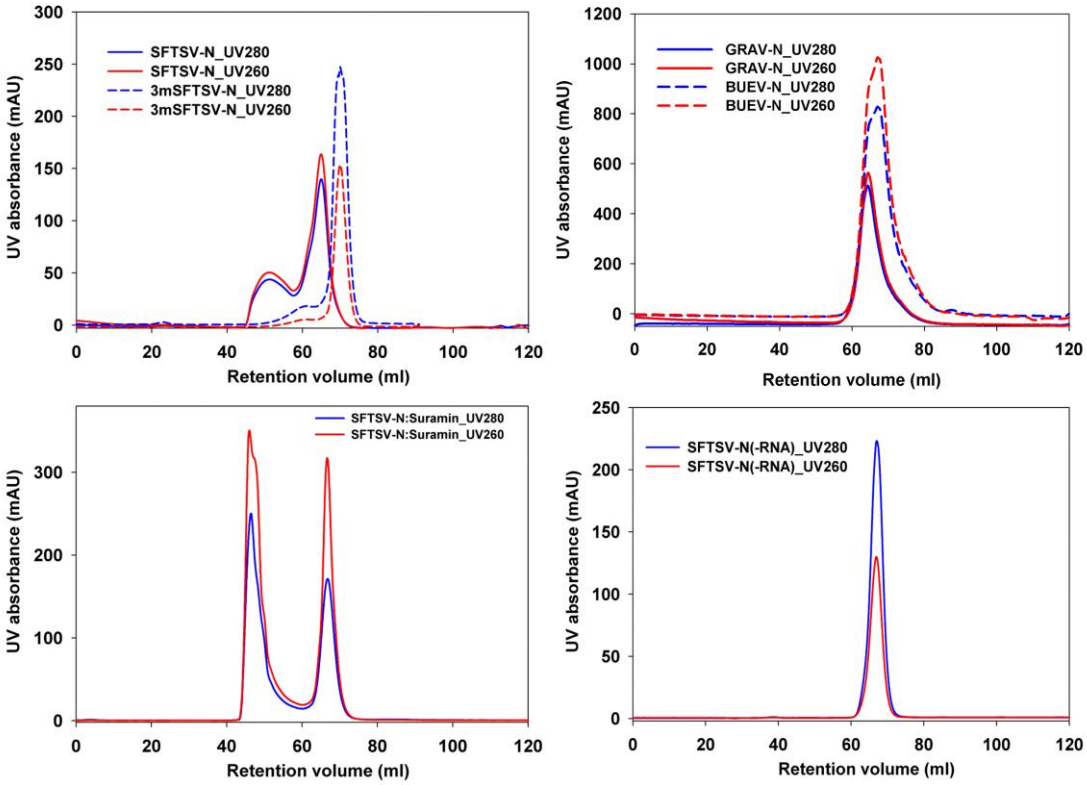
A



B

Mutations		A_{260}/A_{280}	Oligomerization state
SFTSV-N	Native WT	1.23	Hexamer/Pentamer
	Partial unfolding	0.6	Hexamer/Pentamer
	K67Q	0.52	Hexamer/Pentamer
	K67D	0.49	Hexamer/Pentamer
	R64D/K67D/K74D	0.48	Tetramer
GRAV-N	WT	1.33	Hexamer/Pentamer
	Partial unfolding	0.62	Hexamer/Pentamer
	R69D/K72E/K79E	0.55	Tetramer
BUEV-N	WT	1.31	Hexamer/Tetramer
	Partial unfolding	0.66	Hexamer/Tetramer
	R63D/K66E/K73E	0.56	Hexamer

Figure S1 Native and mutant Ns bind with various amount of RNA from *E. coli*. (A) Size exclusion chromatography (SEC) of SFTSV-N, GRAV-N and BUEV-N. Samples of SFTSV-N, triple mutant SFTSV-N (3mSFTSV-N), GRAV-N and BUEV-N in complex with *E.coli* RNA, SFTSV-N in complex with Suramin and RNA-free SFTSV-N were injected, respectively, onto a Superdex G200 (120 ml) column. (B) A_{260}/A_{280} ratios and corresponding oligomeriztion states of native and mutant Ns bind with RNA from *E. coli*.

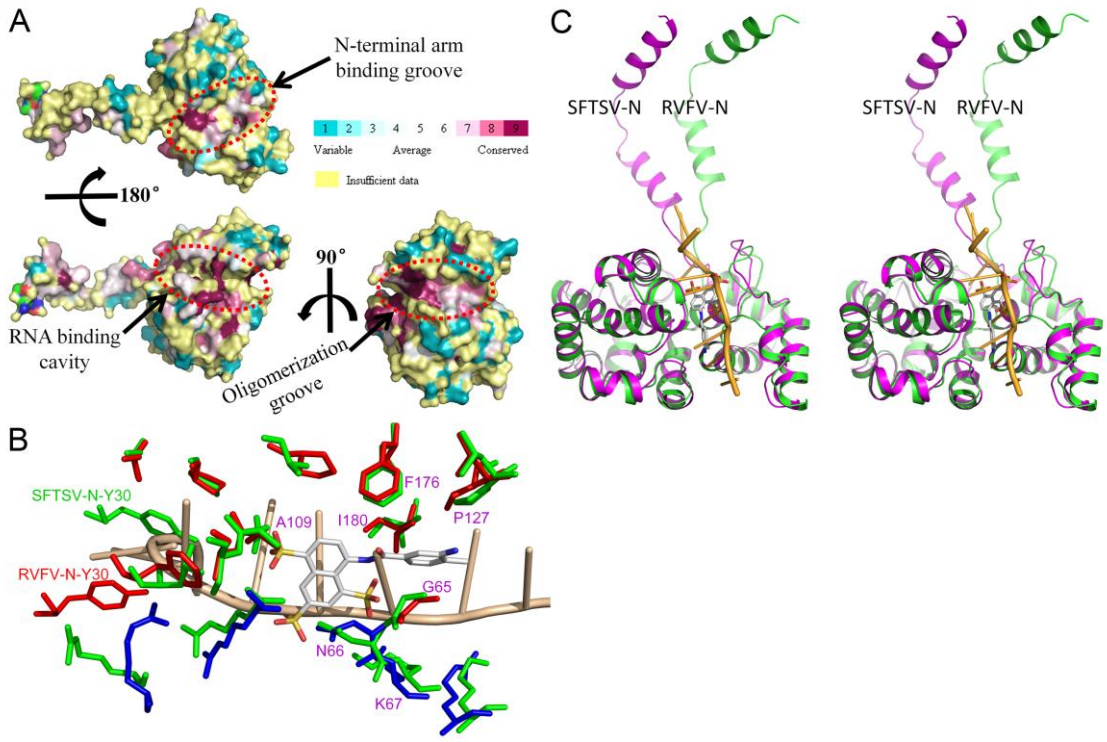
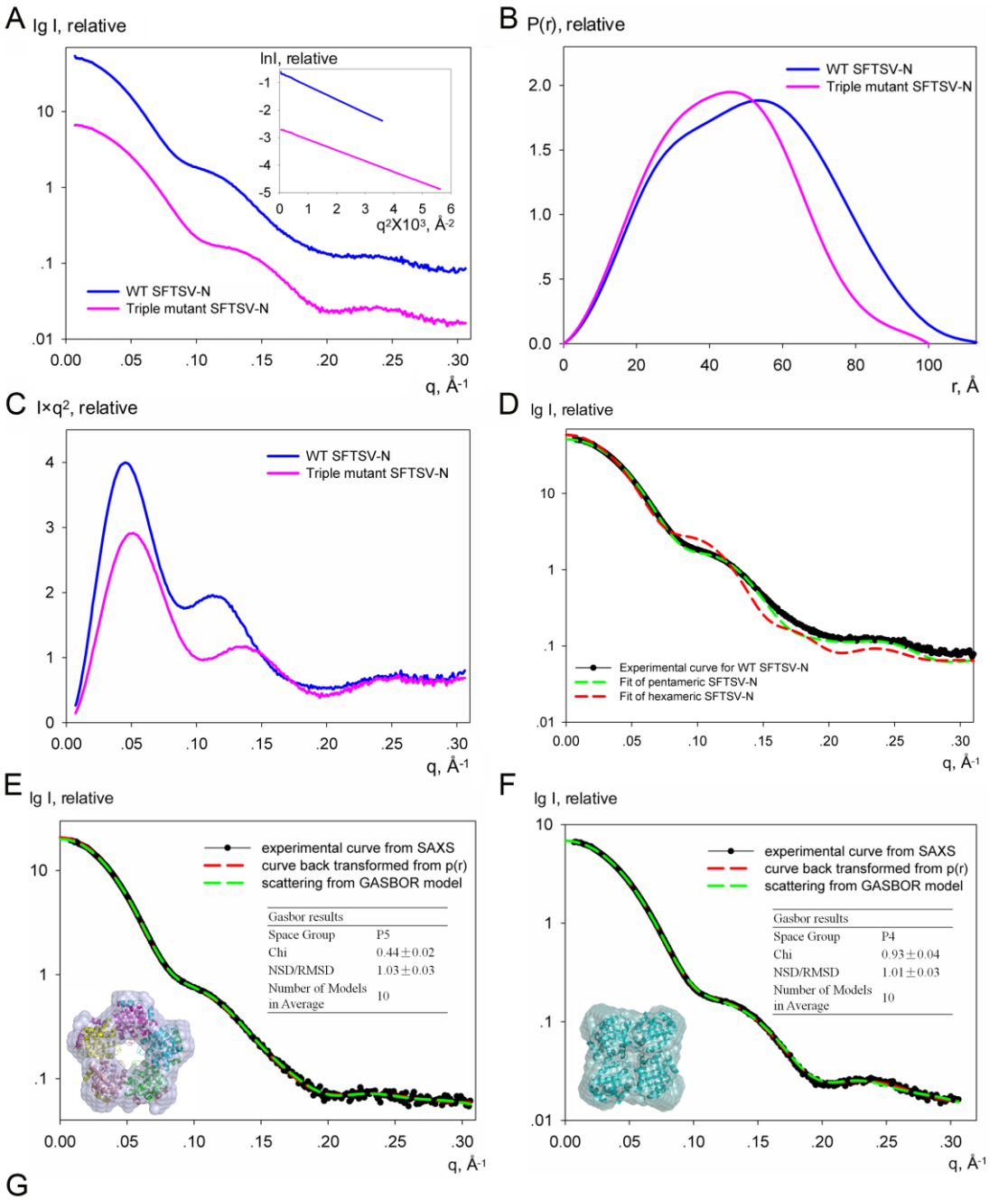


Figure S2 Conserved structures of phleboviral Ns and comparison between RVFV-N:RNA and SFTSV-N:Suramin complexes. (A) Residues on the surface of RNA binding cavity, oligomerization groove, interfaces between N-terminal arm and its binding groove are highly conserved. Amino acid conservation of phleboviral Ns is displayed on the WT SFTSV-N structure by ConSurf (http://consurf.tau.ac.il/). (B) Residues in the RNA binding cavity of RVFV-N (PDB code: 4H5O, residues contributing to hydrophobic interactions are shown in red, while those involved in formation of polar contacts are shown in blue) and their comparison with corresponding residues on SFTSV-N (green). Suramin and RNA are showed in white and light orange, separately. The magenta labels indicate the six RNA binding residues in RVFV-N which are identical with the corresponding conserved residues found in SFTSV-N for binding Suramin. Side chain orientation of Y30 is dependent on the N-terminal arm flexibility. (C) Stereo view of superimposed structures of RVFV-N: RNA complex (PDB code: 4H5O) in magenta and light orange onto SFTSV-N: Suramin complex in green and white.



G

Protein sample	R_g (Å)	D_{max} (Å)	V_{Porod} (Å^3)	MW(kDa)	Calculated shape	Rg (Å)	Oligomeric state
WT SFTSV-N	38.46±0.16	115±3	194,199±1,666	121.37±1.04	Pentamer	35.78	√
					Hexamer	40.50	
Triple mutant SFTSV-N	34.60±0.10	100±2	159,018±396	99.38±0.25	Tetramer	30.56	√

Figure S3 SAXS analysis of WT SFTSV-N and triple mutant SFTSV-N. (A) Scattering curves of SFTSV-N generated by averaging nine experimental datasets, including: WT SFTSV-N (blue) and triple mutant SFTSV-N (magenta). The inset figure is Guinier plots. The wide q range linearity in the plots, ln[I(q)] vs q^2, suggests that there was neither detectable inter-particle interactions nor radiation damage

during collection of SAXS data. (B) Pair distance distribution function, P(r), curves. (C) Kratky plots. The bell-shaped kratky curves demonstrate the proteins were well folded. (D) Fits generated by CRYSOL between experimental scattering curve and pentamer or hexamer observed in crystal packing. GASBOR models for WT SFTSV-N (E) and triple mutant SFTSV-N (F) were obtained from averaging 10 models calculated by GASBOR individually. The crystal structures of pentameric WT SFTSV-N and tetrameric triple mutant SFTSV-N were superimposed into the GASBOR models by SUPCOMB. (E) Pentameric model for WT SFTSV-N with P5 symmetry as a restraint. (F) SAXS model for triple mutant SFTSV-N using P4 symmetry. The goodness-of-fitness parameter Chi and the NSD/RMSD of 10 models are listed in the table (inset). (G) Parameters derived from SAXS curves. R_g and D_{max} are estimated by calculating P(r) using GNOM. V_{Porod} is calculated using PRIMUS. The calculated shape R_g was generated by CRYSOL. The molecular weights (MWs) were predicted using the web server (http://www.if.sc.usp.br/~saxs/saxsmow.html). All the values with error bars are from three replicate samples.

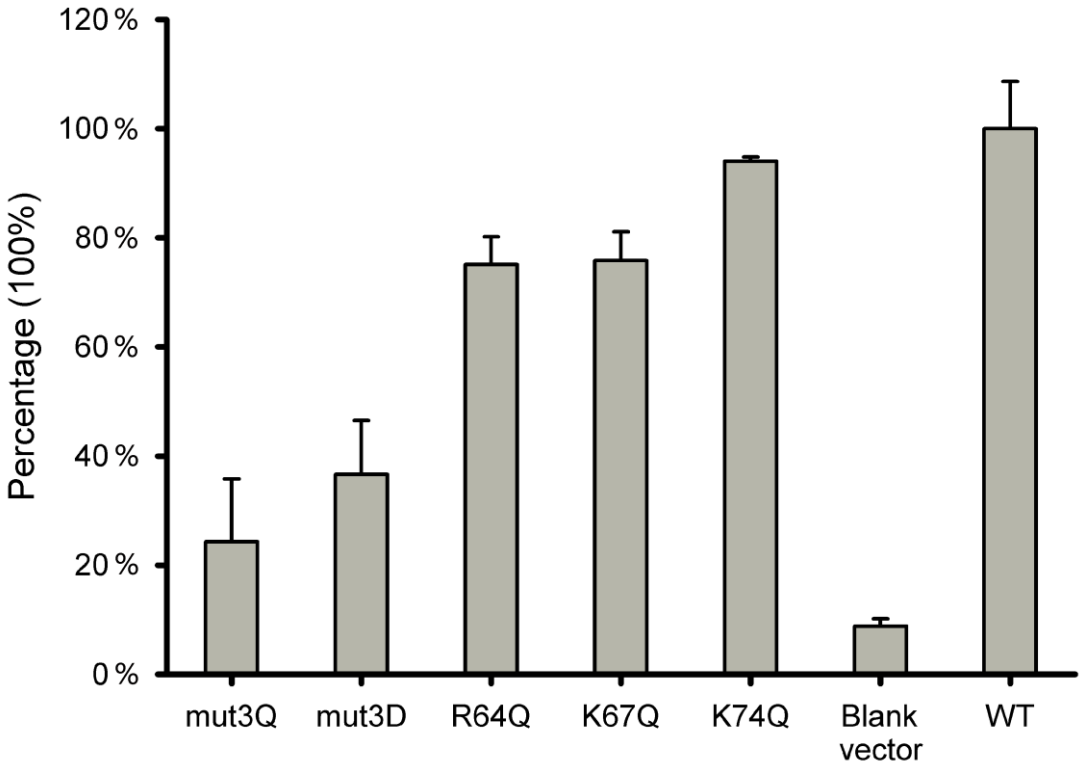


Figure S4 Ability of WT SFTSV-N and a panel of N mutants to transcribe the M reporter gene were evaluated by the luciferase assay in 293T cells. mut3Q: R64Q/K67Q/K74Q; mut3Q: R64D/K67D/K74D.

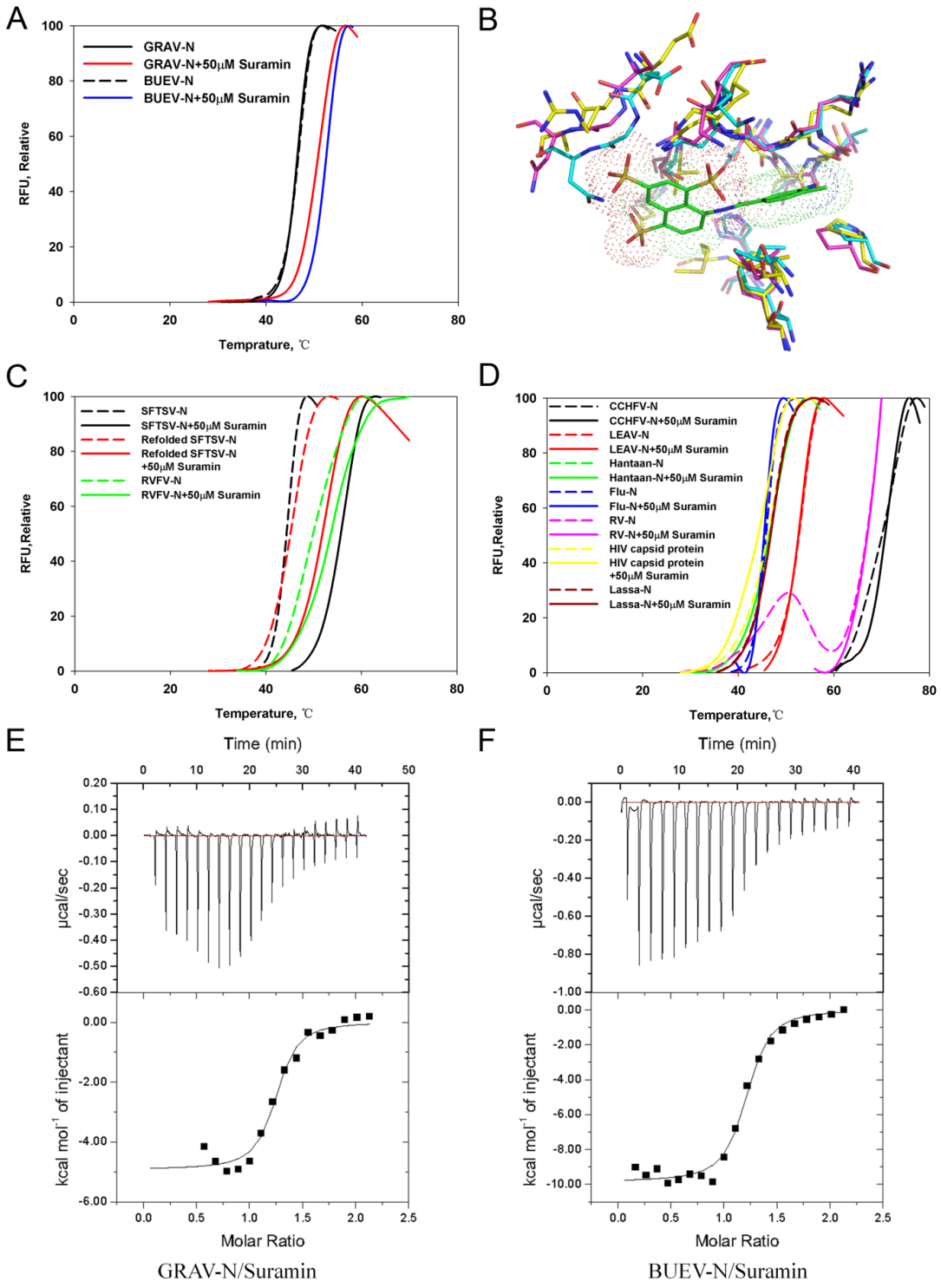


Figure S5 Suramin specifically binds phleboviral Ns. (A) GRAV-N and BUEV-N bind Suramin as measured by thermal shift assays. (B) The residues within 5 Å of Suramin derived from WT SFTSV-N: Suramin complex and the corresponding residues from GRAV-N and BUEV-N. Specificity of Suramin for (C) Bunyavirus Ns and (D) N from other viruses as measured by thermal shift assays. LEAV-N: Leayer virus N (*Orthobunyavirus* genus); Hantaan-N: Hantaan virus N (*Hantavirus* genus);

flu-N: Influenza A virus N; RV-N: Rabies virus N; Lassa-N: Lassa virus N. (E) GRAV-N and (F) BUEV-N bind with Suramin as confirmed by ITC.

TABLE S1 SFTSV N-N protomer interface hydrogen bonds (distances $\leqslant$ 3.2 Å)

N-N protomer	Chain A residue/atom	Chain B residue/atom	Hydrogen bond Distance (Å)
WT SFTSV-N (P6)	SER5/OG	PHE209/O	2.53
	GLN14/OE1	LYS55/NZ	2.77
	GLU20/OE2	LYS52/NZ	2.28
	ARG26/NH1	TYR125/OH	3.07
	GLU27/O	SER76/OG	2.74
	LEU29/O	ARG64/NH1	2.69
	GLU31/N	LYS74/O	2.96
	ARG99/NH1	MET75/O	2.45
	ARG99/NH2	MET75/O	2.77
	ARG99/NH2	SER72/O	3.00
Triple mutant SFTSV N	ASP64/OD2	LEU28/O	2.76
	ASP74/O	ARG99/NH2	2.79
	ASP74/OD1	GLY32/O	3.00
	SER76/OG	GLU27/O	2.55
	LYS184/O	ASN182/ND2	2.71
	PHE209/O	SER5/OG	2.80

TABLE S2 Residues in the RNA binding cavity of RVFV-N and their comparison with corresponding residues on SFTSV-N, GRAV-N and BUEV-N.

SFTSV-N	GRAV-N	BUEV-N	RVFV-N
Y30	Y32	Y29	Y30
L33	F35	F32	F33
G65	G70	G64	G65
Q109	A114	A108	A109
S110	A115	A109	A110
L126	L131	L125	L126
P127	P132	P126	P127
A148	P153	P146	P147
F177	F182	F175	F176
I181	I186	I179	I180
P200	P205	P198	P199
A203	A208	A201	A202
N66	N71	N65	N66
K67	K72	K66	K67
K70	K75	K69	R70
R99	R104	R98	R99
R106	R111	R105	R106

The six residues in red color are responsible to bind both Suramin and RNA potentially.

Strictly conserved residues.

Conserved residues.

TABLE S3 Residues of SFTSV-N contact with Suramin within 5.0 Å and their comparison with corresponding residues on GRAV-N, BUEV-N and RVFV-N.

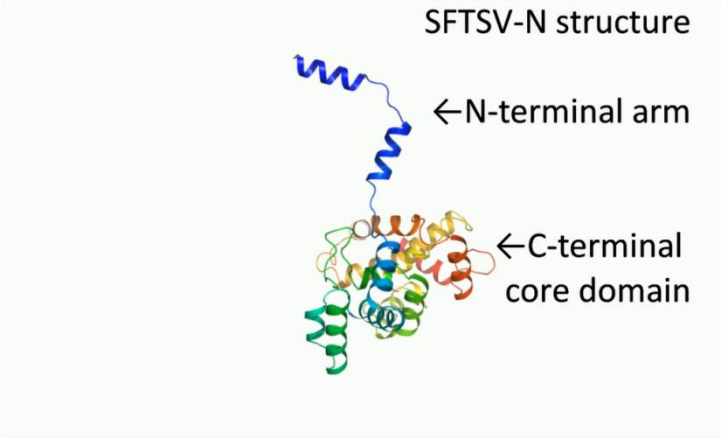
SFTSV-N	GRAV-N	BUEV-N	RVFV-N
A61	N66	S60	A61
L62	L67	L61	L62
T63	V68	T62	T63
R64	R69	R63	R64
G65	G70	G64	G65
N66	N71	N65	N66
K67	K72	K66	K67
E94	E99	S93	E94
R95	G100	G94	G95
A96	N101	N95	N96
V105	S110	S104	S105
Q109	A114	A108	A109
P127	P132	P126	P127
M147	H152	H145	H146
F177	F182	F175	F176
I181	I186	I179	I180

The six residues in red color are responsible to bind both Suramin and RNA potentially.

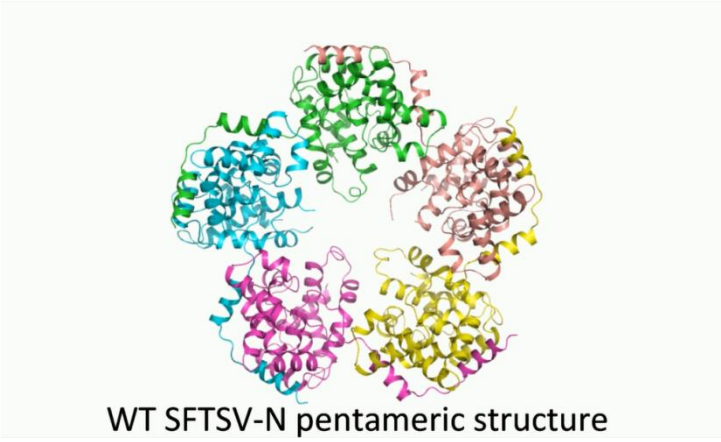
Strictly conserved residues.

Conserved residues.

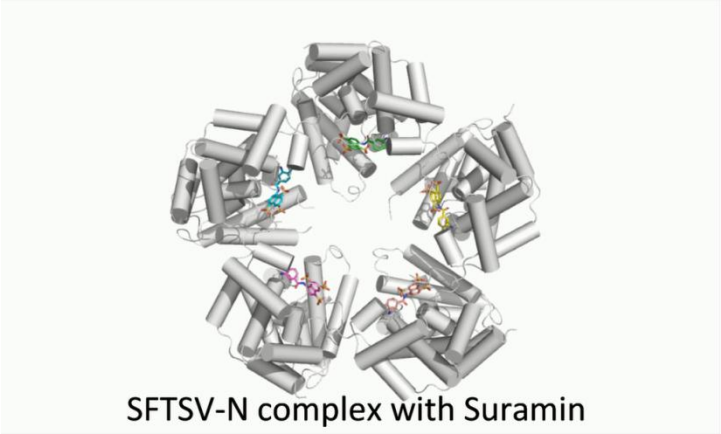
Movie legends



Movie S1: SFTSV-N wild type and triple mutant monomer structures. Three mutated residues are shown as sticks.



Movie S2: The wild type SFTSV-N pentamer structure is shown as ribbon and electrostatic potential surface representation.



Movie S3: SFTSV-N complex with Suramin and detailed interactions are shown.