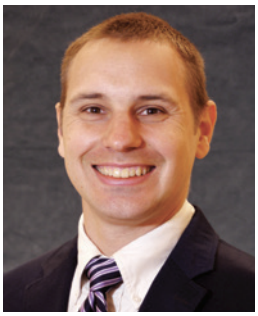

Letter from the Editor

Peter D. Kazarinoff

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Keywords: J ATE, technician education, undergraduate research

Community and Technical College Education Community,

I'm pleased to present the Journal of Advanced Technological Education (J ATE) Vol 2, Issue 2, on behalf of the J ATE Editorial Board and Staff. The theme for this edition is undergraduate research at community and technical colleges across the US. Community college students, along with their mentors, wrote many of the articles in this special J ATE edition! Think about that for a second–community college students now have a peer-reviewed publication where they can publish the research they've been working on and that they can include as part of their resume, job applications, and education journey.

I want to acknowledge the students' courage to move research and a new understanding of subjects impacting and exciting them to a peer-reviewed article. Embracing the process is not easy; challenges and errors were made and worked through until the final published article. These students not only took their classroom and lab experiences and translated them into a written document but then submitted their manuscripts to be put through a process with corresponding editors and reviewers. They took their review feedback and either accepted and made changes or provided acceptable reasoning in a rebuttal letter to their corresponding editor before again working on perfecting their manuscripts to be finally accepted and published for our readers to appreciate. These efforts were a tremendous amount of work, and I want to thank all the students, mentors, and corresponding editors who took valuable time to make this such a special J ATE issue.

In this spirit, J ATE is embarking on exciting programming for the academic year 2023-2024. We are implementing a special program called J ATE Connects that pairs community college faculty and coaches in teams for six months, demonstrating to writers how to publish in peer-reviewed journals. Our J ATE URE program will guide community college students with faculty mentors over nine months to learn how to publish their work, expanding their classroom/lab experiences to share their research in peer-reviewed articles. In addition, J ATE is also working on a new online submission and review tool to streamline the work of our editors, reviewers, and staff. Finally, we are also working on a new public-facing website where you can read and download all J ATE articles.

These are exciting and ambitious new programs. J ATE is dedicated to being your peer-reviewed journal focused on technician education at community and technical colleges. As we grow, we are committed to being a journal that is free to submit, free to publish, and open-access.

Help us by reading J ATE, submitting your manuscript, and serving as a co-author or reviewer. J ATE is our journal, and we value our community.

In Teaching and Learning,

Peter

Peter D. Kazarinoff

Editor-in-Chief

Invited Letter: Greetings from the National Science Foundation!

Keywords: NSF, ATE, technician education

Hello from the National Science Foundation!

It is my pleasure to support the Journal of Advanced Technological Education (JATE) and its success in reaching community and technical colleges since its introduction in 2022. I am excited to contribute to this second issue of Volume 2 of the JATE. As a Program Officer at NSF from a community college, an author of several publications focused on innovations in technician education, and a proud member of the NSF ATE leadership team, I am aware of the importance of community and technical colleges publishing their work in the JATE. As an engineer who worked in industry prior to my teaching career, I understand that our nation's two-year schools are keystones of the community in preparing students to enter directly into the workforce and contribute to our nation's economy. The JATE provides a voice for these institutions to share their research and accomplishments with a national audience.

The theme of this issue, undergraduate research in technician education, is closely aligned with the current and future needs of industry and with the missions of NSF and ATE. Community and technical colleges are known for serving a higher percentage of students from diverse backgrounds, which is important for the future of our economy. I was a Principal Investigator on two ATE grants that focused on enhancing the curriculum for technician education programs and creating pathways for workforce development certification programs into credit technician education majors. I was also the advisor to two teams that made the final round of the NSF, AACC Community College Innovation Challenge (CCIC). Through these ATE grants, the CICC, and private grants, my students researched important national interest areas such as additive and subtractive manufacturing, drone technology, alternative energy, and cybersecurity. They disseminated their research at local and national conferences through presentations, competitions, panel discussions, and casual conversations, contributing to the advancement of knowledge. They met with industry leaders, faculty, and students locally and nationally. My students developed valuable research, communication, and networking skills that instilled confidence and helped prepare them for industry careers.

I am aware of the importance of cultivating student engagement, empowerment, and a sense of belonging through undergraduate research. These valuable research experiences that greatly benefitted my students have informed my strong belief that improving undergraduate research in technician education is crucial for preparing students to enter into industry careers in areas of national need. These research experiences align with the mission of ATE in that they help to create pathways into industry for a diverse population of students, including a larger percentage of groups historically underrepresented in STEM. It helps lead to retention, success, and degree completion for these students. Undergraduate research in technician education aligns with the mission of NSF in that it promotes the progress of science, helps to secure our national defense, and contributes to the health and welfare of our nation through discovery.

I am proud to serve my country as a Program Officer from a community college for NSF and ATE and to share with you the importance of undergraduate research in technician education. It is crucial for faculty members from community and technical colleges to publish their work and disseminate their research. I strongly encourage all community college faculty and leaders on ATE grants, including all projects and centers, to submit their research and accomplishments to JATE for publication. It is important for the future of our nation to reach a wider audience through continued communication and nationwide connections.

Christine Delahanty
Program Officer, ATE Program
The National Science Foundation

Invited Letter: Undergraduate Research as a Means to Build a Creative, Resilient, and Highly Skilled Biomanufacturing Workforce

Keywords: NSF, ATE, future manufacturing, biomanufacturing, STEM education, workforce development, synthetic biology, DNA nanotechnology, outreach, teacher education, undergraduate research, art in science, green chemistry, systems thinking, active learning, equity in education

As a principal investigator of an undergraduate research group at Pasadena City College (PCC) for the past ten years, I have witnessed the profound impact that the research experience has had on our students. I have observed students as they develop the ability to carry out authentic research projects from the inception to dissemination, design and carry out thoughtful experiments, write meaningful conclusions in light of relevant literature, and present science to peers and at conferences. Undergraduate research gives students the opportunity to work in a laboratory setting early in their career and collaborate with scientists as they learn how to do science themselves. It lays the foundation for future experiences in research; it informs their decision to pursue science; it excites, motivates, and inspires them; it contextualizes what they learn in courses and applies interdisciplinary scientific concepts to emerging technologies.

Currently, as Co-Principal Investigator of an NSF Future Manufacturing grant in collaboration with UCLA, UCSB and Caltech entitled "DNA & RNA Condensate Droplets for Programmable Separation and Manufacture of Biomolecules," I lead the education and workforce development aspect of this effort. Through this undergraduate research program in DNA nanotechnology, PCC students are trained in research methods, laboratory techniques and scientific theory to prepare them for the future biomanufacturing workforce. They apply this knowledge to authentic research projects, exploring aspects of DNA nanotechnology while developing important character traits and skills that will enable their success as students, scholars, and technicians. As advisors in undergraduate research, we have the privilege of serving as mentors to students, and mentorship can have a significant and lasting impact on students' lives and careers.

Creative ideas are the centerpiece of my research program at PCC that focuses on bio/nanotechnology. We have made natural paints through green chemistry, biodiesel from algae, nanostructures using DNA origami, and we are now working with DNA nanostars and DNAzymes. Artistic themes permeate our scientific research, which inspires creativity in the students and allows them to learn complex scientific concepts in a fun and engaging way. We love to create art through microscopy, craft paintings with themes at the art-science interface, and use graphic design to illustrate scientific concepts. Students pursue their passions and interests, which motivates and inspires them. Throughout the research experience, students collaborate with professors, graduate students and post-docs, attend seminars, and carry out scientific work; they begin to identify as scientists as they develop confidence in their application of the scientific method and in their ability to communicate effectively. Students learn how to think through problems, troubleshoot instruments, and persevere through challenges. They cultivate important characteristics, such as resilience, ethics, and leadership, and skills such as critical thinking, creativity, and collaboration, which are desirable traits to any future employer.

Course-based undergraduate research affords research experiences for a wider range of students, and it is an equitable, effective way to teach science that engages students from diverse backgrounds. My research group has designed lesson plans to teach students about green chemistry, sustainability, and renewable energy; and we are working to translate our current research into projects focused on DNA condensates. PCC researchers teach these engaging and interactive lessons with me in outreach to underrepresented communities and as

learning assistants in my courses, and this experience inspires everyone involved. Excellent science teachers are necessary in the development of a creative and resilient future workforce, and providing opportunities for students to engage in science teaching can identify talent and passion for teaching science and technology. In summary, undergraduate research is a highly impactful experience for students, and equitable opportunities must be created for more students to participate in the research process.

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Contextualization of Soft Skills in a Biotechnology Course with Project-Based Learning

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Abstract: A shortage in the science, technology, engineering, and mathematics (STEM) workforce has made it clear that STEM educators should adjust their teaching pedagogy to engage and retain students by improving student interest in STEM fields. Furthermore, in addition to discipline-based knowledge, students must learn the soft skills employers value to increase employability. Project-based learning (PBL), a student-centered teaching method in science labs, is a strategy that encourages students to create their projects while applying soft skills. This article demonstrates that integrating and implementing PBL in a biotechnology lab enhances knowledge and increases student retention and employment rates.

Keywords: project-based learning, biotechnology

Introduction

In September 2022, the White House issued an executive order on advancing Biotechnology and Biomanufacturing Innovation for a sustainable, safe, and secure American Bioeconomy to "train and support a diverse, skilled workforce and a next generation of leaders from diverse groups to advance biotechnology and biomanufacturing" [1]. It is known that the science, technology, engineering, and mathematics (STEM) workforce makes essential contributions to improving the nation's living standards, economic growth, and global competitiveness. However, economic projections predict a workforce shortage in the STEM fields to meet the demands in the labor market [2], which will have a high impact on the economy.

An analysis of the leading causes of this shortage indicates that younger generations are not pursuing careers in STEM compared to previous generations. Many factors contribute to the issue, including but not limited to access to higher education, high tuition, and decreased interest in the STEM disciplines [3]. These factors, especially the lack of interest in the STEM disciplines, have contributed to increased dropout rates among underserved minorities. It was also suggested that one of the reasons why students withdraw is that they find their classes boring and therefore become disengaged [4].

Additional studies have demonstrated that the best science training programs have a solid connection to industry and provide sufficient services to help engage adult students and complete the program [5]. Reports also indicate that successful programs offer adult learners (e.g., underrepresented and low-income students) access to essential services that remove barriers to entering and completing a training program. Hess et al. reported that programs with training developed with local employers' needs in mind and with their input result in improved employment opportunities and increased wages [6]. It has also been documented that successful interventions start with strong connections with employers [7]. However, very little detailed information is available about interventions that can make science programs in higher education more effective in training students for high-demand jobs while increasing program completion and job placement rates.

Traditionally, science labs have followed a protocol written in traditional lab manuals and mainly demanded discipline-specific knowledge. This method may not be suitable for science labs with the goal of training students for careers and better employability, where employers value skills such as communication, analytical thinking, and problem-solving [8]. Project-Based Learning (PBL), a student-centered instructional method, has been widely shown to successfully encourage students to direct their learning through inquiry and, at the same time, apply knowledge to develop real-world projects [9]. PBL strategies empower students to work together and develop their approach to scientific methods, where they begin with a clear objective; instructors facilitate and support the learning process. This strategy is effective for facilitating knowledge acquisition and retention [10]. In fact, Movahedzadeh et al. have reported that students in a biology course showed improvement in self-confidence and lab skills, in addition to high levels of satisfaction [11]. Another study by Yeh reported the

effectiveness of PBL in retaining adult learners [12]. Further, using PBL, students acquire and develop soft skills, such as solving complex problems, thinking critically, analyzing, and evaluating information, working cooperatively, and communicating effectively [13, 14].

This study aims to explore the integration and implementation of PBL in a biotechnology laboratory setting and assess its potential impact on various important factors, including student retention rate, development of essential skills, and subsequent employment rates. Based on previous research and theoretical frameworks supporting the effectiveness of PBL, we hypothesize that incorporating this active learning approach into the biotechnology lab curriculum will yield positive outcomes in terms of student retention, skill acquisition, and employment prospects. The utilization of PBL is expected to enhance student engagement, critical thinking, problem-solving abilities, and teamwork skills, all of which are highly valued in the biotechnology industry, making the graduates potentially more competitive and marketable in the job market. Furthermore, this study holds significant potential for informing educational practices and curriculum development in biotechnology programs. The outcomes will provide compelling evidence for the integration of PBL in laboratory settings, serving as a valuable resource for educators and institutions seeking to enhance student learning experiences and improve post-graduation outcomes. Moreover, the findings may contribute to broader discussions on the effectiveness of active learning strategies in STEM education and serve as a catalyst for further research.

Methods

Experimental design

Data was collected over five semesters (two and a half years; 2019-2022) from 52 students (age range: 18-42 years old; average age: 25 years old; over 75% Hispanic - the main underrepresented group) to test the effectiveness of PBL strategy in biotechnology labs. In this 16-week training program, students learn discipline-based knowledge in the lecture and technical skills in the lab in the first eight weeks of the course. In the second eight weeks of the course, students continue with lectures while they apply acquired skills by designing lab projects with real-world applications.

Students were grouped into diverse teams of 3-4 members to design the projects. They were then asked to propose a project relevant to them and their communities. Examples of projects that the students designed were "production and characterization of green nanoparticles," "testing immunoglobulins in saliva," and "reducing bioburden in wastewater." Students were asked to formulate a hypothesis and then propose their experimental design to begin the process. After iterative discussions to optimize the experimental procedure, students conducted the experiments by applying the lab skills learned in the first eight weeks of the semester. Techniques used included enzyme-linked immunoassay (ELISA), polymerase chain reaction (PCR), spectrophotometry, etc. The group projects engaged students and encouraged the use of soft skills as they collaborated to complete projects in a timely and professional manner through guided facilitation by the instructor. At the end of the semester, students were asked to prepare a research poster on their findings. The posters, presented at a poster exhibition organized by the instructor, provided students with the opportunity to showcase their work to industry partners, peers, family, and community members.

In addition to the integration of the PBL, industry partners were invited to the class to speak about job opportunities and required skills. The instructor also had the opportunity to work with the students to prepare their resumes and conduct mock interviews. Actual interviews were set up with the industry partners before the semester ended on campus.

Assessment of student outcomes

A comprehensive survey was administered to assess student outcomes at the conclusion of each semester. The surveys encompassed various aspects such as critical thinking skills, teamwork abilities, motivation to learn, enhanced communication, effective time management, a sense of accomplishment, and improved problem-solving capabilities. The survey questions utilized in the assessment were carefully selected from a diverse range of general surveys to ensure comprehensive coverage of the specific needs and requirements of the PBL design for this particular project. By drawing upon a variety of survey sources, the questions were designed to capture the multifaceted aspects of student learning and to identify the specific areas where PBL could effectively address those needs. This approach ensured that the survey instrument was robust and aligned with the goals of implementing PBL and its impact on student development and achievement.

In addition, after each semester, detailed records were gathered to obtain accurate student attendance and retention rates. Furthermore, as part of the assessment process, students were also given the opportunity to express their preferences regarding the instructional approach. Specifically, they were asked to provide feedback on their preference for PBL as opposed to traditional laboratory exercises. This additional input from students allowed for a more holistic understanding of their learning experiences and provided valuable insights into the effectiveness and impact of different instructional methods.

Results

Fifty-two students were surveyed with 100% return on responses. The survey questions rated their critical thinking skills, teamwork, motivation to learn, improved communications, time management, sense of accomplishment, and enhanced problem-solving skills. Students were asked to reflect on the criteria above at the end of the semester and report how much they enhanced each skill. The majority of students reported that they have improved "exceptionally" in all criteria, and fewer students reported their improvement as good or fair (Figure 1).

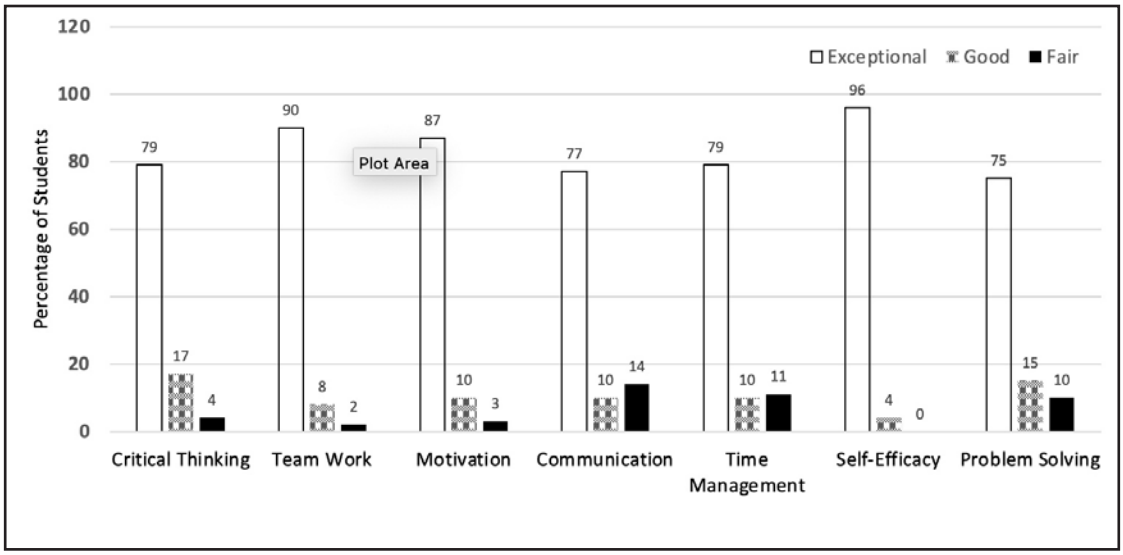

Fig. 1. Skill sets of students developed during PBL included critical thinking, teamwork, motivation, effective communication, time management, sense of accomplishment reported as self-efficacy, and problem-solving skills. The numbers represent the percentage of students grouped as exceptional, good, and fair. As shown, 75% of students (n=52) rated exceptional improvement in all criteria.

Furthermore, 94% of the students indicated their preference with PBL over their previous traditional science labs. Additionally, as shown in Figure 2, the "perfect attendance" data was 89%, and the "student retention" rate was 94%.

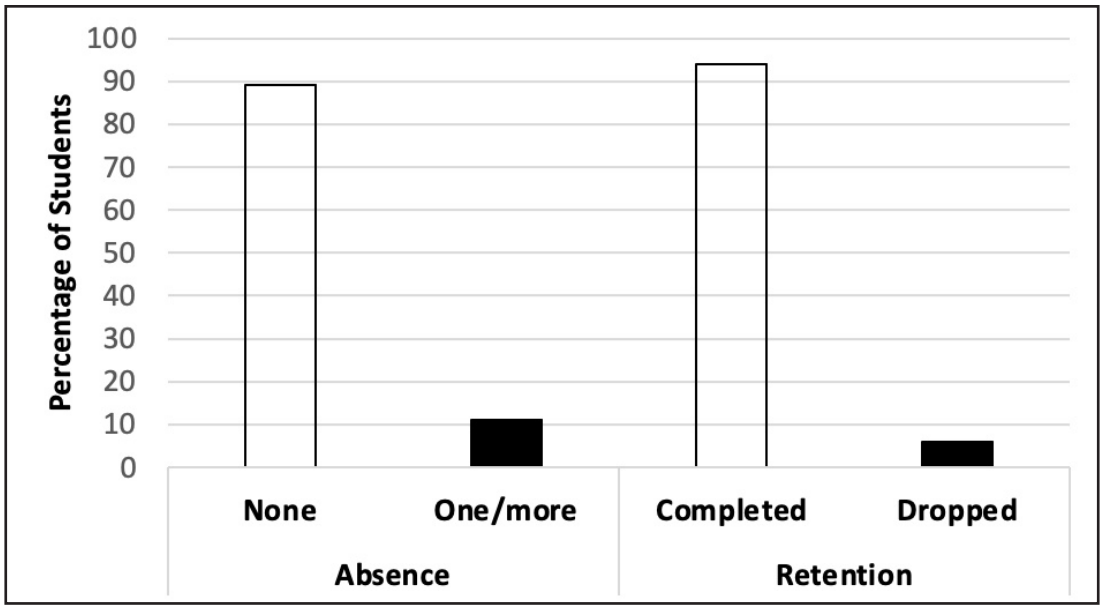

Fig. 2. Effect of PBL on attendance and retention of students in the class. The absence reported was 1-3 during the semester. Retention reflects the percentage of students with completion of the course. (n=52)

Implementing PBL fostered a more dynamic and interactive relationship between students and instructors, facilitating a thorough evaluation of individual students' skills and enabling better preparation for interviews with industry partners. This approach enhanced retention rates and resulted in a notable improvement in students' skill sets. Furthermore, the employment rate following each semester remained consistently high, with a minimum of 90% of students securing employment within two and a half years. Remarkably, even during the challenging times of the pandemic, the employment rate surged to an exceptional 100%. This remarkable achievement speaks to the effectiveness of the PBL approach in equipping students with the necessary knowledge and practical skills sought by employers, ensuring their successful transition into the professional world.

Discussion

Project-based learning is an active learning strategy that has been demonstrated to increase student motivation and self-efficacy [15]. Self-efficacy is a key element of the social cognitive theory that appears to be an important variable because it affects students' motivation and learning [16]. Consistent with results published by Shin, in our study, the self-reflection outcomes on contextualized skill set (critical thinking, teamwork, motivation, improved communication, time management, sense of accomplishment, and problem-solving) were also rated mostly exceptional by the students indicating a sense of enhanced self-efficacy and motivation [15]. In addition to the inclusion of some survey questions adapted from Shin [15] and incorporating feedback from industry partners gathered during advisory board meetings, it is essential to emphasize the limitations of the survey used in this study and the critical need to assess the reliability and validity of the survey in future studies.

PBL also created an opportunity for students to collaboratively engage in a time-limited science experiment and be able to communicate and provide feedback to their team members. Basic knowledge and skills are identified to be essential by employers [17], which our industry partners also emphasized. In fact, according to the National Association of Colleges and Employers, soft skills, in particular critical thinking skills, are the top priority for an employer to hire someone [18].

Motivation and retention were additional factors rated very high in our study. Our results support the findings of Beier et al., where utility value of PBL in STEM courses was examined [19]. They found that PBL in at least one course affects the utility value of participating in STEM courses and STEM career aspirations. In addition to gaining skills required by employers, our findings show that PBL strategies enhance the students' interest in the sciences, which also resulted in a very high employment rate. Our placement has been above 90% within two and a half years. Due to a shortage of workers in STEM, community colleges are being called on to address the persistence of minorities in the STEM fields [3]. Palmer and Wood further report a low number of underrepresented minorities in these fields [3]. The community colleges with primarily enrolled underrepresented minorities are in a great position to train students in STEM disciplines. They can make a meaningful impact on student interest, as well as retention, in the sciences.

Conclusion

Project-based learning in the college science labs provides students the opportunity to approach coursework with curiosity and motivation for the sciences in a collaborative, team-based approach. This strategy will result in skills valued by the employers and hence an improved job placement rate. This study demonstrates that the PBL approach addresses the skills gap in science labs, providing a bridge from classroom to career. In addition, this approach will enhance the interest of underrepresented minorities in STEM disciplines, which may lead to closing the equity gap.

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Disclosures. The authors declare no conflicts of interest.

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Igniting Creativity: Hackathons for Developing Undergraduate Research Projects in Antibody Engineering

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Abstract: Life science organizations are increasingly using hackathons to bring communities together to tackle shared problems, teach skills, and develop new resources. In this study, we explored the potential benefits of hackathons for the biotechnology workforce education community by organizing two hackathons centered around developing research projects in antibody engineering—a practice widely employed in the biotechnology industry but uncommon in biotechnology education. To integrate antibody engineering into courses, instructors need protocols for both computational and laboratory methods. Developing and testing these protocols provides rich opportunities for undergraduate research, allowing students to learn industry-relevant skills and contribute to creating materials for the community. During the hackathons, teams of faculty, students, and industry partners collaborated to generate several new research projects. Each hackathon was only a few days, yet student participants reported benefits similar to those attributed to traditional undergraduate research experiences. We share lessons learned from these hackathons and provide insights for the workforce education community for hosting similar events.

Keywords: antibody, CURE, hackathon, SARS-CoV-2, machine learning, epitope, iCn3D, undergraduate research

Introduction

Undergraduate research enhances student retention and graduation rates, especially for minority students in STEM fields [1-3], but community college students often lack opportunities for such valuable experiences due to limited resources. With 38% of undergraduate students attending community college [4], it is important to find ways to address this disparity.

Course-based undergraduate research projects (CUREs) have been proposed as a method for increasing undergraduate research experiences (UREs). Including research projects in courses enables students to use scientific practices, make new discoveries, and contribute to knowledge [5, 6]. Implementing CUREs, however, requires funding, professional development for instructors, and course modifications.

In the context of college biotechnology and biomanufacturing programs, CUREs also need to align with learning goals designed to meet the demands of local industries. For simplicity, we refer to these programs as biotechnology programs in the rest of this article. Students in biotechnology programs require UREs or CUREs that offer opportunities to develop skills essential for the workforce. Research projects focused on antibody engineering can effectively address this need, as they involve both bioinformatics and laboratory techniques.

To develop antibody-engineering research projects while providing the professional development needed for implementation, we looked for a cost-effective platform that could be scalable, national, and informed by current industry needs and practices. We also wanted to start building a community of instructors with a shared knowledge base, both to improve sustainability and provide a way for instructors to get help.

Using Hackathons to develop CUREs for Antibody Engineering

We are piloting hackathons as a new approach to enlist the community in developing biotechnology-related undergraduate research experiences (UREs) and course-based undergraduate research projects (CUREs) that align with industry needs. Research projects in engineering antibodies are particularly relevant to the industry since they provide opportunities to engage in industry practices.

Hackathons are intensive, short-term events where teams collaborate to generate solutions or create prototypes. Hackathons have become increasingly popular in bioscience communities for fostering collaboration, inspiring creative thinking, building community, and tackling significant challenges. They are a regular feature at scientific conferences like BioIT World [7] and ISMB [8]. Government institutions, including the National Institutes of Health (NIH), routinely use hackathons to develop web applications and facilitate large-scale collaborations [9]. Since 2016, the NIH has organized over 40 hackathons with over 3000 participants, resulting in over 20 publications (Allissa Dillman, personal communication).

Despite their effectiveness, hackathons have not been explored in the ATE (Advanced Technological Education) community. We wanted to determine if hackathons could help us meet our goals of building community, providing professional development, and catalyzing the development of innovative projects around antibody engineering. Since collaboration is already the norm in biotech companies and a growing practice in the bioscience community [10], we also sought to foster teamwork and model a collaborative and supportive environment.

Why focus on Antibody Engineering?

Antibodies are one of the most important types of molecules that biotechnology programs address. These proteins, made by animal immune cells, bind to specific three-dimensional shapes (epitopes) on proteins and other molecules. By fusing an antibody-producing cell with a tumor cell, an immortal cell line capable of secreting identical antibodies (monoclonal antibodies) can be generated. Antibodies can also be produced by introducing plasmids with antibody genes into bacteria, yeast, and mammalian cells.

In biotechnology and biology research, antibodies are ubiquitous. In research, antibodies are used as reagents for detecting and/or purifying other molecules. In biotechnology, antibodies are made into drugs and diagnostic products such as home pregnancy tests [11] and tests for COVID-19 [12].

When it comes to therapeutics, monoclonal antibodies are the largest class of biopharmaceuticals on the market. Over 165 antibody-based drugs have either been approved by the FDA or EU regulatory agencies or were undergoing regulatory review in March 2023 [13]. All therapeutic antibodies have been engineered [14]. Daclizumab, one of the first engineered antibodies, received FDA approval in 1997 [15, 16]. This antibody was derived from mice but modified to resemble a human antibody by substituting mouse amino acids with their human counterparts, a process known as humanization. Humanization is used to minimize the risk of an immune response against the drug.

Antibody engineering encompasses making amino acid substitutions to improve physical characteristics like solubility, alterations in the binding site to improve specificity and affinity, conjugation with toxic molecules to enhance drug activity, and changes designed to optimize antibody manufacturing [14]. Additionally, many new antibody drugs are engineered to be bispecific, enabling them to bind two different antigens [17]. Engineered antibody genes can also be introduced into T cells as chimeric antigen receptors (CARs), designed to trigger an immune response against tumors.

Tools for Antibody Engineering

Web-based computational tools and databases have become valuable resources for structural biology research and education. Tools like iCn3D [18], the Immune Epitope Database (IEDB) [19], and SAbPred [20] offer molecular modeling, epitope analysis, and antibody structure prediction capabilities, respectively. These tools are freely accessible online and provide researchers with visualization and manipulation capabilities, as well as analysis and prediction tools. iCn3D, from the National Center for Biotechnology Information (NCBI, https://www.ncbi.

nlm.nih.gov/), is a web-based molecular modeling tool for visualizing and manipulating molecular structures. IEDB offers analysis tools for studying the molecules recognized by antibodies [19]. SAbPred, developed by the Oxford Protein Informatics Group (OPIG), utilizes deep learning to predict antibody and nanobody structures, facilitating model generation, prediction, mutation analysis, and structure comparisons [20].

On the laboratory side, non-profit organizations like AddGene (Addgene.org) and government entities like the National Institute for Standards and Technology (NIST, NIST.gov) offer biological materials such as plasmids with antibody genes [21] and standardized cell lines [22] that can provide starting materials for antibody projects. AddGene has over 1362 plasmids with antibody genes, including genes for nanobodies. Nanobodies are stable, low molecular weight proteins (~15 kilodaltons), with a single protein chain derived from antibodies found in camels, llamas, and alpacas [23]. NIST recently developed a standardized version of the Chinese Hamster Ovary (CHO) cell line. CHO cells are the most common industrial system for manufacturing antibodies [24].

Not only are biological materials and computational tools available for educational use, but the process of antibody engineering offers multiple steps that can serve as starting points for research projects. Computational projects can involve molecular modeling, prediction, and plasmid design, while laboratory projects can explore purification methods, detection techniques, screening, assay development, specificity, binding strength, glycosylation, and more.

Computational projects also offer flexibility, as they can be carried out remotely, making them suitable for online courses or students unable to attend in-person classes. Furthermore, the low start-up costs for computational projects enable colleges with limited resources to engage students in cutting-edge research. Projects can also be divided into phases, with some work conducted in a physical lab and computational work carried out remotely.

Antibodies in Biotechnology Education

At least 364 US employers in 579 locations list antibodies as a key business area (https://biotech-careers.org/company-core-activity/antibodies), so it is not surprising that many biotech programs teach antibody-related laboratory skills. These include culturing mammalian cells, upstream processing (producing antibodies from CHO cells and monitoring fermentation), and downstream processing (antibody purification). These skills are important for biomanufacturing technicians who manufacture antibodies for therapeutics, diagnostics, and reagents. Analytical skills such as protein gel electrophoresis, protein assays, Western blots, enzyme-linked immunoassays (ELISAs), fluorescent antibody staining, and, more recently, flow cytometry [25] are also taught. These types of analytical skills are used by laboratory technicians in both biotechnology companies and research labs.

Antibody engineering, however, has not been part of two-year college biotech programs. Given the large number of companies that manufacture antibody-related products and the increasing use of antibody engineering in industry, it is important for instructors to learn more about these technologies. The rapid development of artificial intelligence and machine learning tools are revolutionizing the antibody development process. Soon it will be possible to create novel antibodies without immunizing animals.

If we are to use these powerful, new computational tools for antibody design in the classroom, instructors will need professional development. Others will need protocols and guidance for implementing laboratory techniques. Creating research projects from new materials and tools requires another level of learning, familiarity, and practice.

Materials and Methods

Participant recruiting

Participants were recruited through the InnovATEBIO newsletter (https://innovatebio.org/newsletters) and website (https://InnovATEBIO.org), our website (Antibody-Engineers.org), and announcements by QUBES (QUBES.org) and BioMolViz (BioMolViz.org). We used MailChimp (MailChimp.com) to set up an embedded form in our website so visitors could subscribe and receive emails about registration and updates. We also gave presentations to faculty through InnovATEBIO's webinar series on ATE projects [26], to students participating in the MNT-CURN research project (DUE 2000281), and to faculty in a weekly teaching discussion hosted by the California Bioscience Workforce Development Hub.

Communication and publishing platforms

We used several Google apps (Google.com) during the events: Google Forms for submitting applications, Google Sheets for reviewing applications and assembling teams, Google Drives for housing collections of materials, Google Slides for presentations, Google Docs for documenting results, and YouTube for sharing videos. Hackathon teams used Slack (Slack.com) for discussions, messaging, and sharing documents. Slack is a communication platform commonly used in the biotech industry and by research labs. We used Zoom (Zoom.com) for meetings with breakout rooms for each team and software demos.

Project records were assembled and published through QUBES (https://qubeshub.org/). QUBES is a content management platform that allows teams to work together and publish their results. Materials from coding-related projects were managed in GitHub (GitHub, https://github.com/AntibodyEngineers).

Machine learning resources and molecular data

We used the NSF-supported Jetstream (https://jetstream-cloud.org/) computing resources for our machine learning project. Jetstream provides eight petaFLOPS of supercomputing power to simplify data analysis, boost discovery, and increase the availability of AI resources. Datasets were from the Oxford Protein Informatics Group (OPIG; opig.stats.ox.ac.uk) CoV-AbDab in addition to the OPIG Ablang ML package. Other molecular data were obtained from the NCBI structure database and IEDB.org. Sequences from SARS-CoV-2 variant spike proteins were obtained from the NCBI. iCn3D and other analysis tools were accessed through the NCBI and IEDB databases.

Laboratory materials

Materials for working with antibodies included a yeast (Saccharomyces cerevisiae) library obtained from Protabit that produced antibodies to the SARS-CoV spike protein, SARS-CoV-2 spike protein (Genscript), protein G magnetic beads (MedChemExpress), a plasmid producing antibodies to GFP (N8his-GFPenhancer-GGGGS4-LaG16) (Addgene), a mouse anti-histidine tag (Bio-Rad), and ELISA reagents (AssayPro and ThermoFisher).

Results from hackathon participants

Hackathon logistics

We hosted two Antibody Engineering hackathons in 2022: the first in January (Thursday, Jan. 13th - Sunday, Jan 16th) and the second in August (Monday, Aug. 8th - Thursday, Aug. 11th). While most of the hackathons were virtual, the Affordable Antibody Engineering project was held in the lab at Los Angeles Pierce College in January and at both LA Pierce College and Pasadena Community College in August. The virtual format made the events cost-effective, with no travel or lodging expenses.

About six weeks before the hackathons began, we reviewed applications, assigned applicants to projects, and set up accounts in Slack. Both hackathons followed similar schedules. Each day began with a full group meeting, followed by guest speakers and software demonstrations. The first day focused on introducing the hackathon, goals, logistics, and team introductions. On the second day, teams presented their project plans, and writers attended a meeting to learn how to document the process and their results. The third day allowed teams to discuss obstacles and seek assistance, with daily meetings for planning, discussion, and coordination. All teams presented their work on the final day.

In January, guest speakers introduced antibodies and discussed course-based undergraduate research experiences, antibody manufacturing, antibody validation, and IEDB. In August, speakers discussed engineering antibodies, engineering antibody-producing cells, and engineering antibody-related careers. Software demonstrations included iCn3D, NextStrain.org, SabDab [20], QUBES, and IEDB.org. We recorded the talks to create a library of materials for future participants (Antibody Engineering Hackathon 2022 Playlist https://www.youtube.com/playlist?list=PLSAXB_etwzD9dl3gN4hgCUtAdspV5FfP9).

A common practice in NIH-sponsored hackathons has been to organize the event around an overarching topic

with multiple subprojects [Allissa Dillman, personal communication]. This structure provides a mechanism for rapid prototyping and testing multiple ideas. Including multiple related projects also provides a broader wealth of information sharing since participants hear presentations on all the projects. A last reason for including multiple projects is to make sure the teams don't get too large. It's important for everyone on a team to have a specific role that gives them a chance to learn, participate, and contribute without the pressure to compete with others for something to do.

The January hackathon offered five projects, while the August hackathon featured six. Teams consisted of 3 to 7 members. Project selection was influenced by team leader interests, input from our industry advisory board, and suggestions from applicants. During the hackathons, team members choose different roles to facilitate collaboration. Roles like leader, writer, technical support, database expert, researcher, subject matter lead, and quality control are suggested, along with roles like artists, slide makers, and technical writers. Having multiple roles makes it possible for all members to contribute no matter what their experience level. Other hackathons, such as the Bio-IT World 2023 Hackathon, use roles such as: Data scientist/Analyst, Researcher, IT developer, Entrepreneur, Policy Change, and Consultant/Advisor [7].

Participants

Most hackathon participants were community college students and faculty, with others from four-year colleges, universities, high schools, and industry advisory board members (Table 1). For this study, we defined "participants" as people who registered and participated directly on a hackathon team. Over half of the participants were women (59% and 55% in January and August, respectively), and 44% of survey respondents identified as non-white.

Two classes of high school students were also part of the January hackathon. They were unable to participate in August due to a conflict with their schedule. Since they were not registered, were unable to attend all the events and interacted with the group through their teachers, their experience was not directly comparable to other participants, therefore we did not include their survey results in this report.

Table 1. Summary of participant demographics

Position	Institutional affiliation	Jan 2022	Aug 2022
Students	Community college	11	17[c]
	University	2	1
	High school	(79, 2 classes)[a]	2
Faculty	Community college	11	11[c]
	University	5	4
	4 yr college	1	
	High school	4	1
	Industry / Research Institute	5	4[c]
	Total registered	39	40
Gender	Woman	23	22
	Man	15	17
	Transgender	1	1
Race / ethnicity[b]	Asian		7
	Black or African American		4
	Hispanic or Latino		5
	Middle Eastern or North African		2
	Multiracial or multiethnic		1
	White		17
	Answered		30
	Skipped		3

[a]Data from the two high school classes were not included elsewhere since their participation was through their teachers. [b]Race and ethnicity data are from the August 2022 post-hackathon survey. We did not collect this type of data in January. [c]Seven participants (two community college students, four faculty, and one industry advisor participated in both January and August hackathons).

What did participants think?

We surveyed participants at the end of each hackathon and obtained 32 responses in January and 30 in August. Participants described positive and negative aspects, identified the top skills they learned, and indicated their interest in continuing to collaborate on their projects. Students were asked if the hackathons allowed them to practice professional skills such as communication, problem-solving, teamwork, and leadership.

Survey respondents from both hackathons listed their favorite aspects in open-ended comments. The most common responses were learning, collaboration, networking, and discovering new resources [Fig. 1]. Working with multi-generational teams with different experience levels was also cited as a positive factor. One participant shared that "My favorite aspect was being able to talk to industry experts and professors with industry experience who could provide real-world experience on each process I didn't understand."

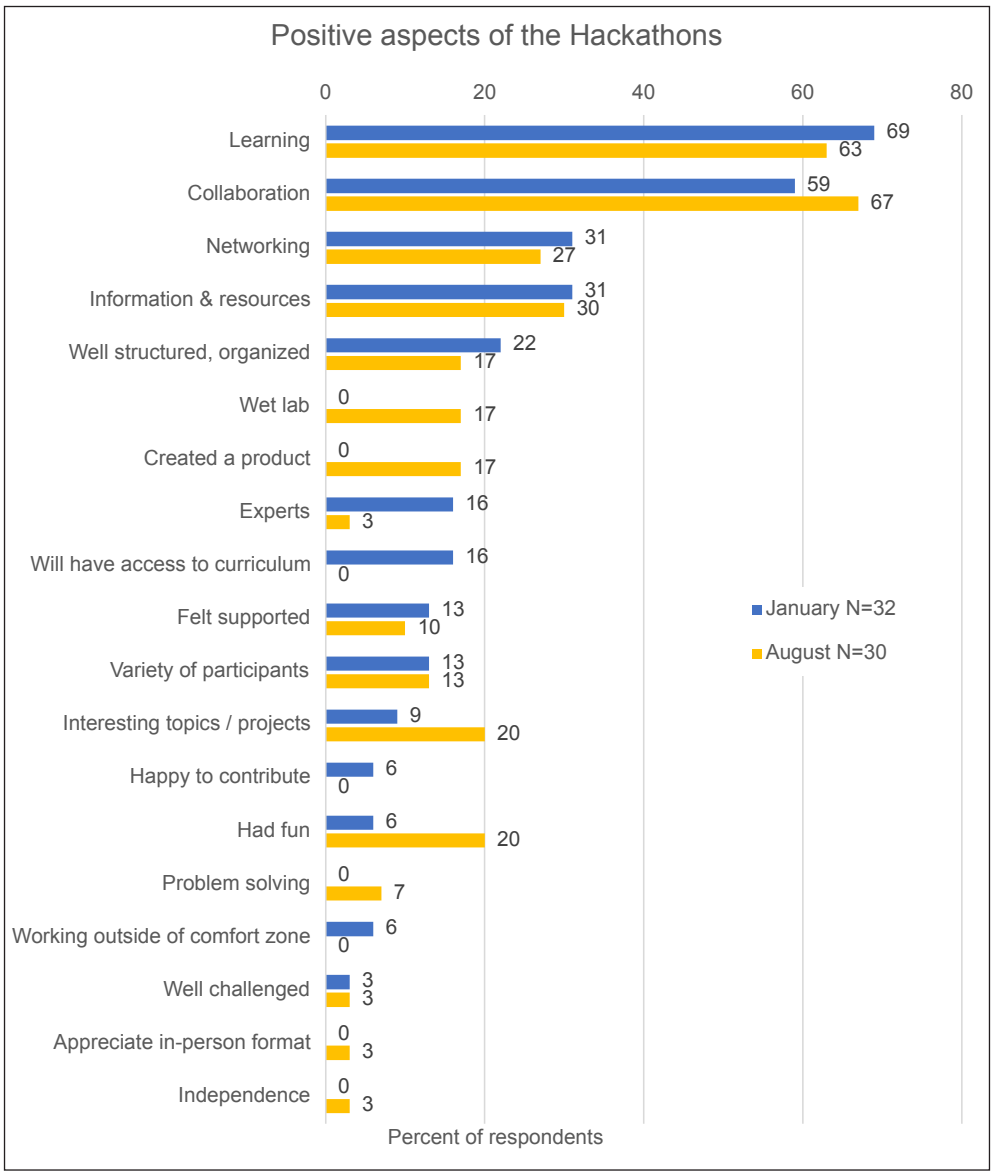

Figure 1. Respondents were asked to identify their favorite aspects of the hackathons. The results are shown as percentages to facilitate comparison.

About a third of the respondents shared negative aspects (Jan N=12, Aug N=11). Some participants found it challenging to manage their time (Jan N=5/32, Aug N=1/30). Some found QUBES confusing (Jan N=4/32). A few respondents [Jan (2), Aug (3)] felt that they were rushed and trying to complete too much work in too short a time and that the material was more advanced than they expected. Two cited a lack of help, and one was disappointed that there wasn't a coding component in their project.

What did hackathon participants learn?

We asked participants to identify the top three things they learned during the events. The software (iCn3D, NextStrain, Slack) and databases (IEDB, OPIG) were listed most often with antibodies and related topics next (Fig. 2). Interestingly, teamwork and collaboration skills were also frequently mentioned as areas of learning [Jan (8), Aug (5)].

Respondents from the January (31/32, 97%) and August (32/33, 97%) hackathons said they learned something new. Additionally, 91% (N=29/32) of the respondents from January and 74% from August (23/31) said they learned new things about themselves. Roughly 20% from both hackathons shared comments related to self-efficacy, stating "I can do this" or that "I love working in a lab." Some participants mentioned discovering potential career paths. Statements to this effect included, "I learned that I could possibly look into protein engineering as a career," and "I learned that Bioinformatics might be a next career for me after my graduation from college." At least one student enrolled in a community college biotechnology program after the January hackathon, suggesting a potential role for hackathons in student recruiting.

Many respondents agreed with statements that indicate a sense of belonging in the scientific community, a positive attitude towards collaborative science, and self-efficacy. Over 90% agreed their input was respected, they were more excited about collaborative science and were more confident about participating in future hackathons (Fig. 3). Over 85% worked outside their comfort zones yet stayed with their projects, demonstrating perseverance.

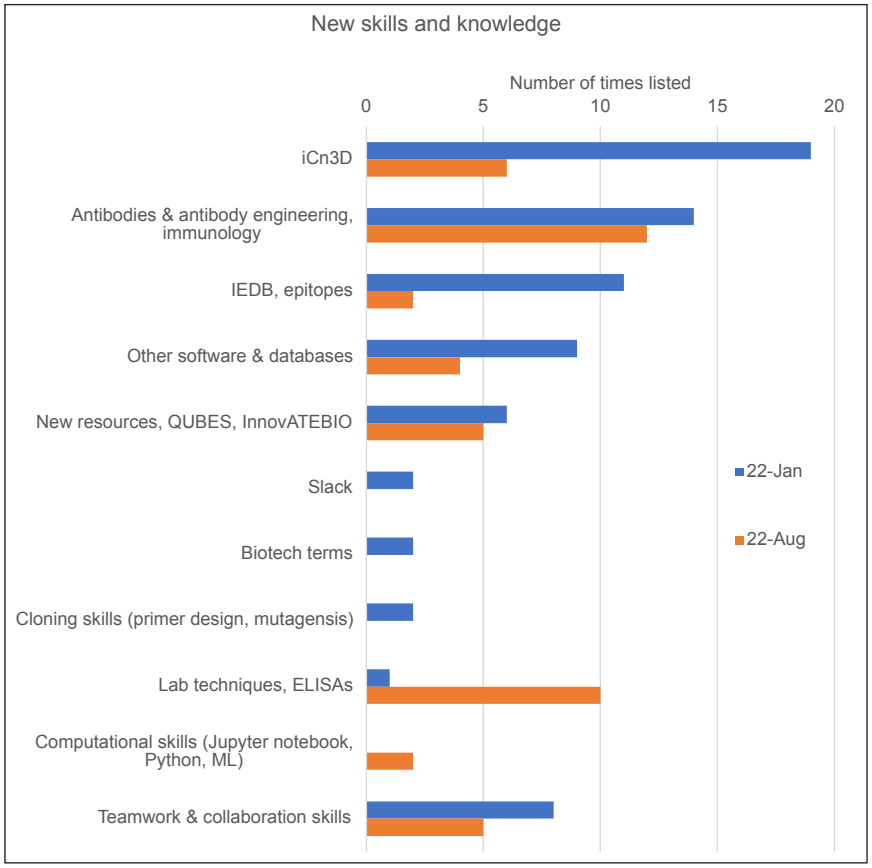

Figure 2. Number of participants listing skills and knowledge learned in the January and August hackathons.

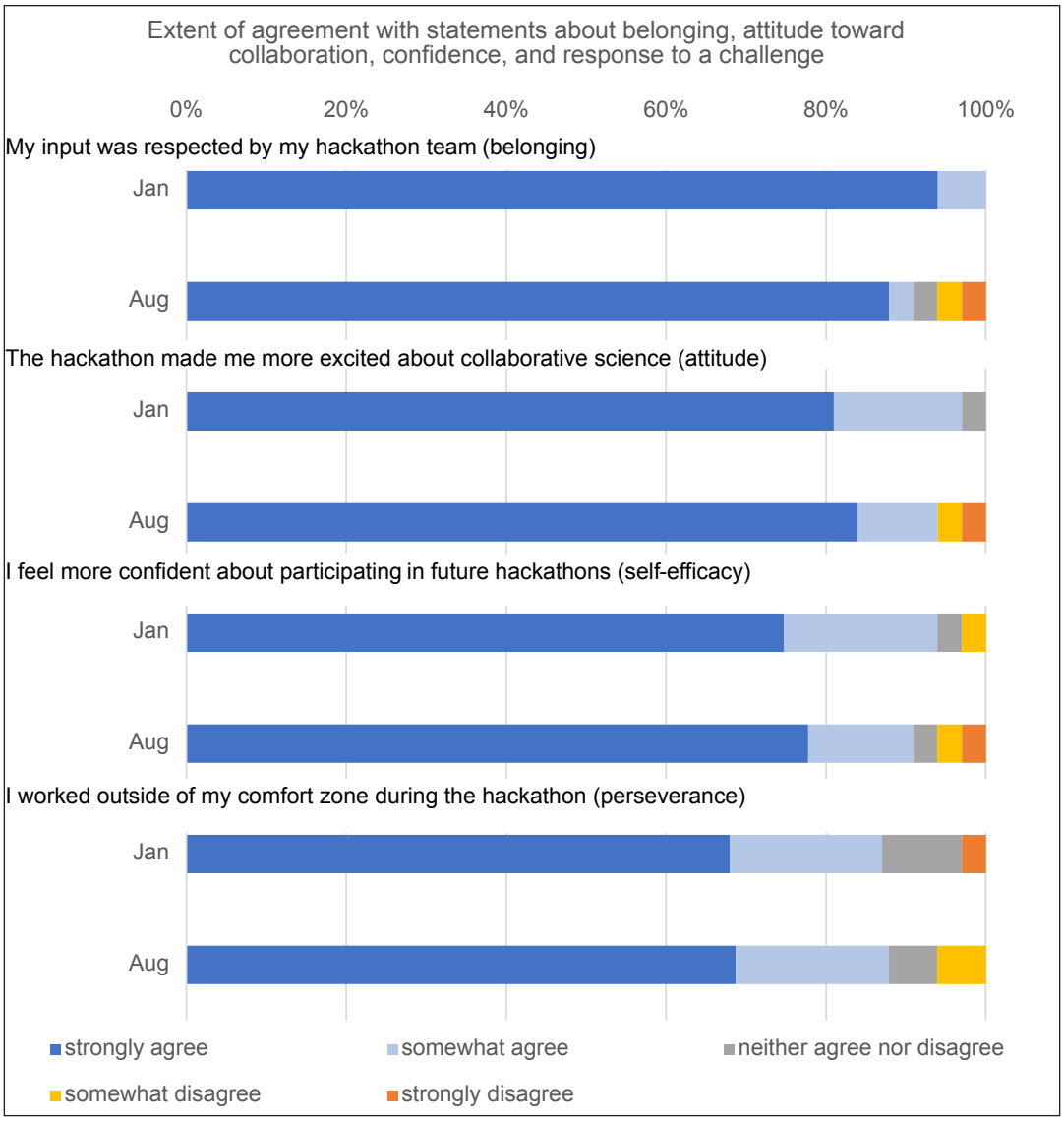

Figure 3. Percent of respondents who agreed or disagreed with statements related to belonging, collaborative science, self-confidence, and working outside their comfort zones (N=31 for both January and August).

Professional skills

Our primary goal for the hackathons was to develop research projects that can be incorporated into courses. Although hackathons are only a few days, we wondered if the research aspects and collaborative nature of working on the projects might provide similar benefits to students as undergraduate research.

We asked student participants whether they agreed with statements about practicing professional skills during the hackathon (Fig. 4). Students from both hackathons agreed they were able to practice communication, teamwork, and problem-solving. Nearly 70% agreed they had practiced leadership skills. All the students from the January hackathon and 69% from August planned to include the event on their resumés.

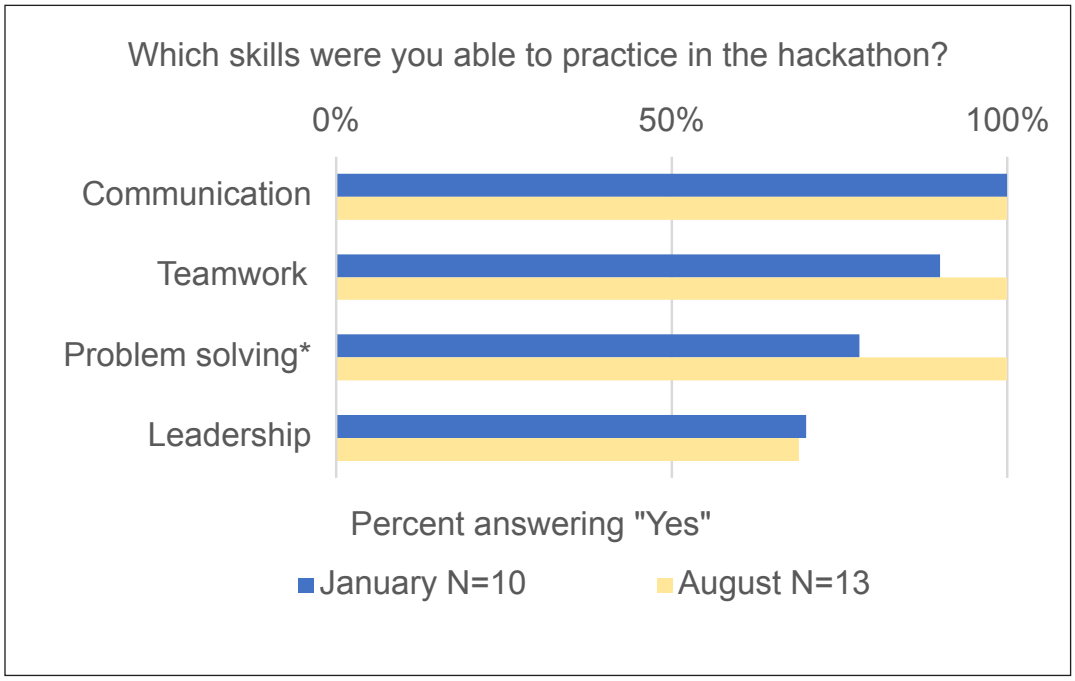

*Figure 4. The percent of students who agreed they had been able to practice professional skills. *Nine students from the January hackathon answered the question about problem solving.*

Results from hackathon projects

The January (5) and August (6) projects are listed in Table 2. Several projects have been used with classes or for undergraduate research as shown in the last two columns.

Table 2. Hackathon projects and outcomes

Hackathon	Project	Project Goal	URE	Used in class
Jan, Aug	Affordable Antibody Engineering	Develop a low-tech method for screening antibodies.	X	X
Jan, Aug	Immune Epitope Database projects	Develop research projects that use IEDB.org.	X	X
Jan, Aug	SARS-CoV-2 vs Antibodies	Predict whether antibodies will neutralize SARS-CoV-2 variants.	X	X
Jan	Break an Antibody	Develop mutagenesis strategies for disrupting antibody binding.	X	X
Jan	iCn3D for Education	Investigate how iCn3D might be used in high school and college.	X	X
Aug	Immuno-Zoo	Create a data set of antibody structures for comparing different features.		
Aug	Machine Learning, Artificial Intelligence (AI) and Antibodies	Develop an antibody data set and classroom examples to help students better understand machine learning and AI.		
Aug	Antibody Company Game	Help develop and test a game based on the process of drug development.		X

Affordable Antibody Engineering

This team explored methods for screening cells to identify high-affinity antibodies. In January, the team used a method where antibodies are displayed on the surface of yeast cells (Saccharomyces cerevisiae). If yeast cells produce high-affinity antibodies, they bind to the antigen and can be captured with iron beads and magnets. Initially, this work focused on identifying antibodies to the SARS-CoV-2 spike protein. In August, the project pivoted to working with nanobodies that bind to Green Fluorescent Protein (GFP). The screening laboratory protocols are currently being developed through undergraduate research by students at LA Pierce College.

Immune Epitope Database (IEDB) projects

This team explored using IEDB to predict good epitopes for creating vaccines and looked at using the Influenza Research Database to find and analyze protein sequences from influenza strains in different parts of the US. They also developed an activity (NetChop, [27]) that uses the IEDB Immunome Browser and an epitope prediction tool, DiscoTope. In the NetChop project, students predict B and T cell epitopes using SARS-CoV-2 as a model. This project is designed to improve student understanding of protein structure and the different responses of B and T cells to epitopes.

Break an Antibody

Our advisory board suggested this project. Successfully engineering an antibody requires modeling the chemical interactions between an antibody and an epitope and evaluating the results of potential changes. Our board suggested it would be easier for students to engineer changes that disrupt binding than it would be to determine if the binding was improved.

In this project, a student identifies key amino acids in the paratope and models the changes in chemical interactions in iCn3D when they are replaced with different amino acids. This process is like the work shown in Fig. 6. The ability of an amino acid substitution to disrupt antibody binding could be tested in vitro by comparing the binding ability of the mutant with the original antibody.

This team used Drugbank.com to identify therapeutic antibodies with available protein sequences and explored three different antibodies: an anti-GFP nanobody and two antibodies that are used as drugs (rituximab and cetuximab). They created drafts of learning objectives and core competencies that would be addressed through these projects.

ImmunoZoo

This team worked to compile a dataset of antibody structures that students could use to compare antibodies from different species. The team located antibodies from llamas and humans but found unanticipated challenges with interpreting database information, making this project more complicated than expected.

SARS-CoV-2 vs. Antibodies

This team developed a CURE based on work from an ISMB hackathon [28], where students would investigate commercially available anti-spike protein antibodies and use iCn3D to determine if they would protect against SARS-CoV-2 variants [29]. Each student has a different antibody. They start by annotating the antibody binding site in iCn3D (Fig. 5B). Then, they find a variant in NextStrain.org. Links from NextStrain to the NCBI are used to get the sequence of the variant spike protein.

The protein BLAST algorithm in iCn3D is used to align the variant sequence to a sequence of an older version of the spike protein bound to an antibody (Fig. 5A). A visual scan of the alignment shows mutations in the antibody binding site. Last, mutation prediction tools in iCn3D are used to model the effects of the mutation on the ability to form chemical interactions with the antibody (Fig. 6A).

In the example (Fig. 5, 6), a student would use the interaction data to create a list of predicted interactions between the original amino acid, in this case, a glutamic acid at position 484 (E484) in the spike protein, and amino acids in the antibody heavy and light chains. They would compare those interactions with the predictions for the A484 variant. Fig. 6A shows that four interactions are potentially lost: a hydrogen bond and contact with R50 (arginine) in the heavy chain, a salt bridge with R96 in the light chain, and a contact with Y101 (tyrosine) in the heavy chain. Losing the two interactions with R50 and the salt bridge, the strongest interaction, is likely to impair binding and allow this variant to escape from this antibody.

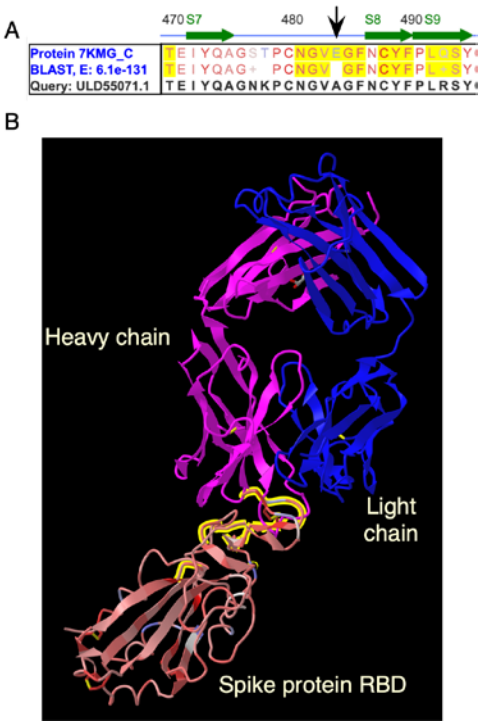

Figure 5. *Antibody binding sites in aligned spike protein sequences and a 3D structure model. Yellow highlights identify amino acids in the antibody binding site. A. Protein BLAST was used in iCn3D to align the spike protein sequence from 7KMG (top) with the sequence from ULD55071.1 (bottom). The black arrow points to a position where glutamic acid (E484 in 7KMG) is replaced by alanine (A484). B. The 3D structure shows the LyCoV555 antibody bound to the receptor-binding-domain (RBD) from a SARS-CoV-2 spike protein. The antibody heavy chain is magenta and the light chain, blue. The spike protein is colored blue, red, or pink depending on the similarity between the two spike proteins. View the annotated structure model in iCn3D using this link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?5hxNQQNL3A6zdNxaA*

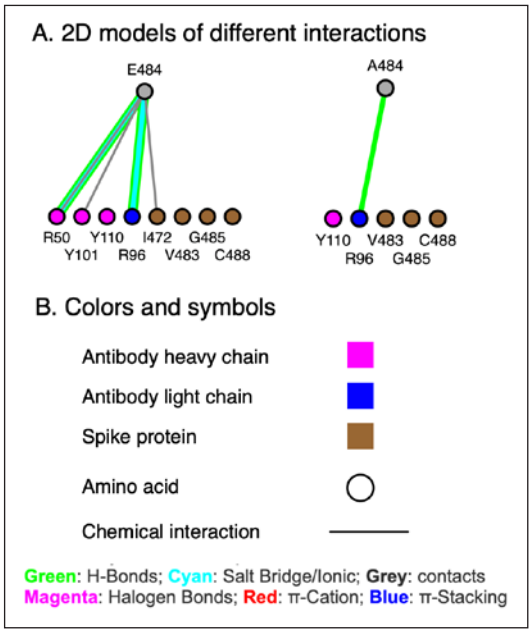

Figure 6. *2D models of chemical interactions between amino acids in iCn3D. A. Chemical interactions that differ between E484 and A484. B. Keys for identifying the symbols, colors, chemical interactions, and bonds. Shareable link: https://structure.ncbi.nlm.nih.gov/icn3d/share.html?zpMVHmnydzc4vuZS8*

iCn3D for Education

This group explored the educational applications of iCn3D for high school and college settings. One activity involved high school seniors using iCn3D to compare two antibody structures and create a Venn diagram noting their similarities and differences. The group also examined how antibody research projects could align with the 5E instruction model [30] (engage, explore, explain, elaborate, evaluate), Next Generation Science Standards [31], and Vision and Change [32]. They compiled datasets of antibodies bound to influenza hemagglutinin and SARS-CoV-2 proteins, as well as structures of Epstein-Barr viral proteins with an antibody.

Machine Learning, Artificial Intelligence, and Antibodies

The project aimed to make machine learning (ML) more accessible and relevant to biotechnology. ML concepts are often communicated at an expert level, using terms like statistical methods, neural nets, convolutional neural nets, and transformers. Moreover, the examples don't apply to biotechnology, often focusing on sorting dogs, cats, and handwriting samples. Our team chose to train an ML model to analyze the protein sequence of an antibody and determine if it can bind to the receptor binding domain of the SARS-CoV-2 spike protein.

For the hackathon, we set up CPU and GPU virtual machines (VMs) with accounts for 10 team members and installed the Python programming language (Python.org) and relevant Python libraries. The Python libraries included machine learning packages such as TensorFlow for computing and Pandas for data preparation. In addition to the libraries, Jupyter notebook software (Jupyter lab) was installed to give team members a web-based interface to develop and share code.

The team obtained protein sequence datasets from the Oxford Protein Informatics Group (OPIG; opig.stats.ox.ac.uk) - CoV-AbDab, which included 9276 relevant COVID sequences. The OPIG Ablang ML package was used for ML-based sequence analysis.

Some members had computing experience and were able to write scripts to clean data and work with the different Python libraries. However, only one team member had enough programming experience to build and test the ML prediction model. Other members were new to this type of computing and using Jupyter Notebooks, which unexpectedly required a significant amount of instruction time.

Regarding ML prediction, we preprocessed a dataset of 4000 sequences containing variable regions from neutralizing and non-neutralizing anti-SARS-CoV-2 spike protein antibodies into 768 attributes using the AbLang library. A first step in ML is to convert data into numerical n-dimensional vectors so that each datum is unique. The data were split into training data (3200 sequences) and test data (800 sequences). The training data were processed in the artificial neural network to build a model that distinguishes neutralizing antibodies from non-neutralizing. The test data were then used to measure the model's predictive quality. With 3200 training sequences, our model had a 71% accuracy. Training the model on a 16-core CPU took only five minutes.

Antibody Company Game

The Antibody company game team collaborated on an early version of a game (created through DUE 1764225) and focused on developing online gameplay mechanics. They designed Career and Data cards, established data costs, defined criteria for progressing through drug development phases, and tested the game. The game, Biotechopoly™ Antibody Edition, will serve as an educational tool for introducing biotechnology careers, business concepts, drug development, and reinforcing knowledge of GMPs and GLPs.

Discussion

We learned that hackathons can be an efficient platform for engaging a community in developing prototypes and testing new ideas. At the same time, the short intense nature of these events can be stressful, both for the organizers and the participants. Four of the organizers had participated in at least one hackathon and had some idea of what to expect. Nevertheless, being a hackathon host comes with a different level of responsibility than being a participant. In this section, we will walk through some challenges, discuss changes we made between the two events, and describe changes we will implement in future events.

Our first surprise was in recruiting participants. Over 70 people signed up for the first event, with 18 outside the US. Given our inexperience and small team, we decided to limit acceptances to US applicants. This helped minimize time zone challenges and allowed us to create smaller teams. We also added text to the application form to indicate eligibility.

We were surprised by the high number of student applicants and initially concerned about the dynamics of mixed student-faculty teams. Fortunately, the mixed teams worked remarkably well. The students' enthusiasm and innovative ideas regarding web technologies pleasantly surprised the faculty. In fact, faculty members considered the collaboration with students to be an unexpected benefit. Students brought great energy to the projects and, in some cases, took the lead in completing most of the work. According to surveys, they also developed a newfound appreciation for faculty efforts in curriculum creation and enjoyed the opportunity to collaborate as peers.

High school students

The most significant challenge arose a week before the first hackathon when we discovered a high school teacher planned to include two classes, totaling 79 seniors. Due to logistical constraints, the high school students had limited participation. School rules prevented the teachers from requiring them to attend during the weekend since those days were outside school hours. The IT policies at the students' high school also prevented them from using Slack.

To overcome these challenges, the high school teachers agreed to be liaisons to their hackathon teams in Slack and assumed leadership roles for their classes. Although the students couldn't fully engage in real-time activities, they were able to attend selected talks and daily Zoom sessions, meet with at least one scientist participant, and had access to session recordings. Additionally, Dr. Porter visited one of the high school classes and demonstrated how to find antibodies in the NCBI structure database and analyze them in iCn3D.

An unexpected benefit emerged when Dr. Menshew's class of 47 high school seniors worked with iCn3D and provided feedback to Dr. Jiyao Wang, iCn3D's lead developer, and a hackathon participant. The students were thrilled to discover that Dr. Wang incorporated some of their suggestions into the iCn3D program during the event. Furthermore, the high school students completed an assignment comparing different antibodies, which eventually led to the development of the ImmunoZoo project in August.

Communication overload

We learned in January that asking our participants to navigate between Slack, Zoom, QUBES, Google Drive, and Google Docs, in addition to learning GitHub, and the science-focused software and databases (iCn3D, IEDB, SabDab, NextStrain.org, and the NCBI) was too overwhelming. We modified our workflow in August by using a Google Drive specifically for the hackathon with a folder for each project. Instead of directing team members to post in QUBES, we had them use their team folder and provided instructions for using Google Drive and Google apps.

Changes between the first and second hackathons

Using the January survey data and our observations, we made several adjustments to the agenda for the second hackathon. These changes included:

- Asking applicants to agree to the time commitment (8 hours per day).
- Having the event take place during the week and scheduling fewer talks and more breaks.
- Adding team meetings to the schedule to ensure availability and help team leaders.
- Setting up Slack accounts for participants a month in advance with preparatory materials.

Despite these changes, the most common feedback from participants was the need for more background information on antibodies and the projects before the hackathon. Therefore, for our upcoming hackathon, we will provide additional background information and hold an orientation session two weeks prior to the event. This session will increase the likelihood that all participants understand how to use Slack and have the information they need to become familiar with antibodies.

The importance of personnel

We learned that hackathons work best when two crucial roles are filled. The first and most vital role is that of the hackathon manager. This individual possesses knowledge of the hackathon's timelines and is responsible for setting up the online environment. They answer questions, provide technical support, and guide participants when they face difficulties during the event. Crucially, the hackathon manager meets with the writers from each team and explains the process of documenting their work. This aspect is particularly helpful since having project information summarized and accessible in the designated Google folders makes the projects easier to complete.

The second key role is that of the team lead. Team leads organize team meetings during the hackathon and make sure that everyone has a voice and an opportunity to present. They assist in assigning team roles and managing the project scope, facilitating the team's ability to achieve results. The significance of team leads was highlighted in August when we attempted to handle too many projects, inadvertently leaving a team of two students without a leader. Although these students persevered, they admitted to feeling lost and frustrated. We learned from this experience and will avoid these situations in the future.

Conclusions

We investigated the use of hackathons as a platform for creating undergraduate research projects, with the accompanying goals of building community and facilitating learning. Through this work, we determined that hackathons are an effective format for achieving these goals. During the two hackathons, we initiated multiple projects. Two projects resulted in curriculum publications in QUBES [27, 29]. Some were paused after a single event, while other projects continued to be developed (Table 2).

In terms of community building, the intense and collaborative nature of the events fostered a shared experience and promoted a sense of community. Participants frequently mentioned learning, collaboration, and networking as highly positive aspects. Almost a quarter of the individuals who participated in the January hackathon returned in August. Moreover, two project teams continued meeting independently over the past year and plan to participate in August 2023.

Survey results indicated that nearly all respondents learned new things, with technical skills being a prominent area of growth. Additionally, participants highlighted learning about teamwork and collaboration. Some expressed surprise at the advanced material but ultimately appreciated the interactions with team members at various levels, demonstrating the value of hackathons as an environment for faculty to prototype new labs and obtain input from a diverse group of team members, from students to scientists.

While our primary focus was creating research projects for undergraduate students, we observed that student participants demonstrated outcomes similar to those attributed to undergraduate research experiences [6]. Their survey responses (Figs 3-4) indicated increased self-efficacy, a sense of belonging, stepping outside of their comfort zones, and enthusiasm for collaborative science, and they could make meaningful contributions. These findings may not be surprising since all the hackathon teams were engaged in short-term research projects. Two notable differences, however, were the short period of time and the focus on collaborative work, as opposed to project ownership. Consequently, hackathons can be a valuable practice for students preparing for careers in the workforce. They offer some of the advantages of undergraduate research while providing a more realistic model of industry practices that prioritize teamwork over individual research.

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How Classroom Gameplay Changes Teachers: Perceptions and Takeaways on the Use of Computer Games After a Classroom Intervention

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Abstract: While teachers are consistently asked to investigate new forms of technology, the use of computer-based games provides additional, unique issues. This research describes the changes in 12 elementary teachers' perceptions of games in the classroom after participating in an early algebra game-based intervention. Teachers implemented two computer-based games and one interactive tool as part of their daily mathematics lesson. They were also asked to guide their students through specific supplemental activities for out-of-game learning, which directly related to the content in the games. Surveys, classroom observations, self-reflection logs, and interviews documented teacher-student interaction during *Math Snacks* games. Findings reflect how the intervention changed teachers' views of games; their orientation to using inquiry in the classroom; their facilitation of technology; and their perception of including students with different abilities in gameplay. Participating teachers saw games as a tool to let students explore and introduce a topic with minimal initial guidance. Some teachers also noted the value of computer-based games in supporting low-performing students' integration and participation with the rest of the class. Teachers reported that students' collaboration and discussion skills were the primary competencies noticed while students were playing. Most of the teachers noted that their role as facilitators is essential n in the students' learning.

Keywords: game-based learning, teaching practices, educational games, mathematics education, learning pedagogies

Introduction

Studies on integrating educational games into the classroom are extensive regarding video games' positive or negative impact as learning tools [1-6]. Research depicts the use of computer games in the classroom as significantly impacting students' cognitive skills for topics such as mathematics and science [7-8]. The suite of games and animations in this research, called *Math Snacks*, were designed to encourage student learning of mathematics content. Research on the impacts of *Math Snacks* games reveals significant pre-post test gains in increasing student understanding of middle school mathematics concepts [9]. Despite the research conducted in the field of educational games, few studies investigate the role of teachers during the game implementation, which is surprising given the purpose of the teachers as true change agents in schools [10].

Researchers consistently suggest that teachers play an important role in facilitating learning with video games, particularly in how teachers guide the use of video games and debrief with students. Yet, teachers are often afraid to integrate the games into lessons even when they intuitively know the importance of their involvement when using video games [11]. Some teachers are uncomfortable moving beyond the simple practice of allowing students to play independently without guidance or lessons or integrating what students learn in games into the classroom [12-13]. Research on the use of commercial off-the-shelf titles documents many challenges teachers face, such as their reluctance to engage with games due to their lack of knowledge of best practices for integrating games within the classroom [14, 12, 15]. However, teachers' role as facilitators of game-based learning can be significant in the success of classroom-based gameplay.

The importance of the role of teachers as facilitators reflects their responsibility to enhance student learning with games and complementary activities, such as post-gameplay exercises, conversations to engage students, and scaffolding the use of educational tools and processes. De Freitas and Jarvis [16] emphasized the importance of the teacher's support in gameplay contexts and indicated the importance of embedding the games into learning contexts using support materials and debriefing or reflection activities to enforce learning outcomes; other activities like discussions and reflections about the learning and playing activities can also enforce students' learning with games. To facilitate these interactions, teachers need to know what students did in the game, what topics were addressed, and how these concepts were presented through the game and

complementary activities [8, 10]. It would follow that giving teachers an opportunity to better understand gameplay is an important part of classroom success.

Research reflects the need to study teachers' experience integrating education games in their classrooms. Important insights would aid in improving teacher training, enhancing in-classroom supporting strategies that accompany the game, and enriching role practices for teachers who want to use computer games in their classrooms [8, 10, 16, 17]. These teaching strategies could support pre-service and current teachers in having a successful game integration as part of their lessons, as research has found that teachers' acceptance and familiarity with games positively impact their role as facilitators [18-20]. In fact, games are usually introduced by the actions of an enthusiastic teacher who is familiar with a particular game and can see the game's potential for a specific subject [21]. If teachers consider digital games tools that can provide learning opportunities to students, teachers will also perceive digital games as useful to enhance job performance [20].

Previous games research presents specific ways in which integrating computer games with different pedagogical practices illustrated important insights, challenges, and changes in teaching practices [22-25]. Even when each study differs in methodology, findings among them relate to pedagogical approaches and challenges collected from self-reported measures indicating a present or future intention.

- The role of the teacher in structuring and framing the learner's activity remains crucial if learning outcomes are to be achieved [22].
- The teacher wishing to use games must know what content particular titles offer. Teachers should be as aware of the content of games as they are of the content of video, television, or film material [22]. Teachers found it difficult to identify how a particular game is relevant to some component of the curriculum and the appropriateness of the content within the game [23].
- The teacher requires some understanding of the controls, menus, and skill levels of the game to use it effectively. Playing the game is the only way to gain these skills [22]. Teachers with little experience in the use of video games are reluctant to use them because they feel insecure and require significant support during the process and available time to familiarize themselves with the game and the methods of producing the best results for its use [23].
- Teachers who reported using games were usually enthusiastic, already familiar with the game, and could foresee the game's learning potential [22]. Teachers with little knowledge showed fundamentally reactive, while the teachers that had expertise in the game were able to assume a proactive role [23]. Teachers took more ownership of the resources, had higher confidence in integrating the unit as a teaching tool, and were more likely to believe that the curriculum resources would have a positive impact on student achievement [25].
- Teachers, who were mainly accustomed to subject matter exposition followed by assigning students worksheets to complete, found they had to work in real-time with ideas that students were contributing based on their gameplay experiences; they found themselves having to think on a mode of behavior they were unaccustomed to. The change triggered reflexivity and prompted the teachers to reflect more deeply on their past and present practices [24].
- Teachers found that using games could provide motivation, develop skills, and encourage collaboration [22].
- It was less common that school policy defined the introduction of games, and the study found no evidence at any State level of the introduction of computer and video games except as part of a research project [22].
- Working with elements or sections of the game may be more beneficial for some games and school contexts than the whole game. Isolating elements of games for use in lessons can be difficult. Most games have been designed for use over an extended time. Some titles offer shortcuts such as scenario builders, pre-defined scenarios, and the facility to save games [22].

Math Snacks Project

The First Suite and Research Study

Math Snacks is a suite of smart educational animations, games, interactive tools, and a series of activities grounded in constructivist learning, designed to support elementary and middle-school students to develop a conceptual understanding of mathematics. The first suite of *Math Snacks* products included six animations and five games, all available in English and Spanish and freely available to play online. Topics in the first suite included number sense, order of operations, placed value, ratios, proportion, scale factor, coordinates, measurement, fractions, and decimals. The products include a series of materials that can be used in various lessons to support teachers and students. Each animation and game have a printable teacher guide that helps teachers to use the tool and offers discussion questions, vocabulary, and companion activities. Each game has a gameplay video that will help teachers understand how the game progresses, even if they don't have time to play it all the way through.

In the research project on the first suite of the *Math Snacks* games in 2014, the research team conducted a randomized control trial study with a delayed intervention with 741 fifth-grade students and 48 teachers. Classrooms were then divided into two groups: teachers in the first set of classes integrating *Math Snacks* into their instruction during the first five months and teachers in the second set continuing instruction as usual. Then, teachers in the second set integrated *Math Snacks* into their instruction, while the first set continued instruction. This delayed intervention model allowed researchers to give all students access to the intervention, control for teacher differences that could influence pre-, post- tests results, and identify any potential drop-off in conceptual understanding from the first group two months after the intervention. This study showed a significant positive learning effect for students who received the intervention compared to students in the second group.

Moreover, when students in the delayed intervention were given the opportunity to use the games in class, they caught up with their peers after receiving the intervention [9, 26]. *Math Snacks* researchers observed classroom integration of the games in all classrooms as part of their fidelity measure (to track teachers' adherence to protocols). Through those observations, they noted anecdotal descriptions of changes in the way teachers used the games and taught math before and after the intervention. However, the research team had no documented evidence about how the use of *Math Snacks* tools impacted teachers' perceptions of games and pedagogical practices of game-based learning. It sought to investigate the impact of using *Math Snacks* tools on how teachers taught math even when not using the tools.

Focus of this Study: Math Snacks Early Algebra Project

In 2015, the Learning Games Lab received further funding from the National Science Foundation (1503507) for the development of three additional multimedia tools targeting early algebra concepts and conducting research on the impacts on 4th-5th graders and their teachers. Content focused on two major content domains: 1) Write and interpret expressions, and 2) Express patterns and relationships between quantities. Their proposed research mirrored the delayed intervention model used in the first study but added an additional objective to better understand how teachers used the games and what changes they made in their teaching.

The *Math Snacks* team developed two games and one interactive tool to support student learning of the two key learning concepts and support materials like those developed for the first suite. Curse Reverse is designed to teach the key learning concept of expressing patterns and relationships between quantities. It is a platform-style game where players travel to various temples and are charged with returning stolen treasures to advance to increasingly more challenging levels to collect up to three stars. As players progress to more advanced locations, they change pillar heights by adjusting different features of algebraic expressions. Agrinautica is a sandbox-style game where players can create a wide variety of numeric expressions using operations and parentheses. By exploring different ways to create expressions given a set of four numbers, they can create artifacts and plants to populate six different planets. The game addresses the primary learning concept by allowing learners to experiment with the syntax of writing expressions that match their original intent. In the Creature Caverns interactive tool, learners analyze the relationship between the creatures' numbers of horns, eyes, brains, and other variables. Students can look at the relationships of these variables through graphs, tables, and expressions. This tool also addresses the principal learning concept of expressing patterns and relationships between quantities.

Math Snacks Early Algebra Intervention

This study focuses on the second suite of *Math Snacks* for Algebra in which teachers were asked to integrate the tools into their existing instruction (like the intervention for research of the first suite). Before the teachers used the tools, the research team modeled the lessons for teachers; teachers then integrated the *Math Snacks* lessons in the classroom with their other instruction. The *Math Snacks* implementation modeled a specific approach to using the tools in lessons, and participants were asked to follow that approach when using the games.

1. *Introduce gameplay with discussion questions and initial play.* Students freely explore the game for about 15 to 20 minutes. The teacher then facilitates a whole class discussion about students' gameplay. Teachers can ask a few students to use the smart board and share game strategies.
2. *Engage students in support activities and discussion questions.* The teacher models and then facilitates a hands-on activity associated with the game's content. Each developed game or tool had its own designed activity in this *Math Snacks* intervention. For Agrinautica, the Sunburst activity was designed to start with a target number on the center of a circle, and different extended-expression forms burst out into multiple layers. For Curse Reverse, the Keys activity was designed to uncover a secret image hidden behind locks. Each lock has a value that must be matched by modifying the expressions on the students' key handouts. For Creature Caverns, the Creatures activity was designed to use different attributes of hand-made creatures to sort by something that can be counted, then think about quantities that are not directly counted, and finally plot the creatures on the coordinate grid, quadrant 1.
3. *Allow students to play again with guided discussion.* Students play the game for another 15 to 20 minutes. This second gameplay session allows students to discover or implement newly learned game strategies and to make use of new content learned from the supporting activity. The teacher then facilitates a class discussion about students' second gameplay strategies and math content.
4. *Engage learners in reflection and assessment.* The *Math Snacks*' teacher guide offers a list of questions that teachers can use for oral discussion, journal entries, or exit tickets. Teachers are encouraged to use the vocabulary words as they ask for any task.

Teachers took up research-based activities while teaching their lessons. An instrument called Observation of Learning Environments (OLE 2) [27], designed to measure teachers' inquiry strategies, was used during classroom observations.

Methods

This study uses a subset of teachers who participated with their students in a more extensive study. To research the experiences of teachers in using games in their classroom more deeply, this qualitative study investigates any changes in their use of game-based teaching strategies and inquiry-based pedagogies.

The following research questions guided this study:

- How did teachers adapt implementation strategies that fit their classroom?
- In what ways do teachers' perceptions of computer games in the classroom change as a result of a *Math Snacks* intervention?

Sampling of Larger Study

For the *Math Snacks* Early Algebra, larger study, researchers contacted principals in a public school district in the Southwestern US, inviting them to recruit 4th and 5th-grade teachers who might be interested in the research project. The research accepted teachers from ten schools representing a range of demographics, and after two teachers withdrew, the final *Math Snacks* research intervention included 28 teachers and approximately 580 students. Teachers agreed to receive a stipend at the end of the study if all activities were completed. For the larger study, researchers randomly assigned one of the three new games (Agrinautica, Curse Reverse, and Creature Interactive) to use in their classroom while being observed by researchers as part of a fidelity measure (to track teachers' adherence to protocols).

Participants for this Study

For this study, researchers sought to collect a subsample of teachers who reflected varying experiences in teaching, a range of skills in inquiry orientation, and grades taught. For the larger study, teachers each taught all three games, but participants were randomly assigned which class would be observed. When selecting the subsample, all three games needed to be represented. Therefore, a subset of 12 teachers was purposefully

selected from this sample of 28, based on the scores of the first classroom observation, the years of teaching experience, the school where they taught, and the assigned game to be observed. Each teacher's OLE 2 score indicated their expertise and inquiry-orientation skills: 0 representing low skill and 4 demonstrating high skill. In the larger study, all teachers scored from 0-3, with no high skill scores. These OLE 2 results provided a way to create three levels of inquiry orientation: low – scores were mainly 0's; medium – scores were mainly 1's; high – scores were mainly 2's and 3's. For the subset of this study, teachers were purposefully selected from each of the three levels. With these considerations, the subgroup of subjects presented diversity in years of teaching experience and expertise in inquiry orientation. It allowed for observation of each game in the study with at least one teacher (see Figure 1). The set of 12 teachers taught grades 4 & 5 across ten different elementary schools. All but one were female. These groups also varied in range of expertise, ethnicity, and age, as well as the different schools participating in the study with at least one teacher from each school in the larger project.

Data Collection

This study focuses on the subset of teachers, using interviews as the primary source of data as well as two OLE 2 classroom observation instances, one pre- and one post-survey, and three self-reflection logs. Over a period of nine months (see Figure 2), teachers in the larger study (including the 12 in, the smaller sample) completed basic assessments on their experience, their comfort with technology, and their knowledge and views on games. Pre- and Post- Surveys were used to assess the teacher's perspective of the value of games in the classroom, if they have used games before as part of their lesson, and in what ways. The teachers then integrated the games into their lessons, following basic protocols set by the project.

Demographics of 12 participant teachers (pseudonyms used for teacher's names)

	Teacher	Grade	Experience			Inquiry orientation as measured by OLE	Game taught while observed
			Yrs. Teaching Exp.	License level	Educ. Level		
1	Adel	5	17	2	Bachelor	Low	Agrinautica
2	Alain	4	15	3	Master	Low	Agrinautica
3	Alba	5	20	2	Bachelor	Low	Agrinautica
4	Casey	4	10	3	Master	Low	Creature Caverns
5	Reagan	5	17	2	Bachelor	Medium	Curse Reverse
6	Cleo	4	12	2	Bachelor	Medium	Creature Caverns
7	Carson	5	15	2	Bachelor	Medium	Creature Caverns
8	Rylee	5	18	2	Master	Medium	Curse Reverse
9	Carly	5	16	2	Master	Medium	Creature Caverns
10	Ryan	5	3	2	Bachelor	High	Curse Reverse
11	Ruby	5	23	2	Bachelor	High	Curse Reverse
12	Cameron	4	15	3	Bachelor	High	Creature Caverns

Fig. 1. Demographic Information of Participants.

Researchers observed one of these lessons to measure teacher's fidelity of implementation (adherence to protocol). Teachers completed three self-reflection logs (one per each game-lesson) on their thoughts and comments about the lesson they taught using each game. A second OLE classroom observation informed researchers about changes in teaching strategies with technology after *Math Snacks* implementation. The subset of teachers for this study also participated in additional semi-structured interviews on how they developed their lessons, what worked and what did not work for them, and how their perspectives about using games have changed after the implementation.

Analysis Procedures

The researcher followed Braun and Clarke's [28] version of the thematic analysis technique in the analysis of the semi-structured interviews to construct a qualitative description of the research data set. When doing transcriptions, the researcher used a side Word document and a whiteboard to take notes about specific things from the interview sessions that could support the analysis, like recalling teachers' specific actions, gestures, and comments. The researcher analyzed all source documents — Pre and post-surveys, observers' notes, reflection

logs, and interview transcripts — as a group using the same – reading sentence by sentence and coding – iteration process with two software MAXQDA 2020 [29] and ATLAS.ti 8 – Mac [30]. Initially, the researcher did not have a specific codebook and started coding data deductively but paid particular attention to features that would result in several themes around Technological-Pedagogical-Game Knowledge, which may include, speak to, or expand on something approximating teaching practices. Some initial codes included: walking around, asking questions, low-performing kids, years of experience, looking at screens, and games being powerful tools.

To determine the pedagogical practices that were used during the implementation of computer-based games, themes were classified concerning strategies teachers used to integrate computer games into their lessons and the different ways to assess the learning practice. To describe the changes in teachers' perceptions of being able to use computer games in the classroom, themes were related to things that teachers modified from the assigned lesson protocol and to teachers' opinions, challenges, and takeaways from the implementation. While searching and reviewing themes, it was important to recall that extra data sources from the *Math Snacks* larger project would support evidence for the emerging themes; therefore, themes were revised iteratively to be useful and accurate data representations. Results from this study are not meant to be read as generalizable but as potentially transferable to other game-learning contexts or technological interactions with similar paradigms.

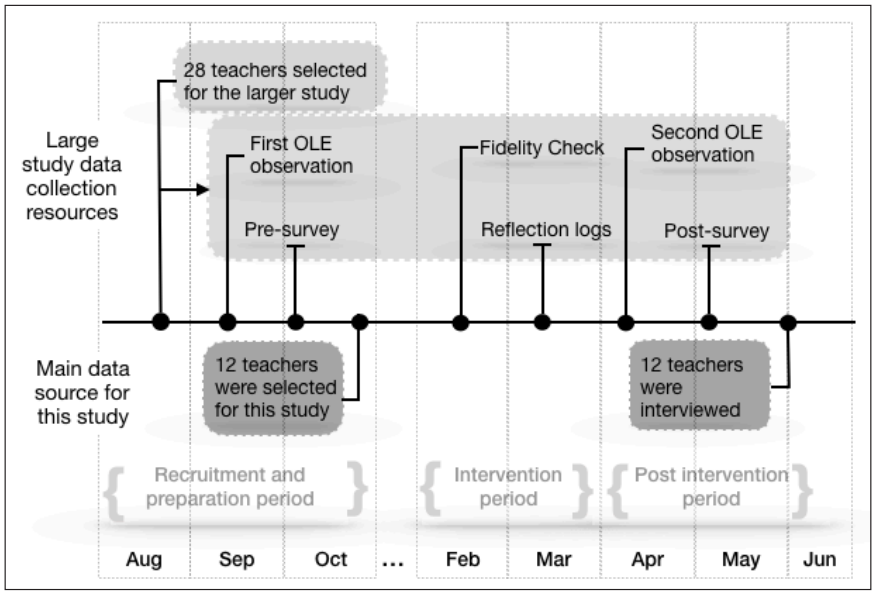

Fig. 2. Data Collection Timeline.

Validity and Reliability

To enhance trustworthiness different data sources were used. Triangulation was ensured by collecting different types of data that could describe different types of things concerning the themes that emerged from the interviews. The researcher consequently summarized the data collected and asked two persons to review the resulting sub-themes. Pieces of transcribed interviews and reflection logs' responses were given to two different persons, and they were asked to code them using an initial set of codes to verify their agreement on using a specific code for a particular piece of the transcript.

Findings and Discussion

Collected data from pre/post-surveys, reflection logs, interviews, classroom observations, and fidelity of implementation observations supported the understanding of how twelve participant teachers developed their lesson with the integration of *Math Snacks* games on.

RQ1:Teachers' Perceptions on the Use of Games in the Classroom

Findings showed that with an experience like the *Math Snacks* implementation, teachers would feel more comfortable and be more skilled in using computer games as part of their lessons. They viewed the introduction to game mechanics as helpful to guide students, especially those with different mathematical skills. Two main themes emerged from the analysis of all data sources:

i) Teachers saw *Math Snacks* games as powerful tools to explore mathematics concepts and to present content in a fun and engaging way

ii) *Math Snacks* games allowed for the inclusion of students with different mathematical skills

Math Snacks games changed teachers' views regarding the power and value of games.

Participant teachers noted that students are motivated when they learn through playing computer-based games. Being able to play and then having a brief discussion was an important contribution to the *Math Snacks* model. Responses to interviews showed that teachers agreed on how students could play the games, connect the mathematics content of the games with the learning in the supporting activity, and later apply these concepts to the reflections they were doing on discussion sessions. Ryan's answer to the question, "How would you describe your experience with *Math Snacks*?" was:

> "it was a good opportunity for us, as teachers, to present a concept without struggling to go and find how I can put all this together. So, it's a great tool to have. It's not limited to just math or that specific skill; it applies to different ones, even for reading. I mean, it is a good way to—or rather, it was a good opportunity to—integrate technology with hands-on activity and with paper and pencil" (Transcribed Interview Ryan, para. 216).

When asked, "Is there one game that sticks out in your mind as being sort of better?" Casey responded,

> "I thought that was a powerful game because they learned something; they were able to apply it, and then they were able to play and have discussions with each other. I'd say that one was, I feel, very beneficial—better—and it was engaging because they liked not only their little activity but also the game" (Transcribed Interview Casey, para. 30).

Teachers' responses to post-surveys showed evidence of positive change in teachers' perceptions on the use of games as there were zero teachers with 'strongly disagree' as their answer to four items related to how teachers view computer games: i) computers/tablets help students to understand mathematics concepts; ii) computers/tablets make sophisticated mathematics concepts accessible to students; iii) computer-based games are a good way for students to explore mathematics concepts, and iv) students are more motivated when they learn through playing computer games. Even after the intervention, teachers would allow students to play games and encourage them to play at home. For observation B, teachers were free to use any technology for their mathematics lesson, and one teacher implemented a different *Math Snacks* game from the intervention. Even when the other teachers did not use *Math Snacks* games for Observation B, they implemented other tools, such as videos, Istation, Kahoot, Online Jeopardy, and Brain Pop. However, they followed the *Math Snacks* intervention model (i.e., explore, discuss, supporting activity, reflection/assessment).

Math Snacks games allow for the inclusion of students with different mathematics skills.

Most teachers thought that the use of computer games was a beneficial tool for students who regularly struggle with paper and pencil activities. These students would often give up easily with paper exercises; however, on the contrary, they would feel motivated to continue trying when they were playing computer games like the ones in the *Math Snacks* intervention. Teachers mentioned being surprised that these low-performing students in a regular mathematics class were pulling above the group during the gameplay sessions. When asked, "Can you describe to me what was happening in the room in the first game session?" Reagan responded,

> "my lowest graded mathematics kids, who struggled, were actually doing better than my high scoring students in class. This pulled them up above that group, where they were actually showing their higher scoring peers shortcuts and how to maneuver through the game, and how to make these cool flowers or these cool trees. So, that was a confidence builder for those kids who struggle with a book, who struggle sitting at a desk with a pencil and paper" (Transcribed Interview Reagan, para. 60).

Other teachers saw implementing *Math Snacks* games as one way to help these low-performing or struggling students. They used the games as a reward whenever students had some free time or when they had finished their assigned work. After the question, "How would you think that the whole experience of *Math Snacks* would help you?" Adel responded,

> "even my B-most behavior kid, my special kid, enjoyed it because he would look at it as a reward for him. So, I would tell him, 'you can get on computer, can I get on *Math Snacks*?' Right away, that was the first question. 'Sure, you can get on *Math Snacks*, but you've got to behave'. It was a reward for him, and he took it as a reward too" (Transcribed Interview Adel, para. 66).

Participants saw *Math Snacks*' game session moments as the moment for struggling students to shine. For the question, "Can you talk to me about the second game session for Creature Caverns?" Casey responded,

> "I was amazed that some of my students who do struggle were able to get down into the medium tasks, even in such a short time" (Transcribed Interview Casey, para. 62).

RQ2: Pedagogical practices in the integration of games

This study extended findings and added further depth to how teachers perceived how gameplay sessions helped students foster deeper learning. Three main themes emerged from the analysis of all data sources:

i) Two separate gameplay sessions resulted in students building confidence and challenging themselves more

ii) Teaching with *Math Snacks* enhances student-to-student collaboration and communication

iii) Teachers integrated different teaching strategies into their *Math Snacks* lessons

Teachers view students building confidence and challenging themselves more because of two separate gameplay sessions.

Results showed how teachers who did two gameplay sessions showed an increase in students' confidence to play the game and take risks to challenge themselves with more complex problems. Interviews responses described what differences teachers saw between the two gameplay sessions. Teachers talked about how students build confidence, use more vocabulary words, and challenge themselves to try more difficult game levels during gameplay session two. According to the teachers' responses, having a second game session triggered students' confidence.

> "In almost every game I made the two sessions, the biggest difference I found is the confidence of the children in the game, feeling more comfortable playing or looking for opportunities to understand the game, and get better scores" (Transcribed Interview Alba, para. 72)

> "they were using different vocabulary, and they were able to key in on those—you know, the specific math terms and stuff that went along with it—and they were making that connection" (Transcribed Interview Carly, para. 38)

> "For the second session, those who had not yet achieved that level in the first session, and yes, they could do more, and they felt a little more confident" (Transcribed Interview Alain, para. 32)

One difference between games sessions was the type of discussions students and teachers had. In the first game session, students talked about basic hints and strategies to play the game. However, in the second game session, students could use mathematics vocabulary they did not use during the first gameplay session (e.g., expression; set of parentheses; and value). Discussions after gameplay sessions also had an impact. During the second game session, students were able to use more mathematics vocabulary words that they learned during that day's lesson.

Teachers enact teaching with Math Snacks games to enhance student-to-student collaboration and communication.

Findings exposed the different ways teachers saw students collaborating with each other. Teachers mentioned that students communicated their strategies while playing to help others get to the same level or achieve the same goals.

When asked, "What were their interactions with the students during the two game sessions and what did they see happening while students were playing?" Some of the responses were,

> "that is the good thing about it: that collaboration. Immediately, when one student would find a solution, they would tell the other student, and then that student would tell another. So, it was a collaboration with all of them" (Transcribed Interview Ryan, para. 28)

> "so, if somebody was struggling, they were able to teach each other how to get to that point. And then they loved when they actually got on the game and saw the plants and the different kinds. They were talking to each other a lot" (Transcribed Interview Casey, para. 28)

> "it was a lot of discussion with the students, to each other, because they were trying to figure out things like: What am I supposed to be doing? What are these numbers? How do I make the numbers move" (Transcribed Interview Cleo, para. 46)

Responses from teachers defined that each game and activity had its own way of integrating collaboration among students. A sandbox-type game like Agrinautica opened the classroom space for teachers to walk around the room and see how students use different methods to share their strategies (e.g., some used Post-it notes, and others explained by showing an example on their screen). It opened the space for student collaboration; they shared strategies for doing a specific plant, and students were not afraid to ask. While observing an Agrinautica lesson, some students were leading the playing strategies and teaching others during the lesson time. These leading students would walk across the room to show others how to execute a specific move. Agrinautica's supporting activity also offered an opportunity to make a gallery walk by hanging their final posters around the classroom. Students would observe all of them, and then the teacher asked students to reflect on what they saw in their classmates' work. In the interactive tool Creature Caverns supporting activity, the students used hand-made creatures to line up according to the number of a specific feature. Students collaborated to develop strategies to line up first by the number of antennae their creature had, then by the number of eyes, and later by the difference in the number of eyes and antennae. Students discussed how uniquely a creature can be created, and the teacher helped the students discuss strategies to make this interactive tool easier and more appealing.

Teachers integrated different teaching strategies to foster students learning.

Teachers could assess learning during gameplay sessions by looking at students' screens, hearing students' discussions, and prompting questions to students about why they were making specific moves in the game. When asked to describe what was happening during gameplay sessions, interview answers revealed different ways teachers assessed students while playing.

> "game session one was pretty much self guided, so I didn't offer assistance. It was more about just going around and listening to what they were saying and how they were helping each other" (Transcribed Interview Casey, para. 134)

> "I would walk and see what they were doing, and allow them to get up and say, 'this is how it is done' or 'look, you are missing this' or 'move here'" (Transcribed Interview Alain, para. 36)

> "The first time, I'm just walking around and kind of monitoring, and just encouraging them to keep trying and to struggle with it" (Transcribed Interview Carly, para. 25)

> "We're just walking around, listening to their conversations, making sure that they were using their math vocabulary. So, just listening, really. I couldn't help; I didn't want to help the first time. It's just listening and repeating after whatever they were saying" (Transcribed Interview Carson, para. 86)

The pedagogical strategy of using games to practice mathematics concepts has been widely used, but *Math Snacks* implementation introduced different ways of using computer games. These were to explore ideas and to launch a lesson for a new mathematics concept. After the *Math Snacks* implementation, all teachers reported exploring concepts as the preferred portion of a lesson where they would use computer-based games. Teachers usually think of using computer games to practice basic mathematics skills, but in this case, teachers started to use and think about computer games differently. Teachers began to see computer-based games as part of their future teaching practice and saw *Math Snacks* games as a new way to build conceptual knowledge.

When asked about their ways to assess learning during the supporting activity, teachers mentioned that they were walking around, ensuring students correctly used the vocabulary. It was mainly during the supporting activity that teachers integrated existing and everyday informal assessment strategies. Four teachers took the reflection assessment in the teacher guide as the only way to assess learning. They even mentioned to students that was the way they would reflect on what they learned. Only one teacher discussed the correctness of the students' answers with the whole class. This teacher reviewed five or six students' responses and asked the entire class to revise the procedure and the result. Teachers had the opportunity to use *Math Snacks* games and materials as homework or practice time after school because they are freely available online. Still, only four participant teachers encouraged their students to play at home.

Conclusion and Future Work

When using computer games in the classroom, teachers need the ability to facilitate the game because their role as facilitators is critical to the success of game integration. This study showed that with an experience like the *Math Snacks* implementation, teachers would feel more comfortable and skilled in using computer games as part of their lessons. After teachers got to experiment with the *Math Snacks* games, they saw these type of games as powerful tools to present mathematics content in a fun and engaging way. Teachers also saw professional learning workshops as a good introduction to game mechanics. These findings imply future research on using this type of computer games in different contexts, such as science, and modification of school policies with the intention of adopting computer games, as these games are believed to be adequate. Results on pedagogical practices in integrating games extend the idea that implementing games in the classroom employs more than one activity type to foster student learning and build student confidence. Teachers found in *Math Snacks* a new tool that can enhance student-to-student collaboration, communication, confidence building, and challenge.

This work confirms the assumption that teachers should explore the game themselves before directing their students to use the game. Participant teachers reported that they regretted not spending more time playing the games prior to implementation. Still, it is unknown whether teachers spending more time playing the game will change the way they interact with students. Future research should study this further by assigning teachers specific times before implementation and then making a comparison with participant teachers from this study. A comparison study of teachers implementing *Math Snacks* games during a specific amount of time vs. teachers using regular teaching materials might confirm what type of teaching skills *Math Snacks* enhance in teachers.

The *Math Snacks* intervention model included four sessions during a lesson: a gameplay introduction, supporting activity, gameplay enrichment, and reflection/assessment time. All participant teachers followed the teacher guide model when implementing their *Math Snacks* game, but only a few could have all four sessions during one class period. It is unknown whether the extended use of *Math Snacks* as homework will have an impact on the type of discussions that could follow a gameplay session. The effects of using *Math Snacks* games as homework can be determined with future research, and studies in other contexts should be conducted to understand whether the major findings extend to different contexts and other types of teachers.

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Invited Letter: iLab at St. Petersburg College Offers Unique Opportunity for Students to Work Collaboratively in a Technology Playground

Keywords: iLab, intelligent cameras, collaborative learning environment, URE

I am writing this letter to express my gratitude for the opportunity to work with Endrit Ngjelina, an exceptional student from St. Petersburg College (SPC), on his research project titled "The Building, Use, and Importance of Intelligent Cameras in the 21st Century." As a Librarian and Assistant Professor managing the Innovation Lab (iLab) on SPC's Seminole Campus, I have been privileged to work with Endrit and witness his growth and dedication throughout this research project. The iLab is a collaborative learning environment, often referred to as a "technology playground," where people with similar interests in science, technology, engineering, digital arts, and math can meet, socialize, and collaborate while sharing ideas and learning new skills. SPC offers an 8-week Undergraduate Research Experience (URE) program facilitated by faculty mentors in the student's field of interest. My role is to guide students through the research process, produce a collaborative and comfortable environment where they can contribute their ideas, provide access to resources and opportunities, and help students refine their research questions and methodologies to ensure they are on a clear path to success.

Endrit's project involved building a device to demonstrate computer vision, a branch of Artificial Intelligence that enables computers to understand and interpret the visual world. Endrit demonstrated a remarkable ability to design appropriate experiments and analyze the device's output. I learn while working with these bright students, and believe that students benefit from working on problems as a team. For example, Endrit could not get the device to work, and we struggled with it, but we finally discovered that we were using the wrong power supply. Sometimes, there is an easy solution sitting behind complex trials and errors that provide that spark of intellect when found. When the device started to function properly, we were ecstatic. Working with students in the URE program has provided rewarding experiences. It has given me opportunities to guide and learn from students as they explore their academic interests, develop crucial research skills, and make meaningful contributions to their fields of study.

To improve successful undergraduate research at community colleges, establishing mentorship programs like this one will provide students with guidance and support from experienced faculty members who have shared interests with the student. Offering writing workshops and presentation skills training so students can showcase their research through events like symposiums or conferences would help create a sense of achievement while motivating others to participate. Finally, community colleges should continue establishing solid partnerships with local industries, governmental organizations, and/or foundations to support these research endeavors financially.

Once again, I want to express my gratitude for the opportunity to work with Endrit. I am confident that he will have a bright future ahead. I also want to extend my appreciation to your publication for encouraging and supporting undergraduate research initiatives.

Sincerely,

Chad Mairn, M.L.I.S.
Librarian | Innovation Lab
St. Petersburg College

The Building, Use, and Importance of Intelligent Cameras in the 21st Century

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Abstract: The field of computer vision has experienced drastic technological breakthroughs since the end of the 20th century. Technology is becoming more prominent in people's lives each year; therefore, intelligent cameras are attracting their deserved attention. Their power and ability are unseen and fit for plenty of demands. The AIY Vision Kit is an intelligent camera that has been experimented with and researched carefully. This research illustrates the fundamentals of intelligent cameras, how they are used, and their importance in the future. Information has been detailed with its respective explanation, picture, and additional knowledge to aid in a deeper sense of understanding of the topic. This paradigm is conducted and concluded through extensive research, examples, and references.

Keywords: artificial intelligence, camera, Python, image recognition

Introduction

In the 21st century, the world is surrounded by astonishing technological advancements, machines, and gadgets that were only thought of as science fiction a century prior. This technical sophistication has prompted the public with innovative devices alongside great features that accompany them. An intelligent camera, just like the name implies, is a camera with versatile functions. Behind the lens that all are familiar with hides complex software that is trained to equip the camera with an array of features. They are customizable, but all fall under the umbrella of computer vision and artificial intelligence. The universal advancement towards automated machines strongly indicates the importance of intelligent cameras in the future.

In [1] the authors note that "The camera is a key sensor to achieve a reliable environment perception," referring to its role in the car's navigation system. In addition, they make work more efficient, precise and they are extremely reliable. Considering regular cameras' impact on all human landscapes, one can expect the same result with intelligent cameras. They are like standard cameras but with tweaks that make them multifunctional and more desirable. This is a great feature because not only does it save storage space and resources, but it also saves time for the appropriate entities. In [2] a surveillance system from intelligent cameras is described as follows, "This system is reliable and meets the aim of a modern intelligent surveillance system by combining multiple approaches to detect intrusions and to inform users effectively ." The camera system would implement face recognition software that would regularly update and have its database for employees. An intruder's face would not be recognized; hence it would be captured and reported to the appropriate authorities. Such technological improvement is escalated by artificial intelligence and its ability to learn, progress, and keep going. While intelligent cameras are still relatively young and have not been fully explored, their influence and ability portray their importance in the future.

To set the stage for anyone interested in computer vision, one must first be familiar with the fundamental concept. Computer vision works by training machines on large amounts of visual data and creating an algorithm through which the computer classifies the object. This concept is based on machine learning, which is the potential or capability of machines to replicate intelligent behavior through extensive training, exploring, and studying.

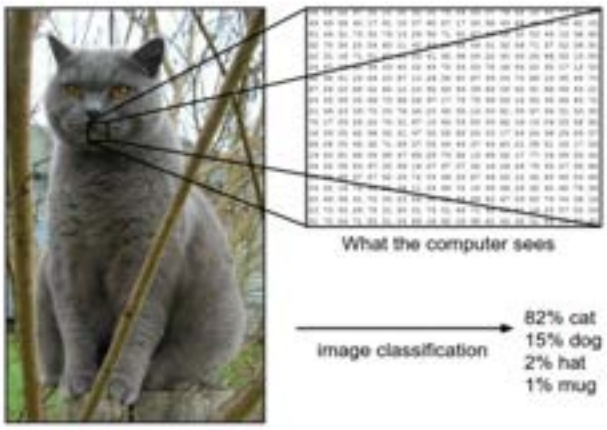

Fig.1. The photo is analyzed, and the computer classifies the image. [3]

The picture above sets an example of how computer vision works. Before classifying the photo, the processing unit examines the pixels, their placement, and other factors. The computer calculates the probability through trial and error and then classifies the object. In [3] the authors note that" Thanks to the use of deep learning in image recognition and classification, computers can automatically generate and learn features – distinctive characteristics and properties. And based on several features, machines predict what is on the image and show the level of probability".

Methods

The AIY Vision Kit is an intelligent camera created by Google LLC. It is crafted in 17 parts that, through careful handling, come together to form an intelligent camera. The main parts of the intelligent camera are the Vision Bonnet, the Raspberry Pi Zero WH, and the Raspberry Pi Camera v2. The Vision Bonnet is a minicomputer specializing in computer vision and artificial intelligence. The Raspberry Pi Zero WH is a tiny board that enables intelligent cameras with wireless and Bluetooth connectivity. The Raspberry Pi Camera v2 is a custom-designed 8-megapixel camera with an image sensor and a fixed focus lens. When powered up, this device can capture and recognize random objects in sight. It distinguishes their color, form, and size. It is embedded with facial recognition software, recognizing when someone is happy, angry, or shows other emotions. The instructions for building this intelligent camera can be found on their dedicated page [4]. Any enthusiastic builder can build and perform the demos available on the web page. They include the Joy Detector, the Image Classification Camera, the Face Detection, and more. Aside from the intelligent camera, one needs a monitor, a smartphone, chargers, and a display port cable to recreate this research. One can use other devices to enhance the research experience, but the devices mentioned above are the minimum requirements.

Results and Discussion

Building an intelligent camera can be challenging. Below are some problems one can face while putting everything together.

The Raspberry Pi Camera v2, the Vision Bonnet, and the Raspberry Pi Zero WH are connected through a long flex cable and a short flex cable, being tightly secured in their respective latches. If this cable is not secured, the Vision Kit will not work. All parts must be connected to communicate with the camera.

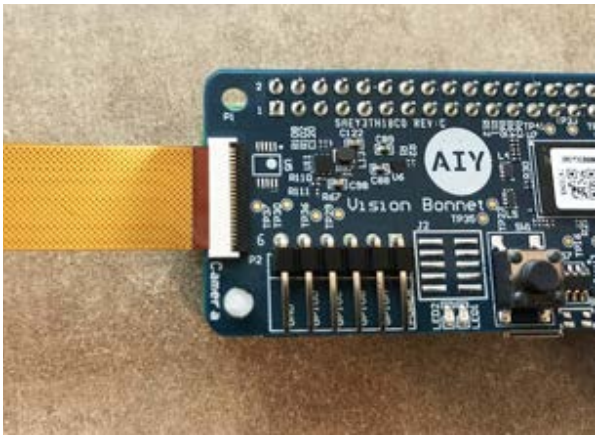

Fig. 2. Cable is not properly secured to the latch.

Fig. 3. Parts are tiny and hard to deal with. [5]

When dealing with the boards or other electronic parts of the Vision Kit, it is suggested to be careful and aware of how fragile they are. Above is a depiction of the Vision Bonnet, the main board which powers up the device, and it is as big as three coins.

Another technical issue that can be expected is a corrupt SD card. The files within it may not run properly; therefore, the Vision Kit does not work. Thankfully, there is an easy solution to this, which is reformatting the SD Card.

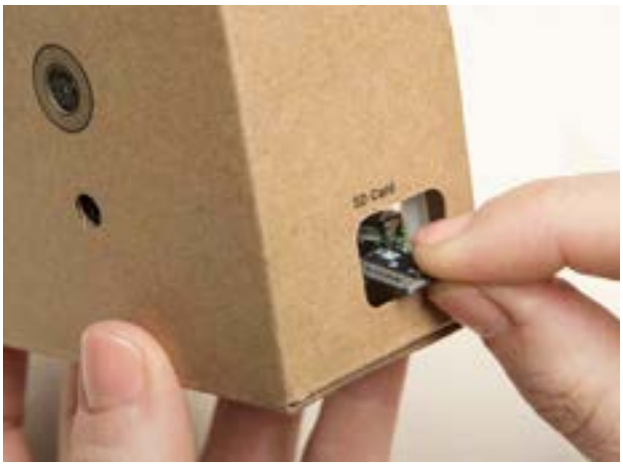

Fig. 4. The SD card is not working. [4]

Fig. 5. Cable flex is turned around.

If one thoroughly follows step three from the dedicated web page, one soon discovers that the kit will not run. To fix this, the copper strip should be turned the other way. This step is incorrectly displayed on the web page, and it should be fixed by the team.

Fig. 6. The power source is not enough.

Fig. 7. The power source is enough.

If the power output is not big enough, the kit will not start. The power plug on the left has an output of 350mA, which was insufficient to start the kit. The kit booted up when switched to the one on the right with an output of 3A.

The first demo that is put to the test is the Joy Detector. As mentioned, the vision kit can detect someone's face and show their feelings. It does so by lighting the main button in different colors based on the person's facial expressions. This effect works on people, and surprisingly it works on photos or videos of people. One must point the camera at anyone and watch the color change according to their joy. Below is an example of this demo in action.

Fig. 8. Button turns bright in color.

Fig 9. Joyful face is detected.

In the picture on the right, someone is depicted through an intelligent camera. On the top left, the program displays the number of faces in the frame and their joy. The joy index is 82% (or 0.82), and when the camera detects a joyful face, the main button changes color to bright pink or yellow, as depicted in the picture to the left.

Fig. 10. Button turns blue color.

Fig. 11. Sad face detected.

The joy index has dropped to 1% (or 0.01) in this example. The intelligent camera quickly reads off the facial expression; when the face is not joyful, the color in the main button shifts to blue or dark blue.

Fig. 12. Button turns bright in color.

Fig. 13. Joyful face detected.

Here, another face is detected but from a photo on the internet. As usual, it detects the joy index, which is 86% or 0.86, and the light correctly turns pink or yellow, to indicate joy.

Another mind-blowing feature of this intelligent camera is a demo called "Image Classification Camera," it identifies, classifies, and displays data about any object just by pointing the camera toward it. This is done through many files in the source code that apply their data set to the input from the camera, and whichever matches is returned as output

in the terminal. In other words, there is already a database for common objects in the source folder, and if you display it in this demo through its shape, size, and color, the database will return those statements that match your object. Below is the output from the terminal when a keyboard was placed in sight.

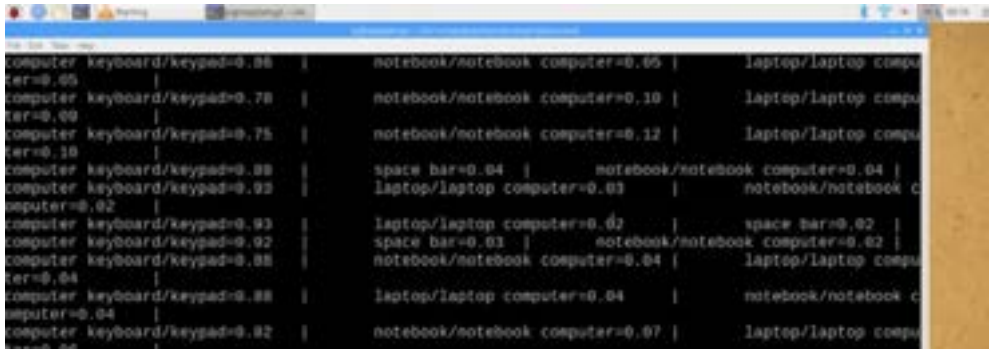

Fig. 14. Terminal outputs correct assumption for object.

On the left side of the screenshot, the type of object is displayed and the certainty of the program. It classified the object as a keyboard with a certainty of 75% (or 0.75) and as high as 93% (or 0.93). On the middle and right side are other options, but they are less likely to be correct since the certainty of the program is extremely low, ranging from 12% down to 1%. This certainty percentage is displayed because the camera does not directly examine the object but compares it to the data of common objects already stored in its source files and then makes an assumption. With some thinking, one might derive that it is possible to display an object not in the database, which is the case for the following example in the terminal.

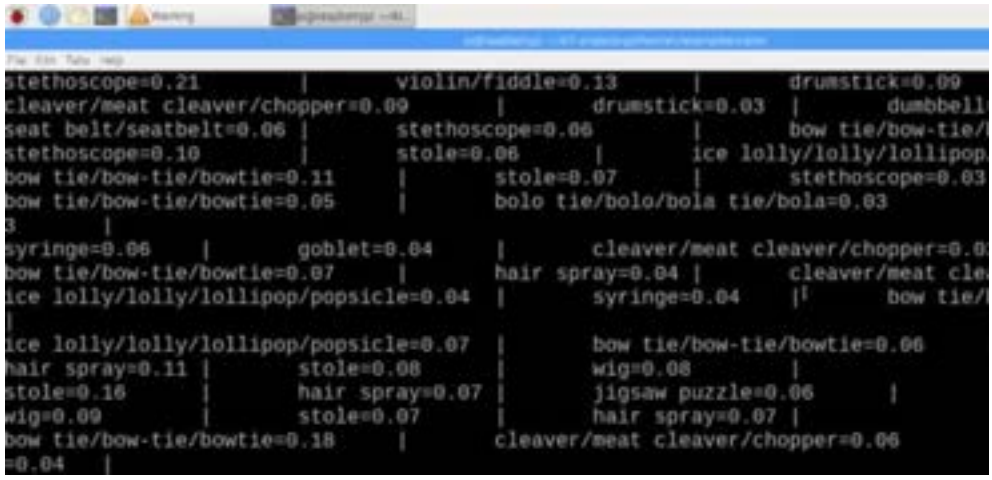

Fig. 15. Terminal outputs incorrect assumption for object.

The object in the instance above is a pair of pliers, but the program does not recognize them. This is most likely since an object named 'pliers' is not in the kit's source code. Since there is not one, the program tries to find similar matches to this object. Pliers, lollipops, bow ties, and seat belts all share a long – vertical shape. Another detail to determine that the program is not sure about the object in question is the certainty that it displays. The most significant value regarding certainty is 21%, assigned to a stethoscope. Compare these answers to those shown in the previous example with the keyboard; drastic differences can be noted.

The last demo tried is the face detection feature of the kit. It may not be as exciting as the one prior, but it plays a significant role in the overall functioning of the camera. Alongside the face detector code, a face tracker feature is programmed in the source files. This enables the vision kit to keep track of the people in the shot and simultaneously record data about their face's geometry and expression. Below, the output of this demo is displayed.

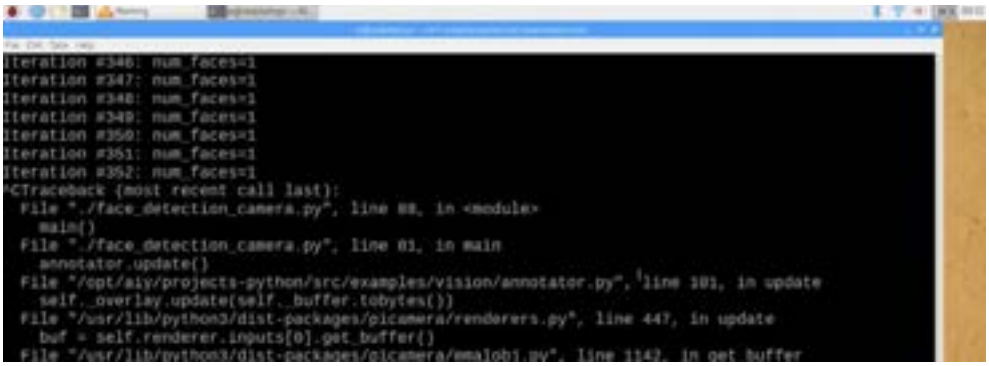

Fig. 16. Terminal outputs correct number of faces detected.

All the lines start with the word iteration, which means that the face is recorded for every frame that the camera captures. The three-digit number next to the iteration is the frame number. After that is displayed the number of faces that were detected. In other words, the intelligent camera does not record a video of what is happening, but rather it captures photos and distinguishes them from one another by iterating through each frame for any changes or abnormalities between them. This is quite effective because it pays great attention to detail and minor things that might go unnoticed.

And lastly, some of the source files were opened in Visual Studio Code, an Integrated Development Environment (IDE) for all programs. While it is difficult to fully understand why and what each command line means, the general purpose for opening it is to see how the software is divided into sections and how they connect. The vision kit is created using Python, which is known for being a versatile and user-friendly programing language. A screenshot of the code is provided below.

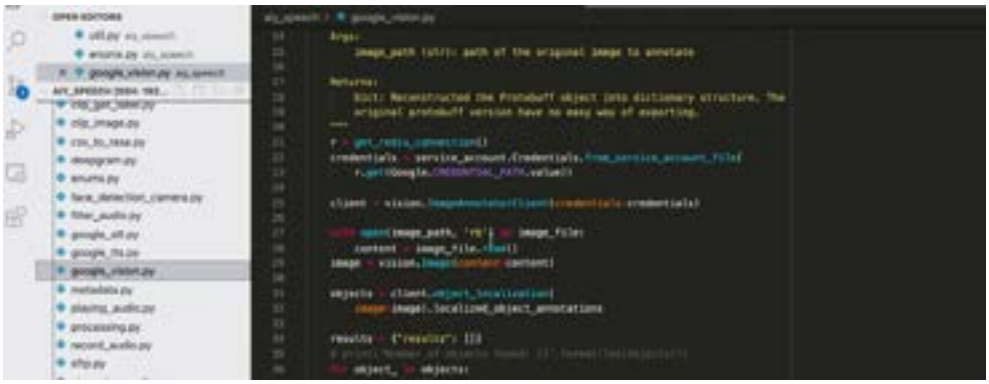

Fig. 17. The source files of the intelligent camera are displayed.

On the left side, all the files comprise the software used in the kit. All the processing and 'intelligent' work is done here. This is what differentiates this camera from a regular plain camera; it contains a lot of power and potential. Inside the dark background, the code of the 'google_vision.py' file is displayed. If one looks at the top of the photo, one notices that this file is located inside the 'aiy_speech' folder. Explained in simple terms, this nesting of files inside another is done to efficiently apply software in any given situation.

Conclusion

Although not widely used, intelligent cameras will soon be almost everywhere due to their incredible performance and the features that they bring along. Casual cameras that are installed on rooftops of banks or traffic lights record footage, but if something noteworthy were to happen, one would need to filter through all that footage and find the accident scene or the crime committed. In comparison, an intelligent camera would be able to filter and delete all the unnecessary footage, separate the noteworthy event, and make it stand out. Artificial intelligence is so good at learning that many of the world's brightest minds fear the

future of technology and the way artificial intelligence will evolve. In the same way, all the magic in the kit is happening between the Raspberry Pi Zero WH and the Vision Bonnet. Those components collect, store, separate, analyze, and portray data. They obtain the capacity and power to analyze and distinguish data in an organized and understandable manner. This is what today's technology can achieve; it is astonishing, to say the least, and the reason why intelligent cameras will be everywhere.

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Disclosures. The authors declare no conflicts of interest.

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Equity Promotion for Underrepresented Community College Students Nationwide: A Case Study of the Micro Nano Technology Collaborative Undergraduate Research Network

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Abstract: There are many individuals from groups traditionally underrepresented in higher education—first-generation, low-income, and Black and Hispanic students—where community college serves as a bridge to entering the technical workforce or pursuing higher education. Most of these students attend community college part-time to accommodate their familial obligations, demanding work schedules, and the budget afforded to their education. Numerous studies indicate that increased financial support, engagement in external experiences, and strong faculty mentorship can promote these students' academic and future professional success. The Micro Nano Technology Education Center aims to increase educational equity and diversity by addressing this need by offering community college students nationwide the opportunity to work with and learn from faculty mentors, baccalaureate research universities, and industry partners from across the nation remotely or in person through the Micro Nano Technology Collaborative Undergraduate Research Network. This network works to prepare underserved students for entering the technical workforce or transferring into higher education through funded academic-year and summer capstone experiences along with faculty mentorship, peer mentorship, and weekly networking opportunities. Our results found no statistically significant difference in student-perceived retention, accessibility, or in students' belief that they belong in laboratories where they may not see representation before and after participating in the MNT-CURN program. However, these results indicate positive trends in these areas, and students self-reported that participating in MNT-CURN increased their confidence that they will complete a STEM-based degree.

Keywords: diversity, equity, inclusion, community college, research, education

Introduction

The current state of the science, technology, engineering, and mathematics (STEM) fields demonstrates disproportionately low African American, Hispanic, Native American & Alaskan Native representation in the workforce and the achievement of bachelor's degrees in STEM [1,2]. In the case of women, underrepresentation in STEM is especially demonstrated in engineering, physics, mathematics, earth science, and computer science [3]. Community colleges serve a vital role in providing opportunities for individuals who are underrepresented and disadvantaged to enter the realm of postsecondary education [4]. Currently, community colleges educate 38% of all U.S. undergraduate students, 52% of Native American students, 48% of Hispanic students, and 39% of African American students. Of students in community college, 30% are first-generation students, 16% are single parents, and 21% are students with disabilities [5]. Open enrollment makes community college more accessible to students with broader variation in academic achievement and preparation [6]. The significantly reduced cost of community college, compared to 4-year schools, makes them accessible to students from low-income backgrounds [7]. However, despite the increased accessibility of community colleges, community colleges in general, still show low STEM degree achievement rates [8,9].

Undergraduate research experiences have been demonstrated to help in increasing STEM excitement and retention rates in underrepresented students [10,11]. Various studies in undergraduate research experiences have shown increased STEM degree achievement rates among undergraduate research students [12,13].

Undergraduate research has also been shown to strengthen positive scientific identities, persistence in pursuing a scientific career, and integral skills in STEM, such as critical thinking, problem-solving, and understanding scientific topics, and persistence in pursuing a scientific career [14,15].

Community college students face a key challenge in the pursuit of undergraduate research, many community colleges have very limited research opportunities, if any at all. These reduced opportunities result in a deficit in undergraduate research culture at community colleges [16,17]. As a result, community college students are at a disadvantage compared to their peers in 4-year research institutions and often miss out on the benefits associated with participation in undergraduate research.

The primary goal of the Micro Nano Technology Collaborative Undergraduate Research Network (MNT-CURN) is to increase educational equity and STEM degree achievement by providing community college students nationwide with research opportunities that are accessible and offer the chance to build valuable STEM workforce skills. Created in 2021, MNT-CURN is a National Science Foundation-funded program based at Pasadena City College in Pasadena, California. Community college students nationwide participate in virtual research-based meeting sessions with experts in various micro-nanotechnology fields throughout the academic year. Ultimately, they gain hands-on research experience at a participating university. This is accomplished by relying on high-impact practices (HIPs) to retain, train, and prepare students for the technical workforce or transfer to a higher education institution. HIPs require students to devote significant time and effort while fostering extensive interaction and feedback among peers and faculty.

Furthermore, students are placed in diverse environments and encouraged to apply their education in different settings [18]. HIPs include academic learning communities, collaborative assignments and projects, common intellectual experiences, undergraduate research, ePortfolios, and internships [18, 19]. They have been shown to increase the likelihood of students completing college within six years [20]. These HIPs have also improved critical thinking skills, more in-depth learning opportunities, and positive attitudes toward interacting with classmates and staff [21]. However, recent research has shown that HIPs, while useful, are often least accessible to historically underserved students. As a result, HIPs have increased inequity rather than decreased it [22]. MNT-CURN strives to address this inequity by utilizing a framework that makes HIPs easily accessible to historically underserved students.

Methods

Evaluation Setup

MNT-CURN's commitment to providing research opportunities to community college students has been evaluated in many ways to observe the program's efficacy. Educators who are not internally affiliated with the program were selected as external evaluators to ensure the goal was met. They were selected due to prior experience evaluating similar scientific programs and equity research. This group of evaluators included Terryl Bailey, the President of The Allison Group, Dr. Jalil Bishop, the co-founder of Equity Research Cooperative (EqRC), and Dr. Antar Tichavakunda, a professor of Race and Higher Education at the University of California at Santa Barbara. Together these expert evaluators used multiple tools to determine the effectiveness of MNT-CURN. These tools included surveys, focus groups, staff interviews, and observations.

Surveys

Surveys that the Allison Group created were administered before, during, and after MNT-CURN to get consistent feedback from the students about their experiences and perceptions of the program. Since the surveys allowed students to answer organically and in a way that can be quantified, they will be the focus of the results in this study. The surveys encouraged students to think about their experiences within STEM, both within the MNT-CURN program and at their home institution. The optional surveys given pre- and post-academic year focused on the student's goals and ideals in STEM fields and whether they planned to continue pursuing STEM-related career paths. In contrast, the bimonthly surveys focused on diversity and inclusion within the program. These surveys aimed to capture a quantified view of how the participants perceived themselves as part of MNT-CURN and STEM as a whole.

Figures 1-5 display the types of questions asked during the surveys. **Figures 1 and 2** show the answers to questions regarding race/ethnicity, gender, and childhood household income. **Figures 3-5** show the answers in pre- and post-cohort of Year Two. These particular questions were chosen as they reflected whether students were likely to continue studying STEM and/or how vital MNT-CURN benefits like stipends and mentorship were to the students.

Results

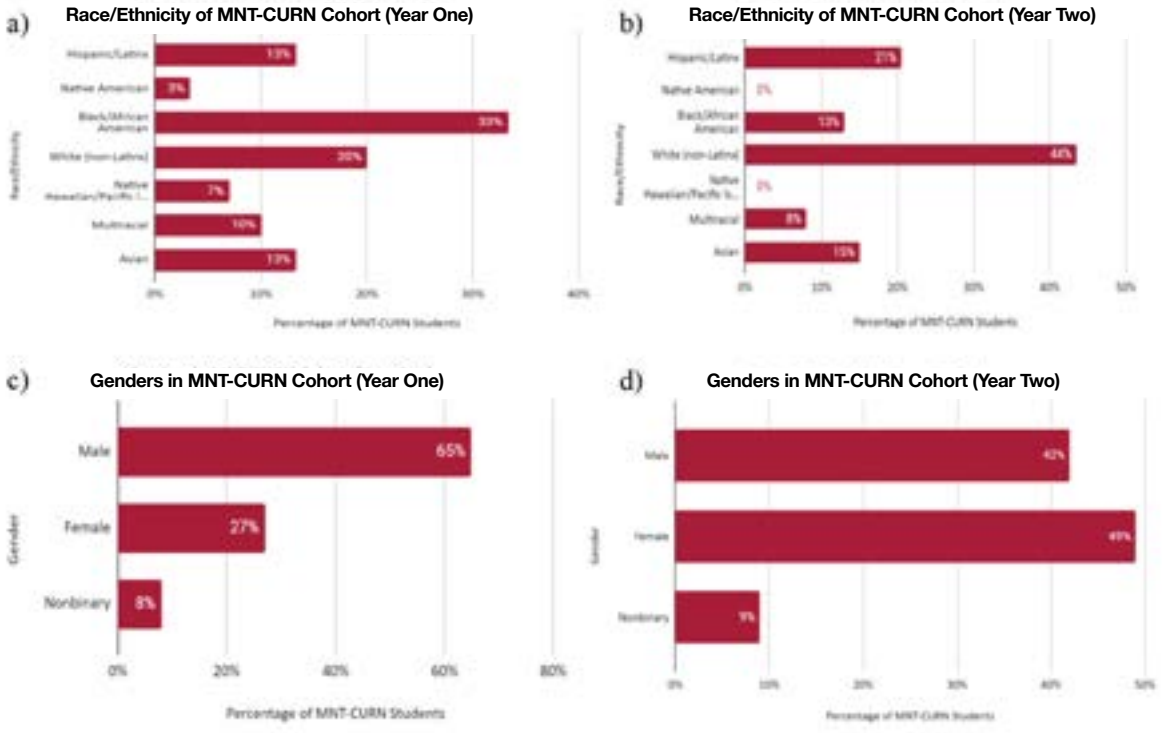

Fig. 1. Race and ethnicity statistics of the MNT-CURN student cohort over the first two years of the MNT-CURN program. (a) Year one N=30, 3 participants skipped (b) Year two N=33, 2 participants skipped. Gender statistics of the MNT-CURN student cohort over the first two years of the MNT-CURN program. (c) Year one N = 30. (d) Year two N=33, 2 participants skipped this question in the survey and the questionnaire.

Figure 1 provides data from the first two years of MNT-CURN cohorts. This portion of the data focused on compiling the racial (a and b) and gender (c and d) distributions from the beginning to the present. This data was used to evaluate the program's equity and inclusion of underrepresented groups.

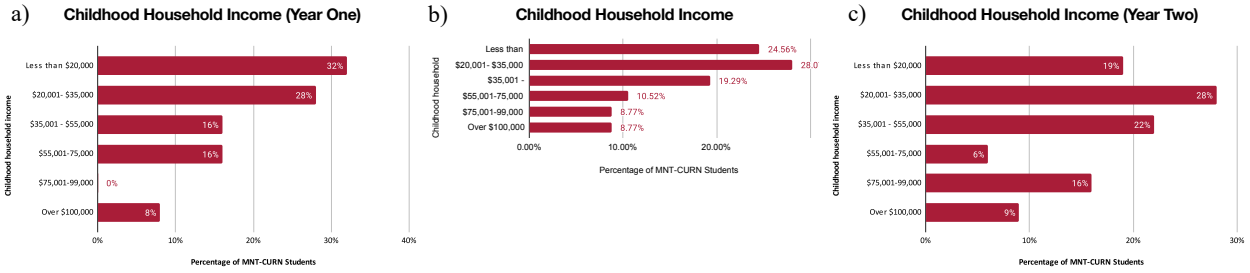

Fig. 2. Childhood household income statistics of MNT-CURN student cohort of the MNT-CURN program, Year One, N= 36(a) Year Two, N=40 (b). Veteran, first generation, rural area, and disability statistics of the MNT-CURN student cohort were collected in Year Two, N=40 (c).

Income data over the two years was also compiled to further evaluate equity and inclusion trends. The statistics of childhood household income allowed analysis of the amount of low-income students that comprised MNT-CURN's student population (a and b). The additional information included aspects of sub-populations amongst students that allowed for the display of diversity among the students (c).

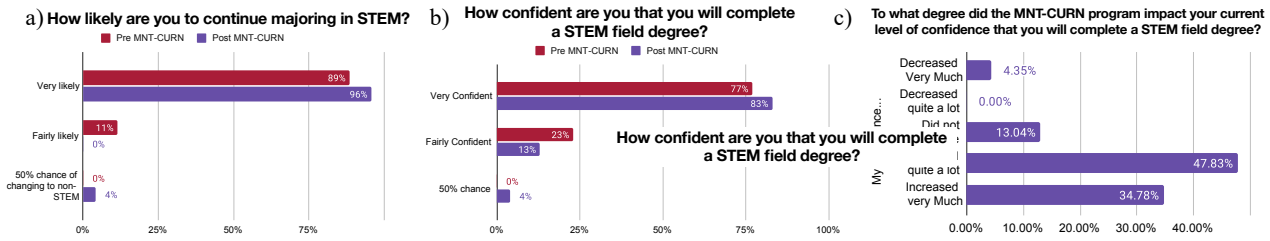

Fig. 3. Percent likelihood of Year Two MNT-CURN cohort students to continue majoring in STEM before and after the MNT-CURN experience (Pre MNT-CURN N=35, Post MNT-CURN N=24) (a). Percent confidence of Year 2 MNT-CURN cohort students to graduate with a degree in STEM before and after the MNT-CURN experience (Pre MNT-CURN N=35, Post MNT-CURN N=23) (b). Students self-declared the impact MNT-CURN had on their level of confidence that they will complete their STEM degree (N=23) (c). Figures a and b showed non-significant results.

To evaluate MNT-CURN's goal of student retention and personal growth, paired T-Test analyses of the questions "How likely are you to continue majoring in STEM?" (a) and "How confident are you that you will complete a STEM field degree?" (b) was completed to see if there were statistical significance between the pre-and post- cohort surveys. For the "How likely are you to continue majoring in STEM?" question, the responses were found to be insignificant, with a p-value of 0.294. Similarly, the "How confident are you that you will complete a STEM field degree?" question had a p-value of 0.270. Despite the non-significant results, the self-report of MNT-CURN's impact on the confidence of students to complete STEM-related degrees displayed that the majority of students had an increase in confidence post-cohort (c). Figure 3c was excluded from paired T-Test analysis because this question only pertains to and is being asked in the post-cohort survey.

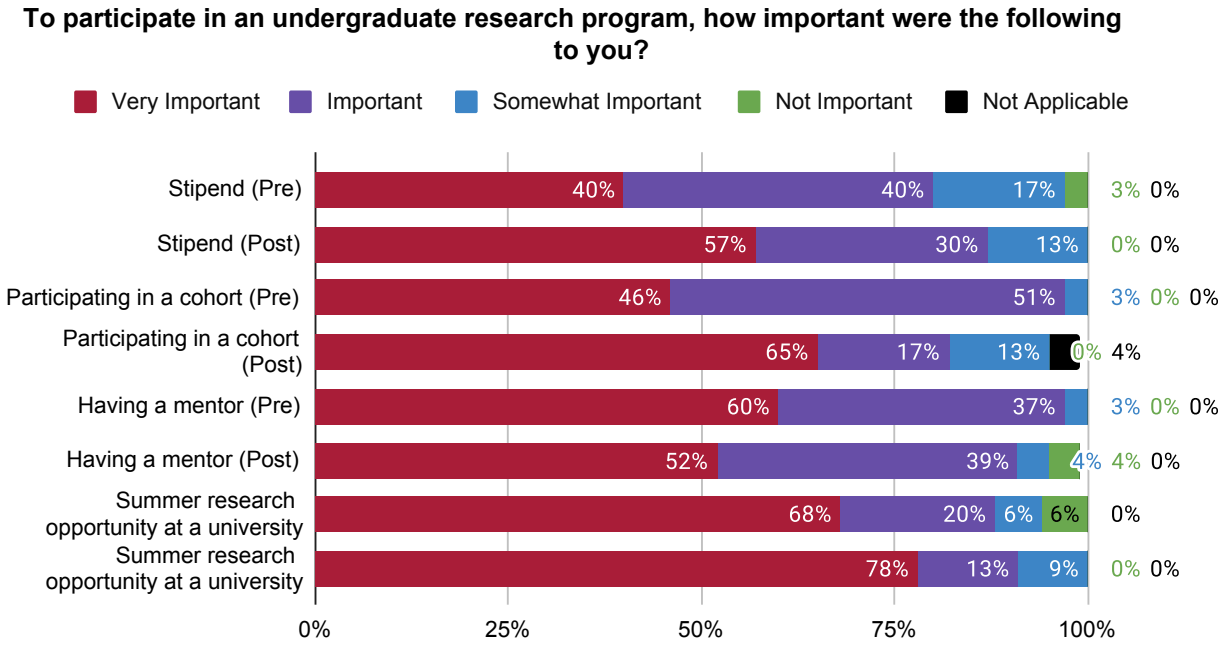

Fig. 4. Student self-declared the importance of various aspects of the MNT-CURN program before and after MNT-CURN program completion. All comparisons were non-significant.

Pre MNT-CURN N=35; Post MNT-CURN N=23.

Due to the noted inaccessibility of programs similar to MNT-CURN that provide research experience and STEM mentorship and the complexity of how each portion may intersect, each aspect of this question was evaluated separately for pre- and post-MNT-CURN cohort response via paired T-Test analyses. All aspects were found to have insignificant differences. The respective p values are: Stipend: 0.127, Participating in a cohort: 0.461, Having a mentor: 0.266, Summer research opportunity at a university: 0.109.

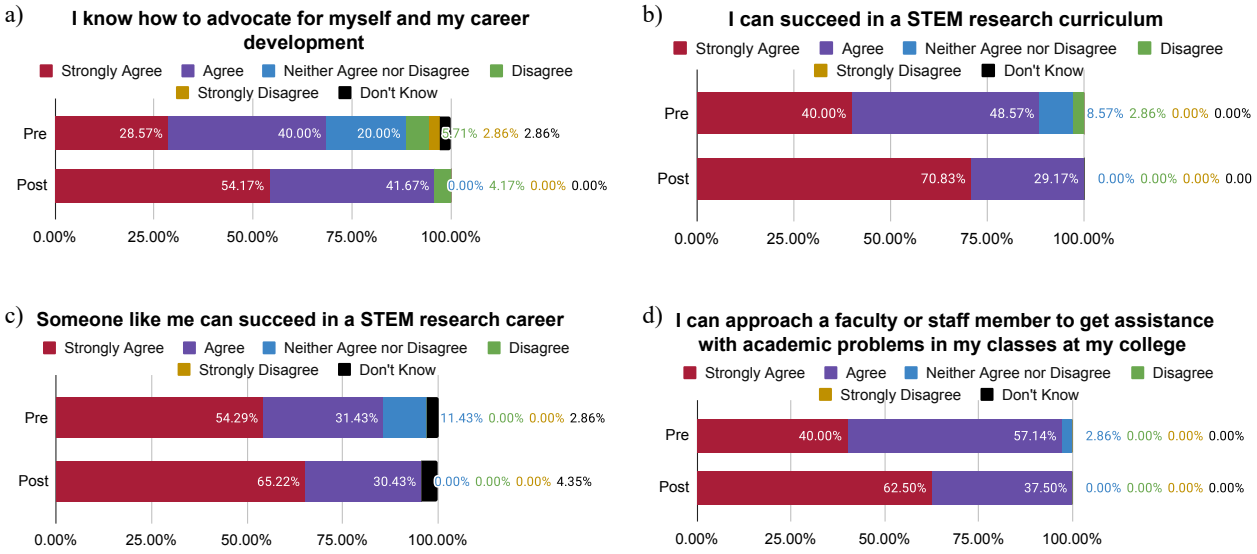

Fig. 5. Student beliefs on various aspects of entering STEM academia and workforce spheres before and after MNT-CURN program completion. Student beliefs regarding their ability to advocate for themselves (a). Student beliefs on their ability to succeed in STEM (b). Student beliefs that someone like them can succeed in STEM (c). Student beliefs that they can approach faculty or staff for help with academic problems (d). All comparisons were non-significant. Pre MNT-CURN N=35; Post MNT-CUTN N=24.

In order to observe MNT-CURN's impact on the student beliefs within the STEM field, paired T-Test analyses were completed to compare the pre-and post-cohort responses. All the aspects resulted in insignificant data. The p values for the separate elements are as follows: Self-Advocacy: 0.240, Succeeding in STEM research curriculum: 0.359, Being able to succeed in STEM research career: 0.076, Approaching Faculty/Staff: 0.368.

Discussion

Equity in STEM

In an effort to execute the goal of promoting equity amongst underrepresented student populations, MNT-CURN invites students from traditionally underrepresented groups to apply. Since its inception, MNT-CURN evaluators surveyed the demographic makeup of the 2022 and 2023 cohorts to observe these efforts. In **Figure 1**, the data shows a shift in racial makeup in the Year One and Year Two cohorts. According to the American Association of Community Colleges (AACC), the reported average racial demographics of community college students enrolled for credit are as follows: Caucasian students - 45%, Hispanic students - 27%, Native American students - 1%, African American students -12% [12]. In our data, we see a shift from Year One to Year Two in terms of the racial makeup of the cohort. In Year One (**Figure 1a**), the majority of the cohort is from an underrepresented group within STEM, though in Year Two (**Figure 1b**), the percentages mirror the AACC data.

Further evaluation would be necessary to uncover how the cohort's racial data shifted to the statistical norm. Further, **Figure 1c-d** displays the data on gender from Year One and Two. A shift from male to female majority can be seen along with increased nonbinary participants in the cohorts. As the goal of MNT-CURN is to promote equity, this trend of racial and gender makeup should be observed for more years for further evaluation.

As the accessibility of community college allows students from impoverished backgrounds a chance to have an education, socioeconomic trends observed show that students from low-income backgrounds will still struggle to attain their associate degree [23]. As represented in **Figure 2a-b**, more than half of the students from both years come from households where the below $55,000. As that would be considered low income, the goal of equity among class is being met as the cohort consistently allows the opportunity of research to lower-income students.

Additionally, of the students in MNT-CURN's year two cohort that answered or skipped the additional information questions in **Figure 2c**, 5% were veterans, 40% were first-generation college students, 12.50% were from a rural area, and 12.50% were disabled. Compared to the representation of first-generation college students in community college as described by the AACC (30%), the MNT-CURN program demonstrated a 33.33% representation of first-generation college students within their program.

Use of HIPs and Their Access:

As previously addressed, research shows that while community colleges create a way for low-income students to gain an education, their financial status and responsibilities will statistically make obtaining degrees harder [23]. While HIPs, like undergraduate research, have been shown to benefit students and bolster STEM retention rates, accessibility for community college students remains limited [10, 11, 22]. MNT-CURN addresses this issue by creating cohorts geared toward community college STEM students and providing resources such as stipends and remote research engagement [24]. As there is a higher prevalence for community college students to experience a lack of basic needs such as food security and housing, the chance to participate in a HIP that provides payment and remote opportunities opens doors for students who would not be able to sacrifice the time or resources to experience undergraduate research [23, 25, 26]. According to our data, **Figure 4** shows that most students pre- and post-cohort considered the stipend payment as "very important" or "important" in participating in an undergraduate research program. The difference between the pre and post surveys were determined to be non-significant; however, the consistency of the majority considering the HIPs available in the program to be of importance, shows the goal of MNT-CURN's accessibility is being met.

Addressing the Student Retention of STEM Majors for Careers

MNT-CURN also has goals to improve STEM student retention and prepare students for technical careers or higher education. According to research, while the majority of community college students aspire to transfer to 4-year universities, only a fraction do so [27]. In the evaluation surveys given at the beginning and end of the academic cohort, students were asked about their plans to major in STEM and their confidence levels for completing a STEM field degree. In **Figure 3a**, the data shows that while 89% of students were most likely planning on continuing STEM-based majors before the cohort, the percentage jumped to 96%. Similarly, the data collected on students' confidence in finishing a STEM degree increased from 77% to 83% (Figure 3b). These observations are supported by **Figure 3c**, where students were asked directly about MNT-CURN's impact on their confidence levels. **Figure 3c** displays that 82.61% of students in the cohorts felt that their confidence in completing a STEM degree was increased due to MNT-CURN. In **Figure 5**, the data shows that students typically displayed a more positive outlook on aspects of belonging and succeeding within STEM after their participation in MNT-CURN. These upward trends in planning to engage with and complete STEM degrees showcase that MNT-CURN's goal of student retention and preparing for future STEM-related endeavors positively influenced the cohort, despite the data being statistically insignificant.

Conclusion

Overall, MNT-CURN's goals for increasing STEM engagement for students statistically underrepresented in STEM fields and undergraduate research will need further evaluation. While the cohorts bridge a gap for students that may benefit from high-impact practices or mentoring, they would otherwise have no accessibility to such until and if they attended a four-year university; our data at this time shows no statistical significance. According to the studies cited in this paper, it is concluded that MNT-CURN's program may see positive trends in the areas of efficacy in student retention, accessibility, and reinforcing the belief in students that they belong in laboratories where they may not see representation, but the data shows no significant increase in these measures and will require more cohorts to see greater impact.

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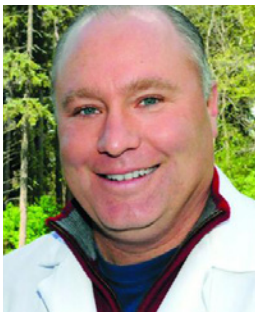

Invited Letter: Primary Focus Is to Work toward Embedding Opportunities within Required Courses

Keywords: undergraduate research models, Finger Lakes Community College, CCURI, NSF, ATE

As a quick introduction, my name is Professor James Hewlett. I am a Professor of Biology at Finger Lakes Community College (FLCC) in Canandaigua, NY. In addition, I serve as the Program Coordinator for our A.S. Biotechnology degree program. Outside of my faculty role, I have served as the Executive Director of the Community College Undergraduate Research Initiative (CCURI) since 2007. With over 140 institutional partners, CCURI has focused on a mission of expanding opportunities for community college students to engage in an undergraduate research experience. The primary focus at Finger Lakes Community College is to work toward embedding these opportunities within the required course sequences and then provide additional opportunities for deeper exploration by participating in an active student research group. Although the current CCURI initiatives and efforts at FLCC are now operating at scale, the journey associated with this scaling effort continues to be defined by what can only be described as modest beginnings.

The word that best captures my initial foray into the undergraduate research space would be serendipity. Early in my career, I was approached by a field scientist to collaborate on a project that required various DNA research techniques to answer questions related to the migratory patterns of Eastern Red-tailed hawks. Without hesitation, I found two biotechnology student volunteers, and the three of us embarked on what eventually became a five-year project involving dozens of students. While we were not intentionally measuring student impacts during this time, the observations that I made concerning these metrics convinced me that this experience needed to be employed at scale. Soon after, we expanded our project portfolio and submitted our first National Science Foundation (NSF) proposal to test a novel model for course-based research experiences (CUREs). The awarding of this grant marked the beginning of what would become CCURI. Through this extensive National network of community colleges, we have found opportunities to study the research cultures at our partner institutions and conduct meta-analyses to capture best practices, institutional barriers, and student opportunities. As a Co-PI on the NSF ATE InnovateBIO National Biotechnology Education Center, I am currently working on undergraduate research models that involve industry-relevant projects that often include direct collaboration with an industry partner. The primary goal of this effort is to explore ways in which a research experience builds workplace skills that enhance the employability of students graduating from technology education programs.

The results from our meta-analyses of CCURI institutional partners suggest that any effort to ensure a successful undergraduate research program at a community college should begin with an understanding of how an institutional culture aligns with the ability of that institution to develop and sustain the research opportunities that they hope to provide for their students. Successful CCURI institutional partners have aligned their efforts with institutional priorities, remained connected to internal and external networks, embedded the experiences in coursework, included a faculty development plan, employed a multi-disciplinary approach, and adhered to a shared strategic vision for undergraduate research on their campus. These institutional characteristics have been shown to be powerful predictors of the ability to scale and sustain research experiences at a community

college. From a broader perspective, enabling our nation's community colleges to become full participants in the active practice of research requires having a better understanding of how to drive institutional-level change that can lead to a significant paradigm shift with respect to how we view the role of the community colleges in STEM educational reform efforts. That being said, it has become clear that the growth of community college participation in the undergraduate research community continues despite the many internal and external challenges these institutions face.

Professor James Hewlett
SUNY Distinguished Service Professor
Coordinator, Natural Sciences
Director, Biotechnology/Biomanufacturing

Mapping Immunogenic Regions In SARS-CoV-2 to Understand Vaccine Design Using Bioinformatics

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Abstract: Disparities in undergraduate STEM degree completions across the United States are a national concern. Undergraduate-level research opportunities are vital for developing future researchers and building their scientific identity. These experiences can help students in community colleges acquire 21st-century skills and build confidence in their ability to do science [1-3]. The development and implementation of guided research experiences provide users with a topic they are familiar with but not necessarily experts in, like SARS-CoV2 infections. In this particular study, the Immune Epitope Database (IEDB) was used to identify amino acid residues located on the immunogenic regions of the spike glycoprotein of SARS-CoV-2 variants: Alpha, Beta, Gamma, Delta, and Omicron. IEDB is a web-based bioinformatics tool that contains published epitope information and prediction aids that can be used as a research platform for studying infectious diseases. The objective of this study aimed to map the immunogenic regions on the spike glycoproteins of the SARS-CoV-2 variants and predict the immune evasion of these variants [4-6]. Identifying the antigenic determinations that bind to the antibodies is essential for designing future candidates for peptide-based vaccines.

This study aims to map the immunogenic regions on the spike glycoproteins of the SARS-CoV-2 variants and predict the immune evasion of these variants [4-6]. Identifying the antigenic determinations that bind to the antibodies is essential for designing future candidates for peptide-based vaccines. This research identifies regions where mutations have occurred in the virus, which are important to study as they can affect the virus's immune evasion and impact available vaccines. Targeting multiple immunogenic regions unaffected by mutations can serve as potential targets for new vaccines, providing better protection against different variants.

Keywords: Immunology, Bioinformatics, Biotechnology, engineering, IEDB, B-cell, peptide-based vaccines, scientific reasoning

Introduction

The adaptive immune system in animals can recognize a wide range of antigens or ligands from various pathogens. The B cell and T cell receptors are responsible for identifying these antigens and triggering an immune response. The specific regions on these antigens are called epitopes, which are recognized by B and T cell receptors. The IEDB database includes validated and benchmarked methods to predict epitope-paratope binding, antigen processing, and T and B cell recognition receptors for infectious diseases, allergens, autoimmune diseases, and transplants.

As part of this research experience, students Brady Anderson, Christopher Salgado, Jatniel Morales Gomez, Brianna Carr, and Joanna Sanchez Rocha were introduced to bioinformatics analytical tools that can help in the development of new vaccines, diagnostics, and therapeutics. They were guided by their mentor, Dr. Sheela Vemu, Associate Professor of Biology at Waubonsee Community College. They had an amazing opportunity to interact with the La Jolla Institute of Immunology team, who provided further clarification on the use of the database and how it can be incorporated into their research.

The Immune Epitope Database Analysis Resource (IEDB) IEDB webpage (www.iedb.org) is a tool created by NIAID (National Institute of Allergy and Infectious Diseases). It is freely available to all to provide access to a variety of epitope analysis and prediction tools [7]. The database consists of experimental published data from humans, non-human primates, mice, and other animal species. This database component can also be queried using a flexible user interface that links pathogen-specific and immunological assays into one set of data [8]. It is populated using information captured and curated from peer-reviewed scientific publications worldwide and contains a vast amount of data.

As of August 2022, there were 1,730,172 peptidic epitopes available on the ImmunomeBrowser section in IEDB. Of that number, 1,119,684 epitopes were mapped on structural complexes leaving 610,488 epitopes not mapped. The mapped epitopes are split with 501,491 on a PDB complex and 618,193 mapped on an Alphafold-modeled structure [9]. As of March 2023, over 100,000 unique epitopes have been put into the IEDB database from cutting-edge scientific research. It is continually updated and is the single most powerful repository in the world for a comprehensive collection of experimental data that can be queried for known epitopes and their immunogenic regions.

The ImmunomeBrowser tool in the IEDB maps epitope recognition information to an antigen and computes an immunogenicity score for each position. This identifies immunogenic hotspots in a protein. So far, this data has only been plotted based on the linear sequence of the proteins. As a part of their research experience, the students wanted to construct a 3D visualization of the data scores and show how immunogenic regions in a protein are located in a 3D space.

Throughout this research experience, the students implemented a series of action steps:

Step 1: Idea development, managing teams in the class with faculty mentorship

Step 2: Learning new resources and databases - gallery walk

Step 3: Ideation, brainstorming of ideas

Step 4: Plan a research project

Step 5: Reaching out to fellow students and regular follow-through

Research experiences such as these students' can provide opportunities for others to participate in consequential research. Which then can increase the science identity of each student and help them feel a sense of belonging to the larger scientific community.

Materials and Methods

ImmunomeBrowser maps and visualizes queries in IEDB linear peptidic epitopes along the length of a protein sequence (a target, or reference, protein). The tool's purpose is to allow users to explore how often each protein region has been studied in immune assays and how many assays the immune response was positive or negative. ImmunomeBrowser provides summarized data by reference antigen because: (i) epitopes reported in IEDB were identified for different strains and protein isoforms – mapping to the reference protein allows to visualize and study of such epitopes as they would have the same antigen; (ii) different mutant variants of the same epitope were tested and reported; and (iii) immune response varies among studies and assays due to heterogeneity of samples and complexity of immune response [11]. Immunome Browser is accessible in IEDB via two entry points (or tabs): the Antigen or Epitope navigation paths [1]. A prominent feature in the IEDB is the ImmunomeBrowser tool [10], which maps epitope recognition information back to an antigen and computes an immunogenicity score for each position in that antigen. This can identify immunogenic hotspots of epitope recognition in a protein as compared to other areas that are not recognized. These data have so far only been plotted based on the linear sequence of the proteins. The ImmunomeBrowser [10] is a tool that retrieves all epitopes available in the IEDB related to a given parent protein and calculates a score called the response frequency (RF). This score attempts to draw attention to regions of the antigen that are more immunogenic. The RF score is based on the number of positive assays at each protein position and uses the lower bound of the 95% confidence interval to provide a conservative estimate.

The resulting RF scores reveal immunogenic hotspots within the protein, providing valuable insights into regions more prone to epitope recognition. The data generated by the ImmunomeBrowser are presented in graphical form, allowing researchers to visually analyze and interpret the immunogenicity profile along the length of the protein. Through these methods, researchers gain a comprehensive understanding of the immune response to specific antigens and can efficiently explore immunogenic regions within the protein. The IEDB web-based interface and the ImmunomeBrowser tool streamline the data analysis process, facilitating informed decision-making and contributing to advancements in immunological research.

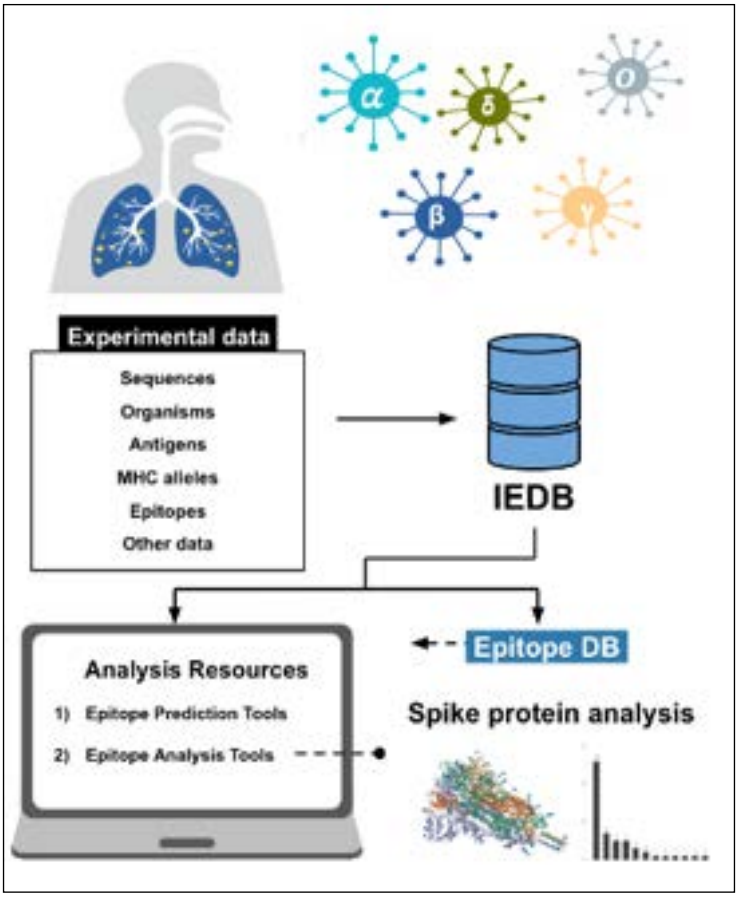

Fig 1. The immune epitope database is composed of real experimental data and provides summarized data by reference antigens. IEDB is composed of the Database and analysis resources.

Results

Analysis of the SARS-CoV-2 spike glycoprotein was achieved through the accession of the IEDB database, specifically the ImmunomeBrowser. Five variants of interest were identified: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529). The IEDB database sourced all scientific literature for positive and negative assay results at the protein residue level [10]. By sorting through the ImmunomeBrowser for data on SARS-CoV-2 spike glycoprotein in human B cells, the students could visualize the frequency of positive assays led by researchers worldwide at specific regions in the spike glycoprotein. Upon ImmunomeBrowser input of various SARS-CoV-2 variants of interest, point mutations from each variant were visualized overlay the region of the spike glycoprotein. In this overlay, the students saw that the variants contained unique mutations in multiple domains of the spike protein but also appeared to share regions of similar point mutation occurrences. Of interest, the Omicron variant appeared to possess the highest frequency of point mutations between 333 to 527 residues, in which the receptor binding domain (RBD) consists.

Overview of The Immune Epitope Database

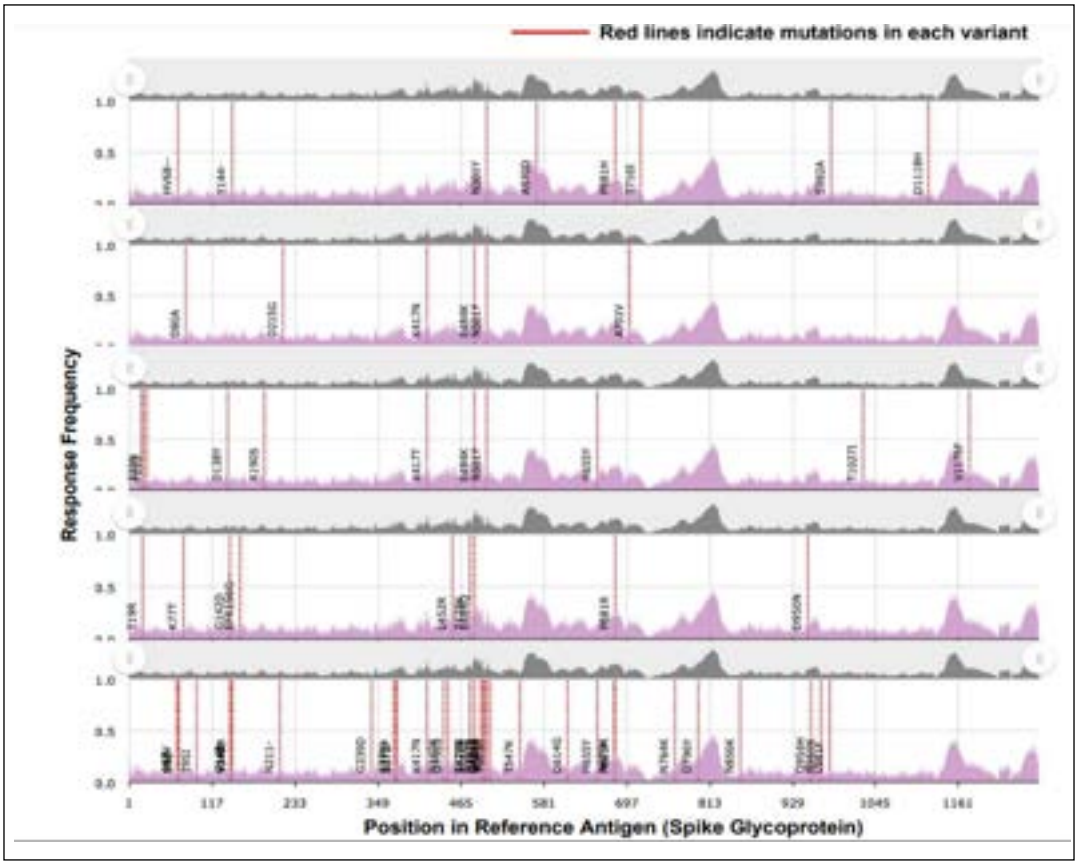

Fig 2: The Immuno Browser graphs above show the map of the response frequency data associated with an amino acid residue. Our results suggest that specific mutations are conserved among these five variants. The immunogenicity hotspots were found in the residue range of 300 - 550, corresponding to the receptor binding domain. The positive epitope assay counts showed activity, while the response frequency exhibited no change when tested against our reference antigen. Omicron displayed the most mutations within the overall range of residues within the hotspot range.

Further analysis of the number of assay counts and associated response frequencies of each residue in the spike protein was plotted and colorimetrically highlighted by each corresponding variant. The students noticed that the Omicron variant had more mutations in residues with increasing response frequencies and increasing assay counts than the other variants of interest.

Next, the students looked at visualizing the spike protein mutations across each of the variants in terms of their unique respective mutations and their shared mutations shown in Fig 2. Using the UpsetR package in RStudio, we saw that Omicron possesses 26 specific mutations on the spike glycoprotein, whereas other variants, such as Gamma, Delta, Alpha, and Beta, have 7, 5, 5, and 3, respectively. Of the residues that shared a mutation with more than one variant, Omicron was common to all. Some residues, such as 417 and 681, had three variants with mutations at these sites. Residues 484 and 501 each contained four of the five variants.

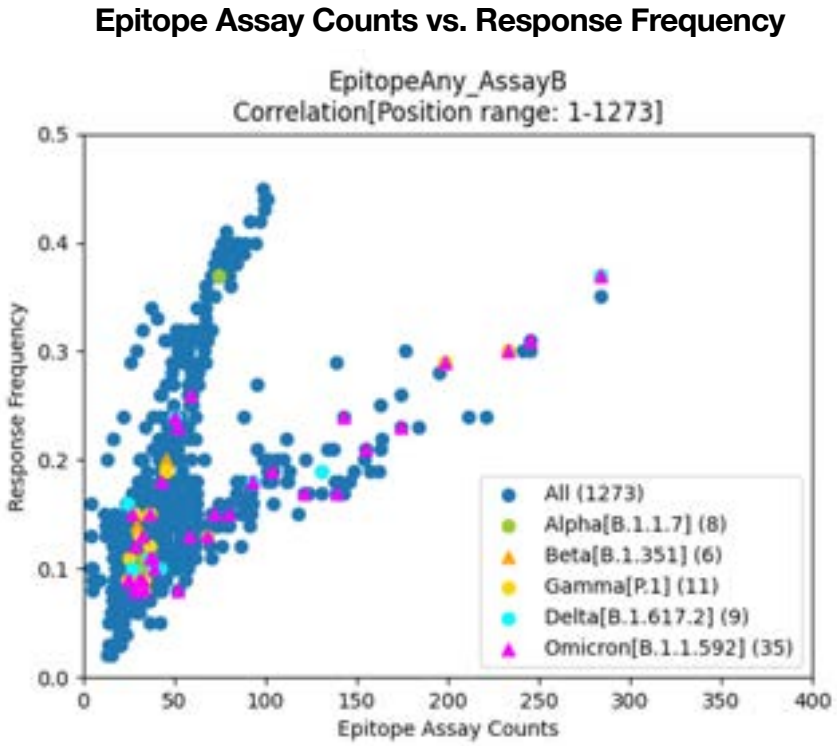

Fig.3: Data from IDEB was analyzed to study correlations between epitope assay counts and response frequency among all five variants. Omicron has the most immunogenic regions and follows the larger count trend concerning higher response frequency. We see two trends: Smaller counts with respect to responses more frequently responded. Larger count with respect to responding less frequently responded. Many mutations in Omicron follow the larger count trend with respect to higher response frequency.

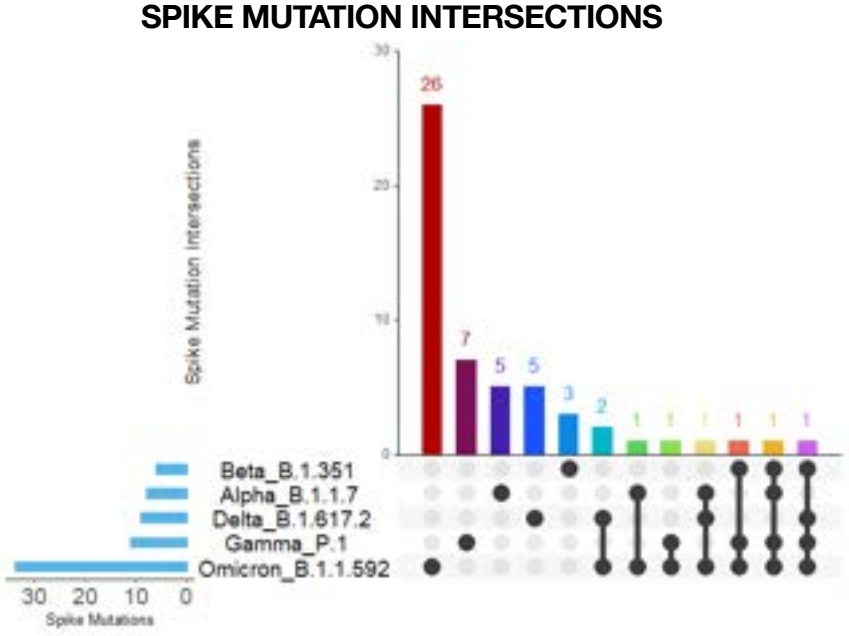

Fig. 4: Shows the shared mutations (amino acid residues) on the spike glycoprotein among the various SARS-CoV-2 variants: Beta, Alpha, Delta, Gamma, and Omicron. Variants with shared mutation residues are depicted by a linking line between each variant(represented as dots). Omicron variants possess 26 unique mutations on the spike glycoprotein and over 30 total spike mutations. Amino acid residues 404 (magenta), 501 (yellow), 417 (orange), and 681 (gold).

All positions in the Spike-Glycoprotein, where Omicron has shared mutations with other variants

Position	142	144	417	478	484	501	655	681
Alpha		o				o		o
Beta					o	o		
Gamma			o		o	o	o	
Delta	o		o	o	o			o
Omicron	o	o	o	o	o	o	o	o

Table 1: Indicates a high degree of overlap (green) as emerging potential hotspots for immune evasion and target for new peptide-based vaccines.

Discussion

The emergence of the Omicron variant has raised numerous concerns, including but not limited to the origin of exposure, the impact of mutations on vaccine efficacy, modifications to host immunity in response to mutations, and the level of lethality and transmissibility of the variant. The student findings suggest that Omicron and Delta have more affinity towards immune escape compared to the other variants due to the receptor binding motif 437-508. This was shown between the amino acid 300 -550 residues. Mutations in this immunogenic region may pose challenges for adaptive immune responses. This could be from the specific protein conformation, which is possibly elusive to antibody and/or vaccine therapies. In the mutation, L452R (Leucine), which is hydrophobic, is bound to a charged arginine in the receptor binding motif of the spike glycoprotein receptor binding domain (RBD). Mutations in this immunogenic region might pose challenges for adaptive immune responses as shown in this data. The increased mutations between Omicron and the other variants may highlight some of the selective pressures on the virus to avoid natural and therapeutically immune detection via vaccines.

This research helps identify specific regions where mutations have occurred. These regions are essential to study because mutations can potentially alter the virus's behavior, including its infectivity and immune system evasion. Knowing which regions have undergone mutations is fundamental to understanding how the virus evolves and how it might impact available vaccines. Also, the approach applied in this study investigates other immunogenic regions that remain unchanged across different variants. By targeting multiple immunogenic regions unaffected by mutations, these regions can serve as potential targets for new vaccines, eliciting a more robust and broad immune response, making it harder for the virus to evade immune detection, and providing better protection against different variants.

Designing broadly applicable epitope-based vaccines against highly variable pathogens, like SARS-CoV-2, often require epitopes to be conserved across variants [12]. Our results can be used to filter possible candidate epitopes by removing residues that are recurrently hypermutated on the spike glycoprotein across globally important variants. Removing such residues may reduce the footprint of vaccine development in silico, in vitro, and in vivo. Other studies utilizing IEDB have successfully collated datasets on multiple epitopes and predicted possible vaccine constructs [13], binding affinity toward T and B cell motifs [14], and immunogenicity effects [15].

Various bioinformatics approaches have been used to expedite the discovery of potential drugs, design vaccines, and understand the concept behind COVID-19 pathogenesis. Bioinformatics has been instrumental in identifying interactions with SARS-CoV-2 proteins, predicting immunogen and antigen epitopes from SARS-CoV-2 proteins, and identifying new potential pathways in COVID-19 progression and pathogenicity. By leveraging these bioinformatics tools, researchers can model and predict complex biochemical processes related to the virus, accelerating the evaluation of existing vaccines against emerging variants. This will aid in timely and effective decision-making to maintain good immunity and combat the disease.

Therefore, this study demonstrated how immune-dominant parts of SARS-CoV-2 proteins have significantly mutated. Due to the need to identify the correct target for vaccines, we were curious about how an algorithm could suggest an epitope that is both highly antigenic and sufficiently conserved. As we've seen over the pandemic period, SARS-CoV-2 strain nomenclature has evolved considerably and continues to do so. Likewise, new mutations continue to be identified for existing or new strains. The IEDB ImmunomeBrowser utilizes SARS-CoV-2 strain and mutation data from NCBI's ACTIV-TRACE program. This information is updated weekly; therefore, a key limitation of the data is the availability of updated SARS-CoV-2 strains and mutations. Therefore, analysis of the ImmunomeBrowser information must be periodically reviewed as SARS-CoV-2 data is updated.

This research study is an excellent example of how exposure to research can create a catalyst for future generations to explore science. Repurposing IEDB can be a valuable learning tool for students interested in immunology. In addition to the research findings, the students gained the confidence & skills like communication & presentation to apply for regional and national internships & fellowships. By participating in this research experience, the students expressed confidence in building a repertoire of skills that are specific to the industry and, in turn, make them more competitive candidates for future jobs.

Acknowledgments. The students would like to thank their faculty advisor, Sheela Vemu, Ph.D., Associate Professor of Biology at Waubonsee Community College. They would like to express their appreciation for the input from Sandy Porter, Ph.D. during the Antibody Hackathons. President Digital World Biology. They would also like to thank Sharon Garcia, Executive Dean for Liberal Arts and Sciences, for advocating the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) institutional membership to support student research fellowships and Waubonsee Community College for extending support to showcase student research on campus. This project was supported by the National Science Foundation (NSF) under the award DUE 2055036.

Disclosures. The authors declare no conflicts of interest.

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Development of Online Modules for Teaching Blockchain

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Abstract: Blockchain technology enables the creation of a distributed and tamper-proof ledger, even in the presence of untrusted agents. While much financial resources and attention are devoted to blockchain tools, the underlying technology is not well understood by the general population. This paper presents a newly developed online tool that allows users to learn and create their own blockchain, with a graphical user interface and code. The module is freely available on nanoHUB.org and describes all components of the blockchain, including the SHA256, Proof of Work, and other features that enable the blockchain to function as a tamper-proof ledger. This tool has been utilized to instruct students without prior knowledge of blockchain technology, and the survey of students' responses demonstrates that this tool is an effective way of teaching the general population about blockchain technology.

Keywords: Blockchain, SHA256, Proof of Work, online education, collaborative research

Introduction

Blockchain technology has garnered significant attention in recent years due to its decentralized and secure approach to recording and verifying transactions, revolutionizing various industries [1]. The importance of blockchain lies in its ability to enhance transparency through decentralization, ensure cryptographic security for immutability, enable the potential of cryptocurrencies like Bitcoin and Ethereum, and streamline transactions for greater speed and efficiency. It can speed up international transactions and can protect against inflation [2-4]. Walmart Canada applied blockchain to solve a common logistics nightmare [5].

To delve deeper into the technical aspects and original concepts behind Bitcoin and Ethereum, it is recommended to refer to their respective white papers. The Bitcoin white paper, titled "Bitcoin: A Peer-to-Peer Electronic Cash System," was published by Satoshi Nakamoto in 2008 [6]. The Ethereum white paper, titled "Ethereum: A Next-Generation Smart Contract and Decentralized Application Platform," was authored by Vitalik Buterin in 2013 [7].

Comprehending blockchain technology can be challenging for the general population [8]. Despite conducting extensive searches, no accessible and interactive learning modules that effectively explain the fundamental mechanisms of blockchain, specifically tailored for the community college community, have been discovered.

To address this gap, an online instructional module has been developed to provide learners with hands-on experience in comprehending blockchain's inner workings. The module offers insights into data structures, transaction verification, consensus mechanisms, and real-world applications beyond cryptocurrencies, empowering individuals to grasp the transformative potential of blockchain in diverse industries. By combining theoretical explanations with interactive coding examples, our objective is to foster a deeper understanding and broader adoption of blockchain in the modern world.

Demonstrating Blockchain Technology Using Python

Our blockchain has two main features: SHA256 and Proof of Work [9]. The SHA, which stands for Secure Hash Algorithms, was created by the National Security Agency (NSA) of the United States. It is a collection of cryptographic hash functions designed to ensure data security [8].

SHA256

The SHA256 function converts input data into a hash of 64 seemingly random numbers and letters. For example, "hi" converted by SHA256 will output "8f434346648f6b96df89dda901c5176b10a6d83961dd3c1ac88b59b2dc327aa4" and this output is affected by factors such as uppercase and lowercase letters, spaces, and other symbols in the input. However, the output hash results are not truly random because each specific input will always have its own unique hash. Crucially, while the SHA256 function easily computes a hash from a given input (See Figure 1), it is extremely challenging to reverse-engineer the original input based solely on the hash result [8]. There are various Secure Hash Algorithms: SHA-1, SHA-224, SHA-256, SHA-384, SHA-512, SHA-512/224, and SHA-512/256 with different levels of security [8]. SHA256 was used to reproduce the demonstration in Blockchain 101 - A Visual Demo [7].

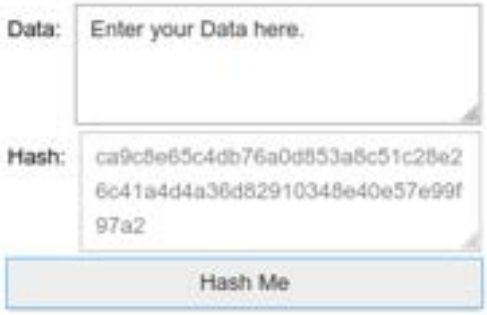

Fig. 1. SHA256 Graphic User Interface [9]

Proof of Work

The Proof of Work is a method of validating a block to be added to the blockchain. It is a consensus mechanism within blockchain and cryptocurrency ecosystems, facilitating transaction verification and block addition. In this process, miners or participants engage in solving intricate puzzles or mathematical problems to validate transactions and solidify them on the network [10, 11].

The blockchain learning module used a simple Proof of Work. A hash that starts with four zeros (0000) indicates that Proof of Work was done on a block. For this demonstration of a simple Proof of Work, the nonce and data are concatenated to generate the hash. The data never changes but the nonce is increment by one until the nonce causes the hash to begin with four zeros. (See Figure 2.)

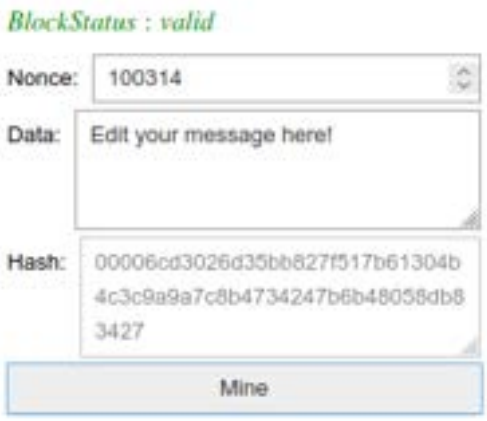

Fig. 2. Proof of Work Graphic User Interface [9]

A Block in a Blockchain

A blockchain consists of valid individual blocks that are linked together. An individual block stores a transaction, so each block has the following: index, nonce, data, previous hash, and hash. The index is the numerical position of the block in the blockchain. For example, the third block in the blockchain will have an index of 2 and not 3. This is because the first block index is 0. The nonce is an integer that changes when performing Proof of Work. The data stores the transaction details. The previous hash is the hash result of the previous block. Importantly, the previous hash makes the blockchain secure; it links the current block to the previous one. The hash is the SHA256 hash result of the concatenation of index, nonce, data, and previous hash.

The Genesis block is the first block in the blockchain, and it has an index of 0. Because there is no block before the Genesis block, the previous hash of the Genesis block is defined to be a sequence of 64 zeros. The system automatically generates the Proof of Work when the Genesis block was created, making it a valid block with the nonce value 58259.

Suppose two transactions will be added to the blockchain: Robert paid John $1000, and Mary paid Tom $1688. Assume the blockchain currently only has the Genesis block created. The following process is performed when adding the transaction "Robert paid John $1000!" to the blockchain as a new block. The new block index is 1, the nonce starts with 1, data is "Robert paid John $1000!" the previous hash has the hash result of the Genesis block, "000045a66e07b1edd4fb65cc5eaf31eca1e2955befbd43975bff6a392164e76f", and hash of the current block is the SHA256 hash result of "11Robert paid John $1000!", which is "1ba761f1dbcf91e0d29e06d14d15d813b3f6d7f54c758c5f617fb289060b3ad3", which may appear random but is actually not random. (See Figure 3.)

Fig. 3-1. Genesis block

Fig. 3-2. Invalid block-1

To validate this transaction, a nonce value must be found, so the hash result will start with four zeros. The nonce is incremented one by one. It will take a long time to find the hash result beginning with four zeros. The probability of getting four zeros at the beginning of the hash result is very small, P = (1/16)^4 because each digit of the hash result could be a number from 0 to 9, or lower-case character from a to f; in total, there are 16 possibilities [8]. In the Python code, a while loop was used to speed up the search. The nonce "48871" was found for this block-1 to become valid, with the hash value "0000a606f7bc866285273e52ffcfc56049fb60c7dd71a398296f929fcf5fa9bc".

(See Figure 4.)

Fig. 4-1. Genesis block

Fig. 4-2. Valid block-1

To add the second transaction, "Mary paid Tom $1688!" to the blockchain, as a new block, the following process is performed. The new block index is 2, the nonce starts with 1, data is "Mary paid Tom $1688!" the previous hash has the hash result of the block 1, "0000a606f7bc866285273e52ffcfc56049fb60c7dd71a398296f929fcf5fa9bc", and hash of the current block is the hash result of "21Mary paid Tom $1688!", which is "fa34602246b194672f6728ab696f044d79ebbf1e8336f35450ed33dce5cd7030". (See Figure 5.)

Fig. 5-1. Genesis block

Fig. 5-2. Valid block-1

Fig. 5-3. Invalid block-2

To validate this transaction, a nonce value needs to be found, so that the hash result will start with four zeros. The nonce is incremented one by one. The nonce "171666" was found for this block-2 to become valid, with the hash value "0000cb8e1e9d0b1b24cc7abdb862dc27d5047fa3fc0620bb6c1204cd21dbf180". (See Figure 6.)

Fig. 6-1. Genesis block

Fig. 6-2. Valid block-1

Fig. 6-3. Valid block-2

Similarly, more blocks can be added to the blockchain, with a lot of computation time and energy.

Why is the Blockchain Tamper-resistant?

The unique property of SHA256 provides the integrity of tamper resistance for the blockchain. To illustrate this, refer to the blockchain with four valid blocks in Figure 7.

Fig. 7-1. Genesis block

Fig. 7-2. Valid block-1

Fig. 7-3. Valid block-2

Fig. 7-4. Valid block-3

If someone wants to tamper the block-1 by changing "1000" to "5000", immediately, the block-1 will become invalid. (See Figure 8.) To make block-1 valid, one must work hard to find the nonce to make it valid. Even then, the new hash result will be passed down to the next block, which makes the following block invalid; similarly, all subsequent blocks become invalid. Unless the person has more than 50% of the world's computing power, it is impossible to make all subsequent blocks valid [10, 11].

Fig. 8-1. Genesis block

Fig. 8-2. Invalid block-1

Fig. 8-3. Invalid block-2

Fig. 8-4. Invalid block-3

Code Development of a Blockchain Learning Module Using Python

The development of our project requires us to research what a blockchain is, how it works, and identify the necessary features needed for a simple blockchain to work. It starts by reviewing Blockchain 101 - A Visual Demo [1][7]. In this Visual Demo, Brownworth presents a graphical user interface for each main concept of a blockchain; SHA256, Proof of Work, and implements them to create an interactive blockchain.

Our project was carried out through remote collaboration. As a result, online tools were necessary for coding, file sharing, and communication. Our team scheduled communication meetings via Cisco Webex and Zoom. The coding was written in Python, and Jupyter Notebook was used to run the scripts. nanoHUB.org is where all the files were hosted, shared, and executed. Our blockchain module is one of many tools available to use on nanoHUB.org.

The flowchart in Figure 9 demonstrates how the blockchain operates and presents the logic of the module, which includes SHA256, Proof of Work, and the complete blockchain.

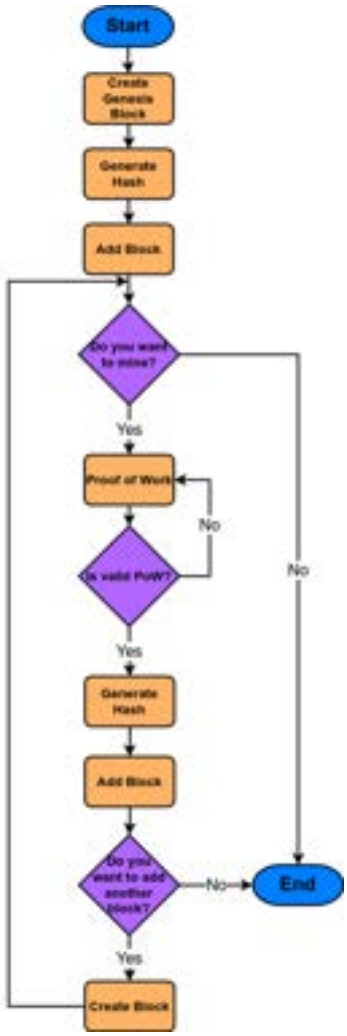

Fig. 9. Flowchart

Create a Blockchain in Python

Naming conventions are rules for how a programmer names the various parts of a computer code to make it easy to read [12]. The authors used the lowerCamelCase naming convention [13]. The lowerCamelCase forms a compound word by uppercase the first letter of each word except the first word and removing the space between each word. For example, naming the previous hash variable as previousHash, or generate hash function as generateHash.

Note: The previousHash is a variable name used in the code to represent the previous hash mentioned in this paper.

The blockchain module uses the string and hashlib libraries:

```
import string
from hashlib import sha256
```

A Blockchain is a chain of linked blocks that were validated by Proof of Work. For this learning module, a valid Proof of Work is when the hash value starts with four zeros; for example, "0000a606f7bc866285273e52ffcfc56049fb60c7dd71a398296f929fcf5fa9bc" is a valid hash. The four zeros for the Proof of Work were chosen because it is manually difficult to find, yet quick for a computer to generate.

Each block has the following variables to store the block details: index, nonce, data, previousHash, and hash. The index and nonce variables are an integer, while the data, previousHash, and hash variables are string.

Note: A string variable stores any character that can be typed from the keyboard.

Here is a code snippet to create a block object:

```
# create new block object to be added to the blockchain
class __block:
    def __init__(self):
        self.index = 0
        self.nonce = 1
        self.data = ""
        self.previousHash = ""
        self.hash = ""
```

The hash is generated by using sha256 from the hashlib library. Here is a code snippet to generate the hash:

```
 # generate hash from block properties: index, nonce, data, previousHash
def generateHash(self, index, nonce, data, previousHash):
    return sha256((str(index) + str(nonce) + str(data) + str(previousHash)).encode()).hexdigest()
```

The Proof of Work function is a simple function that uses a Python while loop: it increments the nonce by one until the sha256 hash result starts with four zeros.

Note: A loop is repeatedly running the same set of code until a specified condition is met.

Here is a code snippet for the Proof of Work:

```
# generate proof of work by computing a hash that begins with mining zero length (four zeros '0000')
def proofOfWork(self, index, nonce, data, previousHash):
    # lending length of zero of hash proof of work
    MINING_ZERO_LENGTH = 4
```

```
    # set nonce to start from 1
    nonce = 1
```

```
   # variable to indicate that proof of work was done
    Check = False
    while check == False:
```

```
        # generate proof of work hash by calling generateHash function
        powHash = self.generateHash(index, nonce, data, previousHash)
```

```
        # validate the proof of work
        if powHash.find('0' *  MINING_ZERO_LENGTH) == 0:
            check = True
        else:
            # increment nonce linearly, nonce is only variable that can be changed to computer proof of work
            nonce = nonce + 1
```

```
    # return proof of work hash and update nonce
    return powHash, nonce
```

Each valid block object is added to a Python list. Here is the code snippet to create a Python list and add block object to it:

```
# blockchain list to store each block
chain = []
```

```
# create a new block
newBlock = self.__block()
```

```
# update a new block object
```

```
newBlock.nonce = 1
newBlock.data = "enter your transaction here"
```

```
# add new block to the chain
self.chain.append(newBlock)
```

Note: When creating a new block, it needs the last index and previousHash from the chain. The hash is generated from the concatenated index, nonce, data, and previousHash.

The content of the blockchain should be immutable (cannot be changed). Here is how to display the content of the blockchain:

```
# display blocks in the blockchain
for x in range(0, 3):
  print(f"Index: {chain[x].index}")
  print(f"Nonce: {chain[x].nonce}")
  print(f"Data: {chain[x].data}")
  print(f"Previous Hash: {chain[x].previousHash}")
  print(f"hash: {chain[x].hash}")
  print()
```

Note: For a Python list, the first index starts from zero.

To create the Graphical User Interface (GUI) in Jupyter Notebook, the following libraries must be called before creating and displaying the widgets:

```
# import GUI libraries
import functools
from IPython.display import display
from ipywidgets import Layout, Label, Text, Textarea, Button, IntText
```

Here is how to create those widgets:

```
# set width of description
styleDescription = {'description_width':'initial'}
```

```
# set width of widgets (Label, Text, IntText, Button)
layoutWidgetWidth = Layout(width="275px")
```

```
# set width for Textarea
layoutTextareaWidth = Layout(width="70px")
```

```
# label
iAmLabel = Label(
    value = 'enter message here',
    style = styleDescription,
    layout = layoutWidgetWidth)
```

```
# change label value
iAmLabel.value = 'new message here'
```

```
# textbox
iAmTextbox = Text(
   description = 'enter input name here:',
   style = styleDescription,
   layout = layoutWidgetWidth,
   disabled = True)
```

```
# change textbox value
iAmTextbox.value = '5'
```

```
# intText, to store only integer
iAmIntText = IntText(
   description = 'enter input name here:',
   style = styleDescription,
   layout = layoutWidgetWidth)
```

```
# change inttext value
IAmIntText.value = '1'
```

```
# textarea
iAmTextarea = Textarea(
   description = 'enter input name here:',
   style = styleDescription,
   layout = layoutTextareaWidth,
   disabled = False)
```

```
# change textarea value
iAmTextarea = 'new content'
```

```
# button
iAmButton = Button(
   description = 'enter button name here',
   style = styleDescription,
   layout = layoutWidgetWidth)
```

```
# display widgets
display(iAmLabel, iAmTextbox, iAmIntText, iAmTextarea, iAmButton)
```

Live Code Examples from the Blockchain Learning Module

To make an effective learning module, two separate functions were created that focus on understanding the two key concepts of SHA256 and Proof of Work, with explanations and live code. The live code allows the user to modify and execute online directly. The code is straightforward, as well as easy to understand and run. (See Figures 10 and 11.)

```
##################################################
# import module
from hashlib import sha256
##################################################

##################################################
## generate hash from user input
##################################################
def generateHash(data):
    # The encode() method returns an encoded version of the given string.
    # The hexdigest() method returns a string object of double length, containing only hexadecimal digits.
    return sha256((data).encode()).hexdigest()

# type your message below in red.
input_data = 'Enter your Data here.'

# this generates the hash from your input_data
hash_result = generateHash(input_data)

# display output
print(f'This is your input data: {input_data}')
print(f'This is your sha256 hash result: {hash_result}')


This is your input data: Enter your Data here.
This is your sha256 hash result: ca9c8e65c4db76a0d853a8c51c28e26c61a6d6a36d8291038e40e57e99f97a2
```

Fig. 10. Python code for SHA256 [9]

```
from hashlib import sha256

# generate hash from block properties: nonce, data
def generateHash(data, nonce):
    return sha256((str(nonce) + str(data)).encode()).hexdigest()

# type your message below in red
data = 'Edit your message here!'

# initialize nonce
nonce = 1

# leading length of zeros of the hash for proof-of-work
MINING_ZERO_LENGTH = 4

# creating an amount of zeros that satisfies computing the proof of work
mine = '0' * MINING_ZERO_LENGTH

# variable to indicate that proof of work was done
is_valid = False

# process of mining
while is_valid == False:
    # new hash for the added block
    hash_result = generateHash(data, nonce)

    # validate the proof of work
    # using string method to search for the MINING_ZERO_LENGTH in beginning of the hash
    if hash_result.find(mine) == 0:
        is_valid = True
    else:
        # increment nonce. nonce is only variable that can be change to compute proof of work
        nonce = nonce + 1

# outputs
print(f'This is your input data: {data}')
print(f'This is your obtained nonce for Proof of Work: {nonce}')
print(f'This is your sha256 result: {hash_result}')
print(f'Is the status valid?: {is_valid}')

This is your input data: Edit your message here!
This is your obtained nonce for Proof of Work: 100314
This is your sha256 result: 00006cd3026d35bb827f517b61304b4c3c9a9a7c8b4734247b6b48058db83427
Is the status valid?: True
```

Fig. 11. Python code for Proof of Work [9]

The Implementation of the Blockchain Development

Ten students from a College Algebra class (Math 3) and eighteen students from a Calculus class (Math 5A) at Pasadena City College and thirty-three students from a Calculus class (Math 190) at El Camino College participated in reviewing the blockchain learning module and then filling out the survey. As a guest speaker, an instructor briefly introduced students to the blockchain and then showed the blockchain video to the class. She encouraged students to participate in interactive activities for testing the tool. The total time spent working on the module was 40 minutes on average.

A sixteen-question survey was designed to capture learning outcomes and experiences. Eighty-one individuals, including instructors and students, completed the survey, and the feedback overall was positive. Figures 12-1, 12-2, 12-3 and 12-4 presents four images of students practicing the developed module. Students were asked to rank their experiences completing the module from 1 to 5, with 5 being the best experience.

Fig. 12-1. Practicing blockchain learning module in a Calculus class at Pasadena City College

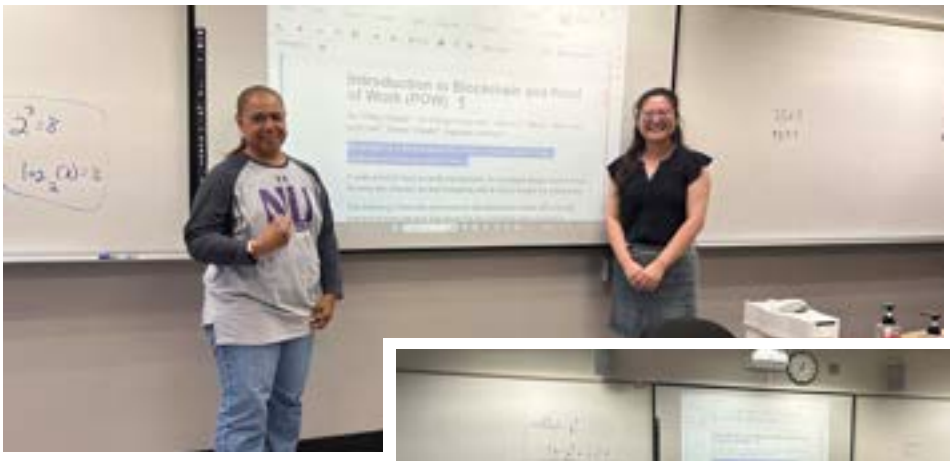

Fig. 12-2. and 12-3. Practicing blockchain learning module in a College Algebra class at Pasadena City College

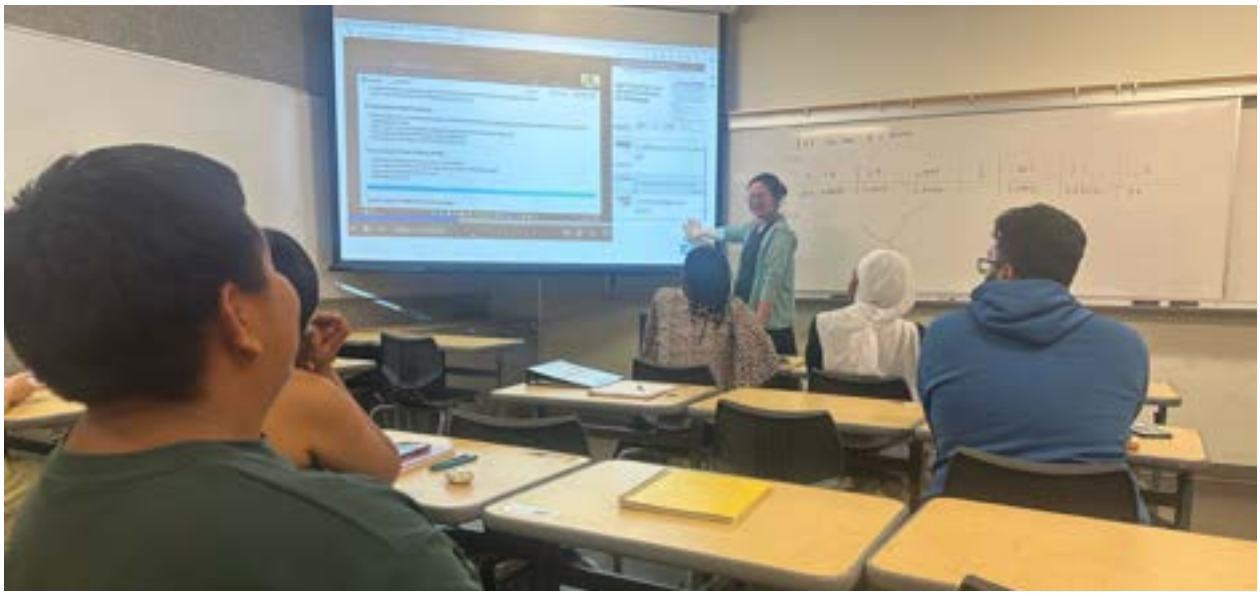

Fig. 12-4. Practicing blockchain learning module in a Calculus class at El Camino College

Students were asked: **"Describe your overall experience using the blockchain tools."** and "**How well did the tool help with understanding general blockchain terminology?"** Most respondents (91%) said they better understood the blockchain technology (rank 3, 4, or 5). Only one student selected rank 1, indicating that almost all respondents (99%) had a positive experience. The responses were encouraging, including "I believe that I have a better understanding of blockchain technology and terminology" and "I feel significantly better being I have had no prior experience with blockchain tools, and now I have a better understanding of the mechanism of blockchain and why it can be used for financial transactions." "I feel, after this tool, my understanding definitely got better, especially about the technical implementation part. I like the process of playing with GUI first, and then look[ing] at the code. This learning experience builds a separation of abstraction, help[ed] me to learn the content step-by-step." "Never heard of blockchain, I like the justification of how secure the blockchain is. I feel the system is thought out well." "I really enjoyed this module! Thank you for organizing this tool and presentation. I've used cryptocurrency years ago, but never really understood how it worked. Now I understand how powerful and secure block chains can be and why people have so much trust in the value of cryptocurrency." "I had no understanding of how blockchain worked beforehand, but this lesson very simply summarized it and explained its functionality using hands on resources. Thank you very much!"

To survey the content usability, students were additionally asked: **"For the content of this tool, please kindly give us your comments and/or suggestions to improve it."** Most respondents (90%) said the content was clear (rank 3, 4, or 5). One respondent emphasized the usefulness of watching the blockchain video before or while testing the learning module, saying: "Once I watched the video [...] everything [was] so clear." Another respondent said, "Put the blockchain videos before [the] introduction." Others provided suggestions to improve users' experience in learning the content. The following are three examples.

> "For the video, perhaps subtitles can be included to help understand what speakers are saying."
>
> "I think that having multiple tabs may make it a bit harder to navigate, especially for those without a larger monitor as they wouldn›t be able to see both the presentation and module at the same time."
>
> "I feel that this tool is an overall good tutorial for technical concepts. If I have any suggestions, I would say that some content, such as the formula for calculating the hash, was explicitly mentioned in the presentation video … but not in the notebook. Personally, I believe it would be helpful to improve the documents by including some information from the video. This will help users review the concepts and follow the flow better. Additionally, I think it would be beneficial to attach a quick, visually appealing, and entertaining YouTube video that talks about blockchain applications and why it works before presenting the entire tool. Through

this, users will be more inclined to learn more about blockchain and, as a result, take a look at this technical tool."

In a continued effort to survey usability, students were asked: **"For the layout or structure of this tool, please kindly give us your comments and/or suggestions to improve it."** One respondent suggested including more pictures, and one respondent recommended the nanoHUB.org administration consider using Jupyter Lab instead of Jupyter Notebook so that there would be a table of contents on the left side which would allow users to navigate among different sessions easily. Additionally, some respondents suggested improving the layout so that it would be easy for users to access the video while using the tool.

> "I strongly believe, with no prior experience, the interface and layout for the tutorial are very approachable. Everything is Clear and Concise as well as the video helps. My only advice is putting the video along with the tool."
>
> "Possibly having the presentation video and tools be usable on the same page."

Most people found the layout reasonably easy to follow and understand, saying:

> "I think it is good because it breaks down to the basic SHA256 and uses it to explain [Proof of Work], so that I can easily understand the whole mechanism of blockchain."
>
> "The layout is fairly easy to understand. The fact that it opens a new window for each notebook or section makes it easier to navigate back to the main page by just exiting out of that one notebook."

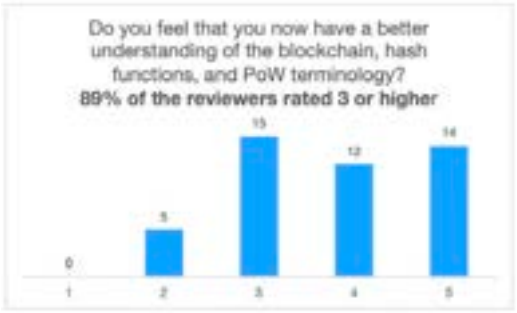

Fig. 13-1. Survey results with sample size 81

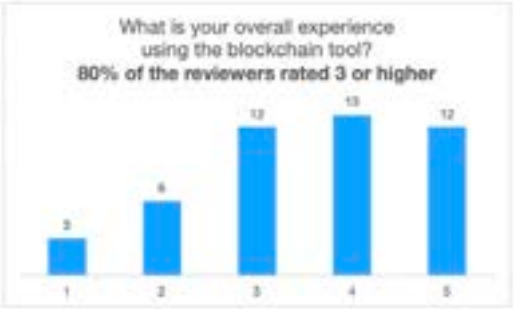

Fig. 13-2. Survey results with sample size 81

Fig. 13-3. Survey results with sample size 81

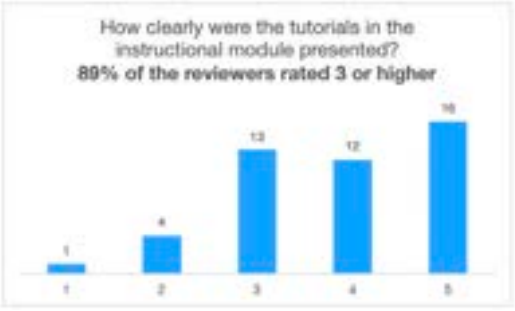

Fig. 13-4. Survey results with sample size 81

Conclusion

This paper presents an original online tool that instructs the mechanism and code behind blockchain technology. The online tool is freely available, and the instructions provide sufficient information to learn and apply blockchain technology. The general reaction from instructors and students was that the modules helped them understand blockchain. It is expected that the tool will be expanded and improved according to the feedback and suggestions and more systematically disseminated to various groups of people beyond students at community colleges, including local middle and high school students, as well as people at work.

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Disclosure. The authors declare no conflicts of interest.

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Introduction to the Chemistry of Alternative Battery Technologies: Survey of Liquid Electrolytes in Next Generation, Fluoride-Ion Batteries

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Abstract: Lithium-ion batteries (LIBs) are central in modern life, where they are found in products from smartphones to laptops to electric vehicles. The demand for efficient and sustainable batteries is higher than ever, with the predicted depletion of lithium sources after 2050 [1-3]. As an alternative to LIBs, next-generation fluoride-ion batteries (FIBs) are now being studied since fluorine is more abundant than lithium. While the majority of FIBs reported use solid electrolytes, liquid electrolytes are of interest for room-temperature applications and they are the focus of this article. This article begins by providing a concise background on specific concepts of battery chemistry that can be used as a basis to expand micro/nanotechnology education curricula to include alternative battery technologies. Key points on defining battery components, battery capacity, and redox reactions at play (including differences between redox reactions in LIBs vs FIBs) are presented. A survey on recent developments of liquid electrolytes in FIBs is derived, where three chemical strategies for designing liquid electrolytes for FIB are determined. This analysis of FIB liquid electrolytes studied so far provides a perspective to holistically improve room-temperature FIBs by tailoring the anode, cathode, and electrolyte combination. Ultimately, the survey of literature developed in the article can have an exemplary role in bibliographic research on alternative battery technologies for students in secondary, two-year, or four-year higher education institutions.

Keywords: chemical education, lithium batteries, fluoride batteries, electrolytes

Introduction

In today's world, energy storage devices are everywhere, from smartphones, laptops, electric vehicles, and power tools. With the increase in technology, the demand for an efficient and sustainable battery is higher than ever. Following the 2019 Nobel Prize in Chemistry to John B. Goodenough, M. Stanley Whittingham and Akira Yoshino, "for the development of lithium-ion batteries," the advancement of novel battery materials is still, more than ever, a hot topic. Lithium-ion batteries (LIBs) have become the top choice for portable electronics. This was preceded by decades of research, starting from their first academic pursuit in the 1970s by the 2019 Nobel Laureates, their first commercial use by Sony in 1991[4], and many years of optimization since then. This has allowed them to attain their current state-of-the-art high energy density, specific power, and cycling ability, making them the most common energy storage systems in the market and in our daily lives.

As a result, it is paramount to integrate the basics of battery chemistry and battery materials into the micro/nanotechnology education of two-year and four-year colleges, especially with the ease of accessibility of these consumer products and the developments in relevant environmental policies. As battery chemistry is an interdisciplinary field, the instruction of various chemistry and engineering subfields can incorporate fundamental battery chemistry topics to be included in course curriculums related to electrochemistry, crystal structure/crystallography, materials chemistry, organic chemistry, and chemical engineering technology [5-11].

While LIBs are the prevailing battery technology today, their high demand has resulted in a depletion of metal lithium resources, which are low in abundance [1]. This means that new generational batteries must be developed to replace LIBs and allow modern life to function with the continuously high technological demand. Other key metal elements used in LIBs are also low in abundance-availability and are highly expensive to mine, such as cobalt and nickel [12]. This issue has motivated research for next-generation battery technologies that are more sustainable and efficient than LIBs. Current research on secondary battery technologies includes sodium batteries [13], magnesium [14], calcium [15], aluminum [16], chloride [17], and fluoride [18]. Fluoride-ion batteries (FIBs) are a highly attractive class of alternative battery technology where monovalent F- anions

have a similar size/ionic radius to Li+ cations. FIBs are of particular importance because they pose sustainable alternatives to lithium, as F- anions are mined from the fluorspar mineral CaF2, a naturally occurring mineral that is abundant on Earth (**Figure 1**) [19, 20]. From **Figure 1**, the global production of fluorine is over 3.5 million tons per year, towering above the production (and consequently availability) of lithium by nearly two orders of magnitude [20]. The massive abundance of fluorine, along with this well-established supply chain are promising for the development of FIBs as a low-cost and sustainable alternative to LIBs. In addition, theoretically, FIBs can even outperform lithium batteries with energy density in the range of 550 Wh/kg for FIBs versus 300 Wh/kg for current LIBs [19]. The majority of battery research has been developed with cations serving as the working ions in batteries since scientists have established the fundamental principles for them and have a better understanding how to harness the electrochemical properties in the respective batteries. In contrast, much is unknown about harnessing anions as working ions in batteries. While LIBs operate with Li+ working cations, FIBs operate with alternative F- working ions inhering different (electro)chemistry from cationic working ions, that has yet to be elucidated and requires fundamental research.

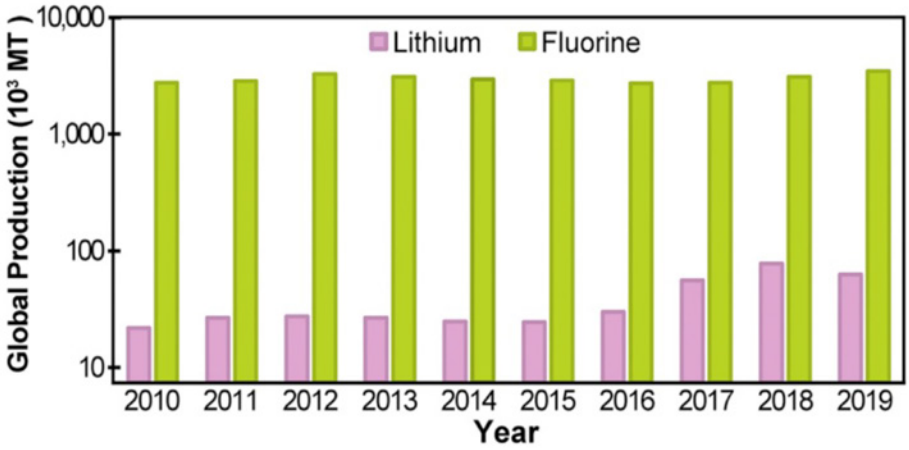


Figure 1. Global production of fluorine and lithium from 2010 to 2019, adapted from Reference [20].

Key Points of Battery Chemistry as Learning Objectives for Developing Lecture Lessons

A key for students' understanding of battery operation is based on understanding the redox processes at play. A battery produces electrical and chemical energy through electrochemical reactions that happen simultaneously at two electrodes, termed the anode and the cathode. An oxidation reaction releases electrons into the external circuit, while a reduction reaction consumes the electrons at the opposite electrode. The gain and loss of electrons is charge balanced by ions that conduct through the electrolyte, which is electronically insulating. The oxidation and reduction reactions that happen respectively at the electrodes are called half reactions. The sum of the two half-reactions together equals the full redox reaction of the battery. If the half-reactions are irreversible, the battery can only be discharged once. Otherwise, if the half-reactions are reversible, then the battery is rechargeable.

Rechargeable batteries are essential because they are vital components in modern-day technology, including electronic devices, electric vehicles, etc. With a rechargeable battery, the same battery can be reused without swapping it with a new one when it "dies," reducing the number of batteries needed. That way, less harm is done to the environment, and helps maintain resources. For any reversible battery, its performance and stability are what is essential. Rechargeable batteries can run into complications such as stability, cycling efficiency (conducting many discharge and charge cycles), high energy density, and manufacture compatibility.

In a rechargeable battery, the electrodes are separated with an electrolyte that transfers ions back and forth between the two electrodes through an external circuit, causing the battery to charge and discharge. The anode is the electrode where oxidation occurs during the discharge, and the cathode is the electrode where reduction occurs. The mnemonics "An Ox" and "Red Cat" can be used as a helpful way to remember this

convention. **Figure 2** illustrates a LIB during discharge, where chemical energy is converted into electrical energy spontaneously, leading to the movement of electrons from the anode to the cathode. During charge, the electrical energy is converted into chemical energy upon applying a voltage to the battery. Now, the oxidation reaction occurs at the cathode and the reduction reaction at the anode (**Figure 2**). Although the opposite reaction occurs at each electrode, the scientific terminology keeps the designation of the electrodes (anode and cathode) as defined for the spontaneous discharge reaction.

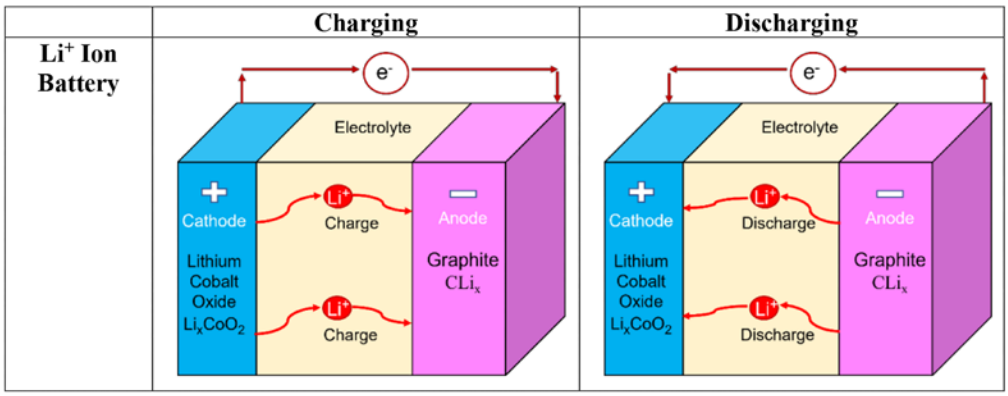


Figure 2. General electrochemical reactions scheme in Lithium-Ion Batteries (LIBs).

The most commercially successful rechargeable Li-ion batteries consist of two electrodes, usually a layered lithium transition metal oxide electrode (LiCoO2) as the cathode and a graphite electrode as the anode (**Figure 2**). Upon charging, the lithium cations travel via the electrolyte from the LiCoO2 cathode to the graphite anode with the simultaneous movement of electrons. The LiCoO2 cathode is oxidized during the charging process, while the graphite anode gets reduced. The oxidation of the cathode is accompanied by delithiation, while the reduction of the anode is accompanied by lithiation. The reverse process will occur upon discharging, and the (negative) graphite electrode will be oxidized, thus losing an electron. Delithiation of the anode occurs as lithium ions are removed and travel to the cathode. The (positive) LiCoO2 electrode will be reduced, thus gaining the electron, and it is lithiated. The redox reactions happening in a lithium-ion battery system upon discharge (**Equation 1**) are as followed:

$$\text{Anode: } CLi_x \rightarrow C + xLi^+ + xe^-$$

$$\text{Cathode: } xLi^+ + Li_{1-x}CoO_2 + xe^- \rightarrow Li_{1-x}CoO_2$$

$$\text{Cell: } CLi_x + Li_{1-x}CoO_2 \rightarrow C + Li_{1-x}CoO_2$$

The cell potential or voltage (E°cell) measures the potential difference between two half-reactions in a battery cell. As the value of E°cell of a redox reaction becomes greater, the driving force of electrons through the system also becomes greater, thus making it more likely that the reaction will proceed more spontaneously. E°cell is measured in volts (V). The overall voltage of the cell (E°cell) is equal to the half-cell potential of the reduction reaction (E°red) plus the half-cell potential of the oxidation reaction (E°ox): E°cell = E°red + E°ox. The standard reduction potentials of many half-reactions are tabulated in Table 1. When viewing a standard potential table, the higher the half-reaction is on the table, the higher its possibility as an oxidizing agent.

Table 1. Standard reduction potentials of selected half-reactions.

Reduction Half-Reaction	Standard Reduction Potential (V)
$F_2(g)+2e^- \rightarrow 2F^-(aq)$	+2.87
$Fe^{3+}(aq)+e^- \rightarrow Fe^{2+}(aq)$	+0.77
$2H^+(aq)+2e^- \rightarrow H_2(g)$	**0.00**
$Na^+(aq)+e^- \rightarrow Na(s)$	-2.71
$Li^+(aq)+e^- \rightarrow Li(s)$	-3.04

The battery capacity or energy density of a battery is a measure of the charge by the battery, and it represents the maximum amount of electrical energy that can be extracted from the battery under specific conditions. The theoretical capacity of a battery rarely matches the practical, actual battery capacity because multiple factors take place primarily (i) related to the internal battery characteristics: active material (that makes up the battery), practical function as an electrode, the age the battery, etc. and (ii) related to the external battery characteristics: the history of the battery functioning, the charging or discharging regimes of the battery and the temperature.

The battery capacity is determined by the mass of active material contained in the battery. In the case of **Equation 1**, the active material of the cathode is LiCoO2. The gravimetric specific capacity of a compound employed as an electrode is given by the following, **Equation 2**:

$$Specific\ Capacity = \frac{F \times x \times n_e}{3.6 \times M}$$

F: Faraday constant, 96485 (Coulomb/mol)

x: number of working ions

ne: Number of electrons involved in each reaction

M: Molecular weight of compound

The theoretical specific capacity can be calculated based on **Equation 2** for the case of LiCoO2 in our discussion, assuming 1e− oxidation per formula unit, as follows:

$$Specific\ Capacity\ for\ LiCoO2 = \frac{96485\ (\frac{c}{mol}) \times 1 \times 1}{3.6\ (\frac{c}{mol}) \times 97.87} = 273.85\ mA\ .\ h/g$$

The key terms of specific battery capacity and battery cell voltage (E°cell) are essential in identifying a material suitable to act as an electrode (anode or cathode). As mentioned, the battery cell voltage (E° cell) is determined by the potential difference between the cathode and the anode. The anodes in LIBs are made from materials such as graphite, silicon, and silicon oxides that can act as hosts for Li cations to be delivered by the cathode. Since only specific materials have been employed as anode in commercial LIBs, the anode materials have a more limited range of cell potentials. Hence, the cathode materials are the key electrode determining the battery's voltage and capacity. The cathode materials in LIBs contain varying amounts of Li in their compound's structure, as seen in **Equation 1**. The higher the Li content (x) in the cathode, the larger the battery capacity.

Finally, as preluded, the electrolyte is a fundamental component of a battery, and it is vital for the functioning of a battery. The electrolyte promotes the movement of ions from the cathode to the anode on charge and in reverse on discharge, enabling the battery to be conductive. Ions are electrically charged atoms that have lost or gained electrons. There are countless electrolytes that scientists have developed for LIBs in the last half-century. Usually, electrolytes of LIBs consist of soluble salts in liquid, polymers, solid ceramics, or molten salts [21]. A liquid electrolyte solution consists of the electrolyte solvent and supporting electrolyte salt. The electrolyte salt is responsible for the ionic conductivity of the overall electrolyte solution, and the electrolyte solvent is responsible for dissolving the electrolyte salt. The ionic conductivity (σ) of an electrolyte solution is a measure of its ability to conduct electricity, and its unit of measurement is Siemens per meter (S/m). The higher the ionic conductivity, the more ions can pass through at a given time, improving the battery capacity at higher discharge rates.

Fluoride Ion Batteries; An Alternative Battery Technology

Having provided a brief overview of the fundamental aspects of battery chemistry, employing the ubiquitous lithium-ion batteries as a reference, we will now expand upon the next generation, alternative battery technology of fluoride-ion batteries.

Both LIBs and FIBs consist of an anode, cathode, and electrolyte. However, how electrons and ions are transferred within a battery differs between the two technologies. Since Li+ ions are cations, they travel to the same electrode the electron travels to. On the other hand, FIBs have F- anions, and those anions travel to the opposite electrode that the electrons are traveling to. Hence, the working principles of the redox processes in FIB (Figure 3) are opposed to LIB since now the working ion is an anion.

During the discharge process of a FIB, electrons are generated at the anode, travel through an external circuit, and reach the cathode. This process causes oxidation of the anode and reduction of the cathode. It also leads to fluorination of the anode and defluorination of the cathode. Charge neutrality is assured by the transport of F- anions through the electrolyte. The opposite occurs for the charging process in a FIB, where electrons are generated at the cathode and reach the anode. This process causes oxidation of the cathode and reduction of the anode. It also leads to fluorination of the cathode and defluorination of the anode. Overall, the movement of electrons and anion charge carrier movements are opposite. The redox reactions happening in a fluoride-ion battery system upon discharge (Equation 3) are as followed:

$$\text{Anode: } xF^- + M' \rightarrow M'F_x + xe^-$$

$$\text{Cathode: } xe^- + MF_x \rightarrow M + xF^-$$

$$\text{Cell: } MF_x + M' \rightarrow M'F_x + M$$

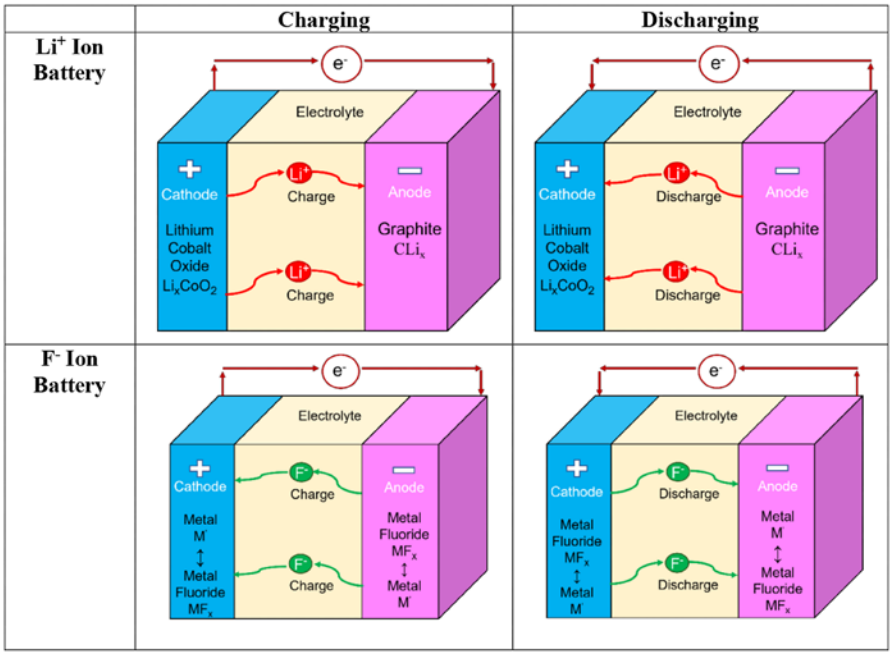


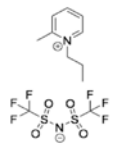
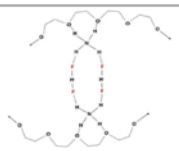
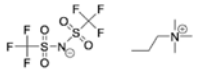
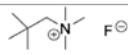
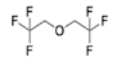
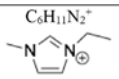
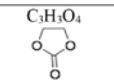
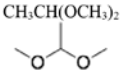
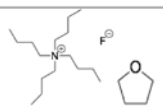
Figure 3. Comparative scheme of electrochemical reactions happening during charge and discharge in (i) Lithium-Ion Batteries (LIBs) and in (ii) Fluoride-Ion Batteries (FIBs), highlighting the movement of the electrons and the working ions during the electrochemical reactions.

Research on FIBs has accelerated since their inception in 2011 [18]. Since then, the majority of FIBs that have been studied employ solid-state electrolytes that require elevated temperatures to operate at around 150ºC [19, 20, 22-25] complicating their application in many daily devices. In addition, liquid electrolytes display higher ionic conductivity than solid electrolytes, thus enabling batteries that can perform more efficiently. Hence, it is of interest to use liquid electrolytes for room-temperature batteries. The following section will discuss current research progress on liquid electrolytes for FIBs, as there has not yet been an established liquid electrolyte for FIBs. At the same time, this survey of the current literature on liquid electrolytes in FIBs can serve as an example of research work that can be conducted by students interested in alternative battery technologies.

Discussion

A primary difference between LIBs and FIBs is the preparation of their liquid electrolytes. The liquid electrolytes for LIBs typically consist of lithium salts such as LiPF6 dissolved in organic solvents. However, in the case of FIBs, metal fluoride salts (e.g., KF, CsF) are not soluble in non-aqueous and organic aprotic solvents. Based on the literature, there are three general strategies for preparing fluoride-ion conducting liquid electrolytes: (i) mixing organic fluoride salts into ionic liquids, (ii) dissolving fluoride salts in organic or aqueous solvents, including the use of anion acceptors and (iii) employing electrolytes that can contain F- ions in true anhydrous form, without transformation of F- into HF2- upon dissolution in solvent. We provide an assessment of recent work on liquid electrolytes in FIBs, concisely summarized in Table 2 with an outline of the anode, cathode, and liquid electrolyte materials to analyze comparatively the developments and improvements of liquid electrolytes for next-generation FIBs.

Table 2. Survey of current liquid electrolytes in FIB with structural formula and electrochemical characteristics.

Electrolyte, Electrolyte Salt, Anion Acceptor (AA)	Structural formula of electrolyte solvent	Ionic conductivity σ (S/cm) at 25°C	Stability-window (V)	Anode/Cathode	Reference
0.1 M TMAF/MPPTFSI	$C_{11}H_{14}F_6N_2O_4S_2$ 	1×10^{-3}	–3.5 to 4.0 (vs. Ag/Ag^+)	PbF_2 /PANI	[26]
0.02 M NH_4F_2-doped PEG		2.1×10^{-3}	—	Mg/BiF_3	[27]
0.35 M MPPF/TMPA-TFSA	$C_8H_{16}F_6N_2O_4S_2$ 	2.5×10^{-3}	0 to 0.7 (vs. Pb/PbF_2)	PbF_2/Bi	[28]
CsF, FBTMPhB, G4	$C_{18}H_{22}BF$	—	–2.2 to –0.3 (vs. BiF_3/Bi)	Pt/BiF_3	[29]
0.75 M Np1F/BTFE	 $(CF_3CH_2)_2O$ 	7.9×10^{-3}	0.7 to 4.8 (vs. Li/Li^+)	Pt/Cu@LaF_3	[30]
$[C_2C_1im][(HF)_{2.3}]$	$C_6H_{11}N_2^+$ 	1×10^{-1}	–0.3 to 0.7 (vs. CuF_2/Cu)	CuF_2/Cu- CuF_2	[31]
1 M $LiPF_6$ in EC/DME	$C_3H_3O_4$  $CH_3CH(OCH_3)_2$ 	—	0 to 3.0 (vs. Mg/MgF_2)	Mg -MgF_2 /BiF_3 Mg - MgF_2 /SnF_2	[32]
0.8 M Aqueous NaF	Na^+ F^-	—	0 to 1.5 (Ag/AgCl)	NMO/BiF_3	[33]
Aqueous CsF	Cs^+ F^-	15.2 10^{-3}	1.9 to 5 (vs. Li/Li^+)	Pb -PbF_2/ Pb-PbF_2 symmetric cell	[34]
TBAF/THF		-	-	Bi -BiF_3/ $CsMnFeF_6$	[35]

The first category of liquid electrolytes for FIBs we will discuss is ionic liquids. An ionic liquid is salt in a liquid state with a melting point below 100ºC [36]. Ordinary liquids like water consist of electrically neutral molecules, while ionic liquids consist of charged ions. Ionic liquids have found wide application as electrolytes for LIB and other battery technologies as a result of their stability, less toxicity, low vapor pressure, and low viscosity that allows favorable ion mobility [36, 37]. As a salt, ionic liquids consist of cations and anions. One of the most common anions in ionic liquids is the bistriflimide group, (formally bis(trifluoromethanesulfonyl) amide or bis(trifluoromethane)sulfonimide) with the chemical formula [(CF3SO2)2N]- abbreviated as TFSI or TFSA [38]. This anion is significant for LIB where LiTFSI is a common electrolyte for lithium batteries and poses as a safer alternative to LiPF6 [39]. From the TFSI anion, ionic liquid electrolytes for FIB draw inspiration for research. An ionic liquid electrolyte for FIB composed of tetramethylammonium fluoride (TMAF) supporting electrolyte salt and 1-methyl-1-propylpiperidinium bis(tri-fluoromethanesulfonyl) imide (MPPTFSI) as the electrolyte solvent was reported with a conductivity of 1.0 mS/cm [26]. In the ionic liquid: 1-methyl-1-propylpiperidinium bis(tri-fluoromethanesulfonyl)imide (MPPTFSI) serving as the electrolyte, the MPP+ species are cationic, and the TFSI- species are anionic. The reported FIB cell with this electrolyte solution TMAF/MMPTFSI was able to perform only two cycles of charge/discharge (**Table 2**) [26]. Another related report studied the electrolyte solution consisting of the organic fluoride salt, 1-methyl-1-propylpiperidinium fluoride (MPPF), and the ionic liquid N,N,N-trimethyl-N-propylammonium bis(trifluoromethanesulfonyl)amide (TMPA/TFSA) as seen in **Table 2** [28]. This electrolyte solution MPPF in TMPA/TFSA with a concentration of 0.35 M was studied to demonstrate a conductivity of 2.5 mS/cm at room temperature. The full-cell assembly utilizing the electrolyte MPPF in TMPA/TFSA demonstrated a few cycles of charge/discharge [28]. Lastly, a fluorohydrogenate ionic liquid: [C2C1im][(HF2)2.3], composed of fluorohydrogenate [HF2]-n anions and 1-ethyl-3-methylimidazolium cations (C2C1im) was reported to exhibit a significantly high conductivity of 100 mS/cm at room temperature, much higher than the prior two electrolytes discussed in this paragraph [31]. In this case, the FIB cell was capable of performing ten reversible cycles of charge/discharge with the [C2C1im][(HF2)2.3] electrolyte (**Table 2**). However, there was gradual performance degradation of this FIB cell as the gravimetric specific capacity of the CuF2 cathode decreased significantly from 500 mA.h g-1 at the first cycle to 264 mA.h g-1 at the 10th cycle. This issue of fully utilizing the specific capacity of a cathode material with liquid electrolytes at room temperature is an existing challenge in developing liquid electrolytes for FIBs.

The second category of liquid electrolytes for FIBs is dissolving fluoride salts in organic solvents. This is the standard preparation method for most liquid electrolytes in FIBs thus far. Initial work on the synthesis of anhydrous, quaternary ammonium salts showed their solubility in tetrahydrofuran (THF) solvent [40, 41]. Quaternary ammonium salts have the structure [NR4]+, where R is an alkyl group or an aromatic group. They are ammonium derivatives where the N atom has a positive charge. Recently, a paper studied the inorganic crystalline compound CsMnFeF6 as a cathode material for FIBs using tetrabutylammonium fluoride (TBAF) in THF as a liquid electrolyte [35]. On the other hand, liquid polymer electrolytes have also been explored as electrolytes for FIB. One of the first reports of FIB utilizing polymer electrolytes was the optimized polymer-based electrolyte: polyethylene glycol (PEG) doped with ammonium bifluoride (NH4F2), with its structural formula depicted in **Table 2** [42]. The chemical rationale for preparing the electrolyte solution of NH4F2 in PEG was to trap NH4+ cations within the extended network of hydrogen bonding in PEG, allowing F- anions into a solution for fluoride conduction [42]. This electrolyte solution of 0.02 M concentration was employed in a cell demonstrating ionic conductivity of 2.1 mS/cm at room temperature. This cell exhibited a good first discharge capacity but could not cycle reversibly. Regarding polymer systems as fluoride ion electrolytes, poly(ethylene)oxide system was studied as a solid polymer electrolyte for FIBs, composed of poly(ethylene) oxide polymer, LiF salt, and trimethoxyboroxine [43].

Furthermore, within the scope of dissolving fluoride salts in organic solvents, there has been a large amount of work from the research groups of Takeshi Abe and Zempachi Ogumi that utilize organic compounds termed “anion acceptors” [29, 44-50]. An anion acceptor is a compound with a positive charge (cation) that can easily bond with anions-compounds with a negative charge. As mentioned, due to the limited solubility of metal fluoride salts in organic aprotic solvents, Abe and Ogumi proposed the addition of the organic compounds named anion acceptors that contain electropositive elements (e.g., B, P, Si). These anion acceptors act as solvating additives to help dissolve the fluoride salt in an organic solvent. The main anion acceptors that have been investigated are phenylboranes [29, 44-50]. A representative example of an electrolyte solution with anion acceptors is bis[2-(2-methoxy ethoxy)ethyl] ether (otherwise known as tetraglyme, abbreviated

as G4) as the electrolyte solvent with the addition of CsF and a boron-based anion acceptor: fluorobis (2,4,6-trimethylphenyl) borane (FBTMPhB) (Table 2) [29]. The anion acceptor FBTMPhB enhanced the solubility of CsF in the tetraglyme solution by reducing the cation–anion interaction between Cs+ and F-. An important observation is that these studied phenylborane anion acceptors were shown to dissolve F- anions from the active cathode material. The dissolution of the cathode material deteriorates the integrity of the electrodes and, thus, the stability of the electrode to last a long time.

Lastly, within this second category of FIB liquid electrolytes, fluoride salts in aqueous solutions can consist as a subcategory of dissolving metal fluoride salts in solvents [33, 51]. A study employing a NaF salt solution as a liquid electrolyte to assemble a FIB cell was studied [51]. The electrochemical data demonstrated stable battery cycling performance, where the authors claim at least 85 cycles of charge/discharge [51]. This year, it was reported that an aqueous CsF electrolyte solution exhibited high solubility, an incredibly high room-temperature conductivity of 152 mS/cm, and (electro)chemical stability, which has been absent in most FIB liquid electrolytes [34]. **Figure 4** depicts comparatively the ionic conductivity and electrochemical stability window of select FIB liquid electrolytes [34]. The electrochemical stability window is the voltage cell potential window in which the electrolyte is stable electrochemically and does degrade to produce byproducts. The high (electro)chemical stability window of 3.1 V of this aqueous CsF electrolyte solution is promising in enabling high operating voltage electrodes that can be maintained stable [34]. Moreover, it is encouraging that the initial cycling performance of FIB cell in a concentrated solution of the aqueous CsF electrolyte demonstrated high retention of its capacity, permitting a more stable cycling performance of FIB at room temperature [34]. Also, it showed suppression of the dissolution of the electrode materials, providing a positive trajectory for the reversible cycling of FIB in liquid electrolytes.

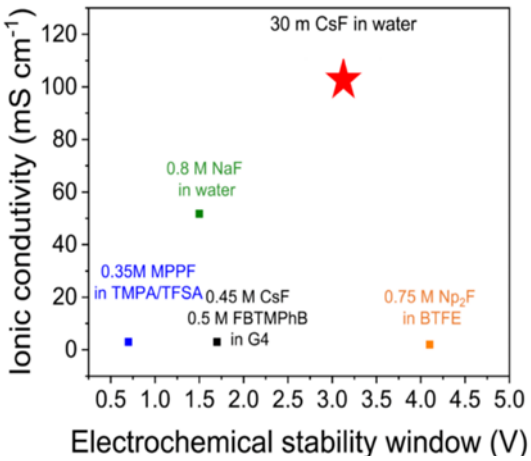


Figure 4. Performance comparison of ionic conductivity and electrochemical stability of selected liquid electrolytes in FIB, adapted from Reference [34].

Finally, our discussion of the literature on liquid electrolytes in FIB culminates with the third category of developing liquid electrolytes, focused on liquid electrolytes that are impervious to protonation of F- ions. The design of liquid electrolytes containing a high concentration of chemically stable F- ions is a challenge in chemical synthesis and electrochemistry, as F- ions are extremely reactive with solvent protons resulting in the formation of bifluoride ions (HF2–) [52]. HF2– lead to electrochemical side-reactions happening upon battery charge/discharge that give electrochemical responses that can be confused to be from the active electrode materials. These electrochemical side reactions deteriorate the battery's performance. One of the few liquid electrolytes to function at room temperature without HF2– formation upon proper handling is the electrolyte solution: Np1F/BTFE where Np1F is N,N,N-trimethyl-N-neopentylammonium fluoride and BTFE is bis(2,2,2-trifluoroethyl) ether [30]. The lack of β-hydrogens is primarily important for preventing decomposition towards the formation of HF2–. The 0.75 M Np1F/BTFE electrolyte solution displayed high ionic conductivity of 7.95 mS/cm (**Table 2**) and a wide electrochemical stability window around 4 V at room temperature. Nevertheless, when subjected to electrochemical cycling using different metallic electrodes (Cu,

Pb, Bi, Ce, and Ca), significant dissolution of the electrodes into the electrolyte was observed, resulting in poor performance [30]. Through the engineering of the electrode, a CuF2@LaF3 core−shell nanoparticle was fabricated, encapsulating the electrodes within the thin layer of LaF3 [30]. This fabricated battery cell made up of the modified electrodes and the Np1F/BTFE liquid electrolyte was able to cycle reversibly at room temperature, although with a gravimetric capacity of around 60 mA h g-1, which is eight times less than the theoretical capacity of CuF2 (528 mA h g-1), indicating an incomplete reaction occurred at the electrodes.

In summary, FIBs are an emerging, sustainable technology that employs highly abundant fluorine instead of lithium, eliminating the dependence on strained metal sources and drastically reducing the cost of energy storage devices. Liquid electrolytes display higher ionic conductivity than solid electrolytes, thus enabling efficient batteries with high energy density. The ability to have FIBs operating at room temperature is highly appealing for commercial applications. Yet, much fundamental research still needs to be pursued to have identifiable principles of efficient and stable liquid electrolytes for FIBs. In this discussion, we have analyzed and developed the ongoing research progress of liquid electrolytes for FIBs. We distinguish three general chemical strategies for designing liquid electrolytes for FIBs. While FIBs have been at an infant stage since their inception almost a decade ago, they are a compelling next-generation battery technology that offers much promise for sustainable batteries. Future research for tailoring the electrolyte chemistry of FIB should also focus on battery safety and manufacturing compatibility that merge the interdisciplinary collaboration of chemists, material scientists, and engineers.

Perspective of a Student from a Community College

The survey of literature developed in the article can have an exemplary role in bibliographic research work on alternative battery technologies for students in secondary, two-year, or four-year higher education institutions. First, one can identify which alternative battery technology to focus on learning (e.g., sodium batteries, magnesium batteries). Within the broader field of alternative battery technology, there is a significant amount of research conducted by scientists on each component of the alternative battery technology (anode, cathode, and electrolyte) component. Instructors and students can work together to determine which component or concept of that battery technology they would like to dive into deeper, as a result expanding dynamic micro/nanotechnology education curricula.

The current research was conducted within the Caltech Connections program, an outreach program that pairs graduate students and postdoctoral scholars from Caltech as mentors with undergraduates from local community colleges. The following paragraph from co-author Pablo A. Romero reflects on his research experience in this outreach program:

> *Besides seeing all the state-of-the-art lab equipment throughout my time at Caltech Connections, I was profoundly impressed by the dedication and time my mentor Dr. Vasileiadou, put into her research and mentorship. Some aspects of battery research I enjoyed were electrode fabrication and battery assembly. It was also the first time I had ever used a glove box, which was pretty remarkable. There was also a great amount of time spent reading the scientific literature on battery chemistry. This included starting off with old and progressing to current literature on Lithium-Ion Batteries, along with Fluoride-Ion Batteries. Throughout the 6-month program, much of the literature read was analyzed and reproduced in the lab. Ipso facto, I feel more confident in my abilities to analyze and interpret scientific literature and, of course, being inside a lab.*
>
> *Additionally, reading papers on battery electrolytes is very dense, but focusing on the chemical molecules (highlighted in the papers' figures) helped connect the compounds to knowledge from my previous chemistry courses. Even though it was my first time in a research lab, I felt comfortable asking Dr. Vasileiadou any questions that came to mind. My questions were always greeted with a detailed answer that made sense to me, and I will always appreciate Dr. Vasileiadou for that.*

Conclusion

In this article, an introduction to battery chemistry is briefly developed to provide background on expanding curriculums toward including alternative battery technologies. The principal topics of battery chemistry are outlined that can be used as learning objectives to develop lecture lessons or research projects for students in secondary, two-year, or four-year higher education institutions. Following the foundation of principal topics in battery chemistry, a concise and comprehensive survey is presented of current literature of liquid electrolytes

in FIBs. Our survey here on the literature of liquid electrolytes in FIBs is an example of bibliographic research work in alternative battery technologies, which targeted student groups can undertake. We identify three chemical strategies for designing liquid electrolytes for FIB: (i) mixing organic fluoride salts into ionic liquids, (ii) dissolving fluoride salts in organic or aqueous solvents, (iii) employing electrolytes that can contain "naked" F- ions in true anhydrous form. A comparative table is generated, including the anode, cathode, and liquid electrolyte materials from our discussion, providing a compass for the future pursuit and design of suitable liquid electrolytes for FIB. This discussion and analysis of liquid electrolytes studied so far in FIBs extends perspective on how to improve room-temperature FIBs holistically by tailoring the anode, cathode, and electrolyte combination.

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Disclosures. The authors declare no conflicts of interest.

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Invited Letter: By Implementing Key Strategies and Providing Necessary Support for Students, Community Colleges Can Better Support Undergraduate Research

Keywords: student learning, undergraduate research, microbiology, molecular biology, dynamic learning environment, research based learning

Hello, my name is Dr. Dominic Salerno and I have served as an assistant professor in the biology department at The Community College of Philadelphia for 12 years. As an instructor, researcher, and vocational training coordinator, my role here contributes to student learning, research, and developing careers in the life sciences. As a professor of microbiology and cell/molecular biology, I attempt to foster a dynamic learning environment with courses that integrate theoretical knowledge with real-world applications. I passionately believe in the value of research-based learning and training for my students. I encourage them to engage in research projects that align with their interests and the fields they desire to work in. Our regional and national partnerships provide technical training through the biomedical technician training program and via original research projects conducted at CCP. I facilitate opportunities for students to participate in meaningful research experiences that enhance their understanding of the life sciences and equips them with the technical skills needed to work in the field.

Working with students in undergraduate research has been an incredibly rewarding experience. I have had the privilege of witnessing students transform from curious learners to confident researchers and observed the numerous ways they have benefited from research experiences. One of the most exciting aspects of undergraduate research is witnessing my students' curiosity ignite. One student who initially seemed reserved became captivated by a research project exploring microbial diversity in the Delaware River Watershed. As the project progressed, their enthusiasm grew, and they started asking questions that pushed their project's boundaries. Their input helped me create a project more sophisticated than I had originally envisioned, and helped the student obtain a prestigious summer URE and pursuing a degree in marine biology. Students have also benefitted through developing critical thinking skills. Initially, many students in our research group were tentative about their ability to conduct research. However, through skills workshops, perseverance, and collaboration with peers, they successfully overcame obstacles in their project, improving their experimental design and generating high-quality data. This experience taught them that setbacks are an inherent part of research and can lead to valuable insights. To overcome these hurdles, students had to think critically about scientific literature and their experiments. This experience honed their analytical skills and highlighted the importance of experiential learning/training.

Community colleges can better support undergraduate research by implementing key strategies and providing necessary support for students. Strategically, community colleges can do a much better job by creating a culture that values and promotes research at the community college rather than focusing solely on instruction and transfer. This might include organizing research seminars and events highlighting student and faculty research projects. This must feature accessible, faculty-mentored research opportunities that enhance learning while promoting technical training and degree completion. Embedding research into the curriculum will better integrate theory and practical skills, leading to employment while not disrupting degree completion. Furthermore, faculty and community colleges need to invest in research and create pathways for students to engage in research over multiple semesters or years so students will delve deeper into their research and make more meaningful contributions. Finally, these programs must foster an inclusive and welcoming environment where students from diverse backgrounds feel comfortable participating in research, and support services should be available to accommodate their different learning styles and needs.

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Electronic Properties of Gold and Silver Nanoparticles Reveal Potential Applications for Medical Treatment

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Abstract: Protein-functionalized nanoparticles introduce a potentially novel drug delivery method for medical therapeutics, including involvement in cancer therapies and as contrast agents in imaging. Gold and silver nanoparticles are of particular interest due to their distinctive properties. Extensive research shows that gold nanoparticles demonstrate incredible photothermal properties and non-toxic behavior, while silver nanoparticles exhibit antibacterial properties but increase toxicity for human use. However, little is known regarding the properties or applications of hybrid silver-gold particles. This study measured the UV-Vis absorbance spectrum for 40 nm diameter Au, streptavidin-conjugated Au, Ag@Au hybrid, Ag nanoparticles, and Transient Absorbance Spectra of Au. Analysis indicates that the hybrid particles exhibit characteristics of both Ag and Au particles, implying potential applications similar to both Ag and Au nanoparticles.

Keywords: nanoparticles, nano-bioconjugates, UV-Vis Spectroscopy, hybrid nanoparticles

Introduction

Current synthetic strategies can be tailored to produce nanoparticles ranging from 1 nm to 100 nm [1]. Among many nanomaterials, metallic nanoparticles have drawn significant attention as they exhibit unique electron interactions known as localized surface plasmon resonance (LSPR) [2]. This interaction occurs when the incident light ray (electric field) excites the valence electrons on the metallic nanoparticles and oscillates electron density. This oscillation will result in a strong light scattering effect, making the different metallic particles have completely different colors than their ordinary states. This property provides potential applications of metallic nanoparticles in cancer therapeutics and medical imaging [3]. Nanoparticles are a rising alternative to enhancing traditional imaging through incorporation into contrast agents for Magnetic Resonance Imaging (MRI). Usage can also be seen in Photoacoustic imaging, which is often used in molecular imaging of cancer and other conditions [4]. Plasmonic nanoparticles are often used as photoacoustic contrast agents, providing high image resolution and greater tissue penetration depths than conventional optical imaging [5]. Nanoparticles are being extensively explored as contrast agents and can potentially benefit imaging modalities, which will in turn, see the rise of nanotechnology in the medical field. Plasmonic metallic nanoparticles also exhibit light scattering and absorbing properties, which some cancer therapies rely on[6]. Traditional photothermal therapy focuses on the absorbing properties of metallic nanoparticles to destroy cancerous tumor cells through hyperthermia therapy from infrared light excitation. Recent research has seen using magnetic nanoparticles to create contrast agents during medical imaging to reduce side effects. Gold nanoparticles (AuNPs), in particular, are ideal for medical purposes due to their biologically inactive nature [7]. In addition, silver nanoparticles (AgNPs) are also heavily investigated as their strong oxidation tendency makes them popular in antibacterial nanomedicine production. Still, their high cytotoxicity makes it difficult to work with [8].

The current focus on the application of AuNPs aims to utilize their unique absorbance patterns and LSPR in plasmonic photothermal cancer therapeutics (PPTT). AuNPs and modified AuNPs are great for this purpose as their absorbance of light and efficient conversion to heat are properties critical for photothermal therapies [9]. Protein-functionalized AuNPs are common modifications as these proteins can mediate

AuNPs entry into specific cells [10]. Combining biotechnology through targeted antibodies in conjunction with nanotechnology involving Au or Ag nanoparticles provides an opportunity to perform cell-specific nano-based medical therapies using the photothermal properties of AuNPs. Including AgNPs in the Au-bionanoconjugates could provide a cell-specific dual therapy utilizing the photothermal properties of Au with the anti-biological properties of Ag [11].

A silver-cored gold hybrid nanoparticle (Ag@AuNPs, Core@Shell) offers an opportunity to study whether the properties of the hybrid nanoparticles are equivalent to Ag or Au nanoparticles or unique to itself. Both AuNPs and AgNPs have significant light scattering and absorption properties and can convert optical energy into heat [12]. AgNPs have a higher photothermal efficiency for radius values lower than 20nm, whereas AuNPs with a larger diameter tend to heat up better [13]. Nevertheless, AgNPs are not as biocompatible as AuNPs while offering great antimicrobial properties. A hybrid Ag@AuNPs is introduced to combine these properties and study their electronic properties.

Understanding the electron activity as excitation occurs in the molecule is crucial for application development. However, the excitation and relaxation of electrons happen on a femtosecond to an attosecond scale, making studying electron activity especially difficult. Remarkably, Transient Absorbance Spectroscopy (TAS) provides an extraordinary path for studying electron activity. A highenergy pumping laser (~400nm) is generally used to excite the sample, and a snapshot of the sample's absorbance profile will be recorded for differences in absorbance measurements. As the excitation decays within the first few hundreds of picoseconds, a second multiwavelength white light will pass through the sample and further excite some excited electrons to an even higher energy level. By controlling the probing light mirror's position to extend a centimeter scale travel distance, a customizable picoseconds delay is achievable in this manner. Through the analysis on the change in probing light's intensity through log based algorithm, an absorbance change in mOD(Optical Density) can be outputted. A three-dimensional map of electron dynamics is generated based on the Wavelength, Decay time, and Decay energy[14]. From this point, TAS is developed and deployed in many studies of electron activity.

In this paper, the Ultraviolet–visible (UV-Vis) spectrum of nanoparticles with the diameter of 40 nm AuNPs, 40 nm protein Streptavidin-conjugated AuNPs, 40 nm AgNPs, and ~42 nm synthesized Ag@AuNPs (30nm diameter Ag core/6nm Au shell) was obtained and compared. Additionally, TAS of AuNPs and its peak specific wavelengths absorbance decay in time are also collected and analyzed. UV-Vis spectrophotometer is an instrument that measures the amount of light absorbed or transmitted by a sample as a function of wavelength. (Figure1) [15].

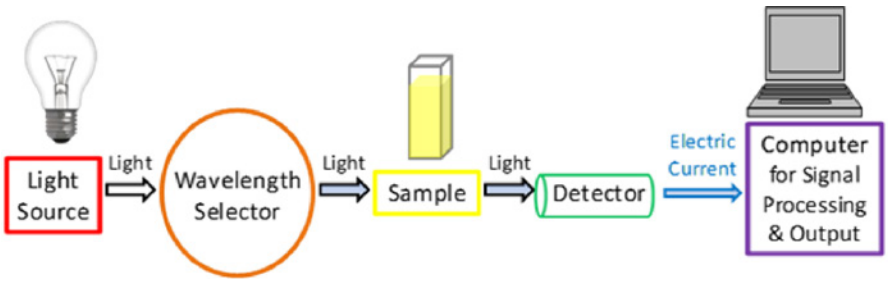

Fig. 1. Schematic of UV-Vis spectroscopy

Methods

UV-Vis spectroscopy measures the absorbance of AuNPs, AgNPs, protein streptavidin conjugated AuNPs, and Ag@AuNPs. Au and Ag nanoparticles were obtained from commercial sources (Sigma Aldrich). Ag@AuNPs were synthesized in Dr. Yadong Yin's lab at the University of California, Riverside[16]. Streptavidin-conjugated AuNPs were obtained from a commercial source (NanoComposix). The colloidal nanoparticles are diluted in distilled water and are analyzed in the UV-Vis Shimadzu UV-2700i Spectrometer from 350 to 650 nm using plastic cuvettes.

The procedure for the conjugation of AuNPs started with pre-wetting the filter device by adding 450 µL of 10 mM potassium phosphate (desalting buffer) to a 2x2 mL housing tube and centrifuging at 5000 RPM for five minutes. The desalting buffer is then aspirated and removed. Next, the antibody solution (Millipore Amicon-Ultra 0.5 mL, 10 kDa, Cat# UFC501096) was added into the filter and concentrated by centrifuge at 5000 RPM for 5 minutes. The filtrate was then removed, and the concentrated antibody was washed five times. In each wash, 350 µL of desalting buffer was added to the filter containing the antibody and centrifuged at 5000 RPM for five minutes. The filtrate was removed and disposed of after each centrifugation. This brings down the antibody concentration to ≥ 1 mg/mL. The antibody solution was conjugated by incubating BioReady™ carboxyl gold with EDC/NHS for thirty minutes at room temperature to activate particles. Activated particles were washed once with reaction buffer, followed by resuspending particles in the reaction buffer and adding antibodies to particles before incubating for an hour. The particles were incubated with hydroxylamine for ten minutes, washed twice with reaction buffer, and resuspended into NCX Conjugate Diluent.

AuNPs were taken to California State University, Northridge (CSUN) for TAS analysis. The data was collected through Femtosecond Laser Spectroscopy. The experimental setup of the femtosecond laser followed a detailed description from Eroglu et al.'s publication [17]. By loading a few drops of AuNPs sample to a well with minor dilution for better signaling, the absorbance change was measured at different time delays between the pump and probe.

Results and Discussion

The UV-Vis spectra from 350 to 650 nm were collected and normalized with respect to their individual maximum measured absorbance for Au, Au-conjugated, Ag, Ag@AuNPs, and streptavidin particles (Fig. 2). The absorbance lambda max (λmax) determined is as 517 nm for Au, 522 nm for Au Conjugate, 404 nm for Ag@Au Hybrid, and 419 nm for Ag. Protein Streptavidin was found to have almost no absorbance for the entire spectrum. The Ag@Au hybrid particles exhibit a broad peak expanding throughout both the ranges of Ag and Au λmax wavelengths, indicating that the Ag@AuNPs have properties inherited from both AuNPs and AgNPs. With a λmax closer to the values of Ag, the properties of AgNPs are expected to be dominant over the properties of Au. However, an increase in the Au peaked absorbance region also shows the influence of the outer gold shell. AuNPs peak at around 517 nm, and Steptavidin-conjugated AuNPs peak at around 522 nm. The small difference between the two λmax demonstrates that the absorbance properties of AuNPs are retained despite the conjugation. This opens the possibility to numerous biomedical applications as protein conjugation can provide selective entrance into cells while keeping the light scattering feature.

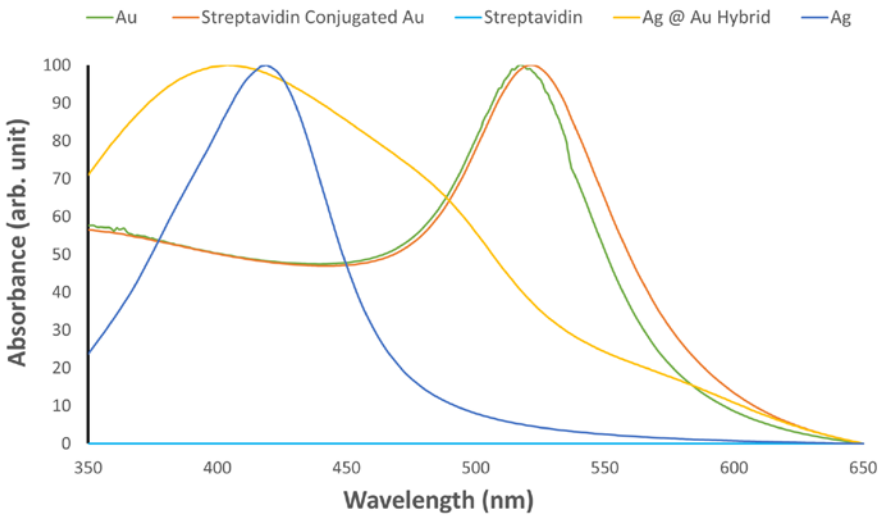

Fig. 2. Normalized UV-Vis absorption spectra of nanoparticles.

The normalized absorption spectra revealed the plasmonic peak of the different nanoparticles. However, the intensity of the light scattering effect for each nanoparticle is a substantial factor in the development of a possible PPTT treatment and effective usage in medical imaging. A simple Mie resonance-based simulation app is used to better understand the light interactions between different nanoparticles. We have used the NFMie program [18], and the results have been plotted in Figure 3. The red plots show the absorption of light impinging upon the nanoparticles, whereas the green plots illustrate the scattering phenomena. The extinction plots account for the total phenomena of absorption and scattering simultaneously. It is obvious that our 40nm diameter particles generate the plasmonic peak locations relatively around the expected wavelength observed in Figure 2 as an outcome of the UV-Vis measurements.

In summary, silver nanoparticles give us a resonance peak around 412nm, whereas gold nanoparticles yield a peak at 527nm (see Figure 3A and 3B). Two different shell thicknesses have been simulated in Figures 3C and D, and it can be concluded that the thinner gold shell lacks the prominent plasmonic peak around 517nm, evident in Figure 3C. Hence, it can be understood that we are losing the gold plasmonic features with the thinner gold shell. In both situations, the silver plasmonic peak still broadens the absorption spectra. In summary, we observe more of the exhibited plasmonic properties from these distinctive metals in Figure 3C.

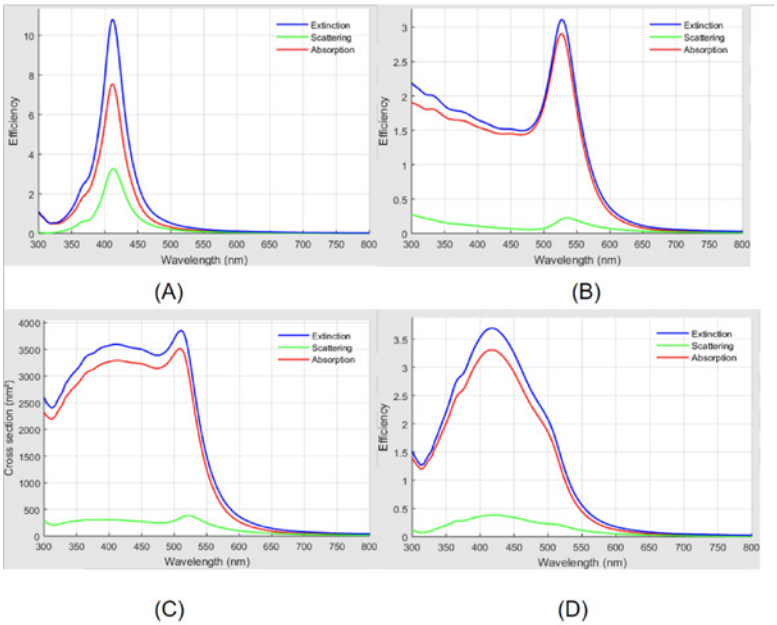

Fig. 3. Simulated Nanoparticles Spectra for (A) 40nm diameter AgNPs, (B) 40nm diameter AuNPs, (C) 30nm-6nm core-shell thickness Ag@AuNPs, and (D) 34nm-3nm core-shell thickness Ag@AuNPs.

The obtained TAS of AuNPs (Figure 4A) presented two induced excited state absorption (ESA) peaks at 475 nm and 571 nm and a ground-state bleaching (GSB) peak at 519 nm. As the pumping laser excites the sample, the ground-state species at the λmax region will experience a decrement in quantity. Thus, as the probing light arrives, since the ground state species at λmax region were depleted by the pump, it is said to leave a hole in this energy level(band). The absorbance measured from probing light will be lower due to decreased absorption activity caused by holes in this band. As a result, a negative peak, referred to as the GSB peak, will be observed in the difference absorbance spectra [19]. ESA, on the other hand, is the transition of the excited electron to continue to absorb photons and promote to an even higher state.

During the process of electron relaxation, the energy is coupled to phonons, vibrational waves that can be quantized in their interaction with the lattice structure. This coupling is crucial to the understanding of photothermal properties in all metallic nanoparticles. Electron-Phonon interaction will last around the first five picoseconds of the electron excitation [20]. However, as the phonon scattering occurs in the lattice structure, which typically happens after the initial 100 picoseconds, the bonds vibrate to relax, releasing the energy

gained in the excitation under the picosecond to nanosecond scale. The energy released during this long-lasting relaxation will express the thermal energy that can be utilized for different purposes, which can be the working principle in the context of PPTT. Thus, TAS can provide a peculiar path for the study of these electron and phonon interactions by measuring the time-resolved energy change (absorption change) within different energy levels.

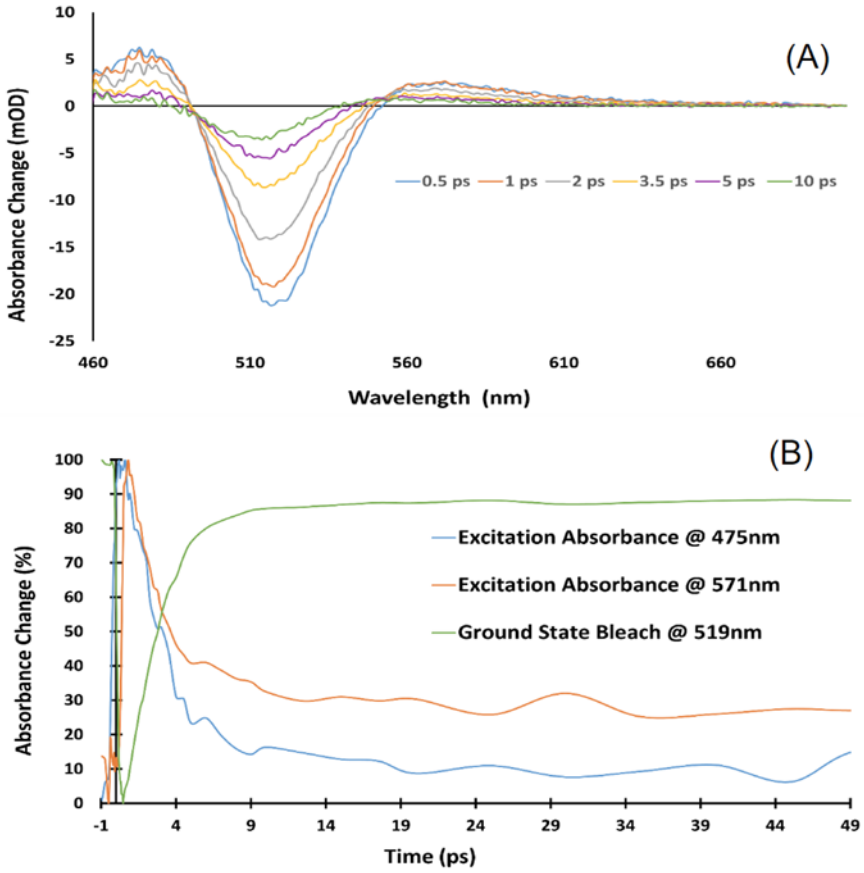

Fig. 4. (A) TAS of AuNPs with different time delays, (B) Time-resolved Peak wavelength absorbance of AuNPs.

A comprehensive comparison of the time-resolved absorption change of all three peaks is presented in Figure 4(B) by comparing the transient absorbance effects in AuNPs at different wavelengths. Similar activities for each peak were also observed in the publication from Zhang [21]. Each peak comprises three stages: initial excitation within one picosecond, rapid energy exchange in different areas due to energy transfer within the initial eight picoseconds, and the long-lasting relaxation caused by the phonon interactions. The ground state bleaching peak shows the fastest response as it corresponds to the direct path for electron-hole recombination and electron-electron and electron-phonon coupling when the AuNPs are excited by the pump. The relaxation finishes within five picoseconds. While the relaxation at 520nm occurs rapidly, a resonance between the excited electron and localized valence electron caused an oscillation of plasmonic nature due to the high energy excitation, thus creating an induced excitation absorption in a broad range of wavelengths between 550nm to 630nm. In the third stage, where long-lasting relaxation takes place, the slow decay of absorption shows a different activity from electron interaction is observed here. The phonon-phonon interaction with the lattice is accountable for long-lasting relaxation. This means the energy from the excitation is coupled and dissipated as heat, which can be utilized in the PPTT or other biomedical photothermal applications. Opposite to the lower energy-induced absorption, the ESA in 480nm shows a more intense energy release. The stronger energy release will result in a faster transition from an excited state to the ground state, reflect as a short phonon-phonon scattering interaction, and reduce the heat generated from this process, which makes it, not an ideal wavelength for the photothermal application as the energy are released more fiercely than the higher wavelength does.

Conclusion

The antibacterial properties of AgNPs and the photothermal properties of AuNPs can be highly beneficial in the nanomedicine industry. Thus, the hybrid particles Ag@AuNPs can potentially provide an alternative path that combines both properties. Meanwhile, antibody-conjugated AuNPs offer a selective cell-entrance advantage over unconjugated AuNPs in medical applications. Studying these nanoparticles through UV-Vis and TAS provides an important understanding of the role electrons play when the system is stimulated through external energy in different scenarios such as PPTT or medical imaging. Through UV-Vis spectra, the hybrid Ag@AuNPs reveal both the properties of AuNPs and AgNPs. The AgNPs core predominates the entire absorbance spectrum, while the AuNPs shell greatly expands the absorption range. The thickness of the shell demonstrates a positive correlation with its Au plasmonic peak region intensity in the absorption spectra based on the Mie scattering simulations. The light extinction effect from the shell can overlay the core and significantly reduce the core absorbance feature as it gets considerably thicker. Compared to AuNPs, streptavidin-conjugated AuNPs demonstrate a similar but shifted absorbance while the general AuNPs absorbance features are retained.

The TAS analysis of AuNPs reveals three different peaks and two different electron coupling mechanisms. The GSB peak demonstrates the formation of an electron-hole pair and its direct interaction with phonons, leading to heat dissipation. On the other hand, the ESA peak displays a rapid and intense relaxation occurring at shorter wavelengths. At higher wavelengths, the relaxation was found to be more gradual, resulting in sustained and enduring heat generation through phonon-phonon scattering while making such longer wavelength energy dissipation channels a better fit for practical applications.

Future research on how the electron activity varies among different nanoparticles has the potential to elucidate a complete image of electron dynamics, establishing a solid groundwork for the utilization of these nanoparticles in actual medical applications. In addition, cell studies on the hybrid Ag@AuNPs will elucidate if the hybrid particles exhibit toxic behaviors more similar to AgNPs or AuNPs.

As an undergraduate research group from a community college, this research experience is definitely a fulfilling and rewarding one. For many of us, this is our very first research experience since PCC does not have an abundance in research resources and opportunities. Throughout this research, we went from learning how to use a micropipette and a centrifuge machine to synthesizing NPs and analyzing the experimental results. There are limitations in a community college lab, such as the lack of proper machines and equipment. In the fortunate scenario where new equipment can be purchased, the operational processes must be mapped out by the group that plans to use it from the very beginning. However, thanks to the MNT-EC led by Dr. Jared Ashcroft and his funding from the NSF, we were able to conduct our experiments and gather significant data using materials and equipment from our partners at CSUN and UC Riverside in a collaborative manner. This development demonstrates how collaboration in academia is extremely important to the success of undergraduate research and how it is necessary to increase research opportunities at the community college level, such as those Dr. Ashcroft offers. From learning how to design an experiment to analyzing data and writing a paper, we learned how these hands-on wet and dry lab skills can benefit and prepare us tremendously. As we prepare for transfer to a 4-year university, we will bring the skills and knowledge that we have obtained throughout this journey. For those of us considering pursuing further education after our bachelor's degree, this experience will propel us toward our academic and future career goals. Since many undergraduates are unaware of the novel nanotechnology research field, these research opportunities at PCC can spark students' interest in studying this field and help pave the way for the next generation of promising and ambitious young scientists. Having the opportunity to perform high-level research with experienced professionals in the field can help develop a confident and inquisitive mindset for budding scientists. Taking the first step into the research field while simultaneously attending a community college can help bridge the institutional gap between 2-year colleges and 4-year universities. More students will be able to explore their interests early on and give themselves a greater chance to flourish brightly in their careers. As students who have benefited from this, we will take the things we have learned with us as we each walk down our chosen paths toward our future.

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Anthropogenic Contaminants Alter Microbial Diversity in Aquatic Ecosystems of the Delaware Watershed

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Abstract: Water quality analysis of Philadelphia County surface waters have indicated that storm events alter the concentrations of pollutants such as polycyclic aromatic hydrocarbons (PAHs), antibiotics, heavy metals, and other pollutants, which could impact aquatic organisms' diversity as well as human health. However, there is limited knowledge regarding the microbial communities in these environments and their responses to these pollutants. To address this knowledge gap, culturing and analysis of genomes isolated from surface water samples was carried out at two different time points: one under average conditions (SW1) and another three days after a storm event (SW2). Colorimetric water quality assays were also employed to assess the levels of common pollutants in waterways and observe alterations in the relative concentrations of various chemicals in the Schuylkill River after storm events. Gram staining, and culture analysis of isolated colonies from surface waters in Philadelphia County waterways was performed to understand microbial diversity and the principles of bacterial identification. Genomic DNA was extracted from bacteria concentrated via filtration. PCR amplification of the 16s rRNA gene was performed in preparation for genomic sequencing. Genomic sequencing of samples from various waterways was performed and analyzed using bioinformatics software to identify microorganisms and classify taxa. The results demonstrate that storm events influence the diversity of microorganisms in the Delaware River Watershed. Further analysis of pollutant levels and the metagenomic data will be needed to further elucidate the correlation between specific pollutants and potential pathogens as well as the influence of said pollutants on microbial diversity.

Keywords: water quality, pollution, genomics, metataxonomics, metagenomics, microbial ecology, microbial diversity, conservation

Introduction

Microorganisms play a fundamental role in maintaining the stability of freshwater ecosystems [1]. Aquatic biofilms and surface waters serve as the foundation for numerous ecosystems, playing a crucial role in stabilizing and maintaining the balance of these systems [2]. Biofilms have the remarkable ability to develop in various interfacial environments and establish themselves on a wide range of organic and inorganic substrates [3, 4]. Biofilm colonization contributes to the stabilization of sediments against re-suspension [5]. The majority of microorganisms in freshwater ecosystems are found in biofilms which increase hydrodynamic transient storage—stream water detained in quiescent zones, removing organic nutrients and influencing biogeochemical processes downstream [6]. The bacterial populations present in freshwater ecosystems have a significant impact on organic matter generation and breakdown, the decomposition of numerous environmental contaminants, and the intricate cycling of elements such as nitrogen, sulfur, and various metals [7].

The biodiversity of natural biofilms and their surface waters are directly linked to the metabolic performance of these communities, influencing the overall functioning of aquatic systems [5]. However, the health and integrity of biofilms and surface water microbial ecosystems can be significantly affected by various factors, including anthropogenic pollution [8-10]. Recently, a study of Singapore rivers indicated the wide occurrence of trace levels of 14 common antibiotics [10]. Climate change and droughts, nutrient enrichment, and alterations stemming from human activities like the release of contaminants from wastewater and stormwater, extraction of natural resources, and runoff from agricultural areas are the primary contributors to the formation of detrimental algal blooms [9]. It is evident that the movement of disease-causing microorganisms across

land through surface runoff is a significant factor contributing to the rise in levels of waterborne pathogens within streams in numerous watersheds [11]. Contaminants in rivers, including drug residues, metals, and different types of waste, contribute to the dissemination of antibiotic resistance genes (ARGs) facilitated by mobile genetic elements (MGEs) [12].

The influx of runoff into surface water systems, as evidenced by heightened turbidity caused by soil particles eroded from the surroundings, is linked to higher levels of bacteria, Giardia, Cryptosporidium, and other microscopic organisms [13]. The pathogens typically associated with waterborne diseases encompass enteric viruses sourced from human fecal contamination, bacterial pathogens typified by Escherichia coli O157:H7, and protozoan pathogens such as Cryptosporidium and Giardia [11]. Pollutants such as polycyclic aromatic hydrocarbons (PAHs) in waterways pose a threat to both freshwater and marine ecosystems [14]. Additionally, the release of hydrocarbons and chemical dispersants into marine environments can disrupt benthic ecosystems, including natural and artificial reefs, as well as the organisms associated with them [15]. Urban environments also contribute to the pollution of aquatic ecosystems, particularly through untreated stormwater. Recent studies in Paris indicated that out of the 88 substances studied, the stormwater was found to be polluted by 55 different chemical compounds [16]. Stormwater runoff, amplified by climate change, poses a significant challenge for cities like Philadelphia, which lies at the bottom of a vast drainage system. Interestingly, urban sampling sites the Code River in Indonesia exhibited reduced bacterial diversity and higher levels of antibiotic resistance genes (ARGs), which showed a correlation with mobile genetic elements (MGEs). This indicates an enhanced potential for gene mobility in response to the impacts of human activities [17]. In Philadelphia, increased turbidity has been associated with increased gastrointestinal illness of both children and the elderly [18, 19]. As a result of climate change, the Philadelphia stormwater management system needs to handle increasingly intense storms, which necessitates costly modernization efforts [20].

From a worldwide perspective, the greatest health threat to humans arises from the presence of disease-causing agents in drinking water. Throughout history, numerous instances of disease outbreaks and poisonings have occurred due to people being exposed to untreated or inadequately treated drinking water [21]. In Europe and North America during the Industrial Revolution, a network of pipes was constructed to divert water from streets, initially to prevent flooding. Later, these pipes were extended to connect houses and businesses, allowing direct disposal of sanitary sewage waste into nearby waterways [21]. Combined sewer systems later connected these pipes to wastewater treatment plants. Over half of the global population resides in urban areas, making urban waters crucial spaces where human activities intersect with the natural environment [22]. Older American cities still possess such combined sewers, which frequently overflow during heavy rainfall, leading to the discharge of untreated sewage mixed with stormwater into nearby rivers [23]. Recent research conducted in Milwaukee suggests that the predominant origin of fecal contamination seems to stem from sewage-related sources rather than non-human sources based on the ratios of human Bacteroides to total Bacteroides spp. from samples at four out of five river locations receiving stormwater discharge [24]. Roughly 60% of Philadelphia's population is serviced by a combined sewer system, which facilitates the transportation of both stormwater runoff and wastewater from residential and commercial structures [25]. In temperate ecosystems, pulsed nutrients from storm events frequently drive macroalgal blooms [26, 27]. Following the cessation of rainfall, it may require a span of up to 48 hours for the water in the vicinity of the Combined Sewer Overflow (CSO) to recover [28]. Sampling SW at 72 hours may be more appropriate for observing the impact on the microbial community present before the discharge event, rather than being masked by the presence of microorganisms present in the CSO discharge event. Urban waters serve as collection basins for various landscape activities and runoff from storm drains, and studying their microbiome can offer valuable information about the effects of pollution, the presence of potential health risks for humans, and the potential long-term consequences for both ecosystems and the communities relying on them.

To assess the impact of pollutants on surface water and the associated microbiomes in surface waters, we adopted a comprehensive biochemical, molecular biological, and metataxonomic approach. By analyzing surface water samples obtained from surface waters at two different time points (one during average conditions and another three days after a storm event), this study aims to investigate the response of aquatic microbiomes to anthropogenic pollution and storm run-off. The utilization of metataxonomics focuses solely on the analysis of the 16S rRNA gene to classify and even identify bacteria to determine the diversity of microorganisms in samples [29]. Work on the earth microbiome project used metataxonomics to confirm the major compositional distinction between saline and non-saline microbial communities throughout the world [30].

Through this research, it is expected that stormwater runoff and raw sewage discharge will lead to alterations in microbial diversity within Philadelphia's waterways. The assessment will encompass direct cell counts, genomics analysis to evaluate species richness, and comparisons between tributaries of The Delaware Watershed. Furthermore, changes in microbial gene expression are anticipated in populations cultured from surface water samples and biofilms after exposure to contaminants. It is also expected that post-storm surface water samples may exhibit virulence factors associated with pathogens.

This study's outcomes will contribute to a better understanding of the impact of stormwater runoff and raw sewage discharge on microbial communities in surface waters. Furthermore, the findings will support the development of controlled laboratory microcosms to further investigate the effects of storm water runoff and raw sewage discharge on microbial communities in surface waters.

Methods

Water Sampling

Surface water samples from various Delaware watershed waterways were collected using sterile Samples were collected for the presence of microorganisms according to protocols used by the PA DEP [31]. Although it is common to sample 1L, when conducting PCR based analyses 150mL are commonly used by the PA DEP and who they reference Wade et. al (2010) [31]. Briefly, 150mL Nalgene plastic bottles were autoclaved prior to sampling. In the field, sterile nitrile gloves were donned prior to sampling flowing surface waters at the sampling sites. Samples were collected by Grab Sampling, by dipping the container in the water and filling it to 150mL. Subsequent to sampling, containers were placed on ice at 4°C prior to transport to the laboratory. Samples were stored at 4°C for 3-5 days prior to filtration and genomic DNA extraction, due to time constraints of the participants. Although this can result in sample degradation, it has been shown that little significant degradation of water samples stored in such a manner occurs between 2-5 days post sampling when stored at 4°C [32]. Pre-storm samples were taken 0n 10/1/2022 where the average temperature was 17°C and skies were cloudy. On 10/02/2022, Philadelphia County and surrounding counties received a reported 2-inch precipitation event, one commonly known to overwhelm combined sewer overflow systems. The post-storm samples were taken on 10/05/2022 where the average temperature was 15°C. The rain proceeded from 10/1-10/5 2022, though at lower levels.

Water Quality Analysis

Prior to the concentration of microorganisms on Filters, SW samples from various sources were tested using the SJ Wave 16 in 1 Drinking Water Test Kit (SJWave.com). The test strips test for Total Hardness: 0-425PPM, Free Chlorine: 0-10PPM, Iron: 0-500PPM, Copper: 0-10PPM, Lead: 0-500PPM, Nitrate: 0-500PPM, Nitrite: 0-80PPM, MPS: 0-20PPM, Total Chlorine: 0-10PPM, Fluoride: 0-100PPM, Cyanuric Acid: 0-250PPM, Ammonia Chloride: 0-500PPM, Bromine: 0-20PPM, Total Alkalinity: 0-240PPM, Carbonate: 0-240PPM, pH: 6-9. Briefly, Test strips were immersed in 5ml of SW sample and the colorimetric changes observed were recorded within 5 minutes. Samples were obtained in triplicate and averaged.

Concentration of Microorganisms on Filters

Filter towers fitted with 0.2µm filter towers (Corning, NY) were used to capture bacteria and other microorganisms from water samples. Surface water samples from the desired locations were collected using sterile containers. Filtration apparatuses were prepared by assembling the filter unit onto the vacuum filtration system and connecting it to the vacuum source. Water samples of volume 150 mL were poured into the filter towers while ensuring that no air bubbles were trapped. After applying vacuum pressure to initiate filtration, water passed through the filter membrane, retaining bacteria and other microorganisms on the filter surface. Filters were carefully removed from the filter tower and placed in a sterile petri dish using sterile forceps. Sterile scalpels were used to mince filters into 1 cm by 0.25 cm strips. Filters were stored in microcentrifuge tubes at -20°C prior to genomic DNA extraction.

Isolation of Genomic DNA from SW Samples

Mixed genomic DNA of microorganisms was isolated from SW samples concentrated onto 0.02 µm filters using The PureLink™ Microbiome DNA Purification Kit (Catalog Number A29790, Thermo Fisher, Waltham, MA). This kit enabled the purification of high-quality microbial DNA from filter paper or environmental swab

samples. It utilizes spin-column technology for robust DNA yields that are suitable for downstream PCR, sequencing, or other applications. The typical DNA recovery from SW or environmental swab samples is 0.1–1 µg. The procedure involved lysing microorganisms using a combination of heat, chemical, and mechanical disruption with specialized beads. Inhibitors were eliminated through precipitation using a proprietary cleanup method. The purified DNA was then bound to a spin column, underwent a single wash step, and was eluted for use or storage.

PCR Amplification of the 16s rDNA gene from mixed genomic DNA samples

The 16s rDNA gene from genomic DNA isolated from Philadelphia Waterways was amplified using Applied Biosystems™ MicroSEQ™ Full Gene 16S rDNA PCR Kit (Thermo Fisher, Waltham, MA) (Catalog number: 4349155). Three PCR reactions were prepared for each sample or control, with one PCR Master Mix for each. The master mix included Taq Polymerase, dNTPs, and primers flanking the beginning, middle, and end of the 16s rDNA gene (530, 742, and 724 bp respectively) at 0.2 uM final concentration. The negative control consisted of 15 µL of PCR Master Mix 1, 2, or 3 along with 15 µL of ultrapure PCR grade water supplied with the kit. Samples included 15 µL of PCR Master Mix 1, 2, or 3 and a 15 µL sample of genomic DNA extracted from Philadelphia Waterways. Positive controls contained 15 µL of PCR Master Mix 1, 2, or 3 and 15 µL of positive-control E. coli DNA. PCR cycling involved setting the thermal cycling conditions, starting with an initial step at 95°C for 10 min, followed by 30 cycles of melting at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extending at 72°C for 45 seconds, concluding with a final extension step at 72°C for 10 min.

Gel electrophoresis of PCR Products

Agarose gels of either 0.5% concentration (for genomic DNA) and 2% (for PCR products), were poured according to standard protocols, and 10 µL of PCR product per lane was loaded into the gel. The gel was placed into a submarine gel electrophoresis chamber containing 1x TBE solution. The samples were electrophoresed at 100 V for 40 min prior to visualization on a BIORAD Chemidoc V3 Western Workflow system (Catalogue # 4561029, Hercules, CA).

DNA Sequencing

Genomic DNA templates (~20 ng/µL per sample) were sent to the GenWiz Agenta facility at (South Plainfield, NJ) for 16S rRNA gene amplification and sequencing. Samples were pooled into a library, quantified, and run on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA), generating 300 bp, paired-end sequences.

Bioinformatics Analysis

Bioinformatics analysis of raw partial 16S rDNA sequences was carried out using QIIME2 pipeline generated through the online resource Nephele [33]. Merged sequences (2.2 M) were quality filtered, discarding reads with a maximum number of expected errors >0.5 (based on Phred quality scores) [34]. A total of 1.08M sequences, with an average sequence length of 434 base pairs, were obtained after quality filtering. Sequences were de-replicated, and remaining sequences assigned to OTUs based on greedy clustering and ≥97% sequence similarity. A table containing the relative abundance of OTUs in each sample was generated. A representative sequence from each OTU was used for taxonomic identification and alignment using UCLUST and the GreenGenes reference database (v 13.8) [35].

Results and Discussion

In order to analyze the microbial diversity of surface waters in response to pollutant exposure and storm run-off, a comprehensive approach was taken. This employed diverse methodologies, including filtering water samples, gram staining isolates from water samples, culturing microorganisms on selective and differential media, genomic DNA extraction, PCR amplification of the 16S rRNA gene, DNA sequencing, and determination of species diversity using bioinformatics/metataxonomics.

Fig. 1. Collection of Surface Waters from Tributaries of The Delaware Watershed

Collection of Surface Waters Pre and Post Storm from Tributaries of The Delaware Watershed (Fig. 1.)

The figure depicts a map illustrating the locations in the Philadelphia region where water samples were collected. The map displays the geographical area of Philadelphia County. Various points or markers (red) are plotted on the map, indicating the specific locations where water samples were taken. Water samples were taken from the same geographic location pre and post storm events. Samples were collected in sterile 150mL bottles prior to processing in the laboratory and were kept at 4°C for no more than 3 days.

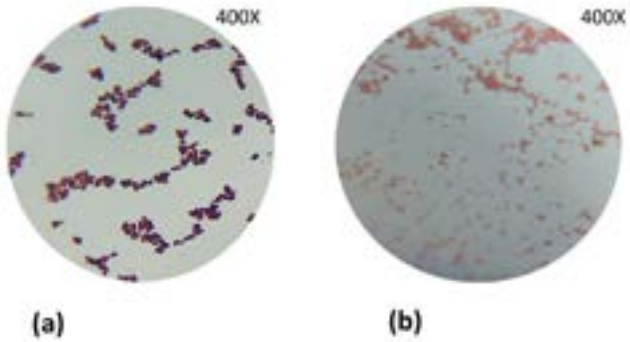

Fig. 2. Gram Stain of Representative Microorganisms from Philadelphia County Surface Waters (a). Gram Positive Organism from Wissahickon Creek. (b). Gram Negative Organism from Wissahickon Creek.

Gram Stain of Representative Microorganisms from Philadelphia County Surface Waters (Fig. 2.)

TSA (Tryptic Soy Agar) plates were inoculated with 100 mL of SW from various samples. Representative colonies were picked from each TSA plate, were fixed to glass slides, and stained using the gram stain protocol [36]. Images were taken at 400x using a Leica fluorescent microscope using brightfield settings. Fig. 2a shows an example of a gram positive bacteria from the Wissahickon Creek. Fig. 2b shows an example of a gram negative bacteria from the Wissahickon Creek.

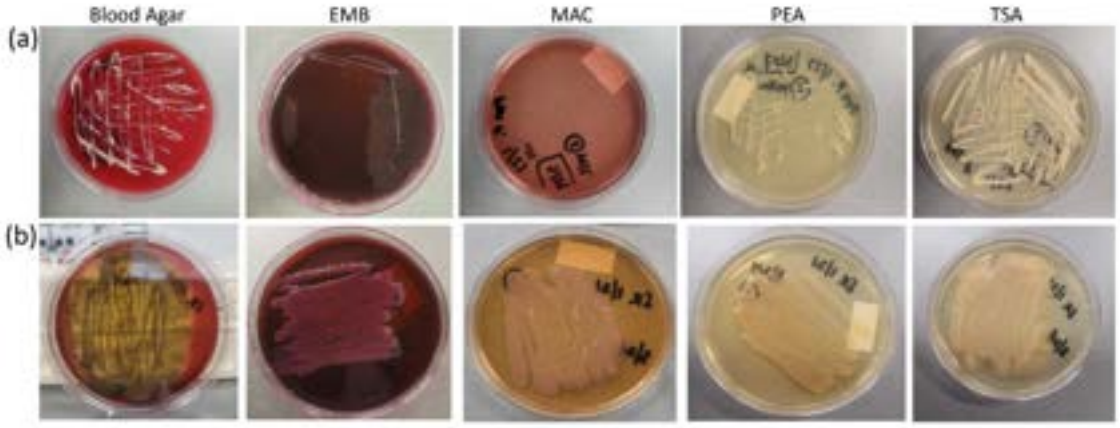

Fig. 3. Microorganisms Isolated From Various Surface Waters Possess Distinct Growth Patterns on Selective and Differential Media. (a). Growth characteristics of the isolated colony from Pennypack Creek. (b). Growth characteristics of isolated colony from Schuylkill River.

Microorganisms Isolated from Various Surface Waters Possess Distinct Growth Patterns on Selective and Differential Media (Fig. 3.)

Figure 3 depicts the growth characteristics of isolated colonies from microorganisms obtained from different water sources (Pennypack Creek (a) and Schuylkill River (b)) when cultured on specific types of media. By utilizing various differential and selective media, one can gain insights into the hemolytic activity, lactose fermentation capabilities, and Gram characteristics of microorganisms. This information is crucial for identification and classification purposes, providing valuable insights into the diversity and potential pathogenicity of the microorganisms in the respective water sources. TSA (Tryptic Soy Agar) is a general-purpose medium that supports the growth of a wide range of microorganisms and serves as a positive control for growth and appearance, allowing for a comparison of growth characteristics between the specific media types mentioned above. The Pennypack Creek sample appeared stark white with heavy growth while the Schuylkill River sample appeared off white with heavy growth. Blood Agar (hemolysis): This medium is used to determine the hemolytic activity of microorganisms. By observing the growth characteristics of the isolated colony from Pennypack Creek on Blood Agar (Figure 3a), no hemolytic activity was observed. The Schuylkill River Sample (Figure 3b) demonstrates B-hemolysis of red blood cells. Eosin Methylene Blue (EMB) Agar (Lactose fermentation): EMB agar is a selective and differential medium used to identify organisms that can ferment lactose. Lactose fermentation can indicate the presence of certain types of bacteria, such as coliforms. By assessing the growth characteristics of the isolated colony from Schuylkill River on EMB agar (Figure 3b), the isolated bacteria could ferment lactose while the sample from Pennypack Creek does not ferment lactose (Figure 3a). MacConkey Agar (Gram isolation): MacConkey agar is both a selective and differential medium used to isolate and differentiate Gram-negative bacteria, particularly those that can ferment lactose. It contains specific indicators that help identify lactose fermentation and distinguish between different types of bacteria. The growth characteristics of the isolated colony from the Schuylkill River showed heavy growth via lactose fermentation (Figure 3b) while the isolated colony from Pennypack Creek did not ferment lactose. PEA (Phenylethyl Alcohol) Agar (Gram isolation): PEA agar is a selective medium used to isolate Gram-positive bacteria while inhibiting the growth of Gram-negative bacteria. By observing the growth characteristics of the isolated colony from Schuylkill River on PEA agar the heavy growth indicates it is Gram-positive (Figure 3b). However, the inhibited growth of the Pennypack isolate compared to TSA growth indicates the organism could be gram negative.

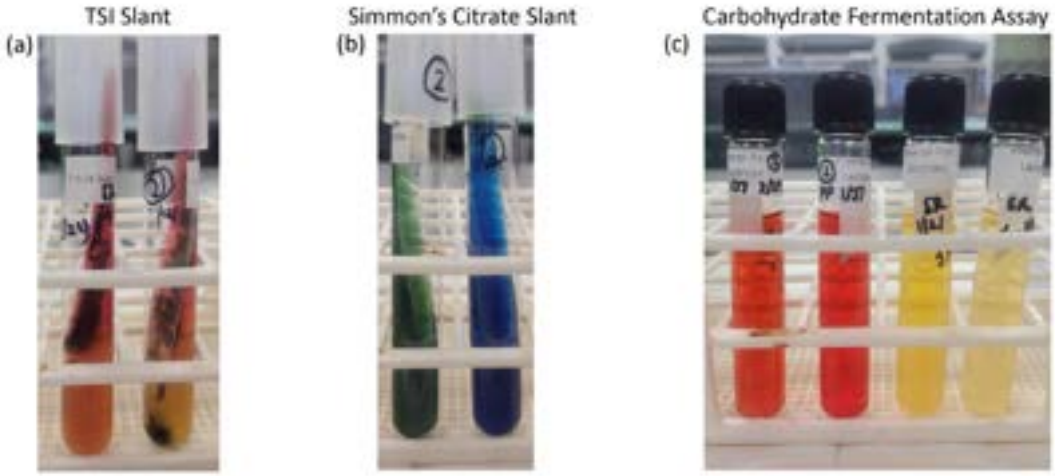

Fig. 4. Microorganisms Isolated From Various Surface Waters Possess Distinct Nutrient Utilization and Fermentation Profiles. Isolated colonies from microorganisms grown on TS agar were cultured in Triple Sugar Iron (TSI). (a). Isolated Colony from Pennypack Creek (Left) and Schuylkill River (Right) cultured in TSI. (b). Isolated Colony from Pennypack Creek (Left) and Schuylkill River (Right) cultured in Simmon's Citrate. (c). Isolated Colony from Pennypack Creek (Left) and Schuylkill River (Right) cultured in Phenol Red Dextrose and Lactose.

Microorganisms Isolated from Various Surface Waters Possess Distinct Nutrient Utilization and Fermentation Profiles (Fig. 4.)

Isolated colonies from SW obtained from Pennypack Creek and Schuylkill River were cultured on specific types of media. (a) Triple Sugar Iron (TSI) is a differential medium commonly used for the identification of enteric bacteria. It contains three sugars (glucose, lactose, and sucrose) and the pH indicator phenol red. The slant portion of the TSI agar allows for the assessment of carbohydrate fermentation, while the butt portion assesses the production of hydrogen sulfide (H2S) gas. The isolated colonies from The Pennypack Creek (a left) showed no fermentation while the isolated colonies from the Schuylkill River (a right) showed glucose fermentation and hydrogen sulfide gas production in TSI agar (Fig. 4a). Simmon's Citrate agar is a selective and differential medium used to identify bacteria based on their ability to utilize citrate as a sole carbon source (Fig. 4b). It contains bromothymol blue as the pH indicator. Bacteria capable of utilizing citrate will produce alkaline byproducts, causing the medium to change from green to blue. The isolated colony from The Pennypack Creek did not utilize citrate while the isolated colony from the Schuylkill River did utilize citrate (Fig. 4b). Phenol Red Dextrose and Lactose: Phenol Red broth with dextrose and lactose is a differential medium used to assess the ability of microorganisms to ferment carbohydrates (Fig. 4c). The phenol red indicator changes color based on the pH of the medium, reflecting the fermentation patterns of the bacteria. By culturing the isolated colonies from both Pennypack Creek and Schuylkill River in Phenol Red Dextrose and Lactose broth (Fig. 4c), it was determined that the Pennypack Creek isolate did not ferment glucose nor lactose (4c left) while the Schuylkill River isolated fermented both. In summary, the growth characteristics of isolated colonies from the two samples on selective and differential media exhibit variations. On Blood Agar, the Schuylkill River sample demonstrates B-hemolysis of red blood cells, indicating the presence of hemolytic bacteria, while the Pennypack Creek sample does not exhibit any hemolytic activity. On Eosin Methylene Blue (EMB) Agar, the Schuylkill River sample shows the ability to ferment lactose, whereas the Pennypack Creek sample does not ferment lactose. Additionally, the growth characteristics on MacConkey Agar and PEA Agar suggest that the Schuylkill River sample may contain both Gram-positive and Gram-negative bacteria, while the Pennypack Creek sample shows inhibited growth on PEA Agar, indicating a possible Gram-negative bacterium in that sample. The nutrient utilization and fermentation profiles further differentiate the Pennypack Creek and Schuylkill River samples. In Triple Sugar Iron (TSI) medium, the Schuylkill River sample demonstrates glucose fermentation and hydrogen sulfide gas production, while the Pennypack Creek sample shows no fermentation. On Simmon's Citrate agar, the Schuylkill River sample utilizes citrate as a carbon source, as indicated by the change in color of the medium, whereas the Pennypack Creek sample

does not utilize citrate. Similarly, in Phenol Red Dextrose and Lactose broth, the Schuylkill River sample ferments both glucose and lactose, while the Pennypack Creek sample does not exhibit fermentation of either carbohydrate.

Table 1: Characterization of Cultured Bacteria from Surface Water Samples from the Pennypack Creek and Schuylkill River

Observation/Test Result	A. Pennypack Isolate	B. Schuylkill Isolate
Gram Result	Gram Positive	Gram Negative
Cellular Morphology	Cocci	Bacilli
Motility	Non-motile	Motile
Mannitol Salt Agar	No Growth, non-halophile	No Growth, non-halophile
EMB	Weak Growth, No Lactose Fermentation	Heavy Pink Growth. Lactose Fermenter
Blood Agar	Gamma-Hemolytic	Beta-Hemolytic
MaConkey's Agar	No Growth, Reinforces gram + and no lactose fermentation	Pink Growth, Reinforces gram – and lactose fermentation
Lactose Fermentation	Negative, no lactose fermentation	Positive, lactose fermentation
Dextrose Fermentation	Negative, no dextrose fermentation	Positive, lactose fermentation
TSI Slant	No fermentation	Glucose fermentation, hydrogen sulfide production
Citrate Slant	No Citrate Utilization	Citrate Utilization

Table 1 Summarizes the data obtained from the culturing of samples taken from the Pennypack Creek (PC) and Schuylkill River (SR). It is evident from the data that the PC Isolate that the organism is a gram positive, non-motile, cocci shaped bacteria (Column A). Furthermore, the PC Isolate is not capable of fermenting either lactose, sucrose, or dextrose (Column A). Finally, the organism is non (Gamma) hemolytic and is unable to utilize citrate as a nutrient (Column A). It is evident from the data that the SR Isolate that the organism is a gram negative, motile, bacilli shaped bacteria (Column B). Furthermore, the SR Isolate is capable of fermenting lactose and dextrose (Column B). Finally, the organism is fully (Beta) hemolytic and can utilize citrate as a nutrient (Column B).

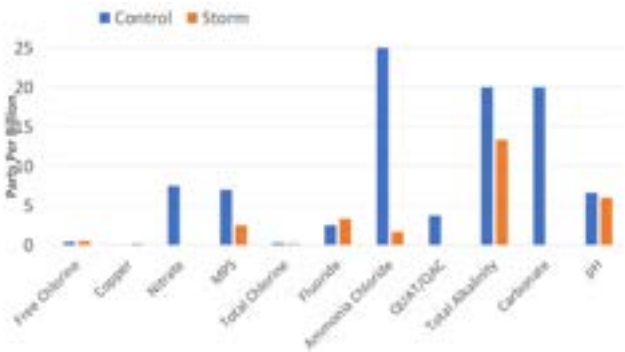

Fig. 5. illustrates the effects of storm events on the levels of chemicals present in the Schuylkill River. Overall, the relative concentrations of chemicals decreased with the influx of water from the storms, and the total hardness (representing all minerals, not shown) experienced a significant decrease. However, two days after the storm, levels of free chlorine, copper, and fluoride showed an increase. Additionally, the pH of the water decreased post-storm.

Storm Events Alter Levels of Chemicals Present in Surface Waters from Schuylkill River (Fig. 5.)

The data indicates that the influx of water from storms leads to a decrease in the relative concentrations of chemicals overall. Additionally, the levels of free chlorine, copper, and fluoride showed an increase two days after the storm. The pH of the water also decreased post-storm. These findings suggest that storm events have a significant impact on the levels of different chemicals present in the Schuylkill River. This alteration in chemical composition may potentially contribute to changes in biodiversity in the river ecosystem following storm events.

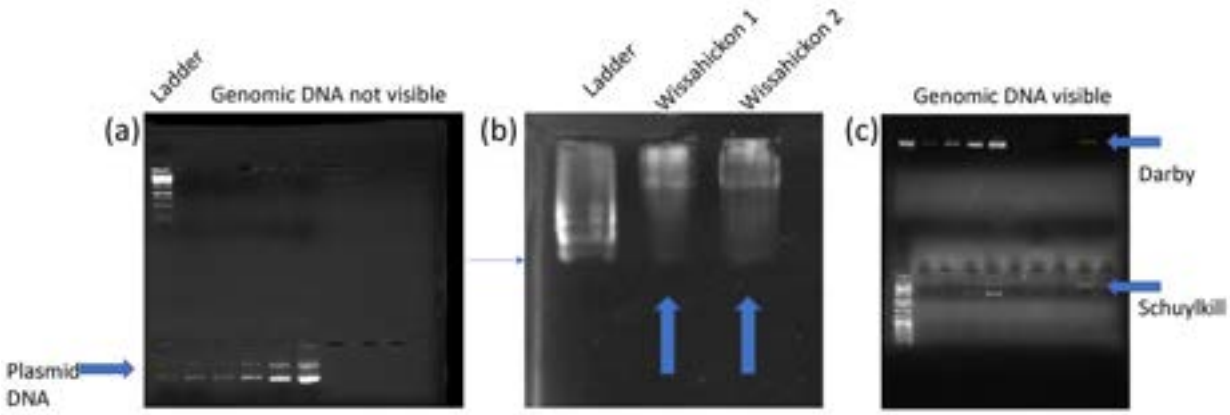

Fig. 6. Genomic DNA Isolation from Surface Waters of Phila. County Waterways (a). This first attempt failed to visibly detect DNA. (b). Genomic DNA was visibly detectable using cultured cells, indicating the genomic DNA extraction protocol/kit was valid. (c). Samples were analyzed as in (a). This attempt was able to visibly detect DNA in some samples.

Genomic DNA Isolation from Surface Waters of Phila. County Waterways (Fig. 6.)

To determine the biodiversity of Philadelphia waterways and to analyze microbial diversity and functional metagenomes, genomic DNA was isolated from various waterways. Firstly, water samples were filtered through 0.2µm filter towers. The filters were sterilely removed, minced, and the genomic DNA was extracted using the PureLink Microbiome DNA Purification kit. Subsequently, the 10µL of extracted DNA was electrophoresed on 0.5% agarose gels along with increasing concentrations of plasmid DNA to serve as a DNA concentration control (Figure 6a) and no genomic DNA was visible. Since no DNA was visible, water was cultured, and bacterial colonies were used to extract genomic DNA from The Wissahickon Creek (Figure 6b). This verified that the genomic DNA extraction kit worked. A repeated attempt was made to extract genomic DNA from water samples (Figure 6c). This demonstrated that for the Darby Creek and Schuylkill River samples, significant DNA was present in the samples (Figure 6c). Subsequent quantification using a NanoDrop 2000 UV Visible Spectrophotometer (Catalogue # ND-2000, Thermo Fisher, Waltham, MA) indicated that genomic DNA was present in all samples in quantities significant enough for metagenomic sequencing (data not shown).

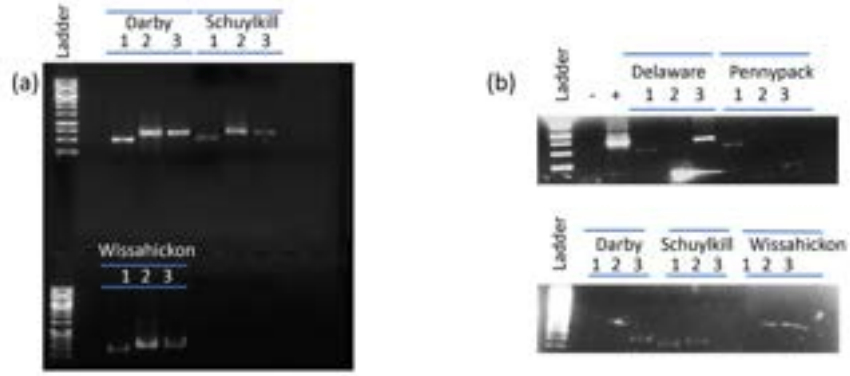

Fig. 7. (a). PCR of Full 16s rDNA gene from SW Samples Taken from Delaware Watershed Tributaries Pre-storm. (b). PCR of Full 16s rDNA from Delaware Watershed Tributaries Post-storm. PCR products of the expected size spanning the 16s rDNA gene were observed (530, 742, and 724 bp respectively).

PCR of Full 16s rDNA from genomic DNA isolated from Philadelphia County Waterways (Fig. 7.)

Figure 7 demonstrates the PCR amplification of the 16s rDNA gene as a preparatory step for DNA sequencing and classification of microorganisms. The 16s rDNA gene serves as a fingerprint for identifying and classifying microbial organisms. In this study, genomic DNA extracted from pre-storm waterways in Philadelphia County was used as the template for PCR amplification. Mastermixes containing primers that span the entirety of the 16s rRNA gene were employed for the PCR reaction. The resulting PCR products were separated by electrophoresis on a 2% Agarose gel and visualized using a V3 Western Workflow System. The full 16s rDNA gene was successfully amplified from the Darby Creek, Wissahickon Creek, and Schuylkill River samples from genomic DNA samples gathered pre-storm event (Figure 7a). The full 16s rDNA gene was successfully amplified from the Darby Creek, Pennypack Creek, Wissahickon Creek, Schuylkill Creek, and Delaware River from genomic DNA samples gathered 2 days post-storm event (Figure 7b). Since we were not able to amplify the 16s rDNA gene for all samples gathered pre and post storm, it was decided that genomic DNA samples would be sent to the sequencing facility (GeneWiz Azenta, South Plainfield, NJ) for 16s rDNA gene amplification prior to sequencing.

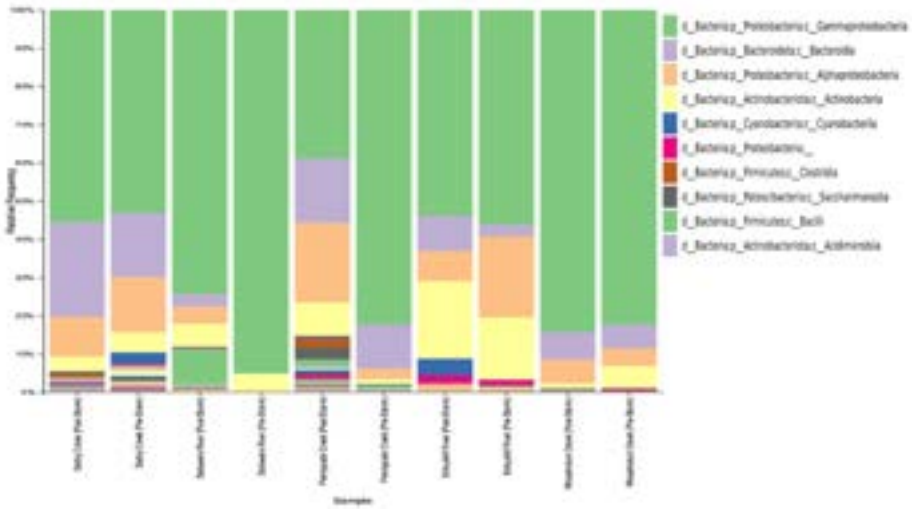

Fig. 8. Microbial Diversity of Philadelphia Surface Waters Pre and Post Storm Events.

Microbial Diversity of Philadelphia Surface Waters Pre and Post Storm Events (Fig. 8.)

Figure 8 shows the bacterial community composition of surface water samples from the Darby Creek, Pennypack Creek, Wissahickon Creek, Schuylkill River, and Delaware River Pre and three days post storm events. Bacteria are identified to the Class taxonomic level. SW samples were collected during periods of no precipitation and three days post significant precipitation. Initial analysis of the data at the Level 3 (Class) taxonomy was used to demonstrate the differences observed between samples. Levels of various taxa changed dramatically between pre and post storm samples, with the least diversity in the Delaware River SW sample. This data correlates with previously published data [12]. where the urban samples demonstrated the least microbial diversity. Furthermore, the most prevalent class of microorganisms present was from the class proteobacteria gamma proteobacteria. This class includes potential pathogens such as Escherichia coli, Yersinia, Salmonella, and Salmonella [37].

This study utilized environmental surface water sampling, genomic DNA isolation from surface water samples, cell culturing of isolates from surface water samples, and PCR of 16s rDNA gene prior to metataxonomic analysis. The data suggests that there is great microbial diversity among the tributaries of the Delaware Watershed which is altered by stormwater runoff. This is supported by studies to catalogue the microbial diversity of The Delaware River using PCR and other non-culturing approaches [38]. Furthermore, the data also suggests that storm and pollution events alter the microbial diversity of the Delaware River Watershed. We used QIIME2, a powerful bioinformatics tool, to perform a metataxonomic analysis of our data. Its tools were used for processing, analyzing and visualizing taxonomic data to identify the composition of the microbial communities [39]. It is interesting to note that the most prevalent class of microorganisms present was from

the class proteobacteria gamma proteobacteria. This class includes potential pathogens such as Escherichia coli, Yersinia, Salmonella, and Salmonella [38]. All these bacterial genera are potential human pathogens.

To our knowledge, much has been done to measure the pollution events and presence of fecal coliforms in these waterways, but no data exists on the impact to microbial diversity at a community level using a metataxonomic/metagenomic approach. And the problem is getting worse. Between July 2017 and June 2018, the combined overflow of the CSOs in Philadelphia County occurred around 6,500 instances, releasing approximately 12.5 billion gallons of untreated wastewater and raw sewage [20]. And with rainfall increasing to an average of 8 inches more per year vs. the 20th century, the problem is likely to get worse [40]. The results suggest that anthropogenic contaminants and storm events can significantly impact microbial diversity in aquatic ecosystems. When sewage discharge enters surface water bodies, it carries an assortment of disease-causing microorganisms that have the potential to cause waterborne illnesses if this contaminated water is utilized for household and other activities [41]. Worldwide, approximately 58% of wastewater generated in urban regions and 81% of industrial waste are directly released into water sources without proper or sufficient treatment, leading to the pollution of approximately 73% of these water bodies [42]. The findings emphasize the need for further investigation into the effects of pollution on microbial communities and highlight the importance of understanding the potential consequences for ecosystem functioning and human health. Therefore, "maintaining the quality of aquatic ecosystems represents one of the most formidable challenges facing global society in the twenty-first century" [43]. Our work to further understand the impact of storm waters/pollution on microbial diversity using a taxonomic approach will further elucidate the scope of the problem and the best means of mitigating it. Our metagenomic study of these same waters will allow us to determine the impact on the functional capacity of these ecosystems.

Conclusion

To better understand the impact of stormwater and pollution events on the microbial diversity of the Delaware River, a more sophisticated approach to identifying and quantifying pollutants such as using mass spectroscopy or HPLC will improve our ability to correlate pollution events with changes in microbial diversity [44]. Furthermore, using more sophisticated tools in the field to measure turbidity, dissolved oxygen, pH and other parameters may also provide a clearer picture of the impact of these storms or pollution events on the microbial community [45].

To better understand the impact of anthropogenic pollution on microbial diversity in Philadelphia waterways, we are constructing recirculating microcosms of the various waterways. The microcosms will incorporate aspects from the designs of two well studied systems [46, 47]. Briefly, an aquarium pump and vinyl tubing will recirculate the water through an acrylic channel lined with unglazed ceramic tiles as a substrate for biofilm growth. Two systems will be inoculated with water from each location of interest, one experimental and one control. They will be inoculated with surface water from the locations being studied. Microcosms are very useful in examining how microbial communities change over time and in response to pollutants at both a species diversity level and functional capacity. For example, through altering the number of species within a pool and adjusting nutrient availability (which impacts species interactions), researchers determined that communities with greater complexity exhibited lower long-term stability [48]. Recently, Other studies have shown that microcosms can mirror the functional capacity of biofilms in aquatic ecosystems. Furthermore, Microcosm studies provide evidence that interactions with biofilms in freshwater ecosystems lead to swift degradation and elimination of widely used herbicides [49]. Other studies have shown bioprecipitation of realgar (As4S4) within a microcosm supplied with natural groundwater and organic material [50]. The ability to manipulate environmental factors and examine their effects on microbial growth patterns within the microcosm will provide insight into the effects of pollutants on microbial diversity and correlate to observed changes in microbial populations observed within the natural environments being studied. The recent Trinseo Altuglas chemical spill in Bristol Pennsylvania will give inspiration for possible experimental contaminants [51]. During this event, 12,000 gallons of latex polymers and trace various acrylates was spilled into the Delaware River. We plan to recreate these conditions using microcosms and compare the biofilm and surface water samples taken at the site the day after the spill in the field with our recreations using the microcosms.

Furthermore, our preliminary data will require further taxonomical, statistical, and metagenomic analysis of the microbial communities present in our water samples, by using taxonomic classification to identify the specific microbial taxa from sequenced data. In addition, a metagenomic approach using full genome sequences will allow us to determine the changes in functional capacity of these microbial communities, identifying enrichment for species with genes capable of metabolizing various pollutants [40]. Further analysis will involve next-generation sequencing, and bioinformatic analysis using tools such as QIIME 2 and Keemei. Of note, there was very little genomic DNA obtained from the Delaware River SW samples post-storm. A switch from SW samples to biofilms should yield much greater concentrations of genomic DNA, allowing for a more robust analysis of taxa and metagenomic analysis. We are also developing a tool written in the python programming language that incorporates the QIIME2 and Sklearn libraries to perform taxonomic classifications. QIIME2 will be used in pre-processing the data by performing tasks such as denoising, filtering, and feature table generation. Sklearn will be used to apply classification models for taxonomic assignment. The goal for this tool will be to help streamline the classification process and to efficiently analyze the data from our water samples. The combinations of surface water, biofilm, and microcosm microbial diversity studies will greatly enhance our understanding of the impact of anthropogenic pollution on microbial communities in our waterways.

Over the past fifty years, significant improvements have been observed in the condition of the Delaware River following the implementation of the Delaware River Basin Commission (DRBC) Compact in 1961 and the enactment of amendments to the Federal Clean Water Act during the 1970s [52]. Yet much work remains to be done to make the river healthier for many threatened species. Researchers from the Delaware Estuary Program and the DRBC are trying to establish stricter dissolved oxygen standards within the tidal Delaware River, aiming for a minimum of 5.0 mg/L to safeguard the continuous reproduction of anadromous fish species like the American shad and Atlantic sturgeon [53]. These efforts will be exacerbated by climate change, with alterations in temperature and precipitation having a potential impact on the occurrence of a water crisis in Philadelphia, should the present management practices of the Delaware River basin be maintained [54]. Much work is being done to understand how the Delaware River and its tributaries influence phytoplankton blooms in the Delaware Bay Estuaries. The abundance of phytoplankton in estuaries is governed by intricate biological and chemical mechanisms that regulate both growth and mortality, as well as by physical processes that oversee the movement and dilution of these organisms [55]. With the continued development of Philadelphia County and climate change likely to have an even greater impact on the Delaware River basin, it is more important than ever to understand the impact of water management and storm/pollution events on the microbial diversity of these waterways. To achieve a better understanding of these dynamics we must employ systems based, metataxonomic and metagenomic approach.

The utilization of whole genome sequencing metagenomics technique allows for the comprehensive study of the complete genetic content of all microbiota members in a natural habitat [56]. This approach offers several benefits in understanding microbial communities. Firstly, it enables the study of highly diverged microbes, including viruses, which may have been challenging to analyze using traditional methods [57]. Secondly, it provides close estimations of microbial diversity by capturing a wide range of microbial species present in the environment. Additionally, it allows for the detection of the abundance of microorganisms across various habitats, shedding light on their distribution patterns [58]. The analysis of unculturable microorganisms, which are difficult to grow in laboratory settings, becomes possible through this technique [59]. Furthermore, whole genome sequencing provides insights into the composition and functional capabilities of an ecosystem, offering a comprehensive understanding of the microbial community. Lastly, it allows for the investigation of functional genes and gene clusters, aiding in the exploration of specific traits and potential metabolic pathways within the microbiota [60]. Overall, the application of metagenomics will greatly enhance our knowledge of microbial ecosystems of the Delaware Watershed and their functional response to storm/pollution events.

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