single cation binding site flanked by two energy barriers, asymmetrically oriented within the electrical field, is sufficient to account for these unique features. Other HypoPP mutations at R666 (S/C) had gating-pore currents with similar ionic selectivity, voltage-dependence and amplitude. In contrast, the R666H HypoPP mutation produced a gating-pore with selectivity for protons over larger monovalent cations, but exhibited similar current amplitude. The estimated inward current flowing through the gating-pores created by all R666 mutants under normal physiological conditions is small (~0.1%) relative to the current flowing through the central Na^+-conducting pore. They are thus similar in magnitude to the gating-pore proton current we previously reported in the R663H HypoPP mutant. The pathological effect of these low-amplitude currents may be to potentiate the sarcolemmal threshold for aberrant depolarization in the setting of reduced extracellular K^+.

Platform AV: Microtubules & Microtubule-Associated Proteins

1869-Plat Effects of Porphyrins on Tubulin Polymerization

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Studies suggest that irradiation of porphyrins produces direct damage of proteins including tubulin. Our previous research group's fluorescence spectroscopy investigation of the interaction of meso-tetrakis(p-sulfonatephenyl)porphyrin (TSPP) and protoporphyrin IX (PPIX) with tubulin showed that binding to the protein occurs. The binding constant for TSPP is approximately one order of magnitude higher than PPIX (3.1 ± 1 x106 M-1 vs. 2.4 ± 0.9 x105 M-1) while the number of binding sites is the same for both (~ 1). Also, the quenching of tubulin shows a larger Stern-Volmer constant for TSPP than PPIX which may indicate a different location of the binding sites. Circular dichroism of both porphyrins also showed that there is no distortion of the porphyrin macrocycle upon binding.

We also investigated the effect of the porphyrins on tubulin polymerization using turbidity assay. Polymerization studies were carried out at a 1:1 ratio of tubulin(~10μM) to porphyrin and showed that in modified MES buffer at 37°C the polymerization of tubulin proceeds very slowly except in the presence of a reagent such as taxol. These studies revealed that polymerization of tubulin occurred more slowly, increasing the nucleation phase and growth phase of microtubules. Simulation of potential porphyrin binding sites using ArgusLab showed that TSPP and PPIX have affinity to sites that do not overlap other anti-microtubule reagents such as taxol, colchicine, and vinblastine.

1870-Plat Buckling And Force Propagation Along Intracellular Microtubules

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The mechanics of most eukaryoric cells is governed by their cytoskeleton, a composite polymeric scaffold made of a variety of protein filaments and crosslinkers. Of all the cytoskeletal filaments, microtubules are the stiffest and play a crucial role in cell mechanics and intracellular transport. Recent experiments [1] have shown that the mechanical reinforcement due to the surrounding cytoskeleton allows microtubules (MTs) to bear very large compressive loads (up to 100pN), and can greatly affect force transmission along MTs. Motivated by this, we study theoretically the mechanical response of and force propagation along these stiff elastic filaments embedded in the non-linearly elastic cytoskeletal matrix. We find that, although embedded microtubules buckle when their compressive load exceeds a critical load found earlier, the resulting deformation is restricted to a finite spatial range that depends on both the non-linear material properties of the surrounding cytoskeleton, as well as the direct coupling of the microtubule to the cytoskeleton possibly through MT-associating proteins (MAPS). This gives rise to a finite, monotonic force-extension behavior.

This work shows how the range of compressive force transmission by microtubules can be as large as tens of microns and is governed by the direct coupling to the surrounding cytoskeleton.

References

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1871-Plat Numerical Modeling Exhibits The Importance Of Microtubule Bundle Formation In The Self-organized Development Of Spindle Poles

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In Xenopus meiotic extracts, despite substantial angular diffusion due to thermal effects, microtubules orient themselves to form spindle poles. This orientation appears to come primarily from the mechanical forces created by crosslinking molecular motor complexes, both plus-directed and minus-directed. Numerical simulations of a biophysical model have shed much light on this process. In our biophysical model, the plus ends of microtubules are attached by plus-directed motors fixed at chromatin surfaces. The attachment points form a random spatial distribution, with rotationally asymmetric statistics. Minus-directed crosslinking motor complexes bring the microtubules minus ends together to form the poles. Our initial hypothesis was that there was a one-stage process where individual microtubules would be captured by crosslinking motors and forced into alignment with one of the developing poles. Numerical simulations showed, however, that the assembly process involves two stages. Initially, microtubules form small bundles of two or three microtubules. These small bundles still exhibit considerable angular diffusion. Over time, these bundles coalesce into larger bundles which show smaller amounts of angular diffusion. In addition, the larger bundles react more strongly to the spherically-asymmetric distribution of chromatin attachment points. This generates a torque on large bundles that moves them toward the two pole positions. The self-similar process of bundle formation and coalescence is important to the formation of spindle poles. Actual angular alignment of microtubules with the spindle becomes important only

after the occurrence of a considerable amount of bundle formation and coalescence. We have found that graph theory, in particular random graph theory, gives us powerful quantitative tools to study spindle bundle formation.

1872-Plat

No Abstract

1873-Plat Free Energy Landscape Of Microtubule Polymerization And Dynamics

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Understanding thermodynamics of microtubule formation can provide critical insights into many biological processes such as cell migration, development, as well as cellular response to external cues. In spite of numerous computational and experimental efforts, the free energy costs of polymerization and depolymerization of microtubules are poorly understood. Current models of microtubule polymerization fail to take into account both solvent and pressure affects. In order to address this problem quantitatively, we have extended Flory-Huggins-type equilibrium polymerization model to determine the free energy of microtubule polymerization. Our work to study microtubule polymerization is based upon previous studies of G-actin polymerization but makes several critical improvements in the existing models. Firstly our model accounts for the lateral interactions along each protofilament caused by each of dimmer subunits (both a- a and B- B interactions). These are be accounted for by the addition of two extra interaction terms X¬¬aa and X¬¬BB for each independently growing protofilament to go along with an interaction term between microtubule dimmers X¬aB. Secondly, the reaction mechanism of microtubule formation is distinct to that of actin as the initiation event, the formation of a GTP-cap, is absolutely necessary to capture microtubule extent of polymerization. In order to capture this, we have separated the reaction mechanism into two separate parts, namely a dynamic GTP cap in which new monomer units are added to the growing microtubule as well as a hydrolysis mechanism in which new monomer units are hydrolyzed to form the bulk of the microtubule and an interaction term between them X¬pp' . Our calculations show good agreement with existing experimental models and by calculating key thermodynamic parameters provides novel insights into the fundamental thermodynamics of microtubule polymerization and dynamics.

1874-Plat Microtubule Architecture Determines Shape Fluctuation Dynamics

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While many aspects of microtubule functionality rely on their mechanical properties, recent experimental studies point at the fact that a concise understanding of microtubule mechanics is still lacking due to the high structural complexity of these filaments. Specifically, little is known about the interplay of relaxation dynamics and molecular architecture. We study the dynamics of thermal shape fluctuations of stabilized, grafted microtubules in the length range of 2–30 μm using high resolution particle tracking on attached fluorescent beads. First mode relaxation times were extracted from the mean square displacement in the transverse coordinate. For short microtubules, the relaxation times were found to follow an L^2 dependence instead of L^4 as expected from the standard wormlike chain model. As these time scales are determined by an interplay of filament stiffness and friction, persistence lengths and drag coefficients were examined. The persistence lengths show a complex dependence on overall filament length and indicate a plateau value of ~600 μm for microtubules shorter than ~5μm. This behavior is consistent with the elastic properties of bundles of wormlike filaments [Heussinger et al., 2007, cond-mat/0702097] and hence suggests modeling microtubules as bundles of their constituent protofilaments [Pampaloni et al., 2006, PNAS 103 (27):10248]. The analysis of the drag coefficients reveals considerable deviations from purely hydrodynamic friction in the short length regime. These deviations are in agreement with theoretical predictions for internal friction arising from fluid flow through narrow pores or conformational changes inside the filament [Poirier and Marko, 2002, PRL *88*(22):228103].

Our results emphasize that microtubule mechanics can be understood as a consequence of their complex protofilament architecture and point out possibilities for natural modulation of mechanical properties in cells.

1875-Plat Anterograde Microtubule Transport Drives Microtubule Bending in LLC-PK1 Epithelial Cells

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Microtubules are proposed to act mechanically as compressive struts that resist both actomyosin forces and polymerization forces to mechanically stabilize cell shape. However, individual microtubules in living cells are often highly bent, suggesting that resistance to bending is weak. To investigate the origin of microtubule bending, we examined microtubule and F-actin dynamics in the periphery of LLC-PK1 epithelial cells. Microtubule bending was characterized by curvature distribution, an approach which has been previously validated using simulations of thermally-driven semiflexible polymers. The experimentally measured microtubule curvature distribution was found to be exponential, rather than the Gaussian expected for a thermally-driven polymer. We found that F-actin remains nearly stationary in these cells even while the microtubules are being deformed, ruling out actomyosin contractility driving bending. In addition, we found through kymographic analysis that microtubule polymerization rarely results in bending; rather the bending is often characterized by transport of the proximal

microtubule towards the periphery with a nearly stationary tip. Interestingly, the curvature distribution of microtubules in an in vitro kinesin-microtubule gliding assay is also exponential. These experimental results are compared with computer simulations of microtubules in an explicit solvent with molecular motors. The primary conclusion of this work is that many of the known mechanisms of microtubule deformation do not play a significant role in mediating microtubule bending in LLC-PK1 cells; rather, molecular motors appear to generate most of the strain energy stored in the microtubule lattice.

1876-Plat Microtubule-driven Multimerization Recruits Ase1 onto Overlapping Microtubules

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Polarity-specific microtubule organization is important both during cell division and interphase. Bipolar microtubule-dependent motor proteins such as Eg5 as well as passive microtubule bundling proteins such as Ase1 play important roles in these ordering processes. Ase1 preferentially crosslinks anti-parallel microtubules and localizes to the zones where microtubules overlap with remarkable prevalence. Here we show that this localization to the overlap zone depends on the capability of Ase1 dimers to form multimers on the microtubule lattice. We find that single dimers diffuse along the microtubule lattice, and can form multimers when concentrated enough. At intermediate concentrations, however, Ase1 multimerization is restricted to regions of microtubule overlap. These findings reveal an intriguing cooperative mechanism that controls targeting of Ase1.

Platform AW: Membrane Fusion

1877-Plat Energetics and Dynamics of SNAREpin Folding Across Lipid Bilayers Invesitgated by Direct Force Measurements

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The core principle of cellular membrane fusion consists of the assembly of cognate SNARE proteins initially residing in opposing membranes to yield a stable, bridging complex (the SNARE-pin) that triggers bilayers to merge. However, the energy released upon SNARE-pin formation, the kinetics of SNARE association and the extent of SNARE-pin assembly prior to fusion remain unknown.

To address these questions, we have used the Surface Force Apparatus to measure in real time the interaction energy versus distance profiles between assemblies of neuronal cognate v- and t-SNARE proteins anchored to lipid bilayers. The energetics and dynamics of SNAREpins formation and the different intermediate structures sampled by cognate SNAREs in the course of their assembly have been determined. The interaction energy versus distance profiles of assembling SNAREpins reveal that SNARE motifs begin to interact when the membranes are 8 nm apart and SNARE-pin formation across lipid bilayers occurs rapidly (less than 1 minute) when the bilayers reach such distance. Even after very close approach of the bilayers (~ 2–4 nm), the SNAREpins remain partly unstructured in their membrane-proximal region. The energy stabilizing a single SNAREpin in this configuration was deduced from these direct force measurements and was found to be about 35 k_BT, which corresponds closely with the energy needed to fuse outer but not inner leaflets (hemifusion) of pure lipid bilayers (40–50 k_BT).

In the presence of complexin, the interaction energy versus distance profiles show striking different features, which reveals its role in the membrane fusion process. How complexin affects the formation of SNAREpins will also be addressed.

1878-Plat Effect of Spontaneous Curvature on the Adsorption of Lipids to the Air/Water Interface

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Prior studies indicate that lipids in the H_{II}-phase mimic the rapid adsorption of pulmonary surfactant in the lung. Structural analysis, however, using small angle X-ray scattering (SAXS) and ^{31}P nuclear magnetic resonance shows that, irrespective of temperature, calf lung surfactant extract (CLSE) forms only lamellar structures. The studies reported here seek to determine the effect of spontaneous curvature on the adsorption of lamellar vesicles. We used SAXS to monitor the structures present, and compared the adsorption of dielaidoyl phosphatidylethanolamine (DEPE), which forms $L_{\beta'}$, L_α, and H_{II} phases at accessible temperatures, with dipalmitoyl phosphatidylcholine (DPPC), which forms only lamellar structures. Below the $L_{\beta'}$-L_α transition temperature, adsorption of DEPE and DPPC failed to lower surface tension below 60mN/m. Vesicles in the L_α phase at 42°C adsorbed faster for both compounds. For DPPC, the increase in rate was limited, and surface tension fell only to ~50 mN/m. DEPE instead adsorbed rapidly to ~25 mN/m. Our results suggest that an equilibrium tightly-curved structure is unnecessary for rapid adsorption. The presence of spontaneous curvature, indicated by the ability to form H_{II} structures at higher temperatures, although unexpressed in the lamellar vesicles, favors formation of curved structure in the presence of an air/water interface, and is sufficient to produce rapid adsorption.

1879-Plat Membrane Hemifusion: Energetics and Growth Kinetics

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