Supplementary Information - Molecular Packing Description

The two crystal forms with determined structures have very large solvent channels and high solvent content. The orthorhombic form is clearly related to the cubic crystals form but is at lower resolution and has more molecules in the asymmetric unit and is not described. The nature of the intermolecular contacts determines the nature of the packing and there are some common features between the cubic and tetragonal forms that are described here for the sake of completion.

1. Cubic crystal form

Cubic crystals contain eight molecules in the asymmetric unit, related by non-crystallographic fourfold, threefold and twofold symmetries. The packing is shown in Figure S1. Analysis with the PISA server revealed four types of intermolecular interfaces that define the packing of molecules in this crystal form.

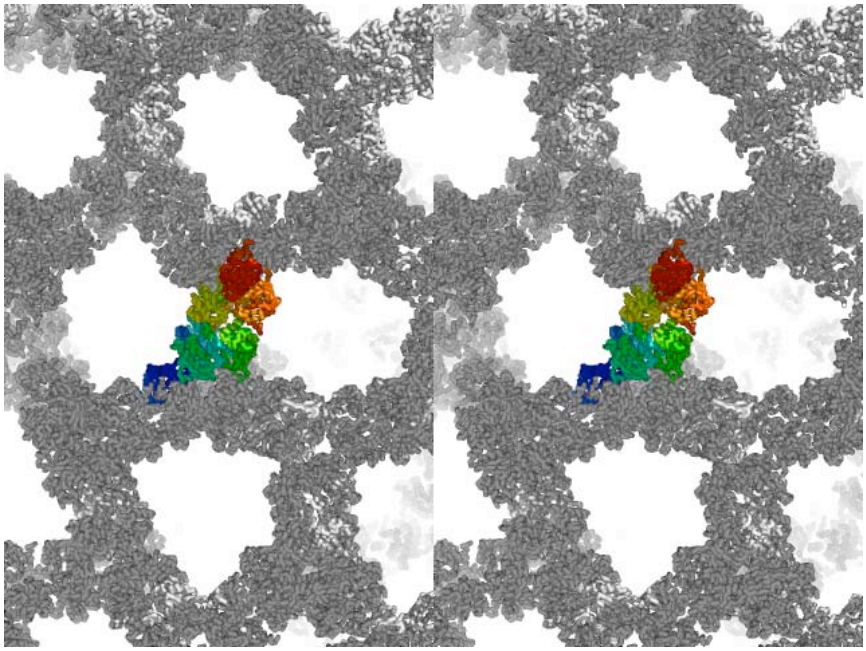

Figure S1. The crystal packing of the FCoV X domain in the cubic crystal form shown in stereo. Molecules representing one asymmetric unit are shown in color.

Some groups of three molecules exhibit similar intermolecular contacts. These are groups (Figure S2a) formed between molecules $ABC_{z,x,y}$, $GHE_{-y,z-1/2,-x+1/2}$, $DD_{y,z,x}D_{z,x,y}$ and $FF_{-z+1/2,-x,y+1/2}F_{-y,z-1/2,-x+1/2}$, where the subscript describes symmetry related molecules. The first two groups have non-crystallographic threefold symmetry and the second two crystallographic threefold symmetry. The biggest interface, with an average area of 450 $Å^2$, is between pairs of molecules in this arrangement. It is

present eight times, between molecules BA, EG, DD, HE, CB, GH, AC and FF. The first member of each pair listed above contributes twelve to fourteen residues that belong to helices α1 and α5, loops $L_{\alpha2\text{-}\alpha3}$ and $L_{\alpha5\text{-}\beta7}$ and region $L_{\beta4a\text{-}\beta4b}$-β4b-$L_{\beta4b\text{-}\beta5}$. The second member of each pair contributes twelve to fourteen interfacing residues from helix α3, loop $L_{\alpha3\text{-}\beta4a}$ and strand β4a. The major interactions at this interface are hydrogen bonds between O(Phe85)-Nζ(Lys99), N(Glu117)-Oε(Glu96) and O(His118)-Oγ(Ser100) (Figure S2b). Van der Waals contacts between residues Ile56-Ser100, Glu60-Ser100, Leu116-Glu96, Glu117-Arg93, His118-Ser100 and His118-Leu111 are also important in defining the interface.

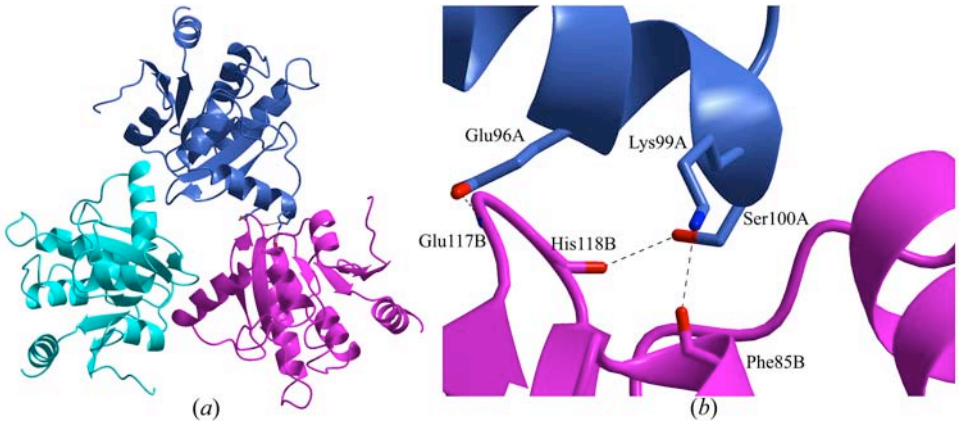

Figure S2. (a) Ribbon representation of trimer1 between molecules ABC. Molecule A shown in light blue, B in magenta and C in cyan. (b) Trimer1 interface between molecules B and A. Hydrogen bonds between O(Phe85)-Nζ(Lys99), N(Glu117)-Oε(Glu96) and O(His118)-Oγ(Ser100) are shown with dashed lines. Molecule A shown in light blue, B in magenta, oxygen atoms in red and nitrogen atoms in blue.

The second interface buries a surface area of 440 $Å^2$. It is present only between molecules C and E. This interface involves twelve residues from chain C, of which eleven belong to the C-terminal alpha helix α6 and one residue belongs to helix α5. Chain E contributes fifteen residues from helices α4, α5 and α6. Interfacing residues form hydrogen bonds between Oδ1(Asp189)-Nδ2(Asn197) and O(Asn200)-Oε1(Glu135). There are also three hydrogen bonds present that involve residues with alternative conformers: Nη1(Arg192)-Oε1(Glu167, conformer A), Nη2(Arg192)-Oε2(Glu167, conformer A) and Nδ2(Asn197, conformer A)-O(Val168) (Figure S3). Close van der Waals contacts were identified between residues Gln190C-Phe198E, Arg192C-Glu167E, conformer A, Val193C-Glu167E, conformer A and Asn200C-Glu135E.

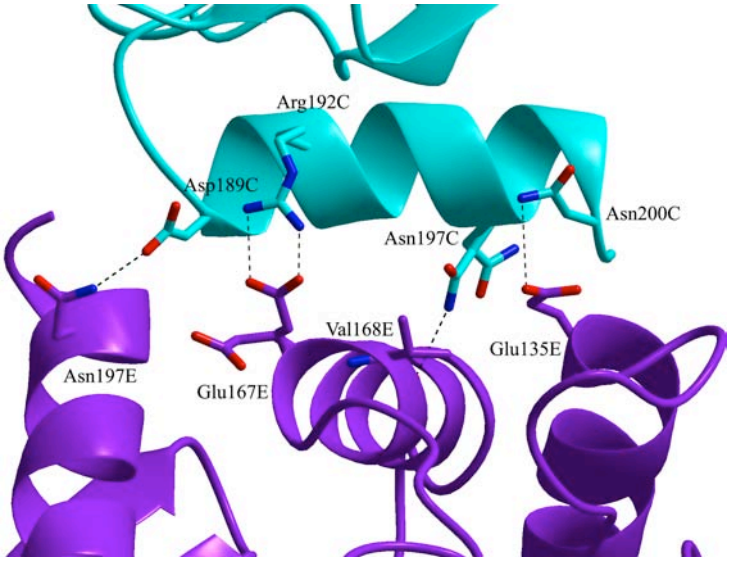

Figure S3. Interface between molecules C and E. Hydrogen bonds between Oδ1(Asp189)-Nδ2(Asn197), O(Asn200)-Oε1(Glu135), Nη1(Arg192)-Oε1(Glu167, conformer A), Nη2(Arg192)-Oε2(Glu167, conformer A) and Nδ2(Asn197, conformer A)-O(Val168) are shown with dashed lines. Molecule C is shown in cyan, E in purple, oxygen atoms in red and nitrogen atoms in blue.

A second constellation of three molecules (Figure S4a), present only in the cubic crystal form, is formed between molecules GEF, BCD, $AA_{z,x,y}A_{y,z,x}$ and $HH_{-y,z-1/2,-x+1/2}H_{-z+1/2,-x,y+1/2}$. The first two groupings have non-crystallographic threefold symmetry. This interface buries an average area of 413 $Å^2$ and is present eight times, between molecules BD, DC, CB, GF, FE, EG, AA and HH. The first member of each pair contributes twelve to fourteen residues from helix α2 and loops $L_{β3-α2}$, $L_{β5-α4}$ and $L_{β6-α5}$. Twelve to fourteen residues from N-terminus and helices α1 and α2 come from the second member of the pair. Hydrogen bonds are formed between Nε(Arg73)-Oδ(Asn57), N(Gly76)-Oδ(Asp53), Nη(Arg80)-Oδ(Asp51), Nη(Arg80)-Oδ(Asp53) and Nζ(Lys163)-O(Leu35) (Figure S4b). Van der Waals contacts occur between residues Val75-Asp53, Ile161-Leu35 and Lys163-Leu35.

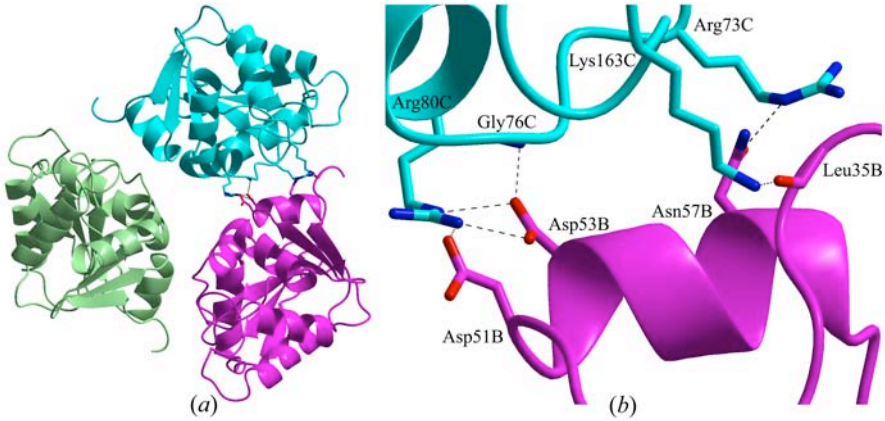

Figure S4. (a) Ribbon representation of trimer2 between molecules BCD. Molecule B is shown in magenta, C in cyan and D in light green. (b) Interface between molecules C and B. Hydrogen bonds between Nε(Arg73)-Oδ(Asn57), N(Gly76)-Oδ(Asp53), Nη(Arg80)-Oδ(Asp51), Nη(Arg80)-Oδ(Asp53) and Nζ(Lys163)-O(Leu35) are shown with dashed lines. Molecule B is shown in magenta, C in cyan, oxygen atoms in red and nitrogen atoms in blue.

The fourth interface buries an area of 377 $Å^2$ and is present only between the pair of molecules A and H ($AH_{-z+1,x+1/2,-y+1/2}$). It involves eleven residues from the N-terminus and helix α6. The major interactions at this interface are hydrogen bonds between Nη1(Arg192)-Oδ1(Asn197), Nη2(Arg192)-Oδ1(Asn197), Nη2(Arg192)-Oε2(Glu196), Oε2(Glu196)-Nη2(Arg192), Oδ1(Asn197)-Nη1(Arg192) and Oδ1(Asn197)-Nη2(Arg192) (Figure S5). Van der Waals contacts between the N-terminus of one molecule and the C-terminus of the other (Leu37 and Phe39 with Asn200 and Gly201).

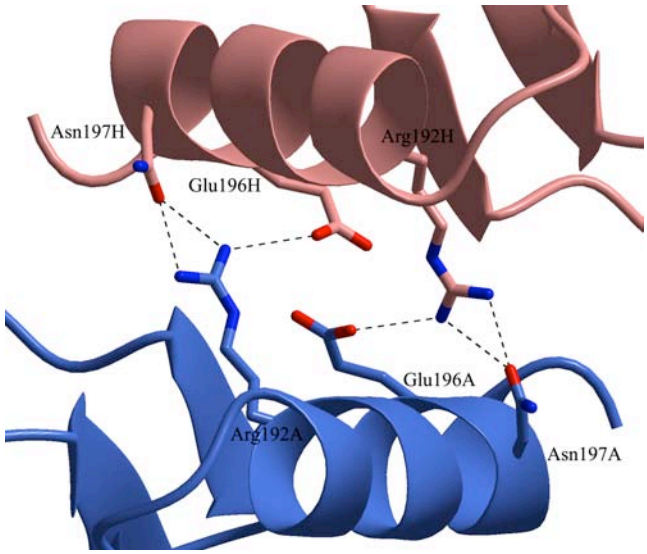

Figure S5. Interface between molecules A and H. Hydrogen bonds between Nη1(Arg192)-Oδ1(Asn197), Nη2(Arg192)-Oδ1(Asn197), Nη2(Arg192)-Oε2(Glu196), Oε2(Glu196)-Nη2(Arg192), Oδ1(Asn197)-Nη1(Arg192) and Oδ1(Asn197)-Nη2(Arg192) are shown with dashed lines. Molecule A is shown in blue, H in pink, oxygen atoms in red and nitrogen atoms in blue.

2. Tetragonal crystal form

Tetragonal crystals of the FCoV X domain contain three molecules per asymmetric unit related by non-crystallographic threefold symmetry. The crystal packing is shown in overview in Figure S6. Analysis using the PISA server revealed three intermolecular interfaces, which effectively determine the packing of the molecules in this crystal form.

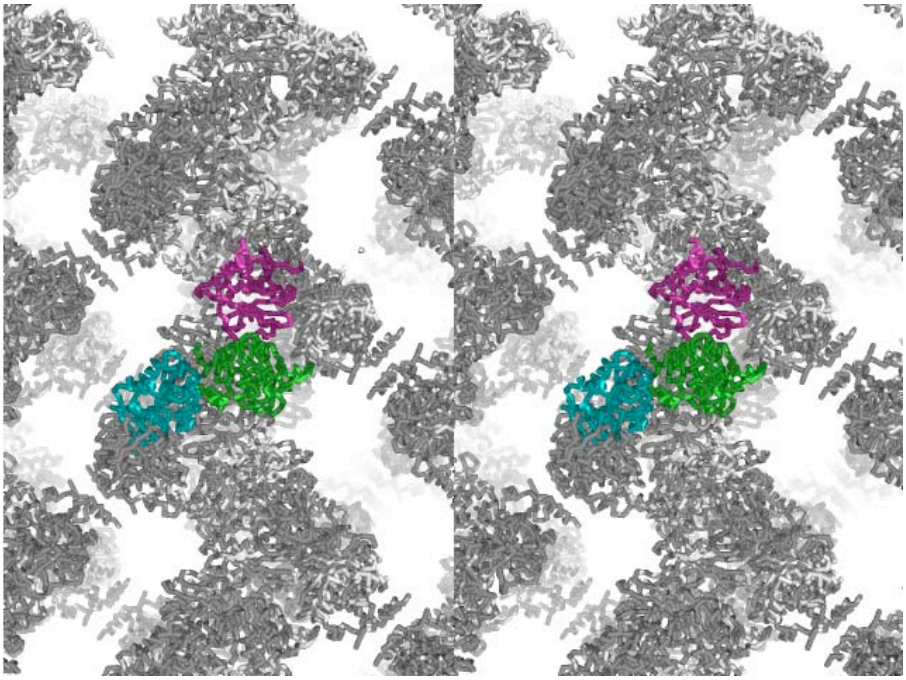

Figure S6. The crystal packing of the FCoV X domain in the tetragonal crystal form shown in stereo. Molecules representing one asymmetric unit are shown in color.

Similar to the cubic crystals, the biggest interface in the tetragonal crystals describes the interactions between pairs of chains in a grouping of three molecules similar to the first grouping of three in the cubic crystal form. The grouping ($AB_{y+1/2,-x+1/2,z-1/2}C$) has non-crystallographic threefold symmetry. The interface buries 446 $Å^2$ on average and is present between three pairs of chains CB, BA and AC. It involves twelve to thirteen residues from the same regions as in the case of cubic crystals. Hydrogen bonds differ depending on molecule pair. In the case of pairs CB and BA hydrogen bonds are present between N(Glu117)-Oε1(Glu96) and O(Phe85)-Nζ(Lys99). The AC pair interacts though hydrogen bonds between N(Glu117)-Oε2(Glu96) and Nδ2(Asn57)-O(Lys99). Van der Waals interactions are essentially the same as in the case of cubic crystals and they involve residues Ile56-Ser100 Asn57-Ser100, Glu60-Ser100, Leu116-Glu96, Glu117-Arg93, His118-Ser100 and His118-Leu111.

The second interface buries an average area of 441 $Å^2$. It is present between the pairs BA and $CC_{y+1/2,-x+1/2,z-1/4}$. The first member of the pair contributes eleven (chain B) or thirteen (chain C) residues from loops $L_{\beta3-\alpha2}$, $L_{\alpha3-\beta4a}$, $L_{\beta5-\alpha4}$ and $L_{\beta6-\alpha5}$. The second member of the pair involves seventeen (chain A) or twelve (chain C) residues from the N-terminus, helix α6 and strands β1 and β2. Hydrogen bonds are formed between Oδ(Asp71)- Nη(Arg192), Nη(Arg73)-Oδ(Asp189), Nη(Arg128)-O(Phe39) and

Nη(Arg128)-Oε(Glu196) (Figure S7). Van der Waals contacts occur between residues Asn69-Arg192, Asp71-Gln49, Arg73-Gln49, Val75-Val193, Arg128-Tyr40 and Lys163-Asn200.

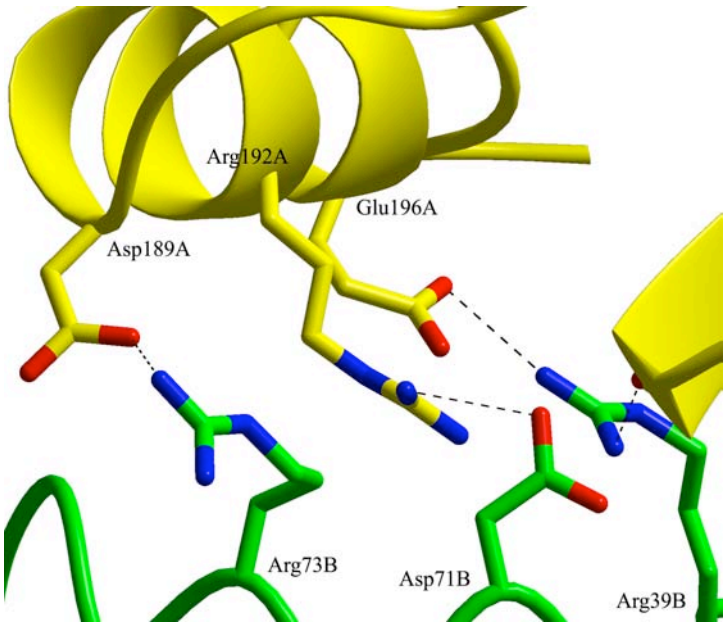

Figure S7. Interface between molecules B and A. Hydrogen bonds between Oδ(Asp71)- Nη(Arg192), Nη(Arg73)-Oδ(Asp189), Nη(Arg128)-O(Phe39) and Nη(Arg128)-Oε(Glu196) are shown with dashed lines. Molecule B is shown in green, A_S in yellow, oxygen atoms in red and nitrogen atoms in blue.

The third interface buries an area of 322 $Å^2$. This interface, between $BB_{y,x,-z+1}$, is similar to the AH dimer interface in the cubic crystal form. It involves nine residues from N-terminus and helix α6. Hydrogen bonds and van der Waals interactions are the same as in the case of cubic crystal form.