Molecular principles underlying dual RNA specificity in the *Drosophila* SNF protein

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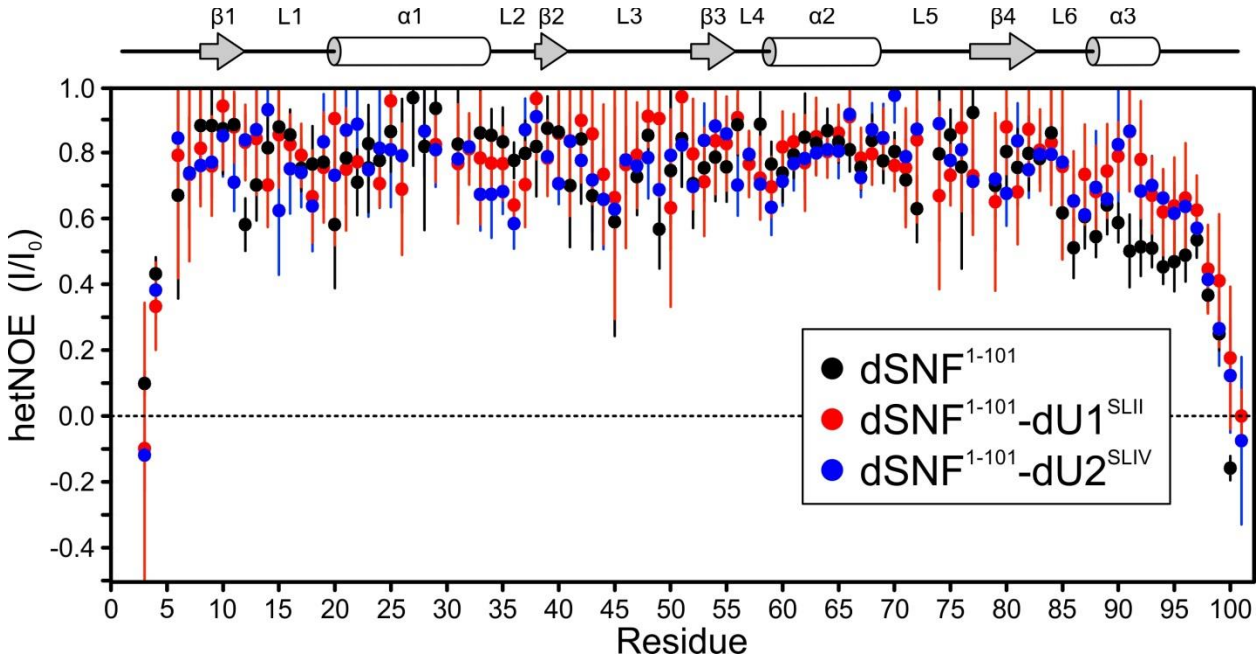
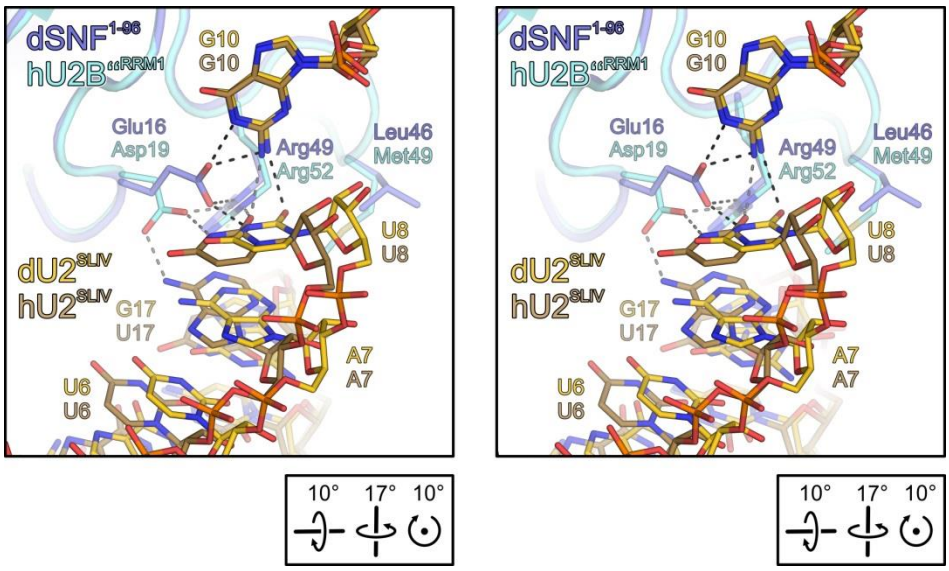
Supplementary Figures



Supplementary Figure 1. NMR $^{15}N/^{1}H$ heteronuclear NOEs.

NMR $^{15}N/^{1}H$ heteronuclear NOEs of dSNF$^{1-101}$, dSNF$^{1-101}$-dU1^{SLII} and dSNF$^{1-101}$-dU2^{SLIV}. $^{15}N/^{1}H$ heteronuclear NOE reports rapid (ps-ns) dynamics of backbone amides. Values < 0.6 indicate significant motion, seen here for the N-terminal and C-terminal residues of free dSNF$^{1-101}$. Addition of RNA reduces dynamics of helix α3. Heteronuclear TROSY ^{15}N-{^{1}H} NOE data were acquired at 300 μM dSNF$^{1-101}$, 300 μM dSNF$^{1-101}$:330 μM dU1^{SLII} and 300 μM dSNF$^{1-101}$:600 μM dU2^{SLIV}. ● - dSNF$^{1-101}$; ● - dSNF$^{1-101}$-dU1^{SLII}; ● - dSNF$^{1-101}$-dU2^{SLIV}. Data are averages of three pairs of on(I)/off(I_o) resonance experiments. Errors were determined form the propagation of base plane rms noise. 50 mM KCl, 20 mM sodium cacodylate, pH 6.5, in 90 % $^{1}H_2O$, 10 % $^{2}H_2O$; 23° C.



Supplementary Figure 2. dSNF$^{1-96}$-dU2^{SLIV} and hU2B''^{RRM1}-hU2^{SLIV} interactions.

hU2A'-hU2B''^{RRM1}-hU2^{SLIV} complex (fern green/cyan/light brown; PDB ID 1A9N [14]) superimposed on the dU2A'-dSNF$^{1-96}$-dU2^{SLIV} complex (green/steel blue/gold) according to the hU2B"^{RRM1}/dSNF$^{1-96}$ subunits.

Supplementary Tables

Supplementary Table 1. Crystallographic data and refinement[a]

	dSNF$^{1-96}$	dSNF$^{1-96}$-dU1^{SLII}	dU2A'-dSNF$^{1-96}$	dU2A'-dSNF$^{1-96}$-dU2^{SLIV}
Data collection				
Wavelength (Å)	0.9184	0.9184	0.9184	0.9184
Temperature (K)	100	100	100	100
Space group	$P2_12_12_1$	$C222_1$	P2	$P2_12_12_1$
Unit cell parameters a/b/c (Å) α/ß/γ (°)	 52.3/119.7/122.8 90/90/90	 56.7/150.0/133.6 90/90/90	 66/53.7/75.8 90/103.1/90	 65/103.9/116.3 90/90/90
Resolution (Å)	50 - 1.49 (1.58 -1.49)	50 - 2.00 (2.12 - 2.00)	50 - 1.42 (1.50 - 1.42)	50 - 1.90 (2.01 - 1.90)
Reflections Unique Completeness (%) Redundancy	 125,106 (5,057) 98.6 (98.4) 4.5 (4.5)	 39,021 (6,164) 99.7 (99.1) 10.9 (10.7)	 97,210 (15,154) 99.0 (96.1) 3.6 (3.26)	 62,952 (9,932) 99.7 (98.5) 5.78 (5.74)
I/σ(I)	22.1 (1.9)	15.8 (1.8)	10.3 (1.5)	11.64 (1.68)
R_{sym}(I)[b]	0.03 (0.94)	0.11 (1.22)	0.07 (0.78)	0.14 (1.20)
$CC_{1/2}$[c]	100 (77.8)	99.9 (66.2)	99.8 (63.6)	99.7 (57.5)
Refinement				
Resolution (Å)	24.32-1.49	38.3 - 2.0	19.90 - 1.42	47.4 – 1.9
Reflections Number Completeness (%) Test set (%)	 125,046 98.6 5.0	 39,017 99.7 5.0	 97,179 99.0 5.0	 62,935 99.7 3.3
R_{work}[d]	0.146	0.169	0.146	0.167
R_{free}[d]	0.188	0.212	0.193	0.218
ESU (Å)[e]	0.15	0.26	0.15	0.22
Contents of A.U.[f] RNA/residues Proteins/residues Water oxygens	 - 6/528 630	 3/66 3/297 378	 - 4/533 665	 2/50 4/535 883
Mean B-factors Wilson (Å^2) Protein (Å^2) Solvent (Å^2)	 22.6 35.1 46.1	 34.8 48.6 43.9	 14.8 23.9 35.6	 24.0 33.1 39.2
Ramachandran plot[g] Favored (%) Allowed (%) Outliers (%)	 99.06 0.94 -	 98.91 1.09 -	 97.48 2.52 -	 97.29 2.53 0.18
Rmsd[h] Bond lengths (Å) Bond angles (°) Dihedral angles (°)	 0.016 1.520 16.4	 0.012 1.259 15.5	 0.007 1.183 14.1	 0.015 1.389 14.5

[a] Data for the highest resolution shell in parentheses.
[b] $R_{sym}(I) = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i |I_i(hkl)|$; for n independent reflections and i observations of a given reflection; $\langle I(hkl)\rangle$ – average intensity of the i observations.
[c] $CC_{1/2}$, correlation factor between random half-datasets as evaluated by XSCALE[1].
[d] $R = \Sigma_{hkl} ||F_{obs}| - |F_{calc}|| / \Sigma_{hkl} |F_{obs}|$; R_{work} – $hkl \notin T$; R_{free} – $hkl \in T$; T – test set (5 %).
[e] ESU – estimated overall coordinate error based on maximum likelihood.
[f] A.U. – asymmetric unit.
[g] Calculated with MolProbity (http://molprobity.biochem.duke.edu/)[2].
[h] Rmsd – root-mean-square deviation from target geometry.

Supplementary Table 2. DNA oligonucleotides used in this study

Name	Sequence
SNF_NcoI_f	TATCCATGGAGATGCTACCCAACC
SNF_96_Xho1_r	GTACTCGAGTTAACCCTTTATCTTGGCCACAATATC
SNF_COEX_96_r	CCTTCTTAAAGTTAAACAAAATTATTTCTAGATGATCTTTAACCCTTTATCTTGGCCACAATATCCG
U2A_COEX_f	GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGTGAAACTAACGCCGGAGCTG
U2A_176_XhoI_r	GTACTCGAGTTACCGGCTAATCTCCTTTAGCACGTCC
U2A'R20A_f	CGATGCAATACATAAACCCAGTCGCGGAGCGCGAGCTGGATTTGCGCGGC
U2A'R20A_r	GCCGCGCAAATCCAGCTCGCGCTCCGCGACTGGGTTTATGTATTGCATCG
U2A'R143AK149A_f	GGCCTACAAGTTCCCGCAGCTGGCCCTACTCGACTTCAGGGCCATCAAGCAGAAGGACCGCCAGGCGG
U2A'R143AK149A_r	CCGCCTGGCGGTCCTTCTGCTTGATGGCCCTGAAGTCGAGTAGGGCCAGCTGCGGGAACTTGTAGGCC
U2A'KQK151-153AAA_f	CTTCAGGGCCATCGCAGCCGCGGACCGCCAGGCGG
U2A'KQK151-153AAA_r	CCGCCTGGCGGTCCGCGGCTGCGATGGCCCTGAAG
SNF_K17A_f	CAATCTGAACGAGGCGATCAAGAAGGAGG
SNF_K17A_r	CCTCCTTCTTGATCGCCTCGTTCAGATTG
T7_promoter	TAATACGACTCACTATAG
T7_Wt_U2_SLIV	GGACCCGGCGGTACTGCAATACCGGCCTATAGTGAGTCGTATTA
T7_Wt_U1_SLII	GGACCCGGCGCGTGGTGCAATGCCGGCCTATAGTGAGTCGTATTA

Supplementary References

1. Kabsch, W. XDS. *Acta Crystallogr. D* **66,** 125–32 (2010).

2. Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D* **66,** 12–21 (2010).