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155 South Grant Street

West Lafayette, IN 47907 -2114

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RPPR Final Report
as of 23-Apr-2021

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Proposal Number: 68392CHDRP **Agreement Number: W911NF-16-2-0020**
INVESTIGATOR(S):

Name: R. Graham Cooks
Email: cooks@purdue.edu
Phone Number: 7654945263
Principal: Y

Organization: **Purdue University**
Address: 155 South Grant Street, West Lafayette, IN 479072114
Country: USA
DUNS Number: 072051394 EIN: 356002041

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Final Report for Period Beginning 18-Jan-2016 and Ending 17-Apr-2021
Title: Analytic-Directed Multi-scale Synthesis System
Begin Performance Period: 18-Jan-2016 **End Performance Period:** 17-Apr-2021
Report Term: 0-Other

Submitted By: R. Cooks Email: cooks@purdue.edu
Phone: (765) 494-5263

STEM Degrees: 7 **STEM Participants:** 36

Major Goals: a. Background: The initial schedule of tasks and milestones as contracted organized the project into three phases, with each phase having two subdivisions (A and B). Phase 1A was completed at month 9, phase 1B at month 18; phase 2A at month 26, phase 2B at month 34, phase 3A at month 41 and phase 3B at month 48. At the end of Phase 1A, following guidance provided by DARPA at the review meeting of August 2016, as well as subsequent internal brainstorming sessions, and interactions with the DARPA program management and with our outside Advisory Committee, a revised list of tasks and milestones was approved. This updated plan reorganized our tasks and milestones to shift effort from the analytic-directed multi-scale synthesis system to a rapid automated reaction screening system, including a commercialization effort. At the end of phase 3B, a 9-month NCE was granted based on the remaining tasks and milestones described below and a second 6-month NCE was granted due to the disruption of experiments caused by the Covid pandemic. Below, due to text limitations, we report the completion % for the tasks and milestones along with a short update on progress over the last project year. Two new tasks, namely enzymatic bioassays and small-scale synthesis and product collection, were added as informal parts of the NCE.

b. Work Accomplished: This section describes the tasks that were contracted, as well as the two additional tasks, not contracted but undertaken during the NCE period.

Original Task 4. Systems: Design, Build, Test, and Operate
4.4. Develop controlled atmosphere for array deposition and data collection to enable air and water sensitive chemistry (100%): The Make It System was used to screen for twelve different air- and water-sensitive palladium catalysts along with 24 copper reagents with 0.5% and 5% loadings in the Sonogashira coupling reaction as part of the effort to optimize anti-cancer alkynylnaphthyridine agent, HSN-608. Results are being prepared for publication.

4.5. Implement new droplet reaction types such as electrochemistry, and heterogeneous reactions (100%): Studies on new reaction types focused on heterogeneous reactions not on electrochemistry. Solid/solution interfacial acceleration was systematically studied using glass nanoparticles using the Purdue Make It system. Accelerated reactions at the glass interface included Knoevenagel condensation, imine formation, elimination of hydrogen halide, ester hydrolysis of acetylcholine and phospholipid cleavage, as well as the oxidation of glutathione. Preliminary results have been published (Y. Li, et al. Angew. Chem. 10.1002/anie.202014613).

4.6. Implement multistep droplet reactions (60%): Preparative reactive extractive electrospray (EESI) for both multistep online and offline synthesis was reported for a two-step atropine synthesis (Falcone, et al. Analyst 10.1039/c7an00622e). Multistep reactions are not conveniently performed, in our system, hence the low %

completion. An alternative, last stage functionalization, begun in Feb. 2021, shows promise.
Original Task 5. Reaction Automation

5.3. Store data in approved data repository (100%): Collected data are stored in the Purdue University Research Repository (PURR) under the project DARPA Make-It: Analytical Multi-Scale Synthesis System (https://purr.purdue.edu/projects/makeit/). All data is classified according to (i) year of collection and (ii) DESI plate information. Data in the repository currently totals 1 TB.

5.6. Record MS/MS and confirm product identity automatically (50%): In-house prepared chemical libraries (ca. 3,000 compounds screened) were added to ca. 6,000 publicly available spectra and used to train an encoder/decoder recurrent neural network (RNN) capable of translating spectra to molecules. The classifier is either a feedforward neural network or a random forest, both model types yielding a cross-validated accuracy of 75%.

5.8. Develop chemical intelligence to ID intermediates/byproducts by MS/MS (80%): We have used and integrated both the RNN (MS/MS spectrum ? molecule) as well as the classifiers (molecule ? MS/MS spectrum) described in section 5.6, to identify intermediates and byproducts of reactions screened with the system, particularly in the case of reductive amination and Sonogashira coupling. This project and 5.6 suffered from the departure of a key person, post-doc Andy Koswara, hence the degree of completion.

Milestone: Demonstrate identification of knowns and unknowns from a complex mixture (60%): The prediction of the molecular structure of knowns and unknowns is described in 5.6 and 5.8, but was implemented for a limited set of chemistries hence the 60% completion. Publications are in preparation.

Original Task 6. Automation of System
6.1. Develop and utilize laboratory information management system (100%) A management system named HELIOS Dashboard (Figure 3) was built to capture experimental information and store it locally before transfer to the PURR repository.

6.4. Integrate fluid handling software with data collection and data analysis software (100%): The HELIOS Dashboard has a reaction configuration and Biomek intelligence module that assists in the preparation of DESI-MS plates. The application takes as inputs the reagents, catalysts, solvents, and replicates. The user enters molecular weights of reagents and expected products. The application searches through a library of prepared i7 methods to locate a method suitable for the experiment. The tool outputs the name of the appropriate method file along with masses/volumes of each chemical to be added to the reservoirs initially. The application also generates the CHRIS input file listing appropriate m/z values to look for in each spot.

6.5. Integrate data system to allow remote control (100%): The system can be remotely controlled through the HELIOS Dashboard.

Deliverable: Engineering diagram for automated synthesis and testing system (100%): The engineering diagram is described at the Purdue Make It Brochure (uploaded 2019 report) and in the software automation map (Attached Figure 3). All software developed under the Make It effort has been copyrighted by the Purdue Research Foundation.

Deliverable: Software for communication protocols between fluid handling, data collection, and data analysis (100%): This has been accomplished by the software integration accomplished using the HELIOS Dashboard.

Milestone: Demonstration of integration of analytics (100%): The current HELIOS Dashboard fully integrates, automates and allows remote control of the system.

Original Task 8. Reaction Scaling
8.3. Validate multistep droplet reactions with multistep microfluidics (100%): The multistep synthesis of Lomustine by microfluidics using the Make It system to optimized the synthesis route has generated a publication and a patent. The optimized synthesis of another lead compound the Purdue anti-cancer agent HSN-608 has also been published.

Original Task 11. Project Administration
11.1 Management of budget (100%): As planned.

11.2 Compilation of technical reports (100%): As planned.

Additional Tasks
NCE New Task 1. Develop and implement the capability of performing enzymatic bioassays: The Make It system is suitable for label-free precise studies of enzyme kinetics directly from the bioassay matrix with an effective analysis time of 0.3 s per sample. A systematic study of the acetylcholinesterase activity is published (N. M. Morato, et al. Angew. Chem. 10.1002/anie.202009598), and other enzymatic assays have also been explored but not yet published.

NCE New Task 2. Small scale synthesis and product collection: Design of a system for small scale synthesis has been completed but not yet tested. The system performs simultaneous collection and MS analysis on a spray plume to allow 'made-to-measure' bioassays of target compounds prepared in ng amounts.

Accomplishments: The original aim of this project was to create an automated system to identify optimum routes to particular target molecules by testing in silico predicted synthetic routes through small scale reactions performed with on-line reaction monitoring.
Feedback of the analytical information to the reactor and small-scale preparation of products purified by on-line crystallization was also proposed. A group of five investigators (Graham Cooks, Chemistry; Ananth Grama, Computer Science; David Thompson, Chemistry, Zoltan Nagy, Chem. Eng.; Eric Barker, Pharmacy) undertook this task.
After Phase 1A (9 months) and in consultation with the new program manager, Anne Fischer, the tasks were modified, some being eliminated, while others were deepened and extended.

The automated prediction of synthetic routes was eliminated as was feedback of analytical information to the reactors. Great emphasis was placed on high throughput by increasing the speed of reaction and automating reaction and product analysis, reaching the speed of 10,000 reactions screened/h. This was achieved by high throughput desorption electrospray ionization (DESI) mass spectrometry using 50nL of reaction solution.

Tests of DESI-MS predictions of optimum reaction conditions in flow reactions was emphasized. The variables available in DESI reactions (stoichiometry, acid/base/catalyst, solvent) were extended to include temperature by performing milliscale reactions in closed vessels while using DESI only for analysis.

Chemical engineering task of on-line crystallization was continued, a reactive DESI system (choice of spray solvents) was built and characterized. Machine learning approaches have been developed to connect mass spectra to product identity and substantial effort has been dedicated to the automation of the MS/MS analysis system. In the middle stages of the project, considerable success was achieved in developing a very high speed automated synthesis and analysis system, operating at beyond the state-of-the-art.

Systematic studies were performed on reactions of a range of types (some twenty different reactions including substitutions, eliminations, condensations, C-C couplings and many others) to characterize product and by-product formation under a variety of conditions. On the basis of this background information, considerable success was also achieved in identifying by DESI, optimum conditions for subsequent small-scale flow synthesis in the case of two drug candidates (lomustine and alkynylnaphthyridine agent, HSN-608).

In the course of this work, the degree of automation of the system was increased to allow remote operation of the system, and a dashboard was implemented to integrate and control subsystems including physical aspects (e.g. plate preparation by robotic pipetter, plate placement on the DESI), data acquisition and interpretation (e.g. heat maps relating products of interest to conditions and reagents) and consequent actions (e.g. MS/MS data to confirm product identity).

These efforts clearly indicate the potential of DESI-MS HT screening to increase the efficiency of drug development through determination of optimum synthetic routes for lead compounds in a fraction of the time and effort normally needed.

In summary, the current system consists of a tailored fluid handling system with a DESI plate shuttle, a robotic arm, two mass spectrometers to perform structural analysis (ion trap) and high mass resolution measurements (QTOF), a 16-channel solvent delivery system and a DESI-MS imaging source which are totally integrated and connected to dedicated software for data processing and interpretation.

In the last stages of the project emphasis was placed on making the community aware of this technology through in-person and on-line lectures and demonstrations of the system, which has been reported in 27 peer-reviewed publications, and is the focus of 7 Ph. D theses and 10 patent applications.

Also, over a dozen graduate students have been trained to perform experiments using the system. Applications of the system to address unusual reactivity were also made. For example, the catalytic reactivity of glass was characterized with a wide range of reagents. Specifically, the Katritzky reaction in bulk solution at room temperature was accelerated by more than two orders of magnitude upon the addition of glass particles. The rate increased linearly with the glass surface area. The reaction acceleration by glass particles involves air/solution phase acceleration (already well known in microdroplets) as well as solid/solution phase, where such acceleration appears to be a new phenomenon. Glass nanoparticles act as a "green" heterogeneous catalyst since they participate as a base in the deprotonation step and are recovered unchanged from the reaction mixture.

Significant attention also went to on-line high throughput bioanalysis, particularly the analysis of enzyme activity, for which we reported characterization of the kinetic parameters of the acetylcholinesterase reaction, inhibitor screening, and inhibition-reactivation assays. Remarkable performance was recorded in terms of speed (0.3 sec/sample), quantitative precision (RSD <10%), and accuracy as judged by agreement with more cumbersome literature methods. Expansion of the Make It platform to other enzymatic systems and further (e.g. receptor binding) assays is continuing. The incorporation of this label-free enzymology capability into an already established platform for HT organic reaction screening opens the door to the implementation of an integrated in situ screening-synthesis-bioassay DESI-based automated system with important implications in drug discovery and design.

Overall, the great advantages of the DESI-MS system are the high speed of analysis 2 Hz (two reaction mixtures per second) and the high precision (typically 10% RSD) in a completely automated workflow, equipped with tailored software for rapid data processing and analysis, and available for complex biological mixtures (that include enzyme, substrate and buffer). Other methods of high-speed analysis bioanalysis (acoustic levitation, MALDI) require sample manipulation and /or dilution by large factors.

A timeline and summary of key publications is included as Report Attachment Figure 1. The medicinal chemistry reactions explored using the Make It System are listed at the table at Figure 2. The schematics of the system's automation is displayed at Figure 3.

Figure captions: Figure 1. Summary of the Purdue DARPA Make It System Tasks, Timeline and Publications. Figure 2. Some medicinal chemistries explored using the Purdue DARPA Make It system with respective publications. Figure 3. Automation schematic of the Purdue DARPA Make It system, showing the integration of data acquisition/visualization program CHRIS and the HELIOS Dashboard.

Training Opportunities: • 2021: One post-doctoral fellow and six additional graduate students were trained on the operation of the Make-It system, with particular emphasis on the utilization of the automated fluidic handling system for the high throughput preparation of microtiter reaction plates. Several publications have been prepared by these students. Several conference presentations have been given.

• 2020: Nine graduate students were trained on the operation of the Make-It system. All of them started research projects using the platform with constant one-on-one mentoring opportunities by experienced peers. Three graduate students were trained on flow synthesis. Four graduate students were trained on the operation of HPLC-MS system for purity analysis of flow products. Three graduate students were trained on the off-line and online operation of the Eveflow microfluidics system for solvent delivery. Training provided skills that all students currently use in daily activities. A number of publications and conference presentations were prepared involving the students.

• 2019: Four undergraduate students worked with four graduate students and one post-doctoral fellow in laboratory work for the project. They were dedicated to flow and batch chemistry, performing halogenation reactions and amide coupling reactions in flow, carried out continuous flow synthesis of Sonogashira coupling. Students also participated in the design of scaled-up accelerated microdroplet synthesis devices and studied accelerated reactions in microdroplets and aerated solutions using the Katritzky reaction as a model. Two graduate students were trained and assigned as main personnel for the operation of the high throughput platform. A number of publications were prepared involving the students. Several conference presentations were also given. Graham Cooks taught a course as the International Mass Spectrometry School, Sitges, Spain, in September 2019, including a module on High Throughput Chemical Reaction Screening.

• 2018: The project involved 7 undergraduate students who have gained deep knowledge of the Make-It platform, acquired research experience and made essential contributions to the project. Four of the undergraduates majored in Computer Science. They were involved in implementing algorithms to detect the locations of rhodamine spots, perform by-product searches, and attempt MS/MS automatic data interpretation. They also helped to set-up the in-house script routine which includes creating sequence files, sequence submission and DESI station position log file. For the CHRIS software, the undergraduates supported the improved algorithm for identifying the next row of rhodamine to locate the spots between the current row and the newly identified row; and improved the by-product search script to show the mass difference between the by-product and the starting materials. The remaining three undergraduates, majoring in Chemistry, worked in the laboratory to support graduate students in flow synthesis, robot method development and setting up of bulk microtiter and DESI experiments. Five graduate students assigned to the project made progress on the project's research, which is strongly thesis related. Three post-doctoral fellows made essential contributions in leading sections of the laboratory work. Constant training opportunities for both undergraduate and graduate students existed during this period. A number of publications and conference presentations involved the students.

• 2017: The project involved nine undergraduate students who gained valuable experience in research and made essential contributions to the project. Eight graduate students assigned to the project made progress on research, being their work strongly thesis related. Two post-doctoral fellows made essential contributions in leading sections of the laboratory work. Constant training opportunities for both undergraduate and graduate students existed during this period. A number of publications were prepared involving the students.

• 2016: The project involved nine undergraduate students working under supervision of graduate student mentors as well as post-doctoral personnel.

• 2016 – 2019 Center for Analytical Instrumentation Development (CAID) Workshop
This lab-based learning experience exposed a group of more than 50 undergraduate and graduate students and some industrial scientists to new analytical instrumentation developed in the Department of Chemistry at Purdue University, including the high throughput DESI experiments.

The CAID workshop (http://www.purdue.edu/discoverypark/caid/) brings together chemists, physicists, engineers, biologists and physicians from multiple departments at Purdue University, other universities in the region and several companies. This annual workshop follows the mission of the Center in developing innovative "machine-tools of science" that enable discoveries across a broad spectrum of life science. In 2020, due to the COVID emergency, the workshop did not take place, however a hybrid (in person and online) format is already being planned for November 2021.

Results Dissemination: In the reporting period mid-2017 through 2018, the Purdue Make-It platform was part of all visitor tours performed daily at the Bindley Bioscience Center (BBC).

Visitors included Purdue Alumni, visiting and new faculty, campus events participants, and also high schoolers. From February to July 2018, there were 63 tours with a total number of 589 visitors to the BBC (data supplied by Kayla Burke, Discovery Park Administrative Assistant).

The system was also demonstrated to 30 students from the Purdue American Institute of Chemical Engineers participating at the North Central Regional Conference. Besides the BBC tours, hands-on demos were made to over 50 CAID (Center for Analytical Instrumentation Development) Workshop participants, as well as to individual visitors, including Dr. Andreas Kaerner (Research Advisor at Eli Lilly), Dr. Shane Tichy (R&D Program Manager at Agilent Technologies), Prof. Nadja Cech (University of North Carolina at Greensboro), Dr. Tom Verhoeven (Purdue Presidential Fellow for Pharmaceuticals Development and Partnerships), Dr. Thomas Sors (Assistant Director, Purdue Institute of Inflammation, Immunology and Infectious Disease), Dr. David Fraley (Georgetown College), and Prof. Junichiro Yamaguchi, Waseda (University of Tokyo, Japan).
In 2019 the Make It system was moved to a dedicated laboratory in the Hall for Discovery and Learning Research. After moving and until early 2020, demos were carried out for diverse companies and researchers, including Dr. Michael T. Rudd (HCV NS3/4a Project, Team Discovery Chemistry, Merck Research Laboratories), Prof. Yousong Ding (University of Florida), Prof. Mike Organ (University of Ottawa), Dr. Jillian True (Indiana University), Prof. Eugene Nikolaev (Skoltech, Moscow, Russia), Prof. Hao Chen (NJIT), Prof. Zheng Ouyang and Prof. Yu Xia (Tsinghua University), Prof. Valerie Gabelica (Inserm, Bordeaux, France), Prof. Jonathan Sweedler (University of Illinois), Prof. Jing-Ke Wang (MIT), Prof. Evan Williams (Berkeley), Mr. Gordon Anderson (GAA Custom Engineering, LLC), Prof. Kerri Pratt (University of Michigan), Dr. Mike Morris (Waters Corporation), Dr. Kevin Wilson (LBNL), Prof. Steve Soper (University of Kansas), Prof. Robert Kennedy (University of Michigan), Dr. Justin Wiseman (Prosolia, Inc.), Dr. Yong Liu and 4 Executives from Merck & Co., Dr. Ben Gaston (Indiana University School of Medicine), Dr. Katherine Hollywood (University of Manchester, UK) and Prof. Roy Goodacre (University of Liverpool, UK), Dr. Steve Pringle (Waters Corporation), Prof. Amir Hoveyda, (Boston College), Dr. Michael McGuire (GSK), Dr. Greg Roman (Waters Corporation), and Harsha Gunawardena (Janssen – J&J) and to prospective graduate students of Purdue's Department of Chemistry during recruitment events in 2019 and 2020. We also acquired test data for Dr. John Masucci (Janssen – J&J) and Dr. Stefan Bauer (Zymergen).

For most of 2020 and 2021 demos were severely restricted due to COVID. In person visitors included Dr. Alexander Godfrey, Dr. G. Sitta Sittampalam and Dr. Marc Ferrer (NCATS, NIH), and Prof. Mingji Dai (Purdue University). Virtual live demos have also been ongoing during this period, with virtual visitors including: Dr. Brian P. Mayer (Forensic Science Center, LLNL), Prof. Arash Zarrine-Afsar (University of Toronto, Canada), Prof. Dame Carol Robinson (University of Oxford), and Dr. Brian Tackett (NRC Postdoctoral Fellow, NIST; Faculty Candidate, Purdue University). A virtual live demo was also presented during the 2021 online graduate recruitment event organized by the Department of Chemistry at Purdue University.

Selected presentations:

- R. Graham Cooks, "Fast-Throughput Strategies for Lipid and Chemical Reaction Screening by Non-Chromatographic Methods" Minnesota Mass Spectrometry Discussion Group, MN, March 11, 2021 (with Christina Ferreira).

- Yangjie Li, Tsdale F. Mehari, Zhenwei Wei, Yong Liu, R. Graham Cooks. "Reaction Acceleration at Solid/Solution and Air/Solution Interfaces: Accelerated Katritzky Reaction by Glass Particulates". Pittcon, Chicago, IL, March 2021.

- R. Graham Cooks, "High throughput Reaction Monitoring and Small-Scale Synthesis" Pittcon, Process Monitoring Symposium, March 8, 2021 (with Nicolás M. Morato).

- R. Graham Cooks, "High throughput screening, synthesis and enzymatic assay system: clinical relevance" Association for Mass Spectrometry and Advances in the Clinical Lab (MSACL) Connect, February 23, 2021 (with Nicolás M. Morato).

- Nicolás M. Morato,. "The Purdue Make-It System: high Throughput DESI-MS for Synthesis, Screening and Bioanalysis" Waters Virtual MS Application Day, February 18, 2021.

- R. Graham Cooks, “High-throughput Mass Spectrometry in Process Development: Screening, Quantitation and Product Isolation” Novartis, Basel, Switzerland, December 11, 2020.

- R. Graham Cooks, Plenary Lecture: “Mass Spectrometers for High-Throughput Analysis, BioAssays and Synthesis” Annual Conference Instrument Chinese Mass Spectrometry, Chinese American Society for Mass Spectrometry, December 7, 2020.

- D. Thompson, Drug Discovery, Development & Lead Optimization 2020, Lecture & Session Chair, Oct 2020.

- D. Thompson, 2020 Nat’l Inst. for Pharmaceutical Tech. & Education Research Conf., Lecture, December 2020.

- R. Graham Cooks, “High Throughput Reaction Screening, Small Scale Synthesis and BioAssays” Pharm. Sci. Colloquium, Merck &Co. Rahway, NJ, October 7, 2020.

- R. Graham Cooks, “Exploratory Analysis of Small Molecules Guided by Chemical Functionalities" Association for Mass Spectrometry and Advances in the Clinical Lab (MSACL) Connect, September 8, 2020 (with Christina Ferreira).

- R. Graham Cooks, “Make It: High Throughput Screening, Synthesis & Bioanalysis System” NCATS, Bethesda, MD, August 31, 2020 (with Nicolás M. Morato).

- D. Thompson, Commercializing Flow Chemistry Summit 2020, Lecture, Aug 2020.

- Yangjie Li, Yanyang Hu, David Logsdon, Yong Liu, Yuejie Zhao and R. Graham Cooks. “Accelerated Forced Degradation of Therapeutic Peptides in Levitated Microdroplets”. Pittcon, Chicago, IL, March 2020.

- R. Graham Cooks, “Accelerated Reactions in Microdroplets” Asia Oceania Mass Spectrometry Conference 2020, Macao, China, January 4, 2020.

- R. Graham Cooks, “Accelerated Reactions in Microdroplets” Tsinghua University, Beijing, China, January 8, 2020.
- R. Graham Cooks, “Speed in Chemical Analysis” International Conference on Emerging Frontiers in Chemical Sciences, Farook College, Kozhikode, Kerala, December 13, 2019.
- R. Graham Cooks, “Mass Spectrometry in Synthesis and Analysis of Nanomaterials” International Conference on Recent Advances in Nanoscience and Nanotechnology, Stella Maris College, Chennai, India, December 11-12, 2019.

- R. Graham Cooks, “Accelerated Reactions in Ambient Microdroplets” Colloquium, Chemistry Department, IIT Madras, December 9, 2019.

- R. Graham Cooks, “Accelerated Reactions for High Throughput Screening, Small-scale Synthesis and Intraoperative Tissue Diagnostics” Genentech, San Francisco, CA, November. 22, 2019.

- R. Graham Cooks, “Accelerated Reactions in Microdroplets and Thin Films” Boston University, Boston, MA, November 11, 2019.

- R. Graham Cooks, “Accelerated Reactions in Microdroplets and Thin Films” Pimentel Lecture, University of California Berkeley, CA, October 22, 2019.

- R. Graham Cooks, “Mass Spectrometry in Synthesis and Reaction Screening” Kolthoff Lecture, University of Minnesota, MN, October 8, 2019.

- R. Graham Cooks, “Accelerated Reactions, High Throughput Screening and Interoperative Tissue Diagnostics” Bindley Omics Symposium, Purdue University, IN, October 4, 2019.
- R. Graham Cooks, “Accelerated Reactions, High Throughput Screening/Synthesis and Intraoperative Tissue Diagnostics” Waters, Milford, CT, August 5, 2019.

- R. Graham Cooks, “Organic Synthesis and Chemical Analysis by Mass Spectrometry” Delaware Valley Mass Spectrometry Discussion Group, Newark, DE, May 13, 2019.

Honors and Awards: •Yangjie Li, Full Member, Sigma Xi, Scientific Research Honor Society, 2021.

- Nicolás M. Morato, Member, Phi Kappa Phi, Honor Society, 2021.
- R. Graham Cooks, Fellow, Hagler Institute for Advanced Study at Texas A&M University, 2020.
- Yangjie Li and R. Graham Cooks, Research highlighted in Purdue Chemistry News: “Professor Cooks and team advancing a Green Chemistry catalyst”, 2020.
- Yangjie Li and R. Graham Cooks, Research highlighted in Chemistry World: “Katritzky reaction rate turbocharged by glassware”, 2020.
- Zoltan K. Nagy, Most Impactful Inventor of Fiscal Year 2020, Purdue University.
- R. Graham Cooks, J. Calvin Giddings Award for Excellence in Education, American Chemical Society, Division of Analytical Chemistry, 2020.
- Yangjie Li, Thomas W. Keough Graduate Scholarship, Department of Chemistry, Purdue University, 2020.
- Shruti Biyani, Robert R. Squires Scholarship Award, Department of Chemistry, Purdue University, February 2020.
- Nicolás M. Morato, Charles H. Viol Memorial Fellowship, Department of Chemistry, Purdue University, 2020.
- Zoltan K. Nagy, Doctor Honoris Causa, Budapest University of Technology and Economics, Budapest, Hungary, 2019.
- R. Graham Cooks, China International Science and Technology Cooperation Award, 2019.
- Zoltan K. Nagy, Pharmaceutical Discovery Development and Manufacturing (PD2M) Forum Award for Outstanding Contribution to QbD for Drug Substance (AIChE), 2019.
- Shruti Byani. Organic Division H. C. Brown Travel Grant Award, Department of Chemistry, Purdue University, 2019.
- Zinia Jaman, H. C. Brown Organic Chemistry Seminar Award, Department of Chemistry, Purdue University, 2019.
- Zoltan K. Nagy, Honorary Professor, Dalian University of Technology, China, 2019.
- Zinia Jaman, Poster award in 9th Chicago Organic Symposium, 2019.
- R. Graham Cooks, Waters Symposium at PittCon recognizing Ambient Ionization, 2018.
- Zoltan K. Nagy, Excellence in Process Development Research Award, American Institute of Chemical Engineers (AIChE), 2018.
- Zinia Jaman, Best Poster Award, Flow Chemistry Congress, Royal Society of Chemistry, 2018.
- Kiran Iyer, Best Poster Award, Dawn or Doom, Purdue University, 2018.
- R. Graham Cooks, Aston Medal, British Mass Spectrometry Society, 2017.
- Zinia Jaman, Cagiantas Fellowship, College of Science, 2017.
- Zoltan K. Nagy, PSE 2017 Model-Based Innovation Prize, American Institute of Chemical Engineers (AIChE), 2017.
- Nagy, Mathematical modelling and experimental validation of a novel periodic flow crystallization process using

MSMPR crystallizers, AIChE J., 63 (4), 1313–1327, 2017.

• R. Graham Cooks, Focus Honor Issue, Journal of the American Society for Mass Spectrometry, vol. 28, no. 6, 2017.

Protocol Activity Status:

Technology Transfer: In the annual report of 2017, the team reported two patent disclosures: “Systems and methods for increasing reaction yield”, and “Carbon fiber flexible tubing heater”. Also, we reported two non-provisional patent applications: “Systems and methods for producing a chemical product” and “Systems and methods for anti-fouling feedback control of plug-flow crystallization in continuous manufacturing of chemical products”.

In 2018, The team add two more patents filed, namely “Devices for heating small-diameter tubing and methods of making and using” and “On-demand Rapid Synthesis of Lomustine under Continuous Flow Conditions”.

In 2018 a large demo experiment was performed for the team of Andreas Kaerner, from Lilly Product and Research Development Department have secured where EE deprotection was pursued.

From 2019 to the present, we have also run samples for a comparative study on arylation reactions shared with the Stanford Research Institute (SRI) Team (Nathan Collins). We had interactions with Klavs Jensen (MIT) on machine learning to the MS/MS data collected from diverse starting materials. We have also run samples for Audrey Williams (Lawrence Livermore National Laboratories - LLNL), Andreas Kaerner (Eli Lilly & Co.), Waters Corporation, Andrew Mesecar (Purdue Biochemistry), Jeremy Walker (Flir Inc.).

In 2020 four new patents were submitted through the Purdue Research Foundation: “Continuous Flow Sonogashira Coupling Synthesis”, “Scale Up Microdroplet Organic Synthesis with Solvent Recycling”, “High-troughput synthesis/screening/bioanalysis automated platform” and “High-throughput label-free enzymatic bioassays using automated DESI-MS”.

In 2021, in addition to demos (in person and over the web) we have worked closely with LLNL running samples for them in the area of acetylcholinesterase inhibition and reactivation. We have also worked closely with Flir Inc. on illicit drugs and have explored the use of the DESI system for fentanyl characterization in complex mixtures. We have obtained DHS and DTRA funding for work on biological agents with Flir, using miniature mass spectrometers and 2D MS/MS and we plan a proposal with them involving the DESI Make it system to study enzyme inhibition.

See section titled System Demos to Visitors and Companies for a listing of companies to whom the DESI Make It system was demonstrated.

PARTICIPANTS:

Participant Type: PD/PI
Participant: R. Graham Cooks
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Staff Scientist (doctoral level)
Participant: Larisa Avramova
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Staff Scientist (doctoral level)

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Participant: Christina Ramires Ferreira
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Staff Scientist (doctoral level)
Participant: Tiago Jose Paschoal Sobreira
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Shruti Biyani
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Damien Edward Dobson
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Harrison Ewan
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Edwin Gonzalez
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Staff Scientist (doctoral level)
Participant: Ryan Hilger
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Yanyang Hu
Person Months Worked: 6.00 **Funding Support:**

Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Kai-Hung Huang
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Kiran Iyer
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Zinia Jaman
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Bharath Keshavamurthy
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Andy Koswara
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: My Phuong Le Thi
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Yangjie Li
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: David Logsdon
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Brett Marsh
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Other Professional
Participant: Brandy McMasters
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Nicolas Morato Gutierrez
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Ahmed Mufti
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Giulia Murbach Oliveira
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: Zoltan Nagy
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

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Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Namita Narendra
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Lingqi Qiu
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Other Professional
Participant: Nicole Remley
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Zachary Struzik
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Botond Szilagyi
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: David Thompson
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Zhenwei Wei
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Staff Scientist (doctoral level)

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as of 23-Apr-2021

Participant: Phillip Wyss
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Zhuoer Xie
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Graduate Student (research assistant)
Participant: Bradley Loren
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: PD/PI
Participant: R. Graham Cooks
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: Y

Participant Type: Graduate Student (research assistant)
Participant: Michael Wleklinski
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

ARTICLES:

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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Angewandte Chemie International Edition
Publication Identifier Type: DOI Publication Identifier: 10.1002/anie.201602270
Volume: x Issue: x First Page #:
Date Submitted: 8/25/16 12:00AM Date Published: 8/17/16 4:00AM
Publication Location: United States
Article Title: Organic Reactions in Microdroplets: Reaction Acceleration Revealed by Mass Spectrometry
Authors: Xin Yan, Ryan M. Bain, R. Graham Cooks
Keywords: mbient ionization · mass spectrometry · micro-droplets · reaction kinetics · spray-based ionization
Abstract: The striking finding that reaction acceleration occurs in confined-volume solutions sets up an apparent conundrum: Microdropletsformed by spray ionization can be used to monitor the course of bulk-phase reactions and also to accelerate reactions between the reagents insuch a reaction. This Minireview introduces droplet and thin-filmacceleration phenomena and summarizes recent methods applied tostudy accelerated reactions in confined-volume, high-surface-areasolutions. Conditions that dictate either simple monitoring or accel-eration are reconciled in the occurrence of discontinuous and completedesolvation as the endpoint of droplet evolution. The contrastingfeatures of microdroplet and bulk-solution reactions are describedtogether with possible mechanisms that drive reaction acceleration inmicrodroplets. Current applications of droplet microreactors are notedas is reaction acceleration in confined volumes and possible futurescale-up.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Angewandte Chemie International Edition
Publication Identifier Type: DOI Publication Identifier: 10.1002/anie.201602270
Volume: 55 Issue: 42 First Page #: 12960
Date Submitted: 8/28/17 12:00AM Date Published: 10/1/16 12:00AM
Publication Location:
Article Title: Organic Reactions in Microdroplets: Reaction Acceleration Revealed by Mass Spectrometry
Authors: Xin Yan, Ryan M. Bain, R. Graham Cooks
Keywords: Organic Reactions, Microdroplets, Reaction Acceleration, Mass Spectrometry
Abstract: The striking finding that reaction acceleration occurs in confined-volume solutions sets up an apparent conundrum: Microdroplets formed by spray ionization can be used to monitor the course of bulk-phase reactions and also to accelerate reactions between the reagents in such a reaction. This Mini review introduces droplet and thin-film acceleration phenomena and summarizes recent methods applied to study accelerated reactions in confined-volume,high-surface-area solutions.Conditions that dictate either simple monitoring or acceleration are reconciled in the occurrence of discontinuous and complete desolvation as the endpoint of droplet evolution. The contrasting features of microdroplet and bulk-solution reactions are described together with possible mechanisms that drive reaction acceleration in microdroplets.Current applications of droplet micro reactors are noted as is reaction acceleration in confined volumes and possible future scale-up.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Angewandte Chemie International Edition
Publication Identifier Type: DOI Publication Identifier: 10.1002/anie.201704520
Volume: 56 Issue: 32 First Page #: 9386
Date Submitted: 8/26/17 12:00AM Date Published: 8/1/17 4:00AM
Publication Location: Weinheim, Germany
Article Title: Reaction Acceleration in Thin Films with Continuous Product Deposition for Organic Synthesis
Authors: Zhenwei Wei, Michael Wleklinski, Christina Ferreira, R. Graham Cooks
Keywords: Reaction Acceleration, Thin Films, Organic Synthesis, Mass Spectrometry
Abstract: hin film formats are used to study the Claisen–Schmidt base-catalyzed condensation of 6-hydroxy-1-indanone with substituted benzaldehydes and to compare the reaction acceleration relative to the bulk. Relative acceleration factors initially exceeded 103 and were on the order of 102 at steady state, although the confined volume reaction was not electrostatically driven. Substituent effects were muted compared to those in the corresponding bulk and microdroplet reactions and it is concluded that the rate-limiting step at steady state is reagent transport to the interface. Conditions were found that allowed product deposition from the thin film to occur continuously as the reaction mixture was added and as the solvent evaporated. Yields of 74?% and production rates of 98?mg?h?1 were reached in a very simple experimental system that could be multiplexed to greater scales.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: European Journal of Organic Chemistry
Publication Identifier Type: DOI Publication Identifier: 10.1002/ejoc.201601270
Volume: 2016 Issue: 33 First Page #: 5480
Date Submitted: 8/28/17 12:00AM Date Published: 11/1/16 4:00AM
Publication Location:
Article Title: Can Accelerated Reactions in Droplets Guide Chemistry at Scale?
Authors: Michael Wleklinski, Caitlin E. Falcone, Bradley P. Loren, Zinia Jaman, Kiran Iyer, H. Samuel Ewan, Seol
Keywords: Reaction acceleration, microdroplets, organic synthesis, mass spectrometry
Abstract: Mass spectrometry (MS) is used to monitor chemical reactions in droplets. In almost all cases, such reactions are accelerated relative to the corresponding reactions in bulk, even after correction for concentration effects, and they serve to predict the likely success of scaled-up reactions performed in microfluidic systems. The particular chemical targets used in these test studies are diazepam, atropine and diphenhydramine. In addition to a yes/no prediction of whether scaled-up reaction is possible, in some cases valuable information was obtained that helped in optimization of reaction conditions, minimization of by-products, and choice of catalyst. In a variant on the spray-based charged droplet experiment, the Leidenfrost effect was used to generate larger, uncharged droplets and the same reactions were studied in this medium. These reactions were also accelerated but to smaller extents than in microdroplets.
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Acknowledged Federal Support: **Y**

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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: The Analyst
Publication Identifier Type: DOI Publication Identifier: 10.1039/C7AN00622E
Volume: 142 Issue: 15 First Page #: 2836
Date Submitted: 8/26/17 12:00AM Date Published:
Publication Location:
Article Title: Reaction screening and optimization of continuous-flow atropine synthesis by preparative electrospray mass spectrometry
Authors: Caitlin E. Falcone, Zinia Jaman, Michael Wleklinski, Andy Koswara, David H. Thompson, R. Graham Cc
Keywords: Reaction screening, preparative electrospray, mass spectrometry
Abstract: Preparative electrospray (ES) exploits the acceleration of reactions in charged microdroplets to perform a small scale chemical synthesis. In combination with on-line mass spectrometric (MS) analysis, it constitutes a rapid screening tool to select reagents to generate specific products. A successful reaction in preparative ES triggers a refined microfluidic reaction screening procedure which includes the optimization for stoichiometry, temperature and residence time. We apply this combined approach for refining a flow synthesis of atropine. A successful preparative ES pathway for the synthesis of the phenylacetyl ester intermediate, using tropine/HCl/phenylacetyl chloride, was optimized for solvent in both the preparative ES and microfluidics flow systems and a base screening was conducted by both methods to increase atropine yield, increase percentage conversion and reduce byproducts.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Chem. Sci.
Publication Identifier Type: DOI Publication Identifier: 10.1039/C7SC00905D
Volume: 8 Issue: 6 First Page #: 4363
Date Submitted: 8/26/17 12:00AM Date Published:
Publication Location:
Article Title: Mass spectrometric directed system for the continuous-flow synthesis and purification of diphenhydramine
Authors: Bradley P. Loren, Michael Wleklinski, Andy Koswara, Kathryn Yammine, Yanyang Hu, Zoltan K. Nagy, D
Keywords: Mass spectrometry, Continuous-flow synthesis, Diphenhydramine
Abstract: A highly integrated approach to the development of a process for the continuous synthesis and purification of diphenhydramine is reported. Mass spectrometry (MS) is utilized throughout the system for on-line reaction monitoring, off-line yield quantitation, and as a reaction screening module that exploits reaction acceleration in charged microdroplets for high throughput route screening. This effort has enabled the discovery and optimization of multiple routes to diphenhydramine in glass microreactors using MS as a process analytical tool (PAT). The ability to rapidly screen conditions in charged microdroplets was used to guide optimization of the process in a microfluidic reactor. A quantitative MS method was developed and used to measure the reaction kinetics. Integration of the continuous-flow reactor/on-line MS methodology with a miniaturized crystallization platform for continuous reaction monitoring and controlled crystallization of diphenhydramine was also achieved.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: IEEE Life Sciences Letters
Publication Identifier Type: DOI Publication Identifier: 10.1109/LLS.2017.2661981
Volume: 3 Issue: 1 First Page #: 1
Date Submitted: 8/28/17 12:00AM Date Published: 3/1/17 5:00AM
Publication Location:
Article Title: ON–OFF Feedback Control of Plug-Flow Crystallization: A Case of Quality-by-Control in Continuous Manufacturing
Authors: Andy Koswara, Zoltan K. Nagy
Keywords: ON–OFF Feedback Control, Plug-Flow Crystallization, pharmaceutical manufacturing
Abstract: Plug-flow crystallization (PFC) is a promising candidate to realizing the paradigm shift from batch-to-continuous pharmaceutical manufacturing. While PFC has been recently touted as the ideal continuous crystallizer due to its compact design, proper mixing, and flexible cooling and antisolvent control, it is prone to surface fouling or encrustation. In this letter, a model of encrustation growth and dissolution dynamics coupled with PFC is discussed and a novel method of ON-OFF feedback control of PFC with antifouling control is proposed. The study illustrates a quintessential example of quality-by-control concept, which is complementary to the quality-by-design and essential in ensuring desired control performance and product quality.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Organic Process Research & Development
Publication Identifier Type: DOI Publication Identifier: 10.1021/acs.oprd.7b00218
Volume: Issue: First Page #:
Date Submitted: 8/28/17 12:00AM Date Published: 8/1/17 4:00AM
Publication Location:
Article Title: Multistep Flow Synthesis of Diazepam Guided by Droplet-Accelerated Reaction Screening with Mechanistic Insights from Rapid Mass Spectrometry Analysis
Authors: H. Samuel Ewan, Kiran Iyer, Seok-Hee Hyun, Michael Wleklinski, R. Graham Cooks, David H. Thompsc
Keywords: Multistep Flow Synthesis, Diazepam, Droplet-Accelerated Reaction Screening, Mass Spectrometry
Abstract: Electrospray and Leidenfrost droplet accelerated reactions were used as a predictive tool for estimating the outcome of microfluidic synthesis as demonstrated by Wleklinski et al. Rapid analysis by electrospray-mass spectrometry (ESI-MS) also provided immediate feedback on reaction outcomes in flow reactions. Significant reaction acceleration was observed in electrospray relative to the corresponding bulk reaction. This rapid reaction screening and analysis method has allowed for the detection of previously unreported outcomes in the reaction between 5-chloro-2-(methylamino)benzophenone and haloacetyl chloride (halo = Cl or Br) in the continuous synthesis of diazepam. In our current study, a more detailed extension of the previous work, we report acceleration factors that are solvent dependent; additional byproducts that were observed on the microfluidic scale that were absent in the droplet reactions.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Chemical Science
Publication Identifier Type: DOI Publication Identifier: 10.1039/C7SC04606E
Volume: 9 Issue: 6 First Page #: 1647
Date Submitted: 8/16/18 12:00AM Date Published:
Publication Location:
Article Title: High throughput reaction screening using desorption electrospray ionization mass spectrometry
Authors: Michael Wleklinski, Bradley P. Loren, Christina R. Ferreira, Zinia Jaman, Larisa Avramova, Tiago J. P. S
Keywords: High-throughput reaction screening, Desorption electrospray ionization, alkylation
Abstract: We report the high throughput analysis of reaction mixture arrays using methods and data handling routines that were originally developed for biological tissue imaging. Desorption electrospray ionization (DESI) mass spectrometry (MS) is applied in a continuous on-line process at rates that approach 104 reactions per h at area densities of up to 1 spot per mm2 (6144 spots per standard microtiter plate) with the sprayer moving at ca. 104 microns per s. Data are analyzed automatically by MS using in-house software to create ion images of selected reagents and products as intensity plots in standard array format. Amine alkylation reactions were used to optimize the system performance on PTFE membrane substrates using methanol as the DESI spray/analysis solvent. Reaction times can be <100 ?s when reaction acceleration occurs in microdroplets, enabling the rapid screening of processes like N-alkylation and Suzuki coupling reactions as reported herein. Products and by-products were confirmed
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Chemistry - A European Journal
Publication Identifier Type: DOI Publication Identifier: 10.1002/chem.201801165
Volume: 24 Issue: 38 First Page #: 9546
Date Submitted: 8/16/18 12:00AM Date Published: 7/1/18 12:00AM
Publication Location:
Article Title: High Throughput Experimentation and Continuous Flow Validation of Suzuki-Miyaura Cross-Coupling Reactions
Authors: Zinia Jaman, Ahmed Mufti, Samyukta Sah, Larisa Avramova, David H. Thompson
Keywords: Suzuki–Miyaura Cross-Coupling, High-throughput reaction screening
Abstract: Traditional methods to discover optimal reaction conditions for small molecule synthesis is a time? consuming effort that requires large quantities of material and a significant expenditure of labor. High?throughput techniques are a potentially transformative approach for reaction condition screening, however, rapid validation of the reaction hotspots under continuous flow conditions remains necessary to build confidence in high throughput screening hits. Continuous flow technology offers the opportunity to upscale the screening hotspots and optimize their output of the target compounds due to the exceptional heat and mass transfer ability of flow reactions that are conducted in a smaller and safer reaction volume. We report a robotic high throughput technique to execute reactions in multi?well plates that were coupled with fast mass spectrometric analysis using an autosampler to accelerate the reaction screening process.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Chemical Engineering Science
Publication Identifier Type: DOI Publication Identifier: https://doi.org/10.1016/j.ces.2018.10.046
Volume: 195 Issue: First Page #: 1010
Date Submitted: 8/27/19 12:00AM Date Published: 2/23/19 5:00AM
Publication Location: United States
Article Title: Piezoelectric-based high performance spray solvent delivery system for desorption electrospray ionization mass spectrometry: Systematic design and case studies for high throughput screening of N-alkylation reactions
Authors: Botond Szilagyi, Andy Koswara, Bradley P. Loren, Christina R. Ferreira, David H.Thompson, Zoltan K.N
Keywords: High throughput screening, DESI-MS Solvent delivery system, Adaptive controller synthesis
Abstract: Desorption electrospray ionization mass spectrometry (DESI-MS) is a powerful tool for ultra-high throughput chemical reaction screening having enormous scientific and practical implications. DESI-MS enables considerably faster chemical reaction and/or product discovery than most current screening methods using very small amount of materials. It has the advantage of bringing the reagents in contact inside charged micro-droplets, that can accelerate reaction by compared to bulk or flow reaction approaches. Since both the desorption efficiency from the surface and the reaction kinetics can be significantly influenced by the DESI spray solvent flowrate and composition, these parameters are expected to play key roles in the overall performance of the ultrahigh-throughput screening. Syringe pumps are frequently used to deliver the DESI spray. They are robust, but inflexible and slow when it comes to manipulating solvent composition and varying flowrate setpoints. This study addresses the imp
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Journal of The American Society for Mass Spectrometry
Publication Identifier Type: DOI Publication Identifier: DOI: 10.1007/s13361-019-02287-3
Volume: 1 Issue: 1 First Page #: 1
Date Submitted: 8/27/19 12:00AM Date Published: 8/8/19 4:00AM
Publication Location: United States
Article Title: Screening of the Suzuki Cross-Coupling Reaction Using Desorption Electrospray Ionization in High-Throughput and in Leidenfrost Droplet Experiments
Authors: Patrick W. Fedick, Kiran Iyer, Zhenwei Wei, Larisa Avramova, Grace O. Capek, R. Graham Cooks
Keywords: Suzuki cross-coupling, Microdroplets, DESI-MS, Reaction acceleration, Leidenfrost Confined volumes, High-throughput screening
Abstract: Suzuki cross-coupling is a widely performed reaction, typically using metal catalysts under heated conditions. Acceleration of the Suzuki cross-coupling reaction has been previously explored in microdroplets using desorption electrospray ionization mass spectrometry (DESI-MS). Acceleration factors greater than 200 were measured for brominated substrates, paralleling the DESI-MS results. Acceleration factors dropped to near unity with highly substituted pyridines, attributable to a steric effect. The reaction proceeded in the absence of a base in Leidenfrost droplets although no product formation was seen without base in the bulk or in the DESI-MS screening experiments. These differences between Leidenfrost chemistry and the bulk and in droplets formed in high-throughput DESI are tentatively attributed to extremes of pH associated with the surfaces of Leidenfrost droplets.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Organic Process Research & Development
Publication Identifier Type: DOI Publication Identifier: 10.1021/acs.oprd.8b00387
Volume: 23 Issue: 3 First Page #: 334
Date Submitted: 8/27/19 12:00AM Date Published: 2/1/19 10:00AM
Publication Location: United States
Article Title: Rapid On-Demand Synthesis of Lomustine under Continuous Flow Conditions
Authors: Zinia Jaman, Tiago J. P. Sobreira, Ahmed Mufti, Christina R. Ferreira, R. Graham Cooks, David H. Thor
Keywords: Lomustine, continuous synthesis reaction, telescoping, desorption electrospray ionization, mass spectrometry, (DESI-MS) high throughput experimentation
Abstract: Lomustine, an important agent for treatment of brain tumors and Hodgkin's lymphoma, has been synthesized using continuous flow methodology. Desorption electrospray ionization mass spectrometry (DESI-MS) was used to quickly explore a large number of reaction conditions for one of the reaction steps and guide the efficient translation of optimized conditions to continuous lomustine production. Using only four inexpensive commercially available starting materials and a total residence time of 9 min, lomustine was prepared via a linear sequence of two chemical reactions performed separately in two telescoped flow reactors. Sequential offline extraction and filtration resulted in a 63% overall yield of pure lomustine at a production rate of 110 mg/h. The primary advantages of this approach are the rapid manufacture of lomustine with two telescoped steps to avoid isolation and purification of a labile intermediate and the mild conditions used in the nitrosylation step.
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Acknowledged Federal Support: **Y**

CONFERENCE PAPERS:

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 21st International Mass Spectrometry Conference
Date Received: 29-Aug-2016 Conference Date: 20-Aug-2016 Date Published: 20-Aug-2016
Conference Location: Toronto, Canada
Paper Title: Analytic Directed Synthesis System for the Manufacture of Pharmaceuticals
Authors: Michael Wleklinski, Bradley P. Loren, Andy Koswara, Caitlin E. Falcone, Zinia Jaman, Adam Hollerbach
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: AIChE Annual Meeting
Date Received: 28-Aug-2017 Conference Date: 13-Nov-2016 Date Published: 28-Aug-2016
Conference Location: San Francisco,CA
Paper Title: Alpha and Beta Glycine Nanocrystal Growth and Dissolution Kinetics in the Presence of an Electric Field through Molecular Dynamics - Towards Electric Field Controlled Crystallization
Authors: Conor Parks, Andy Koswara, Nandkishor Nere, Shailendra Bordawekar, Hsien-Hsin Tung, Zoltan K. Na
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: AIChE Annual Meeting
Date Received: 28-Aug-2017 Conference Date: 13-Nov-2016 Date Published: 13-Nov-2016
Conference Location: San Francisco, CA
Paper Title: Process Intensification through Continuous Spherical Crystallization Using an Oscillatory Baffled Crystallizer
Authors: Ramon Pena, Zoltan K. Nagy, Christopher L. Burcham and Daniel Jarmer
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: 2nd Annual Process Analytical Technologies Symposium
Date Received: 28-Aug-2017 Conference Date: 07-Sep-2016 Date Published: 07-Sep-2016
Conference Location: Indianapolis, IN
Paper Title: Monitoring and Control of Pharmaceutical Crystallization Systems in the Presence of Impurities Using Online UPLC Technology
Authors: Yang Yang, Chuntao Zhang, Kanjakha Pal, Andy Koswara, Justin Quon, Rahn McKeown, Charles Goss
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: AIChE Annual Meeting
Date Received: 28-Aug-2017 Conference Date: 13-Nov-2016 Date Published: 28-Aug-2016
Conference Location: San Francisco, CA
Paper Title: A Kinetic Approach to Polymorph Prediction: Polymorph Specific Alpha and Beta Glycine Nucleation Rates and Solubility Curves from Molecular Dynamics
Authors: Conor Parks, Andy Koswara, Hsien-Hsin Tung, Nandkishor Nere, Shailendra Bordawekar, Zoltan K. Nag
Acknowledged Federal Support: **Y**

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Conference Name: 65th ASMS Conference on Mass Spectrometry & Allied Topics, Indianapolis
Date Received: 30-Aug-2017 Conference Date: 04-Jun-2017 Date Published: 04-Jun-2017
Conference Location: Indianapolis, IN
Paper Title: Preparative Electrospray and Preparative Reactive Extractive Electrospray for Route Prediction and Optimization of Atropine Synthesis in Microfluidics
Authors: Caitlin E. Falcone, Zinia Jaman, Michael Wleklinski, Andy Koswara, David H. Thompson, R. Graham Co
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: AIChE Annual Meeting
Date Received: 28-Aug-2017 Conference Date: 17-Nov-2016 Date Published: 17-Nov-2016
Conference Location: San Francisco, CA
Paper Title: Miniaturized Purification Platform for Automated Screening of Flow Synthesis Using Extraction and Crystallization
Authors: Edward Barks, Claire Yiqing Liu, Joseph Oliva, Bradley P. Loren, Michael Wleklinski, Zinia Jaman, Ryan
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Chemistry Europe 2017
Date Received: 29-Aug-2017 Conference Date: 07-Feb-2017 Date Published: 07-Feb-2017
Conference Location: Cambridge University, Cambridge, UK
Paper Title: Analytic-Directed System for the Production of Diphenhydramine
Authors: Brad P. Loren, Michael Wleklinski, Andy Koswara, K. Yammine, Y. Hu, Zoltan Nagy, David Thompson, R
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Cal Meyers Memorial Organic Chemistry Symposium
Date Received: 29-Aug-2017 Conference Date: 27-Apr-2017 Date Published: 27-Aug-2017
Conference Location: Southern Illinois University, Carbondale, IL
Paper Title: Continuous-flow Synthesis of Atropine and Optimization of the Reaction Conditions Utilizing both Organic and Inorganic Bases
Authors: Zinia Jaman, Caitlin E. Falcone, Michael Wleklinski, Andy Koswara, R. Graham Cooks, David H. Thomp
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 23-Apr-2021

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Flow Chemistry Europe
Date Received: 16-Aug-2018 Conference Date: 06-Feb-2018 Date Published: 06-Feb-2018
Conference Location: Cambridge, UK
Paper Title: High-Throughput Screening and Microfluidic Validation of Suzuki-Miyaura Cross-Coupling Reactions
Authors: Zinia Jaman, Ahmed Mufti, Samyukta Sah, , Larisa Avramova, R. Graham Cooks, David H. Thompson
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Green Chemistry Gordon Research Conference
Date Received: 16-Aug-2018 Conference Date: 29-Jul-2018 Date Published: 29-Jul-2018
Conference Location: Castelldefels, Spain
Paper Title: High Throughput Experimentation Using DESI-MS to Guide Continuous-Flow Synthesis
Authors: Bradley P. Loren, H. Samuel Ewan, Larisa Avramova, Christina R. Ferreira, Tiago J. P. Sobreira, Kathry
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: American Chemical Society National Meeting and Exposition 2018
Date Received: 16-Aug-2018 Conference Date: 21-Aug-2018 Date Published: 21-Aug-2018
Conference Location: Boston, MA
Paper Title: Make-it and screen-it using high-throughput experiments at Purdue University
Authors: Zoltan Nagy , Christina Ferreira, Bradley Loren, David Thompson, Robert Graham Cooks
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Gordon Research Conference & Seminar, High Throughput Chemistry and Chemical Biology, Colby-Sawyer College
Date Received: 30-Aug-2019 Conference Date: 01-Jun-2019 Date Published: 01-Jun-2019
Conference Location: New London, NH
Paper Title: High Throughput Experimentation and Continuous Flow Synthesis of Sonogashira Coupling Reactions
Authors: Shruti Biyani, Prof. David H. Thompson, Prof. Graham Cooks, Tiago Sobreira, Larisa Avramova, David I
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Dawn or Doom Research Symposium and Poster Competition
Date Received: 30-Aug-2019 Conference Date: 05-Nov-2018 Date Published: 05-Nov-2018
Conference Location: Purdue University, West Lafeytte, IN
Paper Title: Suzuki Cross-coupling Reaction Screening Using Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) and Reaction Acceleration in Leidenfrost Droplets
Authors: Kiran Iyer, Patrick Fedick, Zhenwei Wei, Grace O. Capek, Larisa Avramova, R. Graham Cooks
Acknowledged Federal Support: **Y**

DISSERTATIONS:

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 28-Aug-2017 Completion Date: 11/30/16 6:15AM
Title: Preparative Mass Spectrometry Applications In Nanomaterials and Organic Synthesis
Authors: Michael Wleklinski, Ph.D. (R. Graham Cooks)
Acknowledged Federal Support: **Y**

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as of 23-Apr-2021

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 29-Aug-2017 Completion Date: 11/30/16 4:15PM
Title: Preparative Mass Spectrometry Applications In Nanomaterials and Organic Synthesis
Authors: Michael Wleklinski, Ph.D. (R. Graham Cooks)
Acknowledged Federal Support: **Y**

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 30-Aug-2017 Completion Date: 3/22/17 8:00AM
Title: Monitoring Of Organic Reactions With And Without Accelerated Rates Using Electrospray And Ambient Ionization Mass Spectrometry
Authors: Ryan Bain
Acknowledged Federal Support: **Y**

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 19-Aug-2018 Completion Date: 5/5/18 4:00AM
Title: Development of an Analytic-Directed Synthesis System
Authors: Brad, Loren
Acknowledged Federal Support: **Y**

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 18-Oct-2019 Completion Date: 5/16/19 10:30AM
Title: HIGH THROUGHPUT EXPERIMENTATION AS A GUIDE TO THE CONTINUOUS FLOW SYNTHESIS OF ACTIVE PHARMACEUTICAL INGREDIENTS
Authors: Zinia Jaman
Acknowledged Federal Support: **Y**

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 21-Aug-2020 Completion Date: 12/16/20 4:10AM
Title: High-throughput Organic Reaction Screening using Desorption Electrospray Ionization Mass Spectrometry
Authors: Logsdon II, David Land
Acknowledged Federal Support: **Y**

Publication Type: Thesis or Dissertation
Institution: Purdue University
Date Received: 21-Aug-2020 Completion Date: 1/2/20 5:00AM
Title: Microdroplets: Chemistry, Applications and Manipulation using Ionization Sources and Mass Spectrometry
Authors: Iyer, Kiran
Acknowledged Federal Support: **Y**

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as of 23-Apr-2021

Publication Type: Thesis or Dissertation
Institution: Purdue
Date Received: 21-Aug-2020 Completion Date: 12/6/19 5:58AM
Title: HIGH THROUGHPUT EXPERIMENTATION WITH DESORPTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY TO GUIDE CONTINUOUS-FLOW SYNTHESIS
Authors: Ewan, Harrison Samuel
Acknowledged Federal Support: **Y**

WEBSITES:

URL: https://www.chem.purdue.edu/thompson/continuous-flow-chemistry.html
Date Received: 25-Aug-2016
Title: Continous Flow Chemistry - David H. Thompson Group
Description: Short definition, importance and overview of flow chemistry
URL: https://www.purdue.edu/newsroom/releases/2018/Q1/a-popular-tool-for-drug-discovery-just-got-10-times-faster.html
Date Received: 16-Aug-2018
Title: Purdue News - A popular tool for drug discovery just got 10 times faster
Description: Press release about Make-it System
URL: https://www.chemistryviews.org/details/ezine/11078348/Finding_Optimal_Reaction_Conditions.html
Date Received: 16-Aug-2018
Title: Chemistry Views - Finding Optimal Reaction Conditions
Description: Press release comments on the manuscript High Throughput Experimentation and Continuous Flow Validation of Suzuki-Miyaura Cross-Coupling Reactions, Zinia Jaman, Ahmed Mufti, Samyukta Sah, Larisa Avramova, David H. Thompson, Chem. Eur. J. 2018. https://doi.org/10.1002/chem.201801165
URL: https://www.purdue.edu/newsroom/releases/2019/Q1/researchers-at-purdue-center-for-cancer-research-develop-innovative,-more-cost-effective-method-to-make-drugs.html
Date Received: 27-Aug-2019
Title: Researchers at Purdue Center for Cancer Research develop innovative, more cost-effective method to make drugs
Description: David H. Thompson, a professor in Purdue's Department of Chemistry and a member of the Purdue University Center for Cancer Research, has written a research paper published in Organic Process Research and Development about how to make a generic form of lomustine, prescribed to people with Hodgkin lymphoma and certain brain cancers. But the continuous manufacturing process described in the paper is not just limited to lomustine. It can be applied to many other products. The ability to reduce production costs has the potential to allow for more agile and cost effective production of many life-saving medicines.
URL: https://www.sciencedaily.com/releases/2019/03/190307131419.htm
Date Received: 27-Aug-2019
Title: Researchers develop innovative, more cost-effective method to make drugs
Description: Continuous manufacturing is a modern process that promises to enable the pharmaceutical industry to scale operations more easily in order to meet demand and help reduce drug shortages. A new research paper has been published about how to make a generic form of lomustine. The continuous manufacturing process can be applied to many other products.
URL: https://www.europeanpharmaceuticalreview.com/news/84750/generic-lomustine/
Date Received: 27-Aug-2019
Title: Continuous manufacturing method used to make generic lomustine
Description: Desorption electrospray ionisation mass spectrometry (DESI-MS) was used to quickly explore a large number of reaction conditions for one of the reaction steps and guide the efficient translation of optimised conditions to continuous lomustine production," write the authors in Organic Process Research and Development.
URL: https://www.eurekalert.org/pub_releases/2019-03/pu-prd030719.php
Date Received: 19-Apr-2021
Title: Purdue researchers develop innovative, more cost-effective method to make drugs
Description: Within six months, Thompson's team developed a method to make lomustine at a rate equivalent to one dose every two hours using continuous manufacture. His group is now developing methods to scale up the production rate

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URL: https://www.eurekalert.org/pub_releases/2019-03/pu-prd030719.php
Date Received: 27-Aug-2019
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URL: https://www.eurekalert.org/pub_releases/2019-03/pu-prd030719.php
Date Received: 27-Aug-2019
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Description: Within six months, Thompson's team developed a method to make lomustine at a rate equivalent to one dose every two hours using continuous manufacture. His group is now developing methods to scale up the production rate
URL: https://cancerletter.com/articles/20191108_2/
Date Received: 19-Apr-2021
Title: Purdue’s Thompson: Lomustine establishes proof of principle for resolving cancer drug shortages
Description: David H. Thompson, a professor at the Department of Chemistry at Purdue University and a member of the Purdue Center for Cancer Research, is working on a better and faster way to produce drugs and eliminate shortages.
URL: https://www.pbiforum.net/mag/featured/purdue-researchers-developing-more-cost-effective-method-to-make-drugs/
Date Received: 19-Apr-2021
Title: Purdue researchers developing more cost-effective method to make drugs
Description: Researchers at Purdue Centre for Cancer Research develop innovative, more cost-effective method to make drugs
URL: https://www.pbiforum.net/mag/featured/purdue-researchers-developing-more-cost-effective-method-to-make-drugs/
Date Received: 19-Apr-2021
Title: Purdue researchers developing more cost-effective method to make drugs
Description: Researchers at Purdue Centre for Cancer Research develop innovative, more cost-effective method to make drugs
URL: https://www.chemistryworld.com/news/katritzky-reaction-rate-turbocharged-by-glassware/4012816.article
Date Received: 19-Apr-2021
Title: Katritzky reaction rate turbocharged by glassware
Description: The rate that an amine transfer reaction proceeds at is boosted when it takes place in a glass vessel, as opposed to plastic one.1 The findings suggest that glass particles catalyse the Katritzky reaction by acting as a base, potentially offering a reusable green catalyst for this and possibly other reactions.
URL: https://www.chem.purdue.edu/media/news/2020/cooks_glass.html
Date Received: 19-Apr-2021
Title: Professor Cooks and team advancing a Green Chemistry catalyst
Description: Catalysts speed up chemical reactions. And many catalysts contain expensive, ‘noble’ metals. For example, a single load of a catalyst for industrial scale processes (e.g. formation of ethylene oxide from ethylene) can cost as much as $1M. As part of the general “Green Chemistry” movement towards more environmentally friendly, recyclable chemicals, there is growing interest in ‘greener’ catalysts. Professor R. Graham Cooks and his team are using glass as an inexpensive 'green' catalyst in the Katritzky reaction.
URL: https://aston.chem.purdue.edu/
Date Received: 19-Apr-2021
Title: Graham Cooks Laboratory
Description: Highlights DARPA Make It Capabilities
URL: https://www.davidthompsonlab.com/
Date Received: 18-Apr-2021
Title: David Thompson Website
Description: Highlights DARPA Make It Capabilities
URL: https://www.wlfi.com/content/news/A-Purdue-University--564736731.html
Date Received: 19-Apr-2021
Title: PURDUE UNIVERSITY CHEMISTRY PROFESSOR CREATES DEVICE TO COMBAT CANCER DRUG COST
Description: A Purdue University professor is working to resolve the issue of cancer drug shortages. This could mean more money in the pocket for cancer patients and their families.

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URL: https://www.wlfi.com/content/news/A-Purdue-University--564736731.html
Date Received: 19-Apr-2021
Title: PURDUE UNIVERSITY CHEMISTRY PROFESSOR CREATES DEVICE TO COMBAT CANCER DRUG COST
Description: A Purdue University professor is working to resolve the issue of cancer drug shortages. This could mean more money in the pocket for cancer patients and their families.

PATENTS:

Intellectual Property Type: Patent Date Received: **16-Aug-2018**
Patent Title: SYSTEMS AND METHODS FOR INCREASING REACTION YIELD
Patent Abstract: The invention generally relates to systems and methods for increasing reaction yield. In certain
Patent Number: 20180043327
Patent Country: USA
Application Date: 10-Aug-2017 Application Status: 2
Date Issued: 15-Feb-2018

Intellectual Property Type: Patent Date Received: **07-Mar-2019**
Patent Title: Devices for heating small-diameter tubing and methods of making and using
Patent Abstract: Heating devices, systems, and methods of making and using a heating device. Such a heating
Patent Number: WO2018140344A1
Patent Country: USA
Application Date: 22-Jan-2018 Application Status: 3
Date Issued: 22-Aug-2018

Intellectual Property Type: Patent Date Received: **07-Mar-2019**
Patent Title: Systems and methods for producing a chemical product
Patent Abstract: The invention generally provides systems and methods for producing a chemical product. In cer
Patent Number: WO2017192286A1
Patent Country: USA
Application Date: 24-Apr-2017 Application Status: 3
Date Issued: 11-Sep-2019

Intellectual Property Type: Patent Date Received: **07-Mar-2019**
Patent Title: SYSTEMS WITH ANTI-FOULING CONTROL AND METHODS FOR CONTROLLING FOULING WITHIN A CHANNEL OF A PLUG FLOW CRYSTALLIZER
Patent Abstract: he invention generally relates to systems with anti-fouling control and methods for controlling fou
Patent Number: US20170312795
Patent Country: USA
Application Date: 25-Apr-2017 Application Status: 3
Date Issued:

Intellectual Property Type: Patent Date Received: **27-Aug-2019**
Patent Title: On-demand Rapid Synthesis of Lomustine under Continuous Flow Conditions
Patent Abstract: Lomustine is a commercially available drug used to treat Hodgkin's lymphoma and brain tumors
Patent Number: US 62746045
Patent Country: USA
Application Date: 16-Oct-2018 Application Status: 1
Date Issued:

Intellectual Property Type: Patent Date Received: **19-Apr-2021**

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as of 23-Apr-2021

Patent Title: Continuous Flow Sonogashira Coupling Synthesis
Patent Abstract:
Patent Number: 2020-THOM-69095
Patent Country: USA
Application Date: 19-May-2020 Application Status: 1
Date Issued:

Intellectual Property Type: Patent Date Received: **19-Apr-2021**
Patent Title: High-Throughput Label-Free Enzymatic Bioassays using Automated DESI-MS
Patent Abstract:
Patent Number: 2020-COOK-69031
Patent Country: USA
Application Date: 05-Mar-2020 Application Status: 1
Date Issued:

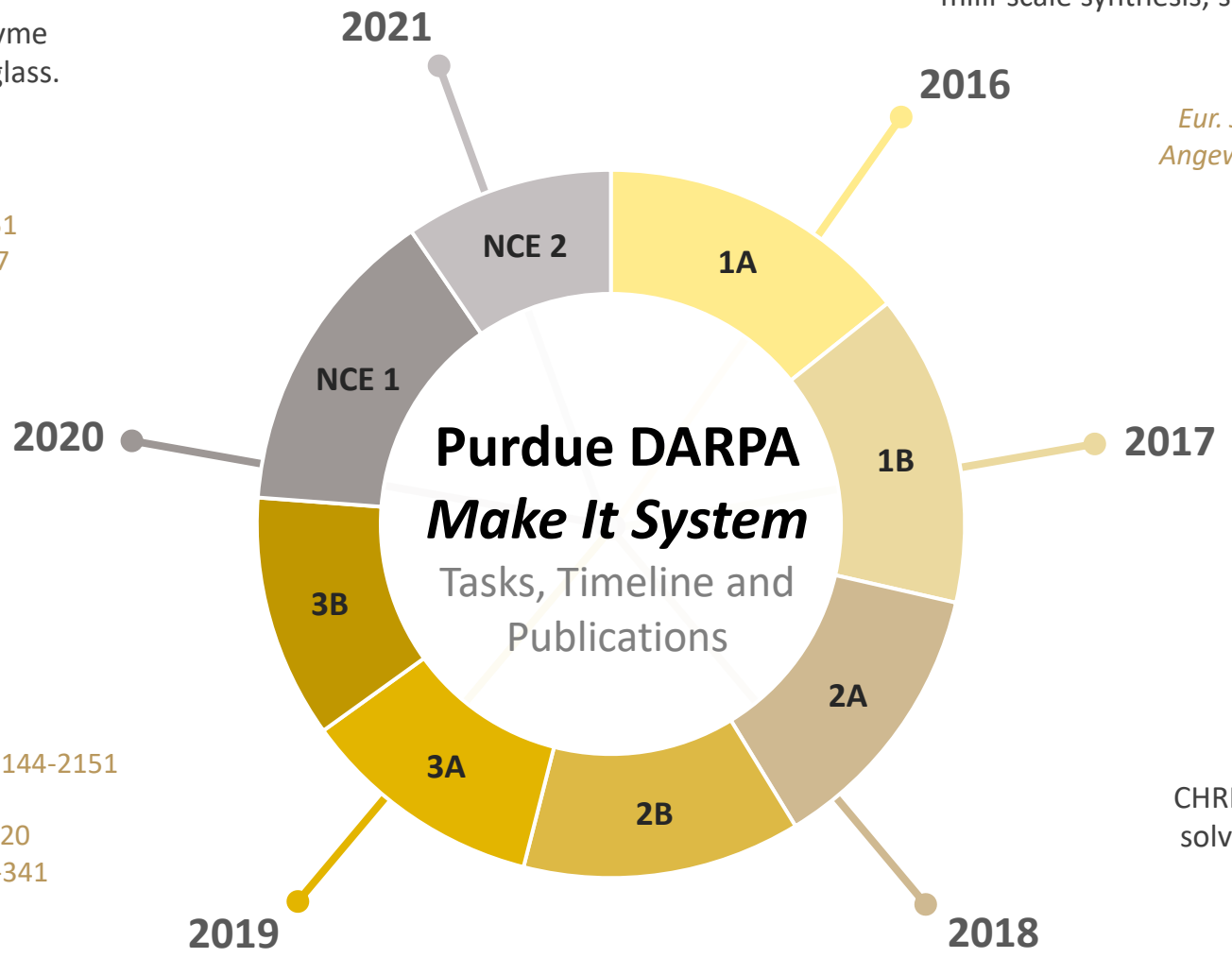
Intellectual Property Type: Patent Date Received: **19-Apr-2021**
Patent Title: Scaled Up Microdroplet Organic Synthesis with Solvent Recycling
Patent Abstract:
Patent Number: 2020-COOK-68885
Patent Country: USA
Application Date: 24-Feb-2021 Application Status: 1
Date Issued:

Partners

,

I certify that the information in the report is complete and accurate:
Signature: Christina R. Ferreira
Signature Date: 4/19/21 10:31PM

Fig. 1



Angew. Chem. Int. Ed. **2021**, 60, 2929–2933
SLAS Tech. **2021**, *Invited Submission*, in press
Chemistry-Methods **2021**, submitted.

Online HT bioanalysis, enzyme assays, catalytic activity of glass.

Anal. Methods. **2020** 12, 28, 3654-3669
Angew. Chem. Int. Ed. **2020**, 59, 20459-64
Org. Process Res. Dev. **2020,** 24, 10, 2240-2251
Org. Process Res. Dev. **2020,** 24, 9, 1647-1657
ACS Comb Sci. **2020**, 22, 4, 184–196
ACS Comb Sci. **2020**, 22, 12, 796-803
Annu. Rev. Phys. Chem. **2020,** 20, 71, 31-51
J. Mass Spectrom. **2020**, 56, 4, e4585
Pharm. Res. **2020,** 37, 7, 138
Chem. Sci. **2020**, 11, 9, 2356-2361

Faster and precise screening, integrative modularity concept, reactor relationship studies. Helios Dashboard.

J. Am. Soc. Mass Spectrom. **2019**, 30, 10, 2144-2151
Sci. Rep. **2019,** 9, 1, 14745
Chem. Eng. Sci. **2019,** 195, 1010-1020
Org. Process Res. Dev. **2019,** 23, 334-341

24-h continuous operation, real-time data analysis, MS and MS/MS data collection, interactive database, SOPs, exploration of 4 more chemistries.

Synthesis of 3 target compounds, online monitoring, oscillatory baffled crystallizer and anti-fouling for milli-scale synthesis, spherical crystallization online.

Eur. J. Org. Chem. **2016,** 33, 5480-5484
Angew. Chem. **2016,** 55, 42, 12960-12972

Implemented DESI, automated reagent preparation and MS analysis, explored four chemistries. Multistep reactions and scale-up using thin film deposition.

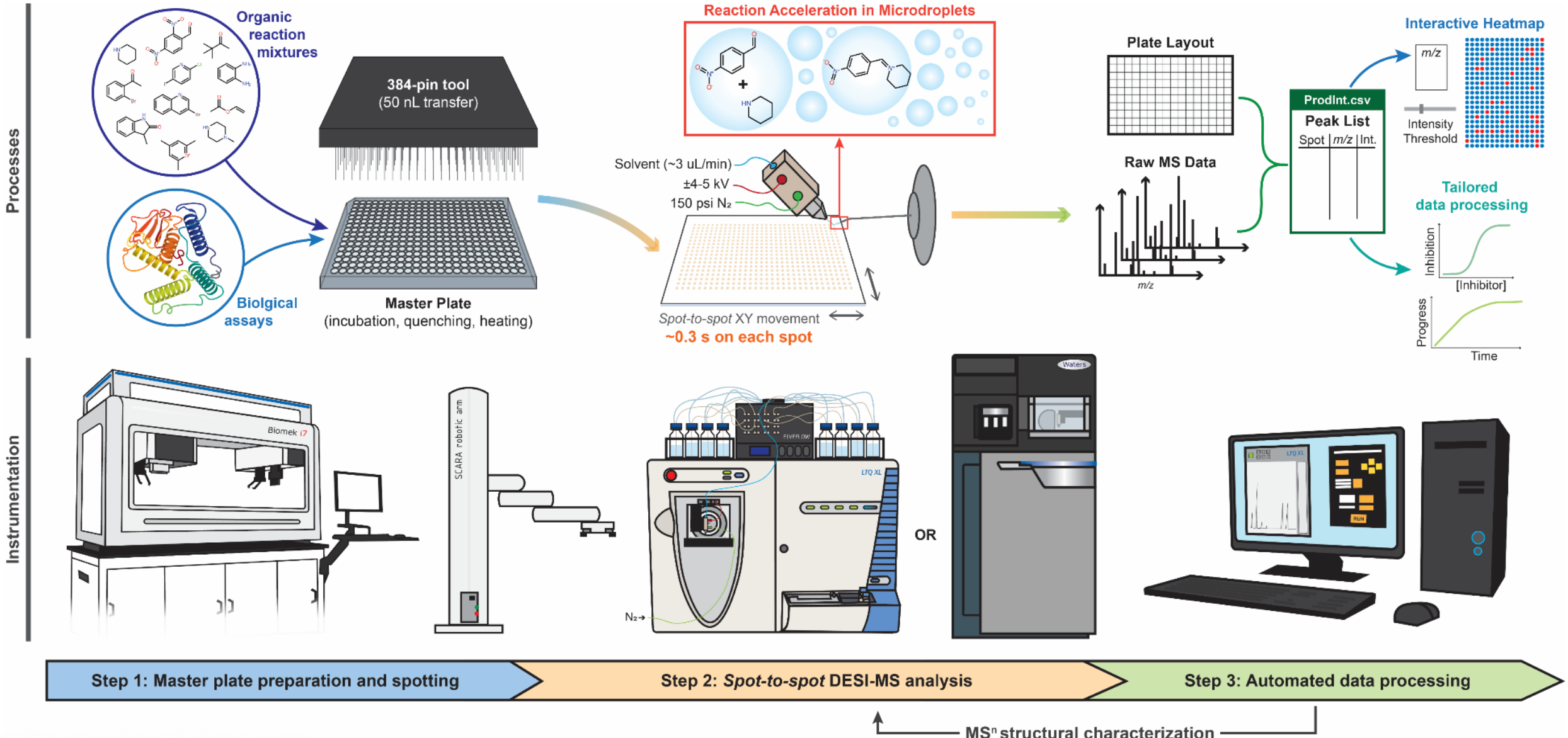
IEEE Life Sciences Letters **2017,** 3, 1, 1-4
Org. Process Res. Dev. **2017,** 21, 10, 1566-70
Analyst **2017,** 142, 2836-2845
Chem. Sci. **2017,** 8, 4363-4370
Angew. Chem. **2017,** 56, 3, 29386-9390

CHRIS software, piezoelectric-based DESI solvent delivery system, integration and automation, scale-up.

Chem. Sci. **2018**, 9, 1647-1653
Chem. Sci. **2018**, 9, 40, 7779-7786
Chem. Eur. **2018**, 24, 9546-9554

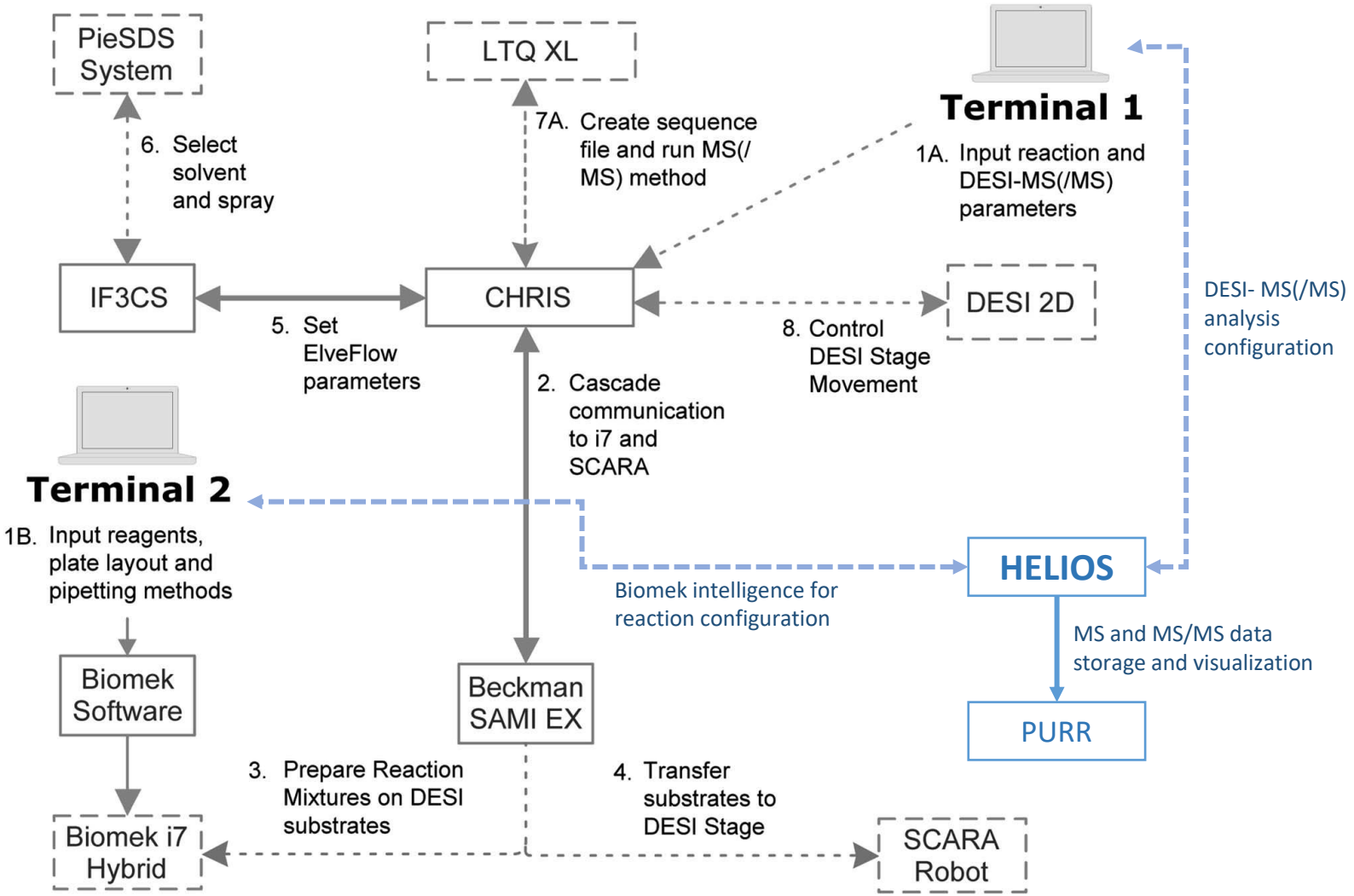
PURDUE UNIVERSITY

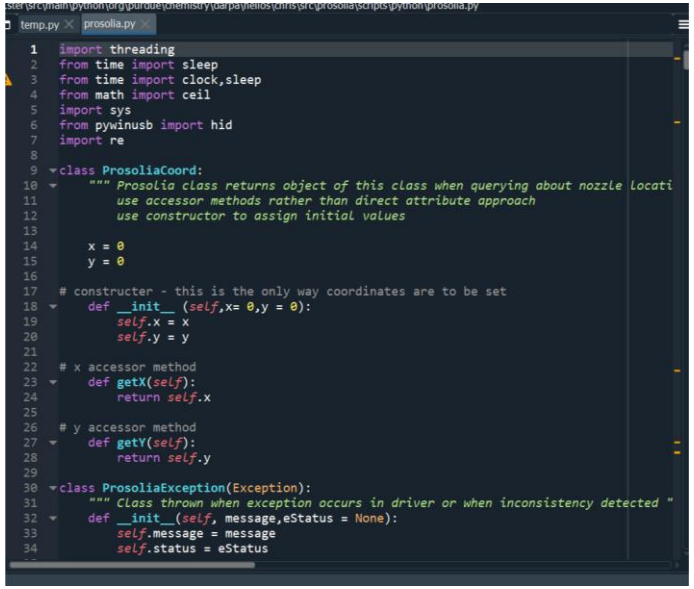
Purdue Make It System: Current Workflow



Slide Credit: ***Nick Morato***

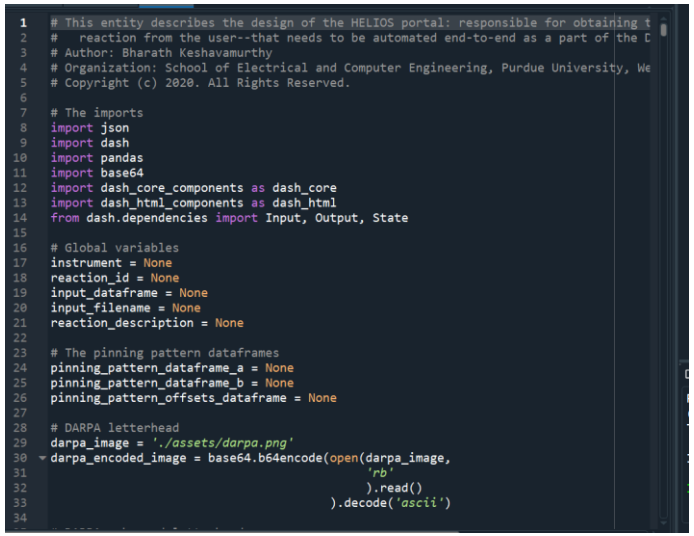
Fig. 3





```python
import threading
from time import sleep
from time import clock,sleep
from math import ceil
import sys
from pywinusb import hid
import re

class ProsoliaCoord:
    """ Prosolia class returns object of this class when querying about nozzle locati
        use accessor methods rather than direct attribute approach
        use constructor to assign initial values

    x = 0
    y = 0

 # constructer - this is the only way coordinates are to be set
    def __init__ (self,x= 0,y = 0):
        self.x = x
        self.y = y

 # x accessor method
    def getX(self):
        return self.x

 # y accessor method
    def getY(self):
        return self.y

class ProsoliaException(Exception):
    """ Class thrown when exception occurs in driver or when inconsistency detected "
    def __init__(self, message,eStatus = None):
        self.message = message
        self.status = eStatus
```

HELIOS (Python/Pearl)



```python
# This entity describes the design of the HELIOS portal: responsible for obtaining t
#   reaction from the user--that needs to be automated end-to-end as a part of the D
# Author: Bharath Keshavamurthy
# Organization: School of Electrical and Computer Engineering, Purdue University, We
# Copyright (c) 2020. All Rights Reserved.

# The imports
import json
import dash
import pandas
import base64
import dash_core_components as dash_core
import dash_html_components as dash_html
from dash.dependencies import Input, Output, State

# Global variables
instrument = None
reaction_id = None
input_dataframe = None
input_filename = None
reaction_description = None

# The pinning pattern dataframes
pinning_pattern_dataframe_a = None
pinning_pattern_dataframe_b = None
pinning_pattern_offsets_dataframe = None

# DARPA letterhead
darpa_image = './assets/darpa.png'
darpa_encoded_image = base64.b64encode(open(darpa_image,
                                            'rb'
                                           ).read()
                                      ).decode('ascii')
```

CHRIS (Python)

PURDUE UNIVERSITY

Automation: CHRIS and HELIOS

Purdue DARPA *Make It System*

Proposed Facility for High Throughput Synthesis and BioAnalysis using DESI-MS

R. Graham Cooks, Department of Chemistry, Bindley BioSciences Center and Center for Cancer Research, Purdue University April 18th, 2021

Summary: A facility is proposed in order to effectively utilize existing capabilities for small-scale, high-throughput molecular synthesis, rapid mass spectrometric analysis of small molecules and high throughput enzyme kinetics measurements. Capabilities developed over the past 5 years take the form of instrumentation, methodology and expertise acquired as the result of a DARPA grant of $8.5M through the "Make It" program (W911NF-16-2-0020 (W911NF-16-2-0020 U.S. Dept. of Defense/DARPA Analytic-directed Multi-scale Synthesis System Purdue University, PI R. G. Cooks). The establishment of this Facility will allow Purdue University scientists, as well as colleagues in the region and nationally, including colleagues in for-profit organizations, to have access to this unique capability with significant advantages in terms of discovering routes to molecular synthesis and of performing high-throughput chemical and biological analyses on multiple samples.

Current Resources, Instruments and Technology: Cooks lab will make available resources to fund the system operation in the six first months of the facility. After that, a gradual transition is proposed, where operational funds are requested (**Table 1**) and recharges are applied. The current system is composed of a i7 Biomek liquid handler (Beckman Coulter), a SCARA robot arm, a linear ion trap mass spectrometer (Thermo Fisher Scientific), a Synapt G2 mass spectrometer (Waters Inc), a DESI imaging source (Prosolia), and a DESI solvent delivery system of 16-channels (Elveflow). The system operation is integrated. Dedicated software for data acquisition and processing is used.

Proposed Organization: The DESI-MS facility would be a part of Bindley Bioscience Center. It is suggested that this facility be operated as a recharge center within MPF. The existing cores Advisory Committee of Bindley would have management and financial oversight over the High Throughput DESI-MS capability.

Request: The complete operational cost of the proposed new service comprises (i) half-time salary support for two graduate students ($64k/year) who will operate and maintain the system and (ii) operating funds of $60k/year (including a service contact of $19.5k/year on the Beckman i7 robot). Total request is $124k/year. We expect a gradual phase-in of activities, with time sharing between research of the Cooks group and service tasks. As a proposed timeline, the initial two years will be a transition period between the Cooks lab and the Bindley Bioscience Center, where in the first 6 months the requested amount is $0 and correspondingly, recharge access time on the system is 0 hours. After that, through year 2, a recharge increase to the full request of $124k and access full time of 40 hours week will occur (**Table 1**). During this 2-year period, the operating costs will be offset by recharges (see scenarios of cost recovery below). Industrial support in the form of a long-term loan of a $550k mass spectrometer has already been obtained from Waters Corp. An LTQ mass spectrometer (ca. $300k) is being provided by the Cooks group. Strong existing relationships with industry/national labs including ICASE, is expected to bring in clients for this unique facility.

After two years of operation, the system will continue to be managed by graduate students or a technician can be hired as management decides. NOTE: In addition to running experiments, the two graduate students are needed for instrumentation maintenance, calibration and occasional troubleshooting. Careful use of this very complex system by skilled operators (experienced grad students) is extremely important. A set aside of 20% of instrumentation time for use of the Cooks group is proposed.

Table 1. Proposed transition timeline for operation of the High Throughput Screening, Synthesis and Bioanalysis service in the Metabolite Profiling Facility. Over the initial period of 2 years, operating costs will be transferred from the Cooks lab to the Bindley Bioscience Center. Each phase will be 6 months in duration. The Bindley Bioscience Center will provide the funds requested and collect the recharge amounts.

Phases	Duration (Months)	Student Support	Operating support	Total funds requested	Expected cost recovery
Phase 1	6	$0	$0	$0	$0
Phase 2	6	$16K	$10K	$26K	$10K
Phase 3	6	$24K	$20K	$44K	$20K
Phase 4	6	$32K	$30K	$62K	*See* scenarios

Cost Recovery: To estimate recharge revenues after Phase 4, rates similar to the ones predicted by the Metabolite Profiling Facility are considered (**Table 2**). Recharge amounts are calculated in experimental units. Each **experimental unit** includes 1 DESI plate, 6h of labor for plate design, reagent preparation and data analysis support, 1h of fluid handling robot time, and 2h for MS data acquisition. For example, a 1-unit experiment, at the internal rate using the Synapt will cost $20 + (6 x $74) + $46 + (2 x $55) = $620. The instrument used for the experiments can also be the linear ion trap, depending on the user needs. Such an experiment would provide data on up to 6,144 reaction mixtures with MS and MS/MS confirmation and heat maps of reactivity. The same time, effort and cost would provide Michaelis-Menton plots on kinetics of reactions of an enzyme with multiple substrates as well as providing screening data on 62 inhibitors. Nonetheless, we predict each user needing multiple experimental units since enzyme activity study projects may include multiple plates to reach the number of needed replicates to get kinetics of a single substrate, IC50, dose-response, inhibition, etc.

Table 2: Possible recharge rates for the liquid handling robot, the two mass spectrometers and the labor and DESI Plate costs for the High Throughput Screening, Synthesis and Bioanalysis service in the Metabolite Profiling Facility.

Service	Unit	Internal Rate	Consortium Rate	ENP Rate (External Non-Profit)	EFP Market Rate (External For-Profit)
Biomek i7 (liquid handler)	hourly	$46	$31	$86	$92
Linear Ion Trap	hourly	$46	$31	$95	$102
Synapt G2	hourly	$55	$40	$85	$91
Labor	hourly	$74	$42	$138	$147
DESI Plate	1	$20	$16	$33	$35

We present here three simulated scenarios, which can be updated as the plan evolves. The first scenario (low level of cost recovery) foresees only 5 experimental units/month (60/year). A low number considering we expect high interest from industry in exploring DESI-MS as high throughput strategy for lead compound route discovery, as well as internal interest for the exploration of the chemical reactivity

space and the development of label-free biological assays. Potential external and internal users are included in **Appendix 1** and **Appendix 4**, respectively.

Table 3. *Scenario 1*: Low (total 60 experiment units/year, 67% internal/consortium usage)

Service	Internal	Consortium	External Non-Profit	External For-Profit	Recharge
Biomek i7	20	20	10	10	$3,320
Linear Ion Trap	10	10	5	5	$1,755
Synapt G2	10	10	5	5	$1,830
Labor	120	120	60	60	$31,020
DESI Plate	20	20	10	10	$1,400
				Total	**$39,325**

The second scenario (ideal cost recovery) is where the operational costs are covered by the usage. This scenario considers more external customers, represented by other research institutions and industry.

Table 4. *Scenario 2*: Optimal (total 120 experiment units/year, 33% internal/consortium usage)

Service	Internal	Consortium	External Non-Profit	External For-Profit	Recharge
Biomek i7	20	20	40	40	$8,660
Linear Ion Trap	10	10	20	40	$6,750
Synapt G2	10	10	20	40	$6,290
Labor	120	120	240	240	$82,320
DESI Plate	20	20	40	40	$3,440
				Total	**$107,460**

The third scenario is where over 15 experimental units would occur every month, with still higher usage by external customers. In this scenario it would be necessary to consider a new hire to provide more technical assistance even though the instrumentation would still be underused.

Table 5. *Scenario 3*: High (total 200 experiment units/year, 40% internal/consortium usage)

Service	Internal	Consortium	External Non-Profit	External For-Profit	Recharge
Biomek i7	40	40	40	80	$13,880
Linear Ion Trap	20	20	20	80	$11,600
Synapt G2	20	20	20	80	$10,880
Labor	240	240	240	480	$131,520
DESI Plate	40	40	40	80	$5,560
				Total	**$173,440**

Support of this Request: This request is being discussed with the director of the Bindley Bioscience Center, Prof. Ramaswamy Subramanian, and with the acting director of the Center for Cancer Research, Prof. Andy Mesecar. Further discussions with leadership of PI4D and the Drug Discovery Center are planned. Several faculty members have also expressed interest as potential users of the system (*see* **Appendix 4**).

Rationale: Considerable gains in efficiency and savings costs are achievable by incorporating the proposed High Throughput Screening, Synthesis and Bioanalysis service within the existing Metabolite Profiling Facility. These savings include those from a single management function and those resulting from sharing of instrumentation scientists who can assist in maintenance and upgrades to multiple instruments. The advantages of establishing this service for future federal and industrial funding opportunities are clear from the interested parties lists given in **Appendix 1** and **4**. The proposed new service will further increase the strength of the programs in measurement science and instrumentation currently being implemented through the Indiana Consortium for Analytical Sciences and Engineering (ICASE), as a regional resource involving Notre Dame and Indiana University as well as Purdue University. The system will be also demonstrated at the annual meeting of the Center for Analytical Instrumentation Development (CAID) (https://www.purdue.edu/discoverypark/caid/events/annual-meeting/index.php) to over 100 participants (most external).

Capabilities offered: Three main capabilities will be offered. These are (i) reaction screening, (ii) small scale synthesis and (iii) bioanalysis. **Reaction screening** uses low nanogram samples of mixtures in arrays (up to 6,144) and automatically records mass spectra which are represented in appropriate formats (e.g. heat maps). Confirmatory MS/MS analysis can be performed on the same spots after the initial MS is recorded. These experiments can cover multiple variables (stoichiometry, solvent, pH, catalyst) and quantitation can be performed using internal standards. Temperature can be used as a variable at lower densities. The reactions occur on the millisecond time scale because of acceleration in droplets, and previous experience suggests that the screening data transfer well to flow and bulk synthesis. **Small scale synthesis** involves collecting the spray from the DESI impact. This made-to-measure experiment can be followed by bioassays on the small reaction product. Alternatively, a larger scale synthesis (e.g. 100 mg) can be performed by DESI spray with re-cycling of solvent. **Bioanalysis** includes several types of bioassays which can be implemented using the DESI system, with enzymatic assays being the best studied. This approach has the advantage of being completely label free and requiring less than 1 second per assay (no sample work-up at all, the entire enzyme/substrate/product/buffer mixture is directly analyzed by DESI). In spite of the small sample sizes coefficients of variation are small (ca. 10 %) and kinetic assays can be completed in times on the order of 25 min for 384 samples, including multiple replicates.

Appendices:

1. Table of Potential Collaborators and Users
2. Development Timeline and Overview of High Throughput System
3. Literature and Capabilities of System
4. Potential Internal Users of the System