Data Collection Report MX1615– ESRF, Grenoble

Noroviruses belong to the *Caliciviridae* family of small positive-sense RNA viruses and are the leading causes of gastroenteritis around the world with an estimated 21 million cases per annum. The error prone replication mechanism of noroviruses generates a high genetic diversity amongst its five genogroups. The rapid emergence of new strains that are antigenically distinct from their predecessors hinders the effective treatment of norovirus outbreaks.

Structurally characterizing the surface architecture of multiple norovirus strains helps in designing superior therapeutics and vaccines to effectively tackle the outbreaks. The virus is structurally viewed as an icosahedron that is composed of 180 copies of capsid protein arranged into 90 dimers. Each capsid protein displays a shell (S) domain and protruding (P) domain. The S domains interlock to form an icosahedron, while the P domains interact with cell receptors and aid in the internalization and pathogenicity of the virus. We are interested in charactering the structural mechanisms employed by the P-domains to bind to their potential partners through X-ray crystallography. These binding partners include naturally occurring histo-blood-group antigens (HBGAs) which are thought to act as co-receptors for internalization and synthetic drug moieties that inhibit norovirus infection.

We have co-crystallized the P-domains of two infectious strains of noroviruses – Snowy Mountain Virus (SMV) and Hawaiian Virus (HV) with HBGAs and synthetic ligands. During this trip to ESRF, we have collected x-ray diffraction data on many co-crystals of P-domains and ligands. The data collection statistics of the crystals, which yielded good diffraction are listed below. We solved all the data-sets by employing the molecular replacement method. Currently we are refining the structures of P-domains and closely inspecting the electron density difference maps for potential ligand binding sites

Datasets collected on the co-crystals of P-domains and ligands.

1. P-dom-SMV-H1 Dataset 1 – This dataset is obtained from a co-crystal of P-domain of SMV with H1 ligand. A complete dataset was collected at a resolution of 2.0 Å in the space group P3 (97.2, 97.2, 64.4; 90° 90° 120°) with a completeness of

98.5% (91.9%), R_{symm} of 6.6% (63.4%), I over sigma of 11.6 (1.82) and multiplicity of 3.4 (3.2). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 24% and 27% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

2. P-dom-SMV-Btri Dataset – This dataset is obtained from a co-crystal of P-domain of SMV with Btri ligand. A complete dataset was collected at a resolution of 1.86 Å in the space group P31 2 1 (96.8, 96.8, 64.4; 90° 90° 120°) with a completeness of 98.8% (89.7%), R_{symm} of 7.0% (100.4%), I over sigma of 14.6 (1.69) and multiplicity of 6.7 (6.1). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 25% and 28% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

3. P-dom-SMV-Leb Dataset – This dataset is obtained from a co-crystal of P-domain of SMV with Leb ligand. A complete dataset was collected at a resolution of 1.7 Å in the space group P31 2 1 (96.2, 96.2, 64.0; 90° 90° 120°) with a completeness of 99.9% (99.8%), R_{symm} of 7.6% (97.6%), I over sigma of 13.0 (1.77) and multiplicity of 6.79 (6.08). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 23.0% and 26.4% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

4. P-dom-SMV-Atri Dataset – This dataset is obtained from a co-crystal of P-domain of SMV with Atri ligand. A complete dataset was collected at a resolution of 1.7 Å in the space group P6 2 2 (97.2, 97.2, 64.4; 90° 90° 120°) with a completeness of 100% (99.8%), R_{symm} of 19.5% (105.3%), I over sigma of 8.5 (1.87) and multiplicity of 11.2 (11.8). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A

solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 25.0% and 27.4% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

5. P-dom-HV-Apo Dataset – This dataset is obtained from an apo-crystal of P-domain of HV virus. A complete dataset was collected at a resolution of 2.0 Å in the space group C2 2 2 (73.1, 96.2, 81.4; 90° 90° 90°) with a completeness of 98.8% (96.8%), R_{symm} of 16.0% (111.6%), I over sigma of 7.7 (1.37) and multiplicity of 4.7 (4.1). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 23.0% and 26.4% respectively.

6. P-dom-HV-Sial-3 Dataset – This dataset is obtained from a co-crystal of P-domain of HV with sial-3 ligand. A complete dataset was collected at a resolution of 1.95 Å in the space group P 2 2 2_1 (73.1, 82.2, 96.4; 90° 90° 90°) with a completeness of 97.8% (86.8%), R_{symm} of 16.0% (111.6%), I over sigma of 7.7 (1.37) and multiplicity of 4.7 (4.1). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 25.0% and 27.4% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

7. P-dom-HV-Leb-2 Dataset – This dataset is obtained from a co-crystal of P-domain of HV with Leb-2 ligand. A complete dataset was collected at a resolution of 2.15 Å in the space group C 1 2 1 (93.1, 73.2, 81.7; 90° 95.14° 90°) with a completeness of 99.6% (99.5%), R_{symm} of 28.2% (120.3%), I over sigma of 3.5 (0.9) and multiplicity of 4.3 (4.3). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 26.0% and 28.4% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

8. P-dom-HV-Lex-2 Dataset – This dataset is obtained from a co-crystal of P-domain of HV with Lex-2 ligand. A complete dataset was collected at a resolution of 2.42 Å in the space group P 1 (60.6 62.4 81.9; 86.3° 86.1° 73.1°) with a completeness of 96.2% (90.9%), R_{symm} of 16.8% (71.7%), I over sigma of 7.4 (1.84) and multiplicity of 3.4 (3.2). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 25.0% and 27.4% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

9. P-dom-HV-Ley-1 Dataset – This dataset is obtained from a co-crystal of P-domain of HV with Ley-1 ligand. A complete dataset was collected at a resolution of 2.42 Å in the space group C 1 2 1 (98.2, 73.0, 81.5; 90.0° 93.9° 90.0°) with a completeness of 97.2% (86.7%), R_{symm} of 17.6% (76.7%), I over sigma of 6.4 (1.54) and multiplicity of 4.1 (3.2). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 24.0% and 26.8% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

10. P-dom-HV-Btri-1 Dataset – This dataset is obtained from a co-crystal of P-domain of HV with Btri-1 ligand. A complete dataset was collected at a resolution of 1.9 Å in the space group C 1 2 1 (99.1, 73.6, 82.0; 90.0° 95.0° 90.0°) with a completeness of 99.6% (97.2%), R_{symm} of 20.3% (114.0%), I over sigma of 5.7 (1.0) and multiplicity of 6.3 (5.7). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 25.0% and 26.9% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.

11. P-dom-G11-H2 Dataset – This dataset is obtained from a co-crystal of P-domain of G11 strain of norovirus with H2 ligand. A complete dataset was collected at a resolution of 1.81 Å in the space group P 3_1 2 1 (82.8, 82.8, 163.8; 90.0° 90.0°

120.0°) with a completeness of 99.8% (99.2%), R_{symm} of 15.2% (105.2%), I over sigma of 13.13 (1.75) and multiplicity of 6.8 (6.9). The data was processed with XDS. A homologous structure of the P-domain was used as a search model for molecular replacement. A solution was obtained and the refinement currently stands at an R_{work} and R_{free} of 24.0% and 27.7% respectively. We are closely inspecting the electron density map currently to discern the binding site and the orientation of the ligand.