Course Syllabus
Chaminade University Honolulu
3140 Waialae Avenue - Honolulu, HI 96816
www.chaminade.edu

Course Number: CH 480 IS (Independent Study)
Course Title: Honors Research
Department Name: Natural Sciences and Mathematics
College/School/Division Name: NSM, Division of Chemistry and Biochemistry
Term: Fall 2025
Course Credits: 3 (IS – Independent Study)
Class Meeting Days: Monday
Class Meeting Hours: 12:40 – 3:40 PM (Face to face meetings and supervised laboratory research.
Class Location: Henry Lab 1

Instructor Name: Joel Kawakami, Ph. D.
Email: jkawakam@chaminade.edu (Best method for contact and I will do my best to get back to you within 24 hours of your received email)
Phone: (808) 739-8576
Office Location: Henry 5
Office Hours: Tuesday through Friday 8:30-9:20am
Instructor Website: https://chaminade.edu/academics/nsm/joel-kawakami/

University Course Catalog Description

Advanced Organic Chemistry is a one-semester course which explores the latest advances in Organic Chemistry. Following an introduction to the course, strategies in advanced organic synthesis and the use of new organometallic reagents and catalysts for the formation of carbon-carbon bonds will be explored. Mechanisms involved in key chemical transformations will be discussed in detail. The students will be exposed to stereochemical aspects of syntheses from the early stages of the course onwards, which will help them analyze new areas of asymmetric synthesis. Biosynthetic and biomimetic routes will be compared to synthetic strategies where applicable. Partial or complete structural elucidation is an integral part of any synthetic endeavor, which does not only prove the presence of the target molecule at the end, but may also shed light into the mechanism of the reactions through an understanding of the intermediates formed. Certain advanced spectroscopic theories and experiments, particularly in nuclear magnetic resonance spectroscopy, will be covered to draw student attention to fine details of structural features.
At the end of the course it is hoped that students have sampled topics at the forefront of modern Organic Chemistry.

Graduate students will be differentiated from undergraduates by having to complete an additional assignment. Each graduate student will be asked to outline the synthesis of a single target compound (natural product or drug substance). Forensic scientists frequently find the need to fully understand the synthetic methods utilized to prepare illicit substances. Drugs from legal or illegal sources are periodically modified structurally for various

reasons. The impetus to modify illegal drugs may stem from a goal to improve potency or evade current methods of drug screening. The additional assignment will not only give to the student an opportunity to apply the knowledge of organic synthesis, but will also focus on the forensic significance of being able to identify the starting material source(s) of an illicit modified drug.

Paste the description from the https://registrar.chaminade.edu/wp-content/uploads/sites/5/2019-2020-UG-Catalog.pdf

Course Overview

Coordinator Dr Joel Kawakami (jkawakam@chaminade.edu)
Office: Henry 5 Tel: (808) 739-8576

Research Mentor

Dr Joel Kawakami (jkawakam@chaminade.edu)Cancer Drug Design/Discovery

Directed Senior Research is a culmination of the course of study in chemistry/biochemistry. The steps that are follow here are quite similar to steps taken by biomedical scientists in a wide variety of research labs, from generating ideas and research proposals to collection and analysis of data and finally to the presentation of results to other scientists (including those at granting agencies) through a written publication and or a public presentation. The weekly meetings with the facilitator, Dr Kawakami, will be used to review project progress and to perform exercises that aim to increase knowledge of topical issues in the realms of biomedical/biochemical discovery, scientific ethics and recent technical advances.

The course has three components:

- **#1. Hands-on Laboratory Research Project**
  You may complete this on or off-campus. Off-campus internships are typically during the summer prior to your registration in BC495. Students who complete this component off campus are still required to perform course components #2 and #3 below. If you wish to perform on campus research you must be accepted by a research mentor from the list above by the end of week 2 of the semester. You should aim to spend at least 10 hours per week on your research project. Be aware that this is a minimum and the nature of biochemistry research means that it is sometimes time-consuming and unpredictable.

- **#2: Weekly class meetings and assignments, including a final research paper.**
- **#3: Poster presentation to faculty and staff in week 13 of the semester.**

A poster documenting your literature research project which you are required to present in our mini-symposium. A single sheet poster will be required. The poster will include title, authors and affiliations, abstract, background, methods, results and data, discussion, literature cited and acknowledgements. PowerPoint templates for poster design are recommended and will be provided on request by Dr Kawakami. At the mandatory poster presentation session you should be prepared to give a brief oral presentation of your poster and answer questions from faculty and your peers. This will be held on campus in week 13 of the semester. The date of this symposium will be announced in class.

All research papers or essays should be formatted in accordance with the guidelines for submission to the Journal of Biological Chemistry (see http://www.jbc.org/site/misc/ifora.xhtml) and Journal of Organic Chemistry (see http://pubs.acs.org/paragonplus/submission/loceah/joceah_authguide.pdf).

Required elements of the paper are:

- **Title page**: title of your research project, your name, course and date of submission.
- **Abstract**: standard abstract form that presents your research (including results) in less than 200 words.
- **Introduction**: a review of literature, hypothesis and rationale of your research project. What is known about your area of interest and about your specific question(s)? What is not known? Where does your work fit in and contribute?
- **Methods and Materials:** a detailed description of techniques, instruments, experimental and control groups and flow-charts if needed.
- **Results:** data tables, figures, photographs and brief narrative of each.
- **Discussion and Conclusion:** a careful analysis of results, error analysis and proposals for additional work.
- **Literature Cited:** provides a complete list of work cited. Comply with the style of the Journal of Biological Chemistry.
- ***The research must comply with the Chaminade University *Writing Across the Disciplines* standards.
- * The research paper must be submitted as hard copy by no later than the end of week fourteen to Dr Kawakami and as a PDF emailed to jkawakam@chaminade.edu by the same deadline.

3.4. How to keep a laboratory notebook

- Completely number pages before recording data/writing in it.
- Use permanent ink.
- Include a complete Table of Contents at the beginning; all experiments should be dated and page numbers indicated. Include your mathematical calculations.
- Cross out errors—do not erase or use Liquid Paper.
- If data for a given experiment is to be collected periodically, leave sufficient space to enter the data over time. A data table might be appropriate in this case.
- Record data directly and do not tear pages out.
- The notebook is the property of the supervising investigator and should be surrendered to them upon completion of the project.

Expectations

I do expect all assignments listed on the module to be done on time (late work is NOT expected). I will assign groups to work together and expect full interaction from everyone within the group. I expect to hear all group assignment communicated to me either on Zoom or Google Doc from all members of the group participating. I expect at all times during one-on-one to large group setting discussions to be civil and respectful to everyone with no vulgar language at any time – that includes my respect to each and every one of you. Thank you all for your dedication to these aspects in advance.

Marianist Values (MVs) for BC/CH480 Special Topics

This class represents one component of your education at Chaminade University of Honolulu. An education in the Marianist Tradition in marked by five principles and you should take every opportunity possible to reflect upon the role of these characteristics in your education and development:

1. Education for formation in faith
2. Provide an integral, quality education
3. Educate in family spirit
4. Educate for service, justice and peace
5. Educate for adaptation and change

In this course, we Provide an Integral, Quality Education (MV No. 2) based on ACS (American Chemical Society) National Norms standard. For further information on this standard, please see: https://uwm.edu/acs-exams/instructors/exam-statistics/national-norms/

Native Hawaiian Values (NHVs) for BC/CH480 Special Topics

Education is an integral value in both Marianist and Native Hawaiian culture. Both recognize the transformative effect of a well-rounded, value-centered education on society, particularly in seeking justice for the marginalized, the forgotten, and the oppressed, always with an eye toward God (Ke Akua). This is reflected in the ʻOlelo Noʼeau (Hawaiian proverbs) and Marianist core beliefs:

1. Educate for Formation in Faith (Mana) E ola au i ke akua (ʻŌlelo Noʻeau 364) May I live by God
2. Provide an Integral, Quality Education (Naʼauao) Lawe i ka maʻalea a kūʻonoʻono (ʻŌlelo Noʻeau 1957) Acquire skill and make it deep
3. Educate in Family Spirit (ʻOhana) ʻIke aku, ʻike mai, kōkua aku kōkua mai; pela iho la ka nohana ʻohana (ʻŌlelo Noʻeau 1200) Recognize others, be recognized, help others, be helped; such is a family relationship
4. Educate for Service, Justice and Peace (Aloha) Ka lama kū o ka noʻeau (ʻŌlelo Noʻeau 1430) Education is the standing torch of wisdom
5. Educate for Adaptation and Change (Aina) ʻAʻohe pau ka ʻike i ka hālau hoʻokahi (ʻŌlelo Noʻeau 203) All knowledge is not taught in the same school

Course Learning Outcomes (CLOs) for BC/CH480 Special Topics

By the end of our course, students will be able to:

LO1: Identify and classify classes of drug-like molecules.
LO2: Design research experiment to elucidate chemical mechanisms.
LO3: Illustrate the chemical mechanism for drug's mode of action at its molecular target.
LO4: Identify thermodynamically favorable conformations in drug-to drug and/or drug-to-receptor interactions.
LO5: Define principles of stereochemistry to explain drug-to drug and/or drug-to-receptor interactions.

Program Learning Outcomes (PLOs) in Chemistry

Upon completion of the undergraduate program in Biochemistry, students will be able to:

1. Appraise and articulate biochemical processes based on the fundamentals of organic chemistry, inorganic chemistry, analytical chemistry, physical chemistry, and biology as part of their integral and quality education.

(This PLO is a link to our Marianist Values of to provide an integral, quality education)

2. Construct and employ effective and safe laboratory skills utilizing modern scientific instrumentation and techniques.

3. Analyze, compare, and formulate an interpretation of biochemical data and problems as applied to living organisms and environment.

4. Assemble and assess biological data and compose a scientific analysis report or presentation.

Alignment of Learning Outcomes

	CLO1	CLO2	CLO3	CLO4	CLO5	CLO6	CLO7
MVs	2, 3, 4 & 5	4 & 5	3, 4 & 5	3, 4 & 5	4 & 5	4	3 & 4
NHVs	2, 3, 4 & 5	4 & 5	3, 4 & 5	3, 4 & 5	4 & 5	4	3 & 4
PLOs	1, 2	1, 2, 3, 4	1, 2, 3, 4	1, 2, 3, 4 & 5	2, 3, 4	1, 2, 4 & 5	1, 2, 3, 4 & 5

Course Prerequisites

Prerequisites: BC 360/L

Required Learning Materials

TBA by the Mentor & Adviser of your research.

Course Website:

https://chaminade.instructure.com/courses/10053

Technical Assistance for Canvas Users:

- Search for help on specific topics at help.instructure.com
- Chat live with Canvas Support 24/7/365
- Watch this video to get you started with online guides and tutorials
- Contact the Chaminade IT Helpdesk for technical issues: helpdesk@chaminade.edu, or call (808) 735-4855

Assessment

Attendance and participation in weekly meetings	100 points
Final Poster Presentation	200 points
Final written paper	100 points
Written assignments	100 Points

A	Excellent	>90%	450 points or more
B	Good	>80%	400-449 points
C	Average	>70%	350-399 points

D	Below Average	>60%	300-349 points
F	Failure	<60%	299 points or less

Student attendance at a weekly meeting with the course coordinator is required, these meetings may be held in a group or individualized format.
Attendance/participation: 20% (100 points). Attendance at weekly meeting with the course coordinator (either in group or individually) as well as participation/engagement during these meetings will be calculated as 20% of the grade. Ten points will be allocated per meeting of which 5 are for attendance and 5 will be allocated on the basis of student participation and engagement. The instructor will provide a rubric for the allocation of these points.

Grading Scale

Letter grades are given in all courses except those conducted on a credit/no credit basis. Grades are calculated from the student's daily work, class participation, quizzes, tests, term papers, reports and the final examination. They are interpreted as follows:

A Outstanding scholarship and an unusual degree of intellectual initiative
B Superior work done in a consistent and intellectual manner
C Average grade indicating a competent grasp of subject matter
D Inferior work of the lowest passing grade, not satisfactory for fulfillment of prerequisite course work
F Failed to grasp the minimum subject matter; no credit given

Credit Hours:

1 hour per supervisory instructions (2.5 hrs per wk for 15 wk).	(Subtotal = 37.5+2)
Minus 0.83 lecture for all holidays (4 Holidays)	(Subtotal = -3.3)
3 hours Research reading/Data Reporting per research day. (10 weeks X 3 hours X 3 days)	(Subtotal = 90)
2 hours preparation of poster per week (10 weeks).	(Subtotal = 20)
3 hours preparation of research article per week (10 weeks)	(Subtotal = 30)
4 hours practice session for poster presentation (3 times)	(Subtotal = 12)
6 hours final practice session & Actual poster presentation.	(Subtotal = 6)

Total Hours = 194.2

Schedule

Week 1 **Orientation and Overview**

Homework: Key Scientific Questions Written Exercise
250 words "What is the most important question scientists should be addressing today?"
Due to Dr. Kawakami (hard copy) by the beginning of week 2.

Week 2 **Discussion of Key Scientific Questions Written Exercise**
Name of Research Project Supervisor due to Dr. Kawakami with project title.

Homework: How do we know what we know? Exercise

Prepare 10 minute white board presentation on the assigned question.

Week 3: **Discussion of <u>How do we know what we know?</u> Presentations**

Homework: Prepare project hypothesis with you research supervisor.

Week 4: **Research hypothesis and experimental plan discussion**
Be prepared to discuss the work you are planning to do or have done in the lab

Week 5: **<u>What makes a good project?</u> Exercise and group discussion**

Week 6: **<u>Role models: the PhD, MD-PhD and postdoctoral experience</u>**
External panel discussion
<u>Homework:</u> prepare project abstract and methods section for poster and paper

Week 7: **Review of Research Progress**
<u>Homework: "Bad apples"</u> exercise
Prepare 10 min whiteboard presentation on you assigned scientific misconduct case

Week 8: **<u>"Bad apples"</u> Research Ethics discussion**
<u>Homework</u>: first drafts of paper and poster

Weeks 9-12 **Paper and Poster preparation**

Week 13: **Poster Symposium, also email poster copy to (jkawakam@chaminade.edu)**
Week 14: **Paper due by the end of this week to Dr Kawakami**
(hard copy and email PDF to jkawakam@chaminade.edu)

12. Alignment of Natural Sciences Courses with Marianist and Hawaiian values of the University.

The Natural Sciences Division provides an *integral, quality education:* sophisticated integrative course content taught by experienced, dedicated, and well-educated instructors.

- We *educate in family spirit* – every classroom is an *Ohana* and you can expect to be respected yet challenged in an environment that is supportive, inclusively by instructors who take the time to personally get to know and care for you.
- We *educate for service, justice and peace*, since many of the most pressing global issues (climate change, health inequity, poverty, justice) are those which science and technology investigate, establish ethical parameters for, and offer solutions to.
- We *educate for adaptation and change*. In science and technology, the only constant is change. Data, techniques, technologies, questions, interpretations and ethical landscapes are constantly evolving, and we teach students to thrive on this dynamic uncertainty.

The study of science and technology can be formative, exploring human creativity and potential in the development of technologies and scientific solutions, the opportunity to engage in the stewardship of the natural world, and the opportunity to promote social justice. We provide opportunities to engage with the problems that face Hawai‘i and the Pacific region through the Natural Sciences curriculum, in particular, those centered around severe challenges

in health, poverty, environmental resilience, and erosion of traditional culture. The Marianist Educational Values relate to Native Hawaiian ideas of *mana, na'auao, ohana*, *aloha* and *aina*. We intend for our Natural Sciences programs to be culturally-sustaining, rooted in our Hawaiian place, and centered on core values of *Maiau,* be neat, prepared, careful in all we do; *Makawalu*, demonstrate foresight and planning; *`Ai,* sustain mind and body; *Pa`a Na`au*, learn deeply.

13.1. Late Work Policy

All overdue assignment not completed or anticipated to be late must have approval from the instructor along with a valid excuse.

13.2. Grades of "Incomplete"

Students and instructors may negotiate an incomplete grade when there are specific justifying circumstances. When submitting a grade the "I" will be accompanied by the alternative grade that will automatically be assigned after 90 days. These include IB, IC, ID, and IF. If only an "I" is submitted the default grade is F. The completion of the work, evaluation, and reporting of the final grade is due within 90 days after the end of the semester or term. This limit may not be extended.

13.3. Writing Policy

Plagiarism will not be tolerated and will be checked.

13.4. Instructor and Student Communication

Questions for this course can be emailed to the instructor at jkawakam@chaminade.edu. Online, in-person and phone conferences can be arranged. Response time will take place up to [1-12 hours].

Cell phones, tablets, and laptops

Out of consideration for your classmates, please set your cell phone to silent mode during class. Students are encouraged to bring laptops or tablets to class as the instructor will assign online activities and readings that will require the use of a laptop or tablet. Laptops and tablets should not be misused, such as checking distracting websites. Use your best judgment and respect your classmates and instructor.

Disability Access

If you need individual accommodations to meet course outcomes because of a documented disability, please speak with me to discuss your needs as soon as possible so that we can ensure your full participation in class and fair assessment of your work. Students with special needs who meet criteria for the Americans with Disabilities Act (ADA) provisions must provide written documentation of the need for accommodations from the Counseling Center by the end of week three of the class, in order for instructors to plan accordingly. If a student would like to determine if they meet the criteria for accommodations, they should contact the Kōkua 'Ike Coordinator at (808) 739-8305 for further information (ada@chaminade.edu).

Title IX Compliance

Chaminade University of Honolulu recognizes the inherent dignity of all individuals and promotes respect for all people. Sexual misconduct, physical and/or psychological abuse will NOT be tolerated at CUH. If you have been the victim of sexual misconduct, physical and/or psychological abuse, we encourage you to report this matter promptly. As a faculty member, I am interested in promoting a safe and healthy environment, and should I learn of any sexual misconduct, physical and/or psychological abuse, I must report the matter to the Title IX Coordinator. If you or someone you know has been harassed or assaulted, you can find the appropriate resources by visiting Campus Ministry, the Dean of Students Office, the Counseling Center, or the Office for Compliance and Personnel Services.

Attendance Policy

The following attendance policy is from the 2019-2020 Academic Catalog (p. 54-55). Faculty members should also check with their divisions for division-specific guidelines.

Students are expected to attend regularly all courses for which they are registered. Student should notify their instructors when illness or other extenuating circumstances prevents them from attending class and make arrangements to complete missed assignments. Notification may be done by emailing the instructor's Chaminade email address, calling the instructor's campus extension, or by leaving a message with the instructor's division office. It is the instructor's prerogative to modify deadlines of course requirements accordingly. Any student who stops attending a course without officially withdrawing may receive a failing grade.

Unexcused absences equivalent to more than a week of classes may lead to a grade reduction for the course. Any unexcused absence of two consecutive weeks or more may result in being withdrawn from the course by the instructor, although the instructor is not required to withdraw students in that scenario. Repeated absences put students at risk of failing grades.

Students with disabilities who have obtained accommodations from the Chaminade University of Honolulu ADA Coordinator may be considered for an exception when the accommodation does not materially alter the attainment of the learning outcomes.
Federal regulations require continued attendance for continuing payment of financial aid. When illness or personal reasons necessitate continued absence, the student should communicate first with the instructor to review the options. Anyone who stops attending a course without official withdrawal may receive a failing grade or be withdrawn by the instructor at the instructor's discretion.

Academic Conduct Policy

From the 2019-2020 Undergraduate Academic Catalog (p. 39):
Any community must have a set of rules and standards of conduct by which it operates. At Chaminade, these standards are outlined so as to reflect both the Catholic, Marianist values of the institution and to honor and respect students as responsible adults. All alleged violations of the community standards are handled through an established student conduct process, outlined in the Student Handbook, and operated within the guidelines set to honor both students' rights and campus values.

Students should conduct themselves in a manner that reflects the ideals of the University. This includes knowing and respecting the intent of rules, regulations, and/or policies presented in the Student Handbook, and realizing that students are subject to the University's jurisdiction from the time of their admission until their enrollment has been formally terminated. Please refer to the Student Handbook for more details. A copy of the Student Handbook is available on the Chaminade website.

For further information, please refer to the Student Handbook: https://chaminade.edu/wp-content/uploads/2019/08/NEW-STUDENT-HANDBOOK-19-20-Final-8.20.19.pdf

Credit Hour Policy

The unit of semester credit is defined as university-level credit that is awarded for the completion of coursework. One credit hour reflects the amount of work represented in the intended learning outcomes and verified by evidence of student achievement for those learning outcomes. Each credit hour earned at Chaminade University should result in 37.5 hours of engagement. For example, in a one credit hour traditional face to face course, students spend 50 minutes in class per week for 15 weeks, resulting in a minimum of 12.5 instructional hours for the semester. Students are expected to engage in reading and other assignments outside of class for at least 2 additional hours per week, which equals an additional 25 hours. These two sums result in

total student engagement time of 37.5 hours for the course, the total engagement time expected for each one credit course at Chaminade.

The minimum 37.5 hours of engagement per credit hour can be satisfied in fully online, internship, or other specialized courses through several means, including (a) regular online instruction or interaction with the faculty member and fellow students and (b) academic engagement through extensive reading, research, online discussion, online quizzes or exams; instruction, collaborative group work, internships, laboratory work, practica, studio work, and preparation of papers, presentations, or other forms of assessment. This policy is in accordance with federal regulations and regional accrediting agencies.

WHAT DOES SUCCESS LOOK LIKE?

Insulin-induced lipid bodies are dynamically regulated in activated mast cells

Christina Linares[1], Carl Sung[1], William E. Greineisen [1,2], and Helen Turner[1,3].

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This project was supported by grants from the National Center for Research Resources (5P20RR016468-12) and the National Institute of General Medical Sciences (8P20GM103466-12) from the National Institutes of Health. The content is solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health. The project was also supported by the General Atlantic Foundation and the Hawaii Community Foundation.

Abstract

We have previously shown that insulin exposure in mast cells (MC) is associated with pronounced lipid body formation, suggesting that immunocytes, like hepatocytes and myocytes, can be sites for ectopic lipid accumulation when insulin levels are systemically dysregulated. Lipid body accumulation in mast cells (MC) is accompanied by suppressed secretory response and cytokine gene induction, but enhanced release of arachidonate-derived bioactive lipids (e.g. leukotriene C4). Our macro-cytochemical staining suggests that LB are dispersed after mast cell activation, but the fate of the LB has not been tracked at the subcellular level or in real time. In this study we tested the hypothesis that lipid bodies will be spatially relocated after stimulation is applied to mast cells (RBL2H3). After being stained with Oil Red-O (ORO) as the identifying marker for lipid bodies, we mimicked antigen receptor ligation using Phorbol 12-Myristate 13- Acetate (PMA) and ionomycin to simulate single live mast cells. Confocal and epi-fluorescent imaging were performed on a Nikon Ti Eclipse fluorescence microscopy system in 2 and 3 dimensions and over a time course of 15 min. Lipid body mobilization was tracked and analyzed in NIS Elements (Nikon). Movement was observed when comparing resting mast cells with PMA/ionomycin treated cells. We observed changes in the relative position, size, and morphology of lipid bodies, apparent dispersal of lipid body contents and both LB fusion and apparent formation of new lipid bodies. Thus lipid bodies in mast cells are highly dynamic, and this study serves as a foundation to evaluate the links between mobilization of pro-inflammatory bioactive lipids in activated mast cells and the microscopic behavior of the lipid bodies themselves.

Methods

Cell culture RBL2H3 were grown at 37 °C, 5% CO_2, in a 95% humidity in DMEM/10% HI FBS /2mM Gln. Lipid body acculumation was initiated by incubation of cells for 6 days with lipogenic stimulus (0.1 ug/ml insulin, 10% FBS, 1µM dexamethasone, 6.25µM IBMX).

Single cell calcium assay RBL2H3 were plated on glass coverslip dishes and incubated with 1µM Fluo-4 for 30 minutes at 37°C in a standard modified Ringer's solution of the following composition (in mM): NaCl 145, KCl 2.8, CsCl 10, $CaCl_2$ 1, $MgCl_2$ 2, glucose 10, Hepes·NaOH 10, pH 7.4, 330 mOsm.

Oil Red Staining in fixed cells. Cells were fixed with PFA (0.4% w/v final in PBS) for 1h RT. Wells were washed twice with dH_2O and stained with 200 µl Hematoxylin-Gill #2 and Oil Red O (0.35% in 6:4 EtOH/H_2O) for 15 minutes.

Oil Red staining in live cells. RBL2H3 were treated with 150µl of ORO (0.35% in 7:3 EtOH:water, 30 min at 37°C followed by two dH_2O washes) for 30 minutes, aspirated, and washed with 1mM Ca Assay Buffer (49.55ml Na Ringers, 50µl 1M Ca, 400µl 250mM Probenecid).

Cell stimulation and Microscopy. Cells were stimulated as indicated on a 37°C heated stage. As a control for activation status, cells were also loaded with 1µM Fluo-4 and calcium signals were acquired simultaneously to confirm that the cell in question had in fact activated in response to stimulation. Fluorescence signals were acquired using a Nikon Ti Eclipse confocal microscopy system at Chaminade University Advanced Instrumentation Facility , using EZ C1 for acquisition and NIS Elements (Nikon) for analysis.

Results

I. Insulin induces lipid body formation in mast cells

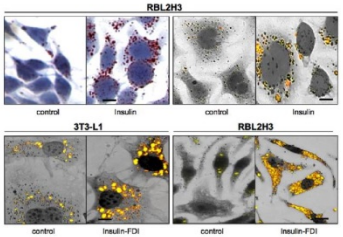


Figure 1. Insulin induced lipid body accumulation in mast cells. Oil Red O staining of RBL2H3 mast cells or 3T3-L1 adipocytes after 6 day treatment with either insulin (1ug/ml) or a lipogenic cocktail comprising insulin with additional FBS, dexamethasone and IBMX. Counterstaining was with hematoxylin. Scale bar is 5µm.

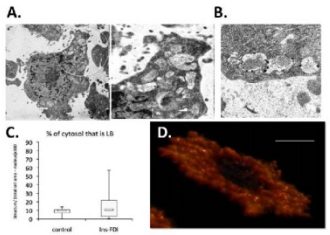


Figure 2. Visualization of ectopic lipid body formation in mast cells. A. SEM of 6d Insulin-treated mast cells at 5,000x (left) and 20,000x zoom (center panel). Examples of insulin-induced cytosolic structures shown in right panel. **B** .Quanitation (NIH Image J) from EM of % cytosol that are apparent *de novo*-formed lipid bodies in control and 6d insulin-treated mast cells. **C.** 3D rendered confocal z-stack of Oil Red O lipid body staining in a 6d insulin-treated mast cell. Scale bar is 10 microns.

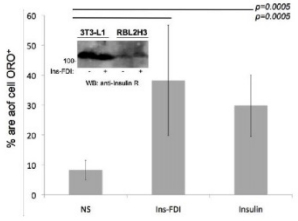


Figure 3. Quantification of Insulin induced lipid body accumulation in mast cells. Image J was used to quantify area of ORO-positive staining per cell in RBL2H3 mast cells stained after 6 day treatment with either insulin (1µg/ml) or a lipogenic cocktail. Inset shows anti-Insulin receptor Western blot in control and treated cells.

II. Cytochemical evidence that lipid bodies are dispersed following antigen or ionophore stimulation of mast cells.

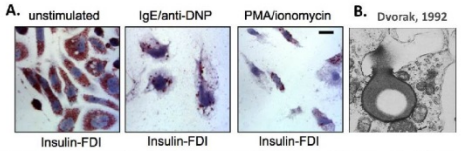
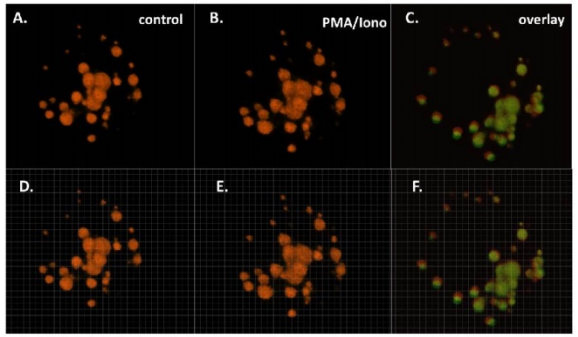
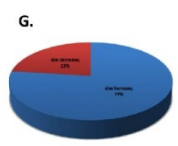


Figure 4. Cytochemical evidence that lipid bodies are mobilized or dispersed after mast cell stimulation. A. Hematoxylin (blue) and Oil Red O (red) staining of 6d insulin-treated RBL2H3 mast cells before or after stimulation with either IgE/antigen (to ligate the FceRI) or a combination of phorbol ester and ionomycin to mimic antigen receptor activation of mast cells. Stimuli were applied for 60 min at 37°C. **B.** Reproduced from Dvorak et al, 1992. this electron micrograph shows the apparent fusion of a lipid body with the plasma membrane of an activated human basophil, and apparent dispersal of LB contents into the extracellular milieu.

III. Live cell fluorescence analysis of lipid body dynamics following activation of mast cells.





H.

Average % increase in LB area (n=21)	Average % decrease in LB area (n=5)
58.67	-15.87
Range of % increase	Range of % decrease
3.4% to 415%	-8 to -22%

Figure 5. Lipid body dynamics after mast cell activation. Lipid body biogenesis was induced in mast cells using 6d insulin treatment. Cells were live cell stained with Oil Red O in a modified Ringer's solution with 1mM calcium. After 5 min equilibration on a 37°C heated stage, cells were imaged **(A, D)** (z-stack) and then stimulated for 15 min with PMA/ionomycin (1µM/1µM) and re-imaged **(B, E)**. Relative position changes in lipid body staining are shown as overlays **(C, F)** with pre-stimulation pseudo-colored red and post-stimulation pseudo-colored green. Lower panels provide gridlines to orient relative positions of lipid bodies. **G.** ROI analysis in 2 dimensions of lipid body apparent area pre- and post stimulation showed that approximately 20% of the lipid bodies displayed an apparent size decrease and 80% displayed a size increase. **H.** Analysis of average and range of size changes in lipid bodies after 15 min stimulation of mast cells with PMA and ionomycin.

IV. Lipid bodies display various changes after mast cell stimulation.

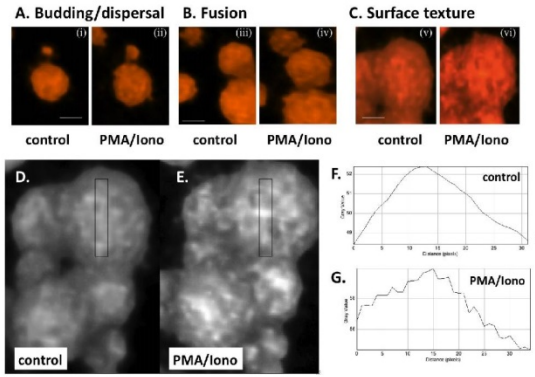


Figure 6. Alterations in lipid body appearance after mast cell stimulation. Lipid body biogenesis was induced in mast cells using 6d insulin treatment. Cells were live cell stained with Oil Red O in a modified Ringer's solution with 1mM calcium. After 5 min equilibration on a 37°C heated stage, cells were imaged **(I, iii, v, D, F)** (z-stack) and then stimulated for 15 min with PMA/ionomycin (1µM/1µM) and re-imaged **(ii, iv, vi, E, G). A, B, C.** We noted examples of apparent lipid body dispersion, fusion and structural changes in lipid body surface morphology. Scale bars are 0.2 microns. **D, E.** High resolution images of lipid body surfaces before and after simulation. NIH Image J was used to plot surface intensity profiles, **(F-H)**, suggesting that an increase in the complexity of the lipid body surface follows stimulation.

Conclusions

- We have established a method for live cell lipid body imaging
- Insulin exposure induces lipid body genesis in mast cells
- Lipid bodies are dynamic after antigen or pharmacological stimulation of mast cells
- Lipid bodies display primarily size increases, as well as fusion, during cellular stimulation
- Slight positional change is observed indicating that the lipid bodies are somewhat motile during stimulation
- The lipid body surface becomes more complex after cellular stimulation

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The Putative Role of TMEM16E in Gnathodiaphyseal Dysplasia

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Abstract

The protein GDD1/TMEM16E is associated with the autosomal dominant bone disorder Gnathodiaphyseal Dysplasia (GDD). GDD is a disease of the skeletal system that results in cemento-fibromas in the mandibular and maxillary regions, a complex distribution of skeletal fragility that includes demineralization in wrists, spine, and pelvis, generalized osteopenia, and a uniquely characteristic bowing and cortical thickening of tubular bones and diaphyseal sclerosis of long bones. Little is known about the biochemical interactions of this protein or its role in GDD. In this study, we aim to investigate the protein interactions of TMEM16E *in vitro* by generating a peptide affinity matrix in order to purify protein interactors, which could identify putative functions TMEM16E.

Introduction

Gnathodiaphyseal dysplasia (GDD) is an autosomal dominant congenital disease of the skeletal system that results in cemento-fibromas in the mandibular and maxillary regions, a complex distribution of skeletal fragility that includes demineralization in wrists, spine, and pelvis, generalized osteopenia, and a uniquely characteristic bowing and cortical thickening of tubular bones and diaphyseal sclerosis of long bones.[1][2] Other characteristics of the disease include bone fragility accompanied by a high frequency of accidental breakage during childhood and adolescence.[3] Patients also experience bacterial infections of the jaw, recurrent purulent osteomyelitis, periodontal inflammation, and mobility of the dentition.[2] The syndrome is process for fractures; there are no symptoms of pseudarthrosis or bone deformity.[5]

The original Japanese family to be diagnosed with this disorder in 1969 was studied in depth, resulting in a mapping of the disease locus to chromosome 11p14.3-15.1.[6] Positional cloning revealed a novel gene encoding a 913 amino acid integral membrane protein termed GDD1, which now has been discovered to be identical to TMEM16E, a member of the largely uncharacterized TMEM16 family of genes.[2][7] All affected members of the Japanese family carry a heterozygous mutation of Cys356Arg caused by a T-to-C transition in exon 11. Notably, a similar mutation in the same codon of Cys356Gly was found in affected members of an African family.[4]