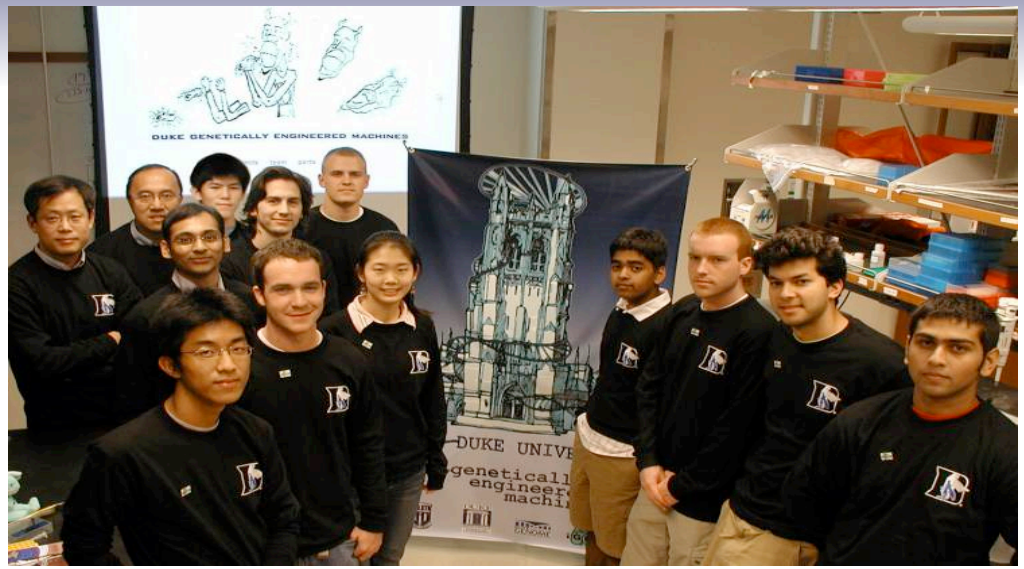
Duke iGEM 2006



Duke University

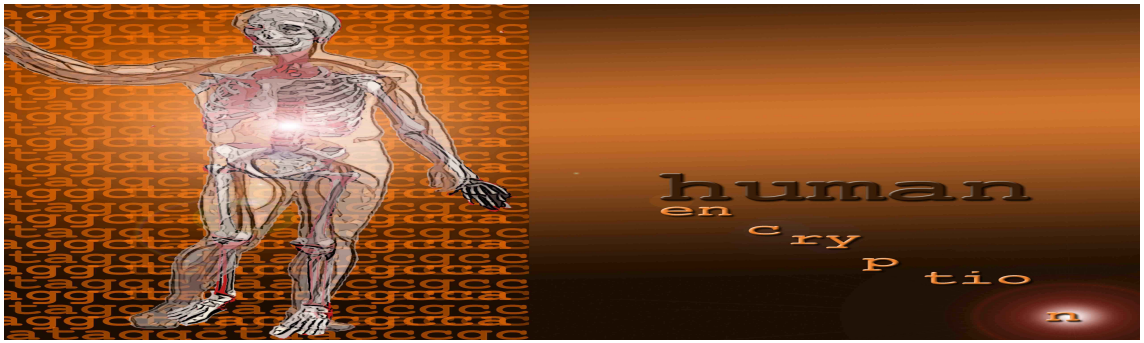
International Genetically Engineered Machines Jamboree 20

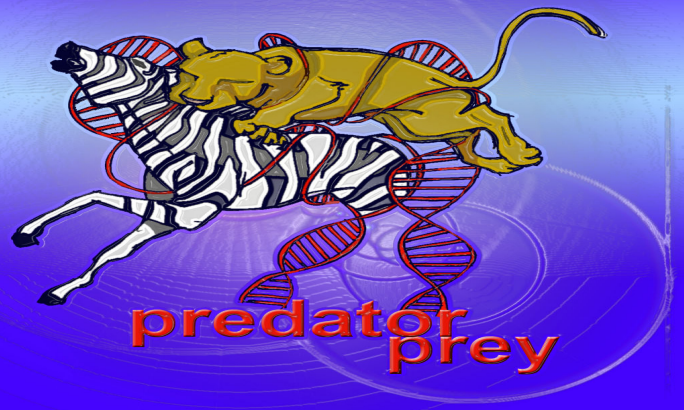
M.I.T., Cambridge MA, 02139 U.S.A.

November 4-5, 2006

staff	students	
Thom LaBean	Keddy Chandran	Nirav Lakhani
Faisal Reza	Hattie Chung	John Lee
Jingdong Tian	Matt Feltz	Steven Lin
Lingchong You	Austen Heinz	Pat O'Brien
Fan Yuan	Sagar Indurkhya	Nicholas Tang
	Eric Josephs	Bryan Van Dyke

2006 Projects

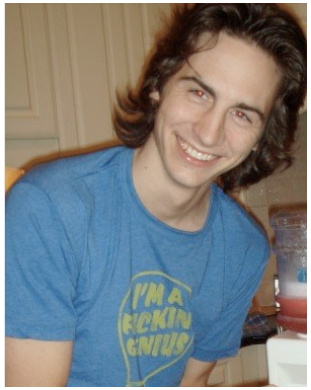




Engineering Synthetic Oscillatory Gene Networks at the Population Level

Sagar Indurkhya

Nicholas Tang



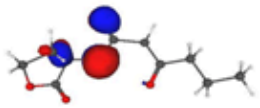
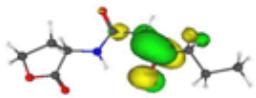
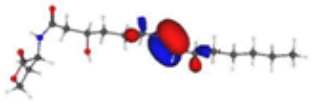
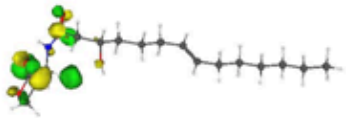
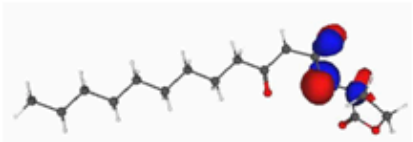
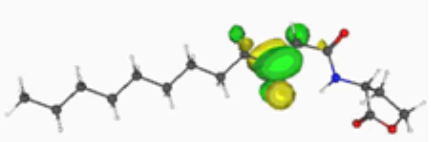
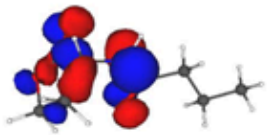
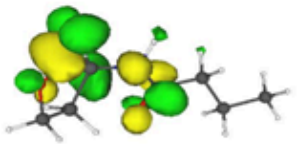
Austen Heinz

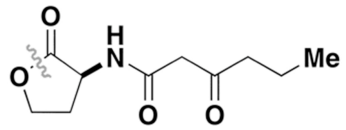
Lingchong You

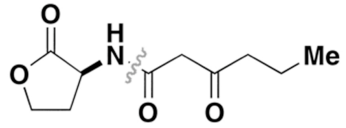
Computational Chemistry

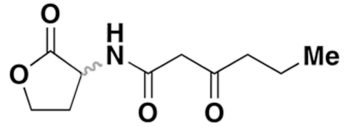
Table 3. HOMO/LUMO Gap Energies

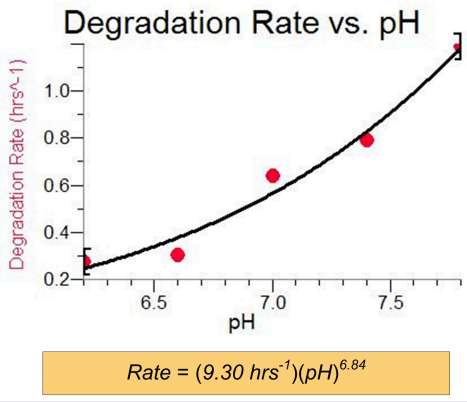
System	Molecular Formula	HOMO (kcal/mol)	LUMO (kcal/mol)	GAP (kcal/mol)
Lux	$C_{10}H_{15}O_4N$	249.51	90.00	339.51
Cin	$C_{18}H_{31}O_4N$	215.87	104.67	320.55
Las	$C_{16}H_{27}O_4N$	249.23	90.52	339.75
Rhl	$C_8H_{13}O_3N$	241.64	114.45	356.10

	HOMO	LUMO
Lux ($C_{10}H_{15}O_4N$)		
Cin ($C_{18}H_{31}O_4N$)		
Las ($C_{16}H_{27}O_4N$)		
Rhl ($C_8H_{13}O_3N$)		

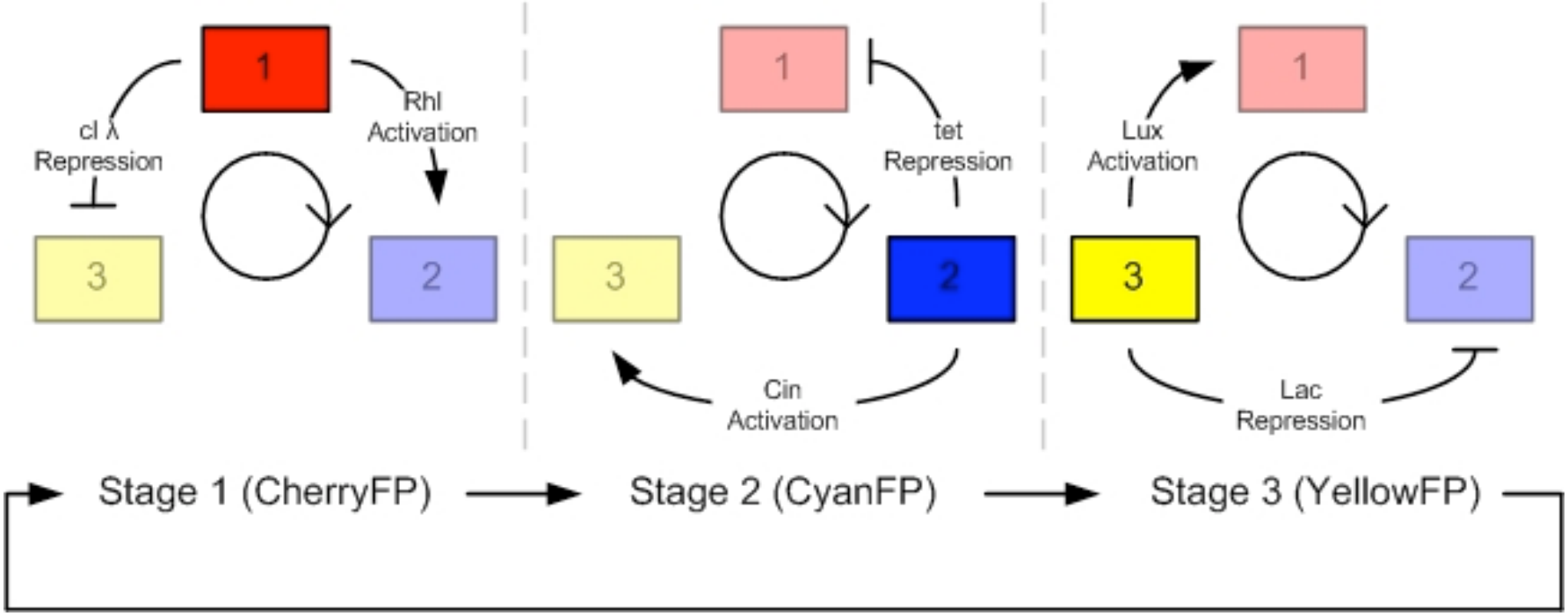
A 

B 

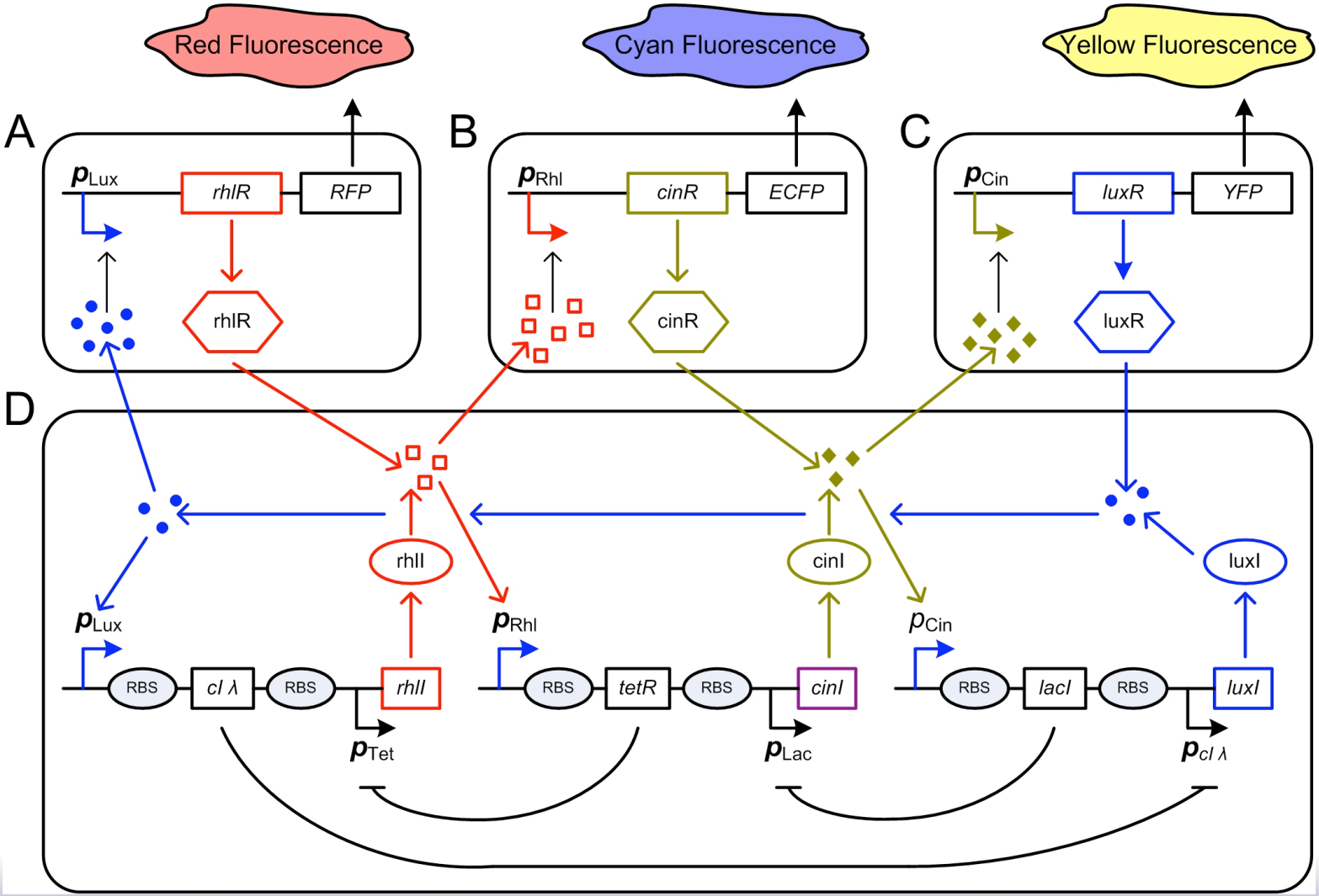
C 



X-Verter


1
Rhl
Activation
cI λ
Repression
3
2
1
tet
Repression
3
2
Cin
Activation
1
Lux
Activation
3
2
Lac
Repression
Stage 1 (CherryFP)
Stage 2 (CyanFP)
Stage 3 (YellowFP)

X-Verter


Red Fluorescence
Cyan Fluorescence
Yellow Fluorescence
A
B
C
D
pLux
rhlR
RFP
rhlR
pRhl
cinR
ECFP
cinR
pCin
luxR
YFP
luxR
rhlI
cinI
luxI
pLux
pRhl
pCin
RBS
cI λ
RBS
rhlI
pTet
RBS
tetR
RBS
cinI
pLac
RBS
lacI
RBS
luxI
pcI λ

X-Verter

X-Verter

Expression by Small Molecule Promotion

$$\frac{d[tetR]}{dt} = V_{max,\,rhl}\frac{[HSL_{Rhl}]}{K_{m,\,rhl}+[HSL_{Rhl}]} \qquad \frac{d[lacI]}{dt} = V_{max,\,cin}\frac{[HSL_{Cin}]}{K_{m,\,cin}+[HSL_{Cin}]} \qquad \frac{d[cI\,1]}{dt} = V_{max,\,lux}\frac{[HSL_{Lux}]}{K_{m,\,lux}+[HSL_{Lux}]}$$

$$\frac{d[cinI_{RBS}]}{dt} = V_{max,\,rhl}\frac{[HSL_{Rhl}]}{K_{m,\,rhl}+[HSL_{Rhl}]} \qquad \frac{d[luxI_{RBS}]}{dt} = V_{max,\,cin}\frac{[HSL_{Cin}]}{K_{m,\,cin}+[HSL_{Cin}]} \qquad \frac{d[rhlI_{RBS}]}{dt} = V_{max,\,lux}\frac{[HSL_{Lux}]}{K_{m,\,lux}+[HSL_{Lux}]}$$

$$\frac{d[cinR]}{dt} = V_{max,\,rhl}\frac{[HSL_{Rhl}]}{K_{m,\,rhl}+[HSL_{Rhl}]} \qquad \frac{d[luxR]}{dt} = V_{max,\,cin}\frac{[HSL_{Cin}]}{K_{m,\,cin}+[HSL_{Cin}]} \qquad \frac{d[cinR]}{dt} = V_{max,\,lux}\frac{[HSL_{Lux}]}{K_{m,\,lux}+[HSL_{Lux}]}$$

$$\frac{d[CFP]}{dt} = V_{max,\,rhl}\frac{[HSL_{Rhl}]}{K_{m,\,rhl}+[HSL_{Rhl}]} \qquad \frac{d[YFP]}{dt} = V_{max,\,cin}\frac{[HSL_{Cin}]}{K_{m,\,cin}+[HSL_{Cin}]} \qquad \frac{d[CFP]}{dt} = V_{max,\,lux}\frac{[HSL_{Lux}]}{K_{m,\,lux}+[HSL_{Lux}]}$$

Expression by Gene Repression

$$\frac{d[rhlI_{mRNA}]}{dt} = \frac{H_{tet}K_{m,\,tet}}{K_{m,\,tet}+[tetR]} \qquad \frac{d[cinI_{mRNA}]}{dt} = \frac{H_{lac}K_{m,\,lac}}{K_{m,\,lac}+[lacI]} \qquad \frac{d[luxI_{mRNA}]}{dt} = \frac{H_{cI\,1}K_{m,\,cI\,1}}{K_{m,\,cI\,1}+[cI\,1]}$$

Formation of HSL Molecule

$$\frac{d[HSL_{Cin}]}{dt} = k_{HSL\ Formation}[cinR][cinI] \qquad \frac{d[HSL_{Lux}]}{dt} = k_{HSL\ Formation}[luxR][luxI] \qquad \frac{d[HSL_{Rhl}]}{dt} = k_{HSL\ Formation}[rhlR][rhlI]$$

$$\frac{d[cinI]}{dt} = k_{Protein\ Formation}[cin_{RBS}][cin_{mRNA}] \qquad \frac{d[luxI]}{dt} = k_{Protein\ Formation}[lux_{RBS}][lux_{mRNA}] \qquad \frac{d[rhlI]}{dt} = k_{Protein\ Formation}[rhl_{RBS}][rhl_{mRNA}]$$

Degradation of Molecules

$$\frac{d[RFP]}{dt} = k_{LVA.\ Protein\ Deg.}[RFP] \qquad \frac{d[cinI]}{dt} = k_{Protein\ Deg.}[cinI] \qquad \frac{d[luxR]}{dt} = k_{Protein\ Deg.}[cinR]$$

$$\frac{d[CFP]}{dt} = k_{LVA.\ Protein\ Deg.}[CFP] \qquad \frac{d[luxI]}{dt} = k_{Protein\ Deg.}[luxI] \qquad \frac{d[luxR]}{dt} = k_{Protein\ Deg.}[luxR]$$

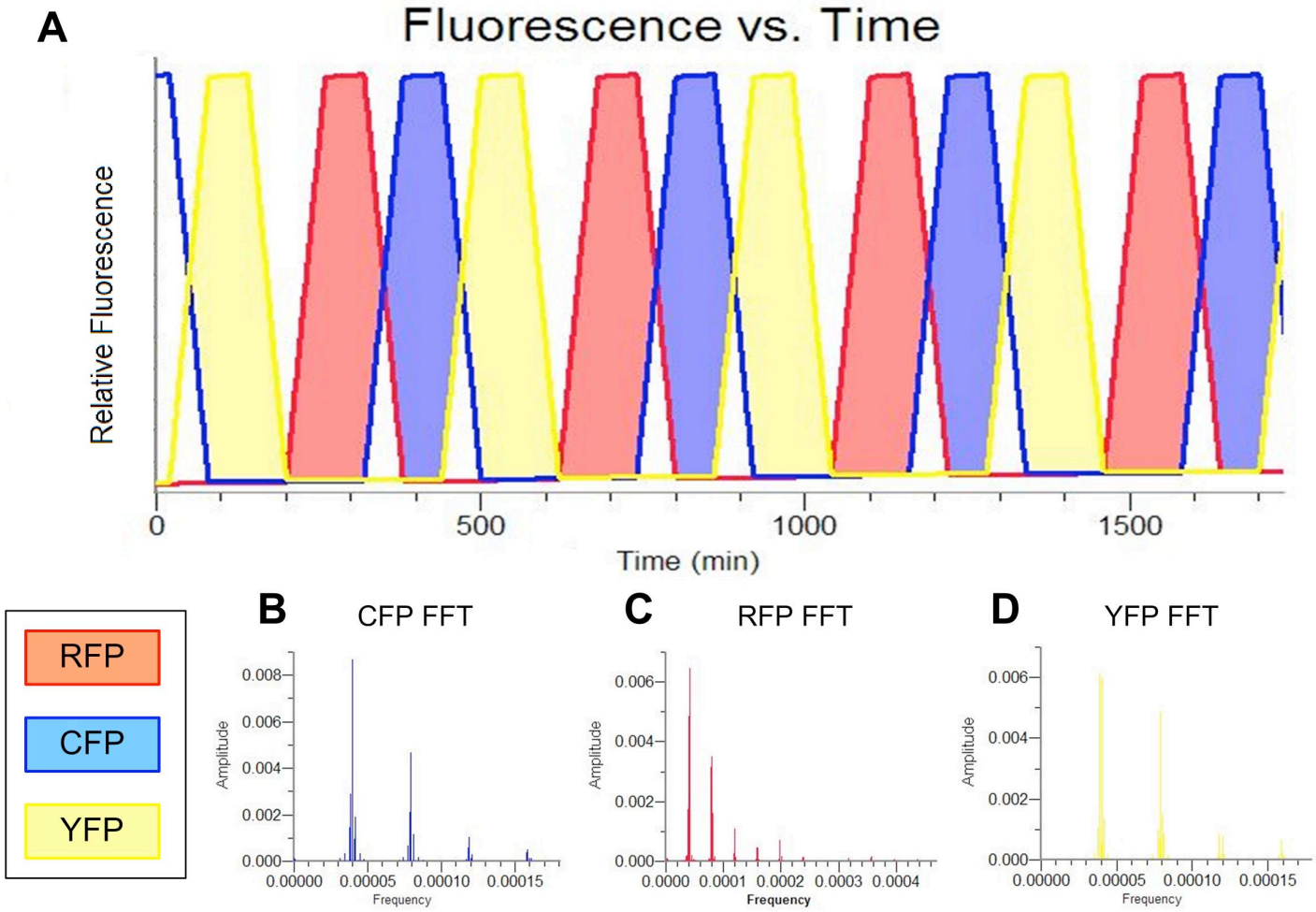
$$\frac{d[YFP]}{dt} = k_{LVA.\ Protein\ Deg.}[YFP] \qquad \frac{d[rhlI]}{dt} = k_{Protein\ Deg.}[rhlI] \qquad \frac{d[rhlR]}{dt} = k_{Protein\ Deg.}[rhlR]$$

$$\frac{d[HSL_{lux}]}{dt} = k_{HSL\ Deg.}[HSL_{lux}] \qquad \frac{d[HSL_{cin}]}{dt} = k_{HSL\ Deg.}[HSL_{cin}] \qquad \frac{d[HSL_{rhl}]}{dt} = k_{HSL\ Deg.}[HSL_{rhl}]$$

$$\frac{d[cinI_{RBS}]}{dt} = k_{Protein\ Deg.}[cinI_{RBS}] \qquad \frac{d[rhlI_{RBS}]}{dt} = k_{Protein\ Deg.}[rhlI_{RBS}] \qquad \frac{d[luxI_{RBS}]}{dt} = k_{Protein\ Deg.}[luxI_{RBS}]$$

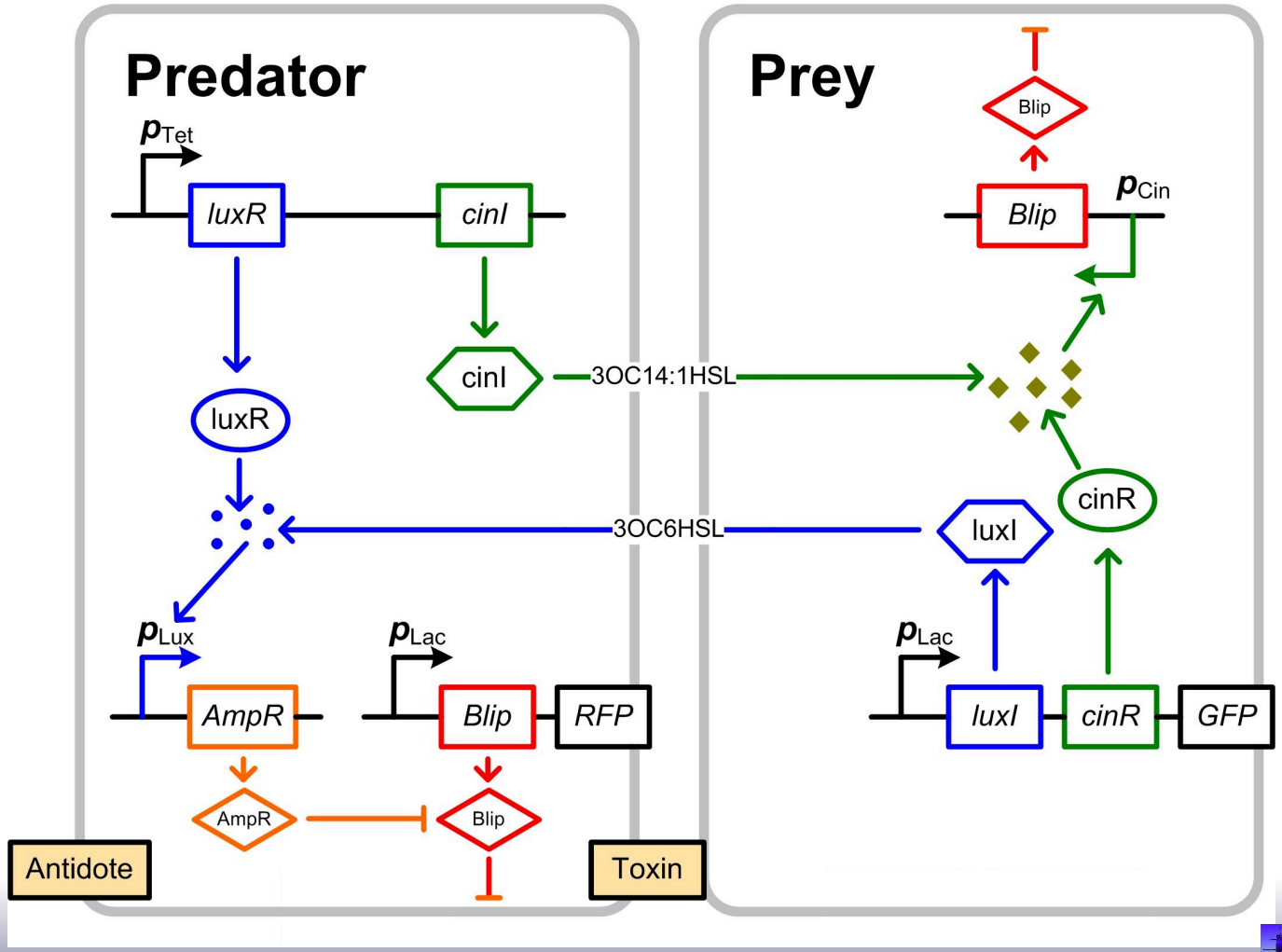
$$\frac{d[cinI_{mRNA}]}{dt} = k_{Protein\ Deg.}[cinI_{RBS}] \qquad \frac{d[rhlI_{mRNA}]}{dt} = k_{Protein\ Deg.}[rhlI_{mRNA}] \qquad \frac{d[luxI_{mRNA}]}{dt} = k_{Protein\ Deg.}[luxI_{mRNA}]$$

X-Verter Modeling Results



Predator-Prey

A Synthetic Predator-Prey Ecosystem



Predator-Prey

Predator-Prey

Expression by Small Molecule Promotion

$$\frac{d[Blip_{Prey}]}{dt} = V_{max,cin}\frac{[HSL_{Cin}]}{K_{m,cin}+[HSL_{Cin}]} \qquad \frac{d[AmpR_{Predator}]}{dt} = V_{max,lux}\frac{[HSL_{Lux}]}{K_{m,lux}+[HSL_{Lux}]}$$

Formation of HSL Molecule

$$\frac{d[HSL_{Cin}]}{dt} = k_{HSL\ Formation}[cinR][cinI] \qquad \frac{d[HSL_{Lux}]}{dt} = k_{HSL\ Formation}[luxR][luxI]$$

Degradation of Molecules

$$\frac{d[GFP]}{dt} = k_{LVA.\,Protein\ Deg.}[GFP] \qquad \frac{d[cinI]}{dt} = k_{Protein\ Deg.}[cinI] \qquad \frac{d[luxR]}{dt} = k_{Protein\ Deg.}[cinR]$$

$$\frac{d[RFP]}{dt} = k_{LVA.\,Protein\ Deg.}[RFP] \qquad \frac{d[luxI]}{dt} = k_{Protein\ Deg.}[luxI] \qquad \frac{d[luxR]}{dt} = k_{Protein\ Deg.}[luxR]$$

$$\frac{d[AmpR]}{dt} = k_{Protein\ Deg.}[AmpR] \qquad \frac{d[HSL_{lux}]}{dt} = k_{HSL\ Deg.}[HSL_{lux}] \qquad \frac{d[HSL_{cin}]}{dt} = k_{HSL\ Deg.}[HSL_{cin}]$$

$$\frac{d[Blip_{Predator}]}{dt} = k_{Protein\ Deg.}[Blip_{Predator}] \qquad \frac{d[Blip_{Prey}]}{dt} = k_{Protein\ Deg.}[Blip_{Prey}]$$

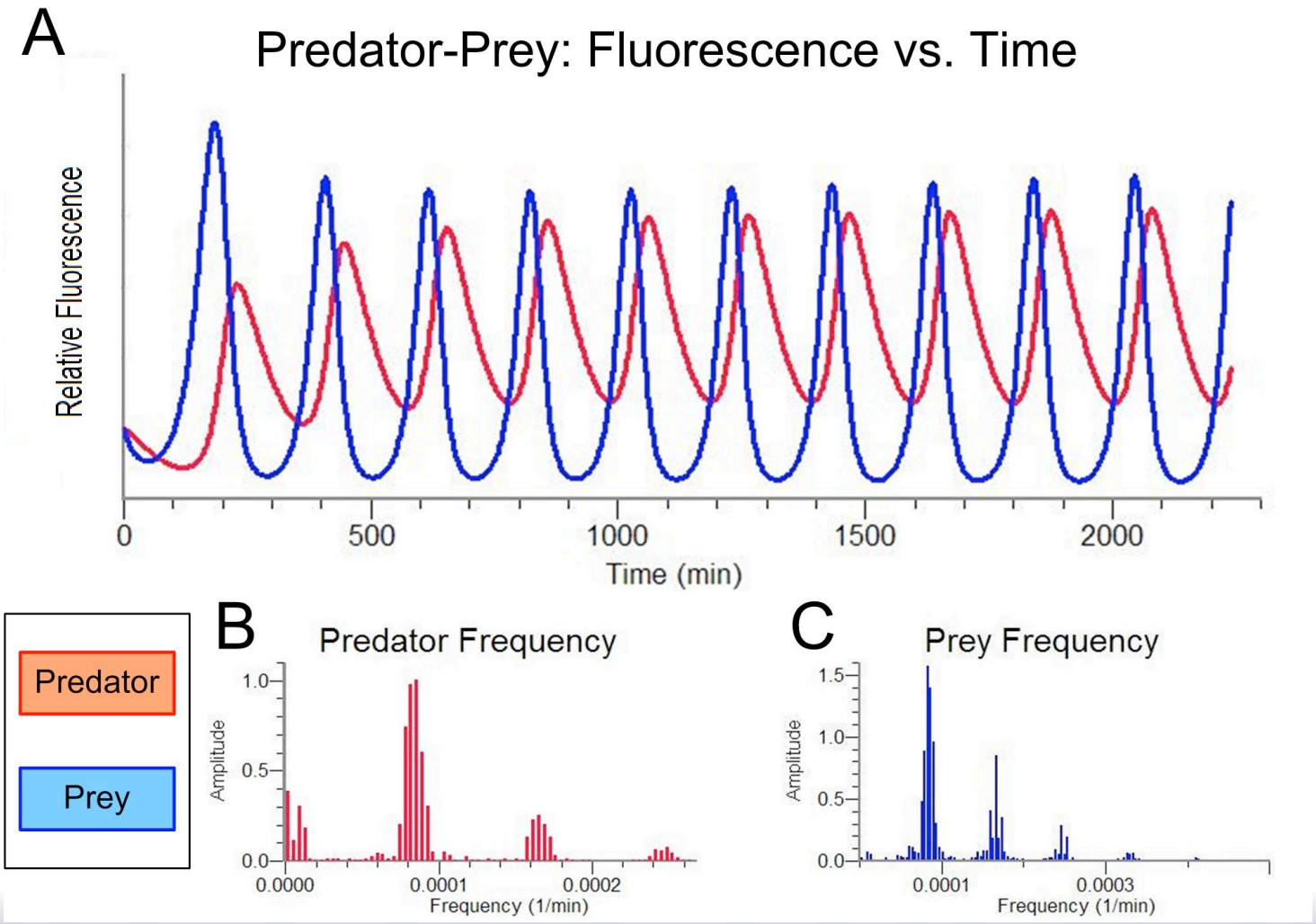
Expression by Gene Repression

$$\frac{d[RFP]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[lacI]} \qquad \frac{d[luxR]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[tetR]} \qquad \frac{d[cinR]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[lacI]}$$

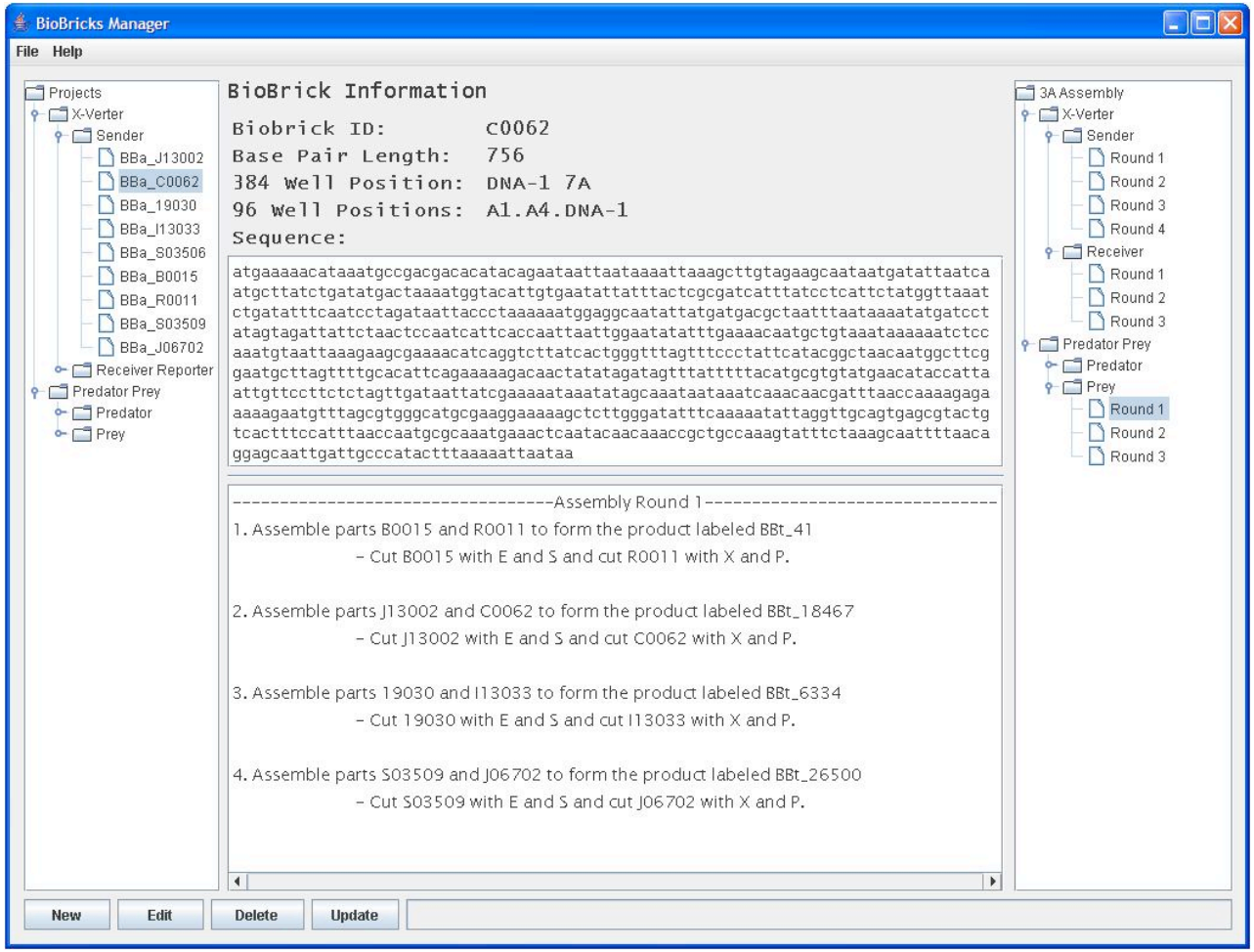
$$\frac{d[GFP]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[lacI]} \qquad \frac{d[luxI]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[lacI]} \qquad \frac{d[cinI]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[tetR]}$$

$$\frac{d[Blip_{Predator}]}{dt} = \frac{H_{lac}K_{lac}}{K_{lac}+[lacI]}$$

Predator-Prey Modeling



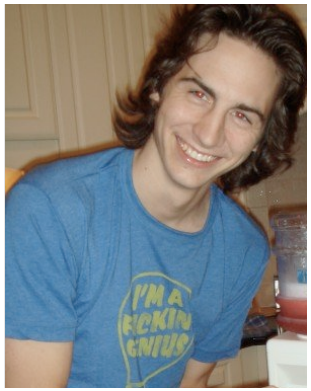
Biobricks Manager



Conclusion

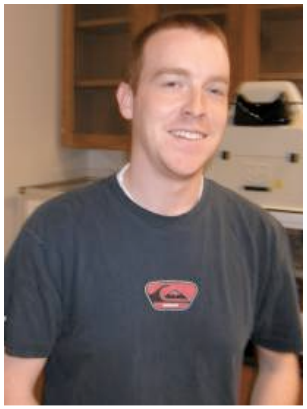
- **Computational Chemistry**
  - Derived pH degradation rates
- **X-Verter (3-Stage Synchronized Oscillator)**
  - Designed and Modeled
- **Predator -Prey (2-Stage Synchronized Oscillator)**
  - Designed and Modeled
  - Nearly Completed with Assembly
- **Biobricks Manager (Biological Circuit IDE)**
  - Soon to be released as open-source
  - Experimental Characterization

Encoding Information In Vivo with DNA and Light

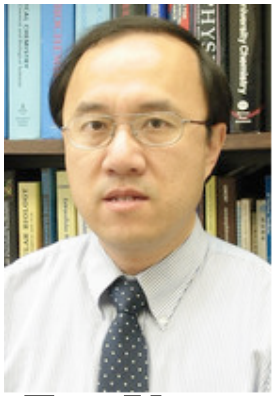


Austen Heinz

Keddy Chandran

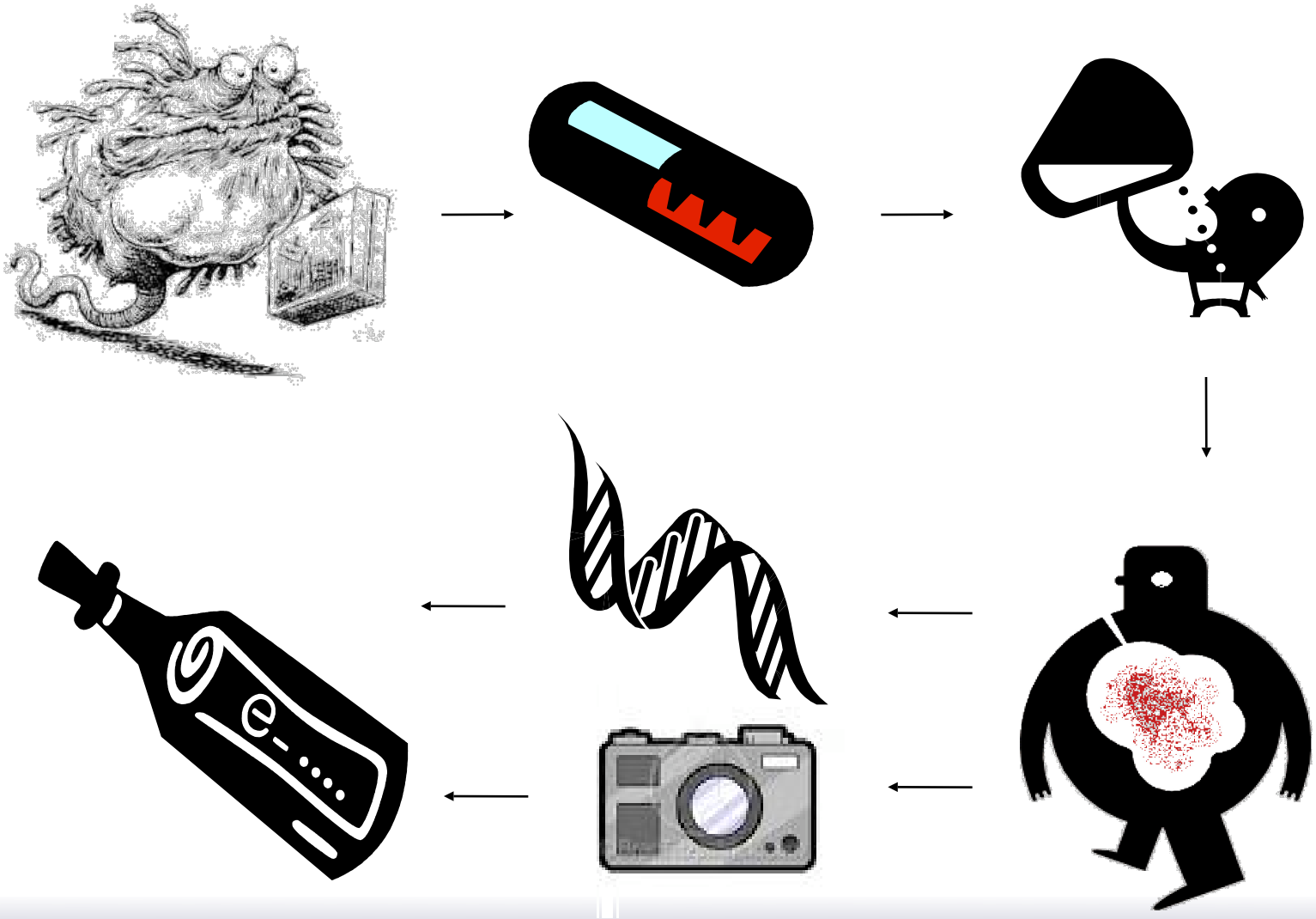


Pat O'Brien



Fan Yuan

Human Encryption System

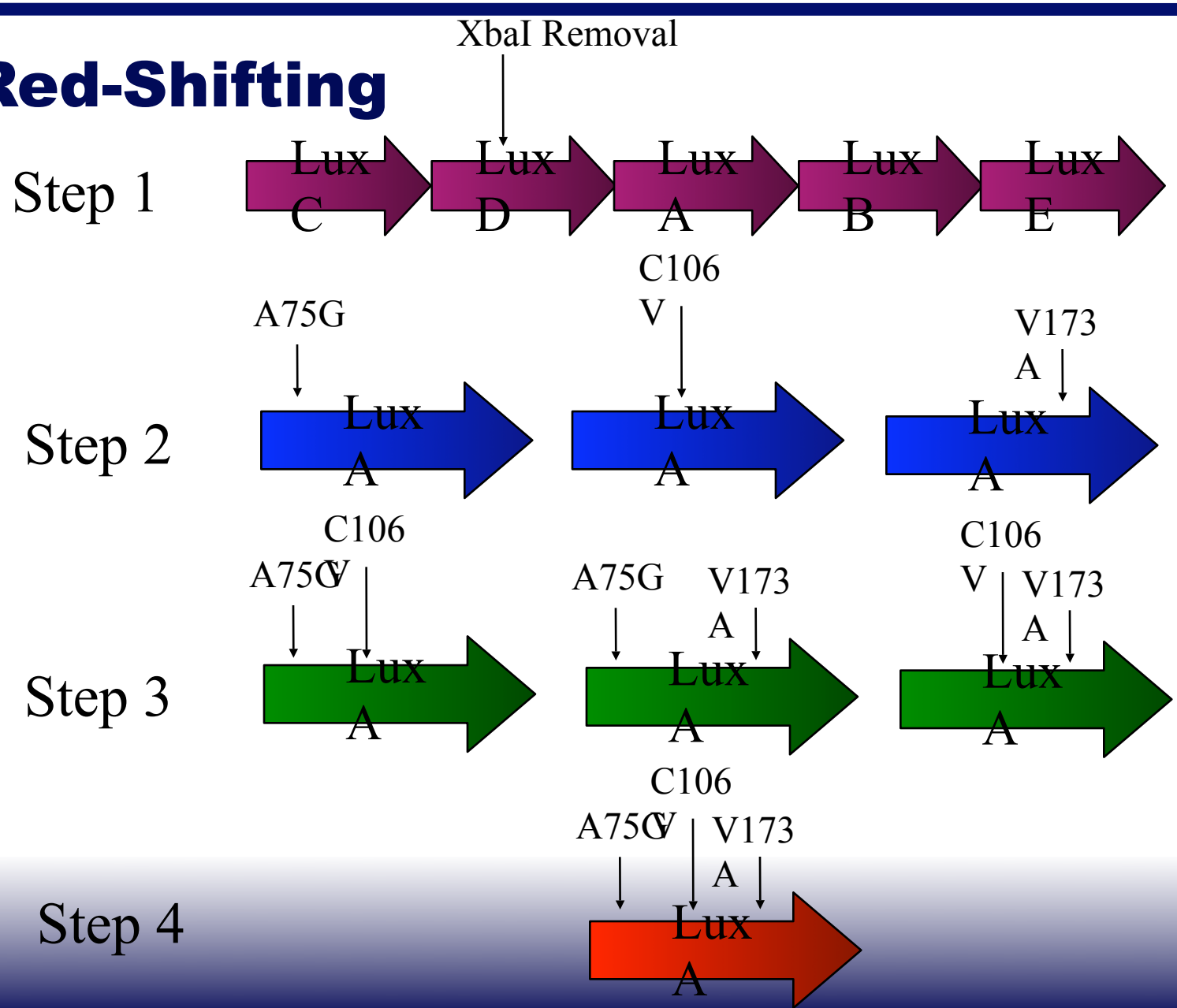


Creating a DNA Alphabet

Symbol	Code	Symbol	Code	Symbol	Code	Symbol	Code
A	AAA	Q	CAC	7	GAG	;	TAG
B	AAC	R	CAG	8	GAT	,	TAT
C	AAG	S	CCA	9	GCA	(	TCA
D	AAT	T	CCC	0	GCC	)	TCC
E	ACA	U	CCG	+	GCG	[	TCG
F	ACC	V	CCT	-	GCT	]	TCT
G	ACG	W	CGA	*	GGA	<	TGA
H	ACT	X	CGC	/	GGC	>	TGC
I	AGA	Y	CGG	=	GGG	@	TGG
J	AGC	Z	CGT	>=	GGT	#	TGT
K	AGG	1	CTA	<=	GTA	^	TTA
L	AGT	2	CTC	!	GTC	&	TTC
M	ATA	3	CTG	?	GTG	%	TTG
N	ATC	4	CTT	.	GTT		TTT
O	ATT	5	GAA		TAA	UNUSED	CAT
P	CAA	6	GAC	"	TAC	UNUSED	ATG

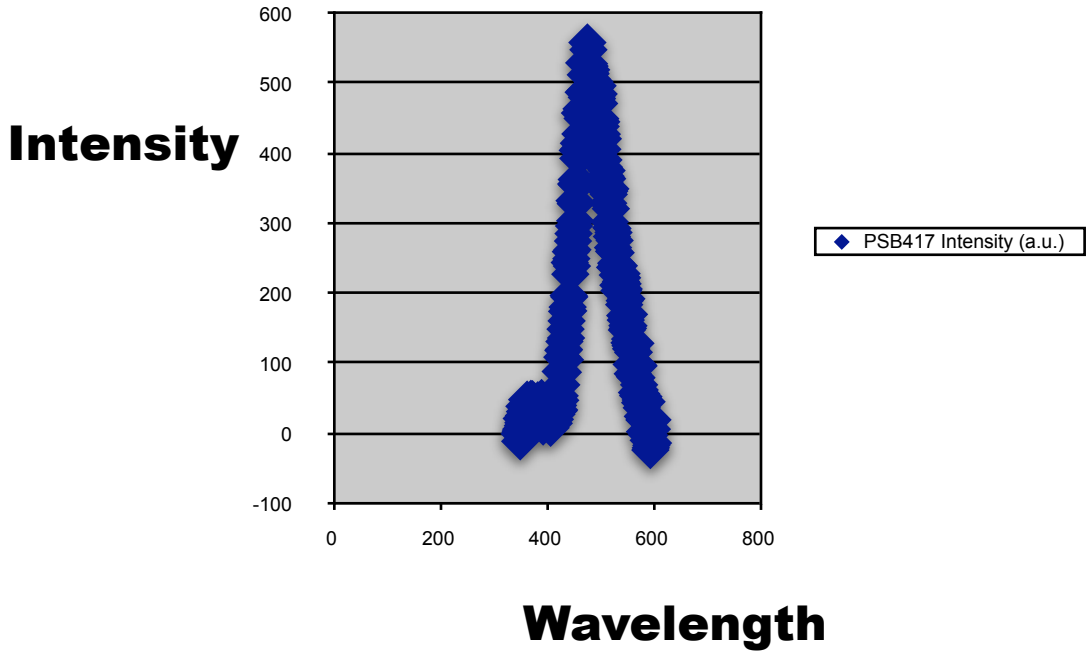
Creating a Light Alphabet

Red-Shifting



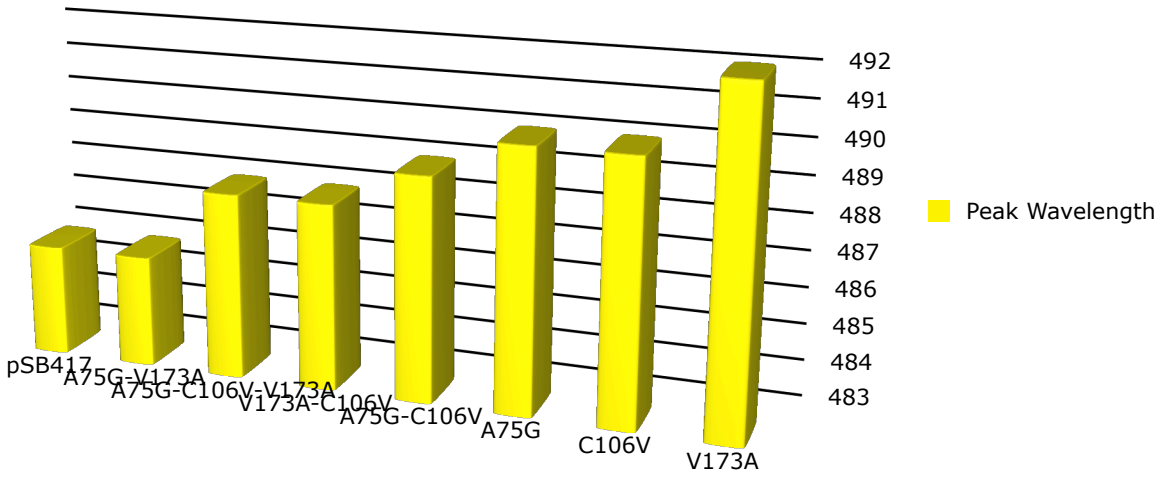
Creating a Light Alphabet

Wavelength Scanning

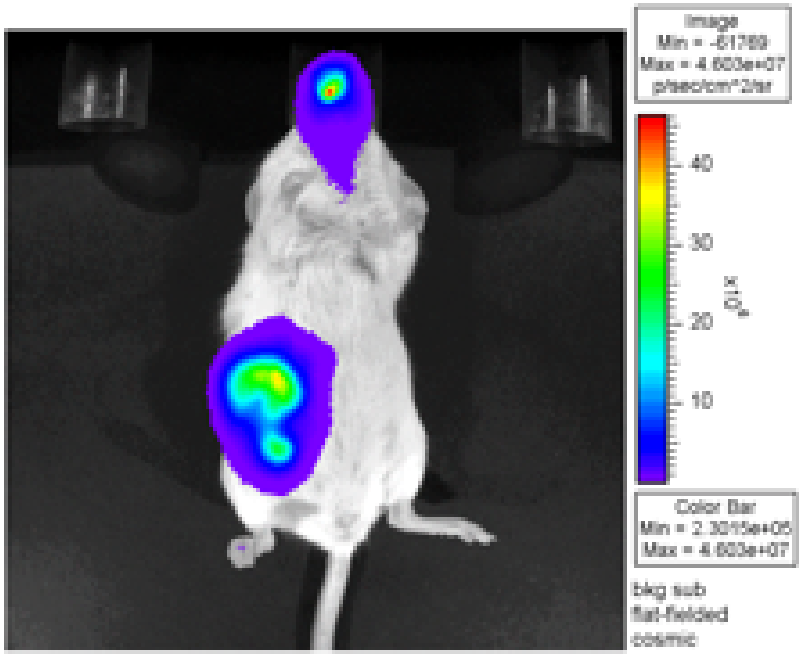


Creating a Light Alphabet

Peak Wavelength



In Vivo Imaging



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Wed, Oct 25, 2006 15:18:44
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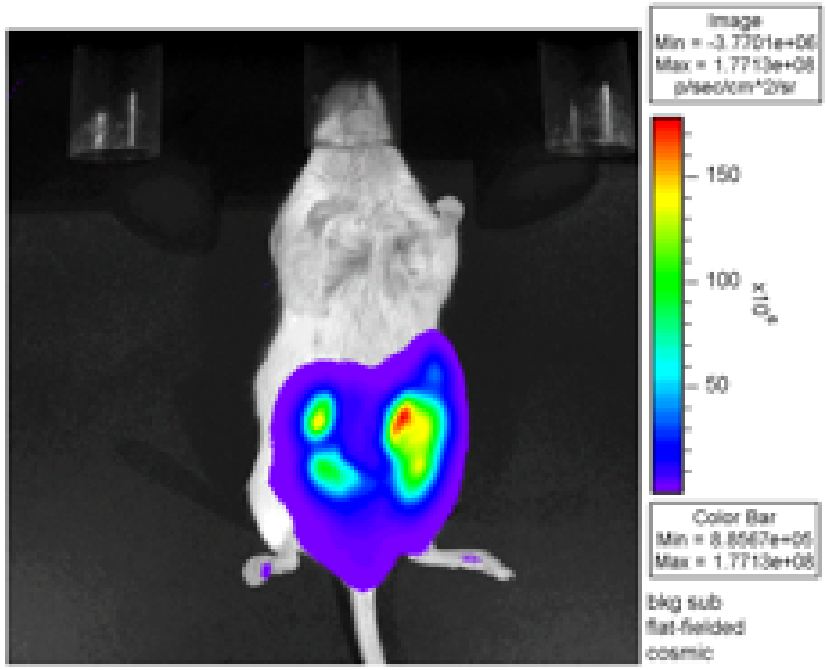
Filter: Open
Camera: IVIS 13062, LN1300EB

Series:
Experiment:
Label:
Comment:
Analysis Comment:



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Thu, Oct 26, 2006 09:28:38
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Filter: Open
Camera: IVIS 13062, LN1300EB

T=0

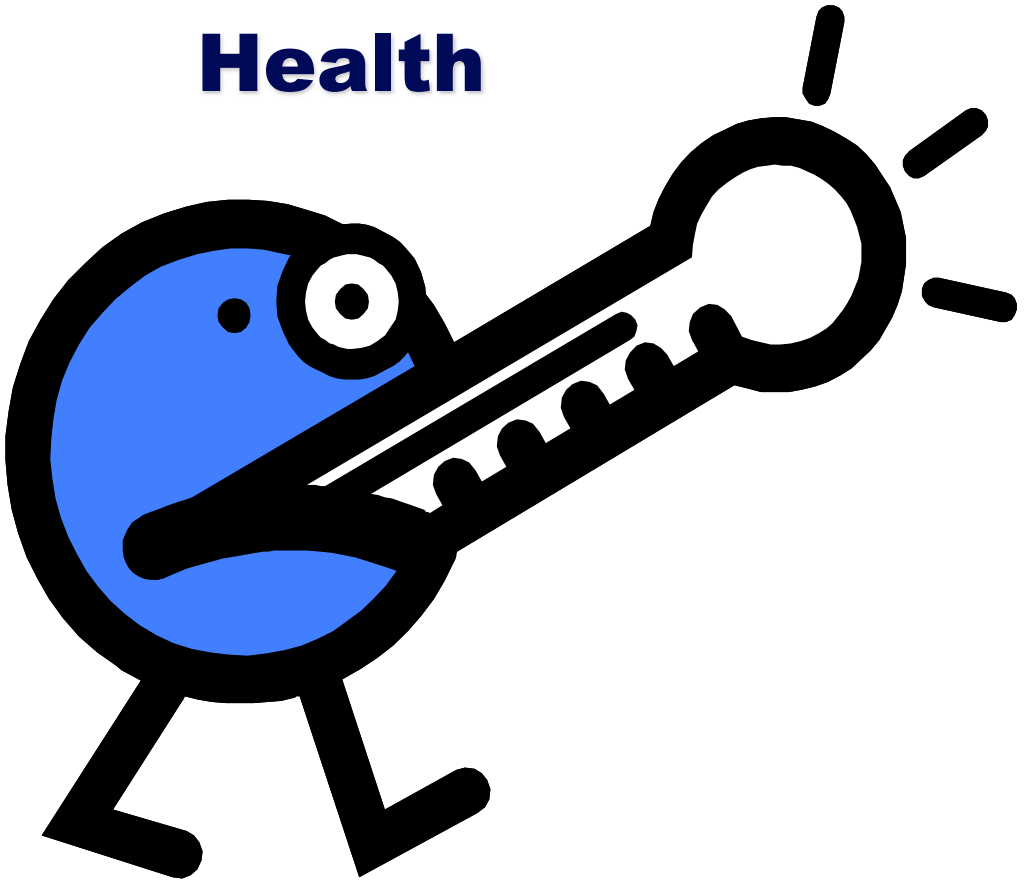
T=17 Hours

Applications

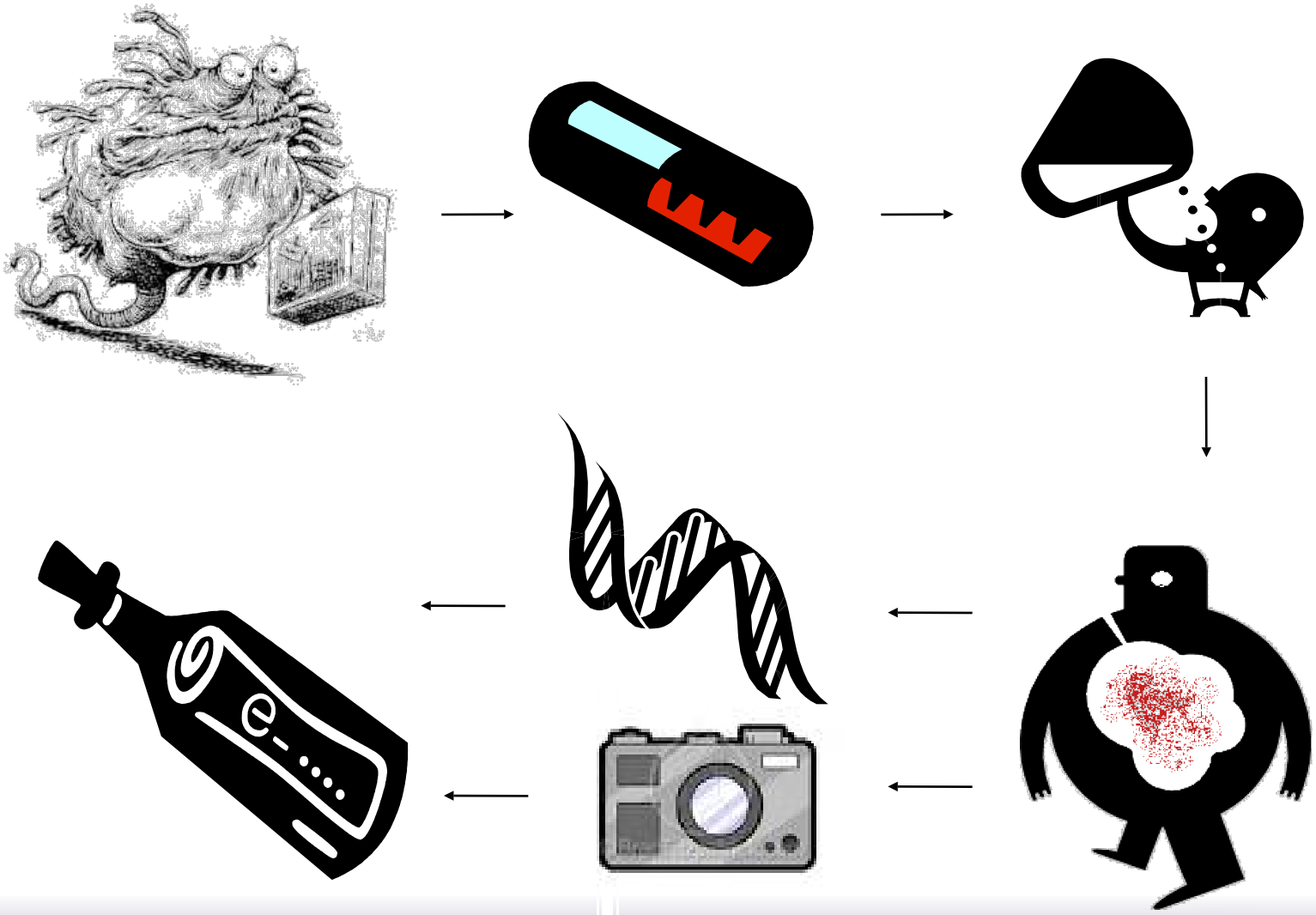
National Security

Applications

Health

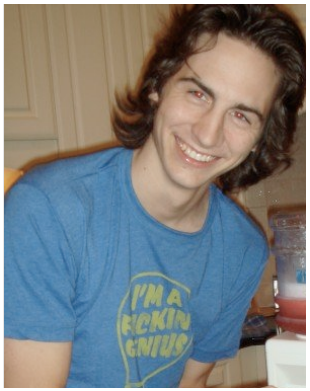


Conclusions



Conclusions

A Novel Suicide Circuit for Tumor Targeting Bacteria

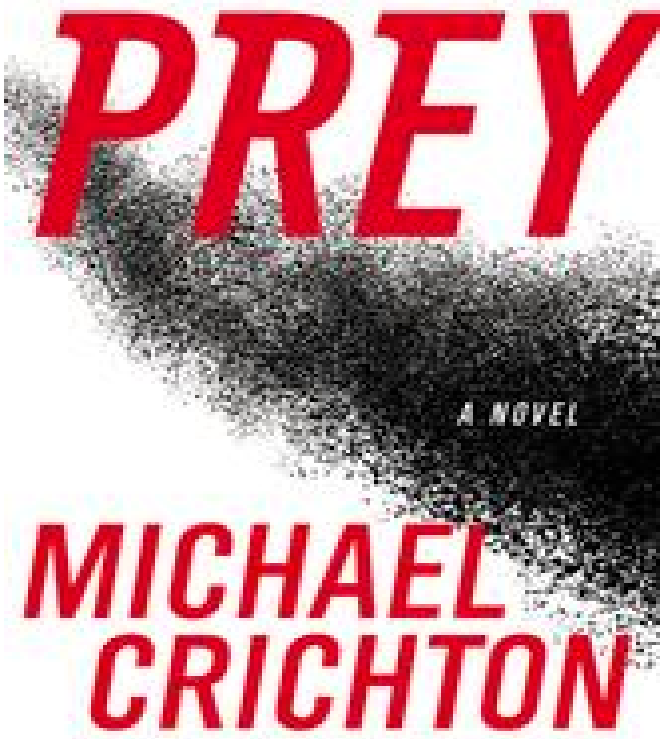


Austen Heinz

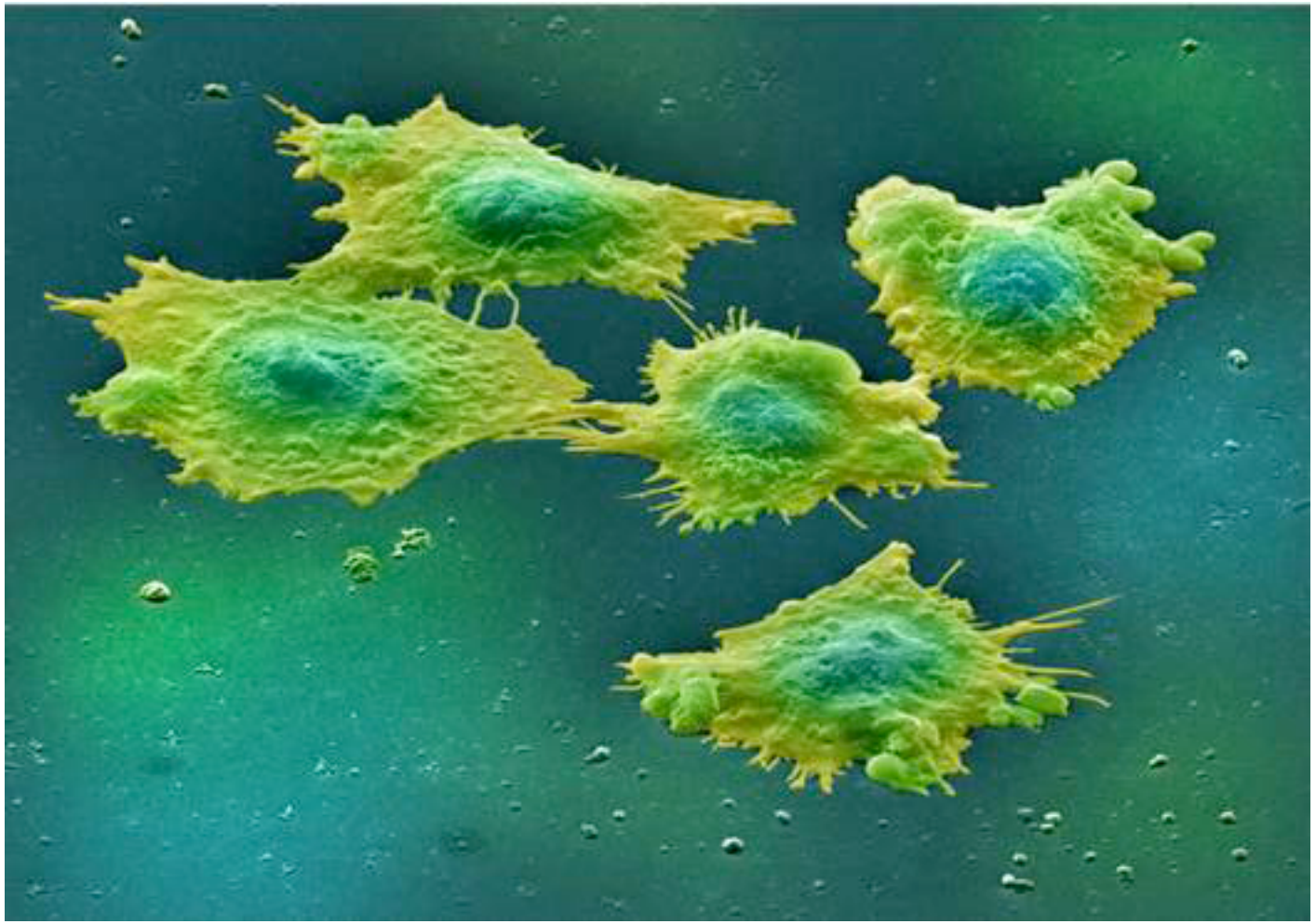
Nirav Lakhani

Lingchong You

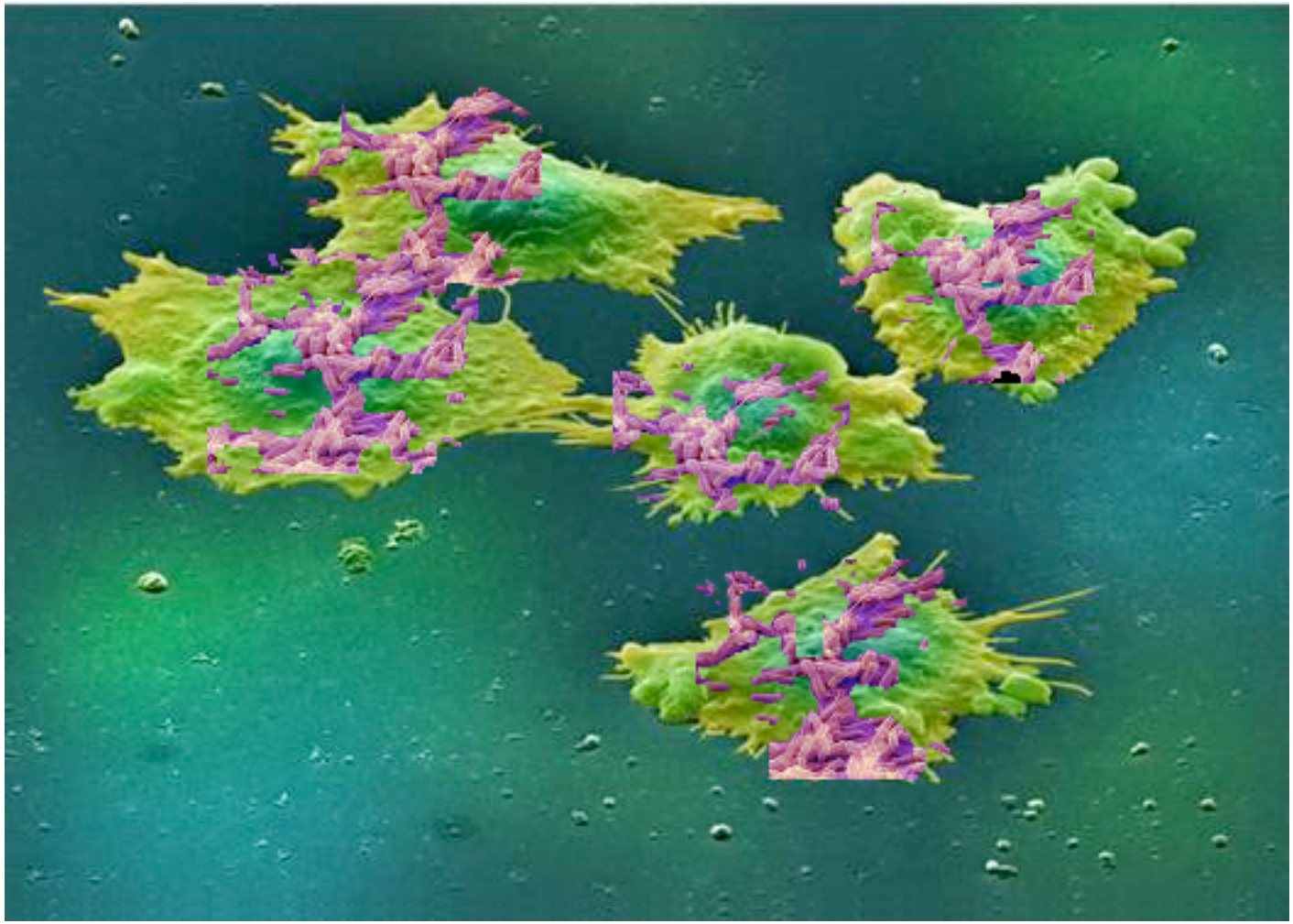
Micromachines and Swarm Behavior



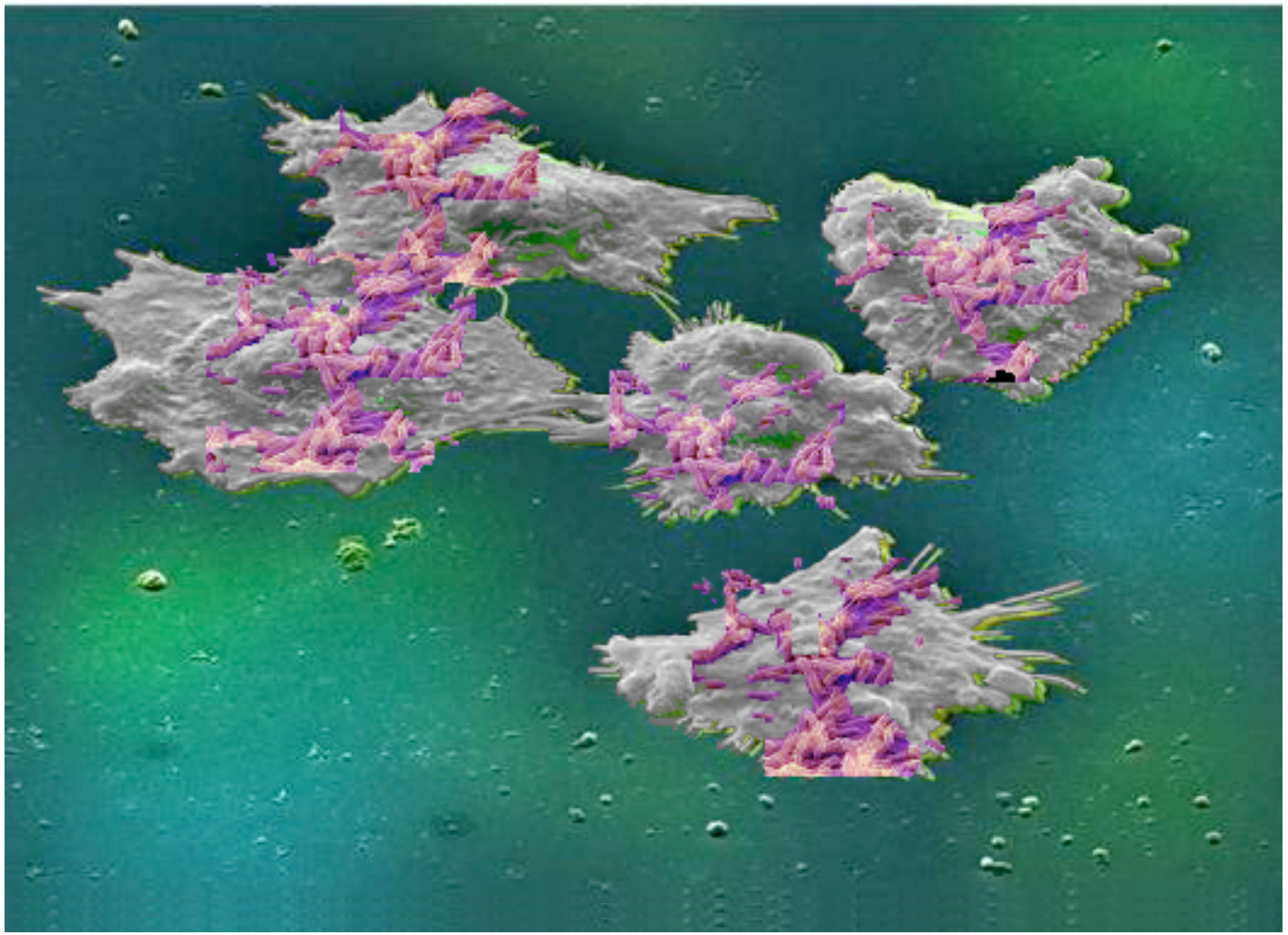
Stickybots



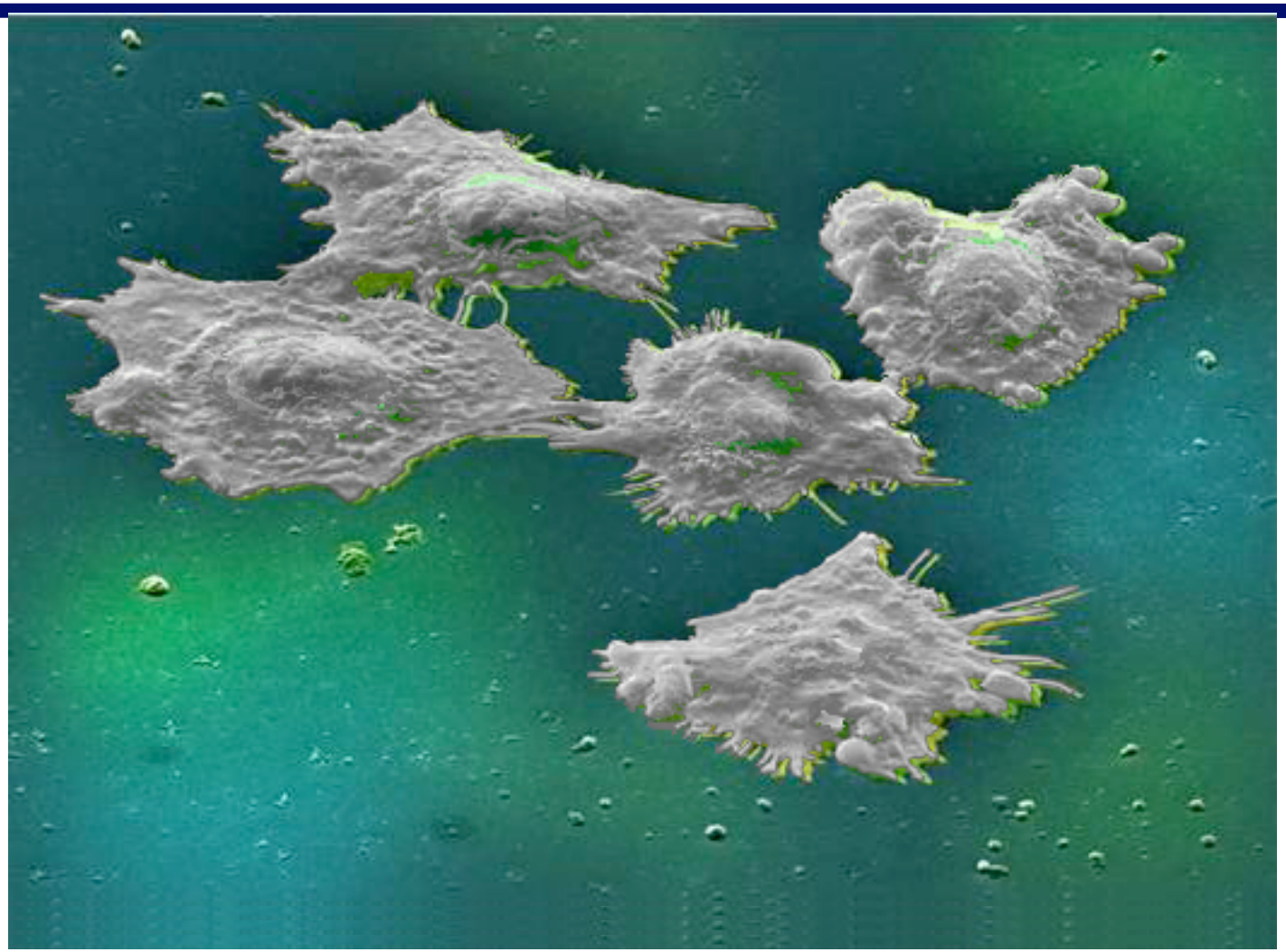
Stickybots



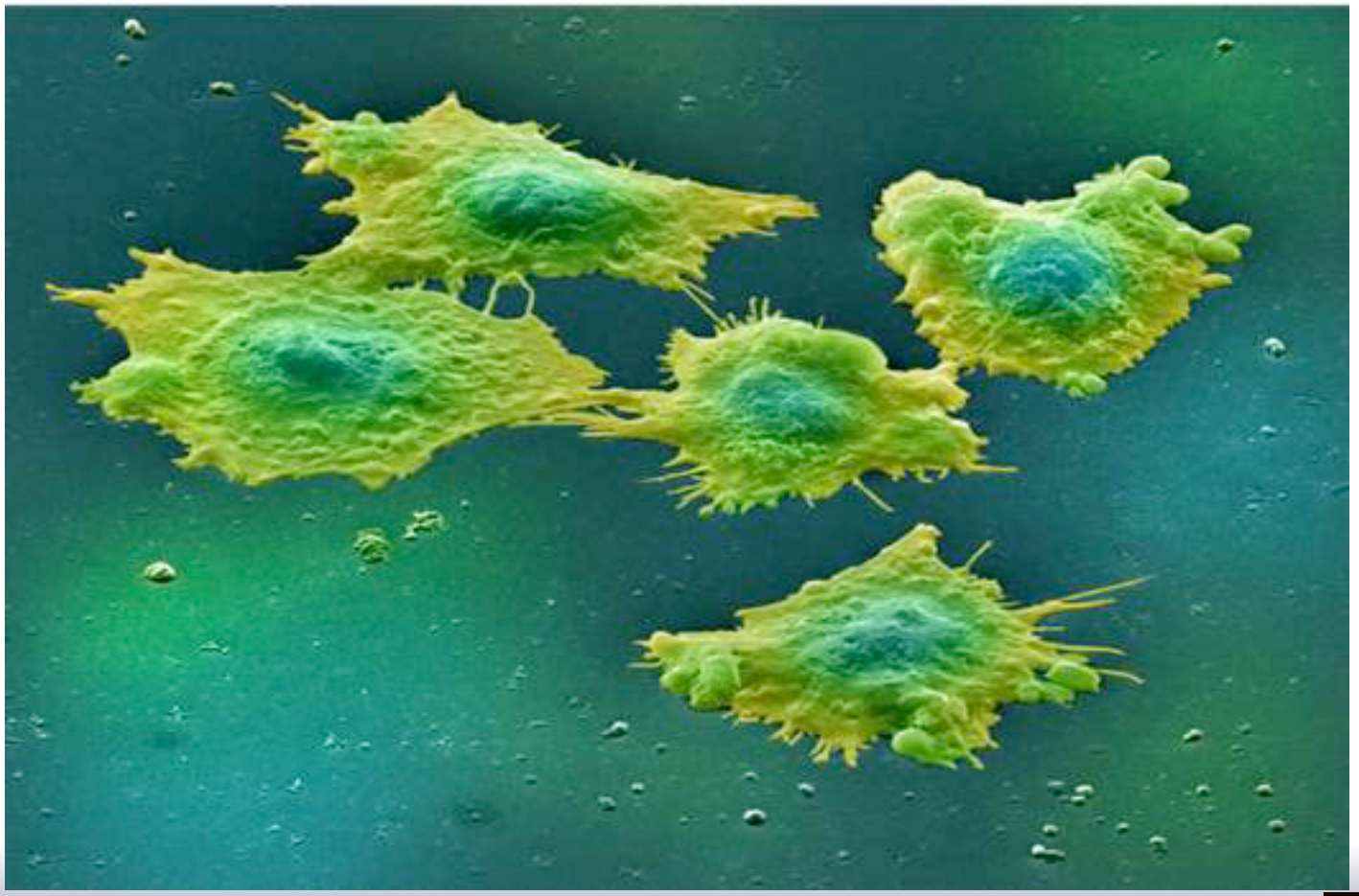
Stickybots



Stickybots



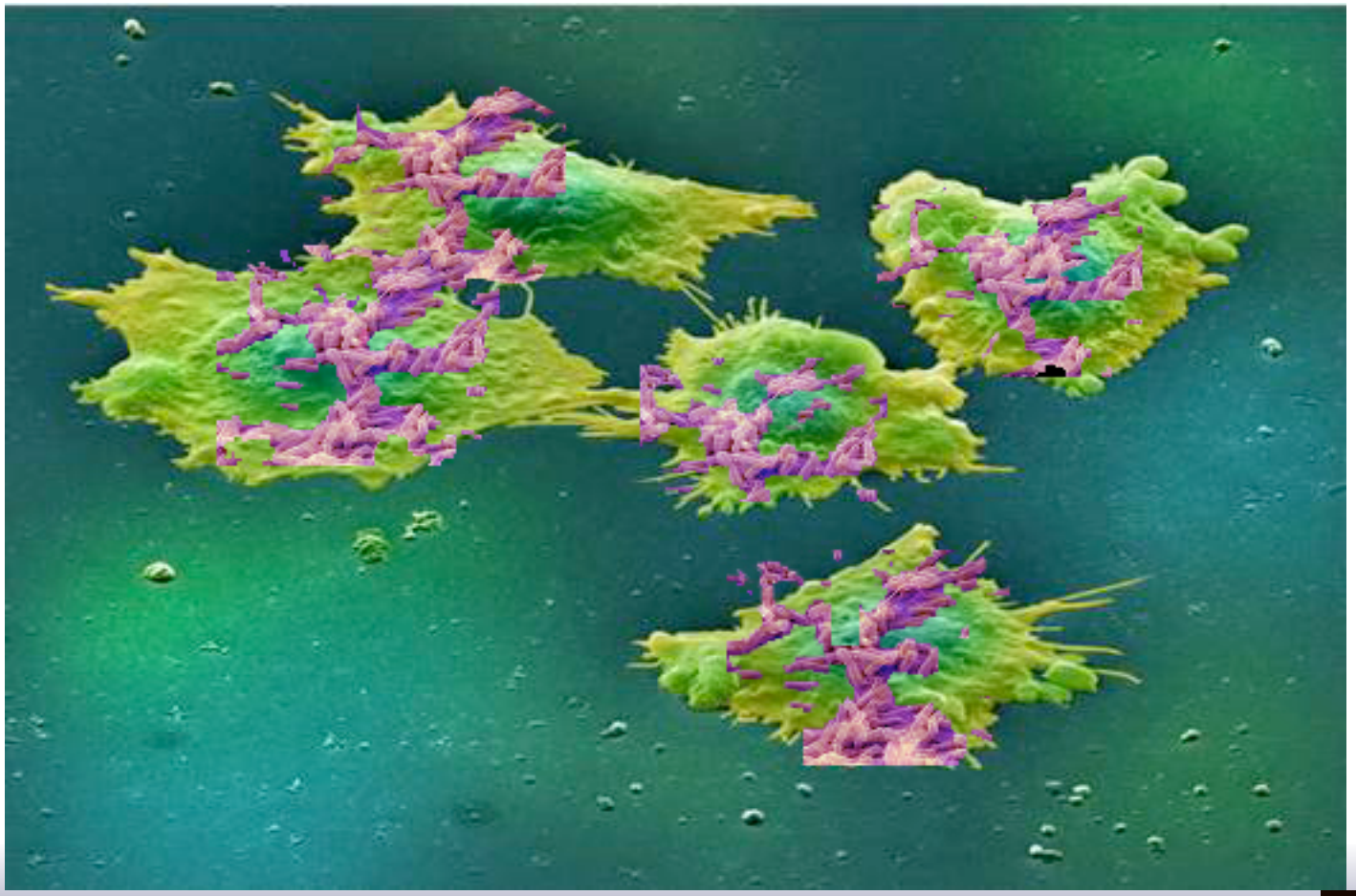
Targeted Localization



cancer stickybots

Targeted Localization

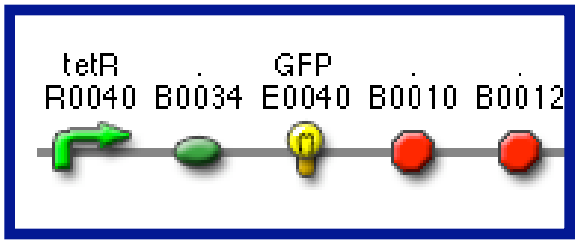
A Sticky Swarm



Targeted Localization

Circuit Function:

Targeted Localization



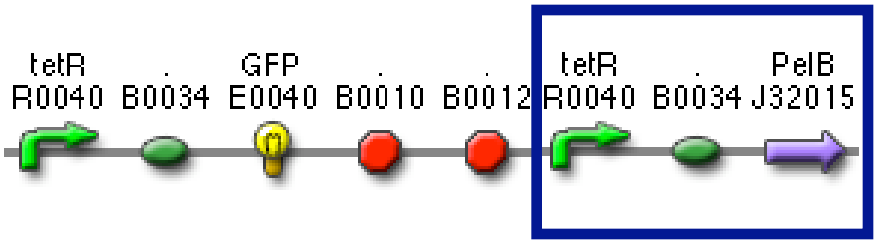
Circuit Function:

- GFP to assess circuit operation

Constitutive Expression of:

- PelB leader sequence-directs the protein to the periplasmic membrane of E.coli
- Surface expression of the cAb-CEA5 Nanobodies™ received from Ablynx® + S*Tag Fusion protein for display detection
- C-IgAP Autotransporter surface display provides a modular scaffold

Targeted Localization



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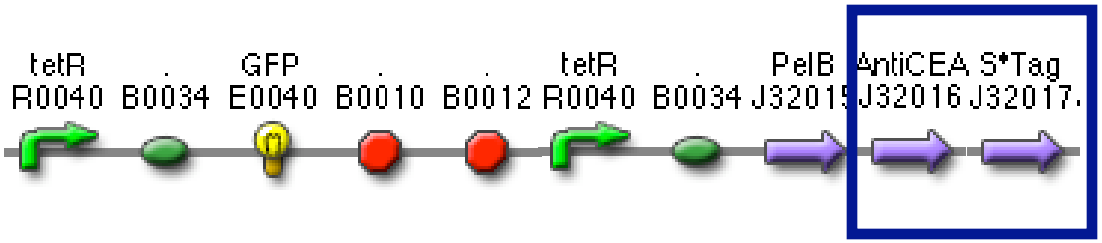

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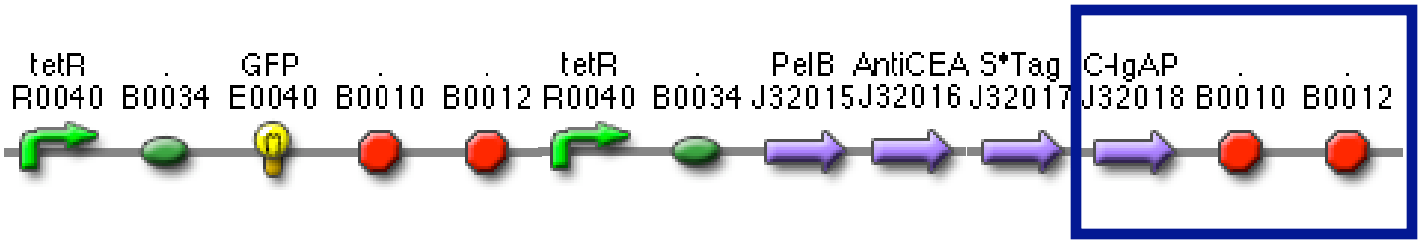

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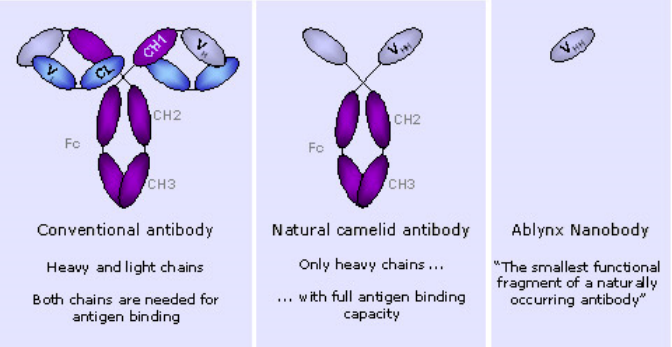
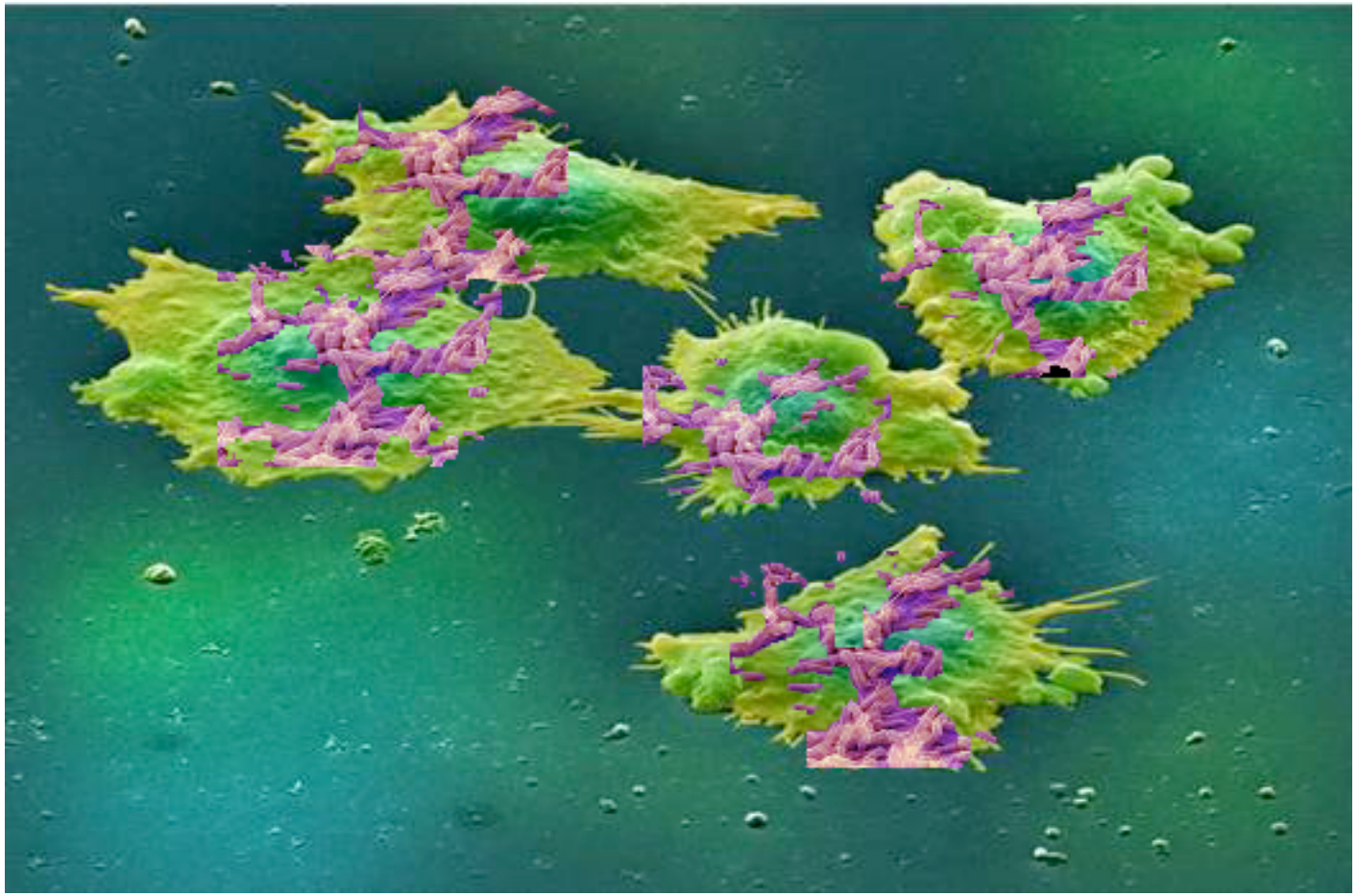
Targeted Localization

Nanobodies



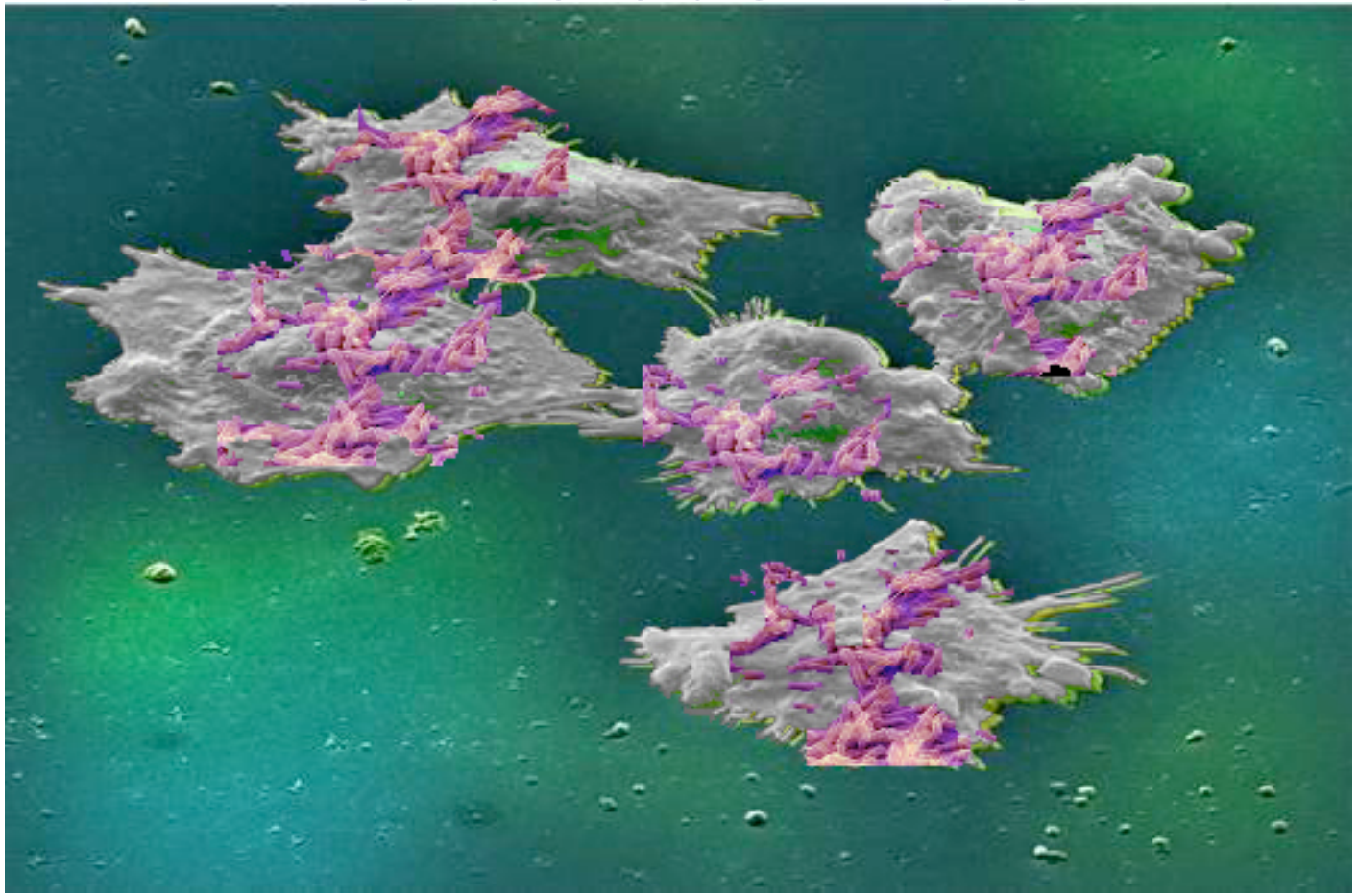
Neisseria gonorrhea IgA Protease

Discriminate Killing



Discriminate Killing

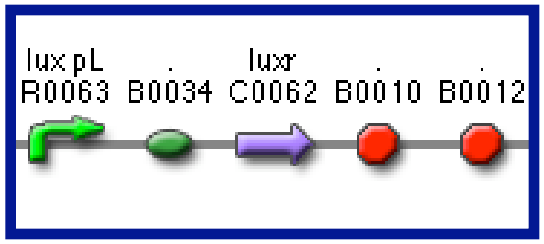
Controllable Killers



Discriminate Killing

Key Characteristics:

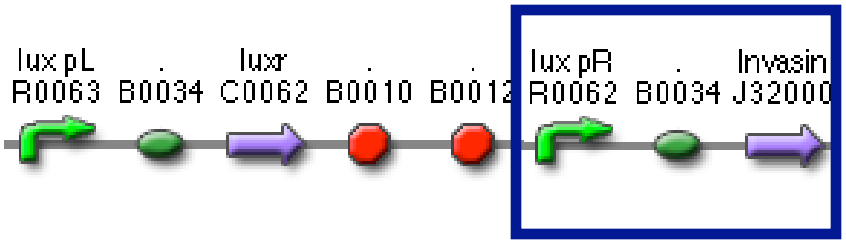
Discriminate Killing



Key Characteristics:

- Quorum sensing receiver device
- Production of Invasin linked to population density
- Production of Cytosine Deaminase, which converts nontoxic 5-Fluorocytosine to 5-Fluorouracil also under control of quorum sensing.
- CFP.

Discriminate Killing



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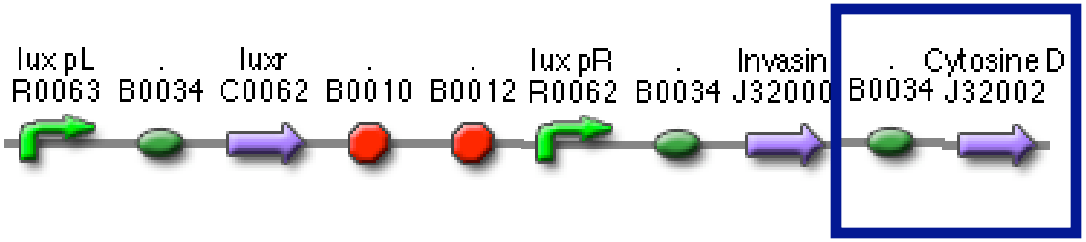

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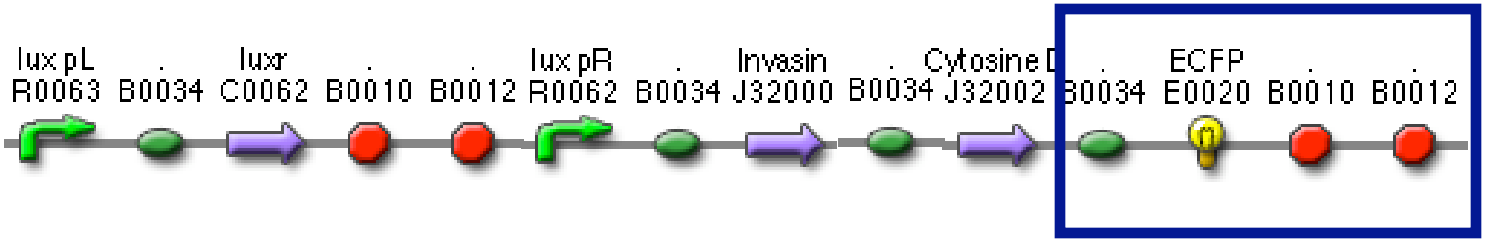

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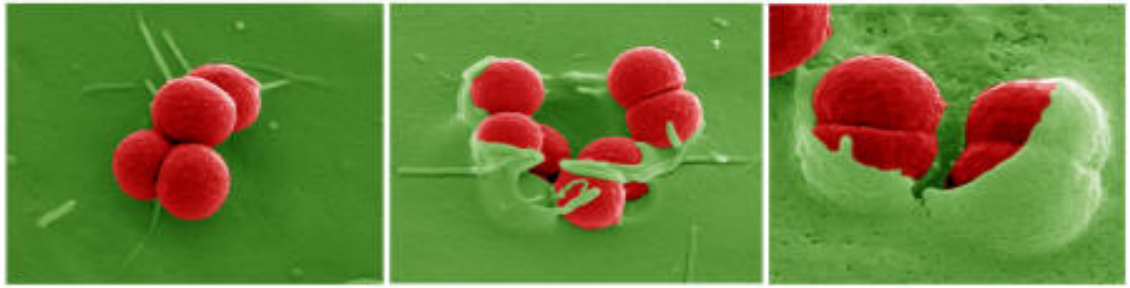
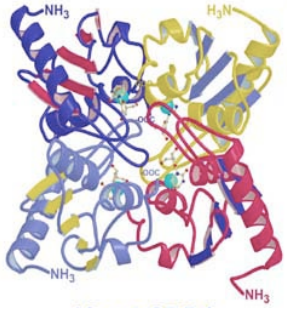
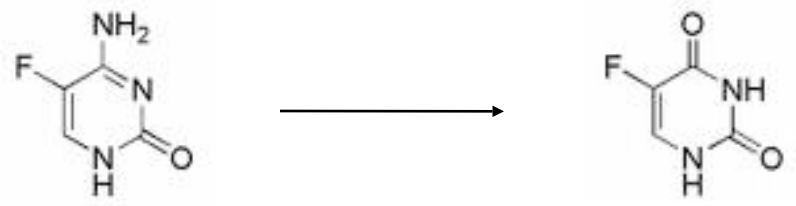
Discriminate Killing

Mammalian Cell Invasion via Invasin



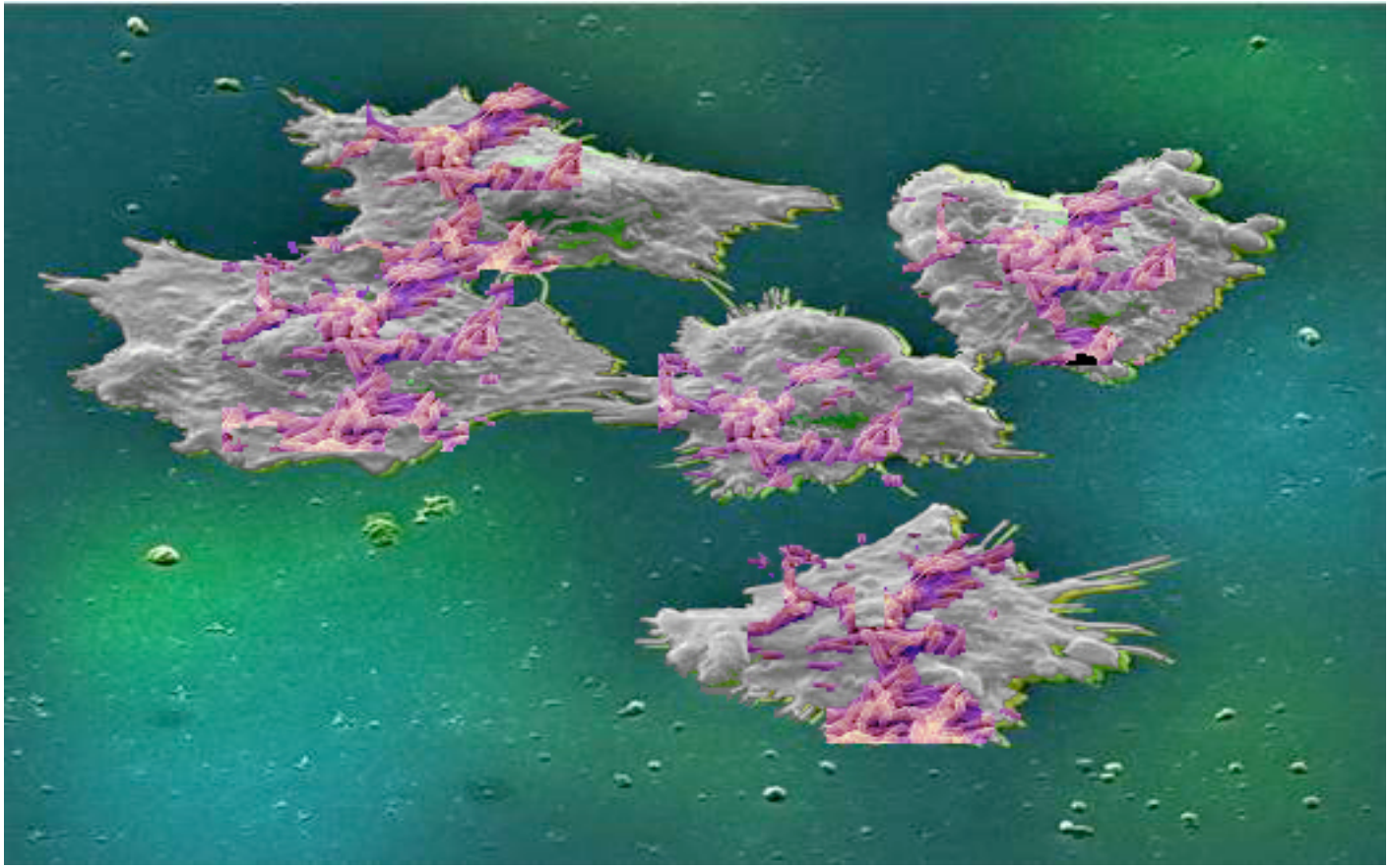
Killing via Cytosine Deaminase conversion of 5-Fluorocytosine to cancer poisoning 5-Fluorouracil





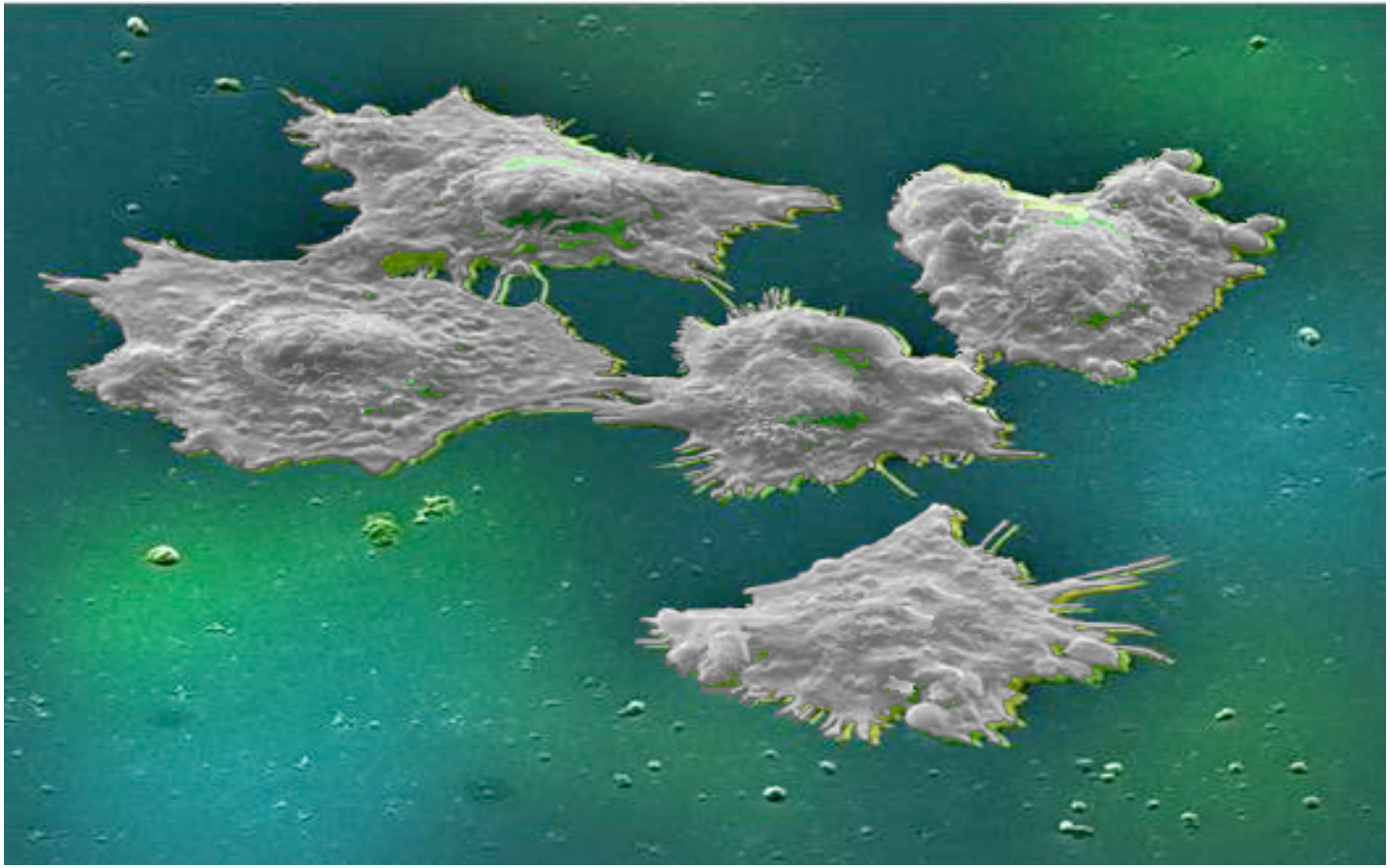
5FC 5FU

Regulated Suicide



Regulated Suicide

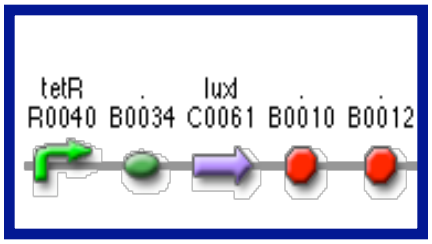
Stickybot Self Destruction



Regulated Suicide

Key Characteristics:

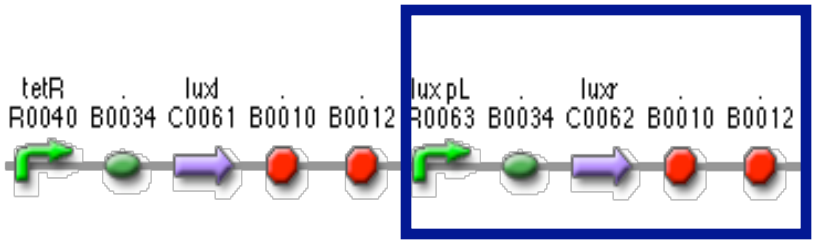
Regulated Suicide



Key Characteristics:

- Quorum sensing receiver device.
- Quorum sensing dependent transcription of anti-toxin protein CcdA
- IPTG activation of CcdB death toxin.
- RFP

Regulated Suicide



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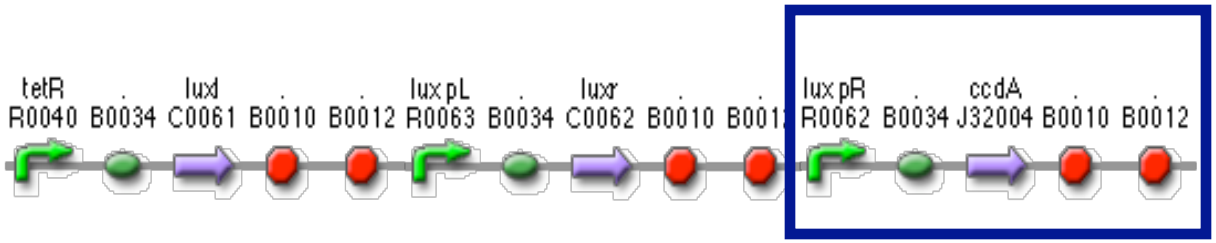

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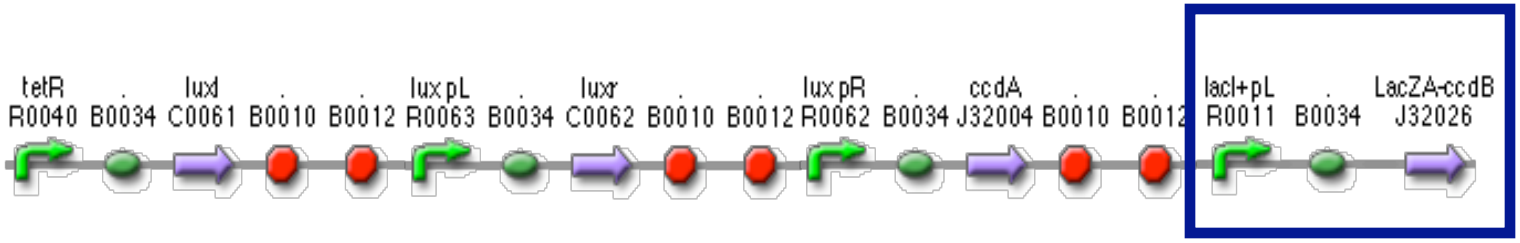

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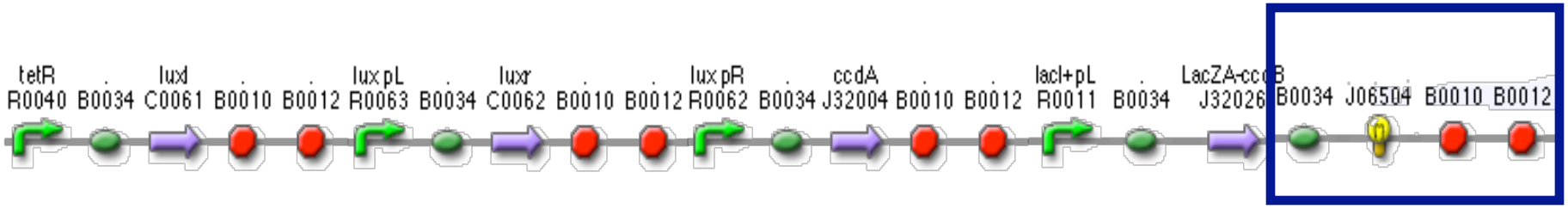

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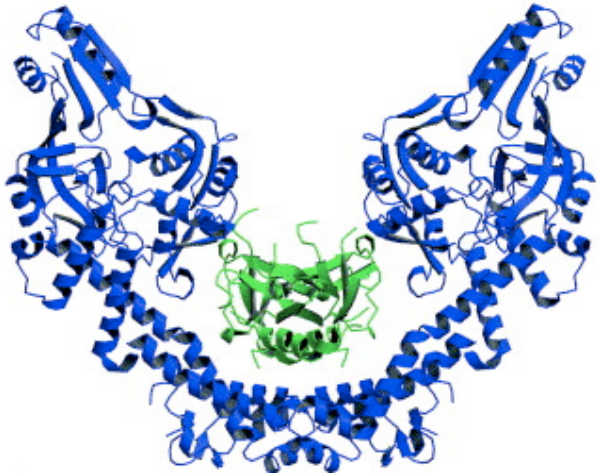
Regulated Suicide

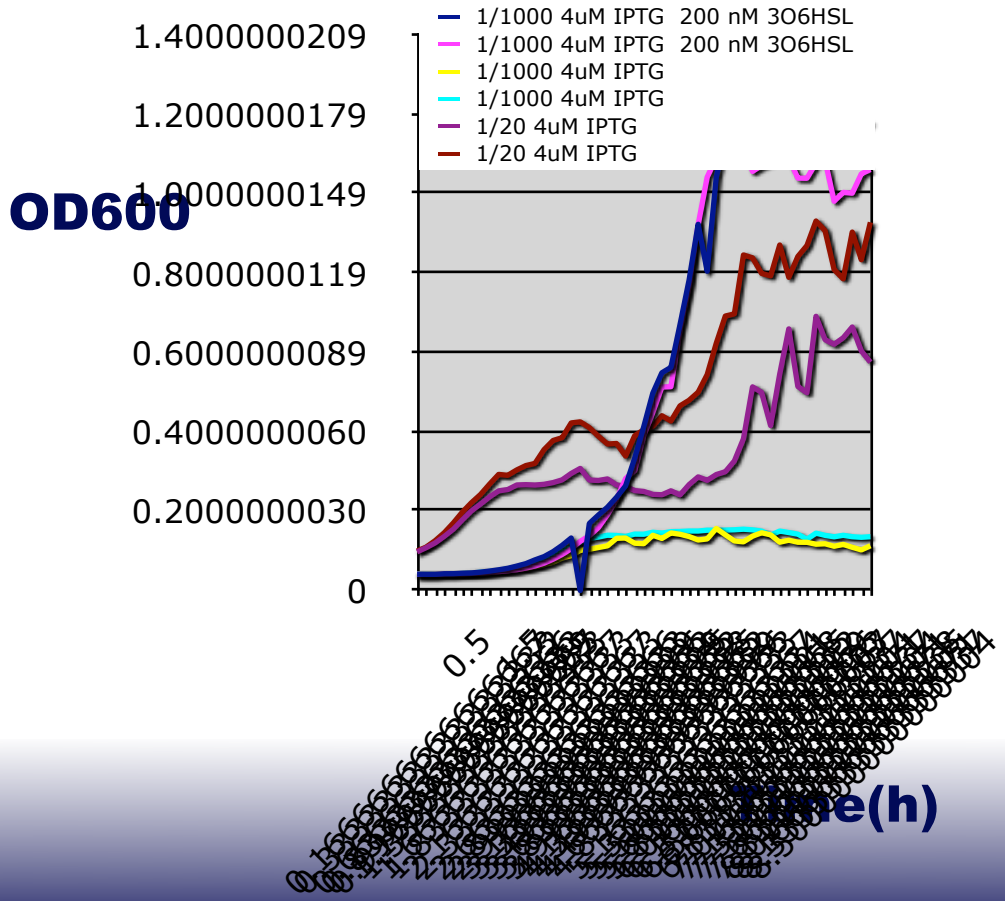
CcdB/GyrA59



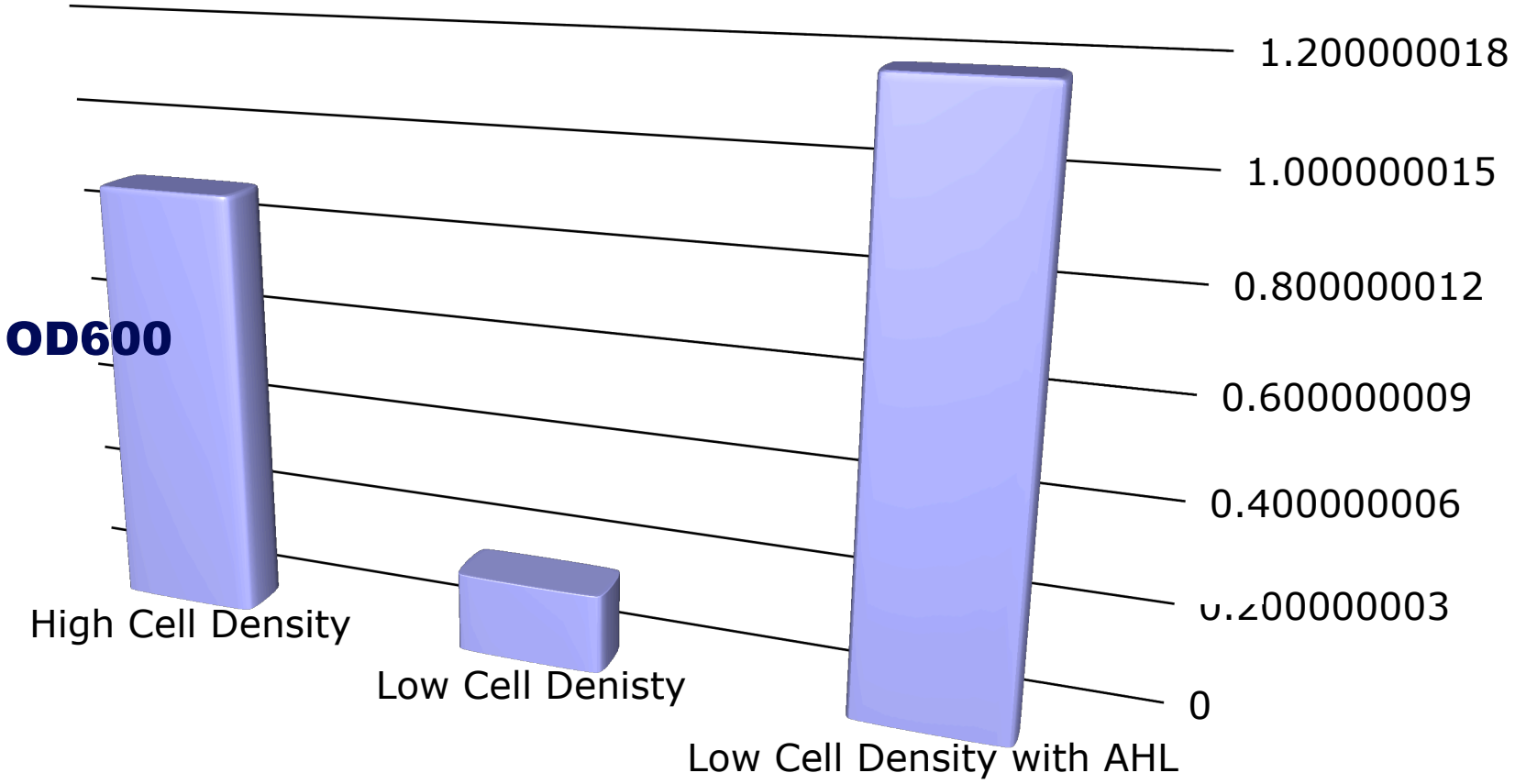
CcdA/CcdB

Regulated Suicide

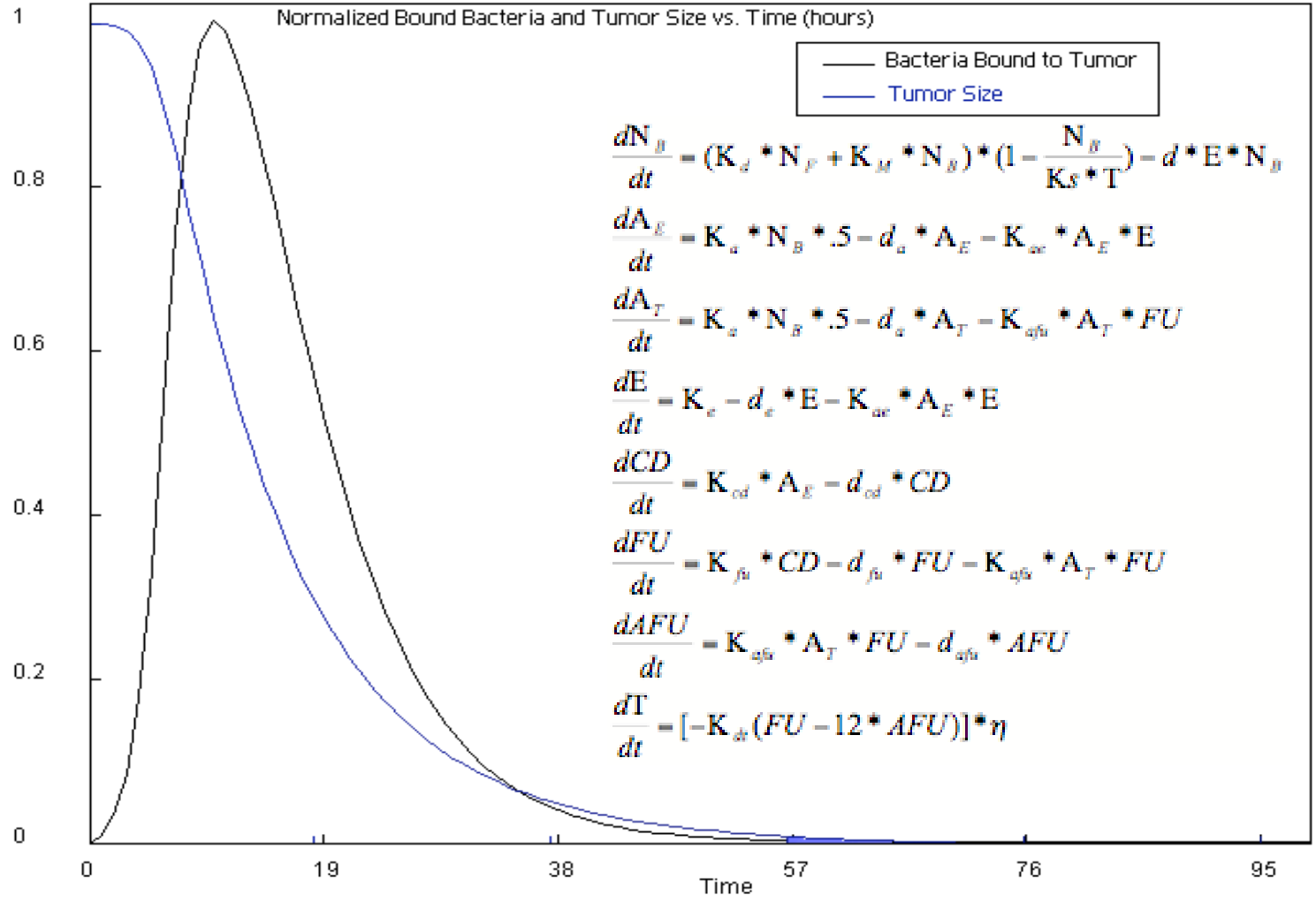
Regulated Suicide



Regulated Suicide



Results



$$\frac{dN_B}{dt} = (K_d * N_P + K_M * N_B) * (1 - \frac{N_B}{Ks * T}) - d * E * N_B$$

$$\frac{dA_E}{dt} = K_a * N_B * .5 - d_a * A_E - K_{ae} * A_E * E$$

$$\frac{dA_T}{dt} = K_a * N_B * .5 - d_a * A_T - K_{afu} * A_T * FU$$

$$\frac{dE}{dt} = K_e - d_e * E - K_{ae} * A_E * E$$

$$\frac{dCD}{dt} = K_{cd} * A_E - d_{cd} * CD$$

$$\frac{dFU}{dt} = K_{fu} * CD - d_{fu} * FU - K_{afu} * A_T * FU$$

$$\frac{dAFU}{dt} = K_{afu} * A_T * FU - d_{afu} * AFU$$

$$\frac{dT}{dt} = [-K_{ft}(FU - 12 * AFU)] * \eta$$

Conclusion

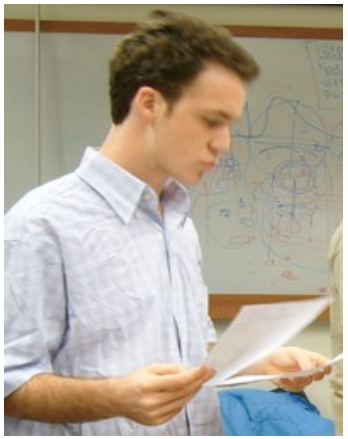
- **Working Regulated Suicide Circuit and System Modeling**

Conclusion

- **Working Regulated Suicide Circuit and System Modeling**

Mission Accomplished

Engineering “Sticky” Magnetic Bacteria for Power Generation


Eric Josephs


Hattie Chung

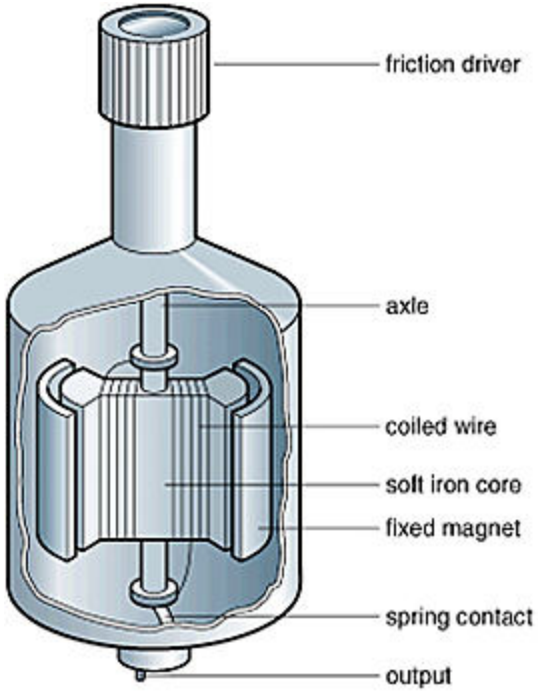

Jingdong Tian


Thom LaBean

Bacterial Dynamo

What's a dynamo?

This is a dynamo:

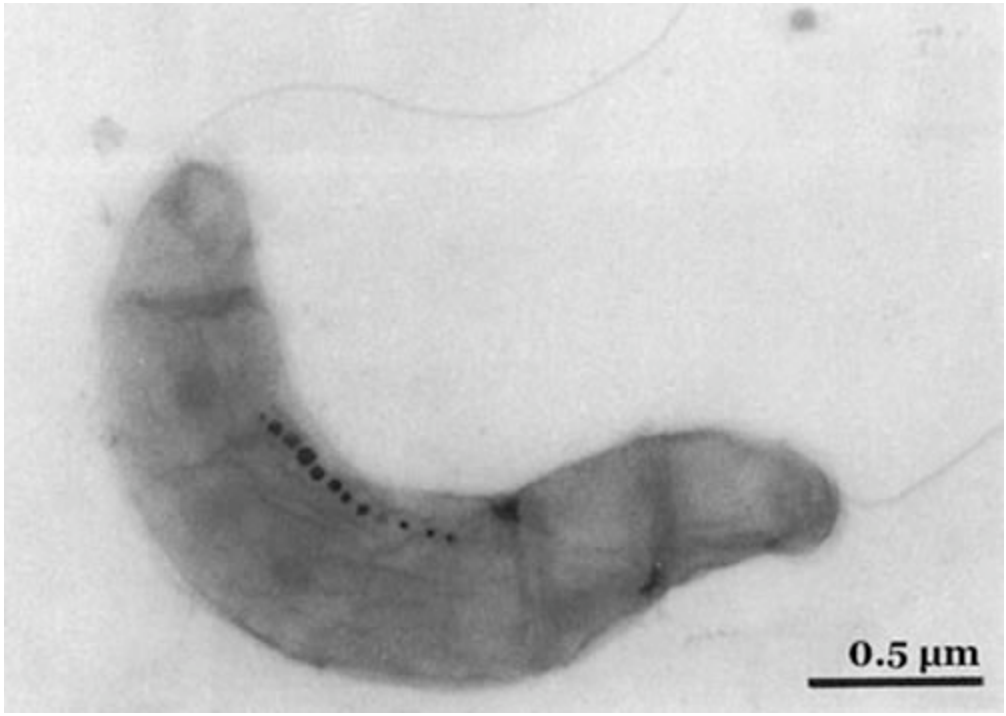


http://www.houseofcuss.com/hocvault/thepipe/archives/2005_09.shtml

But if we want to make this out of bacteria, where are we going to find magnets? Looks like we're going to have to ask our good friend...

Bacterial Dynamo

MAGNETOSPIRILLUM SP. AMB-1!



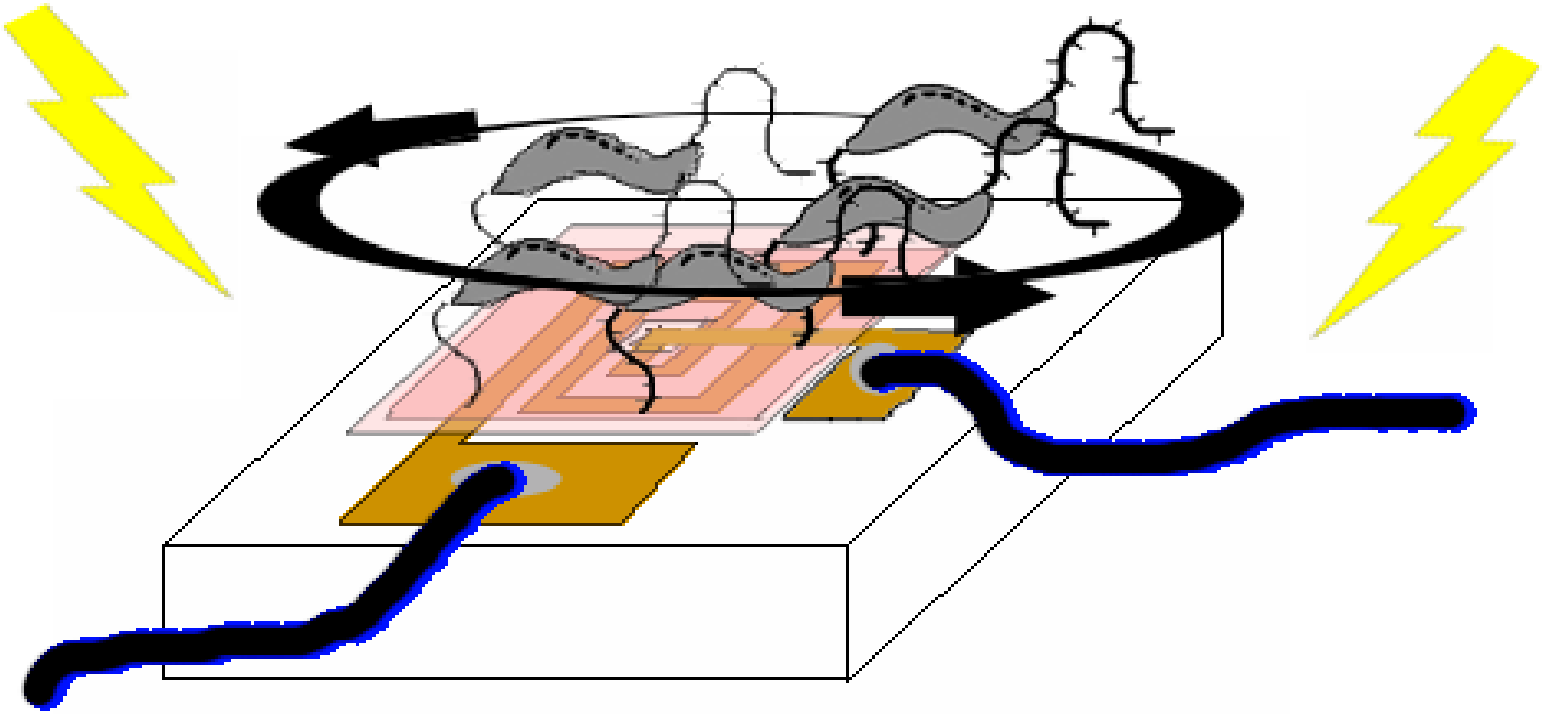
http://magnum.mpi-bremen.de/magneto/research/index.html

Spinning Tethered Bacteria

Bacterial Dynamo

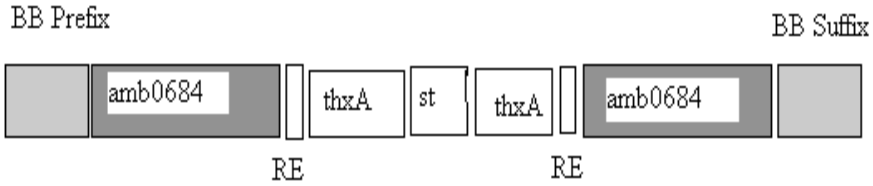
- Bacterial flagellar proteins are easy to modify.
- If a flagella sticks to a surface, it will cause the cell body to spin.
- Some bacteria grow chains of nano-sized magnets in their cell bodies.
- A spinning magnet field will induce a voltage in a coil.
- **If we engineer the flagella of magnetic bacteria to stick to a surface above a coil, we can get a dynamo powered by flagellar motors.**
- This concept has been proven before by sticking magnetic bacteria to coils with anti-flagellin antibodies and the system fell apart after a few hours (days?). If we genetically engineer the bacteria to produce a flagellin protein that sticks to an easily patterned surface, the system will 'self-assemble' and could continue indefinitely.

Bacterial Dynamo



Making ‘sticky’ flagella

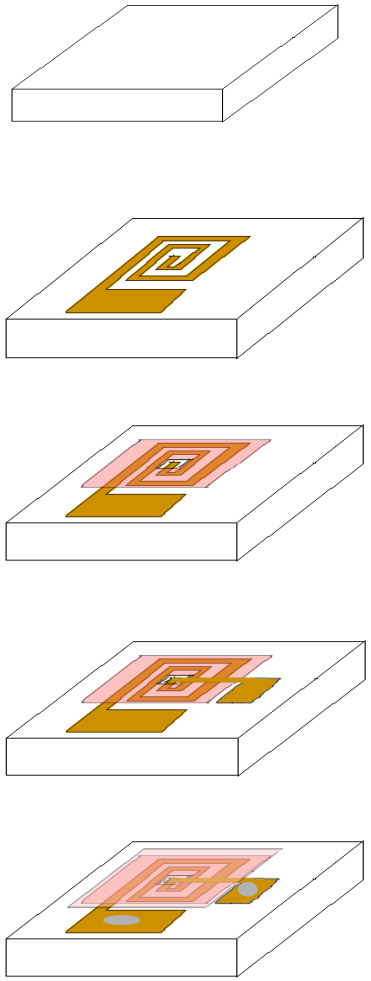
- Find protein which binds to hard-baked S-1813 positive photoresist by screen ~10^8 12-aa random peptides expre ssed on flagellar exterior to see which bind to photoresist.



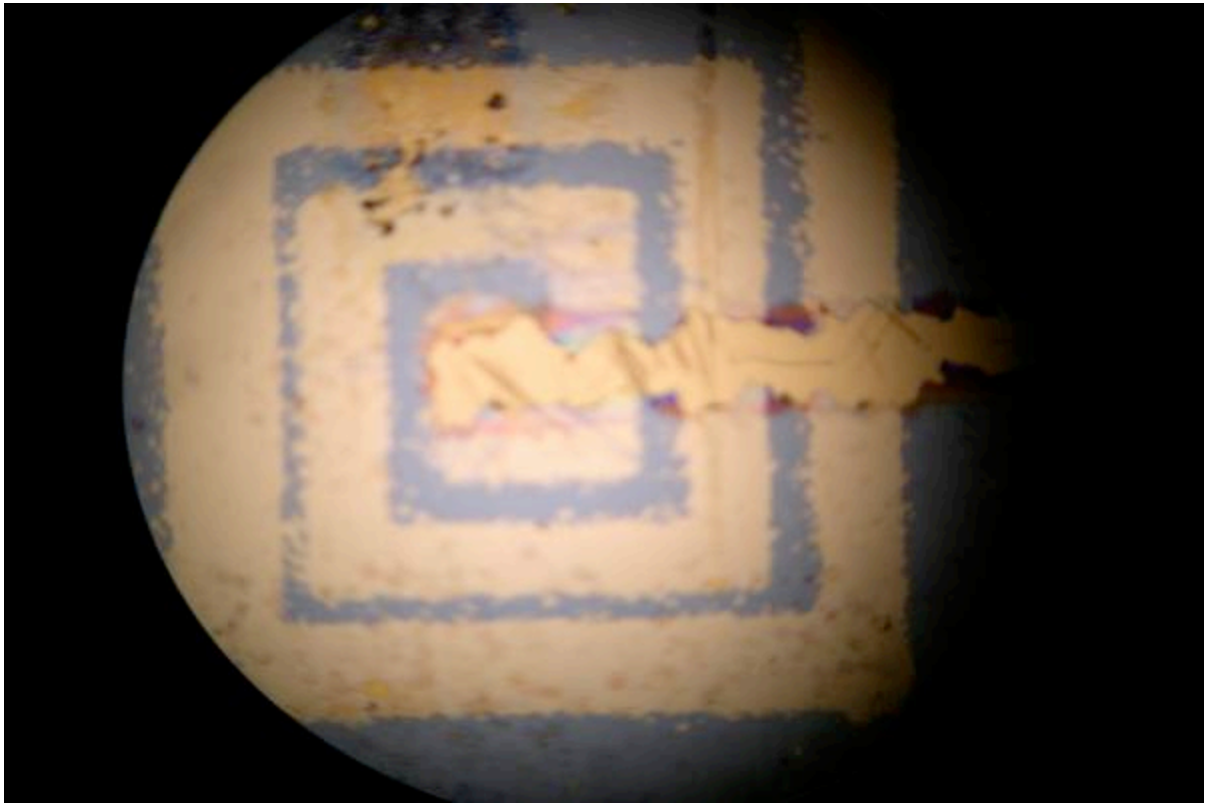
- Cut out amb0684, AMB-1 flagellin gene, split it in two, splice in the sticky gene with thioredoxin structure.
- Put it back into AMB-1, get STICKY MAGNETIC BACTERIA.

Making the dynamo

- Fabricate a little coil for magnetic bacteria to grow on, seal it with positive photoresist so the bacteria will stick.
- More details at poster.



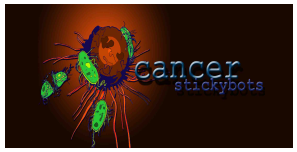
Coil

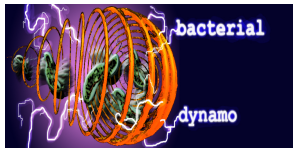


Conclusions

- Our coil is completed, we isolated a ‘sticky’ peptide, and are currently working on PCRing out the AMB-1 flagellin gene.
- Possible applications of this project include ‘natural’ batteries and, since research is being conducted in using bacteria to convert the chemical energy of many different sources (contaminants, pollution, nuclear waste) into energy the bacteria can use, this dynamo could possibly be engineered to convert almost anything as fuel

Duke iGEM 2006 Conclusions





- Testing and characterization of multiple bacteria small molecule communication systems. Modeling and construction of two synthetic artificial ecosystems in bacteria: **X-Verter** a three stage population level oscillator and **Predator Prey** a two stage two population oscillator. Created an open source gene circuit IDE called Biobrick Manager.
- Creation and characterization in a mammalian system of a DNA and light "alphabet" for **Human Encryption**. Future uses include national security and health detection applications
- Development of a working bacteria circuit that causes the bacteria to self-destruct when outside the cancer environment for the **Cancer Stickybots** project and system modeling.
- Evolving E coli bacteria to stick to positive photoresist and micromachining of an apparatus for future use as a **Bacterial Dynamo**: magnetic bacteria that spin above a coil and produce electricity via Faraday's law.

Acknowlegements

- Jingdong Tian (Duke)
- Fan Yuan (Duke)
- Thom LaBean (Duke)
- Lingchong You (Duke)
- Faisal Reza (Duke)
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- Margaret Black (Washington State)
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- Chandra Drennen (USC)
- Andrew Simnick (Duke)
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- Mike Winson (University of Wales Aberystwth)
- Eric Metcalf (University of Illinois)