Supporting Information

Reconstitution and functional characterization of SARS-CoV-2 proofreading complex

Zhijun Ma[1], Yasin Pourfarjam[1], and In-Kwon Kim[1,*]

From the [1] Department of Chemistry, University of Cincinnati, 301 Clifton Ct, Cincinnati, Oh 45221, USA

Running title: *Reconstitution of SARS-CoV-2 proofreading complex*

* To whom correspondence should be addressed: In-Kwon Kim, Department of Chemistry, University of Cincinnati, 301 Clifton Ct, Cincinnati, OH 45221, USA; E-mail: kimiw@ucmail.uc.edu; Tel.: (513) 556-1909; Fax.: (513) 556-9239.

Supporting information contents:

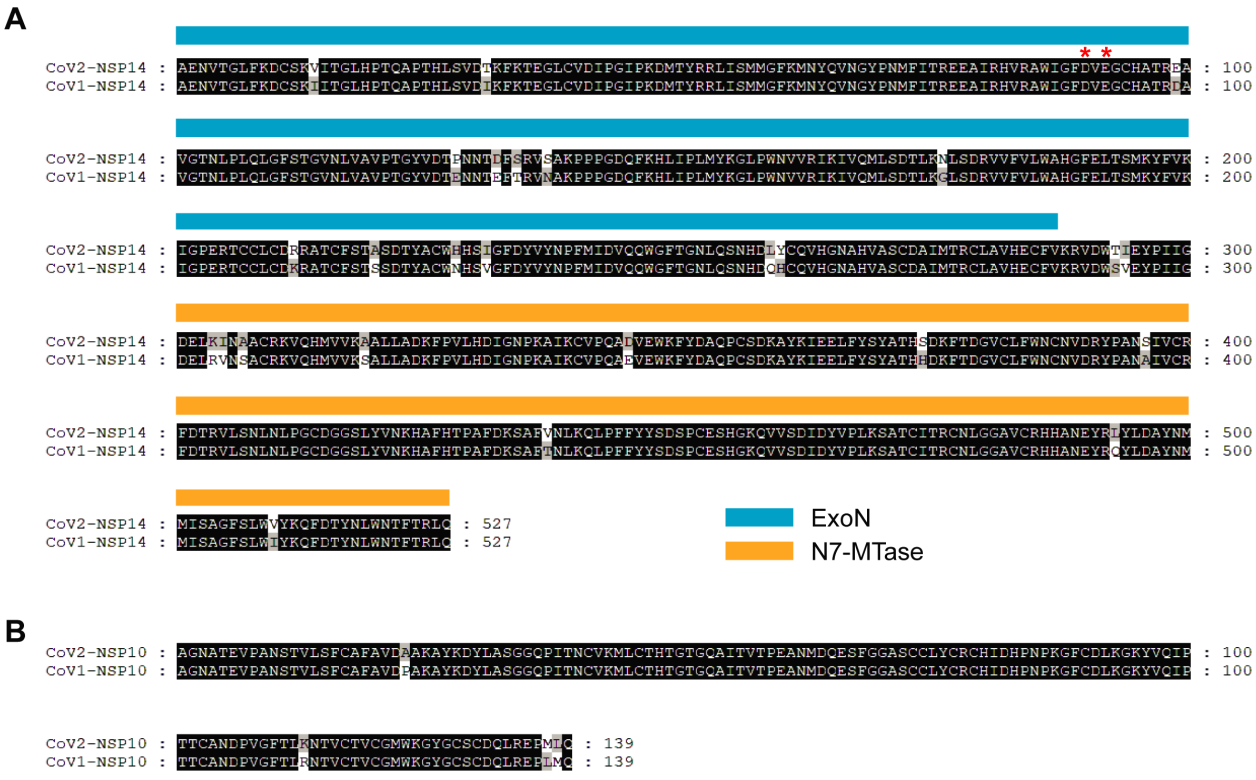


Figure S1. Sequence alignment of nsp14 and nsp10 of SARS-CoV-1 and SARS-CoV-2. (A) SARS-CoV-2 nsp14 shows 95% sequence identity to SARS-CoV-1 nsp14. Two domains of nsp14 (ExoN nad N7-MTase) are shown with sequence. Essential catalytic residues in SARS-CoV-2 nsp14 (1) are shown with red asterisks. (B) SARS-CoV-2 nsp10 shows 97% sequence identity to SARS-CoV-1 nsp10.

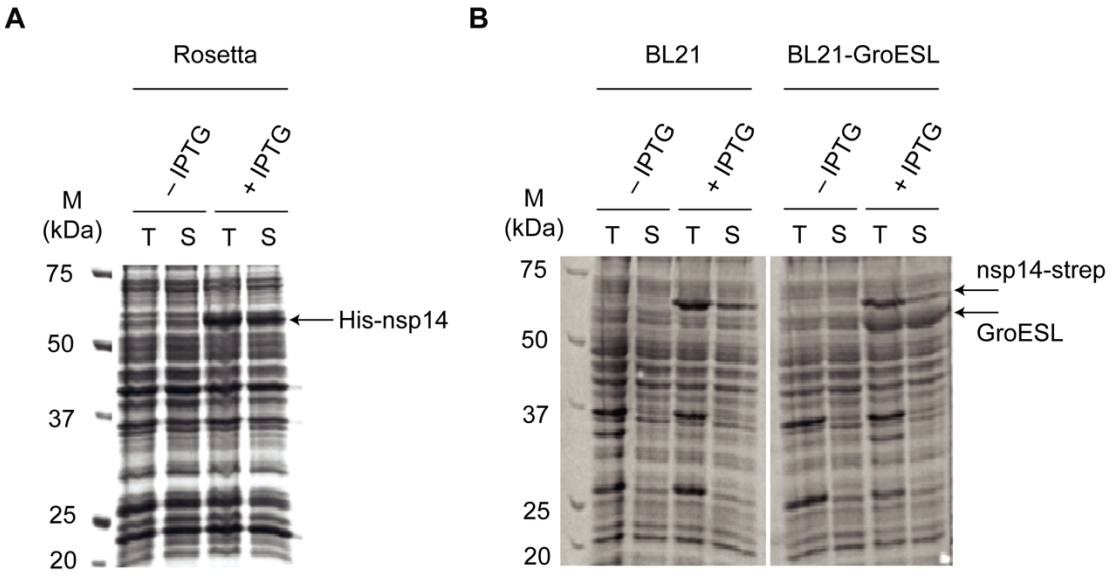


Figure S2. Expression of SARS-CoV-2 nsp14. His-tagged (A) and C-terminal strep-tagged (B) SARS-CoV-2 nsp14 was expressed in *E. coli* BL21(DE3) or Rosetta cells.

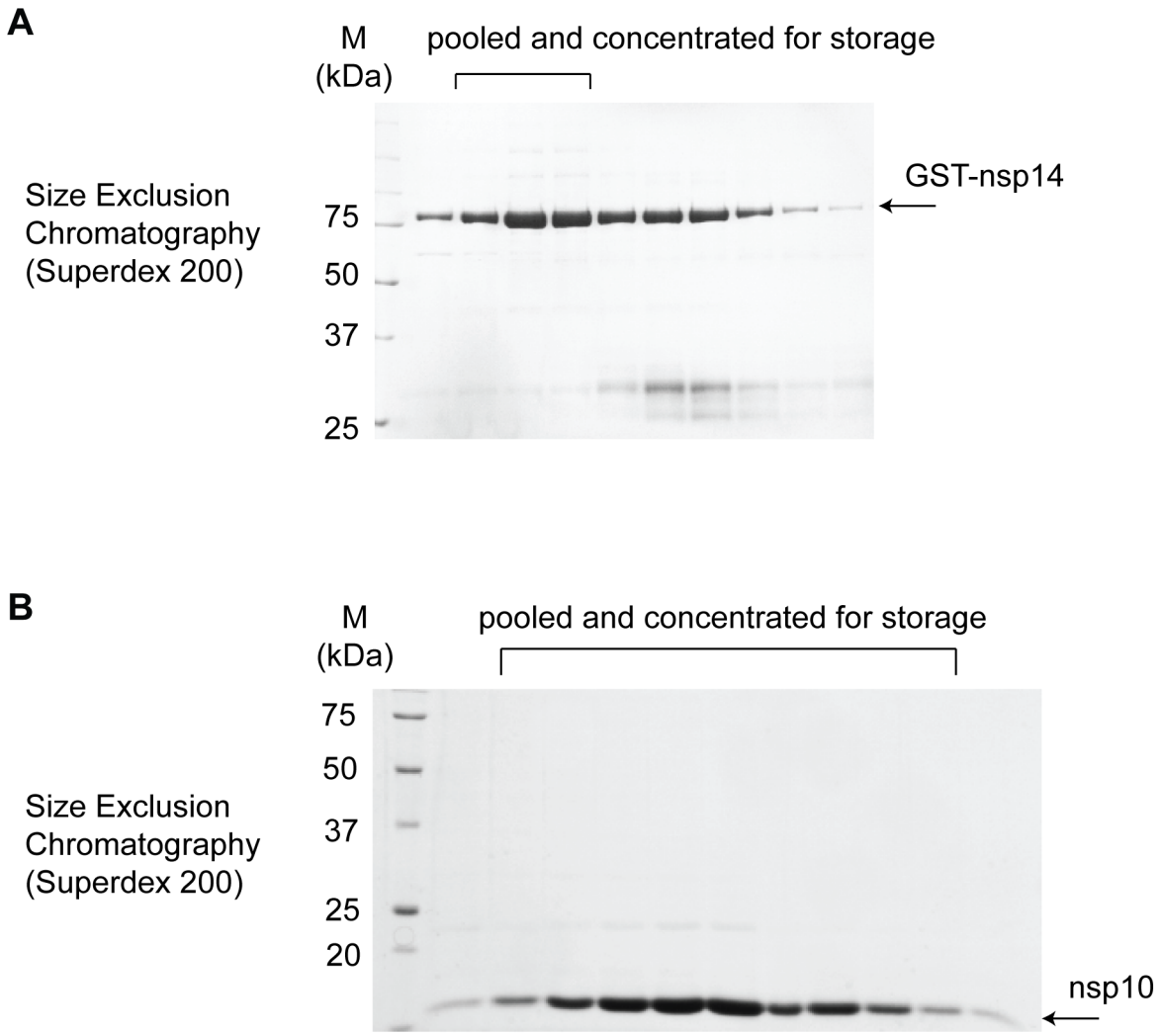
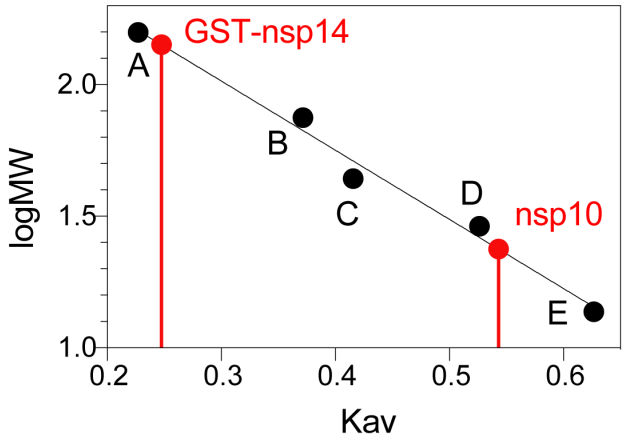


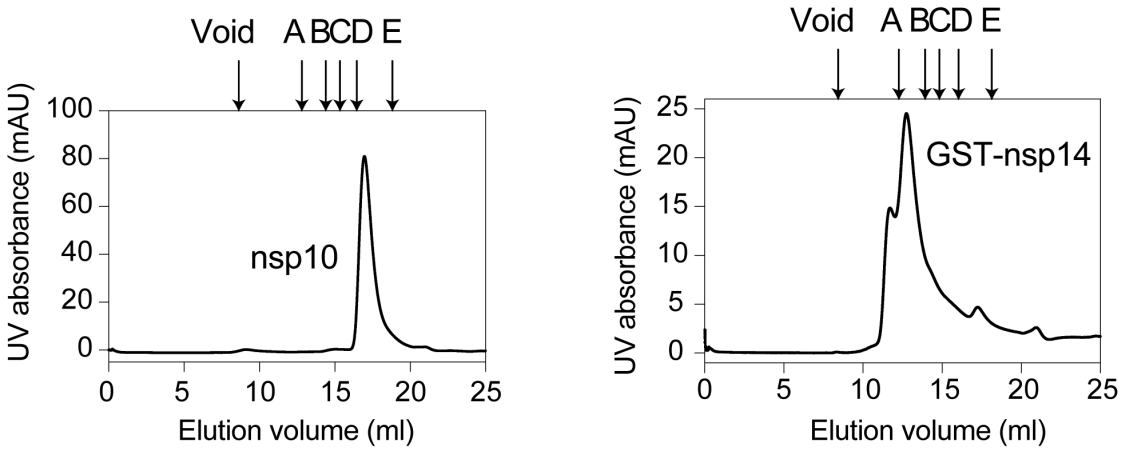
Figure S3. Purification of recombinant SARS-CoV-2 nsp14 and nsp10 proteins. (A) GST-nsp14 was purified to near homogeneity using a three-step protocol (Glutathione-agarose, Q anion exchange, and size exclusion chromatography). A gel image after size-exclusion chromagrography is shown. (B) SARS-CoV-2 nsp10 was purified to near homogeneity using three-step protocol (Ni-NTA, Q anion exchange, and size exclusion chromatography). A gel image after size-exchlusion chromatography is shown.

A



A: Aldolase (158 kDa)
B: albumin (75 kDa)
C: Ovalbumin (44 kDa)
D: Carbonic anhydrase (29 kDa)
E: ribonuclease (13.7 kDa)

B



C

protein	Elution volumn (ml)	MW^{SEC} (kDa)	$MW^{sequence}$ (kDa)
nsp10	17.0	23.7	19.0
GST-nsp14	12.7	141.8	90.4

Figure S4. Size exclusion chromatography of recombinant SARS-CoV-2 nsp14 and nsp10 proteins. (A) A calibration curve using size exclusion calibration standards. The elution volumes (Kav) and molecular weights (logMW) of SARS-CoV-2 nsp14 (GST-nsp14) and nsp10 (His-nsp10) that are calculated from this standard curve are shown. (B) Size-exclusion chromatograms for SARS-CoV-2 nsp10 (left) and nsp14 (right). GST-nsp14 appears to be in equilibrium between different oligomeric states with the dimeric form as the predominant species. (C) A table showing the molecular weights estimated from the size-exclusion standard.

Table S1. RNA and DNA oligonucleotides used in this study.

Oligo name	Sequence 5′ → 3′
5′-FAM-20mer-RNA primer	5′-FAM-GUCAUUCUCCUAAGAAGCUA-3′
40mer-RNA template	5′-CUAUCCCCAUGUGAUUUUAAUAGCUUCUUAGGAGAAUGAC-3′
5′-FAM-21mer-RNA-A (for A:A mismatch)	5′-FAM-GUCAUUCUCCUAAGAAGCUAA-3′
5′-FAM-20mer-DNA primer	5’-FAM-GTCATTCTCCTAAGAAGCTA-3′
40mer-DNA template	5′-CTATCCCCATGTGATTTTAATAGCTTCTTAGGAGAATGAC-3′

REFERENCES

1. Baddock, H. T., Brolih, S., Yosaatmadja, Y., Ratnaweera, M., Bielinski, M., Swift, L. P., Cruz-Migoni, A., Morris, G. M., Schofield, C. J., Gileadi, O., and McHugh, P. J. (2020) Characterisation of the SARS-CoV-2 ExoN (nsp14ExoN-nsp10) complex: implications for its role in viral genome stability and inhibitor identification. *bioRxiv*, 2020.2008.2013.248211