ELECTRONIC SUPPLEMENTARY INFORMATION

Origins of the enhanced affinity of RNA-protein interactions triggered by RNA phosphorodithioate backbone modification‡

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Materials.

5′-DMT-2′-*O*-TBDMS nucleoside (A^{Bz}, C^{Ac}, G^{iBu}, and U) phosphoramidite monomers and 5′-DMT-2′-*O*-methyl nucleoside (A^{Bz}, C^{Ac}, G^{iBu}, and U) phosphoramidite monomers were purchased from Hongene Biotechnology USA, Inc. The biotinTEG phosphoramidite was purchased from Glen Research. The bacteriophage 6X HIS-Tag MS2 phage coat protein with V75E; A81G mutant (sequence below) (MW: 13744.4609) was obtained from GenScript USA, Inc.

Protein sequence:

ASNFTQFVLV DNGGTGDVTV APSNFANGVA EWISSNSRSQ AYKVTCSVRQ SSAQNRKYTI	60
KVEVPKVATQ TVGGEELPVA GWRSYLNMEL TIPIFATNSD CELIVKAMQG LLKDGNPIPS	120
AIAANSGIY	129

These proteins were handled according to manufacturers' recommendations and aliquots were stored at -80 °C. All other chemicals and buffer components were obtained from Sigma-Aldrich. All solutions for *in vitro* assays and purifications were made using deionized/diethylpyrocarbonate (DEPC) treated water filtered through a 0.22 μm filter (Millipore).

RNA and modified RNA synthesis.

Modified and unmodified RNAs were synthesized on the 1 μmole scale on an Expedite 8909 DNA/RNA Synthesizer using commercially available monomers as well as in house produced 5′-DMT-2′-*O*-TBDMS nucleoside (A^{Bz}, C^{Ac}, G^{iBu}, and U) thiophosphoramidite monomers or 5′-DMT-2′-*O*-methyl nucleoside (A^{Bz}, C^{Ac}, G^{iBu}, and U) thiophosphoramidite monomers (1,2). For binding assays leading to affinity ranking, the RNAs were biotinylated at their 5′-end using BiotinTEG (Glen Research), allowing immobilization onto a streptavidin (SA) coated sensor surface (this step enables kinetic characterization of crude RNA binding to proteins on a forteBIO Octet Red96 instrument). After completion of the synthesis, the solid support was suspended in ammonium hydroxide/methylamine (AMA) solution (prepared by mixing 1 volume of ammonium hydroxide (28%) with 1 volume of 40% aqueous methylamine) and heated at 65 °C for 15 min to release the product from the support and to complete the removal of all protecting groups except the

TBDMS group at the 2′-position. The solid support was filtered, and the filtrate was concentrated to dryness. For 2′-*O*-TBDMS RNA, the obtained residue was re-suspended in 115 µL of anhydrous DMF and then heated for 5 min at 65 °C to dissolve the crude product. Triethylamine (TEA, 60 µL) was added to each solution, and the solutions were mixed gently. TEA · 3HF (75 µL) was added to each solution, and the tubes were then sealed tightly and incubated at 65 °C for 2.5 h. The reaction was quenched with 1.75 mL of DEPC-treated water. Following deprotections, the oligonucleotides were desalted/buffer exchanged into DEPC-treated H_2O (using 3000 MWCO Amicon filters) and lyophilized to dryness. The mass of the modified RNAs were confirmed by ESI-MS.

Supplementary Table 1 （ST-1）: The RNA sequences for MS2 coat protein with PS2 substitutions. Each RNA sequence AF147-1 and AF151-2 to AF151-19 and AF151UB (2'-OMe-PS2 modified) is labeled with Biotin at the 5'-end using the BiotinTEG phosphoramidite (Glen Research). AF147-1 is the known RNA sequence that binds to MS2-mutant coat protein. The sequences, AF151-2 to AF151-19 are synthesized by systematically substituting PS2 onto each residue (red).

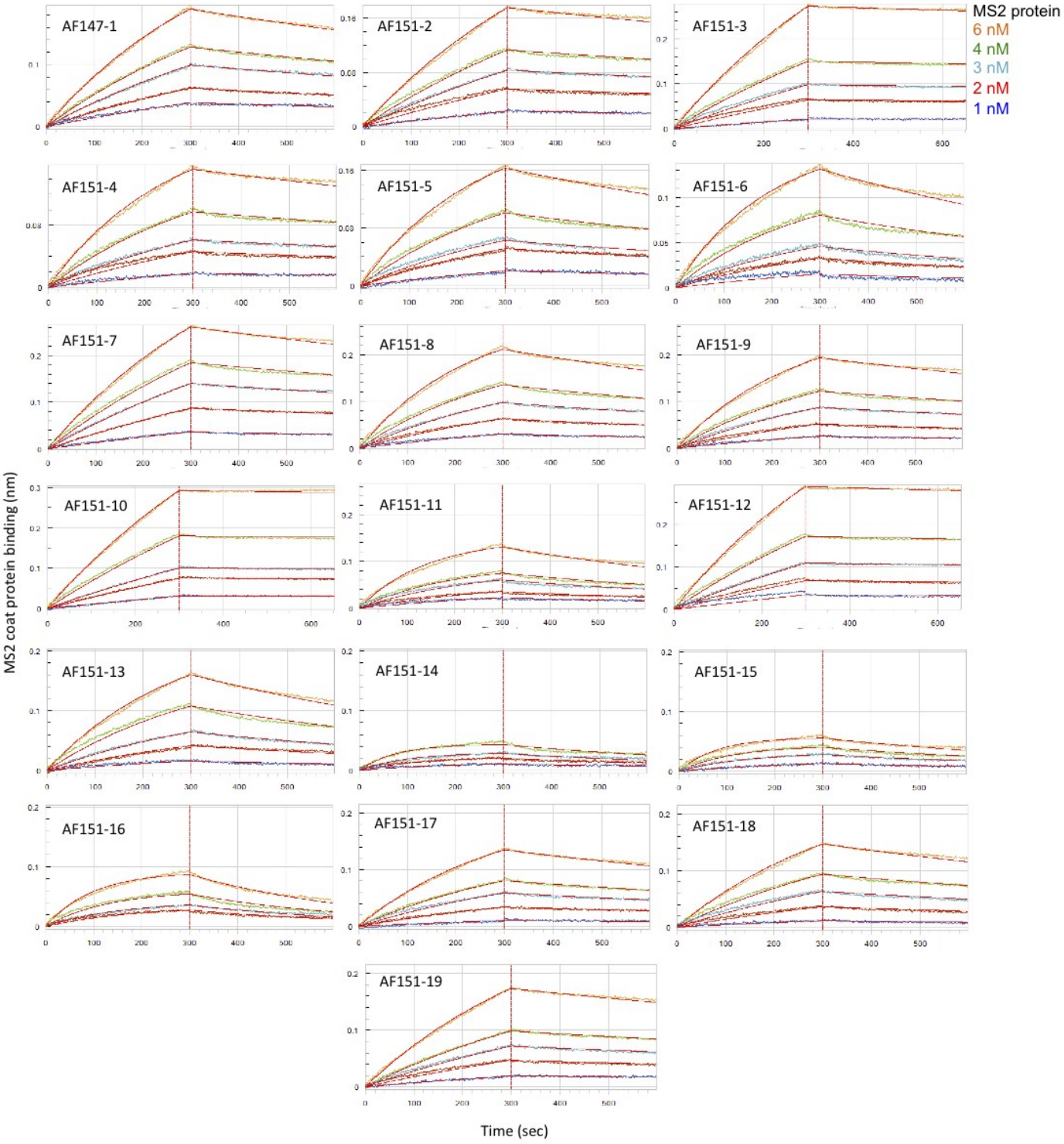
#	Modified sequence information	RNA-ID	Phosphate #
1	5'-ACA UGA GGA UUA CCC AUG U-3'	AF147-1	
2	5'-A_{ps2}CA UGA GGA UUA CCC AUG U-3'	AF151-2	P4
3	5'-AC_{ps2}A UGA GGA UUA CCC AUG U-3'	AF151-3	P5
4	5'-ACA_{ps2} UGA GGA UUA CCC AUG U-3'	AF151-4	P6
5	5'-ACA U_{ps2}GA GGA UUA CCC AUG U-3'	AF151-5	P7
6	5'-ACA UG_{ps2}A GGA UUA CCC AUG U-3'	AF151-6	P8
7	5'-ACA UGA_{ps2} GGA UUA CCC AUG U-3'	AF151-7	P9
8	5'-ACA UGA G_{ps2}GA UUA CCC AUG U-3'	AF151-8	P10
9	5'-ACA UGA GG_{ps2}A UUA CCC AUG U-3'	AF151-9	P11
10	5'-ACA UGA GGA_{ps2} UUA CCC AUG U-3'	AF151-10	P12
11	5'-ACA UGA GGA U_{ps2}UA CCC AUG U-3'	AF151-11	P13
12	5'-ACA UGA GGA UU_{ps2}A CCC AUG U-3'	AF151-12	P14
13	5'-ACA UGA GGA UUA_{ps2} CCC AUG U-3'	AF151-13	P15
14	5'-ACA UGA GGA UUA C_{ps2}CC AUG U-3'	AF151-14	P16
15	5'-ACA UGA GGA UUA CC_{ps2}C AUG U-3'	AF151-15	P17
16	5'-ACA UGA GGA UUA CCC_{ps2} AUG U-3'	AF151-16	P18
17	5'-ACA UGA GGA UUA CCC A_{ps2}UG U-3'	AF151-17	P19
18	5'-ACA UGA GGA UUA CCC AU_{ps2}G U-3'	AF151-18	P20
19	5'-ACA UGA GGA UUA CCC AUG_{ps2} U-3'	AF151-19	P21
20	5'-ACA UGA GGA UU_{Ms2}A CCC AUG U-3'	AF151UB	P14

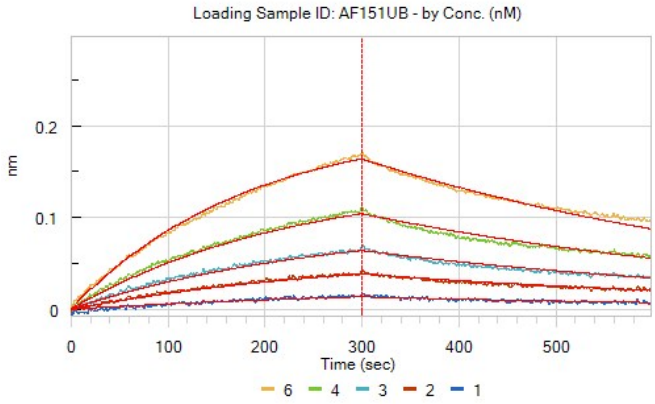
Supplementary Table 2 （ST-2）: Affinity ranking of the RNA sequences (with PS2 substitutions) for MS2 coat-protein (3). The RNA immobilization onto streptavidin-coated sensors was achieved by dipping into 250 nM RNA solution for 1 min at a stir rate of 1,000 rpm. When low affinities are observed (AF151-3, AF151-10, and AF151-12), those samples were purified and confirmed by mass spectrometry. The immobilization of purified RNA was achieved by dipping into a solution of 50 nM pure-RNA for 1 min at a stir rate of 1,000 rpm. A stock of 50.0 nM MS2-mutant in MKMTB buffer (100 mM $MES^{-}Na^{+}$ pH 6.2, 10 mM $MgCl_2$, 80 mM KCl, 0.05% Tween 20, 0.01 mg/mL BSA) was prepared as a dilution series (0, 1.0, 2.0, 3.0, 4.0, 6.0 nM). Association was monitored for 300 sec and the dissociation was followed for 300 sec on a FortéBIO Octet Red 96 instrument. When it was necessary, the dissociation was stretched to at least 900 sec to verify tight binding. The data were fit to a 1:1 binding model using fortéBIO Octet data analysis software. Kinetic constants were determined by integration of the experimental data using the differential rate equation $dR/dt = k_{on} \cdot C \cdot (R_{max}-R) - k_{off} \cdot R$ to obtain both the k_a and k_d values (R = observed response, R_{max} =maximum response upon saturation, C = analyte concentration, k_{on} = association rate constant, k_{off} =dissociation rate constant). The ratio between k_{off} and k_{on} corresponds to the reported dissociation constants ($k_{off}/k_{on}=K_D$). The goodness of the global fitswas judged by the reduced χ^2 and R^2values.RelativeK_D values are obtained as the ratio of K_D of the unmodified RNA and that of the modified one (Relative $K_D = K_D^{unsubstituted}/K_D^{substituted}$).

RNA- ID	Phosphate #	KD (M)	KD Error	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	Full X^2	Full R^2
AF147-1		1.54E-09	3.72E-11	2.46E+05	5.58E+03	3.79E-04	3.14E-06	0.052	0.997
AF151-2	P4	1.53E-09	4.74E-11	2.83E+05	7.33E+03	4.33E-04	7.29E-06	0.025	0.997
AF151-3	P5	2.86E-11	1.44E-12	6.11E+06	1.81E+05	1.75E-04	7.09E-06	0.026	0.998
AF151-4	P6	8.76E-10	1.85E-11	5.83E+05	8.30E+03	5.10E-04	7.96E-06	0.021	0.996
AF151-5	P7	1.56E-09	3.21E-11	5.53E+05	9.60E+03	8.60E-04	9.54E-06	0.031	0.995
AF151-6	P8	1.78E-09	3.92E-11	6.64E+05	1.27E+04	1.19E-03	1.28E-05	0.032	0.991
AF151-7	P9	1.68E-09	3.43E-11	3.09E+05	5.46E+03	5.18E-04	5.33E-06	0.031	0.998
AF151-8	P10	1.32E-09	2.16E-11	6.08E+05	8.01E+03	8.03E-04	7.72E-06	0.037	0.996
AF151-9	P11	1.12E-09	1.95E-11	5.64E+05	7.44E+03	6.33E-04	7.20E-06	0.027	0.997
AF151-10	P12	9.82E-11	1.30E-11	2.49E+05	3.33E+03	2.44E-05	3.22E-06	0.015	0.999
AF151-11	P13	1.42E-09	2.71E-11	9.40E+05	1.50E+04	1.33E-03	1.39E-05	0.038	0.988
AF151-12	P14	2.18E-11	1.62E-12	7.23E+06	3.23E+05	1.58E-04	9.34E-06	0.072	0.997
AF151-13	P15	3.21E-09	8.26E-11	4.00E+05	9.78E+03	1.29E-03	1.04E-05	0.032	0.994
AF151-14	P16	6.14E-10	1.34E-11	2.83E+06	4.89E+04	1.74E-03	2.33E-05	0.009	0.967
AF151-15	P17	8.55E-10	1.45E-11	1.80E+06	2.35E+04	1.54E-03	1.67E-05	0.009	0.983
AF151-16	P18	2.15E-09	3.75E-11	1.25E+06	2.00E+04	2.69E-03	1.87E-05	0.017	0.983
AF151-17	P19	1.77E-09	3.19E-11	4.45E+05	6.96E+03	7.87E-04	7.07E-06	0.012	0.997
AF151-18	P20	2.51E-09	8.20E-11	3.24E+05	9.78E+03	8.11E-04	1.02E-05	0.028	0.994
AF151-19	P21	1.52E-09	3.28E-11	3.37E+05	6.05E+03	5.13E-04	6.17E-06	0.015	0.998
AF151UB	P14	3.77E-09	5.88E-11	5.57E+05	8.34E+03	2.10E-03	9.18E-06	0.020	0.996

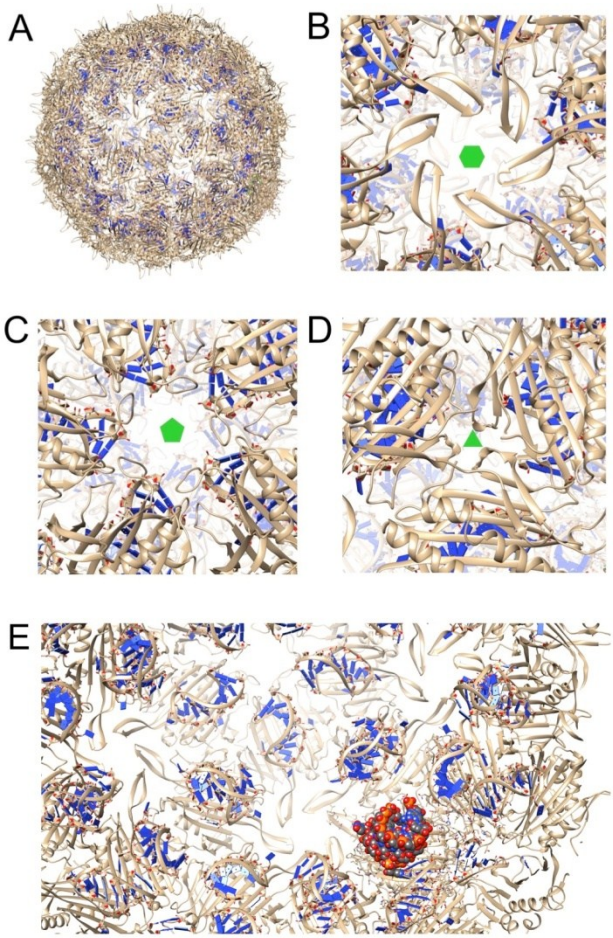
Supplementary Figure 1 (SF-1): BLI analysis of the RNA sequences (with PS2 substitutions) for MS2-coat-protein. The sequences used in this analysis are shown in **Supplementary Table 1** and the kinetic parameters corresponding to the global fits are given in **Supplementary Table 2**. Association was

monitored for 300 sec and the dissociation was followed for 300 sec on a FortéBio Octet Red 96 instrument. Whenit was necessary, the dissociation was stretched to at least 900 sec to verify tight binding. The data were fit to a 1:1 binding model using FortéBio Octet data analysis software.

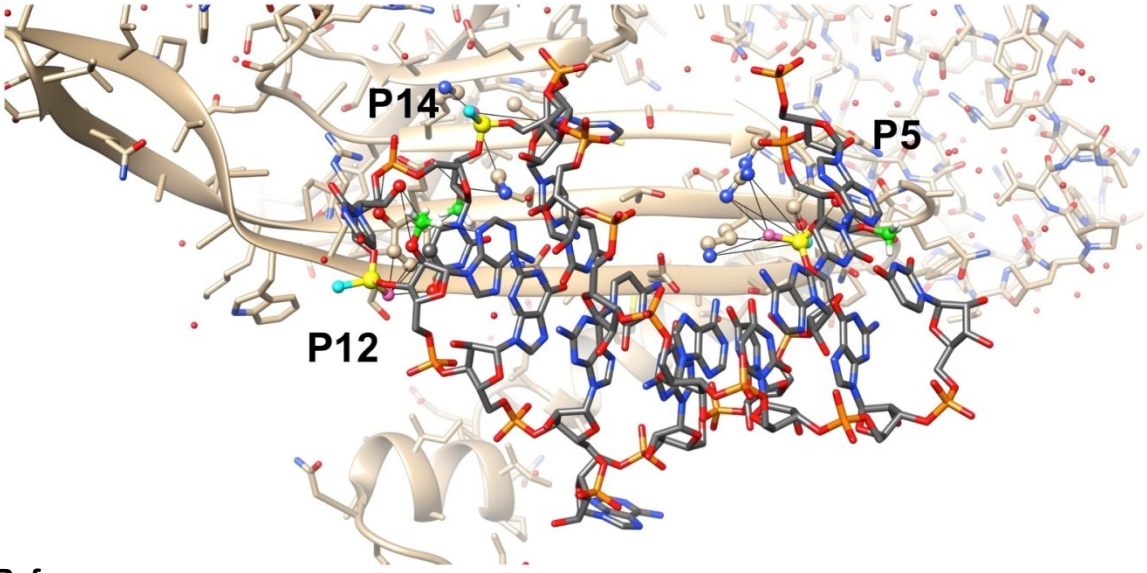




Supplementary Figure 2 (SF-2). Structure of the MS2 phage coat protein-RNA complex [PDB 1zdh] (Valegård et al, 1997). (A) Overall view of the icosahedral capsid. Protein and RNA are shown in ribbon mode and RNA bases are highlighted as blue boxes. (B) – (D) The capsid viewed along one of the 6-fold, 5-fold and 3-fold axes, respectively. Symbols indicating the rotation axes are shown in green. (E) View from inside the capsid with one RNA hairpin shown in space-filling mode and the various rotational symmetries clearly visible.



Supplementary Figure 3 (SF-3). Overall view of interactions between MS2 coat protein (beige ribbon) and RNA hairpin (gray carbons). Points of contact that result in enhanced affinity upon PS2 modification are limited to the base of the stem (P5) and the loop region (P12, P14). P5, P12 and P14 are highlighted as filled circles in yellow along with selected side chains. Rose- and cyan-colored spheres indicate the pro-*R*p and -*S*p sulfurs, respectively, and calculated positions of 2′-OMe carbon atoms are light green.



References

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