Intrinsic microtubule GTP-cap dynamics in semi-confined systems: kinetochore–microtubule interface

Vlado A. Buljan · R. M. Damian Holsinger ·
Brett D. Hambly · Richard B. Banati · Elena P. Ivanova

Received: 21 March 2012 / Accepted: 7 September 2012 / Published online: 18 October 2012
© Springer Science+Business Media B.V. 2012

Abstract In order to quantify the intrinsic dynamics associated with the tip of a GTP-cap under semi-confined conditions, such as those within a neuronal cone and at a kinetochore–microtubule interface, we propose a novel quantitative concept of critical nano local GTP-tubulin concentration (CNLC). A simulation of a rate constant of GTP-tubulin hydrolysis, under varying conditions based on this concept, generates results in the range of $0\text{--}420\text{ s}^{-1}$. These results are in agreement with published experimental data, validating our model. The major outcome of this model is the prediction of 11 random and distinct outbursts of GTP hydrolysis per single layer of a GTP-cap. GTP hydrolysis is accompanied by an energy release and the formation of discrete expanding zones, built by less-stable, skewed GDP-tubulin subunits. We suggest that the front of these expanding zones within the walls

V. A. Buljan (✉) · R. M. Damian Holsinger · R. B. Banati
Brain and Mind Research Institute, Sydney Medical School, The University of Sydney,
Sydney, NSW 2050, Australia
e-mail: bvlado@physiol.usyd.edu.au

V. A. Buljan
Department of Anatomy and Histology, Sydney Medical School, The University of Sydney,
Sydney, NSW 2006, Australia

R. M. Damian Holsinger
Discipline of Biomedical Science, School of Medical Sciences, Sydney Medical School,
University of Sydney, Sydney, NSW 1825, Australia

B. D. Hambly
Discipline of Pathology, School of Medical Sciences, Sydney Medical School, Bosch Institute,
University of Sydney, Sydney, NSW 1825, Australia

R. B. Banati
Discipline of Medical Radiation Sciences, Faculty of Health Sciences, University of Sydney,
Cumberland, NSW, Australia

E. P. Ivanova
Faculty of Life and Social Sciences, Swinburne University of Technology,
Hawthorn VIC, 3122, Australia

of the microtubule represent soliton-like movements of local deformation triggered by energy released from an outburst of hydrolysis. We propose that these solitons might be helpful in addressing a long-standing question relating to the mechanism underlying how GTP-tubulin hydrolysis controls dynamic instability. This result strongly supports the prediction that large conformational movements in tubulin subunits, termed dynamic transitions, occur as a result of the conversion of chemical energy that is triggered by GTP hydrolysis (Satarić et al., *Electromagn Biol Med* 24:255–264, 2005). Although simple, the concept of CNLC enables the formulation of a rationale to explain the intrinsic nature of the “push-and-pull” mechanism associated with a kinetochore–microtubule complex. In addition, the capacity of the microtubule wall to produce and mediate localized spatio-temporal excitations, i.e., soliton-like bursts of energy coupled with an abundance of microtubules in dendritic spines supports the hypothesis that microtubule dynamics may underlie neural information processing including neurocomputation (Hameroff, *J Biol Phys* 36:71–93, 2010; Hameroff, *Cognit Sci* 31:1035–1045, 2007; Hameroff and Watt, *J Theor Biol* 98:549–561, 1982).

Keywords Critical nano local GTP-tubulin concentration • Hydrolysis • Solitons • Kinetochore outer domain • Molecular emergency compartment

1 Introduction

Recent evidence clearly illustrates the importance of quantitative analysis in studies of the dynamics of the microtubule GTP-cap under *semi-confined* conditions such as those that occur within the neuronal cone and the mitotic spindle [1]. Of particular importance to the development of an intrinsic dynamic model representing the tip of the GTP-cap is a recent study [2] that clearly demonstrates that tubulin dimmers influence each other within the structure of the GTP-cap. This work highlights the importance of mutual interactions between GTP/GDP-tubulins sitting in neighboring locations within the GTP-cap. It also draws attention to the fact that, in future models, an understanding of non-linear effects relative to microtubule growth dynamics, such as non-linear dependence of microtubule growth to relative concentration of tubulin isotopes ($\alpha\beta_{II}/\alpha\beta_{III}$), must be taken into account.

We have undertaken a quantitative analysis of the intrinsic dynamics of the GTP-cap observed at its tip under semi-confined conditions of the kinetochore–microtubule interface (KMI). The KMI is embedded within the outer domain of the kinetochore. The theoretical framework for our work is based on a hybrid concept of a critical nano local GTP-tubulin concentration (CNLC). It is based on the coupling of *bulk* solutions and *local* structural features involved in microtubule assembly. Historically, this concept has its roots in seminal works that have demonstrated that linear models of microtubule growth fail to fully reproduce the dynamics of growth in biological systems, mainly due to the fact that this growth is based on non-equilibrium processes [3]. It has been shown that whenever local fluctuations of tubulin concentration occur, the local rather than the bulk concentration of tubulin plays a critical role in the dynamics of microtubule growth [3–5].

Microtubule growth consists of associations and dissociations of GTP-tubulin accompanied by a somewhat delayed hydrolysis of GTP-tubulin subunits. As a result of this hydrolysis, microtubules are heterogeneous polymers. They consist of intermediate microtubule walls, composed predominantly of less stable, hydrolysed GDP-tubulin subunits, while their ends contain stable GTP-tubulin subunits collectively referred to as the microtubule

“GTP-cap”. Due to its intrinsic heterogeneity, microtubule growth is endowed with exceptional dynamic features termed “dynamic instability”, which refers to the coexistence of both assembly and disassembly phases at the same microtubule end and includes random alternation between these two phases [6]. The question regarding the relationship between hydrolysis of the GTP-cap and dynamic instability has been extensively debated and some consider it as the “holy grail” of the field [6, 7]. However, the picture is incomplete. Indeed, it has been suggested that a comprehensive model that incorporates both the mechanical properties and molecular aspects of microtubule growth may complete the picture [8].

Owing to the great extent of dynamic instability, microtubules play a critical role in crucial cellular events such as dislocation of intracellular components, signaling during cellular division and movement [9–13].

Microtubules perform multiple tasks during mitosis as they transduce the force generated by dynamic instability in the GTP-cap into chromosome movement and segregate the chromosomes with high accuracy by synchronizing and fine-tuning their length and direction [14]. The failure in mitotic microtubule dynamics may result in an uneven segregation of genetic material leading to catastrophic consequences known as “chromosomal chaos”, or better known as aneuploidy, which may further lead to speciation or carcinogenesis [15–19].

It is well documented that the growth of individual microtubules is extremely sensitive to local fluctuations in the concentration of GTP-tubulin, especially when free diffusion of GTP-tubulin is impaired [15, 20]. Notably, it has been discovered that local fluctuations of GTP-tubulin concentrations may be directly caused by microtubule growth and shrinkage in small *semi-confined* compartmental volumes, which in turn cause local fluctuations that influence the dynamic instability of the microtubule [1]. Thus, semi-confined conditions, such as within the outer domain of kinetochores, may significantly influence the intrinsic dynamics of the GTP-cap.

In order to quantify the mutual effects of fluctuations in the local concentration of GTP-tubulin and structural changes within the tip of the GTP-cap on overall GTP-cap dynamics under semi-confined conditions, we will formulate a novel concept of critical nano local GTP-tubulin concentration (CNLC). In this concept, the rate of GTP hydrolysis will be determined explicitly by the local fluctuations of GTP-tubulin concentration and GTP-cap structural parameters.

2 Intrinsic dynamics of the GTP-cap within the KMI

2.1 KMI as a bifurcation and emergency stage in mitosis

Our primary interest lies in the emergent events related to the dynamics of the GTP-cap that occur imminently prior to and during the addition of GTP-tubulin to the cap. The direction of movement of a single microtubule (extension or shortening) depends on the positioning of GTP-tubulin at the point of addition onto the cap as well as on the stability of the GTP-cap. Therefore, minute random changes in any of these factors may lead to gross qualitative changes in mitosis. The stage of mitosis at the KMI may be described as a point of *bifurcation*: the course of mitosis may proceed further along one of two pathways. The first pathway is the formation of unpredictable (chaotic) structures including aneuploidy and possibly cancer [16], while the other may lead toward a more predictable, highly ordered structure (a new daughter cell). Therefore, collectively, this micro compartmental

volume, which encompasses the KMI, could be considered as a “molecular emergency compartment” (MEC) of the KMI.

2.2 Semi-confined conditions at the KMI

The tip of the GTP-cap, which constitutes an essential part of the KMI, is deeply embedded in the dense filamentous protein architecture of the outer domain. Therefore, prior to being positioned for addition onto the tip of the GTP-cap, GTP-tubulin moves through the dense network of filamentous proteins of the outer domain [17, 18]. As such, the motion of GTP-tubulin is fairly restricted. Hence, the dense filamentous network constitutes the initial front of the semi-confined KMI [19]. It is likely that these semi-confined conditions lead to lower microtubule growth and shrinking rates compared to those outside the outer plate [3].

2.3 Presence of proteins within the MEC and their effect on the motion of GTP-tubulin

The state of motion of single GTP-tubulin molecules within the MEC may be affected by a multitude of non-tubulin as well as other GTP-tubulin molecules. To date, approximately 60 different classes of proteins have been identified within the outer plate [21]. Ten classes of non-tubulin proteins, including check-point, cross-bridge, and MAPs, interact directly with the wall of the microtubule. Although all outer domain proteins may directly interact with GTP-tubulin and in this way influence its incorporation into the GTP-cap, we will only consider their influence in the first approximation. Their exclusive presence within the outer domain will reduce the space for accommodating GTP-tubulin (to a nanoscale) during its positioning for addition. Additionally, heterogeneity in the size of non-tubulin proteins may create *local heterogeneities* in the effective size of the MEC.

2.4 Critical nano local concentration (CNLC) of GTP-tubulin

Recent calls for a comprehensive model of microtubule growth have indicated that it should incorporate two key aspects: its mechanical properties and molecular kinetics [8]. The lack of such a model may stem from a limitation of our molecular understanding of dynamic instability. Following this line, we formulate a simple concept based on the modification of bulk critical concentration due to modification of kinetic rates by the influence of local structural features of the microtubule lattice.

Due to heterogeneity in the spatial distribution of outer domain proteins, the local concentration of GTP-tubulin is not uniform but heterogeneous. It changes dynamically throughout the volume of the MEC. Therefore, due to the dynamics of heterogeneity of the MEC, it is conceivable that different part(s) of the GTP-cap tip may sense different local GTP-tubulin (at a nanoscale) concentrations. At a certain point in time, at certain location(s), the GTP-tubulin concentration may be above the threshold, enabling addition and growth, while at other location(s) they may be below the threshold for growth and dynamic instability may occur locally (one or a few microtubule protofilaments included selectively). The differentiated dynamics of local heterogeneity and the selectivity of the tip of a GTP-cap are likely rationales for important findings indicating that the distal ends of microtubules embedded within the outer plate are fluidic [22].

In support of the concept of local GTP-tubulin concentrations, we have already discussed the possibility that local GTP-tubulin concentrations may play critical and selective roles in microtubule growth in a semi-confined space and even under bulk conditions [1, 15, 20, 23, 24].

2.5 The effect of CNLC on the push/pull mechanism of the kinetochore–microtubule

In Fig. 1 we demonstrate the applicability of the CNLC concept in the rationale of the intrinsic push/pull mechanism of the kinetochores–microtubule action. We have considered, in previous sections, how the intrinsic dynamics of the GTP-cap is driven by changes in the local concentration of GTP-tubulin. In this instance, the term “intrinsic dynamics of the

Fig. 1 A model of the intrinsic microtubule GTP-cap mechanism of pulling sister chromatids during mitosis. For simplicity, we illustrate the model with one microtubule per outer plate. Two outer plates, i.e., two microtubules, are positioned at opposite ends of sister chromatids. **a** Microtubule assembly phase. The free GTP-tubulin concentration within the space of the kinetochore outer plate is above critical nano local concentration (CNLC). It enables the microtubule to grow. **b** Microtubule critical phase. The microtubule tip reaches the bottom of the outer plate. As such, no GTP-tubulin can reach the tip in order to be associated with the microtubule. Concurrently, the local concentration of GTP-tubulin is depleted, i.e., GTP-tubulin concentration is below CNLC. **c** Microtubule disassembly phase. Conditions at **b** cause the tip of the microtubule to swell and initiate microtubule endwise disassembly. The length of the microtubule became shorter. The swelling tip presses into the wall and the curling proto-filaments are entangled with fibrous proteins of the wall of the outer plate. When this event is synchronized with the identical one on the microtubule counter positioned on the opposite side of sister kinetochores, it will create tension between the two sister kinetochore outer plates. Consequently, the sister kinetochores and the chromatids are pulled away

GTP-cap” refers solely to the changes in the GTP-cap caused by fluctuations in the local concentration of GTP-tubulin.

For simplicity, we describe this model when there is only one microtubule per one outer plate [11]. Briefly, as long as the local concentration of GTP-tubulin within the space of the kinetochore outer plate is above the critical nano local concentration (CNLC), microtubules will grow (Fig. 1a). Gradually, the tip of the growing microtubule GTP-cap will approach the bottom of the outer plate. At this stage, two critical events occur: (a) the concentration of GTP-tubulin molecules rapidly decreases in the space of the outer plate to the extent of depletion and most likely below the CNLC and (b) the space between the bottom of the outer plate and the tip of the GTP-cap is narrowed to the extent that additional GTP-tubulin molecules are excluded from approaching the tip and therefore cannot associate with the tip. These conditions halt microtubule growth (Fig. 1b). Indeed, due to the two factors listed above, the GTP-cap may be lost instantly. Once the GTP-cap is lost, the microtubule begins rapid endwise disassembly at the fast (outer plate embedded) end. Endwise disassembly is accompanied by an enlargement of the tip of the microtubule and a curling out of microtubule protofilaments (rams horn formation) [12]. The enlarged tip of the GTP-cap and the ram horns entangle within the network of fibrous proteins of the outer plates, enabling microtubules to be firmly hooked onto the outer domain fibrous network (see Fig. 1b). Since the walls of the outer domain are flexible, they are not irreversibly stretched due to microtubule “hooking” [12, 25]. However, since the centrosomal microtubule end is changing very slowly or even in a static state, a tensional stress develops in the microtubule in response to the compressed fibrous network and microtubule shortening [12]. The tensional stress formed in the microtubules creates a pulling stress between the two outer plates such that further shortening of the microtubules will pull apart two sister kinetochores in opposite directions and will eventually lead to the separation of the sister chromatids (Fig. 1c).

3 GTP-cap size in terms of the CNLC of GTP-tubulin

To address the effects of structural changes that occur within the GTP-cap during fluctuations in local GTP-tubulin concentrations in the vicinity of the GTP-cap tip, we will define and use the term “ m -coefficients”. These m -coefficients are particular numbers that quantitatively reflect the extent of GTP-tubulin subunits within the tip of the GTP-cap where association, dissociation, and hydrolysis occur.

3.1 The m -coefficients

In order to account for the influence of neighboring tubulin subunits within the tip of the GTP-cap, as was demonstrated in the stochastic model of microtubule dynamics [2], we introduce a concept of m -coefficients.

At a moment (t) a microtubule consists in GTP-tubulin subunits that constitute part of the microtubule known as the GTP-cap and subunits that constitute the rest of microtubule body. For the entire microtubule this can be represented as:

$$m = m_{GTPcap} + m_{GDP} \quad (1)$$

where m denotes the total number of tubulin subunits that constitute the entire microtubule; m_{GTPcap} denotes the number of GTP-tubulin subunits that constitute only the GTP-cap;

m_{GDP} is the number of hydrolyzed GDP-tubulin subunits, which constitute the microtubule body excluding the GTP-cap.

Generally, the numbers m_{GTPcap} and m_{GDP} are defined within a maximal range

$$0 \leq m_{GTPcap} \leq m \quad (2)$$

$$0 \leq m_{GDP} \leq m \quad (3)$$

Although the equations defined above are in relation to the time (t), for simplicity, we have omitted the index related to time (t).

Our goal is to formulate a simple theoretical framework within which it would be possible to study the mutual relationship between the changes in local GTP-tubulin concentration and the GTP-cap size. For this purpose, it is necessary to explicitly define the quantities that represent how the GTP-cap is constituted by quiescent GTP-tubulin subunits, then subunits undergoing association, dissociation, and hydrolysis at time (t). At the first step, for the sake of generality, we define these numbers in terms of probabilities. For a given GTP-cap size (m_{GTPcap}), we denote $(m_{i,GTP}^0)$, $(m_{j,GTP}^a)$, $(m_{k,GTP}^d)$ and $(m_{l,GTP}^h)$ as the probabilities of GTP-tubulin subunits undergoing specific events at time (t), i.e., quiescence (no reaction, association, dissociation, or hydrolysis occurs) at a location associated with index (i), then association, dissociation, and hydrolysis at locations associated with indices (j), (k) and (l), respectively. In terms of the number of events, at time (t) the indices (i, j, k, l) are counters of related events. In terms of the spatial distribution of events, each index from the set (i, j, k, l) stands for the set (x, y, z) of Cartesian coordinates. The maximal ranges of the indices (i, j, k, l) are: $0 \leq i \leq m_{GTPcap}$; $0 \leq j \leq N$; $0 \leq k \leq N$; $0 \leq l \leq m_{GTPcap}$. N is the number of microtubule protofilaments. Let us suppose that quiescence, association, disassociation, and hydrolysis certainly occur but exclusively at locations (i), (j), (k) and (l) respectively, at time (t). Exclusivity means that two different events cannot take place at the same time at the same location, i.e., a different combination of indices belongs to each of two different events. Then, if we count separately all subunits where each kind of event (quiescence, association, dissociation, and hydrolysis) occurs across all its locations, we will obtain the numbers that indicate how many subunits are totally quiescent, or undergoing association, disassociation, and hydrolysis at time (t). These total numbers we call m -coefficients and denote them as (m^0, m^a, m^d, m^h) . They represent measures of the spatio-temporal extent of particular phenomena, quiescence, association, dissociation and hydrolysis, which dynamically constitute the GTP-cap at time (t). As such, they reflect the fundamental structural-dynamic (steric) constraining conditions of the microtubule wall lattice within the GTP-cap. An illustration of the physical representation of m -coefficients in a small GTP-cap is shown in Fig. 2.

For a given GTP-cap size (m_{GTPcap}), the maximal ranges of m -coefficients (extents) are given as follows

$$0 \leq m^0 = \sum_{i=1}^{m_{GTPcap}} m_{i,GTP}^0 \leq m_{GTPcap} \quad (4)$$

$$0 \leq m^a = \sum_j^N m_{j,GTP}^a \leq N \quad (5)$$

$$0 \leq m^d = \sum_k^N m_{k,GTP}^d \leq N \quad (6)$$

Fig. 2 The single layer GTP-cap—an example. A single layer of the GTP-cap comprises 13 GTP-tubulin subunits. The rest of the microtubule (core) consists in GDP-tubulin molecules. Four GTP-tubulin subunits undergo association. They constitute the extent of association m^a and have a value of 4 ($m^a = 4$). Two GTP-tubulin subunits undergo dissociation. This constitutes the extent of dissociation m^d , which has a value of 2 ($m^d = 2$). Four GTP-tubulin subunits undergo hydrolysis and the extent of hydrolysis, m^h , has a value of 4 ($m^h = 4$). Three GTP-tubulin subunits are silent. They constitute the extent of silence m^0 and have a value of 3 ($m^0 = 3$)

$$0 \leq m^h = \sum_{l=1}^{m_{GTPcap}} m_{l,GTP}^h \leq m_{GTPcap} \quad (7)$$

The m -coefficients entail important physical and mathematical features: they are discrete functions of time and space. Indeed, each m -coefficient has a non-zero value in a particular location(s) defined by the indices (i, j, k, l) within the GTP-cap at a particular time (t), while at all other locations it has the value of zero. Thus, loosely speaking, m -coefficients have the character of delta functions. Importantly, if a continuous function is multiplied by a delta function, its value will be non-zero only where the delta function has a non-zero value, otherwise it will be zero. We will employ this delta-function feature of m -coefficients to modify the continuous function of the bulk concentration of GTP-tubulin into locally discrete functions of space and time.

3.2 Critical nano local concentration (CNLC) - quantification

As per quantification of CNLC of GTP-tubulin, we propose that the local concentration threshold is made up of a number of GTP-tubulin molecules that may collectively access the tip of the GTP-cap of a single microtubule in order to be incorporated. Importantly, this concept of local concentration includes both the number of available GTP-tubulin molecules in a solution and the structural-dynamic capacity of the tip of the GTP-cap that would incorporate the GTP-tubulin molecules.

When microtubule growth is described in terms of an Oosawa condensation polymerization model, as an equilibrium process in a bulk solution, then an appropriate linear differential equation is used [20, 26, 27]. Consequently, the critical bulk GTP-tubulin concentration (C_c), which is equal to an equilibrium dissociation constant (K_{diss}), is determined as the ratio of dissociation (k_d) and association (k_a) rate constants, i.e., ($C_c = k_d/k_a = K_{diss}$). Since (k_d) is constrained by equilibrium values of (k_a) and (K_{diss}), it is too small to properly reproduce the mass of microtubules within the cell [20]. The

main drawback of the equilibrium model is that irreversible GTP hydrolysis in GDP is not included at all. However, hydrolyzed GDP-tubulin dissociates rapidly and independently of GTP-tubulin association and dissociation events. Because of irreversible GTP hydrolysis, microtubule growth is truly a non-equilibrium process in which GTP-tubulin association is separated from dissociation by the same irreversible hydrolysis [20]. Thus, once hydrolysis occurs at the GTP-tubulin subunits at the tip of the GTP-cap, the whole process of microtubule growth/shrink will be driven by the ratio between the rate of hydrolysis and the rate of association. This will have an impact on the GTP-tubulin critical bulk concentration. Therefore, to invoke the effects of irreversible GTP-hydrolysis on the bulk GTP-tubulin critical concentration (C_c), in the first approximation, one can formally replace the dissociation rate constant (k_d) with hydrolysis rate constant (k_h), so that the bulk GTP-tubulin critical concentration (C_c) may be defined by the ratio of hydrolysis and the association rate constant

$$C_c = \frac{k_h}{k_a} \quad (8)$$

For justification of formula (8), one can compare it with a related formula in a special case: indeed, when the size of the GTP-cap is constituted by one GTP-tubulin subunit, formula (8) is commensurable with relevant formulae of other authors [28].

The critical bulk concentration (C_c) is, by definition, a continuous (constant) function of space and time throughout the solution space. This is valid as long as solution homogeneity conditions hold. As the GTP-tubulin molecule approaches the tip of the GTP-cap, its state of motion will be affected by the local fluctuations and the state of motion of subunits at the GTP-cap tip, so that homogeneity conditions may be broken in the vicinity of the GTP-cap, i.e., within the nanoscale. Therefore, in this case, the continuity of the bulk critical concentration may be disrupted and the local value of the critical concentration has to be considered. To include local effects, we can transform the bulk critical concentration into a discrete function of space and time. This can be done by multiplication of (8) by the combination of m -coefficients, such as

$$C_c \frac{m^h}{m^a} = \frac{k_h m^h}{k_a m^a} \quad (9)$$

Since m -coefficients are dimensionless functions of space and time, expression (9) retains the dimension of GTP-tubulin concentration. Therefore, since the m -coefficients are delta functions of space and time, expression (9) will have non-zero values where the m -coefficients are not zero. Since the value of expression (9) is uniquely determined by the m -coefficients (m^h , m^a) for each microtubule tip configuration, quantitatively this number may represent the “critical nano local concentration” (CNLC) in the locations, positioned in the vicinity of GTP-tubulin subunits of the GTP-cap tip, defined by the m -coefficients (m^h , m^a) i.e.,

$$CNLC = \frac{k_h m^h}{k_a m^a} \quad (10)$$

We use the term “nano” to depict the concentration immediately surrounding the tip of the GTP-cap and as such imply that the size of volume to which the concentration is associated is measured in nanometers. Loosely speaking, one can say that CNLC represents the critical concentration of GTP-tubulin molecules at the tip of the microtubule GTP-cap. Obviously, assembled formula (10) integrates bulk solution parameters (k_a and k_h) together with local microtubule nano structural parameters (m -coefficients).

Specific cases can be derived from formula (10). For example: if the extent of hydrolysis is equal to the extent of association, i.e.,

$$m^h = m^a \quad (11)$$

the critical nano local concentration (CNLC) can be represented as

$$CNLC = \frac{k_h}{k_a} \quad (12)$$

i.e., CNLC is identical to the bulk GTP-tubulin concentration.

If the extent of hydrolysis is greater than the extent of association, i.e.,

$$m^h > m^a \quad (13)$$

then, CNLC is greater than the bulk critical concentration. If the extent of hydrolysis is less than the extent of association,

$$m^h < m^a \quad (14)$$

then, CNLC is less than the bulk concentration.

3.3 The rate of hydrolysis in terms of GTP-cap size and CNLC

The size of a GTP-cap may be empirically defined by the help of m -coefficients

$$m_{GTPcap} = m_{GTP}^0 + m_{GTP}^a - m_{GTP}^d - m_{GTP}^h \quad (15)$$

For simplicity, we omit spatial indices (i, j, k, l).

Using (10) and applying simple mathematical rearrangements of (15), the relationship between GTP-cap size (m_{GTPcap}) and CNLC can be stated as

$$m_{GTPcap} = m^0 + m^a \left(1 - \frac{m^d}{m^a} - \frac{k_a}{k_h} CNLC \right) \quad (16)$$

Here, we omitted the index (GTP) for all extent coefficients.

In order to test the model, it is convenient to express the rate of hydrolysis (k_h) in terms of GTP-cap size (m_{GTPcap}) and CNLC, such as

$$k_h = \frac{m^a k_a}{m^0 + m^a - m^d - m_{GTPcap}} CNLC \quad (17)$$

Model (17) integrates micro and macro features of the microtubular system, i.e., structural and solution properties of the GTP-cap. More specifically, it integrates structural GTP-cap constraints defined by m -coefficients with the bulk rate constants (k_a and k_h) and CNLC. Formulae (16) and (17) can be used for the simulation of different aspects of GTP-cap dynamics wherever the concept of CNLC is applicable, i.e., within a self-confined solution volume such as the kinetochore outer domain or in a bulk solution.

4 Application of the model

Formula (17) may be helpful to shed light on a fundamental question regarding the relationship between hydrolysis of the GTP-cap and dynamic instability. This question has

been extensively debated [6, 7]. It has been proposed that the force necessary to perform the work of kinetochores could be generated directly by the thermodynamic drive due to microtubule depolymerization [29]. It has been suggested that GTP-hydrolysis may be the key element controlling the spatio-temporal characteristics of GTP-cap instability [7, 30].

5 Simulation results and discussion

We will first test formula (17) to determine whether simulation can reproduce experimental data for the rate of hydrolysis (k_h). If successful, then both formulae (16) and (17) can be employed for the calculation of other parameters under different conditions.

During the simulation, we will treat k_a and k_h as true rate constants of association and hydrolysis expressed in $[M^{-1}s^{-1}]$ and $[s^{-1}]$, respectively. Hence, CNLC will be expressed in moles $[M]$.

Concentration measurements typically obtain average values and as such there is no data from direct measurements of CNLC for a single microtubule. However, data regarding GTP-tubulin concentrations based on microtubule nucleation by kinetochores in vitro have been published [31]. The minimal critical concentration of GTP-tubulin at which the first microtubule appears has been reported to be $10\text{ }\mu\text{M}$ [31]. Conversely, the bulk (average) critical concentration of GTP-tubulin, in vitro (but without kinetochores) at which microtubule nucleation commences, was found to be $2\text{--}5\text{ }\mu\text{M}$ [27, 32, 33]. The difference between critical GTP-tubulin concentration in the presence and absence of kinetochores is an important finding. It is possible to understand these results in terms of the restrictions placed on the local movement of GTP-tubulin by the boundaries (fibrous protein wall) of the kinetochore outer plate that restrict local movement of GTP-tubulin in order to nucleate the bottom of the outer plate. Thus, one can speculate that the concentration of GTP-tubulin outside the outer plate has to be significantly higher in order for a microtubule to commence growth. For our simulation, we will choose a number within the range of $2\text{--}5\text{ }\mu\text{M}$ as our CNLC, similar to that measured in vitro.

Experimental data regarding the critical concentration of GTP-tubulin are usually obtained as an average over an assembly of hundreds of kinetochores and thousands of microtubules. As such, data relating to a single microtubule are implicitly hidden in the average. As long as the system is homogenous, as an approximation the averaging will match reality; i.e., CNLC will closely match bulk concentration. However, if the system becomes heterogeneous (in vivo as well as in vitro), the averaging will lead to significant discrepancies and the concept of nano local concentration will become more relevant.

For the purpose of the current simulation, we will use the experimental value of $5\text{ }\mu\text{M}$, obtained for the bulk critical concentration of homogenous systems in vitro [27], as a CNLC for microtubule assembly. We will use $k_a = 7 \times 10^6\text{ M}^{-1}\text{ s}^{-1}$ as the rate of GTP-tubulin association (the on-rate) [4, 20, 32].

Using the MATLAB 7.9.0 (R2009b) software package and (17) we calculate the rate of hydrolysis for all possible combinations of m -coefficients of extensions m^0 , m^a , m^d and m^h for a small GTP-cap (i.e., a GTP-cap made up of 13 GTP-tubulin subunits). The results are shown in Fig. 3a and b.

We calculate the rate of hydrolysis to be in the range of $0\text{--}420\text{ s}^{-1}$ (Fig. 3a). This result is highly comparable with the experimental results [5, 34–36] and can explain apparent differences among the experimental results. This result serves as an excellent test of the

Fig. 3 **a** The rate of hydrolysis (k_h) versus the extents (m^0, m^a, m^d and m^h). Global view: throughout the GTP-cap. The rate of hydrolysis is a sensitive function, including 11 outbursts, of relative size of the extents (m^0, m^a, m^d and m^h). The graph is constructed in terms of constantly decreasing values of the extent of quiescence (m^0). $*k_h$ = true rate constant $k_h/10$. The GTP-cap is constituted of 13 GTP-tubulin subunits. m^0 is the extent of quiescence, i.e., the number of GTP-tubulin subunits that undergo no association, dissociation, or hydrolysis. m^a is the extent of association, i.e., the number of GTP-tubulin subunits that undergo association. m^d is the extent of dissociation, i.e., the number of GTP-tubulin subunits that undergo dissociation and m^h is the extent of hydrolysis, i.e., the number of GTP-tubulin subunits that undergo hydrolysis. **b** Detail from **a**: a randomly chosen peak of hydrolysis outburst and related events concerning extents (m^0, m^a, m^d and m^h). The apparent rate of hydrolysis at the peak of an outburst is $k_h = 350 \text{ s}^{-1}$. The peak coincides with a particular combination of relative sizes of extents, which may be described as: local minima of quiescence extent (m^0), local maxima of association extent (m^a), local minima of dissociation extent (m^d) and local minima of hydrolysis extent, which is in this case ($m^h = 1$)

model. Therefore, we can proceed with the interpretation of new theoretical predictions using this model.

The most striking prediction of the model is the presence of 11 peaks of GTP-tubulin hydrolysis outbursts for the small GTP-cap consisting in 13 subunits (Fig. 3a). This non-linearity in the rate of hydrolysis is directly controlled by the m -coefficients. Thus, this result is in good agreement with the predictions pertaining to the mutual influence among GTP-cap tubulin subunits [2]. Since the graphs are drawn in terms of decreasing silent GTP-tubulin subunits (m^0), the peaks of hydrolysis outburst appear in a subsequently increasing trend. However, in reality, irrelevant to the size of the GTP-cap, the peaks of hydrolysis may occur randomly and furthermore, due to the growth of microtubules, the zones they occupy may expand and migrate toward the end of the microtubules. Particular nano local conditions at which an outburst of hydrolysis may occur are shown in Fig. 3b and Fig. 4a, b. These outbursts appear to occur synergistically at the local maximum of the association extent (m^a), the local minimum of the dissociation extent (m^d), hydrolysis extent (m^h) and quiescence extent (m^0), respectively. This model establishes an inverse relationship between the rate of hydrolysis and its extent (10): a lower extent of hydrolysis corresponds

to the higher rate of hydrolysis and vice versa. While this relationship could be difficult to reconcile with the hydrolysis in a solution, it may be likely in the case with hydrolysis in the microtubule wall, where neighboring tubulin subunits may mutually and strongly influence each other [37]. Therefore, it is not unlikely that the outburst of hydrolysis occurs at the smallest hydrolysis extent, which in this case corresponds to one tubulin subunit ($m^h = 1$). Thus, the occurrence of an outburst at the lowest hydrolysis extent ($m^h = 1$) indicates that the state of neighboring GTP-tubulin subunits may critically influence the rate of hydrolysis in the microtubule wall.

Since we did not consider m -coefficients as explicit functions of time, the bursts occur randomly. While the model imposes spatial restrictions on m -coefficients at which bursts may occur, it does not impose any limitations in terms of time of occurrence. Therefore, they may occur synchronously, resulting in coherent phenomena [38]. If so, then no matter how fast they are, the associated portions of released energies can be integrated to give the energy to the soliton-like (kink) motion along the microtubule wall, as has been proposed in some works [39–41].

The outbursts of hydrolysis may have critical importance for dynamic instability. They are accompanied by rapid energy release and formation of expanding zones in the wall of the microtubule built by less stable, skewed GDP-tubulin subunits. Cumulatively, these events constitute dynamic instability. When the less stable zones reach a critical size at the tip of microtubules these structures will undergo a final act of dynamic instability—catastrophic endwise depolymerization. Inversely, microtubule degradation may be halted when depolymerization approaches zones of the wall lattice where combinations of m -coefficients m^0 , m^a , m^d , m^h (steric coefficients) do not produce any outburst and microtubule regrowth will be resumed. The finding that bursts of hydrolysis directly underlie dynamic instability strongly supports the predictions that hydrolysis of GTP is followed by the conversion of

Fig. 4 The highest rate of GTP-tubulin subunit hydrolysis (k_h) at the peak of its outburst versus the extent of GTP-tubulin association (m^a). **a** The high rate of hydrolysis (k_h), which strictly corresponds to hydrolysis peak outbursts, is a linear function of the extent of GTP-tubulin association (m^a). It occurs in the absence of dissociation ($m^d = 0$) and at the lowest hydrolysis extent ($m^h = 1$). **b** The extent of silent (quiescent) GTP-tubulin subunits (m^0) decreases linearly compared to increases in the extent of GTP-tubulin association (m^a), which are strictly related to the peaks of hydrolysis outbursts

chemical energy into large conformational movements that have been termed “dynamic transitions” [42].

In order to gain deeper insight into the phenomena of hydrolysis outbursts, it is important to recognize that the associated local energy release and the formation of expanding zones built by skewed GDP-tubulin molecules represent solitons or solitary waves in the microtubule wall. Solitons have been predicted and used in the rationalization of a variety of microtubule related phenomena, including the downstream modulation of neurotransmitter signaling, ionic transport throughout the cytoplasm, learning and memory, local information transfer, bioenergetic transformations within the cytoplasm, chromosome separation, polar vibrations in the wall lattice, microtubule growth and energy-transfer mechanism in microtubules [40, 43–50].

Importantly, the powerful and synchronized bursts of energy in the microtubule wall, coupled with the abundance of microtubules at locations, such as in dendritic spines, may strongly support the hypothesis that solitons, or their higher synchronizing wave compositions produced and mediated by microtubules, may directly underlie higher neural informational processing such as neurocomputation [51–53].

A long-standing question is how the energy of GTP hydrolysis could be used to generate dynamic instability [6]. Utilizing the outburst of hydrolysis discussed above, we conclude that the energy of GTP hydrolysis may serve to switch the soliton motion in the wall of the microtubule. Therefore, we conclude that solitons may serve as a physical rationale for dynamic instability.

In order to further validate our model, we illustrate particular situations that demonstrate the compatibility of some aspects of our model with previous models. These results are illustrated in Figs. 5a, 6a and b.

Fig. 5 The low rate of GTP-tubulin subunit hydrolysis (k_h) compared to the extent of hydrolysis (m^h). **a** The low rate of hydrolysis (k_h) is a *non-linear* function of the GTP-tubulin subunit hydrolysis extent (m^h). It occurs at the lowest association extent ($m^a = 1$) and in the absence of dissociation ($m^d = 0$). **b** The extent of silent GTP-tubulin subunits (m^0) decreases linearly compared to increases in the extent of GTP-tubulin hydrolysis (m^h)

Fig. 6 The low rate of GTP-tubulin subunit hydrolysis (k_h) compared to the extent of dissociation (m^d). **a** The low rate of hydrolysis (k_h) is a *non-linear* function of the GTP-tubulin dissociation extent (m^d). It occurs at the lowest extent of association ($m^a = 1$) and hydrolysis ($m^h = 1$). **b** The extent of silent GTP-tubulin subunits (m^0) decreases linearly compared to increases in the extent of GTP-tubulin dissociation (m^d)

The graph in Fig. 5a demonstrates that the maximal rate of hydrolysis (k_h) of GTP-tubulin, i.e., the rate of hydrolysis during outbursts, is a linear function based on the increase in the extent of association sites (m^a). This example closely resembles that which has been formulated as the “uncoupled stochastic hydrolysis” model [4, 32, 35]. According to our model (10) this occurs at the lowest value of dissociation and hydrolysis ($m^d = 0$, $m^h = 1$). The graph shown in Fig. 5b demonstrates that silent GTP-tubulin units were consumed by hydrolysis. This corresponds well with the graph in Fig. 5a.

In the simulation carried out in Fig. 6a and b, hydrolysis proceeds under the conditions of a small GTP-cap (13 GTP-tubulin subunits) where the extent of association is low ($m^a = 1$) and dissociation is absent ($m^d = 0$). As shown, the rate of hydrolysis is far lower than the outbursts of hydrolysis seen in Fig. 3b. This demonstrates that hydrolysis depends on the concentration of local GTP-tubulin subunits within the GTP cap rather than on the rate of association (m^a) [20, 54]. This is a new aspect of the rate of hydrolysis. In this scenario, only one GTP-tubulin subunit (of 13) undergoes association ($m^a = 1$). The extent of dissociation is assumed to be zero ($m^d = 0$). The remaining GTP-tubulin subunits are “silent”, although it is possible that they may undergo hydrolysis, as revealed in Fig. 6b. The first of the 12 GTP-subunits undergoing hydrolysis proceeds at a very fast rate ($k_h = 35 s^{-1}$) while the remaining subunits proceed at progressively slower rates. As such, the twelfth subunit undergoes the slowest hydrolysis ($k_h = 2.5 s^{-1}$). Hence, the presence of hydrolyzed neighboring GTP-tubulin subunits slows hydrolysis. This scenario does not apply to the hydrolysis outbursts mentioned above. In the simulation demonstrated in Fig. 6a, we assumed that the extent of dissociation was zero ($m^d = 0$). However, the result will not qualitatively change if the extent of dissociation is higher than, albeit close to, zero. Therefore, we conclude that the results of this simulation are commensurable with the proposal that hydrolysis slows down when only a single ring of subunits is present, a phenomenon known as “stochastic dissociation-coupled hydrolysis” [55].

According to our assumption, the rate of hydrolysis at the twelfth GTP-subunit is slower than the rate of association observed at the 13th GTP-subunit. This observation implies the possibility of the existence of a GTP-cap comprised of a single GTP-tubulin subunit. The probability of the existence of a GTP-cap comprised of only one GTP-tubulin subunit has been suggested previously [56]. This prediction is very close to recent experimental results obtained for microtubule assembly [57]. Schek and colleagues [57] were able to demonstrate that the GTP-cap has only three subunits at the point when catastrophic microtubule disassembly occurs. These results strongly demonstrate the applicability of our model in predicting events that occur during microtubule formation.

The results of a simulation using a low number of associations (one GTP-subunit undergoing association, $m^a = 1$) and hydrolysis (one GTP-subunit undergoing hydrolysis, $m^h = 1$) demonstrate that the rate of hydrolysis (k_h) is a non-linear function (Fig. 6a). This is an example in which the rate of hydrolysis changes based on changes in the local dissociation in the GTP-cap even though there is very little or no change in the extent of association (m^a). Here we see that the rate of hydrolysis at an arbitrarily chosen GTP-tubulin subunit within the GTP-cap is higher if the number of dissociating subunits in its immediate environment is lower and vice versa. If no GTP-tubulin subunits undergo dissociation ($m^d = 0$) within a GTP-cap, then the rate of hydrolysis at the chosen GTP-tubulin subunit is 35 s^{-1} . If 11 GTP-tubulin subunits undergo dissociation ($m^d = 11$), then the rate of hydrolysis at one chosen GTP-tubulin subunit is 1.81 s^{-1} . This phenomenon may be described as a particular aspect of our model, which emphasizes the sensitivity of the rate of hydrolysis to dissociation events, i.e., “stochastic dissociation-coupled hydrolysis” [55].

6 Concluding remarks

Although in the current instance we have not explored all the possibilities using our model, we have demonstrated that hydrolysis is a key event in the formation of the GTP-cap and that it may critically affect the intricate regulation of kinetochore–microtubule interplay. Future investigations using our model to predict experimental results are imperative.

Acknowledgements We express our sincere gratitude to Professor Maxwell Bennett, AO (Professor of Neuroscience, University Chair and Scientific Director, Brain and Mind Research Institute, The University of Sydney) for continued support.

References

1. Janulevicius, A., van Pelt, J., van Ooyen, A.: Compartment volume influences microtubule dynamic instability: a model study. *Biophys. J.* **90**, 788–798 (2006)
2. Rezania, V., Tuszynski, J.A.: A stochastic model for microtubule dynamicity involving mixtures of tubulin isotypes. *Int. J. Quantum Chem.* **109**, 3430–3440 (2009)
3. Rieder, C.L., Salmon, E.D.: The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* **8**, 310–318 (1998)
4. Mitchison, T., Kirschner, M.: Dynamic instability of microtubule growth. *Nature* **312**, 237–242 (1984)
5. O’Brien, E.T., Voter, W.A., Erickson, H.P.: GTP hydrolysis during microtubule assembly. *Biochemistry* **26**, 4148–4156 (1987)
6. Erickson, H.P., O’Brien, E.T.: Microtubule dynamic instability and GTP hydrolysis. *Annu. Rev. Biophys. Biomol. Struct.* **21**, 145–166 (1992)
7. Needleman, D.J., Groen, A., Oh, R., Maresca, T., Mirny, L., Mitchison, T.: Fast microtubule dynamics in meiotic spindles measured by single molecule imaging: evidence that the spindle environment does not stabilize microtubules. *Mol. Biol. Cell.* **21**, 323–333 (2010)

8. Schek, H.T., III, Hunt, A.J.: Microtubules: mechanical meets chemical. *Biophys. J.* **89**, 2909–2910 (2005)
9. Kamath, K., Oroudjev, E., Jordan, M.A.: Determination of microtubule dynamic instability in living cells. *Methods Cell. Biol.* **97**, 1–14 (2010)
10. Kapitein, L.C., Yau, K.W., Hoogenraad, C.C.: Microtubule dynamics in dendritic spines. *Methods Cell. Biol.* **97**, 111–132 (2010)
11. Kitamura, E., Tanaka, K., Kitamura, Y., Tanaka, T.U.: Kinetochore microtubule interaction during S phase in *Saccharomyces cerevisiae*. *Genes Dev.* **21**, 3319–3330 (2007)
12. Suzuki, A., Hori, T., Nishino, T., Usukura, J., Miyagi, A., Morikawa, K., Fukagawa, T.: Spindle microtubules generate tension-dependent changes in the distribution of inner kinetochore proteins. *J. Cell Biol.* **193**, 125–140 (2011)
13. Tolic-Norrelykke, I.M.: Push-me-pull-you: how microtubules organize the cell interior. *Eur. Biophys. J.* **37**, 1271–1278 (2008)
14. DeLuca, J.G.: Kinetochore–microtubule dynamics and attachment stability. *Methods Cell Biol.* **97**, 53–79 (2010)
15. Tabony, J.: Microtubules viewed as molecular ant colonies. *Biol. Cell* **98**, 603–617 (2006)
16. Duesberg, P.: Chromosomal chaos and cancer. *Sci. Am.* **296**, 52–59 (2007)
17. McEwen, B.F., Arena, J.T., Frank, J., Rieder, C.L.: Structure of the colcemid-treated PtK1 kinetochore outer plate as determined by high-voltage electron microscopic tomography. *J. Cell Biol.* **120**, 301–312 (1993)
18. Cleveland, D.W., Mao, Y., Sullivan, K.F.: Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell* **112**, 407–421 (2003)
19. Maddox, P., Straight, A., Coughlin, P., Mitchison, T.J., Salmon, E.D.: Direct observation of microtubule dynamics at kinetochores in *Xenopus* extract spindles: implications for spindle mechanics. *J. Cell Biol.* **162**, 377–382 (2003)
20. Kirschner, M., Mitchison, T.: Beyond self-assembly: from microtubules to morphogenesis. *Cell* **45**, 329–342 (1986)
21. Cheeseman, I.M., Desai, A.: Molecular architecture of the kinetochore–microtubule interface. *Nat. Rev. Mol. Cell Biol.* **9**, 33–46 (2008)
22. Maiato, H., DeLuca, J., Salmon, E.D., Earnshaw, W.C.: The dynamic kinetochore–microtubule interface. *J. Cell Sci.* **117**, 5461–5477 (2004)
23. Clarke, P.R.: Cell biology. A gradient signal orchestrates the mitotic spindle. *Science* **309**, 1334–1335 (2005)
24. Fuller, B.G.: Self-organization of intracellular gradients during mitosis. *Cell Div.* **5**, 5 (2010)
25. Dong, Y., Vanden Beldt, K.J., Meng, X., Khodjakov, A., McEwen, B.F.: The outer plate in vertebrate kinetochores is a flexible network with multiple microtubule interactions. *Nat. Cell Biol.* **9**, 516–522 (2007)
26. Oosawa, F., Asakura, S.: *Thermodynamics of the Polymerization of Protein*. Academic Press, London (1975)
27. Bergen, L.G., Borisy, G.G.: Head-to-tail polymerization of microtubules in vitro. Electron microscope analysis of seeded assembly. *J. Cell Biol.* **84**, 141–150 (1980)
28. Pantaloni, D., Carlier, M.F.: Involvement of guanosine triphosphate (GTP) hydrolysis in the mechanism of tubulin polymerization: regulation of microtubule dynamics at steady state by a GTP cap. *Ann. New York Acad. Sci.* **466**, 496–509 (1986)
29. Coue, M., Lombillo, V.A., McIntosh, J.R.: Microtubule depolymerization promotes particle and chromosome movement in vitro. *J. Cell Biol.* **112**, 1165–1175 (1991)
30. Howard, J., Hyman, A.A.: Dynamics and mechanics of the microtubule plus end. *Nature* **422**, 753–758 (2003)
31. Mitchison, T.J., Kirschner, M.W.: Properties of the kinetochore in vitro. I. Microtubule nucleation and tubulin binding. *J. Cell Biol.* **101**, 755–765 (1985)
32. Mitchison, T., Kirschner, M.: Microtubule assembly nucleated by isolated centrosomes. *Nature* **312**, 232–237 (1984)
33. Summers, K., Kirschner, M.W.: Characteristics of the polar assembly and disassembly of microtubules observed in vitro by darkfield light microscopy. *J. Cell Biol.* **83**, 205–217 (1979)
34. Burns, R.G.: Kinetics of GTP hydrolysis during the assembly of chick brain MAP2-tubulin microtubule protein. *Biochem. J.* **277**(Pt 1), 239–243 (1991)
35. Carlier, M.F., Pantaloni, D.: Kinetic analysis of guanosine 5′-triphosphate hydrolysis associated with tubulin polymerization. *Biochemistry* **20**, 1918–1924 (1981)
36. Vandecandelaere, A., Brune, M., Webb, M.R., Martin, S.R., Bayley, P.M.: Phosphate release during microtubule assembly: what stabilizes growing microtubules? *Biochemistry* **38**, 8179–8188 (1999)

37. Rezanian, V., Tuszyński, J.A.: A stochastic model for microtubule dynamicity involving mixtures of tubulin isotopes. *Int. J. Quantum Chem.* **109**, 3430–3440 (2009)
38. Frohlich, H.: *Modern Biochemistry*. Plenum Press, New York (1996)
39. Trpisova, B., Tuszyński, J.A.: Possible link between guanosine 5' triphosphate hydrolysis and solitary waves in microtubules. *Phys. Rev. E* **55**, 3288–3305 (1997)
40. Satačić, M.V., Tuszyński, J.A., Zakula, R.B.: Kink-like excitations as an energy-transfer mechanism in microtubules. *Phys. Rev. E* **48**, 589–597 (1993)
41. Satačić, M.V., Zakula, R.B., and Tuszyński, J.A.: A model of the energy transfer mechanisms in microtubules involving a single soliton. *Nanobiology* **1**, 445–456 (1992)
42. Satačić, M.V., Satačić, B.M., Tuszyński, J.A.: Nonlinear model of microtubule dynamics. *Electromagn. Biol. Med.* **24**, 255–264 (2005)
43. Gardiner, J., Overall, R., Marc, J.: The microtubule cytoskeleton acts as a key downstream effector of neurotransmitter signaling. *Synapse* **65**, 249–256 (2011)
44. Sekulic, D.L., Satačić, B.M., Tuszyński, J.A., Satačić, M.V.: Nonlinear ionic pulses along microtubules. *Eur. Phys. J. E Soft Matter* **34**, 1–11 (2011)
45. Craddock, T.J., Tuszyński, J.A., Priel, A., Freedman, H.: Microtubule ionic conduction and its implications for higher cognitive functions. *J. Integr. Neurosci.* **9**, 103–122 (2010)
46. Lizier, J.T., Prokopenko, M., Zomaya, A.Y.: Local information transfer as a spatiotemporal filter for complex systems. *Phys. Rev. E* **77**, 026110 (2008)
47. Tirosh, R.: Ballistic protons and microwave-induced water solitons in bioenergetic transformations. *Int. J. Molec. Sci.* **7**, 320–345 (2006)
48. Pokorný, J., Jelinek, F., Trkal, V., Lamprecht, I., Holzel, R.: Vibrations in microtubules. *Astrophys. Space Sci.* **23**, 171–179 (1997)
49. Chou, K.C., Zhang, C.T., Maggiora, G.M.: Solitary wave dynamics as a mechanism for explaining the internal motion during microtubule growth. *Biopolymers* **34**, 143–153 (1994)
50. Davydov, A.S.: Solitons and energy transfer along protein molecules. *J. Theor. Biol.* **66**, 379–387 (1977)
51. Hameroff, S.: The “conscious pilot”-dendritic synchrony moves through the brain to mediate consciousness. *J. Biol. Phys.* **36**, 71–93 (2010)
52. Hameroff, S.R.: The brain is both neurocomputer and quantum computer. *Cognit. Sci.* **31**, 1035–1045 (2007)
53. Hameroff, S.R., Watt, R.C.: Information processing in microtubules. *J. Theor. Biol.* **98**, 549–561 (1982)
54. Pantaloni, D., Carlier, M.F., Korn, E.D.: The interaction between ATP-actin and ADP-actin. A tentative model for actin polymerization. *J. Biol. Chem.* **260**, 6572–6578 (1985)
55. Walker, R.A., O'Brien, E.T., Pryer, N.K., Soboeiro, M.F., Voter, W.A., Erickson, H.P., Salmon, E.D.: Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. *J. Cell Biol.* **107**, 1437–1448 (1988)
56. Voter, W.A., O'Brien, E.T., Erickson, H.P.: Dilution-induced disassembly of microtubules: relation to dynamic instability and the GTP cap. *Cell Motil. Cytoskelet.* **18**, 55–62 (1991)
57. Schek, H.T., III, Gardner, M.K., Cheng, J., Odde, D.J., Hunt, A.J.: Microtubule assembly dynamics at the nanoscale. *Curr. Biol.* **17**, 1445–1455 (2007)