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Outer Membrane Permeabilizer – SPR741
Aileen Rubio, Head of Biology

Acknowledgements

Spero Therapeutics

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Agenda

- Brief background on Spero
- Ways to permeabilize the outer membrane
- Concept of potentiation – case study using SPR741
 - Mechanistic insights
 - *In vitro* potentiation with multiple antibacterial classes
 - *In vivo* potentiation in mouse models of infection
 - Current status



Background on Spero Therapeutics

First novel programs to address unmet needs in 40 years



SPR994: Oral Gram-negative



SPR741: Potentiator Platform



SPR720: NTM Program

Financial Strength

\$52M Series C advances runway through 2017-18 inflection points; \$15M additional in non-dilutive funding

atlas venture



LUNDBECKFONDEN



MERCK

RACAPITAL

THE KRAFT GROUP



ROCK SPRINGS CAPITAL



SR-one

Strong team and track record



...of combined experience on core team

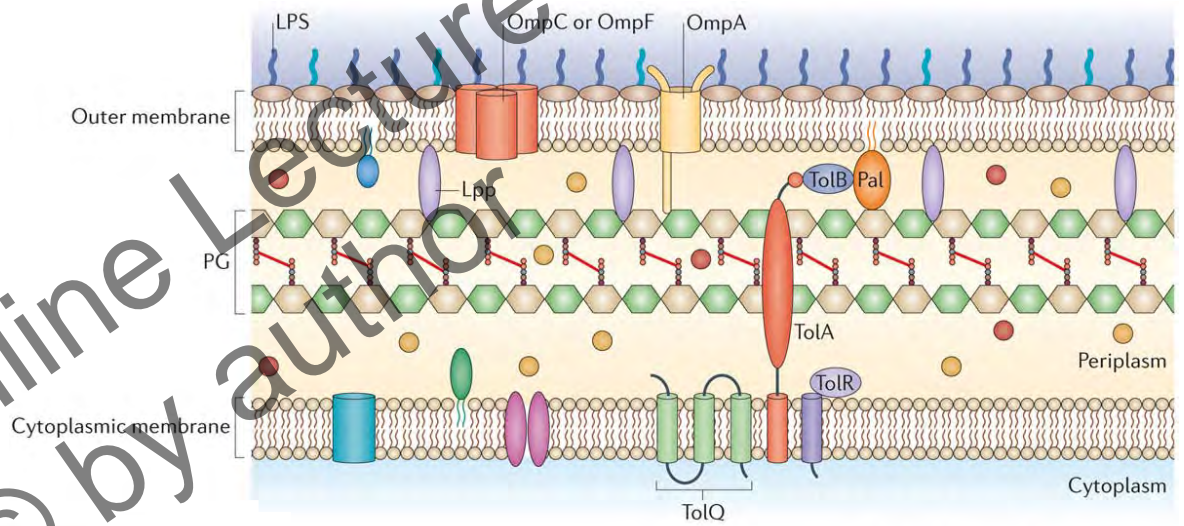


approved drugs

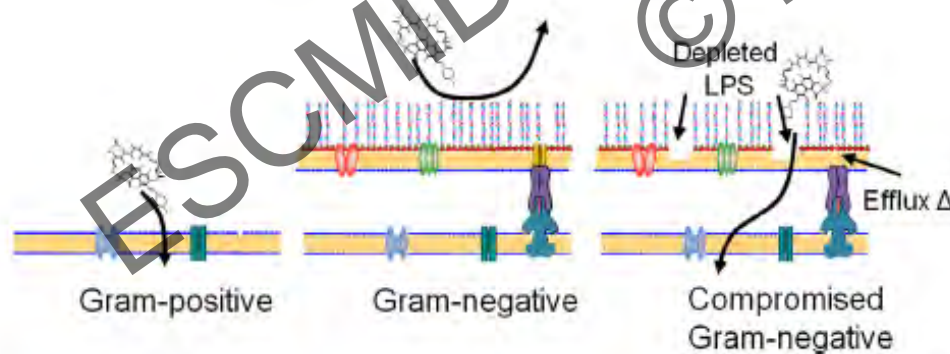


Compounds Known to Permeabilize the Outer Membrane

- Chelators and detergents
- Antimicrobial peptides (AMPs)
- PMB derivatives



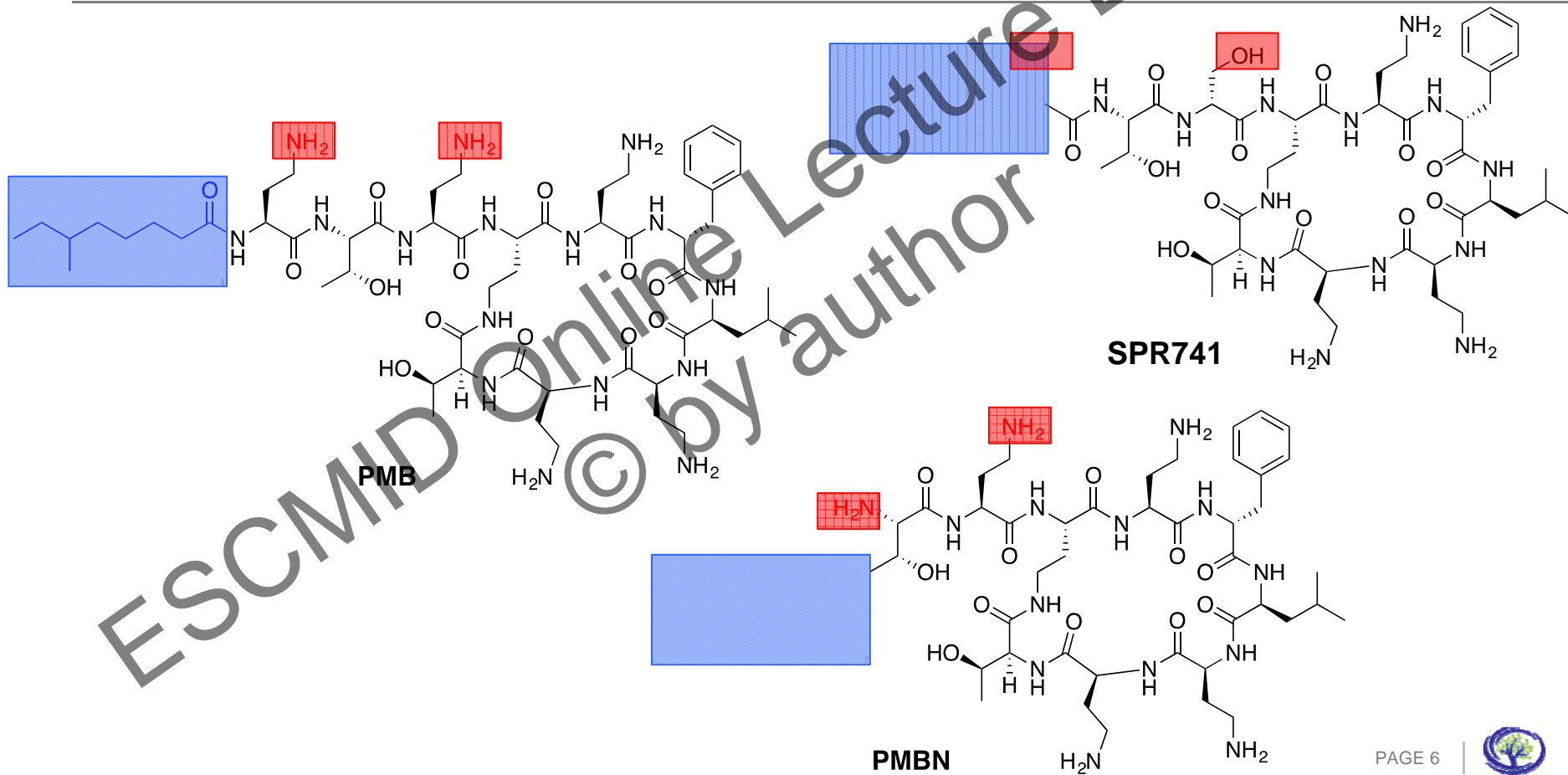
Nature Reviews | Microbiology



Vaara (1992) *Microbiol Rev* 56:395
 Silver (2016) *Bioorg Med Chem* 24:6379
 Zgurskaya et al. (2015) *ACS ID* 1:512
 Zabawa et al. (2016) *Curr Opin Micro* 33:7

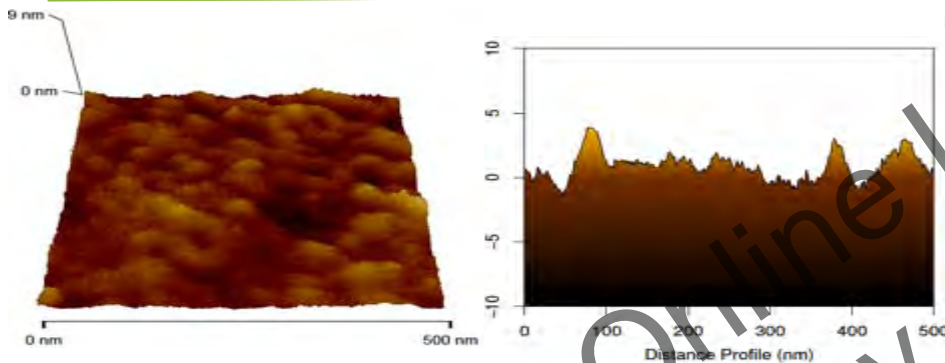


Chemical Structure Comparisons of PMB Derivatives

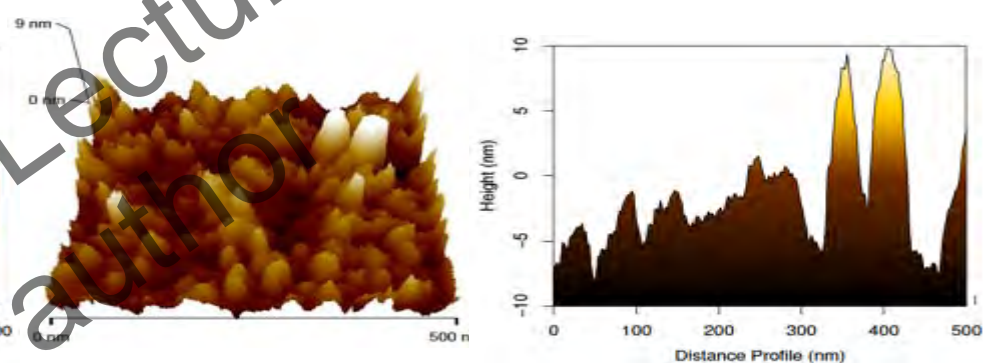


SPR741 Perturbation of *E. coli* Outer Membrane

0 $\mu\text{g}/\text{mL}$ SPR741




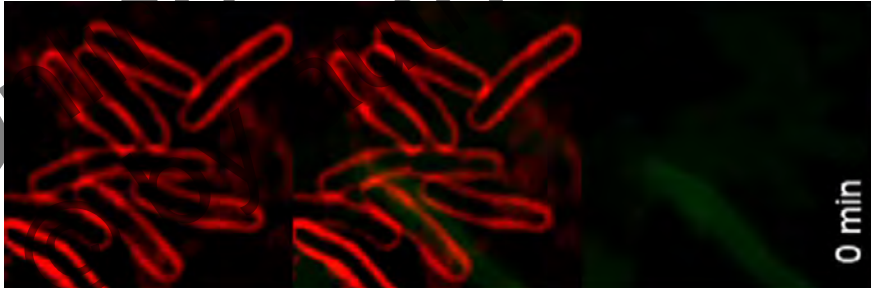

8 $\mu\text{g}/\text{mL}$ SPR741



- Mid-log culture of WT *E. coli* K12 was treated for 5h followed by AFM
- 8 $\mu\text{g}/\text{mL}$ SPR741 caused undulations and structural breaches in the outer membrane of WT *E. coli*

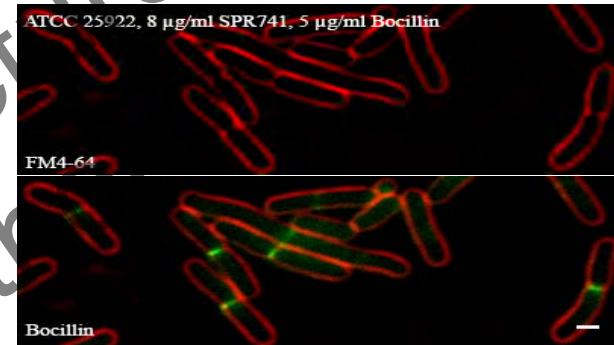
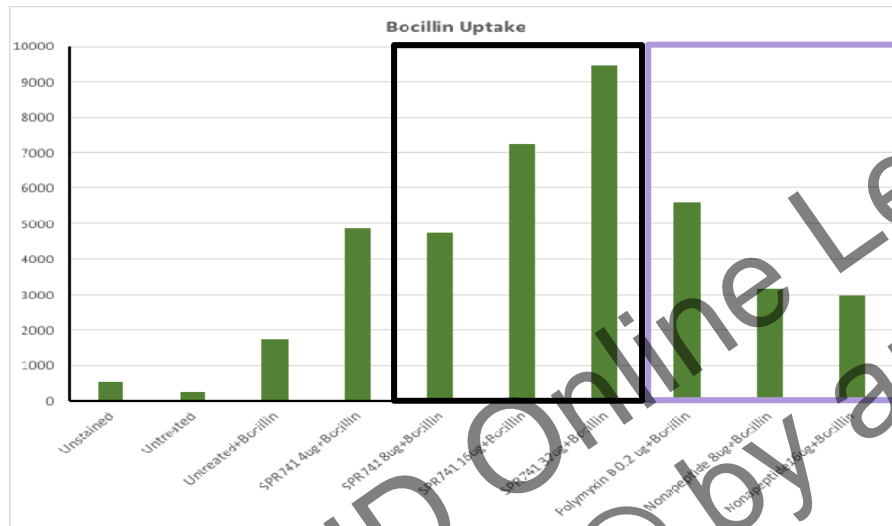


SPR741 Allows Entry of NBD-Azithromycin into G-

SPR741	NBD-AZM		
No	Yes		No accumulation through intact OM
Yes	Yes		Significant accumulation through OM
No	No		Control



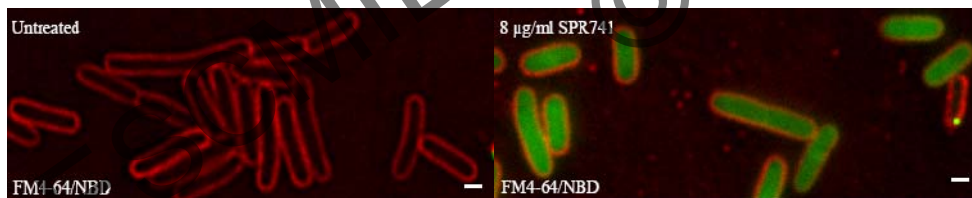
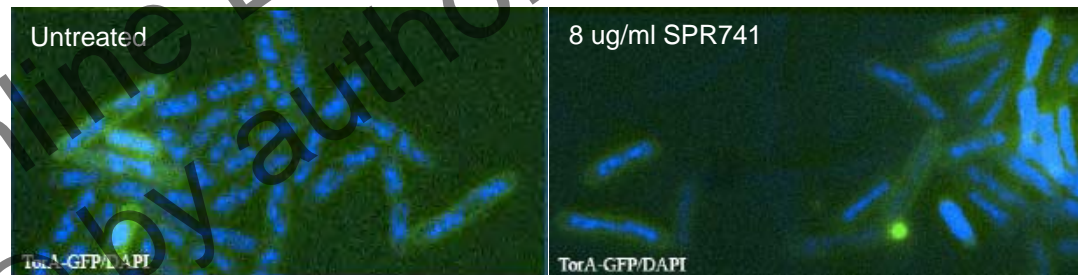
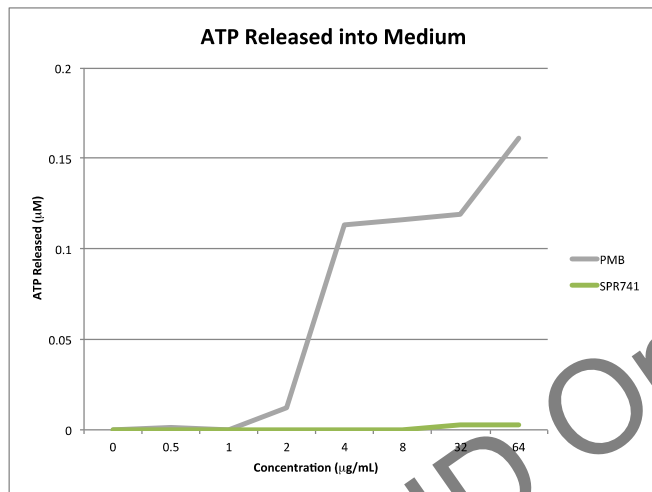
SPR741 Allows Increased Entry of β -Lactams



- Quantitative flow cytometry and microscopy show that *E.coli* ATCC 25922 is largely impermeable to fluorescently labeled Bocillin
- Addition of 4 $\mu\text{g}/\text{mL}$ or 8 $\mu\text{g}/\text{mL}$ SPR741 increased the uptake of fluorescent Bocillin by 2.5-fold
- Higher doses of SPR741 (up to 32 $\mu\text{g}/\text{mL}$) increased Bocillin uptake more than 5-fold
- PMB and PMBN also permeabilize, but less efficiently than SPR741



Mechanistic Insights

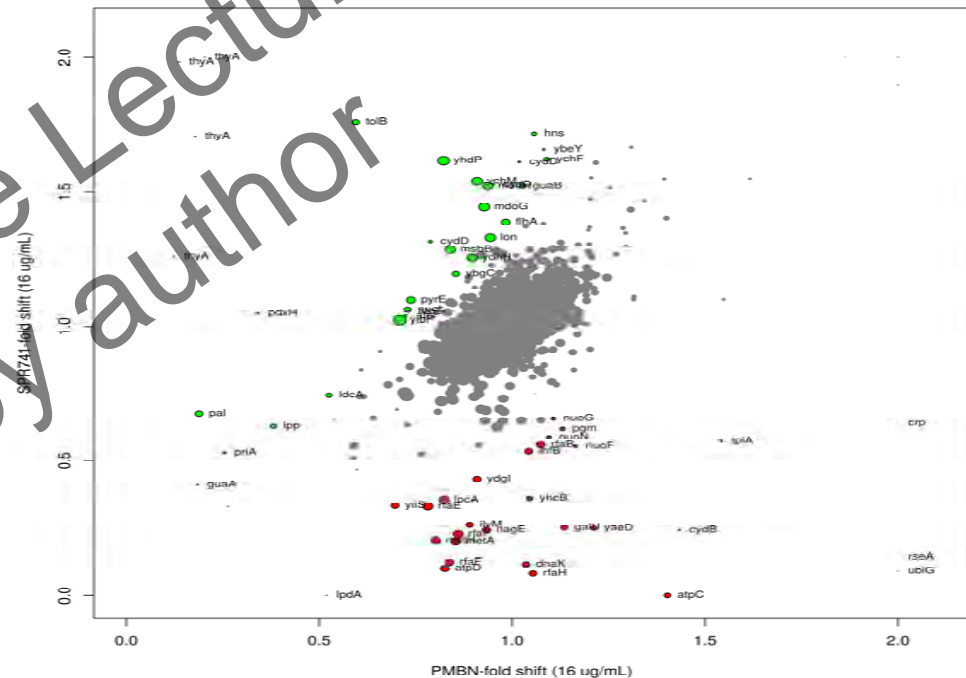


SPR741 or Molecular weight (kDa)



Chemical Genomics Studies to Identify a Molecular Target for SPR741

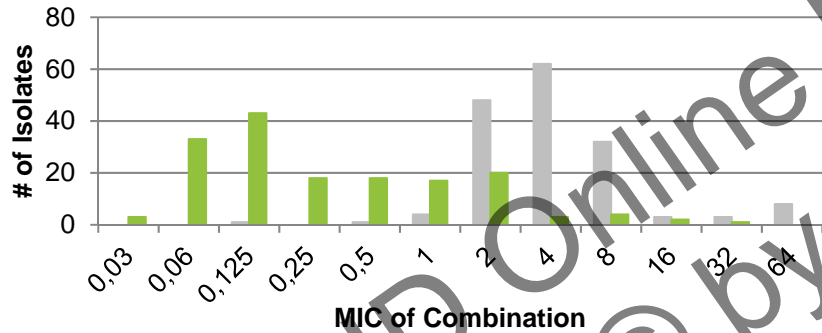
- Keio collection used to look for impact on SPR741 or PMBN activity
- For SPR741, genes found involved in outer membrane processes
- Distinct genes found for PMBN
- Further characterization is in progress



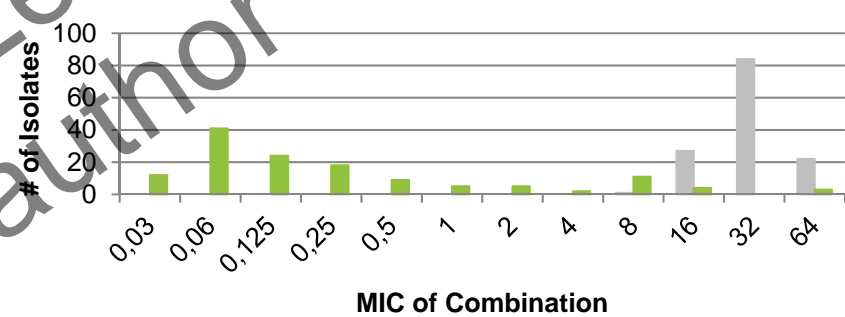
In vitro Potency Expanded into G- with SPR741 / RIF

- RIF MIC shown in grey
- RIF with SPR741 at 8 ug/ml shown in green

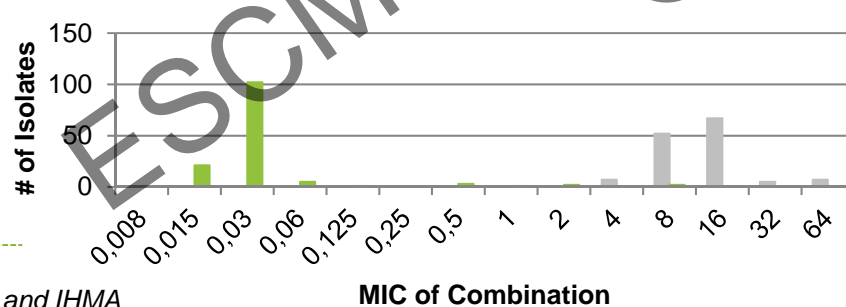
***A. baumannii* (n = 162)**



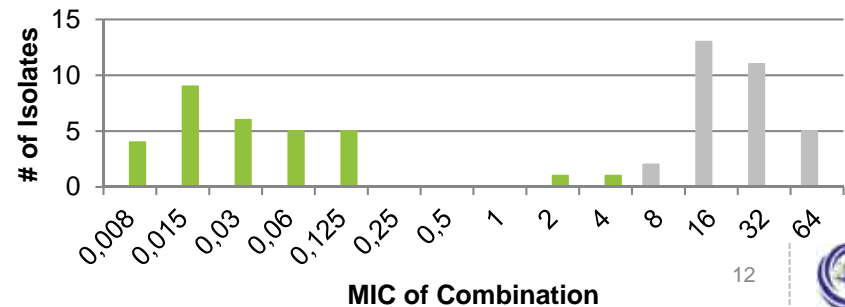
***K. pneumoniae* (n = 136)**



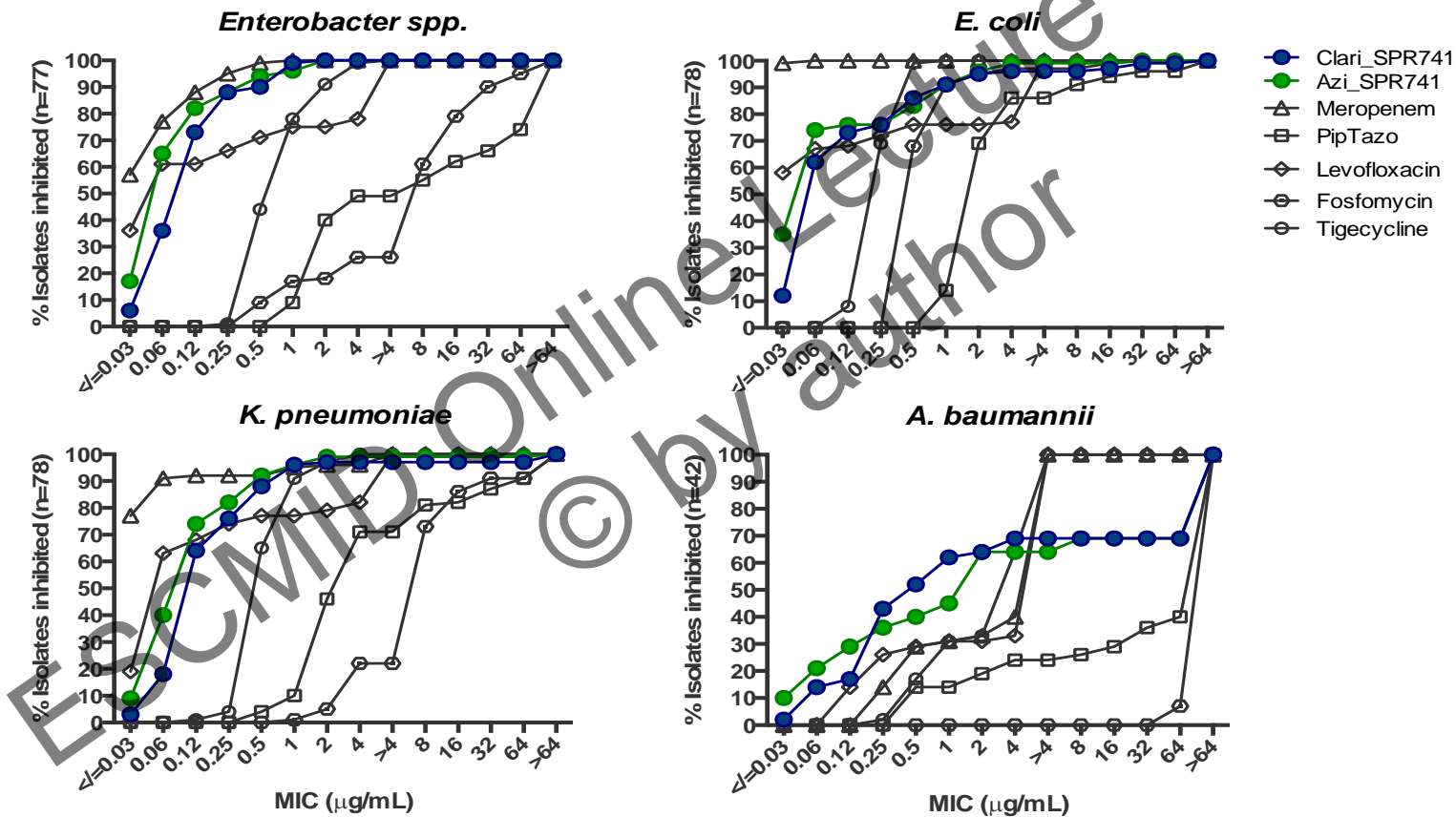
***E. coli* (n = 138)**



***Enterobacter cloacae* (n = 31)**



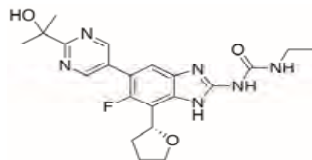
In vitro Potency Expanded into G- with SPR741 / Macrolides



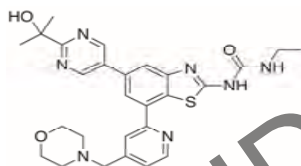
In vitro Potency Expanded into G- with SPR741 / Novel Gyrase Inhibitors

GyrB/ParE ATPase inhibitors

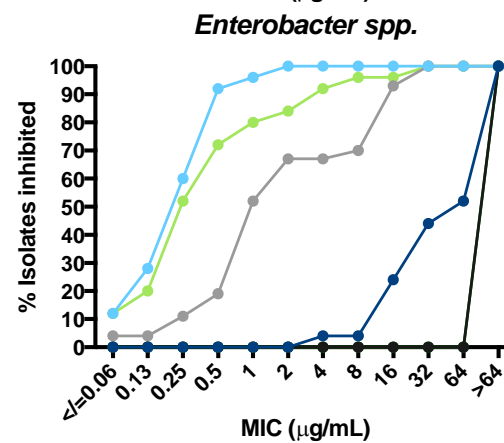
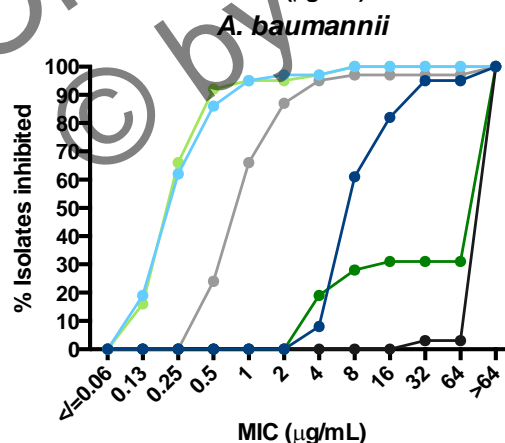
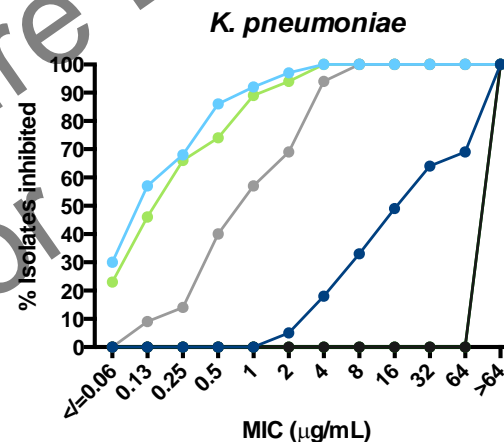
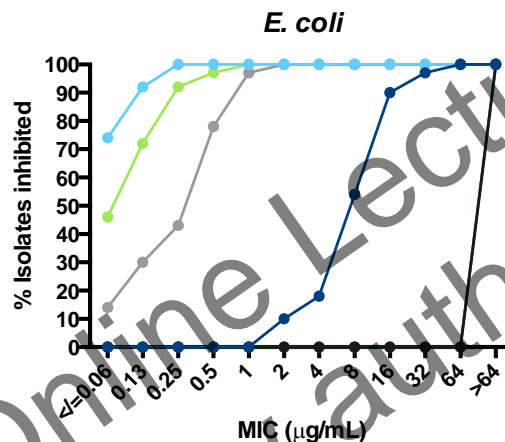
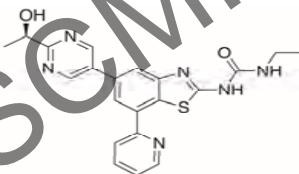
SPR719



SPR750



SPR751



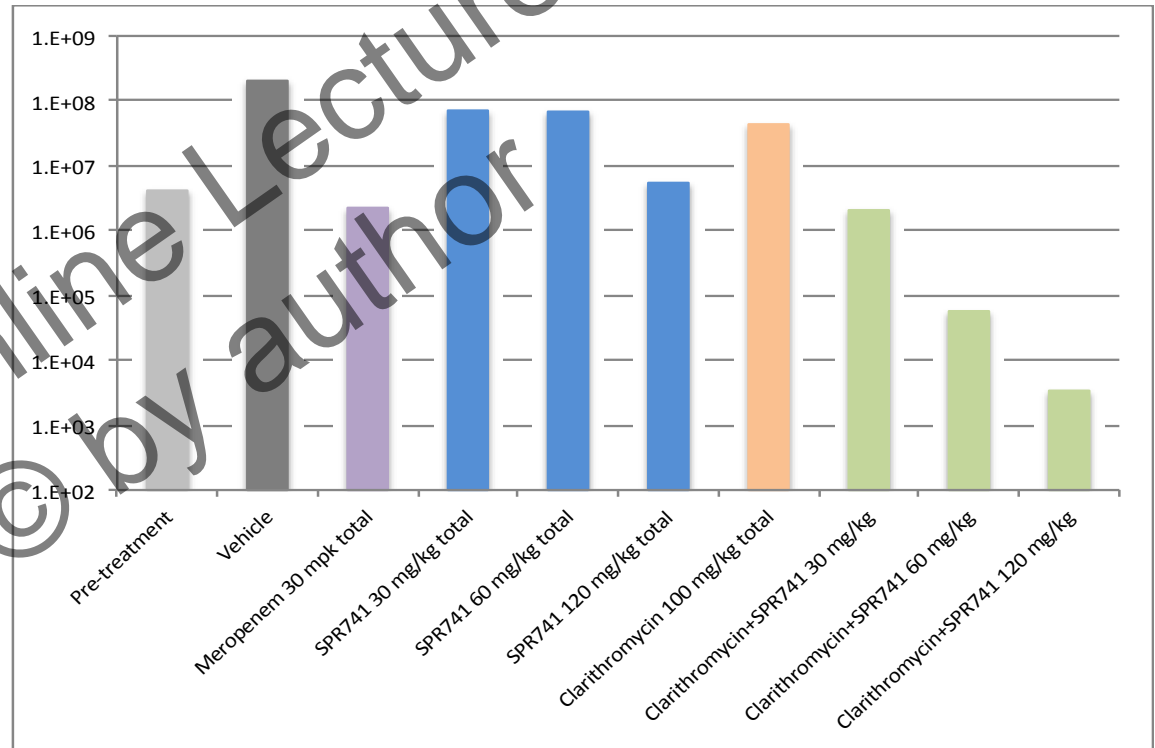
● 719
 ● 719 + 741@8
 ● 750
 ● 750 + 741@8
 ● 751
 ● 751 + 741@8

Nicole Cotroneo at SRL and Youming Zou at HDB – see poster 116 for more information



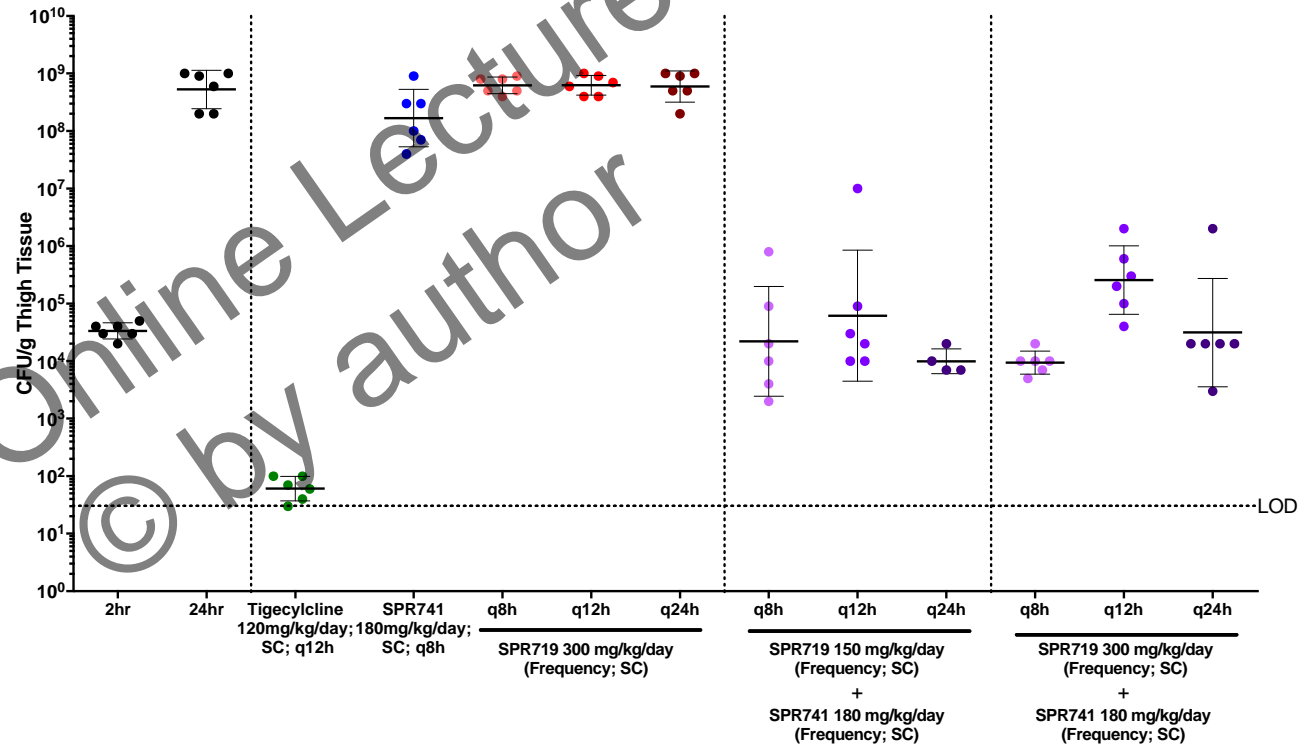
SPR741 / CLR Combination Active *In Vivo* Efficacy – Thigh

- Neutropenic mouse thigh infection, *E. coli* ATCC25922
- SPR741 administered SC at 1, 3.5 and 7h post infection
- CLR administered IV at 1h post infection
- 9h duration



SPR741 / SPR719 Combination Active *In Vivo* – Thigh

- Neutropenic mouse thigh infection, *E. coli* BAA2469 (NDM-1+)
- SPR741 administered SC at 1, 9 and 17h post infection
- SPR719 administered SC at 1, 9 and 17h post infection
- 24h duration

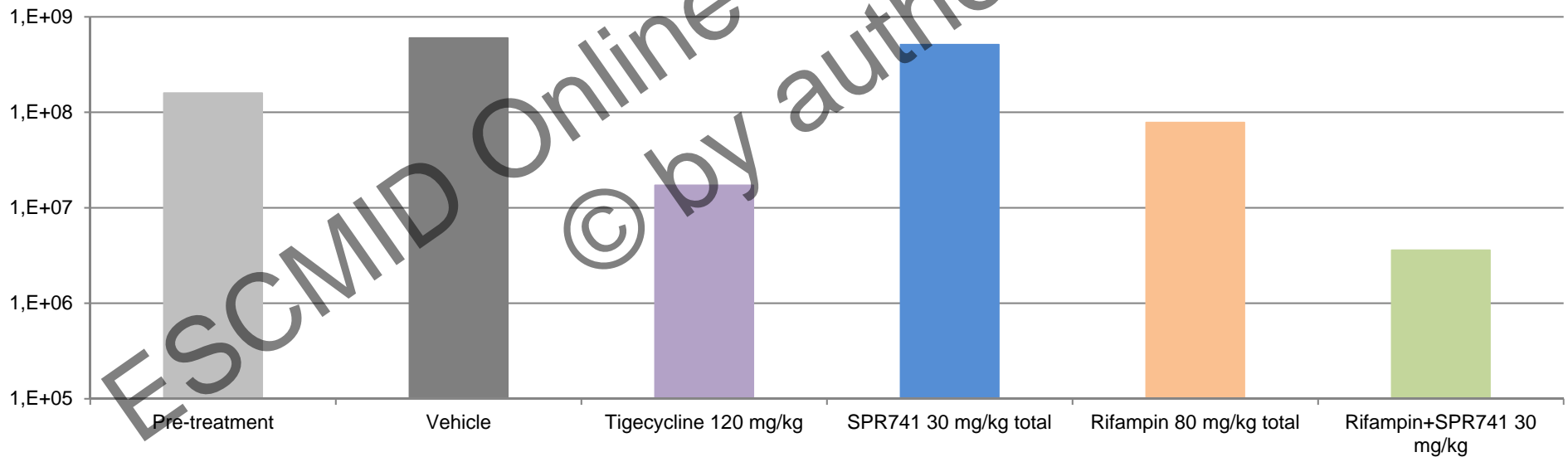


David Corbett at Evotec – see poster 121 for more information



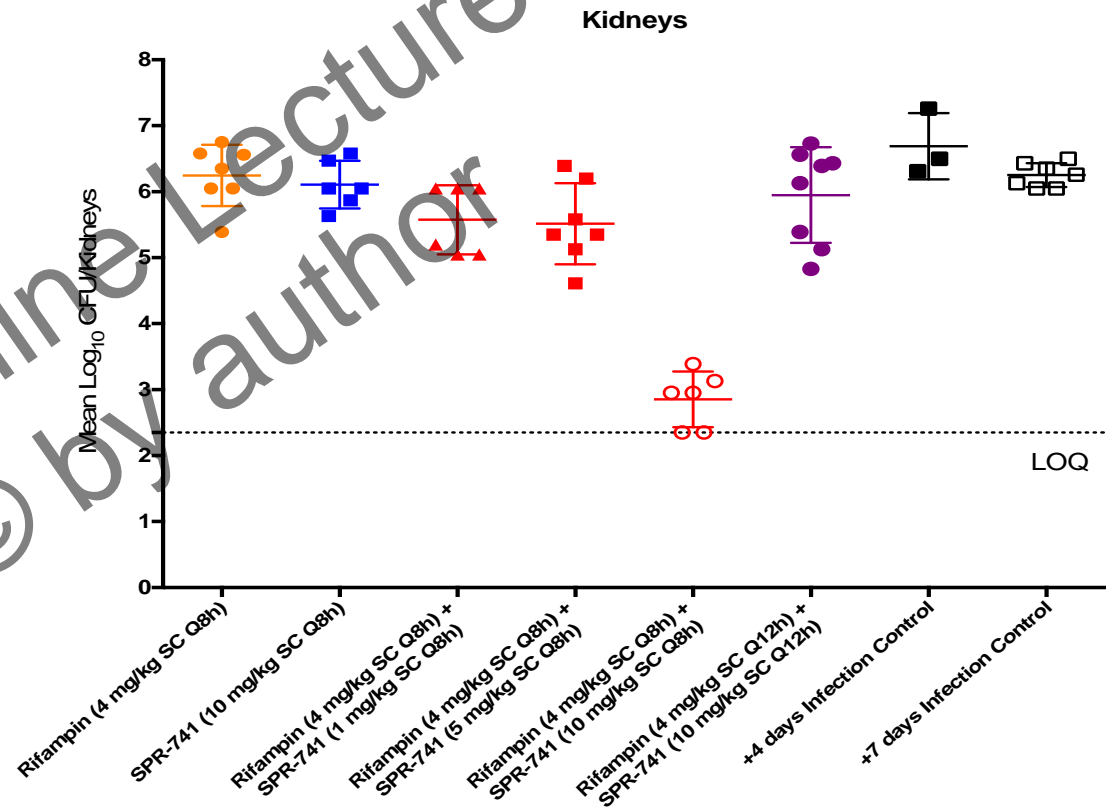
SPR741 / RIF Combination Active *In Vivo* – Lung

- Neutropenic mouse lung infection, *E. cloacea* KP114
- SPR741 administered SC at 1, 3.5 and 7h post infection
- RIF administered SC 1h post infection
- 9h duration



SPR741 / RIF Combination Active *In Vivo* – UTI

- Immunocompetent mouse UTI, *K. pneumoniae* KPC2+ (UNT170-1)
- Trans-urethral infection set up for 4 days
- SPR741 and RIF administered SC q8h for 3 days



SPR741 Associated With No Toxicity at Highest Dose Studied



NHP is the most appropriate non-rodent species based upon work in chemical class across the industry



Non-GLP range finding toxicology study with Polymyxin B (PMB) positive control

- 7 Day study, TID dosing by 1 hour infusion
 - Doses: 15, 30, 60 mg/kg/d for SPR741; 12 mg/kg/d for PMB
-



Summary: SPR741 associated with no toxicity at highest dose tested

- PMB showed anticipated nephrotoxicity at 12 mg/kg/d
- 741 exposures provide >4-fold window as compared to PMB
- 741 was not associated with any toxicity – **NOAEL >60 mg/kg/d**
 - ✓ No histopathological findings in the kidney
 - ✓ No significant changes in clinical chemistry
 - ✓ No changes in hematology, coagulation
 - ✓ No clinical signs throughout the study



SPR741 Safety Supported Progression to Phase I



ADME studies completed



Genotoxicity studies completed



7 and 14-day repeat dose IV toxicology studies in rat & monkey completed

- 7 day study Monkey NOAEL >60 mpk/day
- 14 day study Monkey NOAEL 40 mpk/day → Plasma AUC ~300 $\mu\text{g}\cdot\text{hr}/\text{mL}/\text{day}$



Safety pharmacology studies completed



Hemolysis, flocculation & local tolerance studies completed

SPR741 First in Human Study

- **Study Initiation:** December 2016
- **Study design:** Single Ascending Dose (SAD)/Multiple Ascending Dose (MAD), with dose escalation following safety analysis between cohorts of normal volunteers; 96 subjects in total – 72 received active
- **Endpoints:** Safety, tolerability and pharmacokinetics
- **Location:** Australia
- **Study Status:** Completed – Stay tuned for data!

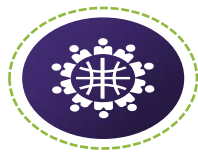


Outer Membrane Permeabilizer – SPR741 Summary

- No significant inherent *in vitro* activity but outer membrane permeability activity retained
- Molecular basis of permeabilization may be distinct from PMBN
- SPR741 has flexibility to potentiate multiple classes of antibiotics both *in vitro* and *in vivo*
 - PK/PD analyses in progress; may differ based on choice of partner antibiotic
- Identified a compound from this class that is safer than PMB
- Current status – PhI completed; partner abx....



Spero Summary



Multiple, novel pipeline programs:
First new therapies for serious
infections in 40 years



World-class team and Advisors



Accelerated path to market based on
unmet need focus



Large market opportunity based on
new paradigm for antibiotics



Acknowledgements

Everyone at Spero Therapeutics

- **Troy Lister**

Collaborators

- Northern Antibiotics
- Evotec
- Hartford Hospital
- HD Bioscience
- ICPD
- IHMA
- JMI
- Linnaeus Bioscience
- Micromyx
- McMaster University
- Pharma Resources
- University of Guelph
- University of North Texas
- Walter Reed

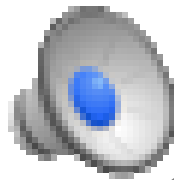




BACKUPs

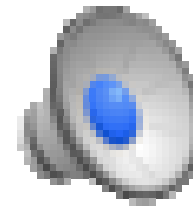
SPR741 Allows Entry of Azithromycin into G-Bacteria

E. coli treated with azithromycin



E. coli bacteria continue to divide during 1 hour period; no entry by labeled azithromycin

E. coli treated with azithromycin and SPR741



Labeled azithromycin enters cell over 1 hour period; cells stop dividing

