SUPPLEMENTARY INFORMATION

Conformational Dynamics in the Interaction of SARS-CoV-2 Papain Like Protease with Human Interferon-Stimulated Gene 15 Protein

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Recombinant PLpro C111S expression

The catalytically inactive C111S variant of PLpro was used for all experiments and was produced using *E. coli* BL21(DE3) cells that had been transformed (pMCSG53), plated on LB agar, and cultivated in a shaking incubator (250 RPM) at 37°C in LB medium using baffled 2.8 L Fernbach flasks. Antibiotic selection with carbenicillin was used throughout. Cell growth was monitored by measuring the absorbance at 600 nm (OD600) to ∼0.7 after which the incubator was set to at 18 °C and the culture induced with 0.2 mM IPTG for ~18 hours. Cells were harvested by centrifugation at 6000xg for 30 minutes. After decanting the supernatant, the pellets were stored at -80°C until needed for protein purification.

Purification of recombinant PLpro C111S

The cell pellet from a 1 L culture were thawed and resuspended in 100 mL of lysis buffer comprised of 50mM Tris, 150 mM NaCl, 50 mM imidazole, 5% glycerol, and 1 mM TCEP, at pH 7.4. Following resuspension, the cells were sonicated on ice at 50% amplitude (2 seconds on and 10 seconds off) for 5 minutes using a Branson 450D Digital Sonifier. After clarification by centrifugation at 38,500xg for 35 minutes at 4 °C, the supernatant was passed through 1.6- and 0.45-micron syringe filters sequentially and kept on ice while loading a 5 mL HisTrap HP column (Cytiva) at 2 ml/min. After washing with 10 CV of lysis buffer, PLpro C111S was eluted and collected in 2 mL fractions using a 20 CV linear gradient of lysis buffer containing 500 mM imidazole. Fractions containing PLpro C111S were identified using SDS-PAGE on a 4–20% Mini-Protean TGX Stain-Free protein gel (Bio-Rad). Pooled fractions containing PLpro C111S were dialyzed overnight at 6 °C in 50mM Tris pH 7.4 with 150 mM NaCl, 5% glycerol, and 1 mM TCEP in the presence of His-tagged TEV protease (1 mg TEV protease:100 mg of PLpro C111S). After confirming His-tag cleavage by SDS-PAGE, the dialyzed protein mixture was passed over a 5 mL HisTrap HP column to remove all his-tagged impurities. The column flowthrough was collected, evaluated using SDS-PAGE, and concentrated with a 10kDa molecular weight cutoff Amicon Ultra-15 ultrafiltration membrane. Upon concentration, partially purified protein was applied 0.5 ml/min to a Superdex 75 10/300 GL size exclusion column (Cytiva) that had been previously equilibrated with dialysis buffer. Fractions (0.5 ml) were collected, pooled, and concentrated for characterization. To prepare the PLpro C111S: hISG15 complex, purified PLpro C111S was gently mixed by pipette with hISG15 (Bio-Techne Minneapolis, MN (Catalog number: UL-601)) at a 1:1 molar ratio in a microcentrifuge tube at room temperature. Using the same protocol as described for PLpro C111S alone, the protein complex was purified using a Superdex 75 size exclusion column. Fractions containing purified complex were identified using SDS-PAGE, pooled, and concentrated for subsequent characterization. For all SANS measurements, concentrated proteins were dialyzed at 6°C overnight in deuterated buffer using Slide-A-Lyzer Mini Dialysis devices (Thermo Fisher Scientific).

Expression of Deuterium Labeled SARS-CoV-2 PLpro C111S
Deuterium labeled SARS-CoV-2 PLpro C111S for contrast variation SANS experiments was expressed using a protocol established by Marley et al. LB starter cultures were inoculated from a frozen glycerol stock and cultivated overnight at 30 °C in a shaking incubator. The following day, the overnight cultures were centrifuged at 4000xg for 10 minutes, resuspended in 25 mL of fresh LB, and split equally when inoculating 4 x 1L of LB medium in baffled 2.8 L Fernbach flasks. Subsequent cell growth was monitored by measuring the absorbance at 600 nm (OD$_{600}$) until it reached ∼0.7. At that point, the cells were pelleted by centrifugation at 4000xg for 10 minutes in pre-sterilized bottles. Each pellet from 1L of culture was then washed in 45mL of deuterated minimal medium and re-pelleted by centrifugation at 4000xg for 10 minutes. The four pellets were then resuspended in 250 mL each of fresh deuterated minimal medium and transferred to four dry, sterile 2.8L Fernbach flasks. After this transfer, the cells were cultivated at 18 °C for 1 hour, induced with 0.2 mM IPTG for ~20 hours, and harvested by centrifugation at 4000xg for 30 minutes. After decanting the supernatant, the pellets were stored at -80°C before purification, which was carried out in the same manner as for unlabeled protein.

**SANS and SEC-SAXS data collection**

Small-angle neutron scattering measurements were performed using the Bio-SANS instrument located at the High Flux Isotope Reactor in Oak Ridge National Laboratory. A single standard configuration of the dual detector system was set with the Panel Scan feature of the instrument with the small-angle detector at 7m and the wide-angle detector at 1.3m and 3.2° to the sample position. Using this configuration, the Q range spanning 0.007 < Q (Å$^{-1}$) < 1 was obtained using 6 Å wavelength neutrons with a relative wavelength spread ($\Delta\lambda/\lambda$) of 15%, ($Q = 4\pi \sin (\theta) / \lambda$, with scattering angle as 2θ and wavelength, $\lambda$). The data were corrected for instrument background, detector sensitivity, and instrument geometry using facility data reduction software, drt-SANS. All SANS measurements were performed in 1 mm path length cylindrical quartz cuvettes (Hellma, Müllheim, Germany) at 10°C.

SAXS coupled with multi-angle light scattering in line with size-exclusion chromatography (SEC-SAXS-MALS) experiments were collected at the Advanced Light Source beamline 12.3.1 in Lawrence Berkley National Laboratory. X-ray wavelength was set at $\lambda=1.240 \text{Å}$, and the sample-to-detector distance was 2077 mm, resulting in scattering vectors spanning from 0.01 < Q (Å$^{-1}$) < 0.35. Briefly, a SAXS flow cell was directly coupled with an online Agilent 1260 Infinity HPLC system using a Shodex KW-802.5 column. The column was pre-equilibrated with buffer (50mM Tris, 150mM NaCl, 1% Glycerol, 1mM TCEP, pH 7.4), samples (55 µl each) were loaded, and three second X-ray exposures were collected continuously during a 30-minute elution at 0.65 ml/min. The SAXS frames recorded before the protein elution peak were used to subtract all other frames. Subsequently, SEC-SAXS frames were deconvoluted in single SAXS profiles using SCÅTTER.

The protein concentrations for SANS measurements were 3.1 mg/ml hISG15; 5.0 mg/ml deuterated PLpro C111S; and 9.1 mg/ml deuterated PLpro C111S:hISG15 complex (1:1 molar ratio). Contrast matching SANS experiments of deuterated PLpro C111S in complex with protiated hISG15 were performed in 100% and 42% D$_2$O buffers to selectively highlight the scattering from hISG15 and deuterated PLpro, respectively. For SEC-SAXS experiments, 55 µL of
each sample was loaded to the SEC-SAXS-MALS setup at various concentrations (6.2 mg/ml hISG15; 6.7 mg/ml PLpro C111S; and 6.2 mg/ml PLpro C111S:hISG15 complex). All SANS measurements were performed using 50mM HEPES pH 7.4 with 150 mM NaCl, 5% Glycerol, and 1 mM TCEP as the buffer solution.

**Data Analysis and Modeling**

Initial SANS data analysis, including Guinier fits and pair-distribution calculations, were performed using the BioXTAS RAW program and ATSAS suite\(^8,9\). The pair distance distribution function \((P(r))\) was calculated using the indirect Fourier transform method implemented in the program GNOM\(^10\). Scattering data over the range \(0.007 < Q (\text{Å}^{-1}) < 8/R_g\) were used for \(P(r)\) analysis and subsequent modeling. The SAXS-derived molecular weight was determined using the volume of Porod method as implemented in RAW\(^11\). SANS data were fitted with the theoretical scattering profiles generated from the coordinates of atomic models using CRYSON\(^12\). Homology structural models were generated using MODELLER\(^13\). Protein conformational sampling was performed using BILBOMD program\(^14\). The experimental SAXS data were compared to theoretical scattering curves generated from atomistic models using the FOXS server\(^15-16\). Chimera was used to visualize PDB coordinates\(^17\). Evaluation of protein-protein interface and contact residues were carried out using PDBePISA server\(^18\).

The SEC-SAXS profile of the PLpro C111S:hISG15 complex was analyzed as follows. First, a model of hISG15 in complex with PLpro was made using MODELLER by using the human ISG15 sequence (Figure S1) and the bound mISG15 from the mISG15:PLpro crystal structure as a template (PDBID: 6YVA)\(^13\). Next, the crystal structure of PLpro C111S in complex with the C-terminal domain of hISG15 (PDB 6XA9) was used to orientate the hISG15 model, described above, in PLpro. This was our starting PLpro:hISG15 model for fitting. BILBOMD was used to perform conformational sampling to determine the structure of the complex that best fits the SAXS of PLpro:hISG15\(^14\). BILBOMD is a program based on CHARMM and allows rapid exploration of conformational space and flexibility. Fixed domains are manually defined as rigid bodies, and intervening regions are allowed to move, keeping bond distances, and preventing steric overlap of atoms. The predicted SAXS curve is calculated for each conformation. The contribution of individual conformations is additive in SAXS, and an associated Minimal Ensemble Search (MES) method selects 2-5 conformations, adds together their SAXS curves, and optimizes the relative % population to fit the experimental data. The linker (V74-P81) between the N- and C-terminal domains was allowed to sample different conformations during the fitting. While the N- and C-terminal domains of ISG15 and PLpro were constrained in the fitting.

The contrast matched SANS experiment was analyzed as follows. To fit experimental contrast matching SANS data of bound PLpro we used the crystal structures of free PLpro C111S and bound PLpro C111S in complex with mISG15 (PDBs 7D6H and 6YVA, respectively). In addition, to take in account the scattering of all particles and reduce discrepancies in the fit, missing regions of PLpro C111S (PDBID: 6YVA, residues between V184-Q196 and L218-V235) were added using MODELLER\(^13\). Finally, to fit experimental contrast matching SANS data of bound hISG15 we used the ISG15 conformers that fit our SEC-SAXS data.
Figure S1: Amino acid sequence alignment and structural comparison of hISG15 and mISG15.

Panel A shows the sequence alignment of hISG15 and mISG15 and its contact residues with PLpro. The hISG15 (PDB 6XA9) and mISG15 (PDB 6YVA) residues in contact with the PLpro:hISG15 C-terminal domain are indicated by black and green arrows, respectively. Panel B shows the superposition of hISG15 and mISG15 structures. The hISG15 structure (PDB code 1Z2M) is shown in orange, while the structure of mISG15 (5TLA) is shown in cyan. The structures are oriented such that the N-terminal domain is located at the bottom right of the panel with the C terminal domain located in the top left.
Figure S2. SANS data for PLpro and ISG15 in solution.

Panel A compares the SANS profile of deuterated PLpro C111S in 42% D₂O buffer and the theoretical SANS curve for the PLpro C111S (PDB 7D6H). Panel B compares the experimental SANS profile of protiated hISG15 in 100% D₂O buffer and theoretical SANS curve for the hISG15 (PDB 1Z2M).

Figure S3. Biochemical characterization of PLpro C111S, hISG15, and the PLpro C111S: hISG15 complex.

Panel A shows the size exclusion chromatography elution profile of hISG15 (orange curve), dPLpro C111S (blue curve), and dPLpro C111S: hISG15 complex (red curve). Panel B shows the SDS-PAGE analysis of dPLpro C111S: hISG15 complex used for SANS experiments after storage for 1 week at 4°C. Lane 1: Molecular mass standards, Lane 2: PLpro C111S:hISG15 complex.
Figure S4. Normalized Kratky analysis of PLpro C111S and hISG15 in the free and bound states from SANS data. Panel A shows the $R_g$ normalized Kratky analysis of free and bound PLpro C111S, colored in blue and pink circles, respectively. Panel B shows the $R_g$-normalized Kratky analysis of free and bound ISG15 colored black and pink circles, respectively.

Figure S5: SANS analysis of bound PLpro.

This figure compares the SANS profile of bound PLpro C111S and the theoretical SANS curve for the PLpro C111S bound to mISG15 (PDB 6YVA). MODELLER$^{13}$ was used to added missing regions of PDB 6YVA chain A prior to data fitting as described in the text.
Figure S6: Comparison of $P(r)$ curves from different species

Panel A: Comparison of the theoretical $P(r)$ curves of mISG15 calculated from PDB 6YVA (red curve) and PDB 5TLA (blue curve) for the free and the protein in complex with PLpro, respectively. Panel B: Comparison of the theoretical $P(r)$ curves for mISG15, hISG15, and bat ISG15 calculated from PDB 5TLA, PDB 1Z2M, PDB 6MDH and colored in red, orange, and green lines, respectively. The experimental $P(r)$ curve of free hISG15 is colored in pink.

Figure S7: Orientation of the ISG15 C and N terminal domains in MERS and SARS-CoV2 PLpro.

Panel A shows the superposition of MERS PLpro:ISG15 (PDB 6BI8) and the SARS-CoV2 PLpro:ISG15 C-terminal domain complex (PDB 6XA9). The MERS-PLpro and SARS-COV-2 PLpro
are colored in semitransparent gray and blue color, respectively. Panel B shows the superposition of SARS-CoV2 PLpro:mISG15 (PDB 6YVA) and SARS-CoV2 PLpro:hISG15 C-terminal domain complex (PDB 6XA9). SARS-CoV-2 PLpro in complex with the mISG15 and hISG15 C-terminal domain are colored in semitransparent pink and blue color, respectively.

**Figure S8: Surface potential representations and contact residues involving the conformer C1.**

Panels A and B show the surface potential of free PLpro C111S (PDB 7D6H) and hISG15 (PDB 1Z2M), respectively. Panel C shows the cartoon representation of conformation C1 determined in the BILBOMD analysis and its surface potential. Panel D shows the contact residues of the N-terminal domain of hISG15 (pink color) with PLpro (blue color) in the conformer C1. The surface potential representations have charge levels from -5kT/e (red) to +5kT/e (blue) and are rotated as indicated by the curved narrows.

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