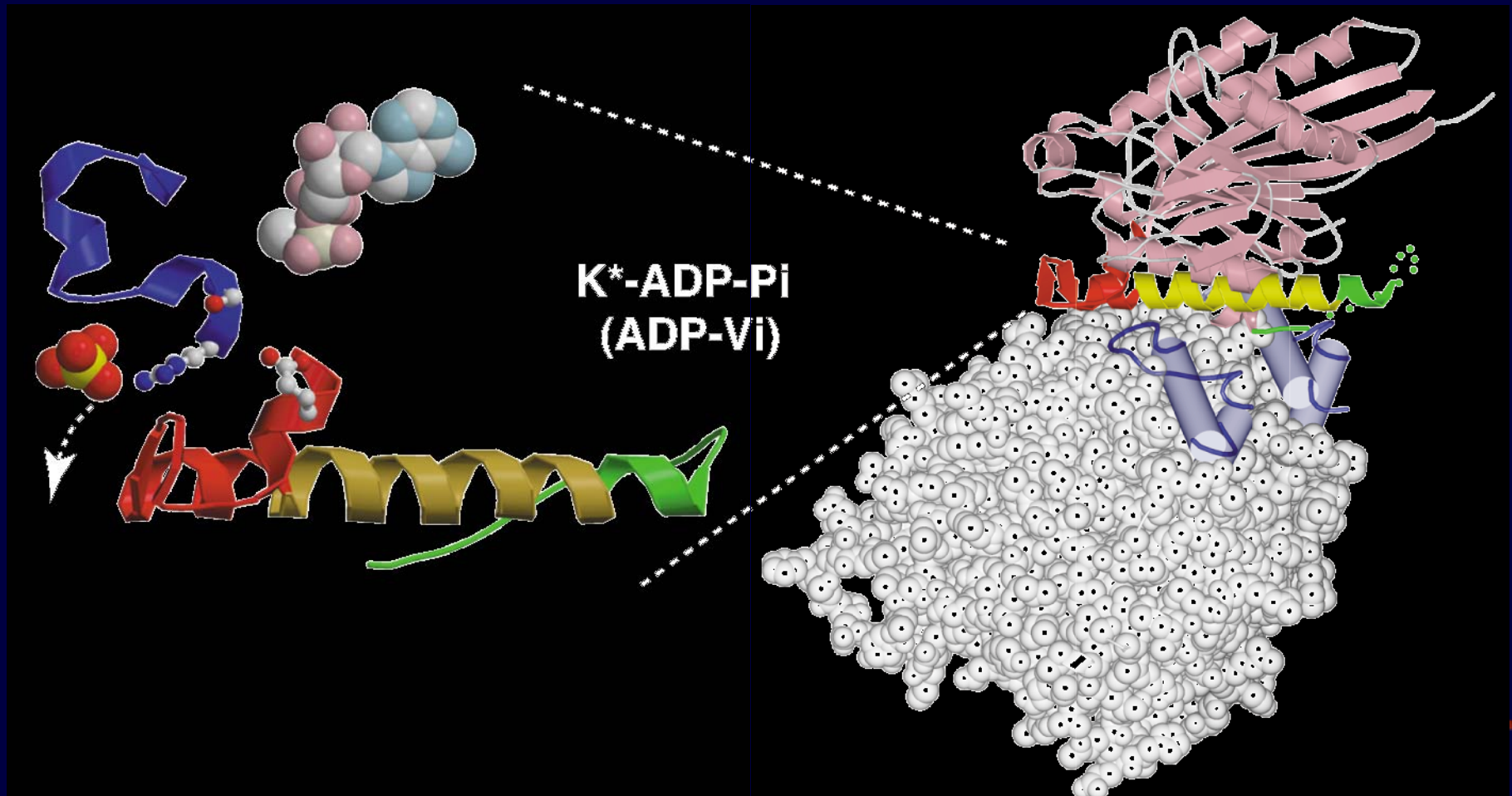
KIF1A*=ATP- Microtubule



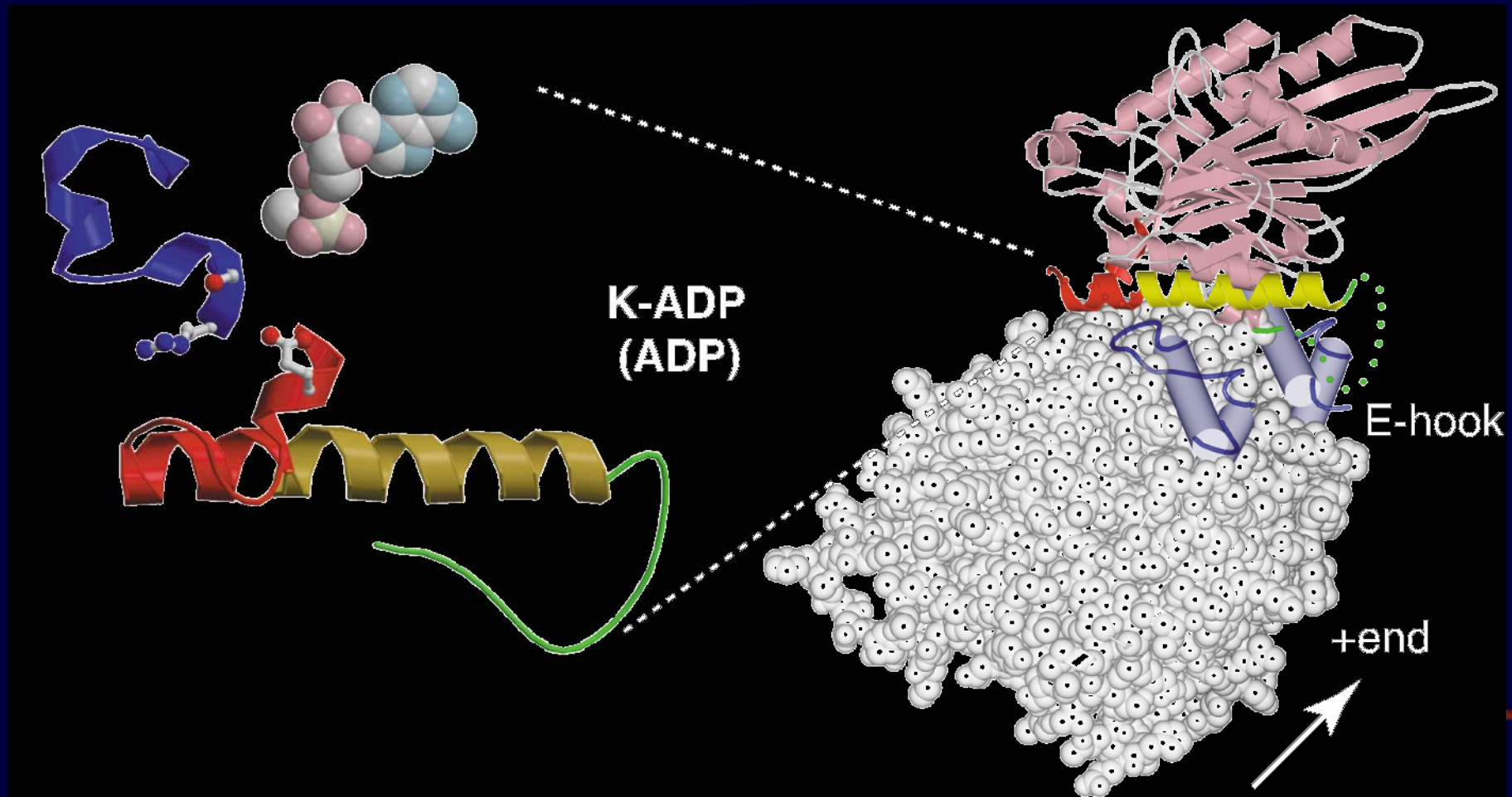
[Home](#)
[About Us](#)
[Contact Us](#)
[Privacy Policy](#)
[Terms of Service](#)



KIF1A*=ADP-Pi -Microtubule



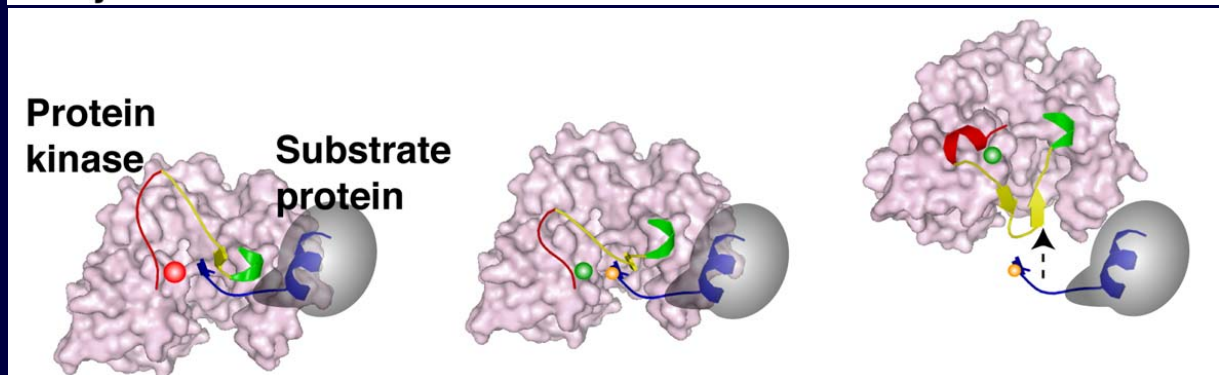
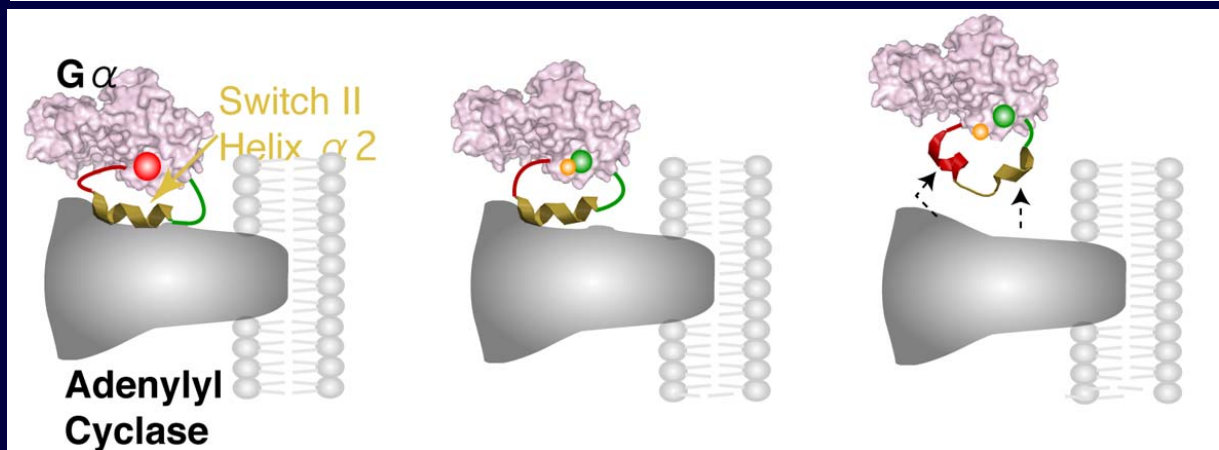
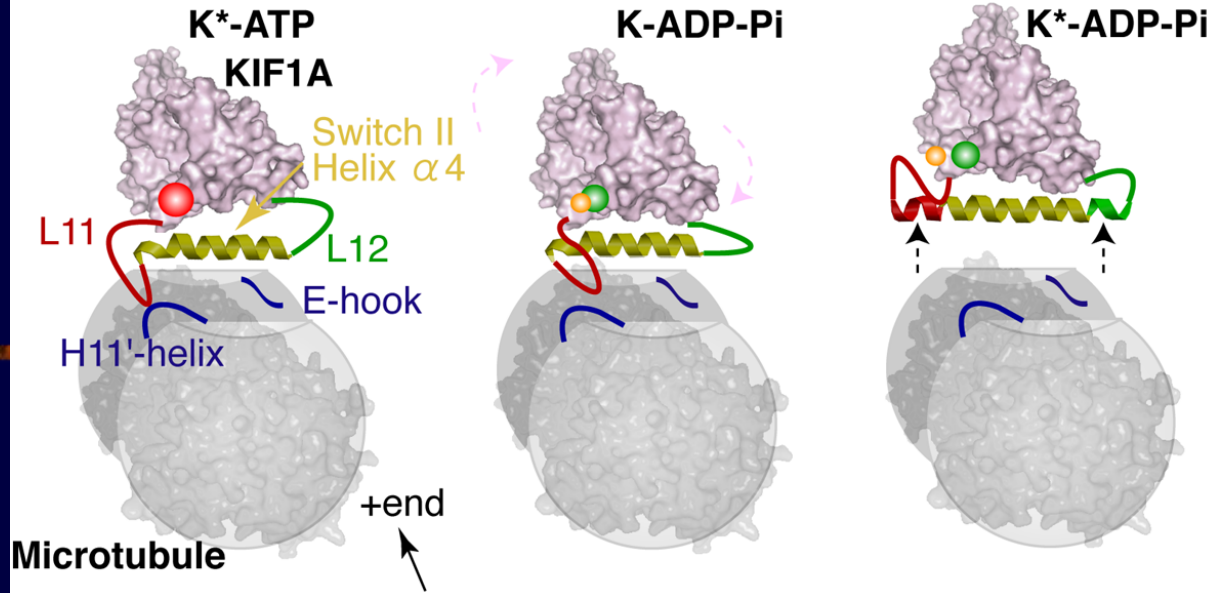
KIF1A=ADP -Microtubule



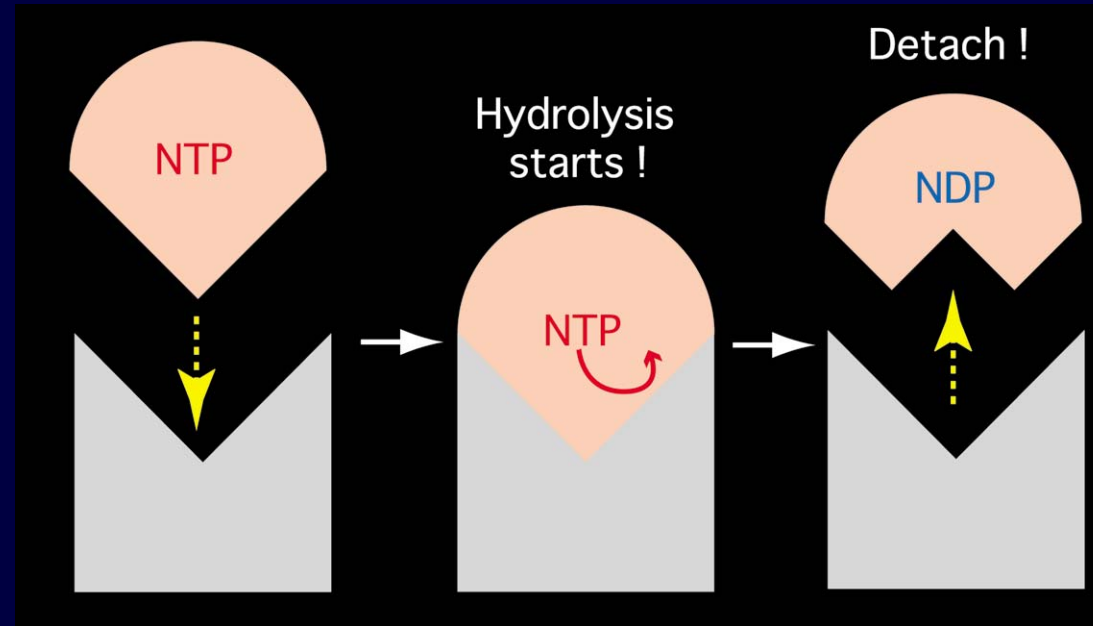
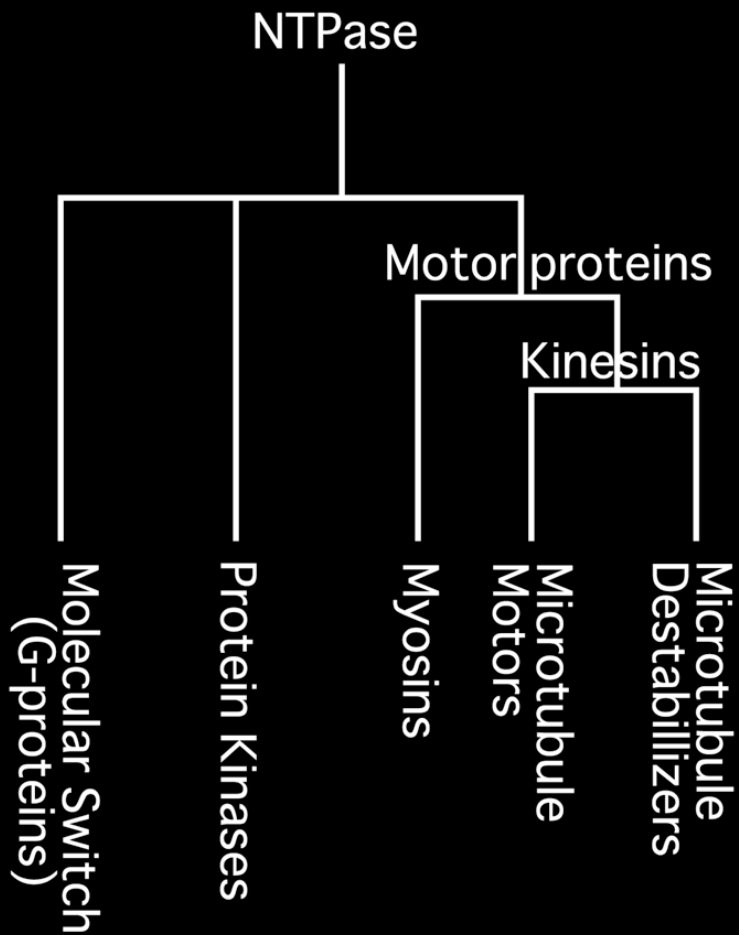
Equilibrium Binding Constants of KIF1A mutant

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

N-terminal Motor
Domain Type
Monomeric Motor

KIF 1A

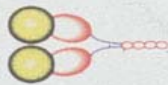


KIF 1B



Central Motor
Domain Type

KIF 2

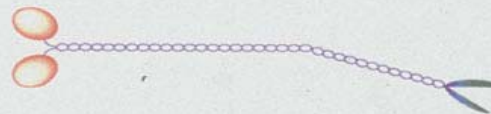


N-terminal Motor
Domain Type
Dimeric Motor

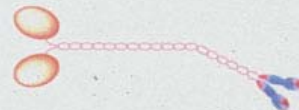
KIF 3A/B



KIF 4



KIF5A
(bKHC-1)



KIF5B
(uKHC)



KIF5C
(bKHC-2)

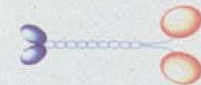


C-terminal Motor
Domain Type

KIFC1



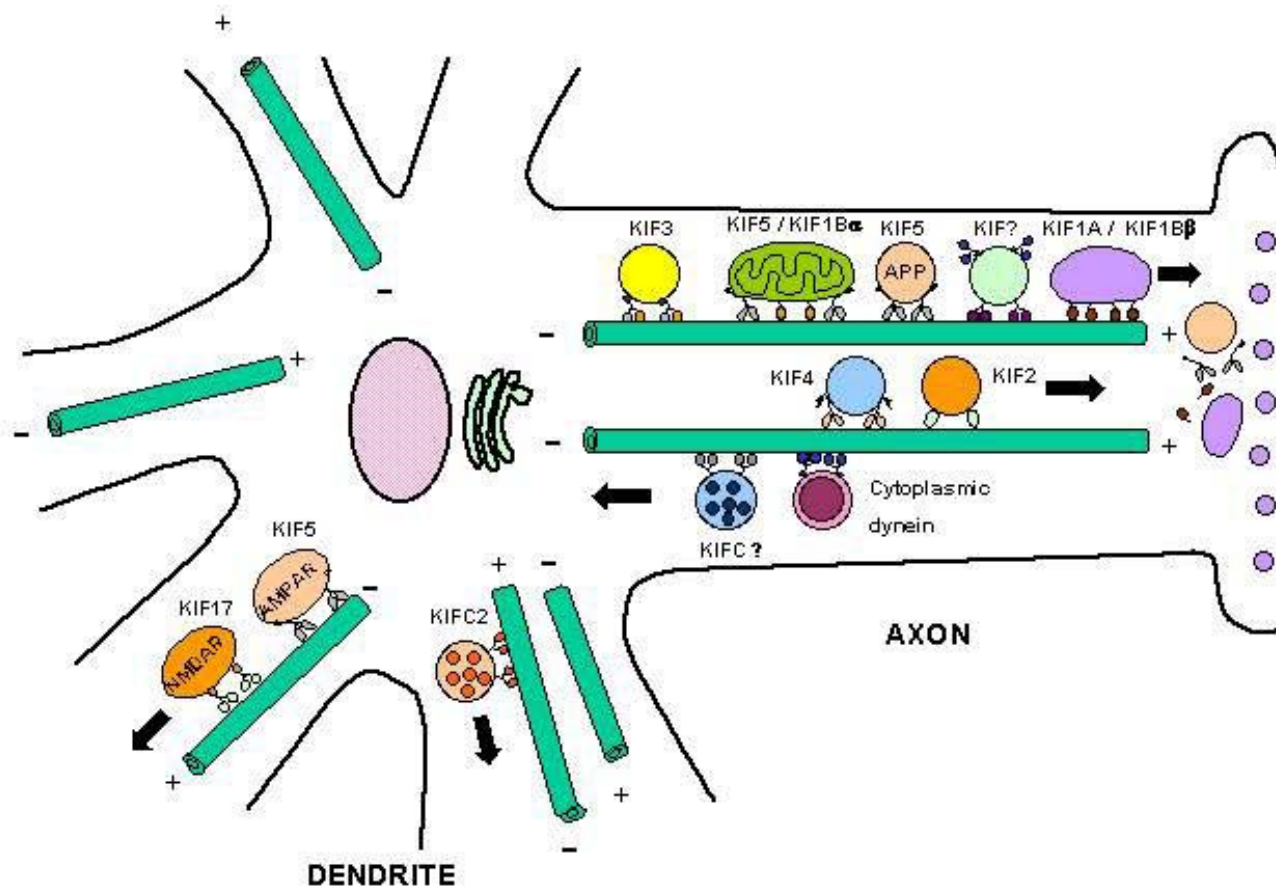
KIFC2



KIFC3



20nm



Univ. Tokyo

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

Laurent GUILLAUD
Richard WONG
Junlin TENG
Harukata MIKI
Dae-Hyung SEO
Chunjie ZHAO
Terunaga NAKAGAWA
Masahide KIKKAWA
Hiroaki YAJIMA
Ryo NITTA
Tadayuki OGAWA

UCSF

Robert J. Fletterick
Elena P. Sabrin

Univ. Hospital Tokyo
Junko TAKITA
Yasuhide HAYASHI

Niigata Univ.
Brain Res. Inst.
Masaaki SAITO
Shoji TSUJI

