

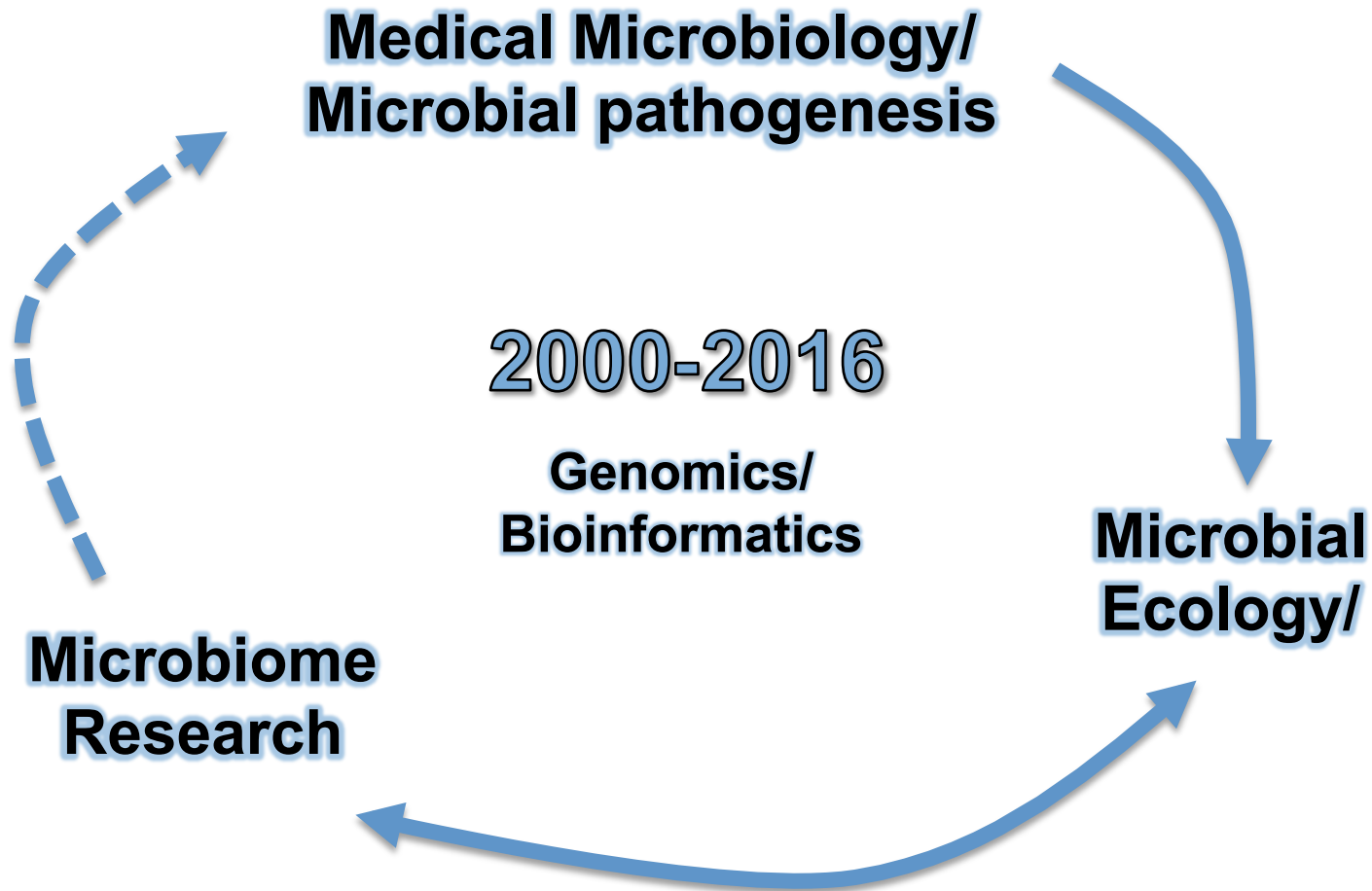
Our **Microbiome** Cloud and Us:

A common Destiny?



Ramy Karam Aziz, PhD
Faculty of Pharmacy, Cairo University

My experience / research cycle



Outline

- **The human microbiome**
 - The human microbiome and microbiome variations
 - What is the microbiome like? How to best describe it/model it?
- **Decoding the human microbiome**
 - Problems / bottlenecks
 - New tools → applications
- **Human microbiome in health, disease, and therapy**
 - Host's health and disease state
 - Drugs (systems pharmacology)
- **What next? Future directions**

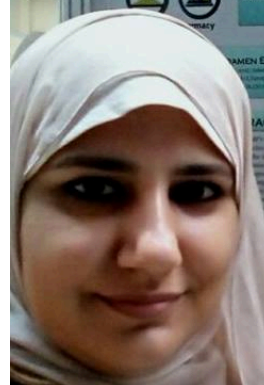
Key players...



Mariam Rizkallah



Rama Saad



Mariam Lotfy

- Marwa ElRakaiby
- Moamen ElMasry
- Soha Gamal-Eldin

& Collaborators:

- Rob Edwards
- Mya Breitbart
- Bas Dutilh, Utrecht
- Jack Gilbert, ANL
- Jarrad Marcel, ANL
- Janet Johnson, PNNL
- Rania Siam, AUC



AbdelRahman Aly



Sherry Aziz

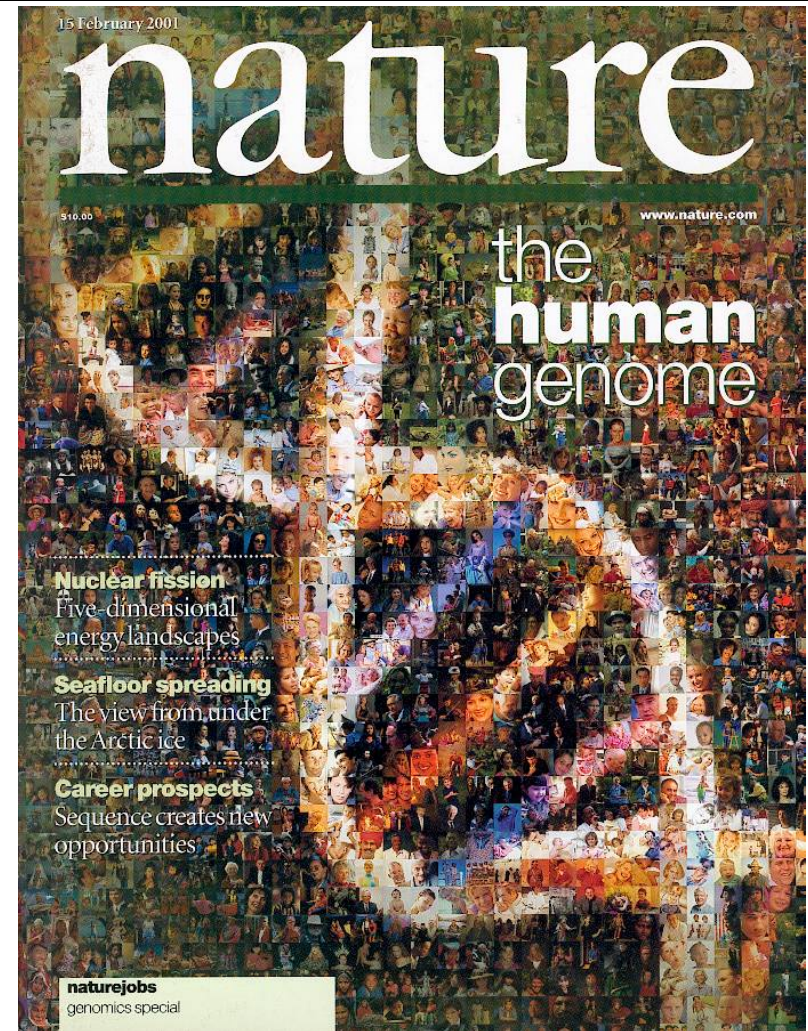


Ali ElBehery

How it all started?

The human microbiome and the HMP

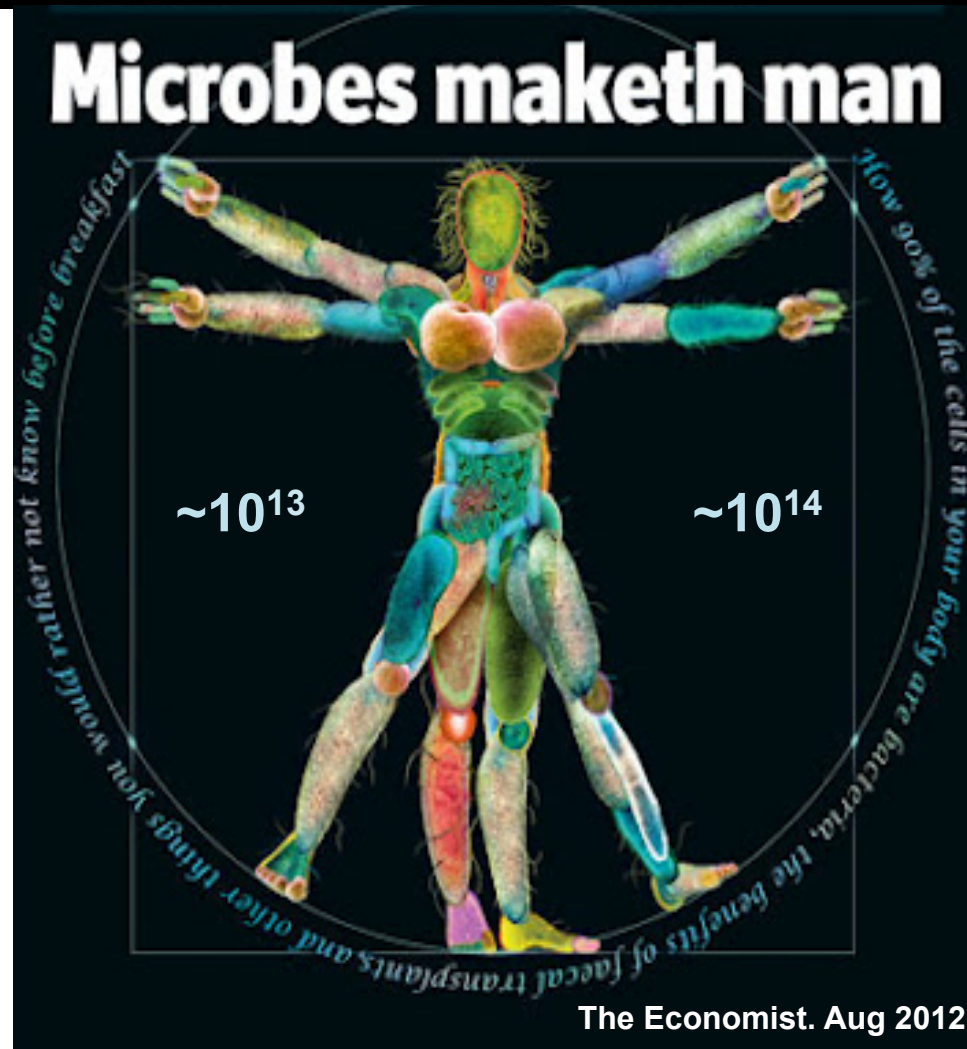
~2001: The human genome



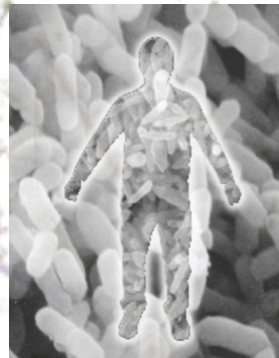
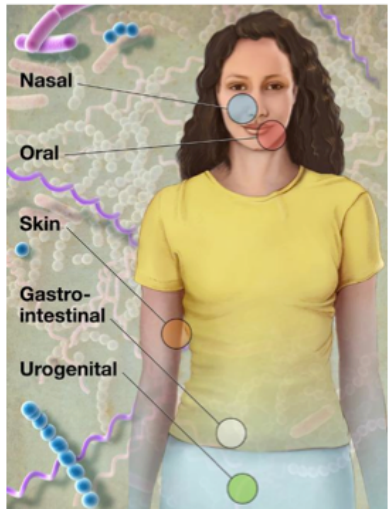
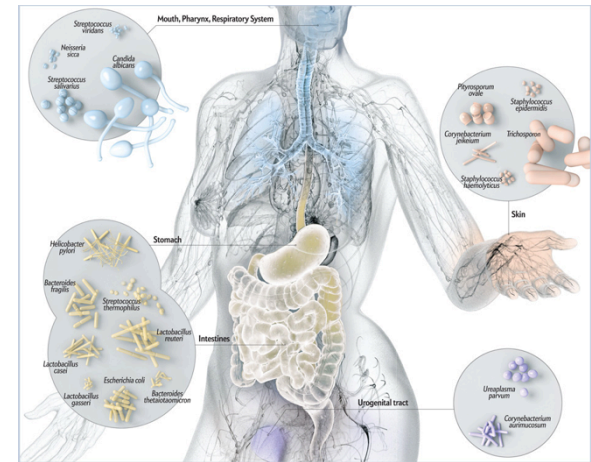
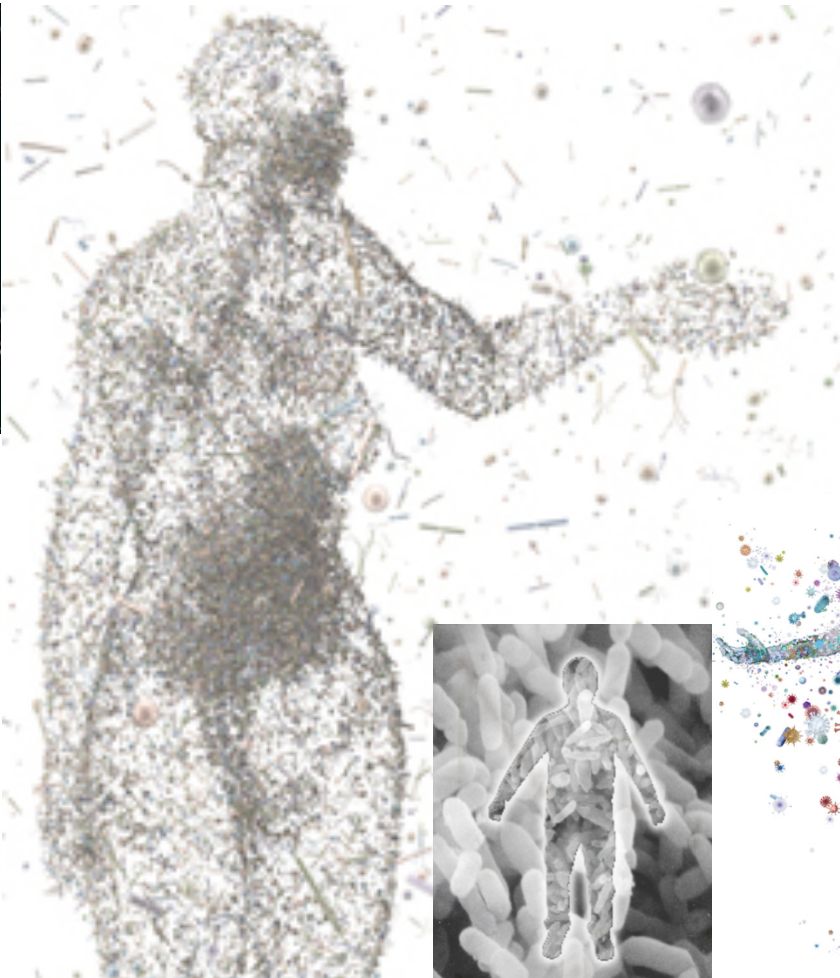
Human genome surprises?!

- Higher-than-expected similarity between humans on the DNA level
- Phenotypic differences are not fully explained by genotypic differences
- What else could introduce variation?
 - regulation
 - epigenetics
 - microbiota (traditionally described as normal flora) → attention towards the ***human microbiome***

~2007: The human microbiome



What's the microbiome really like?





review

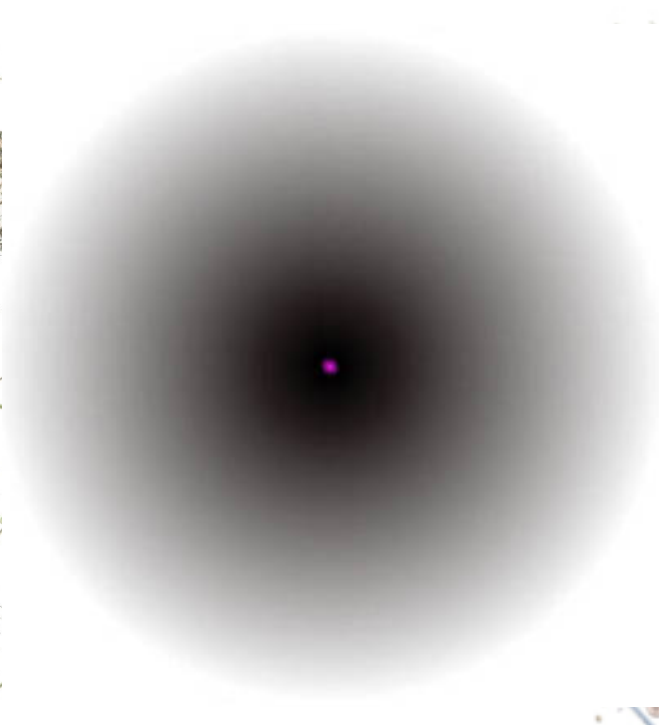
Ann M. O'Hara¹ & Fergus Shanahan^{1,2+}

Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland

... or a cloud of uncertainty?



The microbiome cloud model

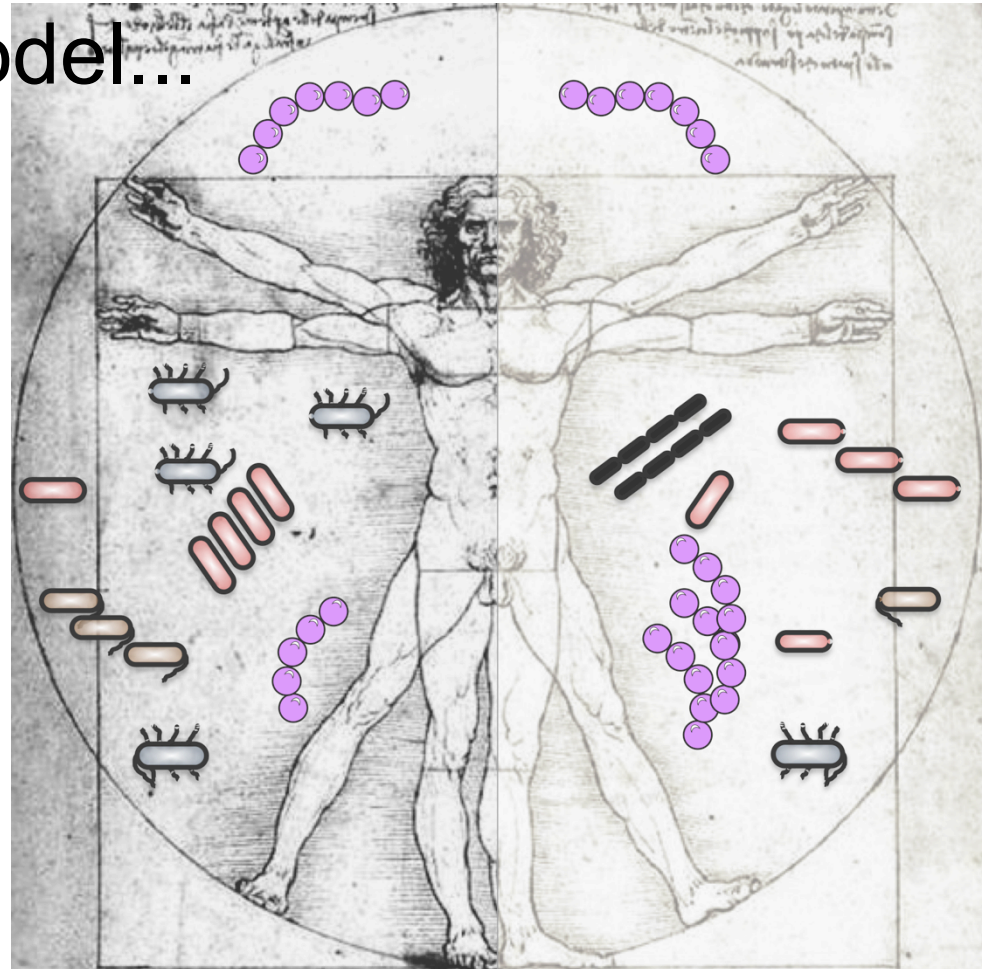


... or a cloud of uncertainty?



Microbiome cloud model...

- Variations:
 - Inter-individual
 - Intra-individual
 - Spatial
 - Temporal
 - Developmental
 - Hormonal
 - Geographical



Message #1

- **We are inhabited by a cloud of gen(om)es** coming mostly from microbes (and viruses) that we have been “domesticating” for long long time.
- **This cloud enriches our gene pool, which, unlike ours, is wrapped in uncertainty.** That’s why the microbiome is exciting.

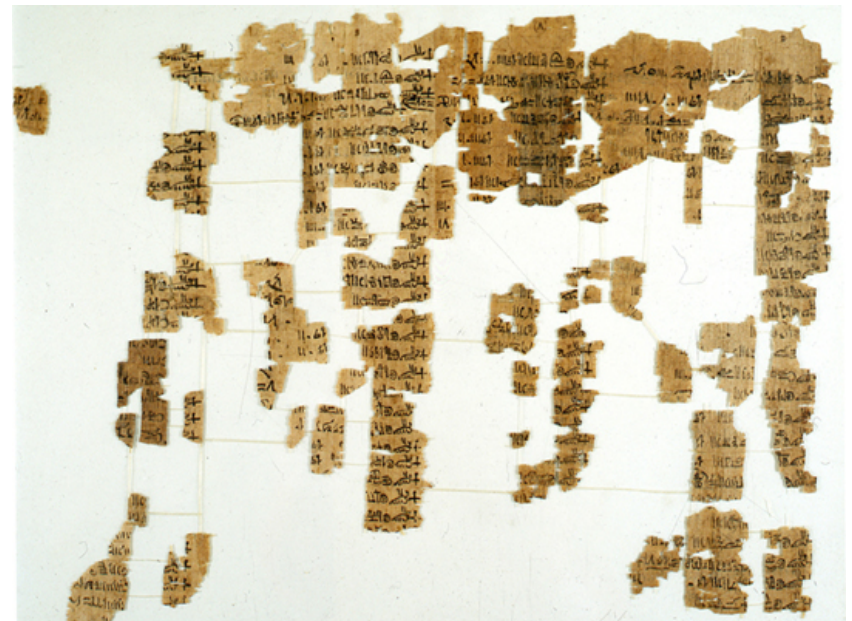


Developing or improving
computational tools to
overcome analysis bottlenecks

Decoding the human microbiome



Bottleneck #1: Decoding and reconstruction



Human expert-based computational annotation



RAST Rapid Annotation using
Subsystem Technology version 4.0

The NMPDR, SEED-based, prokaryotic genome annotation service.

For more information about The SEED please visit theSEED.org.

Info: To monitor RAST's load and view other news and statistics for RAST and the SEED, please visit "[The Daily SEED](#)."

RAST (Rapid Annotation using Subsystem Technology) is a fully-automated service for annotating bacterial and archaeal genomes. It provides high quality genome annotations for these genomes across the whole phylogenetic tree.

As the number of more or less complete bacterial and archaeal genome sequences is constantly rising, the need for high quality automated initial annotations is rising with it. In response to numerous requests for a SEED-quality automated annotation service, we provide RAST as a free service to the community. It leverages the data and procedures established within the [SEED framework](#) to provide automated high quality gene calling and functional annotation. RAST supports both the automated annotation of high quality genome sequences AND the analysis of draft genomes. The service normally makes the annotated genome available within 12-24 hours of submission.

This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272200900040C.

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**The RAST Server: Rapid Annotations using Subsystems
Technology**

Ramy K Aziz^{8,9}, Daniela Bartels³, Aaron A Best⁷, Matthew DeJongh⁷, *et al.*

Human expert-based computational annotation



RAST

The NMPDR, SEED-based,
For more information about The SEED please v

Info: To monitor RAST's load and view other

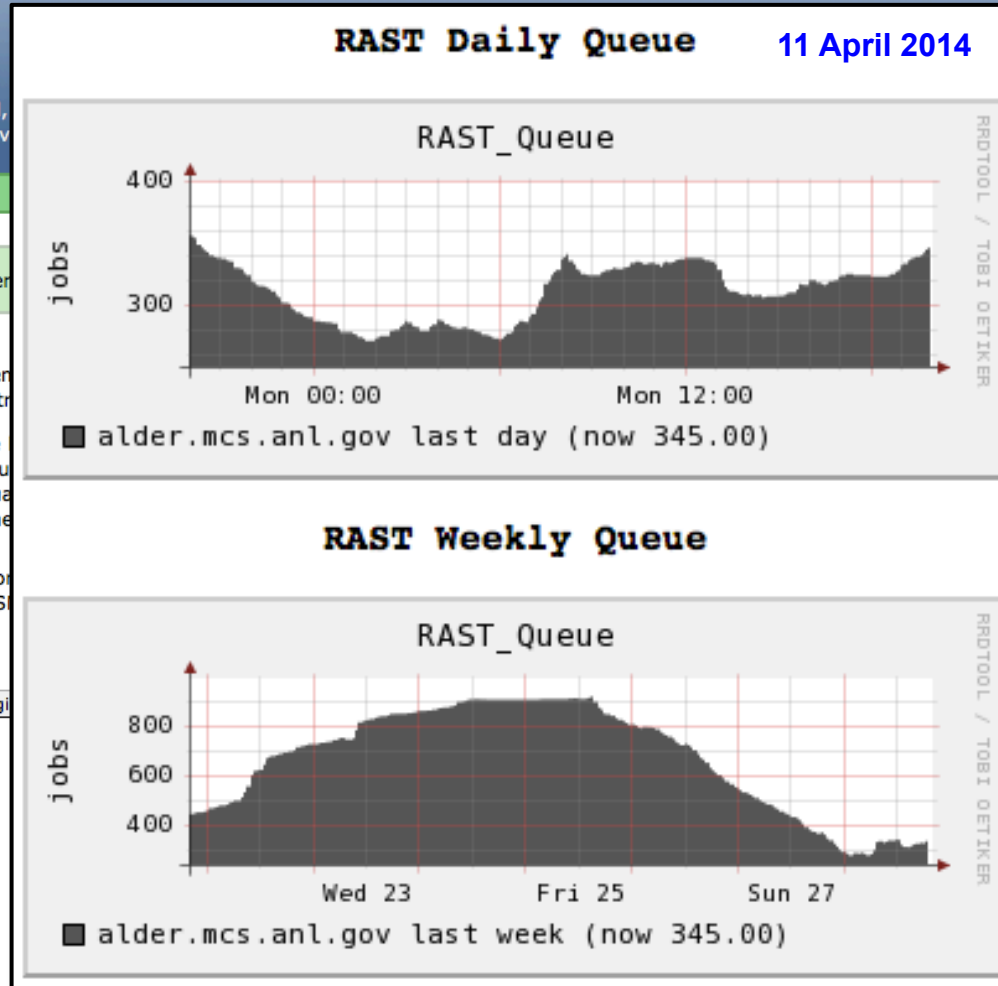
RAST (Rapid Annotation using Subsystem) genomes across the whole phylogenetic tr

As the number of more or less complete numerous requests for a SEED-quality au [framework](#) to provide automated high qua genomes. The service normally makes the

This project has been funded in whole or Human Services, under Contract No. HHS

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high quality genome annotations for these

annotations is rising with it. In response to and procedures established within the [SEED](#) genome sequences AND the analysis of draft

itutes of Health, Department of Health and

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Open Access

ms

Jongh⁷, et al.

Ramy K AZIZ^{1,2}, Daniela Bartsch, Aaron A. ...

Bottleneck #2:

Mobile elements

- Plasmids/ Transposons/ Insertion elements
- Transposases: Jumping genes
 - They make assembly difficult
 - Raison d'être?

Published online 9 March 2010

Nucleic Acids Research, 2010, Vol. 38, No. 13 4207–4217
doi:10.1093/nar/gkq140

SURVEY AND SUMMARY

Transposases are the most abundant, most ubiquitous genes in nature

Ramy K. Aziz^{1,2,*}, Mya Breitbart³ and Robert A. Edwards^{4,5}

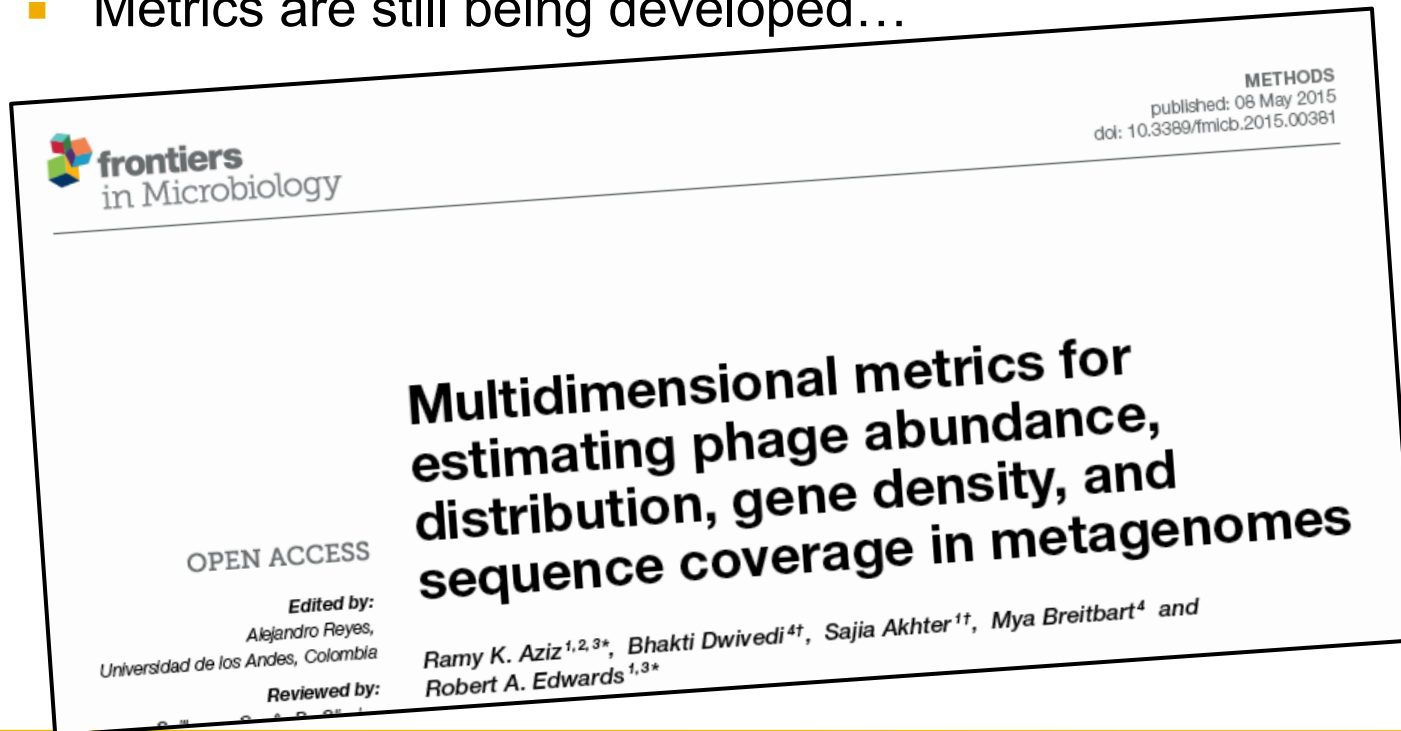
¹Computation Institute, University of Chicago, Chicago, IL 60637, USA, ²Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt, ³College of Marine Science, University of South Florida, ⁴Department of Computer Science, San Diego State University, San Diego, CA 92182 and ⁵Mathematics and Computer Science Division, Argonne National Laboratory, Argonne, IL 60439, USA

Received December 21, 2009; Revised and Accepted February 16, 2010

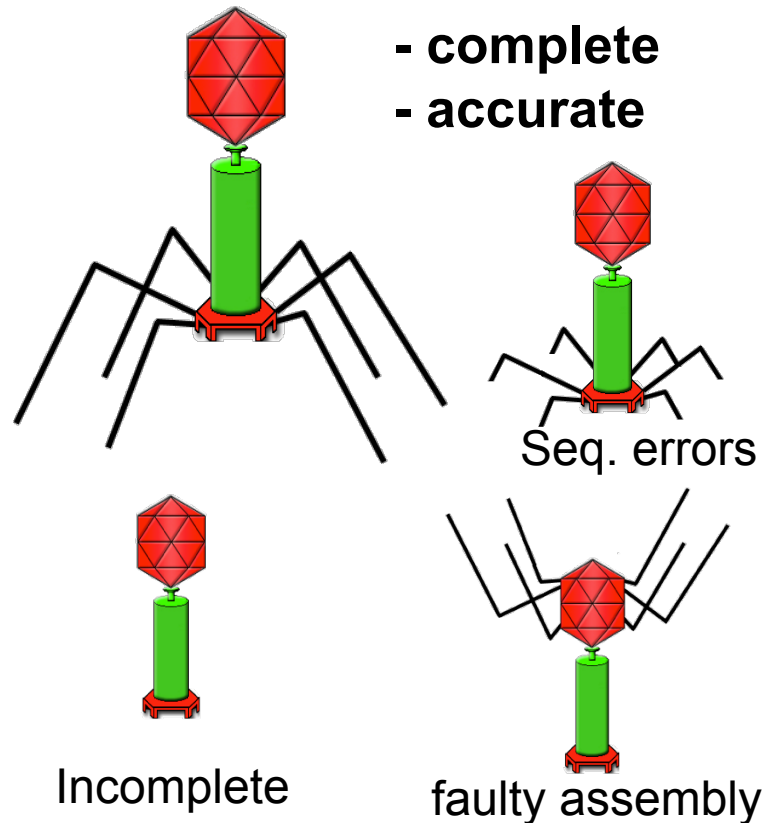


Bottleneck #3: Phages

- **Problem:**
 - No marker gene (16S equivalent):
no gene is conserved in all viruses
 - Highly mosaic genomes
- Metrics are still being developed...



Bottleneck #4: (mis-)Assembly

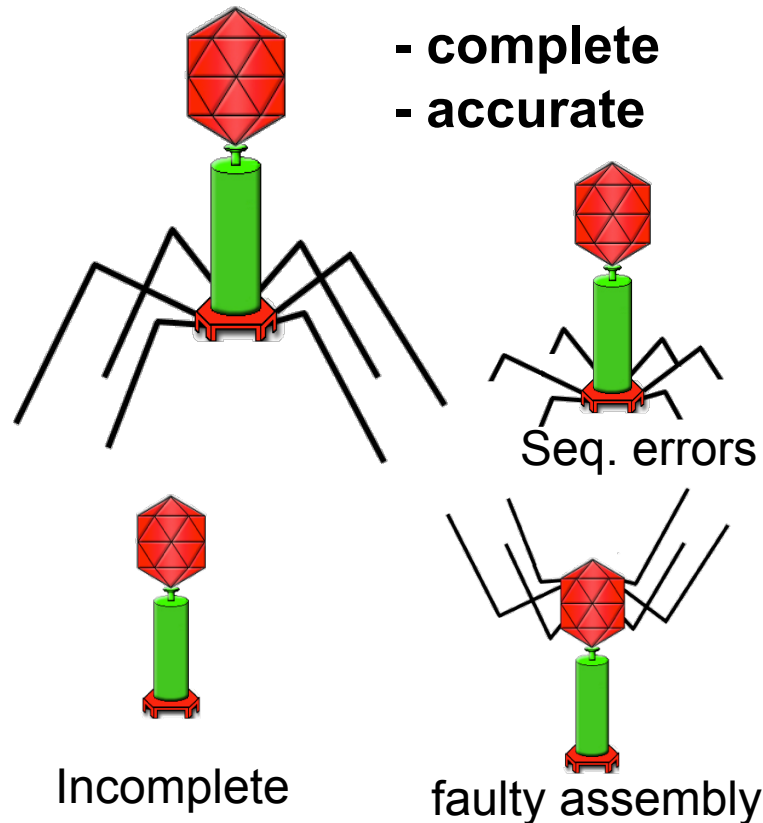


Credit: Andrew Kropinski



Credit: Bas Dutilh

Bottleneck #4: (mis-)Assembly



Credit: Andrew Kropinski



Credit: Bas Dutilh

We are learning more about how little we know!

■ crAssphage

Nat Commun. 2014 Jul 24;5:4498.

ARTICLE

Received 9 Apr 2014 | Accepted 25 Jun 2014 | Published 24 Jul 2014

DOI: 10.1038/ncomms5498

OPEN

A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes

Bas E. Dutilh^{1,2,3,4}, Noriko Cassman^{3,†}, Katelyn McNair², Savannah E. Sanchez³, Genivaldo G.Z. Silva⁵, Lance Boling³, Jeremy J. Barr³, Daan R. Speth⁶, Victor Seguritan³, Ramy K. Aziz^{2,7}, Ben Felts⁸, Elizabeth A. Dinsdale^{3,5}, John L. Mokili³ & Robert A. Edwards^{2,4,5,9}

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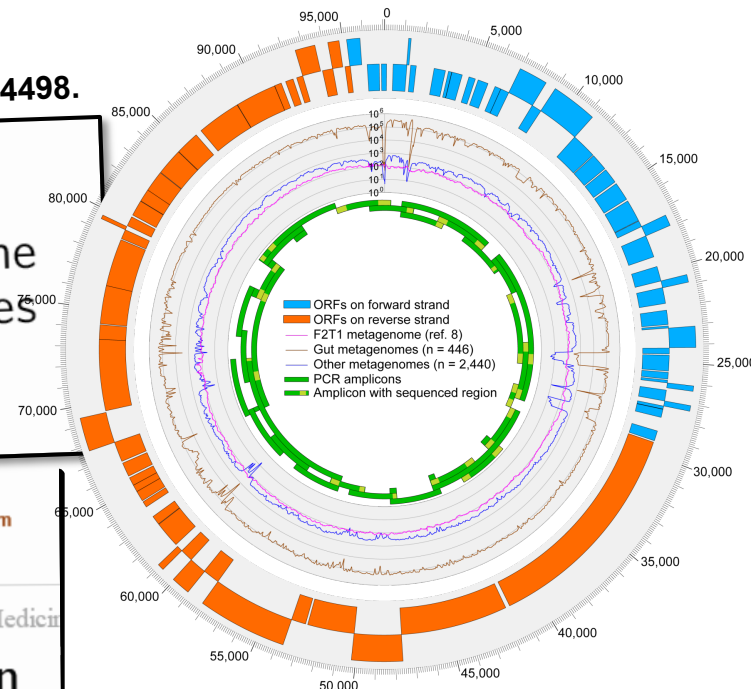
Health & Medicine

New Gut Virus Lives in Half the World's Population

NOT EXACTLY ROCKET SCIENCE:

NATIONAL GEOGRAPHIC

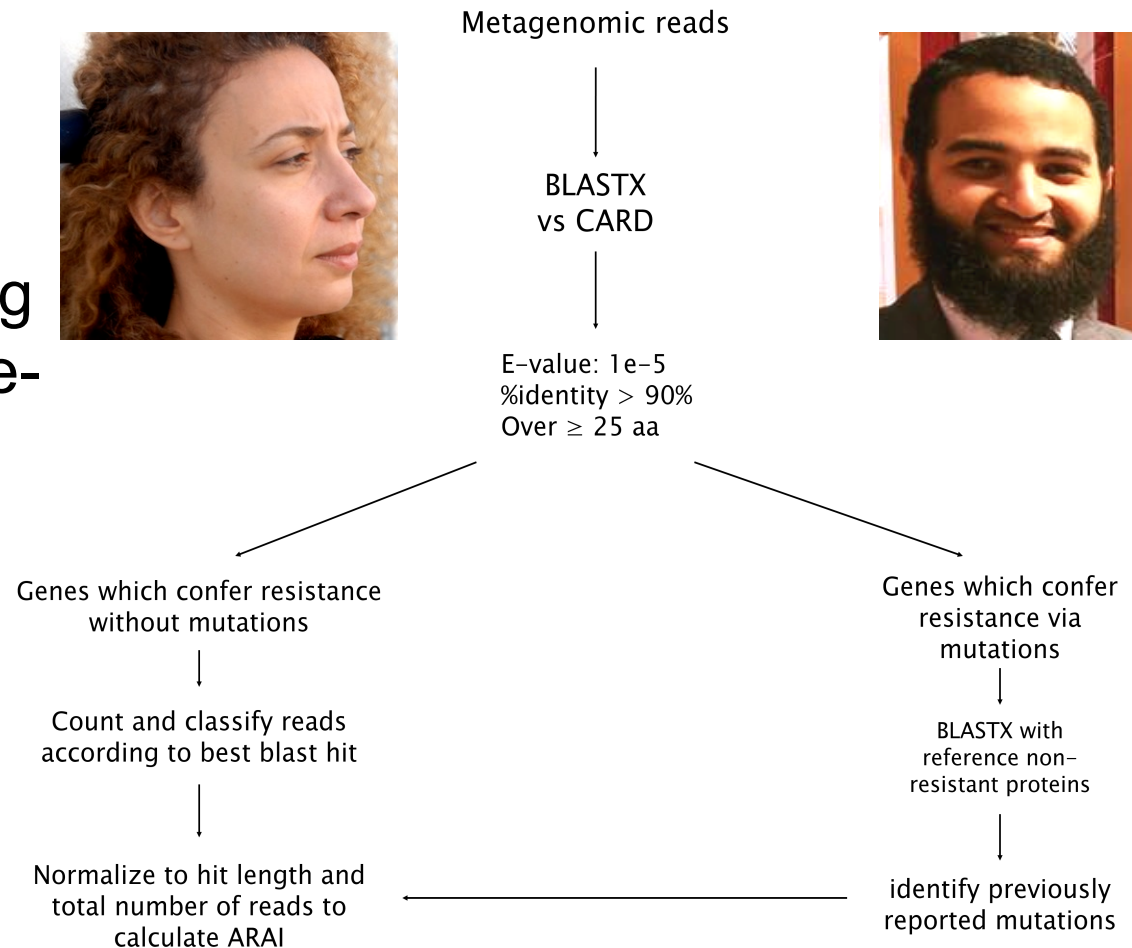
Why Has This Really Common Virus Only Just Been Discovered?



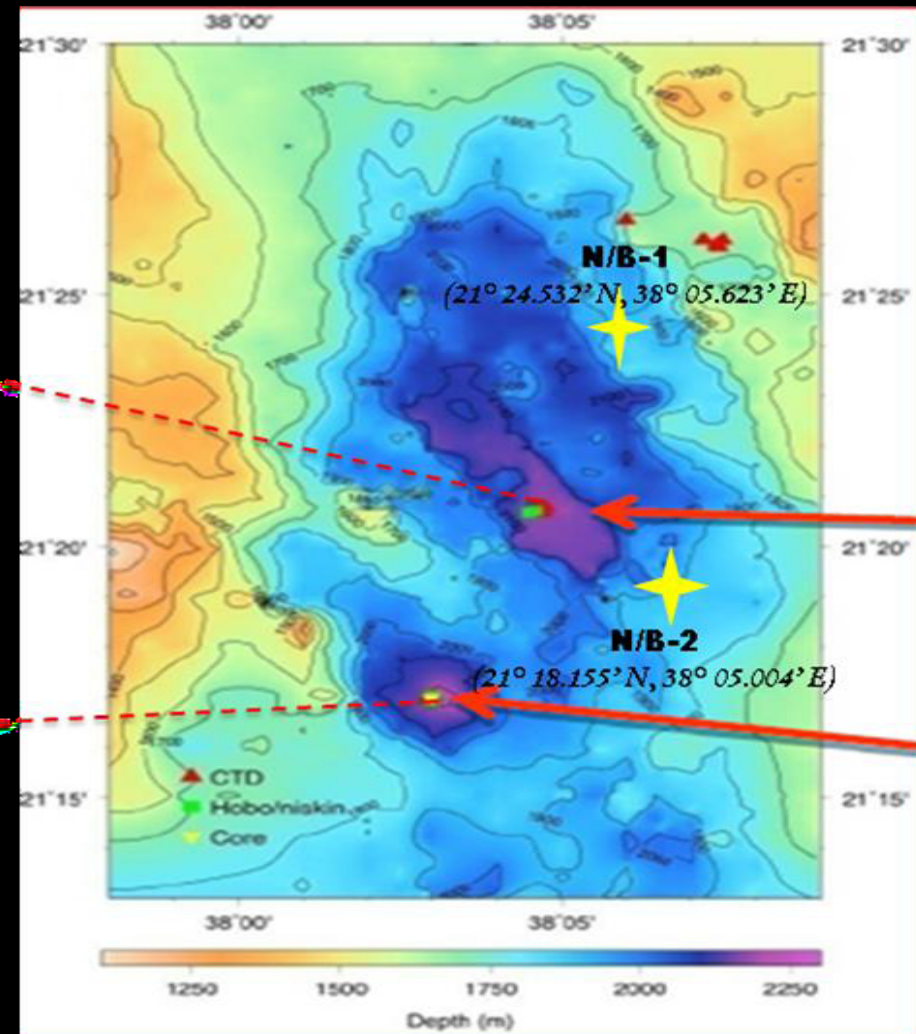
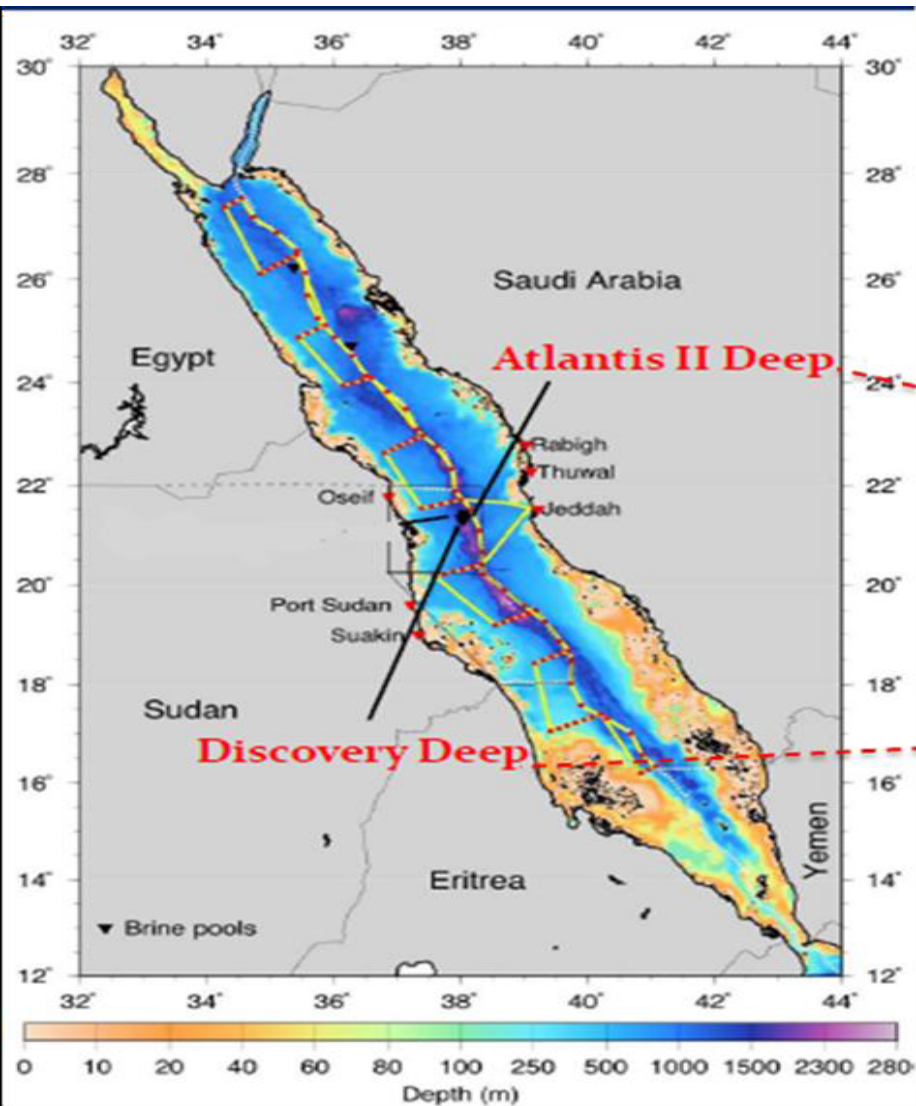
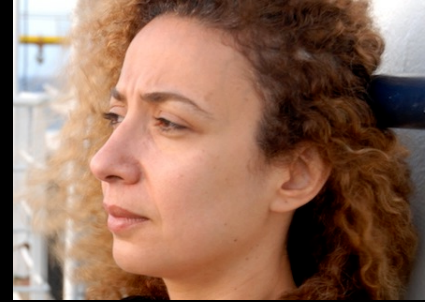
Bottleneck #5:

Antibiotic resistome estimation

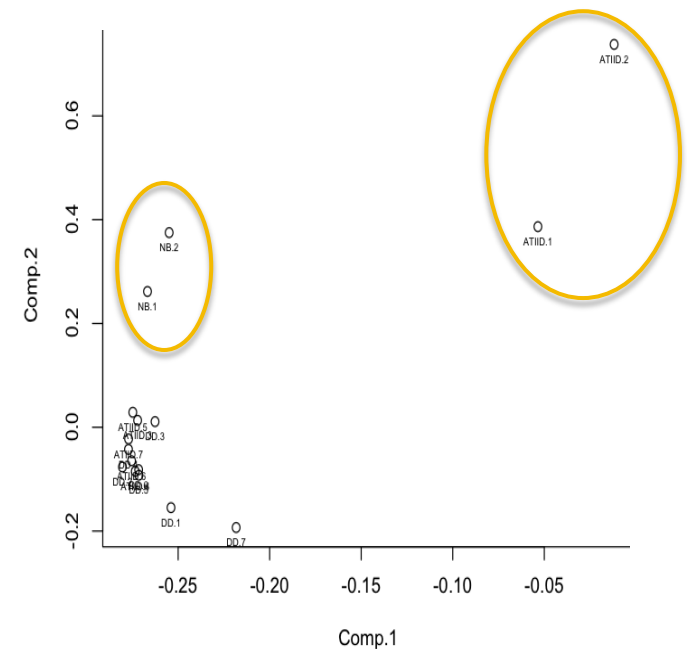
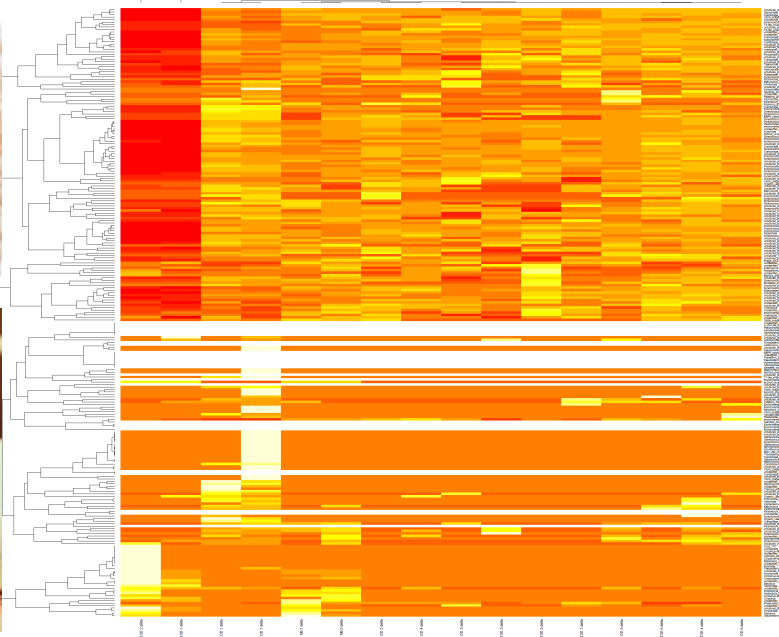
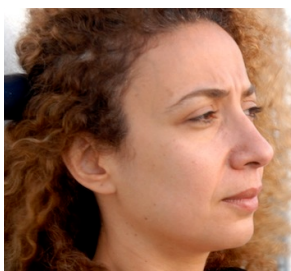
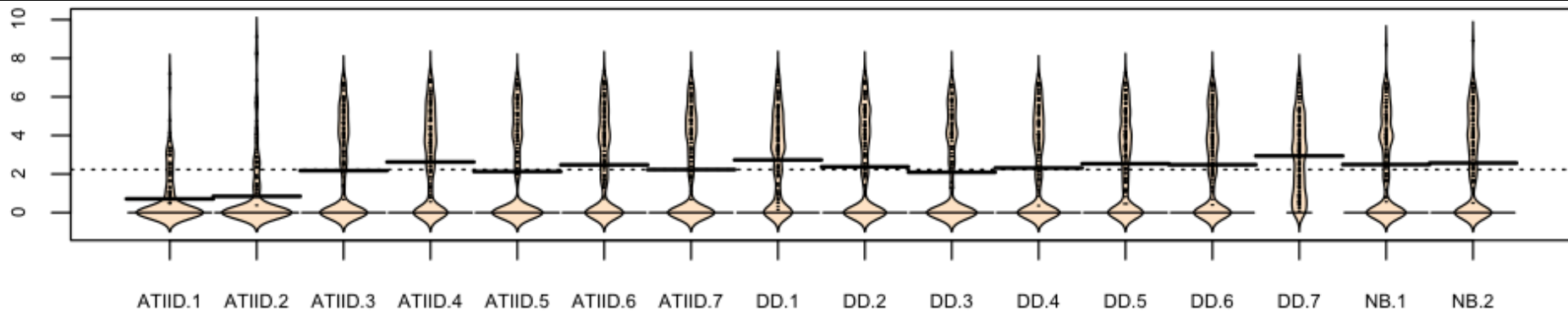
- Resistome = complete set of antibiotic resistance-encoding genes or resistance-generating mutations
- Many efforts in literature but also many erroneous results...



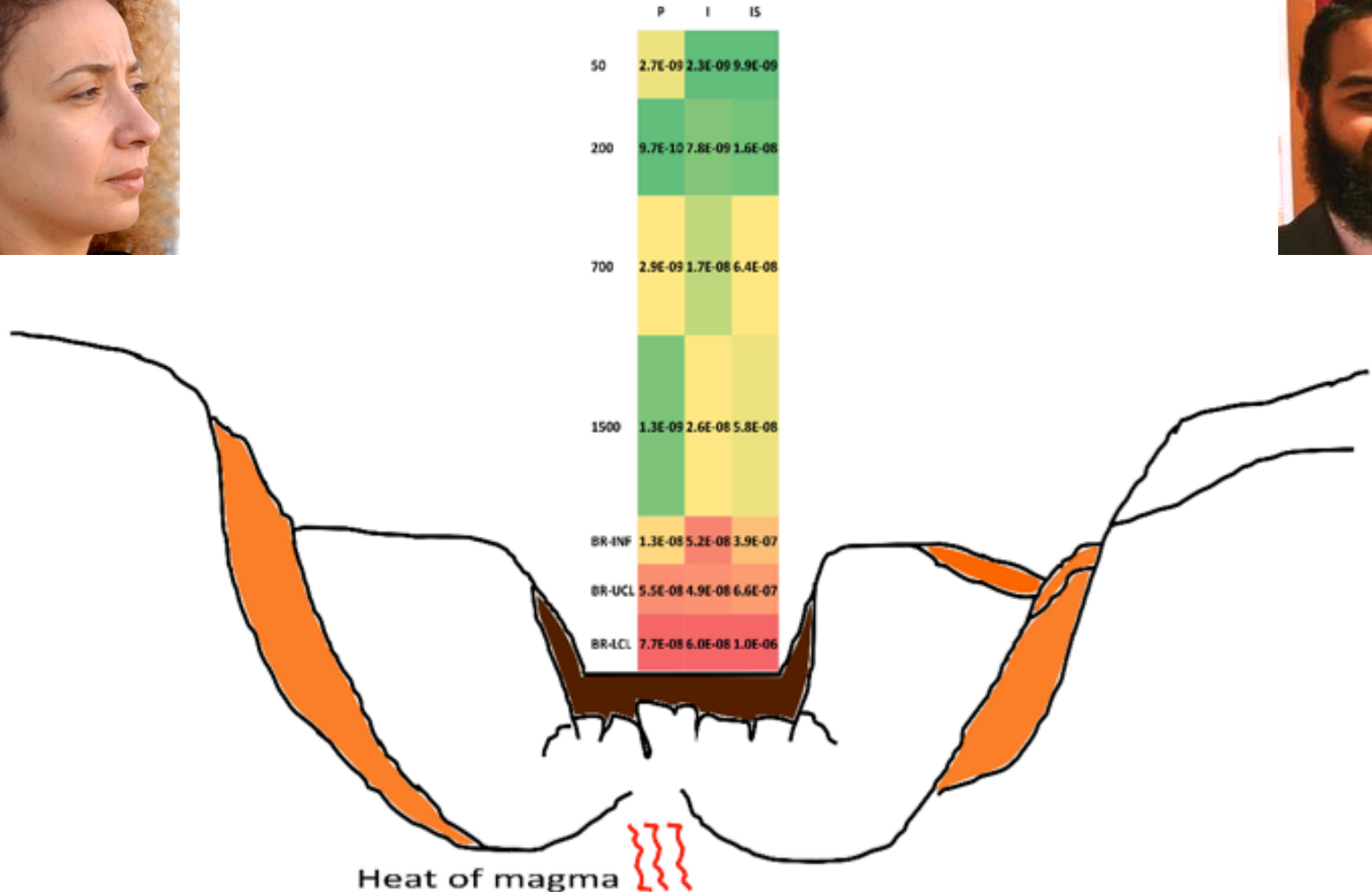
Application: Red Sea brine pools



Two deepest layers stand out!



Mobile elements are abundant in deepest layers



The decoding debate: Who they are vs. what they do

Character: It's Who You Are, Not What You Do

BY RICK WARREN — MAY 21, 2014

Sociological Forum, Vol. 12, No. 4, 1997

It's Not What You Do, But Who You Are: Informal Social Control, Social Status, and Normative Seriousness in Organizations¹

Calvin Morrill,^{2,3} Ellen Snyderman,⁴ and Edwin J. Dawson⁵



Batman Begins (2005)

Quotes

Rachel Dawes: Deep down you may still be that same great kid you used to be. But it's not who you are underneath, it's what you do that defines you.

51 of 53 found this interesting | Share this

TRUE SELF it's Who You Are not What You Do!



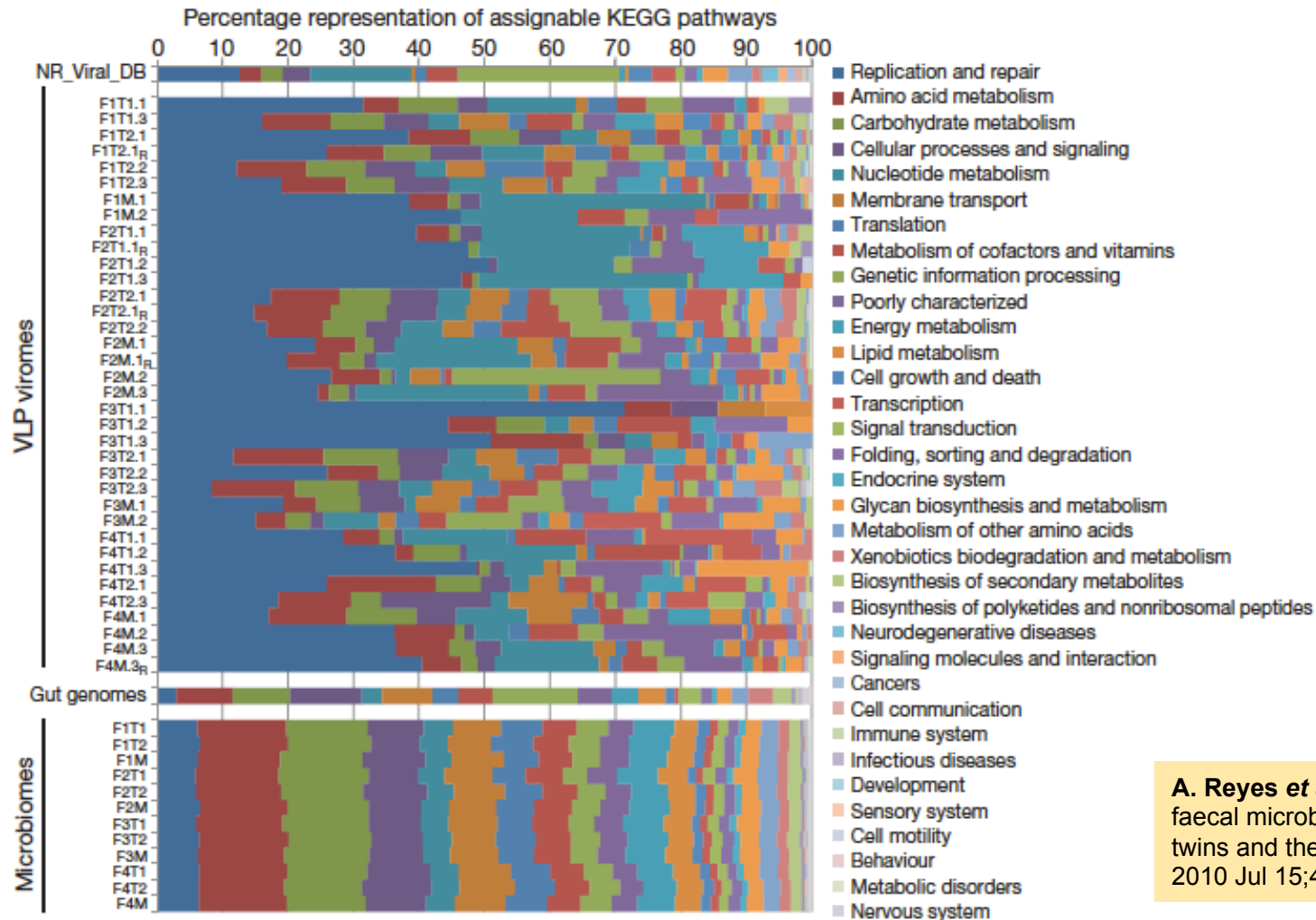
The biggest identity crisis going seems to exist in the blurry zone of confusion between what we do and who we actually are as a person. That's because there is a very strong tendency to identify ourselves with our primary activities in life. Let's look at that.



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Identity vs. function: Gut microbiomes vs. Gut viromes



A. Reyes *et al.* Viruses in the faecal microbiota of monozygotic twins and their mothers. **Nature.** 2010 Jul 15;466(7304):334-8.

100-yr-old insight

- The microbiome topic is certainly trendy now;
- But, the interest & debate are > 100 yr old

SOME OBSERVATIONS ON THE STUDY OF THE INTESTINAL BACTERIA.

By ARTHUR I. KENDALL.

(From the Laboratory of Dr. C. A. Herter, New York.)

(Received for publication, September 14, 1909.)

Gut Pathogens

Commentary

A hundred-year-old insight into the gut microbiome!

Ramy Karam Aziz

An idea of the truly enormous daily bacterial proliferation which takes place in the intestinal tract may be obtained if one remembers that **a considerable portion of the fecal mass is made up of the bodies of bacteria, dead and living. At the same time the multiplicity of types and variety of physiological requirements of this intestinal flora are indications of the excellence of the incubator and a strong reminder of the influence which the unrestrained activity of these organisms might conceivably exercise upon the general condition of the host."** (p. 499)

100-yr-old insight

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Dr. Theobald Smith¹ has stated the case admirably in the following terms: **'It is what bacteria do rather than what they are that commands attention, since our interest centers in the host rather than in the parasite.'**

¹ Theobald Smith: Some Problems in the Life-history of Pathogenic Microorganisms, Amer. Med., viii, pp. 711-718, 1904." (p. 500-501)

Message #2

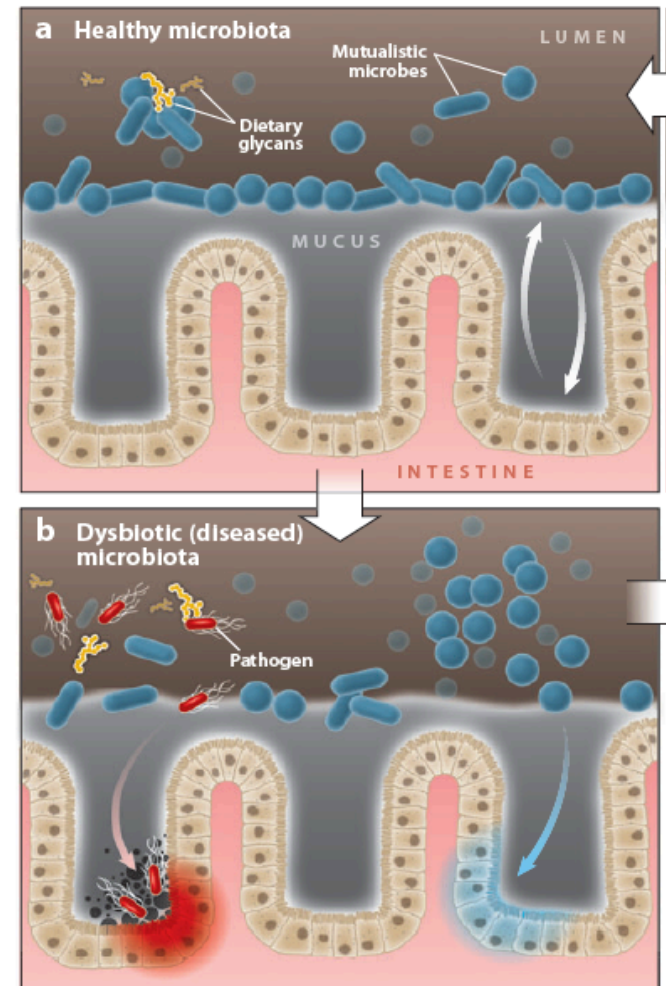
- **Decoding the microbiome is an art and science:**
 - Using computational resources is appealing but error prone.
 - Human expertise is indispensable but slow.
 - Combining human expertise and computer propagation is priceless (e.g., RAST, resistome analysis)!
 - Thinking outside the “one microbiome box” → e.g., crAss
- **A good question is half the way to the answer:**
 - Defining the question helps selecting the tool and strategy.
 - Cataloguing species is tempting; but with function-related questions, there's no way around analyzing genes and pathways.

Human Microbiome in Health and Disease



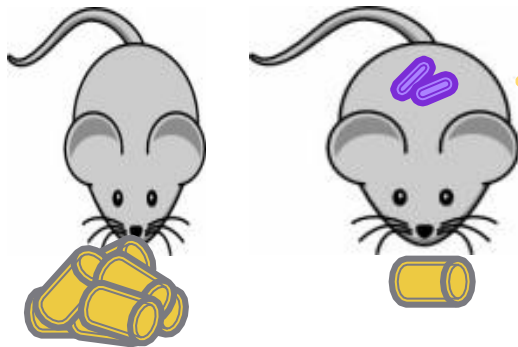
Microbiota in health & disease

- The interaction between a host and its **associated microbiota** mostly plays a beneficial role in one's health.
- The microbiota has a valuable role during normal **homeostasis**, modifying the host's immune system and prompting host development and physiology.
- Any disruption of this homeostatic coexistence leads to **dysbiosis**, which has been powerfully associated with human disease.



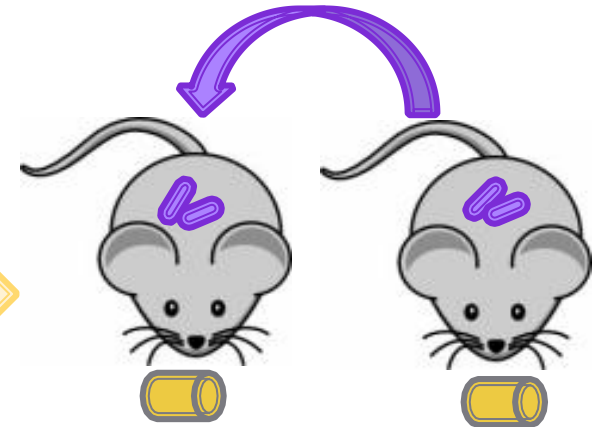
Source: Pflughoeft, K.J. and J. Versalovic. Annu. Rev. Pathol. Mech. Dis. 2012. 7:99–122

Gut microbiota affect nutrient assimilation



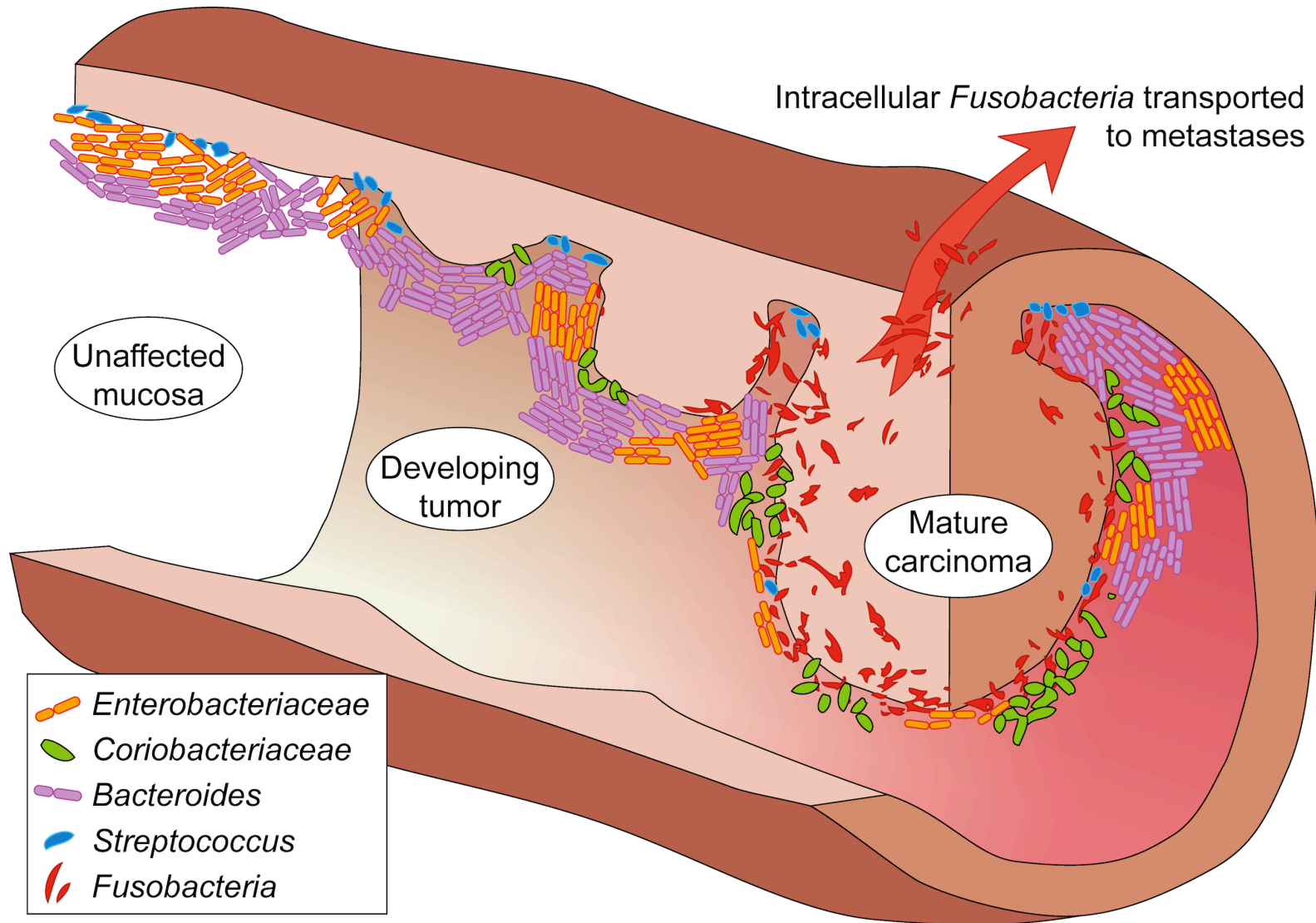
Germ-free mice need more food to take in enough calories because they are less efficient at assimilating food. Even though they eat less, normal mice have 40% more body fat than germ-free mice

Transferring bacteria from normal to germ-free mouse restores its ability to assimilate food, and increases its body fat



Ley, R.E. et al.. (2005). Obesity alters gut microbial ecology. PNAS USA. 102: [11070-11075](#).
Turnbaugh, et al. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 444: [1027-1131](#).

Gut microbiota and colorectal cancer

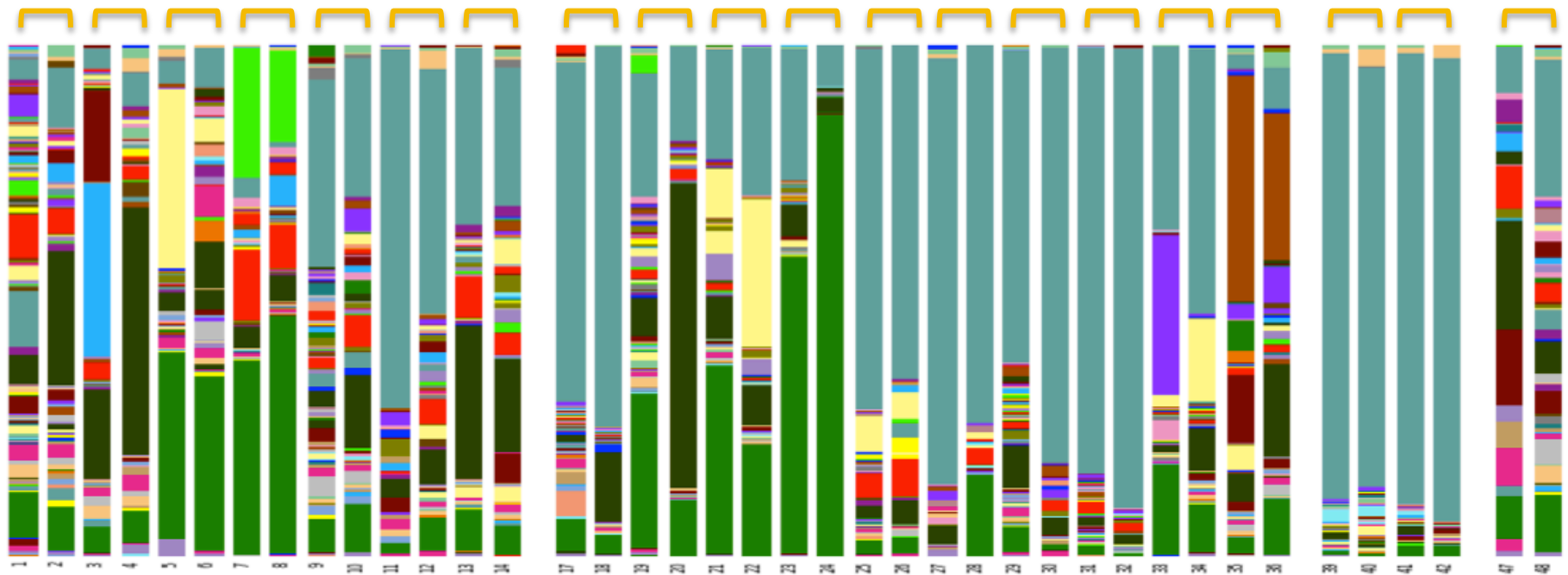


Application: The eye microbiome



Application: The eye microbiome

Bacterial communities in eye swabs from the two eyes of 18 patients + 2 controls



Recap ...

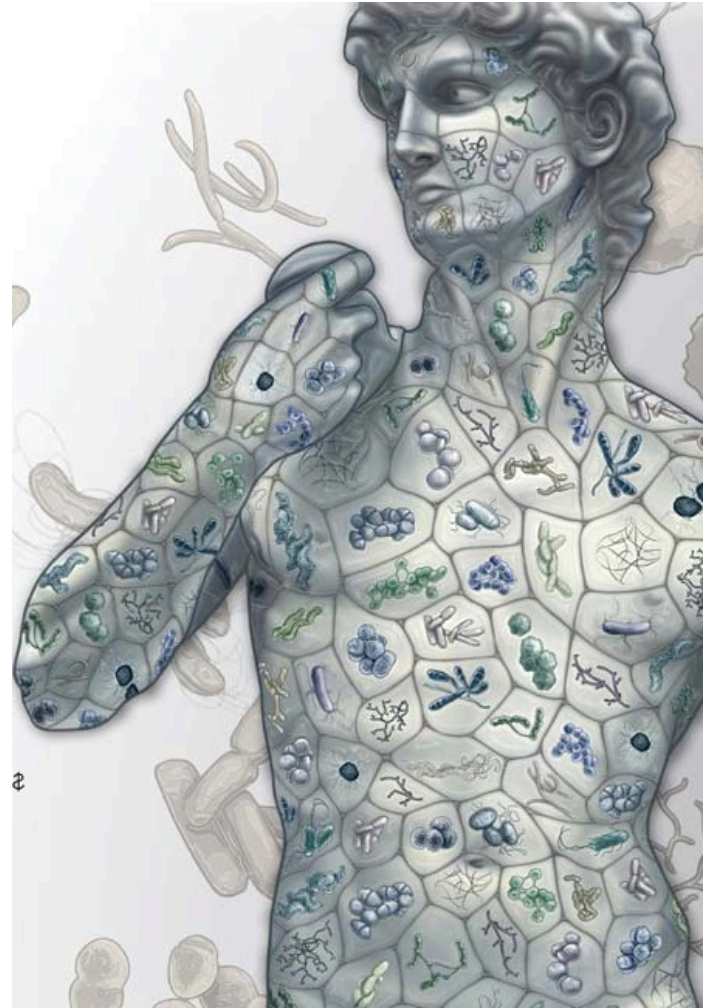
1. Microbiome = clouds of microbes, their genomes, viruses, plasmids, mobile elements, etc. with different levels of variability.
2. *What they do* is not always correlated with *who they are*.
3. Microbes play major functions that affect health, disease, and nutrition.

Last, but not least, how about their effect on pharmacotherapy (drugs)?

PharmacoMicrobiomics

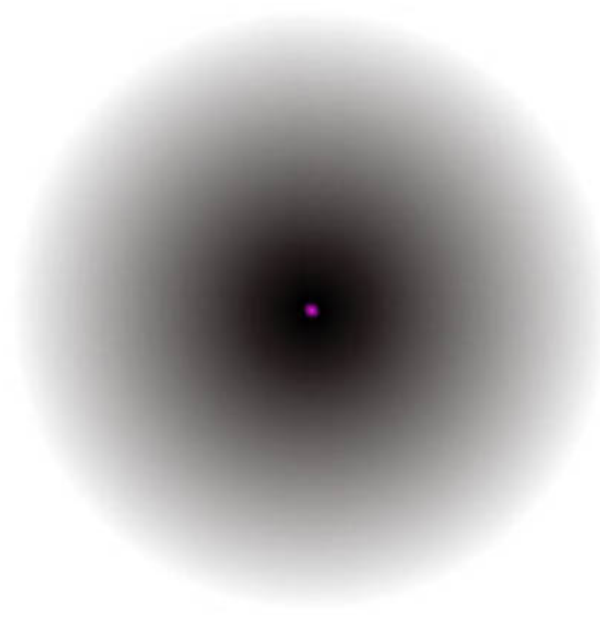
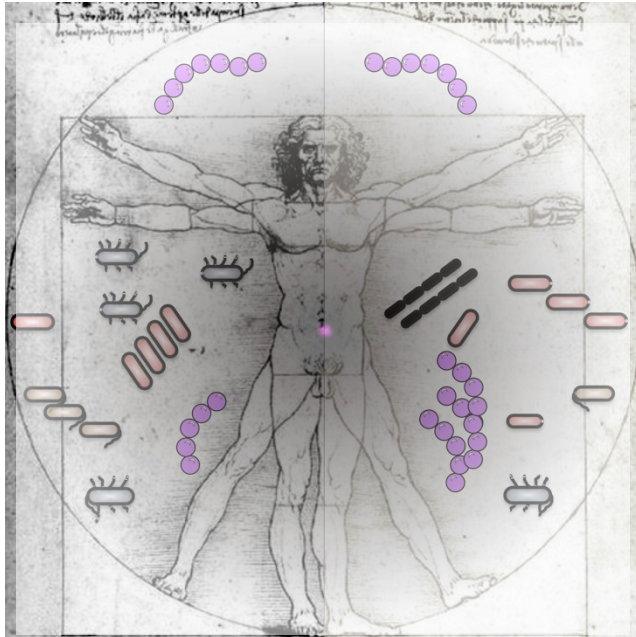
Drug-Microbiome Interactions

Bugs vs. Drugs

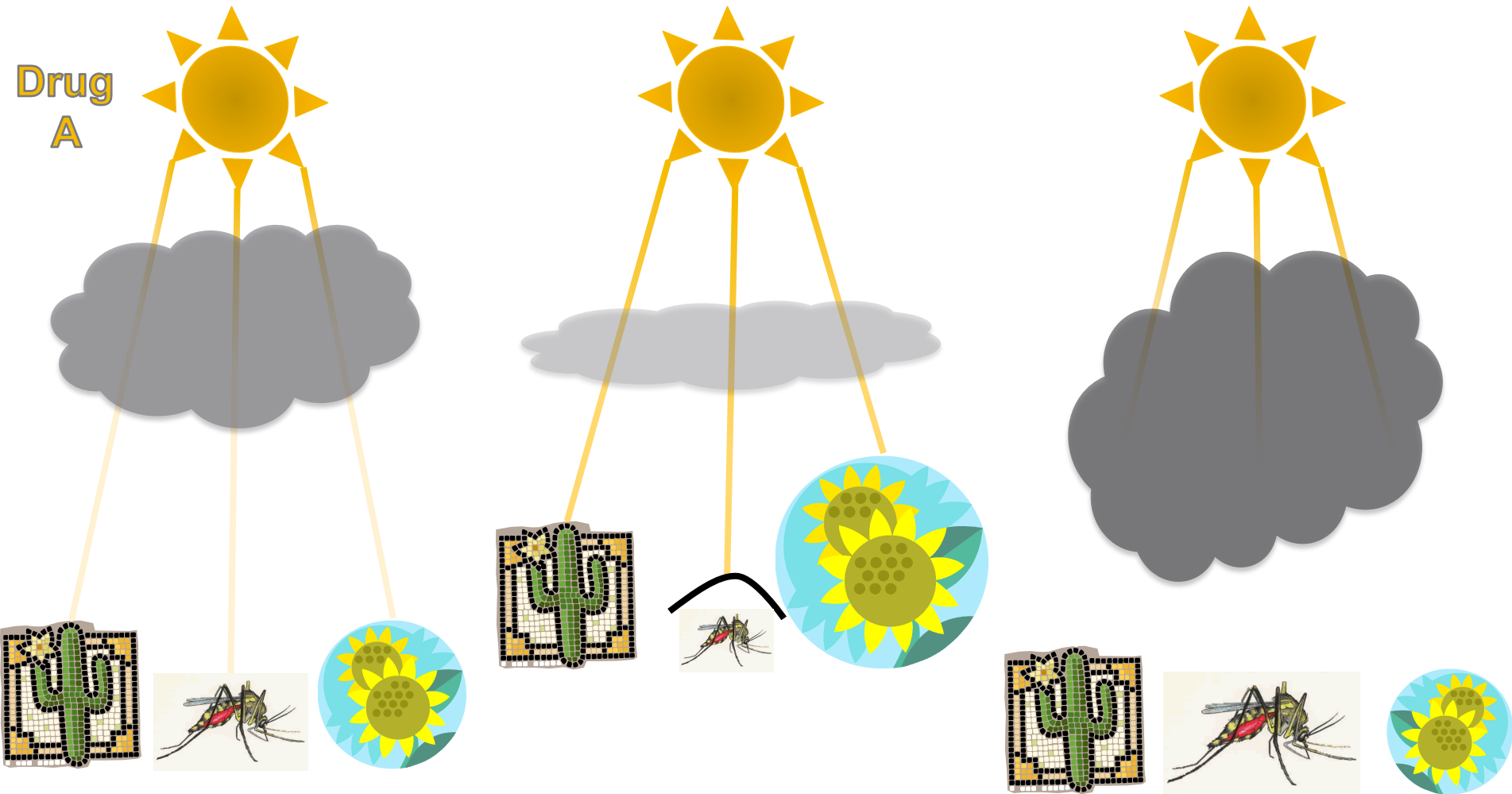


A moving (drug) target!

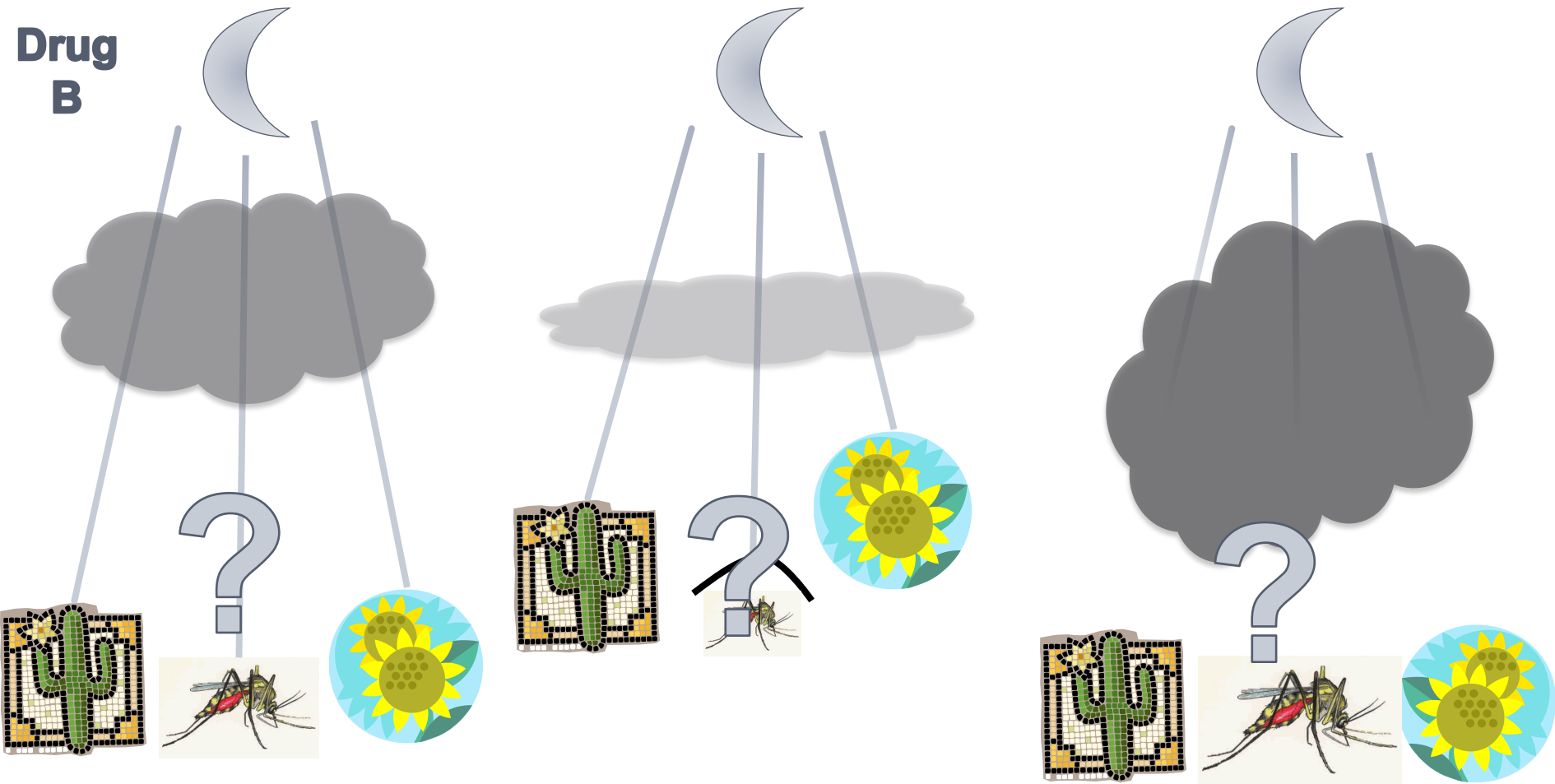
The microbiome cloud model



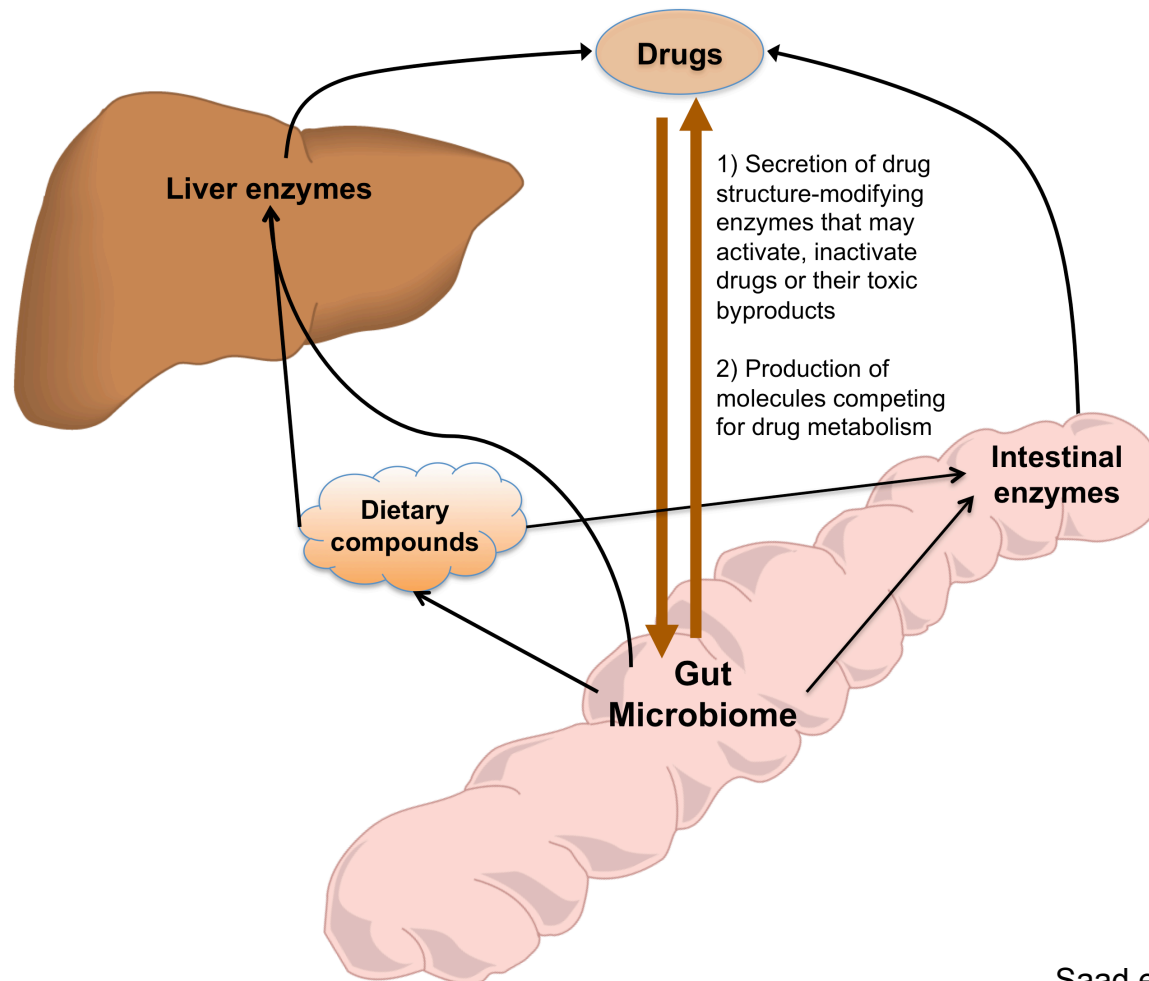
A simplistic *cloud model* for *systems pharmacology*



A simplistic *cloud model* for *systems pharmacology*

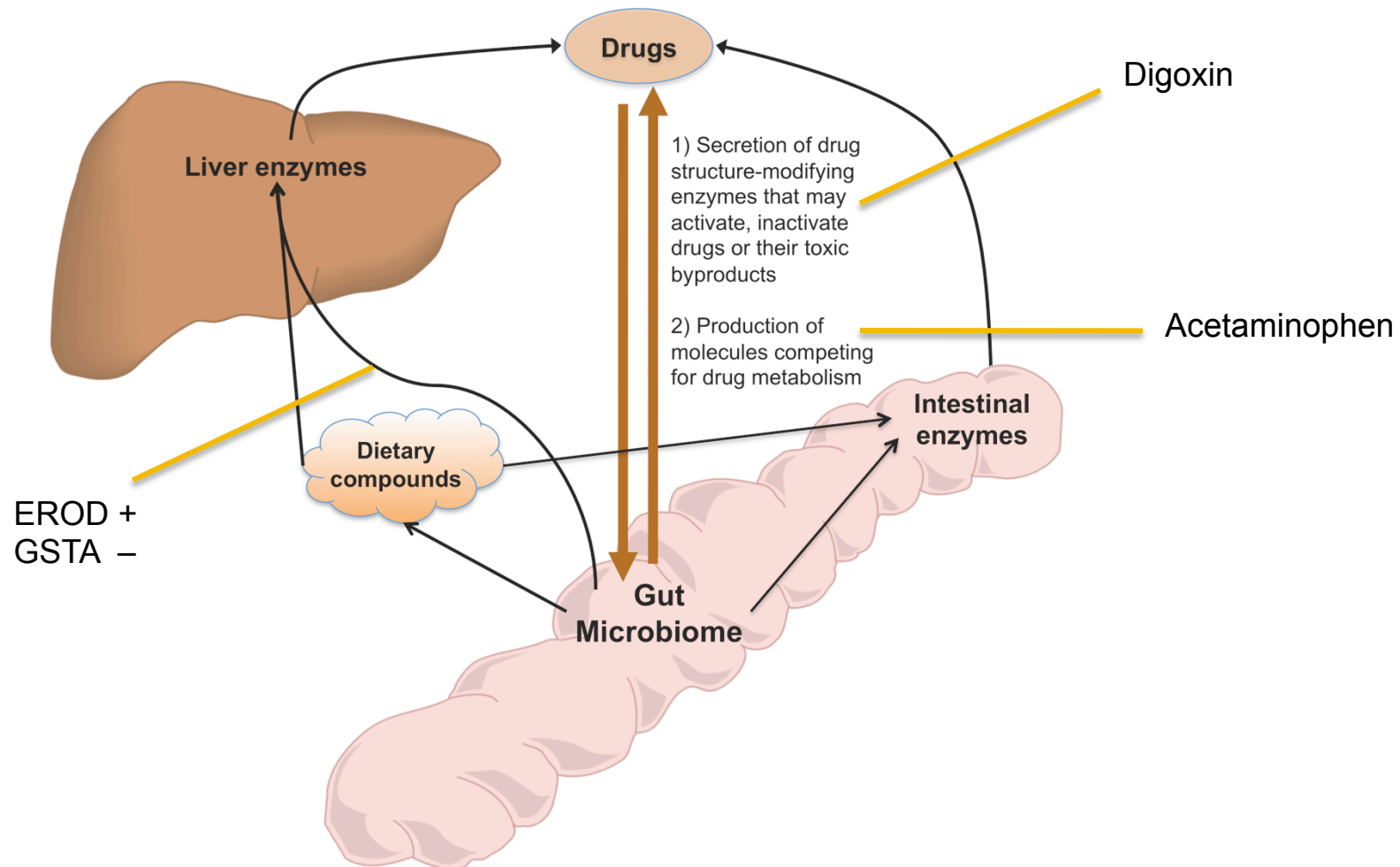


Drug-microbiome interactions



Saad et al. Gut Pathogens 2013

Drug-microbiome interactions



Drug-microbiome interactions

TABLE 2. EXAMPLE DRUG–MICROBE INTERACTIONS WHOSE MOLECULAR MECHANISMS HAVE BEEN DELINEATED

<i>Drug (PubChemID)</i>	<i>Microbe (TaxID)</i>	<i>Pathway/ Reaction</i>	<i>Gene (s)</i>	<i>Interaction</i>	<i>Reference</i>
Acetaminophen (1983)	Unknown	O-sulfonation	Unknown	Some gut microbes may increase acetaminophen toxicity by producing p-cresol, which competes with acetaminophen metabolism.	(Clayton et al., 2009),
Digoxin (2724385)	<i>Eggerthella lenta</i> (84112)	Cytochrome-encoding operon	Cardiac glycoside reductases: <i>cgr1</i> , <i>cgr2</i>	<i>cgr</i> gene products, homologous to bacterial cytochromes, reduce digoxin by using it as an alternative electron acceptor.	(Haiser et al., 2013)
Cyclophosphamide (2907)	Firmicutes (1239)	Immune modulation	Unknown	Cyclophosphamide translocates firmicutes, thus altering the TH1 immune response, leading to synergistic anticancer activity.	(Viaud et al., 2013)

Data compiled from: <http://pharmacomicrobiomics.org>.

The PharmacoMicrobiomics portal

PharmacoMicrobiomics: The Drug-Microbiome Portal

How Bugs Modulate Drugs?

Release 1.0 (on 11/11/11)

<http://pharmacomicrobiomics.org>

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- [Drug-Microbiome Relationship](#)
- [Publication](#)
- [Microbiome Body Site](#)

Relations:

Body site	Drug	Microbe	Publication	Effect	Effect description	Details
Gut	baicalin	unknown	11197087	increase hydrolysis	Gut microbiota normally hydrolyze baicalin into its corresponding aglycone baicalein ...	Go
Gut	digoxin	Eggerthella lenta	2759492	decrease metabolic processing	Altered concentration of E. lentum between populations has been correlated ...	Go

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About

The PharmacoMicrobiomics Web Portal is an [EgyBio](#) "Virtual Lab" project. It started as a research-based educational initiative to introduce bioinformatics and microbial genomics to pharmacy students by exploring drug-microbe and drug-microbiome interactions. The current web portal is the first product of this project and is continuously being updated. (Read more ...)

PharmacoMicrobiomics Links

The PharmacoMicrobiomics portal

PharmacoMicrobiomics: The Drug-Microbiome Portal

How Bugs Modulate Drugs?

Release 1.0 (on 11/11/11)

<http://pharmacomicrobiomics.org>

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- [Publication](#)
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Body site	Chemical	Drug-Microbiome Relationship	Publication	Microbiome Body Site	Description	Details
Gut	baicalin	unknown	11197087	increase hydrolysis	Gut microbiota normally hydrolyze baicalin into its corresponding aglycone baicalein ...	Go
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PharmacoMicrobiomics
Links

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How Bugs Modulate Drugs?

Launched on 11/11/11; Current Release 1.2 (01 Sep 12)

<http://pharmacomicrobiomics.org>

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Simple Search

Q:

You searched for: **Digoxin**

In relations:

Found 3 relations.

Body site	Drug	Microbe	Publication	Full record
Gut	digoxin	Eggerthella lenta	2759492	Go
Gut	digoxin	unknown	7246583	Go
Gut	digoxin	unknown	7104181	Go

Search

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([Read more ...](#))

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The PharmacoMicrobiomics portal

PharmacoMicrobiomics: The Drug-Microbiome Portal
How Bugs Modulate Drugs?
Launched on 11/11/15

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Simple Search
Q:
You searched for **digoxin**
In relations:
Found 3 relations

Body site	Drug	Microbiome	PMID	Go
Gut	digoxin	unknown	7104181	Go

[Home](#)

In publications:
Found 3 publications.

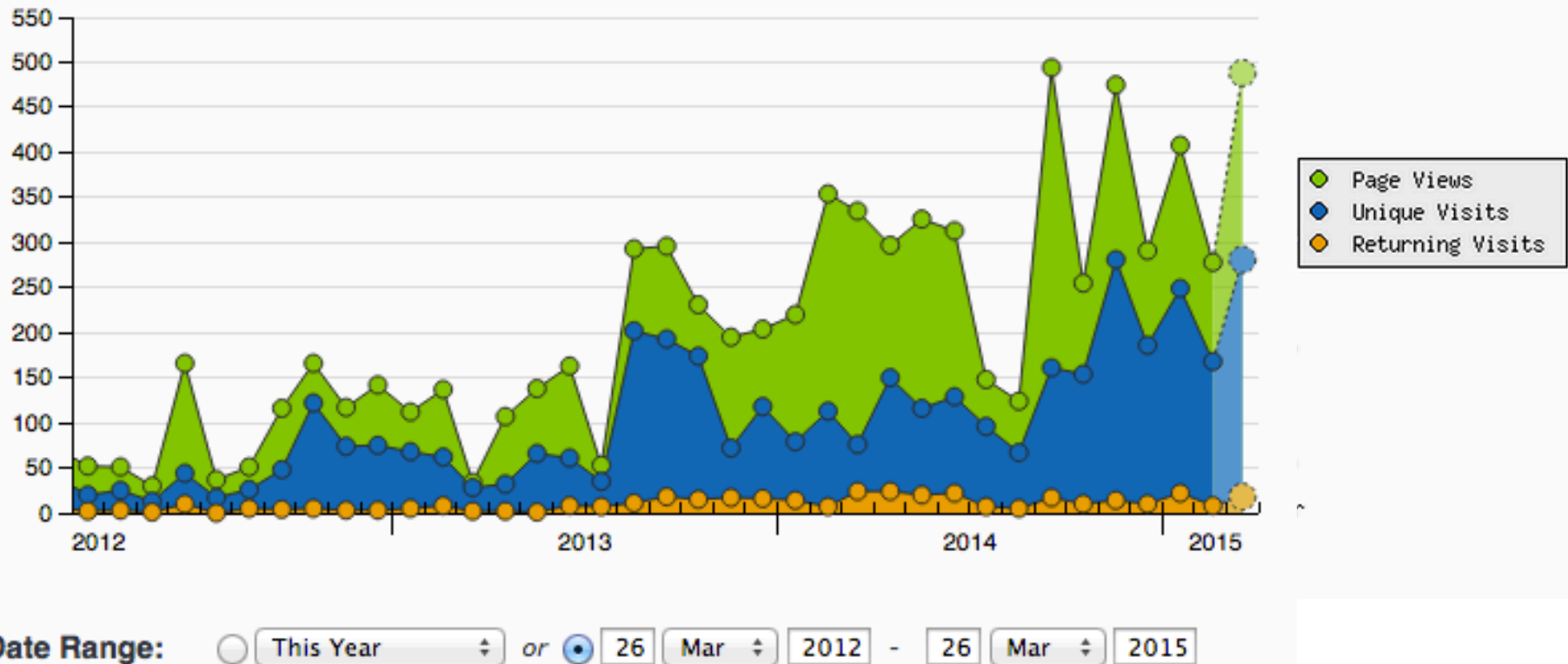
ID	Publication title	PMID	Publication year	Publication type
2	Geographic differences in digoxin inactivation, a metabolic activity of the human anaerobic gut flora.	2759492	1989	Research
62	Metabolism of digoxin and absorption site.	7104181	1982	Research
5	Urinary excretion of reduced metabolites of digoxin.	7246583	1981	Research

[\(Read more ...\)](#)

PharmacoMicrobiomics Links

The PharmacoMicrobiomics portal

■ 3-year performance assessment



PharmacoMicrobiomics

What next?

Ongoing projects

IN VITRO/ IN VIVO

- Pharmacomicrobiomics of Voriconazole in cancer patients
- Azodye degraders from skin and gut
- Nasal microbiota competition with *Staphylococcus aureus*
- How bacterial pathogens react to sugars of the gut environment (rhamnose, fucose, etc.)

IN SILICO

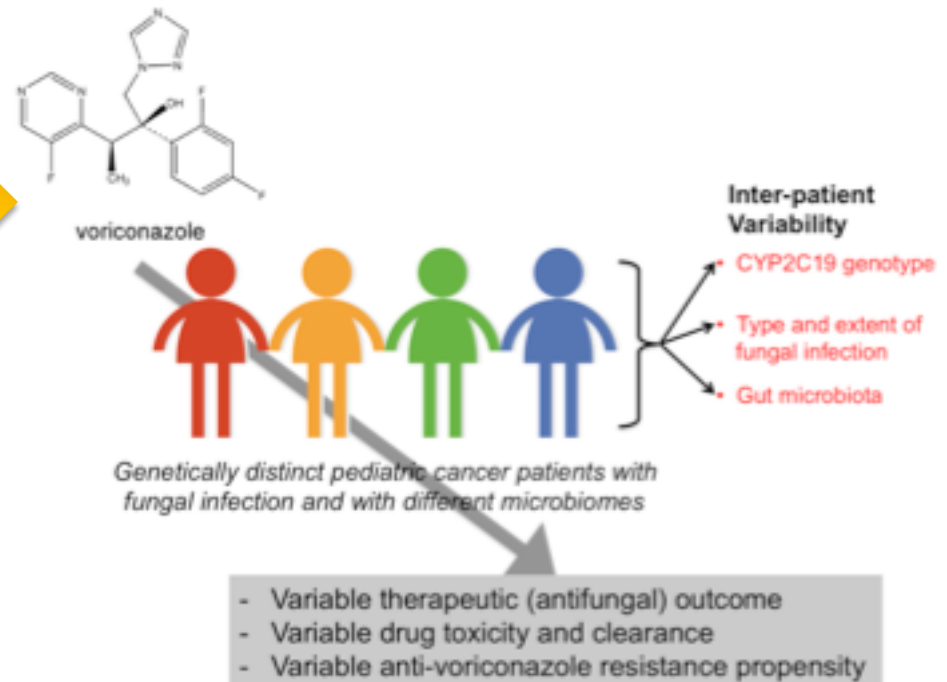
- Azo-reductases and dye peroxidase mining tools
- Beta glucuronidase data mining tools

Ongoing projects

IN VITRO/ IN VIVO

- Pharmacomicrobiomics of Voriconazole in cancer patients
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Final message

- Our cells are surrounded by a microbiome cloud that interacts with us and affects our health, disease, nutrition, and therapy.
- Decoding this microbiome cloud is not easy and is sometimes challenging.
- The future of “our” biology is eternally connected to those *intimate strangers*. We have a **common destiny!**

Questions?

