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Supplemental Information

Refolding of lid subdomain of SARS-CoV-2 nsp14 upon nsp10 interaction releases exonuclease activity

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Supplemental information

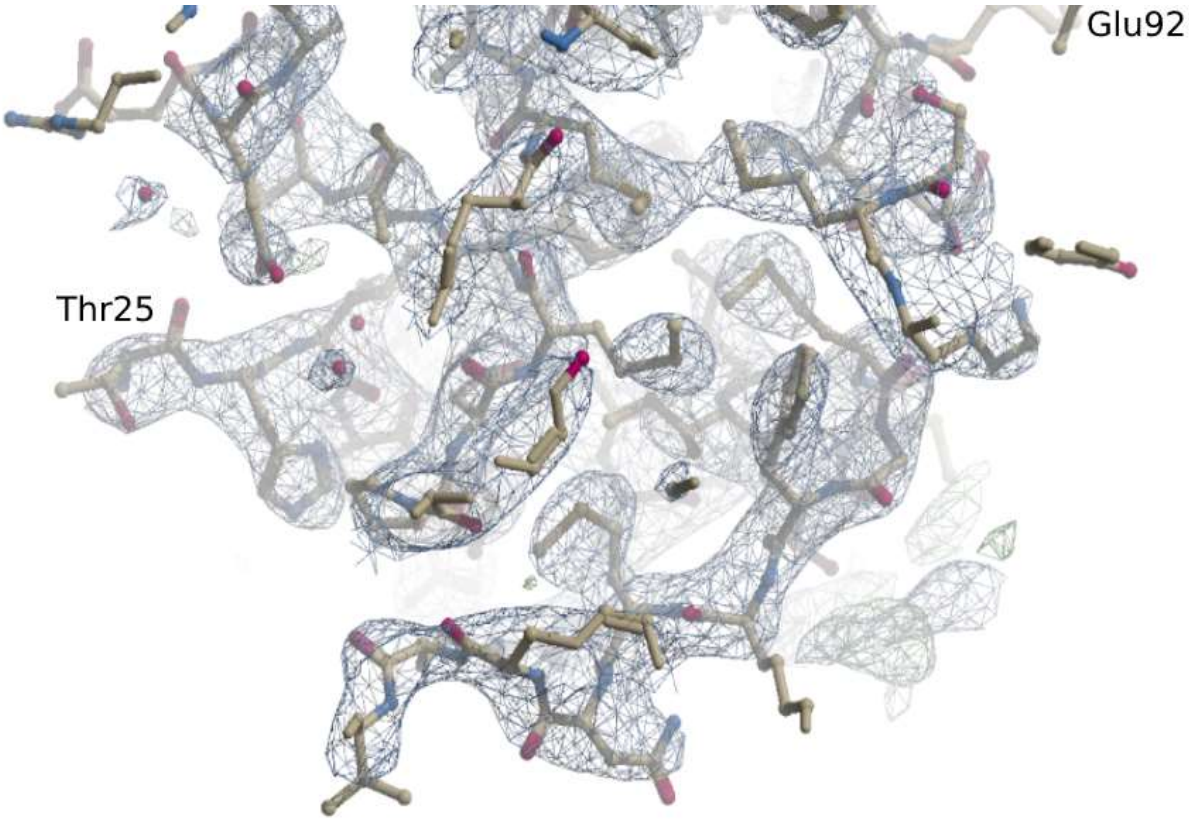

Figure S1: Related to Figure 1. Overview of the electron density defining the SARS-CoV-2 nsp14 N terminal lid region. The $2F_o$-Fc map (blue mesh) is contoured at 1σ. The final model is depicted in stick representation (beige). The density allows for unambiguous determination of residues from 25 to 39 and 45-92. Other residues of the lid region were poorly defined or undefined by the electron density and were not included in the model.

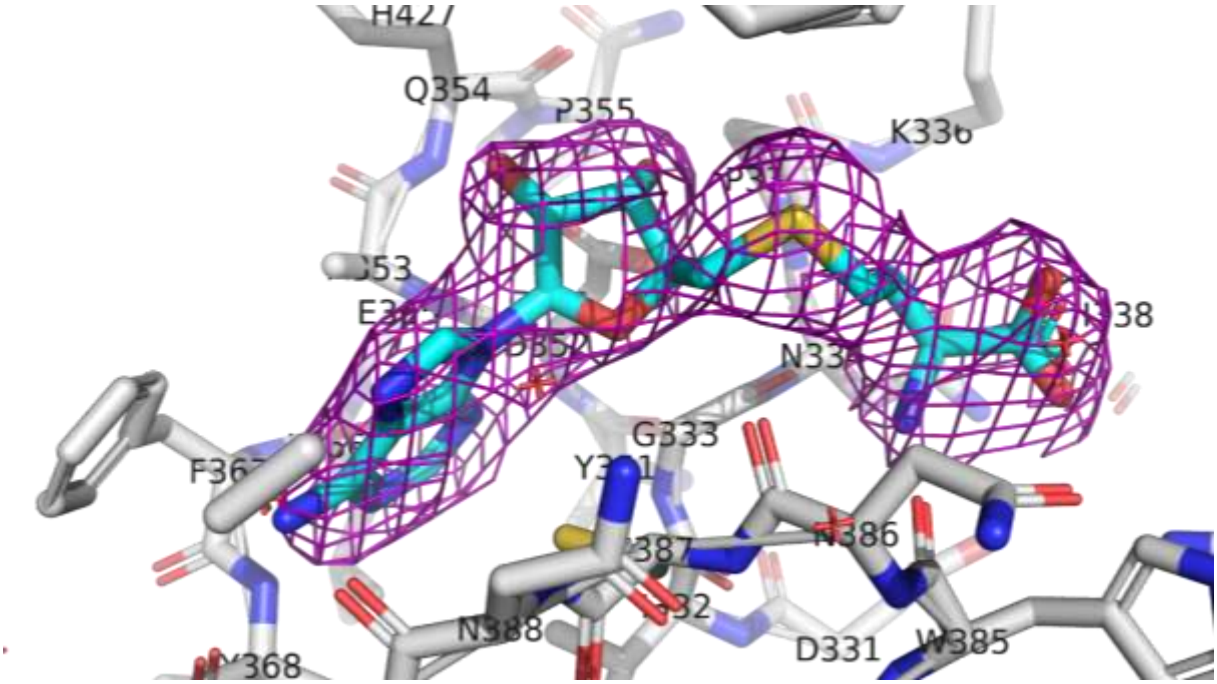

Figure S2: Related to Figure 2. Overview of the SAM/SAH binding site. The bound cofactor (SAH) is shown in stick model (blue). The electron density describing the cofactor is shown as the omit map (2Fo-Fc) with contour level 1σ (magenta mesh). No density describing the methyl moiety at sulphur atom is evident allowing to identify SAH (and not SAM) as a cofactor. Protein model shown in stick representation (gray).

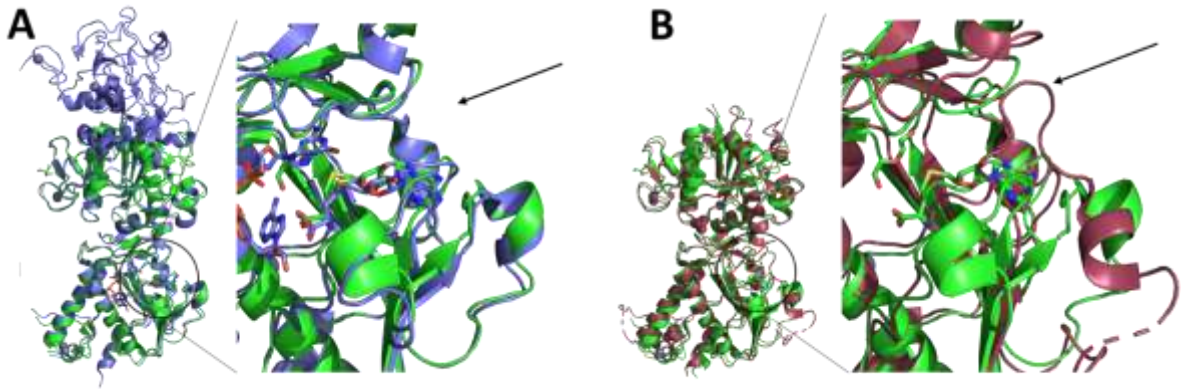

Figure S3: Related to Figure 2. Overlay of selected nsp14 structures. (**A**) Overlay of SARS-CoV-2 nsp14 structure containing SAH (green; this study) and SARS-CoV-1 nsp14/10 complex containing SAH and GpppA (violet; PDB ID:5C8S). No occlusion of the active site is seen. (**B**) Overlay of SARS-CoV-2 nsp14 structure containing SAH (green; this study) and SARS-CoV-2 nsp14 with no cofactor (brown; PDB ID: 7QGI). Significant differences in the orientations of loop connecting β2' and β3' strands blocking the active site in the structure not containing the cofactor. (**A, B**) Insets show close-ups of SAM binding pocket (indicated by an arrow). The structures of PDB ID: 7R2V and PDB ID:5C8S overlap ideally with RMSD of 0.802 for 362 atoms showing no occlusion to the active site for both nsp10-free and nsp10-bound structures (green and violet structures in S2 A, respectively). The structures of PDB ID: 7R2V and PDB ID:7QGI have a significant difference with a loop connecting β2' and β3' strands blocking the active site (indicated with the arrow) and RMSD of 1.440 for 399 atoms.

Table S1. Related to STAR★Methods. Nucleotide and amino acid sequences of SARS-CoV-2 nsp14 used in this study.

Nucleotide sequence:
(ggc)atggctgaaaatgtaacaggactctttaaagattgtagtaaggtaatcactgggttacatcctacacaggcacctacacac ctcagtgttgacactaaattcaaaactgaaggtttatgtgttgacatacctggcatacctaaggacatgacctatagaagactcatc tctatgatgggttttaaaatgaattatcaagttaatggttaccctaacatgtttatcacccgcgaagaagctataagacatgtacgtg catggattggcttcgctgtagctgggtgtcatgctactagagaagctgttggtaccaatttacctttacagctaggtttttctacaggt gttaacctagttgctgtacctacaggttatgttgatacacctaataatacagatttttccagagttagtgctaaaccaccgcctggag atcaatttaaacacctcataccacttatgtacaaaggacttccttggaatgtagtgcgtataaagattgtacaaatgttaagtgaca cacttaaaaatctctctgacagagtcgtatttgtcttatgggcacatggctttgagttgacatctatgaagtattttgtgaaaatagg acctgagcgcacctgttgtctatgtgatagacgtgccacatgcttttccactgcttcagacacttatgcctgttggcatcattctattg gatttgattacgtctataatccgtttatgattgatgttcaacaatggggttttacaggtaacctacaaagcaaccatgatctgtattgt caagtccatggtaatgcacatgtagctagttgtgatgcaatcatgactaggtgtctagctgtccacgagtgctttgttaagcgtgttg actggactattgaatatcctataattggtgatgaactgaagattaatgcggcttgtagaaaggttcaacacatggttgttaaagctg cattattagcagacaaattcccagttcttcacgacattggtaaccctaaagctattaagtgtgtacctcaagctgatgtagaatgga agttctatgatgcacagccttgtagtgacaaagcttataaaatagaagaattattctattcttatgccacacattctgacaaattcac agatggtgtatgcctattttggaattgcaatgtcgatagatatcctgctaattccattgtttgtagatttgacactagagtgctatcta accttaacttgcctggttgtgatggtggcagtttgtatgtaaataaacatgcattccacacaccagcttttgataaaagtgcttttgtt aatttaaaacaattaccatttttctattactctgacagtccatgtgagtctcatggaaaacaagtagtgtcagatatagattatgtac cactaaagtctgctacgtgtataacacgttgcaatttaggtggtgctgtctgtagacatcatgctaatgagtacagattgtatctcga tgcttataacatgatgatctcagctggctttagcttgtgggtttacaaacaatttgatacttataacctctggaacacttttacaagac ttcag
Amino acid sequence
(G)MAENVTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGLCVDIPGIPKDMTYRRLISMMGFKMNYQ VNGYPNMFITREEAIRHVRAWIGFAVAGCHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTDF SRVSAKPPPGDQFKHLIPLMYKGLPWNVVRIKIVQMLSDTLKNLSDRVVFVLWAHGFELTSMKYFVKIG PERTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNPFMIDVQQWGFTGNLQSNHDLYCQVHGNAH VASCDAIMTRCLAVHECFVKRVDWTIEYPIIGDELKINAACRKVQHMVVKAALLADKFPVLHDIGNPKAI KCVPQADVEWKFYDAQPCSDKAYKIEELFYSYATHSDKFTDGVCLFWNCNVDRYPANSIVCRFDTRVLS NLNLPGCDGGSLYVNKHAFHTPAFDKSAFVNLKQLPFFYYSDSPCESHGKQVVSDIDYVPLKSATCITRC NLGGAVCRHHANEYRLYLDAYNMMISAGFSLWVYKQFDTYNLWNTFTRLQ

The initial glycine residue in parentheses is a part of TEV recognition site and remains appended to nsp14 sequence in the purified nsp14. Table with nucleotide and amino acid sequence for SARS-CoV-2 nsp14 used in in this study.