Synthesis and Toxicity of Lipid-Coated-Titanium Oxide Nanoparticles

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Abstract

Nanoparticles have a broad range of applications in novel materials and consumer products. Due to the unique properties of nanoscale materials, the toxic effects of various nanoparticles are largely unknown. Surface modifications to nanoparticles, such as membrane or lipid coatings, may reduce immunogenicity and environmental toxicity, but these effects remain largely uncharacterized. The synthesis of lipid-coated titanium oxide nanoparticles was optimized and toxicity was evaluated. Thermogravimetric analysis showed that 5 μM of tricarboxylic amphiphile sufficiently generated uniformly coated nanoparticles. Toxicity studies on *Zea mays* (corn) revealed that uncoated titanium oxide nanoparticles exhibited phytotoxicity, while lipid-coated nanoparticles had effects resembling deionized water. Scanning electron microscopy displayed visual evidence of nanoparticle absorption into the corn seedlings in experimental groups.

Keywords: Nanoparticle, titanium, oxide, titania, tricarboxylic, amphiphile, lipid, coating, toxicity, phytotoxicity, nanotechnology, scanning, electron, microscopy

1. Introduction

The development of nanotechnology is rapidly increasing, with an enormous number of applications in material science, consumer products, medical diagnosis and treatment, and drug delivery vehicles.[1] Currently, there are more than 1600 nanotechnology products available for consumer use logged in the Nanotechnology Consumer Products Inventory (CPI).[2] Research and commercial use necessitate disposal, thus, nanoparticles will inevitably be released into the environment. Questions arise as to the safety of such nanoparticles, especially concerning their effects on plant life consumed by humans.[3] Further, even less is known in regards to the effects of functionalized nanoparticles; functionalization of nanoparticles for specific commercial uses or to reduce environmental impact may be induced by surface modification. Thus, this project aims to test the phytotoxicity of coated nanoparticles.

1.1 Coating

Metal oxide (Iron oxide, titanium oxide, etc.) nanoparticles have nearly unlimited potential for research applications.[1] Applications of these nanoparticles range from solar cells to MRI contrast agents and drug delivery vehicles.[3,4] Challenges present themselves in developing reliable methods to uniformly coat metal oxide nanoparticles; the coating offers the advantage of controlling the spacing between the solid particle and the pliable outside surface. Compounds with a tricarboxylic "head" group (Figure 1) can attach to iron oxide nanoparticles.[5] In this manuscript we demonstrate that the same head group, when conjugated to a long-chain lipid (4-(2-Carboxyethyl)-4-(3-octadecyloxycarbonylamino) heptanedioic acid (3CCb18), Figure 1), can attach to titanium oxide nanoparticles (TiNPs). These TiNPs can be suspended in aqueous solution and subsequently used for further experimentation.

1.2 Toxicity

Due to their unique properties such as enhanced catalysis, lowered melting point, and increased conductivity, nanoparticles like TiNPs have potentially harmful effects to many organisms in the global ecosystem and ultimately pose a threat to humans.[1,3] Preliminary studies on the impact of nanoparticles on plants have shown both positive and negative effects. In one study, nano-titanium oxide (TiO_2) promoted the growth of spinach; however, the majority of phytotoxicity studies identified solid metal nanoparticles as toxic.[6] Nano-aluminum oxide inhibits root elongation in corn, cucumber, soybean, cabbage, and carrots, while nano-zinc oxide terminates the growth of various plant species.[7,8]

Although there have been many toxicity experiments conducted with uncoated nanoparticles, there has been little evaluation of functionalized or coated nanoparticles. Studies on the toxicity of nanoparticles and the effects of various coatings will elucidate the dangers of this technology and provide guidance for disposal and efficacious drug design. Phytotoxicity experiments aim to determine the effect of 3CCb18 coated (Figure 2) and uncoated TiNPs on the root development, seed germination, and overall germination index of *Zea mays* (corn). *Z. mays* was chosen based on availability, worldwide consumption, and previously conducted toxicity experiments.[7-9] Toxicity was quantified using the germination index, a simple metric reported in nanotoxicology text that takes into account both total germination and early seedling development.[9]

$$\text{Germination Index} = \frac{\textit{Experimental seeds germinated}}{\textit{Control seeds germinated}} * \frac{\textit{Experimental average root length}}{\textit{Control average root length}} * 100 \quad (1)$$

2. Methods

2.1 Nanoparticle Coating

Optimum modification of the TiO_2 surface was achieved by heating TiNPs (Sigma, 21 nm diameter) in a mixture of chloroform ($CHCl_3$), toluene (PhMe), 3CCb18, and triethylamine. 3CCb18 (5 µM, previously synthesized in lab) was stirred in a 250-mL round bottom flask with PhMe/$CHCl_3$ 1:1 v:v (40 mL). Triethylamine (9 µL) was added to the mixture to neutralize the carboxyls. Next, a suspension of aqueous TiNPs (0.3 mL) was added dropwise, and the mixture was stirred at 80 °C for 24 h. The final mixture had a milky white color with noticeable precipitate at the bottom of the round bottom flask. Isolation of suspended nanoparticles was best achieved by rotary evaporation.

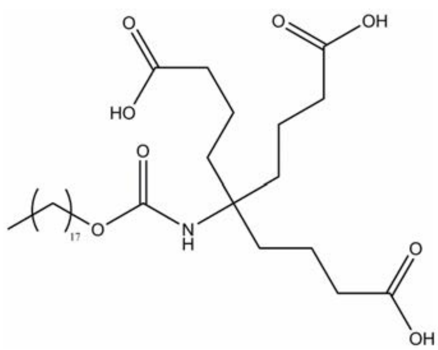

Figure 1: 3CCb18 unit for TiNP surface modification.

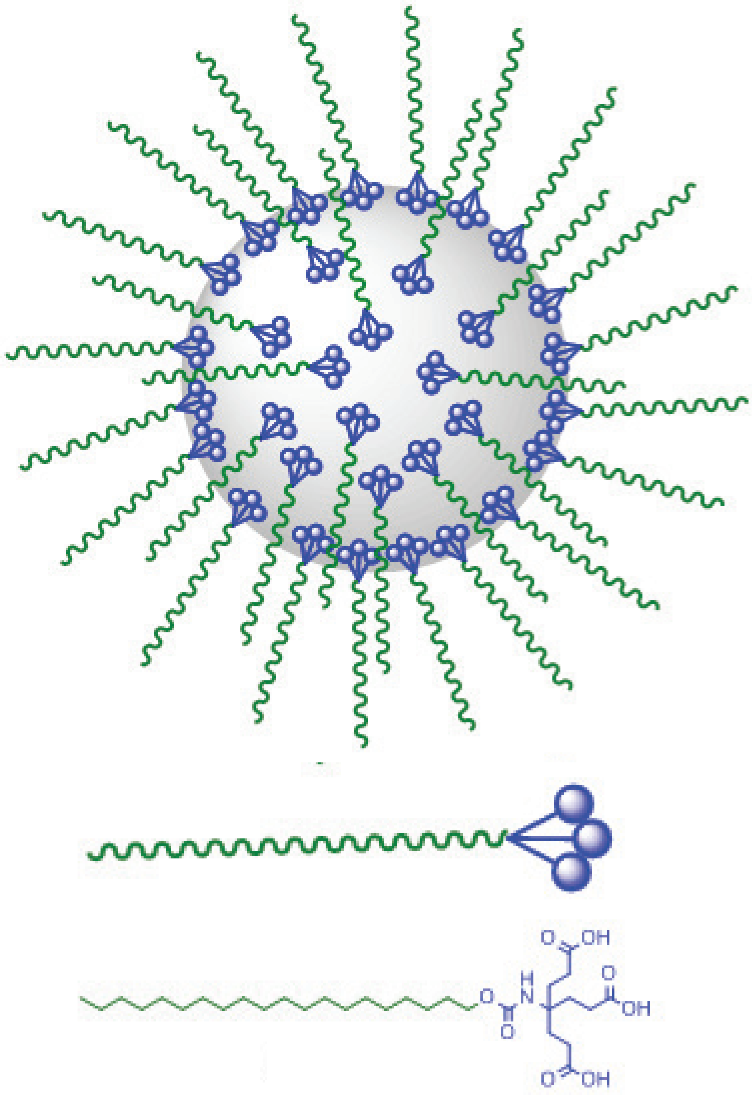

Figure 2: Graphic of 3CCb18-coated-TiNP.

The solvent was removed from the round bottom flask with rotary evaporation, and the nanoparticles were transferred to a small vial and dried under high vacuum for 24 h to give a cream-colored powder.

2.2 Thermogravimetric Analysis

The surface modification of the TiNPs was analyzed with thermogravimetric analysis (TGA) from 0 to 600 °C at 10 °C/min. Samples of TiNP powder, 3CCb18 lipid, and 3CCb18-coated-TiNPs (5-10 mg) were analyzed with a TA Instruments TGA Q500.

2.3 Phytotoxicity

To evaluate toxicity, the growth media of germinating *Z. mays* seeds was varied between three groups: coated and uncoated TiNPs as well as a DI water control. A modified procedure as published previously was followed.[9] Prior to

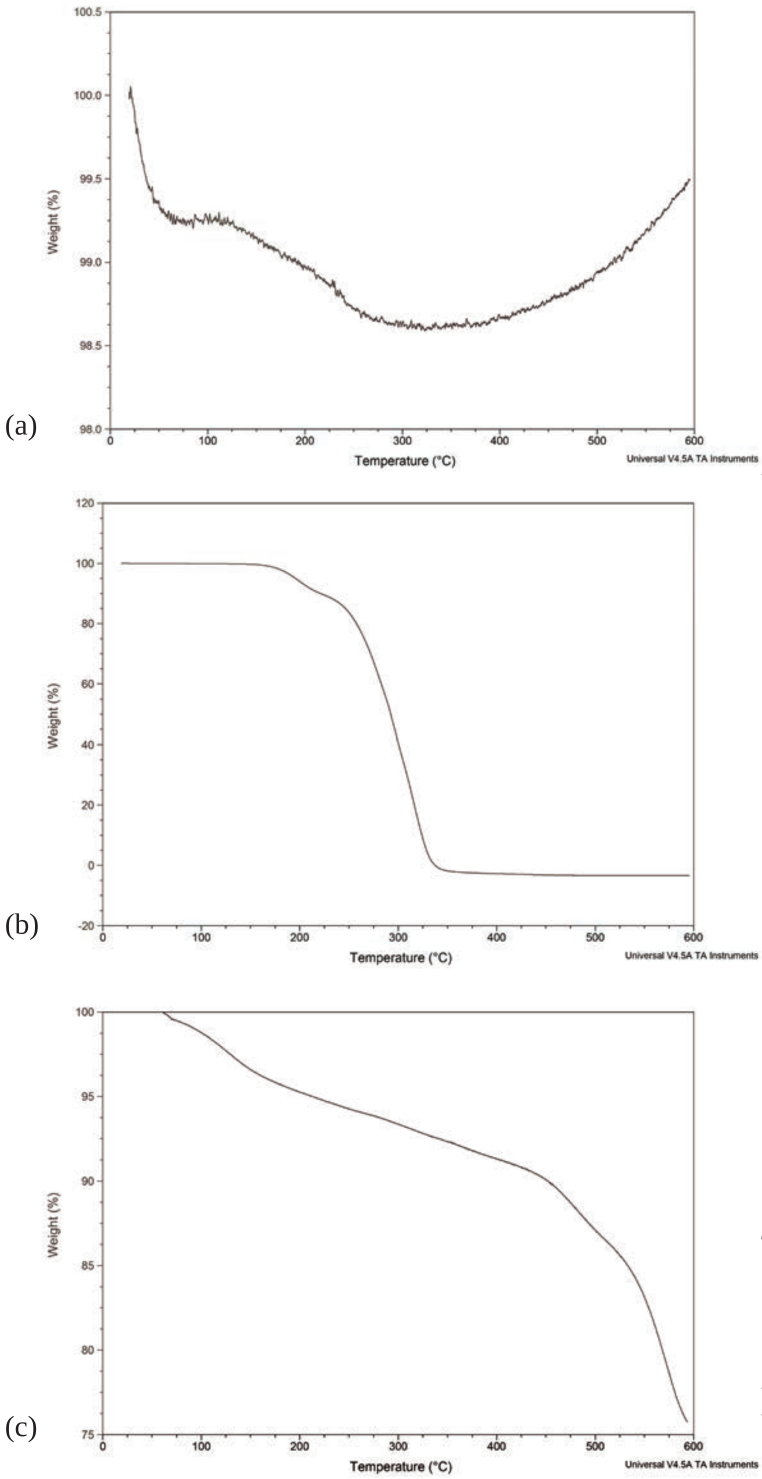

Figure 3: TGA analysis of various TiNPs. (a) Unmodified TiNPs show no significant weight loss. (b) 3CCb18 lipid-coated-TiNPs show 100% weight loss by 350 ^{o}C. (c) 3CCb18-coated-TiNPs show 25% weight loss by the end of the experiment, implying the surface of the nanoparticles was modified.

conducting experimentation, nanoparticle solutions were prepared by adding TiNPs (0.04 g) to deionized (DI) water (20 mL) and sonicating for 1 h. Afterwards, the solutions were diluted further to 100 μg/mL by adding DI water (380 mL) and stirring thoroughly. The viability of the *Z. mays* seeds (Hirt's Gardens, Medina Ohio) was verified by suspending the seeds in DI water and selecting the seeds that settled to the bottom. The seeds were then soaked in 10% sodium hypochlorite solution, which acts as a surface sterilizing agent; after 10 min they were rinsed thoroughly in DI water. After rinsing, the seeds were sorted into three groups of thirty, which would be used for each nanoparticle solution. The three solutions evaluated were DI water (control), 3CCb18-coated-TiNPs, and uncoated TiNPs. Each group consisting of thirty seeds was then placed in solution and stirred for 2 h. Subsequently, nine petri dishes were prepared with Whatman filter and soaked with the corresponding nanoparticle suspension (5 mL) or DI water (5 mL). The seeds were drained, patted dry, and subsequently transferred to the petri dishes containing the filter paper, which were then sealed with parafilm and placed in a dark environment for 4 d. Root length and total germination measurements were taken, and germination index was calculated (Eq. 1).

2.4 Scanning Electron Microscopy

Following procedure *2.3*, several samples were taken at random from each experimental group (DI water control, uncoated TiNP, coated TiNP) and analyzed with Scanning Electron Microscopy (SEM). *Z. mays* seeds were sliced open and sputter coated in Au/Pd 60:40. Samples were loaded into a LEO (Zeiss) 1550 field-emission SEM and electron micrographs were obtained. Visual analysis was used to identify TiNPs embedded in the *Z. mays* seedlings.

3. Results and Discussion

3.1 Coating and Thermogravimetric Analysis

The TGA of TiNPs and 3CCb18 lipid provide controls for comparison with coated TiNPs. TiNPs do not vaporize and lose zero weight percent (Figure 3a), whereas 3CCb18 lipid vaporizes completely at 350 ^{o}C and loses 100% of its weight by the end of the experiment (Figure 3b). With these control TGA experiments, it may be inferred that any lipid conjugated to a TiNP should vaporize by 350 ^{o}C, leaving uncoated TiNPs for the remainder of the experiment. Further TGA experiments were then conducted on coated TiNPs that utilized 5 μM of 3CCb18 during synthesis (Figure 3c); the loss of 7% of the initial mass and retention of 93% of the input weight at 350 ^{o}C shows that lipid molecules successfully coated the TiNPs.

Table 1: Germination rates, average root lengths of the seeds that germinated from each experimental group, and calculated germination index (Eq. 1) for each experimental group.

Group	Seeds Germinated (of 30)	Average Root Length of Germinated Seeds (cm)	Germination Index
Control	27	2.3 ± 1.5	100
TiNPs	23	1.7 ± 1.5	63
Coated TiNPs	21	2.9 ± 1.1	96

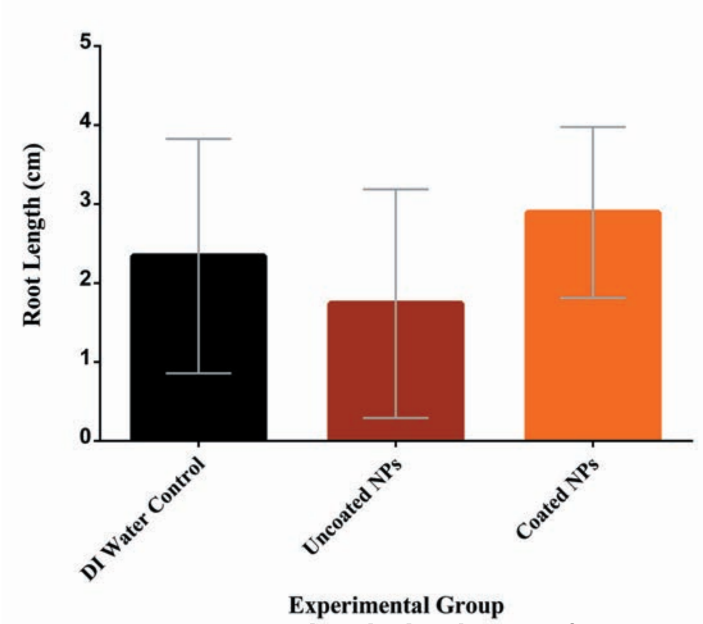

Figure 4: Average root length distribution of DI water, uncoated nanoparticle, and coated nanoparticle-treated experimental groups. Standard errors are displayed with error bars.

3.2 Phytotoxicity Assay

$p < 0.025$ using paired single factor ANOVA analysis. Uncoated nanoparticles had a germination index (Eq. 1) of 63.3 compared to 100 for the DI water control; hence, uncoated TiNPs have detrimental effects on *Z. mays* during early development. According to the literature, concentrations of 100 µg/mL of various nanoparticles begin to show negative effects on root growth and overall germination on different varieties of seeds.[8] The TiO_2 data presents similar results: an uncoated nanoparticle concentration of 100 µg/mL displayed prominent phytotoxicity. Both coated and uncoated nanoparticles showed a reduced percentage of seeds germinated compared to the control; yet, the coated nanoparticles' germination index was very close to 100, comparable to the control's growth rate. These results suggest that coated nanoparticles had little or no phytotoxic effects on seed germination, whereas uncoated nanoparticles exhibited prominent phytotoxicity. Additionally, average root growth was enhanced in coated nanoparticles (Figure 4); this positive effect may be explained by the lipid coating. This coating may have helped hydrophobic molecules absorb more effectively into corn, increasing nutrient uptake and providing for better growth. Alternatively, the lipid may have been hydrolyzed and removed from the coating on the TiNPs, creating a fatty alcohol that provided the *Z. mays* seeds with a source of energy and a component utilized in cell membranes. Experimental results are summarized in Table 1.

3.3 Microscopy

SEM micrographs show the presence of TiNPs in both coated and uncoated experimental groups, suggesting that their uptake contributed to experimental results (Figures 5a, 5b, and 5c). Elemental analysis was conducted in tandem to SEM spectroscopy to confirm the presence of titanium (data not shown)

4. Conclusions

The TGA data suggest that there is a strong interaction between the tricarboxylic acid head group and the TiO_2 core. This shows that the coating procedure and tricarboxylic head group can be used to build novel uniformly coated metal oxide nanoparticles, and a tricarboxylic-acid head group will associate strongly with TiO_2.

Phytotoxicity evaluation used *Z. mays*, a worldwide staple of food consumption. SEM showed that *Z. mays* uptakes nanoparticles (Figures 5b and 5c). Alarmingly, if the seeds carry these TiNPs to their gametes, they may pass into the human body if ingested. Uncoated TiNPs hindered root growth at a concentration of 100 µg/mL, while a solution of 3CCb18-coated-TiNPs at the same concentration promoted root growth when compare to a DI water control (Table 1 and Figure 4). Collectively, these results show that precautions must be taken to prevent the release of TiNPs into the environment. Further analysis of environmental retention must be conducted in order to better assess the direct implications of nanoparticles on the environmental health and human safety.

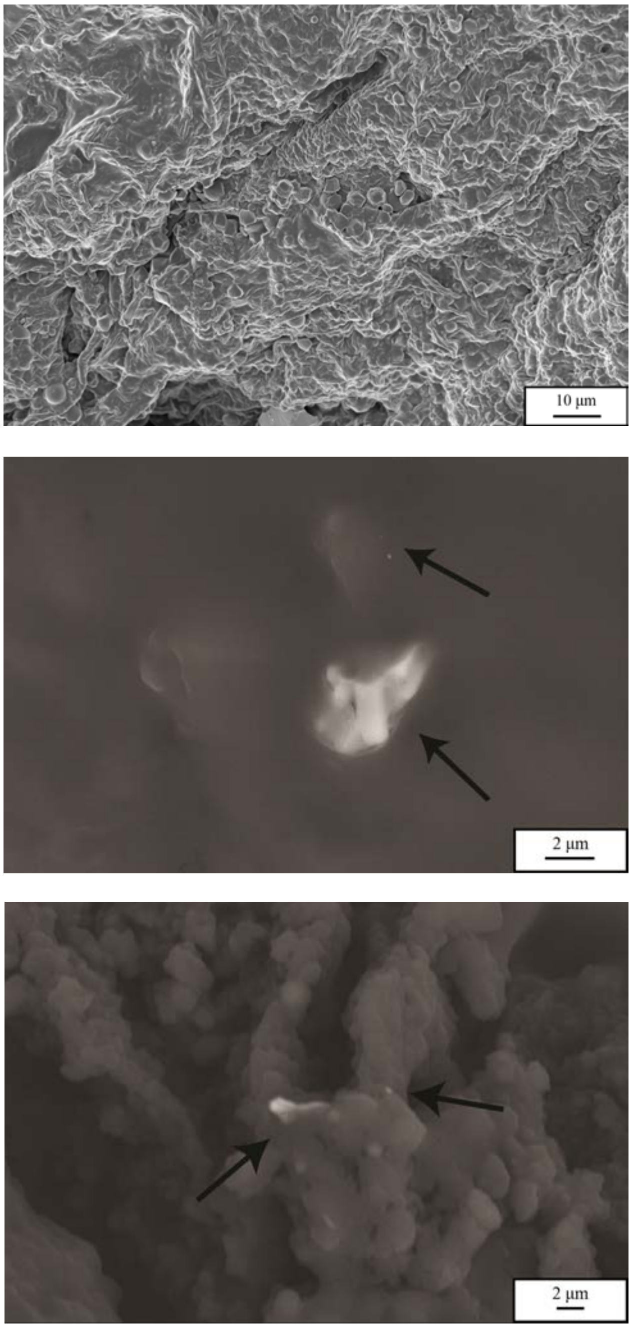

Figure 5: SEM micrographs displaying cross sections of the corn seedlings from the experimental groups. Areas of interest are highlighted with arrows. (a) Control seedlings with no nanoparticles visible in the root's wide-field cross section. (b) Uncoated TiNP experimental group showing a large aggregate of TiNPs present in the seed tissue. (c) Coated TiNP experimental group showing a small aggregate of TiNPs present in the seedlings.

5. Future Work

This paper details initial synthesis and toxicity data for a lipid-coated-nanoparticle; more experiments are necessary to elucidate the long-term exposure effects on plant life and mechanism of toxicity. Additionally, we aim to use the knowledge gained from this study to ultimately create and optimize a minimally toxic nanoparticle for drug delivery applications.

5.1 Nanoparticle Synthesis

Surface modifications of nanoparticles enable unique properties for functionalization. We will synthesize and conjugate polymer linker molecules consisting of a polyethylene glycol spacer and a cholestanol anchor to the solid metal nanoparticle. Hydrophobic interactions with the cholestanol anchor will enable the spontaneous formation of a phospholipid bilayer around the nanoparticle, allowing the incorporation of both hydrophobic and hydrophilic compounds for drug delivery purposes. Further tests on the uniformity of coatings are also necessary before proceeding to the encapsulation of compounds.

5.2 Biological Evaluation

Ongoing biological evaluation is necessary to assess the environmental and human impacts of nanoscale devices. Several different coatings on many types of nanoparticles should be assessed with a variety of concentrations of nanoparticles. Further phytotoxicity and cytotoxicity studies using a broad array of plant and animal models for a statistically representative conclusion on nanoparticle toxicity. Further understanding of toxicity mechanisms will help in designing more effective nanoparticles for commercial use and in shaping public policy regarding nanoparticles utilized in consumer products.

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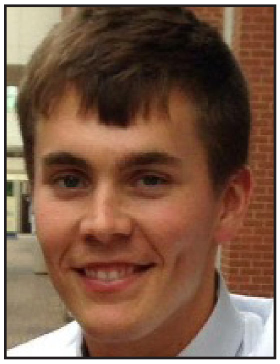
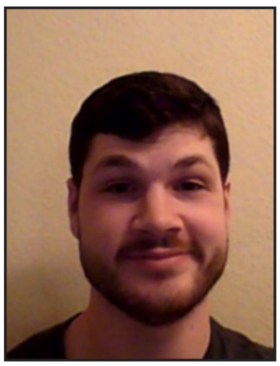
About the Authors

Louis Hollingsworth is a junior obtaining degrees in chemical engineering, biochemistry, and chemistry. He has extensive research experience, including internships at a bioengineering laboratory at George Washington University (2013), a structural biology laboratory at the National Institutes of Health (2014), and a biomedical laboratory at Harvard University (2015). Additionally, Bobby currently conducts chemical synthesis with Professor Richard Gandour in the Department of Chemistry at Virginia Tech and is a member of Virginia Tech's chemical engineering design team, Chem-e-Car. He intends to pursue a Ph.D. in a field related to cancer drug design at a university at the forefront of translational medicine. Contact at: bobbyh11@vt.edu



Joseph Conduff graduated from Virginia Tech in 2014 with a B.S. in Biochemistry. He worked in the laboratory of Dr. Richard Gandour for five semesters during his undergraduate career, conducting research on chemical synthesis and the surface modification of nanoparticles. Joey is currently a first year medical student at Virginia Commonwealth University School of Medicine.



In addition to his contributions to the Gandour lab at Virginia Tech, Connor Balzer worked for the International Petroleum Products and Additives Company (IPAC) Technical Center developing petroleum products as well as in-house petroleum specification tests. He graduated summa cum laude from Virginia Tech in May of 2014 with an honors degree in Biochemistry and a minor in Chemistry. He is currently attending graduate school at the University of Oregon working on a Ph.D. in their Chemistry and Biochemistry program. His research interests include drug development and protein evolution.

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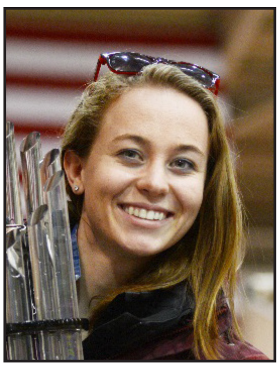

Lauren Rakes is a sophomore from Blacksburg, Virginia currently pursuing two degrees in Biology and Psychology. In addition to her academics, she works as a Hokie Sports photographer for Virginia Tech Athletics. In her spare time, Lauren regularly volunteers as a track coach for Blacksburg Middle School, and is an official partner of the Special Olympics. After college, Lauren wishes to go to medical school and pursue pediatrics.