**CryoEM Structure and Assembly Mechanism   
of a Bacterial Virus Genome Gatekeeper**

Igor Orlov, Stéphane Roche, Sandrine Brasiles, Natalya Lukoyanova,Marie-Christine Vaney, Paulo Tavares, Elena V Orlova

**Supplementary Tables**

**Supplementary Table 1 |** **Cryo-EM analysis of the SPP1 connector: data collection, refinement and validation statistics.**

|  |  |
| --- | --- |
| EMDB, PDB | EMD-14509, 7Z4W |
| Microscope (Voltage) | Titan Krios (300kV) |
| Pixel size (Å/pixel) | 0.69 Å |
| Camera | Falcon III |
| Defocus range (μm) | -1.2 to -2.5 |
| Overall electron dose (e-/ Å2) | 45 |
| Symmetry | C6 |
| Number of micrographs | 3,876 |
| Initial number of particles | 524,494 |
| Final number of particles | 401,807 |
| Map res. range (threshold 0.143) | 2.27-3.0 |
| Map Sharpening B Factor | -90 |
| **Refinement** | |
| Initial Model (PDB) | *ab initio* modelling |
| Model Resolution (Å) | 2.7 |
| CC Model vs. Data (Mask) | 0.83 |
| **Model composition** | |
| Non-hydrogen atoms | 17298 |
| Protein residues | gp6 447; gp15 102; gp16 109 |
| **RMSD** | |
| Bond Lengths | 0.008 |
| Bond angles | 1.117 |
| **Validation** | |
| Molprobity Score | 1.21 |
| Clashscore | 1.86 |
| **Ramachandran plot** |  |
| Favoured (%) | 96.19 |
| Outliers (%) | 0.08 |

**Supplementary Table 2 | Single amino acid substitutions in SPP1 gp6 impairing specifically viral DNA packaging.** Their position and structural effects in elements of gp6 in the connector are presented. The phenotype associated to each mutation previously reported is also described. The phenotypes strength is based on results of a trans-complementation assay: very strong < 2 %; strong 2-5 %; moderate 5-10 % complementation.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mutation** | **Group** | **Structural effect** | **Phenotype** |
| Tyr52Cys | *β*-hairpin anchor | Buried, intrachain H-bond to Asp58. Local destabilization by mutation. | Very strong phenotype, no DNA packaging1. |
| Asp58Asn | Exposed to the portal exterior, intrachain H-bond to Tyr52. | Strong phenotype, no DNA packaging1. |
| Asn82Asp | Interchain bonds to Asn266 in helix α3, possibly positioning the *β*-hairpin relative to the stem. | Strong phenotype, no DNA packaging1. |
| His89Tyr | Buried. Local destabilization by mutation. | Very strong phenotype, no DNA packaging1. |
| Lys96Arg | Buried. Local destabilization by mutation. | Strong phenotype, no DNA packaging1. |
| Gly153Arg | wing | Facing Lys232 and Gly233 main chain. Local destabilization by mutation. | Very strong phenotype, no DNA packaging1. |
| Gly153Ala | Facing Lys232 and Gly233 main chain. Local destabilization by mutation. | Strong phenotype, no DNA packaging1. |
| Ala180Thr | Buried. Local destabilization. | Moderate phenotype, low DNA packaging efficiency1. |
| Phe198Leu | Intrachain salt bridge to Arg182, H-bond to Tyr200. Local destabilization. | Strong phenotype, no DNA packaging1. |
| Asn290Thr | clip interface | Exposed at the gp6 bottom, interchain bond to Val311. Interacts with gp15: main chain H-bond to gp15 Arg98 and side chain H-bond to gp15 Asn95. | Very strong phenotype, no DNA packaging1. No DNA packaging in vitro, no stimulation of gp2 ATPase activity2 but interacts with gp23 . |
| Gly293Arg | Exposed at the gp6 bottom. Interacts with gp15: main chain H-bond to carbonyl of gp15 Arg98. | Strong phenotype, low DNA packaging efficiency1,4. |
| Glu294Lys | Exposed at the gp6 bottom. Interchain H-bond of its main chain with Lys310 but mutation does not disrupt bond. Interacts with gp15: side chain bond to gp15 Met100 . | Very strong phenotype, no DNA packaging1. No stimulation of gp2 ATPase activity2. |
| Glu294Gly | Exposed at the gp6 bottom. Interchain H-bond of its main chain with Lys310 but mutation does not disrupt bond. Interacts with gp15: side chain bond to gp15 Met100. Mutation disrupts this interaction. | Very strong phenotype1. Packaged DNA leaks from capsid. No interaction with gp154. |
| Pro325Leu | clip hinge | Hinge position of the clip. Interchain van der Waals contacts with Arg281 to Glu283 | Strong phenotype, no DNA packaging1. |
| Val347Met | tunnel loop | Buried facing the bend of helix α6. Local destabilization. Mutation might displace tunnel loop. | Moderate phenotype, no DNA packaging1. |
| Ser350Pro | Exposed to the tunnel in the tunnel loop. Side chain H-bond to Asp348 (intrachain). Mutation disrupts this bond leading to a possible destabilization of the gp6 tunnel helix. | Strong phenotype, no DNA packaging1. |
| Glu352Gly | Residue in the tunnel loop helix. Mutation to glycine could affect the helix stability. | Moderate phenotype, low DNA packaging efficiency in vivo and in vitro1,2. Reduced stimulation of gp2 ATPase activity2 but interacts with gp23. |
| Gly360Val | At the N-ter of helix α6. Substitution by a bulkier residue might affect position of helix α7 the lines the portal tunnel in the crown region. | Moderate phenotype, no DNA packaging1,4. |
| Lys373Glu | Near the helix α6 distortion position. Side chain H-bond with the carbonyl of Ala344. Mutation disrupts this interaction and may lead to displacement of the tunnel loop. | Strong phenotype, no DNA packaging1. |
| Ser428Gly | crown | Establishes helices α7- α7 intersubunit bonding: side chain H-bonds with Ser423 and Gln427 (interchain). Mutation disrupts both contacts. | Strong phenotype, no DNA packaging1. |
| Ser428Asn | Establishes helices α7- α7 intersubunit bonding: side chain H-bonds with Ser423 and Gln427 (inte5rchain). Mutation disrupts both contacts. | Moderate phenotype, no DNA packaging1. |
| Ile437Val | Buried in a hydrophobic pocket build together with Val426, Val430 and Val444 of the neighbour subunit, Mutation disturbs crown intersubunit interactions. | Moderate phenotype, no DNA packaging1. |
| Ala443Thr | Buried in the hydrophobic core of the crown. Mutation probably disturbs the domain. | Strong phenotype, no DNA packaging1. |
| Phe449Leu | Found in a hydrophobic interface with Leu429 and Arg446 of the neighbour interface. Mutation disturbs crown intersubunit interactions. | Strong phenotype, no DNA packaging1 |

**References**

1. Isidro, A., Santos, M. A., Henriques, A. O. & Tavares, P. The high-resolution functional map of bacteriophage SPP1 portal protein. *Mol. Microbiol.* **51**, 949–962 (2004).

2. Oliveira, L., Henriques, A. O. & Tavares, P. Modulation of the viral ATPase activity by the portal protein correlates with DNA packaging efficiency. *J. Biol. Chem.* **281**, 21914–21923 (2006).

3. Oliveira, L., Cuervo, A. & Tavares, P. Direct Interaction of the Bacteriophage SPP1 Packaging ATPase with the Portal Protein. *J. Biol. Chem.* **285**, 7366–7373 (2010).

4. Isidro, A., Henriques, A. O. & Tavares, P. The portal protein plays essential roles at different steps of the SPP1 DNA packaging process. *Virology* **322**, 253–263 (2004).

**Supplementary Table 3 | Intersubunit contacts in the SPP1 connector complex**

**gp6 - gp15 bonding**

|  |  |  |
| --- | --- | --- |
| **gp6 residue, atom** | **gp15 residue (chain), atom** | **type of interaction** |
| D68, O | R102 (*j+2*), Nh1 | hydrogen bond |
| N290, O | R98 (*j*), Ne | hydrogen bond |
| N290, Od1 | N95 (j), Nd2 | hydrogen bond |
| D292, N | R98 (*j*), O | hydrogen bond |
| D292, Od2 | H37 (*j+1*), Ne2 | salt bridge |
| G293, N | R98 (*j*), O | hydrogen bond |
| E294, Oe1 | M100 (*j*), N | hydrogen bond |
| E298, Oe1 | R102 (*j*), Nh1 | salt bridge |
| E298, Oe1 | R102 (*j*), Nh2 | salt bridge |
| N302, Od1 | R102 (*j*), Ne | hydrogen bond |
| K310, Nz | R102 (*j*), O | hydrogen bond |
| S312, O | R98 (*j*), Nh2 | hydrogen bond |

**Supplementary Table 3 (cont.) | Intersubunit contacts in the SPP1 connector complex**

**gp15 - gp15 intersubunit bonding**

|  |  |  |
| --- | --- | --- |
| **gp15 chain *j*, residue, atom** | **gp15 chain *j-1*, residue, atom** | **type of interaction** |
| R5, Ne | E23, Oe1 | salt bridge |
| R5, Ne | E23, Oe2 | salt bridge |
| R8, Ne | E23, Oe2 | salt bridge |
| R8, Nh2 | E23, Oe2 | salt bridge |
| K59, Nz | Y64, O | hydrogen bond |
| K59, Nz | T66, O | hydrogen bond |
| Q62, Oe1 | A68, N | hydrogen bond |
| T77, O | D76, N | hydrogen bond |
| S79, N | S74, O | hydrogen bond |
| S79, O | S74, N | hydrogen bond |
| N81, N | G72, O | hydrogen bond |
| N81, O | T71, N | hydrogen bond |
| N81, O | T71, Og1 | hydrogen bond |
| N81, O | G72, N | hydrogen bond |
| A83, N | G69, O | hydrogen bond |
| S88, N | E85, Oe1 | hydrogen bond |
| S88, N | E85, Oe2 | hydrogen bond |
| S88, Og | E85, Oe1 | hydrogen bond |
| T89, N | E85, Oe1 | hydrogen bond |
| T89, Og1 | E85, Oe1 | hydrogen bond |
| T89, Og1 | I86, N | hydrogen bond |
| Y97, Oh | D34, Od2 | hydrogen bond |

**Supplementary Table 3 (cont.) | Intersubunit contacts in the SPP1 connector complex**

**gp15 - gp16 bonding**

|  |  |  |
| --- | --- | --- |
| **gp15 residue (chain), atom** | **gp16 residue, atom** | **type of interaction** |
| M65 (*j-1*), Sd | M1, N | hydrogen bond |
| N67 (*j-2*), Nh1 | Y2, O | hydrogen bond |
| R73 (*j-1*), Nh1 | M3, O | hydrogen bond |
| Y80 (*j*), Oh | E4, Oe1 | hydrogen bond |
| M75 (*j-1*), O | Q39, Oe1 | hydrogen bond |
| T77 (*j-1*), N | Q39, Oe1 | hydrogen bond |
| T77 (*j-1*), Og1 | Q39, Ne2 | hydrogen bond |
| D76 (*j-2*), Od2 | Q43, N | hydrogen bond |
| T77 (*j*), Og1 | Y61, Oh | hydrogen bond |
| T77 (*j+1*), Og1 | S91, Og | hydrogen bond |
| R73 (*j*), Nh1 | E95, Oe1 | salt bridge |
| R73 (*j*), Nh2 | E95, Oe1 | salt bridge |
| D76 (*j*), Od2 | R98, Nh1 | salt bridge |
| T77 (*j*), Og1 | R98, Nh1 | hydrogen bond |
| D76 (*j*), Od2 | R98, Nh2 | salt bridge |

**gp16 - gp16 intersubunit bonding**

|  |  |  |
| --- | --- | --- |
| **gp16 chain *k*, residue, atom** | **gp16 chain *k-1*, residue, atom** | **type of interaction** |
| H37, Ne2 | S91, O | hydrogen bond |
| Q43, Ne2 | E45, Oe2 | hydrogen bond |
| Q43, Ne2 | N59, Od1 | hydrogen bond |
| Q43, Oe1 | Y61, Oh | hydrogen bond |
| Q43, Oe1 | R98, Nh1 | hydrogen bond |
| Y47, Oh | E45, Oe2 | hydrogen bond |
| R77, Nh1 | G92, O | hydrogen bond |

**Supplementary Table 4 | Surface, energetics and bonding of connector interchain interfaces**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **subunit 1** | **subunit 2** | **surface (Å²)** | **ΔG**  **(kcal/mol)** | **number of hydrogen bonds** | **number of salt bridges** |
| gp6 (*i*) | gp6 (*i+1*) | 3814.9 | -38.8 | 43 | 11 |
| gp6 (*i*) | gp6 (*i+2*) | 367.0 | -3.4 | 4 | 0 |
| gp6 (*i*) | gp15 (*j-1*) | 27.7 | 0.0 | 0 | 0 |
| gp6 (*i*) | gp15 (*j*) | 526.4 | 0.2 | 8 | 2 |
| gp6 (*i*) | gp15 (*j+1*) | 77.0 | -0.8 | 0 | 1 |
| gp6 (*i*) | gp15 (*j+2*) | 82.3 | -0.4 | 1 | 0 |
| gp15 (*j*) | gp15 (*j*) | 1239.3 | -13.6 | 18 | 4 |
| gp15 (*j-2*) | gp16 (*k*) | 64.8 | -0.9 | 0 | 0 |
| gp15 (*j-1*) | gp16 (*k*) | 472.5 | -8.2 | 6 | 0 |
| gp15 (*j*) | gp16 (*k*) | 307.0 | -3.5 | 4 | 3 |
| gp15 (*j+1*) | gp16 (*k*) | 143.6 | -1.1 | 2 | 0 |
| gp15 (*j+2*) | gp16 (*k*) | 29.3 | 0.7 | 0 | 0 |
| gp16 (*k*) | gp16 (*k+1*) | 512.7 | -1.6 | 7 | 0 |