Models and Speculations

Why Is the Microtubule Lattice Helical?

Viktória Hunyadi\(^1\), Denis Chrétien\(^2\), Henrik Flyvbjerg\(^3\), and Imre M. Jánosi\(^1\),* 

\(^1\)Department of Physics of Complex Systems, Eötvös University, P.O. Box 32, H-1518 Budapest, Hungary; 
\(^2\) UMR CNRS 6026, Université de Rennes 1, Campus de Beaulieu, Bt 13, 35042 Rennes Cedex, France; 
\(^3\) Biosystems Department and Danish Polymer Centre, Risø National Laboratory, P.O.Box 49, DK-4000 Roskilde, Denmark. 

*Corresponding author: e-mail: janosi@lecso.elte.hu, Tel: +36-1-372-2878, Fax: +36-1-372-2866

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Abstract

Microtubules polymerize from identical tubulin hetero-dimers, which form a helical lattice pattern that is the microtubule. This pattern always has left-handed chirality. It is not known why. But since tubulin, like other proteins, evolved for a purpose, the question of the title seems meaningful. In a computer simulation that explores the “counterfactual biology” of microtubules without helicity, we demonstrate that these have the same mechanical properties as nature’s microtubules with helicity. Thus, only a dynamical reason for helicity is left as potential explanation. We find that helicity solves “the problem of the blind mason”: how to correctly build a structure, guided only by the shape of the bricks. This answer in turn raises some new questions for experimenters to address.

1. Introduction

Nature’s many different crystal lattices did not evolve. They just happened. They reflect properties of atoms that are dictated by quantum mechanics, so each one of them looks the same today as it did when it formed for the first time in the history of the universe. The helical “crystal” lattice of microtubules (MTs), in contrast, has proteins for “atoms” and evolved for well-defined purposes. Thus one may ask which purpose its lattice structure serves, and is optimized for, among the many alternative structures that one can easily imagine. Is it in growth and shrinking dynamics, or in mechanical properties, or in other aspects of microtubule functioning (Howard and Hyman, 2003) one must find the answer?

For example, helicity may promote MT growth because the edge of the tip in a helical wall always contains sites for dimer binding with one or more lateral bonds, which decreases the probability of subsequent dissociation. However, helical ordering might also decrease the attainable rate of growth, because the successful docking of an incoming dimer demands the proper orientation of both longitudinal and lateral bonds. As another example, helicity might prevent critical buckling of microtubules under extreme load, because the spiral structure of the helix is not commensurate with the geometry of a buckling tube.

In this work we discuss available results on MT structure and functions, and formulate an explanation of the observed helicity. We investigate critical deformations, for which local bond orientation may be important for MT mechanical properties. We show that the difference between helical and non-helical wall lattices is negligible from this point of view, as well. This leads to the conclusion that helicity should have a dynamic origin.

We then make the very plausible assumption that MT growth is a fully local process, in which attaching dimers have no information about the lattice structure of the MT wall away from their own location. So the individual hetero-dimer and its immediate environment at the time of its attachment must have encoded in their geometry all necessary information to form a polar, spontaneously closing tube. Obviously, the dimer building blocks must be somewhat wedge-shaped for a tube to form by their lateral bonding. But what ensures that the thin ends of neighboring wedges point in the same direction, so a tube is formed? The same lateral bonds might form between oppositely oriented wedges, with the result that a flat sheet is formed instead. What ensures the
correct radial orientation of the docking dimer? Whatever it is, it does its job while the dimer’s longitudinal orientation is ensured by its hetero-dimer nature. We argue that nature solved this problem in an optimal way by coding all the necessary information in the geometry of the tubulin dimers. This yields a helical MT wall structure. However, it does not explain why only left-handed chirality is observed in all known wall configurations. We discuss this and other questions in the closing section, with an emphasis on the link to the explanation of the helical capsid structure of tobacco mosaic virus by Crick and Watson (1956).

2. Global mechanical properties depend weakly on helical bond orientation

2.1 Dimer size and bond lengths are constants, lattice geometry is variable

Tubulin α-β dimers in a microtubule are most frequently arranged in 13 protofilaments aligned parallel to the central axis. Neighboring protofilaments are shifted relatively to each other longitudinally, which results in a (left-handed) helical order in the lateral direction (Fig. 1). Various lattice configurations are observed with different protofilament number (8 ≤ N ≤ 17) and helix start number (2 ≤ S ≤ 4). (S is the longitudinal displacement achieved in one full lateral turn, measured in units of monomer size, see Fig. 1.) Far the most common configuration is the N_S = 13_3 type (Chrétien and Wade, 1991; Hyman et al., 1995, Chrétien and Fuller, 2000).

All the various observed structures were accurately described by the “lattice accommodation model” (Chrétien and Fuller, 2000; Hunyadi et al., 2005). Its assumptions are that dimers are rigid and the bonding regions have very limited flexibility, so a lattice of given, energetically non-optimal N- and S-values, accommodates these values through a geometric skew in the longitudinal direction, see Fig. 1. Indeed, lattice spacings are found to be very similar for different lattice configurations with different skew, both in the longitudinal and in the transverse directions, where it is, respectively, 4.05 nm and 5.13 nm (Chrétien and Fuller, 2000; Hunyadi et al., 2005). Possible relocations of the lateral bonds are measured to be very limited as well (Chrétien et al., 1998). Strong skew can shift adjacent protofilaments longitudinally (Chrétien and Fuller, 2000), but this shift is “quantized” and small (± 0.15 nm ≈ typical C-C bond distance). Large-scale atomistic computer simulations of protofilament-protofilament interactions support that a longitudinal shift is energetically unfavorable (Sept et al., 2003). All this indicates that both the core of the protein and the tubulin-tubulin bonds are rather stiff.

The nature of the distortions of the MT lattice, which are necessary to accommodate different configurations, is apparently elastic. Direct experimental evidence for this elasticity is found in the fact that protofilament number and helix-start number can vary within individual microtubules, which obviously are made from the same material throughout (Chrétien and Fuller, 2000). Further evidence, albeit indirect, is found in the fact that the frequency distribution of the various lattice types can be well described as energetic consequences of elastic deformations only (Hunyadi et al., 2005). The same results also suggest that the existence of the seam (where lateral contacts between tubulin α and β monomers close the tube) is irrelevant: seamless wall structures with helix start numbers 2 or 4 show no sign of an enhanced stability (Hunyadi et al., 2005).

2.2 Microtubule as an elastic tubular sheet

Since microtubules are the stiffest element of the cytoskeleton, their elastic properties have been measured many times (Gittes et al., 1993; Venier et al., 1994; Kurachi et al., 1995; Mickey and Howard, 1995; Felgner et al., 1996, 1997; Cassimeris et al., 2001; Kis et al., 2002; Janson and Dogterom, 2004). One of the key parameters, the flexural rigidity, parameterizes the resistance to bending forces. The values obtained with various methods in different environments cover a rather wide interval: [1-40]×10⁻²⁰ Nm². It was demonstrated very recently that the flexural rigidity of a MT depends on its length. This
result may seem strange, but it has the most natural, simple physical explanation, and it also explains the large range of observed rigidities (Pampaloni et al., 2006).

Electron micrographs of tapered ends on growing microtubules (Chrétien et al., 1995) revealed another mechanical feature of the MT wall, namely that it has an intrinsic negative Gaussian curvature (Jánosi et al., 1998). This is the mathematical term for the fact that in its longitudinal direction, the material wants to curve one way (outward, away from the MT axis), and in its lateral direction it wants to curve the other way (inward, to form the tube). This curvature is commonly ascribed to a conformational change induced by the hydrolysis of guanosine triphosphate (GTP) bound to β-tubulin, and indirect apparent evidence for this scenario was recently presented by Wang and Nogales (2005).

FIGURE 2 Top: A small piece of a continuous, elastic and flexible sheet with intrinsic negative Gaussian curvature. It displays a saddle shape, an energy-minimizing compromise in the tug-of-war caused by the opposing curvatures. The continuous sheet shown was modeled numerically by triangulating it as shown. Bottom: Simple explanation of the many different shapes observed for microtubule ends: They are all made from identical material, but different amounts of material bend to different shapes, as demonstrated here in a computer simulation. Protofilament numbers are indicated (Jánosi et al., 1998).

The above observations led to the elastic sheet model of MT wall material (Jánosi et al., 1998; 2002), which improved the earlier descriptions based on solid rod elasticity (Fig. 2). This model’s reproduction of various MT morphologies yielded the first estimates on longitudinal and lateral bond strengths, and intrinsic curvatures. Recent modeling development, especially by Molodtsov et al. (2005a; 2005b) and VanBuren et al. (2005), incorporated the three-dimensional nature of tubulin building blocks, which allows a description on a higher level of complexity.

2.3 Lattice structure has no effect on flexural rigidity

All the structural models have common ingredients. Longitudinal and lateral bonds obeying some force law are parameterized by preferred lengths and orientations. Intrinsic curvatures are modeled by prescribed bond angles and bending stiffness values. The simplicity of such models is due to the fact that continuum elasticity works even on atomic length scales, e.g., for carbon nanotubes (Yakobson et al., 1996; Wong et al., 1997).

FIGURE 3 Electron micrograph of a strongly bent microtubule pulled by an extension of a few protofilaments, and elastic sheet model tube pulled by a point force at the top end (clamped at the bottom). The shape can be reproduced only with proper ratio of longitudinal and lateral bond strengths, magnitudes can be estimated by matching measured and model flexural rigidities.

The task is then to tune the model parameters until the best reproduction of measured features is achieved, an illustration is shown in Fig. 3. It is relatively easy to change lattice orientations in an MT model and check the possible effects. By means of the elastic sheet model (Jánosi et al., 1998), we found that helical bond orientation in the lateral direction has negligible (negative) contribution to the flexural rigidity. This is in complete agreement with the molecular-mechanical simulations by Molodtsov et al. (2005a), where they compared shape and stability...
of 13_3 tubes with non-helical 13_0 analogues. They found that the helical 13_3 configuration is slightly less stable, mostly because several dimers at the terminals have one-sided lateral bonds, but the difference is unimportant.

We repeat that these results are not surprising, because the local deformations are very moderate even at the strongly bent tube shown in Fig. 3.

3. Helicity is without effect on critical local buckling

It is far less obvious, what might be the implication of lattice structure for critical deformations. Figure 4 shows an unusual configuration, in which the compression in the concave side of the bent MT wall was probably strong enough to initiate local buckling and breaking protofilaments.

Local buckling is not to be confused with Euler buckling, which refers to the elastic instability of a thin rod subject to longitudinal compressing forces (Landau and Lifshitz, 1986). Local buckling can be demonstrated simply by bending a drinking straw: as its overall curvature increases, its cross-section changes from circular to increasingly elliptic, until a kink forms and the tube collapses (see also Fig. 5). This phenomenon is referred to as the Brazier effect in the engineering literature (Brazier, 1927). Brazier buckling is a rather complex phenomenon (Calladine, 1983). It depends not only on macroscopic properties like flexural and bending rigidities, geometry, deformation history, or internal pressure. In real materials, local (microscopic) parameters, such as residual stresses or structural irregularities (buckling seeds), can play an equally important role.

We did a series of buckling simulations with different parameters in the elastic sheet model (Jánosi et al., 1998). In general, we observed the well-known characteristics of local buckling: an increasing bending force leads to significant eccentricity of the cross-section together with a gradual decrease of global rigidity, until the tube collapses. We obtained the same critical eccentricity, 0.790, for all values of model parameters. This result agrees remarkably well with the theoretical estimate of Reissner (1961), who solved the nonlinear local buckling problem for an infinitely long thin-walled tube, giving also a correction to the linear solution of Brazier (1927).

We found that wall anisotropy has a dramatic effect on how cylindrical shells buckle. A non-helical lattice develops a sharp kink, like a straw does, as demonstrated in Fig. 5, bottom. In contrast, a helical lattice buckles in a way that leaves its sides smooth, as illustrated in Fig. 5, top. At this point, however, the sheet model turns unrealistic as a model for real microtubules, because its wall is infinitely thin: the strong local deformation requires us to consider the effects of finite wall thickness.

Accordingly, we implemented a 3d finite element model of microtubule walls, which is very similar to the model of Molodtsov et al. (2005a; 2005b). Monomers are represented as homogeneous elastic spheres, stretching and bending properties are parameterized by linear “springs” of prescribed orientations between neighboring elements. Longitudinal bonds are set to be 2-5 times stronger
than lateral ones. The monomers are impenetrable, spatial overlap is not permitted. Structural relaxation can be performed either by global conjugate gradient minimization, or by a standard molecular dynamics procedure solving Newtonian equations of motion with hydrostatic damping. Further details will be published elsewhere.

The most important result for the present analysis is that the drastic difference between helical and non-helical configurations disappeared (Fig. 6), irrespective of the particular parameter values. Intuitively, the strongly deformed local configurations at critical buckling are determined by excluded volume interactions in a neighborhood of a few adjacent dimers. The shape of the buckled zone clearly reflects the lattice geometry. However, the stress distribution that determines stability, is very similar for helical and non-helical lattices (Fig. 6).

FIGURE 6 Kink configurations after local buckling for a non-helical and helical 3d MT model. Colors indicate local relative stresses, similarly to Fig. 5.

Since Brazier buckling is the prototypical critical deformation where local bond orientation might play an essential role, we do not expect that different experiments, such as local indentation by a scanning force microscope tip (de Pablo et al., 2003; Schaap et al., 2006) or uniform radial buckling under osmotic pressure (Needleman et al., 2004; 2005) would lead to different conclusions.

Based on the facts and results listed in Sections 2 and 3, we conclude that helicity is at most only weakly coupled to the mechanical properties of microtubules. Consequently, it is not in these mechanical properties we find the raison d’être for the helicity of microtubules.

4. Dynamical origin of the helicity

Dissociation of the tubulin dimer is extremely slow, thermodynamically it is very unfavorable (Caplow and Fee, 2002). The dimer can therefore unambiguously be regarded as the basic building block of MT walls.

The dynamic microtubule network maintains cell shape and promotes motility (Huitorel, 1988; Sammak and Borisy, 1988). The crucial role of MTs in mitosis is well-known: searching, docking and pulling chromosomes (Mitchison, 1988; Inoué and Salmon, 1995; Howard and Hyman, 2003; Scholey et al., 2003). Target (chromosome) localization by continuous growth and random switch to continuous shrinkage (dynamic instability) is faster than equilibrium polymerization by several orders of magnitude (Holy and Leibler, 1994; Desai and Mitchison, 1997; Hyman and Karsenti, 1998; Schuyler and Pellman, 2001).

Continuous MT growth is possible only if dimer attachment is absolutely favored (high affinity), or the process is driven by energy consumption (active growth). Tubulin polymerizes in the presence of non-hydrolysable GTP analogue and forms stable MTs (Hyman et al., 1992). Thus energy consumption is not strictly required for growth. These MTs, however, cannot shrink continuously in a constant environment. Fast and continuous disassembly is promoted by the destabilization of the microtubule lattice resulting from impaired lateral bonds (Krebs et al., 2005; Wang and Nogales, 2005; Kerssemaker et al., 2006). This process is powered by GTP hydrolysis, and measurements show that a large part of the released energy is stored in the microtubule lattice in the form of mechanical stress (Caplow et al., 1995). Therefore, the existence of a GTP cap (Drechsel and Kirschner, 1994; Caplow and Shanks, 1996, Huyadi and Jánosi, 2006), or a conformational cap (Chrétien et al., 1999; Arnal et al., 2000; Jánosi et al., 2002) is inevitable in the growth phase. When depolymerization is hindered, normal microtubule functioning fails: the presence of MT stabilizing agents (e.g. taxol) inhibits mitotic progression and cell proliferation (Yvon et al., 1999).

Thus, basic requirements for a well functioning MT are:
1. A robust mechanism of persistent growth, in order to promote, e.g., chromosome localization during mitosis;
2. Strong lateral curvature of the polymerization product, so that a tube forms spontaneously;
3. Opposite longitudinal curvature, so that protofilaments spontaneously curve away from the MT axis when free to do so.

Next we show that these requirements are fulfilled by evolution’s design of the shape of tubulin dimers.
5. The architecture of the MT wall lattice

Consider the following solution to “the problem of the blind mason”. Clearly, bricks with parallel flat surfaces and right-angled edges will spontaneously form only flat walls. For such bricks to form a tubular structure, extra information, a blueprint, is needed. If the sides of these bricks are not parallel, however, the wall will have built-in curvature because its bricks have that. But this is not sufficient to close the wall into a tube, as illustrated in Fig. 7: the curvature must, optimally, have the same sign everywhere in the wall for this to happen, and certainly cannot have arbitrary sign.

The surfaces of laterally bonding regions in a single tubulin dimer are not parallel, but form a wedge, and this favors ring formation. However, an upside-down rotation of a dimer does not exclude lateral attachment, proven by the existence of tubulin “zinc sheets”, in which protofilaments are aligned antiparallel (Nogales et al., 1998a, 1999). Clearly, the extra Zn ions alter the protein’s configuration, but not by much, because the structural models reconstructed from zinc sheet crystallography fit almost perfectly into the lower resolution electron density maps of whole MTs (Nogales et al., 1998a, 1999; Inclán and Nogales, 2001; Löwe et al., 2001; Meurer-Grob et al, 2001; Wang and Nogales, 2005).

Another asymmetry of the dimer building block solves two other problems, as illustrated in Fig. 8. When the two lateral bonding regions of a dimer are shifted longitudinally relatively to each other, lateral bonding can be realized only in one way. Such an asymmetric dimer cannot establish proper bonds if it is rotated 180° about its long or short axis, with the result that its inside points out of the MT.

The measure of asymmetry depicted in Fig. 8 seems close to optimal. It shows that a dimer that is rotated by 180° will have at most ~2 nm mismatch between its monomers and the monomers of its non-rotated neighbors. This may be compared with the result of atomic level simulations by Sept et al. (2003), which predicts that the relative shift of two dimers that least favors lateral bonding between them, is a shift of 2 nm.

Note also that a dimer that fits correctly into its diamond-shaped slot in Fig. 8 in the helical structure formed by its neighbor dimers in the MT wall, such a dimer will automatically point the same side outwards as its neighbors do, hence automatically form a tube with its neighbors if they all are wedge-shapes. Also, protofilaments formed over this helical pattern will all curve the same way.
6. Discussion and open questions

Although many eukaryotes encode multiple α and β tubulin genes, the amino acid sequences of the variants are generally well conserved (McKean et al., 2001). The sequence conservation among diverse species is also remarkable (Little and Seehaus, 1988; Burns and Surridge, 1990). Atomic level reconstruction of tubulin and MT structure (Nogales et al., 1998a, 1999; Inclán and Nogales, 2001; Löwe et al., 2001; Meurer-Grob et al, 2001; Wang and Nogales, 2005) helped to identify the contacts between neighboring dimers. The lateral contact regions involve less conservative segments, their flexibility is larger, and their strength is lower than that of longitudinal contacts.

It is clear that an unambiguous dimer alignment could be achieved in several different ways. For example, a more sophisticated, more specific surface of contact in the longitudinal and/or lateral direction would do the same job of ensuring tube formation, even from an otherwise perfectly symmetrical protein building block (Van Workum and Douglas, 2006). But elaborated designs of surfaces and/or bonds are more complicated solution to the same problem, and might result in less physically robust and slower assembling MTs by its higher requirements for docking of an incoming dimer. The geometrically asymmetric building blocks that dimers obviously are, is the simplest solution to the problem of the blind mason, and probably the most efficient one.

If one accepts these simple arguments as an explanation, the simplest, of why the MT lattice is helical, several questions follow, which have no simple answers.

First of all, why is the helicity of all known microtubule lattices left-handed? If our explanation is correct, a right-handed helical orientation would work equally well. A mixture of tubulin dimers destined to form left- and right-handed helical lattices would not work, obviously. This explains why the helicity must be one or the other in a cell. This then explains, through evolution, why the helicity must be one or the other in an organism, hence in related organisms. But it does not explain why only one helicity occurs, if that is the case, nor why, when, and how left-handed helicity was chosen.

There are hints that mutant tubulins might form tubes of right-handed helicity. However, direct evidence has not been given. Wild-type Arabidopsis grows straight, but two mutations, “lefty1” and “lefty2”, grow with a right-handed twist (Furutani et al., 2000, Thitamadee et al., 2002). One suggested explanation is that the helical sign of the cortical microtubule array (determining the orientation of cells in the outer epidermis) is a magnified reflection of the chirality of the microtubules. It is not known whether these mutant tubulins alter the helical sign of the MT lattice. If this proves correct, then explanations will be required of how the helicity of the MT lattice is transferred to the helicity of a thick MT bundle (Lloyd and Chan, 2002).

Secondly, we can ask whether helical architecture is used by Nature in other (filamentary) constructions. The main ingredients of the MT design are: (i) unique building blocks, which possess (ii) longitudinal polarity, and (iii) radial polarity. (By radial polarity we refer to the fact that the building block has a face that must face out of the final tube and an opposite face that must point inwards.

Since the closest homolog to tubulin is the bacterial FtsZ (a major septum-forming component of bacterial cell division), and an evolutionary link between FtsZ and tubulin is assumed (Margolin et al., 1996; van den Ent et al., 2001), it is worth to check its polymerization. It has been shown that the protofilaments formed by FtsZ are completely homologous to tubulin protofilaments in their longitudinal contacts (Nogales et al., 1998b; Löwe and Amos, 1999; Oliva et al., 2004). However, the lateral contacts between protofilaments in a microtubule wall involve protein surfaces that are not conserved in FtsZ (Nogales et al., 1998b). FtsZ protofilaments form tubular structures in special circumstances (Lu et al., 2000). Unfortunately, it is unclear whether any of the lateral FtsZ contacts in vivo are relevant in vivo, and how the protofilaments in the Z ring are associated (Romberg et al., 2001).

Probably the closest architectural parallel to microtubules is the helical capsid structure of some viruses, first identified for tobacco mosaic virus (TMV) by Watson (1954) and Franklin (1955). Based on these structures, Crick and Watson (1956) formulated a general principle, supposed to be applicable whenever a structure of definite size and shape is formed from identical smaller units. The principle states that every unit should experience the same arrangement of neighboring units, no matter where in the structure a given unit is located. The principle is realized by helical ordering of units in a cylinder wall. But it does not explain helicity, since non-helical ordering in a cylinder wall also is a realization of the principle. Later, it turned out that purely geometrical principles are insufficient to explain TMV’s helical capsid ordering: the protein unit is actually not designed to form an endless helix, but a closed two-layer disc (For reviews, see Klug, 1993; 1999). When this “protohelix” binds to RNA, a conformational switch occurs in the protein, and this is what promotes addition of more protein units in a helical pattern. Though spontaneous growth of long helices is possible without RNA, as is the growth of
stacks of disks, this growth is extremely slow. This shows that one should distinguish between the shape of building blocks in an existing structure and the shape they had when it was constructed (Klug, 1993, 1999). This is the essential difference between MT and virus growth: the viral coat protein has a strong and highly specific interaction with the RNA that it is to coat, to avoid spontaneous formation of purposeless empty tubes and coating of strange RNA. Microtubules in vivo simply polymerize from gamma-TuRC templates (see e.g. Moritz et al., 2000).

As for other similar architectures, helical morphology is abundant in nature. However, the search for analogues of MT architecture is limited by the fact that probably all of the spiraling constructions on larger scales (membranes, tubes, fibers, etc.) are composed of a number of different protein building blocks, so the interactions are presumably much more complex than in the case of tubulin dimers.

Thinking in evolutionary terms, further questions arise. If right-handed microtubules would serve equally well, what prevents evolutionary drift to zero helicity and on to opposite, right-handed chirality? Obviously, the helical structures we observe in MTs are not the result of evolutionary lock-in of a random initial design, with irrelevant properties inherited from the predecessor of tubulin. This we can conclude because a whole spectrum of left-handed helical structures occurs. They are not locked in, evolution has something to work with.

So what is the evolutionary barrier ensuring the lock-in of left-handedness? We already mentioned that a mixture of left- and right-handed building blocks would not work, according to our understanding of the usefulness of the helical lattice structure. So a spectrum of helicities around zero helicity would not work. Also, our arguments for helicity are arguments why zero helicity is inefficient or does not work in an organism. Mutants with low or no helicity might confirm this, if discovered.

Furthermore, irrespectively of whether it has a purpose, only chirality begets chirality, so where and what is the chiral template for tubulin? Is it the left-handed chirality of the amino acids (e.g., Keszthelyi, 1995) from which tubulin is built?

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