

# T4ファージ溶菌制御タンパク質 gp61.3の立体構造解析

生物プロセス・助教

金丸周司

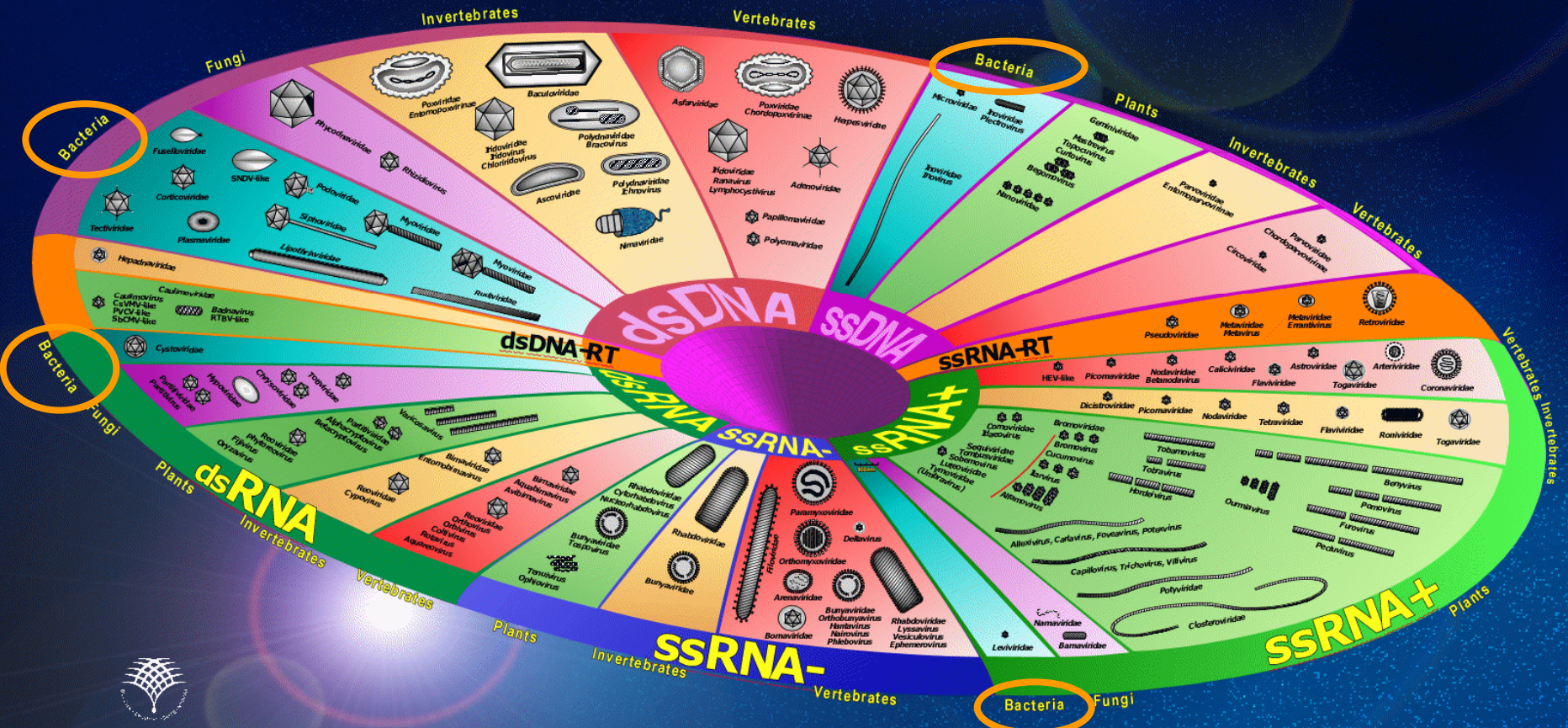
派遣先: EPFL, Petr Leiman研究室

2009. Jul. 15 ~ Oct. 15

# Today's talk

- Crystal structure determination of gp61.3 from bacteriophage T4.
- Life in Lausanne.

# Virosphere 2002



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PLANT SCIENCE CENTER  
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International Committee on Taxonomy of Viruses

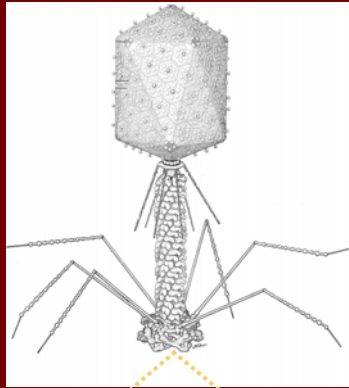


# Environment and phage

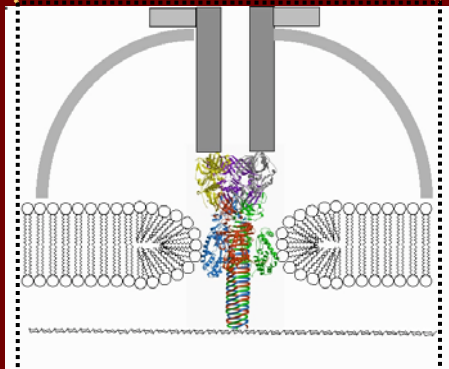
- Several phages can infect one bacterium.
- $10^5 \sim 10^7$  phages are found in 1mL of sea water.
- Phages are most biggest biomass in the world.
- Phage might control the whole environment?

# Cell puncturing device gp5

T4 phage

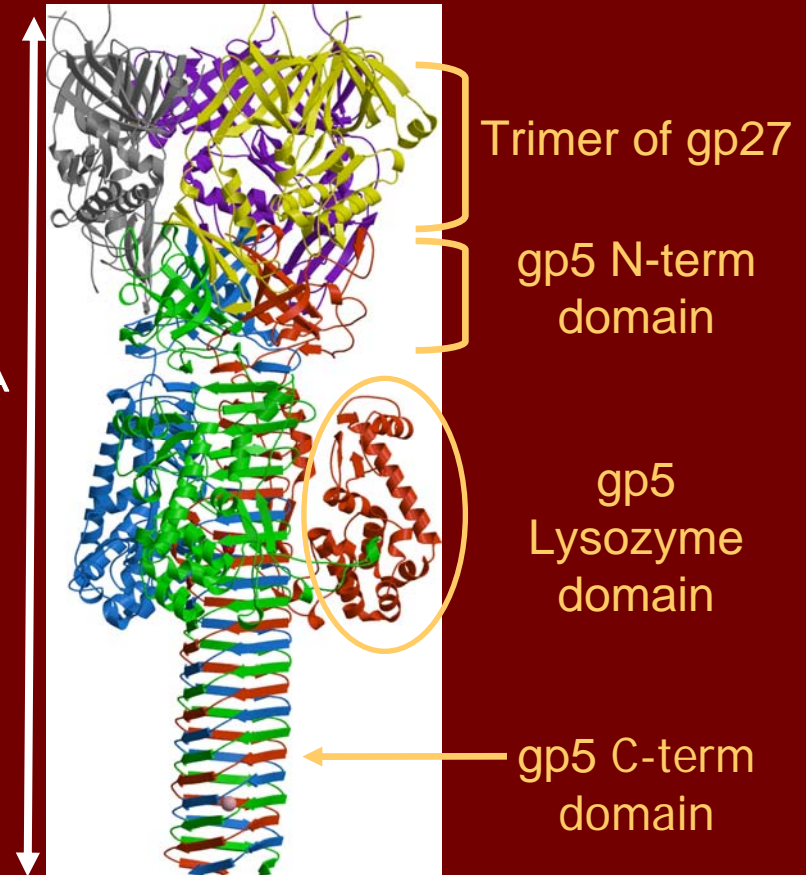


gp5 functions as cell puncturing device



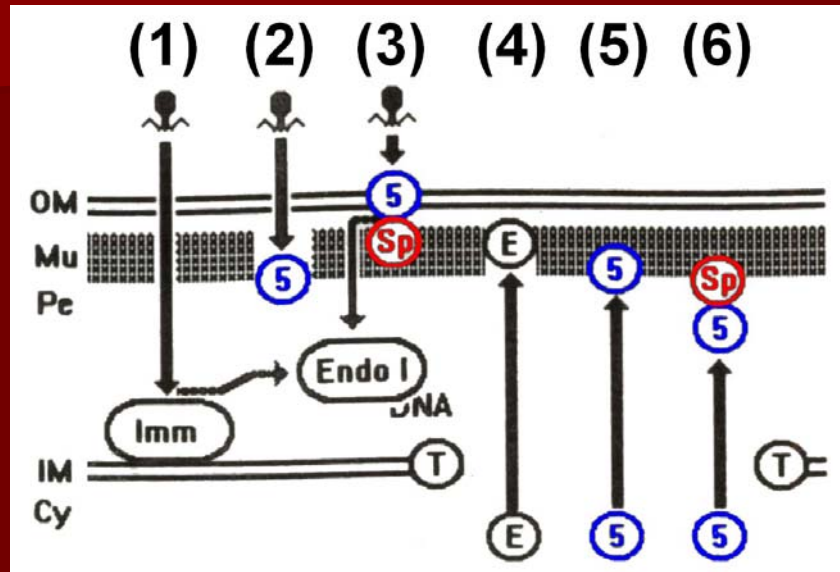
Outer membrane  
Peptidoglycan

190 Å



Kanamaru *et al.* Nature (2002)

# Proteins related to lysis



Sp = gp61.3

- (1) Imm protein perhaps inhibits the interaction of secondary phage with inner membrane
- (2) gp5 puncture both outer membrane and peptidoglycan layer while infection.
- (3) Sp protein inhibits gp5 lysozyme activity while multiple infection takes place.
- (4) gpE goes into periplasm through the hole of gpT and digest peptidoglycan layer when daughter phage is ready to release.
- (5) Free gp5 in cytoplasm also goes into periplasm through the hole of gpT and digest peptidoglycan layer..
- (6) Sp protein also inhibits newly produced gp5 lysozyme activity.

Phage proteins : Imm, 5, Sp, T, E  
*E. Coli* protein : Endo I (endonuclease I)

# gp61.3 inhibits gp5 lysozyme

## Lysozyme activity assay by spot test

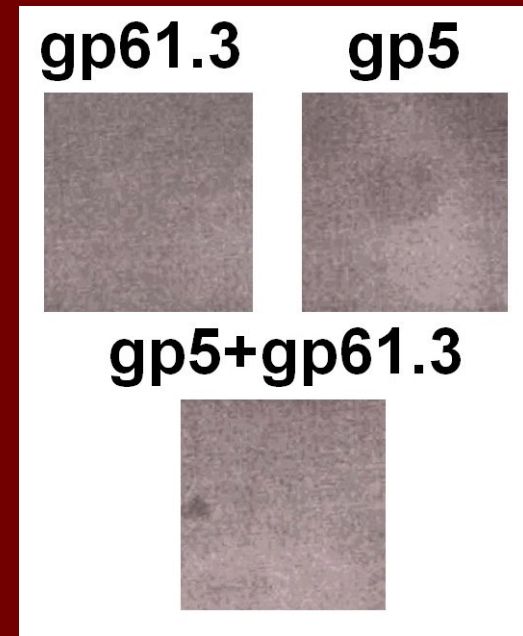
Samples are spotted on chloroform vapor treated *E. coli* layer on LB soft agar plate. If the sample has lysozyme activity, the spot is forming transparent “Halo” on the plate.

## Results

Purified gp5 -> making halo (top right)

Purified gp61.3 -> no halo was observed. (top left)

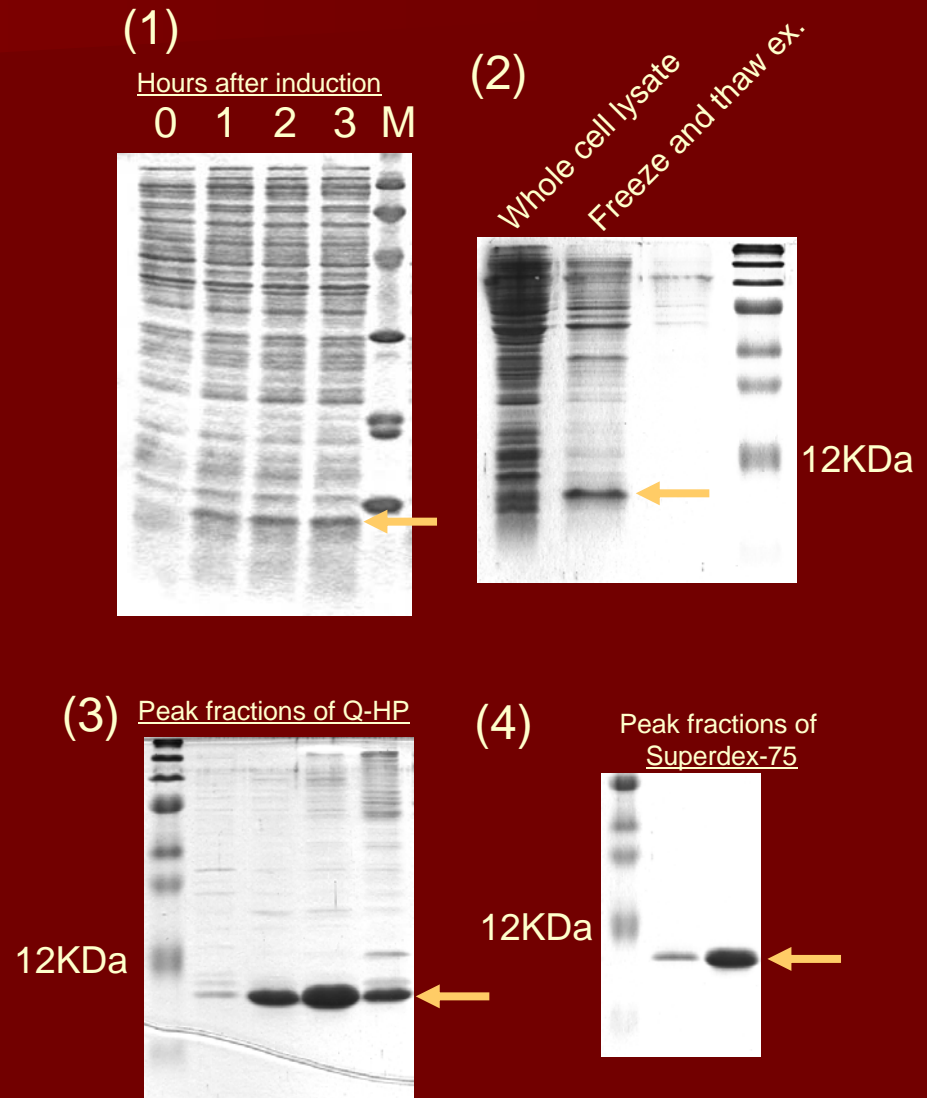
gp5 + gp61.3 -> no halo was observed. (bottom)



**gp5 lysozyme activity was inhibited by gp61.3.**

# Purification of over-expressed gp61.3

1. Gene 61.3 was amplified and cloned into pUC19 vector and the gene product was expressed in *E. coli* BL21 with IPTG induction at 37 °C.
2. Over-expressed gp61.3 was extracted by freeze and thaw.
3. The extracted sample was applied to HiTrap Q-HP anion exchange column. Then gp61.3 was eluted by NaCl gradient.
4. The peak fractions contains gp61.3 from Q-HP column was pooled and purified by Superdex-75 gel filtration column.





# Crystallization of gp61.3



gp61.3 can be crystallized by hanging drop vapor diffusion with:



~1000 conditions were tested.

# Data collection

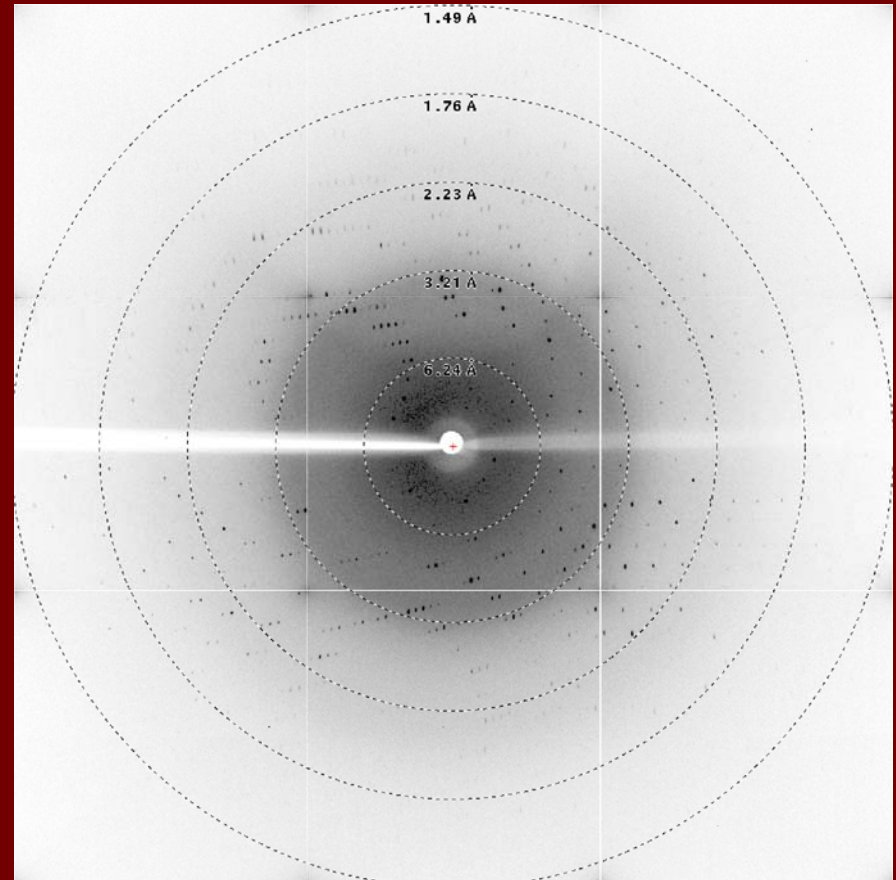


Both SeMet and Native data set was collected at ESRF beam line BM30.

Crystals diffract  $\sim 1.5\text{\AA}$  resolution .

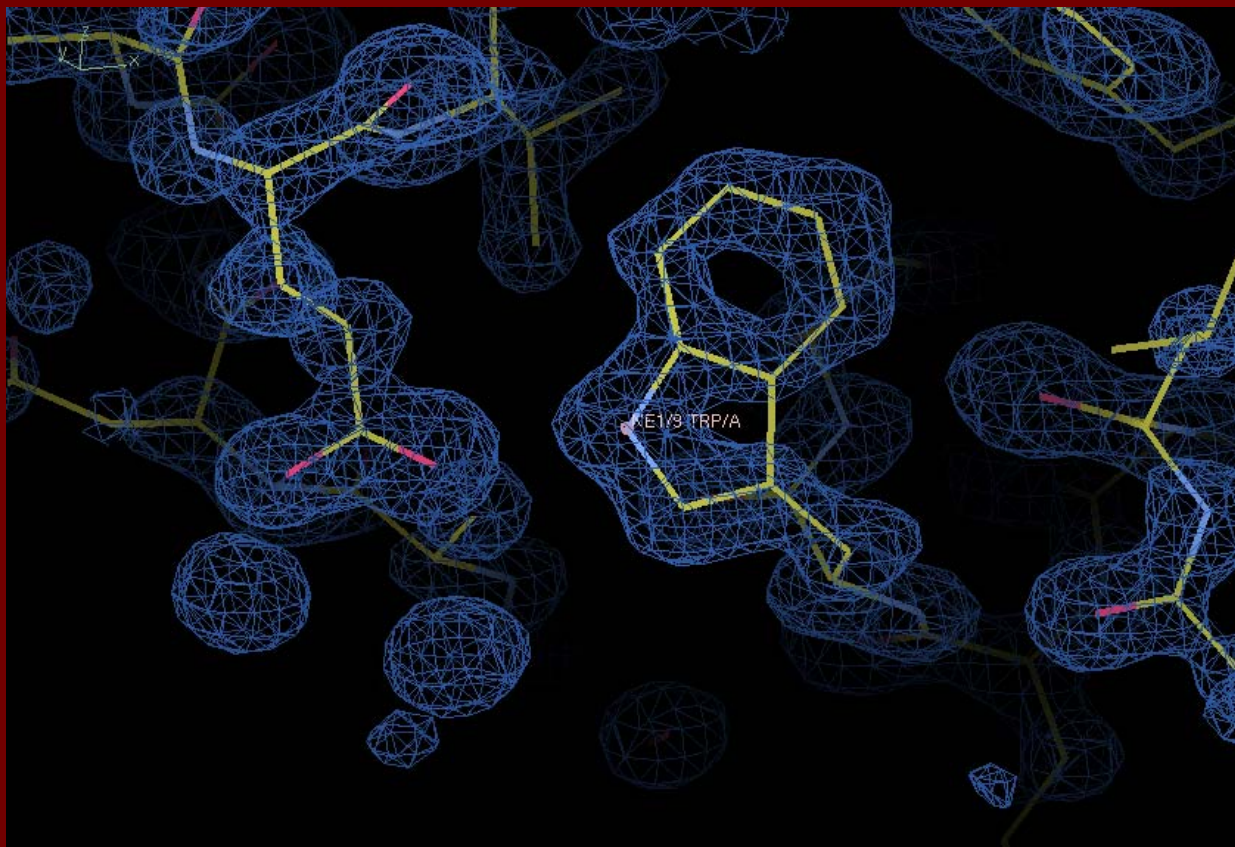
Space group: P21

$a=30.37$   $b=46.92$   $c=75.29$   
 $a=90.00$   $b=93.08$   $g=90.00$



# Structure determination

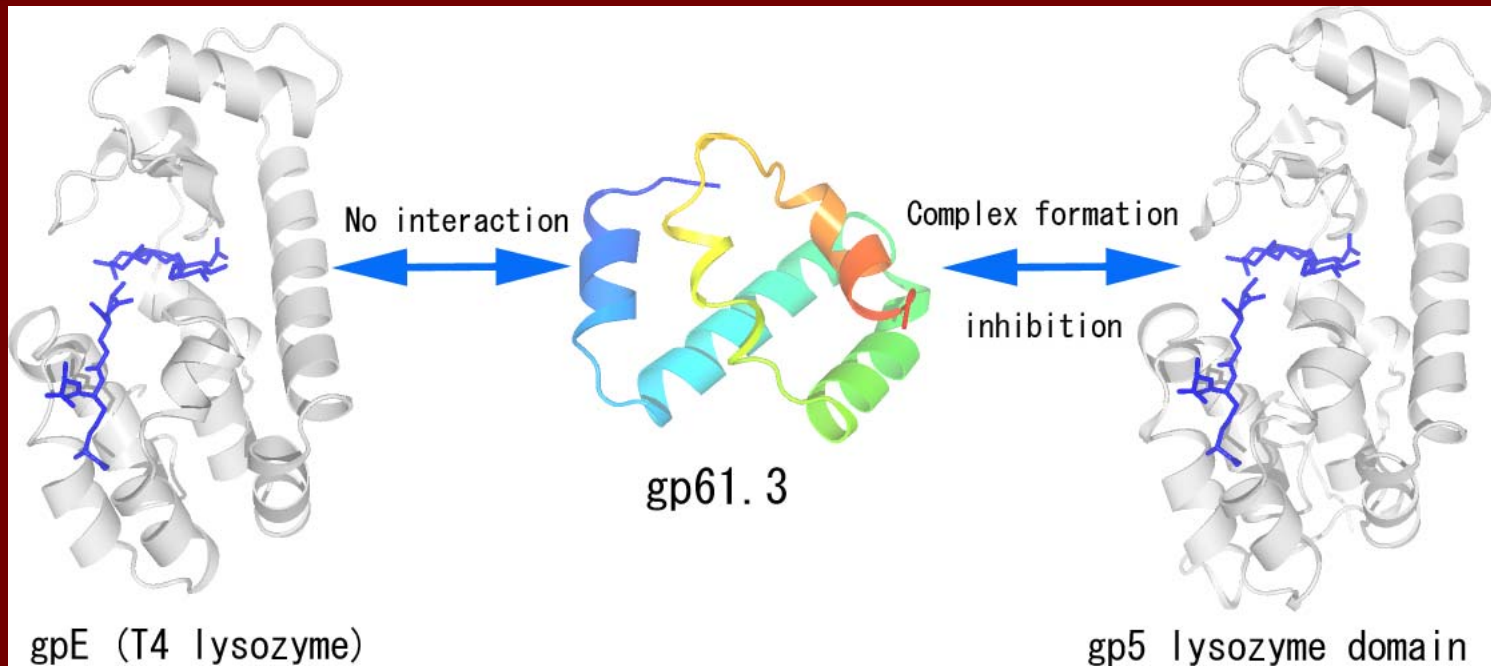
- Data sets were indexed, integrated and scaled using program XDS.
- Selenium atoms' positions of SeMet were determined with the program SHELXD.
- Subsequently, initial atomic model was built automatically with ARP/wARP program.



Typical electron density map at  $1.5\sigma$



# Gp61.3 inhibits gp5 lysozyme, but not soluble T4 lysozyme



# Conclusions and perspectives

- Over-expressed gp61.3 was purified and it is monomer in solution.
- Gp61.3 inhibits gp5 lysozyme activity.
- High resolution crystal structure of gp61.3 was solved.
- Preliminary experiment indicates gp61.3 does not interact with gpE (T4 lysozyme).
- The crystal structure of gp5\* - gp61.3 complex is necessary to understand their interaction.



# Phage Virus assembly meeting 2009 (Annecy)

## Spackle protein (gp61.3) and tail lysozyme (gp5) of bacteriophage T4

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<sup>2</sup> Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

**Abstract**

After T4 phage infection, host E. coli is usually lysed by T4 lysozyme (gp5) about 25 to 30 min. However, its secondary adsorption takes place within 10 min after first infection, the lysis period is extended up to several hours and the burst size is increased. This phenomena has been known as "lysis inhibition". Little detailed mechanism is not known. It is reported that spackle protein is one of the proteins which participate in the process. In 1959, Youssari and co-workers have been the first to clone gp61.3 as the spackle gene. In the present study, to identify the structure and function of the spackle protein, we cloned and over-expressed gene 61.3. Over-expressed gp61.3 was purified with several column chromatographies, and its oligomerization state, its interaction with gp5 and inhibition ability to gp5 lysozyme activity were examined. Gp61.3 was over-expressed in E. coli as a soluble protein, the N-terminal signal sequence of which is removed, indicating that the protein is localized in the periplasmic space. Sequence homology analysis revealed that the monomeric protein with the  $\alpha$ -chain of gp5. We also found that the gp5 associates with gp61.3 and make a stable complex in solution (3.5S). A spot test against bromelain-activated E. coli indicated that gp61.3 inhibits gp5 lysozyme activity. Crystallography analysis revealed the high resolution atomic model structure of gp61.3. The crystal structure of gp61.3 consists of  $\alpha$ -helix with a loop.

### Proteins related to lysis

(1) gp5 gene product inhibits the function of bacteriophage T4 tail lysozyme.  
 (2) gp61.3 gene product overexpressed in Escherichia coli inhibits the function of gp5.  
 (3) gp61.3 gene product binds to gp5 and inhibits its lysozyme activity.  
 (4) gp61.3 gene product binds to gp5 and inhibits its lysozyme activity.  
 (5) gp61.3 gene product binds to gp5 and inhibits its lysozyme activity.  
 (6) gp61.3 gene product binds to gp5 and inhibits its lysozyme activity.

### Purification of over-expressed gp61.3

- 1) Over-expressed gp61.3 was purified by Ni-NTA affinity chromatography.
- 2) The purified gp61.3 was further purified by ion exchange chromatography.
- 3) The purified gp61.3 was further purified by size exclusion chromatography.
- 4) The purified gp61.3 was further purified by gel permeation chromatography.

### gp61.3 inhibits gp5 lysozyme

Lysozyme activity assay by spot test

Over-expressed gp61.3 inhibits gp5 lysozyme activity. The spot test results are shown in the figure. The spot test results are shown in the figure.

gp61.3 + gp5: no lysis observed (dark spot)  
 gp5: lysis observed (clear spot)

### Crystal structure of gp61.3

**CrySTALLIZATION**

Diffraction data were collected on a Bruker AXS D8 Advance X-ray diffractometer with a graphite monochromator. The data were indexed, integrated, and scaled using the program XDS. The structure was solved by molecular replacement using the program PHENIX. The structure was refined using the program PHENIX. The final R-factor was 0.182 (0.200 for the highest resolution shell).

**Data collection**

Beamline: BL44XU (SPring-8)  
 Wavelength: 0.10000 nm  
 Detector: Rayonix RAXIS G2  
 Resolution: 1.80 Å  
 Completeness: 99.8%  
 Redundancy: 10.0  
 I/sigma: 18.0  
 R-merge: 0.040  
 CC1/2: 0.999  
 CC3/4: 0.999  
 CC5/6: 0.999  
 CC7/8: 0.999  
 CC9/10: 0.999  
 CC11/12: 0.999  
 CC13/14: 0.999  
 CC15/16: 0.999  
 CC17/18: 0.999  
 CC19/20: 0.999  
 CC21/22: 0.999  
 CC23/24: 0.999  
 CC25/26: 0.999  
 CC27/28: 0.999  
 CC29/30: 0.999  
 CC31/32: 0.999  
 CC33/34: 0.999  
 CC35/36: 0.999  
 CC37/38: 0.999  
 CC39/40: 0.999  
 CC41/42: 0.999  
 CC43/44: 0.999  
 CC45/46: 0.999  
 CC47/48: 0.999  
 CC49/50: 0.999  
 CC51/52: 0.999  
 CC53/54: 0.999  
 CC55/56: 0.999  
 CC57/58: 0.999  
 CC59/60: 0.999  
 CC61/62: 0.999  
 CC63/64: 0.999  
 CC65/66: 0.999  
 CC67/68: 0.999  
 CC69/70: 0.999  
 CC71/72: 0.999  
 CC73/74: 0.999  
 CC75/76: 0.999  
 CC77/78: 0.999  
 CC79/80: 0.999  
 CC81/82: 0.999  
 CC83/84: 0.999  
 CC85/86: 0.999  
 CC87/88: 0.999  
 CC89/90: 0.999  
 CC91/92: 0.999  
 CC93/94: 0.999  
 CC95/96: 0.999  
 CC97/98: 0.999  
 CC99/100: 0.999

**Structure determination**

The structure was determined by molecular replacement using the program PHENIX. The structure was refined using the program PHENIX. The final R-factor was 0.182 (0.200 for the highest resolution shell).

**Overall structure of gp61.3**

The overall structure of gp61.3 is shown in the figure. The structure consists of an  $\alpha$ -helix and a loop.

### Interaction analysis of gp61.3 and gp5\*

Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) analysis of gp61.3 and gp5. The results are shown in the figure. The results are shown in the figure.

gp61.3 + gp5: elution volume 1.8 ml  
 gp5: elution volume 2.2 ml

### Conclusions and perspectives

- Over-expressed gp61.3 was purified and its monomeric form was identified.
- Gp61.3 inhibits gp5 lysozyme activity.
- Gp61.3 and gp5 make 1:1 stable complex in solution.
- High resolution crystal structure of gp61.3 was solved.
- The structure of  $\alpha$ -helix and one short loop, (the loop may inhibit gp5 lysozyme activity).
- Preliminary experiment indicates gp61.3 does not interact with gp6 (T4 lysozyme).
- To understand the mechanism of "lysis inhibition", we need more biological experiments.
- The crystal structure of gp61.3-gp5 complex is necessary for their interaction.



# EPFL (Ecole Polytechnique Federale de Lausanne)



# le Cubotron





# Wet lab





# Synchrotrons





# Preparing for visit

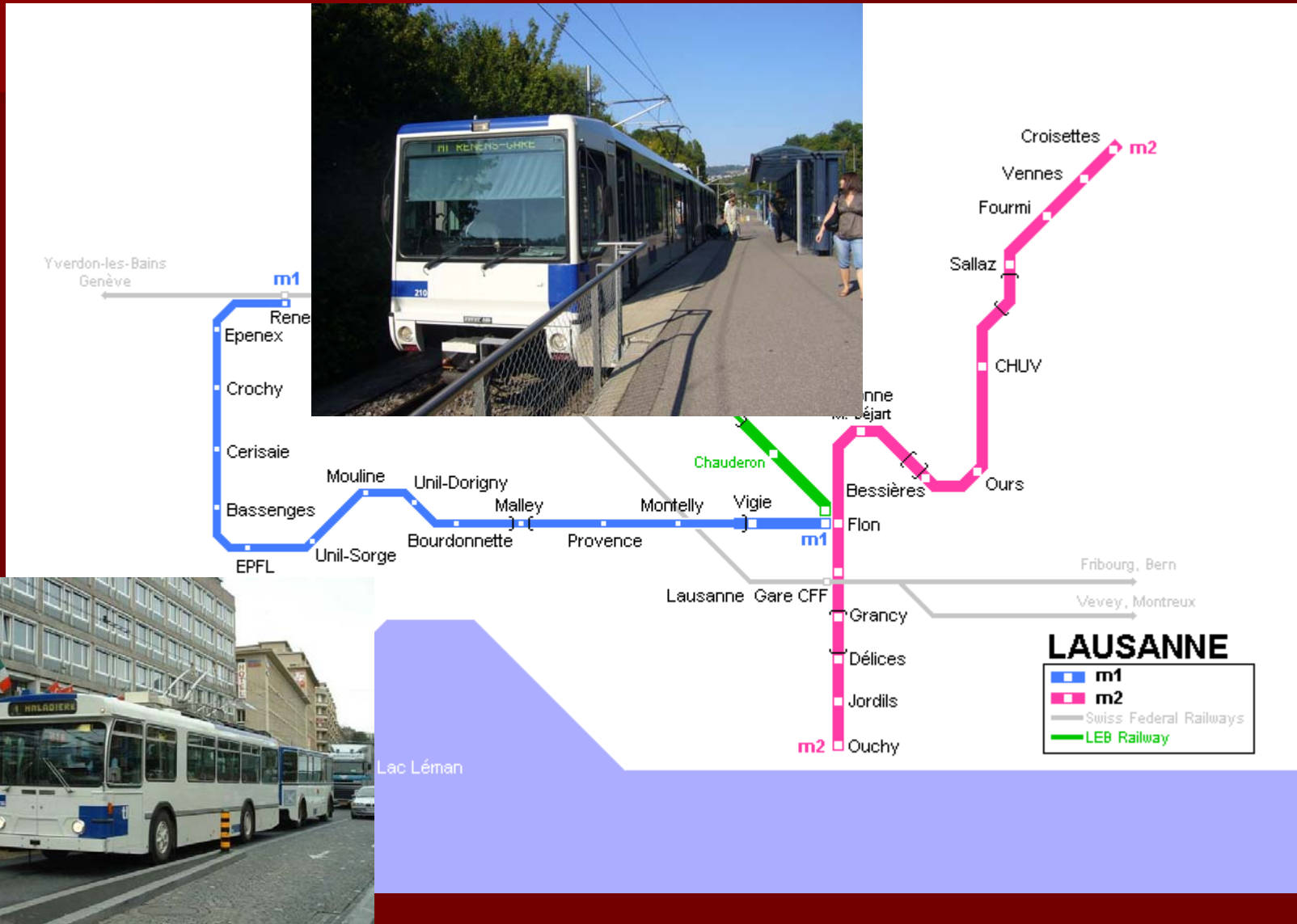
- April: sending CV, apartment reservation
  - May: sending proof documents of ITP program
  - June: airplane and hotel reservation
  - July: insurance, sending money
- After arrival,
- registration, ID card application, open bank account

# Grocery stores



- Foods at restaurant are very expensive.
- All the shops and stores are closed on Sunday.

# Transportation, metro & bus



# Apartment



2,080CHF / month





# Apartments are not enough...



**Lunettes de soleil ou parapluie?**  
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### Les étudiants transpirent pour se dégoter un appart

par Joël Burri

**Les hautes écoles en appellent à la solidarité pour trouver un toit aux étudiants, toujours plus nombreux.**

«Maintenant que je ne vis plus chez eux, mes parents louent ma chambre à un étudiant», raconte Liz, Genevoise de 22 ans inscrite en droit à Lausanne. C'est le réflexe que le Service des affaires socio-culturelles de l'UNIL et de l'EPFL (SASC) souhaiterait développer chez les Vaudois.

La rentrée académique de septembre s'annonce en effet difficile. «Environ 500 étudiants cherchent toujours une chambre alors que nous n'en avons plus qu'une soixantaine dans notre base de données», déplore Gilberte Isler, cheffe du SASC.



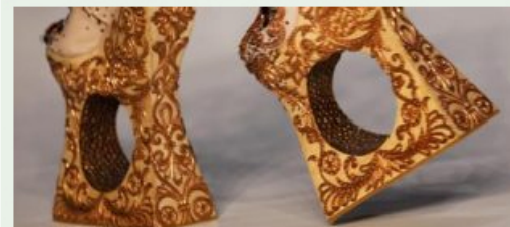
Les passants ont été sensibilisés à la pénurie de logements pour les étudiants. (Photo: Reuters)

#### Link-Box

Site du Service du logement de l'Université de Lausanne et de l'EPFL



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Diaporama



# What else you can do?



# Leiman lab member

