

generation by molecular motors The role of thermal activation in motion and force

R. Dean Astumian

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 The role of thermal activation in motion and force generation by molecular motors

R. Dean Astumian

Department of Surgery and Department of Biochemistry and Molecular Biology, MC 6035, University of Chicago, 5841 S. Maryland Ave, Chicago, IL 60637, USA (*dastumia@surgery.bsd.uchicago.edu*)

5841 S. Maryland Ave, Chicago, IL 60637, USA (dastumia@surgery.bsd.uchicago.edu)
The currently accepted mechanism for ATP-driven motion of kinesin is called the hand-over-hand
model where some chemical transition during th The currently accepted mechanism for ATP-driven motion of kinesin is called the hand-over-hand
model, where some chemical transition during the ATP hydrolysis cycle stretches a spring, and motion
and force production resul model, where some chemical transition during the ATP hydrolysis cycle stretches a spring, and motion and force production result from the subsequent relaxation. It is essential in this mechanism for the model, where some chemical transition during the ATP hydrolysis cycle stretches a spring, and motion
and force production result from the subsequent relaxation. It is essential in this mechanism for the
moving head of kine and force production result from the subsequent relaxation. It is essential in this mechanism for the moving head of kinesin to dissociate, while the other head remains firmly attached to the microtubule. Here we propose a moving head of kinesin to dissociate, while the other head remains firmly attached to the microtubule.
Here we propose an alternative Brownian motor model where the action of ATP modulates the inter-
action potential betwe Here we propose an alternative Brownian motor model where the action of ATP modulates the inter-
action potential between kinesin and the microtubule rather than a spring internal to the kinesin
molecule alone. In this mod action potential between kinesin and the microtubule rather than a spring internal to the kinesin
molecule alone. In this model neither head need dissociate (which predicts that under some circumstances
a single-headed kin best described as thermally activated steps. This model is consistent with a wide range of experimental a single-headed kinesin can display processive motion) and the transitions by which the motor moves are
best described as thermally activated steps. This model is consistent with a wide range of experimental
data on the fo best described as thermally activated
data on the force–velocity curves, the
stochastic properties of the stepping. stochastic properties of the stepping.
 Keywords: molecular motors; ion pumps; Brownian ratchets; thermal activation

1. INTRODUCTION

1. **INTRODUCTION**
An important question for understanding biomolecular
actors is whether these proteins are best modelled as motors is whether these proteins are best modelled as
injutive versions of macroscopic deterministic devices In important question for understanding biomolecular
notors is whether these proteins are best modelled as
niniature versions of macroscopic deterministic devices,
r whether they are intrinsically Brownian motors that notors is whether these proteins are best modelled as uniature versions of macroscopic deterministic devices, r whether they are intrinsically Brownian motors that iniature versions of macroscopic deterministic devices,
r whether they are intrinsically Brownian motors that
vork on the very different principle of biasing thermal
oise. A common misconception is that this question boils r whether they are intrinsically Brownian motors that
 ork on the very different principle of biasing thermal

oise. A common misconception is that this question boils

own to whether a conformational change of the motor for to the very different principle of biasing thermal
oise. A common misconception is that this question boils
own to whether a conformational change of the motor
polecule is required for motion, or whether motion takes oise. A common misconception is that this question boils
own to whether a conformational change of the motor
olecule is required for motion, or whether motion takes
lace by simple diffusion of the protein motor as a whole. own to whether a conformational change of the motor nolecule is required for motion, or whether motion takes
lace by simple diffusion of the protein motor as a whole.
This is a red herring—it seems almost certain that some
or formational change is involved. The critical iss lace by simple diffusion of the protein motor as a whole.

This is a red herring—it seems almost certain that some

onformational change is involved. The critical issue is

the conformational change requires thermal actio This is a red herring—it seems almost certain that some onformational change is involved. The critical issue is ϕ the conformational change requires thermal acti-
action with a Poisson-distributed stochastic completion ation, with a Poisson-distributed stochastic completion r elaxation with a deterministic completion time \rightarrow a `power stroke'. axation with a deterministic completion time—a evaluation exponent of the mechanism
To highlight this point, we first consider the mechanism
which an ion pump is able to use chemical or electrical

by ower stroke'.

To highlight this point, we first consider the mechanism

y which an ion pump is able to use chemical or electrical

lnergy to drive transport of ions against an electro-To highlight this point, we first consider the mechanism
y which an ion pump is able to use chemical or electrical
length of ions against an electro-
bemical gradient. It is very well established that this v which an ion pump is able to use chemical or electrical
Jnergy to drive transport of ions against an electro-
phemical gradient. It is very well established that this
wolves a conformational change of the nump protein Internal gradient. It is very well established that this
subveys a conformational change of the pump protein
tween states each of which is close to thermal equilibetween states each of which is close to the pump protein
etween states each of which is close to thermal equili-
rium. Transitions between the states are activated by provides a conformational change of the pump protein
etween states each of which is close to thermal equili-
rium. Transitions between the states are activated by
ermal poise and are well modelled by chemical kinetic etween states each of which is close to thermal equili-
rium. Transitions between the states are activated by
nermal noise, and are well modelled by chemical kinetic
recent work in rium. Transitions between the states are activated by
nermal noise, and are well modelled by chemical kinetic
neory. This picture is able to explain recent work in
thich externally applied oscillating or fluctuating electr not applied on the modelled by chemical kinetic
heavy. This picture is able to explain recent work in
thich externally applied oscillating or fluctuating electric
elds substitute for the energy normally provided by ATP elds substitute is able to explain recent work in
the externally applied oscillating or fluctuating electric
dels substitute for the energy normally provided by ATP
verolysis to drive ion transport hich externally applied oscillatin
dels substitute for the energy nor ydrolysis to drive ion transport.
Lising the concents developed Ids substitute for the energy normally provided by ATP
drolysis to drive ion transport.
Using the concepts developed for ion pumps, we
scribe a simple model for the molecular motors

ydrolysis to drive ion transport.
Using the concepts developed for ion pumps, we escribe a simple model for the molecular motors inesin and Ncd. These motors have a similar structure but se chemical energy from ATP hydrolysis to drive motion

onformational change is involved. The critical issue is characteristics of kinesin: one-to-one stoichiometry,

thether the conformational change requires thermal acti-

and a stopping

ation, with a Poisson-distributed s in opposite directions along microtubules. The model is in opposite directions along microtubules. The model is
based on a 'Brownian ratchet' in which the direction of
motion of the motor is controlled by the chemical in opposite directions along microtubules. The model is
based on a 'Brownian ratchet' in which the direction of
motion of the motor is controlled by the chemical
mechanism of ATP bydrolysis and is an inherent propbased on a 'Brownian ratchet' in which the direction of
motion of the motor is controlled by the chemical
mechanism of ATP hydrolysis and is an inherent prop-
erty of a single head. In contrast to conventional 'nower motion of the motor is controlled by the chemical mechanism of ATP hydrolysis and is an inherent property of a single head. In contrast to conventional 'power stroke' models, dissociation of the individual heads is not mechanism of ATP hydrolysis and is an inherent property of a single head. In contrast to conventional 'power
stroke' models, dissociation of the individual heads is not
obligatory in the chemomechanical cycle, and the steps
during which motion and force generation occur ar stroke' models, dissociation of the individual heads is not
obligatory in the chemomechanical cycle, and the steps
during which motion and force generation occur are best
described as one-dimensional thermally activated tr obligatory in the chemomechanical cycle, and the steps
during which motion and force generation occur are best
described as one-dimensional thermally activated transi-
tions that take place while both heads are attached to during which motion and force generation occur are best
described as one-dimensional thermally activated transi-
tions that take place while both heads are attached to the microtubule. The predictions of this model are tions that take place while both heads are attached to
the microtubule. The predictions of this model are
consistent with all major experimentally observed
characteristics of kinesin: one-to-one stoichiometry the microtubule. The predictions of this model are
consistent with all major experimentally observed
characteristics of kinesin: one-to-one stoichiometry,
maximum velocity of about 1 u m s^{-1} and a stopping consistent with all major experimentally observed
characteristics of kinesin: one-to-one stoichiometry,
maximum velocity of about $1 \mu m s^{-1}$, and a stopping
force of about 5 pN Eurthermore, the thermodynamic characteristics of kinesin: one-to-one stoichiometry,
maximum velocity of about $1 \mu m s^{-1}$, and a stopping
force of about 5 pN. Furthermore, the thermodynamic
efficiency for this Brownian motor can approach unity maximum velocity of about $1 \mu m s^{-1}$, and a stopping
force of about 5 pN. Furthermore, the thermodynamic
efficiency for this Brownian motor can approach unity,
even at finite velocity. We also discuss how in singleefficiency for this Brownian motor can approach unity, even at finite velocity. We also discuss how in singleefficiency for this Brownian motor can approach unity,
even at finite velocity. We also discuss how in single-
molecule experiments the variance of the distance
moved in a given time is expected to depend on conceneven at finite velocity. We also discuss how in single-
molecule experiments the variance of the distance
moved in a given time is expected to depend on concen-
tration of fuel ATP and compare this model with the molecule experiments the variance of the distance
moved in a given time is expected to depend on concen-
tration of fuel, ATP, and compare this model with the
observed behaviour of kinesin moved in a given time is expected to depend on concentration of fuel, ATP, and compare this model with the observed behaviour of kinesin.

2. THERMAL NOISE AND ACTIVATION OVER ENERGY BARRIERS

A particle in solution is subject to random collisions **EXEMPLE SOFTLE SOF** A particle in solution is subject to random collisions
with solvent molecules giving rise to the erratic
'Brownian' motion first observed and reported by Robert
Rrown in 1826. This dynamic behaviour was described with solvent molecules giving rise to the erratic

"Brownian" motion first observed and reported by Robert

Brown in 1826. This dynamic behaviour was described

theoretically by I angevin, who hypothesized that the 'Brownian' motion first observed and reported by Robert
Brown in 1826. This dynamic behaviour was described
theoretically by Langevin, who hypothesized that the
forces on the particle due to the solvent can be split into Brown in 1826. This dynamic behaviour was described
theoretically by Langevin, who hypothesized that the
forces on the particle due to the solvent can be split into
two components: (i) a fluctuating force that changes theoretically by Langevin, who hypothesized that the forces on the particle due to the solvent can be split into two components: (i) a fluctuating force that changes magnitude and direction very frequently compared to any forces on the particle due to the solvent can be split into
two components: (i) a fluctuating force that changes
magnitude and direction very frequently compared to any other time-scale of the system; and (ii) a viscous drag

Force that always acts to slow the motion induced by the
uctuation term. Einstein derived a (fluctuation-dissinathat always acts to slow the motion induced by the uctuation term. Einstein derived a (fluctuation-dissipa-
(on) relationship between the magnitude of the fluctuaorce that always acts to slow the motion induced by the uctuation term. Einstein derived a (fluctuation-dissipa-
ion) relationship between the magnitude of the fluctua-
ion term and the viscous drag coefficient that damnen uctuation term. Einstein derived a (fluctuation-dissipation) relationship between the magnitude of the fluctuation term and the viscous drag coefficient that dampens s effect. Because the strength of the fluctuation increa ion) relationship between the magnitude of the fluctuation term and the viscous drag coefficient that dampens
is effect. Because the strength of the fluctuation increases ion term and the viscous drag coefficient that dampens
s effect. Because the strength of the fluctuation increases
ith temperature, the fluctuating force is often called
hermal noise. If the particle is a molecule bombardm is effect. Because the strength of the fluctuation increases
ith temperature, the fluctuating force is often called
hermal noise. If the particle is a molecule, bombardment
is well also allows exploration of the different The solvent also allows exploration of the different
of the solvent also allows exploration of the different
of the different
of the different
of the arrangements of the hermal noise. If the particle is a molecule, bombardment
y the solvent also allows exploration of the different
plotcular configurations, i.e. the arrangements of the
toms of the molecule relative to each other. Biological y the solvent also allows exploration of the different
abecular configurations, i.e. the arrangements of the
toms of the molecule relative to each other. Biological
and many other) macromolecules often have only a few obecular configurations, i.e. the arrangements of the total to the molecule relative to each other. Biological
and many other) macromolecules often have only a few
check configurations called conformations with large toms of the molecule relative to each other. Biological
and many other) macromolecules often have only a few
cable configurations, called conformations, with large and many other) macromolecules often have only a few able configurations, called conformations, with large nergy barriers separating them. Thermal noise 'activates' transitions of the configurations, called conformations, with large
 Exercy barriers separating them. Thermal noise 'activates'
 Exercise barriers, allowing passage from one
 Position to another Almost all chemical r - cansitions over these barriers, allowing passage from one cells, and for using the metabolic energy of ATP hydro-

another. Almost all chemical reaction lysis to form the Na and K ion gradients rapidly depleted

during t \Box onformation to another. Almost all chemical reaction Information to another. Almost all chemical reaction
athways are described in terms of rate constants that
 $\sum_{n=1}^{\infty}$ exists the probability that thermal noise will provide
 $\sum_{n=1}^{\infty}$ existing the surmount partiers subsequently a
subsequently that thermal noise will provide
the probability that thermal noise will provide
the provide infinity of surmount barriers separating
the price of surmount barriers separating ecify the prob.

Ifficient energy

Internal states.

Despite sharing metricant energy to surmount barriers separating
emical states.
Despite sharing the similar function of using chemical
ergy to drive vectorial transport the effect of thermal

Dhemical states.

Despite sharing the similar function of using chemical

nergy to drive vectorial transport, the effect of thermal

oise on molecular motors and numes is typically Despite sharing the similar function of using chemical
nergy to drive vectorial transport, the effect of thermal
oise on molecular motors and pumps is typically
enited from entirely different standpoints. Molecular nergy to drive vectorial transport, the effect of thermal
oise on molecular motors and pumps is typically
epicted from entirely different standpoints. Molecular oise on molecular motors and pumps is typically
epicted from entirely different standpoints. Molecular
jumps are most often modelled in terms of chemical
inetics where ATP energy is used to change the relative epicted from entirely different standpoints. Molecular

Jumps are most often modelled in terms of chemical

inetics, where ATP energy is used to change the relative

finities of and barrier heights between different bindin are most often modelled in terms of chemical
inetics, where ATP energy is used to change the relative
finities of and barrier heights between different binding
its by sequentially favouring different conformational inetics, where ATP energy is used to change the relative
finities of and barrier heights between different binding
ites by sequentially favouring different conformational
ates of the protein as ATP is bound bydrolysed and finities of and barrier heights between different binding
its by sequentially favouring different conformational
ates of the protein as ATP is bound, hydrolysed, and the
reducts released. The conformational relaxation and ites by sequentially favouring different conformational
ates of the protein as ATP is bound, hydrolysed, and the
roducts released. The conformational relaxation and
plecular transport across the membrane are treated as ates of the protein as ATP is bound, hydrolysed, and the roducts released. The conformational relaxation and indecular transport across the membrane are treated as hermally activated steps roducts released. The conducts remains across
hermally activated steps.
Models for molecular mo blecular transport across the membrane are treated as
ermally activated steps.
Models for molecular motors, on the other hand, have
cused on an ATP-driven 'power stroke' a viscoelastic

formally activated steps.
Models for molecular motors, on the other hand, have
ocused on an ATP-driven 'power stroke', a viscoelastic
elaxation process where the protein starts from a non-Models for molecular motors, on the other hand, have
beyond on an ATP-driven 'power stroke', a viscoelastic
elaxation process where the protein starts from a non-
quilibrium 'strained' conformation following product equilibrium, and ATP-driven 'power stroke', a viscoelastic elaxation process where the protein starts from a non-
quilibrium, 'strained' conformation following product
elease. The subsequent relaxation does not require elaxation process where the protein starts from a non-
quilibrium, 'strained' conformation following product
elease. The subsequent relaxation does not require
hermal activation and can be visualized much as the quilibrium, 'strained' conformation following product
elease. The subsequent relaxation does not require
hermal activation and can be visualized much as the
outraction of a stretched rubber band. In many ways elease. The subsequent relaxation does not require
hermal activation and can be visualized much as the
ontraction of a stretched rubber band. In many ways
rotein motors have been modelled as miniature versions hermal activation and can be visualized much as the ontraction of a stretched rubber band. In many ways
rotein motors have been modelled as miniature versions
f macroscopic devices, employing springs, cogs, levers,
nd the like, to effect motion and force generation, where rotein motors have been modelled as miniature versions
f macroscopic devices, employing springs, cogs, levers,
nd the like, to effect motion and force generation, where
he inescanable molecular fluctuations arising from in f macroscopic devices, employing springs, cogs, levers,

and the like, to effect motion and force generation, where

the inescapable molecular fluctuations arising from inter-

ction with the medium are viewed as a nuisanc and the like, to effect motion and force generation, where
the inescapable molecular fluctuations arising from inter-
ction with the medium are viewed as a nuisance to be
vercome rather than as an essential feature that c be inescapable molecular fluctuations arising from inter-
ction with the medium are viewed as a nuisance to be
vercome rather than as an essential feature that can be
arnessed to allow for regulation of the timing between ction with the medium are viewed as a nuisance to be
vercome rather than as an essential feature that can be
arnessed to allow for regulation of the timing between
hemical and mechanical steps vercome rather than as an essex
arnessed to allow for regulation
hemical and mechanical steps.
At first it may seem that the hemical and mechanical steps.
At first it may seem that the mechanism for using

hemical and mechanical steps.

At first it may seem that the mechanism for using

hemical energy to allow molecular motors to move

ver great distances and exert large forces must indeed At first it may seem that the mechanism for using
hemical energy to allow molecular motors to move
ver great distances and exert large forces must indeed
the fundamentally different from the way that molehemical energy to allow molecular motors to move
ver great distances and exert large forces must indeed
be fundamentally different from the way that mole-The set of the set of th endamentally different from the way that mole-

a membrane and use

energy from ATP hydrolysis to bias the diffusion of

and molecules and ions and do work against an elecular pumps sit in place in a membrane and use
hergy from ATP hydrolysis to bias the diffusion of
nall molecules and ions, and do work against an elec-
cochemical gradient. However, the physics of motion holds are in ATP hydrolysis to bias the diffusion of nall molecules and ions, and do work against an electrochemical gradient. However, the physics of motion f small things in viscous solution (low Reynolds) nall molecules and ions, and do work against an elec-
cochemical gradient. However, the physics of motion
f small things in viscous solution (low Reynolds
umber motion) shows that these processes may not be rochemical gradient. However, the physics of motion

f small things in viscous solution (low Reynolds

umber motion) shows that these processes may not be

s different as our macroscopically based intuition \int f small things in viscous solution (low Reynolds
umber motion) shows that these processes may not be
solifferent as our macroscopically based intuition
and suggest and that perhaps the functions of moleumber motion) shows that these processes may not be
solutions of mole-
and suggest and that perhaps the functions of mole-
alar motors and numns share a common mechanism s different as our macroscopically based intuition
by a common share a common mechanism.
clue that work on 'Brownian ratchets' (Astumian & Bier Ω rould suggest and that perhaps the functions of mole-
ular motors and pumps share a common mechanism.
'ecent work on 'Brownian ratchets' (Astumian & Bier
994: Astumian 1997: Hänggi & Bartussek 1996: ular motors and pumps share a common mechanism.

1994; Astumian 1997; Hänggi & Bartussek 1996; lecent work on 'Brownian ratchets' (Astumian & Bier 994; Astumian 1997; Hänggi & Bartussek 1996; ülicher *et al.* 1997; Prost *et al.* 1994) may provide the nifving link 994; Astumia
ülicher *et al*. 1
nifying link. *Phil. Trans. R. Soc. Lond.* B (2000)

3. BROWNIAN RATCHETS AND ION PUMPS

Perhaps the strongest direct evidence for a ratchet S. BROWNIAN RATCHETS AND TON PUMPS
Perhaps the strongest direct evidence for a ratchet
mechanism for free energy transduction by a biomolecule
comes from recent experiments showing that the Na K-Perhaps the strongest direct evidence for a ratchet
mechanism for free energy transduction by a biomolecule
comes from recent experiments showing that the Na,K-
ATPase a biomolecular ion nump can use an external mechanism for free energy transduction by a biomolecule
comes from recent experiments showing that the Na,K-
ATPase, a biomolecular ion pump can use an external
oscillating (Liu *et al.* 1990) or randomly fluctuating (Xie comes from recent experiments showing that the Na,K-
ATPase, a biomolecular ion pump can use an external
oscillating (Liu *et al.* 1990) or randomly fluctuating (Xie
et al. 1994–1997) electric field to drive unidirection *et* ATPase, a biomolecular ion pump can use an external oscillating (Liu *et al.* 1990) or randomly fluctuating (Xie *et al.* 1994, 1997) electric field to drive unidirectional transport transport. *et al.* 1994, 1997) electric field to drive unidirectional transport.
Much work has been done on characterization of the

transport.

Much work has been done on characterization of the

Na,K-ATPase pump (Skou 1957; Läuger 1990). This

enzyme is found in almost all mammalian cells, and is Much work has been done on characterization of the
Na,K-ATPase pump (Skou 1957; Läuger 1990). This
enzyme is found in almost all mammalian cells, and is
important in the maintenance of the osmotic balance of enzyme is found in almost all mammalian cells, and is important in the maintenance of the osmotic balance of enzyme is found in almost all mammalian cells, and is
important in the maintenance of the osmotic balance of
cells, and for using the metabolic energy of ATP hydro-
lysis to form the Na and K ion gradients rapidly denlete important in the maintenance of the osmotic balance of
cells, and for using the metabolic energy of ATP hydro-
lysis to form the Na and K ion gradients rapidly depleted
during the action potential in excitable cells. Much cells, and for using the metabolic energy of ATP hydro-
lysis to form the Na and K ion gradients rapidly depleted
during the action potential in excitable cells. Much of the
modelling of the data has revolved around refine modelling of the data has revolved around refinement of during the action potential in excitable cells. Much of the modelling of the data has revolved around refinement of a kinetic mechanism first proposed by Albers (1967) and Post (1989). The essential feature of this mechani modelling of the data has revolved around refinement of
a kinetic mechanism first proposed by Albers (1967) and
Post (1989). The essential feature of this mechanism is the
idea that the pump can assume two principal confor a kinetic mechanism first proposed by Albers (1967) and
Post (1989). The essential feature of this mechanism is the
idea that the pump can assume two principal conforma-
tions E. (with inward facing ion binding sites) and Post (1989). The essential feature of this mechanism is the
idea that the pump can assume two principal conforma-
tions, E_1 (with inward facing ion binding sites). E_1 has a high
(with outward facing ion binding site tions, E_1 (with inward facing ion binding sites) and E_2
(with outward facing ion binding sites). E_1 has a high
affinity for Na⁺ and/or ATP and is stabilized by these
ligands, while E, has a high affinity for K (with outward facing ion binding sites). E_1 has a high
affinity for Na⁺ and/or ATP and is stabilized by these
ligands, while E_2 has a high affinity for K⁺ and/or
inorganic phosphate (Pi) and is stabilized by the affinity for Na⁺ and/or ATP and is stabilized by these ligands, while E_2 has a high affinity for K^+ and/or ligands. inorganic phosphate (Pi) and is stabilized by these ligands.
Läuger (1990) has proposed a simple four-state minimal

ligands.

Läuger (1990) has proposed a simple four-state minimal

mechanism for the similar (but simpler) p-type proton

ATPase shown in figure 1h illustrating this principle. A key Läuger (1990) has proposed a simple four-state minimal
mechanism for the similar (but simpler) p-type proton
ATPase shown in figure 1*b* illustrating this principle. A key
feature is that phosphorylation-dephosphorylation mechanism for the similar (but simpler) p-type proton
ATPase shown in figure $1b$ illustrating this principle. A key
feature is that phosphorylation-dephosphorylation of the
enzyme serves to switch the protein between the ATPase shown in figure 1 b illustrating this principle. A key feature is that phosphorylation-dephosphorylation of the enzyme serves to switch the protein between the two conformational states shown in ¢gure 1*a*. In the enzyme serves to switch the protein between the two
conformational states shown in figure $1a$. In the
phosphorylated state, the enzyme binds proton tightly,
with easy access to the binding site from the outside (left) conformational states shown in figure 1*a*. In the phosphorylated state, the enzyme binds proton tightly, with easy access to the binding site from the outside (left). In the dephosphorylated state proton binds much more phosphorylated state, the enzyme binds proton tightly,
with easy access to the binding site from the outside (left).
In the dephosphorylated state proton binds much more
weakly and access is easiest from the inside (right) with easy access to the binding site from the outside (left).
In the dephosphorylated state proton binds much more
weakly, and access is easiest from the inside (right). This
picture also explains how an external perturbat In the dephosphorylated state proton binds much more
weakly, and access is easiest from the inside (right). This
picture also explains how an external perturbation can
drive directed transport, even without energy from ATP weakly, and access is easiest from the inside (right). This
picture also explains how an external perturbation can
drive directed transport, even without energy from ATP
hydrolysis hydrolysis. If the enzyme is caused by an external field to alter-
If the enzyme is caused by an external field to alter-

hydrolysis.
If the enzyme is caused by an external field to alternate between the E and E^{*} states sufficiently slowly, the
system seeks its lowest free energy in each state—proton If the enzyme is caused by an external field to alternate between the E and E^* states sufficiently slowly, the system seeks its lowest free energy in each state—proton bound in the E^* nate between the E and E^* states sufficiently slowly, the system seeks its lowest free energy in each state—proton bound in the E^* state. The most likely path is that which presents the system seeks its lowest free energy in each state—proton
bound in the E state and proton not bound in the E^*
state. The most likely path is that which presents the bound in the E state and proton not bound in the E^*
state. The most likely path is that which presents the
lowest energy barrier—binding from the exterior in the
E state and release to the cytosol in the E^* state. T state. The most likely path is that which presents the lowest energy barrier—binding from the exterior in the E* state. The net E* state and release to the cytosol in the E* state. The net result is that on average one pro lowest energy barrier—binding from the exterior in the E^* state. The net result is that on average, one proton is pumped across the membrane for each cycle of the field if the proton E state and release to the cytosol in the E^* state. The net result is that on average, one proton is pumped across the membrane for each cycle of the field if the proton electrochemical gradient is not too big. As the result is that on average, one proton is pumped across
the membrane for each cycle of the field if the proton
electrochemical gradient is not too big. As the frequency
increases, the number of protons pumped per unit time the membrane for each cycle of the field if the proton increases. At very large frequencies, however, the conforincreases, the number of protons pumped per unit time
increases. At very large frequencies, however, the confor-
mational transition $E = E^*$ cannot keep up and the
number rate decreases with further increase in the increases. At very large frequencies, however, the conformational transition $E \rightleftharpoons E^*$ cannot keep up and the pumping rate decreases with further increase in the frequency frequency. mping rate decreases with further increase in the
equency.
The way that we have drawn the mechanism in figure
implies that proton transport is completely coupled to

frequency.

The way that we have drawn the mechanism in figure

1*b* implies that proton transport is completely coupled to

ATP bydrolysis. This is, of course, only an approxima-The way that we have drawn the mechanism in figure $1b$ implies that proton transport is completely coupled to ATP hydrolysis. This is, of course, only an approximation and in principle it is always possible (though $1b$ implies that proton transport is completely coupled to ATP hydrolysis. This is, of course, only an approximation, and in principle it is always possible (though perhaps not likely) for proton to leak across the ATP hydrolysis. This is, of course, only an approximation, and in principle it is always possible (though perhaps not likely) for proton to leak across the membrane through the protein without hydrolysis of tion, and in principle it is always possible (though
perhaps not likely) for proton to leak across the
membrane through the protein without hydrolysis of
ATP or for ATP to be hydrolysed without numning a perhaps not likely) for proton to leak across the
membrane through the protein without hydrolysis of
ATP, or for ATP to be hydrolysed without pumping a
proton. We can see the connection between this type of membrane through the protein without hydrolysis of alternating access model for membrane transport and a

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Figure 1. Ratchet model for ion transport by a molecular pump. (*a*) Cartoon illustration of a protein with two conformational states—one with a high affinity and easy access from the left (exterior), and one with low affinity and easy access from the right Figure 1. Ratchet model for ion transport by a molecular pump. (a) Cartoon illustration of a protein with two conformational attaction in the right state is the enzyme of the enzyme.
The systeplasm side). Switching betwee The intervals of a high affinity and easy access from the left (exterior), and one with low affinity and easy access from

a proportion side). Switching between the two conformations is induced by phosphorylation-dephospho $\begin{pmatrix} \text{c}^{\text{c}} \text{y}^{\text{c}} \text{y}$ b) How this can be incorporated into a four-state mechanism for active proton transport driven by ATP hydrolysis.
 σ) Illustration of a more general model for the proton transporter that includes slip transitions. As e between the proton transporter that includes slip transitions. As explained in the text, the
 Ω referred pathway is controlled either by switching the for binding ATP and releasing Pi depending on whether the proton

i Free particles is ontrolled either by switching the for binding ATP and releasing Pi depending on whether the proton inding site is occupied, or by using differences in the affinities for proton binding in the two states s inding site is occup
re slow compared
ne high barriers.

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 $\frac{1}{2}$
in the ratchet by rewriting the mechanism in figure
be to explicitly incorporate the possibility of a leak as Frownian ratchet by rewriting the mechanism in figure $\frac{1}{b}$ to explicitly incorporate the possibility of a leak, as nown in figure $\frac{1}{c}$. Here, we have written all the Frownian ratchet by rewriting the mechanism in figure \bar{b} to explicitly incorporate the possibility of a leak, as nown in figure 1 c . Here, we have written all the hemical transitions along the vertical axis and the $\frac{b}{c}$ to explicitly incorporate the possibility of a leak, as nown in figure 1c. Here, we have written all the hemical transitions along the vertical axis, and the cansitions in which proton moves across the membrane hown in figure 1 c . Here, we have written all the hemical transitions along the vertical axis, and the cansitions in which proton moves across the membrane a the horizontal axis. This emphasizes the fact that the hemical transitions along the vertical axis, and the ransitions in which proton moves across the membrane
n the horizontal axis. This emphasizes the fact that the wo processes are *a priori* independent, and that coupling is mediated by the conformational switching of the conformational switching of the conformational switching of the rotein between two states with different affinities and wo processes are *a priori* independent, and that coupling
inediated by the conformational switching of the
rotein between two states with different affinities and
cess. The mechanism in figure 16 is a Brownian access. The mechanism in the mechanism in the mechanism in $\frac{1}{2}$ is a Brownian atchet. The mechanism in figure 1*c* is a Brownian atchet. The protein conformational changes are driven ccess. The mechanism in figure $1c$ is a Brownian let
atchet. The protein conformational changes are driven
y ATP hydrolysis, but the transition of the proton from
ulk solution to the binding site requires thermal activaatchet. The protein conformational changes are driven
y ATP hydrolysis, but the transition of the proton from
ulk solution to the binding site requires thermal activa-
on over an energy barrier y ATP hydrolysis, but the tr
ulk solution to the binding s
on over an energy barrier.
The preferred (coupled) pa In the preferred (coupled) pathway is shown as the white
The preferred (coupled) pathway is shown as the white

on over an energy barrier.
The preferred (coupled) pathway is shown as the white
igzag, and follows the same sequence of states as the four-
rate cycle in figure 1b. In order to achieve tight coupling it The preferred (coupled) pathway is shown as the white igzag, and follows the same sequence of states as the four-
tate cycle in figure 1*b*. In order to achieve tight coupling it
the recessary for two 'rules' to be follow igzag, and follows the same sequence of states as the four-
ate cycle in figure 1*b*. In order to achieve tight coupling it
incressary for two 'rules' to be followed (Jencks 1989*a*).
irst the binding of proton from the ex First, the binding of proton from the external solution in
its, the binding of proton from the external solution in
the external solution in
the external solution in
the external solution in
the First compared to the phos irst, the binding of proton from the external solution in \mathbf{F} are E state must be fast compared to the phosphorylation
f the enzyme by ATP, and second, the dissociation of
a ound proton to the inside must be faster than release of
a paranic phosphate in the $\mathbf{F}^* \mathbf{P}$ inorganic phosphate in the E^*P state.
In One way this can be achieved is for the transition und proton to the inside must be faster than release of
organic phosphate in the E^*P state.
One way this can be achieved is for the transition
tween the E and E^* states to be slow compared to the

forganic phosphate in the E^*P state.
One way this can be achieved is for the transition
etween the E and E^* states to be slow compared to the
longing of the proton over the low energy barrier, but The way this can be achieved is for the transition
etween the E and E^* states to be slow compared to the
opping of the proton over the low energy barrier, but
wet compared to bopping of the proton over the higher Fast compared to the Left and E^* states to be slow compared to the opping of the proton over the higher state compared to hopping of the proton over the higher arrier. This situation can be achieved only if there is a beging of the proton over the low energy barrier, but
situation can be achieved only if there is a
arrier. This situation can be achieved only if there is a
arrea difference in proton binding energy (affinity) Last compared to hopping of the proton over the higher arrier. This situation can be achieved only if there is a tree difference in proton binding energy (affinity) arrier. This situation can be

expected in proton

etween the E and E* states.

A second possibility is the

A second possibility is the control of the chemical secificity of the reactions by allosteric interactions etween the protein and its ligands. If the protein can be A second possibility is the control of the chemical
pecificity of the reactions by allosteric interactions
etween the protein and its ligands. If the protein can be
phosphorylated by ATP (or transfer Pi to ADP) only pecificity of the reactions by allosteric interactions
 \geq etween the protein and its ligands. If the protein can be
 \geq hosphorylated by ATP (or transfer Pi to ADP) only

then the proton binding site is occupied, a etween the protein and its ligands. If the protein can be
binding site is occupied, and can be
chosen box transfer of P_i to water (or phosen hosphorylated by ATP (or transfer Pi to ADP) only
then the proton binding site is occupied, and can be
ephosphorylated by transfer of Pi to water (or phos-
horylated by Pi from water) only when the proton ephosphorylated by transfer of Pi to water (or phos-
horylated by Pi from water) only when the proton
inding site is unoccupied, essentially complete coupling
f proton transfer to ATP hydrolysis occurs horylated by Pi from water) only when
inding site is unoccupied, essentially compl
f proton transfer to ATP hydrolysis occurs. *F* proton transfer to ATP hydrolysis occurs.
hil. Trans. R. Soc. Lond. B (2000)

rotein between two states with different affinities and figure $1c$, but the number of transitions from the upper ccess. The mechanism in figure $1c$ is a Brownian left corner to the lower right corner would exactly equal Both of these mechanisms for enforcing a sequential
estic pathway can be achieved by purely structural Both of these mechanisms for enforcing a sequential
kinetic pathway can be achieved by purely structural
features of the enzyme—no continual energy input is kinetic pathway can be achieved by purely structural features of the enzyme—no continual energy input is kinetic pathway can be achieved by purely structural
features of the enzyme—no continual energy input is
required. But these considerations only provide a
preferred pathway and not directionality. If the proton features of the enzyme—no continual energy input is
required. But these considerations only provide a
preferred pathway, and not directionality. If the proton
electrochemical gradient would be zero, and the ATP required. But these considerations only provide a
preferred pathway, and not directionality. If the proton
electrochemical gradient would be zero, and the ATP
hydrolysis reaction at equilibrium, most of the kinetic preferred pathway, and not directionality. If the proton
electrochemical gradient would be zero, and the ATP
hydrolysis reaction at equilibrium, most of the kinetic electrochemical gradient would be zero, and the ATP
hydrolysis reaction at equilibrium, most of the kinetic
traffic would indeed be along the zigzag white path in
figure 10 but the number of transitions from the unner hydrolysis reaction at equilibrium, most of the kinetic
traffic would indeed be along the zigzag white path in
figure *Lc*, but the number of transitions from the upper
left corner to the lower right corner would exactly e traffic would indeed be along the zigzag white path in figure $1c$, but the number of transitions from the upper left corner to the lower right corner would exactly equal the number of transitions from the lower right cor figure $1c$, but the number of transitions from the upper left corner to the lower right corner would exactly equal the number of transitions from the lower right corner to left corner to the lower right corner would exactly equal
the number of transitions from the lower right corner to
the upper left corner. The directionality is specified by
the signs of the chemical and osmotic free energi the number of transitions from the lower right corner to
the upper left corner. The directionality is specified by
the signs of the chemical and osmotic free energies—if
the ΔG for ATP bydrolysis is greater than the el the upper left corner. The directionality is specified by
the signs of the chemical and osmotic free energies—if
the ΔG for ATP hydrolysis is greater than the electro-
chemical potential of proton, there will be more t the signs of the chemical and osmotic free energies—if
the ΔG for ATP hydrolysis is greater than the electro-
chemical potential of proton, there will be more transithe ΔG for ATP hydrolysis is greater than the electro-
chemical potential of proton, there will be more transi-
tions from upper left to lower right, and ATP-driven
numning of proton chemical potential of
tions from upper let
pumping of proton.
Because the indiv In the individual steps of ATP-driven
Because the individual steps of ATP hydrolysis are
chastic it has long been held that strictly regulated

the individual steps of ATP hydrolysis are

irst, the binding of proton from the external solution in

its, the binding of proton from the external solution in

its, it has long been held that strictly regulated

its are E pumping of proton.
Because the individual steps of ATP hydrolysis are
stochastic, it has long been held that strictly regulated
coupling between the chemical events of ATP hydrolysis Because the individual steps of ATP hydrolysis are
stochastic, it has long been held that strictly regulated
coupling between the chemical events of ATP hydrolysis
and mechanical events of ion transport is essential for th stochastic, it has long been held that strictly regulated function of an ion pump (Jencks 1989*b*). Allosteric interand mechanical events of ion transport is essential for the
function of an ion pump (Jencks 1989b). Allosteric inter-
actions between the protein and ligands could ensure that
neither ATP hydrolysis nor transport can be co function of an ion pump (Jencks 1989b). Allosteric inter-
actions between the protein and ligands could ensure that
neither ATP hydrolysis nor transport can be completed
without the other process occurring resulting in a s actions between the protein and ligands could ensure that
neither ATP hydrolysis nor transport can be completed
without the other process occurring, resulting in a strictly
ordered sequential kinetic mechanism neither ATP hydrolysis nor transport can be completed
without the other process occurring, resulting in a strictly
ordered sequential kinetic mechanism. thout the other process occurring, resulting in a strictly
dered sequential kinetic mechanism.
The rigid requirements for such clock-like coupling have
cently been challenged by experiments of Topg and

phorylated by transfer of Pi to water (or phos-
horylated by Pi from water) only when the proton the sexternal fields are able to drive significant
horylated by Pi from water) only when the proton theless, these external f ordered sequential kinetic mechanism.
The rigid requirements for such clock-like coupling have
recently been challenged by experiments of Tsong and
colleagues (Liu et al. 1990: Xie et al. 1994) on Na K-The rigid requirements for such clock-like coupling have
recently been challenged by experiments of Tsong and
colleagues (Liu *et al.* 1990; Xie *et al.* 1994) on Na,K-
ATPase In these experiments ATP hydrolysis is suppres recently been challenged by experiments of Tsong and
colleagues (Liu et al. 1990; Xie et al. 1994) on Na.Kcolleagues (Liu *et al.* 1990; Xie *et al.* 1994) on Na,K-ATPase. In these experiments ATP hydrolysis is suppressed (either by low temperature or by depletion of ATP concentration) and energy for uphill transport provided ATPase. In these experiments ATP hydrolysis is suppressed

(either by low temperature or by depletion of ATP concentration) and energy for uphill transport provided by exter-

rally, applied, oscillating, or, fluctuating, (either by low temperature or by depletion of ATP concentration) and energy for uphill transport provided by externally applied oscillating or fluctuating electric fields.
Because the fields are external, there is no mecha tration) and energy for uphill transport provided by externally applied oscillating or fluctuating electric fields.
Because the fields are external, there is no mechanism
whatsoever for control of the timing of an electric pulse by
the occupancy of the ion binding site of the pro Because the fields are external, there is no mechanism
whatsoever for control of the timing of an electric pulse by
the occupancy of the ion binding site of the protein. Never-
theless, these external fields are able to dr whatsoever for control of the timing of an electric pulse by
the occupancy of the ion binding site of the protein. Never-
theless, these external fields are able to drive significant
upped the significant
upped to drive si the occupancy of the ion binding site of the protein. Nevertheless, these external fields are able to drive significant
uphill transport. This has been described in terms of a
mechanism known as electroconformational coupl theless, these external fields are able to drive significant
uphill transport. This has been described in terms of a
mechanism known as electroconformational coupling

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Figure 2. Ion pumping by the Na,K-ATPase. (*a*) Electrostatic model for Na,K-ATPase (Wuddel & Apell 1995). In the ATPriven coupled cycle, the step $P-E_2Na_3 \rightleftharpoons P-E_2Na_2$ is the most electrogenic, while $P-E_2Na_2 \rightleftharpoons P-E_2$ and $P-E_2 \rightleftharpoons P-E_2K_2$ are less igure 2. Ion pumping by the Na,K-ATPase. (a) Electrostatic model for Na,K-ATPase (Wuddel & Apell 1995). In the ATP-
riven coupled cycle, the step P-E₂Na₃ \rightleftharpoons P-E₂Na₂ is the most electrogenic, while P-E₂Na₂ riven coupled cycle, the step P-E₂Na₃ \rightleftharpoons P-E₂Na₂ is the most electrogenic, while P-E₂Na₂ \rightleftharpoons P-E₂ and P-E₂-E₂K₂ are less
lectrogenic, and E₁ \rightleftharpoons E₁K₂ and E₁ \rightleftharpoons E₁Na₂ are not e lectrogenic, and $E_1 \rightleftharpoons E_1K_2$ and $E_1 \rightleftharpoons E_1Na_2$ are not electrogenic, indicating that the access channel for E_2 is more resistive t
at for E_1 . The transition $E_1Na_2 \rightleftharpoons E_1Na_3$ is moderately electrogenic, at for E₁. The transition $E_1Na_2 \rightleftharpoons E_1Na_3$ is moderately electrogenic, showing that the binding sites for Na are not equivalen

The net transition $P-E_2 \rightarrow P-E_2K_2 \rightarrow E(K_2) \rightarrow E_1K_2 \rightarrow E_1$, in which two K are transporte lectrogenic, suggesting that the binding site itself bears a charge of -2 . The direct transition $E_1 \rightleftharpoons P-E_2$, while not directly ccessible to measurement using the technique of Wuddel & Apell, is predicted to be very strongly electrogenic. (b) Data
nowing the effect of an AC electric field on the ion transport modes of the Na,K-ATPase (Liu *et al.* nowing the effect of an AC electric field on the ion transport modes of the Na,K-ATPase (Liu et al. 1990) where Rb^+ and Na⁺ data as predicted by a nonlinear extension of relaxation kinetic theory (Robertson & Astumian 1991).

(Tsong & Astumian 1986). The key feature of this ypothesis is that the field alters the relative energy Isong & Astumian 1986). The key feature of this
pothesis is that the field alters the relative energy
are set of the different conformational states of the
potein thus enforcing an external switching between prothesis is that the field alters the relative energy
protein, thus enforcing an external switching between
the two states in figure 1 even without phosphorylation The two states in the different conformational states of the rotein, thus enforcing an external switching between
two states in figure 1 even without phosphorylation.
The rate of movement of ions across the membrane otein, thus enforcing an external switching between
e two states in figure I even without phosphorylation.
The rate of movement of ions across the membrane
duced by the AC electric field is independent of ATP

The two states in figure 1 even without phosphorylation.

The rate of movement of ions across the membrane

and duced by the AC electric field is independent of ATP

and the frequency ω of the The rate of movement of ions across the membrane
duced by the AC electric field is independent of ATP
oncentration, but does depend on the frequency ω of the
led as shown in figure 2*b* where the solid lines are the reduced by the AC electric field is independent of ATP oncentration, but does depend on the frequency ω of the eld as shown in figure 2*b*, where the solid lines are the oncentration, but does depend on the frequency ω of the
eld as shown in figure 2*b*, where the solid lines are the
t curves calculated from an extension of relaxation
inetic theory (Robertson $\&$ Astumian 1991). The n eld as shown in figure 2b, where the solid lines are the t curves calculated from an extension of relaxation inetic theory (Robertson & Astumian 1991). The net cannot was in the direction stimulated by \triangle TP t curves calculated from an extension of relaxation
inetic theory (Robertson & Astumian 1991). The net
ransport was in the direction stimulated by ATP
 α and the direction stimulated by ATP inetic theory (Robertson & Astumian 1991). The net

ransport was in the direction stimulated by ATP

) ydrolysis *in vivo* in both cases, and from low to high

oncentration under the experimental conditions This can
sport was in the direction stimulated by ATP \bigcup ydrolysis in vivo in both cases, and from low to high
oncentration under the experimental conditions. This
chaviour can be understood in terms of the recently behaviour can be understood in terms of the recently
concentration under the experimental conditions. This
chaviour can be understood in terms of the recently
conosed electrostatic model of the N₃ K-ATPase shown e haviour can be understood in terms of the recently Iechanistically the effect of the field can be interpreted

as stimulation of non-canonical flux modes of the enzyme, slip cycles that operate when either Na^+ or K^+ as stimulation of non-canonical flux modes of the enzyme, slip cycles that operate when either Na^+ or K^+ are omitted from the medium. The energy from the field drives the ^{slip}} cycles in a direction opposite to t enzyme, slip cycles that operate when either Na^+ or K^+
are omitted from the medium. The energy from the field
drives the 'slip' cycles in a direction opposite to that
predicted based on the chemical driving force of are omitted from the medium. The energy from the field
drives the 'slip' cycles in a direction opposite to that
predicted based on the chemical driving force of the
cycle The 'slin' transitions are shown as dashed arrows drives the 'slip' cycles in a direction opposite to that
predicted based on the chemical driving force of the
cycle. The 'slip' transitions are shown as dashed arrows.
The conformational transition $F \cong P.F$ confers the el predicted based on the chemical driving force of the cycle. The 'slip' transitions are shown as dashed arrows.
The conformational transition $E_1 \rightleftharpoons P-E_2$ confers the elec-
trical sensitivity on these processes. Althoug cycle. The 'slip' transitions are shown as dashed arrows.
The conformational transition $E_1 \rightleftharpoons P-E_2$ confers the electrical sensitivity on these processes. Although the charge movement is minimal, the electric work is The conformational transition $E_1 \rightleftharpoons P-E_2$ confers the electrical sensitivity on these processes. Although the charge movement is minimal, the electric work is $2e\Delta\psi$ (where *e* is the elementary charge) because the trical sensitivity on these processes. Although the charge
movement is minimal, the electric work is $2e\Delta\psi$ (where e
is the elementary charge), because the access of the nega-
tively charge binding site is changed from movement is minimal, the electric work is $2e\Delta\psi$ (where e is the elementary charge), because the access of the negatively charge binding site is changed from the outside to the cytosol and so the charge effectively mo is the elementary charge), because the access of the negatively charge binding site is changed from the outside to the cytosol and so the charge effectively moves through the entire membrane potential difference $\Delta \psi$. tively charge binding site is changed from the outside to
the cytosol and so the charge effectively moves through
the entire membrane potential difference $\Delta \psi$.
For large fields, the thermodynamic efficiency of the

oncentration under the experimental conditions. This For large fields, the thermodynamic efficiency of the electrostatic model of the Na,K-ATPase shown maximum gradient that can be supported is given by a figure 2*a* (Wud the entire membrane potential difference $\Delta \psi$.
For large fields, the thermodynamic efficiency of the external pumping can approach 100% , and the maximum gradient that can be supported is given by For large fields, the thermodynamic efficiency of the external pumping can approach 100% , and the maximum gradient that can be supported is given by the ratio of the affinity in the birsh and low affinity states external pumping can approach 100% , and the maximum gradient that can be supported is given by the ratio of the affinity in the high and low affinity states (Markin *et al* 1990) maximum gradient that can be supported is given by
the ratio of the affinity in the high and low affinity states
(Markin *et al.* 1990).

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MOTORS

Now, let us apply the principles discussed for ion

mps to consideration of the mechanism of mechano-Now, let us apply the principles discussed for ion
umps to consideration of the mechanism of mechano-
hemical energy conversion by the molecular motors Now, let us apply the principles discussed for ion
umps to consideration of the mechanism of mechano-
hemical energy conversion by the molecular motors
inesin and Ncd umps to consider
hemical energy
inesin and Ncd.
Kinesin and Ncd. emical energy conversion by the molecular motors
nesin and Ncd are two members of the kinesin super-
nily of microtubule (MT)-based molecular motors

inesin and Ncd.
Kinesin and Ncd are two members of the kinesin super-
imply of microtubule (MT)-based molecular motors. Kinesin and Ncd are two members of the kinesin super-

unily of microtubule (MT) -based molecular motors.

owered by ATP hydrolysis, these two molecules move in

posite directions along a MT They are however strucmily of microtubule (MT)-based molecular motors.

by ATP hydrolysis, these two molecules move in

pposite directions along a MT. They are, however, struc-

rally very similar (Kull *et al.* 1996: Sablin *et al.* 1996) Vowered by ATP hydrolysis, these two molecules move in pposite directions along a MT. They are, however, structurally very similar (Kull *et al.* 1996; Sablin *et al.* 1996), and bind with similar crientations on MTs elimi pposite directions along a MT. They are, however, struc-
trally very similar (Kull *et al.* 1996; Sablin *et al.* 1996),
and bind with similar orientations on MTs, eliminating The possibility very similar (Kull *et al.* 1996; Sablin *et al.* 1996),
And bind with similar orientations on MTs, eliminating
An epossibility that the origin of the oppositely directed
aboution comes about because the m In the motor comes about because the motors bind facing oppositely directed
a notion comes about because the motors bind facing oppo-
the directions (Hirose *et al.* 1996). The mystery is deepended **Solution** comes about because the motors bind facing oppositely directed
a position comes about because the motors bind facing opposite directions (Hirose *et al.* 1996). The mystery is deepened
by a recent elegant experi **Compose the metallical state is a recent elegant experiment** in which a chimera was a recent elegant experiment in which a chimera was a chimeral was the metally represent the metallical state in \mathbb{R} and \mathbb{R} ar te directions (Hirose *et al.* 1996). The mystery is deepened
y a recent elegant experiment in which a chimera was
) rmed by attaching the motor domain of Ncd to the neck
 $\sum_{n=1}^{\infty}$ y a recent elegant experiment in which a chimera was
formed by attaching the motor domain of Ncd to the neck
for S egion of *Neurospora* kinesin (Henningsen & Schliwa 1997;
give *et al.* 1997). Surprisingly, the resulting Ω egion of Neurospora kinesin (Henningsen & Schliwa 1997; ments show that at low ATP concentration the motion is

Ormed by attaching the motor domain of Ncd to the neck

egion of *Neurospora* kinesin (Henningsen & Schliwa 1997;

lase *et al.* 1997). Surprisingly, the resulting motor cata-
 $\frac{d}{dt}$ /sed the '+' end-directed motion ch from which the neck (and not the motor) region was aken. In addition to structural studies, there has been an sed the '+' end-directed motion characteristic of kinesin

om which the neck (and not the motor) region was

aken. In addition to structural studies, there has been an
 \bullet -valosion of work on the mechanical behaviour of om which the neck (and not the motor) region was
aken. In addition to structural studies, there has been an
syplosion of work on the mechanical behaviour of kinesin,
ading to a consensus in the field that with saturating aken. In addition to structural studies, there has been an syplosion of work on the mechanical behaviour of kinesin, ading to a consensus in the field that with saturating Γ P the velocity of a single kinesin dimer movi Explosion of work on the mechanical behaviour of kinesin,
ading to a consensus in the field that with saturating
TP the velocity of a single kinesin dimer moving proces-
velv on MTs is between 0.5 and 1 u m s^{-1} and th straining in the field that with saturating TP the velocity of a single kinesin dimer moving proces-
vely on MTs is between 0.5 and $1 \mu m s^{-1}$, and that the
vertex (site of the single state of the single state of the sing For the velocity of a single kinesin dimer moving proces-
vely on MTs is between 0.5 and $1 \mu m s^{-1}$, and that the
orce (either elastic (Svoboda & Block 1994; Coppin *et al.*
997; Meyerhofer & Howard 1995) or viscous (Hunt 997; Meyerhofer & Howard 1995) or viscous (Hunt *et al.* 994)) needed to stop the forward progress is around pN . Furthermore, single-molecule studies of kinesin motion have shown that the motor moves in single steps of pN. Furthermore, single-molecule studies of kinesin otion have shown that the motor moves in single steps of bout 8 nm (Svoboda *et al.* 1993), corresponding well with relative spacing $d \approx 8$ nm of tubulin monomers along totion have shown that the motor moves in single steps of
bout 8 nm (Svoboda *et al.* 1993), corresponding well with
the lattice spacing $d \approx 8$ nm of tubulin monomers along
the same stablished that bout 8 nm (Svoboda *et al.* 1993), corresponding well with
ne lattice spacing $d \approx 8$ nm of tubulin monomers along
ne axis of the MT. Recently, it has been established that
nthe absence of a load the stoichiometry is one in the absence of a load the stoichiometry is one ATP per any step of the motor (Schnitzer & Block 1997; Hun *et al.*) 1e axis of the MT. Recently, it has been established that 1 the absence of a load the stoichiometry is one ATP per nm step of the motor (Schnitzer & Block 1997; Hua *et al.* 1997). here we discuss a model, based on a 'Brownian ratchet'
Here we discuss a model, based on a 'Brownian ratchet'

Huxley 1957; Hänggi & Bartussek 1996; Astumian 1997; Here we discuss a model, based on a 'Brownian ratchet'
Huxley 1957; Hänggi & Bartussek 1996; Astumian 1997;
ilicher *et al.* 1997) where the direction of motion is
ontrolled by the chemical mechanism of ATP hydrolysis Huxley 1957; Hänggi & Bartussek 1996; Astumian 1997;

Lilicher *et al.* 1997) where the direction of motion is

ontrolled by the chemical mechanism of ATP hydrolysis

Astumian & Derényi 1999) A key assumption is that the Filicher *et al.* 1997) where the direction of motion is ontrolled by the chemical mechanism of ATP hydrolysis Astumian & Derényi 1999). A key assumption is that the TP bound state has a large one-dimensional diffusion Astumian & Derényi 1999). A key assumption is that the TP bound state has a large one-dimensional diffusion officient for lateral motion along the MT backbone, though this state has a very small dissociation constant TP bound state has a large one-dimensional diffusion
oefficient for lateral motion along the MT backbone,
though this state has a very small dissociation constant
lowing the motor to retain energetic contact with its oefficient for lateral motion along the MT backbone,
lthough this state has a very small dissociation constant
llowing the motor to retain energetic contact with its
olymeric track while undergoing motion. In contrast to Ithough this state has a very small dissociation constant
Ilowing the motor to retain energetic contact with its
olymeric track while undergoing motion. In contrast to
the standard 'hand-over-hand' mechanism, the model Ilowing the motor to retain energetic contact with its
abymeric track while undergoing motion. In contrast to
be standard 'hand-over-hand' mechanism, the model
loes not require either head of the motor to dissociate at obymeric track while undergoing motion. In contrast to
the standard 'hand-over-hand' mechanism, the model
of the motor to dissociate at
the motor to dissociate at
we time during a mechanochemical cycle. The steps in are standard 'hand-over-hand' mechanism, the model

(b) oes not require either head of the motor to dissociate at

(b) my time during a mechanochemical cycle. The steps in

(b) motion and force production occur are pictur which motion and force production occur are pictured as
a phich motion and force production occur are pictured as
a permally activated transitions over an energy barrier on thich motion and force production occur are pictured as

ermally activated transitions over an energy barrier on
one-dimensional potential between molecular states,
ach of which is close to thermal equilibrium even in the
resence of large $(5-10 \text{ pN})$ external forces. The syst one-dimensional potential between molecular states,
ach of which is close to thermal equilibrium even in the
resence of large $(5-10 \text{ pN})$ external forces. The system is
aus appropriately modelled by chemical kinetics, a ach of which is close to thermal equilibrium even in the
resence of large $(5-10 \text{ pN})$ external forces. The system is
aus appropriately modelled by chemical kinetics, and no
down stroke (i.e., a viscoelastic relaxation f resence of large $(5-10 \text{ pN})$ external forces. The system is
aus appropriately modelled by chemical kinetics, and no
ower stroke (i.e. a viscoelastic relaxation from a nonall appropriately modelled by chemical kinetics, and no

solver stroke (i.e. a viscoelastic relaxation from a non-

quilibrium conformation) is involved. This mechanism is

undamentally similar to that used to describe th Solution and the content of the content of the method is involved. This mechanism is indicamentally similar to that used to describe the continuous of ATP hydrolysis to drive unbill transport of quilibrium conformation) is involved. This mechanism is
indamentally similar to that used to describe the
oupling of ATP hydrolysis to drive uphill transport of
one by ion numes (Läuger 1990: Astumian & Derényi indamentally similar to that used to describe the oupling of ATP hydrolysis to drive uphill transport of ons by ion pumps (Läuger 1990; Astumian & Derényi 998) discussed above. ons by ion pumps (Läuger 1990; Astumian & Derényi

Thermal activation in molecular motors R. D. Astumian 515
To compare our model with experimental results for the To compare our model with experimental results for the
effect of external force on the velocity of dimeric kinesin
(few data are available for Ncd) we provide an extension To compare our model with experimental results for the
effect of external force on the velocity of dimeric kinesin
(few data are available for Ncd), we provide an extension
to a two-beaded model, and incorporate alternatin effect of external force on the velocity of dimeric kinesin
(few data are available for Ncd), we provide an extension
to a two-headed model, and incorporate alternating site
kinetics for the ATP hydrolysis because this see (few data are available for Ncd), we provide an extension
to a two-headed model, and incorporate alternating site
kinetics for the ATP hydrolysis because this seems to be
well established experimentally. In this extended p to a two-headed model, and incorporate alternating site
kinetics for the ATP hydrolysis because this seems to be
well established experimentally. In this extended picture
the mechanical motion is still described in terms o kinetics for the ATP hydrolysis because this seems to be
well established experimentally. In this extended picture
the mechanical motion is still described in terms of
thermal activation on a one-dimensional potential. The well established experimentally. In this extended picture
the mechanical motion is still described in terms of
thermal activation on a one-dimensional potential. The
presence of the second head significantly stabilizes the the mechanical motion is still described in terms of
thermal activation on a one-dimensional potential. The
presence of the second head significantly stabilizes the
overall interaction between the kinesin and MT so that thermal activation on a one-dimensional potential. The
presence of the second head significantly stabilizes the
overall interaction between the kinesin and MT, so that
highly processive motion is possible. In addition to r presence of the second head significantly stabilizes the
overall interaction between the kinesin and MT, so that
highly processive motion is possible. In addition to repro-
ducing quantitative aspects of the effect of an e overall interaction between the kinesin and MT, so that
highly processive motion is possible. In addition to repro-
ducing quantitative aspects of the effect of an external force on the velocity of the motor, and the stoichiometry of ducing quantitative aspects of the effect of an external
force on the velocity of the motor, and the stoichiometry of
one ATP per step at zero load, our picture is consistent
with four key observations: (i) a force annuled force on the velocity of the motor, and the stoichiometry of
one ATP per step at zero load, our picture is consistent
with four key observations: (i) a force applied in the direc-
tion of motion increases the velocity of t one ATP per step at zero load, our picture is consistent
with four key observations: (i) a force applied in the direc-
tion of motion increases the velocity of the motor but the
effect saturates (Comin et al. 1997); (ii) with four key observations: (i) a force applied in the direction of motion increases the velocity of the motor but the effect saturates (Coppin *et al.* 1997); (ii) although the motor seems to be completely counled at zero tion of motion increases the velocity of the motor but the effect saturates (Coppin *et al.* 1997); (ii) although the motor seems to be completely coupled at zero load, experiments show that at low ATP concentration the m effect saturates (Coppin *et al.* 1997); (ii) although the motor seems to be completely coupled at zero load, experiments show that at low ATP concentration the motion is more random even than predicted based on a single motor seems to be completely coupled at zero load, experiments show that at low ATP concentration the motion is
more random even than predicted based on a single rate-
limiting step (Schnitzer & Block 1997); (iii) increasing
significantly the strength of the coiled-coil interact more random even than predicted based on a single rate-
limiting step (Schnitzer & Block 1997); (iii) increasing
significantly the strength of the coiled-coil interaction
between the two necks of a kinesin dimer does not a limiting step (Schnitzer & Block 1997); (iii) increasing significantly the strength of the coiled-coil interaction between the two necks of a kinesin dimer does not abolish significantly the strength of the coiled-coil interaction
between the two necks of a kinesin dimer does not abolish
processive motion (Romberg *et al.* 1998); and (iv) the
motion driven by single-headed kinesin seems to be between the two necks of a kinesin dimer does not abolish processive motion (Romberg *et al.* 1998); and (iv) the motion driven by single-headed kinesin seems to be consistent with a small duty cycle motor while that driv processive motion (Romberg *et al.* 1998); and (iv) the motion driven by single-headed kinesin seems to be consistent with a small duty cycle motor, while that driven by dimeric kinesin is consistent with a large duty cyc motion driven by single-headed kinesin seems to be consistent with a small duty cycle motor, while that driven by dimeric kinesin is consistent with a large duty cycle motor (Young *et al* 1998; Hancock & Howard 1998) tent with a small duty cycle motor, while tha
dimeric kinesin is consistent with a large duty
(Young *et al.* 1998; Hancock & Howard 1998). **5. A CHEMICALLY REVERSIBLE BROWNIAN MOTOR**
5. A CHEMICALLY REVERSIBLE BROWNIAN MOTOR

Consider the model shown in figure 3*a*, which describes **S. A CHEMICALLY REVERSIBLE BROWNIAN MOTOR**
Consider the model shown in figure 3*a*, which describes
the energy profile for movement of a single motor head
along a MT in each of four different chemical states Consider the model shown in figure $3a$, which describes
the energy profile for movement of a single motor head
along a MT in each of four different chemical states.
Transitions between chemical states of the motor are the energy profile for movement of a single motor head
along a MT in each of four different chemical states.
Transitions between chemical states of the motor are
shown on the x-axis along a MT in each
Transitions between
shown on the *y*-axis.
In the E state where Ansitions between chemical states of the motor are
 $\sum_{n=1}^{\infty}$ is the E state where nucleotide phosphate is not bound,
 $\sum_{n=1}^{\infty}$ motor is tightly ninned to one binding site on the

ontrolled by the chemical mechanism of ATP hydrolysis tightly associated to MT. This makes the prediction that Astumian & Derényi 1999). A key assumption is that the one-dimensional diffusion coefficient will increase TP b shown on the y -axis.
In the E state where nucleotide phosphate is not bound,
the motor is tightly pinned to one binding site on the
MT When ATP binds, the activation energy for lateral In the E state where nucleotide phosphate is not bound,
the motor is tightly pinned to one binding site on the
MT. When ATP binds, the activation energy for lateral
movement is decreased and transitions to the monomer the motor is tightly pinned to one binding site on the MT. When ATP binds, the activation energy for lateral movement is decreased and transitions to the monomer on the left or right are fairly fast, but the motor is still MT. When ATP binds, the activation energy for lateral movement is decreased and transitions to the monomer
on the left or right are fairly fast, but the motor is still
tightly associated to MT. This makes the prediction th movement is decreased and transitions to the monomer
on the left or right are fairly fast, but the motor is still
tightly associated to MT. This makes the prediction that
the one-dimensional diffusion coefficient will incr on the left or right are fairly fast, but the motor is still
tightly associated to MT. This makes the prediction that
the one-dimensional diffusion coefficient will increase
upon binding ATP to the motor even though the mo tightly associated to MT. This makes the prediction that
the one-dimensional diffusion coefficient will increase
upon binding ATP to the motor even though the motor
remains tightly bound to the MT the one-dimensional diffusion coefficient will increase

The during a mechanochemical cycle. The steps in asymmetrical—transition from the H to the L position

thermally activated transitions over an energy barrier on

the right is much faster than transition to the L posi-

erm Hydrolysis of ATP at the active site changes the interaction between the motor and track such that there are Hydrolysis of ATP at the active site changes the inter-
action between the motor and track such that there are
two ways the motor can bind in the $E^{ADP\times Pi}$ state—a rela-
tively high-energy (H) position and a lower-energy action between the motor and track such that there are
two ways the motor can bind in the $E^{ADP\times Pi}$ state—a rela-
tively high-energy (H) position and a lower-energy (L)
nosition. The barriers between the H and I position two ways the motor can bind in the $E^{ADP\times Pi}$ state—a relatively high-energy (H) position and a lower-energy (L) position. The barriers between the H and L positions are asymmetrical—transition from the H to the L positio tively high-energy (H) position and a lower-energy (L)
position. The barriers between the H and L positions are
asymmetrical—transition from the H to the L position
on the right is much faster than transition to the L p position. The barriers between the H and L positions are
asymmetrical—transition from the H to the L position
on the right is much faster than transition to the L posi-
tion on the left. Dissociation of Pi again changes th asymmetrical—transition from the H to the L position on the right is much faster than transition to the L posibinding positions on the one-dimensional coordinate are action between the motor and the MT such that the
binding positions on the one-dimensional coordinate are
shifted in the E^{ADP} state, and the barriers are inter-
changed such that a transition from the H to the L posibinding positions on the one-dimensional coordinate are shifted in the E^{ADP} state, and the barriers are inter-
changed such that a transition from the H to the L posi-
tion on the left is much more rapid than a transit shifted in the E^{ADP} state, and the barriers are inter-
changed such that a transition from the H to the L posi-
tion on the left is much more rapid than a transition to
the L position on the right. Release of ADP compl changed such that a transition from the H to the L position on the left is much more rapid than a transition to
the L position on the right. Release of ADP completes a
chemical cycle of ATP hydrolysis, returning the motor tion on the left is much more rapid than a transition to
the L position on the right. Release of ADP completes a
chemical cycle of ATP hydrolysis, returning the motor to
the tightly ninned E state the tightly pinned E state.
One simple possibility for controlling the direction of chemical cycle of ATP hydrolysis, returning the motor to

motion in this model is by the relative rates for release of

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igure 3. Ratchet mechanism for chemically reversible
totion. (*a*) The chemically reversible Brownian motor. The
ratein concomitantly cycles through its chemical states while igure 3. Ratchet mechanism for chemically reversible
iotion. (a) The chemically reversible Brownian motor. The
rotein concomitantly cycles through its chemical states while
atalysing ATP hydrolysis (on the *x*-coordinate) otion. (*a*) The chemically reversible Brownian motor. The rotein concomitantly cycles through its chemical states where at alysing ATP hydrolysis (on the *y*-coordinate) and trans-cates through space along a MT (possibly rotein concomitantly cycles through its chemical states v
atalysing ATP hydrolysis (on the *y*-coordinate) and tran
ocates through space along a MT (possibly varying its
origination in the process) as plotted on the *x*-c atalysing ATP hydrolysis (on the *y*-coordinate) and trans-
ocates through space along a MT (possibly varying its
onformation in the process) as plotted on the *x*-coordinate.
b) Coupled transport Illustration of how this % between the process of MT (possibly varying its
onformation in the process) as plotted on the *x*-coordinate.
b) Coupled transport. Illustration of how this mechanism
could work with a two-beaded motor. We show only the % onformation in the process) as plotted on the *x*-coordinate.

b) Coupled transport. Illustration of how this mechanism

vould work with a two-headed motor. We show only the case

v coupled motion directed to the right. b) Coupled transport. Illustration of how this mechanism
vould work with a two-headed motor. We show only the case
or coupled motion directed to the right. Initially, either head an bind ATP (T) and the interaction of that head with the or coupled motion directed to the right. Initially, either head
an bind ATP (T) and the interaction of that head with the
1T is weakened. This is followed by hydrolysis of ATP at the
ctive site changing the interaction w an bind ATP (T) and the interaction of that head with the
1T is weakened. This is followed by hydrolysis of ATP at the
ctive site changing the interaction with MT, and inducing
inding of ATP to the other head. As the ca IT is weakened. This is followed by hydrolysis of ATP at
citive site changing the interaction with MT, and inducing
inding of ATP to the other head. As the catalytic and
leader incoherence is the second head. ctive site changing the interaction with MT, and inducing
inding of ATP to the other head. As the catalytic and
rechanical cycle of the first head proceeds, the second head inding of ATP to the other head. As the catalytic and

i echanical cycle of the first head proceeds, the second head

illows along. Finally, ADP dissociates from the first head and

new cycle begins by hydrolyging the ATP The new cycle of the first head proceeds, the second head
allows along. Finally, ADP dissociates from the first head an
new cycle begins by hydrolysing the ATP in the second
ead head.

'i and ADP (Astumian & Derényi 1998). This is similar ti and ADP (Astumian & Derényi 1998). This is similar
a recent models for physical ratchets where a position-
dependent modulation of the potential coupled with in and ADP (Astumian & Derényi 1998). This is similar
absorption models for physical ratchets where a position-
dependent modulation of the potential coupled with
absorption absorption coupled with
absorption absorption o spatial anisotropy allows directed motion (Astumian & charged motion (Astumian & charged motion (Astumian & charged motion (Astumian & charged motion (Astumian 1996). If dependent modulation of the potential coupled with

batial anisotropy allows directed motion (Astumian &

ier 1994; Prost *et al.* 1994; Bier & Astumian 1996). If

elease of Pi is slow and release of ADP fast compared to Formula anisotropy allows directed motion (Astumian &

Sier 1994; Prost *et al.* 1994; Bier & Astumian 1996). If

clease of Pi is slow and release of ADP fast compared to

be H \rightarrow I transition, the motor will probably m ier 1994; Prost *et al.* 1994; Bier & Astumian 1996). If
elease of Pi is slow and release of ADP fast compared to
he H \rightarrow L transition, the motor will probably make a
cansition to the L position while Pi is bound but wi Elease of Pi is slow and release of ADP fast compared to

the H \rightarrow L transition, the motor will probably make a

cansition to the L position while Pi is bound, but will

elease ADP while in the transient H position foll he $H \rightarrow L$ transition, the motor will probably make a cansition to the L position while Pi is bound, but will elease ADP while in the transient H position, following be trajectory outlined by the solid arrows. In contrast ransition to the L position while Pi is bound, but will elease ADP while in the transient H position, following he trajectory outlined by the solid arrows. In contrast, if lease of Pi is fast and release of ADP slow compa elease ADP while in the transient H position, following
he trajectory outlined by the solid arrows. In contrast, if
elease of Pi is fast and release of ADP slow compared to
be $H \rightarrow I$ transition, the motor will most probab he trajectory outlined by the solid arrows. In contrast, if
elease of Pi is fast and release of ADP slow compared to
be $H \rightarrow L$ transition, the motor will most probably
elease Pi in the transient H position but will make a release of Pi is fast and release of ADP slow compared to
the H \rightarrow L transition, the motor will most probably
elease Pi in the transient H position, but will make a tran-
tion to the L position before release of ADP fol She $H \rightarrow L$ transition, the motor will most probably elease Pi in the transient H position, but will make a transition to the L position before release of ADP, following the calendary outlined by the dashed arrows. Sadly t elease Pi in the transient H position, but will make a tran-
tion to the L position before release of ADP, following the
rajectory outlined by the dashed arrows. Sadly, this
legant mechanism alone is not sufficient to expl tion to the L position before release of ADP, following the rajectory outlined by the dashed arrows. Sadly, this legant mechanism alone is not sufficient to explain the rechanical data—it predicts that application of a mod rajectory outlined by the dashed arrows. Sadly, this legant mechanism alone is not sufficient to explain the rechanical data—it predicts that application of a modest

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external force opposing the ATP-driven motion should cause the motor to begin stepping backward, and this is external force opposing the ATP-driven motion should
cause the motor to begin stepping backward, and this is
not seen. Experimentally, a force of 5 pN is sufficient to
halt kinesin, but the motor remains fixed and does not cause the motor to begin stepping backward, and this is
not seen. Experimentally, a force of 5 pN is sufficient to
halt kinesin, but the motor remains fixed and does not
undergo significant backwards motion even when chalnot seen. Experimentally, a force of 5 pN is sufficient to
halt kinesin, but the motor remains fixed and does not
undergo significant backwards motion even when chal-
lenged by forces as large as 12 pN (Coppin *et al* halt kinesin, but the motor remains fixed and does not undergo significant backwards motion even when challenged by forces as large as 12 pN (Coppin *et al.* 1997). dergo significant backwards motion even when chal-
19ed by forces as large as 12 pN (Coppin *et al.* 1997).
A second possibility, on which we focus here, is that the
rection is controlled by the specificities for rel

lenged by forces as large as 12 pN (Coppin *et al.* 1997).
A second possibility, on which we focus here, is that the direction is controlled by the specificities for release of ADP and Pi from the H and L positions. A second possibility, on which we focus here, is that the direction is controlled by the specificities for release of ADP and Pi from the H and L positions. This is closely related to Huylev's model for muscle contraction, direction is controlled by the specificities for release of
ADP and Pi from the H and L positions. This is closely
related to Huxley's model for muscle contraction, where
the rate constants for the chemical transitions are ADP and Pi from the H and L positions. This is closely related to Huxley's model for muscle contraction, where the rate constants for the chemical transitions are aniso-
tronic along the reaction coordinate but the potenti related to Huxley's model for muscle contraction, where
the rate constants for the chemical transitions are aniso-
tropic along the reaction coordinate but the potential
itself can be symmetrical (Huyley 1957) Once again t the rate constants for the chemical transitions are aniso-
tropic along the reaction coordinate but the potential
itself can be symmetrical (Huxley 1957). Once again this
closely parallels ideas taken from the coupling mec tropic along the reaction coordinate but the potential
itself can be symmetrical (Huxley 1957). Once again this
closely parallels ideas taken from the coupling mechan-
isms of ion numns (Jencks 1989 a) itself can be symmetrical (Huxley
closely parallels ideas taken from
isms of ion pumps (Jencks 1989*a*).
Consider that the L. position of bely parallels ideas taken from the coupling mechan-
ns of ion pumps (Jencks 1989*a*).
Consider that the L position of the E^{ADP×Pi} state is
ecific for release of Pi and that the H position of the

isms of ion pumps (Jencks 1989*a*).
Consider that the L position of the $E^{ADP \times Pi}$ state is
specific for release of Pi and that the H position of the
 E^{ADP} state is specific for release of ADP (solid arrows) Consider that the L position of the $E^{ADP\times Pi}$ state is specific for release of Pi and that the H position of the E^{ADP} state is specific for release of ADP (solid arrows). First ATP binds to the motor decreasing the in specific for release of Pi and that the H position of the E^{ADP} state is specific for release of ADP (solid arrows).
First, ATP binds to the motor, decreasing the interaction
energy holding the motor to a fixed site. E^{ADP} state is specific for release of ADP (solid arrows).
First, ATP binds to the motor, decreasing the interaction
energy holding the motor to a fixed site. Most probably,
ATP is hydrolysed before a transition to the First, ATP binds to the motor, decreasing the interaction
energy holding the motor to a fixed site. Most probably,
ATP is hydrolysed before a transition to the left or right
occurs. Because the H position is not specific f energy holding the motor to a fixed site. Most probably,
ATP is hydrolysed before a transition to the left or right
occurs. Because the H position is not specific for release
of Pi a transition to the L position on the rig ATP is hydrolysed before a transition to the left or right occurs. Because the H position is not specific for release of Pi, a transition to the L position on the right most occurs. Because the H position is not specific for release
of Pi, a transition to the L position on the right most
probably occurs, triggering release of Pi. The motor then
rapidly equilibrates in the H position in which i of Pi, a transition to the L position on the right most
probably occurs, triggering release of Pi. The motor then
rapidly equilibrates in the H position in which it finds
itself. Now, ADP release most probably occurs from probably occurs, triggering release of Pi. The motor then
rapidly equilibrates in the H position in which it finds
itself. Now, ADP release most probably occurs from the
ADP-specific H position, completing a chemical cycle rapidly equilibrates in the H position in which it finds
itself. Now, ADP release most probably occurs from the
ADP-specific H position, completing a chemical cycle.
Rapid equilibration in the tight binding site completes itself. Now, ADP release most probably occurs from the ADP-specific H position, completing a chemical cycle.
Rapid equilibration in the tight binding site completes a mechanical cycle of movement one period to the right of ADP-specific H position, completing a chemical cycle.
Rapid equilibration in the tight binding site completes a
mechanical cycle of movement one period to the right of
the starting point Rapid equilibration in the tight binding site completes a mechanical cycle of movement one period to the right of the starting point.

If the H position of the $E^{ADP\times Pi}$ state is specific for the starting point.
If the H position of the E^{ADP×Pi} state is specific for
release of Pi, and the L position of the E^{ADP} state is
specific for release of ADP the direction is reversed If the H position of the $E^{ADP\times Pi}$ state is specific for
release of Pi, and the L position of the E^{ADP} state is
specific for release of ADP, the direction is reversed
(dotted arrows) ATP hydrolysis is followed by rele release of Pi, and the L position of the E^{ADP} state is
specific for release of ADP, the direction is reversed
(dotted arrows). ATP hydrolysis is followed by release of
inorganic phosphate from the Pi-ppecific H position specific for release of ADP, the direction is reversed (dotted arrows). ATP hydrolysis is followed by release of inorganic phosphate from the Pi-specific H position. (dotted arrows). ATP hydrolysis is followed by release of inorganic phosphate from the Pi-specific H position.
Then, because the H position is not specific for release of APP a transition over the low barrier to the L po inorganic phosphate from the Pi-specific H position.
Then, because the H position is not specific for release of
ADP, a transition over the low barrier to the L position
on the left is quite likely. The L position is speci Then, because the H position is not specific for release of ADP, a transition over the low barrier to the L position
on the left is quite likely. The L position is specific for
ADP release, thus completing one chemical cyc ADP, a transition over the low barrier to the L position
on the left is quite likely. The L position is specific for
ADP release, thus completing one chemical cycle of ATP on the left is quite likely. The L position is specific for ADP release, thus completing one chemical cycle of ATP hydrolysis, and the motor equilibrates in the tight binding site one period to the left of where it started ADP release, thus completing one chemical cycle of ATP
hydrolysis, and the motor equilibrates in the tight binding
site one period to the left of where it started, completing a
mechanical cycle hydrolysis, and the
site one period to th
mechanical cycle. **6. KINETIC MECHANISM FOR A SINGLE-HEADED**

MOTOR

IF THE CONSUMED MOTOR

If the local equilibration within a state is fast compared

any chemical transitions and to relaxation between the **THE SET ASSEM STAR SET ASSEM**
The local equilibration within a state is fast compared
to any chemical transitions and to relaxation between the
H and I positions we can rewrite the model in terms of If the local equilibration within a state is fast compared
to any chemical transitions and to relaxation between the
H and L positions, we can rewrite the model in terms of
chemical kinetics (Astumian & Bier 1996) (forme to any chemical transitions and to relaxation between the H and L positions, we can rewrite the model in terms of chemical kinetics (Astumian & Bier 1996) (figure 4*a*). For simplicity we assume that ATP hydrolysis is irre H and L positions, we can rewrite the model in terms of chemical kinetics (Astumian & Bier 1996) (figure 4*a*). For simplicity we assume that ATP hydrolysis is irreversible. With this assumption, the steady-state rate of A chemical kinetics (Astumian & Bier 1996) (figure 4*a*). For
simplicity we assume that ATP hydrolysis is irreversible.
With this assumption, the steady-state rate of ATP hydro-
lysis is $\mathcal{F} = -k - P(F^{ATP})$, where $P(F$ simplicity we assume that ATP hydrolysis is irreversible.
With this assumption, the steady-state rate of ATP hydro-
lysis is $\mathcal{J}_{ATP} = k_{\text{hyd}} P(E^{ATP})$, where $P(E^{ATP})$ is the steady-
state, probability for the motor to be With this assumption, the steady-state rate of ATP hydro-
lysis is $\mathcal{J}_{ATP} = k_{\text{hyd}} P(E^{ATP})$, where $P(E^{ATP})$ is the steady-
state probability for the motor to be in the weakly
constrained ATP-bound state E^{ATP} We assum lysis is $\mathcal{J}_{\text{ATP}} = k_{\text{hyd}} P(\text{E}^{\text{ATP}})$, where $P(\text{E}^{\text{ATP}})$ is the steady-
state probability for the motor to be in the weakly
constrained ATP-bound state E^{ATP} . We assume that the
transition over the state probability for the motor to be in the weakly
constrained ATP-bound state E^{ATP} . We assume that the
transition over the high barrier in the E, $E^{ADP \times Pi}$, and
 E^{ADP} states is essentially precluded. The constant E^{ADP} states is essentially precluded. The constant s paraboth states is essentially precluded. The constant *s* para-
ansition over the high barrier in the E, $E^{ADP\times Pi}$, and
 E^{ADP} states is essentially precluded. The constant *s* para-
neterizes the specificity difference f transition over the high barrier in the E, $E^{ADP \times Pi}$, and E^{ADP} states is essentially precluded. The constant *s* parameterizes the specificity difference for Pi and ADP release for the H and I positions When $s \gg 1$ t E^{ADP} states is essentially precluded. The constant *s* para-
meterizes the specificity difference for Pi and ADP release
for the H and L positions. When $s \gg 1$, the L position is
highly specific for release of Pi and meterizes the specificity difference for Pi and ADP release
for the H and L positions. When $s \gg 1$, the L position is
highly specific for release of Pi, and the H position is
highly specific for release of ADP and vice v for the H and L positions. When $s \gg 1$, the L position is highly specific for release of Pi, and the H position is highly specific for release of ADP, and vice versa when highly specific for release of Pi, and the H position is
highly specific for release of ADP, and vice versa when
 $s \ll 1$. The parameter *K* is the equilibrium constant for
transition from the H to the L position and α highly specific for release of ADP, and vice versa when $s \ll 1$. The parameter K is the equilibrium constant for transition from the H to the L position, and α and β are

igure 4. Kinetic mechanisms for kinesin. (*a*) Kinetic rechanism for a chemically reversible ratchet. k_{on} is a implecular rate constant which when multiplied by the igure 4. Kinetic mechanisms for kinesin. (a) Kinetic
rechanism for a chemically reversible ratchet. k_{on} is a
imolecular rate constant which when multiplied by the
oncentration of ATP ([ATP]) gives the on rate for ATP k **BIOLOGICAL**
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oncentration of ATP ([ATP]) gives the on rate for ATP, k_{off}
and k_{hyd} are unimolecular rate constants and represent the
off rate and hydrolysis rate for ATP, respe nd k_{hyd} are unimolecular rate constants and represent the
off rate and hydrolysis rate for ATP, respectively, and k_{diff} is
a transition to the binding site on the monomer to
the monomer to
the monomer is also th nd k_{hyd} are unimolecular rate constants and represent the off rate and hydrolysis rate for ATP, respectively, and k_{diff} is
the rate for a transition to the binding site on the monomer to
the left or right while in the weakly attached ATP bound
at k is the equilibrium const state for a transition to the binding site on the monomerate.
 K is the equilibrium constant for the H to the L ansition α and β are rate constants that set the relative - ne left or right while in the weakly attached ATP bound
ate. K is the equilibrium constant for the H to the L
ansition. α and β are rate constants that set the relative
mescales for the mechanical and chemical tra the same is the equilibrium constant for the H to the L
 α ansition. α and β are rate constants that set the relative

me-scales for the mechanical and chemical transitions,

sensetively and f parametrizes the ef respectively, and β are rate constants that set the relative
me-scales for the mechanical and chemical transitions,
espectively, and f parametrizes the effect of external elastic
vectors of β . me-scales for the mechanical and chemical transitions,
spectively, and f parametrizes the effect of external elastic
rce. (*b*) Reaction along the predominant pathway for a
nonomer showing the side reaction of dissociatio expectively, and f parametrizes the effect of external elastic
orce. (b) Reaction along the predominant pathway for a
nonmer showing the side reaction of dissociation in the ADP-
and state (c) Reaction along the predomina $\begin{bmatrix} \text{bmatrix.} (b) \text{ Reaction along the predominant pathway for a} \\ \text{nonomer showing the side reaction of dissociation in the ADP-} \text{ound state.} (c) \text{ Reaction along the predominant pathway for a} \end{bmatrix}$ is a non-
non-constant parameter of dissociation in the ADP-
ound state. (c) Reaction along the predominant pathway for
 $\frac{1}{2}$ imer showing the side reaction of dissociation in the ADP
and state. Here, two sequential s $\begin{bmatrix} \text{ound state.} (c) \text{ Reaction along the predominant pathway for} \ \text{inner showing the side reaction of dissociation in the ADP} \ \text{ound state. Here, two sequential steps are required—dissociation of one head followed by dissociation of the second.} \end{bmatrix}$ imer showing the side reaction of dissociation in the ADP

ound state. Here, two sequential steps are required—disso-

iation of one head followed by dissociation of the second

and hefore the dimer can be considered disso ound state. Here, two sequential steps are required—disso-
iation of one head followed by dissociation of the second
ead—before the dimer can be considered dissociated.

the rate constants for the translocation and chemical
rations respectively the rate constants for the constants of the constants of the constant of the system of the constant of the con Externally applied homogeneous force *F* can be equalized as superimposing a net tilt on each of the

consistions, respectively.
An externally applied homogeneous force F can be
isualized as superimposing a net tilt on each of the
Dnergy profiles in figure 1 $(I/(x) \rightarrow I/(x) + Fr$ where the An externally applied homogeneous force *F* can be
isualized as superimposing a net tilt on each of the
 Ω nergy profiles in figure 1 ($U(x) \rightarrow U(x) + Fx$, where the
rigin is arbitrary). The energy difference between neighisualized as superimposing a net tilt on each of the

pregy profiles in figure 1 $(U(x) \rightarrow U(x) + Fx$, where the

rigin is arbitrary). The energy difference between neigh-

ouring binding sites in both the E and E^{ATP} states is between profiles in figure 1 $(U(x) \rightarrow U(x) + Fx$, where the rigin is arbitrary). The energy difference between neigh-
ouring binding sites in both the E and E^{ATP} states is
an *Ed*. If we assume that the physical distance betw rigin is arbitrary). The energy difference between neigh-
ouring binding sites in both the E and E^{ATP} states is
ne *Fd*. If we assume that the physical distance between
ne H and I positions is $d/2$ and that the barrie ouring binding sites in both the E and E^{ATP} states is

ien *Fd*. If we assume that the physical distance between

ie H and L positions is $d/2$, and that the barrier is half-

in the energies of the H and L positions nen Fd . If we assume that the physical distance between
ne H and L positions is $d/2$, and that the barrier is half-
ay between them, the energies of the H and L positions

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change relative to each other by $Fd/2$ due to the force, change relative to each other by $Fd/2$ due to the force,
and the effect of the external force on the transition rates
can be parameterized by $f = \exp(-Fd/(4k \cdot T))$. In our change relative to each other by $Fd/2$ due to the force,
and the effect of the external force on the transition rates
can be parameterized by $f = \exp(-Fd/(4k_BT))$. In our
model the effect of an external force appears only in th and the effect of the external force on the transition rates
can be parameterized by $f = \exp(-Fd/(4k_B T))$. In our
model the effect of an external force appears only in the
lateral transitions between the H and L positions and can be parameterized by $f = \exp(-Fd/(4k_B T))$. In our model the effect of an external force appears only in the lateral transitions between the H and L positions, and the model the effect of an external force appears only in the lateral transitions between the H and L positions, and the diffusive step (dashed arrows) in the weakly pinned ATP-
bound state. The force dependencies of the chemi lateral transitions between the H and L positions, and the diffusive step (dashed arrows) in the weakly pinned ATP-
bound state. The force dependencies of the chemical steps
required by thermodynamics are subsumed in the r diffusive step (dashed arrows) in the weakly pinned ATP-
bound state. The force dependencies of the chemical steps
required by thermodynamics are subsumed in the rate
constants for binding ADP and Pi Far from equilibrium bound state. The force dependencies of the chemical steps
required by thermodynamics are subsumed in the rate
constants for binding ADP and Pi. Far from equilibrium required by thermodynamics are subsumed in the rate
constants for binding ADP and Pi. Far from equilibrium
we can assume that Pi and ADP release are irreversible,
and that these binding steps do not occur. This reflects a constants for binding ADP and Pi. Far from equilibrium
we can assume that Pi and ADP release are irreversible,
and that these binding steps do not occur. This reflects a
minimal mechanochemical coupling (Duke & Leibler we can assume that Pi and ADP release are irreversible,
and that these binding steps do not occur. This reflects a
minimal mechanochemical coupling (Duke & Leibler
1996) This apportionment of the external force, while by and that these binding steps do not occur. This reflects a
minimal mechanochemical coupling (Duke & Leibler
1996). This apportionment of the external force, while by
no means unique seems to be the simplest possibility minimal mechanochemical coupling (Duke & Leik
1996). This apportionment of the external force, while
no means unique, seems to be the simplest possibility.
The kinetic equations for the model can be easy 96). This apportionment of the external force, while by
means unique, seems to be the simplest possibility.
The kinetic equations for the model can be easily
pried out in terms of the time-scales of the individual

no means unique, seems to be the simplest possibility.
The kinetic equations for the model can be easily
worked out in terms of the time-scales of the individual The kinetic equations for the model can be easily
worked out in terms of the time-scales of the individual
steps to obtain the net rate of ATP hydrolysis, and the
welocity of the motor along the MT For sufficiently large worked out in terms of the time-scales of the individual
steps to obtain the net rate of ATP hydrolysis, and the
velocity of the motor along the MT. For sufficiently large
values of s the stoichiometry approaches unity and steps to obtain the net rate of ATP hydrolysis, and the velocity of the motor along the MT. For sufficiently large values of *s* the stoichiometry approaches unity and ATP hydrolysis is described by the closed Markoy chain velocity of the motor along the MT. For sufficiently large
values of s the stoichiometry approaches unity and ATP
hydrolysis is described by the closed Markov chain
 $(0) \rightarrow (1) \rightarrow (2) \rightarrow (3) \rightarrow (4) \rightarrow (1)$. The rate of ATP values of *s* the stoichiometry approaches unity and ATP
hydrolysis is described by the closed Markov chain
 $(0) \rightarrow (1) \rightarrow (2) \rightarrow (3) \rightarrow (4) \rightarrow (1)$. The rate of ATP
hydrolysis can then be written in Michaelis-Menten hydrolysis is described by the closed Markov chain $(0) \rightarrow (1) \rightarrow (2) \rightarrow (3) \rightarrow (4) \rightarrow (1)$. The rate of ATP hydrolysis can then be written in Michaelis–Menten form: form:

$$
\mathcal{J}_{\text{ATP}} = \frac{k_{\text{cat}} \times [\text{ATP}]}{K_{\text{M}} + [\text{ATP}]},\tag{1}
$$

with

$$
k_{\text{cat}} = \frac{1}{\frac{1}{k_{\text{hyd}}} + \frac{1}{f \alpha K} + \frac{2 + Kf^{-2}}{s\beta}},
$$

and

$$
K_{\rm M} = \frac{(k_{\rm hyd} + k_{\rm off})}{k_{\rm hyd}} \times \frac{k_{\rm cat}}{k_{\rm on}}.\tag{2}
$$

For $s \ll 1$, the equations are the same except with the For $s \ll 1$, the equations are the same except with the transformation $f \rightarrow f^{-1}$. For large values of *s*, the stoi-
chiometry is +1 step for each ATP hydrolysed so the For $s \ll 1$, the equations are the same except with the transformation $f \rightarrow f^{-1}$. For large values of s , the stoichiometry is +1 step for each ATP hydrolysed, so the ATP driven mechanical velocity is $r = -d\mathcal{I}$ where transformation $f \rightarrow f^{-1}$. For large values of *s*, the stoi-
chiometry is +1 step for each ATP hydrolysed, so the
ATP-driven mechanical velocity is $v_{ATP} = d\mathcal{J}_{ATP}$, where *d*
is the step size (8 pm for kinesin) However chiometry is +1 step for each ATP hydrolysed, so the
ATP-driven mechanical velocity is $v_{\text{ATP}} = d\mathcal{J}_{\text{ATP}}$, where d
is the step size (8 nm for kinesin). However, in the weakly
nimed ATP-bound state, an applied force ATP-driven mechanical velocity is $v_{\text{ATP}} = d\mathcal{J}_{\text{ATP}}$, where d
is the step size (8 nm for kinesin). However, in the weakly
pinned ATP-bound state, an applied force can cause slip
via the transition indicated by the d is the step size (8 nm for kinesin). However, in the weakly
pinned ATP-bound state, an applied force can cause slip
via the transition indicated by the dashed line in the
kinetic mechanism (foure 4*a*). For a single head pinned ATP-bound state, an applied force can cause slip
via the transition indicated by the dashed line in the
kinetic mechanism (figure 4*a*). For a single head, or two
independent heads the term $dk = a(f^2 - f^{-2})P(F^{ATP})$ via the transition indicated by the dashed line in the
kinetic mechanism (figure 4*a*). For a single head, or two
independent heads, the term $dk_{\text{diff}} g(f^2 - f^{-2}) P(E^{\text{ATP}})$
would have to be added to v_{max} to obtain the kinetic mechanism (figure 4*a*). For a single head, or two
independent heads, the term $dk_{\text{diff}}(f^2 - f^{-2})P(E^{\text{ATP}})$
would have to be added to v_{ATP} to obtain the net velocity,
predicting that a force applied in the d independent heads, the term $dk_{\text{diff}} g(f^2 - f^{-2}) P(E^{\text{ATP}})$
would have to be added to v_{ATP} to obtain the net velocity,
predicting that a force applied in the direction of ATP-
catalysed motion would increase the observ would have to be added to v_{ATP} to obtain the net velocity,
predicting that a force applied in the direction of ATP-
catalysed motion would increase the observed velocity
without bound. Compined all (1997) carried ou predicting that a force applied in the direction of ATP-
catalysed motion would increase the observed velocity
without bound. Coppin *et al.* (1997) carried out such an experiment and found that while a force applied in the without bound. Coppin *et al.* (1997) carried out such an experiment and found that while a force applied in the direction of motion does in fact increase the velocity of the motor the effect saturates. This can be evolus experiment and found that while a force applied in the
direction of motion does in fact increase the velocity of
the motor, the effect saturates. This can be explained by a
cooperative two-headed model (Hackney 1994: Peski direction of motion does in fact increase the velocity of
the motor, the effect saturates. This can be explained by a
cooperative two-headed model (Hackney 1994; Peskin &
Oster 1995) where only one head can bind ATP at a t the motor, the effect saturates. This can be explained by a cooperative two-headed model (Hackney 1994; Peskin & Oster 1995) where only one head can bind ATP at a time, as schematically shown in figures $3b$ and 5. cooperative two-headed model (Hackney 1994; Peskin &

7. COOPERATIVE TWO-HEADED MOTOR

In our two-headed model (see figure 5), we consider that the heads can either be together (the minimum In our two-headed model (see figure 5), we consider
that the heads can either be together (the minimum
energy configuration, where the heads occupy neigh-
houring subunits) or apart (where the heads occupy subthat the heads can either be together (the minimum
energy configuration, where the heads occupy neigh-
bouring subunits) or apart (where the heads occupy sub-
units that are displaced relative to each other). We assume bouring subunits) or apart (where the heads occupy sub-
units that are displaced relative to each other). We assume

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igure 5. Pattern of kinesin stepping during normal coupled cycle (middle column), when a diffusive step to the left occurs efore hydrolysis at the active site (left column), and when a diffusive step to the right occurs be igure 5. Pattern
efore hydrolysis
right column).

hat ATP hydrolysis at the active site of one head cohat ATP hydrolysis at the active site of one head co-
peratively induces binding of ATP to the other, but that
TP hydrolysis at the second head cannot proceed until hat ATP hydrolysis at the active site of one head co-
peratively induces binding of ATP to the other, but that
TP hydrolysis at the second head cannot proceed until
.DP dissociates on the first head This ensures alternatin peratively induces binding of ATP to the other, but that
TP hydrolysis at the second head cannot proceed until
DP dissociates on the first head. This ensures alternating
the kinetics for the ATP hydrolysis which is well es ITP hydrolysis at the second head cannot proceed until
IDP dissociates on the first head. This ensures alternating
ite kinetics for the ATP hydrolysis which is well estab-LDP dissociates on the first head. This ensures alternating
ite kinetics for the ATP hydrolysis which is well estab-
shed experimentally (Gilbert *et al.* 1998). In this case,
here are three possibilities following ATP bin te kinetics for the ATP hydrolysis which is well estab-
shed experimentally (Gilbert *et al.* 1998). In this case,
here are three possibilities following ATP binding to the
rst head (i) ATP hydrolysis occurs while the hea shed experimentally (Gilbert *et al.* 1998). In this case, here are three possibilities following ATP binding to the rst head. (i) ATP hydrolysis occurs while the heads are verther (figure 5 middle column) inducing bindin here are three possibilities following ATP binding to the rst head. (i) ATP hydrolysis occurs while the heads are ogether (figure 5, middle column), inducing binding of rst head. (i) ATP hydrolysis occurs while the heads are
ogether (figure 5, middle column), inducing binding of
TP to the second head. The first head completes its
secondized and chemical cycle hydrolysing one ATP and % by sether (figure 5, middle column), inducing binding of Γ P to the second head. The first head completes its
rechanical and chemical cycle, hydrolysing one ATP and
sporting the motor one period to the right (ii). The nechanical and chemical cycle, hydrolysing one ATP and noving the motor one period to the right. (ii) The first rechanical and chemical cycle, hydrolysing one ATP and

poving the motor one period to the right. (ii) The first

and might diffuse a period to the right before ATP

vdrolysis at the active site occurs and induces ATP to hydrolysis at the active site occurs and induces ATP drawing the second bead which then rapidly moves to a ead might diffuse a period to the right before ATP
ydrolysis at the active site occurs and induces ATP to
ind to the second head which then rapidly moves to a
esition adjacent to the first head (figure 5, right column) ydrolysis at the active site occurs and induces ATP to ind to the second head which then rapidly moves to a osition adjacent to the first head (figure 5, right column). At this point, the motor is one period to the right of its station adjacent to the first head (figure 5, right column).
Let this point, the motor is one period to the right of its
arting position. Completion of the mechanical and
hemical cycle of the first head results in movement at this point, the motor is one period to the right of its
arting position. Completion of the mechanical and
hemical cycle of the first head results in movement an
distinct head results in movement and
distinct here is th a arting position. Completion of the mechanical and
hemical cycle of the first head results in movement an
ditional period to the right. Thus, the motor will have
a power while hydrolysing only one ATP movement and the first head results in movement and distributed by distributed by distributed period two steps while hydrolysing only one ATP. (iii) diditional period to the right. Thus, the motor will have
(iii) The first head might diffuse a period to the left
(iii) The first head might diffuse a period to the left
(efter ATP hydrolysis at the active site occu before two steps while hydrolysing only one ATP.

ii) The first head might diffuse a period to the left

efore ATP hydrolysis at the active site occurs (figure 5,
 $\frac{1}{2}$ fr column) Hydrolysis induces ATP to bind to the (iii) The first head might diffuse a period to the left efore ATP hydrolysis at the active site occurs (figure 5, $\frac{1}{2}$ ft column). Hydrolysis induces ATP to bind to the econd head and rapidly move to a position adjac efore ATP hydrolysis at the active site occurs (figure 5, $\frac{1}{2}$ ft column). Hydrolysis induces ATP to bind to the scond head and rapidly move to a position adjacent to be first head. At this point, the motor is one per ϵ ft column). Hydrolysis induces ATP to bind to the ϵ cond head and rapidly move to a position adjacent to he first head. At this point, the motor is one period to he left of its starting position. Completion of the the first head and rapidly move to a position adjacent to
the first head. At this point, the motor is one period to
the left of its starting position. Completion of the mechan-
and chemical cycle of the first head results if he first head. At this point, the motor is one period to the left of its starting position. Completion of the mechan-
Cal and chemical cycle of the first head results in movehe left of its starting position. Completion of the mechan-
Cal and chemical cycle of the first head results in move-
net one period to the right, back to the starting
osition. Thus, the motor will have moved zero steps If the move included a set of the move the move into one period to the right, back to the starting osition. Thus, the motor will have moved zero steps this hydrolysing one ATP. In the absence of an applied hile hydrolysing one ATP. In the absence of an applied not contribute to the net rate. These possibilities are

consistent with the observations that occasionally a motor consistent with the observations that occasionally a motor
may step back and then forward, but almost never takes
two steps backwards in a row (Schnitzer & Block 1997consistent with the observations that occasionally a motor
may step back and then forward, but almost never takes
two steps backwards in a row (Schnitzer & Block 1997;
Connin et al. 1997) may step back and t
two steps backwards
Coppin *et al.* 1997).
An external force l two steps backwards in a row (Schnitzer & Block 1997;
Coppin *et al.* 1997).
An external force biases the diffusive steps, making one

Coppin *et al.* 1997).
An external force biases the diffusive steps, making one
more likely than the other. The effect on the net velocity
can easily be calculated in terms of the splitting probabil-An external force biases the diffusive steps, making one
more likely than the other. The effect on the net velocity
can easily be calculated in terms of the splitting probabil-
ities at the branch point F^{ATP} . more likely than the other. The
can easily be calculated in term
ities at the branch point E^{ATP} :

$$
P_{\text{right}} = \frac{k_{\text{diff}} f^2}{k_{\text{diff}} (f^2 + f^{-2}) + k_{\text{hyd}}},
$$

$$
P_{\text{left}} = \frac{k_{\text{diff}} f^{-2}}{k_{\text{diff}} (f^2 + f^{-2}) + k_{\text{hyd}}}.
$$
 (3)

These probabilities are the fraction of molecules that, These probabilities are the fraction of molecules that,
having bound ATP, diffuse to the right or left before
hydrolyging ATP and are thus the fraction of events in These probabilities are the fraction of molecules that,
having bound ATP, diffuse to the right or left before
hydrolysing ATP and are thus the fraction of events in
which the motor moves two steps for one ATP and zero having bound ATP, diffuse to the right or left before
hydrolysing ATP and are thus the fraction of events in
which the motor moves two steps for one ATP and zero
steps for one ATP respectively. The net velocity can be hydrolysing ATP and are thus the fraction of events in which the motor moves two steps for one ATP and zero steps for one ATP, respectively. The net velocity can be written as which the m
steps for one
written as

$$
v_{\text{net}} = L\mathcal{J}_{\text{ATP}}(1 + P_{\text{right}} - P_{\text{left}}),\tag{4}
$$

net one period to the right, back to the starting $K_M = 60 \mu M$ and $k_{cat} = 100 s^{-1}$, in good agreement with osition. Thus, the motor will have moved zero steps experimental evidence (Schnitzer & Block 1997; Hua *et al.*) th $v_{\text{net}} = L \mathcal{J}_{\text{ATP}} (1 + P_{\text{right}} - P_{\text{left}}),$ (4)
where $(1 + P_{\text{right}} - P_{\text{left}})$ is the average number of steps
per ATP Figure 6*g* shows a plot of the velocity versus where $(1 + P_{\text{right}} - P_{\text{left}})$ is the average number of steps
per ATP. Figure 6*a* shows a plot of the velocity versus
external force at various ATP concentrations calculated where $(1 + P_{\text{right}} - P_{\text{left}})$ is the average number of steps
per ATP. Figure 6*a* shows a plot of the velocity versus
external force at various ATP concentrations calculated
using equations (1) (3) and (4) With the paramete per ATP. Figure 6*a* shows a plot of the velocity versus external force at various ATP concentrations calculated using equations (1) , (3) and (4) . With the parameters used, the Michaelis-Menten constants at zero force are using equations (1), (3) and (4). With the parameters used,
the Michaelis-Menten constants at zero force are
 $K_M = 60 \mu M$ and $k_{cat} = 100 s^{-1}$, in good agreement with
experimental evidence (Schnitzer & Block 1997; Hua *et a* the Michaelis-Menten constants at zero force are $K_M = 60 \mu M$ and $k_{\text{cat}} = 100 \text{ s}^{-1}$, in good agreement with experimental evidence (Schnitzer & Block 1997; Hua *et al.* 1997). The velocity is a nearly linear function o experimental evidence (Schnitzer & Block 1997; Hua et al. 1997). The velocity is a nearly linear function of the applied elastic force, and the extrapolated intercept $\frac{\text{(stopping force')}}{\text{time}}$ 1997). The velocity is a nearly linear function of the applied elastic force, and the extrapolated intercept ('stopping force'), above which no further forward

ig. 6. (*a*) Plot of velocity versus external elastic force at the TP concentrations, with $s = 10^5$, $K = 1000$, $\alpha = 10 s^{-1}$, $k = 1.1 s^{-1}$, $k_{\text{max}} = 25 s^{-1}$, $k_{\text{max}} = 125 s^{-1}$, $k_{\text{max}} = 2.0 \text{ M}^{-1} s^{-1}$, and , ig. 6. (*a*) Plot of velocity versus external elastic force at

TP concentrations, with $s = 10^5$, $K = 1000$, $\alpha = 10 s^{-1}$,
 $= 1 s^{-1}$, $k_{\text{diff}} = 25 s^{-1}$, $k_{\text{hyd}} = 125 s^{-1}$, $k_{\text{on}} = 2 \mu \text{M}^{-1} s^{-1}$, $a = 100 s^{-1}$ (*b* at three
¹,
, and
of **ATP** TP concentrations, with $s = 10^5$, $K = 1000$, $\alpha = 10 \text{ s}^{-1}$,
 $k = 1 \text{ s}^{-1}$, $k_{\text{diff}} = 25 \text{ s}^{-1}$, $k_{\text{hyd}} = 125 \text{ s}^{-1}$, $k_{\text{on}} = 2 \mu \text{M}^{-1} \text{ s}^{-1}$, and
 $\text{if } k = 100 \text{ s}^{-1}$. (*b*) Plot of the randomness as $c_{\text{off}} = 100 \text{ s}^{-1}$. (*b*) Plot of the randomness as a function of ATP pncentration for zero load (black curve), a force of 3 pN $\mu_{\text{eff}} = 100 \,\text{s}^{-1}$. (*b*) Plot of the randomness as a function of ATP oncentration for zero load (black curve), a force of 3 pN pposing ATP catalysed motion (dashed curve), and a force f 3 pN in the direction of ATP oncentration for zero load (black curve), a force of 3 pN
pposing ATP catalysed motion (dashed curve), and a force
f 3 pN in the direction of ATP catalysed motion (dotted
urve). We used the same parameters as in (a) with pposing ATP catalysed motion (dashed curve), and a force
f 3 pN in the direction of ATP catalysed motion (dotted
urve). We used the same parameters as in (*a*), with $r_{\infty} = 0.5$.
list reflects two approximately equal ra f 3 pN in the direction of ATP catalysed motion (dotted urve). We used the same parameters as in (*a*), with $r_{\infty} = 0.5$. This reflects two approximately equal rate-controlling steps in he chemical cycle at large [ATP]. In our model with the his reflects two approximately equal rate-controlling steps in

1 e chemical cycle at large [ATP]. In our model with the

arameters used these are ATP hydrolysis $k_{\text{hyd}} = 125 \text{ s}^{-1}$ and

DP release, with an effective re chemical cycle at large [ATP]. In our model with the arameters used these are ATP hydrolysis $k_{\text{hyd}} = 125 \text{ s}^{-1}$.

DP release, with an effective off rate $\beta s/K \approx 100 \text{ s}^{-1}$. DP release, with an effective off rate $\beta s/K \approx 100 s^{-1}$.

progress can be observed, is around 5 pN and indepen-
ent of ATP concentration, consistent with experimental rogress can be observed, is around 5 pN and independent of ATP concentration, consistent with experimental evalues (Syaboda & Block 1994). This stopping force is regress can be observed, is around 5 pN and independent of ATP concentration, consistent with experimental positive (Svoboda & Block 1994). This stopping force is mited by the free energy available from ATP bydrolysi ent of ATP concentration, consistent with experimental
absorbed & Block 1994). This stopping force is
mited by the free energy available from ATP hydrolysis.
The actual intercent where the velocity crosses zero and The actual intercept, where the velocity crosses zero and $\sum_{n=1}^{\infty}$ actual intercept, where the velocity crosses zero and $\sum_{n=1}^{\infty}$ comes negative can be arbitrarily large limited only mited by the free energy available from ATP hydrolysis.

The actual intercept, where the velocity crosses zero and

comes negative, can be arbitrarily large, limited only

v the largest kinetic barrier to motion found in a The actual intercept, where the velocity crosses zero and

acomes negative, can be arbitrarily large, limited only

by the largest kinetic barrier to motion found in any

bemical state. This is consistent with the results ecomes negative, can be arbitrarily large, limited only
y the largest kinetic barrier to motion found in any
hemical state. This is consistent with the results of loppin *et al.* (1997) who found that even at forces as high

8. STOCHASTIC BEHAVIOUR OF SINGLE-MOTOR STEPPING

Recently, several groups have studied the stepping notion of single motors (Svoboda et al. 1993; Vale et al. 996; Higuchi *et al.* 1997). Because the individual transions are stochastic, the displacement of a motor in a

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given time is characterized by an average value and a given time is characterized by an average value and a
variance. If the stepping is controlled by a single rate-
limiting process, the variance is large, but if a step is given time is characterized by an average value and a
variance. If the stepping is controlled by a single rate-
limiting process, the variance is large, but if a step is
made up of many discrete sub-transitions each of whi variance. If the stepping is controlled by a single rate-
limiting process, the variance is large, but if a step is
made up of many discrete sub-transitions each of which
on average takes about the same time, the variance limiting process, the variance is large, but if a step is
made up of many discrete sub-transitions each of which
on average takes about the same time, the variance is made up of many discrete sub-transitions each of which
on average takes about the same time, the variance is
much smaller. Svoboda *et al.* (1994) defined a randomness
parameter *r* in terms of the variance in the displace on average takes about the same time, the variance is
much smaller. Svoboda *et al.* (1994) defined a randomness
parameter r in terms of the variance in the displacement
of the motor due to ATP bydrolysis the average di much smaller. Svoboda *et al.* (1994) defined a randomness
parameter r in terms of the variance in the displacement
of the motor due to ATP hydrolysis, the average displace-
ment, and the step size d evaluated in the parameter r in terms of the variance in the displacement
of the motor due to ATP hydrolysis, the average displace-
ment, and the step size d evaluated in the limit of very
long observation time of the motor due to ATI
ment, and the step size
long observation time.
For a completely cour For a completely coupled kinetic cycle where hydrolysis
For a completely coupled kinetic cycle where hydrolysis
one ATP always produces one mechanical step of fixed

long observation time.
For a completely coupled kinetic cycle where hydrolysis
of one ATP always produces one mechanical step of fixed For a completely coupled kinetic cycle where hydrolysis
of one ATP always produces one mechanical step of fixed
length, the randomness varies between zero if many tran-
sitions of similar lifetime make up a single step (a of one ATP always produces one mechanical step of fixed
length, the randomness varies between zero if many tran-
sitions of similar lifetime make up a single step (a clock-
like mechanism), and unity if there is a single r length, the randomness varies between zero if many transitions of similar lifetime make up a single step (a clock-
like mechanism), and unity if there is a single rate--
limiting process (a 'Poisson' stepper). Thus for any sitions of similar lifetime make up a single step (a clock-
like mechanism), and unity if there is a single rate-
limiting process (a 'Poisson' stepper). Thus for any model, like mechanism), and unity if there is a single rate-
limiting process (a 'Poisson' stepper). Thus for any model,
r depends on ATP concentration (Schnitzer & Block
1997) At very low ATP concentration ATP binding must limiting process (a 'Poisson' stepper). Thus for any model,
 r depends on ATP concentration, ATP binding must

1997). At very low ATP concentration, ATP binding must

be the single rate-limiting step in the reaction and r depends on ATP concentration (Schnitzer & Block 1997). At very low ATP concentration, ATP binding must
be the single rate-limiting step in the reaction and r is
unity At intermediate ATP concentration, the number of 1997). At very low ATP concentration, ATP binding must
be the single rate-limiting step in the reaction and r is
unity. At intermediate ATP concentration, the number of
rate-controlling transitions is maximum because AT be the single rate-limiting step in the reaction and r is
unity. At intermediate ATP concentration, the number of
rate-controlling transitions is maximum because ATP
binding and other relatively slow steps will have sim unity. At intermediate ATP concentration, the number of
rate-controlling transitions is maximum because ATP
binding and other relatively slow steps will have similar
characteristic times thus minimizing the randomness rate-controlling transitions is maximum because ATP
binding and other relatively slow steps will have similar
characteristic times, thus minimizing the randomness. binding and other relatively slow steps will have similar
characteristic times, thus minimizing the randomness.
Finally, at very high ATP concentration, ATP binding no
longer plays any rate-controlling role and the randomn characteristic times, thus minimizing the randomness.
Finally, at very high ATP concentration, ATP binding no
longer plays any rate-controlling role and the randomness
annroaches a value r , characteristic of the number Finally, at very high ATP concentration, ATP binding no
longer plays any rate-controlling role and the randomness
approaches a value r_{∞} characteristic of the number of
rate-limiting transitions in the mechanism longer plays any rate-controlling role and the randomness
approaches a value r_{∞} characteristic of the number of
rate-limiting transitions in the mechanism. proaches a value r_{∞} characteristic of the number of
te-limiting transitions in the mechanism.
If the pathway is not completely coupled, hydrolysis of
 Γ **P** can sometimes produce more or less than one

rate-limiting transitions in the mechanism.

If the pathway is not completely coupled, hydrolysis of

ATP can sometimes produce more or less than one

mechanical step as described above. This situation is If the pathway is not completely coupled, hydrolysis of
ATP can sometimes produce more or less than one
mechanical step as described above. This situation is
somewhat more complicated and the randomness can be ATP can sometimes produce more or less than one mechanical step as described above. This situation is somewhat more complicated, and the randomness can be mechanical step as described above. This situation is
somewhat more complicated, and the randomness can be
larger than unity. For the kinetic model in figure 6, *r* can
be derived to be somewhat more col
larger than unity. I
be derived to be

be derived to be
\n
$$
r = \frac{1 - P_{\text{left}} + 3P_{\text{right}}}{1 - P_{\text{left}} + P_{\text{right}}} + (r_0 - 1)(1 - P_{\text{left}} + P_{\text{right}}),
$$
\n(5)

where

$$
r_0 = \frac{r_{\infty} + \frac{K_M^2}{[\text{ATP}]^2}}{\left(1 + \frac{K_M}{[\text{ATP}]}\right)^2},\tag{6}
$$

 $\left(1+\frac{\text{A}}{\text{ATP}}\right)$
is the randomness for the completely coupled cycle. A
plot of r versus [ATP] is shown in figure 6*b* for several is the randomness for the completely coupled cycle. A
plot of r versus [ATP] is shown in figure 6*b* for several
values of applied force. The black line is that for zero is the randomness for the completely coupled cycle. A plot of r versus [ATP] is shown in figure $6b$ for several values of applied force. The black line is that for zero force and is consistent with the experiments of S plot of r versus [ATP] is shown in figure $6b$ for several values of applied force. The black line is that for zero force and is consistent with the experiments of Schnitzer values of applied force. The black line is that for zero
force and is consistent with the experiments of Schnitzer
& Block (1997). The dashed and dotted lines are for
 -3 pN and $+3 \text{ pN}$ applied force, respectively. Block (1997). The dashed and dotted lines are for
 β pN and +3 pN applied force, respectively.

An important point to note is that in the limit of very
 α let k be model is very tightly counled and slowing

y the largest kinetic barrier to motion found in any of the motor is accompanied by a commensurate decrease hemical state. This is consistent with the results of in the rate of ATP hydrolysis, analogous to the Fenn effect -3 pN and $+3$ pN applied force, respectively.
An important point to note is that in the limit of very
small k_{diff} the model is very tightly coupled and slowing
of the motor is accompanied by a commensurate decrease An important point to note is that in the limit of very
small k_{diff} the model is very tightly coupled and slowing
of the motor is accompanied by a commensurate decrease
in the rate of ATP hydrolysis, analogous to the small k_{diff} the model is very tightly coupled and slowing
of the motor is accompanied by a commensurate decrease
in the rate of ATP hydrolysis, analogous to the Fenn effect
in myosin (Fenn 1924). In this limit the ran of the motor is accompanied by a commensurate decrease
in the rate of ATP hydrolysis, analogous to the Fenn effect
in myosin (Fenn 1924). In this limit the randomness
cannot be greater than unity Schnitzer & Block (1997) in the rate of ATP hydrolysis, analogous to the Fenn effect
in myosin (Fenn 1924). In this limit the randomness
cannot be greater than unity. Schnitzer & Block (1997),
however found a randomness of about 1.25 for kinesin a in myosin (Fenn 1924). In this limit the randomness cannot be greater than unity. Schnitzer & Block (1997), however, found a randomness of about 1.25 for kinesin at cannot be greater than unity. Schnitzer & Block (1997),
however, found a randomness of about 1.25 for kinesin at
low ATP concentration. With larger k_{diff} the motor is not
completely coupled, and at low ATP the randomn however, found a randomness of about 1.25 for kinesin at
low ATP concentration. With larger k_{diff} the motor is not
completely coupled, and at low ATP the randomness can
be greater than unity. Also, at large force, sig low ATP concentration. With larger k_{diff} the motor is not completely coupled, and at low ATP the randomness can
be greater than unity. Also, at large force, significant slip
occurs, and ATP hydrolysis, continues, even completely coupled, and at low ATP the randomness can
be greater than unity. Also, at large force, significant slip
occurs and ATP hydrolysis continues even when the motor comes to a halt. As seen in figure 6*b*, the randomness depends strongly on the applied force for motor comes to a halt. As seen in figure 6*b*, the randomness depends strongly on the applied force for $k_{\text{diff}} = 25 \text{ s}^{-1}$. However, for $k_{\text{diff}} < 1 \text{ s}^{-1}$ (not shown), the

randomness is far less sensitive to the applied force. Thus
reasuring the randomness at several forces will allow andomness is far less sensitive to the applied force. Thus
reasuring the randomness at several forces will allow
irect determination of k_{tot} and discrimination between andomness is far less sensitive to the applied force. Thus
reasuring the randomness at several forces will allow
irect determination of k_{diff} and discrimination between
while and loosely counled models % reasuring the randomness at several irrect determination of $k_{\rm diff}$ and disapility and loosely coupled models. ghtly and loosely coupled models.
9. PROCESSIVITY

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THE

Dimeric kinesin is highly processive, and can move for ver a hundred steps before dissociating from MTs. Dimeric kinesin is highly processive, and can move for

lead apparently also Ncd) is much

Monomeric kinesin (and apparently also Ncd) is much

also Ncd) is much

also neglective moving at most two to four steps before ver a hundred steps before dissociating from MTs.

Monomeric kinesin (and apparently also Ncd) is much

ess processive, moving at most two to four steps before

issociating In experiments, where the motors are Io domination (and apparently also Ncd) is much
so processive, moving at most two to four steps before
issociating. In experiments where the motors are
described onto a surface MT motion driven by monoass processive, moving at most two to four steps before issociating. In experiments where the motors are \blacktriangleright dsorbed onto a surface, MT motion driven by monoissociating. In experiments where the motors are

disorbed onto a surface, MT motion driven by mono-

different than that of

dhe dimeric wild-type The MT velocity increases almost In disorbed onto a surface, MT motion driven by monostreric kinesin is also qualitatively different than that of
The dimeric wild-type. The MT velocity increases almost
Integrally with increasing surface density of monomer learly kinesin is also qualitatively different than that of
the dimeric wild-type. The MT velocity increases almost
enearly with increasing surface density of monomers,
and is effectively zero in the limit that only one The dimeric wild-type. The MT velocity increases almost

rearly with increasing surface density of monomers,

and is effectively zero in the limit that only one

concept interacts with the MT This is similar to the mearly with increasing surface density of monomers,
and is effectively zero in the limit that only one
pronomer interacts with the MT. This is similar to the
property of myosin and is consistent with a motor that nd is effectively zero in the limit that only one

(behaviour of myosin, and is consistent with a motor that

(behaviour of myosin, and is consistent with a motor that

(between pulling nor offering appreciable resistance If also needs to the MT. This is similar to the chaviour of myosin, and is consistent with a motor that is neither pulling nor offering appreciable resistance to action a large fraction of the time i.e. a small duty

explored a motor that
in either pulling nor offering appreciable resistance to
ion a large fraction of the time, i.e. a small duty
atio (Howard 1997) Dimeric kinesin, however catalyses ration a large fraction of the time, i.e. a small duty
atio (Howard 1997). Dimeric kinesin, however, catalyses processive motion in the limit of very small surface atio (Howard 1997). Dimeric kinesin, however, catalyses
rocessive motion in the limit of very small surface
sensity, and the velocity quickly saturates with increasing
reface density of motors. This is consistent with a hi rocessive motion in the limit of very small surface

sensity, and the velocity quickly saturates with increasing

urface density of motors. This is consistent with a high

uty ratio motor that spends most of the time eithe ensity, and the velocity quickly saturates with increasing
arface density of motors. This is consistent with a high
uty ratio motor that spends most of the time either
ulling or immobile on the surface This is consistent with a high uty ratio motor that spends most of the time either ulling or immobile on the surface. ty ratio motor that spends most of the time either
Illing or immobile on the surface.
This behaviour is most often interpreted in terms of a
and-over-hand' mechanism for motion of dimers, where

ulling or immobile on the surface.
This behaviour is most often interpreted in terms of a
nand-over-hand' mechanism for motion of dimers, where
ne head dissociates and swings forward while the other This behaviour is most often interpreted in terms of a
and-over-hand' mechanism for motion of dimers, where
ne head dissociates and swings forward while the other
ead remains attached This swinging head then hinds head over-hand' mechanism for motion of dimers, where ne head dissociates and swings forward while the other ead remains attached. This swinging head then binds, ne head dissociates and swings forward while the other
ead remains attached. This swinging head then binds,
llowing the other head to release and swing forward.
The process continues with the heads strictly alternating ead remains attached. This swinging head then binds,
llowing the other head to release and swing forward.
The process continues, with the heads strictly alternating
alse as swinging arm and anchor. Because one head is llowing the other head to release and swing forward.
The process continues, with the heads strictly alternating
oles as swinging arm and anchor. Because one head is
lways firmly attached the duty cycle is very high and he velocity saturates at low motor surface density. lways firmly attached, the duty cycle is very high, and
he velocity saturates at low motor surface density.
Aotion catalysed by single-headed kinesin is pictured as
curring in a much more happeared fashion where an he velocity saturates at low motor surface density.

Aotion catalysed by single-headed kinesin is pictured as

ccurring in a much more haphazard fashion, where an

adividual motor must release the MT altogether before fotion catalysed by single-headed kinesin is pictured as
ccurring in a much more haphazard fashion, where an
idividual motor must release the MT altogether before
poving forward (Young et al. 1998). In the detached state ccurring in a much more haphazard fashion, where an idividual motor must release the MT altogether before ioving forward (Young *et al.* 1998). In the detached state in individual motor offers no resistance to motion cause by order to more provided in the set of the modern control of the motor molecules, so the velocity increases with χ or the velocity increases with χ or the velocity increases with χ or the velocity increases with In individual motor offers r

y other motor molecules,

acreasing surface density.

Our mechanism is entirely other motor molecules, so the velocity increases with
creasing surface density.
Our mechanism is entirely different. Neither head need
ssociate at all during a chemomechanical cycle

discording the density.

The correct at all during a chemomechanical cycle.

Intervalse at all during a chemomechanical cycle. However, in the ATP-bound state (in which an individual ead spends about 50% of the time) a monomer offers I dowever, in the ATP-bound state (in which an individual
read spends about 50% of the time) a monomer offers
it is attached,
it in the case of dimers at least one of the heads is tightly ead spends about 50% of the time) a monomer offers
the resistance to lateral motion even though it is attached,
it in the case of dimers at least one of the heads is tightly
linned reproducing the observed dependence o If the resistance to lateral motion even though it is attached,
it in the case of dimers at least one of the heads is tightly
inned, reproducing the observed dependence of velocity
in surface density of the motor. Dissocia \Box ut in the case of dimers at least one of the heads is tightly \bigcup inned, reproducing the observed dependence of velocity \bigcap n surface density of the motor. Dissociation is a side reac-) inned, reproducing the observed dependence of velocity

in surface density of the motor. Dissociation is a side reac-

ion and not an essential element of the chemomechanical

velocities for the picture is apploased to n surface density of the motor. Dissociation is a side reaction and not an essential element of the chemomechanical yele (see figure $4*b*,*c*$). This picture is analogous to the reatment of Young *et al.* (1994) for ion and not an essential element of the chemomechanical
ycle (see figure $4b$,*c*). This picture is analogous to the
reatment of Young *et al.* (1994) for processivity of ATP-
riven translocases such as DNA belicase If di ycle (see figure $4b$, c). This picture is analogous to the reatment of Young *et al.* (1994) for processivity of ATP-
riven translocases such as DNA helicase. If dissociation is llowed mainly from the ADP-bound state, the probability hat a monomeric motor (figure $4b$) dissociates in a given riven translocases such as DNA helicase. If dissociation is
llowed mainly from the ADP-bound state, the probability
hat a monomeric motor (figure 4*b*) dissociates in a given
 $\sum_{i} \text{FP}$ hydrolysis cycle is $\frac{P}{P} = k / [k$ Howed mainly from the ADP-bound state, the probability
hat a monomeric motor (figure 4b) dissociates in a given
 $\mathcal{P}(\mathbf{P}|\mathbf{R})$ and $\mathcal{P}(\mathbf{R})$ are *k*_d is the rate constant for dissociation in the ADP-
hand sta TP hydrolysis cycle is $P_{\text{mon}} = k_d/[k_d + \beta s/(1 + Kf^{-2})]$,
there k_d is the rate constant for dissociation in the ADP-
ound state. The average number of steps per encounter
ith the MT is $N = P^{-1} - 1 - \beta s/[(1 + Kf^{-2})k]$ where k_d is the rate constant for dissociation in the ADP-
ound state. The average number of steps per encounter
ith the MT is $\mathcal{N}_{\text{mon}} = P_{\text{mon}}^{-1} - 1 = \beta s/[(1 + Kf^{-2})k_d]$.
Vith $k = 100 s^{-1}$ and the parameters used to ob ound state. The average number of steps per encounter
 *i*th the MT is $N_{\text{mon}} = P_{\text{mon}}^{-1} - 1 = \beta s/[(1 + Kf^{-2})k_d]$.

Vith $k_d = 100 s^{-1}$ and the parameters used to obtain the

t shown in figure 6 $N \approx 2$ *i*th the MT is $\mathcal{N}_{\text{mon}} = P_{\text{mon}}^{-1}$
Vith $k_d = 100 \text{ s}^{-1}$ and the param t shown in figure 6, $\mathcal{N}_{\text{mon}} \approx 2$. *t* shown in figure 6, $\mathcal{N}_{\text{mon}} \approx 2$.
hil. Trans. R. Soc. Lond. B (2000)

Dissociation of a dimer, in contrast, requires two Dissociation of a dimer, in contrast, requires two
sequential dissociation events. Following dissociation of
the ADP-bound head, the other head remains tightly Dissociation of a dimer, in contrast, requires two
sequential dissociation events. Following dissociation of
the ADP-bound head, the other head remains tightly
bound The effective rate constant for dissociation of this sequential dissociation events. Following dissociation of
the ADP-bound head, the other head remains tightly
bound. The effective rate constant for dissociation of this
tightly bound head is probably much smaller than k the ADP-bound head, the other head remains tightly
bound. The effective rate constant for dissociation of this
tightly bound head is probably much smaller than k_d and
we label it k^* . While the one head is bound, the bound. The effective rate constant for dissociation of this
tightly bound head is probably much smaller than k_d and
we label it k_d^* . While the one head is bound, the disso-
ciated ADP-bound head has a high local conc we label it k_d^* . While the one head is bound, the disso-
ciated ADP-bound head has a high local concentration
(of order 1M), and the recombination rate constant is
 $k \cdot \exp\left(\frac{\Delta U}{k_a T}\right)$ where ΔU is the binding energ tightly bound head is probably much smaller than k_d and
we label it k_d^* . While the one head is bound, the disso-
ciated ADP-bound head has a high local concentration
(of order 1M) and the recombination rate constant ciated ADP-bound head has a high local concentration
(of order 1M), and the recombination rate constant is
 $k_d \exp(\Delta U / k_B T)$, where ΔU is the binding energy. For (of order 1M), and the recombination rate constant is k_d exp ($\Delta U / k_B T$), where ΔU is the binding energy. For this mechanism the probability per cycle that the dimer dissociates can be calculated from k_d exp ($\Delta U / k_B T$), where ΔU is the bithis mechanism the probability per cyclissociates can be calculated from

$$
P_{\text{dim}} = P_{\text{mon}} \frac{k_{\text{d}}^{*}}{k_{\text{d}}^{*} + k_{\text{d}} \exp[\Delta U/(k_{\text{B}} T)]} + \left| 1 - \frac{k_{\text{d}}^{*}}{k_{\text{d}}^{*} + k_{\text{d}} \exp[\Delta U/(k_{\text{B}} T)]} \right| P_{\text{dim}} \right|, \tag{7}
$$

and thus the number of steps before dissociation is

and thus the number of steps before dissociation is
\n
$$
\mathcal{N}_{\text{dim}} = P_{\text{dim}}^{-1} - 1 = \mathcal{N}_{\text{mon}} \frac{k_d^* + k_d \exp[\Delta U/(k_B T)]}{k_d^*}.
$$
\n(8)

We see that with very reasonable values for the binding We see that with very reasonable values for the binding
energy of only $10-20$ kJ mol⁻¹ a dimer can take a hundred
steps per encounter even if the monomer takes only two We see that with very reasonable values for the binding
energy of only $10-20 \text{ kJ} \text{ mol}^{-1}$ a dimer can take a hundred
steps per encounter even if the monomer takes only two
with $k \geq k^*$ with $k_d \geq k_d^*$.

10. DISCUSSION AND CONCLUSIONS

The process continues, with the heads strictly alternating ities of different binding states for ADP and Pi release.

Les as swinging arm and anchor. Because one head is This mechanism is very similar to that for how ion p dividual motor must release the MT altogether before relative affinities and barrier heights between neigh-
noving forward (Young *et al.* 1998). In the detached state bouring binding sites. The timing and regulation is
n We have discussed a `Brownian ratchet' mechanism for motion of motor proteins in the kinesin family where the We have discussed a 'Brownian ratchet' mechanism for
motion of motor proteins in the kinesin family where the
direction of motion is governed by the rates and specific-
ities of different binding states for ADP and Pi rele motion of motor proteins in the kinesin family where the direction of motion is governed by the rates and specificities of different binding states for ADP and Pi release.
This mechanism is very similar to that for how ion direction of motion is governed by the rates and specific-
ities of different binding states for ADP and Pi release.
This mechanism is very similar to that for how ion pumps
counle ATP hydrolysis to ion transport across me ities of different binding states for ADP and Pi release.
This mechanism is very similar to that for how ion pumps
couple ATP hydrolysis to ion transport across membranes.
Motion and force generation involve transitions be This mechanism is very similar to that for how ion pumps
couple ATP hydrolysis to ion transport across membranes.
Motion and force generation involve transitions between
states that are close to thermal equilibrium even at couple ATP hydrolysis to ion transport across membranes.
Motion and force generation involve transitions between
states that are close to thermal equilibrium even at a very
large driving force. ATP energy is used to change Motion and force generation involve transitions between
states that are close to thermal equilibrium even at a very
large driving force. ATP energy is used to change the
relative affinities and barrier beights between neig states that are close to thermal equilibrium even at a very large driving force. ATP energy is used to change the large driving force. ATP energy is used to change the relative affinities and barrier heights between neighbouring binding sites. The timing and regulation is controlled by thermally activated steps from the H to I relative affinities and barrier heights between neigh-
bouring binding sites. The timing and regulation is
controlled by thermally activated steps from the H to L
sites and the H sites act as switching stations where the bouring binding sites. The timing and regulation is controlled by thermally activated steps from the H to L sites, and the H sites act as switching stations where the chemical rates are compared to the mechanical $H \rightarrow I$ controlled by thermally activated steps from the H to L
sites, and the H sites act as switching stations where the
chemical rates are compared to the mechanical $H\rightarrow L$
transition rate. The H and L sites may represent eith sites, and the H sites act as switching stations where the
chemical rates are compared to the mechanical $H\rightarrow L$
transition rate. The H and L sites may represent either
different physical locations along the MT or differen chemical rates are compared to the mechanical $H\rightarrow L$
transition rate. The H and L sites may represent either
different physical locations along the MT or different
conformations of the kinesin head. This simple model transition rate. The H and L sites may represent either
different physical locations along the MT or different
conformations of the kinesin head. This simple model
shows that Brownian ratchet mechanisms can have a stoidifferent physical locations along the MT or different conformations of the kinesin head. This simple model shows that Brownian ratchet mechanisms can have a stoi-chiometry very close to unity and offers a new way of conformations of the kinesin head. This simple model
shows that Brownian ratchet mechanisms can have a stoi-
chiometry very close to unity and offers a new way of
thinking about the how molecular motors work shows that Brownian ratchet mechanisms can have a stoi-
chiometry very close to unity and offers a new way of
thinking about the how molecular motors work. iometry very close to unity and offers a new way of
inking about the how molecular motors work.
Our picture of how ATP hydrolysis causes directed
pation is entirely different from the mechanical hand-

thinking about the how molecular motors work.
Our picture of how ATP hydrolysis causes directed
motion is entirely different from the mechanical hand-
over-hand model often used to interpret the observation Our picture of how ATP hydrolysis causes directed
motion is entirely different from the mechanical hand-
over-hand model often used to interpret the observation
that kinesin dimers can move many steps along a MT motion is entirely different from the mechanical hand-
over-hand model often used to interpret the observation
that kinesin dimers can move many steps along a MT over-hand model often used to interpret the observation
that kinesin dimers can move many steps along a MT
without dissociating. The hand-over-hand model requires
each head to successively detach from the MT swing that kinesin dimers can move many steps along a MT
without dissociating. The hand-over-hand model requires
each head to successively detach from the MT, swing
forward, and reattach to it. In contrast, our mechanism without dissociating. The hand-over-hand model requires
each head to successively detach from the MT, swing
forward, and reattach to it. In contrast, our mechanism
does not require dissociation as an obligatory step in the each head to successively detach from the MT, swing
forward, and reattach to it. In contrast, our mechanism
does not require dissociation as an obligatory step in the forward, and reattach to it. In contrast, our mechanism
does not require dissociation as an obligatory step in the
mechanochemical cycle, but does require relatively free
lateral diffusion of a head while in the tightly as does not require dissociation as an obligatory step in the
mechanochemical cycle, but does require relatively free
lateral diffusion of a head while in the tightly associated
ATP-bound state. The dissociation due to ADP bi mechanochemical cycle, but does require relatively free
lateral diffusion of a head while in the tightly associated
ATP-bound state. The dissociation due to ADP binding
observed experimentally is viewed as a side reaction lateral diffusion of a head while in the tightly associated
ATP-bound state. The dissociation due to ADP binding
observed experimentally is viewed as a side reaction.

To directly compare our model with mechanical experiments on kinesin in which the effect of external

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prce on the velocity of motion was studied, we integral of the velocity of motion was studied, we introduced a cooperative two-headed model. In this nodel one head of kinesin at random binds ATP Hydroordeed a cooperative two-headed model. In this nodel one head of kinesin at random binds ATP. Hydro-
vis of ATP induces binding of ATP to the other head it is not a cooperative two-headed model. In this noted one head of kinesin at random binds ATP. Hydrosis of ATP induces binding of ATP to the other head, educing the activation barrier for transition to a neighreducible one head of kinesin at random binds ATP. Hydro-
rsis of ATP induces binding of ATP to the other head,
educing the activation barrier for transition to a neighvsis of ATP induces binding of ATP to the other head,
educing the activation barrier for transition to a neigh-
ouring binding site. As the first head continues through
solutionally continues through
solutionally continues educing the activation barrier for transition to a neigh-
ouring binding site. As the first head continues through
s catalytic cycle, moving a period to the right in
 $\frac{1}{2}$ and $\frac{1}{2}$ are $\frac{1}{2}$ and $\frac{1}{2}$ and ouring binding site. As the first head continues through
s catalytic cycle, moving a period to the right in
gure $3b$, the second head is more or less 'dragged' along
our the ride This model is able to explain how a rando is catalytic cycle, moving a period to the right in gure $3b$, the second head is more or less 'dragged' along gure $3b$, the second head is more or less 'dragged' along

and γ is obtained, and predicts that a

and predicts that a

and predicts that a

are opposing the ATP-driven motion will decrease the or the ride. This model is able to explain how a random-
ess greater than unity is obtained, and predicts that a
orce opposing the ATP-driven motion will decrease the
andomness at low ATP concentration and increase it at ess greater than unity is obtained, and predicts that a
prec opposing the ATP-driven motion will decrease the
andomness at low ATP concentration and increase it at high ATP-driven motion will decrease the
andomness at low ATP concentration and increase it at
 \blacktriangleleft igh ATP concentration, and that a force acting in the
sirection of ATP-driven motion will increase the randomandomness at low ATP concentration and increase it at

igh ATP concentration, and that a force acting in the

irection of ATP-driven motion will increase the random-

less at all ATP concentrations let igh ATP concentration, and the irection of ATP-driven motion
and the set all ATP concentrations.
An interesting prediction of rection of ATP-driven motion will increase the random-
ss at all ATP concentrations.
An interesting prediction of the model is that if the
reraction between the heads were stiffened by substi-

Figure 2 css at all ATP concentrations.

An interesting prediction of the model is that if the

different neck region the motor could still work

different neck region, the motor could still work An interesting prediction of the model is that if the
traction between the heads were stiffened by substi-
ling a different neck region, the motor could still work
left or right The vector of the probability for different out of substitution of the motor could still work that the probability for diffusion to the left or right at the F^{ATP} state would be significantly reduced. This

The EATP state would be significantly reduced. This is the EATP state would be significantly reduced. This could cause a more complete coupling resulting in a Well, but the probability for diffusion to the left or right
 $\frac{1}{1}$ the E^{ATP} state would be significantly reduced. This
 $\frac{1}{2}$ coupling cause a more complete coupling, resulting in a

verbolic flow-force curve hence E^{ATP} state would be significantly reduced. This vould cause a more complete coupling, resulting in a yperbolic flow–force curve, and the randomness would be decreased. This should be testable using the construct be decreased. This should be testable using the construct of Romberg *et al.* (1998) yperbolic flow–force curve, and the randomness would

e decreased. This should be testable using the construct
 $\bigcap_{n=0}^{\infty}$ f Romberg *et al.* (1998). decreased. This should be testable using the construct
Romberg *et al.* (1998).
We made several simplifying assumptions to allow us to
press the chemical and mechanical rates in terms of only

We made several simplifying assumptions to allow us to appress the chemical and mechanical rates in terms of only few parameters not taken directly from experiment: K , We made several simplifying assumptions to allow us to appress the chemical and mechanical rates in terms of only few parameters not taken directly from experiment: *K*, \lim_{diff} , α , β and *s*. Nevertheless, the m few parameters not taken directly from experiment: K ,
 $_{\text{diff}}$, α , β and s. Nevertheless, the model fits experimental

ata on kinesin for velocity as a function of external force,

nd the observed stochlometry an α , β and *s*. Nevertheless, the model fits experimental ata on kinesin for velocity as a function of external force, nd the observed stoichiometry and statistical behaviour f single-molecule stepping extremely well. ata on kinesin for velocity as a function of external force, nd the observed stoichiometry and statistical behaviour f single-molecule stepping extremely well. We anticipate nd the observed stoichiometry and statistical behaviour
f single-molecule stepping extremely well. We anticipate
at transient experiments on the biochemical mechanisms
f ATP hydrolysis by kinesin and Ncd (Gilbert *et al.* f single-molecule stepping extremely well. We anticipate
at transient experiments on the biochemical mechanisms
f ATP hydrolysis by kinesin and Ncd (Gilbert *et al.* 1995;
*A*₂ & Taylor 1997: Pechatnikova & Taylor 1997) at transient experiments on the biochemical mechanisms
f ATP hydrolysis by kinesin and Ncd (Gilbert *et al.* 1995;
Ia & Taylor 1997; Pechatnikova & Taylor 1997) can be
sed to further constrain the rate constants f ATP hydrolysis by kinesin and Ncd (Gilbert *et al.* 1995; *Ia & Taylor 1997*) can be sed to further constrain the rate constants.

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Discussion

Discussion

L. Cruzeiro-Hansson (*Department of Mathematics*, *Heriot-Watt University*, *Edinburgh*, *UK*). Ratchets are a clever way of explaining directional motion However I L. Cruzeiro-Hansson (Department of Mathematics,
Heriot-Watt University, Edinburgh, UK). Ratchets are a
clever way of explaining directional motion. However, I
have a bit of difficulty in manning them to the realities Heriot-Watt University, Edinburgh, UK). Ratchets are a clever way of explaining directional motion. However, I have a bit of difficulty in mapping them to the realities of kinesin and Ncd. It would be simple if we could th clever way of explaining directional motion. However, I
have a bit of difficulty in mapping them to the realities
of kinesin and Ncd. It would be simple if we could think of MT as providing a ratchet potential in which kinesin of kinesin and Ncd. It would be simple if we could think
of MT as providing a ratchet potential in which kinesin
and Ncd were the moving particles. But even if we did
not know any better, just the fact that kinesin and Ncd of MT as providing a ratchet potential in which kinesin
and Ncd were the moving particles. But even if we did
not know any better, just the fact that kinesin and Ncd
move in different directions means that the shape of the and Ncd were the moving particles. But even if we did
not know any better, just the fact that kinesin and Ncd
move in different directions means that the shape of the
notential, which determines these directions, is define not know any better, just the fact that kinesin and Ncd
move in different directions means that the shape of the
potential, which determines these directions, is defined
by the interaction between kinesin and Ncd with MT. move in different directions means that the shape of the potential, which determines these directions, is defined
by the interaction between kinesin and Ncd with MT.
But, as we heard yesterday, the direction of motion is
determined by a part of kinesin and Ncd that does not by the interaction between kinesin and Ncd with MT.
But, as we heard yesterday, the direction of motion is
determined by a part of kinesin and Ncd that does not
interact with MT. So, the shape of the potential is deter-But, as we heard yesterday, the direction of motion is
determined by a part of kinesin and Ncd that does not
interact with MT. So, the shape of the potential is deter-
mined by parts of the molecules that are relatively fa determined by a part of kinesin and Ncd that does not
interact with MT. So, the shape of the potential is deter-
mined by parts of the molecules that are relatively far
from MT. interact with MT. So, the shape of the potential is deterined by parts of the molecules that are relatively farm MT.
There is the idea that if you have a kinetic or a ther-
polynamic model for a conformational change, and

from MT.
There is the idea that if you have a kinetic or a thermodynamic model for a conformational change, and
measure or calculate rate constants or dissociation There is the idea that if you have a kinetic or a thermodynamic model for a conformational change, and
measure or calculate rate constants or dissociation
constants that necessarily means that the conformamodynamic model for a conformational change, and
measure or calculate rate constants or dissociation
constants that necessarily means that the conformameasure or calculate rate constants or dissociation
constants that necessarily means that the conforma-
tional change takes place by thermal activation, i.e.
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random fluctuations. But kinetic or thermodynamic
models apply equally if the conformational change tional change takes place by thermal activation, i.e.
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models apply equally if the conformational change
takes place in a more deterministic fashion. The only random fluctuations. But kinetic or thermodynamic
models apply equally if the conformational change
takes place in a more deterministic fashion. The only
way we can distinguish is by measuring bow fast a models apply equally if the conformational change
takes place in a more deterministic fashion. The only
way we can distinguish is by measuring how fast a
conformational change takes place after the action of takes place in a more deterministic fashion. The only
way we can distinguish is by measuring how fast a
conformational change takes place after the action of
the trigger If it is say nanoseconds then it is not by way we can distinguish is by measuring how fast a conformational change takes place after the action of the trigger. If it is say nanoseconds, then it is not by thermal activation. conformational change takes place after the action of

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