Supplementary Information for

Inhibitory antibodies identify unique sites of therapeutic vulnerability in rhinovirus and other Enteroviruses

Bing Meng\textsuperscript{a}, Keke Lan\textsuperscript{a,b}, Jia Xie\textsuperscript{c}, Richard A. Lerner\textsuperscript{c}, Ian A. Wilson\textsuperscript{a,d,*} and Bei Yang\textsuperscript{a,*}

Ian A. Wilson; Bei Yang
Email: wilson@scripps.edu; yangbei@shanghaitech.edu.cn

This PDF file includes:

- Supplementary Materials and Methods
- Figures S1 to S7
- Tables S1 to S3
- SI References
Supplementary Materials and Methods

Gene cloning, protein expression and purification. The gene encoding full-length HRV14 3C protease was cloned into a modified pMCSG7 vector fused with an N-terminal His\textsubscript{10} tag followed by a thrombin cleavage site (pMCSG7_3C). The DNA of YDF antibody was cloned into the vector pMCSG7 with an N-terminal His\textsubscript{6} tag followed by a tobacco etch virus (TEV) protease cleavage site (pMCSG7_YDF). The DNA of GGVV antibody was cloned into a modified pFastBac-HTA vector with an N-terminal honeybee melittin (HBM) signal peptide followed by a His\textsubscript{6} tag and TEV protease cleavage site (pFastBac-HTA_GGVV). Mutants were constructed by site-directed mutagenesis and verified by DNA sequencing. Expression and purification of the wildtype proteins and mutants were performed using the method described below.

The recombinant plasmids pMCSG7_3C and pMCSG7_YDF were transformed into \textit{Escherichia coli} BL21 (DE3) cells for protein expression. The cells were cultured in Luria-Bertani (LB) medium at 37 °C until the OD\textsubscript{600} reached 0.8, and the temperature was then decreased to 16 °C. The cultures were then incubated for 16-20 h in the presence of 0.2 mM isopropylthio-beta-D-galactoside (IPTG) before being harvested by centrifugation at 5,000 g for 5 min. The cells were then re-suspended in buffer A (50 mM Tris-HCl, pH 8.0, 250 mM NaCl, 10% glycerol) for YDF or buffer B (50 mM Tris-HCl, pH 8.0, 250 mM NaCl, 10% glycerol, 1 mM TCEP) for 3C, lysed by sonication, and cleared by centrifugation at 80,000 g for 60 min. After the supernatants were loaded onto the TALON column (Clontech), proteins were eluted with a linear gradient of 5–500 mM imidazole. Elution fractions were then examined by SDS-PAGE, and target fractions were pooled before being processed with thrombin or TEV protease to remove the N-terminal His tags. The protein samples were further purified by passing through the TALON column again. YDF antibody in the flow through of the TALON column was then purified using size exclusion chromatography (SEC) with a Superdex S200 column (GE Healthcare) equilibrated with buffer A. Elution fractions containing the YDF antibody were pooled, concentrated, and stored at -80 °C until further use. HRV14 3C protease in the flow through of the TALON column was concentrated and dialyzed against buffer C (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 10% glycerol, 1 mM TCEP) for 3C, lysed by sonication, and cleared by centrifugation at 80,000 g for 60 min. After the supernatants were loaded onto a Hitrap heparin column (GE Healthcare), proteins were eluted with a linear gradient of 5–500 mM imidazole. Elution fractions were then examined by SDS-PAGE, and target fractions were pooled before being processed with thrombin or TEV protease to remove the N-terminal His tags. The protein samples were further purified by passing through the TALON column again. YDF antibody in the flow through of the TALON column was then purified using size exclusion chromatography (SEC) with a Superdex S200 column (GE Healthcare) equilibrated with buffer A. Elution fractions containing the YDF antibody were pooled, concentrated, and stored at -80 °C until further use. HRV14 3C protease in the flow through of the TALON column was concentrated and dialyzed against buffer C (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 10% glycerol, 1 mM TCEP) before being applied to a HiTrap heparin column (GE Healthcare) to remove contaminating nucleic acids. The protease was then similarly purified on a Superdex S200 column (GE Healthcare) equilibrated with buffer B. Elution fractions containing 3C protease were concentrated and immediately used for enzymatic assay or stored at -80 °C for ITC and crystallization experiments.

For assembly of the 3C-Rupintrivir complex, HRV14 3C protease was first buffer exchanged to HEPEES buffer (50 mM HEPEES, pH 7.4, 250 mM NaCl, 10% glycerol). Then, it was incubated with Rupintrivir (Tocris Bioscience\textsuperscript{TM}) in a molar ratio of 1:10. After overnight incubation at 4 °C, the mixture was clarified by centrifugation and loaded onto a Superdex S75 column (GE Healthcare) to remove excess Rupintrivir. Elution fractions containing 3C-Rupintrivir complex were then concentrated and immediately used for enzymatic assay or stored at -80 °C for ITC experiments. Covalent binding of Rupintrivir to HRV14 3C was further confirmed by enzymatic assays where the reconstituted 3C-Rupintrivir complex exhibited no enzymatic activity.

GGVV antibody was expressed in insect cells using the Bac-to-Bac system (Invitrogen) according to the manufacturer’s protocol. Sf9 cells at 70% confluency were transfected with 10 µg freshly prepared bacmids that encode GGVV antibody using FuGene HD (Promega) in 6-well plate. The supernatant was harvested after 72 h, and P2 virus was produced using the supernatant as P1 stock. Harvested P2 virus was stored at 4 °C in the dark until further use. For GGVV antibody expression, 1 L High Five cells at 2x10\textsuperscript{6} cells/ml were infected with 20 ml P2 virus and harvested at 60 h by centrifugation at 5,000 g for 10 min. The supernatant was then supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) and loaded onto an Excel Ni-NTA column (GE Healthcare) equilibrated with buffer A. GGVV antibody were eluted with a linear gradient of 5–500 mM imidazole. The N-terminal His\textsubscript{6} tag was removed by TEV protease and subsequent purification was performed as for the YDF antibody.
For assembly of the antigen-antibody complexes, HRV14 3C protease was incubated with YDF antibody or GGVV antibody at a molar ratio of 1.2:1 for 4 h. The 3C-YDF or 3C-GGVV complex was separated from excess 3C protease via SEC in buffer B.

**In vitro transcription and purification of RNA.** The transcription template which encodes the T7 promoter and 1-72 nt of the 5’ NCR of HRV14 genomic RNA was synthesized and amplified by PCR. The 72-nt ssRNA was transcribed *in vitro* using the HiScribe™ T7 High Yield RNA Synthesis kit (New England Biolabs) according to the manual. Target ssRNA was then purified by the RNAiso for Small RNA kit (TaKaRa) and isopropanol precipitation. Purified RNA was re-suspended in RNase-free water and stored at -80 °C.

**Enzymatic assay.** The enzymatic activity of the HRV 3C protease and 3C-Rupintrivir complex was measured using the HRV 3C Protease Activity Assay kit (Abcam). Briefly, different concentrations of substrate were prepared in a 96-well plate with a total reaction volume of 100 μl at 25 °C. The reaction was initiated by the addition of HRV 3C protease or 3C-Rupintrivir complex at a final concentration of 0.5 μM, and the absorbance at 405 nm was monitored using a SpectraMax i3x spectrophotometer (Molecular Devices). The data were analyzed by Michaelis-Menten curve fitting with Origin software (OriginLab). For the kinetic assay of HRV 3C protease in the presence of YDF antibody, HRV 3C protease was first incubated with YDF antibody for 2 min at final concentrations of 0.5 μM and 0.05-0.6 μM, respectively. The enzyme kinetics were then measured as described above.

To compare the inhibitory effect of YDF and GGVV antibody on the enzymatic activity of HRV 3C protease, 0.5 μM HRV 3C protease was incubated with YDF antibody or GGVV antibody at different molar ratios. The enzymatic activity of the mixtures was then measured in similar ways at a substrate concentration of 200 μM. The initial velocity of HRV 3C protease in the presence of either antibodies were normalized against the initial velocity of HRV 3C protease alone to reflect its relative activity in different conditions.

**Isothermal titration calorimetry (ITC) assay.** ITC experiments were performed using a MicroCal iTC200 or PEAQ-ITC instrument (Malven) at 25 °C. All of the protein samples were prepared in assay buffer containing 50 mM Tris-HCl (pH 8.0) and 50 mM NaCl. The assay was performed in a multi-injection mode by titrating 40 μl of titrant in the syringe into 200 μl of titrate in the cell. To characterize the interaction between either antibody and HRV 3C protease or 3C-Rupintrivir, titrations were conducted with 20 injections of 0.5 mM YDF antibody or 0.5 mM GGVV antibody into 50 μM HRV 3C protease or 3C-Rupintrivir at intervals of 120 s. The titrations of mutants were performed in a similar way.

For the binding of 72-nt ssRNA, titrations were conducted with 20 injections of 100 μM 72-nt ssRNA into 10 μM HRV 3C protease or 10 μM HRV 3C protease-antibody complex at intervals of 120 s. The thermodynamic parameters were obtained by nonlinear least-squares fitting of the data using MicroCal PEAQ-ITC analysis software (Malven). Heat changes of the reactions were corrected for the effect of dilution by buffer subtraction and final figures were prepared with the Origin software (OriginLab). The results are an average of at least two independent repeats.

**Size exclusion chromatography with multi-angle static light scattering (SEC-MALS).** The SEC-MALS measurements were carried out using the Agilent 1260 infinity II HPLC system coupled to a DAWN HELEOS-II light scattering detector (Wyatt Technology) and an Optilab T-rEX refractive index monitor (Wyatt Technology). 100 μl protein sample of 3C-YDF or 3C-GGVV complex was loaded onto a WTC-030S5 column (Wyatt Technology) equilibrated with buffer A running at a flow rate of 0.5 ml/min. The SEC-MALS/RI data were collected and analyzed with the ASTRA software (Wyatt Technology) and the Origin software (OriginLab).

**Crystallization and data collection.** Protein complexes were screened for crystallization using the hanging drop vapor diffusion method at 20°C. Diffraction quality crystals of HRV 3C protease in complex with YDF antibody were obtained in condition containing 0.1 M citrate (pH 5.5) and 40% PEG600. Crystals of HRV 3C protease in complex with GGVV antibody were obtained in conditions containing 0.1 M Tris-HCl (pH 8.6), 25% MPEG5000, and 0.2 M Li2SO4. Crystals were harvested with 25% (v/v) glycerol as a cryoprotectant and flash-cooled in liquid nitrogen for data
collection. X-ray diffraction data were collected on beamline BL18U1 at the Shanghai Synchrotron Radiation Facility (SSRF) or beamline BL41XU of the Super Photon Ring-8 GeV (Spring-8), and processed using HKL2000 or HKL3000 software (46).

**Structure determination and refinement.** The crystal structures of HRV 3C protease in complex with YDF (3C-YDF) or GGVV (3C-GGVV) were solved by molecular replacement with the Phaser program in PHENIX (47), using the HRV 3C protease structure (PDB: 2IN2) and an scFv structure (PDB: 2GHW) as the search models. The initial models were further improved by cycles of manual building using COOT (48) and refinement using PHENIX (49). The quality of the final models was analyzed with MolProbity (50). Data collection and refinement statistics are outlined in Table 1. Figures were prepared using program Pymol (The PyMOL Molecular Graphics System, Version 2.1 Schrödinger, LLC). Electrostatic calculations were performed with PDBePISA (51). The buried solvent-accessible surface area was calculated with PDB2PQR (52).

**Molecular simulation.** Molecular dynamics simulations were all performed using Desmond from Schrodinger Suite 2019-1 (http://www.schrodinger.com). For comparison of HRV14 3C in the presence and absence of YDF, either the coordinates of 3C from 3C-YDF or the coordinates of 3C-YDF were used as the initial models for the simulation. For the comparison of HRV14 3C in the presence and absence of GGVV, either the coordinates of 3C from 3C-GGVV or the coordinates of 3C-GGVV were used as the initial models for the simulation. The models were first optimized using Protein Preparation Wizard following default settings. The simulation systems were solvated with SPC water and neutralized, containing Cl- and Na+ ions at 0.15 M to mimic physiological ionic strength. Prepared structures were then subjected to 200 ns molecular dynamics simulation under OPLS3 force field following Desmond implemented multi-stage MD simulation protocol with temperature T and pressure P kept constant at 310 K and 1 atm, respectively. The RMSD and RMSF, as well as distances, angles and dihedral angles of related residues over the simulation trajectory were calculated with Simulation Events Analysis from Schrodinger Suite 2019-1 (http://www.schrodinger.com), and statistically analyzed and plotted with Prism 8 (GraphPad). To compare the binding energy between 3C and substrate analogue 'Ace-LEALFQ-ethylpropionate' in the absence and presence of YDF, molecular simulations of 3C-'Ace-LEALFQ-ethylpropionate' complex structure (PDB:2B0F) and 3C-YDF-'Ace-LEALFQ-ethylpropionate' complex structure (prepared by docking) were performed in similar ways as described above. The 200 ns simulation trajectories were then calculated with third-party script thermal_mmgbsa.py from Schrodinger, with a step size of 10.

**Hydrogen–deuterium exchange mass spectrometry (HDX-MS).** Amide hydrogen exchange of HRV 3C protease alone were started by diluting 2 μl protein sample at 38 μM into 18 μl D2O buffer (50 mM Tris, pH 8.0, 50 mM NaCl) at 25 °C. At different time points (0 s, 10 s, 100 s, 1000 s and 10000 s), the labeling reaction was quenched by the addition of chilled quench buffer (400 mM KH2PO4/K2HPO4, pH 3.0, 50 mM TCEP) and immediately frozen in liquid nitrogen. For the HDX-MS of HRV 3C protease in the presence of 72-nt ssRNA, 1.5 μl HRV 3C protease at 50 μM was first mixed with 0.5 μl 72-nt ssRNA at 0.6 mM. The mixture was then labeled by adding 18 μl D2O buffer before being quenched at different time points and flash-frozen. All frozen samples were stored at -80 °C until analysis.

The thawed samples were immediately injected into HPLC-MS (Agilent 1100) system equipped with in-line peptic digestion and desalting. The desalted digestes were then separated with a Hypersil Gold™ analytical column (Thermo) over an 18 min gradient and directly analyzed with an Orbitrap Fusion mass spectrometer (Thermo). The HPLC system was extensively cleaned with blank injections between samples to minimize any carryover. Peptide identification was performed by tandem MS/MS under orbi/orbi mode. All MS/MS spectra were analyzed using the MASCOT program, and final PSMs were filtered with an FDR of 1%. Initial analysis of the peptide centroids was performed with HD-Examiner v2.3 (Sierra Analytics) and then manually verified for every peptide to check retention time, charge state, m/z range and the presence of overlapping peptides. The peptide coverage of HRV 3C protease was 92% and the relative deuteration levels (%) of each peptide were automatically calculated by HD-Examiner with the assumption that a fully deuterated sample retains 90% D in current LC setting.
Fig. S1. Sequence alignment of YDF scFv and GGVV scFv. The heavy chain is connected to the light chain in both scFvs by a ‘GGGSGGGSGGGSGS’ linker. CDRs are marked by black rectangles and numbered according to Kabat numbering.
Fig. S2. YDF and GGVV scFv inhibit HRV through different mechanisms. (A) ITC results of YDF or GGVV scFv titration into HRV14 3C protease. (B) ITC results of YDF or GGVV scFv titration into 3C-Rupintrivir complex. (C) SEC-MALS profiles of HRV14 3C protease in complex with YDF scFv (left panel) and GGVV scFv (right panel). The theoretical molecular weights (MW) of HRV14 3C protease, YDF scFv and GGVV scFv are 20.1 kDa, 25.9 kDa and 26.2 kDa, respectively. (D) Enzymatic activity of HRV14 3C protease in the presence of YDF or GGVV. The normalized enzymatic activity of HRV14 3C was plotted against different molar ratios of antibody/HRV14 3C protease. Values are means ± S.D. (n=3 assays).
Fig. S3. Conformational changes of HRV14 3C upon YDF binding. (A) The substrate cleft of HRV14 3C in the presence of YDF can readily accommodate the shape and volume of a substrate analogue, such as ‘Ace-LEALFQ-ethylpropionate’. Left panel: surface representation of HRV14 3C in complex with this substrate analogue (PDB: 2B0F) with 3C colored dark grey and substrate analogue colored yellow. Right panel: the same substrate analogue (yellow) was docked onto the 3C structure in the 3C-YDF complex, with 3C shown as light grey surface and YDF shown as ribbons). The three active site catalytic residues of 3C are highlighted as a red surface in both panels, and the accessible ‘Ace-’ and ‘-ethylpropionate’ moieties are not shown. (B) Computational binding energies (dG_binding) between substrate analogue ‘Ace-LEALFQ-ethylpropionate’ and 3C (without YDF) or 3C-YDF (with YDF) was calculated along the 200 ns molecular simulation trajectory with a step-size of 10. n = 2000 frames. (C) The crystal structure of HRV14 3C in complex with YDF (3C-YDF, cyan) was superimposed on the NMR solution structures of apo HRV14 3C (PDB: 2IN2, grey) and HRV14 3C in complex with a peptide inhibitor (PDB:2B0F, pink). The three structures align well with overall RMSDs in the range of 0.9-1.0 Å for 178 Cα atoms of 3C. The 3C catalytic residues (H40, E71 and C146) are shown as stick models and color coded as above to illustrate their similar positions and conformations in all three structures. The CDRs from YDF are shown below as a molecular surface to identify the interface between HRV14 3C and YDF.
Fig. S4. Molecular simulation identifies regions on HRV14 3C that manifest flexibility change upon YDF or GGVV binding. (A) and (D) Control plots representing the stability of the HRV14 3C models (A: +/- YDF; D: +/- GGVV) during the molecular dynamics run. The RMSD of the non-hydrogen atoms of 3C is smoothed by Savitzky-Golay filter and shown as a function of time. Color code: HRV14 3C - black, HRV14 3C in the presence of YDF - red, and HRV14 3C in the presence of GGVV - green. (B) and (E) RMSF of atomic positions computed for the backbone atoms is smoothed by Savitzky-Golay filter and shown as a function of HRV14 3C residues. The color coding is the same as in (A) and (D). Note that for (B), residue 1 is absent in the initial models and for (E), residues 64-69, 122-132, 180-181 are absent in the initial models of simulation; hence, the corresponding parts are missing in the RMSF plots. The insets in (B) and (E) are box plots depicting the means (denoted with +), quartiles (box), min and max (whisker) of per-residue RMSF changes upon YDF or GGVV binding. (C) and (F) The per-residue RMSF changes upon YDF (C) or GGVV (F) binding are mapped onto the HRV14 3C structure. Residues that manifest the highest 10% of RMSF change are colored dark brown to indicate decreased RMSF or pink to indicate increased RMSF, respectively. Other residues with no significant RMSF changes are colored grey. The C\(_{\alpha}\) positions of the catalytic triad are indicated with yellow spheres, and the interacting CDRs of YDF or GGVV are shown as ribbons. Note that the catalytic Cys146 and flanking 141-145 residues (highlighted with red box) exhibit significant flexibility decrease upon YDF binding (C).
Fig. S5. HDX-MS peptide coverage of HRV14 3C. The sequence coverage map for HRV14 3C in the HDX-MS study. Green lines above the protein sequence represent the digested peptides that were identified and analyzed in this study.
Fig. S6. Additional peptides whose hydrogen-to-deuterium exchange rate decreased considerably in the presence of 72-nt RNA. (A-C) Deuterium uptake plots and mass spectra of indicated peptides from HRV14 3C in the absence and presence of 72-nt RNA. Left: The deuterium uptake data are plotted as percent deuterium uptake versus time on a logarithmic scale. Right: Mass spectra of indicated peptides at different labeling time points, with the mass spectra of undeuterated samples shown as controls.
Fig. S7. Deuterium uptake plots and mass spectra of indicated peptides from HRV14 3C.
(A-D) Uptake plots and mass spectra of additional peptides whose hydrogen-to-deuterium exchange rate generally manifested no difference in the presence of 72-nt RNA. Left: The deuterium uptake data are plotted as percent deuterium uptake versus time on a logarithmic scale. Right: Mass spectra of indicated peptides at different labeling time points with the mass spectra of undeuterated samples shown as controls.
| Protein                        | $N$       | $K_d$ (M)            | Relative affinity |
|-------------------------------|-----------|----------------------|-------------------|
| Wild-type YDF scFv            | 0.99 ± 0.01 | $8.23 ± 0.82 \times 10^{-8}$ | 100%              |
| CDRH3                         |           |                      |                   |
| K97A                          | 0.93 ± 0.01 | $9.13 ± 1.06 \times 10^{-8}$ | 90.1%             |
| Y100A                         | 0.96 ± 0.01 | $1.29 ± 0.13 \times 10^{-7}$ | 63.8%             |
| D100A                         | 1.01 ± 0.01 | $1.50 ± 0.16 \times 10^{-7}$ | 54.9%             |
| F100A                         | 0.96 ± 0.01 | $1.11 ± 0.14 \times 10^{-7}$ | 74.1%             |
| Y100A/D100A/F100A             | 0.97 ± 0.01 | $4.23 ± 0.42 \times 10^{-6}$ | 2.0%              |
| CDRL1                         |           |                      |                   |
| Y32A                          | NB*       | NB*                  | 0                 |
| CDRL2                         |           |                      |                   |
| Q50A                          | 1.01 ± 0.01 | $9.76 ± 1.32 \times 10^{-8}$ | 84.3%             |
| D51A                          | NB*       | NB*                  | 0                 |
| K53A                          | 1.03 ± 0.01 | $9.36 ± 1.34 \times 10^{-8}$ | 87.9%             |
| CDRL3                         |           |                      |                   |
| W91A                          | NB*       | NB*                  | 0                 |
| D92A                          | 1.03 ± 0.01 | $3.05 ± 0.34 \times 10^{-7}$ | 27.0%             |
| S93A                          | 0.96 ± 0.01 | $2.35 ± 0.21 \times 10^{-7}$ | 35.0%             |

* NB indicates no binding detected
Table S2. ITC results of wild-type GGVV scFv and its mutants with 3C protease

| Protein                  | N       | $K_d$ (M)       | Relative affinity |
|--------------------------|---------|-----------------|-------------------|
| Wild-type GGVV scFv      | 0.96 ± 0.01 | (9.11 ± 1.06) x 10^{-9} | 100%              |
| **CDRH2**                |         |                 |                   |
| V53A                     | 1.02 ± 0.01 | (1.41 ± 0.16) x 10^{-8} | 64.6%             |
| F54A                     | 1.03 ± 0.01 | (1.67 ± 0.17) x 10^{-6} | 0.6%              |
| D55A                     | 1.05 ± 0.01 | (1.05 ± 0.11) x 10^{-8} | 86.8%             |
| T56A                     | 0.97 ± 0.01 | (9.90 ± 0.12) x 10^{-9} | 92.0%             |
| V53A/F54A/D55A/T56A      | NB*     | NB*             | 0                 |
| **CDRH3**                |         |                 |                   |
| V100A                    | 0.97 ± 0.01 | (4.39 ± 0.52) x 10^{-8} | 20.8%             |
| V100A/V100A              | 1.01 ± 0.01 | (1.40 ± 0.16) x 10^{-8} | 65.1%             |
| V100A/V100A              | 1.04 ± 0.01 | (5.04 ± 0.55) x 10^{-6} | 0.2%              |

* NB indicates no binding detected
Table S3. Enzymatic parameters of HRV14 3C in the presence of different concentrations of YDF.

| YDF concentration (μM) | Molar ratio (3C:YDF) | $K_m$ (μM) | $V_{max}$ (μM s$^{-1}$) |
|------------------------|----------------------|------------|------------------------|
| 0                      | 1:0.0                | 93.4 ± 8.0 | 0.48 ± 0.02            |
| 0.05                   | 1:0.1                | 98.9 ± 4.9 | 0.45 ± 0.01            |
| 0.1                    | 1:0.2                | 96.9 ± 5.4 | 0.42 ± 0.01            |
| 0.2                    | 1:0.4                | 91.0 ± 10.3| 0.37 ± 0.02            |
| 0.4                    | 1:0.8                | 84.7 ± 8.4 | 0.22 ± 0.01            |
| 0.6                    | 1:1.2                | 85.4 ± 10.8| 0.11 ± 0.01            |
References

46. Minor W, Cymborowski M, Otwinowski Z, Chruszcz M. HKL-3000: the integration of data reduction and structure solution--from diffraction images to an initial model in minutes. Acta Crystallogr D Biol Crystallogr 62, 859-866 (2006).

47. McCoy AJ. Solving structures of protein complexes by molecular replacement with Phaser. Acta Crystallogr D Biol Crystallogr 63, 32-41 (2007).

48. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr 60, 2126-2132 (2004).

49. Adams PD, et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr D Biol Crystallogr 66, 213-221 (2010).

50. Chen VB, et al. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 66, 12-21 (2010).

51. Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA. PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations. Nucleic Acids Res 32, W665-667 (2004).

52. Krissinel E, Henrick K. Inference of macromolecular assemblies from crystalline state. J Mol Biol 372, 774-797 (2007).