Kinesin Motor Enzymology: Chemistry, Structure, and Physics of Nanoscale Molecular Machines

JC Cochran

Received: 15 September 2014 / Accepted: 16 November 2014 / Published online: 13 February 2015
© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag Berlin Heidelberg 2015

Abstract Molecular motors are enzymes that convert chemical potential energy into controlled kinetic energy for mechanical work inside cells. Understanding the biophysics of these motors is essential for appreciating life as well as apprehending diseases that arise from motor malfunction. This review focuses on kinesin motor enzymology with special emphasis on the literature that reports the chemistry, structure and physics of several different kinesin superfamily members.

Keywords Molecular motors · Kinesin motor enzymology · Kinesin superfamily · Chemical models · Enzymes

Abbreviations

AlF _x	Aluminum fluoride
AMPPCP	Adenosine 5'-(β,γ-methylene)triphosphate
AMPPNP	Adenosine 5'-(β,γ-imido)triphosphate
ATP	Adenosine 5'-triphosphate
ATPγS	Adenosine 5'-(γ-thio)triphosphate
BeF _x	Beryllium fluoride
E	Kinesin
FRET	Förster resonance energy transfer
mant-ATP	2'-(or-3')-O-(N-Methylantraniloyl) adenosine 5'-triphosphate
MDCC-PBP	7-Diethylamino-3-(((2-maleimidyl)ethyl)amino)carbonyl coumarin)-labeled phosphate binding protein

Electronic supplementary material The online version of this article (doi:10.1007/s12551-014-0150-6) contains supplementary material, which is available to authorized users.

J. Cochran (✉)

Department of Molecular & Cellular Biochemistry, Indiana University, Simon Hall Room 405C, 212 S. Hawthorne Dr., Bloomington, IN 47405, USA
e-mail: jcc6@indiana.edu

MT	Microtubule
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SO ₄ ⁻	Sulfate
VO ₄ ⁻	Vanadate

Introduction

Molecular motors are enzymes that convert chemical potential energy into controlled kinetic energy for mechanical work (i.e., motion or movement) inside cells. These biological motors come in a variety of flavors, including cytoskeletal motors, rotary motors, nucleic acid motors, protein synthesis and translocation machinery, and dynamic biopolymers. Each motor is designed to perform various tasks inside cells, such as intracellular transport, signal transduction, oxidative phosphorylation, cell motility, nucleic acid translocation, nucleic acid synthesis, endocytosis, protein synthesis, protein translocation across membranes, and cell division. Understanding the chemistry, structure, and physics of these biological motors is essential for appreciating life, as well as for apprehending diseases that arise from malfunction of these motors.

Since the discovery of the first force-generating, microtubule-dependent ATPase that drives fast axonal transport in neurons (Brady 1985; Vale et al. 1985), a wealth of research has focused on characterizing the structure and function of various members of the kinesin superfamily. These motors convert the free energy of nucleoside 5'-triphosphate hydrolysis (typically ATP) into directed mechanical motion during their interaction with a microtubule track. Most kinesins contain: (1) a catalytic domain (also known as a motor or head domain) responsible for nucleotide catalysis, microtubule binding, and force production, (2) a central α-

helical stalk domain involved in coiled–coil interactions to organize the motor into higher order oligomers (e.g., dimers, tetramers), and (3) a tail domain responsible for various subfamily specific functions [e.g., light chain binding domain for kinesin-1 (Hackney 2007), DNA binding for kinesin-10 (Vanneste et al. 2011), ATP-independent microtubule binding domain for kinesin-8 (Weaver et al. 2011) and kinesin-14 (Karabay and Walker 1999)]. Several reviews have been written that summarize the various features of the kinesin superfamily (Hirokawa and Takemura 2004; Marx et al. 2009; Sack et al. 1999; Vale and Fletterick 1997).

Studying the biophysics of molecular motors has been the focus of many laboratories for decades. The fundamental understanding of enzyme biochemistry, structure, and (particularly for motors) mechanics has been investigated, yet networking the results from these scientific disciplines into a coherent model is often a difficult task. This review focuses on kinesin enzymology with special emphasis on the literature that reports the chemistry, structure and physics of several different kinesin molecular motors. In the first section, I summarize the biochemistry of kinesins, with a focus on the types of kinetic and thermodynamic experiments that researchers have used to study these motors. In this section I also discuss how the interpretation of these results lead to a chemical model for how kinesins catalyze nucleotide hydrolysis and how their partner filaments (microtubules) stimulate their ATPase cycle. In the second section, I provide a detailed summary of the current knowledge of kinesin structure, highlighting the conserved and divergent structural characteristics among the kinesin superfamily, as well as recent structures that have provided much insight into the function of molecular motors. In the third section, I describe the physics of kinesin motors, focusing on the movements and mechanics that are driven by the biochemical reactions in kinesin enzymes for force generation. A dominant feature of this section is the connection of biochemical free energies with the work performed by the motors. In the fourth and final section, several conclusions and future directions for the study of molecular motors are presented.

Chemistry

Although the kinesin superfamily consists of 14–17 different subfamilies with several individual members per subfamily (Dagenbach and Endow 2004; Lawrence et al. 2004; Miki et al. 2005; Wickstead et al. 2010), only a select few have been studied extensively using a combination of biophysical, biochemical, and structural methodologies. Based on a series of elegant kinetic, thermodynamic, and structural studies, ATPase mechanisms of truncated, recombinant monomeric (Fig. 1a) and dimeric (Fig. 1b) kinesin motors have been defined (note: the minimal mechanisms presented here are

Fig. 1 Minimal ATPase mechanisms for monomeric and dimeric kinesins. Monomeric (**a**) and dimeric (**b**) kinesin ATPase mechanisms are shown. *E* kinesin, *M* microtubule, *T* ATP substrate, *D* ADP product, *P_i* inorganic phosphate product, *apo* nucleotide-free/rigor. For dimeric kinesins that have two inter-molecularly regulated active sites, each site is depicted as either a *superscript* or *subscript* (for example: $\text{E} \cdot \text{D}^{\text{ADP}}$ indicates that both sites of the dimeric kinesin (*E*) are bound to ADP)

simplified in order to highlight the dominant steps in the cycle and for the purpose of comparing many different members of the superfamily; Tables 1 and 2). For monomeric kinesins, the minimal mechanism consists of four consecutive biochemical reactions [ATP binding, ATP hydrolysis, inorganic phosphate (H_2PO_4^- ; P_i) release, and ADP release]. These four reactions can occur in either the microtubule-bound or unbound state. Depending on the family member, the motor affinity for the microtubule varies with the nucleotide state at the kinesin active site (e.g., kinesin-1 binds the microtubule tightly in the nucleotide-free/apo/rigor, ATP-like, and ADP· P_i -like states, but binds the microtubule weakly in the ADP state). Therefore, the detachment/dissociation and attachment/association rate constants define the four equilibrium constants at each nucleotide state in the cycle ($K_{d,A}$ = nucleotide-free, $K_{d,B}$ = ATP-like, $K_{d,C}$ = ADP· P_i -like, $K_{d,D}$ = ADP). The ATP-like and ADP· P_i -like states are typically achieved with nucleotide and P_i analogs, respectively (e.g., AMPPNP, AMPPCP, ATPγS as non-hydrolyzable or slowly hydrolyzable ATP analogs, and ADP with BeF_x , AlF_x , VO_4^- , SO_4^- as ADP· P_i analogs). These nucleotide states have revealed structural details for a variety of different ATPases, including kinesins (Table 3), myosins (Coureux et al. 2004; Gulick et al. 1997; Menetrey et al. 2008; Smith and Rayment 1996), helicases (Bernstein et al. 2003; Halbach et al. 2012; Luo et al. 2008; Schutz et al. 2010; Xiol et al. 2014), AAA^+ motors

Table 1 Comparison of rate and equilibrium constants for truncated monomeric kinesins in published studies^a

Constant (units)	Reaction	Kinesin-1		Kinesin-5	Kinesin-7	Kinesin-10	Kinesin-13	Kinesin-14
		<i>Hs/BtkHC</i> ^{1,3}	<i>DmKHC</i> ^{4,5}					
$K_{1'}$, $k_{+2'}$ ($\mu\text{M}^{-1} \text{s}^{-1}$)	ATP Binding	300–400	ND	0.08±0.01	ND	13.8±0.6	ND	ND
$k_{+2'}$ (s^{-1})	Isomerization	170±30	ND	0.5–1.2	ND	1800±500	ND	ND
$k_{-2'}$ (s^{-1})	ATP release	<1	ND	<0.01	ND	5±1	ND	ND
$k_{+3'}$ (s^{-1})	Hydrolysis	7	ND	>10	ND	0.016±0.002	ND	ND
$k_{+4'}$ (s^{-1})	P _i release	>0.1	ND	>10	ND	ND	ND	ND
$k_{+5'}$ (s^{-1})	ADP release	0.003	0.025	0.02±0.001	ND	0.23±0.004	ND	0.003±0.0006
$K_{-6'}$, $k_{-5'}$ ($\mu\text{M}^{-1} \text{s}^{-1}$)	ADP binding	333	ND	0.07±0.01	ND	19.7±0.4	ND	ND
$k_{-5'}$ (s^{-1})	Isomerization	>300	ND	0.6–1.0	ND	1400±220	ND	ND
$K_{-7'}$, k_{+2} ($\mu\text{M}^{-1} \text{s}^{-1}$)	ATP binding	19±3	20±5	3.4±0.3	4.9±0.7	5.1±0.2	ND	1.1±0.1
k_{+2} (s^{-1})	isomerization	>800	565±50	21.2±1.3	28.7±3.9	900±120	ND	>400
k_{-2} (s^{-1})	ATP release	105±11	113±60	<1	123±51	0.02±0.004	ND	7±7
k_{+3} (s^{-1})	Hydrolysis	ND	>300	10.2±0.7	ND	ND	ND	11.0±2.3
k_{+4} (s^{-1})	P _i release	92±1	ND	6.0±0.1	11.4±0.9	ND	ND	ND
k_{+5} (s^{-1})	ADP release	300	303±23	43.3±0.2	193±35	0.60±0.01	ND	3.9±0.4
K_{-6} , k_{-5} ($\mu\text{M}^{-1} \text{s}^{-1}$)	ADP binding	3.3	11±1	2.8±0.5	1.4±0.4	10.6±0.4	ND	0.6±0.04
k_{-5} (s^{-1})	Isomerization	>300	ND	>300	ND	760±120	ND	>100
k_{cat} ($\text{s}^{-1} \text{site}^{-1}$)	Basal ATPase	0.005±0.0004	ND	0.02±0.003	ND	0.001±0.0001	0.03	0.004
$K_{m,ATP}$ (μM)		ND	ND	0.17±0.03	ND	14.1±2.6	ND	ND
$k_{cat} / K_{m,ATP}$		ND	ND	0.12±0.01	ND	5 × 10 ⁻⁵	ND	ND
k_{cat} ($\text{s}^{-1} \text{site}^{-1}$)	MT-ATPase	60±7	81±2	5.5±0.3	21.0±0.7	2.5±0.1	0.49±0.06	2.1±0.1
$K_{m,ATP}$ (μM)		ND	91±10	9.5±0.4	ND	40.9±9.3	34±6	28±4
$k_{cat} / K_{m,ATP}$		ND	0.9	0.6	ND	0.06	0.014	0.075
$K_{0.5,MT}$ (μM)		3.4±1.5	0.2±0.01	0.71±0.60	1.9±0.2	38.5±3.3	0.01±0.01	9.6±1.6
$k_{cat} / K_{0.5,MT}$		18	405	8	11	0.07	49	0.22
$K_{d,A}$ (μM)	Apo	0.4±0.2	ND	0.03–0.05	ND	0.07±0.02	0.7±0.1	0.2±0.06
$K_{d,B}$ (μM)	AMPPNP	1±0.1	ND	0.03–0.05	ND	9.4±0.3	ND	ND
$K_{d,C}$ (μM)	ADP•P _i	7±0.6	ND	ND	ND	8.0±0.7	ND	ND
$K_{d,D}$ (μM)	ADP	22±2	ND	ND	ND	11.6±0.8	ND	ND

See Fig. 1 for basal (i.e. no microtubules) and microtubule-simulated ATPase mechanisms

ND Not determined

^{a1} Rosenfeld et al. (2001), ² Sadhu and Taylor (1992), ³ Cochran et al. (2011), ⁴ Moyer et al. (1998), ⁵ Jiang et al. (1997), ⁶ Cochran et al. (2004), ⁷ Cochran and Gilbert (2005), ⁸ Cochran et al. (2006), ⁹ Rosenfeld et al. (2009), ¹⁰ Cochran et al. (2009), ¹¹ Sontag et al. (unpublished), ¹² Hertz et al. (2006), ¹³ Mackey and Gilbert (2000), ¹⁴ Song and Endow (1998), ¹⁵ Mackey and Gilbert (2003)

Table 2 Comparison of rate and equilibrium constants for truncated dimeric kinesins in published studies^a

Constant (units)	Reaction	Kinesin-1		Kinesin-5		Kinesin-7		Kinesin-14	
		<i>HsKHC</i> ¹⁻⁴	<i>DmKHC</i> ⁵⁻⁸	<i>RnKHC</i> ^{9,10}	<i>HsEg5</i> ¹¹⁻¹⁴	<i>HsCENP-E</i> ¹⁵	<i>XlCENP-E</i> ^{16,17}	<i>DmNCD</i> ¹⁸⁻²³	<i>ScKar3Vik1</i> ^{24,25}
k_0 (s ⁻¹)	ADP release from head 1	ND	306±25	>1000	28±0.5	0.9±0.01	ND	17.8±1.6	110±8
K_{+1}/k_{+2} (μM ⁻¹ s ⁻¹)	ATP Binding	2.6±0.3	2.0±0.8	1.7	1.2±0.1	1.4±0.1	1.7±0.3	2.3±0.1	8.4±0.02
k_{+2} (s ⁻¹)	Head isomerization	457±56	>200	210±25	50±3	47.5±0.8	13.7±2.7	>200	69±1.4
k_{-2} (s ⁻¹)	ATP dissociation	55–135	71±9	18	1.4±0.6	1.6±0.7	101±13	14±5	16.6±1.0
k_{+3} (s ⁻¹)	ADP release from head 2	170±17	300±100	>1000	0.62±0.04	19.4±0.6	56.8±3.5	1.4±0.02	N/A
k_{+4} (s ⁻¹)	Hydrolysis	79±4	100±30	500	5–10	24.7±0.5	ND	35±5	26±0.8
k_{+5} (s ⁻¹)	P _i Release / Dissociation	48–55	50±8	80	6.6±3.0	1.4±0.01	4.7±0.4	12.2±0.3	11.5±0.3
k_{cat} (s ⁻¹ site ⁻¹)	MT-ATPase	20±2	20±2	40.3±1.1	0.5±0.02	0.9±0.02	12.1±0.8	2.1±0.02	5.0±0.1
$K_{m,ATP}$ (μM)		23±9	61±8	53.6±4.8	7.9±2.4	18.3±2.5	35±5	23.1±1.5	65.2±4.1
$k_{cat} / K_{m,ATP}$		0.87	0.33	0.72	0.06	0.05	0.35	0.09	0.08
$K_{0.5,MT}$ (μM)		0.3±0.1	0.9±0.3	ND	1.8±0.2	1.5±0.2	0.10±0.04	18.3±5.1	0.7±0.05
$k_{cat} / K_{0.5,MT}$		66.7	22.2	ND	0.3	0.6	121	0.1	7.1
K_0 (μM)	No added nucleotide	ND	ND	ND	1.0±0.1 ^a	0.9±0.1	ND	0.22±0.05	0.6±0.1
V_{max} (μm/min)	MT gliding velocity	42.2±4.4	51.2±1.5	39–48	2.8±0.1	0.66±0.003	20.5±0.6	10.2±0.7	2.7±0.1
	MT end directionality	Plus	Plus	Plus	Plus	Plus	Plus	Minus	Minus
Stall force (pN)		6±1	7±1	ND	5±1	ND	6±1	1±0.5 ^c	ND

ND Not determined; N/A Not applicable

^a Published studies: ¹ Rosenfeld et al. (2002), ² Rosenfeld et al. (2003), ³ Clancy et al. (2011), ⁴ Schmitzer et al. (2000), ⁵ Gilbert et al. (1998), ⁶ Coy et al. (1999), ⁷ Moyer et al. (1998), ⁸ Higuchi et al. (2004), ⁹ Auerbach and Johnson (2005a), ¹⁰ Mazumdar and Cross (1998), ¹¹ Krzyziak et al. (2006), ¹² Kikkawa and Hirokawa (2006), ¹³ Krzyziak et al. (2008), ¹⁴ Valentine et al. (2006), ¹⁵ Sardar and Gilbert (2012), ¹⁶ Yardinci et al. (2008), ¹⁷ Rosenfeld et al. (2009), ¹⁸ Chandra et al. (1993), ¹⁹ Foster et al. (1998), ²⁰ Foster and Gilbert (2000), ²¹ Foster et al. (2001), ²² Furuta et al. (2013), ²³ Heuston et al. (2010), ²⁴ Chen et al. (2011), ²⁵ Sproul et al. (2005), ²⁶ Allingham et al. (2007), ²⁷ Chen et al. (2012)

^b K_{fill} value is the product of the K_d values for cooperative sites in the Eg5 dimer (Krzyziak et al. 2006)

^c Stall force determined for 2–4 NCD motors linked via a rigid DNA scaffold with an ~6 or ~22-nm spacing (Furuta et al. 2013)

Table 3 X-ray crystallographic structural summary for kinesin motors

Family	Name	PDB ID	Notes	Nucleotide	Ligand(s)	Resolution (Å)	Reference
Kinesin-1	<i>HsKHC</i>	1bg2		MgADP	Acetate	1.8	Kull et al. 1996
	<i>HsKHC</i>	1mkj		MgADP	SO ₄	2.7	Sindelar et al. 2002
	<i>RnKHC</i>	2kin		MgADP	SO ₄	2.0	Sack et al. 1997
	<i>NcKHC</i>	1goj		MgADP		2.3	Song et al. 2001
	<i>RnKHC</i>	3kin	Dimer	ADP		3.1	Kozielski et al. 1997
	<i>DmKHC</i>	2y5w	Dimer	MgADP		2.7	Kaan et al. 2011
	<i>DmKHC</i>	2y65	Dimer; Tail	MgADP		2.2	Kaan et al. 2011
	<i>HsKHC</i>	4hna		MgADP·AlF _x	Tubulin	3.2	Gigant et al. 2013
Kinesin-2	<i>GiKIN2A</i>	2vvg		MgADP		1.6	Hoeng et al. 2008
Kinesin-3	<i>MmKIF1A</i>	2zfi		MgADP		1.6	Nitta et al. 2008
	<i>MmKIF1A</i>	1i6i	Collision	MgAMPPCP		2.0	Kikkawa et al. 2001
	<i>MmKIF1A</i>	1vfv	Isomerization	MgAMPPNP		1.9	Nitta et al. 2004
	<i>MmKIF1A</i>	1vfx		MgADP·AlF _x		2.6	Nitta et al. 2004
	<i>MmKIF1A</i>	1vfz		MgADP·VO ₄		2.2	Nitta et al. 2004
	<i>MmKIF1A</i>	2zfm		ADP		2.3	Nitta et al. 2008
	<i>NcKIN3</i>	2owm		MgADP		3.3	Nitta et al. 2008
	<i>HsKIF3B</i>	3b6u		MgADP		1.8	Zhu et al. (SGC)
	<i>HsKIF3C</i>	3b6v		MgADP		2.7	Tempel et al. (SGC)
	<i>HsKIF13B</i>	3gbj		MgADP		2.1	Tong et al. (SGC)
	<i>MmKIF14</i>	4ozq	β-sheet twist	ADP		2.7	Arora et al. 2014
	<i>MmKIF4A</i>	3zfd		MgAMPPNP		1.7	Chang et al. 2013
	<i>HsKIF7</i>	4a14		MgADP		1.6	Klejnot and Kozielski 2012
	<i>HsKIF7</i>	2xt3		MgADP		1.9	Klejnot and Kozielski 2012
Kinesin-5	<i>HsEg5/KSP</i>	1ii6		MgADP	NO ₃	2.1	Turner et al. 2001
	<i>HsEg5/KSP</i>	3hqd	Isomerization	MgAMPPNP	PO ₄	2.2	Parke et al. 2010
	<i>HsEg5/KSP</i>	1q0b	L5 Inhibitor	MgADP	Monastrol	1.9	Yan et al. 2004
	<i>HsEg5/KSP</i>	4ap0	L5 Inhibitor	MgADP	Ispinesib	2.6	Talapatra et al. 2012
	<i>HsEg5/KSP</i>	4as7	L5 Inhibitor	CoADP	SB743921	2.4	Talapatra et al. 2013
	<i>HsEg5/KSP</i>	3zcw	α4 Inhibitor	MgADP	4A2	1.7	Ulaganathan et al. 2013
Kinesin-7	<i>HsCENP-E</i>	1t5c		MgADP	NO ₃	2.5	Garcia-Saez et al. 2004
Kinesin-8	<i>HsKIF18A</i>	3lre		MgADP		2.2	Peters et al. 2010
Kinesin-9	<i>HsKIF9</i>	3nwn		MgADP		2.0	Zhu et al. (SGC)
Kinesin-10	<i>HsKIF22</i>	3bfm		MgADP		2.3	Zhu et al. (SGC)
	<i>DmNOD</i>	3 dc4		MgADP		1.9	Cochran et al. 2009
	<i>DmNOD</i>	3deb		MgAMPPNP		2.5	Cochran et al. 2009
	<i>DmNOD</i>	3pxn		MnADP		2.6	Cochran et al. 2011
Kinesin-12	<i>HsKIF15</i>	4bn2		MgADP		2.7	Klejnot et al. 2014
Kinesin-13	<i>MmKIF2C</i>	1v8j		MgADP		3.2	Ogawa et al. 2004
	<i>MmKIF2C</i>	1v8k		MgAMPPNP		2.3	Ogawa et al. 2004
	<i>PfKINI</i>	1ry6		SO ₄		1.6	Shipley et al. 2004
	<i>HsKIF2A</i>	2gry		MgADP		2.4	Wang et al. (SGC)
	<i>HsKIF2C</i>	2heh		MgADP		2.2	Wang et al. (SGC)
Kinesin-14	<i>DmNCD</i>	2ncd	Dimer	ADP	SO ₄	2.5	Sablin et al. 1998
	<i>DmNCD</i>	1cz7	Dimer	MgADP		2.9	Kozielski et al. 1998
	<i>DmNCD</i>	1n6m	Dimer	MgADP		2.5	Yun et al. 2003

Table 3 (continued)

Family	Name	PDB ID	Notes	Nucleotide	Ligand(s)	Resolution (Å)	Reference
	<i>Dm</i> NCD	3l1c	Dimer; T436S	MgADP		2.8	Heuston et al. 2010
	<i>Dm</i> NCD	3u06	Dimer; G347D	MgADP		2.4	Liu et al. 2012b
	<i>Sc</i> KAR3	3kar		MgADP		2.3	Gulick et al. 1998
	<i>Sc</i> KAR3	1f9t	WT	MgADP		1.5	Yun et al. 2001
	<i>Sc</i> KAR3	1f9u	N650K	MgADP		1.7	Yun et al. 2001
	<i>Sc</i> KAR3	1f9v	R598A	MgADP		1.3	Yun et al. 2001
	<i>Sc</i> KAR3	1f9w	E631A	MgADP		2.5	Yun et al. 2001
	<i>Sc</i> KAR3/VIK1	4etp		MgADP		2.3	Rank et al. 2012
	<i>Cg</i> KAR3	4gkr		MgADP		2.7	Duan et al. 2012b
	<i>Ca</i> KAR3	4h1g	MBP fusion	MgADP		2.2	Delorme et al. 2012
	<i>Ag</i> KAR3	3t0q		MgADP		2.4	Duan et al. 2012a
	<i>St</i> KCBP	1sdm		MgADP		2.3	Vinogradova et al. 2004
	<i>St</i> KCBP	3cob	Extended α 4	MgADP		2.2	Vinogradova et al. 2008
	<i>St</i> KCBP	3h4s	Regulator KIC	MgADP		2.4	Vinogradova et al. 2009
	<i>At</i> KCBP	4fz		MgADP		2.4	Vinogradova et al. 2013
	<i>Hs</i> KIFC1	2rep		MgADP		2.6	Zhu et al. (SGC)
	<i>Hs</i> KIFC3	2 h58		MgADP		1.9	Wang et al. (SGC)
	<i>Sc</i> VIK1	2o0a	Nonmotor	N/A		1.6	Allingham et al. 2007

SGC Structural Genomics Consortium Toronto; N/A Not applicable

(Schmidt et al. 2012; Stinson et al. 2013; Yamada et al. 2001), and RecA (Datta et al. 2003; Rajan and Bell 2004).

For dimeric kinesins, the ATPase mechanism is more complicated due to the intramolecular communication between the two heads of the dimer (Fig. 2). This intramolecular communication (aka gating) is both chemical and mechanical in nature and functions to ensure one head is tightly bound to the filament at any given point in the cycle for processive motility (Block 2007). Historically, kinesins have been purified with ADP bound to the active site (Muller et al. 1999); and upon interaction with the microtubule, the first head (head 1) tightly binds the filament and releases its bound ADP very rapidly, while the second head (head 2) remains tightly bound to ADP and weakly bound to the microtubule (Ma and Taylor 1997). Upon ATP binding to head 1, a conformational change in the motor domain induces the neck linker to dock (Asenjo et al. 2006; Khalil et al. 2008; Rice et al. 1999; Rosenfeld et al. 2001; Skiniotis et al. 2003; Sugata et al. 2004; Sugata et al. 2009) and thus bias the diffusional search by head 2 toward its next microtubule binding site in the direction of the plus-end. Head 2 will tightly bind the microtubule and rapidly release its ADP, leading to a “strained” complex with two heads tightly bound to the microtubule, thus resulting in significant head–head tension (Hariharan

and Hancock 2009; Hyeon and Onuchic 2007). This intramolecular strain is thought to decrease the affinity of the front head (head 2) for ATP until the rear head (head 1) hydrolyzes ATP and detaches from the microtubule (Guydosh and Block 2006; Klumpp et al. 2004b; Rosenfeld et al. 2003), as well as to trigger ATP hydrolysis and accelerate detachment of the rear head (Crevel et al. 2004; Farrell et al. 2002; Schief et al. 2004; Yildiz et al. 2008). After ATP hydrolysis and P_i release from the rear head (head 1), this head detaches and effectively brings the motor to the beginning of the cycle, with the exception that the heads have swapped position (i.e., head 1 is now tightly bound to ADP and weakly bound to the microtubule, and head 2 is tightly bound to the microtubule where it awaits ATP binding). This finely tuned mechanochemical cycle maintains head–head coordination in order to keep each head’s ATPase cycle out of phase so that the motor is able to take hundreds of steps (for kinesin-1, kinesin-7 and kinesin-8), five to ten steps (for kinesin-5), or thousands of steps (for kinesin-3) along the microtubules without completely detaching and diffusing away from the track (this topic is discussed in more detail in the “Physics” section of this review).

In the absence of microtubules, most purified kinesin motors are unstable and will denature when the nucleotide is removed

Fig. 2 Model for the kinesin-1 “hand-over-hand” walking mechanism. Molecular representations of the dimeric kinesin-1 motor and microtubule (MT) are shown in each of four states (labeled numbers to the left of figure). The coiled-coil stalk (gray) and neck linker (red) are highlighted in each state. Coloring of the kinesin motor core represents three structural states: dark blue strongly ADP-bound, weakly microtubule-bound, neck linker undocked; cyan weakly nucleotide-bound, strongly microtubule-bound, neck linker docked; magenta strongly ATP- or ADP·P_i-bound, strongly microtubule-bound, neck linker docked. Dimeric kinesin-1 motors are purified with ADP tightly bound to both active sites. The first head to strongly interact with the microtubule (head 1) will rapidly release its bound ADP to reach the nucleotide-free/apo/rigor state (state 1). Since head 2 remains unattached to the microtubule, head 1 is able to bind ATP, which docks the neck linker of head 1, biasing head 2 forward (i.e., toward the MT plus-end) to the next binding site on the same MT protofilament (state 2). ATP hydrolysis on head 1 is concomitant with tight microtubule-binding by head 2 to rapidly release its ADP product (state 3). Upon P_i release from head 1, intramolecular strain coupled to conformational changes in the ADP state of head 1 lead to weakening of head 1’s interaction with the MT (state 3). After detachment of head 1 from the MT, the kinesin effectively reaches state 1 with the exception that the heads trade places (i.e., head 2 is nucleotide-free/apo/rigor while head 1 is ADP-bound and detached from the MT). The transition from state 3 to state 4 leads to a release of the intramolecular tension that is thought to provide a mechanical gating of ATP binding to the front head

from its active site (Pechatnikova and Taylor 1997; Sadhu and Taylor 1992). Past studies with monomeric kinesin-5 (Eg5) have demonstrated that the motor domain is stable and active when purified away from the bound ADP (Cochran and Gilbert 2005). Using kinetic (Cochran and Gilbert 2005; Cochran et al. 2006; Cochran et al. 2004; Rosenfeld et al. 2005) and thermodynamic (Zhao et al. 2010) methodologies, the energy landscape has been defined in the absence and presence of

microtubules (note: a more thorough discussion of this energy landscape is found in the “Physics” section of this review). The microtubule modulates the energy landscape in three major ways: (1) by accelerating the rate of ATP binding, (2) by weakening ADP binding, and (3) by accelerating the rate of ADP release. Thus, the microtubule acts as an ATPase-activating protein as well as an ADP-exchange factor, analogous to GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) for small G proteins (Bos et al. 2007).

Steady-state ATPase

The kinetic study of kinesin family members has been made possible through the purification of truncated constructs from a recombinant source (usually *Escherichia coli* or baculovirus). After preparation, the protein concentration must be established using available methods (e.g. Bradford assay or ultraviolet light absorbance at 280 nm). The first step in understanding the kinetic properties of the motor is to determine the microtubule-activated Michaelis–Menten steady-state ATPase activity of kinesin, which simply requires measuring the products of ATP hydrolysis (ADP or P_i) produced as a function of time [note: see De La Cruz and Ostap (2009); Gilbert and Mackey (2000) for methods to monitor hydrolysis product formation]. The aim of such experiments is to characterize the steady-state kinetic parameters (k_{cat} , $K_{m,ATP}$, $K_{0.5,MT}$) using a set of reciprocal experiments. In the first experiment, the microtubule concentration is kept constant and in excess (ideally 10-fold higher than the $K_{0.5,MT}$) and the ATP concentration is varied. The initial ATPase velocity (i.e., v_o , slope of a plot of product formed over time) is then plotted against ATP concentration and the data fit to the Michaelis–Menten equation (Michaelis and Menten 1913; Michaelis et al. 2011):

$$v_o = \frac{[E]_o k_{cat} [ATP]}{K_{m,ATP} + [ATP]} \quad (1)$$

where $[E]_o$ is the total concentration of kinesin [which ideally is 100- to 1,000-fold lower than the lowest ATP concentration ($[ATP]$) in the experiment], k_{cat} is the maximum rate of ATP turnover per enzyme active site at infinite $[ATP]$, and $K_{m,ATP}$ is the Michaelis constant, which is defined as the $[ATP]$ which yields $\frac{1}{2} [E]_o k_{cat}$.

In the second experiment, the ATP concentration is kept constant and in excess (ideally 10-fold higher than $K_{m,ATP}$) and the microtubule concentration is varied. The initial ATPase velocity is plotted against microtubule concentration and the data fit to the following quadratic equation since (1) $[E]_o$ is rarely 100- to 1,000-fold lower than the lowest microtubule concentration in the experiment, (2) the microtubules are not consumed in the reaction (whereas ATP does get consumed as a

substrate), and (3) microtubules typically bind very tightly to kinesin (Morrison 1969). Thus,

$$v_o = k_{cat} \left\{ \frac{([E]_o + K_{0.5,MT} + [MT]) - \sqrt{([E]_o + K_{0.5,MT} + [MT])^2 - 4[E]_o[MT]}}{2} \right\} \quad (2)$$

where $[E]_o$ is the total concentration of kinesin (typically 2- to 10-fold lower than the lowest microtubule concentration ($[MT]$)), k_{cat} is the maximum rate of ATP turnover per enzyme active site at infinite $[MT]$, and $K_{0.5,MT}$ is the $[MT]$ that yields $\frac{1}{2} [E]_o k_{cat}$.

It has been observed that unpolymerized tubulin heterodimers stimulate the ATPase activity of various kinesin motors (Alonso et al. 2007; Arora et al. 2014; Friel and Howard 2011; Gigant et al. 2013; Hertz et al. 2006). Tubulin-stimulated ATPase activity in kinesin can be assayed in a similar manner as described above [i.e., with $[ATP]$ held constant and in excess (10-fold higher than $K_{m,ATP}$) while the concentration of unpolymerized tubulin is varied]. One must be thorough and also perform an ATP-concentration dependence test with excess tubulin heterodimer (ideally 10-fold higher than the $K_{0.5,tub}$) to test if the substrate binding affinity is different when kinesin is bound to tubulin heterodimers rather than polymerized microtubules. An important consideration for this analysis would be to test that the tubulin is not polymerizing under the experimental conditions. Sedimentation and light microscopy using fluorescently labeled tubulin are two commonly used experimental strategies to provide evidence that tubulin remains soluble and unpolymerized. Also, recent studies have demonstrated a designed ankyrin repeat protein (DARPin) that blocks longitudinal interaction between tubulin dimers along the protofilament axis, thus preventing microtubule polymer formation (Gigant et al. 2013; Pecqueur et al. 2012). This biochemical reagent can be useful to ensure non-polymerizable tubulin conditions.

Steady-state ATPase assays have also been useful for assessing the binding affinity of allosteric inhibitors of multiple different kinesin motors (Catarinella et al. 2009; Cochran et al. 2005; DeBonis et al. 2003; Maliga et al. 2002; Skoufias et al. 2006; Tcherniuk et al. 2010; Wood et al. 2010). Typically, these steady-state assays are designed by varying the inhibitor concentration in the presence of excess ATP and microtubule concentrations. However, with this added reagent to the ATPase reaction, one can determine the ATP

affinity (vary $[ATP]$ while keeping microtubule and inhibitor concentrations constant) and microtubule affinity (vary microtubule concentration while keeping $[ATP]$ and inhibitor concentration constant) in the presence of the inhibitor (Cochran et al. 2005). Results from these experiments are important for deciphering the mechanism of kinesin inhibition and establish the foundation for the design of subsequent kinetic and thermodynamic experiments.

Together, these steady-state assays provide information on important properties of the kinesin enzyme, including the apparent binding affinities for ATP ($K_{m,ATP}$) and microtubules ($K_{0.5,MT}$), as well as the quantitative measure of the maximum rate of ATPase activity (k_{cat}). However, since the Michaelis–Menten theory does not adequately model even the minimal ATPase mechanism for kinesin (Fig. 1), one must be careful in the interpretation of the meaning of these steady-state kinetic parameters. Also, the determination of the k_{cat} requires an accurate measure of the active enzyme population, which can be a very challenging task for a protein enzymologist. Monomeric kinesins have been shown to have a range of microtubule-stimulated k_{cat} values, i.e., 0.5–80 s^{-1} , $K_{m,ATP}$ values of between 9 and 90 μM , and $K_{0.5,MT}$ values of between 0.01 and 10 μM (Table 1). The enzymatic efficiency ($k_{cat}/K_{m,ATP}$) and the biochemical “processivity” [$k_{bi} = k_{cat}/K_{0.5,MT}$; (Hackney 1995a; Hackney 1995b)] of the motor can be derived from the steady-state constants, which range from 0.04 to 0.9 $\mu M^{-1} s^{-1}$ and from 0.07 to 405 $\mu M^{-1} s^{-1}$, respectively. For dimeric kinesin motors (Table 2), k_{cat} values range from 0.5 to 40 s^{-1} , $K_{m,ATP}$ values range from 8 to 65 μM , and $K_{0.5,MT}$ values range from 0.1 to 20 μM , with the enzymatic efficiency and biochemical processivity ranging from 0.05 to 0.9 $\mu M^{-1} s^{-1}$ and from 0.1 to 121 $\mu M^{-1} s^{-1}$, respectively. Despite the relatively minimal information attained through these assays, this information is essential for the design of more complicated pre-steady-state experiments to measure intrinsic rate constants for ATP binding/release, microtubule association/dissociation, P_i release, and ADP binding/release, as well as for the design of parameters for cosedimentation experiments.

Microtubule-kinesin cosedimentation

Cosedimentation experiments have historically been performed to quantify the concentration of kinesin that pellets (or sediments) with microtubule polymers after ultra-high speed centrifugation. Since this methodology provides the effective dissociation constant (K_d) of an ensemble of kinesin states, various nucleotides (or nucleotide analogs) can be added in excess to the reaction to assess how different nucleotide states can influence microtubule binding affinity. For this assay, the kinesin concentration is typically kept constant, and microtubules are titrated into the reaction where they are allowed to equilibrate prior to centrifugation at ultra-high speed. Samples of the supernatant and pellet are prepared for analysis by SDS-PAGE. The fraction (f) of total kinesin $[E]_0$ bound to microtubules $[E \cdot MT]$ is plotted as a function of

microtubule concentration, and the data are fit to a quadratic equation (Eq. 6):

$$K_{d,MT} = \frac{[E][MT]}{[E \cdot MT]} \quad (4)$$

$$f = \frac{[E \cdot MT]}{[E]_0} \quad (5)$$

$$f = \frac{([E]_0 + K_{d,MT} + [MT]) - \sqrt{([E]_0 + K_{d,MT} + [MT])^2 - 4[E]_0[MT]}}{2[E]_0} \quad (6)$$

To ascertain mechanistic information for the kinesin affinity for microtubules at various nucleotide states (nucleotide-free/apo/rigor: apyrase or alkaline phosphatase treatment; ATP-like: AMPPNP, AMPPCP, ATP γ S; ADP $\cdot$ P $_i$ -like: ADP + BeF $_3$, AlF $_3$, VO $_4^{3-}$, SO $_4^{2-}$; ADP; steady-state ATP hydrolysis), care must be taken to ensure that the “equilibrium population” being studied demonstrates a valid biochemical state that will lead to sound interpretation. For example, if you want to characterize the fraction of kinesin motors bound to microtubules under steady-state ATP hydrolysis conditions, the concentrations of reagents and timing for incubation prior to sedimentation must be correct to monitor the binding under “steady-state” ATP turnover conditions. Often an ATP-regeneration system [pyruvate kinase/phospho(enol)pyruvate or creatine kinase/phosphocreatine] will assist in keeping ATP concentrations “constant,” but may interfere with densitometry analysis of Coomassie-stained SDS-polyacrylamide gels. Overall, this cosedimentation methodology is very powerful in elucidating the mechanochemistry of a kinesin motor and is a relatively simple experiment to perform in a biochemistry laboratory.

Care must be taken in the design and interpretation of cosedimentation results. Typically, these experiments rely on Coomassie-stained SDS-polyacrylamide gel and densitometry analysis, which sets the lower limit for the concentration of kinesin used in the experiment. Under most nucleotide conditions, kinesin binds tightly to microtubules (usually a 10- to 100-fold lower K_d than the kinesin concentration) and renders an inaccurate measurement of the binding constant.

Alternative SDS-gel staining methods (e.g., Sypro Ruby; Life Technologies, Carlsbad, CA) have been utilized to perform the cosedimentation experiments at lower kinesin concentrations (Allingham et al. 2007), however the error in the standard curve using low quantities of protein (5–20 ng) renders experimental reproducibility difficult, ultimately leading to large standard deviations in observed binding constants (± 10 –30 %). It is possible that emerging technologies, such as microscale thermophoresis (Seidel et al. 2013) or isothermal titration calorimetry (Devred et al. 2010; Tsvetkov et al. 2013), will provide a means to accurately determine the kinesin–microtubule binding constant under various nucleotide conditions.

ATP binding

The kinetic mechanism of substrate binding to an enzyme is very important in enzymology. This is especially true for nucleotide binding to kinesin motors since conformational changes during MgATP binding are central to harnessing of the free energy of ATP hydrolysis for force production (Rice et al. 1999). Depending on how the observed rate of the reaction changes as a function of ATP concentration, the data can reveal the presence of intermediate steps after (or before) ATP binding that limit the rate of this reaction. Several methodologies are available for measuring the presteady-state ATP binding kinetics for kinesins, including fluorescent nucleotide binding experiments (mant-ATP), radiolabeled pulse-chase methodologies, FRET methods, and intrinsic protein

fluorescence experiments (in the absence of microtubules). All kinesins studied to date have demonstrated (at least) a two-step rapid equilibrium binding mechanism in the presence or absence of microtubules. This binding mechanism can follow two similar (yet distinct) pathways:

In the first mechanism (Eq. 7), the ATP substrate collides with the kinesin motor (E) and weakly interacts. Then, upon a reversible isomerization (i.e., change in conformational state) of the E-S complex, the motor tightens its interaction with ATP to form the $E^* \cdot S$ intermediate. In the second mechanism (Eq. 8), the motor undergoes a reversible isomerization prior to binding the ATP substrate, which is followed by tight ATP binding. The difference between these two mechanisms centers on the timing of the reversible isomerization step (i.e., a conformation change after or before ATP binding, respectively). Either mechanism satisfies the curvature in the concentration dependence of the observed reaction rate, which is indicative of (at least) a two-step mechanism approaching a maximum rate that is limited by a first-order isomerization of the E-S complex (or E prior to binding ATP).

Monomeric kinesin motors have shown a wide range of ATP binding affinities in the absence and presence of microtubules (K_{+1} , k_{+2} = 0.08–400 $\mu M^{-1} s^{-1}$ and 1–20 $\mu M^{-1} s^{-1}$, respectively; Table 1) as well as a range of isomerization rates (k_{+2} = 0.5–1800 s^{-1} and 20–900 s^{-1} , respectively; Table 1). Dimeric kinesins have also demonstrated a wide range of ATP binding kinetics when bound to microtubules ($K_{+1}k_{+2}$ = 1–20 $\mu M^{-1} s^{-1}$ and k_{+2} = 10–500 s^{-1} ; Table 2). These kinetic constants indicate that kinesin motors have diverse ways of interacting with ATP substrate and that the structural interpretation for these kinetics may not be as straightforward as some have suggested (Chang et al. 2013).

ATP hydrolysis

After ATP binds tightly to the kinesin core, the motor can adopt the necessary active site configuration that promotes rapid hydrolysis of the γ -phosphate from ATP. In certain kinesins [e.g., monomeric *Drosophila* kinesin-10 in the absence and presence of microtubules (Cochran et al. 2009); monomeric human kinesin-13 in the absence of microtubules (Friel and Howard 2011)] and microtubule-stimulated dimeric human kinesin-5 (Krzysiak and Gilbert 2006)], this conformational change in the core represents the slow, rate-limiting step in the pathway. For other well-characterized kinesins, this

reaction is relatively fast during the ATPase cycle (Tables 1 and 2), so the time spent in the ATP-bound state is transient. Subsequent to the characterization of ATP binding kinetics, rapid acid-quench-flow methodologies are used to measure the kinetics of the ATP hydrolysis reaction (Gilbert and Mackey 2000; Johnson 1992). However, since the kinetics of ATP binding precede the hydrolysis reaction, care must be taken in the interpretation of observed kinetics determined by acid-quench experiments. Normally kinetic simulation and global data fitting software are utilized to fit presteady-state data to the minimal mechanism based on numerical integration of the rate equations [e.g. DynaFit (Kuzmic 1996), KINSIM/FITSIM (Barshop et al. 1983; Zimmerle and Frieden 1989), or Global Kinetic Explorer (Johnson et al. 2009)].

ATP hydrolysis is a thermodynamically favorable reaction ($\Delta G^\circ = -35.6$ kJ mol $^{-1}$; see Appendix A), yet, when uncatalyzed, is a very slow reaction ($\Delta G^\ddagger = 115$ kJ mol $^{-1}$, $k_{uncat} = 3.8 \times 10^{-8}$ s $^{-1}$, $t_{0.5} = 211$ days; Stockbridge and Wolfenden 2009). ATPases catalyze this reaction by lowering the energy of the transition state relative to the reactants through either a “dissociative” mechanism, an “associative” mechanism, or a “concerted” mechanism between these two extremes (Admiraal and Herschlag 1995; Khan and Kirby 1970; Thatcher and Kluger 1989). A substantial amount of data supports a largely “dissociative” mechanism for phosphoanhydride hydrolysis in which bond cleavage precedes nucleophilic attack by the lytic water molecule, thus leading to a metaphosphate-like transition state (Bunton and Chaimovi 1965; Halkides and Frey 1991; Lightcap and Frey 1992; Miller and Ukena 1969; Miller and Westheim 1966a; Miller and Westheim 1966b). Recent studies using combined quantum-mechanical/molecular-mechanical metadynamics simulations support the “dissociative” model of ATP hydrolysis by kinesin whereby two water molecules participate in proton transfer to ultimately the P_i product (McGrath et al. 2013). These two water molecules (one “lytic” water molecule located proximal to the γ -phosphate and a second “relay” water molecule positioned between the lytic water molecule and the switch-2 glutamic acid that forms a salt bridge with the switch-1 arginine; for more detail, see the “Structure” section of this review) establish a network of hydrogen bonds in the hydrolysis-competent conformation of motor ATPases (Dittrich et al. 2003; Dittrich et al. 2004; Grigorenko et al. 2011; Grigorenko et al. 2007; Hayashi et al. 2012; Onishi et al. 2004; Parke et al. 2010; Schwarzl et al. 2006).

This “hydrolysis-competent” conformational state of the motor must be a very transient (i.e., short-lived) structural intermediate that rapidly isomerizes to a state that is not conducive for ATP synthesis for monomeric kinesins in the absence and presence of microtubules, yet the trailing head in a dimeric kinesin does show propensity for ATP synthesis (Hackney 2005). Despite having an abundant quantity of data

available on the ATP hydrolysis reaction and various conformational states of motor ATPases, it remains unknown how sequential conformational changes in hydrolases catalyze nucleotide bond cleavage.

P_i release

After ATP hydrolysis produces its reaction products (ADP and P_i), P_i product release occurs first, followed by ADP release. For most kinesins, P_i release leads to an allosteric change in the microtubule-binding region that weakens the motor's affinity for the microtubule (see below). Therefore, P_i release is one of the pivotal reactions in the kinesin ATPase cycle, yet arguably the most difficult to experimentally study since it is a transient step buried deep within the mechanism. Although P_i release is typically a rapid reaction in the basal ATPase cycle, monomeric kinesin-1, kinesin-5, and kinesin-7 motors demonstrate rate-limiting P_i release for their microtubule-stimulated ATPase cycles [(Cochran and Gilbert 2005; Cochran et al. 2006; Cochran et al. 2004; Cochran et al. 2011; Moyer et al. 1998; Rosenfeld et al. 2001; Rosenfeld et al. 2009); Table 1].

Experimental strategies have been developed to directly measure the rapid kinetics of P_i release from kinesin in the absence and presence of microtubules using a fluorescence-coupled assay (Brune et al. 1994; White et al. 1997). This strategy relies on the very rapid (i.e., near diffusion-limited reaction) and tight ($K_{d,P_i} \sim 100$ nM) binding of P_i to a modified phosphate-binding protein with the MDCC fluorophore (MDCC-PBP; Phosphate Sensor[®] from Life Technologies). This reagent can be rapidly mixed in a kinesin-catalyzed ATPase reaction using a stopped-flow fluorimeter, and fluorescence can be monitored over time to measure the kinetics of P_i release. After ATP hydrolysis, P_i is released from the kinesin active site, followed by rapid and tight P_i binding to MDCC-PBP, thus triggering a five- to sixfold fluorescence enhancement of the MDCC-PBP-Pi complex. Since this assay is very sensitive to P_i in solution, a “P_i mop” consisting of 7-methylguanosine and purine nucleoside phosphorylase must be added to effectively eliminate background P_i before the ATPase reaction is initiated (Cochran and Gilbert 2005). Depending on the intrinsic rate constant for P_i release in relation to the other steps in the ATPase cycle, various outcomes are possible for the observed rate of reaction (e.g., linear kinetics if P_i release is the rate-limiting step or burst kinetics if a step after P_i release is rate-limiting). Also, the presence or absence of an initial exponential lag phase can provide insight into the rate of a given reaction prior to P_i release, which can be compared with observed rates of ATP binding or ATP hydrolysis reactions. Kinetic simulation and global data

fitting software ought to be utilized to model the intrinsic rate constant for P_i release since both the ATP binding and ATP hydrolysis steps precede this reaction (note: P_i rebinding to kinesin can be ignored due to the P_i binding to MDCC-PBP rendering the reaction kinetically irreversible).

MgADP release

The final reaction that completes the kinesin ATPase cycle is MgADP release to reach the nucleotide-free/apo/rigor state. For the basal ATPase cycle, this step represents the rate-limiting reaction for most (but not all) kinesin motors (Table 1). The observed presteady-state rate of ADP release in the absence of microtubules is typically similar to the measured basal ATPase activity, strengthening the argument for ADP release being rate-limiting in the cycle (Table 1). However, in the presence of microtubules, the rate of ADP release is dramatically accelerated for most kinesins (3–5 orders of magnitude). For kinesin-14 motors, the rate of MgADP release remains the rate-limiting step in the mechanism, but for other kinesins, the rate of this reaction no longer limits steady-state ATPase activity (Tables 1 and 2).

The mechanism of MgADP release has been the subject of multiple investigations since the discovery of kinesin, yet it remains a poorly understood step in the kinesin ATPase cycle. Several techniques are available to measure the presteady-state rate constants for MgADP binding and release, including mant-ADP fluorescence (both direct excitation/emission and FRET from protein tryptophans and tyrosines), intrinsic tryptophan fluorescence (in the absence of microtubules), and radiolabeled ADP chase experiments using a rapid ATP regeneration system. One can also measure the kinesin or microtubule-kinesin complex binding affinity for ADP using mant-ATP binding competition assays (De La Cruz and Ostap 2009; Zhao et al. 2010). Each method has advantages and limitations that must be taken into consideration when determining the mechanism of ADP product release. If nucleotide-free kinesin can be attained, the kinetics of MgADP binding can be measured in the absence of microtubules (Cochran et al. 2009; Zhao et al. 2010). Most kinesins are stable in the nucleotide-free state when bound tightly to microtubules. Therefore, presteady-state MgADP binding kinetics can be measured for the microtubule-kinesin complex (Table 1). The mechanism of MgADP binding ought to provide insight into the pathway for ADP release from kinesins since the intrinsic rate constants that govern the minimal mechanism can be derived from the fit of the ADP concentration dependence of the observed reaction rate (Johnson 1992).

All kinesins studied to date have demonstrated (at least) a two-step rapid equilibrium binding mechanism

for MgADP binding in the presence or absence of microtubules. As with MgATP binding, this binding mechanism can follow two similar (yet distinct) pathways:

The difference between these two mechanisms centers on the timing of the reversible isomerization step (i.e., a conformation change after or before ADP binding, respectively). Although the reversible isomerization of the kinesin motor domain has been demonstrated using enzyme kinetics, the details of the structural changes that drive this isomerization remain mysterious.

Monomeric kinesin motors have shown a wide range of ADP binding affinities in the absence and presence of microtubules (K_{-6} , k_{-5} = 0.07–330 $\mu\text{M}^{-1} \text{s}^{-1}$ and 0.6–11 $\mu\text{M}^{-1} \text{s}^{-1}$, respectively; Table 1), as well as a range of isomerization rates (k_{-5} = 0.6–1400 s^{-1} and 100–760 s^{-1} , respectively; Table 1). As with MgATP binding, the variability of rate constants governing MgADP binding suggests that the conformational changes occurring in each motor extend beyond the conserved active site motifs. What remains an unexplainable phenomenon is the symmetry of the rate constants that limit the isomerization reactions for MgATP and MgADP binding. For example, these rates were measured at 0.5–1 s^{-1} for monomeric kinesin-5 in the absence of microtubules using multiple different methodologies (Cochran and Gilbert 2005), suggesting the rate-determining structural change is dictated by a reaction that does not require the presence of the nucleotide γ -phosphate.

Microtubule detachment and binding

How kinesin motors convert the energy of ATP hydrolysis into directed mechanical work largely relies on allosteric changes in the region of the motor that interacts with the microtubule in order to modulate binding affinity. A wealth of data supports the notion that the kinesin nucleotide state modulates the microtubule binding affinity and vice versa. Thus, not only measuring the equilibrium binding constants under different nucleotide conditions (as described above), but also measuring the kinetics of microtubule binding (aka microtubule association) and microtubule detachment (aka microtubule dissociation) is important for deciphering the kinesin ATPase mechanism.

These presteady-state experiments have been adopted from similar methodologies from the actomyosin (Finlayson et al. 1969; Finlayson and Taylor 1969) and axonemal dynein

systems (Porter and Johnson 1983). Solution turbidity (λ = 340–350 nm at 180° to the incident beam) and light scattering (λ = 420 nm at 90° to the incident beam) provide optical signals for kinesin binding to or detaching from microtubules, which can be monitored over time in a rapid mixing stopped-flow fluorimeter. This experimental design depends on: (1) the turbidity/light scattering signal from the microtubule-kinesin complex is greater than the sum of turbidity/light scattering signals from the individual components; (2) the formation of or dissociation of the microtubule-kinesin complex follows simple exponential kinetics; (3) the initial and final equilibrium states can be characterized using other biophysical methodologies, such as cosedimentation or analytical ultracentrifugation, to confirm either complex formation or dissociation (Finlayson et al. 1969). For measuring the kinetics of microtubule-kinesin complex formation, kinesin motors under various nucleotide conditions are rapidly mixed with increasing concentrations of taxol-stabilized microtubules, and the observed rate of the change of turbidity/light scattering is plotted as a function of microtubule concentration. The fit of the data provides the apparent second-order rate constant for kinesin association with microtubules. For measuring the kinetics of microtubule-kinesin complex dissociation, the microtubule-kinesin complex is rapidly mixed with increasing concentrations of MgATP + extra salt (typical ionic strength 100–200 mM). The MgATP triggers the kinesin to detach from the microtubule during the ATPase cycle, whereas the increased ionic strength promotes weak microtubule-kinesin interaction, thus shifting the kinesin population towards its detached state. The observed rate of turbidity/light scattering is plotted as a function of MgATP concentration, and the hyperbolic fit provides the maximal rate of kinesin dissociation and the relative binding affinity of kinesin for MgATP.

Structure

The very first glimpse into how the kinesin motor is built came from determination of the high-resolution structure of the kinesin-1 motor domain based on by X-ray crystallography [Fig. 3; (Kull et al. 1996)], which showed that the kinesin-1 motor has many similarities with the related myosin motor domain (Rayment et al. 1993). Since then, over 100 kinesin structures from multiple families have been deposited into the Protein Data Bank (PDB; Table 3). and detailed reviews of these structures have been published (Kull 2000; Kull and Endow 2002; Marx et al. 2009; Marx et al. 2005; Woehlke 2001).

The kinesin motor domain contains: (1) the motor core consisting of an $\alpha\beta\alpha$ fold with three α -helices on each side of an eight-stranded β -sheet; (2) a family-specific neck-linker sequence immediately preceding or following the core in its primary sequence; (3) a motif called the cover strand at the

Fig. 3 Kinesin structure and topology. The monomeric human kinesin-1 structure (IBG2; Kull et al. 1996) is depicted in four distinct orientations: **a** front, **b** back, **c** left side, **d** right side. Secondary structure elements (α -helices, β -strands, and loops) are colored blue, gold, and red, respectively. Mg^{2+} and ADP are shown as space-fill model and are colored magenta and cyan, respectively. **e** Topology diagram of the kinesin motor domain shows α -helices (circles; shaded blue; up/down direction indicated by loop attachment), β -strands (triangles; shaded gold; up/down to depict orientation), and loops (red lines) as rendered from (Kull et al. 1998)

opposite terminus relative to the neck linker (e.g., kinesin-5 has its neck linker at the C-terminus and the cover strand has its neck-linker at the N-terminus relative to the core). The neck linker is central for energy transduction in kinesin motors (Rice et al. 1999; Schief and Howard 2001) and is also important for communicating strain between the two motor domains of dimeric kinesins to chemically and physically gate the mechanochemical cycle (Guydosh and Block 2006; Hwang and Lang 2009; Hyeon and Onuchic 2007; Jaud et al. 2006; Miyazono et al. 2010; Rosenfeld et al. 2003; Shastry and Hancock 2010; Yildiz et al. 2008). Although the neck linker and the cover strand are at opposite ends of the core in the primary sequence, they are in close tertiary-structural proximity due to the physical location for where the N- and C-termini enter and exit the core, respectively (Fig. 3). The cover strand interacts with the neck linker to form the cover-neck bundle, which is thought to participate in force generation (Hwang et al. 2008; Khalil et al. 2008).

The core contains several key structural motifs present in all kinesin motors. First, located on opposite sides of the eight-stranded β -sheet are the nucleotide binding pocket and the microtubule-binding interface (β 5-L8 lobe, L11, and the “Sw2 cluster” comprised of α 4-L12- α 5; Sosa et al. 1997; Woehlke et al. 1997). Second, conserved motifs called the phosphate binding loop (P-loop: Walker A motif consensus GxxxxGKT/S located at L4), switch-1 (Sw1: NxxSSR located at the β 6-L9 junction), and switch-2 (Sw2: Walker B motif, DxxGxE located at the β 7-L11 junction) undergo conformational changes during the ATPase cycle in response to the presence (e.g., ATP-bound state) or absence (e.g., ADP-bound or nucleotide-free/apo/rigor states) of the γ -phosphate. These structural changes are reciprocally related to—and thus modulate—the conformations of the neck linker/cover strand, and vice versa (Block 2007; Hwang et al. 2008; Khalil et al. 2008; Rice et al. 1999; Zhao et al. 2010). Third, a conserved motif called N-4 (consensus: RxxP) establishes interaction with the purine nucleotide base.

Central to the kinesin motor domain is an eight-stranded β -sheet (topology β 2- β 1- β 8- β 3- β 7- β 6- β 4- β 5; Fig. 3e) which serves as the core element that structurally connects the nucleotide-binding pocket and the microtubule-binding region. This central β -sheet has shown slightly variable degrees of right-handed twisting, which is thought to be important for the geometry and function of the motor domain (Kull and Endow 2013). Since most kinesins have been crystallized with bound nucleotide (Table 3), the degree of twisting has been very similar. However, recent crystal structures have demonstrated significant increases in right-handed twist or “over-twisting” of the kinesin β -sheet (Fig. 4; (Arora et al. 2014; Gigant et al. 2013), similar to the “over-twisting” seen in myosin’s β -sheet as it transitions from the nucleotide-bound state to the nucleotide-free state (Cecchini et al. 2008; Coureux et al. 2003; Reubold et al. 2003) [note: the “nucleotide-free” state has historically been called the “rigor” state since myosin binds very tightly to actin filaments in this nucleotide state]. The degree of “over-twisting” ($\tau_{\text{avg}}=9.1\pm4.3^\circ$; range $3.9\text{--}13.2^\circ$; Fig. 4f) observed in kinesins transitioning between the nucleotide-bound to nucleotide-free rigor states was comparable to that seen in myosin-V ($\tau_{\text{avg}}=6.7\pm4.8^\circ$; range $1\text{--}14^\circ$; Cecchini et al. 2008), which supports the hypothesis for common allosteric communication in cytoskeletal motors via the twisting of the central β -sheet. Indeed, theoretical studies on the origin of β -sheet twisting have demonstrated that the conformational energy associated with perturbing the structure of a β -sheet (through “twisting”, “bulging,” and “bending”) provide physical “reservoirs” of conformational free energy in globular proteins (Shamovsky et al. 2000a; Shamovsky et al. 2000b). Examination of the number of hydrogen bonds formed between residues of the central β -sheet reveals that five more hydrogen bonds are formed in the “under-twisted” nucleotide-bound state compared to

Fig. 4 Structural changes in the eight-stranded central β -sheet of kinesin. Side (a) and end (b) views of the kinesin β -sheet after a nucleotide-bound structure (1BG2; Kull et al. 1996) was aligned with a rigor-like structure (4OZQ; Arora et al. 2014) using the main chain atoms of the P-loop. Mg^{2+} and ADP are shown as space-fill model and are colored magenta and cyan, respectively. **c** The root-mean-square deviation (RMSD) of complementary residues is plotted for each individual β -strand. **d** Individual β -strand curvature based on the sum of sequential C_{α} distances for each β -strand residue (i =initial residue, $i+x$ =final residue) divided by the total distance from initial to final C_{α}

$\left[\frac{\sum_i^{i+x} (D_{i \rightarrow i+1})}{D_{i \rightarrow i+x}} \right]$ plotted for nucleotide-bound and rigor-like structures. **e** β -strand torsion angles (τ) relative to β_1 are plotted to quantify the degree of twisting within the entire β -sheet. Torsion angles were calculated using the following equation for each individual β -strand: $\tau_{\beta 2} = \tau_{\beta 1, \beta 2} = \cos^{-1}(\hat{\beta}_1 \cdot \hat{\beta}_2)$, where $\hat{\beta}_1$ and $\hat{\beta}_2$ are the unit vectors for each respective β -strand (Cecchini et al. 2008). **f** The change in each individual β -strand torsion angle ($\Delta\tau$) as the kinesin central β -sheet transitions from nucleotide-bound (1BG2) to rigor-like (4OZQ) states

the “over-twisted” rigor-like state, which amounts to 10–50 kJ mol⁻¹ bond energy. This “stored” bond energy may provide the biophysical basis whereby kinesin motors can accelerate their rates of product release during their ATPase cycles (for more details, see the “Physics” section of this review). Interestingly, Hsp70 ATPases have also demonstrated nucleotide-dependent conformational changes in their central β -sheet (Zhuravleva et al. 2012; Zhuravleva and Gierasch 2011), suggesting this means of allosteric communication in proteins may be quite prevalent.

Structural differences among kinesin members result in differences in the intrinsic rate and equilibrium constants that

govern the ATPase cycle. Although the detailed structural mechanism of the nucleotide- and microtubule-driven conformational changes remains largely unknown, a current model exists for processive kinesins and is largely based on studies of kinesin-1. The nucleotide state at the active site is thought to trigger a conformational change that is transmitted to the adjacent microtubule-binding regions of the core. Communication between the active site and the microtubule binding region is achieved through two prominent structural pathways: (1) Sw1 to $\alpha 3$ to the $\beta 5$ -L8 lobe (Cochran et al. 2009; Ogawa et al. 2004) and (2) Sw2 to L11 to helix $\alpha 4$ to the remainder of the “Sw2 cluster” (Rice et al. 1999; Sindelar and Downing 2007; Vale and Milligan 2000). The Sw2 cluster also controls the orientation of the neck linker in alternate conformations that result in directed force production and motility stabilization (Gigant et al. 2013; Kikkawa et al. 2001). Cryo-electron microscopy (cryo-EM) studies have provided key insights into the conformational changes that kinesin motor domains undergo during their ATPase cycle, and recent advances in image processing algorithms have provided moderate/high resolution structures of these complexes (Table 4).

Family-specific motifs modulate the structural communication of various kinesins during their mechanochemical cycles. For example, L5 plays an important role during the kinesin ATPase cycle and has been the subject of several studies due to its location in the motor domain (it interrupts the long $\alpha 2$ helix immediately downstream of the P-loop; Fig. 3), variability in length across the superfamily (6–21 residues), and forming part of the binding pocket for various kinesin-5/Eg5 inhibitors (Brier et al. 2004; Kaan et al. 2010; Maliga et al. 2006; Talapatra et al. 2012; Yan et al. 2004). L5 was proposed to first undergo conformational changes upon nucleotide binding during the kinesin-5 ATPase cycle (Cochran et al. 2005) and has since been found to function as a “conformational latch” to structurally and kinetically regulate its stepping behavior (Behnke-Parks et al. 2011; Parke et al. 2010; Waitzman et al. 2011). Specific interactions of the “microtubule-sensing latch” L7 with Sw1 and Sw2 have been implicated in the mechanism of MgADP product release from the active site of the kinesin-3 motor KIF1A (Nitta et al. 2008). For kinesin-13 motors, microtubule depolymerization activity relies on an extended L2 that contains the sequence “KVD” (also called the “KVD finger”), which has been shown to be necessary for microtubule depolymerization activity of these motors (Ogawa et al. 2004). This extended L2 is located within the conserved β -lobe near $\alpha 6$ (Fig. 3); however, the actual function of this β -lobe has remained elusive. The authors of a recent molecular dynamics simulation study aimed at investigating the role of this β -lobe in the mechanical stepping behavior in kinesin-1 suggested that it functions to amplify movements in the neck linker for processive motility (Geng et al. 2014).

Table 4 Summary for kinesin-microtubule interactions via cryo-electron microscopy methodologies

Family	Name	PDB ID	Nucleotide	Notes	Resolution (Å)	Reference
Kinesin-1	<i>HsKHC</i>	2p4n	Free/Rigor		9.0	Sindelar and Downing 2007
	<i>RnKHC</i>	4atx	Free/Rigor	Dcx ^a ; Pseudo model	8.2	Liu et al. 2012b
Kinesin-3	<i>MmKIF1A</i>	2hxf	MgAMPPNP		10.0	Kikkawa and Hirokawa 2006
	<i>MmKIF1A</i>	2hxx	MgADP		11.0	Kikkawa and Hirokawa 2006
	<i>MmKIF1A</i>	1ai0	MgAMPPCP		15.0	Kikkawa et al. 2001
Kinesin-5	<i>HsEg5/KSP</i>	4aqw	Free/Rigor		9.5	Goulet et al. 2012
	<i>HsEg5/KSP</i>	4aqv	MgAMPPNP		9.7	Goulet et al. 2012
	<i>DmKLP61F</i>	2wbe	MgAMPPNP	KLP61F homology model	9.4	Bodey et al. 2009
Kinesin-10	<i>DmNOD</i>	3dco	Free/Rigor		11.0	Cochran et al. 2009
Kinesin-13	<i>DmKLP10A</i>	3j2o	Unknown		10.8	Asenjo et al. 2013
	<i>DmKIF2C</i>	3edl	MgAMPPNP		28	Tan et al. 2008

^a Dcx, Microtubule-associated protein called doublecortin

Most kinesins walk along the microtubule lattice in a directional manner, either moving towards the plus-end or minus-end of the microtubule (Wade and Kozielski 2000). Traditionally, kinesins which have their motor domain located at the N-terminus of the heavy chain (i.e., N-class kinesins; e.g., kinesin-1) have shown plus-end directed motility, while kinesins with their motor domain located at the C-terminus (i.e., C-class kinesins; e.g., kinesin-14) have demonstrated minus-end directed motility. It was originally thought that directionality was caused by inherent differences in the structure of the motor domains of N-class and C-class kinesins (Henningsen and Schliwa 1997). However, crystal structures of the motor domains of kinesin-1 and kinesin-14 show very few structural differences (Kull et al. 1996; Sablin et al. 1996), while dimeric structures obtained through cryo-EM show significant differences in the orientation of the two motor domains relative to one another, with both having their unattached heads pointing in the direction they move along the microtubule (Arnal et al. 1996; Hirose et al. 1996; Kozielski et al. 1999; Sablin et al. 1998). Furthermore, dimeric chimeras with the motor core from kinesin-14 and stalk and neck regions from kinesin-1 show plus-end directed movement, despite the fact that the motor domains are from a minus-end directed motor (Henningsen and Schliwa 1997). Likewise, chimeras with the kinesin-1 motor core and kinesin-14 neck and stalk regions show minus-end motility (Endow and Waligora 1998). Taken together, these results suggest that directionality is determined primarily by structures outside the conserved motor core (Case et al. 1997).

The neck linker, which connects the motor core to the stalk, has been shown to reorient in response to the nucleotide state of the motor domain (Rice et al. 1999) and is thought to be responsible for positioning the detached motor head in the dimers towards either the plus- or minus-end of the microtubule, as seen in the dimeric structures of kinesin-1 and

kinesin-14 (Rice et al. 1999). Additionally, mutant studies of kinesin-14 chimeras have also indicated that interactions between the neck region and the stalk are critical for maintaining minus-end directed movement. Mutant motors that lack critical core-interacting residues result in the slowing of kinesin-14 walking towards the plus-end of microtubules (Sablin et al. 1998). Disrupting the interaction between the neck linker and the motor domain in kinesin-1 also significantly impairs motility (Case et al. 2000), suggesting that interactions of the motor core with both the neck linker and neck region are responsible for determining directional movement. Lastly, the finding that kinesin-14 moves towards the plus-end of microtubules when interactions between the motor core and neck are interrupted suggests that the catalytic motor core inherently has an slight plus-ended directionality (Sablin et al. 1998), which in plus-ended motors is amplified by their neck linker and neck interactions, and in minus-ended motors is over-ridden by stronger interactions resulting in minus-directed movement.

Recently, the kinesin-5's isolated from *Saccharomyces cerevisiae* (Cin8p and Kip1p; Fridman et al. 2013; Gerson-Gurwitz et al. 2011; Roostalu et al. 2011; Thiede et al. 2012) and from *Schizosaccharomyces pombe* [Cut7p; (Edamatsu 2014)] have been shown to be able to switch directionality between slow plus-end movement to rapid minus-end movement. *In vitro*, the switch to plus-end directionality for both Cin8 and Kip1 can be induced by motor coupling when they bind two antiparallel microtubules or by decreasing the ionic strength. At high (physiological) ionic strength, or when on single microtubules, they act as rapid minus-end directed motors, while at low ionic strength, or when cross-linking two antiparallel microtubules, they act as slow plus-end directed motors. This ability for single kinesin motors to change directionality based on environmental conditions provides an opportunity to better understand the underlying factors that

Table 5 Residue Some of the WT sequence is in bold. Should the bold font not be explained in a footnote? substitutions in kinesin motor domain with reported effects relative to the respective WT construct

Family	Name	Substitution	WT Sequence	Structure	Effects	Reference
Kinesin-I	<i>HsKHC</i>	R14A	MCRFR	β 1/N-4	R14A/R14A homodimer; 35-fold tighter K_m ; 18-fold slower k_{cat} ; 18-fold slower gliding V_{obs}	Kaseda et al. 2003
		R14A	MCRFR	β 1/N-4	WT/R14A heterodimer; 2-fold tighter K_m ; 9-fold slower k_{cat} ; 8-fold slower gliding V_{obs} ; similar stall force	Kaseda et al. 2003
		D140A	YLDKI	L7	3.5-fold tighter K_m ; 1.7-fold slower k_{cat} ; 1.8-fold slower gliding V_{obs}	Woehlke et al. 1997
		K141A	LDKIR	L7	1.8-fold weaker K_m ; 1.1-fold faster k_{cat} ; 1.1-fold slower gliding V_{obs}	Woehlke et al. 1997
		K159A	EDKNR	L8	2.3-fold weaker K_m ; similar k_{cat} ; 1.3-fold slower gliding V_{obs}	Woehlke et al. 1997
		E170A	CTERF	L8	2.6-fold tighter K_m ; 1.2-fold slower k_{cat} ; 1.6-fold slower gliding V_{obs}	Woehlke et al. 1997
		S202C	HSSRS	L9/Sw1	10-fold slower MgATPase; similar MnATPase; 100-fold slower MgATP gliding V_{obs} ; 10-fold slower MnATP gliding V_{obs}	Cochran et al. 2011
		E236A	GSEKV	L11/Sw2	No MT gliding; > 1000-fold slower k_{cat} ; similar ATP binding kinetics; increased ADP release kinetics; can take one ATP-dependent step	Rice et al. 1999
		K240A	VSKTG	L11	4.2-fold weaker K_m ; similar k_{cat} ; 1.6-fold slower gliding V_{obs}	Woehlke et al. 1997
		L248A	AVLDE	L11	3.1-fold weaker K_m ; 1.3-fold slower k_{cat} ; 1.1-fold faster gliding V_{obs}	Woehlke et al. 1997
		E250A	LDEAK	L11	1.9-fold tighter K_m ; 1.3-fold slower k_{cat} ; 1.8-fold slower gliding V_{obs}	Woehlke et al. 1997
		K252A	EAKNI	α 4 ext.	4.4-fold weaker K_m ; 2.7-fold slower k_{cat} ; 1.1-fold faster gliding V_{obs}	Woehlke et al. 1997
		K256A	INKSL	α 4	2.9-fold weaker K_m ; 1.4-fold slower k_{cat} ; similar gliding V_{obs}	Woehlke et al. 1997
		E270A	LAEGS	L12	2.1-fold tighter K_m ; 1.1-fold faster k_{cat} ; 1.1-fold slower gliding V_{obs}	Woehlke et al. 1997
		Y274A	STVVP	L12	3.2-fold weaker K_m ; 1.3-fold slower k_{cat} ; 1.2-fold slower gliding V_{obs}	Woehlke et al. 1997
		R278A	PYRDS	L12	15.5-fold weaker K_m ; 1.5-fold slower k_{cat} ; 1.3-fold slower gliding V_{obs}	Woehlke et al. 1997
		D279A	YRDSK	L12	2.3-fold tighter K_m ; 1.3-fold slower k_{cat} ; 1.2-fold slower gliding V_{obs}	Woehlke et al. 1997
		K281A	DSKMT	L12/ α 5	2.4-fold weaker K_m ; 1.1-fold slower k_{cat} ; 1.2-fold slower gliding V_{obs}	Woehlke et al. 1997
		R284A	MTRIL	α 5	2.2-fold weaker K_m ; 2.3-fold slower k_{cat} ; 1.1-fold slower gliding V_{obs}	Woehlke et al. 1997
		E311A	ESETK	α 6	3.1-fold tighter K_m ; 2.9-fold slower k_{cat} ; 3.2-fold slower gliding V_{obs}	Woehlke et al. 1997
<i>DmKHC</i>	W340F	EQWKK		Neck	Similar k_{cat} ; 4-fold tighter $K_{0.5,MT}$; Cys-light K413-C333 construct	Rosenfeld et al. 2001
	T94S	GQTSS				Higuchi et al. 2004

Table 5 (continued)

Family	Name	Substitution	WT Sequence	Structure	Effects	Reference
		E164A	VHEDK	L4/P-loop β5a/L8	3.6-fold faster basal ADP release; 1.8-fold faster MT-stimulated ADP release; 3.3-fold slower MT gliding V_{obs} ; similar stall force 4.2-fold slower k_{cat} ; 4-fold faster ATP hydrolysis; no observed MT dissociation; 2–3-fold slower ADP release	Klump et al. 2004a; Klump et al. 2004b; Klump et al. 2003
		E164K	VHEDK	β5a/L8	3-fold slower k_{cat} ; 2-fold faster ATP hydrolysis; 3–4-fold slower ADP release	Klump et al. 2004a; Klump et al. 2004b
		E164N	VHEDK	β5a/L8	2.6-fold slower k_{cat} ; 3-fold faster MT dissociation; 3-fold slower MT association; 2–3-fold slower ADP release	Klump et al. 2004a
		E164D	VHEDK	β5a/L8	1.5-fold slower k_{cat} ; 4-fold faster MT dissociation	Klump et al. 2004a
		E164Q	VHEDK	β5a/L8	1.4-fold slower k_{cat} ; 3-fold faster MT dissociation; 2-fold slower MT association; 2-fold slower ADP release from both heads	Klump et al. 2004a
		D165A	HEDKN	β5a/L8	1.7-fold slower k_{cat} ; 1.5-fold slower ADP release	Klump et al. 2004a
		R210A	SSRSH	L9/Sw1	>500-fold slower ATP hydrolysis; 350-fold slower k_{cat} ; 12–25-fold slower MT association; 5–6-fold slower ADP release from both heads	Farrell et al. 2002
		R210K	SSRSH	L9/Sw1	>800-fold slower ATP hydrolysis; 350-fold slower k_{cat} ; 1.3-fold slower MT association; processive motility by WT/R210K heterodimer	Klump et al. 2003; Thoresen and Gelles 2008
		T291M	KLTRI	α5	3.8-fold slower MT gliding V_{obs} ; 2.5-fold weaker K_{in} ; 4-fold weaker $K_{0.5,MT}$; 2.5-fold faster ATP hydrolysis; 3-fold faster MT dissociation	Brendza et al. 1999; Brendza et al. 2000
		E200A	MNEHS	L9/Sw1	1.4-fold slower k_{cat} ; 5–10-fold slower MT-stimulated ADP release	Auerbach and Johnson 2005b
<i>RnKHC</i>		E200D	MNEHS	L9/Sw1	1.7-fold slower k_{cat} ; 2-fold faster mantATP binding; 4.3-fold slower MT-stimulated mantADP release	Auerbach and Johnson 2005b
		E237A	GSEKV	L11/Sw2	>400-fold slower k_{cat} ; >500-fold faster basal ADP release; 3–4-fold slower MT-stimulated ADP release	Auerbach and Johnson 2005b
		E237D	GSEKV	L11/Sw2	9.1-fold slower k_{cat} ; 2.7-fold slower ATP binding; 4.4-fold slower MT-stimulated ADP release	Auerbach and Johnson 2005b
		N256K	NINKS	α4 ext.	133-fold slower k_{cat} ; 1.3-fold faster ATP binding; 400–500-fold slower MT-stimulated ADP release	Auerbach and Johnson 2005b
		V240Q/S241K	EKVSKT	L11	Double substitution; similar k_{cat} ; ~2-fold weaker $K_{0.5,MT}$	Chang et al. 2013
		S258G	NKSLS	α4	2-fold faster k_{cat} ; 10-fold weaker $K_{0.5,MT}$	Chang et al. 2013
		GDDKKG	AEGTK	L12	Chimeric construct; 2-fold faster k_{cat} ; 1.6-fold tighter $K_{0.5,MT}$; 3.4-fold increase in $k_{cat}/K_{0.5,MT}$	Chang et al. 2013
		E148A	YMEIY	β4	37-fold slower k_{cat} ; 2.9-fold slower basal ATPase	Nitta et al. 2008
		Y150F	EIYCE	β4/L7	5.3-fold slower k_{cat} ; 2.1-fold slower basal ATPase	Nitta et al. 2008
		E152A	YCERV	L7	11.5-fold slower k_{cat} ; 8.1-fold tighter $K_{0.5,MT}$; >7-fold faster MT-stimulated ADP release; 1.5-fold slower basal ATPase	Nitta et al. 2008
Kinesin-3	<i>MmKIF1A</i>	R153A	CERV/R	L7/β5	2.4-fold slower k_{cat} ; similar basal ATPase	Nitta et al. 2008
		K161A/R167A		L8	Quadruple substitution; 6-fold weaker $K_{d,MT}$ with AMPPNP; 3.9-fold weaker $K_{d,MT}$ with ADP	Nitta et al. 2004
		R169A/K183A				

Table 5 (continued)

Family	Name	Substitution	WT Sequence	Structure	Effects	Reference
Kinesin-4		R203A	KARTV	$\alpha 3$	15-fold slower k_{cat} ; 3.3-fold slower basal ATPase	Nitta et al. 2008
		R216A	SSRSH	L9/Sw1	240-fold slower k_{cat} ; 8.3-fold slower basal ATPase	Nitta et al. 2008
		D248A	LVDLA	$\beta 7$ /Sw2	1670-fold slower k_{cat} ; 3.1-fold slower basal ATPase	Nitta et al. 2008
		K261A/R264A/ K266A		L11	Triple substitution; 4.8-fold weaker $K_{d,MT}$ with AMPPNP; 1.8-fold weaker $K_{d,MT}$ with ADP	Nitta et al. 2004
		E267A	LKEGA	$\alpha 4$ ext.	22-fold slower k_{cat} ; 5.6-fold slower basal ATPase	Nitta et al. 2008
		GTKT	MDSGPNKN(K) ₆ TD	L12	Chimeric construct; 3.5-fold weaker $K_{d,MT}$ with ADP; 3.8-fold weaker $K_{d,MT}$ with ATP; 7.8-fold weaker $K_{d,MT}$ with ADP+VO ₄	Nitta et al. 2004
		Q248V/K249S	ERQKKT	L11	Double substitution; Slightly increased k_{cat} and $K_{0.5,MT}$; similar $k_{cat}/K_{0.5,MT}$	Chang et al. 2013
	<i>Mm</i> KIF4A	G267S	NRGLL	$\alpha 4$	Slightly decreased k_{cat} and $K_{0.5,MT}$; similar $k_{cat}/K_{0.5,MT}$	Chang et al. 2013
		AEGTK	GDDKKG	L12	Chimeric construct; 2-fold decreased k_{cat} ; similar $K_{0.5,MT}$; 2.3-fold decrease in $k_{cat}/K_{0.5,MT}$	Chang et al. 2013
		P121A	RSPNE	L5	2-fold slower neck-linker isomerization; 4.3-fold slower ADP binding; 5.1-fold slower ADP release	Behnke-Parks et al. 2011
Kinesin-5		P131A	EDPLA	L5	50-fold slower ATP binding; 5-fold slower neck-linker isomerization; 7.7-fold slower ADP release; 3.5-fold slower MT dissociation	Behnke-Parks et al. 2011
		S233C	YSSRS	L9/Sw1	5–6-fold slower MgATPase; similar MnATPase; ATP hydrolysis slowed for MgATPase; similar ATP binding and ADP release kinetics	Cochran et al. 2011
		R234K	SSRSH	L9/Sw1	125-fold slower ATP hydrolysis; 10-fold faster MT association; similar AMPPNP binding affinity	Zhao et al. 2010
		SC/RK	YSSRSH	L9/Sw1	Double substitution; similar ATP binding, complete loss of ATP hydrolysis	Krzyśiak et al. 2008; Zhao et al. 2010
	<i>Dm</i> NOD	S203C	NSSRS	L9/Sw1	~20-fold slower basal MgATPase; similar basal MnATPase	Cochran et al. 2011
	<i>Mm</i> KIF2C	K293A	KLKVD	L2	~50 % MT depolymerization activity	Ogawa et al. 2004
		V294A	LKYDL	L2	~55 % MT depolymerization activity	Ogawa et al. 2004
		D295A	KVDLT	L2	~65 % MT depolymerization activity	Ogawa et al. 2004
		K293A/V294A/ D295A		L2	Triple substitution; ~35 % MT depolymerization activity	Ogawa et al. 2004
		G489A	LAGNE	L11/Sw2	Defective in tubulin detachment; very slow ATP hydrolysis	Wagenbach et al. 2008
<i>Pf</i> KINI		E491A	GNERR	L11/Sw2	Defective in tubulin detachment; 20-fold tighter $K_{d,tubulin}$; catalytically depolymerizes MTs in ADP	Wagenbach et al. 2008
		K40A/V41A/ D42A		L2	Triple substitution; 10-fold reduction in MT depolymerization; ~10-fold slower	Shipley et al. 2004
		R242A	SERGA	L11	MT-stimulated k_{cat} ; similar tubulin-stimulated k_{cat}	Shipley et al. 2004
					~2-fold reduction in MT depolymerization; ~2-fold slower MT-stimulated	
		D245A	GADTV	L11	k_{cat} ; ~3-fold slower tubulin-stimulated k_{cat}	Shipley et al. 2004
		K268A/E269A/ C270A		$\alpha 4$	Similar MT depolymerization; ~1.5-fold faster MT-stimulated k_{cat} ; ~1.2-fold faster tubulin-stimulated k_{cat}	Shipley et al. 2004

Table 5 (continued)

Family	Name	Substitution	WT Sequence	Structure	Effects	Reference
Kinesin-14	<i>Dm</i> NCD	R272A	CIRAM	$\alpha 4$	Triple substitution; no MT depolymerization; ~20-fold slower MT-stimulated k_{cat}	Shipley et al. 2004
		R335A/K336A	MERKEL	Neck	~4-fold slower tubulin-stimulated k_{cat}	Sablin et al. 1998
		H339A/N340A	ELHNTV	Neck	~2-fold reduction in MT depolymerization; ~3-fold slower MT-stimulated k_{cat} ; ~3-fold slower tubulin-stimulated k_{cat}	Sablin et al. 1998
		N340K	LHNTV	Neck	Double substitution; 2.0-fold slower MT gliding V_{obs}	Endow and Higuchi 2000; Endres et al. 2006
					Double substitution; 1.3-fold slower MT gliding V_{obs}	
					Bidirectional MT gliding; similar MT gliding velocities in either direction;	
					unstable neck position with AMPPNP	
					Double substitution; bidirectional MT gliding	Endow and Higuchi 2000
		N340K/K640N	RKELHN	Neck	WT (aa 335-346); 50-fold slower MT gliding V_{obs}	Sablin et al. 1998
		ESGAKQ	TVMDLR	Neck	Dimer X-ray structure (PDB ID: 3u06); 1.5-fold slower basal ADP release and ATPase; no MT gliding observed;	Liu et al. 2012b
		GEKGES	LRGNI		6-fold weaker $K_{0.5MT}$	
		G347D			Double substitution; 2.3-fold slower MT gliding V_{obs}	Sablin et al. 1998
		Q420A/S421A	LIQSAL	$\alpha 1$	1.8-fold slower MT gliding V_{obs}	Sablin et al. 1998
		Y426A	DGYNI	L3	Triple substitution; 10-fold slower MT gliding V_{obs}	Heuston et al. 2010
		Q420A/S421A/Y426A	GQTGS	$\alpha 1/L3$	Dimer X-ray structure (PDB ID: 3l1c); 10-fold faster basal ADP release; similar MT-stimulated ADP release; 2-fold faster k_{cat} ; 1.5-fold slower	
		T436S		L4/P-loop	MT gliding V_{obs}	
		Y485E	EIYNE	$\beta 4/L7$	3.8-fold faster basal ADP release; 1.5-fold weaker $K_{d,MT}$; similar MT-stimulated ATPase; similar MT gliding V_{obs}	Liu et al. 2012a
		Y485N	EIYNE	$\beta 4/L7$	10-fold faster basal ADP release and ATPase; 1.9-fold weaker $K_{d,MT}$	Liu et al. 2012a
		Y485K	EIYNE	$\beta 4/L7$	2-fold faster MT-stimulated ATPase; 1.3-fold faster MT gliding V_{obs}	Liu et al. 2012a
					26-fold faster basal ADP release; 3-fold weaker $K_{d,MT}$; 2-fold faster k_{cat}	
		S551C	RSSRS	L9/Sw1	10-fold tighter $K_{0.5MT}$; 2-fold faster MT gliding V_{obs}	Cochran et al. 2011
		R552A	SSRSH	L9/Sw1	~6-fold slower basal MgATPase; similar basal MnATPase	Kocik et al. 2009
					4-fold weaker $K_{d,MT}$ (no nucleotide); 70-fold weaker $K_{d,MT}$ (AMPPNP); >9-fold weaker $K_{d,MT}$ (ADP); 2-fold slower k_{cat} ; 3-fold weaker $K_{0.5MT}$	
		V556F	HAVTK	$\beta 6$	5-fold slower MT gliding V_{obs}	Moore et al. 1996
					3-fold slower k_{cat} and weaker $K_{0.5MT}$; 2-fold weaker $K_{d,MT}$; 3-fold weaker $K_{d,MT}$ in pyrase; 4-fold tighter $K_{d,MT}$ in AMPPNP	
		E567A/K568A	HA EKQE	L10	Double substitution; similar MT gliding V_{obs}	Sablin et al. 1998
		E585A	GSESK	L11/Sw2	3-fold weaker $K_{d,MT}$ (no nucleotide); 5–7-fold weaker $K_{d,MT}$; 7-fold slower k_{cat} ; 28-fold slower MT gliding V_{obs}	Kocik et al. 2009
		E585D	GSESK	L11/Sw2		Kocik et al. 2009

Table 5 (continued)

Family	Name	Substitution	WT Sequence	Structure	Effects	Reference
	N600K		NINRS	$\alpha 4$ ext.	3-fold weaker $K_{d,MT}$ (no nucleotide); 5–7-fold weaker $K_{d,MT}$; 7-fold slower k_{cat} ; 28-fold slower MT gliding V_{obs}	Kocik et al. 2009; Song and Endow 1998
					8-fold weaker $K_{d,MT}$ (no nucleotide); 17-fold weaker $K_{d,MT}$ (AMPPNP); 2-fold slower k_{cat} and weaker $K_{0.5,MT}$; 1.4-fold slower MT gliding V_{obs}	
					Double substitution; 1.3-fold slower MT gliding V_{obs}	
	G636A/G637A	K640A	SLGGNS	L13	3-fold slower MT gliding V_{obs}	Sablin et al. 1998
					Bidirectional MT gliding	Sablin et al. 1998
					X-ray structure (PDB ID: 1f9v); similar basal ATPase; complete loss of MT-stimulated ATPase	Endow and Higuchi 2000
					X-ray structure (PDB ID: 1f9w); similar basal ATPase; complete loss of MT-stimulated ATPase	Yun et al. 2001
	R598A	E631A	GSERR	L11/Sw2	X-ray structure (PDB ID: 1f9u); similar basal ATPase; complete loss of MT-stimulated ATPase; complete loss of MT gliding; mant-ADP release not stimulated by MT binding	Song and Endow 1998; Yun et al. 2001
	S6KAR3	N650K	NINKS	$\alpha 4$ ext.		

The bold font indicates the amino acid position that was substituted

regulate directional movement of all members of the kinesin superfamily.

Kinesin motors are allosteric enzymes by nature. Therefore, amino acid residue substitutions in the motor domain can yield expected results, yet often produce unexpected or unpredicted pleiotropic effects (Table 5). Many different substitutions have been discovered in kinesin motors that have been implicated in diseases such as cancer (Rath and Kozielski 2012) or motor neuron degeneration [e.g., hereditary spastic paraplegia; (Ebbing et al. 2008; Fuger et al. 2012)]. As a general trend, but one not yet considered to be universal across all kinesin members, most tested substitutions lead to a reduced turnover number (k_{cat}), altered binding affinity for microtubules, and slower *in vitro* microtubule gliding velocity (Table 5). Substitutions in the conserved active site motifs lead to drastically reduced (if not completely abolished) ATPase activity, whereas substitutions in adjacent loops involved in the structural communication pathways between the active site and the microtubule binding region lead to disruption of allosteric regulation of the motor. Recently, the conserved switch-1 serine residue that physically interacts with the divalent metal ion in the active site was substituted with a cysteine; this rendered several kinesin family members inactive with Mg^{2+} but swapping metals with Mn^{2+} led to the restoration of activity (Cochran et al. 2011). Interestingly, single substitutions in the neck of kinesin-14 leads to bidirectional motility (Endow and Higuchi 2000), which should shed light on how the yeast kinesin-5 motors (Cin8p, Kip1p, and Cut7p) show directionality switching behavior (Fridman et al. 2013; Gerson-Gurwitz et al. 2011; Roostalu et al. 2011; Thiede et al. 2012). Despite the numerous residue substitutions that have been characterized, researchers still have only scratched the surface for using site-specific mutagenesis techniques to probe kinesin structure and function.

Physics

Kinesin-1 is a processive translocase that can take hundreds of steps along the microtubule while consuming 1 ATP molecule per 8-nm (approx.) step (Carter and Cross 2005; Coy et al. 1999; Hua et al. 1997; Iwatani et al. 1999; Ray et al. 1993; Schnitzer and Block 1997; Schnitzer et al. 2000; Svoboda et al. 1993; Vale et al. 1996). Using the law of mass action and standard thermodynamic equations, the average available free energy of ATP hydrolysis under typical *in vitro* steady-state ATP turnover conditions equals $-74.9 \text{ kJ mol}^{-1}$ or -124 pN nm (see Appendix A for details). The stall force for kinesin-1 has been measured at 5–7 pN using optical trapping methodologies (Schnitzer and Block 1997; Svoboda and Block 1994; Visscher et al. 1999); therefore, with an average step size at 8.3 nm (Yildiz et al. 2004), kinesin-1 is 33–47 % efficient (e.g. $5 \text{ pN} \times 8.3 \text{ nm} = 42 \text{ pN nm} = 33 \text{ \% efficiency}$) in

its use of ATP hydrolysis-free energy for moving along the microtubule.

The stall force in other dimeric kinesin motors has been determined: kinesin-2 (KLP11/KLP20 heterodimer) at ~4 pN (Brunnbauer et al. 2010), kinesin-3 (Unc104/KIF1A) at ~6 pN (Tomishige et al. 2002), kinesin-5 (Eg5/KSP) at ~5 pN (Valentine et al. 2006), kinesin-7 (CENP-E) at ~6 pN (Yardimci et al. 2008), kinesin-8 (KIF18A/Kip3) at ~1 pN (Jannasch et al. 2013), and kinesin-14 (Ncd) at ~1 pN for two to four motors linked via a rigid DNA scaffold with ~6- or ~22-nm spacing (Furuta et al. 2013). The motility velocities for these different kinesin family members were typically much slower than that for kinesin-1, and their run lengths were much shorter. Therefore, kinesin motors appear to have a wide range of efficiencies (namely, 9–64 %) for converting the energy of ATP hydrolysis to mechanical motion, as well as for using this energy to carry out different functions in cellular processes.

Processivity is a characteristic of most motor proteins that is defined by the distance traveled along its track before it detaches and diffuses away. Several classes of kinesin motors are quite processive, traveling from several microns through hundreds of ATP hydrolysis cycles before dissociating from their microtubule track. These families include kinesin-1 (Howard et al. 1989), kinesin-2 (Muthukrishnan et al. 2009), kinesin-3 (Soppina et al. 2014), kinesin-5 (Valentine et al. 2006), kinesin-7 (Yardimci et al. 2008), and kinesin-8 (Varga et al. 2006). Early experiments designed to measure the movement of single dimeric kinesin motors tethered to a bead provided the first direct evidence of kinesin processivity (Svoboda et al. 1993). Presteady-state kinetic analyses of the dimeric kinesin-1 ATP hydrolysis cycle subsequently provided additional evidence for chemical processivity (Gilbert et al. 1995; Hackney 1995a). Single fluorescently labeled kinesin-1 motors were shown to demonstrate processive motility in solution (Vale et al. 1996), again supporting this important biophysical characteristic for transport kinesins. The molecular basis for kinesin processivity is chiefly thought to stem from an asymmetric hand-over-hand mechanism that keeps the catalytic cycles in each head “out-of-phase” with its partner, thus allowing one head to remain tightly bound to the microtubule at any given point along a processive run (Toprak et al. 2009). It still remains a mystery why certain kinesin members take a few steps during a processive run (5–10 steps for kinesin-5; Valentine et al. 2006), while other kinesin members can take thousands of steps during a processive run (e.g., kinesin-3; Soppina et al. 2014; Soppina and Verhey 2014). It has been postulated that increased positive charge in the neck region and/or the C-terminal region functions to enhance kinesin processivity (Grissom et al. 2009; Kim et al. 2008; Mayr et al. 2011; Rosenfeld et al. 2009; Stumpff et al. 2011; Su et al. 2011; Thorn et al. 2000; Varga et al. 2009; Weaver et al. 2011). Another prevailing mechanism for modulating

kinesin processivity centers on the length of the neck linker, which is thought to affect mechanochemical gating between partner heads during a processive run (Clancy et al. 2011; Duselder et al. 2012; Miyazono et al. 2010; Shastry and Hancock 2010; Shastry and Hancock 2011; Yildiz et al. 2008). However, recent work from the Verhey laboratory on kinesin-3 motors clearly shows that these so-called “superprocessive” motors utilize an unknown molecular mechanism to enable this level of processivity (Soppina et al. 2014; Soppina and Verhey 2014). It is also important to note that not all kinesins are processive (e.g., kinesin-10/Kid and kinesin-14/Ncd are non-processive mitotic motors), and that in fact some kinesins are not transport motors at all (e.g., kinesin-10/NOD is a non-motile microtubule plus-end tracker and kinesin-13/MCAK is a potent microtubule depolymerase). This variation highlights the diversity of ways that the energy of ATP hydrolysis can be converted into mechanical motion for a variety of biophysical tasks.

Cytoskeletal molecular motors such as kinesin, myosin, and dynein have demonstrated the ability to generate force tangential to the direction of movement along their tracks, thus producing “torque” in the motor–filament interaction (Nishizaka et al. 1993; Vale and Toyoshima 1988; Walker et al. 1990). Kinesin-1 has been shown to processively step along a single protofilament of a microtubule consisting of 13–16 protofilaments (Mori et al. 2007; Ray et al. 1993; Ray et al. 1995; Schaap et al. 2011), whereas kinesin-2 and kinesin-5 have demonstrated an ability to step along adjacent protofilaments of the microtubule, thus causing the motors to spiral around the microtubule (Brunnbauer et al. 2012; Pan et al. 2010; Yajima et al. 2008). It was initially thought that the degree of processivity was inversely proportional to torque generation (i.e., that increasing torque generation decreases processivity; Yajima et al. 2008); however, the combination of results from several studies strongly disfavor this hypothesis (Brunnbauer et al. 2012; Brunnbauer et al. 2010; Muthukrishnan et al. 2009; Yildiz et al. 2008). Indeed, recent results suggest that the stability of the neck (not that of the neck linker with the motor core) is responsible for conferring the side-stepping behavior (Brunnbauer et al. 2012). Understanding how conformational changes in the kinesin dimer’s motor domains and the directionally biased selection of microtubule binding sites are biophysically linked during asymmetric hand-over-hand stepping remains a subject for future investigation.

Analysis of the energy landscape of kinesin-5 using isothermal titration calorimetry (ITC) (Fig. 5) revealed an the observed free energy of ATP binding and hydrolysis ($\Delta G_1 + \Delta G_2$) of -31 kJ mol^{-1} (-51 pN nm), which represents an energy efficiency of 43 % based on the conditions used in the ITC experiment ($\Delta G_{\text{rxn}} = -72.4 \text{ kJ mol}^{-1}$ or -120 pN nm ; Zhao et al. 2010). This observed free energy is comparable to the range of work performed by dimeric kinesin-5 (-42 to 49

Fig. 5 Kinesin energy landscape in the absence and presence of microtubules. The energy landscapes for human monomeric kinesin-5 (Eg5) in the absence (black) and presence (red) of microtubules are shown. The thermodynamically and kinetically defined intermediate states are defined in the table. The magnitude of energy for each transition state was calculated from the Arrhenius/Eyring/Kramer equation [$k = \frac{k_B T}{h} e^{-\frac{\Delta G^\ddagger}{RT}}$; k_B = Boltzmann constant, T = temperature, h = Planck's constant, R = gas constant, $\Delta G^\ddagger$ = Gibbs transition state free energy] using previously determined kinetic rate constants for the monomeric kinesin-5 ATPase mechanism (Table 1). Adapted and updated from Zhao et al. (2010)

pN nm; Valentine et al. 2006), suggesting that the observed thermodynamics of a single kinesin head provides valuable insight into dissecting the structure and function of the motor. One outstanding question: where is the remainder of free energy made available from the hydrolysis of ATP (i.e., approximately $-41.4 \text{ kJ mol}^{-1}$ missing free energy)? This remaining chemical free energy that is not converted to mechanical work could get rapidly exchanged (within picoseconds) as entropy production (i.e., dissipated “heat”) in the surrounding physiological milieu (Ge and Qian 2013; Henry et al. 1986). If this hypothesis were true, the excess heat should be “observed” in the ITC experiment since the instrument physically measures the heat associated with a biochemical reaction. Yet this heat was not observed (Zhao et al. 2010), therefore refuting the “dissipated heat” hypothesis. Another hypothesis centers on the “deposition” or “storage” of the energy into the motor as conformational potential energy, which is used by the motor to promote ADP product release from the active site at a much faster rate than theoretically possible (see below). To put the quantity of missing energy into perspective, this represents a net gain of two to six hydrogen bonds (or some other combination of non-covalent interactions within the motor). It is worth speculating that the source of this conformational potential energy resides within the “under-twisted” central β -sheet seen in nucleotide-bound kinesin structures (Fig. 4).

Nevertheless, the accounting of the free energy from the reaction remains a mystery.

Kinesin motors typically bind P_i very weakly and ADP very tightly (Hackney 1988). Consequently, the release of these products is thermodynamically unfavorable—but necessary—to get the enzyme to the beginning of the next cycle. The calculated free energy of P_i binding to kinesin-5 ($-\Delta G_3$) was -3.3 kJ mol^{-1} , which equates to a binding affinity (K_{d,P_i}) of 260 mM. The observed ADP binding free energy ($-\Delta G_4 + -\Delta G_5$) was $-27.6 \text{ kJ mol}^{-1}$, which equates to a binding affinity ($K_{d,ADP}$) of 7–8 μM . As the rates of ATP and ADP binding to kinesin-5 have been determined in the absence of microtubules (k_{+2} and $k_{-5} \approx 1 \text{ s}^{-1}$, respectively; Cochran and Gilbert 2005), the Arrhenius/Eyring/Kramer equation can be used to calculate the free energy of these transition states ($\Delta G^\ddagger_1$ for ATP binding and $\Delta G^\ddagger_5$ for ADP binding $= +73 \text{ kJ mol}^{-1}$). The transition state free energy for ATP hydrolysis ($\Delta G^\ddagger_2$) and P_i release ($\Delta G^\ddagger_3$) are modeled using the minimum intrinsic rate constants for both steps at 10 s^{-1} [see Cochran and Gilbert (2005) for details on how this minimum rate was determined].

Once kinesin-5 releases the bound P_i product and reaches the ADP-bound state (state “d” in Fig. 5), a very large thermodynamically unfavorable transition must occur for ADP to be released from the active site (i.e., to reach state “a” in Fig. 5). The height of this energy barrier is approximately $+101 \text{ kJ mol}^{-1}$, which means that if the mechanism of ADP release happened in a single step (where the motor utilizes thermal energy from the surrounding environment), then the rate of the reaction (determined using Arrhenius/Eyring/Kramers rate theory) should be $7.2 \times 10^{-6} \text{ s}^{-1}$, which is approximately 2,900-fold slower than the observed rate of ADP release from kinesin-5 ($k_{+5} = 0.02 \text{ s}^{-1}$; Cochran and Gilbert 2005; Cochran et al. 2004; Zhao et al. 2010). Therefore, another intermediate state along the pathway must exist (state “e” in Fig. 5), and this state must somehow avoid microscopic reversibility (i.e., when the enzyme is in state “e”, the probability of returning to state “d” rather than proceeding to state “a” is 671,000:1 under the fundamental principles of microscopic reversibility) and thus act as a molecular thermal ratchet. It has been postulated that the release of Mg^{2+} and ADP from the kinesin-MgADP intermediate (state “d”) occurs sequentially along the ATPase pathway. For kinesin-1, this takes the form of slow, rate-limiting Mg^{2+} release followed by rapid ADP release (Hackney and Stock 2008). For kinesin-5, it appears that a similar mechanism of MgADP release is utilized, but the intrinsic rate constants which govern these reactions lead to relatively rapid Mg^{2+} release followed by slow, rate-limiting ADP release (unpublished data). The existence of isomerization state “e” on the reaction path, as presented in Fig. 5, is supported since the concentration of Mg^{2+} modulates the observed rate of ADP product release in kinesin-5 (unpublished data), as well as in other kinesins (Hackney and Stock 2008; Nitta et al. 2008). Also, kinesin-5 shows very weak

ADP binding in the absence of Mg^{2+} (unpublished data), which is consistent with the notion that the free energy of state “e” is linked to the occupancy of the divalent ion in the active site. Future biophysical investigations surrounding these reactions in the kinesin ATPase cycle will be central to gaining an understanding of force generation and mechanochemical gating in kinesin motors.

Where is the mechanical motion produced in the kinesin ATPase cycle? Even for kinesin-1, this question has been answered differently and the issue remains controversial (Block 2007). Nevertheless, this is a very important and outstanding question for other kinesin families, particularly for the mitotic kinesins, since inhibition of force production would provide a plausible therapeutic strategy. Mechanical output requires communication between the two motor domains in a dimeric kinesin-1 molecule; yet just how this communication occurs is still unclear. Most theories suggest that communication occurs through a “gating” mechanism where a mechanistic step in one motor domain is blocked until a certain step is taken in the other motor domain (Block 2007). This gating mechanism could be chemical in nature, such that ATP binding to the one head is prohibited until the other head dissociates from, or attaches to, the microtubule (Block et al. 2003; Guydosh and Block 2006; Klumpp et al. 2004b; Rosenfeld et al. 2003), or mechanical in nature, such that a conformational change in one head pulls the other head off the microtubule (Hancock and Howard 1999; Spudich 2006; Yildiz et al. 2008). Of course, it is likely that the actual mechanism of communication utilizes both types of gating, as they are not mutually exclusive.

Conclusions

As we near the 30th anniversary of the discovery of kinesin motors, we have uncovered many interesting chemical, structural, and physical insights into several members of the superfamily. Yet a series of important outstanding questions remain unanswered:

- *What are the biophysical characteristics of “unconventional” kinesins?* When you compare the number of kinesin motors that have been studied to date and the number of kinesins that exist in nature (1,624 putative members; Wickstead et al. 2010), it is clear that much work is ahead of us. Several studies of various “conventional” kinesins (not to be confused with conventional kinesin, kinesin-1) have provided a biochemical/structural/mechanical model with variations between the different families studied (e.g., similarities and differences between kinesin-1, kinesin-5, kinesin-13, and kinesin-14 families). However, it remains to be seen how long this model will persist. Indeed, with the characterization of

“unconventional” kinesins, researchers are led back to their favorite kinesin and design a series of experiments to test various aspects of the already well-characterized family members. As just one example, when we solved the crystal structure of one of the most distant “unconventional” kinesins in the superfamily (*Drosophila melanogaster* NOD), we identified two proline residues in L5 that physically interacted with helix $\alpha 3$ (Cochran et al. 2009). Another research group proceeded to mutate one of these conserved L5 proline residues in human kinesin-5 to demonstrate that L5 acts a conformational latch in kinesins (Behnke-Parks et al. 2011).

- *How do different kinesins utilize distinct nucleoside triphosphorylase mechanisms to either promote MT motility or to regulate MT polymerization dynamics for force generation?* Differences in the kinesin superfamily show that some kinesins walk along microtubules (e.g., kinesin-1, kinesin-5, and kinesin-7), some take a few steps along microtubules in order to establish tension in the mitotic spindle (e.g., kinesin-14), while some kinesins are not motile, but instead use this energy to destabilize microtubule polymers (e.g., kinesin-13) or promote microtubule polymerization (e.g., kinesin-10). How are these vastly different outputs generated from a common motor core? Structures of these motor cores are known, but it is still unclear how subtle differences give rise to these different functions.
- *How can the same kinesin walk in different directions on the microtubule?* At the turn of the century, the kinesin community would have overwhelmingly agreed that regions outside of the kinesin motor core determine the direction of movement (for review, see Wade and Kozielski 2000). The experiments that generated chimeric versions of kinesin-1 (plus-end directed) and kinesin-14 (minus-end directed) demonstrated that the linker sequences immediately adjacent (~2–3 residues) to the motor core ($\beta 1$ – $\alpha 6$) dictate directionality along the microtubule. However, recent studies with native yeast kinesin-5 motors (i.e., not chimeric constructs) show directional switching that is dependent on motor–microtubule density, ionic strength, and/or parallel *versus* antiparallel microtubule orientation (Fridman et al. 2013; Gerson-Gurwitz et al. 2011; Roostalu et al. 2011; Thiede et al. 2012). It will be interesting to develop a better understanding of the molecular mechanism that dictates this biophysical conundrum.
- *Why does the β -sheet “twist” during the ADP–ATP exchange process?* Study of the twisting of the central β -sheet during the ADP–ATP exchange steps of the cycle is an emerging trend in kinesin biophysics. How does “twisting” of the β -sheet in molecular motors facilitate mechanochemistry and force production? Does the twisting provide a mechanism for storing conformational potential

energy in order for kinesins to rapidly release ADP product and start a new cycle with ATP binding? Does the “over-twisting” and “under-twisting” of the β -sheet contribute to the thermodynamics and mechanics of force generation in kinesins? These questions will be important to answer in the near future, yet the appropriate experimental design will be essential to narrow the interpretation of data in supporting or refuting these hypotheses.

- *How do specific macromolecular interactions between kinesin and microtubules (or soluble tubulin for some kinesins) stimulate enzymatic activity?* Although we have very solid experimental support for microtubule stimulation of the kinesin ATPase cycle, we still lack a detailed structural model that answers this question. Cryo-EM structures and X-ray crystal structures of kinesin–tubulin complexes have advanced our understanding of the molecular interface, although much work remains to complete our understanding of the molecular dynamics of this interaction. Given the large number of atoms that comprise the kinesin–tubulin complex, technological advances in computational power will be necessary to simulate this interaction in order to generate detailed structural hypotheses that can be tested in the laboratory.
- *How are kinesin, myosins, and G proteins related?* Since the structures of myosin (Rayment et al. 1993) and kinesin (Kull et al. 1996) were initially determined, many structural and functional similarities have been observed to be shared between the motor proteins and small G proteins. These similarities include common nucleotide binding motifs (mentioned earlier), common active site chemistry, and common secondary structure topology in the catalytic domain (Kull et al. 1998). G proteins show exclusive preference for using GTP hydrolysis for its cellular function (GTP binds $\sim 10^7$ -fold tighter than ATP; Goody et al. 1992), while kinesins have historically been thought to use ATP hydrolysis (ATP binds $\sim 10^2$ -fold tighter than GTP; Gilbert and Johnson 1993). Nevertheless, given the vast wealth of independent studies on molecular motors and small G proteins, there remain many unanswered questions on the conservation of base specificity and nucleotide hydrolysis mechanisms for these hydrolases. Given the allosteric nature of kinesin enzymes and the relatively “loose” pocket for the nucleotide base, can certain kinesin family members use either GTP or ATP for energy transduction? If so, what are the unique features in the catalytic domain that provide a structural basis to explain these differences in the catalytic mechanism? Future research will provide insight into the conserved and non-conserved mechanisms between molecular motors and molecular switch proteins.

Acknowledgments I would like to thank Joseph Eskew for his help with Appendix A, and Kayla Bell, Benjamin Walker, and Jeff Ewer for

helpful discussions. I am grateful for comments on the manuscript provided by Claire Walczak and F. Jon Kull.

Compliance with Ethical Standards

Conflict of interest Jarod C Cochran declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

References

- Admiraal SJ, Herschlag D (1995) Mapping the transition state for ATP hydrolysis: implications for enzymatic catalysis. *Chem Biol* 2:729–739
- Allingham JS, Sproul LR, Rayment I, Gilbert SP (2007) *Vik1* modulates microtubule-Kar3 interactions through a motor domain that lacks an active site. *Cell* 128:1161–1172. doi:10.1016/j.cell.2006.12.046
- Alonso MC, Drummond DR, Kain S, Hoeng J, Amos L, Cross RA (2007) An ATP gate controls tubulin binding by the tethered head of kinesin-1. *Science* 316:120–123. doi:10.1126/science.1136985
- Arnal I, Metoz F, DeBonis S, Wade RH (1996) Three-dimensional structure of functional motor proteins on microtubules. *Curr Biol* 6:1265–1270
- Asenjo AB, Chatterjee C, Tan D, et al (2013) Structural model for tubulin recognition and deformation by kinesin-13 microtubule depolymerases. *Cell Rep* 3:759–768. doi:10.1016/j.celrep.2013.01.030
- Arora K, Talje L, Asenjo AB, et al (2014) KIF14 binds tightly to microtubules and adopts a rigor-like conformation. *J Mol Biol*. doi:10.1016/j.jmb.2014.05.030
- Asenjo AB, Weinberg Y, Sosa H (2006) Nucleotide binding and hydrolysis induces a disorder-order transition in the kinesin neck-linker region. *Nat Struct Mol Biol* 13:648–654. doi:10.1038/nsmb1109
- Auerbach SD, Johnson KA (2005a) Alternating site ATPase pathway of rat conventional kinesin. *J Biol Chem* 280:37048–37060. doi:10.1074/jbc.M502984200
- Auerbach SD, Johnson KA (2005b) Kinetic effects of kinesin switch I and switch II mutations. *J Biol Chem* 280:37061–37068. doi:10.1074/jbc.M502985200
- Barshop BA, Wrenn RF, Frieden C (1983) Analysis of numerical methods for computer simulation of kinetic processes: development of KINSIM—a flexible, portable system. *Anal Biochem* 130:134–145
- Behnke-Parks WM, Vendome J, Honig B, Maliga Z, Moores C, Rosenfeld SS (2011) Loop L5 acts as a conformational latch in the mitotic kinesin Eg5. *J Biol Chem* 286:5242–5253. doi:10.1074/jbc.M110.192930
- Bernstein DA, Zittel MC, Keck JL (2003) High-resolution structure of the *E. coli* RecQ helicase catalytic core. *EMBO J* 22:4910–4921. doi:10.1093/emboj/cdg500
- Block SM (2007) Kinesin motor mechanics: binding, stepping, tracking, gating, and limping. *Biophys J* 92:2986–2995. doi:10.1529/biophysj.106.100677
- Block SM, Asbury CL, Shaevitz JW, Lang MJ (2003) Probing the kinesin reaction cycle with a 2D optical force clamp. *Proc Natl Acad Sci USA* 100:2351–2356. doi:10.1073/pnas.0436709100
- Bodey AJ, Kikkawa M, Moores CA (2009) 9-Angstrom structure of a microtubule-bound mitotic motor. *J Mol Biol* 388:218–224. doi:10.1016/j.jmb.2009.03.008

- Bos JL, Rehmann H, Wittinghofer A (2007) GEFs and GAPs: critical elements in the control of small G proteins. *Cell* 129:865–877. doi:10.1016/j.cell.2007.05.018
- Brady ST (1985) A novel brain ATPase with properties expected for the fast axonal transport motor. *Nature* 317:73–75
- Brendza KM, Rose DJ, Gilbert SP, Saxton WM (1999) Lethal kinesin mutations reveal amino acids important for ATPase activation and structural coupling. *J Biol Chem* 274:31506–31514
- Brendza KM, Sontag CA, Saxton WM, Gilbert SP (2000) A kinesin mutation that uncouples motor domains and desensitizes the gamma-phosphate sensor. *J Biol Chem* 275:22187–22195. doi:10.1074/jbc.M001124200
- Brier S, Lemaire D, Debonis S, Forest E, Kozielski F (2004) Identification of the protein binding region of S-trityl-L-cysteine, a new potent inhibitor of the mitotic kinesin Eg5. *Biochemistry* 43:13072–13082. doi:10.1021/bi049264e
- Brune M, Hunter JL, Corrie JE, Webb MR (1994) Direct, real-time measurement of rapid inorganic phosphate release using a novel fluorescent probe and its application to actomyosin subfragment 1 ATPase. *Biochemistry* 33:8262–8271
- Brunnbauer M, Dombi R, Ho TH, Schliwa M, Rief M, Oken Z (2012) Torque generation of kinesin motors is governed by the stability of the neck domain. *Mol Cell* 46:147–158. doi:10.1016/j.molcel.2012.04.005
- Brunnbauer M, Mueller-Planitz F, Kosem S, et al (2010) Regulation of a heterodimeric kinesin-2 through an unprocessive motor domain that is turned processive by its partner. *Proc Natl Acad Sci USA* 107:10460–10465. doi:10.1073/pnas.1005177107
- Bunton CA, Chaimovi H (1965) Acid-catalyzed hydrolysis of pyrophosphoric acid. *Inorgan Chem* 4:1763–1766. doi:10.1021/ic50034a020
- Carter NJ, Cross RA (2005) Mechanics of the kinesin step. *Nature* 435:308–312. doi:10.1038/nature03528
- Case RB, Pierce DW, Hom-Booher N, Hart CL, Vale RD (1997) The directional preference of kinesin motors is specified by an element outside of the motor catalytic domain. *Cell* 90:959–966
- Case RB, Rice S, Hart CL, Ly B, Vale RD (2000) Role of the kinesin neck linker and catalytic core in microtubule-based motility. *Curr Biol* 10:157–160
- Catarinella M, Gruner T, Strittmatter T, Marx A, Mayer TU (2009) BTB-1: a small molecule inhibitor of the mitotic motor protein Kif18A. *Angew Chem Int Ed Engl* 48:9072–9076. doi:10.1002/anie.200904510
- Cecchini M, Houdusse A, Karplus M (2008) Allosteric communication in myosin V: from small conformational changes to large directed movements. *PLoS Comput Biol* 4:e1000129. doi:10.1371/journal.pcbi.1000129
- Chandra R, Salmon ED, Erickson HP, Lockhart A, Endow SA (1993) Structural and functional domains of the *Drosophila* *ncd* microtubule motor protein. *J Biol Chem* 268:9005–9013
- Chang Q, Nitta R, Inoue S, Hirokawa N (2013) Structural basis for the ATP-induced isomerization of kinesin. *J Mol Biol* 425:1869–1880. doi:10.1016/j.jmb.2013.03.004
- Chen CJ, Porche K, Rayment I, Gilbert SP (2012) The ATPase pathway that drives the kinesin-14 Kar3/Vik1 powerstroke. *J Biol Chem* 287:36673–36682. doi:10.1074/jbc.M112.395590
- Chen CJ, Rayment I, Gilbert SP (2011) Kinesin Kar3/Cik1 ATPase pathway for microtubule cross-linking. *J Biol Chem* 286:29261–29272. doi:10.1074/jbc.M111.255554
- Clancy BE, Behnke-Parks WM, Andreasson JO, Rosenfeld SS, Block SM (2011) A universal pathway for kinesin stepping. *Nat Struct Mol Biol* 18:1020–1027. doi:10.1038/nsmb.2104
- Cochran JC, Gatial JE 3rd, Kapoor TM, Gilbert SP (2005) Monastrol inhibition of the mitotic kinesin Eg5. *J Biol Chem* 280:12658–12667. doi:10.1074/jbc.M413140200
- Cochran JC, Gilbert SP (2005) ATPase mechanism of Eg5 in the absence of microtubules: insight into microtubule activation and allosteric inhibition by monastrol. *Biochemistry* 44:16633–16648. doi:10.1021/bi051724w
- Cochran JC, Krzysiak TC, Gilbert SP (2006) Pathway of ATP hydrolysis by monomeric kinesin Eg5. *Biochemistry* 45:12334–12344. doi:10.1021/bi0608562
- Cochran JC, Sindelar CV, Mulko NK, Collins KA, Kong SE, Hawley RS, Kull FJ (2009) ATPase cycle of the nonmotile kinesin NOD allows microtubule end tracking and drives chromosome movement. *Cell* 136:110–122. doi:10.1016/j.cell.2008.11.048
- Cochran JC, Sontag CA, Maliga Z, Kapoor TM, Correia JJ, Gilbert SP (2004) Mechanistic analysis of the mitotic kinesin Eg5. *J Biol Chem* 279:38861–38870. doi:10.1074/jbc.M404203200
- Cochran JC, Zhao YC, Wilcox DE, Kull FJ (2011) A metal switch for controlling the activity of molecular motor proteins. *Nat Struct Mol Biol* 19:122–127. doi:10.1038/nsmb.2190
- Coureux PD, Sweeney HL, Houdusse A (2004) Three myosin V structures delineate essential features of chemo-mechanical transduction. *EMBO J* 23:4527–4537. doi:10.1038/sj.emboj.7600458
- Coureux PD, Wells AL, Menetrey J, Yengo CM, Morris CA, Sweeney HL, Houdusse A (2003) A structural state of the myosin V motor without bound nucleotide. *Nature* 425:419–423. doi:10.1038/nature01927
- Coy DL, Wagenbach M, Howard J (1999) Kinesin takes one 8-nm step for each ATP that it hydrolyzes. *J Biol Chem* 274:3667–3671
- Crevel JM, Nyitrai M, Alonso MC, Weiss S, Geeves MA, Cross RA (2004) What kinesin does at roadblocks: the coordination mechanism for molecular walking. *EMBO J* 23:23–32. doi:10.1038/sj.emboj.7600042
- Dagenbach EM, Endow SA (2004) A new kinesin tree. *J Cell Sci* 117:3–7. doi:10.1242/jcs.00875
- Datta S, Krishna R, Ganesh N, Chandra NR, Muniyappa K, Vijayan M (2003) Crystal structures of *Mycobacterium smegmatis* RecA and its nucleotide complexes. *J Bacteriol* 185:4280–4284
- De La Cruz EM, Ostap EM (2009) Kinetic and equilibrium analysis of the myosin ATPase. *Methods Enzymol* 455:157–192. doi:10.1016/S0076-6879(08)04206-7
- DeBonis S, Simorre JP, Crevel I, et al (2003) Interaction of the mitotic inhibitor monastrol with human kinesin Eg5. *Biochemistry* 42:338–349. doi:10.1021/bi026716j
- Delorme C, Joshi M, Allingham JS (2012) Crystal structure of the *Candida albicans* Kar3 kinesin motor domain fused to maltose-binding protein. *Biochem Biophys Res Commun* 428:427–432. doi:10.1016/j.bbrc.2012.10.101
- Devred F, Barbier P, Lafitte D, Landrieu I, Lippens G, Peyrot V (2010) Microtubule and MAPs: thermodynamics of complex formation by AUC, ITC, fluorescence, and NMR. *Methods Cell Biol* 95:449–480. doi:10.1016/S0091-679X(10)95023-1
- Dittrich M, Hayashi S, Schulten K (2003) On the mechanism of ATP hydrolysis in F1-ATPase. *Biophys J* 85:2253–2266. doi:10.1016/S0006-3495(03)74650-5
- Dittrich M, Hayashi S, Schulten K (2004) ATP hydrolysis in the betaTP and betaDP catalytic sites of F1-ATPase. *Biophys J* 87:2954–2967. doi:10.1529/biophysj.104.046128
- Duan D, Hnatchuk DJ, Brenner J, Davis D, Allingham JS (2012a) Crystal structure of the Kar3-like kinesin motor domain from the filamentous fungus *Ashbya gossypii*. *Proteins* 80:1016–1027
- Duan D, Jia Z, Joshi M, et al (2012b) Neck rotation and neck mimic docking in the noncatalytic Kar3-associated protein Vik1. *J Biol Chem* 287:40292–40301. doi:10.1074/jbc.M112.416529
- Duselder A, Thiede C, Schmidt CF, Lakammer S (2012) Neck-linker length dependence of processive Kinesin-5 motility. *J Mol Biol* 423:159–168. doi:10.1016/j.jmb.2012.06.043
- Ebbing B, Mann K, Starosta A, Jaud J, Schols L, Schule R, Woehlke G (2008) Effect of spastic paraplegia mutations in KIF5A kinesin on

- transport activity. *Hum Mol Genet* 17:1245–1252. doi:[10.1093/hmg/ddn014](#)
- Edamatsu M (2014) Bidirectional motility of the fission yeast kinesin-5, Cut7. *Biochem Biophys Res Commun* 446:231–234. doi:[10.1016/j.bbrc.2014.02.106](#)
- Endow SA, Higuchi H (2000) A mutant of the motor protein kinesin that moves in both directions on microtubules. *Nature* 406:913–916. doi:[10.1038/35022617](#)
- Endow SA, Waligora KW (1998) Determinants of kinesin motor polarity. *Science* 281:1200–1202
- Endres NF, Yoshioka C, Milligan RA, Vale RD (2006) A lever-arm rotation drives motility of the minus-end-directed kinesin Ncd. *Nature* 439:875–878. doi:[10.1038/nature04320](#)
- Farrell CM, Mackey AT, Klumpp LM, Gilbert SP (2002) The role of ATP hydrolysis for kinesin processivity. *J Biol Chem* 277:17079–17087. doi:[10.1074/jbc.M108793200](#)
- Finlayson B, Lymn RW, Taylor EW (1969) Studies on the kinetics of formation and dissociation of the actomyosin complex. *Biochemistry* 8:811–819
- Finlayson B, Taylor EW (1969) Hydrolysis of nucleoside triphosphates by myosin during the transient state. *Biochemistry* 8:802–810
- Foster KA, Correia JJ, Gilbert SP (1998) Equilibrium binding studies of non-claret disjunctional protein (Ncd) reveal cooperative interactions between the motor domains. *J Biol Chem* 273:35307–35318
- Foster KA, Gilbert SP (2000) Kinetic studies of dimeric Ncd: evidence that Ncd is not processive. *Biochemistry* 39:1784–1791
- Foster KA, Mackey AT, Gilbert SP (2001) A mechanistic model for Ncd directionality. *J Biol Chem* 276:19259–19266. doi:[10.1074/jbc.M008347200](#)
- Fridman V, Gerson-Gurwitz A, Shapira O, Movshovich N, Lakamper S, Schmidt CF, Gheber L (2013) Kinesin-5 Kip1 is a bi-directional motor that stabilizes microtubules and tracks their plus-ends in vivo. *J Cell Sci*. doi:[10.1242/jcs.125153](#)
- Friel CT, Howard J (2011) The kinesin-13 MCAK has an unconventional ATPase cycle adapted for microtubule depolymerization. *EMBO J* 30:3928–3939. doi:[10.1038/emboj.2011.290](#)
- Fuger P, Sreekumar V, Schule R, et al (2012) Spastic paraplegia mutation N256S in the neuronal microtubule motor KIF5A disrupts axonal transport in a *Drosophila* HSP model. *PLoS Genet* 8:e1003066. doi:[10.1371/journal.pgen.1003066](#)
- Furuta K, Furuta A, Toyoshima YY, Amino M, Oiwa K, Kojima H (2013) Measuring collective transport by defined numbers of processive and nonprocessive kinesin motors. *Proc Natl Acad Sci USA* 110:501–506. doi:[10.1073/pnas.1201390110](#)
- Garcia-Saez I, Yen T, Wade RH, Kozielski F (2004) Crystal structure of the motor domain of the human kinetochore protein CENP-E. *J Mol Biol* 340:1107–1116. doi:[10.1016/j.jmb.2004.05.053](#)
- Ge H, Qian H (2013) Dissipation, generalized free energy, and a self-consistent nonequilibrium thermodynamics of chemically driven open subsystems. *Phys Rev E Stat Nonlin Soft Matter Phys* 87:062125
- Geng YZ, Liu SX, Ji Q, Yan S (2014) Mechanical amplification mechanism of kinesin's beta-domain. *Arch Biochem Biophys* 543:10–14. doi:[10.1016/j.abb.2013.12.017](#)
- Gerson-Gurwitz A, Thiede C, Movshovich N et al (2011) Directionality of individual kinesin-5 Cin8 motors is modulated by loop 8, ionic strength and microtubule geometry. *EMBO J* 30:4942–4954. doi:[10.1038/emboj.2011.403](#)
- Gigant B, Wang W, Dreier B, et al (2013) Structure of a kinesin-tubulin complex and implications for kinesin motility. *Nat Struct Mol Biol* 20:1001–1007. doi:[10.1038/nsmb.2624](#)
- Gilbert SP, Johnson KA (1993) Expression, purification, and characterization of the *Drosophila* kinesin motor domain produced in *Escherichia coli*. *Biochemistry* 32:4677–4684
- Gilbert SP, Mackey AT (2000) Kinetics: a tool to study molecular motors. *Methods* 22:337–354. doi:[10.1006/meth.2000.1086](#)
- Gilbert SP, Moyer ML, Johnson KA (1998) Alternating site mechanism of the kinesin ATPase. *Biochemistry* 37:792–799. doi:[10.1021/bi971117b](#)
- Gilbert SP, Webb MR, Brune M, Johnson KA (1995) Pathway of processive ATP hydrolysis by kinesin. *Nature* 373:671–676. doi:[10.1038/373671a0](#)
- Goody RS, Pai EF, Schlichting I, Rensland H, Scheidig A, Franken S, Wittinghofer A (1992) Studies on the structure and mechanism of H-ras p21. *Philos Trans R Soc Lond B Biol Sci* 336:3–10. doi:[10.1098/rstb.1992.0037](#). discussion 10–11
- Goulet A, Behnke-Parks WM, Sindelar CV, Major J, Rosenfeld SS, Moores CA (2012) The structural basis of force generation by the mitotic motor Kinesin-5. *J Biol Chem* 287:44654–44666. doi:[10.1074/jbc.M112.404228](#)
- Grigorenko BL, Kaliman IA, Nemukhin AV (2011) Minimum energy reaction profiles for ATP hydrolysis in myosin. *J Mol Graph Model* 31:1–4. doi:[10.1016/j.jmgm.2011.07.005](#)
- Grigorenko BL, Rogov AV, Topol IA, Burt SK, Martinez HM, Nemukhin AV (2007) Mechanism of the myosin catalyzed hydrolysis of ATP as rationalized by molecular modeling. *Proc Natl Acad Sci U S A* 104:7057–7061. doi:[10.1073/pnas.0701727104](#)
- Grissom PM, Fiedler T, Grishchuk EL, Nicastro D, West RR, McIntosh JR (2009) Kinesin-8 from fission yeast: a heterodimeric, plus-end-directed motor that can couple microtubule depolymerization to cargo movement. *Mol Biol Cell* 20:963–972. doi:[10.1091/mbc.E08-09-0979](#)
- Gulick AM, Bauer CB, Thoden JB, Rayment I (1997) X-ray structures of the MgADP, MgATP γ S, and MgAMPPNP complexes of the *Dictyostelium discoideum* myosin motor domain. *Biochemistry* 36:11619–11628. doi:[10.1021/bi9712596](#)
- Gulick AM, Song H, Endow SA, Rayment I (1998) X-ray crystal structure of the yeast Kar3 motor domain complexed with Mg $\cdot$ ADP to 2.3 Å resolution. *Biochemistry* 37:1769–1776. doi:[10.1021/bi972504o](#)
- Guydosh NR, Block SM (2006) Backsteps induced by nucleotide analogs suggest the front head of kinesin is gated by strain. *Proc Natl Acad Sci USA* 103:8054–8059. doi:[10.1073/pnas.0600931103](#)
- Hackney DD (1988) Kinesin ATPase: rate-limiting ADP release. *Proc Natl Acad Sci USA* 85:6314–6318
- Hackney DD (1995a) Highly processive microtubule-stimulated ATP hydrolysis by dimeric kinesin head domains. *Nature* 377:448–450. doi:[10.1038/377448a0](#)
- Hackney DD (1995b) Implications of diffusion-controlled limit for processivity of dimeric kinesin head domains. *Biophys J* 68:267S–269S. discussion 269S–270S
- Hackney DD (2005) The tethered motor domain of a kinesin-microtubule complex catalyzes reversible synthesis of bound ATP. *Proc Natl Acad Sci USA* 102:18338–18343. doi:[10.1073/pnas.0505288102](#)
- Hackney DD (2007) Jump-starting kinesin. *J Cell Biol* 176:7–9. doi:[10.1083/jcb.200611082](#)
- Hackney DD, Stock MF (2008) Kinesin tail domains and Mg $^{2+}$ directly inhibit release of ADP from head domains in the absence of microtubules. *Biochemistry* 47:7770–7778. doi:[10.1021/bi8006687](#)
- Halbach F, Rode M, Conti E (2012) The crystal structure of *S. cerevisiae* Ski2, a DEXH helicase associated with the cytoplasmic functions of the exosome. *RNA* 18:124–134. doi:[10.1261/ma.029553.111](#)
- Halkides CJ, Frey PA (1991) The mechanism of the hydrolysis of mu-monothiopyrophosphate. *J Am Chem Soc* 113:9843–9848. doi:[10.1021/Ja00026a020](#)
- Hancock WO, Howard J (1999) Kinesin's processivity results from mechanical and chemical coordination between the ATP hydrolysis cycles of the two motor domains. *Proc Natl Acad Sci USA* 96:13147–13152
- Hariharan V, Hancock WO (2009) Insights into the mechanical properties of the kinesin neck linker domain from sequence analysis and

- molecular dynamics simulations. *Cell Mol Bioeng* 2:177–189. doi:10.1007/s12195-009-0059-5
- Hayashi S, Ueno H, Shaikh AR, et al (2012) Molecular mechanism of ATP hydrolysis in F1-ATPase revealed by molecular simulations and single-molecule observations. *J Am Chem Soc* 134:8447–8454. doi:10.1021/ja211027m
- Henningsen U, Schliwa M (1997) Reversal in the direction of movement of a molecular motor. *Nature* 389:93–96. doi:10.1038/38022
- Henry ER, Eaton WA, Hochstrasser RM (1986) Molecular dynamics simulations of cooling in laser-excited heme proteins. *Proc Natl Acad Sci U S A* 83:8982–8986
- Hertzer KM, Ems-McClung SC, Kline-Smith SL, Lipkin TG, Gilbert SP, Walczak CE (2006) Full-length dimeric MCAK is a more efficient microtubule depolymerase than minimal domain monomeric MCAK. *Mol Biol Cell* 17:700–710. doi:10.1091/mbc.E05-08-0821
- Heuston E, Bronner CE, Kull FJ, Endow SA (2010) A kinesin motor in a force-producing conformation. *BMC Struct Biol* 10:19. doi:10.1186/1472-6807-10-19
- Higuchi H, Bronner CE, Park HW, Endow SA (2004) Rapid double 8-nm steps by a kinesin mutant. *EMBO J* 23:2993–2999. doi:10.1038/sj.emboj.7600306
- Hirokawa N, Takemura R (2004) Kinesin superfamily proteins and their various functions and dynamics. *Exp Cell Res* 301:50–59. doi:10.1016/j.yexcr.2004.08.010
- Hirose K, Lockhart A, Cross RA, Amos LA (1996) Three-dimensional cryoelectron microscopy of dimeric kinesin and ncd motor domains on microtubules. *Proc Natl Acad Sci U S A* 93:9539–9544
- Hoeng JC, Dawson SC, House SA, et al (2008) High-resolution crystal structure and in vivo function of a kinesin-2 homologue in *Giardia intestinalis*. *Mol Biol Cell* 19:3124–3137. doi:10.1091/mbc.E07-11-1156
- Howard J, Hudspeth AJ, Vale RD (1989) Movement of microtubules by single kinesin molecules. *Nature* 342:154–158. doi:10.1038/342154a0
- Hua W, Young EC, Fleming ML, Gelles J (1997) Coupling of kinesin steps to ATP hydrolysis. *Nature* 388:390–393. doi:10.1038/41118
- Hwang W, Lang MJ (2009) Mechanical design of translocating motor proteins. *Cell Biochem Biophys* 54:11–22. doi:10.1007/s12013-009-9049-4
- Hwang W, Lang MJ, Karplus M (2008) Force generation in kinesin hinges on cover-neck bundle formation. *Structure* 16:62–71. doi:10.1016/j.str.2007.11.008
- Hyeon C, Onuchic JN (2007) Internal strain regulates the nucleotide binding site of the kinesin leading head. *Proc Natl Acad Sci USA* 104:2175–2180. doi:10.1073/pnas.0610939104
- Iwatani S, Iwane AH, Higuchi H, Ishii Y, Yanagida T (1999) Mechanical and chemical properties of cysteine-modified kinesin molecules. *Biochemistry* 38:10318–10323. doi:10.1021/bi9904095
- Jannasch A, Bormuth V, Storch M, Howard J, Schaffer E (2013) Kinesin-8 is a low-force motor protein with a weakly bound slip state. *Biophys J* 104:2456–2464. doi:10.1016/j.bpj.2013.02.040
- Jaud J, Bathe F, Schliwa M, Rief M, Woehlke G (2006) Flexibility of the neck domain enhances Kinesin-1 motility under load. *Biophys J* 91:1407–1412. doi:10.1529/biophysj.105.076265
- Jiang W, Stock MF, Li X, Hackney DD (1997) Influence of the kinesin neck domain on dimerization and ATPase kinetics. *J Biol Chem* 272:7626–7632
- Johnson KA (1992) Transient-state kinetic analysis of enzyme reaction pathways. In: *The Enzymes*, vol 20. Academic Press, New York, pp 1–61
- Johnson KA, Simpson ZB, Blom T (2009) Global kinetic explorer: a new computer program for dynamic simulation and fitting of kinetic data. *Anal Biochem* 387:20–29. doi:10.1016/j.ab.2008.12.024
- Kaan HY, Hackney DD, Kozielski F (2011) The structure of the kinesin-1 motor-tail complex reveals the mechanism of autoinhibition. *Science* 333:883–885. doi:10.1126/science.1204824
- Kaan HY, Ulaganathan V, Rath O, Prokopcova H, Dallinger D, Kappe CO, Kozielski F (2010) Structural basis for inhibition of Eg5 by dihydropyrimidines: stereoselectivity of antimitotic inhibitors enastron, dimethylenastron and fluorastrol. *J Med Chem* 53:5676–5683. doi:10.1021/jm100421n
- Karabay A, Walker RA (1999) Identification of microtubule binding sites in the Ncd tail domain. *Biochemistry* 38:1838–1849. doi:10.1021/bi981850i
- Kaseda K, Higuchi H, Hirose K (2003) Alternate fast and slow stepping of a heterodimeric kinesin molecule. *Nat Cell Biol* 5:1079–1082. doi:10.1038/ncb1067
- Khalil AS, Appleyard DC, Labno AK, et al (2008) Kinesin's cover-neck bundle folds forward to generate force. *Proc Natl Acad Sci U S A* 105:19247–19252. doi:10.1073/pnas.0805147105
- Khan SA, Kirby AJ (1970) Reactivity of phosphate esters - multiple structure reactivity correlations for reactions of triesters with nucleophiles. *J Chem Soc B*:1172–1182. doi:10.1039/j29700001172
- Kikkawa M, Hirokawa N (2006) High-resolution cryo-EM maps show the nucleotide binding pocket of KIF1A in open and closed conformations. *EMBO J* 25:4187–4194. doi:10.1038/sj.emboj.7601299
- Kikkawa M, Sablin EP, Okada Y, Yajima H, Fletterick RJ, Hirokawa N (2001) Switch-based mechanism of kinesin motors. *Nature* 411:439–445. doi:10.1038/35078000
- Kim Y, Heuser JE, Waterman CM, Cleveland DW (2008) CENP-E combines a slow, processive motor and a flexible coiled coil to produce an essential mitotic kinetochore tether. *J Cell Biol* 181:411–419. doi:10.1083/jcb.200802189
- Klejnot M, Fahnrikar A, Ulaganathan V, Cross RA, Baas PW, Kozielski F (2014) The crystal structure and biochemical characterization of Kif15: a bifunctional molecular motor involved in bipolar spindle formation and neuronal development. *Acta Crystallogr D Biol Crystallogr* 70:123–133. doi:10.1107/S1399004713028721
- Klejnot M, Kozielski F (2012) Structural insights into human Kif7, a kinesin involved in Hedgehog signalling. *Acta Crystallogr D Biol Crystallogr* 68:154–159. doi:10.1107/S0907444911053042
- Klumpp LM, Brendza KM, Gatial JE 3rd, Hoenger A, Saxton WM, Gilbert SP (2004a) Microtubule-kinesin interface mutants reveal a site critical for communication. *Biochemistry* 43:2792–2803. doi:10.1021/bi035830e
- Klumpp LM, Hoenger A, Gilbert SP (2004b) Kinesin's second step. *Proc Natl Acad Sci U S A* 101:3444–3449. doi:10.1073/pnas.0307691101
- Klumpp LM, Mackey AT, Farrell CM, Rosenberg JM, Gilbert SP (2003) A kinesin switch I arginine to lysine mutation rescues microtubule function. *J Biol Chem* 278:39059–39067. doi:10.1074/jbc.M304250200
- Kocik E, Skowronek KJ, Kasprzak AA (2009) Interactions between subunits in heterodimeric Ncd molecules. *J Biol Chem* 284:35735–35745. doi:10.1074/jbc.M109.024240
- Kozielski F, Arnal I, Wade RH (1998) A model of the microtubule-kinesin complex based on electron cryomicroscopy and X-ray crystallography. *Curr Biol* 8:191–198
- Kozielski F, De Bonis S, Burmeister WP, Cohen-Addad C, Wade RH (1999) The crystal structure of the minus-end-directed microtubule motor protein ncd reveals variable dimer conformations. *Structure* 7:1407–1416
- Kozielski F, Sack S, Marx A, et al (1997) The crystal structure of dimeric kinesin and implications for microtubule-dependent motility. *Cell* 91:985–994
- Krzysiak TC, Gilbert SP (2006) Dimeric Eg5 maintains processivity through alternating-site catalysis with rate-limiting ATP hydrolysis. *J Biol Chem* 281:39444–39454. doi:10.1074/jbc.M608056200
- Krzysiak TC, Grabe M, Gilbert SP (2008) Getting in sync with dimeric Eg5. Initiation and regulation of the processive run. *J Biol Chem* 283:2078–2087. doi:10.1074/jbc.M708354200

- Krzysiak TC, Wendt T, Sproul LR, Tittmann P, Gross H, Gilbert SP, Hoenger A (2006) A structural model for monastrol inhibition of dimeric kinesin Eg5. *EMBO J* 25:2263–2273. doi:[10.1038/sj.emboj.7601108](https://doi.org/10.1038/sj.emboj.7601108)
- Kull FJ (2000) Motor proteins of the kinesin superfamily: structure and mechanism. *Essays Biochem* 35:61–73
- Kull FJ, Endow SA (2002) Kinesin: switch I & II and the motor mechanism. *J Cell Sci* 115:15–23
- Kull FJ, Endow SA (2013) Force generation by kinesin and myosin cytoskeletal motor proteins. *J Cell Sci* 126:9–19. doi:[10.1242/jcs.103911](https://doi.org/10.1242/jcs.103911)
- Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD (1996) Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature* 380:550–555. doi:[10.1038/380550a0](https://doi.org/10.1038/380550a0)
- Kull FJ, Vale RD, Fletterick RJ (1998) The case for a common ancestor: kinesin and myosin motor proteins and G proteins. *J Muscle Res Cell Motil* 19:877–886
- Kuzmick P (1996) Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV proteinase. *Anal Biochem* 237:260–273
- Lawrence CJ, Dawe RK, Christie KR, et al (2004) A standardized kinesin nomenclature. *J Cell Biol* 167:19–22. doi:[10.1083/jcb.200408113](https://doi.org/10.1083/jcb.200408113)
- Lightcap ES, Frey PA (1992) Discrete Monomeric Metaphosphate Anion as an Intermediate in the Hydrolysis of Mu-Monothiotriphosphate. *J Am Chem Soc* 114:9750–9755. doi:[10.1021/Ja00051a004](https://doi.org/10.1021/Ja00051a004)
- Liu HL, Hallen MA, Endow SA (2012a) Altered nucleotide-microtubule coupling and increased mechanical output by a kinesin mutant. *PLoS One* 7:e47148. doi:[10.1371/journal.pone.0047148](https://doi.org/10.1371/journal.pone.0047148)
- Liu HL, Pemble IV CW, Endow SA (2012b) Neck-motor interactions trigger rotation of the kinesin stalk. *Sci Rep* 2:236. doi:[10.1038/srep00236](https://doi.org/10.1038/srep00236)
- Luo D, Xu T, Watson RP, et al (2008) Insights into RNA unwinding and ATP hydrolysis by the flavivirus NS3 protein. *EMBO J* 27:3209–3219. doi:[10.1038/emboj.2008.232](https://doi.org/10.1038/emboj.2008.232)
- Ma YZ, Taylor EW (1997) Interacting head mechanism of microtubule-kinesin ATPase. *J Biol Chem* 272:724–730
- Mackey AT, Gilbert SP (2000) Moving a microtubule may require two heads: a kinetic investigation of monomeric Ncd. *Biochemistry* 39:1346–1355
- Mackey AT, Gilbert SP (2003) The ATPase cross-bridge cycle of the Kar3 motor domain. Implications for single head motility. *J Biol Chem* 278:3527–3535
- Maliga Z, Kapoor TM, Mitchison TJ (2002) Evidence that monastrol is an allosteric inhibitor of the mitotic kinesin Eg5. *Chem Biol* 9:989–996
- Maliga Z, Xing J, Cheung H, Juszczak LJ, Friedman JM, Rosenfeld SS (2006) A pathway of structural changes produced by monastrol binding to Eg5. *J Biol Chem* 281:7977–7982. doi:[10.1074/jbc.M511955200](https://doi.org/10.1074/jbc.M511955200)
- Marx A, Hoenger A, Mandelkow E (2009) Structures of kinesin motor proteins. *Cell Motil Cytoskeleton* 66:958–966. doi:[10.1002/cm.20392](https://doi.org/10.1002/cm.20392)
- Marx A, Muller J, Mandelkow E (2005) The structure of microtubule motor proteins. *Adv Protein Chem* 71:299–344. doi:[10.1016/S0065-3233\(04\)71008-6](https://doi.org/10.1016/S0065-3233(04)71008-6)
- Mayr MI, Storch M, Howard J, Mayer TU (2011) A non-motor microtubule binding site is essential for the high processivity and mitotic function of kinesin-8 Kif18A. *PLoS One* 6:e27471. doi:[10.1371/journal.pone.0027471](https://doi.org/10.1371/journal.pone.0027471)
- Mazumdar M, Cross RA (1998) Engineering a lever into the kinesin neck. *J Biol Chem* 273:29352–29359
- McGrath MJ, Kuo IF, Hayashi S, Takada S (2013) Adenosine triphosphate hydrolysis mechanism in kinesin studied by combined quantum-mechanical/molecular-mechanical metadynamics simulations. *J Am Chem Soc* 135:8908–8919. doi:[10.1021/ja401540g](https://doi.org/10.1021/ja401540g)
- Menetrey J, Llinas P, Cicolari J, et al (2008) The post-rigor structure of myosin VI and implications for the recovery stroke. *EMBO J* 27:244–252. doi:[10.1038/sj.emboj.7601937](https://doi.org/10.1038/sj.emboj.7601937)
- Michaelis L, Menten ML (1913) Die kinetik der invertinwirkung. *Biochem Z* 49:333–369
- Michaelis L, Menten ML, Johnson KA, Goody RS (2011) The original Michaelis constant: translation of the 1913 Michaelis-Menten paper. *Biochemistry* 50:8264–8269. doi:[10.1021/bi201284u](https://doi.org/10.1021/bi201284u)
- Miki H, Okada Y, Hirokawa N (2005) Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol* 15:467–476. doi:[10.1016/j.tcb.2005.07.006](https://doi.org/10.1016/j.tcb.2005.07.006)
- Miller DL, Ukena T (1969) P1, P1-Diethyl Pyrophosphate. *J Am Chem Soc* 91:3050–3053. doi:[10.1021/Ja01039a038](https://doi.org/10.1021/Ja01039a038)
- Miller DL, Westheim FH (1966a) Enzymic Hydrolysis of Gamma-Phenylpropyl Di- and Triphosphates. *J Am Chem Soc* 88:1511–1513. doi:[10.1021/Ja00959a035](https://doi.org/10.1021/Ja00959a035)
- Miller DL, Westheim FH (1966b) Hydrolysis of Gamma-Phenylpropyl Di- and Triphosphates. *J Am Chem Soc* 88:1507–1511. doi:[10.1021/Ja00959a034](https://doi.org/10.1021/Ja00959a034)
- Miyazono Y, Hayashi M, Karagiannis P, Harada Y, Tadokuma H (2010) Strain through the neck linker ensures processive runs: a DNA-kinesin hybrid nanomachine study. *EMBO J* 29:93–106. doi:[10.1038/emboj.2009.319](https://doi.org/10.1038/emboj.2009.319)
- Moore JD, Song H, Endow SA (1996) A point mutation in the microtubule binding region of the Ncd motor protein reduces motor velocity. *EMBO J* 15:3306–3314
- Mori T, Vale RD, Tomishige M (2007) How kinesin waits between steps. *Nature* 450:750–754. doi:[10.1038/nature06346](https://doi.org/10.1038/nature06346)
- Morrison JF (1969) Kinetics of the reversible inhibition of enzyme-catalysed reactions by tight-binding inhibitors. *Biochim Biophys Acta* 185:269–286
- Moyer ML, Gilbert SP, Johnson KA (1998) Pathway of ATP hydrolysis by monomeric and dimeric kinesin. *Biochemistry* 37:800–813. doi:[10.1021/bi9711184](https://doi.org/10.1021/bi9711184)
- Muller J, Marx A, Sack S, Song YH, Mandelkow E (1999) The structure of the nucleotide-binding site of kinesin. *Biol Chem* 380:981–992. doi:[10.1515/BC.1999.122](https://doi.org/10.1515/BC.1999.122)
- Muthukrishnan G, Zhang Y, Shastry S, Hancock WO (2009) The processivity of kinesin-2 motors suggests diminished front-head gating. *Curr Biol* 19:442–447. doi:[10.1016/j.cub.2009.01.058](https://doi.org/10.1016/j.cub.2009.01.058)
- Nishizaka T, Yagi T, Tanaka Y, Ishiwata S (1993) Right-handed rotation of an actin filament in an in vitro motile system. *Nature* 361:269–271. doi:[10.1038/361269a0](https://doi.org/10.1038/361269a0)
- Nitta R, Kikkawa M, Okada Y, Hirokawa N (2004) KIF1A alternately uses two loops to bind microtubules. *Science* 305:678–683. doi:[10.1126/science.1096621](https://doi.org/10.1126/science.1096621)
- Nitta R, Okada Y, Hirokawa N (2008) Structural model for strain-dependent microtubule activation of Mg-ADP release from kinesin. *Nat Struct Mol Biol* 15:1067–1075. doi:[10.1038/nsmb.1487](https://doi.org/10.1038/nsmb.1487)
- Ogawa T, Nitta R, Okada Y, Hirokawa N (2004) A common mechanism for microtubule destabilizers-M type kinesins stabilize curling of the protofilament using the class-specific neck and loops. *Cell* 116:591–602
- Onishi H, Mochizuki N, Morales MF (2004) On the myosin catalysis of ATP hydrolysis. *Biochemistry* 43:3757–3763. doi:[10.1021/bi040002m](https://doi.org/10.1021/bi040002m)
- Pan X, Acar S, Scholey JM (2010) Torque generation by one of the motor subunits of heterotrimeric kinesin-2. *Biochem Biophys Res Commun* 401:53–57. doi:[10.1016/j.bbrc.2010.09.007](https://doi.org/10.1016/j.bbrc.2010.09.007)
- Parke CL, Wojcik EJ, Kim S, Worthylake DK (2010) ATP hydrolysis in Eg5 kinesin involves a catalytic two-water mechanism. *J Biol Chem* 285:5859–5867. doi:[10.1074/jbc.M109.071233](https://doi.org/10.1074/jbc.M109.071233)
- Pechatnikova E, Taylor EW (1997) Kinetic mechanism of monomeric non-claret disjunctional protein (Ncd) ATPase. *J Biol Chem* 272:30735–30740

- Pecqueur L, Duellberg C, Dreier B, et al (2012) A designed ankyrin repeat protein selected to bind to tubulin caps the microtubule plus end. *Proc Natl Acad Sci USA* 109:12011–12016. doi:[10.1073/pnas.1204129109](#)
- Peters C, Brejc K, Belmont L, et al (2010) Insight into the molecular mechanism of the multitasking kinesin-8 motor. *EMBO J* 29:3437–3447. doi:[10.1038/emboj.2010.220](#)
- Porter ME, Johnson KA (1983) Transient state kinetic analysis of the ATP-induced dissociation of the dynein-microtubule complex. *J Biol Chem* 258:6582–6587
- Rajan R, Bell CE (2004) Crystal structure of RecA from *Deinococcus radiodurans*: insights into the structural basis of extreme radioresistance. *J Mol Biol* 344:951–963. doi:[10.1016/j.jmb.2004.09.087](#)
- Rank KC, Chen CJ, Cope J, Porche K, Hoenger A, Gilbert SP, Rayment I (2012) Kar3Vik1, a member of the Kinesin-14 superfamily, shows a novel kinesin microtubule binding pattern. *J Cell Biol* 197:957–970. doi:[10.1083/jcb.201201132](#)
- Rath O, Kozielski F (2012) Kinesins and cancer. *Nat Rev Cancer* 12:527–539. doi:[10.1038/nrc3310](#)
- Ray S, Meyhofer E, Milligan RA, Howard J (1993) Kinesin follows the microtubule's protofilament axis. *J Cell Biol* 121:1083–1093
- Ray S, Wolf SG, Howard J, Downing KH (1995) Kinesin does not support the motility of zinc-macro tubules. *Cell Motil Cytoskeleton* 30:146–152. doi:[10.1002/cm.970300206](#)
- Rayment I, Rypniewski WR, Schmidt-Base K, et al (1993) Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* 261:50–58
- Reubold TF, Eschenburg S, Becker A, Kull FJ, Manstein DJ (2003) A structural model for actin-induced nucleotide release in myosin. *Nat Struct Biol* 10:826–830. doi:[10.1038/nsb987](#)
- Rice S, Lin AW, Safer D, et al (1999) A structural change in the kinesin motor protein that drives motility. *Nature* 402:778–784. doi:[10.1038/45483](#)
- Roostalu J, Hentrich C, Bieling P, Telley IA, Schiebel E, Surrey T (2011) Directional switching of the kinesin Cin8 through motor coupling. *Science* 332:94–99. doi:[10.1126/science.1199945](#)
- Rosenfeld SS, Fordyce PM, Jefferson GM, King PH, Block SM (2003) Stepping and stretching. How kinesin uses internal strain to walk processively. *J Biol Chem* 278:18550–18556. doi:[10.1074/jbc.M300849200](#)
- Rosenfeld SS, Jefferson GM, King PH (2001) ATP reorients the neck linker of kinesin in two sequential steps. *J Biol Chem* 276:40167–40174. doi:[10.1074/jbc.M103899200](#)
- Rosenfeld SS, van Duffelen M, Behnke-Parks WM, Beadle C, Correia J, Xing J (2009) The ATPase cycle of the mitotic motor CENP-E. *J Biol Chem* 284:32858–32868. doi:[10.1074/jbc.M109.041210](#)
- Rosenfeld SS, Xing J, Jefferson GM, Cheung HC, King PH (2002) Measuring kinesin's first step. *J Biol Chem* 277:36731–36739. doi:[10.1074/jbc.M205261200](#)
- Rosenfeld SS, Xing J, Jefferson GM, King PH (2005) Docking and rolling, a model of how the mitotic motor Eg5 works. *J Biol Chem* 280:35684–35695. doi:[10.1074/jbc.M506561200](#)
- Sablin EP, Case RB, Dai SC, Hart CL, Ruby A, Vale RD, Fletterick RJ (1998) Direction determination in the minus-end-directed kinesin motor ncd. *Nature* 395:813–816. doi:[10.1038/27463](#)
- Sablin EP, Kull FJ, Cooke R, Vale RD, Fletterick RJ (1996) Crystal structure of the motor domain of the kinesin-related motor ncd. *Nature* 380:555–559. doi:[10.1038/380555a0](#)
- Sack S, Kull FJ, Mandelkow E (1999) Motor proteins of the kinesin family. Structures, variations, and nucleotide binding sites. *Eur J Biochem* 262:1–11
- Sack S, Muller J, Marx A, Thormahlen M, Mandelkow EM, Brady ST, Mandelkow E (1997) X-ray structure of motor and neck domains from rat brain kinesin. *Biochemistry* 36:16155–16165. doi:[10.1021/bi9722498](#)
- Sadhu A, Taylor EW (1992) A kinetic study of the kinesin ATPase. *J Biol Chem* 267:11352–11359
- Sardar HS, Gilbert SP (2012) Microtubule capture by mitotic kinesin CENP-E. *J Biol Chem* 287(30):24894–904. doi:[10.1074/jbc.M112.376830](#)
- Schaap IA, Carrasco C, de Pablo PJ, Schmidt CF (2011) Kinesin walks the line: single motors observed by atomic force microscopy. *Biophys J* 100:2450–2456. doi:[10.1016/j.bpj.2011.04.015](#)
- Schief WR, Clark RH, Crevenna AH, Howard J (2004) Inhibition of kinesin motility by ADP and phosphate supports a hand-over-hand mechanism. *Proc Natl Acad Sci USA* 101:1183–1188. doi:[10.1073/pnas.0304369101](#)
- Schief WR, Howard J (2001) Conformational changes during kinesin motility. *Curr Opin Cell Biol* 13:19–28
- Schmidt H, Gleave ES, Carter AP (2012) Insights into dynein motor domain function from a 3.3-Å crystal structure. *Nat Struct Mol Biol* 19(492–497):S491. doi:[10.1038/nsmb.2272](#)
- Schnitzer MJ, Block SM (1997) Kinesin hydrolyses one ATP per 8-nm step. *Nature* 388:386–390. doi:[10.1038/41111](#)
- Schnitzer MJ, Visscher K, Block SM (2000) Force production by single kinesin motors. *Nat Cell Biol* 2:718–723. doi:[10.1038/35036345](#)
- Schutz P, Karlberg T, van den Berg S, et al. (2010) Comparative structural analysis of human DEAD-box RNA helicases. *PLoS One* 5. doi:[10.1371/journal.pone.0012791](#)
- Schwarzl SM, Smith JC, Fischer S (2006) Insights into the chemomechanical coupling of the myosin motor from simulation of its ATP hydrolysis mechanism. *Biochemistry* 45:5830–5847. doi:[10.1021/bi052433q](#)
- Seidel SA, Dijkman PM, Lea WA, et al (2013) Microscale thermophoresis quantifies biomolecular interactions under previously challenging conditions. *Methods* 59:301–315. doi:[10.1016/j.ymeth.2012.12.005](#)
- Shamovsky IL, Ross GM, Riopelle RJ (2000a) Ab initio studies on the origin of beta-sheet twisting. *Abstr Pap Am Chem Soc* 219:U599–U599
- Shamovsky IL, Ross GM, Riopelle RJ (2000b) Theoretical studies on the origin of beta-sheet twisting. *J Phys Chem B* 104:11296–11307. doi:[10.1021/jp002590t](#)
- Shastri S, Hancock WO (2010) Neck linker length determines the degree of processivity in kinesin-1 and kinesin-2 motors. *Curr Biol* 20:939–943. doi:[10.1016/j.cub.2010.03.065](#)
- Shastri S, Hancock WO (2011) Interhead tension determines processivity across diverse N-terminal kinesins. *Proc Natl Acad Sci U S A* 108:16253–16258. doi:[10.1073/pnas.1102628108](#)
- Shikama K (1971) Standard free energy maps for the hydrolysis of ATP as a function of pH, pMg and pCa. *Arch Biochem Biophys* 147:311–317
- Shipley K, Hekmat-Nejad M, Turner J, et al (2004) Structure of a kinesin microtubule depolymerization machine. *EMBO J* 23:1422–1432. doi:[10.1038/sj.emboj.7600165](#)
- Sindelar CV, Budny MJ, Rice S, Naber N, Fletterick R, Cooke R (2002) Two conformations in the human kinesin power stroke defined by X-ray crystallography and EPR spectroscopy. *Nat Struct Biol* 9:844–848. doi:[10.1038/nsb852](#)
- Sindelar CV, Downing KH (2007) The beginning of kinesin's force-generating cycle visualized at 9-Å resolution. *J Cell Biol* 177:377–385. doi:[10.1083/jcb.200612090](#)
- Skinotiotis G, Surrey T, Altmann S, Gross H, Song YH, Mandelkow E, Hoenger A (2003) Nucleotide-induced conformations in the neck region of dimeric kinesin. *EMBO J* 22:1518–1528. doi:[10.1093/emboj/cdg164](#)
- Skoufias DA, DeBonis S, Saoudi Y, et al (2006) S-trityl-L-cysteine is a reversible, tight binding inhibitor of the human kinesin Eg5 that specifically blocks mitotic progression. *J Biol Chem* 281:17559–17569. doi:[10.1074/jbc.M511735200](#)

- Smith CA, Rayment I (1996) X-ray structure of the magnesium(II)-ADP-vanadate complex of the Dictyostelium discoideum myosin motor domain to 1.9 Å resolution. *Biochemistry* 35:5404–5417. doi:10.1021/bi952633+
- Song H, Endow SA (1998) Decoupling of nucleotide- and microtubule-binding sites in a kinesin mutant. *Nature* 396:587–590. doi:10.1038/25153
- Song YH, Marx A, Muller J, et al (2001) Structure of a fast kinesin: implications for ATPase mechanism and interactions with microtubules. *EMBO J* 20:6213–6225. doi:10.1093/emboj/20.22.6213
- Soppina V, Norris SR, Dizaji AS, Kortus M, Veatch S, Peckham M, Verhey KJ (2014) Dimerization of mammalian kinesin-3 motors results in superprocessive motion. *Proc Natl Acad Sci USA* 111:5562–5567. doi:10.1073/pnas.1400759111
- Soppina V, Verhey KJ (2014) The family-specific K-loop influences the microtubule on-rate but not the superprocessivity of kinesin-3 motors. *Mol Biol Cell* 25:2161–2170. doi:10.1091/mbc.E14-01-0696
- Sosa H, Dias DP, Hoenger A, et al (1997) A model for the microtubule-Ncd motor protein complex obtained by cryo-electron microscopy and image analysis. *Cell* 90:217–224
- Sproul LR, Anderson DJ, Mackey AT, Saunders WS, Gilbert SP (2005) Cik1 targets the minus-end kinesin depolymerase kar3 to microtubule plus ends. *Curr Biol* 15:1420–1427. doi:10.1016/j.cub.2005.06.066
- Spudich JA (2006) Molecular motors take tension in stride. *Cell* 126:242–244
- Stinson BM, Nager AR, Glynn SE, Schmitz KR, Baker TA, Sauer RT (2013) Nucleotide binding and conformational switching in the hexameric ring of a AAA+ machine. *Cell* 153:628–639. doi:10.1016/j.cell.2013.03.029
- Stockbridge RB, Wolfenden R (2009) The intrinsic reactivity of ATP and the catalytic proficiencies of kinases acting on glucose, N-acetylglucosamine, and homoserine: a thermodynamic analysis. *J Biol Chem* 284:22747–22757. doi:10.1074/jbc.M109.017806
- Stumpff J, Du Y, English CA, et al (2011) A tethering mechanism controls the processivity and kinetochore-microtubule plus-end enrichment of the kinesin-8 Kif18A. *Mol Cell* 43:764–775. doi:10.1016/j.molcel.2011.07.022
- Su X, Qiu W, Gupta ML Jr, Pereira-Leal JB, Reck-Peterson SL, Pellman D (2011) Mechanisms underlying the dual-mode regulation of microtubule dynamics by Kip3/kinesin-8. *Mol Cell* 43:751–763. doi:10.1016/j.molcel.2011.06.027
- Sugata K, Nakamura M, Ueki S, Fajer PG, Arata T (2004) ESR reveals the mobility of the neck linker in dimeric kinesin. *Biochem Biophys Res Commun* 314:447–451
- Sugata K, Song L, Nakamura M, Ueki S, Fajer PG, Arata T (2009) Nucleotide-induced flexibility change in neck linkers of dimeric kinesin as detected by distance Measurements using spin-labeling EPR. *J Mol Biol* 386:626–636. doi:10.1016/j.jmb.2008.12.079
- Svoboda K, Block SM (1994) Force and velocity measured for single kinesin molecules. *Cell* 77:773–784
- Svoboda K, Schmidt CF, Schnapp BJ, Block SM (1993) Direct observation of kinesin stepping by optical trapping interferometry. *Nature* 365:721–727. doi:10.1038/365721a0
- Talapatra SK, Anthony NG, Mackay SP, Kozielski F (2013) Mitotic kinesin Eg5 overcomes inhibition to the phase I/II clinical candidate SB743921 by an allosteric resistance mechanism. *J Med Chem* 56:6317–6329. doi:10.1021/jm4006274
- Talapatra SK, Schuttelkopf AW, Kozielski F (2012) The structure of the ternary Eg5-ADP-ispinesib complex. *Acta Crystallogr D Biol Crystallogr* 68:1311–1319. doi:10.1107/S0907444912027965
- Tan D, Rice WJ, Sosa H (2008) Structure of the kinesin13-microtubule ring complex. *Structure* 16:1732–1739. doi:10.1016/j.str.2008.08.017
- Tcherniuk S, Skoufias DA, Labriere C, Rath O, Gueritte F, Guillou C, Kozielski F (2010) Relocation of Aurora B and survivin from centromeres to the central spindle impaired by a kinesin-specific MKLP-2 inhibitor. *Angew Chem Int Ed Engl* 49:8228–8231. doi:10.1002/anie.201003254
- Thatcher GRJ, Kluger R (1989) Mechanism and catalysis of nucleophilic-substitution in phosphate-esters. *Adv Phys Org Chem* 25:99–265
- Thiede C, Fridman V, Gerson-Gurwitz A, Gheber L, Schmidt CF (2012) Regulation of bi-directional movement of single kinesin-5 Cin8 molecules. *Bioarchitecture* 2:70–74
- Thoresen T, Gelles J (2008) Processive movement by a kinesin heterodimer with an inactivating mutation in one head. *Biochemistry* 47:9514–9521. doi:10.1021/bi800747e
- Thom KS, Ubersax JA, Vale RD (2000) Engineering the processive run length of the kinesin motor. *J Cell Biol* 151:1093–1100
- Tomishige M, Klopfenstein DR, Vale RD (2002) Conversion of Unc104/KIF1A kinesin into a processive motor after dimerization. *Science* 297:2263–2267. doi:10.1126/science.1073386
- Toprak E, Yildiz A, Hoffman MT, Rosenfeld SS, Selvin PR (2009) Why kinesin is so processive. *Proc Natl Acad Sci USA* 106:12717–12722. doi:10.1073/pnas.0808396106
- Tsvetkov PO, Barbier P, Breuzard G, Peyrot V, Devred F (2013) Microtubule-associated proteins and tubulin interaction by isothermal titration calorimetry. *Methods Cell Biol* 115:283–302. doi:10.1016/B978-0-12-407757-7.00018-9
- Turner J, Anderson R, Guo J, Beraud C, Fletterick R, Sakowicz R (2001) Crystal structure of the mitotic spindle kinesin Eg5 reveals a novel conformation of the neck-linker. *J Biol Chem* 276:25496–25502. doi:10.1074/jbc.M100395200
- Ulaganathan V, Talapatra SK, Rath O, Pannifer A, Hackney DD, Kozielski F (2013) Structural insights into a unique inhibitor binding pocket in kinesin spindle protein. *J Am Chem Soc* 135:2263–2272. doi:10.1021/ja310377d
- Vale RD, Fletterick RJ (1997) The design plan of kinesin motors. *Annu Rev Cell Dev Biol* 13:745–777. doi:10.1146/annurev.cellbio.13.1.745
- Vale RD, Funatsu T, Pierce DW, Romberg L, Harada Y, Yanagida T (1996) Direct observation of single kinesin molecules moving along microtubules. *Nature* 380:451–453. doi:10.1038/380451a0
- Vale RD, Milligan RA (2000) The way things move: looking under the hood of molecular motor proteins. *Science* 288:88–95
- Vale RD, Reese TS, Sheetz MP (1985) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 42:39–50
- Vale RD, Toyoshima YY (1988) Rotation and translocation of microtubules in vitro induced by dyneins from *Tetrahymena* cilia. *Cell* 52:459–469
- Valentine MT, Fordyce PM, Krzyziak TC, Gilbert SP, Block SM (2006) Individual dimers of the mitotic kinesin motor Eg5 step processively and support substantial loads in vitro. *Nat Cell Biol* 8:470–476. doi:10.1038/ncb1394
- Vanneste D, Ferreira V, Vernos I (2011) Chromokinesins: localization-dependent functions and regulation during cell division. *Biochem Soc Trans* 39:1154–1160. doi:10.1042/BST0391154
- Varga V, Helenius J, Tanaka K, Hyman AA, Tanaka TU, Howard J (2006) Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. *Nat Cell Biol* 8:957–962. doi:10.1038/ncb1462
- Varga V, Leduc C, Bormuth V, Diez S, Howard J (2009) Kinesin-8 motors act cooperatively to mediate length-dependent microtubule depolymerization. *Cell* 138:1174–1183. doi:10.1016/j.cell.2009.07.032
- Vinogradova MV, Malanina GG, Reddy AS, Fletterick RJ (2009) Structure of the complex of a mitotic kinesin with its calcium binding regulator. *Proc Natl Acad Sci USA* 106:8175–8179. doi:10.1073/pnas.0811131106
- Vinogradova MV, Malanina GG, Reddy VS, Reddy AS, Fletterick RJ (2008) Structural dynamics of the microtubule binding and

- regulatory elements in the kinesin-like calmodulin binding protein. *J Struct Biol* 163:76–83. doi:[10.1016/j.jsb.2008.04.004](https://doi.org/10.1016/j.jsb.2008.04.004)
- Vinogradova MV, Malanina GG, Waitzman JS, Rice SE, Fletterick RJ (2013) Plant kinesin-like calmodulin binding protein employs its regulatory domain for dimerization. *PLoS One* 8:e66669. doi:[10.1371/journal.pone.0066669](https://doi.org/10.1371/journal.pone.0066669)
- Vinogradova MV, Reddy VS, Reddy AS, Sablin EP, Fletterick RJ (2004) Crystal structure of kinesin regulated by Ca(2+)-calmodulin. *J Biol Chem* 279:23504–23509. doi:[10.1074/jbc.M400741200](https://doi.org/10.1074/jbc.M400741200)
- Visscher K, Schnitzer MJ, Block SM (1999) Single kinesin molecules studied with a molecular force clamp. *Nature* 400:184–189. doi:[10.1038/22146](https://doi.org/10.1038/22146)
- Wade RH, Kozielski F (2000) Structural links to kinesin directionality and movement. *Nat Struct Biol* 7:456–460. doi:[10.1038/75850](https://doi.org/10.1038/75850)
- Wagenbach M, Domnitz S, Wordeman L, Cooper J (2008) A kinesin-13 mutant catalytically depolymerizes microtubules in ADP. *J Cell Biol* 183:617–623. doi:[10.1083/jcb.200805145](https://doi.org/10.1083/jcb.200805145)
- Waitzman JS, Larson AG, Cochran JC, et al (2011) The loop 5 element structurally and kinetically coordinates dimers of the human kinesin-5, Eg5. *Biophys J* 101:2760–2769. doi:[10.1016/j.bpj.2011.10.032](https://doi.org/10.1016/j.bpj.2011.10.032)
- Walker RA, Salmon ED, Endow SA (1990) The *Drosophila* claret segregation protein is a minus-end directed motor molecule. *Nature* 347:780–782. doi:[10.1038/347780a0](https://doi.org/10.1038/347780a0)
- Weaver LN, Ems-McClung SC, Stout JR, LeBlanc C, Shaw SL, Gardner MK, Walczak CE (2011) Kif18A uses a microtubule binding site in the tail for plus-end localization and spindle length regulation. *Curr Biol* 21:1500–1506. doi:[10.1016/j.cub.2011.08.005](https://doi.org/10.1016/j.cub.2011.08.005)
- White HD, Belknap B, Webb MR (1997) Kinetics of nucleoside triphosphate cleavage and phosphate release steps by associated rabbit skeletal actomyosin, measured using a novel fluorescent probe for phosphate. *Biochemistry* 36:11828–11836. doi:[10.1021/bi970540h](https://doi.org/10.1021/bi970540h)
- Wickstead B, Gull K, Richards TA (2010) Patterns of kinesin evolution reveal a complex ancestral eukaryote with a multifunctional cytoskeleton. *BMC Evol Biol* 10:110. doi:[10.1186/1471-2148-10-110](https://doi.org/10.1186/1471-2148-10-110)
- Woehlke G (2001) A look into kinesin's powerhouse. *FEBS Lett* 508:291–294
- Woehlke G, Ruby AK, Hart CL, Ly B, Hom-Booher N, Vale RD (1997) Microtubule interaction site of the kinesin motor. *Cell* 90:207–216
- Wood KW, Lad L, Luo L, et al (2010) Antitumor activity of an allosteric inhibitor of centromere-associated protein-E. *Proc Natl Acad Sci USA* 107:5839–5844. doi:[10.1073/pnas.0915068107](https://doi.org/10.1073/pnas.0915068107)
- Xiol J, Spinelli P, Laussmann MA, et al (2014) RNA clamping by vasa assembles a piRNA amplifier complex on transposon transcripts. *Cell* 157:1698–1711. doi:[10.1016/j.cell.2014.05.018](https://doi.org/10.1016/j.cell.2014.05.018)
- Yajima J, Mizutani K, Nishizaka T (2008) A torque component present in mitotic kinesin Eg5 revealed by three-dimensional tracking. *Nat Struct Mol Biol* 15:1119–112. doi:[10.1038/nsmb.1491](https://doi.org/10.1038/nsmb.1491)
- Yamada K, Kunishima N, Mayanagi K, et al (2001) Crystal structure of the Holliday junction migration motor protein RuvB from *Thermus thermophilus* HB8. *Proc Natl Acad Sci USA* 98:1442–1447. doi:[10.1073/pnas.031470598](https://doi.org/10.1073/pnas.031470598)
- Yan Y, Sardana V, Xu B, et al (2004) Inhibition of a mitotic motor protein: where, how, and conformational consequences. *J Mol Biol* 335:547–554
- Yardimci H, van Duffelen M, Mao Y, Rosenfeld SS, Selvin PR (2008) The mitotic kinesin CENP-E is a processive transport motor. *Proc Natl Acad Sci USA* 105:6016–6021. doi:[10.1073/pnas.0711314105](https://doi.org/10.1073/pnas.0711314105)
- Yildiz A, Tomishige M, Gennerich A, Vale RD (2008) Intramolecular strain coordinates kinesin stepping behavior along microtubules. *Cell* 134:1030–1041. doi:[10.1016/j.cell.2008.07.018](https://doi.org/10.1016/j.cell.2008.07.018)
- Yildiz A, Tomishige M, Vale RD, Selvin PR (2004) Kinesin walks hand-over-hand. *Science* 303:676–678. doi:[10.1126/science.1093753](https://doi.org/10.1126/science.1093753)
- Yun M, Bronner CE, Park CG, Cha SS, Park HW, Endow SA (2003) Rotation of the stalk/neck and one head in a new crystal structure of the kinesin motor protein, Ncd. *EMBO J* 22:5382–5389. doi:[10.1093/emboj/cdg531](https://doi.org/10.1093/emboj/cdg531)
- Yun M, Zhang X, Park CG, Park HW, Endow SA (2001) A structural pathway for activation of the kinesin motor ATPase. *EMBO J* 20:2611–2618. doi:[10.1093/emboj/20.11.2611](https://doi.org/10.1093/emboj/20.11.2611)
- Zhao YC, Kull FJ, Cochran JC (2010) Modulation of the kinesin ATPase cycle by neck linker docking and microtubule binding. *J Biol Chem* 285:25213–25220. doi:[10.1074/jbc.M110.123067](https://doi.org/10.1074/jbc.M110.123067)
- Zhuravleva A, Clerico EM, Gierasch LM (2012) An interdomain energetic tug-of-war creates the allosterically active state in Hsp70 molecular chaperones. *Cell* 151:1296–1307. doi:[10.1016/j.cell.2012.11.002](https://doi.org/10.1016/j.cell.2012.11.002)
- Zhuravleva A, Gierasch LM (2011) Allosteric signal transmission in the nucleotide-binding domain of 70-kDa heat shock protein (Hsp70) molecular chaperones. *Proc Natl Acad Sci USA* 108:6987–6992. doi:[10.1073/pnas.1014448108](https://doi.org/10.1073/pnas.1014448108)
- Zimmerle CT, Frieden C (1989) Analysis of progress curves by simulations generated by numerical integration. *Biochem J* 258:381–387