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Work, dissipation, and fluctuations in nonequilibrium physics

Bidirectional control—a unifying view of the structure and function of proteins

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Abstract

The chemo-mechanical cycles of a single head of myosin and that of kinesin are analyzed qualitatively, based on the framework of the bidirectional control for autonomous systems working under thermal and chemical fluctuations [K. Sekimoto, Physica D 205 (2005) 242]. This framework supposes two allosteric coordinates each joining a sensor with a gate. The analysis shows a fundamental parallelism between these motors, despite the apparent differences, with a common hidden symmetry-breaking of the chemo-mechanical cycles. The results give insights into the structure–function relationship of these motor proteins. ***To cite this article: K. Sekimoto, C. R. Physique 8 (2007).***

Résumé

Contrôle bidirectionnel : une vue unifiée de la structure et de la fonction des protéines. Les cycles chimio-mécaniques d'une seule tête de myosine et ceux de la kinésine sont analysés qualitativement sur base du schéma du contrôle bidirectionnel pour les systèmes autonomes fonctionnant sous des fluctuations thermiques et chimiques [K. Sekimoto, Physica D 205 (2005) 242]. Ce schéma suppose deux coordonnées allostériques chacune joignant un senseur avec une porte. L'analyse montre un parallélisme fondamental entre ces moteurs en dépit de différences apparentes, avec une commune brisure de symétrie cachée des cycles chimio-mécaniques. Les résultats donnent une perspective sur la relation entre la structure et la fonction de ces protéines motrices. ***Pour citer cet article : K. Sekimoto, C. R. Physique 8 (2007).***

Keywords: Autonomous system; Molecular motor; Bidirectional control

Mots-clés : Système autonome ; Moteur moléculaire ; Contrôle bidirectionnel

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1. Introduction

Since the end of the last century, the far from equilibrium physics of *externally controlled* systems has been very much advanced, as shown by the articles in these proceedings. The biophysics of molecular machines has also much developed over a similar period, mainly through single-molecule experiments and three-dimensional structural analysis of proteins. Molecular machines can, however, run their chemo-mechanical cycle autonomously *without external control*. Therefore, the far from equilibrium physics of autonomous systems deserves interest, as a still new field of research. In the present article a concept of 'bidirectional control' is proposed as a framework to look into the structure-function relationship of autonomous molecular machines, with special reference to the two molecular motors, myosins and kinesins [1].

Fig. 1(a) shows a simple schema of the molecular transporter which pumps up a load (L; blue) particle from its low-chemical potential reservoir (L, ℓ) to the high-chemical potential one (L, h), at the cost of the passive diffusion of a fuel (F; red) particle from its high-chemical potential reservoir (F, h) to the low-chemical potential one (F, ℓ). We especially focus on the process drawn in Fig. 1(b), where 1_{F} and 1_{L} indicate the 'presence' (see below) of the fuel and load particles, respectively, in the machine, while 0_{F} and 0_{L} indicate their 'absence'. In the presence of a load particle without a fuel particle ($(0_{\mathsf{F}}, 1_{\mathsf{L}})$ in the figure), the fuel particle can arrive from the (F, h) reservoir to realize $(1_{\mathsf{F}}, 1_{\mathsf{L}})$. From this state the departure of a load particle is constrained to the (L, h) reservoir, to realize $(1_{\mathsf{F}}, 0_{\mathsf{L}})$. This state permits the departure of the fuel particle to the (F, ℓ) reservoir, to realize $(0_{\mathsf{F}}, 0_{\mathsf{L}})$. The last state in turn allows the arrival of a new load particle from (L, h). The system then returns the state $(0_{\mathsf{F}}, 1_{\mathsf{L}})$, and the new cycle starts. In the figure, on-site (free) energy levels ($\epsilon^{0_{\mathsf{F}}}_{1_{\mathsf{L}}}$, etc.) and rate constants ($k_{\mathsf{F},h}$, etc.) are also assigned. The transporter works, i.e. performs the average counterclockwise cycles on the diagram of Fig. 1(b), as far as the chemical potentials of the particles in each reservoir, $\mu_{\mathsf{F},h}$ etc. satisfy the thermodynamical requirement: $\mu_{\mathsf{F},h} - \mu_{\mathsf{F},\ell} \geqslant \mu_{\mathsf{L},h} - \mu_{\mathsf{L},\ell}$. In fact, the steady-state circulation can be written

$$\text{(positive factor)} \times \left(\mathrm{e}^{(\mu_{\mathsf{F},h}-\mu_{\mathsf{F},\ell})/k_{\mathrm{B}}T} - \mathrm{e}^{(\mu_{\mathsf{L},h}-\mu_{\mathsf{L},\ell})/k_{\mathrm{B}}T}\right)$$

The chemical kinetics approach has recently been put forward to aid the understanding of single-molecule experiments, taking account of applied forces [2] and intermediate steps [3,4]. This approach postulates a set of chemical states and the rates of transitions among them, and studies the global statistics of the probability flow on the network of transitions [5]. In order to understand the structure-function relationship of molecular machines, we need to go a step further to know how those chemical states are organized. For example, if a machine has n independent molecular switches, they give rise to 2^n states. The question is to identify those switches. The approach of the present article is to

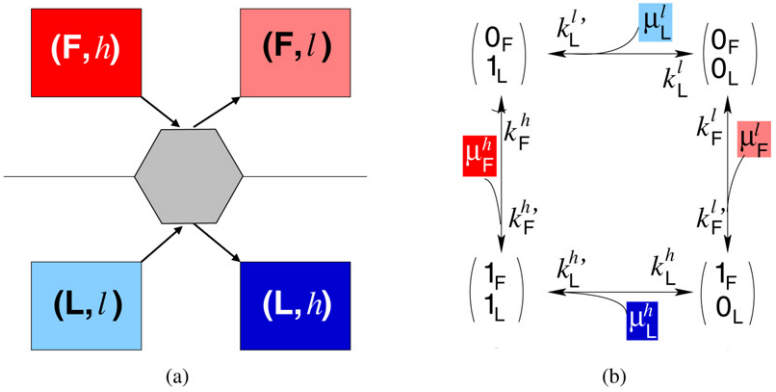


Fig. 1. (a) A simple schema for the function of a molecular transporter. Two reservoirs of fuel (F) of high (h) [low (ℓ)] densities are shown in dark [light] blue, and those corresponding to load (L) particles are shown in dark read [pink], respectively. The arrows indicate the direction of the average particle fluxes. The temperature T is supposed to be constant. (b) Chemical kinetic diagram specifying the molecular transporter of (a). The side branches indicate the export or import of particles between the transporter and the particle reservoirs, which are indicated by their chemical potential, e.g. μ^h_{F} for the (F, h) reservoir, etc. The rate constants (ks and k's) of the state transitions are defined in the figure. They obey the following detailed-balance relationships [7–9]: $k^{\ell\prime}_{\mathsf{L}} = k^{\ell}_{\mathsf{L}} \exp[\beta(\mu^{\ell}_{\mathsf{L}} + \epsilon^{0_{\mathsf{F}}}_{0_{\mathsf{L}}} - \epsilon^{0_{\mathsf{F}}}_{1_{\mathsf{L}}})]$, $k^{h\prime}_{\mathsf{F}} = k^{h}_{\mathsf{F}} \exp[\beta(\mu^{h}_{\mathsf{F}} + \epsilon^{0_{\mathsf{F}}}_{1_{\mathsf{L}}} - \epsilon^{1_{\mathsf{F}}}_{1_{\mathsf{L}}})]$, $k^{h\prime}_{\mathsf{L}} = k^{h}_{\mathsf{L}} \exp[\beta(\mu^{h}_{\mathsf{L}} + \epsilon^{1_{\mathsf{F}}}_{0_{\mathsf{L}}} - \epsilon^{1_{\mathsf{F}}}_{1_{\mathsf{L}}})]$, and $k^{\ell\prime}_{\mathsf{F}} = k^{\ell}_{\mathsf{F}} \exp[\beta(\mu^{\ell}_{\mathsf{F}} + \epsilon^{0_{\mathsf{F}}}_{0_{\mathsf{L}}} - \epsilon^{1_{\mathsf{F}}}_{0_{\mathsf{L}}})]$, where the ϵ's indicate the (free) energy associated to each state.

postulate the principle of the bidirectional control as a working hypothesis, and to see to what extent this principle can adapt to the chemical kinetics of myosin and kinesin molecules. The article is organized as follows: a brief summary of the basic principle [6] is given in Section 2. The model is then applied to the chemo-mechanical cycles of single heads of myosin and of kinesin on the formal functional level (Section 3) and on the structural level (Section 4). In the last section, Section 5, we conclude and some perspectives are given.

2. Bidirectional control

The chemical kinetics of Fig. 1(b) is reproduced by the following simple principle, called bidirectional control [6], see Fig. 2(a):

(i) In the molecular transporter there are two internal degrees of freedom ('allosteric coordinates'), each of which links between the interface with fuel reservoirs and that with load reservoirs. In this paper they are assumed to be single-bit Boolean variables.
(ii) The two links are arranged bidirectionally: the one combines a 'sensor' of fuel particles at the fuel–machine interface (F-sensor, for short) and a 'gate' of load particles at the load–machine interface (L-gate), and the other combines a 'sensor' of load particles at the load–machine interface (L-sensor) and a 'gate' of fuel particles at the fuel–machine interface (F-gate).
(iii) Each sensor is supposed to admit, at most, one particle at a time, by steric exclusion.
(iv) Each gate regulates the access of particles to the sensor at the same interface (e.g. the F-gate controls the access to the F-sensor).
(v) The links obey a single rule: "the detection [non-detection] by the sensor at one interface imposes the gate at the opposite interface to allow the access exclusively by the high-density [low-density] reservoir, respectively" (e.g. when the L-sensor detects a L-particle, the action of the F-gate is such that the F-particle's access to the F-sensor is limited by the (F, h) reservoir).

With these two allosteric coordinates undergoing stochastic transitions, the diffusion of a fuel particle, $(\mathsf{F}, h) \to (\mathsf{F}, \ell)$, induces the upward transport of a load particle, $(\mathsf{L}, \ell) \to (\mathsf{L}, h)$, through the cycle, $(0_\mathsf{F}, 1_\mathsf{L}) \to (1_\mathsf{F}, 1_\mathsf{L}) \to (1_\mathsf{F}, 0_\mathsf{L}) \to (0_\mathsf{F}, 0_\mathsf{L})$, see Figs. 2(b) and (c). The schema of Fig. 1(b) is thus constructed.

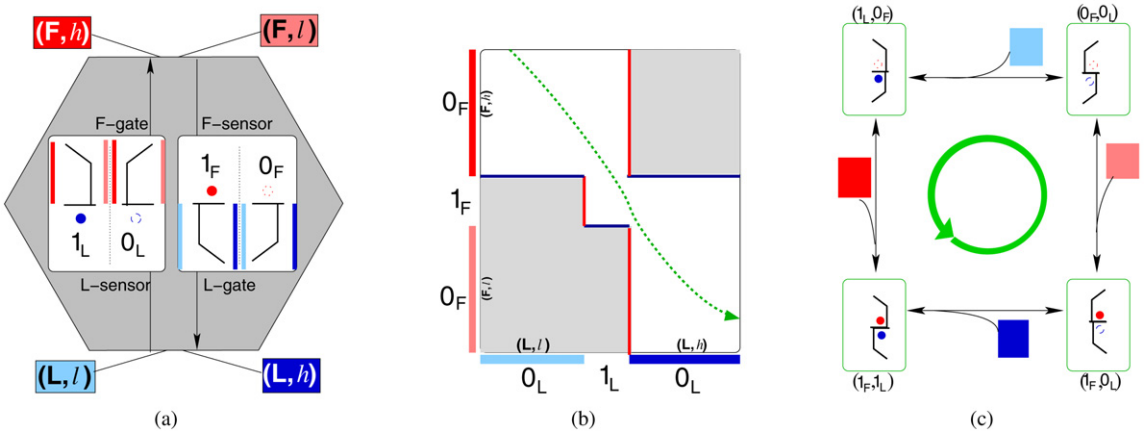


Fig. 2. (a) Function of the two allosteric coordinates, each connecting a sensor of fuel [load] particles to a gate of load [fuel] particles, respectively. For example, at the interface with the load reservoirs a load particle (filled blue dot) is detected by L-sensor with the left coordinate ('LF degree of freedom') on the one hand, and the access to this sensor from the reservoirs (L, ℓ) and (L, h) is controlled by the L-gate with the right coordinate ('FL degree of freedom') on the other hand. (b) Schematic representation of combined function of the two allosteric coordinates. The horizontal axis distinguishes the location of an anonymous load particle among (L, ℓ)-reservoir, detection site, and (L, h)-reservoir, from left to right, while the vertical axis distinguishes the location of an anonymous fuel particle among (F, h)-reservoir, detection site, and (F, ℓ)-reservoir from top to bottom. The blue horizontal and red vertical bars indicate, respectively, the constraints by the F-gate and L-gate. As the result of the combination of these bars, a channel is formed between the $(\mathsf{F}, h)(\mathsf{L}, \ell)$-quadrant (top-left) and the $(\mathsf{F}, \ell)(\mathsf{L}, h)$-quadrant (bottom-right). (c) Demonstration of how the rules of (a) reconstitute the cycle of Fig. 1(b).

In fact the alternative rule (v′) in which 'high-density [low-density]' of (v) is replaced by 'low-density [high-density]' works as well through a different cycle, $(1_{\mathsf{F}}, 0_{\mathsf{L}}) \to (1_{\mathsf{F}}, 1_{\mathsf{L}}) \to (0_{\mathsf{F}}, 1_{\mathsf{L}}) \to (0_{\mathsf{F}}, 0_{\mathsf{L}})$ [6]. The choice between the rules (v) and (v′) reveals a hidden symmetry that the simultaneous replacement of the rule (v) by the rule (v′) for the F- and L-gates leaves the schema Fig. 1(a) unchanged. It is shown below that the protein motors like myosin and kinesin have chosen rule (v) rather than (v′). A scenario for this coincidence is based on 'symmetry breaking': that a gate can switch the accessibility from two reservoirs, e.g. (F, h) and (F, ℓ), in a synchronized manner might seem too fortuitous. In fact, the primitive form of the gate could be much less selective. However, it is also natural that the accessibilities from the two reservoirs are modulated in an asymmetric manner by an allosteric degree of freedom. Once such (allosteric) change of biased accessibilities works favorably through natural selection, the refinement of the function is a matter of evolutional time. Seeing that the bidirectional control can *in principle* be achieved in two complementary manners (i.e. the choice between the rule (v) and (v′)), we may imagine that the evolution that realized myosin and kinesin have chosen the former rule by breaking the symmetry. If this symmetry breaking is not due to some other selective pressure which we have overlooked, the same type of symmetry breaking in myosin and kinesin suggests that the event of symmetry breaking had occurred in some ancestor before it underwent differentiation into (what are now) myosin and kinesin.

In general the sensor may be defined as a device to *correlate* uncontrollable stochastic events to an (allosteric) coordinate of the device. The uncontrollability of thermal and/or chemical environments and the reliability of sensors are compatible: in [6] it was shown that, through the mechanism of compensation of attractive and repulsive (restoring) interactions (like the 'induced fit' [10]), an ideal sensor of particles is, in principle, conceivable such that it practically never responds to the absence of particles even at finite temperature. Moreover, such sensor can function athermally. (In [6] the special case has been studied where all the chemical transition occurs without energy exchange: $\epsilon^{0_{\mathsf{F}}}_{1_{\mathsf{L}}} = \epsilon^{1_{\mathsf{F}}}_{1_{\mathsf{L}}} = \epsilon^{1_{\mathsf{F}}}_{0_{\mathsf{L}}} = \epsilon^{0_{\mathsf{F}}}_{0_{\mathsf{L}}}$.) It was also shown there that the action of the sensor requires three constitutive elements: the sensing tip and the backbone, these two belonging to the sensor, and the ligand. The attraction between the sensing tip and the ligand can be cancelled by the repulsion between the sensing tip and the backbone.

The gate may be defined as a device for *filtering* uncontrollable stochastic events. So-called the ratchet models [11] use gates [12]. The gate does not specifies the moment of time when the admissible events occur. In [13] it was shown that an ideal gate is, in principle, conceivable such that it costs arbitrarily small irreversible work. Such gates or filters in an autonomous machine do not lead to the paradox of Maxwell's demon [14]: the gate can rectify the fluctuations only if it is coupled to a sensor, which itself is a part of the autonomous system. The stochastic process and its energetics of Fig. 2 can be explicitly formulated by what has been called stochastic energetics [15].

For systems working in thermal and chemical fluctuations, the distinction between the roles of sensor and gate seems to be a key to understanding molecular motors. A phenomena called the 'exchange of binding' is a simple type of allosteric link between a sensor and a gate: for example, in the 'rigor' state of a myosin head, the head without nucleotide strongly binds an actin filament. Upon binding an ATP molecule, the head allows the actin filament to detach. This process can be regarded as an action of the F-sensor which admits the ATP molecule, and the linked action of the L-gate which weakens the myosin–actin interaction. For the F-sensor to admit an ATP molecule, the F-gate should admit the nucleotide. And this gate does not change immediately when the F-sensor admits an ATP molecule. The exchange of binding is a special case of combination of the sensor and the gate. In general, the sensors and gates do not push forward the cycle process but this is especially so when the process is nearly athermal. For example, the intramolecular hydrolysis in molecular motors is an approximately reversible reaction and the back and forth reactions continue until the product, especially the phosphate, diffuses out of the protein (see [16] and the references cited therein).

The background idea of the bidirectional control of autonomous systems is not new: it can be found in other practical or theoretical instances. In practice, the public payphone, for example, functions with two lines of control; one for checking the credit (sensor) and for admitting the conversations (gate), and the other for detecting the communication (sensor) and for debiting the credit (gate). In theory, a sort of bidirectional control is implicitly assumed in those thermal ratchet models having two or more potential landscapes: the transition rates controls the transitions among these potentials (gates) and these rates are made to depend on the position, implying the sensors of position [17–19]. The oscillations in reaction–diffusion systems such as FitzHugh Nagumo oscillations might also be interpreted as bidirectional control.

Is there an autonomous machine with a single allosteric coordinate, rather than two? In fact, it is possible [20]. However, this involve more subtle aspects than the bidirectional control: (i) the assignment of the roles of sensor

and gate depends on the external environment; (ii) the allosteric coordinate does not undergo a cycle while the chemical states of whole system do; (iii) the sureness and rapidity of the cyclic function are complementary. The chemo-mechanical cycles of myosins and kinesins adapts much better to the bidirectional control, as will be shown below.

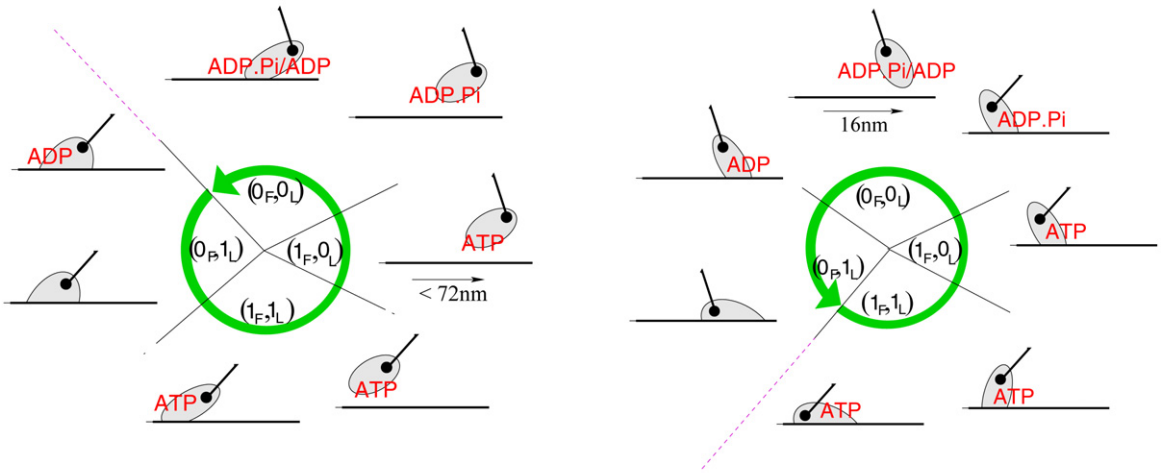
3. Application to motors: function

Figs. 3(a) and (b) summarize the putative and typical chemo-mechanical cycles of a single head of conventional myosin [21,22] and of kinesin [1], respectively. Viewed from the motor heads, filaments (actin for the myosin head and microtubule for the kinesin head) are translocated to the left in the course of an ATP hydrolysis cycle. In particular, for the myosin-II or kinesin-1 (KHC) families, the plus (+) end of the filaments is at the rightmost side. Detailed processes during the period around the so-called isomerization (conformational changes associated to intramolecular hydrolysis reaction) is still controversial because of its transient character. The schemas marked with ADP.Pi and ADP.Pi/ADP in Fig. 3 show the order of transitions assumed by the present paper. The detailed account of the lever's stroke of myosin and the tilting of kinesin's head will be given in the next section.

Apparently, the relative timing of the chemical versus mechanical cycles is quite different between myosin and kinesin. The myosin head in the rigor state detaches upon binding an ATP molecule and the isomerisation takes place while the head interacts with the actin filament only weakly. By contrast, the kinesin head with ATP binds microtubule rather strongly, and the isomerisation occurs while the head is ready to detach from the microtubule.

The approach of the present article is to generalize the bidirectional control introduced in the previous section so that the two allosteric degrees of freedom, a load(L)-sensor–fuel(L)-gate coordinate (LF-coordinate, for short) and a fuel(F)-sensor–load(L)-gate coordinate (FL-coordinate, for short), adapts to the cyclic process shown in Fig. 3. The details of the energetics and kinetics of the chemo-mechanical cycle will not be discussed. Instead, the analysis is focused on the assignment of the sensors and gates that reproduces the schema Fig. 3. The quantitative energetics and kinetics will be discussed in the future.

We separate the analysis into two levels: first the allosteric coordinates are assigned on the functional level (in this section) and then the intra-molecular structures that support the functions will be discussed (Section 4). For the



(a) Myosin's chemo-mechanical cycle (b) Kinesin's chemo-mechanical cycle

Fig. 3. Putative and typical chemo-mechanical cycle of a single head of myosin (a) and kinesin (b). In both figures the interaction strength between the head (gray) and an actin filament (thick horizontal bar) is represented by the length of contact. Across the dashed line (magenta) counter-clockwise, the view-frame is shifted towards the right. In (a) the conformations of the lever-arm and the converter region of myosin head are symbolically represented by an oblique bar with a thick black dot. For example in the rightmost head (ATP bound, actin detached), the lever-arm is in the 'pre-stroke/priming' position. (cf. The words 'stroke' or 'pre-stroke' refers to their geometrical characteristics with no presumption of 'power stoke'.) In (b) the clockwise rotation an oval found in the bottom right symbolically indicates the 'upright' kinesin head. See the text for the assignments of the allosteric coordinates, $(1_F, 0_L)$, etc.

molecular motors the F-particle and L-particle need not be true particles. Rather, what is truly consumed by the hydrolysis is the bond between β- and γ-phosphates ($\beta\gamma$-Pi bond, for short). The interpretation of the L-particle should also be generalized so that the active transport from L_ℓ to L_h amounts to the filament translocation against a load. Bearing these observations in mind, the time-ordering of the arrival and departure of F- and L-particles in Fig. 3 is found to be the same as that of Fig. 2(c), but different from the cycle with the alternative rule (v′) (see Section 2). That the myosin and kinesin share a common *broken symmetry* about rules may support, from the behavioral viewpoint, the notion of the common ancestor [23] for these proteins, as well as G-proteins, the reason why the rule (v) has been chosen instead of the rule (v′) is still unknown.

Being encouraged by the above correspondence, we propose the following assignment for the sensors and gates:

Myosin:

(M-LF) **L-sensor–F-gate**: L-sensor detects the 'stroke' conformation of the lever-arm.The detection (1_L) allows the arrival and departure of the nucleotide without cleaving/hydrolysing the $\beta\gamma$-Pi bond. The non-detection (0_L) (i.e. the detection of 'pre-stroke/priming' conformation) allows the cleavage of the $\beta\gamma$-Pi, and constraints the exchange of the nucleotide.

(M-FL) **F-sensor–L-gate**: F-sensor detects the $\beta\gamma$-Pi bond of ATP. The detection (1_F) *allows* to it exit from the rigor binding with the actin filament. The non-detection (0_F) *allows* to it enter into the rigor binding with actin.

Kinesin:

(K-LF) **L-sensor–F-gate**: L-sensor detects the presence of rigor binding. The detection (1_L) allows the arrival and departure of the nucleotide without cleaving the $\beta\gamma$-Pi bond. The non-detection (0_L) allows the cleavage of the $\beta\gamma$-Pi bond, and constraints the exchange of the nucleotide.

(K-FL) **F-sensor–L-gate**: The same as M-FL except that the 'actin' is replaced by the 'microtubule'.

Inside the circles of Fig. 3, the chemo-mechanical cycles of the motors are mapped onto the cycles of bidirectional control. As the gates do not impose a single state, the two stages of ATP-bound myosin, for example, are found under the same category, (1_F, 1_L). The result shows a close correspondence between the chemo-mechanical cycles of myosin and kinesin on the functional level.

Several comments could be made on the variety of the motor's behavior in the context of bidirectional control. The directionality of the motor, which can vary within each superfamily, is the matter of implementation either of the L-sensor, as proposed for a mutated minus-end directed myosin-II [24], or of the L-gate, as proposed for the myosin-VI (minus-end directed myosin) [25]. That a single head of myosin can bind to actin and exert a force even at 100 msec after the ADP dissociation [26] is understandable if the FL-coordinate of the motor remains in its 0_F state while the ADP has released eventually by a rare thermal fluctuation. Different types of mutants could be conceived based on the present scheme: a mutant deficient, for example, in the FL-coordinate may still hydrolyze nucleotide in the way that the hydrolysis rate depends on the interaction with filaments [6].

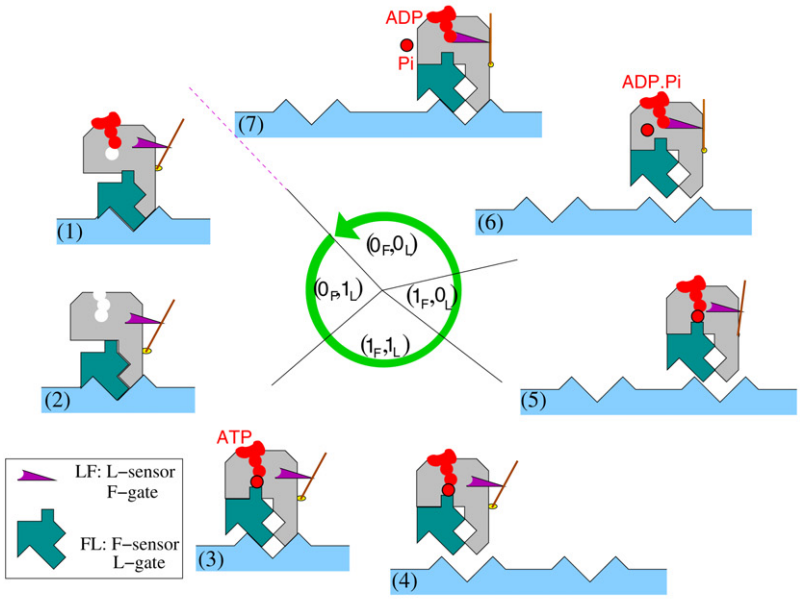
4. Application to motors: structure

Figs. 4(a) and (b) illustrate schematically the proposed intramolecule cycles of myosin and kinesin, respectively. Although we have taken into account the literature on the three-dimensional structures of myosin [27–30,16] and of kinesin [31–34]. The schema is still tentative, to be confirmed or modified by future experimental results. The correspondence of the stages along the chemo-mechanical cycles is made between Fig. 3(a) [Fig. 3(b)] and Fig. 4(a) [Fig. 4(b)] for myosin [kinesin] cycles, respectively. In the representation the three-dimensional geometry of the constituent elements are not observed. In particular, the exit pathway of the γ-phosphate ('back-door' [28]) is deviated toward upwards from the switch 1 and switch 2 elements. The inserts indicates the symbols representing the LF-coordinate and FL-coordinate.

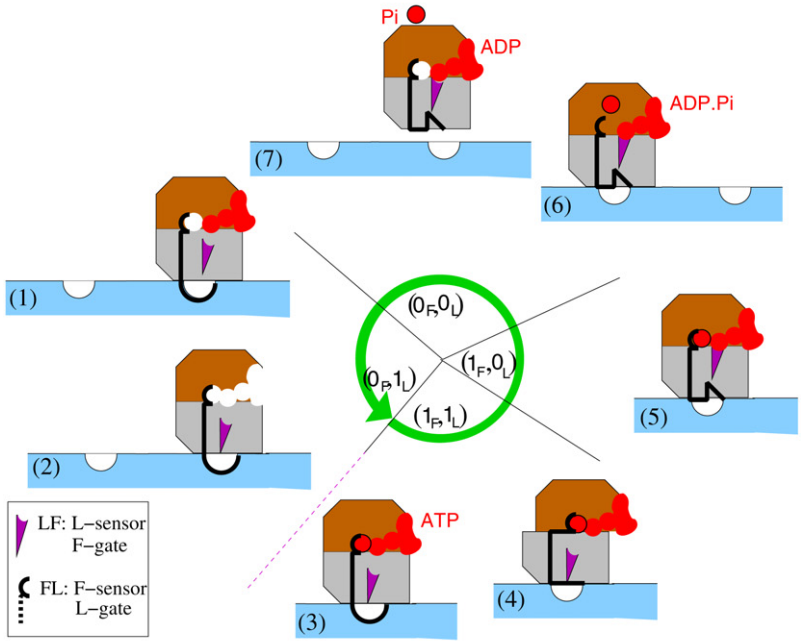
The following is the proposition of the constitutive elements of LF- and FL-coordinates. Within parentheses their states in the 'detected' states, 1_L or 1_F, are indicated:

Myosin:

(M-LF) – lever-arm (after-stroke/down position); nucleotide binding pocket [NBP] (open); switch-2 [sw 2] (open); inner-cleft of 50 kDa domain (open); kink in relay helix (present); 60°-rotation of converter (absent).



(a) Myosin's intramolecular cycle



(b) Kinesin's intramolecular cycle

Fig. 4. Proposed intramolecular processes along the chemo-mechanical cycles of (a) myosin and (b) kinesin single head. Light blue base lines represent filaments. Inserts indicates the LF-coordinate and FL-coordinate. Gray bodies represent the 'core' of the motor head. Important mechanical signals/fluctuations for the LF-coordinate are filtered by a lever in myosin or by head's tilting in kinesin (brown).

(M-FL) – switch-1 [sw-1] and P-loop (closed); (outer-) cleft of 50 kDa domain (open); twists in β-sheets of upper 50 kDa domain (absent).

Kinesin:

(K-LF) – sw-1/loop-9 [L9] (open); NBP (open); extension of sw-2 helix [α4] (absent).

(K-FL) – $\beta\gamma$-Pi detecting part of sw-2 loop [L11] (closed).

Below the detailed account, are given, stage after stage, starting with the $(0_{\mathsf{F}}, 1_{\mathsf{L}})$-stage. The numbers in parenthesis refer to those in Figs. 4(a) and (b).

(1) $(0_{\mathsf{F}}, 1_{\mathsf{L}})$: For *both proteins* the FL-degree of freedom to bind strongly the filament with specific interaction, as is allowed by the non-detection of ATP on the active site (0_{F}). The filament binding (1_{L}) by the L-sensor opens NBP, so that the ADP molecule can diffuse out.
For *myosin* this stage is the 'rigor' state [21].
For *kinesin* the 1_{L} the L11 is specifically bound to the microtubule (with a high energy $\sim 10 k_{\mathrm{B}}T$ [35,36]), and that F-gate is at the non-hydrolysing position. In fact the sw-1 appears to be open [34]. (This is, however, somewhat contradictory to the EPR results [37] that is interpreted as the 'closure' of the sw-1 upon microtubule binding. The meaning of 'closure' needs to be clarified.) The rate of ADP release in this stage has been shown to increase under a backward force (to the left in Fig. 4(b)) [38,39]. This result is postulated as a mechanism of 'biased capturing' for an ADP bearing head to bind ahead of its cargo or the partner head [34].

(2) Ibid.: For *both proteins* this stage after the departure of ADP allows an ATP molecule to enter the NBP. (The return of ADP is much less likely than the arrival of ATP due to their concentration difference.)

(3) $(1_{\mathsf{F}}, 1_{\mathsf{L}})$: For *both proteins* the arrival of an ATP molecule, stage (2) $\rightarrow$ (3), causes a strong and attractive stabilisation of the FL-degree of freedom, $0_{\mathsf{F}} \rightarrow 1_{\mathsf{F}}$.
For *myosin* this stage is the 'post-rigor' state [21]. The binding of ATP causes the breaking of the specific binding to the actin (exchange of binding) accompanied by the opening of the 50 kDa cleft. The L-gate then allows the detachment of actin.
For *kinesin* the spatial coordination of FL is such that the stabilisation of the 1_{L} does not appreciably change the filament binding interface as illustrated in the schema. But this state now allows the eventual weakening of the microtubule binding (at the stage (4)). The docking of the 'neck-linker' is not emphasised in the present analysis. It is true that the docking is favored by 1–$2k_{\mathrm{B}}T$ [40] in this stage, and that the docked geometry favors the throwing forward of its partner head. However, a head can occasionally make ATP-consuming backsteps under high backward applied force [41,42], when the docking of the neck-linker is seemingly impossible.

(4) Ibid.: For *myosin* the head diffuses off the actin filament because the open 50 kDa cleft disables the specific binding to the actin filament. If weak electrostatic interactions between the head and actin filament have a spatial gradient, the diffusion can be biased [43].
For the *kinesin* head in this stage, once an appreciable forward force is applied to it, either by the leading partner head for two headed kinesin or by a big cargo placed ahead of this head, this head can be rotated towards forward (shown as the displacement rightwards of the orange block). Under this strong rotation (which may correspond to the 'upright' position in [34]), the FL-degree of freedom must follow the $\beta\gamma$-Pi until it ruptures the strong microtubule binding. At this stage, the L-sensor could detect some change in L11 (see the end of the section.)

(5) $(1_{\mathsf{F}}, 0_{\mathsf{L}})$: For *both proteins* the L-sensor detects the change of its target and this is linked to the approach of the F-gate close to the nucleotide. The closing of NBP prevents the release of ADP part of the nucleotide, even after the $\beta\gamma$-Pi bond is lost (in stage (6)).
For *myosin* this is the 'pre-power-stroke' state [21]. The L-sensor detects the priming position of the lever arm through the rotation of the converter region [16]. To have a strong enough rotation, the head will need to interact fairly strongly to the actin. This condition may impose a bias for the head to make a forward displacement and to take a good orientation [25]. The role of the correlation between the head's displacement and the internal deformations has been suggested some time ago [44]. If the geometrical arrangement between the lever and the converter domain is altered, the L-sensor may detect the displacements in the opposite direction, as is proven by using a mutant my-II [24].
For *kinesin*, recovery of deformation from the previous stage (4) seems to be a branched process, either back to the stage (3) reversibly, or to a new stage, (5). In other words, the stages (3) and (5) are separated by the activation barrier (4). The transition (4) $\rightarrow$ (5) may be the process in which the sw-2 helix ($\alpha 4$) winds up the part of the sw-2 loop (L11) while the latter is released in the course of deformation recovery [34]. A small kink in the FLin Fig. 4(b)(5)–(7) represents the extended part of sw-2 helix. This part in turn pushes LF(sw-1/L9) toward the bound ATP molecule.

(6) $(0_{\mathsf{F}}, 0_{\mathsf{L}})$: For *both proteins* the stages (6)–(7) include the cleavage of ATP and the release of Pi ($1_{\mathsf{F}} \rightarrow 0_{\mathsf{F}}$). The transition (5)–(6) requires a sufficient and correct change in LFdegrees of freedom. Microscopically, the

cleavage requires that a lytic water becomes in-line attack position [45]. This stage is usually an ephemeral one and, therefore, most difficult to assess by crystallography, cryo-microscopy or single molecule measurements. An experimentally ambiguous point is whether the γ-phosphate release precedes the actin binding (for myosin) or the microtubule unbinding (for kinesin). A too early release of the γ-phosphate would cause the dysfunction of hand-over-hand coordination [53,54]. In view of the fact that the FL degree of freedom participates in constituting the phosphate 'back-door' in conjunction with the LF degree of freedom [28], the too early phosphate release might actually be avoided by a supplementary conformational changes of the FL degree of freedom when it interacts weakly with the filament. This point should be made clearer through biochemical [46] and bio-engineering [47, 48] approaches.

For *myosin* this is the 'top-of-power-stroke' state [21]. The transition from weak non-specific binding to actin becomes replaced by a stronger (but not yet rigor) and more specific interaction with actin once the head finds the 'right position'. When failed, the state returns reversibly to the stage (5). Only sufficient change in the LF degree of freedom allows it to move onto the next stage (7). The observation that the length of the lever arm linearly defines the step-size [49,50] may be explained by this criteria, since the 60°'s rotation of the converter region corresponds to the displacement proportional to the arm's length. (An estimation indicates [25] that the power-stroke hypothesis would require too high torque at the neck region of myosin-VI.) An X-ray structural analysis [29] suggests that the twist occurs in some β-sheets in the upper 50 kDa domain at this stage. Considering this flexibility, the authors of [29] propose two modes of intramolecular conformational change; see also [16].

For *kinesin* a single motor kinetic analysis [51] suggests that the phosphate release is load-independent, implying the release after detaching of microtubule. If the microtubule-binding surface is modified genetically [47,48], the phosphate release can precede the detachment.

(7) Ibid.: For *both proteins* the ADP molecule is kept on the motor head by the F-gate of the LF degree of freedom. After the release of γ-phosphate, the non-detection of $\beta\gamma$-Pi (0_F) implies the L-gate to allow the future strong binding to the filament ($\rightarrow$(1)).

For *myosin* the head will bound specifically, most likely at the 'good positions' where the lever arm can take its pre-stroke position.

For *kinesin* the head is diffusing or only weakly interacting with the microtubule via non-specific interactions (K-loop within the loop L12 interacts electrostatically with the E-hook of the microtubule [52,33]).

It would be interesting to compare the mechanism that holds an ATP molecule in myosin in its weak binding state (4) and the one that holds an ADP molecule in kinesin in (7). Fig. 4 shows that the sw-I plays the main role in both case one as F-sensor the other as F-gate. The ATP binding by myosin is dominant over its actin binding. By contrast the ADP binding by kinesin is metastable, being kept only before the L11 finds the strong binding site on the microtubule. In this sense the switch-2 (L11-α4) is a plastic memory of the single head kinesin.

An alternative pathway for the kinesin's chemomechanical cycle is that the transition, $(1_{\mathsf{F}}, 1_{\mathsf{L}}) \rightarrow (1_{\mathsf{F}}, 0_{\mathsf{L}})$ occurs already at stage (4) and it directly proceeds to the stage (6). Experiments with keeping forward force at the stage (3) may decide which is the more likely.

A recent single-molecule experiment suggests that the synchronisation is not strict [55]. The above schema does not take account of a precise synchronisation between the two heads moving by the hand-over-hand mechanism [53,54].

5. Conclusion and perspectives

Sensors and gates are basic concepts in standard textbooks of control theory. An information theoretical approach to the observability and controllability has been made recently [56,57]. A single pair of sensor and gate may not be sufficient, however, for the study of autonomous systems or processes mediating two different interfaces: the bidirectional control seems to be a useful concept. This concept includes the feed-back loop as a special case, where the sensor and gate at one interface are bound. The crystal structures show detailed interaction among modules, but the crystal structures are not the snapshots of chemo-mechanical process. The single molecule experiments show real-time functions but the detailed intramolecular processes are, at present, only partially accessible. Viewing these complementary aspects of the two experimental approaches, both structural biologists and single motor experimentalists are now more than ever conscious about the progress of each other (see, for example [52,38,39,16]).

The present study aimed to synthesize the achievements of these experimental approaches and to point out certain general features of molecular motors. The broken symmetry shared by myosin and kinesin (Section 3) is an example. The main assertion is the presence of (at least) two non-localized (allosteric) principal components of intramolecular coordination in molecular motors. The structural biologists have also suggested two coordinates ([29], [16], p.18).

Application and generalisation of the bidirectional control to other systems is conceivable, such as to Ca^{2+}-ATPase [58], kinases [59], or ABC-transporters [60,61]. The possibility of interaction between the two allosteric coordinates was completely ignored in the present analysis. This limitation could be removed to have more quantitative model. The usage of Boolean variables can be replaced by continuous stochastic dynamics. From the schema Fig. 4 many qualitative tests are conceivable, which, together with more quantitative analysis, will be performed in future.

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