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How cells exploit tubulin diversity to build functional cellular microtubule mosaics

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Cellular microtubules are mosaic polymers assembled from multiple αβ-tubulin isoforms bearing chemically diverse posttranslational modifications. This tubulin diversity constitutes a combinatorial code that regulates microtubule interactions with cellular effectors and alters their intrinsic dynamic and mechanical properties. Cells generate stereotyped and complex tubulin modification patterns that are important for their specialized functions. Here we give a brief overview of the tubulin genetic and chemical diversity and highlight recent advances in our understanding of how the tubulin code regulates essential biological processes ranging from intracellular cargo transport, to cell division and cardiomyocyte contraction. Finally, we speculate on the molecular mechanisms for the generation and maintenance of the complex stereotyped modification patterns that form cellular microtubule mosaics.

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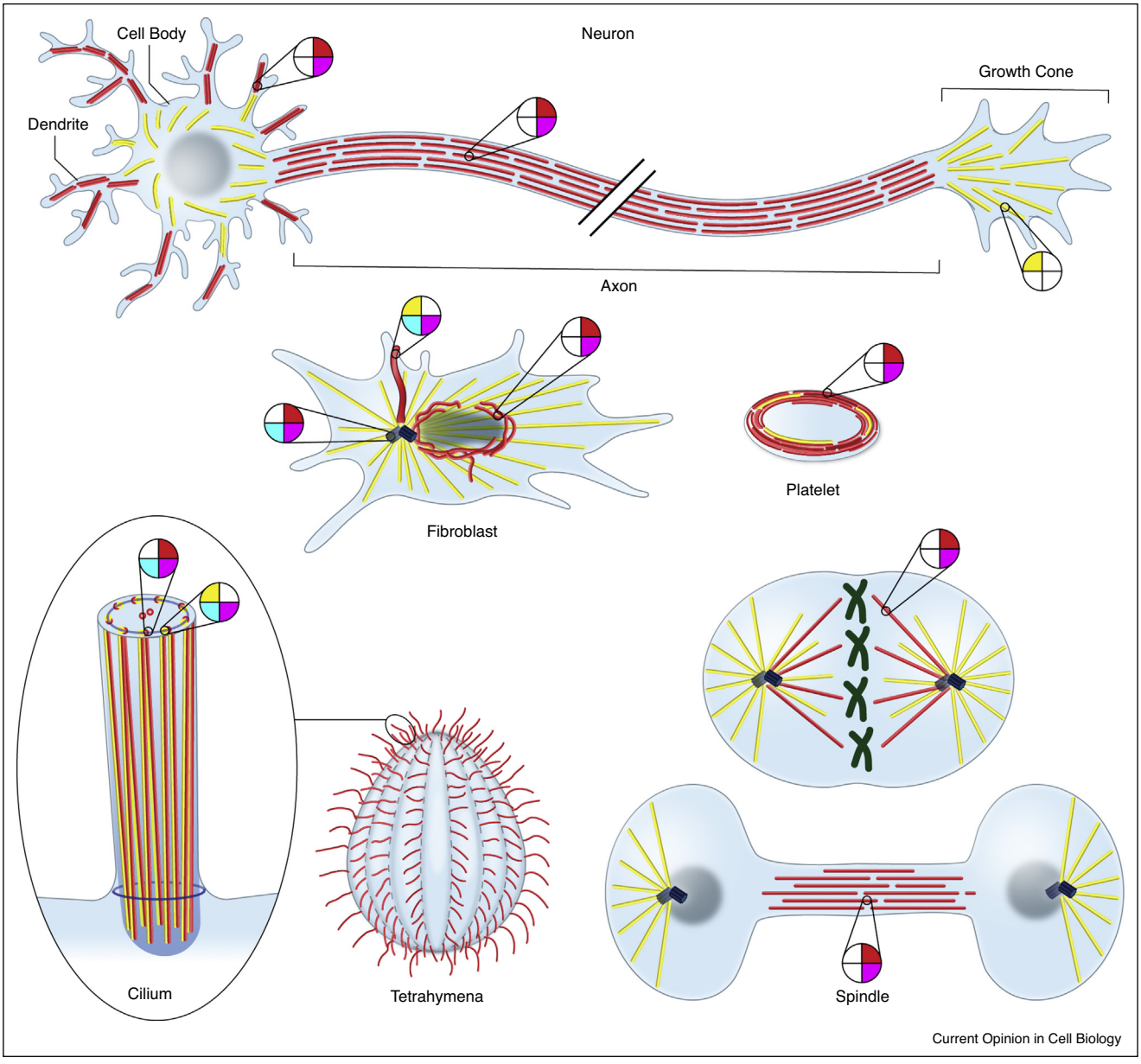
Together with actin and intermediate filaments, microtubules form one of the three cytoskeletal filament systems in eukaryotic cells. They are non-covalently linked cylindrical polymers assembled from αβ-tubulin heterodimers. Microtubules provide mechanical support for cells, function as highways for the delivery of cargo and segregate chromosomes during cell division. In order to perform these disparate functions, cells build microtubule arrays with diverse morphologies and dynamics. These include the stable bundled microtubule arrays in neurons to facilitate efficient cargo transport by molecular motors, the dynamic bipolar spindle to capture chromosomes and generate the forces needed to separate them, the ring of stable microtubules beneath the membrane of platelets that gives these cells their characteristic discoid shape or the highly stable nine-fold symmetric axonemes that are optimized for high-flux cargo movement and built to withstand the high mechanical forces generated during ciliary or flagellar beating (Figure 1).

Microtubule mosaics are formed by diverse tubulin isoforms

Microtubule arrays display a remarkable diversity in the chemical composition of their constituent subunits, the αβ tubulin heterodimers. This diversity is generated through the expression of different tubulin isoforms as well as posttranslational modifications. Most eukaryotes possess multiple tubulin genes (reviewed in Ref. [1]). Humans have eight α-tubulin and nine β-tubulin genes. Some cell types have restricted tubulin isotype expression, while others express multiple, thus creating many combinations of αβ heterodimers [1]. Most microtubules are made of mixtures of isotypes, while some contain predominantly a single isotype. For example, the incorporation of the most divergent β-tubulin isoform, βVI (TUBB1) in platelet microtubules is essential for the formation of the platelet marginal band that gives these cells their discoid shape. Loss of this tubulin isoform results in thrombocytopenia [2]. βIII tubulin (TUBB3) is specific for neurons [3] and is expressed in non-neuronal cells only during tumorogenesis [3,4]. Mutations in βIII are linked to polymicrogyria [5] and axon guidance disorders ([6], reviewed in Ref. [7]). βIVa and IVb tubulins (TUBB4A and B) are important for the formation of cilia and flagella [8]. They contain an axonemal-specific motif on the C-terminal tail identified as important for the generation of the central pair in the motile axonemes of fly sperm [9]. Remarkably, we do not know the exact stoichiometry of tubulin isoforms in most cell types and we have a poor understanding of how tubulin isoform expression levels are regulated throughout development. We also currently lack an understanding of the basic biophysical properties of most tubulin isoforms: their kinetics of incorporation into microtubules and their effect on microtubule structure, dynamics and mechanical properties. Recent studies using recombinant tubulin isoforms and tubulin isolated from an embryonic kidney cell line using an affinity approach revealed that tubulin isoform composition can proportionally tune microtubule dynamic parameters [10••,11••]. Thus, changes observed in tubulin isoform expression during differentiation or tumorogenesis can directly control microtubule dynamics in addition to possibly serving

Figure 1



Cells have microtubule arrays with diverse geometries and chemical compositions.
Microtubule organization in a neuron, fibroblast, platelet, spindle and the motile axoneme of a ciliated protozoan. Dynamic (tyrosinated) microtubules shown in yellow; stable microtubules shown in red. The magnifying glass shows the enrichment in modifications on microtubule subpopulations: tyrosination (yellow), glutamylation or detyrosination (red), glycylation (cyan) and acetylation (magenta). We note that the abundance of these modifications on microtubules varies. For example, the abundance of glutamylated tubulin is higher in axonemes than in the axon, however comprehensive quantitative measures of modification levels on different microtubule subpopulations have yet to be undertaken.

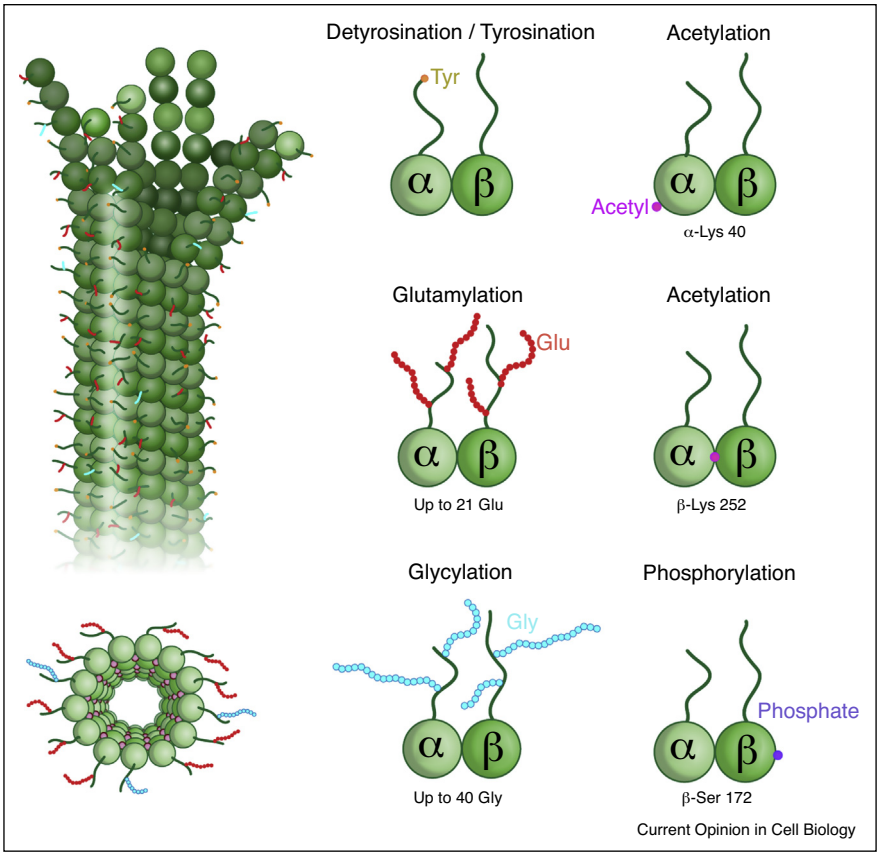
to recruit isoform-specific effectors *in trans*. We also do not know whether tubulin isoforms incorporate in precise patterns in microtubule arrays and whether such patterns matter for the recruitment of cellular effectors. A comprehensive understanding of tubulin isoform distributions in cells and tissues has been so far hampered by a scarcity of tubulin isoform-specific antibodies and difficulties with systematic tagging of tubulin isoforms in many organisms because of adverse effects on folding or microtubule function. The recent advances in producing recombinant tubulin isoforms [10••,11••,12] should catalyze the isolation of high-affinity affimers and antibodies with high specificity for different tubulin isoforms to facilitate such efforts.

Cells generate temporally and spatially regulated mosaic patterns of tubulin posttranslational modifications

Tubulin is further diversified through a large number of chemically diverse posttranslational modifications (reviewed in Refs. [13,14]). Together with tubulin isoforms they are thought to constitute an evolutionarily conserved 'tubulin code' that regulates intrinsic microtubule properties and their recognition by effectors. These modifications include acetylation of α and β-tubulin, phosphorylation of α and β-tubulin, polyamination, detyrosination/tyrosination (the removal and addition of the genetically encoded C-terminal tyrosine on α-tubulin), Δ2-tubulin (the removal of the last and penultimate glutamates in α-tubulin), glutamylation (the reversible addition of chains of glutamates of variable lengths to α or β-tubulin at internal glutamates in the C-terminal tails) and glycylation (the addition of chains of glycines of variable lengths to α or β-tubulin at internal glutamates in the C-terminal tails) (Figure 2). Mass spectrometric studies of tubulin isolated from cilia have identified varieties with as many as 21 glutamates [15] and 40 glycines [16], while tubulin isolated from brain tissue is functionalized with an average of 3–6 glutamates, with as many as eleven and seven detected on α-tubulin and β-tubulin, respectively [17]. To put these numbers in perspective, the C-terminal tails of tubulin that decorate the microtubule surface and to which these amino acids chains are added, range between 10–15 residues in length [18]. Thus, these posttranslationally added amino acid chains can drastically change the electrostatic properties of the microtubule surface and also modify the bulk properties of the cytoplasm around this polymer.

Tubulin posttranslational modifications vary widely between cell types and their patterns are stereotyped, indicative of roles in spatial and temporal control. For example, microtubules oriented towards a wound in a confluent cell monolayer are enriched in detyrosination, microtubules in the growth cone are tyrosinated, microtubules in the axon are predominantly glutamylated, detyrosinated, acetylated and enriched in Δ2-tubulin (reviewed in Ref. [13], references within), microtubules in the platelet marginal band are acetylated, detyrosinated and enriched in Δ2-tubulin [19], kinetochore

Figure 2



Microtubule structure and posttranslational modifications.
Left, microtubule structure showing tubulin C-terminal tails emanating from the microtubule surface and functionalized with various posttranslational modifications. The cross-section shows acetylated Lys40 in the microtubule lumen. Right, cartoon representation of αβ-tubulin heterodimers functionalized with chemically diverse posttranslational modifications.

microtubules are detyrosinated, astral microtubules are tyrosinated, midbody microtubules are detyrosinated, glutamylated and acetylated and microtubules in centrioles, cilia and flagella are acetylated as well as heavily glutamylated and glycylated (reviewed in Ref. [13], references within; Figure 1). Currently we do not understand how the cell achieves the selective enrichment of modifications on these subsets of microtubules. Early studies in cells revealed a correlation between microtubule lifetimes and posttranslational modifications: dynamic microtubules with lifetimes on the order of tens of seconds to minutes are enriched in tyrosinated tubulin while microtubules that persist for hours and are resistant to cold or drug-induced depolymerization are enriched in acetylated, detyrosinated and glutamylated tubulin [20–22]. These subsets of stable microtubules are used for endoplasmic reticulum sliding and are preferred sites of contacts between the endoplasmic reticulum and mitochondria [23]. They are also used for the selective transport of many viruses (reviewed in Ref. [24]). Axonemes offer the most dramatic example of specialization of closely positioned microtubule tracks with different posttranslational modifications. A recent elegant study using correlative light and cryo-electron microscopy of the *Chalmydomonas* flagellum revealed that the A-tubule, enriched in tyrosination, is dedicated to retrograde intraflagellar transport (IFT), while the B-tubule, which is enriched in detyrosination, is used for anterograde transport [25••]. A more recent study revealed that rapid depletion of tubulin glutamylation in the cilium through rapamycin induced targeting of the deglutamylase CCP5 preferentially impaired kinesin-2 anterograde IFT [26•]. This study also demonstrates the utility of the rapamycin dimerization system to interrogate the effects of acute changes in tubulin modifications in cells. The use of photo-regulated modules [27] will allow in the future the investigation of acute changes in modification levels with higher spatial resolution than those achievable with the rapamycin dimerization system.

Cis and *trans* regulation of microtubule functions by tubulin modifications

While the effects of tubulin posttranslational modifications on microtubule dynamic parameters have still yet to be quantitatively characterized, recent studies show that these modifications regulate the recruitment and activity of cellular effectors and can affect the mechanical properties of microtubules directly. Tyrosination regulates the recruitment of Clip-170 to microtubule tips [28]. It also recruits the motor dynein through its regulator dynactin which interacts directly with the α-tubulin tail and prefers its tyrosinated form over the detyrosinated one [29••,30•]. Tyrosination also enhances the depolymerization activity of kinesin-13 MCAK [31]. Detyrosination was recently shown to guide chromosomes towards the cell equator during mitosis by allowing the chromosome associated CENP-E motor to remain attached to microtubules for longer times when subjected to high loads. Disrupting the detyrosination pattern in the spindle caused the chromosomes to be transported in random directions from the spindle poles [32••]. A fascinating recent study reports that asymmetric tyrosination in the mammalian oocyte spindle allows preferential meiotic segregation [33••]. The asymmetric tyrosination is dependent on signaling by the small GTPase cdc42 from the oocyte cortex which increases tubulin tyrosination activity (or inhibits detyrosination) in the half-spindle close to the cortex compared to the half-spindle facing the egg cytoplasm. Detyrosination also regulates the interaction between sarcomeres and microtubules in cardiomyocytes and this interaction is mediated through desmin intermediate filaments [34••]. Microtubules provide mechanical resistance during cardiomyocyte contraction through their interaction with the sarcomere. Disrupting this interaction by decreasing detyrosination reduces stiffness and allows sarcomeres to contract further and faster. Notably, cardiomyopathy patients with increased tubulin detyrosination levels showed a more pronounced functional decline [34••], suggesting therapeutic interventions through the detyrosination/tyrosination cycle. Indeed, a recent study shows that cardiomyocytes from failing hearts show a dense network of detyrosinated microtubules that provide increased viscoelastic resistance and reduce contractility. A decrease in stiffness and partial recovery in contractile function can be achieved by inhibiting detyrosination with parthenolide or destabilizing microtubules with colchicine [35].

Glutamylation is ubiquitous in axons and flagella and *in vitro* studies show that this modification tunes the motility of motors that function in these compartments. For example, glutamylation results in modest increases in the run length of kinesin-1 and 2, but not that of cytoplasmic dynein [36]. Microtubule glutamylation controls the activity of microtubule severing enzymes spastin and katanin [37••,38,39]. *In vitro* studies using microtubules with quantitatively defined levels of glutamylation revealed a graded control of spastin microtubule severing by tubulin glutamylation. This mechanism has parallels to the one recently discovered for the actin severing protein cofilin where stereospecific oxidation of actin filaments by MICAL family proteins enhances the binding of cofilin and promotes rapid filament severing [40]. Notably, the microtubule binding affinity of spastin increases linearly with the number of glutamates added to tubulin [37••] indicating that glutamylation of the tubulin tails can function as a linear affinity tuner for cellular effectors. Such a graded modulation of microtubule binding affinity is likely at play for many microtubule associated proteins (MAPs) that depend on the C-terminal tails of tubulin for their binding to the microtubule, as hinted by early qualitative experiments using blot overlays [41].

Recent studies demonstrated for the first time that a tubulin posttranslational modification can affect intrinsic

properties of the microtubule polymer: acetylation of α-tubulin on Lys40, a residue situated in the microtubule lumen, was shown to mechanically stabilize long-lived microtubules and prevent their breakage in cells [42••]. Complementary *in vitro* experiments showed that acetylation in fact decreases the flexural rigidity of the microtubule, but increases its resilience to repeated deformation, possibly by weakening inter-protofilament contacts and allowing protofilaments to slide with respect to each other when the microtubule bends [43••]. Interestingly, acetylated microtubules are often highly curved [23], suggesting that they are subject to high compressive forces in cells. In the mechanosensory neurons of *Caenorhabditis elegans*, loss of the tubulin acetyltransferase produces microtubule structures that are splayed open [44], presumably because of their decreased resilience to the mechanical stress experienced by the worm during its sinusoidal movement. Intriguingly, molecular dynamics simulations proposed that acetylation at Lys40 favors the formation of a stabilizing salt bridge between α-tubulin subunits from neighboring protofilaments [44], offering a possible molecular explanation for the observed changes in mechanical properties. Since tubulin sequence variation also concentrates at polymerization interfaces (in addition to the tubulin C-terminal tails) and structures of microtubules with different tubulin isoform compositions show subtle structural variations at lateral interfaces between protofilaments [45•], it will be interesting to establish whether isoform composition impacts microtubule mechanics directly.

Tubulin posttranslational modifications also act on soluble tubulin, affecting its ability to incorporate into microtubules. For example, acetylation of β-tubulin on Lys 252 by the San acetyltransferase slows the kinetics of tubulin incorporation into microtubules by possibly affecting the intradimer interface [46]. Recent work shows that phosphorylation by the minibrain kinase MNB/DYRK1 of Ser172 in β-tubulin inhibits tubulin polymerization and affects dendrite morphology in *Drosophila* [47]. This residue is located at the tubulin interdimer interface. Proteomic studies reveal many other modification sites on the tubulin dimer whose physiological relevance is not yet clear.

Towards understanding and reconstituting complex tubulin modification patterns

Clearly cells expand a considerable portion of their genetic and energetic capital to regulate tubulin isoform and posttranslational diversity. However, we currently lack a comprehensive and mechanistic understanding of how tubulin isoforms are distributed in cells and how tubulin posttranslational patterns are generated and maintained. The length-scales of the mosaic blocks are still to be determined at high-resolution as well as the fidelity in generating patterns. For function, is only the enrichment of a particular type of modification or isoform important on a microtubule or set of microtubules or does the precise patterning of the mosaic blocks matter? The complex microtubule modification patterns observed in cells result from the interplay between microtubule dynamics and the subcellular localization, substrate specificity and kinetics of the tubulin modification enzymes. Kinetic control that is the intersection between the turnover rates of the microtubules themselves and the catalytic rates of the modification enzymes, is likely an important mechanism for generating complex spatial patterns. Interestingly, the catalytic rates of tubulin modification enzymes in isolation span a broad range, from sec^{-1} in the case of the tubulin tyrosine ligase TTL [48], min^{-1} in the case of the glutamylase TTLL7 [49] and the glycylase TTLL3 [50], to $hours^{-1}$ in the case of the tubulin acetyltransferase [51,52], and thus can selectively modify microtubules with a broad range of turnover rates as previously hypothesized [53]. In this scenario, an external factor (a MAP or anchoring by a motor to the plasma membrane or kinetochore, for example) acts as the initial stabilizing cue for the microtubule, thus prolonging its lifetime to match that of a modification enzyme. The introduced posttranslational modification can then further reinforce the initial stabilizing cue by specifically recruiting cellular effectors and/or directly affecting the intrinsic stability of the polymer. To make matters more complex, microtubule severing enzymes have been shown to remodel the microtubule lattice by promoting the exchange of lattice GDP-tubulin with that from the soluble GTP-tubulin pool *in vitro* [54]. In addition to introducing GTP islands in microtubules, this mechanism could potentially also edit locally the posttranslational modification status of the lattice by exchanging tubulin with different modifications or tubulin isoforms.

To understand the mechanisms used by cells to selectively modify microtubules will require first their characterization at high spatial and temporal resolution in various cell types to compare and contrast patterns with enzyme expression and localization. *In vivo* perturbation analysis by manipulating modification enzymes and their regulators will have to go hand in hand with *in vitro* reconstitution with physiologically relevant tubulin isoform mixtures. Can complex spatial distributions of modified microtubules arise *ex nihilo* from the specificity, kinetics and dynamics of the modification enzymes and microtubules alone? Or do they depend on a pre-existing scaffold generated through nucleation and motor and MAP-dependent microtubule organization? *In vitro* microtubule assays utilize a nucleating structure, in many cases a stabilized GMPCPP microtubule seed, to grow single microtubules without higher order organization. While these experimental setups have been instrumental in studying the basic properties of microtubule regulators, they do not capture the geometric constraints of microtubule effectors in cells. The continuous advancements in micropatterning makes the creation of more

physiologically relevant microtubule geometries in confined environments possible [55]. Wider adoption of these technologies coupled with advances in the biochemical characterization of the modification enzymes and the signaling cascades that regulate them may get us closer to recapitulating *in vitro* the chemical and geometric diversities of cellular microtubule arrays.

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Conflict of interest statement

Nothing declared.

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