20.020/20.902/20.947
Brain/Machine Hybrids
Studio Meeting Notes/Project Log

Overview:
The Brain/Machine Hybrids project log is a compilation of notes taken at our group meetings as well as notes from separate interviews and research done individually. Most of our meeting time was in class or studio time, and we shared most of our research material via the Facebook group, email, and Google Docs. Studio days with no explicit log entry were spent on individual research.

More than providing a breakdown/trail of work or comprehensive body of research, this project log should provide an idea of our ideas, considerations, and thought processes throughout the semester as we tackled the challenge our group was given.

BRAIN/MACHINE HYBRIDS - Studio 1 (02/27/2008)
Advisors (20.902/20.947)
Kate Broadbent, Han Zhu, Sophia Roosth

20.020 Students

Anonymous student JE

Charlotte Yang

Alex Rodriguez

Angela Chang

Introductions
Angela - chose this project because it seemed like a good intersection between Courses 6, 9, 20; background in Course 6
Charlotte - Project Seastar, Course 9 --> 20
Alex - interested in neuroengineering, tagging neurons, current UROP work (Ed Boyden), resources
JE - interested in neurology, psychology, changed from studying epilepsy to cancer studies

What is the importance of brain/machine hybrids?

- Allows for greater control (eg. surgery)
- Helps people with disabilities (eg. prosthetics)
- Applications help extend functionality, go places where you normally can't or do things you can't normally do
- Cognition/Information processing
- What exactly defines a machine and what counts as biological tissue? Scaffolding? Radioelectronics? Genetic encoding?
    - Can they contain biological material?
    - Does genetic engineering count?
    - Are engineered biological systems machines or not?
        - Philosophy: Dualism
            - Living is a combination of the physical and the spiritual
    - Drew says that there has to be a piece of genetic engineering in it, because we're engineering biology
        - Solution isn't just an external stimulus
        - Cells receive stimulus automatically
    - What do we mean by "interface" between biology and the mechanics?
- Difference between bacterial computing and brain/machine hybrids
    - Biological computing is trying to recreate the computer in a biological way to perform machine processes
    - We are trying to accept/send signals to and from the brain and translate them into electrical signals to perform tasks

Which way are we going with this topic?

- Synthetic biology? (viruses, cells, bacteria)
- Prosthetics?
- Technology? (eg. brain imaging)
- Neurons? Reading input, stimulate neurons for a response
- Methods of delivery? Problems: resistance
    - We need to know more about neurons/connections
    - Blood/brain barrier
    - Then what about encephalitis? Meningitis? Bacteria can cross the barrier
- Create some sort of control over a system and being able to deliver stimuli
- Brain also affects hormones (how much is known about this?) Target pituitary/hypothalamus?
- Engineer a bacteria that can take up and release neurotransmitters (NT)
- Experiments have shown neurons to accept certain types of light and respond differently
- Main challenge: converting neural signals into electrical signals
    - Make this as minimally intrusive as possible
- Distinct approaches:
    1. Reading brain signals and translating them to control technological devices
        - Example: prosthetic limb that accepts brain signals and translates them to motion. Could incorporate living tissue (tissue engineering?)
          Issues: too Course 6? Too wide a scope for our topic and abilities?
    2. Engineering biological elements to alter/fix/enhance brain function
        - Example: A bacteria or engineered cell that could replace radiation chemotheraphy by targeting certain areas or stimuli in the brain
          Issues: not truly or uniquely a brain-machine interaction; the use of engineered biology to affect the brain, but how is this a hybrid of brain/machine?
- Grafted neurons/rewiring the human body (for prosthetics, movement and sensory) -- should these be artificial or grafted?
- What purpose is there for reading neural signals over a lifetime? Is there any? Because genetically engineered [cells, etc] would have to remain in the brain/body/genetic code for the rest of your life. Just applying it to brain imaging and research doesn't seem to be worth the genetic/biological alteration.

Three Ideas Presentation
- Needs to be pretty detailed
- Essentially pitching three ideas: to ourselves and the class
- Must be interesting and realistic
- We're basically asking for resources and feedback so that we can just run with one idea
- Choose a positive decision vs. just falling into one (should feel confident about your idea!)

Suggestions
- Check the new Facebook group for discussions, communication of ideas:
http://mit.facebook.com/group.php?gid=22104395636
- Look at iGEM videos/look up research to see where the world is at this point/how much has been done; we want to start at the right place
- For next time, bring in 3-5 concrete ideas/topics/areas of research that interest you
    - What do you think the impact would be?
    - What form can the solution possibly take?
    - How plausible is this idea given the technology we have today? It's okay to not know, but we have to ask.
- Very little is known about how neural networks actually work right now

Contacts - MIT
Ed Boyden
Karl Deisseroth

BRAIN/MACHINE HYBRIDS - Class (03/04/2008)
Prosthetic limbs/muscle implants
Brain imaging – more specific application?
Artificial neurons
Visual neuroprosthetics

Why would we want to grow things in our brains?
Insert something into our genome that would kick in if anything catastrophic happened (like a stroke)

But machine?
Reads and analyzes hormone activity in the brain? Kicks in if we need anything supplementary? But this would change the human being species…

Brain pacemakers for depression, epilepsy, Parkinson's, neural disorders etc

Help people whose brains have degraded
Insert a simple logical circuit (biological hardware)
Motor strip
Use existing technology, like simple on/off switch

Focus on neurons in a specific area (such as motor strip)

BRAIN/MACHINE HYBRIDS - Studio 2 (03/05/2008)

Neuroscience: Converting Thoughts into Action
http://www.nature.com/nature/journal/v442/n7099/full/442141a.html

[individual research]

Team Log - 03/13/08
Present: Angela, Charlotte, Alex

> DREW/NATALIE FEEDBACK:

All of our projects are about getting info in/out of neural system

Looking at EM/RF (RF = Radio frequency)

Real projects: want a generic input/output device
    Using magnetic fields, whatever that can pick up and send

Go meta - there are myriad applications
    But make a generic device
    Fast time scale
    Input/output

Hone down on the practical details of implemention!
    Don't get too bogged down in one application
    Don't forget about the real technical difficulties of implementation

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> DISCUSSION

Meta-solution
It's easier to read the brain than communicate to the brain

Idea #3-oriented approach

Read NT levels? If we can figure out where NTs are being sent, we can do something about it... practically anything.

How about not reading to an external device... keep it internal.

OR how about engineering a purely external device and not have to worry about touching the brain?

Tag NTs with radioactive or fluorescent markers. Can we read these somehow?

Glia - not-neurons that we can use?

Time-scales
- Magnetotactic bacteria
    - Too slow
    - Too much noise
    - Can we modify them?
- Yeast cells?

Radiofrequency would be hard to detect. How would that work?

Everyone should do a little reading on neuroscience... it may help.

Brain/Machine Hybrids - Brainstorming with Mom 03/17/2008
- Angela

NOTE: My mother is kind of ridiculous. There are wild brainstorms in these notes that are not meant to

be taken seriously! However, she did teach me a lot about neuron signaling and the anatomy of the nervous system.

Blind kid can navigate by echolocation

Sound waves, machine will have vibrations. If vibrations and sound waves match up, communicate with the brain.
Voice control comes from the brain

Pre/post synapse at cleft between nerve terminals
Intercept with pre-synapse
Produce a post-synapse wave
Machine to read wavelength

Can't possibly plant millions of bacteria at each nerve terminals/heads.

How do psychics read brain waves?

Deal with peripheral nerves/spinal cord instead?

There's no central connection location between the brain/spinal cord/rest of the body. The brain is like a network of highways.

Pupils dilate/shrink
Read pupil size
---> too machine-like

Glia cells are support structures... but too many glia is bad! Creates glioblastoma, stage 4 astrocystoma -- Brain becomes like a thicket, loses elasticity, plasticity. Skull is very rigid so there's not as much space to work with, but the brain itself is very elastic. So we can't plant too many of our own things!

The more folds (gyrus) there are, the smarter (human vs. apes, etc).

Hearing/Speech patients. Can we narrow focus?

Heat -- bacteria/cells be able to detect the difference and transmit that information

Central, peripheral, autonomic nerve systems

Autonomic system (involuntary):
Sympathetic - fight and flight (e.g. pupils constricting, involuntary urination)
Parasympathetic - relaxation (e.g. heat)

Reflexes: not totally controlled by the brain; controlled by spinal cord.
Automatically, one synapse, in order not to hurt yourself.

Alphamotorneuron > gammamotorneuron
If not sharp stimulus, there's time to go to the brain (e.g. hot/cold)
Different stimuli transmit to different parts of the spinal cord ascending to the brain (e.g. hot/cold travels the spinalthalamic track), then the brain sends a descending signal down one cortical spinal track

Nerves have branches, filaments, branch out.

Actual impulses... are they electrical or chemical? Neurotransmitters are chemical compounds... but are electrolytes electrical or chemical? Brain impulses are not necessarily neurotransmitters.

"You know what, why don't you just clone a creature?"

PET machine
Like regular MRI, but inject a dye into your blood vessels, it goes to the brain, make the person think about something, or rehearse a mental exercise, and observe brain colors... some kind of biological dye with a trace/something magnetic/fluorescent/something SMALL but that you can see.
Fades away in time.

What about creating neural impulses?
Alzheimer's -- forgetting things, create neural impulses to help. Stimulate neurons, create neural networks, bridges over gaps.
Parkinson's -- movement initiation; can't walk immediately, can't stop immediately
Aphasia (can't speak, stroke patients) create a palm-sized brain that's mapped to a specific person's brain or thinking, use communication tools (eyes, etc) transmit impulses, or touch to some part of the body, transmit the impulse, and do something with it

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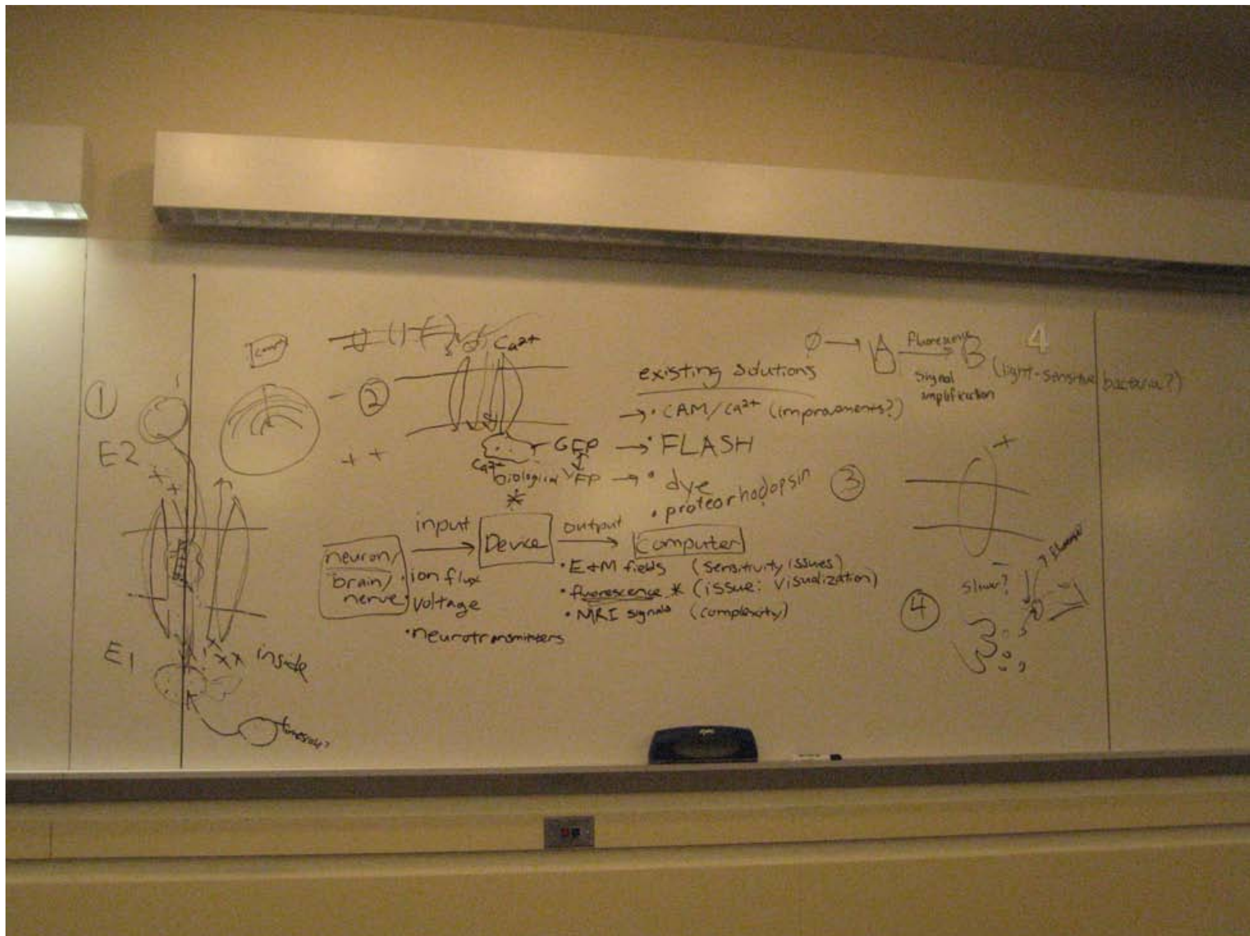
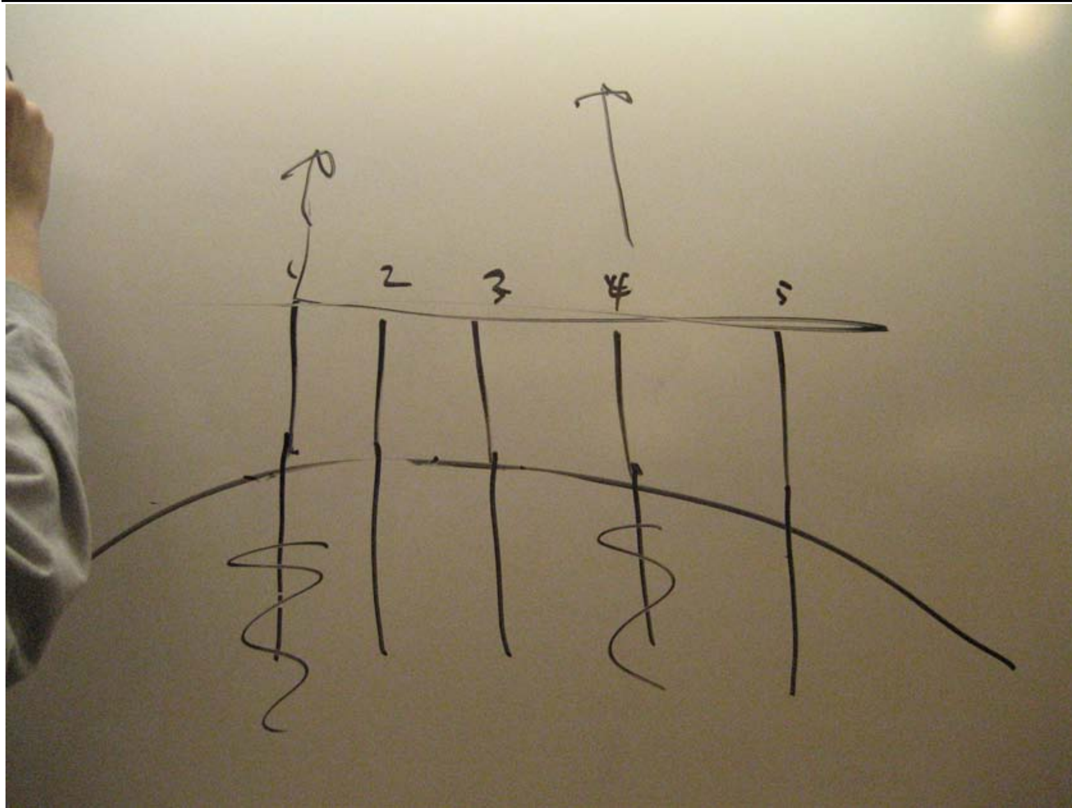
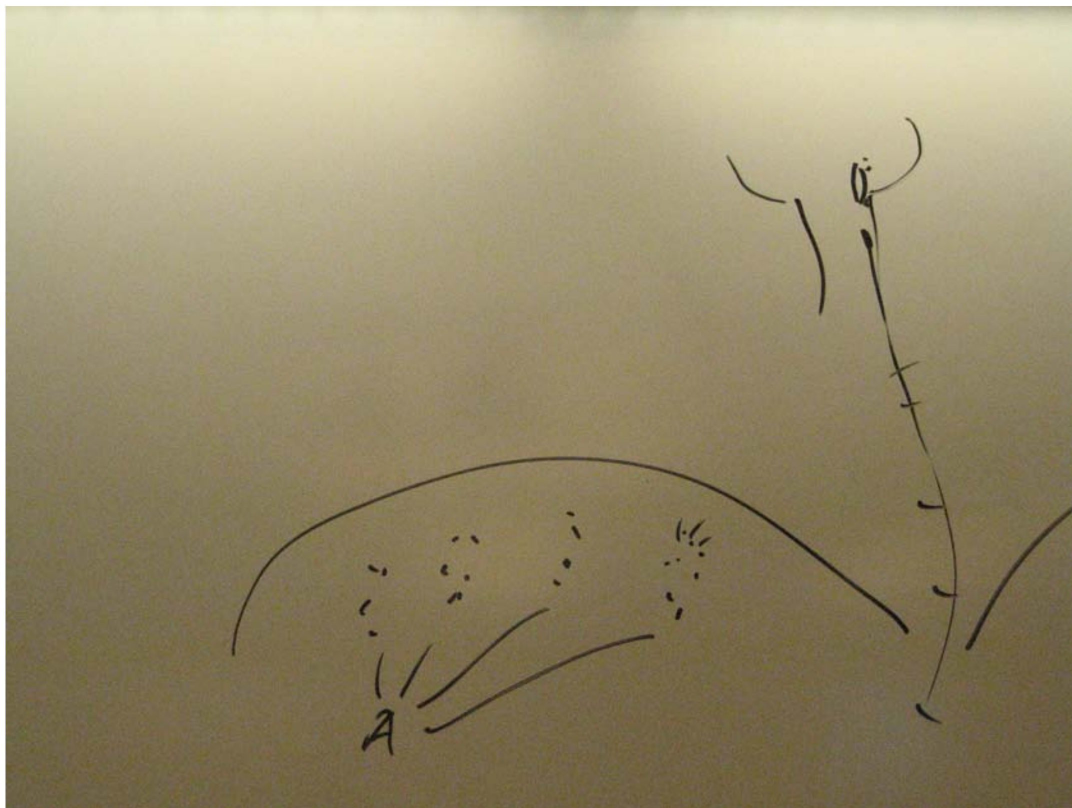
BRAIN-MACHINE HYBRIDS: Studio Log (in images)



Overview of issues still needing to be resolved (and possible solutions)



How electrodes plug into the brain



Neurotransmitters

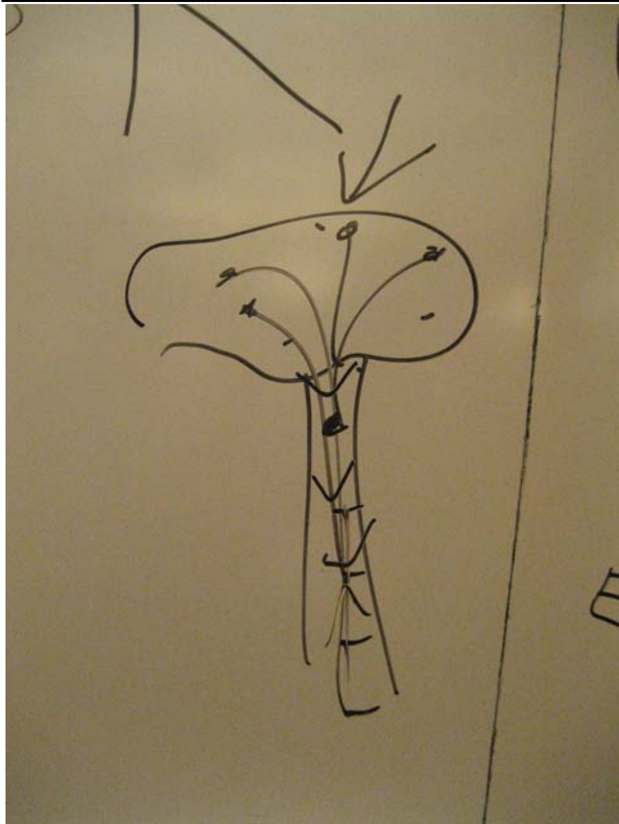
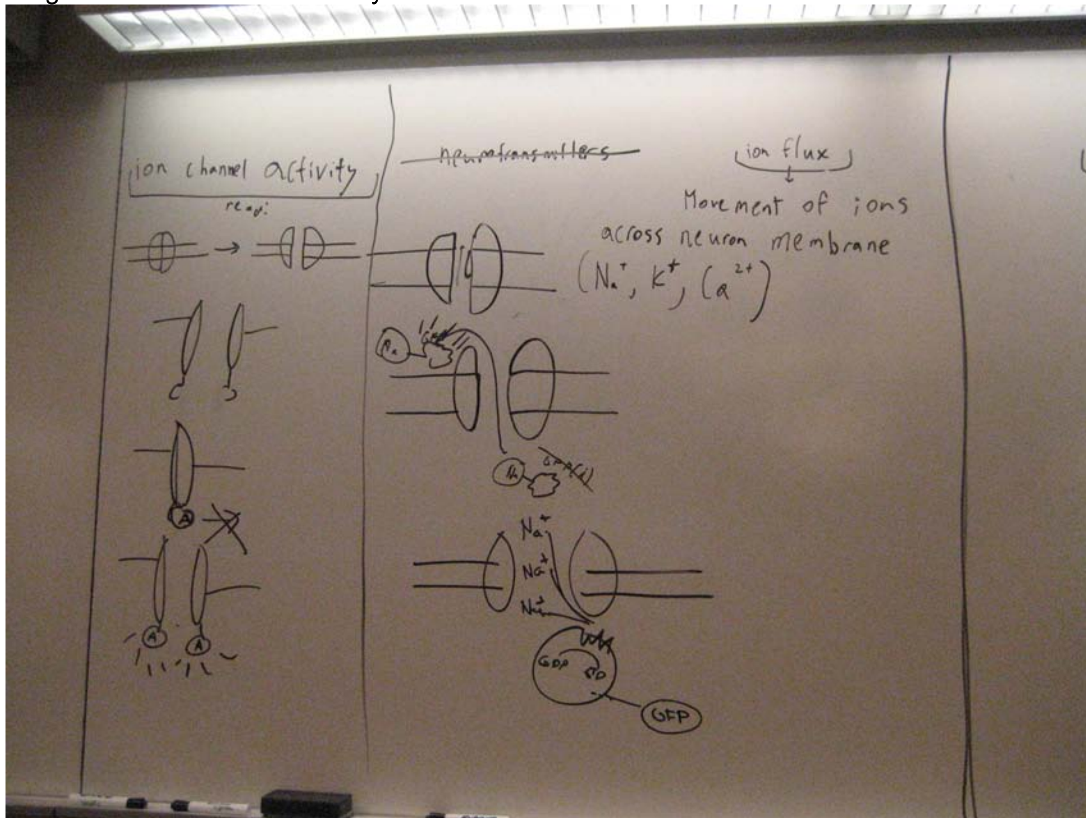
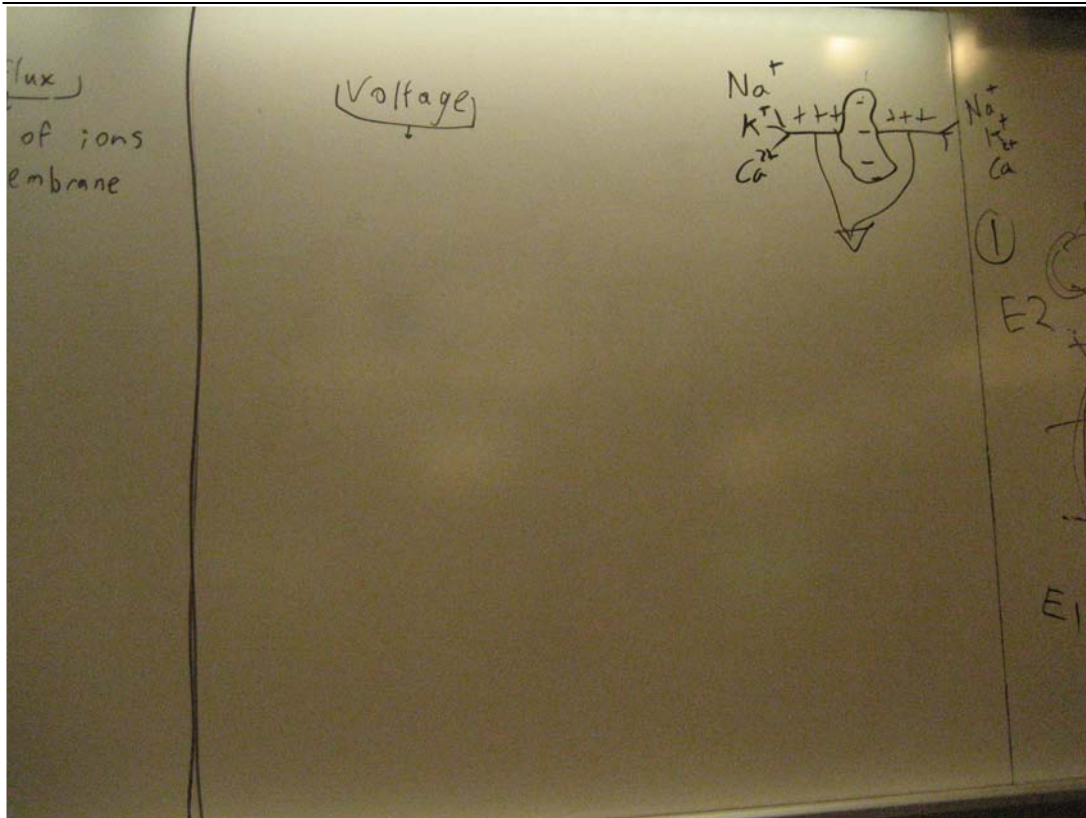


Diagram of the brain/nervous system



How we would handle ion channels



Still unsure of how we would handle voltage-gated channels

Individual Research: Physiology (ion channels, biochemistry, neurophysiology), Basic Neuroanatomy
Angela

Physiology
Sensory systems pg. 32
- Different receptors (e.g. light, touch) receive different types of signals... this is the beginning of how they are differentiated
- Pgs. 34-35: transmission of and how sensory receptors are consciously perceived

Endocrine Physiology pg. 211
- Hormone concentrations can be measured by radioimmunoassay (radioactively labeled vs. unlabeled hormone concentration)
- Regulation of hormone secretion pg. 213
    - Negative feedback is most common
        - Develop something that creates negative feedback when too much of something undesired is created?

Neuroanatomy
Chapter 22: Neurotransmitters and Pathways pg. 275

The Human Brain
Chapter 6: Sensory Receptors
- "The basic task of a receptor is to monitor some aspect of its environment by converting and amplifying part of the stimulus energy into an electrical signal that is meaningful to the nervous system."

The brain knows what happens to the body because specialized receptors do all the measuring and converting of information and converts it to electrical signals that it can understand. We can't really optimize this intermediary process any further. There's no real need to measure it, because the intermediary steps are just a means to the end. Measuring them doesn't really provide a "snapshot" of the final effect or response, because sometimes many signals act on the same synapse, for example, or signals travel down different pathways that are hard to predict and vary widely. What we can control are the external stimuli. Machines can do this. However, we have to make sure that we are not just "stimulating the body and watching what happens". We can also try to intercept signals that are being transmitted with our own. The problem is how to determine which signals to intercept, because since all signals have a specialized purpose, there's not really such thing as a "bad" signal.

Brain/Machine Hybrids - Notes 04/01/2008

Voltage Sensors
- FlaSh protein is extremely competitive
    - Can already measure electric potentials of each neuron
    - Specific enough to bind to each one

How FlaSh protein works
- Takes Ca^{2+} concentrations
- Change in potential is measured
- Read out by GFP
- Emits flash through gates

(See Facebook for link: second Research discussion)

(Discussion: Ion flux, how signals are transmitted)

FlaSh is directly injected in vitro, and it works (right now with K^+)
Don't want to just use FlaSh because we need some sort of innovation
Better way to inject FlaSh?
- Vector?
- Taking from phage therapy... virus? Viruses in the brain?

The necessity of having something in the brain?
- Signals are sent from sensory neurons to brain and brain sends signals to motor neurons.
- We may only need the "downward-traveling" signals. Kind of how prosthetics work, except we can read specific signals at the connecting nodes. Wouldn't have to worry about taking measurements in the brain.

Timescale of Na^+: 2 ms

So: we're going to use some sort of variation of FlaSh
- Faster

Input > Output device
- Ion concentrations > DEVICE > Fluorescence

Ion: (Try not to use Na^+ or K^+)
Na^+ is pretty fast, but not very accurate (because they'll open even if nothing's fired)
K^+ is slower, but more accurate

Device:
Include amplification?
- How much stronger does it need to be?

We're focusing a lot on input right now. Remember that we need the device.
- Simpler may be better
- There may be proteins that we can use/that do what we want
- We can make combinations/chimeric proteins
- To keep it simple, amplification may not be necessary

Brain/Machine Hybrids - 04/02/2008

Want to use fluorescence, chromophores

Bioluminescence
- Two luminescence components on the receptor
- When brought close together, will luminesce

For our project:
- Let a receptor have a half of the component
- Create a ligand that has the other half
- e.g. dopamine (a stronger binder) is released, and there would be no more fluorescence
- Measure negative change in fluorescence

Would help differentiating between different kinds of neurons
- Each releases a different NT (e.g. acetylcholine)
- (FlaSh can change the protein/color)

On the other hand, if we choose to focus on a specific kind of neuron (sensory or motor), we won't have the differentiation problem

Which ion to use? Ca2+ is probably the best choice... but look at Chameleon, has it already been done?

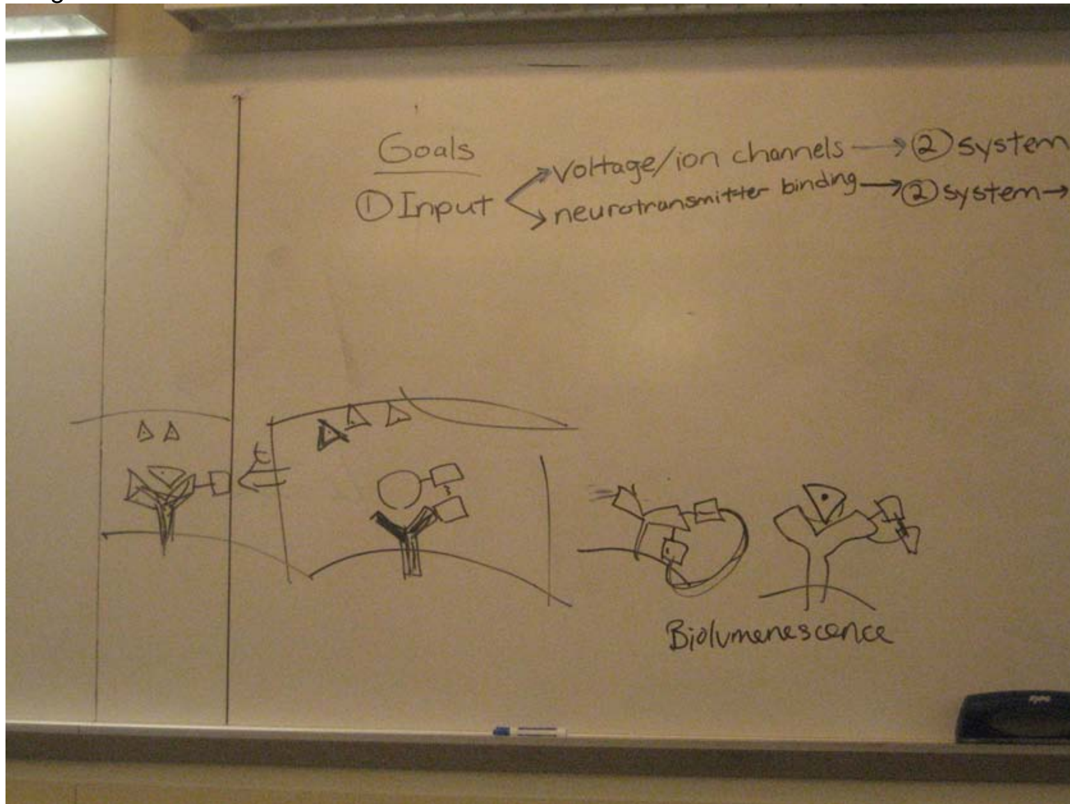
FRET (fluorescence resonance energy transfer)

Figuring out how FlaSh works, exactly

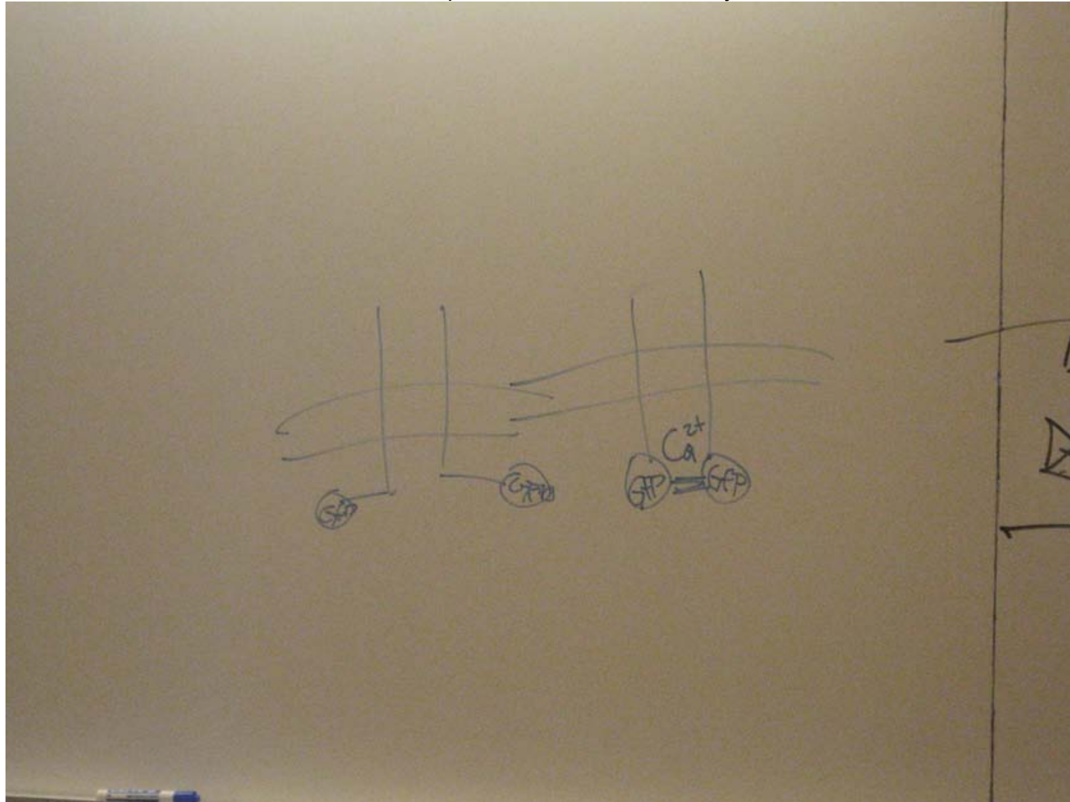
>>> bioluminescent FlaSh!

Target specific neurons by finding promoter sequence

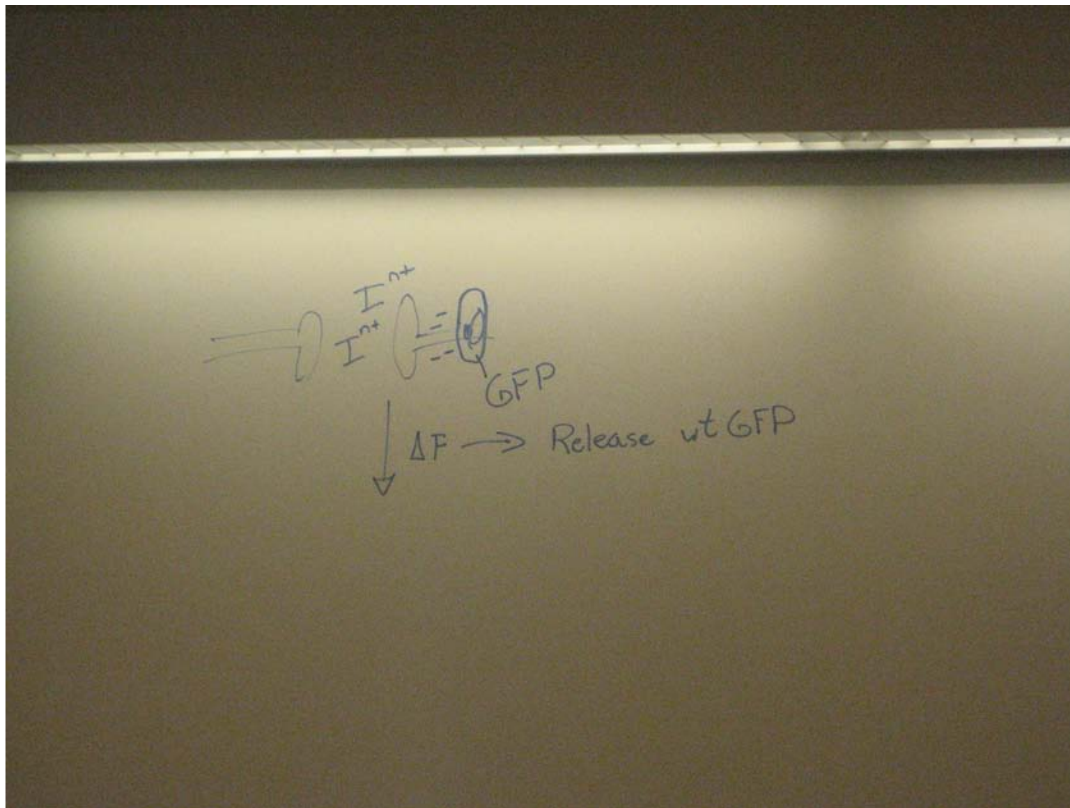
Images from this studio:



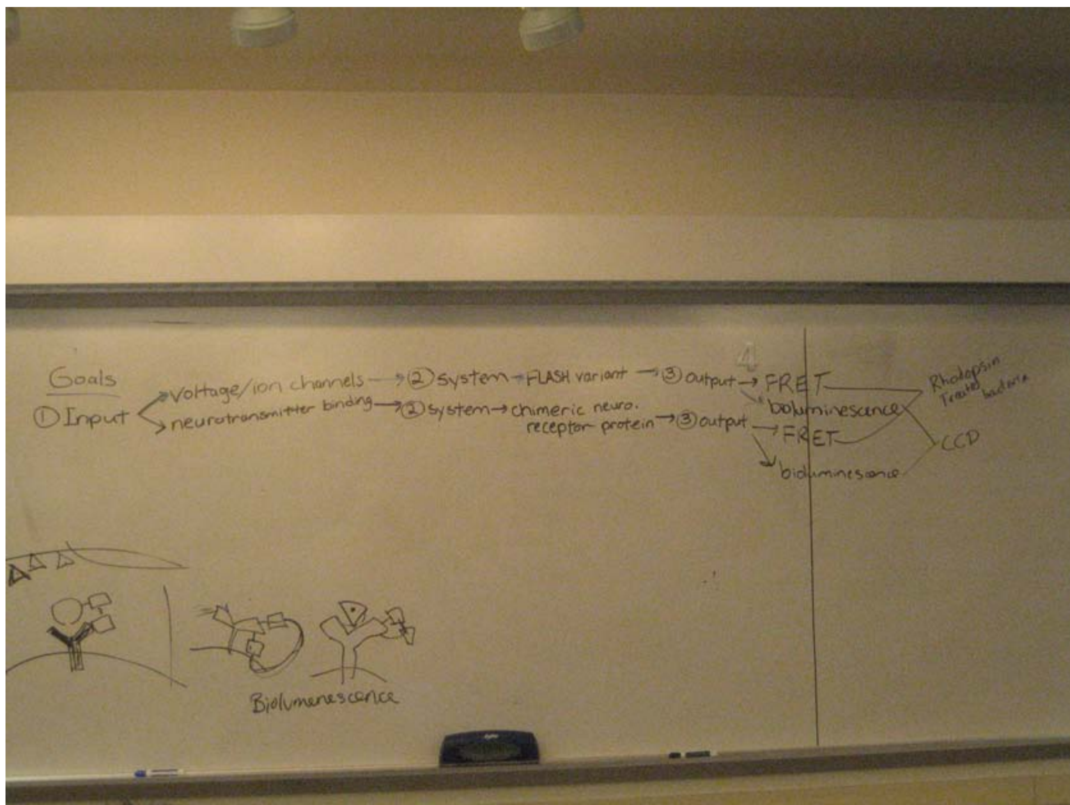
Overview of bioluminescence and a split luciferase/luciferin system



Split luciferase/luciferin system with as-yet-unknown ion channel



Overview of FRET and fluorescence-based voltage-gated ion channel sensors



Beginnings of a general diagram/system overview

Tech Spec Review Expectations

Be sure to briefly re-introduce everyone. You can start with your team/project name(s) and the project idea (i.e. what you'll be addressing and, very roughly speaking, how). Then you can spend the bulk of your time getting down to the brass tacks.

Each project idea must have

* A description of your system's design in terms of devices
  Bioluminescent FlaSh variant
  Input:
  voltage/ion channels on FlaSh variant

  Device 1: Ion channel detector
  Device 2: Bioluminescence

  Output: Biolumnescence
  Rhodopsin-treated bacteria? (necessary?)

  Device 3:
  CCD camera

* A description of your system's design in terms of parts
  How is FlaSh made?
  - Patented in Berkeley
  Parts list for bioluminscence

* A timing diagram to show anticipated system operation

* A plan for testing and debugging your first generation system
  In vitro:
  Dump in something with an ion gradient. It should light up and be detected by the camera.
  - Test over time? Different temperatures?

  Debugging:

* A description of the impact you envision for your system

* A description of any concerns raised and open issues within your team

* A "GO/NO GO" decision

The format for this technical specification is likely to be powerpoint but other platforms for describing the work may also be approved. Each person in your group should plan to speak for some of the time.

Brain/Machine Hybrids -- Studio Log 04/03/2008

Discussion: Whether to stay with K+ or switch to Ca2+
Probably going to stay with K+

FlaSh is just GFP attached to a Shaker K+ ion channel
We can just attach a luciferase to the ion channel and create our own version.
Where to find the sequence?

Luciferase + Luciferin glows
We can attach one to each side, when they come together, they glow.
Bioluminescence is easier to image in vivo
Nothing in the body produces visible light, so there won't be noise

Bioluminescence:
http://www.diagnosticimaging.com/molecularimagingoutlook/2004jun/03.jhtml
http://www.diagnosticimaging.com/molecularimagingoutlook/2005mar/02.jhtml

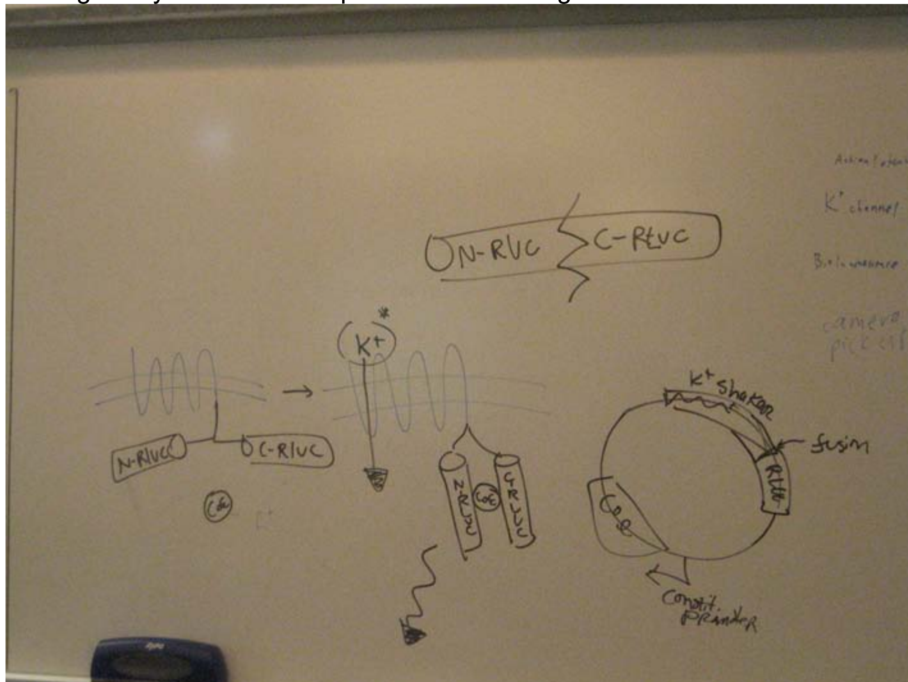
Can change colors (e.g. for motor or sensory neurons) but probably doesn't quite work the same way for bioluminescence.

Ca2+ concentrations are assigned musical notes and creates MIDI files
- Another variation on our project
- Works with EEG (electroencephalography)?
- We probably don't know enough about this to go with it...

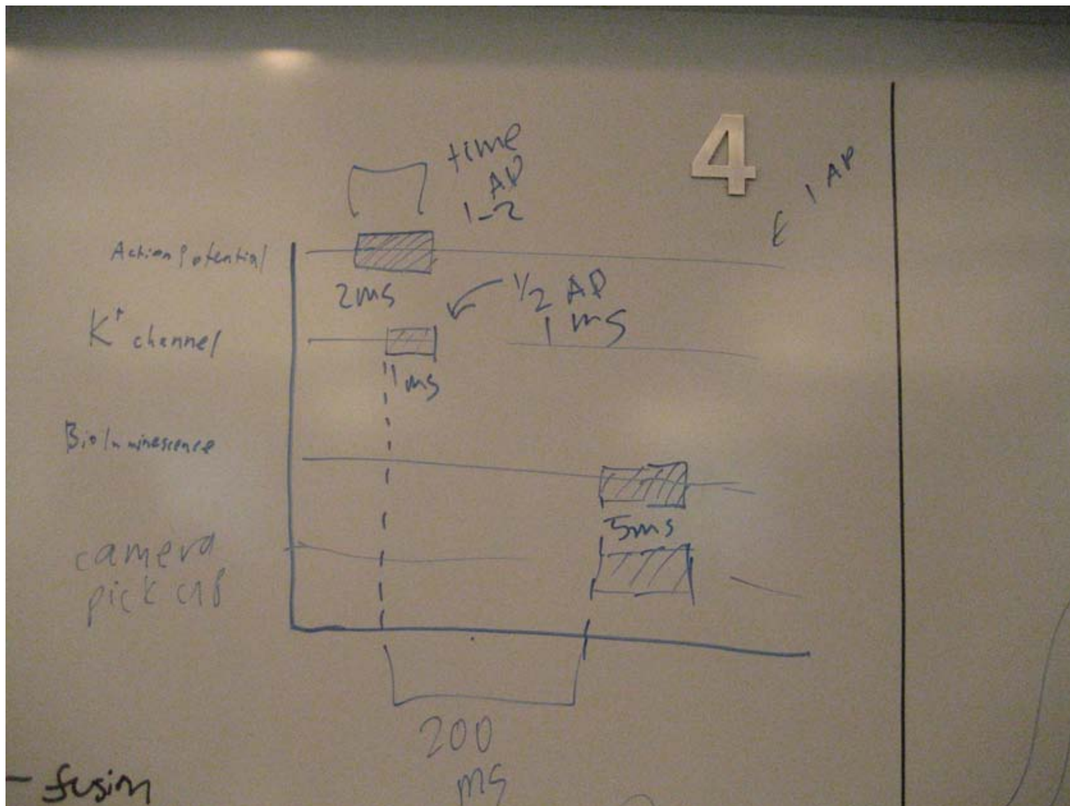
Began powerpoint of Tech Spec Review

Brain-Machine Hybrids – Studio Log 04/08/2008

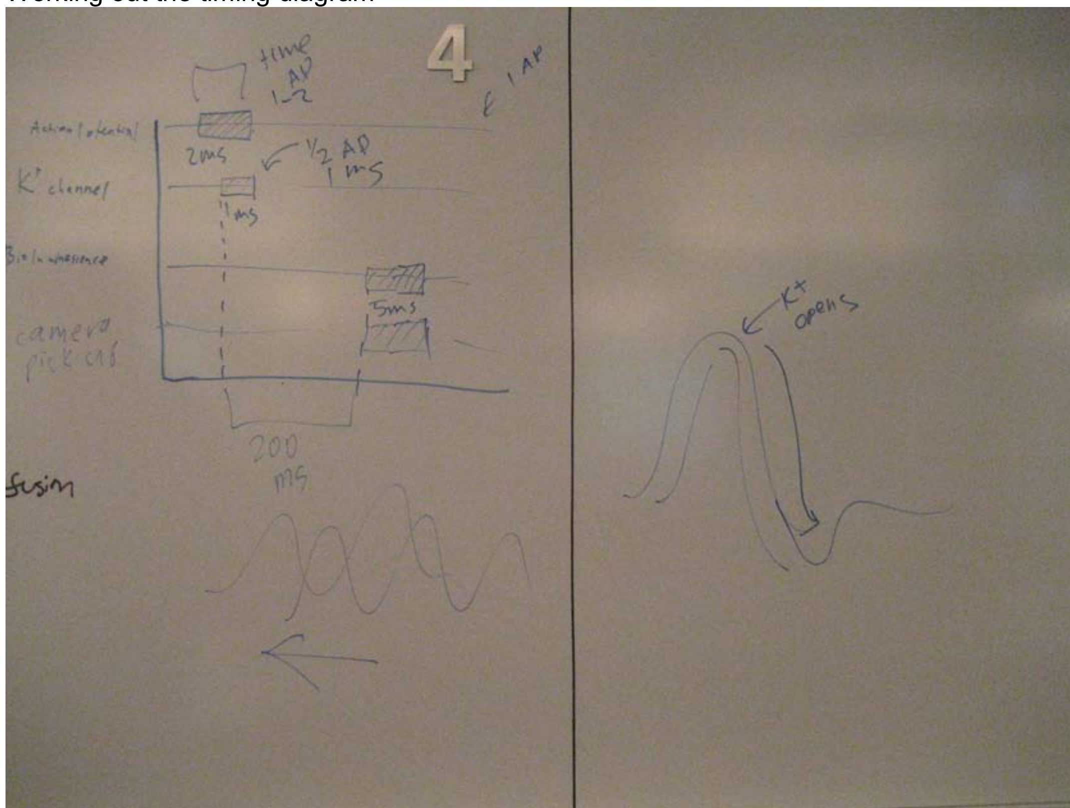
Getting ready for the Tech Spec Review in images:



Detailed diagram of split *Renilla* luciferase attached to ion channel; beginning of a plasmid sequence



Working out the timing diagram



Propagation of an action potential; voltage-sensor relationship

Brain-Machine Hybrids - Studio Log 04/16/2008

Applications:
Can we pick one of the original three ideas presentation ideas?
Neurotransmitters seems most accessible... neurodisorders are pretty straightforward... imbalance of NTs
Align with purposes of current research.. imaging?

Specificity?

CoE from small sea creatures
Larger creatures get CoE from small shrimps, etc. that they eat

Even if we just do imaging, it hasn't been done with bioluminescence before and we'd also be introducing specificity.

Brain/Machine Hybrids - Studio Log 04/17/2008

Continued research/individual reading and posting articles to Facebook to share.

Solution!: CCD Camera
- Signals are electrical signals that can be interpreted by any video or imaging device
- Tutorial posted on Facebook

Solution!: Synthesizing CTZ
- A process has been patented that uses a gene-encoded peptide
- Link posted on Facebook

Open questions:
- Lentivirus gene delivery

Over the long weekend, everyone should familiarize themselves with the articles on the Facebook group.
Next week, begin work on testing/debugging and DNA sequences

After incubation with CTZ for 1 h, luminescence signals were clearly observed inside cells (Fig. 2D) and continued after removing CTZ from the cultured medium. The luminescence image of cells might be explained by continuous replenishment of CTZ remaining in intracellular organelles. These observations suggest that CTZ is imported into the membrane traffic network for protein secretion and the luminescence image shows the lumen of secretory organelles in this network.

Brain-Machine Hybrids -- Studio Log 04/23/2008

NOTE: The individual assignments have changed since these notes were taken!

Technical Documentation

You will need TWO hard copies of these documents to turn in. You must also upload a copy to the class Stellar site here These technical documents must inclue:

1. A narrative that is ~1-2 pages long that includes > or = a paragraph for each of the following:
   * the problem, challenge, or opportunity your project has addressed
   * the existing or developing alternative technologies that address this problem, challenge or opportunity

    * a system description
    * a description of the impact your solution will have if implemented
    * a safety/security analysis
    * a plan of work for the first 6 months for 6 people working full time on this project
2. A Device-level system diagram
3. A Timing Diagram
4. A Parts list (table)
5. A paragraph description of your spotlighted Part and where to find it in the Registry of Standard Biological Parts
6. The DNA sequence for your key new part or device
7. A test/debug plan for that part and for your system or key intermediate device

These technical documents are worth 20% of your team's final grade and must be turned in at the time of your final presentation. You should also turn in your project design notebook if you have not been keeping an online version.

Final Presentation

You will have 20 minutes to present your project and there will be time for 10 minutes of questions. You are expected to follow all the best practices you've learned for oral presentation planning and delivery. This oral presentation is worth 10% of your team's final grade.

ALL:
1. Narrative
    * Problem - JE
    * Alternative technologies - Alex
    * System description - Angela
    * Impact - Charlotte
    * Safety/security analysis - Alex
        - lentivirus
        - bioluminescence forever?
        - CCD camera
    * 6-month plan of work - All

Add CoE and lentivirus to system diagrams (Angela has files):
2. Device-level system diagram (minor modification)
3. Timing Diagram (minor modification)
4. Parts list (table)

5. Paragraph desc. of Part (Shaker K channel) - Charlotte
6. DNA sequence - Charlotte
    - Shaker protein
    - R-luciferase (2 parts)
    - CoE peptide (patented)
    - Plasmid/restriction enzymes (lentivirus)
7. Test/debug plan - JE
8. Project Design Notebook - Angela

Calendar
RR = Reading Room
M 4/28 3-6 RR - Group mtg for parts 1-4
W 4/30 in class - Meeting with Ed Boyden
Sun 5/4 3-6 RR - Final group mtg for presentation and polishing

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Read up on Facebook articles: Patented CoE peptide
Check GoogleDocs

Brain/Machine Hybrids -- Studio Log 04/28/2008

Charlotte, JE, Alex, Angela

What is our general overview?

Via lentivirus vector, introduce new DNA sequence to neurons (later on, target to a specific type. For right now, we can target neurons and add specificity in a later project. Neurons code for pre-CoE + BlaSh. Pre-CoE converts to CoE [spontaneously?] When repolarization occurs, [K+] change is detected by Shaker channel. This causes luciferases to come together and bioluminesce. Bioluminescence is picked up by external CCD camera.

Unknowns

- Specific neuron types: how to determine/localize? Will lentiviruses be able to do this?
- What processes are involved in transforming neuron with lentivirus DNA?
- How does pre-CoE become CoE?

Add to timing diagram (?)

- How long it will take lentivirus to reach neuron? (determined experimentally?)
- How long will it take to produce initial sufficient concentration of pre-CoE?
- How long will the reaction from pre-CoE to CoE take?

Answers to questions

- Working with low-to-medium copy number plasmid (experimentally determine ideal copy number)
- Will create new protein channels along with normal functioning ones. Because of low copy number, will hopefully not inhibit natural neuron function.
- Max gene expression occurs within 2-6 weeks

Safety

- There is no immune response to lentiviruses
- HIV/retroviruses can be rendered replication-incompetent (doesn't transfer viral genes, just our desired gene)
- K channels remain separate from existing ones

Slides to add

- How a lentivirus works (ties into safety); its role as a host of our vector
- How luciferase is split
- CCD camera: we can convert any output to any other form of output

Testing/Debugging

TEST: Luciferase/CoE binding *(in vitro)*

- Inject CoE to location of modified Shaker channel, introduce K+ ions and see if bioluminescence changes.

Pass: Negative bioluminescence change detected
Fail: No change

TEST: Whether Shaker channel (BlaSh) works in neuron *(in vitro)*

- Allow our BlaSh and CoE to be expressed in a neuron in vitro. Stimulate with voltage see if bioluminescence changes.

Pass: Negative bioluminescence change detected
Fail: No change

TEST: Does our device REALLY work? *(in vivo)*

- Introduce our gene to plasmid and insert to lentivirus. Allow to transform neuron genome in vivo and see if bioluminescence changes over time (as correlated to voltage detectors/brain images?)

TODO:
--- Documentation is on GoogleDocs! Update it! Make changes in color and the original person can approve them by making them black again.
--- Everyone just keep reading and working.
--- Finish Parts table (begun in MS Excel today)

Brain/Machine Hybrids -- Studio Log 04/30/2008
Visit from Ed Boyden

- How does pre-CoE become CoE?
    - Happens in the presence of oxygen
    - Plasmid can be ordered online within the ATCC

Ed Boyden
- Find papers that talk about pre-CoE peptide, not just patent
    - Patents talk mostly about what is possible, not what has been done
    - Patents cite other patents
- How do you know this is going to be fast enough? Think about Kinetics
    - FlaSh lags a little
    - This will probably lag more
    - Sustained/amplified signal
        - Need to worry about saturation
            - Problem is plateau, will never go any higher
            - Look at molecules similar to this one
    - 1996-2007 FRET -- ?
    - Don't need to completely unfold protein to stop bioluminescence, just need to tweak
    - Model with proteins of similar size and sanity test to see if we're totally off or not
    - Neuron will fire 300-400 times a second, while FlaSh takes a few seconds to plateau
    - Come up with a plan to make it faster
        - Do less FRET
            - 2 molecules have to change orientation w.r.t. each other
            - Figure out how to take a small amount of energy and warp it
- Bioluminescence is a chemical property; requires molecules to move, which is why it takes longer than fluorescence
- KINETICS:
    - How do we know the thing can actually warp at all?
    - How do we make it a useful sensor?
        - Fireflies blink on/off at Hz
        - Check latency to turn off
- Many different substrates
    - Luciferin/aequorin relationship
- If the time scale is slower than we make it, we could just use existing sensors. What applications would there be?
    - You want better than 20 ms resolution
    - Don't want a plateau after a few ms and hit a plateau
- Look up Friedrich, Liam Paninski
- Could come up with a screening for drugs
    - Don't need real time
- Neurons are not dividing cells
    - e.g. don't get cancers from neurons
    - Could probably put a lentivirus and get away with it
    - Might inject 10^10 viruses and hit an oncogene
- AAV (adeno-associated) viruses can't hold as many 3kb (~700aa, 2100 bases), but is safer
    - 5 – 10 years down the road, it might be possible

- Very difficult to do in real life
    - Just hangs out in the nucleus
- Gene gun, blast gold particles with a little gold gun
- Dozens of other methods, but stick with viruses
- Bind luciferase to most delicate helix
    - When it gets tweaked, just move a helix, don't completely split
    - If split, you may have long dark periods
    - Need to worry about whether it will recombine or work after recombining
    - Recommends using just one molecule
- Neuron targeting
    - DNA regulator
    - Put into cells that can only activate that specific promoter
- Chromosome 19 is a safe part to land on
    - Find out what makes it target?
    - AAV doesn't go to chr19 in mice b/c they don't have it
    - Problem: how to you make sure it doesn't go to other places?
- Adenovirus is even bigger and more toxic than the other two
    - Not many more great options
- Channels are huge
    - May not be able to fit into AAV
- Look up Ci-VSP
    - Not a channel; a voltage-sensing something else
- "Optics is a whole other ball of wax"
    - Best way is to assume that microscopes work
    - Find vendor that sells microscopes that use CCD cameras
    - Look at specs, sample pictures
    - Do a sanity check with kinetics
- If we're concerned with not seeing, then:
    - Do tests, you may be fine
    - Increase number of molecules
    - Maybe you can't see single cells, but you can see clumps of cells
    - Do calculations
    - Can illumination of X cells be seen Y cm away from the brain
    - If it doesn't work out, can still find a new way to image/drug screening, etc.
    - Sanity check of the resolution

1 - Check patents
2 - Consider kinetics
3 - Don't worry about viruses
4 - Look at systems, check out some real pictures

Alex's Ed Notes

Look up latency times for bioluminescence.

Can it warp, and what is the latency for it?

Signals:
friederick - germany
paninski - columbia

<20 ms to be useful.

AAV - |Adenone associated virus| 3 kb after promoter.

Gene gun.

cell-specific promoters

CI-VFP

Bioluminescence resolution of cells through the brain and skull.

New Drug Screening platform if original idea fails.

patent
kinetics
NOT VIRUSES
photon emission

Post-Interview Notes

- Time of gene expression from injection of virus to expression is ~ 2 weeks

- Use GFP kinetics data

- If we use a tweaked luciferase, we'd be looking for a flash of light rather than loss of light
    - Can save substrate that way
    - More intuitive
    - To find which area to tweak, screen
        - Look at crystal structure (X-ray crystallography)
        - GFP was tested with screening

- Ci-VSP
    - Just the voltage-sensing section of a voltage-gated channel
    - Significantly smaller
    - Would make it more likely to fit into an AAV
    - Ci-VSP (Ciona voltage-sensor containing phosphatase) link:

http://www.ionchannels.org/showabstract.php?pmid=17615106

- Liam Paninski, Columbia University
    - Research on statistics of neuroresponse; probabilities, tracking, regression, etc.

- Read Tuning FlaSh

NOTE: Notes from 04/30/2008 were the last formal notes taken. Meetings afterwards involved everyone in preparing the final presentation.

Remaining images from final few meeting days:

Kinetics

luciferase (A) [enzyme] + luciferin (B) [CoE] ⇌ (O_2, k' / k^{-1}, RDS) $[enzyme \cdot CoE]^{\neq}$ —k_2→ light

$rate_f = k' [luciferase]^x [luciferin]^y$ slow step

$rate_r = k^{-1} [enzyme \cdot CoE]$ <<0

$rate_2 = k_2 [enzyme \cdot CoE]$ >>0 fast step

A + B → C → D → E (10s, 1ms, 1ms)

$K_d = \frac{[C]}{[A][B]}$

Kd ↓ tighter nM–μM

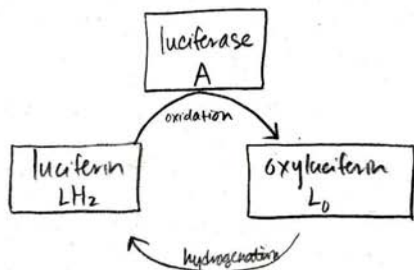
Kd ↑ weaker >1mM

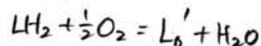
$\Delta [Ca^{2+}]_{out}$ Ca^{2+} $\Delta [Ca^{2+}]_{in}$

Beginning to work out kinetics

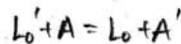
oxidation: loss of electrons

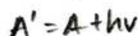
luciferase: destroyed by heat
- colloidal in solution
- Roles:
  1. catalyst (luciferin oxidation)
  2. supplies molecules
     ↳ luminescent!
     excited by energy from oxidizing luciferin





(' = excited)

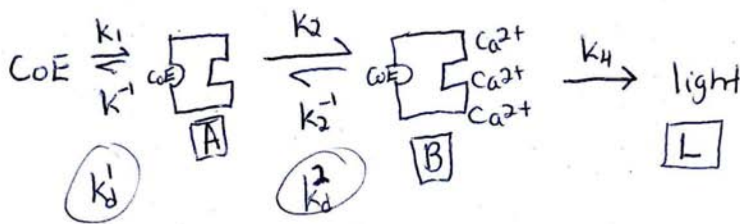


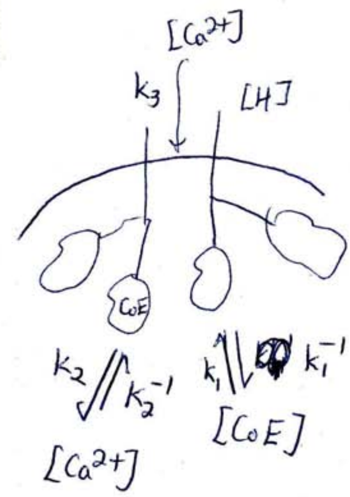


Light intensity (assume sufficient $[O_2]$)
- Initial flash
- decay curve ∝ rxn velocity of oxidizing luciferase
- total light slightly >∝ [luciferase], [luciferin]
- logarithmic decay

Keep [luciferin] : [luciferase] small

Ordering of actions during bioluminescence for kinetics analysis





$$\frac{dA}{dt} = k_1[CoE] - k_1^{-1}[A] + k_2^{-1}[B] - k_2[A]$$

$$\frac{dB}{dt} = k_2[A] - k_2^{-1}[B] - k_4[B]$$

$$\boxed{\frac{dL}{dt} = k_4[B]}$$

$$k_d^1 = \frac{k_1^{-1}}{k_1} \qquad k_d^2 = \frac{k_2^{-1}}{k_2}$$

$$\frac{dL}{dt} = k_2[A] - k_2^{-1}[B] - \frac{dB}{dt}$$

$$\frac{dL}{dt} = k_1[CoE] - k_1^{-1}[A] + k_2^{-1}[B] - \frac{dA}{dt} - k_2^{-1}[B] - \frac{dB}{dt}$$

$$\boxed{\frac{dL}{dt} = k_1[CoE] - k_1^{-1}[A] \cancel{- 2k_2^{-1}[B]} - \frac{dA}{dt} - \frac{dB}{dt}}$$

Assume:

$k_2 \gg k_1$

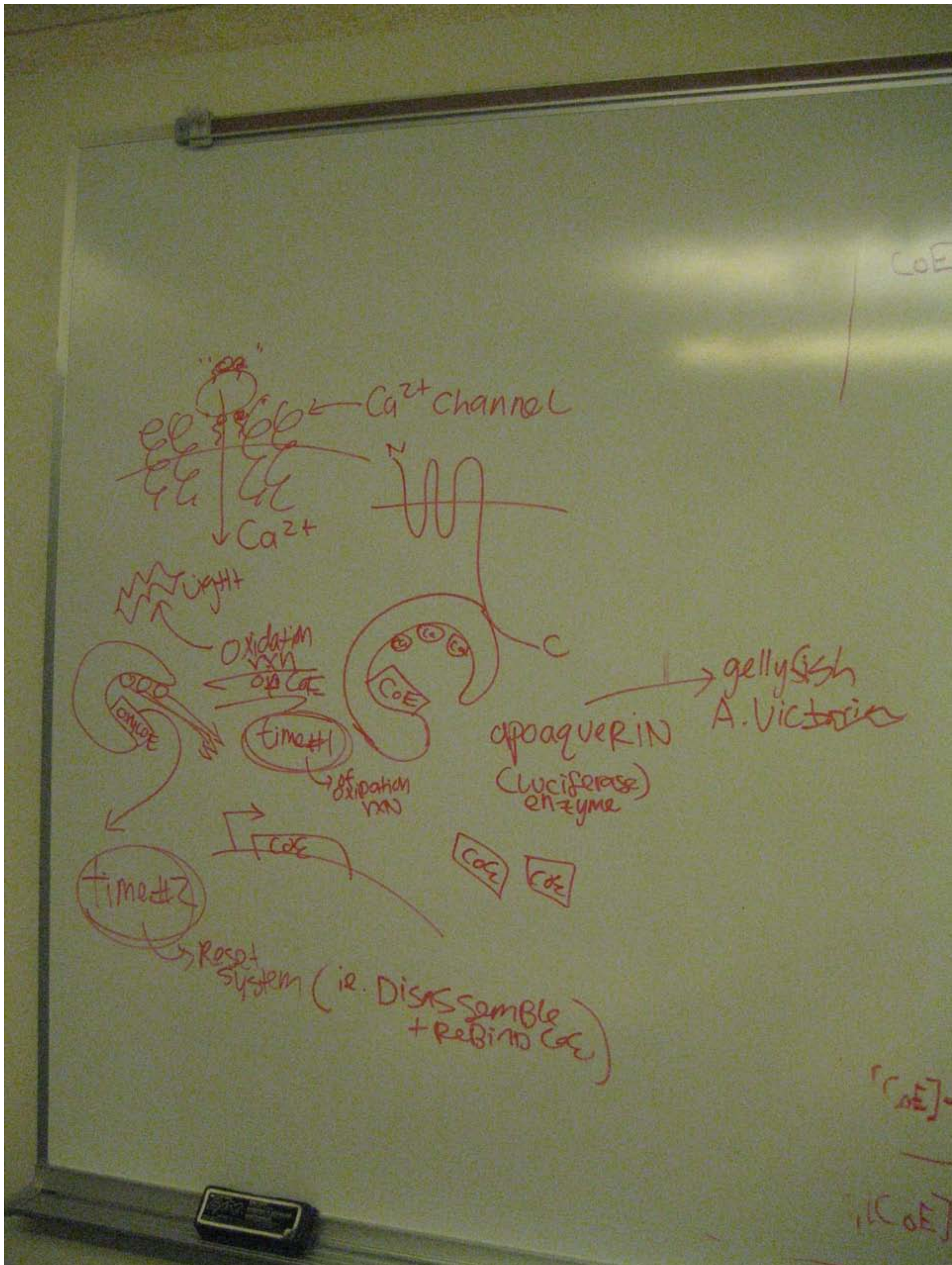
$k_2 \sim k_3$

k_2^{-1}

reaction-limited

$$\left.\begin{matrix} K_m \\ V_{max} \end{matrix}\right\} A$$

Final kinetics overview



Final system overview

END OF PROJECT LOG

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Spring 2009

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