

# Small Molecules from the Human Microbiota

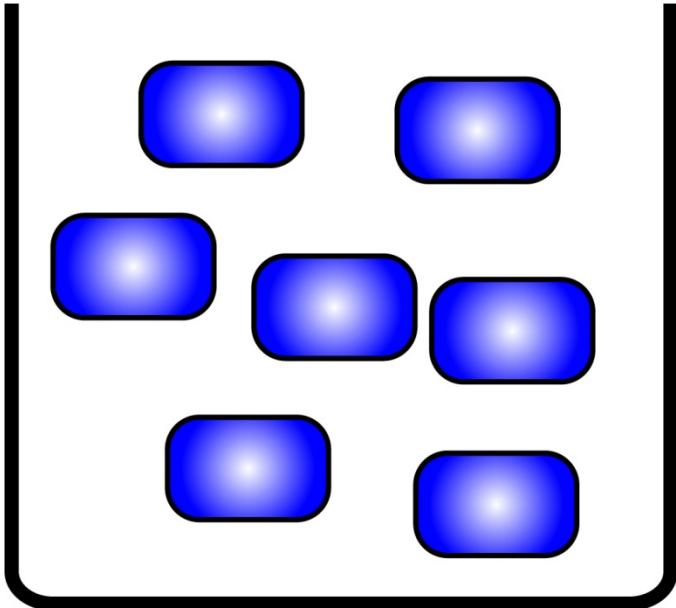
Michael Fischbach, PhD

University of California, San Francisco

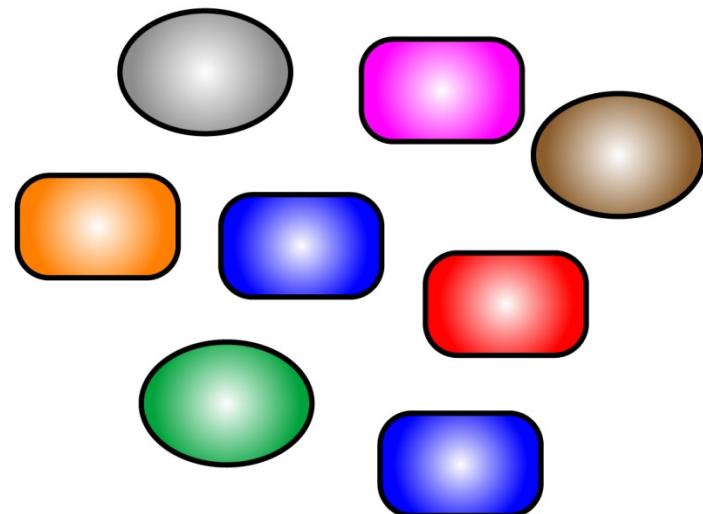


**One handful of soil:  
10,000,000,000 microbes  
100,000 different species**

# 1. How do microbes interact with each other, and with multicellular organisms?



**Microbes in the  
laboratory**



**Microbes in the  
real world**

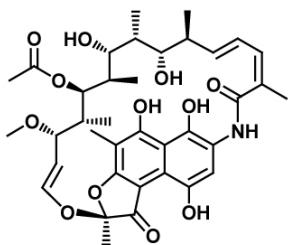
# Natural products: Medicinal relevance

Natural products as drugs (1981-2002)

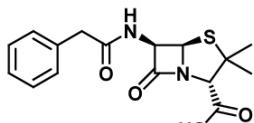
70% of new antibiotics

60% of new anticancer drugs

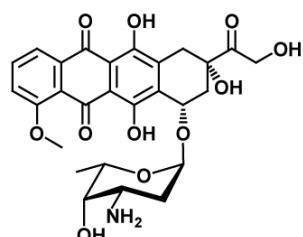
50% of new immunosuppressants



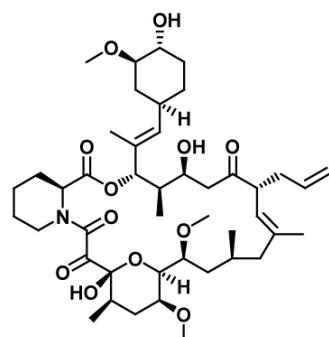
rifamycin  
antitubercular



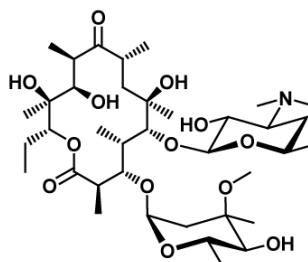
penicillin  
antibacterial



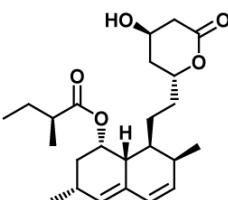
doxorubicin  
anticancer



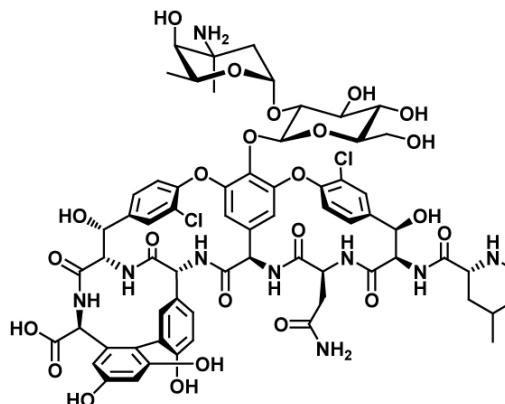
FK506  
immunosuppressant



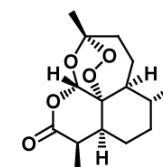
erythromycin  
antibacterial



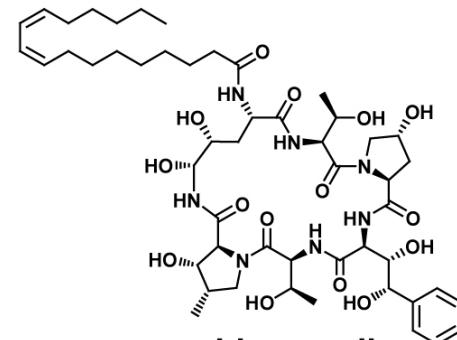
lovastatin  
antihyper-  
cholesterolemic



vancomycin  
antibacterial

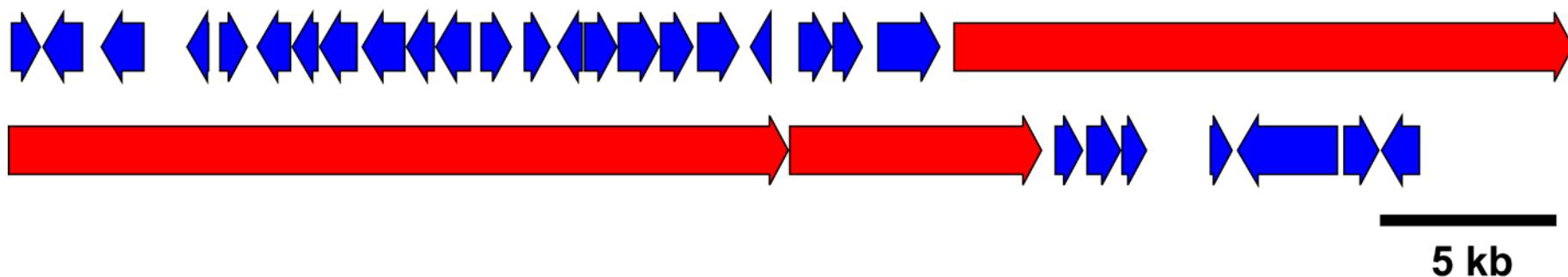
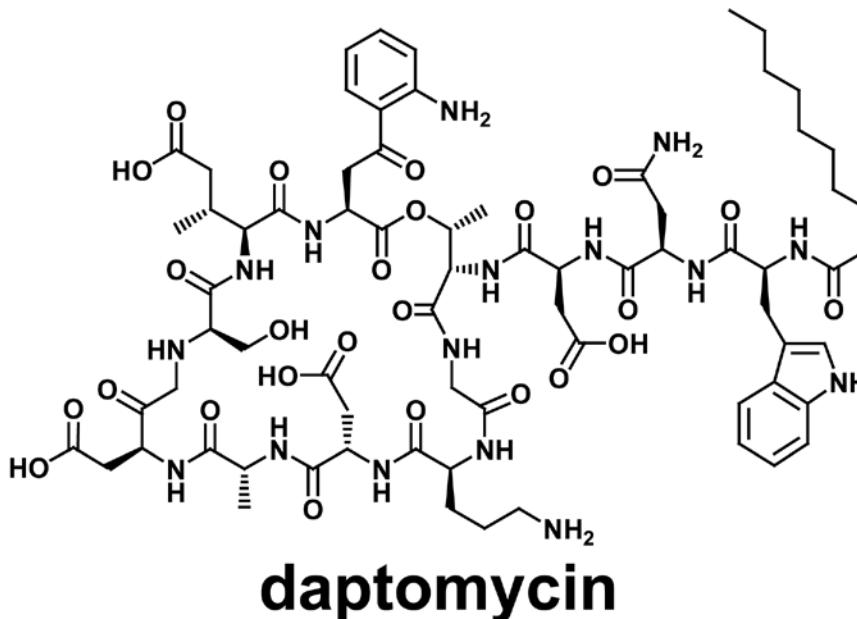


artemisinin  
antimalarial

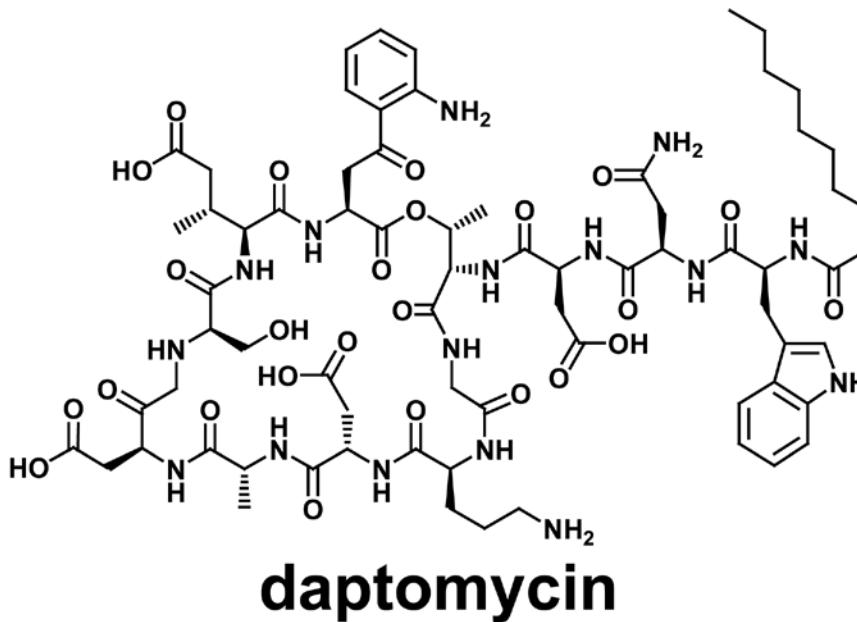


echinocandin  
antifungal

# The metabolic cost of a natural product

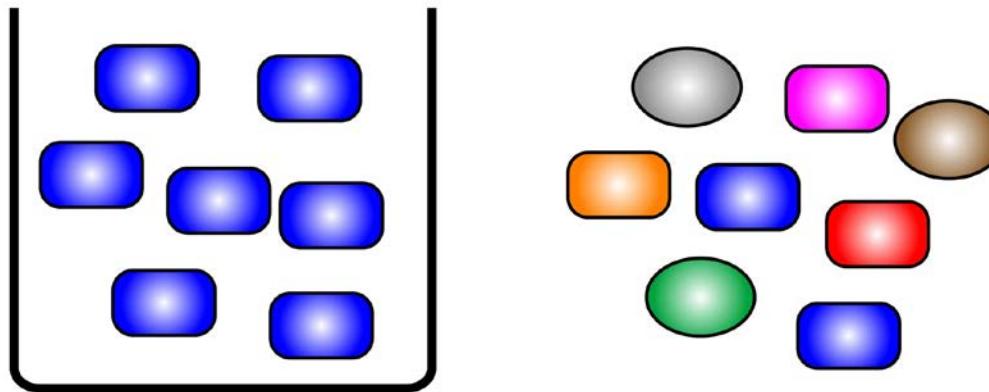


# The metabolic cost of a natural product

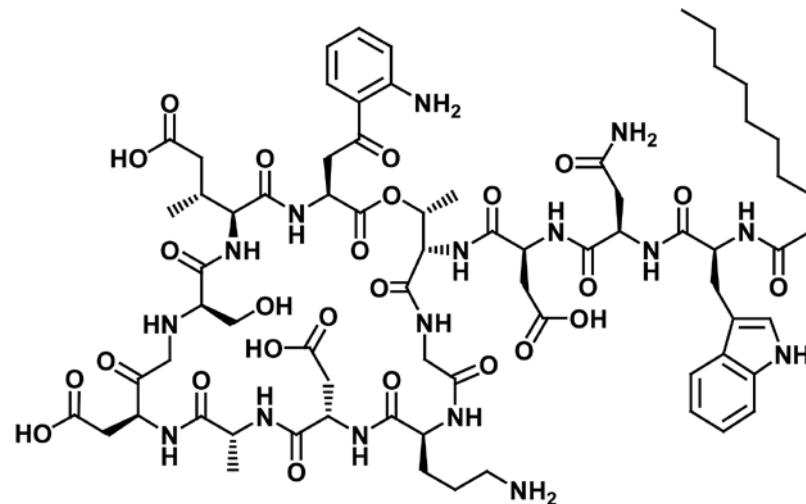


	Ribosome	Daptomycin synthetase
Parts	<i>55 proteins, 3 RNAs</i>	<i>37 proteins</i>
Size	$\sim 2600 \text{ kDa}$	$\sim 3000 \text{ kDa}$
Product	<i>Thousands of proteins</i>	<i>One small molecule</i>

# 1. How do microbes interact with each other, and with multicellular organisms?

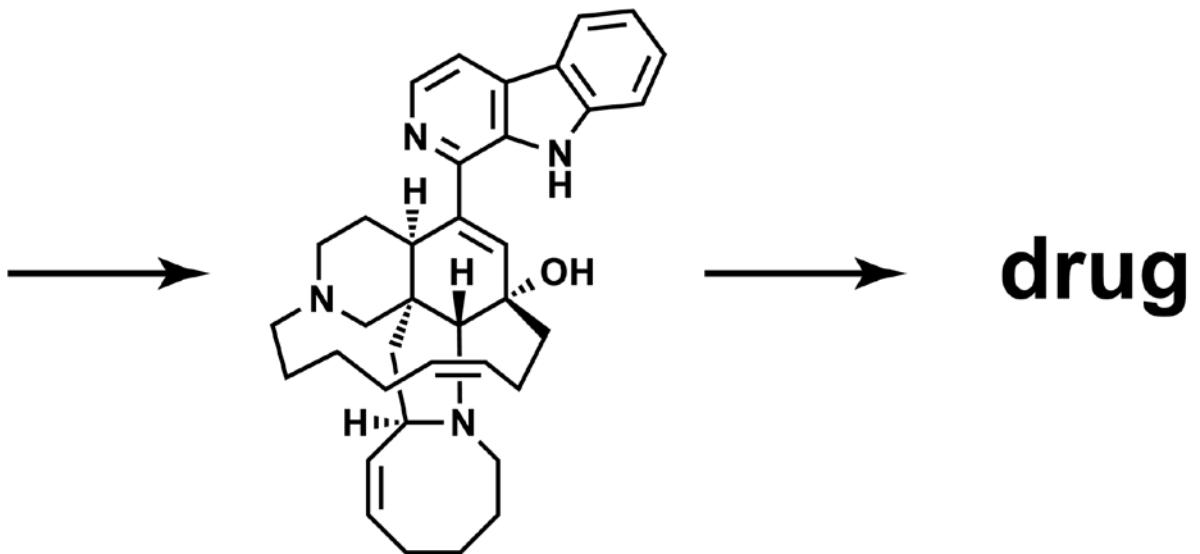
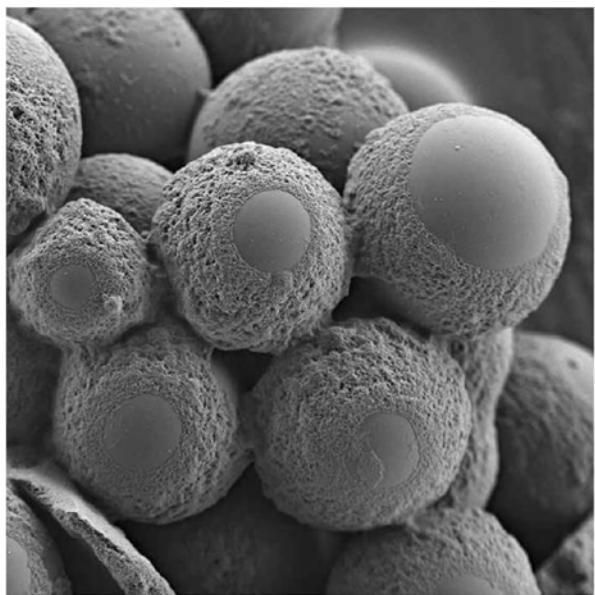


# 2. What are the natural roles of natural products?

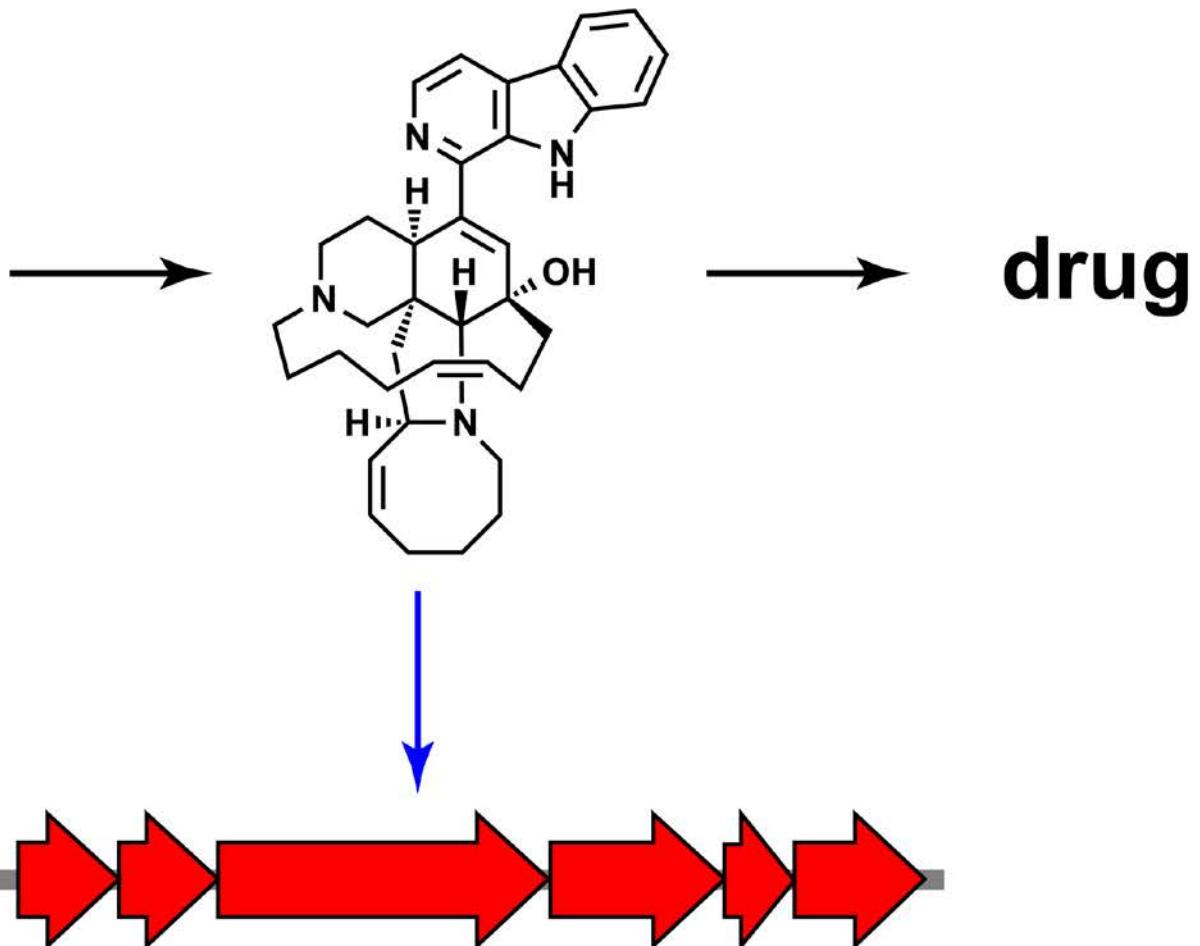
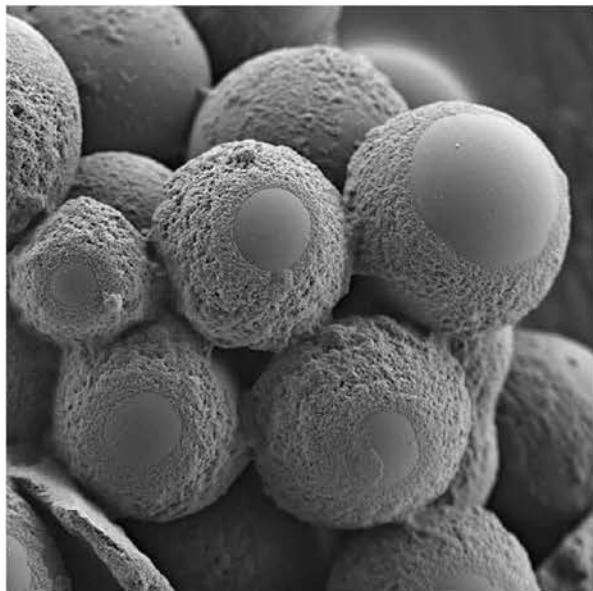


***Natural products mediate interspecies interactions.***

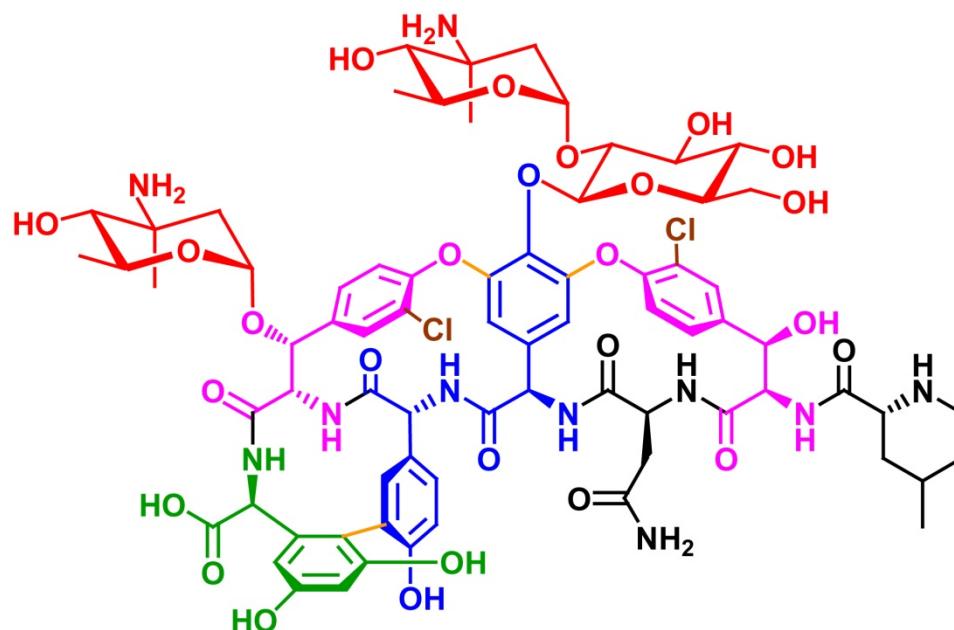
# Old paradigm of natural product discovery



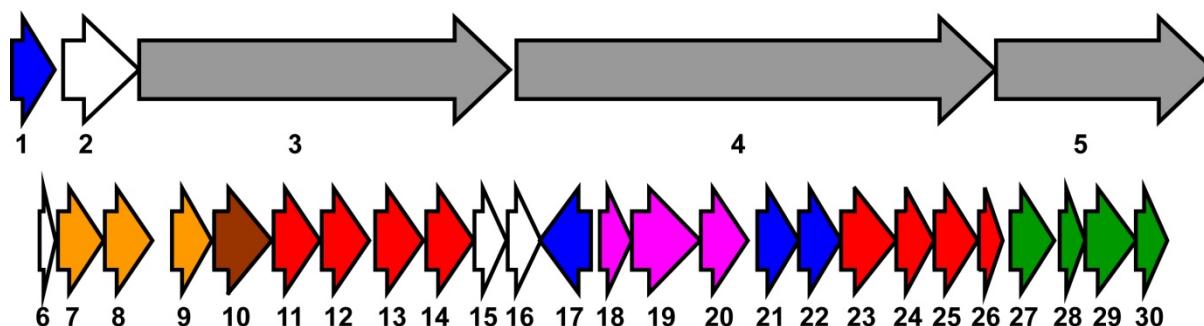
# Connecting molecules to genes



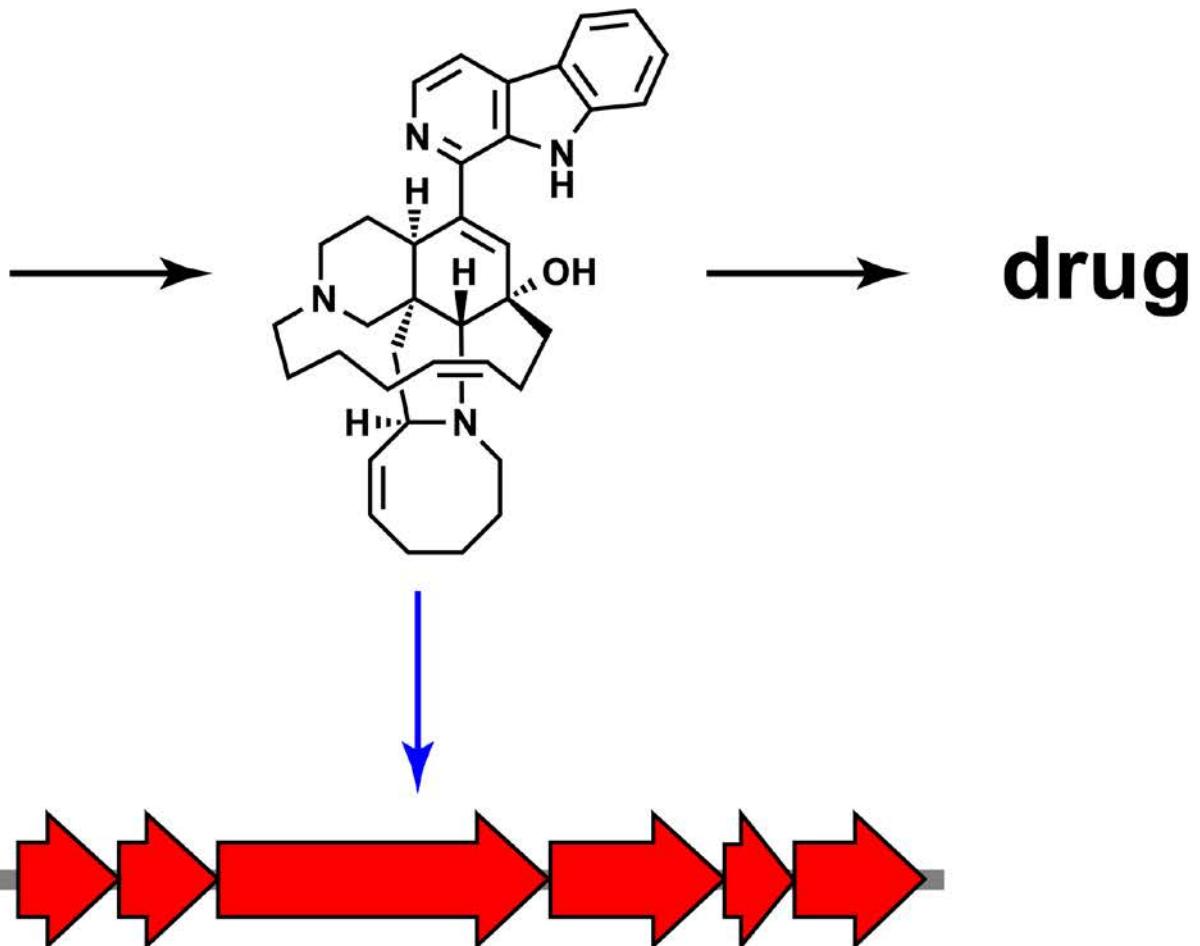
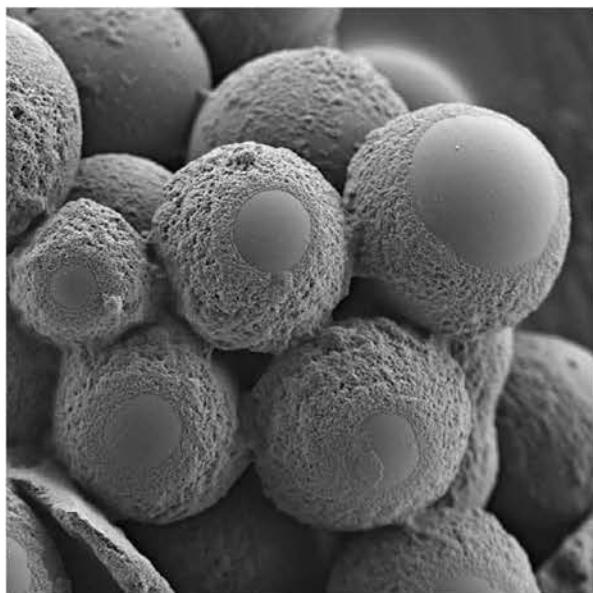
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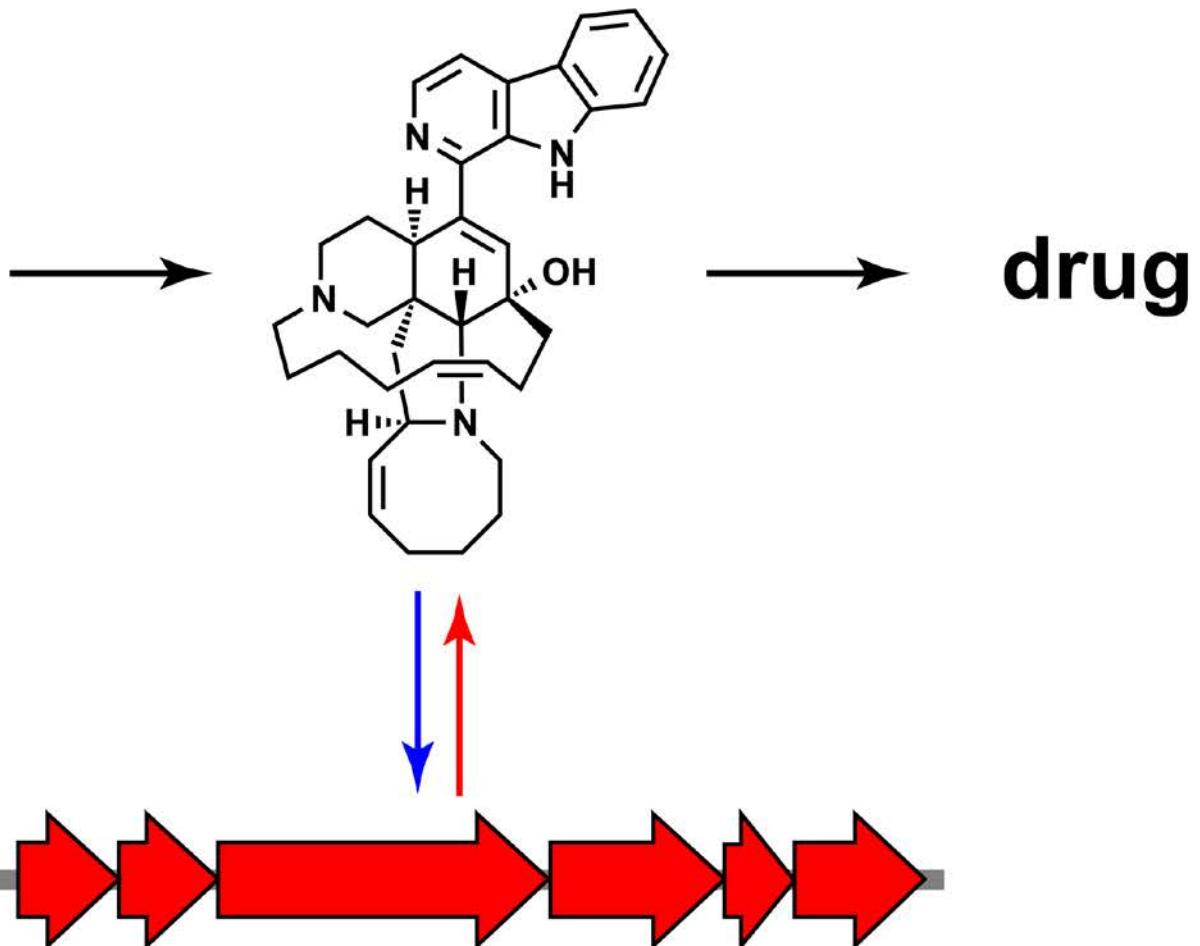
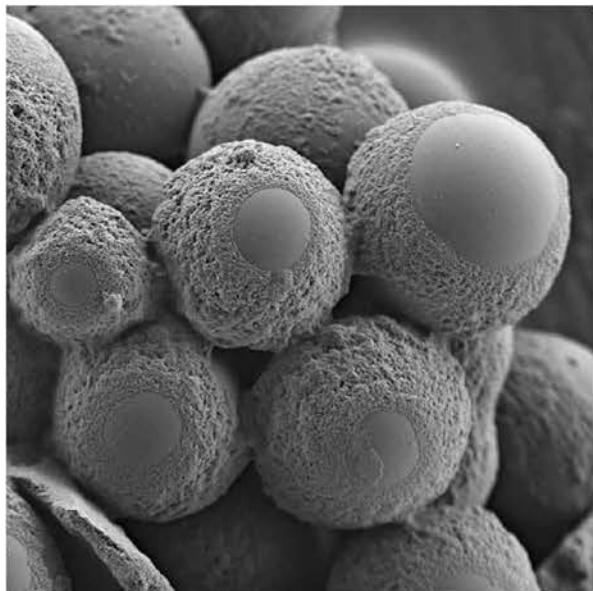
chloroeremomycin



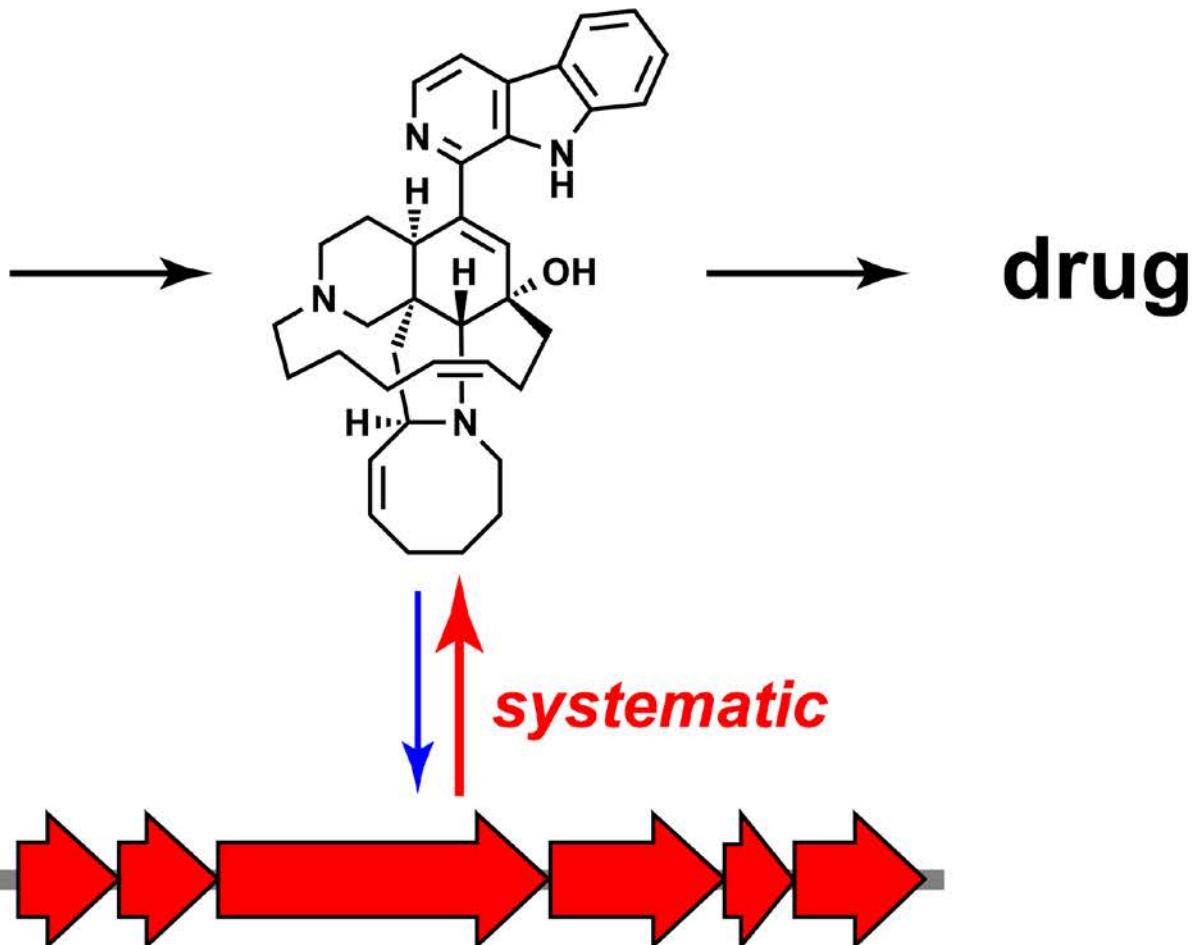
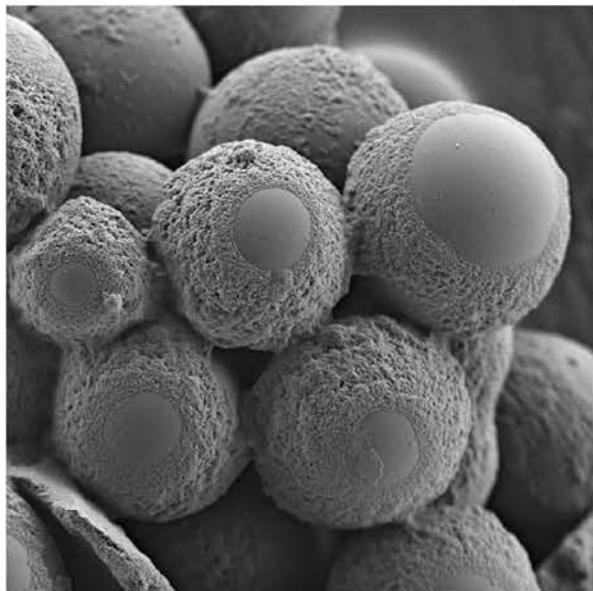
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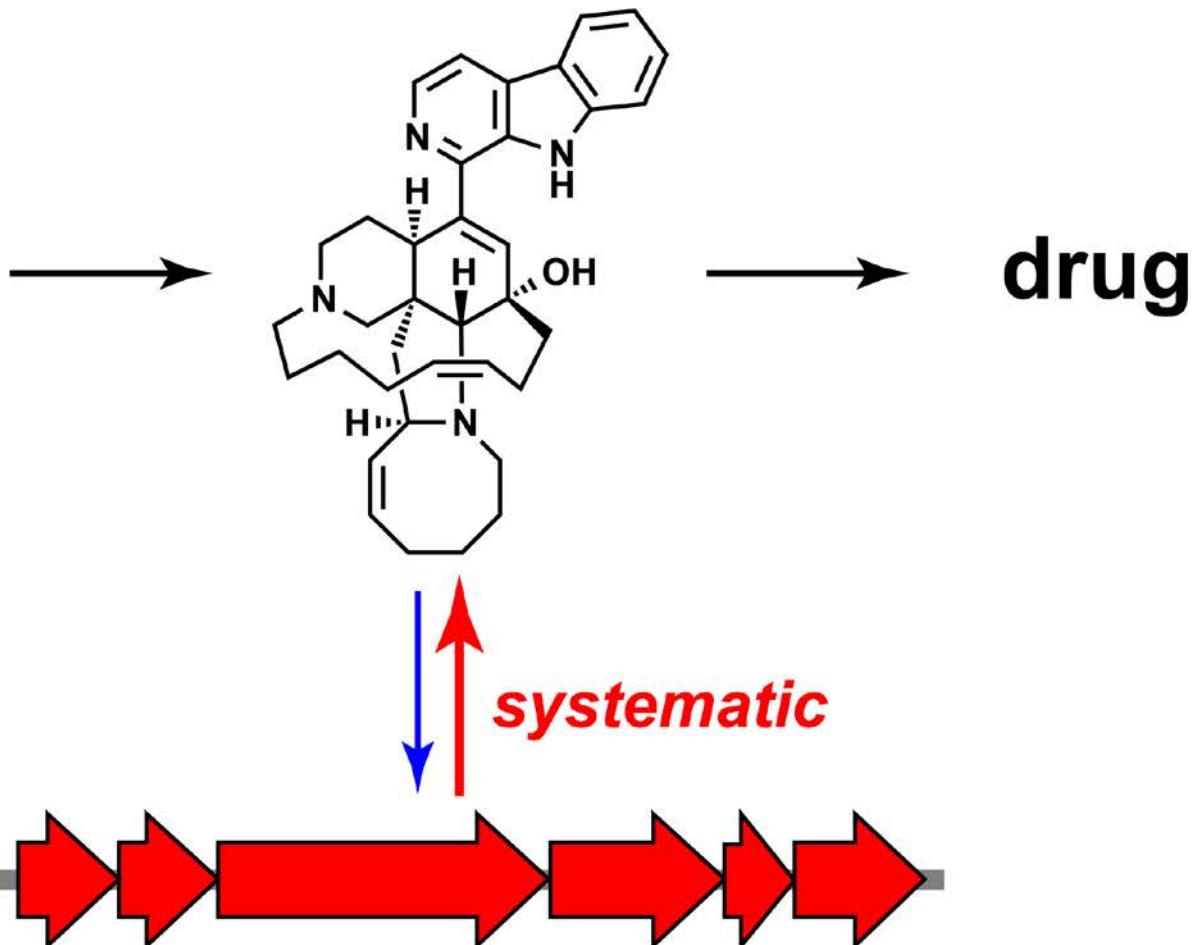
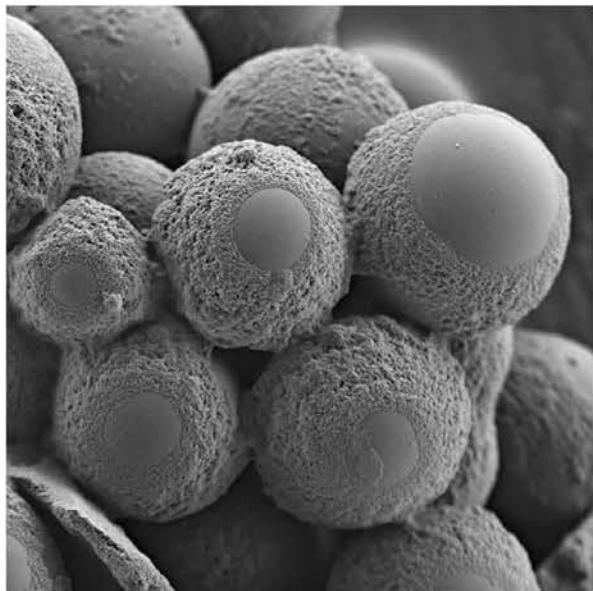
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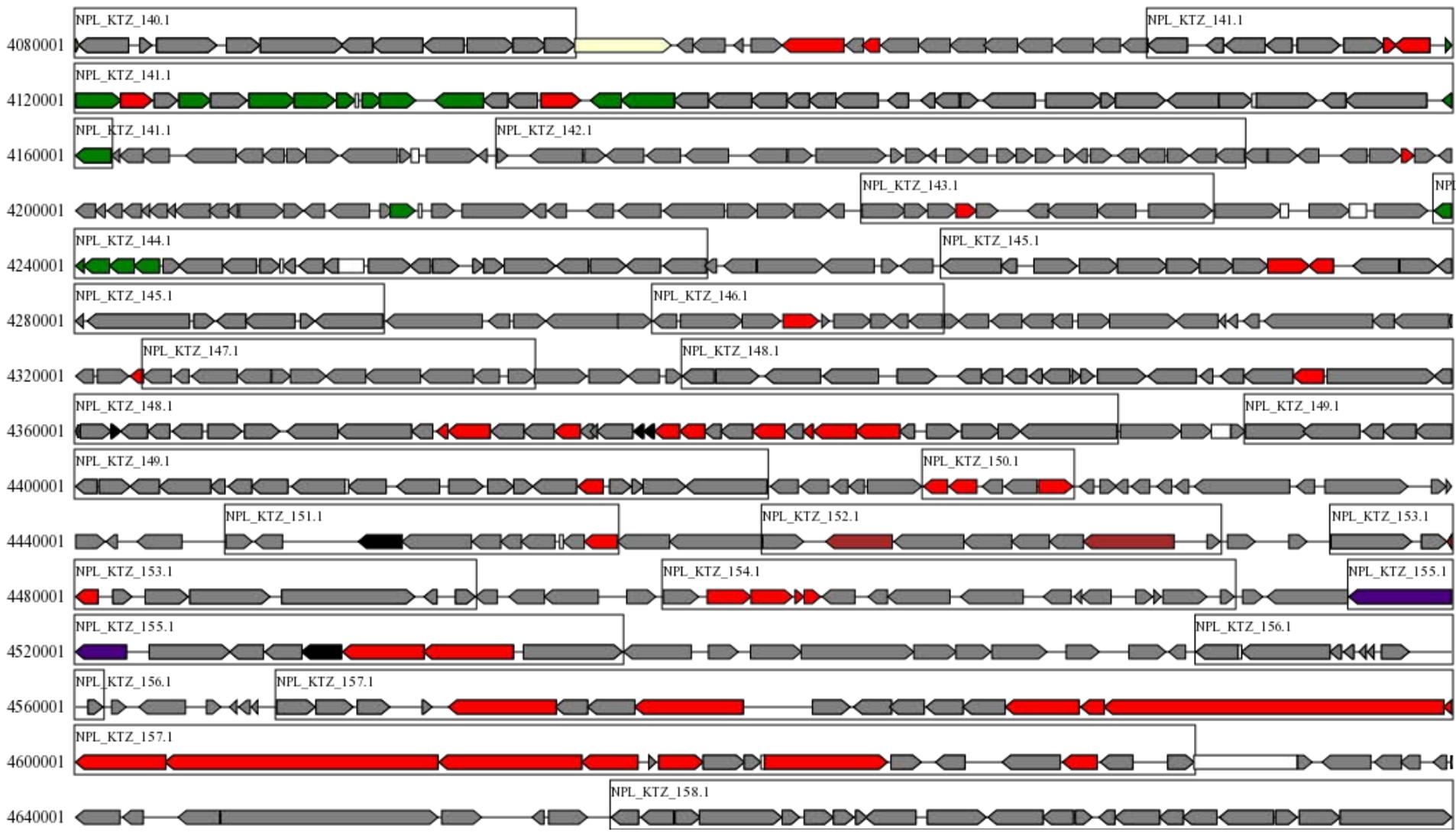
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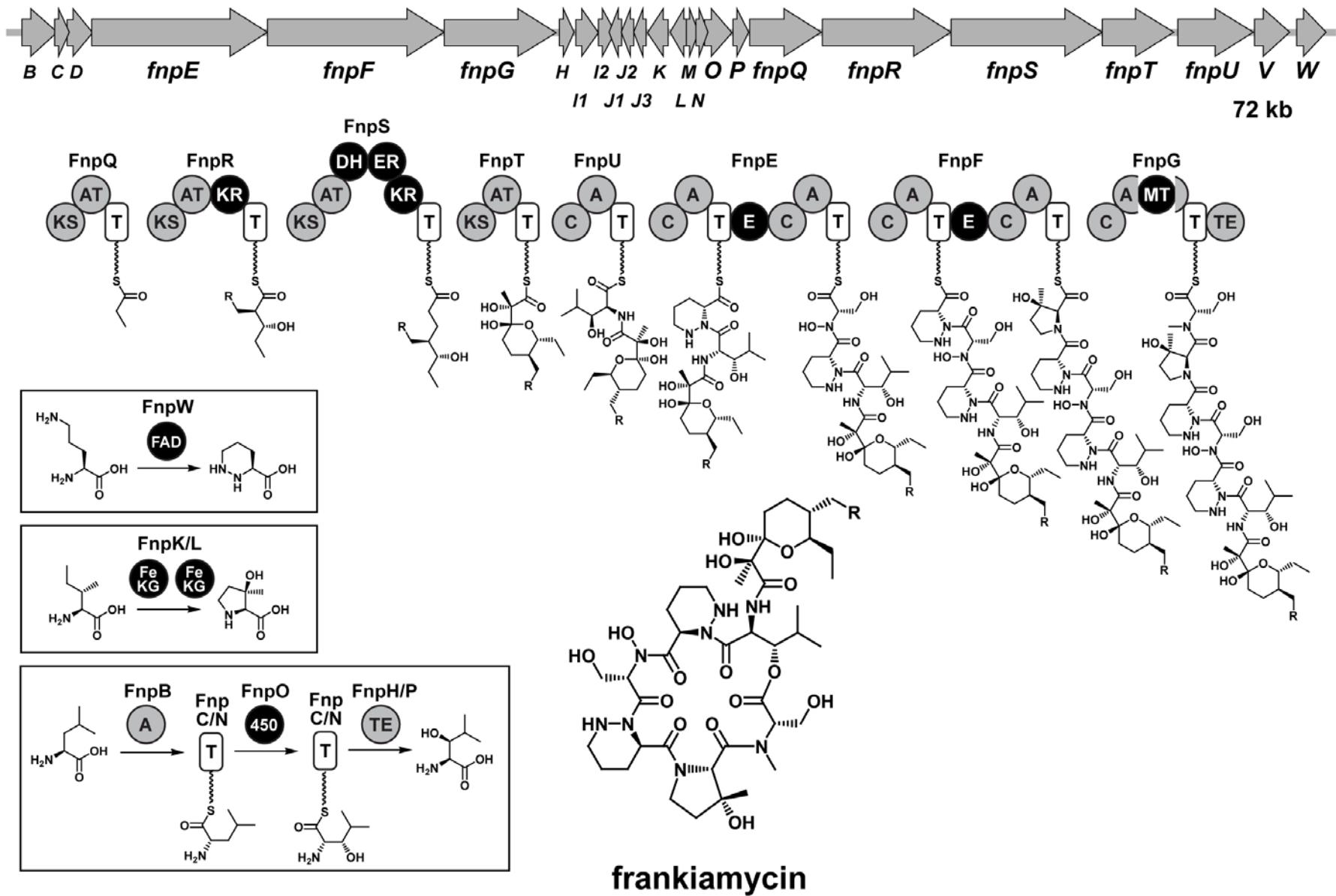
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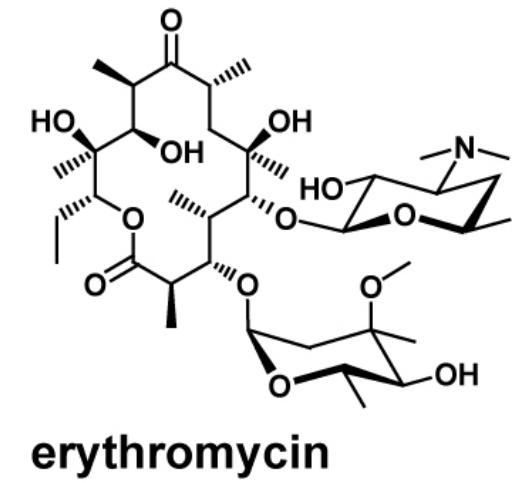
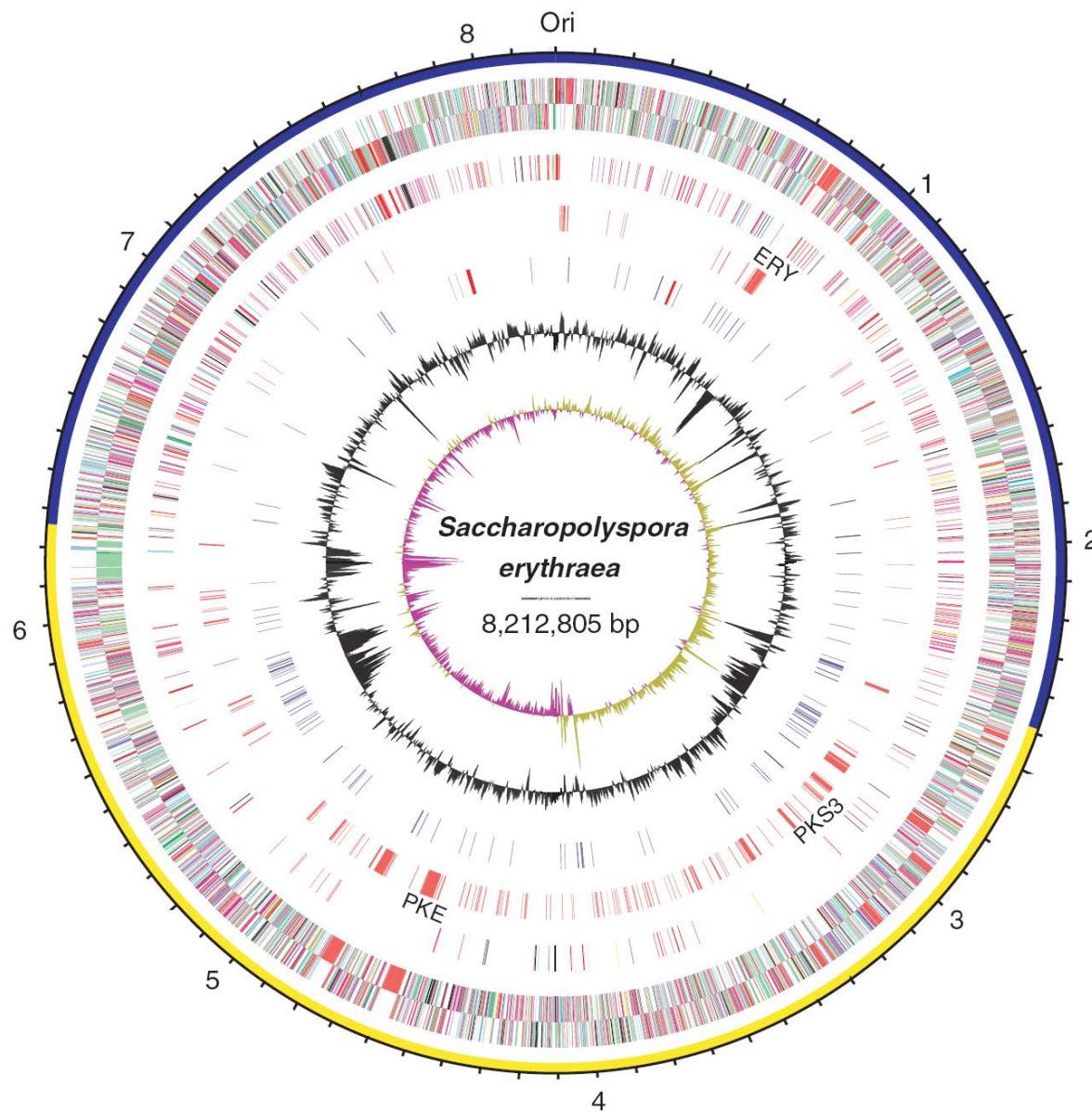
# From genome to biosynthetic gene clusters



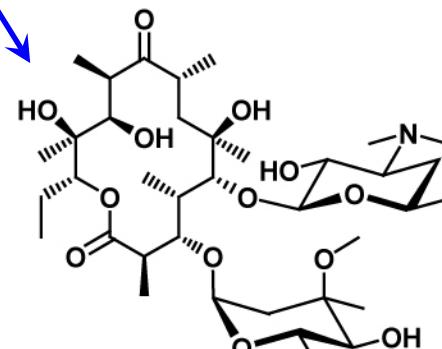
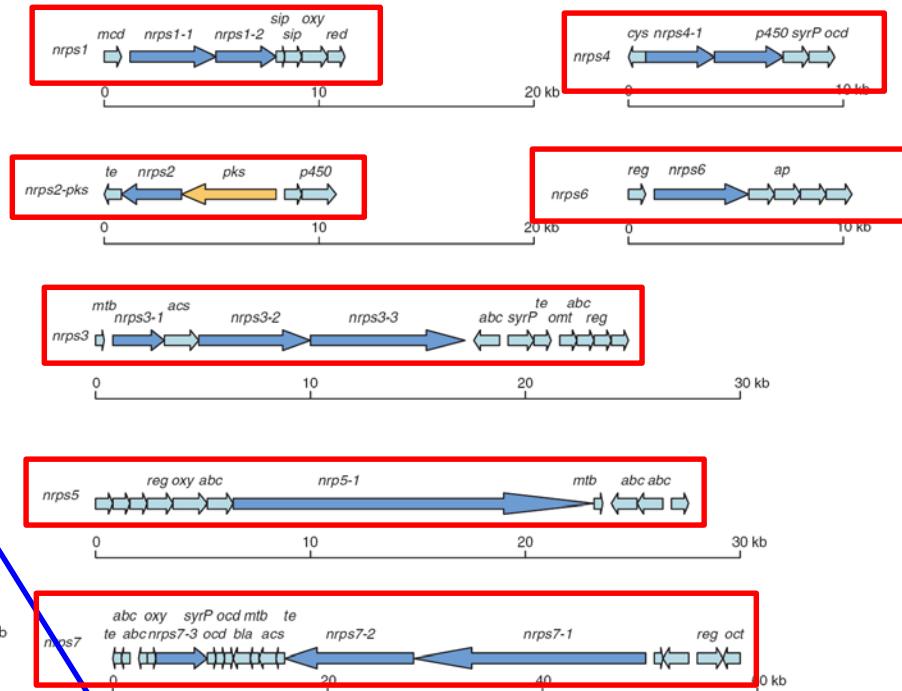
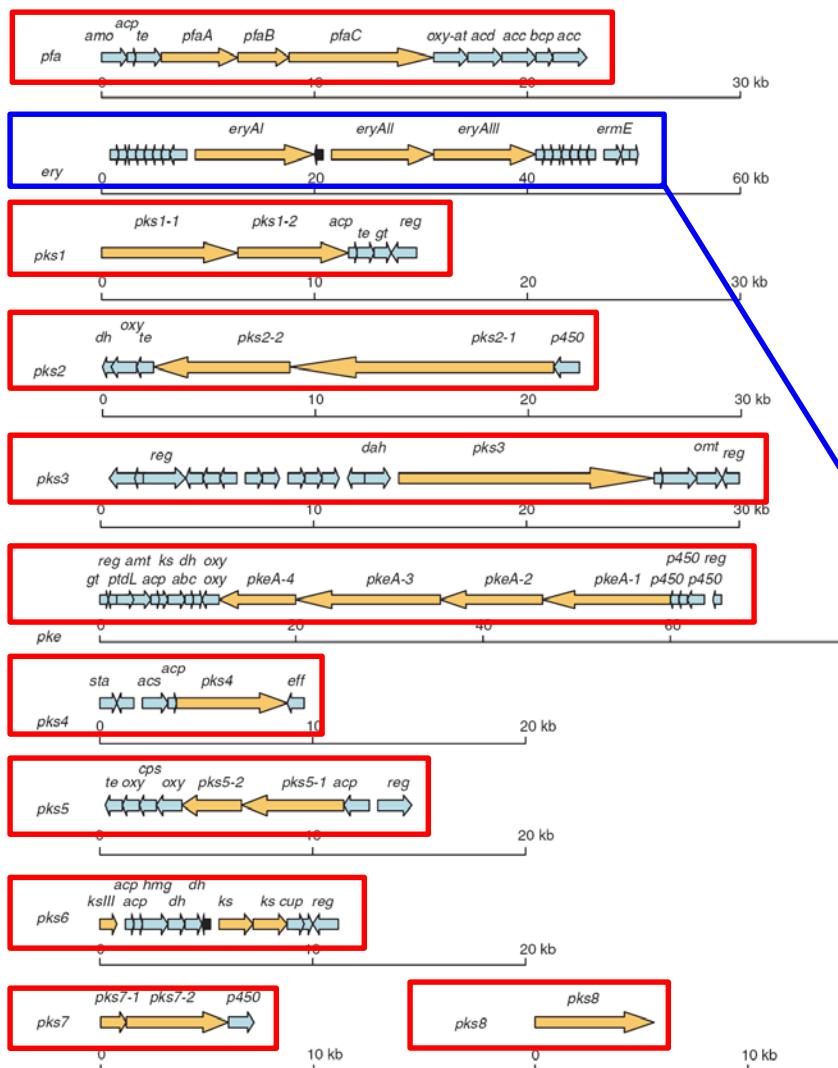
# From gene cluster to molecule



# Genomics reveals cryptic natural products



# Genomics reveals cryptic natural products



erythromycin

# Automating gene cluster identification



**Peter  
Cimermancic**

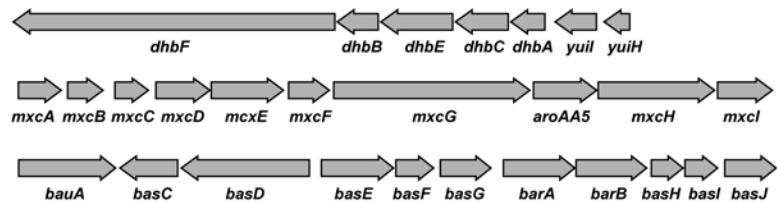


**Marnix  
Medema**



**Jan  
Claesen**

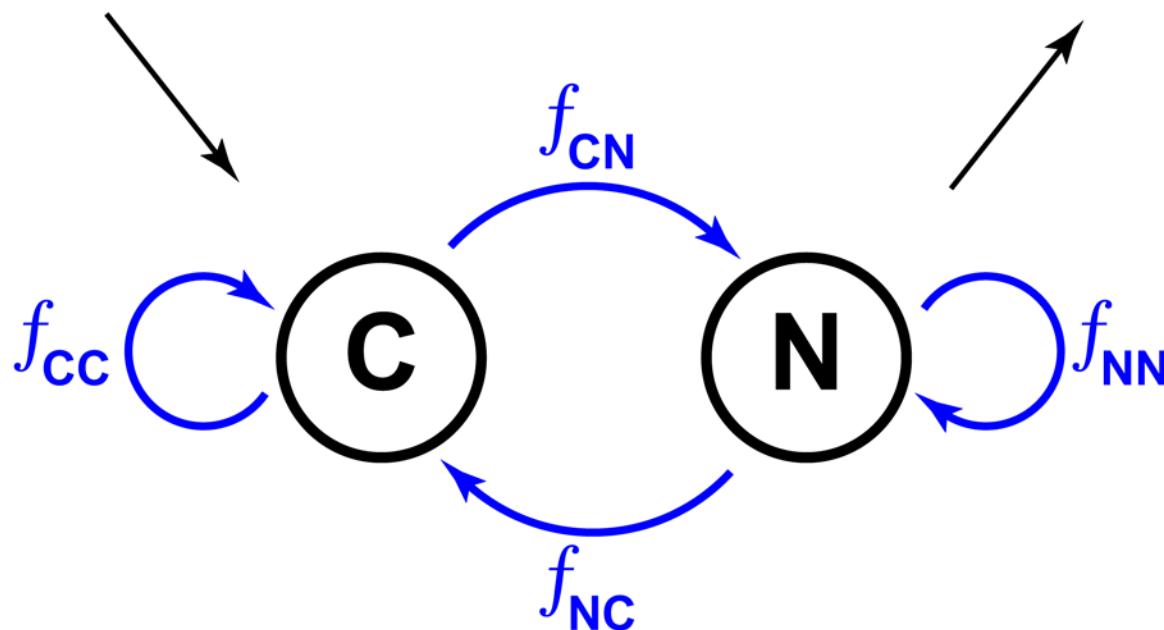
# Automating gene cluster identification



Training Set

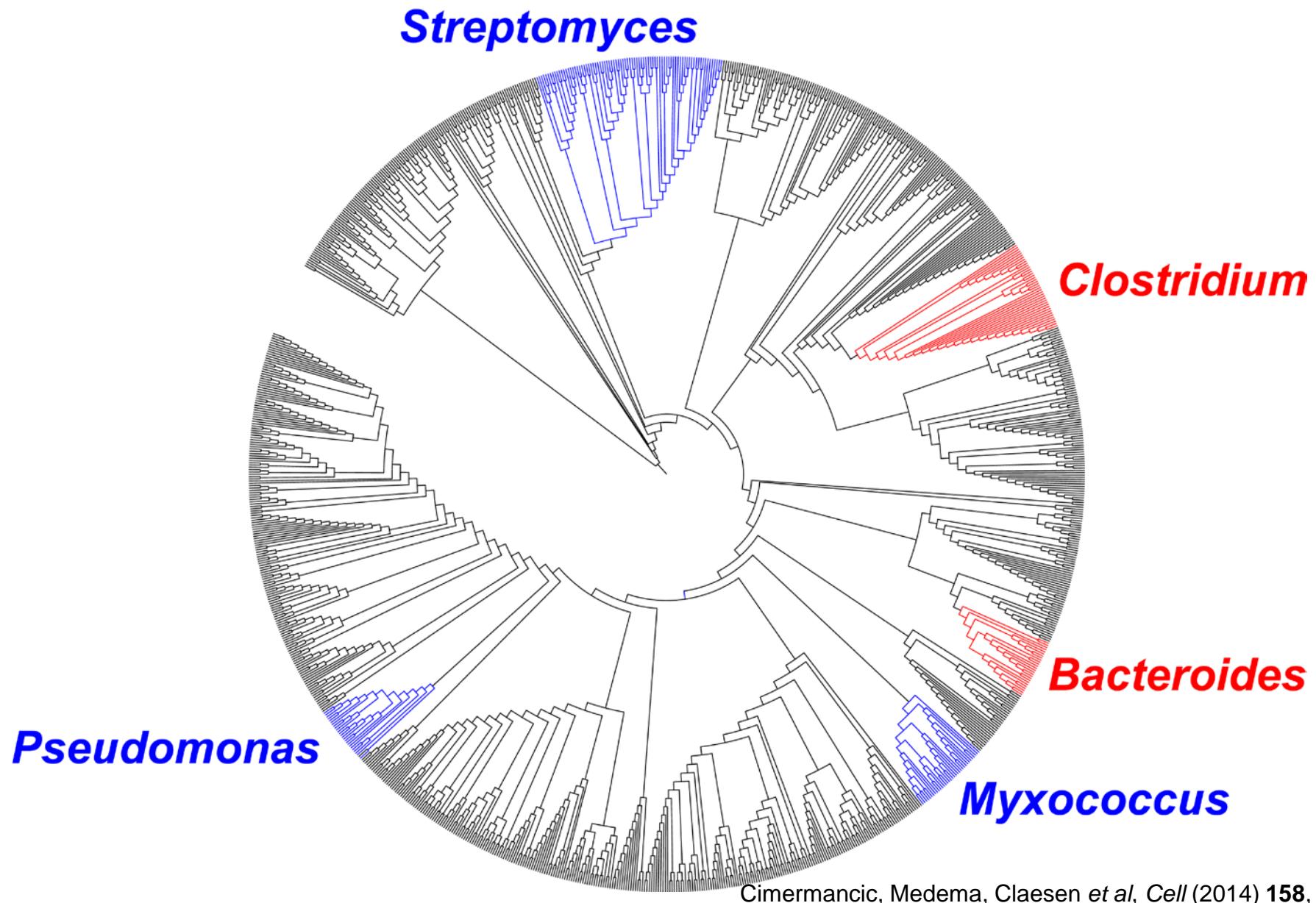


Probability Scores  
Across Genomes



Hidden Markov Model

# Biosynthetic genes in the human microbiome



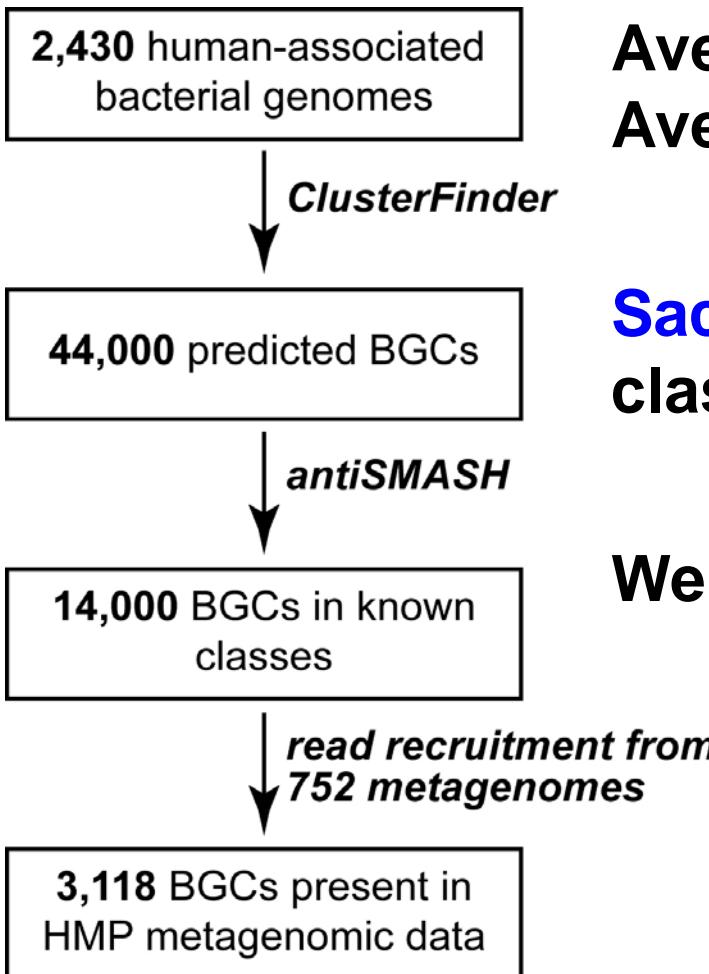
# Gene clusters in the human microbiome

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**Mohamed Donia**

# Microbiome gene clusters: Facts and figures

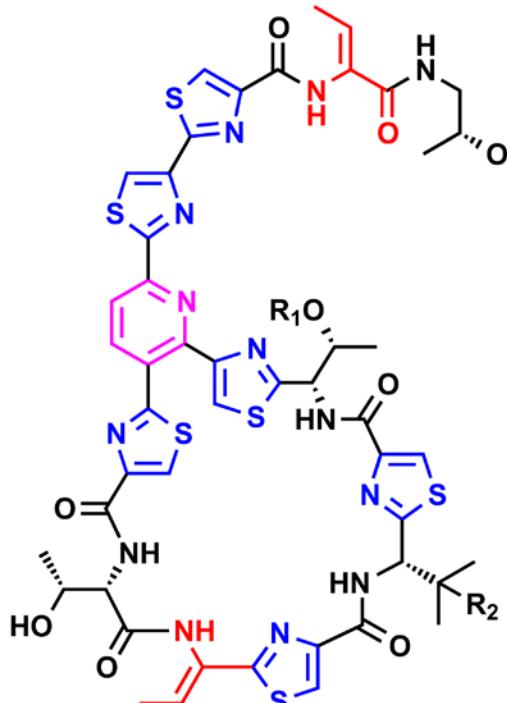


Average gut: **599** gene clusters  
Average oral cavity: **1,061** gene clusters

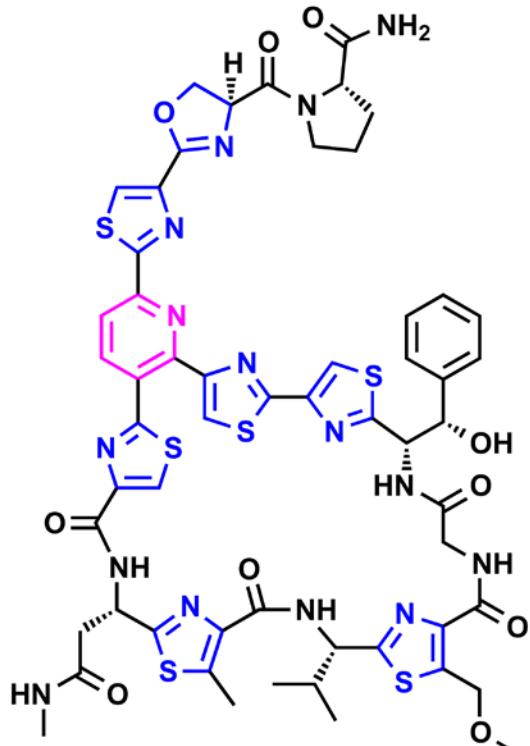
**Saccharides** predominate, but other classes well represented

Well-studied gene clusters are **rare**

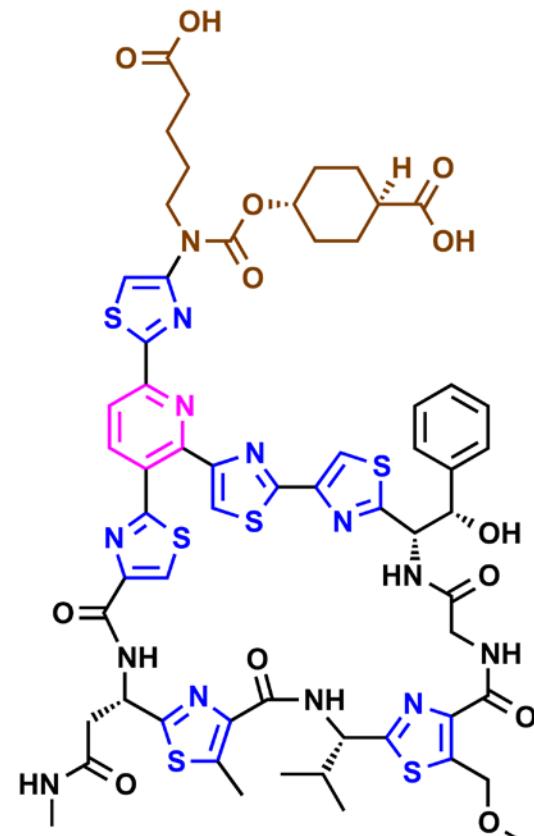
# Thiopeptide antibiotics



**thiocillin**  
*Bacillus cereus*

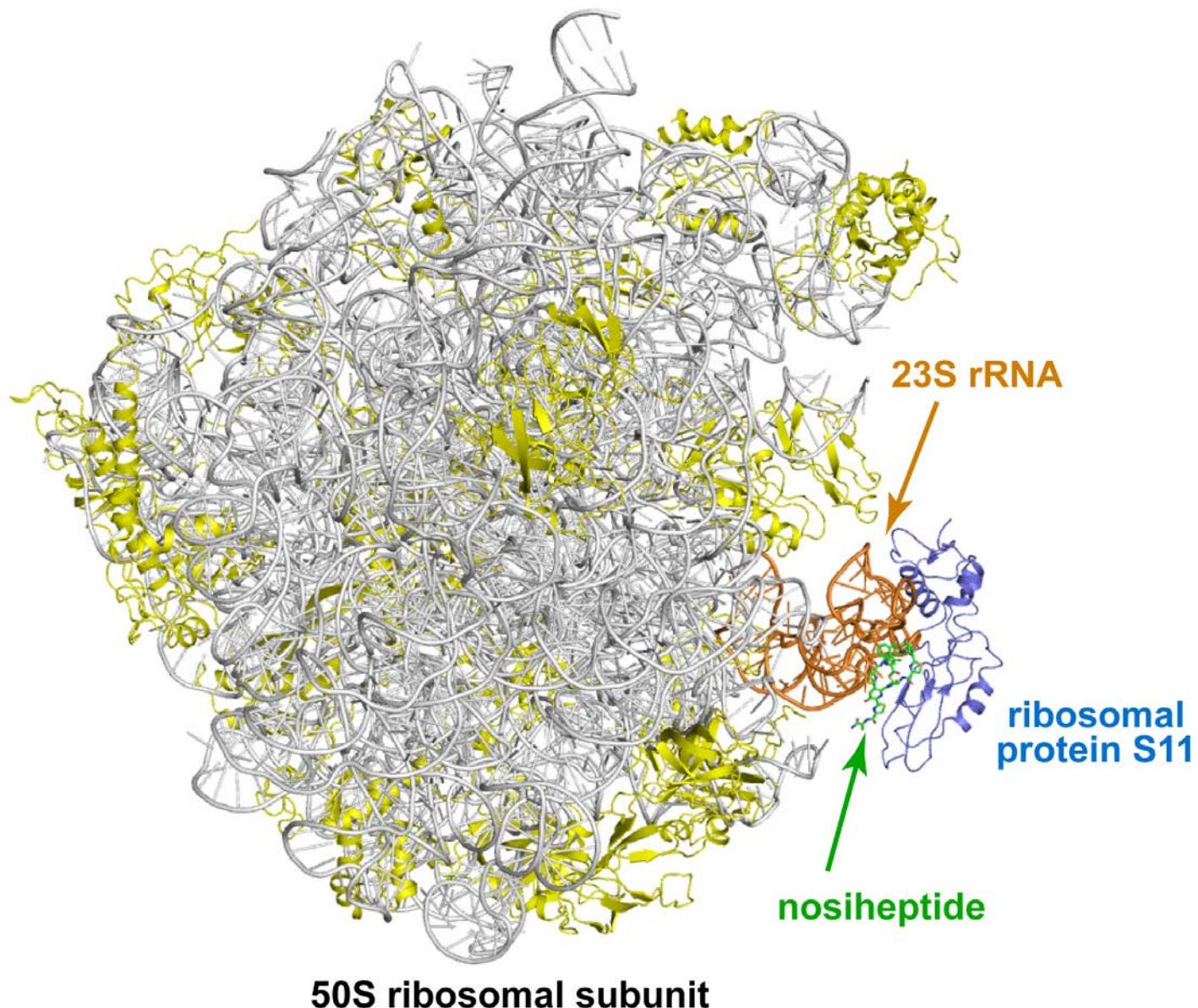


**GE2270**  
*Planobispora rosea*

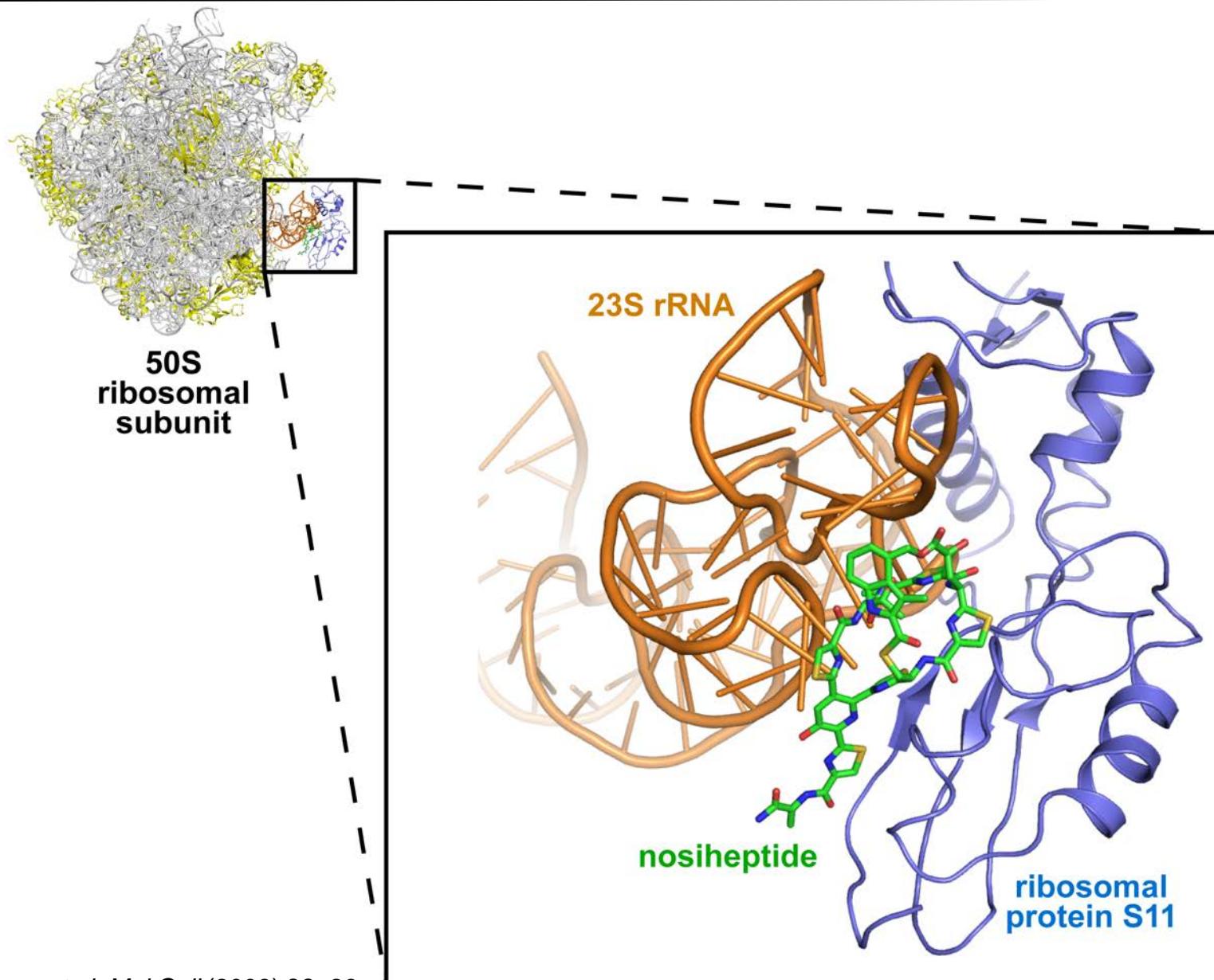


**LFF571**  
Novartis, Phase II

# Thiopeptides bind the 50S subunit

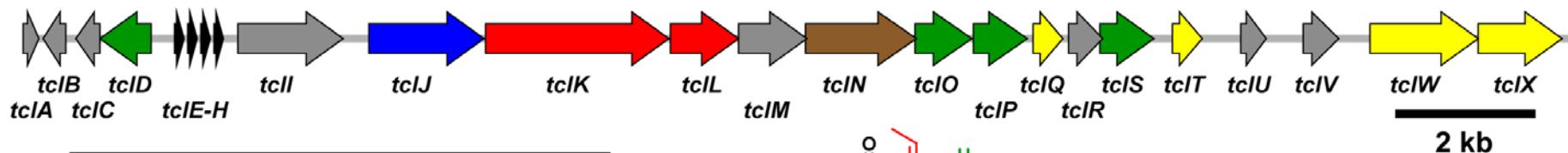


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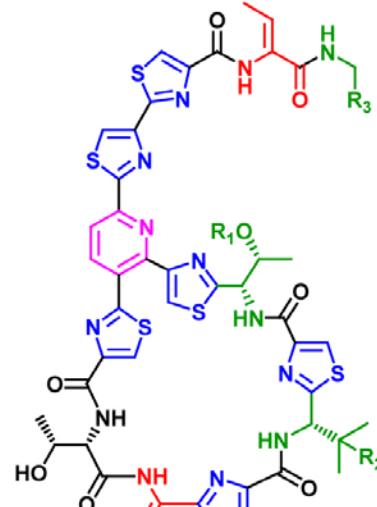


# Gene clusters for thiopeptide antibiotics

*Bacillus cereus* ATCC 14579

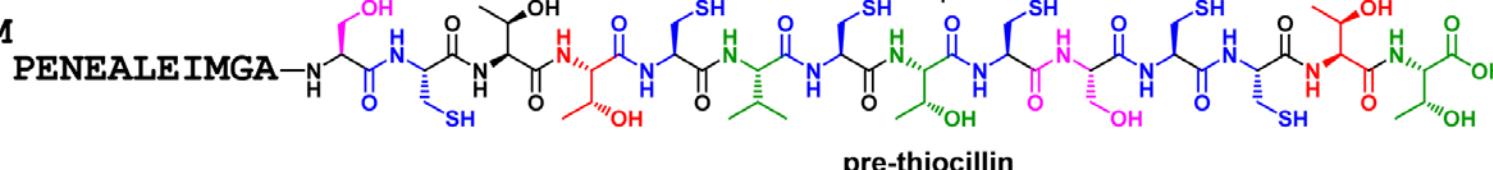


- YcaO (DUF181)
- Lantibiotic dehydratase
- McbC
- Other posttranslational modifications
- Structural gene
- Efflux and host resistance
- Other



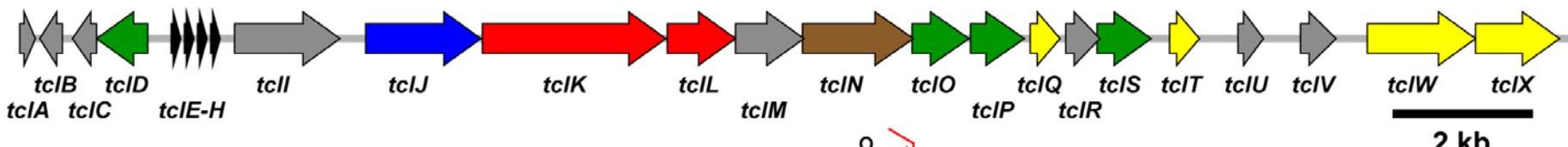
13 posttranslational modifications

DVME IADFDEIELTNLAKKIESM—NH<sub>2</sub>  
V  
D  
A  
M

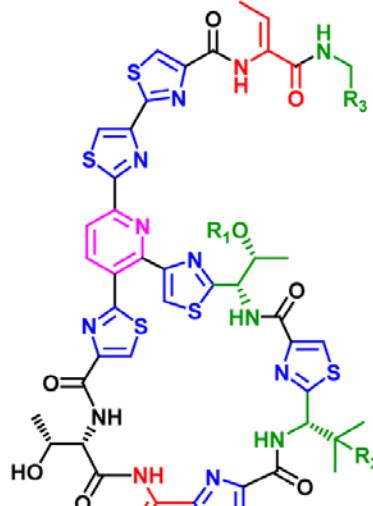


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*Bacillus cereus* ATCC 14579



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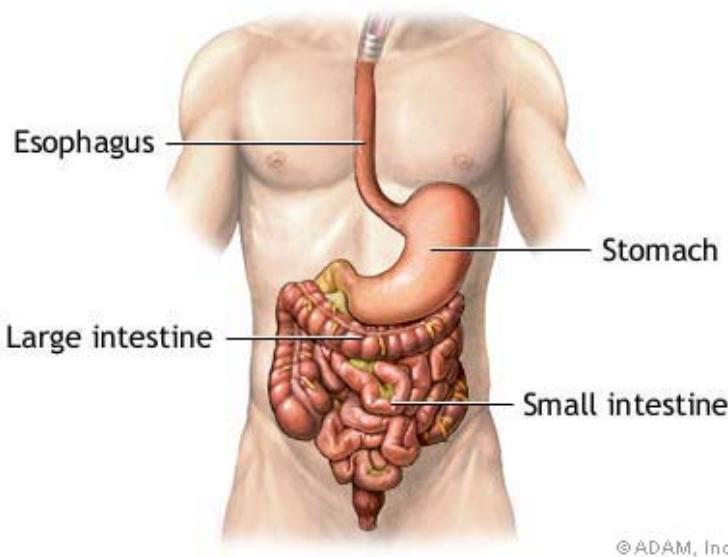
13 posttranslational modifications

pre-thiocillin

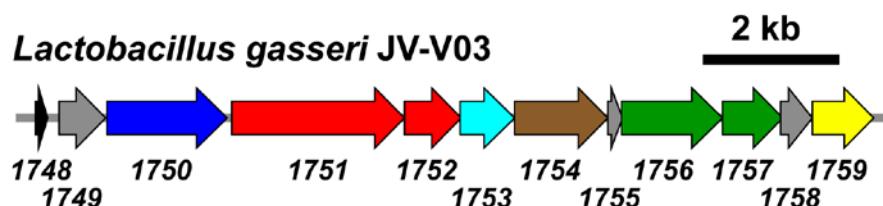
micrococcin P1  
micrococcin P2  
thiocillin I  
thiocillin II  
thiocillin III  
YM-266183  
YM-266184  
thiocillin IV

	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
micrococcin P1	H	CH <sub>3</sub>	H
micrococcin P2			OH
thiocillin I			
thiocillin II			
thiocillin III			
YM-266183			
YM-266184			
thiocillin IV			

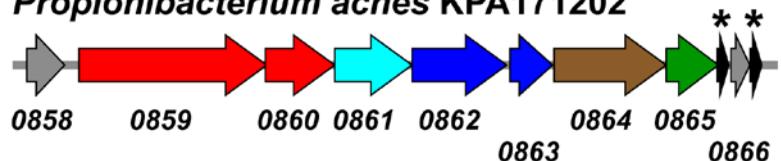
# Thiopeptides from the human microbiota



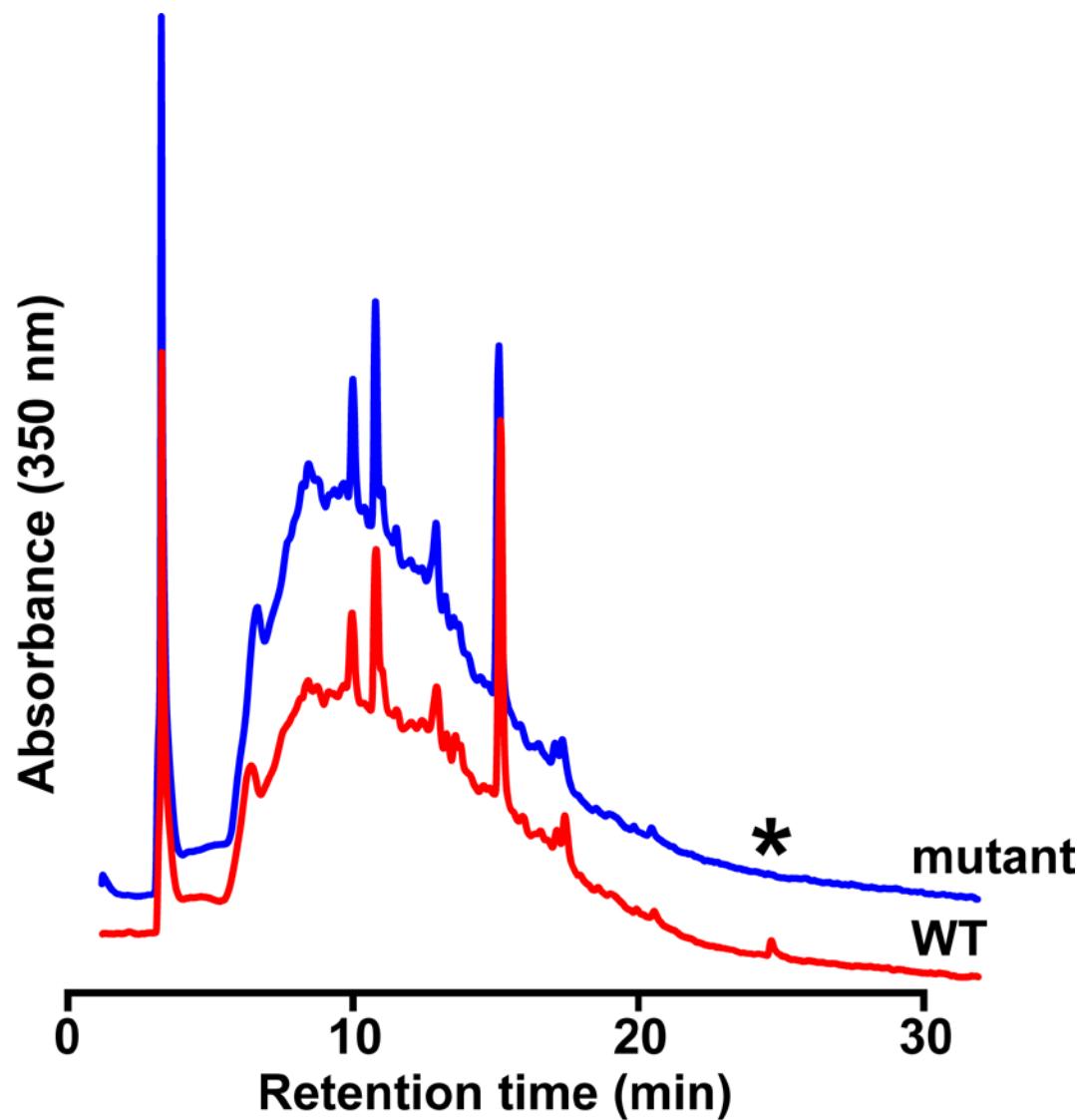
*Lactobacillus gasseri* JV-V03



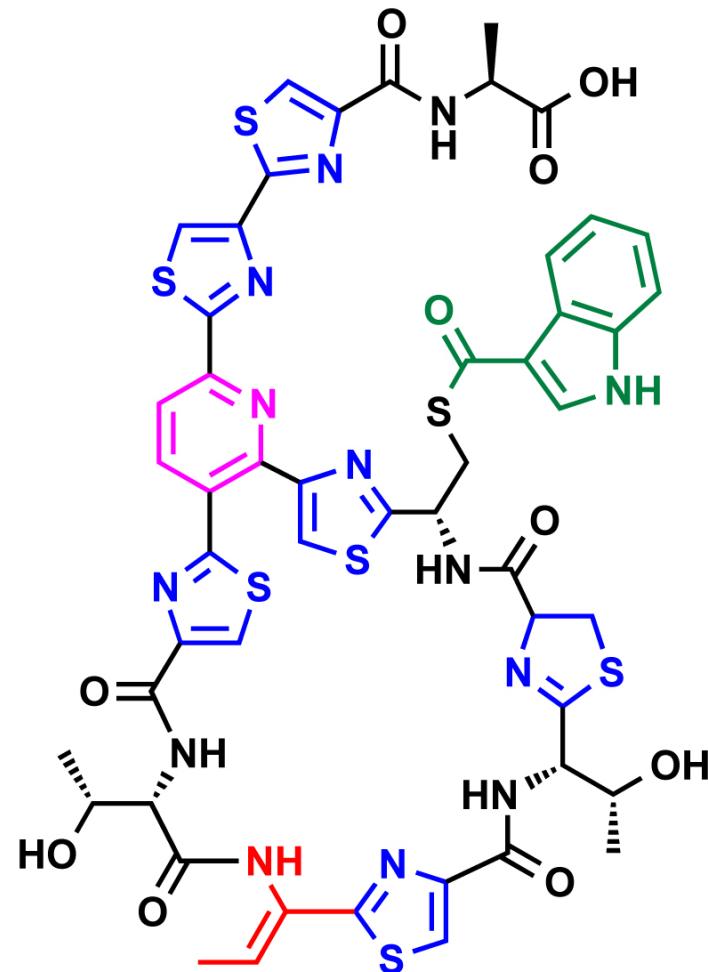
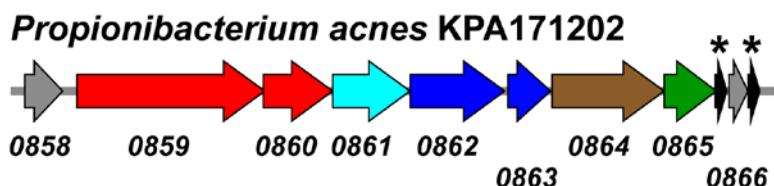
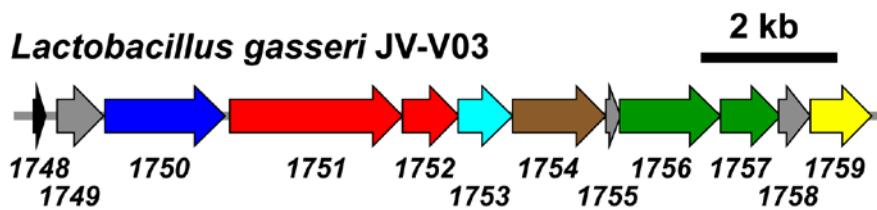
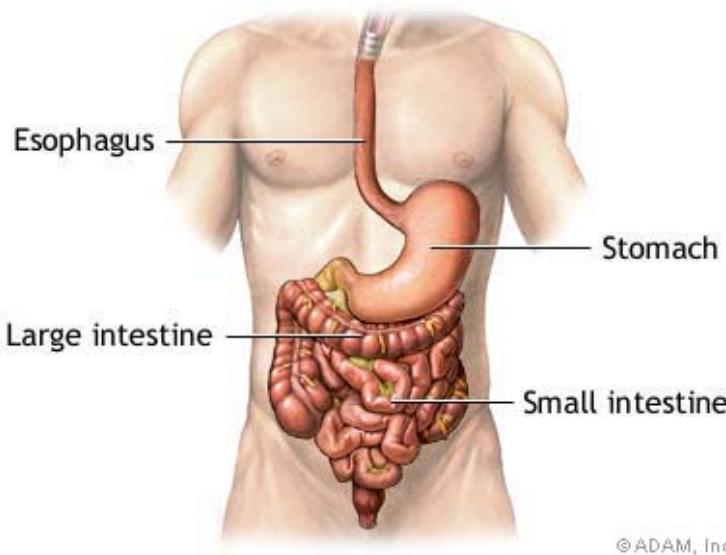
*Propionibacterium acnes* KPA171202



# Thiopeptides from the human microbiota



# Thiopeptides from the human microbiota



**lactocillin**  
*Lactobacillus gasseri*

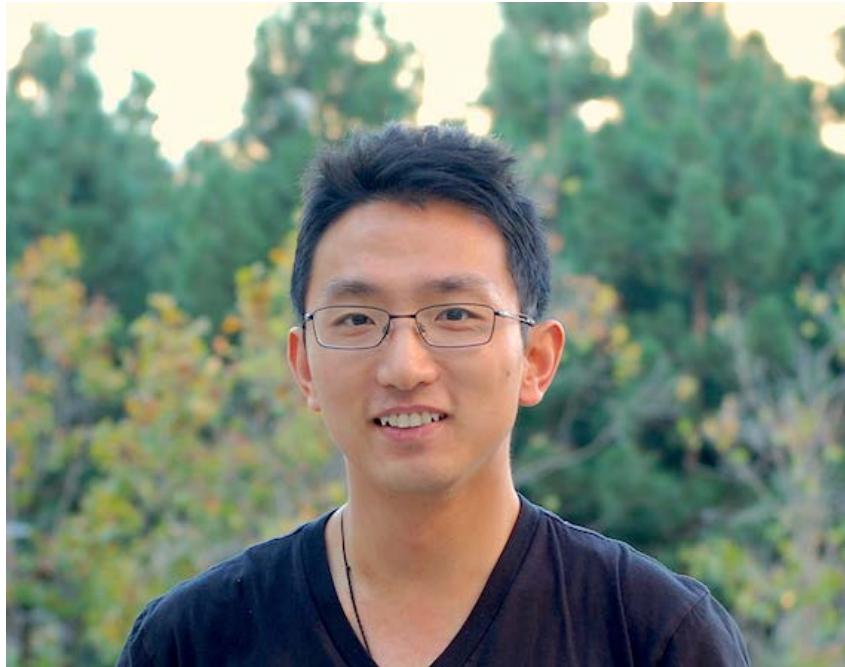
# Other thiopeptide clusters in the microbiota

	Body Site	Prevalence in HMP samples
<i>pac</i>	skin	30%
<i>sdo</i>	oral	-
<i>ot4</i>	oral*	25%
<i>ot5</i>	oral*	8%
<i>Ijo</i>	gut	-
<i>gt1</i>	gut*	9%
<i>ot7</i>	oral*	10%
<i>sso</i>	oral	-
<i>ot3</i>	oral*	1%
<i>ot1</i>	oral*	34%
<i>ot2</i>	oral*	1%
<i>Icl</i>	vaginal	-
<i>ot6</i>	oral*	22%

\*Discovered first in metagenomic data

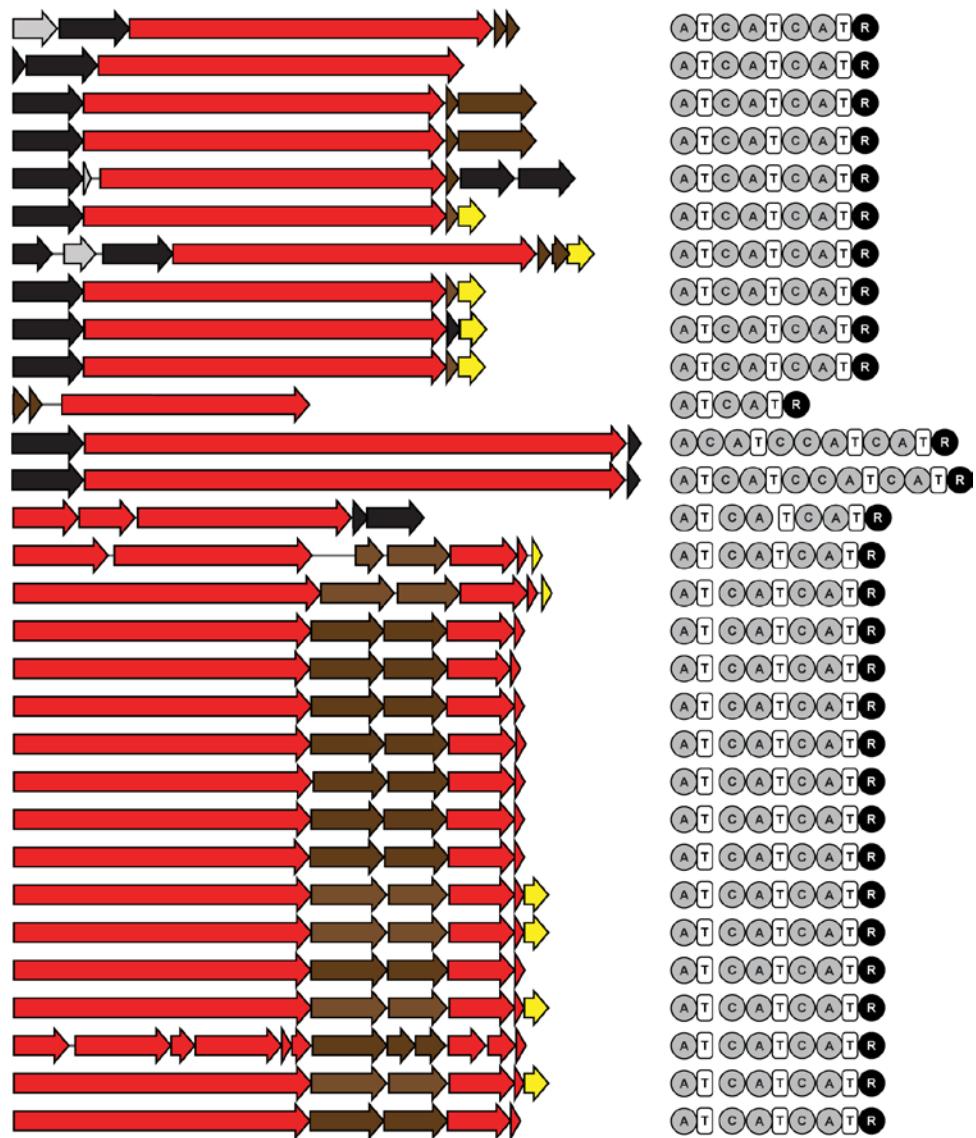
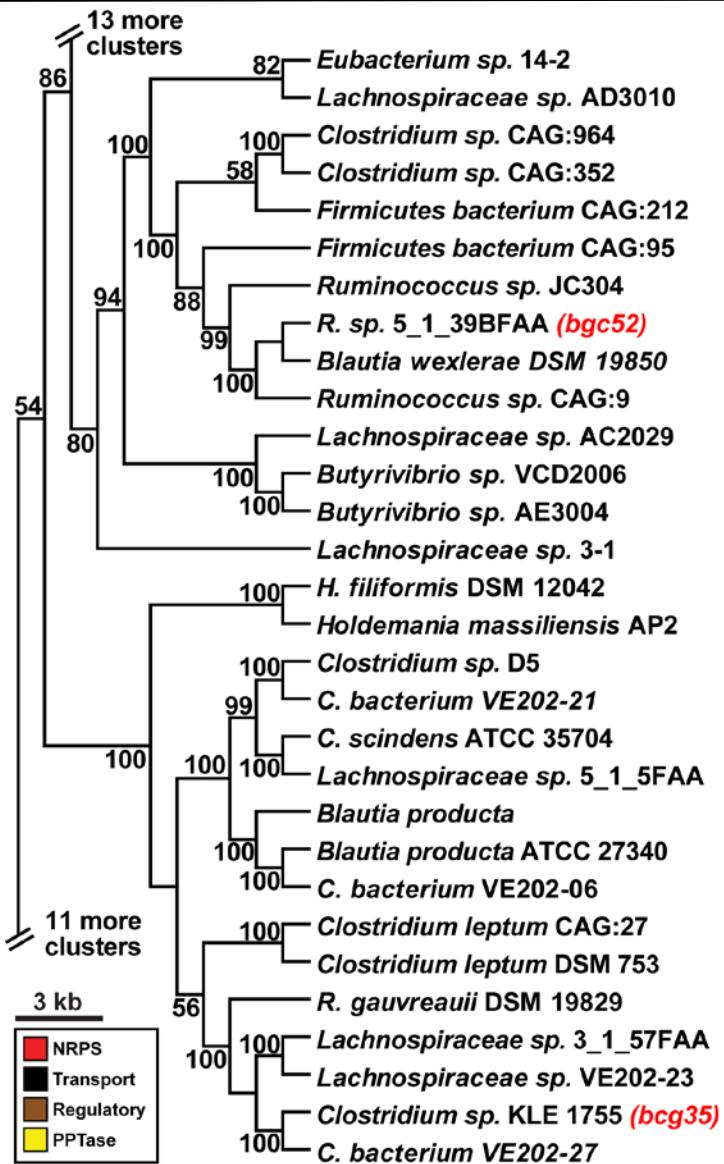
# A widely distributed gut gene cluster family

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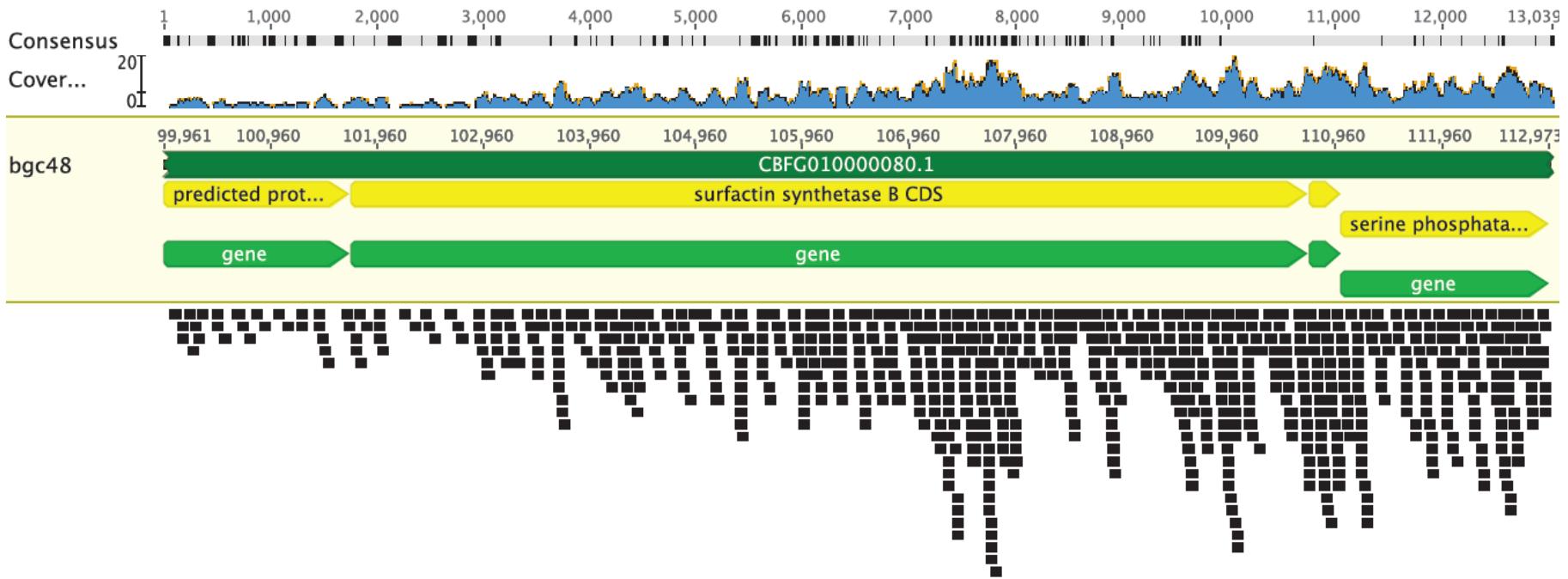
**CJ Guo**

# A widely distributed gut gene cluster family

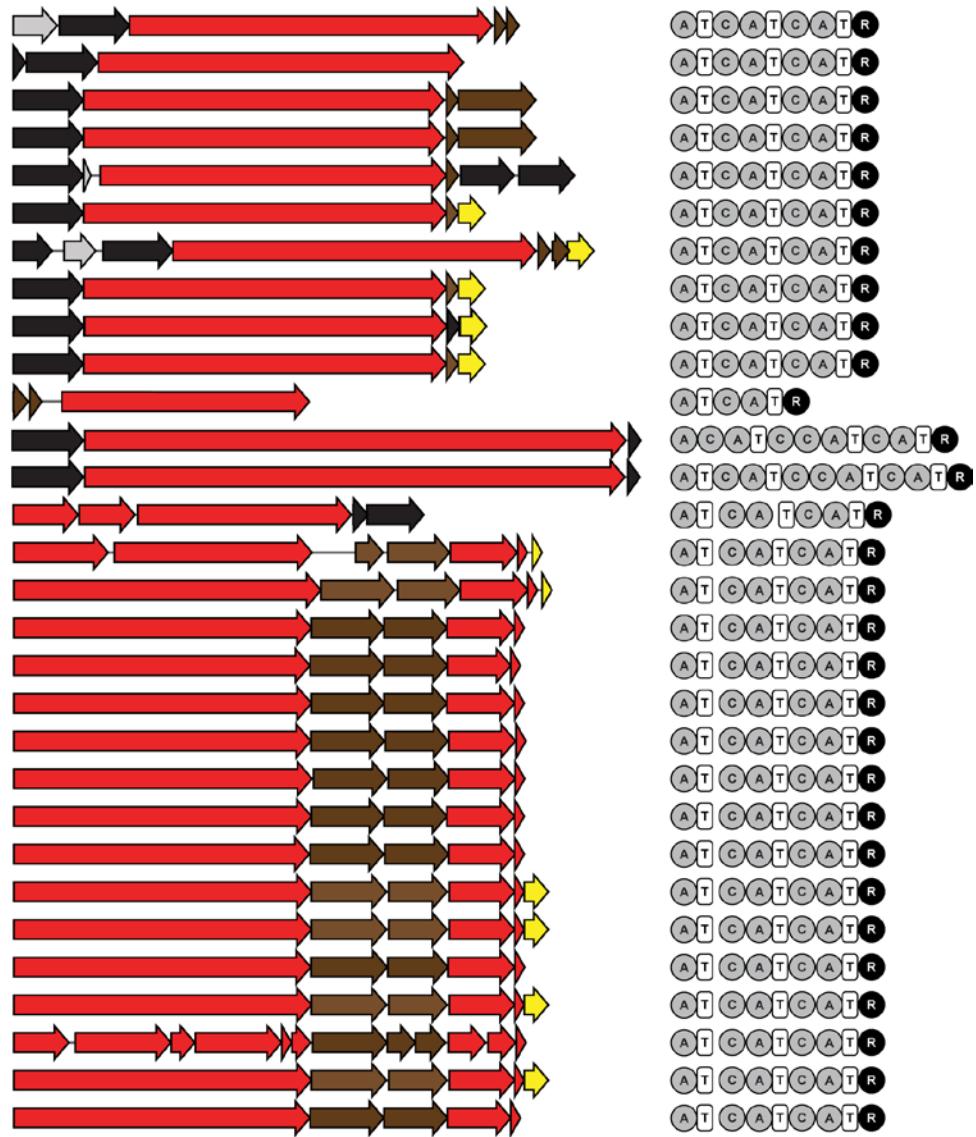
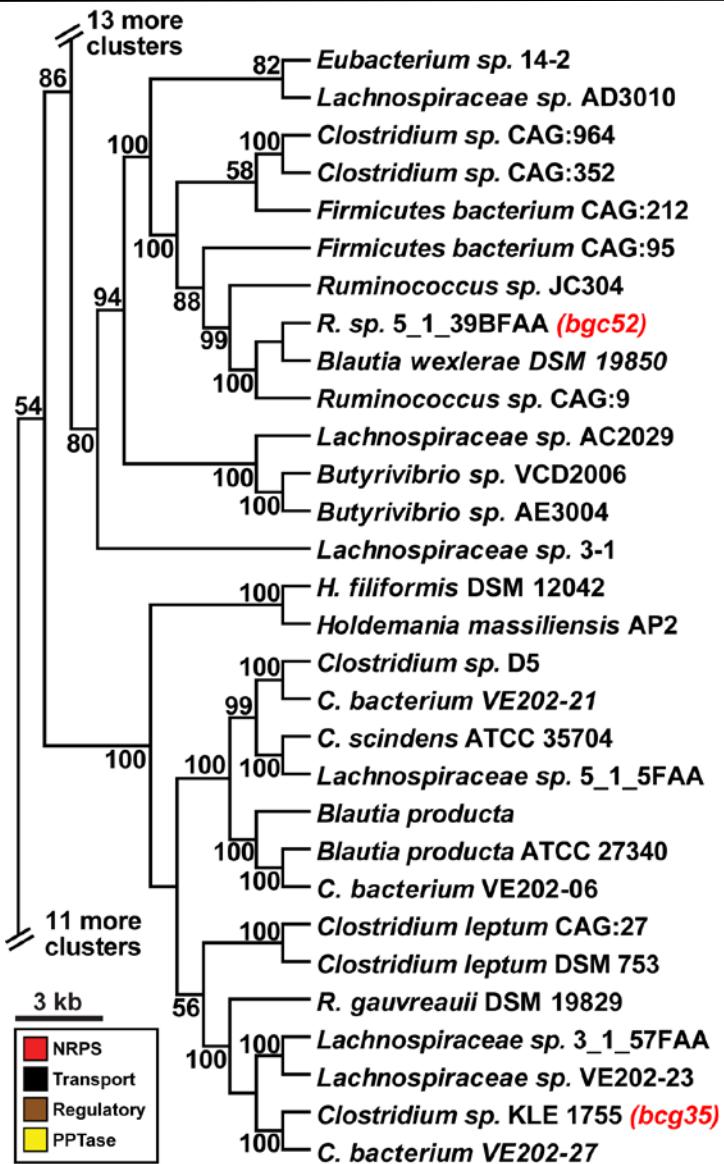


Present in **92%** of gut samples from HMP

# A widely distributed gut gene cluster family

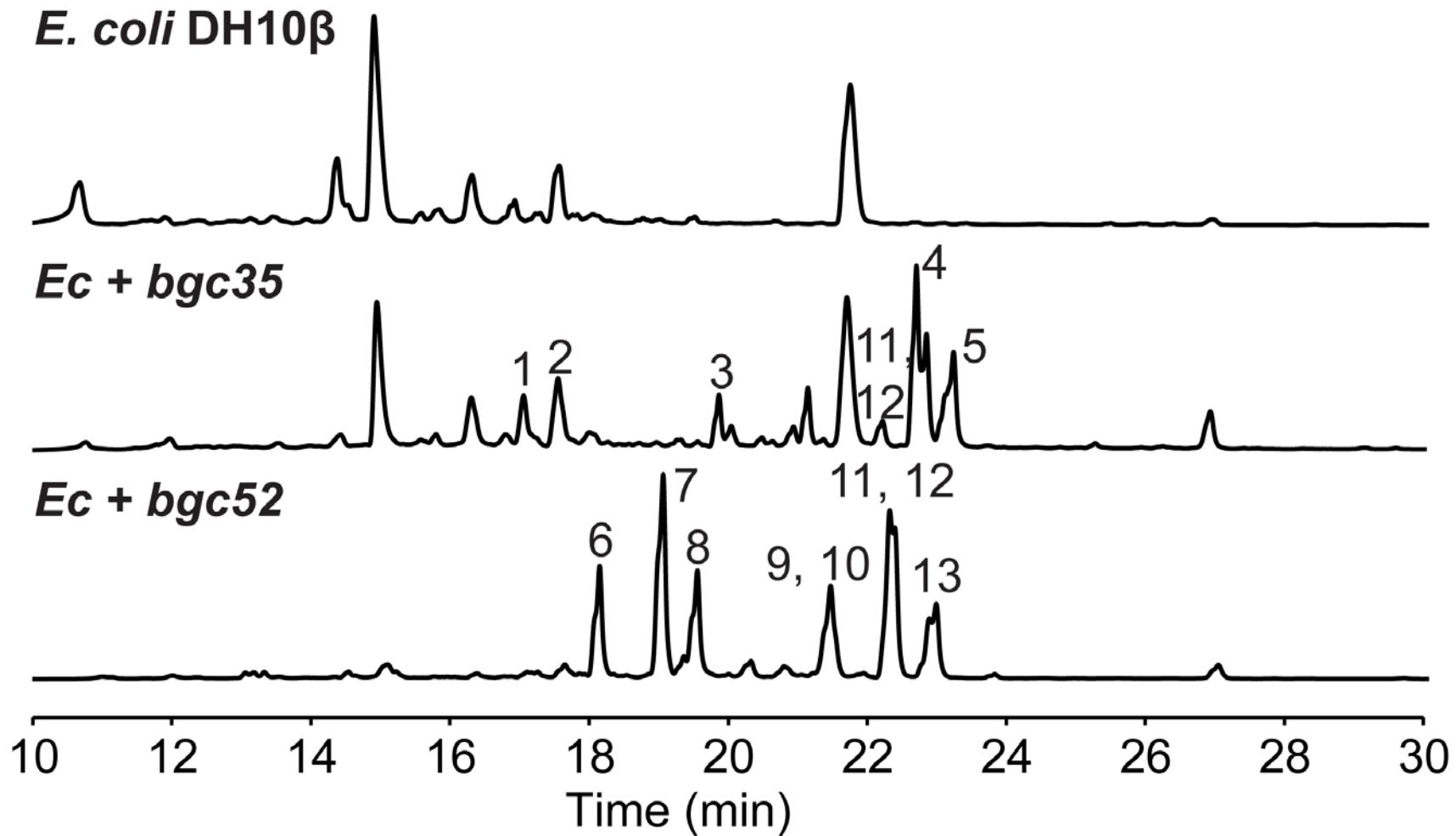


# A widely distributed gut gene cluster family

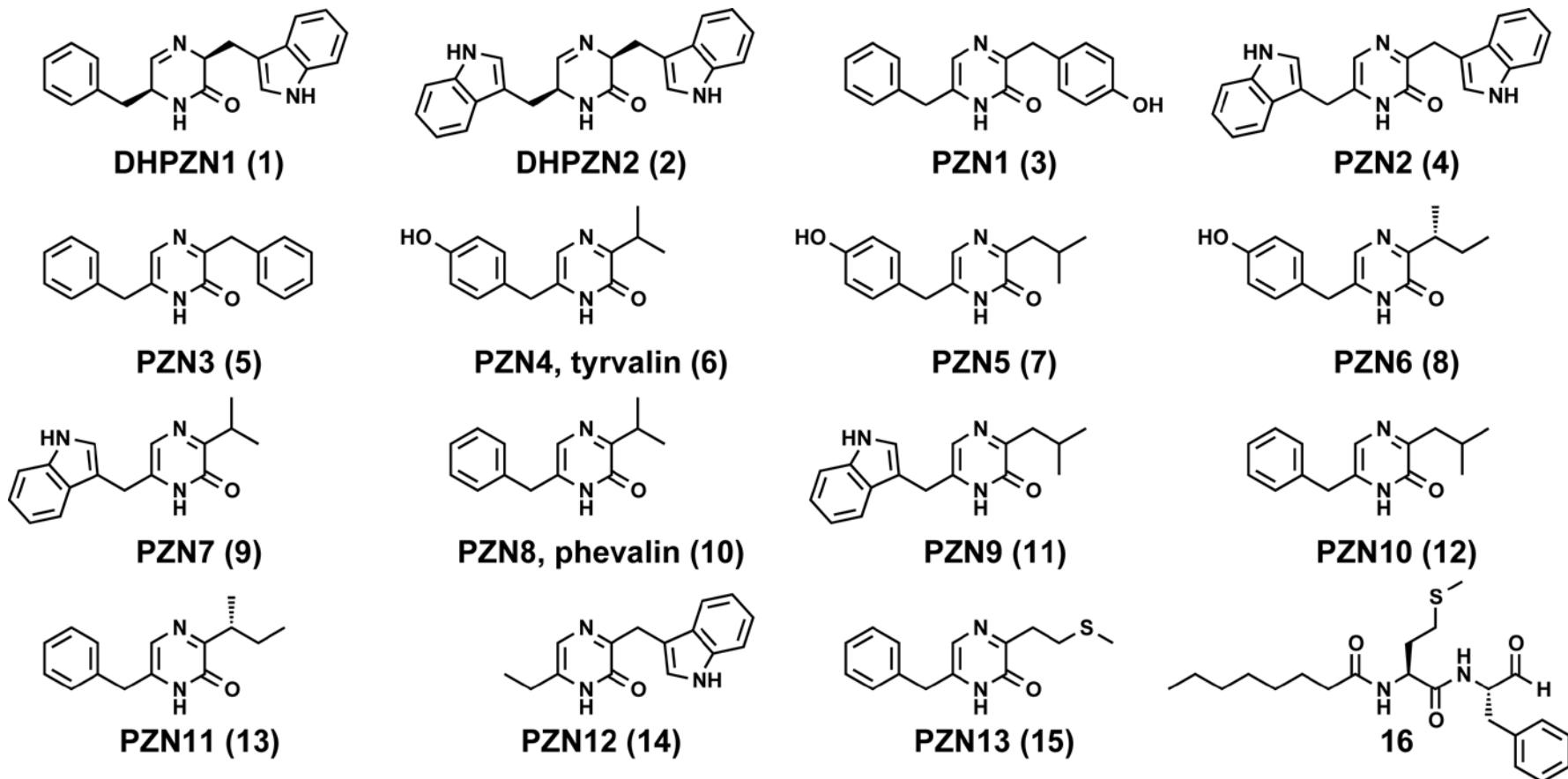


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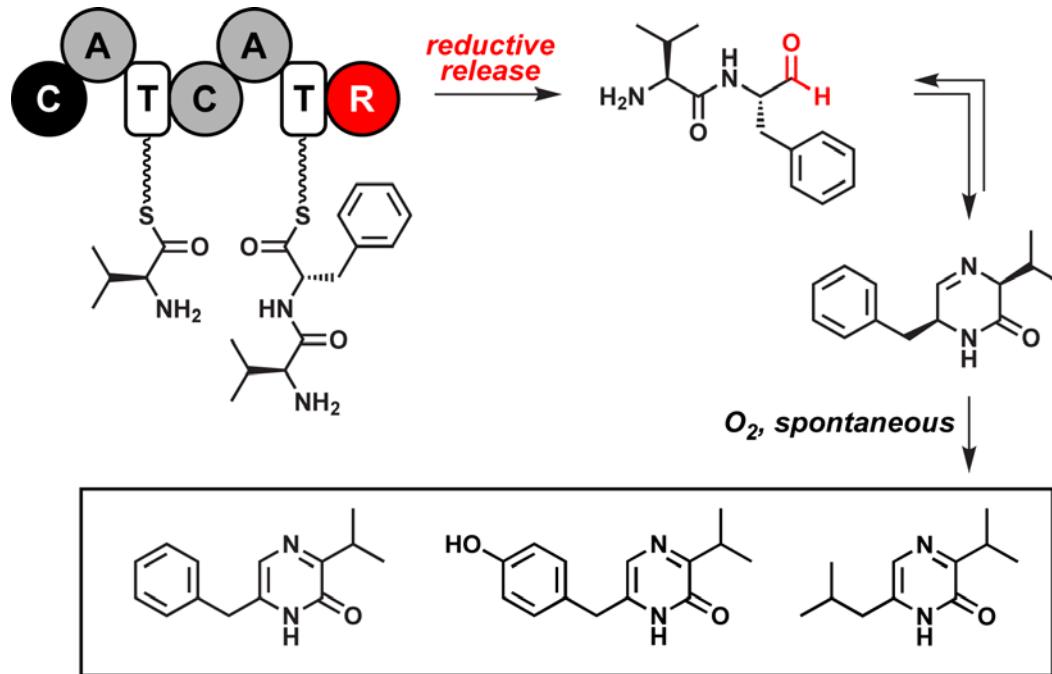


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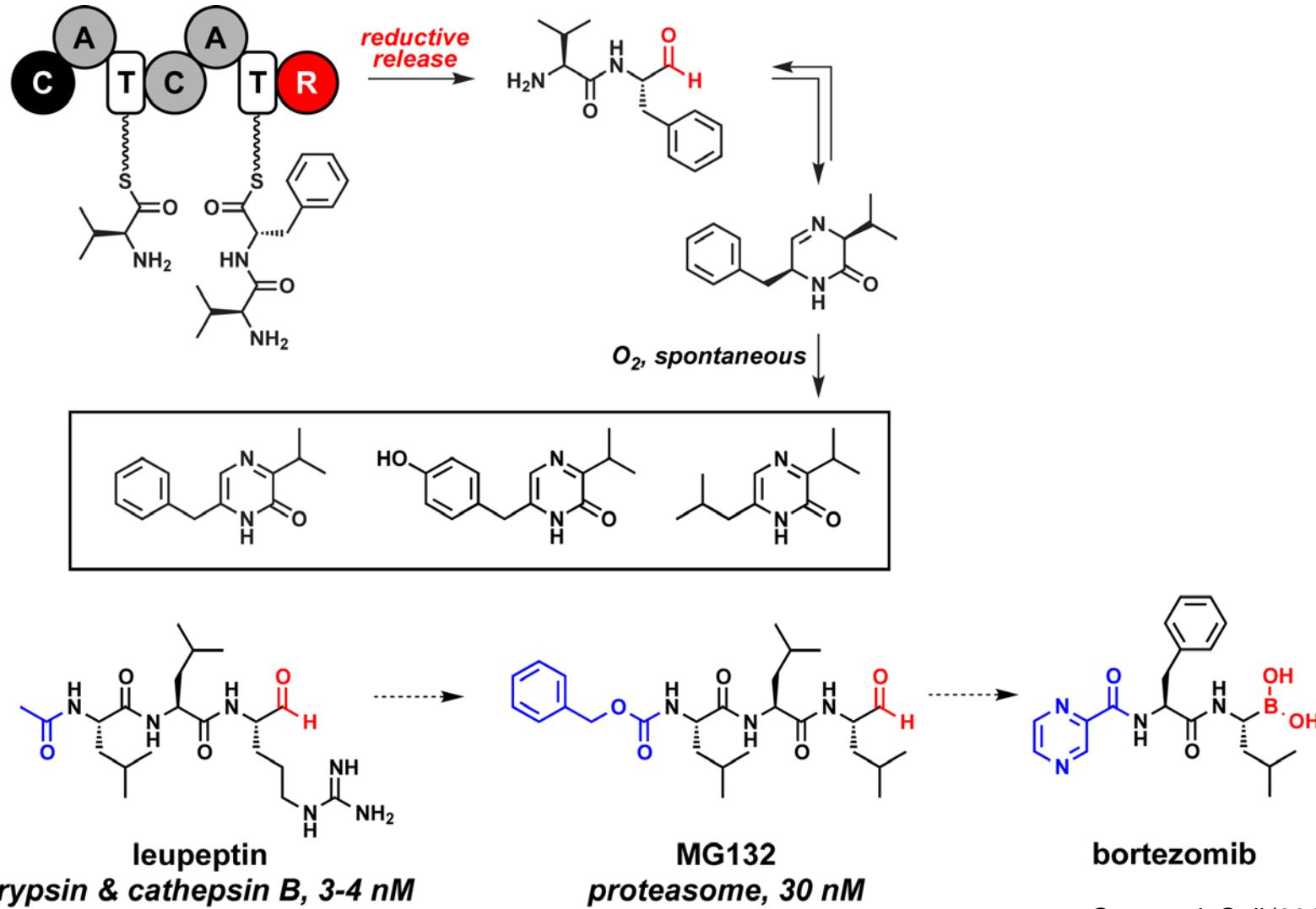


From 6 clusters in *E. coli* and *B. subtilis*, 32 molecules (all but two are new)

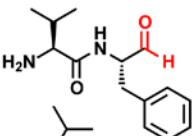
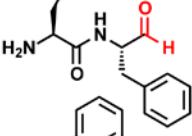
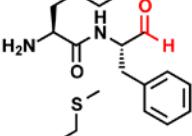
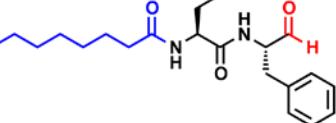
# A widely distributed gut gene cluster family



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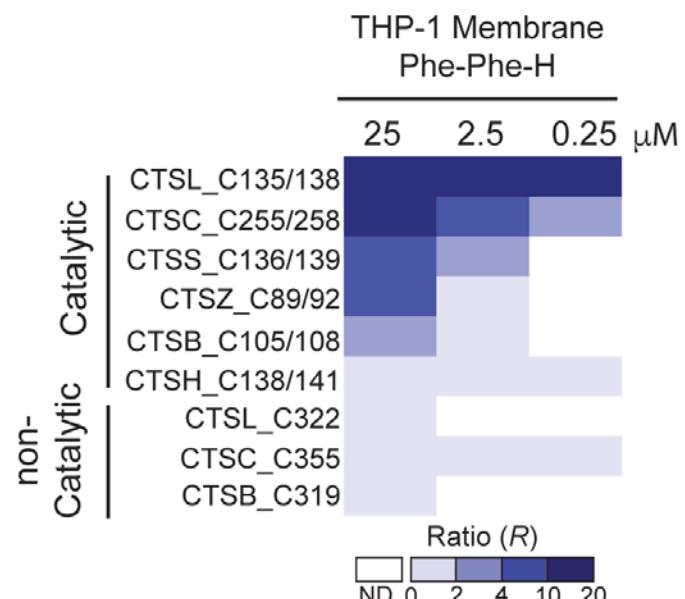
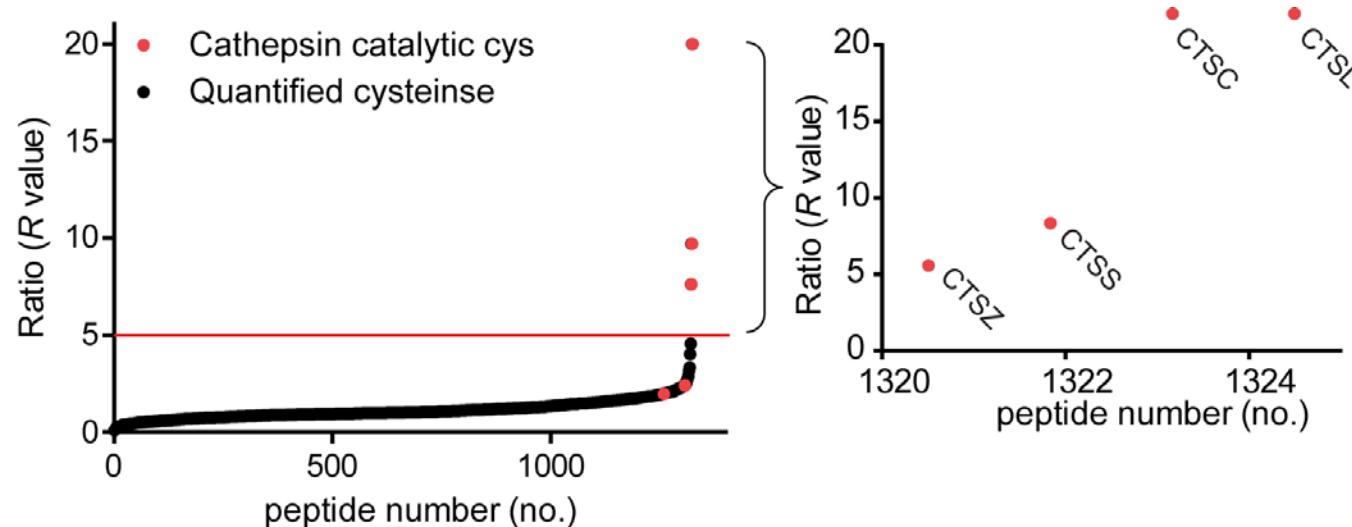


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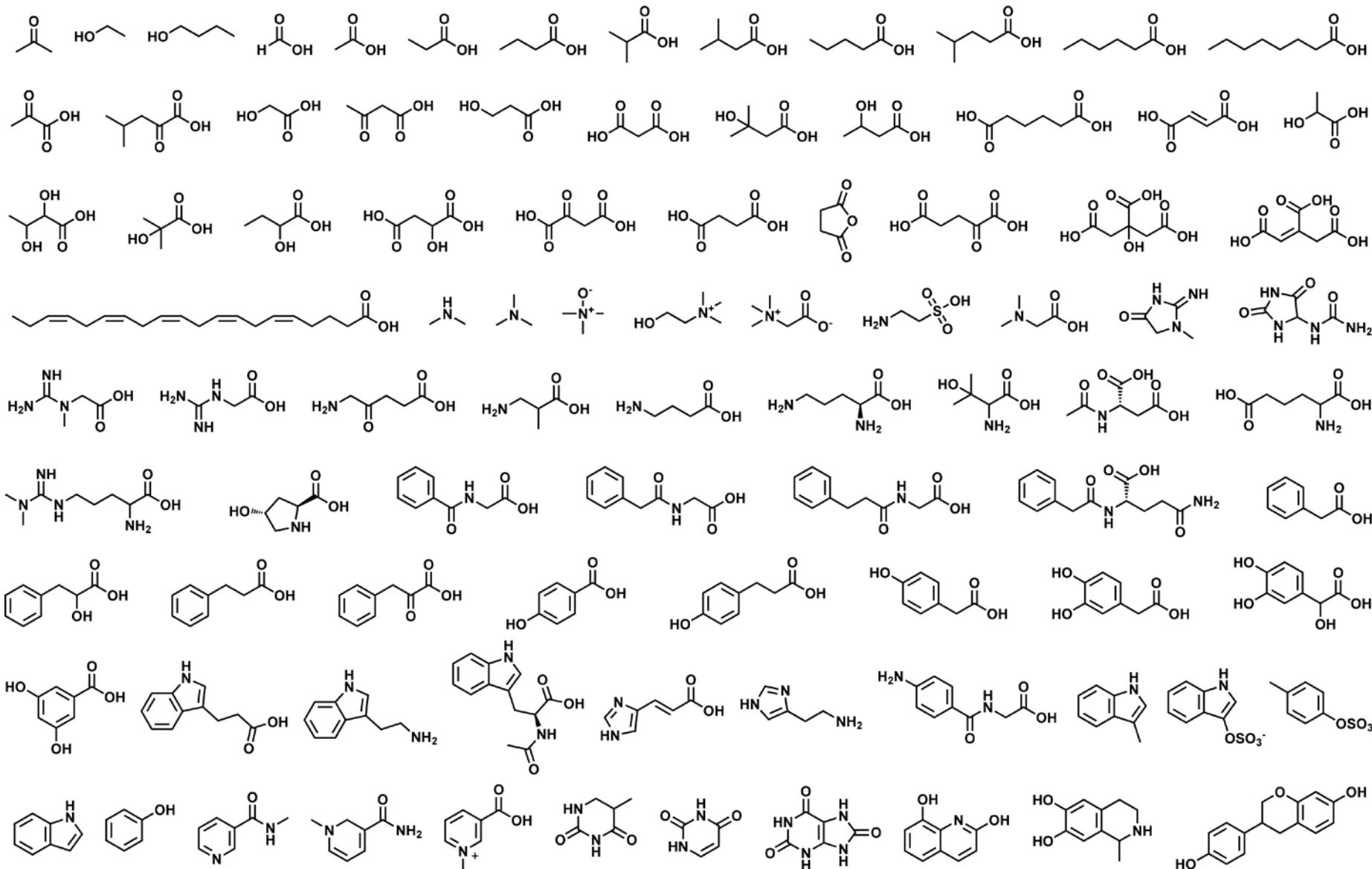
	Cat B	Cat L	Cat C	Cat S	Calpain	Proteasome	Trypsin	Chymotrypsin
	2.8	0.006	0.5	2	1.0	30	N/O	>100
	46.0	0.006	8	0.9	1.5	>30	N/O	N/O
	9.4	0.005	>20	0.6	>20	30	N/O	N/O
	0.376	N/O	0.37	0.013	1.2	2.8	N/O	N/O

IC50 values in micromolar  
N/O: no inhibition observed

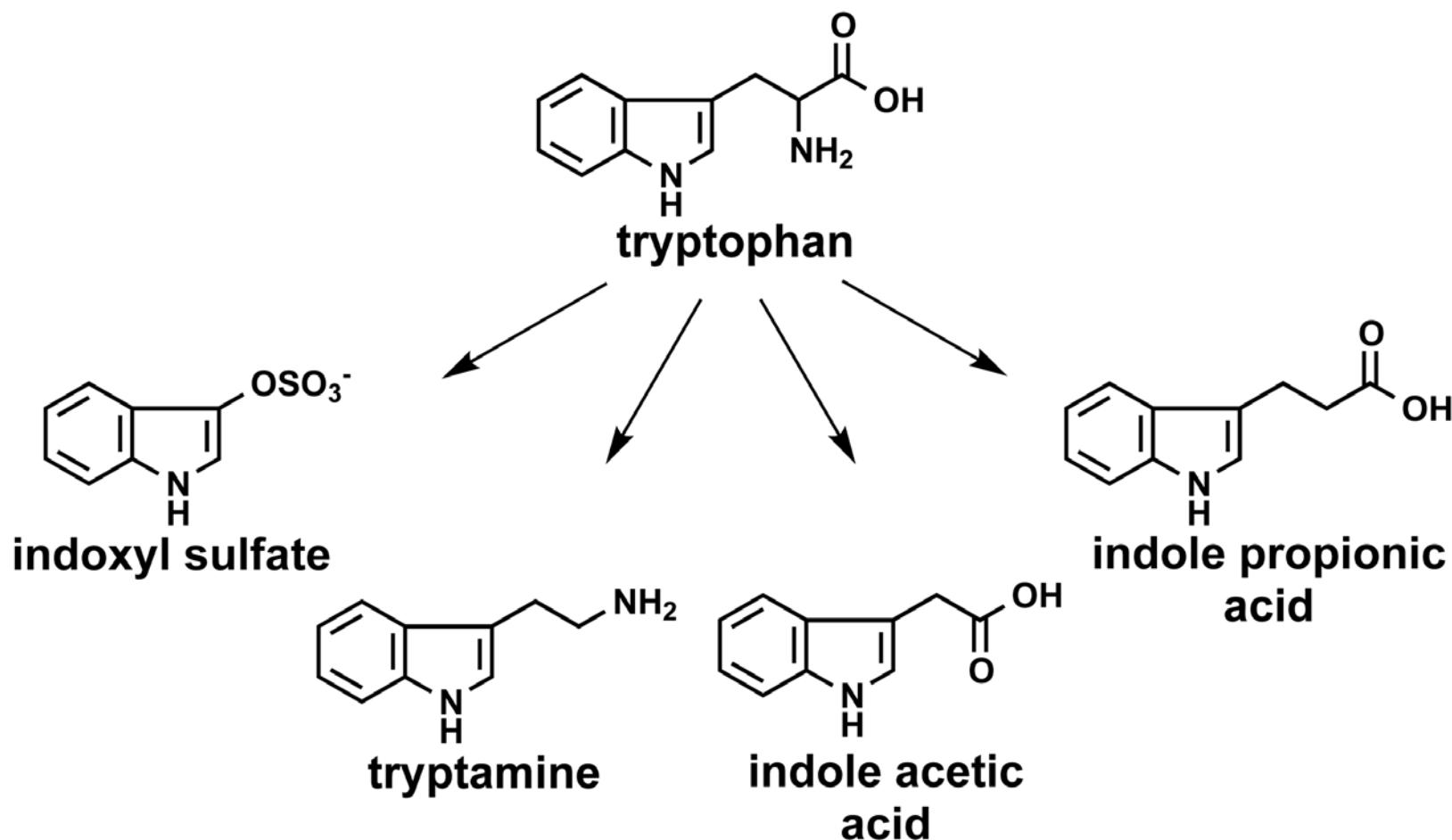
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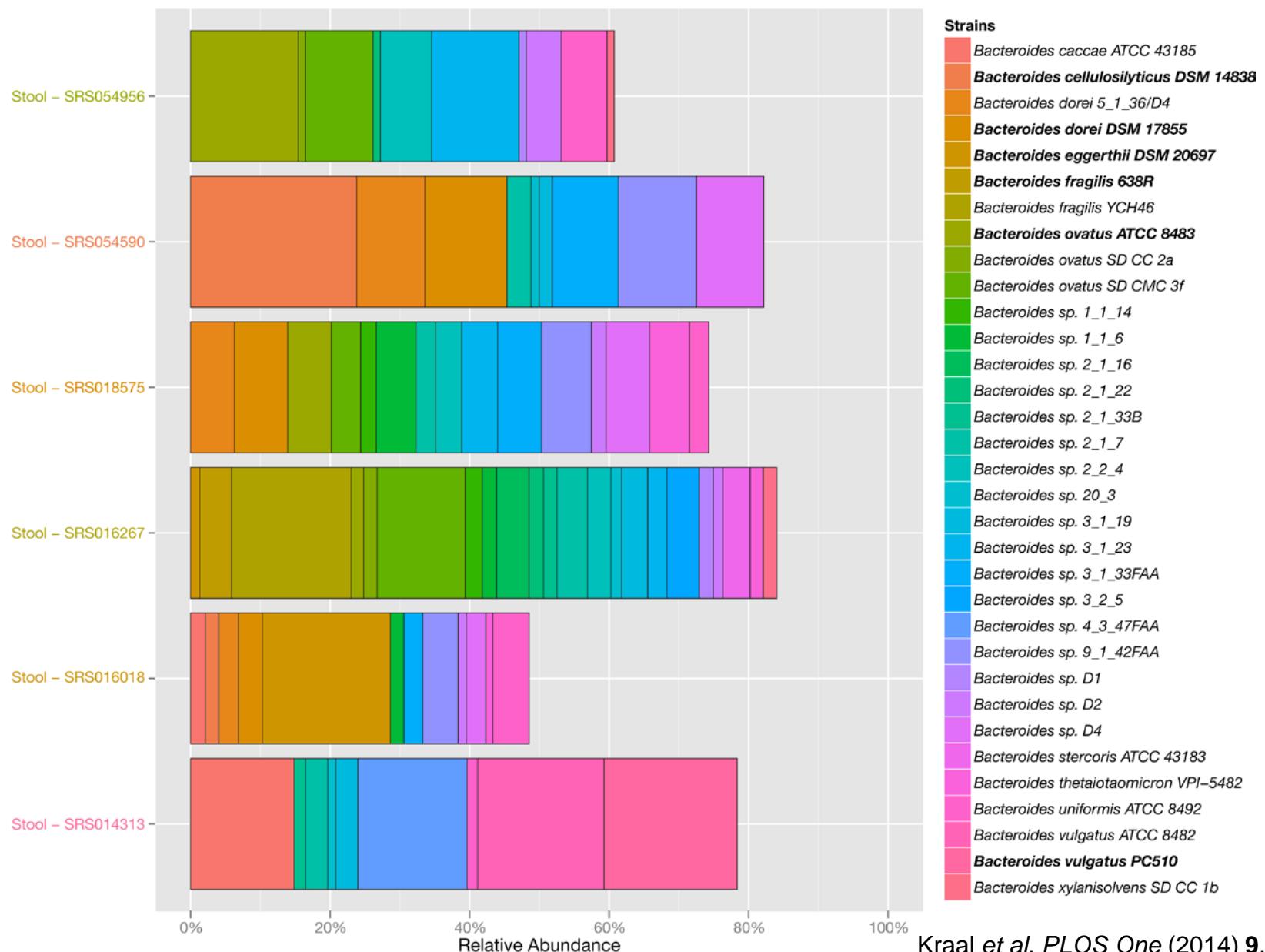
# Microbiota: Largest endocrine organ?



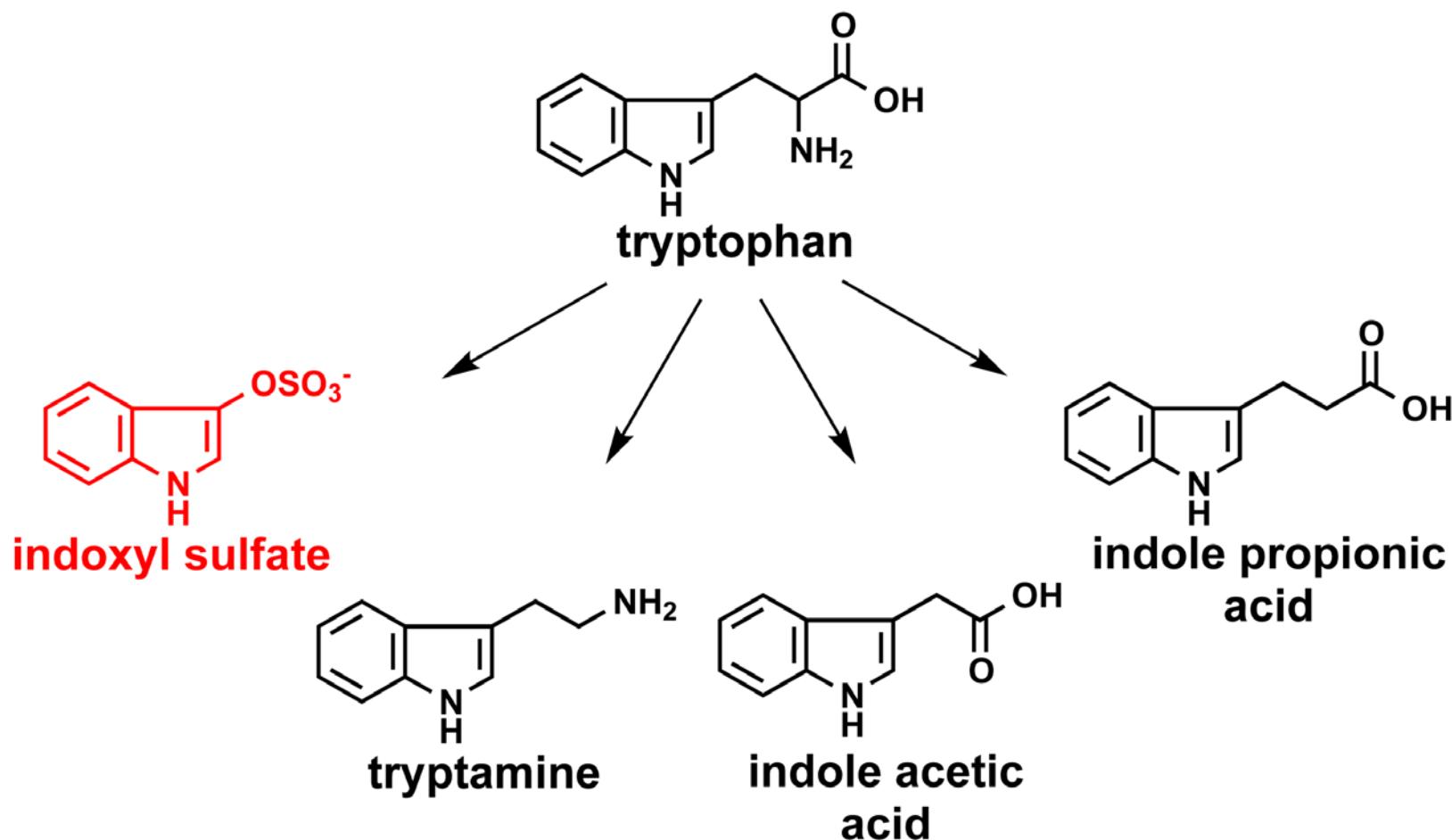
# Trp metabolism: Different end products



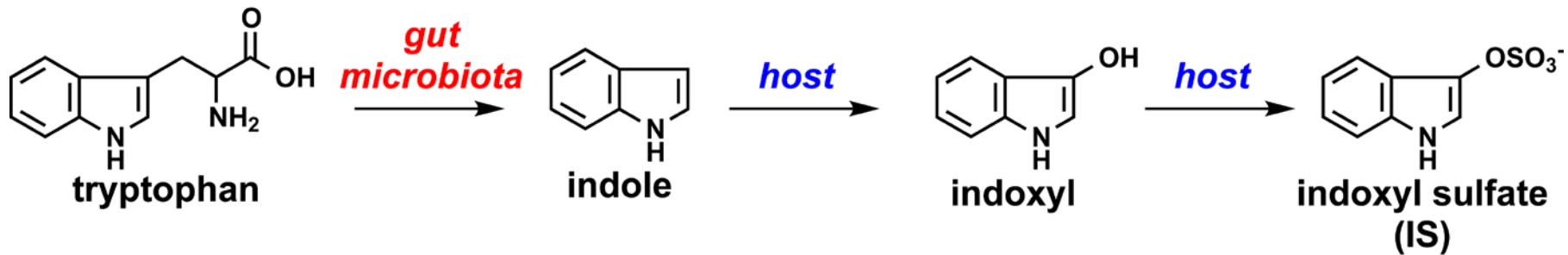
# Large differences at the strain level



# Trp metabolism: Different end products



# IS: Uremic solute from the gut microbiota

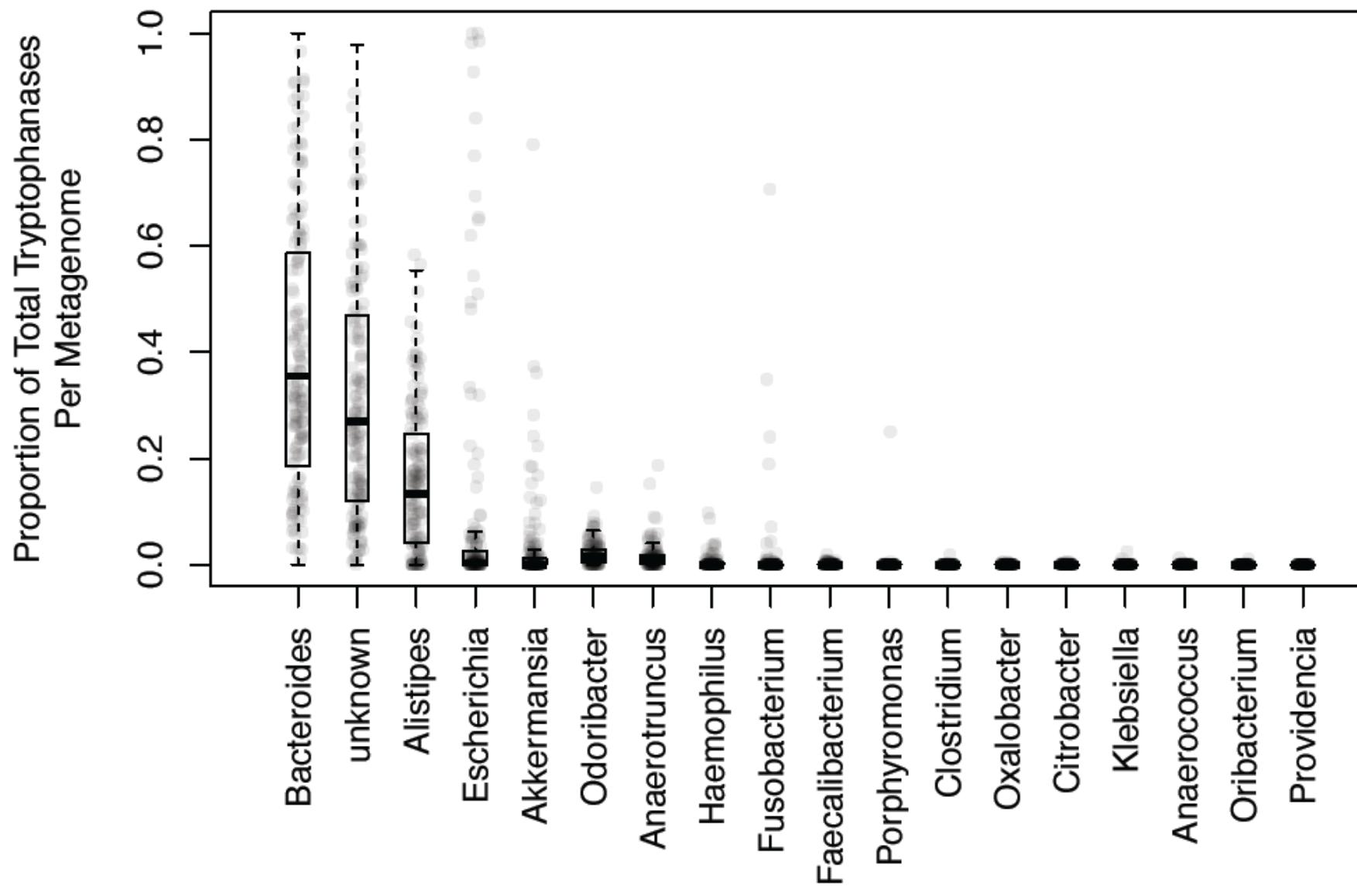


Level in humans: 10-200 mg/day

Strongly correlated with disease

A single gene responsible for its production; its levels vary widely among people

# Single gene responsible for IS production



# Single gene responsible for IS production

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## Producers

*Bacteroides thetaiotaomicron* VPI-5482

*Bacteroides ovatus* ATCC 8483

*Bacteroides uniformis* ATCC 8492

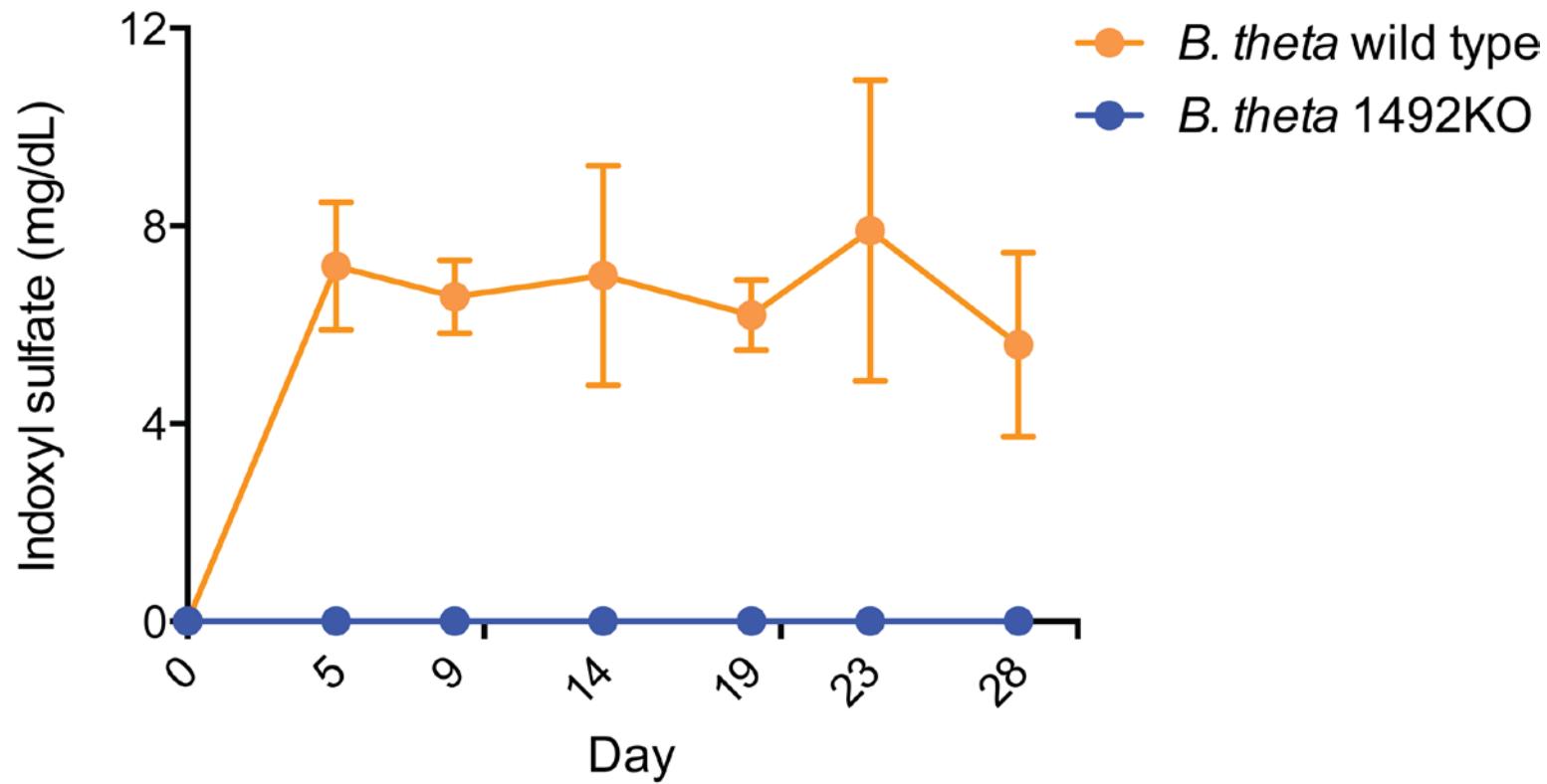
## Non-Producers

*Bacteroides fragilis* NCTC 9343

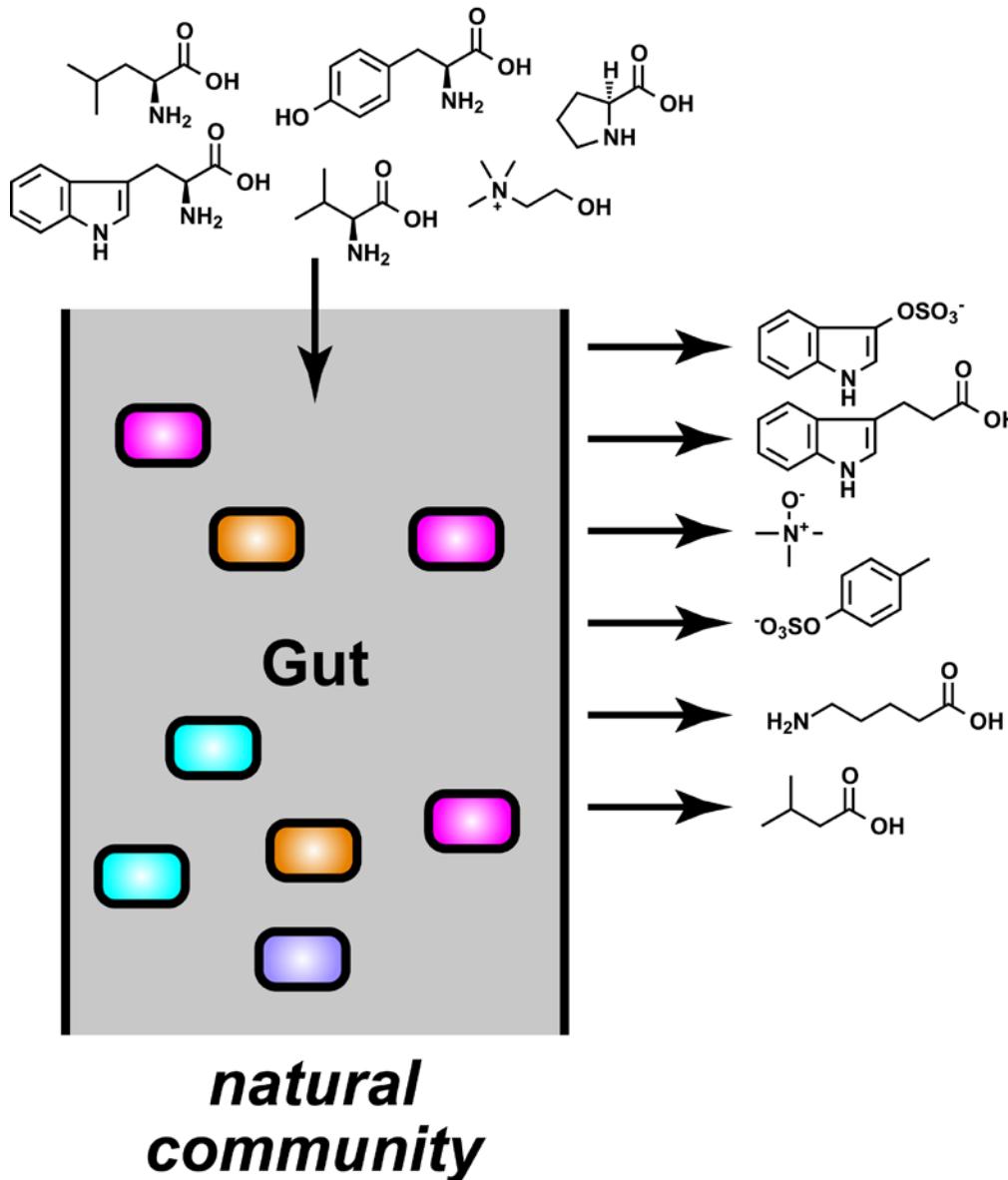
*Bacteroides vulgatus* ATCC 8482

*Bacteroides caccae* ATCC 43185

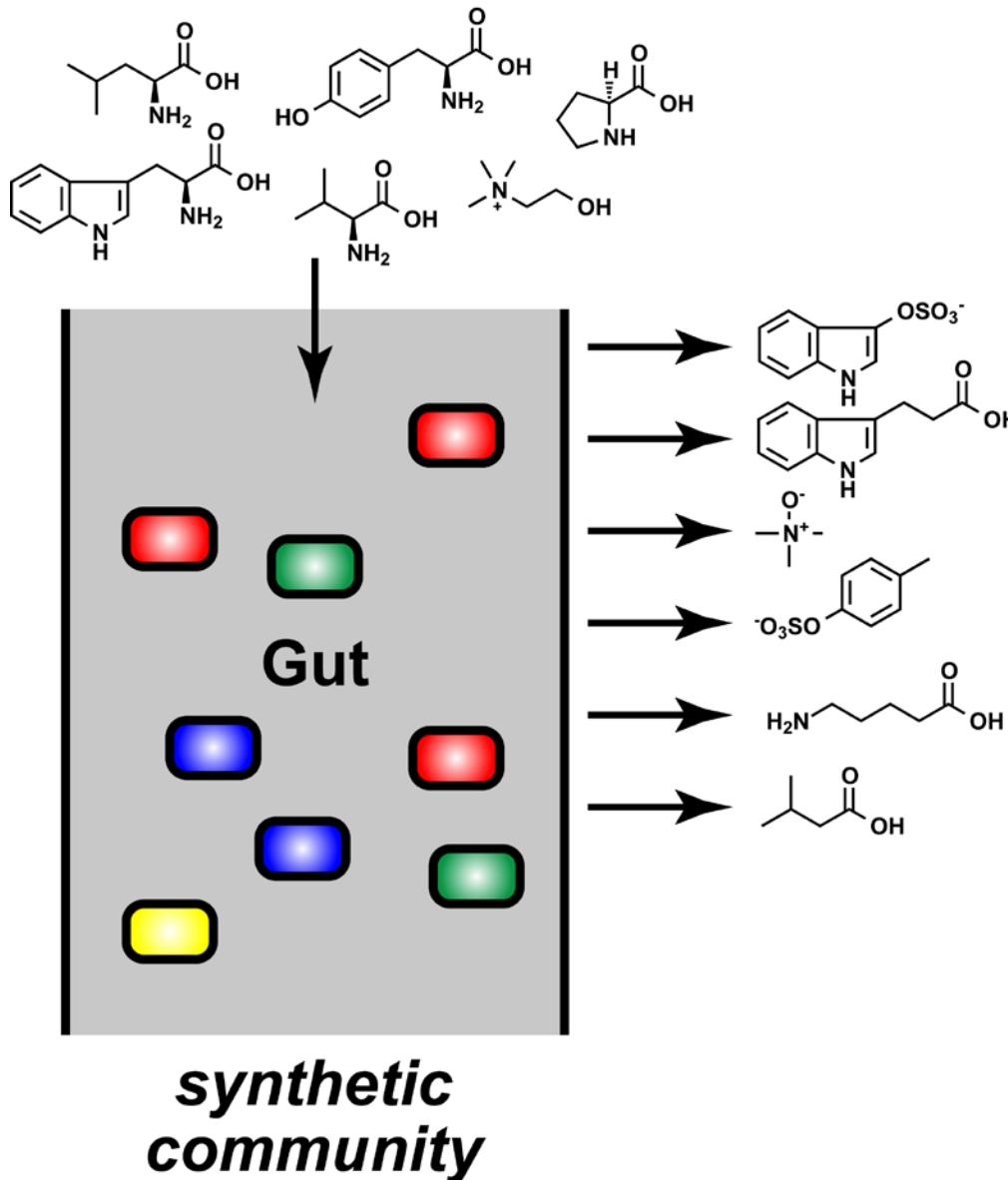
# Modulating IS production in mice



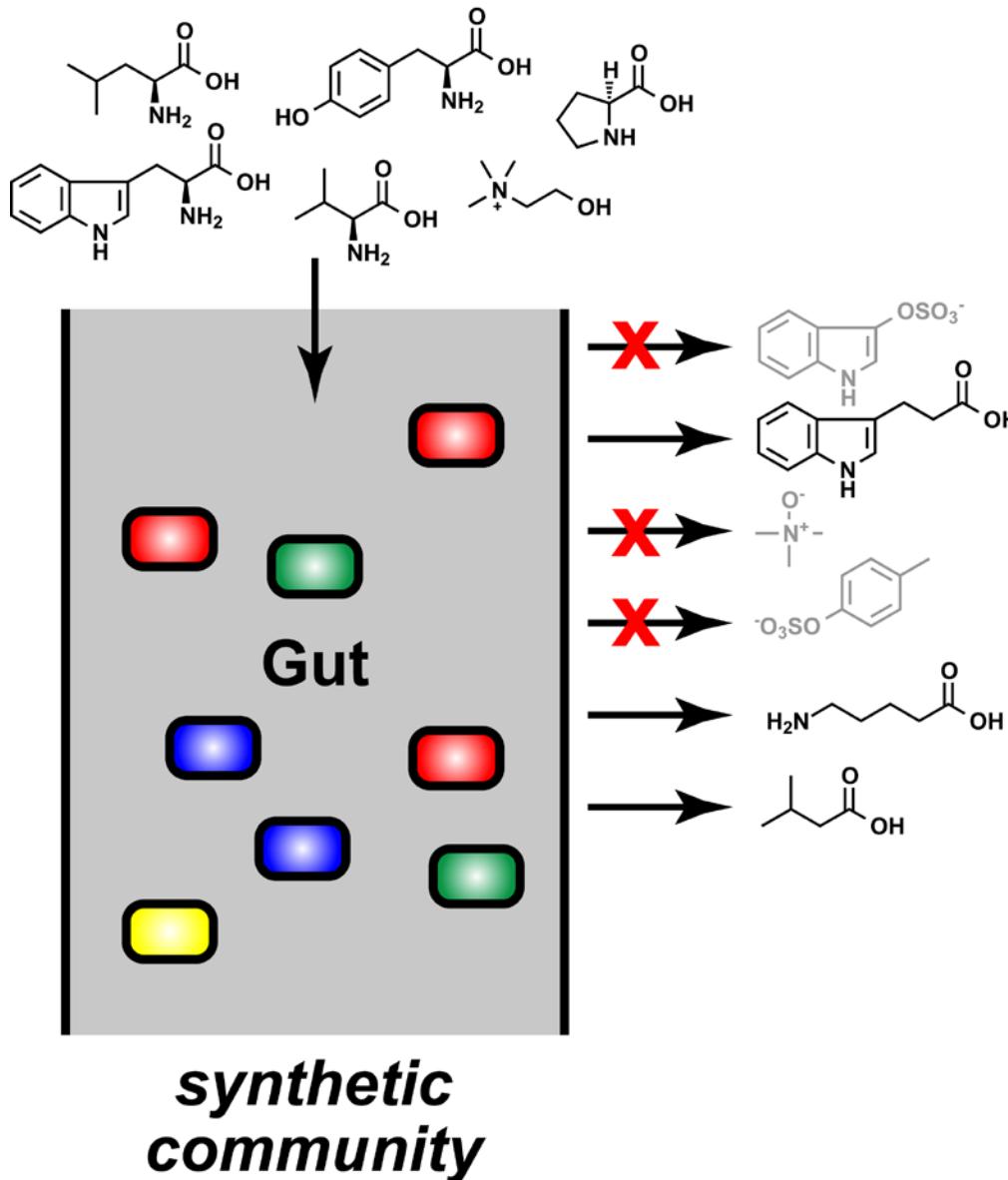
# Synthetic communities with defined output



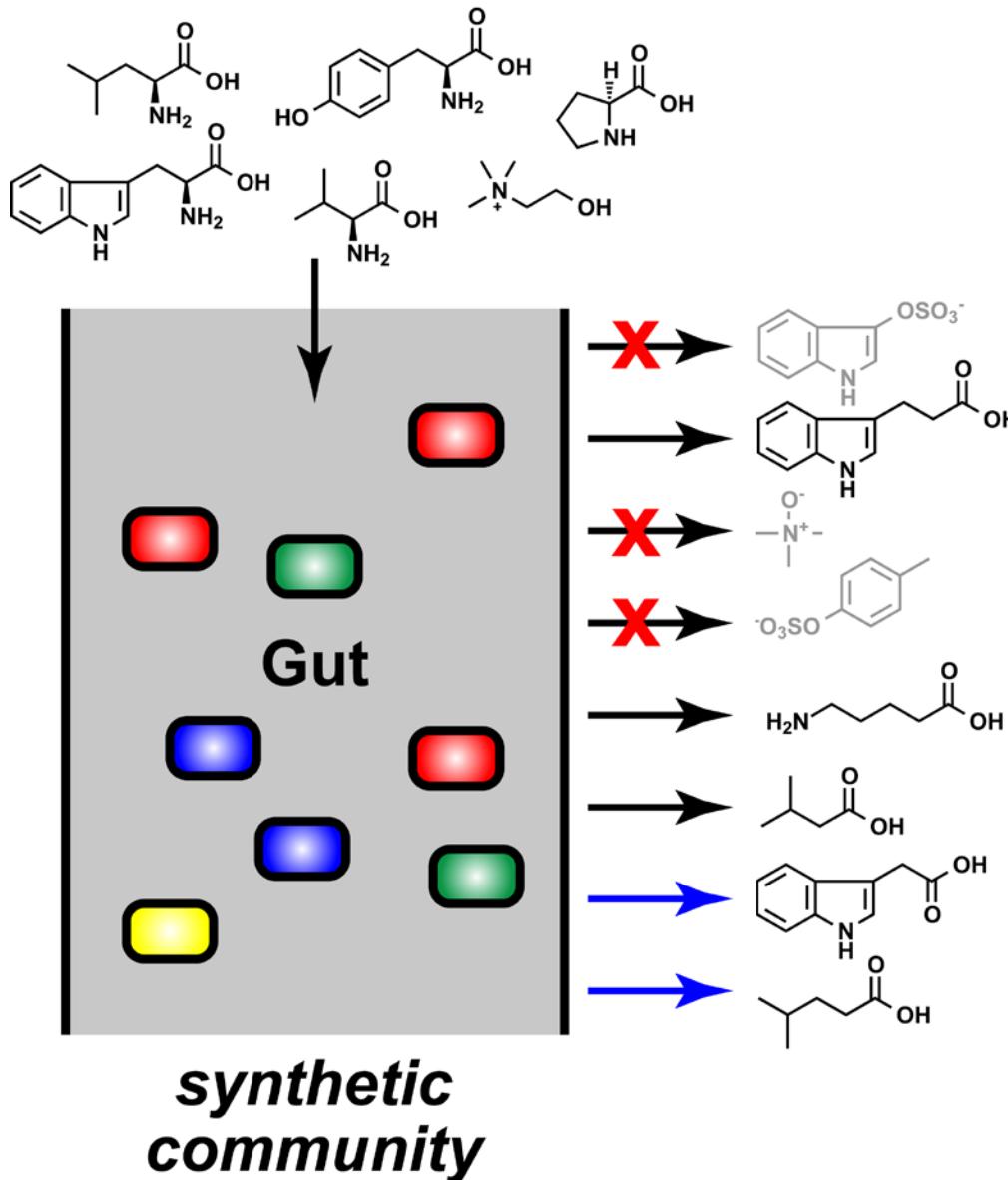
# Synthetic communities with defined output



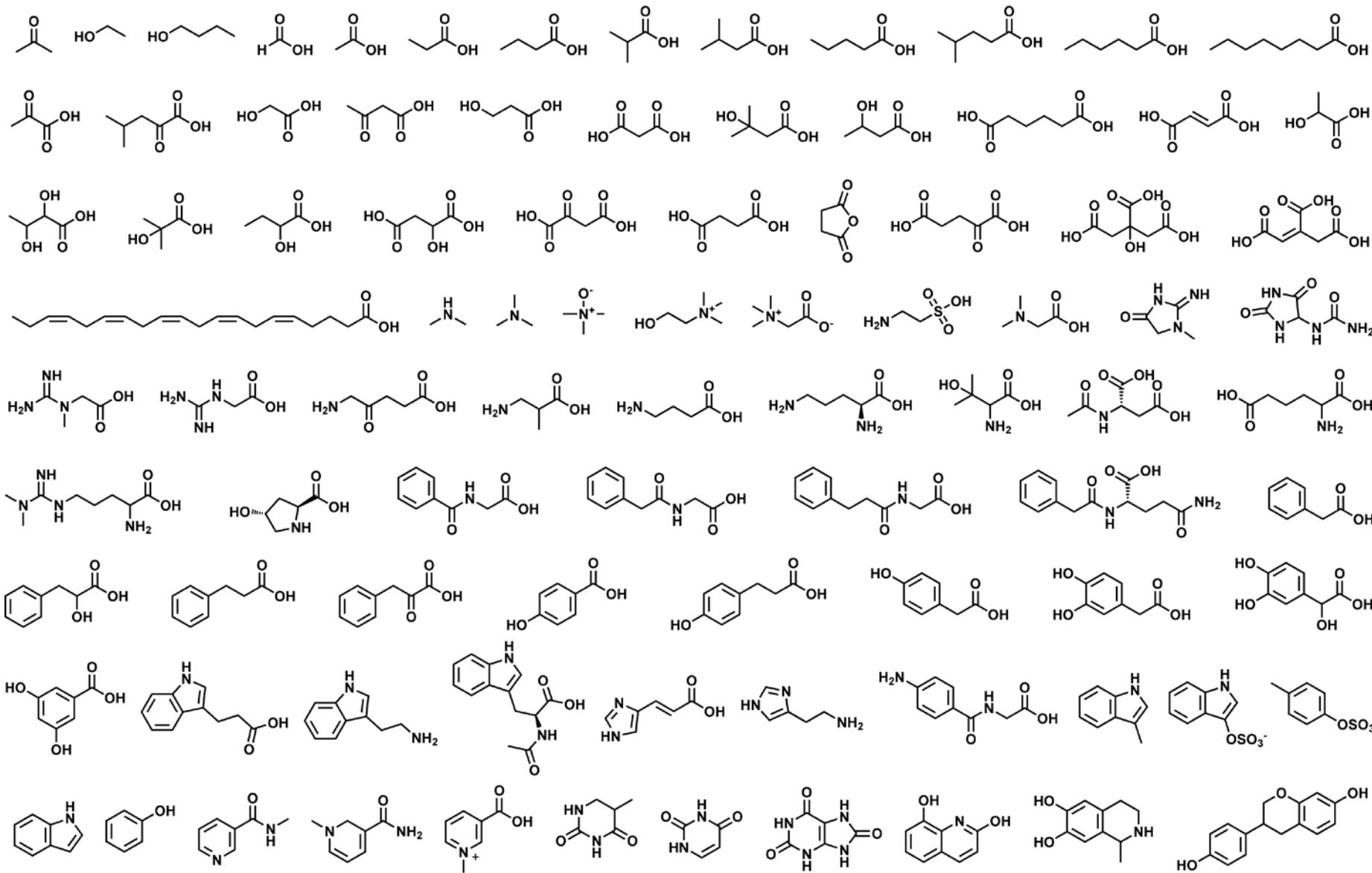
# Synthetic communities with defined output



# Synthetic communities with defined output



# Synthetic communities with defined output



# Acknowledgements

## Fischbach Group

Ariel Brumbaugh  
Erin Chen  
**Masanori Funabashi**  
Chunjun (CJ) Guo  
Amy Jacobson  
Isabel Kolinko  
Aedan Liu  
Eric Lubeck  
Colleen O'Loughlin  
Jakub Rajniak  
Anthony Rush  
Brianna Williams

*Laura Brown*  
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*Peter Cimermancic*  
*Sloan Devlin*  
*Mohamed Donia*  
*Laurens Kraal*  
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*Mao Taketani*  
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Averil Ma  
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Marnix Medema  
Tim Meyer  
David Relman  
Justin Sonnenburg  
Peter Turnbaugh  
Chris Voigt

## Funding

Agilent  
BASF  
Burroughs Wellcome Fund  
David and Lucille Packard Foundation  
DARPA  
Glenn Foundation  
HHMI-Simons Faculty Scholar  
NIH (Pioneer, New Innovator, NIDDK, NIAID)  
Novartis  
Pfizer  
W.M. Keck Foundation  
UCSF Center For Systems/Synthetic Biology