Development of β-Lactamase Therapies to Protect the Gut Microbiome from Antibiotics

Michael Kaleko, MD, PhD

Senior Vice President of Research and Development

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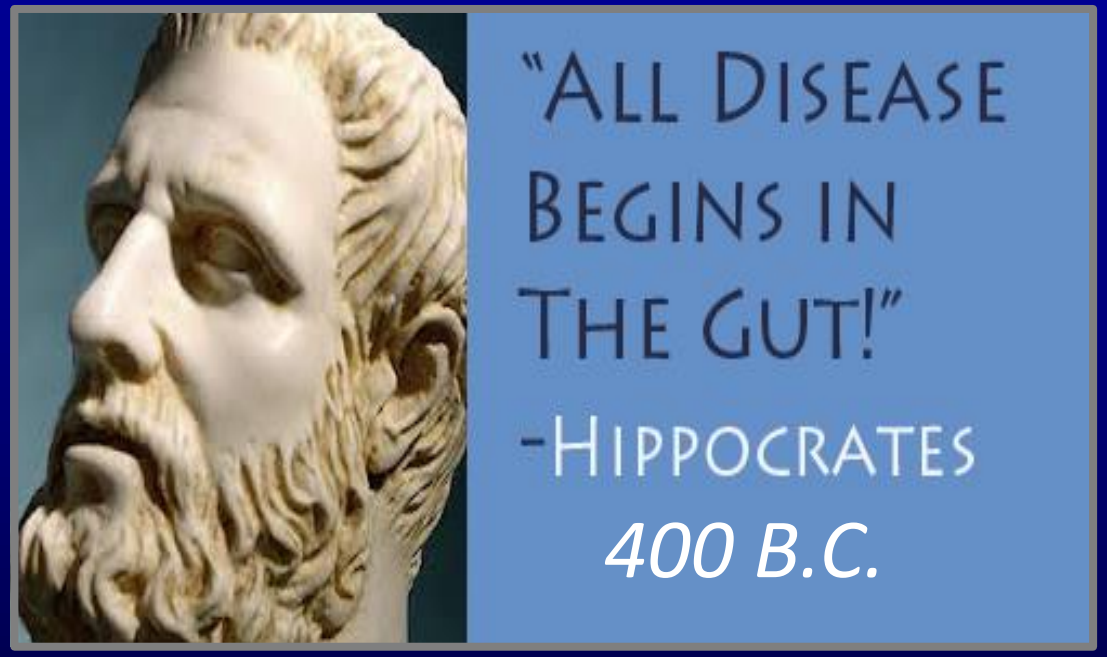
Importance of Intestinal Health Has Long Been Recognized



Gut Microflora Involved in

- Digestion
- Nutrient absorption
- Vitamin synthesis
- Bile salt metabolism
- Stimulation of immune system

Disrupted by

- Antibiotic use

Synthetic Biologics is developing therapies to protect the gut microflora from the damage caused by antibiotic use

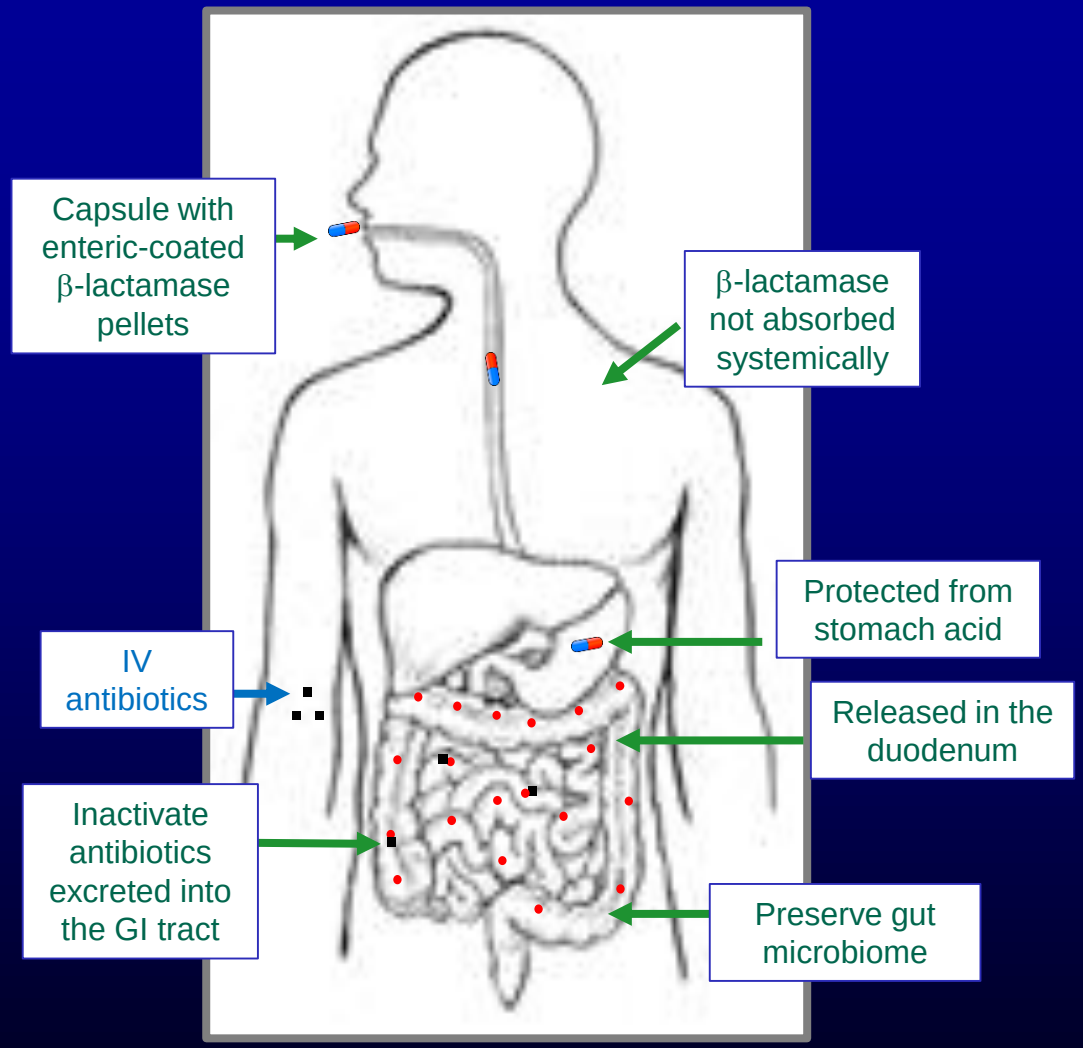
SYNTHETIC BIOLOGICS

β-Lactamases: From Enemies to Therapies

Strategy: Orally administered β-lactamase to degrade residual antibiotics in the GI tract without affecting systemic antibiotic efficacy

Product: Capsule with enteric-coated enzyme

Outcome: Prevention of *Clostridium difficile* infection and antibiotic-associated diarrhea



SYNTHETIC BIOLOGICS

Issues to Consider in Developing β-Lactamases as Therapeutics

Choice of β-Lactamase

Can it be manufactured

Does it have a suitable degradation profile

Is it stable in chyme

Is it compatible with enteric coating

Is it efficacious and safe in animal models

Advance to human clinical trials

SYNTHETIC BIOLOGICS

P1A Was Efficacious in Clinical Trials

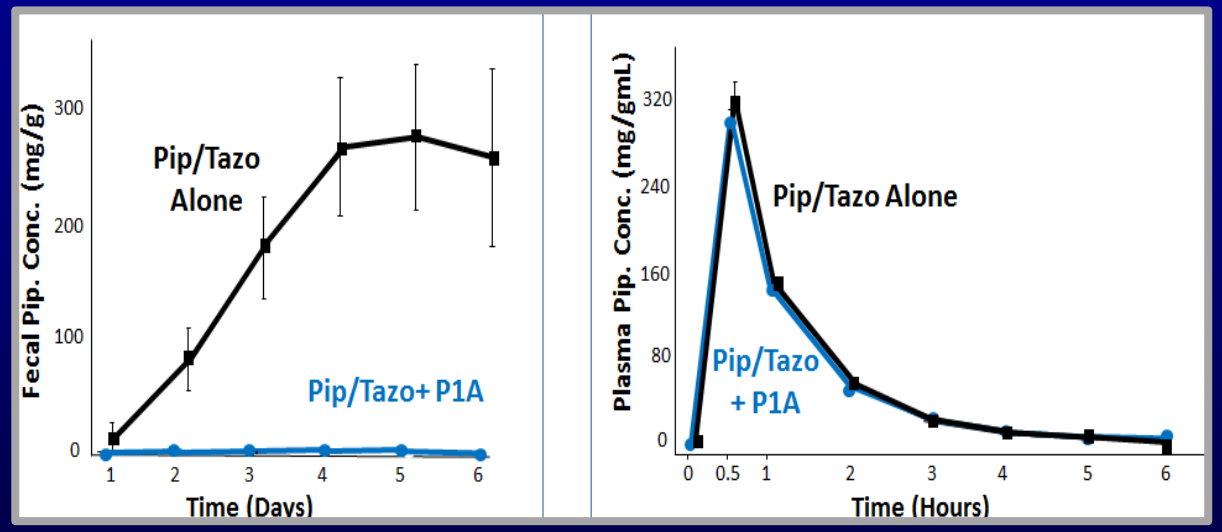
Isolated from *Bacillus licheniformis*

Class A serine β–lactamase

Degrades penicillins

GI Tract

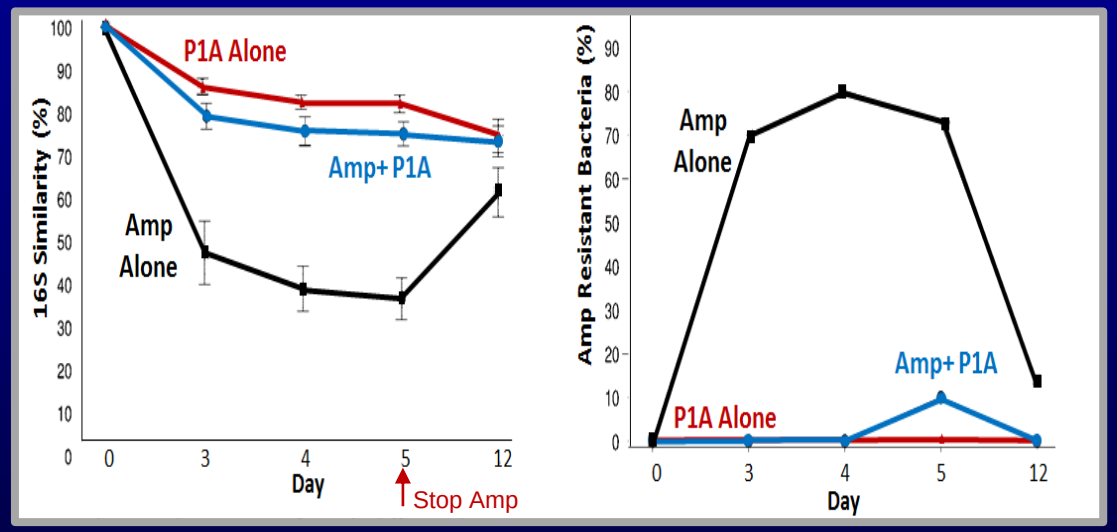
Systemic



Pitout (2009). Curr. Opin. Investig. Drugs 10:838

Similarity Index

Amp-Resistant Bac



Tarkkanen et al. (2009). Antimicrob. Agents Chemother. 53:2455

However, P1A does not degrade cephalosporins, a major risk factor for *Clostridium difficile* infection

SYNTHETIC BIOLOGICS

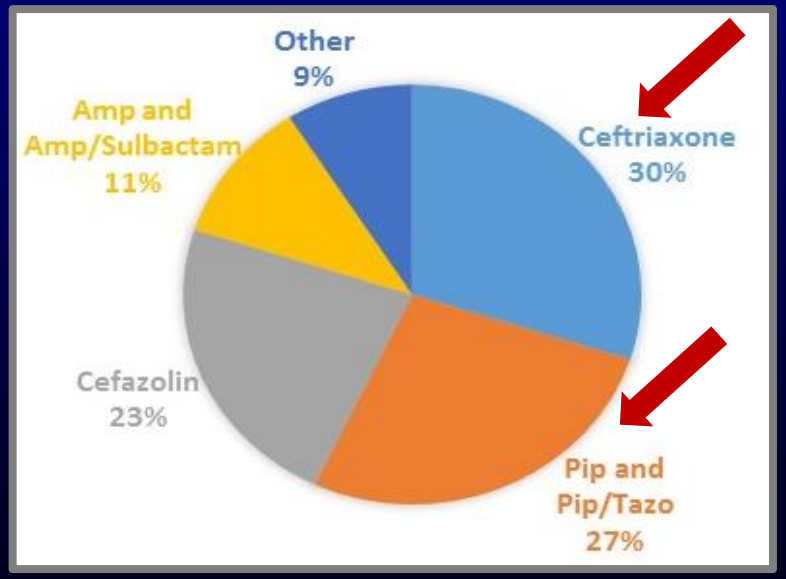
Ceftriaxone Is an Important Antibiotic Target

IV β-Lactam Use

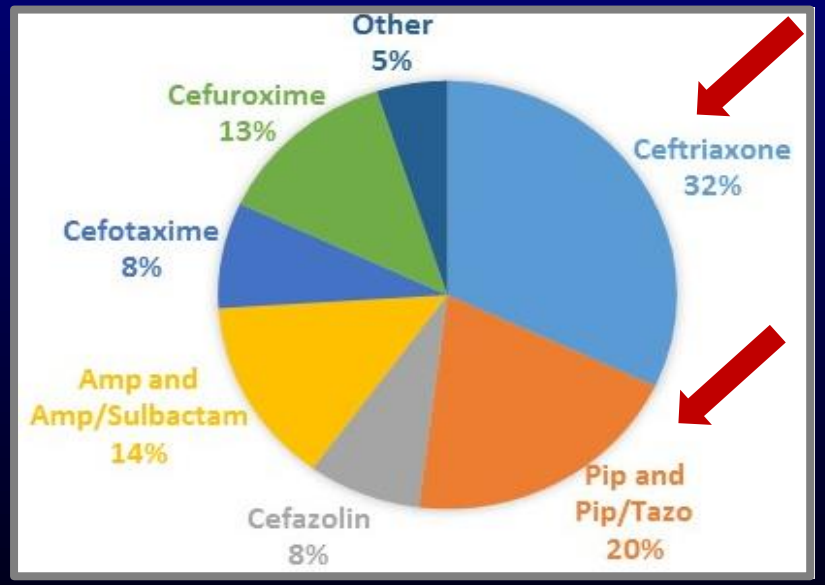
	US		EU	
	Patients	**Days on Therapy**	**Patients**	**Days on Therapy**
Total IV Abx	23 million	170 million	17 million	115 million
IV β–lactams	17 million	73 million	10 million	46 million
% of Total	72%	43%	70%	40%

Individual β-Lactam Antibiotics: Days on Therapy

US



EU



Arlington Medical Resources (AMR), a Decision Resources Group Company 2014 audits of acute care hospital antibiotic utilization

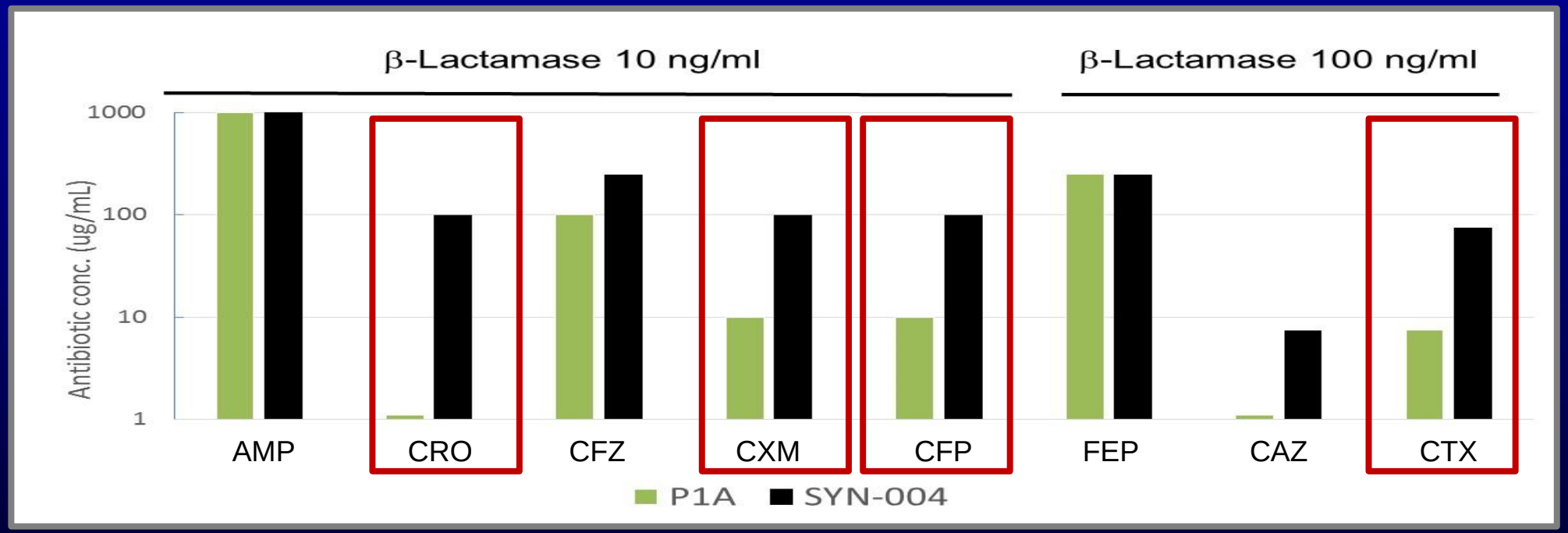
SYNTHETIC BIOLOGICS

SYN-004 Degrades Cephalosporins

SYN-004 was engineered from P1A

One amino acid substitution: D276N

E. coli growth microtiter plate assay



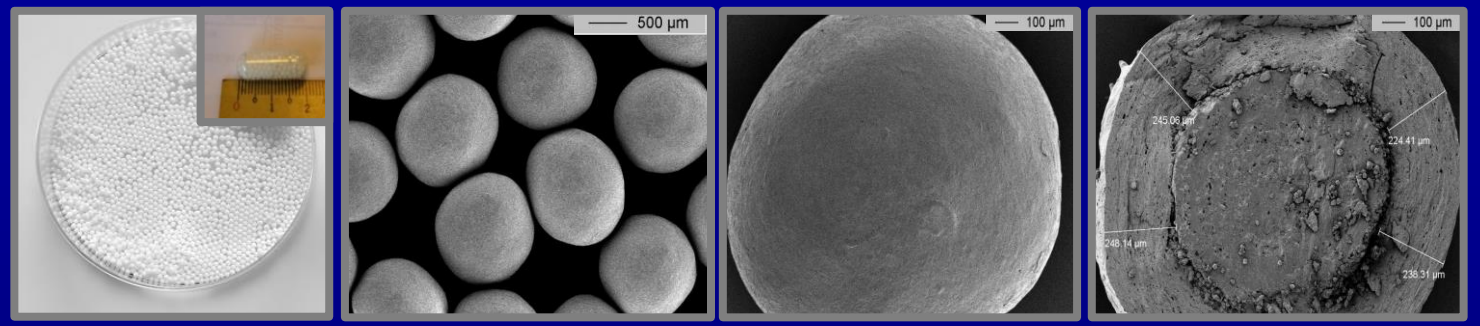
Amp: ampicillin
CRO: ceftriaxone
CFZ: cefozolin
CXM: cefuroxime
CFP: cefoperazone
FEP: cefepime
CAZ: ceftazidime
CTX: cefotaxime

SYN-004 efficiently degrades cephalosporins

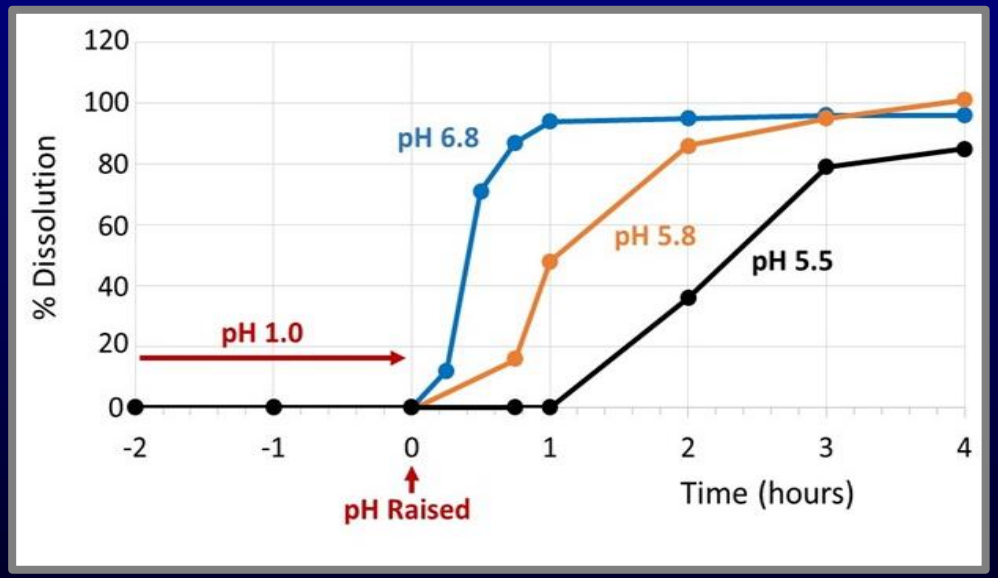
SYNTHETIC BIOLOGICS

SYN-004 is Stable in Human Chyme

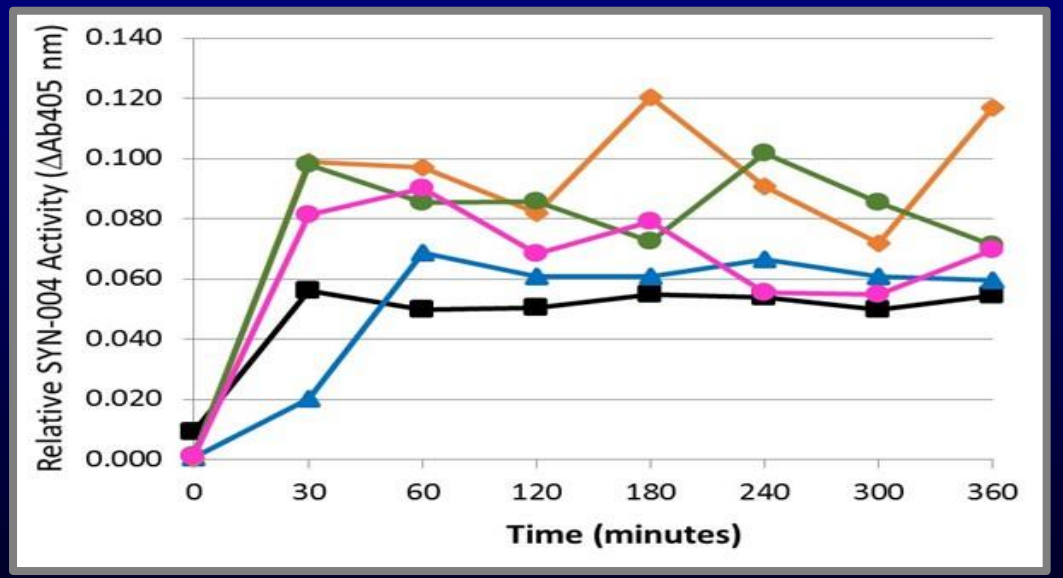
SYN-004 Enteric-Coated Pellets



pH Dissolution Profile



Stability in Human Chyme



SYN-004 Degrades CRO in the Dog GI Tract

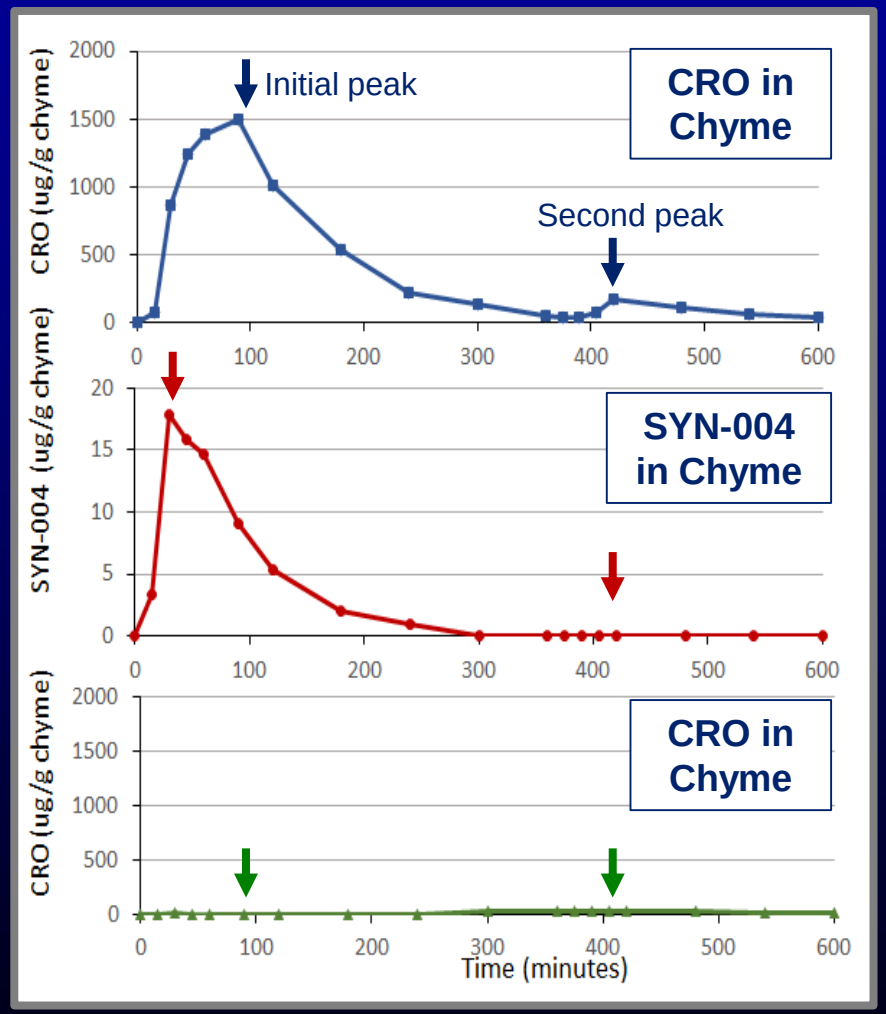
Six fistulated dogs received IV ceftriaxone +/- oral SYN-004

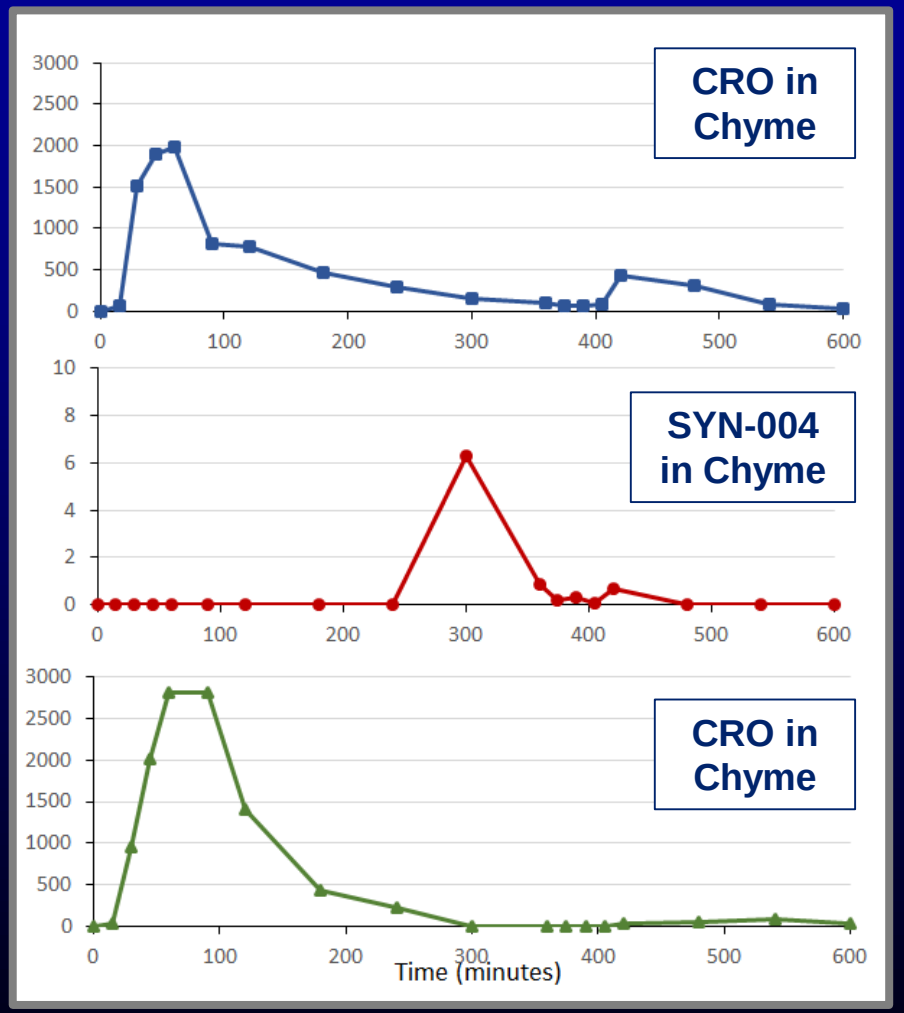
Chyme was collected and assayed for ceftriaxone (CRO) and SYN-004

CRO Alone

SYN-004 + CRO

SYN-004 + CRO





SYN-004 Dog Toxicity Studies

Study Design

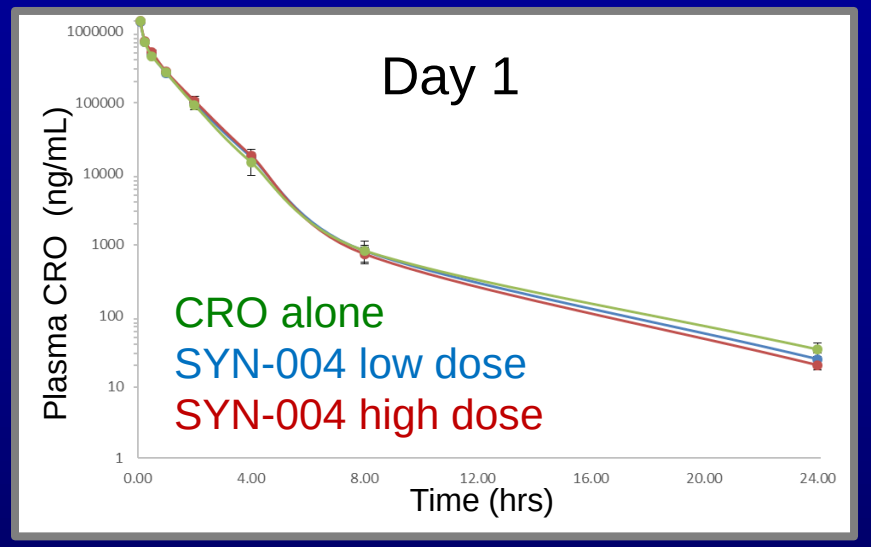
Ceftriaxone intravenously 1X per day for 14 days
SYN-004 orally 3X per day
Three cohorts (n=6)

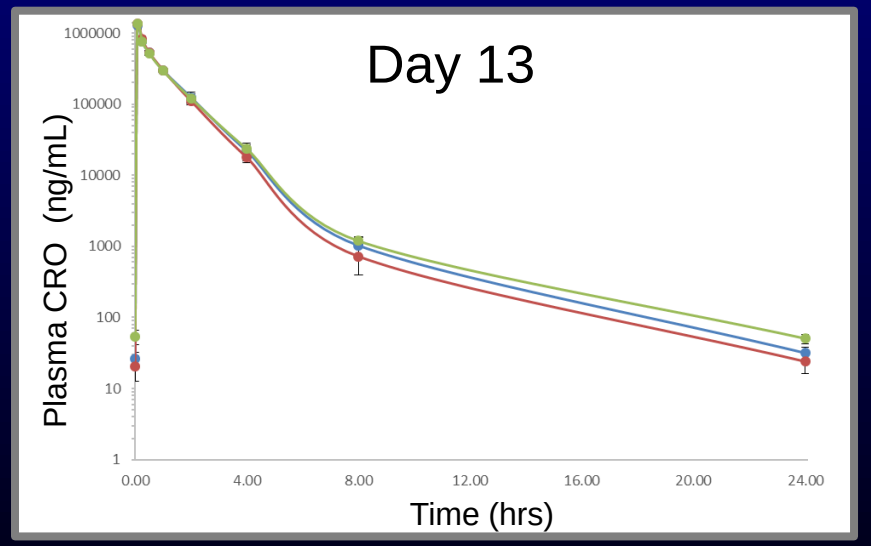
- CRO alone
- CRO plus SYN-004 (6.6 mg/kg/day)
- CRO plus SYN-004 (57 mg/kg/day)

Results

Safe and well tolerated
NOAEL of 57 mg/kg/day, highest dose tested
Not detected systemically
Did not affect ceftriaxone blood levels

Plasma CRO





SYNTHETIC BIOLOGICS

SYN-004 is in Phase 2 Clinical Trials

Clinical Results

Phase 1 clinical studies demonstrated SYN-004 was safe and well tolerated with a single dose of up to 750 mg and multiple doses of 300 mg 4X per day for 7 days

Phase 2a clinical studies were initiated in 1H 2015

- Ileostomy studies
- To confirm that SYN-004 removes CRO from the chyme without altering CRO plasma levels

A Phase 2b clinical study is on track to be initiated in 3Q 2015

- Endpoints include CDI

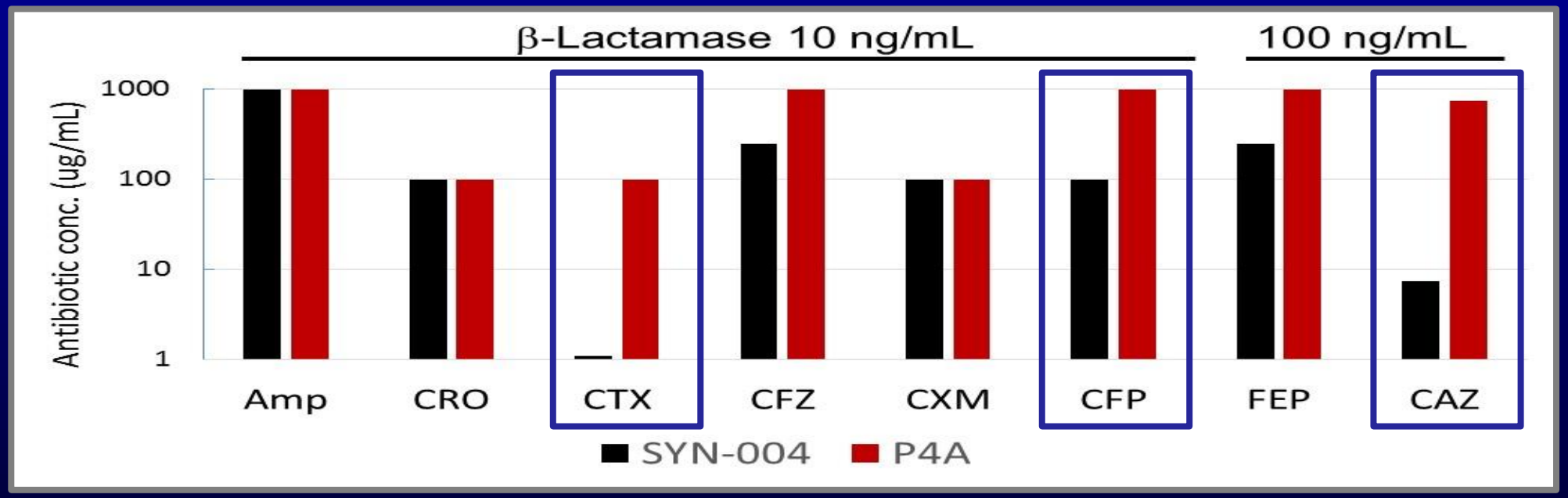
SYNTHETIC BIOLOGICS

P4A Further Expands the Antibiotic Degradation Profile

P4A was engineered from SYN-004 using random mutagenesis and rational design

Contains 4 aa substitutions: A232G, A237S, A238G, and S240D

E. coli growth microtiter plate assay



Amp: ampicillin
CRO: ceftriaxone
CTX: cefotaxime
CFZ: cefozolin
CXM: cefuroxime
CFP: cefoperazone
FEP: cefepime
CAZ: ceftazidime

P4A further improves the degradation of cephalosporins

However, P4A does not degrade carbapenems

SYNTHETIC BIOLOGICS

P2A, NDM, and KPC are Broad-Spectrum Carbapenemases

P2A

Isolated from *Bacillus cereus*
Class B metallo β-lactamase
Requires Zn^{2+} for activity
Resistant to β-lactamase inhibitors

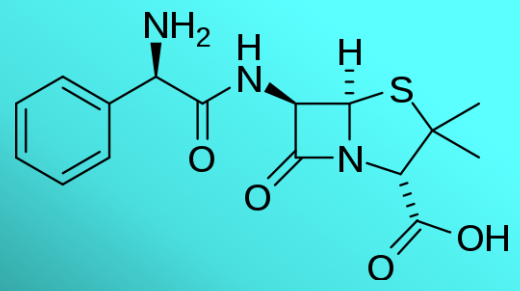
NDM

New Delhi Metallo-β-lactamase
Class B metallo β-lactamase
Requires Zn^{2+} for activity
Resistant to β-lactamase inhibitors

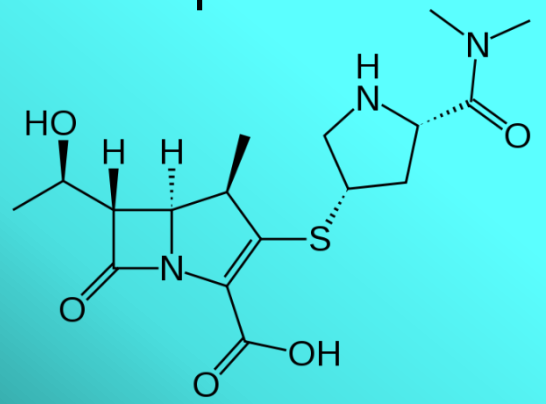
KPC

Klebsiella pneumoniae carbapenemase
Class A serine β-lactamase

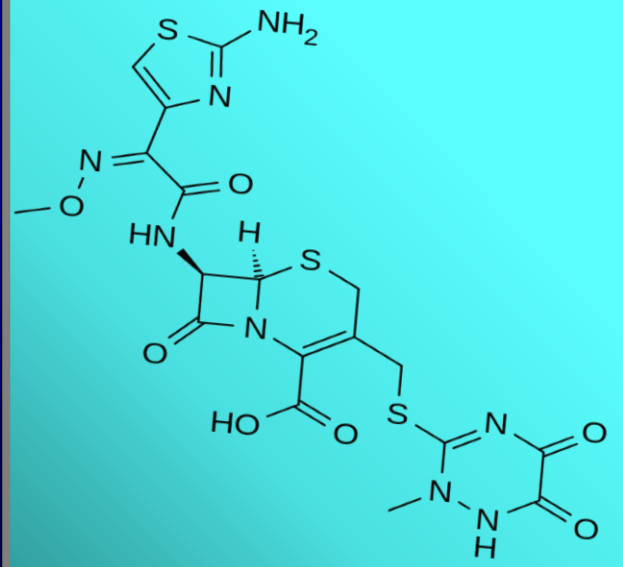
Ampicillin



Meropenem



Ceftriaxone



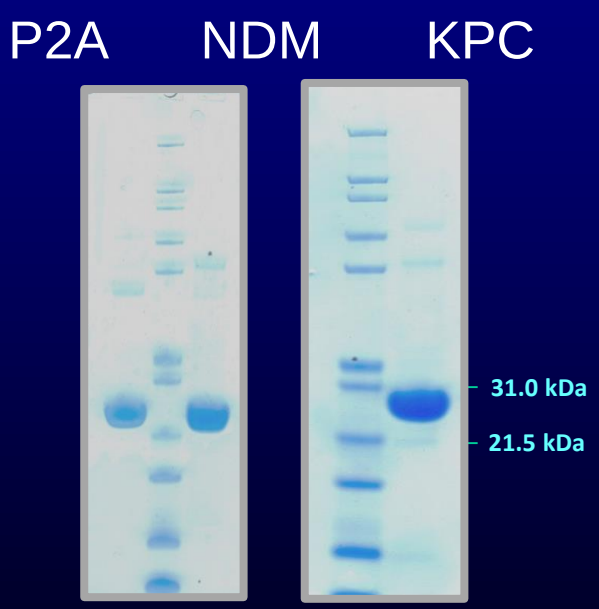
P2A, NDM, and KPC Were Produced in *E. coli*

Over 100 *E. coli* strains were generated

P2A and NDM were caught in inclusion bodies

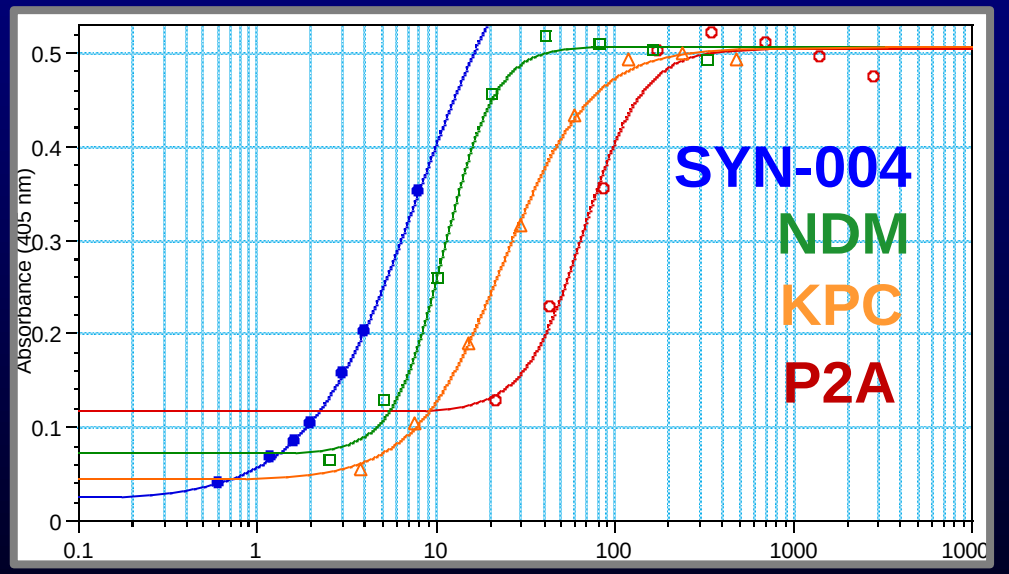
But Zn^{2+} shifted their expression to the soluble cytoplasmic fraction

Purification



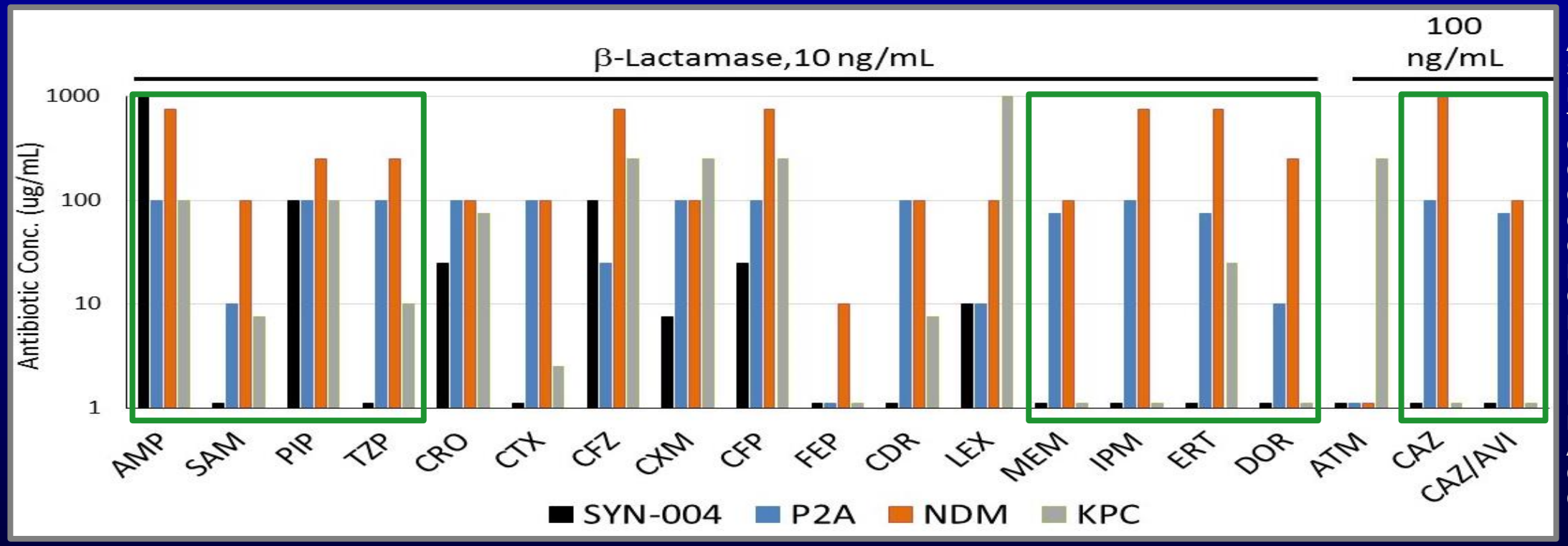
Activity

CENTA Chromogenic Assay



Antibiotic Degradation Profiles of the Purified Carbapenemases

P2A, NDM, and KPC were compared to SYN-004



AMP:ampicillin
SAM:amp/sulbactam
PIP:piperacillin
TZP:pip/tazobactam
CRO:ceftriaxone
CTX:cefotaxime
CFZ:cefozolin
CXM:cefuroxime
CFP:cefoperazone
FEP:cefepime
CDR: cefdinir
LEX: cephalexin
MEM:meropenem
IPM:imipenem
ERT:ertapenem
DOR:doripenem
ATM:aztreonam
CAZ:ceftazidime
CAZ/AVI: caz/avibactam

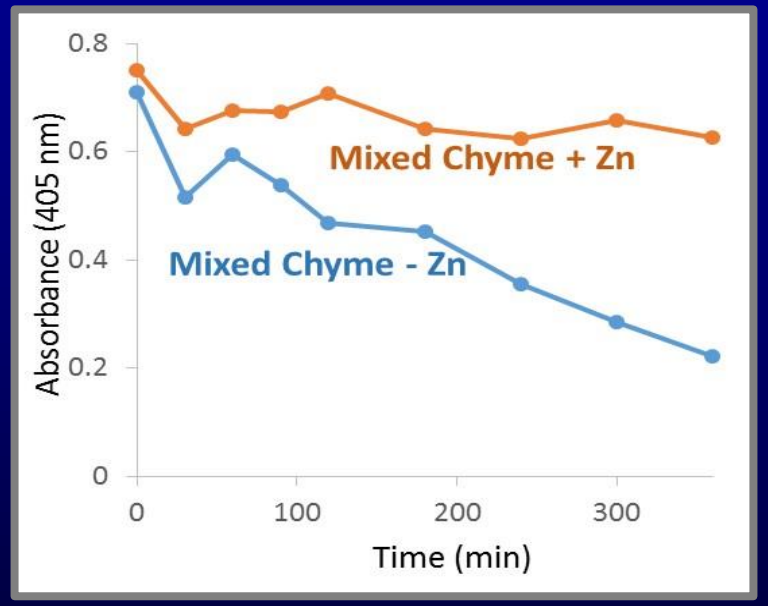
NDM displayed the broadest antibiotic degradation profile. P2A was a close second.

SYNTHETIC BIOLOGICS

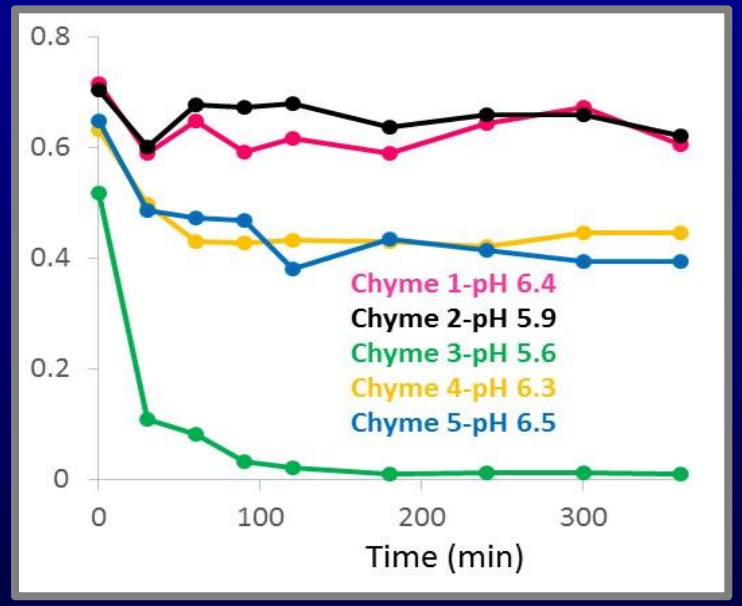
P2A is Stable in Human Chyme

P2A was incubated in human chyme and activity was assessed with the CENTA assay

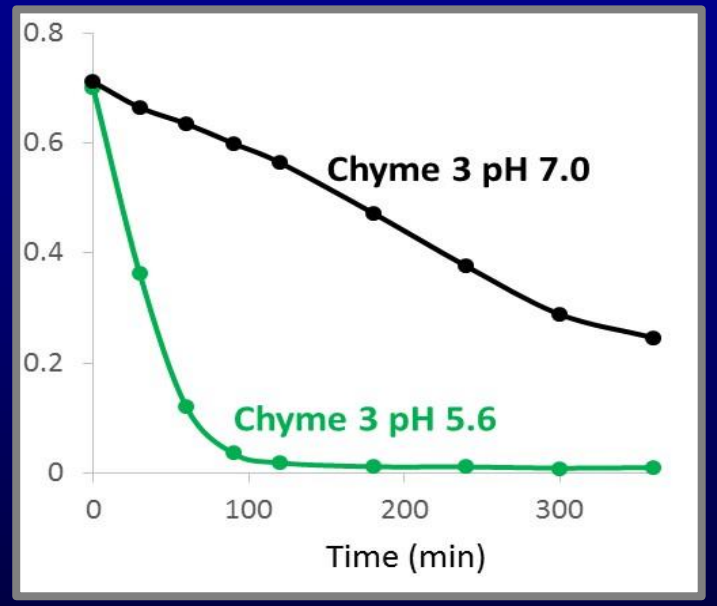
Mixed Chyme



Individual Chyme



pH-Adjusted Chyme 3



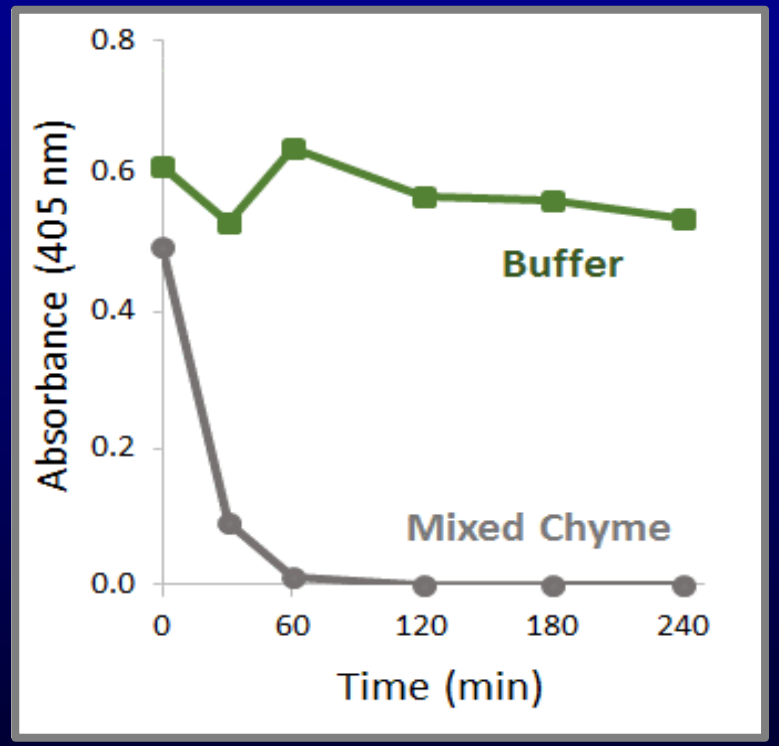
P2A displayed sustained biological activity in human chyme but was sensitive to low pH

SYNTHETIC BIOLOGICS

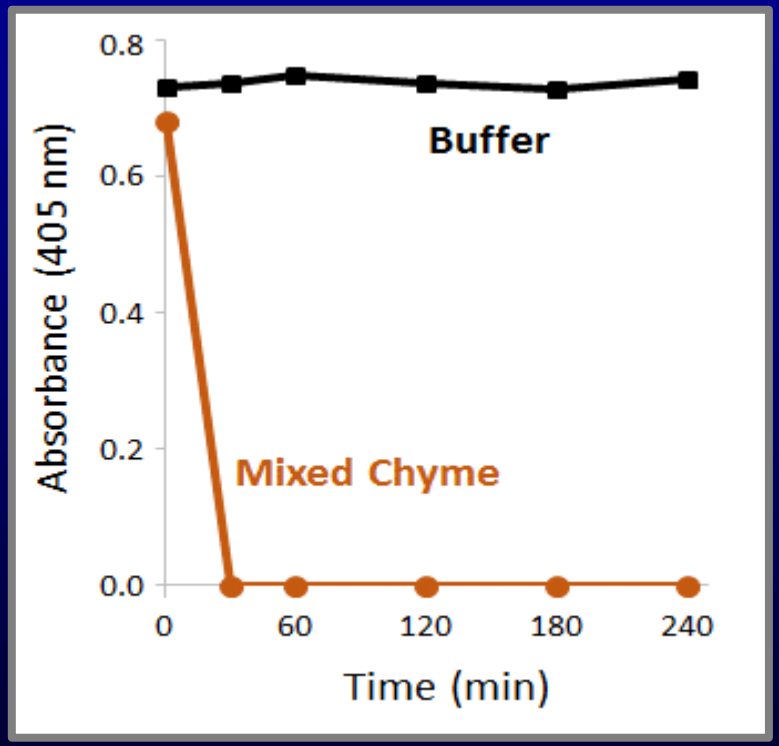
NDM and KPC Were Less Stable in Human Chyme

NDM and KPC were incubated in human chyme and activity was assessed with the CENTA assay

KPC



NDM

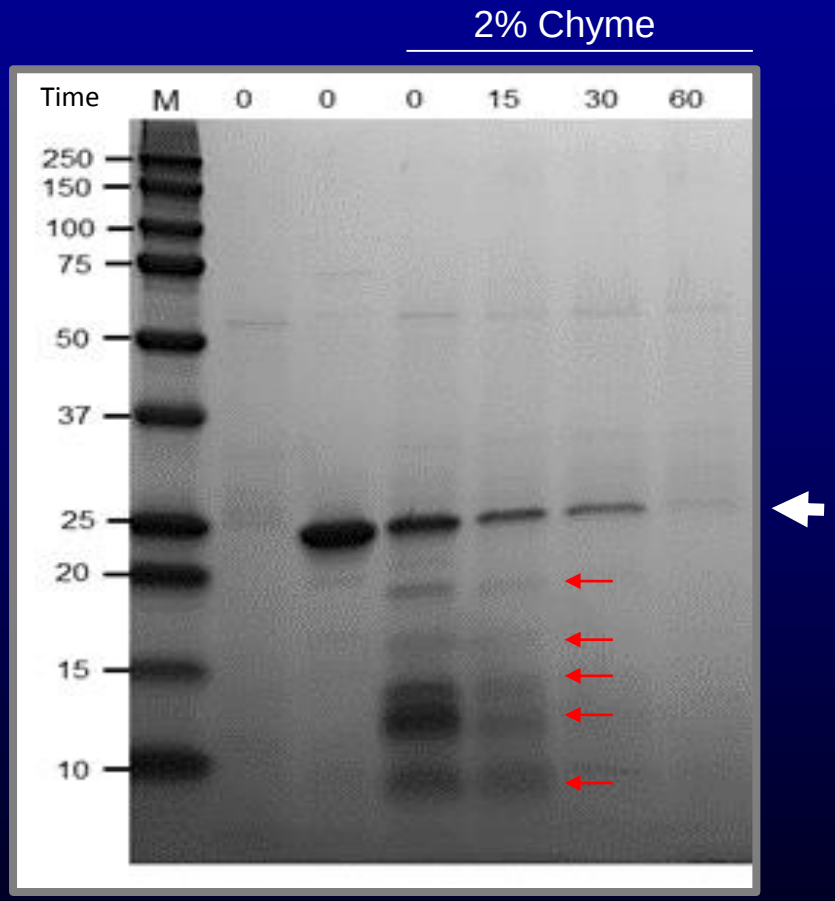


Why was NDM less stable in chyme?

NDM Cleaved by Human Chyme

Purified NDM was incubated in 2% human chyme and the initial cleavage sites were mapped

SDS/PAGE



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ANALSNQLAPQEGMVAAQHSLTFAANGWVEPATAP
NFGPLKVFYPGPGHTSDNITVGIDGTDIAFGGCLI
KDSKAKSLGNLGDADTEHYAASARAFGAAFPKASM
IVMSHSAPDSRAAITHTARMADKLR

NDM contains a limited number of sites
at which protease digestion starts

P2A Degrades Meropenem in the Dog GI Tract

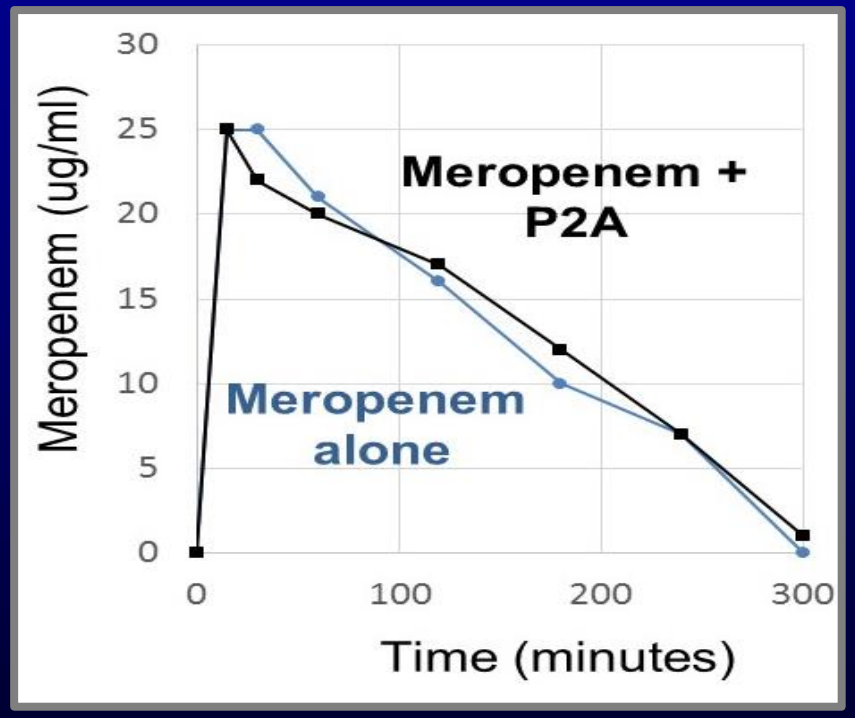
6 fistulated dogs received IV meropenem 3 received oral P2A in liquid formulation

Chyme was assayed for meropenem and P2A and serum was assayed for meropenem

Chyme Meropenem and P2A

Treatment (n=3)	Dog	P2A (U/g)	Meropenem (ug/g)
Meropenem Alone	1	NA	3.0
	2	NA	3.2
	3	NA	3.0
Meropenem + P2A	4	80	0
	5	0.5	0
	6	0.2	2.0

Serum Meropenem



P2A degraded meropenem in the dog GI tract without altering the systemic levels

SYNTHETIC BIOLOGICS

Conclusions

SYN-004 is intended as an orally-delivered β-lactamase to protect the gut microbiome from IV penicillins and cephalosporins to prevent *C. difficile* infection

Clinical validation was achieved with the SYN-004 precursor, P1A

SYN-004 is progressing though Phase 2 clinical trials

SYN-004 and P4A are broadly acting cephalosporinases that do not degrade carbapenems

P2A, NDM, and KPC were evaluated as pipeline candidates

P2A was chosen based on broad antibiotic degradation and stability in human chyme

Currently formulating P2A for evaluation in a pig model

Acknowledgements

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