

gpD, the Capsid-Stabilizing Protein of Bacteriophage Lambda

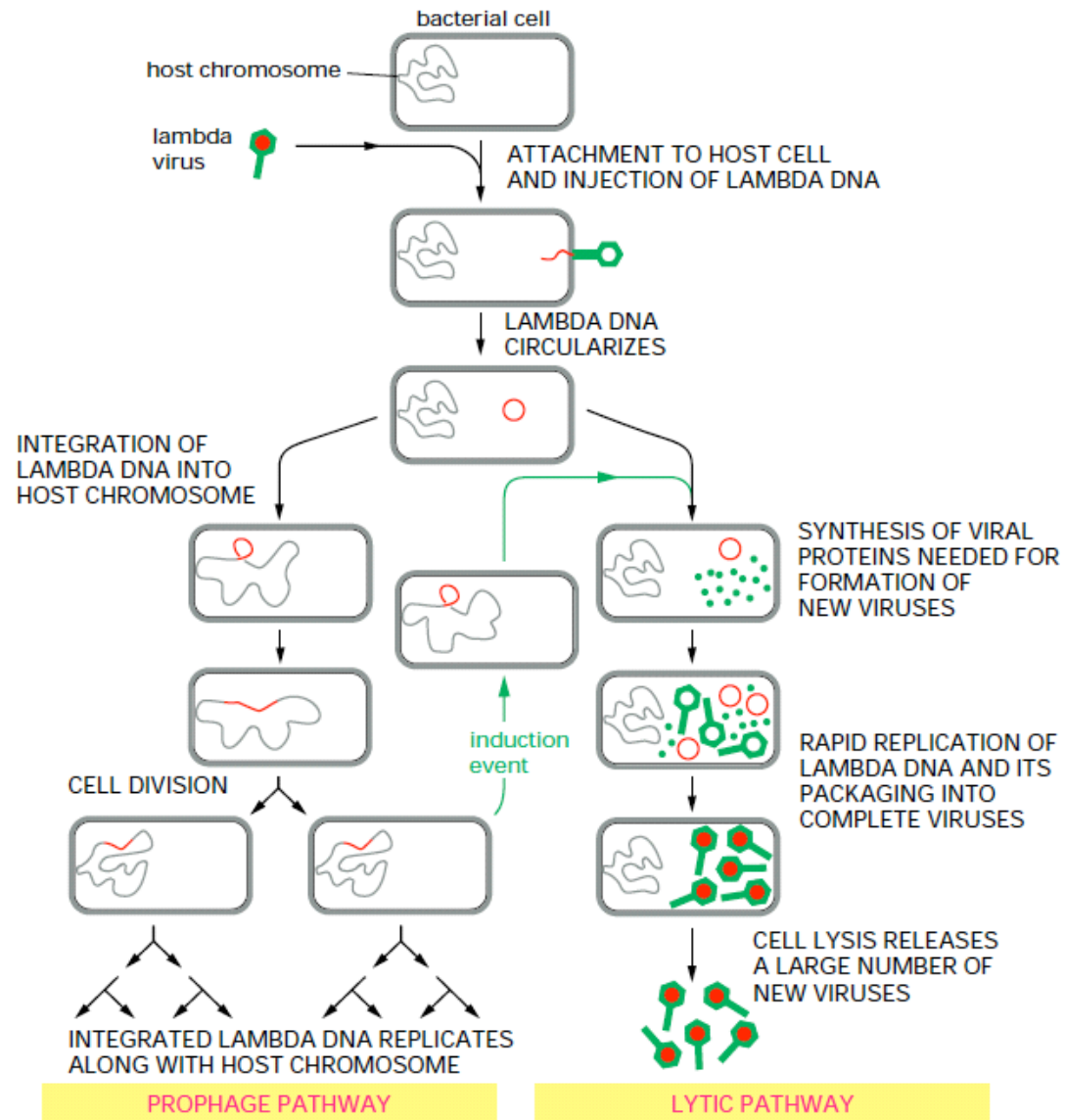
Jenny Lynn Maki

Biomolecular Structure Presentation

November 30, 2005

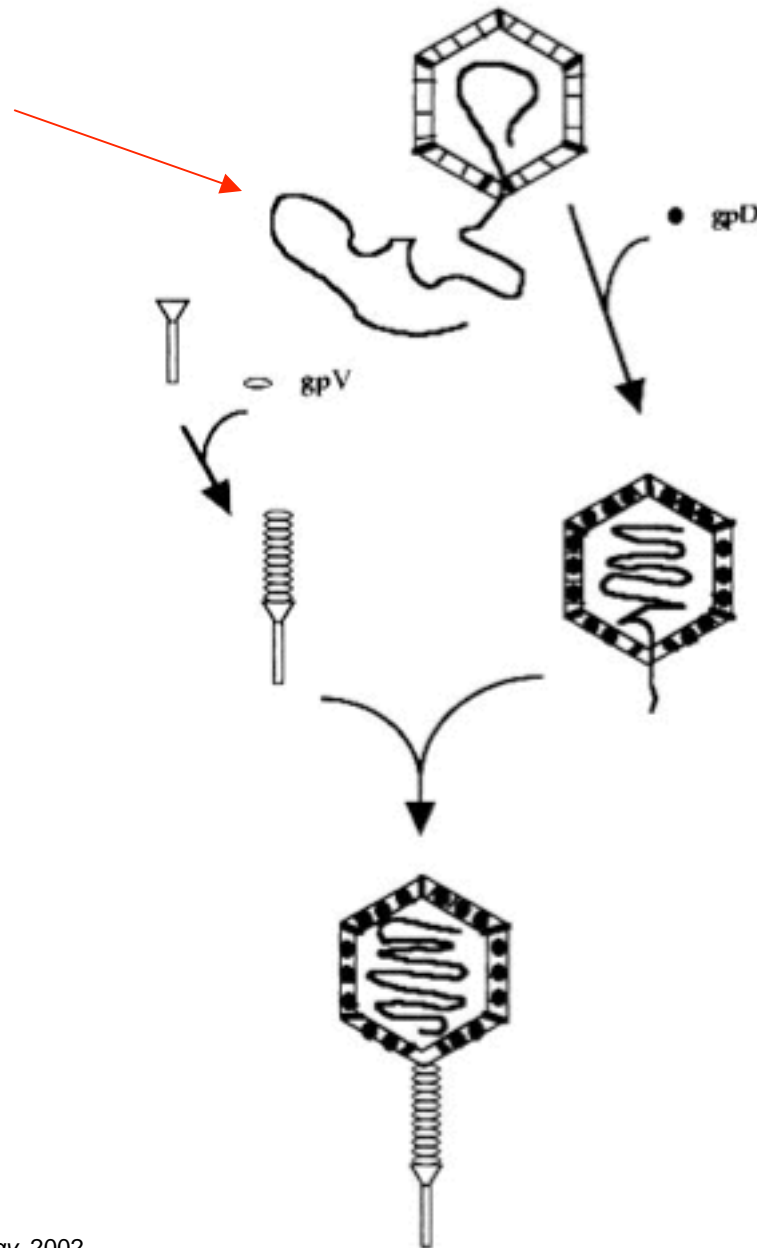
The Life Cycle Of Bacteriophage Lambda

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Bacteriophage Lambda Assembly

dsDNA genome



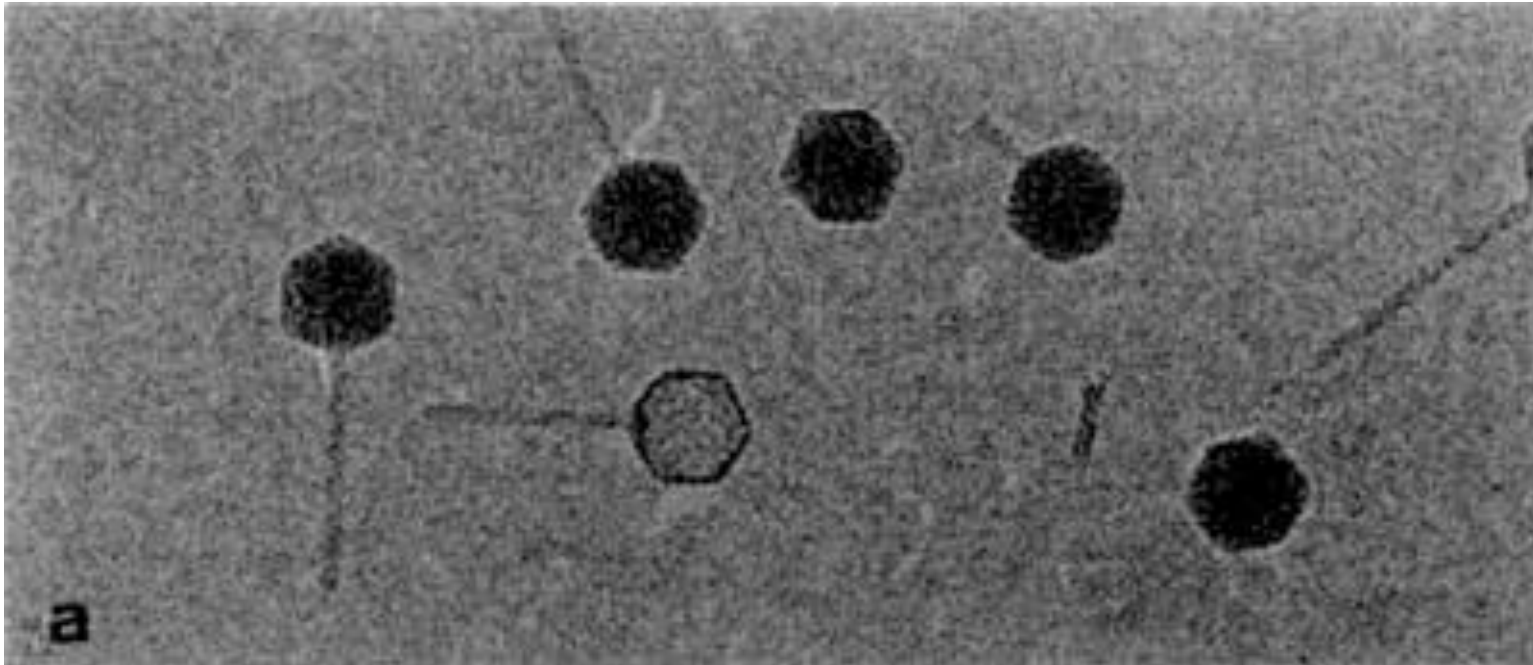
Contains 405-420 copies of gpD
Contains 415 copies of gpE

Overview of Cryo-EM

- 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 (1).
- 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(2).
- In preparing for cryo-EM, the complex is immersed in water and then immediately froze in supercold 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 (3).

1. <http://jiang.bio.purdue.edu/research.php>
2. Saibil, *Acta Crystallogr D Biol Crystallogr*, 2000.
3. <http://www.sciencedaily.com/releases/2005/11/051120234914.htm>

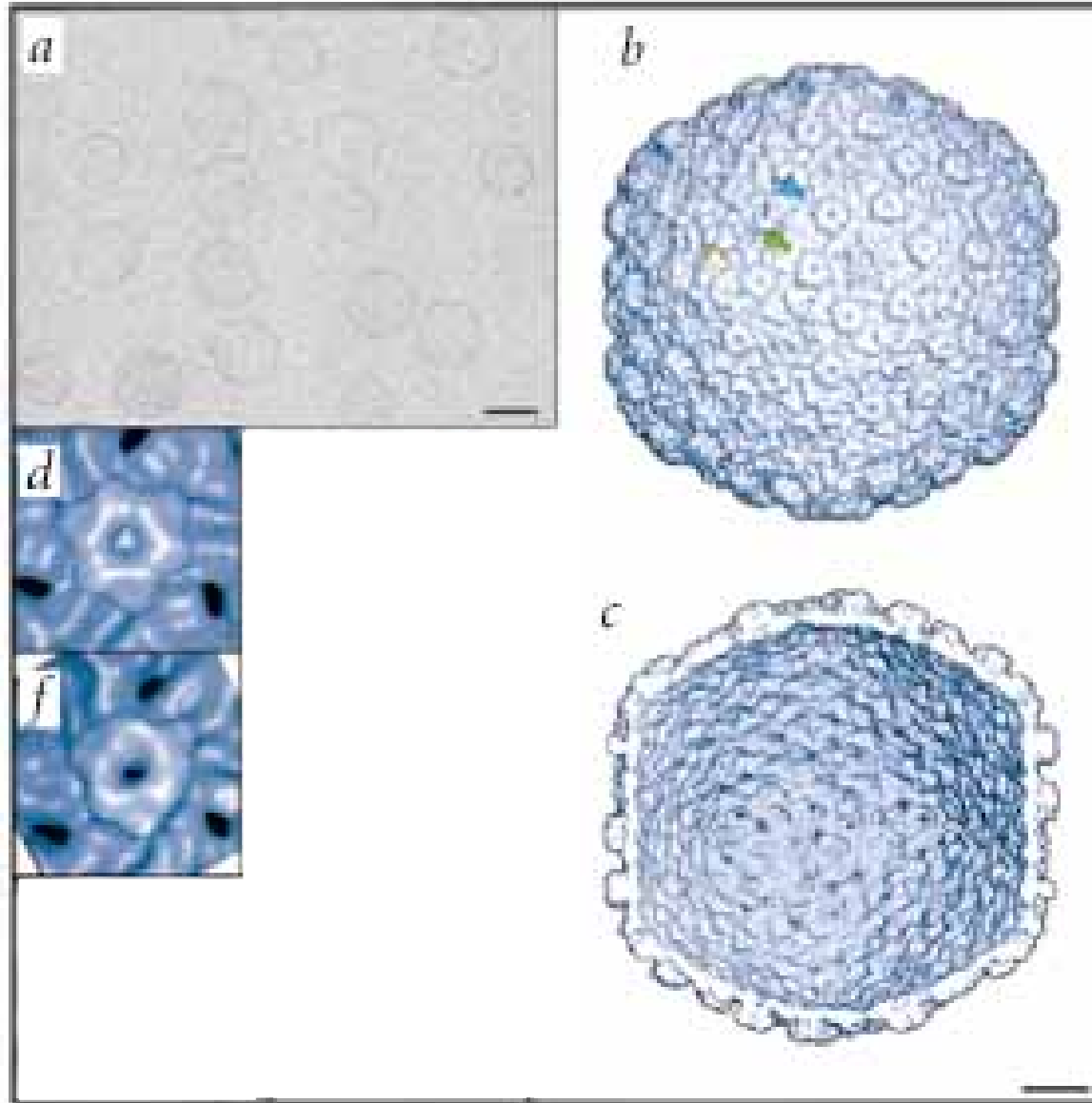
Cryo-EM (Negative Staining) of Bacteriophage Lambda at 34 Å



The lambda capsid is icosahedral - approximately spherical.

Electron Micrographs of Phage λ Capsids at 15 Å

EM of phage λ capsids



Outer surface of the capsid

Enlargement of blue arrow in b

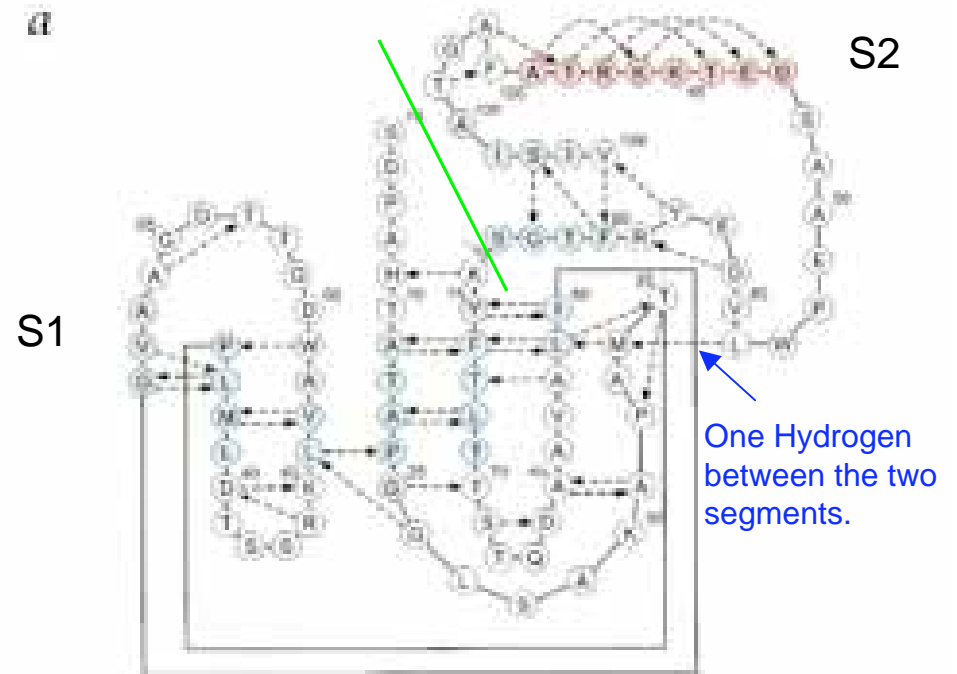
Enlargement of yellow arrow in b

Inner surface of the capsid

Primary and Secondary Structures of gpD

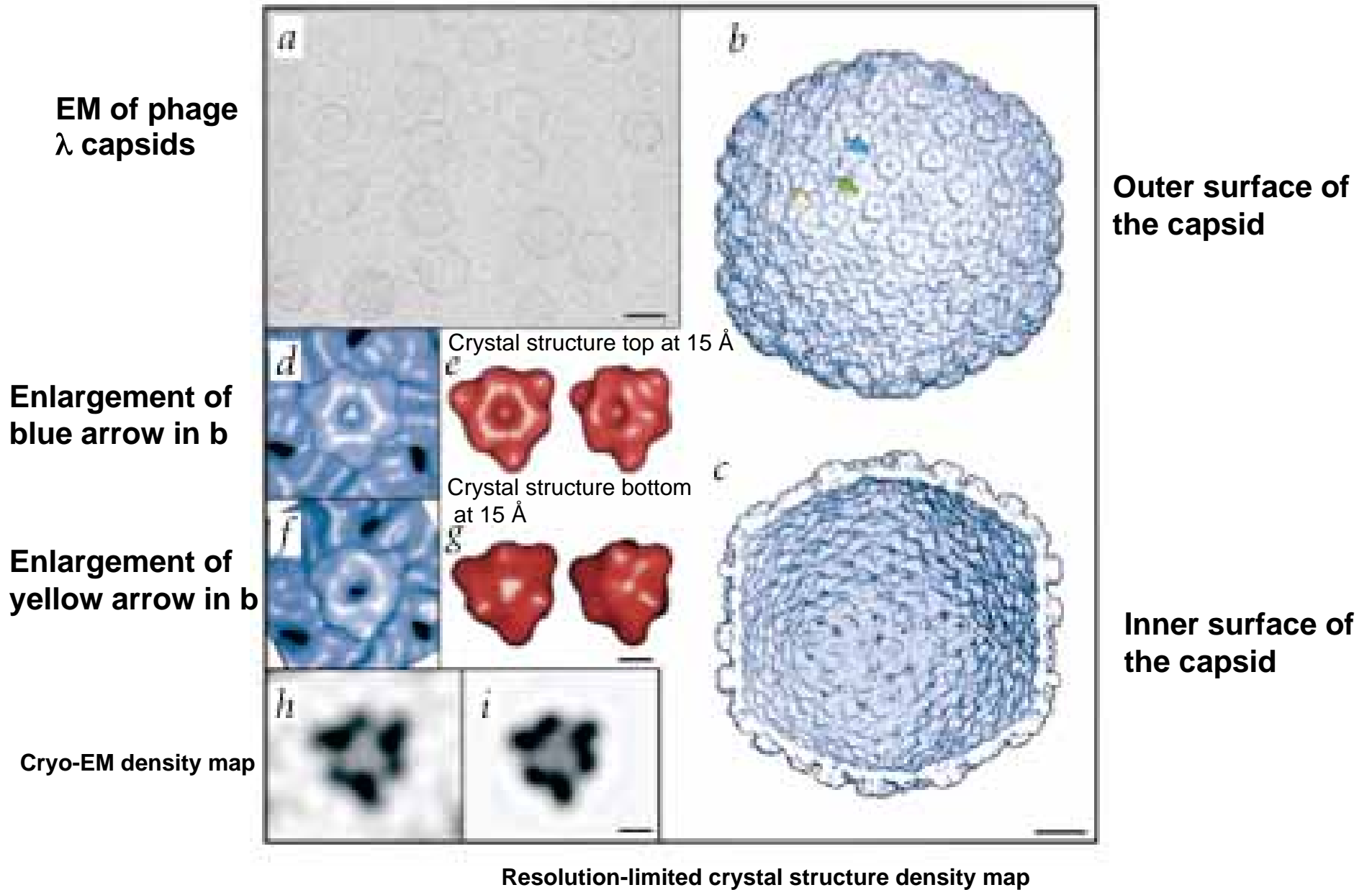
Intramolecular hydrogen bonds in gpD
Blue colored residues are β -strands
Red colored residues are α -helix

-----> Main chain H-bonds from
amide nitrogen atoms to
carbonyl oxygen atoms



PyMOL Presentation of gpD

Electron Micrographs of Phage λ Capsids at 15 Å



Alignment of gpD and SHP Primary Protein Sequences

```

gpD      ---TSKETFTHYQPQNSDPAHTATAPGGLSAKAPAMTPLMLDTSSRKLVAWDGTTDGAA
SHP      VTKTITEQRAEVRIFAGNDPAGTATGSSGISSPTPALTPMLMLDEATGKLVVWDGQKAGSA
          *  .*   :.  :   ...***  **  ...*:*:  :*:*****  ::  ***  .***  .  *:*
    
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gpD      VGILAVAADQTSSTLTFYKSGTFRYEDVLWPEAASDETKKRTAFAGTAISIV---
SHP      VGILVLPLEGTEETALTYKSGTFATEAIHWPEV-DEHKKANAFAGSALSHAALP
          ****  .:.  :  *.*:*:*:*****  *  :  ***:.  **  **  .*****:*:*  .
    
```

* = single, fully conserved residue
 : = conservation of strong groups
 . = conservation of weak groups

Interesting Aspects of SHP Folding

- 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.
- Kinetic 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.
- SHP possess a high kinetic stability with a unfolding half life of 25 days at 25°C.
- If the His from the Pro-His ring is deleted 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.

Conclusions

- The gpD protein from bacteriophage λ possess 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.
- 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.
- The proposed site of interaction with the gpE hexamer and pentamers is the hydrophobic conserved 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.
- gpD can also be used as a fusion protien in phage display assays.