Selective targeting of bad bugs in the gut may have many health benefits

Nebojsa Janjic, Ph.D.

Crestone, Inc.

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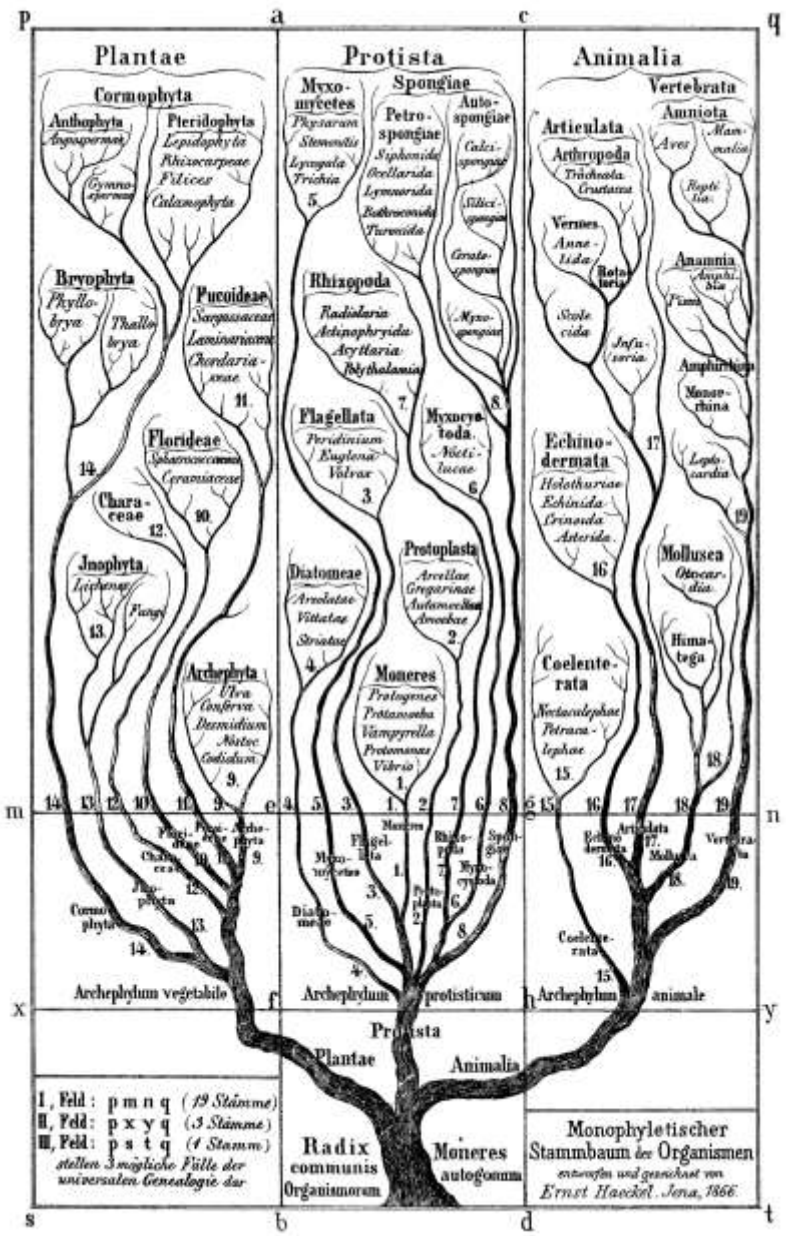
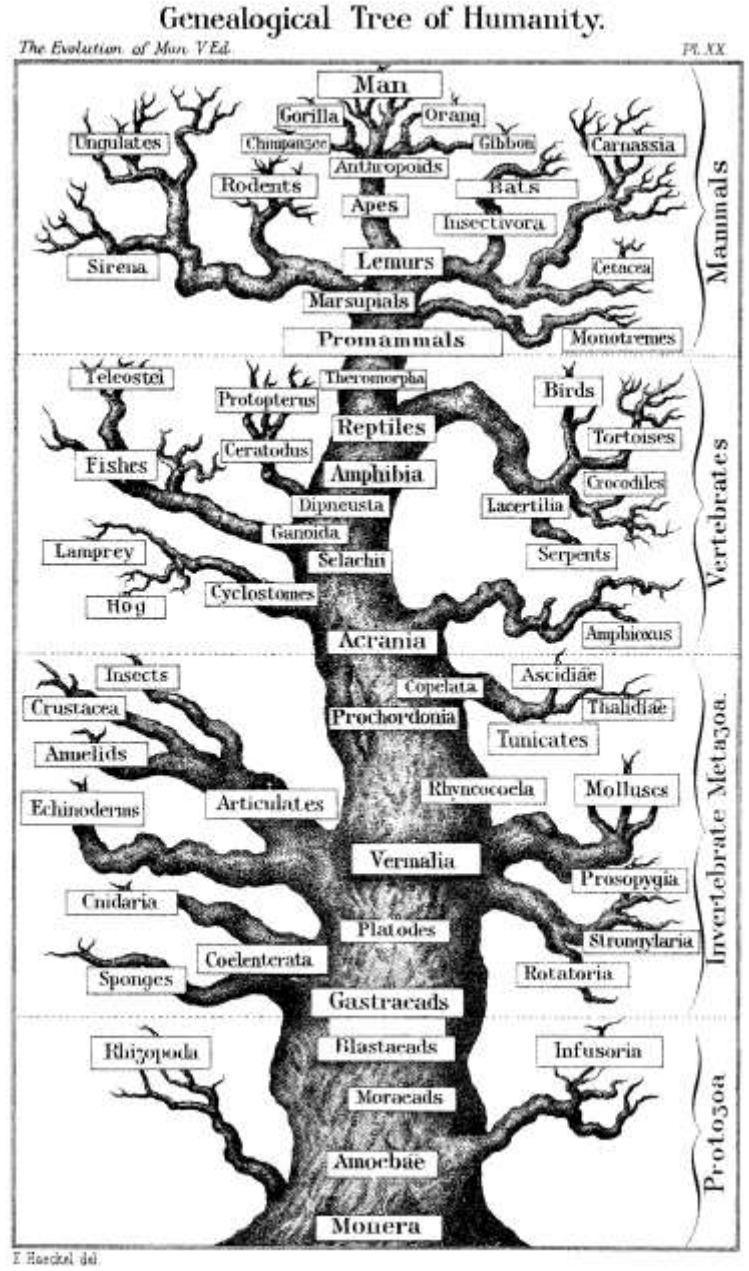
Life, sorted

- Species in the context of evolution: phylogeny
- Sorted based on appearance





1866

1879

Haeckel (1866) General Morphology of Organisms
Haeckel (1879) The Evolution of Man

Life, rearranged

- All life has similar molecular machinery
- DNA → RNA → Protein
- Phylogeny based on ribosomal RNA sequence, rather than appearance

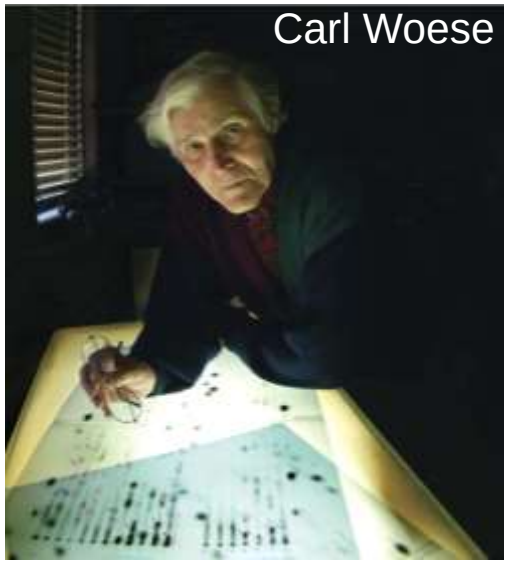
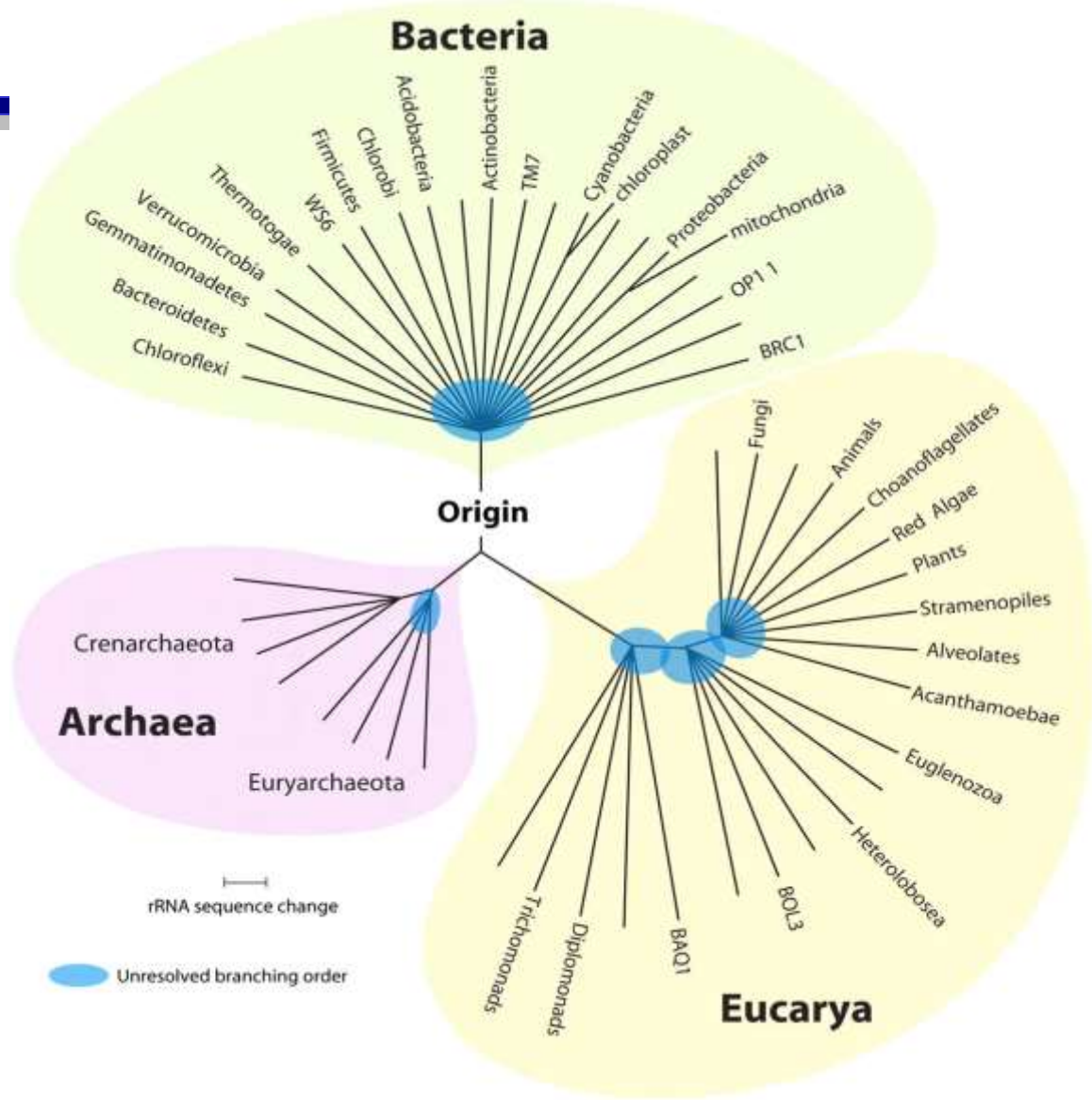


Photo by Jason Lindley

…AAUGCCGAUCAAGUAUCG…
…AAUGCCGAUCGAGAAUCG…

…AAUGCCGAUGGUCCGAUG…



Woese and Fox (1977) *Proc. Natl. Acad. Sci*. **74**, 5088
Nair (2012) *Proc. Natl. Acad. Sci*. **109**, 1021
Pace (2009) *Microbiol. Mol. Biol. Rev*. **73**, 565

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We can now detect organisms we can't culture

- Discovery of rich bacterial communities in:
  - Thermal vents (like Yellowstone)
  - Caves
  - New York subways
  - Shower curtains
  - Intestines

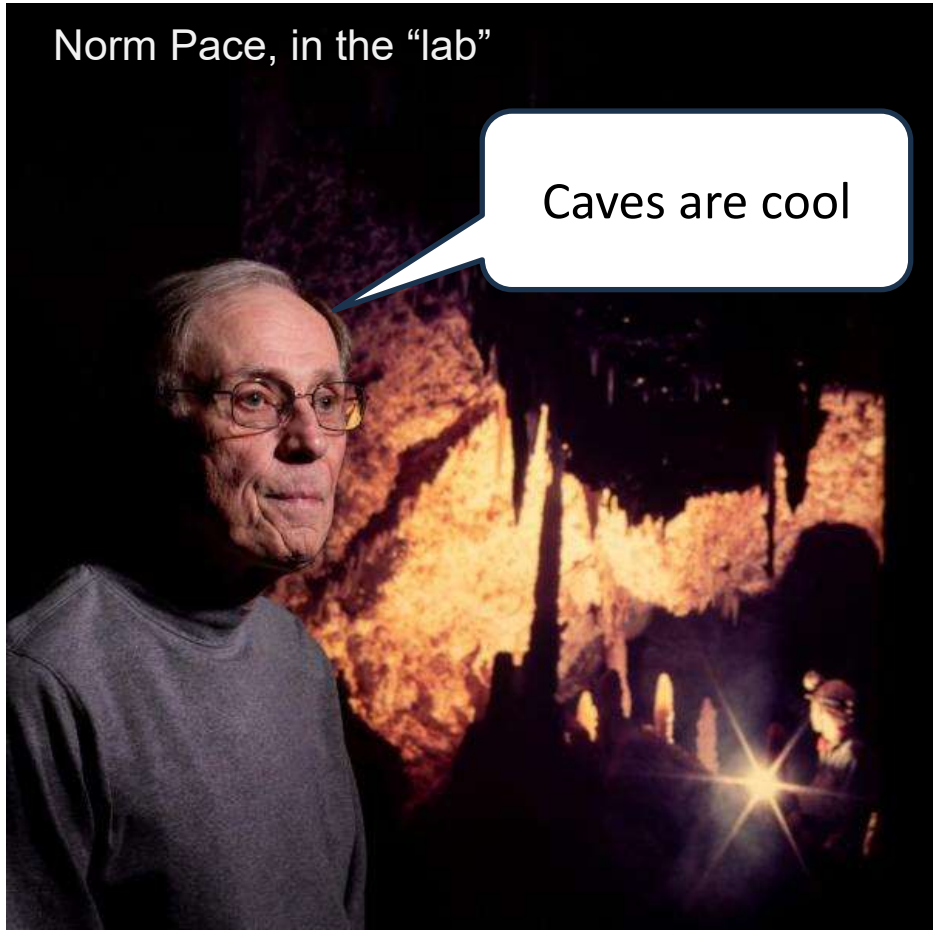

Photo by Glenn Asakawa

Stahl *et al*. (1984) *Science* **224**, 409
Pace (2009) *Microbiol. Mol. Biol. Rev*. **73**, 565

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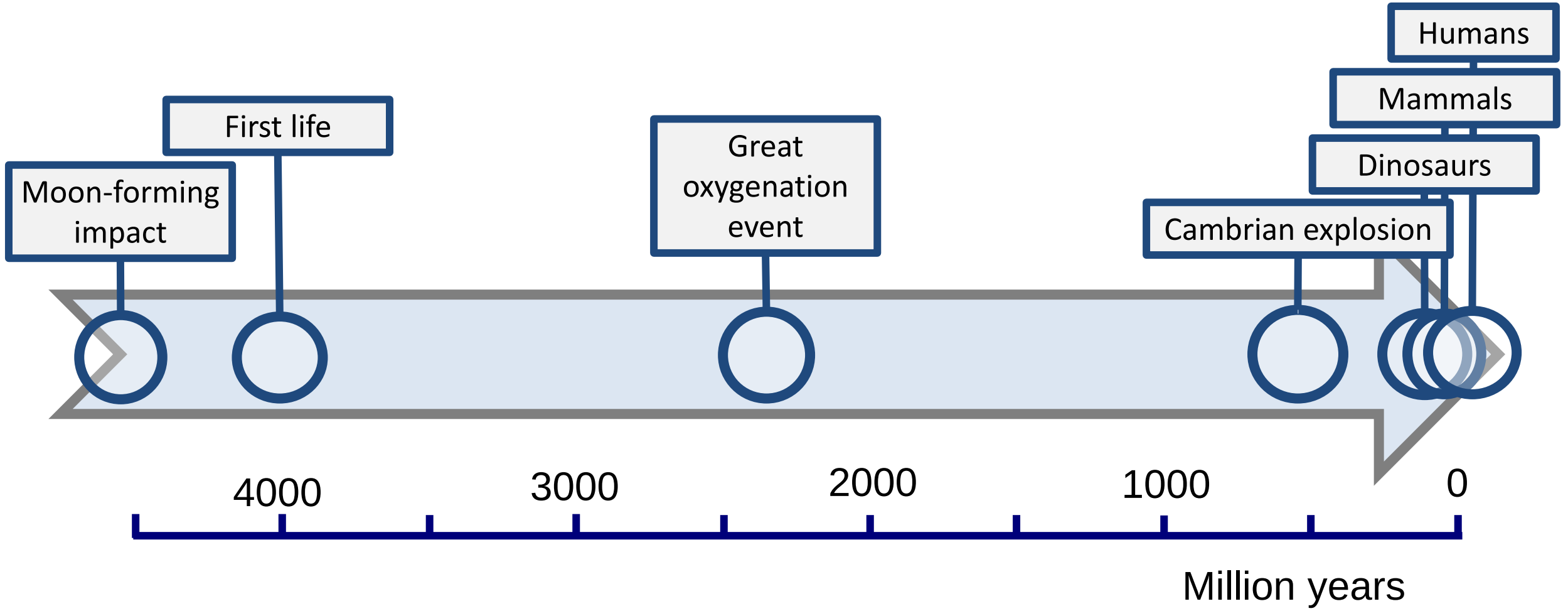
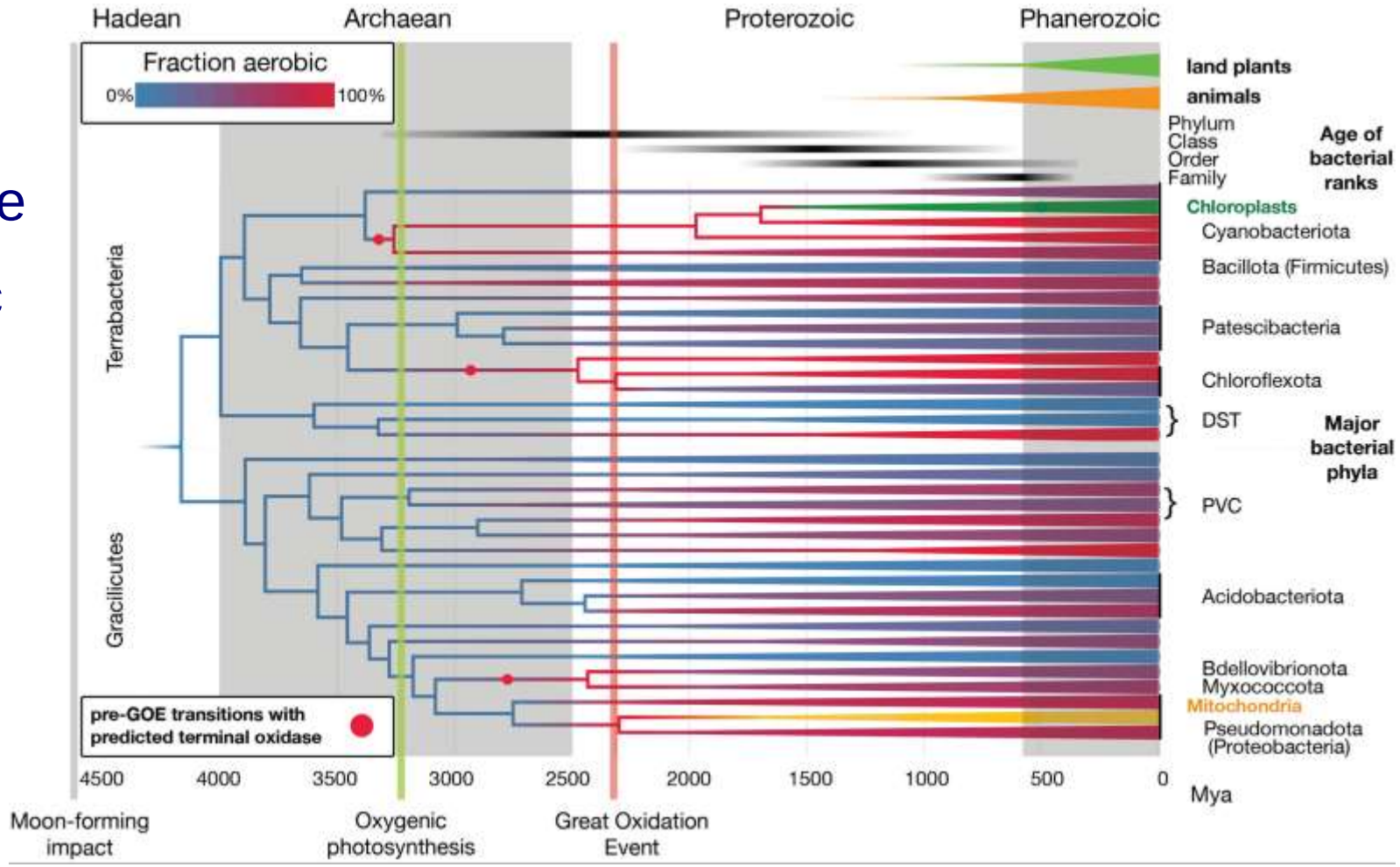
Adding time: abridged history of the Earth



Anaerobic organisms were the first lifeforms on Earth

- Oxygen emerged late
- Humans emerged really late
- GI tract is mostly anaerobic
- More than 99% of gut bacteria are obligate anaerobes



Davín *et al*. (2025) *Science* **388**, 50

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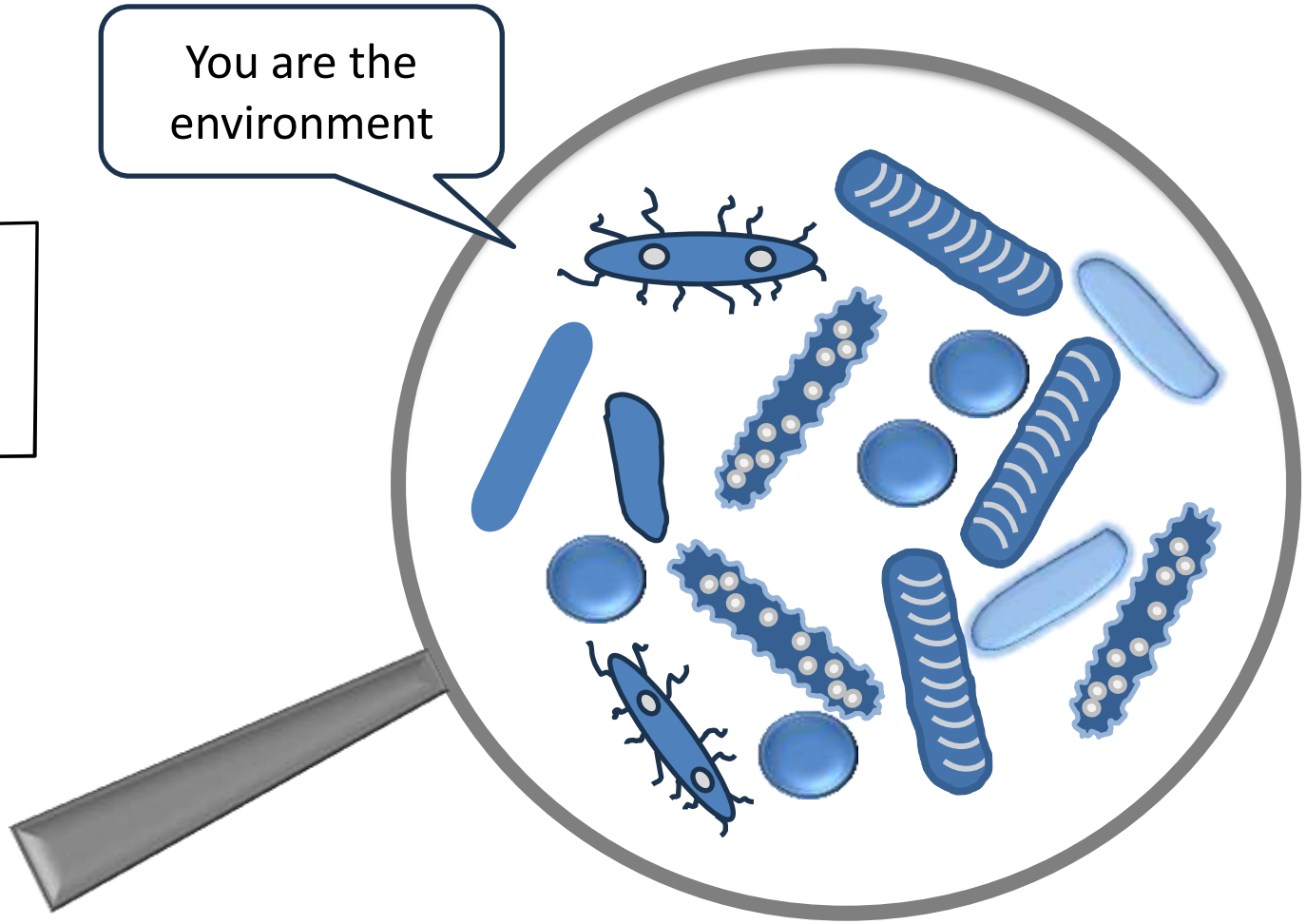
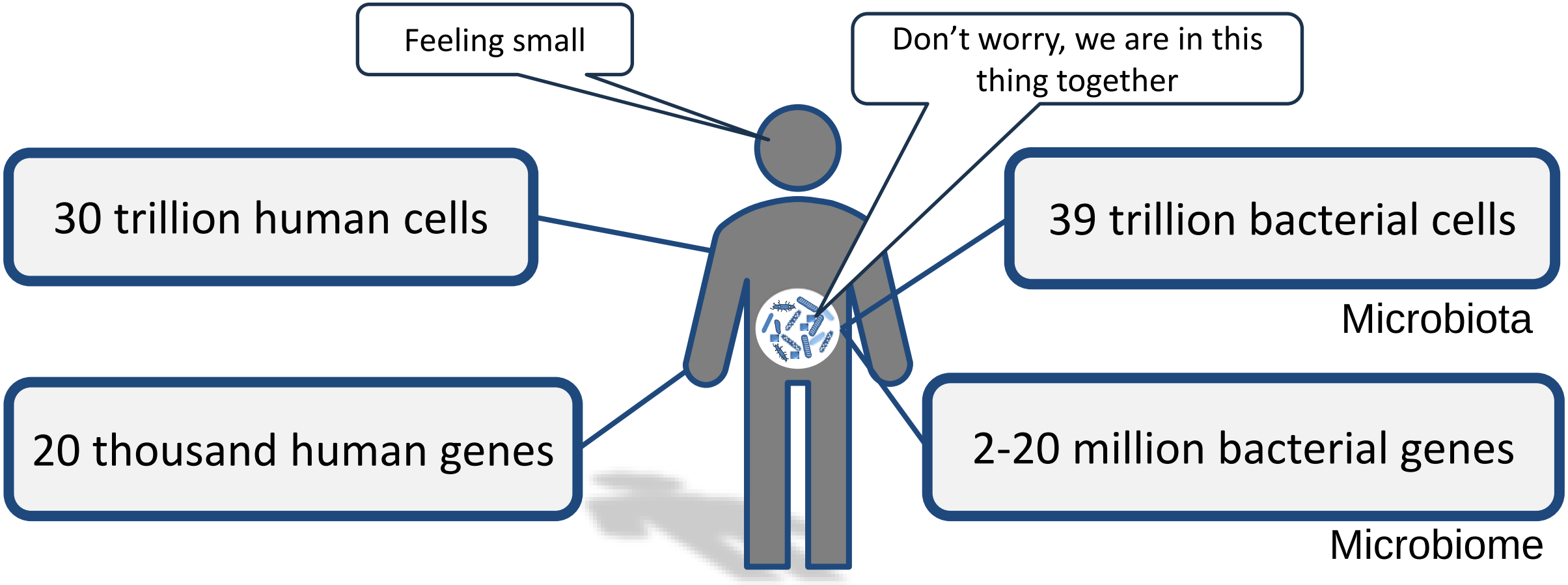
Humans host many bacteria



Bacterial cells and genes outnumber ours



Sender *et al*. (2024) *Cell* **164**, 337

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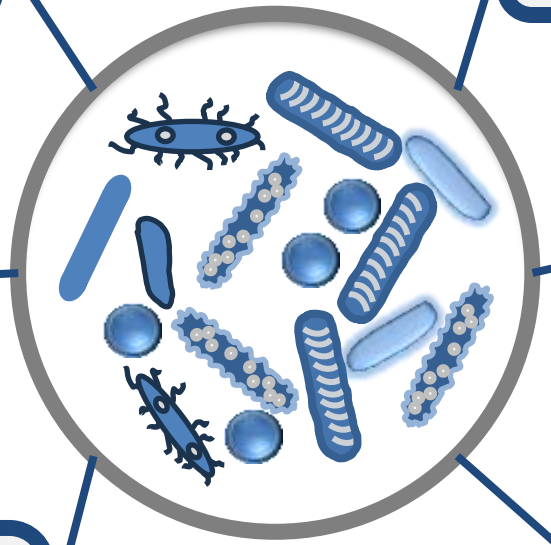
Most gut bacteria are beneficial

Immune system stimulation

Nutrient metabolism and fermentation

Protection against pathogens (colonization resistance)



Extraction of energy from food

Short-chain fatty acid synthesis

Maintenance of colon epithelium

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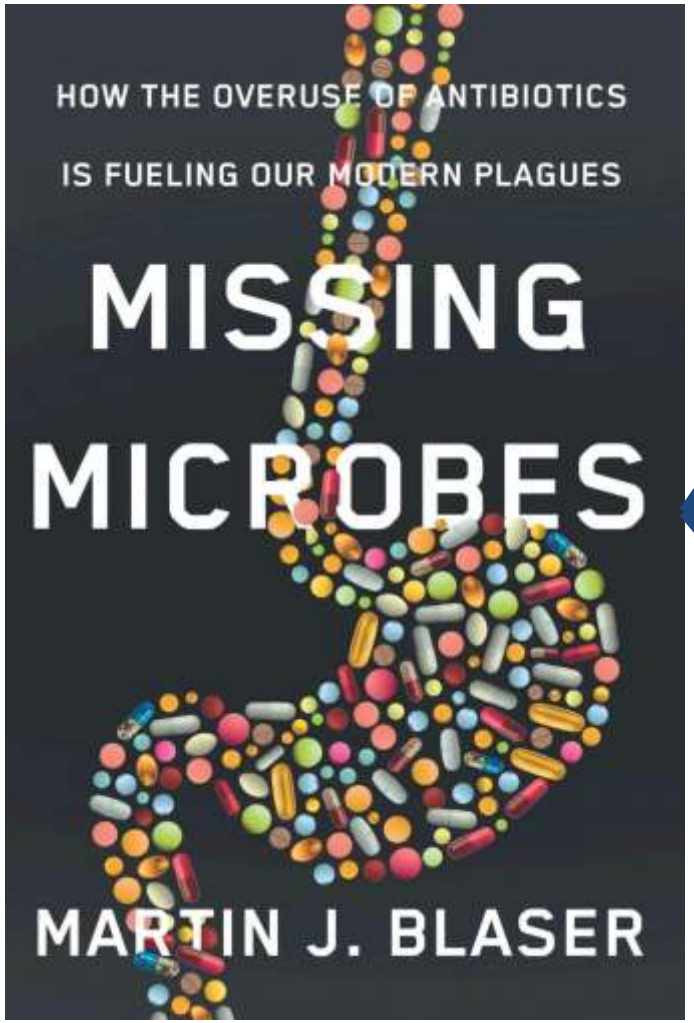
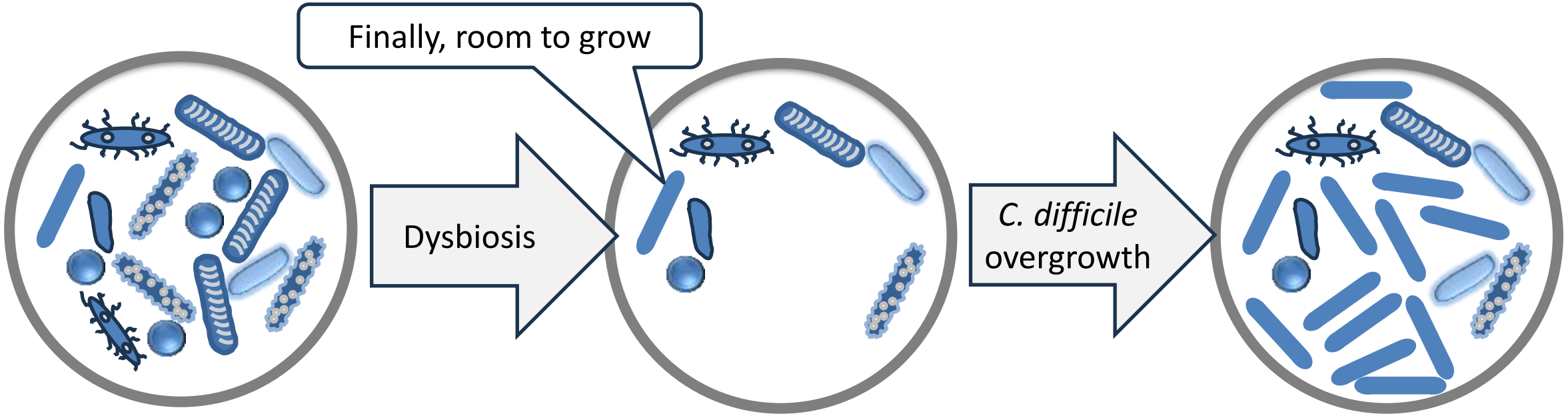
Modern life has compromised microbial diversity



- ↑ Processed food
- ↓ Fiber consumption
- ↑ Antibiotics use
- ↓ Family size
- ↓ Breast feeding
- ↑ C-sections

Damage to gut flora weakens colonization resistance

- Most infections are treated empirically (with broad-spectrum antibiotics)
- This causes massive damage to gut flora
- And antibiotic-associated diarrhea, mostly caused by *Clostridioides difficile* ( )



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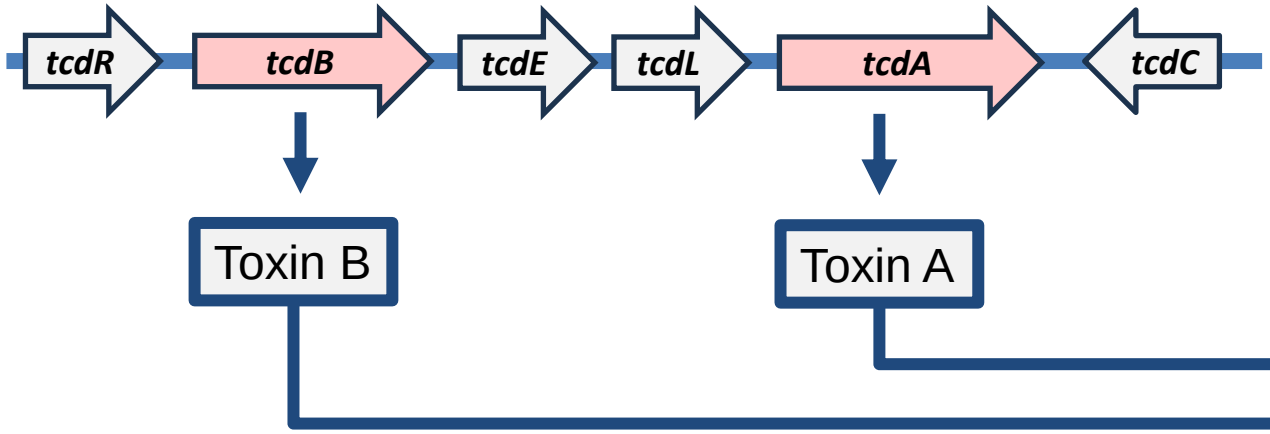
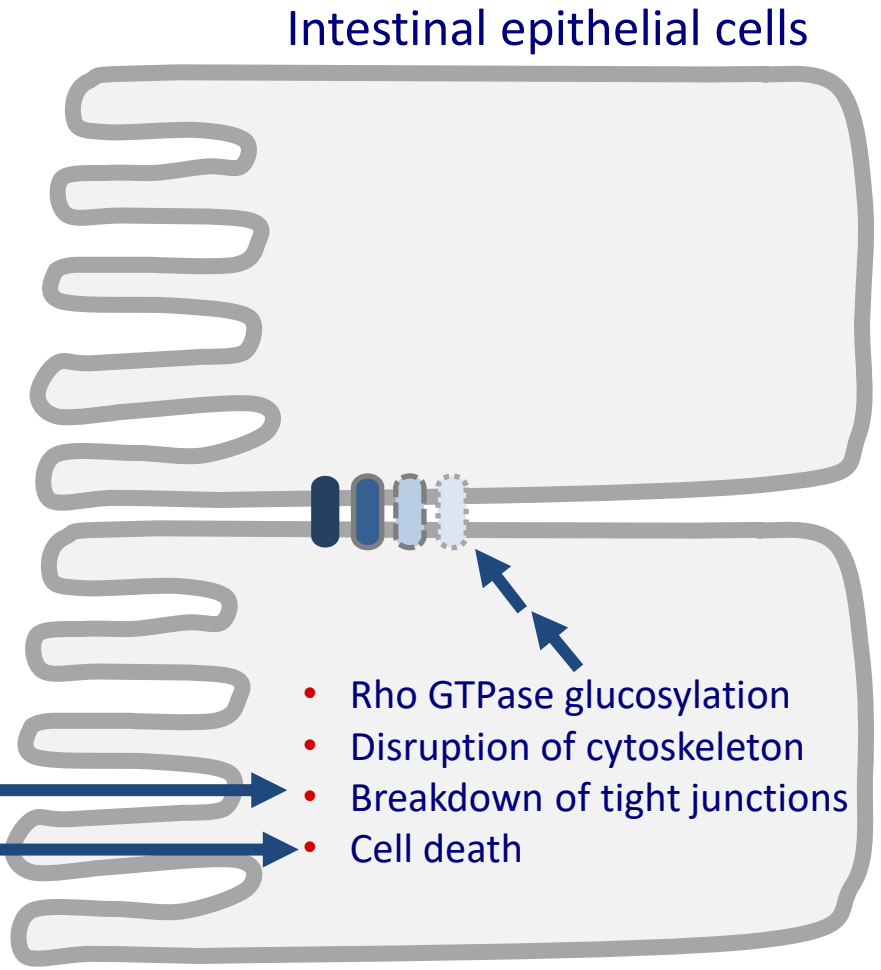
Toxinogenic *C. difficile* is a bad bug

- Toxins A and B (and other toxins) encoded on pathogenicity island (PaLoc) cassette
  - Cause severe damage to intestinal epithelium





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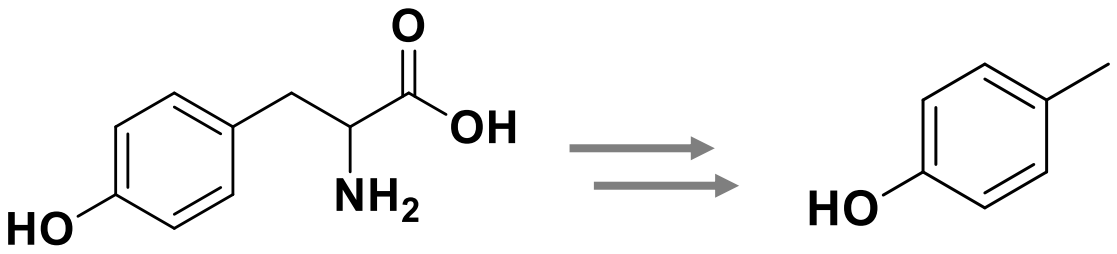
C. difficile also actively suppresses other gut bacteria

- *C. difficile* metabolizes tyrosine to produce *p*-cresol
  - Found in mM levels in stool
- Used as a biological warfare agent



tyrosine *p*-cresol

RESEARCH ARTICLE

Para-cresol production by *Clostridium difficile* affects microbial diversity and membrane integrity of Gram-negative bacteria

Ian J. Passmore[1], Marine P. M. Letertre[2], Mark D. Preston[3], Irene Bianconi[4], Mark A. Harrison[1], Fauzy Nasher[1], Harparkash Kaur[1], Huynh A. Hong[4], Simon D. Baines[5], Simon M. Cutting[4], Jonathan R. Swann[2], Brendan W. Wren[1], Lisa F. Dawson[1]*

Production of *p*-cresol by Decarboxylation of *p*-HPA by All Five Lineages of *Clostridioides difficile* Provides a Growth Advantage

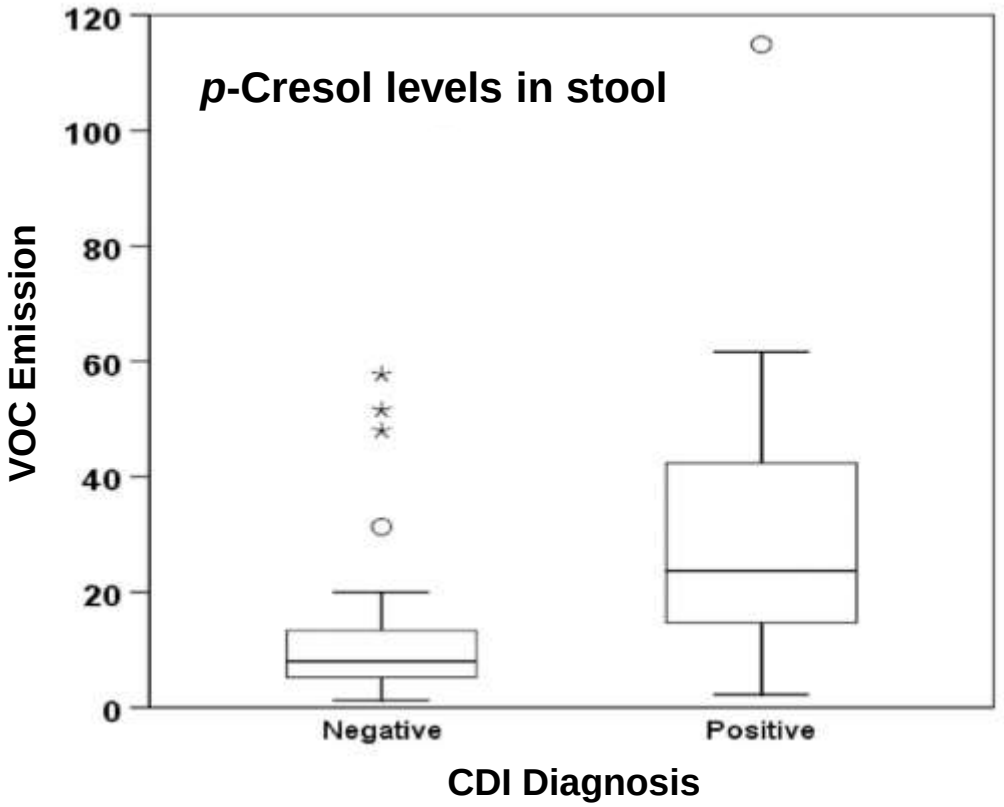
*Mark A. Harrison[1], Harparkash Kaur[2], Brendan W. Wren[1] and Lisa F. Dawson[1]**

Passmore *et al*. (2018) *PLoS Pathog*. **14**, e1007191
Harrison *et al*. (2021) *Front. Cell. Infect. Microbiol*. **11**, 757599

CRESTONE

Some nurses claim they can smell *C. difficile* on patients

- Not a great stand-alone diagnostic test
- Maybe a rule-out test
- But there are definitely higher levels of *p*-cresol in CDI patients

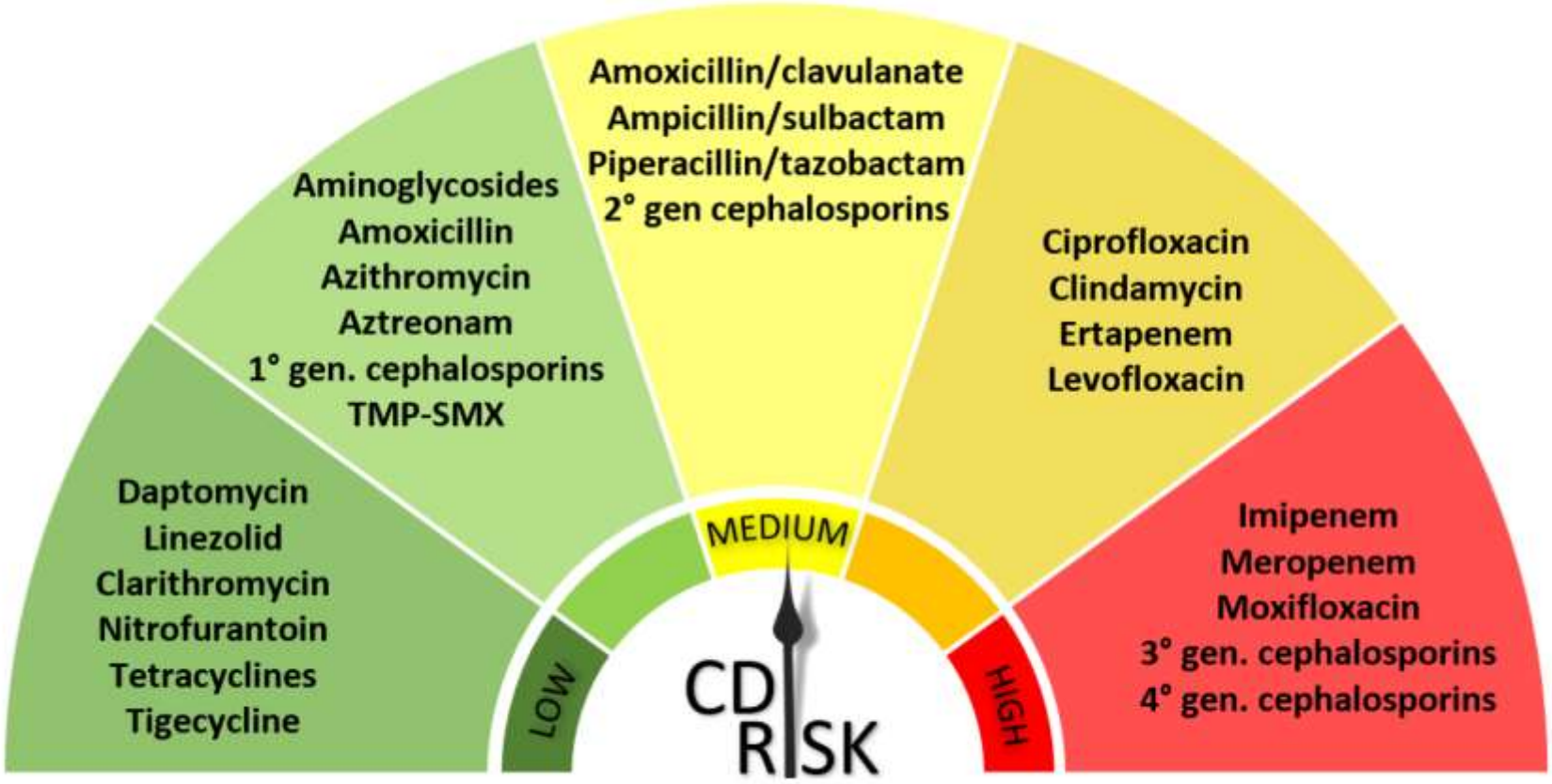


Burdette et al. (2007) *Clin. Infect. Dis*. **44**, 1142
Rao *et al*. (2013) *Clin. Infect. Dis*. **56**, 615
Patel *et al*. (2019) *PLoS ONE* **14**, e0215256

CRESTONE

Broad-spectrum antibiotics increase risk of CDI

- Some are worse than others



Di Bella *et al*. (2024) *Clin Microbiol Rev* **37**, e00135-23

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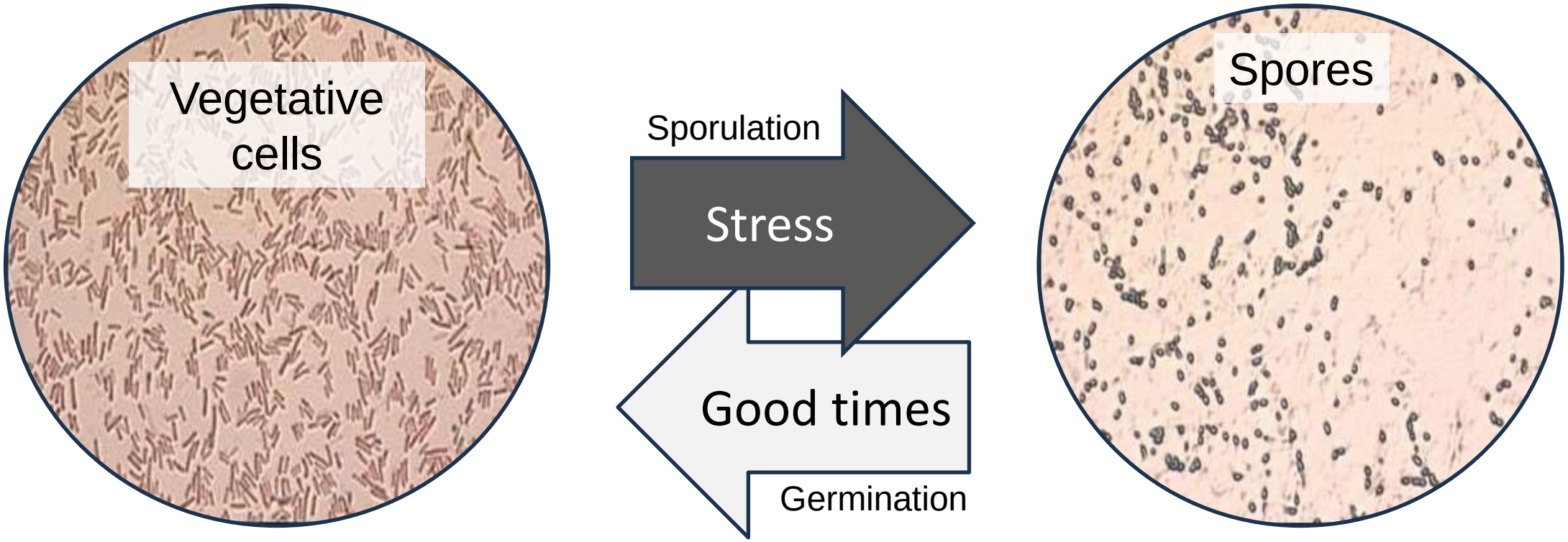
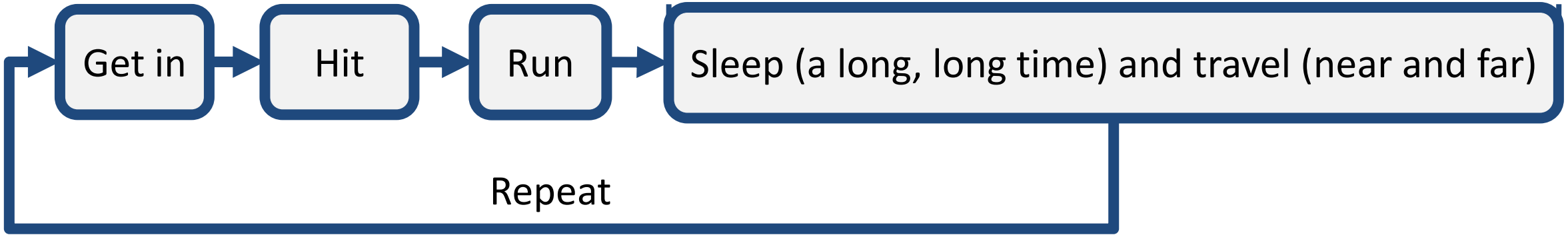
Under stress, *C. difficile* forms spores

- Spores are resistant to heat, alcohol, antibiotics, lack of nutrients

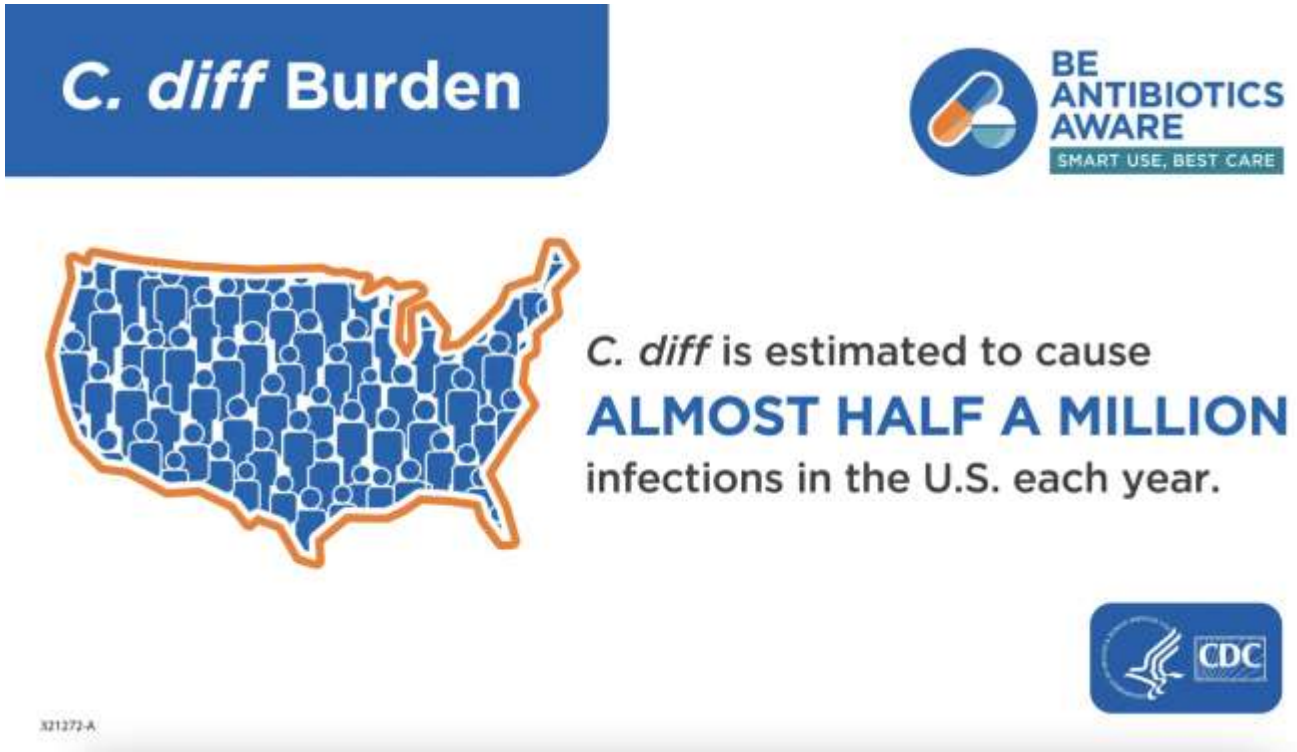


Spore formation is an evolutionary superpower

- No need to be nice
  - To your host
  - Or your neighbors
- Easy spread
- Survival in harsh conditions



C. difficile infection (CDI) remains an urgent threat (CDC)



https://www.cdc.gov/cdiff
https://www.cdc.gov/c-diff/media/images/USBurden.jpg

- High death rate (1 in 11 patients)
- *C. difficile* spores are everywhere

Bobulsky *et al*. (2008) *Clin. Infect. Dis*. **46**, 447

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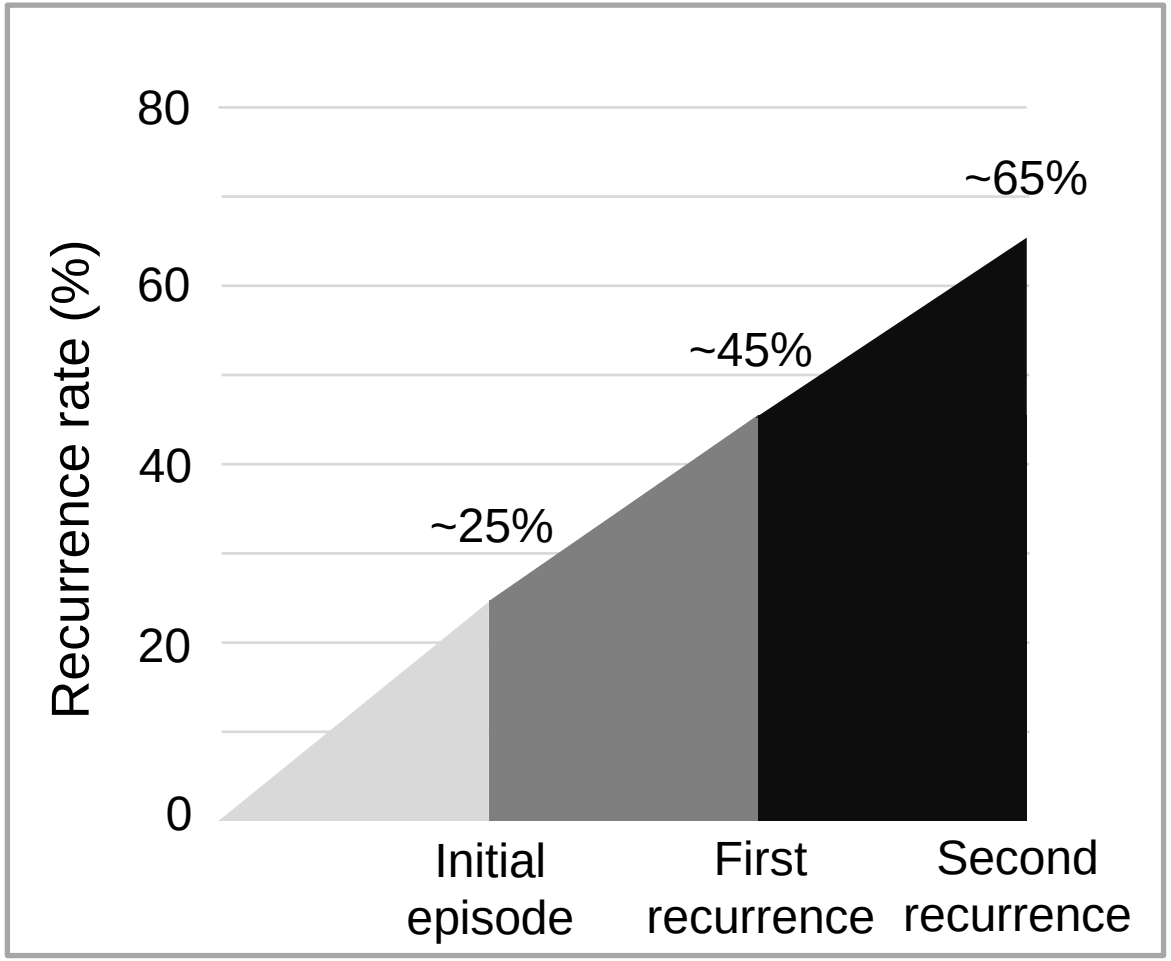
The biggest clinical problem with CDI is recurrence

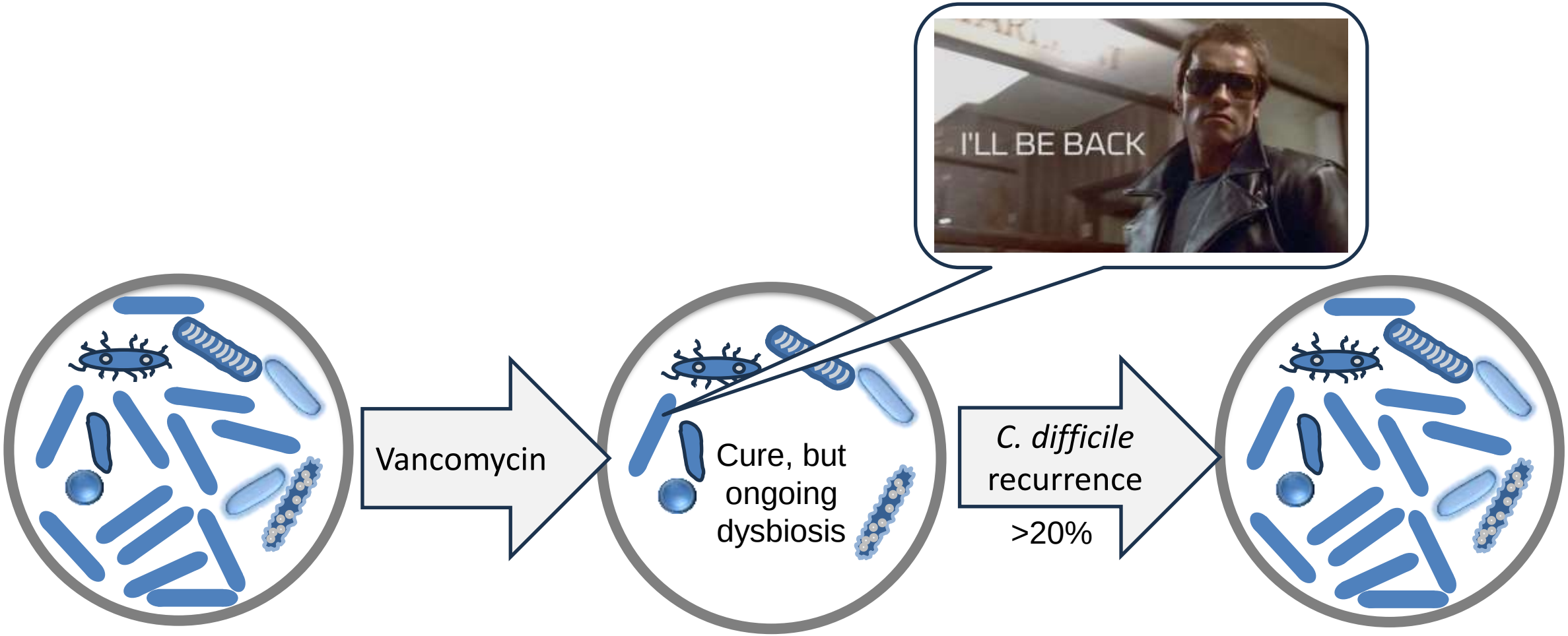
- Initial episode of CDI is successfully treated with one of several available antibiotics
  - Metronidazole (now discouraged)
  - Vancomycin
  - Fidaxomicin
- But recurrence rates remain high



Kelly (2012) *Clin. Microbiol. Infect. (Suppl. 6)* **18**, *21*

CRESTONE

Vancomycin, the most commonly-used treatment for CDI, is also a broad-spectrum antibiotic



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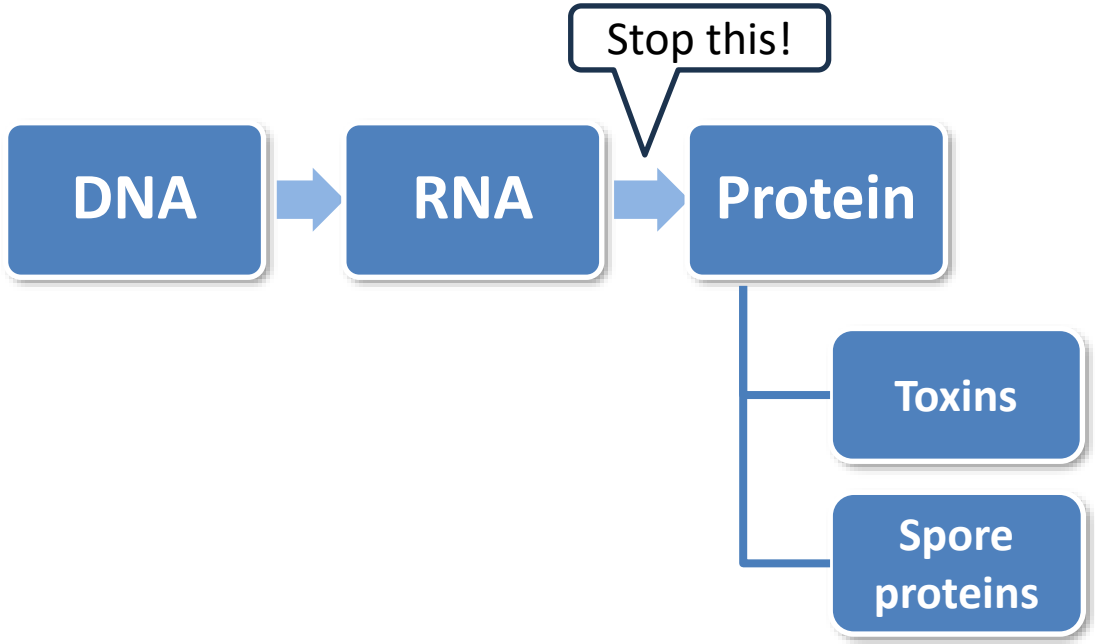
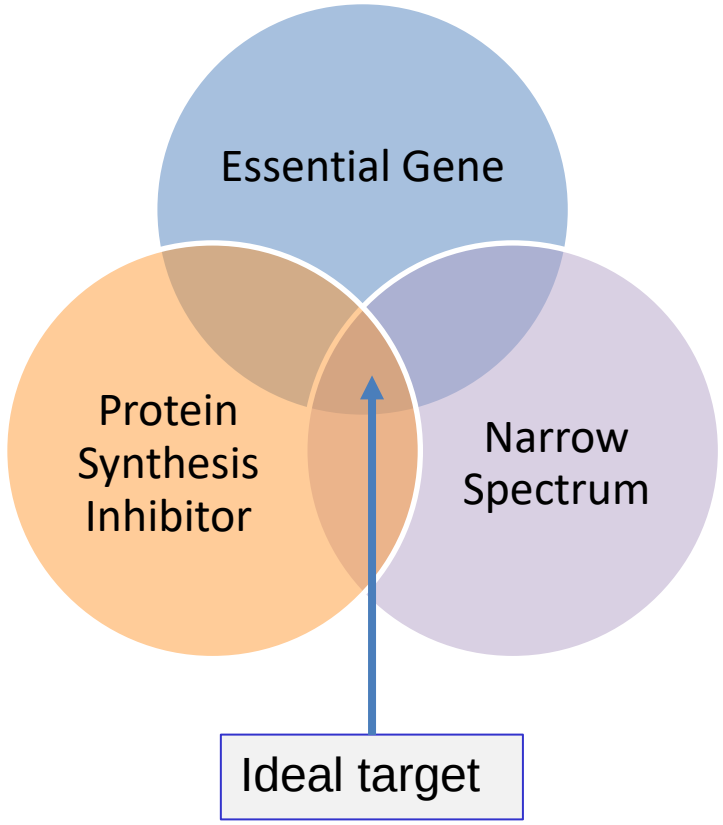
Toward optimal CDI treatment

- Stop toxin production and sporulation (rapidly)
- Cause minimal disruption of beneficial gut flora





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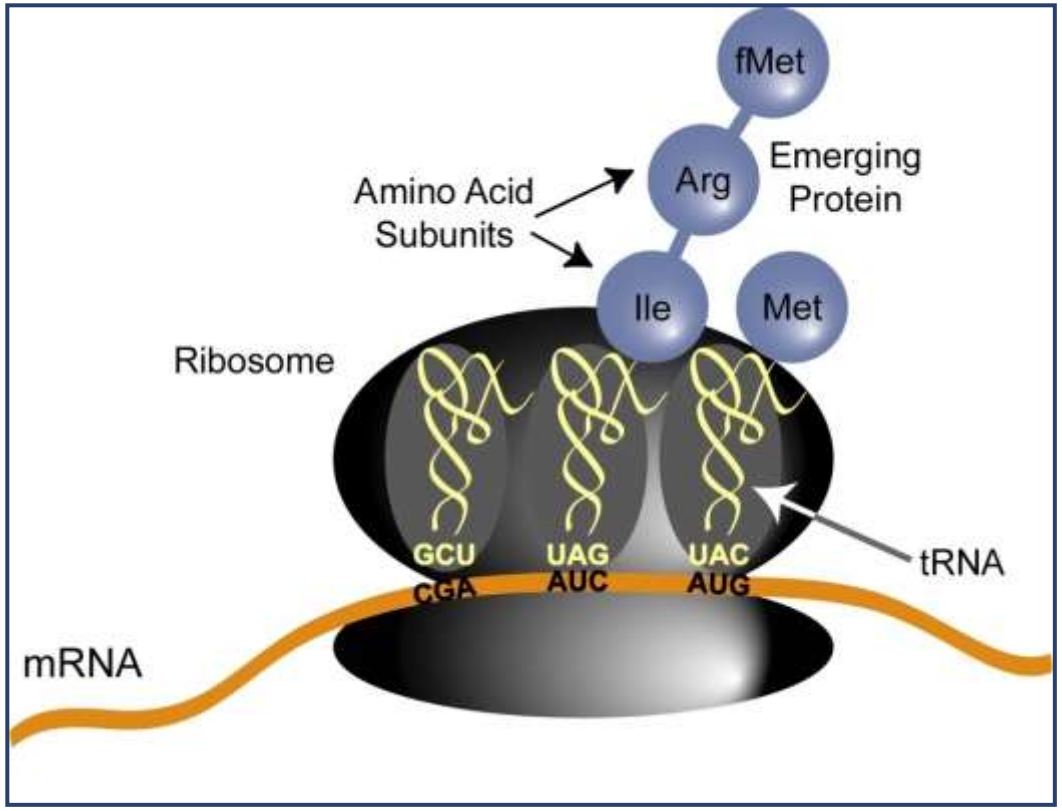
Methionyl tRNA synthetase (MetRS) is novel drug target

- Loads methionine to $tRNA^{fMet}$ and $tRNA^{Met}$
- Essential enzyme in all bacteria
- Unique among tRNA synthetases
  - Needed for both initiation (fMet) and elongation (Met) steps
- Bacterial and eukaryotic enzymes are different
  - Large safety window

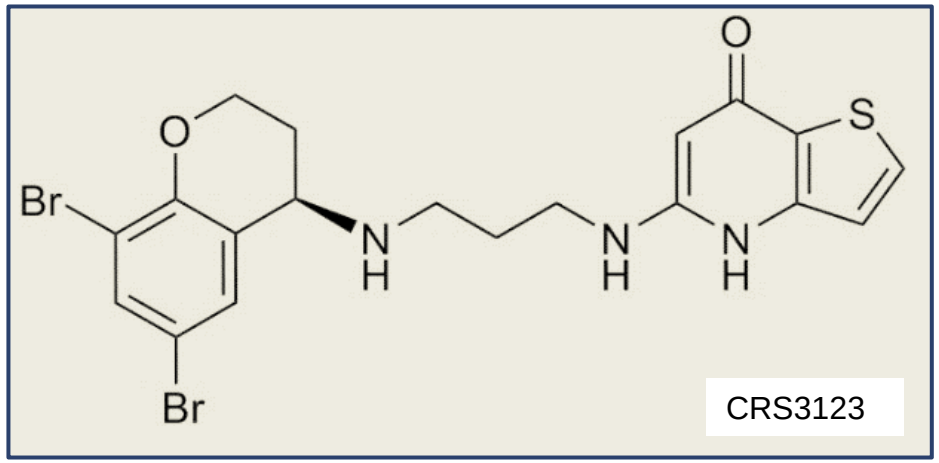


CRESTONE

CRS3123 is a potent inhibitor of *C. difficile* MetRS

- Identified from diaryldiamine SAR series
  - Originally licensed from GSK
- Small molecule, fully synthetic
- One chiral center
- Only R-enantiomer is active

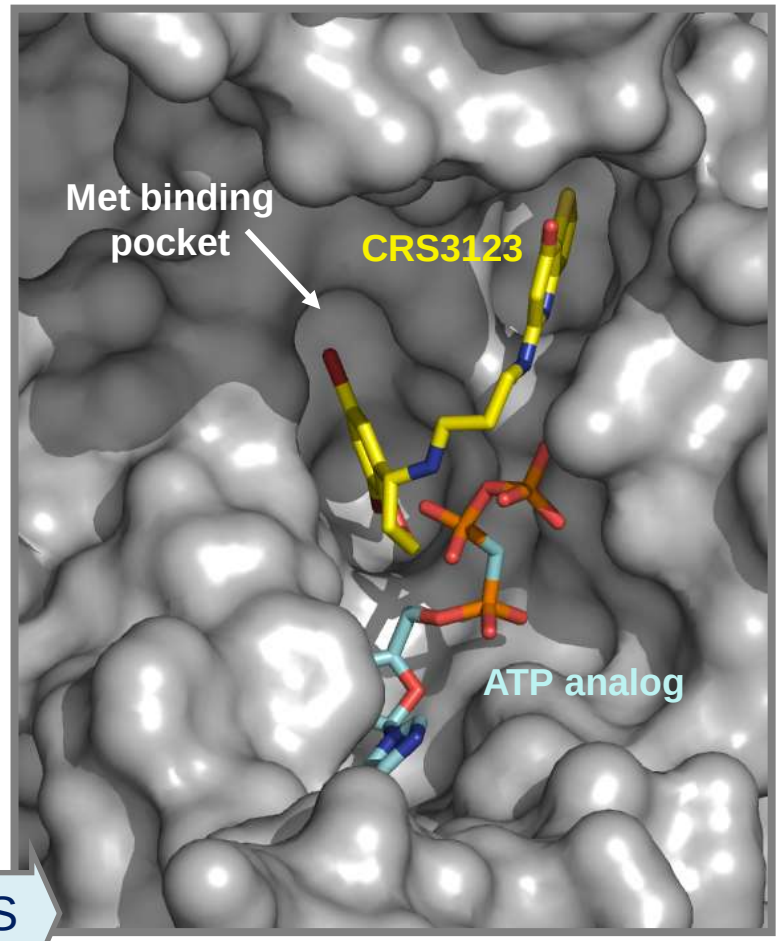
	CRS3123 R-Enantiomer	CRS3124 S-Enantiomer
K_i, pM	20	80,000
MIC, μg/mL	0.5	>32



CRESTONE

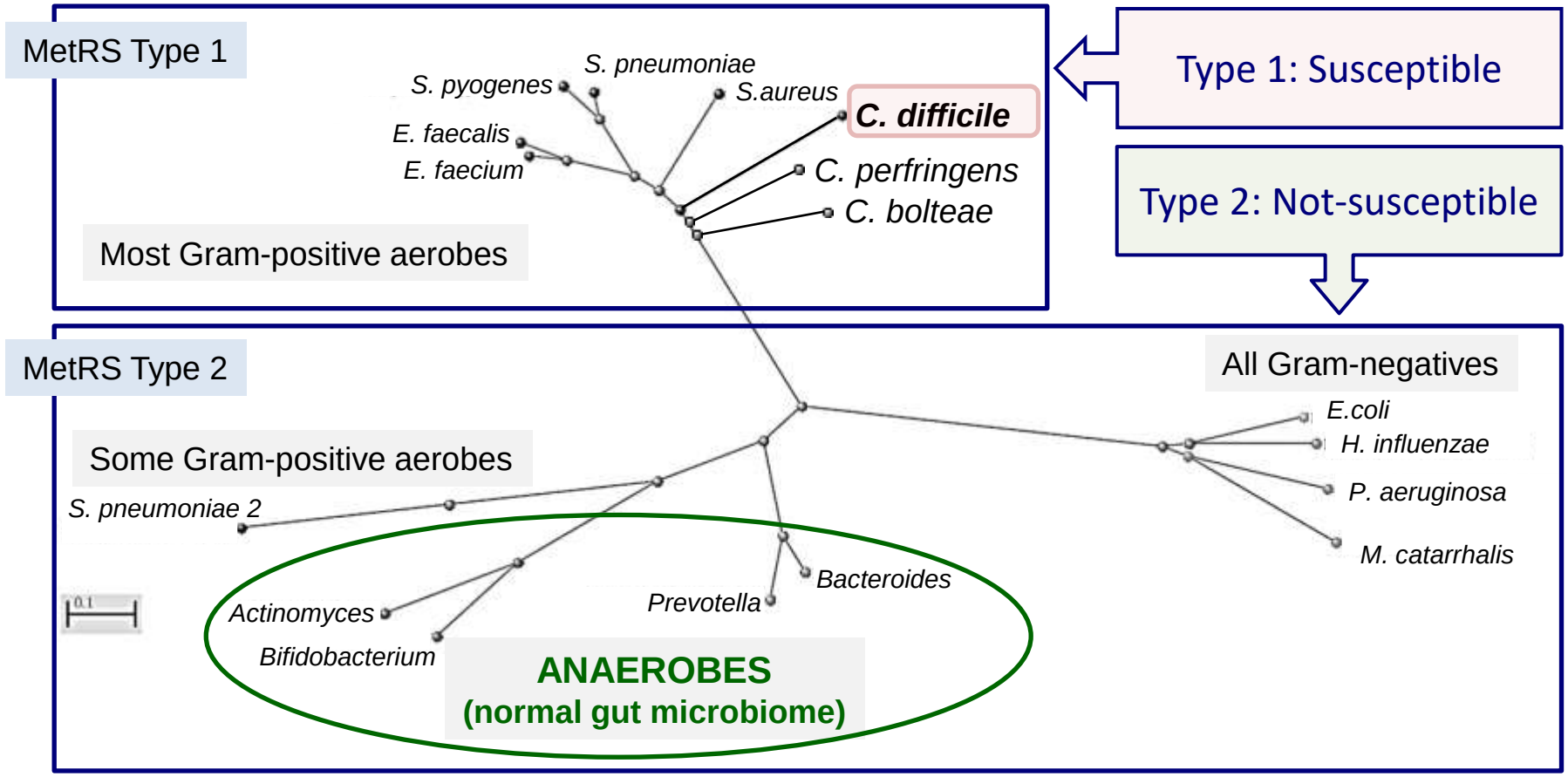
CRS3123 binds to active site of *C. difficile* MetRS

- CRS3123 occupies methionine binding pocket
  - Competitive inhibitor of methionine binding
  - Cooperative (uncompetitive) binding with ATP
    - This is important because there is a lot of ATP inside cells



CRS3123 and ATP analog bound to *C. difficile* MetRS

MetRS phylogeny explains narrow spectrum of CRS3123



CRS3123 showed favorable safety and tolerability in two phase 1 studies

- Single ascending dose (DMID10-0008)
  - 5 cohorts: 100, 200, 400, 800, 1200 mg
  - https://clinicaltrials.gov/ct2/show/NCT01551004
- Multiple ascending dose (DMID10-0009)
  - 3 cohorts: 200, 400, 600 mg; twice daily (BID) for 10 days
  - https://clinicaltrials.gov/ct2/show/NCT02106338
- Most adverse events mild, no serious adverse events

Nayak *et al*. (2017) *Antimicrob. Agents Chemother.* **61**, e02760
Lomeli *et al*. (2019) *Antimicrob. Agents Chemother.* **64**, e01395

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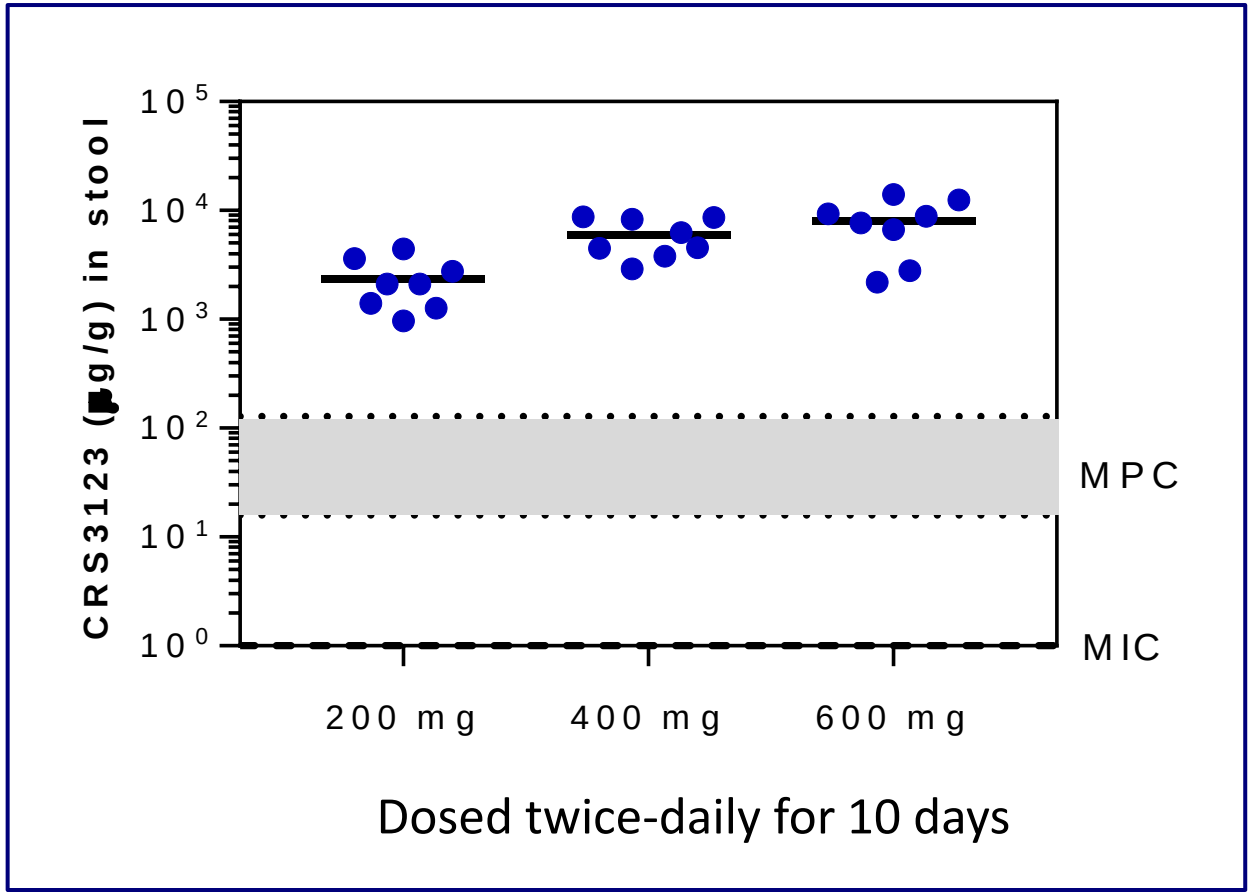
CRS3123 has low oral bioavailability

- High intestinal drug levels (above 1,000 µg/ml) observed at all three doses



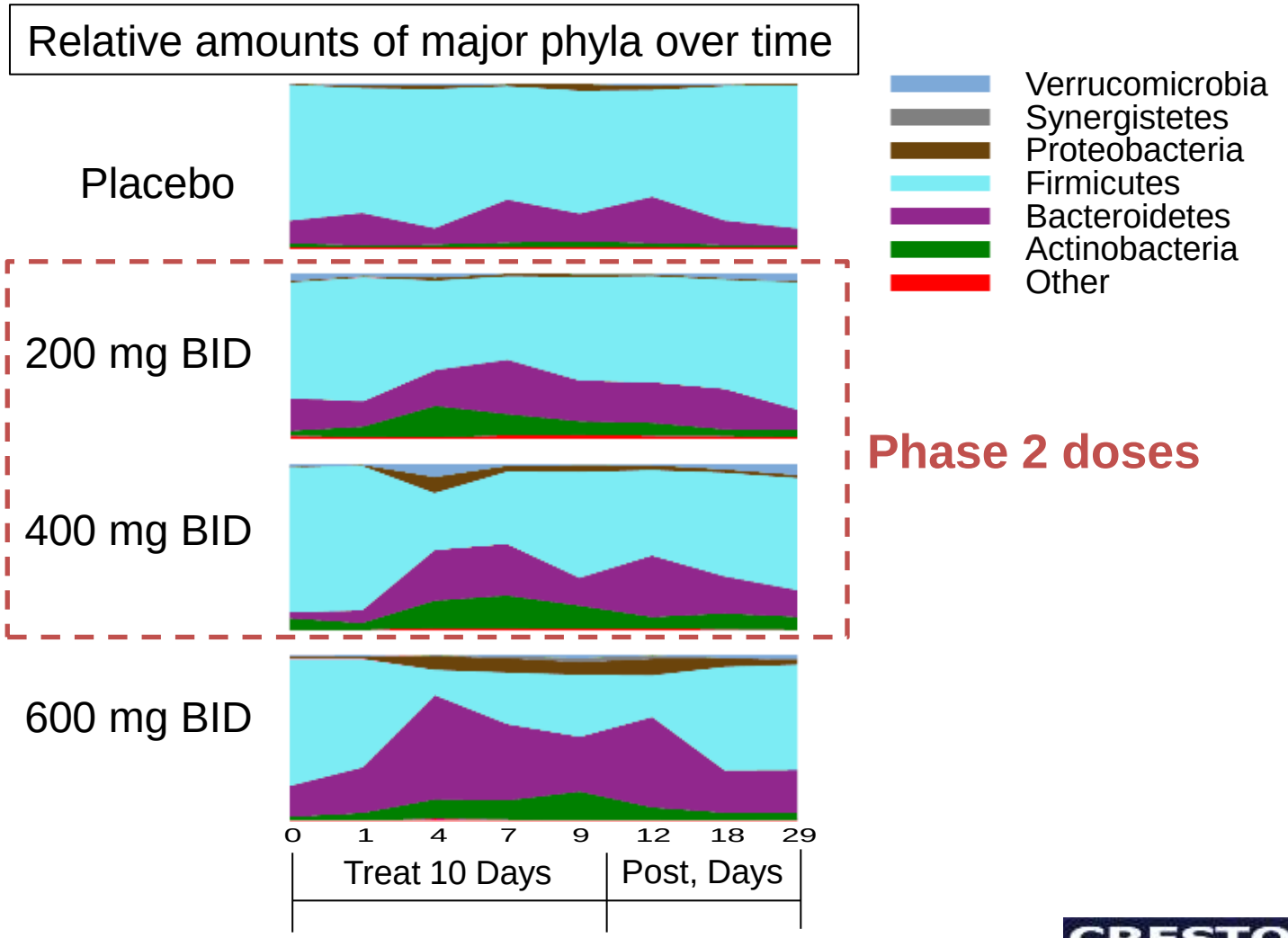
MIC: minimum inhibitory concentration
MPC: mutant prevention concentration

Lomeli *et al*. (2019) *Antimicrob. Agents Chemother.* **64**, e01395

CRESTONE

CRS3123 shows dose-dependent, minimal to moderate effect on normal gut microbiota

- Gut microbiome data from Phase 1 study, 10-day, twice-daily (BID) dosing
  - Analyzed by 16S rRNA sequencing
- No major phyla lost



Lomeli *et al*. (2019) *Antimicrob. Agents Chemother.* **64**, e01395

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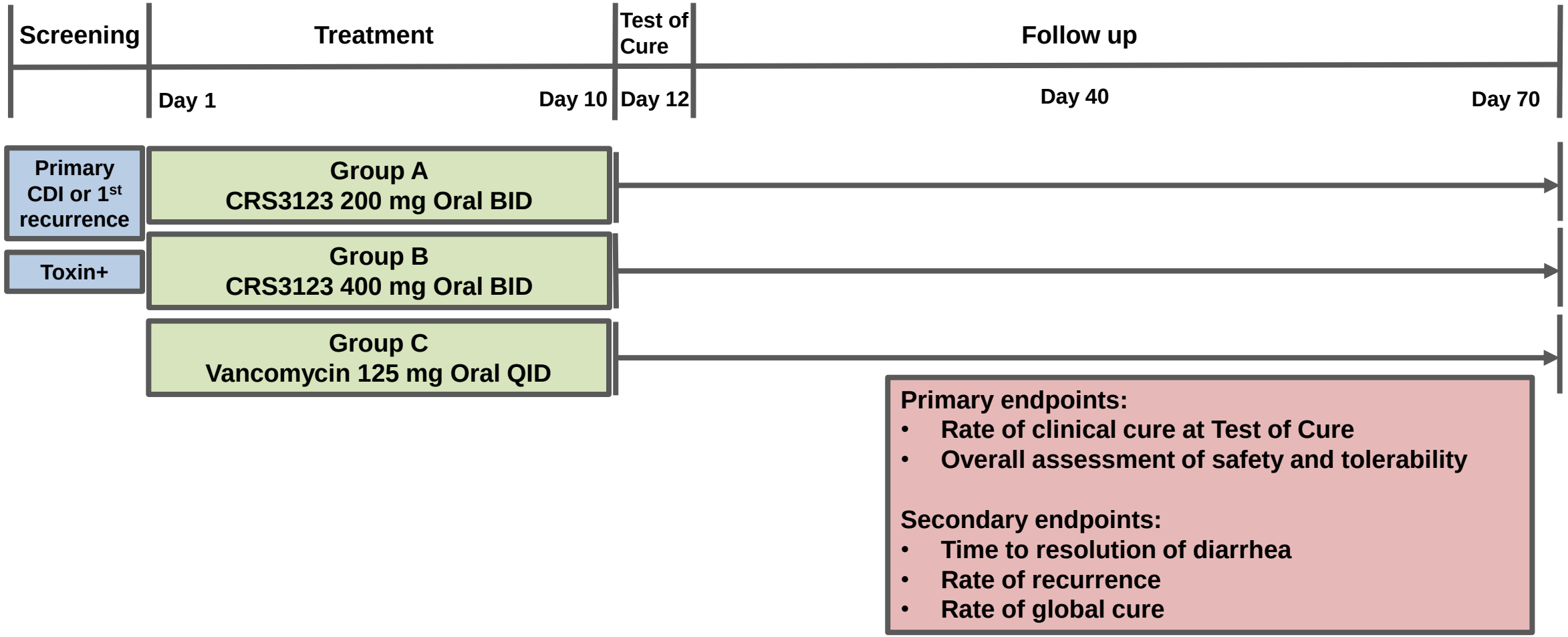
CRS3123 for CDI: phase 2 study design



Primary endpoints:

- Rate of clinical cure at Test of Cure
- Overall assessment of safety and tolerability

Secondary endpoints:

- Time to resolution of diarrhea
- Rate of recurrence
- Rate of global cure

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CRS3123 shows high initial cure rates and low recurrence rates

- Clinical cure rates at test-of-cure (day 12)
  - CRS3123 200 mg and 400 mg BID dosages combined 97%
  - Vancomycin 125 mg QID 93%
- Recurrence rates (day 40)
  - CRS3123 200 mg and 400 mg BID dosages combined 4%
  - Vancomycin 125 mg QID 23%
- Well-tolerated at both doses of CRS3123
  - https://clinicaltrials.gov/study/NCT04781387

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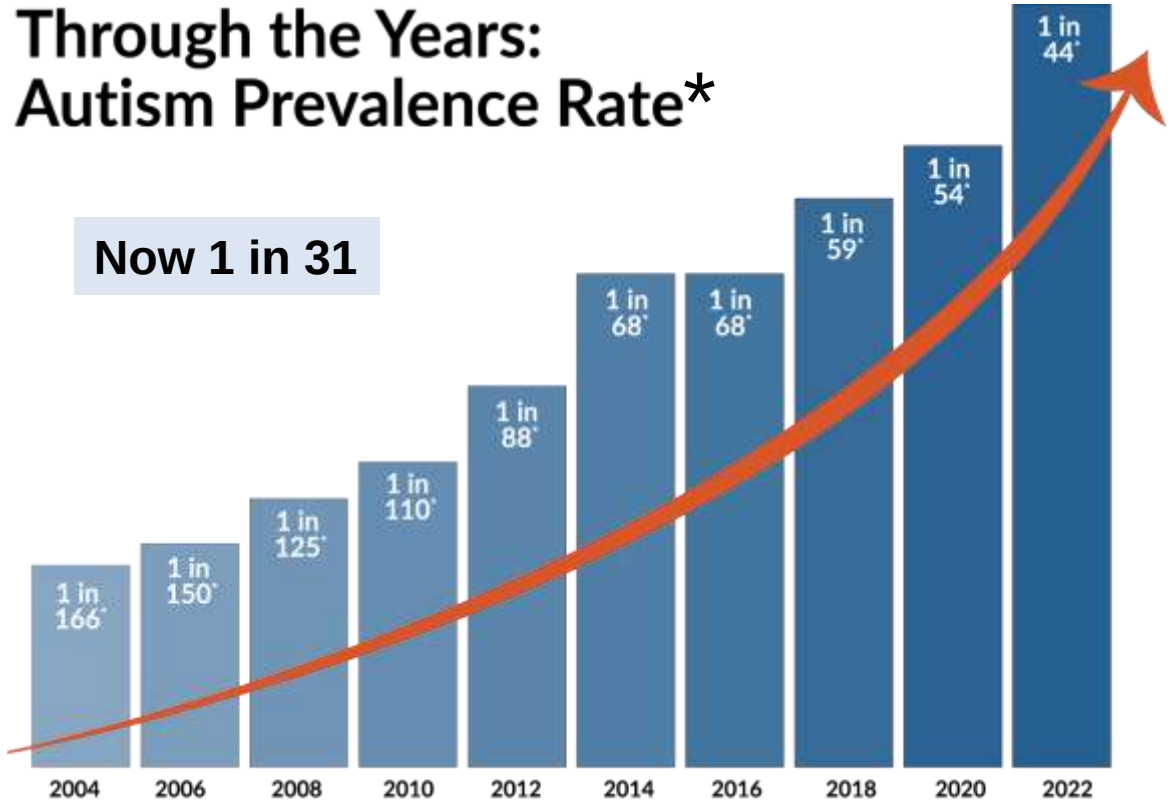
Autism: a large unmet need with limited treatment options

- Increasing prevalence, reasons unclear
- Affects boys 4 times more often than girls
- Occurs in all racial, ethnic, and socioeconomic groups
- Genetic predisposition poorly understood
- No FDA-approved treatments for core symptoms
- Improvement in symptoms would be highly meaningful



*CDC prevalence estimates for four years prior to report date

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Gastrointestinal (GI) problems are common in autism

- GI symptoms are at least 3-fold more frequent in autistic compared to neurotypical individuals
  - Constipation, diarrhea, bloating, pain
  - Strongly correlated with severity of autism
- Gut microbiome is less diverse
- Reduced GI motility and increased permeability (“leaky gut”) are common

Bresnahan *et al*. (2015) *JAMA Psychiatry* **72**, 466-74
McElhanon *et al*. (2014) *Pediatrics* **133**, 872-83
Chaidez *et al*. (2014) *J. Autism Dev. Disord.* **44**, 1117-27
Adams *et al*. (2011) *BMC Gastroenterol.* **11**, 22
Kang *et al*. (2013) *PLoS One* **8**, e68322
Kang *et al*. (2017) *Microbiome* **5**, 10
Wang *et al*. (2019) *Brain Behav. Immun*. **75**, 1921
Ma *et al*. (2019) *Front. Cell. Infect. Microbiol*. **9**, 40
Hsiao *et al*. (2013) *Cell* **155**, 1451-63

Enter Ellen Bolte

- Her son, Andrew, was developing normally until 18 months of age (mid 1990's)
- He got an ear infection for which he was treated with multiple courses of broad-spectrum antibiotics
- He then developed diarrhea
- And rapidly descended into severe autism

Bolte (1998) *Med. Hypotheses* **51**, 133

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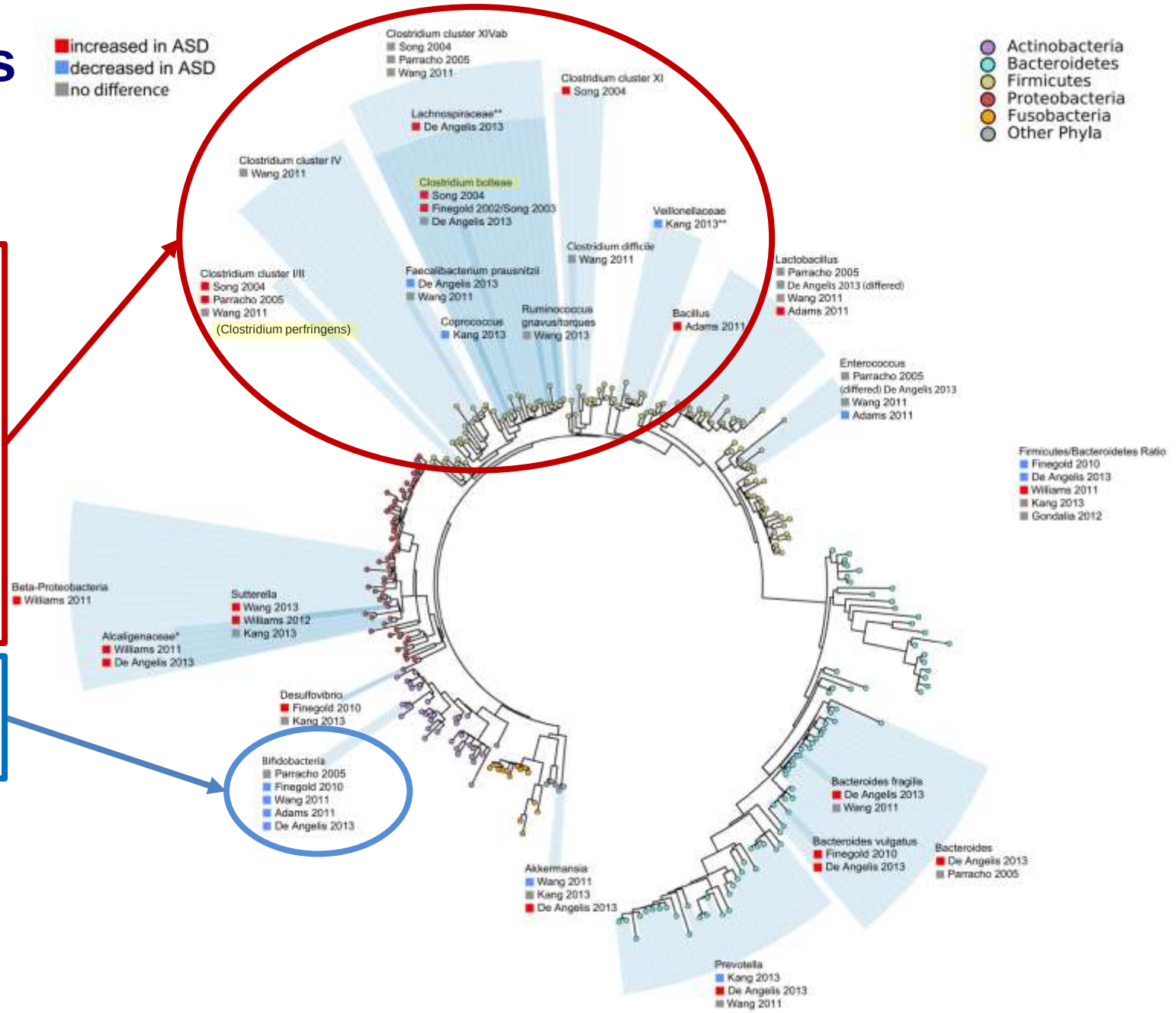
Late-onset autism is often preceded with GI symptoms

- Late-onset (regressive) autism occurs in about 1/3 of the cases
- History of increased antibiotics use
- Onset of symptoms often follows antimicrobial therapy
- GI symptoms are common at the onset and often persist

Sandler *et al*. (2000) *J. Child Neurol*. **15**, 429-35
Niehus *et al*. (2006) *J. Dev. Behav. Pediatr*. **27**, S120-7
Finegold (2008) *Med. Hypotheses* **70**, 508-11

Dysbiosis in autism shows directional trends

- Increased *Clostridium* clusters
  - Most are spore-formers
  - Principal producers of enterotoxins, neurotoxins, and toxic metabolites (phenols and indole derivatives)
- Decreased *Bifidobacterum* clusters



Ding *et al*. (2017) *J. Autism Dev. Disord.* **47**, 480

CRESTONE

From Ellen to Sid Finegold

- Ellen reached out to Sydney Finegold (UCLA)
  - A leading expert in anaerobic bacteria
- Together, they tested oral vancomycin in late-onset autistic children (including Andrew)
- 8 out of 10 children improved on treatment
- But the effect did not last
- In 2010, Sid called us

Sandler *et al*. (2000) *J. Child Neurol*. **15**, 429-35

CRESTONE

CRS3123 has activity against select *Clostridium* species implicated in autism

Organism	MIC (µg/mL)	
	CRS3123	Vancomycin
(*C. difficile*)	1	1
C. perfringens	0.25	1
C. bolteae	0.125	0.5
C. histolyticum	0.5	1
C. ramosum	>32	4
Actinomyces spp.	>32	0.5
Bifidobacterium spp.	>32	0.5

Pathogens

Beneficial

Critchley *et al*., (2009) *J. Antimicrob. Chemother.* **63,** 954
Ochsner *et al*. (2009) *J. Antimicrob. Chemother.* **63,** 964
Citron *et al*. (2009) *J. Antimicrob. Chemother.* **63,** 972

CRESTONE

p-Cresol is elevated in autistic individuals

- Elevated in 17 of 17 studies
- Increased levels correlate with autism symptoms
- Just correlation?

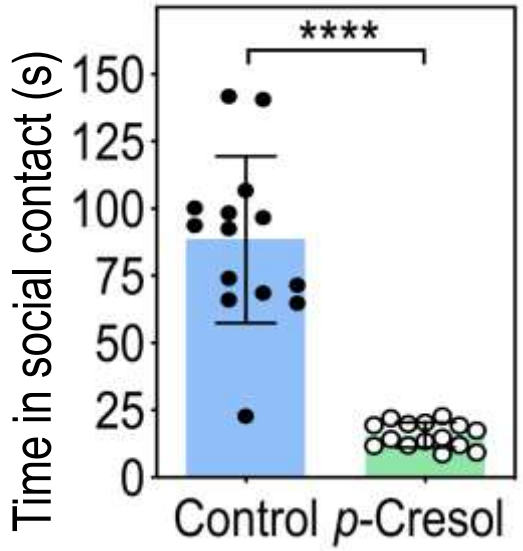
Study	ASD Participants	TD Participants	Country of Origin	Findings
Altieri, 2011 [29]	59	59	Italy	Significantly ↑
Chen, 2013 [41]	156	64	China	Significantly ↑
Daneberga, 2021 [42]	44	**	Latvia	**
Diémé, 2015 [31]	30	32	France	Significantly ↑
Emond, 2013 [32]	26	24	France	Significantly ↑
Gabriele, 2014 [30]	33	33	France	Significantly ↑
Gevi, 2016 [43]	30	30	Italy	↑
Gevi, 2020 [40]	40	40	Italy	Significantly ↑
Li, 2018 [36]	33	44	China	Significantly ↑
Mussap, 2020 [39]	31	26	Italy	Significantly ↑
Noto, 2014 [33]	30	28	France	↑
Osredkar, 2023 [38]	143	48	Slovenia	Significantly ↑
Perisco, 2012 [37]	59	59	Italy	Significantly ↑
Piras, 2022 [34]	13	14	Italy	Significantly ↑
Timperio, 2022 [44]	14	14	Italy	Significantly ↑
Tevzadze, 2017 [35]	14	14	Georgia	Significantly ↑
Zhang, 2020 [45]	39	40	China	↑

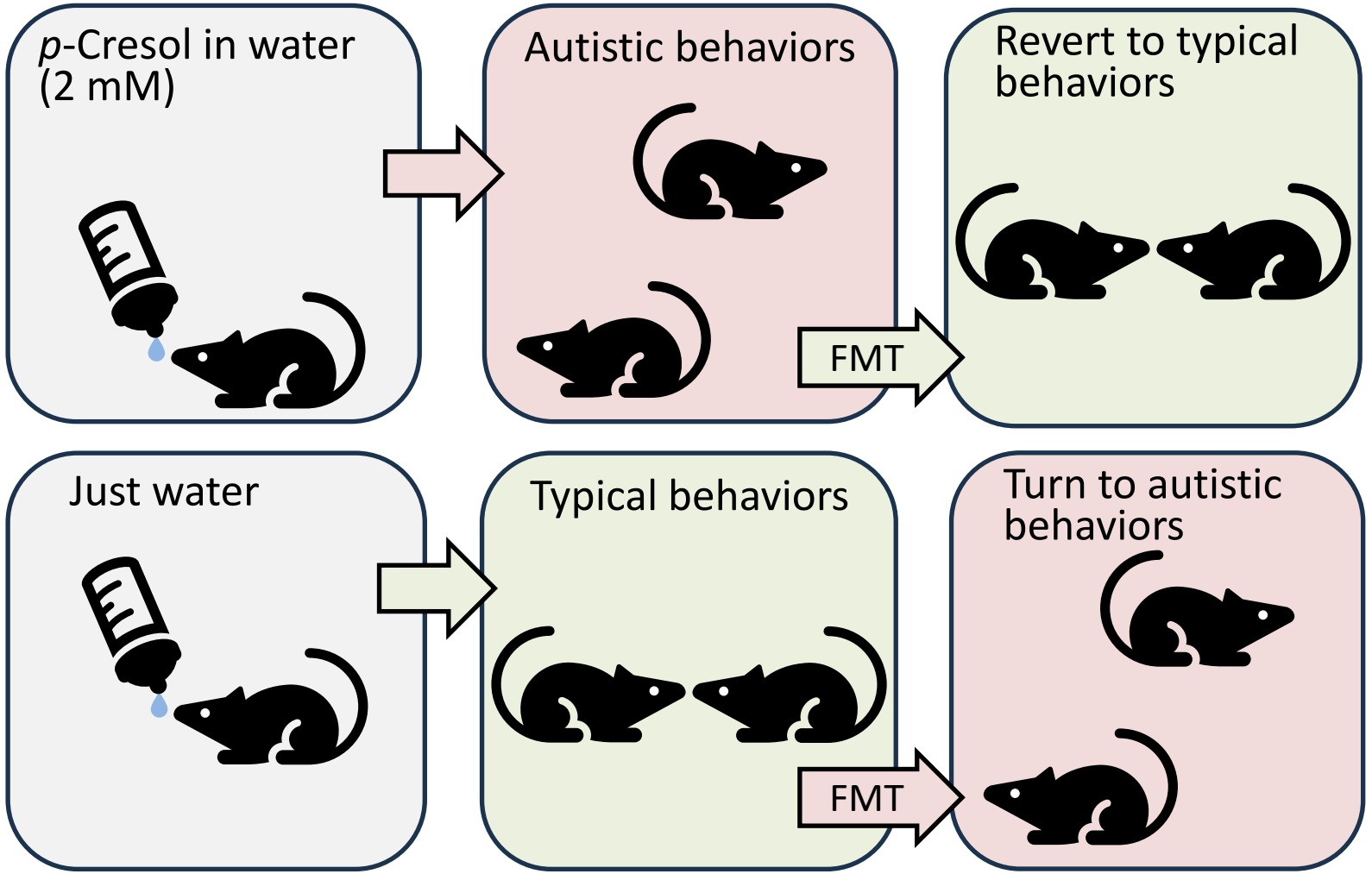
** Daneberga 2021 examined microbial community determination and divided the group into "High P-cresol" and "Low P-cresol".

Flynn *et al*. (2025) *Int. J. Mol. Sci*. **26** 1513

p-Cresol causes autism symptoms

- p-Cresol in drinking water makes mice less social
- Phenotype is transplantable
- The effect is not subtle





FMT: fecal microbiota transplant

Bermudez-Martin *et al*. (2021) *Microbiome* **9**, 157

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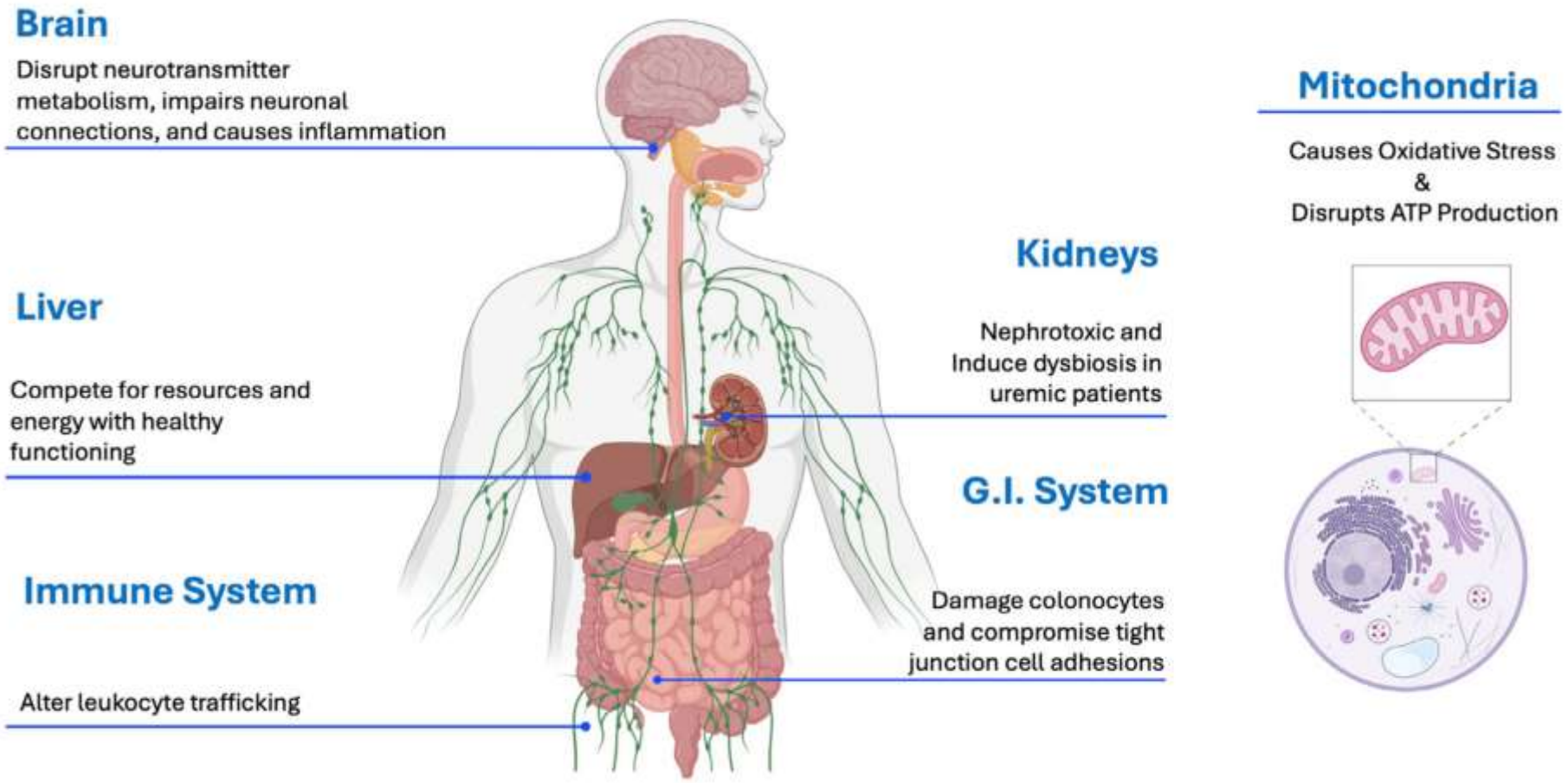
p-Cresol is toxic to multiple organs



Flynn *et al*. (2025) *Int. J. Mol. Sci*. **26**, 1513

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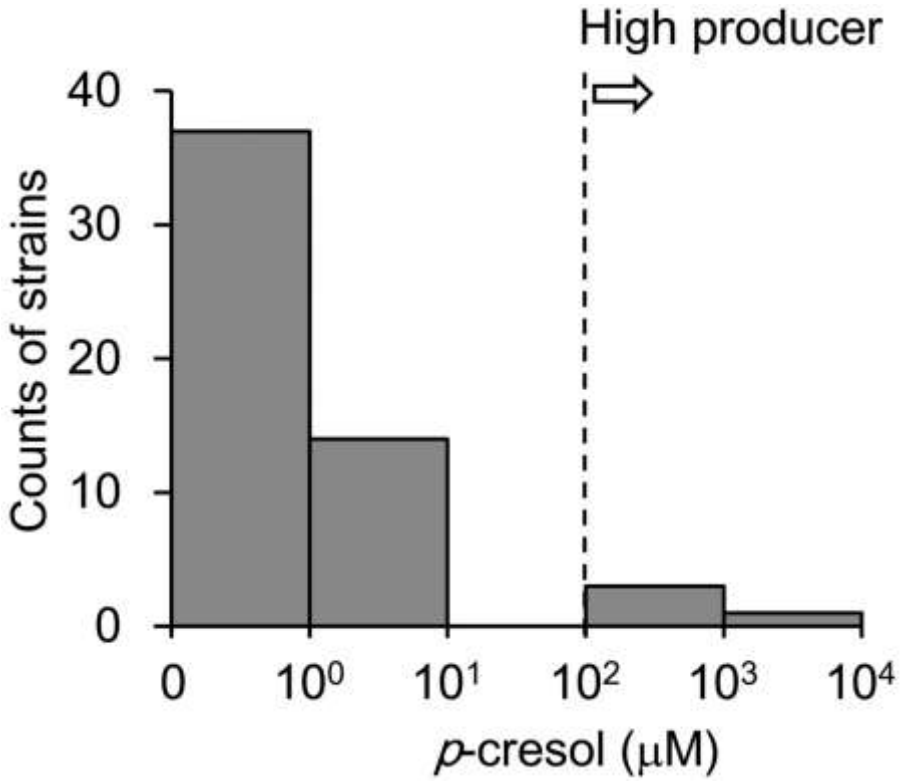
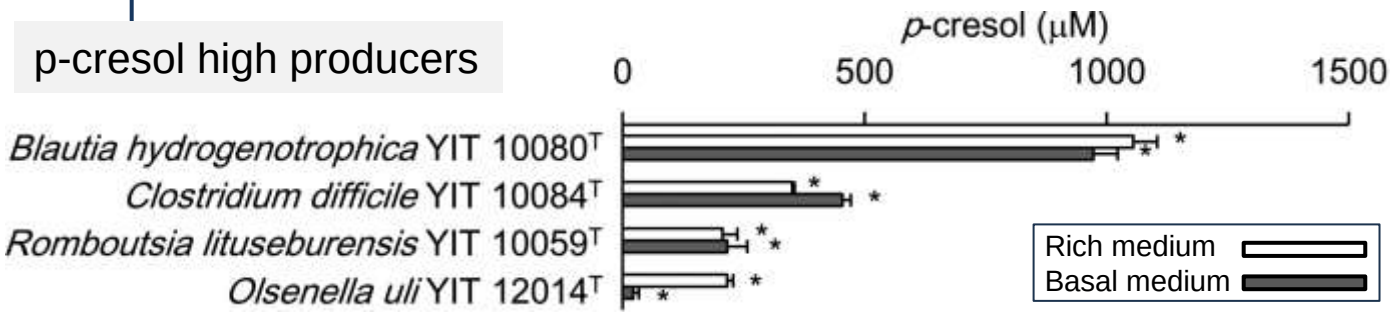
CRS3123 is active against highest producers of *p*-cresol



- Among 55 bacteria known to produce p-cresol, 4 are markedly higher producers

All high producers have MetRS1 (but not MetRS2) gene, CRS3123 is active against all

p-cresol high producers



Saito *et al*. (2018) *FEMS Microbiol Ecol* **94**, fiy125
Passmore *et al*. (2018) *PLoS Pathog*. **14**, e1007191

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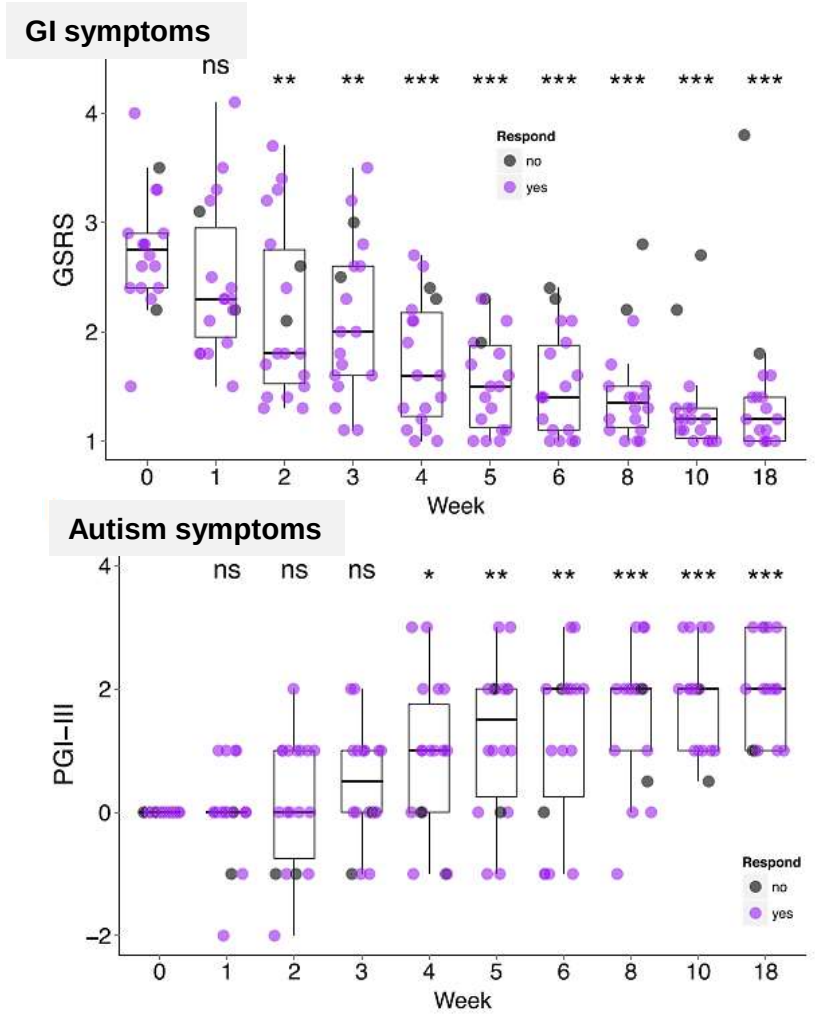
Vancomycin treatment followed by microbiota transplant improves both GI and autism symptoms

- Eighteen autistic children 7-16 years of age
  - Oral vancomycin for 2 weeks
  - Bowel cleanse
  - Fecal microbiota transplant (FMT) therapy for 8 weeks



Kang *et al*. (2017) *Microbiome* **5**, 10

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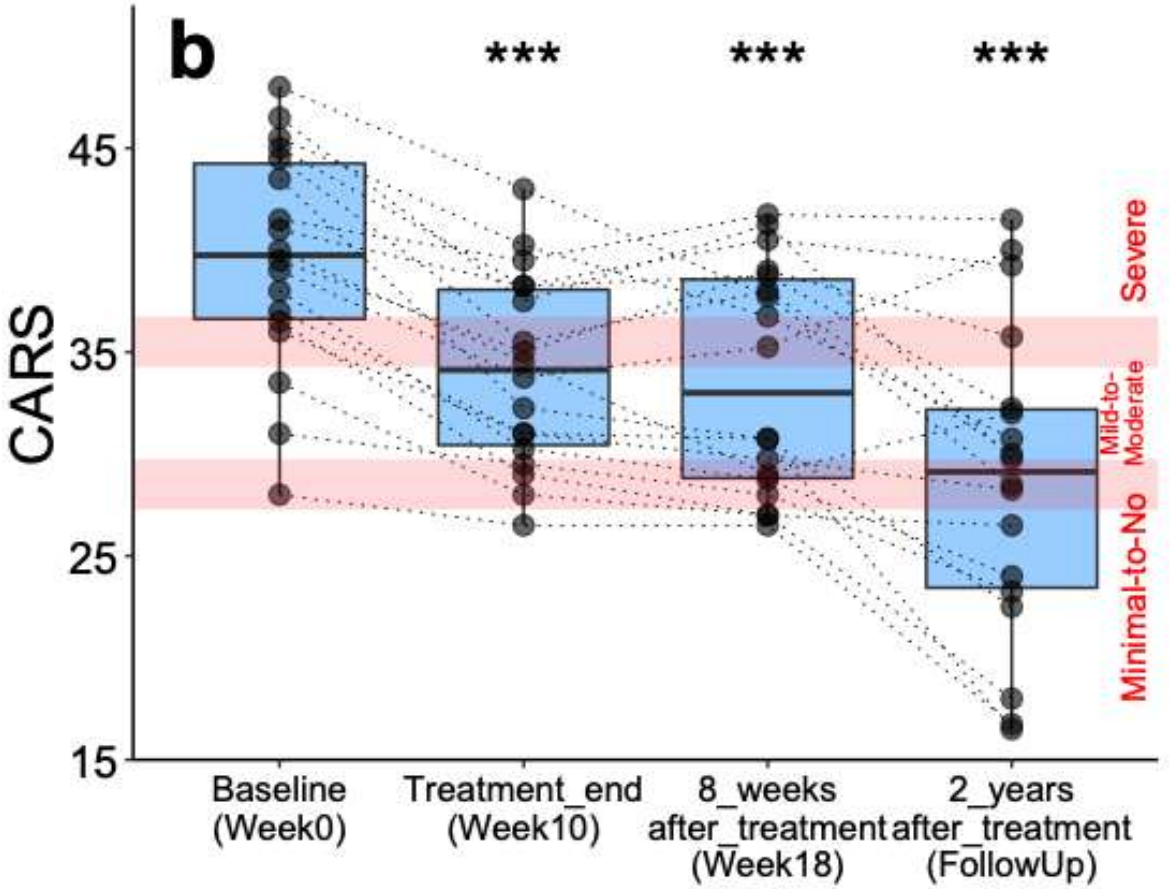
With lasting effect

- Reduction in autism symptoms maintained through 2-year follow-up



Kang *et al*. (2017) *Microbiome* **5**, 10

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Can CRS3123 achieve a similar, lasting effect?

- We now have an IND for autism, with a “study may proceed” letter from FDA
- Enroll autistic individuals with
  - GI symptoms
  - Elevated levels of *C. perfringens*, *C. bolteae* and *C. histolyticum*
  - Also elevated p-cresol levels?
- What is the effect size (autism behavior and GI symptoms)?
- Does narrow spectrum = less dysbiosis = longer lasting effect?

Pitt Hopkins Syndrome (PTHS): a rare, genetic, autism-like disorder

- ASD-like behavioral symptoms, unique facial features, intellectual disability, speech delay
- Initially described in 1978
- Caused by mutation in *tcf4* gene (Chr 18)
- Very high incidence of GI dysfunction (~70%)
  - Major clinical problem

PITT HOPKINS RESEARCH FOUNDATION

Goodspeed *et al*. (2018) *J. Child Neurol*. **33**, 233
Pitt & Hopkins (1978) *Aust. Paediatr. J*. **14**, 182
Amiel *et al*. (2007) *Am. J. Hum. Genet*. **80**, 988

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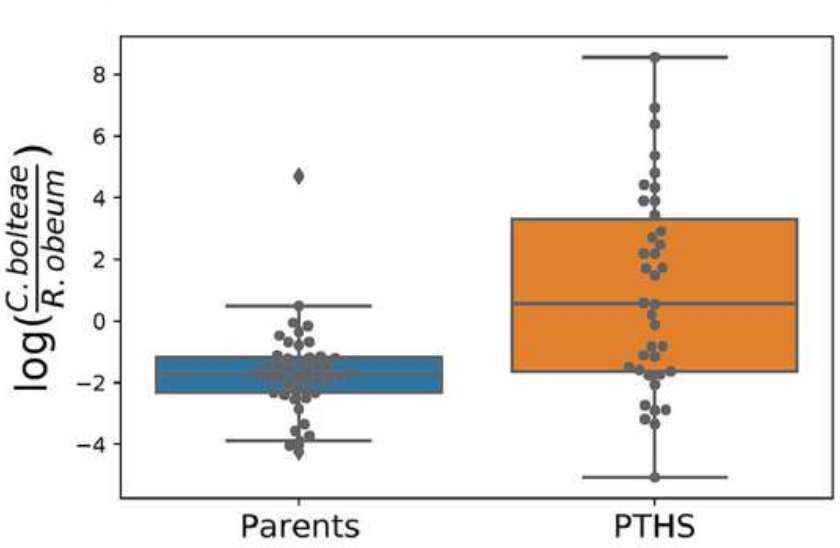
CRS3123 for PTHS: rationale

- Like with autism, *C. bolteae*, which is susceptible to CRS3123, is often elevated in PTHS individuals
- A small phase 2 study in PTHS (*e.g.*, 15 individuals) could inform further development
- FMT treatment is known to improve symptoms
- Accelerated, rare disease path to FDA approval



Arizona State University

Biodesign Center for Health Through Microbiomes

Dilmore *et al*. (2021) *mSystems.asm.org* **6**, e01006-21
Adams, J. (2025) unpublished observations

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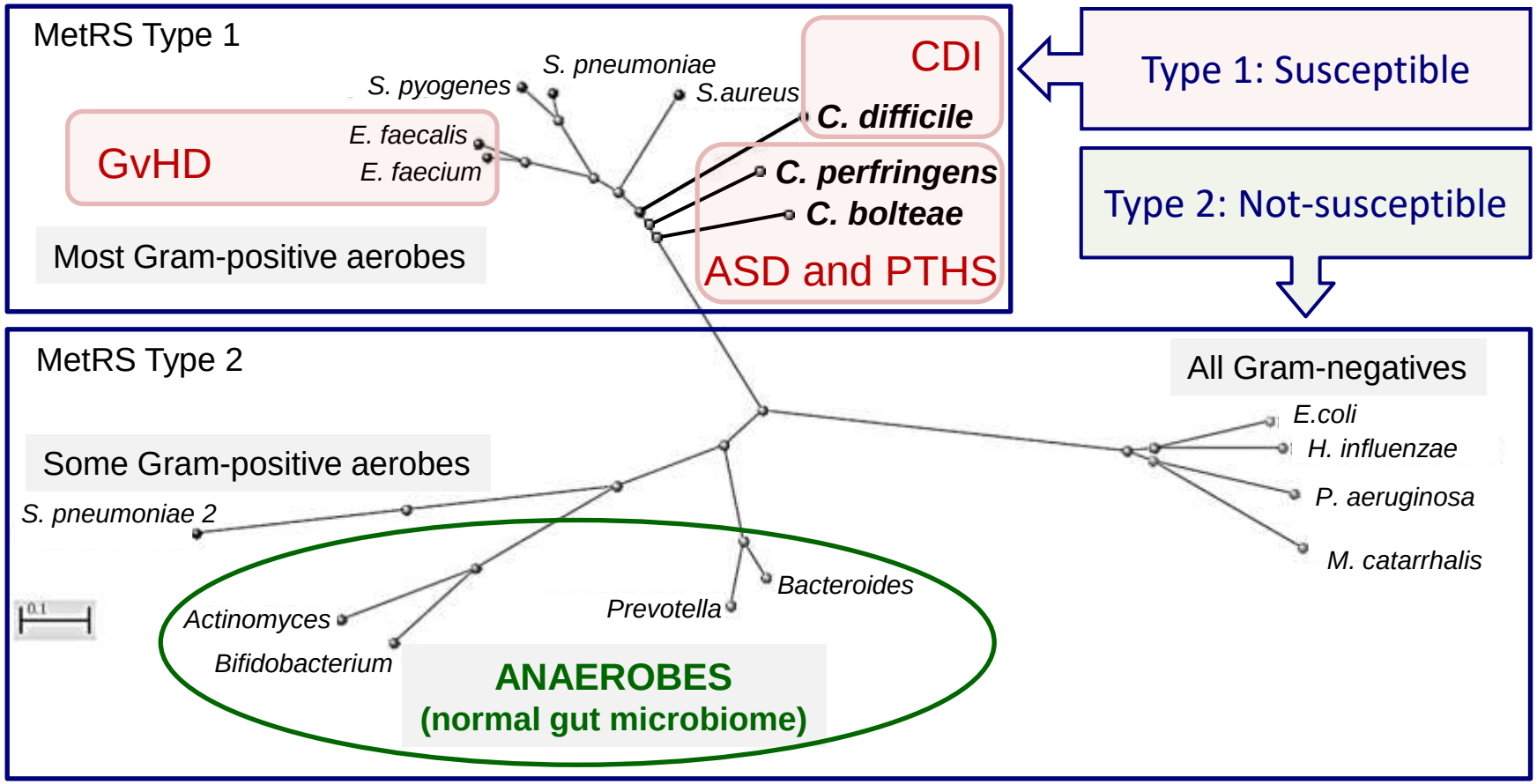
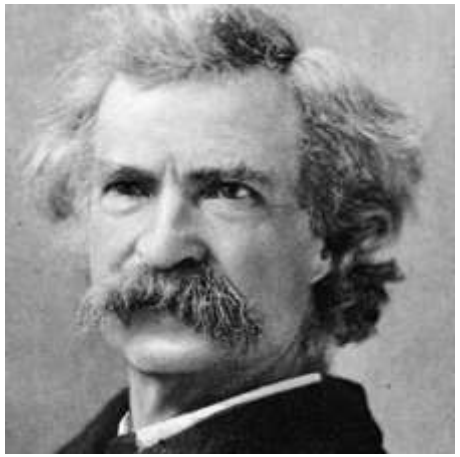
Target phylogeny explains narrow spectrum of CRS3123 and its multiple indications

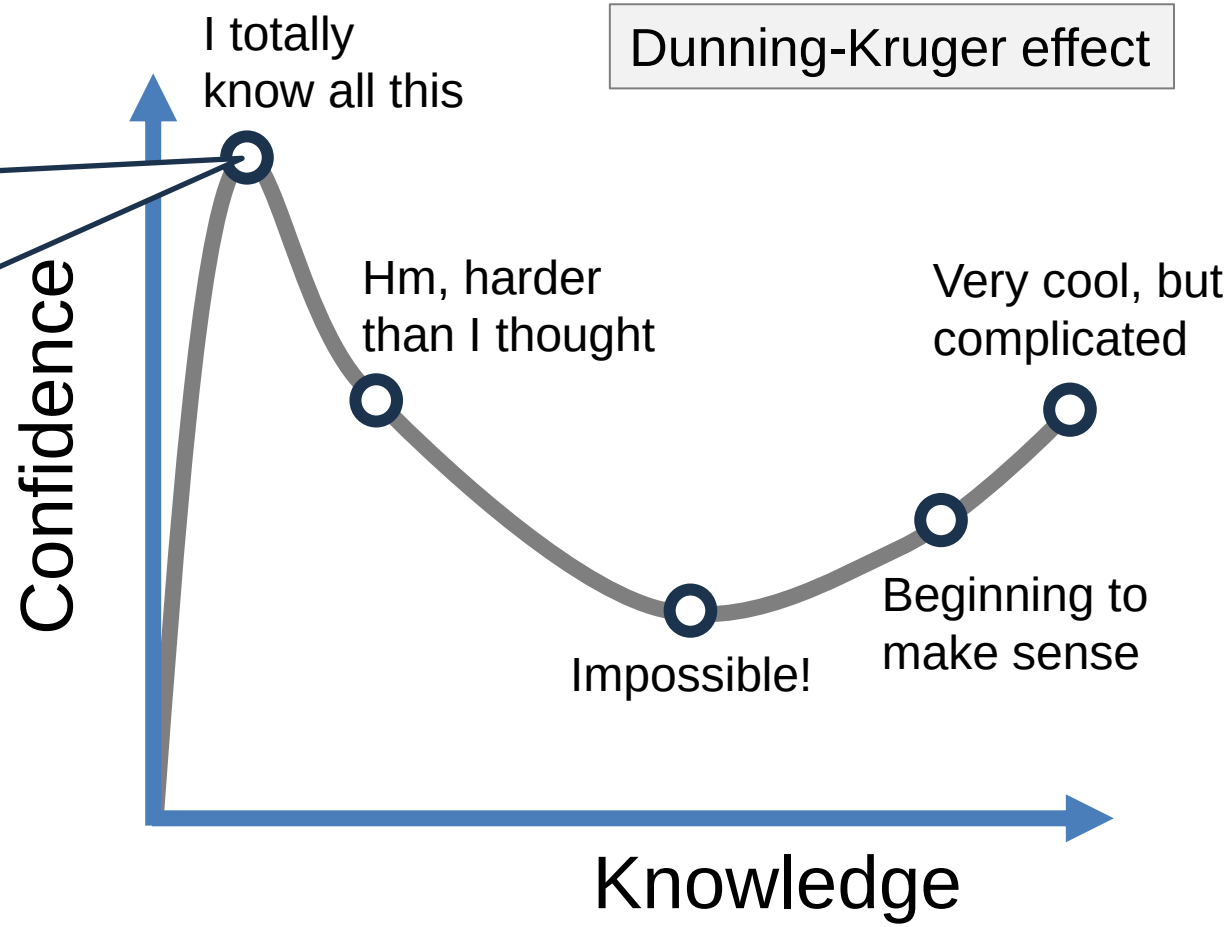


When do we know enough to do something?

It ain't what you don't know that gets you into trouble, it's what you know for sure that just ain't so.



Mark Twain



Kruger & Dunning (1999) *J. Personality Soc. Psych* **77**, 1121

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It takes a (large) village, thank you all

Crestone
Urs Ochsner
Thale Jarvis
John Rohloff
Xicheng Sun
Wendy Ribble
Joshua Day
Cliff Mason
Mary Ann De Groote
Jon Bruss
Hang Liu
Aline Oliver
William Bracken
Teresa Hoang
Lou Boccumini
Katie Johnson
Stacie Bell
Dorothy Colagiovanni
Don Morrissey

NIAID
Seema Nayak
Carol Ostrye
Kenan Gu
Chen Pi
Michael Kozar
Ryan Ranallo
Rick Sciotti
Patricia Gottdiener
Shahida Baqar
Marian Wachtel
Stephanie Zafonte
Blaire Osborn
Mohamed Elsafy
Gabriele Feolo
Richard Gorman

Full support for phase 1 and phase 2 studies

Clinical Support
Jonathan Zenilman
J. McLeod Griffiss
Jeffrey Blumer
Melissa Le
Mary Ann O'Riordan
Wesley Gray
Robin McKenzie
Robert Jurao
Amanada An
Barbara Lomeli
Wael El-Amin
George Saviolakis
Hal Galbraith
Jared Schettler

University of Colorado
Catherine Lozupone

CDI Advisors
Tom Louie
Mark Wilcox
Jane Freeman
Glenn Tillotson
Mike Corrado

Structural Biology
Doug Davies
Alex Burgin

Replidyne
Ian Critchley
Joe Guiles
Dan Drolet
Jian Qui
Albert Gyorkos
Sarah Strong
Ron Evans
Louis Green
James Bullard
Casey Young
Kimberly Stone
Melissa Price

Arizona State University
James Adams
Rosa Krajmalnik-Brown

Phoenix Children's Hospital
Richard Frye

In memoriam
Sydney Finegold

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