

Supplemental Information

Quantitative Structural Analysis

of Importin- β Flexibility: Paradigm

for Solenoid Protein Structures

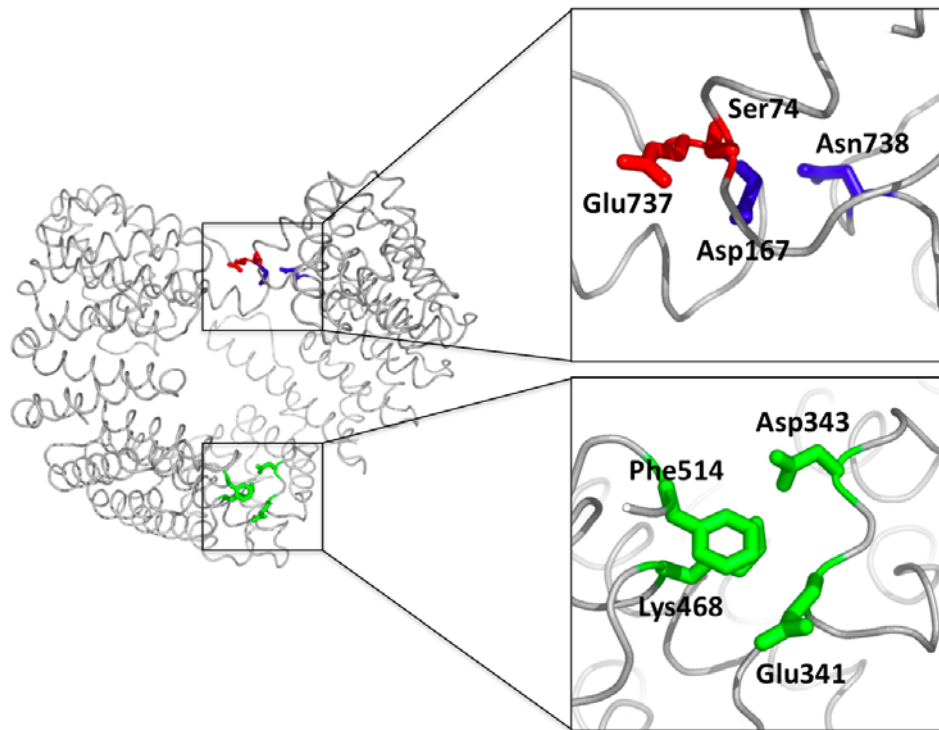
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Figure S1. Residues targeted in mutagenesis studies; relates to Figure 1

(A) Close-up views of residues targeted in mutagenesis studies. The structure of unliganded γ Imp β is shown in ribbon representation (grey), with the residues targeted in mutagenesis studies in stick representation and colored: red, Ser74 (HEAT repeat H2)/Glu737 (H17); blue, Asp167 (H4)/Asn738 (H17); green, Glu341 (H8)/Phe514 (H12) and Asp343 (H8)/Lys468 (H11). The structure is shown in the analogous orientation as in Figure 1.

(B) Alignment of human, mouse and *S. cerevisiae* (yeast) Imp β amino acid sequences. Highlighted are *S. cerevisiae* Imp β residues involved in the principal sequence-distal contacts between HEAT repeats H2/H4 and H17 (Ser74 [red], Asp167 [blue], Arg696 [red], Glu737 [red], Asn738 [blue], Gly739 [blue]), and between H8 and H11/H12 (Pro340, Glu341, Asp343, His466, Lys468, Phe514 [green]). The analogous residues in human and mouse Imp β are highlighted in the same colours if conserved, and with grey background if not conserved. The sequences were aligned using ClustalW (Larkin et al., 2007).

Figure S1A



Yeast	QNTKP ENG TLEALDYQIKVLEAVLDAYVGVIVAGLHDKPE-----ALFP 774
Human	RVEFILSFIDHIAGDE--DHTDGVVACAAGLIGDLCTAFGKDVLLKLVEAR 840
Mouse	RVEFILSFIDHIAGDE--DHTDGVVACAAGLIGDLCTAFGKDVLLKLVEAR 840
Yeast	YVGTIFQFIAQVAEDPQLYSEDATSRRAVGLIGDIAAMFPDGSIKQFYGQ 824
Human	PMIHELLTEGRRS--KTNKAKTLARWATKELRKLKNQA 876
Mouse	PMIHELLTEGRRS--KTNKAKTLATWATKELRKLKNQA 876
Yeast	DWVIDYIKRTRSGQLFSQATKDTARWAREQQKRQLSL- 861

Figure S2. Additional molecular dynamics simulations of yImp β and variation of inter-repeat angles along the polypeptide chain for simulated structures; relates to Figure 2

(A) Evolution of radius of gyration of during an additional molecular dynamics simulation of yImp β . The starting condition of the simulation was the crystal structure. The behaviour of the protein during the simulation resembles the behaviour during the original simulation (Figure 3), but switches more frequently between the extended and compact conformations.

(B) Variation of inter-repeat angles along the polypeptide chain for simulated structures. Top row: curvature (left), twist (middle) and lateral bending angles (right panels) for the original state and 30 structures after each ns along the molecular dynamics trajectory shown in (A). Bottom row: standard deviations for curvature, twist and lateral bending angle values.

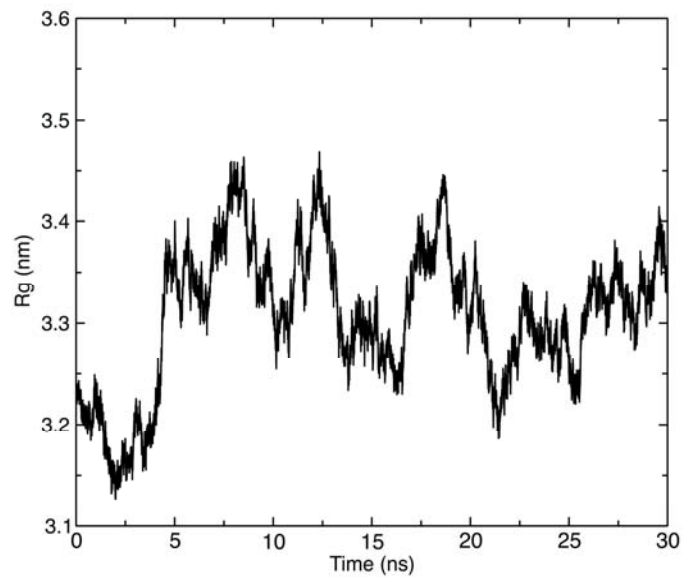
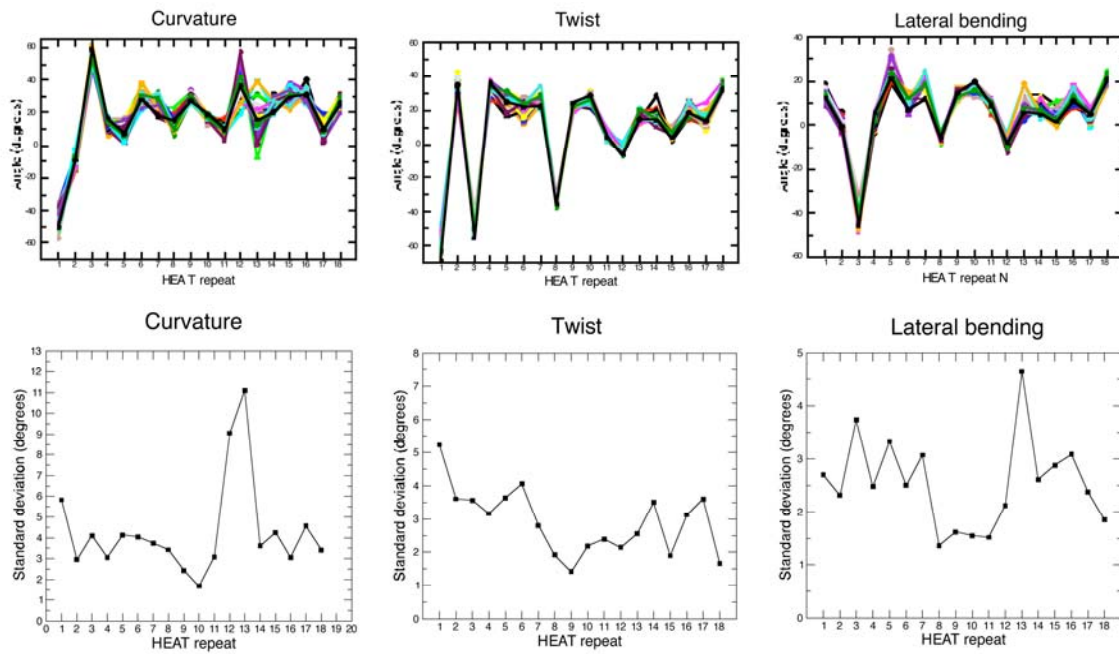
A**B**

Table S1. Yeast strains and plasmids used in this study; relates to Figure 4

Strain/Plasmid	Description	Origin
FY23 (ACY192)	Wild type, <i>MATa ura3-52 leu2Δ1 trp1</i>	(Winston et al., 1995)
PSY884 (ACY209)	<i>ΔRSL1::HIS3 [RSL1 CEN URA3 AMP^R], MATa ade2 leu2 trp1 ura3</i>	(Koepp et al., 1996)
ACY639	<i>srp1-31, MATα trp- ura- leu- lys- his-</i>	(Loeb et al., 1995)
pRS315 (pAC3)	<i>CEN LEU2 AMP^R</i>	(Sikorski and Hieter, 1989)
pAC62	<i>RSL1, CEN LEU2 AMP^R</i>	This Study
pAC511	<i>RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC1065	<i>SV40TA_gNLS-GFP-GFP, CEN URA3 AMP^R</i>	(Hodel et al., 2006)
pAC1069	<i>GFP-GFP, CEN URA3 AMP^R</i>	(Hodel et al., 2006)
pAC2460	<i>S74K RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2461	<i>D167K RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2462	<i>E341A RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2463	<i>K468D RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2464	<i>F514K RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2465	<i>E737K RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2466	<i>S74K RSL1, CEN LEU2 AMP^R</i>	This Study
pAC2467	<i>D167K RSL1, CEN LEU2 AMP^R</i>	This Study
pAC2468	<i>E341A RSL1, CEN LEU2 AMP^R</i>	This Study
pAC2469	<i>K468D RSL1, CEN LEU2 AMP^R</i>	This Study
pAC2470	<i>F514K RSL1, CEN LEU2 AMP^R</i>	This Study
pAC2471	<i>E737K RSL1, CEN LEU2 AMP^R</i>	This Study
pAC2536	<i>S74K/F514K RSL1-GFP, CEN TRP1 AMP^R</i>	This Study
pAC2537	<i>S74K/F514K RSL1, CEN LEU2 AMP^R</i>	This Study

SUPPLEMENTAL REFERENCES

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