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Three-State Model for One-Dimensional Brownian Motion of Charged Nanoparticles Along Microtubules

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A variety of motor proteins, such as dynein, KIF1A and MCAK, are known to exhibit one-dimensional (1D) Brownian motion along microtubules. The electrostatic interaction between the proteins and microtubules appears to be crucial for 1D Brownian motion, although the underlying mechanism has not been fully clarified. We examined the interactions of positively-charged nanoparticles composed of polyacrylamide gels with microtubules. These hydrophilic nanoparticles bound to the microtubules and displayed 1D Brownian motion in a charge-dependent manner, which indicates that nonspecific electrostatic interaction is sufficient for 1D Brownian motion. While the diffusion coefficient decreased exponentially with an increasing particle charge (with the exponent being 0.10 $k_BT$per charge), the duration of the interaction increased exponentially (with an exponent of 0.22 k_BT per charge). These results can be consistently explained if one assumes that a particle repeats a cycle of 'binding' and 'diffusion' along a microtubule until it finally 'dissociates' from the microtubule. This entire process can be described by a three-state model analogous to the Michaelis-Menten scheme, in which two parameters - the equilibrium constant between 'binding' and 'diffusion', and the rate of 'dissociation' from the microtubule - are derived as a function of the particle charge density. Further, to understand the molecular basis for this 'binding' and 'diffusion' mechanism, we engineered microtubules with variable charges at the tubulin C-terminal tail (CTT) and measured 1D Brownian motion of the particles along these mutant microtubules. The measurements revealed an unexpected result: the negative charges in the CTT did not significantly affect the diffusion coefficient of the particles, but the equilibrium between 'binding' and 'diffusion' shifted more towards 'diffusion' with increasing charges of the CTT. These results indicate that the negatively-charged CTT provides a field that facilitates the 'diffusion' of charged particles.

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Mechanism of Unidirectional Move of KIF1A Motor Studied by Coarse-Grained Simulations

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KIF1A is a single-headed motor which can move unidirectionally along a microtubule (MT) using the chemical energy produced by ATP hydrolysis. Several experimental studies revealed that KIF1A makes the biased Brownian movements (Okada et al., 2000). Fortunately, two major structures (ATP type and ADP type) are available. However, how KIF1A generates the translational movement from chemical reaction cycle still remains to be elucidated.
To address this question we try to reproduce translational movement of KIF1A by coarse-grained simulation of the multiple-basin model (Okazaki et al., 2006) that realizes conformational change (Kikkawa et al., 2006) during ATP hydrolysis cycle.
With a first set of simulations, ADP-type KIF1A detached from MT, diffused along MT, and attached to MT, but we did not find any forward bias in the stepping. We then found one condition that reproduces the biased Brownian movement. Namely, when a cargo (or a bead) with sufficiently large radius is attached to the C terminus of KIF1A, as in the in vivo situation, KIF1A exhibited the forward-biased Brownian movement along MT, in a consistent manner to experiments. In the presentation, we will also suggest the similarity of stepping mechanism between one-headed KIF1A motor and two-headed conventional kinesin.

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Protein Motors Induced Enhanced Diffusion In Intracellular Transport

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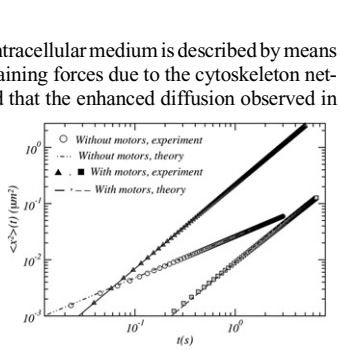
Diffusion of transported particles in the intracellular medium is described by means of a generalized diffusion equation containing forces due to the cytoskeleton network and to the protein motors. We find that the enhanced diffusion observed in experiments depends on the nature of the force exerted by the protein motors and on parameters characterizing the intracellular medium which is described in terms of a generalized Debye spectrum for the noise density of states. The model can be used to account for endocrine exocytosis. Comparison between theory and experiment are in good agreement.



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Synthesis of Photochromic ATP Analogue and its Interaction with Motor Proteins

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Azobenzene is one of the photochromic molecules, which undergoes rapid and reversible transitions between the cis isomer and trans isomer by visible and ultra-violet light irradiation. We have been trying to control the activities of motor proteins using the photochromic molecules as photo-regulatory devices. We have recently demonstrated that microtubules dependent ATPase activity of the kinesin modified by azobenzene derivative was regulated by UV-VIS light irradiation. However, it was not so easy to incorporate the photochromic molecules into the functional site of motor proteins without altering the native enzymatic properties.
In the present study, we have design the ATP analogues consist photochromic molecules in order to photo-regulate the motor proteins without their chemical modification. It is expected that the ATP analogues induce the reversible conformational change in the active site by alternate UV-VIS light irradiation. We have synthesized non-nucleotide ATP analogue composed of azobenzene derivative, Phenylazobenzoic-aminoethyl -triphosphate (PABATP). PABATP showed UV/VIS light absorption spectral change accompanied by transition between cis and trans similar to that seen with azobenzene. Cis isomer of PABATP was hydrolyzed by skeletal muscle myosin in the presence of Mg2+, Ca2+ or EDTA-K+ much faster than trans isomer. It has been demonstrated that the cis isomer and trans isomer perform differently as a substrate of myosin. Currently we are studying the conformational changes of myosin head induced by cis isomer and trans isomer of PABATP.

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Synthesis of Novel Fluorescent ATP Analogue and its Interaction with Nucleotide Dependent Motor Proteins

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Previously several kind of fluorescent ATP analogues have been synthesized for the application to the kinetic studies of ATPases. However, some of the ATP analogues exist as a mixture of isomers. For instance, 2′(3′)-*O*-Mant-ATP has isomer of 2′ and 3′ in its ribose moiety and each isomer performs differently as substrate for the ATPases. In the present study, we have tried to synthesize novel fluorescent ATP analogues that have no isomer. The fluorescent ATP analogue 6-(N- (7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino) ethyl triphosphate (NBDTP) and N-methylanthraniloyl amino ethyl triphosphate (MANTTP) have been designed and synthesized, which are similar to non-nucleotide ATP analogue 2-[(4-azido-2-nitrophenyl) amino] ethyl triphosphate (NANTP). It is known that NANTP are good substrate for skeletal myosin and induces actin gliding in vitro motility assay. Excitation and emission maximums in the fluorescence spectrum of the ATP analogues were 474nm and 533nm for NBDTP, and 374nm and 430nm for MANTTP, respectively. In addition, molar absorbance coefficients of ATP analogues were 40274 $M^{-1}cm^{-1}$ for NBDTP in 478nm, and 3730 $M^{-1}cm^{-1}$ for MANTTP, respectively. The analogues showed ATP hydrolysis for conventional kinesin and skeletal myosin at the almost same level to that of ATP. Moreover, the analogues induced dissociation of acto-myosin. The ADP form of the fluorescent ATP analogues showed the formation of skeletal muscle myosin/ADP analogues/BeFn complexes which mimic the transient state in ATPase cycle.

Ion Motive ATPases

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Nonequilibrium Energetics of a Single F_1-ATPase Molecule

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Energetics of a rotary molecular motor F_1-ATPase was studied by applying recent developments in nonequilibrium physics. Since molecular motors are engines that transduce chemical energy to mechanical motions, it is essential to focus on their energetics. Here, for a single F_1-ATPase molecule, we have evaluated the amount of heat dissipation Q_{rot} through its rotational degree of freedom as well as the work W against external load. Q_{rot} was estimated using a new nonequilibrium equality connecting the heat dissipation to the violation of the fluctuation dissipation theorem. External torque was applied using the electrorotation method. We found a nontrivial energy balance relation that $W+Q_{rot}$ per 120° rotation was almost equal to the free energy change in a single ATP hydrolysis $\in''\in 1/4$ under various conditions. This implies that F_1-ATPase focuses the free energy consumption toward rotations with an efficiency of nearly 100%.

Moreover, we found that under a sufficiently strong torque in the opposite direction of ATP hydrolytic rotations, it rotated in the opposite direction, or the ATP synthetic direction, in a stepwise manner. The torque necessary for rotations in the synthetic direction times 120° was nearly equal to $\in'' \in 1/4$ under various conditions except for conditions at sufficiently low ADP concentrations.

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Spatial Distribution of Elasticity in the F1 Motor of ATP Synthase Reveals the Microscopic Nature of the Coupling Between the Central Shaft and the Catalytic Subunit

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F_OF_1-ATPase is a rotary motor protein that synthesize ATP from ADP using the proton gradient across a membrane as a free energy source. The proton flow through the membrane-embedded portion, F_O, is thought to generate the rotary torque that drives the rotation of the asymmetric shaft in the cylinder of hexagonally arranged alpha and beta chains forming the catalytic subunit of the F_1 portion. Mechanical energy of the rotating shaft is used by the active sites of F_1 to synthesize ATP against thermodynamic potential gradient. The microscopic mechanism of this energy conversion is still not fully understood. It was suggested that elastic power transmission with transient storage of energy in some compliant part of the common shaftis required for the high turnover rate to occur [1].

Here we use fully atomistic molecular dynamics simulation to study the spatial distribution of torsional elasticity in the F_1 motor on the 500-ns timescale. The overall range of angular fluctuations of the central shaft with respect to the symmetry axis of the catalytic subunit is consistent with the results of the corresponding experimental study [1]. The detailed analysis of the rotational mobility reveals, however, that the measured range of fluctuations results from two different effects: the internal elasticity of the shaft itself and the effective load imposed on it by the catalytic subunit. Separation of these two effects has led to the detailed description of the dynamic coupling between the shaft and the catalytic subunit. We also propose a simple model of the F_1 motor that might be a useful tool in future studies of the energy transfer in F_OF_1-ATPase.

[1] Sielaff et al. PNAS 105:17760-17765 (2008)

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Structure of CopA from Archaeoglobus Fulgidus by Cryoelectron Microscopy

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CopA, a bacterial transporter of Cu^+ and Ag^+ from *Archaeoglobus fulgidus* , was cloned, overexpressed, purified, reconstituted into lipid bilayers and crystallized into tubular crystals in the presence of Cu^+ chelator BCDS by dialysis at 50°C. Three Cryo-EM Structures were obtained with different constructs and lipids 1) dNdC-CopA with DOPC, N and C terminal cytoplasmic peptides truncated of CopA reconstituted into 1,2-dioleoyl-sn-glycero-3-phosphocholine, 2) dC-CopA with DOPC, C terminal cytoplasmic peptide truncated of CopA reconstituted into 1,2-dioleoyl-sn-glycero-3-phosphocholine, 3) dC-CopA with DMPC/DOPE, C terminal cytoplasmic peptide truncated of CopA reconstituted with 1,2-dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine. All reconstituted proteoliposomes retain their functionality with V_{max} ranging from 1.14 to 2.03 nmol/μg/min and $K_{0.5}$ ranging from 0.1 to 0.8 μM, depending on the reconstituted proteoliposomes and ion substrates. The optimal temperature for enzyme assays of these reconstituted proteoloposomes are located between 65°C to 75°C and these activity measurements were conducted at the temperature of 70°C. Three cryo-EM structures obtained by frozen-hydrated tubular crystals and Fourier processing have resolutions from 12.5 to 17.5 é…. Based on the difference map and the modeling between dC-CopA and dNdC-CopA, N-terminal metal binding domain (MBD) of CopA appears to lie between the ATP binding domain and Actuator domain and has an inhibitory role, which is relieved by receiving Cu^+ from the soluble metals chaperon. Efforts for higher resolution as well as computational modeling of CopA are underway in order to investigate the relative position of cytoplasmic domains with respect to transmembrane helices, in which the transport sites and ion gateway are located.

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Sodium Pump A1 And A3 Subunit Isoforms Mediate Distinct Responses To Ouabain And Are Both Essential For Human Neuroblastoma

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The sodium pump (Na^+,K^+-ATPase) maintains the sodium gradient across plasma membranes of animal cells. By hydrolyzing ATP the enzyme transports 3 Na^+ ions out of the cell in exchange for 2 K^+ ions that are taken into the cytosol. This activity can be interrupted by cardiotonic steroids (CTS). Recent publications have, however, established that CTS not only inhibit the sodium pump but that they also induce signalling cascades that influence the physiology of cells in various ways.

Sodium pumps are composed of α and β subunits (and additionally in some tissues of γ subunits) that appear is several isoforms. In some cells different α subunit isoforms are coexpressed, giving rise to the question about the need for their co-existence.

Using human neuroblastoma cells SK-N-AS that co-express α1 and α3 isoforms of the sodium pump α subunit, we selectively silenced either the α1 or α3 subunit by means of small interfering RNA and investigated cell survival and the cellular response to ouabain, a widely used CTS. We find that both of the two α subunit isoforms are essential for cell survival, indicating that substitution of one subunit for the other is not sufficient. In the presence of both α subunits ouabain causes a sustained Erk1/2 activation. This activation is not affected when the α1 subunit is silenced. When α3 expression is silenced, ouabain-induced activation of Erk1/2 does not occur, even at a high concentration of ouabain (1 μM). Thus, ouabain-induced Erk1/2 activation is mediated in SK-N-AS cells by α3 only, and α1 does not participate in this event. This is the first demonstration of selective involvement of a specific sodium pump α subunit isoform in ouabain-induced signaling.

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Ion-Selectivity of Externally Facing Na^+-Exclusive and Na^+/K^+-Shared Sites in the Na/K-Pump

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The Na/K-pump extrudes 3 Na^+ in exchange for 2 K^+ across the plasmalemma of animal cells. Two-out-of-three ion binding sites in the protein can be occupied by either Na^+ or K^+, whereas another site exclusively binds Na^+. At maximally activating [K^+_o], Na^+_o binding to the Na^+-exclusive site (first site to open in sequential Na^+ release) is manifested as [Na^+_o]- and voltage-dependent inhibition of outwardly-directed (due to the 3:2 stoichoimetry) pump current (I_P). Guanidinium$^+$ can also backward-transit this Na^+-release channel inhibiting I_P at negative voltages (Yaragatupalli et al. (2009) PNAS **106**:15107-15512). To study the ion-selectivity of this Na^+-release channel we measured voltage-dependent inhibition of I_P with external solutions containing different cations (120-125 mM). This inhibition followed the sequence: $Na^+_o > Li^+_o >$ guanidinium^+_o >aminoguanidinium^+_o > acetamidinium^+_o ~diaminoguanidinium^+_o ~formamidinium^+_o ~Cs^+_o ~K^+_o ~N-Methyl-D-Glucamine (NMG^+_o).

Ouabain-sensitive currents in the absence of Na^+_o and K^+_o were also measured. An inward current (possibly representing leakage through the Na-exclusive site when the shared sites are empty) was observed in NMG^+_o, guanidinium^+_o and aminoguanidinium^+_o. The other cations tested induced ouabain-sensitive outward currents at all voltages. Ouabain-sensitive current amplitude in 120 mM acetamidinium^+_o was similar to maximal K^+_o-induced I_P. Without Na^+_o, [acetamidinium ^+_o] of outwardly-directed current gave $K_{0.5acet+}$ ~10 mM indicating that this ion acts as a low-affinity K^+ surrogate. Consistently, in sheep kidney purified enzyme preparations, both acetamidinium$^+$ and formamidinium$^+$ induced ouabain-dependent ATPase activity ($K_{0.5acet}$ = 80 mM, $K_{0.5form}$ = 113 mM, $K_{0.5K+}$ = 0.9 mM). Transport was confirmed by means of ouabain-sensitive C^{14}-acetamidinium uptake. Our results indicate that acetamidinium$^+$ and formamidinium$^+$ can be transported like K^+ by the Na/K pump. Molecular dynamics simulations based on an atomic model are used to explain organic cation coordination in the occluded form. Supported by NIH DK083859 and GM062342.

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The Route and Mechanism of Uncoupled Current Flow through Na/K-ATPase Pumps Lacking the Two COOH-Terminal Tyrosines

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Na/K-ATPase pumps generate outward current during ATP-driven stoichiometric exchange of three intracellular Na ions for two extracellular K ions. At acidic pH, in the absence of extracellular Na and K ions, an uncoupled current flows through wild-type Na/K pumps at large negative membrane potentials, believed carried by protons. Both currents are abolished by the specific Na/K pump inhibitor ouabain. In *Xenopus*α1 pumps made less sensitive to ouabain by mutations Q120R/N131D or C113Y we observed a similar uncoupled current in the absence of extracellular Na and K ions even at physiological pH