Supporting Information

**The Naturally Occurring m1A RNA Modification Can Be Efficiently Incorporated into RNA by SARS-CoV-2 RdRp**

Alexander Apostlea, Reed Arnesonb, Yinan Yuanb\* and Shiyue Fanga\*

a Department of Chemistry and Health Research Institute, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA

b College of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA

Emails: yinyuan@mtu.edu and shifang@mtu.edu

**Experimental Details**

**Materials:** RdRp, also referred to as SARS-CoV-2 replication complex (SC2RC), which consists of NSP12/NSP7/NSP8 (BPS Bioscience); ATP, CTP, GTP and UTP (Fisher Scientific); m1ATP (TriLink Biotechnologies); RNA loading dye (New England Biolabs, NEB); NovexTM TBE-Urea Gel (10%, Fisher Scientific); and RNA **1** (20-mer, FAM+, RP HPLC purified, Integrated DNA Technologies, IDT) were purchased from commercial sources. RNA **2** (30-mer, FAM-) was synthesized and purified with RP HPLC in house. The RNAs were characterized with MALDI MS.

**SARS-CoV-2 RdRp catalyzed RNA synthesis:** RNA duplex was prepared by annealing RNAs **1** and **2**.The solution ofRNAs **1** (20 μM, all concentrations are final) and **2** (20 μM) in Tris-HCl (10 mM), and KCl (100 mM) was heated at 94 oC for 5 min. The solution was then cooled to RT slowly.

 To five PCR tubes containing MgCl2 (5 mM, all concentrations are final), NaCl (50 mM), HEPES (pH 7.5, 20 mM), RNA duplex (1.5 μM), and SARS-CoV-2 RdRP (1 μM) were added, respectively, the following NTPs with each NTP having a final concentration of 0.5 mM. The five tubes correspond to the experiments for lanes 2 to 6 in Figure 1. Tube for lane 2: ATP, CTP, GTP, and UTP. Tube for lane 3: m1ATP, CTP, GTP, and UTP. Tube for lane 4: m1ATP only, after incubating the mixture at 37 oC for 15 min., added ATP, CTP, GTP, and UTP. Tube for lane 5: CTP, GTP, and UTP. Tube for lane 6: ATP, m1ATP, CTP, GTP, and UTP. The final volumes in each tube were 10 μL. The five tubes were incubated at 37 oC for a total of 120 min. The tubes were cooled on ice immediately following incubation, whereupon 5 μL of a 150 μM EDTA solution and 15 μL of 2X RNA loading dye were added sequentially achieving a final volume of 30 μL. To another tube containing only the RNA duplex in 10 μL nuclease free water (1.5 μM, corresponding to the experiment of lane 1 in Figure 1) were also added the EDTA and loading dye solutions (same quantities as above, final volume 30 μL). Aliquots of 10 μL solution from each of the six tubes were loaded onto a 10% PAGE-Urea gel. Electrophoresis was run at 200 V for 35 min. The gel was stained with GelRed (final concentration 300X) for 35 min., and then rinsed with DI water for 5 min. Gel image (Figure 1) was obtained using a UVP GelDoc-IT Imaging System 2UV Transilluminator at 302 nm.