Supplementary Information

Rational design of cyclic peptide inhibitors of U2AF homology motif (UHM) domains to modulate pre-mRNA splicing

Pravin Kumar Ankush Jagtap, Divita Garg, Tobias G. Kapp, Cindy L Will, Oliver Demmer, Reinhard Lührmann, Horst Kessler and Michael Sattler

Contents

Table S1. Dissociation constants (K_D) of the SPF45 UHM domain determined by ITC

Peptide	K_D[a] (μM)	ΔH[a] (kcal/mol)	-TΔS[a] (kcal/mol)
RKSRWDETP	0.70±0.11	-18.18±0.26	9.77±0.51
RKARWDETP	5.46±0.03	-17.64±0.48	10.45±0.45
RWDET	40.93±1.25	-16.59±0.88	10.58±0.86
SRWDET	19.73±2.73	-9.3745±1.55	2.94±1.46

[a]Errors represent standard deviation of the fitting errors calculated by error propagation

Table S2. Crystallographic data collection and refinement statistics for the SPF45-UHM/peptide 3 complex

Parameter	**Value**[a]
Wavelength	1.0 Å
Resolution range	37.24 - 2.22 (2.30 - 2.22)
Space group	P 1 21 1
Unit cell	37.6 73.59 45.01 90 97.89 90
Total reflections	60259 (4142)
Unique reflections	11806 (1033)
Multiplicity	5.1 (4.0)
Completeness (%)	98 (88)
Mean I/σ(I)	14.61 (5.29)
R_{merge}	0.07603 (0.2116)
R_{work}	0.1812 (0.2704)
R_{free}	0.2444 (0.3842)
RMS(bonds)	0.019
RMS(angles)	1.87
Ramachandran favoured (%)	99
Ramachandran allowed (%)	0.95
Ramachandran outliers (%)	0
Rotamer outliers (%)	1.7
Average B-factor	21.04
macromolecules	20.32
solvent	27.38

[a]Statistics for the highest-resolution shell are shown in parentheses.

Table S3. Dissociation constants (K_D) for peptide binding to the U2AF65 UHM domain determined by ITC

Peptide	K_D(μM) [a]	Selectivity[b]
RKSRWDETP	6.46±0.29	9.1
(Peptide **3**)		
[sc,sc(KSRWDE)]	7.2±0.36	5.1
(Peptide **10**)		270.4
[*sc,sc*(KSRWDE)]-K	49±1.58	

[a]Errors represent standard deviation of the fitting errors calculated by error propagation

[b]$K_D^{\text{U2AF65-UHM}}/K_D^{\text{SPF45-UHM}}$

Table S4: Analytical data of compounds

Sequence[a]	Peptide	*m*/*z*[a]	t_R,[b] min
RWDET[b]		706.5	3.18
SRWDET[b]		793.4	3.66
RKARWDETP[b]		1158.7	3.01
KSRWDE[b]	**0**	820.6	2.52
sc,bb(KSRWDE)[b]	**1**	802.5	3.88
sc,sc(OrnSRWDE)[b]	**2**	788.5	4.10
sc,sc(KSRWDE)[b]	**3**	802.5	4.23
[*sc,sc*(KSRWDE)]-H[c]	**4**	939.5	4.67
[*sc,sc*(KSRWDE)]-Y[c]	**5**	949.5	4.95
[*sc,sc*(KSRWDE)]-W[c]	**6**	988.7	4.26
[*sc,sc*(KSRWDE)]-R[c]	**7**	958.7	4.95
[*sc,sc*((*N*ε-Me)KSRWDE)]-K[c]	**8**	944.6	4.57
[*sc,sc*(KSRWDE)]-*homo*R[c]	**9**	972.5	4.87
[*sc,sc*(KSRWDE)]-K[c]	**10**	930.5	4.21

[a]Found ESI-MS, $[M+H]^+$

[b]Retention time, analytical HPLC (5−95%, 8 min)

[c]Retention time, analytical HPLC (10−90%, 15 min)

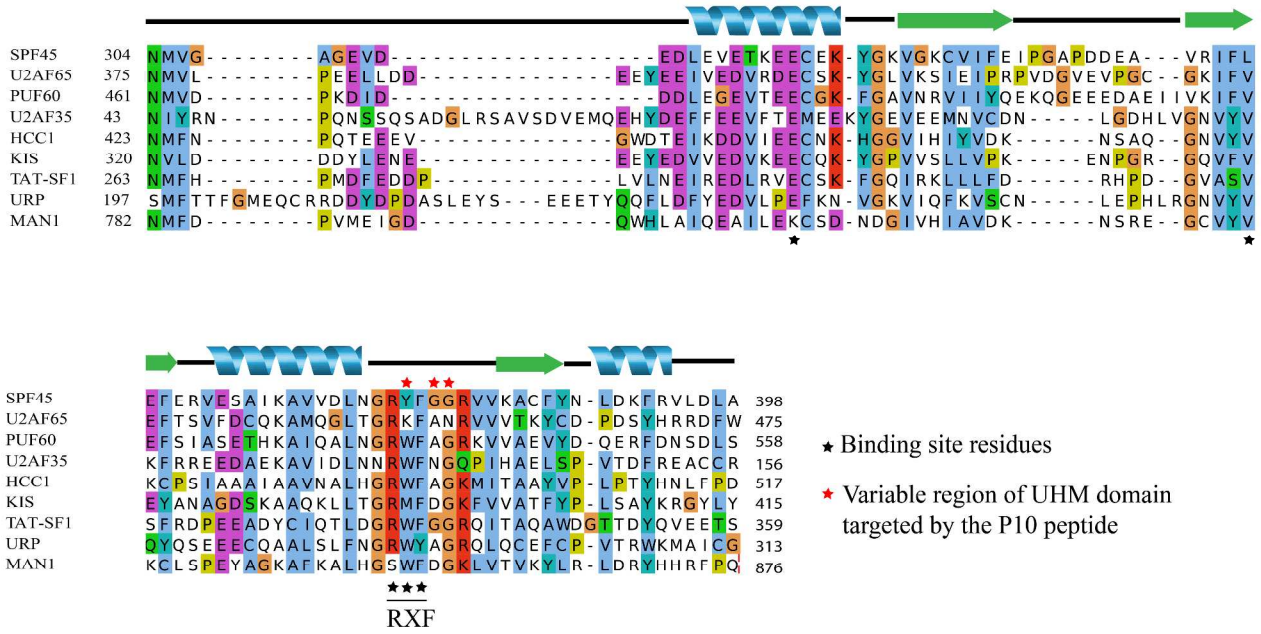

Figure S1

Sequence alignment of UHM domains. Key residues for ULM recognition are indicated by "*". RXF motif is underlined. Secondary structure of the SPF45 domain is indicated on top of the sequence alignment.

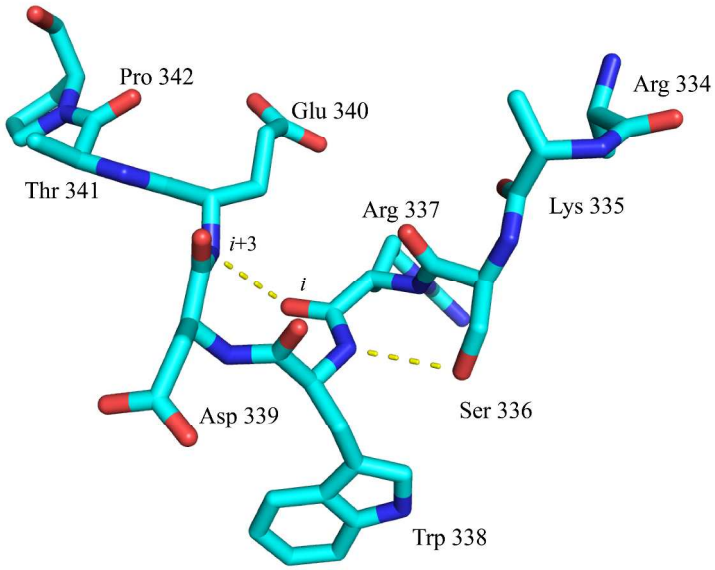

Figure S2

Conformation of SF3b155-ULM5 bound to the SPF45 UHM domain (PDB id: 2PEH). The ULM peptide has a β-turn formed by the hydrogen bond between carbonyl oxygen of Arg337 (*i*) and amide of Glu340 (*I+3*). The sidechain of Ser 336 from the ULM forms an intra-molecular hydrogen bond with the backbone amide of Trp 338 and helps to stabilize the β-turn.

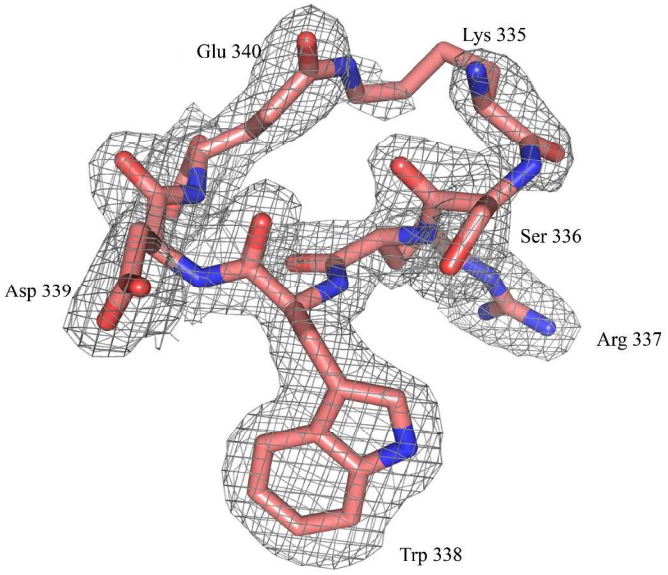

Figure S3

Electron density of the cyclic peptide **3** contoured at 1σ.

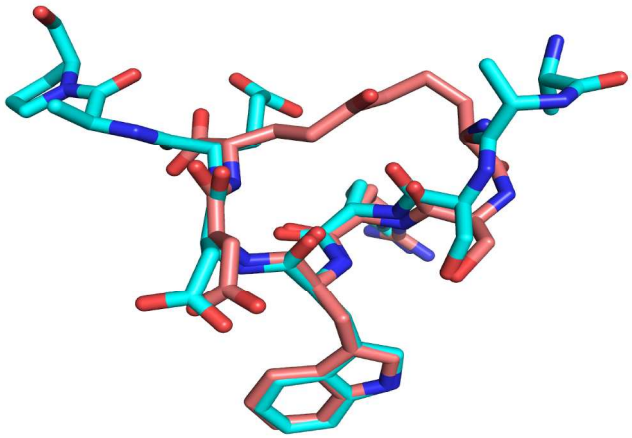

Figure S4

Structural comparison of the SF3b155 ULM peptide and cyclic peptide **3** bound to the SPF45 UHM domain. Peptide **3** shows an RMSD of 0.97 Å with differences limited to Lys 335 and Glu340 arising from the cyclization.

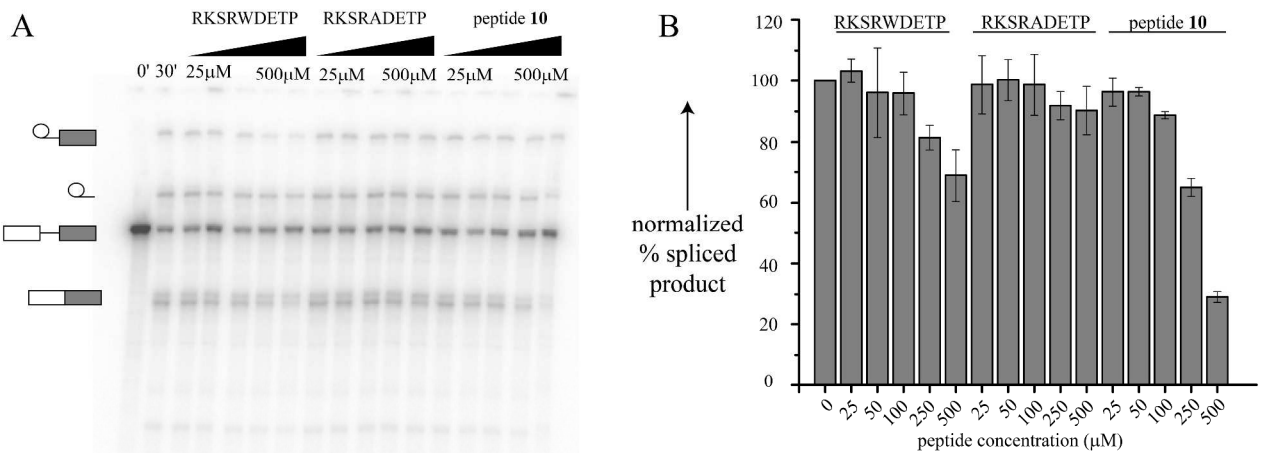

Figure S5.

In vitro splicing assays with MINX pre-mRNA. A) ^{32}P-labelled MINX pre-mRNA was incubated in HeLa nuclear extract for 0 or 30 min in the presence of increasing amounts of the indicated peptides, and splicing intermediates and products were analyzed by denaturing PAGE. RKSRWDETP and RKSRADETP were used as positive and negative controls, respectively. Peptide **10** shows significant inhibition of MINX splicing after 30 minutes compared to the linear peptide. The inhibition by peptide **10** is weaker with MINX compared to IgM pre-mRNA, which could be rationalized, based on the differential requirement of UHM-ULM interactions for the splicing of pre-mRNAs with strong (MINX) versus weak (IgM) 3' splice sites/polypyrimidine tracts. B) Quantification of the percent of spliced product formed, normalized to the 0 μM peptide control (set to 100%). Error bars represent standard deviation of the % spliced product measured in two independent experiments.