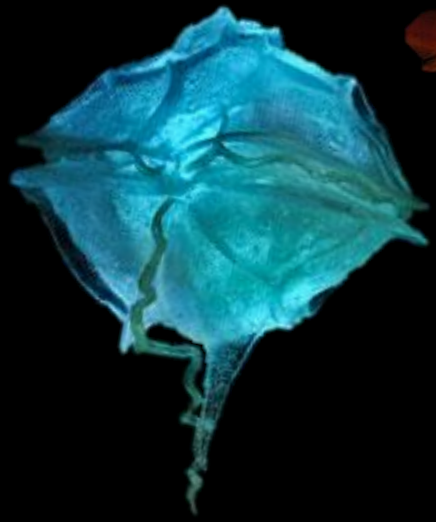
Battling the Superbugs: New Drugs from the Sea

Ecologically-inspired drug discovery

Dr. Kristen Whalen

Marine Chemistry & Geochemistry

Woods Hole Oceanographic Institution



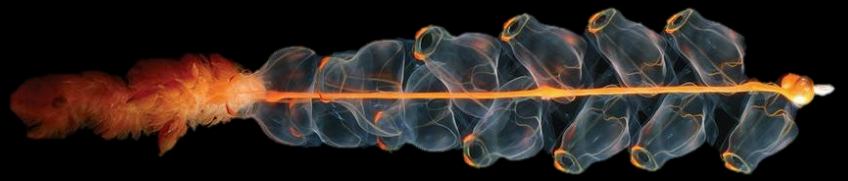
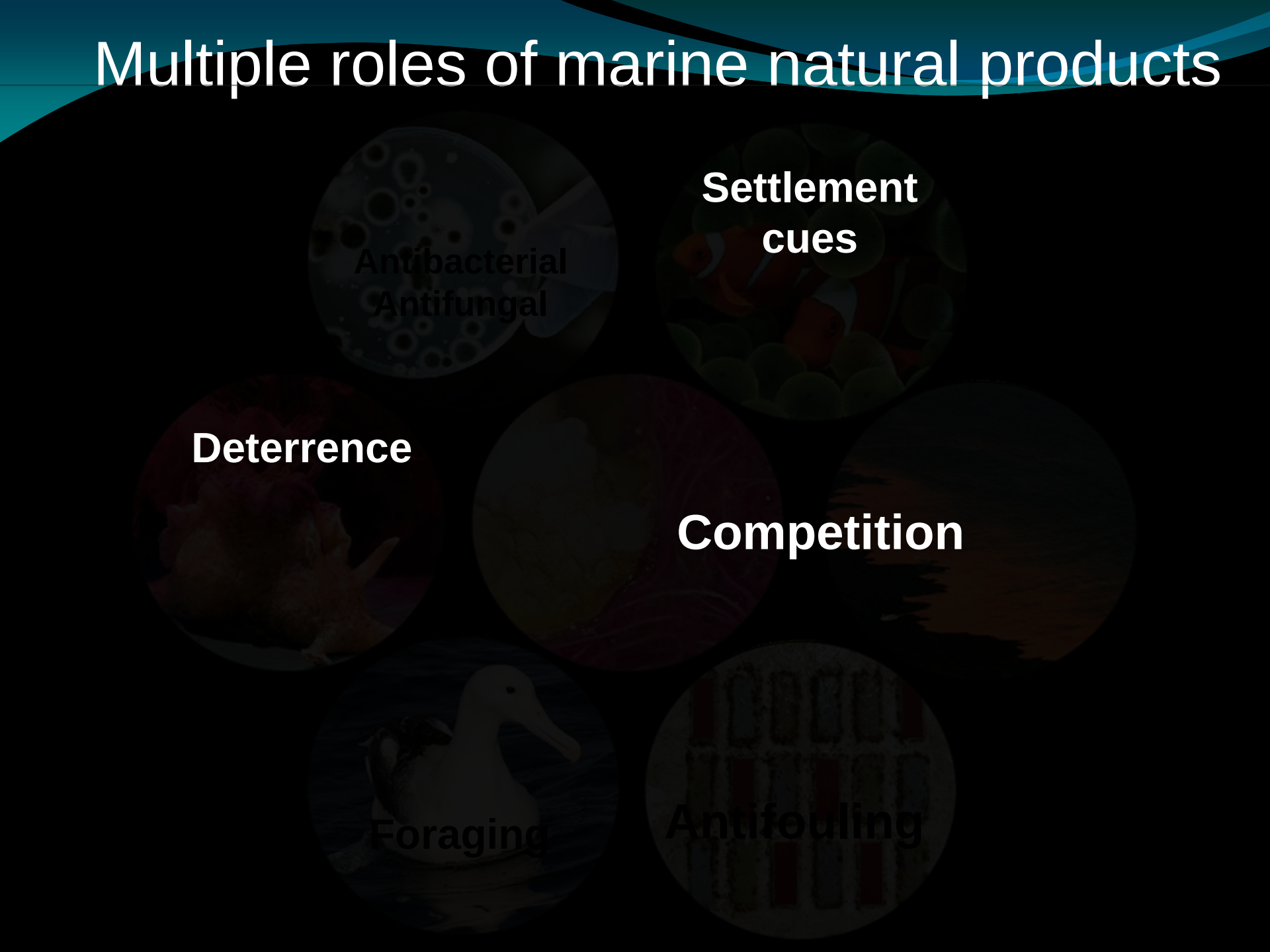


Photo credit: Thomas Vignaud, www.oceanexplorer.noaa.gov, www.postermagnet.com, www.masterfile.com, Kevin Raskoff, www.news.discovery.com

Multiple roles of marine natural products



MARCH 2012
VOLUME 75
NUMBER 3
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JOURNAL OF NATURAL PRODUCTS

75th Anniversary

Fungus
(*Penicillium wortmanii*)
Cancer

Bacteria
(*Streptomyces* sp.)
Anthracyclines
antibiotics

Tree
(Taxus sp.)
Cancer,
mitotic inhibitor

Mollusc
Jorumycin
Anti-tumor
Cancer

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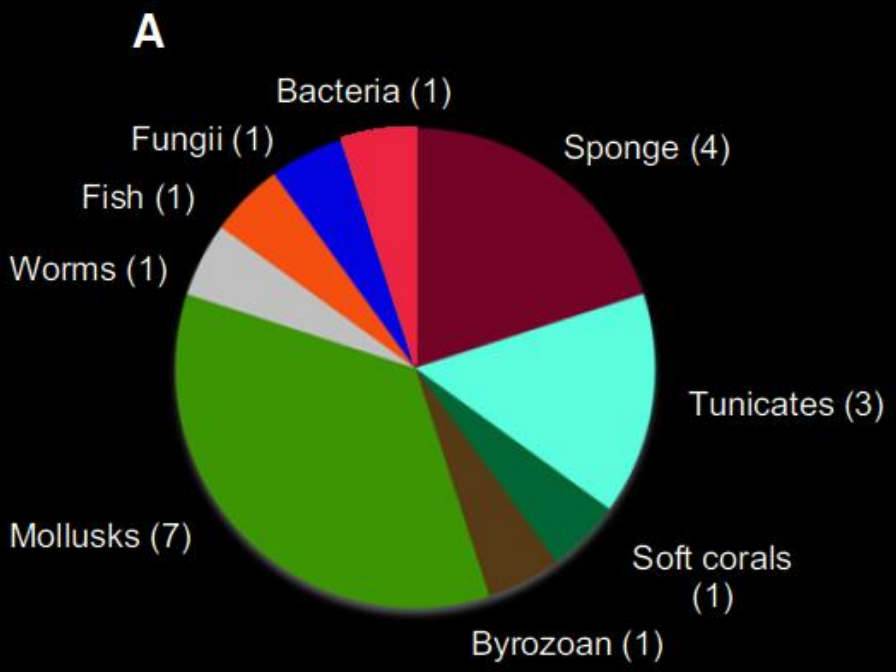
AMERICAN SOCIETY OF PHARMACOGNOSY

Partial list of the 20 MNP (derived or inspired) in use or in trials

Clinical Status	Compound name	Disease	Molecular Target	Collected
FDA approved	Cytarabine (Ara-C)	Cancer	DNA polymerase	Sponge
FDA approved	Vidarabine (Ara-A)	Antiviral	Viral DNA polymerase	Sponge
FDA approved	Eribulin Mesylate (E7389)	Cancer	Microtubules	Sponge
FDA approved	Trabectedin (ET-743)	Cancer	Minor groove of DNA	Tunicate
FDA approved	Brentuximab vedotin	Cancer	Microtubules	Mollusc
Phase II	Plinabulin (NPI 2358)	Cancer	Microtubules and JNK stress protein	Fungus
Phase II	Elisidepsin	Cancer	DNA binding	Nudibranch
Phase I	Bryostatin 1	Cancer, Alzheimer’s	Protein kinase C	Bryozoan
Phase I	Pseudopterosins	Pain, wound healing	Eicosanoid metabolism	Soft Coral

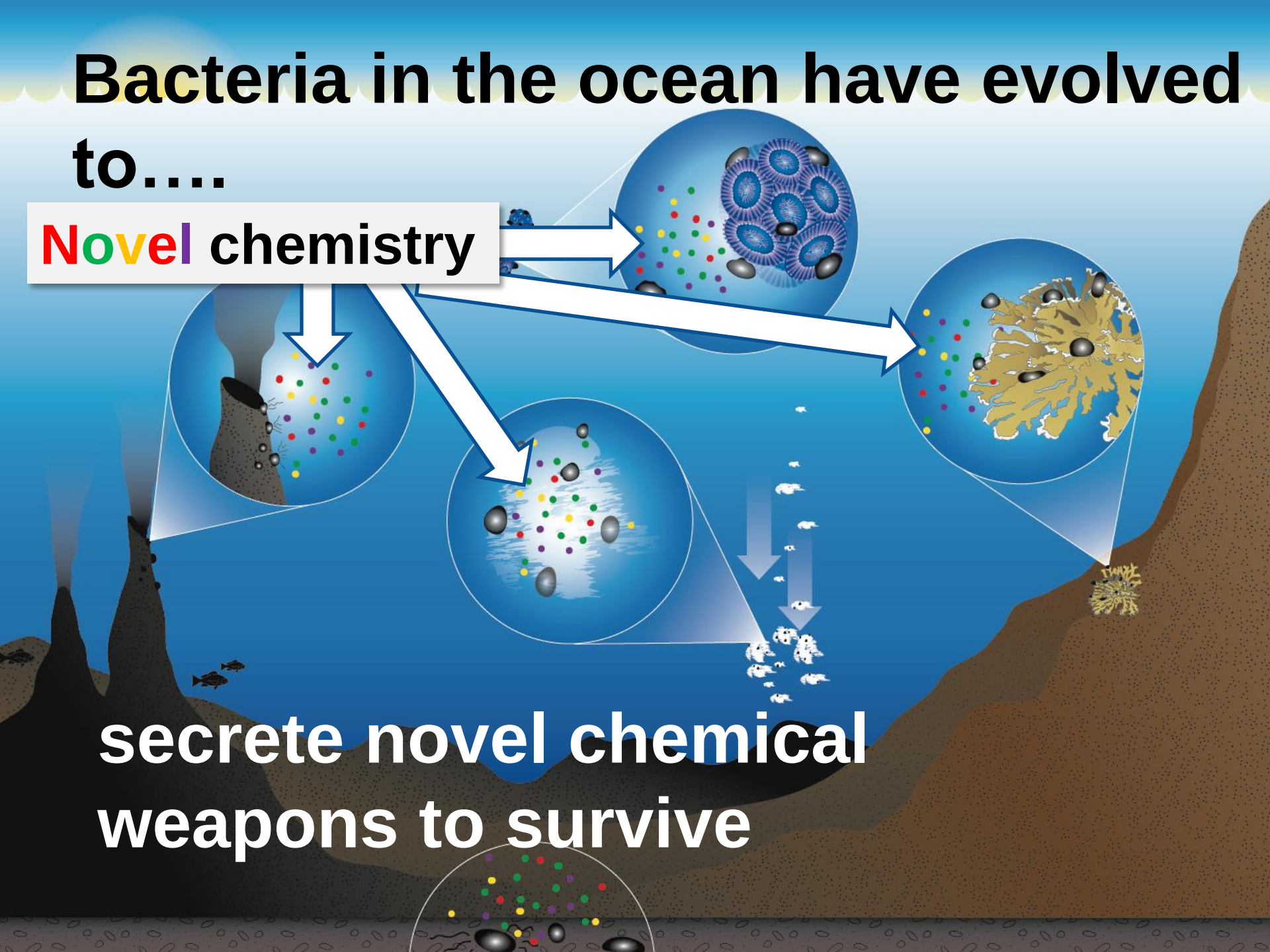
Where should we focus our effort?



Collected sources

Bacteria in the ocean have evolved to….

Novel chemistry



secrete novel chemical weapons to survive

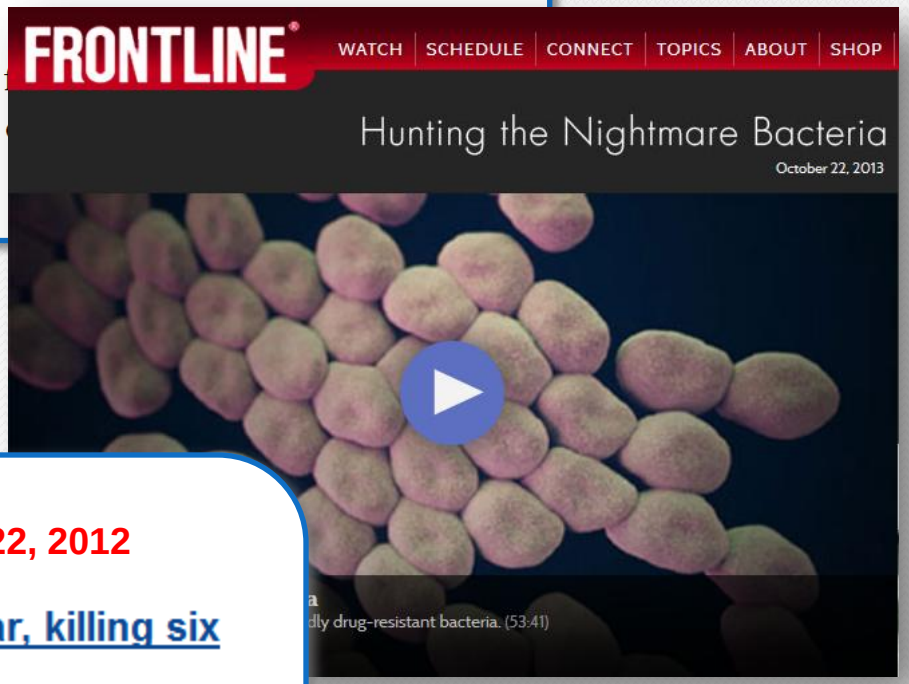

At WHOI,
we are leveraging
the chemical diversity
produced by marine microbes
to help us develop new drugs

The New York Times June 2, 2013

Pressure Grows to Create Drugs for 'Superbugs'

By BARRY MEIER

Government officials, drug companies and medical experts, f
resistant "superbugs," are pushing to speed up the approval
raising safety concerns among some critics.



The Washington Post August 22, 2012

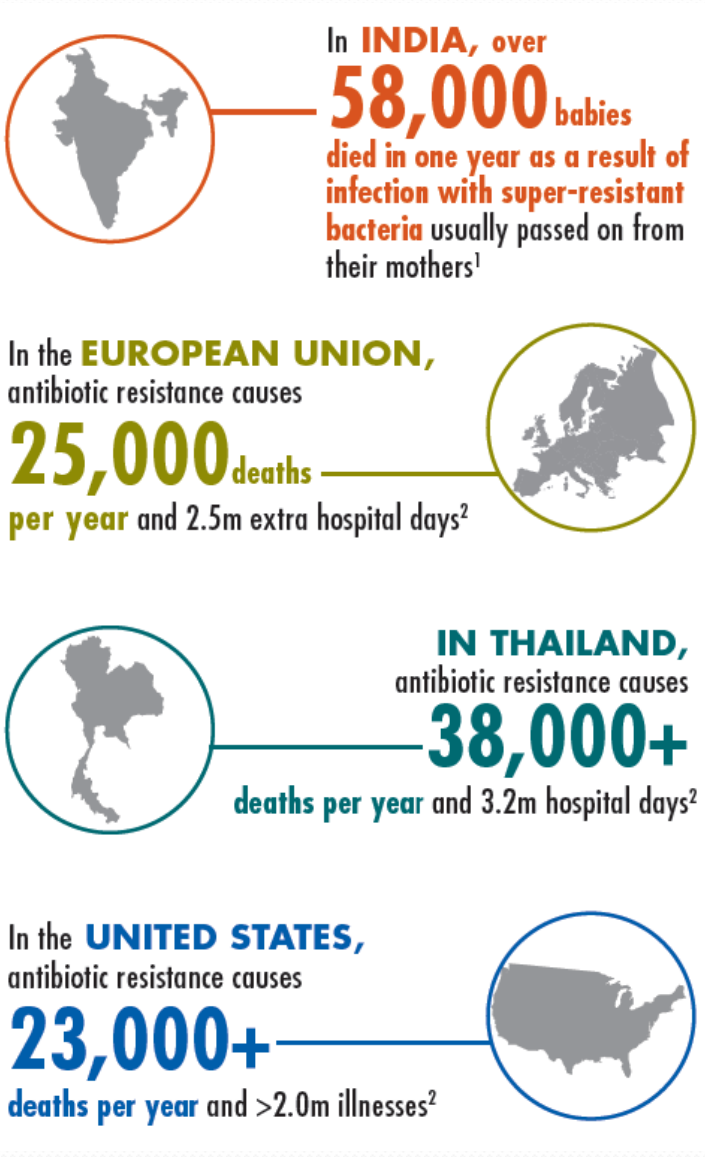
'Superbug' stalked NIH hospital last year, killing six

By Brian Vastag

As a deadly infection, untreatable by nearly every antibiotic, spread through the National Institutes of Health's Clinical Center last year, the staff resorted to extreme measures. They built a wall to isolate patients, gassed rooms with vaporized disinfectant and even ripped out plumbing. They eventually used rectal swabs to test every patient in the 234-bed hospital.

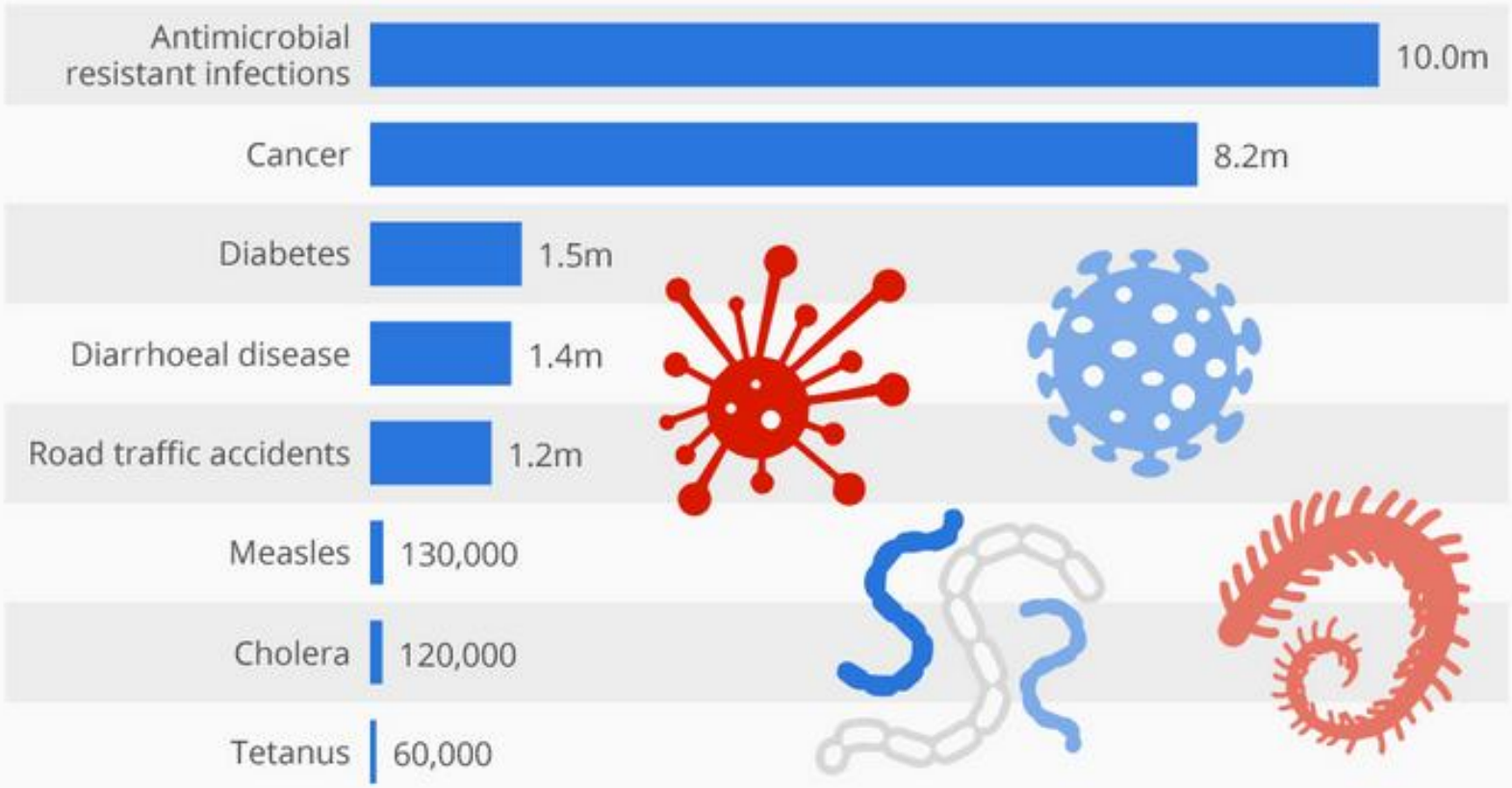
Infection Control

- 6th leading cause of death worldwide
- Gram-negatives limited antibiotics
- Drug-resistant gonorrhea
  - 820,000 infections/yr (U.S.)
  - Antibiotic resistance (30% cases)
  - Additional $235 million to treat
- Carbapenem-resistant Enterobacteria (CRE)
  - 44 states (CDC)
  - Resistant to nearly all antibiotics
  - 50% mortality

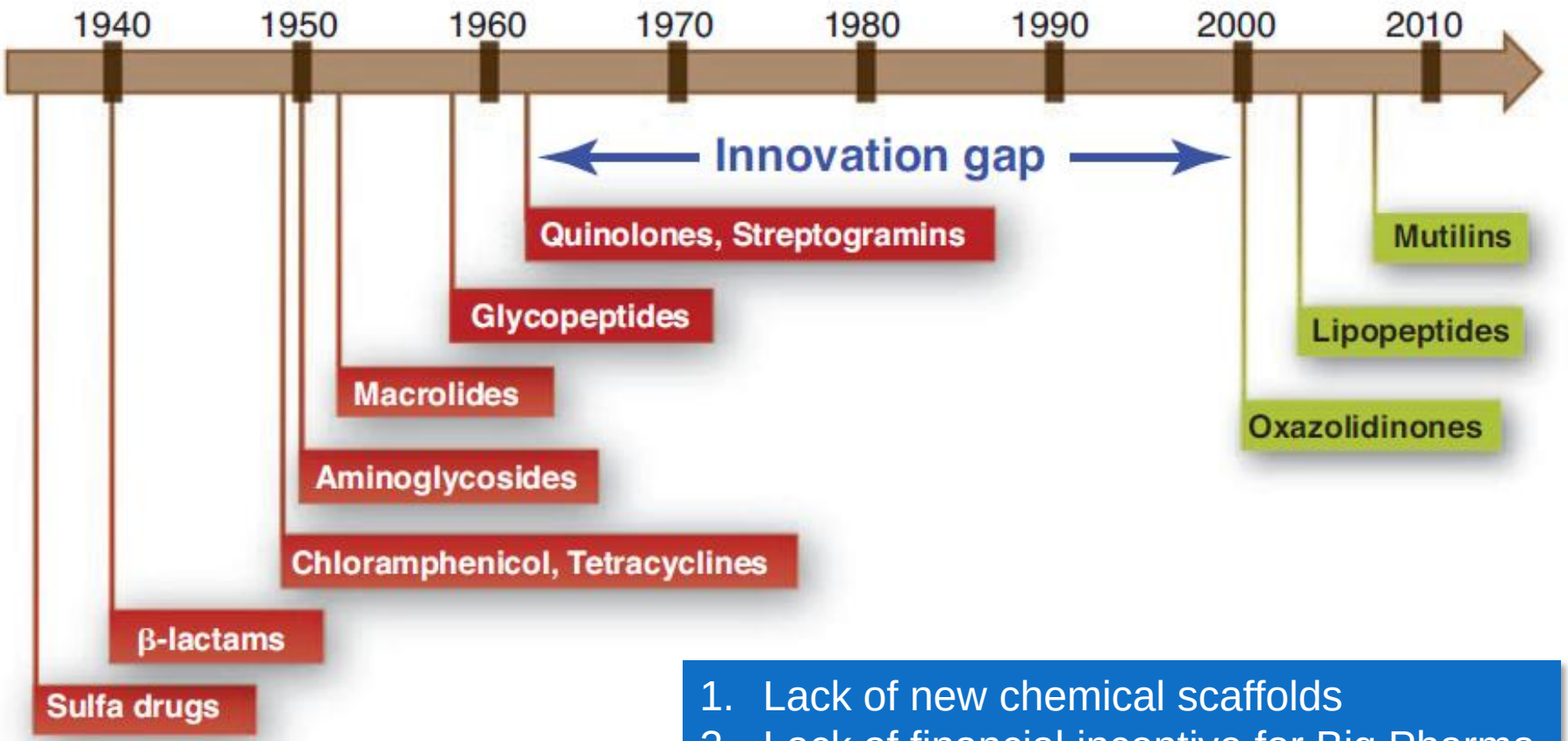


Deaths From Drug-Resistant Infections Set To Skyrocket

Deaths from antimicrobial resistant infections and other causes in 2050



Source: Review on Antimicrobial Resistance



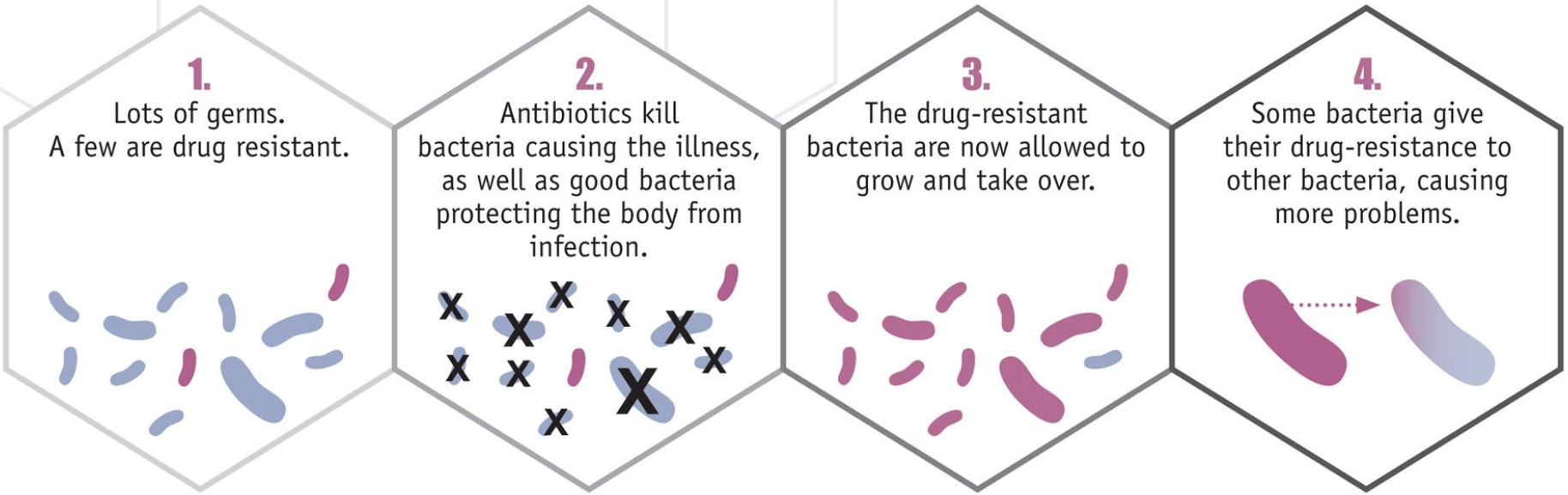
1. Lack of new chemical scaffolds
2. Lack of financial incentive for Big Pharma

Fig. 3. Between 1962 and 2000, no major classes of antibiotics were introduced.

Our Target:

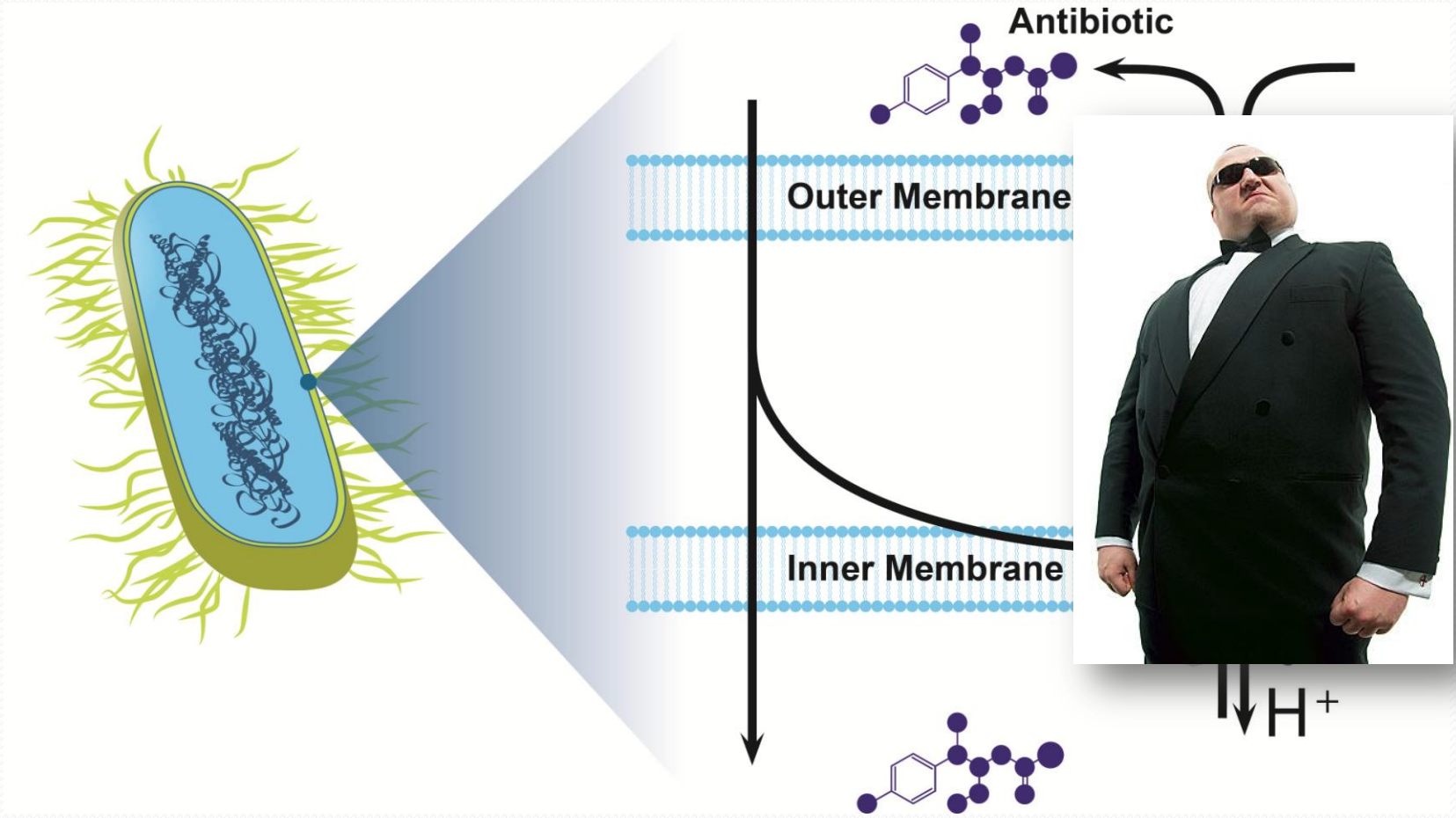
Multidrug transporters of the pathogen

How Antibiotic Resistance Happens



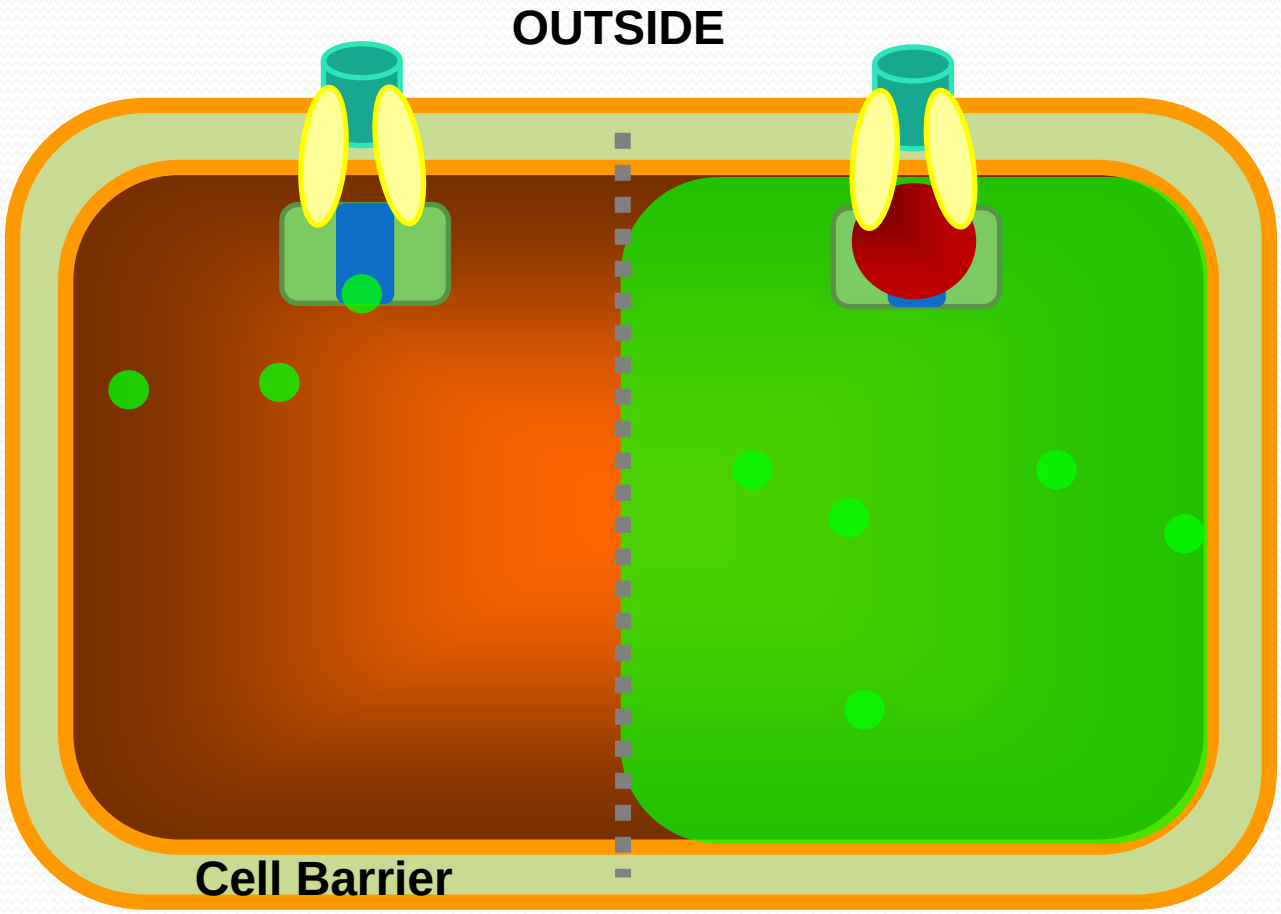
Source: Centers for Disease Control

Strategy: Targeting resistance mechanisms



Re-sensitize cells to existing arsenal of antibiotics

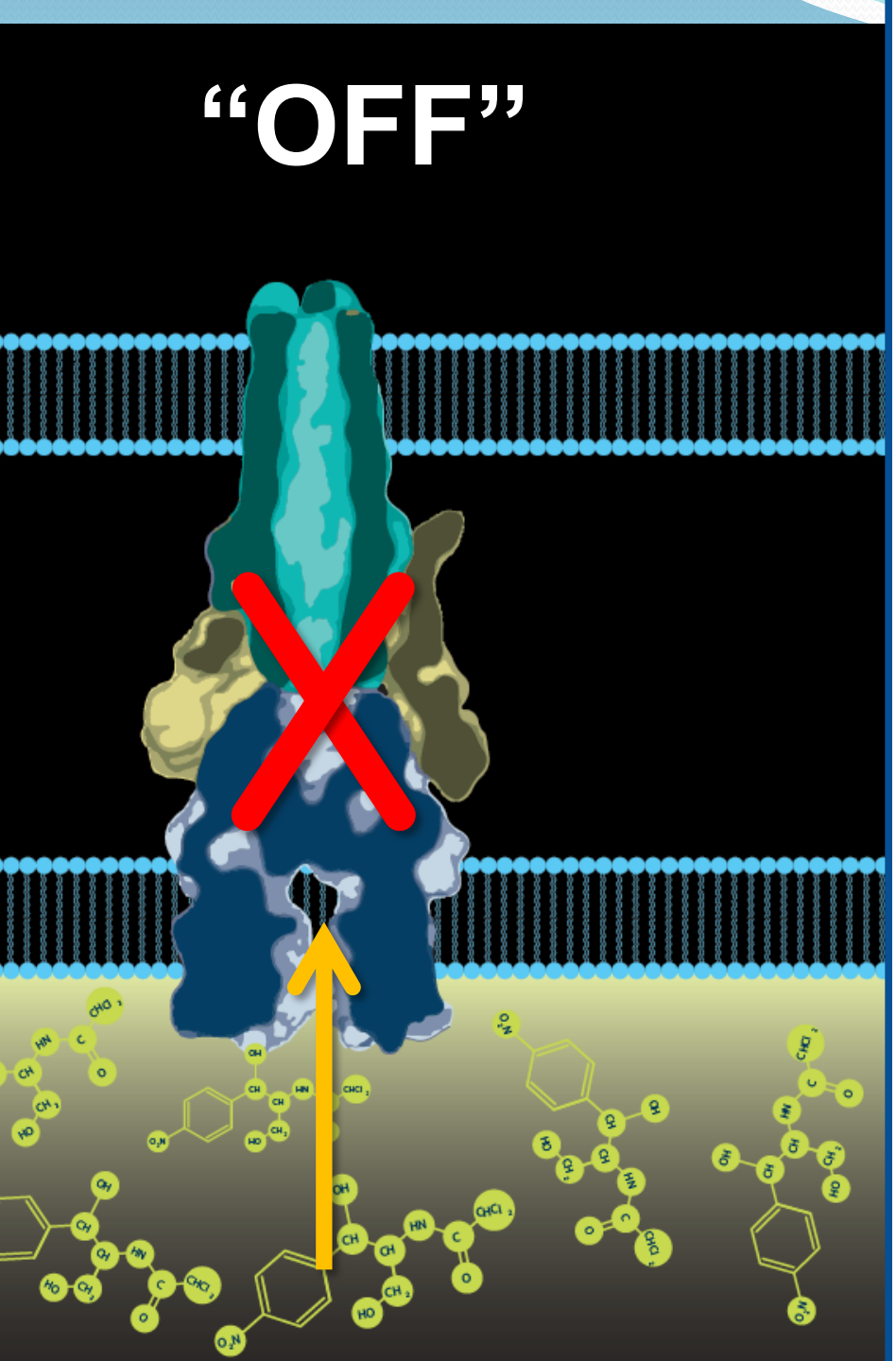
Blocking the pump



Antibiotic

Inhibitor

“ON” “OFF”



Chemical Plug

Inhibit Assembly

Competition with antibiotic

Energy Collapse

Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor

Frank R. Stermitz*, Peter Lorenz*, Jeanne N. Tawara*, Lauren A. Zenewicz†, and Kim Lewis†‡

*Department of Chemistry, Colorado State University, Fort Collins, CO 80523; and †Biotechnology Center, Tufts University, Medford, MA 02155

Communicated by Arnold L. Demain, Massachusetts Institute of Technology, Cambridge, MA, December 13, 1999 (received for review October 6, 1999)

PNAS | February 15, 2000 | vol. 97 | no. 4 | 1433–1437

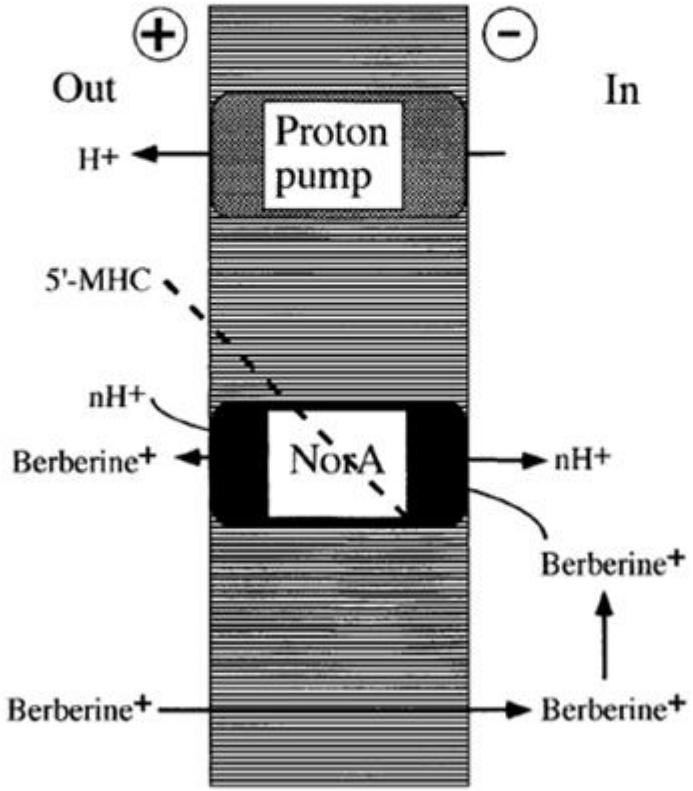
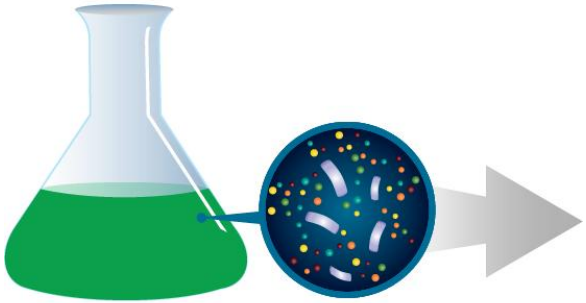


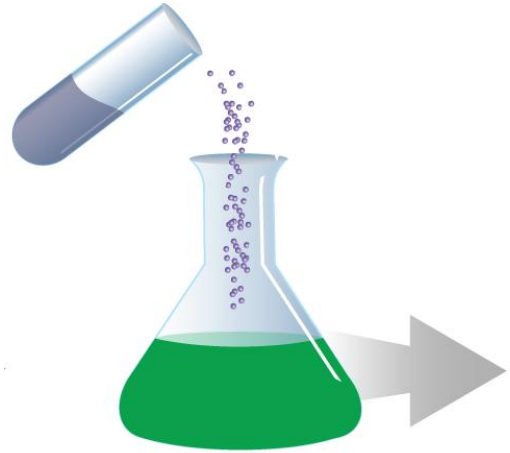
Fig. 4. A model of synergistic action of berberine and an MDR inhibitor that are both produced by *B. fremontii*. Berberine accumulates in the cell driven by the membrane potential. The NorA pump extrudes berberine. The MDR inhibitor 5′-MHC blocks the NorA pump, potentiating the antibiotic action of berberine.

Fig. 2. Medicinal plants producing berberine and the MDR inhibitor 5′-MHC. (*Top*) *B. fremontii*. (*Middle*) *B. repens*. (*Bottom*) *B. aquifolia*.

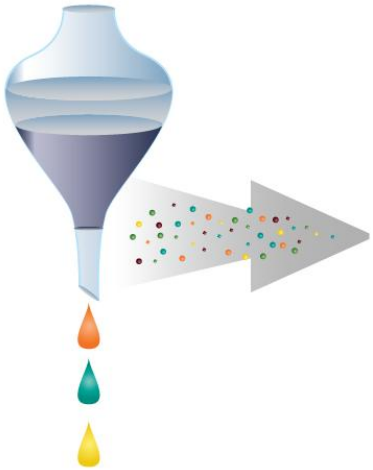
What is the process of chemical discovery?



Culture marine microbes



Absorb compounds to resin

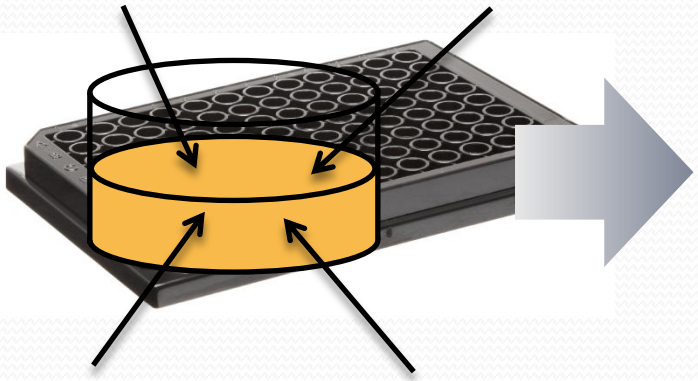


Extract compounds

Screening for Efflux Pump Inhibitors (EPIs)

Multi-drug resistant bacteria (3 strains)

Culture media

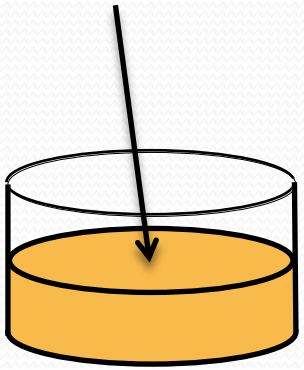


Very dilute antibiotic

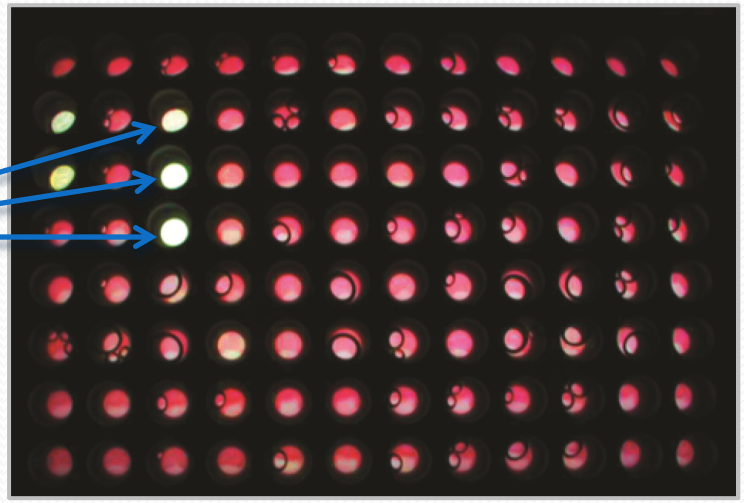
Chemical extract from marine microbe

18 hr.

colorimetric reagent

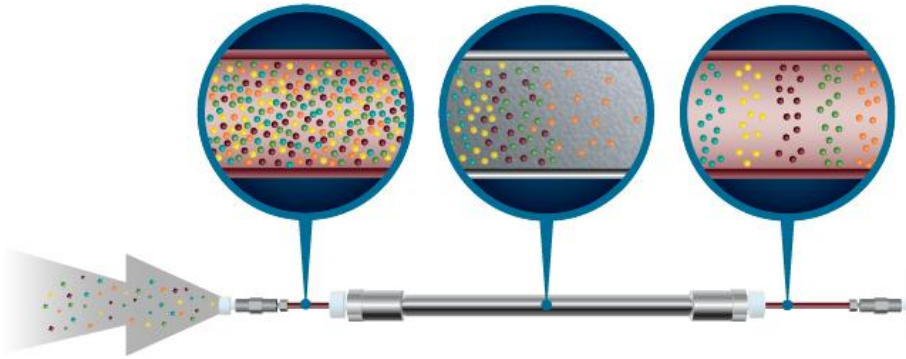


Absorbance 490nm

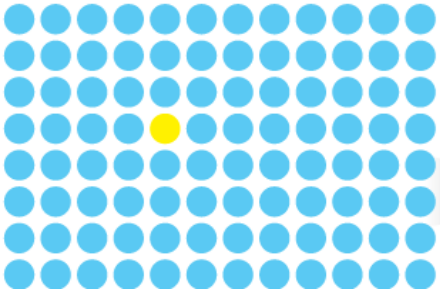


Active extracts

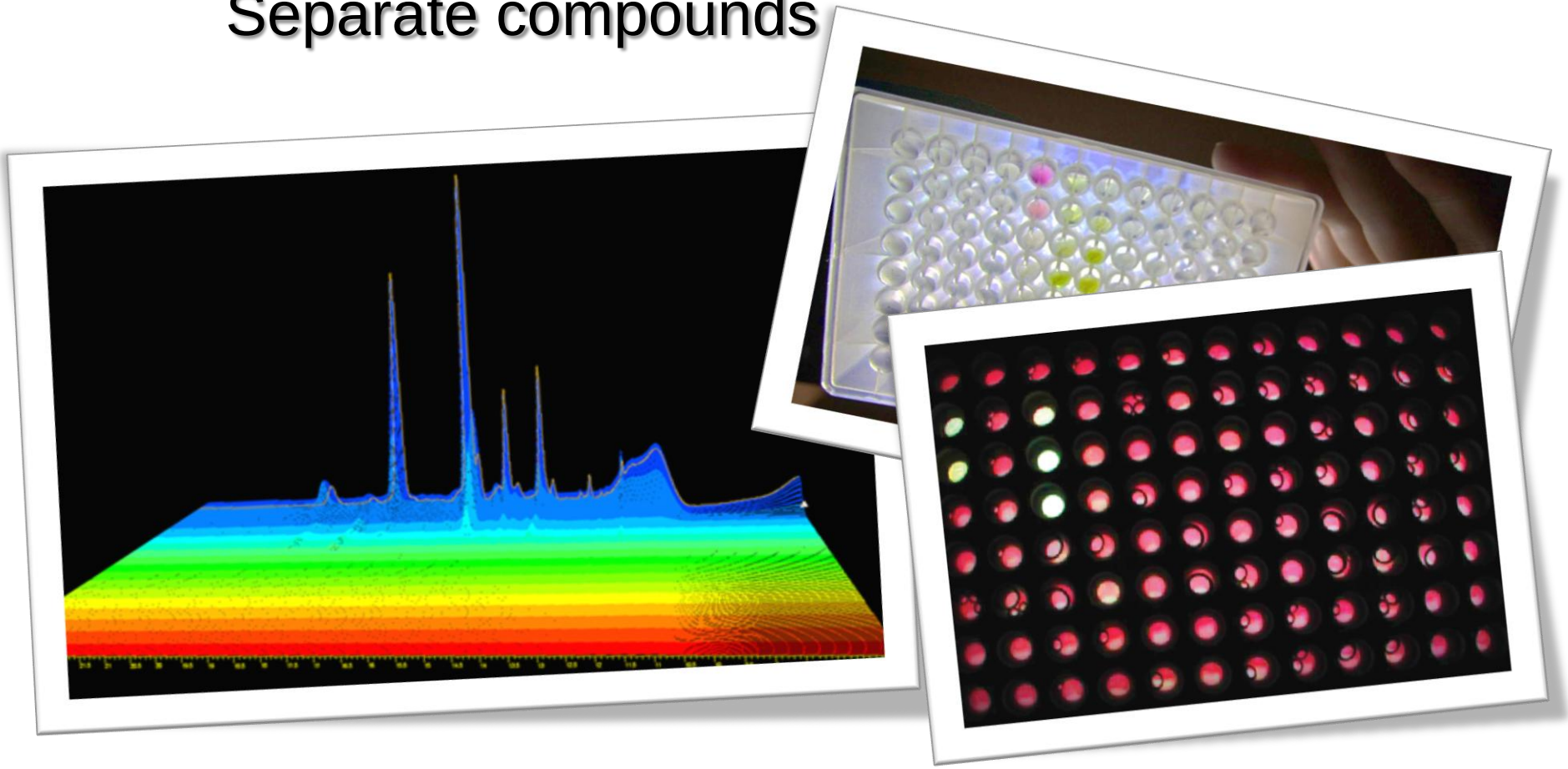
Elena Perry, WHOI SSF



then test for activity

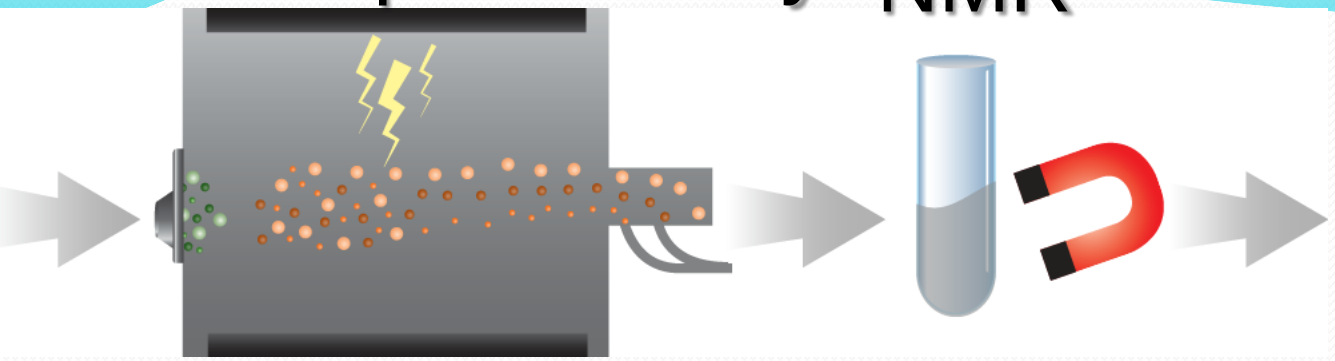


Separate compounds



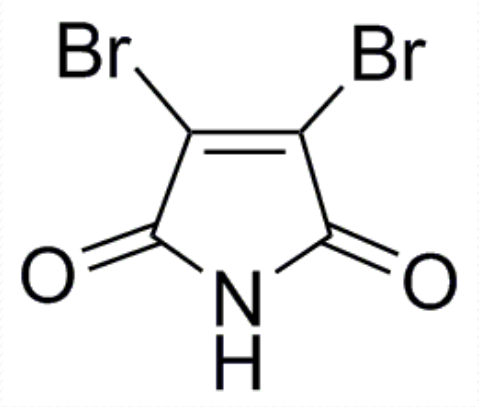
Mass Spectrometry

NMR



Structure determination

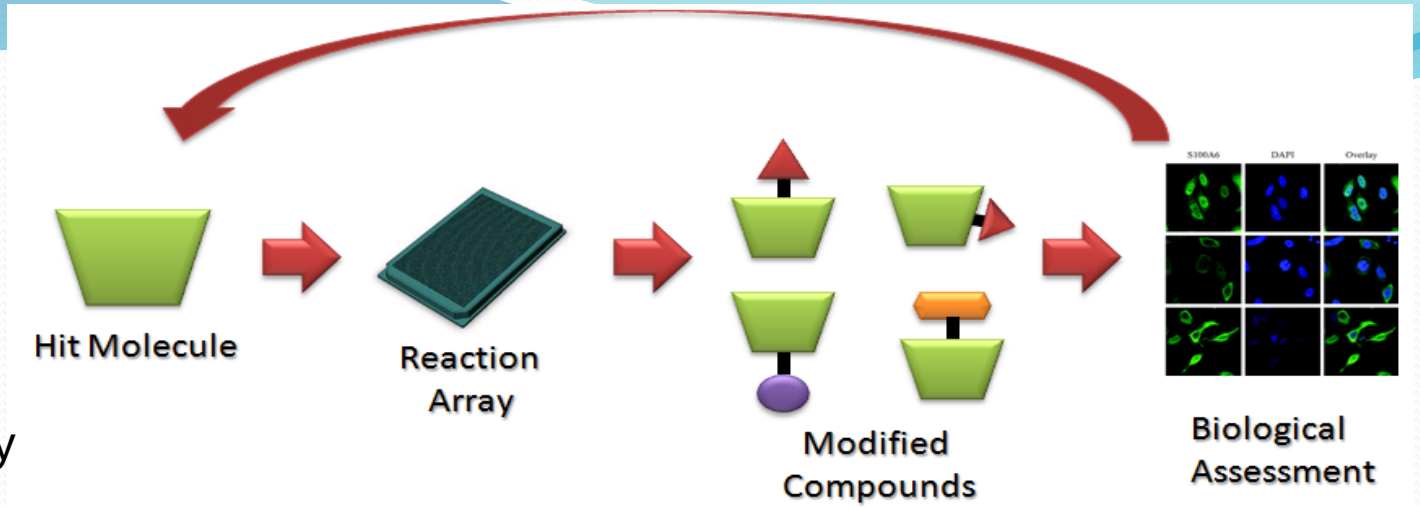
Rob Deering
Dave Rowley
University of Rhode Island



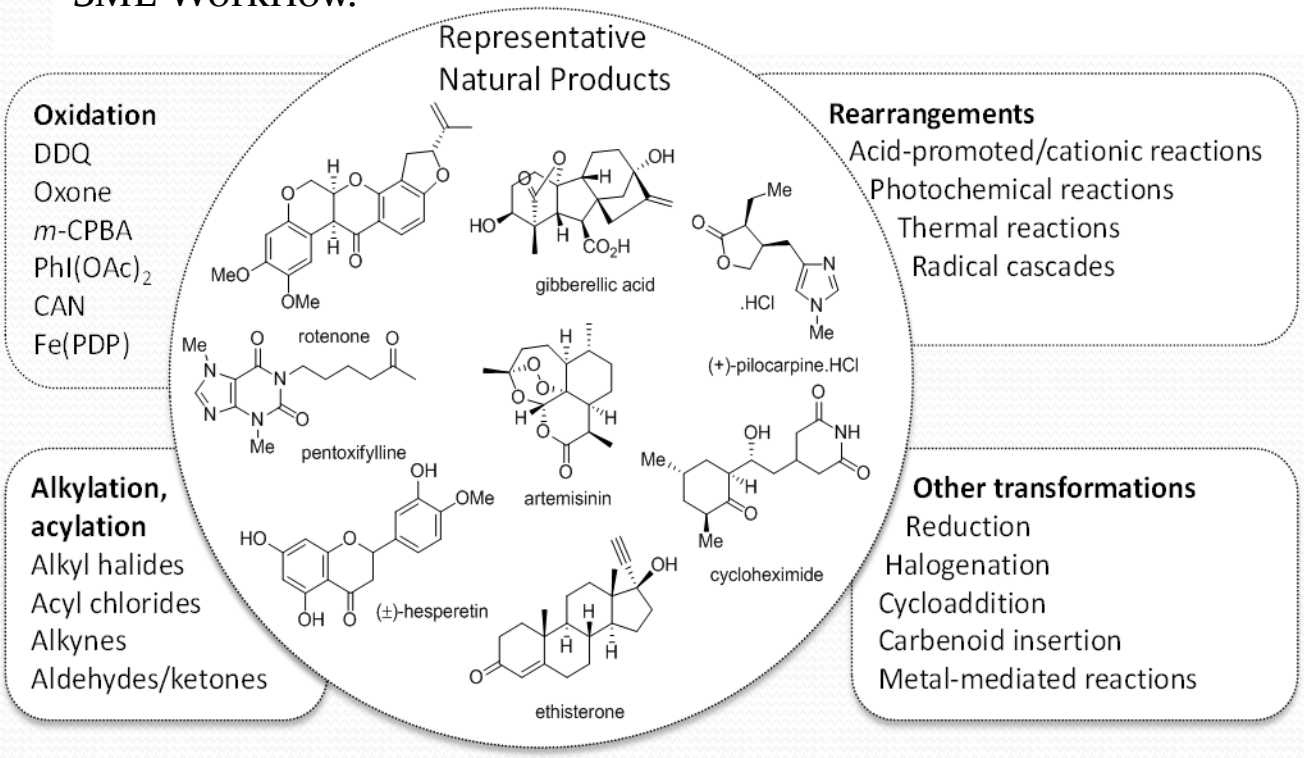
3,4-dibromopyrrole-2,5-dione
Pseudoalteromonas piscicida
Gamma Proteobacteria

Whalen et al. 2015, *Journal of Natural Products*
El Gamal et al. 2016, *PNAS*

Aaron Beeler
Boston University



SME Workflow.



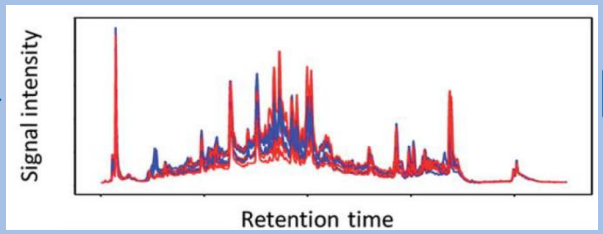
SME reaction toolbox and representative natural products that have been utilized.

Can we accelerate the process of novel compound discovery?

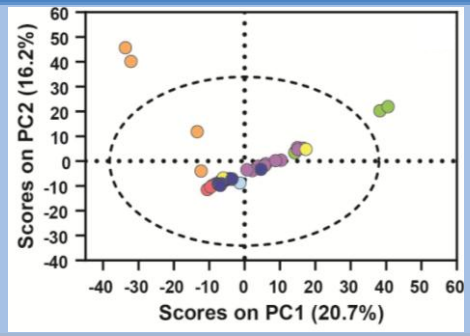
Comparative metabolomics

Induce stress conditions

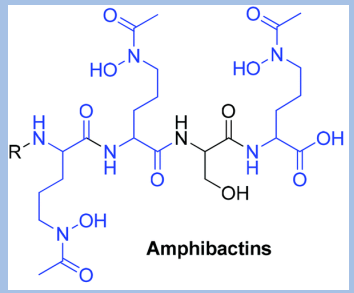
LC-MS profiling



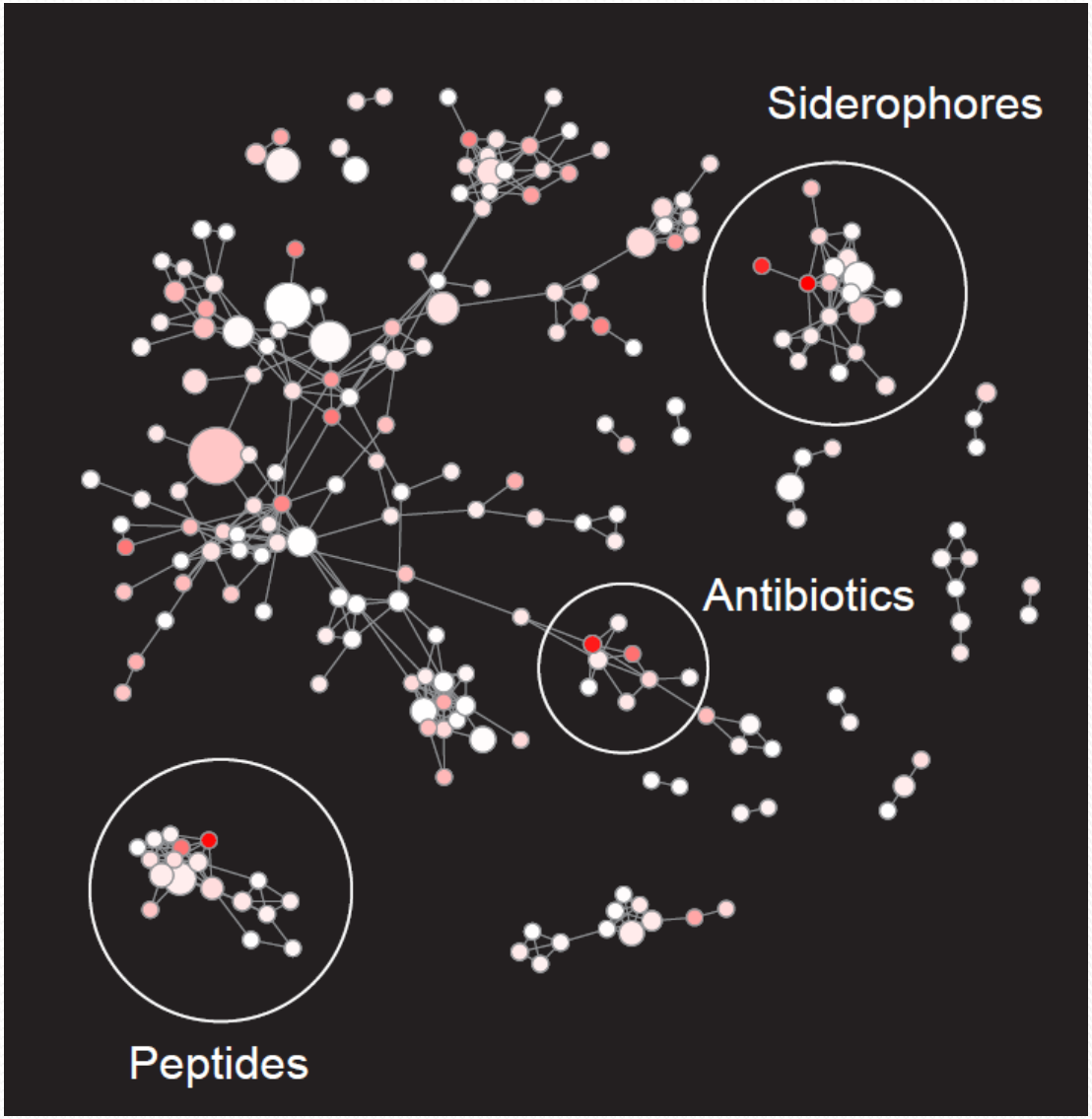
Statistical analysis



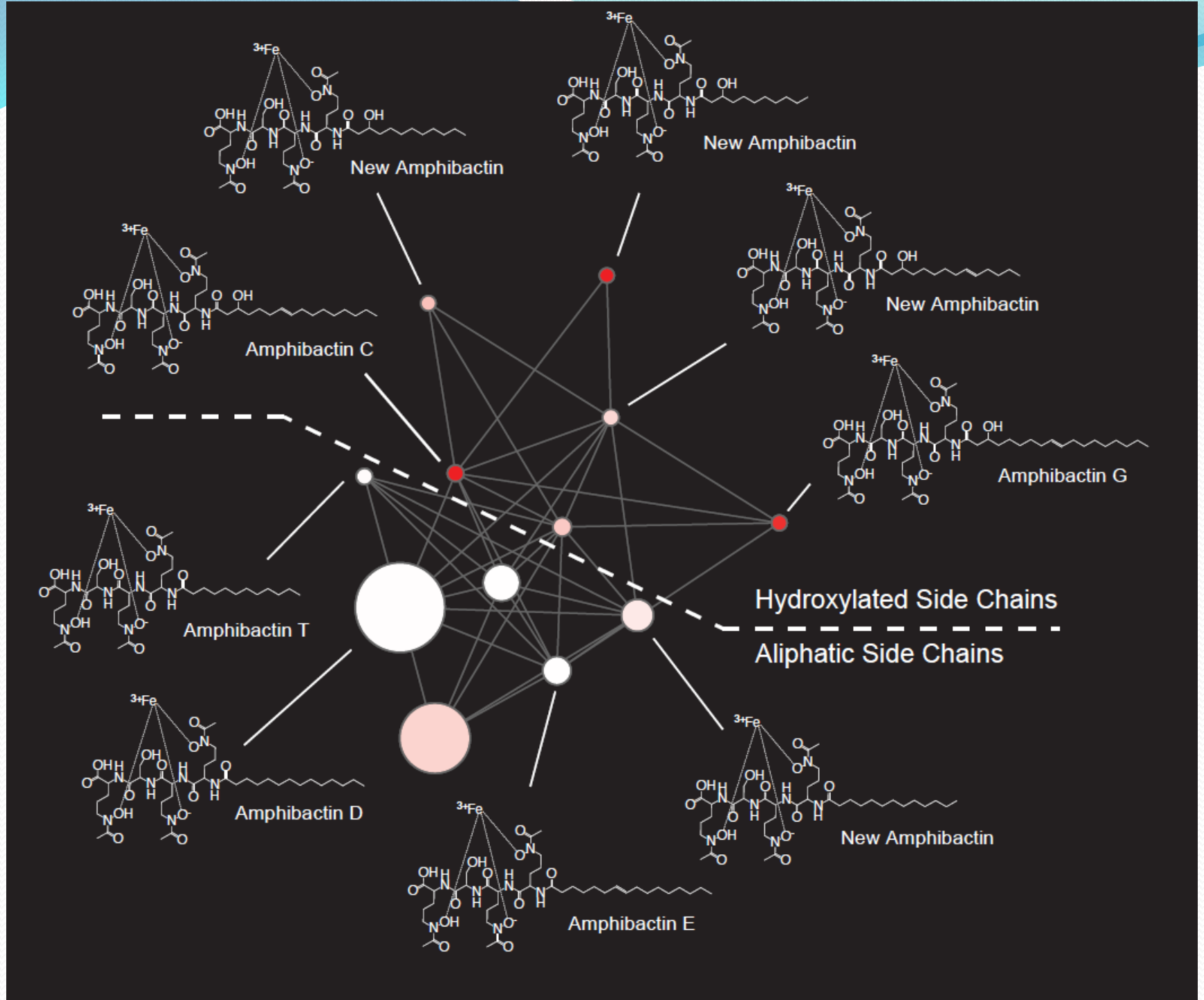
Compound identification

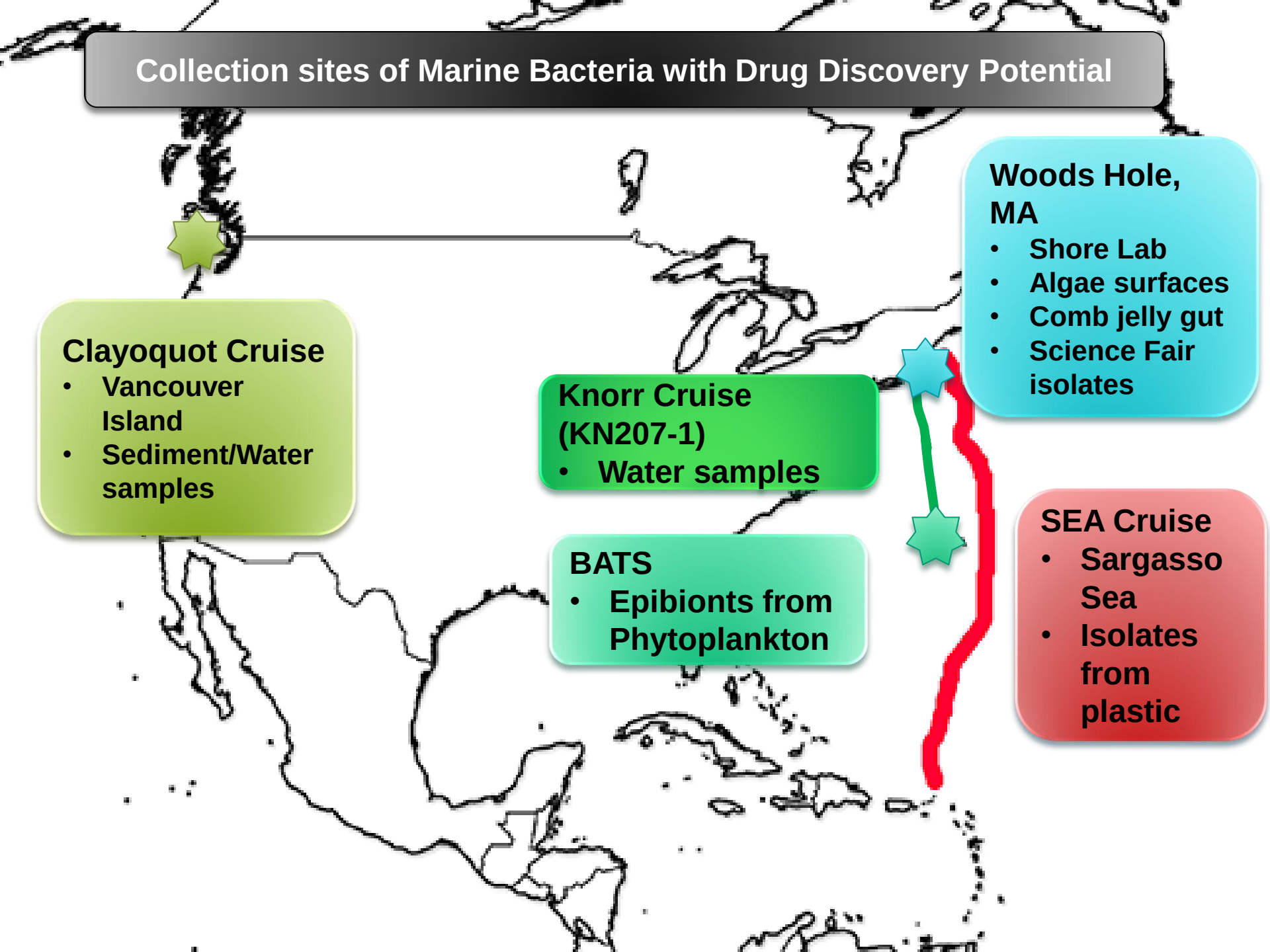


Emerging tool: MS-based molecular networking

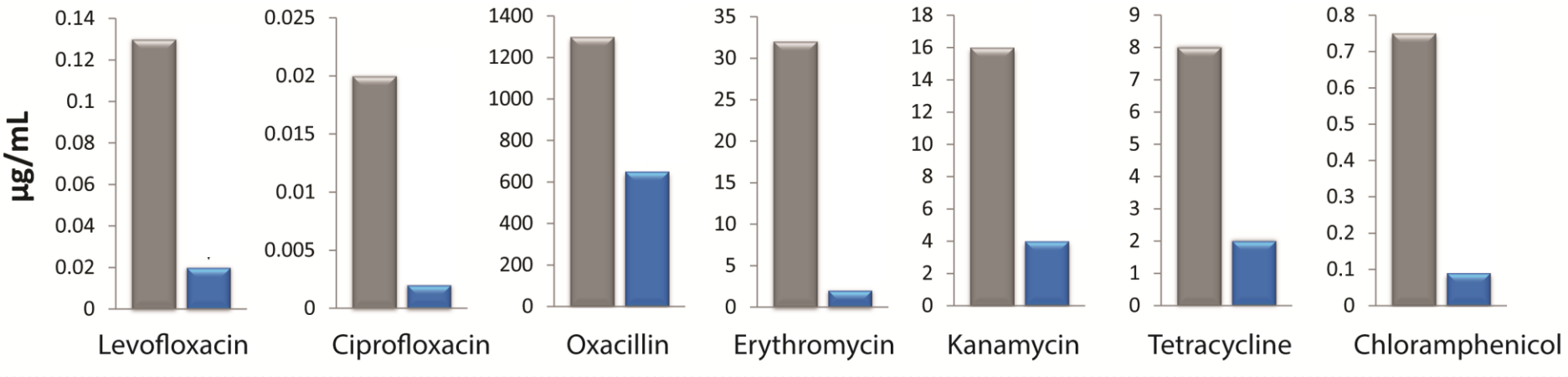


Rene Boiteau
WHOI


New Amphibactin
New Amphibactin
Amphibactin C
New Amphibactin
Amphibactin G
Amphibactin T
Hydroxylated Side Chains
Aliphatic Side Chains
Amphibactin D
New Amphibactin
Amphibactin E

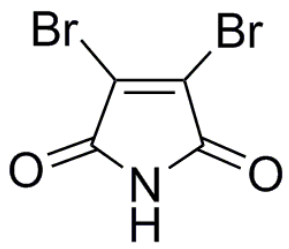

Collection sites of Marine Bacteria with Drug Discovery Potential
Woods Hole, MA
• Shore Lab
• Algae surfaces
• Comb jelly gut
• Science Fair isolates
Clayoquot Cruise
• Vancouver Island
• Sediment/Water samples
Knorr Cruise (KN207-1)
• Water samples
BATS
• Epibionts from Phytoplankton
SEA Cruise
• Sargasso Sea
• Isolates from plastic

Our 1st drug candidate enhances the effectiveness across antibiotic classes



Antibiotic alone

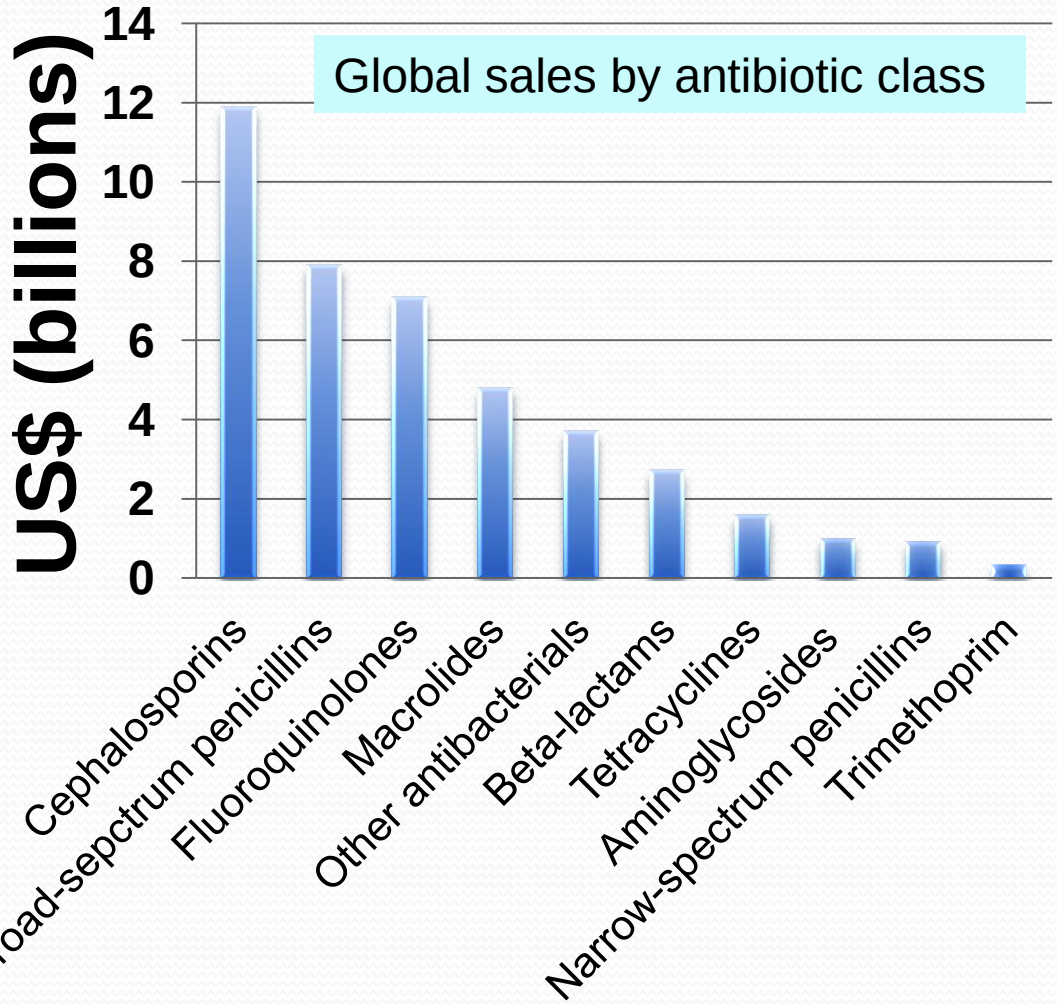
Antibiotic + Inhibitor



Dibromopyrroledione

Pump inhibitors rescue our most valuable antibiotics

- Bacteria have developed resistance to all antibiotic classes
- Restoring antibiotic potency
- Reducing spread and resistance development



Source: Global Antibiotic Market, Technavio (2014); Nature Reviews: Drug Discovery (2010)

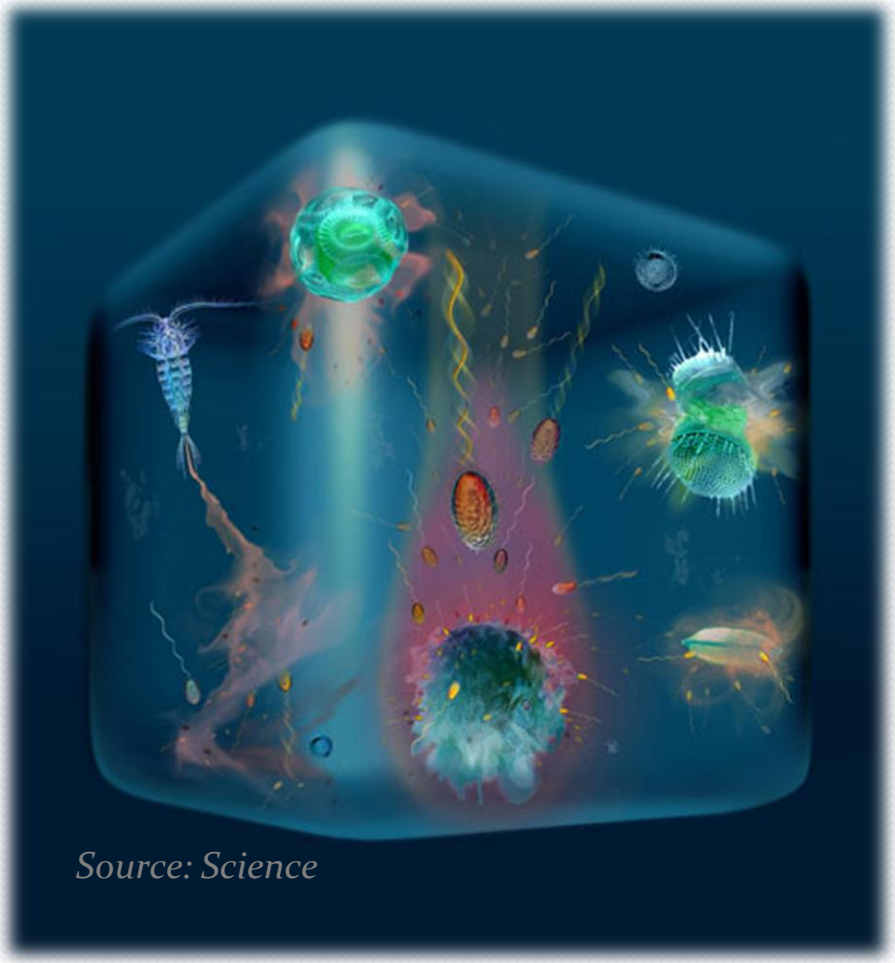
Competitive Advantage

- Robust pipeline
  - 2.8% hit rate, 36 marine isolates
  - Screening: fast, reproducible, inexpensive
- Accelerated discovery of new inhibitors
  - Marine environment = untapped chemical reservoir
  - Water soluble; stable; halogenated; easily derivatized for optimization
- Broad therapeutic applicability
  - Rescue of shelved antibiotics
  - Combination approach does not rely on developing new classes of antibiotics
  - Antibiotic safety previously determined
- IP protection of derivatives, combinations with other antibiotics & utility (Tech Transfer, WHOI)
  - 1 U.S. Patent Pending

Efflux inhibitors offer promising new avenue to treat antibiotic resistant superbugs

- Dibromo compound ready for preclinical stage
- Opportunity to rescue failing antibiotics
  - Applies to multitude of antibiotic classes
  - Reduce resistance development
  - Clinical precedent
    - Antibiotic + Resistance Blocker
    - 2 combination drugs approved by FDA in last 6 months
    - Zerbaxa™ (Cubist/Merck) revenue of >$1 billion/yr
    - Avycaz™ (Actavis/Astra Zeneca)

Search for new therapeutics to treat multidrug resistance



- Teasing apart the role of MNP
- Microbial warfare b/w competitors
- Cell-cell communication
- Biofilm formation
- Mutualistic interactions between micro/macroorganisms
- Integrate aspects of ecology, cell biology, and chemistry

Dr. Kristen Whalen
Research Scientist
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