Nonlinear Dynamics of Microtubules: Biophysical Implications

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Abstract. A recently developed model of nonlinear dynamics for microtubules is further expanded based on the biophysical arguments involving the secondary structure of the constitutive protein tubulin and on the ferroelectric properties of microtubules. It is demonstrated that kink excitations arise due to GTP hydrolysis that causes a dynamical transition in the structure of tubulin. The presence of an intrinsic electric field associated with the structure of a microtubule leads to unidirectional propagation of the kink excitation along the microtubule axis. This mechanism offers an explanation of the dynamic instability phenomenon in terms of the electric field effects. Moreover, a possible elucidation of the unidirectional transport of cargo *via* motor proteins such as kinesin and dynein is proposed within the model developed in this paper.

Key words: microtubule, tubulin, ferroelectricity, kink propagation, GTP hydrolysis, dynamic instability

1. Introduction

Microtubules (MTs) are major cytoskeletal proteins assembled from the protein called tubulin that plays a crucial role in all eukaryotic cells. The structure of microtubules is cylindrical and it typically involves 13 protofilaments (see Figure 1).

The building block of a microtubule is a tubulin dimer that contains approximately 900 amino acids comprising some 14 000 atoms with a combined mass of 110 kDa. The tubulin dimer is made up of two slightly different monomers, called α and β tubulin, respectively.

Tubulin's secondary structure is of crucial importance to the model proposed in this paper. The α -monomer contains mostly α -helices terminated with loops protruding partially through the globular structure of the protein. These loops are important for the formation of weak bonds between neighbouring proteins, enabling polymerization of tubulin into microtubules. Additionally, they provide a binding site for the energy-giving molecule GTP that is non-exchangeable in the α -monomer while exchangeable in the β -monomer. The flexible loops are ideally suited for adjusting the positions of the many amino acid residues that participate

Figure 1. A cartoon showing the construction of a MT from individual tubulin dimers.

in the binding between two neighbouring dimers. The core part of the β -monomer consists of β -sheets of peptide chains surrounded by α -helices that terminate with protruding flexible loops whose properties are similar to those in the α -monomer. Functionally, the β -monomer plays a more important role than the α -monomer owing to its exchangeable GTP binding site as well as many other ligand-binding sites.

Microtubules form an important part of the cellular scaffold providing the required rigidity and plasticity in addition to creating a network of “railroad tracks” for active intracellular transport *via* motor proteins. Active transport is critical for cellular organization and the multitude of functions performed by sub-cellular structures. Impaired transport has been linked to a range of diseases such as neuronal degeneration [1]. Long-distance transport in cells uses opposite-polarity molecular motors of the kinesin and dynein super-families that move cargo along MTs.

MTs are structurally rigid against thermal fluctuations since their persistence length is in the mm-range, far exceeding that of either actin or DNA. On the other

hand, MTs are flexible when subjected to bending and buckling forces. MTs rupture when stretched beyond 50% of their original length [2]. Their flexibility can be attributed to the presence of flexible protruding loops that contribute to the protein polymerization binding forces.

MTs also play a crucial role during cell division forming a dynamic structure that spatially segregates the duplicated chromosomes. *In vitro*, MTs exhibit dynamic instability that was discovered some 20 years ago [3] and so far has been proven unique to MTs only. During this process, MTs switch stochastically between periods of persistent assembly and catastrophic disassembly. This paper develops a physical model that is an attempt to explain the possible trigger mechanism for the seemingly random catastrophe events.

The mechanism of initiating tubulin assembly into MTs depends strongly on the binding of a Mg^{2+} ion to tubulin. It has been recently shown [4] that the six-coordinated Mg^{2+} complex with water, glutamic acid and GTP in singlet and triplet states is formed. In the triplet state, the magnesium complex concentrates its spin density toward one of the coordinated water molecules. Within that molecule, a redox reaction occurs and one of the hydrogen atoms is released from the complex at a speed of 330 m/s. The released hydrogen moves over a distance of 1.5 nm through water molecules surrounding the GTP centre. Eventually, the hydrogen atom collides inelastically with one water molecule, producing H_3O^+ and a free electron. Free electrons have been shown to have a tendency to accumulate in the bound water shell surround the tubulin dimer. This process results in the formation of a local electric field in the form of a Faraday cage for the intrinsic dipole moment of tubulin, protecting it from the ions in solution.

The role of electrons in the bound water shell surrounding tubulin has been confirmed experimentally [4] whereby a buffer solution containing non-polymerized tubulin was irradiated by a beam of electrons and subjected to laser impulses in the femtosecond range. Upon irradiation, tubulin began to assemble in the absence of Mg^{2+} ions.

2. Ferroelectric Properties of Microtubules

Our departure point in this section is the secondary structure of tubulin. Virtually every peptide group in an α -helix possesses a considerable dipole moment, on the order of $p_0 = 1.2 \times 10^{-29}$ Cm = 3.5 D. All these dipoles are almost parallel to the helical axis, giving rise to an overall dipole moment of this particular helix. It is generally accepted that this large dipole moment of an α -helix has an important biological role [5]. Since the tubulin dimer contains several α -helices that are not oriented randomly, it is not surprising that each tubulin dimer possesses a large net dipole moment p . On the other hand, closed loops lend themselves to the cancellation of an overall dipole moment.

The idea that MTs are ferroelectric was proposed some 30 years ago [6] on the basis of their piezoelectric properties. It is apparent that due to the strong

Figure 2. A map of the surface electric potential on a tubulin dimer with an elongated C-terminal conformation. The “+” and “-” symbols indicated the local field strengths. Figure prepared using MolMol.

curvature of an MT cylinder (see Figure 1), the inner parts of the dimer structure are compressed in order to fit into a microtubule while the outer ones are stretched by a substantial amount of tension. This additional redistribution of excess negative charge enhances the transverse component of the net dipole moment of every dimer comprising a MT. This effect is very analogous to that observed for the cylindrical hair cells [7]. This has also been corroborated by a detailed map of the electric charge distribution for the tubulin dimer [8].

As shown in Figure 2, it is clear that the C-termini that extend outwardly from the MT surface carry a significant amount of negative electric charge (as much as 40% of the total monomer charge can be attributed to a C-terminus). These negative charges on each C-terminus are responsible for its extended conformational state since the surface charge is also negative leading to Coulomb repulsion.

Taking into account the bound water layer with electrons released due to the Mg^{2+} complex activity, it is highly plausible to envisage the MT structure as a ferroelectric system with the dipole moments of tubulin oriented as shown in Figure 3.

Figure 3. An individual dimer with its dipole decomposed, a protofilament with the dipoles of individual dimers, and a MT with the giant dipole due to these individual contributions.

The ends of a MT therefore possess a different amount of net charge as was recently demonstrated through massive molecular dynamics computations [9]. We therefore conclude that the MT cylinder supports an intrinsic electric field E [10].

3. Role of GTP Hydrolysis in MT Dynamics

Conformational flexibility of proteins refers to molecular motions on a time scale ranging from picoseconds (thermal fluctuations) to large-scale conformational motions important for functional dynamics occurring over microseconds or even milliseconds. Neutron scattering experiments [11] showed that at lower temperatures proteins are biologically inactive since thermal motions are basically harmonic. At higher temperatures, activation energy becomes accessible and thermal motions are predominantly anharmonic since molecules can make transitions between two different equilibrium states. The protein becomes structurally “soft” and biologically active. This phenomenon is called a dynamical transition.

Non-thermal energy sources are important for proteins in terms of facilitating these dynamical transitions. In the present case of tubulin and microtubules, a β -tubulin monomer in the dimeric structure has an exchangeable GTP site that is

an active domain. The chemical potential energy of GTP hydrolysis is converted into mechanical motion when some key chemical bonds are broken. The loss of a phosphate group occurs when the bound GTP molecule is hydrolysed to GDP in a reaction that is catalyzed by tubulin.

The dissociation of the inorganic phosphate group (P_i) in the reaction

causes a shift of a few angstroms in the GTP binding site that contains a polypeptide loop called a sensor loop. This small displacement causes a further conformational change that propagates along an associated α -helix (called a switch helix).

The switch helix serves as a “piston” that transmits a stimulus to adhere to a specific site of the same β -tubulin latching it in a “closed” conformation. This state of the α -helix pertains to a pre-stressed spring to use a mechanical analogy. The next stage of the conformational change triggered by the release of GDP causes the switch helix to unlatch allowing tubulin to tilt, rotating through an angle θ in the (r, x) -plane (in the filament direction as shown in Figure 4). This transformation is consistent with the conformational changes exhibited by motor proteins due to ATP hydrolysis [12]. The main difference between them is that in the case of motor proteins, their conformational changes are much larger due to their overall geometry and the need to overcome the viscosity of the medium while tubulin is incorporated in the cylindrical structure of an MT.

Next, we estimate the magnitude of the force associated with the conformational change due to the effect of GTP hydrolysis in tubulin. We deduce that the switch

Figure 4. Characteristic dimensions of an MT cylinder with wedge-like dimers as its building blocks. The parameters are $d = 5$ nm, $R = 10$ nm, $r_1 = 7.5$ nm, $r_2 = 12.5$ nm.

helix has the approximate length of $l = 3$ nm. The force exerted by the helix and involving a translation by length Δl is [13]:

$$F = 3EI l^{-3} \Delta l = \kappa \Delta l \quad (2)$$

where EI is the flexural rigidity of the helix which is related to the persistence length l_p according to the formula:

$$l_p = EI/(k_B T) \quad (3)$$

where k_B is the Boltzmann constant and T the temperature in degrees Kelvin. The stiffness parameter κ can be estimated as

$$\kappa = 3EI l^{-3} = 3k_B T l_p l^{-3}. \quad (4)$$

Taking the persistence length of the switch to be $l_p = 100$ nm and its length as $l = 3$ nm one obtains

$$\kappa = 19 \text{ pN/nm}. \quad (5)$$

If the expected conformational change that occurs during GTP hydrolysis is of the order of $\Delta l = 1$ nm, the force generated by it is $F = 19$ pN. This force results in the tilt of the tubulin dimer by an angle $\theta = (1 \text{ nm}/2.5 \text{ nm}) = 0.4$ rad where 2.5 nm is the approximate radius of the β -tubulin monomer.

The above discussion provides a strong argument in favour of expanding the elastic potential energy of the dimer-dimer interaction to the fourth order with respect to the tilt angle since it implies a double-well situation with two local minima. Consequently, we calculate the work done by the force in Eq. (2) over the displacement of $\Delta l = 1$ nm to be $W = 1.9 \times 10^{-20} \text{ J} = 0.12 \text{ eV}$. Since the energy released in one GTP hydrolysis event is known to be approximately 0.15 eV, it follows that a large part of this energy is utilized in generating the elastic deformation, while the remainder is dissipated into the surroundings. The elastic deformation following GTP hydrolysis will also affect the inter-dimer bonds between neighbouring tubulin units in a microtubule. The resultant displacements may lead to a dynamic collective phenomenon of a traveling wave of conformational changes propagating down the length of an MT. This effect is discussed in the next section.

4. The Nonlinear Model of a Propagating Conformational Tilt

The two main aspects of our theory are the nonlinear character of the inter-dimer potential energy and the ferroelectric behaviour of an MT. In an earlier publication

Figure 5. The ferroelectric geometry of a tubulin dimer; x is in the MT direction.

[14] we have provided a detailed justification of the free energy functional for an MT in cylindrical co-ordinates (r, θ, φ) that are schematically illustrated in Figure 5. Since the azimuthal angle φ is largely restricted due to the pronounced curvature of the MT cylinder, here we pay special attention to the tilt angle θ that dominates the overall conformational dynamics.

The free-energy density in the MT model presented here consists of the following four terms.

- a) The elastic energy of the dimer-dimer interaction within a protofilament expands in terms of the tilt angle θ up to the fourth order as:

$$W_{el} = (-A\theta^2 + B\theta^4)/r^2 \quad (6)$$

where A and B are coefficients that are expressed in terms of the cylindrical components of the elasticity tensor [14].

- b) The splay energy density has the form:

$$W_{sp} = k/2(\partial\theta/\partial x)^2 \quad (7)$$

where the coefficient k denotes the smectic splay elastic modulus. The presence of splay deformation has been experimentally demonstrated by bending MT filaments [15].

- c) The polarization energy (see Figures 3 and 5 for illustration) takes the form:

$$W_{pol} = (p_t^2/\chi_t + p_\ell^2/2\chi_\ell)/2 - Ep_\ell - \mu_p p_t \theta \quad (8)$$

where the longitudinal and transverse dielectric susceptibilities are denoted by χ_ℓ and χ_t , respectively. The intrinsic electric field E is directed along the long axis of the MT cylinder (x -axis), and μ_p is a phenomenological constant that depends on various model parameters such as the anisotropic polarizability of tubulin and the chirality effect due to C-termini.

d) The rotational kinetic energy of the tilting motion of the tubulin dimers is:

$$W_{\text{kin}} = \frac{1}{2} J (\partial \theta / \partial t)^2 \quad (9)$$

where J stands for tubulin's rotational moment of inertia divided by its volume (specific rotational inertia).

The total free energy functional of the system can be written as

$$F = 2\pi \int_{R-d/2}^{R+d/2} r \, dr \int_0^L (W_{\text{el}} + W_{\text{sp}} + W_{\text{pol}} + W_{\text{kin}}) \, dx. \quad (10)$$

Before a free-energy minimization procedure is performed, we comment here on the necessity of accounting for the presence of viscosity in the medium surrounding the MT (cytosol). This effect can be modeled by including a friction term in the equation of motion that is subsequently derived using the Euler–Lagrange procedure. Hence a viscous torque is added

$$\tau_{\text{vis}} = -\Gamma \partial \theta / \partial t \quad (11)$$

where Γ depends both on the viscosity coefficient and the structural details of the MT through the Stokes–Einstein formula.

Combining the torque in Eq. (11) with the Euler–Lagrange equation stemming from the free-energy functional in Eq. (10) leads to a nonlinear differential equation of motion for the tilt angle θ . Using scaled variables and adopting a traveling wave form one obtains the following ordinary differential equation:

$$d^2 \eta / d\xi^2 + \beta \, d\eta / d\xi + \eta - \eta^3 + \varepsilon = 0 \quad (12)$$

where we have introduced the following notation:

$$\eta = \theta / \theta_0; \quad \theta_0 = (a/b)^{1/2}; \quad \xi = \sqrt{a / [RdJ\pi(v_0^2 - v^2)]} (x - vt) \quad (13a)$$

$$\beta = \Gamma R v / \sqrt{RdJ(v_0^2 - v^2)} \quad (13b)$$

$$\varepsilon = a^{-3/2} b^{1/2} c = a^{-3/2} b^{1/2} R q_{\text{eff}} E \quad (13c)$$

$$a = 2\pi \ln(5/2) A d R^{-1} - 2\pi R \, d\psi \mu_p^2 \cos^2(\gamma) \quad (13d)$$

$$b = 4\pi \ln(5/3) B \, d R^{-1} \quad (13e)$$

$$c = \pi R d \psi \sin(2\gamma) \mu_p E = q_{\text{eff}} R E \quad (13f)$$

and

$$\psi = [\sin^2(\gamma) / \chi_\ell + \cos^2(\gamma) / \chi_t]^{-1} \quad (13g)$$

where v_0 is the sound velocity along the MT filament and v stands for the kink propagation velocity.

The most important result coming from Eq. (12) is the existence of an analytical solution in the form of a kink-type domain wall given by

$$\eta(\xi) = \eta_2 + (\eta_1 - \eta_2)/[1 + \exp \xi(\eta_1 - \eta_2)/\sqrt{2}] \quad (14)$$

with the asymptotic values of η_1 and η_2 depending on E via ε as follows:

$$\eta_1 = (2/\sqrt{3}) \cos[\cos^{-1}(3\sqrt{3}\varepsilon/2)] \quad (15)$$

$$\eta_2 = (2/\sqrt{3}) \cos[2\pi/3 + \cos^{-1}(3\sqrt{3}\varepsilon/2)/3]. \quad (16)$$

The maximum value of the field strength has been estimated earlier [16] and is expected to reach $E_{\max} = 2.6$ MV/m. Note that even in this extreme case the condition $\varepsilon < 1$ still holds. It implies a simplified formula for the kink's propagation velocity as

$$v = 3\sqrt{JbdR/2v_0q_{\text{eff}}E/(aR\Gamma)} = \mu_{\text{kink}}E \quad (17)$$

representing the so-called terminal velocity for the given constant electric field E . It is analogous to the drift velocity of electrons as described by Ohm's law. The kink's mobility μ_{kink} depends on the model parameters including the ferroelectric character of the MT via the effective charge q_{eff} that enters into the parameter c and elastic properties of the MT that are reflected in the form of the coefficients a and b . The role of viscosity is reflected in the damping coefficient Γ that reduces the kink's propagation velocity.

The analysis of the kink's stability in the absence of an electric field [14] indicates random motion of the kink under the influence of thermal fluctuations. The mean free path of the kink's translational motion is given by the formula:

$$\lambda = bv_0a^{-\frac{3}{2}}\sqrt{3/2\pi RdJ}. \quad (18)$$

This demonstrates that the strength of nonlinearity manifested by the parameter b increases the length of translational motion. Figure 6 illustrates the kink's spatial dependence.

Figure 6. The kink's spatial dependence in terms of the local dipole moment's orientation.

In the following section we will comment on the linear response mechanism for the kink's terminal velocity electric field dependence as a possible control mechanism for a number of intra-cellular processes.

5. Summary and Conclusions

In this paper we argued that GTP hydrolysis, taking place as a result of tubulin assembly into MTs, leads to a combination of mechanical and electrical energy propagating along the MT axis in the form of a kink excitation. The first point made in this connection was that the difference in the electrical properties of the two MT ends leads to the emergence of piezoelectric properties of MTs, creating an intrinsic electric field along the MT axis. The intensity of this field depends on the length of the MT as well as on the physiological conditions of the solution (pH, ion concentration and temperature) due to screening effects by counter-ions. The shorter the MTs, the stronger the field (potential gradient) and the greater the kink's propagation velocity. Faster kinks possess a greater kinetic energy and hence a greater impact is expected on the MT structure that may become destabilized by this energy deposition at the distant end. If the distant tip is already weakened by either mechanical tension or a diminished lateral cap, the effects of kink energy can cause a catastrophic disassembly of the entire MT. Below we list some experimental evidence in support of this hypothesis:

- a) If tubulin used in assembly experiments contains a non-hydrolysable analog of GTP, very stable MTs are polymerized. This precludes the formation of kink excitations [17].
- b) Conversely, the addition of Ca^{2+} ions to the solution leads to an increased rate of GTP hydrolysis and hence a higher frequency of catastrophes [18] which would indicate a faster production of kink excitations.
- c) Adding vinblastine to the solution results in the capping of MT tips and an increased MT stability despite GTP hydrolysis [19]. We postulate that the kinks can still be formed but that their effects are eliminated by the capping process.
- d) Similarly, microtubule-associated proteins (MAPs) stabilize MTs, especially in neurons, by interconnecting them and forming a scaffold-like structure. The presence of MAPs reduces dramatically the frequency of catastrophes and we suggest that part of their role is to impede the generation and movement of kinks by forming inhomogeneities and structural obstacles to their propagation. The scattering potentials created in this manner lead to either trapping or reflection of propagating kinks preventing them from reaching the MTs tips [16]. Consequently, increasing the number of MAPs enhances the stability of interconnected MTs.

We now turn our attention to the role played by MTs in motor protein transport involving the kinesin and dynein super-families. Note that the terminal velocity of a propagating kink is an average velocity due to electric field effects (see Eq. 17),

combined with random effects due to thermal fluctuations (see Eq. 18). If a propagating kink encounters a motor protein bound to the filament, it may cause a tug-of-war effect on the motor [1], disrupting the otherwise unidirectional movement of the kink. In this connection, it is instructive to estimate the kink's propagation velocity. For a stable MT with an approximate length of $L = 4 \mu\text{m}$, the intrinsic electric field in the central region can be calculated to be:

$$E = Q/(\pi \epsilon_0 \epsilon_r L^2). \quad (19)$$

Taking $Q = 13 e$, i.e. one excess charge per protofilament, and estimating the relative dielectric constant to lie in the range $10 < \epsilon_r < 80$, we find that the electric field is expected to lie in the range $0.5 \text{ V/m} < E < 5 \text{ V/m}$ resulting in the window of kink propagation velocities being

$$0.2 \mu\text{m/s} < v_{\text{kink}} < 2 \mu\text{m/s}. \quad (20)$$

It is interesting to note that, depending on the ATP and salt concentrations, and the load placed on the kinesin molecule, kinks propagate along MTs with velocities ranging between $0 \mu\text{m/s} < v_{\text{kinesin}} < 0.9 \mu\text{m/s}$, i.e. there is a strong overlap in the velocities of the two types of biological motions that may interact with each other. Using an easy-to-grasp analogy, the kinesin motor can be viewed as a surfer and the moving kink as a water wave. Thus, when properly matched, the motor may be able to “catch the wave” and travel on its crest for a while. Conversely, when the two objects travel in opposite directions, the motor protein may experience a “tug-of-war” effect in trying to overcome the disruptive influence of the wave impacting on its motion.

How are proteins, with their very diverse functional roles in the cell, able to produce mesoscopic and even macroscopic movements in a co-ordinated fashion? The answer to this question is still largely unknown. For example, the formation of the mitotic spindle apparatus and the process of chromosome segregation require a concerted effort of force generation by kinetochore microtubules and a simultaneous action of kinesin and dynein motors. In a process as important as cell division, mistakes that would be a frequent occurrence if random thermal fluctuation were to rule, must be necessarily eliminated. Within the framework of our model proposed in this paper, MTs are not only passive tracks for active transport in the cell but also signal relays for electrical, mechanical and chemical stimuli that may be transduced over distances compared to the cell size. Additionally, the cell can control the magnitude of the electric field induced by a MT by changes in the salt and ion concentration as well as pH values. In summary, the present hypothesis and the model developed in this paper offers new insights into intracellular molecular processes related to MT dynamics. Some of the predictions made here can be tested with experimental tools such as Mössbauer spectroscopy [20] or neutron scattering with deuterium labelling that would enable the verification of

the magnitude of tubulin conformational changes and their propagation due to GTP hydrolysis [11].

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