M3D1:Growth of phage materials

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

Announcements

- Extra office hours:
  - 11/10 (Sat): 1-3 pm, 56-302
  - 11/11 (Sun): 2-7 pm, 56-302
- Mod 2 research article due 11/12, 10pm
- Blogpost due 11/13, 10pm
- No Lecture or Lab on Tues 11/13
- ***Spend time to think about/read papers for research proposals***
- re-download WF acetate data

Thank you, Jifa Q. (Belcher Laboratory)!

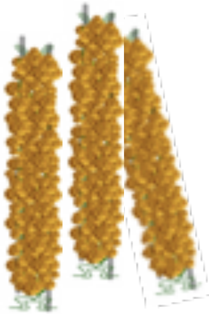
Module 3: biomaterials engineering

Do gold nanoparticles improve battery capacity?

Purify
M13 phage
Add AuNP

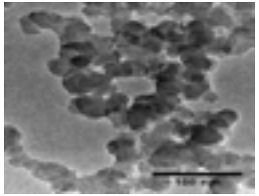
DAY 1

Biomineralize
Fe(III)-
phage-AuNP



Visualize
nanowires
on TEM

DAY 4



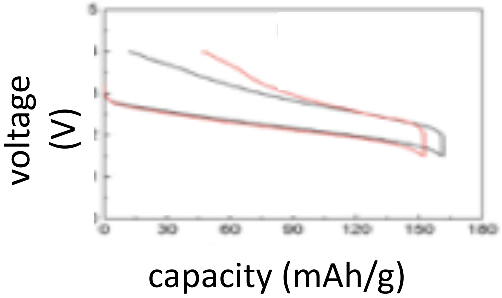
Purify
active material
DAY 2

Construct
cathode
(and battery)

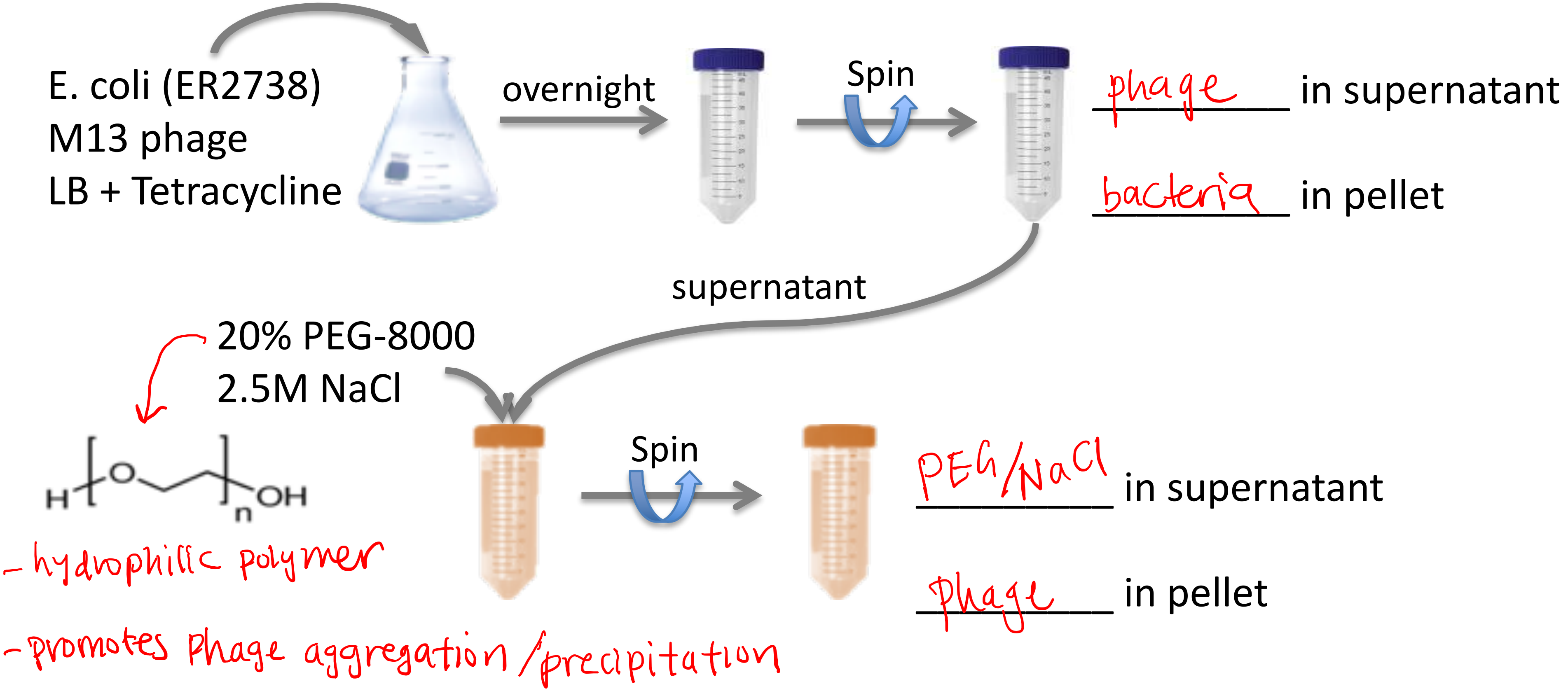
DAY 3

Measure battery
capacity

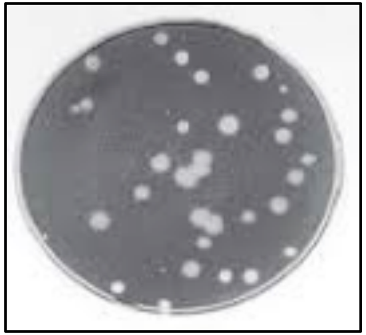
DAY 5



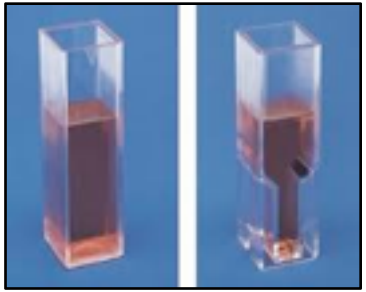
Phage purification using polyethylene glycol (PEG) in 2.5M NaCl



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

– molar extinction coefficient
– protein & DNA
– baseline

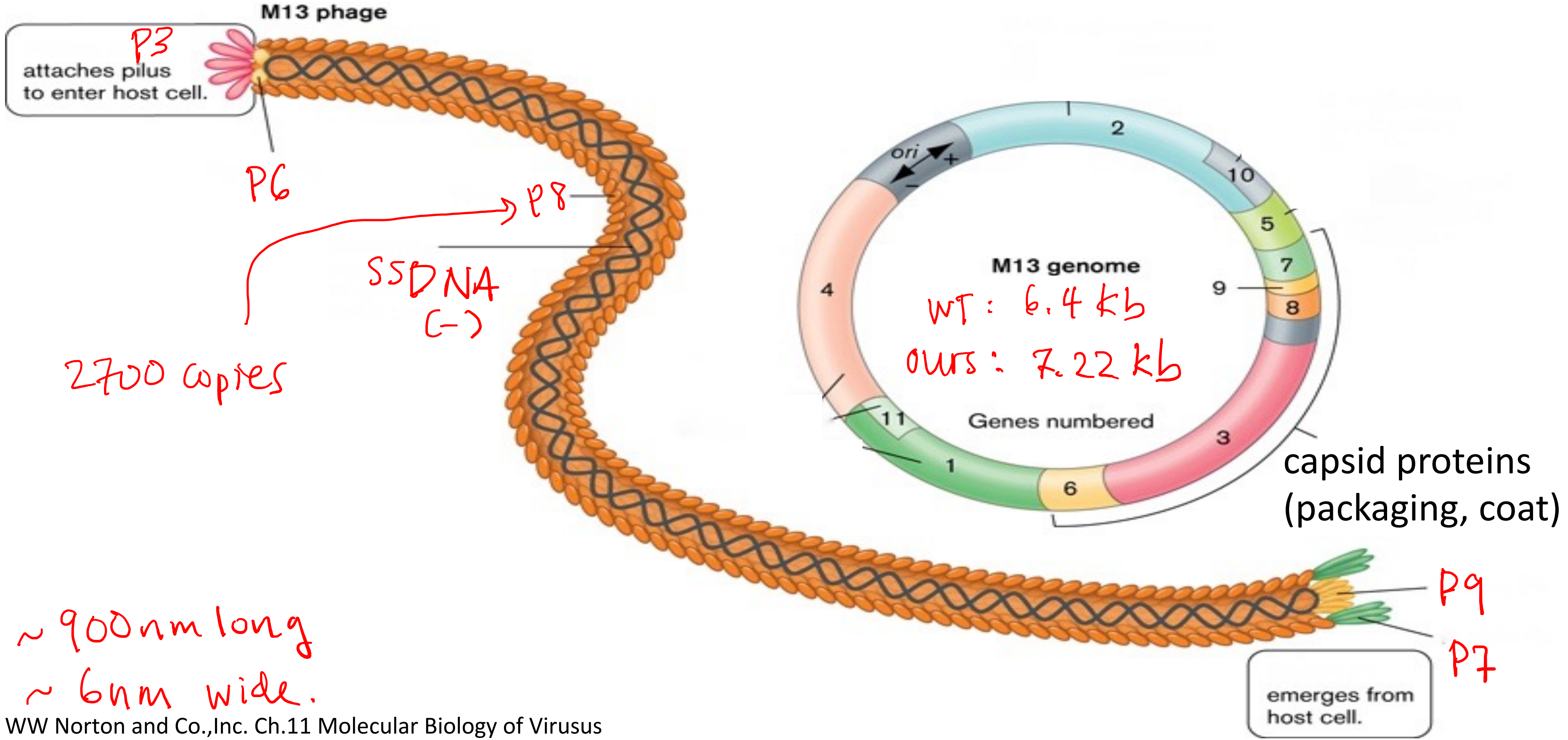
$$\# \text{ phage / mL} = \frac{(6 \times 10^{16})\ (A269 - A320)}{\# \text{ bases in phage genome}}$$

* dilution factor

~7220

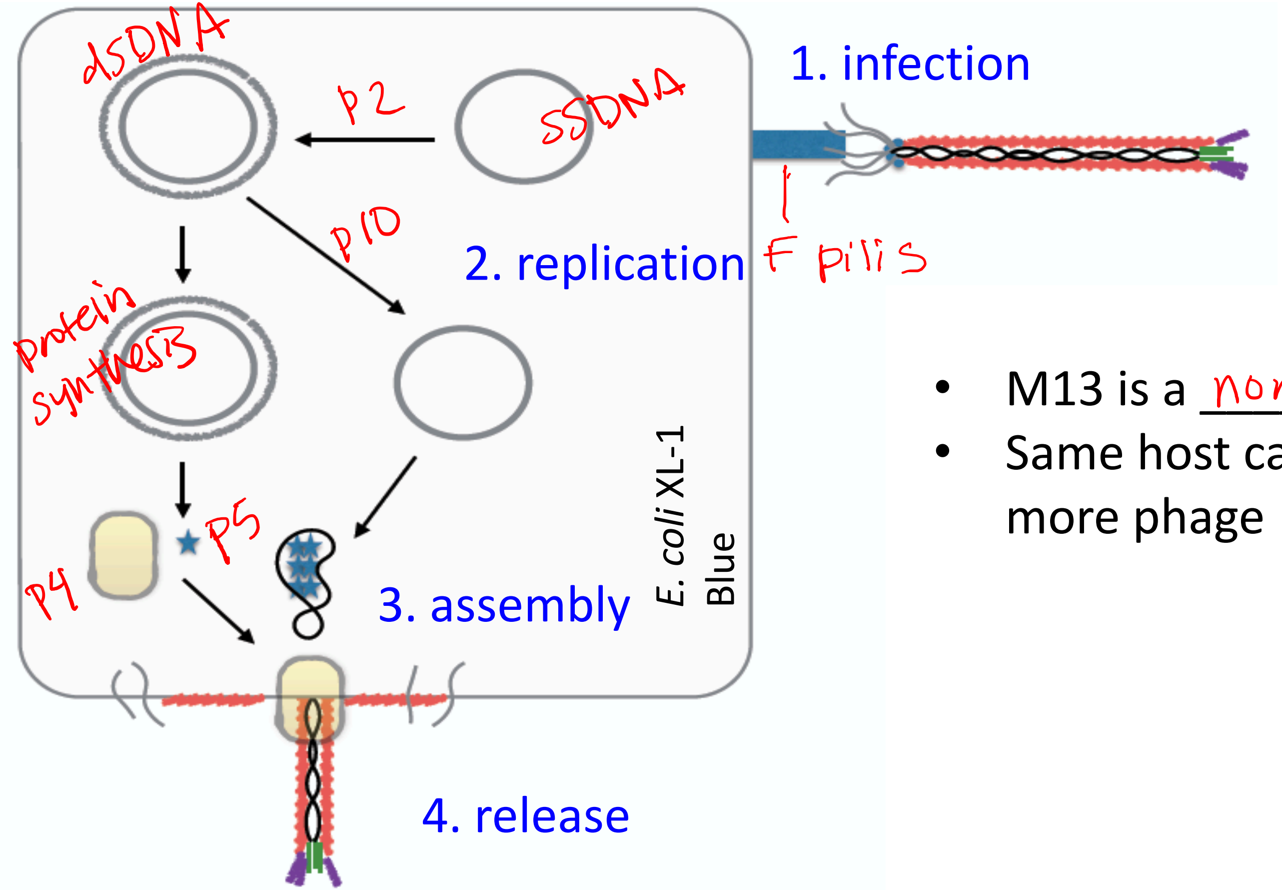
❖ Quartz cuvettes are expensive!

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



© WW Norton and Co.,Inc. Ch.11 Molecular Biology of Virusus

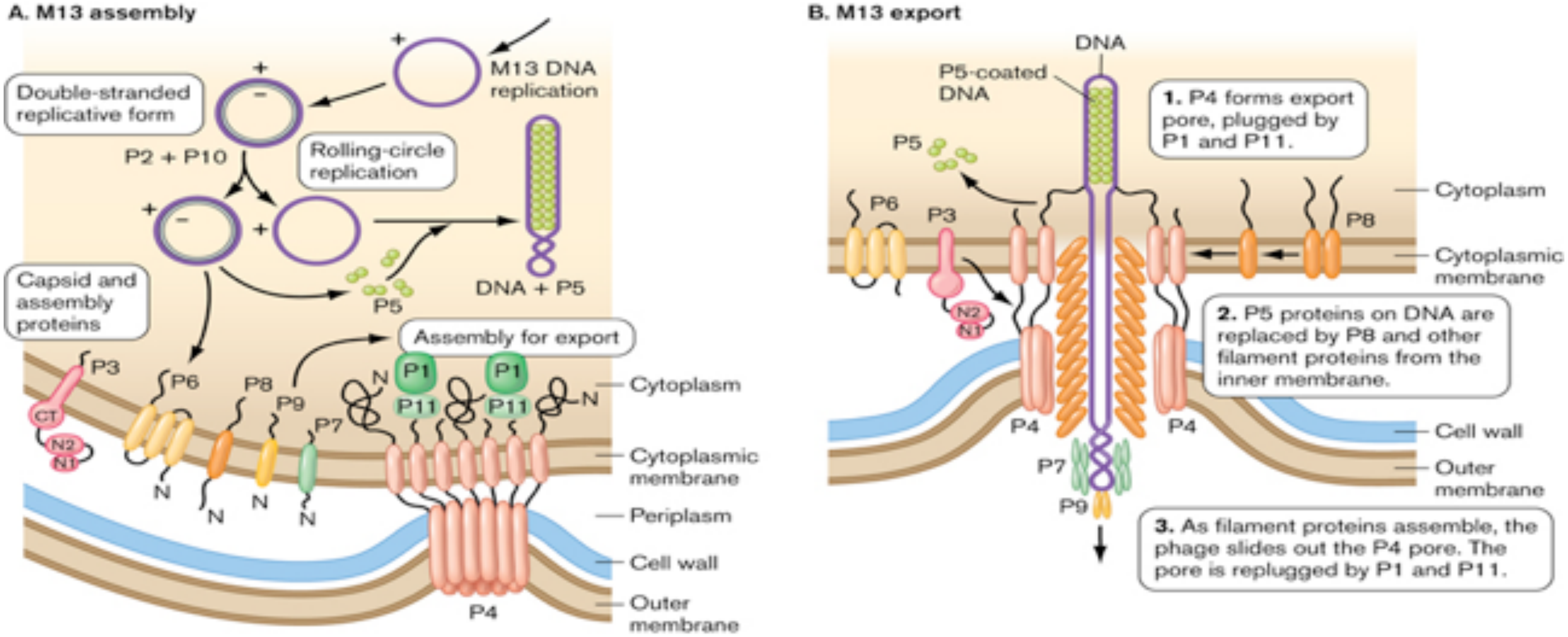
M13 virus life-cycle has four essential steps



- M13 is a nonlytic phage
- Same host can keep producing more phage

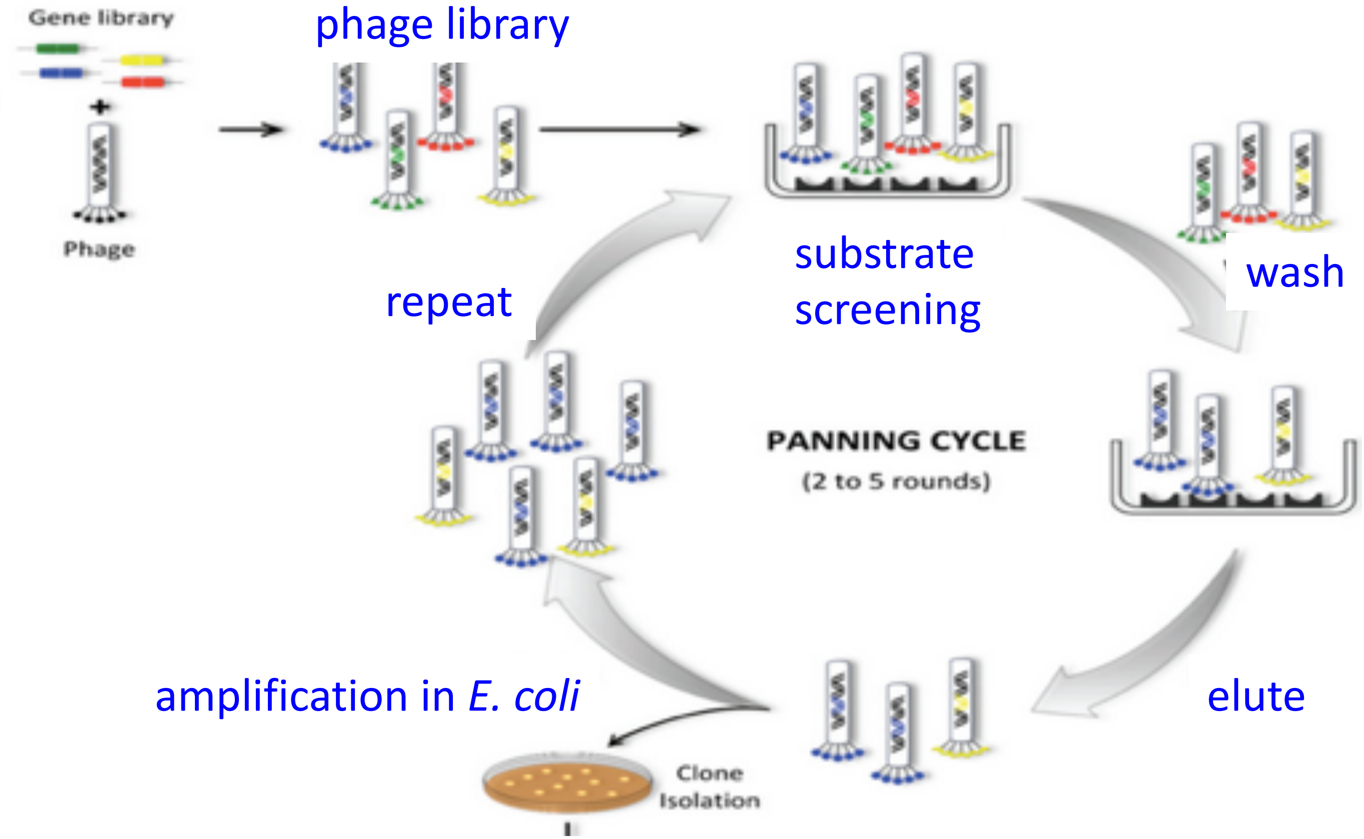
M13 is a nonlytic bacteriophage

(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)

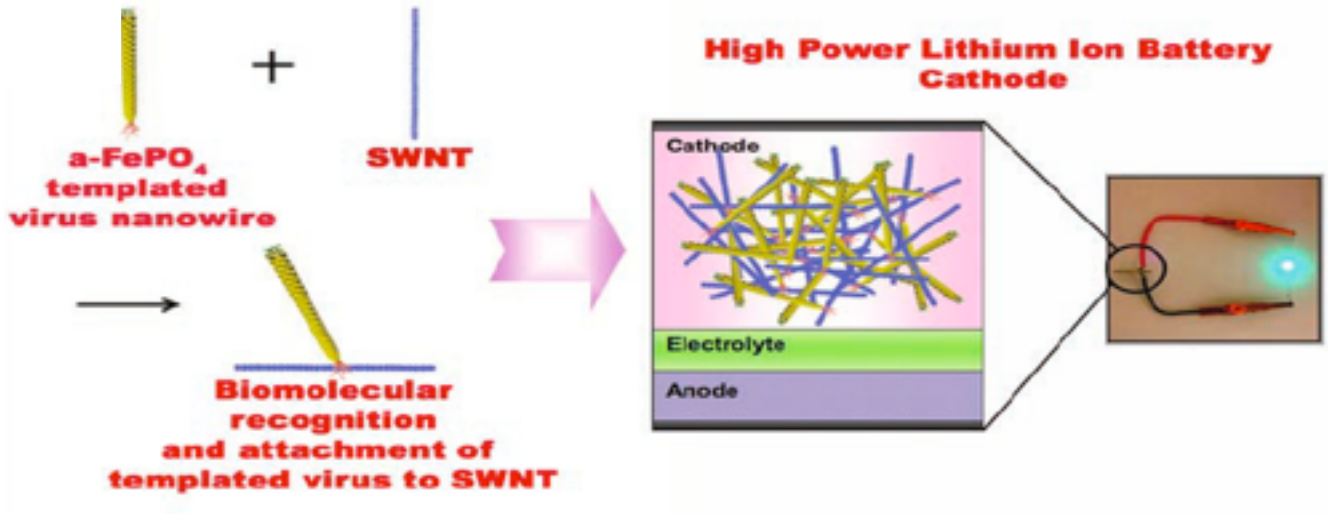


Biomolecules 2015, 5(3), 1783-1809;
doi:10.3390/biom5031783

M13 are engineer-able biomaterials

negatively charged

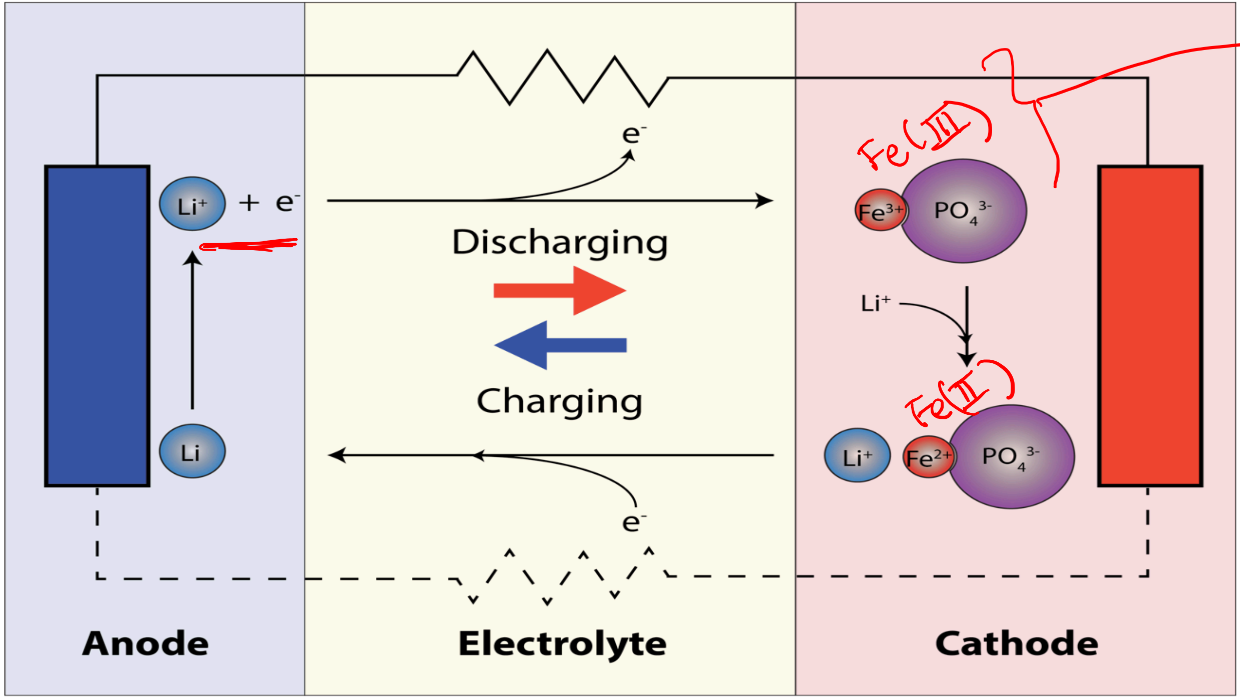
- Our p8 coat protein was mutated to contain sequence DSPHTELP
- Modified p8 proteins bind single wall carbon nanotubes (SWCNT), iron, gold, and other cationic metals
- Example of this virus in literature (Science, 2009):



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

M13 nanowires as battery cathode

bound to phage



oxidation

reduction

Cathode needs to be a good conductor of:

- <u>ions</u>
- <u>electrons</u>

Image: George Sun

You will choose an experimental condition—quantity of gold nanoparticles

- Control made by instructors: no gold (0 AuNP/phage)
- Experimental: choice of quantities
  - Size: 4 nm AuNP
  - Quantity of AuNPs: _______ AuNP/phage (≤40 AuNP/phage)
  - Constraint: up to 50 mL total volume (phage + NPs) per flask
- Make two flasks of experimental condition

Considerations for experimental battery: nanoparticle material and size

- Total volume of gold
  - Gold is conductive
  - Surface of gold may be beneficial if Au has a catalytic function (Au may facilitate intercalation of Li^+ in $FePO_4$ cathode)
  - But too much gold may act as anode
- Phage surface area available for Au and Fe binding
  - Too many AuNPs may reduce # binding sites for $FePO_4$

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

Make two flasks of the experimental condition

Control—

1 flask made by instructors

1) 4e13 Phage

\+ 2) Water

Final volume 50 mL

Experimental—

2 flasks made by your team

1) 4e13 Phage/flask

2) 4 nm Au NPs

(_______NPs/phage)

\+ 3) Water

Final volume 50 mL/ flask

Today in lab

Spend time to think about/read papers for research proposals

1. Finish phage purification
2. Calculate phage number
3. Begin construction of phage-NP-$FePO_4$ nanowires (2 flasks, one per battery)
   - **Choose Au NP quantity (≤40 NP/phage)**

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