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BACTERIOPHAGE MULTIFUNCTIONAL POLYPEPTIDES AS BIO-TETHERING FOR IMPROVED LITHIUM-ION BATTERY FUNCTION

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ABSTRACT

High internal resistance acts as a major barrier in the development of **safe Lithium-ion batteries** which offer both **high capacity and voltage** from their battery cathodes. Linking of cathode nanoparticles with single wall carbon nanotubes through **bifuctional peptide nanobridges** may allow for **faster charge/discharge speeds, decreased internal resistance and higher cyclability. Phage display** is a combinatorial approach that utilizes **M13 bacteriophage** that have been engineered to express a random 12 amino acid sequence on one end of each virus. These random sequences can be exposed to inorganic materials like **lithium ion battery cathode materials.** If there is some **specific interaction** between the phage and the inorganic material, then the polypeptides responsible for that interaction can be identified through a process of artificial selection. **Polypeptides** isolated from M13 bacteriophage Phage Display serve as a useful component in a **'biological toolbox'**, functioning to **bio-tethering electrodes** to other components inside a battery cathode such as **conducting carbon nanotubes.** In this project, M13 bacteriophages which bind to various battery components will be identified by Phage Display bio-panning and their performance will be investigated in Li-ion coin cells.

INTRODUCTION

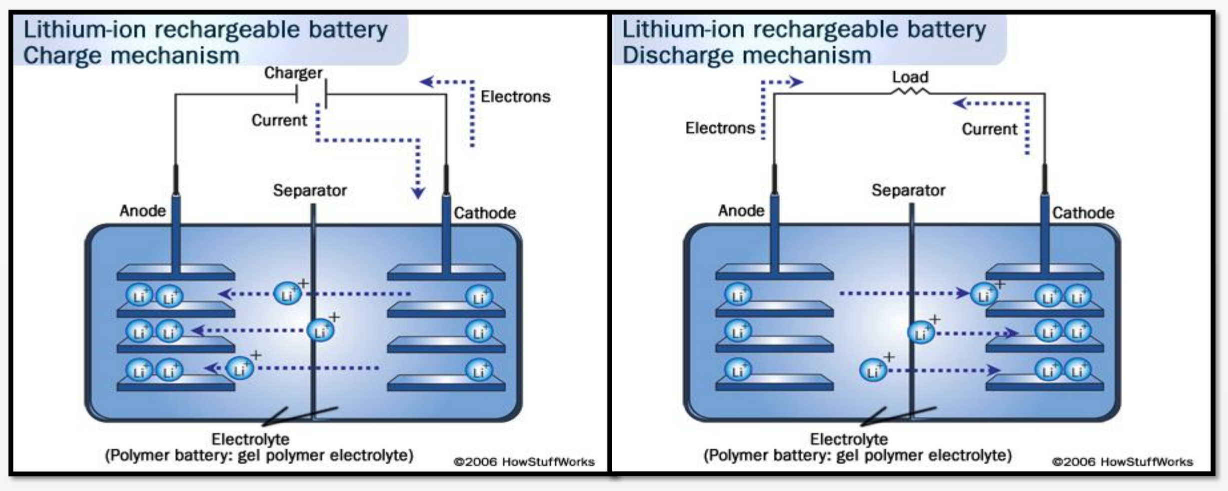


Figure 3: Lithium-ion Battery Operation
During charging Li-ions move from the cathode through the electrolyte to the anode, when discharging Li-ions move from the anode to the cathode. Presence of positive Li-ions induces electron flow - Marshall Brain "How Lithium-ion Batteries Work" 14 November 2006.

- **Li-ion batteries composed of a lithium containing cathode, anode (usually graphite), a liquid or polymer electrolyte and separator**
- **Their benefits include their relatively high capacity, cyclability (charge/discharge cycles) and voltage compared to other battery technologies**
- **Typical commercial 4-V Li-ion rechargeable batteries utilize oxides such as $LiCoO_2$ and $LiNiO_2$ and $LiMn_2O_4$ as their electroactive cathode material**
- **Li-ion batteries suffer from their own limitations such as their high cost (800 to 300 $ kWh-1, as opposed to about 50–100 $ kWh-1 for lead-acid battery technology[3]**
- **Li-ion batteries take up around 63% of worldwide sales for portable batteries[2]**

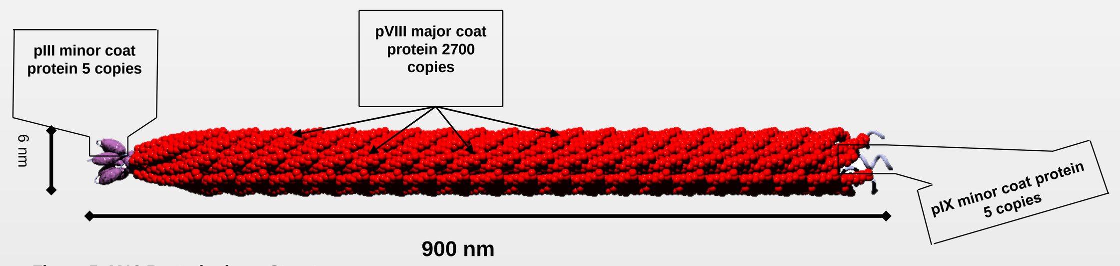


Figure 5: M13 Bacteriophage Structure
Structure of M13 phage indicating proteins of interest - Mark Allen Ph.D *A Biological Toolbox: Making a Connection*

- **M13 Bacteriophage are viruses that only infect E.coli and utilize the host cells internal processes to reproduce.**
- **'Biological Toolboxes' refers to taking advantage of normal biological processes and entities and using them in outside scientific applications as tools**
- **Bacteriophage are a useful tool for 'biological toolboxes' due to the many forms and structures they can take on and their numbers which dwarf bacteria.**
- **pVIII major coat proteins can be modified to bind to and synthesize metal oxides and phosphates**
- **pIII and pIX Minor coat proteins can be modified to bind to single wall carbon nanotubes**

MOTIVATION

Li-ion Battery Limitations

- **Internal resistance is an issue with many types of battery technologies**
- **Internal resistance results in heating during charging and discharging**
- **Battery heating slows charging speed, so if internal resistance could be reduced it would be possible to make batteries capable of faster charging**
- **Heating results in another limitation with regards to Li-ion batteries whish is safety**
- **Li-ion batteries are composed of the highly reactive metal Lithium which along with many of the used electrolytes are flammable**

Figure 4: SAMSUNG Li-ion battery explosion
Li-ion battery ignition can cause major damage to both property and those in the vicinity - (Image: SWNS) http://www.mirror.co.uk/news/uk-news/mums-samsung-phone-explodes-bursts-7575275

- **Improving cathode materials present the best option for increasing the performance of Li-ion batteries**
- **Certain desirable cathode materials sufferer from limitations such as thermodynamics and high internal-resitance**
- **If these cathode materials could effectively and safely be used li-ion battery capacity would be greatly increased and make more ecofriendly applications such as solar power and electric vehicles more viable**

Theoretically Increasing Performance ↓

Material	Potential (V)	Specific capacity (Ah/kg)	Energy (Wh/kg)
$LiCoO_2$	3.9	130	507[1]
$LiMn_2O_4$	3.95	148	585[1]
FeF_3	2.74	712	1951[2]
BiF_3	3.13	302	945[2]
MnF_3	2.65	719	1905[2]
CuF_2	3.55	528	1874[2]
$LiFePO_4$	3.4	170	578[3]
$LiMnPO_4$	4.1	171	701[3]
$LiCoPO_4$	4.8	167	802[3]
$LiNiPO_4$	5.1	167	852[3]
Li_2FeSiO_4	3.3	328	1082
Li_2MnSiO_4	4.0	333	1332
Li_2CoSiO_4	4.3	325	1397
Li_2NiSiO_4	4.7	325.5	1530

Cathode material of interest to us in this study ← ($LiMnPO_4$)

Figure 4: List of possible some cathode materials
Cathode materials with high voltages, capacities and energy densities in theory present the best possibility for Li-ion batteries in theory. These materials are however limited by their high internal resistance and other factors. - Mark Allen Ph.D *A Biological Toolbox: Making a Connection*

OBJECTIVE

Bio-Tethering

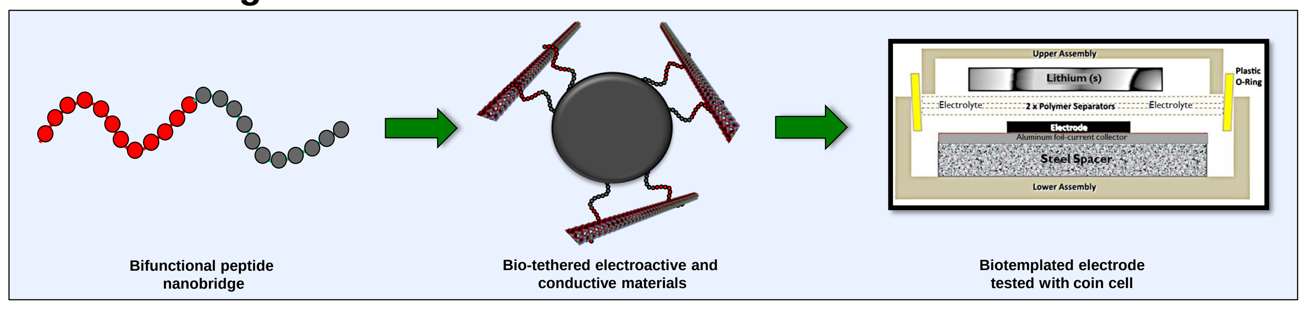
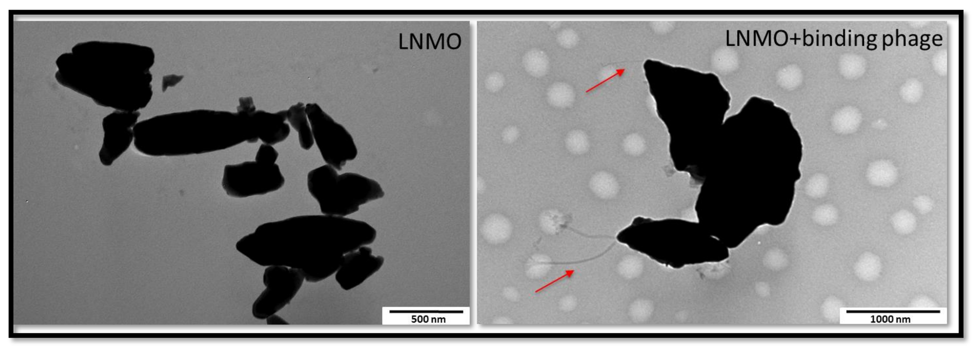


Figure 6: Bio-tethering cathodes
Electroactive material Specific Binding Polypeptides isolated from phage joined to mono-wall carbon nanotube SBP to form a bifuctional nanobridge to connect cathode material and carbon nanotubes to improve electron flow- Mark Allen Ph.D *A Biological Toolbox: Making a Connection*

Apply a biological toolboxing approach to identify polypeptide sequences with specific binding with battery materials and test their efficacy in improving battery performance and technology



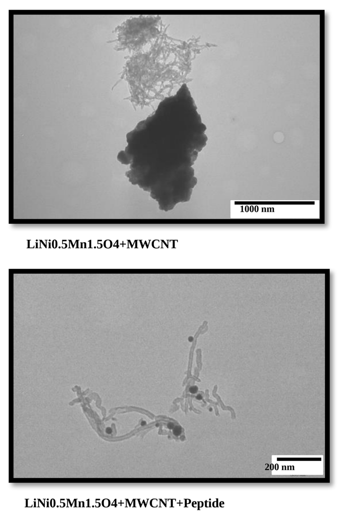


Figure 7: Prior Allen Lab work Bio-tethering cathode materials
TEM images of $LiMn_{1.5}Ni_{0.5}O_4$ from Ph.D. student Evgenia Barannikova shows successful binding of phage with single wall carbon nanotubes. Electrochemical Impedance Spectroscopy verifies that the internal resistance is decreased in cathodes that include both carbon nanotubes and the bifunctional polypeptides.

Electrode composition	R_{ct} , Ω
LNMO	570
LNMO+CNT	391
LNMO+CNT+Peptide	251

METHODS

Materials

- ER 2738 Strain E.coli
- M13 Phage Library
- LB Media
- LB Agarose Top
- LB Plate Agar
- Yeast Extract
- NaCl
- Bacto-tryptone
- Agarose
- Tetracycline
- IPTG (for induction)
- X-GAL
- TWEEN 20
- $Mn(NO_3)_2 \cdot 4H_2O$ (Merck, 98.5%)
- H_3PO_4 (Baker, 85%)
- $LiNO_3$ (Alfa Aesar, 99%)
- $LiOH \cdot H_2O$ (sigma Aldrich, 99%)
- Tris-buffered Saline (TBS)
- Polyethylene Glycol/NaCl (PEG/NaCl)
- Zymo Plasmid Isolation Kit
- Milli-Q Deionized water
- Whatman® Cellulose Paper
- Coin cell Stainless steel spacers
- Aluminum tape (EM Sciences)
- Polypropylene Film (Celgard)

Phage display

- Material of interest exposed to M13 Bacteriophage library. Non/weakly binding phage removed by progressive washing with 0.2, 0.4, 0.6% Tween 20 TBS (Tris-buffered Saline) solution
- 0.8% Tween 20 TBS eluent and pH 2.2 buffered solution eluent collected
- Eluents amplified with ER 2738 E.coli and biopanning repeated at least 3 times
- Amplified phage isolated, DNA isolated with Zymo Plasmid Isolation Kit and sent to Genewiz for Sanger sequencing

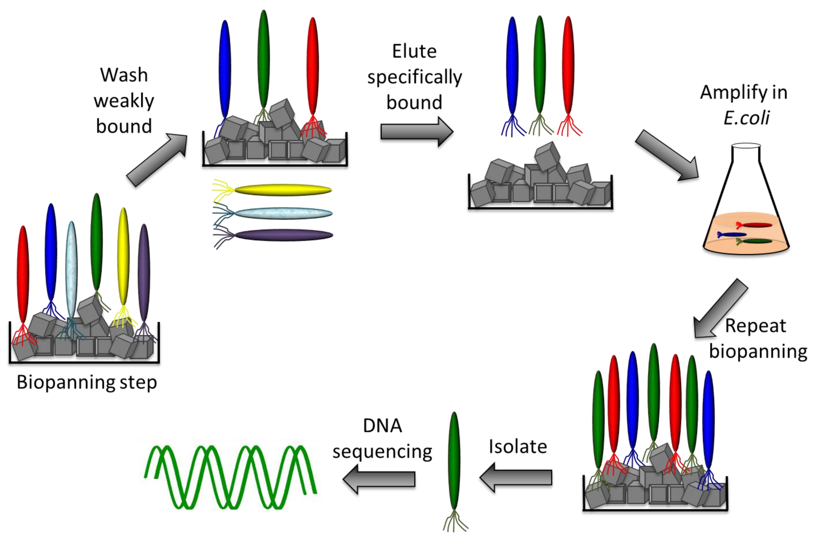


Figure 9: Phage display Process
Sequence of bio-panning and amplification in Phage display - Ph.D.-12TM Phage Display Library Kit, New England Biolabs, Inc.

Titering

- Phage present in eluents were quantified by titering at each biopanning step.
- 200μL of ER 2738 E. coli culture in LB media exposed to 10μL of 1x, 100x and 10000x dilutions of Phage display eluent, suspended in LB Agarose Top and plated
- Plates allowed to grow to 1 day at 37° C, blue plaques indicating infected E.coli due to the presence of X-Gal are then counted
- Round 3 Phage Display titering plaques extracted and DNA isolated

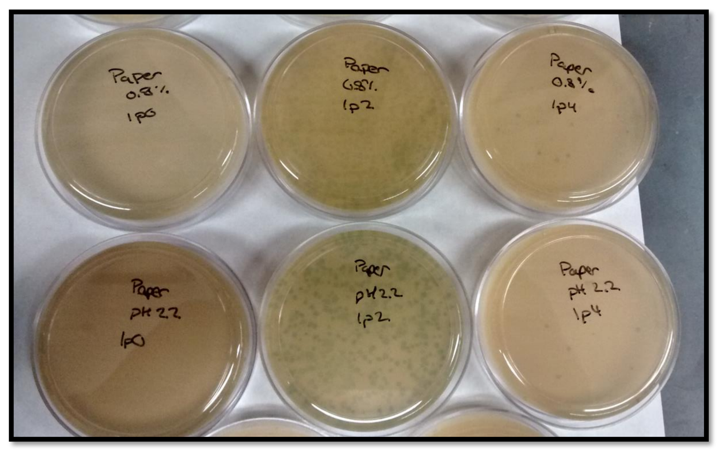


Figure 14: Results of Round 1 Titering
Titering to quantify amount of phage. Infected E.coli indicated by blue plaques from presence of X-GAL

Synthesis

- Electroactive cathode material $LiMnPO_4$ was synthesized by published methods[3].
- Aqueous solutions of 1 M Mn(II) and 1 M phosphate with excess Li^+ ions brought to pH 10.7 at room temp using 1 M LiOH
- Aged in reflux for 5 days
- Brown precipitate recovered by subsequent centrifugation and progressive washing with deionized Milli-Q water and lastly acetone. Precipitate was then allowed to dry for 1 day at 55ºC

Figure 12: Current lab setup to replicate LiMnPO4 synthesis
LEFT: a chime RT synthesis of $LiMnPO_4$ Right: Reflux apparatus at 100ºC

RESULTS

Specific Binding Polypeptide Sequences Identified by Phage Display	
Whatman® Cellulose Paper	Stop N V L V K Q Y D L A R
	H K Y I Q G P F Q L E R
Polypropylene Separator	H Y V Met Y P S F P I S Q
	D P F F L Met T P Q S N F
Stainless Steel Spacer	A P A V H V V Q T G Q P
	G A N S P V S T A K N K
Aluminum Tape	G Q S I G S T N F T E P
	F Y S H S A E T V E S

Figure 14: 12 Amino Acid Specific Binding Polypeptide sequences
Specific binding polypeptide sequences from M13 Bacteriophage minor coat protein PIII from phage identified by Phage Display

Table shows translated DNA sequence from Genewiz
Amino Acid sequence translated with web.expasy.org/translate/ at 3'5' Frame 2

- **pIII minor coat protein code initiated by amino acid sequence FYSHS**
- **pIII minor coat protein ends with amino acid sequence AETVES**
- **M13 Wild-type sequence given by FYSHSAETVES**
- **M13 Modified sequence given by FYSHS – 12 amino acid insert – GGG – AETVES**

FYSHS, GGG, and AETVES amino acid sequence removed for ease of viewing

- **Second Aluminum polypeptide sequence matches Wild-type**
- **Other sequences appear specific based on comparison to prior phage display sequences on other materials completed in the Allen lab**

- **$LiMnPO_4$ Synthesis resulted in 0.0007g of recovered precipitate material**
- **Material recovered not enough to create an functioning coin cell that can be analyzed**

CONCLUSION / FUTURE DIRECTION

- Phage display has shown that there exists certain bacteriophage with strong binding behavior with steel, aluminum, polypropylene, and cellulose paper and are not wild-type M13 bacteriophage

This project is still ongoing

- Further testing is needed to confirm the specific binding of the identified amino acid sequences.
- Next step is to complete DNA sequencing of more specific binding phage plaques to identify more specific binding polypeptide sequences
- Restart synthesis of $LiMnPO_4$ with higher concentrations. 10x the prior concentration will be used
- Begin phage display of $LiMnPO_4$ and identify its specific binding polypeptide sequences
- Utilize Transmission Electron Microscopy to view material interactions with carbon nanotubes and bacteriophage specific binding polypeptides
- Synthesize specific binding phage sequences as bifunctional polypeptides
- Analyze $LiMnPO_4$ performance in coin cell with carbon nanotubes and with identified specific binding polypeptides

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