National Cancer Institute

The NCI Natural Product Library for Antimicrobial Discovery

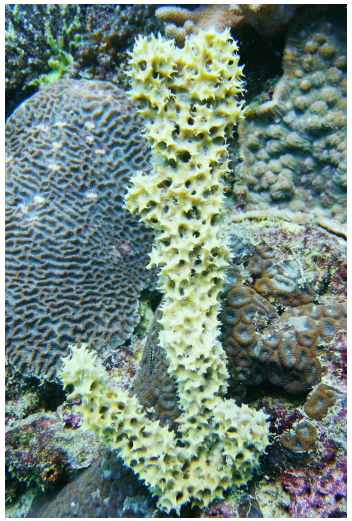
Barry R. O'Keefe

Chief, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis and Acting Chief, Molecular Targets Program, Center For Cancer Research, National Cancer Institute, National Institutes of Health, USA

NIAID, Rockville, MD, August 27, 2018

National Cancer Institute

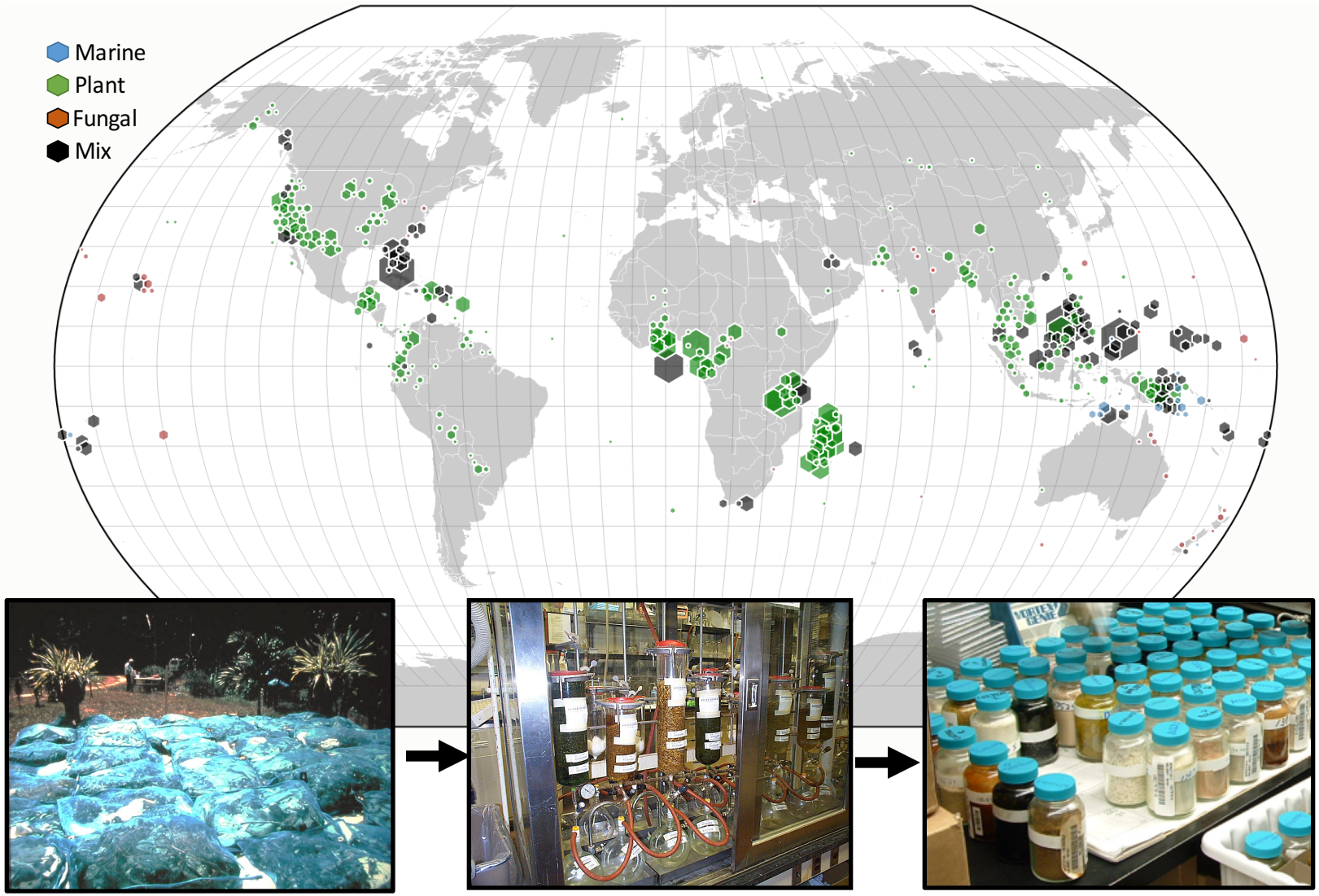
Natural Products Branch Responsibilities



- Collection of Source Organisms
- Growth of Microbial Source Organisms
- Sample Processing and Extraction
- Provision of Vialed and Plated Extracts
- Preparation of Test Samples for NCI-60 Screening
- Isolation and Identification of Active Compounds from Selected Extracts
- Re-isolation of Bulk Quantities of Natural Products
- Maintenance of Appropriate Data Fields on Source Organisms and Transferred Extracts

National Cancer Institute

NCI Natural Product Collections



National Cancer Institute

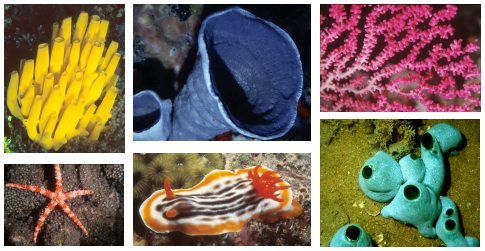
NCI Natural Products Repository

The NCI has one of the world's largest, most diverse collections of natural product extracts (>230,000 extracts).

Plant Extract Library

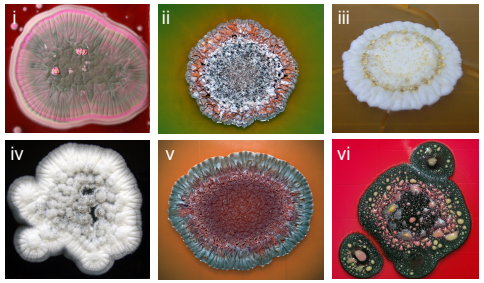
- **~161,000** extracts (organic + aqueous)
- **~44,000** plants, including 81,400 raw materials (leaves, roots, fruit, etc.) collected from Africa and Madagascar; North, Central and South America; and Southeast Asia.

Marine Extract Library



- **~41,000** extracts (organic + aqueous)
- **~20,500** organisms collected from the Indo-Pacific region.

Microbial Extract Library

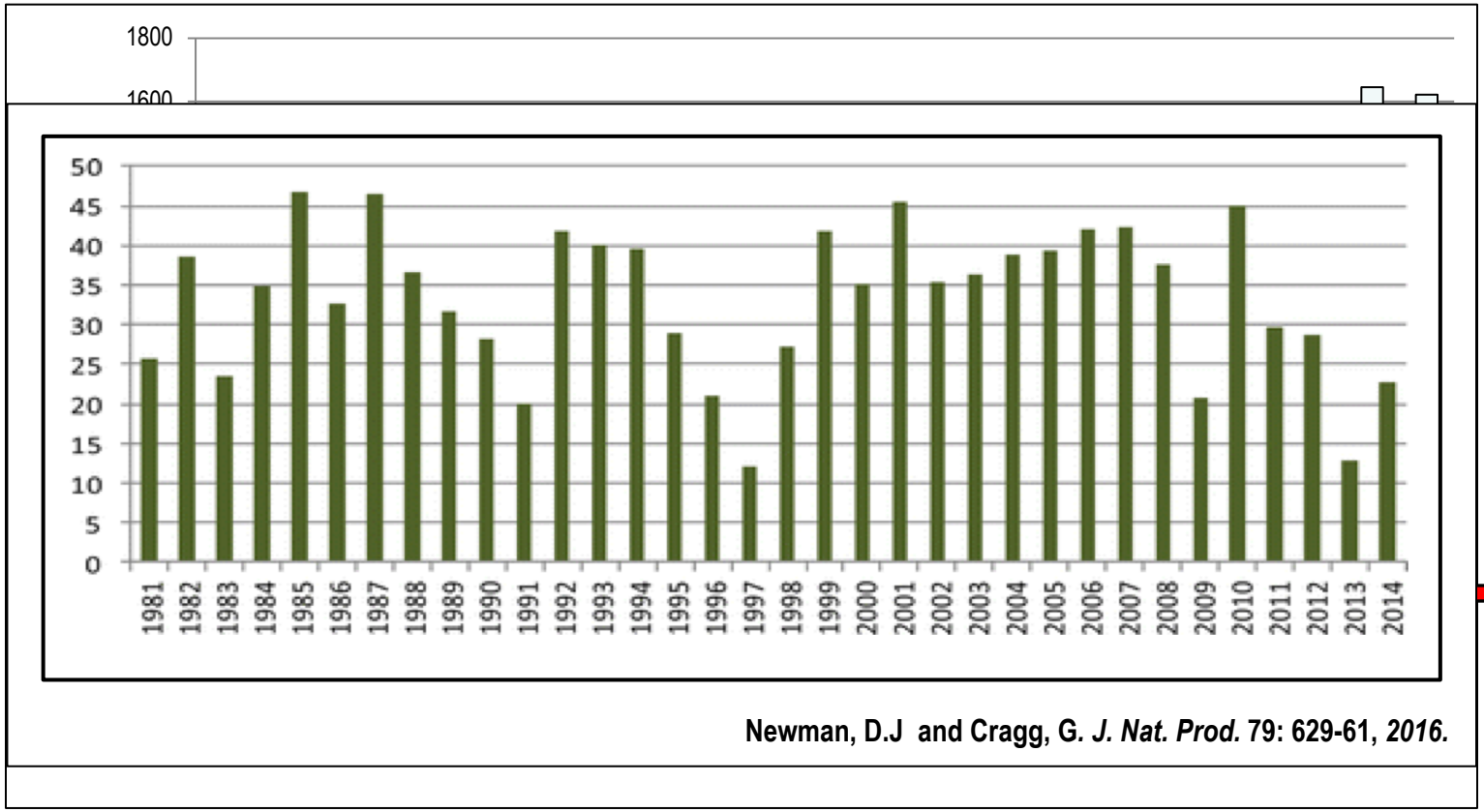


- **~30,000** extracts (organic + aqueous)
- **~16,000** organisms:
  - 4,160 unique genus and species
  - 7,600 unknown organisms

National Cancer Institute

Natural Product-derived Drugs and Screening

PubMed Citations for HTS and Natural Products



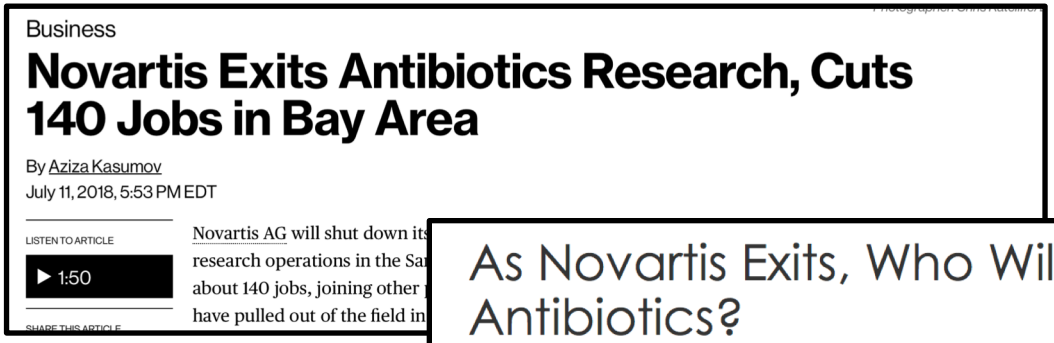
Newman, D.J and Cragg, G. *J. Nat. Prod.* 79: 629-61, *2016.*

Henrich, C.J., Beutler, J.A. *Nat Prod Rep*. 30(10):1284-98, 2013.

National Cancer Institute

Antibiotic and Antiviral Research Increasingly needs to be Supported

- The pharmaceutical industry continues to reduce its efforts in anti-infection research and development



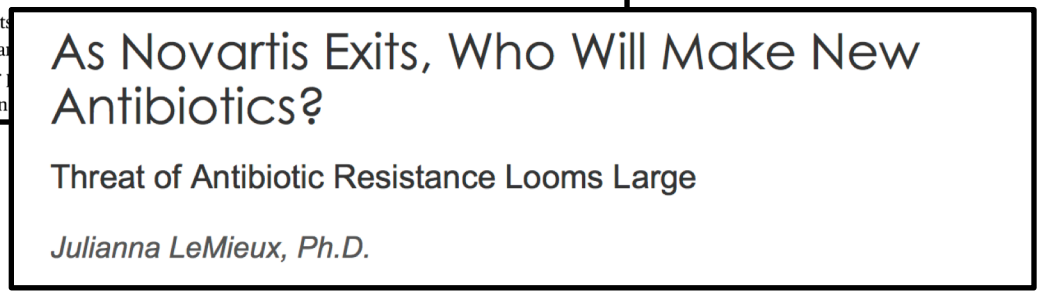
Business

Novartis Exits Antibiotics Research, Cuts 140 Jobs in Bay Area

By Aziza Kasumov
July 11, 2018, 5:53 PM EDT

LISTEN TO ARTICLE
▶ 1:50
SHARE THIS ARTICLE

Novartis AG will shut down its research operations in the San about 140 jobs, joining other have pulled out of the field in



As Novartis Exits, Who Will Make New Antibiotics?

Threat of Antibiotic Resistance Looms Large

Julianna LeMieux, Ph.D.

Novartis is not the first big pharma company to leave antimicrobial research, with similar decisions having been made in the past by Bristol-Myers Squibb and AstraZeneca. Even Eli Lilly, one of the first companies to manufacture penicillin in the 1940s, followed by decades of continued success with the development of top-selling antibiotics such as vancomycin, erythromycin, Keflex, and Ceclor, has gotten out of the game.

National Cancer Institute

Need for Improved Capabilities for Natural Product Research

- How to best use the NCI natural products repository
  - Over 40% of clinically used anti-cancer chemotherapeutics are natural products or derived from natural products (1981-2014)[1]
  - Less than 1% of high-throughput screening programs reported over the last 15 years have screened natural product extracts.
  - NCI natural product repository currently underutilized for drug discovery due to extract complexity and outdated technologies.
  - How best to increase utilization of the NCI repository of extracts and encourage inclusion of natural products in high-throughput screening?
    - Screening challenges
    - Isolation, structure elucidation
    - Resupply

- **Over 50% of clinically used antimicrobials are natural products or derived from natural products (1981-2014)[1]**

[1]Newman, D.J and Cragg, G. *J. Nat. Prod.* 79: 629-61, *2016.*

National Cancer Institute

Natural Product Research and the NIH Intramural Program

- Natural product Research has been declared a “Core Scientific Opportunity for the Future”

To remain a leading academic center of excellence, and to clearly define the long-term vision for the IRP, we have identified 12 core scientific opportunities for the future. These opportunities were selected following an extensive review process conducted by investigators from across the IRP, and were chosen based on their potential to transform the healthcare field and take advantage of the diversity and scope of the IRP’s cross-disciplinary resources, infrastructure, and scientific expertise.

Uncovering New Opportunities for Natural Products

National Cancer Institute

NCI Program for Natural Products Discovery

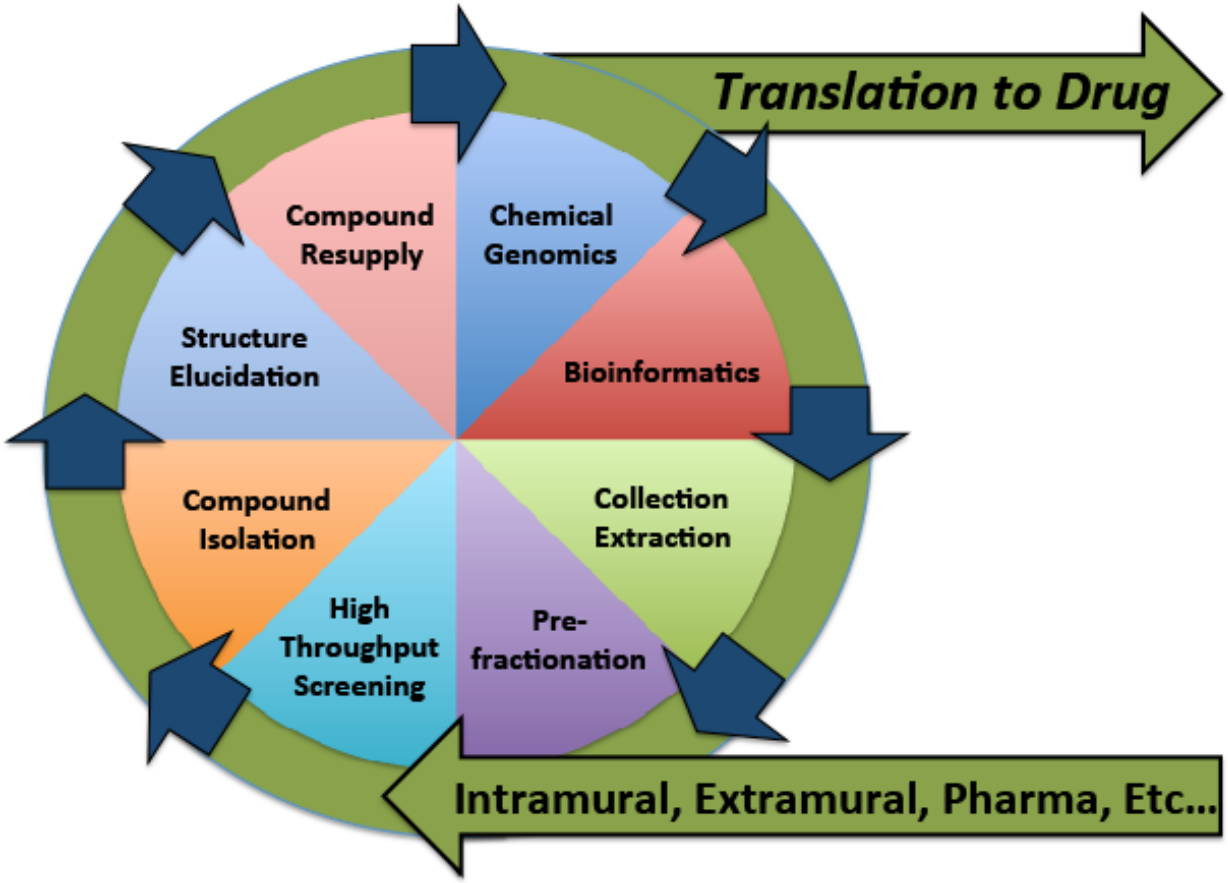
- **The NCI Program for Natural Products Discovery (NPNPD) is a joint effort of the Division of Cancer Treatment and Diagnosis and the Center for Cancer Research.**

- The NPNPD is designed to facilitate both intramural and extramural research and address current challenges in natural product based drug discovery.

National Cancer Institute

NPNPD Design

Functional Scope of the NCI Program for Natural Product Discovery



National Cancer Institute

NPNPD Cancer Moonshot Project Specific Aims

Co-PIs: Drs. Joel Schneider and Barry O'Keefe

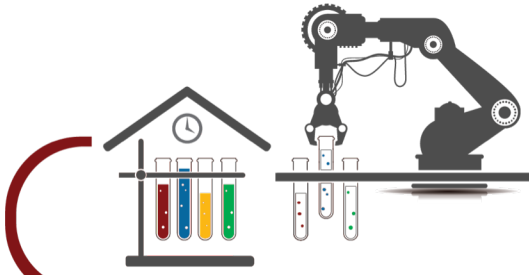
Specific Aims

- Aim 1. Create new technologies to build an enhanced NP pre-fractionated library amenable to modern high-throughput targeted screening programs.
- Aim 2. Expand the chemical diversity available to the public from culturable microorganisms with new methods and libraries.
- Aim 3. Provide the pre-fractionated library to screening centers worldwide to accelerate drug discovery.
- Aim 4. Encourage high throughput screening support for researchers to enable targeted discovery efforts.
- Aim 5. Provide faster analytical resources (isolation, structure elucidation, re-supply) to expedite translational pipelines.
- Aim 6. Establish a public database and bioinformatics platform to broaden input and expand impact.

National Cancer Institute

Prefractionation Plans

- Creation of a ~1,000,000 fraction library of semi-pure natural product samples more amenable to modern screening technologies.
- Provide a “value added” screening library unlike anything currently publicly available to encourage increased use of natural product chemical diversity.
- Use of the pre-fractionated library should improve the efficiency of both high throughput screening and subsequent chemistry efforts.
- Practical considerations
  - Need to produce >150,000 fractions per year
  - Fractions must have a defined weight
  - Sufficient mass to support screening programs for 10 years
  - Storage must allow for rapid automated access



NPNPD Fraction Library

National Cancer Institute

Prefractionation Method Development

I. Extract Sources and Fractionation System

- **Plant Organic (4):**
  - *Rubiaceae Exostema caribaeum* (stem bark)
  - *Euphorbiaceae Fluegga virosa* (root)
  - *Olacaceae Olax scandens* (leaf)
  - Proteaceae Conospermum stoechadis (leaf & stem)
- **Marine Aqueous (3):**
  - *Poritidae Goniopora lobata* (stony coral)
  - *Desmacellidae Biemna* sp. (sponge)

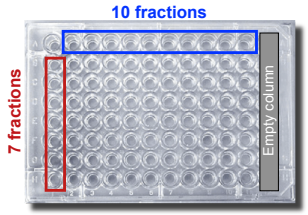
- **Marine Organic (3):**
  - Codiaceae Codium fragile (seaweed)
  - Blue-green algae (cyanobacterium)
  - Plakortis lita (sponge)
- **Microbial Organic (3):**
  - Actinomycete (bacterium)
  - *Penicillium duclauxi* (fungus)

- The result was >2000 individual fractions that were all tested in 3 biochemical assays, the NCI-60 cancer cell cytotoxicity assay and a >200 parameter high content imaging assay.
- Fractions were tested for mass, % phenolics and total yield.
- Fractions were also analyzed by LC/MS for principal component analysis.

Layered SPE column (C4/HLB) showing differential binding of analytes.

III. Solvent Schemes Investigated

- **Normal Phase**
  Hexanes, CH_2Cl_2, EtOAc, MeOH:
  - 2 solvent systems for 7 total fractions
  - 2 solvent systems for 10 total fractions



Number of fractions were determined by 96-well and 384-well plate formats.

- **Reverse Phase**
  MeOH / H_2O vs MeCN / H_2O:
  - 2 solvent systems for 7 total fractions
  - 2 solvent systems for 10 total fractions

National Cancer Institute

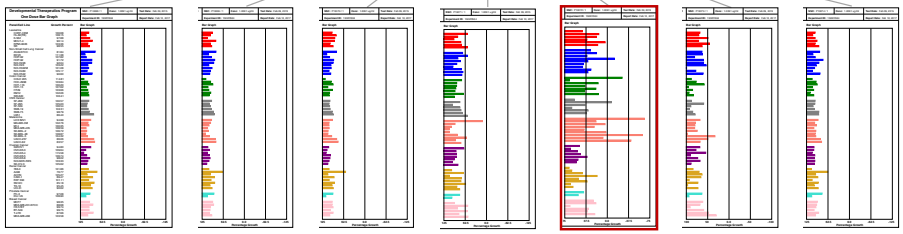
Pre-fractionation Method Evaluation

1 Mass Distribution

***Fluegga virosa* (N15197)**

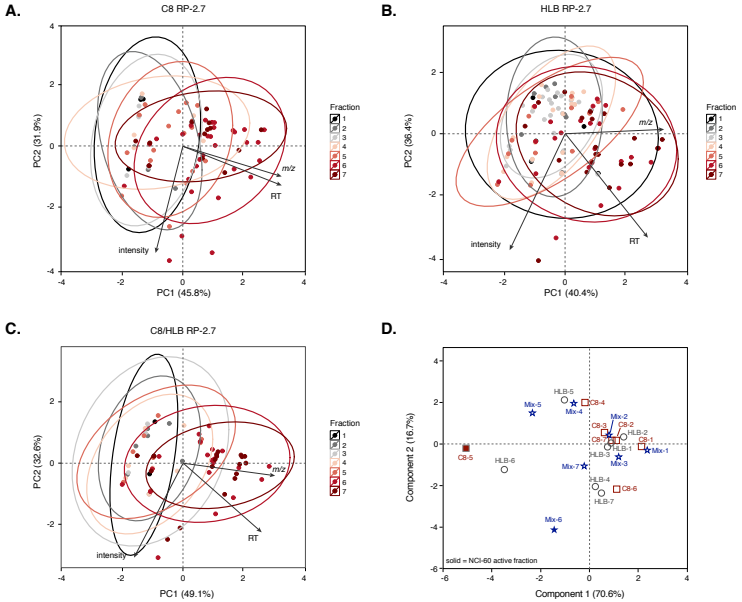
	F1	F2	F3	F4	F5	F6	F7
Diol NP-1.7	5.6	4.0	15.2	3.6	13.2	75.5	32.3
Diol NP-2.7	0.3	6.5	7.2	3.6	3.9	29.1	97.8
HLB RP-1.7	52.6	18.2	18.8	7.5	4.7	9.2	11.6
HLB RP-2.7	45.9	4.2	4.2	8.5	10.3	13.6	19.8
C8/HLB RP-1.7	39.4	44.0	19.1	6.9	1.7	7.3	9.3
C8/HLB RP-2.7	36.0	3.8	18.5	19.4	14.5	18.4	14.8
C8 RP-1.7	71.7	60.4	6.0	3.7	4.1	7.7	11.6
C8 RP-2.7	66.4	10.8	41.1	22.0	7.4	18.3	7.9

2 Bioassay Data



C8 RP-2.7 / F 1 | F 2 | F 3 | F 4 | F 5 | F 6 | F 7

3 LC/MS-PCA

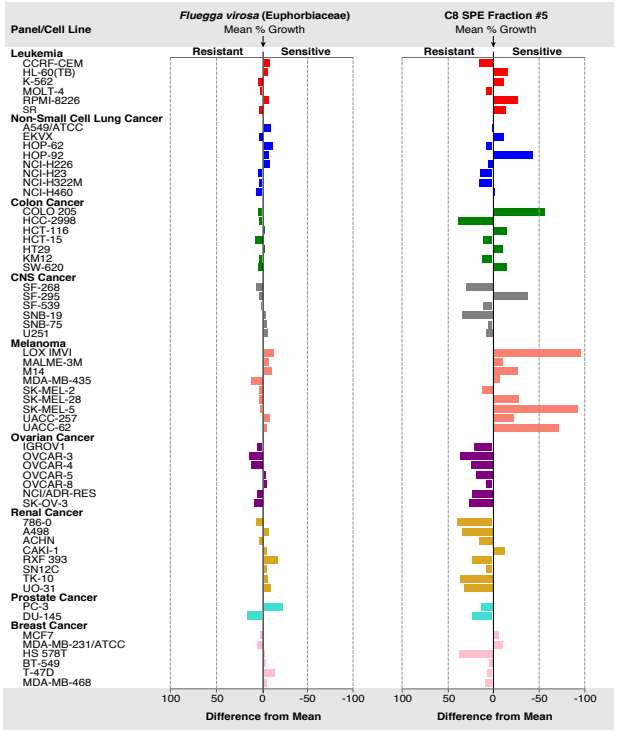


National Cancer Institute

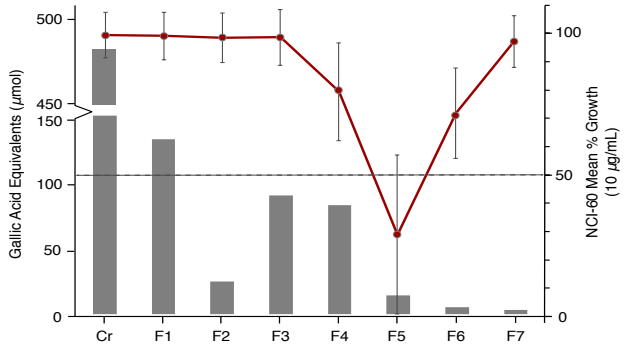
Pre-fractionation Method Outcomes

A.

***Fluegga virosa* (plant)**

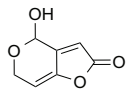


B.



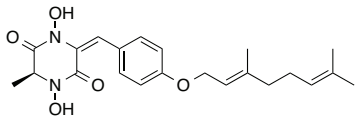
***Penicillium griseofulvum* (fungus)**

F1, F2 and F3

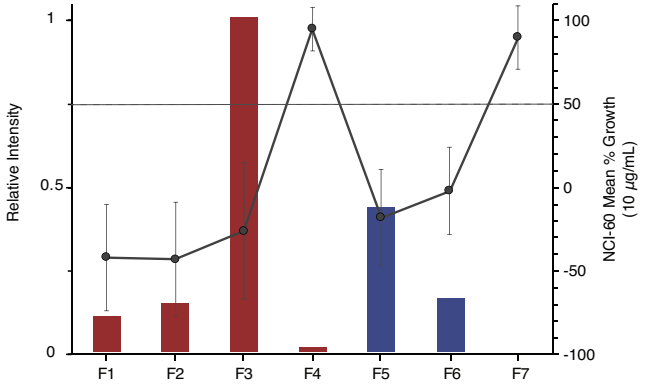


3

F5 and F6



4

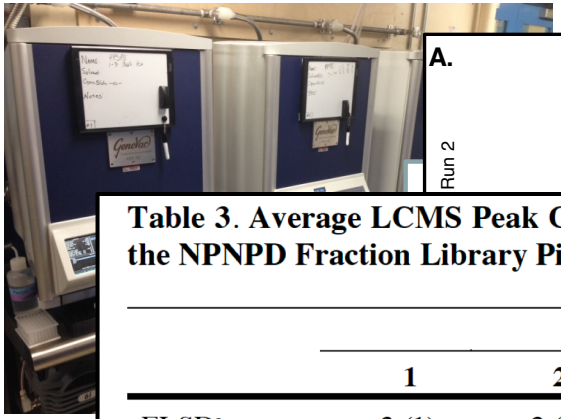


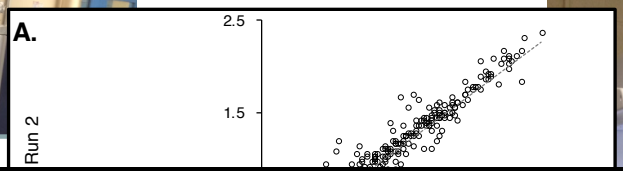
Thornburg, C. *et al. ACS Chem. Biol.*. 2018.

National Cancer Institute

NPNPD Pre-fractionation Automation

Initial load of 250 mg organic extracts, 500 mg of aqueous extracts
~90% of resulting fractions contain >2 mg mass
~90% recovery of mass overall for organic extracts





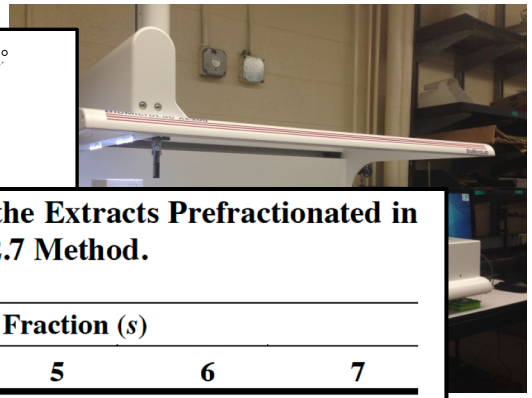
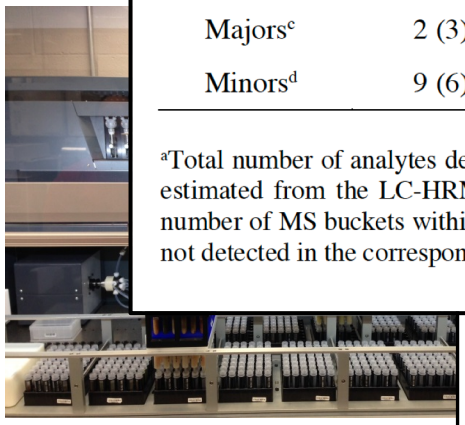
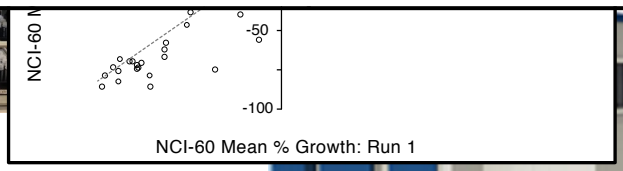


Table 3. Average LCMS Peak Count by Fraction for All of the Extracts Prefractionated in the NPNPD Fraction Library Pilot Study Using the C8, RP-2.7 Method.

	Avg Peak Count per C8 SPE Fraction (*s*)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
ELSD[a]	2 (1)	2 (2)	2 (2)	4 (3)	7 (3)	11 (8)	9 (8)
Total MS[b]	11 (7)	9 (8)	12 (8)	22 (16)	43 (44)	40 (34)	23 (12)
Majors[c]	2 (3)	2 (3)	3 (3)	7 (7)	17 (21)	12 (12)	6 (3)
Minors[d]	9 (6)	8 (5)	9 (6)	15 (13)	26 (25)	28 (27)	17 (11)

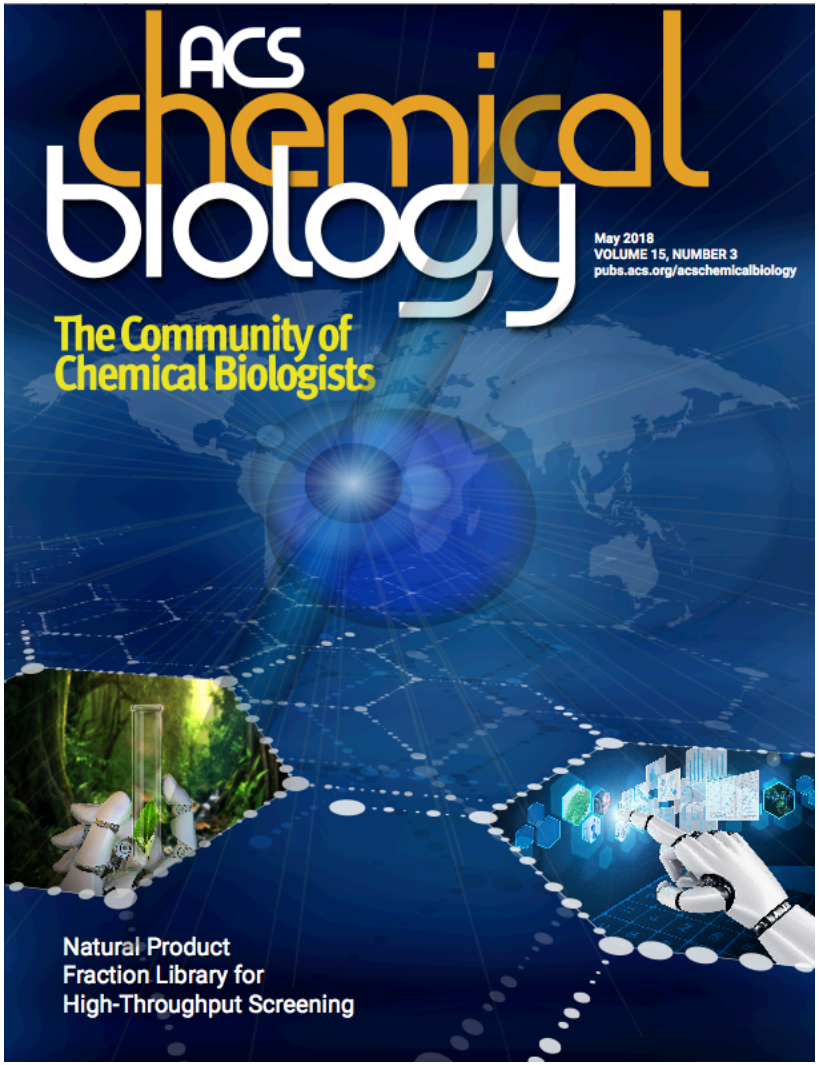
[a]Total number of analytes detected using an evaporative light scattering detector (ELSD). [b]Total number of analytes estimated from the LC-HRMS data and defined by *m/z* value, retention time and intensity (MS buckets). [c]Total number of MS buckets within each detectable ELSD retention time window. [c]Total number of MS buckets that were not detected in the corresponding ELSD chromatogram. Standard deviation (*s*).





National Cancer Institute

NPNPD Pre-fractionation

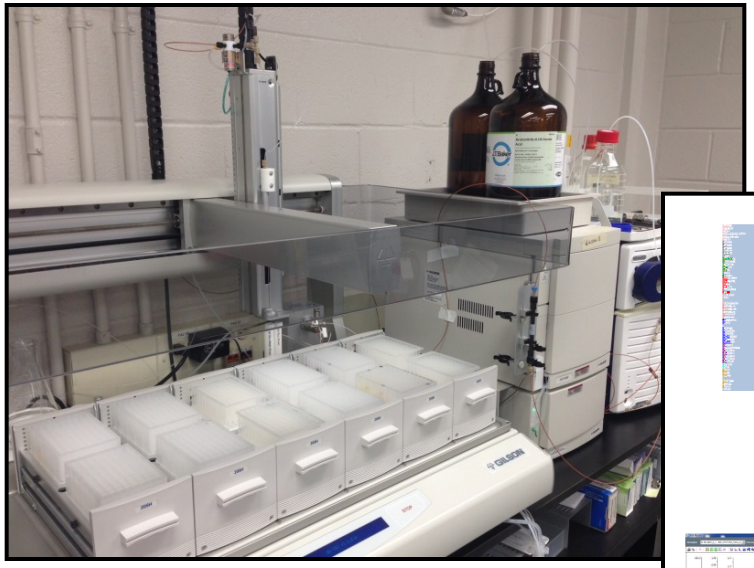


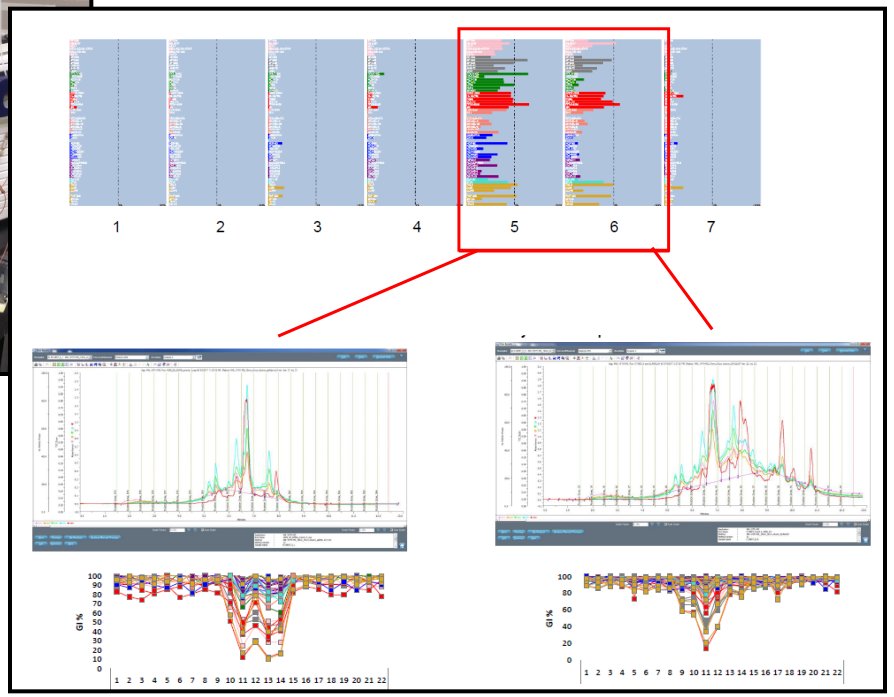
September 2018, ACS Chemical Biology Cover Article

National Cancer Institute

NPNPD High Throughput Secondary Separations

- Goal is to be able to get sub-fractions back to screening labs quickly (>200/week)
- Each active fraction (1 mg) will be further separated into 22 sub fractions
- One system run can purify 44 fractions in 13 hrs (~1000 sub-fractions/day)
- Analytical data (UV-MS and ^{1}H NMR) gathered and stored for active samples
- Allows for rapid de-replication and compound identification

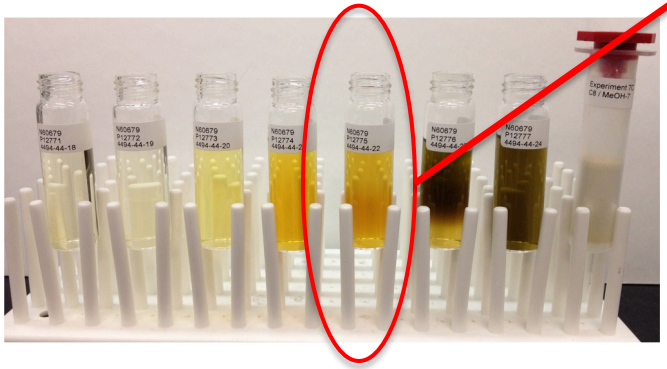


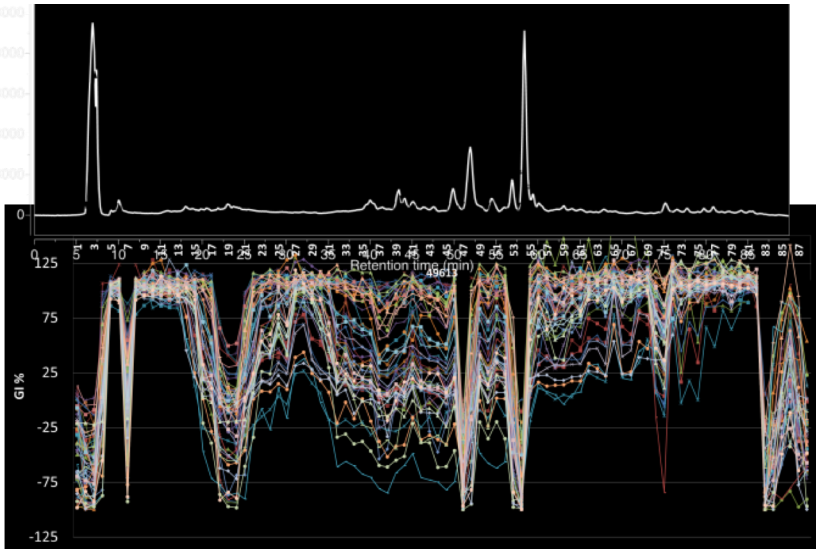


National Cancer Institute

Advantages of Rapid Secondary Active Fraction Purification

- Automated 2nd–step chromatography systems will process >200 samples per week, gather necessary analytical data and return further fractionated samples to screening laboratories.
- Improve speed and efficiency of “hit” confirmation by screening laboratories.
- Assist both intramural and extramural screening laboratories in the identification of active constituents.
- Add valuable chemical information to annotation of active samples in NCI repository
- Minimize waste of extracts.





National Cancer Institute

New Collections Coming to the NCI Repository

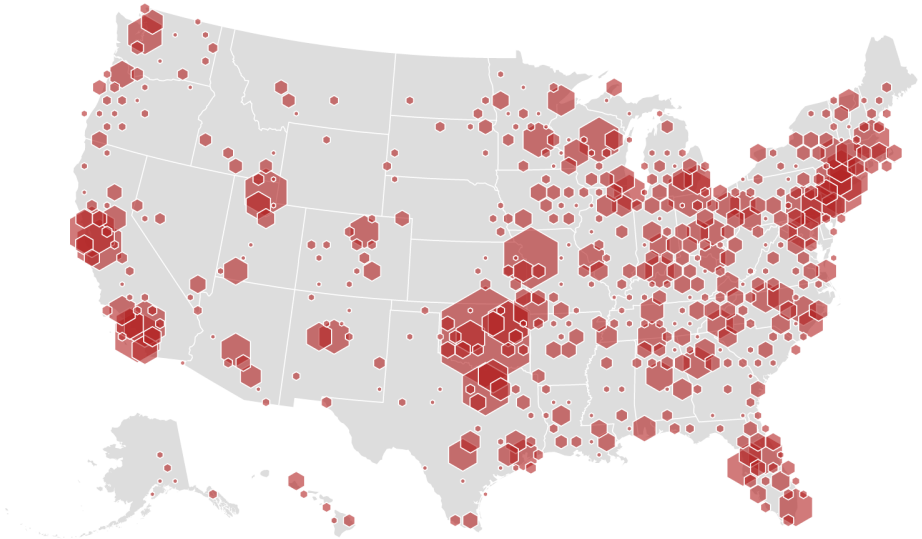
- **Recent Agreement with the Australian Institute of Marine Science (AIMS)**

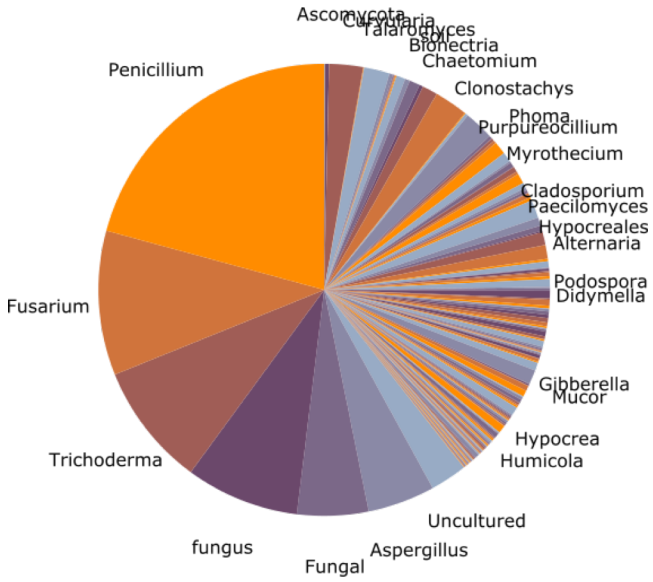
  - >4000 new marine macro-organisms from Australian waters have been shipped to the NCI (~2000 porifera)
  - NCI will extract source material and split extracts between NCI collection and AIMS-selected Australian research institute (Griffith Institute for Drug Discovery - GRIDD)
  - Extracts will be made available to researchers under the normal benefit sharing requirements for access to NCI collections
  - ~7700 marine microorganisms will also be brought to the NCI
    - ~1100 actinomycetes, 1450 fungi, 5000 other eubacteria
    - These will need to be sequenced for taxonomy
    - NCI will culture and extract the organisms and again split the resulting extract with GRIDD

National Cancer Institute

New NPNPD Microbial Collections

- Citizen Science partnership with the University of Oklahoma
- Up to 4000 new, ITS sequenced, fungi per year from around the United States
- Improved biological diversity for extract preparation
- Source organisms as well as prefractionated extracts provided
- Improved automated culture conditions to increase throughput and yield

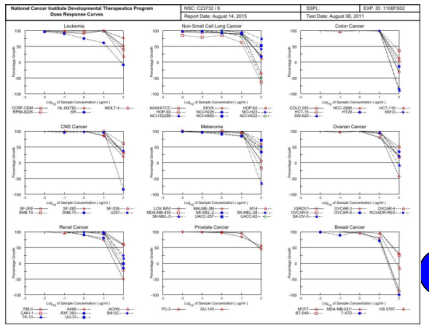


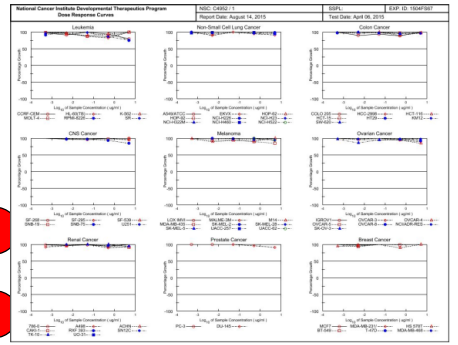


National Cancer Institute

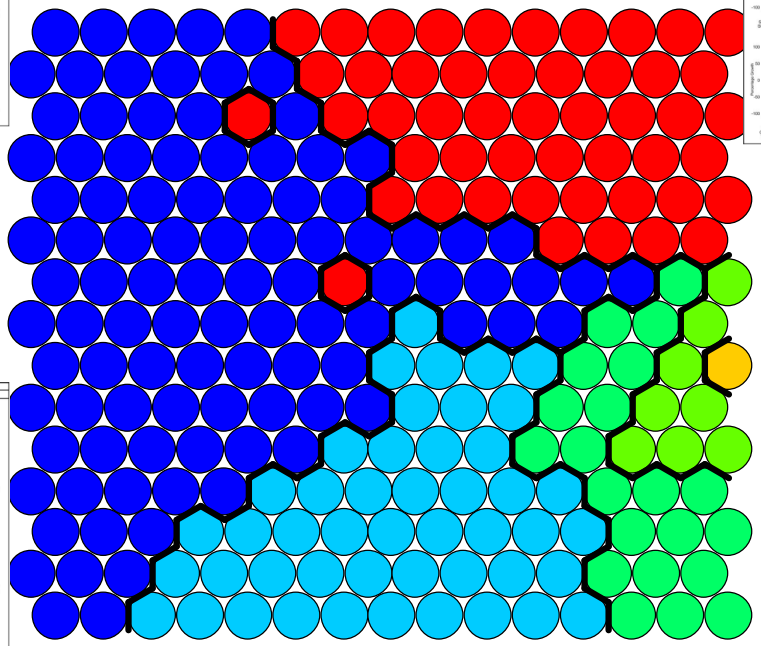
NPNPD Bioinformatics and Self-organizing Map (SOM) Technologies

- 15 x 15 matrix of nodes
- 2205 data points (~10/node)

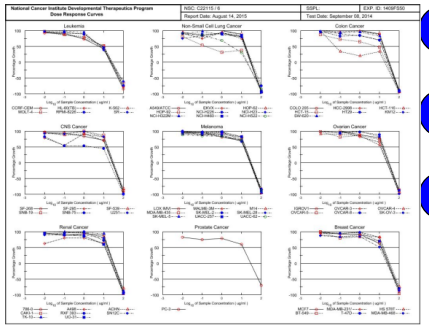


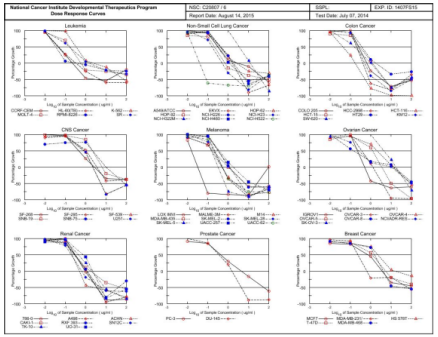


Clusters



Increased lethal response



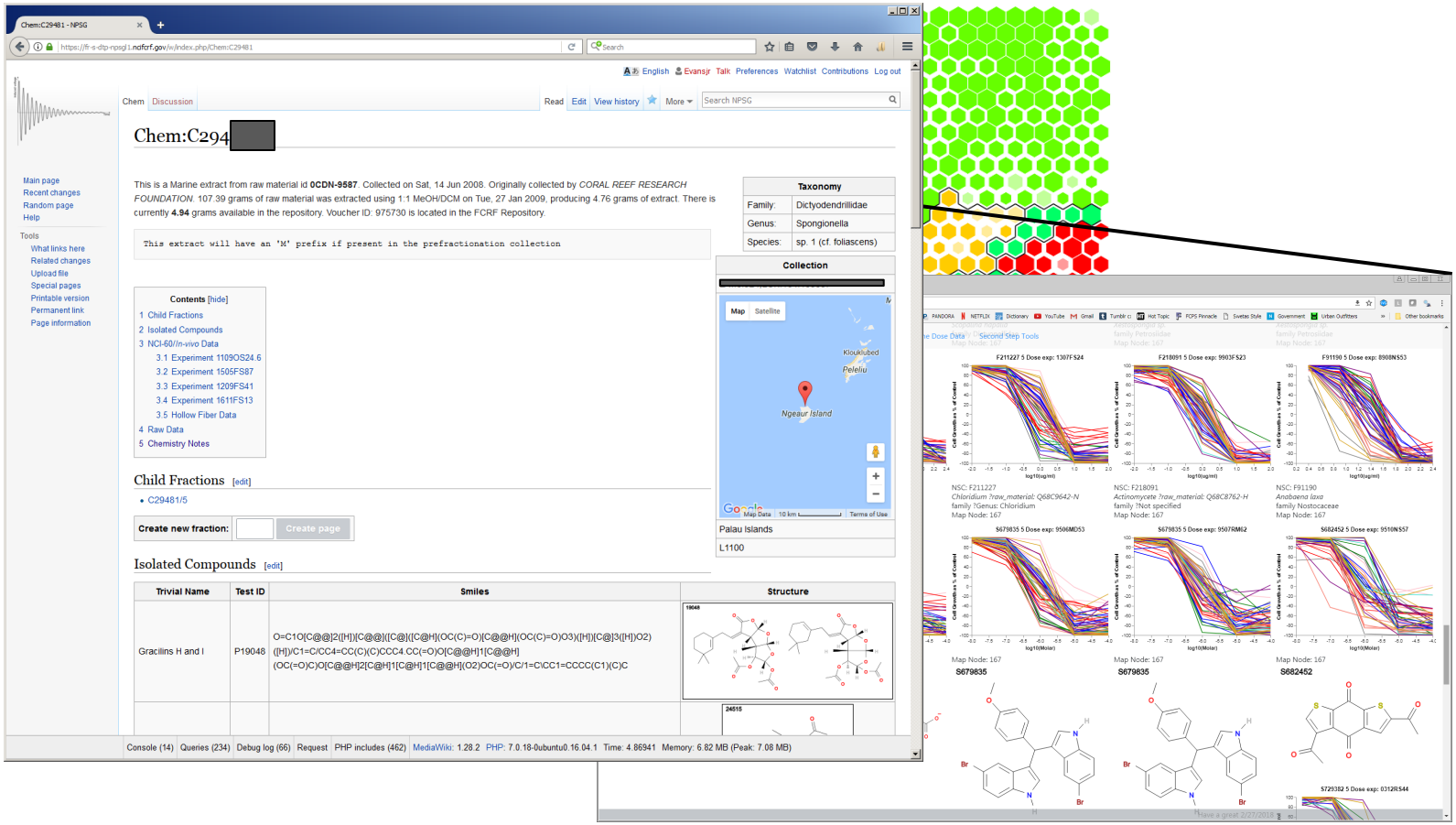


Lower initial concentration for response

National Cancer Institute

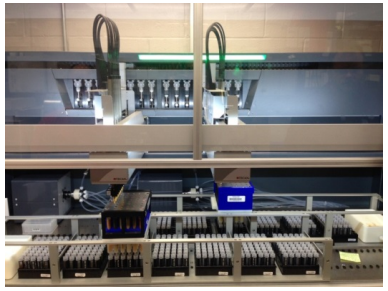
NPNPD Bioinformatics and Self-organizing Map (SOM) Technologies

- Places natural product samples in “biological and chemical space”
- Can be parsed by assay, cell type, source organisms or chemical structure
- Will be broadly available on line through a web interface
- Extract Wiki pages with all pertinent information including data files



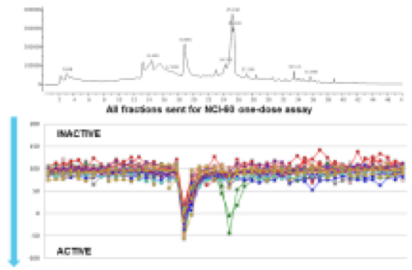
National Cancer Institute

NPNPD Compound Isolation/Identification

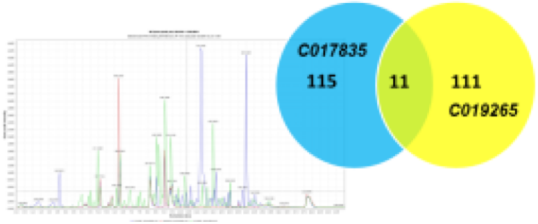


Prefractionation and activity assessment

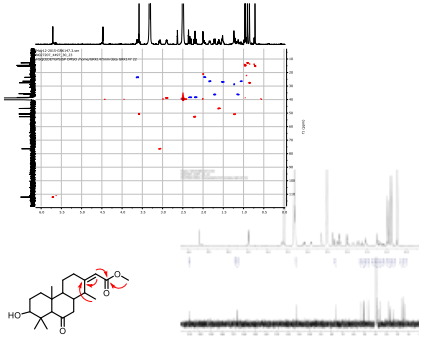
SOM-based analysis



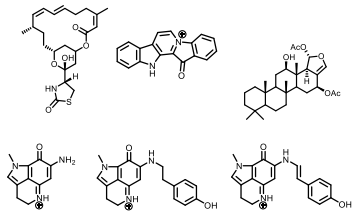
2nd stage HPLC/MS and activity screening



Dereplication and spectral analysis



Structure elucidation

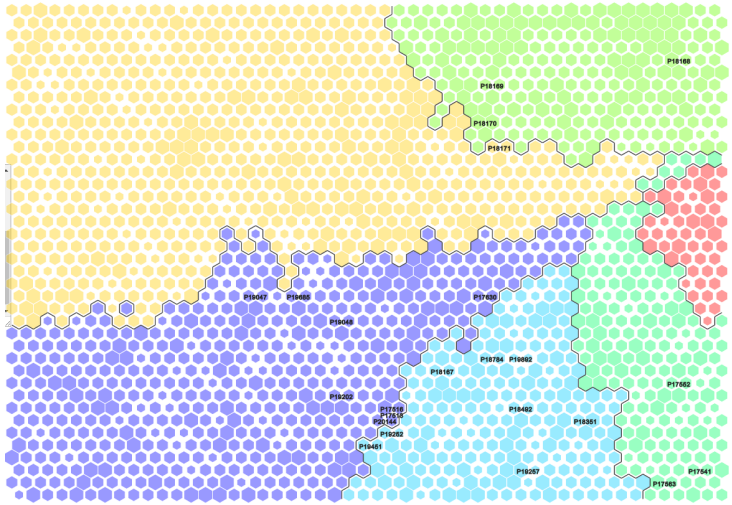


Pure compounds

National Cancer Institute

A NPNPD Pilot Study

34 Marine Organic Extract Projects Selected Based on NCI-60 SOM

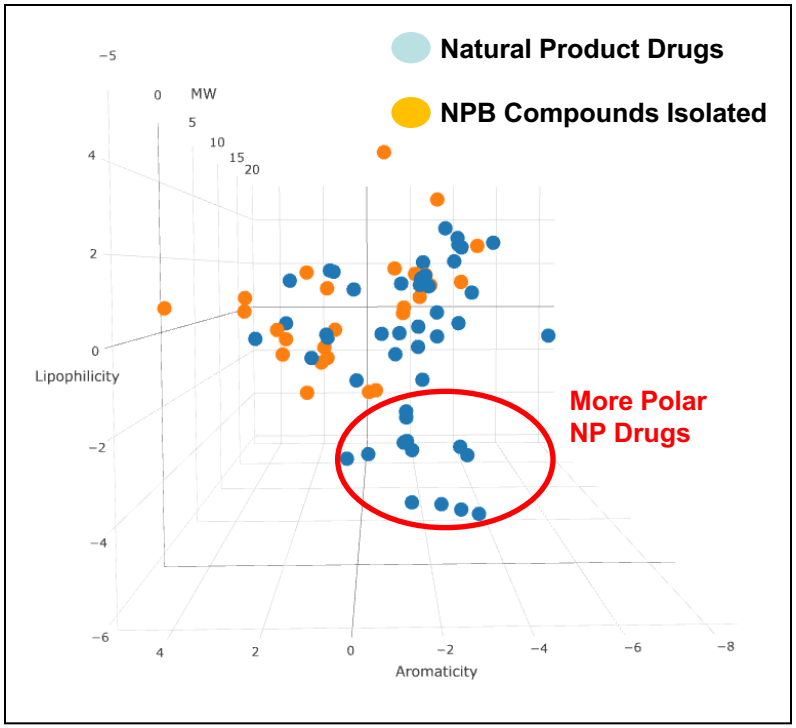


~3 Months

- **26 bioactive natural products identified**
- **3 new natural product structures determined**

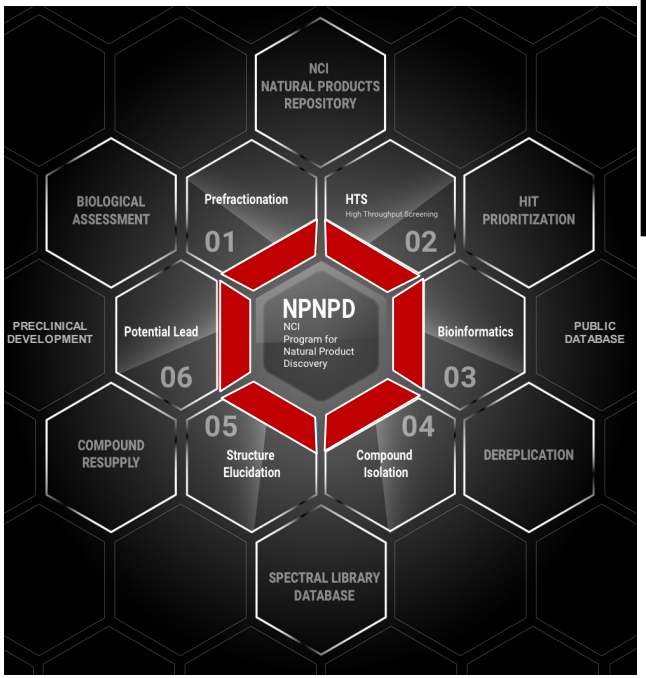
Us of ChemGPS* to compare chemical space between FDA approved natural products and compounds identified by NPNPD modernized infrastructure

***Larsson et al., J. Nat Prod. 2007**



National Cancer Institute

NCI Program for Natural Product Discovery



Initial prefractionated samples (~150,000) and secondary fractionation of NCI fractions should become available in January 2019.

National Cancer Institute

NCI Program for Natural Product Discovery

- Antimicrobial discovery and development has been and continues to be a unique area of productivity for natural product research.
- To get the maximum yield form NIH's investment in natural product research the involvement of scientists working on infectious disease targets is important.
- **So, how can we work together with NIAID to encourage the use of this new NCI resource to identify new antibacterial and antiviral agents?**
  - Extramural funding opportunities for NIAID grantees
  - Intramural research efforts