# System Engineering

20.109(F10)
M2D6 lecture
11.02.10

**Rational protein design:**

Knowldege-based, deterministic engineering of proteins with novel characteristics

design/modeling (often computer-aided)
↓
generate required DNA constructs
↓
express proteins
↓
purify proteins
↓
assess proteins for desired characteristics

**"Irrational" high throughput protein engineering:**

Selection for desired properties from libraries of random variants

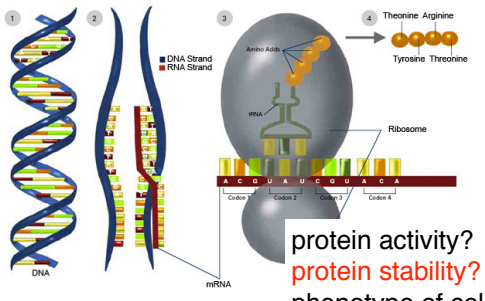
design/modeling (often computer-aided)
↓
generate library of DNA constructs
↓
express proteins
↓
screen proteins in high throughput assay
↓
assess "hits" for desired characteristics

Slide from Alan Jasanoff

## Design: rational vs "irrational"

| Cph1/EnvZ | A553 | G554 | V555 | S556 | H557 |
|---|---|---|---|---|---|
| EnvZ | **A239T** | **G240E** | **V241G** | **S242D** | **H243A** |
| | | | | | |
| wt seq | GCG | GGG | GTA | AGT | CAC |
| **oligo seq** | **RNS** | **RNS** | **RNS** | **RNS** | **SNW** |
| poss aa | Val | Val | **Val** | Val | Val |
| | **Ala** | Ala | Ala | Ala | **Ala** |
| | Asp | Asp | Asp | **Asp** | Asp |
| | Glu | **Glu** | Glu | Glu | Glu |
| | Gly | **Gly** | **Gly** | Gly | Gly |
| | Ile | Ile | Ile | Ile | Leu |
| | Met | Met | Met | Met | Pro |
| | **Thr** | Thr | Thr | Thr | **His** |
| | Asn | Asn | Asn | Asn | Gln |
| | Lys | Lys | Lys | Lys | Arg |
| | Ser | Ser | Ser | **Ser** | |
| | Arg | Arg | Arg | Arg | |
| NOTE: no stop codons should be in mix | | | | | |

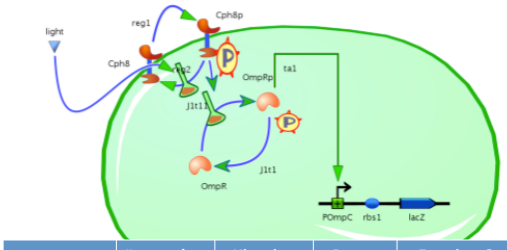
## Beyond the C-dog




protein activity?
protein stability?
phenotype of cell?

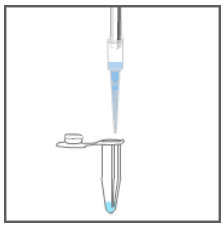
http://publications.nigms.nih.gov/thenewgenetics/images/ch1_trans.jpg

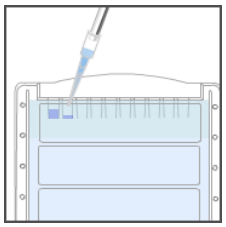
## How could a change in protein stability affect β-gal?




| | # autophos Cph8 | Kinasing rate | P-tase rate | Fraction OmpR+P |
|---|---|---|---|---|
| Wild type | 10 | 1 | 0.5 | 1 of 10 |

## Part 1: SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

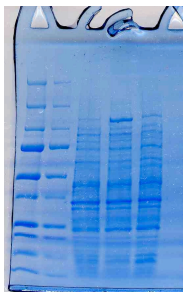


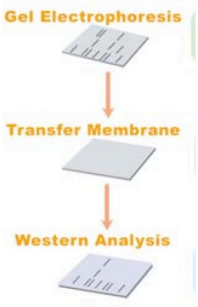


Loading dye has glycerol, SDS, reducing agent

Samples boiled before loading

## Part 2: Transfer for Western






Thursday: probe membrane with an antibody

http://www.genscript.com/product_001/western_application/grp_id/60065/op/detail/Uvrag_Antibody_Analysis.html

## 20.109(F10): Laboratory Fundamentals of Biological Engineering

In lab you will:

- Measure OD600 of 1:10 of bacterial photography strain, Candidate 1, Candidate 2
- Harvest 4 OD
  e.g. if 0.5 OD, harvest 8 ml of 1:10 or 0.8 ml of undiluted
- Isolate protein with lysis kit (enzymatic lysis of cells, spin out debris)
- Mix supernatant with loading dye
- Boil
- Load for SDS-PAGE along with markers, + control lysate

| Order# | Req# | SeqId | Download | View | Sample | Primer | UID | Date | Phred Q20 | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| 31477 | 379712 | 4491-1 | Text Chromat | View | Red-334 | NO289 | 1742 | Oct 28 2010 | 0 | being repeated-failed |
| 31477 | 379713 | 4491-2 | Text Chromat | View | Red-cand1 | NO289 | 1742 | Oct 28 2010 | phd qual 189 fasta | being repeated-noisy/failed |
| 31477 | 379714 | 4491-3 | Text Chromat | View | Red-cand2 | NO289 | 1742 | Oct 28 2010 | phd qual 17 fasta | being repeated-noisy/failed |
| 31477 | 379715 | 4491-4 | Text Chromat | View | Orange-334 | NO289 | 1742 | Oct 28 2010 | 0 | being repeated-failed |
| 31477 | 379716 | 4491-5 | Text Chromat | View | Orange-cand1 | NO289 | 1742 | Oct 28 2010 | 0 | being repeated-failed |
| 31477 | 379717 | 4491-6 | Text Chromat | View | Orange-cand2 | NO289 | 1742 | Oct 28 2010 | phd qual 812 fasta | Results Available |
| 31477 | 379718 | 4491-7 | Text Chromat | View | Yellow-334 | NO289 | 1742 | Oct 28 2010 | phd qual 1000 fasta | Results Available |
| 31477 | 379719 | 4491-8 | Text Chromat | View | Yellow-cand1 | NO289 | 1742 | Oct 28 2010 | phd qual 946 fasta | Results Available |
| 31477 | 379720 | 4491-9 | Text Chromat | View | Yellow-cand2 | NO289 | 1742 | Oct 28 2010 | 0 | being repeated-failed |
| 31477 | 379721 | 4491-10 | Text Chromat | View | Green-334 | NO289 | 1742 | Oct 28 2010 | phd qual 987 fasta | Results Available |
| 31477 | 379722 | 4491-11 | Text Chromat | View | Green-cand1 | NO289 | 1742 | Oct 28 2010 | 0 | being repeated-failed |
| 31477 | 379723 | 4491-12 | Text Chromat | View | Green-cand2 | NO289 | 1742 | Oct 28 2010 | phd qual 12 fasta | being repeated-noisy/failed |
| 31477 | 379724 | 4491-13 | Text Chromat | View | Blue-334 | NO289 | 1742 | Oct 28 2010 | phd qual 992 fasta | Results Available |
| 31477 | 379725 | 4491-14 | Text Chromat | View | Blue-cand1 | NO289 | 1742 | Oct 28 2010 | phd qual 922 fasta | Results Available |
| 31477 | 379726 | 4491-15 | Text Chromat | View | Blue-cand2 | NO289 | 1742 | Oct 28 2010 | phd qual 104 fasta | being repeated-noisy/failed |

## First place to look: your notebook!

6-4-76 UV. Spectrum of Guanine from Adduct

Introduction: The experiment on p. 17 failed to produce an interpretable UV spectrum. The Gua peak was evaporated to dryness as described ~~above~~ and dissolved in $H_2O$ (100µl) as described above.

- Inject into HPLC eluted under tRNA conditions
- Collect peak in Cuvette
- Obtain UV. spectrum

Chromatographic Results:

1. Gua from adduct : 95% on X.1 (254 nm)
2. Gua standard : 60% on X.2

Spectroscopic Results : facing page.

Conclusions: The adduct contains a base with an identical UV. spectrum to guanine.

## Today in lab

1. Lab treat
2. SDS-PAGE + blot
3. β-gal assay for cells grown in light and dark
4. Analysis of sequence data

BEFORE LEAVING LAB today:

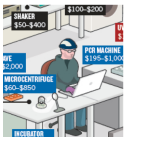
5' with NK, NT and/or MS to discuss what you and your partner would like to do….

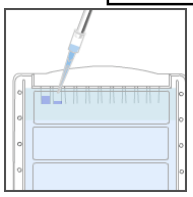
Consider repeating experiments that need to be repeated

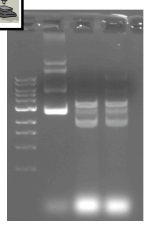
Consider collaborating with other groups to get more interpretable/robust data

| Cph1/EnvZ | A553 | G554 | V555 | S556 | H557 |
|---|---|---|---|---|---|
| EnvZ | A239T | G240E | V241G | S242D | H243A |

## Summary





**20.109(F10): Laboratory Fundamentals of Biological Engineering**