

THE NUCLEUS

January 2018

Vol. XCVI, No.5

Monthly Meeting

ACS 2018 President, Peter K. Dorhout, to Speak at Nova Biomedical

National Chemistry Week 2017

A report by Raymond Lam

2018 Chair's Statement

By Mindy Levine

Summer Scholar Report

By Abraham Bayer, Caitlin Hill and Rebecca Scheck, Tufts University



National Chemistry Week 2017 Chemistry Rocks!

By Raymond Lam, Massachusetts Maritime Academy



Photo Credit: Alissa Daniels

The theme for National Chemistry Week (NCW) 2017 was “Chemistry Rocks!” The Northeastern Section of American Chemical Society (NESACS) teamed up with Boston Children’s Museum and the Museum of Science-Boston once again to celebrate NCW with our community. Prior to the events, a guest educators’ orientation day was held at the Museum of Science-Boston on Sunday, October 1. Approximately 20 volunteers came and got an early preview of the activities offered this year; many also offered suggestions for improvements. In order to better communicate with our volunteers and increase our social media presence, a new public Facebook group (NESACS National Chemistry Week) was created and our volunteers were encouraged to join. Periodic updates of our events, including important dates and photos, were posted on our page and shared with our group members. All volunteers were given NCW 2017 t-shirts and Dr. Jayashree Ranga from Salem State University also distributed Chemistry Ambassador ribbons at the Museum of Science.



Visitor at Boston Children’s Museum investigating the conductivity of rocks and minerals. Photo Credit: Alissa Daniels

Museum of Science-Boston

Our kick-off event this year was the High School Science Series at the Museum of Science on Friday, October 13. Approximately 500 students participated in a number of hands-on activities and demonstrations facilitated by Museum of Science staff. Two lecture demonstrations were given by David Sittenfeld from the Museum of Science.

On Sunday October 15, NESACS sponsored the public event at the Museum of Science. Over 80 NCW volunteers ensured that visitors to the daylong event enjoyed the hands-on theme-related activities. Approximately 300 visitors attended our event and many also had detailed discussions with our volunteers on the science behind each activity. Among the highlights of the day were the two Phyllis A. Brauner Memorial Lectures, presented by Prof. Bassam Z. Shakhshiri, Professor of Chemistry at the University of Wisconsin-Madison.

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NCW volunteer with visitor at the “Sink or Float” activity at Museum of Science, Boston. Photo Credit Emily Hostetler



Rock band “Nakedly” performing at Boston Children’s Museum. Photo Credit: Alissa Daniels

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Cover: Peter K. Dorhout, 2018 American Chemical Society President. Photo Courtesy of Professor Dorhout.

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Chemistry Rocks

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Boston Children's Museum

Our last NCW 2017 event was held at Boston Children's Museum on Saturday, October 22. Approximately 500 visitors of all ages joined us for the day-long event. Visitors were once again given a stamp sheet as they entered our activity area in order to collect chemistry-related stamps at each station. ACS NCW promotional materials and bookmark magnifiers were given as prizes upon completion.

In addition to all the hands-on activities related to the yearly theme, Boston Children's Museum also worked with ZUMIX, an East Boston-based nonprofit organization dedicated to building community through music, to provide a live rock concert alongside our event. The rock concert was sponsored by Massachusetts Maritime Academy and featured a group of high school students performing two 25-minute sessions. The concert was well received and small 2-minute clips of the concert were streamed live on our Facebook page.

The activities and demonstrations that were performed throughout NCW 2017 events included: Testing for Radioactivity; Painting with Soil; Rocky Reactions; Instant Crystals; Sink or Float; What's in the Soil?; FluoROCKscent; Cleaning Oil Spills with Chemistry; One Metal, Many Colors; Igneous, Sedimentary or Metamorphic; What to do about CO₂?; Sharklet/Biomimicry; Mineral & Metals; and Harvesting Fossils from Rocks.

Illustrated Poem Contest &



NCW volunteers at Boston Children's Museum.
Photo Credit: Alissa Daniels

Acknowledgments

The success of our events would not have been possible without the effort of our contributors and volunteers. Special thanks to Boston Children's Museum and Museum of Science-Boston for hosting the events, and to our sponsors, Massachusetts Maritime Academy and Millipore Sigma, for their generous donations. The events would not have been possible without the help of the volunteers from Alnylam Pharmaceuticals, Beyond Benign, Emmanuel College, Gordon College, Malden Catholic High School, Massachusetts Maritime Academy, Nipmuc Regional High School, Northeastern University, Salem State University, NSYCC, Stonehill College, Suffolk University, Toxikon Corp. and UMass Boston. The author apologizes in advance if anyone has been inadvertently omitted from these acknowledgments.

Children, grades K-12, were able to participate in the national poster competition. Congratulations to Cosette Cummins for winning the 3rd – 5th Grade category and Hannah Pais for winning the 9th – 12th Grade category.

The 2018 theme for NCW is "Chemistry Is Out of this World!" Join our Facebook group (NESACS National Chemistry Week) for the latest news and updates! ♦

ResMed: Residential School on Medicinal Chemistry and Biology in Drug Discovery
June 10-15, 2018
Drew University, Madison, NJ

This graduate level course concentrates on the fundamentals that are useful in drug discovery spanning initial target assay evaluation through clinical development. Case histories of recent successful drug development programs will also be presented. The five-day program covers:

Principles of Med Chem	DMPK
Cheminformatics	Toxicophores
Lead ID & Optimization	GPCRs
Epigenetics	Kinase Inhibitors
Fragment-based Drug Design	Ion Channels
Structure-based Drug Design	Enzyme Inhibitors
Drug-like Properties	Bioisosteres
Protein-Protein Interactions	Preclinical Toxicology
Molecular Modeling	Clinical Development
Antibody-Drug Conjugates	

Bill Greenlee, Vince Gullo & Ron Doll – Co-organizers

Attendees will be staying at the Madison Hotel

www.drew.edu/resmed
e-mail: resmed@drew.edu
phone: 973/408-3787; fax: 973/408-3504

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Monthly Meeting

The 975th Meeting of the Northeastern Section of the American Chemical Society

Sponsored by Nova Biomedical

Thursday, January 11, 2018

Nova Biomedical

200 Prospect Street, Waltham, MA 02453

4:00 pm NESACS Annual Meeting

4:30 pm NESACS Board Meeting

5:30 pm Social Hour

6:30 pm Dinner

7:30 pm **Keynote Presentation:** Professor Peter Dorhout, President of the American Chemical Society, Vice-President for Research, Kansas State University

Title: *Challenges with and for Chemistry – Being Prepared*

**YOU MUST REGISTER IN ADVANCE TO ATTEND THE MEETING
THERE IS NO REGISTRATION FEE TO ATTEND THE MEETING
DINNER RESERVATIONS ARE REQUIRED.**

PUBLIC IS INVITED

- For those who would like to join us for dinner, register by noon, Thursday, January 4, at <https://peter-dorhout-president-ac.s.eventbrite.com>. Cost: Members, \$30; Non-members, \$35; Retirees, \$20; Students, \$10. Dinner reservations not cancelled at least 24 hours in advance must be paid.
- If you wish to join us for this meeting and not eat dinner, please register by noon, Thursday, January 4 at <https://peter-dorhout-president-ac.s.eventbrite.com> Select "Seminar only".

If you have any questions or require additional information, contact the Administrative Coordinator, Anna Singer, via email at secretary@nesacs.org. ◇

Biography:

Dr. Peter K. Dorhout is a Professor of Chemistry and was appointed in 2016 as Vice President for Research at Kansas State University, where he had also served four years as dean of the College of Arts & Sciences. Prior to coming to Kansas State in 2012, he served as the Interim Provost at Colorado State University-Pueblo, preceded by 20 years at Colorado State University-Fort Collins as Vice Provost for Graduate Studies, Assistant Vice President for Research, and Professor of Chemistry. He has served as a collaborator at Los Alamos National Laboratory since 1987.

Peter is the 2018 President of the American Chemical Society, where he has also served as a member of the Board of Directors, Chair of the Committee on Committees, Chair of the International Activities Committee, and member of Budget & Finance, Divisional Activities Committee, the Younger Chemists Committee, and served on numerous task forces. He has been a councilor for the Inorganic Division and the Colorado Local Section, where he also served as Local Section Chair.

He currently serves on the Board of Directors for Research Corporation for Science Advancement, the Kansas State University Research Foundation, and the Coronado Council BSA Executive Board.

Dr. Dorhout is a recognized expert in solid state and nuclear materials science and environmental chemistry. He has had active research programs in solid-state f-element and radiochemistry, and nanomaterials science. He has published over 115 peer-reviewed journal articles, book chapters, and reviews while presenting over 130 international and national invited lectures on his chemistry. Dr. Dorhout earned a bachelors degree in chemistry from the University of Illinois at Urbana-Champaign and a doctorate in inorganic chemistry from the University of Wisconsin-Madison. His list of professional awards includes Fellow of the American Chemical Society, Fellow of the AAAS, Research Corporation Cottrell Scholar, Camille Dreyfus Teacher-Scholar, A. P.

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Abstract:

Challenges with and for Chemistry – Being Prepared

As I have been getting prepared for my year as ACS President, I have been reminded by the many challenges facing our members and the discipline of chemistry today. In keeping with my Boy Scout motto: Be Prepared, I wanted to highlight a few challenges that I've heard the past few years and present some opportunities for ACS and its members to work together to address those challenges. It begins with re-establishing our credibility as scientists in the eyes of the public, which starts with educating the next generations of

chemists. In particular, promoting the responsible conduct of research, advocating for enhancements to safety training, and enriching our communications with the public will continue to be part of my activities over my three years in the Presidential succession. Being prepared for global challenges in chemistry includes developing skills for life-long learning, building a portfolio of global experiences and perspectives, and learning to work on teams with diverse members. How we infuse these skills and perspectives into our undergraduate, graduate, early-career, and mid-career members will depend on us and our creativity to work as a broader Society to enhance our members and their professional experiences. ◇

American Chemical Society 254th ACS National Meeting Washington, District of Columbia August 20-24, 2017

Councilor Talking Points:

Summary of Governance Issues and Actions

The following summary is provided to help Councilors report to their Local Sections and Divisions on key actions of the ACS Council meeting held August 23, 2017 and the Board of Directors meetings held August 18-19, 2017, at the 2017 ACS fall national meeting in Washington, District of Columbia. Full reports will be posted on the ACS Website as they become available.

Actions of the Council

Election Results: Elected Committees of Council

- By electronic ballot, the Council elected Karl S. Booksh, Mark D. Frishberg, Zaida C. Morales Martinez, and Linette M. Watkins for three-year terms (2018-2020), and Ella L. Davis for a one-year term (2018) on the Council Policy Committee (CPC).

*Karl S. Booksh	209
James C. Carver	180
Dwight W. Chasar	140
*Ella L. Davis	203
*Mark D. Frishberg	207
Lydia E.M. Hines	165
Will E. Lynch	190
*Zaida C. Morales Martinez	215
Barbara P. Sitzman	150
*Linette M. Watkins	260

- By electronic ballot, the Council elected Michael Appell, Neil D. Jespersen, Mamie W. Moy, Eleanor D. Siebert, and Julianne M.D. Smist for three-year terms (2018-2020) on the Committee on Nominations and Elections (N&E).

Anthony W. Addison	159
Joe D. Allison	103
*Michael Appell	165
Mark A. Benvenuto	149
Arindam Bose	140
*Neil D. Jespersen	237
*Mamie W. Moy	269

*Eleanor D. Siebert	252
*Julianne M.D. Smist	228
Keith R. Vitense	134

- By electronic ballot, the Council elected Mitchell R. M. Bruce, Jetty Duffy-Matzner, Martha G. Hollomon, Diane Krone, and Robert A. Pribush for three-year terms (2018-2020) on the Committee on Committees (ConC).

*Mitchell R. M. Bruce	225
*Jetty Duffy-Matzner	223
Rick Ewing	177
Barbara R. Hillery	115
*Martha G. Hollomon	187
Judith M. Iriarte-Gross	163
Russell W. Johnson	123
*Diane Krone	192
*Robert A. Pribush	227
Susan M. Schelble	184

Other Council Actions

Amendments to the ACS Bylaws

- A recommendation by the Committee on Membership Affairs that Council approve the Petition on International Chemical Sciences Chapters narrowly failed to achieve the two-thirds majority required to amend the Bylaws. The proposal would have amended Bylaw IX, Section 4, to permit financial support for International Chemical Sciences Chapters and to remove language from the Bylaws prohibiting Chapters from having representation on Council.

Probationary Division of Space Chemistry

- The Council defeated a proposal from the Committee on Divisional Activities that it establish a probationary Division of Space Chemistry, effective January 1, 2018.

Change in Local Section Territory

- On the recommendation of the Com-

mittee on Local Section Activities, the Council approved a petition from the South Jersey Local Section to annex the unassigned and adjacent territory of Ocean County, New Jersey.

Resolutions

- The Council passed resolutions in memory of deceased Councilors; acknowledging President Allison A. Campbell's service as presiding officer of the Council; and in gratitude for the officers and members of the Chemical Society of Washington, the host Section for the 254th National Meeting, the divisional program chairs and symposium organizers, and ACS staff.

Highlights from Committee Reports

Nominations and Elections

The Committee on Nominations and Elections solicits Councilors' input of qualified individuals for President-Elect and/or Directors for future consideration. Suggestions may be sent to nom-elect@acs.org.

Ballots for the 2017 fall national election will be distributed on September 29, with a voting deadline four weeks later, on October 27. In a change of procedures, all members with an email address on file and eligible to vote will receive an electronic ballot with the option to request a paper ballot. Those members with no email address on file will be sent a paper ballot with the option to still vote electronically. The ACS election vendor, Survey & Ballot Systems, will send three email reminders during the voting period to those who have not voted as of the reminder date.

Budget and Finance

The Society's 2017 Probable 1 Projection calls for a Net from Operations of \$25.3 million. This is \$2.1 million fa-

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ACS Meeting 2017

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avorable to the Approved Budget and \$1.6 million higher than 2016. Total revenues are projected to be \$553.0 million, which is \$2.4 million unfavorable to the budget, but 5.0% higher than the prior year. Total expenses are projected at \$527.6 million, which is \$4.5 million favorable to the budget, and 4.9% higher than 2016.

The Committee considered several program funding requests for 2018, and on its recommendations, the Board subsequently approved funding for the ACS Online Course in Laboratory Safety and the New Faculty Workshop Series for inclusion in the 2018 Proposed Budget and the 2019-2020 Forecast.

Additional information can be found at www.acs.org, at the bottom of the page, click 'About ACS', then 'Financial'. There you will find several years of the Society's audited financial statements and IRS 990 filings.

Washington Meeting Attendance

The theme of the 254th ACS National Meeting was "Chemistry's Impact on the Global Economy." As of Tuesday evening, August 22, attendance was:

Attendees	7,938
Students	2,997
Exhibitors	1,068
Expo only	475
Guest	426
Total	12,904

Petitions to Amend the Constitution and Bylaws

New petitions to amend the Constitution or Bylaws must be received by the Executive Director no later than November 29 to be included in the Council agenda for consideration at the spring 2018 meeting in New Orleans. Contact the Committee on Constitution and Bylaws with any questions or requests for information at bylaws@acs.org

Actions of the Board of Directors

The Board's Executive Session

At this meeting, the ACS Board of Directors considered a number of key strategic issues and responded with several actions.

The Board's Committees

The Board of Directors received and

discussed reports from its committees on the Petroleum Research Fund, Strategic Planning, Corporation Associates, Executive Compensation, Professional and Member Relations, the Society Committee on Budget and Finance, the ACS Governing Board for Publishing, and the Joint Board-Council Task Force on Governance Design.

- On the recommendation of the Committee on Professional and Member Relations, the Board voted to approve the Society's nominees for the 2018 Perkin Medal, and the 2018 National Science Board Public Service Award.
- On the recommendation of the Joint Board-Council Committee on Publications and an Editor Selection Committee, the Board voted to approve the appointment and reappointment of several editors-in-chief for ACS journals.
- On the recommendation of the Society Committee on Budget and Finance, the Board voted to approve the advance member registration fee for national meetings held in 2018 at \$475; and to authorize two new program funding requests: an ACS Online Course in Laboratory Safety, and a New Faculty Workshop Series.

The Executive Director/CEO Report

- The Executive Director/CEO and his direct reports provided updates to the Board on the activities of Chemical Abstracts Service (CAS) and the ACS Publications Division. He offered current and proposed strategies to increase membership in the Society; reported on safety initiatives, resources and security; and provided an update on the Atlantic Basin Conference on Chemistry (ABCCChem) scheduled for January 2018. As part of his report, he invited the Treasurer to brief the Board on the Enterprise Financial Systems Program (EFSP), which is unifying several financial operations for Society staff; the Financial Planning Conference in early November; and ACS Development Activities.

Other Society Business

- The Board held discussions with members of the Presidential Succession on their current activities and those planned for 2018.

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From the 2018 Chair



Hello NESACS members! What an exciting time to be a chemist here in the Northeastern Section of the American Chemical Society. 2018 promises to be an extremely exciting and jam-packed year, including the National Meeting in Boston in August. We are extremely interested in showcasing how strong our local section is and in facilitating a positive meeting experience for everyone in the wonderful city of Boston. Please reach out to a board member if you are interested in helping with this important task. At the same time, I will be chairing the section remotely for much of the first couple of months, during my sabbatical appointment in Israel, and will look forward to using all available electronic tools to facilitate this important, 21st century flexibility in the workforce. Happy to talk more about this workplace flexibility and how we can leverage it to help all of our volunteers participate more freely in the NESACS event. Remember that we as a section are only as good as our volunteers, and if you are a member who is not yet involved in NESACS, we would benefit tremendously from your participation and involvement. Reach out to me (mindy.levine@gmail.com or 516-697-9688) or contact any of the other board members for more information. Looking forward to a great year working with everyone!

Mindy Levine
Associate Professor of Chemistry
University of Rhode Island ◇

Summer Scholar Report

The Targeted Charging of Ubiquitin to E2: Developing a New Tool to Study the Ubiquitin Proteasome System

Abraham Bayer, Caitlin Hill, Rebecca Scheck, Tufts University Chemistry Department

Abstract:

Here we present initial *in vitro* work on a new tool to study the ubiquitin-proteasome system (UPS), named “The Targeted Charging of Ubiquitin to E2” or “tCubE.” This approach will allow us to uncover the complex enzymatic cascades of the UPS by connecting ubiquitinated target proteins to the activity of a specific ubiquitin-conjugating enzyme, or E2, of interest. While developing this assay in mammalian cells, we worked to purify each engineered protein construct for use in complementary *in vitro* studies, as well as mutated constructs for control experiments. These *in vitro* assays allow us to confirm that each component of tCubE is functioning as designed and can interact with native UPS enzymes.

Introduction:

The ubiquitin-proteasome system (UPS), most well-known for its role in protein degradation, is also a dynamic signaling pathway that regulates a variety of cellular processes, including cell cycle control and the immune response.^{1,2} The UPS mediates these essential functions by modifying target proteins with ubiquitin, a small 76 residue protein, through a three-enzyme cascade (E1-E2-E3).¹ Proper function of the UPS requires the coordination of hundreds of enzymes and thousands of specific protein-protein interactions. Due to its importance and complexity, malfunction of this system has been linked to multiple cancers, heart disease, and neurodegenerative disorders.² A variety of anti-cancer therapeutics that target the UPS have been approved or are in development, but these are limited to a few well characterized E3-target interactions.^{3,4} A better understanding of the protein network in the UPS would give insight into the mechanisms and limitations of current cancer therapies, as well as uncover opportunities for novel, specific drug targets.

A UPS cascade begins with an E1 enzyme activating ubiquitin in an ATP dependent process.^{1,2} Then, a ubiquitin-conjugating E2 enzyme catalyzes a transthioesterification reaction to carry ubiquitin to an E3 ligase, which recognizes a variety of both E2 and target proteins, resulting in the transfer of ubiquitin to a final substrate.^{1,2} A protein can be mono-ubiquitinated, or poly-ubiquitinated through an iterative process, leading to ubiquitin chains which can vary in both length and topology.^{1,2} In humans, there are 2 main E1s, about 40 E2s, and well over 600 known E3s, making it difficult to link final ubiquitinated products to specific E1-E2-E3 pathways.² At the center of each ubiquitination cascade, E2 enzymes are known to play an essential role in determining substrate specificity, multiplicity, and chain topology. Further study of this key point is needed to deconvolute the complexity of the UPS.^{5,6} Here, we propose a new tool that will allow us to study the UPS from the center, by targeting a tagged ubiquitin to an E2 of interest *in vivo* and monitoring the products that arise from

the activity of this E2.

Our tool, called “The Targeted Charging of Ubiquitin to E2” (tCubE), will allow us to delineate specific E2-E3-substrate interactions that lead to distinct ubiquitinated products in living cells. This approach differs from traditional methods because it focuses on the upstream components of the UPS and introduces a new point of control. As seen in Figure 1A, using the addition of a small molecule, specific E2-ubiquitin interactions can be induced *in vivo*.

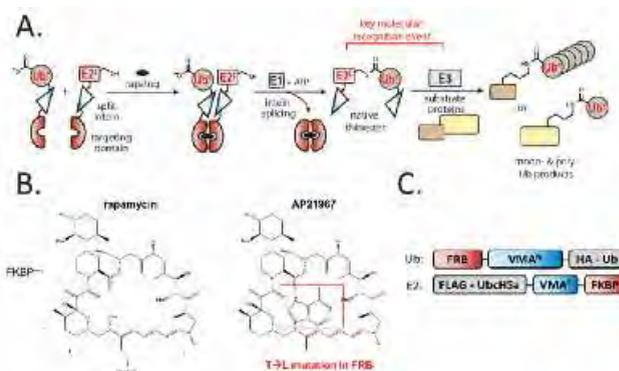


Figure 1: The Targeted Charging of Ubiquitin to E2. (A) Overview of tCubE and designed constructs interacting with the native UPS enzymes. (B) Structures and binding properties of small molecule dimerizers rapamycin and AP21967 (Rapalog). (C) Design of the protein constructs

To accomplish this, we use the conditional protein splicing method developed by Mootz *et al.* that combines complementary binding domains with self-splicing “split inteins.”^{7,8} Upon addition of the small molecule rapamycin, binding domains fused to the E2 of interest and tagged ubiquitin construct associate. This subsequently causes conditional protein splicing to remove the bulky binding domains, allowing the charged E2 to continue through the native UPS cascade to interact with endogenous E3s and transfer the tagged ubiquitin to substrate proteins.^{7,8} These products can be monitored, identified, and connected to specific E2-E3 interactions.

tCubE will overcome many drawbacks of current methods to study the UPS, which are limited by its complexity, redundancy, and the transient nature of these protein-protein interactions *in vivo*. One main advantage is the ability of tCubE to operate within cells, enabling us to monitor the UPS in a native environment. Another advantage is the power to “turn on” tCubE, through the inducible targeting step. Finally, tCubE focuses on the upstream components of UPS cascades, allowing us to visualize more complete and previously unstudied pathways. Long term, tCubE can be used to profile the activity of all known human E2’s.

Currently, we are developing tCubE in mammalian cells.

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Summer Scholar

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However, because of the complexity of the tCubE system and the UPS overall, we also created a version of this system that can be used for complementary studies *in vitro*. This will allow us to better analyze how tCubE functions in the context of a cell, and confirm that each component of tCubE is working as designed. Here we present the design, expression, and purification process of our novel ubiquitin and E2 constructs, as well as *in vitro* studies that support the initial steps of tCubE.

Results:

Construct Design & Cloning:

When designing our parent constructs (Figure 1C), we chose Ubch5a as our initial E2 to study. This enzyme has been previously studied and is known to promote ubiquitination of p53, a clinically relevant target.⁹ The split intein halves for conditional protein splicing are derived from the vacuolar ATPase subunit of *S. Cerevisiae* (VMA), and have little native affinity for each other. Their association is then reliant on the Rapamycin-dependent dimerization of complementary FRB and FKBP binding domains, fused to each half of the intein. The binding domains are positioned as exteins, so that they are removed and linked together by a peptide bond after the inteins become active.⁸⁻⁹

The ubiquitin construct contains the FRB binding domain at its N-terminus, one half of the split VMA intein, an HA epitope tag for detection, then ubiquitin, leaving the C-terminus free for conjugation. The E2 construct contains Ubch5a with a FLAG epitope tag at its N-terminus, followed by the other half of the VMA intein, and then the FKBP binding domain. To avoid any off target effects of rapamycin interacting with the human m-TOR protein (target of rapamycin), we decided to use a synthetic rapamycin derivative, rapalog, as our dimerizing agent (Figure 1B). This required a complementary mutation in the FRB domain at threonine 2098 to leucine for binding compatibility.⁸ We termed this construct rbUb (rapalog binding ubiquitin). The original ubiquitin construct could then be used as a targeting control, as the wtFRB domain cannot accommodate rapalog. We also designed intein inactive control constructs by mutating the key intein residues that catalyze the splicing reaction to alanine (Cys in VMA^N and Asn in VMA^C). Finally, we made a catalytically inactive E2 variant, replacing the cysteine used for thioester linkage to ubiquitin with alanine.

We cloned the two parent constructs into the vector pDEST17TM for bacterial protein expression using GatewayTM cloning. This vector contains a T7 promoter for IPTG induction and an N-terminal (His)₆ tag for affinity purification by Nickel column. Each mutant construct was made using PCR-based mutagenesis with the parent constructs as templates.

Protein Expression:

To start, we performed small scale pilot expressions in BL21(DE3) E. Coli cells. The initial attempts showed expression of each parent construct at the correct molecular weight,

but almost exclusively in the total lysate rather than soluble fraction, as seen in Figure 2A. To optimize for expression of soluble proteins, first we screened a variety of cell lines for higher levels of each protein in the soluble fraction. Cells with increased expression of tRNAs used for difficult codons (BL21(DE3) RIL), with a different induction system (BL21 A.I.), or even enhanced expression systems (C41(DE3)), all resulted in high levels of insoluble protein for both constructs.

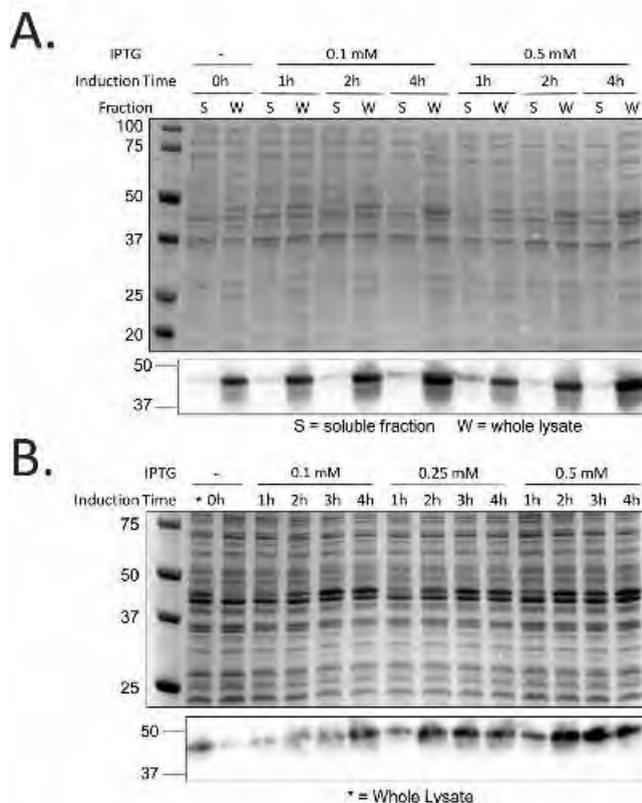


Figure 2: Protein Expression (A) Expression of insoluble rbUb in BL21(DE3) cells. Expected MW: 47.5 kDa. Top: Coomassie stained gel, Bottom: Anti-HA WB. (B) Expression of rbUb in the soluble fractions of BL21(DE3) PLysS cells, Top: Coomassie stained gel, Bottom: Anti-HA WB.]

We noticed that these experiments showed very high levels of basal protein expression, which could result in subsequently overexpressed protein after induction remaining insoluble as well. This led us to try BL21(DE3) pLysS cells, which co-express T7 lysozyme to inhibit leaky expression of the T7 RNA polymerase. This resulted in much higher levels of soluble protein (Figure 2B). Using this cell line, the induction was optimized for the highest level of soluble protein. For both constructs, inducing at 16-18 °C improved solubility when compared to induction at room temperature (25°C) or 37 °C. We varied IPTG concentrations, finding 0.5 mM IPTG showing the greatest expression for both constructs. Finally, we tried induction times ranging from 1 hour to overnight. We found that overexpression peaked between 2 and 4 hours, then declined with longer expression times.

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Summer Scholar

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Protein Purification:

Using the N-terminal (His)₆ tag added by pDEST17™, we aimed to purify each protein construct using immobilized metal affinity chromatography (IMAC).

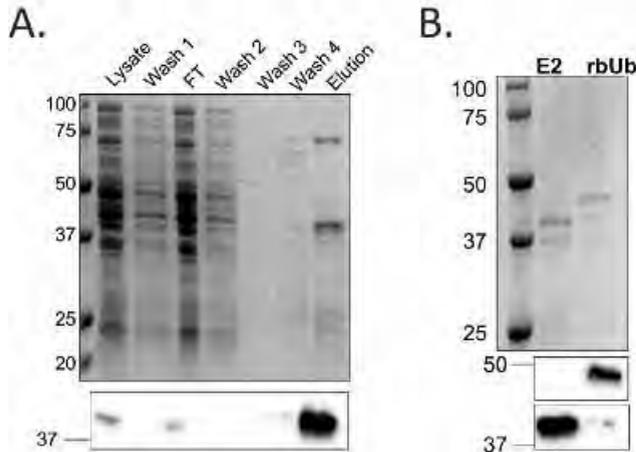


Figure 3: Protein Purification (A) Analysis of early protein purification of E2 construct by gravity flow IMAC. Expected MW: 40 kDa. Top: coomassie stained gel, Bottom: Anti-FLAG WB. (B) Purified protein constructs during optimization process. Top: coomassie stained gel, Middle: Anti-HA WB, Bottom: Anti-FLAG WB]

We attempted purification using two nickel affinity column methods: a gravity flow column and a column for fast protein liquid chromatography (FPLC). Initial attempts showed the gravity flow nickel column to have purer protein and higher yields. Continuing with this method, we optimized the lysis, wash, and elution conditions of the purification to remove as many contaminants as possible without sacrificing protein yield (Figure 3). High levels of protein have been continuously seen in the flow through from each affinity column, leading us to believe that the His tag is not properly exposed in the native protein structures of our protein constructs. While we obtained pure enough samples to begin *in vitro* work and have identified nonspecific bands, we are continually working to optimize the purification for purer product.

Activation of rbUb by E1 *In Vitro*:

The first step of a UPS cascade is the ATP-dependent activation of ubiquitin by an E1 enzyme, forming a reducible thioester linkage between an active site cysteine and the C-terminus of ubiquitin. Before testing the targeted charging steps of tCUBE, we wanted to ensure that the fused intein and binding domain would not interfere with the recognition or activation of rbUb. Ube1 is the ubiquitin-activating enzyme for most UPS cascades in humans, so we purchased active, His-tagged, recombinant Ube1 to test with rbUb.

The active E1~rbUb complex can be observed as the appearance of a high molecular weight band only when ATP is included in the reaction and the gel is run under non-reducing

conditions (Figure 4). While the band appears higher than the molecular weight that we expected, both the dependence on ATP and non-reducing conditions combined with the observed decrease in unreacted E1 and rbUb signal support our identification of E1~rbUb. We predict that a different gel will better resolve this high molecular weight protein complex.

Wild type ubiquitin has been shown to be fully activated in 30 minutes in similar experiments, so we included a longer time point to see if the added domains slowed this reaction.¹⁰ Interestingly, we observed the most active E1~rbUb signal after 30 minutes, decreasing slightly in two hours. Further experiments will be done to test the competition between wild type ubiquitin and rbUb in E1 activation, as both will be present in the cellular environment, and background ubiquitin transfer can potentially interfere with tCUBE.

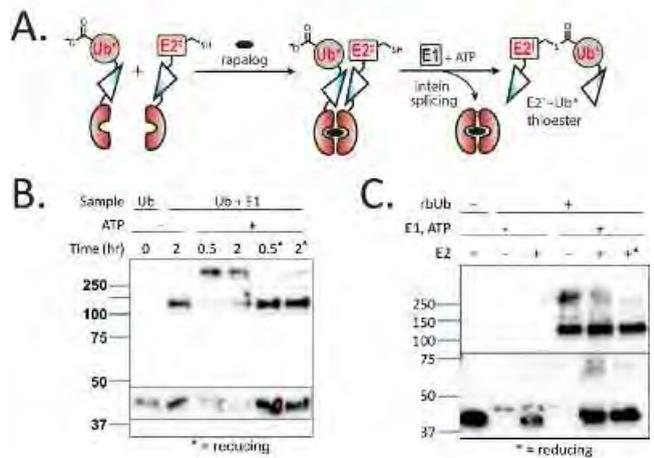


Figure 4: Initial *In Vitro* work (A) Overview of initial steps of the UPS and tCUBE. (B) rbUb activation by E1. Anti-His tag WB, with higher exposure inlay. High molecular weight bands, in addition to a decrease in E1 (121 kDa) and rbUb (47.5 kDa) are observed with ATP under non-reducing conditions. (C) rbUb transfer to E2. Anti-His tag WB, with higher exposure inlay. Spliced E2~rbUb appears under non-reducing conditions at 60 kDa.]

Transfer of rbUb to E2 *in vitro*:

After activating ubiquitin, E1 enzymes recognize multiple E2 enzymes and transfer ubiquitin to another active site cysteine through a transthioesterification reaction. To assess this step, we included our E2 construct with the activated E1~rbUb complex. This also allows for the analysis of the splicing functionality induced by proximity, rather than Rapalog treatment. We observed a decrease in the high molecular weight signal from the E1 activation, as well as the appearance of a band at the molecular weight of the post-splicing rbUb~E2 complex under only non-reducing conditions. While the FRB-FKBP extein fragment was not observed, additional experiments are underway to confirm the identity of the rbUb~E2 complex and test the splicing functionality by Rapalog treatment.

Conclusion:

Here we present the design, expression, and purification, and initial *in vitro* testing of protein constructs used tCUBE, a new

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tool to study the UPS. We have screened expression conditions to optimize expression, and used IMAC to purify our ubiquitin and E2 constructs. Initial results have showed that our rbUb can be activated by recombinant E1, and can be transferred to our E2 construct. Moving forward, additional *in vitro* experiments will be run to demonstrate conditional protein splicing by Rapalog, transfer to E3 and target proteins, and finally competition between rbUb and wtUb. We will also express and purify the mutated constructs for additional control experiments.

By confirming and characterizing the functionality of each step of tCUBE *in vitro*, we will be better able to interpret and support our results *in vivo*. The development of tCUBE will provide us with a powerful new tool to study ubiquitination cascades in living cells. By connecting ubiquitinated products with the activity of specific E2 enzymes, we will better understand the regulation of vital cellular processes, as well as uncover new opportunities for drug development targeting many cancers and chronic diseases.

Methods:

Cloning:

All plasmids were prepared using Gateway™ cloning, and listed protocols. Entry clones were generated in the donor pDONR221 from either attB flanked PCR fragments or expression clones. attB recombination sites were added by PCR using specially designed primers and Phusion® DNA polymerase. Expression clones were generated in the destination vector pDEST17.

PCR Site Directed Mutagenesis:

All mutations were created in expression clones using the QuikChange II Site-Directed Mutagenesis Kit™ according to provided protocols, and primers designed according to given guidelines.

Protein Expression:

50 ng of plasmid was transformed into each cell line according to the given protocol, and plated on agar plates with Ampicillin for all cell lines except for B121(DE3)PLysS, which required Ampicillin & Chloroamphenicol. 5 mL cultures for pilot expressions and 1L cultures for full-scale expressions were grown in LB with the appropriate antibiotics. Cultures were grown until an OD of 0.5 – 0.6, then induced with IPTG and moved to the desired induction temperature. All cultures were spun down and frozen at -20 °C until lysis. Cells pellets were thawed on ice, resuspended in 50 mM phosphate buffer with 150 mM NaCl, 1 mM DTT, ½ Pierce™ Protease Inhibitor Tablet, and Benzonase™ Nuclease. Samples were lysed by 10 minutes sonication (15s on, 15s off).

Gravity Flow Affinity Purification:

Bacterial pellets were lysed as described above and cleared by centrifugation. 20 mM imidazole was added directly to the supernatant. 10 mL of binding buffer (20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.5) was used to

equilibrate the column (GE Healthcare His GraviTrap™) then the lysate was applied to the column. 10 x 10 mL wash buffer (binding buffer with 65 mM imidazole) was used to wash the column, then 2.5 mL elution buffer (binding buffer with 500 mM imidazole) was used to elute bound protein. A GE Healthcare Nap25™ column was equilibrated with 5 x 5 mL 50 mM Tris-HCl, 300 mM NaCl, pH 7.5. The eluate from the His GraviTrap column was applied to the Nap25 column. 3.5 mL equilibration buffer was used to elute the purified protein, which was quantified by Bradford Assay, 5% glycerol was added, then samples were aliquoted and flash frozen.

In Vitro Assays:

Protein samples were thawed on ice. E1 activation experiments were run in 50 mM Tris-HCl with 300 mM NaCl, using 0.25 μM E1, 2-4 μM rbUb, 0.5 mM DTT, 5 mM ATP, 10 mM MgCl₂ and incubated at 37 °C for 30 – 120 minutes. E2 transfer experiments were run with the above conditions plus 2 μM E2. Reactions were quenched with SDS-PAGE loading dye, with or without DTT.

SDS-PAGE & Western Blotting:

Samples were boiled at 95°C for 5 minutes, then centrifuged for 2 minutes. Samples were loaded onto BioRad Mini-Protein TGX™ any kD gels, and run for 35 minutes at 200 V. For Coomassie staining, gels were exposed to Coomassie blue for 1 hour to overnight, then destained until minimal background was left. These were imaged on a ChemiDoc XRS+ system. For western blotting, gels were transferred onto PVDF membranes at 4 °C for 1 hour at 100 V. Membranes were blocked in 5% milk in TBST, then all primary antibodies were added to a final concentration of 1:1000 and incubated at 4 °C overnight. Membranes were washed 3 x 5 min with TBST. Secondary antibody, either anti-Mouse IgG or anti-Rabbit IgG was added to a final concentration of 1:2000 and incubated at room temperature for 1-2 hours. Membranes were washed 3 x 5 min with TBST, then exposed to BioRad Clarity Max Western ECL Blotting Substrates™, then imaged on a ChemiDoc XRS+ system.

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Photos from the November Meeting

By Morton Z. Hoffman



Sandy Hoffman (at left) with Anna Singer (NESACS Administrative Coordinator).



The 2017 U.S. Chemistry Olympiad Team: (l-r) Brendan Yap, Steven Liu, Harrison Wang, Joshua Park at the reception.



Displaying their NESACS certificates of recognition: (l-r) Joshua Park, Harrison Wang, Steven Liu, Brendan Yap.



Joshua Park with Janice Compton, his chemistry teacher at Lexington High School.



Timothy Haeg (at left) and Tony Hill.



Robert Carter (University of Massachusetts Boston) speaks about his colleague, Marietta Schwartz.

Photos from the November Meeting

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Celebrating the Hill Award: (l-r) Ruth Tanner (Acting Chair, NESACS Education Committee), Lee Johnson (NESACS Chair), Timothy Haeg (brother of the Awardee, Marietta Schwartz), Dorothy Phillips (Chair, NESACS Awards Committee), Michael Filosa (Editor, The Nucleus), Michael Singer (NESACS Secretary).



(l-r) Chris Bauer (University of New Hampshire), Marcy Towns (Purdue University), Melanie Cooper (Michigan State University), Tom Gilbert (Northeastern University).



Ken Mattes (NESACS Archivist) presents the history of the James Flack Norris Award.



Melanie Cooper (Michigan State University) introduces the Norris Awardee.



Marcy Towns (Purdue University) gives the Norris Award Address, "Improving Student Hands-on Laboratory Skills Through Digital Badging."



Presentation of the James Flack Norris Award for Outstanding Achievement in the Teaching of Chemistry: (l-r) Mark Tebbe (Chair, NESACS Norris Award Committee), Marcy Towns (Purdue University), Lee Johnson (NESACS Chair).



Norris Awardees: (l-r) Morton Hoffman (Boston University), 2005; Marcy Towns (Purdue University), 2017; Melanie Cooper (Michigan State University), 2013.

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ACS Meeting 2017

Continued from page 7

- The Board also held a discussion with officers and members of the board of directors of the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) on what governance participation might look like at the organizations' annual meetings, possible meetings between ACS and NOBCChE at the ACS Leadership Institute, dual membership between both organizations, strategic alliances of student chapters at the local section and regional levels, and ACS Board participation at NOBCChE Annual Conferences.

The Board's Regular Session

The Board held a well-attended regular session which featured a discussion on the role ACS and its members play in advocating for adoption of important public policy priorities to foster scientific advancement and innovation. Mr. Glenn Ruskin, Director, External Affairs & Communications in the Office of the Secretary and General Counsel, and Mr. Anthony Pitagno, Director of Government Affairs and Outreach, External Affairs & Communications, spoke about the critically important role federal investment in basic research plays in driving U.S. innovation, job creation and economic growth. A question and answer session followed the presentation, first with the presenters, and then with the Board for general concerns and comments.

Prior to the presentation, members of the presidential succession and the Executive Director and CEO offered brief reports on their activities. The officers provided more extensive reports on their activities and/or future plans as part of their written and oral reports to the Council.

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Biography

Continued from page 5

Sloan Foundation Fellow, National Science Foundation CAREER Fellow, and the ACS-ExxonMobil Faculty Award in Solid State Chemistry. ◇

Summer Scholar

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January 4

Prof. Noah Burns (Stanford)
MIT, Room 6-120
4:00 pm

January 17

Prof. Kuo-Wei Huang, (King Abdullah U of Science and Technology)
Boston College, Merkert 130
4:00 pm

January 18

Prof. Richard Anderson (University of Wisconsin)
WPI, Gateway Park Room 1002
12:00 noon

January 22

Dr. Lisa Olshansky (UCal-Irvine)
"Proton-Coupled Electron Transfer in Natural and Artificial Metalloproteins"
MIT, Room 4-270
4:00 pm

Prof. Bjoern Reinhard (Boston University)
Boston College, Merkert 130
4:00 pm

January 25

Dr. Marco A. Allodi (U. of Chicago)
"Visualizing Chemical Dynamics Across Nanoscale Interfaces"
MIT, Room 6-120
2:30 pm

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Announcing the Vote

January 11, 2018

The NESACS Board of Directors invites all members of the American Chemical Society from the Northeastern Section (NESACS) to attend our 2017 NESACS Annual Meeting at Nova Biomedical in Waltham, MA on January 11th in order to discuss and vote on the 2017 Revised Bylaws for the Northeastern Section of the American Chemical Society. We have undertaken the revision of our bylaws to enable electronic voting, change some of the vocabulary used, modernize, and comply with the recommendations from the Committee on Constitution and Bylaws from the American Chemical Society.

Please see the November 2017 issue of *The Nucleus* and refer to the website (www.nesacs.org) for the proposed version of the NESACS Bylaws (2017) for consideration as well as our past version of our Constitution and Bylaws (1998).

If you have any questions, please contact Leland Johnson (chemlee@yahoo.com). ◇