MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments

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MAP2 and tau exhibit microtubule-stabilizing activities that are implicated in the development and maintenance of neuronal axons and dendrites. The proteins share a homologous COOH-terminal domain, composed of three or four microtubule binding repeats separated by inter-repeats (IRs). To investigate how MAP2 and tau stabilize microtubules, we calculated 3D maps of microtubules fully decorated with MAP2c or tau using cryo-EM and helical image analysis. Comparing these maps with an undecorated microtubule map revealed additional densities along protofilament ridges on the microtubule exterior, indicating that MAP2c and tau form an ordered structure when they bind microtubules. Localization of undecagold attached to the second IR of MAP2c showed that IRs also lie along the ridges, not between protofilaments. The densities attributable to the microtubule-associated proteins lie in close proximity to helices 11 and 12 and the COOH terminus of tubulin. Our data further suggest that the evolutionarily maintained differences observed in the repeat domain may be important for the specific targeting of different repeats to either α or β tubulin. These results provide strong evidence suggesting that MAP2c and tau stabilize microtubules by binding along individual protofilaments, possibly by bridging the tubulin interfaces.

Introduction

The microtubule cytoskeleton plays a fundamental role in a variety of cellular processes. Microtubules assemble by lateral association of protofilaments generated from head to tail polymerization of αβ tubulin dimers. The tendency of tubulin to switch stochastically between polymerization and depolymerization phases, termed dynamic instability (Mitchison and Kirschner, 1984), facilitates remodeling of the microtubule cytoskeleton for its various roles. Microtubule-associated proteins (MAPs) influence dynamic instability by binding and stabilizing microtubules (Cleveland et al., 1977). The MAP2/tau family is a unique class of structural MAPs that modulate microtubule dynamics in neurons during the development of dendrites and axons (for reviews see Drewes et al., 1998; Goldstein and Gunawardena, 2000). Phosphorylation of MAP2/tau proteins at specific sites induces dissociation from microtubules (Drewes et al., 1997; Ozer and Halpain, 2000). However, hyperphosphorylation and/or specific mutations can promote aggregation of the dissociated tau into paired helical filaments, which are a hallmark of certain neurodegenerative diseases such as familial tauopathies and Alzheimer’s disease (Crowther and Goedert, 2000; Garcia and Cleveland, 2001).

MAP2/tau proteins have dissimilar NH2-terminal “projection” domains and homologous COOH-terminal microtubule binding domains. Studies investigating the cytoskeleton of neuronal processes showed that microtubules with MAP2 or tau bound are organized in parallel arrays in which the spacing between microtubules correlates with the length of the projection domains of the MAP (Hirokawa, 1982; Chen et al., 1992). Rotary shadowing experiments and circular dichroism studies on isolated MAP2/tau proteins indicate that they are highly extended polypeptides with little or no detectable secondary structure (Voter and Erickson, 1982; Schweers et al., 1994).

The MAP2/tau microtubule binding domain contains three or four 18-residue microtubule binding repeats (MTBRs) separated by 13–14-residue inter-repeats (IRs) (Lewis et al., 1988; Himmler et al., 1989). Neighboring MTBRs within a given MAP share moderate homology; however, sequence alignment shows that repeats at identical positions in different MAP2s and taus are highly conserved (Fig. 1 A). There is evidence that IRs as well as MTBRs con-
The prevailing model to explain how MAP2/tau family proteins interact with microtubules presumes that each MTBR-IR module interacts with a separate, but adjacent, tubulin monomer within the microtubule wall (Butner and Kirschner, 1991; Gustke et al., 1994). Despite considerable study, the structural basis for MAP2/tau stabilization of microtubules is not understood. It remains unclear whether increased microtubule stability is achieved by MAPs binding along protofilaments (a longitudinal binding model) or wrapping around the microtubule (a lateral binding model). As microtubule disassembly proceeds by protofilament separation and curling outwards at the ends of the microtubule (Mandelkow et al., 1991), longitudinal binding could account for increased microtubule stability by strengthening tubulin interactions along protofilaments and preventing outward curling. Alternatively, in the lateral binding model, the wrapped MAPs would prevent protofilament separation (Ichihara et al., 2001).

Here we have used cryo-EM and helical image analysis to determine the geometry of MAP2c- and tau binding to microtubules. We show that MAP2c- or tau-decorated microtubules have additional ordered density along protofilament ridges compared with undecorated microtubules. We used undecagold labeling to show that the IRS lie along the ridges and not between protofilaments. The gold labeling data suggest that the MTBR-IR modules may be uniquely targeted to α or β tubulin. Taken together, our results suggest that MAP2 and tau proteins reduce microtubule depolymerization by bridging and stabilizing the tubulin–tubulin interfaces along protofilaments.

Results
Cryo-EM of MAP2c and tau-decorated microtubules
To investigate how MAPs stabilize microtubules, we have analyzed two distinct proteins using cryo-EM and helical image analysis: a recombinant three-repeat rat MAP2c (termed MAP2c throughout) and a recombinant four-repeat human tau (termed tau throughout). The MAP2c and tau proteins are homologous in their repeat domain, but tau includes an additional MTBR-IR module (Fig. 1 A; see Materials and methods).

We used cosedimentation assays to determine conditions for full decoration of microtubules by MAP2c and tau. Under saturating conditions, MAP2c bound microtubules at a ratio of one molecule for every 2.4 tubulin monomers (Fig. 1 B, I), and tau at one molecule for every 3.8 tubulin monomers (Fig. 1 B, II). These stoichiometry values are very similar to those determined by others (Gustke et al., 1994; Coffey and Purich, 1995). We used the conditions found for maximal binding to prepare decorated microtubules for cryo-EM.

Images of microtubules decorated with either MAP2c or tau were visually indistinguishable from those of undecorated microtubules (Fig. 2 A). Both the diameter and moiré (super-twist) repeat length of decorated 15-protofilament helical microtubules were similar to those of undecorated microtubules (Fig. 2 B), indicating that binding of MAP2c or tau does not induce large changes in the microtubule structure.
structure. Although individual images of decorated and undecorated microtubules look similar, differences were evident after averaging the layer line data from a number of images. In particular, layer line 1 amplitudes were stronger for decorated microtubules (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200201048/DC1) with minor changes in other layer lines, consistent with an increase in protofilament density. There were no additional layer lines in the decorated microtubules, as would be expected if the MAP projection domains were contributing to off-equatorial diffraction (Amos, 1977).

**MAP2 and tau are ordered on microtubule protofilaments**

From the averaged layer lines, we calculated 3D maps of MAP2c-decorated, tau-decorated, and undecorated microtubules. All three maps show the same general features as those previously reported for microtubules (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200201048/DC1) with minor changes in other layer lines, consistent with an increase in protofilament density. There were no additional layer lines in the decorated microtubules, as would be expected if the MAP projection domains were contributing to off-equatorial diffraction (Amos, 1977).

**MAP2c and tau form ordered densities protofilament ridges.** (A) En face view of the undecorated microtubule map showing four protofilaments. A tubulin monomer is outlined by a dotted line. Bar, 300 Å. (B) Number of moiré repeats and asymmetric units contributing to the final data are listed together with average microtubule diameter and average moiré length (in angstroms) for each dataset.

| Data Sets | Repetes | Asymmetric units | Diameter | Moiré length |
|-----------|---------|-----------------|----------|--------------|
| MTs       | 285     | 220±2.4         | 159±184  |
| MAP2c-MTs | 194     | 221±3.3         | 158±284  |
| tau-MTs   | 129     | 223±3.3         | 160±203  |

The binding of MAPs to microtubules does not induce changes to their architecture. (A) Images and power spectra (FFT) of MAP2c-decorated microtubules (MAP2c-MT), tau-decorated microtubules (tau-MT), and undecorated microtubules (MT) are visually indistinguishable. Only one half of each power spectrum (FFT) is shown and it is compressed 16-fold in the equatorial direction. Bar, 300 Å. (B) Number of moiré repeats and asymmetric units contributing to the final data are listed together with average microtubule diameter and average moiré length (in angstroms) for each dataset.

Figure 2.

Figure 3.

**MAP2c and tau proteins bind longitudinally along protofilaments**

To distinguish between lateral and longitudinal binding geometries, we used undecagold labeling to locate IR-3/4 of MAP2c. The single natural cysteine (Cys 348) present in MAP2c was first replaced by a serine, thereby generating a cysteine-free MAP2c (cf-MAP2c). This mutant protein
bound microtubules with the same stoichiometry as MAP2c (Fig. 4 A). We then introduced a cysteine in place of lysine 364 (Fig. 1 A, asterisk) in IR-3/4 of cf-MAP2c generating a cysteine-IR-MAP2c mutant (cIR-MAP2c), which we conjugated to undecagold (Au_{11}) (Milligan et al., 1990; Safer, 1999; Rice et al., 1999). We confirmed that neither the mutation nor Au_{11} attachment interferes with cIR-MAP2c binding to microtubules (Fig. 4 B). The 3D map calculated from Au_{11}–cIR-MAP2c–decorated microtubules showed an additional knob-like density on the microtubule surface. Difference mapping and statistical analysis showed that this density was statistically significant at the P/H110050.001 level (Fig. 5 and Fig. S3, E and F).

These experiments allow us to distinguish between the longitudinal and the lateral binding models by localization of the undecagold and by inference the IR-3/4 to which it is attached. The two possible positions of the IR–Au_{11} would either be on the protofilament ridges, if MAP2c binds microtubules longitudinally (Fig. 5 A, I), or in the valleys between protofilaments, if it binds microtubules laterally (Fig. 5 A, II). The undecagold difference map clearly indicates that IR-3/4 lies on top of the MAP2c difference densities along the protofilament ridges (Fig. 5 A, III). Thus, the IRs lie between bound MTBRs along the ridges (Fig. 5 A, III, arrows), consistent with a longitudinal binding model.

The repeat domain recognizes \( \alpha \) and \( \beta \) tubulins uniquely

Unexpectedly, we observed an additional degree of specificity in the Au_{11}–IR-3/4 localization. As \( \alpha \) and \( \beta \) tubulins are indistinguishable at the resolution of this study, either tubulin monomer (40-Å repeat) or dimer symmetry (80-Å repeat) may be imposed during averaging. With longitudinal binding and nonspecific MTBR-IR module attachment to \( \alpha \) or \( \beta \) tubulins, image analysis and averaging would yield an undecagold difference peak at every tubulin monomer irrespective of whether monomer or dimer symmetry is applied (Fig. 5 B, II). On the other hand, if MTBR-IR modules uniquely target \( \alpha \) or \( \beta \) tubulin, the undecagold difference peak will only be visualized when dimer symmetry is imposed (Fig. 5 B, I). The repeat domain recognizes \( \alpha \) and \( \beta \) tubulins uniquely.
We analyzed our data imposing either monomer or dimer symmetry and were only able to visualize the undecagold when we used dimer symmetry (Fig. 5B, III). These data strongly argue in favor of specific binding of IR-3/4 to either α or β tubulin, but not to both (Fig. 5B, III).

The repeat domains bind helix 11 (H11), helix 12 (H12), and the COOH termini of tubulin

Although there is considerable evidence showing that MAP2 and tau are unstructured in solution (Voter and Erickson, 1982; Schweers et al., 1994), the data presented here show that they form ordered densities along the protofilament ridges when they bind microtubules. Thus, it seems likely that specific residues on the protofilament surface are required to fold the MTBR-IR modules and induce the bound density that we observe. To investigate which structural elements on the tubulin surface are likely to induce the IRs and MTBRs to fold, we manually docked the atomic coordinates of the αβ tubulin dimer into the microtubule map and displayed the result with the MAP2c and undecagold difference maps. As the tubulin COOH termini (18 and 10 residues for α and β tubulins, respectively) are disordered and not resolved in the structure (Nogales et al., 1998), we modeled these parts of the molecule to show where they exit the protofilament surface (Fig. 6A).

MAP2c difference densities are closely apposed to helices 11 (H11) and 12 (H12), which are the prominent structural elements of tubulin exposed on the outer surface of microtubules. The last resolved residues of the tubulin COOH terminus are positioned directly below the MAP2c difference map (Fig. 6A).

The undecagold difference map lies directly over the tubulin–tubulin interface close to the COOH-terminal end of H11 (Fig. 6B and C). This implies that the IR, to which the undecagold is attached (Fig. 1A, asterisk), also binds to the interface, the region of the structure that has been shown to directly modulate the protofilament conformation in growth and catastrophe (Mandelkow et al., 1991; Nogales et al., 1999; Gigant et al., 2000). In light of this localization of the IR, the MTBR portion must lie centrally on the tubulin monomer in close proximity to the end of H12 and the exit site of the tubulin COOH terminus. It is attributable to the MTBR and the tubulin COOH terminus (red dotted lines).

Discussion

The binding of each 31–32-residue MTBR-IR module of the MAPs to a single tubulin monomer is supported by our data on the stoichiometry of binding as well as peptide and
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IRs bridge the tubulin interfaces. The MAP2c projection domain is not has a specific orientation with respect to the tubulin dimer and the Ludin et al., 1996). In both binding geometries, the repeat domain and there are gaps (one unbound tubulin monomer) between adjacent MAP2c molecules. In III, only two of the MAP2c repeats are bound and there are gaps between successive MAP molecules along the protofilament. (Fig. 7 II), and one in which only two of the three MTBR-IR repeats are attached (Fig. 7 III). This model satisfies both the observed stoichiometry and our data on specific targeting.

MAP2 and tau may stabilize protofilaments by bridging tubulin interfaces

Our data support a previously proposed model that longitudinal binding of the MAP2/tau proteins to protofilaments leads to microtubule stabilization by bridging the tubulin interfaces (Dye et al., 1993). It is likely that the longitudinal MAP2/tau binding stabilizes a specific conformation of the tubulin interdimer interface. The conformation of this interface depends on the state of guanosine nucleotide in β tubulin. The GTP-bound state mediates straight interdimer interfaces and maintains straight protofilaments (Muller-Reichert et al., 1998). In the absence of the GTP cap, the GDP-bound state leads to curved protofilaments (Gigant et al., 2000), which promote microtubule catastrophe. By bridging tubulin–tubulin interfaces axially, we suggest that MAP2/tau binding along protofilaments may stabilize a straight GDP conformation and produce a cumulative effect along their length. Furthermore, longitudinal binding is consistent with evidence showing that the binding of MAP2c and tau decreases flexibility of microtubules (Dye et al., 1993; Felgner et al., 1997). In contrast, taxol-induced stabilization slightly increases microtubule flexibility (Mickey and Howard, 1995; Felgner et al., 1997). This distinction may underlie the observed differences between the action of taxol and MAP2/tau proteins in vivo (Spero et al., 1985; Leclerc et al., 1996).

The effect of MAP2/tau on stabilizing straight protofilaments by longitudinal binding is consistent with their observed effects on microtubule dynamics. At low concentrations, MAP2/tau proteins decrease the frequency of catastrophes and dramatically increase the frequency of rescues (Joly et al., 1989; Kowalski and Williams, 1993; Gam-
blin et al., 1996). Furthermore, rescues tend to occur at regions in microtubules where MAP2 and tau proteins are bound, suggesting that the protofilaments are less likely to curl at such regions (Ichihara et al., 2001). Below the critical concentrations of tubulin, MAP2/tau proteins inhibit microtubule catastrophes and lead to “stalled” microtubule ends, which do not shrink or grow (Panda et al., 1995).

At high concentrations, MAP2/tau proteins moderately increase the rate of tubulin polymerization and slightly lower its critical concentration (Drechsel et al., 1992), effects that resemble the consequences of subtilisin cleavage of the tubulin COOH termini or increasing the divalent cation concentration. These “charge-shielding” effects enhance polymerization presumably by altering the conformation or charge of the tubulin COOH termini at the growing ends (Wolff et al., 1996). We suggest that the latter effects are distinct from those observed on microtubule dynamics, because they are observed at a different concentration range (Drechsel et al., 1992). The effects on microtubule dynamics were observed with low MAP2 or tau concentrations, whereas effects on the polymerization rate at such concentrations were negligible (Gamblin et al., 1996). Microtubule flexibility also decreased when MAP2c and tau bound to microtubules at low concentrations (Felgner et al., 1997). The convergence of the effects on microtubule dynamics and flexibility at similar concentrations of bound MAPs suggests that both are a result of the MAPs acting on the protofilament conformation.

**MTBRs bind tubulin COOH termini and H12, whereas IRs bind H11**

We observed the MTBR peaks in the MAP2c difference map to lie along H12. These MTBR peaks contain higher density compared with neighboring regions in the MAP2c difference map and colocalize with the tubulin COOH termini exit sites. Thus, they are likely to be composed of MTBRs and the tubulin COOH termini, both of which presumably become ordered upon binding. This evidence is consistent with NMR data suggesting that MTBR peptides fold when they bind microtubules, interacting with tyrosines within H12 and the tubulin COOH terminus (Kotani et al., 1990). The Au11 localization indicates that IRs are bound to the end of H11, suggesting that the sequences of the IRs, not just their lengths (as suggested by Butner and Kirshner, 1991), are crucial for repeat domain function. A combination of recent mutagenesis, peptide, and cross-linking studies has suggested that IR sequences contribute to the microtubule affinity and stabilizing activity of the repeat domain (Goode and Feinstein, 1994; Chau et al., 1998; Goode et al., 2000). Our data are consistent with the idea that IRs and MTBRs bind at adjacent sites and that both are required for stabilizing protofilaments (Fig. 7).

We hypothesize that both IR and MTBR binding to H11 and H12 would influence the conformation of longitudinal tubulin interfaces via the H11-H12 loop. The H11-H12 loop participates in the outer region of the longitudinal tubulin interfaces (Nogales et al., 1999). There are two possible explanations for how the MTBR-IR binding arrangement on tubulins might lead to increased stability at the interfaces. First, MTBR-IR binding to H12 and H11 may possibly influence the flexibility of the intervening loop, in turn affecting the longitudinal interface. Second, the MTBR-COOH terminus complex could act as a physical wedge that stabilizes tubulin interfaces by preventing the straight to curved protofilament conformation. Validation of either possibility must await a high-resolution structure of the MAP2/tau–microtubule complex.

**MTBR-IR modules specifically target α or β tubulins**

The specific targeting of MAP2c to αβ tubulin dimers is suggested by the localization of Au11–IR-3/4 near only one of the two possible tubulin–tubulin interfaces. As we cannot distinguish α and β tubulin in our 3D maps, we are unable to say whether the IR-3/4 is at the interdimer interface or at the intradimer interface. However, our results suggest that IRs bind to either β–α (interdimer) or α–β (intradimer) regions. Such specific targeting is consistent with the evolutionarily maintained divergence between neighboring IRs and MTBRs (Fig. 1 A). It is notable that many charged residues that lie on the surfaces of H11 and H12 are different in α and β tubulin and this distribution is highly conserved across species (Villasante et al., 1986; Wang et al., 1986; Nogales et al., 1998, 1999). Specificity in targeting requires such differences in the residue distribution on α and β tubulins and in different MTBRs and IRs within the repeat domains.

**Comparison with kinesin–microtubule interactions**

As suggested here for MAPs, the kinesin motor domain effectively distinguishes between α and β tubulin, because only one motor binds per tubulin heterodimer (Sosa et al., 1997). Specific targeting of α or β tubulin is attained by both kinesin and MAP2c using completely different microtubule binding domains. Furthermore, a class-specific lysine-rich insertion in KIF1A motor domain (termed the K-loop) binds the β tubulin COOH terminus, forming an ordered complex that was observed at medium resolution (Kikkawa et al., 2000), suggesting that the COOH termini induce K-loop folding in the KIF1A. This phenomenon, ordering of the COOH termini and K-loop, is analogous to what we suggest is occurring with the MTBRs in MAP2c. Finally, the overlap between MAP and kinesin binding sites on the protofilament surface suggests that steric interference would occur between these two classes of molecules when both attempt to bind microtubules simultaneously. Such interference probably accounts for the observed effects of MAPs in inhibiting kinesin-based motility, observed in vivo (Ebneth et al., 1998; Trinczek et al., 1999). Thus, the ordered longitudinal binding of MAPs along the outside of the protofilament ridges not only stabilizes microtubules, but also may regulate kinesin-based motility along microtubules.

**Materials and methods**

**Preparation and mutagenesis of recombinant tau and MAP2c**

The 467-residue three-repeats MAP2c (MAP2c) and the 441-residue four-repeat human tau (tau) cDNAs were inserted in PET3a and PET30 expression vectors as previously described (Lewis et al., 2000; Lim and Hallpain, 2000). Proteins were expressed according to previously published methods (Gamblin et al., 1996). MAP2/tau-containing extracts were dialyzed into 50 mM sodium acetate, pH 5.5, 1 mM EDTA, 1 mM EGTA, and 1 mM DTT (dialysis buffer). The dialyzed extracts were loaded onto a HiTrap SP column preequilibrated with 10 vol of dialysis buffer, and washed...
with 5 vol of dialysis buffer. Protein was eluted with a continuous (50–700 mM) NaCl gradient. Fractions eluting at 250 mM NaCl were analyzed by SDS-PAGE and then pooled. Each purified full-length protein fraction was dialyzed against 50 mM Tris, pH 7.6, 1 mM EGTA, 1 mM DTT at 4°C, divided into small aliquots, and stored at −80°C.

To generate a cysteine-free mutant of MAP2c, cysteine 348 in MAP2c R3 (Fig. 1 A) was mutated to serine (cf-MAP2c) by site-directed mutagenesis using the primer 5′-GTCTTTAGAGAGCCAGATTTGGAAGTC-3′ (Strategene) with the previously published orientation (Nogales et al., 1999). The primer 5′-GTCTTTAGAGAGCCAGATTTGGAAGTC-3′ and its complementary strand (Operon). All point mutagenesis steps were confirmed by DNA sequencing. The cf-MAP2c and cIR-MAP2c mutants were bacterially expressed and purified as described above.

**Microtubule binding assays**

Microtubules were polymerized by incubating phosphocellulose purified bovine tubulin (Cytoskeleton Co.) at 5 mg/ml in 80 mM Pipes, pH 6.8, 4 mM MgCl2, 1 mM EGTA, 6 mM GTP, 8% DMSO, and 50 mM Taxol for 20 min at 34°C. Polymerized microtubules were then diluted to 0.5 mg/ml into binding buffer (50 mM Pipes, pH 7.6, 1 mM MgCl2, 1 mM EGTA) containing various concentrations of freshly dialyzed MAP2/tau proteins. MAP2/tau proteins with and without microtubules were incubated at 30°C for 1 h to induce microtubule binding, and then spun at 100,000 g for 10 min. Supernatants and pellets were then analyzed on SDS-PAGE. Molar ratios of tubulin monomer to MAP2/tau proteins were determined by scanning gel bands using a personal densitometer (Molecular Dynamics) equipped with ImageQuant software.

**Undecagold cluster labeling**

Monomaleimide undecagold clusters were synthesized and attached to Cys 364 of cIR-MAP2c as previously described (Safer et al., 1986; Safer, 1999). We used a ratio of 10 mol of activated undecagold to 1 mol of cIR-MAP2c to ensure full labeling. The extent of labeling was confirmed by comparing protein concentration determined by SDS-PAGE to undecagold cluster concentration determined by its absorbance at 420 nm (undecagold extinction coefficient ε = 470 M⁻¹ cm⁻¹).

**Sample preparation for electron microscopy**

Microtubule-saturating MAP2/tau protein concentrations were determined by microtubule binding assays as described above. The MAP2/tau saturated microtubule mixtures (4–5 μl aliquots) were then applied to glow discharged 400-mesh Quantifoil grids with uniform 2–3 nm hole carbon support films (Signal Probe Co.). After 2 min, the grids were washed with binding buffer containing MAP2/tau protein, which prevents the dissociation of bound MAP2/tau protein, blotted, and frozen by rapidly plunging them into liquid ethane slush (Duboc et al., 1988). Frozen grids were stored under liquid nitrogen.

**Cryo-EM and helical image analysis**

A Gatan cryo-stage was used for transfer and observation of frozen grids in a Philips CM200 TWIN electron microscope. Electron micrographs were recorded under low-dose conditions (<10 e/Å² total dose) at an operating voltage of 120 kV and 40,000 nominal magnification. Images of 15-protofilament, two-start helical microtubules (assuming tubulin dimer symmetry) were chosen for image analysis (Sosa et al., 1997). Selected micrographs were digitized on a flatbed microdensitometer (PDS 1010G; Perkin-Elmer Corp.) with spot and step sizes equivalent to 4.97 Å at the specimen. The digitized images were analyzed by standard helical reconstruction procedures (DeRosier and Moore, 1970) on Silicon Graphics workstations using the program software package PHOELIX (Whittaker et al., 1995; Carragher et al., 1996). An integral number of microtubule moiré repeats (three to eight) were masked off and Fourier transforms were calculated. Near- and far-side layer lines with Bessel orders up to 19, 35; (19, 35), 21, 53; (21, 53), 17, 17); (17, 17), 21, 53; (21, 53), 28, 20; (28, 20), −19, 35; (−19, 35), 36; (11, 37), 26, 38; (−21, 53); (6, 54), 9, 55; (24, 56) showed 3D maps of MAP2c-decorated, tau-decorated, and undecorated microtubules. Fig. S3 shows the MAP2c, tau, and undecagold statistical difference maps. Online supplemental materials are available at http://www.jcb.org/cgi/content/full/jcb.20021048/DC1.

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