Supporting Information for:

2'-C-Methylated nucleotides terminate virus RNA synthesis by preventing active site closure of the viral RNA-dependent RNA polymerase

Alyson K. Boehr[1], Jamie J. Arnold[2], Hyung S. Oh[2], Craig E. Cameron[2] and David D. Boehr[1]*

From the [1]Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802; [2]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania16802

Supporting Information Figures S1 and S2, and Table S1

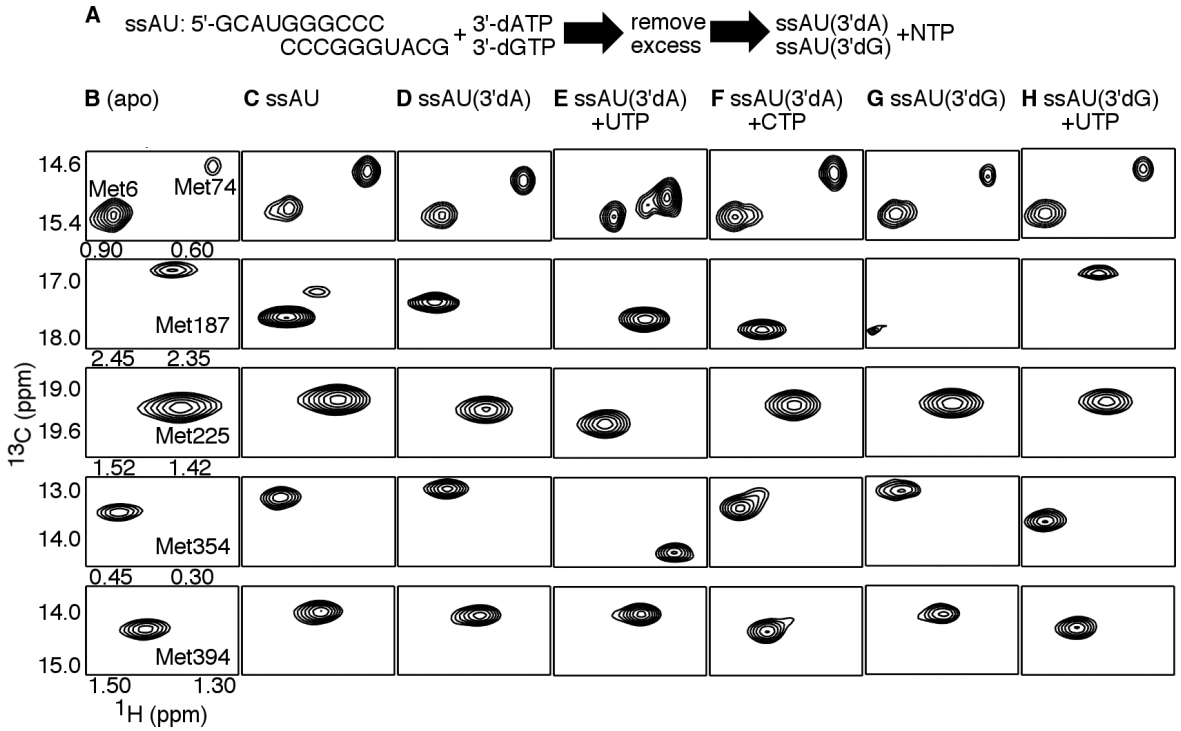

Figure S1. Generation of RdRp-RNA binary and RdRp-RNA-NTP ternary complexes. (**A**) Experimental design. PV RdRp (250 μM) is first incubated with 500 μM ssAU duplex RNA and 4 mM 3'-dATP or 4 mM 3'-dGTP such that the 3'-deoxyribonucleotides become incorporated, but the lack of the 3'-hydroxyl prevents further nucleotide addition. Excess 3'-dATP/3'dGTP are removed through a desalting column, before the addition of the second NTP (4-12 mM) to generate the ternary RdRp-RNA-NTP complexes. The D_2O-based buffer consisted of 10 mM HEPES, pH 8.0, 200 mM NaCl, 0.02% NaN_3, 5 mM $MgCl_2$ and 10 μM $ZnCl_2$. (**B-H**) [^{13}C-*methyl*]Met ^{1}H-^{13}C HSQC NMR spectra of different RdRp complexes, including the (**B**) ligand-free RdRp, (**C**) RdRp:ssAU, (**D**) RdRp:ssAU(3'dA) (i.e. ssAU with incorporated 3'-dAMP), (**E**) RdRp:ssAU(3'dA):UTP, (**F**) RdRp:ssAU(3'dA):CTP, (**G**) RdRp:ssAU(3'dG) (i.e. ssAU with incorporated 3'-dGMP) and (**H**) RdRp:ssAU(3'dG):UTP. Resonances belonging to the ε-$^{13}CH_3$ groups of Met6, Met74, Met187, Met225, Met354 and Met394 are highlighted. NMR spectra were collected at 293 K using a Bruker Avance III 600 MHz spectrometer. Note that information presented in panels S1B and S1C is the same as the information presented in Figure 2 panels S2B and S2C. Here it was important to make spectral comparisons between PV RdRp in the absence of RNA and NTP (apo) and when bound to ssAU RNA prior to nucleotide incorporation of cognate and noncognate nucleotides to form the binary RdRp-RNA and ternary RdRp-RNA-NTP complexes. These data show diagnostic chemical shift changes upon binding RNA, and successful formation of the binary and ternary complexes.

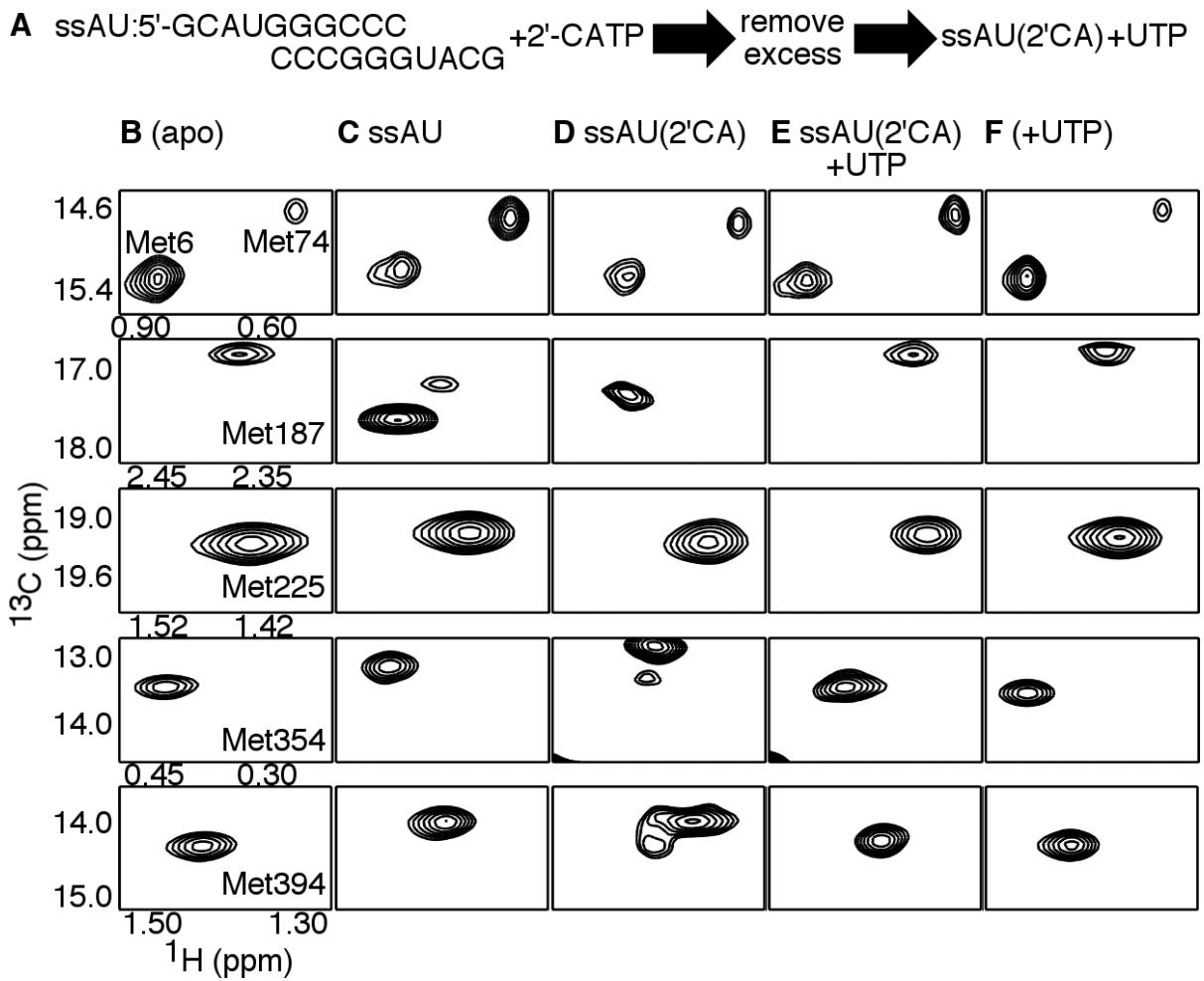

Figure S2. Structural and dynamics changes to PV RdRp bound RNA with incorporated 2'-C-Me-AMP. **A**) Experimental design. PV RdRp (250 μM) is first incubated with 500 μM ssAU duplex RNA and 4 mM 2'-C-Me-ATP (also abbreviated in the figure as 2'-CATP) such that 2'-C-Me-AMP is incorporated, and terminates RNA synthesis (i.e. ssAU(2'CA)). Excess 2'-C-Me-ATP is removed through a desalting column, before the addition of the second NTP to generate the ternary RdRp-ssAU(2'CA):UTP complex. The D_2O-based buffer consisted of 10 mM HEPES, pH 8.0, 200 mM NaCl, 0.02% NaN_3, 5 mM $MgCl_2$ and 10 μM $ZnCl_2$. (**B-H**) [^{13}C-*methyl*]Met ^{1}H-^{13}C HSQC NMR spectra of different RdRp complexes, including the (**B**) ligand-free RdRp, (**C**) RdRp:ssAU, (**D**) RdRp:ssAU(2'CA) (i.e. ssAU with

incorporated 2'-C-Me-AMP), (**E**) RdRp:ssAU(2'CA):UTP and (**F**) RdRp bound with UTP (no RNA). Resonances belonging to the ε-$^{13}CH_3$ groups of Met6, Met74, Met187, Met225, Met354 and Met394 are highlighted. NMR spectra were collected at 293 K using a Bruker Avance III 600 MHz spectrometer. Note that information presented in panels S2B and S2C is the same as the information presented in Figure 1 panels S1B and S1C. Here it was important to make spectral comparisons between PV RdRp in the absence of RNA and NTP (apo) and when bound to ssAU RNA prior to nucleotide incorporation of the ribonucleotide analog 2'-C-Me-ATP and formation of the corresponding RdRp-RNA-NTP complex. The chemical shift changes indicate that 2'-C-Me-ATP was incorporated, but binding of the next correct NTP did not result in active site closure.

Table S1. ^{1}H and ^{13}C chemical shifts for [*methyl*-^{13}C]Met groups in PV RdRp bound with different RNA and nucleotides. All chemical shifts are reported in ppm.

Complex	Met6		Met74		Met187		Met225		Met354		Met394	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
"apo"	0.85	15.26	0.53	14.60	2.37	16.79	1.43	19.28	0.43	13.46	1.43	14.32
ssAU	0.79	15.16	0.53	14.67	2.42 2.38	17.63 17.17	1.42	19.16	0.42	13.15	1.39	13.99
ssAU(3'dA)	0.80	15.26	0.53	14.79	2.43	17.36	1.43	19.31	0.40	12.98	1.38	14.06
ssAU(3'dA)+UTP	0.74	15.31	0.57	15.05	2.36	17.67	1.48	19.51	0.27	14.29	1.37	14.03
ssAU(3'dA)+2'dUTP	0.94	15.40	0.55	14.78	2.40 2.36	17.80 17.54	1.45	19.25	0.43	13.58	1.43 1.38	14.33 14.08
ssAU(3'dA)+CTP	0.86	15.30	0.54	14.71	2.41	17.86	1.44	19.24	0.43	13.55	1.43	14.35
ssAU(3'dG)	0.83	15.29	0.54	14.68			1.44	19.20	0.43	13.59	1.38	14.05
ssAU(3'dG)+UTP	0.86	15.27	0.54	14.67	2.38	16.84	1.44	19.19	0.45	13.65	1.43	14.30
ssAU(2'3'ddA)	0.78	15.17	0.50	14.68			1.40	19.19	0.38	12.90	1.34 1.39	13.92 14.18
ssAU(2'3'ddA)+UTP	0.77 0.87	15.21 15.32	0.50	14.60	2.30	16.92	1.40	19.13	0.39	13.63	1.33	13.97
ssAU(2'CA)	0.76	15.24	0.50	14.72	2.41	17.31	1.40	19.27	0.35 0.37	12.85 13.33	1.35 1.40	13.98 14.32
ssAU(2'CA)+UTP	0.85	15.27	0.50	14.64	2.34	16.80	1.40	19.18	0.39	13.46	1.39	14.25
ssUU(3'dA)	0.76	15.15	0.50	14.71			1.40	19.22	0.35	12.82	1.34 1.41	13.97 14.19
ssUU(3'dA)+ATP	0.88 0.72	15.34 15.26	0.50	14.74	2.33	17.63	1.40	19.20	0.38 0.28	13.39 14.31	1.38 1.33	14.19 13.97
ssUU(3'dA)+2'CATP	0.75	15.21	0.50	14.70			1.40	19.22	0.35 0.37	12.87 13.43	1.33 1.39	13.98 14.22
ssUU(2'CA)+ATP	0.76 0.87	15.18 15.32	0.50	14.71			1.40	19.23	0.35 0.38	12.85 13.37	1.34 1.39	13.99 14.19