

Supplementary Information for
The structure of a Tau fragment bound to tubulin prompts new hypotheses on Tau mechanism and oligomerization.

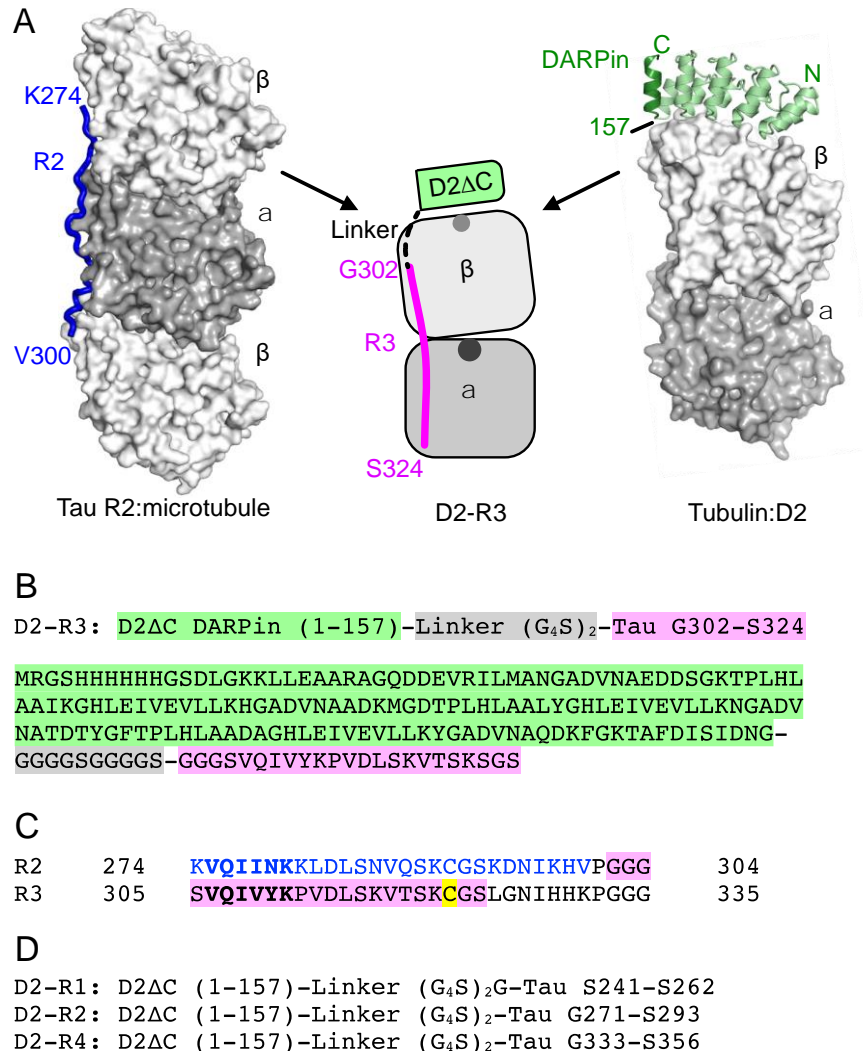


Fig. S1. Design of DARPin-Tau fusion proteins. (A) Design of D2-R3. The superposition of a β subunit of the microtubule decorated with Tau R2 (Left; pdb id 6CVN) to that of the tubulin:D2 DARPin complex (Right; pdb id 4F6R) leads to the model shown in the middle. (B) Schematic view and sequence of D2-R3. (C) Sequence alignment of R2 and R3 MTBRs (definition of the repeats as in Kellogg et al, Science, 360:1242–1246 (2018)). The Tau residues that are traced in the R2: microtubule cryo-EM model are in blue, those present in D2-R3 are highlighted in pink. The PHF6* (in R2) and PHF6 (in R3) hexapeptides are in bold letters. Residue Cys322 (yellow) has been replaced by a Ser residue in D2-R3. (D) D2-R1, D2-R2 and D2-R4 were designed by replacing the R3 moiety in D2-R3 with the equivalent region of R1, R2 or R4 MTBR, respectively.

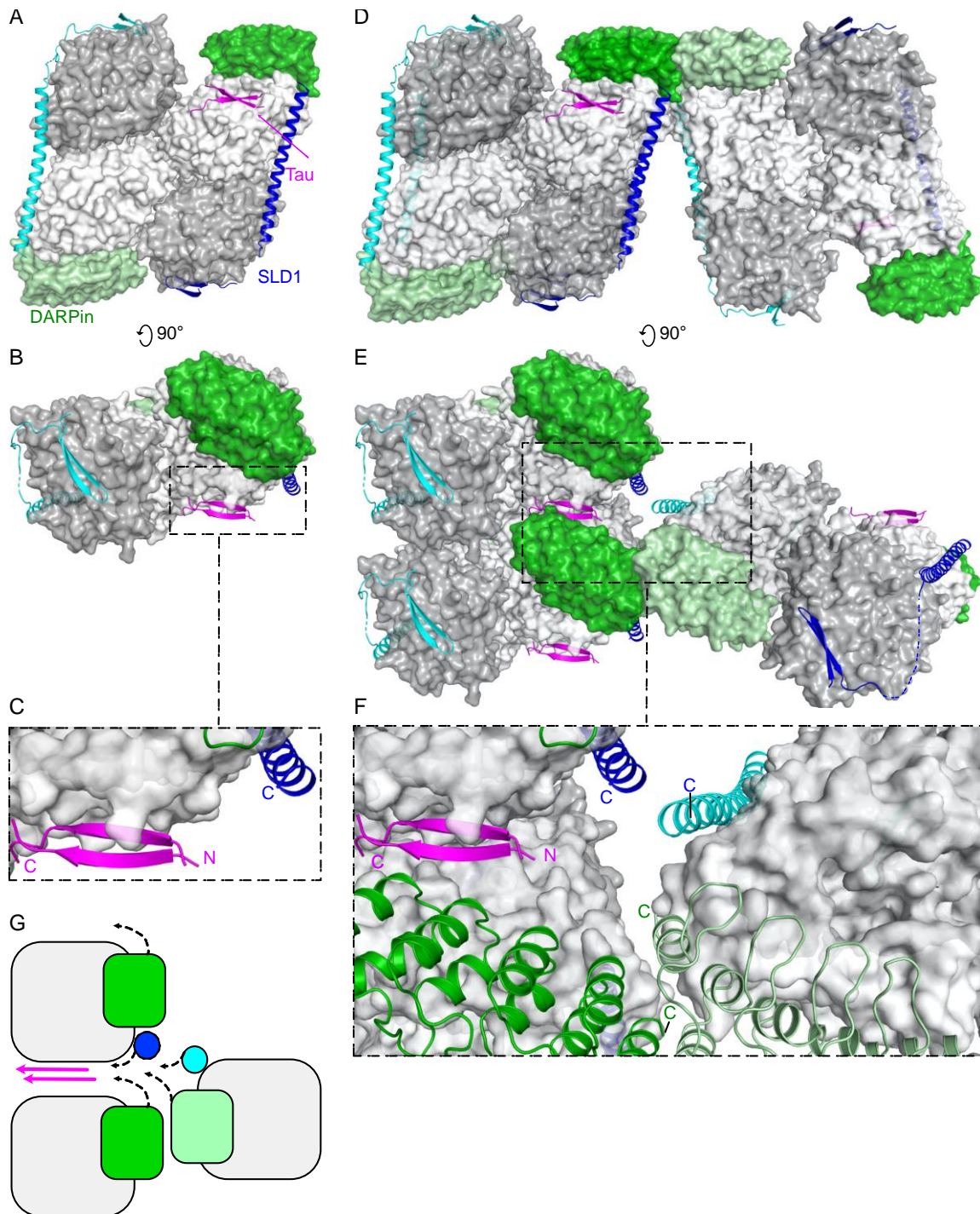


Fig. S2. Crystal packing of SLD1:tubulin:D2-R3. (A-C) The 2 complexes of the asymmetric unit. (A) View as in Fig. 1A. (B) View rotated 90°. (C) Close-up image of the part framed in panel B. (D-F) Same views but with two additional crystal-related asymmetric units. By virtue of crystal symmetry, the C-termini of the DARPins and of the SLD1 molecules (labeled in panel F) are in proximity to the N-termini of the Tau R3 stretches (schematized in panel G).

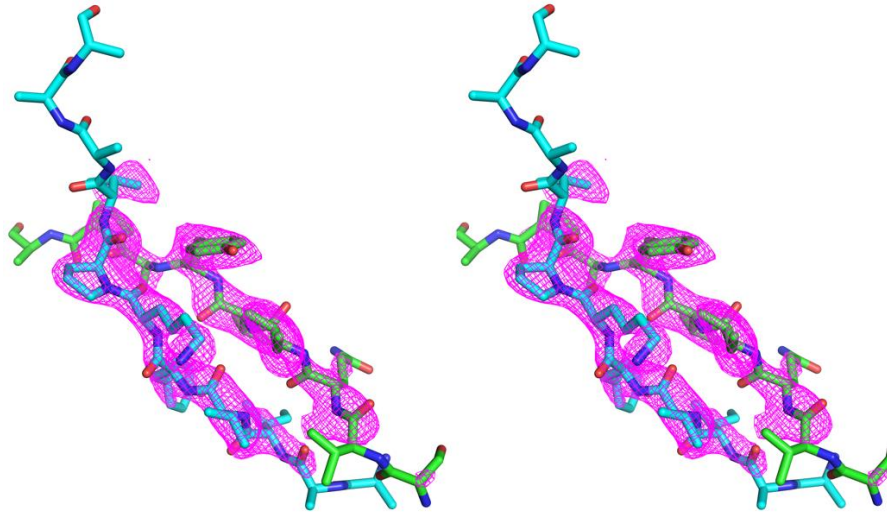


Fig. S3. Stereo view of the $F_{\text{obs}}-F_{\text{calc}}$ omit map contoured at the 3σ level (magenta). The final model for the Tau part is shown in cyan and green as in Fig. 1B.

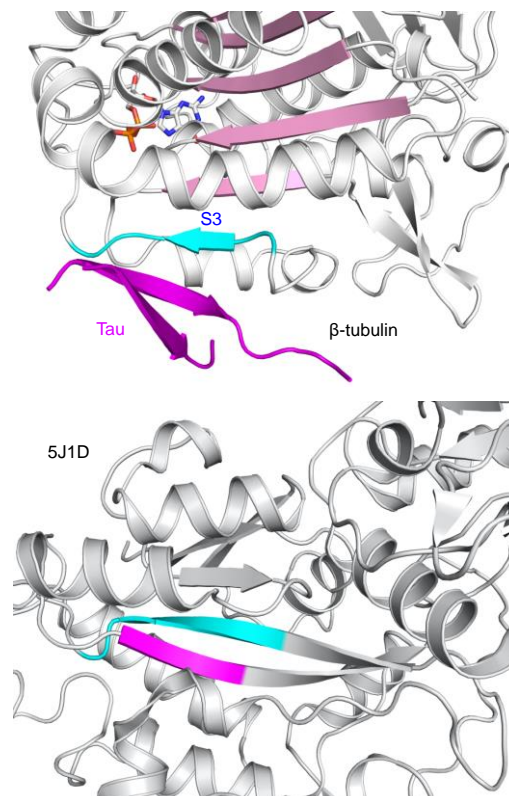


Fig. S4. The NFVFGQ β -tubulin sequence, encompassing its S3 β -strand, is also found in a bacterial phosphate-binding protein. (Top) Antiparallel interaction between the β -tubulin S3 β strand and Tau R3. (Bottom) Antiparallel interaction of the NFVFGQ motif with a LVQI motif (magenta) in a *Stenotrophomonas maltophilia* phosphate-binding protein (pdb id 5J1D). The NFVFGQ motif is in cyan in both panels.

Table S1. Data collection and refinement statistics.

D2-R3:tubulin:SLD1-R3	
Data collection^(a)	
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
a, b, c (Å)	52.30, 222.25, 227.66
α , β , γ (°)	90, 90, 90
Resolution (Å)	159-2.21 (2.51-2.21)
Anisotropy resolution limits ^(b)	2.18, 2.63, 2.97
R _{meas}	0.124 (3.96)
I / σ (I)	9.4 (1.8)
CC _{1/2}	0.996 (0.607)
Completeness	
spherical (%)	60.6 (9.7)
ellipsoidal (%)	95.0 (68.4)
Multiplicity	27.4 (23.4)
Refinement	
Resolution (Å)	2.21
No. reflections	81,231
R _{work} / R _{free}	0.200 / 0.234
No. atoms	
Protein	16,810
Ligands	263
Solvent	328
B factors (Å ²)	
Protein	45.1
Ligands	49.3
Solvent	30.0
Coordinate error (Å)	0.31
R.m.s.d.	
Bond lengths (Å)	0.008
Bond angles (°)	0.93
Ramachandran (%)	
Favored region	97.62
Allowed region	2.38
Outliers	0

^(a) Two dataset collected on the same crystal were merged. Values in parentheses are for the highest-resolution shell. ^(b) Determined by STARNISO using a local mean I / σ (I)=1.2.