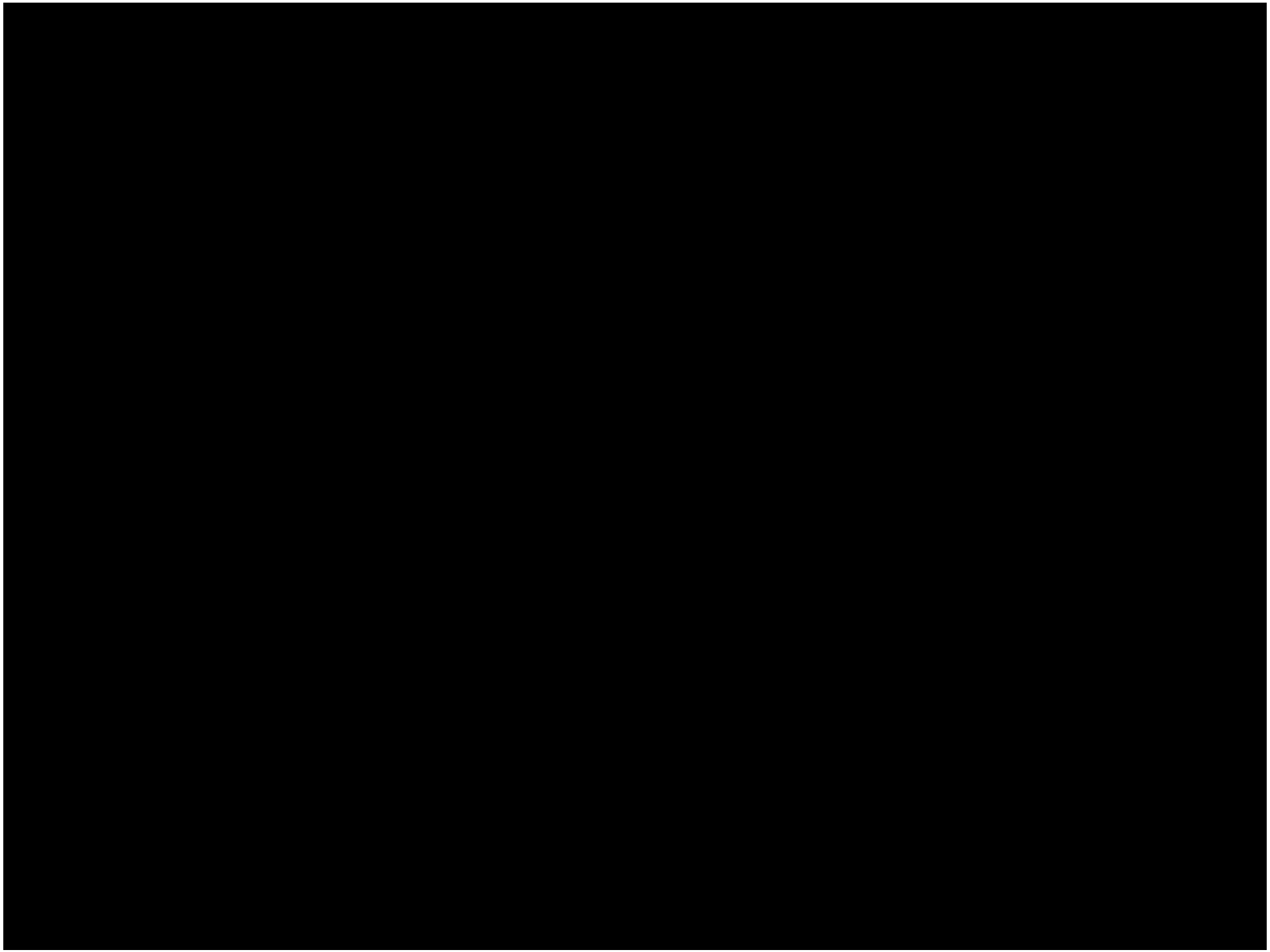
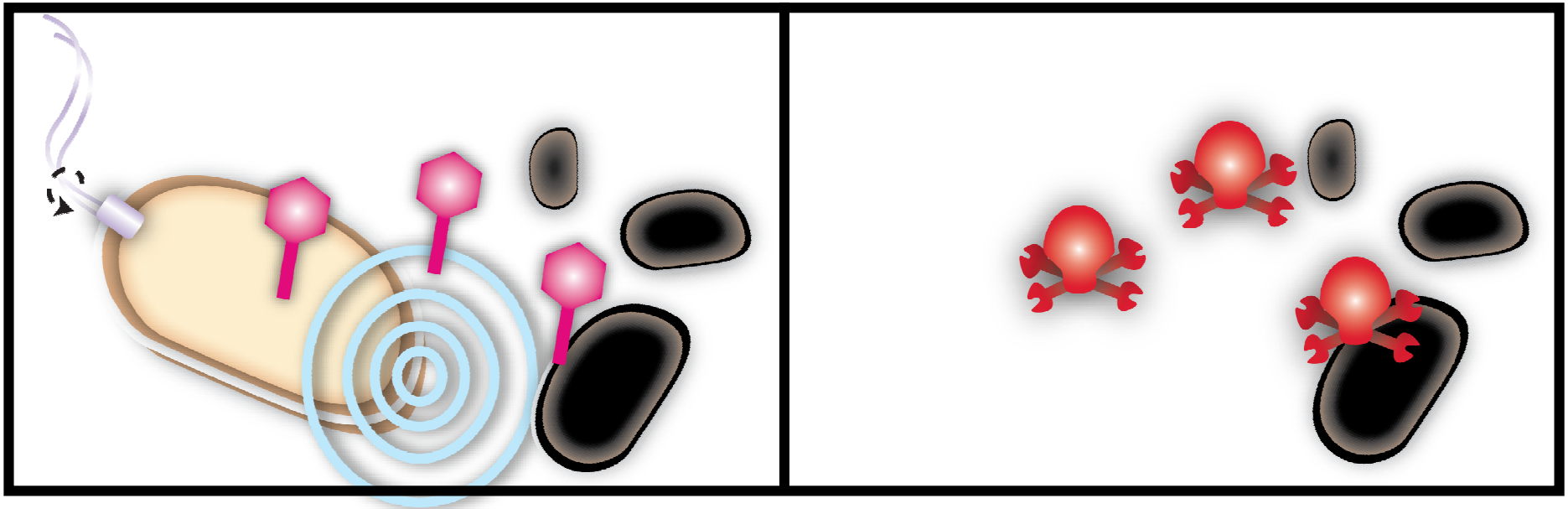


iGEM TEAM
HEIDELBERG

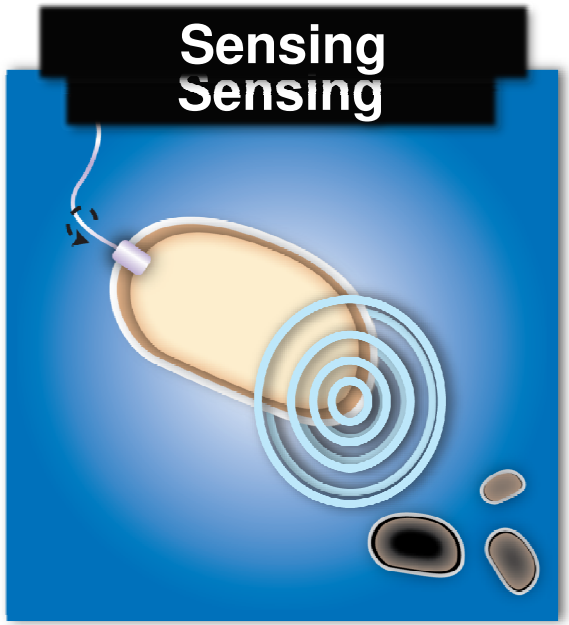
08 ECOLICENCE
TO KILL

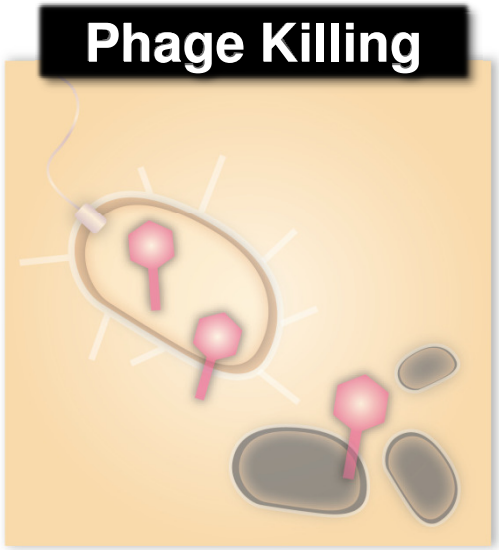
Project Overview

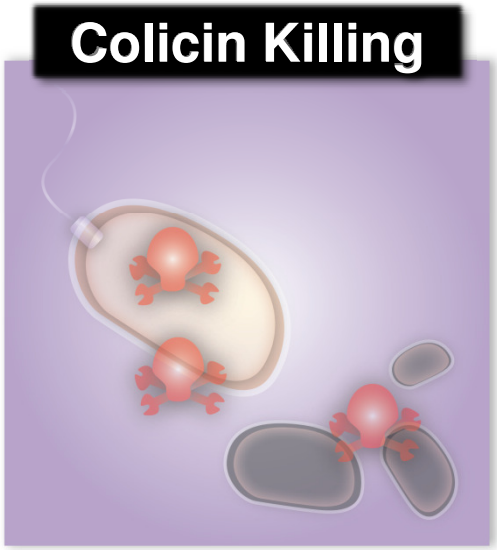
- Target and kill antibiotic resistant pathogenic bacteria and cancer cells
- Implementing a killer-prey-system with two different *E.coli* strains



Overview

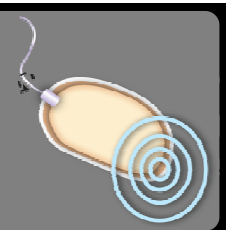


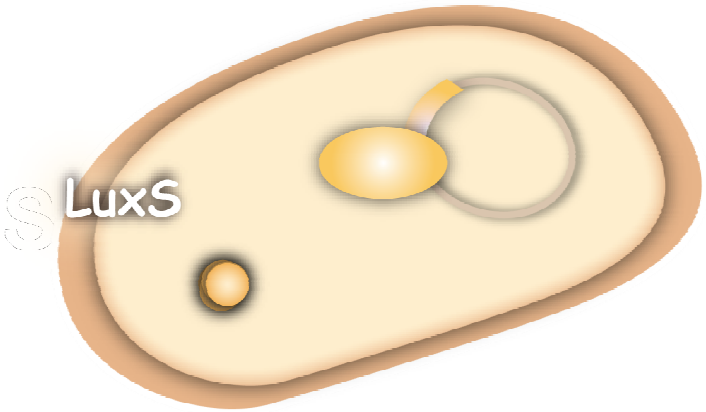


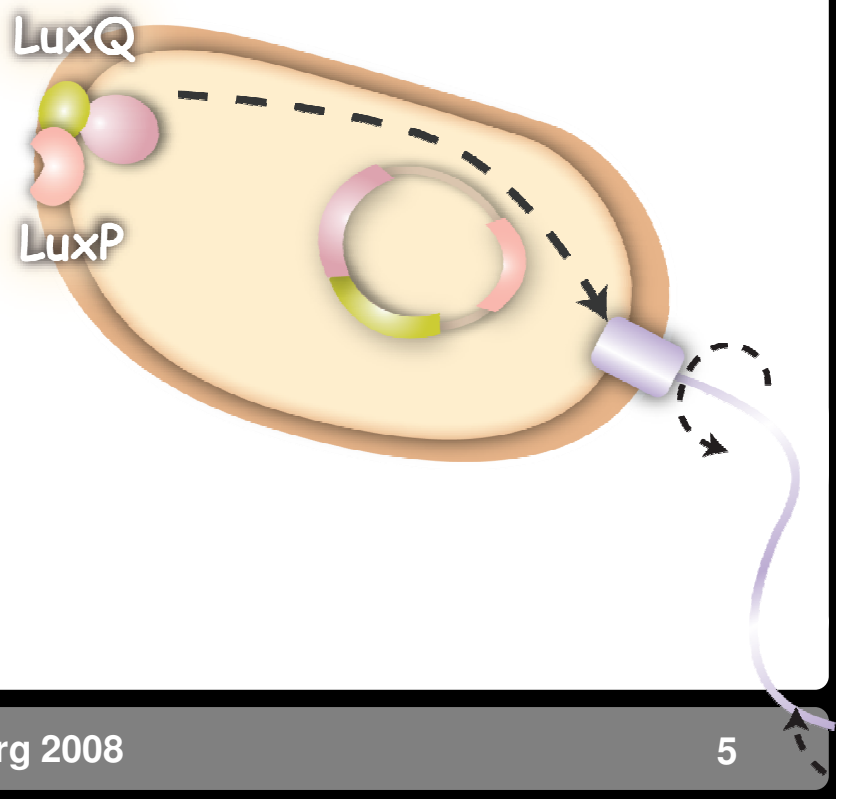


Sensing System

Overview



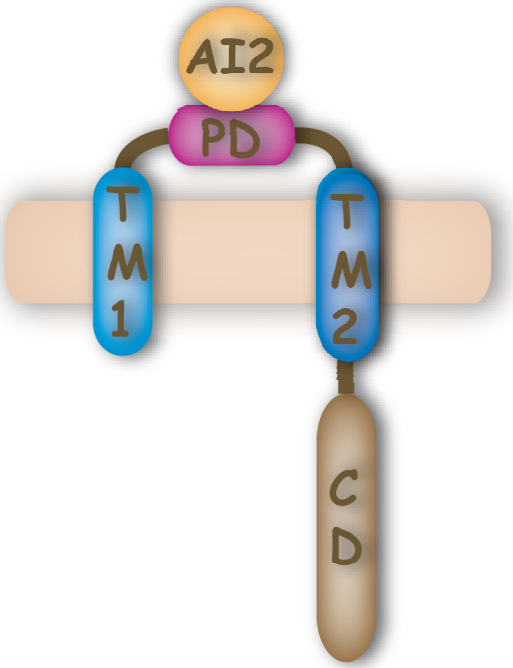




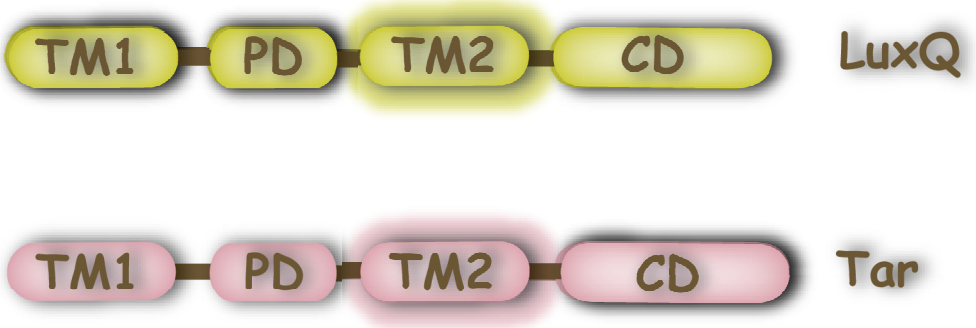
Chemoreceptor triggers chemotaxis
→ AI-2 secretion

Sensing System

LuxQ-Tar Fusion Protein



General structure of LuxQ and Tar



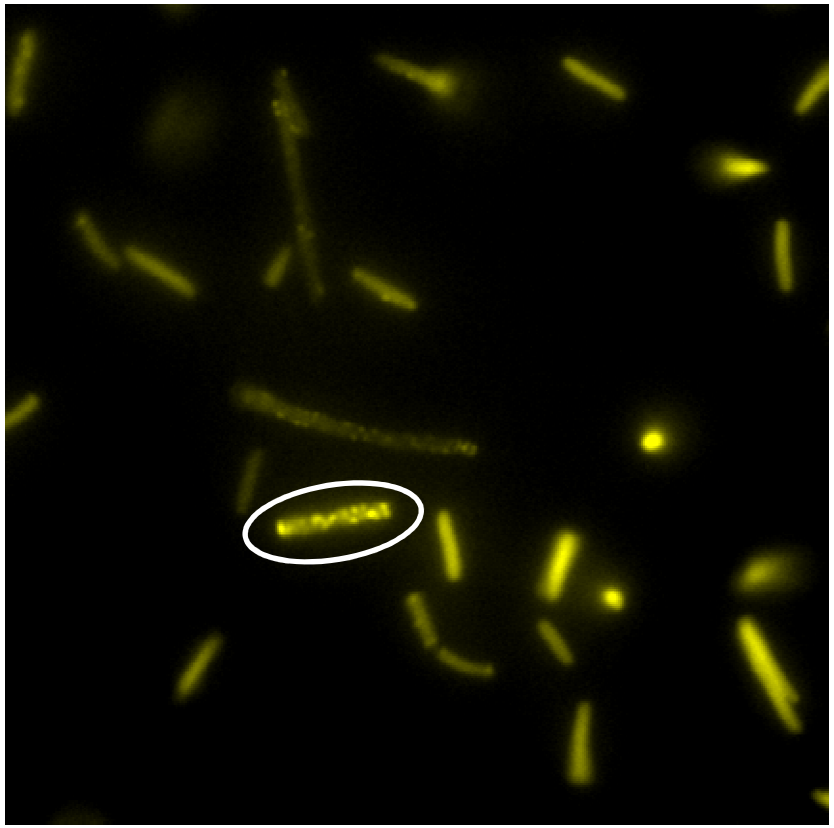
Construct 1: TM2 from LuxQ

Construct 2: TM2 from Tar

Legends:
TM: transmembran domain PD: periplasmic domain CD: cytoplasmic Domain

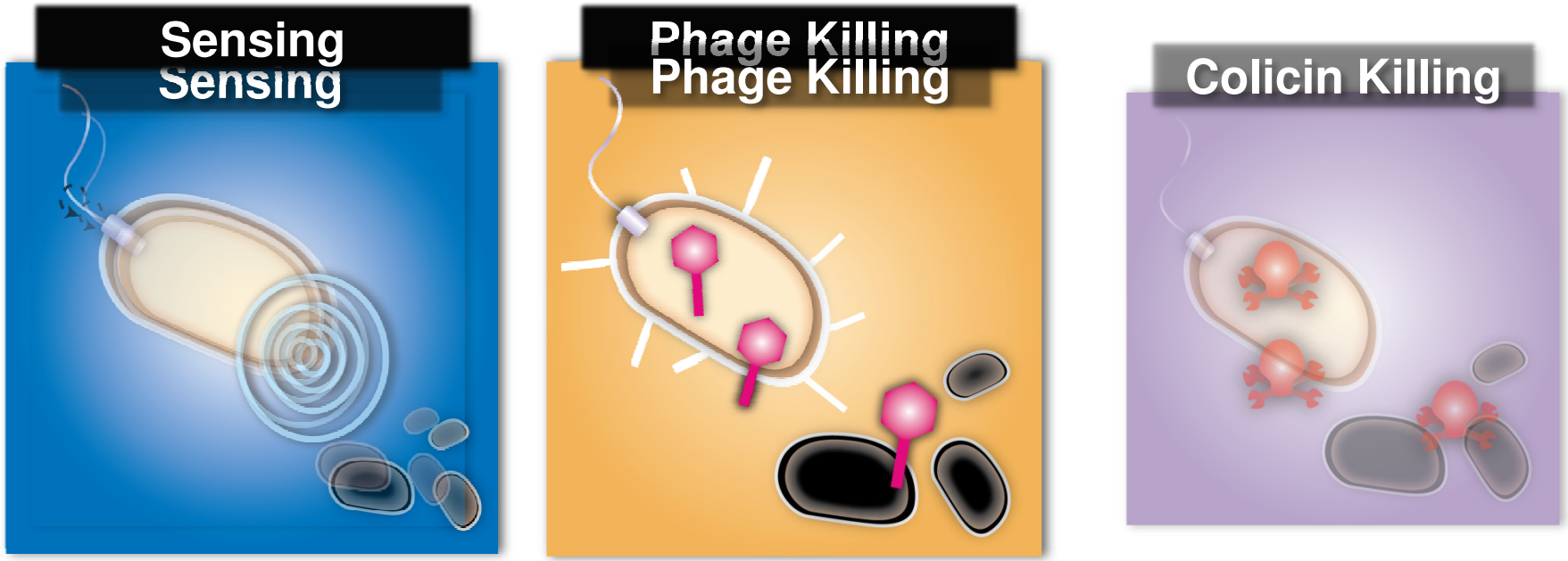
Results

Expression Test with Fusion-YFP constructs



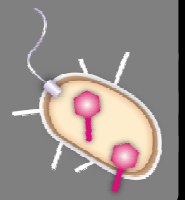
➡ LuxQ-Tar fusion protein is expressed and located in the membrane

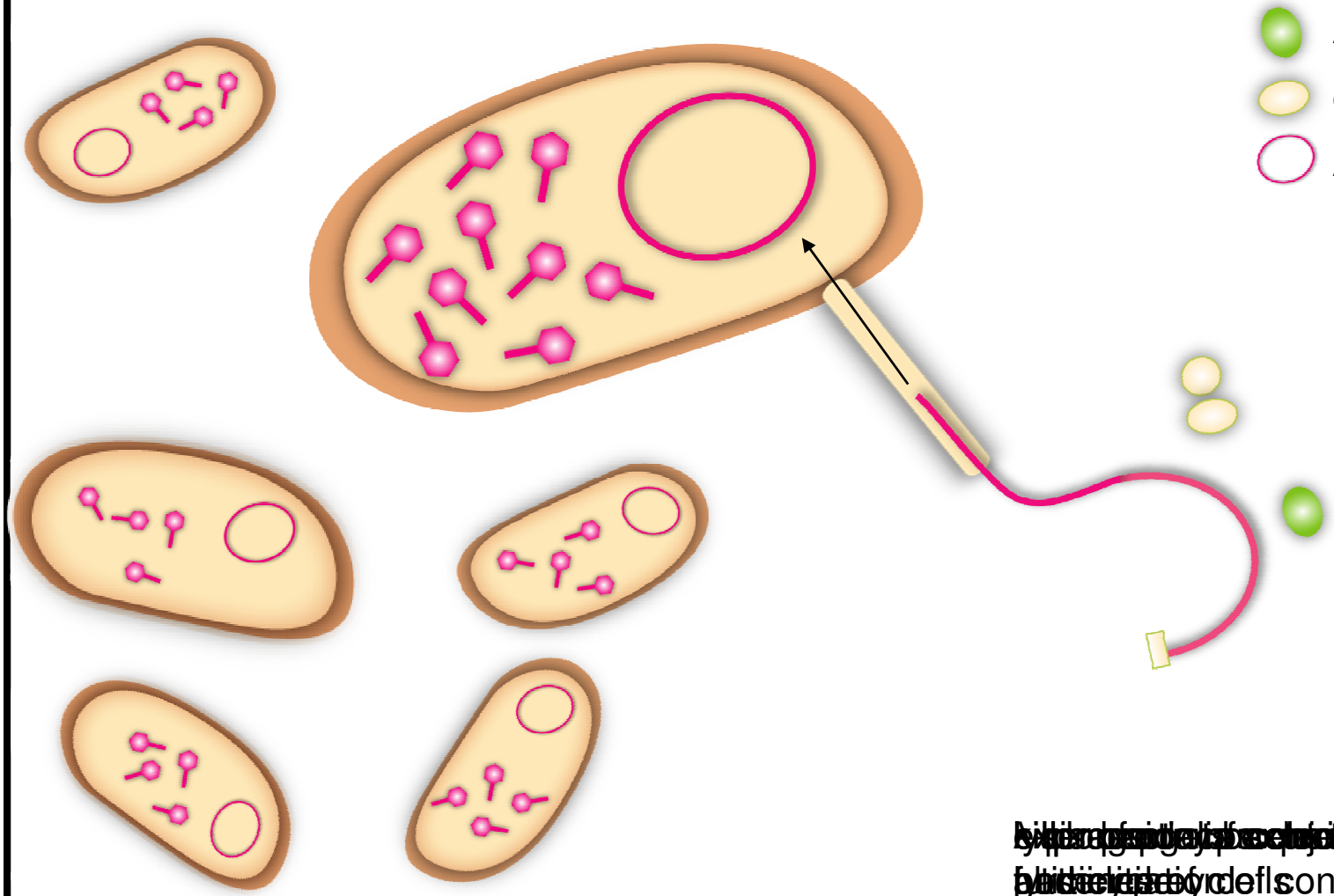
Overview


Sensing
Sensing
Phage Killing
Phage Killing
Colicin Killing

Killing System I – Killing by Phages

Overview

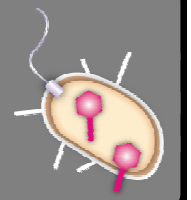




conjugation

Phage-Killing Modeling

Model overview



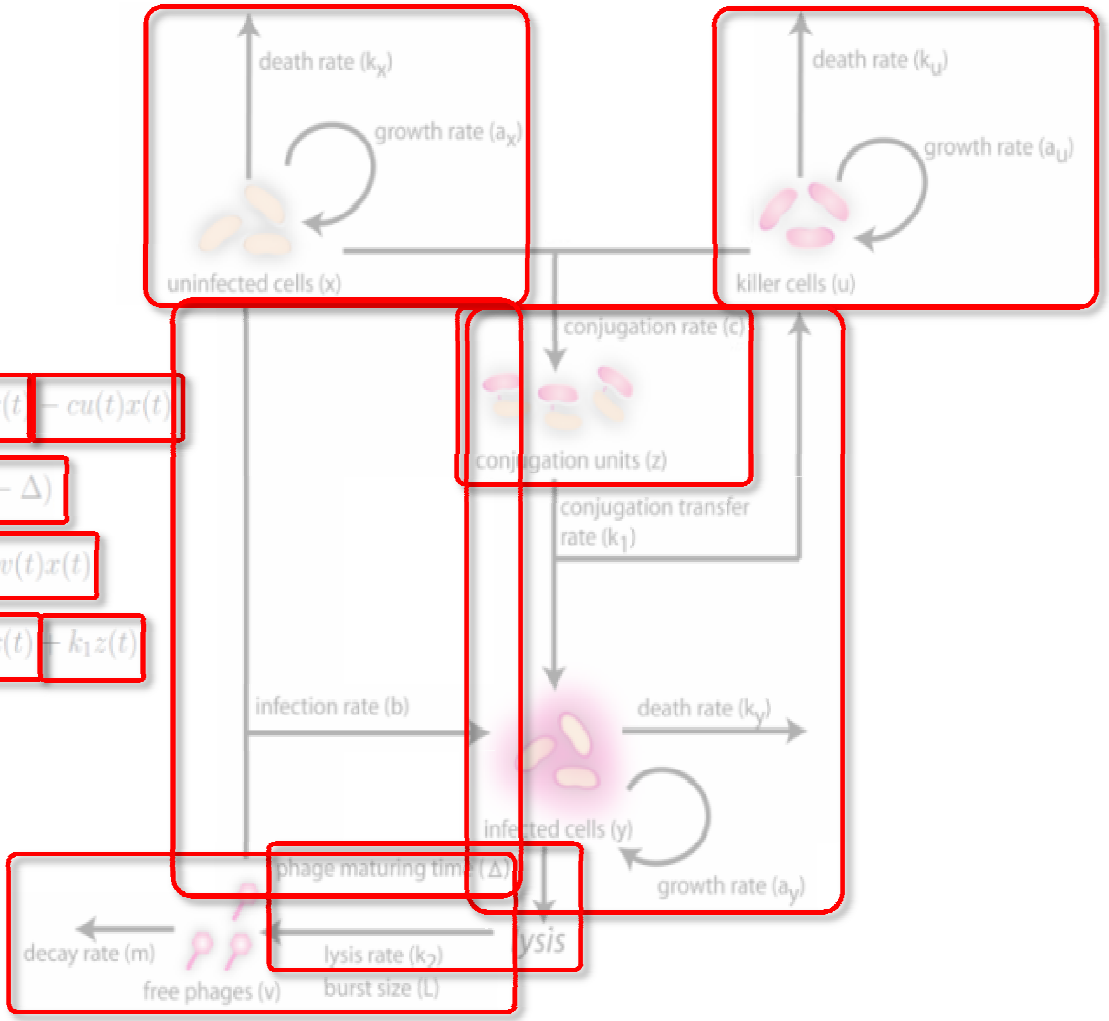
$$\frac{dx}{dt} = a_x\left(1 - \frac{x(t)+y(t)+u(t)+z(t)}{\kappa}\right)x(t) - bv(t)x(t) - cu(t)x(t)$$

$$\frac{dy}{dt} = bv(t)x(t) + k_1 z(t) - bv(t-\Delta)x(t-\Delta) - k_1 z(t-\Delta)$$

$$\frac{dv}{dt} = L(bv(t-\Delta)x(t-\Delta) + k_1 z(t-\Delta)) - mv(t) - bv(t)x(t)$$

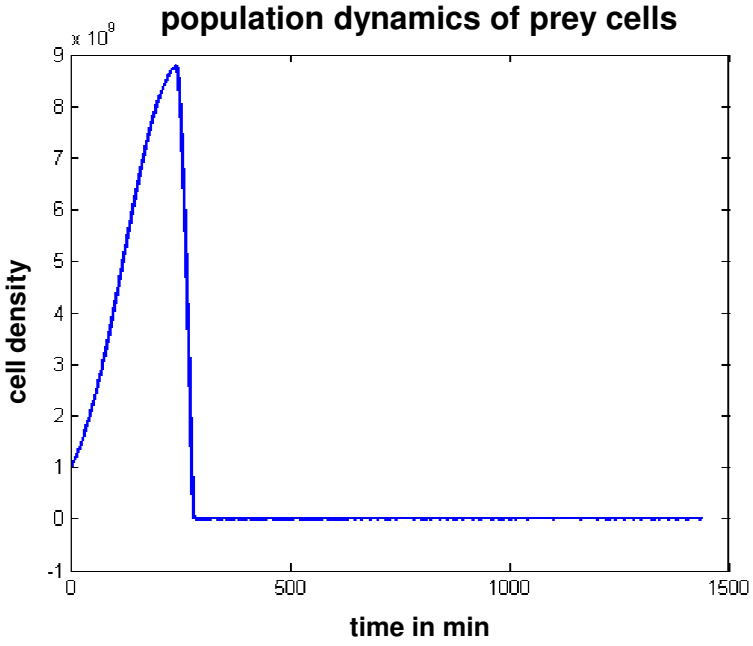
$$\frac{du}{dt} = a_u\left(1 - \frac{x(t)+y(t)+u(t)+z(t)}{\kappa}\right)u(t) - cu(t)x(t) + k_1 z(t)$$

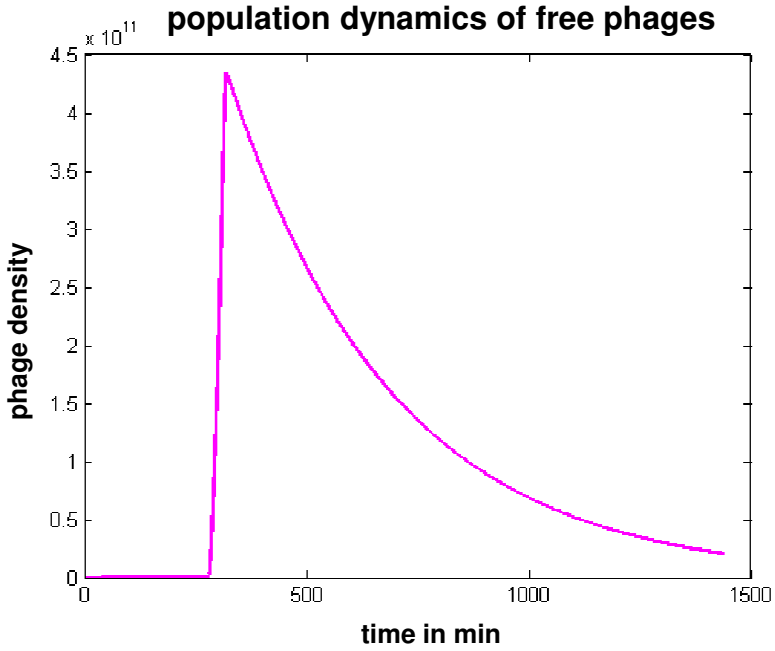
$$\frac{dz}{dt} = cu(t)x(t) - k_1 z(t)$$



Phage-Killing Modeling

Simulation Results

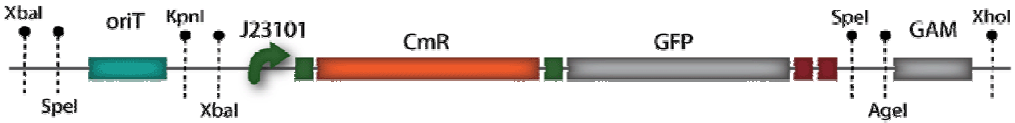


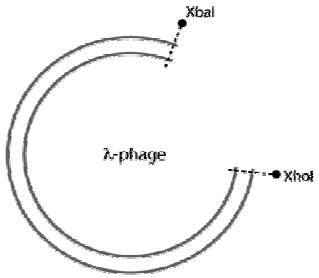


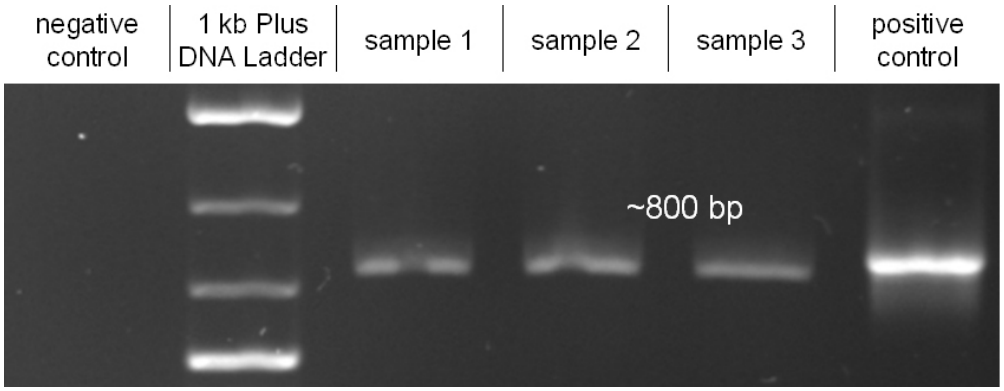
High initial killer cell density is not necessary for efficiency of the system: 10 killer cells are able to kill 10^9 prey cells *in silico*

Killing System I – Killing by Phages

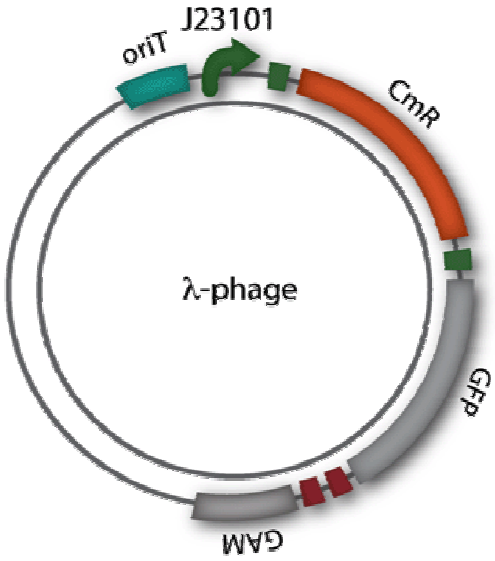
Cloning strategy







PCR amplification with CmR specific primers from the λ- genome

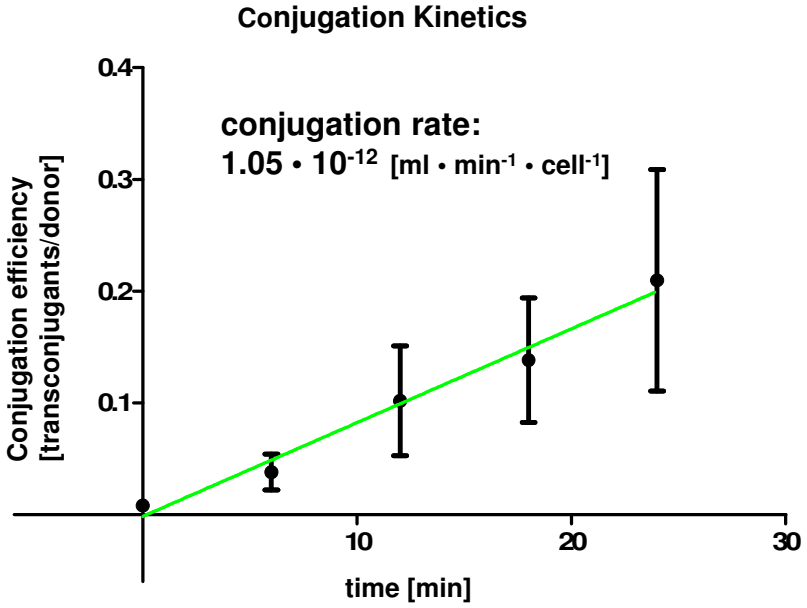


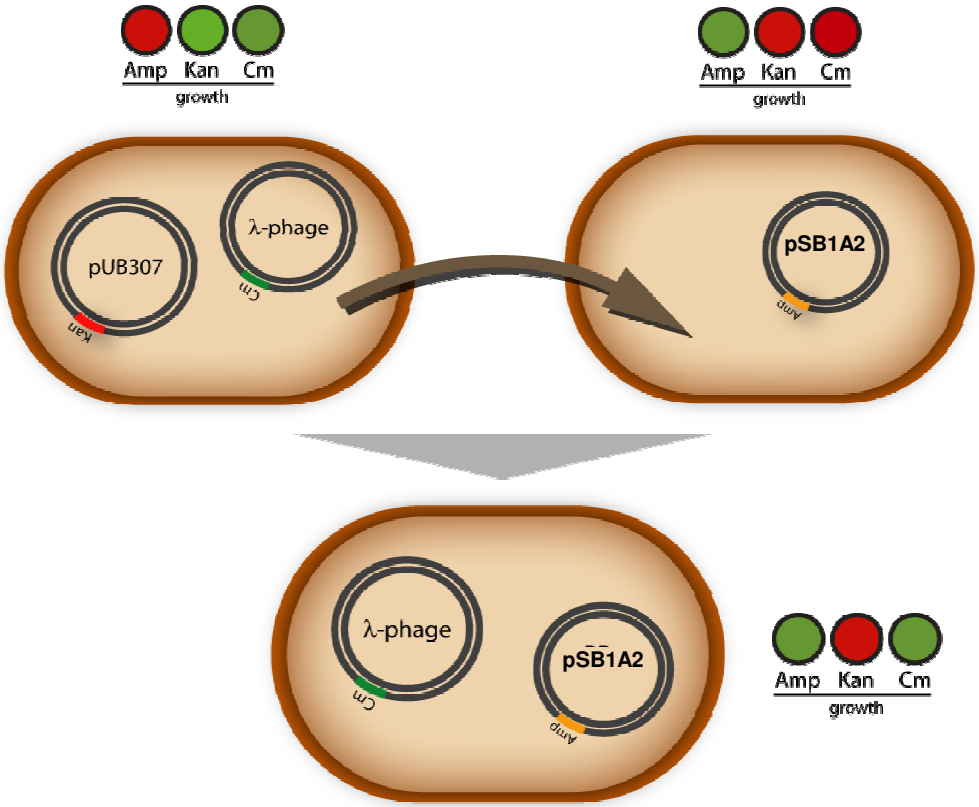
Engineering of the λ-phage succesful

Killing System I – Killing by Phages

Conjugation Results

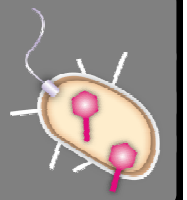
- Conjugation tests with engineered λ- phages
- Numerical values for conjugation rate obtained for modeling

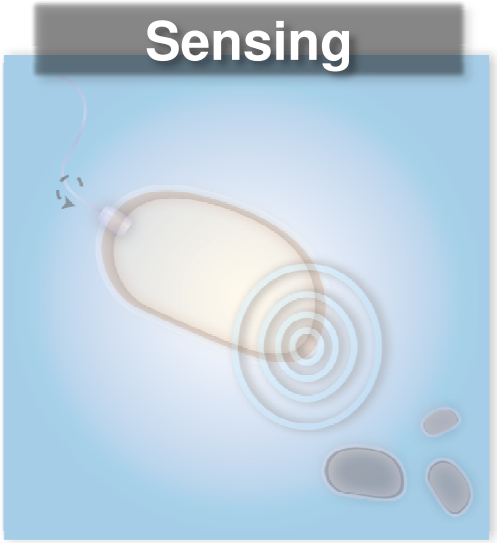


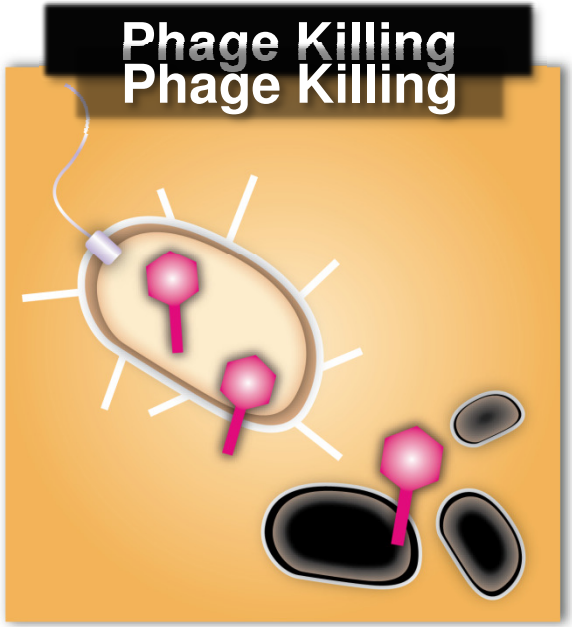


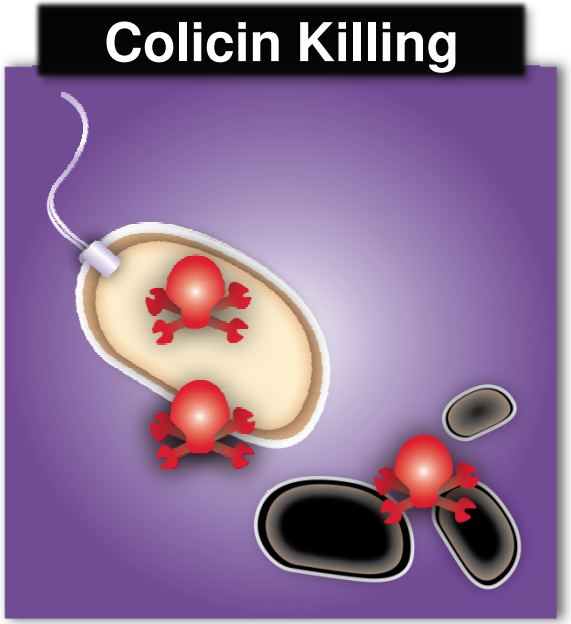
System is functional

Overview



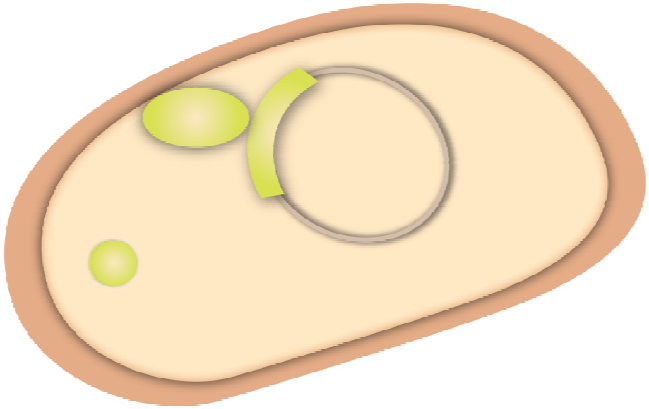


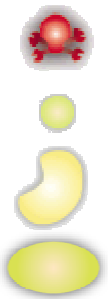


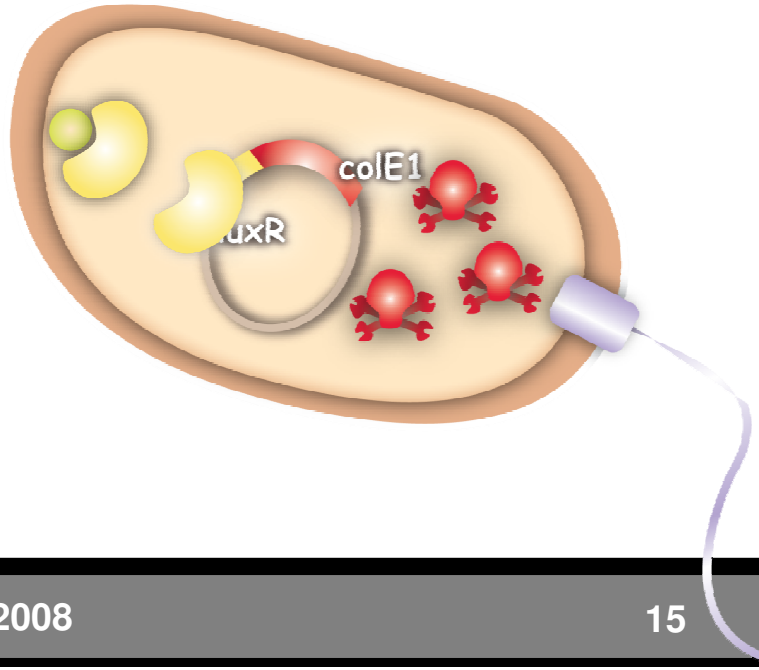


Killing System II – Killing by Colicins

Overview







[illegible]

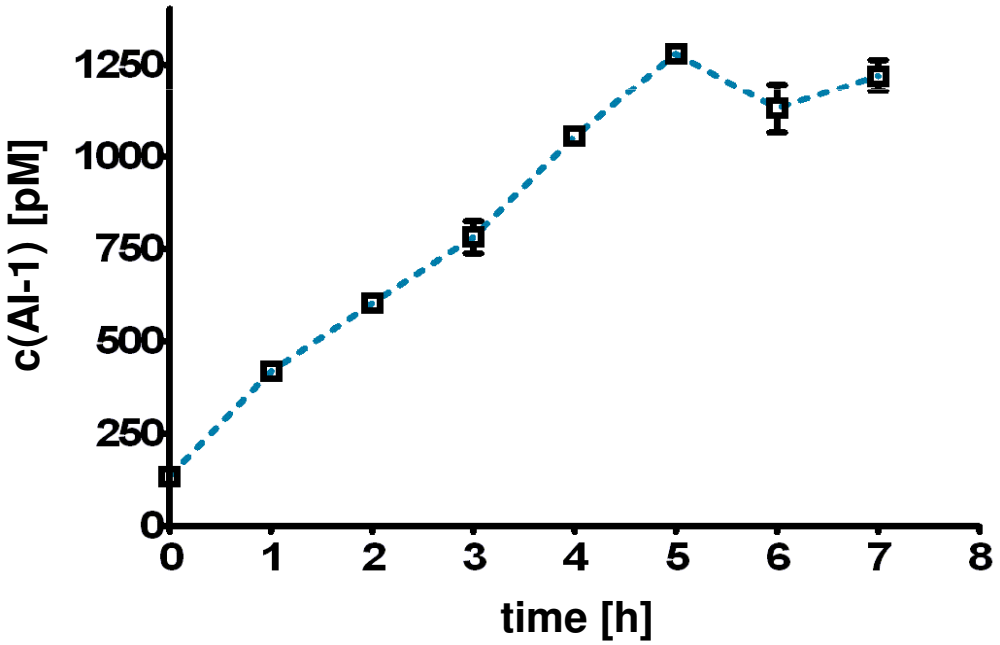
AI-1 producing Sender Part

Construction and Characterization

Construction of Sender part for Prey bacterium producing constitutively AI-1

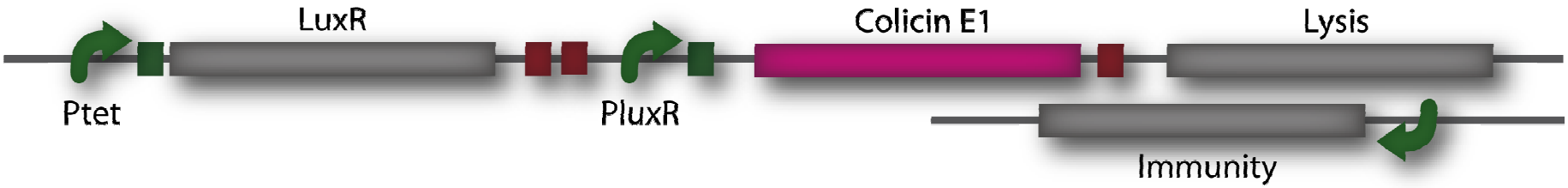
- Characterization: Determination of AI-1 concentration in culture supernatants

  ➡ **Maximum c(AI-1) ≈ 1250 pM**



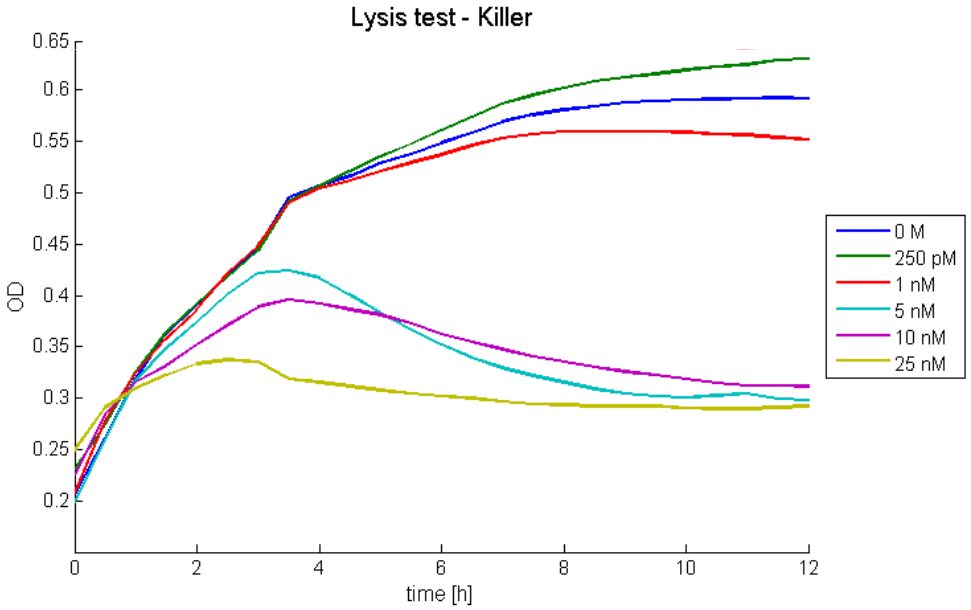
Colicin Production by Killer Strain

Construction and Characterization



Construction of AI-1 inducible colicin producing Killer strain

- Characterization: Determination of killing efficiency for different AI-1 induction levels and prey-killer ratios



⇨ Lysis effect observed for…

…c(AI-1) ≥ 5 nM

…c(AI-1) > 500 pM

…prey-killer ratios up to 100:1

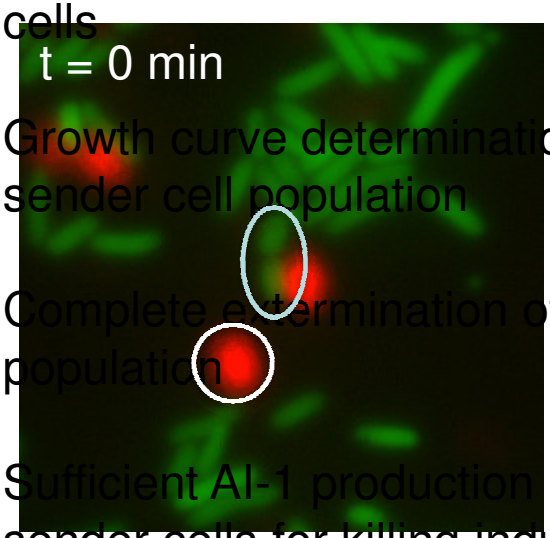
System Test

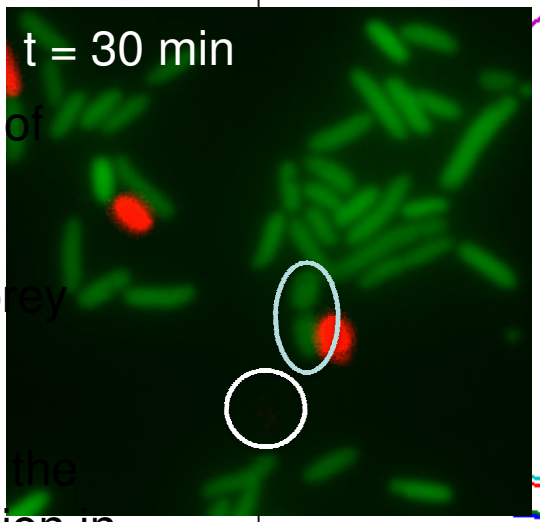
Sender cells induce colicin production by killer cells

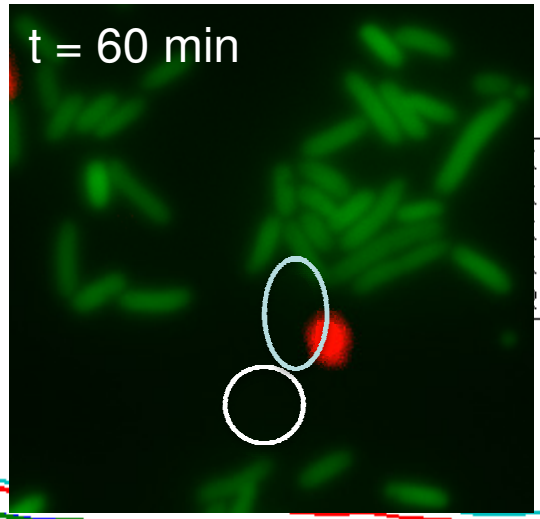
- Killer cells prepared in different ratios with AI-1 producing sender cells
- Growth curve determination of sender cell population

➡ Complete determination of prey population

➡ Sufficient AI-1 production by the sender cells for killing induction in ratios up to killer:prey 1:25



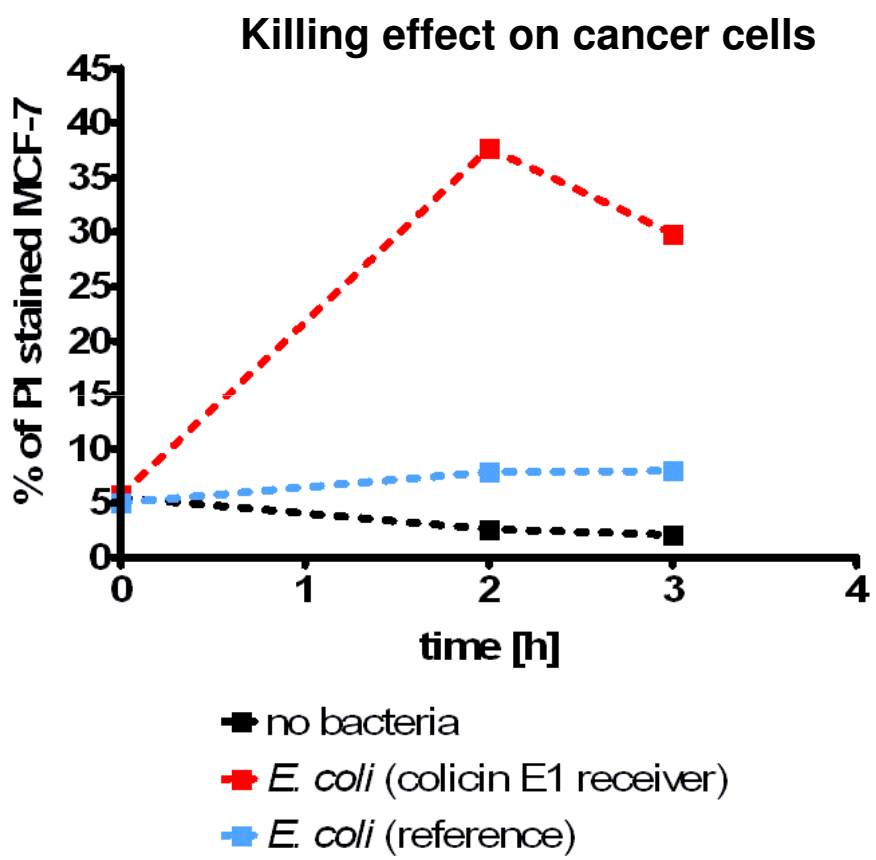




➡ **Constructed Killer-Prey-System is functional with high efficiency**

Cancer Cell Test

Toxic Effect on eukaryotic cancer cells



- Cancer cells (MCF-7) treated with colicin E1 producing killer cells
- Propidium iodide (PI) used to detect dying cells
- Percentage of PI stained cancer cells, thus dying ones were measured over time

⇨ Colicin E1 producing killer cells are able to kill eukaryotic cancer cells

⇨ Approach for medical treatment

Sensing-Colicin-Killing Modeling

Model overview

Studied spatio-temporal behaviour of predator-prey system using Partial Differential Equations (PDE)

... towards them by using the AI-2 gradient.

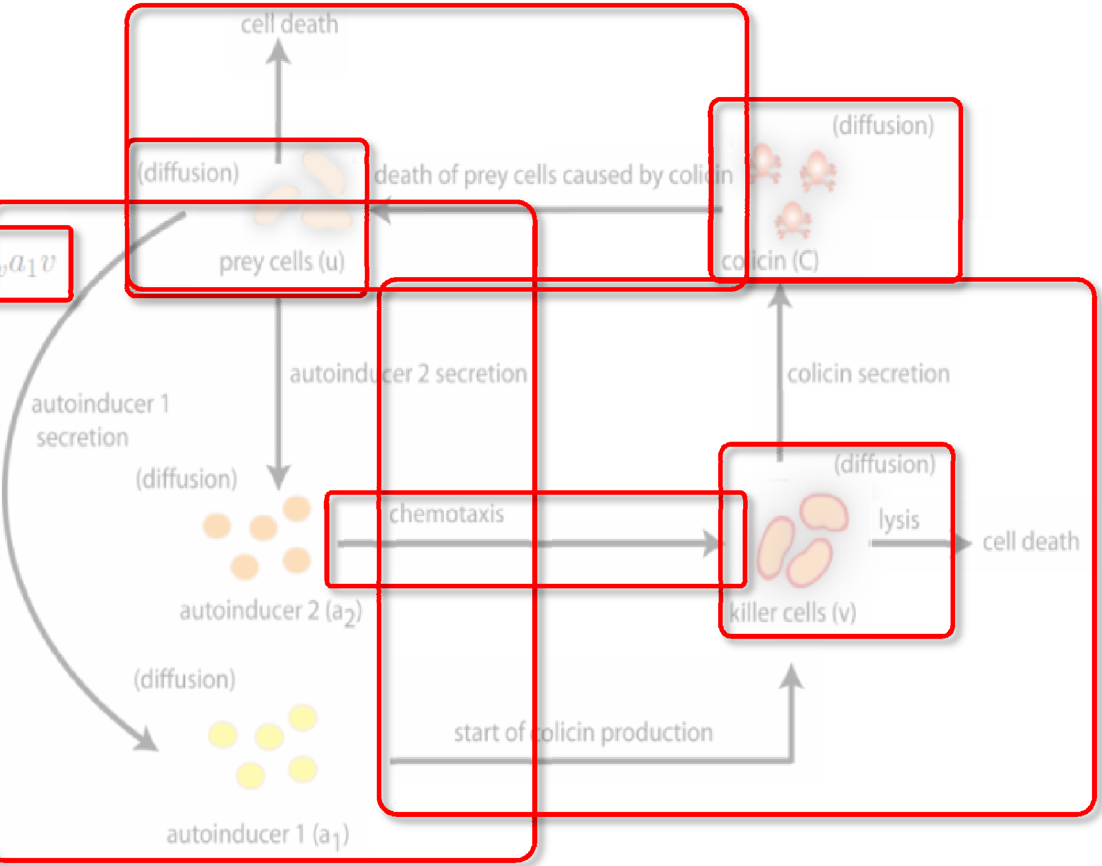
$$\frac{\partial u}{\partial t} = D_u \Delta u - \delta_u c u$$

$$\frac{\partial v}{\partial t} = D_v \Delta v - \alpha \nabla (\chi(a_2) v \nabla a_2) + \delta_v a_1 v$$

$$\frac{\partial c}{\partial t} = D_c \Delta c + \beta_c \delta_v a_1 v$$

$$\frac{\partial a_1}{\partial t} = D_{a_1} \Delta a_1 + \beta_{a_1} u$$

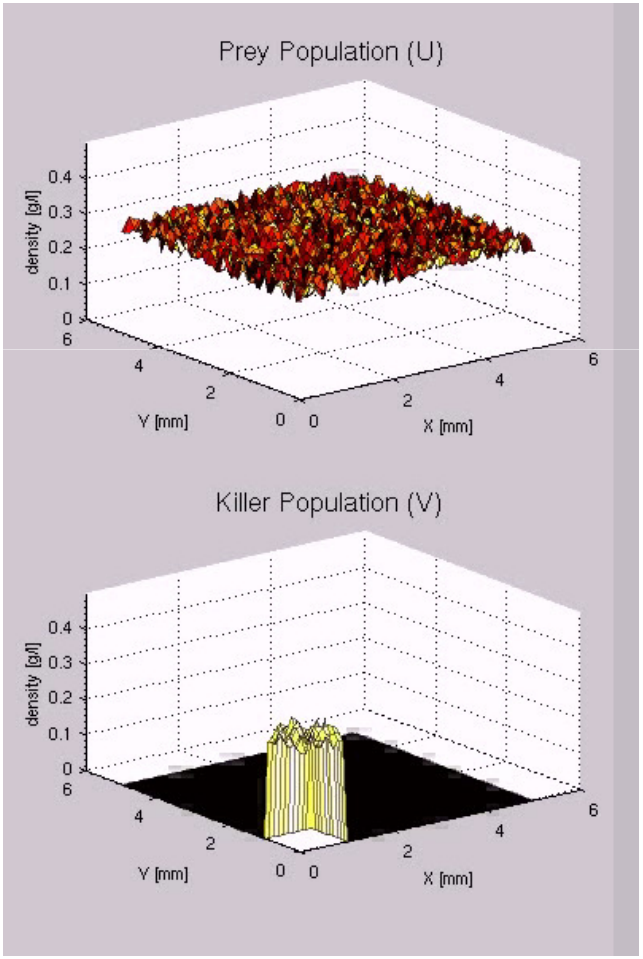
$$\frac{\partial a_2}{\partial t} = D_{a_2} \Delta a_2 + \beta_{a_2} u$$



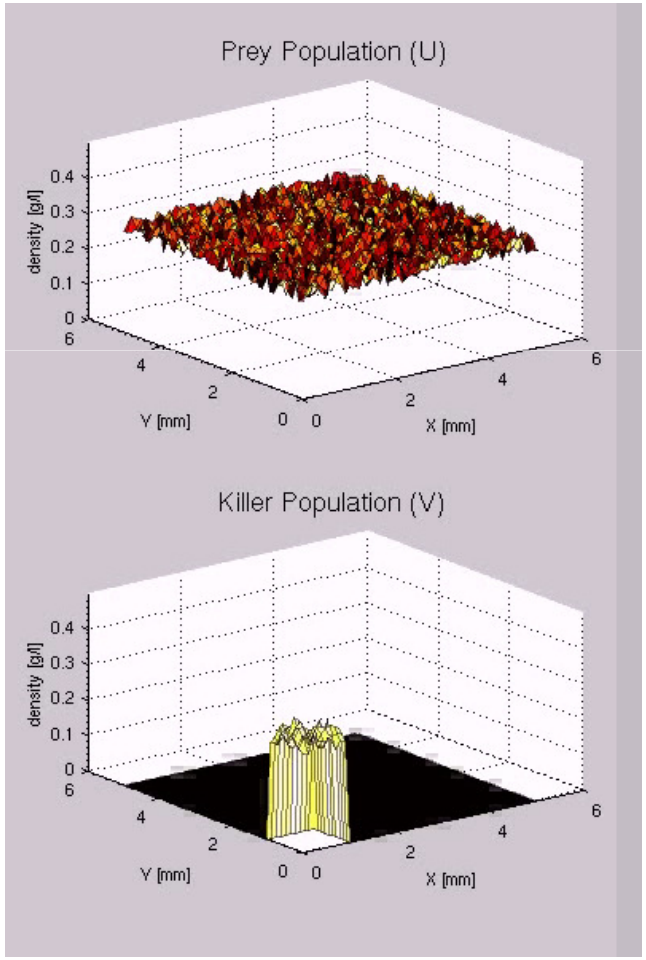
Sensing-Colicin-Killing Modeling

Simulation Results

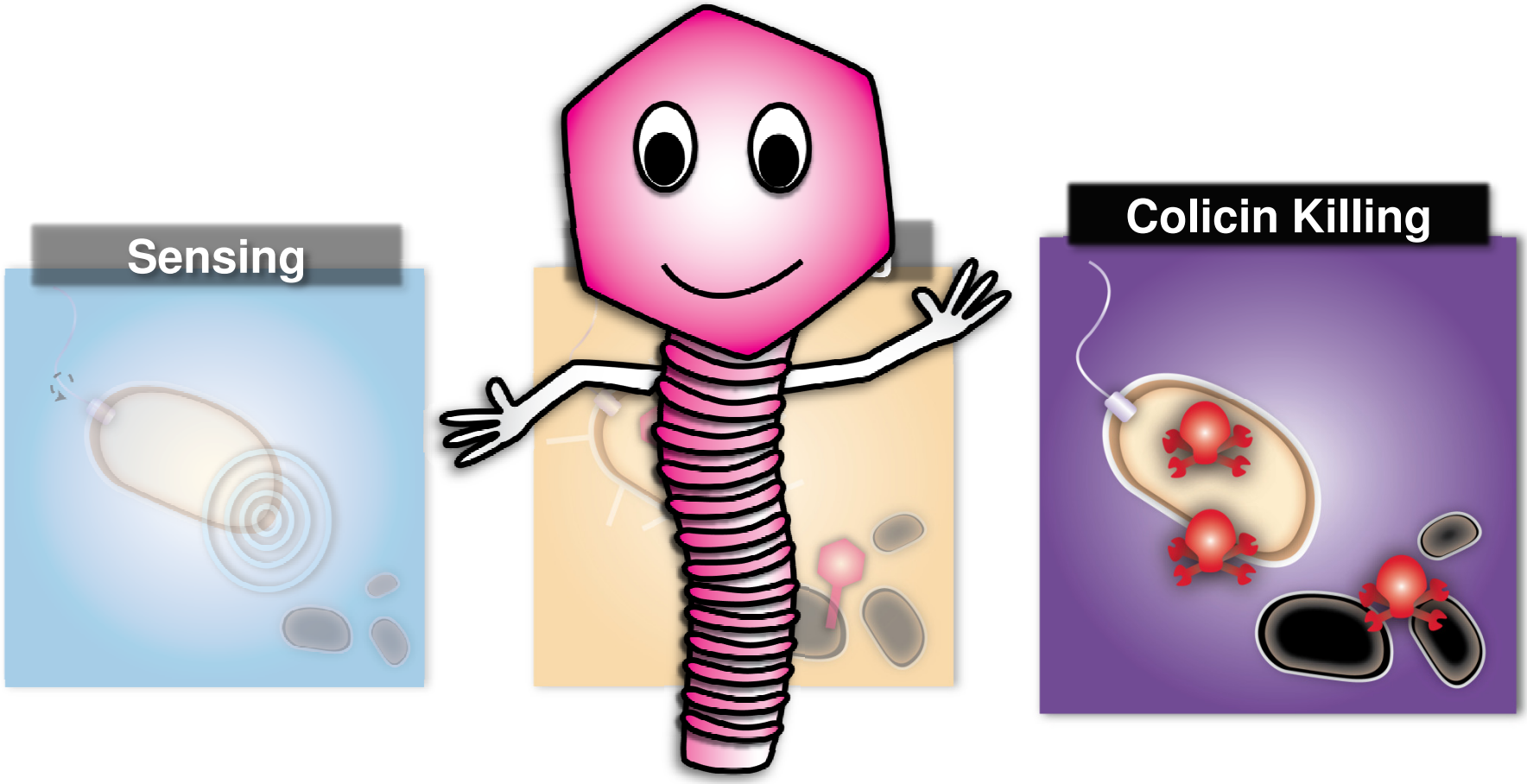
low chemotactic activity



high chemotactic activity



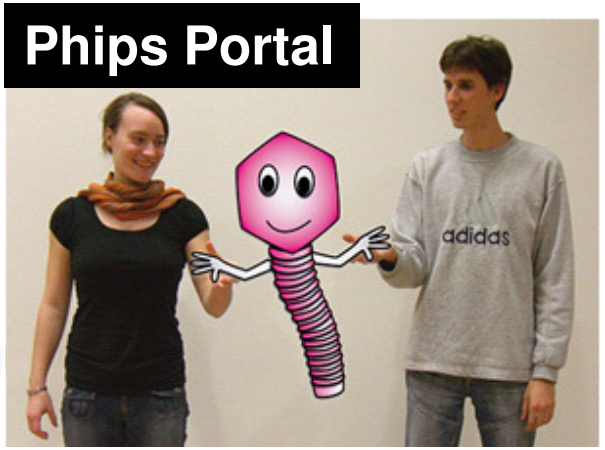
Overview Human Practice - Science Communication

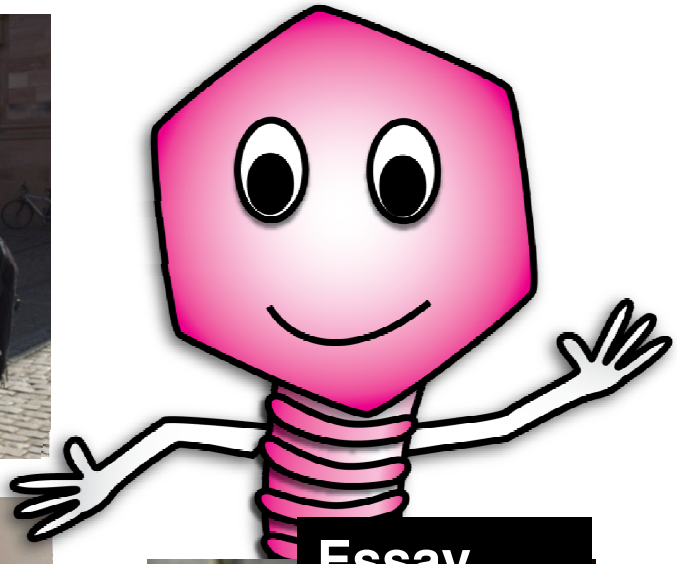


Human Practice - Science Communication

Surveys

Phips Portal





Open Day

Essay

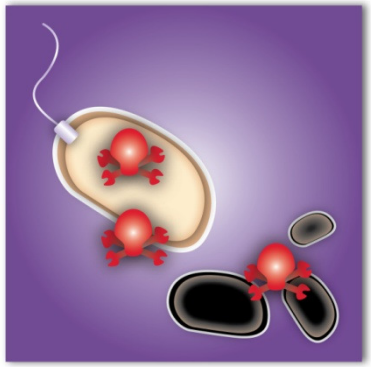
Media Work

http://2008.igem.org/Team:Heidelberg

Summary

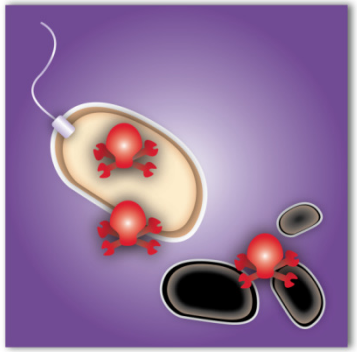
- ☑ Creation of AI-2 Sender Part
- ☑ Creation of chemotaxis LuxQ-Tar fusion receptor
- ➡ Expressed and located in Membrane

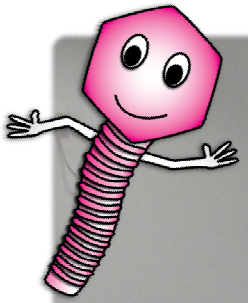
- ☑ Cloning and Characterization of bacteriophage λ cI
- ☑ Assembly of modified bacteriophage λ
- ➡ Conjugation of modified bacteriophage λ



- ☑ Creation of AI-1 Sender Part
- ☑ Creation of AI-1 inducible colicin producing killer part
- ➡ Verification of the colicin prey-killer system
- ➡ Killer activity on eukaryotic cancer cells

At the end of the day…





Acknowledgements

We thank all our supporters

BioQuant
MODEL base of LIFE

HELMHOLTZ ASSOCIATION
Alliance on Systems Biology

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

ViroQuant

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KTF
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LANGE + PFLANZ

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Marcus Kaufhold
Photojournalist

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