A Teaching Innovation at Chemistry

Integrating Undergraduate Curriculum with Research-based Laboratory Courses

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Course Description

- Department of Biological Sciences
- Department of Chemistry
- Antibiotics Discovery

Small World Initiative (1 credit)

Honors Organic Chemistry Lab (2 credits)

- Strains discovery
- Control Strain: Lysobactor Antibioticus
- Unknown Strain

Antibiotics Discovery
Antibiotic Crisis Statistics

2,049,442 illnesses, 23,000 estimated deaths per year nationally as a result of antibiotic resistance - (CDC).

If no action is taken between now and 2050, the true cost of antimicrobial resistance is estimated to be $100 trillion and 300 million premature deaths - (WHO)

MRSA kills more Americans each year than HIV/AIDS, Parkinson’s disease, emphysema, and homicide combined - (NIH)

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Goals and Rationales

• Create an innovative curriculum to integrate frontier discovery-based scientific research with undergraduate laboratory courses.
• Promote cross-department efforts to integrate curriculum and offer an interdisciplinary research experience for students.
• Form an undergraduate workforce from prominent research projects.
• Foster creative research mentor and teacher training for graduate students.
• Enhance and diversify the chemistry curriculum to attract more high quality students to Pitt.
Laboratory Dynamics Led by Students

Global Project Planning

Experimental Design

Discussion/Peers Feedback

Bench Experiments

Data Analysis

Capstone Poster Presentation
Strategies for Antibiotics Discovery

Initial Investigations and Purification Procedures

Incubation of Bacteria and Liquid-Liquid Extraction

L. antibioticus grown in NB broth, and extracted with ethyl acetate

Thin-Layer Chromatography

Used to quickly observe how many compounds in crude sample

Reverse-phase column chromatography and disc assay

Separation of compounds by polarity, eluting from most non-polar to most polar

Normal-phase column chromatography and disc assay

Separation of compounds in active fraction by polarity, eluting from most polar to most non-polar

High Performance Liquid Chromatography

Separation of the individual compounds present in the active fraction and detection of UV spectra

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Characterization Procedures and Strategies

Mass Spectrometry
Determines the molar mass of the compound and possible molecular formulas

Infrared Spectroscopy
Determines various identities of functional groups in a molecule

1H NMR
Determines the physical and chemical properties of the hydrogen atoms in a molecule

Carbon-13 NMR
Determines the physical and chemical properties of the carbon atoms in a molecule

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Purification with High-Pressure Liquid Chromatography

Figure 4: HPLC spectra results for 254 nm wavelength. Unique compounds indicated by peaks on the graph.

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Paper disc assay to monitor antibiotic activity throughout purification

Figure 3: Results of paper disc assay of fractions and control obtained from the normal phase chromatography dissolved in methanol

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High Resolution Mass Spectrometry Results

Conclusions:
- Mass of active antibiotic compound = 256.09158 g/mol.
- Suspected molecular formula: $C_{14}H_{12}O_3N_2$

Figure 6. Mass spectroscopy spectrum for fraction 5. The mass of the cation of the molecule from fraction 5 is 257.09158.

Gabriela Galli & Alina Quach
$^1$H NMR Results

Figure 9. Fraction 5 proton NMR spectrum.

Gabriela Galli & Alina Quach
Carbon NMR Results

**Conclusions:**
- There are a total of 14 unique carbons
- All carbons are in different chemical environments
- The molecule is **not** symmetrical

*Figure 10.* Carbon NMR Results. 13 unique peaks can be discerned from this spectrum.

Gabriela Galli & Alina Quach
Structure of Antibiotics

1,6-dimethoxyphenazine 5-oxide
256 amu

1-hydroxy-6-methoxyphenazine N10-oxide
242 amu

1,6-dimethoxyphenazine
240 amu

Analysis will focus on compound above. The two compounds to the left were extracted by other lab members.

Forrest Wang & Santina Possanza
Course Transformation: Honors Organic Chemistry Lab vs Organic Chemistry Lab

- Authentic research on a real-world problem
- One central research project
- All techniques are centered around the research project
- Techniques are used in modern day research
- Students lead the research

- Experiments on known projects
- Each lab session is an independent experiment
- One technique per lab
- Techniques are traditional
- Research is pre-designed for students
# Students’ feedback

**Based on interview:** Ownership, Relevance, Connection, Positive experience

**Retention from Fall 2015 to Spring 2016:** 100%

## Reference Letters for students:

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<tr>
<th>Students</th>
<th>Summer Program 2015</th>
<th>Summer Program 2016</th>
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<tr>
<td>Matt Greenwald</td>
<td>Amgen Scholar: Columbia University</td>
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<td>Forrest Wang</td>
<td>NSF REU Program: DePaul University and University of Chicago</td>
<td>UPMC</td>
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<tr>
<td>Coleman Pinkerton</td>
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<td>Gabriela Galli</td>
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<td>Emily Crane</td>
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<td>Amy Zahn</td>
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<tr>
<td>Jessica Canoge</td>
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Spectroscopy Society of Pittsburgh
Scientists Promoting Education in Science for Over 50 Years

Society for Analytical Chemists of Pittsburgh
Our Research Journey: Adventures & Discoveries!