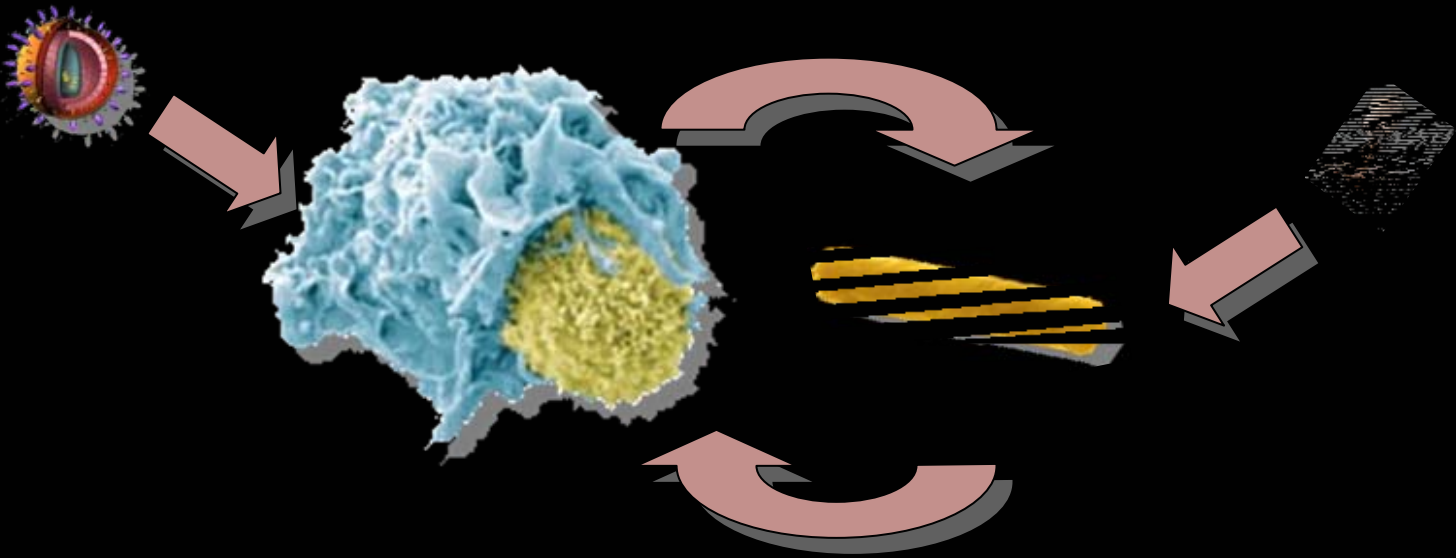


Adversity, Diversity and Design

Signal Processing in Cellular Regulatory Networks: Evolution, Design, Control and Physics



Prediction, Control and Design

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AGGCTTTTTCTT

Design of Therapeutic Bacteria

J. Christopher Anderson,
Christopher Voigt, Adam Arkin

UC-Berkeley
UC-San Francisco

Biological Attacks on Cancer

Gene Therapy

- Suicide *Listeria* used to deliver plasmids to macrophage genome

(Dietrich et al., 1998)

Therapeutic Bacteria

Live Vaccine

- *Shigella*, *V. cholerae*, Hepatitis B, *P. falciparum*
- *Salmonella*: Direct delivery of peptide antigens to immune cells

(Russman et al., 1998)

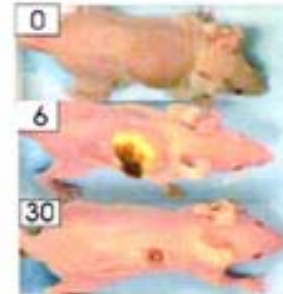
Immunotherapy

- *Mycobacterium bovis* BCG is common treatment of bladder cancer
- Engineered to release IL-18, improves macrophage cytotoxicity against cancer cells

(Luo et al., 2004)

Cancer

- Many bacteria localize to tumors
Clostridium novyi spores



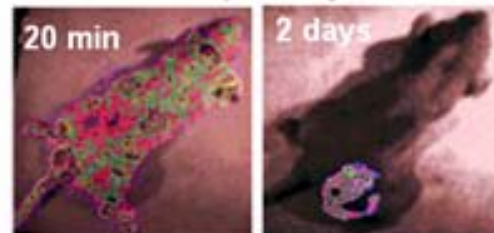
(Dang et al., 2001)

- *Salmonella* in phase I trials
- Live bacteria iv administered
- Single gene knockout to reduce septic shock

(Low et al., 1999)

Imaging

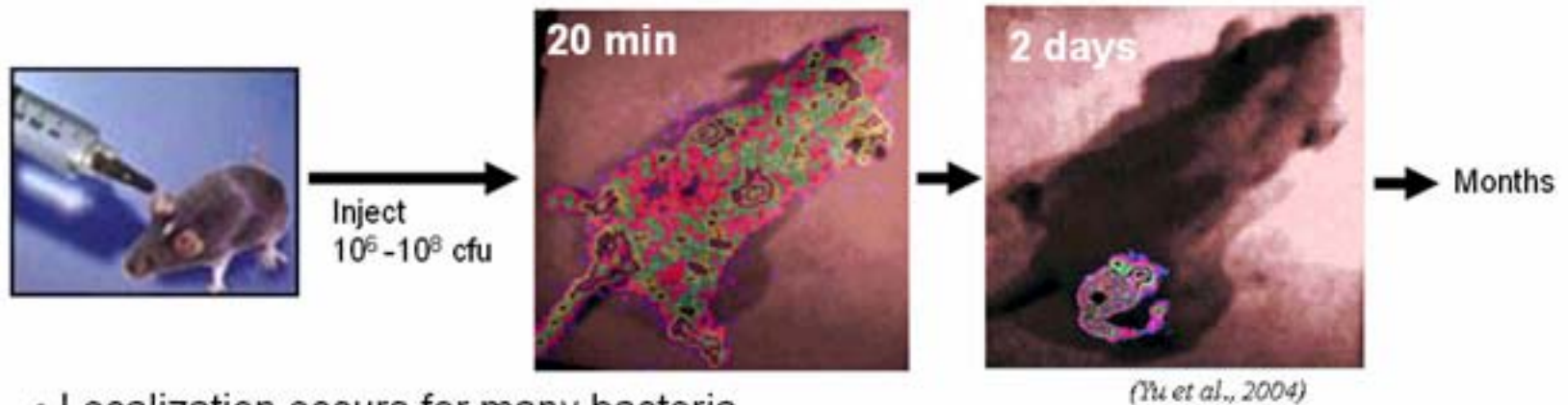
- *Salmonella* expressing luciferase



(Yu et al., 2004)

Many bacteria localize to tumors

- Intravenous injection of *Salmonella typhimurium*



- Localization occurs for many bacteria

Vibrio cholerae

Clostridium

Bifidobacterium

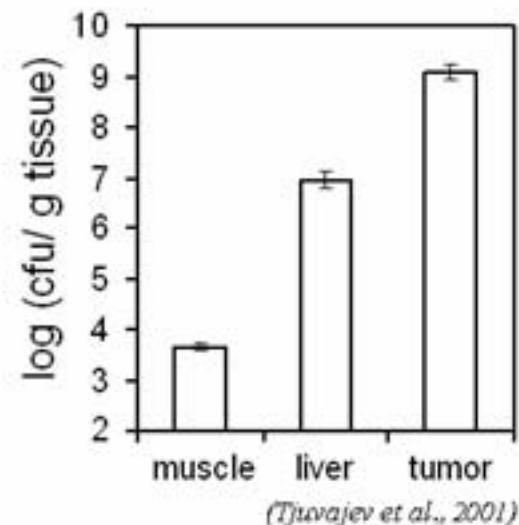
Listeria monocytogenes

Bordetella pertussis

Escherichia coli DH5a

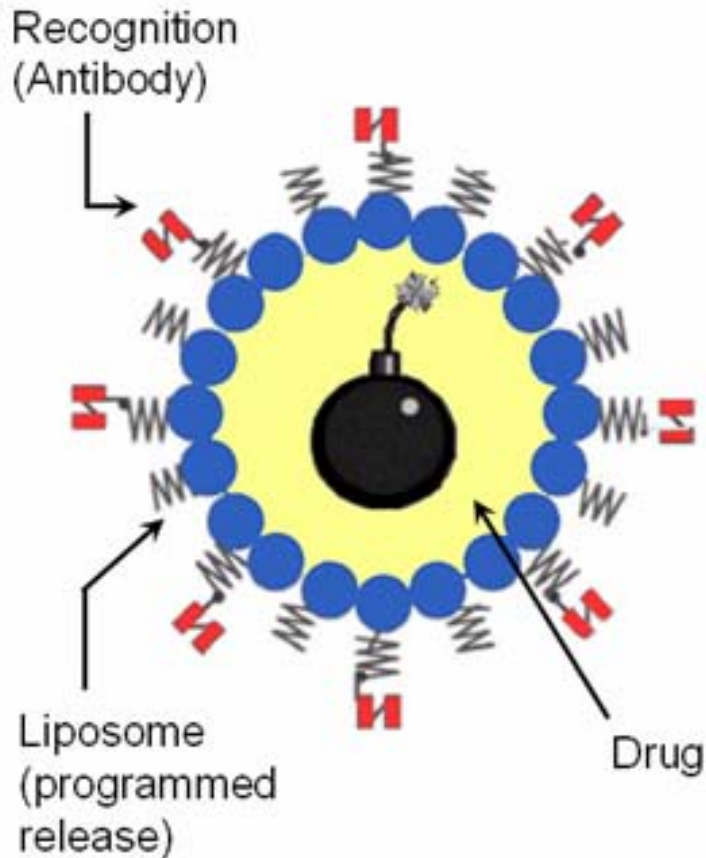
(Dang et al., 2001; Low et al., 1999; Yu et al., 2004)

- Phase I clinical trials in humans
- High doses have toxic side effects



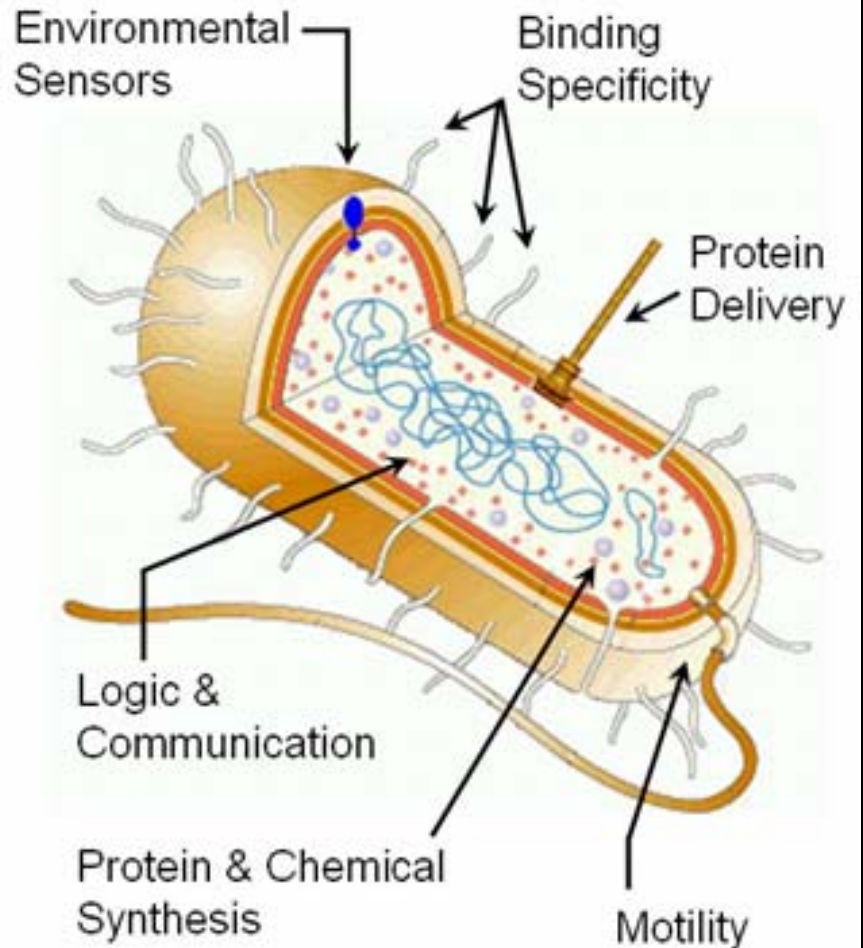
Really smart drugs

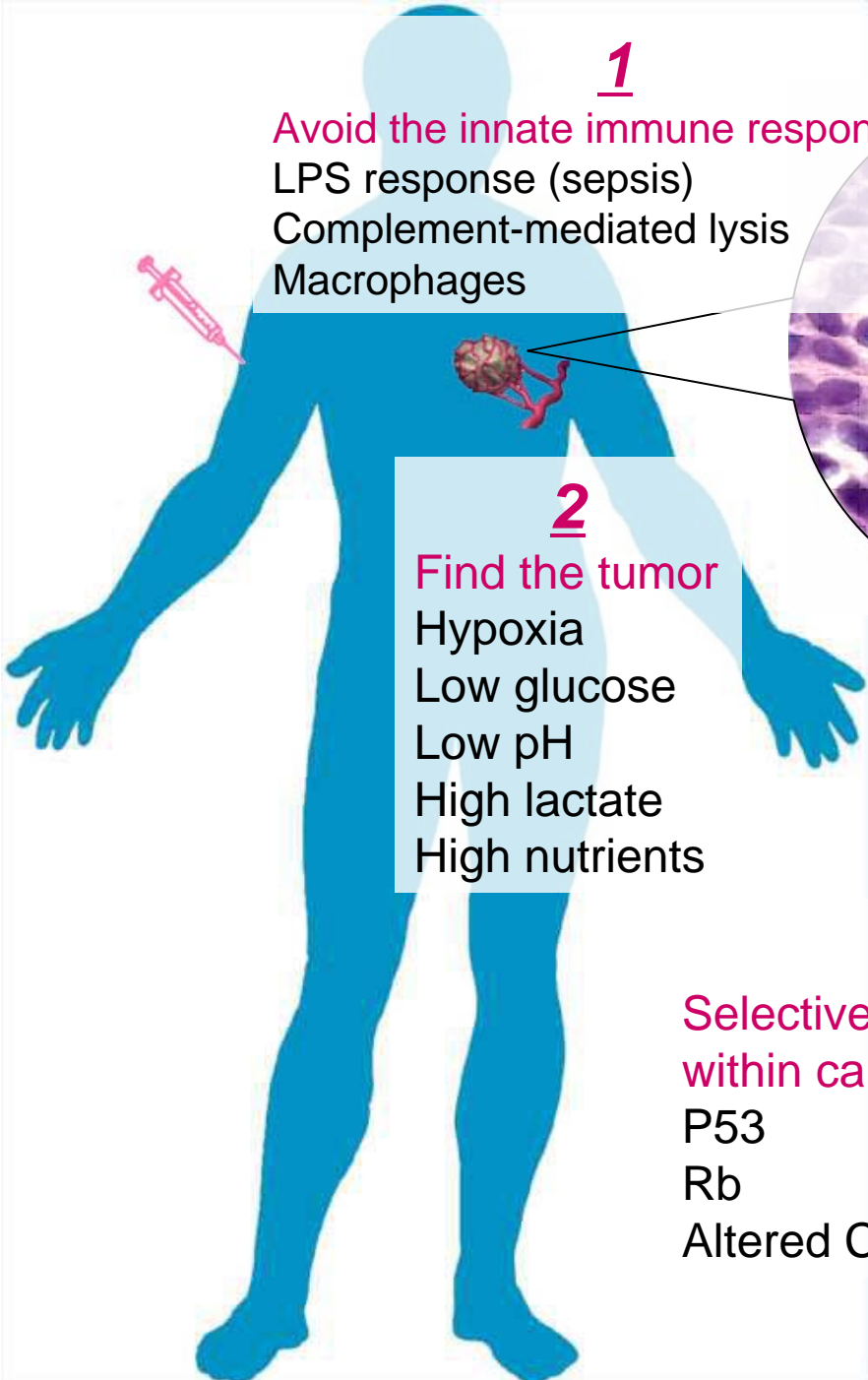
"Smart" Liposome



(Park et al., *J. Controlled Release*, 2001)

Bacteria



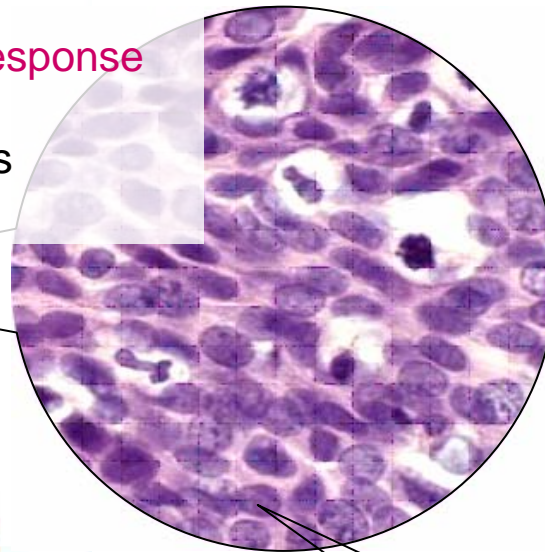


1

Avoid the innate immune response
LPS response (sepsis)
Complement-mediated lysis
Macrophages

2

Find the tumor
Hypoxia
Low glucose
Low pH
High lactate
High nutrients

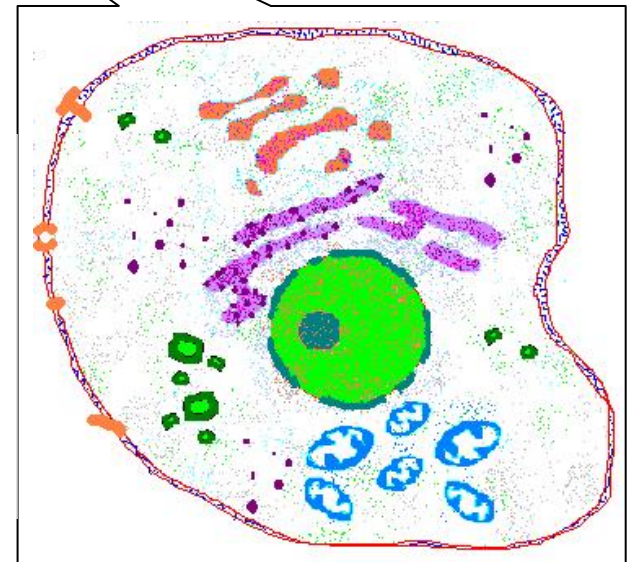


3

Selectively invade
cancer cells
TF-Antigen
Sialyl Lewis X
Her2

4

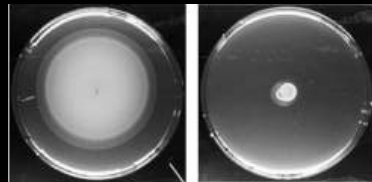
Selectively kill or spread
within cancer cells
P53
Rb
Altered Chromatin



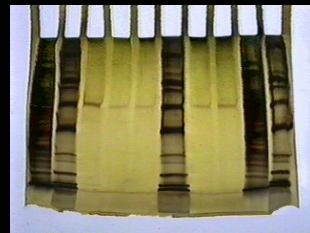
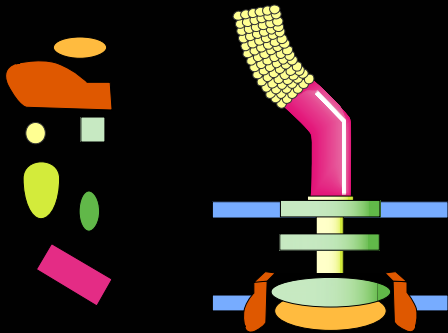
The Abstraction Hierarchy

E. coli strain JCA0934893

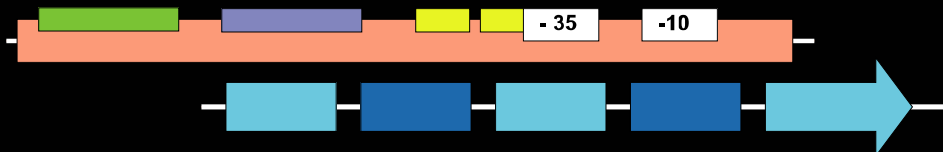
The System



Processes

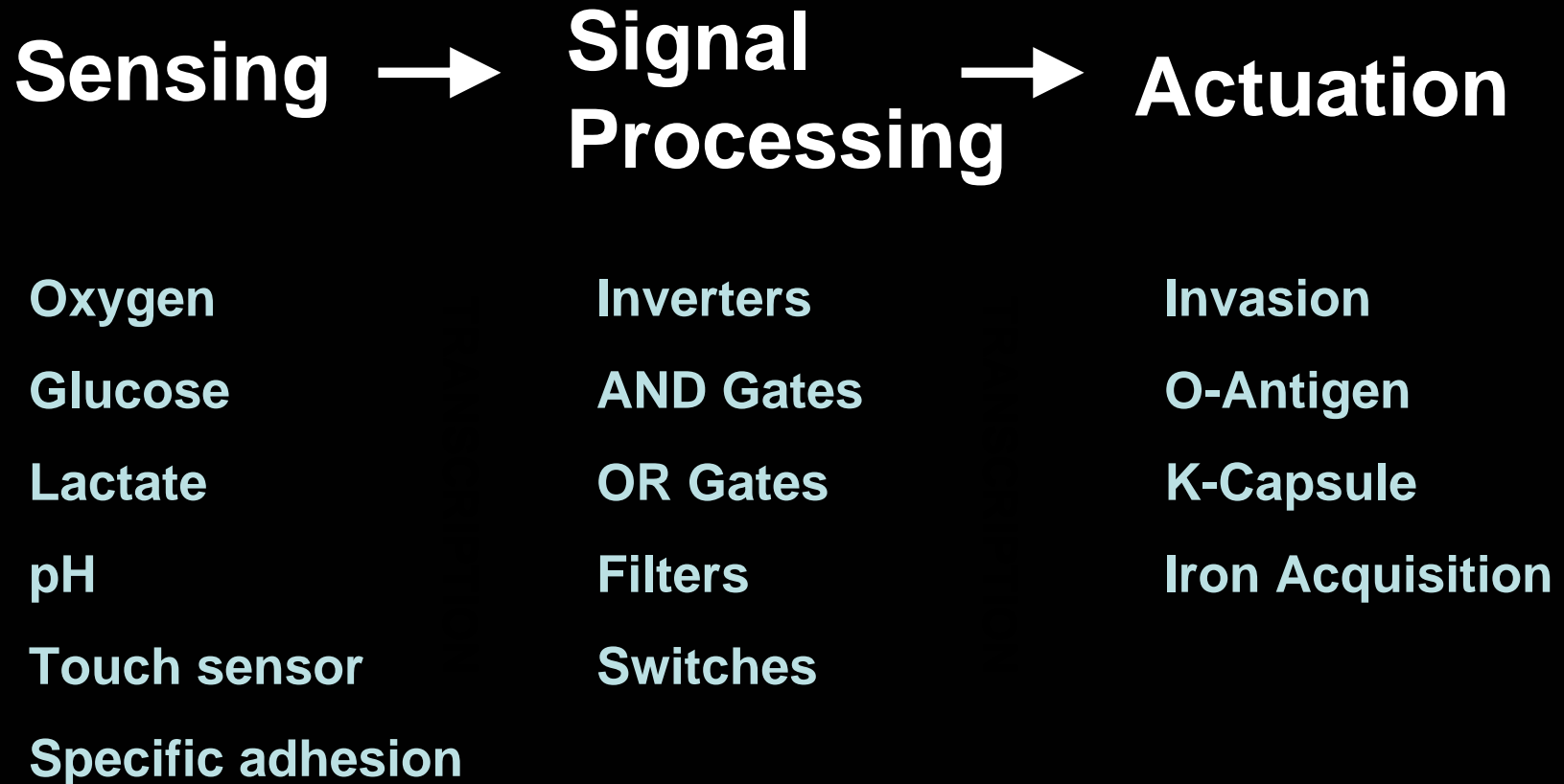


Modules (Devices) 1
Modules (Devices) 2
Modules (Devices) ..n

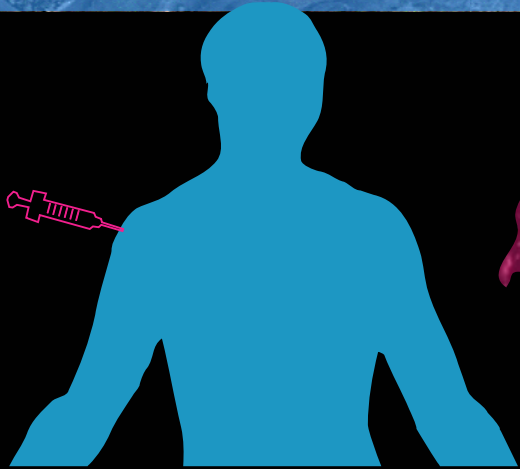


Parts

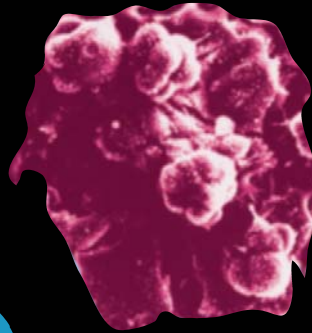
What's a Process?



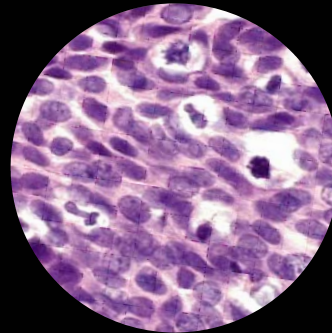
Engineering for Distinct Environments



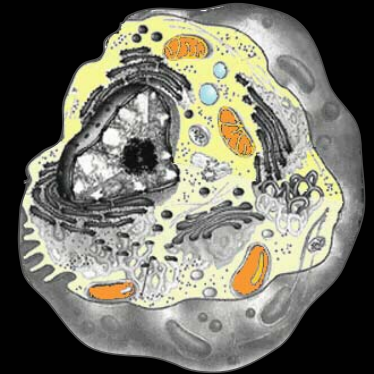
Bloodstream



Tumor

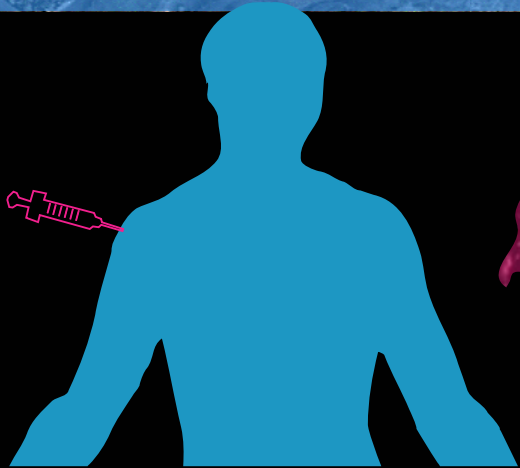


Cell Surface



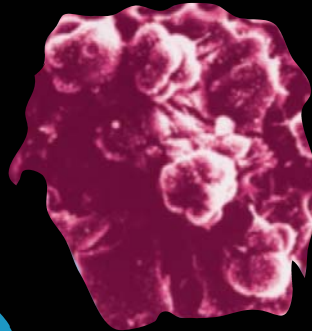
Intracellular

Engineering for Distinct Environments



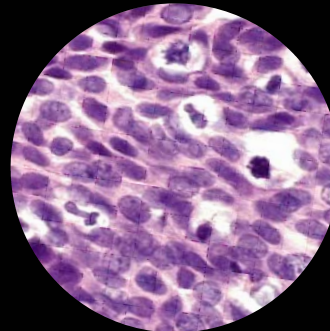
Bloodstream

High Glucose
High O₂
Low lactate



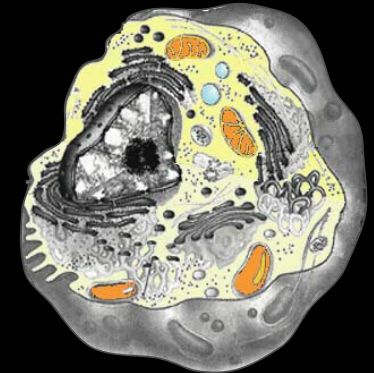
Tumor

Low Glucose
Low O₂
Low lactate
High Density



Cell Surface

β 1-Integrin
TF Antigen
Her2
Sialyl Lewis X

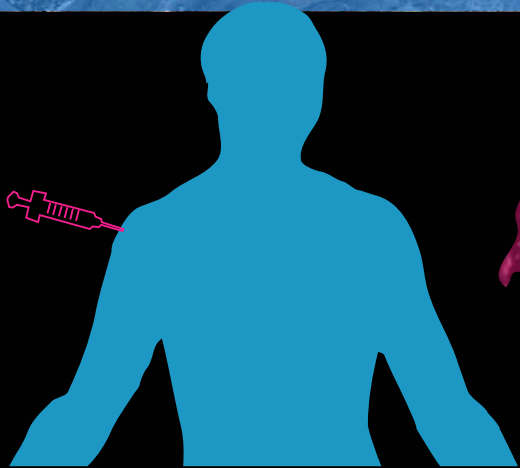


Intracellular

Vacuole
Metabolites

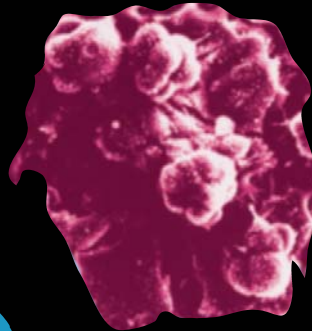
Sensing

Engineering for Distinct Environments



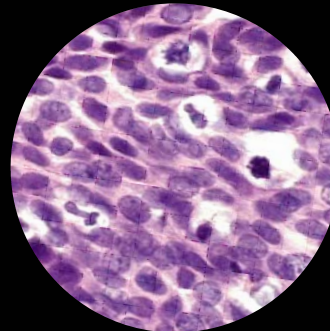
Bloodstream

Shielding
No Growth



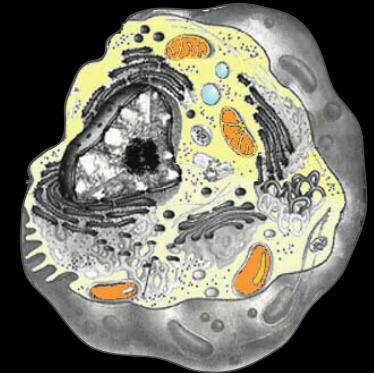
Tumor

Activation
Deshielding
No Growth



Cell Surface

Adhesion
Secretion
invasion
No Growth



Intracellular

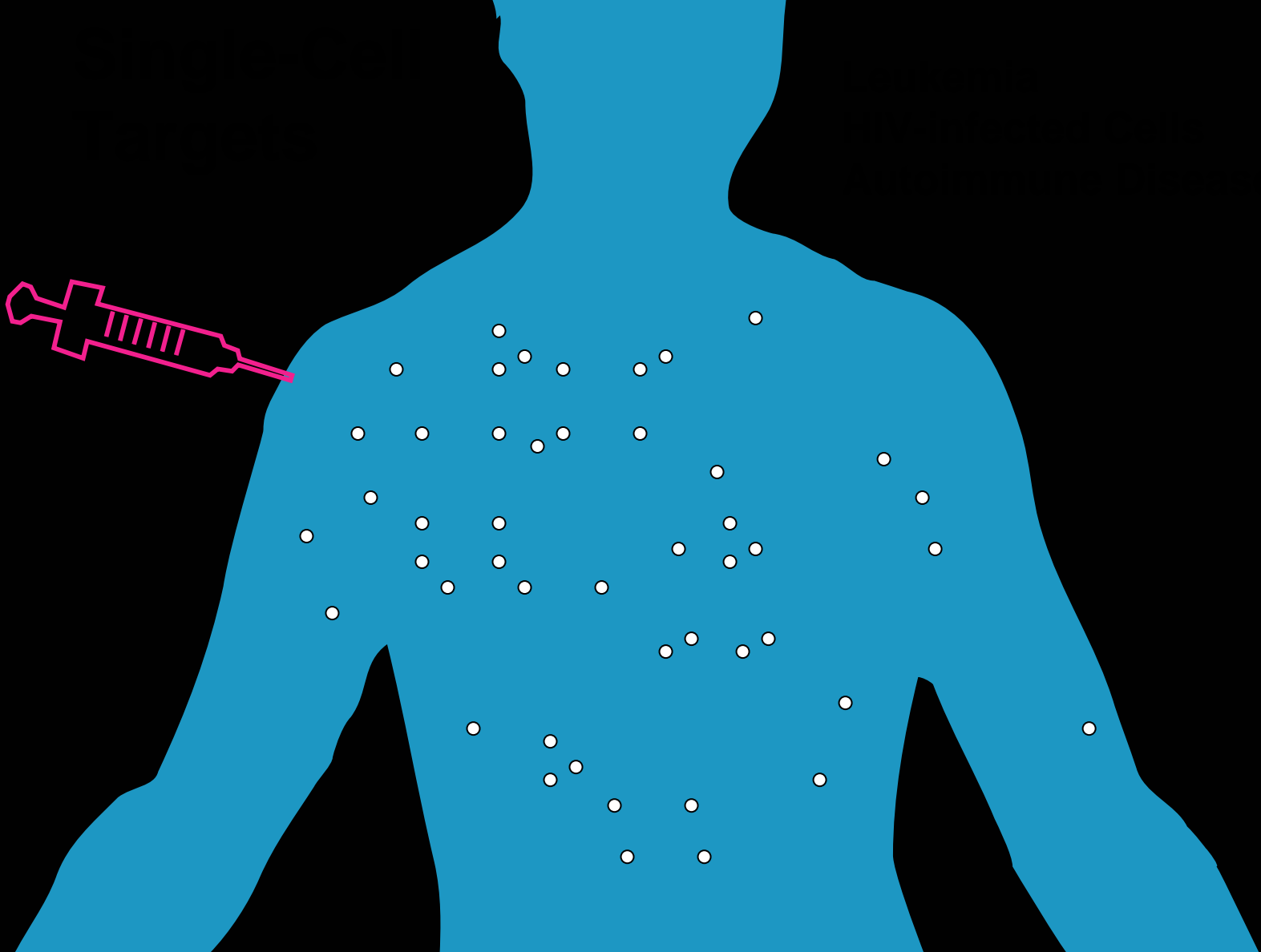
Growth
Lysis
Killing

Actuation



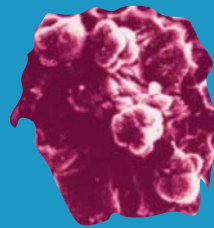
Environment 1:

The Bloodstream





**Solid
Tumor**

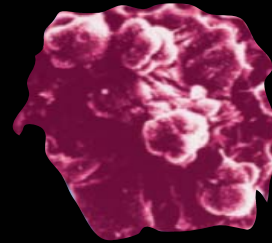
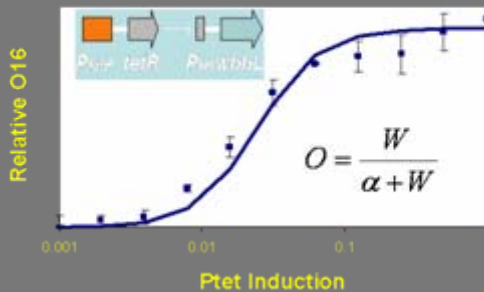
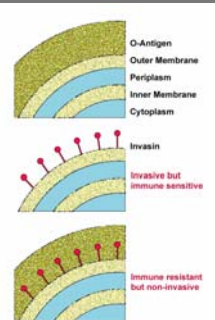
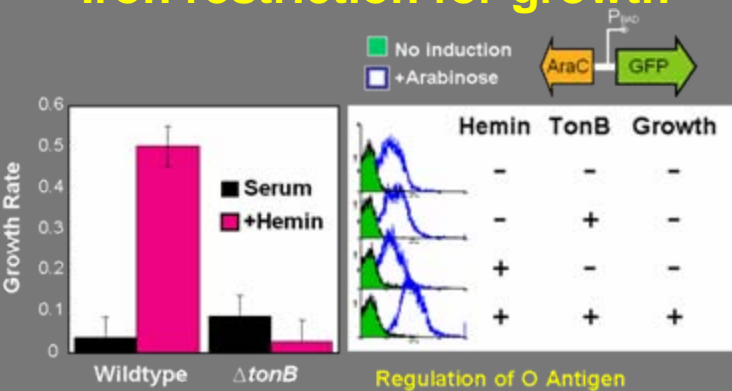


Design: Interfaces, Logic, Transfer Functions

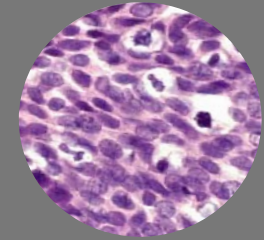
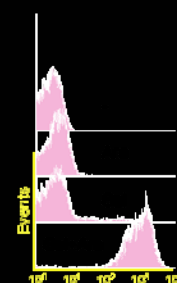
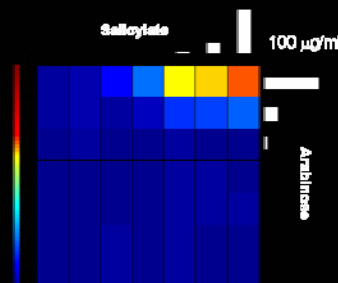
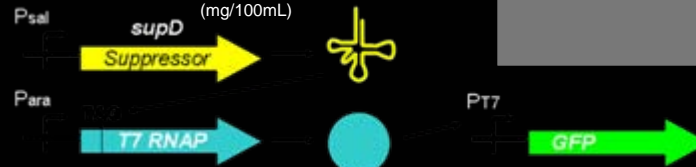
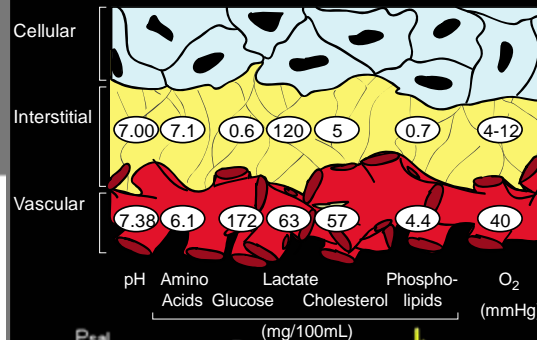


Blood stream

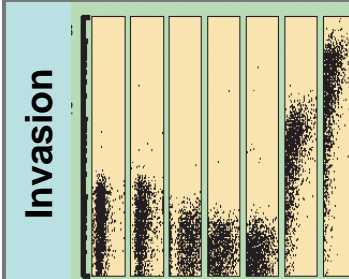
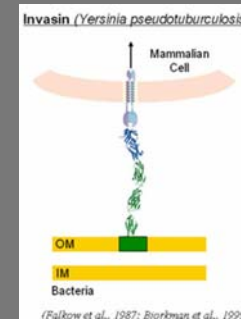
Iron restriction for growth



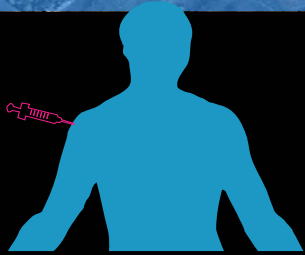
Tumor



Cell Surface



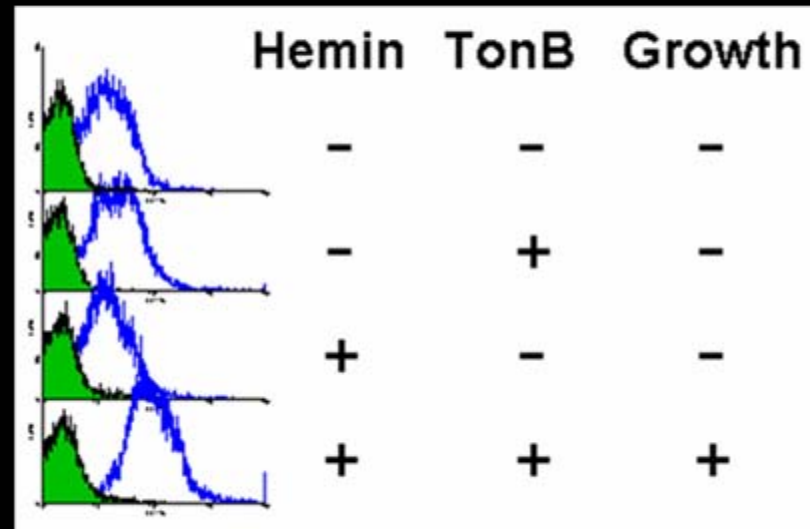
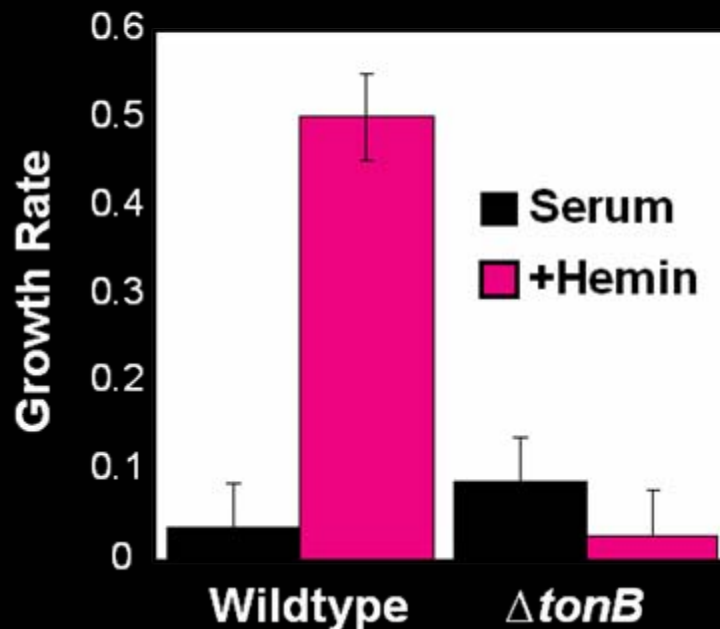
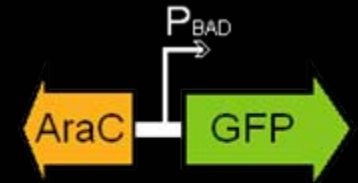
Design: Interfaces, Logic, Transfer Functions



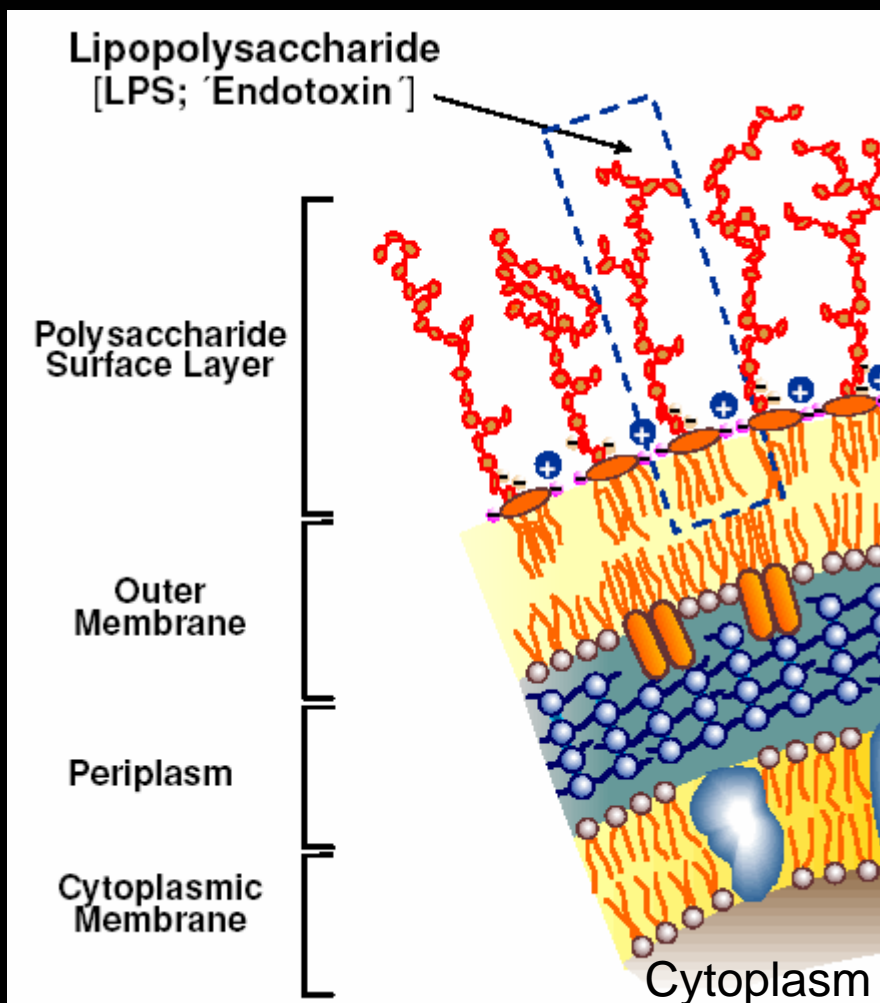
Blood stream

Iron restriction for growth

■ No induction
■ +Arabinose

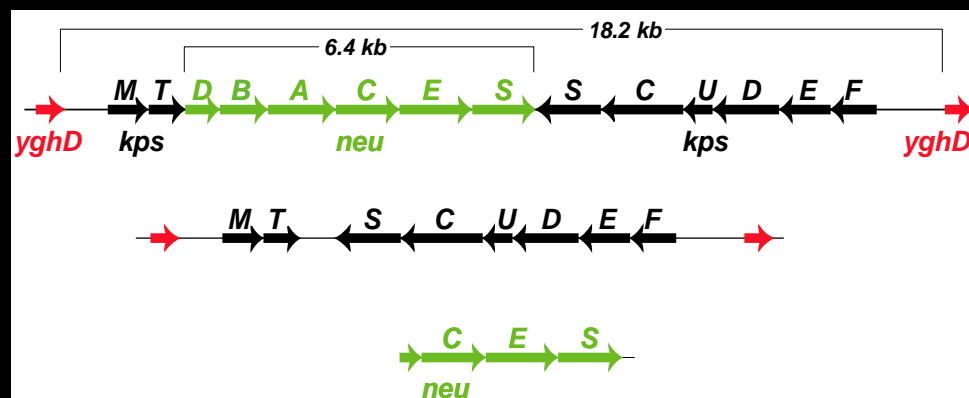
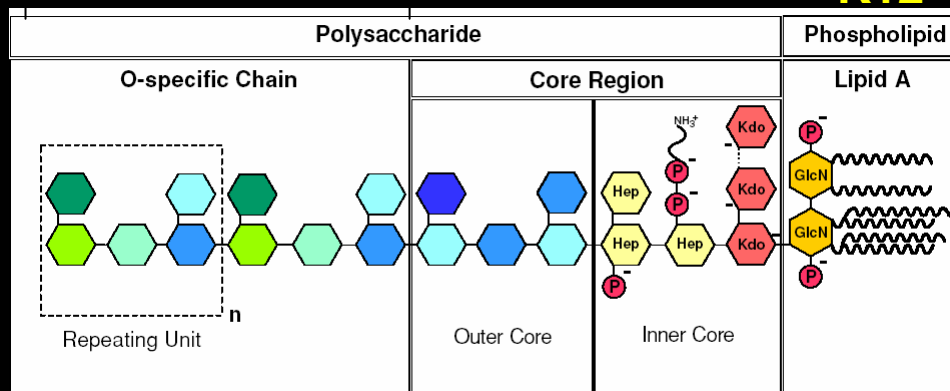


The bacterial surface Engineering the capsule



Salmonella, Shigella EHEC, EPEC, UPEC, etc.

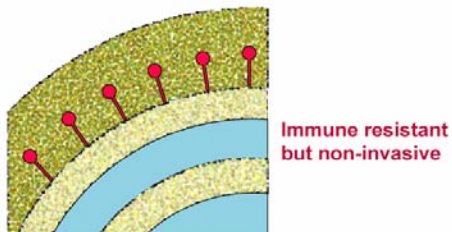
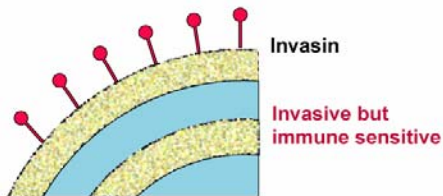
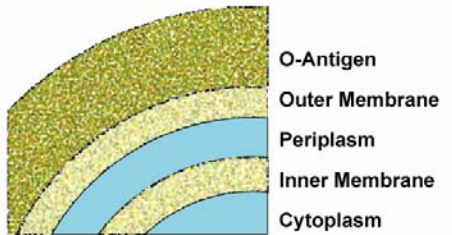
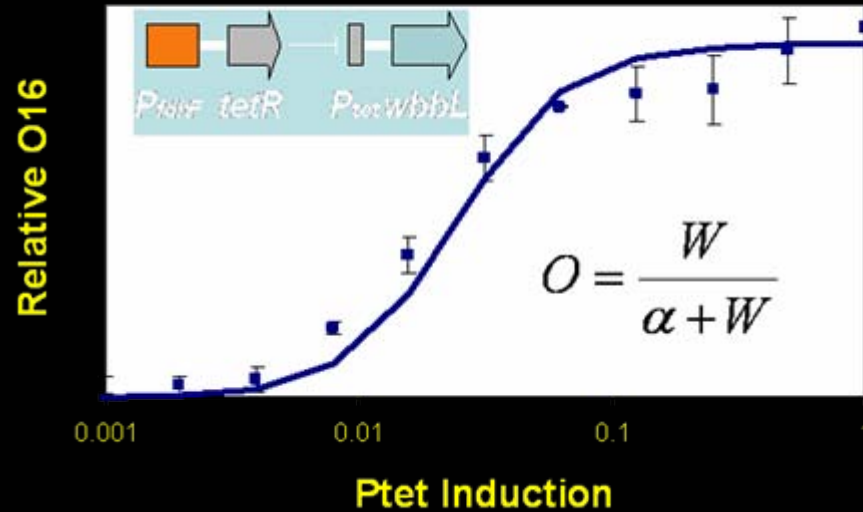
K12



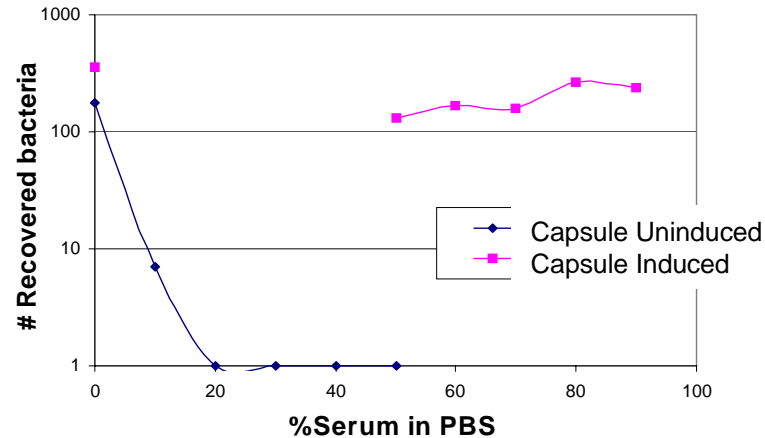
Design: Interfaces, Logic, Transfer Functions



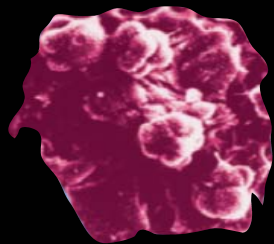
Regulation of O Antigen



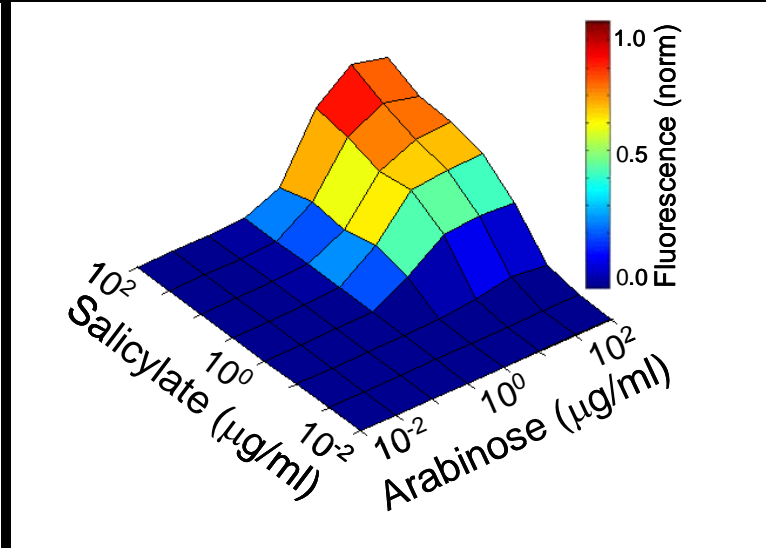
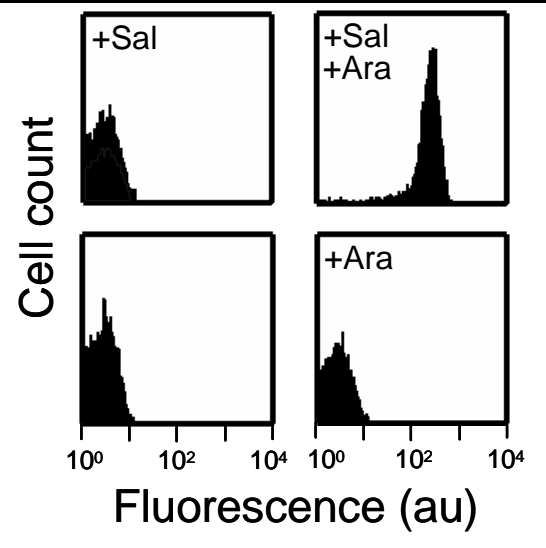
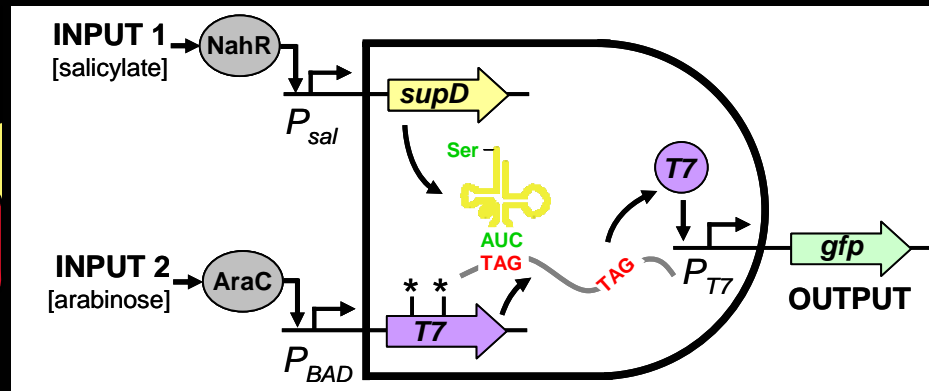
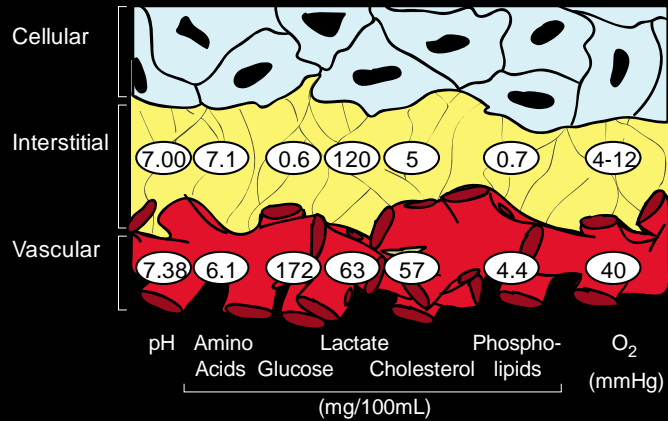
Serum resistance of capsulated bacteria



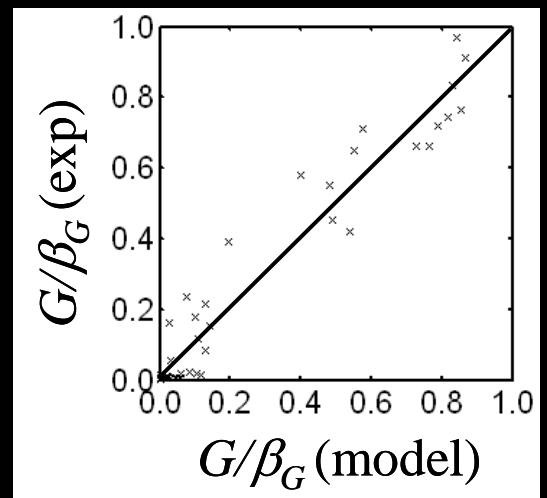
Design: Interfaces, Logic, Transfer Functions



Tumor

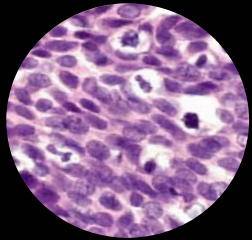


$$\frac{G}{G_{\max}} = \frac{I_1 I_2^2}{a(b + I_2)^2 + I_1 I_2^2}$$

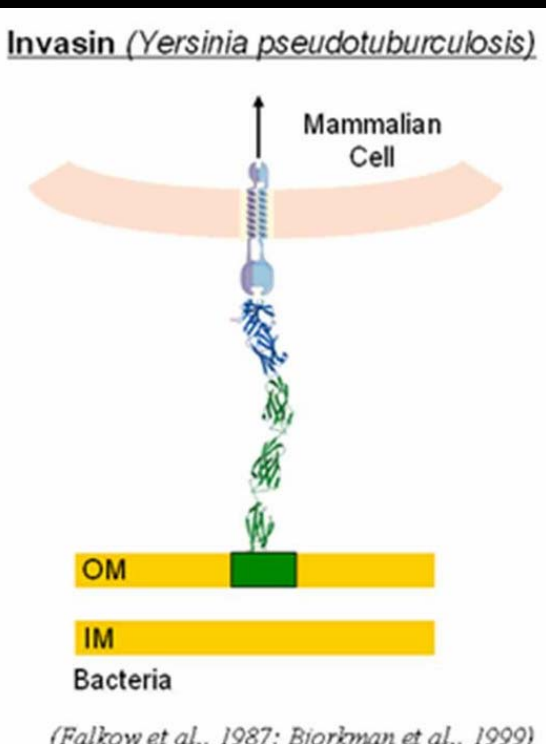


Transfer Function

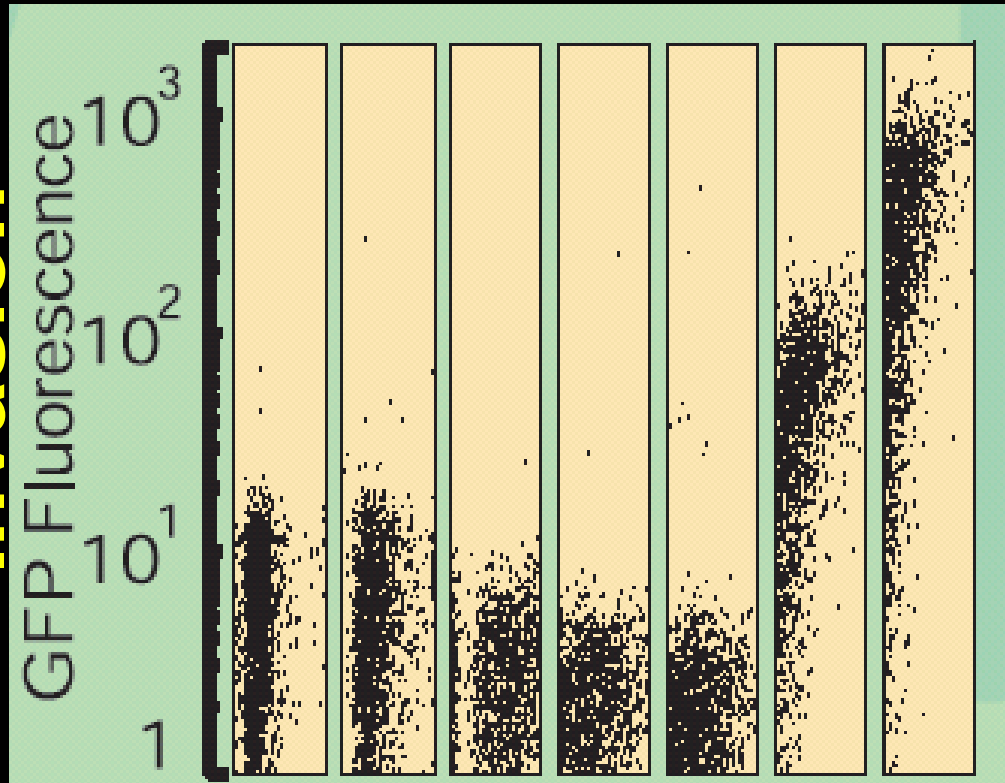
Design: Interfaces, Logic, Transfer Functions



Cell Surface

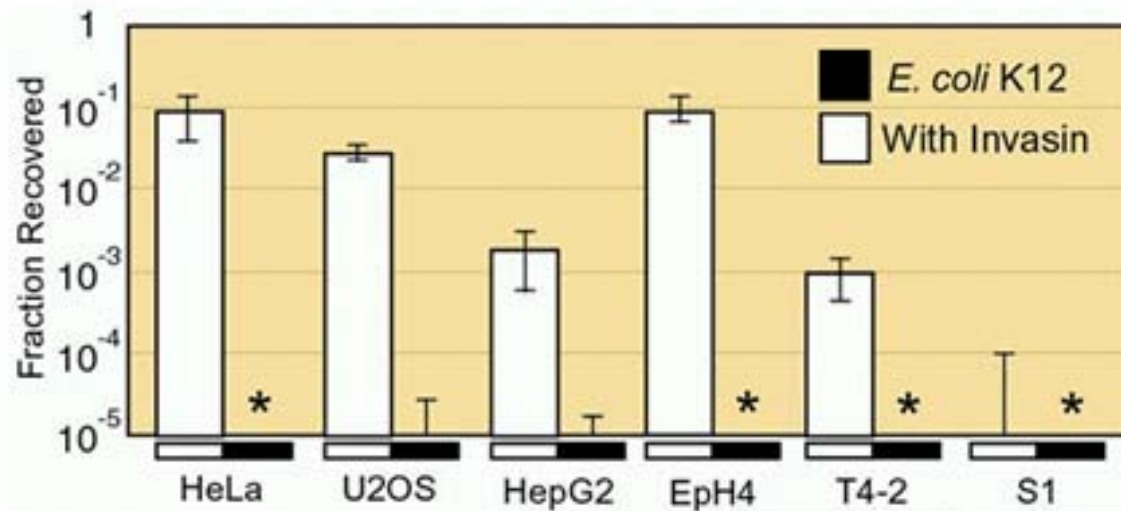


Invasion

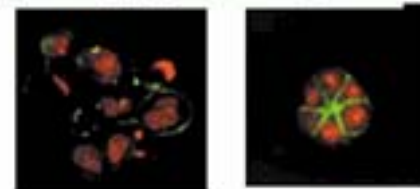


Cell density

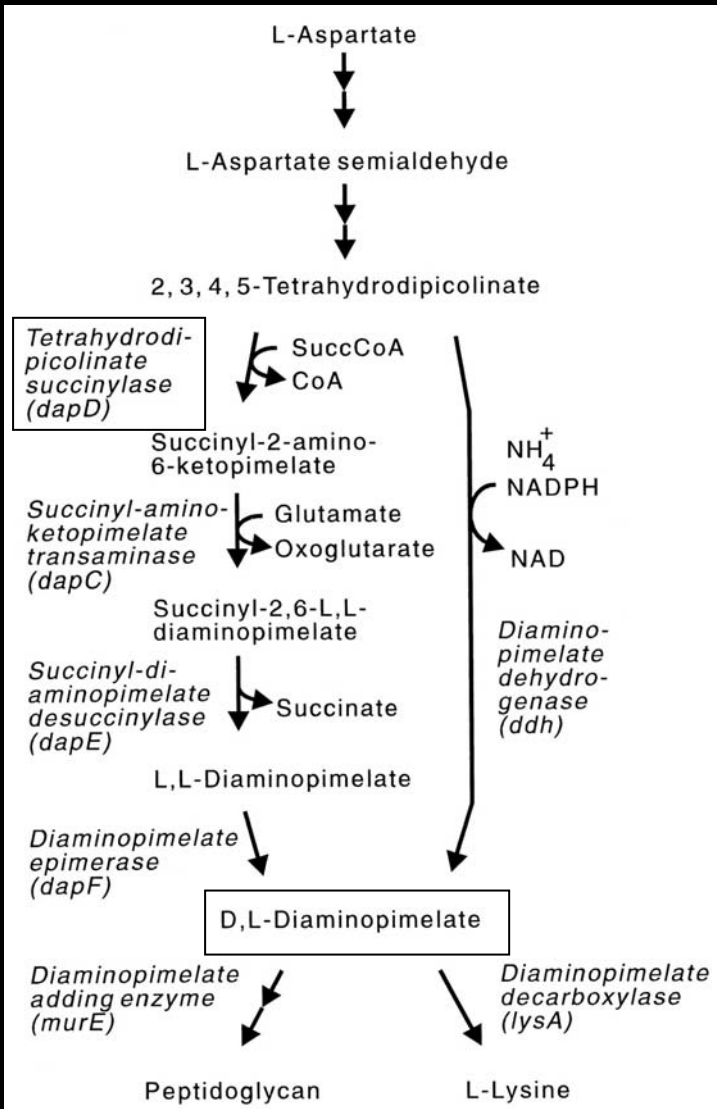
Invading cancer cell lines



HeLa (cervical carcinoma)
 U2OS (osteosarcoma)
 HepG2 (hepatocarcinoma)



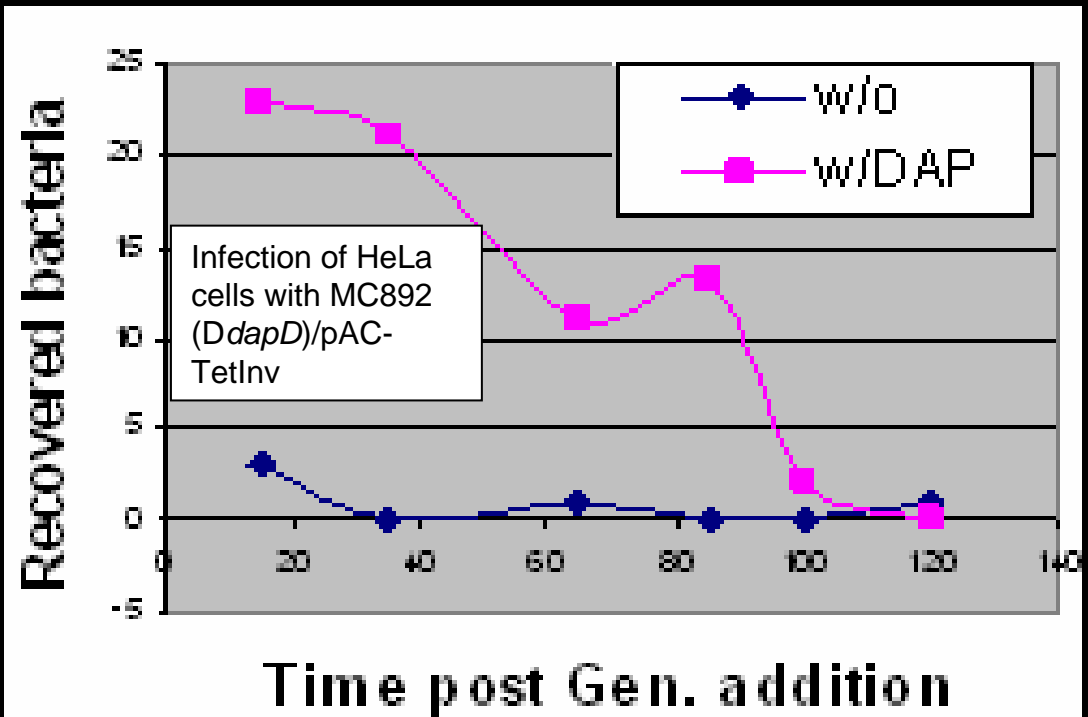
Metabolic safety catch



DAP Necessary for cell-wall biosynthesis and lysine production

No DAP in mammalian metabolism

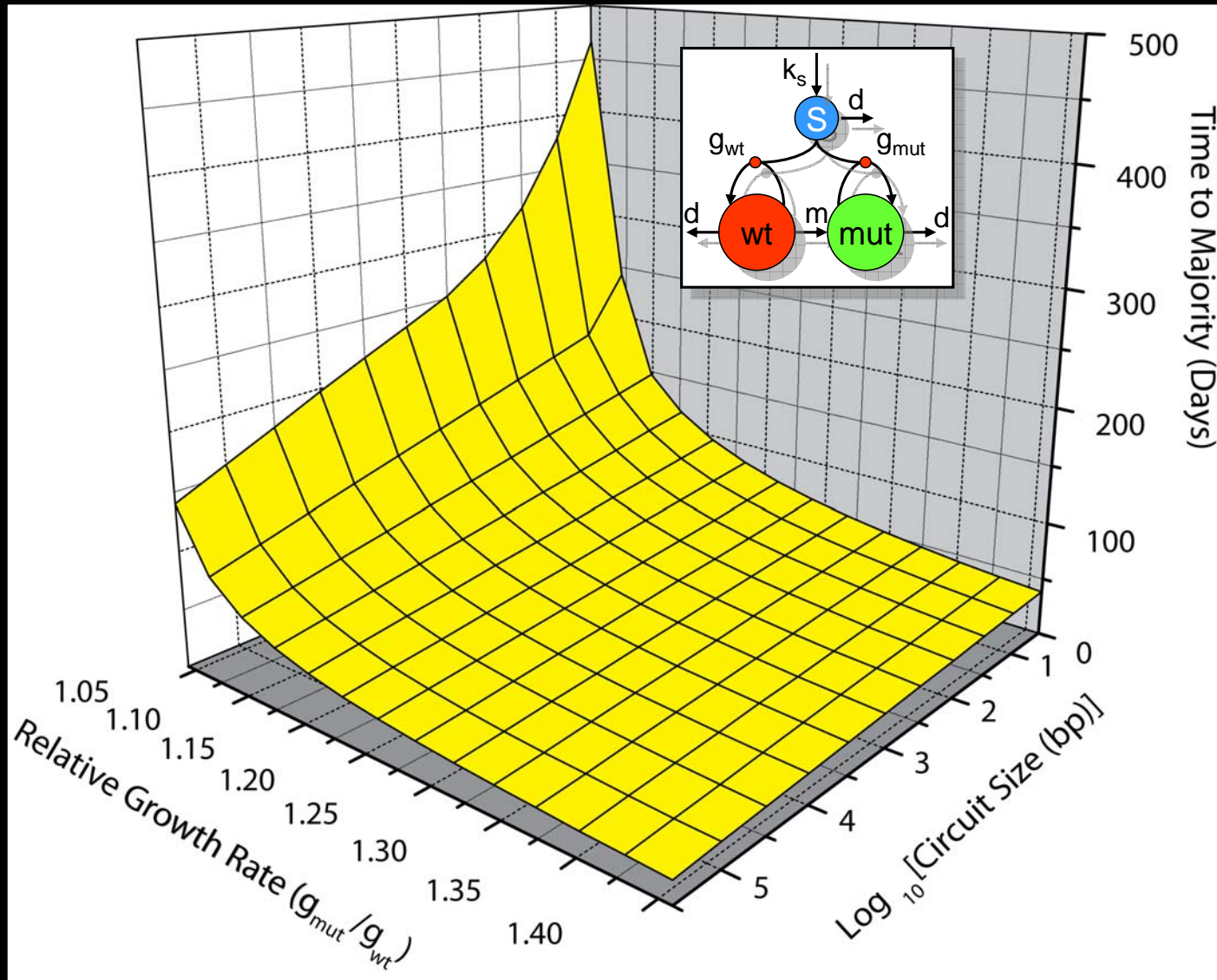
Tight auxotroph; requires 40 ug/mL in LB



Challenges

- Specificity of function
 - Selecting for sensors
 - Evolving optimal thresholds in the switches
 - Combining multiple signals at the right thresholds
- Preservation of function
 - Predicting load of circuit components on cell growth/fitness
 - Robust Design
 - Harmless failure modes
- Engineering for safety
 - Multiple sensitivities/dependencies
 - Genetic incompatibility?

Maximizing Efficacy: Time to take-over of dysfunctional mutants



Summary cont.

- The TKB provides a test bed for our ability to predict, control and design behaviors.
- It test our ability to understand
 - Specific sensing of the microenvironment
 - Engineering for both robustness and sensitivity
 - Engineering against evolution
 - Engineering for game with the host immune system.
 - How to build a real synthetic biology application

Acknowledgements

- **J. Chris Anderson**
- *Elizabeth Clark*
- *Christopher Voigt*

Funding

- Damon Runyan Cancer Research Foundation
- HHMI

