Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Microtubule lattice plasticity

Robert A Cross

In classical microtubule dynamic instability, the dynamics of the built polymer depend only on the nucleotide state of its individual tubulin molecules. Recent work is overturning this view, pointing instead towards lattice plasticity, in which the fine-structure and mechanics of the microtubule lattice are emergent properties that depend not only on the nucleotide state of each tubulin, but also on the nucleotide states of its neighbours, on its and their isotypes, and on interacting proteins, drugs, local mechanical strain, post translational modifications, packing defects and solvent conditions. In lattice plasticity models, the microtubule is an allosteric molecular collective that integrates multiple mechanochemical inputs and responds adaptively by adjusting its conformation, stiffness and dynamics.

Address
Centre for Mechanochemical Cell Biology, Warwick Medical School, Gibbet Hill, Coventry CV4 7AL, UK

Corresponding author: Cross, Robert A (r.a.cross@warwick.ac.uk)

Current Opinion in Cell Biology 2019, **56**:88–93

This review comes from a themed issue on **Cell signalling**

Edited by **Johanna Ivaska** and **Manuel Théry**

For a complete overview see the Issue and the Editorial

Available online 8th November 2018

https://doi.org/10.1016/j.ceb.2018.10.004

Introduction

The assembly properties of tubulin heterodimers, the building block of microtubules (MTs), are controlled to a large extent by their exchangeable guanosine nucleotide. GTP-tubulin polymerises readily, whilst GDP-tubulin does not. Polymerisation of GTP-tubulin catalyses GTP hydrolysis, so that in growing MTs, a stabilising, newly-polymerised GTP-tubulin cap typically overlies a metastable GDP-tubulin core. Stochastic breaches in the stabilising GTP-cap expose the unstable GDP-core, triggering rapid endwise depolymerisation. This overall scheme was discovered some time ago [1], but the detailed molecular mechanisms of MT dynamics remain an important unsolved problem [2]. Can polymerised tubulin in the cap and/or the core change conformation? Does tubulin exchange only at MT tips, or can it exchange into and out of the built lattice? Do lattice dislocations and vacancies occur? And if any of these things do occur, how do they influence MT function? To address these questions, recent work has sought to visualise large-scale conformational changes, subunit exchange, packing defects and modes of mechanical failure within the lattice of assembled MTs. What is emerging is an increasing body of experimental evidence for structural plasticity models [3], in which the conformation, mechanics and dynamics of the MT lattice are sensitive not just to GTP and GDP, but also to a variety of other factors.

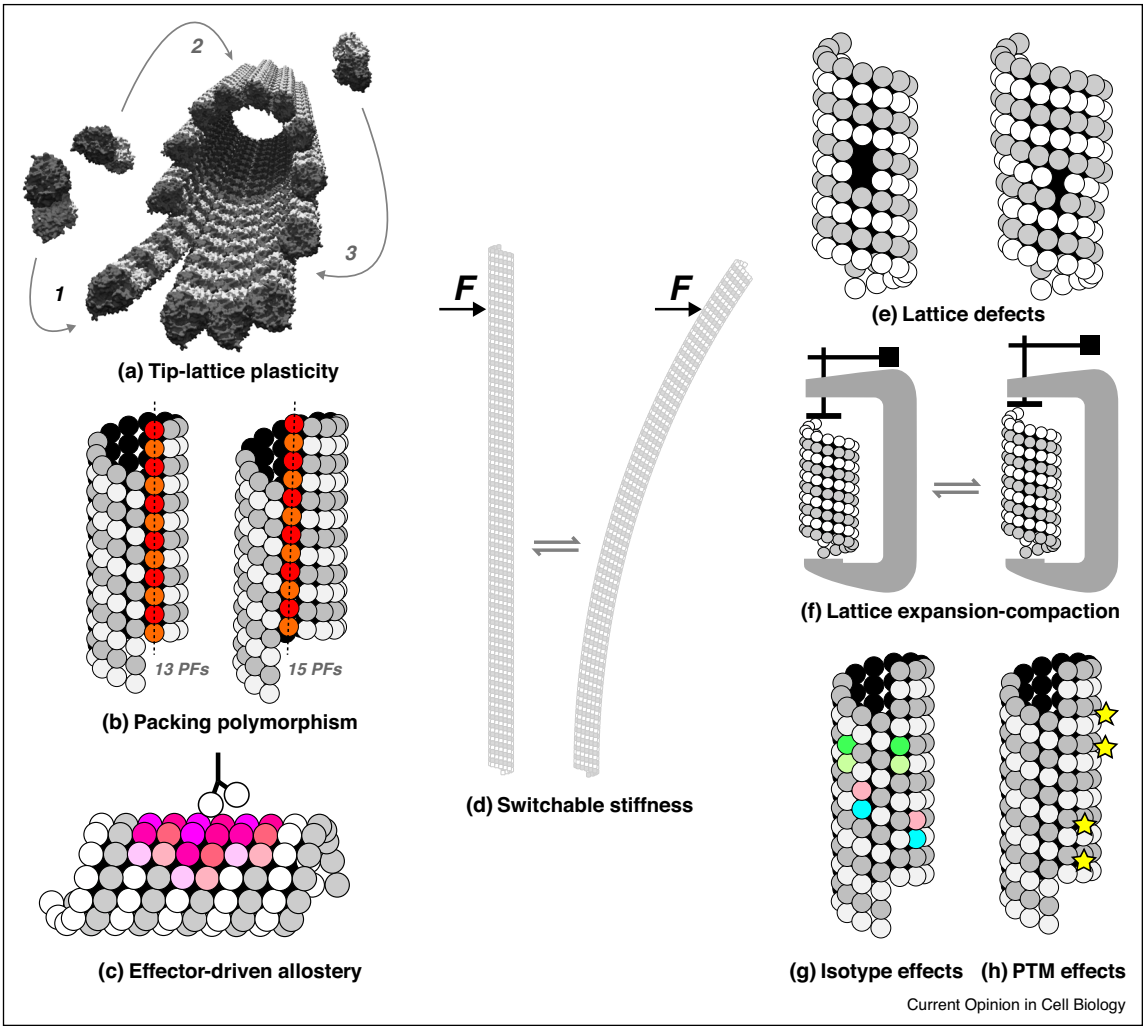
Plasticity of the tip lattice

The potential for lattice plasticity in dynamic microtubules begins at their tips. It remains tantalisingly unclear exactly how GTP-tubulins incorporate into the growing tip-lattice. GTP-tubulins interacting with the ends of exposed single protofilaments (PFs) will form longitudinal contacts but not lateral contacts (Figure 1a). If such single PFs are sufficiently stable, they can go on to zipper into the growing lattice progressively and relatively slowly and the MT growth rate will approximate the rate of PF growth. However there is *in vitro* evidence suggesting that such single PFs are unstable [4], so that only those that rapidly go on to make lateral contacts will survive. If so, then growth must instead occur predominantly via GTP-tubulins fitting into niches at the exposed leading edge of an already-built sheet or flared split tube of PFs.

Which view is correct? The answer to this question is unlikely to be simple. Nonetheless, recent electron microscopy of growing plus ends adds substantial weight to the view that incoming GTP-tubulins add to short, separate PFs with an appreciable outwards curvature and that these then anneal progressively into the growing lattice [5••], which in turn serves to straighten them and their subunits and propagate tubulation [6,7]. Computational simulation [8•] can provide insight into processes that are inaccessible to wet experiments. Recent simulations [9••] argue firmly for a lattice-driven conformational change in GTP-tubulin.

In this picture, the assembled tip-lattice functions as an allosteric effector [10] that remodels GTP-tubulin subunits after they land, whilst the key property that GTP confers on tubulin is to make it more flexible, so allowing it to be readily reshaped by incorporation into the lattice and to store thereby relatively little strain energy. Conversely, GDP-tubulin would store appreciable strain-energy when forced into shape by the lattice, effectively becoming spring-loaded [11•]. What then happens if GTP-tubulins pack alongside GDP-tubulins?

Figure 1



Types of microtubule lattice plasticity. **(a)** Plasticity in tip-lattice structure and conformation. Tubulin molecules landing from solution might add to the ends of single PFs (*1*), or fit into niches in the built lattice (*2,3*). **(b)** Two examples of polymorphic protofilament (PF) number and packing. In 13-PF MTs (*left*), PFs lie exactly parallel to the MT axis and there is a single A-lattice seam, in which beta tubulin binds laterally to alpha tubulin. The rest of the tube is built from the B-lattice, in which beta binds laterally to beta and alpha to alpha. Supernumerary A-lattice seams can occur (*not shown*). 15 PF MTs (*right*) are entirely built from the B-lattice, lack seams, and have their PFs skewed relative to the MT long axis [16]. **(c)** Binding of interactors (motors, MAPS, drugs) can drive the lattice to change conformation. The range of such allosteric effects (purple patch) is so far little-studied. **(d)** MTs can change their stiffness, for example via lumenal acetylation [30••]. **(e)** Lattice defects support rapid exchange of tubulin and attract specific interactors. At vacancies (left) one or more subunits is missing. At dislocations (right) the PF number changes at the defect. **(f)** Reversible shifts in lattice spacing can occur, for example on kinesin binding [39••], raising the possibility of a mechanical work-cycle. **(g)** MTs containing different tubulin isotypes can have different dynamics [23••]. Isotypes copolymerise and dose-response in copolymers [23••] is of great interest. **(h)** Post-translational modifications (PTMs), both at C-termini and within the core sequence, can change lattice mechanics and dynamics, but can also have indirect effects, by re-setting the affinity for particular interactors.

Biochemical kinetics shows that GDP-and GTP-tubulin can copolymerise, provided GTP-tubulin is in sufficient excess, and that the resulting MTs show intermediate dynamics [12]. This suggests that GTP and GDP allosterically influence not only the mechanics of the tubulin molecule to which they are bound, but also the mechanics of its neighbours. One consequence is that the spatial extent of the stabilising cap of dynamic microtubules may not map straightforwardly to the GTP-tubulin distribution [13]. Further, there is evidence that GTP and GDP molecules can exchange at the MT tip [14].

Plasticity of the core lattice

Whilst the typical number of PFs per MT (Figure 1b) varies appreciably across the tree of life [15], so far all eukaryotic microtubules appear based on just two packing diagrams, the B-lattice, in which neighbouring PFs are staggered by 0.9 nm, and the A-lattice, with a stagger of

4.9 nm [16]. Within this framework, can the built lattice change conformation? Direct comparison by electron microscopy of the GDP, GMPCPP lattice and GTPγS lattices of mixed-isoform brain tubulin reveals that it can, with the most obvious hydrolysis-dependent change being a reduction in the longitudinal lattice spacing [17] due to compaction of alpha-tubulin immediately adjacent to its longitudinal intersubunit contact. Remarkably however, hydrolysis-dependent lattice compaction is not seen in MTs built from either *Saccharomyces cerevisae* or *Schizosaccharomyces pombe* tubulins, even though these microtubules show canonical dynamic instability [18•,19•,20]. Clearly lattice compaction, when seen, signals an underlying conformational change that stores strain-energy, but equally clearly, this conformational change only registers as a spacing change in brain MTs. Strain energy is still stored in yeast GDP-MTs, but its effects on the lattice spacing are too slight to detect. This is explicable if the material properties of the lattice (its stiffness) are changing according to its nucleotide state, and differently for different isotypes. Considerable progress has recently been made in isolating single tubulin isotypes in workable quantities [21,22] and the way is open now for the field to decipher the roles of tubulin isotypes [22], blends of isotypes [23••], isotype-specific post-translational modifications [24] and mutations [25] in the dynamics [26••] and mechanics of the lattice. CryoEM will be central [27].

Mechanical plasticity

MTs with different nucleotides but nominally similar (B-lattice) structure have different mechanical stiffnesses [28]. Post-translational modifications (PTMs) can also change stiffness-1acetylation of lysine 40, which projects into the MT lumen, protects mixed isoform brain MTs from mechanical damage [29,30••], and signals to kinesin and dynein motility [31]. Recent optical trapping work shows that MTs that soften under strain show flattening of their cross section [32••] and that their bending stiffness varies depending on the magnitude of the applied force. Length-dependent bending stiffness has previously been noted [33], but this new work [32••] shows that stiffness varies according to load. Complex mechanics is emerging as an important aspect of lattice plasticity [2].

Lattice defects can enhance plasticity

Lattice vacancies and dislocations (Figure 1e) weaken the MT and accelerate tubulin exchange. Microtubules 'soften' in response to repeated bending at the site of a defect, but can self-repair by incorporating fresh tubulin into the damaged site, which in turn can favour rescue [34,35••,36]. Under particular growth conditions, defects are incorporated with a fixed probability, so that the total number of defects increases with age. This effect has been proposed progressively to favour catastrophe as the MT grows longer [13]. Defects are recognised by specific interactors — for example, katanins, which are enzymes that catalyse subunit exchange and so can either enlarge or repair defects [37]. Defects occur in cells [16] and there is increasing evidence to suggest that they are exploited as control points within the built lattice at which fresh GTP tubulin can be introduced and rescue/regrowth events can nucleate [35••,36].

The assembled lattice can split

MTs sliding across a kinesin surface can split [38], perhaps at a defect site. However, splitting of the GDP lattice can also be induced purely by binding of a GDP-MT to a kinesin-coated surface [39••], because the kinesin-bound lower part of the MT expands its lattice spacing, generating shear force (see below). A-lattice seams (Figure 1b) have been suggested to be more susceptible to splitting than the rest of the lattice, albeit this remains controversial. Seams have been proposed to be a control point for dynamics — for example, a trigger-point for catastrophes [40,41]. Rapid changes in PF number can occur in MTs *in vitro*, at rates too fast to correspond to disassembly/reassembly [20]. Perhaps splitting and reannealing might be involved here also.

Allosteric effectors can shift lattice conformation

Allostery within individual tubulin heterodimers is required for, but distinct from, lattice allostery — most obviously, the allosteric effectors GTP and GDP influence not just the stability of the intersubunit interface surrounding the E-site, but also the stability of lateral contacts and the bending stiffness of the MT. A point mutant in helix H7 of beta tubulin has been described that suppresses the allosteric response to the GTP → GDP transition [42]. Several drugs that influence polymer stability bind well away from the longitudinal and lateral intersubunit interfaces [43]. How far can the actions of allosteric effectors be transmitted? Polymer allostery [44] can allow information to be transmitted over substantial distances. An important outstanding problem is to map the range and mechanisms of larger-scale allosteric effects in the MT.

MAPs can shift lattice conformation

The potentially complex effects of MAPs can be illustrated by considering EB proteins. EBs bind tightly to both straight and curved regions [45] of growing MT tips, and more weakly to the core lattice. At growing tips, they diminish their own high-affinity binding site, by driving GTP processing [46]. Different EB isotypes bind differently e.g. Bim1 binds *S. cerevisae* MTs at double the stoichiometry that it binds brain MTs [18•]; Mal3 tip-tracks on both brain and *S. pombe* MT tips, but under different conditions [19•]. EBs focus the PF number to 13, de-skew the PF axes [18•,19•,20], stabilise A-lattice seams, and generate ectopic seams [18•,41]. In mixed

isoform brain MTs, EBs drive lattice compaction [17], but again, not in yeast MTs [18•,19•,20].

Motors can shift lattice conformation

My own lab recently showed that kinesin binding expands the lattice spacing of mixed isoform GDP-brain MTs by about 1.6%, and simultaneously inhibits their depolymerisation [39••]. Releasing the kinesin, by adding ADP, causes the lattice to recoil rapidly to its original length. Much-earlier work already showed that kinesin can prefer and propagate particular lattice states [47]. A preference for GMPCPP MTs has also been seen [48]. Recent evidence hints at the corollary, whereby different tip-lattice conformations can switch a kinesin-8 between two different functional modes [49••]. Thus, the MT lattice can sense kinesin engagement and respond by altering its conformation and dynamics, raising the possibility of a feedback loop linking motor activity to lattice dynamics. Aside from the forces developed by kinesins, measurements of strain energy in depolymerising tips [11•] emphasise that shifts in lattice conformation can also in principle do work (Figure 1c).

Drugs can shift lattice conformation

Tubulin binds MTAs (microtubule targeting agents) at five different sites [43], and all can have allosteric as well as local effects. The classic example is taxol, which binds at a luminal site adjacent to the M-loop and stabilises lateral contacts, but also acts as a remote lever [50] to influence the GTP-site and thereby longitudinal contacts. Taxol expands the lattice spacing (the more so if added after MT assembly) and can straighten isolated PFs [51,52]. It also influences lattice stiffness [32••], and tends to restrict the PF number to 12–13 [52]. Recent simulations [53•] suggest that taxol nearly eliminates the energy difference between GTP- and GDP-tubulin.

Prospects

The study of lattice plasticity is in its infancy. For example, we are only just beginning to explore the influence of different tubulin isotypes, and isotype mixtures [23••], on lattice dynamics. But it is already clear that microtubules can, in principle, serve the cell not just as structural-mechanical scaffolding poles and rails and springs, but as sensory antennas that accept multiple chemical and mechanical inputs, store, integrate and transmit their effects, and respond by adjusting their own conformation, mechanics, assembly dynamics and interactor-affinities. In this emerging new picture, the MT lattice is an allosteric collective that continually responds to its environment. One consequence is that the structure and dynamics of particular MTs at particular times will be history-dependent, extending lattice plasticity into the time domain.

Very interesting new work delineates the residue-level mechanisms by which kinesin can drive the GDP MT lattice into a more GTP-like conformation, merging results from an array of different biophysical and cell biological experiments [54].

Conflict of interest statement

Nothing declared.

Acknowledgement

RAC is funded by a Wellcome Trust Senior Investigator Award (grant number 103895/Z/14/Z).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Mitchison T, Kirschner M: **Dynamic instability of microtubule growth**. *Nature* 1984, **312**:237-242.

2. Brouhard GJ, Rice LM: **Microtubule dynamics: an interplay of biochemistry and mechanics**. *Nat Rev Mol Cell Biol* 2018, **13**:1-13.

3. Kueh HY, Mitchison TJ: **Structural plasticity in actin and tubulin polymer dynamics**. *Science* 2009, **325**:960-963.

4. Coombes CE, Yamamoto A, Kenzie MR, Odde DJ, Gardner MK: **Evolving tip structures can explain age-dependent microtubule catastrophe**. *Curr Biol* 2013, **23**:1342-1348.

5. •• McIntosh JR, O'Toole E, Morgan G, Austin J, Ulyanov E, Ataullakhanov F, Gudimchuk N: **Microtubules grow by the addition of bent guanosine triphosphate tubulin to the tips of curved protofilaments**. *J Cell Biol* 2018, **265** http://dx.doi.org/10.1083/jcb.201802138.
Carefully-controlled study showing that growing MT tips in a wide variety of*in vitro* and *in vivo* situations display a crown of outwardly curved single PFs, implying that MT growth in these situations occurs predominantly by the extension of single PFs, which then anneal progressively into the growing tube.

6. Buey RM, Díaz JF, Andreu JM: **The nucleotide switch of tubulin and microtubule assembly: a polymerization-driven structural change**. *Biochemistry* 2006, **45**:5933-5938.

7. Brouhard GJ, Rice LM: **The contribution of -tubulin curvature to microtubule dynamics**. *J Cell Biol* 2014, **207**:323-334.

8. • Hemmat M, Castle BT, Odde DJ: **ScienceDirect Microtubule dynamics: moving toward a multi-scale approach**. *Curr Opin Cell Biol* 2018, **50**:8-13.
Simulation is an increasingly important route to discovering and testing mechanisms. This admirably clear review discusses strategies to integrate all-atom simulations of fast, localized dynamics with larger scale approaches.

9. •• Igaev M, Grubmüller H: **Microtubule assembly governed by tubulin allosteric gain in flexibility and lattice induced fit**. *Elife* 2018, **7**:403-426.
Atomistic computational simulations providing strong support for models in which GTP-tubulin heterodimers are more flexible than GDP-tubulin heterodimers, allowing them to be readily straightened by incorporation into the growing lattice.

10. Rice LM, Montabana EA, Agard DA: **The lattice as allosteric effector: structural studies of alphabeta- and gamma-tubulin clarify the role of GTP in microtubule assembly**. *Proc Natl Acad Sci USA* 2008, **105**:5378-5383.

11. • Driver JW, Geyer EA, Bailey ME, Rice LM, Asbury CL: **Direct measurement of conformational strain energy in protofilaments curling outward from disassembling microtubule tips**. *Elife* 2017, **6**:576.
Quantification of the off-axis force exerted on an optically trapped bead by the outward curling of a depolymerizing protofilament that is linked to the bead by a single antibody.

12. Valiron O, Arnal I, Caudron N, Job D: **GDP-tubulin incorporation into growing microtubules modulates polymer stability**. *J Biol Chem* 2010, **285**:17507-17513.

13. Bowne-Anderson H, Zanic M, Kauer M, Howard J: **Microtubule dynamic instability: a new model with coupled GTP hydrolysis and multistep catastrophe**. *Bioessays* 2013, **35**:452-461.

14. Piedra F-A, Kim T, Garza ES, Geyer EA, Burns A, Ye X, Rice LM: **GDP-to-GTP exchange on the microtubule end can contribute to the frequency of catastrophe**. *Mol Biol Cell* 2016, **27**:3515-3525.

15. Chaaban S, Brouhard GJ: **A microtubule bestiary: structural diversity in tubulin polymers**. *Mol Biol Cell* 2017, **28**:2924-2931.

16. Chrétien D, Fuller SD: **Microtubules switch occasionally into unfavorable configurations during elongation**. *J Mol Biol* 2000, **298**:663-676.

17. Zhang R, Alushin GM, Brown A, Nogales E: **Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins**. *Cell* 2015, **162**:849-859.

18. • Howes SC, Geyer EA, LaFrance B, Zhang R, Kellogg EH, Westermann S, Rice LM, Nogales E: **Structural differences between yeast and mammalian microtubules revealed by cryo-EM**. *J Cell Biol* 2017, **216**:2669-2677.

Demonstrates that*S. cerevisiae* MTs do not display hydrolysis-related lattice compaction, except in the case of GTPγS MTs decorated with Bim1, the *S. cerevisae* EB protein. Remarkably, Bim1 binds at both the interdimer and intradimer longitudinal interfaces of *S. cerevisiae* MTs.

19. • Loeffelholz von O, Venables NA, Drummond DR, Katsuki M, Cross R, Moores CA: **Nucleotide- and Mal3-dependent changes in fission yeast microtubules suggest a structural plasticity view of dynamics**. *Nat Commun* 2017, **8**:2110.

Demonstrates that*S. pombe* MTs do not display hydrolysis-related lattice compaction, with and without decoration by Mal3, the *S. pombe* EB protein.

20. Howes SC, Geyer EA, LaFrance B, Zhang R, Kellogg EH, Westermann S, Rice LM, Nogales E: **Structural and functional differences between porcine brain and budding yeast microtubules**. *Cell Cycle* 2018, **17**:278-287.

21. Minoura I, Hachikubo Y, Yamakita Y, Takazaki H, Ayukawa R, Uchimura S, Muto E: **Overexpression, purification, and functional analysis of recombinant human tubulin dimer**. *FEBS Lett* 2013, **587**:3450-3455.

22. Vemu A, Atherton J, Spector JO, Szyk A, Moores CA, Roll-Mecak A: **Structure and dynamics of single-isoform recombinant neuronal human tubulin**. *J Biol Chem* 2016, **291**:12907-12915.

23. •• Vemu A, Atherton J, Spector JO, Moores CA, Roll-Mecak A: **Tubulin isoform composition tunes microtubule dynamics**. *Mol Biol Cell* 2017, **28**:3564-3572.

Shows clearly not only that MT dynamics depends heavily on tubulin isotype, but also that the minor component in a mix of isotypes can potentially dominate.

24. Magiera MM, Singh P, Gadadhar S, Janke C: **Tubulin posttranslational modifications and emerging links to human disease**. *Cell* 2018, **173**:1323-1327.

25. Ti S-C, Pamula MC, Howes SC, Duellberg C, Cade NI, Kleiner RE, Forth S, Surrey T, Nogales E, Kapoor TM: **Mutations in human tubulin proximal to the kinesin-binding site alter dynamic instability at microtubule plus- and minus-ends**. *Dev Cell* 2016, **37**:72-84.

26. •• Fees CP: **Regulation of microtubule dynamic instability by the carboxy-terminal tail of β-tubulin**. *Life Sci Alliance* 2018, **1** e201800054–13.

Isotype-specific sequence differences at the extreme C-terminus of ß-tubulin can profoundly influence dynamics, despite the C-terminus being remote from both the lateral and longitudinal intersubunit contacts.

27. Nogales E, Kellogg EH: **Challenges and opportunities in the high-resolution cryo-EM visualization of microtubules and their binding partners**. *Curr Opin Struct Biol* 2017, **46**:65-70.

28. Mickey B, Howard J: **Rigidity of microtubules is increased by stabilizing agents**. *J Cell Biol* 1995, **130**:909-917.

29. Portran D, Schaedel L, Xu Z, Thery M, Nachury MV: **Tubulin acetylation protects long-lived microtubules against mechanical ageing**. *Nat Cell Biol* 2017, **19**:391-398.

30. •• Xu Z, Schaedel L, Portran D, Aguilar A, Gaillard J, Marinkovich MP, Thery M, Nachury MV: **Microtubules acquire resistance from mechanical breakage through intralumenal acetylation**. *Science* 2017, **356**:328-332.

With 29, reveals that acetylation of Lys40 profoundly stabilizes the lattice, showing that the long-established link between acetylation and MT stability is causal.

31. Sirajuddin M, Rice LM, Vale RD: **Regulation of microtubule motors by tubulin isotypes and post-translational modifications**. *Nat Cell Biol* 2014, **16**:335-344.

32. •• Memet E, Hilitski F, Morris MA, Schwenger WJ, Dogic Z, Mahadevan L: **Microtubules soften due to cross-sectional flattening**. *Elife* 2018, **7**:1.

Careful study combining optical trapping, electron microscopy and quantitative modelling to show that a MT that is bent with sufficient force softens via collapse of the tube.

33. Pampaloni F, Lattanzi G, Jonás A, Surrey T, Frey E, Florin E-L: **Thermal fluctuations of grafted microtubules provide evidence of a length-dependent persistence length**. *Proc Natl Acad Sci USA* 2006, **103**:10248-10253.

34. Schaedel L, John K, Gaillard J, Nachury MV, Blanchoin L, Thery M: **Microtubules self-repair in response to mechanical stress**. *Nat Mater* 2015, **14**:1156-1163.

35. •• Aumeier C, Schaedel L, Gaillard J, John K, Blanchoin L, Thery M: **Self-repair promotes microtubule rescue**. *Nat Cell Biol* 2016, **18**:1054-1064.

With 34, establishes that lattice defects promote rapid subunit exchange, leading to self-repair via the incorporation of fresh GTP-tubulin, and a related propensity to rescue episodes of shrinkage.

36. de Forges H, Pilon A, Cantaloube I, Pallandre A, Haghiri-Gosnet A-M, Perez F, Poüs C: **Localized mechanical stress promotes microtubule rescue**. *Curr Biol* 2016, **26**:3399-3406.

37. Valenstein ML, Roll-Mecak A: **Graded control of microtubule severing by tubulin glutamylation**. *Cell* 2016, **164**:911-921.

38. VanDelinder V, Adams PG, Bachand GD: **Mechanical splitting of microtubules into protofilament bundles by surface-bound kinesin-1**. *Sci Rep* 2016, **6**:39408.

39. •• Peet DR, Burroughs NJ, Cross RA: **Kinesin expands and stabilizes the GDP-microtubule lattice**. *Nat Nano* 2018, **112**:1065.

Shows that strong-state binding of kinesin-1 to GDP-MTs expands their lattice and suppresses their shrinkage. Binding of GDP-MTs to a kinesin-coated surface not only stabilises the tube, it also causes it to split, presumably because the kinesin side expands whilst the other side does not.

40. Sandblad L, Busch KE, Tittmann P, Gross H, Brunner D, Hoenger A: **The Schizosaccharomyces pombe EB1 homolog Mal3p binds and stabilizes the microtubule lattice seam**. *Cell* 2006, **127**:1415-1424.

41. Katsuki M, Drummond DR, Cross RA: **Ectopic A-lattice seams destabilize microtubules**. *Nat Commun* 2014, **5**:3094.

42. Geyer EA, Burns A, Lalonde BA, Ye X, Piedra F-A, Huffaker TC, Rice LM: **A mutation uncouples the tubulin conformational and GTPase cycles, revealing allosteric control of microtubule dynamics**. *Elife* 2015, **4** e10113.

43. Steinmetz MO, Prota AE: **Microtubule-targeting agents: strategies to hijack the cytoskeleton**. *Trends Cell Biol* 2018 http://dx.doi.org/10.1016/j.tcb.2018.05.001.

44. Motlagh HN, Wrabl JO, Li J, Hilser VJ: **The ensemble nature of allostery**. *Nature* 2014, **508**:331-339.

45. Guesdon A, Bazile F, Buey RM, Mohan R, Monier S, García RR, Angevin M, Heichette C, Wieneke R, Tampé R *et al.*: **EB1 interacts with outwardly curved and straight regions of the microtubule lattice**. *Nat Cell Biol* 2016, **18**:1102-1108.

46. Duellberg C, Cade NI, Holmes D, Surrey T: **The size of the EB cap determines instantaneous microtubule stability**. *Elife* 2016, **5**:711.

47. Muto E, Sakai H, Kaseda K: **Long-range cooperative binding of kinesin to a microtubule in the presence of ATP**. *J Cell Biol* 2005, **168**:691-696.

48. Nakata T, Niwa S, Okada Y, Perez F, Hirokawa N: **Preferential binding of a kinesin-1 motor to GTP-tubulin-rich microtubules underlies polarized vesicle transport**. *J Cell Biol* 2011, **194**:245-255.

49. •• Arellano-Santoyo H, Geyer EA, Stokasimov E, Chen G-Y, Su X, Hancock W, Rice LM, Pellman D: **A tubulin binding switch underlies Kip3/Kinesin-8 depolymerase activity**. *Dev Cell* 2017, **42**:37-51.e8.
Shows that Kip3 mechanochemistry changes profoundly when it binds to curved PFs at MT tips, compared to when bound to the lattice.

50. Amos LA, Löwe J: **The subtle allostery of microtubule dynamics**. *Nat Struct Mol Biol* 2014, **21**:505-506.

51. Elie-Caille C, Severin F, Helenius J, Howard J, Müller DJ, Hyman AA: **Straight GDP-tubulin protofilaments form in the presence of taxol**. *Curr Biol* 2007, **17**:1765-1770.

52. Kellogg EH, Hejab NMA, Howes S, Northcote P, Miller JH, Díaz JF, Downing KH, Nogales E: **Insights into the distinct mechanisms of action of taxane and non-taxane microtubule stabilizers from Cryo-EM structures**. *J Mol Biol* 2017, **429**:633-646.

53. • Castle BT, McCubbin S, Prahl LS, Bernens JN, Sept D, Odde DJ: **Mechanisms of kinetic stabilization by the drugs paclitaxel and vinblastine**. *Mol Biol Cell* 2017, **28**:1238-1257.
Simulation evidence arguing that vinblastine works by suppressing tubulin exchange at MT tips, whereas paclitaxel (taxol) works partly by suppressing exchange, but largely by reducing the strain energy stored in GDP-tubulin.

54. Shima T *et al.*: **Kinesin-binding–triggered conformation switching of microtubules contributes to polarized transport**. *J Cell Biol* 2018, **68** jcb.201711178-20.