

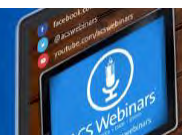
Type them into questions box!

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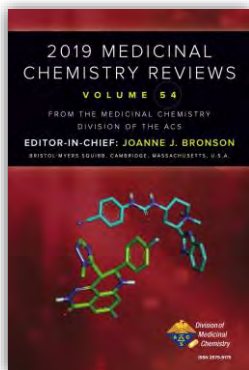
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<https://www.acs.org/content/acs/en/acs-webinars/drug-discovery/covid-19.html>



Join Research Fellow Li Di of Pfizer as she discusses why design principles that increase passive permeability are effective approaches to increase oral bioavailability, enhance brain penetration, and reduce renal clearance.

<https://www.acs.org/content/acs/en/acs-webinars/drug-discovery/passive-permeability.html>



Join Douglas Kell, Research Chair in Systems Biology at the University of Liverpool to discover how drugs pass through cell membrane solely by hitchhiking on membrane transporters and why so-called "passive diffusion" through any bilayer in real cells is negligible.

<https://www.acs.org/content/acs/en/acs-webinars/drug-discovery/so-lute-carriers.html>

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Friday, July 31, 2020 at 2:30pm ET  
 Speaker: Barry Weinert, Barry M. Weinert & Associates  
 Moderator: Bryan Tweedy, American Chemical Society

[Register for Free!](#)

#### What You Will Learn

- Become familiar with the planning activities required for successful team projects
- Understand key aspects of executing a team project, measuring success in terms of cost, schedule and performance criteria
- Learn key ways to successfully transition from your former job to a project management position

Co-produced with: ACS Professional Education



Tuesday, August 4, 2020 at 6-7pm IST (8:30am ET)  
 Speaker: Sandeep Verma, Science and Engineering Research Board, DST, Govt. of India  
 Moderator: Dreetisha Gupta, ACS International

[Register for Free!](#)

#### What You Will Learn

- Become familiar with the planning activities required for a successful project implementation
- Learn methods to successfully communicate and collaborate
- Understand key aspects of executing a project, measuring success in terms of cost, schedule and performance criteria

Co-produced with: ACS International and CBEN Jobs

\* This special broadcast has been created for a target audience in India.



Thursday, August 6, 2020 at 2-3:30pm ET  
 Speakers: Corey Colina, University of Florida / Theresa Reineke, University of Minnesota and ACS Macro Letters  
 Moderator: Laura Stratton, Polymer Chemistry Innovations

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#### What You Will Learn

- How biological performance significantly influenced by the bioconjugation architecture
- Why noncovalent conjugation is a stable way to assemble polymers with biological payloads
- State-of-the-art of molecular simulations for bioconjugation
- The effect of a variety of polymer architectures (PEI-based and beyond), grafting densities and polymer lengths on the bioconjugates structure, dynamics and function

Co-produced with: ACS Division of Polymer Chemistry

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Nature's  
Dirty Little  
**SECRET**

The Diverted Total Syntheses of  
Rhizosphere Natural Products as  
Targeted Antibacterial Agents

THIS ACS WEBINAR WILL BEGIN SHORTLY...

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Nature's Dirty Little Secret: The Diverted Total Syntheses of  
Rhizosphere Natural Products as Targeted Antibacterial Agents



**Bill Wuest**

GRA Distinguished Investigator &  
Associate Professor, Department of  
Chemistry, Emory University



**Cassandra Quave**

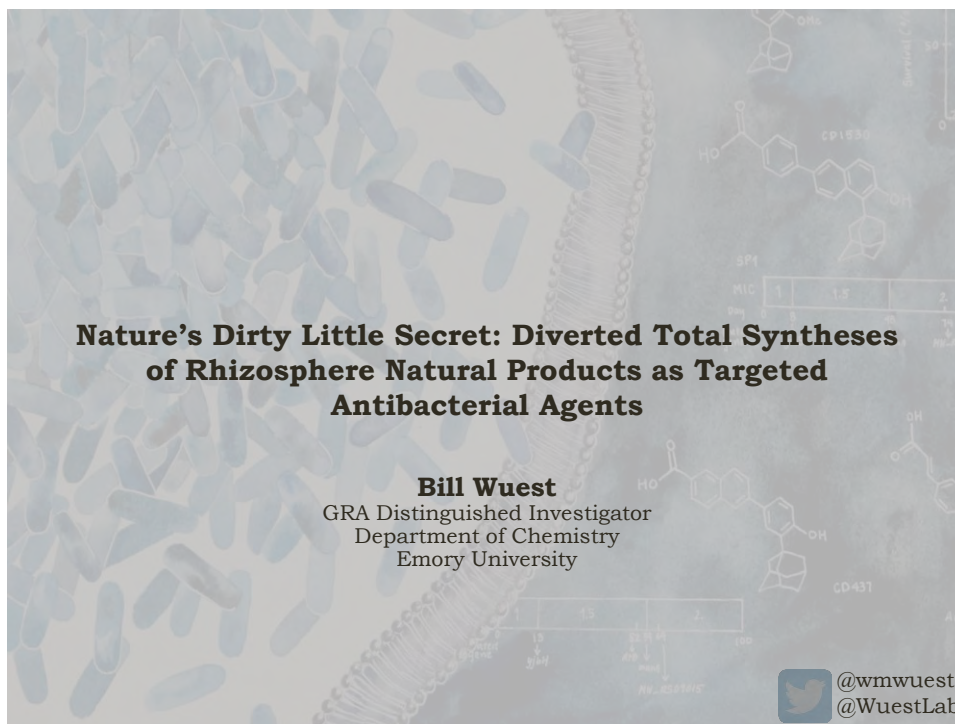
Assistant Professor, Department of Dermatology, Emory  
University School of Medicine; Assistant Professor,  
Center for the Study of Human Health, Emory College of  
Arts and Sciences; Curator, Emory University Herbarium

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The background of the slide features a microscopic view of numerous rod-shaped bacteria on the left. On the right, there are several chemical structures, including a complex polycyclic molecule with a carboxylic acid group and a hydroxyl group, and a smaller molecule with a carboxylic acid group. A scale bar is visible in the lower right quadrant of the image.

**Nature's Dirty Little Secret: Diverted Total Syntheses  
of Rhizosphere Natural Products as Targeted  
Antibacterial Agents**

**Bill Wuest**  
GRA Distinguished Investigator  
Department of Chemistry  
Emory University

 @wmmwuest  
@WuestLab

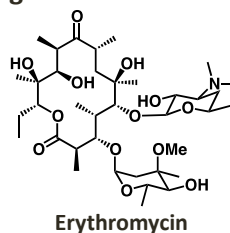
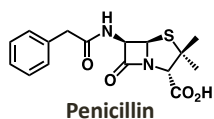
## Today's Webinar Learning Objectives

### At the end of this webinar you will be able to:

- List the “pros” and “cons” for broad-spectrum and narrow spectrum antibiotics
- Describe what the rhizosphere is
- Define diverted total synthesis
- Describe the type of research conducted in the Wuest Lab
- Propose a proteomics experiment
- Explain how promysalin elicits its narrow-spectrum response

## Rethinking Antibiotic Development

- Incredibly effective broad-spectrum agents with wide scope and usage

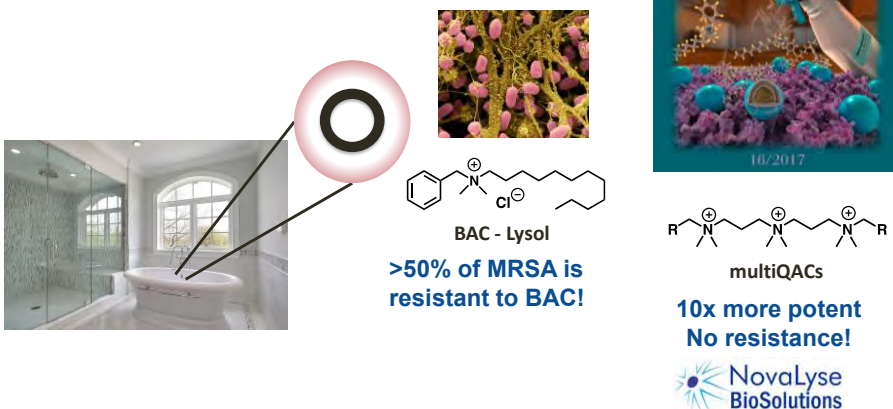


Rossiter, S.E.; Fletcher, M.H.; Wuest, W.M. *Chem. Rev.* **2017** *117*, 12415

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## Rethinking Antibiotic Development

- Incredibly effective broad-spectrum agents with wide scope and usage
- These benefits come at a cost:
  - Antibiotic resistance



**BAC - Lysol**

**>50% of MRSA is resistant to BAC!**

**multiQACs**

**10x more potent  
No resistance!**

**Novalyse  
BioSolutions**

Jennings, M.C.; Minbiole, K.P.C.; Wuest, W.M. *ACS Inf. Dis.* **2015** *1*, 288  
 Jennings, M.C.; Buttaro, B.; Minbiole, K.P.C.; Wuest, W.M. *ACS Inf. Dis.* **2015** *1*, 304  
 Jennings, M.C.; Minbiole, K.P.C.; Wuest, W.M. *ChemBioChem* **2017** *18*, 1573

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## Rethinking Antibiotic Development

- Incredibly effective broad-spectrum agents with wide scope and usage
- These benefits come at a cost:
  - Antibiotic resistance
  - Collateral damage

Over 1/2 of our cells are bacteria!



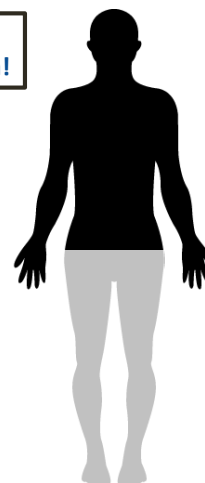
### Human Microbiomes



Oral



Gut



What effects do these broad-spectrum agents have on these environments?

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### Audience Challenge Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



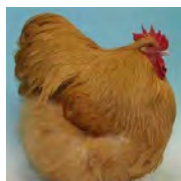
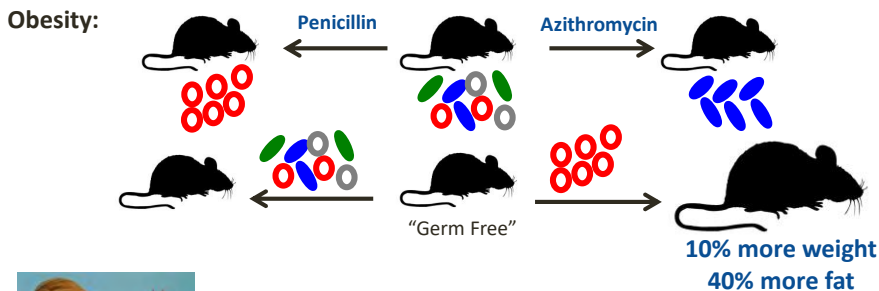
**Broad-spectrum antibiotics have been connected to which of the following?** (Select all that apply)

- Weight Gain
- Humor
- Allergies
- Celiac Disease
- Baldness

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## Collateral Damage of Broad-Spectrum Antibiotics



This is why livestock are given low-dose antibiotics!

### Antibiotic Usage in 2010

Humans: 70B daily doses

Livestock: 63B daily doses

Antibiotics have also been connected to allergies, Crohn's and Celiac Disease

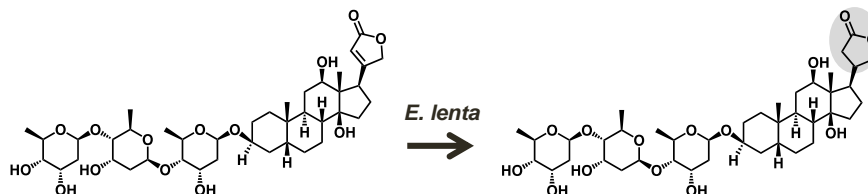
What if we instead focused on pathogen-specific agents?

Cox, L. *et al. Cell* 2014 158, 705

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## Potential Benefits of Pathogen-Specific Agents

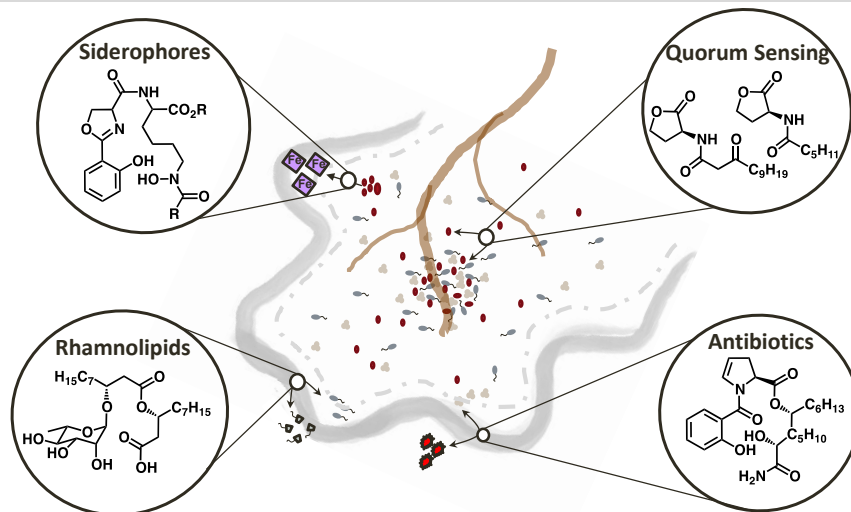
- Specific pathogenic bacteria are responsible for broad-spectrum usage
  - **Pregnancy: Group B Strep-(+)** results in prophylactic antibiotic use
    - Antibiotics used in 40% of pregnancies (20-25% in 1996)
  - **Oral care: *S. mutans*** causative agent for caries
  - **Cystic Fibrosis: 50% of patients have *MRSA* and/or *P. aeruginosa***
- Microbiome-bacteria mediated interactions within the host
  - ***E. lenta* inactivates Digoxin (heart disease) by selective degradation**



Can we identify, and improve on, natural products that have been tuned for these specific purposes?

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## Rhizosphere Microbiome

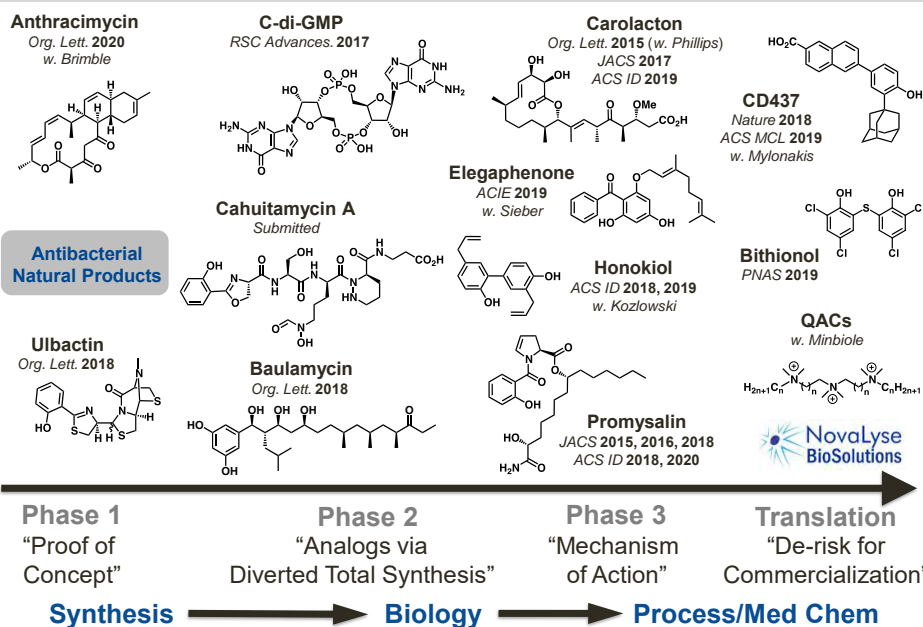


**Potential to discover narrow-spectrum Gram-negative agents with unique mechanisms of action!**

Keohane, C.E.; Steele, A.D.; Wuest, W.M. *Synlett* **2015** 26, 2739

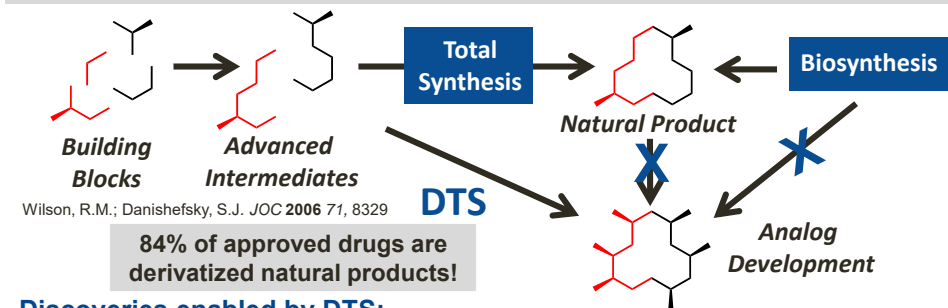
19

## Wuest Group Research Pipeline

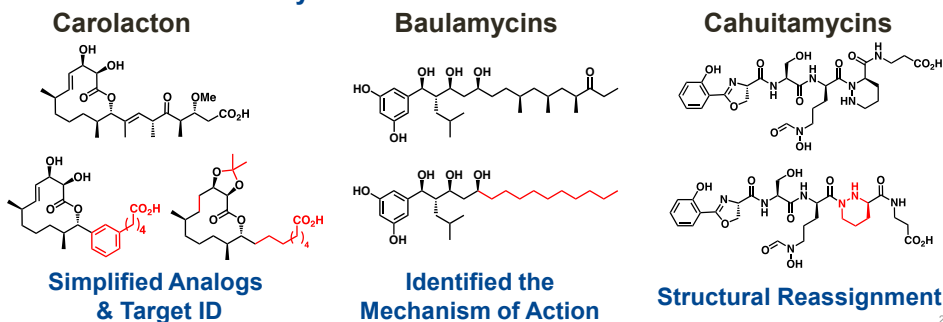


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## Diverted Total Synthesis

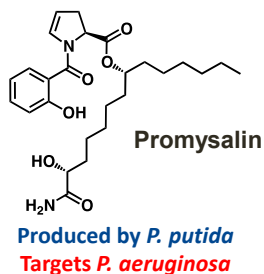


### Discoveries enabled by DTS:



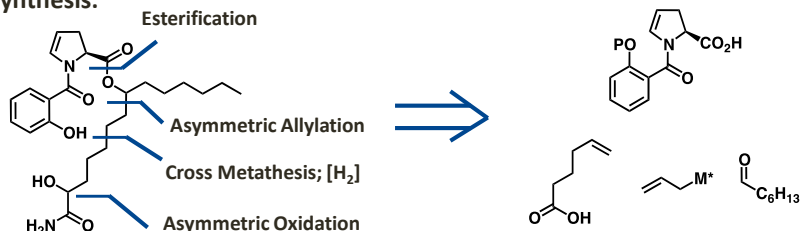
## Rhizosphere Natural Products that Target Pathogens

- “Ultra narrow-spectrum”: Species-specific inhibition



- Specifically targets *PA14* ( $IC_{50} = 64$  nM) and *PAO1* ( $2 \mu M$ ) over other bacteria
- Promotes swarming in *P. putida*
- **Key unanswered questions:**
  - What is the absolute structure?
  - Can we make simplified analogs?
  - What is the mechanism of action?
  - Why is the molecule species-specific?

### Retrosynthesis:

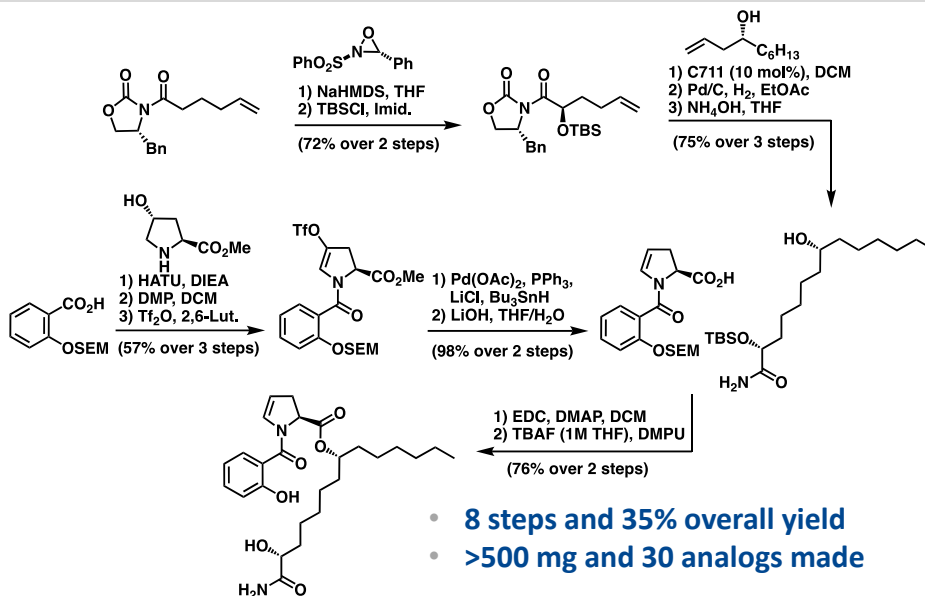


**Established, flexible chemistry that completely controls stereochemistry!**

De Mot, R. et al. *Chem. Biol.* 2011 18, 1320

22

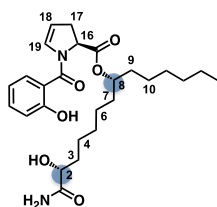
## Efficient Total Synthesis of Promysalin



Steele, A.D.; Knouse, K.W.; Keohane, C.E.; Wuest, W.M. *J. Am. Chem. Soc.* **2015** *137*, 7314

23

## Confirmation of Structure and Activity



<sup>1</sup>H NMR confirmed  
relative stereochemistry

PAO1: (IC<sub>50</sub> = NR)

PAO1	IC <sub>50</sub>
(2 <i>R</i> ,8 <i>R</i> )	4.1
(2 <i>R</i> ,8 <i>S</i> )	46
(2 <i>S</i> ,8 <i>S</i> )	90
(2 <i>S</i> ,8 <i>R</i> )	33

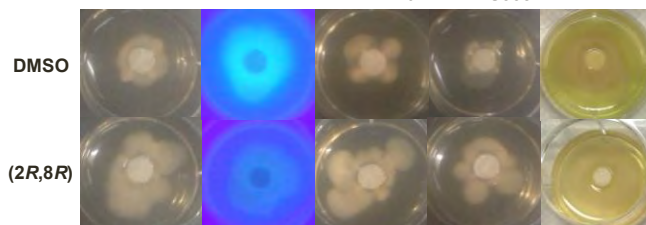
PA14: (IC<sub>50</sub> = 1.8)

PA14	IC <sub>50</sub>
(2 <i>R</i> ,8 <i>R</i> )	0.063
(2 <i>R</i> ,8 <i>S</i> )	6.6
(2 <i>S</i> ,8 <i>S</i> )	22
(2 <i>S</i> ,8 <i>R</i> )	4.3

Inhibitory activity supported  
absolute confirmation

PPRW10S1

PPKT2440 PPWCS358

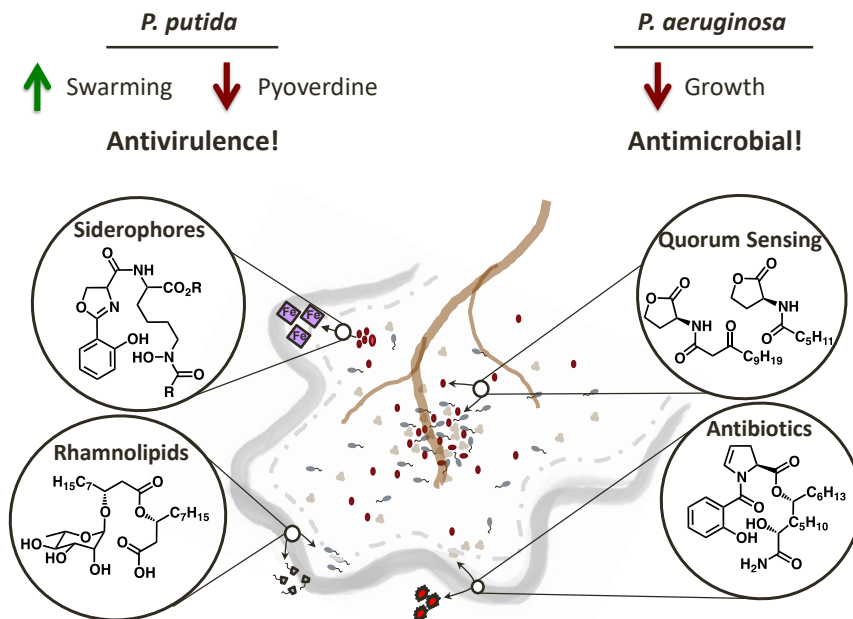


Inhibits the production of pyoverdine, a virulence factor but not growth!

Steele, A.D.; Knouse, K.W.; Keohane, C.E.; Wuest, W.M. *J. Am. Chem. Soc.* **2015** *137*, 7314

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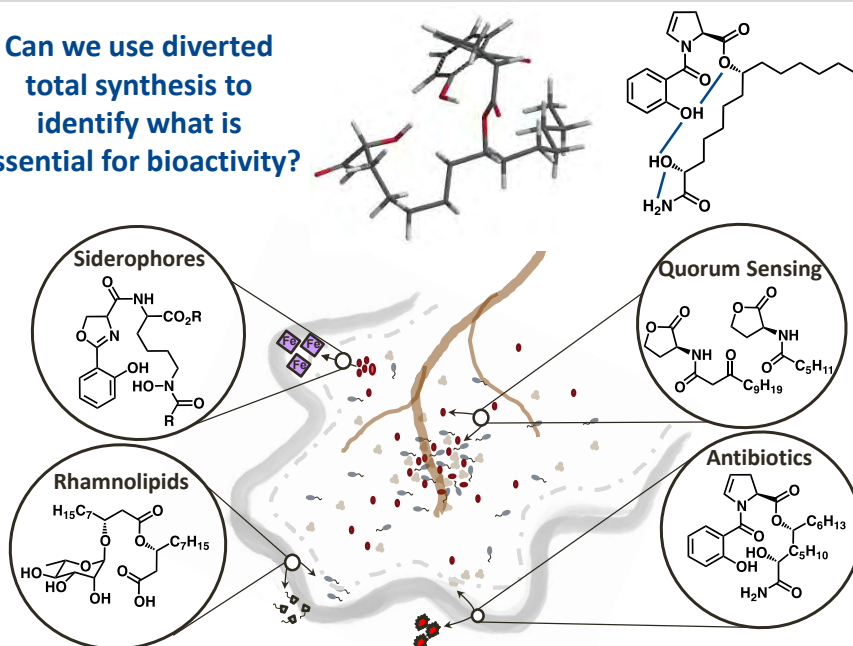
## Promysalin – The “Swiss Army Knife” for *P. putida*



25

## Promysalin – The “Swiss Army Knife” for *P. putida*

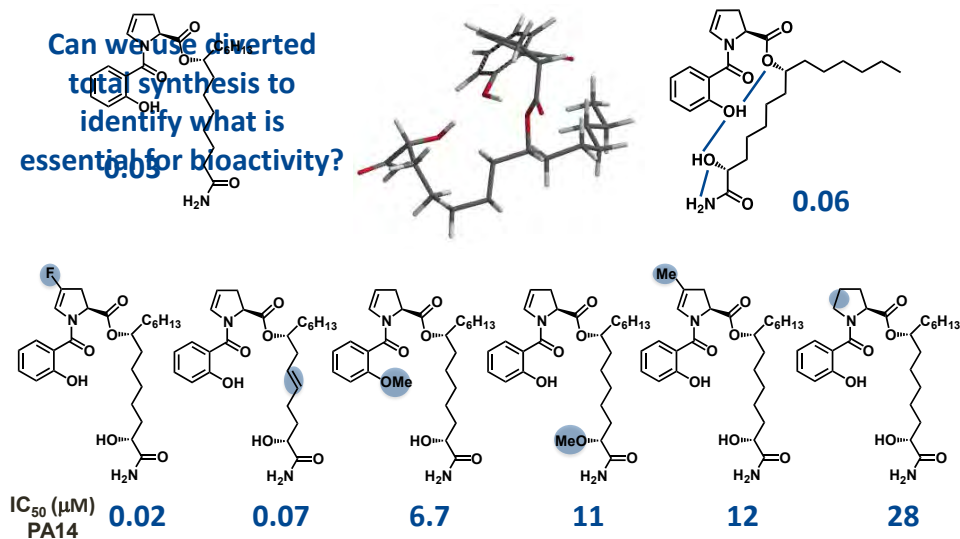
Can we use diverted total synthesis to identify what is essential for bioactivity?



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## Promysalin – The “Swiss Army Knife” for *P. putida*

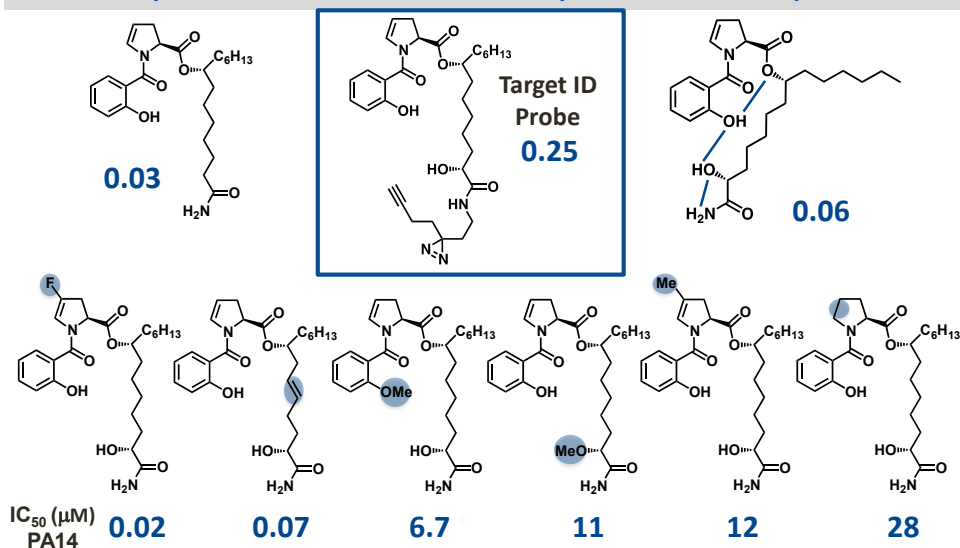
Can we use diverted  
total synthesis to  
identify what is  
essential for bioactivity?



Steele, A.D.; Keohane C.E.; Knouse, K.W.; Rossiter, S.E.; Williams, S.J.; Wuest, W.M. *J. Am. Chem. Soc.* **2016** 138, 5833

27

## Promysalin – The “Swiss Army Knife” for *P. putida*



What is the target of promysalin?

Steele, A.D.; Keohane C.E.; Knouse, K.W.; Rossiter, S.E.; Williams, S.J.; Wuest, W.M. *J. Am. Chem. Soc.* **2016** 138, 5833

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## Audience Challenge Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT



### What do you think is the biological target of Promysalin?

- A siderophore transporter that shuttles iron
- A virulence factor that controls biofilm formation
- An essential enzyme in primary metabolism
- The ribosome

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### Affinity-based Protein Profiling

**Target ID Probe**  
0.25  $\mu\text{M}$

1) Probe + Light

HC  $\xrightarrow{\text{H}}$

2) Lyse

3) Tag-N<sub>3</sub>

4) Pull-down

5) Analyze

**Issues to Consider:**

- 1) Non-specific binding  
**Competitive Inhibitor**
- 2) How to quantify and rank hits  
**Stable isotope dimethyl labeling**

**Probe**

HC  $\xrightarrow{\text{H}}$

**Steps 2-4**

**5) Digest**

**Inhibitor + Probe**

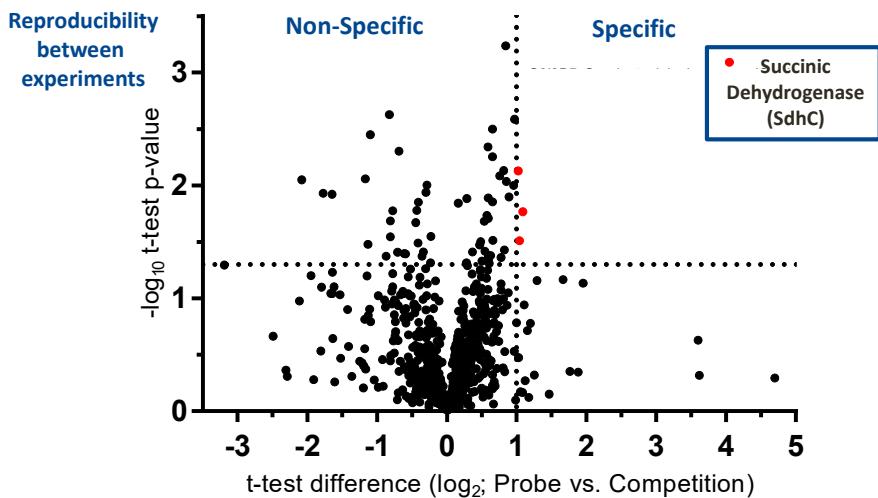
**Analyze by HRMS**

$\Delta$  Mass Shift = 4

1) Compare differential  
2) Run in triplicate

30

## Proteomic Profiling of Promysalin in PAO1

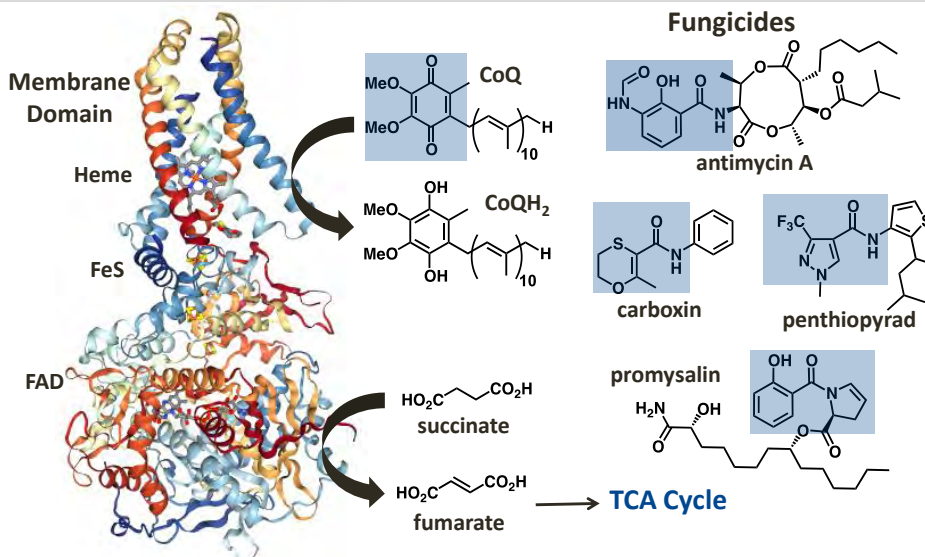


### SdhC: Quinone-oxidoreductase site of succinic dehydrogenase

Keohane C.E.; Steele, A.D.; Sieber, S.; Wuest, W.M. *et al.* JACS 2018 140, 1174

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## Proteomics Identifies Succinic Dehydrogenase as Target

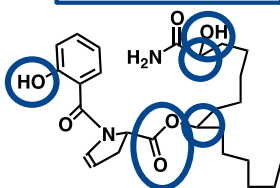
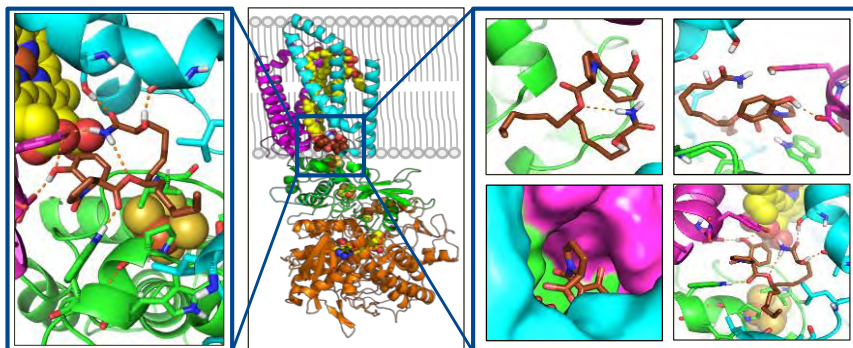


Can we use docking to identify where promysalin binds?

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## Computational Docking of Promysalin with SdhC



### SAR Findings:

- Chirality Matters
- Ester Needed
- Phenol
- Alcohol Necessary?

Can we confirm target by selecting for resistant mutants?

	E. coli	PAO1	PA14	KT2440
E. coli	100.00	55.47	55.47	55.47
PAO1	55.47	100.00	100.00	84.38
PA14	55.47	100.00	100.00	84.38
KT2440	55.47	84.38	84.38	100.00

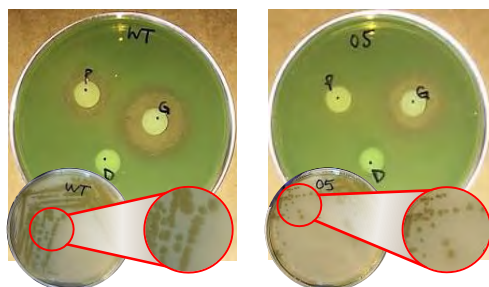
High sequence identity!  
Does not explain selectivity!

with J. Karanicolas (FCCC)

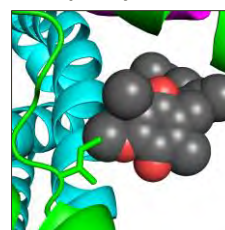
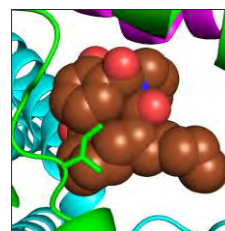
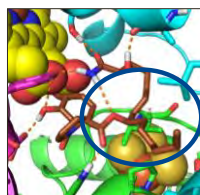
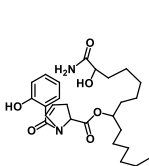
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## Sub-Inhibitory Dosing Provides Resistant Mutants

P = Promysalin; G = gentamicin (+ ctrl); D = DMSO (- ctrl)



SdhCDAB operon:



613nt: GGA ATC ATG Wild-type  
205aa: GGA AIC AMG  
GGA GTC ATG Mutant  
GGA AVC AMG

What is the role of Sdh in TCA Cycle?  
How does this explain the selectivity?

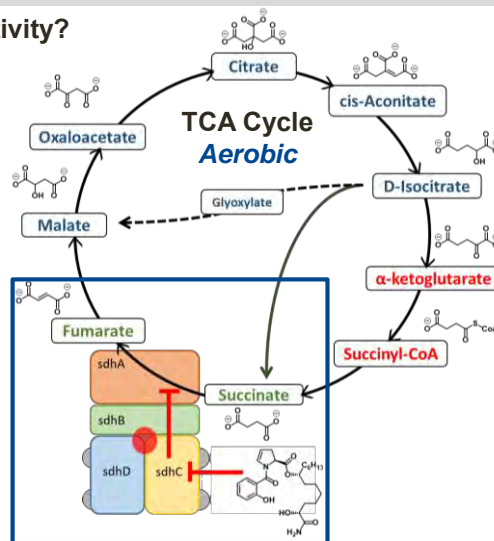
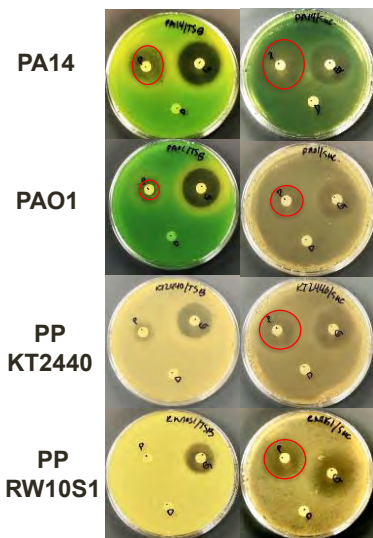
Keohane C.E.; Steele, A.D.; Sieber, S.; Wuest, W.M. *et al.* JACS 2018 140, 1174

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## Biochemistry 101: Primary Metabolism

Will feeding studies explain selectivity?

TSB Media Succinate

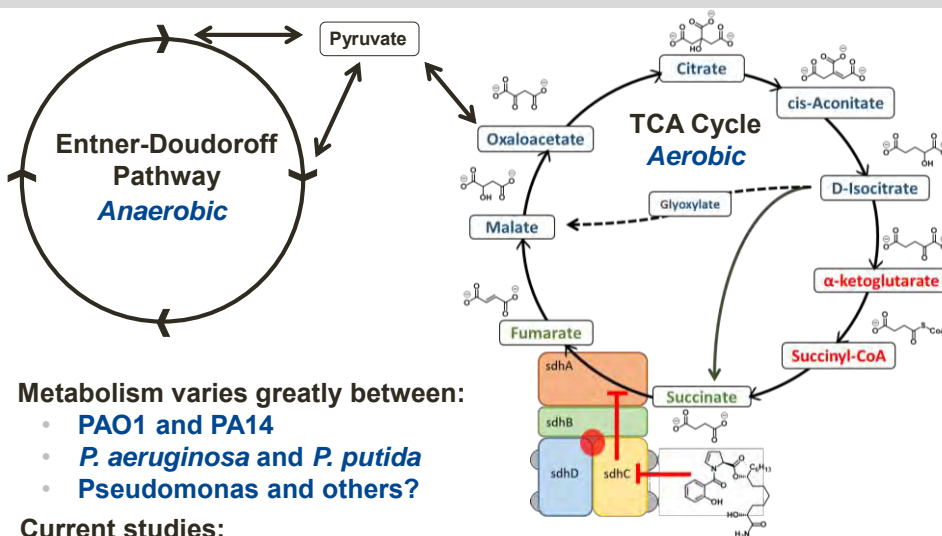


Toxic to PP including producing strain!

Keohane C.E.; Steele, A.D.; Sieber, S.; Wuest, W.M. *et al.* JACS 2018 140, 1174

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## Differences in Metabolism Drive Selectivity



Metabolism varies greatly between:

- PAO1 and PA14
- *P. aeruginosa* and *P. putida*
- *Pseudomonas* and others?

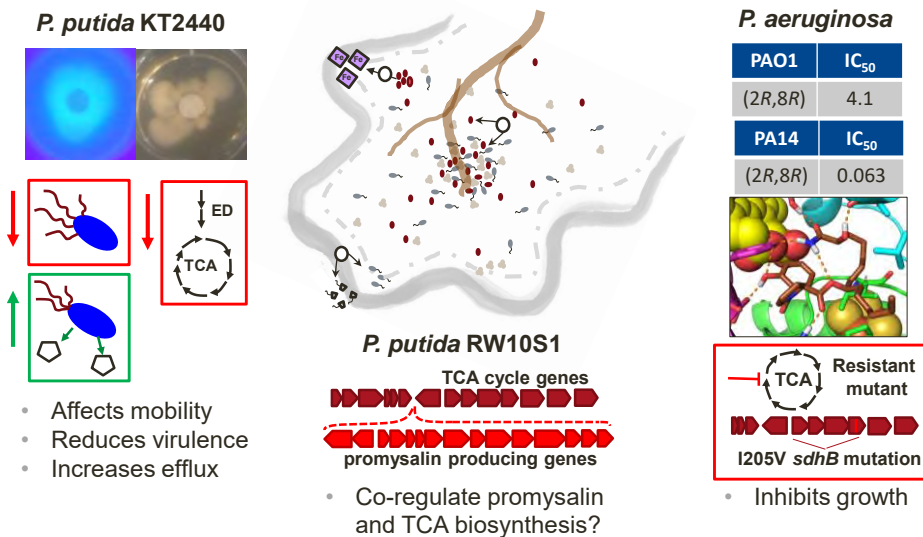
Current studies:

- Transcriptomics in *P. putida* – Effects metabolism and iron transport
- Develop a best in class compound – Synergistic w/ antibiotics?

Keohane C.E.; Steele, A.D.; Sieber, S.; Wuest, W.M. *et al.* JACS 2018 140, 1174  
Giglio, K. M.; Keohane, C.E.; Steele, A.D.; Wuest, W.M.; Filiatrault, M. *et al.* ACS Infect. Dis. 2018 4, 1179

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## Promysalin modulates the rhizosphere microbiome



### Targeting primary metabolism – new strategy!

Giglio, K. M.; Keoghane, C.E.; Steele, A.D.; Wuest, W.M.; Filiatrault, M. *et al.* *ACS Infect. Dis.* **2018** *4*, 1179  
 Shapiro, J.A.; Kaplan, A.R. Wuest, W.M. *ChemBioChem* **2019** *20*, 34

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## Nature's Dirty Little Secret: The Diverted Total Syntheses of Rhizosphere Natural Products as Targeted Antibacterial Agents



**Bill Wuest**

GRA Distinguished Investigator & Associate Professor, Department of Chemistry, Emory University



**Cassandra Quave**

Assistant Professor, Department of Dermatology, Emory University School of Medicine; Assistant Professor, Center for the Study of Human Health, Emory College of Arts and Sciences; Curator, Emory University Herbarium

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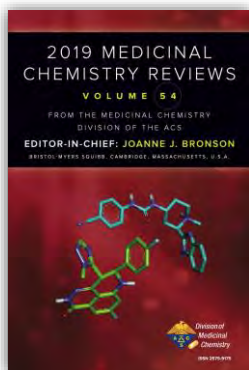
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