New and Notable

Microtubules: Mechanical Meets Chemical

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Although the cylindrical shape and regular lattice of microtubules is beguilingly simple, the polymerization dynamics have proven aggravatingly complex, and the molecular basis of dynamic instability, the behavior whereby microtubules alternate between periods of slow growth and rapid shortening, has eluded accurate mechanistic description. In part this is due to insufficient experimental resolution—light microscopy is limited by the light resolution limit, approximately the length of 30 tubulin subunits (1,2)—but is also due to the lack of a comprehensive mechanistic model tying mechanical properties with molecular kinetics.

Cryoelectron microscopy has revealed details of the structure of individual protofilaments of the microtubule lattice, but only with frozen specimens that are no longer dynamic (3). These images show great variability in the structures at the microtubule tip, and the shapes of these structures have been reproduced by modeling the microtubule wall as an elastic sheet with two competing intrinsic curvatures (4). But the role of these structures in dynamic instability is difficult to predict, because connecting the mechanical aspects of microtubule structure with the kinetics of growth and shortening has proven theoretically challenging, and thus far experimentally intractable.

In this issue, VanBuren et al. present a model and simulation, founded on earlier work (5,6), which explicitly links microtubule mechanics with chemical kinetics and thermodynamics. With this model they are able to estimate important thermodynamic parameters such as the free energy of tubulin-tubulin interactions, important mechanical parameters such as the flexural rigidity of tubulin subunits, and provide a framework to predict the influence that mechanical properties of microtubules and their dimeric tubulin subunits have on dynamic instability.

By explicitly including mechanical parameters, this work extends previous modeling that lay the foundation for the approach to the kinetics. The kinetics are addressed by calculating probabilities for the addition or loss of a subunit to specific positions in the microtubule lattice, based on the free-energy change associated with formation of lateral and longitudinal bonds between tubulin subunits. Because the number of lateral and longitudinal bonds depends on the specific site where a subunit adds at a microtubule tip, varying the energy in these bonds shifts the probability of subunits adding to specific sites, and thus influences the tip structures that evolve during microtubule growth. At each step, a roll of the dice determines which event (loss or gain of a single subunit at a single position in the lattice) takes place. Unfortunately, the free energies of these interactions haven't been measured. The authors cleverly overcome this by using their model to estimate the energies: simulations are run with the free energies as variable parameters. For a given tubulin concentration the various combinations of values that give the experimentally observed growth rates are determined. Because the energies of the bonds themselves (i.e., the standard free energies) are independent of the tubulin concentration, repetition at several free tubulin concentrations produces a consensus on the correct values; these are the longitudinal and lateral bond energies that uniquely predict growth rates at all tubulin concentrations. By constructing a model based on experimentally determined structure, and choosing an important measurable parameter (e.g., growth rate) the simulation predicts important parameters (interaction energies in this case) that are not measurable.

In this issue's article, the authors apply this approach to estimate the flexural rigidity of microtubules. The measured parameter in this case is the rate that microtubules shrink after transitioning into a distinct state called ''rapid shortening''. The rate of rapid shortening depends strongly on the mechanical strain that destabilizes the microtubule, which is in turn dependent on the flexural rigidity of the strained components. By varying the flexural rigidity over the range of the experimentally determined values, they identify the value that predicts the experimentally observed shortening rate. This is possible because the model explicitly connects the mechanical parameters (such as flexural rigidity) to chemical kinetics (rate and equilibrium constants). This is accomplished by considering how the free energy associated with mechanical strain in the microtubule lattice influences the detailed balance of tubulin binding and unbinding events modeled in the earlier work.

This approach models microtubule growth appropriately as a nonequilibrium process, and effectively captures many of the complexities of dynamic instability. Notably, it recognizes that the initial addition of tubulin to a microtubule is path dependent. Furthermore, the model is extended to predict the relative probability of different end structures undergoing ''catastrophe'': that is a transition to rapid shortening. Specifically it is predicted that a sheet-like tip is more likely to undergo a catastrophe than a blunt end. An intriguing further prediction is that the probability of catastrophe is reduced if the tubulin compliance is increased, and the flexural rigidity of the microtubule thereby decreased: this finding

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may explain how taxol, which in many studies (summarized in this issue's article by VanBuren et al.) reduces microtubule stiffness, stabilizes the microtubule lattice.

The authors have done an admirable job carefully building their model based on the microtubule literature. The incorporation of recent findings, such as more exact treatment of tubulin-tubulin interactions (7), may improve the predictive power of the model. The authors have used average growth and shortening rates to tie their model's predictions to the microtubule literature, but the model also holds promise for predicting growth rate variability. Microtubule growth rates are surprisingly variable (1,8) on the timescales of seconds and minutes even for a single microtubule, and the model may be able to capture this behavior by relating polymerization kinetics to the evolving structure at a microtubule tip. Such simulations would provide an additional test of the model, and perhaps reveal how growth-rate variability is generated. With improved biochemical characterization of the myriad of cellular factors that interact with microtubules, it should be fairly direct to extend the model to describe the behavior of microtubules in less reduced environments, e.g., Arnal et al. and others (9–11).

As important as any specific predictions is the model's general approach, which connects mechanics to kinetics and cleverly allows estimation of parameters that are difficult to directly measure. The model goes beyond descriptive to provide new mechanistic insights and physical predictions about the behavior of microtubules. This work is an excellent example of how theory and computational experimentation can cast new light on existing data to reveal new insights and provide guidance for future experiments.

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