Plans for today:

1. Lab 
2. Written proposal demonstration
3. Jackie pre-lab lecture and demo
4. Teams Blue, Yellow, Red go to Belcher lab
5. Teams Purple, Green, Orange go to Belcher lab
6. Work with your co-PI(s) to develop your proposal.
7. Atissa here at 4pm to discuss partner presentations

Project Title: Engineered bacteria for the conversion of amyloid plaques to dark chocolate.

Principal Investigators: Shannon K. Hughes and Butterstick C. Puppydog

Summary: Amyloid plaques that form in the brain of Alzheimer's patients contribute to degeneration of nerve communication leading to the physical manifestations of the disease. The symptoms of Alzheimer's disease might be alleviated through elimination of the beta-amyloid protein accumulation in the brain. We propose to address this devastating disease by engineering e.coli to convert beta-amyloid protein to dark chocolate, which is then easily consumed by microglia. Our approach involves expression of a novel protein, ADC…[Two more succinct sentences that describe the experimental approach and one sentence stating the novelty of the research]

Introduction: Alzheimer's disease affects 5.4 million Americans [1]……[1-2 sentences about the disease]. The aberrant deposit of beta-amyloid plaques in the brain of Alzheimer's patients significantly affects neurological function by blocking cell-cell communication, inducing apoptosis, and leading to a general degeneration of the brain tissue [2]. [1-2 more sentences about the structure of plaques and where they originate]

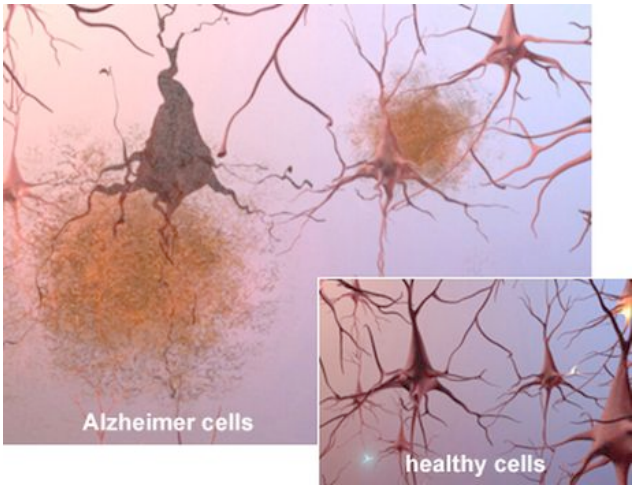


Figure 1: Some important background on amlyoid plaques in Alzheimer's Disease.

Removal of amyloid plaques might significantly improve the quality of life for Alzheimer's patients. Several research groups have attempted to eliminate amyloid plaque formation by….[an entire paragraph about what other people have done].

Although some progress has been made to reduce amyloid plaques, there have been no attempts to convert plaques to the alternative, degradable form of dark chocolate [statement of where the gap in knowledge is]. To

address this shortcoming, we propose to engineer a non-toxic strain of e.coli to produce a new protein recently discovered in our laboratory through a yeast two-hybrid screen, amyloid-to-dark-chocolate (ADC). As the initial steps to accomplish our long-term goal of conversion of amyloid plaque to dark chocolate in the human brain, we will:

1. **Optimize the production of genetically engineered ADC using the non-toxic e.coli strain BL21(DE3).**
2. **Determine the enzymatic efficiency of engineered ADC *in vitro* utilizing amyloid plaques harvested from murine models of Alzheimer's disease available from Jackson Laboratory.**
3. **Measure the efficacy of engineered ADC *in vivo* by quantifying the conversion of amyloid plaque to dark chocolate via MRI in a murine model of Alzheimer's disease.**

Research Plan: Our research proposal encompasses three major milestones; optimization of expression and secretion of the ADC protein, *in vitro* evaluation of ADC enzymatic efficiency, and proof-of-concept *in vivo* testing in mice. The experiments required to accomplish the stated research goals are outlined below for each milestone and a workflow diagram is shown in Figure 2.

1. **Optimize the production of genetically engineered ADC using the non-toxic e.coli strain BL21(DE3).**

The ADC protein was originally cloned from a 35-year woman with an intense craving for dark chocolate. Normally secreted in the brain, ADC contains a sequence recognition motif for extracellular export, but it is unknown if the e.coli strain BL21(DE3) is able to efficiently produce and secret ADC. Therefore, we will….[outline the experiments and supplies necessary to test this].

Anticipated Results and Alternative Paths: The literature-derived data on ADC suggests that it will be efficiently produced and secreted by e. coli [citation]. However, it is possible that the protein will be contained within inclusion bodies, thereby lowering the efficiency of production. If this issue arises, we will examine other possible non-toxic organisms that can be engineered to secrete ADC, such as small kittens or fireflies.

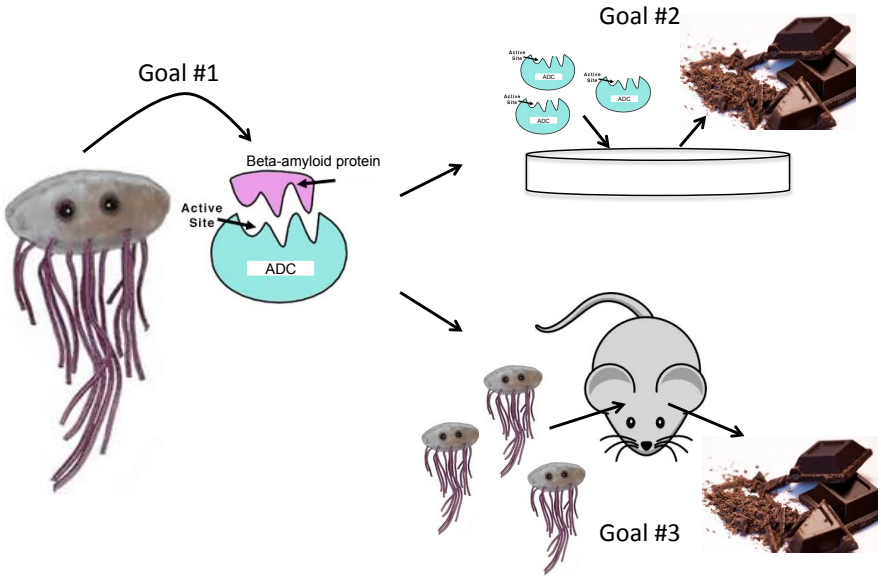


Figure 2: A thoughtful, informative, well-drawn schematic of our research plan. The figure legend should explain the diagram such that a reviewer might not even need to read the text of the proposal.

[The rest of this section should look similar to the first research goal. Outline the experiments that you will complete to test your hypothesis / reach your research goals. Follow each with an alternative approach and an honest evaluation of your chance of success].

Budget: This section should contain a detailed explanation of how much money you need to accomplish your research. This could be in table form. Prof. Belcher suggested that one graduate student = $50K, one post-doctoral research assistant = $100K, and UROPs = $5K. This is a 3 Yr, $500K call for proposals, so you'll want to stick within those guidelines.

Bibliography: Your references should be numbered in [brackets] within the text and be listed in the bibliography in the order that they appear within the text. Here is an example of a well-referenced journal article:

1. Meyer AS, Hughes-Alford SK, Kay JE, Castillo A, Wells A, Gertler FB, Lauffenburger DA. 2D protrusion but not motility predicts growth factor-induced cancer cell migration in 3D collagen. J Cell Biol. 2012 Jun 11; 197(6): 721–9. PMCID: PMC3373410

Max length = 5 pages.

M3D4 : Solar cell assembly

What are we doing today?

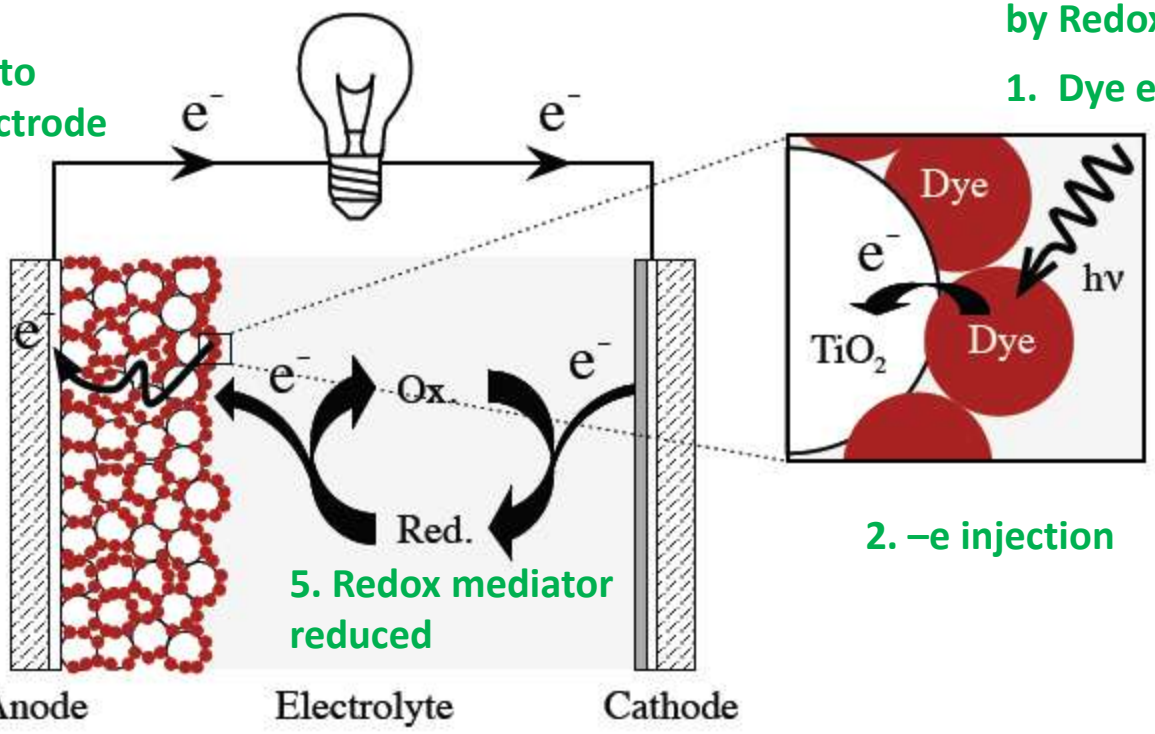
- DSSC summary and review
  - Solar cell function: electron flow and kinetics
  - Main components of a DSSC / specific components in our DSSC's
- Doctor blading demo
- Build solar cells in Belcher lab

DSSC function: -e flow diagram

6. Dye replenished by Redox mediator

1. Dye excitation

4. –e travel to counter electrode



2. –e injection

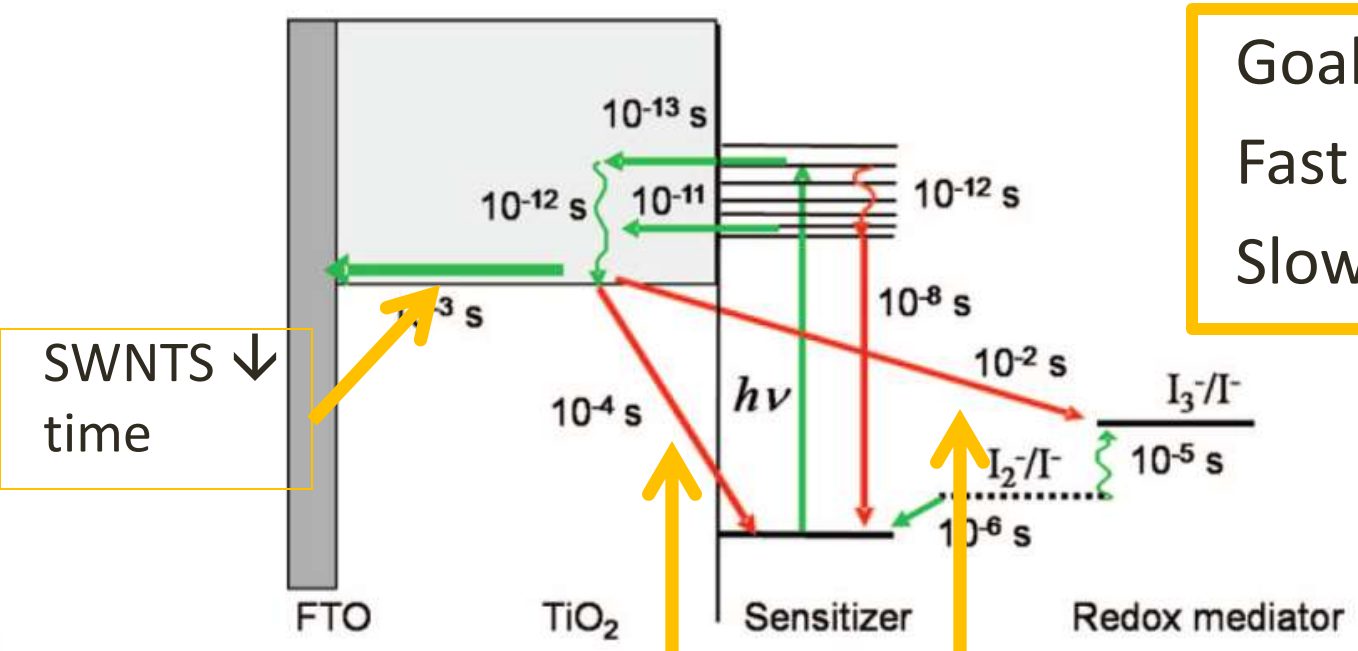
5. Redox mediator reduced

3. –e collection

http://www.solaronix.com

DSSC function: kinetics

Directional electron flow occurs due to kinetics of electron injection and recombination events:



SWNTS ↓ time

Goal:

Fast green steps

Slow red steps

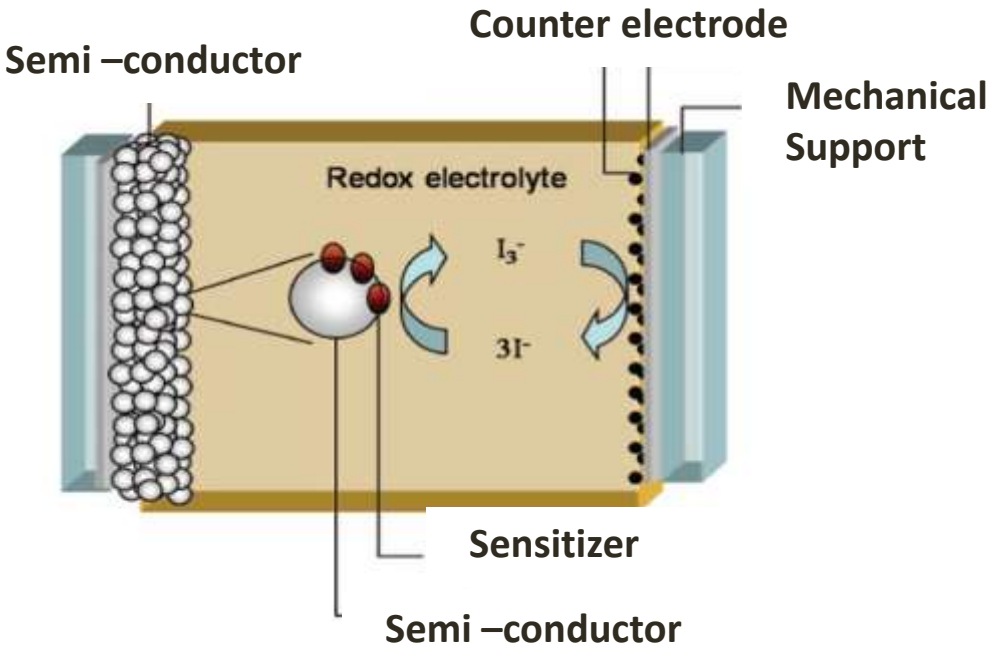
SWNTS ↑ time

Semi-conductors ↑ time

Advanced Materials, 2010

DSSC components

General

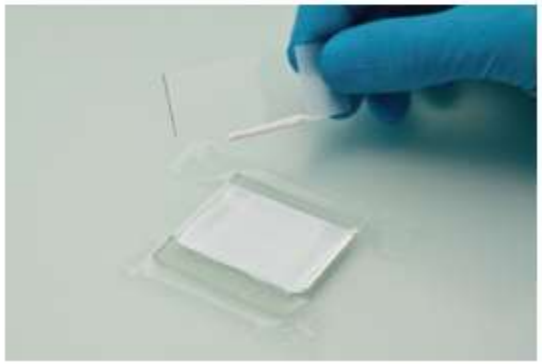


Our DSSC's

- **Semi –conductor:** TiO2
- **Sensitizer (dye):** N719 dye
- **Electrolyte and redox mediator:** I_3- / I-
- **Counter electrode:** Platinum
- **Mechanical support:** FTO glass with TiO2 coating

Constructing solar cells…

Identifying the conductive side of the TCO (transparent conductive oxide)



"Doctor-blading" the titania (TiO_2) paste

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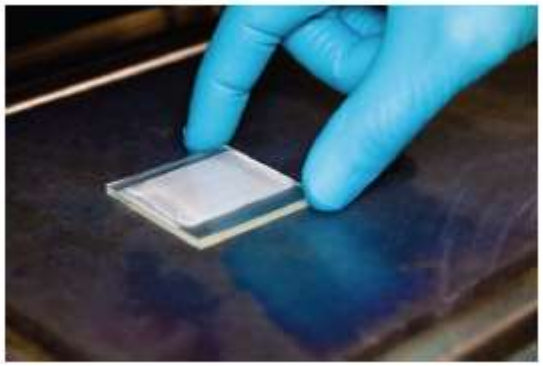

Heat treatments:

(1) Burn off polymer binder in paste to create pores for the dye. (Must be in air)

(2) Sinter nano-particles . Must be in argon, here particles are connected in a conducting network

Dyeing the film

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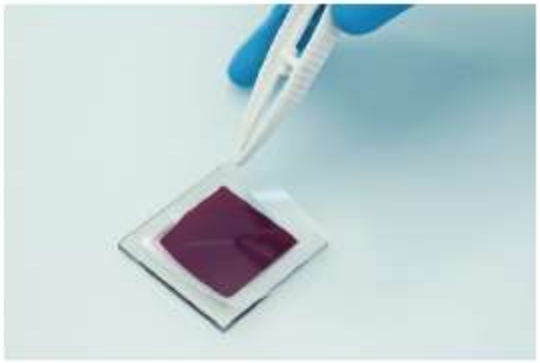
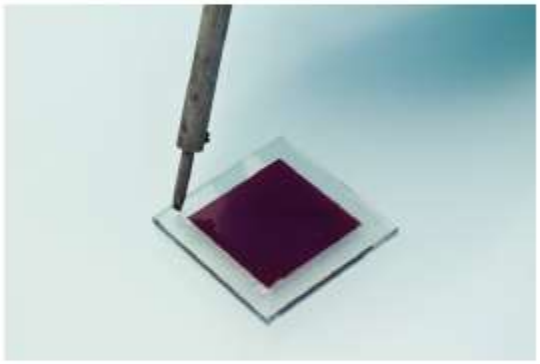
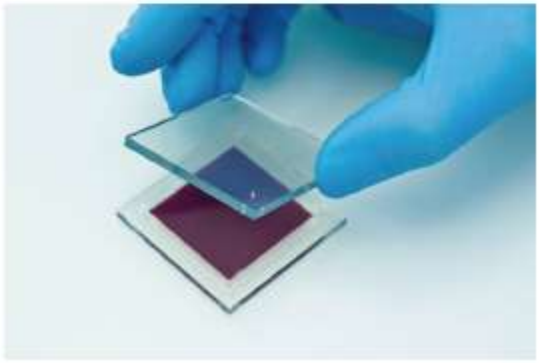
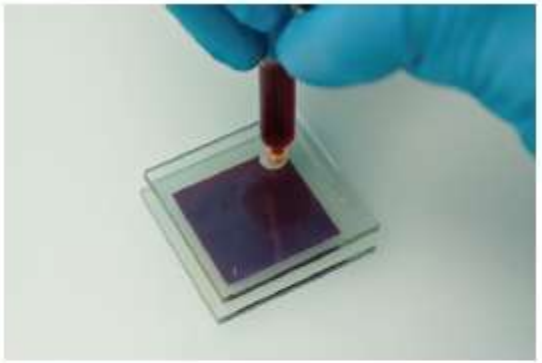
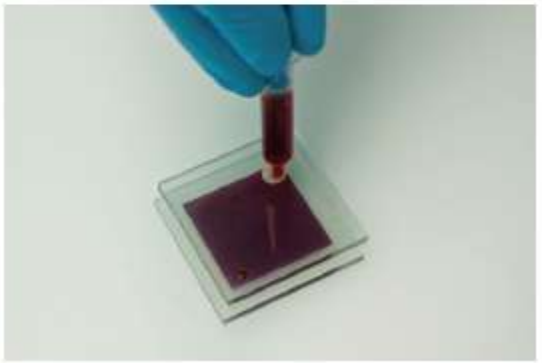






Assembling the device with another electrode





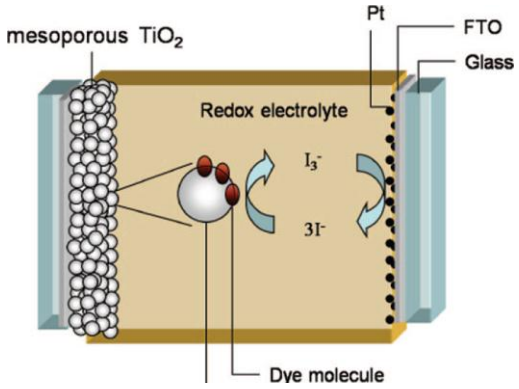
Filling the electrolyte

Testing the device

Overview of Dye-Sensitized Solar Cells (DSSC): Key components and basic operating principle

Key components in our DSSC:

(1) Semi –conductor: TiO2
(2) Sensitizer (dye): N719 dye
(3) Redox mediator: I_3- / I-
(4) Counter electrode: Platinum
(5) Mechanical support: FTO glass coated with TiO_2



DSSC schematic; Chemical Rev. 2010, 110, 6595-6663

Electron flow in the DSSC:

1. Dye becomes excited by light.

2. Dye injects an electron very rapidly to the TiO_2* (the conduction band), dye is oxidized in the process.

3. Electrons are transported through the semi-conducting TiO_2, move through the load, and eventually reach the counter electrode.

4. At counter electrode, normally platinum, the electrons reduce the redox mediator located in the electrolyte of the DSSC.

5. Redox mediator diffuses to meet and regenerate oxidized dye molecules.

* The TiO_2 (or other semiconductor used in the DSSC) promotes directional flow of electrons in the solar cell. This is due to kinetics of electron movement. Once injected quickly to the TiO_2(10^-12 seconds), electrons are not as easily recombined with the sensitizer or redox mediator (which occurs on a 10^-2, 10^-3second time frame). If instead, the electrons entered a metal, recombination events would be much more frequent.