Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

I. Project Title

Marine Virus Isolation for Use in Phage Templating of Nanoparticles

II. Abstract

Here we propose to use a bio-panning technique to isolate unharnessed and potentially yet-unidentified viruses that may be able to play a pivotal role in the novel field of phage-templating of nanoparticles. Applications for phage templating currently include solar cells and fuel cells optimized using nanoparticles, environmental sensor arrays, diagnostic tools, and nano-self-assembly procedures for very small electronics among others. The most pressing bottleneck in making use of developments from the rapidly growing nanoscience field is in the difficulty and tediousness in assembly of nanoparticles at scales as small as a billionth of a meter. A relatively new approach to this problem made use of a laboratory modified filamentous m13 phage to bind and organize carbon nanotubes and titanium dioxide nanoparticles in dye synthesized solar cells. This method helped manage otherwise difficult to manipulate nanotubes by noncovalently binding them to the phage and assembling them into easier to manage units. However, this approach is limited in scope by the need to genetically engineer phage. Our goal is to bio-pan marine water samples to isolate specific phage-nanoparticle interactions yielding an unlimited number of viral morphologies. Once isolated, we will utilize a metagenomic approach to genotype these viruses by shotgun sequencing. An online data-bank of these phage-nanoparticle interactions will be provided to the research community, thus enhancing the field of nanoscience.

III. Introduction

Nanoscience has been touted as the scientific field of the future. It has captured the attention of many existing fields from renewable energy to medicine [1,2,3]. The amount of research and resources invested in the field has only grown in the past years, with close to $1.5 billion going into various research as part of the National Nanotechnology Initiative for the year of 2016 [4]. A fundamental problem with the widespread utilization of nanomaterials in products has been with the manipulation and organization of individual nanoparticles. For example, one attempt at modifying existing solar cell technology using nanomaterials was made by introducing carbon nanotubes, known for their uncannily high conductivity, into the layers of the solar cell to aid electron transport from source to sink [5]. This method increased the electron collection and efficiency of the solar cell type from 2.13% to 2.87%, but it was understood that organizing and directionalizing the randomly dispersed nanotubes could lead to even greater increases in efficiency. This was partially achieved by utilizing linear phage to bind carbon nanotubes into parallel bundles on the

nanoparticle-bound phage.

- *Propagating isolated phage*
  Propagation of bio-panned bacteriophage will be achieved by growth in concentrated bacterial pellets, derived from seawater sources and maintained in growth media.
- *Phage identification*
  Bio-panned and selected phage will be identified with a metagenomic approach via shotgun sequencing. Briefly, phage genetic material will be isolated, sheared and fragmented, followed by the insertion into a cloning vector and sequenced with established primers. Every phage readout will be compared with known phage libraries. All phage-nanoparticle interactions will be recorded and available to the research community via an online data-bank.

References:

1. Dong, P. *The Journal of Physical Chemistry.* **2014**. 118, 25863–25868.
2. Sharma, A. *Nature Nanotechnology.* **2015**. Published Online, 1-7.
3. Wei, G. *Advanced Functional Materials.* **2008**. 18, 3568–3582.
4. Roco, M. AAAS National Nanotechnology Investment in the FY 2016 Budget. **2016**. Online.
5. Jorio, A. Springer. **2008**. 1-45.
6. Dang, X. *Nature Nanotechnology.* **2011**. 6, 377
7. Dong, P. *The Journal of Physical Chemistry.* **2014**. 118, 25863–25868.
8. Alharbi, F. H. *Renewable Energy.* **2014**. Preprint.
9. Clockie, MR. *Bacteriophage.* **2011**. 2, 31-45
10. John, SG. *Environmental Microbiology Reports.* **2011**. 3, 195-202
11. Yamamoto, KR. *Virology.* **1970**. 40, 734-744
12. Ferguson, A. *Materials Letters.* **2012**. 90, 115-125

VI. Timeline

Milestone:	Date:
Develop protocol for phage isolation	September 2016
Develop working bio-panning assay	February 2017
Identification of novel phage-nanoparticle interactions	June 2017

VII. Final Products and Dissemination

Given the novel approach of this proposal, we envision the following outcomes from this project:

1) Published data in a peer reviewed journal such as: *Advanced Materials* or *Bioconjugate Chemistry*
2) An online data-bank with identified phage-nanoparticle interactions will be available to the research community via an online data-bank.
3) We expect to present our work at international nanotechnology conferences such as: *International Conference of Nanotechnology, Rome, 2017*
4) We expect to present our work at local undergraduate scientific conferences such as: *COSAM Student Research Conference*
5) Senior Project: topic and data for Pranav Santan & Morgan Smith-Boeck's senior project.

VIII. Budget Justification

1. **Equipment:** The Martinez laboratory has all the necessary equipment for this project.
2. **Materials & Supplies:** Single Walled CNTs (2g_Thermoscientific_$600), Multi-walled CNTs (2g_Thermoscientific_$600), gold nanoparticles (2g_Sigma_$774), Fullerenes C60 (1g_Thermoscientific_$500), Fullerenes C70 (1g_Thermoscientific_$500), PEG 6000 (5Kg_Thermoscientific_$183), $FeCl_3$ (2.5Kg_Thermoscientific_$106), Restriction Enzymes (~$110/each), Cloning Vector (~$200), Sequencing (~$160/assay)
3. **Journal Publication:** We expect our research to be published in a peer-reviewed journal. Based on current publication fees (*Advanced Materials)*, a scientific paper of five pages in length and three colored figures will total ~$879.

surface of the virions on which titanium dioxide nanoparticles and photosensitive dye were deposited [6]. Phage provided two key advantages to the previous method; firstly, groups of nanotubes shared the same orientation from parallel binding on the virion surface minimizing the probability of short-circuited paths and secondly, the titanium dioxide nanoparticles were able to interface directly with the electron highways, the carbon nanotubes, to rapidly transport the electrons to the sink. This approach increased the solar cells' efficiency from 8.3% to 10.6% [7]. The first method increased the efficiency by about 30% of the cell's baseline, whereas the second method increased the efficiency by 27% of the cell's baseline. Considering that increasing the performance for a cell with a higher baseline is more difficult than for one with a lower baseline the increased efficiency in the second method is far more impressive than in the first method. This is because there is a theoretical maximum for simple solar cell performance of around 50%, which is increasingly difficult to reach, as a cell becomes more efficient [8].

Bacteriophages are viruses that specifically infect bacteria. Bacteriophages are most abundant in the ocean, with an estimated concentration of 1 million phage particles per milliliter of water [9]. Given the abundance and diversity of phage in seawater, we propose that a relatively straightforward bio-panning protocol with concentrated seawater phage, against a library of nanoparticles will yield an extraordinary number of phage-nanoparticle interactions. Every interaction may prove to be functional in templating nanoparticles for a variety of nanotechnology applications. .

IV. Objective(s)

- *Isolate and concentrate marine virions from sea water samples*
- *Phage bio-panning against a library of nanoparticles (SWNTs, fullerenes, gold nanoparticles, etc.)*
- *Identification of phage, resulting in the creation of a phage-nanoparticle data bank*

V. Methodology

- *Isolating Marine Virus*

  We will concentrate viruses from seawater samples, obtained at the CalPoly pier, using polyethylene glycol precipitation and iron chloride ($FeCl_3$) flocculation. Several previously published filtering, centrifugation, and precipitation steps will be utilized to clarify, separate, and isolate viruses from the water samples [10,11].
- *Phage bio-panning of nanoparticles*

  A variety of nanoparticles will be tested. Beginning with carbon nanotubes of multi-walled, single-walled, metallic, and semiconducting varieties; fullerenes of C60, C70, and functionalized varieties such as [6,6]-phenyl-C61-butyric acid methyl ester (PCBM) often used in organic solar cell technologies [12]; and quantum dots of various varieties. The particles will be tested in two ways.
  1. *Bio-panning*: Binding of the nanoparticles to a glass substrate using high concentration sulfuric and nitric acids. These slides will be placed in virus solutions and rocked for a period of time. Any bound virions will be dislodged via pH manipulation and propagated.
  2. *Homogenous suspension*: Homogenous solutions of nanoparticles will be suspended in the virus solutions which will then be agitated for some time. The nanoparticles will be removed and any bound virions will be dislodged and propagated. This second method will be ideal for metallic nanotubes and metallic

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PROPOSAL BUDGET

Student Applicant(s):	
Faculty Advisor:	
Project Title:	**Requested Endowment Funding**
Travel *subtotal*	**$0**
Travel: In-state	**$N/A**
Travel: Out-of-state	**$N/A**
Travel: International	**$N/A**
Operating Expenses *subtotal*	**$ 5000**
Non-computer Supplies & Materials	**$4121**
Computer Supplies & Materials	**$**
Software/Software Licenses	**$**
Printing/Duplication	**$**
Postage/Shipping	**$**
Registration	**$**
Membership Dues & Subscriptions	**$**
Multimedia Services	**$**
Advertising	**$**
Journal Publication Costs	**$879**
Contractual Services *subtotal*	**$0**
Contracted Services	**$**
Equipment Rental/Lease Agreements	**$**
Service/Maintenance Agreements	**$**
TOTAL	**$5000**

CAL POLY
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April 22nd, 2016

Re: Letter of Support for Pranav Santan, Morgan Smith-Boeck & Siddarth Prabhu (Baker & Koob Endowment)

Dear Endowment Committee,

With this letter I enthusiastically support Mr. Pranav Santan, Ms. Morgan Smith-Boeck and Mr. Siddarth Prabhu in their pursuit for funding of the proposed project "Marine Virus Isolation for Use in Phage Templating of Nanoparticles". I have had the pleasure of working with each of these students in the laboratory and in the classroom setting, and I have no reservations that they have the knowledge and enthusiasm to achieve the specific aims of this project.

The field of nanotechnology is rapidly developing and it is quite evident that discovering methods for uniquely organizing these particles in very specific 3-dimensional orientations is going to be crucial to the advancement of this field. This project is brilliant in its experimental simplicity (collecting seawater, concentrating phage, biopaning against nanoparticles of interest, identifying the phage and developing an interaction databank), with a very high potential for gleaning valuable information for the scientific community. I am extremely excited to pursue this project with our CalPoly students.

During the course of this proposed project, I will serve as a PI and mentor for these three students. I will ensure that proper techniques are learned and performed, while delivering sound and meaningful scientific data. My laboratory will be at these student's full disposal, as will be my time and input. This is a wonderful project that truly deserves start-up funding.

Please do not hesitate in contacting me should you have any questions regarding Pranav, Morgan & Sid's candidacy for this wonderful award.

Best regards,

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