Engineering Bacteria to Target Tumors

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Workshop on controllable cell-based therapies

City University London

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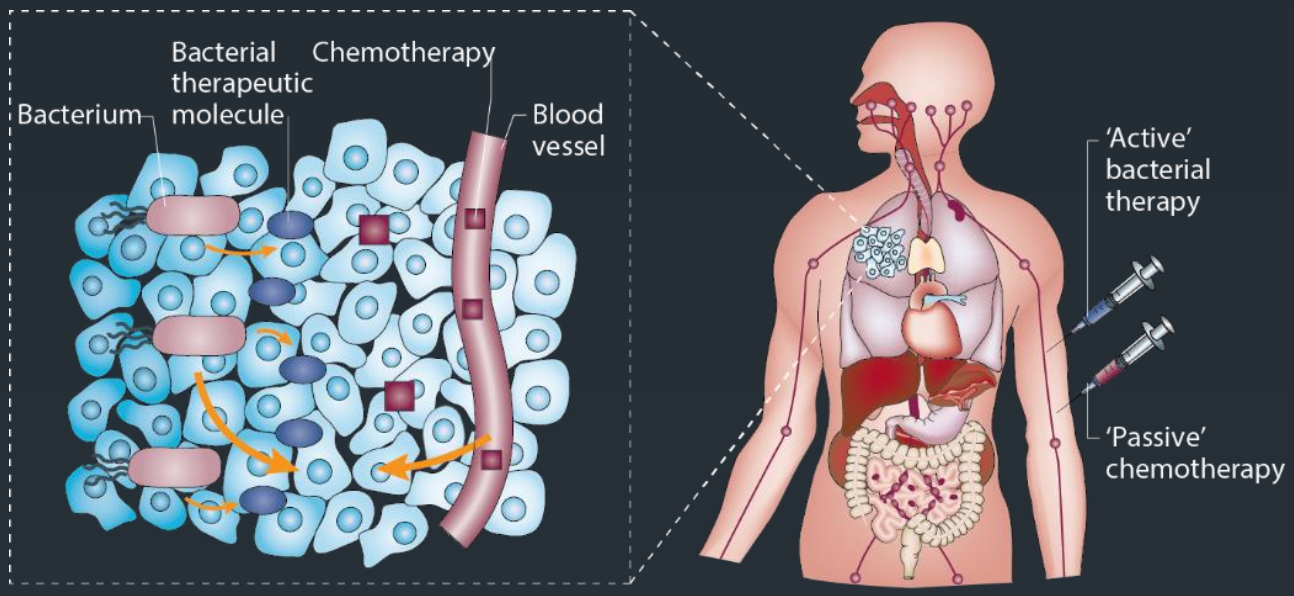
University of Massachusetts
Chemical Engineering

What is Bacterial Anticancer Therapy?

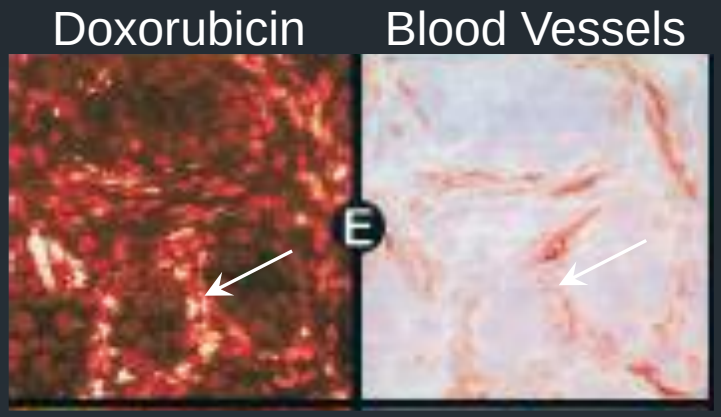


- Overcomes limitations of small-molecules and biologics

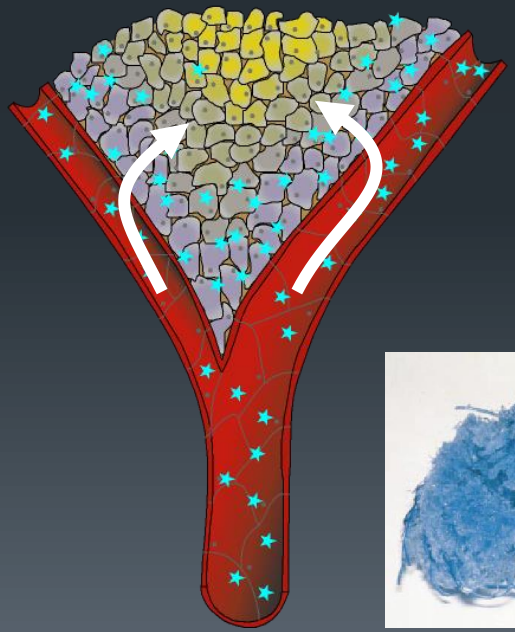
- Engineered bacteria are injected intravenously
- Specifically accumulate in tumors and metastases
- Penetrate tumor interstitium
- Deliver molecules intra- and extracellularly

Limitations of small molecules and biologics

- Many tumors are resistant to hormone therapy and chemotherapy
  - e.g. Triple-negative breast cancer does not respond to Herceptin or Tamoxifen
  - Intravenous delivery leads to systemic toxicity
  - Poor penetrations leaves regions untreated
  - Exacerbated by chaotic vessels
- Metastatic disease is hard to treat
- Difficult to design biologics that cross the cell membrane

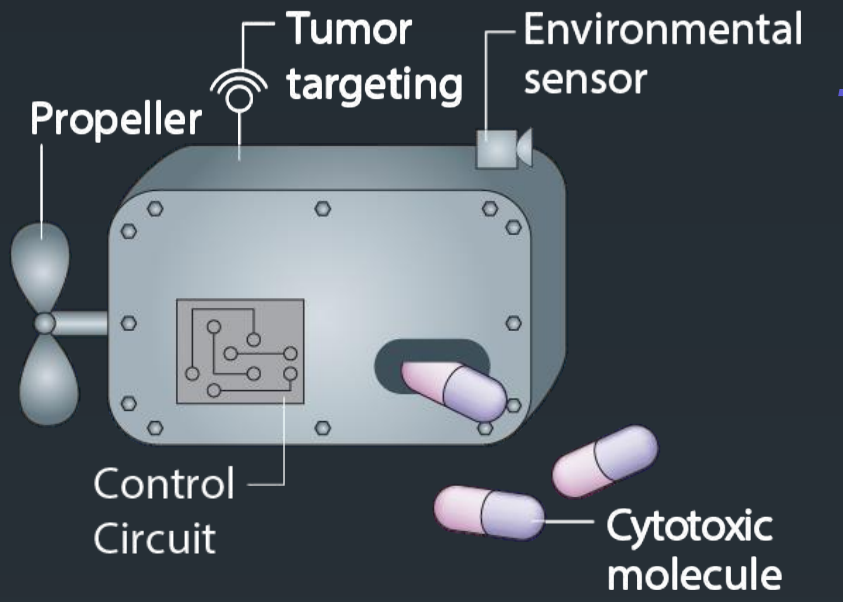


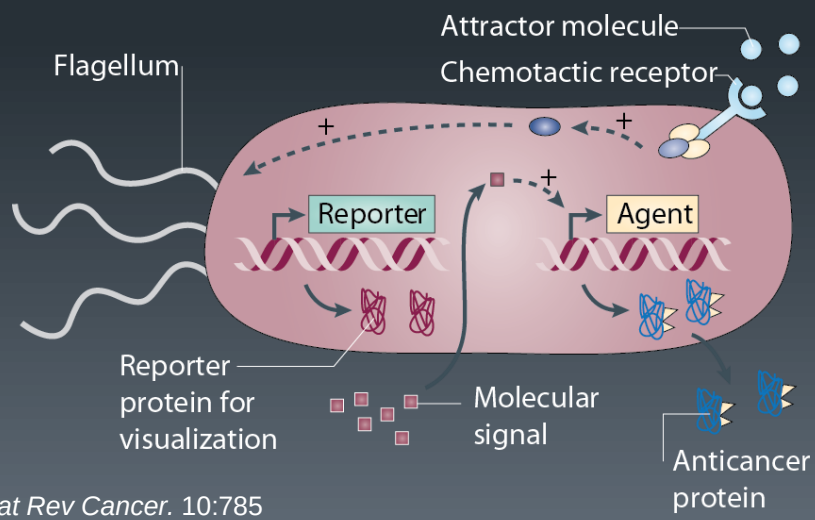
Human breast cancer biopsies



Lankelma et al. 1999. *Clinical Caner Res*. 5:1703
Minchinton and Tannock 2006 *Nat Rev Cancer* 6:583
Jain and Forbes 2001 *PNAS* 98:14748; Less et al. 1997 *Microcirculation* 4:25

Bacteria as *Tiny Robot Factories*





The ideal cancer therapy:

- Target tumors and metastases
- Penetrate tissue
- Deliver therapeutics
- Sense the environment
- Intelligent expression

Bacteria are a platform for an array of therapies

Forbes. 2010. *Nat Rev Cancer.* 10:785

Advantages over Small Molecules and Biologics

Bacterial Therapeutics:

- Target tumors and metastases
- Penetrate interstitial tumor tissue
- Delivery of therapeutic proteins extracellularly
- Delivery of proteins, DNA and siRNA intracellularly
- Have controllable expression and release
- Produce therapeutics *in situ* continuously
- Would be cost effective to manufacture

Who would benefit from bacterial therapy?

- Patients with late-stage, metastatic cancer
- Patients with drug-resistant cancer
  - For example, triple-negative breast cancer
- Tumors that can't be treated in any other way

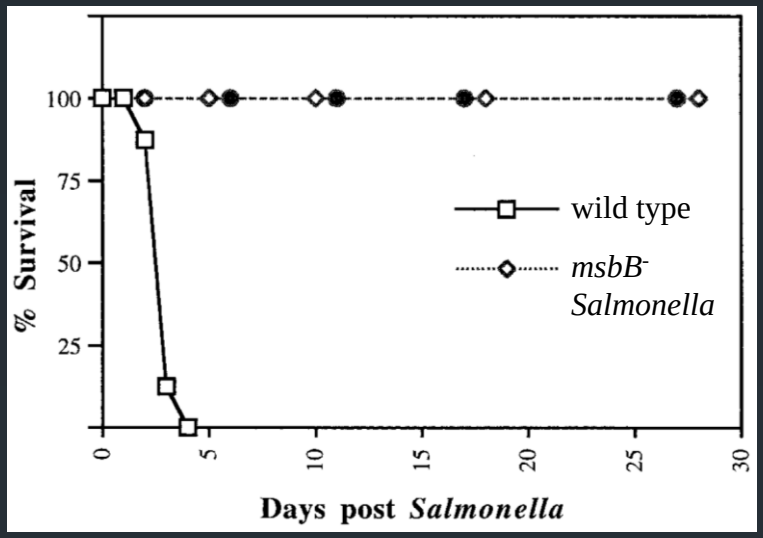
Risks and Methods to Ensure Safety

What are the risks?

- Immune response and septic shock
  - Excessive TNF production can cause an immune reaction and potentially death
- Infection
- Release into the environment

Bioengineering methods to ensure safety

- *msbB*$^{-}$ deletion alters lipid A, reduces TNF and prevents sepsis. LD_{50} is 10,000-fold greater
- Auxotrophic attenuation (*aroA*$^{-}$, *purA*$^{-}$)
- Density sensing focuses production to tumors
- Failsafe gene circuit
  - Controlled lysis without maintenance molecule
  - Would prevent unintentional infection and escape



- *Salmonella* have test tried in human clinical trials with minimal toxicity

Toso et al. 2002. *J Clin Oncol*. 20:142; Low et al. 1999. *Nat Biotech. 17:37*

Demonstration of Advantages

Targeting of tumors

- Breast tumors
- Others have demonstrated accumulation in colorectal, cervical, glioma, Lewis-lung ovarian, pancreatic, and prostate cancer

Targeting of metastases

- Pulmonary and hepatic metastases

Continuous production

- TRAIL (TNF-related apoptosis-inducing ligand)
- SAH (*Staphylococcus aureus* α-hemolysin)

Intracellular delivery

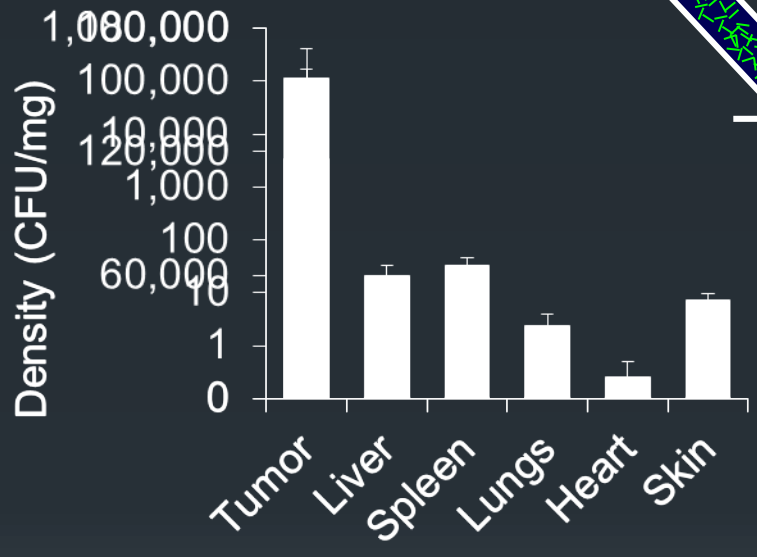
- Interaction of PP1 and NIPP1
- Example of targeting a signal transduction pathway

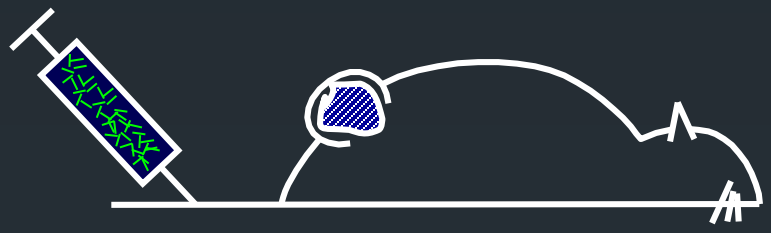
Delivery of genetic material

Control of production

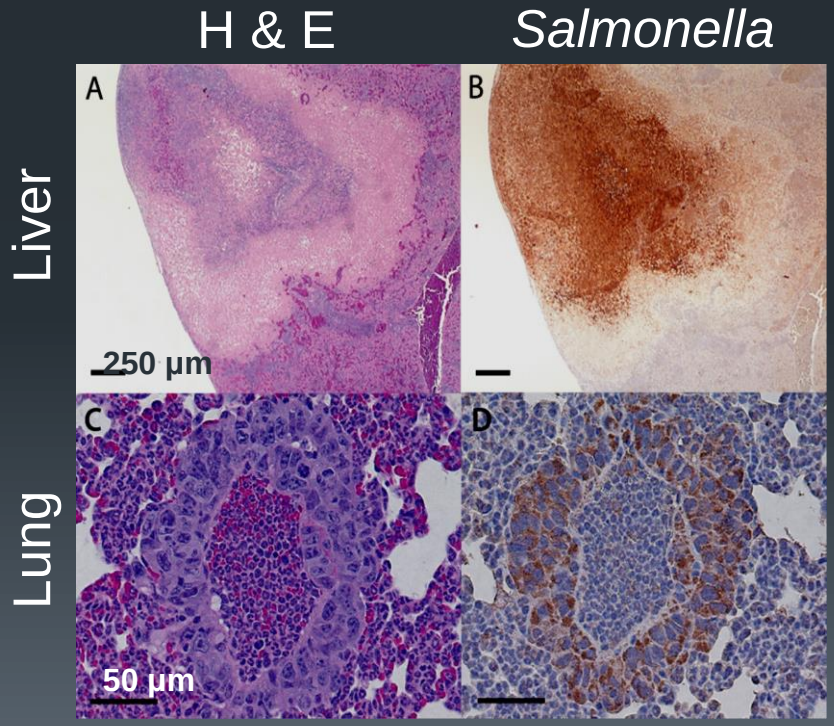
- Quorum Sensing
- Cell-cell communication

Salmonella accumulate in tumors and Hepatic and Pulmonary metastases





Biodistribution, one week following systemic tail vein injection



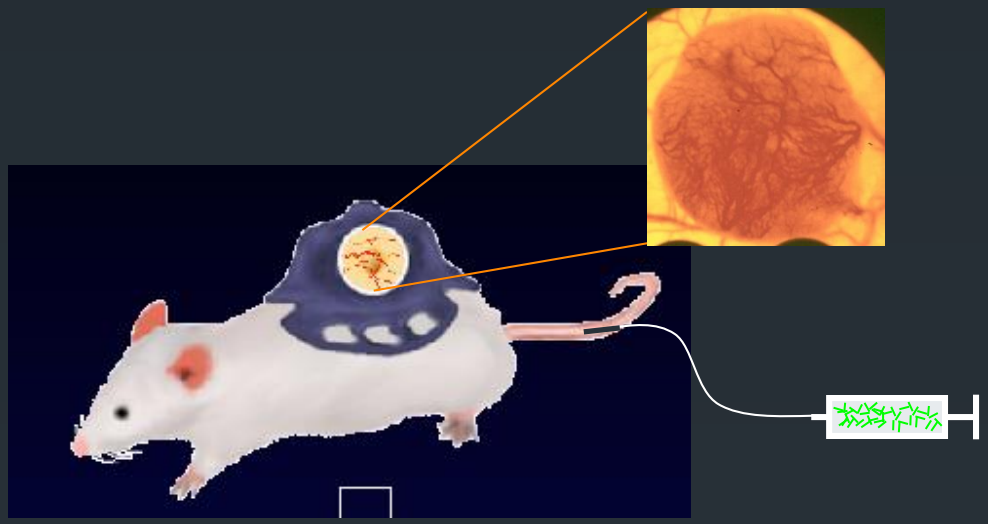
Forbes et al. 2003. *Cancer Res*. 63:5188

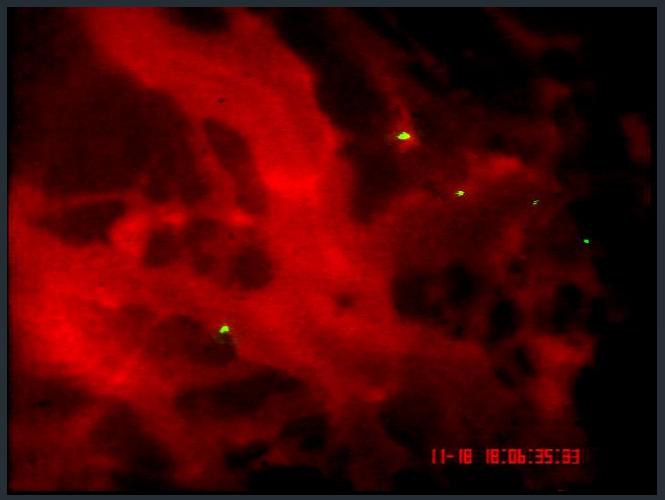
Mechanisms of Bacterial Accumulation

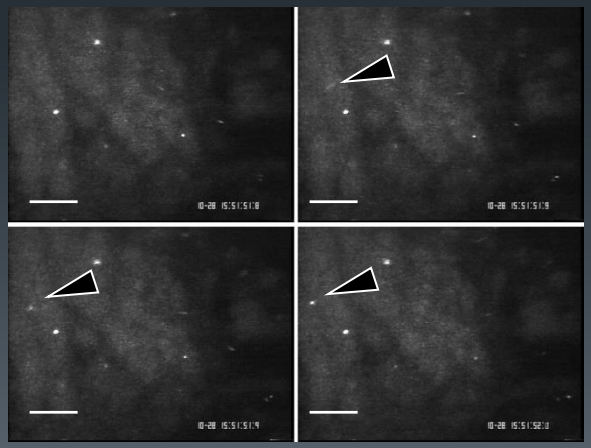
- Inflammation induced blood influx
- Filtration by vessels
- Immune-privileged environment of tumors
- Chemotaxis
- Preferential growth

Zhang et al. 2014. *Int J Cancer*. 135:647
Ganai et al. 2011. *Cancer Gene Ther*. 18:457
Kasinskas and Forbes. 2007. *Cancer Res*. 67:3201
Kasinskas and Forbes 2006. *Biotechnol Bioeng*. 94:710
Forbes et al. 2003. *Cancer Res*. 63:5188

Bacteria Adhere Sparsely to Tumor Vasculature





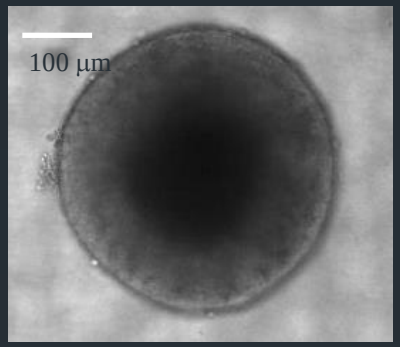


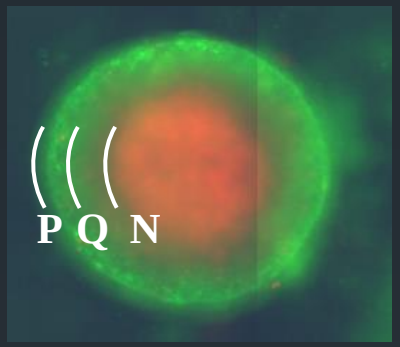
- Dorsal Skin Fold Chamber
- 2 million *Salmonella* injection per mouse
- Tumor vasculature observed for 1 hour

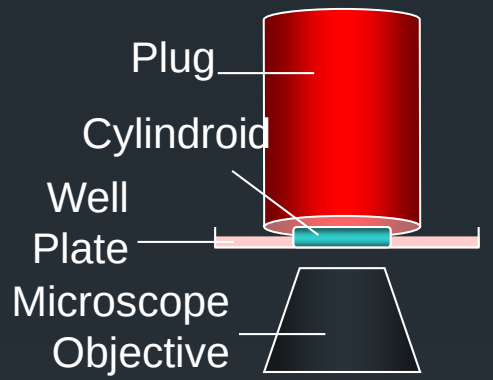
- **4 in 10,000 Adhere**

Forbes et al. 2003. *Cancer Res*. 63:5188

Bacteria Accumulate in Cylindroids



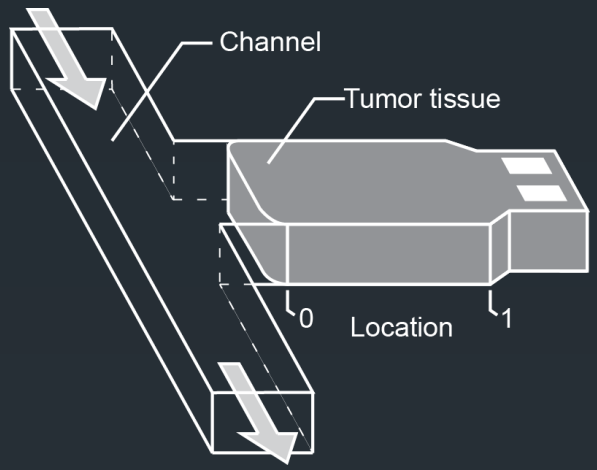


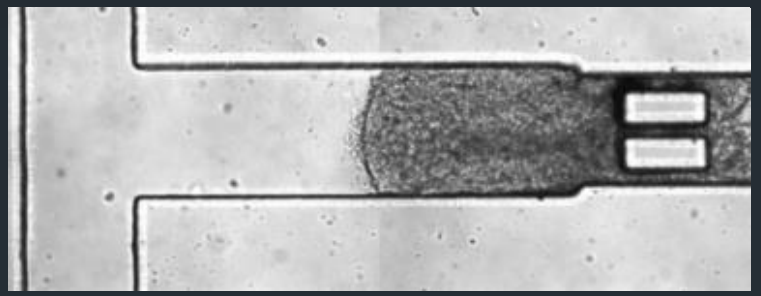


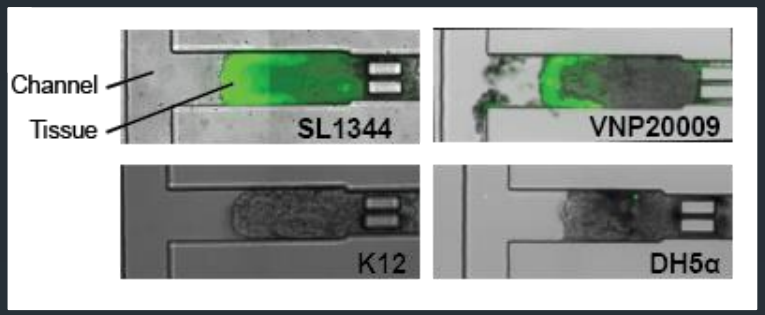
- *Salmonella* are attracted to dying cells
- Penetrate through tissue, between cells
- Proliferate in tissue

Kasinskas, Forbes. 2006. *Biotech Bioeng*, 94:710

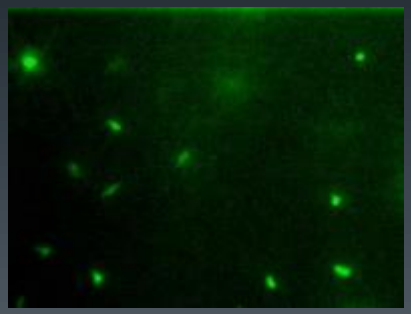
Motility Increases Accumulation



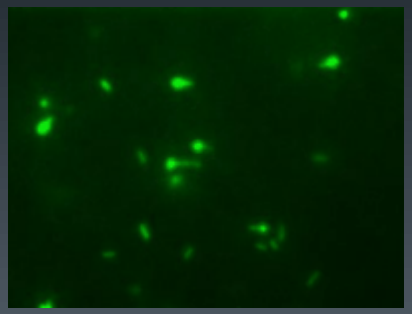


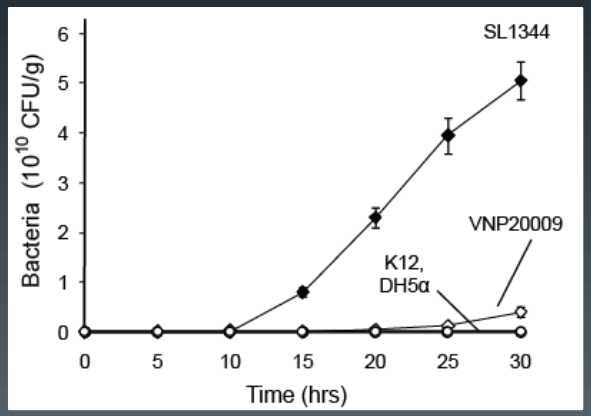


Salmonella



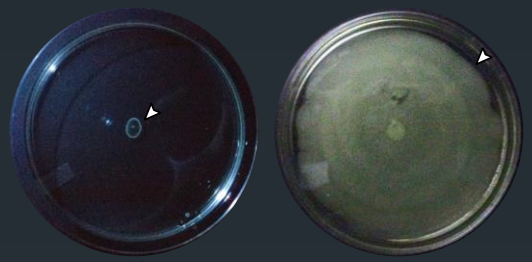
E. coli

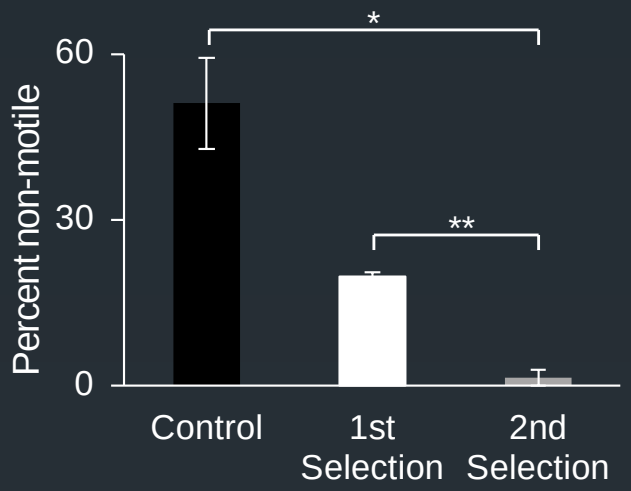


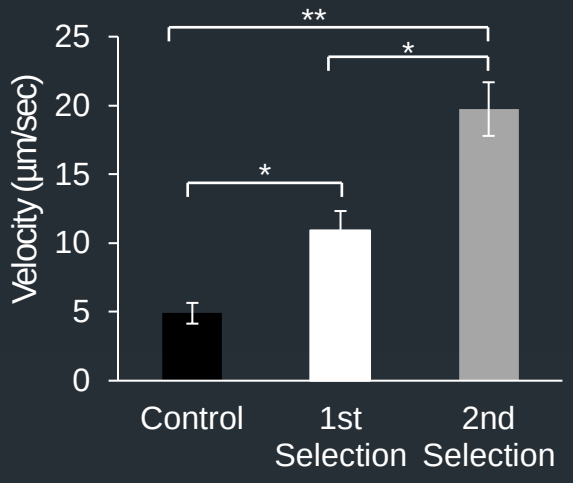


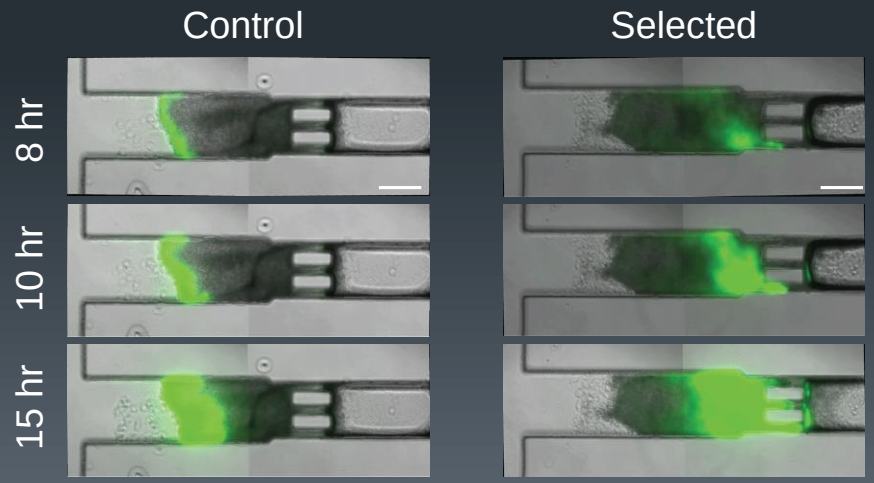
Toley BJ, Forbes NS. 2012. *Integr Biol.* 4:165-76
Walsh CL et al. 2009. *Lab on a Chip*. 9:545
Toley BJ et al. 2011. *J Vis Exp* DOI: 10.3791/2425

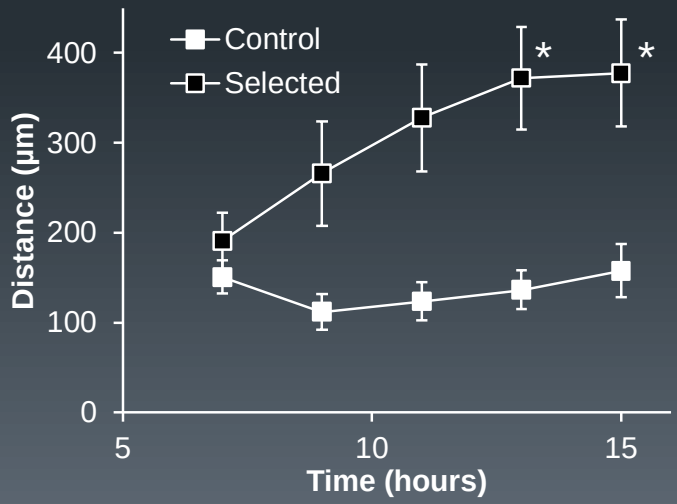
Selection Increases Penetration







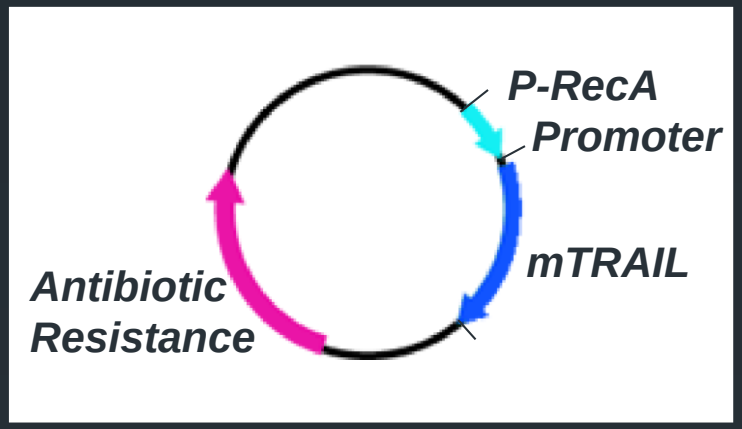


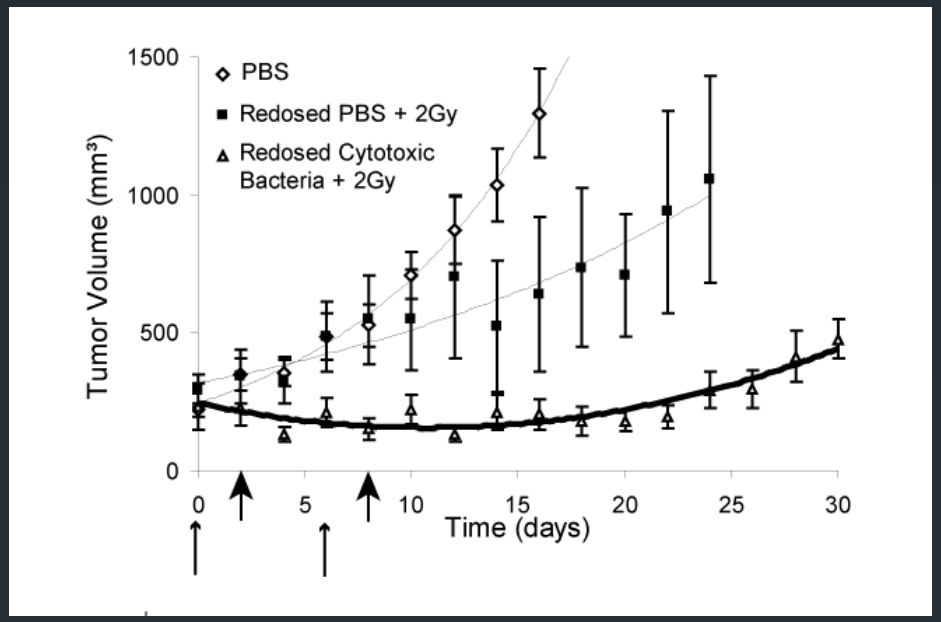


Thornlow et al. 2015. *Biotechnol Bioeng*. 112:2397

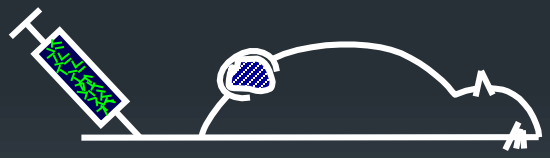
Extracellular therapeutic delivery

Delivery of TRAIL

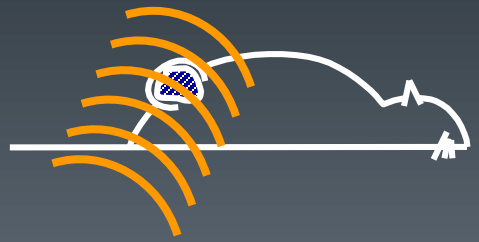




Inject



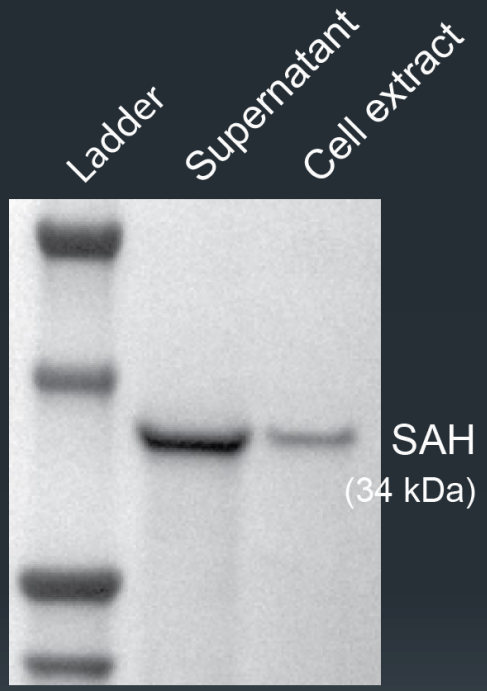
Induce with irradiation

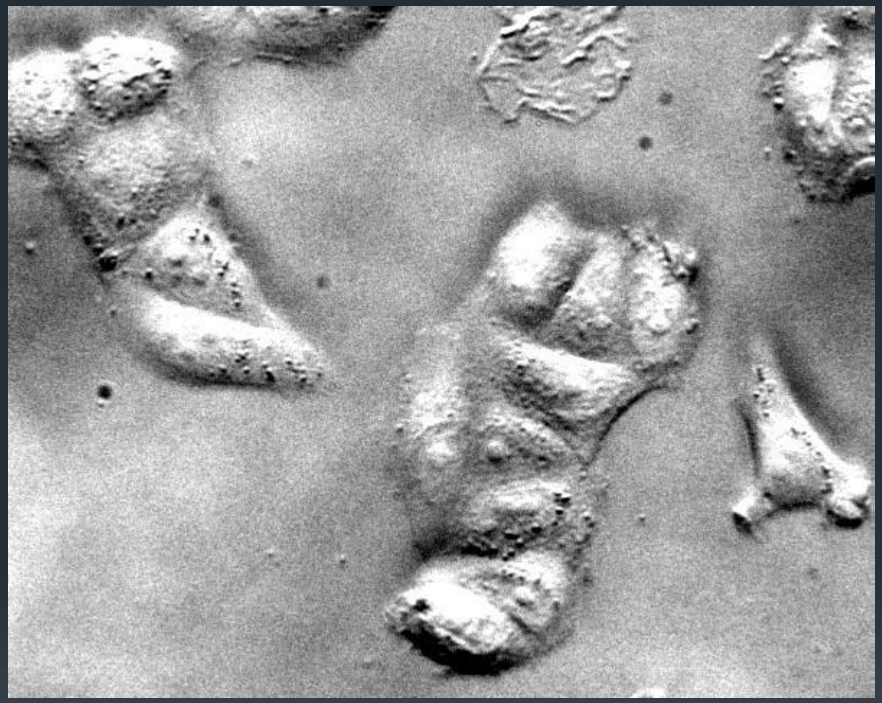


- *P(RecA)* promoter is activated by DNA damage and irradiation
- mTRAIL induces apoptosis
- Delayed growth 30.3 days

Ganai et al. 2009. *Brit J Cancer* 101:1683-91

Staphylococcus aureus α-hemolysin



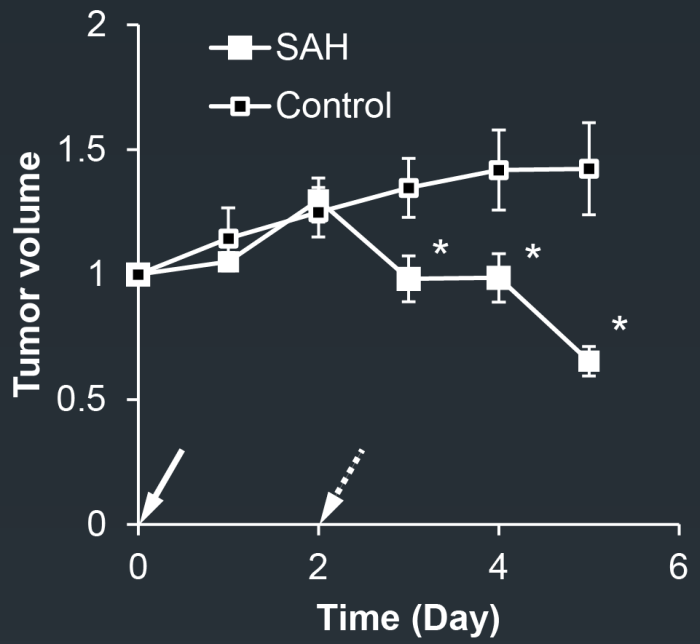


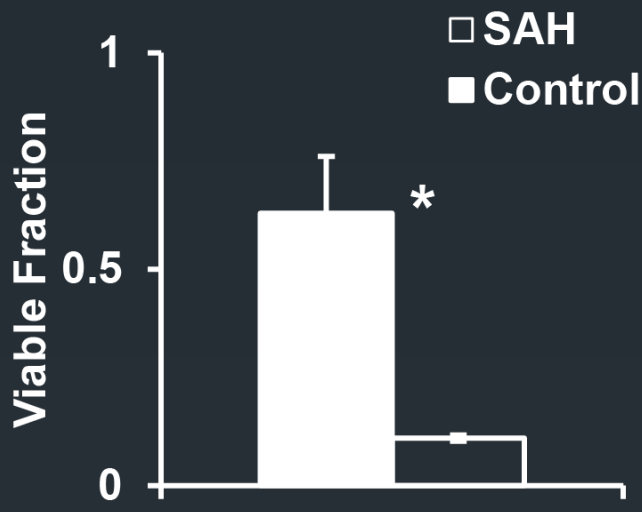
- Naturally secreted by *E. coli* and *Salmonella*
- Begins killing cells in less than 5 minutes

150 min movie, 5 minute intervals

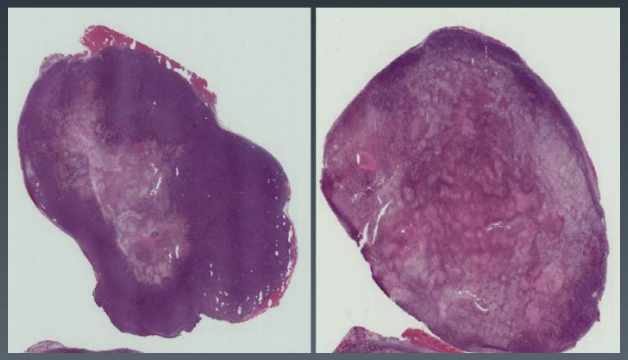
St. Jean et al. 2014. *Mol Ther*. 22:1266

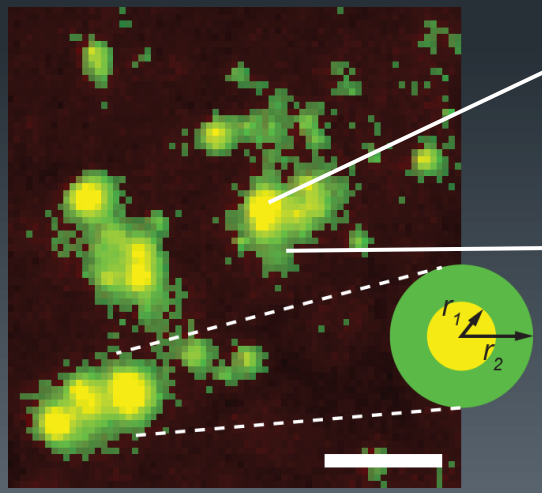
SAH Shrinks Tumors





Control SAH

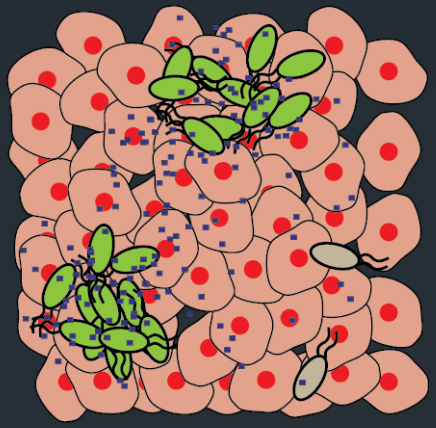




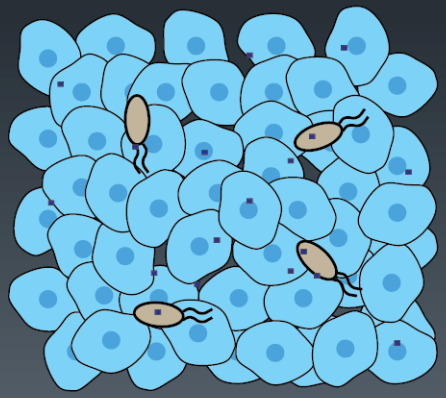
St. Jean et al. 2014. *Mol Ther*. 22:1266

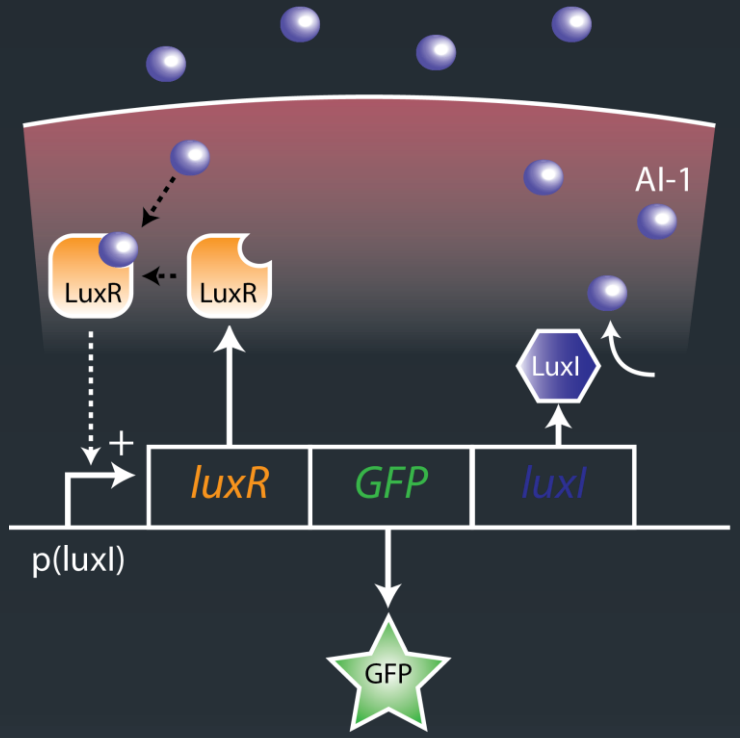
Density Sensing Activates Expression in Tumors

Tumor Tissue



Healthy Tissue

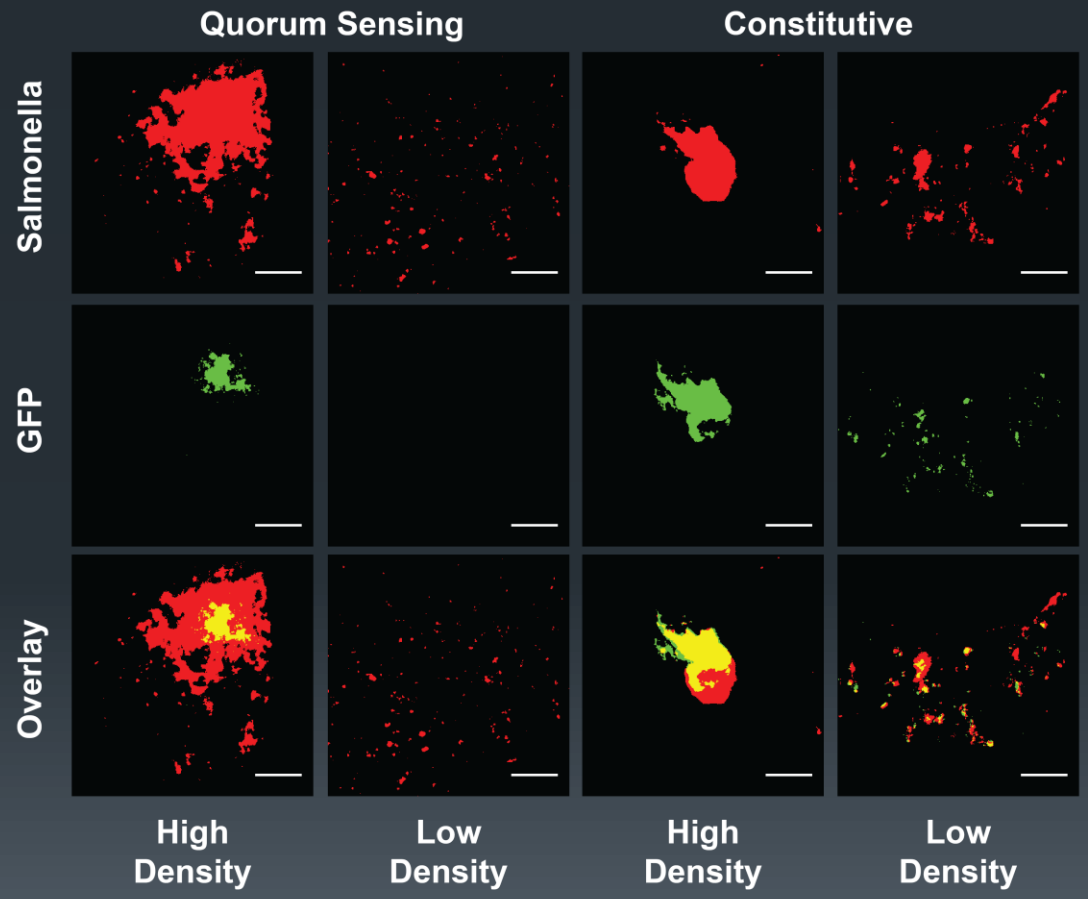


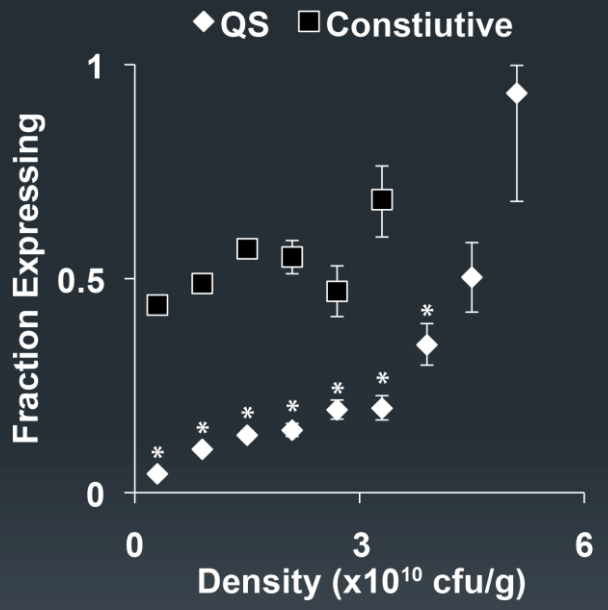


- Quorum sensing circuit dependent on density
- No inducer required
- Inactive at low density
- Activation occurs at densities only seen within tumor tissue

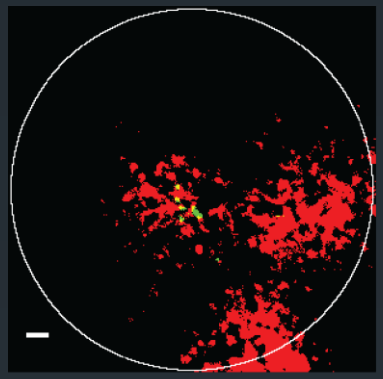
Swofford et al. 2015. *PNAS.* 112:3457

Density Controls Expression



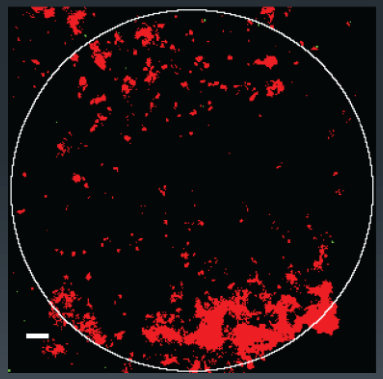


Distance also Controls Expression



ON

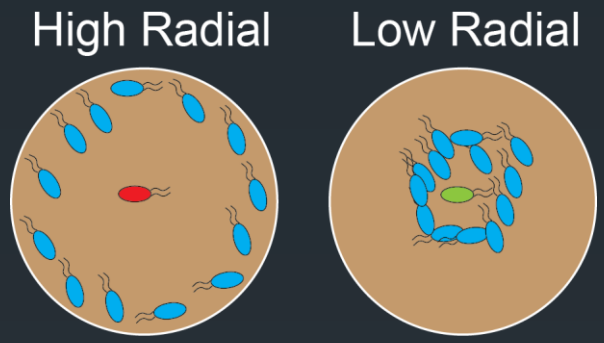
r = 74µm

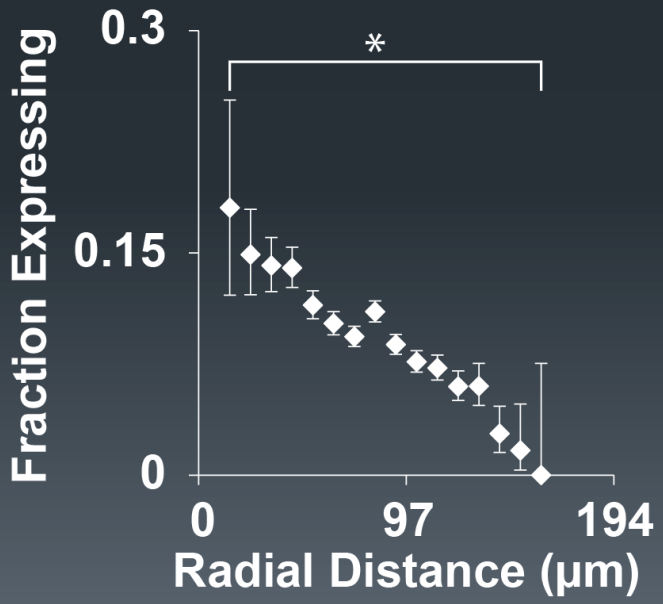


OFF

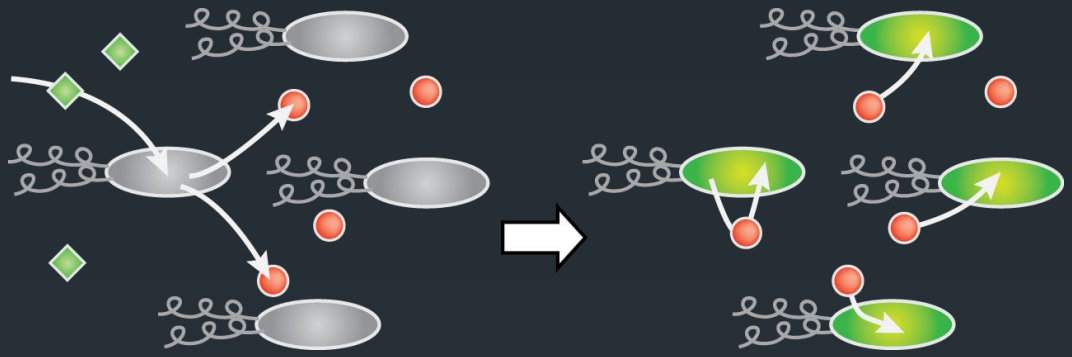
r = 145µm

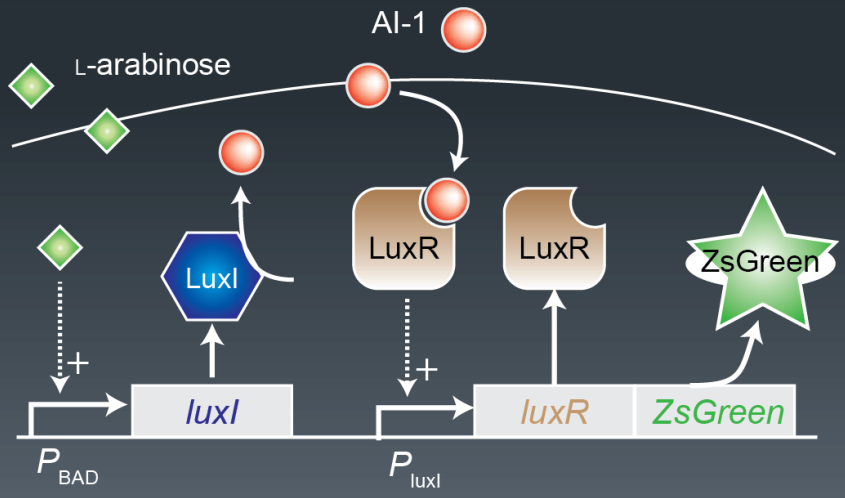
Density = 1.2x1010 cfu/g





Amplification with Cell-Cell Signaling



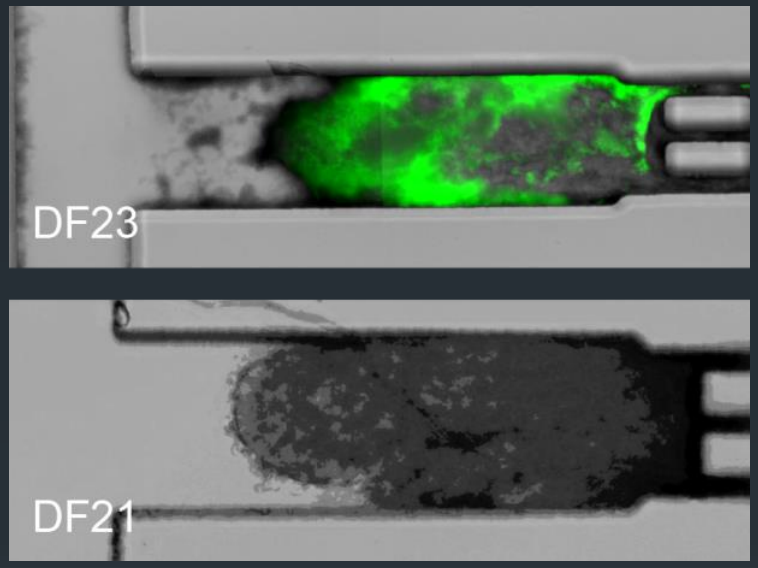


Activate Neighbors to:

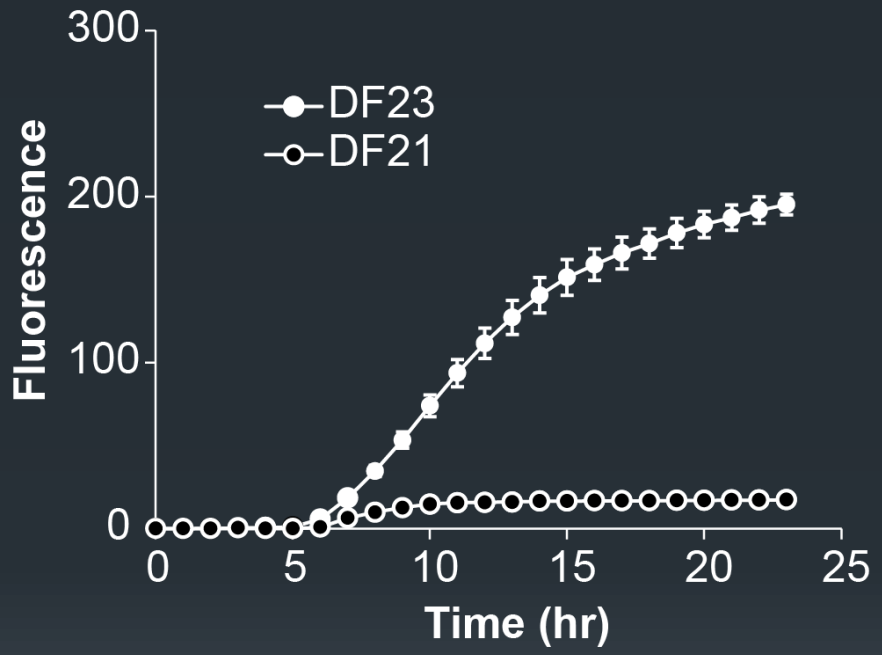
- Amplify production
- Increase sensitivity
- Prolong expression

Dai et al. 2013. *Biotechnol Bioeng*. 110:1769

Communication in Tissue



0.02% L-arabinose, 4hr induction



- Increased production in tissue 200 fold
- Increased sensitivity 10,000 fold

Dai et al. 2013. *Biotechnol Bioeng*. 110:1769

Summary

- There are many advantages of bacterial therapy over small molecules and biologics
- Synthetic genetic tools can enhance these mechanisms and reduce risks

- *Salmonella* accumulate in tumors
- Delivery of therapeutic molecules kills cancer cells and reduces tumor burden
- Tumor-specific targeting enables use of strong therapeutics
- Quorum sensing focusses expression to tumors

- We are close to clinically relevant *Salmonella*

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