



A de novo designed coiled coil-based switch regulates the microtubule motor kinesin-1

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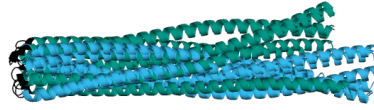
Table S1: Coiled coil register assignment of elbow designs used in this study		
Kif5C CC2 674→ <i>abcdefg abcd</i> EKMHEVS FQDK	Elbow	CC3 → 698 <i>cd abcdefg</i> TR LQDAEEV
Kinesin ^{WT}	EKEHL	
Kinesin ^{Elbow Lock}	-	
Register ^{CC2/CC3/AB}	<i>ef gabcdef gabcdef gabcdef gab</i>	
Kinesin ^{EL-CC-Di-IR}	KQ EIAAIKK ENAALKW EIAALKQ EIA	
Kinesin ^{EL-A-IR}	EQ ELAALDQ EIAAAEQ ELAALDW QIQ	
Kinesin ^{EL-A-Gly}	GGG QLEQ ELAALDQ EIAAAEQ ELAALDW QIQ GGG	
Register ^{CC2/CC3}	<i>efgabcd efgabcd efgabcd efgabcd efgab</i>	
Kinesin ^{EL-A-Ala}	AAAQLEQ ELAALDQ EIAAAEQ ELAALDW QIQAA	
Register ^{AB}	<i>cdef gabcdef gabcdef gabcdef gab</i>	
Peptide-B	G QLKQ RRAALKQ RIAALKQ RRAALKW QIQ G	

Table S2. Size distribution parameters D_{max} and R_g values obtained from pair distance distribution ($P(r)$) analysis		
Elbow Design	D_{max} (nm)	R_g (nm)
Kinesin ^{WT: Lamda}	45.0	13.8
Kinesin ^{WT: Open}	72.9	20.8
Kinesin ^{Delta Elbow}	74.5	22.2
Kinesin ^{Elbow Lock}	38.3	10.6
Kinesin ^{EL-CC-Di-IR}	70.0	21.5
Kinesin ^{EL-A-Ala}	40.0	13.1
Kinesin ^{EL-A-Ala + Peptide B}	69.7	20.9

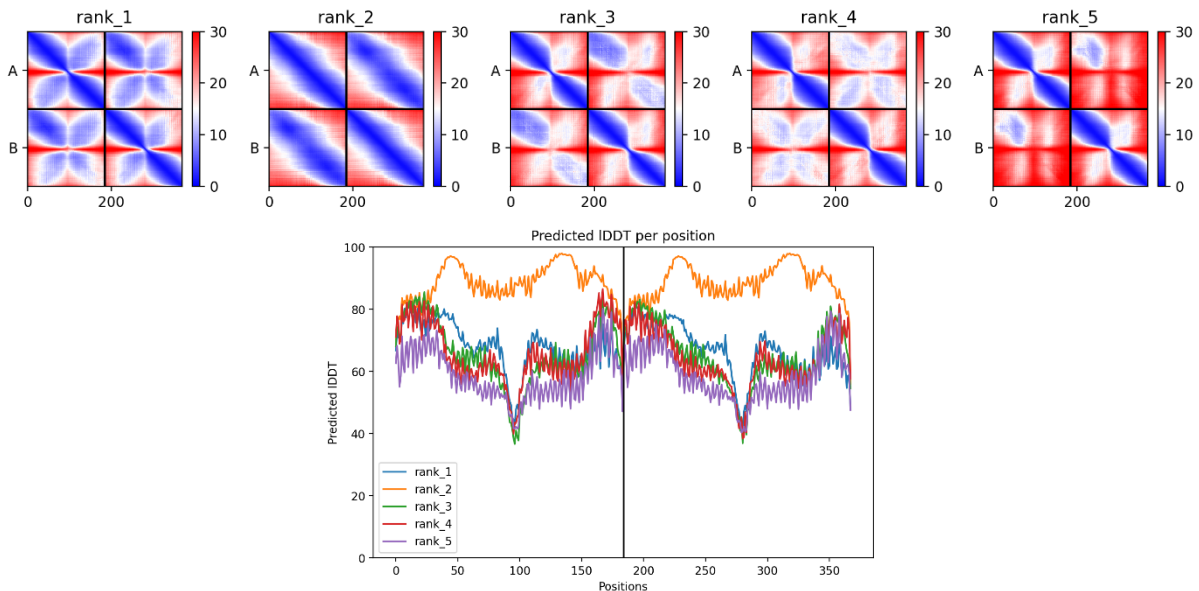
Table S3. SEC-SAXS Data collection parameters

Source Type	Bending magnet
Beamline	B21, biological X-ray scattering
Detector	Eiger 4M (Dectris)
Beam size at the focal point at detector (FWHM)	34 x 40 μm (horizontal vertical)
Beam size at sample	01102 x 240 μm
Wavelength range	0.89 – 1.3 \AA
Mode/Column	SEC/Superose 6 (10/300)
Exposure time	3 s

Ranks 1,3,4,5



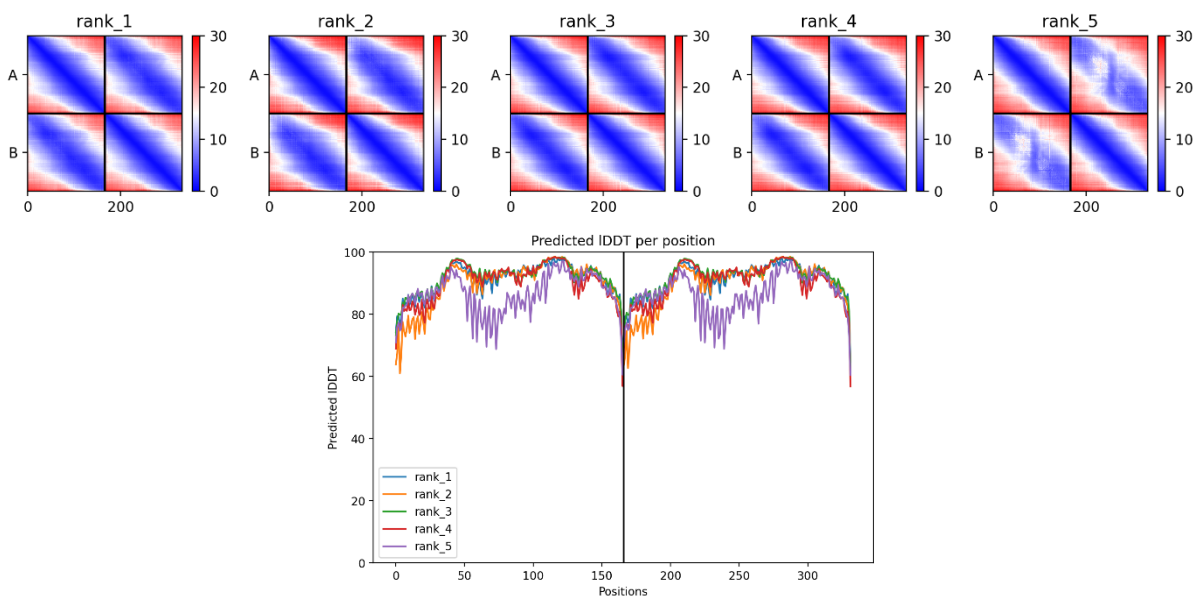
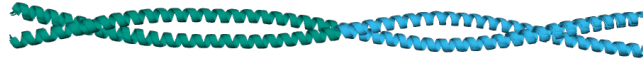
Rank 2



Sequence:

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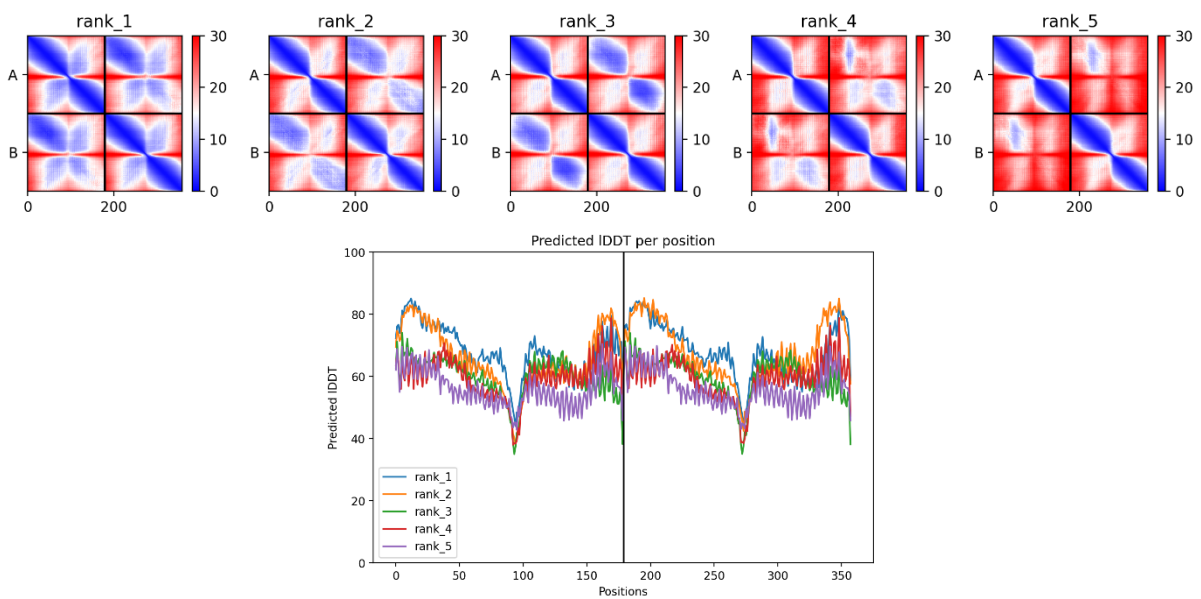
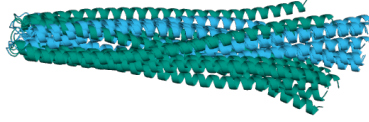
Fig. S1. Alignment of AlphaFold2 models of Kinesin-1^{WT} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. 4 aligned models (ranks 1,3,4,5) of Kinesin-1^{WT} CC2-elbow-CC3 have a folded structural prediction with a flexible elbow (black) between CC2 (teal) and CC3 (cyan) The remaining model predicts an extended coiled coil conformation (rank 2).



Sequence:

```
iskmksevkslvnrskqlesaqtdsnrkmnaserelaacqllisqheakiksltdymqnmeqkrrqle
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eqerlssdynklkiedqerevkleklillln
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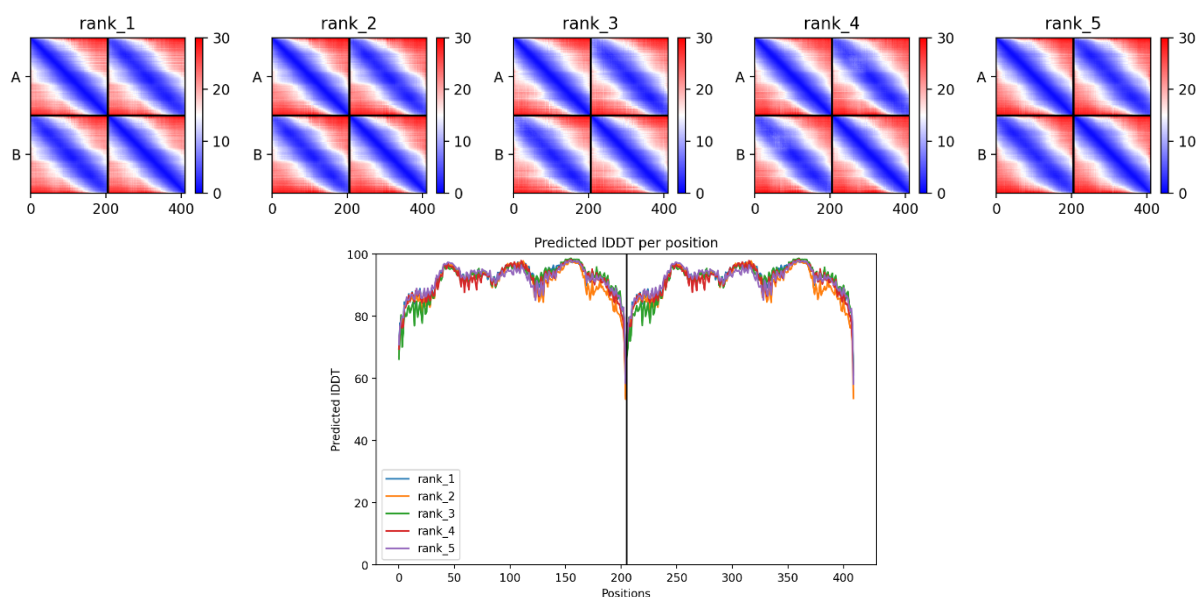
Fig. S2. Alignment of AlphaFold2 models of Kinesin-1^{Delta Elbow} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. All five models closely overlay with the same extended coiled coil structural prediction, CC2 (teal) and CC3 (cyan).



Sequence:

```
iskmksevkslvnrskqlesaqtdsnrkmnaserelaacqllisqheakiksltdymqnmeqkrrqle
esqdslseelaklraqekmhevsvfdktrlqdaeevkkaleqqmeshreahqkqlsrlrdeieekqri
ideirdlnqklqleqerlssdynklkiedqerevklekl1111n
```

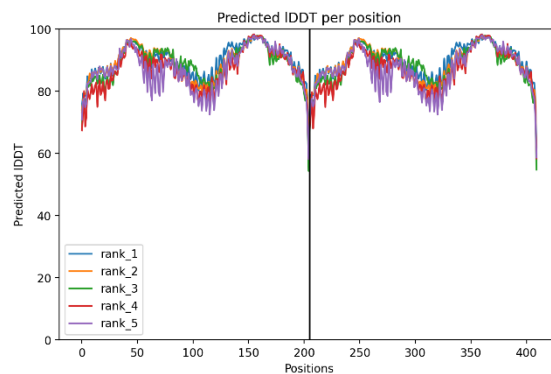
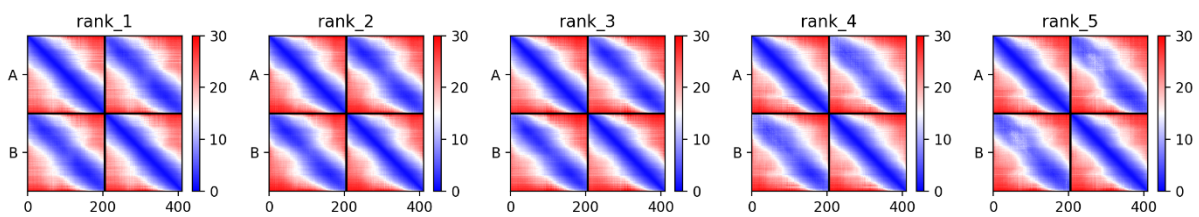
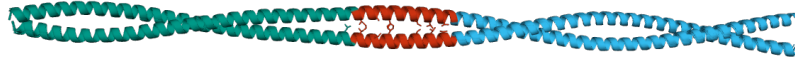
Fig. S3. Alignment of AlphaFold2 models of Kinesin-1^{Elbow Lock} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. All five models have a folded structural prediction with a flexible hinge between CC2 (teal) and CC3 (cyan).



Sequence:

```
iskmksevkslvnrskqlesaqtdsnrkmnaserelaacqlisqheakiksltdymqnmeqkrrqle
esqdslseelaklraqekmhevsvqdkKQEIAAIAIKKENAALKWEIAALKQEIAtrlqdaeevkkaleq
qmeshreahqkqlsrldrdeieekqriideirdlnqklqleqerlssdynkklkiedqerevklekl111
n
```

Fig. S4. Alignment of AlphaFold2 models of Kinesin-1^{EL-CC-Di-IR} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. All five models closely overlay with the same extended coiled coil structural prediction, CC2 (teal) and CC3 (cyan) with CC-Di in grey. Side chains are shown as sticks for the hydrophobic core of CC-Di.



Sequence:

```
iskmksevkslvnrskqlesaqtdsnrkmnaserelaacqllisqheakiksltdymqnmegkrrqle
esqdslseelaklraqekmhevsvfjdkEQELAALDQEIAAAEQELAALDWQIQtrlqdaeevkkaleq
qmeshreahqkqlsrldrdeieekqriideirdlnqklqleqerlssdynklkiedqerevklekl111
n
```

Fig. S5. Alignment of AlphaFold2 models of Kinesin-1^{EL-pA-IR} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. All five models closely overlay with the same extended coiled coil structural prediction, CC2 (teal) and CC3 (cyan) with pA in red. Side chains are shown as sticks for the hydrophobic core of pA.

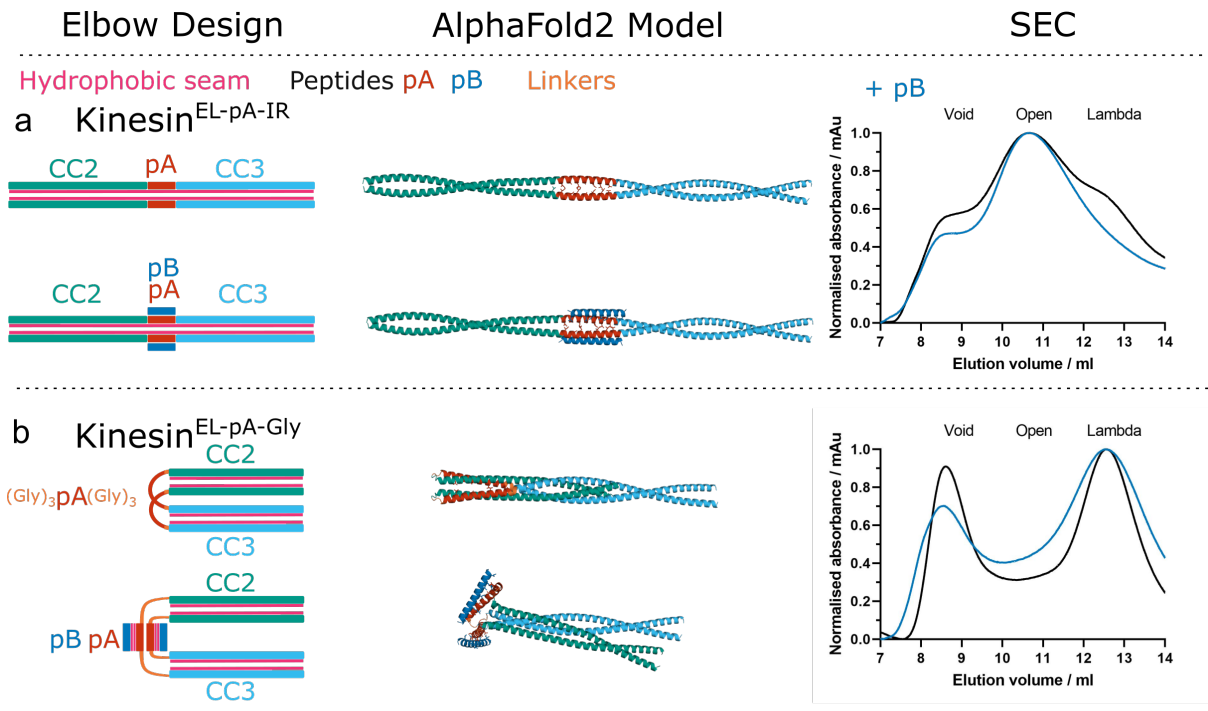
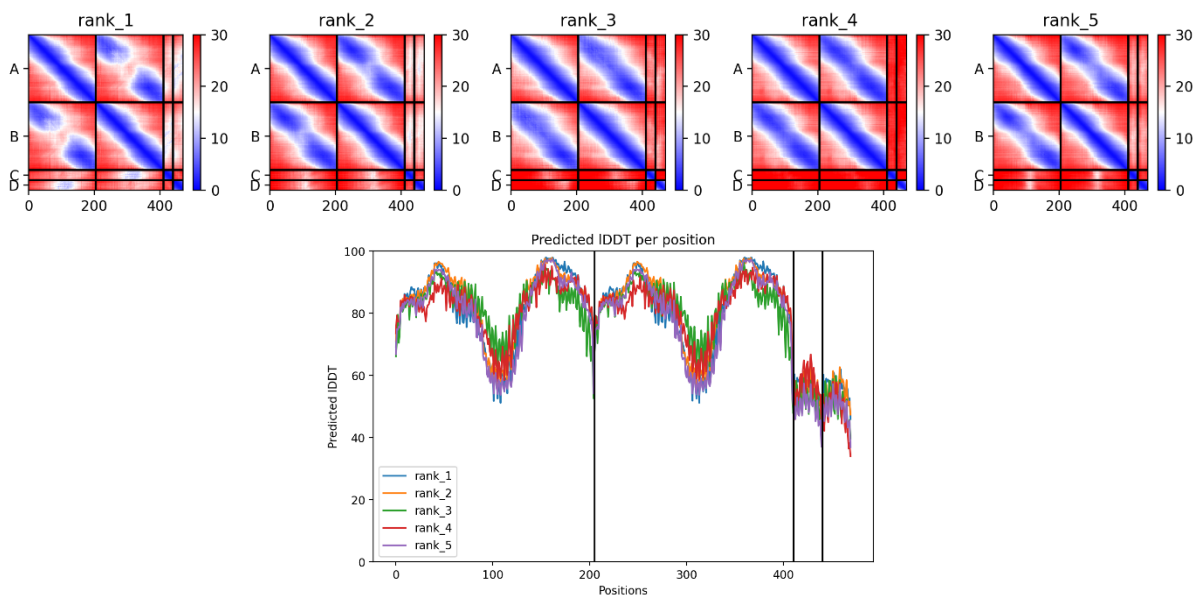
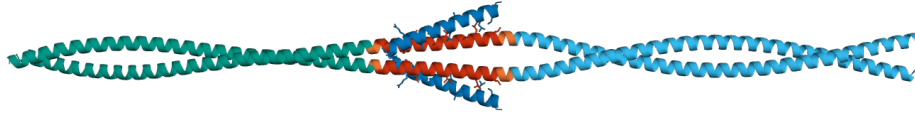


Fig. S6. Designing an allosteric switch into kinesin-1 that is activated by a *de novo* designed peptide. From left to right: cartoon illustrations of each elbow design without (top) and with (bottom) peptide pB; AlphaFold2 models of KHC for the CC2-linker-CC3 region without (top) and with (bottom) peptide pB; and size-exclusion chromatography (SEC) elution profiles of purified heterotetrameric KHC-KLC complexes without (black) and with (blue) peptide pB. Colour scheme for the structural cartoons: KHC CC2 teal, CC3 cyan; hydrophobic seams/cores, magenta; peptide pA, red; linkers, orange; and peptide pB, blue. **a.** Insertion of pA in register (IR) between CC2 and CC3 drives helical read through and favours the open state without or with pB. **b.** Insertion of pA between flexible (Gly)₃ linkers after CC2 and before CC3 favours the lambda particle without or with pB.



Sequence:

iskmksevkslvnrskqlesaqtdsnrkmnaserelaacqllisqheakiksltdymqnmeqkrrqle
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 qmeshreahqkqlsrlrdeieekqriideirdlnqklqleqerlssdynklkiedqerevklekl
 llll
 n

Peptide pB: [GQLKQRRRAALKQRIAALKQRRRAALKWQIQG](#)

Fig. S7. Alignment of AlphaFold2 models of Kinesin-1^{EL-pA-IR} CC2-elbow-CC3 with peptide pB with corresponding PAE and pLDDT plots and sequences. All five models of Kinesin-1^{EL-pA-IR} CC2-elbow-CC3 closely overlay with the same extended coiled coil structural prediction, CC2 (teal) and CC3 (cyan) with pA in red. The placement of peptide pB (blue) is more varied. Side chains are shown as sticks for the hydrophobic core of pA and pB.

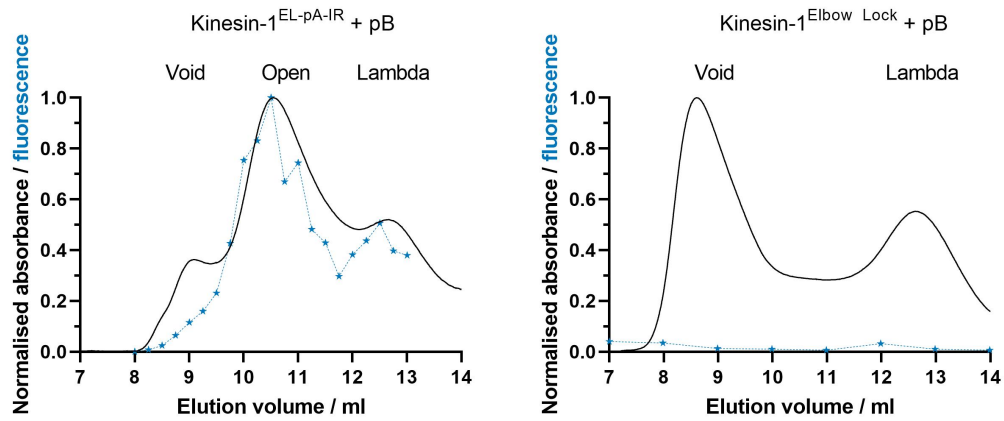
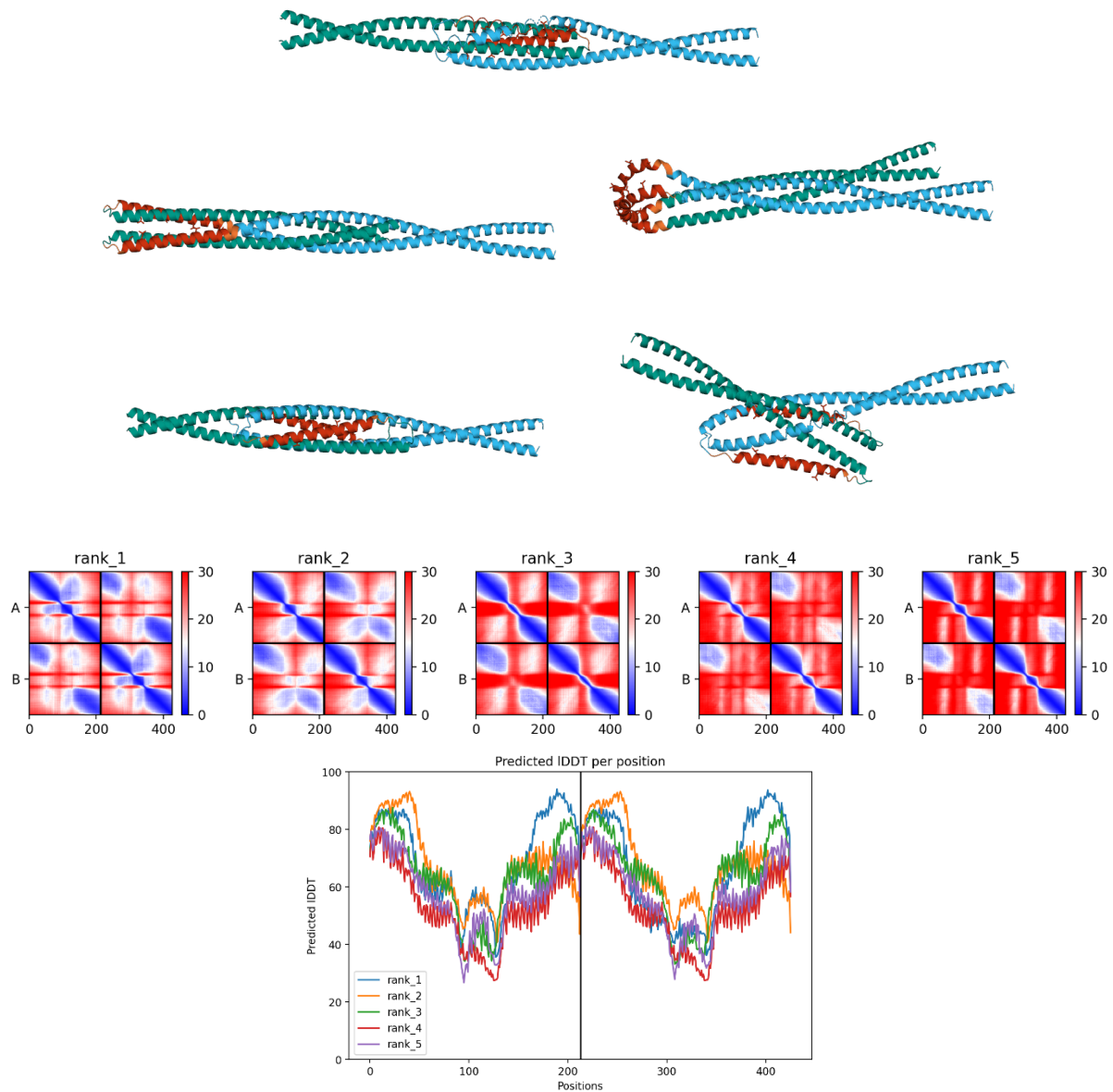


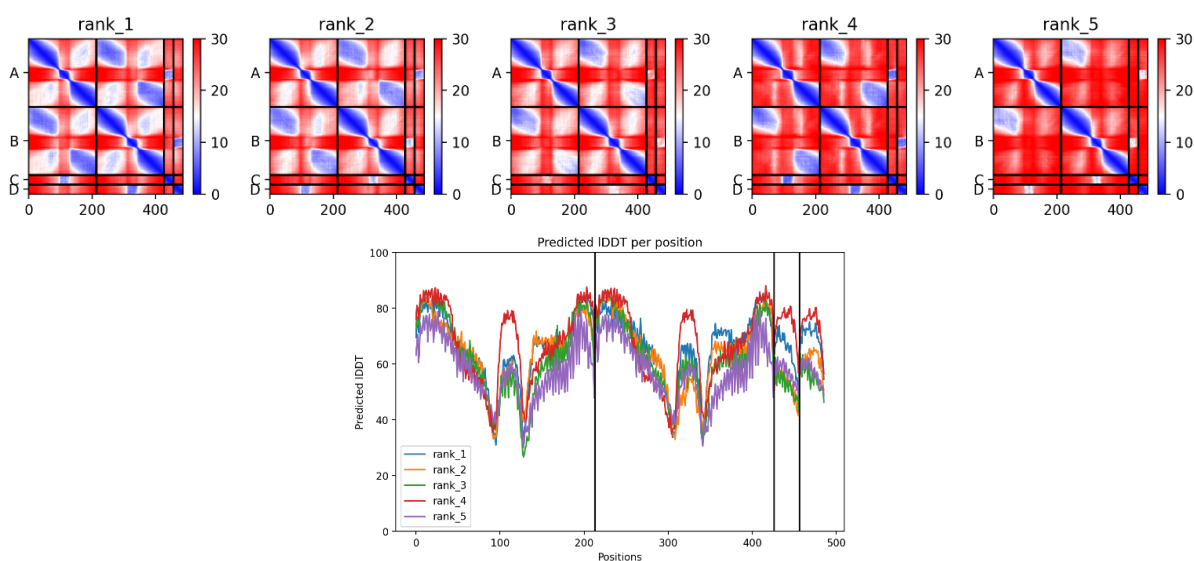
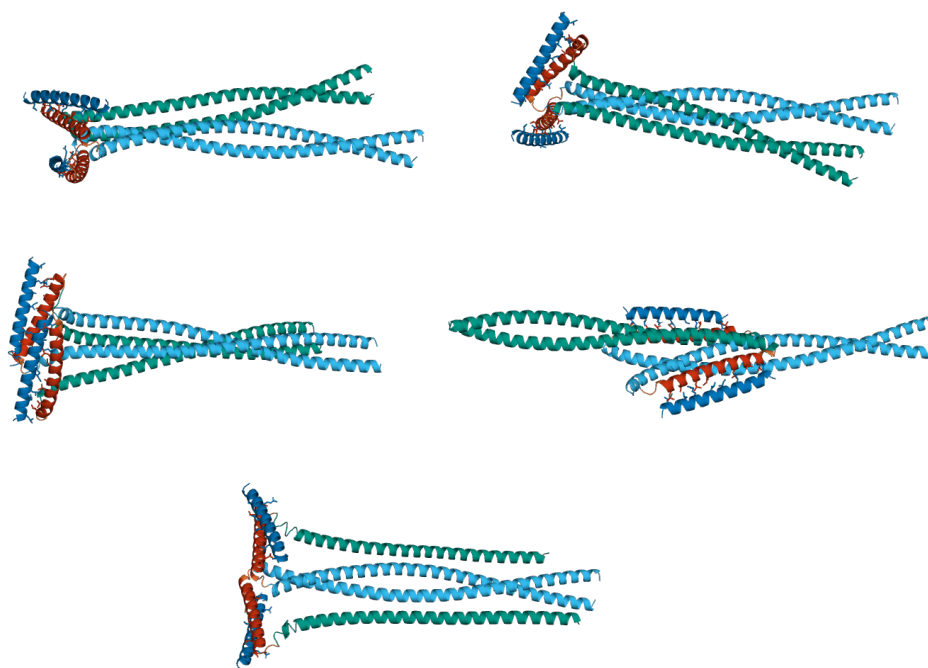
Fig. S8. Binding of peptide pB to Kinesin-1^{EL-pA-IR} is observed by fluorescence in SEC (left) compared to no binding to Kinesin-1^{Elbow Lock} (right). Peptide pB was added to purified Kinesin-1 (Kif5C, KLC1) protein at 4°C for 1h with agitation and then subject to SEC. Elution of protein in SEC was monitored by absorbance at 280 nm and normalised (black curve). Presence of peptide-B was monitored by fluorescence of TAMRA at 555 nm in individual fractions using a plate reader and normalised (blue stars). Coelution of peptide pB and Kinesin-1^{EL-pA-IR} indicates binding.



Sequence:

ismksevkslvnrskqlsaqtdsnrkmnaserelaacqllisqheakiksltdymqnmeqkrrqle
 esqdslseelaklragekmhevsvfkdGGGQLEQELAALDQEIAAAEQELAALDWQIQGGGtrlqdae
 evkkaleqqmeshreahqkqlsrldrdeieekqrideirdlnqklqleqerlssdynklkiedqerev
 klekl111n

Fig. S9. AlphaFold2 models of Kinesin-1^{EL-pA-Gly} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. The five models vary but all have a folded structural prediction with a flexible hinge between CC2 (teal) and CC3 (cyan) with pA in red, and GGG linkers in yellow. Side chains are shown as sticks for the hydrophobic core of pA.



Sequence:

iskmksevkslvnrskqlesaqtdsnrkmnaserelaacqllisqheakiksltdymqnmeqkrrqle
 esqdslseelaklragekmhevsvfdkGGGQLEQELAALDQEIAAAEQELAALDWQIQGGGtrlqdae
 evkkaleqqmeshreahqkqlsrlrdeieekgriideirdlnqklqleqerlssdynkklkiedqerev
 klekl1111n

Peptide pB: [GQLKQRRRAALKQRIAALKQRRRAALKWQIQG](#)

Fig. S10. AlphaFold2 models of Kinesin-1^{EL-pA-Gly} CC2-elbow-CC3 with peptide B with corresponding PAE and pLDDT plots and sequences The five models vary but all have a folded structural prediction with a flexible hinge between CC2 (teal) and CC3 (cyan) accommodating pA (red) binding to pB (blue). GGG linkers are in yellow and side chains are shown as sticks for the hydrophobic core of pA and pB.

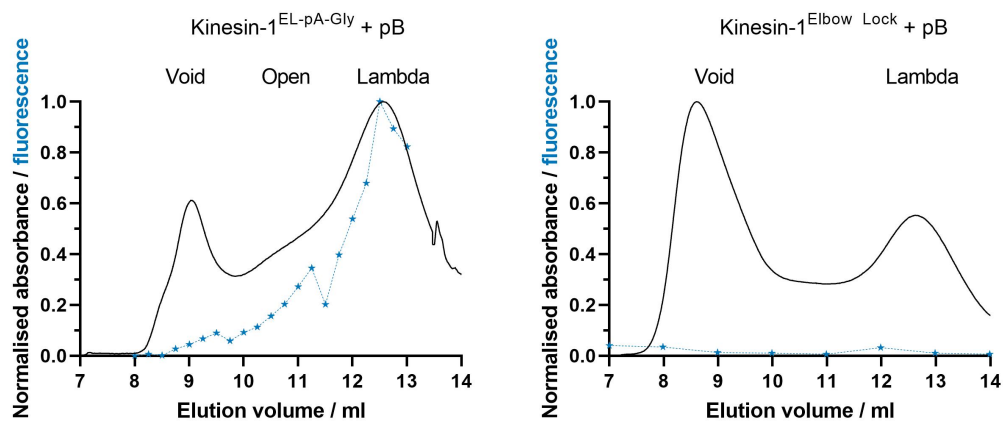
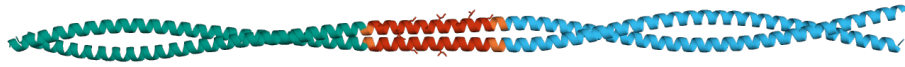
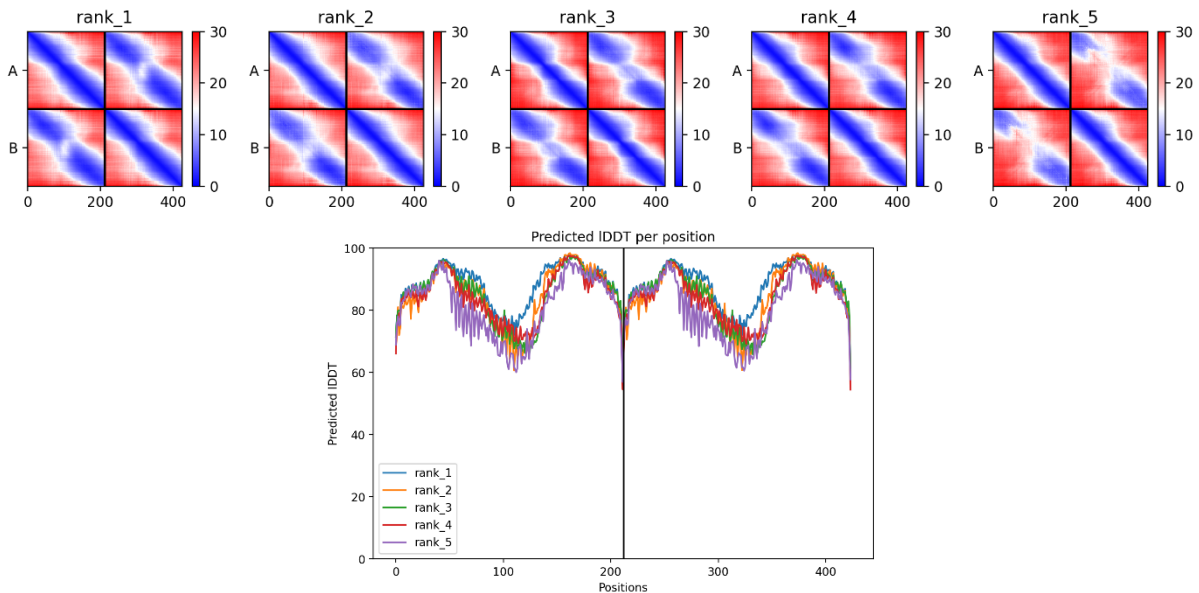
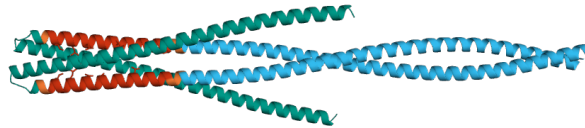


Fig. S11. Binding of peptide pB to Kinesin-1^{EL-pA-Gly} is observed by fluorescence in SEC (left) compared to no binding to Kinesin-1^{Elbow Lock} (right). Peptide pB was added to purified Kinesin-1 (Kif5C, KLC1) protein at 4°C for 1h with agitation and then subject to SEC. Elution of protein in SEC was monitored by absorbance at 280 nm and normalised (black curve). Presence of peptide-B was monitored by fluorescence of TAMRA at 555 nm in individual fractions using a plate reader and normalised (blue stars). Coelution of peptide pB and Kinesin-1^{EL-pA-Gly} indicates binding.

Ranks 1-4



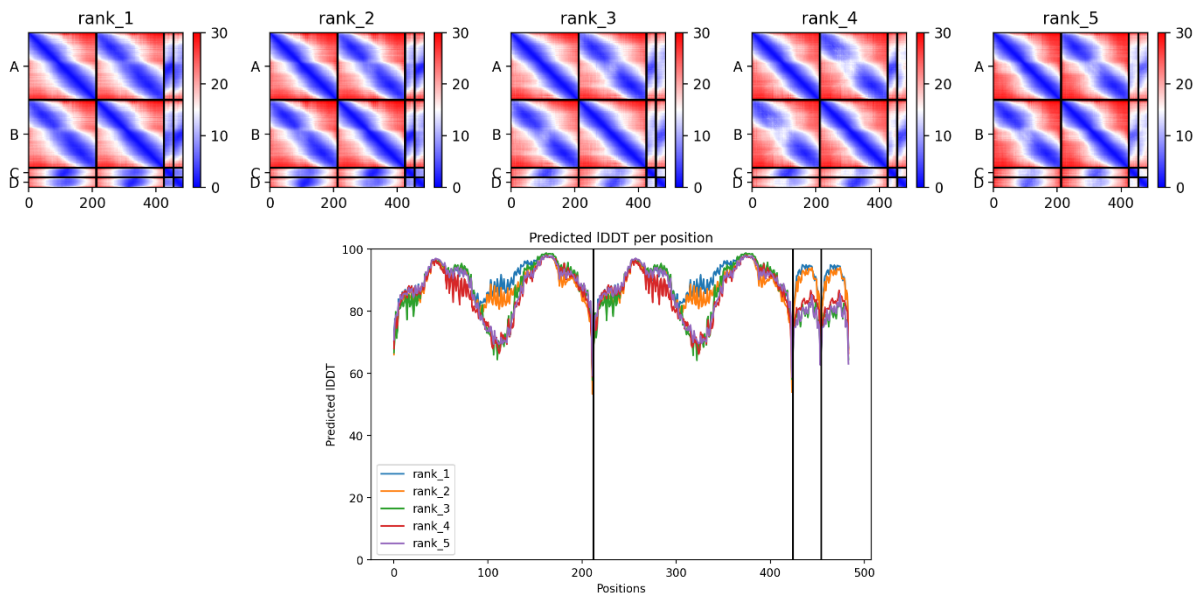
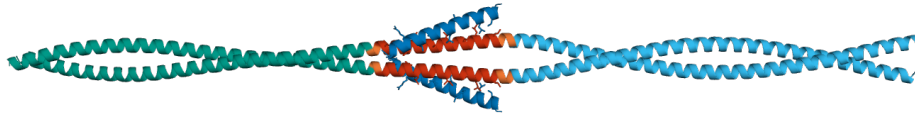
Ranks 5



Sequence:

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 vkkaleqqmeshreahqqlsrlrdeieekqriideirdlnqklqleqerlssdynklkiedqerevk
 lekllllln

Fig. S12. Alignment of AlphaFold2 models of Kinesin-1^{EL-pA-Ala} CC2-elbow-CC3 with corresponding PAE and pLDDT plots and sequence. 4 aligned models (ranks 1-4) of Kinesin-1^{EL-pA-Ala} CC2-elbow-CC3 closely overlay with the same extended coiled coil structural prediction of CC2 (teal) and CC3 (cyan) with peptide-A in red and AAA/AA linkers in yellow. The final model (rank 5) predicts a folded conformation. Side chains are shown as sticks for the hydrophobic core of pA.



Sequence:

Iskmksevkslvnrskqllesaqt dsnrkmnaserelaacql lisqheakiksltdymqnmeqkrrqle
 esqds lseelaklraqekmhev sfqdk **AAAQLEQEL AALDQEIAAAEQEL AALDWQIQAA**trlqdaee
 vkkaleqqmeshreahqqlsr lrdeieekqriideirdlnqklqleqerlssdynk lkiedqerevk
 leklllln

Peptide pB: [GQLKQRRRAALKQRIAALKQRRRAALKWQIQG](#)

Fig. S13. Alignment of AlphaFold2 models of Kinesin-1^{EL-pA-Ala} CC2-elbow-CC3 with peptide B with corresponding PAE and pLDDT plots and sequences. All five models of Kinesin-1^{EL-pA-IR} CC2-elbow-CC3 and peptide pB closely overlay with the same extended coiled coil structural prediction of CC2 (teal) and CC3 (cyan) with pA in red, peptide pB in blue and AAA/AA linkers in yellow. Side chains are shown as sticks for the hydrophobic core of pA and pB.

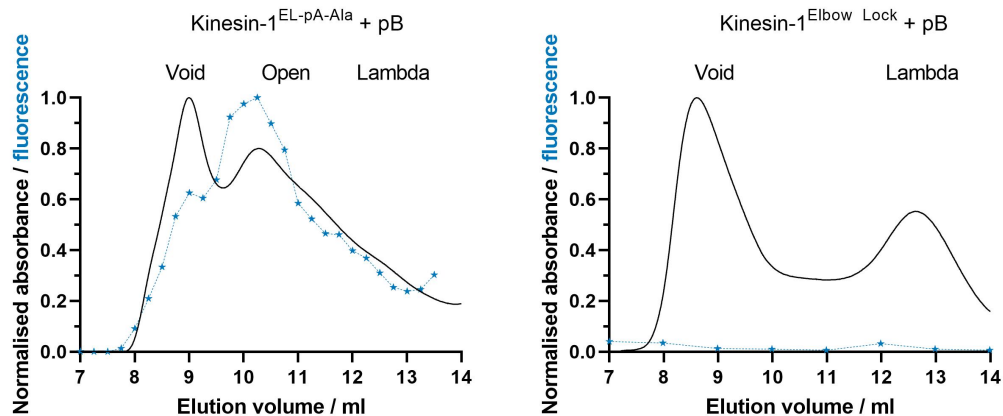


Fig. S14. Binding of peptide pB to Kinesin-1^{EL-pA-Ala} is observed by fluorescence in SEC (left) compared to no binding to Kinesin-1^{Elbow Lock} (right). Peptide-B was added to purified Kinesin-1 (Kif5C, KLC1) protein at 4°C for 1h with agitation and then subject to SEC. Elution of protein in SEC was monitored by absorbance at 280 nm and normalised (black curve). Presence of peptide pB was monitored by fluorescence of TAMRA at 555 nm in individual fractions using a plate reader and normalised (blue stars). Coelution of peptide pB and Kinesin-1^{EL-pA-Ala} indicates binding.

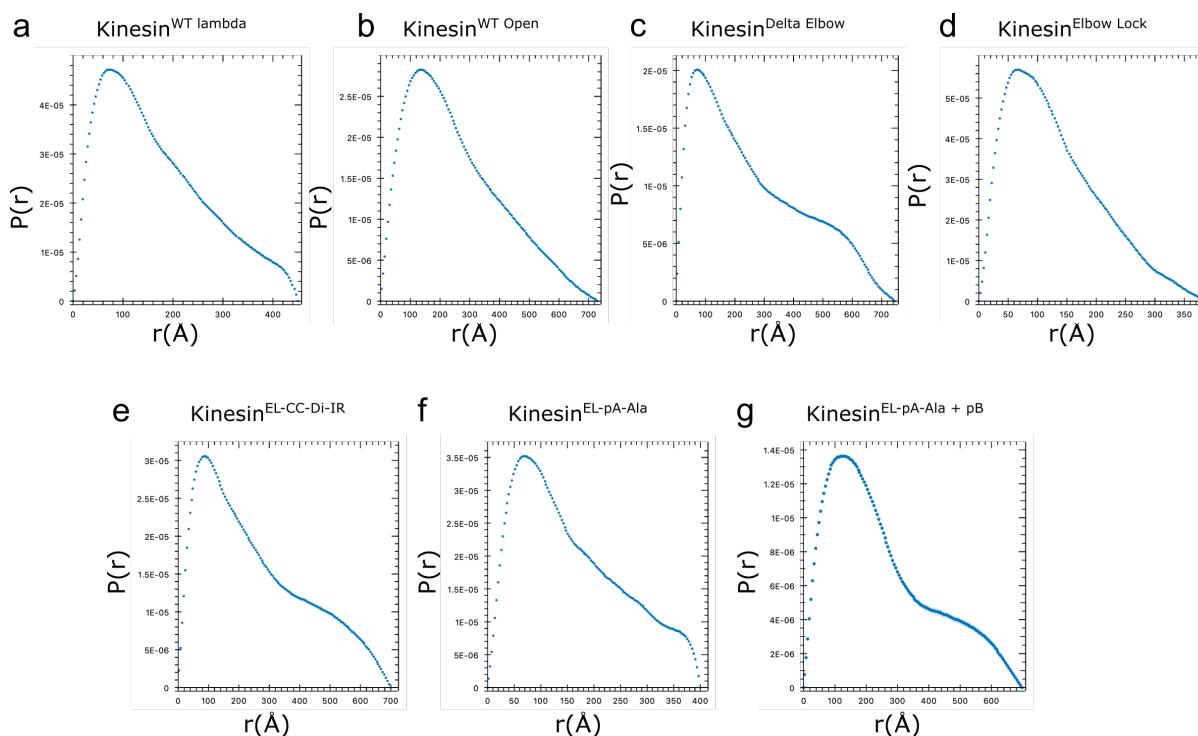


Fig. S15. Pair distance distribution plots ($P(r)$) for kinesin-1 variants from SEC-SAXS scattering data. a. Kinesin-1^{WT} Lambda, b. Kinesin-1^{WT} Open, c. Kinesin-1^{Delta} Elbow, d. Kinesin-1^{Elbow Lock}, e. Kinesin-1^{EL-CC-Di-IR}, f. Kinesin-1^{EL-pA-Ala}, g. Kinesin-1^{EL-pA-Ala + pB}.

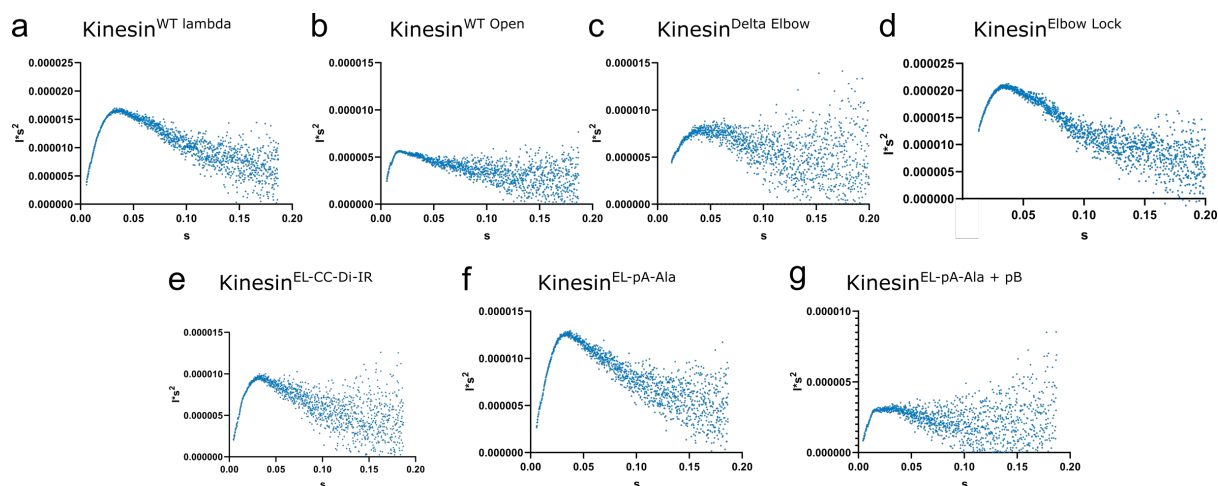


Fig. S16. Kratky plot indicated flexibility in all the kinesin-1 complexes from SEC-SAXS scattering data. Kratky plots give a qualitative assessment of globularity or flexibility in samples. Whilst globular proteins are expected to converge with a gaussian peak, these plots show a plateau at high s indicating flexibility in the complex. **a.** Kinesin-1^{WT} Lambda, **b.** Kinesin-1^{WT} Open, **c.** Kinesin-1^{Delta} Elbow, **d.** Kinesin-1^{Elbow Lock}, **e.** Kinesin-1^{EL-CC-Di-}, **f.** Kinesin-1^{EL-pA-Ala}, **g.** Kinesin-1^{EL-pA-Ala + pB}.

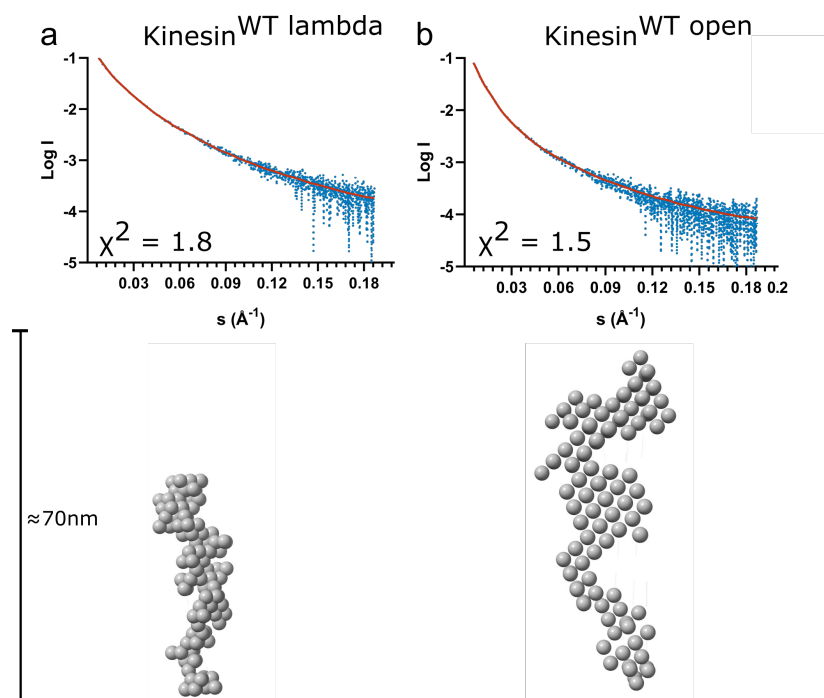


Fig. S17. SEC-SAXS in-solution shape determination and conformational states of Kinesin-1^{WT}. Top: Comparison of trimmed experimental scattering profiles (blue) with theoretical scattering profiles (red) generated from ab-initio models using DAMMIN. The quality of fit is assessed using the χ^2 statistic. Bottom: Representative models obtained from DAMMIN illustrating the shapes of the kinesin-1 variants, **a.** Kinesin-1^{WT} lambda is in the lambda state $D_{\max} = 45.0$ nm $\chi^2=1.8$, **b.** Kinesin-1^{WT} open is in the open state $D_{\max} = 72.9$ nm $\chi^2 = 1.5$.

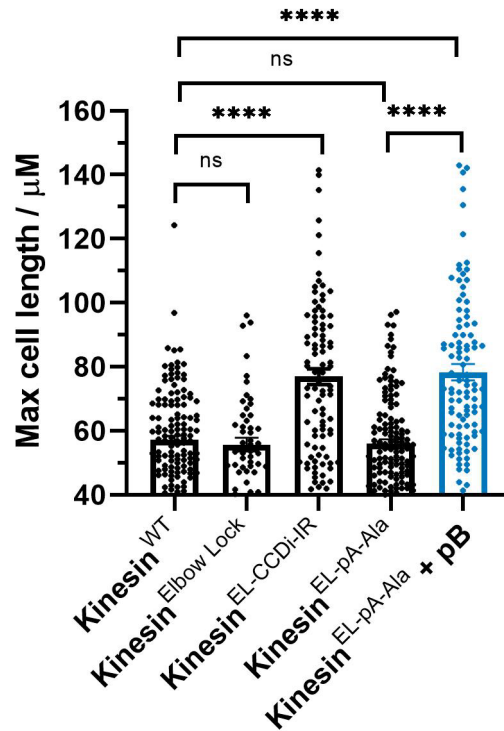


Fig. S18. Quantification of HeLa cell length in cells transfected with kinesin constructs. As in figure 4, HeLa cells were transfected with GFP-KLC2 and Ha-Kif5C (Kinesin-1^{WT}, Kinesin-1^{Elbow Lock}, Kinesin-1^{EL-CC-Di-IR} or Kinesin-1^{EL-pA-Ala}) with (blue) /without (black) treatment with 2 μM TAMRA-labelled pB for 1 h. The maximum length of each transfected cell was measured for a minimum of 52 cells for each construct, pooled across 3 independent experiments. There is no significant difference between the length of cells transfected with Kinesin-1^{WT}, Kinesin-1^{Elbow Lock} and Kinesin-1^{EL-pA-Ala}. By contrast, cells transfected with Kinesin-1^{EL-CC-Di-IR} or Kinesin-1^{EL-pA-Ala} and treated with peptide B are extended with a longer maximum length. Data are presented as individual values with bars showing mean ± S.E.M. and an unpaired two-tailed t-test is used for statistical analysis. ns: not significant ****: Kinesin-1^{WT} vs Kinesin-1^{EL-pA-Ala + pB} $P = 1.87 \times 10^{-13}$, Kinesin-1^{WT} vs Kinesin-1^{EL-CC-Di-IR} $P = 6.55 \times 10^{-13}$, Kinesin-1^{EL-pA-Ala} vs Kinesin-1^{EL-pA-Ala + pB} $P = 4.09 \times 10^{-15}$.