Jenny Maki
Biomolecular Structures
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gpD – the Capsid-Stabilizing Protein of Bacteriophage λ

Bacteriophage λ (λ phage) is a double-stranded DNA virus, which infects bacteria. During infection the λ phage can either become incorporated into the bacterial genome or multiple in number until bacterial cell lysis (1). The genome of the λ phage is packaged into capsids (heads), which is composed of two proteins gpD (11.4 kD) and gpE (40 kD). GpD is not an essential protein for λ phage propagation but is needed for complete genome packaging (only ~85% of the genome can be packaged without gpD). The capsid of λ phage consists of 415 copies of gpE and 405-420 copies of gpD (2).

The cryo-EM structure at 15Å resolution of the capsid revealed that gpD was trimeric (2). Electron cryomicroscopy (cryo-EM) is a structure determination technique for solving structures of large macromolecular assemblies. A crystalline order is not needed for the studied object. This unique capability makes cryo-EM extremely valuable in imaging and solving the structures and the dynamics of macromolecular machines (3). Cryo-EM provides complementary information to the other methods such as NMR and crystallography, with the added feature of being able to tackle very large assemblies and transient or mixed species, and usually requires small amounts of material. Although most of the structures so far determined are not at atomic resolution (best resolution I found was 6Å), cryo-EM information can be combined with partial atomic structure detail to give new detailed information on large complexes. Additionally, cryo-EM is a powerful tool for studying conformational changes in large assemblies such as the GroEL/ES (4). In preparing for cryo-EM, the complex is immersed in water and then immediately froze in super cold liquid ethane. The complex is imprisoned in vitreous ice, a glassy non-crystalline form of ice, which preserves its native structure. Low-intensity

beam EM avoids damaging the molecules and allows for the imaging of thousands of captive protein complexes. Computer image analysis produces detailed, three-dimensional maps from the images produced by the EM (5).

The subsequent crystal structure of gpD was solved at 1.1Å resolution and was also determined to be trimeric as shown in Figure 1. The first 14 amino acids of this protein were not resolved in the crystal structure and the overall fold of this protein is novel. The individual monomers posses a very low content of regular secondary structure as illustrated in Figure 2. About 65% of the protein is composed of coils (shown in green in Figure 2) and 27% is made up of β-sheets (shown in yellow in Figure 2) but even these sheets are unusual since none of the sheets are longer than 5 residues. The remaining 8% of the structure is α-helix (shown in red in Figure 2). Another striking feature of this protein is that there are a small number of hydrogen bonds even between the β-sheets. Sheet 1 and sheet 5 are only connected by four hydrogen bonds. The hydrophobic core of gpD consists of Leu 27, Ala 31, Pro 36, Leu 37, Trp 49, Ala 57, Ile 60, Leu 72, Phe 80, Val 85, Trp 87, Ala 100, Phe 101, Ile 106, and Ile 108. These residues form a tight core, which does not have any cavities. These hydrophobic residues are highlighted in blue and represented as spheres in Figure 3. The three monomers come together due to the interaction of hydrophobic residues. These residues are the primary interactions except for a few hydrogen bonds (2). These interactions are illustrated in Figure 4.

Additionally, the trimers have a 'top' side, which is slightly concave and contains a central cavity at the center, which is solvent accessible. On the 'bottom' side of the trimer there are 10 hydrophobic residues most of which are also conserved in the gpD homolog SHP. These hydrophobic residues, which are shown as blue spheres in Figure 5

are proposed to interact with the hexamers and pentamers of gpE. An additional feature of the 'bottom' side of the trimer is a ring formed by the presence of two ring-containing residues, Pro 17 and His 19. One of these residues from one monomer interacts with the other residue on another monomer and may aid in stabilizing the orientation of the neighboring residues. Another stabilizing factor in this same region is the salt bridge between Asp 16 on one monomer and Lys 79 on the neighboring monomer (2). The Pro-His interaction and the Asp-Lys interactions are depicted in Figure 6.

Using the density map generated from the crystal structure and comparing it to the density map generated from the cryo-EM structure, it was determined that the trimeric structure is indeed the physiologically relevant structure.

Since the initial crystal structure of gpD, there has been another crystal structure of gpD solved at a lower resolution, 1.8Å. This crystal structure was solved at a lower resolution in order to resolve the N-terminal amino acids of the protein but was a truncated protein lacking the first amino acid. Unfortunately, this crystal structure also had these amino acids unresolved (6).

Recently, the solution NMR structure of gpD was solved from 20 structures. This structure has the first 14 amino acids of gpD resolved. In this structure these residues point away from the rest of the structure (7). This structure is shown in Figure 7 and alignment of the two crystal structures and the NMR structure are shown in Figure 8. The main differences between the structures are the N-terminal residues are resolved in the NMR and residues 15-17 are in different positions and also two of the β-sheets from the crystal structures are shown as extended structures in the NMR structure.

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SHP shares over 50% identity with gpD. It also has a similar fold to gpD as shown from the crystal structure. In folding experiments showed that SHP does not possess a high thermodynamic stability but does have a high kinetic stability. Kinetic stability is due to an energy barrier that exists between the folded and unfolded states. This stability is important for protein stability and plays a major role in many biological functions. Virus particles, as in SHP or gpD, must endure the harsh conditions of the extracellular environment before infecting a new host. Therefore, SHP possess a high kinetic stability with an unfolding half-life of 25 days at 25°C. If the His from the Pro-His ring is deleted from SHP then the protein stability is decreased dramatically. The folding pathway of SHP is $3D \leftrightarrow 3M \leftrightarrow N_3$ where D represents the unfolded monomer, M is the monomeric intermediate and N is the native trimeric protein (8).

The gpD protein from bacteriophage λ possesses a novel fold, which has a low content of regular secondary structure, but the first 14 residues were disordered in the crystal structure. GpD has a low content of intermolecular hydrogen bonds and the oligomeric status of gpD has been determined to be trimeric by both crystallography and cryo-EM. The trimer interface on each monomer is mostly composed of hydrophobic interactions and also the proposed site of interaction with the gpE hexamer and pentamers are conserved hydrophobic residues found on the 'bottom' of the trimeric structure. The functional homolog of gpD, SHP has been shown to possess a high kinetic stability, which would correspond to the need for the virus to survive the harsh conditions of the extracellular environment until a new host can be infected. Interestingly, GpD can also be used as a fusion protein in phage display assays, an experiment method for the detection of interacting proteins.

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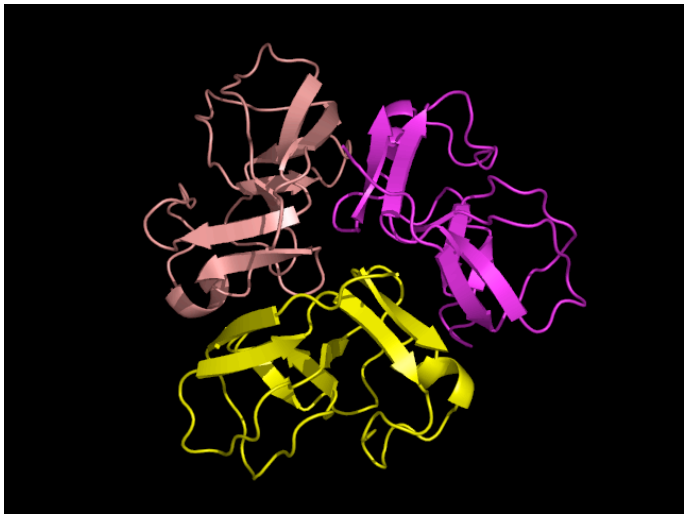

Figure 1: Trimeric crystal structure of gpD. Each monomer of gpD is represented by a different color. The image was constructed in PyMOL with PBD ID: 1C5E. **PyMOL Presentation 1-Scene F1**

Figure 2: Secondary Structure of gpD. The coils and loops are represented in green, the β-sheets are represented in yellow, and the α-helix is represented in red. Note that the majority of the protein is coils and loops. PDB ID 1C5E. **PyMOL Presentation 1-Scene F2**

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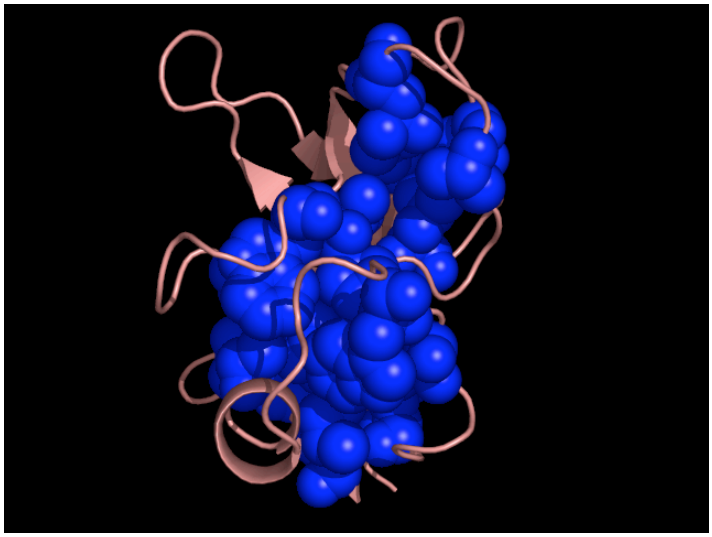

Figure 3: The Hydrophobic Core of gpD. The residues, which make up the hydrophobic core of gpD are shown as blue spheres. Notice that these residues form a tight core without any cavities. PDB ID 1C5E. **PyMOL Presentation 1-Scene F4**

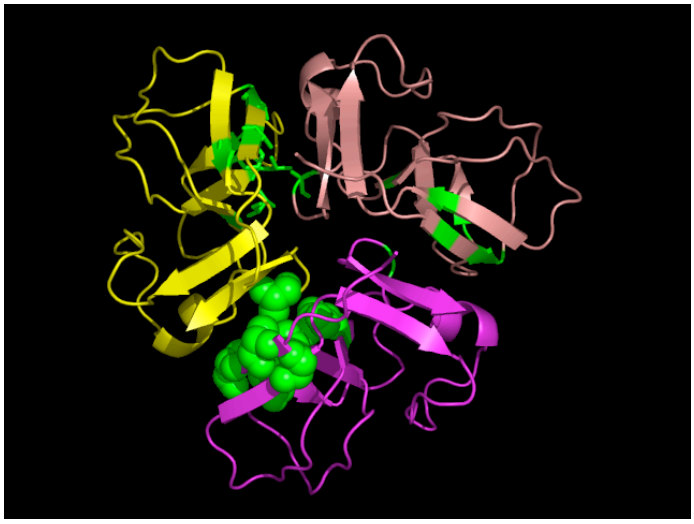

Figure 4: The Trimer Interaction Sites. Each monomer is tightly associated to its neighboring monomer through the interaction of hydrophobic surface residues. The residues are painted green on structure. The yellow monomer shows the side chain interactions while the purple monomer shows the interactions by spheres. PDB ID 1C5E. **PyMOL Presentation 1-Scene F6**

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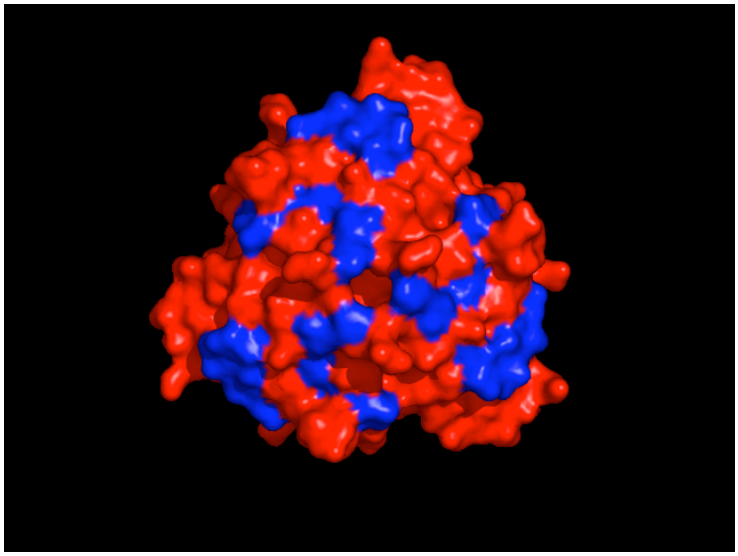

Figure 5: Hydrophobic Residues Involved in gpE Binding. The residues proposed to interact with gpE are shown in blue on this 'bottom' side surface view of gpD. These residues are hydrophobic indicating that the interaction between the two proteins is mostly hydrophobic in nature. PDB ID 1C5E. **PyMOL Presentation 1-Scene F11**

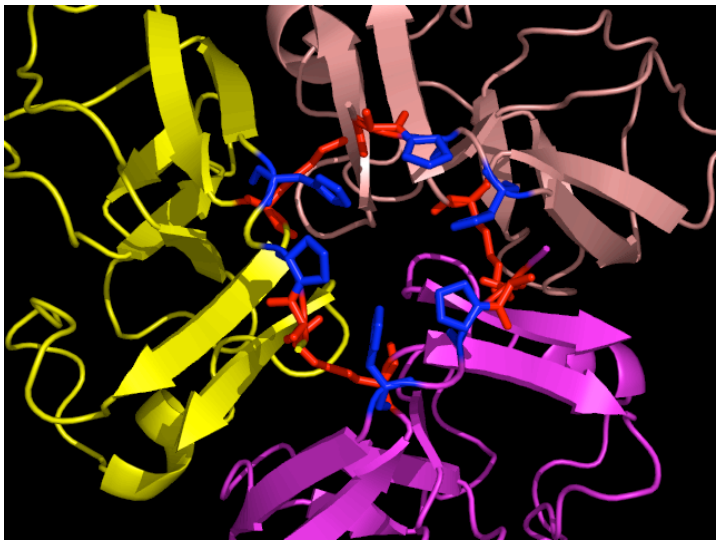

Figure 6: Pro-His Ring and Asp-Lys Salt Bridge. Pro17 and His 19 shown in blue interact with each other on neighboring monomers. Lys 76 and Asp 19 form a salt bridge between two monomers. These interactions may help stabilize the neighboring residues, which form the interface with the neighboring monomer. PDB ID 1C5E. **PyMOL Presentation 2-Scene F2**

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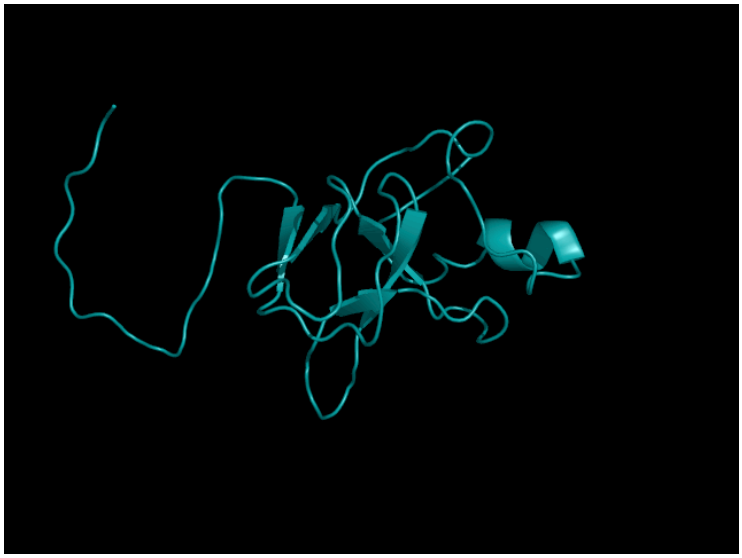

Figure 7: The NMR structure of gpD. The solution NMR structure of gpD, which includes the first 14 amino acids not resolved in the crystal structure. These residues are found away from the rest of the structure (see left hand side) and have a coil structure. PDB ID 1VD0. **PyMOL Presentation 2- Scene F12**

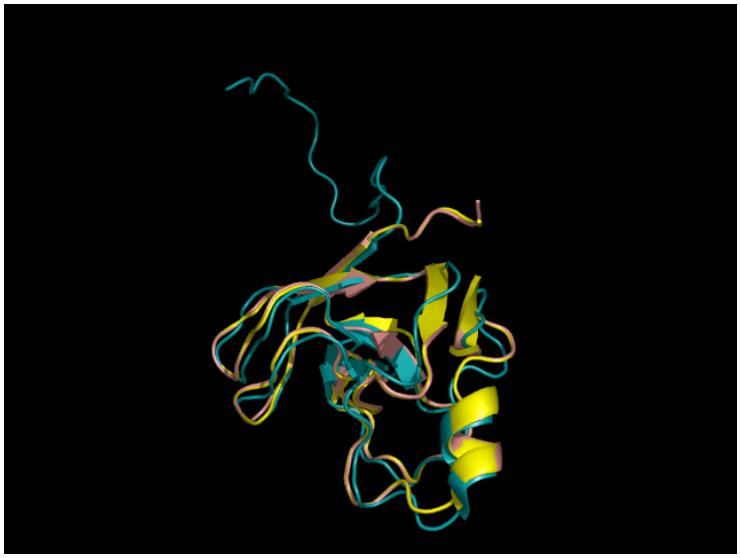

Figure 8: Alignment of Three Structures. Monomers from the two crystal structures (salmon color PDB ID 1C5E, yellow color PDB ID 1TCZ) are aligned with the monomeric NMR structure (PDB ID 1VD0). The structures are similar except for the NMR structure lacks two β-sheets located directly above the α-helix. Additionally the N-terminal residues in the NMR structure are positioned away from the rest of the structure. **PyMOL Presentation- Scene F11**