Drug discovery strategies: Focusing on synthetic compounds and natural products

Guest speakers: Heike Brötz-Oesterhelt & Scott Hecker

Moderator: Kim Lewis

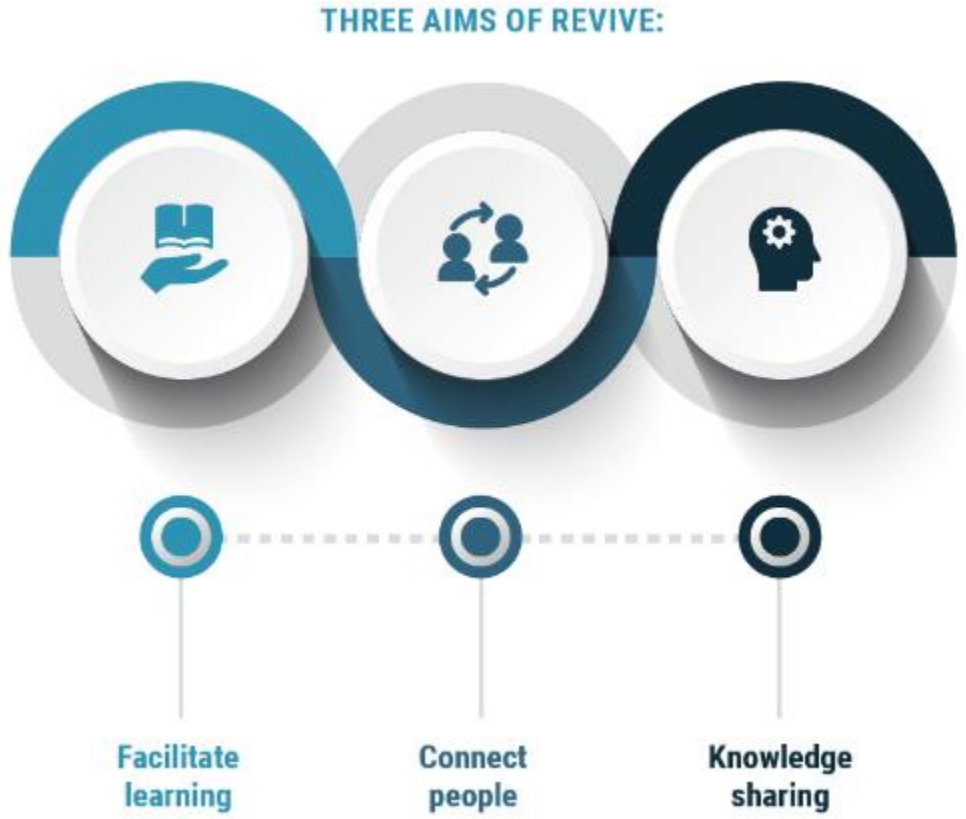
Host: Astrid Pentz-Murr (GARDP)

11 October 2022

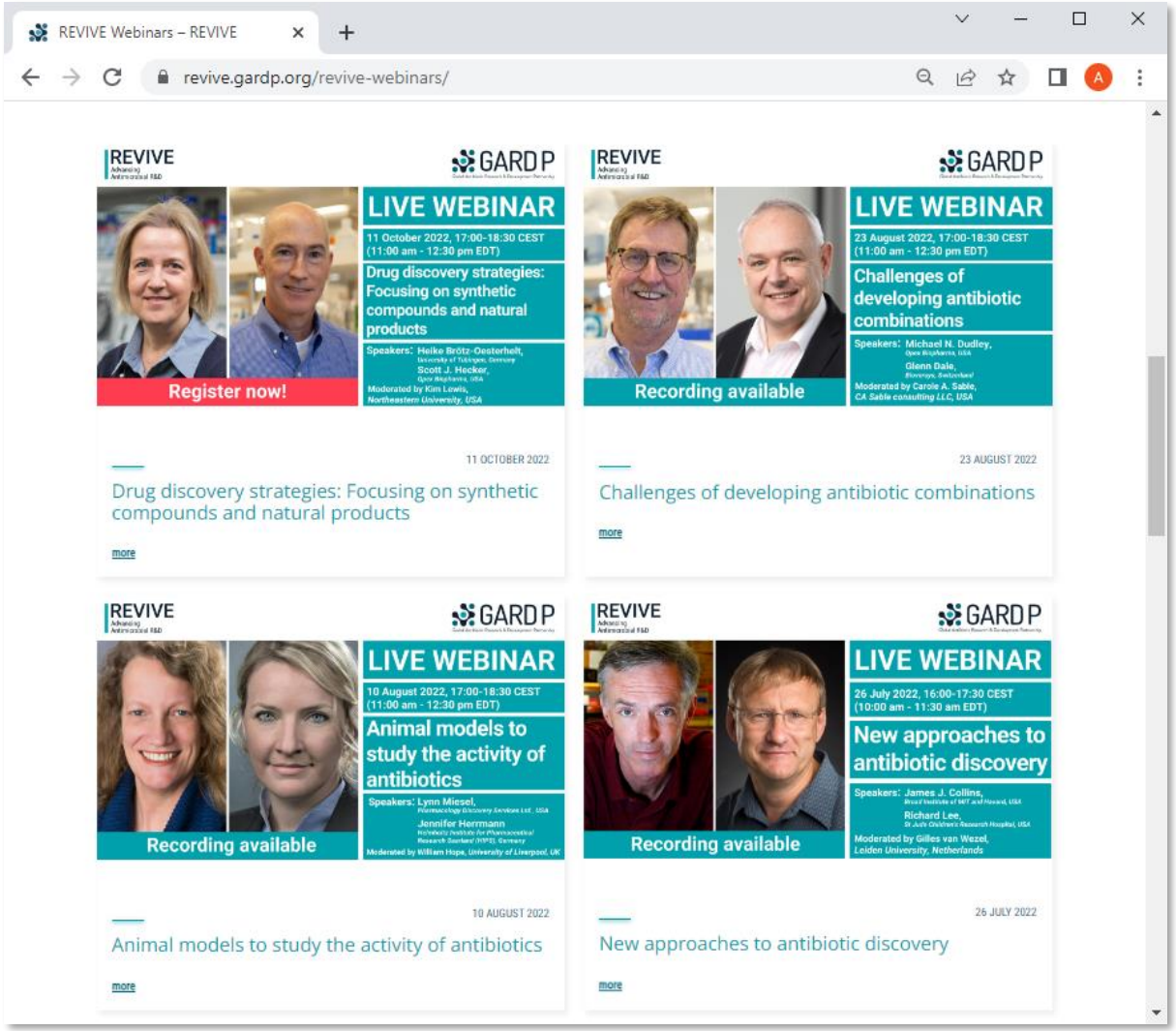
GARD P
Global Antibiotic Research & Development Partnership

REVIVE

An online space to connect and support the antimicrobial discovery, research, and development community

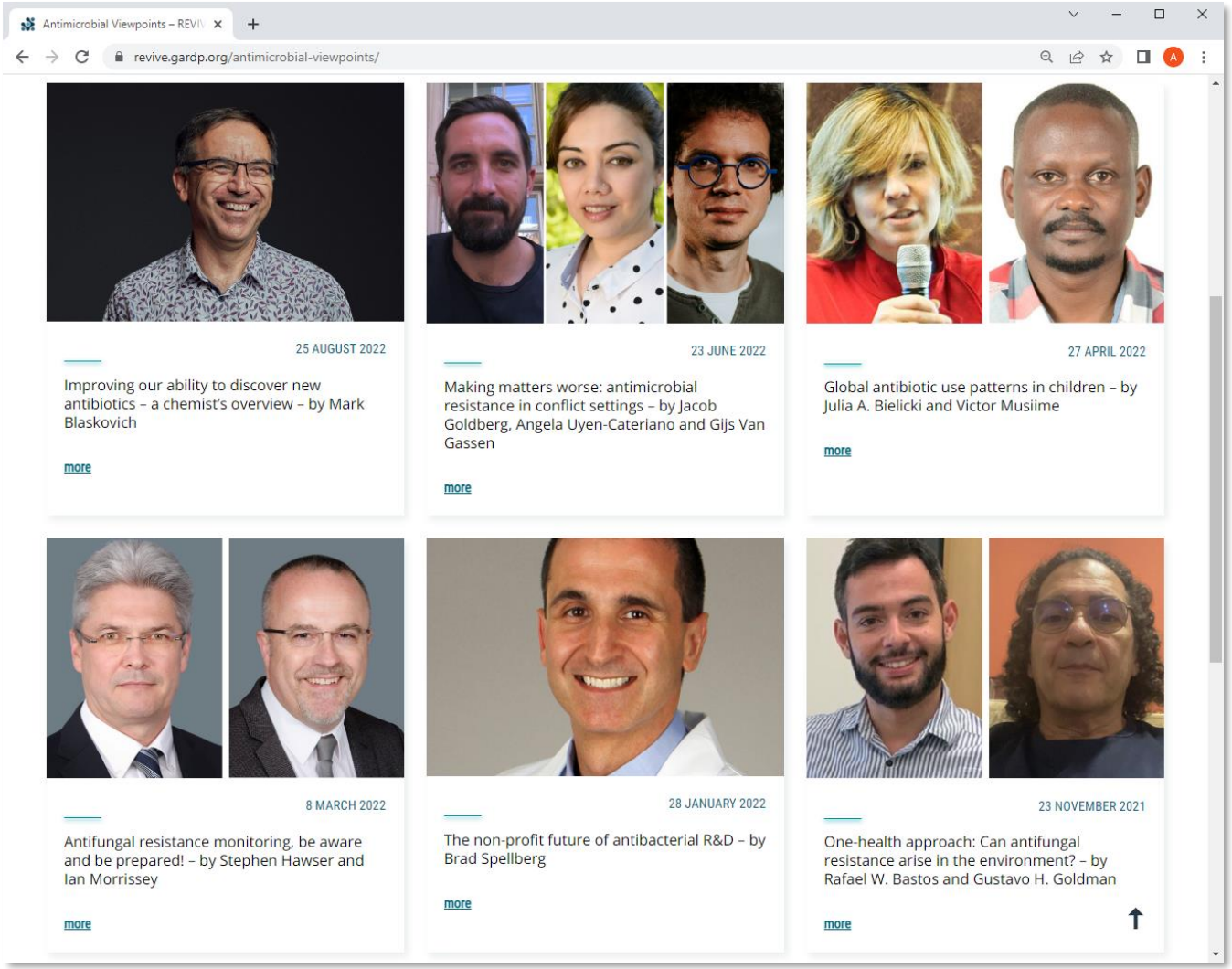


Webinar recordings



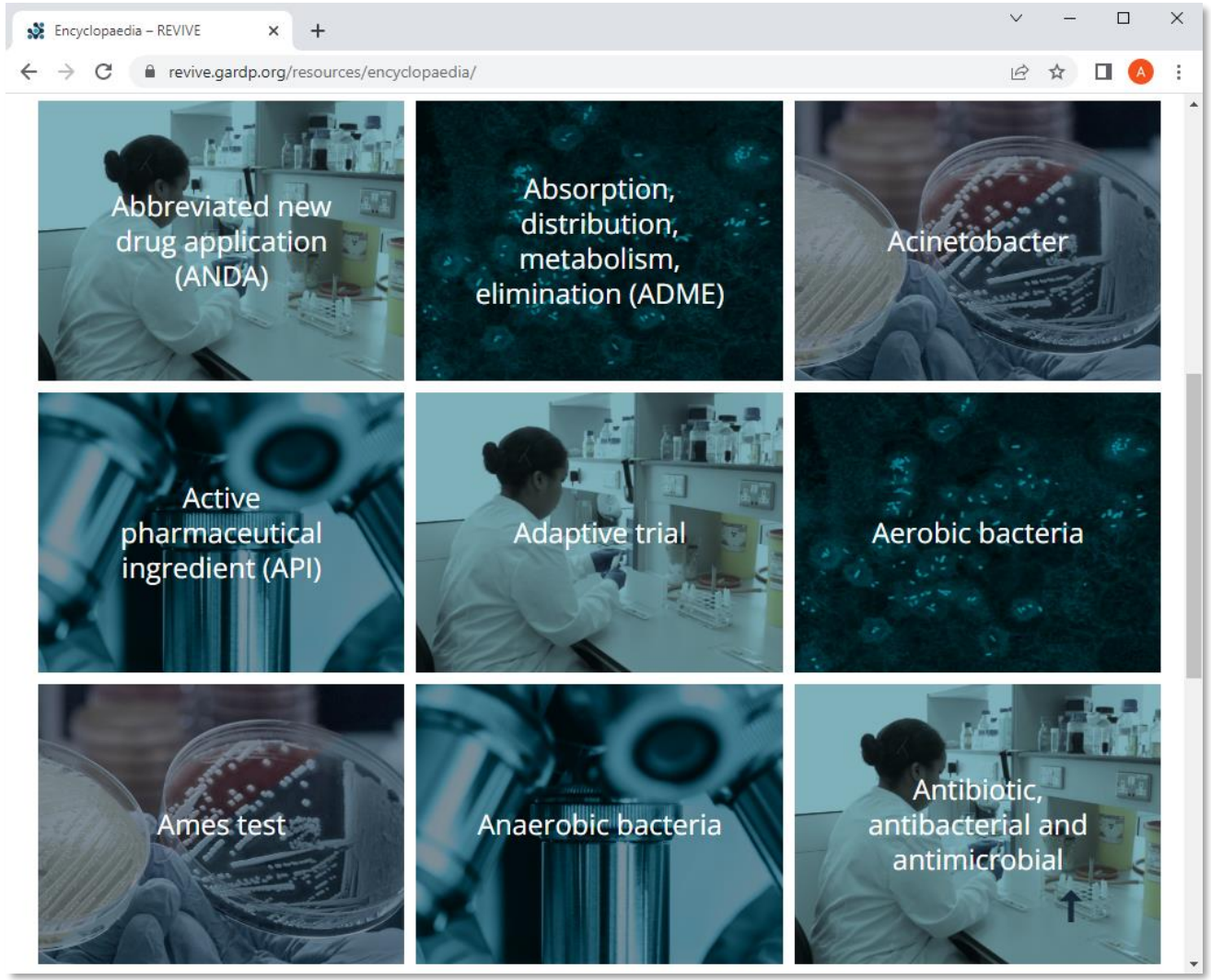
revive.gardp.org/webinars

Antimicrobial Viewpoints



revive.gardp.org/antimicrobial-viewpoints

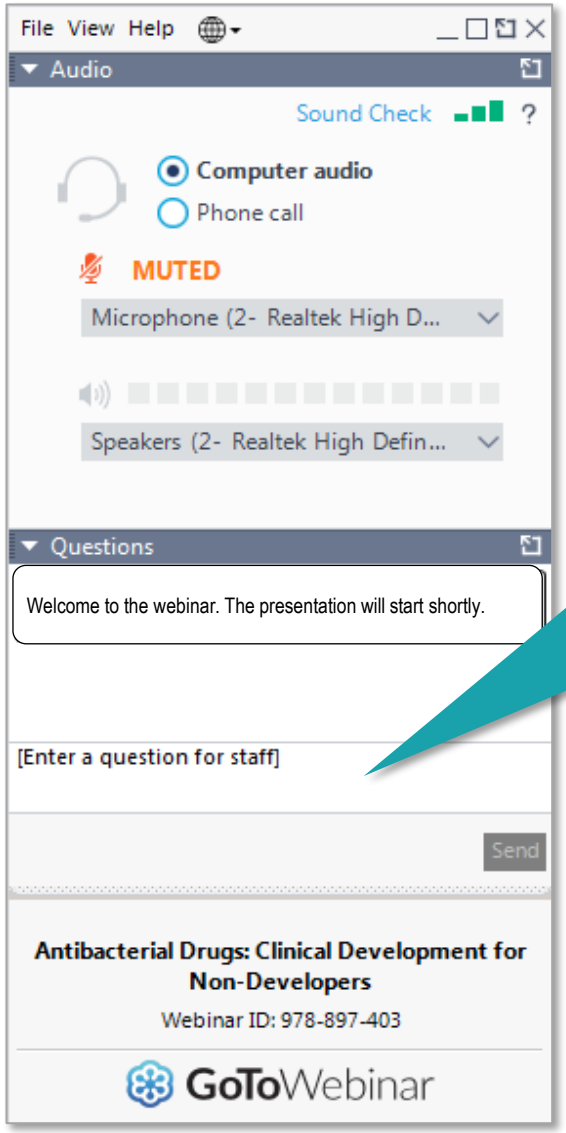
Antimicrobial Encyclopaedia



revive.gardp.org/resources/encyclopaedia

How to submit your questions

If your question is addressed to a specific speaker, please include their name when submitting the question.

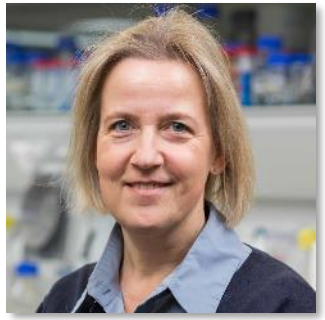


The presentation will be followed by an interactive Q&A session.

Please submit your questions via the ‘questions’ window. We will review all questions and respond to as many as possible after the presentation.

Today’s speakers

Drug discovery strategies: Focusing on synthetic compounds and natural products

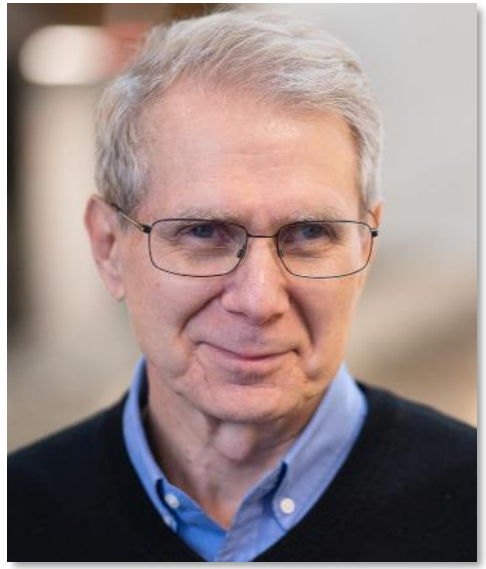


Heike Brötz-Oesterhelt
Chair of Dept. “Microbial Bioactive Compounds”, Interfaculty Institute of Microbiology and Infection Medicine
University of Tuebingen (Germany)

Scott Hecker
Senior Vice President, Chemistry and Chemical Development
Qpex Biopharma (USA)

Moderator:
Kim Lewis
Northeastern University Distinguished Professor
Director, Antimicrobial Discovery Center (USA)

Kim Lewis

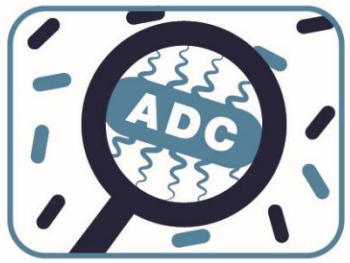


Kim Lewis is a University Distinguished Professor and Director, Antimicrobial Discovery Center at Northeastern University in Boston. He obtained his Ph.D. in Biochemistry from Moscow University in 1980, and has been on the Faculty of MIT, University of Maryland, and Tufts University prior to coming to Northeastern.

Kim has authored over 100 papers and is an inventor on several patents. His notable findings include the development of general methods to grow previously uncultured bacteria that make up >99% of biodiversity on the planet, the discovery of the culprit of recalcitrant biofilm infections, drug-tolerant persister cells; and several novel antibiotics.

Apart from his work in Academia, Kim has served as a consultant to the pharmaceutical industry, biotech, and is a co-founder of NovoBiotic Pharmaceuticals, Arietis Pharma, Holobiome, Flightpath and Odyssey Therapeutics.

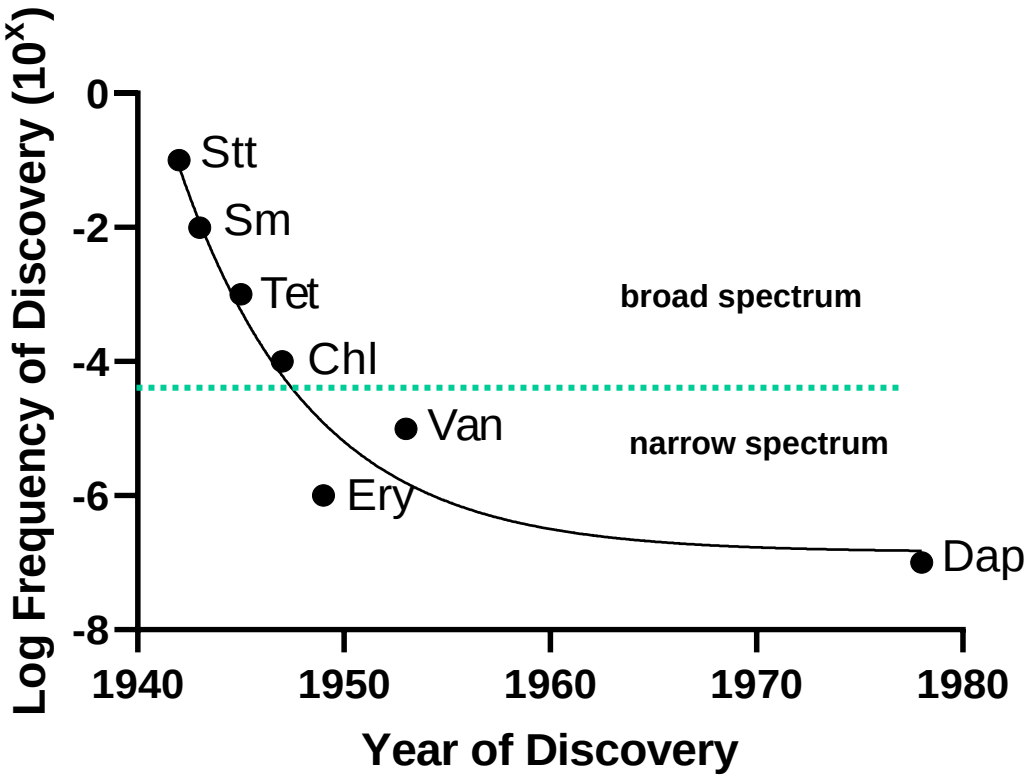
Drug Discovery Strategies: Focusing on Synthetic Compounds and Natural Products



Northeastern University

Antimicrobial Discovery Center

The Probability of Discovery



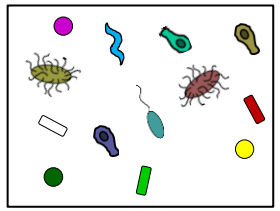
Richard Baltz, Microbe 2007

K. Lewis, Cell 2020

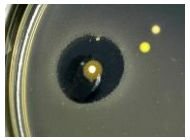
Pipeline for Drug Discovery

Soil Sample

Environmental bacteria



Screening



Petri dish

Chemical Dereplication

-purification
-structure elucidation

Novel?

In vitro/In vivo Validation

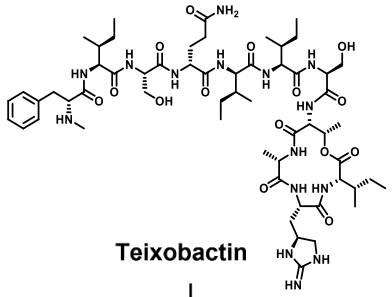
Mechanism?

IND

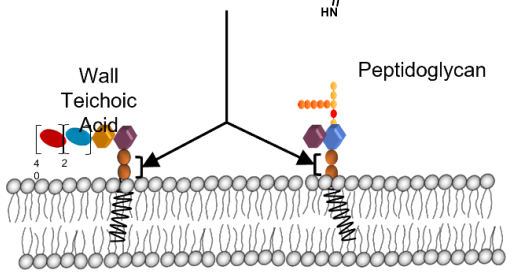
Clinical Studies

Prospecting Outside of Actinomycetes

Eleftheria terrae



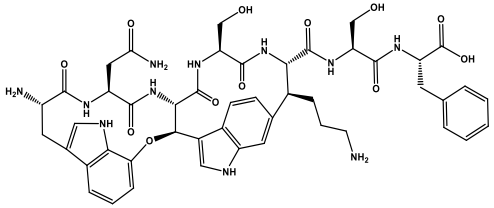
Teixobactin

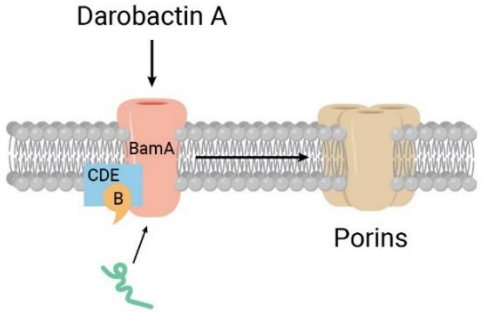


Ling et al., Nature 2015

Shukla et al., Nature 2022

Photorhabdus khanii

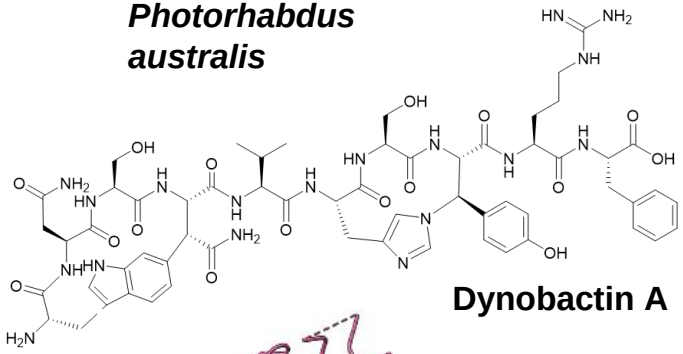




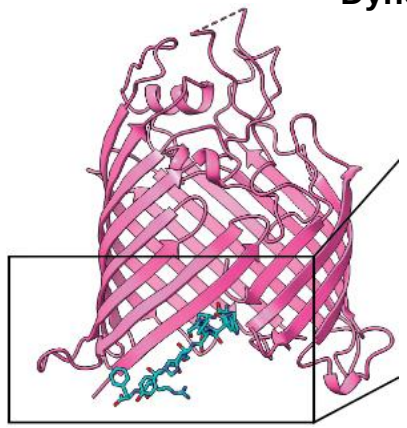
Imai et al., Nature 2019

Kaur et al., Nature 2021

Photorhabdus australis



Dynobactin A

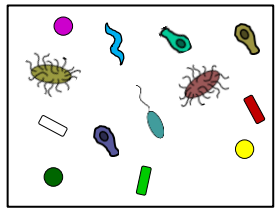


Miller et al., Nature Microbiol. 2022

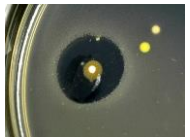
Pipeline for Drug Discovery

Soil Sample

Environmental bacteria



Screening



Petri dish

Chemical Dereplication

-purification
-structure elucidation

Novel?

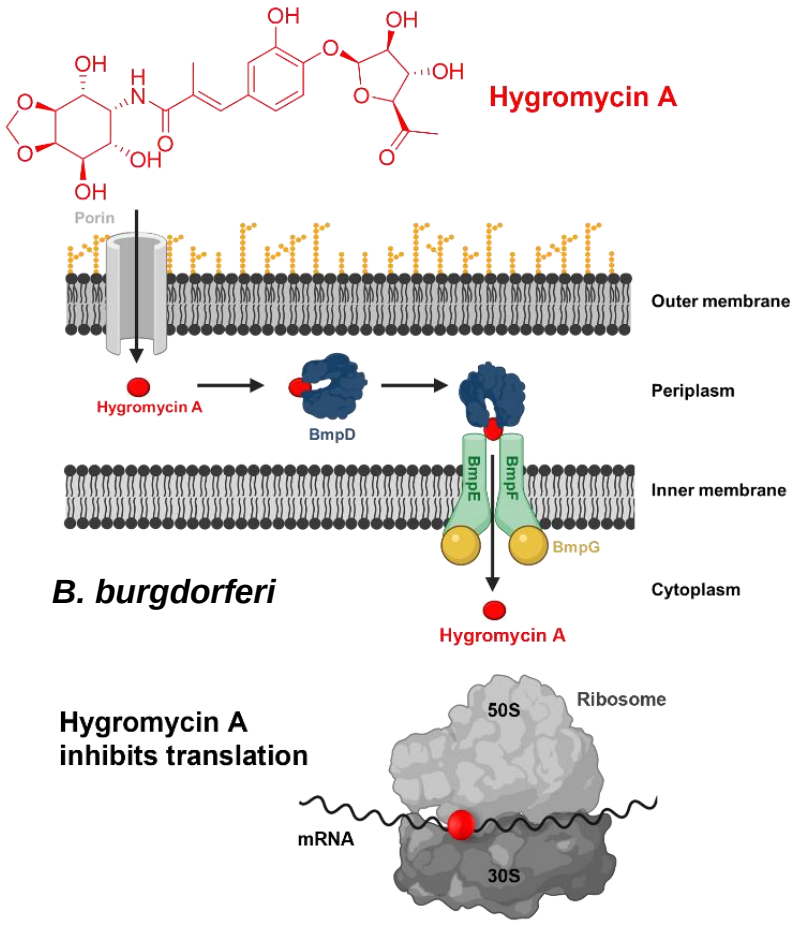
In vitro/In vivo Validation

Mechanism?

IND

Clinical Studies

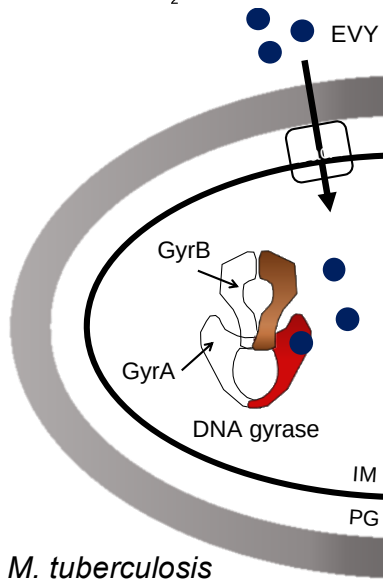
Differential Screening



Leimer et al., CELL 2021

Photorhabdus noenieputensis

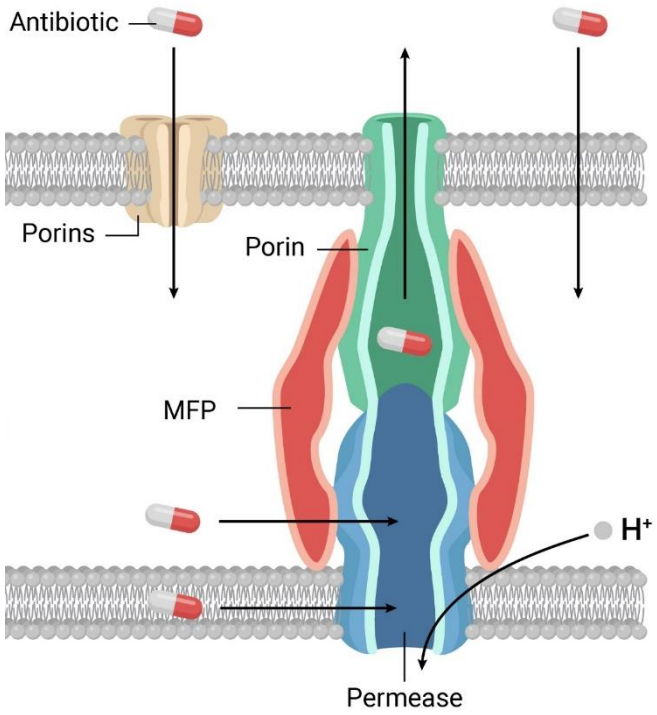
Evybactin



M. tuberculosis

Imai et al., Nat Chem Biol., 2022

Synthetic Compounds, The Penetration Bottleneck



Payne et al., Nature Rev. Drug Disc., 2007

Tommasi et al., Nat. Rev. Drug Discovery 2015

Med Chem Challenge: SAR Target + Permeation

Emerging rules of permeation:

Cutoff ≤ 600 Da
(O'Shea, Moser J. Med Chem 2008)
LogP ~0.1

Low number of rotatable bonds

Flat **(Richter…Hergenrother Nature 2017)**
(Parker… Hergenrother Nature Microbiol. 2020)
-NH3+

Helen Zgurskaya and colleagues:
Hengartner et al., 2020 *J Chem Inf Model* 60: 2838-2847.
Zhao et al., 2020 *Nat Chem Biol* 16: 1293-1302.
Mehla et al., 2021 *mBio* 12.

SAR Target + Porin:

ETX0462 Durand-Reville… Tommasi Nature 2022

Heike Brötz-Oesterhelt

Heike Brötz-Oesterhelt is a microbiologist with extensive experience in antibacterial drug discovery in the academic and pharmaceutical industry settings. After her PhD, she joined the Anti-Infectives Department of Bayer HealthCare and later co-founded the anti-infective biotech company AiCuris. In 2010, she returned to academia as a professor for Pharmaceutical Biology at the University of Duesseldorf, and since 2014 she is professor for Microbiology at the University of Tübingen heading the Department of Microbial Bioactive Compounds. Her scientific focus is unraveling the molecular mechanisms of new antibiotic lead structures and operation modes of novel antibiotic targets.

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Natural Products in Antibacterial Drug Discovery

Heike Brötz-Oesterhelt

Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen
Chair: Dept. Microbial Bioactive Compounds

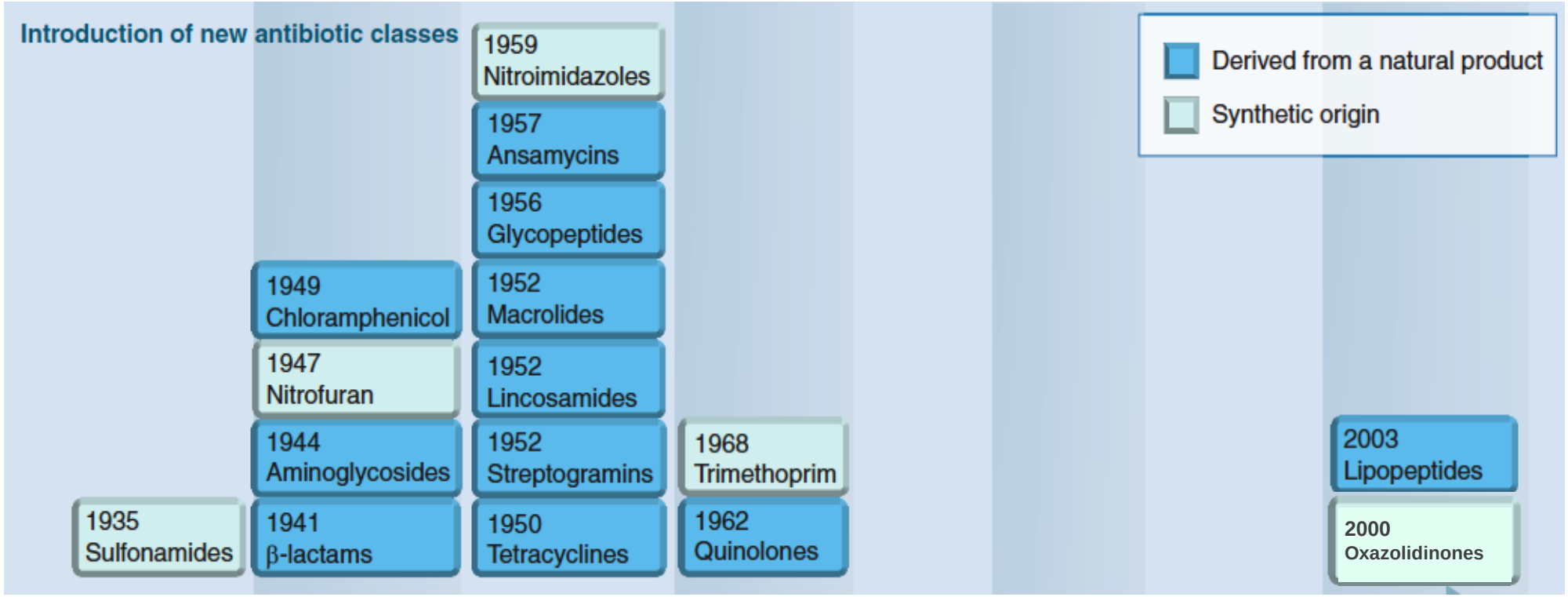
antibiotic cellMAP
CELLULAR MECHANISMS OF ANTIBIOTIC ACTION AND PRODUCTION

cmfi Cluster of Excellence
Controlling Microbes to Fight Infections

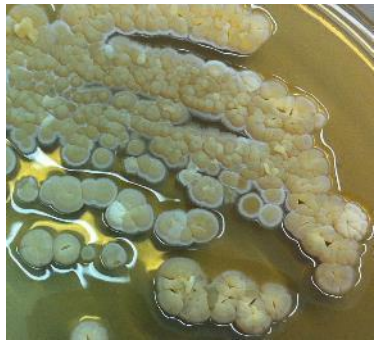
DZIF

REVIVE webinar, Oct. 11th, 2022

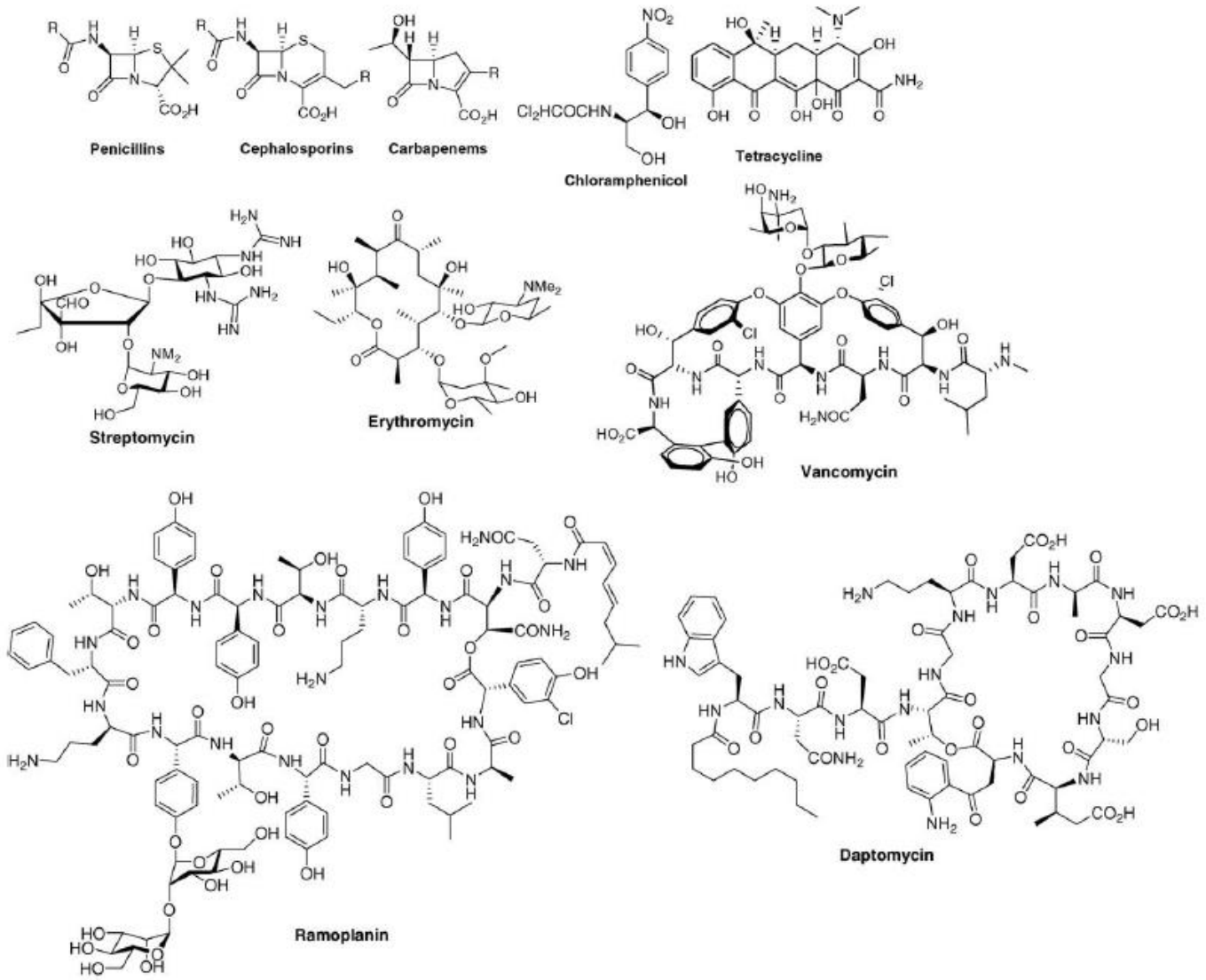
Learn from nature



Future Medicine, 5, 1553 ff. (2010)



Antibiotics differ from standard synthetic drugs



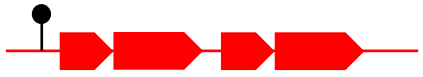
Biochem. Pharmacol. 2006, *71*, 1006

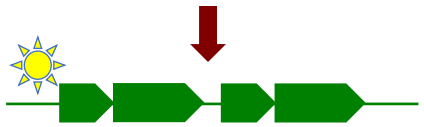
Dept. of Microbial Bioactive Cpds. (IMIT)

Natural product bioengineering

Evi Stegmann

Antibiotic gene cluster analysis
Producer strain engineering
Gene cluster activation
Heterologous expression

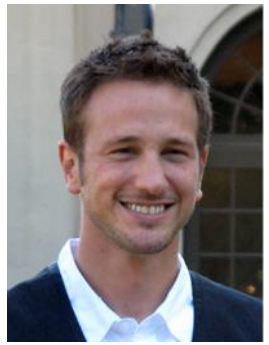




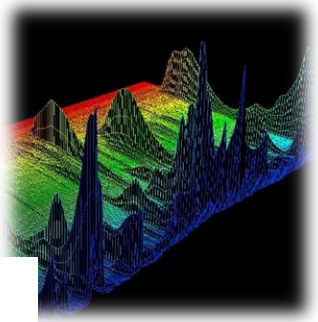
Active biosynthetic gene cluster

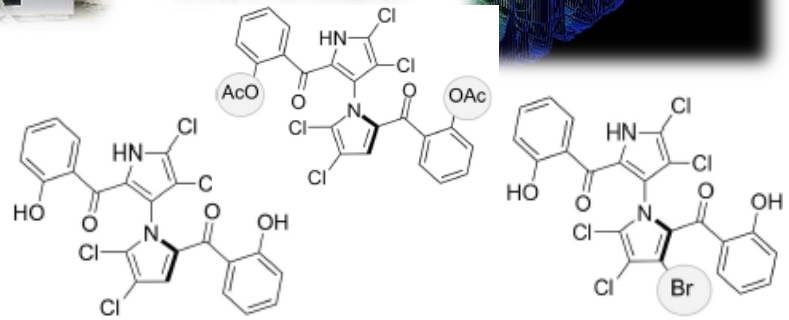
Natural product chemistry

Compound production
Isolation (targeted, untargeted)
Structure elucidation
Synthesis (derivatives, probes)



Chambers Hughes



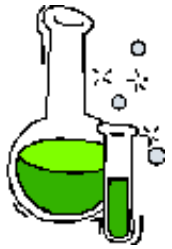


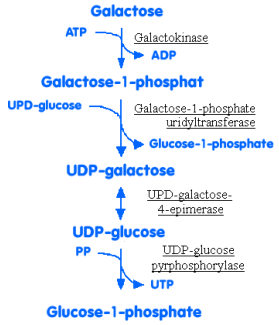
Dept. of Microbial Bioactive Cpds. (IMIT)

Mode of action – discovery platform

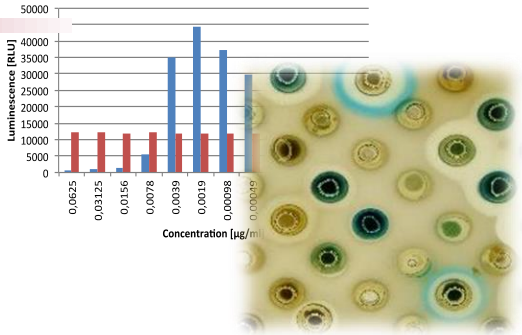
Assays for
cell envelope integrity,
membrane function,
protease function,
metabolic pathways

Incorporation of labeled metabolites

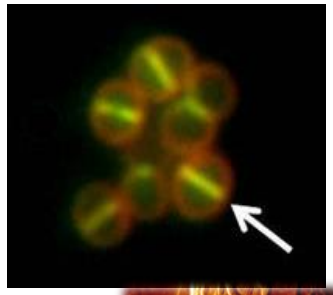




Reporter constructs



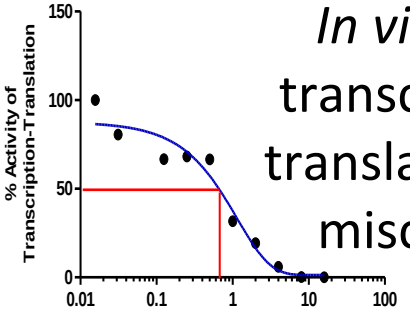
Microscopy: super resolution, time-lapse, microfluidics / AFM



Mode of action

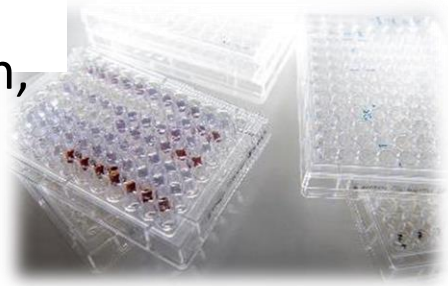
Reference compound collection:
antibiotics, antibacterial agents,
natural products

Strain collection for profiling:
clinical isolates (full antibiograms)
uptake, efflux mutants etc.

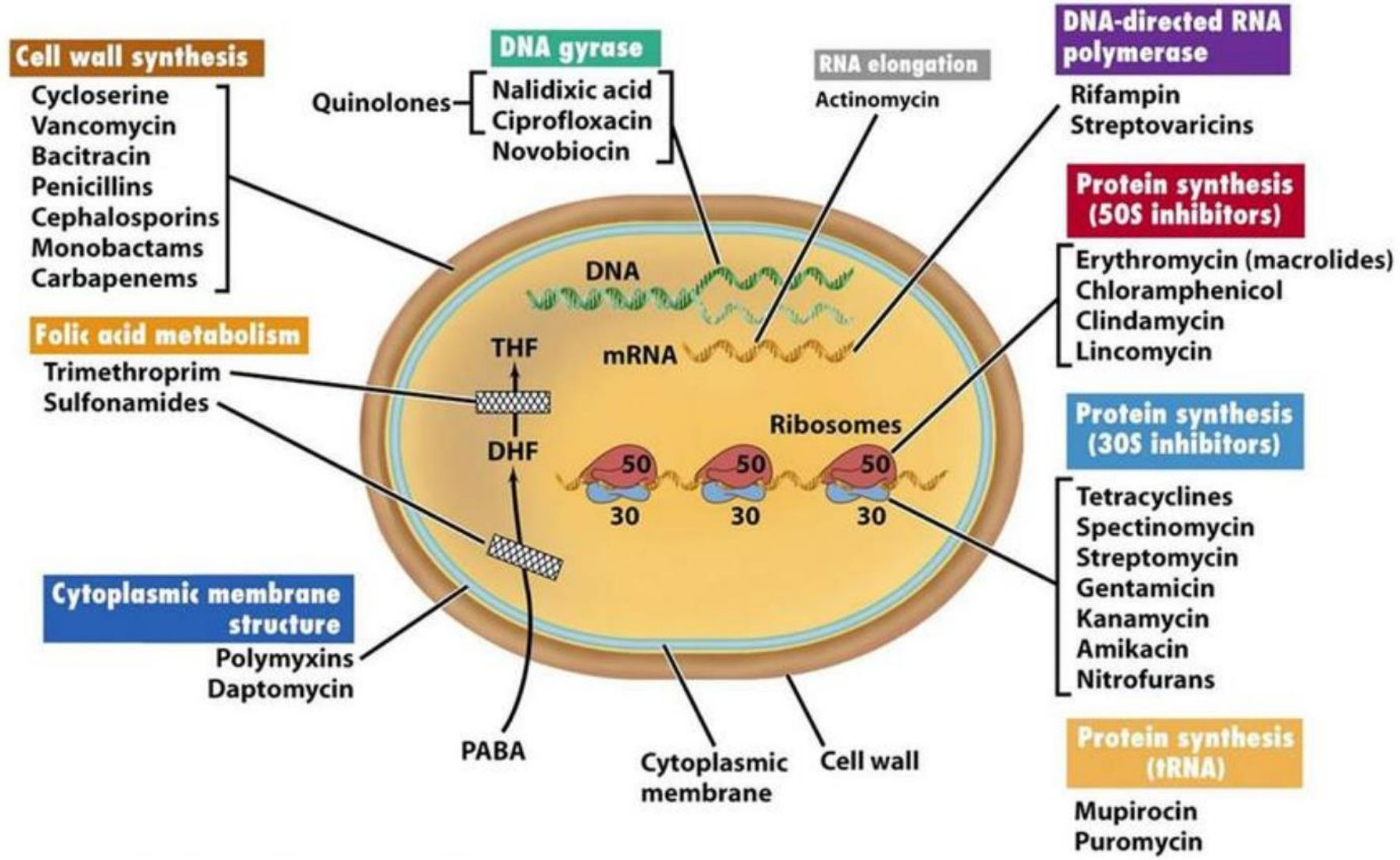


In vitro
transcription,
translation,
miscoding

Whole genome knock-out, knock-down libraries



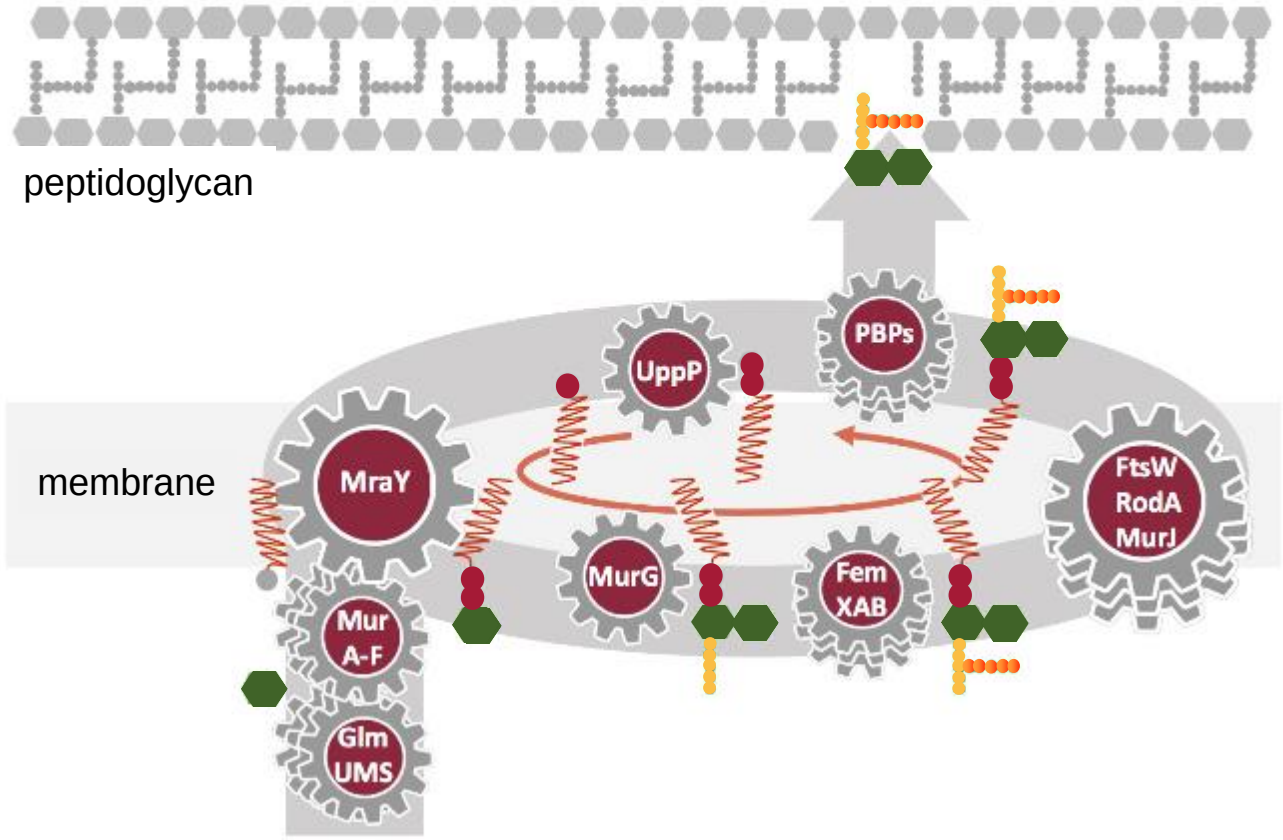
What can we learn from nature regarding targets?



Antibiotic target sites (Madigan and Martinko, 2006).

Complex machineries make good targets

Multi-enzyme machineries require functional coordination in space and time

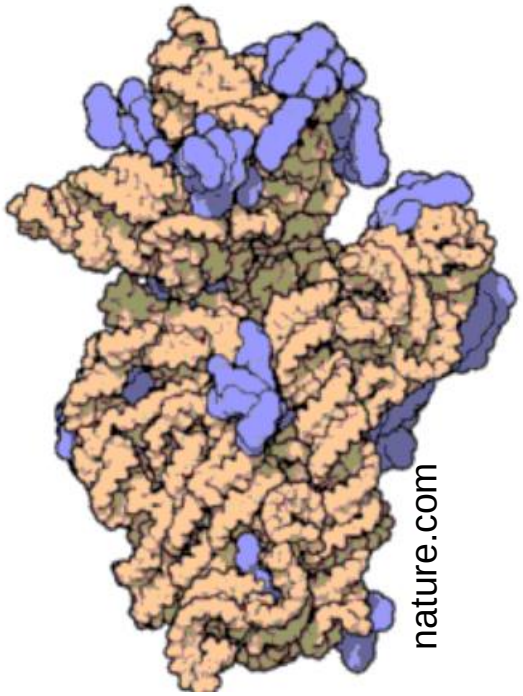


Many successful antibiotics target large machineries

Powerful MoAs can also work by disturbing
- spacial order
- protein or lipid microenvironments
- protein – protein interaction

Multiple target gene copies and complex mechanisms are advantageous

Binding to ribosomal RNA



30S ribosomal subunit
A-site inhibitors

tetracyclines	**aminoglycosides**
translation arrest	mistranslation
bacteriostatic	bactericidal

mass of defective proteins

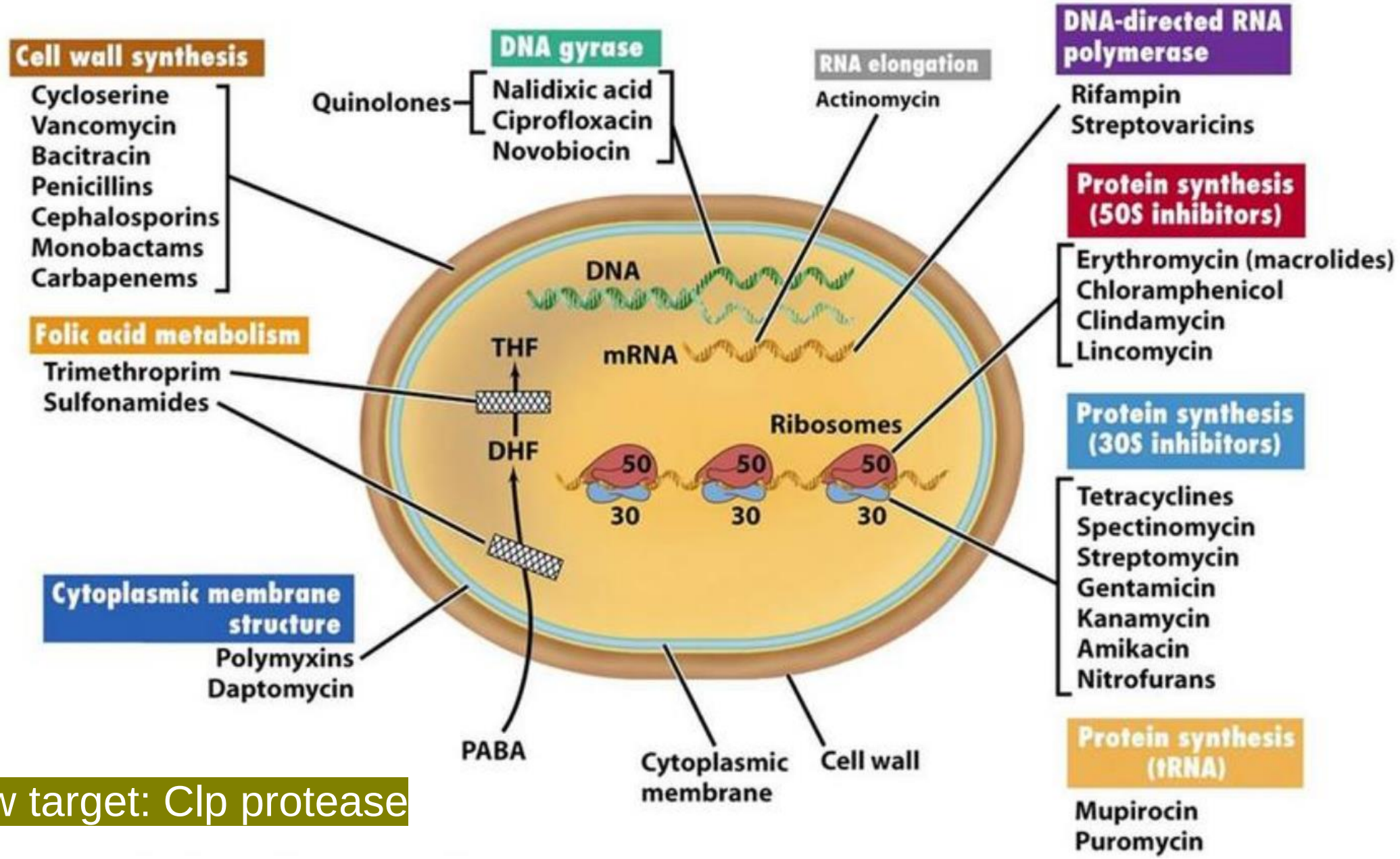
Pleiotropic destructive events following target interaction are powerful

Lessons from therapeutically validated targets of natural products

- Targets of successful antibiotics used for a long time as monotherapeutics are **not encoded by single genes**
  - e.g. b-lactams, quinolones, glycopeptides, ribosomal inhibitors
- Single gene targets in therapeutic applications need to be protected by **combination therapy**
  - e.g. sulfonamides, trimethoprim, fusidic acid, novobiocin, rifampicin
- Small molecules can target **several orthologs and/or paralogs** simultaneously
  - e.g. fosfomycin, D-cycloserine, b-lactams
- **Polypharmcology** reduces resistance development
  - e.g. additional membrane interactions, target families
- **Complex mechanisms** are successful in achieving bactericidal activity
  - e.g. quinolones vs. novobiocin, aminoglycosides vs. other ribosomal inhibitors

Natural products can show us

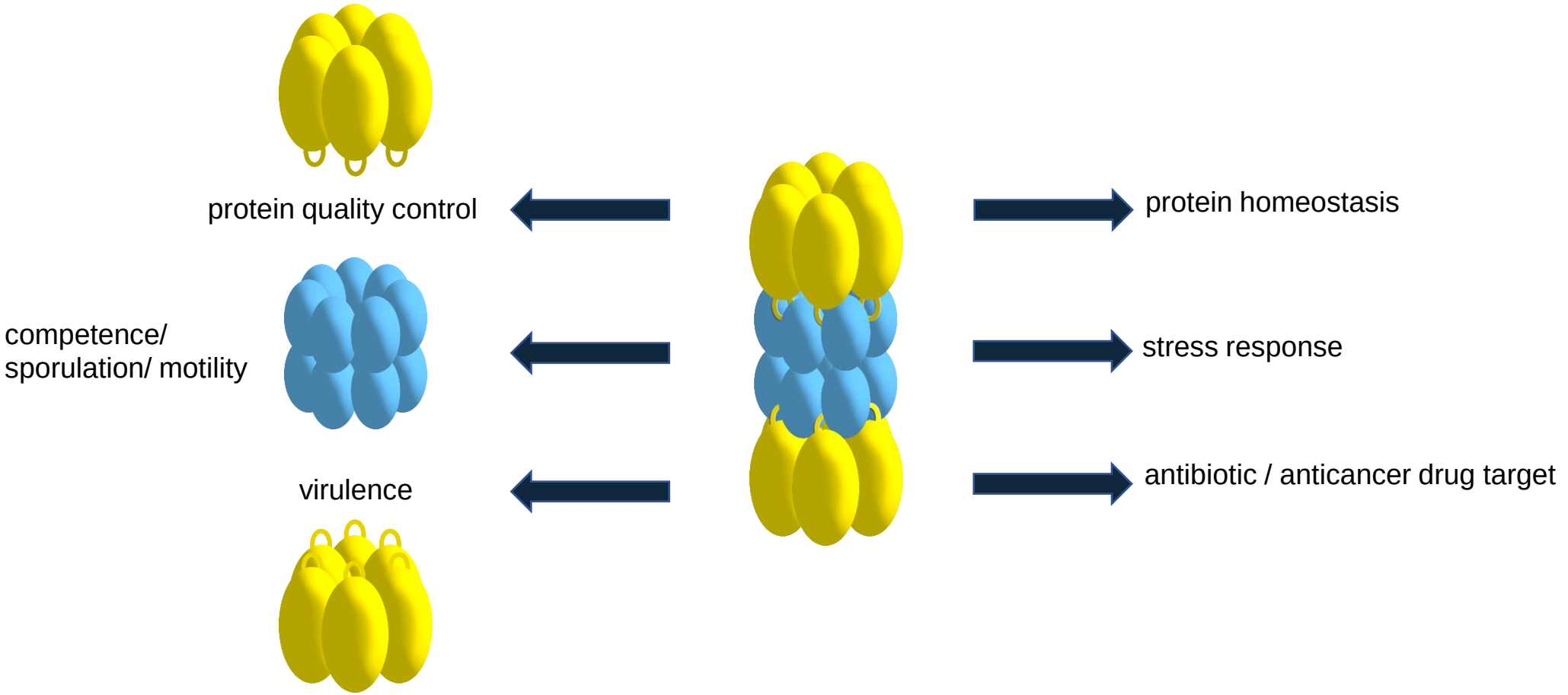
which mechanisms work for successful antibiotic drugs



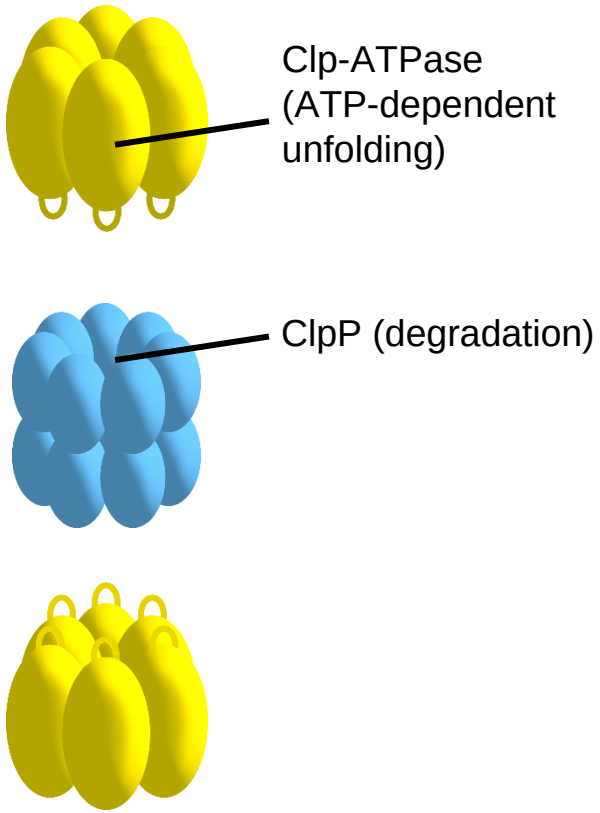
New target: Clp protease

Antibiotic target sites (Madigan and Martinko, 2006).

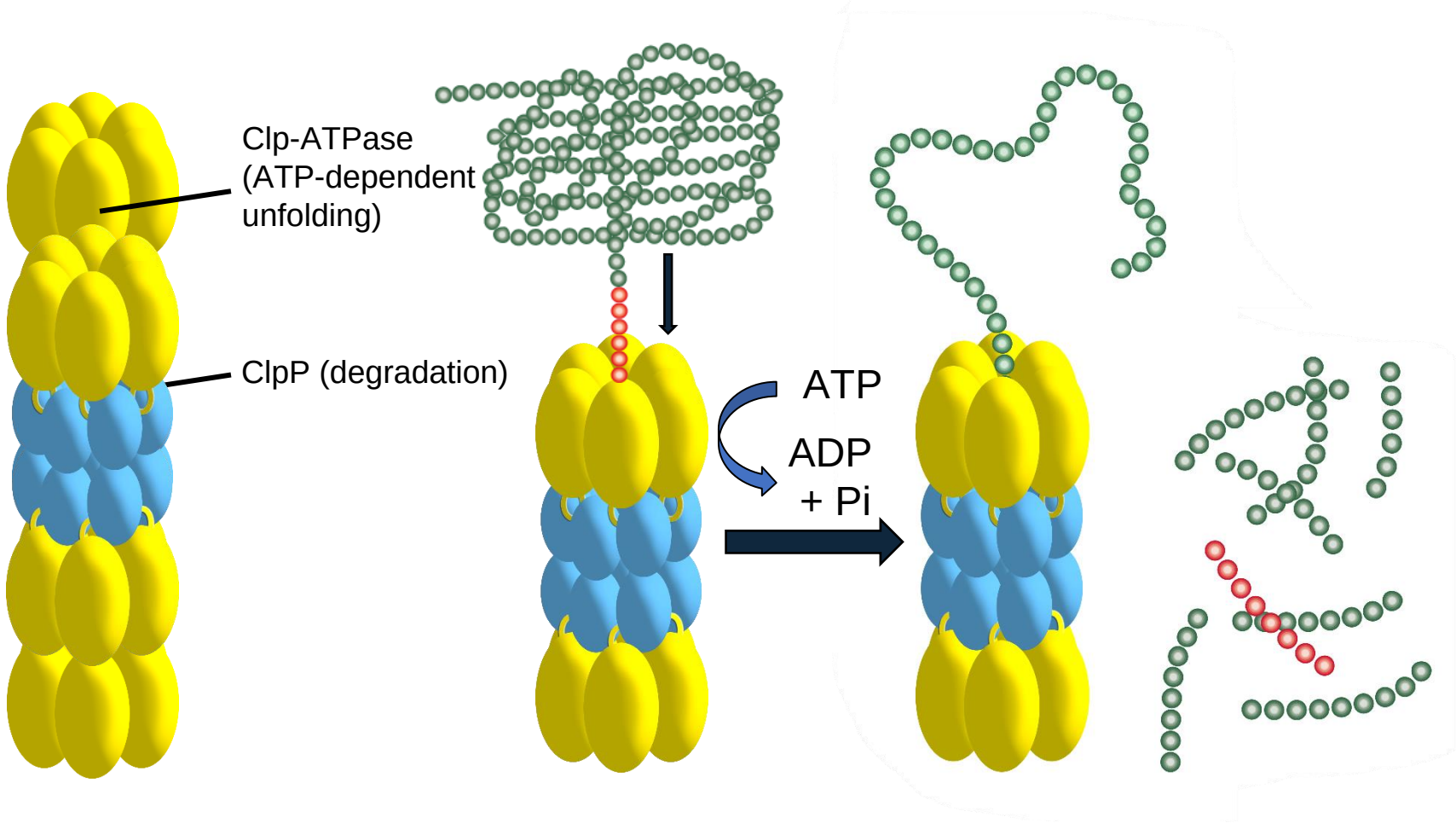
Clp protease – natural functions & mechanics



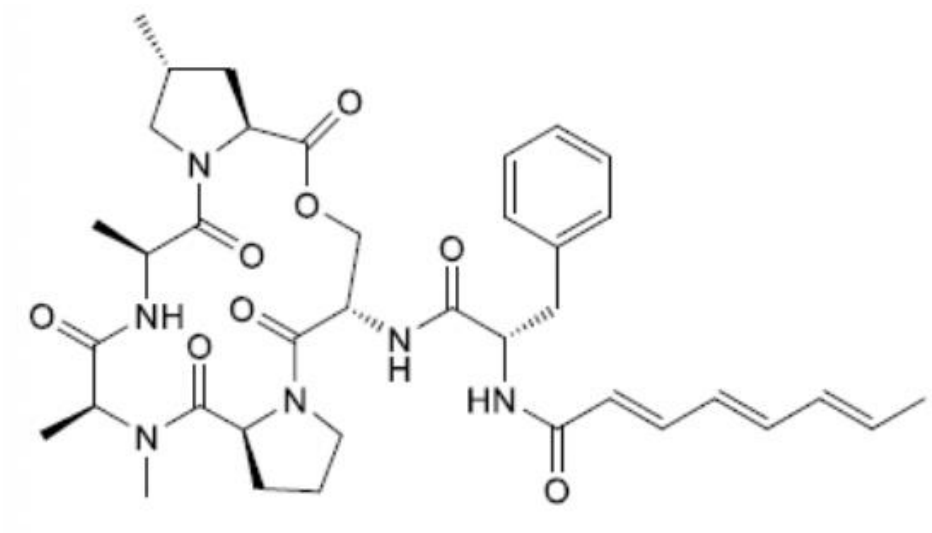
Clp protease – natural functions & mechanics



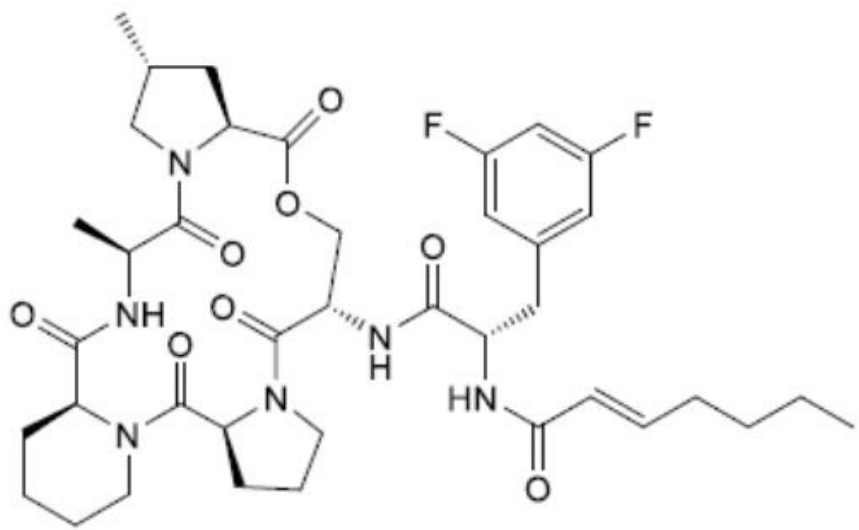
Clp protease – natural functions & mechanics



ADEP antibiotics activate and deregulate ClpP



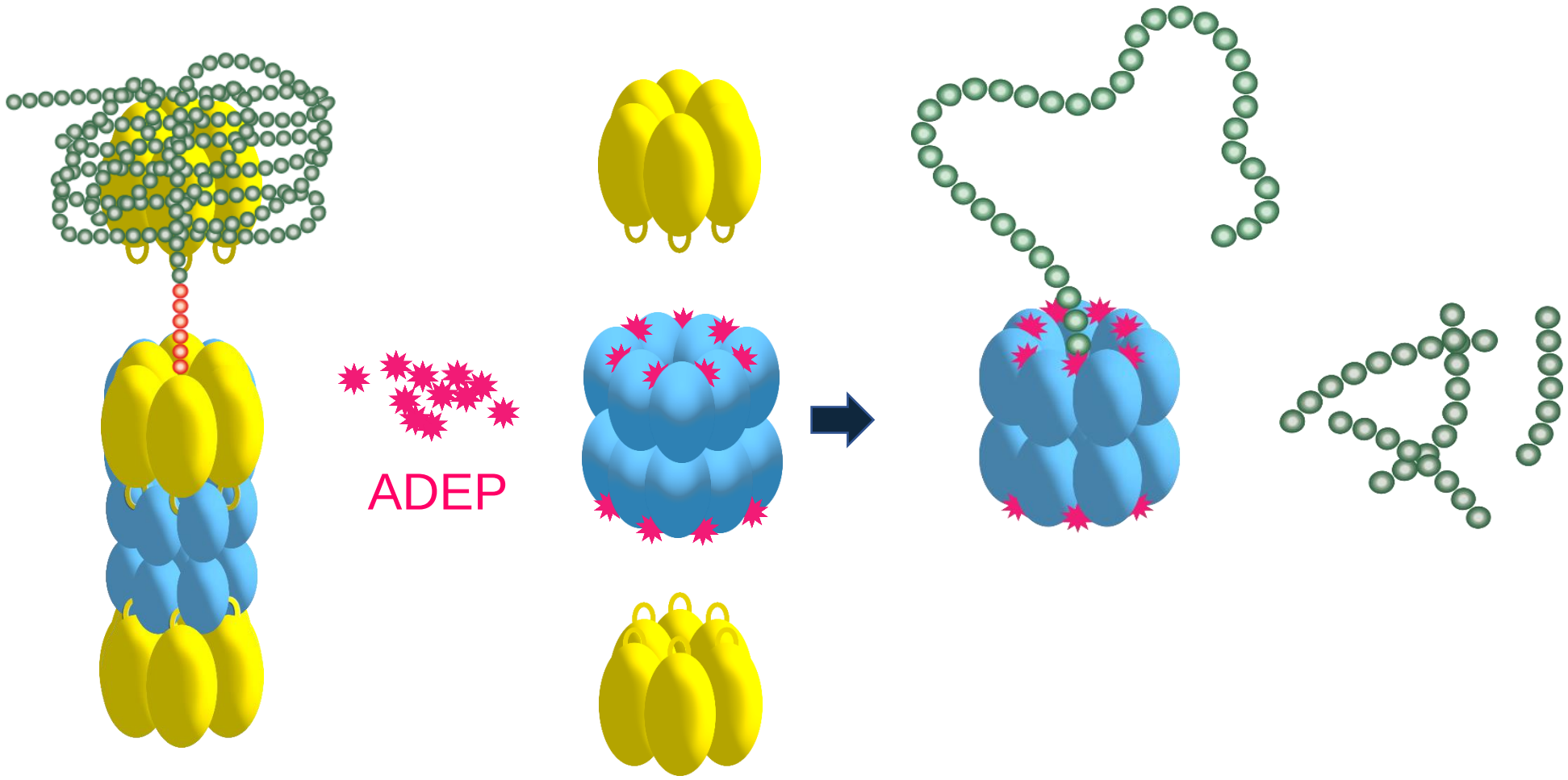
ADEP1



ADEP4

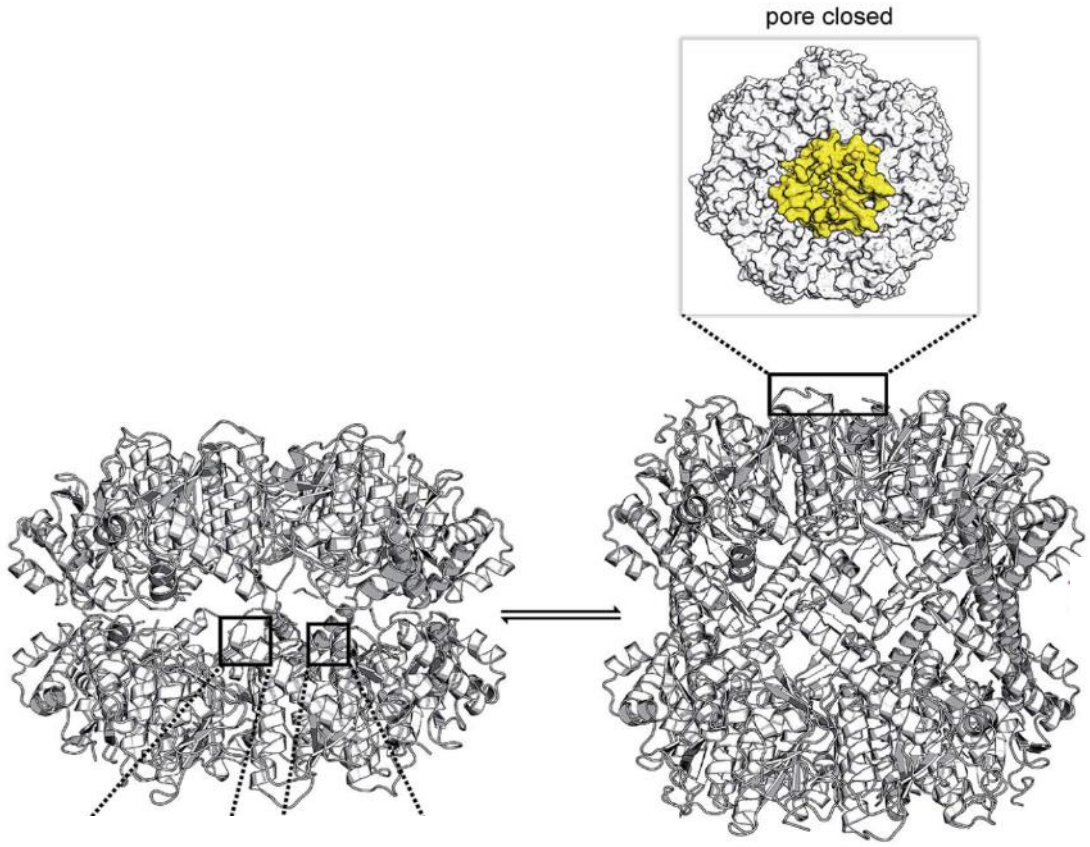
MIC *S. aureus*	6.3 µg/ml	0.05 µg/ml

ADEP antibiotics activate and deregulate ClpP



Brötz-Oesterhelt *et al.*, Nature Med. (2005), Kirstein *et al.* EMBO Mol. Med. (2009)

ADEP antibiotics activate and deregulate ClpP



<u>ClpP activation on several levels</u>

Intra-ring connection

Inter-ring connection

Extended conformation

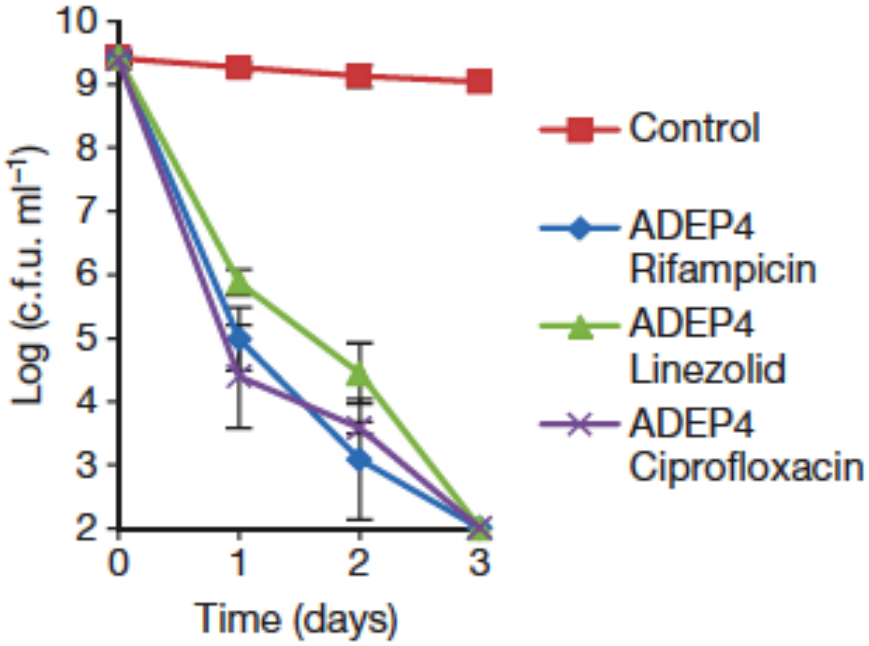
Gate opening

Allosteric activation of catalysis

Lee *et al.* Nat. Struct. Mol. Biol. (2010)
Gersch *et al.*, Nat.Commun., (2015)
Malik *et al.*, Chem.Bio.Chem., (2020)
Kim *et al.*, EMBO J., (2022)

ADEP's potential as a drug lead

- Strong **antibacterial activity against Gram-positive bacteria**
- Activity **against multi-resistant pathogens**
- Exceptional **activity against persister cells** *in vitro* and *in vivo*
- **Combination therapy** recommended



Growth of *S. aureus* in biofims, treatment by various antibiotics

Conlon *et al.*, Nature (2013)
Griffith et al., ACS InfDis (2019)

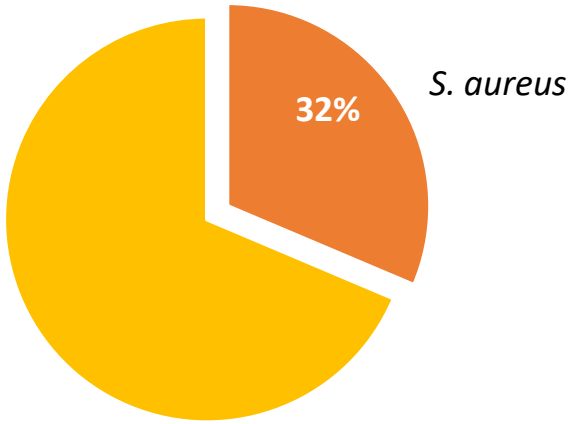
Natural products can lead us to

- new targets,
- new binding sites at established targets,
- powerful polypharmacology

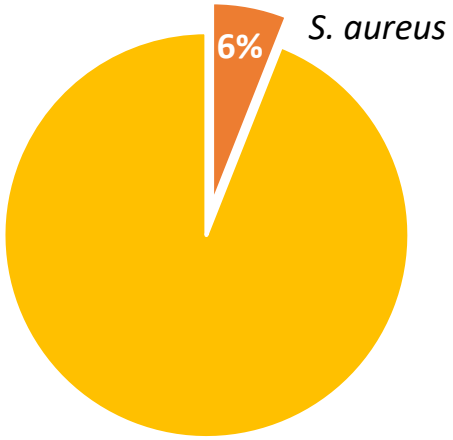
Antibiotics are ideal TOOLS for studying antibacterial targets and bacterial cell biology

Microbiom-derived lugdunin is the first member of a new class

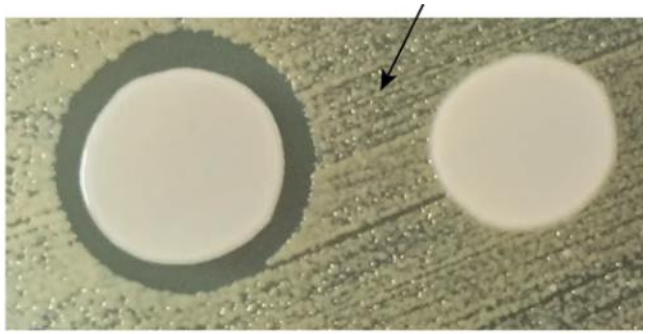
187 hospitalized patients



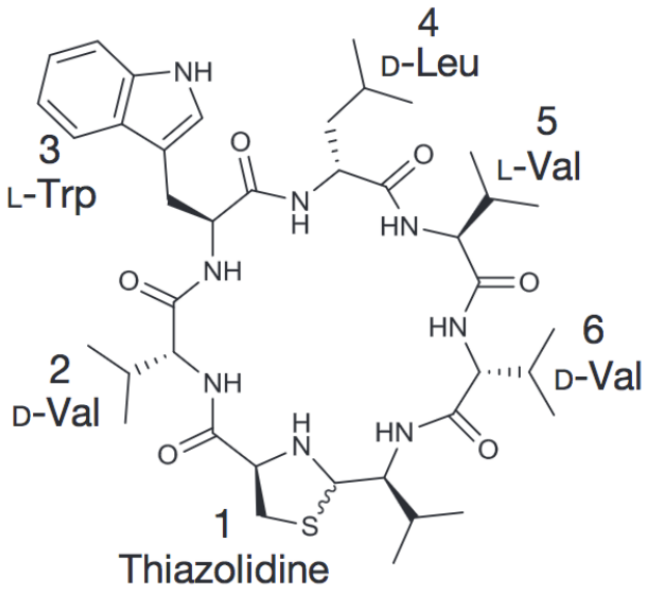
S. lugdunensis carriers



S. aureus USA 300



S. lugdunensis wt *ΔlugD*



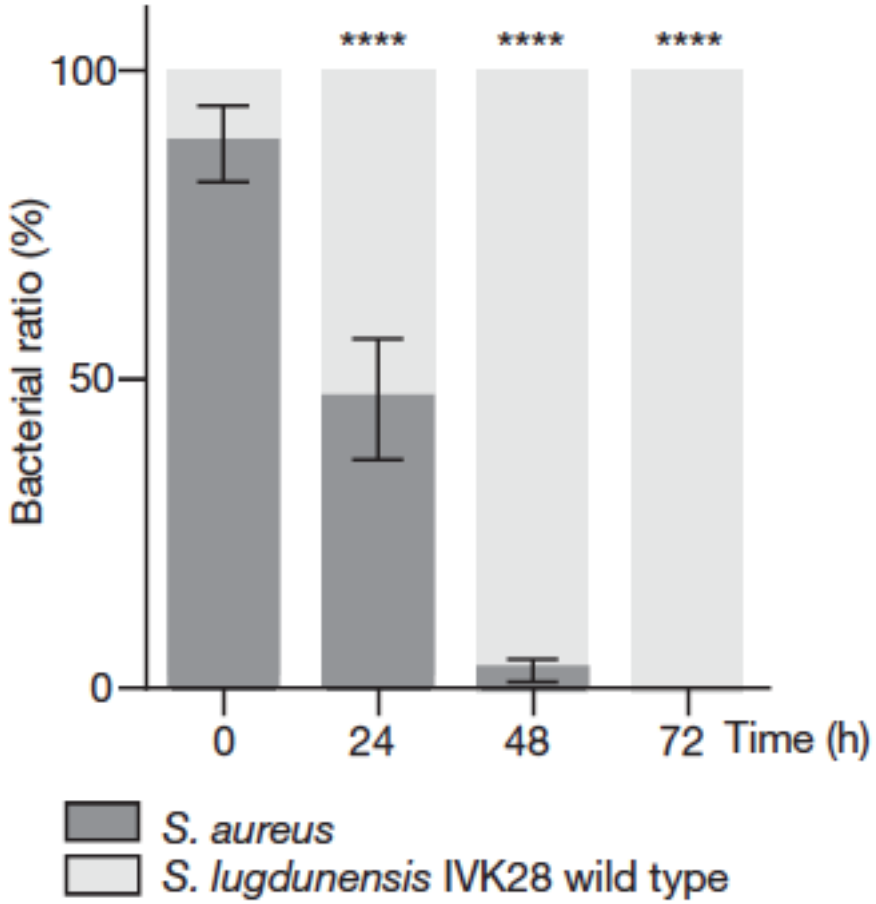
New class of „fibupeptides“

First NRPS-produced bacteriocin isolated from the human nose

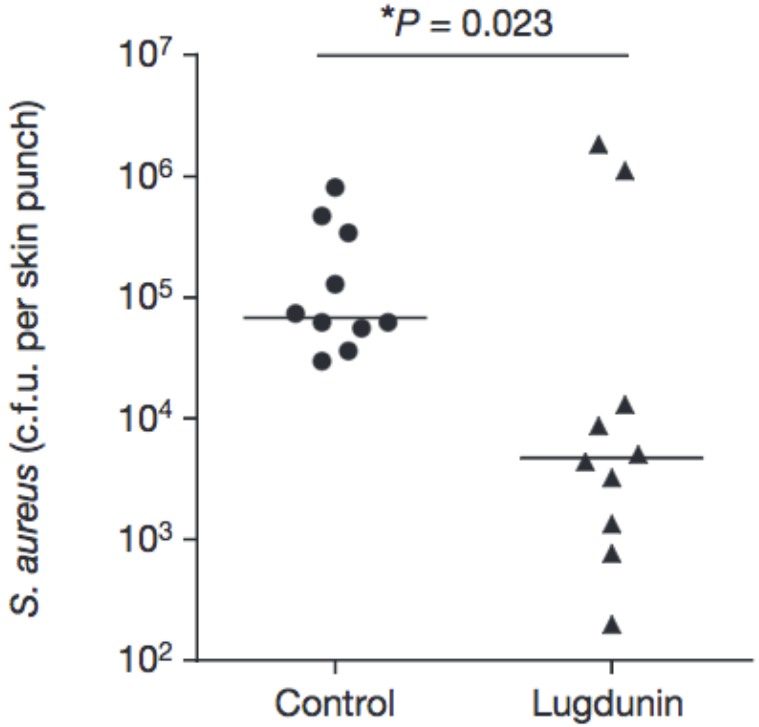
Peschel, Grond groups

Zipperer *et al.*, Nature (2016)

S. lugdunensis outcompetes *S. aureus* and shapes the microbiome



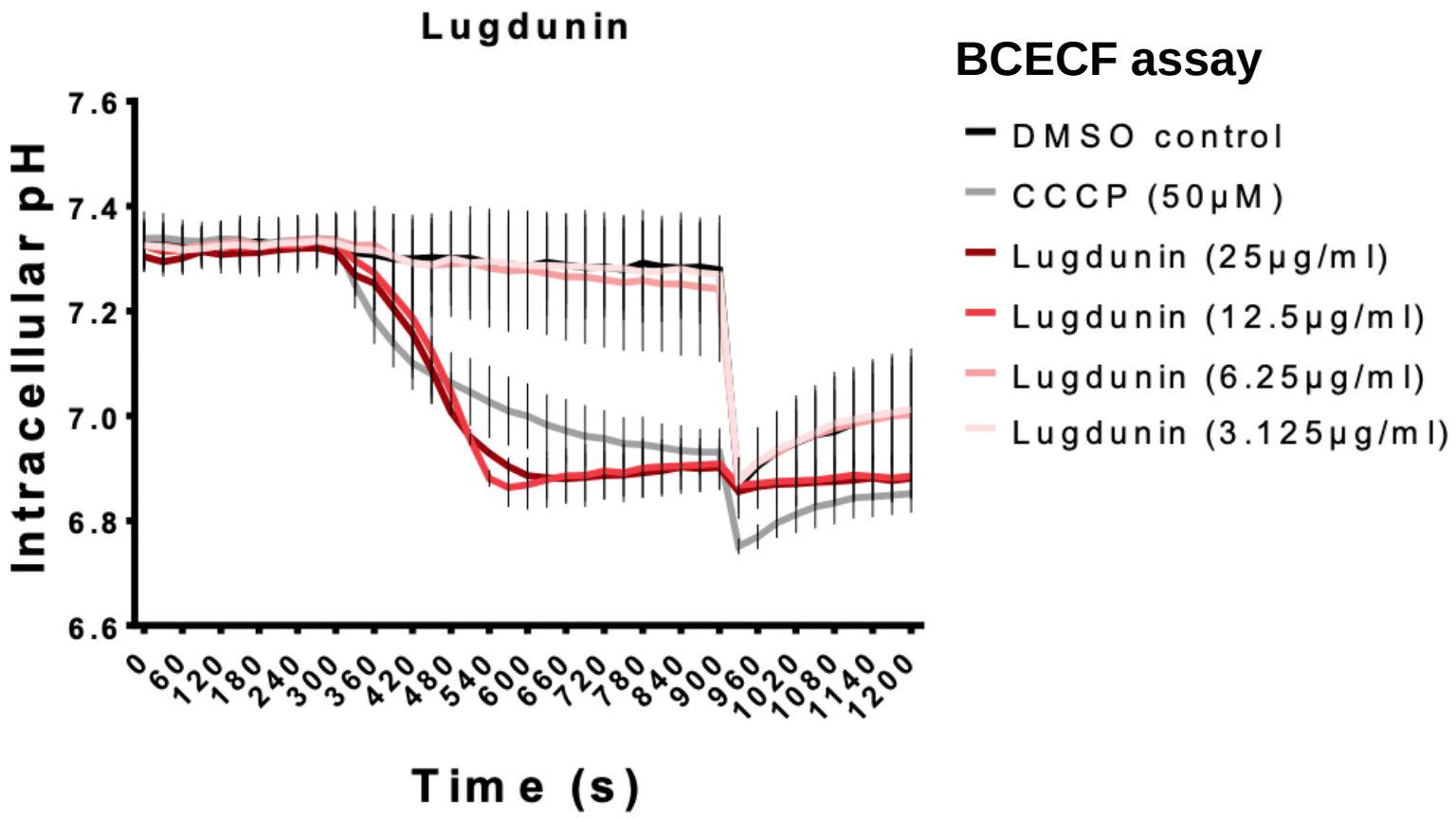
S. aureus
mouse deep skin infection



Peschel group

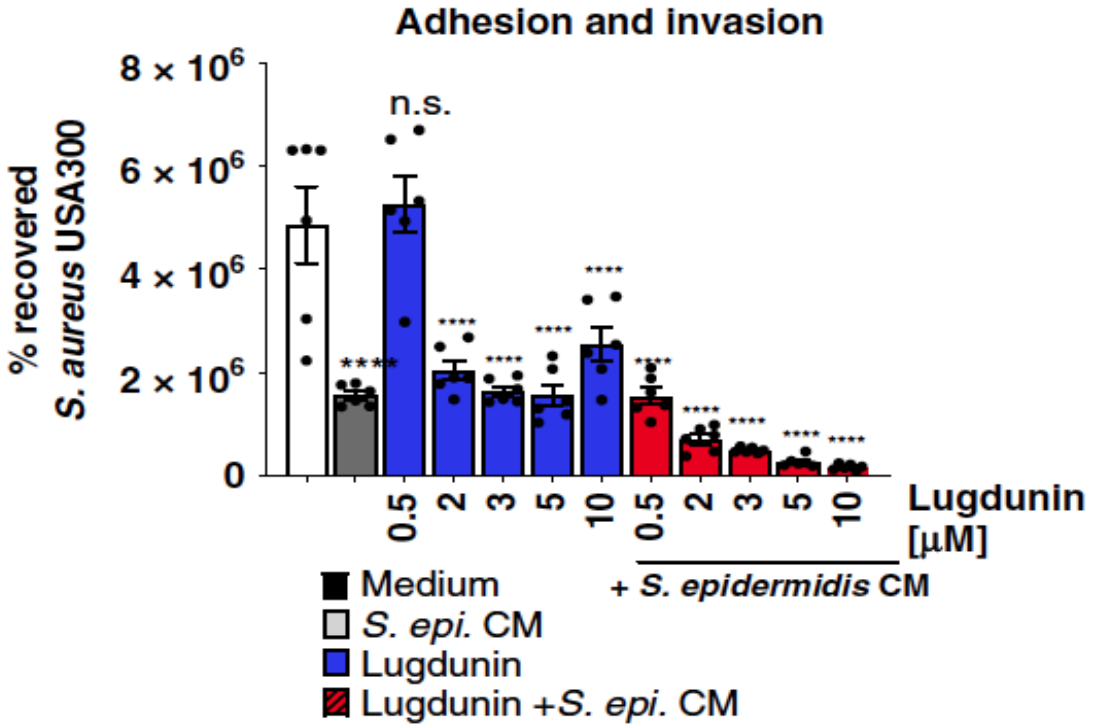
Zipperer *et al.*, Nature (2016)

Lugdunin triggers proton influx into *S. aureus* cells



Diverse beneficial effects of lugdunin on skin

- Lugdunin, in **synergy with further commensal factors**, **reduces *S. aureus* infection** of primary human keratinocytes
- Lugdunin triggers production and release of **host defense peptides**
- Lugdunin triggers **recruitment of monocytes and neutrophils** in skin



Schittek group

Bitschar *et al.*, Nat. Commun. (2019)

Natural products can show us

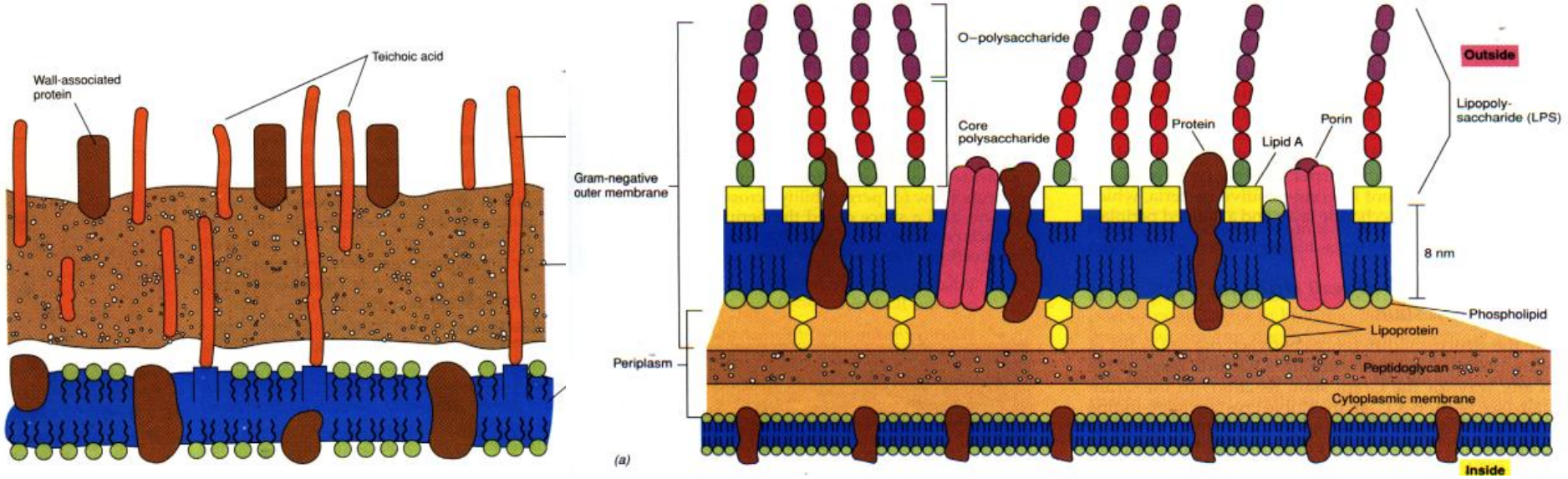
- which modes of action and polypharmacology

work at the natural site of pathogen colonisation

Bacterial cell envelope – effective permeation barrier

Gram+

Gram-

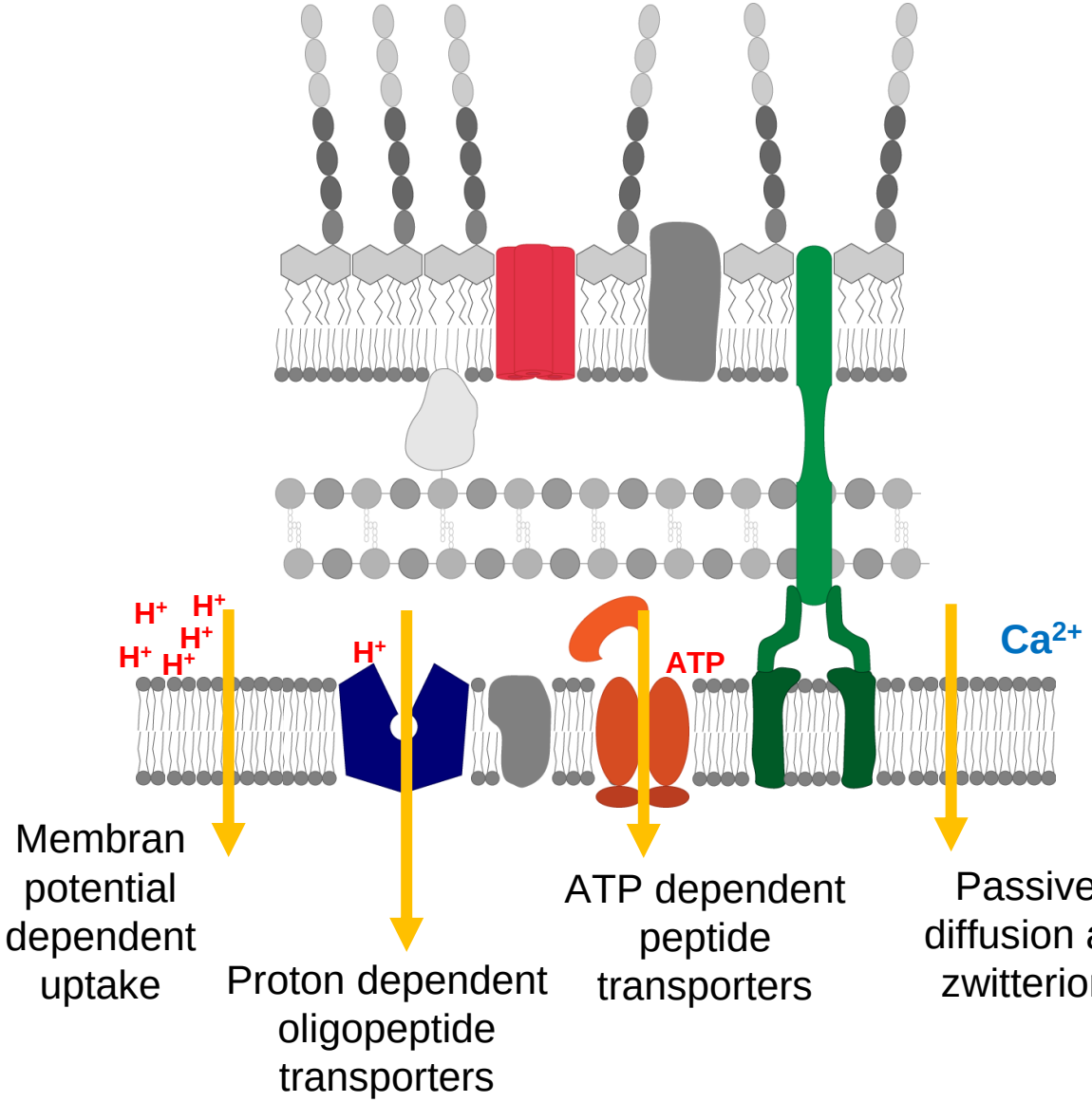


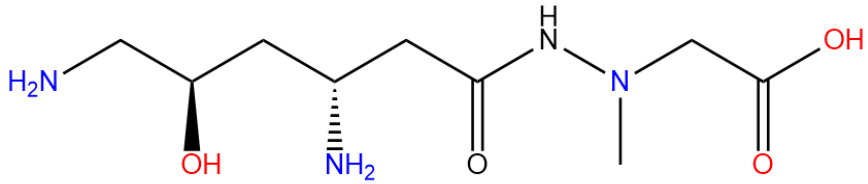
- Outer membrane: Porins prefer hydrophilic, (positively) charged molecules < 600 Da. The lipid regions are largely impenetrable.

- Cytoplasmic membrane: only uncharged, lipophilic molecules can penetrate passively

Passage into the Gram- cytoplasm requires special characteristics

Multiple uptake routes slow down resistance development





Negamycin
natural product,
pseudopeptide

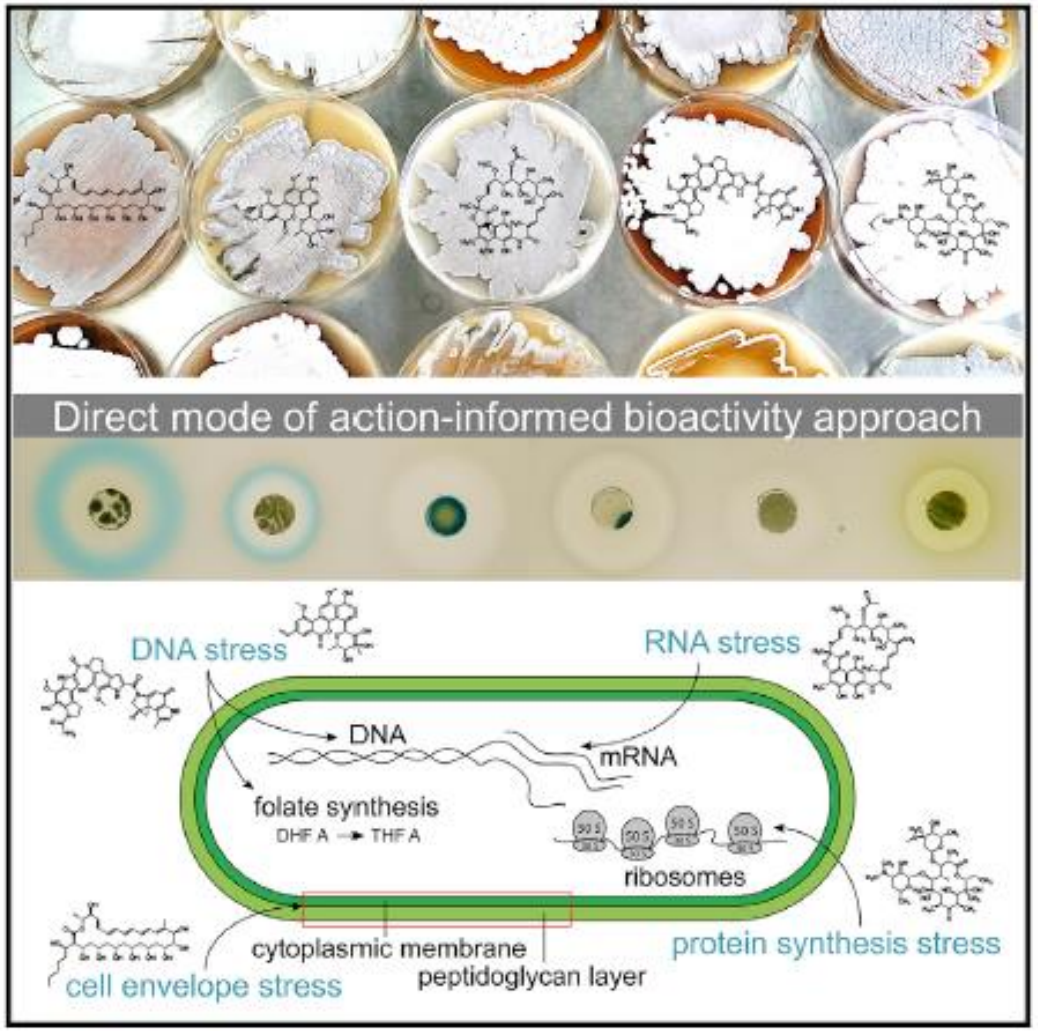
Phospholipid interaction facilitated by calcium

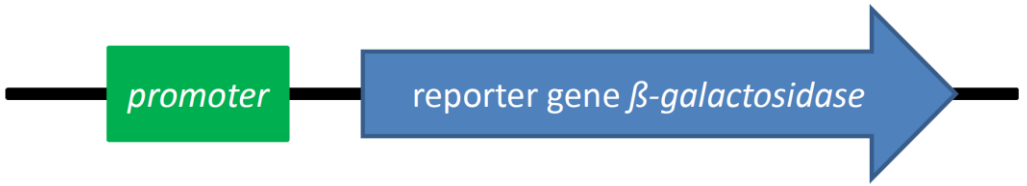
Hörömpöli et al., Antimicrob. Agents Chemother., 2021

Natural products can show us

new routes of uptake and
combinations of uptake routes that reduce resistance

Development of bioreporters for direct, mode of action-informed producer strain screening



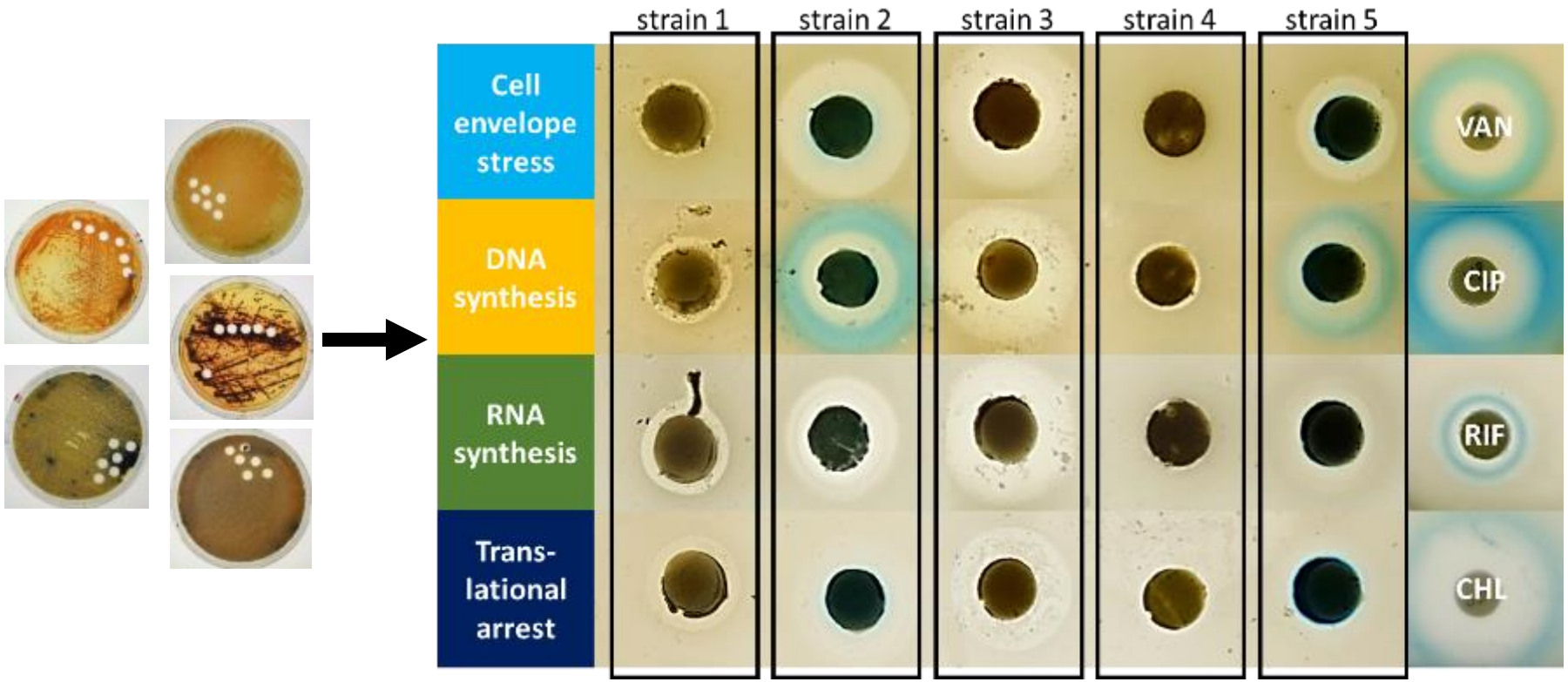


Wex et al., Cell Chem. Biol., 2021

Bioreporter-based screening of the Tübingen strain collection

~300 Tü- producer strains screened (preselected for activity against *S. aureus*)

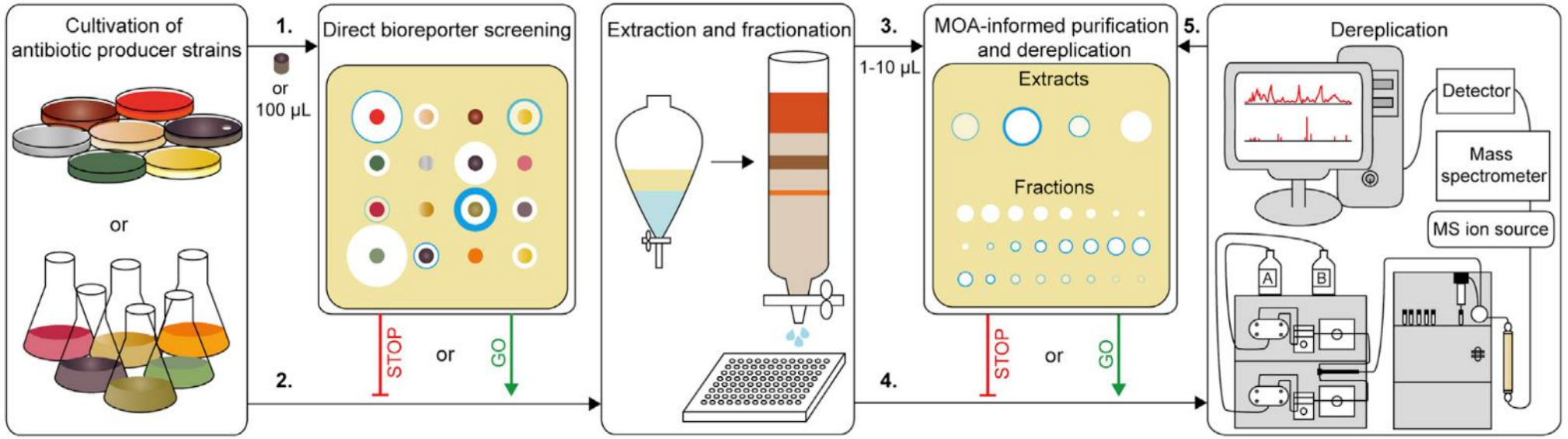
- Producer strains grown on 2 different media
- tested against 5 different reporters, 3 replicates each

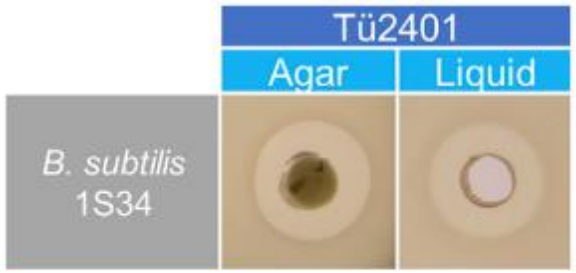


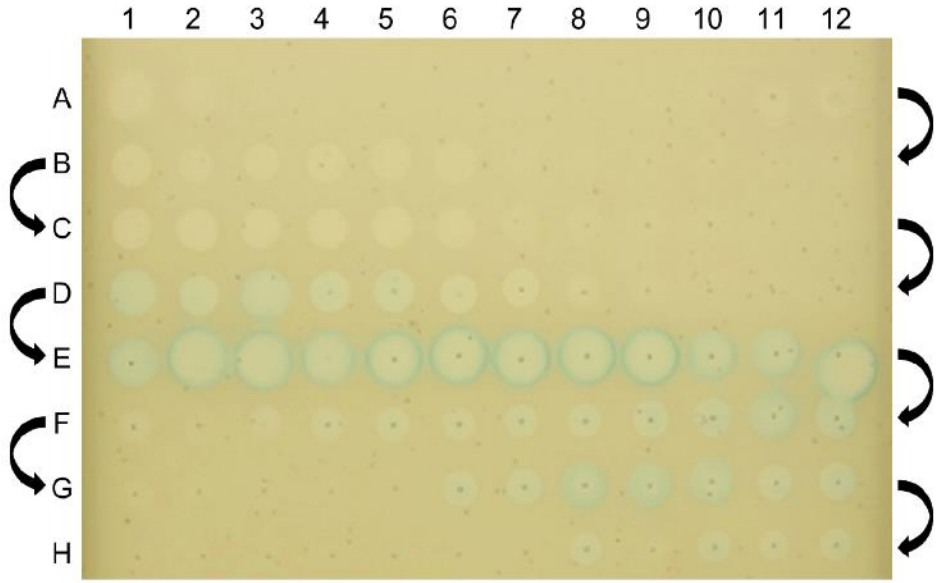
Bioreporters work directly with producer strains, no compound purification required

Wex et al., Cell Chem. Biol., 2021

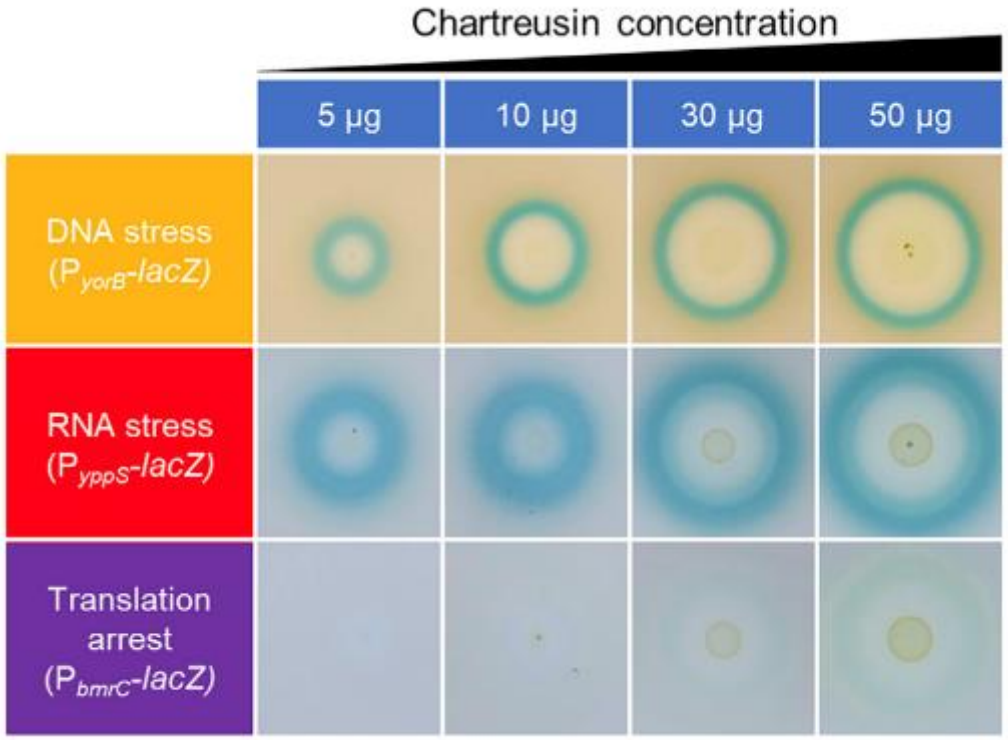
Useful along the entire purification pipeline



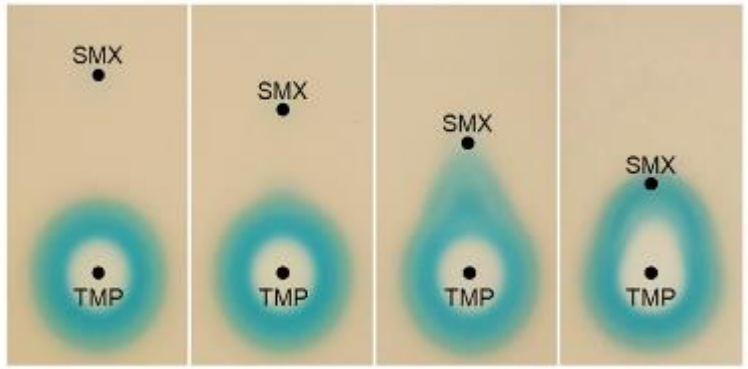




Useful to detect polypharmacology and synergism



Trimethoprim & sulfamethoxazole



Wex et al., Cell Chem. Biol., 2021

Technological progress

in chemical analytics and
in biological screening

is important to speed up deconvolution and
avoid rediscovery of known natural products

Nature provides good starting points

Nature has **pre-optimized for antibacterial potency**

Complex and efficient mechanisms of action have been realized

However, nature has no need to optimise for

- Safety / tolerability
- Absorption
- Distribution
- Metabolic stability
- Chemical accessibility

Matterhorn wikipedia.de

Most natural products require extensive optimization (understanding of biology and chemistry)

and PATIENCE

Microbial Bioactive Compounds (Mode of Action subteam)

Interfaculty Institute for Microbiology and Infection Medicine,
Department of Microbial Bioactive Compounds
University of Tuebingen

www.imit.uni-tuebingen.de/bioactcompounds

Peter Sass
Anne Berscheid
Filipp Oesterhelt
Lina Thoma
Jan Bornikoel
Imram Malik
Dhana Thomy
Laura Reinhardt
Christian Mayer
Nadine Silber
Katharina Wex
Daniel Hörömpöli
Stephan Pan
Astrid Illigmann
Andreas Vohrbach
Yvonne Thoma
Julian Schubert
Lidia Alejo
Elisa Liebhardt
Laura Reinke
Vladislav Mokeev
Franziska Höhn
Dana Schneider

Steffen Drees
Linda Westermann

Jan Straetener
Tatjana Wommer
Anne Wochele

CMFI, TRR261,
GRK 1708,
FOR 854, SFB 766

Federal Ministry of Education and Research

Thanks to all of our great collaborators

Kirsten Famulla
Catherine Ciglia

Markus Korkowski
Holger Paulsen,
Siegfried Raddatz,
Berthold Hinzen,
Rainer Endemann, etc.

Leendert Hamoen

University of Berlin
Janine Kirstein,
Kürsad Turgay

University of Heidelberg
Anja Hoffmann,
Axel Mogk

University of Halle
Hauke Lilie

Ute Sauer
Guido Schiffer
Cuong Vuong
Helga Rübsamen-Waigmann

Michaele Josten,
Hans-Georg Sahl

Julia Bandow

Byung-Gil Lee
Hyun-Kyu Song

Hyesung Jeon

Malte Gersch
Stephan Sieber
Marie-Theres Vielberg
Michael Groll

Alfred Goldberg
Tatos Akopian,
Olga Kandror

University of Marburg
Julian Hegemann
Mohamed Marahiel

Joaquin Ortega

Mc Master University
Gerry Wright

Eric Cheng

Scot Oullette

Richard Lee (Memphis)
Mathias Winterhalter (Bremen)
Matteo Ceccarelli (Cagliari)
Lars Haustedt (Analyticon)
Karl-Heinz Glüsenkamp (Squarix)

Funding: German Research Foundation, FOR 854, SFB 766, GRK 1708, TRR261, EXC 2124

Cluster of Excellence
Controlling Microbes to Fight Infections

The lugdunin team

Peschel lab
Bernhard Krismer
Alex Zipperer
Andreas Peschel
et al.

Grond lab
Martin Konneth
Nadine Schilling
Stephanie Grond
et al.

Hubert Kalbacher

Fritz Götz

Christopher Weidenmaier

Matthias Willmann

Brötz-Oesterhelt lab
Anne Berscheid
Jan Straetener
Heike Brötz-Oesterhelt
et al.

Schittek lab
Katharina Bitschar
Birgit Sauer
Birgit Schittek
et al.

Steinem lab (University of Göttingen)
Johannes Schumacher
Claudia Steinem
et al.

DFG

FOR 854, SFB 766,
GRK 1708, TRR 261

DZIF cmfi

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

antibiotic cellMAP

TRR261 CELLULAR MECHANISMS OF ANTIBIOTIC ACTION AND PRODUCTION

www.trr261.de

@TRR261 twitter

Cluster of Excellence

Controlling Microbes to Fight Infections

@CoE_CMFI

CMFI YouTube-Kanal

DZIF

German Center for Infection Research

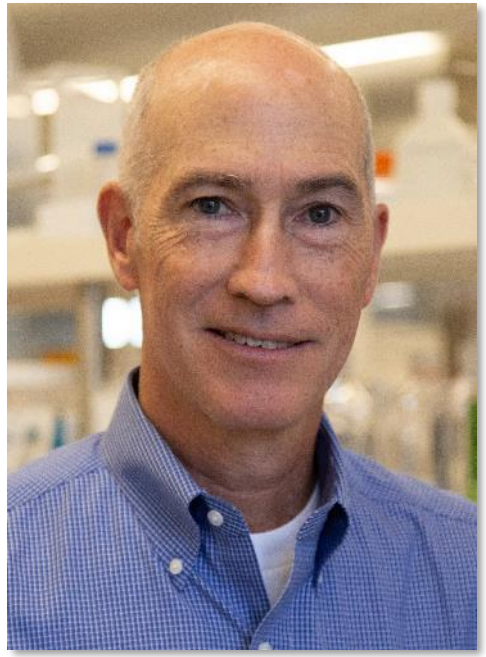
TTU Novel Antibiotics

DFG

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

Federal Ministry of Education and Research

Scott Hecker



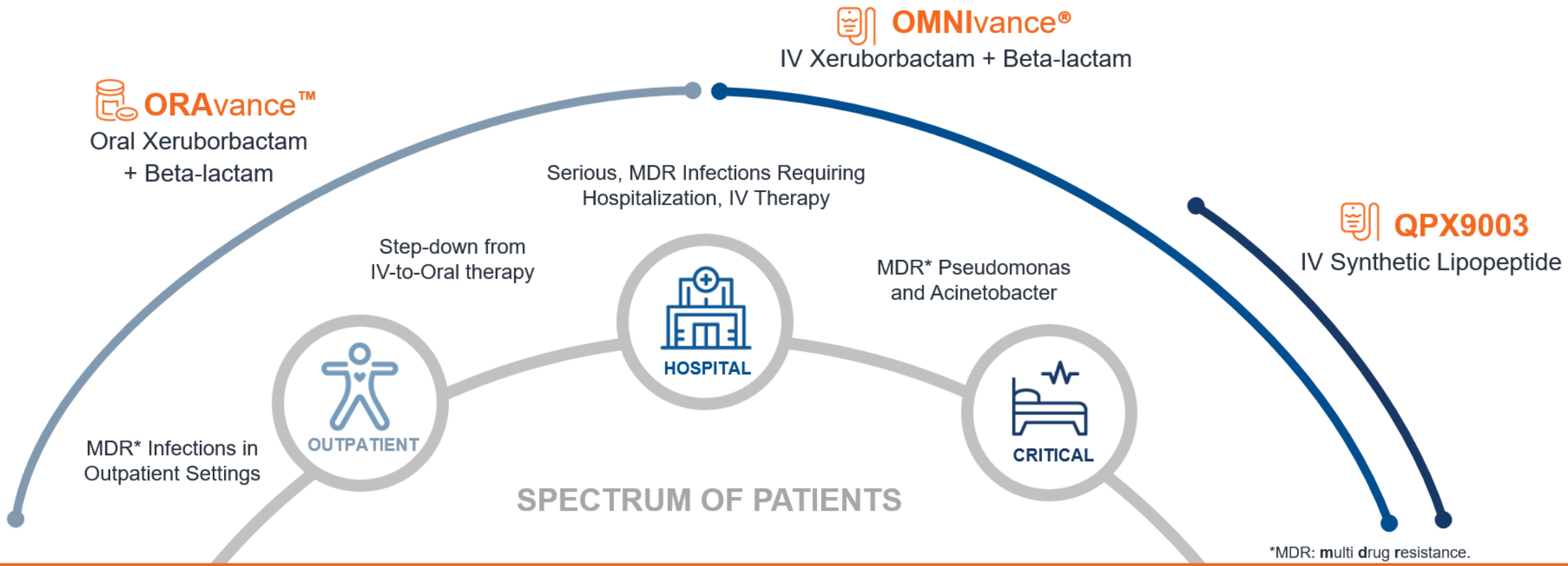
Scott Hecker has over 35 years of industry experience in leading medicinal chemistry and manufacturing in antibacterial drug discovery and development. He began his industrial career at Pfizer as a medicinal chemist in antibacterial drug discovery and then joined Microcide/Essential Therapeutics, where he led teams in the discovery of novel anti-infective drug candidates. He then moved to Metabasis Therapeutics where he oversaw the discovery and early development of drug candidates for diabetes, hyperlipidemia and hepatitis C. He later joined Mpex/Rempex Pharmaceuticals, where he was a founding member of the beta-lactamase inhibitor program that discovered the now FDA-approved agent vaborbactam. Upon acquisition of Rempex by The Medicines Company, he oversaw the API manufacturing of vaborbactam as well as leading medicinal chemistry efforts to identify next-generation agents that are now being developed by Qpex Biopharma.

Discovery of Xeruborbactam (QPX7728), an Ultra-broad-spectrum Boronic Acid Inhibitor of Serine and Metallo Beta-lactamases

Scott J. Hecker, PhD
Sr. Vice President, Chemistry and Chemical Development
Qpex Biopharma, Inc.

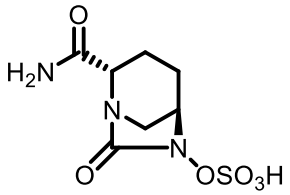
Qpex Designed a Portfolio of Products That Addresses Critical Needs for New Antibiotics

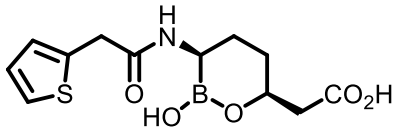
Full Spectrum of Gram-Negative Infections in Multiple Patient Types and Care Settings

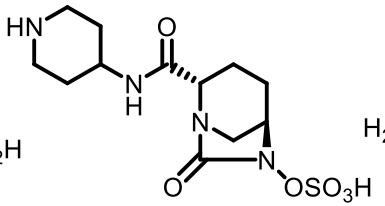


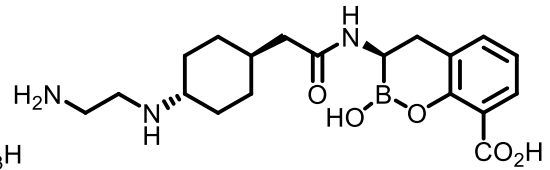
*MDR: **m**ulti **d**rug **r**esistance.

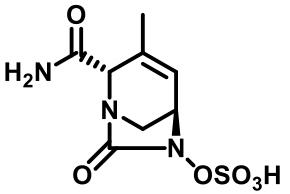
Where Do the New BL-BLI Combos Fall Short?


avibactam


vaborbactam


relebactam


taniborbactam

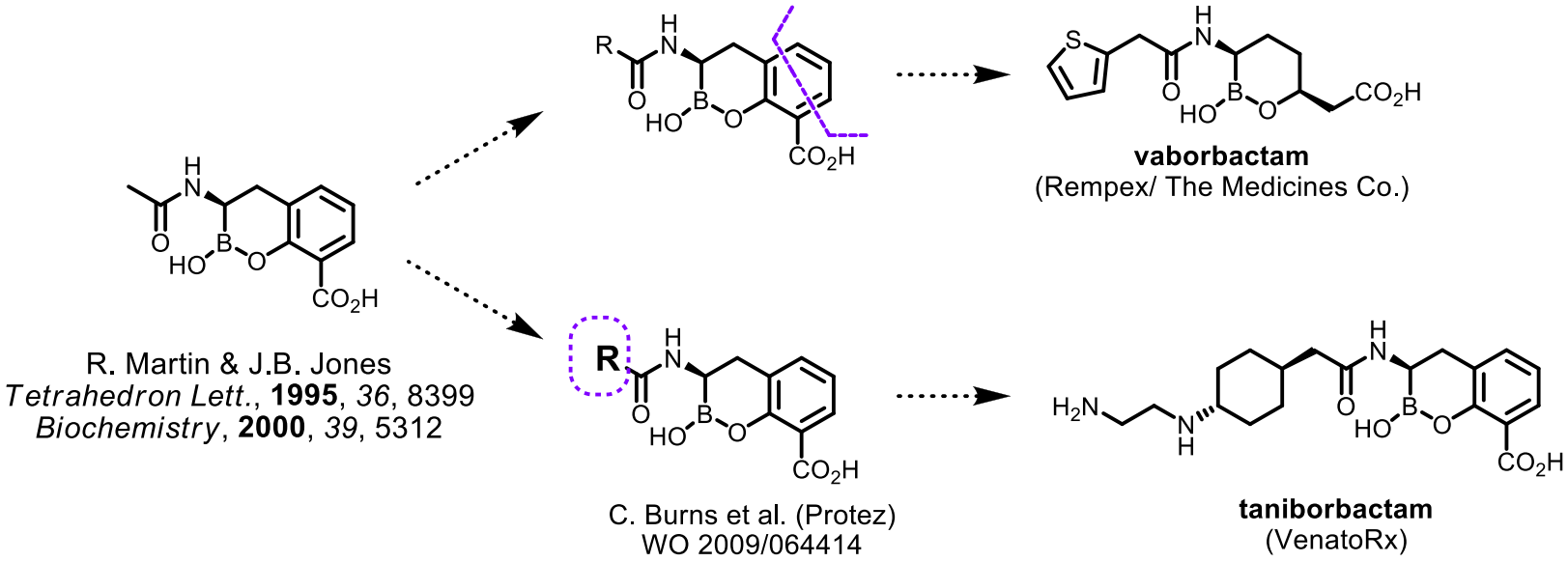

durlobactam

Inhibitor Combo	ESBLs	CRE Serine	CRE Metallo	Pseudomonas	Acinetobacter
Tazobactam/ Ceftolozane *(Zerbaxa™; Merck)*				***Resistance Emerging***	
Relebactam/ Imipenem *(Recarbrio™;Merck)*		***No OXA***			
Avibactam / Ceftazidime *(Avycaz™; Allergan / Pfizer)*		***Resistance Emerging***			
Vaborbactam / Meropenem *(Vabomere™; Melinta)*		***No OXA***			
Taniborbactam / Cefepime *(Investigational; VenatoRx)*			***No IMP***		
Durlobactam / Sulbactam *(Investigational; Entasis)*					***OXA only (no NDM)***

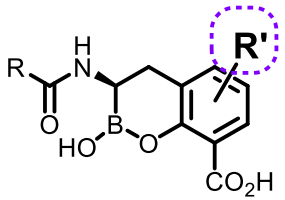
QPEX BIOPHARMA

The Benzoxaborinine Nucleus

- Vaborbactam and taniborbactam originate from discovery of a potent pharmacophore in the labs of J. Bryan Jones at the University of Toronto



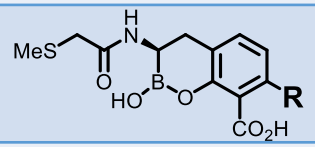
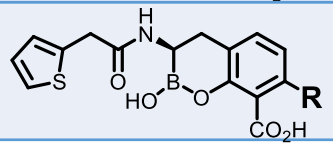
- After launching vaborbactam into development, we returned to the bicyclic structure and explored substitution on the aromatic ring



QPEX BIOPHARMA

Substitution Reduces MexAB-OprM Dependent Efflux

- Small substituents ortho to the carboxylate are generally consistent with good activity
- Reduced impact of efflux is modest but consistent

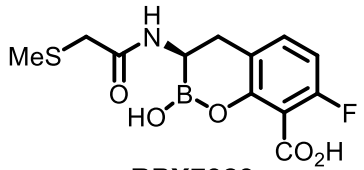
Structure	R	PAM1032/KPC-2 (overexpressor)	PAM1154/KPC-2 (efflux deficient)	Efflux Index
	H	80	5	8
	F	20	5	4
	OMe	10	5	2
	H	80	2.5	32
	F	40	10	4
	OMe	40	5	8

MIC (µg/mL) of BLI in presence of 1 µg/mL of biapenem

- An optimization program produced RPX7323
  - Much broader spectrum than vaborbactam (broad Class A/C vs. KPC only)
  - Prodrugs with 100% oral bioavailability (overall profile very similar to VenatoRx' ledaborbactam)

	KPC-2	CTX-M-15	SHV-12	CMY-6	OXA-48	NDM-1
Class	A	A	A	C	D	B
vaborbactam	1.25	20	5	20	>40	>40
RPX7323	1.25	0.3	0.3	1.25	10	>40

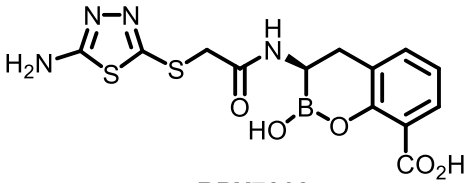
MIC (µg/mL) of BLI in presence of 4 µg/mL of aztreonam



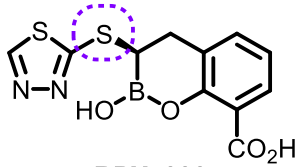
RPX7323

QPEX BIOPHARMA

A Simple Replacement Dramatically Improves Potency Against Metalloenzymes and Brings Whole-cell Activity



RPX7262



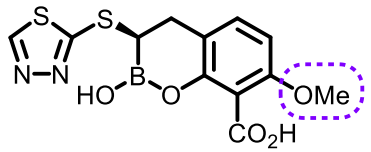
RPX7282

	Avibactam		RPX7262		RPX7282	
	K_i (µM)	MIC* (µg/mL)	K_i (µM)	MIC* (µg/mL)	K_i (µM)	MIC* (µg/mL)
KPC-2	0.0026	≤0.04	0.0003	0.08	0.01	0.16
OXA-48	0.0001	1.25	0.004	2.5	0.01	5
NDM-1	>80	>20	7.4	>20	0.024	0.6

MIC*: MIC of BLI in presence of 1 µg/mL of biapenem

- An optimization program produced RPX7546
  - Potent, broad-spectrum activity including NDM, VIM, IMP as well as OXA-48
  - Prodrugs with 100% oral bioavailability

Compound	KPC* (µg/ml)	NDM-1* (µg/ml)	VIM-1* (µg/ml)	IMP-26* (µg/ml)	OXA-48* (µg/ml)	Rat Cl free (L/hr/Kg)
RPX7282	0.3	0.6	2.5	40	5	5.88
RPX7546	0.16	0.3	0.3	5	5	0.8



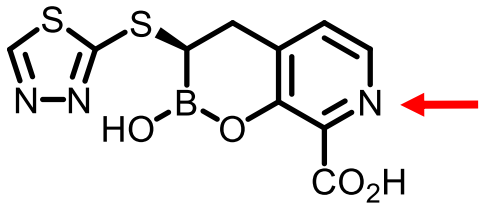
RPX7546

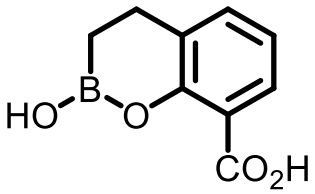
* MIC of BLI in presence of 1 µg/mL of biapenem

QPEX BIOPHARMA

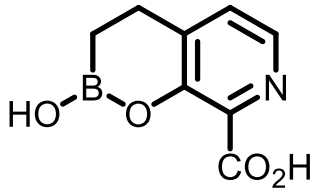
Next: Install Heteroatoms in the Aromatic Ring

- Target:





RPX7388



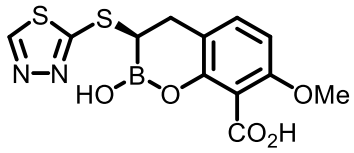
pyridyl analog

- Problem: making this is likely to take a long time, and in the end the compound may have poor activity

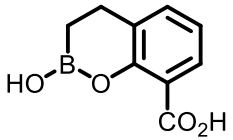
- Approach:
  - We had previously made RPX7388, which had some activity but was unimpressive
  - We could make the corresponding pyridyl analog to see whether it has comparable activity; if so, it would be worth the investment to make the original target

A Substituent Next to Boron is Not Required!

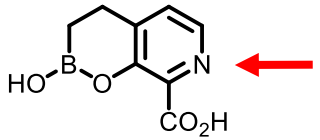
- The pyridyl analog, unsubstituted next to boron, has remarkably potent broad-spectrum activity, including OXA enzymes of *Acinetobacter baumannii*



RPX7546



RPX7388



QPX7609

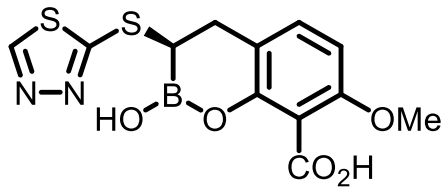
							MIC (µg/mL) of BLI in presence of stated amt of β-lactam (β-L)		
Organism	Strain	BL	Class	β-L	MIC β-L alone	β-L amt (µg/mL)	**RPX7546**	**RPX7388**	**QPX7609**
K. pneumoniae	KP1009	CTX-M-15	A	ATM	>128	4	2.5	20	**2.5**
E. coli	ECM6625	SHV-5	A	ATM	>128	4	0.3	20	**5**
E. coli	ECM6619	TEM-10	A	ATM	128	4	2.5	10	**0.08**
K. pneumoniae	KP1004	KPC-2	A	ATM	>128	4	1.25	0.6	**0.16**
E. cloacae	ECL1002	chAmpC	C	ATM	64	4	10	5	**1.25**
P. aeruginosa	PAM 2035	AmpC	C	ATM	64	4	10	0.6	**0.3**
A. baumannii	AB1053	OXA-72	D	MP	64	8	>20	>20	**1.25**
A. baumannii	AB1054	OXA-23	D	MP	64	8	>20	20	**2.5**
K. pneumoniae	KP1081	NDM-1	B	BP	16	1	0.3	1.25	**1.25**
K. pneumoniae	KP1054	VIM-1	B	BP	16	1	0.3	10	**20**

Abbreviations: β-L, beta-lactam; ATM, aztreonam; MP, meropenem; BP, biapenem

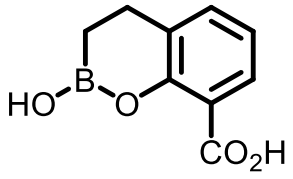
QPEX BIOPHARMA

Other Substituents Next to the Carboxylate

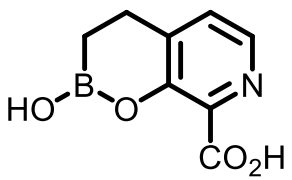
- Adding substituents next to the carboxylate makes things even better



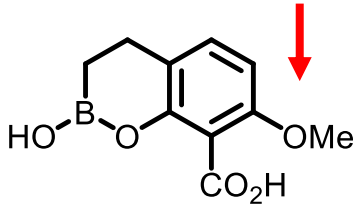
RPX7546



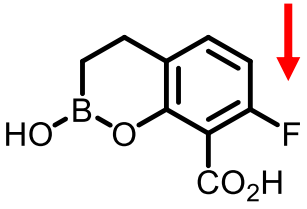
RPX7388



QPX7609



QPX7610



QPX7614

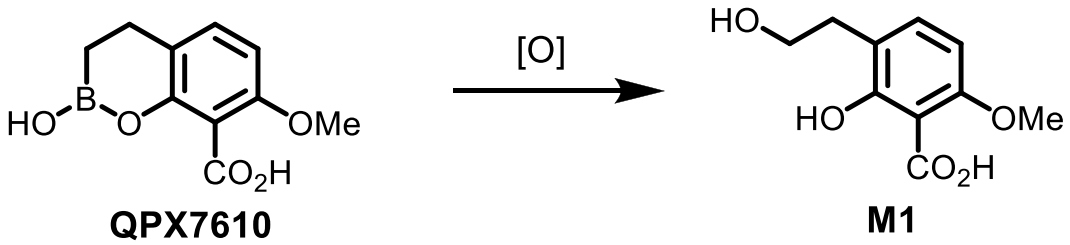
							MIC (µg/mL) of BLI in presence of stated amt of β-lactam (β-L)				
Organism	Strain	BL	Class	β-L	MIC β-L alone	β-L amt (µg/mL)	**7546**	**7388**	**7609**	**7610**	**7614**
K. pneumoniae	KP1009	CTX-M-15	A	ATM	>128	4	2.5	20	2.5	**0.6**	0.6
E. coli	ECM6625	SHV-5	A	ATM	>128	4	0.3	20	5	**0.6**	0.6
E. coli	ECM6619	TEM-10	A	ATM	128	4	2.5	10	0.08	**0.08**	0.08
K. pneumoniae	KP1004	KPC-2	A	ATM	>128	4	1.25	0.6	0.16	**0.16**	0.16
E. cloacae	ECL1002	chAmpC	C	ATM	64	4	10	5	1.25	**0.3**	0.16
P. aeruginosa	PAM 2035	AmpC	C	ATM	64	4	10	0.6	0.3	**1.25**	0.6
A. baumannii	AB1053	OXA-72	D	MP	64	8	>20	>20	1.25	**0.6**	2.5
A. baumannii	AB1054	OXA-23	D	MP	64	8	>20	20	2.5	**0.6**	2.5
K. pneumoniae	KP1081	NDM-1	B	BP	16	1	0.3	1.25	1.25	**0.6**	0.6
K. pneumoniae	KP1054	VIM-1	B	BP	16	1	0.3	10	20	**0.6**	5

Abbreviations: β-L, beta-lactam; ATM, aztreonam; MP, meropenem; BP, biapenem

QPEX BIOPHARMA

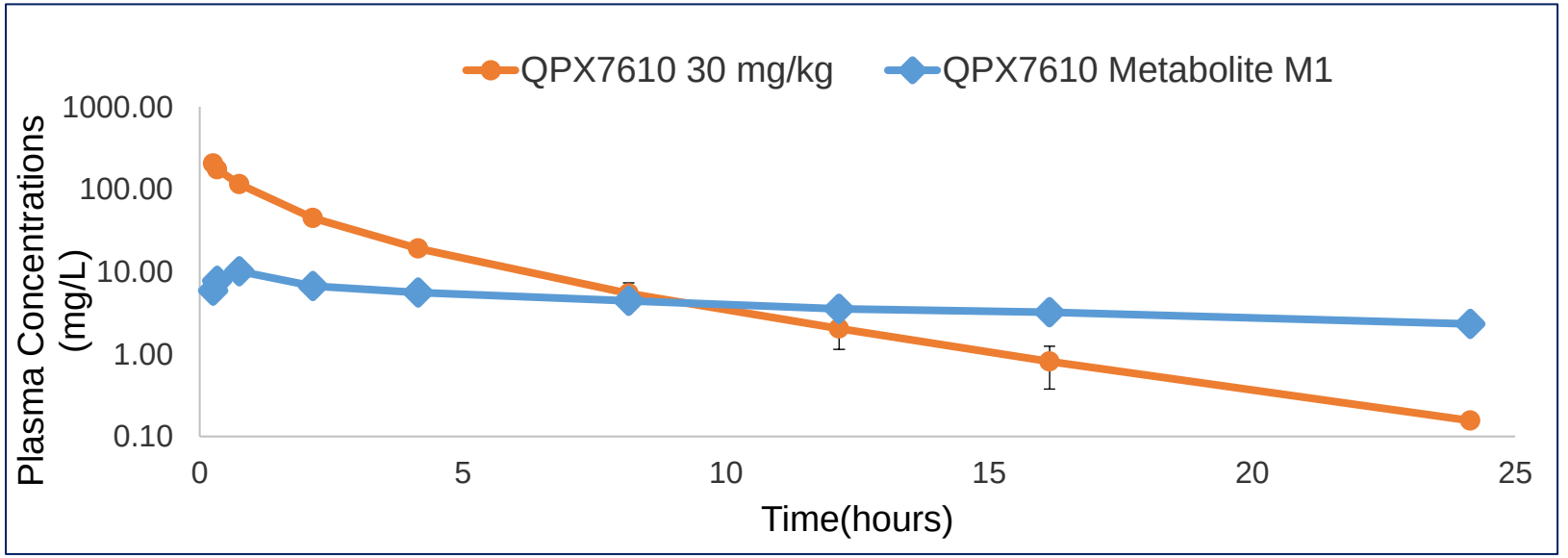
QPX7610: Almost a Development Candidate

- Great compound – until we did PK in monkeys
- Product of oxidative deboronation is a long-lived metabolite



QPX7610 **M1**

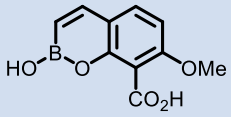
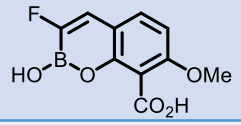
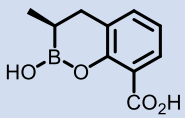
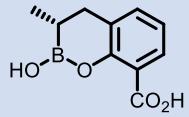
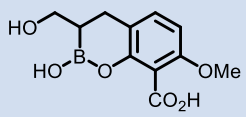
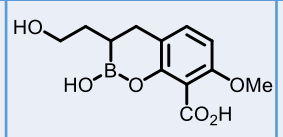
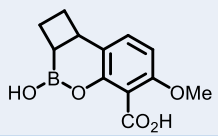
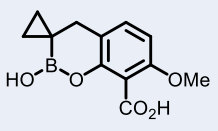
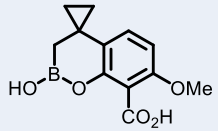
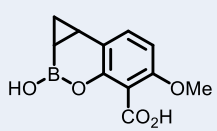
Pharmacokinetics of RPX7610 after a 30 mg/kg 15 min IV Infusion in Cynomolgus Monkeys



QPEX BIOPHARMA

Next: Block Oxidative Metabolism

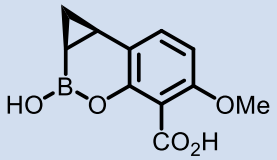
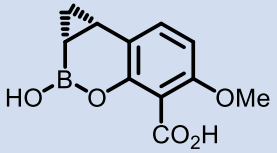
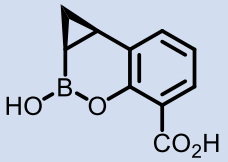
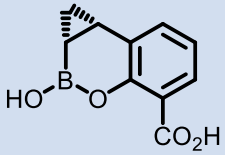
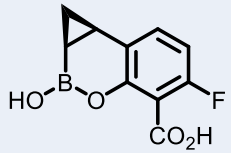
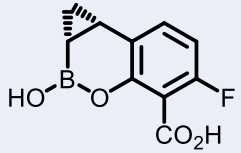
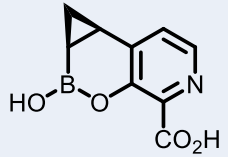
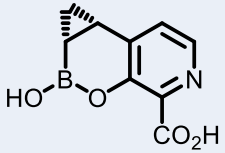
- Oxidative deboronation had not been an issue with previous analogs substituted next to the boron atom
- Therefore, we sought to introduce carbon substitution near boron

			MIC (μg/mL) of BLI in presence of β-lactam (β-L)				
Organism	BL	Class					
E. coli	TEM-10	A	20	1.25	>20	>20	>20
E. cloacae	AmpC	C	10	0.3	20	>20	20
A. baumannii	OXA-23	D	>20	5	>20	>20	>20
K. pneumoniae	NDM-1	B	>20	5	1.25	10	>20
Organism	BL	Class					
E. coli	TEM-10	A	>20	10	>20	5	0.3
E. cloacae	AmpC	C	10	10	10	2.5	0.3
A. baumannii	OXA-23	D	10	10	>20	2.5	1.25
K. pneumoniae	NDM-1	B	2.5	10	10	2.5	0.6

QPEX BIOPHARMA

Cyclopropyl is the Best

- "Alpha" (down) stereochemistry is consistently better

			MIC (μg/mL) of BLI in presence of β-lactam (β-L)			
Organism	**BL**	**Class**				
E. coli	TEM-10	A	0.6	0.08	5	0.16
E. cloacae	chAmpC	C	1.25	0.3	10	0.3
A. baumannii	OXA-23	D	1.25	1.25	20	10
K. pneumoniae	NDM-1	B	0.6	0.3	1.25	0.6
Organism	**BL**	**Class**				
E. coli	TEM-10	A	1.25	0.16	5	0.3
E. cloacae	chAmpC	C	2.5	0.6	20	5
A. baumannii	OXA-23	D	10	1.25	10	1.25
K. pneumoniae	NDM-1	B	5	0.6	20	2.5

QPX7728

QPEX BIOPHARMA

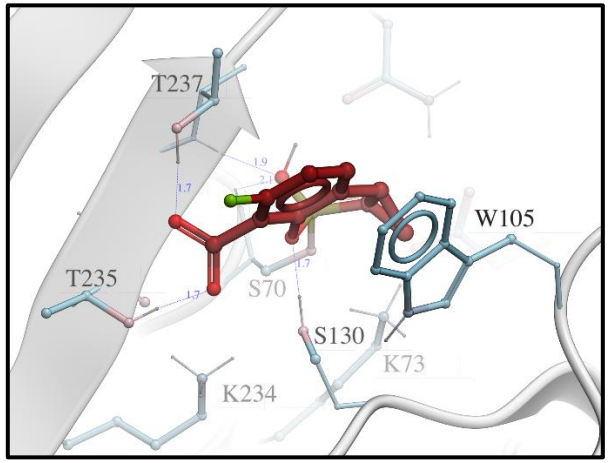
Structural Biology of QPX7728

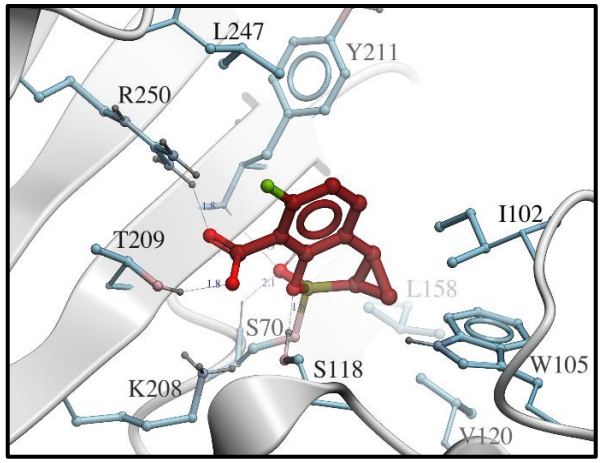
- In all cases, the nucleophilic moiety is covalently bound to the boron atom
  - For KPC-2 and OXA-48, it is the serine hydroxyl
  - For NDM-1 and VIM-2, it is the catalytic water molecule

S. J. Hecker, et al., "Discovery of QPX7728, an Ultra-broad-spectrum Inhibitor of Serine and Metallo Beta-lactamases."J Med Chem 2020;63:7491-7507.

KPC-2

(1.30 Å)



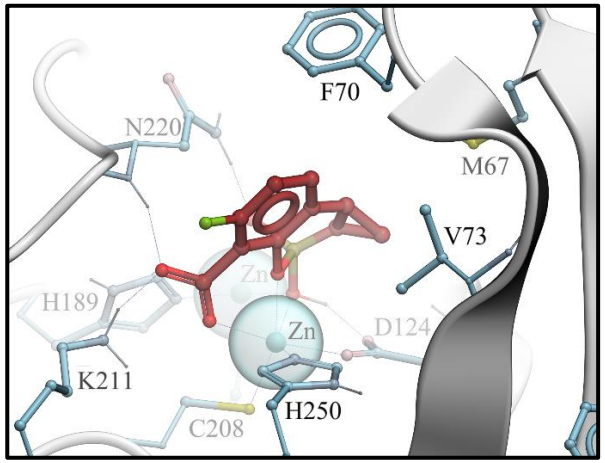


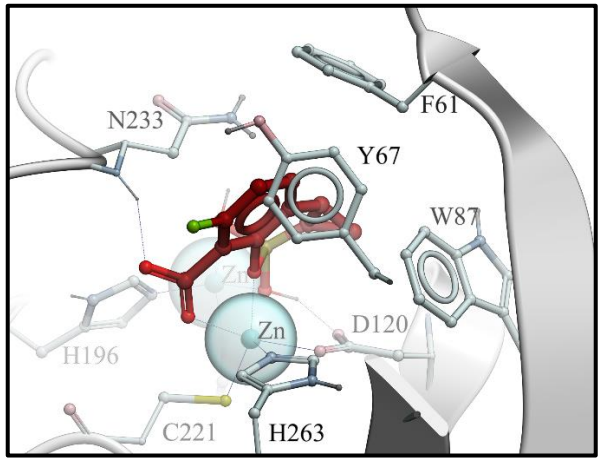
OXA-48

(1.80 Å)

NDM-1

(1.05 Å)





VIM-2

(1.20 Å)

Yu Chen
Orville Pemberton
(Univ. of S. Florida)

QPEX BIOPHARMA

Comparison of Inhibition of Purified Beta-lactamases in Cell-Free Preparations

Xeruborbactam has the broadest spectrum of inhibition of Class A-D serine and metallo enzymes

K_i (nM) of inhibition of nitrocefin hydrolysis

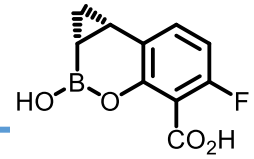
Enzyme	Class	CARB	Xeruborbactam	Vaborbactam	Avibactam	Relebactam	Taniborbactam	Durlobactam
KPC-2	A	+	1.4 ± 0.2	56 ± 15	11 ± 3	40 ± 8	32 ± 0.009	1.7 ± 0.4
CTX-M-14	A	-	0.29 ± 0.06	33 ± 13	0.41 ± 0.13	10 ± 3	1.2 ± 0.5	0.37 ± 0.16
CTX-M-15	A	-	0.37 ± 0.01	30 ± 4	0.18 ± 0.08	24 ± 1	2.6 ± 0.1	0.21 ± 0.12
SHV-12	A	-	0.74 ± 0.21	21 ± 4	0.24 ± 0.07	36 ± 6	0.15 ± 0.03	0.74 ± 0.21
TEM-10	A	-	0.66 ± 0.23	140 ± 40	1.4 ± 0.4	48 ± 7	2.8 ± 0.8	0.99 ± 0.44
P99	C	-	8.5 ± 2.4	35 ± 15	10 ± 2	14 ± 2	2.4 ± 0.5	0.17 ± 0.08
OXA-48	D	+	0.22 ± 0.08	$1.4 \pm 0.5 \times 10^4$	36 ± 10	$1.8 \pm 0.1 \times 10^3$	6.1 ± 0.7	0.72 ± 0.27
OXA-23*	D	+	0.74 ± 0.28	$6.6 \pm 1.1 \times 10^3$	$1.7 \pm 0.4 \times 10^3$	ND	$>2 \times 10^4$	94 ± 32
NDM-1	B	+	32 ± 14	$>1.6 \times 10^5$	$>1.6 \times 10^5$	$>1.6 \times 10^5$	31 ± 3	$>1.6 \times 10^5$
VIM-1	B	+	7.5 ± 2.1	$>1.6 \times 10^5$	$>1.6 \times 10^5$	$>1.6 \times 10^5$	4.5 ± 1.3	$>1.6 \times 10^5$
IMP-1	B	+	240 ± 30	$>1.6 \times 10^5$	$>1.6 \times 10^5$	$>1.6 \times 10^5$	$>2 \times 10^4$	$>1.6 \times 10^5$

ESBL, extended spectrum beta-lactamase; CP, carbapenemase
*The most prevalent carbapenemase from carbapenem-resistant Acinetobacter

Reference:Tsivkovski & Lomovskaya, *Antimicrobial Agents and Chemotherapy 2020; e00130-20*
(Data for taniborbactam and durlobactam unpublished)

QPEX BIOPHARMA

Microbiology of QPX7728



QPX7728

- QPX7728 combinations with different beta-lactam partners each show advantages

Organism	No. of strains	MIC$_{90}$ (µg/ml)							
		FEP	FEP-QPX	CZN	CZN-QPX	MEM	MEM-QPX	CAZ-AVI	MEM-VAB
All Enterobacterales	1015	>32	0.25	>32	8	>32	0.125	>32	>32
ESBLs (No CRE)*	507	32	0.03	>32	0.25	0.125	0.03	0.5	0.06
CRE KPC	157	>32	0.25	>32	0.5	>32	0.125	4	2
CRE OXA-48	150	>32	0.5	>32	0.5	>32	0.125	1	>32
Non-CP CRE*	51	>32	0.5	>32	1	16	0.25	2	4
CRE MBL (NDM, VIM)	150	>32	1	>32	>32	>32	2	>32	>32
Carbapenem-res. A. baumannii	503	>32	32	>32	32	>32	4	>32	>32
P. aeruginosa	500	32	8	4	1	16	8	4	16

Abbreviations: FEP, cefepime; CZN, ceftolozane; MEM, meropenem; CAZ-AVI, ceftazidime-avibactam; MEM-VAB, meropenem-vaborbactam.

QPEX BIOPHARMA

Ultra-Broad-Spectrum: Broad Combinations with Xeruborbactam (QPX7728)

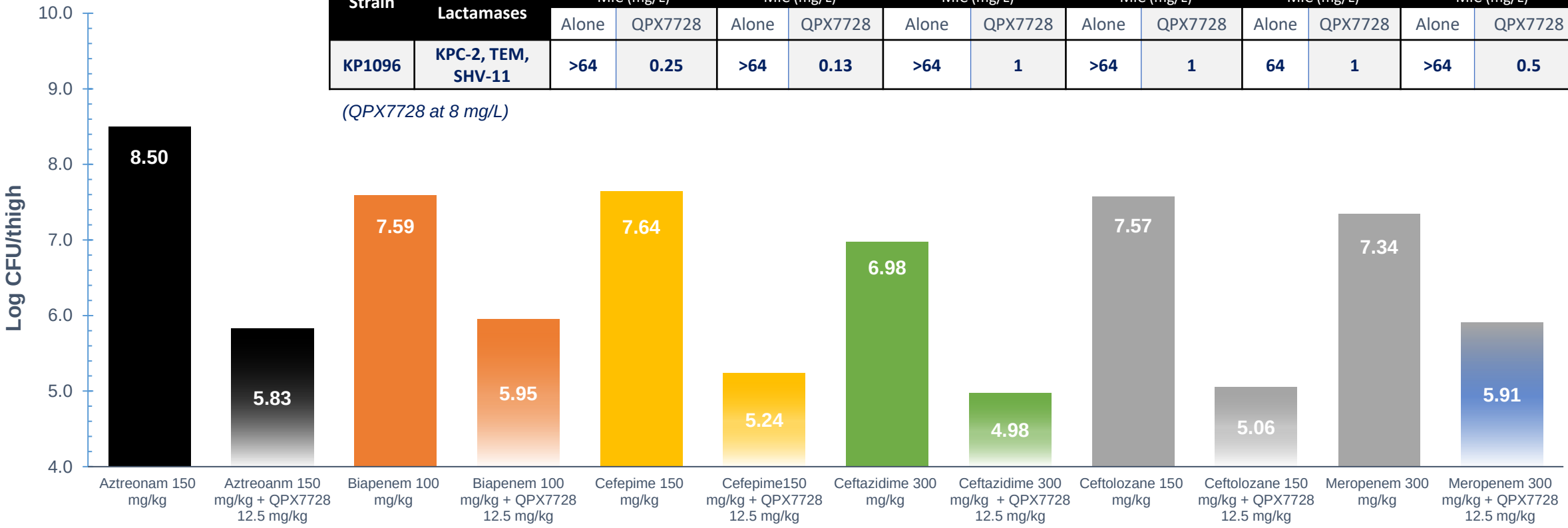
In Vivo Efficacy in Neutropenic Mouse Thigh Infection Model

➢ Carbapenem-Resistant *K. pneumoniae* KP1096; Humanized Beta-lactam Dosing

(Sabet et. al. Antimicrob Agents Chemother 2020;64:11(November)

Strain	Beta-Lactamases	Aztreonam MIC (mg/L)		Biapenem MIC (mg/L)		Cefepime MIC (mg/L)		Ceftazidime MIC (mg/L)		Ceftolozane MIC (mg/L)		Meropenem MIC (mg/L)	
		Alone	QPX7728	Alone	QPX7728	Alone	QPX7728	Alone	QPX7728	Alone	QPX7728	Alone	QPX7728
KP1096	KPC-2, TEM, SHV-11	>64	0.25	>64	0.13	>64	1	>64	1	64	1	>64	0.5

(QPX7728 at 8 mg/L)

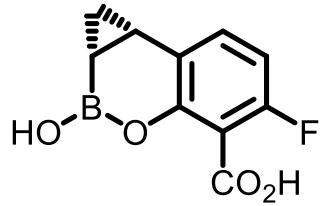


QPX7728 potentiates multiple beta-lactam antibiotics in vivo against carbapenem-resistant *K. pneumoniae*

QPEX BIOPHARMA

Why is QPX7728 So Good?

- Small size: MW 222
- Polar: logD @ pH 7.4: -2.85
- Reduced effect of porins and efflux



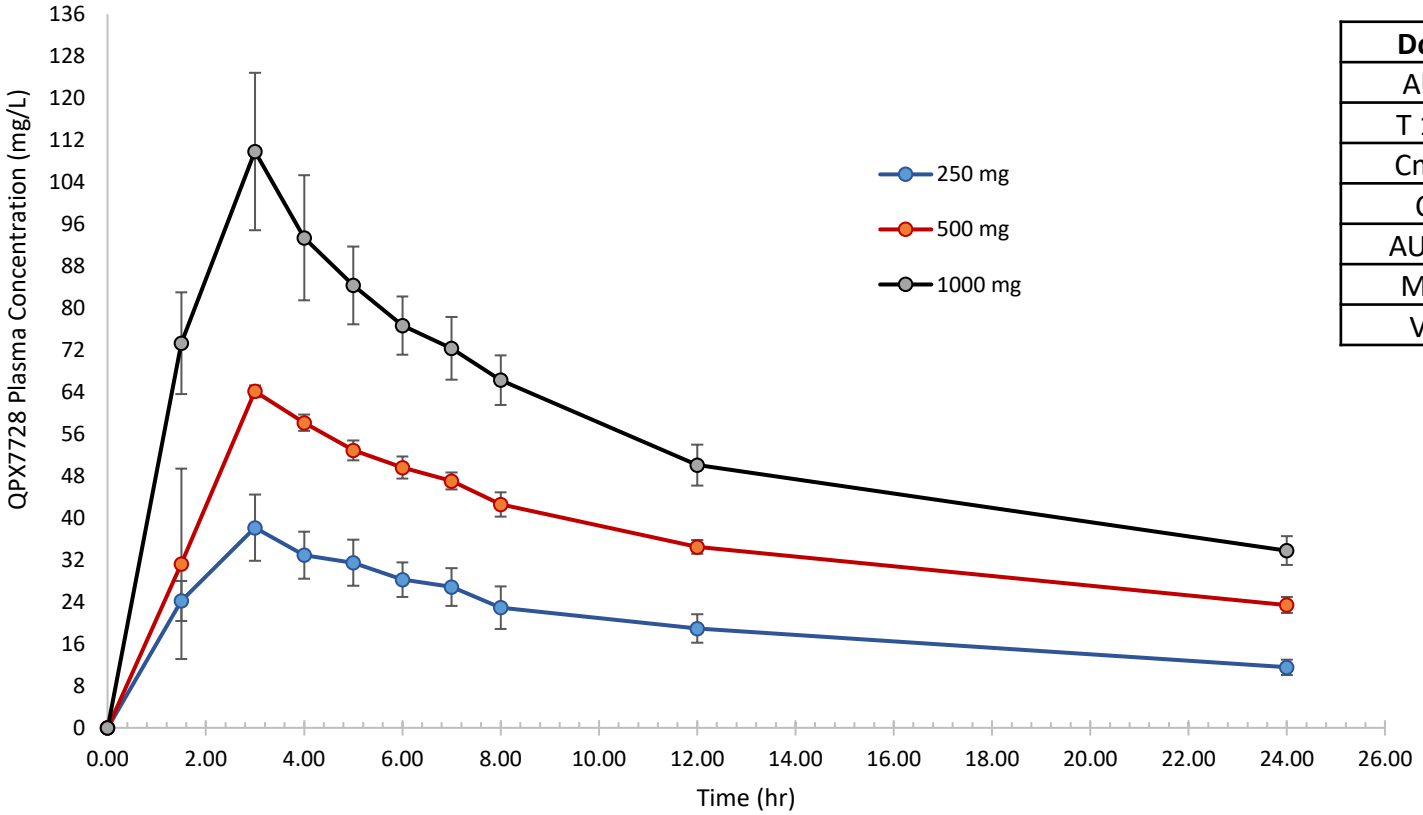
QPX7728

Strain	OmpK35	OmpK36	AcrAB	Beta-lactamase	Meropenem MIC (µg/ml)		
					Alone	+ VAB	+ QPX7728
KPM1271	Wild type	Wild type	Wild type	KPC-3	16	≤0.06	≤0.06
KPM2631	Non-functional	Non-functional	Wild type	KPC-3	256	8	0.25
KPM2965	Non-functional	Non-functional	Up-regulated	KPC-3	256	16	0.5

QPEX BIOPHARMA

QPX7728 Human Plasma PK Profile (Total drug)

Mean QPX7728 Plasma Concentrations Following a 250, 500, or 1000 mg 3-hour IV infusion to Healthy Subjects



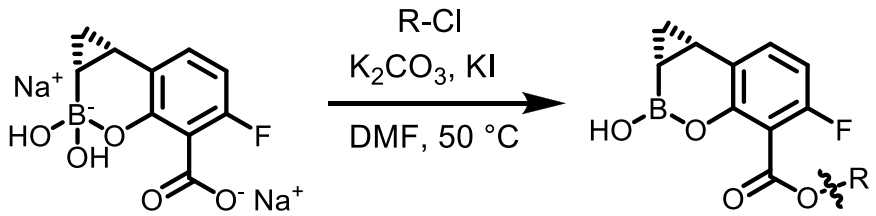
Dose	mg	250	500	1000
AUC	hr*mg/L	659.42	1346.82	1854.45
T 1/2	hr	11.35	14.84	11.22
Cmax	mg/L	36.80	58.70	104.57
CL	L/hr	0.38	0.37	0.54
AUMC	hr*hr*mg/L	11785.96	30856.12	32799.68
MRT	hr	16.37	21.41	16.19
Vss	L	6.21	7.95	8.73

- Plasma half-life 12-14 hrs
  - ~24 hrs with longer sampling
- Human protein binding is ~90%

David C. Griffith, et al., "A Phase 1 Study of the Safety, Tolerability, and Pharmacokinetics of the Beta-Lactamase Inhibitor QPX7728 [xeruborbactam] Following IV Dosing in Healthy Adult Subjects." 32nd European Congress of Clinical Microbiology & Infectious Diseases, Lisbon, 2022

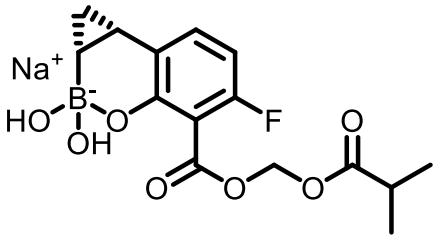
QPEX BIOPHARMA

Selection of Oral Prodrug QPX7831

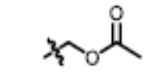
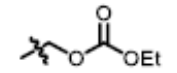
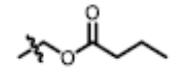
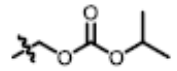
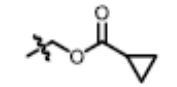
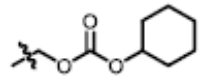
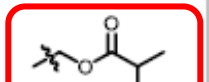
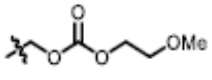
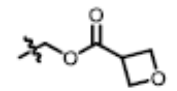
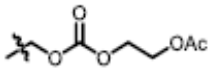
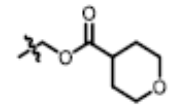
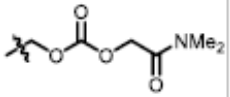
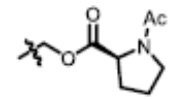
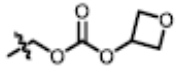
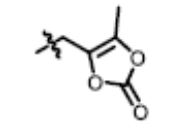
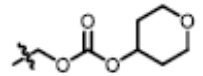
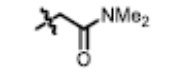


QPX7728 Disodium **2 - 18**

- Numerous prodrugs synthesized and evaluated in vitro for half-lives (min) in human serum (HS) and human liver microsomes (HLM)
  - Surprisingly low cleavage in HS, but several compounds rapidly cleaved in HLM
- Selected compounds followed up in
  - LM from multiple species
  - PK in rat, dog and monkey
  - Crystallinity studies of sodium salts
- QPX7831 emerged as the winner
  - ~100% oral bioavailability in rat and monkey; ~60% in dog



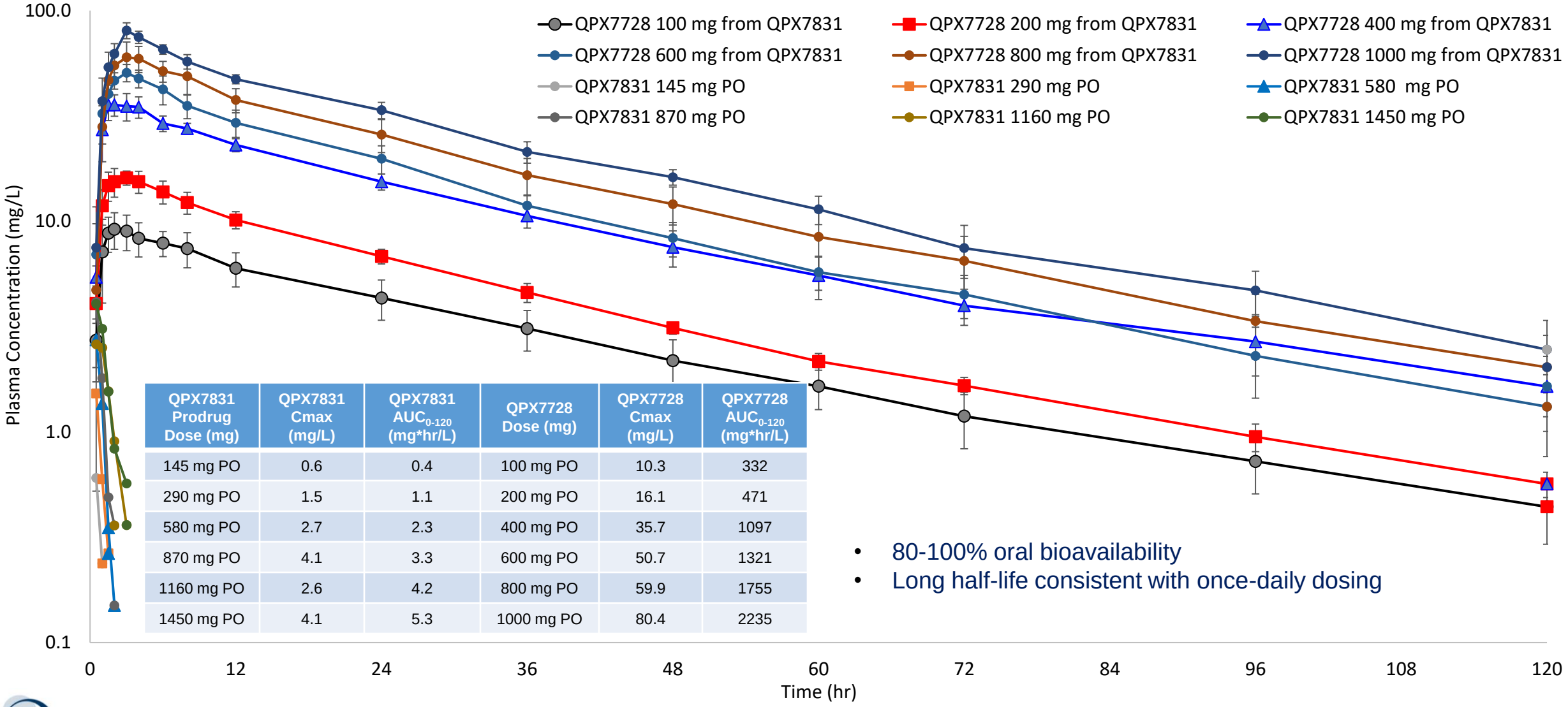
QPX7831 Sodium

Cpd	R	HS	HLM	Cpd	R	HS	HLM
2		>30	8	11		>60	>30
3		>60	28	12		NT	>30
4		>60	14.3	13		>60	27
5		>60	9	14		>60	>30
6		3.9	NT	15		>30	24
7		26	23	16		>60	>30
8		>30	>30	17		11	>30
9		3.7	>30	18		>30	>30
10		>60	>30				

QPEX BIOPHARMA

"Selection of QPX7831, an Orally Bioavailable Prodrug of Boronic Acid β-Lactamase Inhibitor QPX7728," *J. Med. Chem.*, **2021**, *64*, 17523.

Pharmacokinetics of QPX7728 following 100, 200, 400, 600, 800, and 1000 mg Oral doses Administered as the Prodrug QPX7831 (145-1450 mg doses) in Normal Human Subjects



QPX7831 Prodrug Dose (mg)	QPX7831 Cmax (mg/L)	QPX7831 AUC_{0-120} (mg*hr/L)	QPX7728 Dose (mg)	QPX7728 Cmax (mg/L)	QPX7728 AUC_{0-120} (mg*hr/L)
145 mg PO	0.6	0.4	100 mg PO	10.3	332
290 mg PO	1.5	1.1	200 mg PO	16.1	471
580 mg PO	2.7	2.3	400 mg PO	35.7	1097
870 mg PO	4.1	3.3	600 mg PO	50.7	1321
1160 mg PO	2.6	4.2	800 mg PO	59.9	1755
1450 mg PO	4.1	5.3	1000 mg PO	80.4	2235

- 80-100% oral bioavailability
- Long half-life consistent with once-daily dosing

QPEX BIOPHARMA

Summary: Competitive Analysis of Beta-lactamase Inhibitor Combinations (JMI Studies: 20-QPX-04)

In Vitro (MIC_{50} /MIC_{90}) Activity of Various Combinations Based on Side-by-Side-Comparisons

	Enterobacteriaceae				Non-Fermenters	
Inhibitor Combo	ESBLs (N=520)	CRE/KPC (N=195)	CRE/OXA-48 (N=96)	CRE/Metallo (N=168)	Pseudomonas (N=506)	Acinetobacter Carbapenem-R (N=506)
Tazobactam/ Ceftolozane *(Zerbaxa™; Merck)*	**0.5 / 8**	**64 / >64**	**64 / >64**	**>64 / >64**	**0.5 / 4**	**>64 / >64**
Avibactam / Ceftazidime *(Avycaz™; Allergan / Pfizer)*	**0.25 / 1**	**1 / 2**	**1 / 2**	**>32 / >32**	**2 / 8**	**32 / >32**
Vaborbactam / Meropenem *(Vabomere™; Melinta)*	**≤0.03 / ≤0.03**	**≤0.03 / 0.5**	**16 / 32**	**64 / >64**	**0.5 / 16**	**>32 / >32**
Relebactam/ Imipenem *(Recarbrio™; Merck)*	**0.125 / 0.5**	**0.125 / 0.5**	**2 / 8**	**32 / >32**	**0.25 / 2**	**>32 / >32**
Taniborbactam / Cefepime *(Investigational; Venatorx)*	**≤0.06 / 0.25**	**0.25 / 1**	**1 / 4**	**1 / 16**	**2 / 8**	**>32 / >32**
Durlobactam / Sulbactam *(Investigational; Entasis)*	not tested	not tested	not tested	not tested	not tested	**1 / 2**
Xeruborbactam / Meropenem ***(Qpex)***	**≤0.03 / ≤0.03**	**≤0.03 / 0.06**	**≤0.03 / 0.125**	**≤0.03 / 1**	**0.25 / 8**	**0.5 / 4**

QPEX BIOPHARMA

Emerging Resistance to Cefiderocol

In Vitro Activity of Cefiderocol (FDC) Alone or with Xeruborbactam (XERU) against:

Review

Cefiderocol: Systematic Review of Mechanisms of Resistance, Heteroresistance and In Vivo Emergence of Resistance

Stamatis Karakonstantis [1,2,*], Maria Rousaki [2] and Evangelos I. Kritsotakis [3]

Antibiotics, **2022**, *11*, 723

- Numerous reports of in vivo emergence of resistance during treatment
- Co-expression of multiple β-lactamases, often in combination with permeability defects, appear to be the main drivers
  - Especially NDM, KPC and AmpC variants conferring resistance to CAZ/AVI; OXA-427, PER- and SHV-type ESBLs), porin mutations, mutations affecting siderophore receptors, efflux pumps and target (PBP-3) modifications

No β-lactam should be developed without a β-lactamase inhibitor to protect it!

Carbapenem- Resistant *A. baumannii* (CRAB) with Cefiderocol MICs ≥ 2 µg/ml

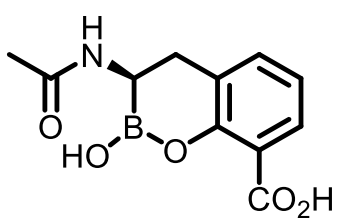
	FDC	FDC + XERU (8)
ALL (CRAB with FDC MIC≥ 2 µg/ml) , N=131		
MIC_{50}	16	0.125
MIC_{90}	>64	2
PER Beta-lactamase Producers (N=36)		
MIC_{50}	>64	0.125
MIC_{90}	>64	0.5
NDM Beta-lactamase Producers (N=17)		
MIC_{50}	16	≤0.06
MIC_{90}	>64	2

Carbapenem Resistant *Enterobacterales* with Cefiderocol MIC ≥ 4 µg/ml

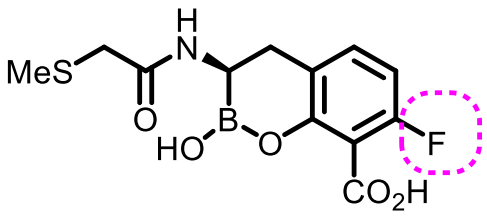
	FDC	FDC + XERU (8)
All (CRE with FDC MIC≥ 4 µg/ml), N=82		
MIC_{50}	16	≤0.06
MIC_{90}	64	0.5
NDM Beta-lactamase Producers (N=55)		
MIC_{50}	16	≤0.06
MIC_{90}	64	0.5

QPEX BIOPHARMA

Conclusions

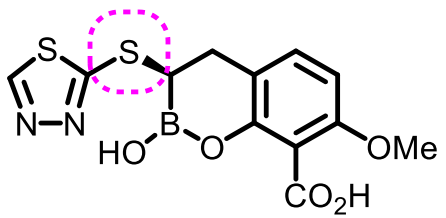


Lead compound



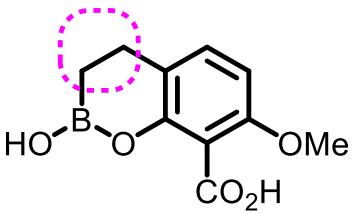
RPX7323

Reduced oligomers and efflux



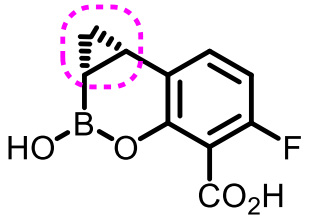
RPX7546

Potent metallo activity



QPX7610

OXA enzymes of Acinetobacter



QPX7728

Metabolically stable

- Status of QPX7728 (*xeruborbactam*): Phase 1 completed
  - Multiple-dose results to be presented next Thursday, October 20 at ID Week in Washington, DC (Poster #216)

D.C. Griffith, et al., *A Phase 1 Study of the Safety, Tolerability, and Pharmacokinetics of Multiple Doses of the Beta-lactamase Inhibitor Xeruborbactam Alone and in Combination with Meropenem in Healthy Adult Subjects*

The Team

Chemistry
Raja Reddy
Tomasz Glinka
Serge Boyer
Acme Bioscience

Biology
Olga Lomovskaya
Kirk Nelson
Debra Rubio-Aparicio
Dongxu Sun
Ruslan Tsivkovski

Pharmacology
David Griffith
Mojgan Sabet
Ziad Tarazi
Jonathan Parkinson
Tom Nolan

Modeling
Maxim Totrov
(Molsoft LLC)

Structural Biology
Yu Chen
Orville Pemberton
(Univ. of S. Florida)

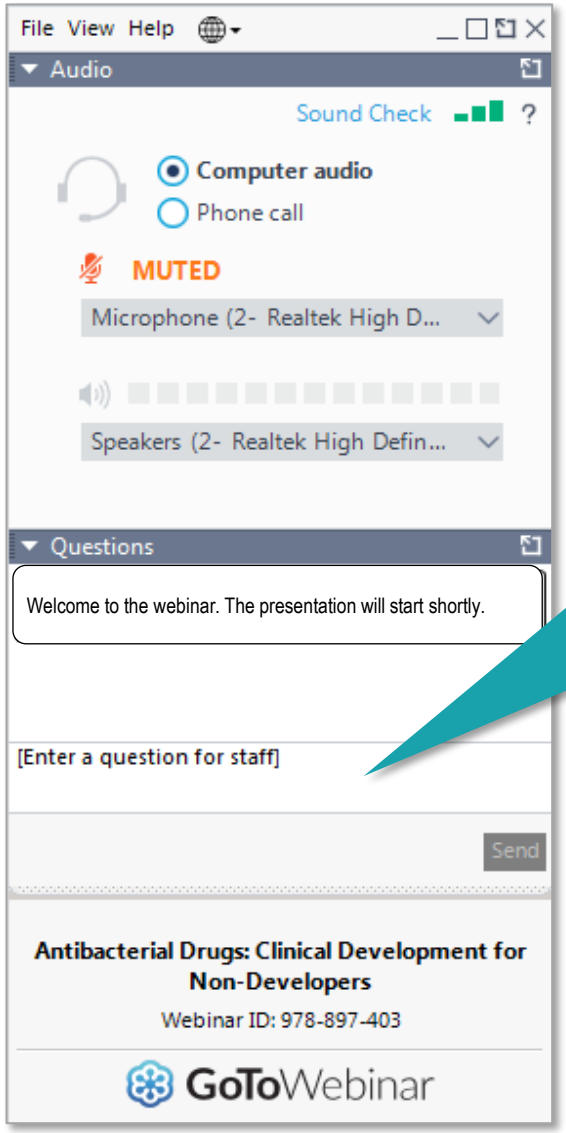
Clinical
Jeff Loutit
Liz Morgan
Shawnee Morgan

Leadership
Mike Dudley

Supported in part with federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority **(BARDA),** under Contract No. HHSO100201600026C

How to submit your questions

If your question is addressed to a specific speaker, please include their name when submitting the question.

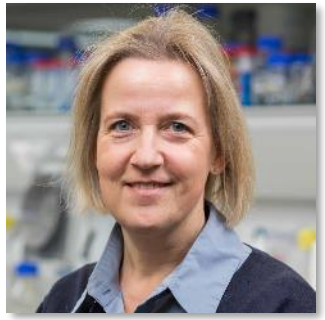


The presentation will be followed by an interactive Q&A session.

Please submit your questions via the ‘questions’ window. We will review all questions and respond to as many as possible after the presentation.

Today’s speakers

Drug discovery strategies: Focusing on synthetic compounds and natural products



Heike Brötz-Oesterhelt
Chair of Dept. “Microbial Bioactive Compounds”, Interfaculty Institute of Microbiology and Infection Medicine
University of Tuebingen (Germany)

Scott Hecker
Senior Vice President, Chemistry and Chemical Development
Qpex Biopharma (USA)

Moderator:
Kim Lewis
Northeastern University Distinguished Professor
Director, Antimicrobial Discovery Center (USA)

Join us for our next webinars

8 November, 17:00-18:30 CET

Regulatory aspects of risks and safety in the clinical development of antibiotics

Speakers:
- Enrica Alteri, former Head of Division EMA
- John Alexander, Deputy Director, Division of Pediatrics and Maternal Health, Center for Drug Evaluation and Research, US FDA

Moderator:
- Radu Botgros, EMA

13 December, 15:30-17:00 CET

Influence of biological sex in infectious disease

Speakers:
- Molly Ingersoll, INSERM, Institut Pasteur
- Sabra Klein, The Johns Hopkins Bloomberg School of Public Health

Moderator:
- Neeloffer Mookherjee, University of Manitoba

Registration links and more information soon available on:
revive.gardp.org/webinars

Thank you for joining us