Bio-templated $FePO_4$ Synthesis Protocol:

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$FePO_4$ Biomineralization**:**

1. **Fe^{+3}-Phage incubation:**
   i. Mix 100 mL of 1mM $(NH_4)_2Fe(SO_4)_2$ with 30 mL of 2×10^8 pfu/µl phage in deionized (DI) water.
   ii. Incubate mixture overnight (~10 – 12 hours) in cold room while on a stir plate

2. **Reaction:**
   i. Add 100 mL of 1 mM Na:PO_4 pH 7.5
   ii. Again, react in cold room (~10 – 12 hours) while on a stir plate
   Solution will turn murky green

   Note: Li:PO_4 or Na:PO_4 can be used as the source of PO_4^{3-}.
   It is easier to pH a 5 mM Na:PO_4 stock, and pH with HCl to 7.5.
   Alternatively, use: http://clymer.altervista.org/buffers/phos2.html calculator

3. **Washing and Collecting:**
   i. Collect the active material using centrifugation. Centrifuge the material at > 5000 x g for 20 min.
   ii. Discard the supernatant and wash the material with ddH_2O. Repeat centrifugation an additional 2 times.

4. **Preparing TEM samples:**
   i. Take a 10 μL pipette and stab the pelleted material. Dilute this material in 10 uL of ddH_2O in a PCR tube.
   ii. Place all 10 μL solution into a TEM Cu-Carbon Fomvar Grid.

iii. Let the sample sit overnight to evaporate the water and settle the material onto the grid.

5. **Drying:**
   i. Dry the collected $FePO_4$ in a vacuum oven for 24 hours at ~80 ^{o}C.

Construct the (cathode) electrode:

1. **Weigh out dried material before constructing the electrode***

2. **Collect the dried material:**
   i. For the remainder of this protocol, the collected material will be referred to as the *active material.*
   ii. As personal preference, fold a piece of weighing paper to form a triangle and cut off all three corners for better handling.
   iii. A typical weight > 20 mg is sufficient to construct 2 – 3 electrodes.

3. **Weigh out electrode material:**
   The electrode will consist of (by weight):
   i. 70% of active material
   ii. 25% of SuperP carbon powder. SuperP increases the conductivity of the electrode when constructing the final battery
   iii. 5% of Teflon 8A (aka *binder*), a fluoropolymer used to bind the carbon powder with the active material.

4. **Crush materials in pestle and mortar:**
   i. Liberally crush the active material, SuperP, and binder together while using a small spatula to push edge material to the center of the mortar.
   ii. The final homogenized material is referred to as the *electrode*.

5. **Roll out & weigh thin film electrode:**
   i. Place a stainless steel slab on a cloth or towel.

ii. Liberally roll out the electrode onto the flat stainless steel slab. The material should slowly condense to a thin glossy film with a thickness of < 1 mm.

iii. Punch out a disk using a 3/8 hole punch.

iv. Weigh the electrode.
A weight of < 5 mg is sufficient. As thicker electrodes will impede electron transfer and promote electron-hole recombination (more to discuss during lab section).

6. **Vacuum dry electrode overnight:**
   i. @ 80 ^{o}C

Battery Construction:

1. **Overview of materials:**
   i. *Large cathode case* – cathode (active material) battery contact
   ii. *Spacer* – Avoids direct contact with active material and probe (when measuring battery performance). In addition, the spacer adds additional space allowing for proper "sandwiching" of subsequent layers.
   iii. *Separator* – A thin semipermeable membrane separating the cathode and anode from touching, i.e. prevents shorting.
   iv. *Electrolyte (EC/DMC $LiPF_6$)* – A concentrated electrolyte solution used to transfer electrons and ions between the cathode and anode through the separator.
   v. *Lithium foil (anode)* – Anode material
   vi. *Small anode case* + *washer* – anode (lithium) battery contact

2. **Purge vacuum inlet and move materials into vacuum chamber**:
   i. Materials that need to be transported into the vacuum chamber are:
      i. Gloves
      ii. Active material in glass vial

iii. Container to place disposable items (do not dispose of lithium foil!)

ii. To further prevent moisture accumulation, heat the active material on a hot plate @ 80 ^{o}C.

3. **Assembly:**
   i. Place the large cathode case facing down on a plastic sheet for support.
   ii. Add the spacer onto the cathode case.
   iii. Gently center the active material on top of the spacer.
   iv. Place a sheet of separator on top of the active material.
   v. Add 50 – 80 μL of electrolyte and soak the separator.
   vi. Repeat steps iv. and v.
   vii. On the side, punch out a 9/16'' sheet of lithium foil. Place the excess in the lithium waste beaker
   viii. Gently center and place the lithium foil on top of the separator.
   ix. Finally, add a washer to the rim of the anode casing, and gently center the anode casing on top of the assembled battery stack.
   x. With tweezers, carefully transfer the assembled battery stack to the crimper.
   xi. Crimp the battery.
   xii. Finally, check for any shortage by measuring the voltage of the battery with the multimeter. Voltages between 2.0 – 4.5 V are OK.

Battery Measurement:

1. **Introduction to Galvnostatic Testing**:
   i. Brief overview can be found here: http://www.ecochemie.nl/download/Applicationnotes/Autolab_Application_Note_BAT02.pdf
   ii. We will apply a constant charging and discharging current on the battery to measure its capacity.

iii. By definition, capacity is the ability to store charge, and is measured in coulombs (the charge of a single electron)

Internally, the battery is undergoing

Half-Cell Reaction

$$\mathrm{Fe(III)PO_4} + \mathrm{Li} + e^- \leftrightarrow \mathrm{LiFe(II)PO_4}$$

The theoretical capacity of $\mathrm{LiFe(II)PO_4}$ is ~178 [mAh/g]. More information can be found here:
https://en.wikipedia.org/wiki/Lithium_iron_phosphate

iv. However, like most experimental results, the actual measured value will likely deviate from the theoretical value. To measure our battery's capacity, we will run multiple charging and discharging events to capture how much charge (electrons, i.e. coulombs) can be stored in our battery. This method is called *Constant Current Galvanostatic* testing, as we will apply a constant charge and discharging current into our battery to measure accumulation and removal of charge.

2. Back of the envelope calculations:

i. In order to optimize the battery's performance measurement, we first have to determine how long we would like to charge and discharge the battery, and at what current.

ii. Current is measured as coulombs per time, or the rate of charge movement. The *Ampere* (A), units of coulombs per second, are the SI unit for current. Therefore we can perform dimensional analysis to determine the optimal current knowing the theoretical capacity of $\mathrm{LiFe(II)PO_4}$.

$$\text{Theoretical Capacity} \approx 178 \left[\frac{mAh}{g}\right]$$

$$\text{Current per Hour} = \text{Theoretical Capacity} \times \text{Active Material Weight } [g]$$

We would like to perform each cycle in N hours

$$\text{Current} = 178 \cdot X\ [mA\cancel{h}]\ /\ N\ [\cancel{h}]$$

iii. For our experiment, we will have a cycle length of 5 hours (N = 5). In jargon, this is referred as *C* over 5, or capacitance in 5 hours.
X is the adjusted weight of the active material measured in step 5 of $FePO_4$ mineralization on your phage*. To be more precise, considering the estimated percent composition of phage, *X adjusted* should be

$$X^* = X \times 90\%$$

Assuming 10% of the mass is contributed by the phage

3. Measurement setup:

i. Note:
The convention for wiring electrodes is
Red = Cathode (+)
Black = Anode (–)
The cathode casing is the layer nearest the active material during battery construction, i.e. the large end of the battery.
The anode casing is the layer nearest the lithium foil during battery construction, i.e. the smaller end of the battery.

ii. Hook up the battery into the CellTest Battery Measurement System in the Belcher Lab

iii. Create a new method with the following parameters:

1. *Wait 1*
   - 5 hours – to equilibrate battery
2. *Discharge*
   - Input the calculated current with a negative sign to signify discharge step.

3. *Wait 2*
    - 1 min – to re-equilibrate battery
4. *Charge*
    - Input the calculated current with a positive sign to signify charge step.
5. *Wait 3*
    - 1 min – to re-equilibrate battery
6. Cycle back to 2 # number of times

Run & Analysis:

A thorough description will be held during lab section

Material	Per Group	Purpose	20.109 Lab	Belcher Lab
LB	1 Liter	E.coli growth	X	
Tetracycline	> 1 g	E.coli growth		X
M13 DPSH Phage Stock	Shared	Phage purification		X
20% PEG-8000	> 20 g	Phage purification		X
2.5 M NaCl	> 16 g	Phage purification		X
10X TBS	100 mL	Phage purification		X
Oakridge tubes	3	Phage purification		X
15 mL and 50 mL conical tubes	> 4	Phage purification	X	
250 mL Erlenmeyer flask	2	Active material synthesis	X	
Magnetic stir bar	1	Active material synthesis	X	
Ammonium Iron (II) Sulfate hexahydrate	~ 1 g	Active material synthesis	X	
Na_2HPO_4	~ 1 g	Active material synthesis		X
NaH_2PO_4	~ 1 g	Active material synthesis		X
pH paper	> 5	Active material synthesis	X	
Stainless steel roller & plate	1 each	Electrode construction	X	
SuperP Carbon powder	~ 50 mg	Electrode construction		X
Binder Flouropolymer Teflon 8A	~ 20 mg	Electrode construction		X
Pestle and Mortar	1	Electrode construction	X	
3/8'' Puncher	1 – Shared	Electrode construction		X
20 mL glass vials	> 1	Electrode construction		X
TEM Cu Grids	1	TEM		X
PCR tube	2	TEM	X	
Cathode casing	1	Battery construction		X
Spacer	1	Battery construction		X
Separator	2	Battery		X

		construction		
EC/DMC $LiPF_6$ electrolyte	~ 200 μL	Battery construction		**X**
Lithium foil	1'' x 1'' sheet	Battery construction		**X**
9/16'' Puncher	1 – Shared	Battery construction		**X**
Washer	1	Battery construction		**X**
Anode casing	1	Battery construction		**X**

Reservations

- *TEM: Dec 1st – 4th from 2 pm – 6 pm (reservations can only be made at most 12 days in advance)*
- *Galvnostat: Dec 2nd – 4th entire day*
- *Professor Belcher: November 17th & 18th or 19th & 20th*

November 11th (Wednesday, or a week prior):
Phage Growth

- *Setup infected cultures for labs tomorrow*
- *~ 100 mL of infected culture per group*

November 12th & 13th (Thursday & Friday):
Phage Purification
Location: 56-322

- Purify phage
- Quantify phage
- Outline following days of experiments
    - Calculate quantities of material to use
    - Calculate theoretical yield
    - Summarize things to expect, and potential modes of failure

November 17th & 18th (Tuesday & Wednesday):
Fe(III)-Phage Biomineralization (Part I)
Location: 56-322

- Weight out and prepare all materials required for synthesis
    - Fe^{3+} source
    - Phosphate source
    - Appropriate volume and concentration of phage
    - pH solutions
- Fe^{3+} - Phage incubation
    - Prepare 2 batches,
        - 1 mL incubation reaction for demonstration purposes*
        - 100 mL for subsequent reactions
- Guest appearance by Angela Belcher *(option 1)*

*November 18th and 19th (Wednesday and Thursday)**:
Fe(III)-Phage Biomineralization (Part II)

- *Add phosphate source to student's incubation reactions in order to start $FePO_4$ – phage synthesis. This will be used tomorrow for purification and cleaning*

November 19th and 20th (Thursday & Friday):

Purify Active Material
Location: 56-322 → 76-589

- Visualize $FePO_4$ – phage reaction (which was done by the TA a day prior) on the small batch Fe^{3+} - phage incubation*.
  This is for visualization purposes only
- Collect and wash material from the larger batch reaction (which was gone to completion a day prior, overnight)
- Spot a small portion of the material, dissolve in water and add to TEM fomvar Cu-grid
- Dry material in 80^{o}C vacuum oven.
- Guest appearance by Angela Belcher *(option 2)*

Thanksgiving Break:

- *Remove dried material and store in 4^{o}C under low hydroscopic conditions*

December 1st and 2nd (Tuesday and Wednesday):
Cathode Construction + TEM
Location: 76-589 → Bldg. 13

- Collect dried material and weigh
- Make electrode material
- Dry electrode material
- TEM Day 1

December 2nd and 3rd (Wednesday and Thursday):
Battery Assembly & Galvanostat Measurement

- *Assemble batteries for the group*
- *Immediately setup galvanostat runs on assembled batteries at 5C (or 10C depending on timing and cycle #).*
- *Leave an unmade battery aside for demo purposes***

December 3rd and 4th (Thursday & Friday):
Battery Assembly & Galvanostat Demo + TEM
Location: 76-589 → Bldg. 13

- Demo assembly of battery**
- Test battery performance on galvanostat
- TEM Day 2
- With remaining time, analyze galvanostat data and submit biomaterials report