

Supporting Information for
The Nup62 Coiled-Coil Motif Provides Plasticity for
Triple Helix Bundle Formation.

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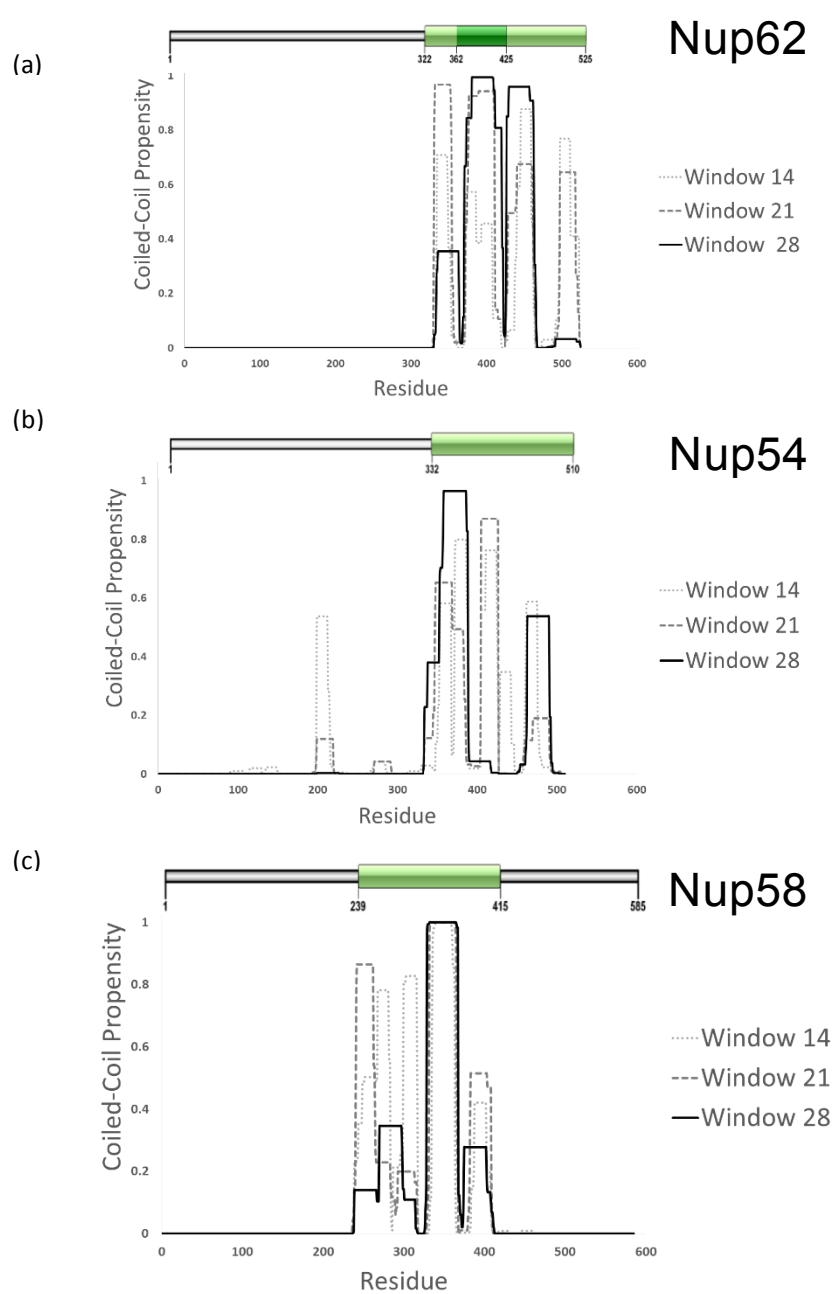


Figure S1: Coiled-coil domains of channel Nups: Domain organization of rat (a) Nup62, (b) Nup54 and (c) Nup58 with the FG/unstructured regions as grey bars and structured region as green bars. The coiled-coil propensity using three different windows (14, 21 and 28) for the proteins were calculated using COILS server.

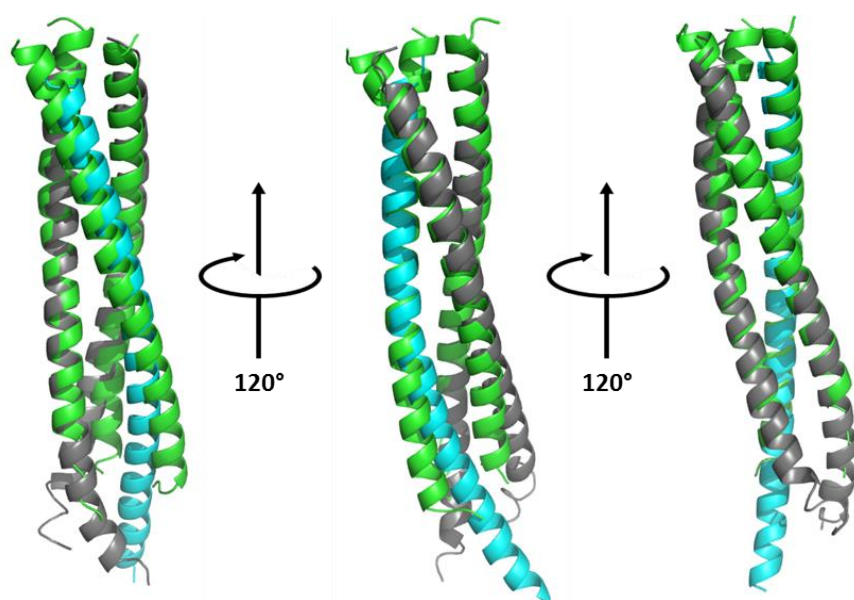


Figure S2: Superimposition of rat Nup62(362-425) homotrimer (PDB:5H1X) on rat Nup62(362-425)•Nup54(346-407) heterotrimer (PDB:3T97). LSQ superimposition of rat Nup62(362-425) homotrimer (Nup62 chains are shown in green) on rat Nup62(362-425)•Nup54(346-407) heterotrimer (Nup62 chains are shown in Gray and Nup54 in light blue) in COOT. 120° left rotated view is depicted. The RMSD of superimposition is 1.93 Å.

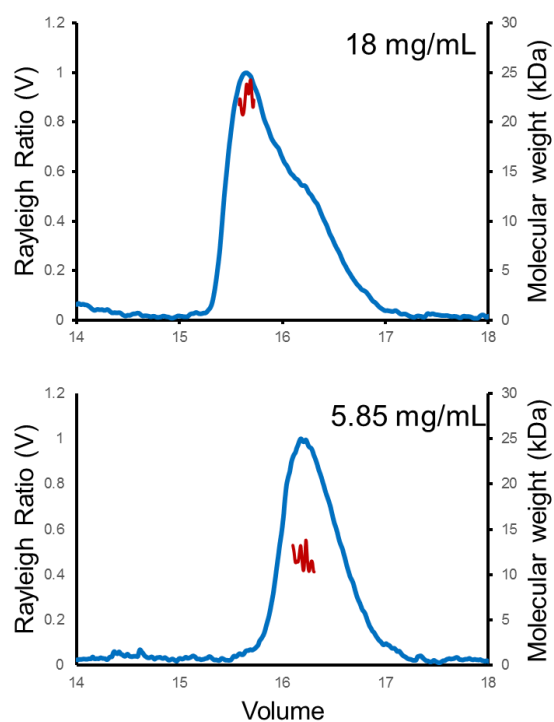


Figure S3: Size exclusion chromatography coupled Multi-angle Light Scattering (SEC-MALS) analysis. SEC-MALS analysis of rat Nup62(362-425) at 18 mg/mL (top) and 5.85 mg/mL (bottom). Red colored lines indicate averaged molecular masses (Mw) of Nup62(362-425) protein at two different concentrations.

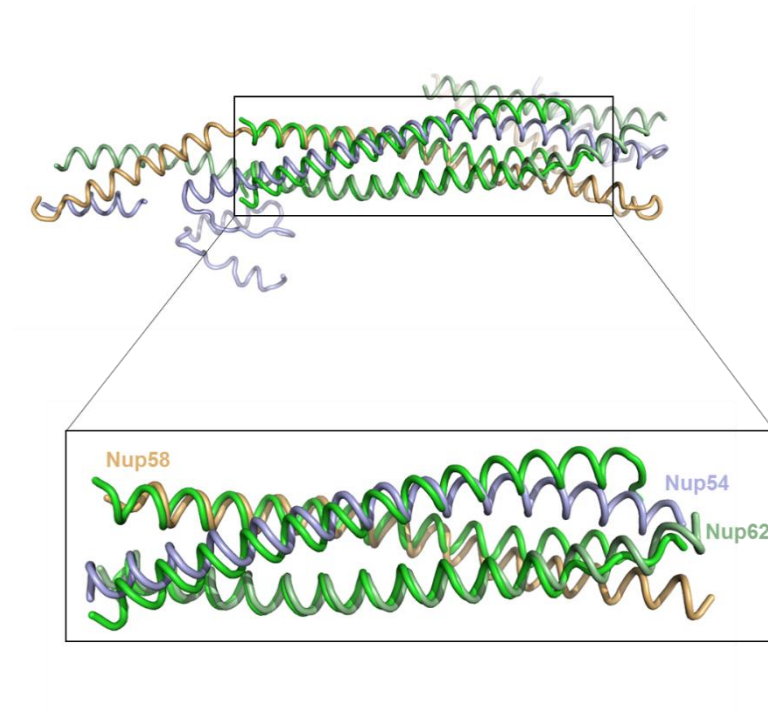


Figure S4: Superimposition of rat Nup62(362-425) homotrimer (PDB ID: 5H1X) on *Xenopus* CTC subcomplex (PDB ID: 5C3L). Nup62(362-425) (shown in green) homotrimer was superimposed (LSQ method) with CTC subcomplex (pale green – Nup62, pale blue – Nup54 and pale orange – Nup58) in COOT. The RMSD of superimposition is 1.94 Å. The names of *Xenopus* Nups are shown in the image.

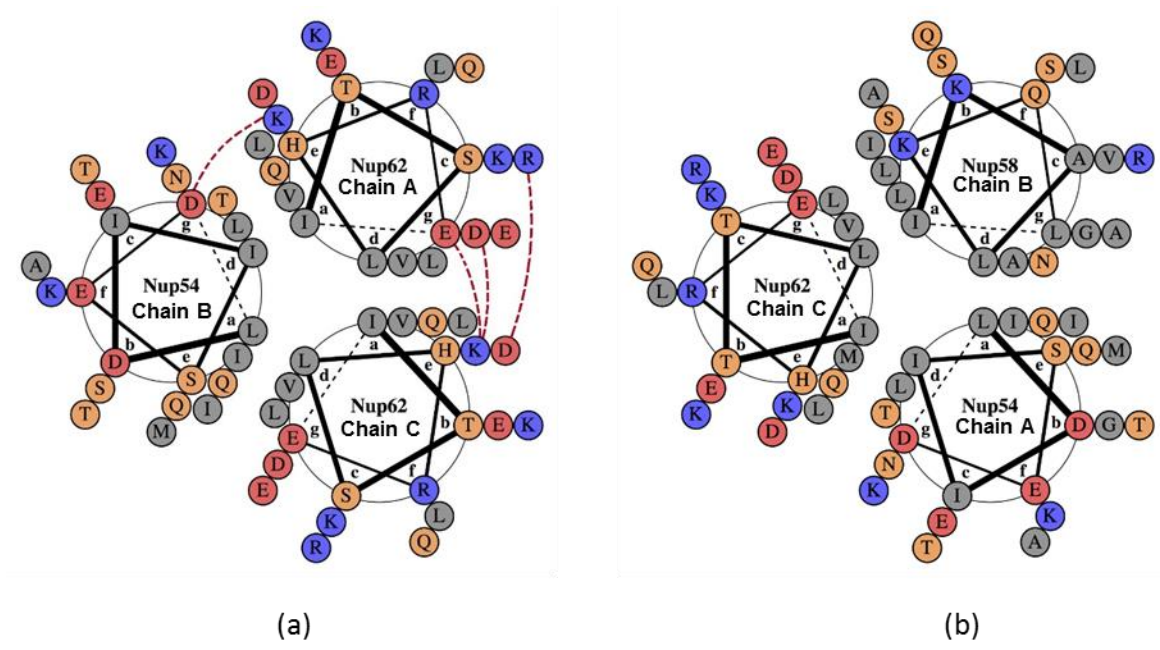


Figure S5: Helical wheel representation of the coiled-coil sequence for rat Nup62(362-425)•Nup54(346-407) heterotrimer and *Xenopus* CTC sub-complex (a) Rat Nup62(362-425)•Nup54(346-407) heterotrimer (adapted from) and (b) *Xenopus* CTC sub-complex. Color coding is same as in Figure 1c.

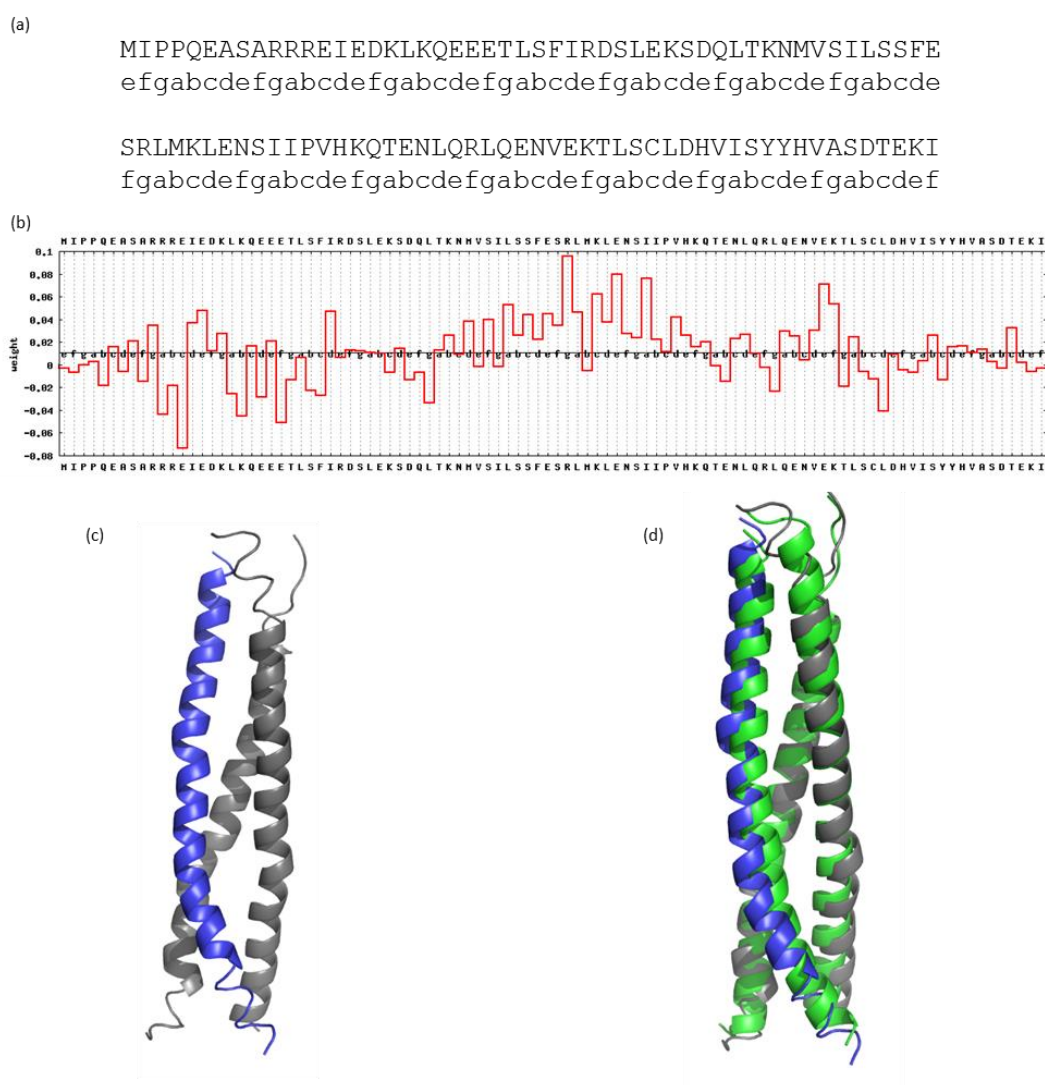


Figure S6: Modelling of rat Exo70(33-81) coiled-coil region with rat Nup62(362-425). (a) Heptad register for rat Exo70(1-100) region. (b) PrOCoil coiled-coil prediction program output for rat Exo70(1-100) region. (c) The modelled structure of Nup62(362-425)•Exo70(33-81) complex. Depicted in grey: rat Nup62(362-425) chains, deep blue: Exo70(33-81) (d) Superimposition of the Nup62(362-425)•Exo70(33-81) geometry minimized model and Nup62(362-425) homotrimer crystal structure (PDB ID: 5H1X). Green: rat Nup62(362-410) homotrimer. Color coding for the Nup62(362-425)•Exo70(33-81) heterotrimer model is same as in ‘c’.

(a) GST-Exo70 (1-100)

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKL
TQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML
KMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKS
SKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPEFPGRQMI PPQEASARRREIEDKLKQEEETLSF
IRDSLEKSDQLTKNMVSISSFESRLMKLENSIIPVHKQTENLQRLQENVEKTLSCLDHVISYYH
VASDTEKIKRPHRD

(b) GST-Exo70 (100-384)

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKL
TQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML
KMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKS
SKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPEFPGRQIREGPTGRLEEYLGSMKIQKAVEYFQ
DNSPDSPENKVKLLFERGKESLESEFRSLMTRHSKVISPVLVLDLISADDELEVQEDVVLEHLP
ESVLQDVIRISRWLVEYGRNQDFMNYYYQIRSSQLDRSIKGLKEHFRKSSSSSGVPYSPAIPNKR
KDTPTKKPIKRPGRDDMLDVETDAYIHCVSFAFVRLAQSEYQLLMGIIPEHHQKKTFDLSLIQDALD
GLMLEGENIVSAARKAIIRHDFSTVLTVPFILRHLKQTKPEFDQVLQGTAASTKNKLPGLIT

Figure S7: Sequences of GST-fusions of Exo70 proteins used in this study. (a) Translated sequence of Exo70(1-100) and (b) Exo70(100-384) are colored in green. Black colored sequence represents GST from the vector, pGEX4T1.

Table S1: PISA analysis indicating salt bridges in various Nup62 complexes.

Nup62(362-425) homotrimer	Chain A (Nup62) with Chain C (Nup62)			Chain B (Nup62) with Chain C (Nup62)			Chain A (Nup62) with Chain B (Nup62)		
	Sr. No.	Chain A (Nup62)	Chain C (Nup62)	Sr. No.	Chain B (Nup62)	Chain C (Nup62)	Sr. No.	Chain A (Nup62)	Chain B (Nup62)
	1	GLU 381	LYS 386	1	ASP 393	ARG 391	1	ASP 393	ARG 391
	2	ARG 391	ASP 393	2	LYS 386	ARG 391	2	LYS 386	GLU 381
Nup62(362-425)•Nup54(346-407) complex	Chain B (Nup54) with Chain A(Nup62)			Chain C (Nup62) with Chain B (Nup54)			Chain A (Nup62) with Chain C (Nup62)		
	Sr. No.	Chain B (Nup54)	Chain A (Nup62)	Sr. No.	Chain C (Nup62)	Chain B (Nup54)	Sr. No.	Chain A (Nup62)	Chain C (Nup62)
	1	ASP 360	LYS 386	1	LYS 374	ASP 355	1	ARG 391	ASP 393
	2	LYS 381	GLU 407	2	GLU 395	LYS 379	2	GLU 381	LYS 386
	3	ARG 388	GLU 407						
	4	ARG 388	GLU 414						
Xenopus Nup62 subcomplex	Chain A (Nup54) with Chain C (Nup62)			Chain B (Nup58) with Chain A (Nup54)			Chain C (Nup62) with Chain B (Nup58)		
	1	ASP 385	LYS 408		-	-	1	GLU 417	LYS 346
	2	GLU 435	ARG 455						
	3	ARG 349	GLU 395						
	4	ARG 364	ASP 387						
	5	LYS 406	GLU 429						
	6	ARG 413	GLU 436						
	7	ARG 413	GLU 429						

Table S2: PISA analysis showing complex formation score and the effective solvation energies for complexes of Nup62.

Molecule	Interface	PISA Complex formation Significance Score (CSS)	Effective Solvation Energy (ΔG) kcal/mol
Rat Nup62 Homotrimer	Chain A (Nup62) – Chain B (Nup62)	0.659	-12.4
	Chain B (Nup62) – Chain C (Nup62)	0.659	-13.0
	Chain A (Nup62) – Chain C (Nup62)	0.341	-12.9
Rat Nup62-Nup54 heterotrimer	Chain A (Nup62) – Chain B (Nup54)	1.0	-14.0
	Chain B (Nup54) – Chain C (Nup62)	1.0	-17.2
	Chain A (Nup62) – Chain C (Nup62)	1.0	-19.2