Biophysical Journal, Volume *111*

Supplemental Information

SF1 Phosphorylation Enhances Specific Binding to U2AF^{65} and Reduces Binding to 3′-Splice-Site RNA

Rakesh Chatrikhi, Wenhua Wang, Ankit Gupta, Sarah Loerch, Alexandre Maucuer, and Clara L. Kielkopf

Supplementary Tables

Table S1. Thermodynamics of SF1 binding $U2AF^{65}$ proteins in different ionic strengths.[a]

Interaction:	**K_D** (nM)	**ΔG**[b] (kcal mol^{-1})	**ΔH** (kcal mol^{-1})	**$-T\Delta S$**[c] (kcal mol^{-1})	**n**[d]
$U2AF^{65}_{R12U}$ in 100 mM NaCl buffer titrated into: [e]					
$SF1_{1-255}$	60 ± 5	-10.1 ± 0.1	-13.5 ± 0.5	3.5 ± 0.1	1.0 ± 0.1
Phospho-$SF1_{1-255}$	40 ± 12	-10.3 ± 0.2	-14.5 ± 0.2	4.2 ± 0.1	0.9 ± 0.1
$U2AF^{65}$ UHM in 100 mM NaCl buffer titrated into: [f,g]					
$SF1_{14-132}$	43 ± 1	-10.2 ± 0.1	-13.5 ± 0.1	3.3 ± 0.1	1.0 ± 0.1
Phospho-$SF1_{14-132}$	24 ± 1	-10.6 ± 0.1	-20.6 ± 0.1	10.0 ± 0.1	0.9 ± 0.1
$U2AF^{65}$ UHM in 250 mM NaCl buffer titrated into: [f]					
$SF1_{14-132}$	940 ± 47	-8.4 ± 0.1	-13.5 ± 0.5	5.1 ± 0.5	0.9 ± 0.1
Phospho-$SF1_{14-132}$	623 ± 20	-8.6 ± 0.1	-14.5 ± 0.1	5.8 ± 0.1	1.0 ± 0.1

[a] Average values and standard deviations of at least two independent titrations.
[b] Calculated using the equation $\Delta G = -RT \ln (K_D^{-1})$ at T=303 K.
[c] Calculated using the equation $-T\Delta S = \Delta G - \Delta H$.
[d] Apparent stoichiometry of $U2AF^{65}$:SF1 proteins (n).
[e] $c = [M]/K_D$ where [M] is the concentration of macromolecule in the sample cell.
[f] 100 mM NaCl and 250 mM NaCl buffers include 25 mM HEPES pH 7.4, 0.2 mM TCEP.
[g] Also shown for comparison in Table 1.

Supplementary Figures

Figure S1. Control titrations of proteins or RNA into buffer, including (**A**) SPF45 UHM (200 μM), (**B**) U2AF^{35} UHM (200 μM), (**C**) 35 μM B3P3 RNA, or (**D**) 35 μM SF1$_{1\text{-}255}$ – U2AF$^{65}_{R12U}$ complex. The control titration of CAPERα UHM into buffer is shown in Loerch *et al*. (2014) *J. Biol. Chem*. 289:17325-17337.

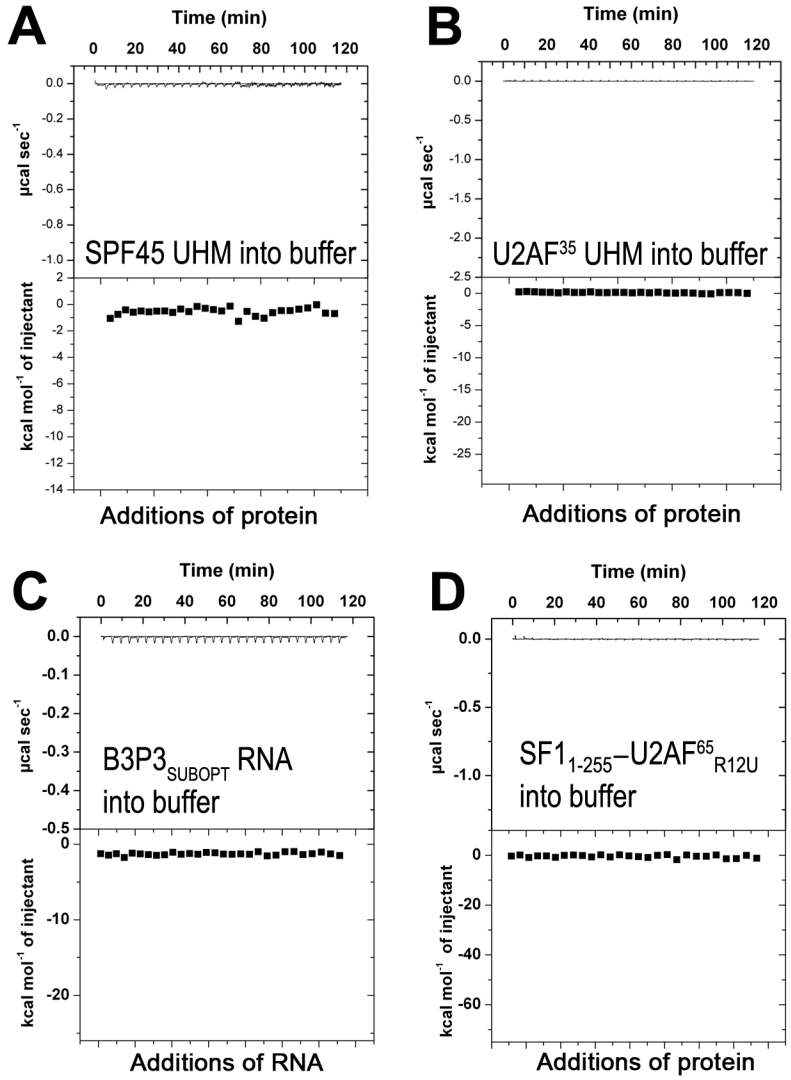


Figure S2. Representative isotherms of SF1 or phospho-SF1 titrated with U2AF^{65} constructs and fit with identical sites binding models. The buffer in (**A-B**) is 100 mM NaCl, 25 mM HEPES pH 7.4, 0.2 mM TCEP. The titrations include (**A**) 48 μM U2AF$^{65}{}_{R12U}$ into 6 μM SF1$_{1-255}$, c=100. (**B**) 32 μM U2AF$^{65}{}_{R12U}$ into 4 μM phospho-SF1$_{1-255}$, c=100. The buffer in (**C-D**) is 250 mM NaCl, 25 mM HEPES pH 7.4, 0.2 mM TCEP. The titrations include (**C**) 120 μM U2AF^{65} UHM into 15 μM SF1$_{14-132}$, c=16. (**D**) 120 μM U2AF^{65} UHM into 15 μM phospho-SF1$_{14-132}$, c=24. The apparent equilibrium dissociation constants (K_D) and standard deviations between two titrations are given.

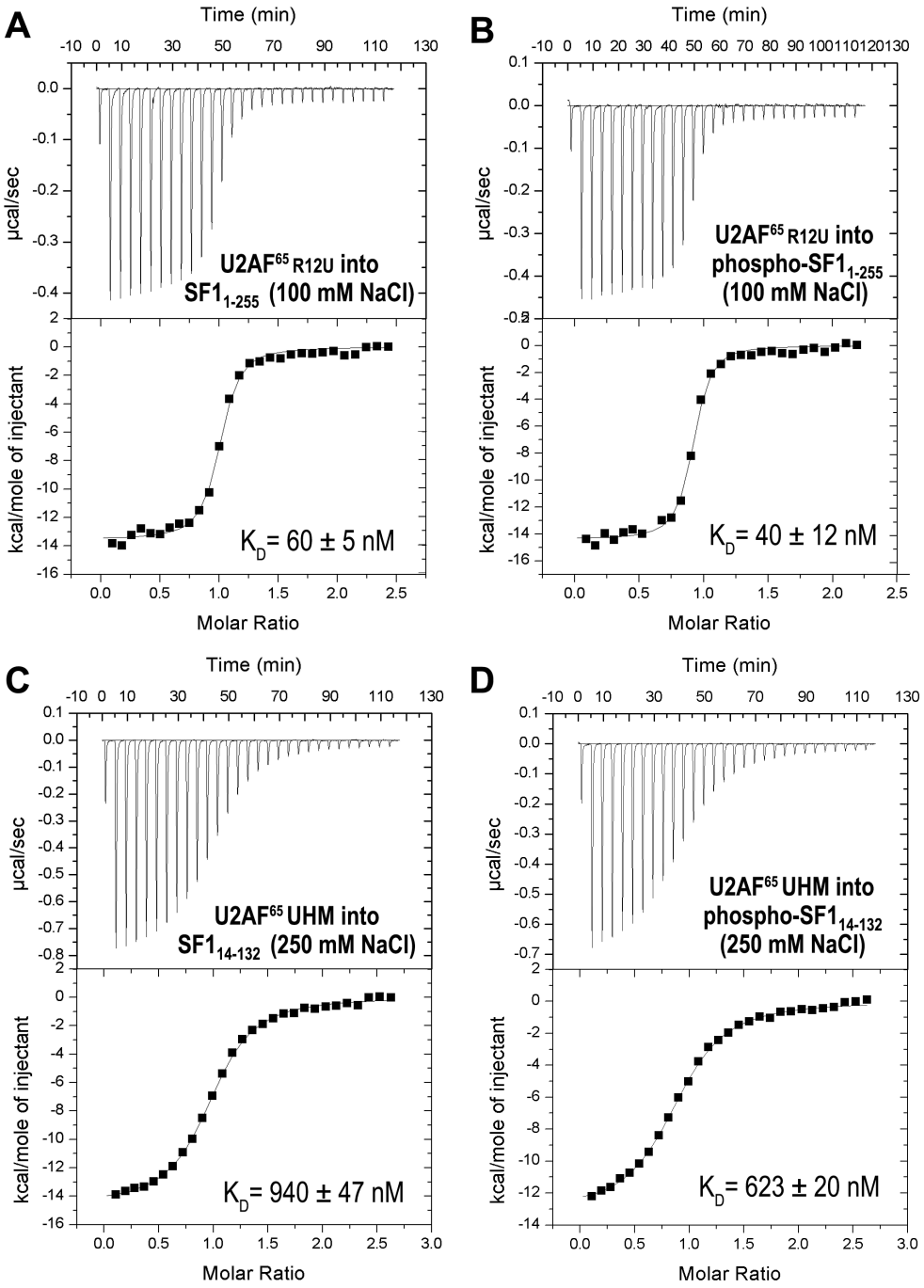


Figure S3. Schematic diagram of binding events during the titration of $SF1_{1\text{-}255}$ or phospho-$SF1_{1\text{-}255}$ into a mixture of $U2AF^{65}$ (or $U2AF^{65}$–$U2AF^{35}$ UHM complex) and fluorescein-labeled AdML RNA, for which apparent K_D's are given in Figure 3. A dashed line separates the titrated $SF1_{1\text{-}255}$ or phospho-$SF1_{1\text{-}255}$ proteins from the mixture in the cuvette. Based on the ~30 nM K_D of the full length $U2AF^{65}$–$U2AF^{35}$ UHM complex binding the AdML Py tract (56) and a 25 nM concentration of $U2AF^{65}$–$U2AF^{35}$ UHM protein and RNA in the sample cell, approximately 8 nM $U2AF^{65}$–$U2AF^{35}$ UHM–RNA complex and 17 nM free $U2AF^{65}$–$U2AF^{35}$ UHM protein and RNA are present in the sample cell at the start of the titration. The K_D's of other binding reactions are not known for the current constructs, RNA sequence, ionic strength, and pH.

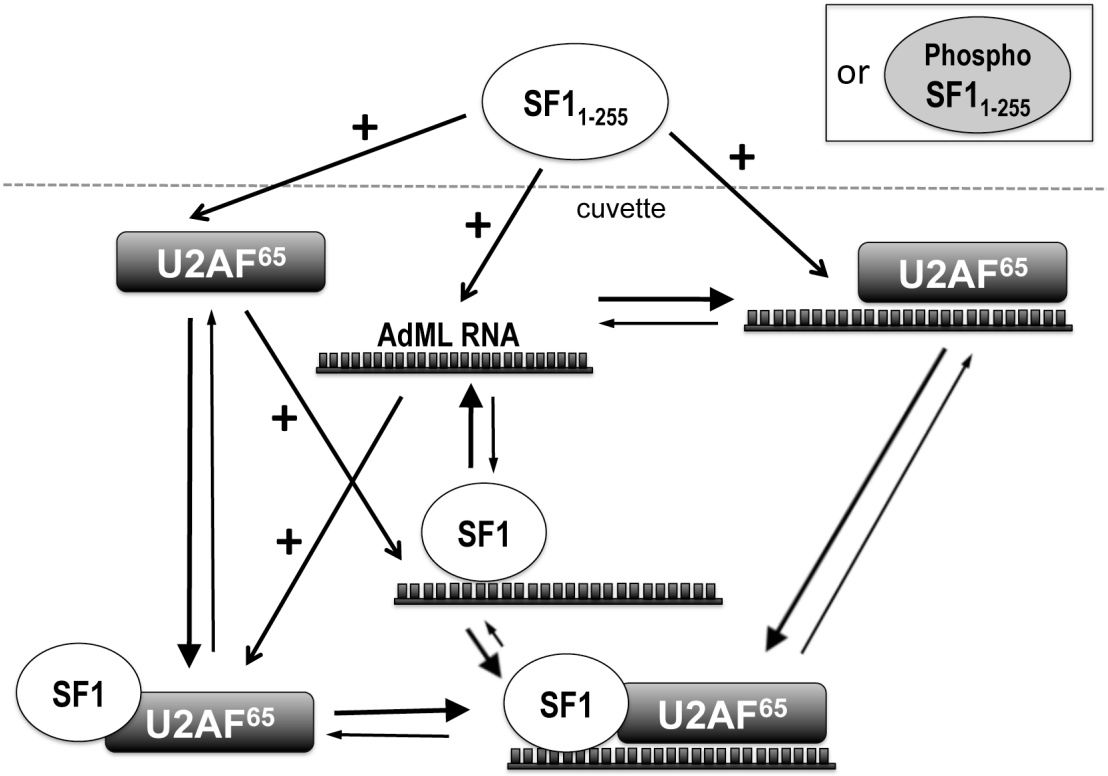
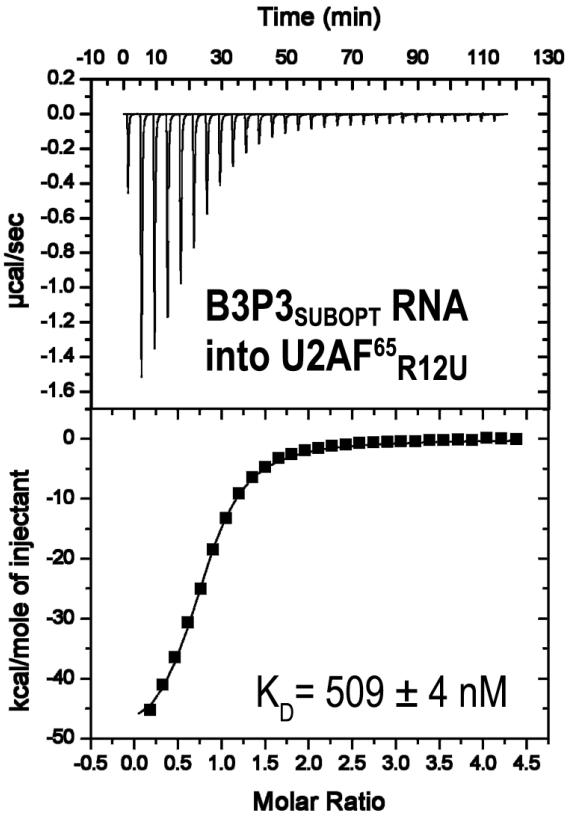


Figure S4. Representative isotherm showing 100 μM B3P3 RNA titrated into 5 μM $U2AF^{65}{}_{R12U}$ (c=10) and fit with an identical sites binding model. The apparent equilibrium dissociation constant (K_D) and standard deviation between two titrations is given.



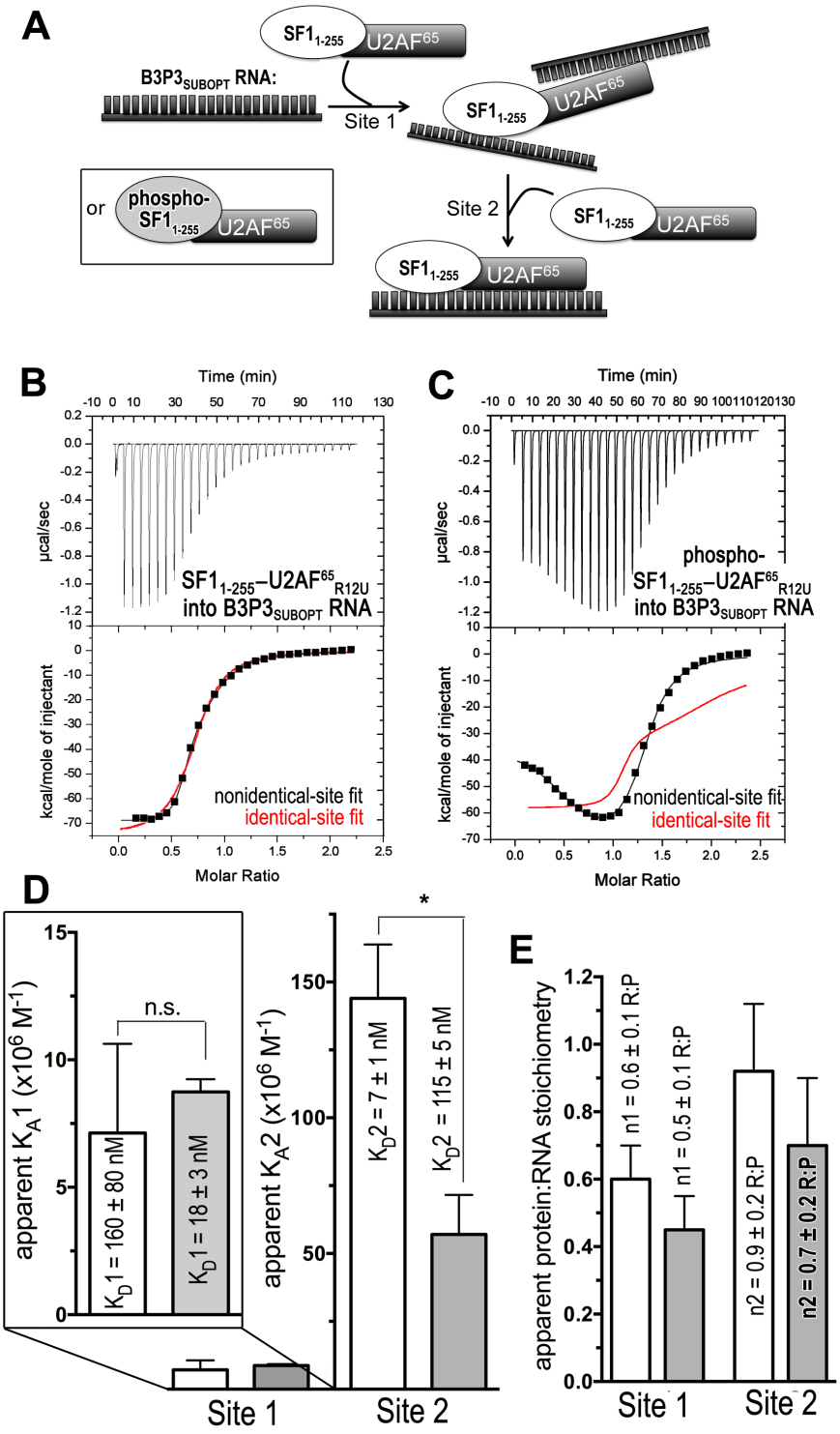


Figure S5. ITC results for $SF1_{1\text{-}255}$ – $U2AF^{65}{}_{R12U}$ or phospho-$SF1_{1\text{-}255}$ – $U2AF^{65}{}_{R12U}$ reverse titrated into B3P3 RNA. (**A**) Schematic diagram of the ITC experiment. (**B**-**C**) Representative isotherms fit with binding models for either nonidentical sites (black lines) or identical sites (red lines). (**B**) 35 μM $SF1_{1\text{-}255}$ – $U2AF^{65}{}_{R12U}$ complex into 5 μM B3P3 RNA (nonidentical site χ^2= 0.30E6 ± 0.20E6; identical sites χ^2 = 1.88E6 ± 0.69E6), c=31 for site 1 and c=714 for site 2. (**C**) 50 μM phospho-$SF1_{1\text{-}255}$ – $U2AF^{65}{}_{R12U}$ complex into 5 μM B3P3 RNA (nonidentical sites (χ^2 = 1.45E6 ± 0.14E6; identical sites χ^2 = 82.3E6 ± 76.0E6), c=43 for site 1 and c=278 for site 2. The identical site models were discarded based on the large χ^2 increase and poor fit. (**D**) Bar graph of apparent affinities (K_A) of the interactions, colored as shown in (A). The apparent equilibrium dissociation constants (K_D) are given. An expanded view of the lower affinity sites is inset to the left. (**E**) Bar graph of apparent protein:RNA stoichiometries (n), colored as for (A).

Figure S6. Representative isotherm showing 50 μM $B3P3_{SUBOPT}$ RNA titrated into 5 μM SF1mut–$U2AF^{65}{}_{R12U}$ in 100 mM NaCl, 25 mM sodium phosphate pH 7.4. The isotherm was fit using an identical sites binding model. The apparent equilibrium dissociation constant (K_D) and standard deviation of two independent titrations is inset.