

Supplementary File

1. Cloning strategies for nucleocapsid proteins (Sup Figure S1)

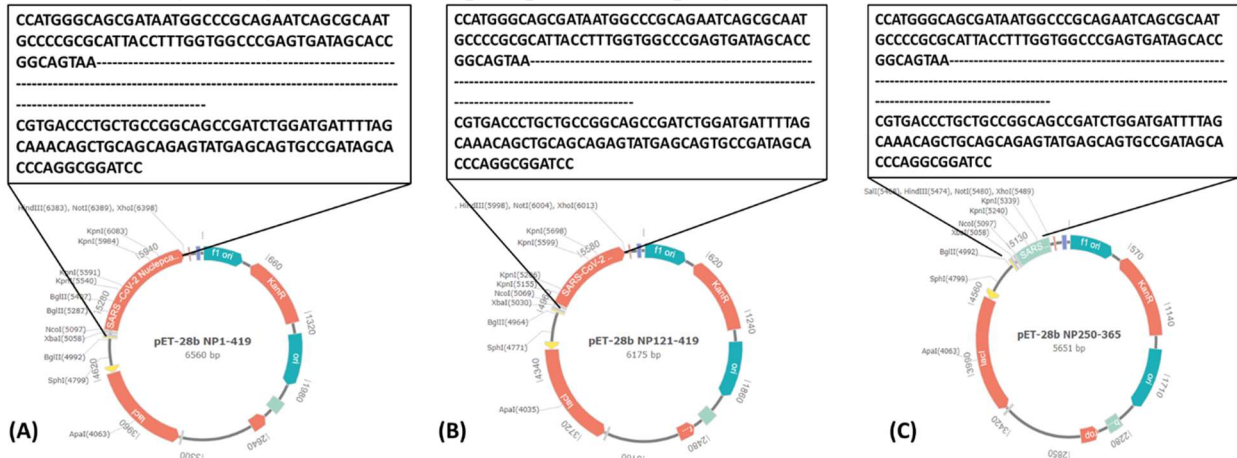


Figure S1. (A): Schematic diagram for SARS-CoV-2 NP₁₋₄₁₉ cloned in pET-28b vector, (B): Schematic diagram for SARS-CoV-2 NP₁₂₁₋₄₁₉ cloned in pET-28b vector and (C): Schematic diagram for SARS-CoV-2 NP₂₅₀₋₃₆₅ cloned in pET-28b vector.

2. Expression and partitioning studies of NP₁₋₄₁₉, NP₁₂₁₋₄₁₉ and NP₂₅₀₋₃₆₅ (Sup Figure S2)

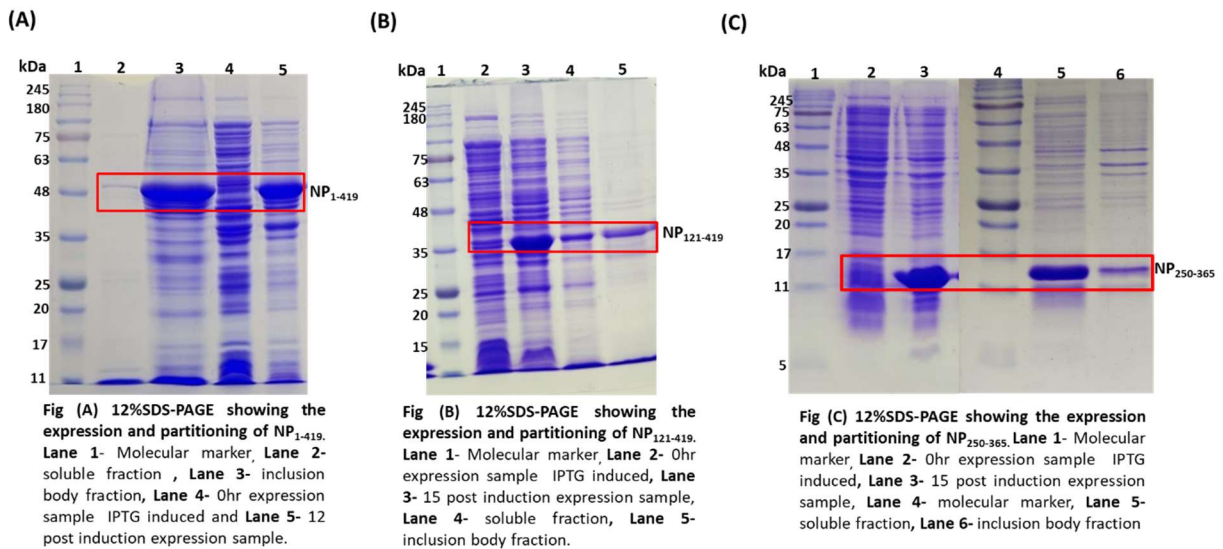


Figure S2. Analysis of Nucleocapsid protein and its variants expression and partitioning studies on 12% tris-glycine SDS-PAGE.

3. Purification studies of NP₁₋₄₁₉, NP₁₂₁₋₄₁₉ and NP₂₅₀₋₃₆₅. (Sup Figure S3)

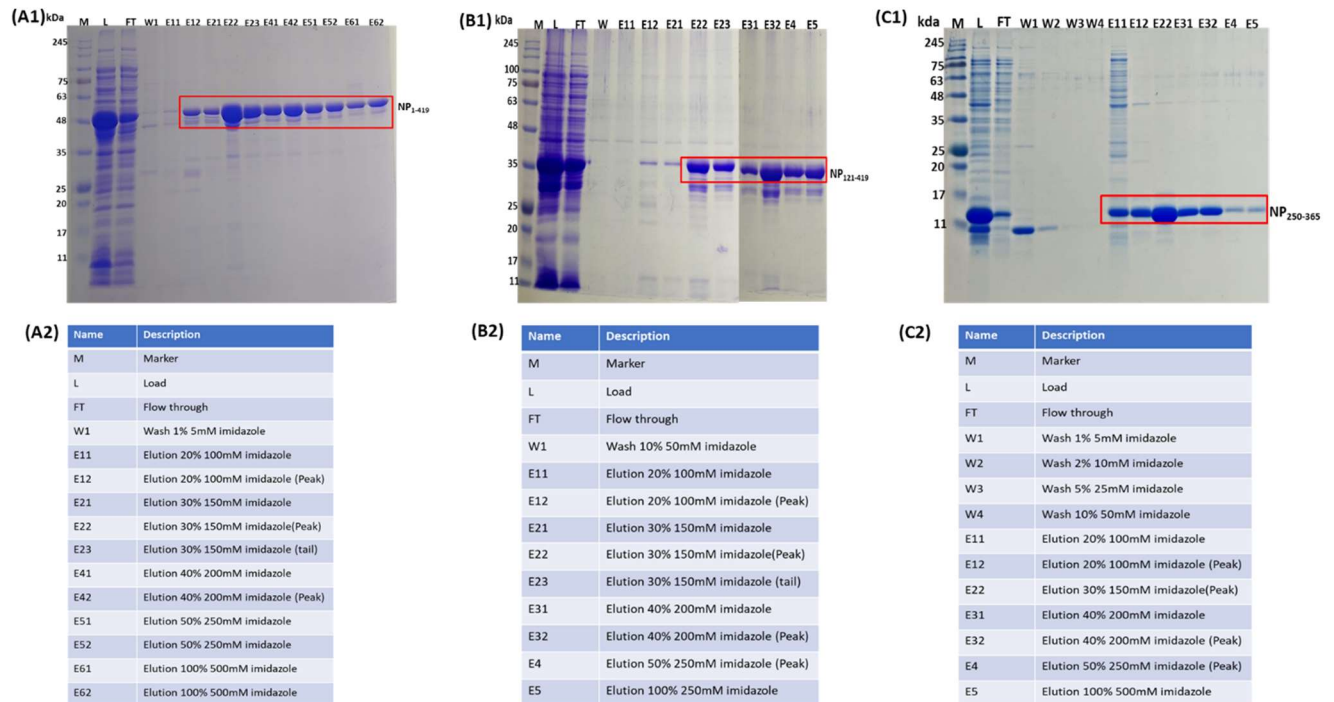


Fig (A1&A2) 12% SDS-PAGE showing the purification of NP₁₋₄₁₉ and its elution gradient description, respectively. **Fig (B1&B2)** 12% SDS-PAGE showing the purification of NP₁₂₁₋₄₁₉ and its elution gradient description, respectively. **Fig (C1&C2)** 12% SDS-PAGE showing the purification of NP₂₅₀₋₃₆₅ and its elution gradient description, respectively.

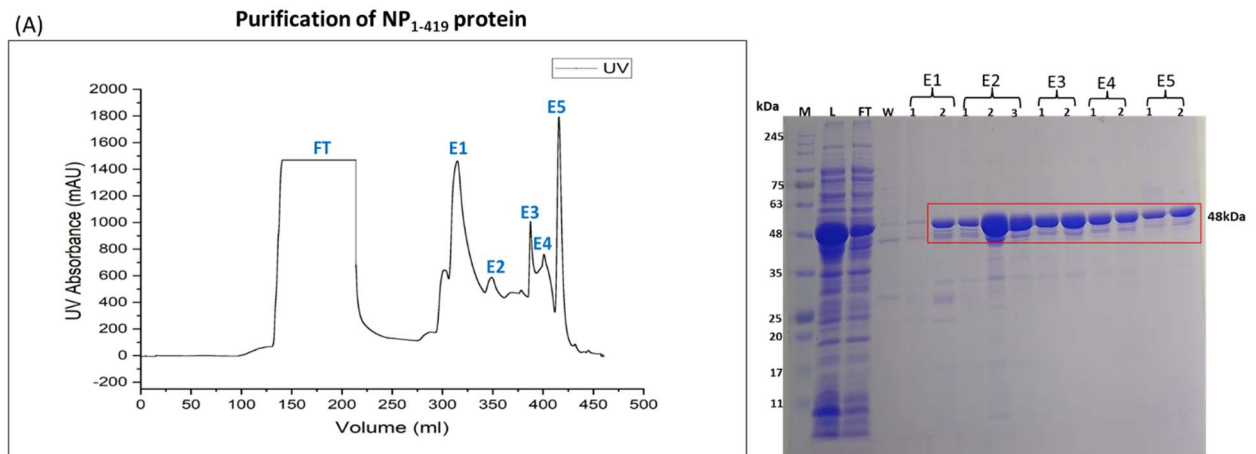


Figure A. Chromatogram and 12% SDS PAGE gel showing purification of NP₁₋₄₁₉ by Ni-NTA resins. M: Molecular weight marker, L: Protein (Supernatant after lysis) loaded, FT: Flow-through, W1: wash with equilibration buffer,, E1: 1st elution with 50mM Imidazole. E2: 2nd elution with 100mM Imidazole, E3: 3rd elution with 200mM Imidazole, E4: 4th peak with 300 mM Imidazole and E5: 5th peak with 500mM Imidazole

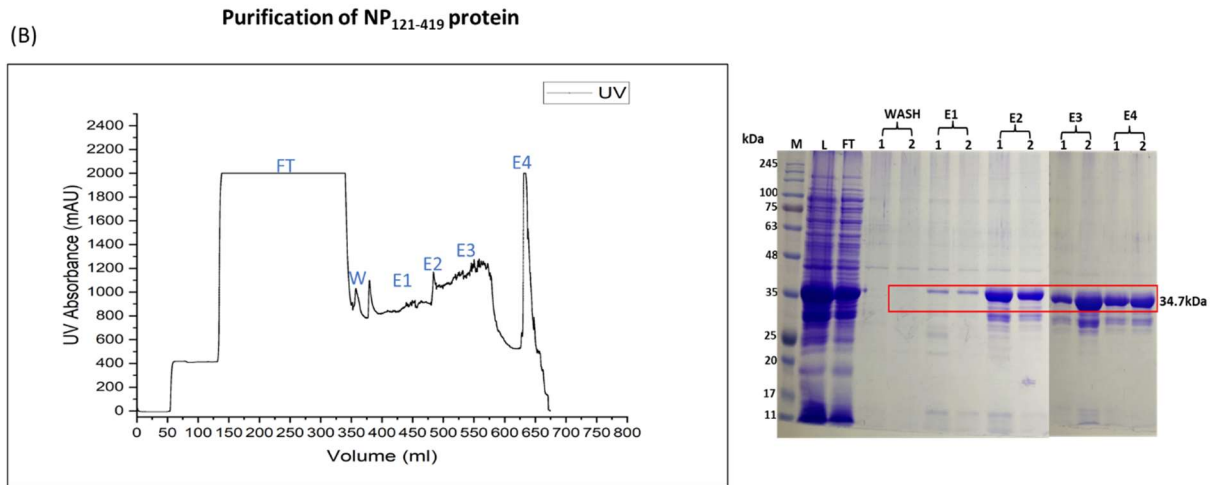


Figure B. Chromatogram and 12% SDS PAGE gel showing purification of NP₁₂₁₋₄₁₉ by Ni-NTA resins. M: Molecular weight marker, L: Protein (Supernatant after lysis) loaded, FT: Flow-through, W1: wash with equilibration buffer,, E1: 1st elution with 50mM Imidazole. E2: 2nd elution with 100mM Imidazole, E3: 3rd elution with 250mM Imidazole and E4: 4th peak with 500 mM Imidazole.

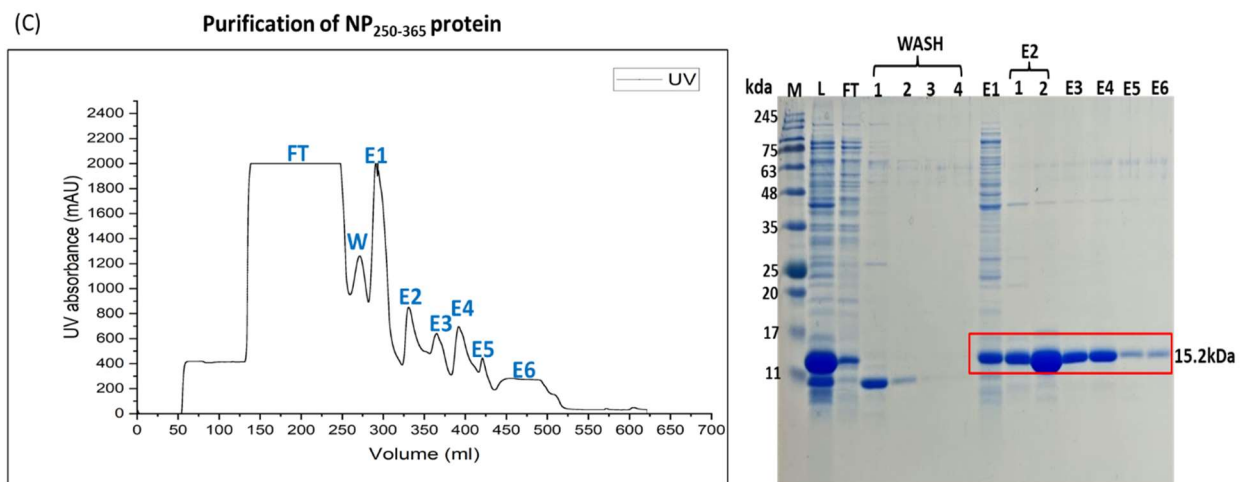
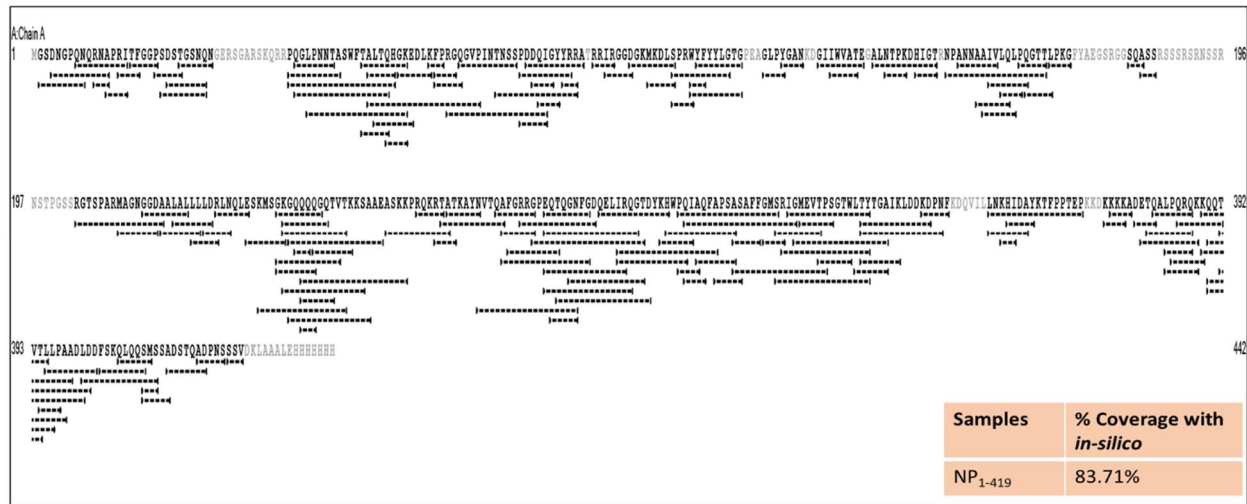


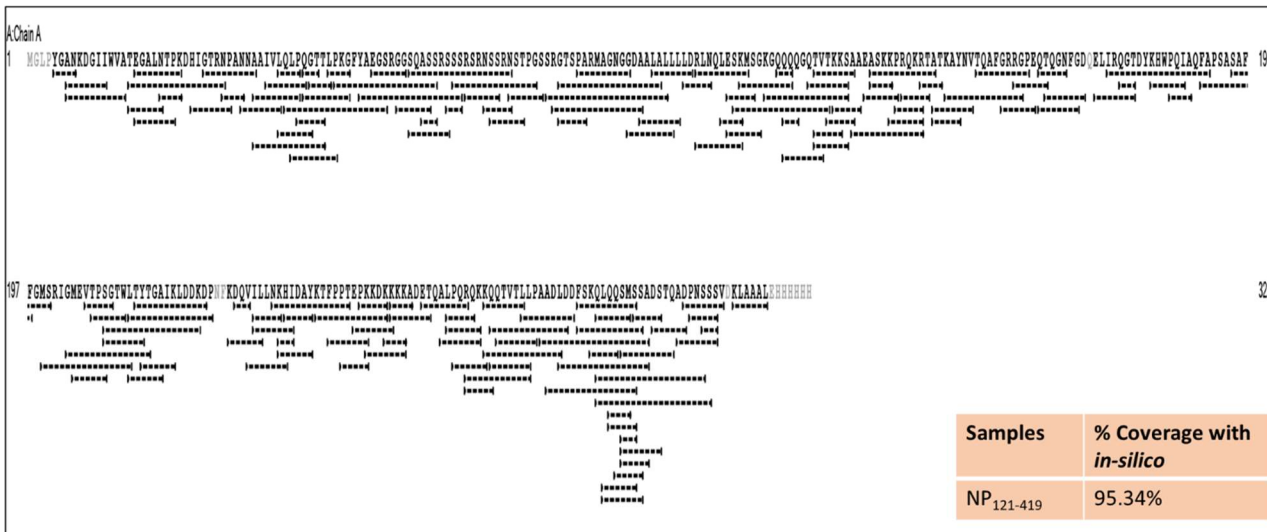
Figure C. Chromatogram and 12% SDS PAGE gel showing purification of NP₂₅₀₋₃₆₅ by Ni-NTA resins. M: Molecular weight marker, L: Protein (Supernatant after lysis) loaded, FT: Flow-through, W1-W4: wash with equilibration buffer,, E1: 1st elution with 50mM Imidazole. E2: 2nd elution with 100mM Imidazole, E3: 3rd elution with 200mM Imidazole, E4: 4th peak with 300 mM Imidazole, E5: 5th peak with 400mM Imidazole and E6: 6th peak with 500mM Imidazole.

4. Peptide mapping of purified proteins (Sup Figure S4)

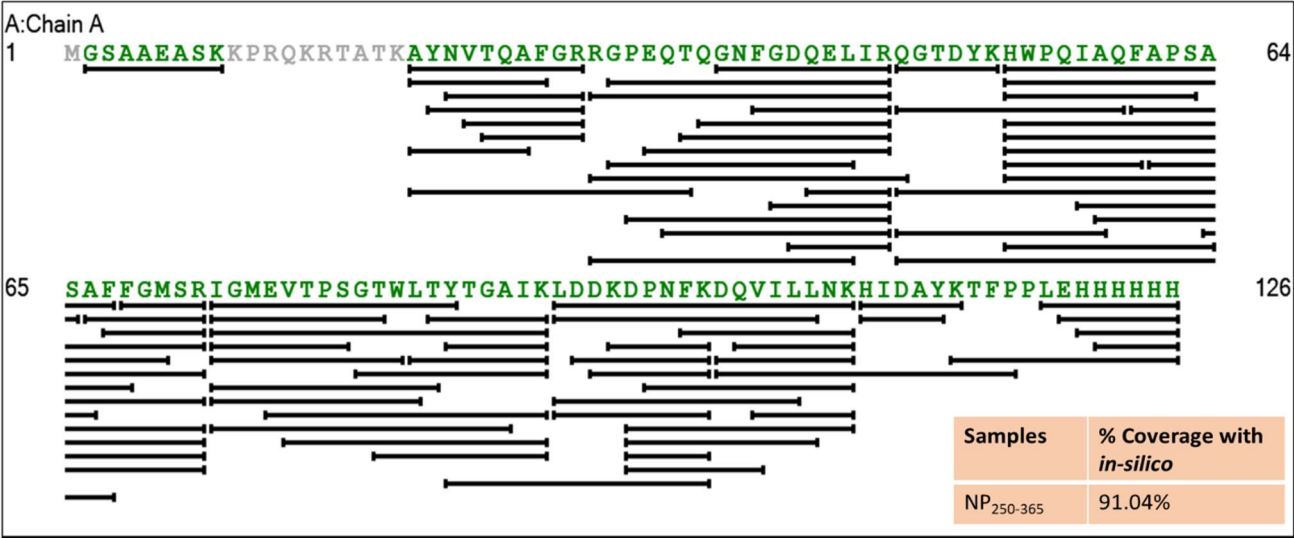
(A) Representative sequence coverage map of NP₁₋₄₁₉ when compared to *in-silico* sequence.



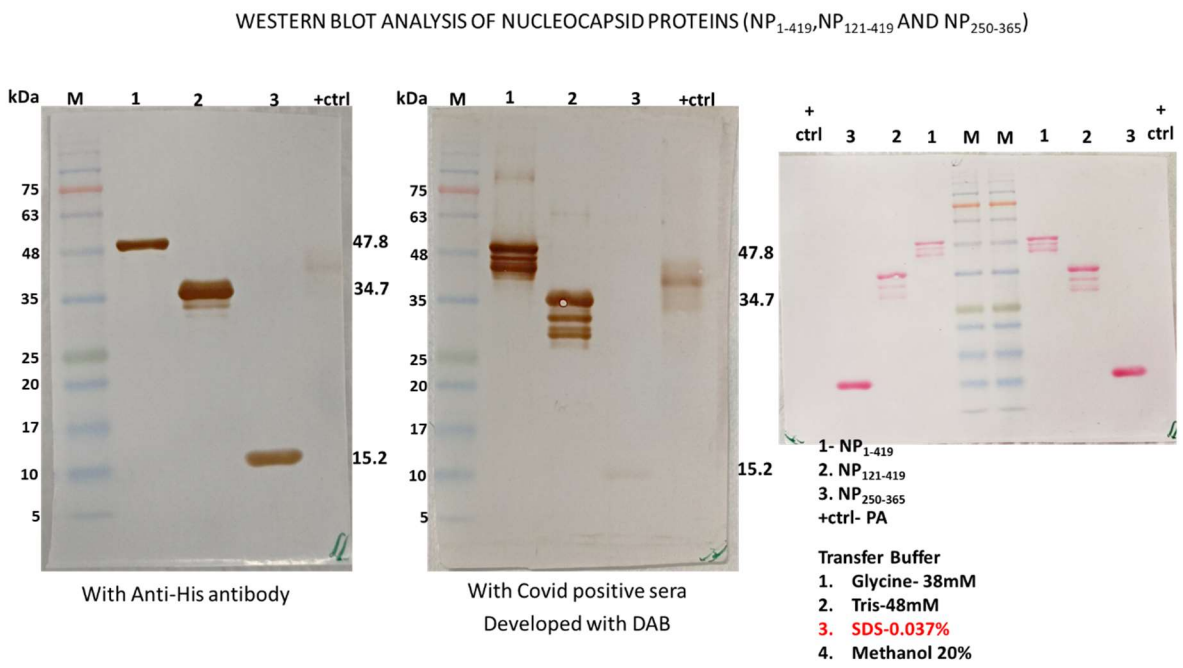
(B) Representative sequence coverage map of NP₁₂₁₋₄₁₉ when compared to *in-silico* sequence.



(C) Representative sequence coverage map of NP₂₅₀₋₃₆₅ when compared to *in-silico* sequence.

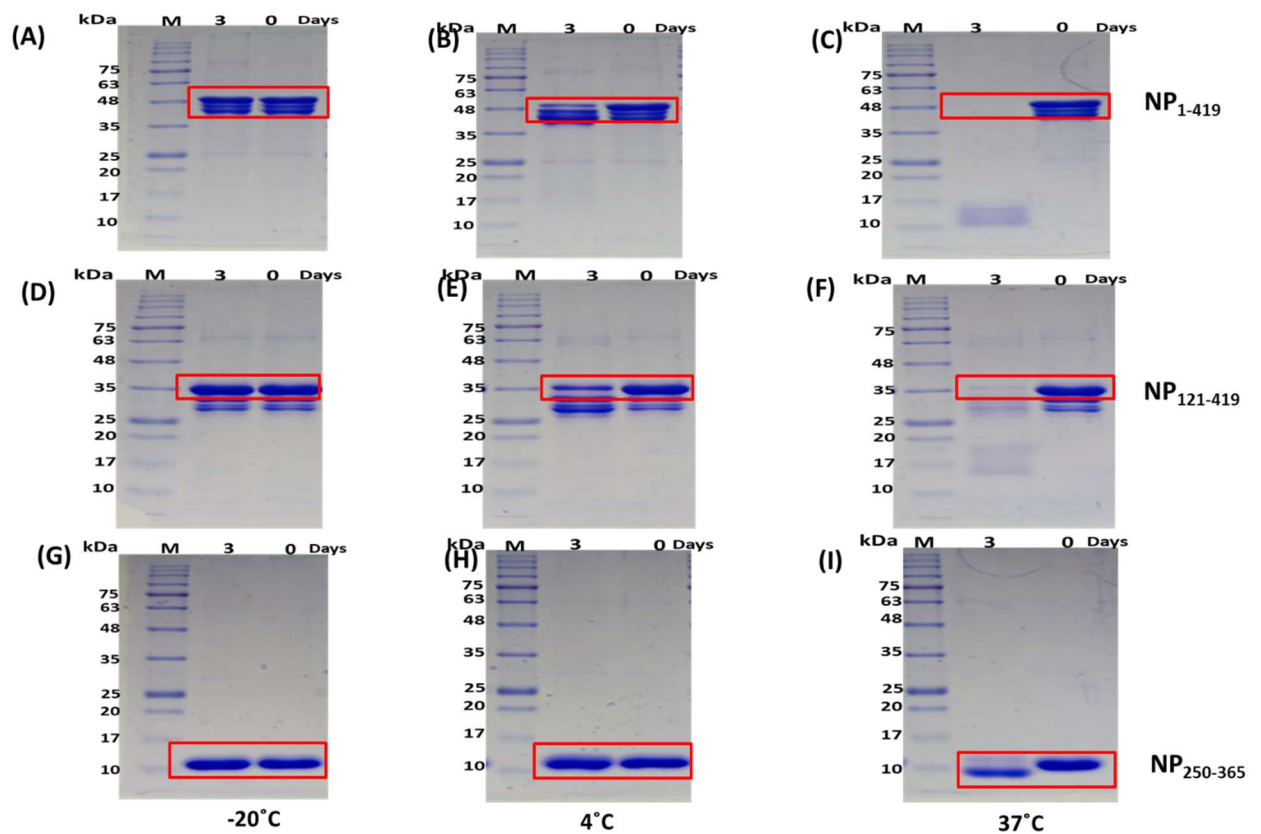


5. Western blot of purified proteins (Sup Figure S5)



SUP-Figure S5: Ponceau stained NC membrane for NP₁₋₄₁₉, NP₁₂₁₋₄₁₉ AND NP₂₅₀₋₃₆₅ . Western blot is done with anti-His antibody as well as SARS-COV-2 positive human sera

6. Stability test of purified proteins (Sup Figure S6) Analysis by SDS-PAGE

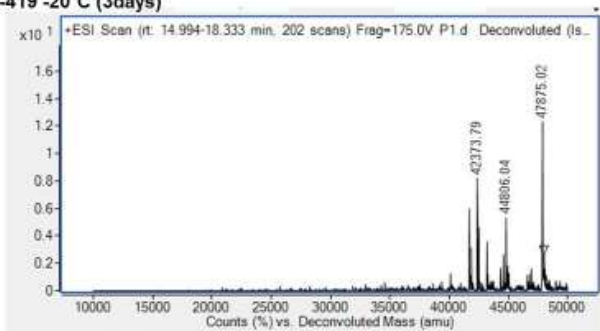


SUP-Figure S6 A: Degradation pattern of NP1-419, NP121-419 and NP250-365 proteins analysed on 12 % SDS-PAGE: In each lane 10 µg of protein charged for stability at different temperatures. NP1-419 Gel (A-C): temperature -20 °C, 4 °C and 37 °C, respectively. NP121-419 Gel (D-F) Temperature -20 °C, 4 °C and 37 °C, re-spectively. NP250-365 Gel (G-I) Temperature -20 °C, 4 °C and 37 °C, respectively. In each gel, Lane 1-3: Molecular marker, Day 3, and Day 0 samples, respectively.

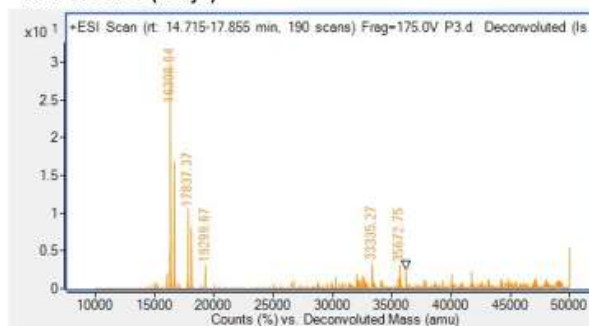
B) Analysis by Mass

(I)

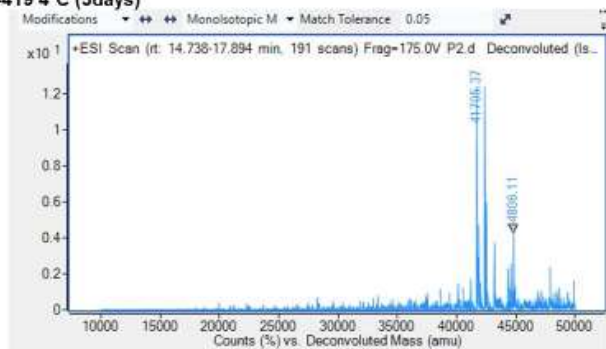
NP1-419 -20°C (3days)



NP1-419 37°C (3days)

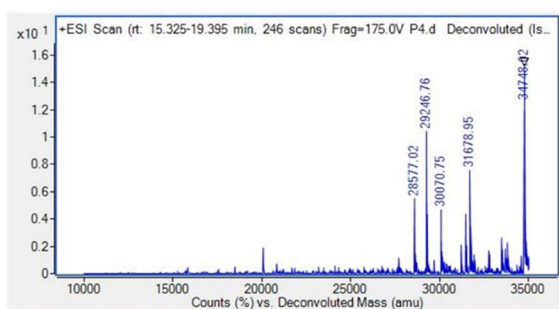


NP1-419 4°C (3days)

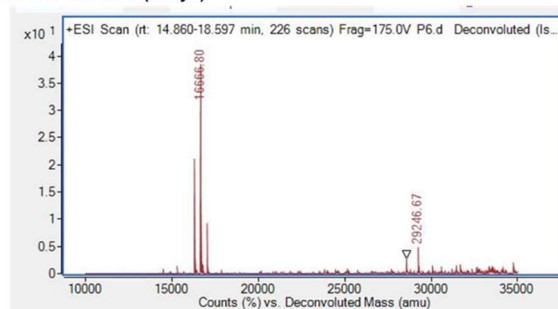


(II)

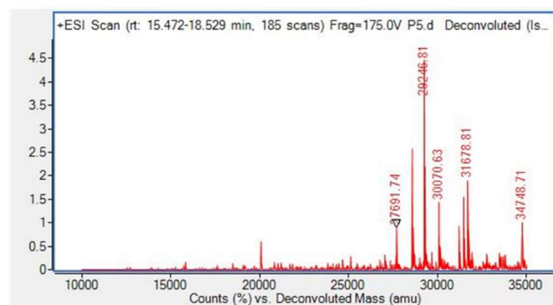
NP121-419 -20°C (3days)



NP121-419 37°C (3days)

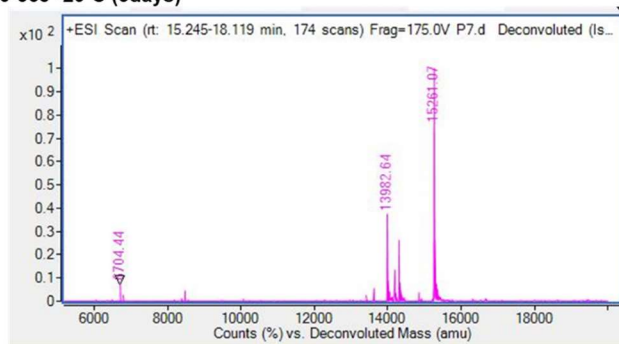


NP121-419 4°C (3days)

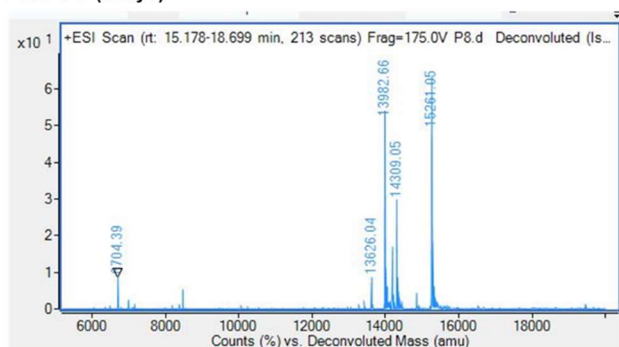


(III)

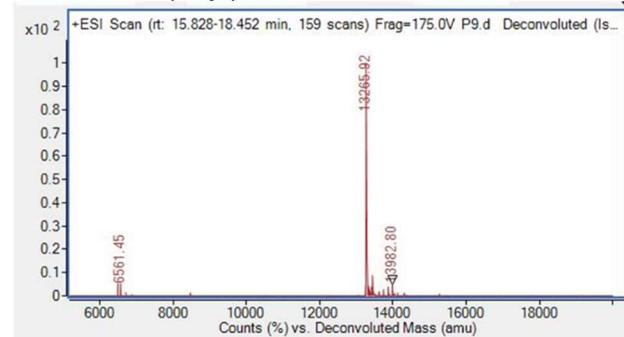
NP250-365 -20°C (3days)



NP250-365 4°C (3days)



NP250-365 37°C (3days)



SUP-Figure S6 B: Degradation pattern of NP1-419, NP121-419 and NP250-365 proteins analysed on LCMS: In each 5 µg of protein charged for stability at different temperatures. (I) NP1-419: temperature -20 °C, 4 °C and 37 °C, respectively. (II) NP121-419: Temperature -20 °C, 4 °C and 37 °C, re-spectively. (III) NP250-365 Temperature -20 °C, 4 °C and 37 °C, respectively. In each panel 3rd day, analysis of 3rd sample is presented