Novel Broad-Spectrum β-Lactamase Therapy to Protect the Gut Microbiome from Antibiotics

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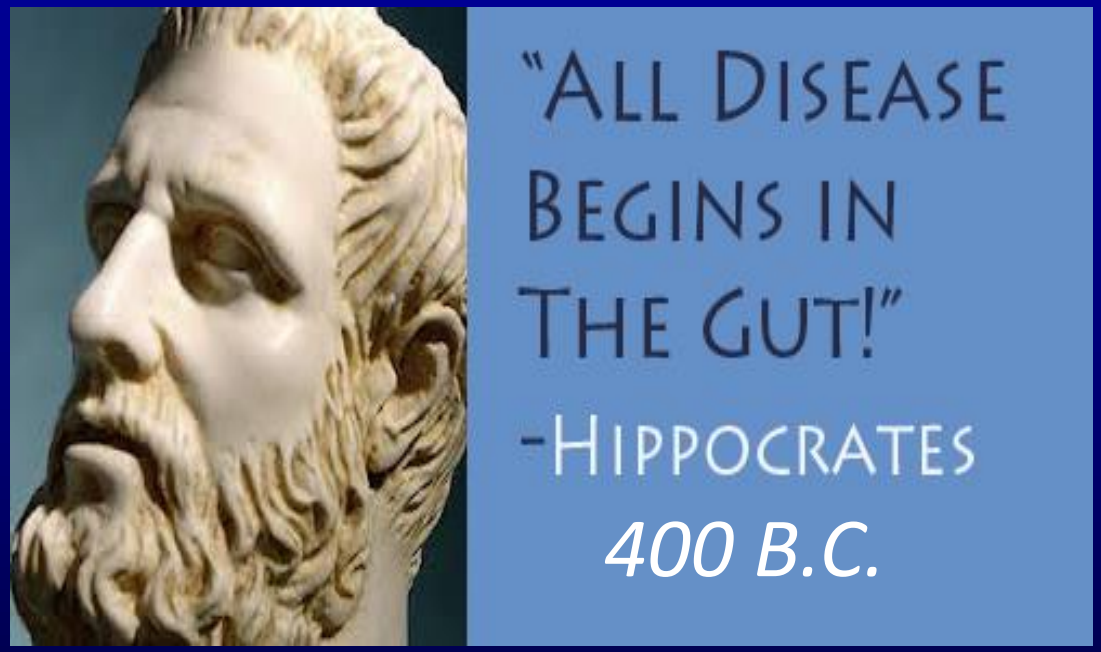
Importance of Intestinal Health Has Long Been Recognized



Gut Microbiome Involved in:

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

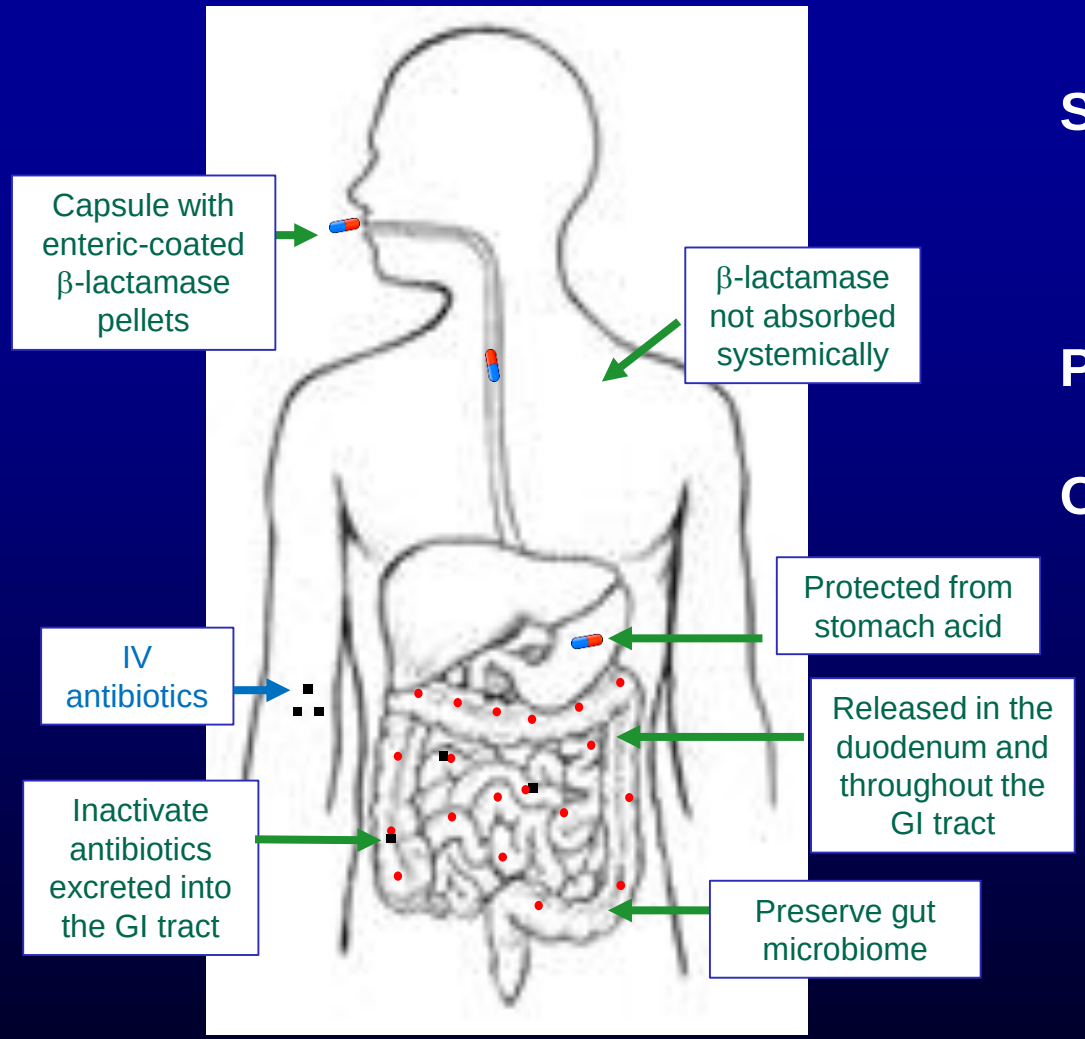
Disrupted by:

- Antibiotic use

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

SYNTHETIC BIOLOGICS

β-Lactamases: From Enemies to Therapies



Strategy: β-lactamase enzyme is intended to degrade residual antibiotics in the GI tract to protect the microbiome

Product: Capsule with enteric-coated enzyme

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

Orally-delivered β–lactamases intended to degrade residual antibiotics in the GI tract to protect the gut microbiome without affecting antibiotic efficacy

SYNTHETIC BIOLOGICS

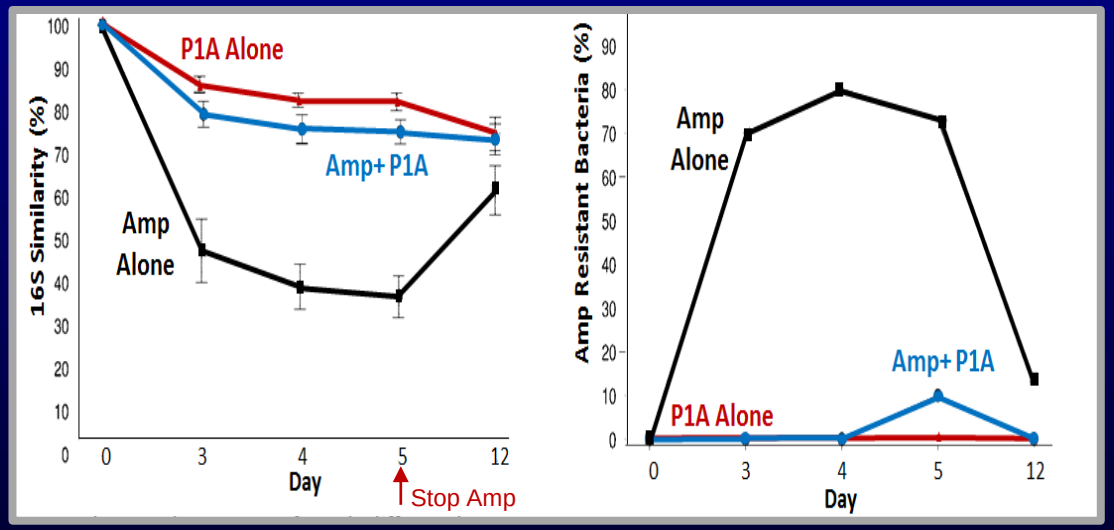
β-Lactamase Clinical Efficacy: Degradation of Intestinal Penicillins

P1A

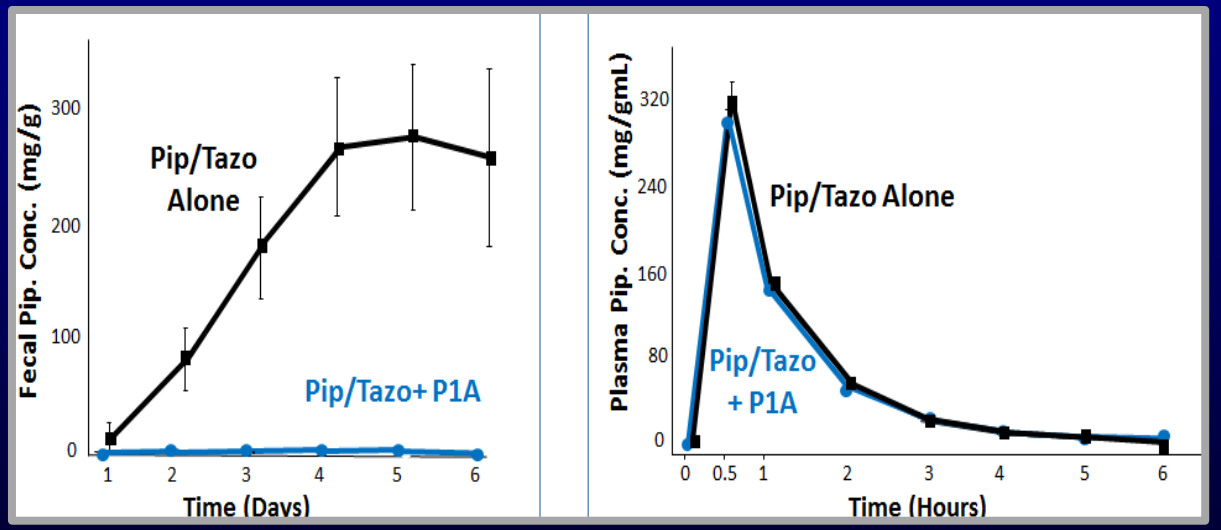
- Clinical isolate from *Bacillus licheniformis*
- Class A serine β–lactamase
- Degrades penicillins

Clinical results

Similarity Index | Amp-Resistant Bac | GI Tract | Systemic



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



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

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

SYNTHETIC BIOLOGICS

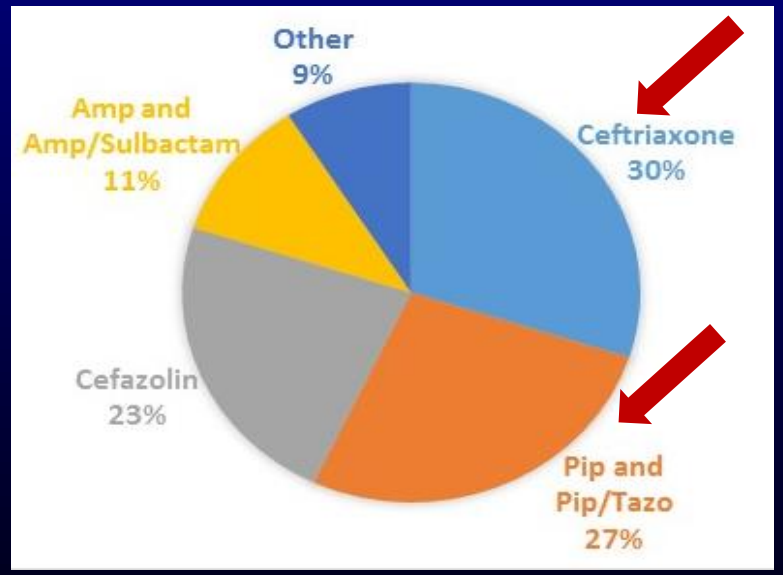
Ceftriaxone and Pip/Tazo are the Most Frequently Used IV β-Lactams

IV β-Lactam Use

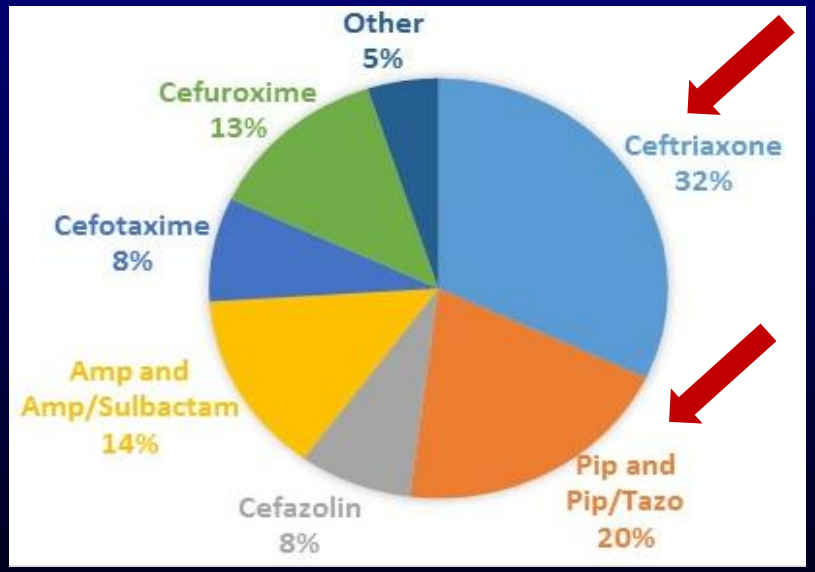
	US		EU	
	Patients	**Days on Therapy**	**Patients**	**Days on Therapy**
Total IV Abx	23 million	170 million	17 million	115 million
IV β–lactams	17 million	73 million	10 million	46 million
% of Total	72%	43%	70%	40%

Individual b-Lactam Antibiotics: Days on Therapy

US



EU



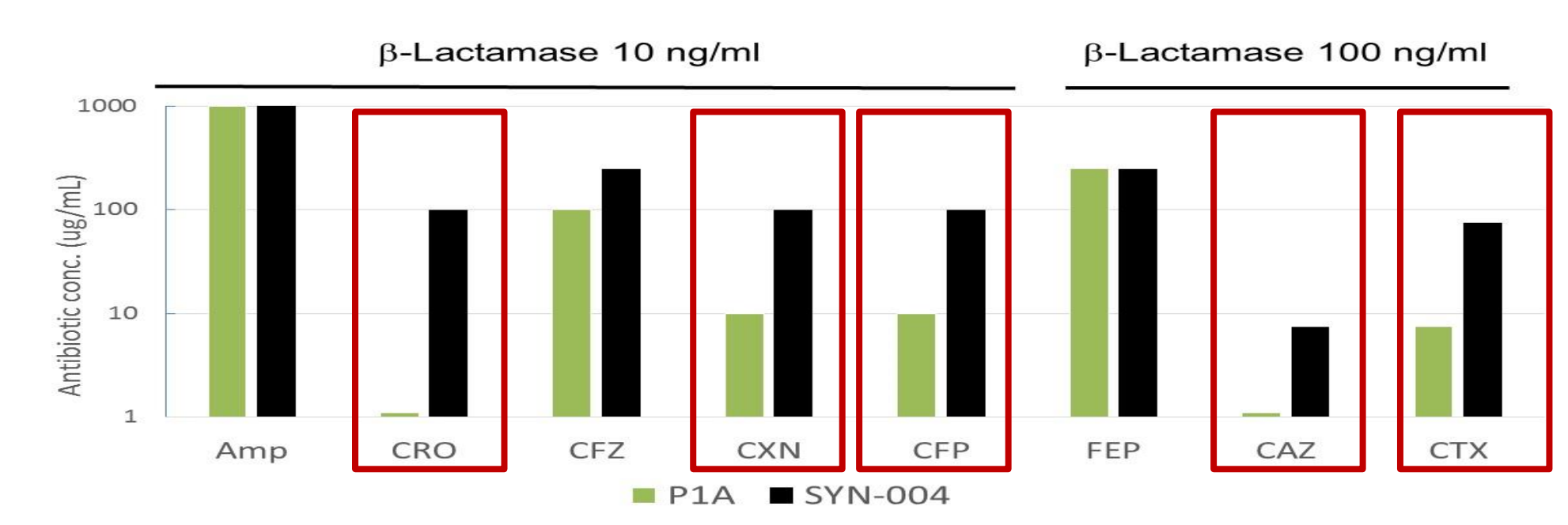
Arlington Medical Resources (AMR), a Decision Resources Group Company 2014 audits of acute care hospital antibiotic utilization

SYNTHETIC BIOLOGICS

SYN-004 Degrades Cephalosporins

- SYN-004 was engineered from P1A
- Contains one amino acid substitution: D276N

E. coli growth microtiter plate assay



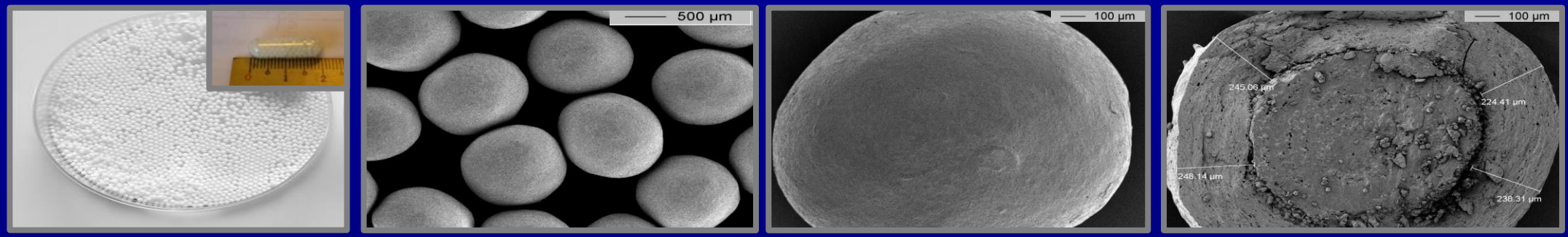
Amp: ampicillin
CRO: ceftriaxone
CFZ: cefozolin
CXM: cefuroxime
CFP: cefoperazone
FEP: cefepime
CAZ: ceftazidime
CTX: cefotaxime

SYN-004 efficiently degrades cephalosporins, including ceftriaxone, cefuroxime, cefoperazone, ceftazidime, and cefotaxime

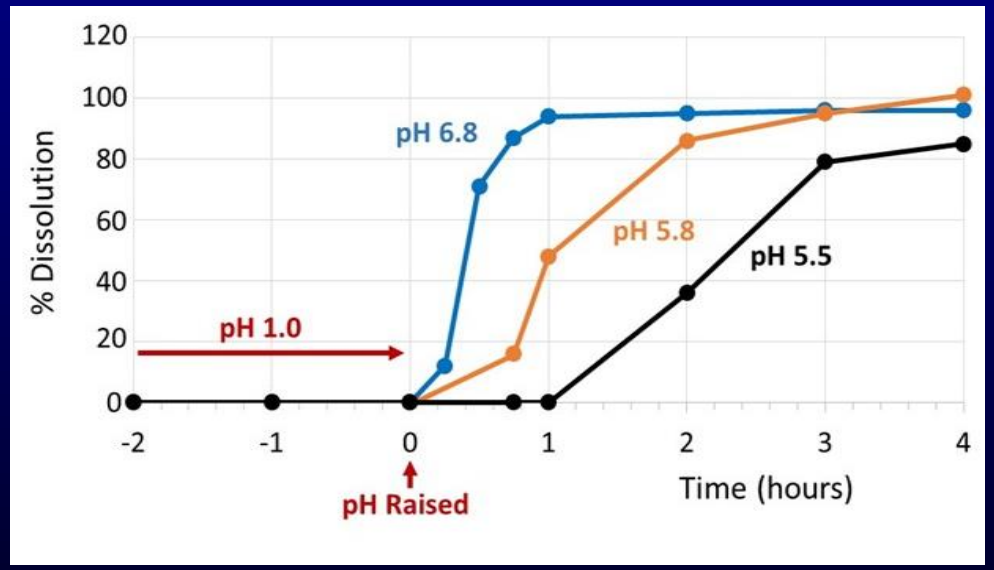
SYNTHETIC BIOLOGICS

SYN-004 Oral Formulation is Stable in Human Chyme

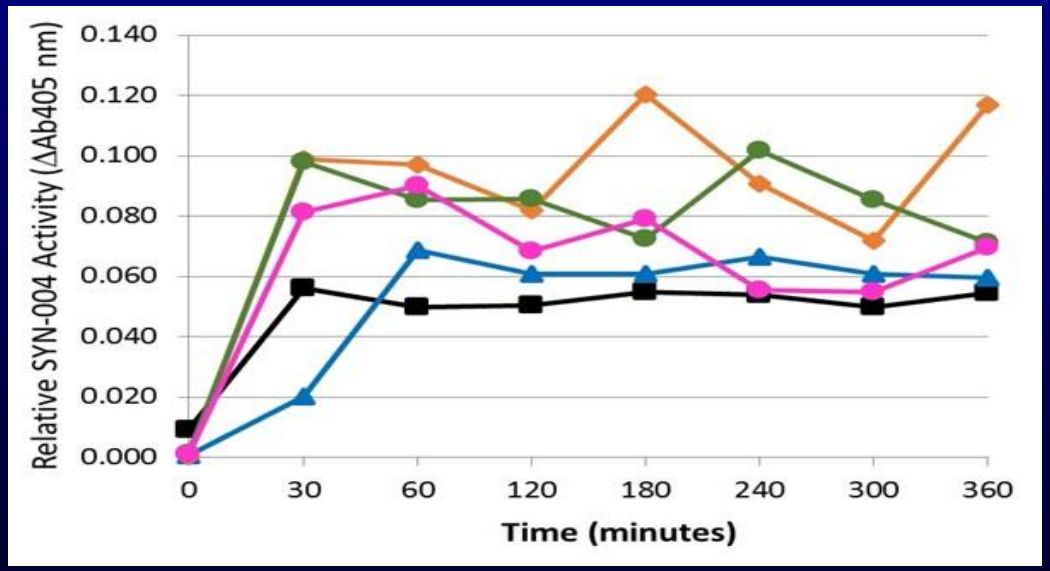
SYN-004 Enteric-Coated Pellets



pH Dissolution Profile



Stability in Human Chyme



Enteric-coated SYN-004 pellets remain intact at low pH and released enzyme retains biological activity for at least 6 hours in human intestinal contents

SYNTHETIC BIOLOGICS

SYN-004 is in Phase 2 Clinical Trials

Preclinical Results

- Safe in two GLP toxicity studies in dogs
- Well tolerated with a NOAEL of 57 mg/kg/day, highest dose tested
- Not detected systemically
- Did not affect ceftriaxone blood levels

Clinical Results

- Phase 1 clinical studies demonstrated SYN-004 safety and tolerability with a single dose of up to 750 mg and multiple doses of 300 mg 4X a day for 7 days
- SYN-004 was neither systemically bioavailable nor immunogenic
- Phase 2a clinical studies were initiated in 1H 2015
- A Phase 2b clinical study is on track to be initiated in 3Q 2015

SYN-004 efficiently degrades penicillins and cephalosporins but does not degrade carbapenems

SYNTHETIC BIOLOGICS

P2A, NDM, and KPC are Broad-Spectrum Carbapenemases

P2A

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

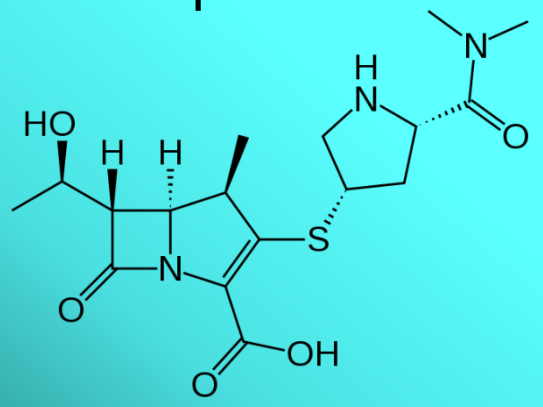
NDM

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

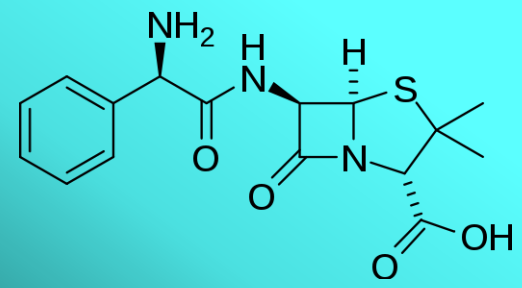
KPC

- *Klebsiella pneumoniae* carbapenemase
- Class A serine β-lactamase

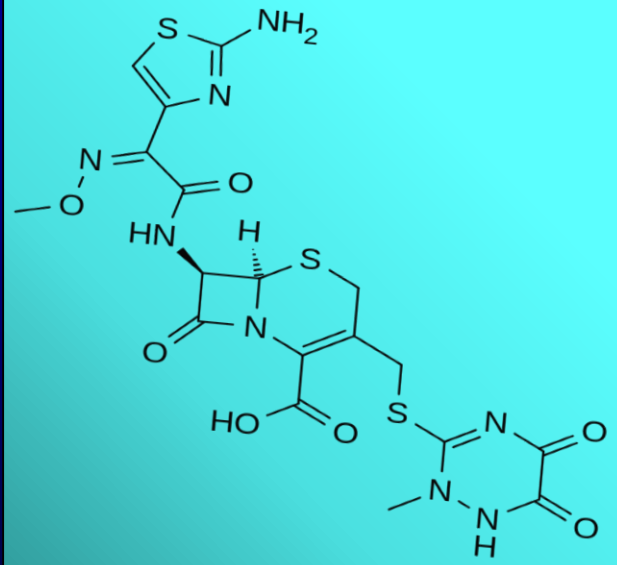
Meropenem



Ampicillin



Ceftriaxone

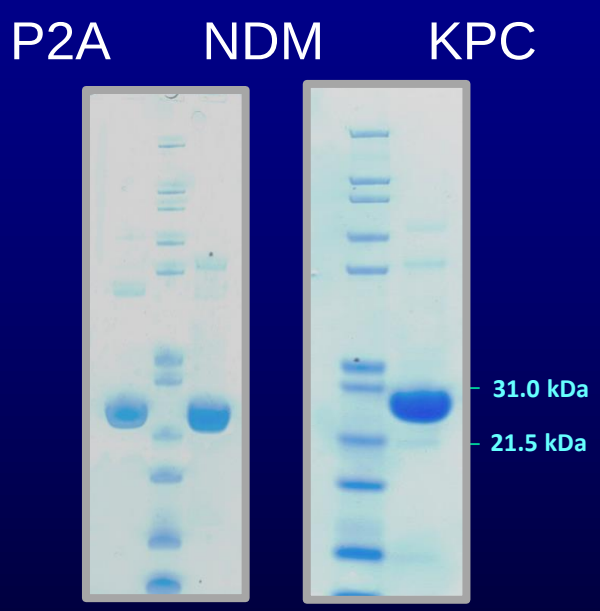


SYNTHETIC BIOLOGICS

Expression of P2A, NDM, and KPC in *E. coli*

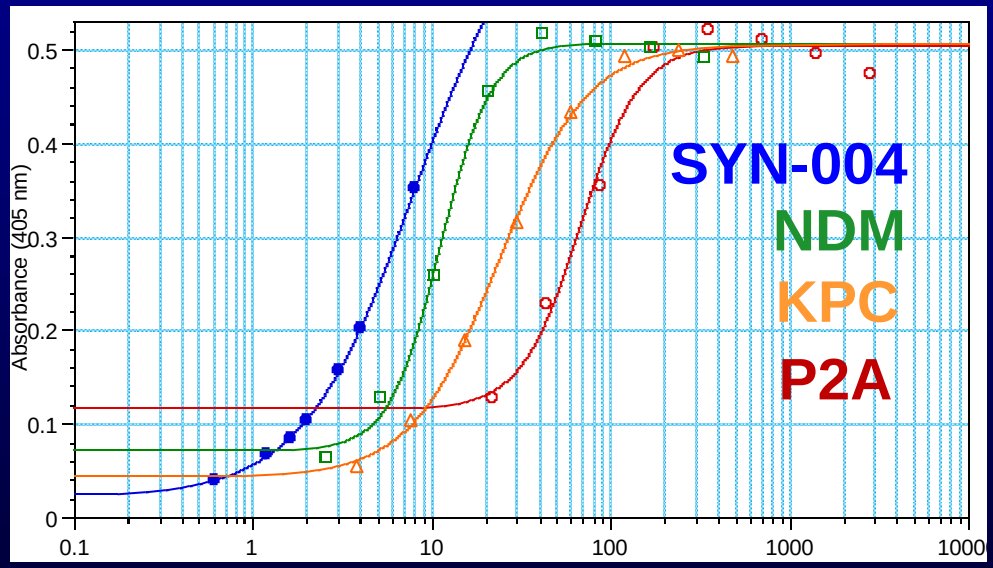
- Over 100 *E. coli* strains were generated
- P2A, NDM, and KPC scaled to 5L bioreactor fermentation

Purification



Activity

CENTA Chromogenic Assay



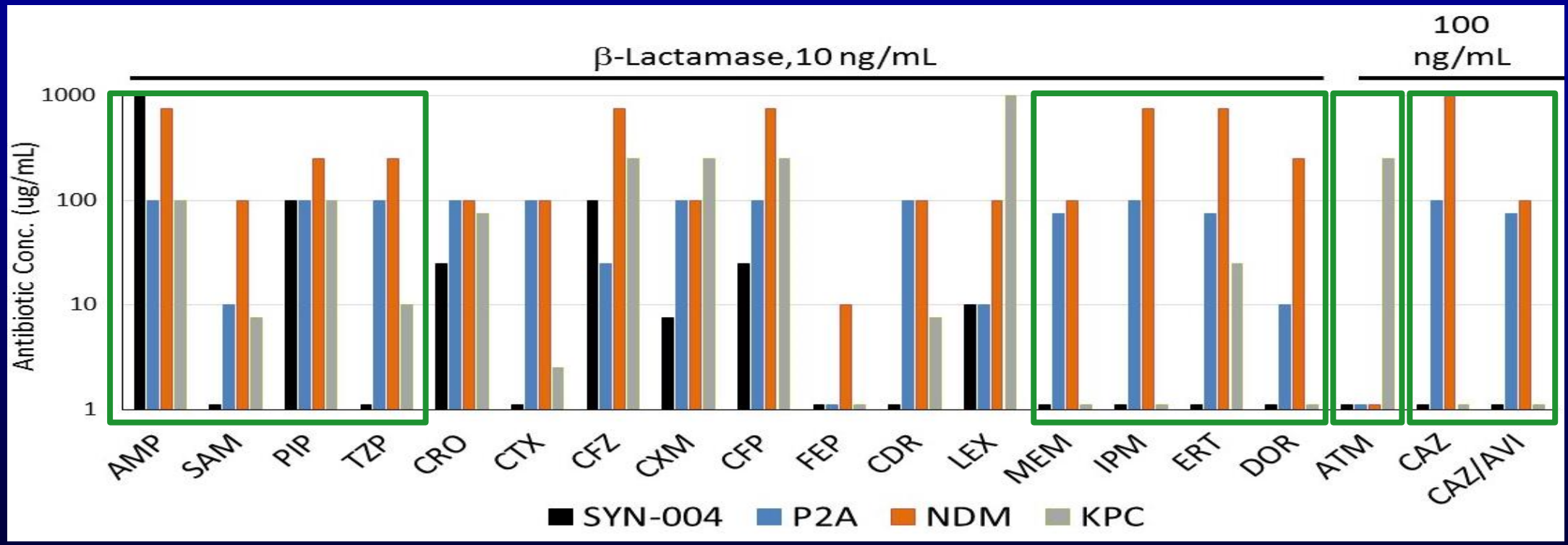
Carbapenemases were efficiently produced in *E. coli* and retained biological activity following purification

SYNTHETIC BIOLOGICS

Antibiotic Degradation Profile of Selected Carbapenemases

P2A, NDM, KPC were compared to SYN-004

E. coli growth microtiter plate assay



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

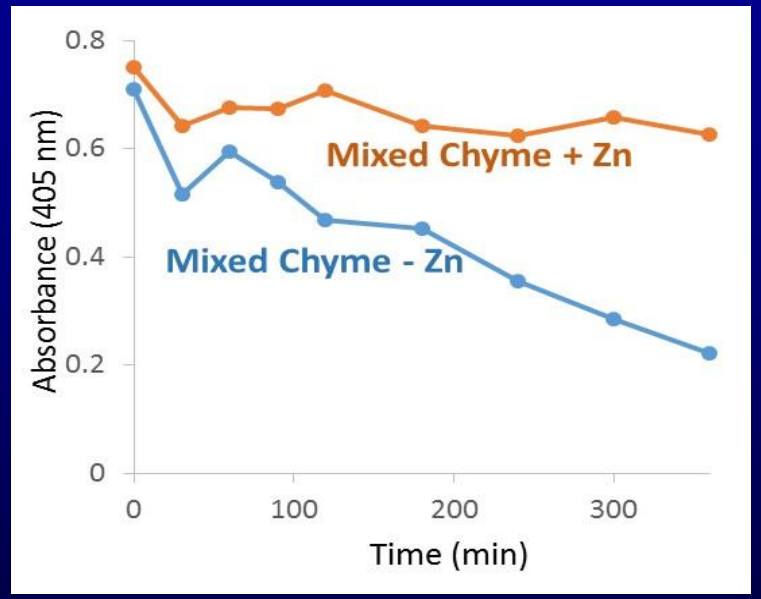
P2A and NDM display the broadest antibiotic degradation profiles including penicillins, cephalosporins, and carbapenems and are resistant to β-lactamase inhibitors

SYNTHETIC BIOLOGICS

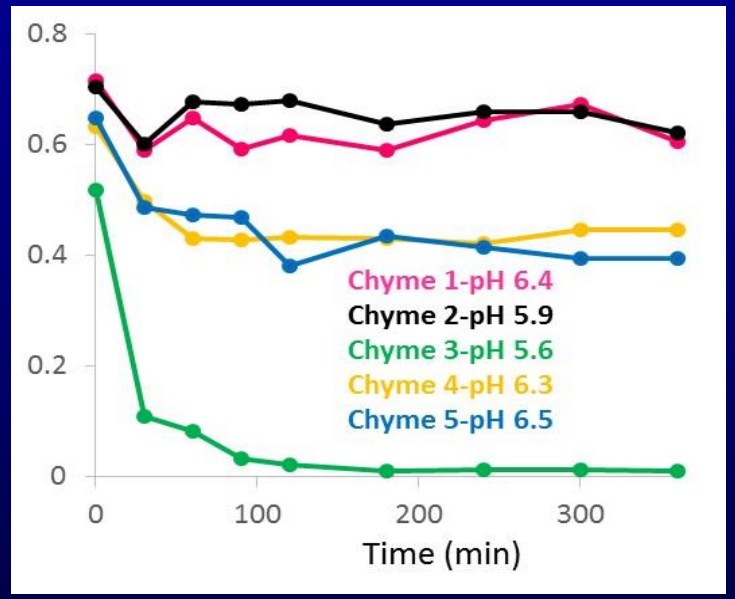
P2A is Stable in Human Chyme

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

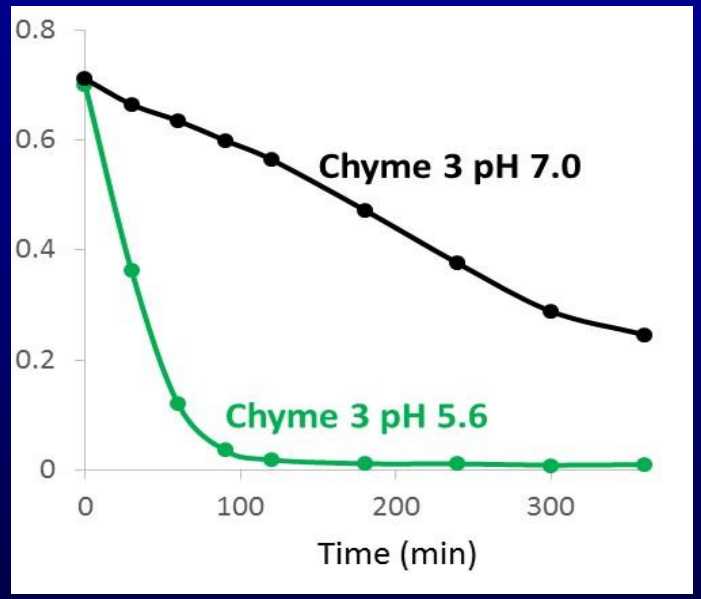
Mixed Chyme



Individual Chyme



pH-Adjusted Chyme 3



- P2A displayed sustained biological activity in human chyme in the presence of Zn^{2+}
- P2A was sensitive to pH as increasing the pH of Chyme 3 improved P2A stability

SYNTHETIC BIOLOGICS

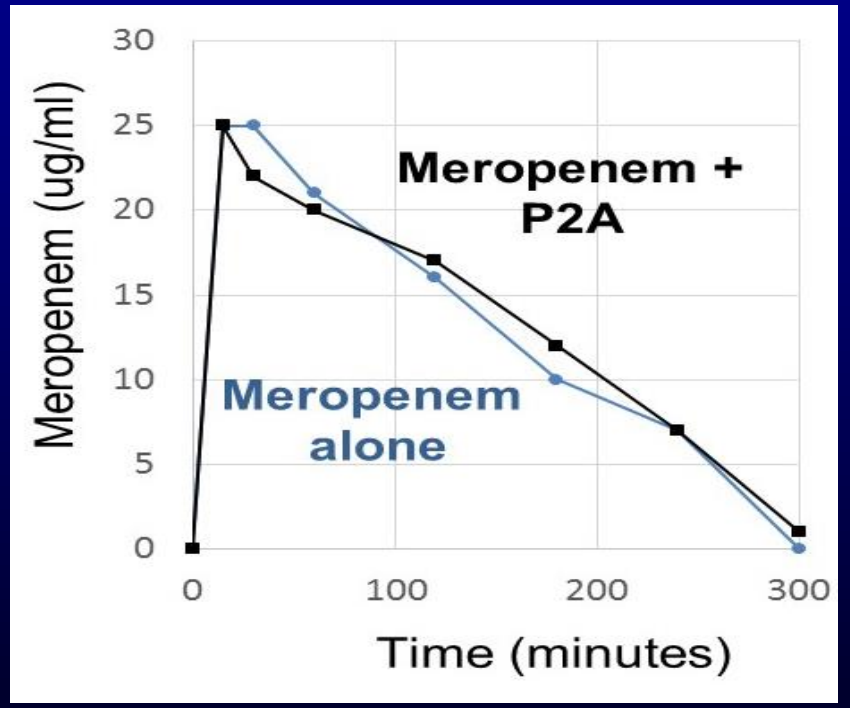
P2A Degrades Meropenem in Dog GI Tract

- Fistulated dogs (n=6) received IV meropenem (30 mg/kg)
- P2A (liquid formulation) was delivered orally (1 mg/kg) following antibiotic injection
- Levels of meropenem and P2A in the jejunual contents and serum were measured

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 the meropenem in the dog GI tract and did not affect meropenem serum 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 *Clostridium difficile* infection
- Clinical validation was achieved with the SYN-004 precursor, P1A
- SYN-004 is progressing though Phase 2 clinical trials
- SYN-004 is a broadly acting cephalosporinase that does 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
- P2A formulation and evaluation in a pig microbiome model is in progress

SYNTHETIC BIOLOGICS

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