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**Supplemental Information**

**Cryo-EM Reveals How Human Cytoplasmic**

**Dynein Is Auto-inhibited and Activated**

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**Table S1.** Cryo-EM data collection and processing summary, Related to Figure 1.

<b>Data Collection and processing</b>					
	<b>Dynein-(1)</b>	<b>Dynein-(2)</b>	<b>Dynein-(3)</b>	<b>DDB-(1)</b>	<b>DDB-(2)</b>
Institution	MRC-LMB	MRC-LMB	DLS	MRC-LMB	CEITEC
Detector	Falcon II	K2 summit	K2 summit	Falcon II	Falcon II
Frames	34	25	20	34	7
Exposure (sec)	2	20	8	2	2
Dose ( $\bar{e}/\text{\AA}^2$ )	54	40	40	54	54
Micrographs	6746	2649	3781	2634	3058
Pixel size ( $\text{\AA}$ )	1.32	1.43	1.06	1.32	1.34
Raw particles	312, 198	187, 830	214, 543	75, 052	41, 222
Final particles	233,227 (motor) / 102,309 (tail)				78,671
Resolution	3.8 $\text{\AA}$ (motor) / 8.4 $\text{\AA}$ (tail)			8.7 $\text{\AA}$ / 12 $\text{\AA}$ (masked tail)	
<b>Refinement summary</b>					
<b>Model composition of all proteins</b>					
Non-hydrogen atoms	46214				
Amino acid residues	5852				
Ligands (ADP/ATP)	6/2				
<b>Refinement statistics</b>					
Resolution	257.28 - 3.80				
sharpening B-factors ( $\text{\AA}^2$ )	-100				
Rfactor*	0.339				
Overall FSC†	0.820				
Correlation coefficient	0.922				
Mean B-factor ( $\text{\AA}^2$ )	157.1				
<b>Rms deviations</b>					
Bond length ( $\text{\AA}$ )	0.0060				
Bond angle ( $^\circ$ )	1.1182				
Chiral volume ( $\text{\AA}^3$ )	0.0699				
<b>Validation</b>					
MolProbity score	2.13 (100 <sup>th</sup> percentile)				
Clashscore, all atom	19.17 (97 <sup>th</sup> percentile)				
Good rotamers	95.22%				
<b>Ramachandran plot</b>					
Favored	94.87%				
Outliers	0.10%				
C $\beta$ deviations >0.25 $\text{\AA}$	0.50%				

\*Rfactor =  $(\sum(|F_{\text{obs}}| - |F_{\text{calc}}|) / \sum |F_{\text{obs}}|)$ , in which  $|F_{\text{obs}}|$  is the amplitude of simulated reflections from EM map and  $|F_{\text{calc}}|$  is the amplitude of calculated reflections from the coordinates.

† Fourier Shell Correlation,  $\text{FSC}_{\text{overall}} = \sum(N_{\text{shell}} \text{FSC}_{\text{shell}}) / \sum(N_{\text{shell}})$ , where  $\text{FSC}_{\text{shell}}$  is the FSC in a given shell,  $N_{\text{shell}}$  is the number of 'structure factors' in the shell.  $\text{FSC}_{\text{shell}} = \sum(F_{\text{model}} F_{\text{EM}}) / (\sqrt{\sum(|F_{\text{model}}|^2)}) \sqrt{\sum(|F_{\text{EM}}|^2)}$ .