Revisiting the microtubule based quantum models of mind: tubulin bound GTP cannot pump microtubule coherence or provide energy for α ⇄ β computation in stable microtubules

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The current paper investigates the biological models of stable brain microtubules as quantum or classical computers whose function is based on electron hopping associated with kinking of the tubulin dimer. Hameroff (1998a, 1998b, 2003a, 2003b), Tuszynski et al. (1998), Hagan et al. (2000), Mershin et al. (1999); Mershin (2003) suppose that the energy needed could be somehow delivered via guanosine diphosphate (GDP) exchange for guanosine triphosphate (GTP) or via cycles of tubulin bound GTP hydrolysis. Here is presented biological and structural data from electron diffraction studies performed by Lowe et al. (2001) and computer simulation with MDL ® Chime Version 2.6 SP4, explaining and visualizing the inconsistency of the proposed tubulin bit (qubit) GTP energized α ⇄ β computation and/or tubulin bound GTP pumped coherence in stable microtubules.

Tubulin structure and GTP binding

The α/β-tubulin heterodimer is the basic structural unit of microtubules. The heterodimer does not come apart, once formed. The α and β tubulins, which are each about 55-kDa, are homologous but not identical. Each tubulin has a nucleotide-binding site. The main difference between α- and β-tubulins is in binding GTP. In the dimer α–bound GTP is effectively sequestered - not exchanged and not hydrolyzed; that's why α-tubulin nucleotide-binding site is labelled as N-site (non-exchangeable site). In contrast β-bound GTP is labile - exchangeable in the free dimer; that's why β-nucleotide-binding site is labelled as E-site (exchangeable site). The E-site bound GTP however is hydrolyzed to non-exchangeable GDP in the protofilament (Weisenberg et al., 1976).

Microtubule assembly starts with formation of protofilaments by the tubulin dimers. GTP must be bound to both α and β-tubulins for a tubulin heterodimer to associate with other heterodimers to form a protofilament or microtubule. During in vitro microtubule assembly, heterodimers join end-to-end to form protofilaments. Protofilaments associate laterally to form sheets, and eventually microtubules. In vitro α/β tubulin heterodimers can add or dissociate at either end of a microtubule, but there is greater tendency for tubulin dimers to add at the plus end, where β-tubulin is exposed.

Tubulin dimer addition brings β-tubulin that was exposed at the plus end into contact with α-tubulin. The minus end of α-tubulin may contribute an essential residue to the catalytic site of β-tubulin. Thus the minus end of the α-subunit may serve as GAP (GTPase activating protein) for β-tubulin of the adjacent dimer in a protofilament and could promote hydrolysis of GTP bound to the now interior β-tubulin. Phosphate group (Pi) dissociates, converting β-tubulin bound GTP into GDP, but the β-tubulin remaining within the microtubule interior cannot exchange its bound GDP for GTP after its integration (Heald & Nogales, 2002).

The protofilament structure has been determined by Lowe et al. (2001) at atomic resolution using cryo-EM (electron diffraction) analysis of 2-D crystals induced by treating tubulin with zinc ions in the presence of a derivative of the drug taxol [1JFF.pdb]. These "zinc sheets" consist of parallel arrays of protofilaments. Each nucleotide in the tubulin protofilament is at an α/β-interface. The inability of GTP to dissociate from the α-subunit is consistent with occlusion by a loop from the β subunit. A similar occlusion by successive α-tubulin loops would account for the inability of β-tubulin within a protofilament to exchange bound GDP for GTP (Diwan, 2002).

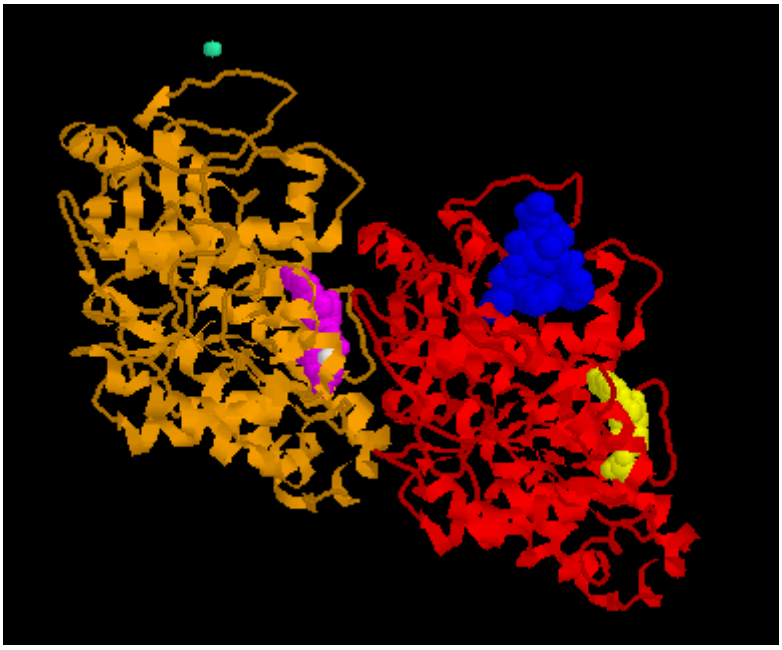
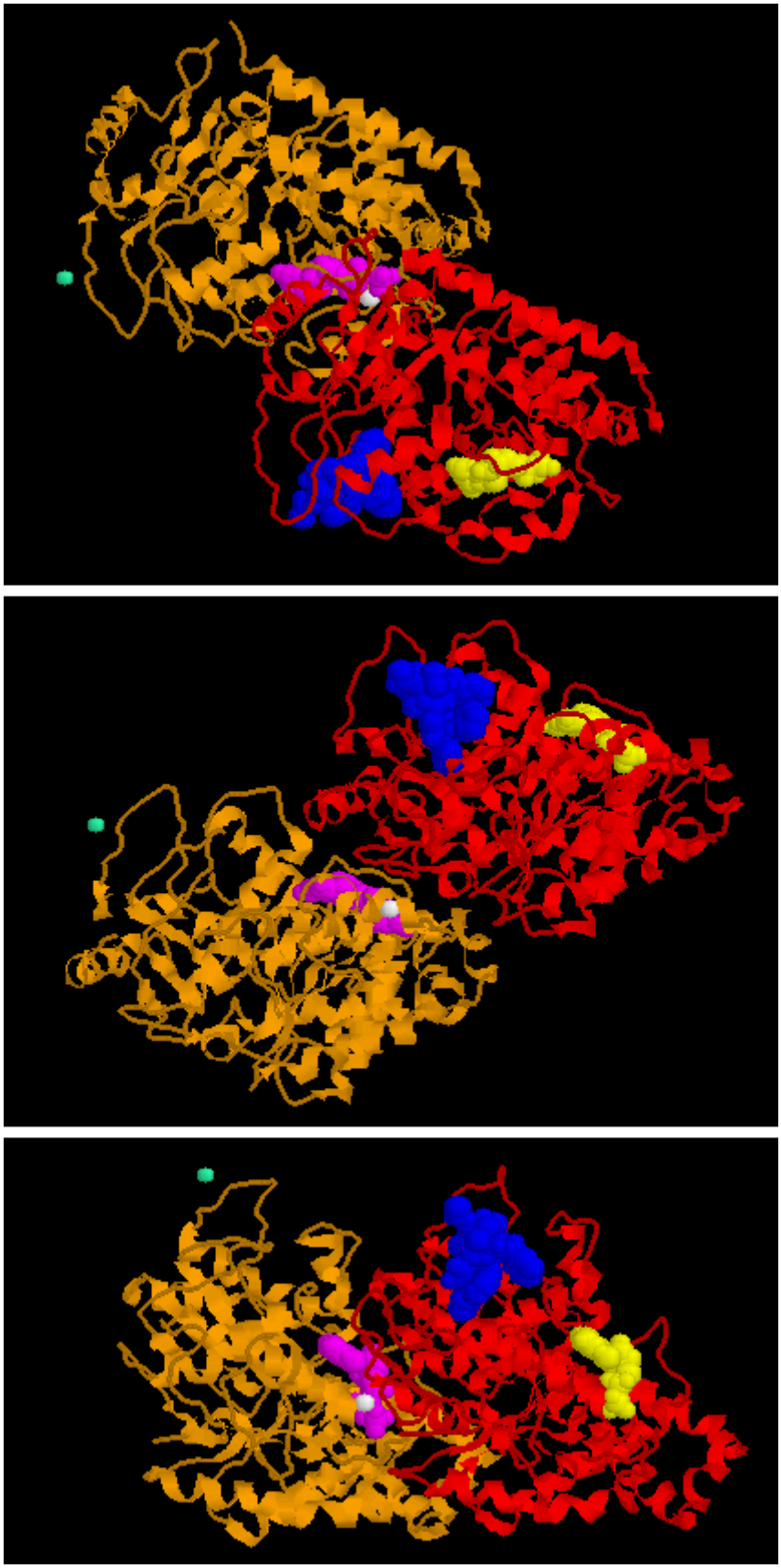
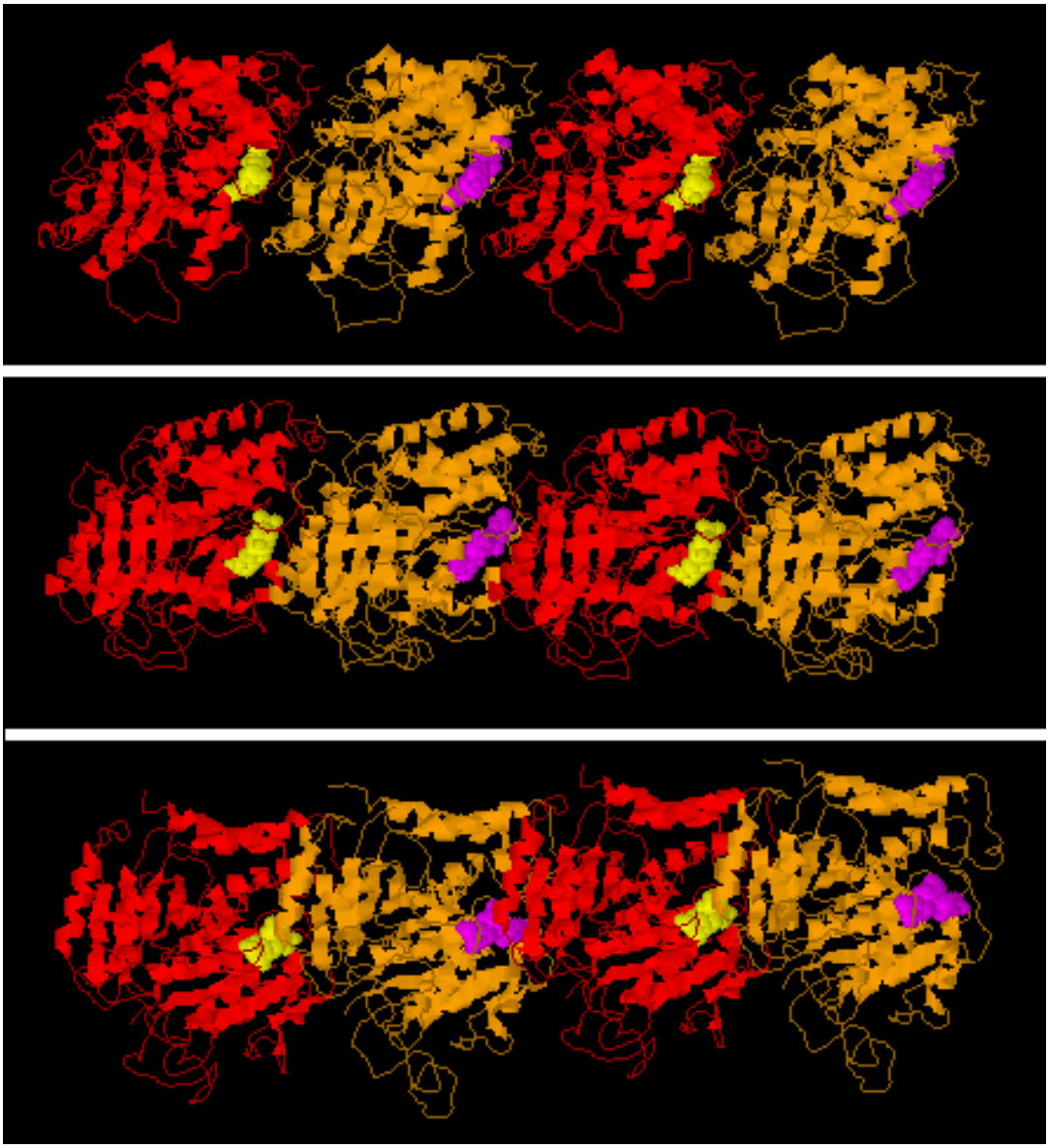


Figure 1. Computer model of the refined structure of α/β tubulin dimer from Zn^{2+}-induced sheets stabilized with taxol. The tubulin bound nucleotides are located in pockets at an α/β-interface. α-bound GTP is occluded and successfully sequestered by β-tubulin loops. The 1JFF.pdb file used in the simulation is obtained from The Protein Data Bank. Legend: α–tubulin in orange, β–tubulin in red, GTP in magenta, GDP in yellow, Zn^{2+} in green blue, Mg^{2+} bound to GTP in white.

The occlusion of α-tubulin bound GTP by β-tubulin loops and the occlusion of β-tubulin bound GDP by successive α-tubulin loops explains why these nucleotides are not exchangeable. Thus no α–GTP-hydrolysis and β-GDP/DTP exchange are possible explaining why the tubulin-bound nucleotides cannot supply energy for computation as suggested by Tuszynski et al. (1998); Hagan et al. (2000); Hameroff (2003b).



Figures 2-4. The α/β tubulin dimer with bound nucleotides [1JFF.pdb].



Figures 5-7. Computer simulated β–α–β–α yeast mini-protofilament visualizing the β-tubulin bound GDP occlusion and sequestration by polypeptide loops of the successive α-tubulin. In the centre of the mini-protofilament is located α/β-tubulin dimer (subunits 2 and 3) linked to β-tubulin (subunit 1) on its minus end and to α-tubulin (subunit 4) on its plus end. Legend: α-bound GTP in magenta, β-bound GDP in yellow, α-tubulin in orange and β-tubulin in red. The proto_yeast.pdb file used in the simulation is from Richards et al. (2000).

Microtubule structure

The microtubule (MT) wall is a 2D polymer of α/β tubulin dimers connected to each other by two types of bonds. Longitudinal bonds connect dimers into protofilaments and lateral bonds connect dimers in adjacent protofilaments. The protofilament consists of alternating α and β subunits, which are spaced 4.0-4.2nm apart (Erickson & Stoffler, 1996). The protofilaments inside MTs are straight and parallel to the MT axis. There are usually 13 protofilaments in a MT, but MTs with 10-16 protofilaments also have been observed. When a flattened MT wall is viewed with the protofilaments vertical, the lateral bonds form a line of subunits with a 10-degree pitch from the horizontal (Erickson, 1974). In the intact MT this line of subunits forms a shallow helix that meets the third subunit up when it has completed the circuit of 13 protofilaments. This is called a 3-start helix, because it is necessary to start three independent helices to cover all the subunits. The helix is left-handed (Erickson, 1974; Mandelkow et al., 1986).

A significant amount of the free energy of the β-tubulin bound GTP hydrolysis during the MT assembly goes into the MT via a conformational change of the tubulin dimer (Caplow et al., 1994). Although the hydrolysis reaction is closely coupled to MT assembly (Carlier & Pantaloni, 1981; Stewart et al., 1990), its consequence is to destabilize the structure. When a GTP molecule is bound at the exchangeable E-site located at the β-tubulin the dimer is supposed to be in “straight” state. If the E-site bound GTP is hydrolysed to GDP and the inorganic phosphate (Pi) is released the dimer changes its conformation into “curved” state (Muller-Reichert et al., 1998). Analysis of tubulin structure by cryo-electron microscopy (EM) has shown that in MTs in the presence of a nonhydrolyzable analogue of GTP, called GMPCPP, the average length of the tubulin monomer is 0.3 nm longer than in MTs consisting of GDP-tubulin (Hyman et al., 1995). The interpretation of the data by *Hyman and co-workers* is that GTP hydrolysis changes the conformation of the tubulin dimers that built up the MT wall into “curved” kidney bean-shaped state. Nicholson et el. (1999) used cryo-electron microscopy to observe GDP-tubulin rings showing

that free GDP-protofilaments have intrinsic curvature. In the stable MTs, however, the GDP-protofilaments have to be straight - phenomenon resulting in mechanical strain between the curved GDP-tubulin dimers. This mechanical strain results in accumulation of elastic energy in the inter-dimer bonds of the MT lattice i.e. transformation of the GTP released chemical energy into “stored” elastic energy (Caplow et al., 1994).

In vitro studies show that GTP hydrolysis is not necessary for polymerization to proceed. Tubulins liganded with slowly hydrolyzable GTP-analogs (GMPCPP) also polymerize (Hyman et al., 1992; Drechsel and Kirschner, 1994). However in vivo GTP hydrolysis occurs very soon after the incorporation of a fresh subunit in the MT (Stewart et al., 1990; Walker et al., 1991; Melki et al., 1996), and GTP hydrolysis keeps pace with the addition of tubulin at various rates (Vandecandelacre et al., 1999). This suggests that the conversion of the GTP stored energy into elastic energy of the MT lattice is biologically important for MT intracellular function - that's why during, or soon after, interdimer bonds have been formed, the unit of GTP liganded to β-tubulin is hydrolyzed and inorganic phosphate (Pi) is released.

Microtubule cap and dynamic instability

The GMPCPP-bound MTs are unable to depolymerize, while GDP-bound MTs could undergo fast depolymerization known as “catastrophe”. The catastrophe event is important in cytoskeletal remodelling and in cell division (mitosis), however the cytoskeleton of the differentiated cells is organized from stable MTs as well. Prevention from rapid depolymerization is achieved via structural cap comprised from GDP-Pi (or GTP) tubulins located at the MT end. Near the end of the elastic MT tube the most favourable configuration, i.e., the one with lowest mechanical energy, represents a compromise between the forces at play: the material bends somewhat, longitudinally, at the cost of stretching around the circumference (Janosi et al., 2002). The net effect is a lower local energy stored in the tube, so in terms of local energy and geometry, this tube is capped by a structure different from its bulk: it

displays a structural cap. Calculations show that the GDP-Pi (or GTP) cap stabilizes the MT simply by being intrinsically straight, and it does this quite efficiently: exponentially as the cap size increases. The structural cap is stretching around the circumference maximal outward bending of a blunt MT end, and a corresponding 0.5 nm increase in the MT radius right at its end.

Inorganic phosphate (Pi) binds to MTs with a low affinity (KD = 25 mM) and slows down the rate of GDP-subunit dissociation by about 2 orders of magnitude. Carlier et al. (1988, 1989) propose that Pi and its structural analogues bind to the site of the γ-phosphate of GTP in the E-site and reconstitute a GDP-Pi-MT, from which tubulin subunits dissociate very slowly. In the literature is widely used the term "GTP cap" but recent evidence suggests that the cap is composed from GDP-Pi tubulins. Panda et al. (2002) performed experiments using [γ(32)P]GTP and have found that the stabilizing cap at MT ends consists of a single layer of tubulin GDP-Pi subunits. The data also support the hypothesis that the mechanism giving rise to the destabilized GDP-tubulin core involves release of Pi rather than hydrolysis of the GTP. Following the GTP cleavage on MTs, Pi release in the medium is accompanied by a structural change resulting in a large destabilization of the polymer - GDP-tubulin takes a "curved" conformation. In contrast GDP-Pi tubulins that form the cap similarly to GTP-tubulins are supposed to have straight conformation (Janosi et al., 2002).

Although Caplow & Fee (2003) criticized the work of *Panda and co-workers* claiming that the used [γ(32)P]GTP probes were hydrolyzed the issue is still not settled. Panda et al. (2002) are confident that (32)Pi is located at the MT ends because colchicine and vinblastine, drugs that suppress tubulin incorporation into the MT by binding specifically at MT ends, reduced the quantity of the stably bound (32)Pi. Taxol, a drug that stabilizes MT dynamics by binding along the MT surface rather than at the ends, did not affect the stoichiometry of the bound (32)Pi. Considering the uncertainty about the chemical nature of the MT cap we will be cautious and will assume that it could be comprised either from GDP-Pi or GTP-tubulins.

Experiments indicate that the GDP-Pi-tubulin cap (or unhydrolyzed GTP-tubulin) is limited to the last layer of subunits at the end of a MT (Voter et al., 1991; Walker et al., 1991). The loss of the GDP-Pi (GTP) cap through dissociation or hydrolysis exposes the unstable GDP core, leading to rapid depolymerization (Mitchison & Kirschner, 1984). Experiments to measure the minimum GDP-Pi (or GTP) cap in MTs assembled from tubulin bound to a slowly hydrolyzed GTP analog (GMPCPP) support a model in which a single GTP subunit at the end of each of the 13 protofilaments of a MT is sufficient for stabilization. Depolymerization of a MT may be initiated by an exposed tubulin-GDP subunit at even a single position (Drechsel & Kirschner, 1994). MT dynamic instability arises from the hydrolysis of the GTP and the dissociation of the Pi from the GDP-Pi cap. The released energy of 0.4 eV from GTP-hydrolysis triggers a conformational change in the tubulin molecule (Hyman et al., 1992) that eventually destabilizes the aggregate (Tran et al., 1997) and causes MT disassemble into protofilaments of GDP-bound tubulin that curve away from the MT axis. Fygenson (2001) suggests that the unfolding of N-terminal domain called entropic bristle domain (EBD) of the tubulin molecule localized in the MT interior leads to disassembly of the MT into protofilaments. The unfolding of EBD could be the cause of tubulin dimer "curving" that destabilizes the aggregate in a manner consistent with structural data (Mandelkow et al., 1991).

Electron hopping and tubulin conformation

Hameroff et al. (1988) suggested that tubulins could compute via electron hopping inside the dimer. The electron could be either in the α-tubulin or in the β-tubulin. When in the α-state the dimer is supposed to have straight conformation, while when in the β-state the dimer is supposed to have outward kink of 29^{o} (Brown & Tuszynski, 2003). The energetic difference between the "curved" and "straight" state is ~ 0.4eV suggesting that spontaneous flipping does not occur frequently. It is safe to assume that the "curved" β-state and the associated elastic strain are important for the biological function of MTs, however it is not clear how a stable MT could

obtain such huge amount of energy needed for electron hopping and associated α (straight) ⇄ β (curved) dimer switching, considering that both α and β bound nucleotides are sequestered. The suggested propagation of conformational soliton along the MT based on α ⇄ β dimer conformational state flipping (Tuszynski et al., 1998) must possess energy Nx0.4eV, where N is the number of flipped dimers. When the soliton reaches the MT end with the GDP-Pi (or GTP) cap it will trigger catastrophe event (rapid depolymerization) if there are not stabilizing the structure MT end-binding MAPs, such as CLIP-170 and EB1 (Heald & Nogales, 2002). In the stable MT the energy needed for such soliton formation could be supplied neither from GTP, nor from the local electromagnetic field (Georgiev, 2003) making the proposed conformational computing via α ⇄ β dimer flipping highly problematic.

In dynamically unstable MT undergoing assembly ⇄ disassembly the energy freed in the GTP hydrolysis, which takes place soon after the addition of each tubulin molecule to the MT end, could propagate along the MT as a solitary wave (Chou et al., 1994; Sataric et al., 1993; Trpisova and Tuszynski, 1997; Tuszynski et al., 1994). This wave may locally alter the elastic state of tubulin, which may consequently lead to a dissociation of tubulin dimers from the MT end or may be involved in the coordinated behaviour of microtubule-associated proteins (MAPs) attaching to the growing or shrinking MT.

For a stable MT, however, there is no possibility for “coherence pumping” or computation via MT wall solitons comprised from α ⇄ β flipped dimers. The only available for hydrolysis GTP molecules form the MT “cap”, and if hydrolyzed, rapid MT disassembly known as catastrophe will occur. Thus no biological computing in the stable brain MTs energized by tubulin-bound GTP is feasible. Great part of the GTP released energy during MT assembly is accumulated inside the MT lattice as elastic strain presumably important for the biological functions of the stable MTs in vivo. Further exploration of the interaction between the local intraneuronal electric field and the charged elastic brain MTs could provide insight into novel ways for subneuronal processing of information.

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