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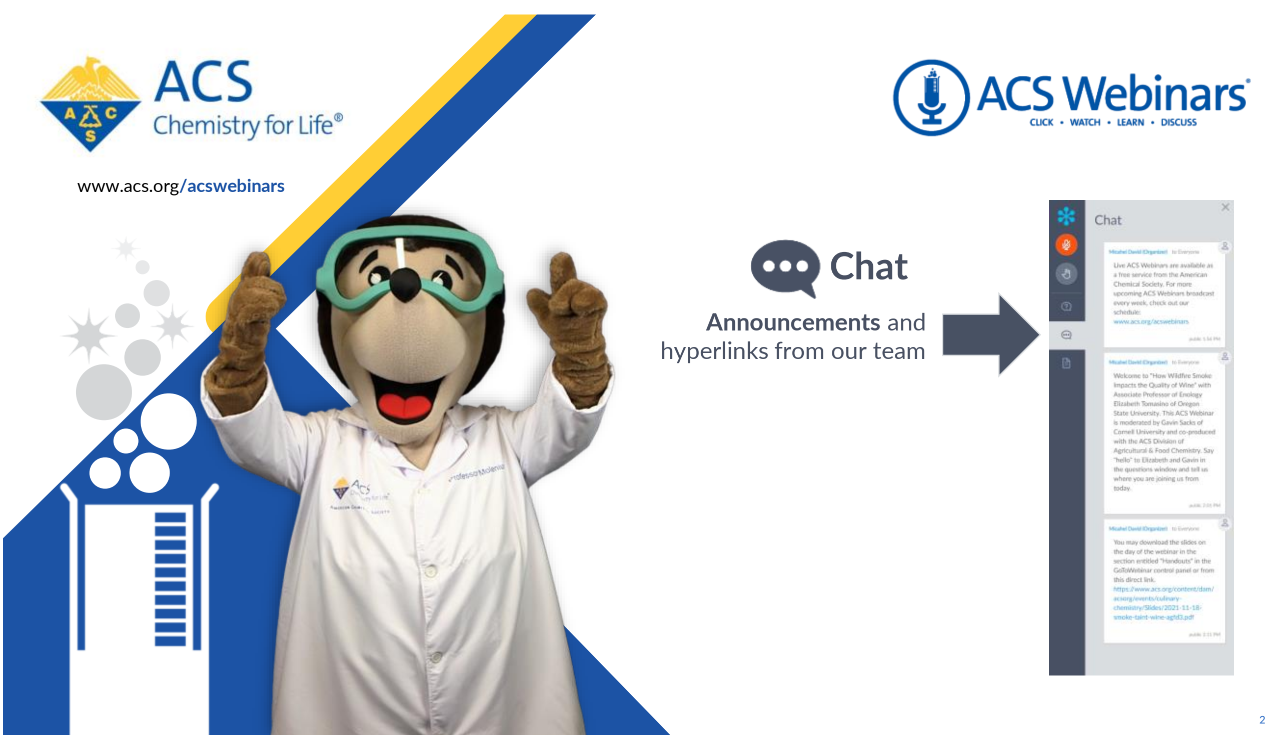
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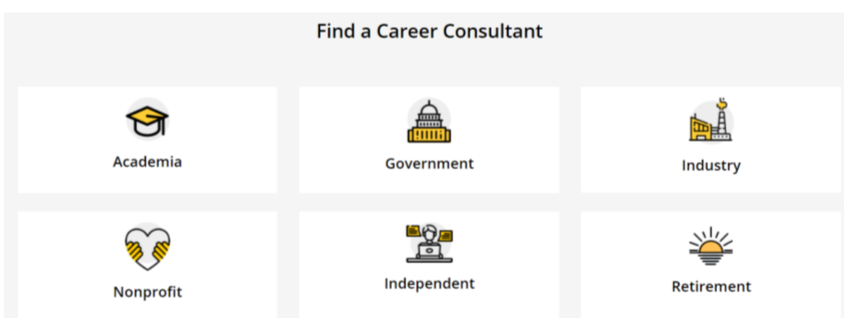

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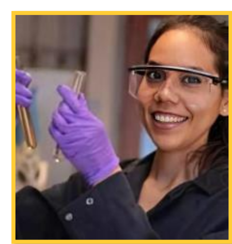

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Covering diversity and inclusion at the workplace

ACS Publications DEIR Hub
See what ACS Publications is doing for fostering inclusivity in scholarly publishing

ACS Volunteer and ACS Meetings Code of Conduct
Fostering a positive and welcoming environment for attendees, volunteers and staff.

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C&EN highlights scientists from different backgrounds who are making an impact in chemistry.

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Download this educational guide for additional recommendations on videos, articles, books, podcasts, and more on diversity, inclusion, and related topics.

Quick Guide: Inclusion Moments
Learn more about what Inclusion Moments are and see ideas to host them during your meetings.

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Recommendations and best practices to ensure that your events can accommodate everyone.

Diversity, Equity, Inclusion, and Respect

**Adapted from definitions from the Ford Foundation Center for Social Justice:

Equity**
Seeks to ensure fair treatment, equality of opportunity, and fairness in access to information and resources for all. We believe this is only possible in an environment built on respect and dignity. Equity requires the identification and elimination of barriers that have prevented the full participation of some groups.

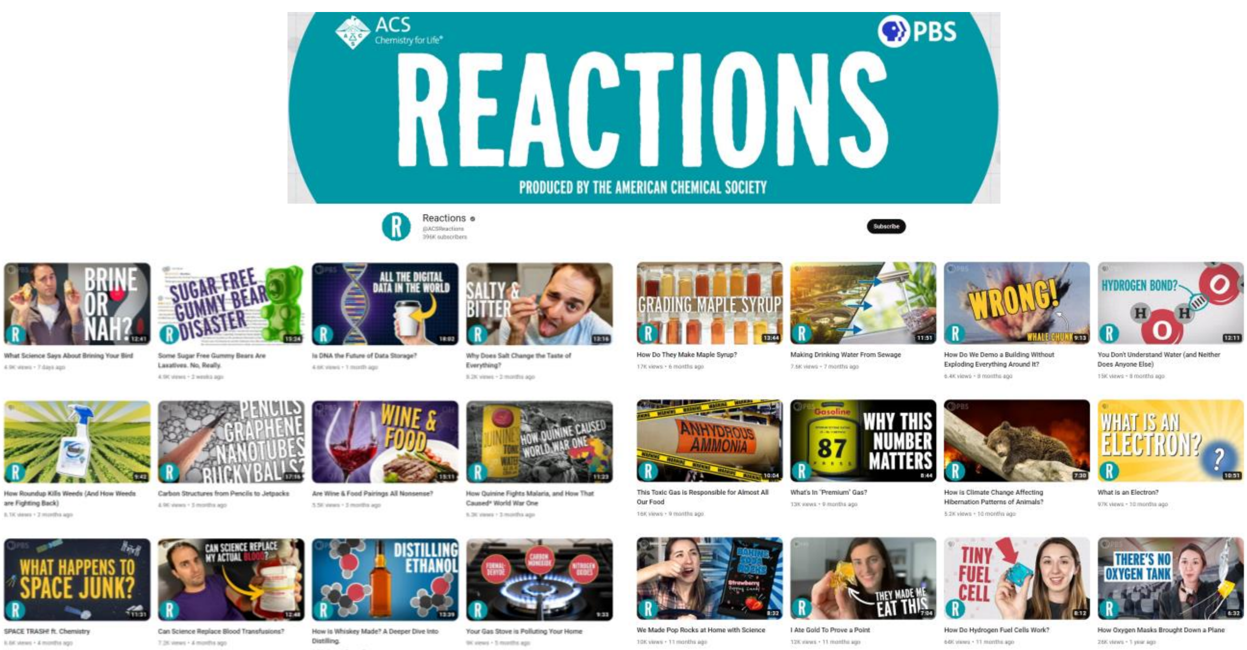
Diversity**
The representation of varied identities and differences (race, ethnicity, gender, disability, sexual orientation, gender identity, national origin, tribe, caste, socio-economic status, thinking and communication styles, etc.), collectively and as individuals. ACS seeks to proactively engage, understand, and draw on a variety of perspectives.

Inclusion**
Builds a culture of belonging by actively inviting the contribution and participation of all people. Every person's voice adds value, and ACS strives to create balance in the face of power differences. In addition, no one person can or should be called upon to represent an entire community.

Respect
Ensures that each person is treated with professionalism, integrity, and ethics underpinning all interpersonal interactions.

https://www.acs.org/diversity

11



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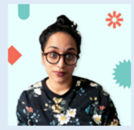
Sam Jones, PhD
Science Writer & Exec Producer



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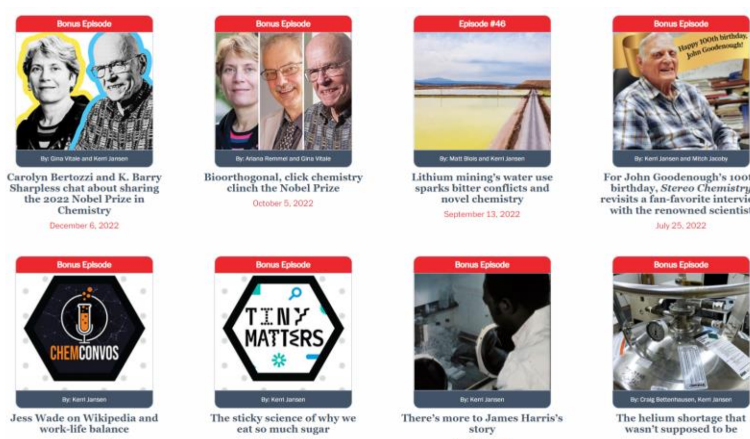

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16

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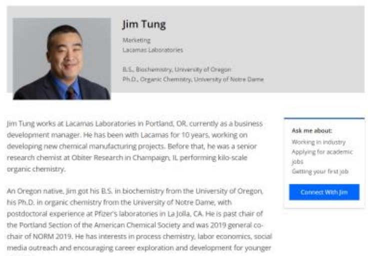

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LinkedIn Learning

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17

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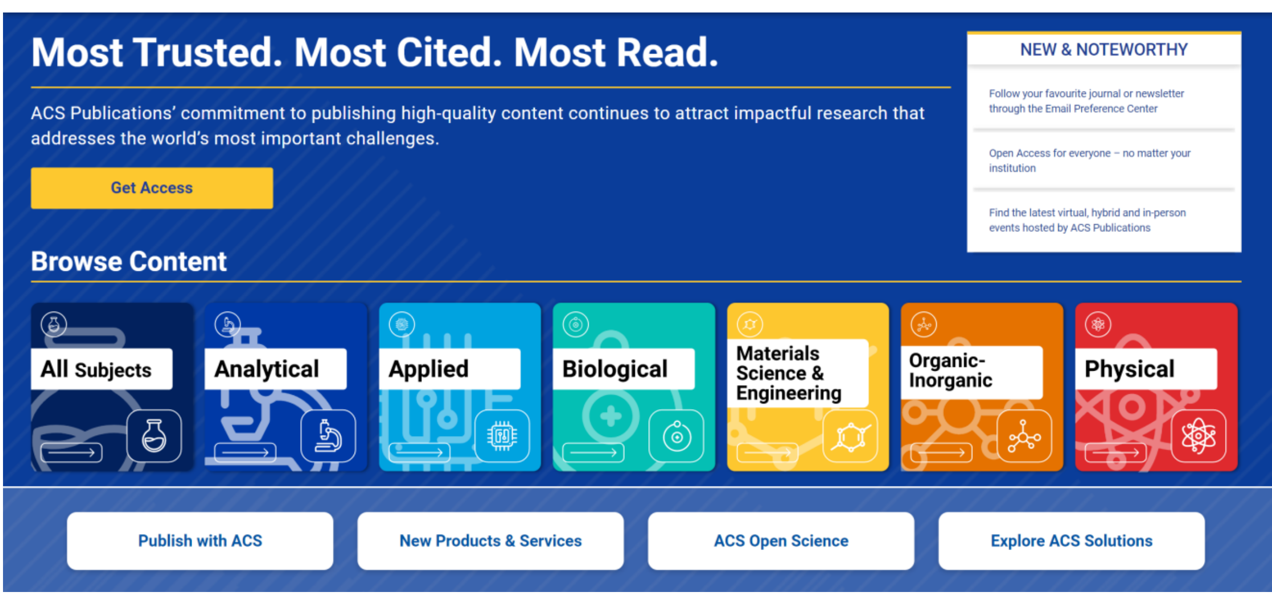

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18

18


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22

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Genome Mining of Fungal Natural Products

Yi Tang
Department of Chemistry and Biochemistry
Department of Chemical and Biomolecular Engineering
University of California, Los Angeles, USA

UCLA

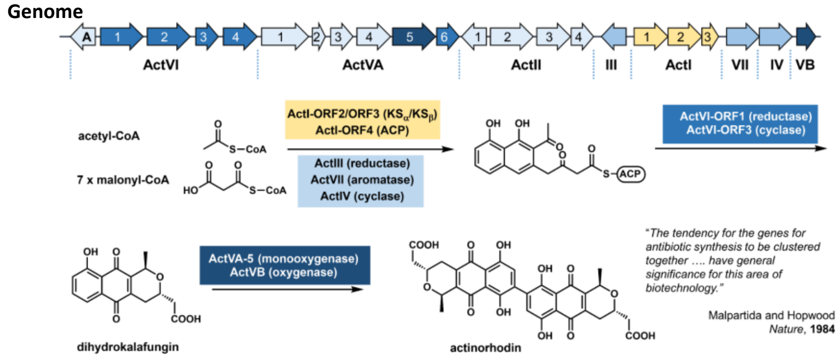
24

NP Biosynthetic Gene Clusters (BGCs)

Central dogma in biology and biosynthesis

DNA → RNA → Protein → NP

Biosynthetic genes are clustered

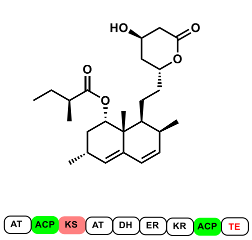


Walsh and Tang, *Natural Product Biosynthesis – Chemical Logic and Enzymatic Machinery* **2017**, *RSC Press*

Major Natural Products Families and BGCs

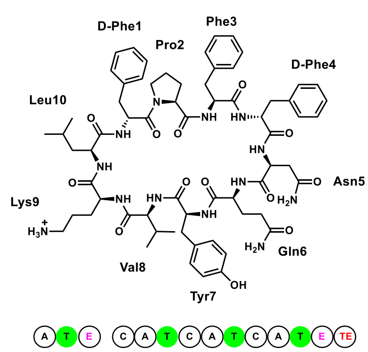
- Major NP families are assembled by "**core, polymerizing**" enzymes, and decorated by "**tailoring**" enzymes.

polyketides



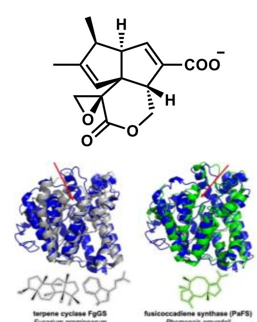
polyketide synthase (PKS)

nonribosomal peptides



Nonribosomal peptide synthetase (NRPS)

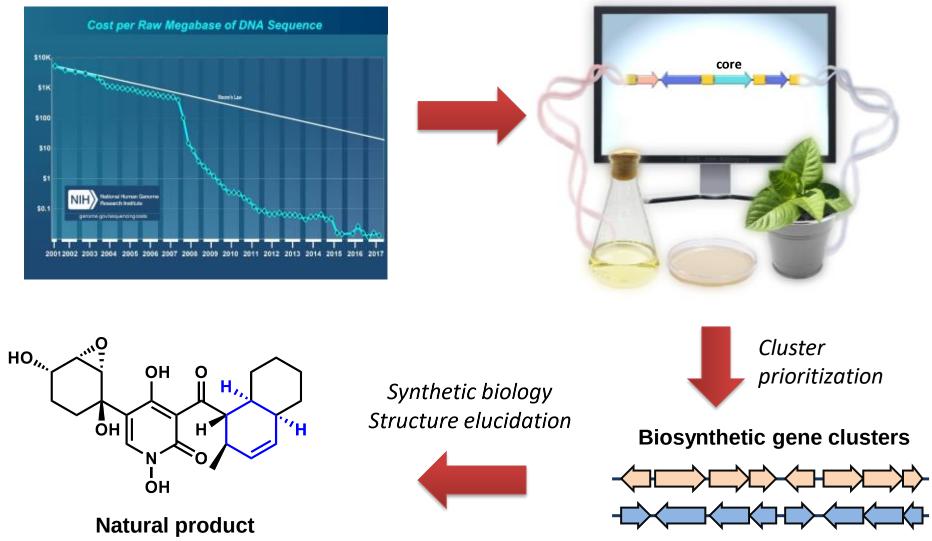
terpenes



terpene synthase/cyclase (TS/TC)

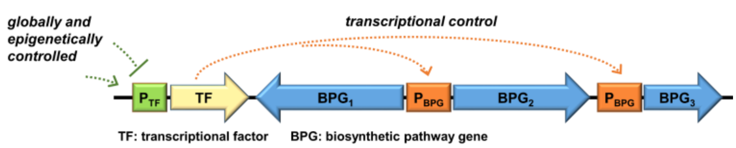
The anchoring core enzymes serve as the starting point for genome-driven NP mining.

Genome Mining of Natural Products



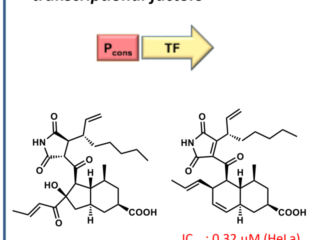
Kim, L., *et al. Nat. Chem. Biol.* **2021**

Genomics Guided Natural Product Discovery



Pathway activation

Constitutively overexpress silent transcriptional factors

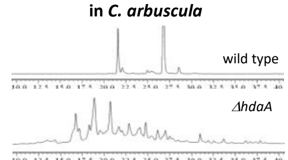


Oxaleimides from *Penicillium oxalicum*

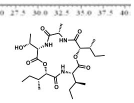
Epigenetic changes

Modify chromatin to make P_{TF} and P_{BPG} more accessible

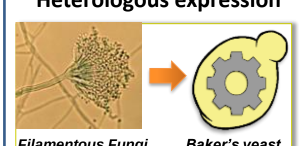
Inactivate HDAC in *C. arbuscula*



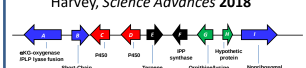
Activated >75% of NP pathways, isolated 10 new compounds



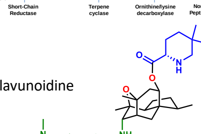
Heterologous expression



Harvey, *Science Advances* **2018**



flavunoidine



Sato, *JACS* **2017** | Mao, *ACIE* **2015** | Yee, *JACS* **2020**

How to mine new NPs from genomes?

~97% of Fungal biosynthetic gene clusters are uncharacterized		
Type of pathway	Characterized	Total
Polyketides	127	4984
Nonribosomal peptides	81	2983
Alkaloids	44	550
Diterpenes	25	336
Total	277 (3.1%)	8853

How to search through genomes for gene clusters of interest?

Can we search gene clusters based on desired biological activity and structural novelty?

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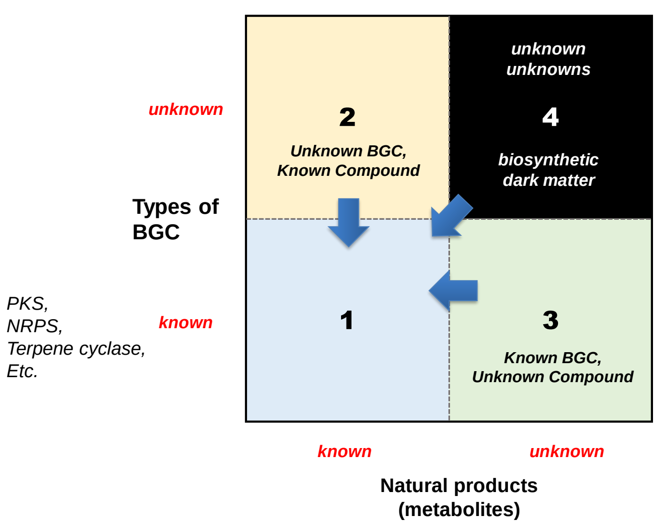
Li and Hillenmeyer, *Fungal Genet Biol*, **2016**; Walsh and Tang, *Natural Product Biosynthesis – Chemical Logic and Enzymatic Machinery* **2017**

29

29

Natural Products (NPs) and BGCs



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Biermann and E. J. N. Helfrich, *mSystems*, **2021**, e0084621.

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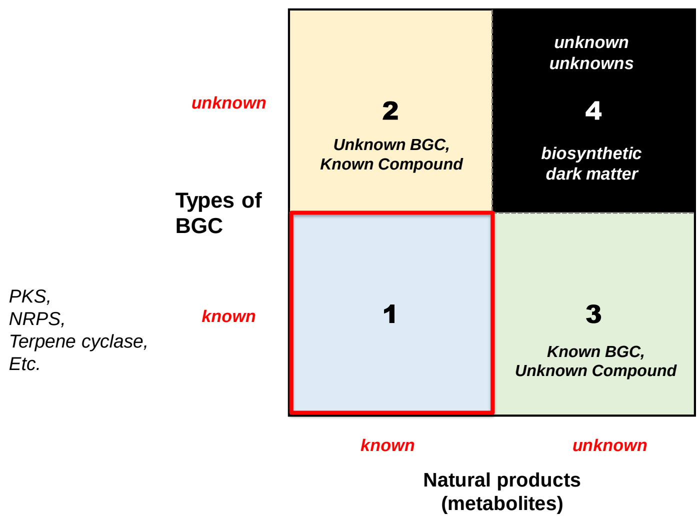
Natural Products (NPs) and BGCs



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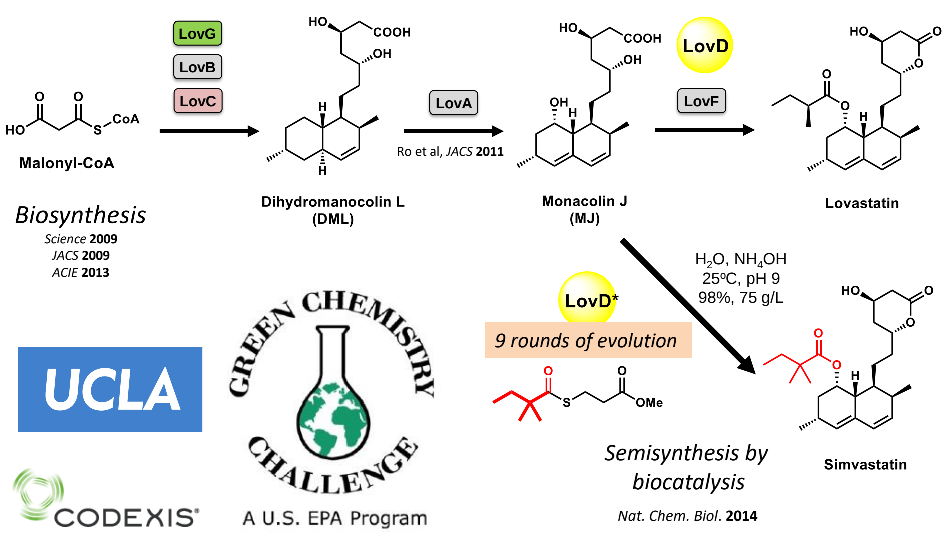
Biermann and E. J. N. Helfrich, *mSystems*, **2021**, e0084621.

From NP Biosynthesis to Biocatalysis



2012

Used in commercial API manufacturing

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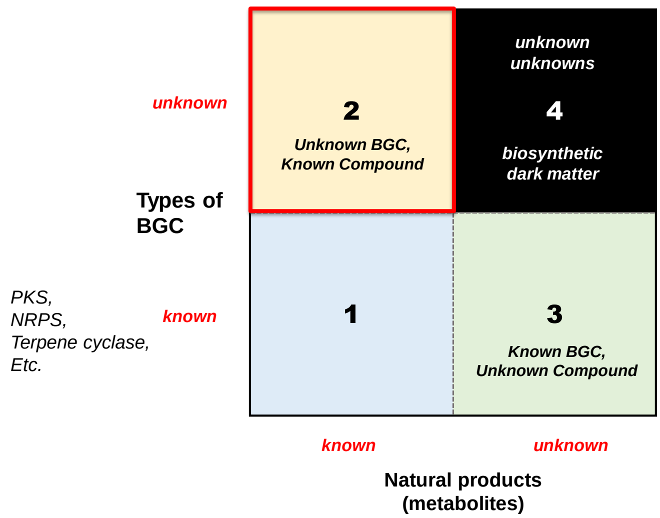
Natural Products (NPs) and BGCs



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Biermann and E. J. N. Helfrich, *mSystems*, **2021**, e0084621.

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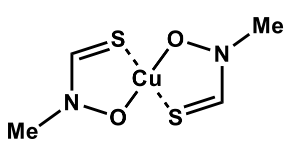
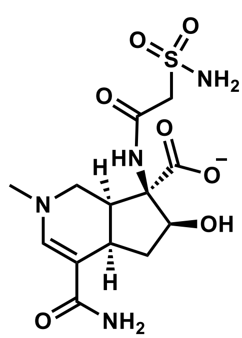
(previously) Unknown BGC-Known Compounds



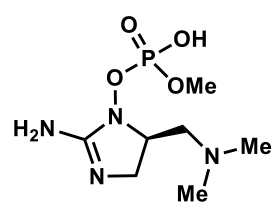
fluopsin

Bo Li and coworkers, *Science* **2021**



altemicidin

Ikuro Abe and coworkers, *Nature* **2022**



guanitoxin

Bradley Moore and coworkers, *JACS* **2022**

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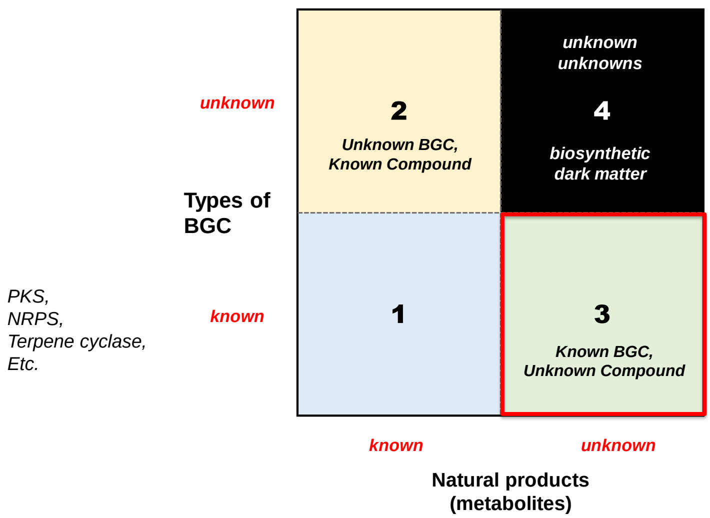
Natural Products (NPs) and BGCs



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Biermann and E. J. N. Helfrich, *mSystems*, **2021**, e0084621.

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Known (BGC) –Unknowns (NPs)

Biosynthetic gene clusters (BGCs) were predicted by AntiSMASH 5.0

Output for a biocontrol fungus *Trichoderma afroharzianum* t-22

Compound family	# of BGC	Reported NPs
Polyketides	16	harzianolide, pachybasin azaphilone
Nonribosomal Peptides	22	peptaibols, gliotoxin
Polyketide-peptide hybrids	8	trichosetin, harzianic acid,
Terpenes	11	abscisic acid*
RiPPs	1	-
Total	**58**	**9**

Most predicted BGCs are cryptic and have no associated NPs

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How to mine new NPs from genomes?

~97% of Fungal biosynthetic gene clusters are uncharacterized		
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Li and Hillenmeyer, *Fungal Genet Biol*, **2016**; Walsh and Tang, *Natural Product Biosynthesis – Chemical Logic and Enzymatic Machinery* **2017**

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What Makes a *Known-Unknown* BGC Novel?

- ***Novel clusters lead to novel natural products***
  - A cluster that offers minimal clue to the structure of NP
  - For fungi, >30 kB of biosynthetic enzymes
  - Abundance of tailoring enzymes (redox enzymes transferases, PLP-dependent, pericyclases, etc)
  - Hypothetical proteins (including DUFs)
  - Atypical core enzyme domain arrangements
  - Combinations of core enzymes in a single cluster
  - Etc.

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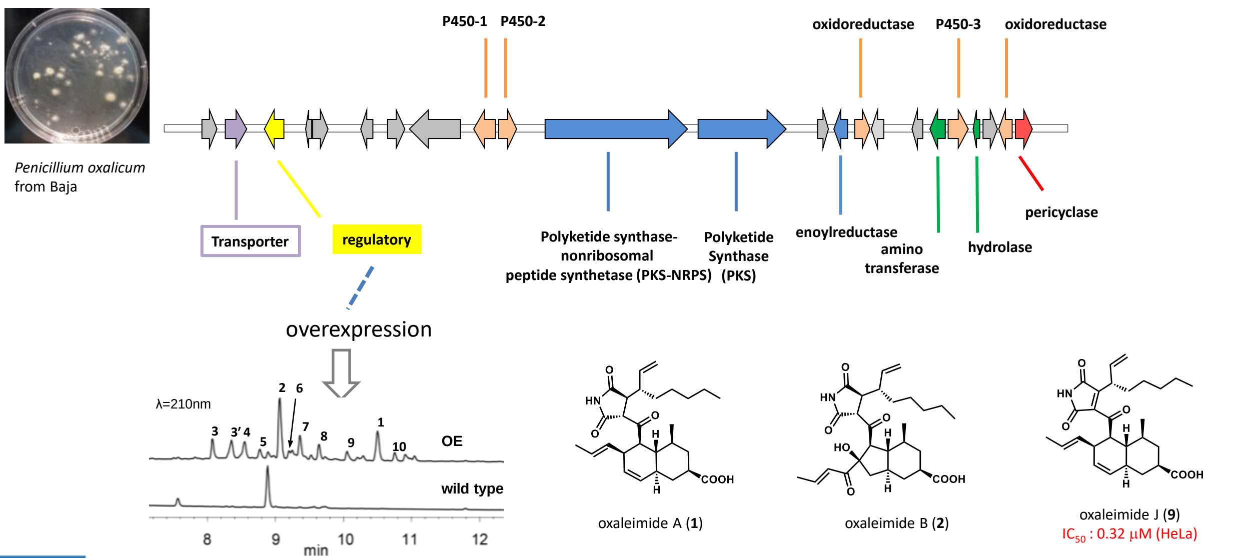
Chiang et al, *Nat. Prod. Rep.* **2022**

38

38

Example of KU Mining from Fungi



Sato *et al.*, *JACS*, **2017**

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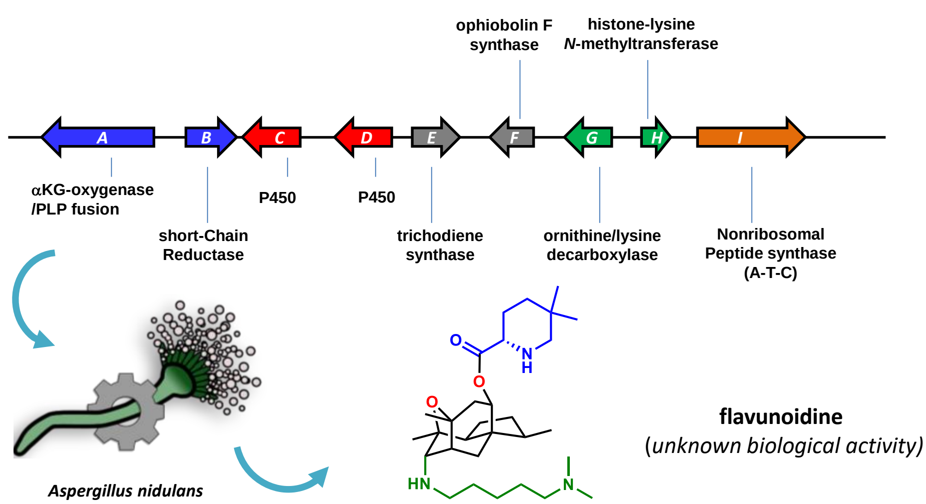
Example of KU Mining from Fungi

The cluster is entirely conserved in *Aspergillus oryzae. Aspergillus turcosus, etc.*



Yee *et al.*, *JACS*, **2020**

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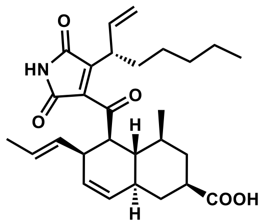
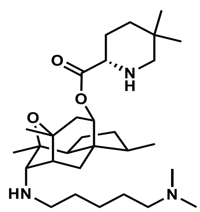
Genome Mining for Desired Activity

		New Compound?	Bioactivity?	Target?
	oxaleimide J	YES	YES	NO
	flavunoidine	YES	NO	NO

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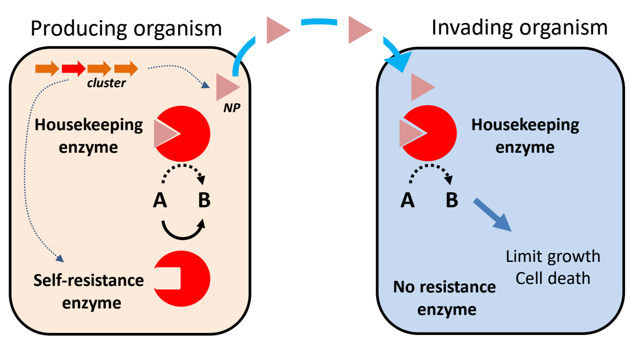
Mining Guided by Self-Resistance Enzyme



Self-resistance enzyme

- provides the essential resistance needed to neutralize the effects of the natural product on the producing host.
- is frequently a mutated version of a housekeeping enzyme that is insensitive to the natural product and performs the same function.
- The encoding gene is colocalized in the natural product biosynthetic gene cluster.

Provides a predictive window to the function of the NP encoded by the gene cluster

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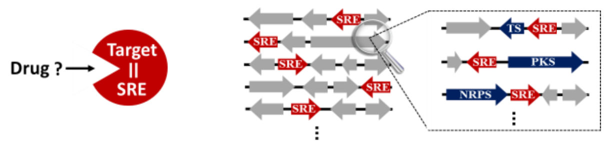
42

Mining Guided by Self-Resistance Enzyme

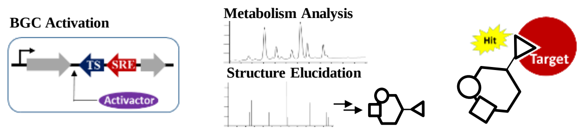
Step 1: Biosynthetic cluster Identification

Target → **Genomics** → **Bioinformatics**



Step 2: NP Production and Target Validation

Molecular Biology → **Analytical Chemistry** → **Bioassay**



Step 3: Biological Activity Demonstration

Lead Verification → **Medicinal Chemistry** → **Activity optimization**

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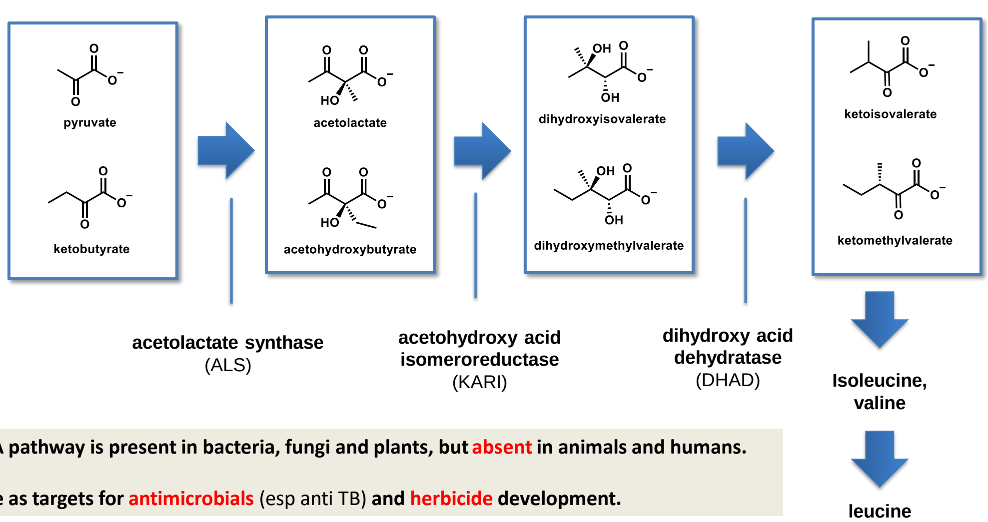
43

Target: Branched Chain Amino Acid Biosynthesis



The BCAA pathway is present in bacteria, fungi and plants, but absent in animals and humans.

Attractive as targets for antimicrobials (esp anti TB) **and herbicide development.**

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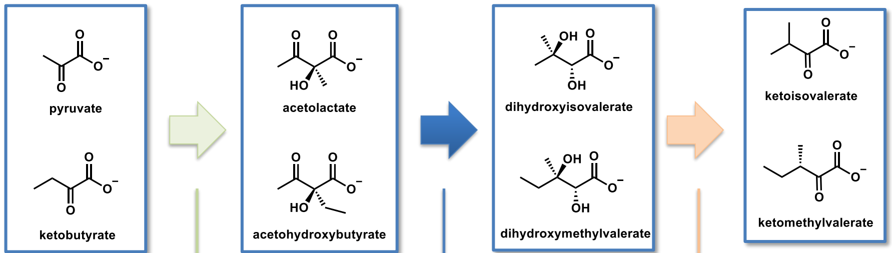
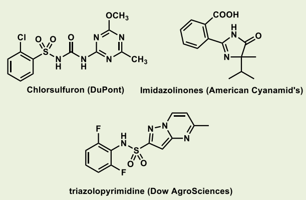
44

BCAA as herbicide targets

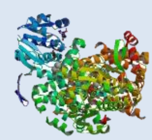


acetolactate synthase (ALS)



ALS is the most common target for registered herbicide

acetohydroxy acid isomeroreductase (KARI)



Successful crystallization of KARI can guide rational design of inhibitors

Acta Crystallogr. D Biol. Crystallogr. **2000**; 56, 389

dihydroxy acid dehydratase (DHAD)

1. Targeted for development of herbicide by major ag chemical companies with no success.
2. No crystal structure available.
3. **No natural product inhibitor of DHAD is known.**

Bioorg. Chem. **1993**; 21, 367.

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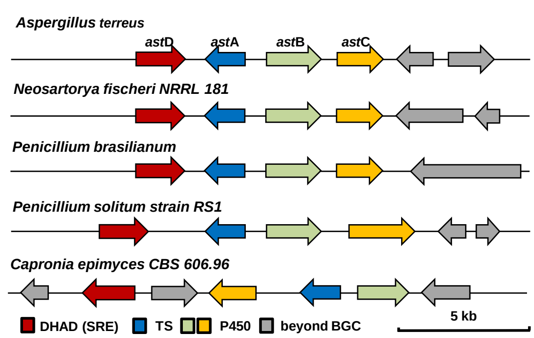
A Potential DHAD Inhibitor BGC

A conserved fungal terpene BGC contains DHAD as second copy (60% identity) **in addition to the housekeeping DHAD**



A. terreus **NIH 2624, scaffold 6 (NT_165929.1, 469,00-486,00), 17 kbp**

Gene	Conserved domain
astA	Trichodiene synthase (TRI5), pfam06330
astB	Cytochrome P450, pfam00067
astC	Cytochrome P450, pfam00067
astD	Dihydroxy-acid dehydratase, PRK00911

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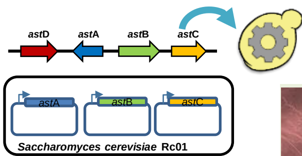
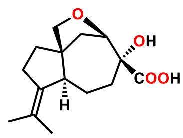
Heterologous Expression of BGC in Yeast





aspterric acid

Titer: ~ 20 mg/L

Original isolation:
J. Chem. Soc., Chem. Commun., ***1978***, *160-161.*

		Kinetic parameters	AA inhibition parameters	AA sensitivity
	***Aspergillus terreus*** **DHAD** (housekeeping)	k_{cat} =3.0 s^{-1} K_m > 20 mM	IC_{50} = 0.31 μM	sensitive
	***Arabidopsis thaliana*** **DHAD** (target)	k_{cat} = 1.2 s^{-1} K_m = 5.7 mM	IC_{50} = 0.50 μM K_i = 0.3 μM *Competitive*	sensitive
	***Aspergillus terreus*** **AstD** (resistance?)	k_{cat} = 0.05 s^{-1} K_m = 5.4 mM	IC_{50} > 8 mM (solubility limit)	**insensitive**

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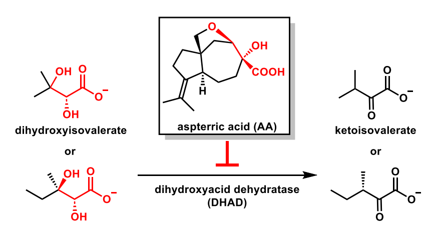
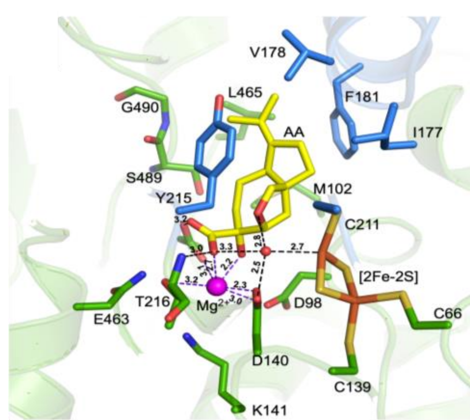
Yan, et al, *Nature* **2018**

Mechanism of Inhibition





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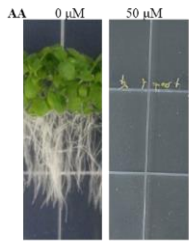
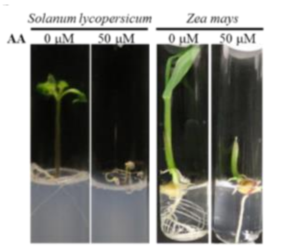
unpublished

Herbicidal Activities of AA

Growth inhibition of plant on agar plate





Top view

Side view

*solvent 250 mM AA + *solvent

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Yan, et al, *Nature* **2018**

49

49

Hexagon Bio

Menlo Park, CA

Genomics	Data Science	Synthetic biology	Drug Discovery

Genomics
- Strain collecting
- Strain dereplication
- ***Genome sequencing***
- Genome assembly & annotation

Data Science
- Genome assembly & annotation
- Gene cluster mining
- ***Target prediction & gene cluster scoring***
- LCMS data processing and analysis

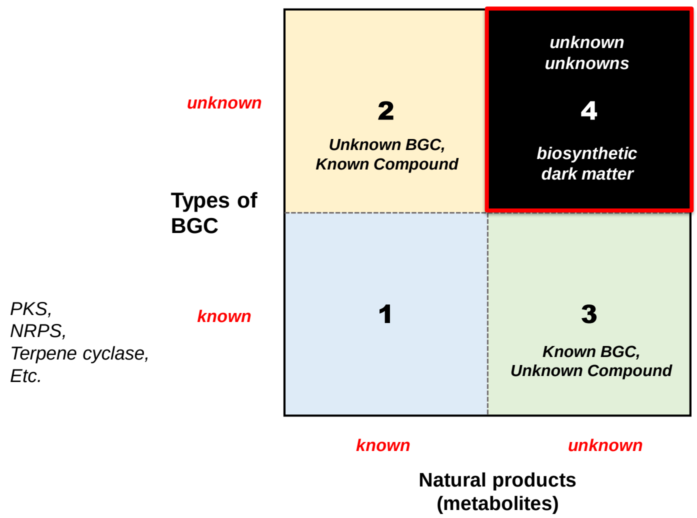
Synthetic biology
- Manual gene cluster curation
- ***Cluster activation and expression***
- Cluster product analysis and characterization

Drug Discovery
- Assay development
- ***Bioactivity screening of cluster products***
- Medicinal chemistry and lead optimization

50

50

Natural Products (NPs) and BGCs



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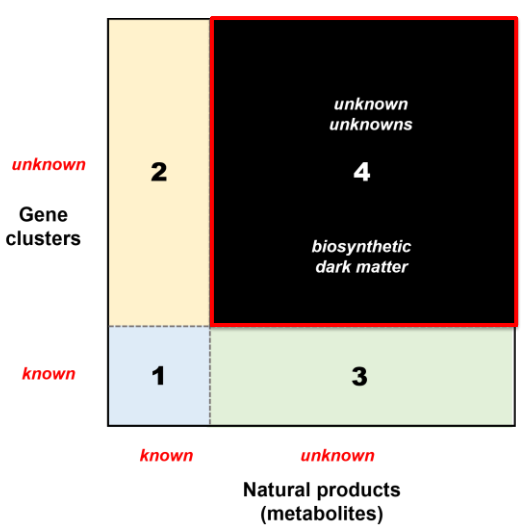
Biermann and E. J. N. Helfrich, *mSystems*, **2021**, e0084621.

Search for the Unknown/Unknown



What makes a cluster UU?

- No predicted core enzymes (PKS, NRPS, TS, Prenyltransferase) → new methods to generate molecular scaffold (C-X bond formation)
- Abundance of modification enzymes (redox enzymes, transferases, PLP-dependent enzymes, pericyclases, etc)
- Hypothetical protein (HP)
- DUFs (proteins with domains of unknown function)
- Etc.

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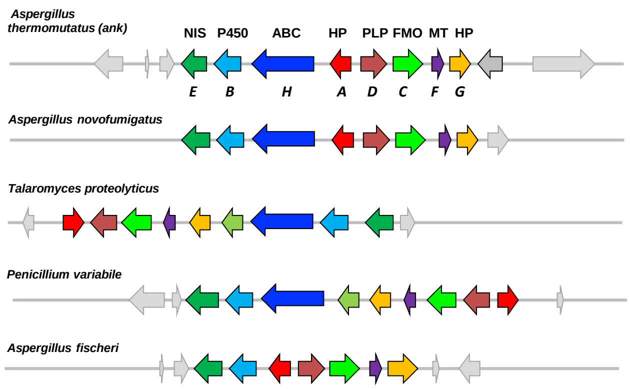
Biermann and E. J. N. Helfrich, *mSystems*, **2021**, e0084621.

Example of Unknown-Unknown BGC Mining



gene	predicted function
E	NIS synthetase
B	cytochrome P450
H	ABC bile acid transporter
A	hypothetical protein
D	PLP-dependent transferase
C	FAD monooxygenase
F	*O*-methyltransferase
G	hypothetical protein

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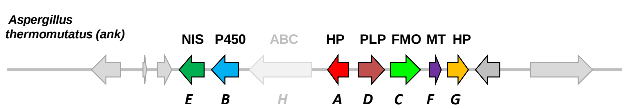
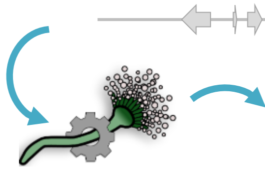
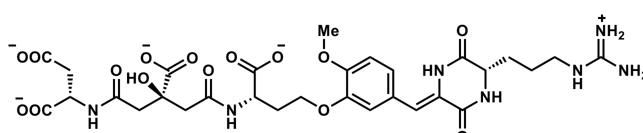
Yee, et al, *Nature Chemical Biology* **2023**

Heterologous recon. of *ank* cluster

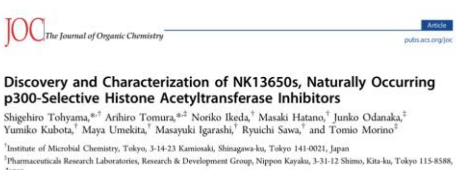




Aspergillus nidulans A1145



NK13650 C


The Journal of Organic Chemistry
Article
pubs.acs.org/joc

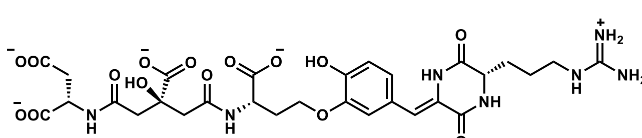
Discovery and Characterization of NK13650s, Naturally Occurring p300-Selective Histone Acetyltransferase Inhibitors

Shigehiro Tohyama,*,† Arihiro Tomura,*,‡ Noriko Ikeda,† Masaki Hatano,† Junko Odanaka,‡ Yumiko Kubota,† Maya Umekita,† Masayuki Igarashi,† Ryuichi Sawa,† and Tomio Morino‡

†Institute of Microbial Chemistry, Tokyo, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan
‡Pharmaceuticals Research Laboratories, Research & Development Group, Nippon Kayaku, 3-31-12 Shimo, Kita-ku, Tokyo 115-8588, Japan

Tohyama, *JOC* **2012**

New compound, but..



NK13650 A

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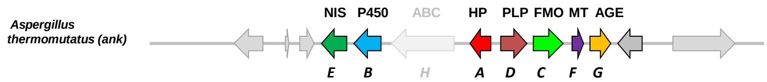
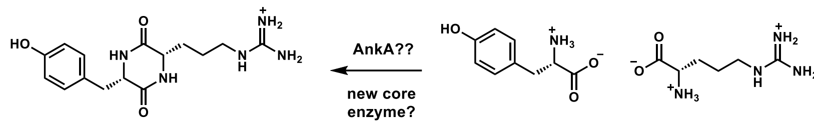
Biosynthesis of NK13650

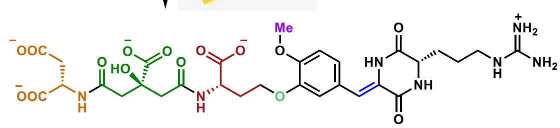




cyclo-Arg-Tyr L-Tyr L-Arg

AnkBCDEFG

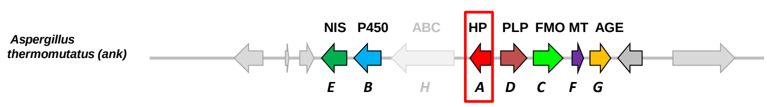
Building molecular complexity without core "polymerization" enzyme

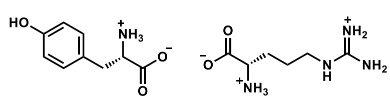


NK13650 C

Ank	verified function
E	citrate ligase
B	cytochrome P450
A	hypothetical protein
D	PLP-dependent C-O forming
C	FAD monooxygenase
F	*O*-methyltransferase
G	ATP grasp enzyme

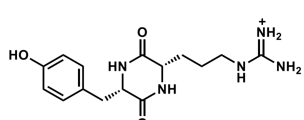
AnkA is the core enzyme?





L-Tyr L-Arg

AnkA



cyclo-Arg-Tyr

Initial assignment

A	hypothetical protein

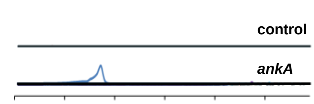
518 aa protein

No predicted function
No characterized sequence homolog
No structural homolog
No sequence resemblance to NRPS or bacterial CDPS.

Verified function in yeast

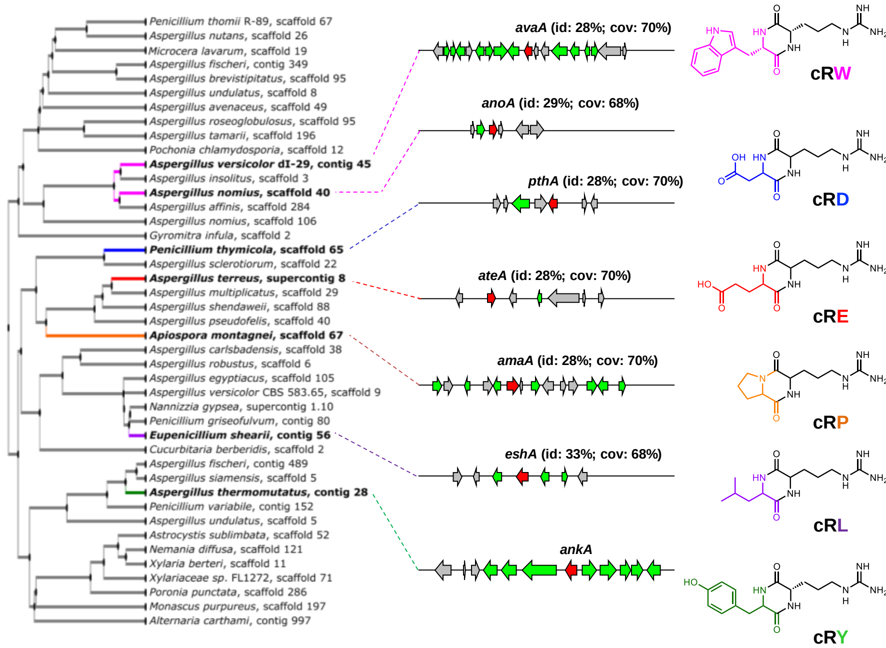
A	Cyclo-Arg-Tyr synthase

AnkA is the core enzyme in this pathway!



Yee, et al, *Nature Chemical Biology* **2023**

MAFFT
phylogenetic tree



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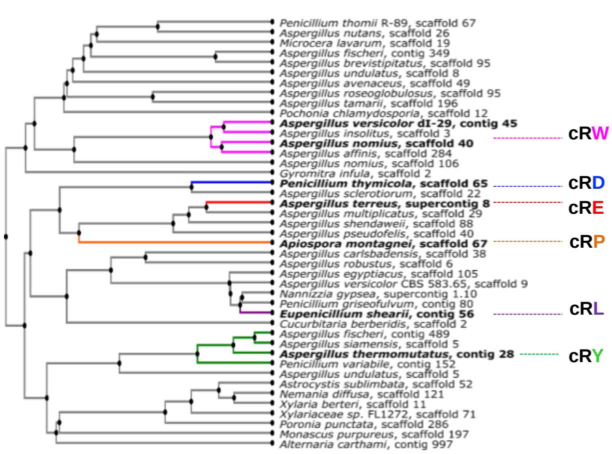
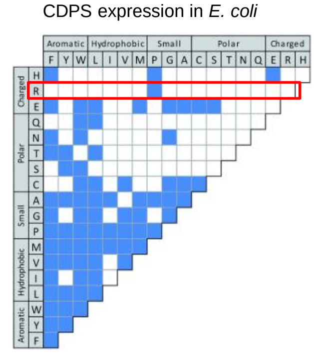
57

Genome Mining of AnkA-like Enzymes

Over 100 homologs of AnkA detected from JGI/NCBI blast search



Cyclodipeptides accessible through CDPS expression in *E. coli*



Canu and Magarvey ***2019***

Fungal AknA homologs (CDRPSs) generate rare and new-to-nature Cyclo-Arg-Xaa didpeptides.

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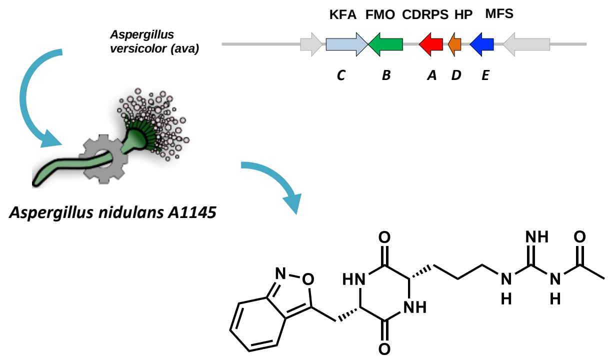
58

Using CDRPS to find UU Natural Products



DY225

gene	predicted function
A	cRW synthase (verified)
B	FAD monooxygenase
C	kynurenine formamidase (KFA)
D	hypothetical protein
E	transporter

New compound identified from UU genome mining

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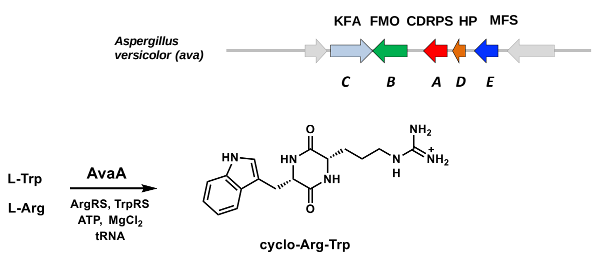
59

Using CDRPS to find UU Natural Products



gene	predicted function
A	cRW synthase (verified)
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C	kynurenine formamidase (KFA)
D	hypothetical protein
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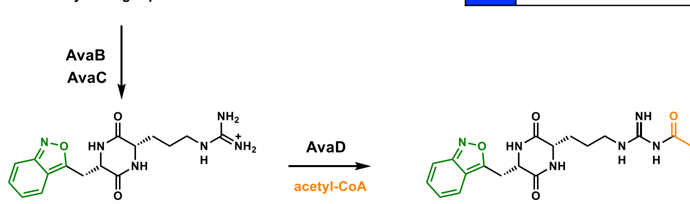

DY225

DY225 may not represent the final NP of the cluster. Surrounding enzyme (including P450s) are currently being tested for function.

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Conclusions

Gene clusters		Natural products (metabolites): known	Natural products (metabolites): unknown
	unknown	*unknown BGC, known Compound* **2** *New Enzymes New Biosynthetic Logic*	*unknown unknowns* **4** *biosynthetic dark matter*
	known	*known BGC, known Compound* **1** *New Enzymes New Biocatalysts*	*known BGC, unknown Compound* **3** *New Structures Targeted Bioactivities*

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61

61

Current Members

Ike Okorafor
Moriel Dror
Yorick Chiang
Abner Abad
Chunsheng Yan
Colin Johnson
Wenyu Han
Theodosia Bartashevitch

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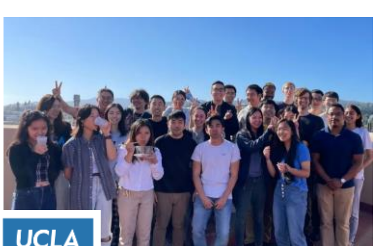
Dr. Zuodong Sun
Dr. Kanji Niwa
Dr. Yalong Zhang
Dr. Mengting Liu

Dr. John Billingsley (VS)



Former Members

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Prof. Neil Garg (UCLA)
Prof. Jose Rodriguez (UCLA)
Prof. Hosea Nelson (Caltech)
Prof. Steve Jacobsen (UCLA)
Prof. Dino Di Carlo (UCLA)
Prof. Ben Tu (UTSW)
Prof. Kenji Watanabe (Shizuoka)
Prof. Jiahai Zhou (SIOC)

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National Science Foundation
National Institute of Health
Department of Agriculture
Packard Foundation
UCLA SEAS Endowment

Conflict of Interest

Y. Tang is a shareholder of Hexagon Bio. Inc.

62

62

63

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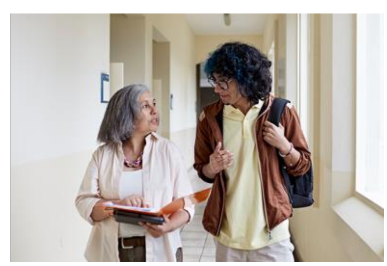

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