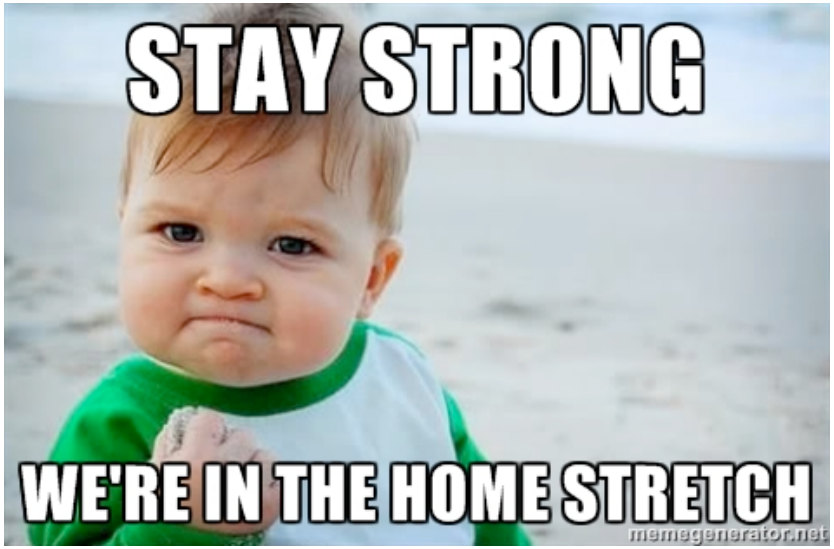
M3D1:Growth of phage materials

11/15/17

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



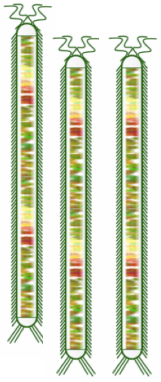
Thank you, Eric L. and Jifa Q. (Belcher Laboratory)!

Module 3: biomaterials engineering

How do material choice and nanoparticle size affect battery capacity?

Purify
M13 phage
Add AuNP/NiNP

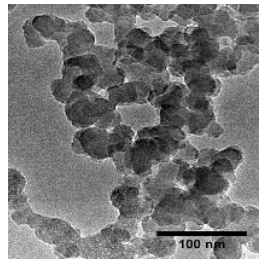
DAY 1



Biomineralize
Fe(III)-
phage-AuNP

Visualize
nanowires
on TEM

DAY 4



Purify
active material

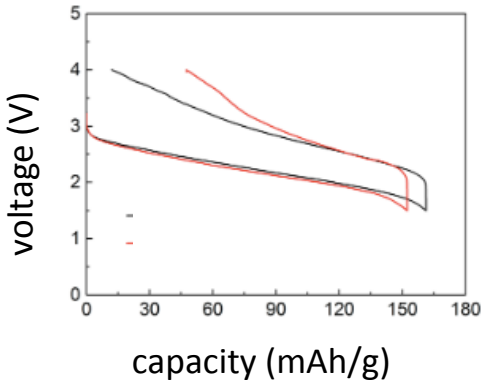
DAY 2

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

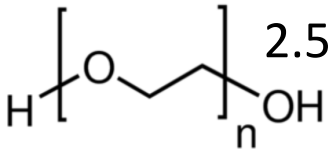
Spin

phage in supernatant

e. coli in pellet

supernatant

20% PEG-8000
2.5M NaCl



-hydrophilic polymer
-promotes phage aggregation/ precipitation

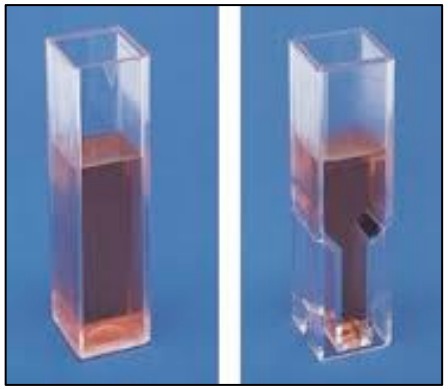
Spin

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

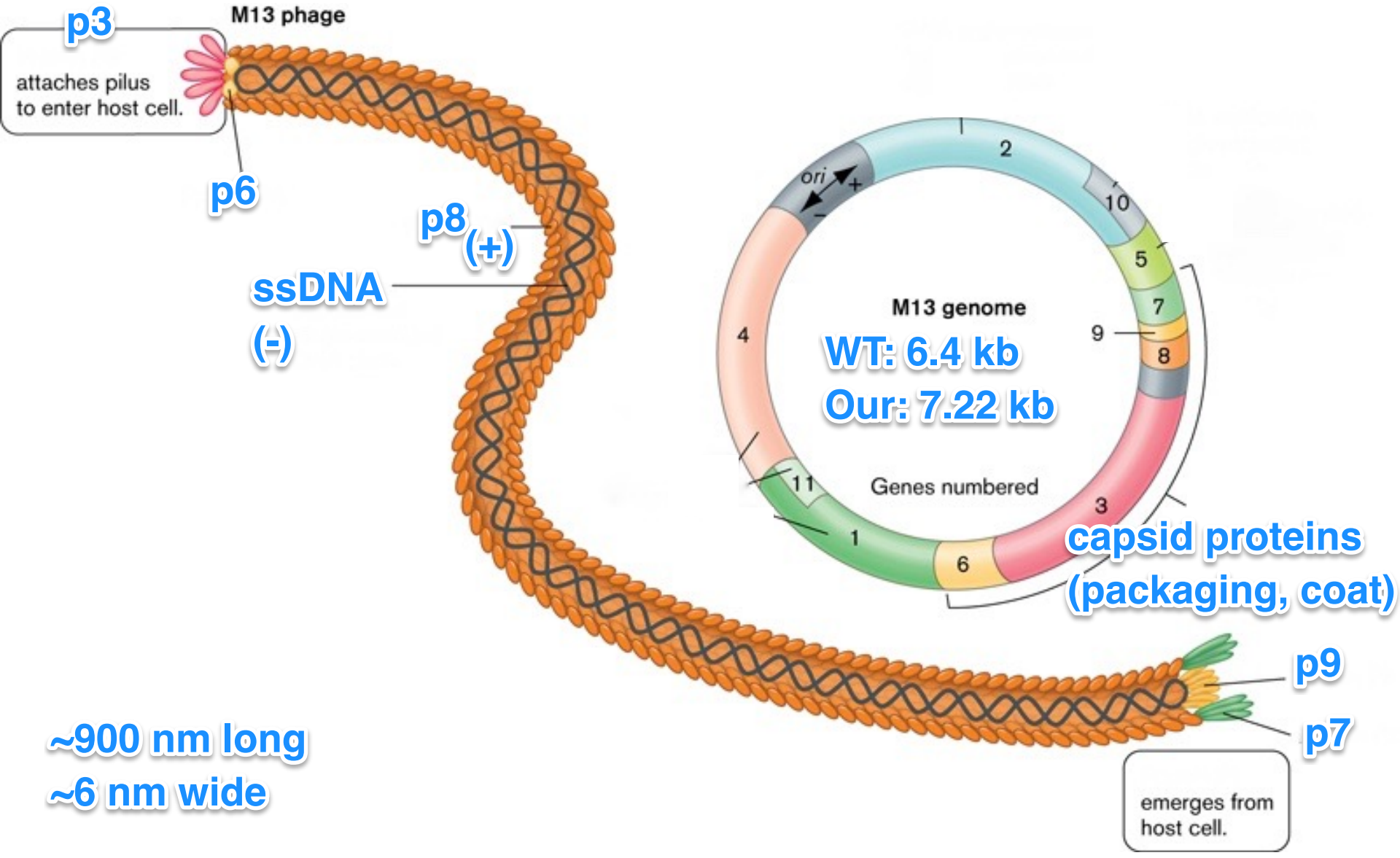


- By spectrophotometry

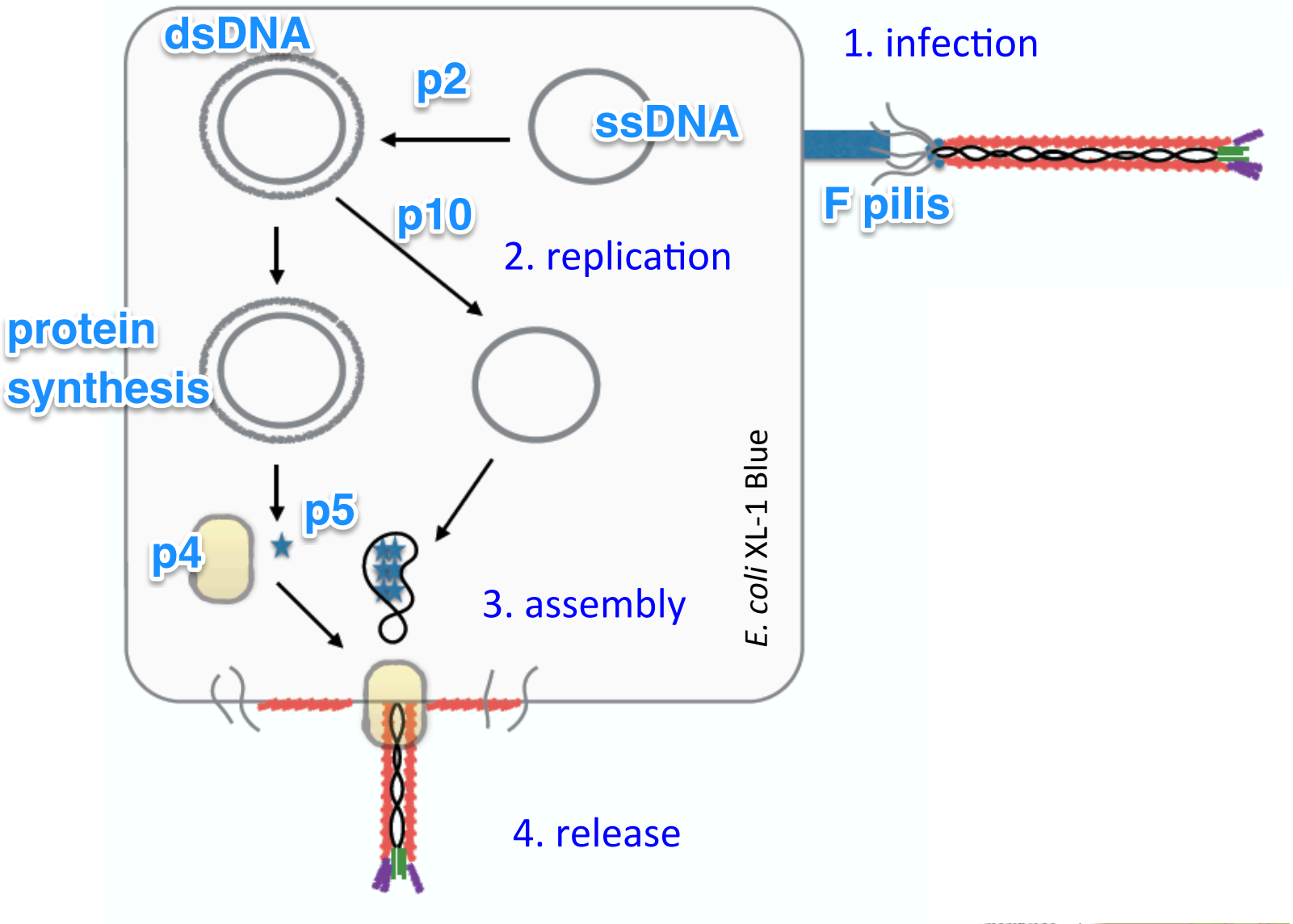
$$\#\ \text{phage / mL} = \frac{(6 \times 10^{16})\ (A269 - A320)}{\#\ \text{bases in phage genome}}$$

- Quartz cuvettes are expensive!

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

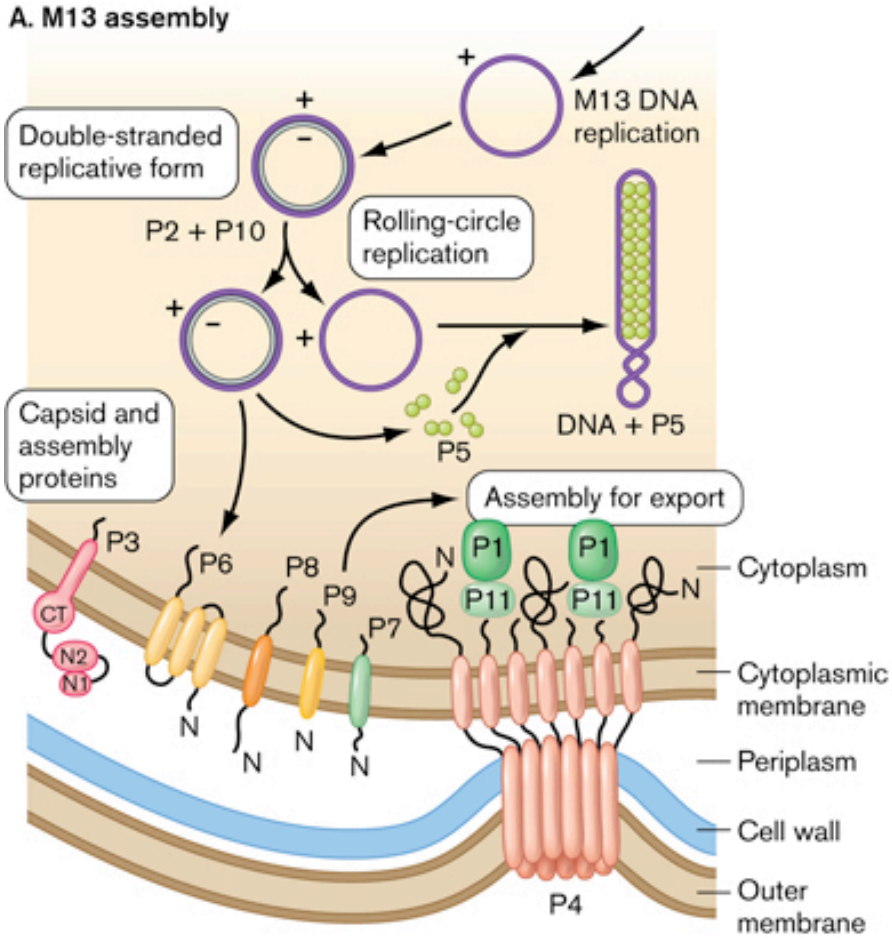


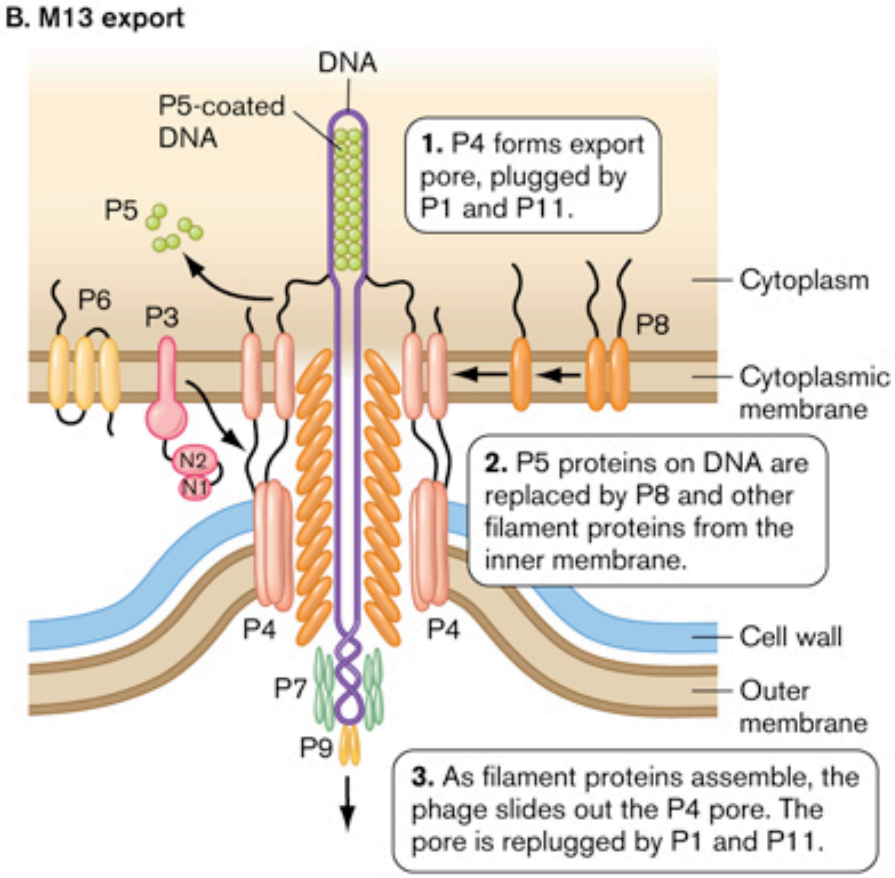
M13 virus life-cycle has four essential steps



M13 is a nonlytic bacteriophage

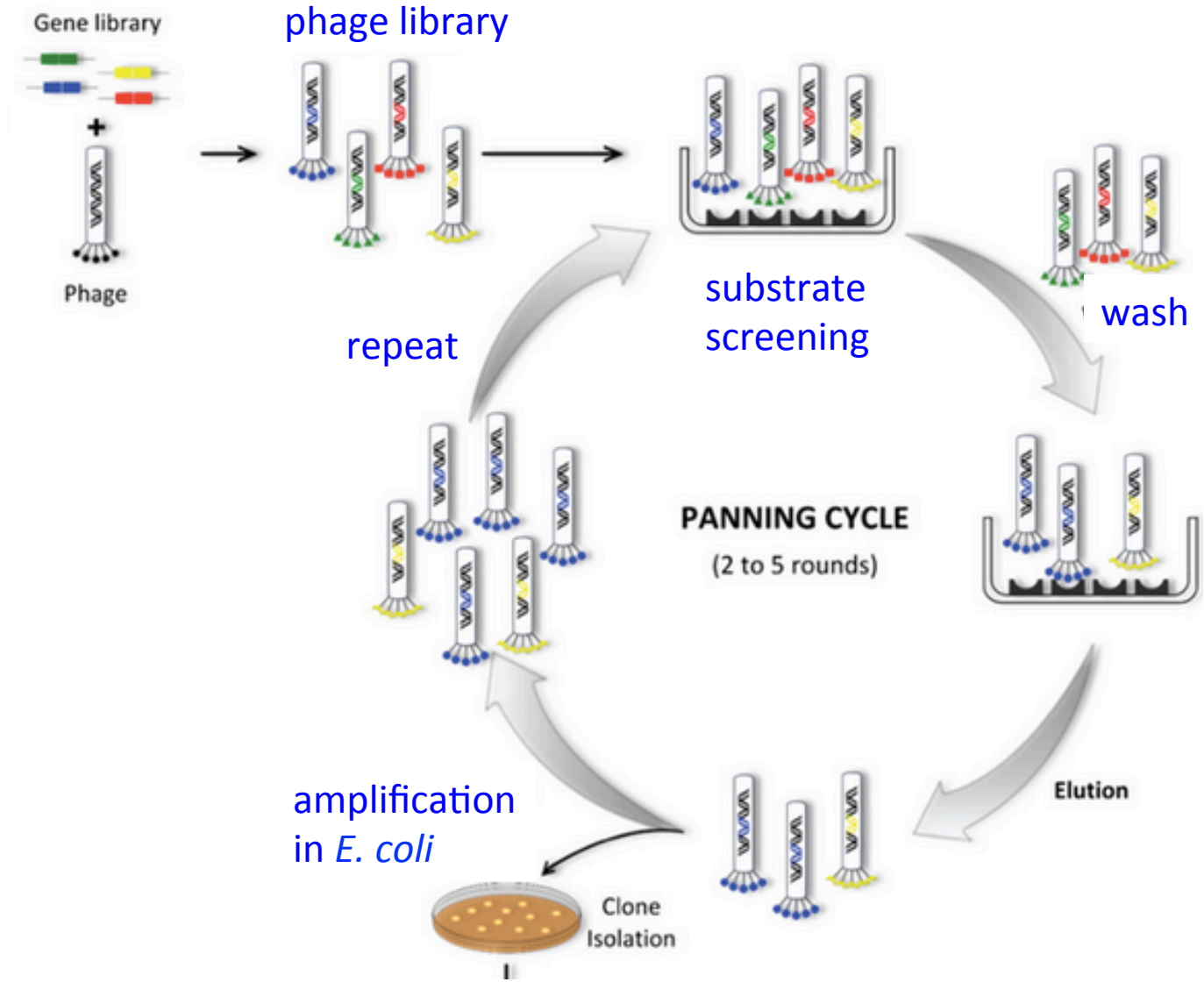
(so we can easily get lots of it)





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

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

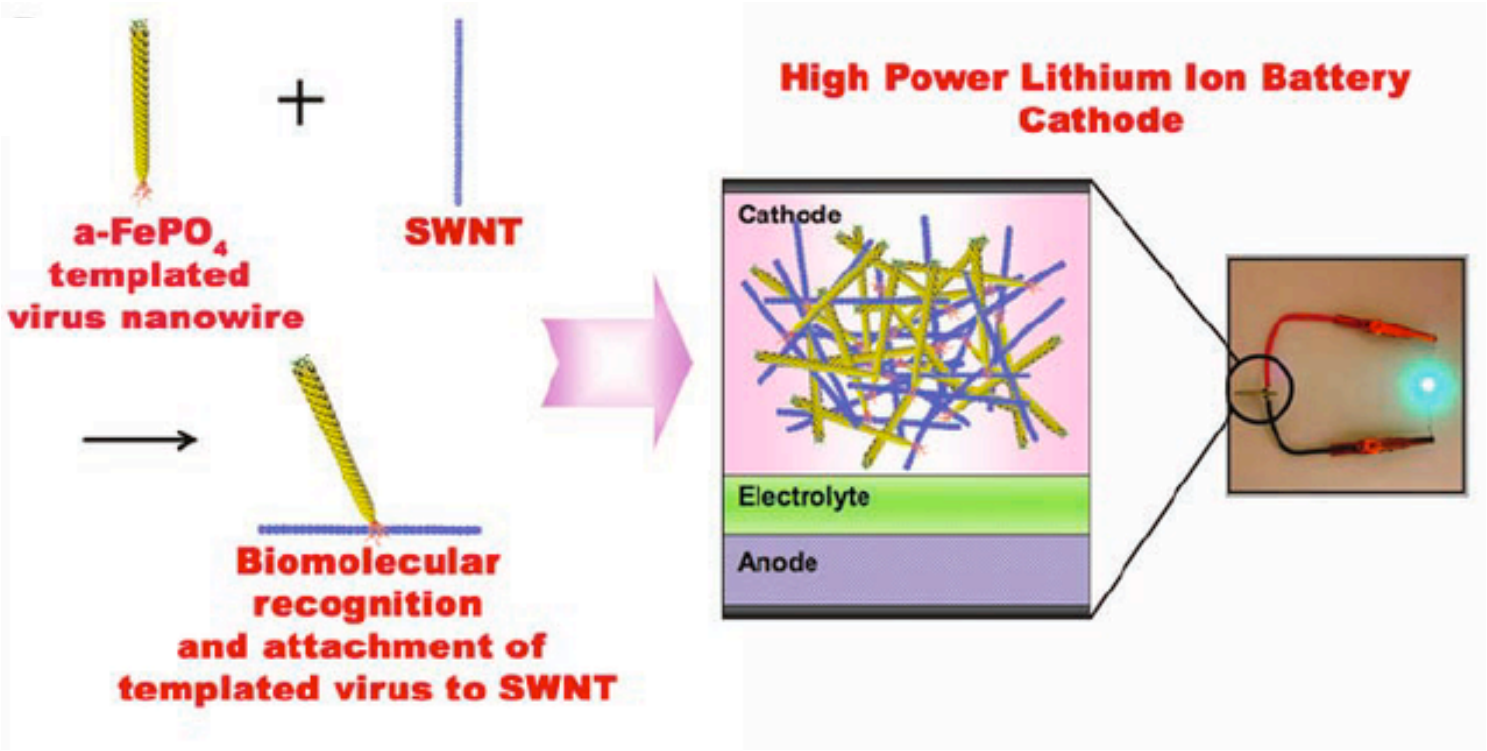


Biomolecules 2015, 5(3), 1783-1809; doi:10.3390/biom5031783

M13 are engineer-able biomaterials

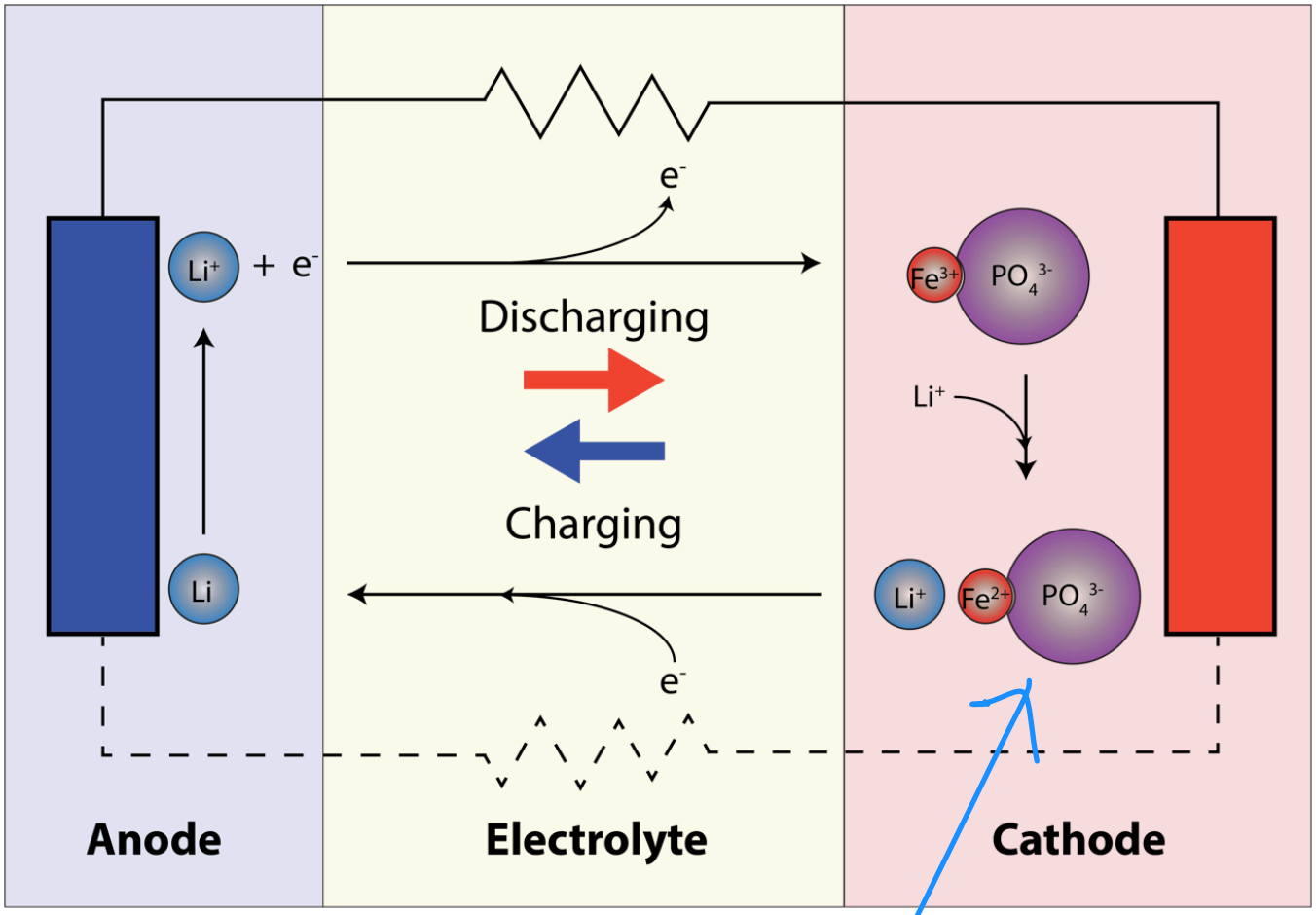
negatively charged

- 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

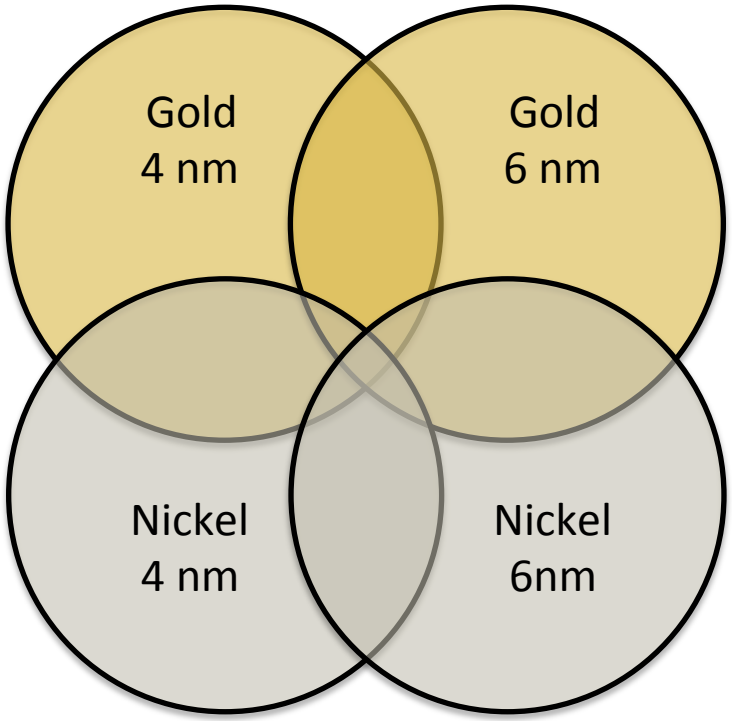


mineralized on phage

Image: George Sun

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

- Gold standard: 4nm AuNPs
- Choice of combination: 4/6nm, Au/Ni and ratio



NP = nanoparticle

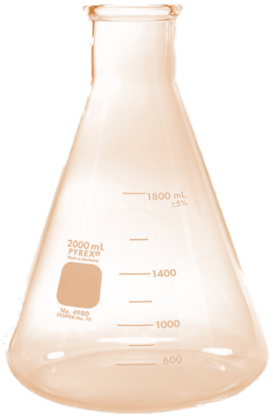
Considerations for experimental battery: nanoparticle material and size

- Redox coupling
  - Li/material interaction: Ni could oxidize at relevant voltage (Au will not)
- Conductivity
  - Au is more conductive than Ni
- Internal battery reaction catalysis
  - Li+ in solution → Li+ embedded
  - Surface area to volume ratio

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

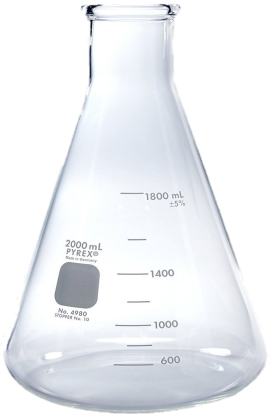
You will make two flasks—one for each battery

Gold standard



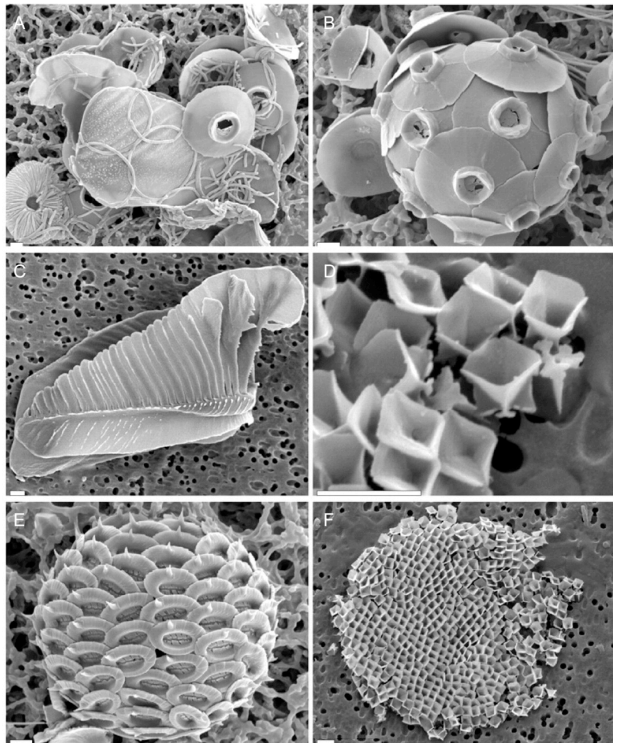
- 4e13 Phage
- 4 nm Au NPs
  (40 NPs/phage)
- Water (final volume 50 mL)

Experimental



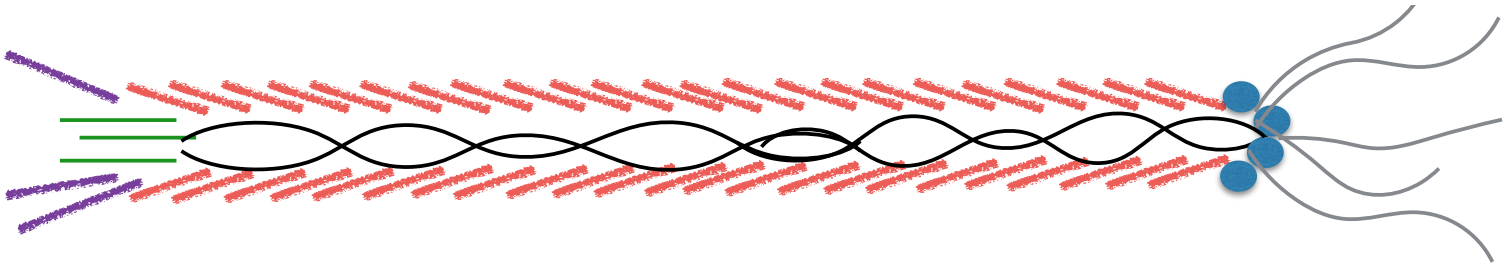
- 4e13 Phage
- 4 nm/6 nm Au/Ni NPs
  (up to 100 NPs/phage)
- Water (final volume 50 mL)

Example biomineralization from nature:



Engineering biomineralization using M13 phage:

- Environmental conditions
  4C, mild buffer, H20
- Structural organization
  wire-like virus
- M13 provides scaffold for Li($FePO_4$) cathode construction
  Fe = Li+ conductor/storage
  Au/Ni = e- conductor



Today in lab

1. Finish phage purification
2. Calculate phage number
3. Begin construction of phage-NP-$FePO_4$ nanowires (2 flasks, one per battery)
   - **Choose gold / nickel size, quantity**

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