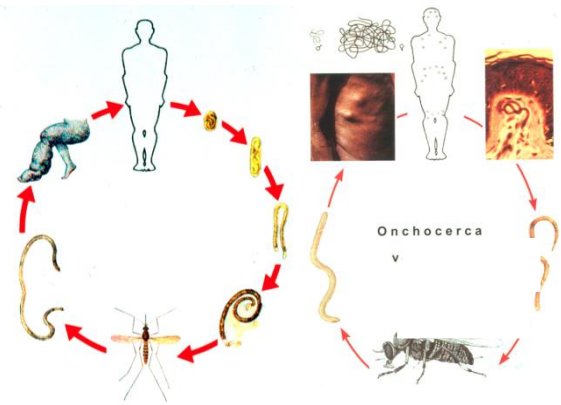
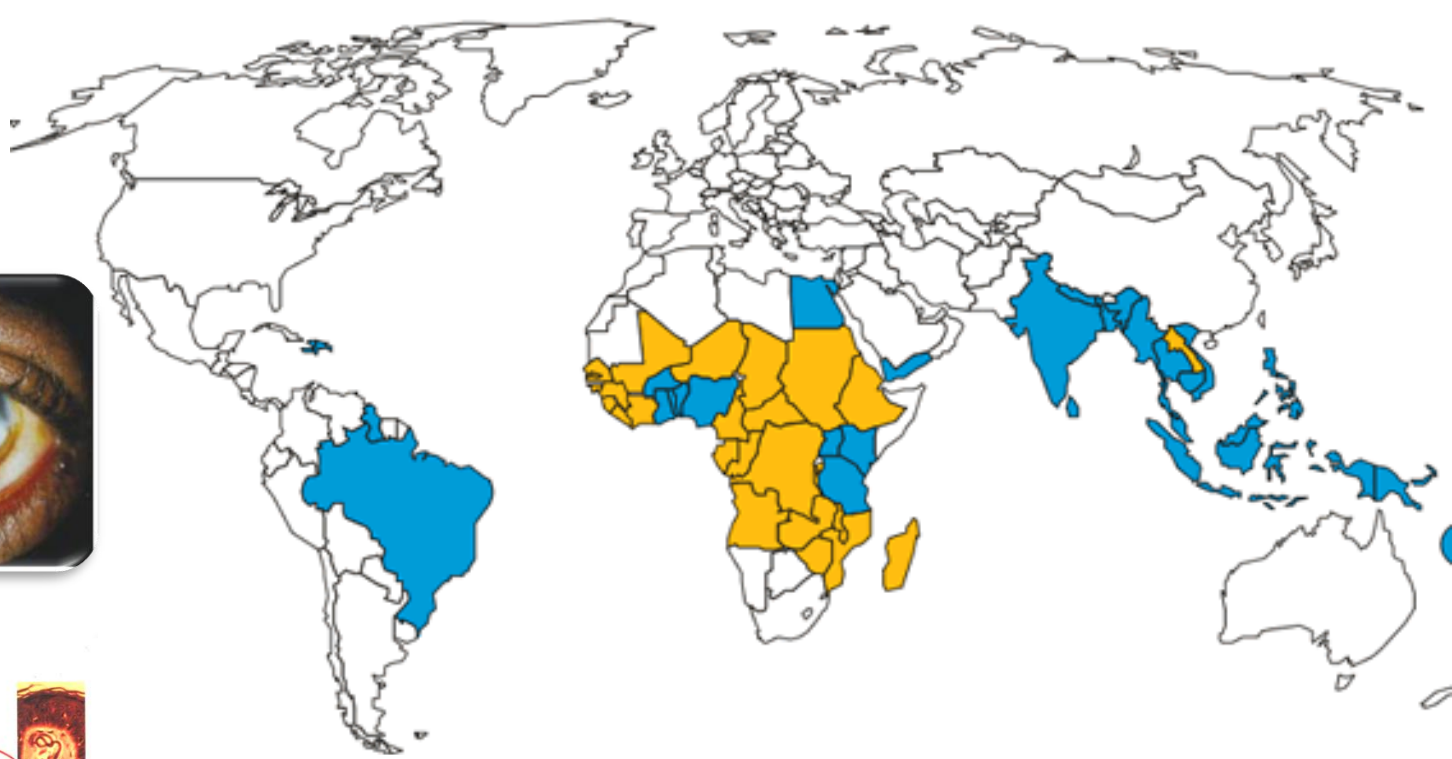


Corallopyronin A: a new anti-filarial drug



Kenneth Pfarr
Institute for Medical Microbiology,
Immunology and Parasitology

Filariasis

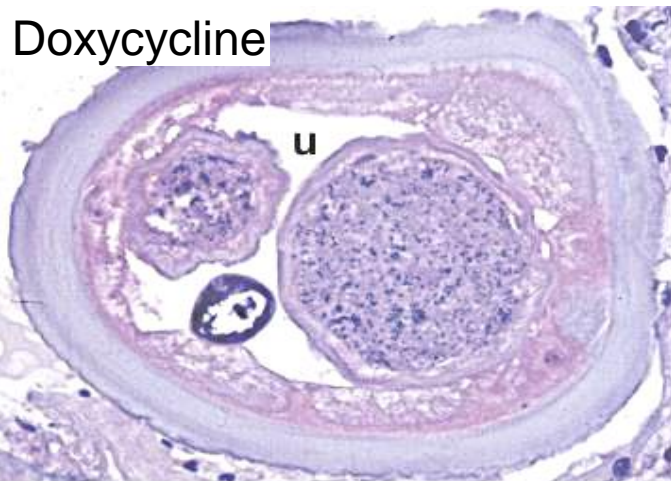
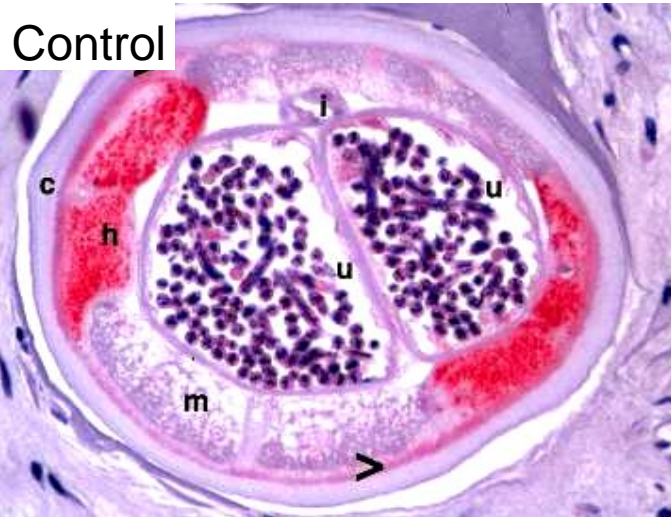


~150 million people infected
>1.3 billion at risk

- Current drugs only kill microfilariae
 - Diethylcarbamazine (+ albendazole)
 - Ivermectin (+ albendazole) in Africa
- Adult worms are long lived (>8 years)
 - Requires years of annual treatment
- Evidence of resistance

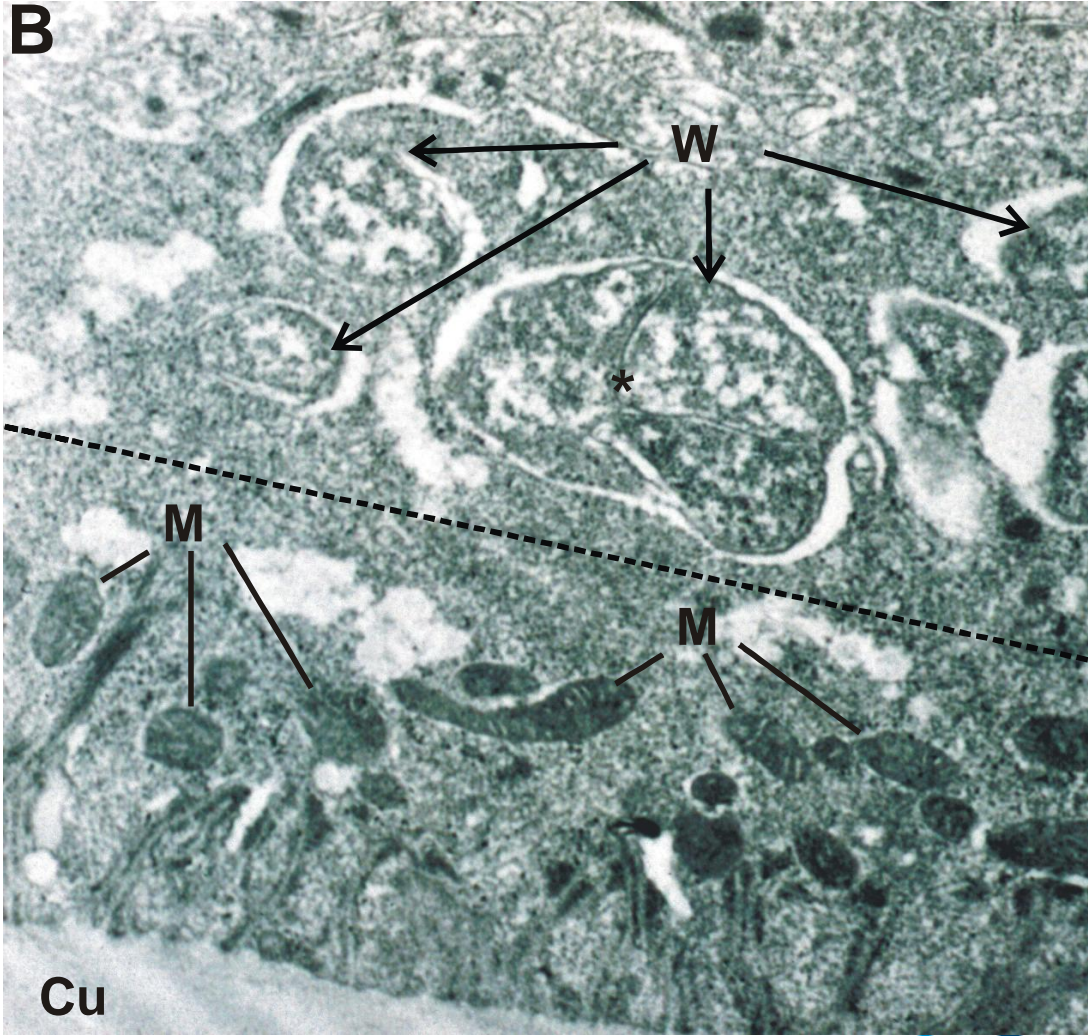
– **A macrofilaricidal drug is needed**

Background (*Wolbachia*)

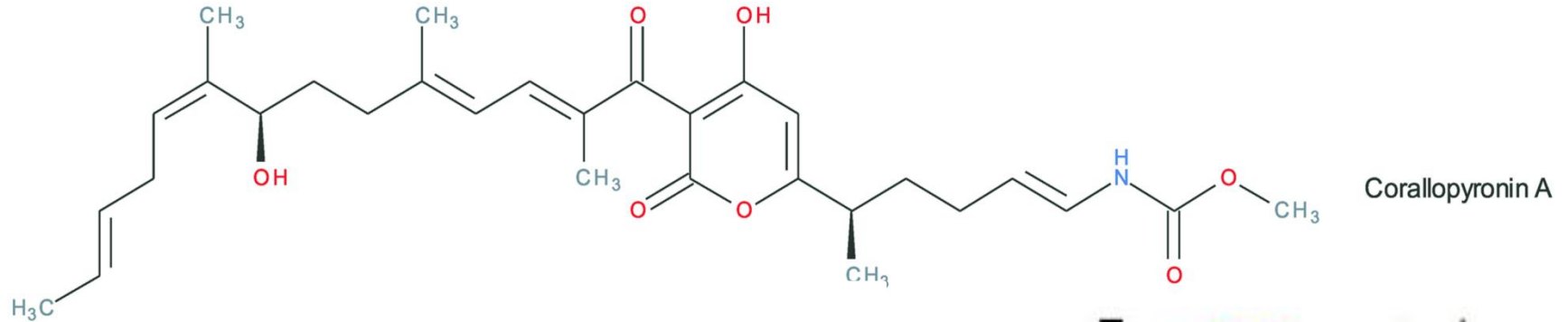


- Symbionts in many arthropods and filariae
- Order: Rickettsiales
- Vertical transmission via oocytes
 - Embryos/developing larvae only survive with *Wolbachia*
- Treatment with tetracyclines/**rifampicin** depletes *Wolbachia*
 - Blocks embryogenesis and molting
 - Kills adult worms (>4 weeks)
 - Fewer adverse reactions to DEC/IVM
 - Reverses early LF pathology

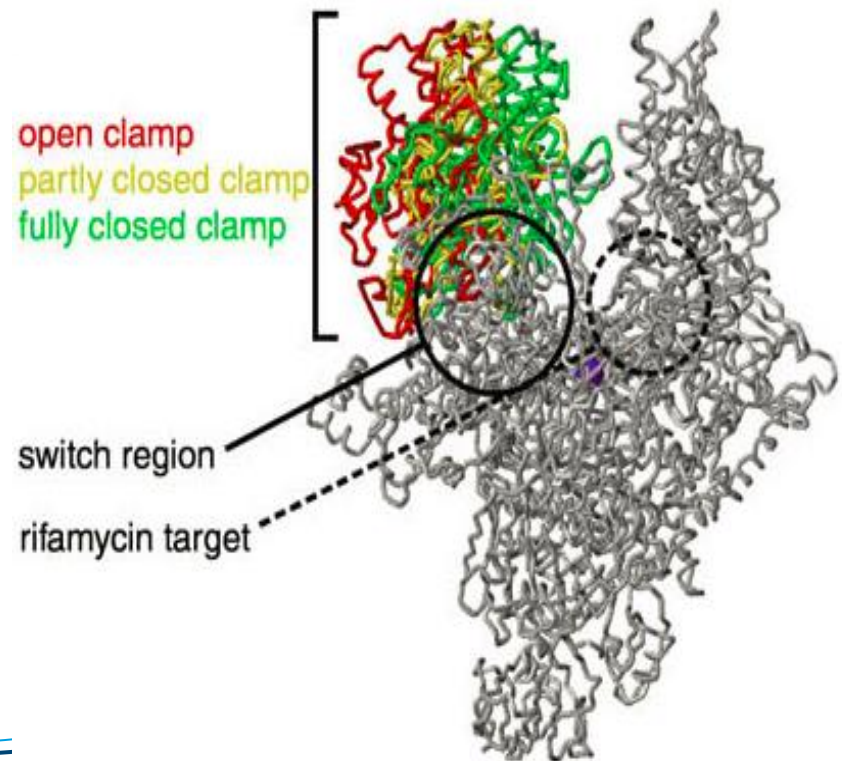
Wolbachia behind multiple physical barriers



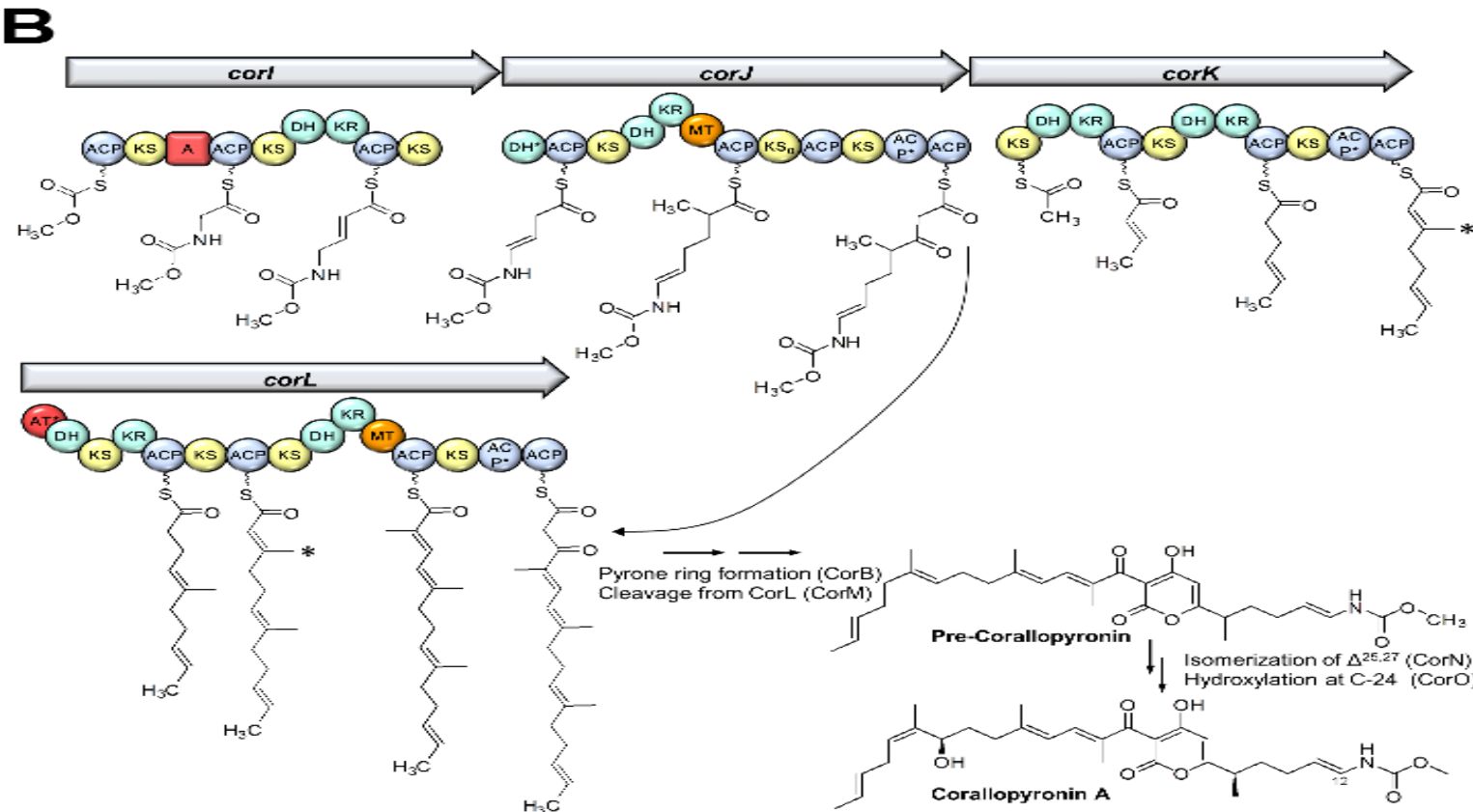
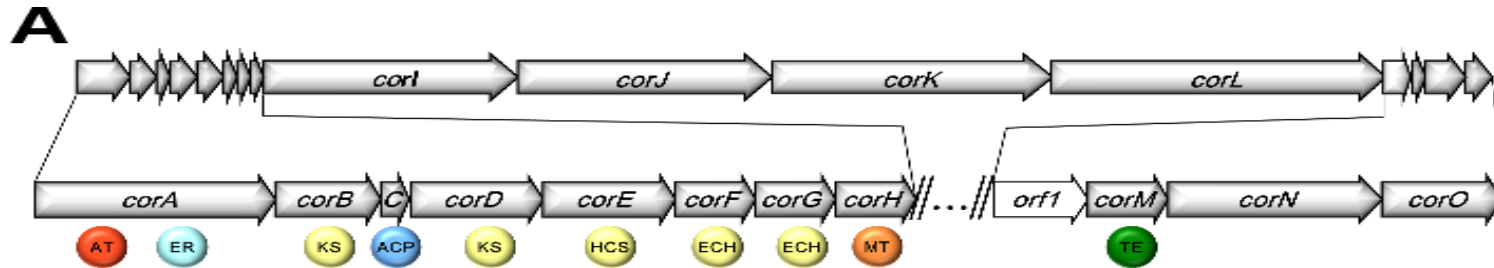
Corallopyronin A (CorA)



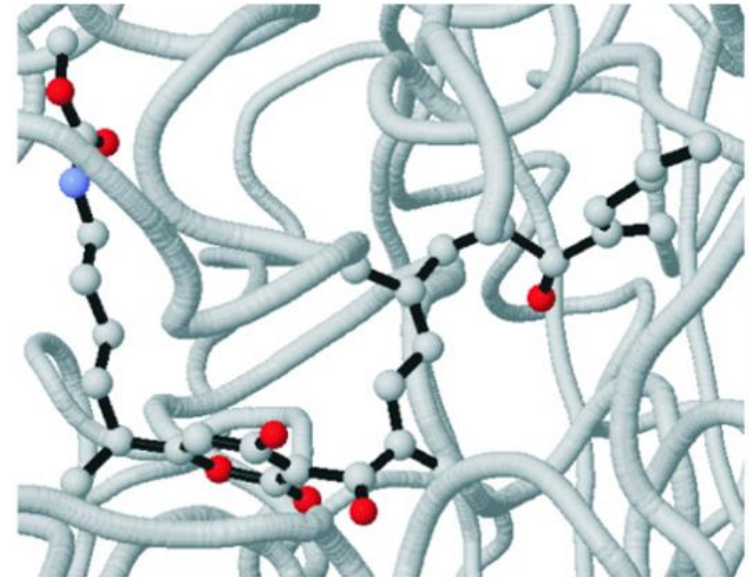
- *Coralloccoccus coralloides*
 - Soil Myxobacteria (gliding bacteria)
- Effective against Gram-positive bacteria
 - Gram-negative $\Delta tolC$ mutants
- Ineffective against *Mycobacterium spp.*
- Non-toxic
- MoA: different from rifamycins
 - Effective against rifampicin-resistant *Staphylococcus aureus*



Corallopyronin A



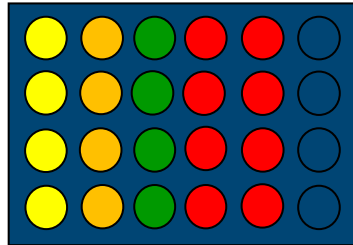
Corallopyronin A binding site is conserved in bacteria



RpoBC wMel	1356	GGQRF	GEMECWALQA	..X ₉ ..	MLT	..X ₂₇ ..	PES	SFNVMIKE
RpoBC wBm	1356	GGQRF	GEMECWALQA	..X ₉ ..	MLT	..X ₂₇ ..	PES	SFNVMIKE
RpoB <i>T. thermophilus</i>	1028	GGQRF	GEMEVWALEA	..X ₉ ..	MLT	..X ₂₇ ..	PES	SFRVLVKE
RpoB <i>E. coli</i>	1266	GGQRF	GEMEVWALEA	..X ₉ ..	MLT	..X ₂₇ ..	PES	SFNVLLKE
RpoB <i>S. aureus</i>	1071	GGQRF	GEMEVWALEA	..X ₉ ..	ILT	..X ₂₇ ..	PES	SFRVLMKE
RpoB <i>M. tuberculosis</i>	1058	GGQRF	GEMECWAMQA	..X ₉ ..	LLT	..X ₂₇ ..	PES	SFKVLLKE
RpoB <i>M. bovis</i>	1058	GGQRF	GEMECWAMQA	..X ₉ ..	LLT	..X ₂₇ ..	PES	SFKVLLKE
RpoB <i>M. smegmatis</i>	1055	GGQRF	GEMECWAMQA	..X ₉ ..	LLT	..X ₂₇ ..	PES	SFKVLLKE
					*		*	*

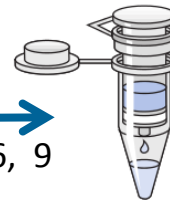
RpoBC wMel	1769	GRFRQNL	LGKRV	..2221	LVDVSQ	..2739	SFISAAS	FQETT	..X ₁₆ ..	GLKENVI
RpoBC wBm	1769	GRFRQNL	LGKRV	..2224	LVDVSQ	..2742	SFISAAS	FQETT	..X ₁₆ ..	GLKENVI
RpoC <i>T. thermophilus</i>	612	GRFRQNL	LGKRV	..1098	LVDVTH	..1433	SWLSAAS	FQNTT	..X ₁₆ ..	GLKENVI
RpoC <i>E. coli</i>	336	GRFRQNL	LGKRV	..800	LVDVAQ	..1318	SFISAAS	FQETT	..X ₁₆ ..	GLKENVI
RpoC <i>S. aureus</i>	325	GRFRQNL	LGKRV	..808	LVDVAQ	..1136	SFLSAAS	FQETT	..X ₁₆ ..	GLKENVI
RpoC <i>M. tuberculosis</i>	411	GRFRQNL	LGKRV	..877	LVDVSQ	..1219	SWLSAAS	FQETT	..X ₁₆ ..	GLKENVI
RpoB <i>M. bovis</i>	411	GRFRQNL	LGKRV	..877	LVDVSQ	..1219	SWLSAAS	FQETT	..X ₁₆ ..	GLKENVI
RpoB <i>M. smegmatis</i>	411	GRFRQNL	LGKRV	..876	LVDVSQ	..1220	SWLSAAS	FQETT	..X ₁₆ ..	GLKENVI
					*		*		*	*

CorA treatment depletes *Wolbachia* from *Aedes albopictus* cells



C6/36 cells infected with *Wolbachia*

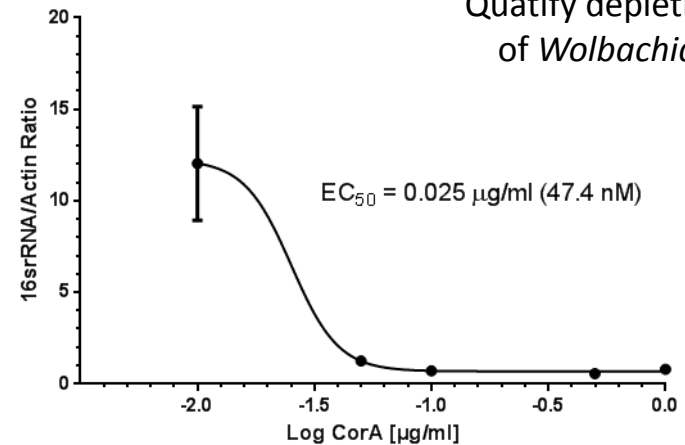
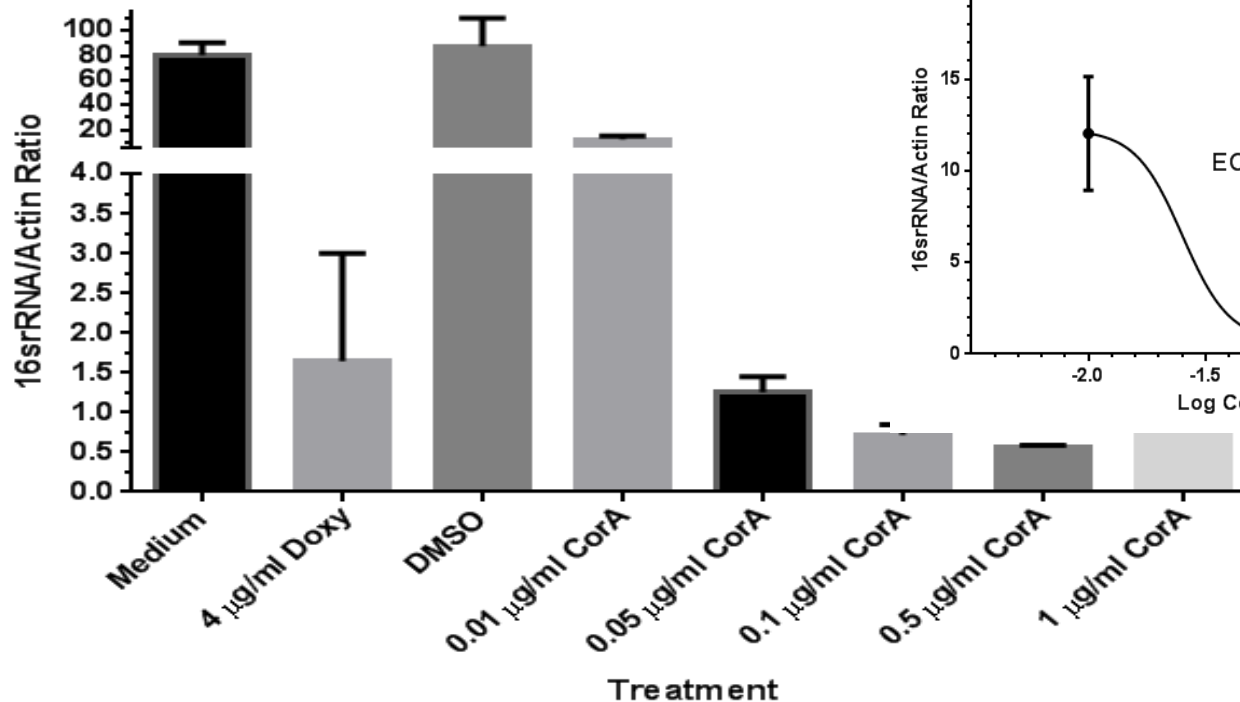
Incubate with antibiotics;
changed every third day
Harvest cells on days 0, 3, 6, 9



Isolate DNA

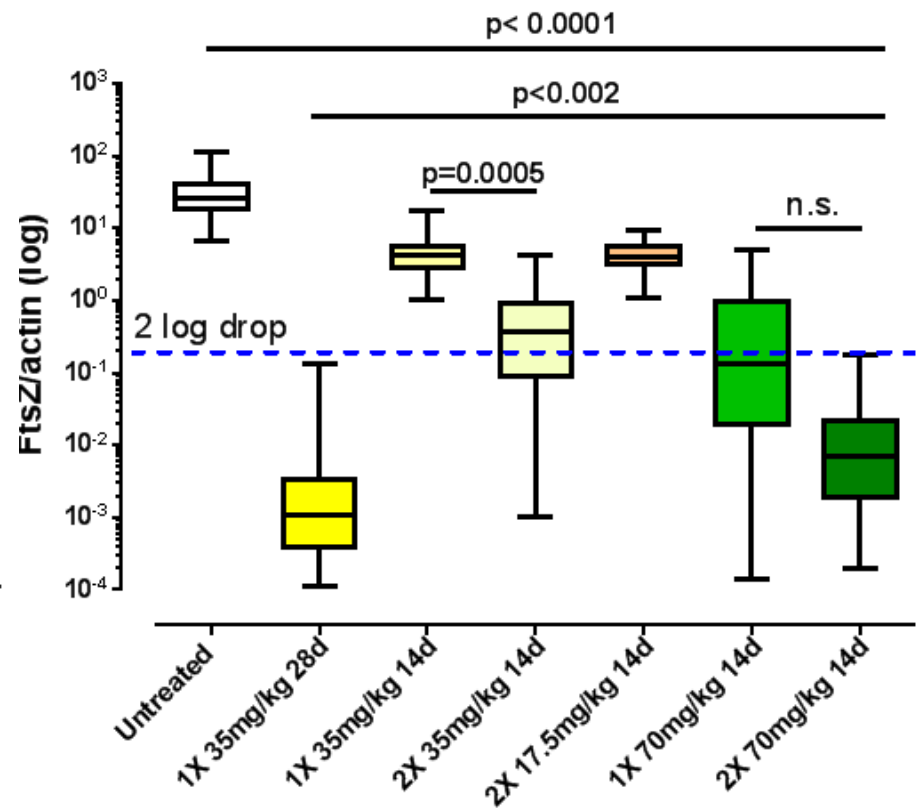
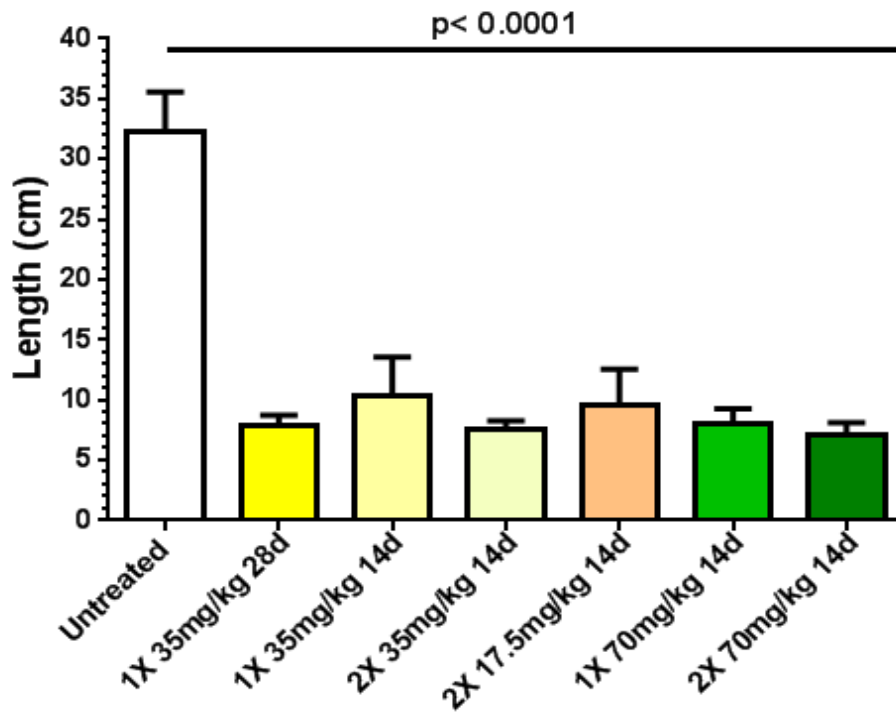


Quantify depletion of *Wolbachia*

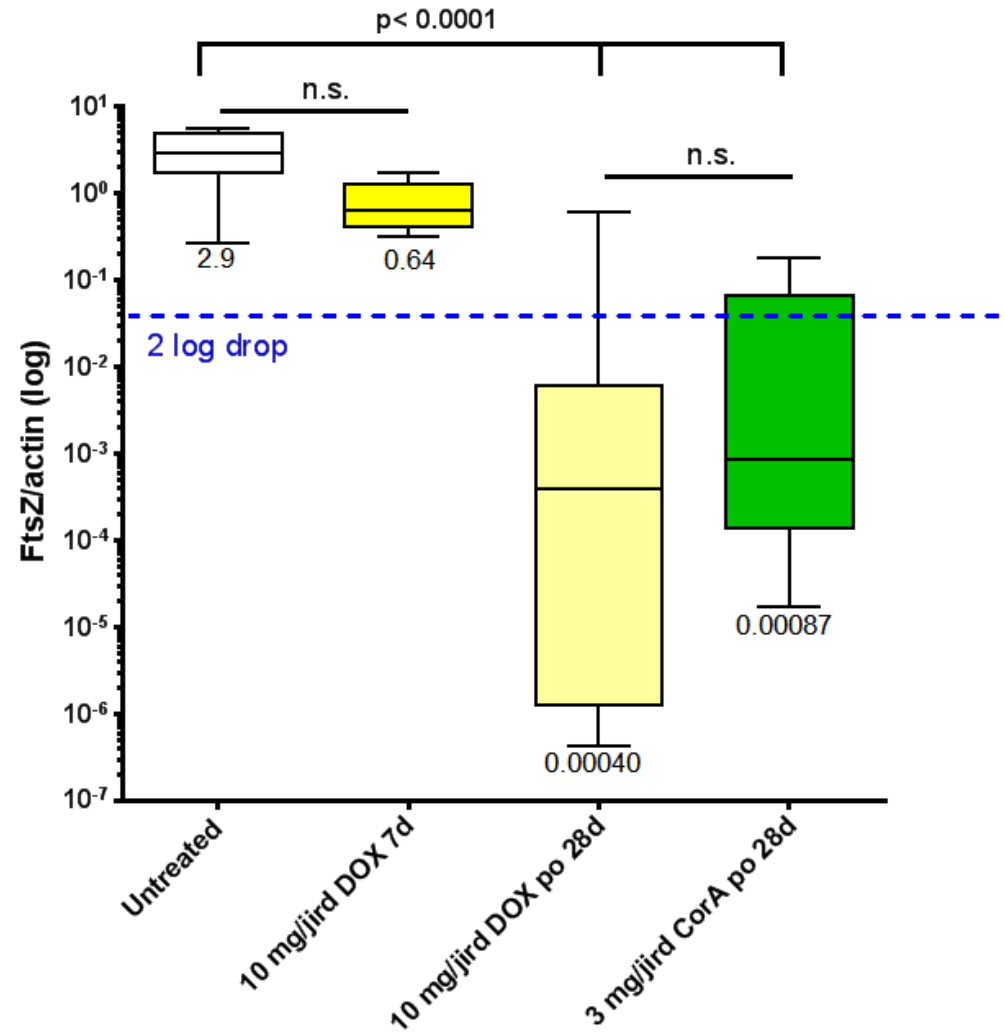
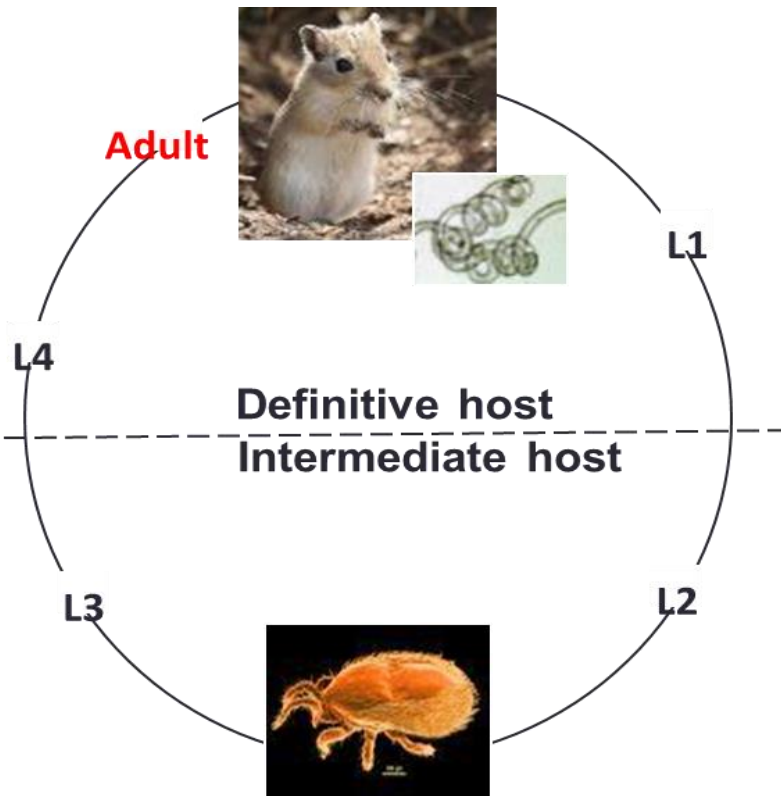


1.89 µM CorA is equivalent to 7.8 µM of doxycycline

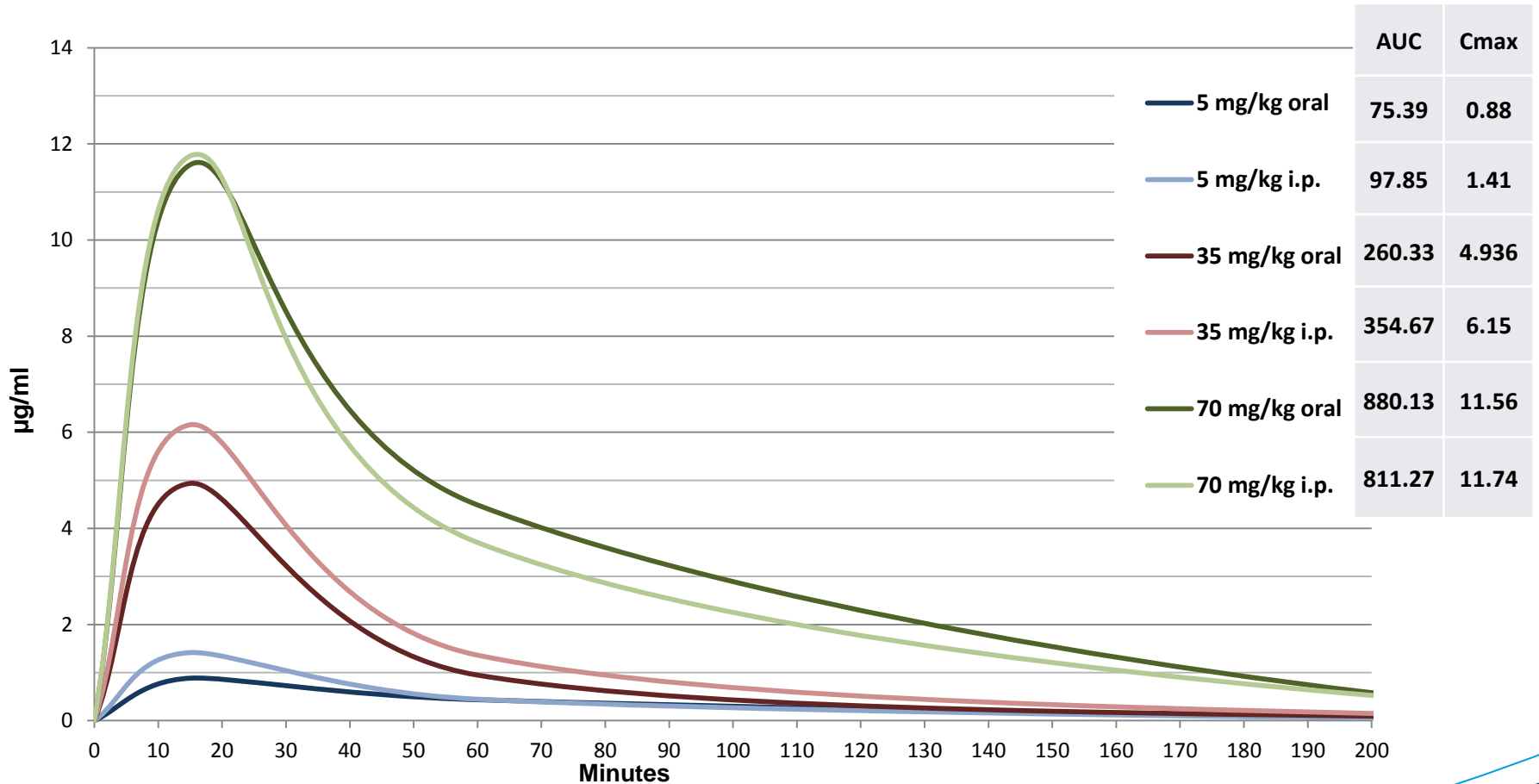
CorA depletes *Wolbachia* from *Litomosoides sigmodontis*



CorA depletes *Wolbachia* from adult worms




CorA PK in mice

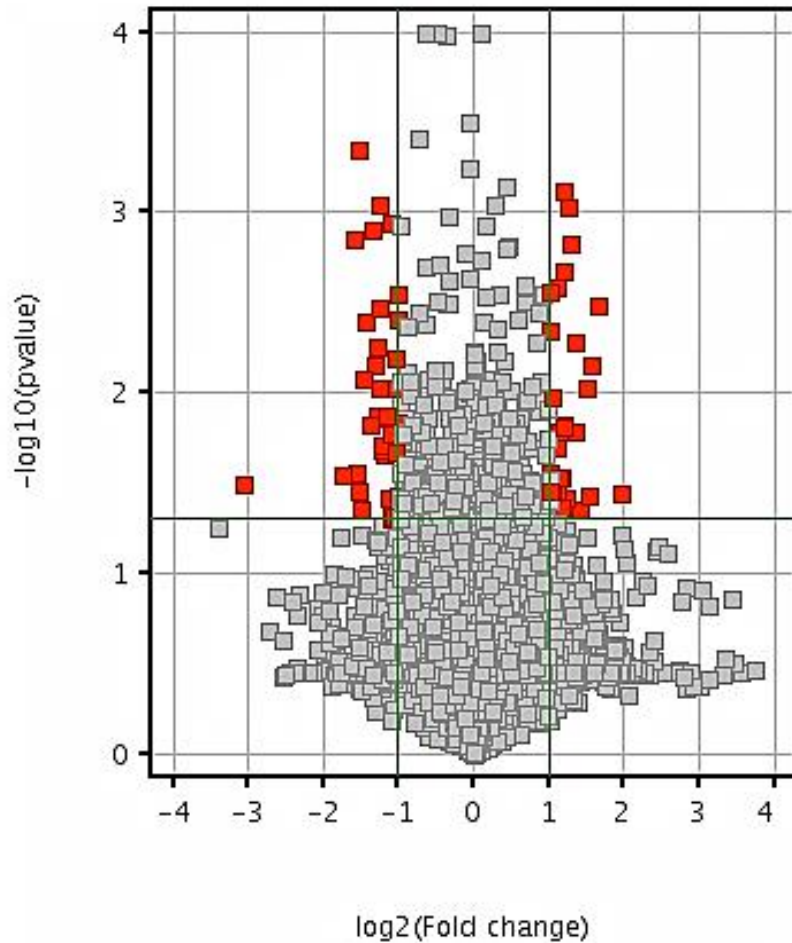


CorA is not cytotoxic

Sample	(+ Strep) ¹		(-Strep) ²	
	xCell	Cedex	xCell	Cedex
	Viable cells in relation to control (%)	Viable cells in relation to control (%)	Viable cells in relation to control (%)	Viable cells in relation to control (%)
DMSO³	100.0	100.0	100.0	100.0
CorA 200 µg/mL	24.2	9.3	11.2	17.4
CorA 20 µg/mL	89.0	81.6	90.8	73.8
CorA 2 µg/mL	102.9	96.1	101.8	100.0
CorA 0.2 µg/mL	98.2	93.0	106.7	104.2
CorA 0.02 µg/mL	100.4	91.6	107.9	101.1

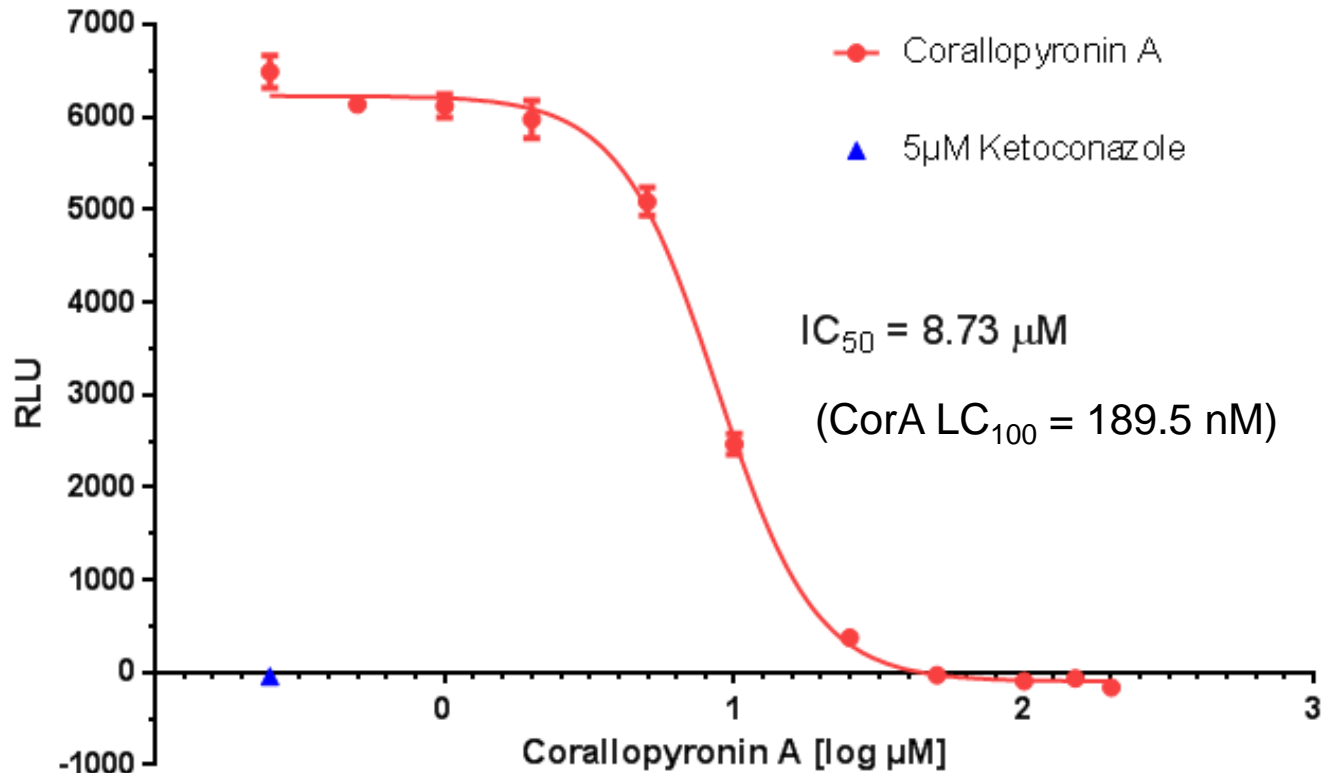
1 µg/mL


CYP450s are minimally regulated

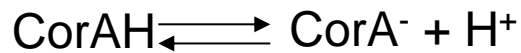
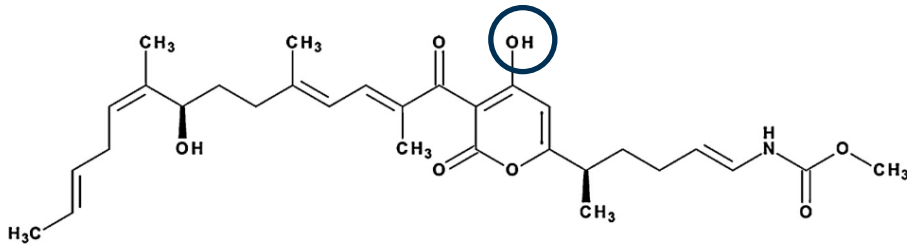


- Microarray of HepG2 treated 6 hours with CorA 15 $\mu\text{g/ml}$
- 32 transcripts up-regulated
- 36 transcripts down-regulated
 - None encode cytochrome P4503A4
 - CYP4504F2 down-regulated 2-fold (CorA 100 $\mu\text{g/ml}$)

CYP450 3A4 is not inhibited



Physicochemical characterization



weak acid: pKa = 3.6

solubility:

pH 1 (stomach) 0.0001 mg/ml

pH 6 (small int.) 0.029 mg/ml

pH 7.4 (blood) 0.723 mg/ml

Despite poor solubility at
absorption site (small intestine)
no issues with oral bioavailability
→ *in vivo* sink of highly permeable
lipophilic CorA

assessed by

Octanol water distribution coefficient

log D:

pH 1 (stomach) 5.42

pH 6 (small int.) 3.00

pH 7.4 (blood) 1.81

$$D = \frac{[\text{CorAH}]_{\text{oct}} + [\text{CorA}^-]_{\text{oct}}}{[\text{CorAH}]_{\text{H}_2\text{O}} + [\text{CorA}^-]_{\text{H}_2\text{O}}}$$

Despite high degree of ionization
still highly lipophilic

Conclusions and Outlook

- *Wolbachia* RNAP is predicted to bind CorA
 - Fills binding pocket of the switch region, prevents closing
 - Limited chance of rifamycin cross-resistance
- CorA depleted *Wolbachia* from a infected insect cell line
 - $EC_{50} = 47.4 \text{ nM}$
- CorA is effective *in vivo*
 - Depletes *Wolbachia* > 2-logs from larvae and adults
 - See expected phenotypes: blocked larval development and Mf release
- CorA is not cytotoxic in the effective dose range
- CYP450 3A4 is not induced nor inhibited
 - Negative drug-drug interaction are limited
- ADME and preclinics within the German Center for Infection Research (DZIF)

Acknowledgements

UKB and Uni Bonn

Prof. Dr. Achim Hoerauf
Prof. Dr. Gabrielle M. König
Prof. Dr. Karl G. Wagner
Dr. Andrea Schiefer
Dr. Sabine Specht
Dr. Till F. Schäberle
Dr. Sarah Bouhired
Christine Lämmer
Alexander Schmitz
Katharina Gorski
Helene Neufeld

External Collaborators:

Dr. Dmitry G. Vassilyev, University of
Alabama at Birmingham

Dr. Martin Roth, Hans-Knöll-Institut für
Naturstoff-Forschung und Infektionsbiologie

Publications:

EU Pat.- Pend.: EP12 721 456.7
USA Pat.-Pend.: US14/115,358
Schiefer, A. *et al.* (2012). *J Infect Dis*

Funding:



For 854

