A Gold Nanoparticle-based Lab Experiment Sequence to Enhance Learning in Biomedical Nanotechnology at the Undergraduate Level

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Abstract:

Introduction: The development of affordable, practical, and real-life hands-on nanotechnology labs for biomedical engineering students is challenging. Here, we present a three-part series of lab experiments that comprise synthesis, characterization, and biomedical application of gold nanoparticles in a logical and sequential order. These experiences were designed as part of a 1 credit hour lab course to complement a traditional style upper-level 3 credit hour "lecture" course titled "Biomedical Micro- Nanotechnology". Synchronization of lecture and lab allows students to directly apply their theoretical knowledge to understand and conduct experiments in biomedical nanotechnology. Participation in the lab course is optional, and all students in the lab course were also enrolled in the traditional style course.

Materials and Methods: Students carry out hands-on experiments to synthesize, modify, and apply gold nanoparticles to solve problems in a biomedical context. They are required to write hypotheses, develop aspects of the experimental plans, analyze data, and draw conclusions from the data.

Assessment of learning was primarily evaluated based on the pre-defined learning objectives related to each of the three lab sequences and student performance on a final exam in the lecture course. The effectiveness of the lab sequence was evaluated in both a qualitative and quantitative manner. The performance of students in the lab course (n=21) can be compared to performance of a control group of students who did not opt into the lab course (n=7) and only attended the traditional lecture course. Assessment of learning was evaluated in three ways: 1) self-perceived accomplishment of lab learning objectives reported by students in the lab course through an anonymous survey, 2) instructor evaluation of learning objectives assessed via lab reports, and 3) student performance on the final exam in the traditional style course, ~10 weeks after the lab experiences concluded. The third assessment technique allows us to evaluate the effect of participating in the lab course, as performance of students that are enrolled in the traditional course but not the lab course can serve as a control.

Results and Discussion: The assessment of these learning objectives indicates that at least 80% of students had satisfactory or exceptional performance on all learning objectives as assessed by an instructor via lab notebook submissions. We also asked students about their own perceived accomplishment of learning objectives, which revealed they believe the labs enhanced their learning of the lecture content. Finally, students in the lab had ~7% increase in correct points in the lecture course's final exam, on questions related to lab topics.

Introduction:

Nanotechnology integrates concepts from various disciplines, including chemistry, physics, engineering, and biology, to design nanomaterials for a wide range of applications, such as catalysis, energy, and medicine. The medical application of nanotechnology for diagnosis and treatment of diseases is referred to as nanomedicine and is a cornerstone of biomedical nanotechnology. This technology has the potential to transform healthcare and clinical outcomes. Due to the impact and potential of nanotechnology on research and society, students in biomedical engineering benefit from training in basic nanotechnology concepts.

There are several examples of nanoparticle labs for undergraduate students in the literature, but these are typically designed for chemistry students rather than focused on biomedical applications [1]–[5]. There are fewer examples of nanotechnology labs with biomedical engineering application [6], [7]. The examples of nanotechnology labs developed within biomedical engineering applications tend to require access to mammalian cell culture [7] or prohibitively expensive equipment such as scanning electron and atomic force microscopes [6]. Access to mammalian cell culture can be both prohibitively expensive and/or time consuming if student access will be hands-on rather than just a demonstration. The lab experiences described here do not require mammalian cell culture and can be accomplished with relatively less expensive equipment. Development of affordable, practical, and real-life nanotechnology labs for biomedical engineering students enhances learning through hands-on experiential approaches. The goal of this paper is to present a relatively affordable novel sequence of nanomedicine-based labs designed for biomedical engineering students.

We present a three-part series of lab experiments designed for upper-level undergraduate biomedical engineering students. The series comprises of synthesis, characterization and modification, and use of gold nanoparticles to detect infectious pathogens. In the first lab, students apply the Beer-Lambert equation to calculate and analyze their results. Part 2 develops experimental design and planning to modify and characterize colloidal stability of nanoparticles treated with biomedically relevant surface modifications. Finally, the third part demonstrates a point of care technology utilizing gold nanoparticles to detect *E. coli*. These experiences were designed as part of a 1 credit hour lab course to complement a traditional style upper-level 3 credit hour "lecture" course titled "Biomedical Micro- Nanotechnology". Here we only present a portion of the lab course related to nanotechnology. Other portions of the lab, focusing on biomedical microdevices, were developed for the remainder of the semester but are not presented in this paper. Synchronization of lecture content with lab experiences allows students to directly apply their theoretical knowledge to understand and conduct experiments in biomedical nanotechnology.

Materials & Methods:

Overview of Lab Sequence: The lab sequence presented in this paper occurs in the first third of the semester. An overview of the general lab lecture schedule appears in Appendix A.

In the first lab of the series, part 1, students synthesize gold nanoparticles with citrate ion surface coating using a straightforward and robust protocol, which is easy for beginners to follow. The learning outcomes of the lab include: 1) use of newly acquired micropipetting techniques to synthesize gold nanoparticles with an average diameter of approximately 14 nm and, 2) an

ability to apply knowledge of Beer-Lambert law to validate synthesis of gold nanoparticles and calculate nanoparticle molar concentration based on absorbance measurements.

The procedure is based on redox reaction involving an aqueous gold(III)chloride solution and sodium citrate. The sodium citrate ions not only act as ligands to ensure colloidal stability of synthesized gold nanoparticles at neutral pH but also reduces Au^{3+} ions to Au^0. The fact that gold atoms are produced by this redox reaction teaches students the concept of bottom-up synthesis, where nanoparticles are formed by clustering of gold atoms into nano-sized crystals, which ultimately grow in size to form nanoparticles. The nanoparticle synthesis can be easily observed by students in real-time due to visual changes of the solution resulting from the size-dependent light interaction behavior of gold nanoparticles (Figure 1). Students' results are validated via absorbance measurements by means of spectral analysis using a UV-vis spectrophotometer.
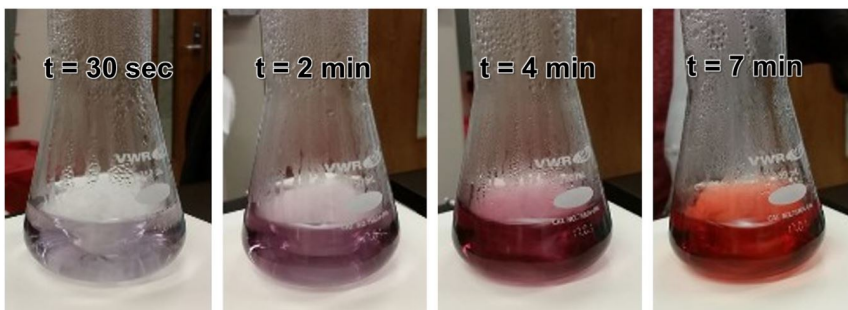

Figure 1: During the synthesis of gold nanoparticles, a continuous color change from violet to red can be observed within ~5 minutes.

In part 2, the second lab session, students use their own synthesized gold nanoparticles from part 1. Learning outcomes of this lab include abilities to: 1) compare different methods of colloidal stabilization of gold nanoparticles, and 2) write and assess a measurable hypothesis based on surface modification of gold nanoparticles. They make hypotheses about two different biomedically relevant surface modifications strategies: PEGylation with methoxy-PEG-thiol (mPEG) vs coating with bovine serum albumin protein due to a protein corona formation. Students design aspects of an experiment to test colloidal stability (Figure 2), by selecting and planning a range of salt solutions to react with their surface-modified gold nanoparticles. Students learn in the lecture course the relevance of colloidal stability when using nanoparticles for therapeutic application in nanomedicine, for example as drug delivery vehicles or imaging contrast agents. Utilization of nanoparticles for medical applications requires control over their colloidal stability to ensure safety and efficacy. In their lab notebook students analyze the effectiveness of either surface engineering treatment to maintain colloidal stability of gold nanoparticles. In addition, students discuss the relative advantages of using mPEG and serum albumin in biomedical applications.

Figure 2: Gold nanoparticle solutions which are colloidally stable (left) and aggregated (right).

In the final lab session, part 3, students use a gold nanoparticle-based nucleic acid detection kit (SensoGold Bacterial Detection kit, Luna Nanotech, Toronto, Canada) to test whether *E. coli* RNA is present in a set of mystery samples (Figure 3). Learning outcomes for this lab session include: 1) purify RNA from mystery bacteria samples and use gold nanoparticle detection kit to detect the presence or absence of *E. coli*, and 2) accurately describe the mechanism of action of a MNAzyme based gold nanoparticle detection system.

Detection of RNA is based on a DNAzyme/MNAzyme assay [8]. This lab is used to represent a real-world application of gold nanoparticles in a biomedical setting which students had previously learned about in the lecture course.

Course Details. Enrollment in the lab course is an elective option. That is, not all students are required to take this particular lab course, although students must take at least three lab courses within the department related to 6 "core areas". The traditional "lecture" course is a co-requisite for the labs. This conveniently provides a control group of (n = 7) students only enrolled in the lecture course to compare to the (n = 21) students who were enrolled in both lecture and lab courses.

Figure 3: Sample results of gold nanoparticle-based MNAzyme Assay indicating the presence (red dots) or absence (blue dots) of *E. coli*.

Experiments can be completed in individual 2 to 2.5-hour lab sessions, are affordable, and only require standard equipment commonly available in many biomedical wet labs (see Appendix B for equipment list and approximate cost). This lab sequence was contained in three 2.5 hour lab sessions spread over a 3 week time period. The 1 credit hour lab is held once per week with students working together in groups of 2 or 3 members per group. The lab course is run by a single faculty member with help of a TA to accommodate ~12 students per lab section. The lecture course is taught by another faculty member.

Necessary Materials. These labs utilized equipment that is available in most biomedical wet labs: stirrer-hot plates, Erlenmeyer flasks, micropipettes, water bath, UV-Vis spectrophotometer, mini-centrifuge, and analytical balance.

The consumable materials required an initial up-front cost of approximately $1,100 (table in Appendix B). However, since many of the materials have relatively long shelf-lives, all three of the labs can be completed with an estimated cost of less than $20 of consumable materials per group of students (table in Appendix B). All of the materials can be kept at least one year, if not longer, if stored properly.

Laboratory Preparation. For the most part, the minimum amount of reagent and equipment preparation is done for the students by the instructional team in advance to allow students to complete the experiments in about 2 hours. Since students do prepare reagents (e.g., create dilutions from stocks), they are given pre-lab assignments to check that they understand the necessary skills, calculations, and safety cautions in advance. There are only about two major things that require preparation from the instructional staff. One is ensuring that glassware is cleaned with aqua regia (3:1 v/v mixture of HCl and HNO_3), to clear any residual metals that may interfere with AuNP synthesis. The second is preparing the mystery bacterial solutions, which can be prepared in bulk. These bacteria solutions can be prepared using standard culture methods, and then be aliquoted and frozen for long-term storage.

Since the lecture course provides necessary background knowledge for the lab, the labs have minimal lecture to allow for hands-on applications. Typically, a short lab lecture (10-20 minutes) is provided to review any concepts that were identified as weak points in the pre-lab
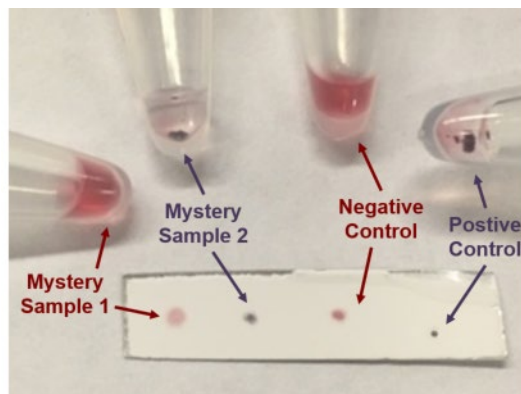
assignments. In addition, the short lecture is used to provide tips for successfully implementing unfamiliar lab techniques.

Self-reported Learning Assessment. Since grades from lab notebooks may be affected by outside factors besides learning (e.g., student effort, time devoted to completing written assignments, inconsistent application of rubrics by graders, etc.), we also considered self-reported assessment of learning. Students completed an anonymous survey where they identified their own assessment of learning objectives on a Likert scale (disagree, somewhat disagree, neutral, somewhat agree, and agree). The format was roughly "I am able to…[lab objective]". For the purposes of presentation and relating to instructor assessment (Figure 4), "disagree" and "somewhat disagree" were coded as unsatisfactory, "neutral" and "somewhat agree" were coded as satisfactory, and "agree" was coded as excellent performance. In addition, we asked students whether or not they thought the "lab experiences enhanced [their] understanding of the lecture course material".

Learning Assessment via Lab notebook. Electronic lab notebook submissions are required for each assignment. In addition to the pre-lab assignment, mentioned earlier, students also complete a "post-lab" assignment. The post-lab assignments are graded for proper formatting, notation, as well as data collection, analysis, and interpretation of data. At the end of the protocols are questions related to data analysis, statistics, and interpretation of results. This section accounts for 30% of the grade. Accomplishment of the lab learning objectives were assessed in written submissions in a lab notebook format of the n=21 students in the lab. Lab notebooks were graded by TAs using a rubric to assess technical writing, analysis of data, and interpretation of data. Components related to listed lab outcomes were pulled from the rubrics. Performance was coded as "unsatisfactory" for < 70%, "satisfactory" for 70% to <90%, and "excellent" for > 90% of the points associated with the lab learning objectives.

Final Exam Performance in lecture course. Final exam questions were drafted in conjunction with the instructor of the lab and the lecture instructor. A set of questions were written from lecture content that related directly to the nanotechnology lab experiences. That is, all questions were based on lecture content, however, questions that also overlapped with concepts from the labs were analyzed for this study. For instance, all students learned conceptually in the lecture about how the Beer-Lambert law is used to evaluate properties of AuNP solutions. However only the students in the lab actually used a spectrophotometer to measure absorbance and evaluate actual AuNP solutions. Questions related to Beer-Lambert, measuring absorbance with spectrophotometer, would be considered "lab focused" questions. The performance on remaining exam questions, not directly related to the nanotechnology lab experiences (for example, a question related to the delivery efficiency of targeted nanoparticle to a tumor), are used for a control comparison. Example questions can be found in Appendix C. The final exam was given approximately 10 weeks after the three-part nanotechnology lab sequence was concluded.

Statistical Analysis. The results of the exam scores were analyzed in GraphPad Prism version 8.0.2, GraphPad Software, La Jolla California USA, www.graphpad.com. An unpaired, two-sample, two-tailed t-test was used to determine differences between students in the lab and students in the lecture only. For comparisons of question difficulty, a paired two-tailed t-test was used. For all tests, statistics were interpreted using a definition of statistical significance at $p < 0.05$.

Results & Discussion

These lab experiments were received with overwhelmingly positive feedback. With all of the students in the anonymous survey agreeing on the Likert scale that the "lab experiences enhanced [their] understanding of the lecture course material". Although the part 3, bacterial detection assay was the most expensive of the lab sessions (~$15 of the total $20 consumables cost), it was also the highest ranked out of the three sessions, in informal course feedback from students in Spring 2018, the previous course implementation before this study.

Overall the lab course objectives were successful. At least 80% of students were able to satisfactorily complete all lab objectives, as assessed by an instructor from lab notebook submissions (Figure 4).

For part 1, all student groups were able to successfully synthesize gold nanoparticles. Students exhibited varying levels of success applying knowledge of Beer-Lambert law and calculating AuNP concentrations. Students used the equation: $A = \varepsilon \cdot c \cdot x$, where A is the absorbance of the solution measured by the spectrophotometer, ε is the given molar extinction coefficient, and x is the calculated path length of light through the sample.

Students that had satisfactory (~14% of students) but not excellent (~77% of students) performance may have incorrectly estimated path length for light traveling through the sample. Since absorbance was measured in well-plates rather than cuvettes, students had to calculate the height of the volume of the liquid in the well plate to estimate path length. Other examples of satisfactory but not excellent performance might be simple calculator or unit errors. For this part, students that did unsatisfactory (~9 % of students) if they incorrectly applied the Beer-Lambert law, for example, by using peak wavelength instead of absorbance to calculate concentrations.
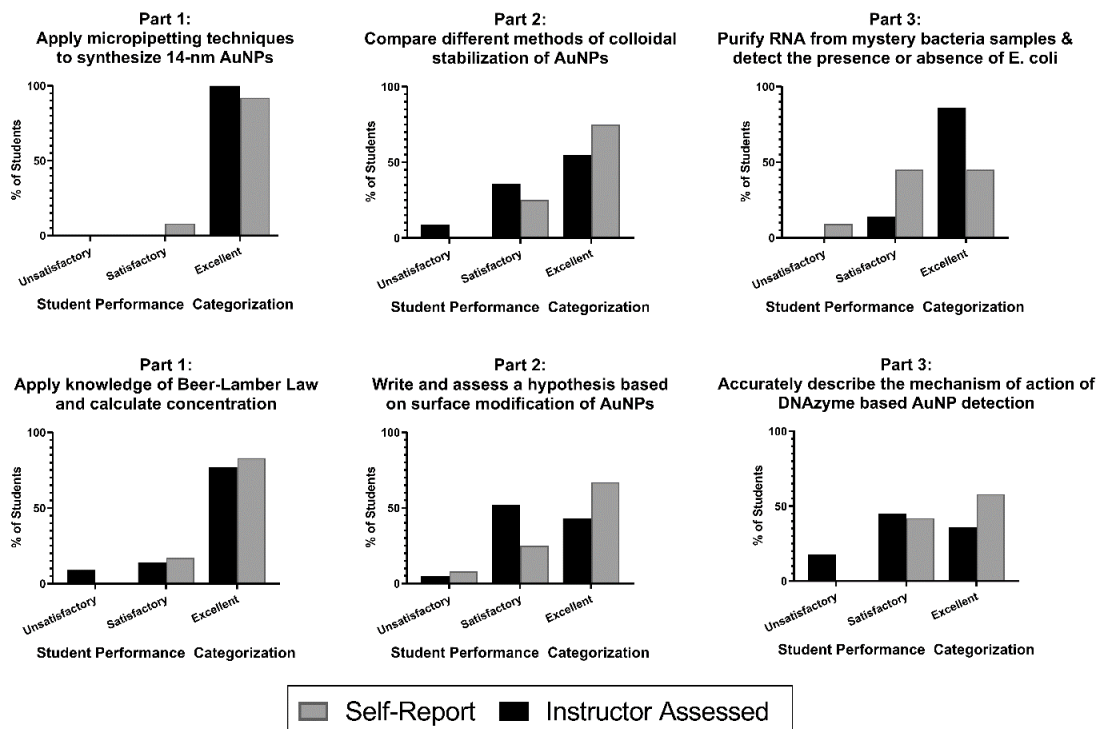

Figure 4: Instructor-Assessed and Self-reports of student performance on lab course objectives.

The part 2 objectives emphasize hypothesis writing and data analysis. Satisfactory performance (~36% of students) was determined by logical conclusions, supported by data, but in some cases the designed experimental parameters were not sufficient to fully compare the two methods of colloidal stabilization. Students that did unsatisfactory (~9% of students) in comparing different methods of colloidal stabilization made conclusions that were not supported by the data they generated.

Most students (~52%) performed satisfactorily on writing and assessing a hypothesis based on surface modification of AuNPs. Unsatisfactory performance (~5% of students) were hypotheses that were illogical or untestable. For instance, "BSA will be better than Pegylation because it is a natural protein". An example of satisfactory (52%) but not excellent performance (43%) is a hypothesis that is testable based on their experimental tests, but perhaps they vaguely referred to "higher absorbance" rather than specifically to an increase in peak absorbance or wavelength.

In part 3, using the MNAzyme to detect *E. coli*, all student groups satisfactorily completed the objective of RNA purification and *E. coli* detection (86% excellent and 14% satisfactory). Students struggled more with accurately describing the mechanism of action of the MNAzyme detection method. Some students struggled with the misconception that RNA binds directly to the gold nanoparticles to provide steric interactions, but this is false. Students that were categorized in the excellent category (36% of students) were able to identify the function of the gold nanoparticles and the linker MNAzyme substrate.

In anonymous student responses, at least 90% agreed that they were able to satisfactorily complete the lab objectives (Figure 4). Here, we present the results of both the student self-report and instructor evaluation because they assess different things. Instructor assessment may underestimate learning if the work they turn in does not reflect their understanding, for example if a student rushed to complete lab notebooks, and their entries do not actually reflect their understanding or actual performance. Whereas, student self-reports of learning, may likely reflect a measure of confidence of the material or some other bias. For instance, self-enhancement and self-diminishment bias may be at play. It has been previously shown that some low-achieving students tend to over-estimate their abilities and high-achieving students tend to under-estimate their performance when compared to the assessment done by a tutor [9]. The self-enhancement bias may be in effect in cases where the instructor rated some of the students' performance as unsatisfactory, but all students assessed themselves as having completed the objective (e.g., accurately describing the mechanism of action of DNAzyme based AuNP detection, Fig. 4).

Performance on the comprehensive final exam of the lecture style course was assessed for students in the lab and also a group of students that did not take the lab (Figure 5). Overall the performance on the entire set of exam questions was similar, with students doing roughly the same if they took the lab class or not (Figure 5A, p=0.14). When looking at questions that were not directly related to topics covered in the lab class, the student performance was no different (Figure 5B, 92.5% correct for students in the lecture only and 93.1% for students also in the lab).

This indicates that all of the students were of roughly the same quality, roughly as motivated, or were able to succeed in the class equally well.
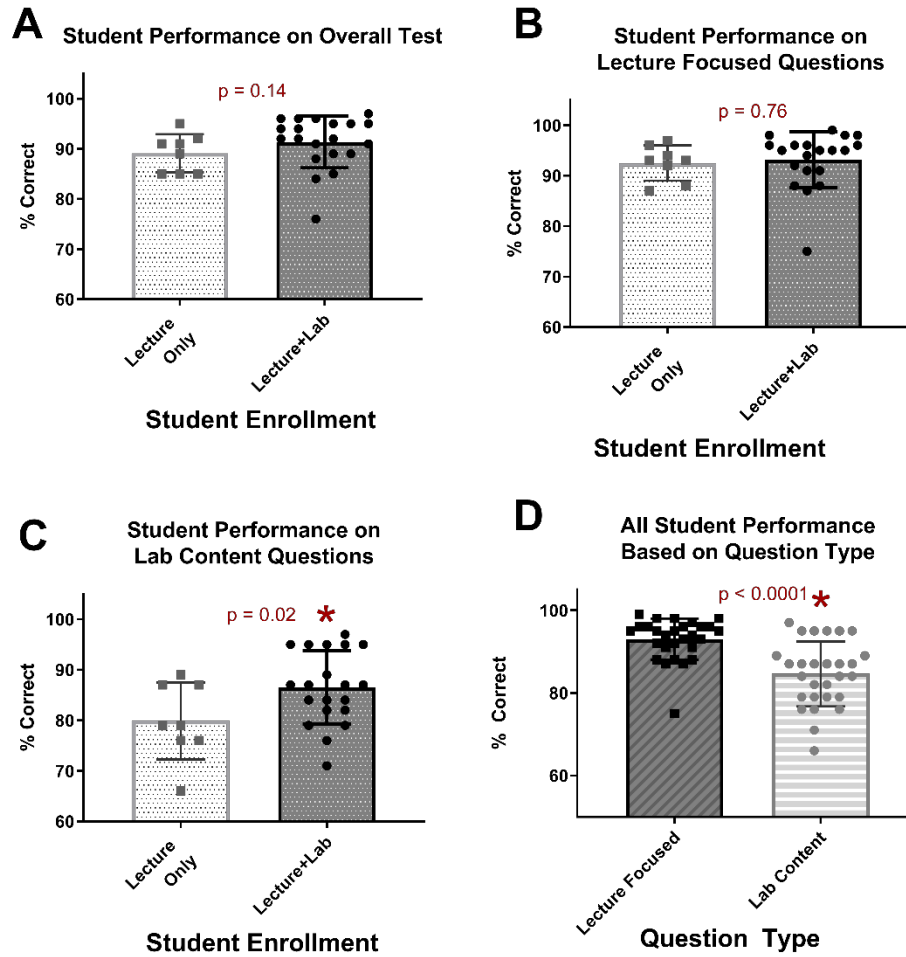

Figure 5: Student Performance on Final Exam.

Students in the lab did significantly better on test questions related to lab content than the students who did not take the lab (an increase of ~7% more points, p=0.02). Since the exam was a comprehensive final, it included questions unrelated to nanotechnology, for example, related to biomedical microdevices, and question related to nanotechnology, but not directly related to anything done in the labs (see Appendix C for example questions). When comparing the difficulty of the questions, it appears that students (in either the lecture only category or lab category) did slightly worse on the questions that were focused on lab content, a difference on average of about 7% of the points for those questions (Figure 5D, p < 0.0001). That is the questions were harder. The instructors did not intentionally make the lab content questions harder, but they did tend to focus on critical thinking questions. It is possible that the lab helped the performance on the exam, by simply allowing students to be in contact with the course

material longer. However, that is one of the goals of the lab, to increase exposure to course material related to biomedical nanotechnology. The results are not surprising, but it does support the idea that students in the lab had improved learning related to the lab content, 10 weeks later during the final exam. Overall, the students benefitted from the lab experiences as they were able to achieve certain laboratory objectives and performed better on the final exam questions compared to students not in the lab.

One goal of the development of these labs was to create a course sequence directed toward biomedical engineering students. While part 1 of the sequence is very chemistry related, the synthesis of gold nanoparticles is a logical first step as these nanoparticles are used in part 2 and part 3 of the study. Part 1 establishes the fundamental concepts of nanoparticle synthesis and characterization that are relevant for successful completion of parts 2 and 3. Part 2 has significant biomedical relevance as colloidal stability of nanoparticles is required for biomedical applications for example, when using nanoparticles as drug delivery vehicles. The use of albumin teaches students that proteins can stabilize nanoparticles by non-specific adsorption onto the nanoparticle surface. This process happens naturally when nanoparticles are administered into the blood stream, which leads to the formation of a so-called protein corona, i.e., serum proteins that adsorb onto the nanoparticle surface.

Future work could be implemented to provide exposure to additional biomedical applications. For instance, including experiments related to targeting of AuNPs to cancer cells as others have [7], but that might not be done in such an affordable setting. Another option would be to have the students design their own gold nanoparticle detection kit. For example, they could first identify a specific pathogen and then design a DNAzyme assay from scratch, including linker design, nanoparticle synthesis, characterization, and validation.

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Appendix A:

Overview of the lab sequence and lecture topics schedule.

Week	Lecture Topics	Lab Topics
1	Examples of nanomaterial properties, synthesis strategies, top down, bottom up, AuNP synthesis	Intro, lab safety, and electronic lab notebooks
2	Organic and Inorganic nanomaterials	Liquid handling and micropipetting
3	Characterization methods, spectroscopy, scattering, microscopy	Synthesis of 14-nm AuNPs
4	Zeta potential, colloidal stability, aggregation, AuNP bio-assays	Colloidal stability and modification of AuNPs
5	Biomolecules, proteins, DNA, virus, identification, characterization	MNA/DNAzyme detection of *E. coli*
…	…	…
16	Final Exam	

Appendix B:

Table 1: Required materials for initial costs and estimated costs per student group.

Lab Experiment	Material	Total Upfront Cost	Estimated Cost / Group
Part 1: Synthesis of AuNPs	Sodium citrate tribasic dehydrate	$ 39.10	< $0.01
	Gold (III) chloride trihydrate	$ 122.00	$ 1.21
	Tween 20	$ 52.00	< $0.01
	transparent 96-well plate	$ 88.10	$ 0.88
Part 2: Modification of colloidal stability	micro-cuvette	$ 21.40	$ 2.57
	mPEG-thiol	$ 120.00	$ 0.03
	BSA	$ 143.38	$ 0.29
	20X PBS	$ 36.88	$ 0.09
Part 3: Bacterial Detection	Luria broth	$ 14.20	< $0.01
	DL Dithiothreitol	$ 64.53	$ 0.05
	Kit	$ 349.00	$ 14.54
	TE	$ 33.10	$ 0.10
	Lysozyme	$ 21.68	$ 0.07
	bacterial solutions	$ -	-
	ethanol	$ 26.73	$ 0.03
		$1,105.37	**$ 19.83**

Table 2: Required equipment

Lab Experiment	Equipment	Cost	# Needed Per Group
Part 1: Synthesis of AuNPs	nanopure water purifier	$ 3,550.10	shared
	250 mL Erlenmeyer flask	$ 4.17	1
	stirring hot plate	$ 220.00	1
	micropipettes set	$ 832.70	1
Part 2: Modification of colloidal stability	UV-Vis spectrophotometer	$ 8,999.00	shared
Part 3: Bacterial Detection	mini-centrifuge	$ 832.00	1
	analytical balance	$ 400.00	shared
	waterbath	$ 288.00	shared
	Initial Shared Equipment Cost	**$13,237.10**	
	Equipment Cost Per Group	**$ 1,888.87**	

Appendix C: Example Exam Questions

Lab content focused:

1. You suspect that your labmate might have mislabeled the tubes of gold nanoparticle samples you hope to use for an experiment. According to your labmate's lab notebook, you expect one tube to be 14-nm gold nanoparticles, another tube to be 60-nm gold nanoparticles, and a third tube to be 100-nm gold nanoparticles. Quantitative analysis of all three tubes results in following the following graphs:
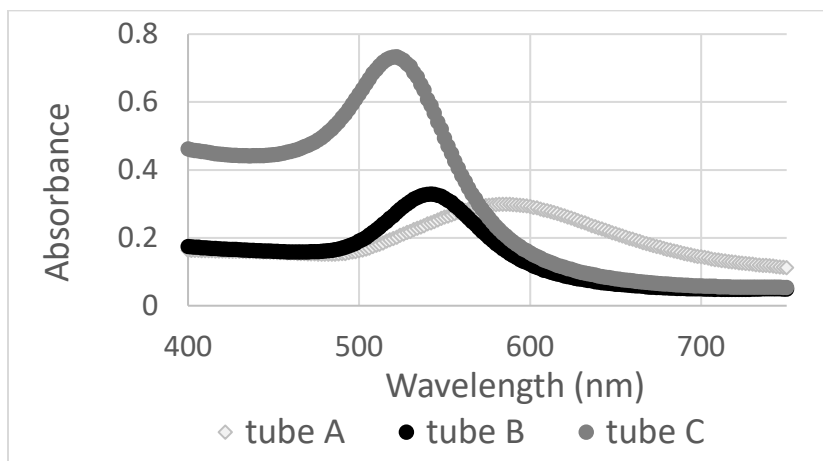
(a) **(3P)** Which quantitative analytical method/instrument has been used to generate the graphs above?
    (i)   UV-Vis spectrophotometry
    (ii)   Fluorescence spectroscopy
    (iii)   Inductively coupled plasma mass spectrometry (ICP-MS)
    (iv)   None of the above

(b) **(6P)** Match the tubes (A, B, and C) with the corresponding gold nanoparticle size of your colloidal dispersions.

_____ 14-nm gold nanoparticles

_____ 60-nm gold nanoparticles

_____ 100-nm gold nanoparticles

(c) **(3P)** What is the appropriate unit label for the y-axis in the figure above?
    (i)   L/(mol·cm)
    (ii) $M^{-1} \cdot cm^{-1}$
    (iii)   μm
    (iv)   no unit

(d) **(3P)** Your labmate claims that based on the graphs shown above tubes A and B have the same molar concentrations of gold nanoparticles.

(i)   The claim is <u>correct</u>, because both tubes (A and B) have similar absorbance values of approximately 0.32 at the corresponding absorbance maximum.

(ii)   The claim is <u>incorrect</u>, because gold nanoparticles in tubes A and B exhibit very different molar extinction coefficients.

(iii)   The analytical method used to generate the above figure does not allow any conclusions about nanoparticle concentrations.

2.   **(4P)** You and your lab partner want to use a gold nanoparticle (AuNPs) based MNAzyme detection kit to detect the presence of *E.coli* RNA in a romaine lettuce sample. Your lab partner accidentally drops the tube with AuNPs from the kit. The kit's AuNPs are no longer usable.
Your lab partner wants to use 14-nm AuNPs synthesized earlier in the lab. These AuNPs are citrate-coated, with no other surface modification. <u>Multiple answers may be correct.</u>

(i)   The experiment <u>will work</u> because your AuNPs naturally clump together and aggregate, if *E. coli* RNA is present. The RNA will sterically interfere with your AuNPs and give the desired result.

(ii)   The experiment <u>will work</u>, because your AuNPs are high quality, monodispersed, and negatively charged due to the citrate surface ligands.

(iii)   The experiment <u>will not work</u> because the AuNPs you synthesized do not have single-stranded DNA sequence that will bind to the complementary linker strand.

(iv)   The experiment <u>will not work</u> because one of the steps of the MNAzyme requires a buffer with high salt concentration. This affects colloidal stability of citrate-coated AuNPs.

<u>Lecture-only content focused:</u>

3.   **(5P)** Bring the following objects in the correct order. Start with the largest object and end with the smallest object.

Largest object?

(a) Herceptin antibody
(b) Toscana virus
(c) Glucose molecule
(d) *E. coli* bacterium
(e) MDA-MB-231 human breast cancer cell

Smallest object?

4.   A CD-1 immunodeficient nude mouse bearing a subcutaneous human SKOV-3 ovarian cancer xenograft tumor was intravenously injected with spherical 55-nm gold nanoparticles that were modified with Herceptin antibodies. These antibodies can selectively bind to ErbB2 surface receptors on SKOV-3 cancer cells.

Quantitative biodistribution analysis based on ICP-MS revealed the following data for the accumulation of 55-nm gold nanoparticles in the tumor over time:

Nanoparticle concentration in tumor [%ID/g]	Time (post injection) [h]
0.00	0.00
0.25	0.30
0.54	2.00
1.02	6.00
0.63	24.00

(20P) Calculate the nanoparticle delivery efficiency to the tumor using a linear trapezoidal analysis model. The dimensions of the ellipsoidal solid tumor are (a = 5.2 mm; b = 1.24 cm; c = 3.8 mm). Assume that the tumor tissue density is 1.21 g/cm^3. Show all of your steps with corresponding units!

5. **(3P)** The strategy of using Herceptin-conjugated gold nanoparticles for targeting ErbB2 positive cancer cells is referred to as:

      (a) Active targeting strategy
      (b) Passive targeting strategy
      (c) None of the above

6. **(10P)** Assume a delivery efficiency of Herceptin-conjugated gold nanoparticles (spherical shape; 55.0 nm in diameter) to the solid tumor of 0.72% ID. The conjugation density of surface-conjugated Herceptin antibodies is $1.0 \cdot 10^{-2}$ Herceptin antibodies per nm^2 of nanoparticle surface area. The total injected dose (ID) of Herceptin-conjugated gold nanoparticles is composed of 150.0 µL with a nanoparticle concentration of $2.21 \cdot 10^{-8}$ M. How many Herceptin antibodies were delivered to the solid tumor?