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Oxidation of Ethidium Using TAML Activators: A Model for High School Research Performed in Partnership with University Scientists

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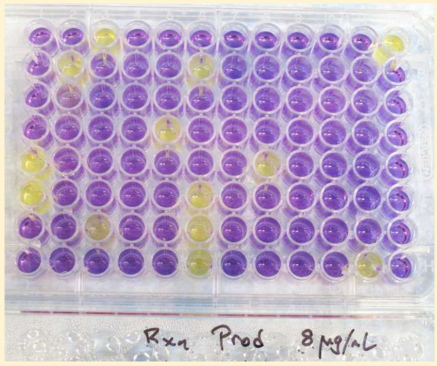
S *Supporting Information*

ABSTRACT: A chemical research program at a public high school has been developed. The full-year Advanced Chemical Research class (ACR) in the high school enrolls 20–30 seniors each year, engaging them in long-term experimental projects. Through partnerships involving university scientists, ACR high school students have had the opportunity to explore a number of highly sophisticated original research projects. As an example of the quality of experimental work made possible through these high school–university partnerships, this article describes the development of a novel method for the oxidation of ethidium bromide, a mutagen commonly used in molecular biology. Data collected from ACR alumni show that the ACR program is instrumental in encouraging students to pursue careers in scientific fields and in creating life-long problem-solvers.



KEYWORDS: *High School/Introductory Chemistry, Curriculum, Interdisciplinary/Multidisciplinary, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Problem Solving/Decision Making, Oxidation/Reduction, Green Chemistry, UV-Vis Spectroscopy*

Much emphasis has been placed on inquiry-based learning in the teaching of science at both high school and college levels. This *Journal* has published hundreds of articles describing the challenges and benefits of inquiry-based science education.[1−4] Some of the benefits of inquiry-based laboratories include greater conceptual understanding, improved thinking skills, and improved communication skills. Inquiry-based laboratories are designed to mimic the reasoning skills that form the basis for scientific research. Whereas a handful of high school students may get a taste of original research through participation in science fairs or college-based summer programs, there are very few high schools that offer research programs as part of their official curricula. This article describes how a chemical research program initiated at a public high school has grown to become a vibrant example of inquiry-based education, including educational partnerships spanning the high school–college interface.

The Advanced Chemical Research course (ACR) at Laguna Beach High School (LBHS) enrolls between 20 and 30 high school seniors each year, engaging these students in authentic research as they pursue long-term experimental projects. The program relies on the collaborative efforts of many university-level scientists, some in the local area and some far across the country. In the six years that it has been offered, ACR enrollment has exceeded prior years' enrollment in advanced placement (AP) chemistry, suggesting that students value the opportunity to engage in a hands-on learning model rather than a textbook-based learning model. ACR alumni cite their ACR experience as a motivation to pursue careers in science. In addition, ACR alumni report enhanced problem-solving skills that are applicable to all fields of college work. We present the success of the ACR program as a model for other educational institutions that may be capable of developing similar high school research programs.

THE RATIONALE AND STRUCTURE OF THE ADVANCED CHEMICAL RESEARCH PROGRAM

In the fall of 2006, the full-year ACR course was conceived with the intention of teaching advanced topics in chemistry by engaging students in long-term laboratory research projects. The hypothesis was that shifting the advanced chemistry course from a static AP curriculum to a dynamic research-based course would provide a more valuable and inspirational capstone course in science, thereby improving the probability of students pursuing a science-based career.

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Table 1. Outlines of the Training Projects Used in the First Semester of ACR

September–October (6 weeks) Training project 1: Isolation of Caffeine from Diet Coke (Each team of 4 students is assigned the task of isolating as much caffeine as possible from 100 mL of Diet Coke.)	October–November (6 weeks) Training Project 2: Ester Syntheses (Each team of 4 students is assigned the task of synthesizing a UV-active ester (e.g., methyl cinnamate) in as high a yield as possible.)	November–January (6 weeks) Training Project 3: Biochemistry (Each team of 4 students is assigned the task of isolating β-lactamase enzyme from antibiotic resistant *E. coli*.)
Techniques[a]	Techniques	Techniques
Using organic solvents	Reflux distillation	Growing bacterial cells
Thin-layer chromatography	Use of catalysts	Protein extraction methods
UV spectroscopy 1: Wavelength Scans	Shifting equilibria	Bradford protein assays
Liquid–liquid extraction	UV spectroscopy 2: Reaction Kinetics	Enzyme assays
Rotary evaporation	Silica column chromatography	Size-exclusion chromatography (gel filtration)
		Polyacrylamide gel electrophoresis (PAGE)
Lectures	Lectures	Lectures
Polarities of organic solvents and their use in chromatography	Reaction mechanisms	Protein structure part 1: amino acids make insulin
UV spectroscopy: wavelengths and photon energies	Kinetics vs Equilibrium	Protein structure part 2: secondary and tertiary structures; enzymes and active sites
Introduction to proton NMR: chemical shifts	NMR 2: integration and splitting	The beta-lactamase enzyme: the “arms race” in antibiotic research
Bonus features	Bonus features	Bonus features
NMR analysis of isolated caffeine at UC Irvine	NMR analysis of synthesized esters at UCI	Field Trip to Chemistry and Biochemistry laboratories at UCSD.
	GC–MS analysis of esters at UCI	Guest lecture on Enzymology by Professor Elizabeth Komives of UCSD.

[a]Safety training is included as each technique is introduced.

The ACR course is structured with a complete emphasis on experimental science. In the first semester, students rotate through a series of three six-week training projects designed to introduce key skills and techniques, including organic extraction, chromatography, spectroscopy, catalysis, proton NMR, and protein chemistry. Extensive use is made of Web-based instructional videos, both “home-grown” and university created (e.g., the MIT Digital Lab Techniques Manual). Each training project has a clearly defined goal (e.g., the synthesis of a particular ester), but nearly all of the work is unscripted, encouraging students to design their own experiments to accomplish the project objectives. During these unscripted experimental projects, students quickly realize that the key question in science is “How do I know?” instead of “What do I know?” Outlines of the training projects used in the first semester of ACR are shown in Table 1.

In the second semester, students are grouped into teams of four. Each student team is presented with an original research project that they investigate for an entire semester. To offer students a wealth of original research projects, the ACR program has enlisted the help of many university-level scientists. An example of the sophisticated science made possible to high school students through partnerships with university scientists is presented below.

■ INVESTIGATIONS INTO THE OXIDATION OF ETHIDIUM BROMIDE: AN EXEMPLARY ACR PROJECT

Initially, when contemplating the creation of the ACR program, one of the authors (S.G.S.) contacted friends and former professors from his graduate school days. These efforts were rewarded when Terry Collins of Carnegie Mellon University offered his enthusiastic support for the program. Collins is the creator of novel tetra-amido macrocylic ligands (TAML activators) that, when complexed with iron atoms (Figure 1A), are capable of catalytically activating hydrogen peroxide to rapidly and completely destroy many environmentally signifi-

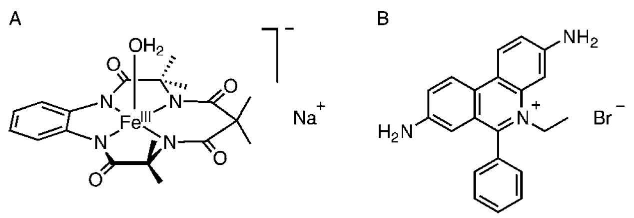

Figure 1. (A) The TAML activator used for this study and (B) the structure of ethidium bromide ($C_{21}H_{20}N_3Br$).

cant pollutants.[5] In 2005, Collins graciously provided access to Fe–TAML catalysts, as well as some preliminary experimental protocols that the Collins research group had developed for use with high school students. After reading Collins’ published papers involving the use of Fe–TAML catalysts to destructively oxidize dyes, such as Orange IV,[6] pesticides, such as fenitrothion,[7] and pharmaceutical wastes, such as estradiol,[8] the author (S.G.S.) decided to investigate the use of Fe–TAML catalysts and household hydrogen peroxide to attack ethidium bromide (Figure 1B), a mutagenic DNA-staining reagent commonly used in molecular biology laboratories. The AP biology classes use ethidium in their laboratories, creating a local need for effective disposal methods. Past literature reported that the oxidation of ethidium utilizing ozone is a highly effective method for detoxifying ethidium.[9] Other literature reported that the oxidation of ethidium using sodium hypochlorite (bleach) created products with enhanced mutagenicity.[10] Upon hearing of the proposed TAML activator approach to oxidizing ethidium, researchers in the Collins group suggested that the catalysts would probably be able to accomplish at least a partial oxidation of ethidium, but the only way to verify this would be to run actual experiments. This presented an exciting opportunity to enable students to embark upon an original research project in the ACR program.

Initially, student investigations into the oxidation of ethidium focused on experiments that could be done in the high school laboratory. Students began with Collins' protocols for using Fe–TAML catalysts to oxidize red dye #40, investigating variables such as pH, temperature, and solution concentrations. After gaining an understanding of how to run these practice oxidations effectively, the students began working with actual ethidium.

Most of the early work on Fe–TAML catalyzed ethidium oxidation was accomplished using UV–vis spectroscopy in the high school. Using a Beckman DU530 scanning spectrophotometer, students investigated spectral changes induced by the Fe–TAML-activated reaction of hydrogen peroxide with ethidium. Initial studies demonstrated a shift in peak absorbance from 480 to 418 nm corresponding to a visible color change from red–orange to yellow. This evidence of reaction next led to kinetic studies measuring the reaction's rate by monitoring absorbance at 480 nm over time (data shown in the Supporting Information).

Toward the end of the first year of student research, the high school team had gathered definitive evidence that TAML activators could catalyze the oxidation of ethidium to form a metastable yellow-colored product. The desire to identify the oxidation product next led the team to The University of California at Irvine (UCI), a 25-min drive from the high school, where they collaborated with Dr. John Greaves, director of the mass spectrometry facility. Mass spectrometry has been utilized as a powerful tool in structure elucidation for proteins, peptides, fatty acids, and other small organic molecules.[11−14] The high school team hoped to elucidate the molecular changes occurring during oxidation by monitoring changes in the mass spectrum of the reaction mixture over time.

Initially, the mass spectral data that students collected was useless due to contamination with salt (resulting in a beautiful spectrum of sodium chloride) and with soap (resulting in a spectrum of polyethylene glycol), but with time and patient instruction from Dr. Greaves, meaningful data began to appear. Eventually, the students acquired exciting data showing that TAML-activated hydrogen peroxide rapidly converts ethidium (molar mass = 314 g/mol) into a short-lived intermediate species (m/z 344) that is subsequently converted to a fairly stable molecule with m/z 364. The increase in mass observed during the reaction provided strong evidence for the addition of oxygen atoms (and possible removal of hydrogen atoms) from ethidium molecular structure, consistent with typical oxidations of organic molecules. High-resolution mass spectra suggested a likely molecular formula for the oxidation product ($C_{20}H_{18}N_3O_4$), but the mass spectra alone were not sufficient to pin down a specific molecular structure.

The high school team then focused on the primary goal of the project—to determine whether Fe–TAML-catalyzed oxidation had eliminated the ethidium mutagenic properties. To investigate the biological activity of the oxidation product, the high school team sought the help of a professional biologist. Their search led them to collaborate with Professor Bruce Blumberg of the UCI Dept of Developmental & Cell Biology. Prof. Blumberg, an expert on endocrine disrupting chemicals (EDCs), had previously collaborated with Collins testing the effects of TAML activators on nuclear receptors.[15] Blumberg's studies showed that TAML activators possess no endocrine disrupting activity and can therefore be considered as viable agents for purification of environmental water samples.

The high school team collaboration with Blumberg began in the spring of 2010 and intensified in the summer of 2010, when four students worked in Blumberg's lab. During their time in the Blumberg lab, the high school team performed experiments to compare the mutagenicity of oxidized ethidium to unreacted ethidium.[16] The results of these experiments, summarized in Table 2, clearly showed that the Fe–TAML-catalyzed oxidation t2

Table 2. Mutagenicity of Ethidium before and after TAML Activator-Catalyzed Oxidation

	Number of Revertants		
Trial	Without Mutagen[a]	With Ethidium Bromide[a]	With TAML Activator-Oxidized Ethidium Bromide[a]
1	9	96	13
2	4	96	6

[a]96 wells plated.

eliminated ethidium's mutagenic properties. This exciting result was a crowning achievement. Not only had the high school students obtained an outstanding science education through their participation in an original research project, but they they had discovered something that might be useful to the scientific community!

STUDENT OUTCOMES

This project

The work described in this article was carried out over three school years and involved a total of nine high school students. Building on previous student work, students in each subsequent year were able to carry the project to new heights. Each student involved in the project had the opportunity to collaborate with professional scientists in a highly meaningful way. The students describe the ACR experience as pivotal in their college career path. The life paths of the nine student authors after graduating from Laguna Beach High School are briefly summarized:[17]

- **Andrew G. Raub** graduated in June 2012 from UC San Diego with a B.S. in Chemistry, where he garnered the Dean's Undergraduate Award for Excellence, which recognizes promise as a research scientist. He is currently applying to Ph.D. programs in chemistry.

  "I am 100% confident ACR is the reason that I even considered chemistry as a career path. I owe my success and happiness to ACR."
- **Allegra Mount** graduated in June 2012 from Northwestern University with a B.S. in Environmental Science, Engineering and Policy, where she was awarded a scholarship from Northwestern University's Women's Board for outstanding female students in environmental fields. She is currently participating in a one year fellowship in Environmental and Natural Science Education at Eagle Bluff Environmental Learning Center in Minnesota.

  "I always knew that research would be a part of my college career, largely because of how ACR enriched my high school experience. I felt confident entering a lab group as a freshman, which provided me invaluable technical experience."
- **Kyle Naughton** graduated in June 2012 from UC Santa Barbara with a B.S. in Physics. During his undergraduate

career, he engaged in research in 4 different laboratories investigating astrophysics and biophysics. He is currently applying to Ph.D. programs in physics.

"ACR was pivotal in my scientific career. Without the class I almost certainly would have gone into experimental science, yet without ACR I surely could not have excelled."

- **Ashley Eaton** graduated in December 2012 from UC Davis with a degree in Biochemistry and Molecular Biology. Her current interests lie in the fields of public and environmental health.

  "My positive experiences in ACR are what compelled me to pursue a science major at UC Davis. I feel very lucky to have been gained insight into scientific research so early on in my education."
- **Sean Jackson** is currently stationed in Afghanistan as a sergeant in the US Marine Corps. He has attended Orange Coast Community College and UC Santa Barbara, where he has maintained a 3.93 GPA. Following his deployment in Afghanistan, Sean will return to UCSB to complete a degree in Political Science.

  "When viewed chronologically it does not appear that ACR had an enduring impact on my course of study. However, ACR was the first class that really taught me to not only ask questions, but also find ways to solve them. I think that lesson will carry over into whatever I decide to do with my life."
- **Madalyn Metz** is a third-year student majoring in Economics at Brown University.

  "Although I did not ultimately pursue chemistry as my major in college, I give an immense amount of credit to my ACR experience in developing a higher level of thinking and curiosity that I have been able to explore while at college. Because of my experience in ACR and the research we did, I am strongly considering pursuing a career in the technology or pharmaceuticals industries so I can apply my skills as a finance major to a science-based company."
- **Natalie Pueyo** is a second-year student majoring in Mechanical Engineering at UC Davis, where she has worked with Professor Kurt Kornbluth to set up a joint research project with a university in Qatar on energy and water efficiency and with a team in Honduras to develop a zeolite refrigeration system that requires no electricity.

  "The most important thing I learned from ACR was that I have to work really hard to get what I want; it does not matter if what I want is a good grade or a spot in a research group."
- **Peter Hastings** is a student at Orange Coast Community College.
- **Nicole Thomas** declined to provide information describing her current status.

These biographies strongly suggest that the ACR program has great value for training both future scientists and societal problem-solvers. It is evident that their ACR experiences motivated the student authors to engage in meaningful learning activities beyond the traditional 4-walled classroom. We suggest that the open-inquiry nature of ACR projects inspired these students not only to take advantage of learning opportunities presented to them, but also to take the initiative to create opportunities.

The current ACR students (in June 2012) participated in an anonymous online survey, providing the following statistical data (19 responses collected from 20 current students):

- 74% are planning to major in science or engineering.
- 79% report that their ACR experience has increased their interest in science whereas 16% report no real change in interest and 5% report a decrease in interest.
- 74% are planning to take chemistry classes in college
- 0% reported that they would have preferred an AP Chem in place of ACR (though 37% reported a desire for AP Chem in addition to ACR)

Alumni

In June 2012, the 124 alumni from the ACR classes of 2007–2011 were asked to complete an online survey. Sixty-six responses were collected (a reply rate of approximately 53%) and the data from this survey are summarized below:

- 62% of ACR alumni are majoring in a science or engineering field (with 5% still undecided).
- 79% of ACR alumni have taken an introductory-level general chemistry course in college.
- ACR alumni who took an introductory-level chemistry course, performed admirably, with 50% earning "A" grades, 40% earning "B" grades, and 10% earning "C" grades.
- ACR alumni who have taken organic chemistry also performed admirably, with 40% earning "A" grades, 44% earning "B" grades, and 16% earning "C" grades.
- 61% of ACR alumni have engaged in or are planning to engage in undergraduate laboratory research.
- 80% of ACR graduates rate their ACR experience as highly valuable in preparing them for college work.

Free-response comments gathered from alumni (collected through the online survey and published in toto in the Supporting Information) further describe the unique opportunities for student growth presented by the ACR learning model.

- It is still today one of the best classes I have ever taken. I ended up with an Art degree, but pursued Geology for a while. It helped me learn valuable problem solving skills that I used in both majors. I still wear my ACR shirt and I still love chemistry.
- ACR was the most inspiring and college level class I took in high school. It allows the student to explore and test something that interests them for the first time in his or her school career.
- In an ACR group I learned how to plan, how to deal with the fact that I had limited knowledge on particular topics, and that doing a little bit of research beforehand always helps. I also learned that results are not always the most important factors, instead, what is important is learning the method and why something might not have worked.

■ DISCUSSION

Although the ethidium oxidation project has been showcased here, it is not an isolated success story. Currently, other exciting ACR research projects include the creation of molecularly imprinted polymers capable of binding snake venom toxins and the investigation of the catalytic properties of a novel metal–ligand complex. Each year in ACR offers new opportunities for student projects and increased interactions with university scientists. The success of this project and the ACR program as a whole has relied on several factors:

1. A high school teacher willing to think beyond "canned" laboratories to involve students in higher levels of inquiry-based science.
2. University scientists who generously shared time, materials, expertise, and facilities with high school students. Although the ACR program was initiated by a high school teacher who reached out to university scientists, it is easily imaginable that a dedicated university professor (or team of professors) could be the initiators of a collaborative program with a talented high school teacher.
3. Funding from a variety of educational and scientific foundations.

Although the combination of a dedicated high school teacher, enthusiastic university collaborators, and strong financial support will not represent the norm at most school sites, the engagement of high school students in experimental research projects has tremendous benefits and should be seriously considered by school sites that may have the necessary ingredients for success. For sites with fewer resources, a research program might focus on projects that are less sophisticated, but that still engage students in meaningful scientific inquiry. The projects listed below represent examples of such investigations.

- Effects of various metal ions on the hydrolysis rate of aspirin—this is a relatively low-tech project that investigates the effectiveness of Lewis acid catalysts.
- Determination of capsacin content in chili peppers—this could become an interdisciplinary project investing biochemical concepts such as whether capsacin content varies depending on growing conditions (e.g., amount of water, sunlight, plant hormones).
- Building a homemade battery pack to power student calculators when their batteries have died. This project requires very little specialized equipment and is filled with challenges for constructing electrochemical cells that can provide adequate voltage and current while maintaining a reasonably long shelf life.

For instructors intrigued by TAML activators, these are now commercially available at a reasonable cost from GreenOx Catalysts, Inc.[18] One can envision a plethora of potential research projects employing these easy-to-use oxidation catalysts. Additionally, this *Journal* has described some research-based laboratory programs developed for college undergraduates that may be suitable models for initiating a high school research program.[19−22]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details on the experimental work performed in the ethidium oxidation project and the data acquired by ACR students over the three years of investigative work summarized in this article; grading schemes used to evaluate students in the ACR program; raw student survey data (including student comments). This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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were inoculated with histidine auxotrophic strain *Salmonella typhimurium TA98* bacteria. Bacterial growth in a well (registered as a color change in the medium) indicates that at least one bacterium in the well has reverted to wild-type. In the presence of ethidium (8 μg/mL), 100% of the wells showed bacterial growth, demonstrating a very high rate of reversion to wild-type. When bacteria were incubated with 8 μg/mL of oxidation products, the number of revertants was only slightly higher than the spontaneous reversion rate (a statistically insignificant increase), demonstrating that the mutagenicity of ethidium has been eliminated by TAML-mediated oxidation.

(17) Two of the student authors did not issue statements about their experience with ACR. A full description of the life paths of the nine student authors after graduating from Laguna Beach High School is available in the Supporting Information.

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