RNA-seq vs. qPCR

RNA-seq:
- Measures every expressed gene
- You *enrich* for mRNA
  And get rid of rRNA
- Requires sequencing

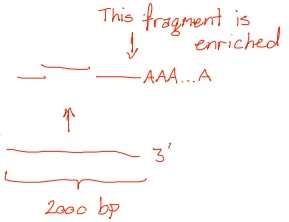
qPCR:
- Measures single gene
- No mRNA enrichment
  Uses primers to amplify GOI
- Does not require sequencing

Q1:

Why are you more likely to observe sequence from the 3' end of a gene in RNA-seq data (relative to sequence from the 5' end)?

Why are you more likely to observe sequence from the 3' end of a gene in RNA-seq data?

- What is special about the 3'-end?
  polyA tail 5' ~~ AAA...A 3'
- What is special about mRNA?
  It has a p(A) tail
- How do you enrich for mRNA?
  Uses oligo(dT)'s



Q2:

To compare two sets of RNA-seq data, you first normalize the results by calculating the RPKM value for each gene. What are the two factors to which you normalize (hint: how do you normalize between experiments AND how do you normalize between genes)?

Calculating the RPKM

- RPKM = Reads Per Kilobase Million

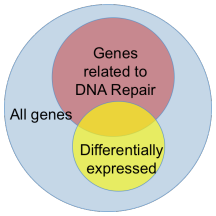
from RNA-seq exp.

Total reads / 1,000,000 = **per million (PM)** scaling factor

Reads / **PM** = RPM

RPM / gene length in Kb = RPKM

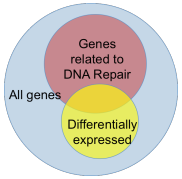
Q3:
When analyzing RNA-seq data you identify a group of differentially expressed genes (yellow circle). You already know which genes are involved in DNA repair (red circle).w



Which probability distribution will tell you the probability of overlap?

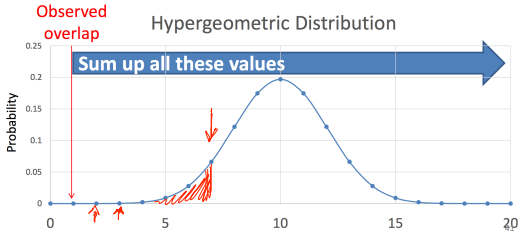
- Hypergeometric distribution

$$P(overlap) = (\blacksquare DNA\ repair Overlap)(\blacksquare Genome - DNA\ repair Diff.\ expr. - overlap) / (\blacksquare Genome Diff.\ expr.)$$



What statistical function can you use to test if the overlap is significant?

- Cumulative density function (CDF)
- Fisher's Exact Test



Q4:
qPCR is used to measure expression levels of specific genes.

qPCR is used to measure expression levels of specific genes

Why measure p21?

Has to do w/ cell cycle.
Stalls cell cycle in response to DNA damage

Why measure GAPDH?

Housekeeping gene
Normalize p21 expression

Q5:
Briefly describe "synthetic lethality" and how it applies to your cell viability experiment

Briefly describe "synthetic lethality" and how it applies to your cell viability experiment

What is synthetic lethality?

The combination of two mutations kills the cell. The individual mutations do not.

How does it apply to our experiment?

Our BRCA -/- mutant is defective in HR.
We treat with a drug that inhibits NHEJ.
We want to see if the combined knockout of both pathways leads to cell death when we cause DNA damage (etoposide)

M3D1:Grow phage-based active (cathode) material

4/19/18

1. Purify M13 bacteriophage (phage)
2. Prelab during 60min incubation
3. Finish M13 purification and measure concentration
4. Incubate phage with gold nanoparticles (AuNP)

Thank you, Jifa Q. (Belcher Laboratory)!

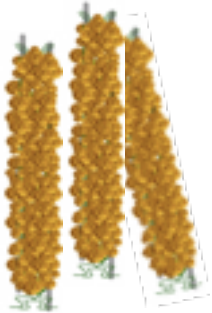
Module 3: biomaterials engineering

How do material choice and nanoparticle size affect battery capacity?

Purify
M13 phage
Add AuNP

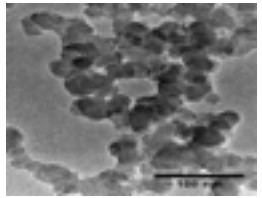
DAY 1

Biomineralize
Fe(III)-
phage-AuNP



Visualize
nanowires
on TEM

DAY 4



Purify
active material

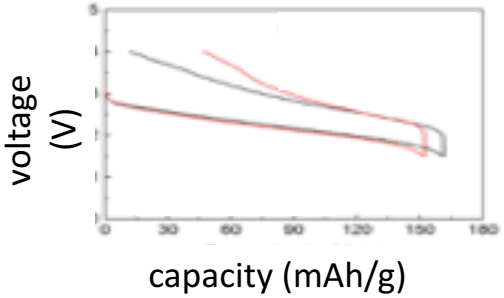
DAY 2

Construct
cathode
(and battery)

DAY 3

Measure battery
capacity

DAY 5



Phage purification using polyethylene glycol (PEG) in 2.5M NaCl

E. coli (ER2738)
M13 phage
LB + Tetracycline

overnight

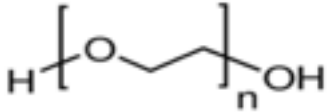
Spin

(virus) Phage in supernatant

(bacteria) E. coli in pellet

supernatant

Polyethylene glycol
20% PEG-8000
2.5M NaCl



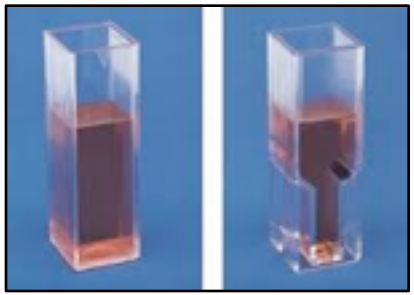
- hydrophilic polymer
- promotes phage aggregation/precipitation

Spin

PEG/NaCl in supernatant

Phage in pellet

Determining phage titer (number of virus):



- By plating: plaque assay
  - Phage slows *E. coli* growth = plaque (cleared zone)
  - Plaque-forming units: PFU/mL

- By spectrophotometry

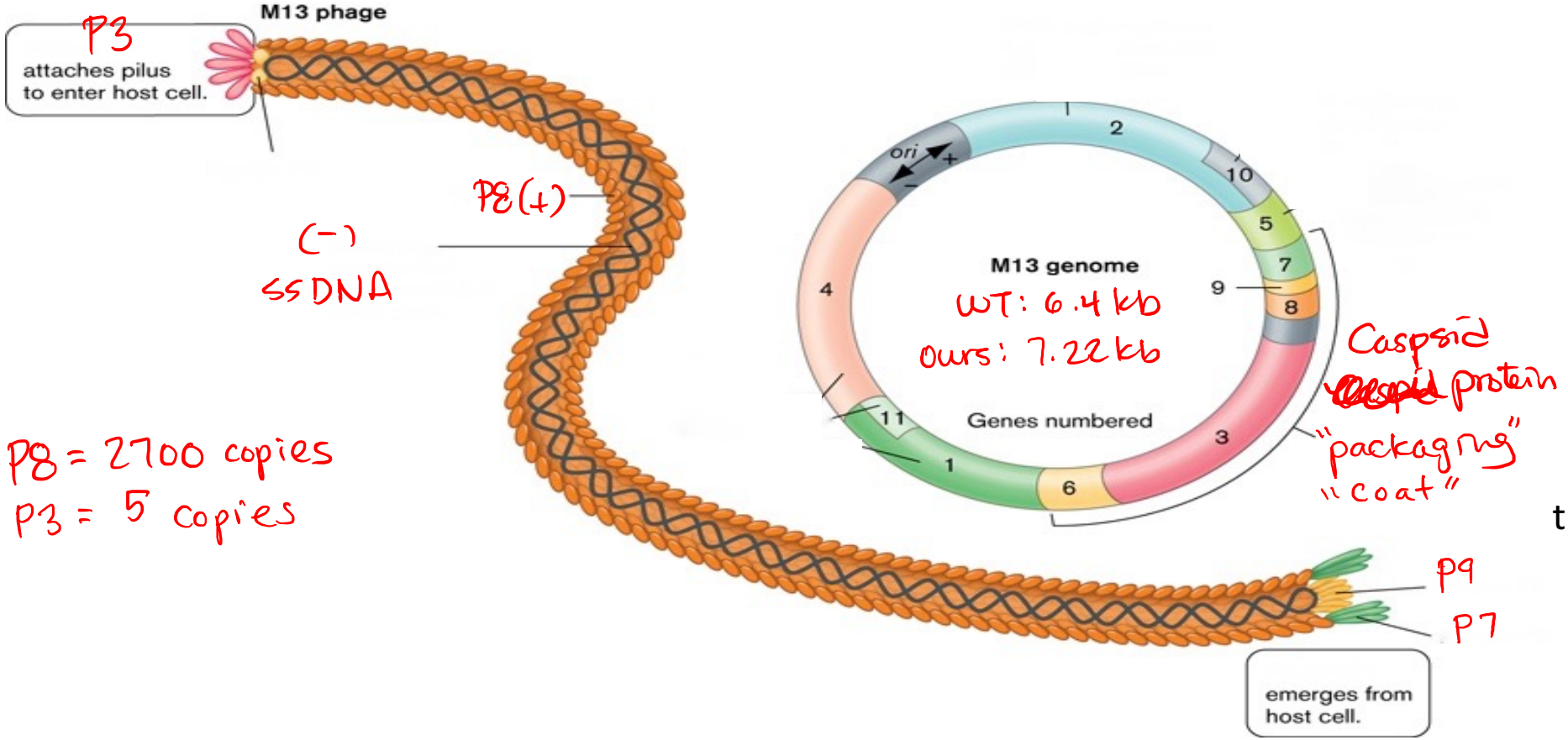
$$\#\ \text{phage / mL} = \frac{(6 \times 10^{16})\,(A269 - A320)}{\#\ \text{bases in phage genome}}$$

* don't forget to consider dilution in this calculation

- Quartz cuvettes are expensive!

M13 is a high aspect ratio phage coated in proteins encoded by ssDNA loop

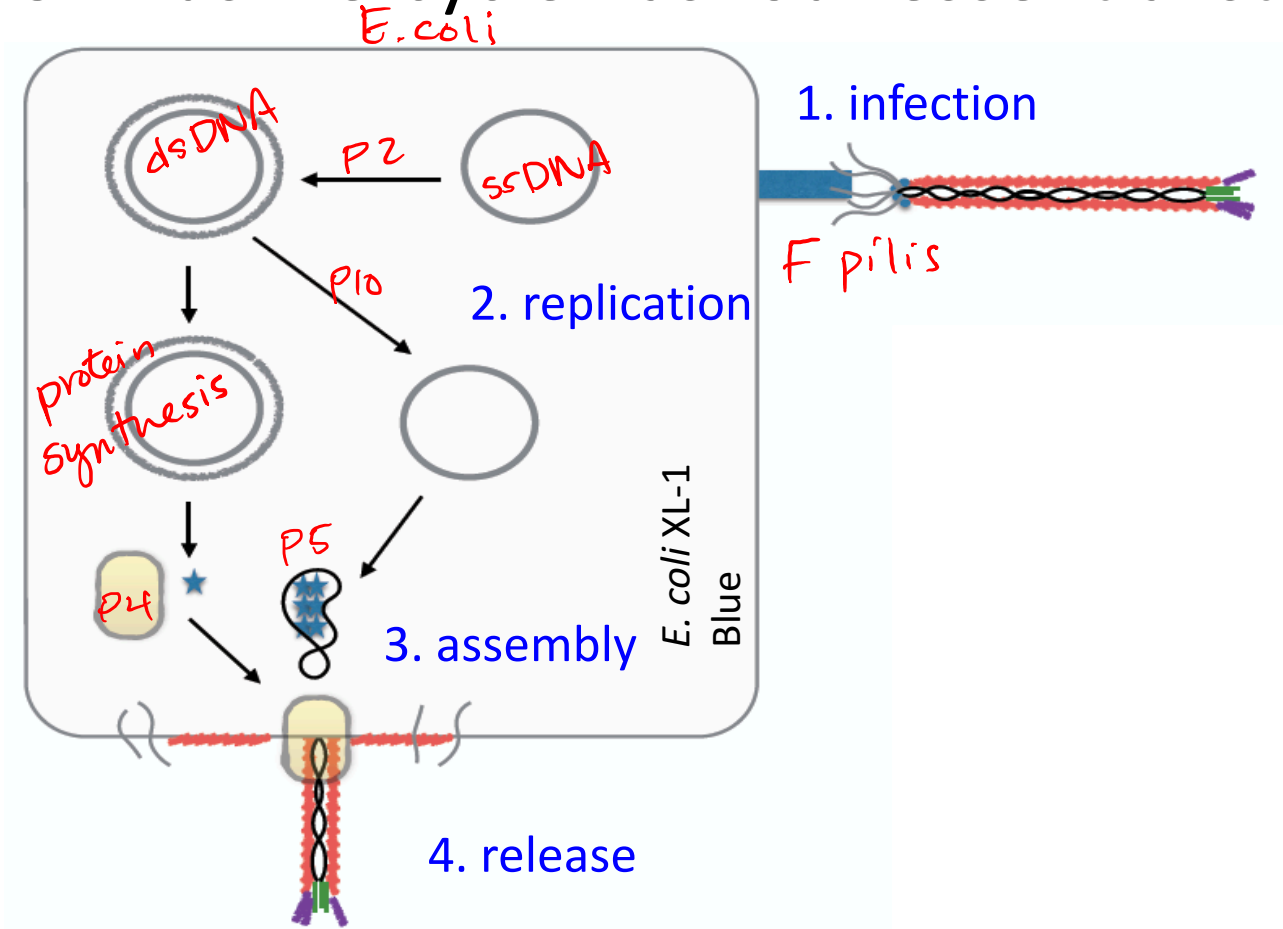
990nm long
6nm wide



P8 = 2700 copies
P3 = 5 copies

t)

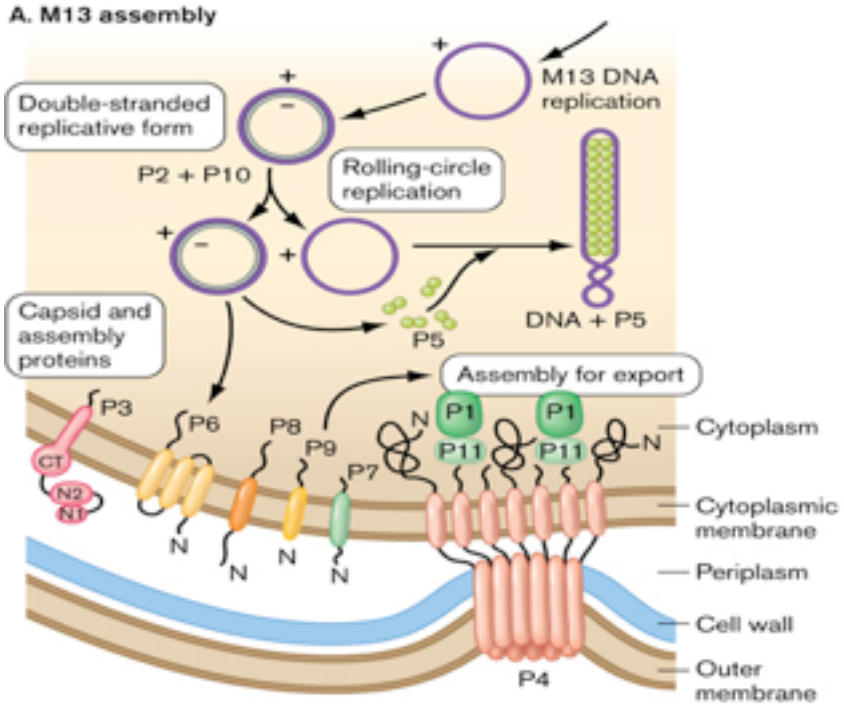
M13 virus life-cycle has four essential steps

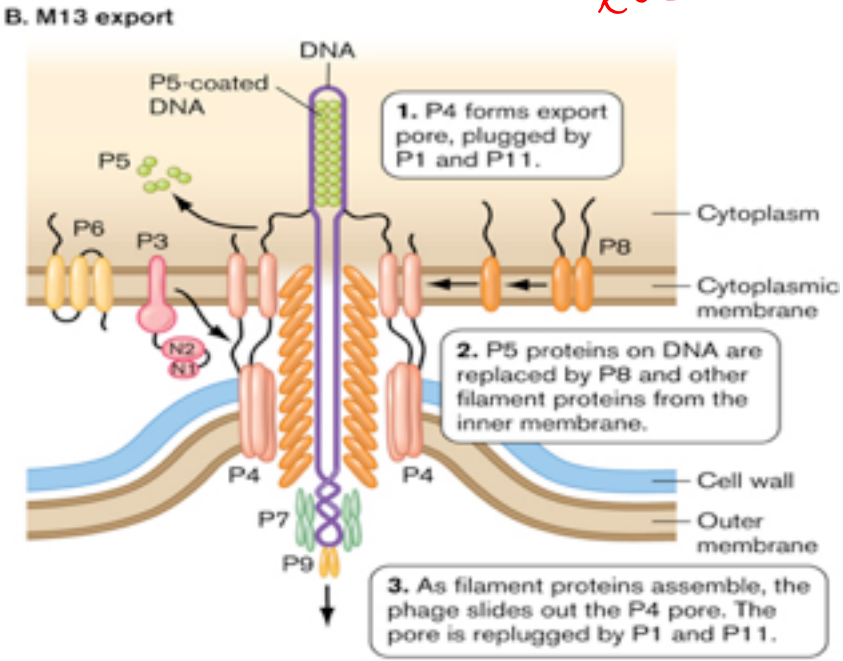


M13 is a nonlytic bacteriophage

(so we can easily get lots of it)

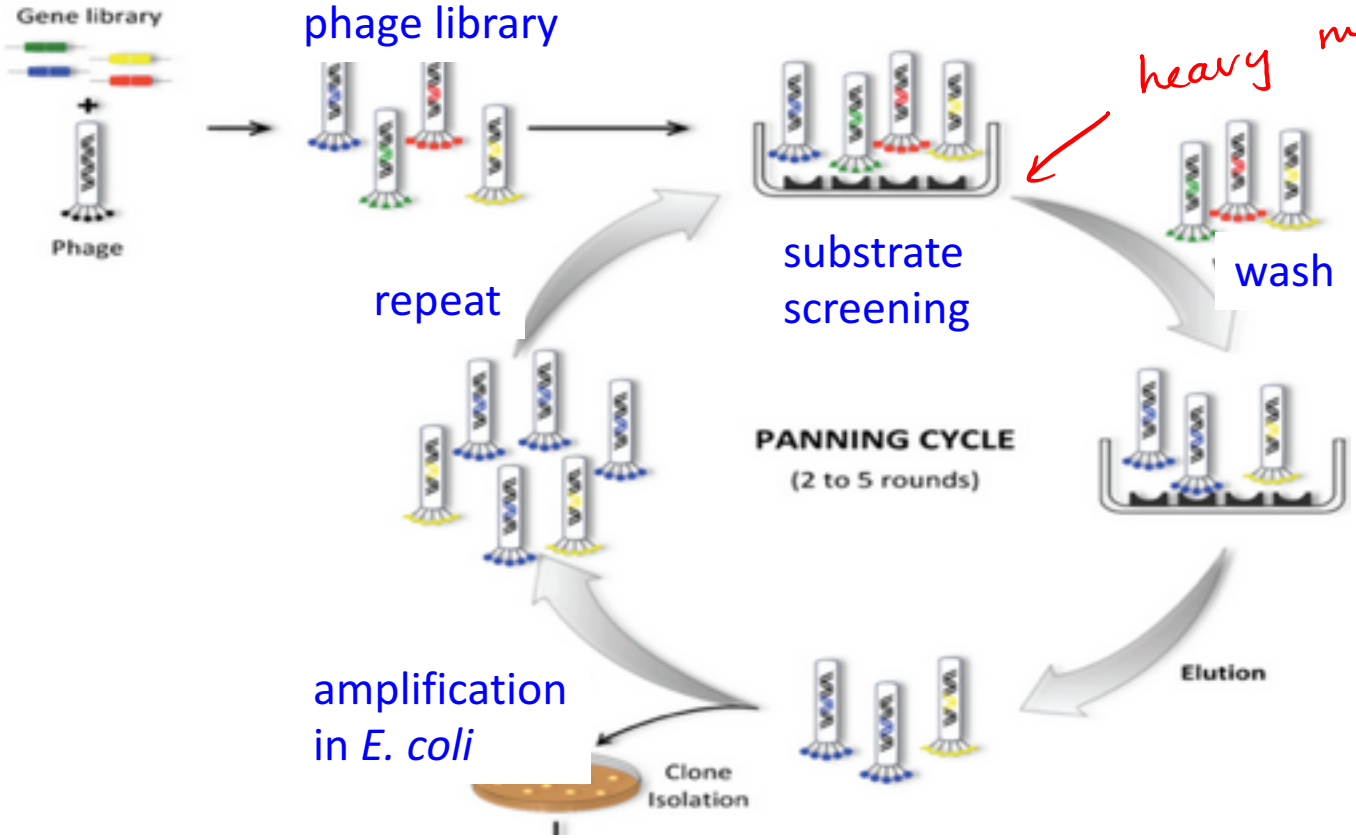
Zoomed in view of escape





http://www.wwnorton.com/college/biology/microbiology2/ch/11/etopics.aspx

Phage display allows unbiased selection of useful peptide sequences (typically binding)

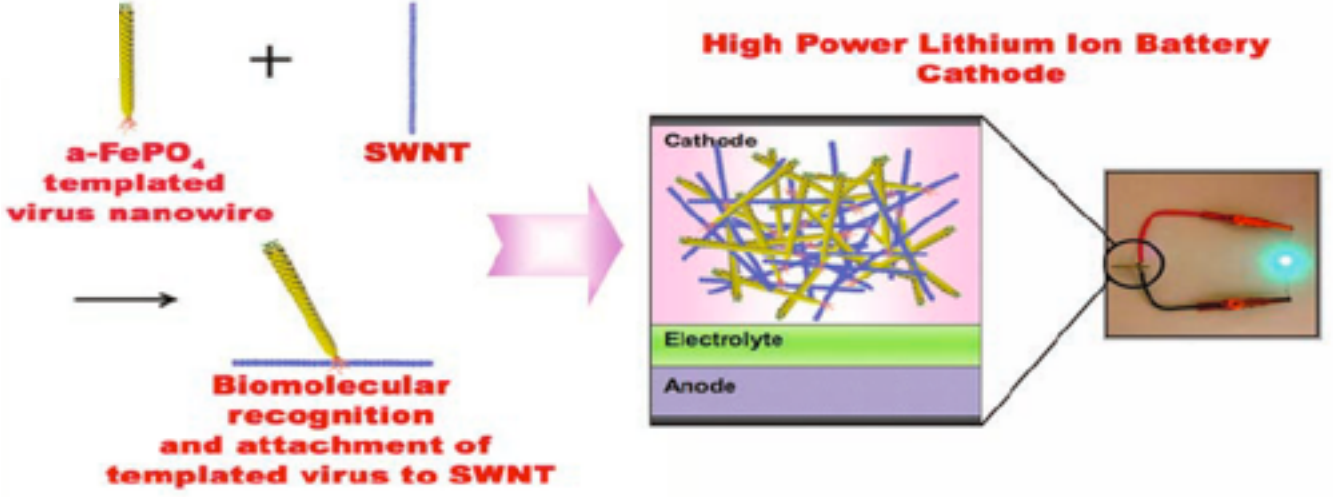


Biomolecules 2015, 5(3), 1783-1809; doi:10.3390/biom5031783

M13 are engineer-able biomaterials

negatively charged

- Our p8 coat protein was mutated to contain sequence DSPHTELP
- Modified p8 proteins bind single wall carbon nanotubes (SWCNT), iron, gold, and other cationic metals
- Example of this virus in literature (Science, 2009):



Lee et al. Fabricating Genetically Engineered High-Power Lithium-Ion Batteries Using Multiple Virus Genes. Science. 2009

M13 nanowires as battery cathode

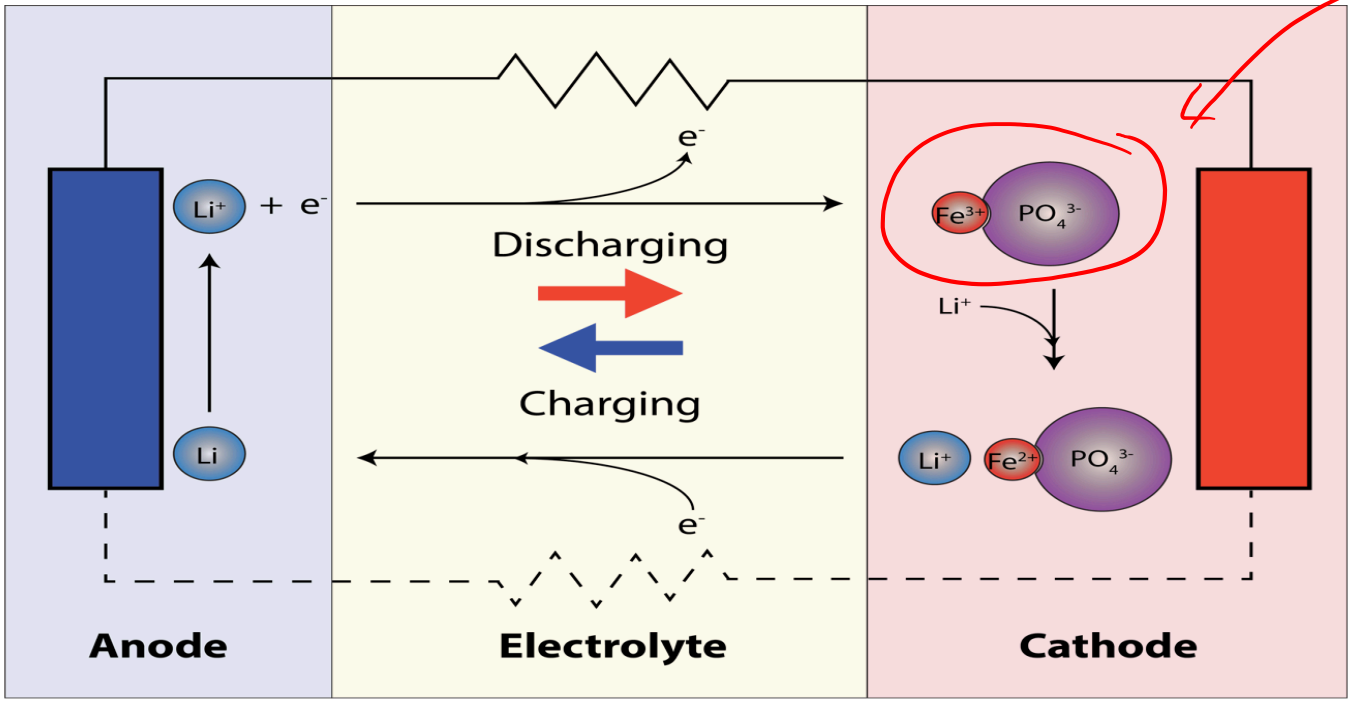


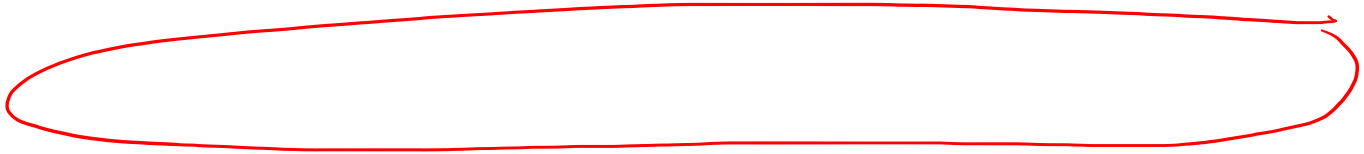
Image: George Sun

You will make a "Gold Standard" battery and an experimental battery

- Gold standard: 3.8nm Gold, 40 AuNP per phage
- Choice of combination: Keep total volume less than 50ml
  - 3.8nm Gold
  - 5nm Gold
  - 9nm Gold

concentrations on wiki

990nm



Considerations for experimental battery: nanoparticle material and size

- Conductivity
  - Au is conductive, how much total gold will be in your cathode?
- Internal battery reaction catalysis
  - Li+ in solution → Li+ embedded
    - lithium embedded in iron phosphate
    - gold may catalyze this interaction
  - Surface area to volume ratio
    - consider surface area of each nanoparticle
    - consider NP binding phage reduces iron phosphate binding sites

Design with your lab partner. What is your **hypothesis**?

You will make two flasks—one for each battery

Gold standard

- 4e13 Phage
- 40 (3.8nm) AuNPs/phage
- Water (final volume 50 mL)

Experimental

- 4e13 Phage
- ? AuNPs
- Water (final volume 50 mL)

Today in lab

1. Finish phage purification
2. Calculate phage number
3. Mix components: phage, AuNP, $FePO_4$ nanowires (2 flasks, one per battery)

M3D2 HW: Describe **FIVE** recent findings that could potentially define an interesting research question.

- Formally cite the finding
- Write 3-5 sentences summarizing the finding