A Mechanism-Based Model of Polymyxin B in Combination with Chloramphenicol against *Klebsiella pneumoniae* based on Multi-Omics Network Analysis

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UNC ESHELMAN SCHOOL OF PHARMACY

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Bad Bugs: The Urgent Need For Drugs

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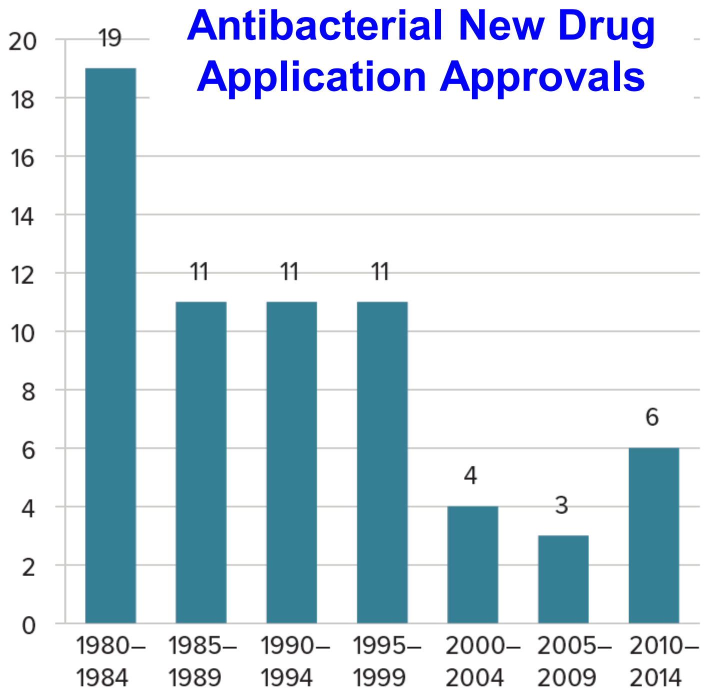
Multi-drug Resistant Pathogens

Enterococcus faecium
Staphylococcus aureus
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacter species

- *Klebsiella pneumoniae* is among the top 3 critical pathogens (WHO)
- High mortality rates reported ranging from 26% to 44%

Bush K et al. *Curr Opin Microbiol*. 2010 Oct;13(5):558-64.
Falgas M et al. Energ Infect Dise. 2014 Jul;20(7):1170-1175
https://www.cdc.gov/drugresistance/biggest_threats.html

Need for Optimized Antibiotic Dosing Strategies



Ventola CL. *P T*. 2015 Apr;40(4):277-83.

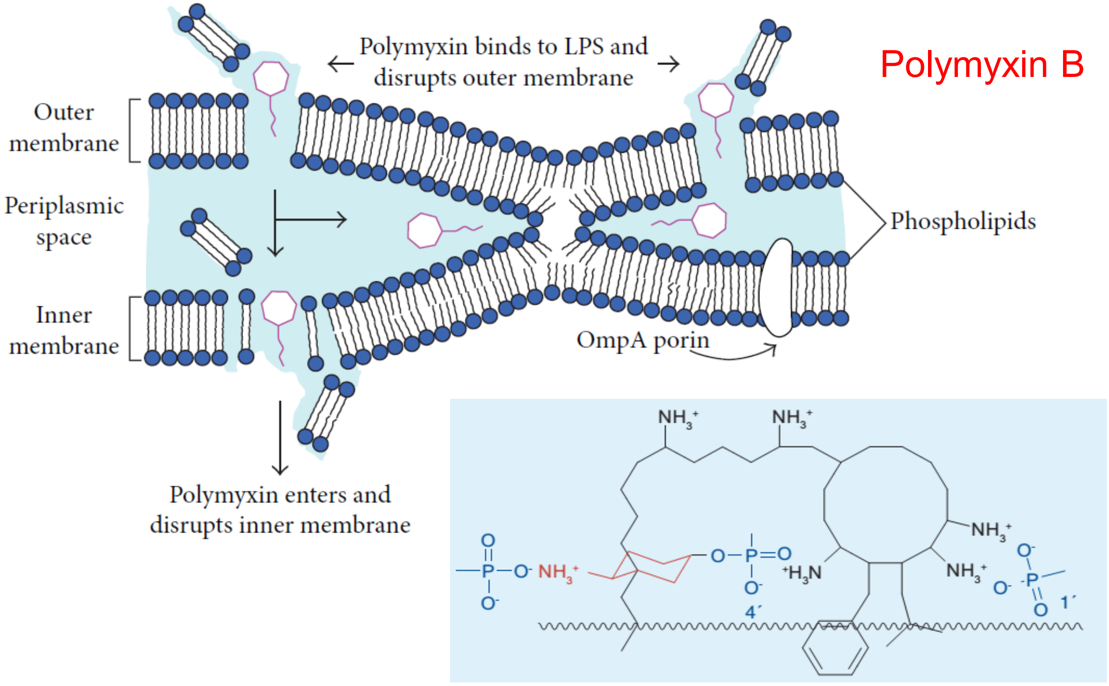
- Current empiric approach to selecting and dosing antibiotics increasingly ineffective
- Combination therapy would be beneficial for the treatment of infections due to 'superbugs', however empiric use of combinations also leads to resistance.

→ Need better approaches to optimise treatment regimens based on bacterial and host characteristics

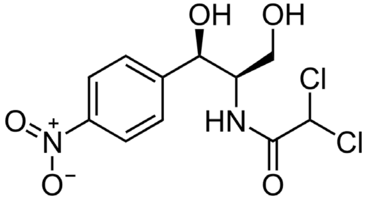
Agyeman AA, Bergen PJ, Rao GG, Nation RL, Landersdorfer CB.(Under review Antimicrob Agents Chemother. Feb 2019)

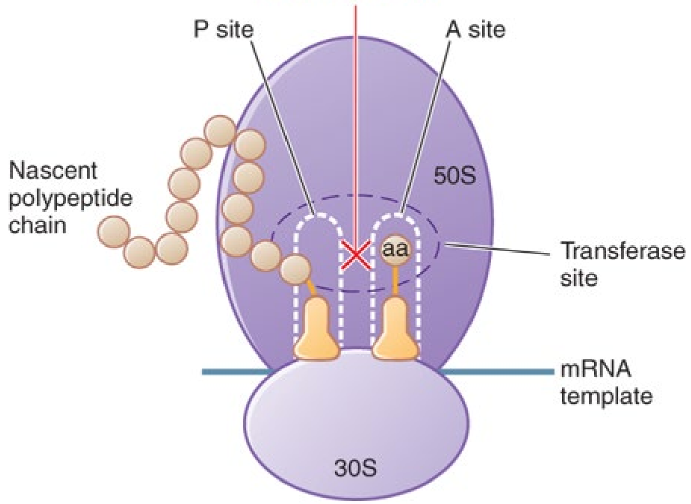
Study Design

Polymyxin B



- Antibiotics:
  - Polymyxin B (PMB)
  - Chloramphenicol (CHL)
- NDM-1 producing *Klebsiella pneumoniae* clinical isolate
  - MIC PMB: 0.5 mg/L
  - MIC CHL: 16 mg/L
- Objective: Develop a mechanism based model describing the impact of PMB and CHL on the bacterial dynamics of NDM-1 *Klebsiella pneumoniae* informed by multi-omics network analysis.

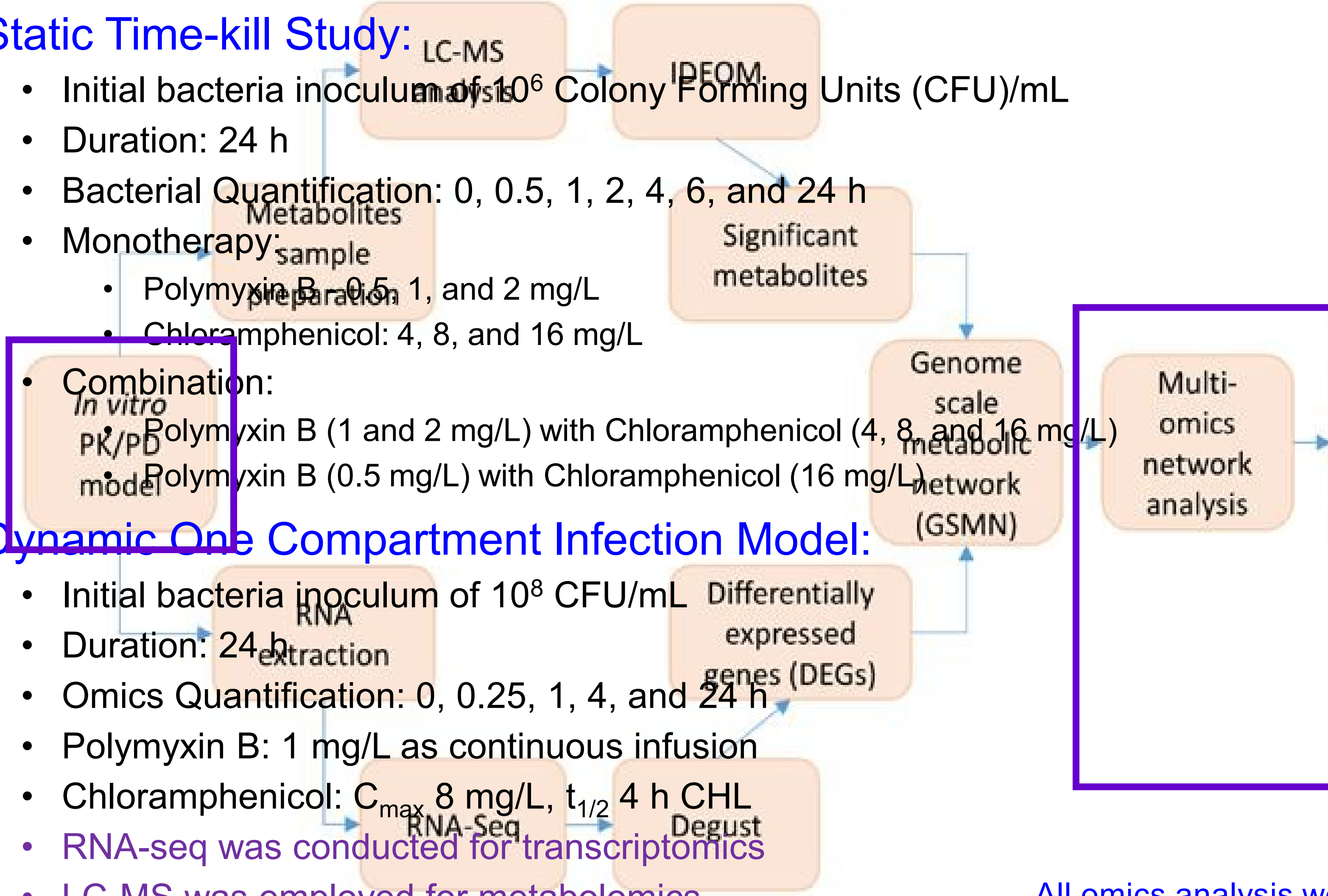




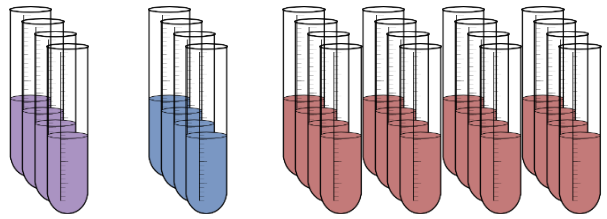
Yu, Z. *Biomed Res Int.* 2015 Jan.
Poirel, L. *Clin Microbiol Rev.* 2017 Apr. 30(2):557-596
Brunton, L.L. *Pharmacological Basis of Therapeutics.* 2016: Chap. 55

Methods

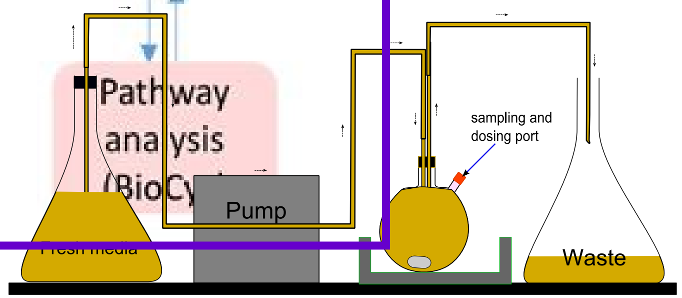
- Static Time-kill Study:
  - Initial bacteria inoculum of 10^6 Colony Forming Units (CFU)/mL
  - Duration: 24 h
  - Bacterial Quantification: 0, 0.5, 1, 2, 4, 6, and 24 h
  - Monotherapy:
    - Polymyxin B – 0.5, 1, and 2 mg/L
    - Chloramphenicol: 4, 8, and 16 mg/L
  - Combination:
    - Polymyxin B (1 and 2 mg/L) with Chloramphenicol (4, 8, and 16 mg/L)
    - Polymyxin B (0.5 mg/L) with Chloramphenicol (16 mg/L)
- Dynamic One Compartment Infection Model:
  - Initial bacteria inoculum of 10^8 CFU/mL
  - Duration: 24 h
  - Omics Quantification: 0, 0.25, 1, 4, and 24 h
  - Polymyxin B: 1 mg/L as continuous infusion
  - Chloramphenicol: C_{max} 8 mg/L, $t_{1/2}$ 4 h CHL
  - RNA-seq was conducted for transcriptomics
  - LC-MS was employed for metabolomics
- Modeling done in ADAPT5



***In vitro* Time-Kill Model**



***In vitro* One Compartment Infection Model**



Abdul Rahim N, Velkov T, Li J.Poster, ESCMID, 2017
https://www.escmid.org/escmid_publications/escmid_elibrary/material/?mid=48911

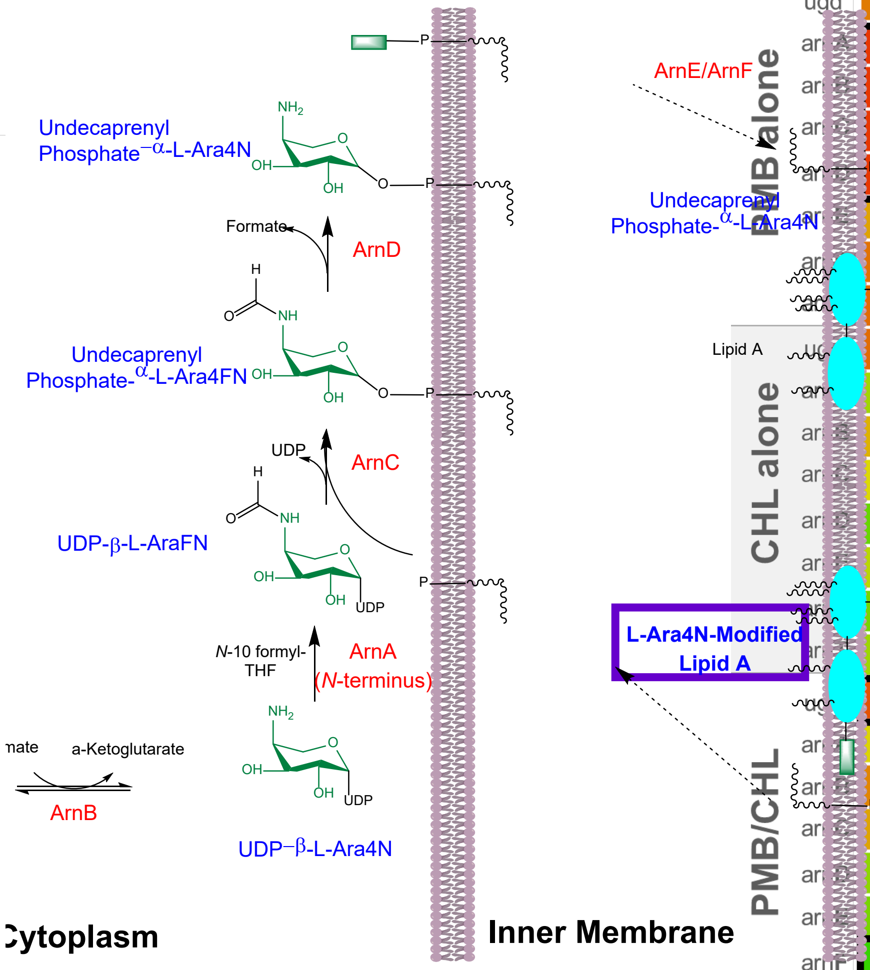
All omics analysis were performed in the laboratory of Dr. Jian Li, Monash University Melbourne, Australia

Images from Rao Lab

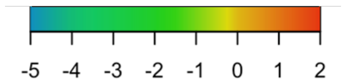
A Key Pathway of PMB Resistance

Metabolomics*

		0.25 h	1 h	4 h	24 h
PMB alone	UDP-glucose	-0.95	0.21	0.39	-0.08
	UDP-glucuronate	-0.46	0.28	0.47	0.18
	UDP-LAra4FN	-0.59	0.45	0.10	0.39
	LAra4N	-0.18	0.24	-0.09	0.12
CHL alone	UDP-glucose	-1.20	0.58	-2.77	-2.83
	UDP-glucuronate	-0.86	0.42	-2.28	-1.56
	UDP-LAra4FN	-1.15	-0.10	-2.60	-2.06
	LAra4N	0.16	-0.38	0.07	0.50
PMB/CHL	UDP-glucose	-0.43	-0.23	-1.97	-4.50
	UDP-glucuronate	0.17	0.11	-1.51	-3.89
	UDP-LAra4FN	-0.10	-0.14	-1.67	-3.42
	LAra4N	0.41	-0.74	0.29	-0.12



Metabolites with ≥2-fold changes in levels (P<0.05) were deemed significant metabolites.



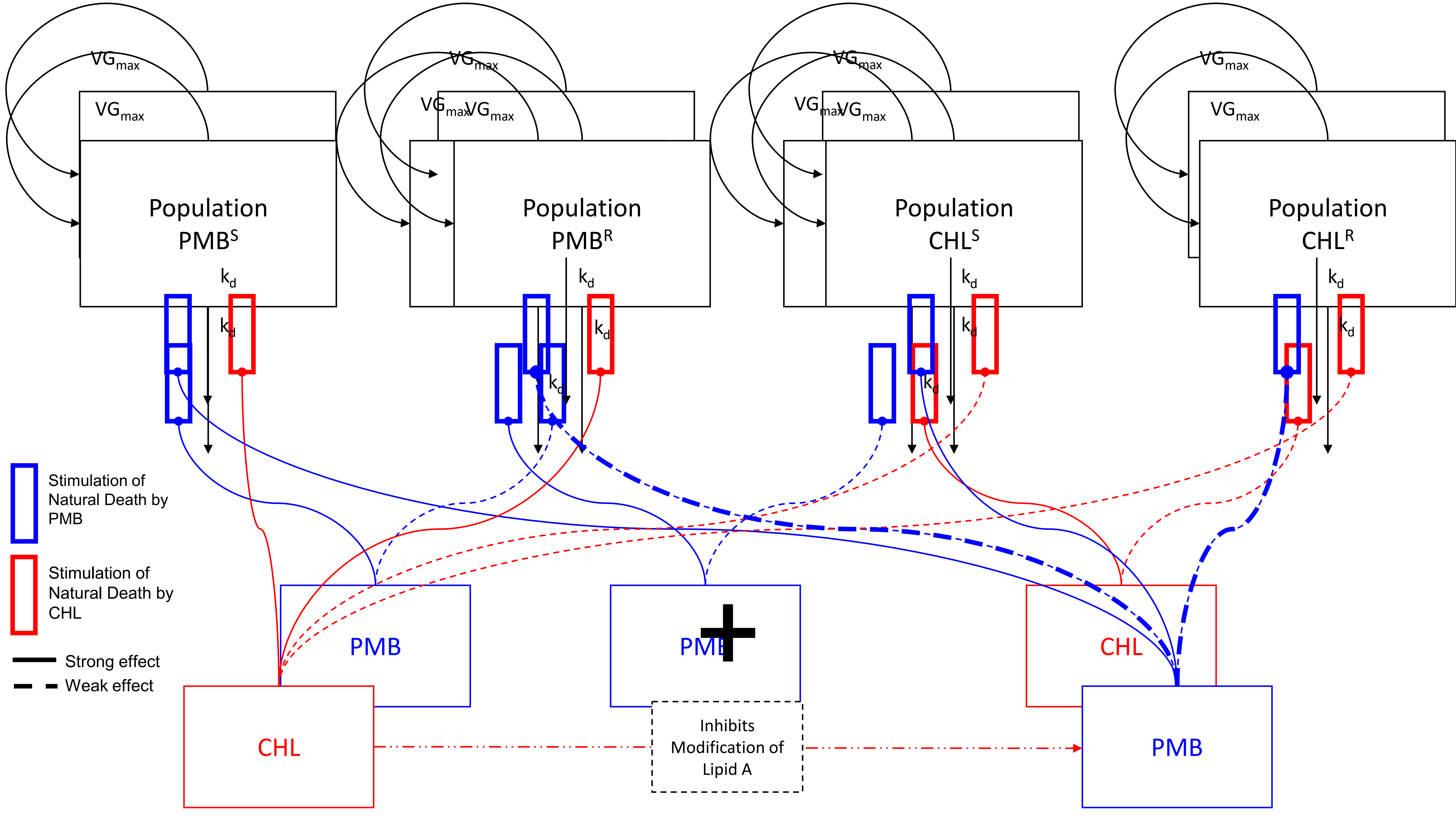
Transcriptomics

		0.25 h	1 h	4 h	24 h
PMB alone	ugd	0.93	0.14	-0.02	0.21
	ar	2.16	2.35	-0.47	0.35
	ar	2.44	1.89	-0.05	0.24
	ar	2.36	1.72	-0.35	0.21
	ar	2.24	2.32	-0.44	0.47
		0.32	0.43	0.04	-0.20
	ar	1.06	0.98	-0.01	0.27
		1.17	1.02	-0.29	0.13
CHL alone	u	1.17	1.09	-0.31	-0.33
		-0.68	-0.73	-1.00	0.07
	ar	0.09	-0.33	-0.54	-0.13
	ar	-0.23	-0.85	-0.95	-0.20
	ar	-1.11	-0.25	-0.62	0.48
	ar	-0.50	0.29	0.20	-0.13
		-1.00	0.08	0.70	0.15
	ar	-0.71	-0.15	-0.15	0.37
PMB/CHL	u	1.55	1.16	-0.05	-0.59
	ar	-0.20	-0.87	-1.66	-1.48
	ar	0.79	-0.03	-1.10	-1.03
	ar	0.31	-0.84	-1.54	-1.63
	ar	-0.82	-1.20	-1.29	-0.99
	ar	-0.99	0.26	0.24	-0.95
	arnF	-1.49	-0.54	-0.46	-0.18
	arnT	-1.05	-0.33	-0.66	-0.83

Genes with log2 fold change in expression>1.0 and false discovery rate(FDR) of <0.05 were considered DEGs.

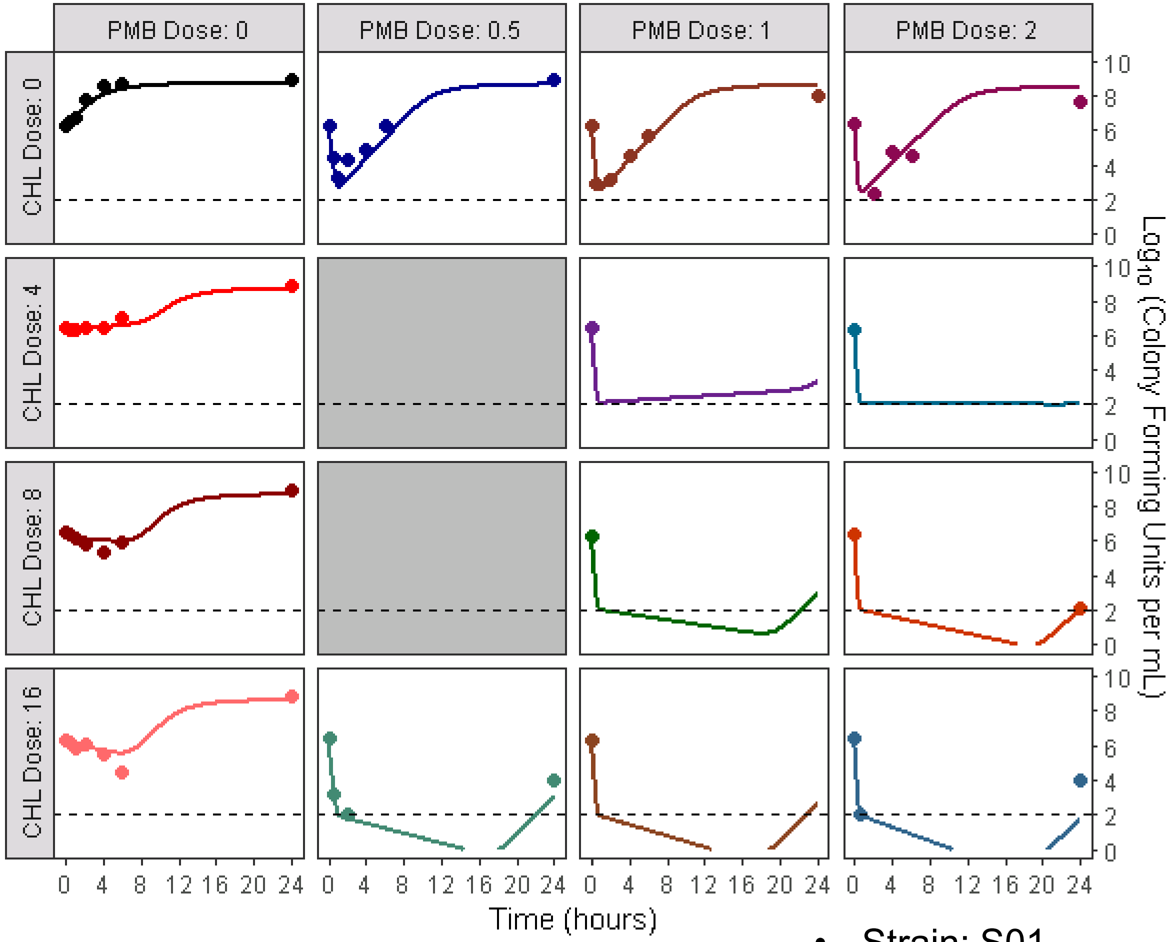
Adapted from Yan, A., Guan, Z., Raetz, C.R., J Biol Chem 282:36077-89 (2007).

* Unpublished data from Rahim, and Li, Monash University



VGmax
VGmax
Population PMB^S
kd
Population PMB^R
Population CHL^S
Population CHL^R
Stimulation of Natural Death by PMB
Stimulation of Natural Death by CHL
Strong effect
Weak effect
PMB
CHL
Inhibits Modification of Lipid A

Results



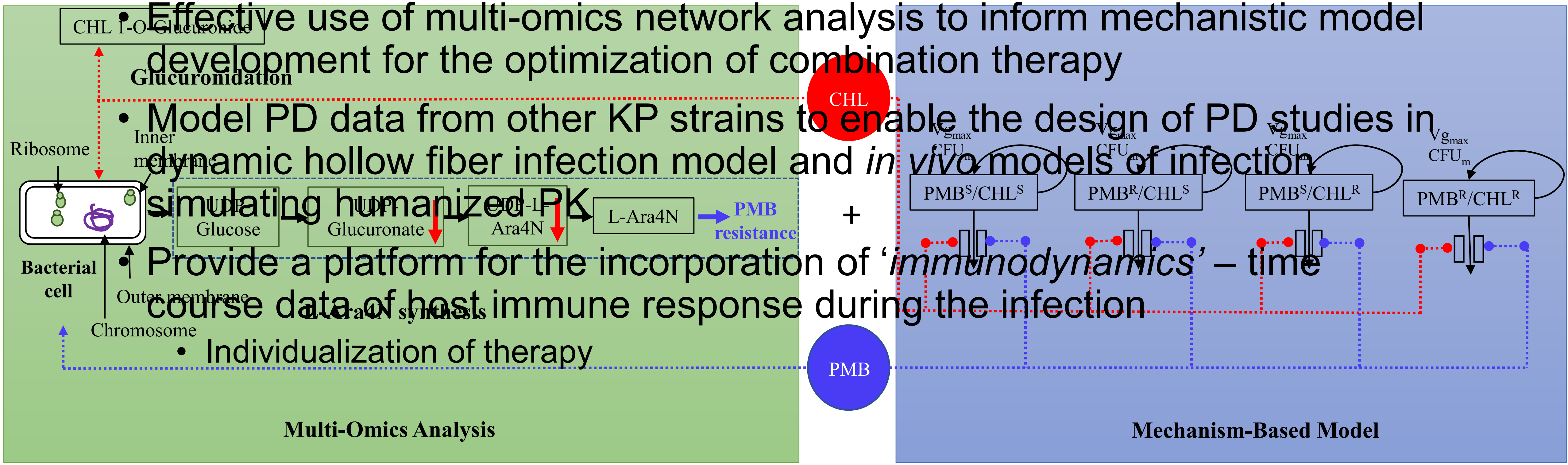
- Strain: S01
- CHL MIC: 16 mg/L
- PMB MIC: 0.5 mg/L

*Unpublished Data Hanafin, Rahim, Jian, Rao, 2019

Parameter	Parameter Description	Estimate	SE (%CV)
Log_{MF_RR}	Log_{10} mutation frequency of population resistant to both PMB and CHL	-16.4	8.1
Log_{MF_RS}	Log_{10} mutation frequency of population resistant to CHL only	-4.67	25.2
Log_{MF_SR}	Log_{10} mutation frequency of population resistant to PMB only	-4.16	2.87
$KC_{50,PMB,S}$	PMB concentration resulting in 50% of $K_{MAX,PMB}$ in the susceptible population	0.826	35.5
$KC_{50,PMB,R}$	PMB concentration resulting in 50% of $K_{MAX,PMB}$ in the resistant population	339	57.0
$KC_{50,PMB,R,COMBO}$	PMB concentration resulting in 50% of $K_{MAX,PMB}$ in the resistant population with PMB in combination with CHL	329	27.9
$KC_{50,CHL,S}$	CHL concentration resulting in 50% of $K_{MAX,CHL}$ in the susceptible population	2.27	34.1
$KC_{50,CHL,R}$	CHL concentration causing 50% of $K_{MAX,CHL}$ in the resistant population	445	153
$K_{MAX,PMB}$	Maximum killing rate constant of PMB	94.6	19.0
$K_{MAX,CHL}$	Maximum killing rate constant of CHL	6.34	9.27

Conclusion and Future Directions

- Effective use of multi-omics network analysis to inform mechanistic model development for the optimization of combination therapy
- Model PD data from other KP strains to enable the design of PD studies in dynamic hollow fiber infection model and *in vivo* models of infection simulating humanized PK
- Provide a platform for the incorporation of '*immunodynamics*' – time course data of host immune response during the infection
  - Individualization of therapy



Acknowledgments

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