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
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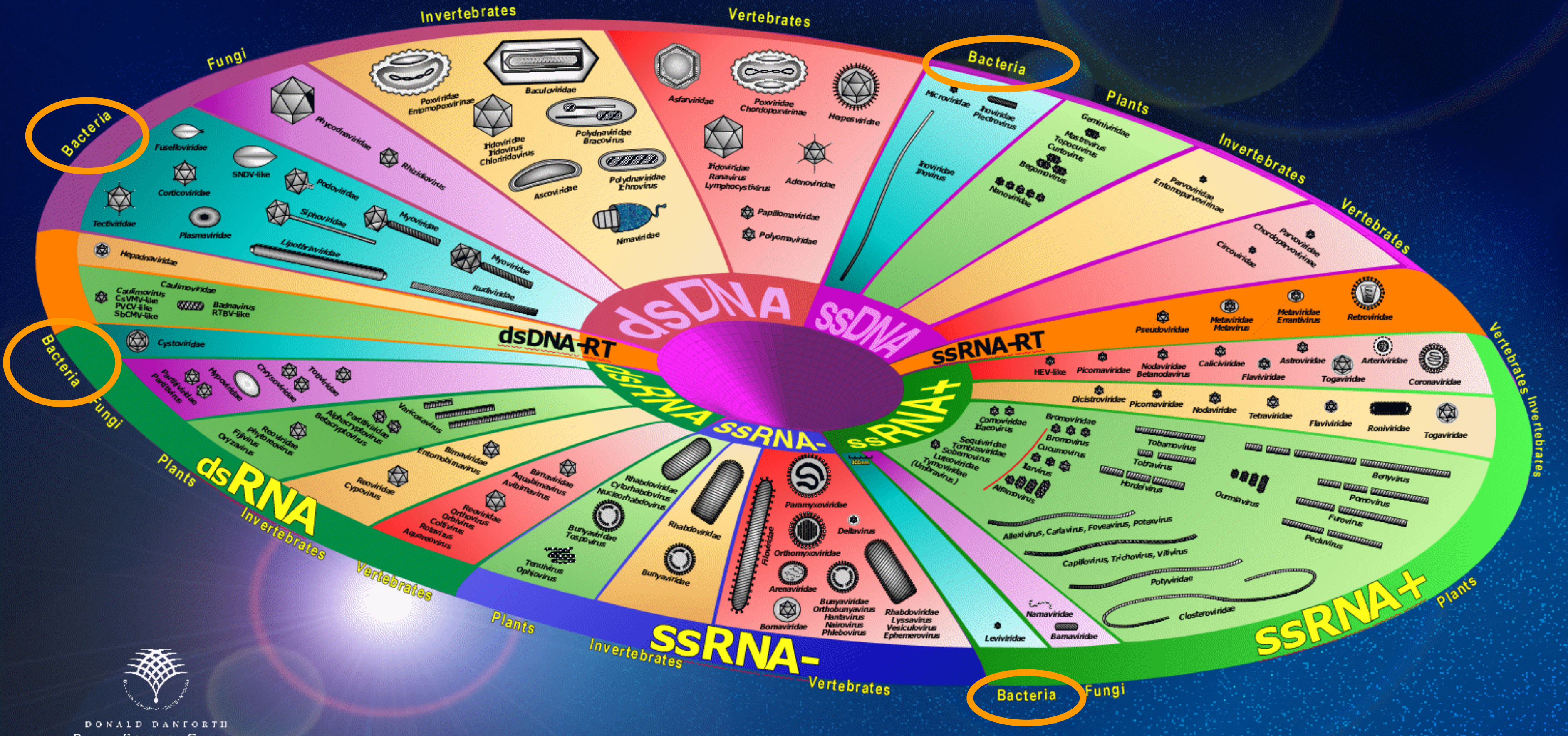
派遣先：EPFL, Petr Leiman研究室
2009. Jul. 15 ~ Oct. 15

Today's talk

- Crystal structure determination of gp61.3 from bacteriophage T4.

- Life in Lausanne.

Virosphere 2002



International Committee on Taxonomy of Viruses

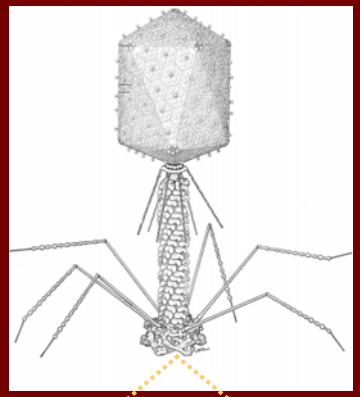
DONALD DANFORTH PLANT SCIENCE CENTER

Environment and phage

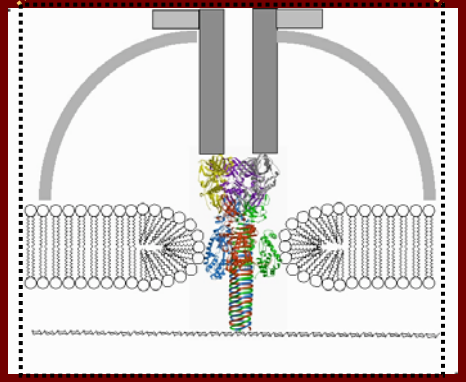
- Several phages can infect one bacterium.
- 10^5~10^7 phages are found in 1mL of sea water.
- Phages are most biggest biomass in the world.
- Phage might control the whole environment?

Cell puncturing device gp5

T4 phage



gp5 functions as cell puncturing device

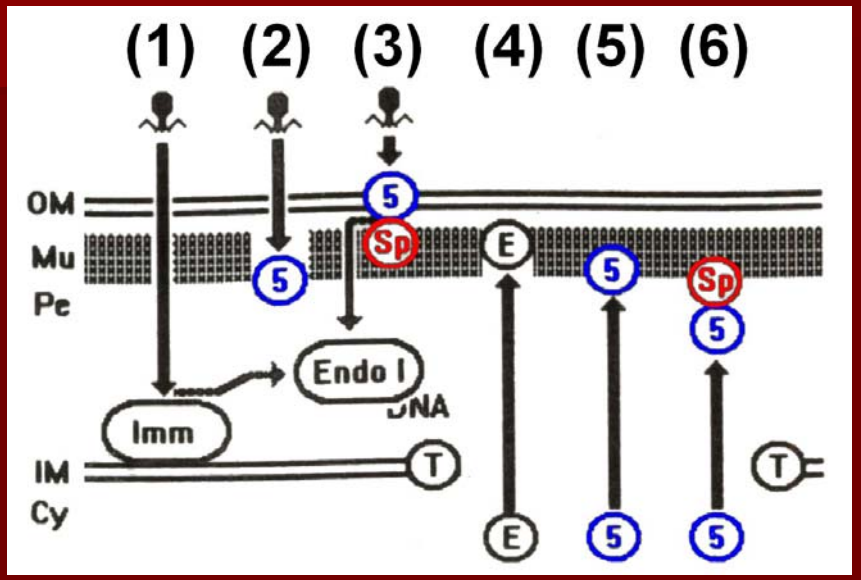


Outer membrane

Peptidoglycan

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..

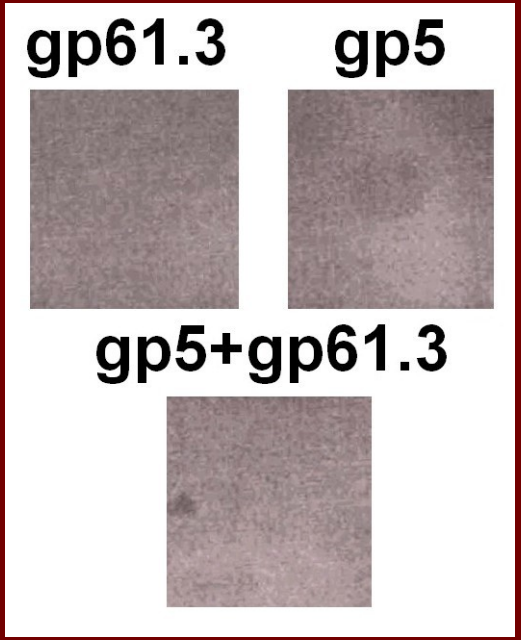
(6) Sp protein also inhibits newly produced gp5 lysozyme activity.

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 ager 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.

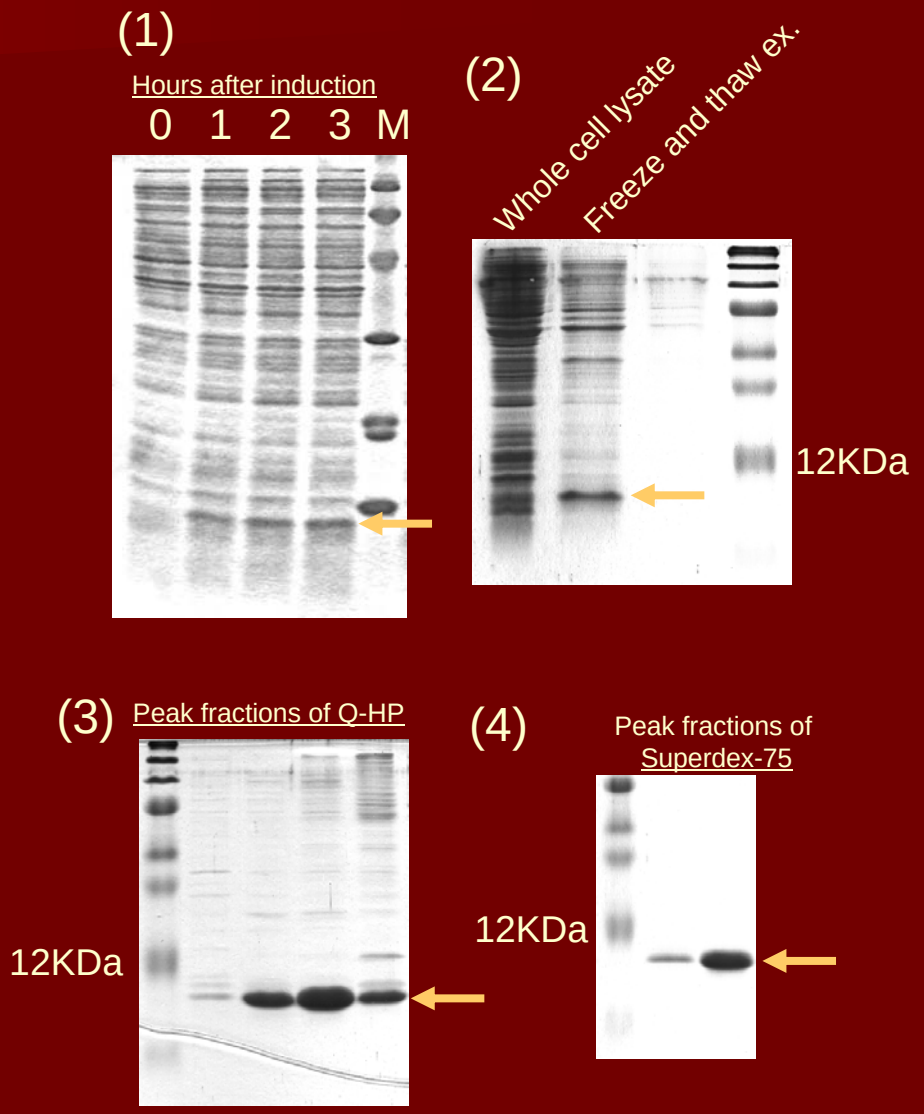
Purification of over-expressed gp61.3

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.

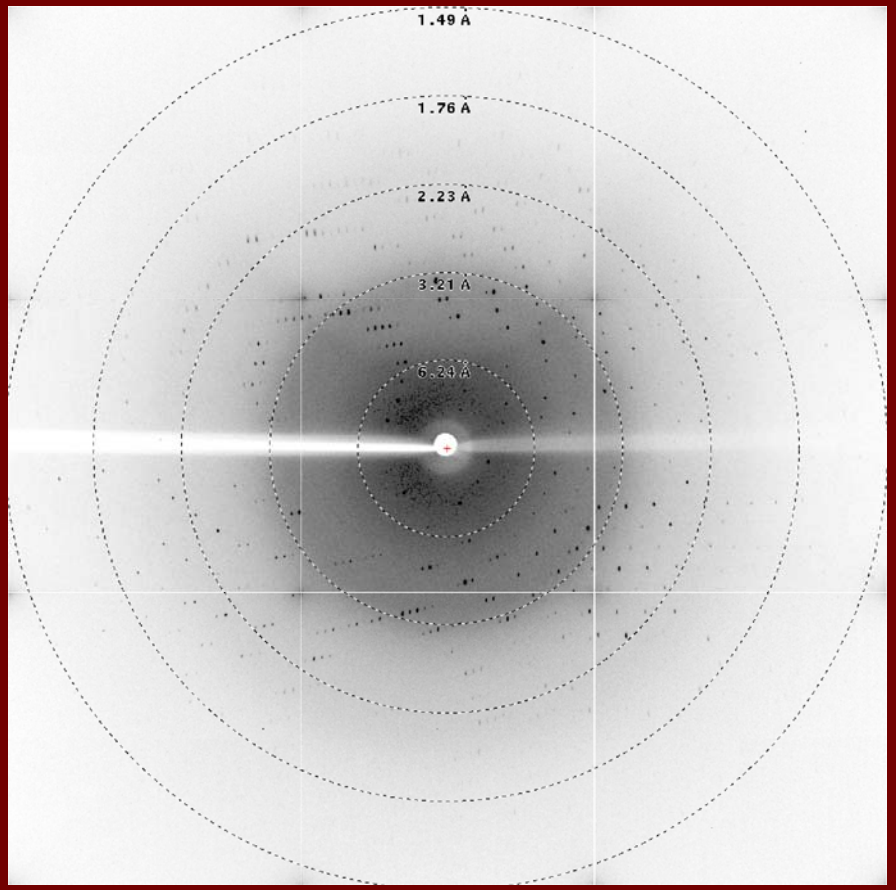


Crystallization of gp61.3

~1000 conditions were tested.

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

Data collection



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

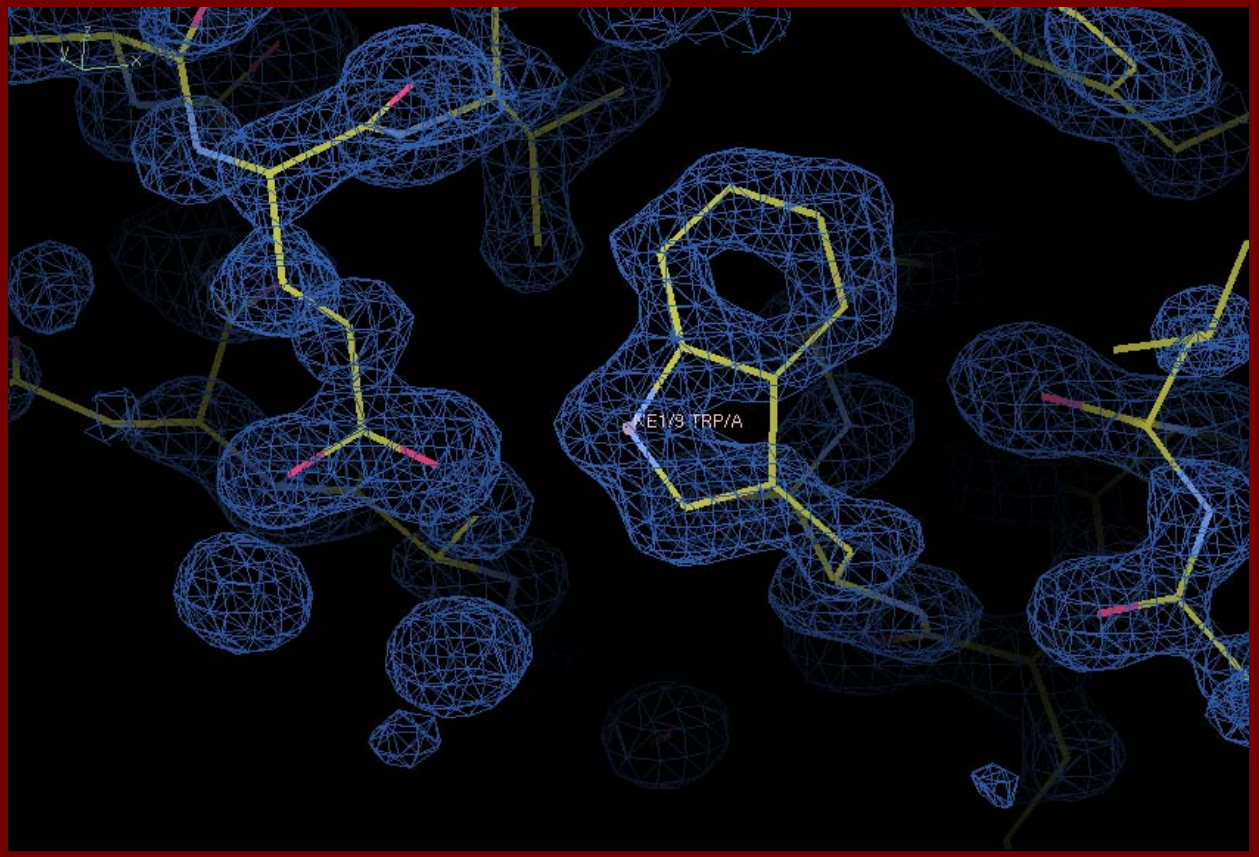
Crystals diffract ~1.5Å resolution .

Space group: P21

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

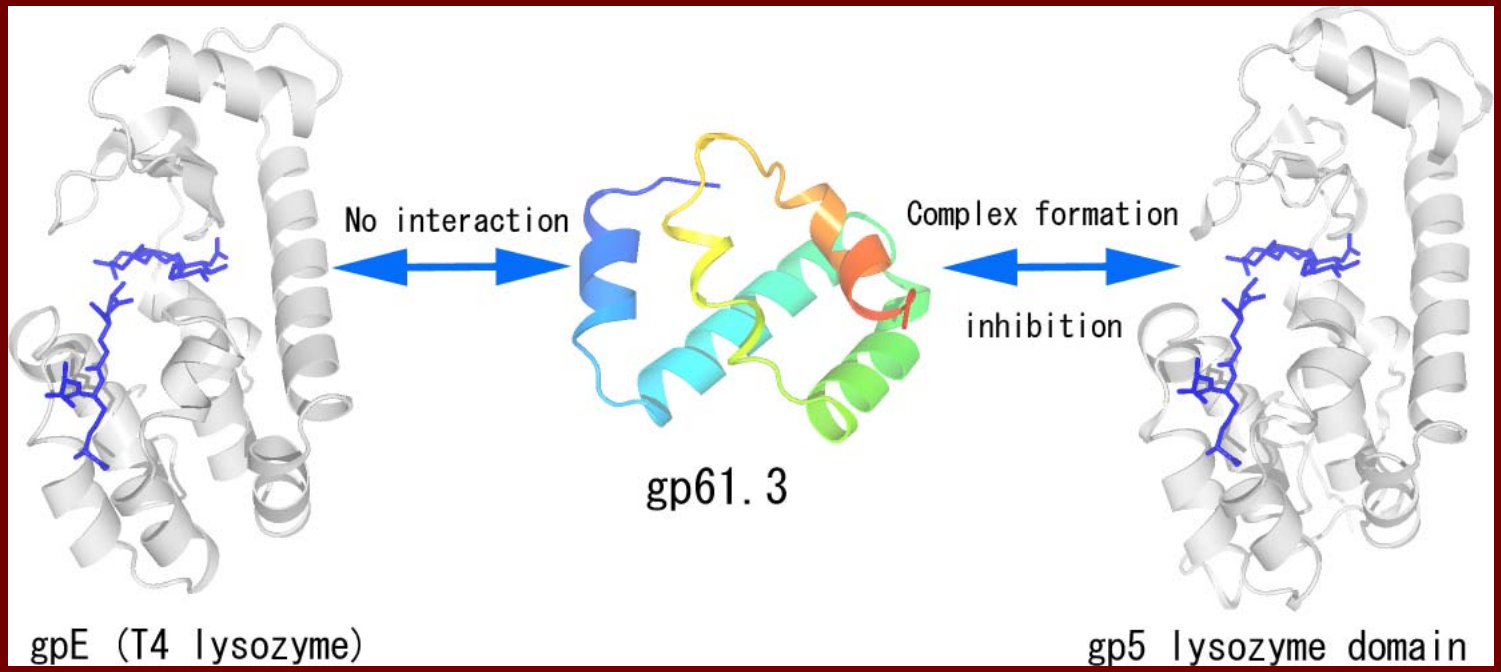
Structure determination

- 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 1.5σ

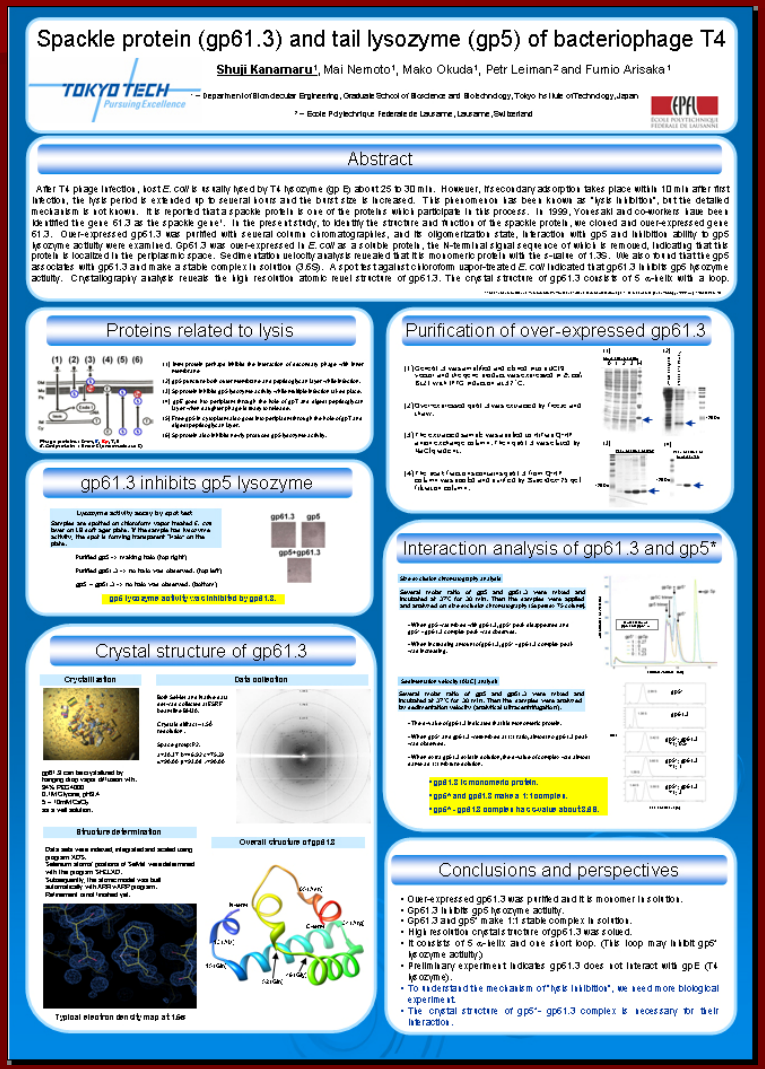
Gp61.3 inhibits gp5 lysozyme, but not soluble T4 lysozyme



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)



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…

NEWS

Act. 27.08.09; 22:26 Pub. 27.08.09; 22:44

LAUSANNE

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

LES IMAGES DU JOUR

Retrouvez chaque jour les meilleures images qui font l'actualité.
Diaporama

What else you can do?

Leiman lab member