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1 **Architecture-Encoded Mechanics and Communication in** 2 **Microtubules: A Multiscale Computational Study**

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11 **Abstract**

12 The mechanical architecture of microtubules (MTs) is crucial for modulating their functions within
13 cells; however, the effect of varying the number of protofilaments (PF) on the propagation of
14 mechanical signals remains largely unexplored. Nevertheless, MTs assembled in vitro exhibit diverse
15 PF numbers depending on the specific tubulin composition, stabilising agents, and cellular context,
16 suggesting a regulated architectural adaptation. Here, we performed a multiscale computational study
17 integrating molecular dynamics, dynamical network analysis, and elastic network modelling to
18 investigate the influence of the MT architecture on structural communication and mechanics. Our
19 results highlight that an increase in PF number alters tubulin-tubulin contact patterns, reshapes lateral
20 surface hydrophobicity, and modulates the dynamics of a specific unstructured region known as the M-
21 loop. Remarkably, we identified a correlation between the PF number, vibrational path length, and
22 bending stiffness, revealing that MTs with larger architectures propagate mechanical information less
23 efficiently, but offer increased structural support. These findings suggest that MT architecture may serve
24 as a design parameter influencing the propagation of mechanical signals across scales. Moreover, they

25 may contribute to the emerging field of neuromechanobiology, where MTs are considered potential
26 integrators of mechanical and informational processes within neurons.

27 **Keywords**

28 microtubules, cytoskeleton mechanics, protofilaments, ENM, protein network modelling, molecular
29 dynamics

30

31 **Introduction**

32 Microtubules (MTs) are hollow tubular assemblies composed of several laterally coupled
33 protofilaments (PFs), which can vary in number based on the tubulin isotype ¹, stabilising agents ^{2,3},
34 cell type, organism, and post-translational modifications ^{4,5}.

35 MTs play essential roles in cell structure, transport, and mechanotransduction, and their architecture is
36 closely linked to these functions. For instance, a recent study showed that the distribution and
37 architecture of MTs change during the life span of *Plasmodium falciparum*, the malarial parasite ⁶,
38 reinforcing the idea that MT architecture is a regulated variable that adapts to cellular needs. In
39 particular, the authors observed how the parasite expresses giant 18PF MTs when it takes an extended,
40 rigid shape. This evidence, coupled with results from investigations of 15PF-shaped MTs in
41 mechanosensory cells ⁴, corroborates the hypothesis that large MTs are stiffer than the canonical 13PF
42 lattice, and can provide structural support for specific cells ⁷. The functional significance of PF
43 architecture is also evident in ciliary axonemes, which are specialized structures found in cilia and
44 flagella whose wall is composed of nine MT doublets, each consisting of a complete 13PF A-tubule and
45 a partial 10PF B-tubule, with a central microtubule pair usually composed of two 13-PF microtubules⁸⁻
46 ¹⁰. This organization ensures both structural stability and mechanical efficiency: the 13PF A-tubule
47 provides a rigid scaffold for anchoring dynein arms and other axonemal complexes, whereas the 10PF
48 B-tubule economizes tubulin usage and contributes to the flexibility required for axonemal beating^{8,9}.

49 In addition, protofilament-specific patterns of tubulin post-translational modifications further fine-tune
50 the mechanical properties of axonemal MTs and regulate their interactions with motor and regulatory
51 proteins, thereby directly influencing ciliary motility^{9,11}. These examples demonstrate how cells control
52 the architecture of their MTs according to specific functional requirements, with larger MTs generally
53 providing enhanced structural support and stiffness, and smaller MTs providing increased flexibility.

54 Despite the recognised variability in PF numbers and their potential impact on cellular functions^{1,12,13},
55 the exact mechanisms by which these variations influence MT mechanics and signal propagation remain
56 unclear¹⁴⁻¹⁶. Most research has concentrated on specific MT states or geometries, lacking a
57 comprehensive analysis that directly compares how different architectures affect the mechanical

58 behaviour across scales. This question is particularly relevant in the context of neuromechanobiology,
59 an emerging field that investigates how mechanical forces and cytoskeletal dynamics contribute to
60 neuronal signalling¹⁷⁻¹⁹, development²⁰, and cognition^{21,22}.

61 In this context, multiscale modelling presents a unique opportunity to bridge the gap between molecular
62 interactions within MTs and their macroscopic mechanical properties, allowing for a detailed
63 exploration of how variations in the number of PFs impact MT function.

64 In the present work, we quantitatively investigate how the MT architecture is linked to its mechanical
65 behaviour through a multiscale computational framework that combines molecular dynamics
66 simulations, dynamic network analysis, and elastic network modelling. Specifically, to cover a wide
67 range of MT sizes including both common MT lattices and rare, specialized assemblies, we focused our
68 study on the 11PF assembly, a specialized structure found e.g. in nematodes and plants⁴, the 13PF
69 assembly, which is the most prevalent lattice in eukaryotic cells²³, and the 16PF assembly, a large and
70 highly specialized MT lattice occurring in protists and insects⁴. This allowed us to span a wide range of
71 PF numbers – and, thus, MT sizes – in our investigation, while also relying on available electron
72 microscopy density (EMD) maps²⁴, which enabled us to reconstruct representative models of MTs
73 characterised by a different numbers of PF starting from experimental data.

74 We recently applied molecular dynamics (MD) simulations to study tubulin-tubulin interactions within
75 the MT lattice^{25,26}, providing a detailed view of the local structural and communication dynamics. In
76 addition, we have previously used elastic network modelling to estimate the mechanical properties of
77 cytoskeletal filaments²⁷⁻²⁹, which agrees with recent experimental studies on microtubule deformation
78 mechanics³⁰. Herein, we investigate MTs with different numbers of PFs to investigate how MT
79 architecture, tubulin-tubulin interactions, and MT mechanics are interlinked.

80 Our results highlight that changes in the MT wall curvature can be associated with differences in the
81 lateral interaction surface and in its hydrophobicity. Moreover, increasing the number of PFs increases
82 the bending stiffness of the assembly, which is consistent with previous hypotheses⁷.

83 Our study advances our current understanding of how MT architecture influences its mechanical
84 properties, with potential implications for understanding how cells adapt MT functions to meet various
85 physiological demands.

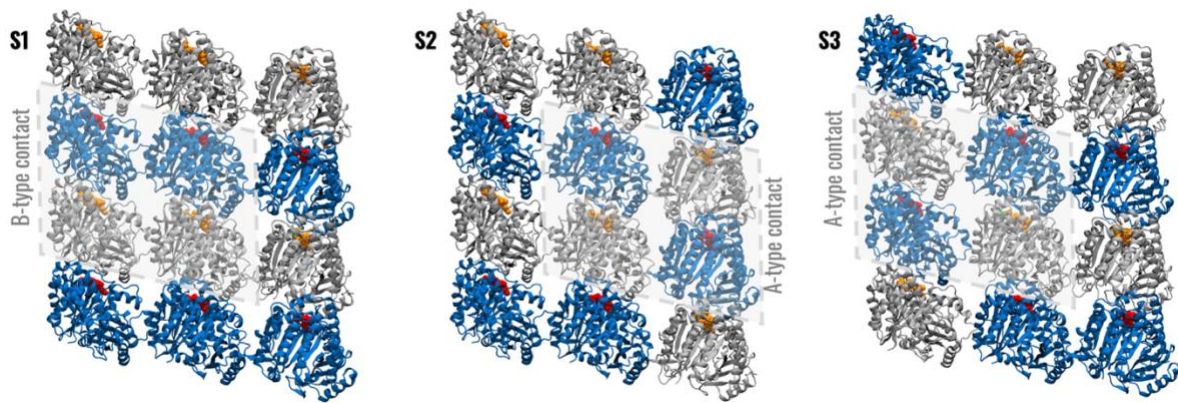
86 **Methods**

87 **Preparation and simulation of the models**

88 To study tubulin dynamics within MT sheets representative of different lattices, we started from our
89 previously published methodology^{25,26}. In greater detail, we used Protein Data Bank structure 3J6F³¹
90 as a template to construct a 3x4 GDP-bound patch of human α Ib- β III tubulin dimers using homology
91 modelling, as performed in an earlier study of our research group²⁵ and further illustrated in the
92 Supporting Information. This molecular configuration was regarded as representative of a 13 PF MT
93 wall and will be referred to as MT^{13PF}. To model MT sheets at 11 and 16 PFs, the curvature of this initial
94 MT sheet has been adapted using the ChimeraX software³² and available EMD maps²⁴ (accession
95 codes EMD-5191 for the 11:3 lattice and EMD-5196 for the 16:4 lattice). In detail, first the whole
96 MT^{13PF} structure has been fit as a rigid body into the EMD maps. Subsequently, the three PFs have been
97 independently fit into the EMD map. The obtained systems will be referred to as MT^{11PF} and MT^{16PF}
98 and represent the initial structure of 11 and 16PF MT patches. Following the same naming convention
99 and rationale used in our previous works^{25,26}, we will distinguish between S1, S2 and S3 systems. S1
100 systems represent MT patches far from the MT seam, i.e. in which the tubulin dimers of the different
101 PFs are laterally aligned. S2 and S3 systems instead refer to MT patches representing the MT seam
102 region, i.e. where one PF (the rightmost in system S2, the leftmost in system S3) is offset by one tubulin
103 subunit with respect to the other two (see Figure 1).

104 In the case of the MT^{11PF}, the S2 and S3 systems have been modelled following the same procedure as
105 above, but starting from the corresponding S2 and S3 systems of MT^{13PF}. These systems have been
106 constructed as models of the MT seam region. In the case of the 16PF lattice, the S2 and S3 systems
107 have not been modelled as the seam is not present in the 16:4 architecture. All assembled molecular
108 systems were setup, equilibrated and simulated using the following Molecular Dynamics (MD)
109 protocol, as already employed in our previous studies^{25,26}: first, we assigned the protonation state of
110 titratable residues using PROPKA³³ predictions corresponding to a pH of 7.4, and parametrized all
111 protein subunits using the AMBER ff99SB-ILDN force field³⁴. Additional ligands, namely GTP and

112 GDP whose coordinates were preserved from the original experimental structure, were parametrized
113 using the General Amber Forcefield (GAFF)³⁵ after assigning atomic partial charges using the AM1-
114 BCC³⁶ methodology. Each molecular system was then positioned into a cubic box of size 20 nm x 18
115 nm x 9 nm filled with explicit TIP3P water and an adequate number of Na⁺ and Cl⁻ ions to ensure system
116 neutralization and a physiological salt concentration of 0.15M. Systems then underwent a 1000-step
117 energy minimization run using the steepest descent algorithm. Finally, three distinct MD simulation
118 replicates were obtained by first equilibrating the systems in the NVT ensemble at 300 K ($\tau = 1$ ps) for
119 500 ps using the Berendsen thermostat³⁷. Then, systems were further equilibrated for 500 ps in the NPT
120 ensemble at a reference temperature of 300K and a reference pressure of 1 bar ($\tau = 5$ ps) using the
121 Berendsen barostat. During both these equilibration runs, we harmonically restrained the positions of
122 all alpha-carbons with a force constant of 1000 kJ/mol nm². After equilibration, we ran production MD
123 simulations in the NPT ensemble, using the Particle Mesh Ewald algorithm with a short-range cutoff of
124 1.2 nm and potential switching at 1.0 nm to treat electrostatic interactions. Van Der Waals interactions
125 were cutoff at 1.2 nm with potential switching at 1.0 nm. To limit the deviation of the sheets from the
126 experimental curvature of the MT during these production runs, we restrained the positions of one β -
127 strand of the tubulin monomers located at the four vertices of the sheets – specifically residues β 132-
128 138, β 163- 170, β 198-203 and α 133-140, α 165-171, α 200-204 – with a force constant of 1000 kJ/mol
129 nm². Unlike similar approaches in previous literature^{27,28,38}, by only restraining the vertices, we did not
130 apply any position restraint to tubulins whose dynamics we analyzed. All simulations were carried out
131 in GROMACS 2021.4³⁹, in three replicates for each molecular system.



132

133 Figure 1 Graphical visualization of the three simulated sheets: S1 is representative of 3 adjacent protofilaments
 134 far from the MT seam; S2 and S3 represent the structural discontinuity between adjacent PFs to the left (S2) and
 135 to the right (S3) of the MT seam.

136 S1 systems, which we focussed on herein to investigate the tubulin dynamics within the MT wall with
 137 different lattice configurations, were simulated for a total of 500 ns per replica. The rationale of focusing
 138 on the dynamics of tubulins in regions far from the seam – represented by the S1 systems – is that no
 139 A-type contacts are present in the 16:4 lattice, and that depolymerization is statistically less favorable
 140 to initiate at the seam ⁴⁰. Also, previous studies have shown that the MT seam does not constitute a
 141 mechanically weaker discontinuity with respect to the rest of the lattice⁴¹. Conversely, in the case of S2
 142 and S3 systems representing regions adjacent to the MT seam, the MD simulations served the purpose
 143 of relaxing the systems before extracting conformations for the subsequent ENM-NMA analysis, and
 144 these systems were thus simulated for 200 ns per replica.

145 **Analysis of MD simulations**

146 The MD simulations of MT^{11PF}, MT^{13PF}, and MT^{16PF} were analysed as follows. The structural stability
 147 of the system in each of the three replicas was quantified by evaluating the root-mean-squared deviation
 148 (RMSD) of the backbone atoms of the sole central tubulin heterodimer as well as of the whole system
 149 with respect to their initial coordinates. Once the equilibrium portions of the simulations were identified,
 150 the respective trajectories were concatenated and sampled every 50 ps to perform further analyses. The
 151 most flexible regions were identified by computing the root-mean-squared fluctuations (RMSF) of Ca
 152 atoms, while per-residue secondary structure probability was computed using the STRIDE software
 153 package ⁴². We subsequently analysed the interactions ⁴² between the central tubulin heterodimer and the

154 ones of adjacent PFs by means of the frequency of contacts^{25,26}, and computed the contact surface
155 between the central tubulin heterodimer and the neighbouring ones²⁶. We also inspected the percentage
156 of the hydrophobic area within the lateral buried surface. In addition, we used the PLIP⁴³ tool to
157 determine the nature of the interactions between specific amino acids of the central tubulin heterodimer
158 and adjacent PFs, and further calculated the electrostatic potential map as follows: first, we extracted
159 frames every 1 ns from the concatenated equilibrium trajectory, and then performed clustering using
160 the gromacs *cluster* tool, using the gromos clustering method and a cutoff of 0.15 nm, after RMSD-
161 fitting the frames based on the positions of alpha carbons of the central tubulin dimer. We then used the
162 centroid of the most populated cluster as the representative equilibrium conformation, and calculated
163 the electrostatic potential map using ABPS⁴⁴, imposing an ionic strength of 150 mM, a solvent dielectric
164 constant of 78.54 and a protein dielectric constant of 1.0.

165 The structural communication within MT^{11PF}, MT^{13PF}, and MT^{16PF} has been investigated using the
166 dynamic network analysis approach⁴⁵, as done in our previous studies^{25,26}. The term structural or
167 mechanical communication refers to the possible ways in which the vibrational stimuli can propagate
168 inside the protein structure thanks to dynamic interaction between amino acids. After obtaining a
169 network representation of the molecular structure, we calculated the following metrics from the network
170 analysis: the *degree of centrality* represents how important a given node is locally in the network, and
171 can be calculated as $dg_i = \sum_{i \neq j} A_{ij} w_{ij}$, where A is the adjacency matrix defining the graph and w_{ij} is
172 the weight of the edge connecting node i with node j. The *betweenness centrality* (of a node or edge) is
173 a metric representing the importance of a given node or edge in connecting distant parts of the network.
174 In other terms, it is related to how many shortest pathways between any two nodes include the given
175 node or edge. It is calculated as $b_i = \frac{1}{C} \sum_{s,t \in V} \frac{\sigma(s,t|i)}{\sigma(s,t)}$ where $\sigma(s,t)$ represents the total number of
176 shortest paths between two nodes s and t , and $\sigma(s,t|i)$ is the number of such shortest paths involving
177 node (or edge) i . C is a normalization factor equal to $\frac{(n_n-1)(n_n-2)}{2}$ for node betweenness centrality and
178 $\frac{n_n(n_n-1)}{2}$ for edge betweenness centrality, where n_n is the number of nodes in the graph. Lastly, the
179 *closeness centrality* of a node i is calculated as the reciprocal of the sum of the length $d(j,i)$ of the

180 shortest paths between node i and all other reachable nodes N , according to the formula $C_i = \frac{N-1}{\sum_{j=1}^{N-1} d(j,i)}$.

181 The closeness centrality of each node in the network has been computed to gain information about the
182 length of paths connecting one node to the rest of the network. In this case, higher average closeness
183 centralities are associated with networks where the transfer of vibrational information is more efficient.
184 Three-dimensional representations of receptor structures were rendered in VMD software ⁴⁶. RMSD,
185 RMSF, and solvent accessible surface area were computed through the GROMACS analysis tools.
186 Contact frequencies and probability of secondary structures were computed through custom-made
187 Python scripts and using the MDAnalysis library.

188 **Elastic network modelling of MT models**

189 We followed the procedure described in our previous study ²⁶ to build three MT models for each of the
190 architectures under study, i.e., 11:3PF, 13:3PF, and 16:4PF, starting from tubulin conformations derived
191 by the MD simulations of MT^{11PF}, MT^{13PF}, and MT^{16PF}, respectively. For this purpose, previously
192 published EMD maps (accession codes EMD-5191 for the 11:3 lattice, EMD-5193 for the 13:3 lattice,
193 and EMD-5196 for the 16:4 lattice ²⁴) were exploited to reconstruct 400 nm-long MTs. One MT model
194 was obtained from each S1-3 replica, obtaining a total of 3 MT models per architecture. The MT models
195 were analysed using ENM-NMA, which was performed using an in-house fortran77 code ²⁷. After a
196 visual inspection of the normal modes, the frequencies corresponding to the first bending, torsion, and
197 stretching motions were considered to predict the bending, torsional, and stretching stiffness, as done
198 in previous studies ^{27,47}. Briefly, the bending stiffness (flexural rigidity, k_{Eb}), stretching stiffness (k_E)
199 and torsion stiffness (k_G) can be extracted starting from the eigenvalues of the corresponding normal
200 modes using the linear elastic beam theory: for small deformations, wave equations can be used to
201 describe the bending, stretching and torsion displacements, leading to the following relationships
202 between the wave numbers (w_n) and the angular frequencies (ω_n) of the n -th mode:

$$203 \quad \rho \omega_n^2 = k_{Eb} w_n^4 \quad (1)$$

$$204 \quad \rho \omega_n^2 = k_E w_n^2 \quad (2)$$

$$205 \quad \rho_v I \omega_n^2 = k_G w_n^2 \quad (3)$$

206 Where I is the second moment of area of the cross-sectional area, and ρ and ρ_v are the mass per unit
 207 length and per unit volume of the filament, respectively. The wave numbers can be then determined for
 208 bending modes as:

$$209 \quad \cos(w_n L_{MT}) + \cosh(w_n L_{MT}) = 1 \quad (4)$$

210 Where L_{MT} is the length of the microtubule.

211 Conversely, for modes corresponding to stretching and torsion, the wave number can be calculated (for
 212 the n -th mode) using:

$$213 \quad w_n = \frac{n\pi}{L_{MT}} \quad (5)$$

214 Once the wave numbers have been calculated, k_{Eb} , k_E and k_G can be calculated using equations (1),
 215 (2) and (3) after calculating the angular frequencies ω_n from the eigenvalues of the modes. Finally, the
 216 stiffnesses can be converted to mechanical moduli using the following relationships: the bending
 217 modulus E_b is given by:

$$218 \quad E_b = \frac{k_{Eb}}{I} \quad (6)$$

219 The stretching modulus, E , is given by the ratio between the stretching stiffness k_E and the cross-
 220 sectional area of the filament, A , multiplied by L_{MT} :

$$221 \quad E = \frac{k_E}{A} L_{MT} \quad (7)$$

222 Lastly, the shear modulus is directly proportional to the torsion stiffness, k_G , and is equal to:

$$223 \quad G = \frac{k_G}{J} \quad (8)$$

224 Where J is the polar moment of inertia. Details of the MT reconstruction and NMA are provided in the
 225 Supporting Information. Compared to previous studies, three models were built for each MT lattice
 226 using different tubulin conformations extracted from MD sampling. A visual representation of the
 227 analyzed molecular systems and methodological approaches is provided in figure S1 of the Supporting
 228 Information.

229 To evaluate possible quantitative relationships between the number of PFs and the mechanical
 230 parameters of the MT described above, we fitted and compared three different scaling models, namely

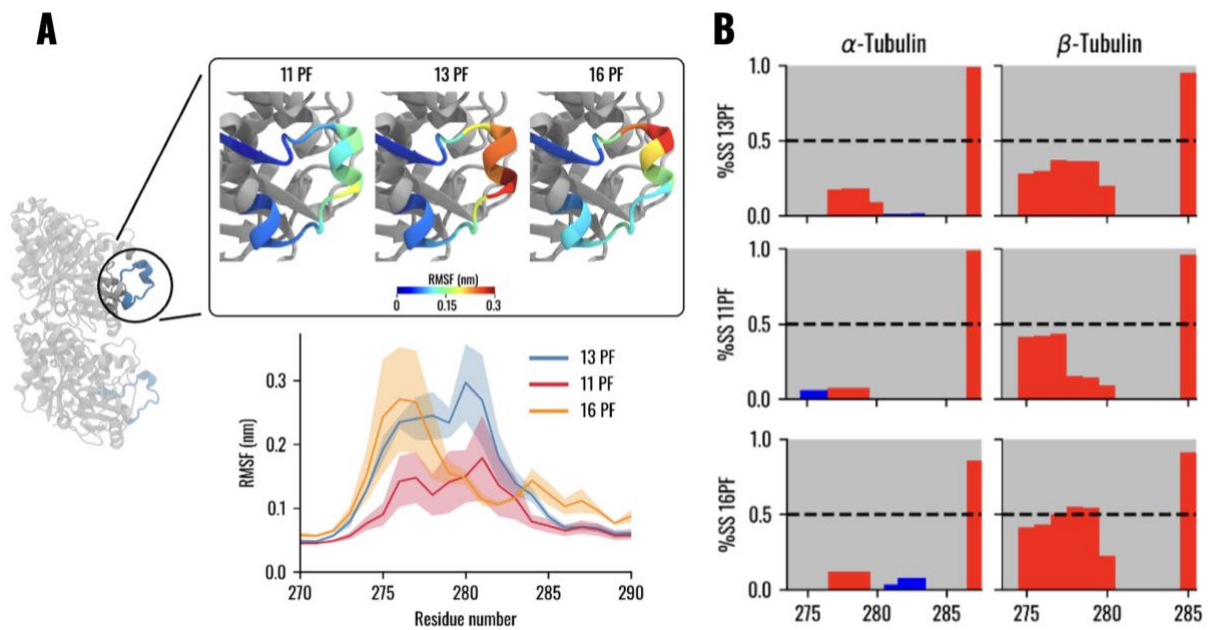
231 a power-law model in the form $Q(N) = \alpha N^b$, a linear model in the form $Q(N) = \alpha N + b$ and an
232 exponential model $Q(N) = \alpha e^{bN}$. The best model was selected based on the log-likelihood. Details on
233 the methodology for determining the best scaling models and estimating uncertainties can be found in
234 the Supporting Information.

235 **Results**

236 The RMSD analysis, representing a quantitative assessment of conformational stability (Figure S2 and
237 S3), led to the first 100 ns of each MD simulation being regarded as structural equilibration, and thus
238 discarded from further analyses; the conformational stability of the central tubulin dimer in the
239 remainder of the MD simulations was confirmed by means of a cluster analysis, performed using the
240 single linkage algorithm and the RMSD between the C _{α} s as the metric (cutoff = 0.15 nm). The
241 conformational stability regions of the S1 systems were finally concatenated to obtain 1.2 μ s long
242 trajectories for MT^{11PF}, MT^{13PF}, and MT^{16PF} that were subsequently analysed further.

243 **M-loop flexibility in the different MT lattices**

244 Since the conformation and flexibility of the M-loop (residues 272 to 288)²⁴ are pivotal for MT assembly
245 and stabilisation, this was the first aspect that we investigated. The RMSF analysis highlighted no
246 particular difference in flexibility in the α M-loop region, whereas a different flexibility profile of the
247 β M-loop was evident (Figure S4).



248

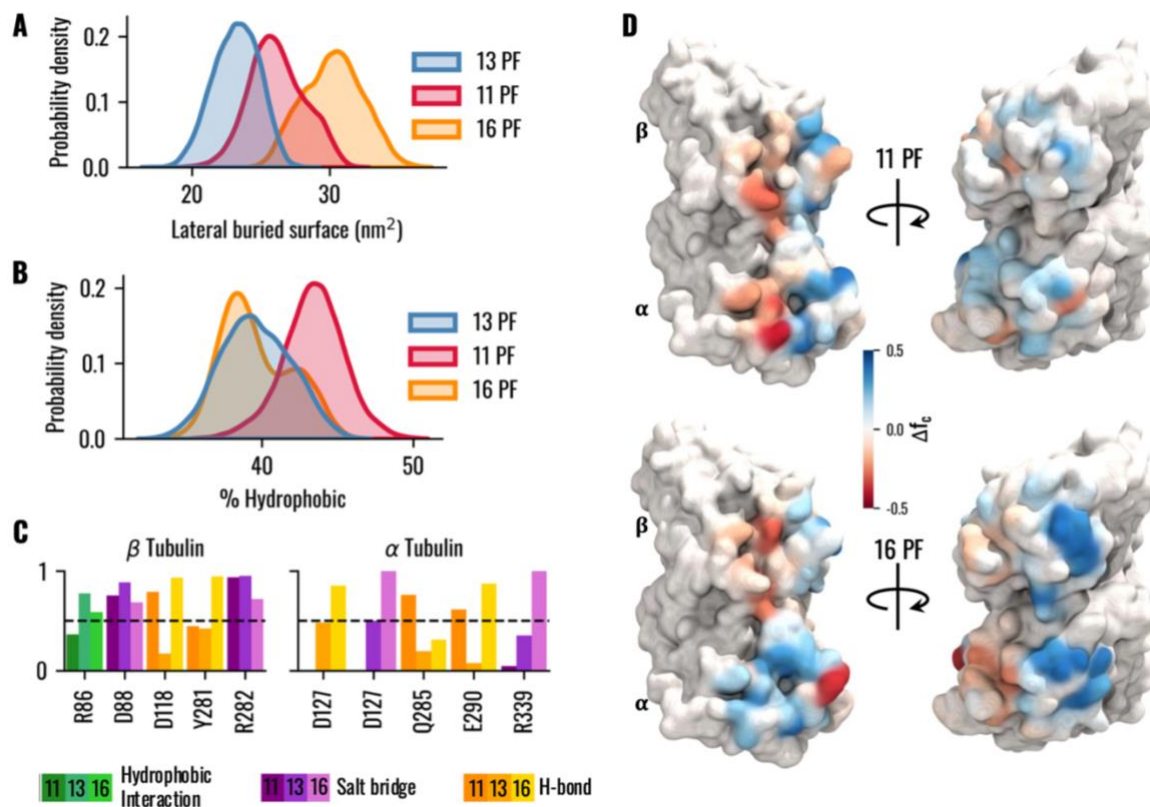
249 **Figure 2.** M-loop flexibility in the three MT lattices. (A) RMSF of the central β -tubulin M-loop in MT^{11PF} , MT^{13PF} ,
 250 and MT^{16PF} . Mean value across the three replicas is represented with a continuous line, whereas the shaded region
 251 represents the region within one standard deviation from the mean. At the top, a visual representation of the mean
 252 RMSF is shown on the cartoon representation of the β M-loop. The structure of the β -tubulin is the starting
 253 conformation of MT^{13PF} . Full tubulin dimer shown in light grey on the left with the location of the M-loops
 254 highlighted for visual reference. (B) Secondary structure probability for M-loop residues. Helices are colored in
 255 red, strands in blue, and coils in grey.

256 In particular, the RMSF of MT^{11PF} was lower than that of MT^{13PF} , especially at the N-terminus. In
 257 contrast, the β M-loop of MT^{16PF} was as flexible as that of MT^{13PF} at the N-terminus, while its
 258 fluctuations were remarkably reduced in the C-terminal region (Figure 2A). In comparison, the H1'-S2
 259 loop (residues 53-64) and the H2-S3 loop (residues 81-91), both known to also be involved in the lateral
 260 PF coupling together with the M-loop²⁴, showed no remarkable differences in secondary structure or
 261 RMSF trends in the different MT architectures (Supporting Figures S5, S6). Instead, while the α M-loop
 262 remaining mainly unstructured in all systems, the β M-loop was characterised by an increased
 263 probability of forming helices (Figure 2B). An increase in the folding probability, especially at the C-
 264 terminus, was found in MT^{16PF} , whereas in MT^{11PF} we noticed a slight increase in the helix content at
 265 residues 275-277 as well as an unfolding of residues 278-280. Interestingly, the residues which
 266 displayed a reduced flexibility in the MT^{16PF} architecture were completely unstructured in all systems.
 267 These findings indicate that variations in protofilament number influence the structural dynamics and
 268 secondary structure propensity mainly of the M-loop, with MT^{16PF} in particular showing enhanced
 269 stabilization at its C-terminal region. Given the established role of the M-loop in mediating lateral

270 interactions between protofilaments^{24,48,49}, these observations provide a structural basis for further
 271 examining the differences in lateral coupling between adjacent PFs across MT^{11PF}, MT^{13PF}, and MT^{16PF}
 272 architectures more in depth.

273 Lateral coupling of PFs

274 Since it has been shown that the main actors of PFs lateral coupling are the M and H2-S3 loops in all
 275 the investigated MT architectures²⁴, we subsequently focused on the lateral interactions between tubulin
 276 dimers in MT^{11PF}, MT^{13PF}, and MT^{16PF}. Interestingly, the laterally buried surface area between tubulins
 277 was not linearly related to the number of PFs.



278

279 **Figure 3** (A) lateral buried surface. (B) percentage of hydrophobic area in the total lateral buried surface. (C)
 280 Probability of interactions formed by specific amino acids of the central tubulin dimer with adjacent PFs.
 281 Hydrophobic interactions shown in green shades, salt bridges shown in purple shades and hydrogen bonds shown
 282 in orange/gold shades. Each group of bars represent the probability of the given interaction in the three systems
 283 (11PF leftmost bar, 13PF central bar, 16PF rightmost bar). (D) visual representation of the difference in frequency
 284 of contacts between MT^{11PF} and MT^{13PF} (top) and between MT^{16PF} and MT^{13PF} (bottom). Positive (blue) values
 285 indicate an increase in frequency with respect to MT^{13PF}, while negative (red) values reduction in frequency. The
 286 side exposing the intermediate domain is shown on the left, and the one exposing the N-terminal domain on the
 287 right.

288 MT^{13PF} showed the lowest amount of buried area, MT^{16PF} the highest (Figure 3A). In terms of surface
289 characteristics, MT^{13PF} and MT^{16PF} showed a comparable fraction of hydrophobic buried surface,
290 whereas in the MT^{11PF} architecture the hydrophobic portion of the lateral surface was higher (Figure
291 3B). Previously, we showed that the H3 and H9 helices, M, H1'-S2, and H2-S3 loops were characterised
292 by high contact frequency in MT^{13PF}²⁵. To also investigate the role of electrostatics in mediating lateral
293 PF-PF coupling, we computed the electrostatic potential map on the surface of the central tubulin dimer
294 in the sheet, in the three architectures (Figure S7). The results highlighted qualitative differences in
295 surface electrostatic potential in the inter-protofilament contact regions between the 11PF, 13PF and
296 16PF configuration: overall, the 11PF configuration featured the largest number of neutral patches in
297 the lateral PF-PF contact region, and the surface of the 11PF β M-loop in particular showed an increased
298 number of locally neutral and positive patches with respect to the 13PF and 16PF configurations,
299 consistent with the reduced flexibility observed in the RMSF analysis. The differences in surface
300 potential between the 13PF and 16PF architectures instead were more subdued, with no evident
301 differences in the β M-loop region.

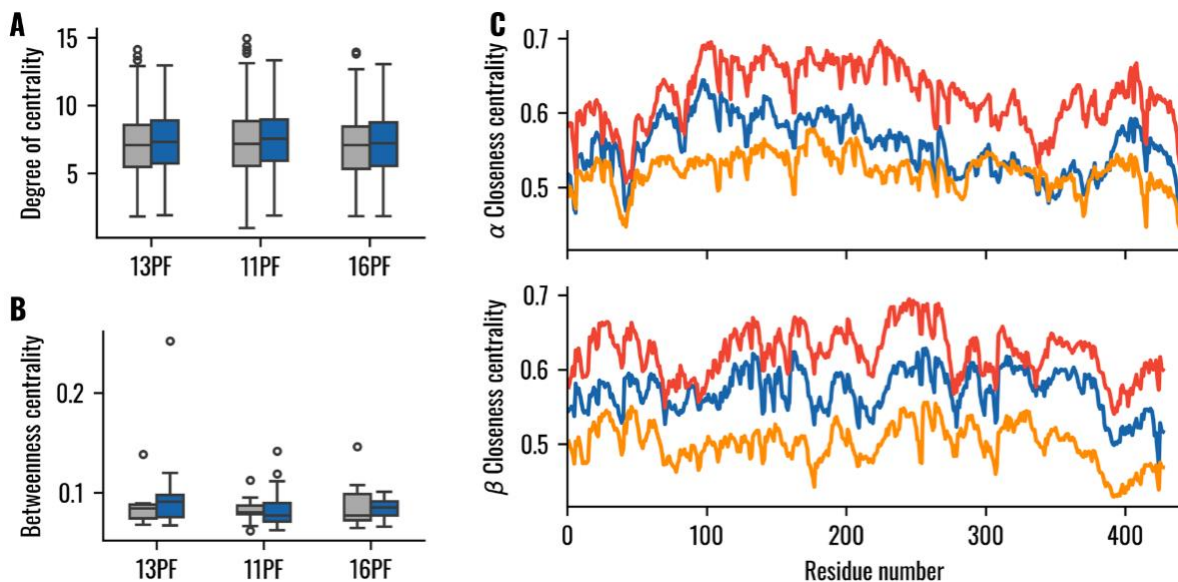
302 The analysis of the contact frequencies in MT^{11PF} highlighted no remarkable differences at the N-
303 terminal domain, whereas at the intermediate domain, an increase in contacts was observed towards the
304 MT lumen, as well as a loss of contacts moving away from the MT lumen. Regarding MT^{16PF}, at the
305 intermediate domain, there were minimal differences in β -tubulin compared to MT^{13PF}, but contact
306 frequencies were generally higher in α -tubulin, with the only exception being a small region of the M-
307 loop. In contrast, at the N-terminal domain, a gain of contacts towards the outside of the MT wall was
308 identified in both α - and β -tubulins. A visual representation of MT^{11PF} and MT^{16PF} contact frequency
309 differences with respect to MT^{13PF} is shown in Figure 3D. The analysis of specific interacting amino
310 acid patches (Figure 3C) revealed a decreased hydrophobic interaction of β Arg86 in the 11PF
311 configuration, increased hydrogen bonding of β Asp118 in the 11PF and 16PF architectures, and
312 enhanced formation of hydrogen bonds of β Tyr281 in the 16PF configuration. On the α -tubulin, the
313 formation of hydrogen bonds of α Asp127 was lost in the 11PF configuration, and greatly reduced for
314 α Glu290 in the 13PF configuration, whereas α Gln285 formed hydrogen bonds with neighbouring PFs
315 mainly in the 11PF configuration compared to other architectures. α Asp127 formed salt bridges with

316 neighbouring PFs mainly in the 16PF architecture, and moderately in the 13PF architecture. No salt
317 bridge was instead observed for this residue in the 11PF configuration. Also, the probability of forming
318 salt bridges of α Arg339 noticeably increased with the number of PFs.

319 Overall, the variations in contact frequencies and interaction probabilities among the MT^{11PF}, MT^{13PF},
320 and MT^{16PF} architectures originate from the distinct packing of tubulin dimers in adjacent
321 protofilaments, driven by the curvature of the MT wall imposed by different protofilament numbers.
322 This structural feature is expected to influence the propagation of mechanical cues within the lattice,
323 which we therefore investigated using dynamic network analysis.

324 Structural communication in the MT lattices

325 To investigate the relationship between MT architecture and mechanical cue propagation within the MT
326 wall, we performed a dynamic network analysis on the three systems. As resulted from earlier studies
327 ²⁵, the degrees of centrality of β -tubulin and α -tubulin are similar, with slightly higher centralities for β -
328 tubulin. In the present investigation, the same pattern was observed in all three MT lattices, indicating
329 that the local dynamic influence of tubulin amino acids was similar in MT^{11PF}, MT^{13PF}, and MT^{16PF}
330 (Figure 4A).



331

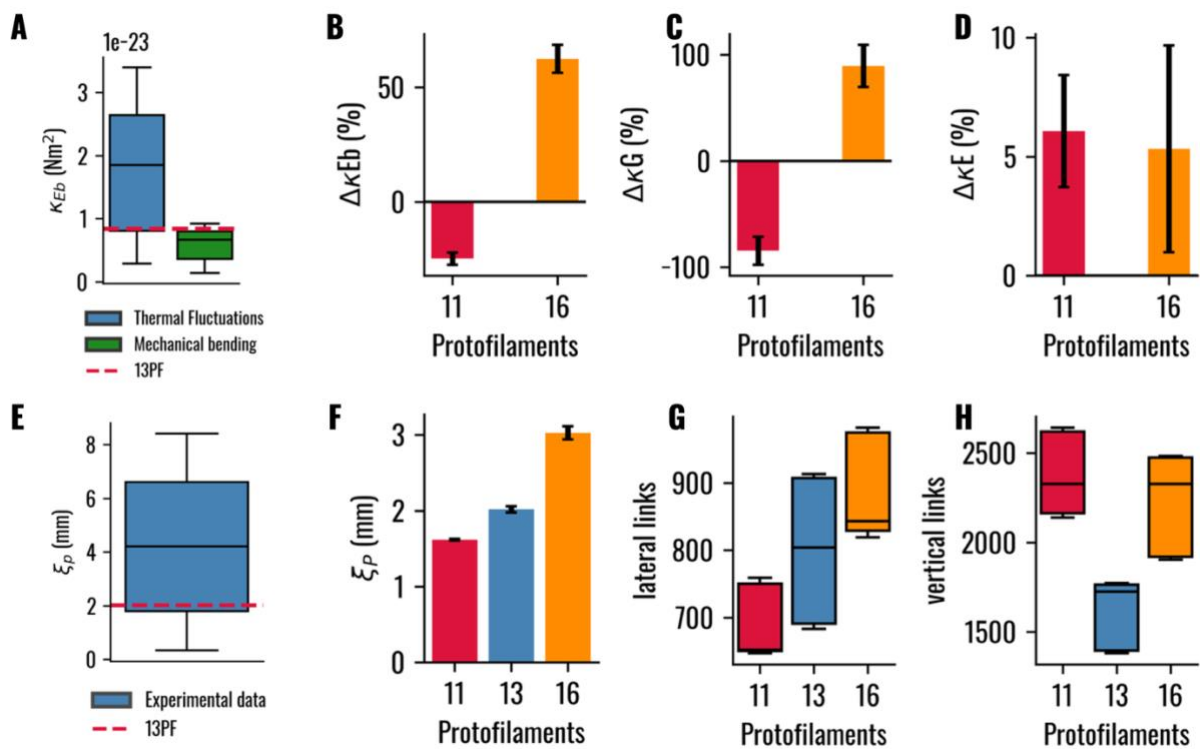
332 **Figure 4** Network analysis of the different MT architectures. (A) Boxplots showing the degree of centrality of the
333 central tubulin heterodimer nodes. Grey boxplots show α -tubulin nodes, while blue boxplots the β -tubulin nodes.
334 (B) Boxplots showing the nodes betweenness centralities for the central tubulin heterodimer. Grey boxplots show
335 α -tubulin nodes, while blue boxplots the β -tubulin nodes. (C) Closeness centrality of the central tubulin
336 heterodimer residues. MT^{16PF}, MT^{13PF}, and MT^{11PF} are coloured in orange, blue, and red, respectively.

337 We subsequently plotted the betweenness centrality of the nodes against the node rank to define a
338 threshold, that is, the knees of the curves for the MT^{11PF}, MT^{13PF}, and MT^{16PF} systems. The three
339 thresholds were similar (Figure S8), and the lower one was selected. Node betweenness centrality values
340 above the selected threshold were considered in the subsequent analyses. First, we inspected the curve
341 of α - and β -tubulin node betweenness centralities versus their rank, and observed that in the case of β -
342 tubulin, the first-ranking nodes had the lowest betweenness in MT^{16PF} (Figure S9). Comparing the
343 distribution of the betweenness centrality of α - and β -tubulins, MT^{11PF} displayed a higher importance
344 of β -tubulin nodes, as in MT^{13PF}. In contrast, in the case of MT^{16PF}, the median of β -tubulin distribution
345 was still higher than that of α -tubulin distribution, but the highest importances were observed for α -
346 tubulin (Figure 4B). Details of the distributions of degrees of centrality and betweenness centralities are
347 reported in the Supporting Information (Figure S10). Finally, we computed the closeness centrality as
348 a metric indicating how “close” a certain node is to the rest of the network. The distribution of the
349 closeness centrality values within tubulins was similar in the three architectures; however, higher
350 centralities were observed in MT^{11PF}, whereas the lowest centralities were observed in MT^{16PF} (Figure
351 4C). Therefore, in general, we found that the shortest communication paths increase with the number
352 of PFs. This was confirmed by the computation of the wiener indexes in the three networks (Figure
353 S11), which also increased with the number of PFs. Interestingly, the M-loops were regions of low
354 centrality in all systems, but the α M-loop importance was higher in MT^{11PF} (Figure S12). Moreover,
355 helix H8, which mediates vertical interactions between tubulins²⁵, was characterised by higher
356 closeness centrality in the intra-dimer interface than in the inter-dimer interface, particularly for MT^{16PF}.

357 **Elastic Network Modelling of the MT models**

358 The mechanical properties of MTs were inferred using the ENM-NMA approach^{27,28}. From the
359 longitudinal bending, torsion, and stretching vibrational modes, mechanical parameters such as bending
360 (κ_{Eb}), torsion (κ_G), and stretching (κ_E) stiffnesses were estimated. The results obtained using this
361 methodology were consistent with previous experimental data found in the literature^{30,50–52} (Figure
362 5A,E). We found that the bending and torsional stiffnesses significantly increased with the number of

363 PFs, whereas minimal differences were observed between the stretching stiffnesses (Figure 5B-D),
 364 significant if $p < 0.05$ with Mann-Whitney U test).



365

366 **Figure 5** Elastic Network Modeling of the three MT lattices. (A) Comparison between the mean bending stiffness
 367 from this work (dashed line) and the experimental data found in ^{30,50-52}. (B-D) Bending (B), torsional (C), and
 368 stretching (D) stiffnesses of the 11PF and 16PF MT architectures expressed as the percentage of variation from
 369 the 13PF MT. The bars show the standard deviation across the three ENMs. (E) Comparison between the mean
 370 persistence length of the 13PF MT from this work (dashed line) and the experimental data found in ^{30,51}. (F)
 371 Persistence length of the 11PF, 13PF, and 16PF MT architectures, where the bars represent the standard deviation
 372 across the three ENMs. (G, H) Number of links, in the ENMs of the 11PF, 13PF, and 16PF MT architectures,
 373 connecting vertically (G) and laterally (H) coupled tubulins.

374 Thus, given the direct proportionality between the bending stiffness and the persistence length of the
 375 MT, the latter also increased with the number of PFs, reaching a value of 3 mm in the case of a 16PF
 376 lattice (Figure 5F). The persistence length value obtained for the 13PF system was also found to be in
 377 good agreement with previously reported data^{30,51} (Figure 5E).

378 We then determined the mechanical moduli, that is, the bending (E_b), shear (G), and Young's (E) moduli,
 379 which characterise the material properties: no significant differences were observed between the shear
 380 moduli, whereas the Young's and bending moduli decreased with the number of PFs (Figure S13).
 381 Interestingly, these findings highlight how geometric effects (increase of the MT radius) dominate over
 382 variations of material properties (increase of the bending stiffness κ_{Eb}) as the number of protofilaments

383 increases: the bending modulus (E_b) decreases with the number of PFs since the second moment of area
384 of the MT, which scales with the fourth power of the radius, increases more rapidly than the bending
385 stiffness κ_{Eb} (see Figure S14).

386 Notably, the bending and Young's moduli were approximately the same in all the architectures; thus,
387 the Euler–Bernoulli approximation could be used to model the MTs at the considered length, under the
388 hypothesis of a limited effect of shear between PFs on the bending deformation. Starting from the
389 mechanical moduli, we inferred the lengths at which the deformation due to shear was 5% and 50% of
390 the total (Figure S15). The length at which the shear contribution was 5% was ~ 460 nm for the 11PF
391 and 13PF lattices, while it reached ~ 550 nm for the 16PF MT. Therefore, the shear contribution of the
392 16PF architecture is significantly higher when considering the same MT length. Interestingly,
393 considering the length/radius ratio at which the shear contribution is 5%, we obtained a value of ~ 43 in
394 the case of the 13PF and 16PF lattices, while it was ~ 52 for the 11PF architecture. While a precise
395 quantification of the shear stresses experienced by MTs *in vivo* remains elusive, earlier studies^{53,54}
396 confirmed that shear stresses in the range of 0.05 to 23 Pa^{54–57} induce microtubule alignment and
397 reorganization, affecting cell shape and migration: for instance, in cardiomyocytes and endothelial cells
398 subjected to shear stresses, MTs act as compression-resistant elements^{58–60}. Taken together, these results
399 further corroborate the hypothesis that PF architecture may play an active role in mechanotransduction.

400 In particular, the enhanced shear contribution of 16PF lattices may amplify deformation under flow,
401 potentially affecting force transduction and remodelling dynamics, whereas 11PF lattices appear
402 intrinsically more resistant to shear deformation.

403 We finally related the differences in the mechanical moduli to the topologies of the ENMs built using
404 the MT models of different lattices. We found that the number of links in the ENMs between laterally
405 coupled tubulins increased with the number of PFs, providing a structural explanation to the observed
406 increase in bending stiffness and persistence length. Conversely, the links between vertically coupled
407 tubulins were similar between the 11PF and 16PF MTs, with an increase for the 13PF lattice (Figure
408 5G, H). Overall, we observed that the mechanical parameters such as κ_{Eb} , κ_G , E_b and E scale differently
409 with increasing numbers of PFs in the microtubule (Figure S16), but further studies are required to

410 precisely determine the nature of such scaling behaviour and to formulate hypotheses regarding the
411 mechanistic underpinnings of the observed trends.

412 **Discussion**

413 Although several studies have documented the existence of MTs with different lattice architectures, the
414 structure–function relationship underlying this variability remains incompletely understood. Previous
415 evidence supports the hypothesis that MT architecture adapts to fulfil specific cellular functions. Given
416 the mechanical support role of MTs, it has been hypothesised that variations in PF number modulate
417 their mechanical properties. In this work, we provided a detailed mechanical characterisation of MTs
418 with distinct architectures, focusing on how tubulin-tubulin interactions vary across lattices and how
419 these variations propagate at higher scales, impacting mechanical behaviour.

420 Our results show that the main changes in tubulin flexibility are localised to the β M-loop (Figure 2).
421 Specifically, we found it to be partially stabilized in the MT^{16PF} architecture, whereas in MT^{11PF} the β M-
422 loop was fully stabilized. Interestingly, the reduction in β M-loop flexibility and its partial structuring
423 have also been observed in the presence of taxol ²⁶, which is known to promote the formation of MTs
424 with less than 13 PFs ³. However, we underline how the partial folding in MT^{11PF} involved different
425 residues than those in the taxol-driven scenario.

426 Our observations are consistent with the idea that MTs may sample a range of accessible architectural
427 states defined at the level of individual tubulin dimers and at the level of their supramolecular lattice
428 architecture. Despite involving different residues, the observed similarities between the MT^{11PF}
429 configuration and the taxol-stabilised state suggest that ligand binding might shift the system toward
430 one of multiple accessible architectural states, a mechanism also known as *conformational selection*.
431 Thus, rather than imposing an artificial conformation, specific stabilising agents may act by selecting
432 and reinforcing specific lattice organisations within this landscape. Distinct protofilament numbers may
433 correspond to discrete, metastable architectural states that differ in local flexibility, inter-dimer contacts,
434 and dynamic communication properties. By stabilising one of these accessible states, ligands such as
435 taxol may shift the equilibrium toward specific MT architectures that would otherwise be transient or

436 energetically disfavoured under physiological conditions. Indeed, studies have shown that the number
437 of protofilaments is set early during MT formation *in vivo*, primarily during the initial tubulin nucleation
438 and sheet closure phase, rather than through remodelling of the conformation of a pre-existing
439 lattice^{61,62}. This has been shown to occur mainly through the stabilisation of early tubulin assemblies by
440 binding agents which promote specific tubulin-tubulin interactions^{26,63,64}, whereas in the absence of such
441 ligands tubulin *in vitro* assembles freely into microtubules composed of 9 to 16 protofilaments⁶⁵.
442 This concept of ligand-guided conformational selection could help explain the context-dependent
443 efficacy or resistance observed with MT-targeting agents, depending on whether a given cell type
444 naturally explores—or avoids—the ligand-favoured state. The β M-loop conformational rearrangements
445 were also associated with differences in the lateral coupling of tubulins, considering different PF
446 numbers (Figure 3). In the largest MT lattice, a wider laterally buried surface was observed due to the
447 involvement of an additional interaction site including helix H3²⁴. However, the contacts were
448 stabilised on average in the intermediate domain of the sole α -tubulin. In this context, this study shows
449 that the recruitment of additional contacts is reflected in the increase in the lateral buried area. Although
450 MT^{11PF} showed loss of contacts in H9 and subtle changes in the N-terminal domain, its laterally buried
451 surface was slightly increased with respect to MT^{13PF}, along with the hydrophobic contribution.
452 Therefore, the width and nature of the lateral tubulin-tubulin contacts change in the different
453 architectures to provide stability to the MT lattice. This is consistent with studies exploring the role of
454 tubulin-tubulin contact discontinuities, arising e.g. due to lattice defects, in altering overall MT
455 mechanics: missing dimers, dislocations and tubulin-tubulin contact mismatches have been shown to
456 weaken the lattice⁶⁶⁻⁶⁸ by locally concentrating mechanical stresses^{69,70}, thereby increasing the
457 susceptibility of the MT to damage. Instead, the discontinuity at the MT seam does not appear to be
458 mechanically weaker than the rest of the lattice^{41,71}.
459 Analysis of the degree of centrality showed that the local influence of residues belonging to α - and β -
460 tubulin was similar in the three architectures (Figure 4A). For MT^{16PF}, the betweenness of nodes in α -
461 tubulin was higher, which can be related to the increase in the contact frequencies of α -tubulin residues,
462 resembling the results obtained in the presence of taxol²⁶. This observation confirms the relationship
463 between the stability of lateral contacts and the transfer of vibration within the MT, but also suggests

464 that taxol might induce lattice stabilisation through a combination of mechanisms, apart from the
465 longitudinal lattice expansion, that are also partially adopted to stabilise MT lattices other than the
466 classical 13:3. Finally, the analysis of the closeness centrality (Figure 4C) highlighted that the
467 vibrational path lengths, i.e. the communication pathways in the MT along which mechanical vibrations
468 can propagate, were on average shorter in MTs with lower PFs numbers.

469 Thus, mechanical communication within lattices with fewer PFs could be said to be more “efficient”,
470 in the sense that vibrational signals propagate through more correlated and shorter pathways. As a result,
471 smaller MT architectures might be better suited for rapid mechanical signalling, while larger MTs are
472 structurally optimized to withstand sustained mechanical loads. In particular, large MTs are
473 hypothesised to be employed by cells to provide mechanical support for maintaining shape ⁶ or resisting
474 deformation under stress ⁴.

475 This dual behaviour reinforces the idea that MT architecture is not a passive structural feature, but rather
476 a tunable parameter that balances mechanical sensitivity and robustness depending on functional
477 demands. This view is consistent with previous proposals that microtubules can act as mechanical force
478 sensors ⁷², and it encourages further investigation into whether architectural tuning may also influence
479 intracellular communication and mechanoresponsive behaviour. Such architectural tuning aligns with
480 the principles of neuromechanobiology, where cytoskeletal elements are increasingly recognised as
481 integrators of mechanical and informational processes in the nervous system ^{21,22}.

482 Our results are consistent with these hypotheses, as the bending stiffness of MTs increased with the
483 number of PFs. This effect appears to stem from an increased resistance to inter-protofilament shear—
484 i.e., torsional stiffness (Figure 5B–D)—rather than differences in intrinsic material properties, as the
485 shear moduli were not statistically different (Figure S13). This mechanical response reflects
486 architectural differences in the topology of the elastic network models (Figure 5G–H).

487 Accordingly, the shear contribution to bending was higher in the MT^{16PF} compared to the 13PF and
488 11PF lattices (Figure S15). However, the increased bending stiffness observed between the 13PF and
489 16PF lattices primarily results from their different diameters, as confirmed by the similar length-to-
490 radius ratio at which the shear contribution accounts for 5% of the total deformation (Figure S15). In

491 contrast, the higher ratio observed in the 11PF architecture indicates a distinct network topology that
492 alters the shearing response.

493 Thus, while the MT^{16PF} is characterised by more inter-PF contacts that enhance lattice stability and
494 bending resistance, its shearing behaviour remains comparable to that of the 13PF lattice. By contrast,
495 the 11PF lattice exhibits a qualitatively different shearing pattern, which aligns with its unique
496 hydrophobic profile in lateral interactions. Interestingly, these findings suggest that MT architectures
497 represent design solutions evolved to optimize specific mechanical tasks: lower PF numbers favour
498 efficient transmission of mechanical signals through correlated pathways, whereas higher PF numbers
499 provide increased resistance to deformation and enhance lattice robustness. Notably, as the number of
500 protofilaments increases, a competing effect emerges between material properties and geometrical
501 properties, whereby the overall bending modulus decreases despite increased structural connectivity
502 and bending stiffness. Such trade-off between stability and flexibility provides a framework for
503 optimizing mechanical properties through structural design. Considering the preliminary trends that we
504 were able to observe in the present study, a more in-depth investigation of how the mechanical
505 characteristics of MTs scale with increasing numbers of PFs warrants further experimental and
506 computational studies, potentially covering a wider range of MT architectures. This would allow to
507 reconstruct quantitative relationships between MT architecture and key mechanical parameters, thereby
508 further elucidating the biophysical underpinnings of MT mechanics.

509 The concept of architecture-encoded function also opens new directions in biological understanding
510 and biomaterials design. From a biological standpoint, cells might leverage PF variability to spatially
511 modulate MT mechanics according to local demands, such as rigidity in the axon shaft versus signal
512 responsiveness in dendritic spines.

513 From an engineering perspective, our approach demonstrates that combining atomistic simulations with
514 ENM/NMA can guide the rational design of bioinspired filaments with tailored mechanical responses.
515 Future extensions of this framework may include ligand binding, isotype substitution, or post-
516 translational modifications to explore how additional regulatory layers integrate into the architecture-
517 function paradigm.

518 **Conclusions**

519 In this study, we conducted a multiscale computational analysis to investigate how MT architecture
520 influences its mechanical properties and internal mechanical cue propagation dynamics. By focusing
521 on experimentally observed MT lattices with varying protofilament numbers, we explored how
522 differences in tubulin-tubulin interactions relate to changes in lattice curvature, structural signalling
523 efficiency, and bending stiffness. Our results support a model in which increased PF number enhances
524 MT rigidity, consistent with previous literature, while reduced PF number favours more efficient
525 mechanical communication.

526 Beyond structural and mechanical implications, these findings may inform emerging perspectives in
527 neuro-mechanobiology, where microtubules are increasingly considered potential mediators of
528 information transfer within cells.

529 Given their highly charged surfaces and intrinsic vibrational dynamics, changes in lattice architecture,
530 such as those driven by PF number, could influence mechanical signalling, electrostatic and field-based
531 interactions.

532 This raises the challenging possibility that MTs might contribute to subcellular information-processing
533 functions, including neuronal signalling and potentially memory-related processes.

534 This line of reasoning aligns with recent biophysical models exploring microtubules' dynamic and
535 vibrational properties as potential conduits for intracellular computation⁷³. Our findings contribute to
536 a growing body of evidence suggesting that MTs possess an architectural and dynamic complexity that
537 could support information-modulatory roles beyond purely structural or mechanical functions. Whilst
538 speculative, this hypothesis opens exciting avenues for future investigation at the interface between
539 mechanics, electrobiology, and cognitive biology. It is worth highlighting, however, that further
540 experimental validation is needed to clarify the mechanistic links between MT architecture, its dynamic
541 behaviour, and its functional role in complex cellular systems.

542 **Data Accessibility**

543 A Supporting Information file is available, containing details on the MD and ENM methodology and
544 supplementary figures S1-S16. In addition, all files necessary to replicate the findings reported herein,
545 namely system topologies, starting structures and parameter files for the MD simulations as well as
546 starting files and templates to reconstruct the MT rings, can be found on Zenodo at
547 <https://doi.org/10.5281/zenodo.16961992> .

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551 **Author Contributions**

552 The manuscript was written through contributions of all authors. EAZ, MC and MM conceptualized the
553 study, carried out the molecular modelling and simulations, analysed and interpreted the results. UM
554 and MAD conceptualized and supervised the work and interpreted the results. All authors have given
555 approval to the final version of the manuscript. †These authors contributed equally.

556 **Conflict of Interest Statement**

557 The authors declare no competing interests.

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