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Structural and Biochemical Characterization of the nsp12-nsp7-nsp8 Core Polymerase Complex from SARS-CoV-2

Graphical Abstract

Highlights
- Cryo-EM structure of SARS-CoV-2 nsp12-nsp7-nsp8 core polymerase complex
- The core complex of SARS-CoV-2 has lower enzymatic activity than SARS-CoV
- SARS-CoV-2 nsp7-8-12 subunits are less thermostable than the SARS-CoV counterpart

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In Brief
Viral polymerase plays a central role in the virus life cycle and is an important antiviral drug target. Peng et al. report the cryo-EM structure of the SARS-CoV-2 core polymerase complex, finding that it has less efficient activity for RNA synthesis and lower thermostability of individual subunits compared with SARS-CoV.

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SUMMARY

The ongoing global pandemic of coronavirus disease 2019 (COVID-19) has caused a huge number of human deaths. Currently, there are no specific drugs or vaccines available for this virus (SARS-CoV-2). The viral polymerase is a promising antiviral target. Here, we describe the near-atomic-resolution structure of the SARS-CoV-2 polymerase complex consisting of the nsp12 catalytic subunit and nsp7-nsp8 cofactors. This structure highly resembles the counterpart of SARS-CoV with conserved motifs for all viral RNA-dependent RNA polymerases and suggests a mechanism of activation by cofactors. Biochemical studies reveal reduced activity of the core polymerase complex and lower thermostability of individual subunits of SARS-CoV-2 compared with SARS-CoV. These findings provide important insights into RNA synthesis by coronavirus polymerase and indicate adaptation of SARS-CoV-2 toward humans with a relatively lower body temperature than the natural bat hosts.

INTRODUCTION

At the end of 2019, a novel coronavirus (2019-nCoV) caused an outbreak of pulmonary disease (Zhu et al., 2020) that was later officially named severe acute respiratory syndrome virus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). The pneumonia disease was named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). The outbreak has developed into a global pandemic affecting most countries in the world (Holtshue et al., 2020; Kim et al., 2020; Shi, 2020). As of May 17, 2020, more than 4,700,000 human infections have been reported worldwide, including over 300,000 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The origin of this virus has not been identified, but multiple origins possibly exist, based on recent bioinformatics analyses of viral isolates from different countries (Andersen et al., 2020; Zhang and Holmes, 2020). So far, there are no specific drugs or vaccines available, which poses a great challenge for the treatment and control of related diseases.

SARS-CoV-2 belongs to the family of Coronaviridae, a group of positive-sense RNA viruses with a broad host spectrum (Vicenzi et al., 2004). Currently, seven human-infecting coronaviruses have been identified, among which SARS-CoV-2 displays the highest similarity in genome sequence to the SARS-CoV emerging in 2002–2003 (Zhong et al., 2003; Zhou et al., 2020). Both viruses utilize the same host receptor, angiotensin-converting enzyme 2 (ACE2), for cell entry and cause respiratory symptoms that may progress to severe pneumonia and lead to death (Lu et al., 2020; Zhou et al., 2020). However, compared with SARS-CoV, SARS-CoV-2 has a much higher transmission rate and lower mortality (Huang et al., 2020; Wang et al., 2020). Most infections result in mild symptoms, and a substantial number of asymptomatic infection cases have also been reported (Rothe et al., 2020). These properties allow SARS-CoV-2 to be transmitted among humans furtively, facilitating quantum leaps of pandemic expansion. Characterizing the infection and replication behavior of SARS-CoV-2 would provide critical information for understanding its unique pathogenesis and host adaptation properties.
The replication of coronavirus is operated by a set of non-structural proteins (nsps) encoded by open reading frame 1a (ORF1a) and ORF1ab in its genome, which are initially translated as polyproteins, followed by proteolysis cleavage for maturation (Ziebuhr, 2005). These proteins assemble into a multi-subunit polymerase complex to mediate transcription and replication of the viral genome. Among them, nsp12 is the catalytic subunit with RNA-dependent RNA polymerase (RdRp) activity (Ahn et al., 2012). nsp12 itself is capable of conducting the polymerase reaction with extremely low efficiency, whereas the presence of nsp7 and nsp8 cofactors remarkably stimulates its polymerase activity (Subissi et al., 2014). The nsp12-nsp7-nsp8 subcomplex is thus defined as the minimal core component for mediating coronavirus RNA synthesis. To achieve complete transcription and replication of the viral genome, several other nsp subunits are required to assemble into a holoenzyme complex, including nsp10, nsp13, nsp14 and nsp16, for which the precise functions in RNA synthesis are not well understood (Adejei et al., 2012; Lehmann et al., 2015; Sevajol et al., 2014; Ziebuhr, 2005). The viral polymerase has shown enormous promise as a highly potent antiviral drug target because of its higher evolutionary stability compared with the surface proteins, which are more prone to drift as a result of selection by host immunity (Shi et al., 2013). Therefore, understanding the structure and function of the SARS-CoV-2 polymerase complex is an essential prerequisite for developing novel therapeutic agents.

In this work, we determined the near-atomic-resolution structure of the SARS-CoV-2 nsp12-nsp7-nsp8 core polymerase complex by cryo-electron microscopy (cryo-EM) reconstruction and revealed its reduced polymerase activity and thermostability compared with the SARS-CoV counterpart. These findings improve our understanding of coronavirus replication and evolution which might indicate clues for the fitness of SARS-CoV-2 to human hosts.

**RESULTS**

**Overall Structure of the SARS-CoV-2 Core Polymerase Complex**

The SARS-CoV-2 nsp12 polymerase and nsp7-nsp8 cofactors were expressed using the baculovirus and *Escherichia coli* expression systems, respectively. The three protein subunits were mixed *in vitro* to constitute the core polymerase complex (Figure S1). The structure of the SARS-CoV-2 nsp12-nsp7-nsp8 complex was determined at 3.7-Å resolution, which clearly resolved the main-chain trace and the most bulky side chains of each subunit (Figures S2 and S3). Similar to the counterpart complex of SARS-CoV (Kirchdoerfer and Ward, 2019), the N-terminal ~110 amino acids of nsp12 as well as the N-terminal ~80 residues of nsp8 and a small portion of the nsp7 C-terminus could not be resolved in the density map (Figure 1A). In total, approximately 80% of the 160-kDa complex was interpreted in the structure.

The SARS-CoV-2 polymerase complex consists of a nsp12 core catalytic subunit bound with a nsp7-nsp8 heterodimer and an additional nsp8 subunit at a different binding site (Figure 1B). The N-terminal portion of the nsp12 polymerase subunit contains a nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain that is shared by all members of the Nidovirales order (Lehmann et al., 2015). This domain binds at the back side of the right-hand-configured C-terminal RdRp. Between them is an interface domain that links the NiRAN domain to the finger subdomain of RdRp (Figures 1B and 1C). The NiRAN and interface domains represent additional features of coronavirus RdRp compared with the polymerase subunit of flaviviruses, which is also a group of positive-sense RNA viruses (Duan et al., 2017; Godoy et al., 2017; Zhao et al., 2017). The C-terminal catalytic domain adopts a conserved architecture of all viral RdRps, composed of the finger, palm, and thumb subdomains (Figure 1C). A remarkable feature of the coronavirus RdRp is the long finger extension that intersects with the thumb subdomain to form a closed-ring structure (Figure 1C), in contrast to the smaller loop in segmented negative-sense RNA virus (sNSV) polymerases that results in a relatively open conformation, such as influenza virus, bunyavirus, and arenavirus polymerases (Figure S4; Gerlach et al., 2015; Peng et al., 2020; Pfug et al., 2014). Similar close contact between the fingers and thumb subdomains is also observed in the structures of poliovirus (PV) and Zika virus (ZIKV) polymerases (Figure S4; Godoy et al., 2017; Gong and Peersen, 2010), which might be a common feature of positive-sense RNA virus polymerases.

**Structural Comparison of the SARS-CoV-2 and SARS-CoV nsp12-nsp7-nsp8 Complexes**

Basically, the structure of the SARS-CoV-2 polymerase complex highly resembles that of SARS-CoV, with a global root-mean-square deviation (RMSD) of ~1 Å for the C-α carbon atoms (Figure 2A). There are 1, 4, and 25 residue substitutions between the two viruses in the structurally visualized regions of the nsp7, nsp8, and nsp12 subunits, respectively (Figure 2B) (there are 1 and 7 additional substitutions in the unresolved regions of the nsp8 and nsp12 subunits, respectively). However, these variations did not result in obvious structural changes in the polymerase complex. During the review process of this manuscript, two other research groups also reported the high-resolution structure of the SARS-CoV-2 core polymerase complex, which revealed structural features similar to the SARS-CoV counterpart, consistent with our observations (Gao et al., 2020; Yin et al., 2020). All three structures reported by our group and others revealed similar structural features and allowed identification of a previously undefined b-hairpin motif in the N terminus of the nsp12 subunit that binds at the interface between the NiRAN domain and the palm subdomain of RdRp (Figure 2C). However, no extra density for this region was observed in the reconstruction of the SARS-CoV polymerase complex (Figure 2D), suggesting a different conformation or flexibility of this motif between the two viruses. Of note, in the structure determined by Gao et al. (2020), the N-terminal residues S1–117 of the nsp12 subunit are clearly resolved to constitute an almost complete NiRAN domain (Gao et al., 2020). In contrast, this region reveals poor density in the reconstructions by our group and Yin et al. (2020), suggesting moderate flexibility of this region, but the functional relevance remains elusive.
Conserved Catalytic Center of nsp12 and Interaction with Cofactors

The catalytic domain of the SARS-CoV-2 nsp12 subunit is arranged following the typical right-hand configuration shared by all viral RdRps, which includes seven critical catalytic motifs (A–G) (Figure 3). Among them, motifs A–F are highly conserved for all viral RdRps, and motif G is defined as a hallmark of primer-dependent RdRp in some positive-sense RNA viruses and interacts with the primer strand to initiate RNA synthesis (Figure S4). Motif C contains the critical 759-SDD-761 catalytic residues, which reside in a β-turn loop connecting two adjacent strands. Motif F forms a fingertip that protrudes into the catalytic chamber and interacts with the finger extension loops and the thumb subdomain (Figure 3B). It has been shown that some sNSV polymerases, e.g., influenza virus and bunyavirus polymerases, require binding of a conserved 5’ RNA hook to activate the activity for RNA synthesis by stabilizing the fingertip, which is otherwise highly flexible in the apo form (Gerlach et al., 2015; Hengrung et al., 2015; Peng et al., 2020; Pflug et al., 2014; Reich et al., 2014). In the structure of coronavirus polymerase, this fingertip loop is stabilized by the adjacent finger extension loops, and sandwiches the finger extension loops in between to stabilize its conformation (Figure 1B). This interaction is mainly mediated by nsp7 within the heterodimer, whereas nsp8 (nsp8.1) contributes few contacts to the nsp12 polymerase subunit (Figures 1B and 3D). The other nsp8 (nsp8.2) subunit clamps the top region of the nsp12 finger subdomain and forms additional interactions with the interface domain (Figures 1B and 3D). The two nsp8 subunits display significantly different conformations with substantial refolding of the N-terminal extension helix region that mutually preclude binding at the other molecular context (Figure 3C). The importance of both cofactor-binding sites has been validated by previous biochemical studies of SARS-CoV polymerase, which revealed their essential roles in stimulating activity of the nsp12 polymerase subunit (Subissi et al., 2014).

Based on the elongation complex of PV polymerase (Gong and Peersen, 2010), we modeled the RNA template and product strands into the catalytic chamber of the SARS-CoV-2 nsp12 subunit. This pseudo-elongation intermediate structure reveals that the template entrance is supported by the finger extension loops and the fingertip to guide the 3’ viral
RNA (vRNA) to the catalytic chamber (Figure 3D). The nucleotide triphosphate (NTP) substrate enters through a channel at the back side of the palm subdomain to reach the active site. The template and product strands form a duplex to exit the polymerase chamber in the front (Figure 3D). Because the viral genome and sub-genomic mRNA products are functional in single-stranded form, it requires further steps assisted by other nsp subunits to separate the duplex for complete transcription and replication processes.

**Reduced Activity of the SARS-CoV-2 Core Polymerase Complex**

Given the residue substitutions between SARS-CoV-2 and SARS-CoV polymerase subunits but the high degree of overall sequence similarity, we compared the enzymatic behaviors of the viral polymerases, aiming to analyze their properties in terms of viral replication. Both sets of core polymerase complex could well mediate primer-dependent RNA elongation reactions templated by the 3' vRNA. Intriguingly, the SARS-CoV-2 nsp12-nsp7-nsp8 complex displayed a much lower efficiency (∼2.35%) for RNA synthesis compared with the SARS-CoV counterpart (Figure 4A). Because all three nsp subunits harbor some residue substitutions between the two viruses, we further conducted cross-combination analysis to evaluate the effects of each subunit on the efficiency of RNA production. In the context of the SARS-CoV-2 nsp12 polymerase subunit, replacement of the nsp7 cofactor subunit with that of SARS-CoV did not result in an obvious effect on polymerase activity, whereas introduction of the SARS-CoV nsp8 subunit greatly boosted the activity to ∼2.1 times of that of the homologous combination. Simultaneous replacement of the nsp7 and nsp8 cofactors further enhanced the efficiency of RNA synthesis to ∼2.2 times of that for the SARS-CoV-2 homologous complex (Figure 4B). Consistent with this observation, combination of the SARS-CoV-2 nsp7-nsp8 subunits with the SARS-CoV nsp12 polymerase subunit compromised its activity compared with the native cognate cofactors, among which the nsp8 subunit exhibited a more obvious effect than nsp7 (Figure 4C). This evidence suggested that the variations in the nsp8 subunit had a significant negative effect on the polymerase activity of SARS-CoV-2 nsp12. The non-significant effect of nsp7 on polymerase activity was quite conceivable because only one residue substitution occurred between the two viruses (Figure 2B). In addition, we also compared the polymerase activity of different nsp12 subunits in the same
context of nsp7-nsp8 cofactors. Combined with either cofactor set, the SARS-CoV-2 nsp12 polymerase showed a lower efficiency (≤50%) for RNA synthesis compared with the SARS-CoV counterpart (Figure 4D). This observation demonstrated that the residue substitutions in nsp12 also contributed to the reduction of its polymerase activity, with a similar effect on the variations in the nsp8 cofactor.

Effects of Amino Acid Substitutions on the Core Polymerase Subunits

Despite the amino acid substitutions in all three subunits of the core polymerase complex between SARS-CoV-2 and SARS-CoV, none of these residues is located at the polymerase active site or the contacting interfaces between adjacent subunits (Figure 2B), suggesting that these substitutions do not affect the inter-subunit interactions for assembly of the polymerase complex. To test this hypothesis, we measured the binding kinetics between different subunits of the two viruses by surface plasmon resonance (SPR) assays. Each interaction pair exhibited similar kinetic features for the two viruses, all with sub-micromolar-range affinities (Figures 5A and 5B). We also tested cross-binding between subunits of the two viruses, which revealed similar affinities for heterologous pairs compared with the native homologous interactions (Figures 5C and 5D).

We then compared the thermostability of each component in the polymerase complex of the two viruses (Figure 6). In contrast, both the nsp8 and nsp12 subunits of SARS-CoV-2 showed lower melting temperature (Tm) values compared with the corresponding subunits of SARS-CoV, suggesting poorer thermostability of SARS-CoV-2 proteins (Figure 6B, 6C, 6E, and 6F).

Taken together, the residue substitutions in the SARS-CoV-2 nsp12 polymerase subunit and nsp7-nsp8 cofactors compromise the efficiency of RNA synthesis by the core polymerase complex and reduce the thermostability of individual protein subunits compared with the counterparts of SARS-CoV. These changes may indicate some clues regarding the adaptive evolution of SARS-CoV-2 in favor of human hosts. It has been shown that humans have a relatively lower body temperature than bats, which are potentially the natural host of a panel of zoonotic viruses, including SARS-CoV and SARS-CoV-2 (O’Shea et al., 2014; Zhou et al., 2020).

DISCUSSION

The structural information of coronavirus polymerase interaction with cofactors suggests a common theme of viral RdRp activation despite being executed by different structural components. The coronavirus polymerase subunit requires multiple cofactors or subunits for complete transcription and replication functions, similar to the related flaviviruses, which also harbor a positive-sense RNA genome (Aktepe and Mackenzie, 2018; Sevajol et al., 2014; Ziebuhr, 2005). In contrast, sNSVs utilize fewer multi-subunit or multi-domain protein
components to accomplish similar processes, which could be activated by RNA segments instead of proteins (Gerlach et al., 2015; Peng et al., 2020; Pflug et al., 2014). As revealed by the coronavirus core polymerase structures, it lacks the essential component for unwinding the template-product hybrid, which is required to release the single-stranded mRNA and viral genome for protein expression and virion assembly. In the structure of sNSV polymerases, a lid domain is present at the intersection region of template and product exit tunnels to force duplex deformation before leaving the polymerase chamber (Gerlach et al., 2015; Peng et al., 2020; Reich et al., 2014). The nsp13 subunit has been shown to have RNA helicase activity, suggesting its involvement in RNA synthesis at the post-catalytic stage (Adedeji et al., 2012). Further investigations are required to understand how this process takes place.

Of note, we demonstrate that the amino acid substitutions in the polymerase and cofactors of SARS-CoV-2 lead to obviously reduced activity for RNA synthesis compared with the SARS-CoV core polymerase complex. Efficient activity of nsp12 polymerase requires the presence of the nsp7 and nsp8 cofactors. Apart from the fully elongated product (green arrowhead), some aberrant termination products were also observed (yellow arrowhead). The excess primer band is indicated by a cyan arrowhead. (B and C) Comparison of the regulatory effects of the nsp7 and nsp8 cofactors in the context of the SARS-CoV-2 (B) and SARS-CoV (C) nsp12 polymerase, respectively. (D) Comparison of the activity of the nsp12 polymerase of different viruses in the same context of cofactors. Polymerase activity was quantified by integrating the intensity of the fully elongated product bands. The results, in histograms, are presented as the means ± SD, where error bars represent SDs. The significance of difference was tested by one-way ANOVA based on the results of four independent experiments (n = 4) using different protein preparations. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. See also Figure S4.

Figure 4. In Vitro Polymerase Activity of nsp12 and Regulatory Effects of Cofactors

(A) Comparison of RNA synthesis activities of the SARS-CoV-2 and SARS-CoV core polymerase complex. Efficient activity of nsp12 polymerase requires the presence of the nsp7 and nsp8 cofactors. Apart from the fully elongated product (green arrowhead), some aberrant termination products were also observed (yellow arrowhead). The excess primer band is indicated by a cyan arrowhead. (B and C) Comparison of the regulatory effects of the nsp7 and nsp8 cofactors in the context of the SARS-CoV-2 (B) and SARS-CoV (C) nsp12 polymerase, respectively. (D) Comparison of the activity of the nsp12 polymerase of different viruses in the same context of cofactors. Polymerase activity was quantified by integrating the intensity of the fully elongated product bands. The results, in histograms, are presented as the means ± SD, where error bars represent SDs. The significance of difference was tested by one-way ANOVA based on the results of four independent experiments (n = 4) using different protein preparations. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. See also Figure S4.

ferases (Sevajol et al., 2014; Ziebuhr, 2005). Among them, nsp14 may directly interact with nsp12 to assemble a processive elongating complex and ensure the fidelity of RNA synthesis (Subissi et al., 2014). Moreover, nsp16 may also be involved in formation of a stable holoenzyme, which, together with nsp10 and nsp14, constitutes the capping modules in transcription (Chen et al., 2011; Sevajol et al., 2014). These steps would also render important determinants for the efficiency and accuracy of RNA synthesis by the coronavirus replication machinery. Thus, the collective behavior of SARS-CoV-2 polymerase complex in the context of an authentic viral replication cycle still remains an open question to be explored further. On the other hand, the lower thermostability of SARS-CoV-2 polymerase subunits might indicate its adaptation to humans, who have a relatively lower body temperature compared with bats, the potential natural hosts of SARS-CoV-2 (O’Shea et al., 2014; Zhou et al., 2020). Interestingly, we also found that the closely related bat coronavirus RaTG13 showed an extremely high sequence identity of core polymerase subunits to SARS-CoV-2, in which the nsp7 and nsp8 cofactors are strictly identical and the nsp12 catalytic subunit harbors only four residue replacements between the two viruses (Figure S5), suggesting similar enzymatic properties and thermostabilities of their polymerase components. This observation indicates that the RaTG13 coronavirus may have been well adapted to human hosts in terms of viral replication machinery and would further support the probable bat origin of SARS-CoV-2 (Zhou et al., 2020).
In summary, our structural and biochemical analyses of the SARS-CoV-2 core polymerase complex improve our understanding of the mechanisms of RNA synthesis by different viral RdRps and highlight a common theme for polymerase activation by stabilizing critical catalytic motifs via diverse means. In addition, the different biochemical properties of the polymerase components of SARS-CoV-2 and SARS-CoV suggest clues for adaptive evolution of coronaviruses in favor of human hosts.

Figure 5. SPR Binding Kinetics of Protein Pairs in the Polymerase Complex of SARS-CoV-2 and SARS-CoV

(A and B) The binding profiles of the homologous protein subunits of SARS-CoV-2 (A) and SARS-CoV (B), respectively.

(C and D) The cross-binding kinetics between protein subunits of SARS-CoV-2 and SARS-CoV. All analytes were measured with serially diluted concentrations as shown in (A).

The title is presented as "analyte/immobilized ligand" to facilitate comparison. The binding between nsp12 and the nsp7L8 fusion protein was fitted with the heterogeneous binding mode because the nsp7-nsp8 heterodimer exhibited non-uniform conformations in solution (Zhai et al., 2005). It can occupy either cofactor binding site as a stable nsp7-nsp8 complex or free nsp8 when nsp7 detaches from the heterodimer. Both equilibrium binding constant values (KD1 and KD2) were calculated in this mode. The data shown are a representative result of three independent experiments using different protein preparations, all of which produced similar results. See also Figure S4.
Detailed methods are provided in the online version of this paper and include the following:

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Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2020.107774.

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Author Contributions

Conceptualization, Y. Shi, Q.P., and R.P.; Methodology, Q.P., R.P., and M.W.; Investigation, Q.P., R.P., B.Y., J.Z., M.W., X.W., Q.W., Y. Sun, Z.F., and J.Q.; Data Curation, Q.P. and R.P.; Writing – Original Draft, Q.P., R.P., M.W., and Y. Shi; Writing – Review & Editing, all authors; Visualization, Q.P., R.P., and M.W.; Supervision, Y. Shi; Project Administration, Q.P. and R.P.; Funding Acquisition, Y. Shi, R.P., and M.W.

Declaration of Interests

The authors declare no competing interests.
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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and Virus Strains** | | |
| E. coli: BL21 (DE3) competent cells | Thermo Fisher | Cat.# EC0114 |
| E. coli: DH10Bac competent cells | Invitrogen | Cat.# 10361-012 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Insect-XPRESS™ Protein-free Insect Cell Medium | Lonza | Cat.# 12-730Q |
| FuGENE 6 Transfection Reagent | Promega | Cat.# E2691 |
| TCEP | Sigma | Cat.# C4706 |
| ATP | Takara | Cat.# 4041 |
| CTP | Takara | Cat.# 4043 |
| UTP | Takara | Cat.# 4044 |
| GTP | Takara | Cat.# 4042 |
| IPTG | Sigma | Cat.# 6758 |
| nsp7 subunit from SARS-CoV-2 | This study | N/A |
| nsp8 subunit from SARS-CoV-2 | This study | N/A |
| nsp12 subunit from SARS-CoV-2 | This study | N/A |
| nsp7L8 from SARS-CoV-2 | This study | N/A |
| nsp7 subunit from SARS-CoV | This study | N/A |
| nsp8 subunit from SARS-CoV | This study | N/A |
| nsp12 subunit from SARS-CoV | This study | N/A |
| nsp7L8 from SARS-CoV | This study | N/A |
| **Deposited Data** | | |
| Cryo-EM map and model of nsp12 bound to nsp7 and nsp8 from SARS-CoV-2 | This study | 7BW4 (PDB); 30226(EMDB) |
| **Experimental Models: Cell Lines** | | |
| High Five cells | Invitrogen | Cat.# B85502 |
| SF9 cells | Invitrogen | Cat.# 11496015 |
| **Oligonucleotides** | | |
| 40-nt Template RNA strand | Takara | N/A |
| CUAUCCCAUGUGAUAUUAAUCUCUUAAGG AGAAUGAC | | |
| 20-nt Primer RNA strand, FAM-GUCAUUCUCUAAGAAGCUA | Takara | N/A |
| **Recombinant DNA** | | |
| Expression plasmid for nsp7 of SARS-CoV-2 | This study | N/A |
| Expression plasmid for nsp8 of SARS-CoV-2 | This study | N/A |
| Expression plasmid for nsp7L8 of SARS-CoV-2 | This study | N/A |
| Expression plasmid for nsp12 of SARS-CoV-2 | This study | N/A |
| Expression plasmid for nsp7 of SARS-CoV | This study | N/A |
| Expression plasmid for nsp8 of SARS-CoV | This study | N/A |
| Expression plasmid for nsp7L8 of SARS-CoV | This study | N/A |
| Expression plasmid for nsp12 of SARS-CoV | This study | N/A |
| **Software and Algorithms** | | |
| Relion 3.0 | Zivanov et al., 2018 | https://github.com/3dem/relion |
| MotionCor 2.0 | Zheng et al., 2017 | https://emcore.ucsf.edu/ucsf-motioncor2 |
| CTFFIND 4.1 | Rohou and Grigorieff, 2015 | https://grigoriefflab.umassmed.edu/ctffind4 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yi Shi (shiyi@im.ac.cn).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
The cryo-EM density map and atomic coordinates have been deposited to the Electron Microscopy Data Bank (EMDB) and the Protein Data Bank (PDB) with the accession codes EMD-30226 and 7BW4, respectively. All other data are available from the authors on reasonable request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

*Escherichia coli* BL21 (DE3) strain (EC0114) and MAX Efficiency DH10Bac Competent cells (10361-012) were incubated in LB medium with corresponding antibiotics at 37°C in a shaking incubator (180 rpm). SF9 (11496015) and High Five (B85502) cells were cultured at 27°C in Insect-XPRESS (12-730Q) with continuous shaking (120-130 rpm).

METHOD DETAILS

**Protein expression and purification**
The codon-optimized sequences of nsp7 and nsp8 were synthesized with an N-terminal 6 × histidine tag and inserted into pET21a vector for expression in *E. coli* (Synbio Tec, Suzhou, China). For the nsp7L8 fusion protein, the sequence was also codon-optimized for *E. coli* expression system and a 6 × histidine linker was introduced between the nsp7 and nsp8 subunits (Genewiz Tec, Suzhou,
China). Protein production was induced with 1 mM isopropylthio-galactoside (IPTG) and incubated for 14-16 hours at 16°C. Bacterial cells were harvested by centrifugations (12,000 rpm, 10 min), resuspended in buffer A (20 mM HEPES, 500 mM NaCl, 2 mM Tris (2-carboxyethyl) phosphine (TCEP), pH 7.5, and lysed by sonication. Cell debris were removed via centrifugation (12,000 rpm, 1 h) and filtration with a 0.22 μm cut-off filter. The supernatant was loaded onto an HisTrap column (GE Healthcare) for initial affinity purification. The target proteins were eluted using buffer A supplemented with 300 mM imidazole. Fractions were pooled and subjected to size-exclusion chromatography (SEC) with a Superdex 200 10/300 increase column (GE Healthcare). The final product was concentrated and stored at −80°C.

For nsp12 proteins, the genes were codon-optimized for Spodoptera frugiperda and incorporated into the pFastBac-1 plasmid with a C-terminal thrombin proteolysis site, a 6 × histidine and two tandem Strep tags. Proteins were expressed with High Five cells at 27°C for 48 h post infection. Cells were collected by centrifugation (3,000 rpm, 10 min) and resuspended in buffer B (25 mM HEPES, 300 mM NaCl, 1 mM MgCl₂, and 2mM TCEP, pH 7.4. The cell suspension was lysed by sonication and the lysate was clarified using ultracentrifugation (30,000 rpm, 2 h) and filtered with 0.22 μm cut-off membranes. The resulting supernatant was applied to a Strep-Trap column (GE Healthcare) to capture the target proteins. The bound proteins were eluted with buffer B supplemented with 2.5 mM desthiobiotin. Target fractions were pooled and subjected to further purification by SEC using a Superdex 200 10/300 increase column (GE Healthcare). The final product was concentrated and stored at −80°C before use.

**Cryo-EM sample preparation and imaging**
An aliquot of 3 μL protein solution (0.6 mg/mL) was applied to a glow-discharged Quantifoil 1.2/1.3 holey carbon grid and blotted for 2.5 s in a humidity of 100% before plunge-freezing with an FEI Vitrobot Mark IV. Cryo-samples were screened using an FEI Tecnai TF20 electron microscope and transferred to an FEI Talos Arctica operated at 200 kV for data collection. The microscope was equipped with a post-column Bioquantum energy filter (Gatan) which was used with a slit width of 20 eV. The data were automatically collected using SerialEM software (https://bio3d.colorado.edu/SerialEM/). Images were recorded with a Gatan K2-summit camera in super-resolution counting mode with a calibrated pixel size of 0.8 Å at the specimen level. Each exposure was performed with a dose rate of 10 e⁻/pixel/s (approximately 15.6 e⁻/Å²/s) and lasted for 3.9 s, resulting in an accumulative dose of ~60 e⁻/Å² which was fractionated into 30 movie-frames. The final defocus range of the dataset was approximately −1.4 to −3.4 μm.

**Image processing**
The image drift and anisotropic magnification was corrected using MotionCor2 (Zheng et al., 2017). Initial contrast transfer function (CTF) values were estimated with CTFFIND4.1 (Rohou and Grigorieff, 2015) at the micrograph level. Images with an estimated resolution limit worse than 5 Å were discarded. Particles were automatically picked with RELION-3.0 (Zivanov et al., 2018) following the standard protocol. In total, approximately 1,860,000 particles were picked from ~4,200 micrographs. After 3 rounds of extensive 2D classification, ~924,000 particles were selected for 3D classification with the density map of SARS-CoV nsp12-nsp7-nsp8 complex (EMDB-0520) as the reference which was low-pass filtered to 60 Å resolution. After two rounds of 3D classification, a clean subset of ~101,000 particles was identified, which displayed clear features of secondary structural elements. These particles were subjected to 3D refinement supplemented with per-particle CTF refinement and dose-weighting, which led to a reconstruction of 3.65 Å resolution estimated by the gold-standard Fourier shell correlation (FSC) 0.143 cut-off value. The local resolution distribution of the final density map was calculated with ResMap (Kucukelbir et al., 2014).

**Model building and refinement**
The structure of SARS-CoV nsp12-nsp7-nsp8 complex (PDB: 6NUR) was rigidly docked into the density map using CHIMERA (Petersen et al., 2004). The model was manually corrected for local fit in COOT (Emsley et al., 2010) and the sequence register was corrected based on alignment. The initial model was refined in real space using PHENIX (Adams et al., 2010) with the secondary structural restraints and Ramachandran restraints applied. The model was further adjusted and refined iteratively for several rounds aided by the stereochemical quality assessment using MolProbity (Chen et al., 2010). The representative density and atomic models are shown in Figure S3. The statistics for image processing and model refinement are summarized in Table S1. Structural figures were rendered by either CHIMERA (Petersen et al., 2004) or PyMOL (https://pymol.org/2/).

**In vitro polymerase activity assay**
The activity of SARS-CoV-2 polymerase complex was tested as previously described for SARS-CoV nsp12 with slight modifications. Briefly, a 40-nt template RNA (5’-CUAUCCCCCAGUGAGUUUAAUGCCUCUUCUAGGACAUGAC-3’, Takara) corresponding to the 3’end of the SARS-CoV2 genome was annealed to a complementary 20-nt primer containing a 5’-fluorescein label (5’-FAM- GUCAUU CUCCUAAGAAGCUA-3’, Takara). To perform the primer extension assay, 1 μM nsp12, nsp7 and nsp8 were incubated for 30 min at 30°C with 1 μM annealed RNA and 0.5 mM NTP in a reaction buffer containing 10 mM Tris-HCl (pH 8.0), 10 mM KCl, 1 mM beta-mercaptoethanol and 2 mM MgCl₂ (freshly added prior usage). The products were denatured by boiling (100°C, 10 min) in the presence of formamide and separated by 20% PAGE containing 9 M urea run with 0.5 × TBE buffer. Images were taken using a Vilber Fusion system and quantified with the ImageJ software.
**SPR assay**
The affinities between nsp12, nsp7 and nsp8 or nsp7L8 proteins were measured at room temperature (r.t.) using a Biacore 8K system with CM5 chips (GE Healthcare). The nsp12 protein was immobilized on the chip with a concentration of 100 µg/mL (diluted by 0.1 mM NaAc, pH 4.0), and the nsp7 protein was immobilized with a concentration of 50 µg/mL (diluted by 0.1 mM NaAc, pH 4.5). For all measurements, the same running buffer was used which consists of 20 mM HEPES, pH 7.5, 150 mM NaCl and 0.005% tween-20. Proteins were pre-exchanged into the running buffer by SEC prior to loading to the system. A blank channel of the chip was used as the negative control. Serially diluted protein solutions were then flowed through the chip surface. The Multi-cycle binding kinetics was analyzed with the Biacore 8K Evaluation Software (version1.1.1.7442) and fitted with a two-state reaction binding model (for ligand nsp8) or heterogeneous ligand binding model (for ligand nsp7L8).

**Circular dichroism measurement**
The thermostability of nsp12, nsp7 and nsp8 were tested by measuring the CD spectra of each protein at different temperatures. The multi-wavelength (215-260 nm) CD spectra of each protein were recorded with a Chirascan spectrometer (Applied Photophysics) using a thermostatically controlled cuvette at rising temperatures from 25 to 99 °C with 0.5 °C intervals and an elevating rate of 1 °C/min. The data were analyzed using Global3 software and the Tm values were calculated for each sample.

**QUANTIFICATION AND STATISTICAL ANALYSIS**
Data are presented as the means ± SD and were analyzed with GraphPad Prism and SPSS programs. The one-way analysis of variance (ANOVA) was used to compare the significance of differences between different groups. The *p* values less than 0.05 were considered significant. Statistical differences are indicated as follows: *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001; ****, *p* < 0.0001. Biological replicates are indicated in the figure legends.