

Evolutionary arms races produce unusual
weapons

or

How I learned to stop worrying and love a
pseudokinase

Michael Reese

<http://www.reeselab.org>

Dept. of Pharmacology

My meandering career path:

- 1995-1999: Worked in X-ray crystallography, on the “postal system” of the cell
- 1999-2001: Taught high school in San Diego;(Chemistry, Math, Physics)
- 2001-2006: Graduate studies at UC San Francisco; NMR methodology to quickly determine how two proteins interact
- 2004-2005: Moved to Germany for my PhD studies
- 2006-2013: Postdoctoral work at Stanford on parasite genetics
- 2013-present: Joined UTSW Pharmacology

Non-biology jobs I have held or considered:

English teacher in Japan

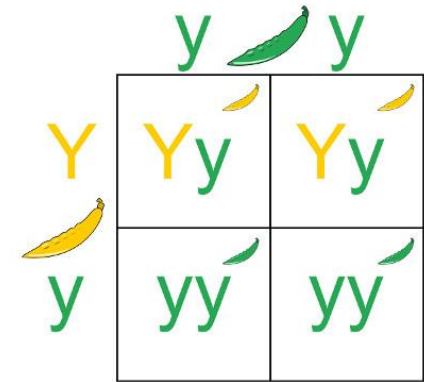
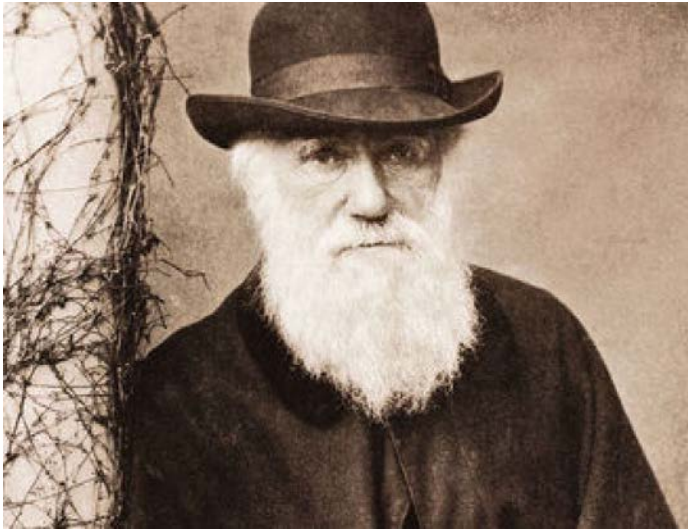
Back-end code maintenance for online gaming
(did this part-time for 10+ years)

Web designer for online TV listings
(before there was youtube, or hulu, or google, or...)

Designer/coder for digital medical record startup

Sake brewer

Evolutionary Theory 101: Individuals in a species vary



Mendel's peas

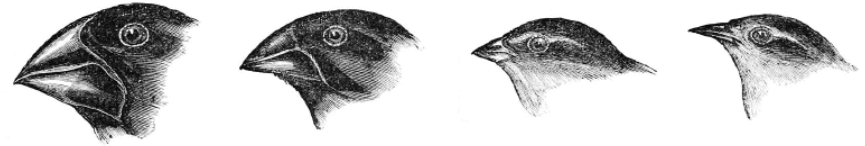
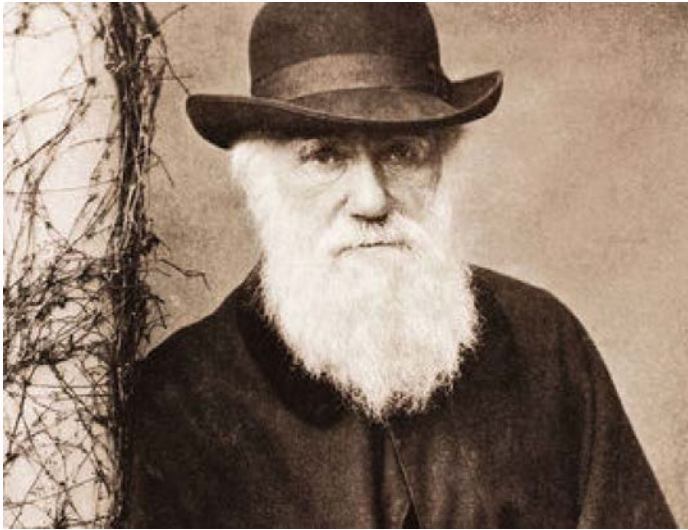
On the origin of species – Charles Darwin 1859

I. Variation under domestication



Dog breeds

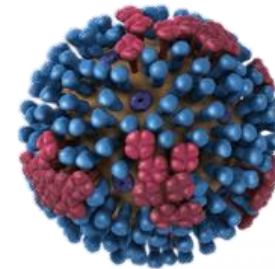
Evolutionary Theory 101: Individuals in a species vary



Galapagos finches

On the origin of species – Charles Darwin 1859

- I. Variation under domestication
- II. Variation under nature
- III. Struggle for Existence
- IV. **Natural Selection**
- V. **Laws of Variation**



Flu virus



Evolutionary Theory 101: What is a selective pressure?

Individuals that produce more viable offspring will have their genetic make-up better represented in future generations

Traits that are beneficial are maintained over time

Traits that are deleterious are lost over time

Traits that are neutral may be randomly lost or gained over time

Evolution is survival of the **just good enough**

Note: If Tom already had kids then he can be lunch and **still** have been “successful”



Molecular evolution: fast vs. slow

To be conserved, genes must encode something that is important (at least some of the time)

Most mutations are **neutral**;
they offer neither benefit nor cause harm

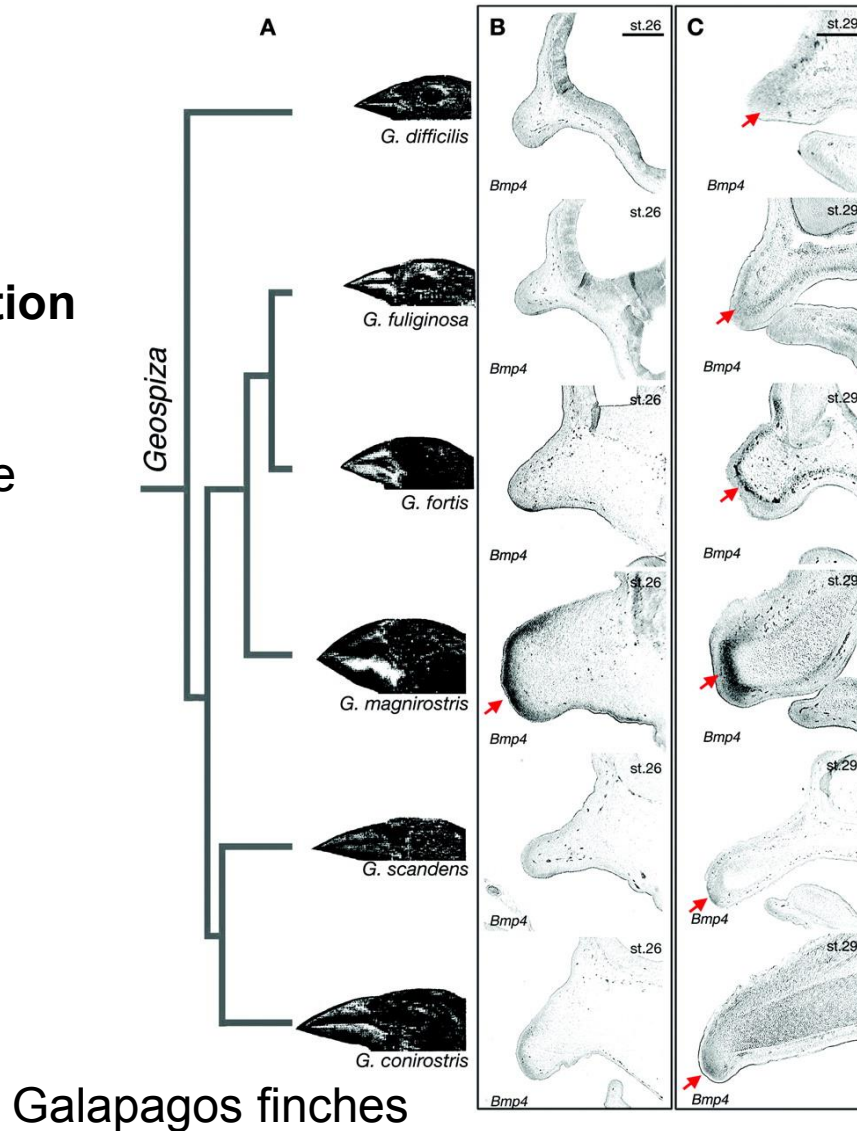
Gaining entirely new functions can be slow
(new tricks take time)

Most genes evolve very slowly...

How can genes evolve more quickly?

Molecular evolution: fast vs. slow

Variation in **Timing** and **location** of Bmp4 (bone growth factor) expression causes major changes in beak size & shape



Molecular evolution: fast vs. slow

What about humans. Are we still evolving?

Starchy diet

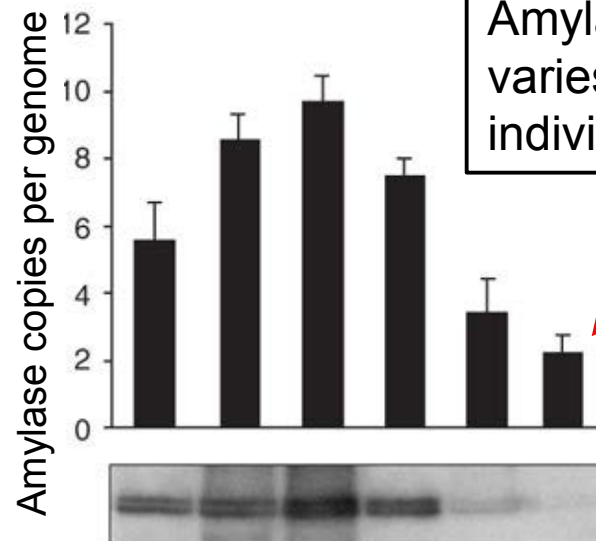


Amylase is an enzyme that breaks starch into simple sugars

It is incredibly close to a “perfect” enzyme...

So how could you get more activity?

Meaty diet



Amylase **copy number** varies depending on an individual's ancestry

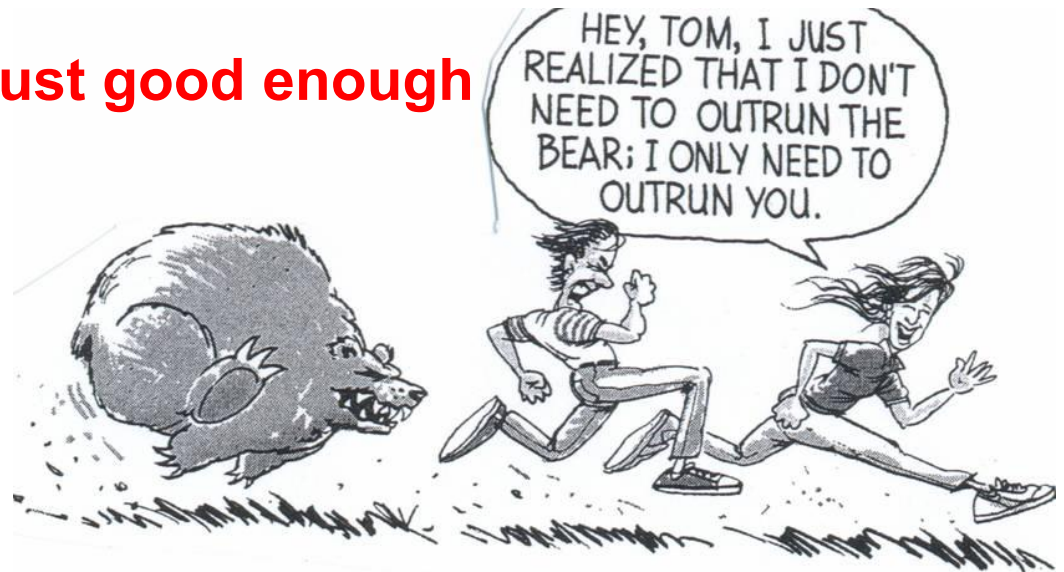
Texan?

Make more of it!

Evolutionary Theory 101: What is a selective pressure?

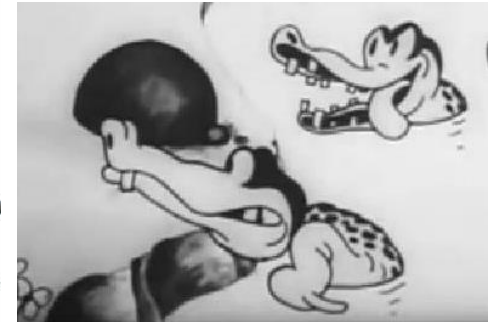
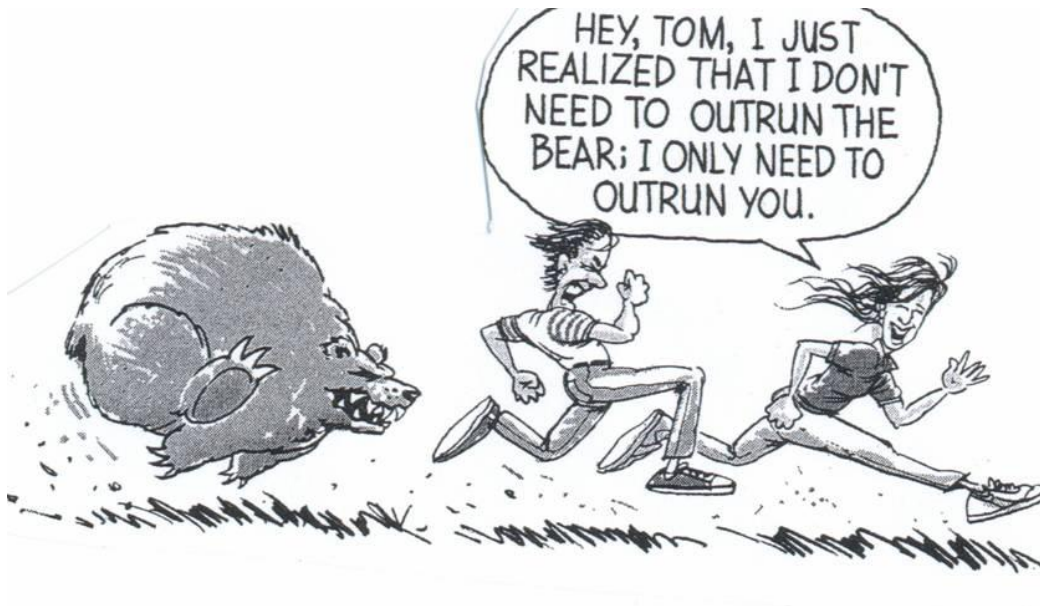
Individuals that produce more viable offspring will have their genetic make-up better represented in future generations

Evolution is survival of the **just good enough**



Evolutionary Theory 101: What is a selective pressure?

What happens when the selective pressure changes?



What happens when a selective pressure is always changing?
(*i.e.*, the selective pressure is, itself, evolving!)

Evolutionary arms races – the game where nobody wins



“It takes all the running you can do to keep in the same place”
– Lewis Carroll, *Through the Looking Glass*

Evolving systems must continuously develop to maintain the status quo.
– Leigh Van Valen

A thought experiment: What happens to the flu virus if it stopped evolving?

Evolutionary arms races – the game where nobody wins



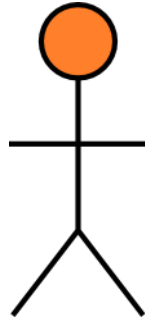
Pathogens and their hosts (that's us), must both constantly evolve to survive

Hosts have a fast evolving immune system to combat disease

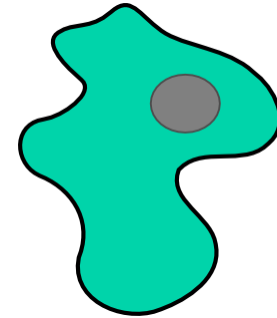
Pathogens use “effectors” to sneak past or disarm the immune system

Most pathogens have a restricted host range; they must specialize in one or two similar host species in order to be able to keep up in the arms race.

Evolutionary arms race: Round 1



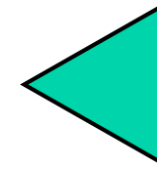
vs.



Winner!

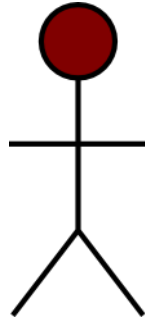


CIP1
(Critical Immune Protein 1)

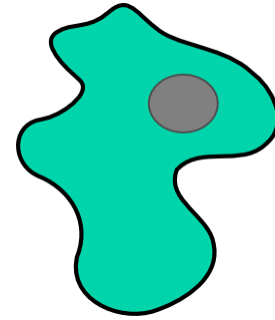


IDP1
(Immune Defeating Protein 1)

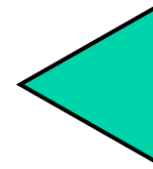
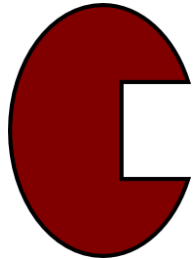
Evolutionary arms race: Round 2



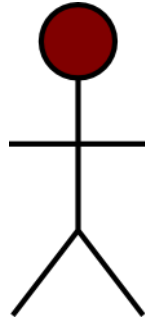
vs.



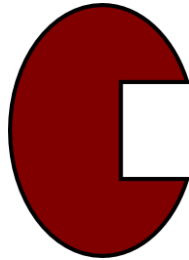
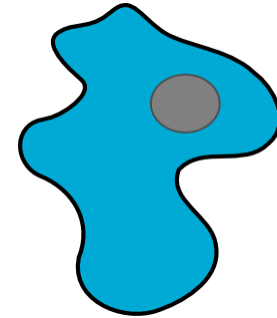
Winner!



Evolutionary arms race: Round 3...etc

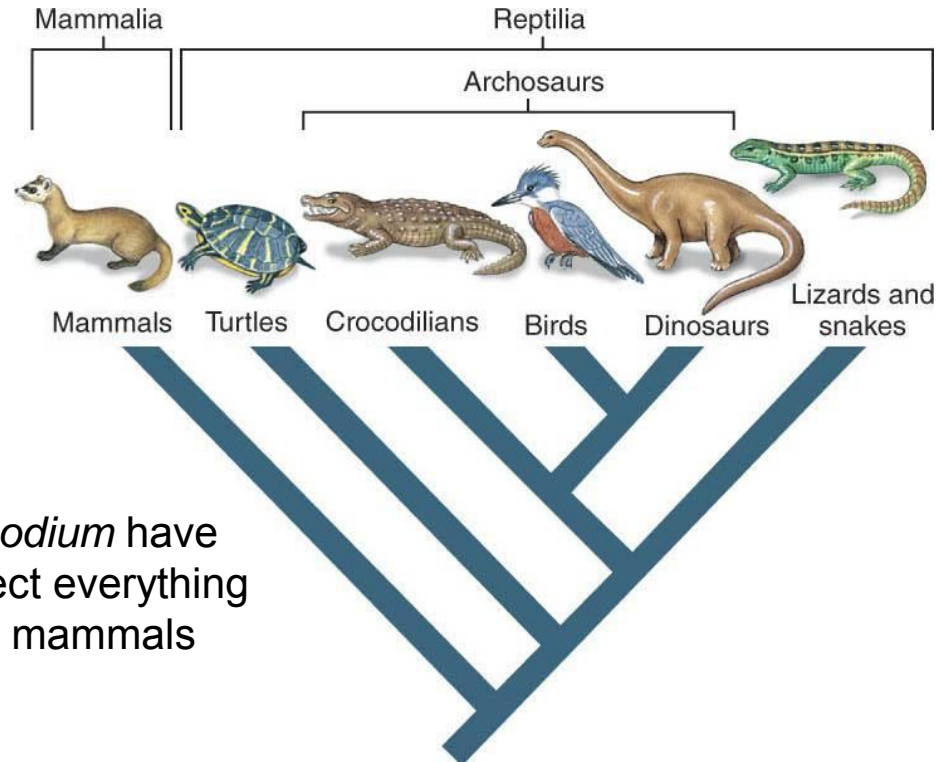


vs.
(and so on...)



The history of this competition is recorded in the genomes of both organisms (host & pathogen)

Plasmodium (malaria) species infect specific hosts



~250 species of *Plasmodium* have been identified and infect everything from snakes to birds to mammals



“Definitive” host

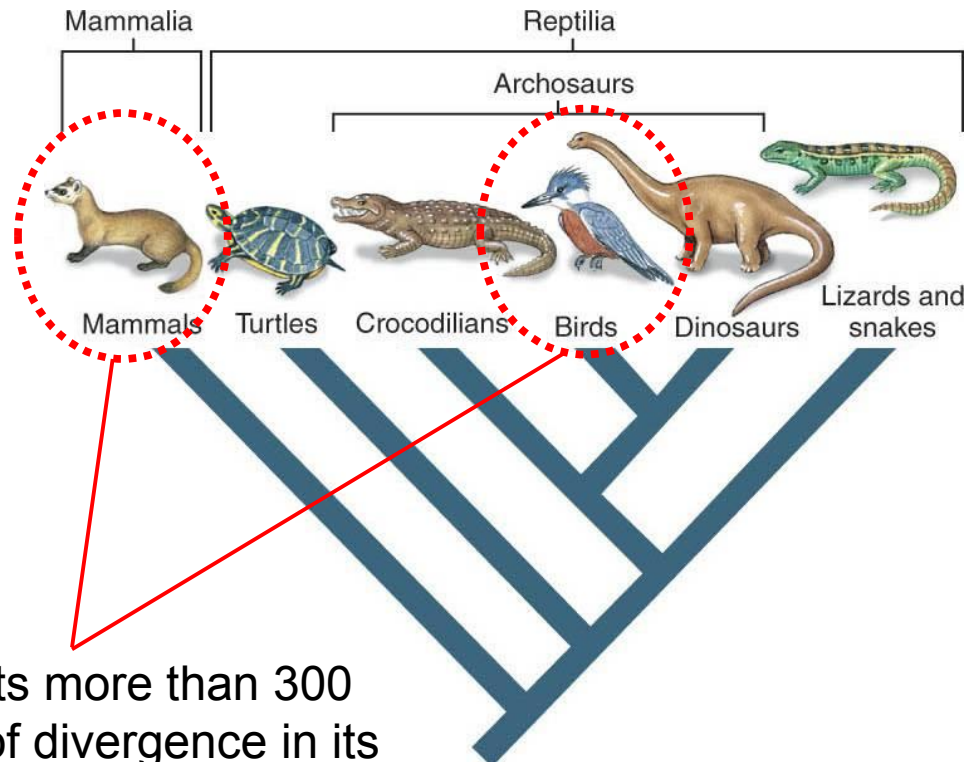
They are specialists:

One parasite species infects handful of species of both their insect and non-insect hosts

Both sets of hosts are trying to kill the parasites (mosquitoes don't like being sick any more than you do!)

Malaria's prolific cousin: *Toxoplasma gondii*

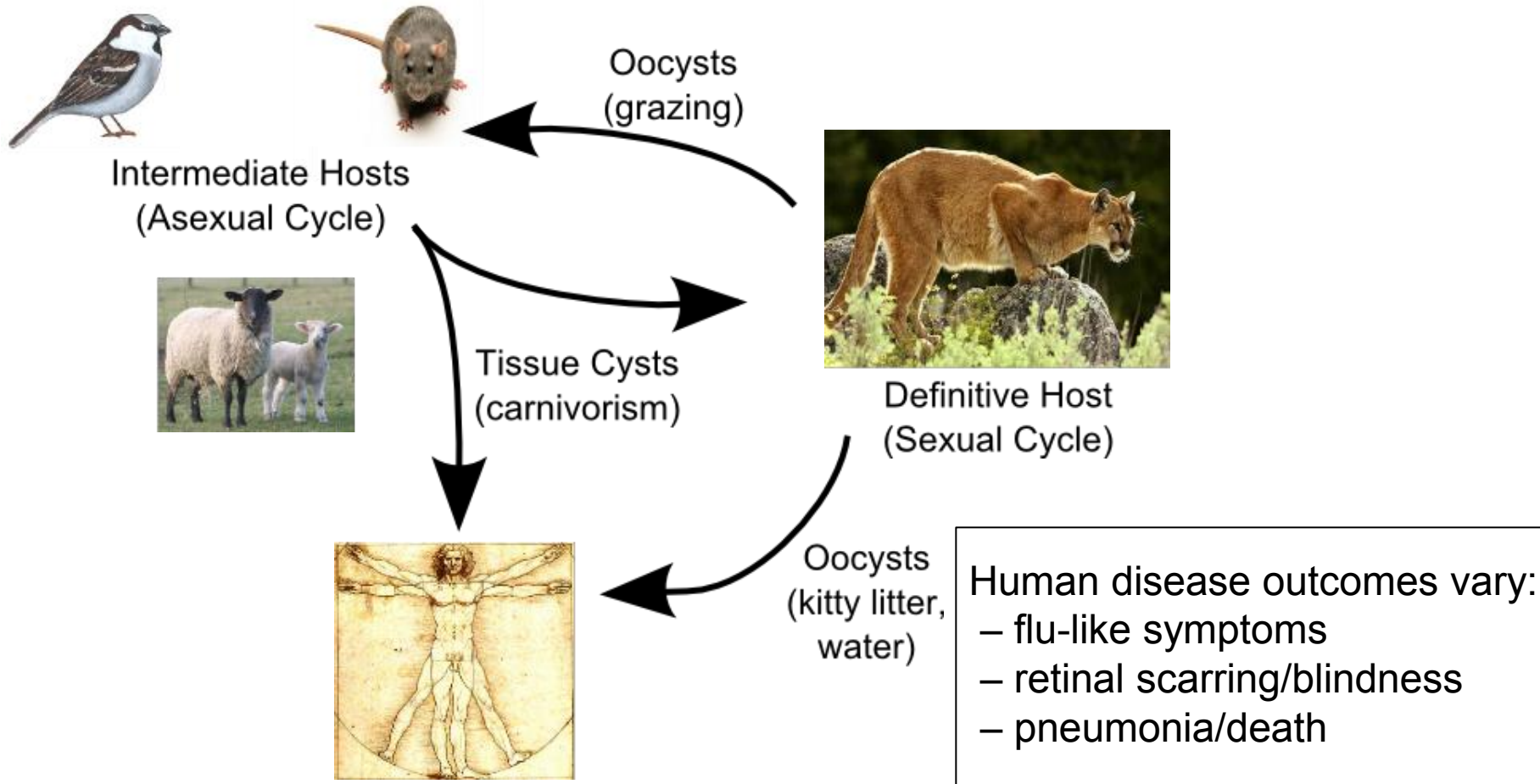
Once species of *Toxoplasma* can infect any warm blood animal



This represents more than 300 million years of divergence in its hosts

Toxoplasma is the most successful parasite in the world!

Toxoplasma infects a broad host range

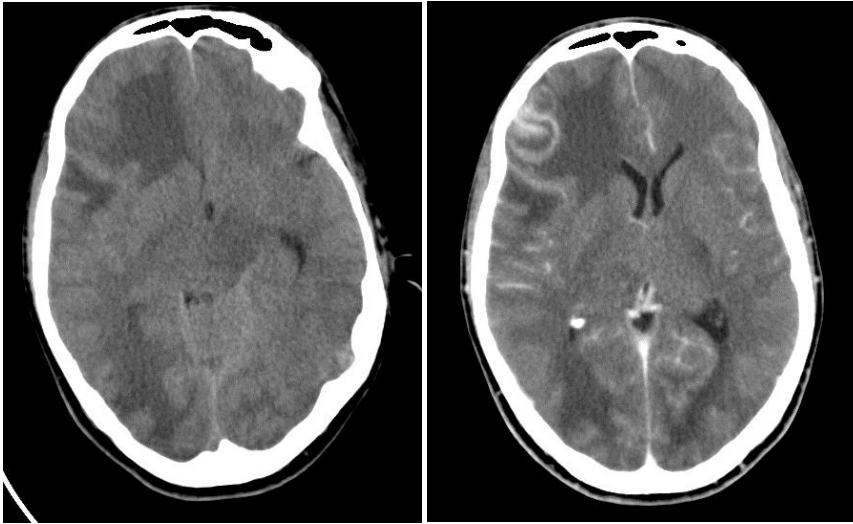


Toxoplasma has evolved the ability to infect any warm-blooded animal

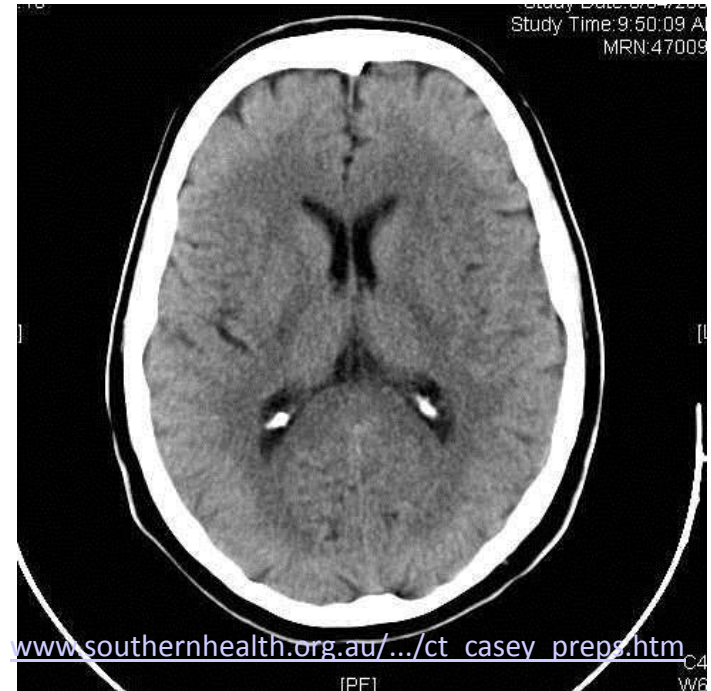
Toxoplasmosis in humans

- Lifelong infection
 - ~30% of humans worldwide are infected (10-15% of Americans)
 - No treatment for latent infection
- Opportunistic / accidental pathogen
 - Developing fetus
 - HIV/AIDS patients
 - Organ transplant patients (e.g. heart, lungs)
 - Bone marrow transplant patients (e.g. non-Hodgkins lymphoma)
- Different strains cause different disease
 - Encephalitis
 - Ocular toxoplasmosis
 - Some “atypical” strains can cause severe disease even in the immunocompetent – Carne, *et al. Emerg. Infect Dis* (2009)

Toxoplasmosis: encephalitis



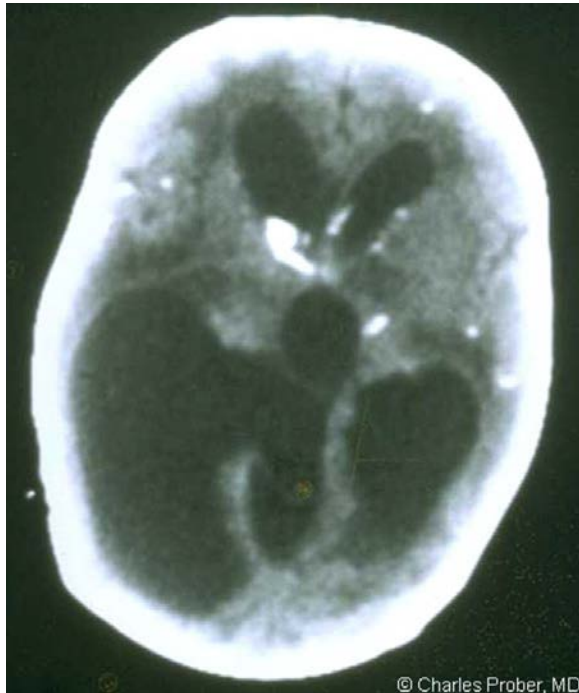
CT from a Toxoplasma-infected AIDS patient
Courtesy of Dr. Anita Koshy, Univ. of Arizona



CT of a normal brain

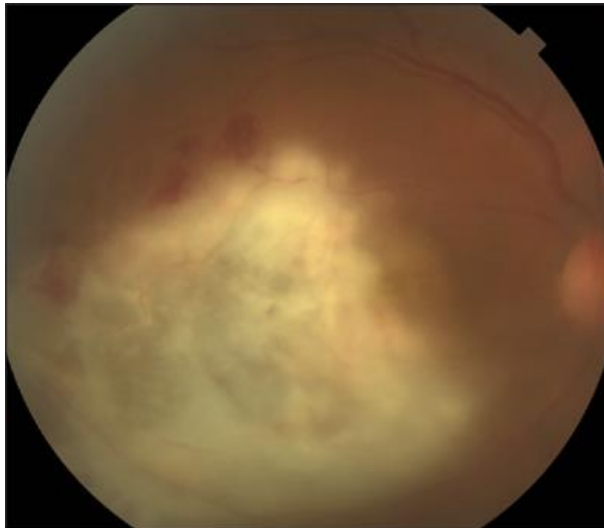
Congenital Toxoplasmosis

The developing fetus does not have a functioning immune system. If newly infected while pregnant, *Toxoplasma* can cross the placenta...

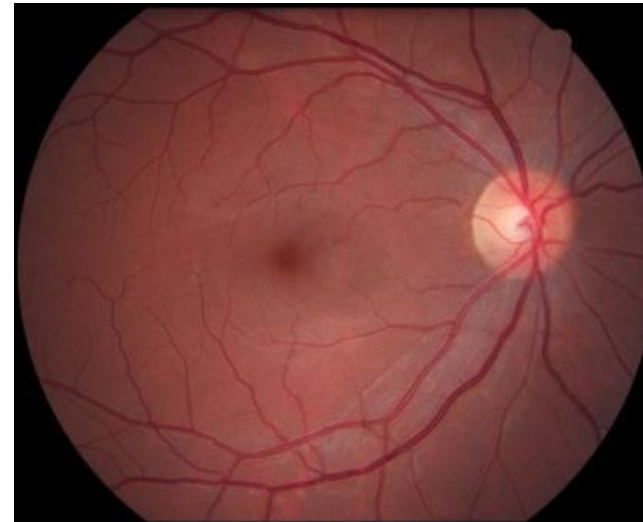


Hydrocephalus in a newborn

Ocular toxoplasmosis



Toxoplasma infection

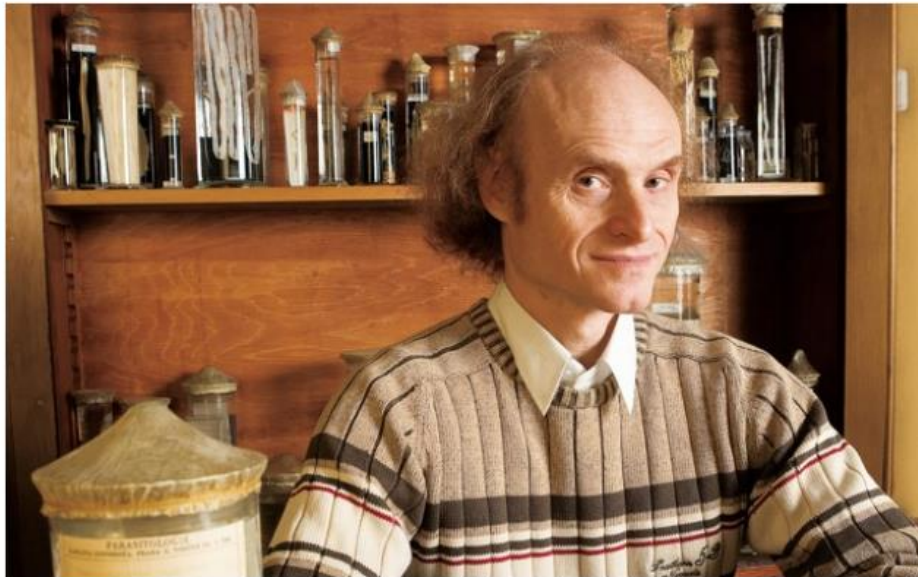


Normal Right Retina

Open to debate: Mind control...

How Your Cat Is Making You Crazy

Jaroslav Flegr is no kook. And yet, for years, he suspected his mind had been taken over by parasites that had invaded his brain. So the prolific biologist took his science-fiction hunch into the lab. What he's now discovering will startle you. Could tiny organisms carried by house cats be creeping into our brains, causing everything from car wrecks to schizophrenia?

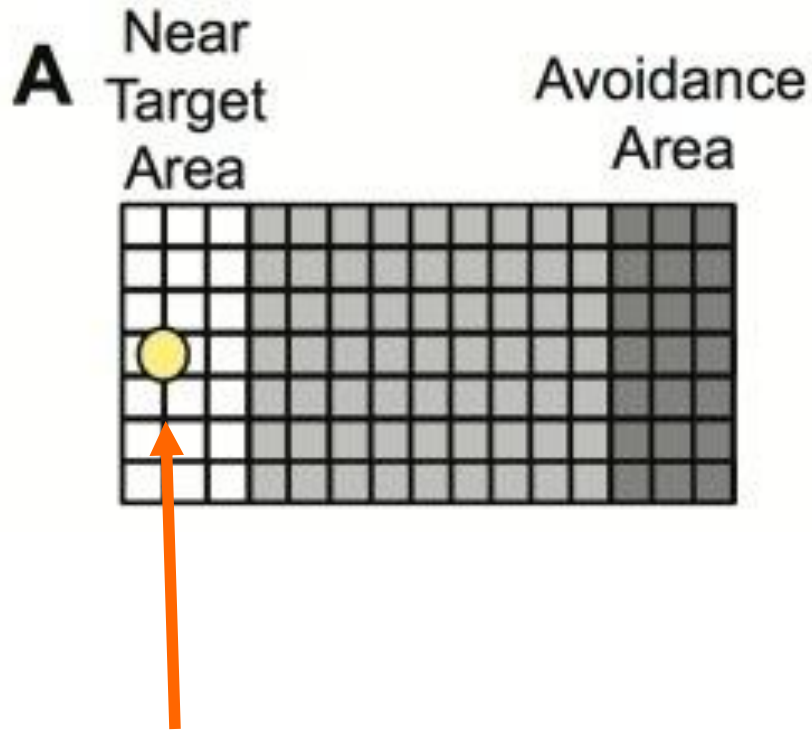


Michal Novotný

<http://www.theatlantic.com/magazine/archive/2012/03/how-your-cat-is-making-you-crazy/308873/>

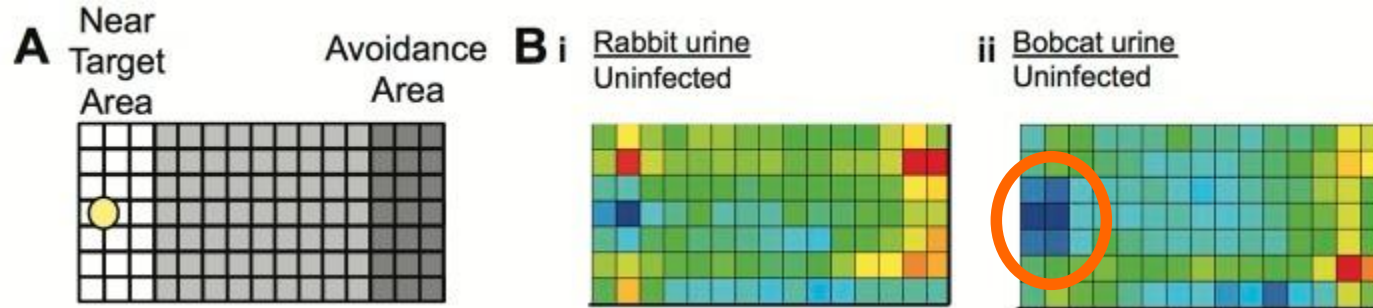
See also: Radiolab episode: <http://www.radiolab.org/story/91689-parasites/>

The dirty truth: *Toxo* alters rodent behavior

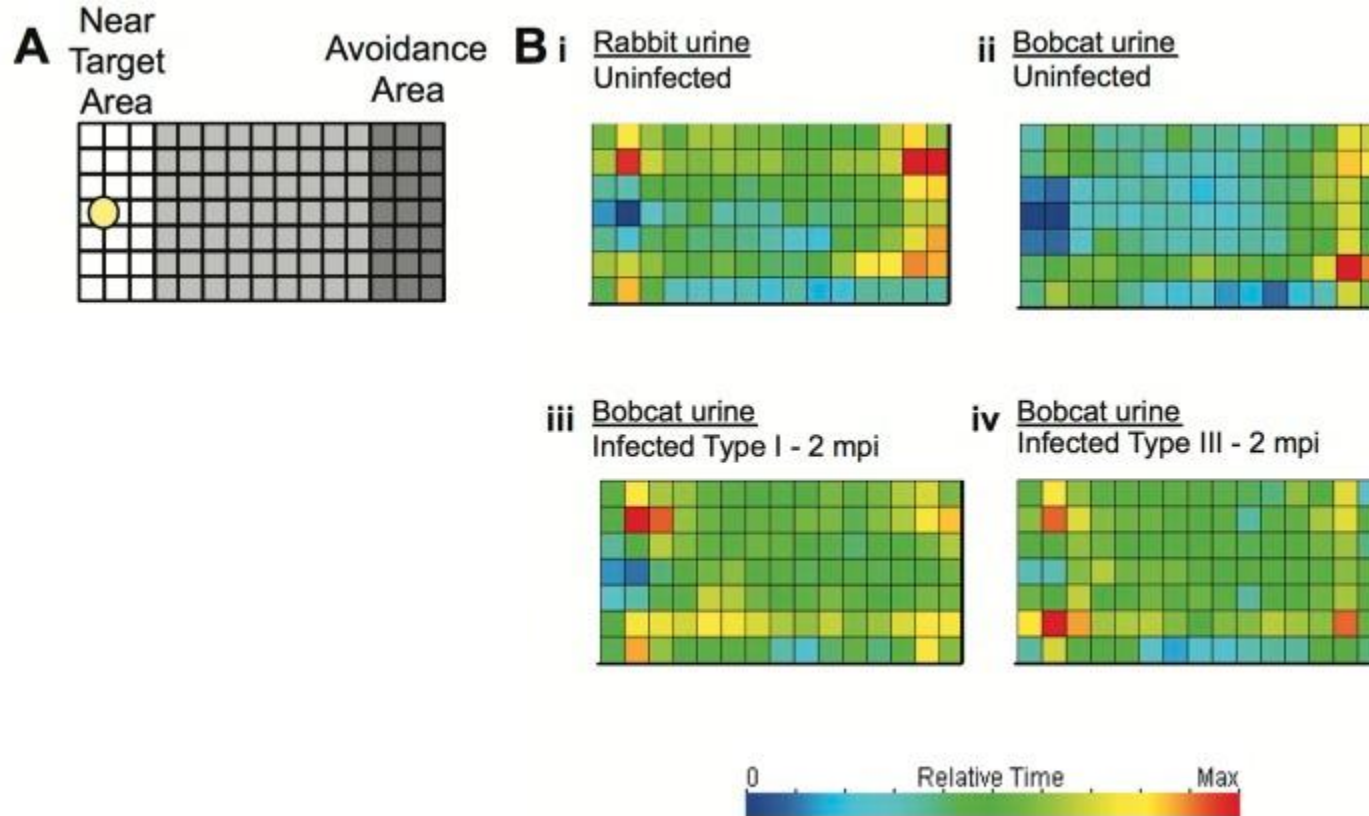


Put urine there (bobcat, rabbit, whathaveyou) and film a mouse or rat to see if it stays away or not...

Odd but true: *Toxo* alters rodent behavior



Odd but true: *Toxo* alters rodent behavior



Behavior is complicated...

It turns out that infected rats/mice are willing to sniff bobcat urine but are still quite afraid of a collar worn by a cat.

What about humans?

Does *Toxoplasma* really cause changes in behavior?

Can it be related to schizophrenia?

I propose to you a control:

In the US, ~10% of people are Toxo-positive

In France & Germany, ~90% of adults are Toxo-positive

Is there a massive difference in schizophrenia in those countries? (No)

To summarize: Behavioral studies are difficult and often suffer from small sample sizes.

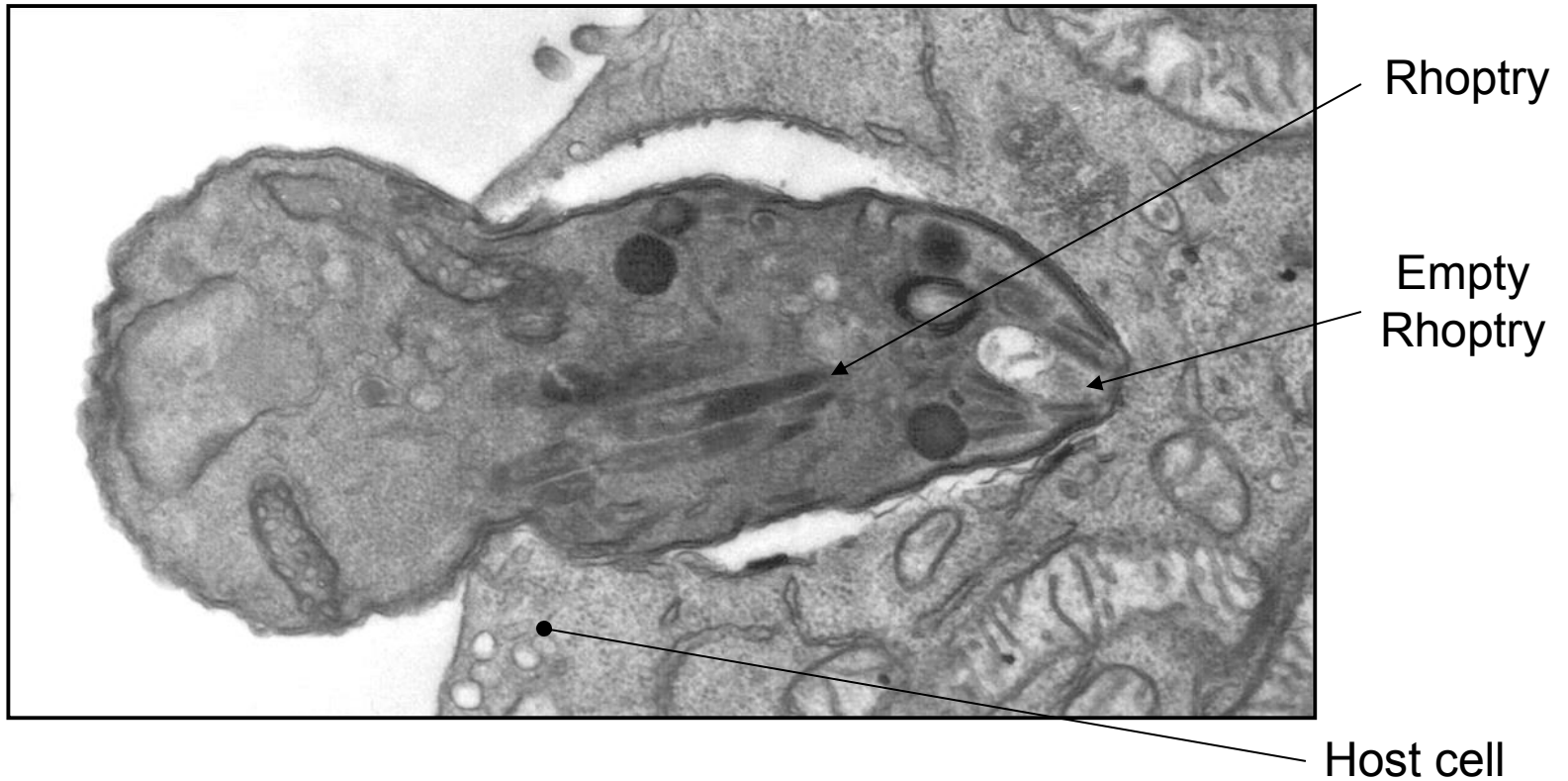
Three major strains of *Toxoplasma*

Type I >> Type II >> Type III

$LD_{100} = 1$ $LD_{50} \sim 1,000$ $LD_{50} \sim 10,000$

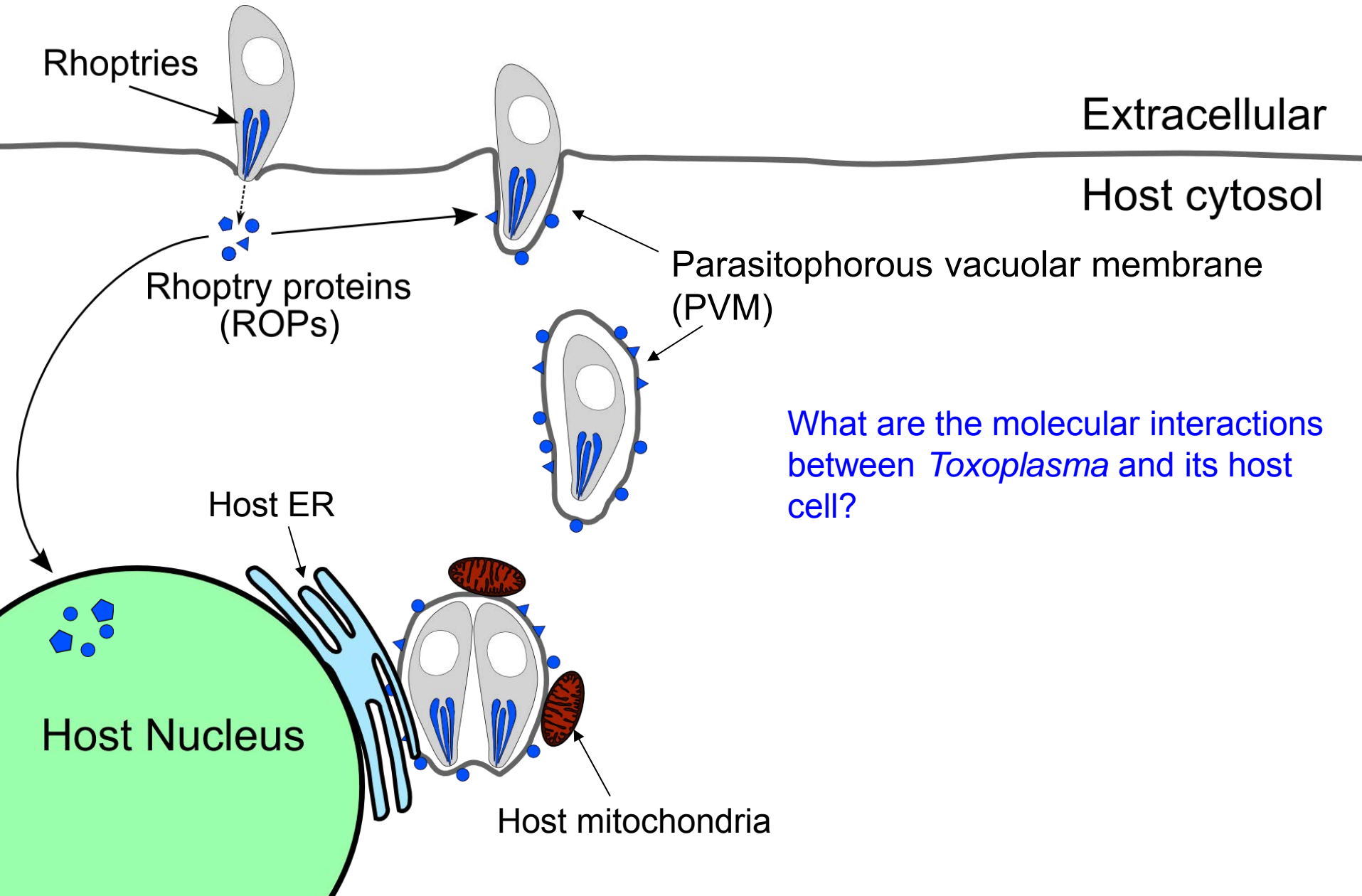
Increasing virulence in mice


Toxoplasma gondii secretes effector proteins upon invasion

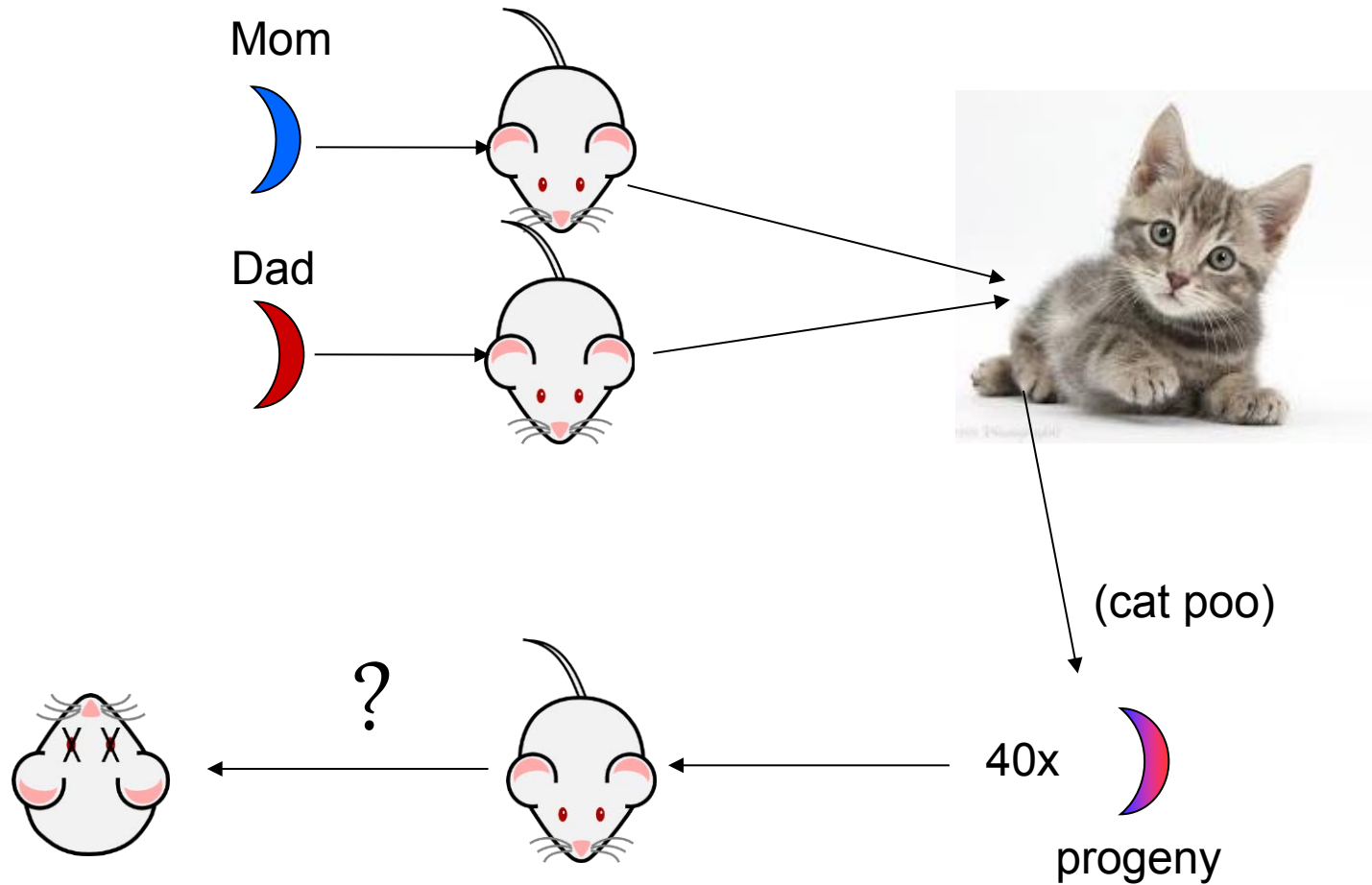


Effectors rewire host signaling: 30% host genes have altered transcription after infection

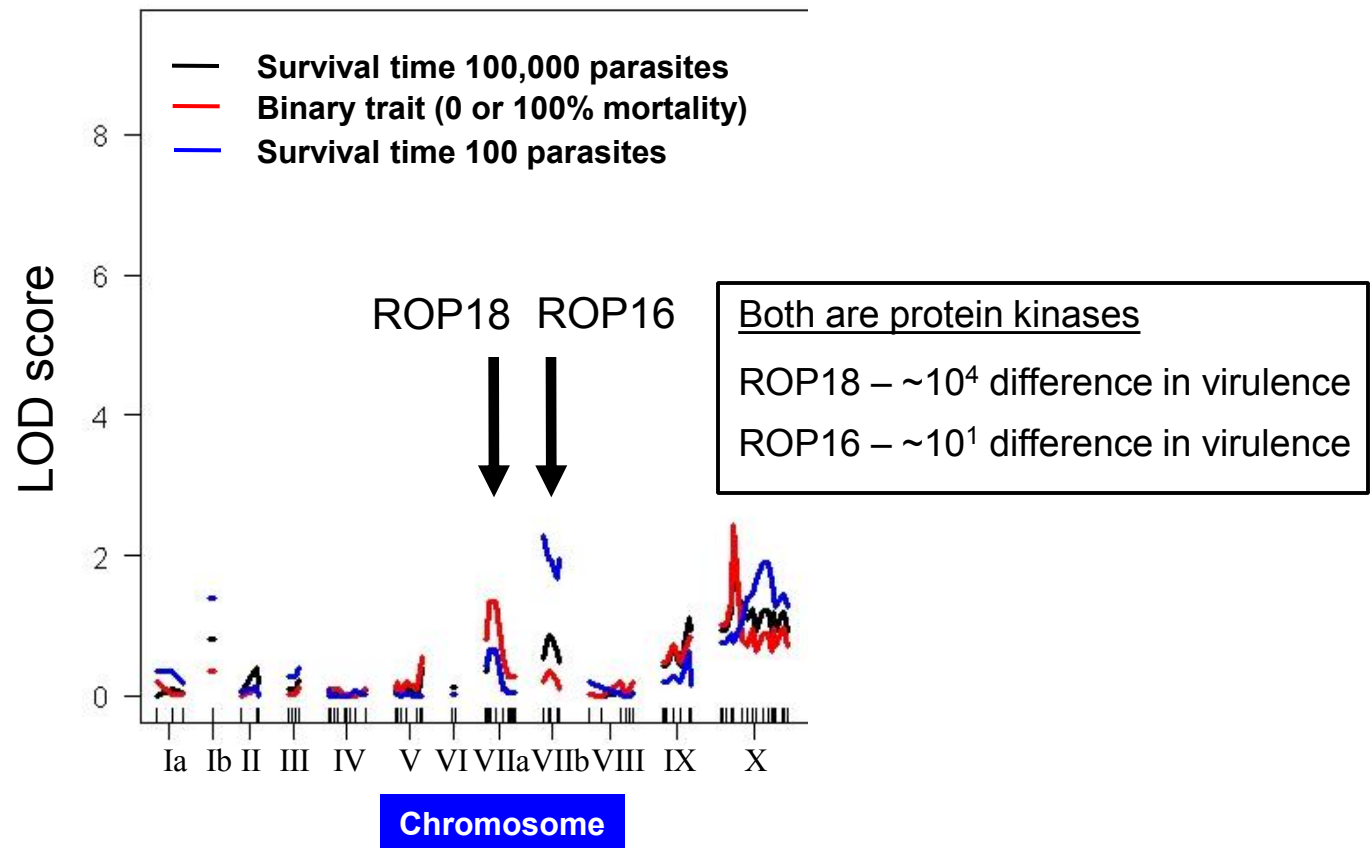
Toxoplasma invasion



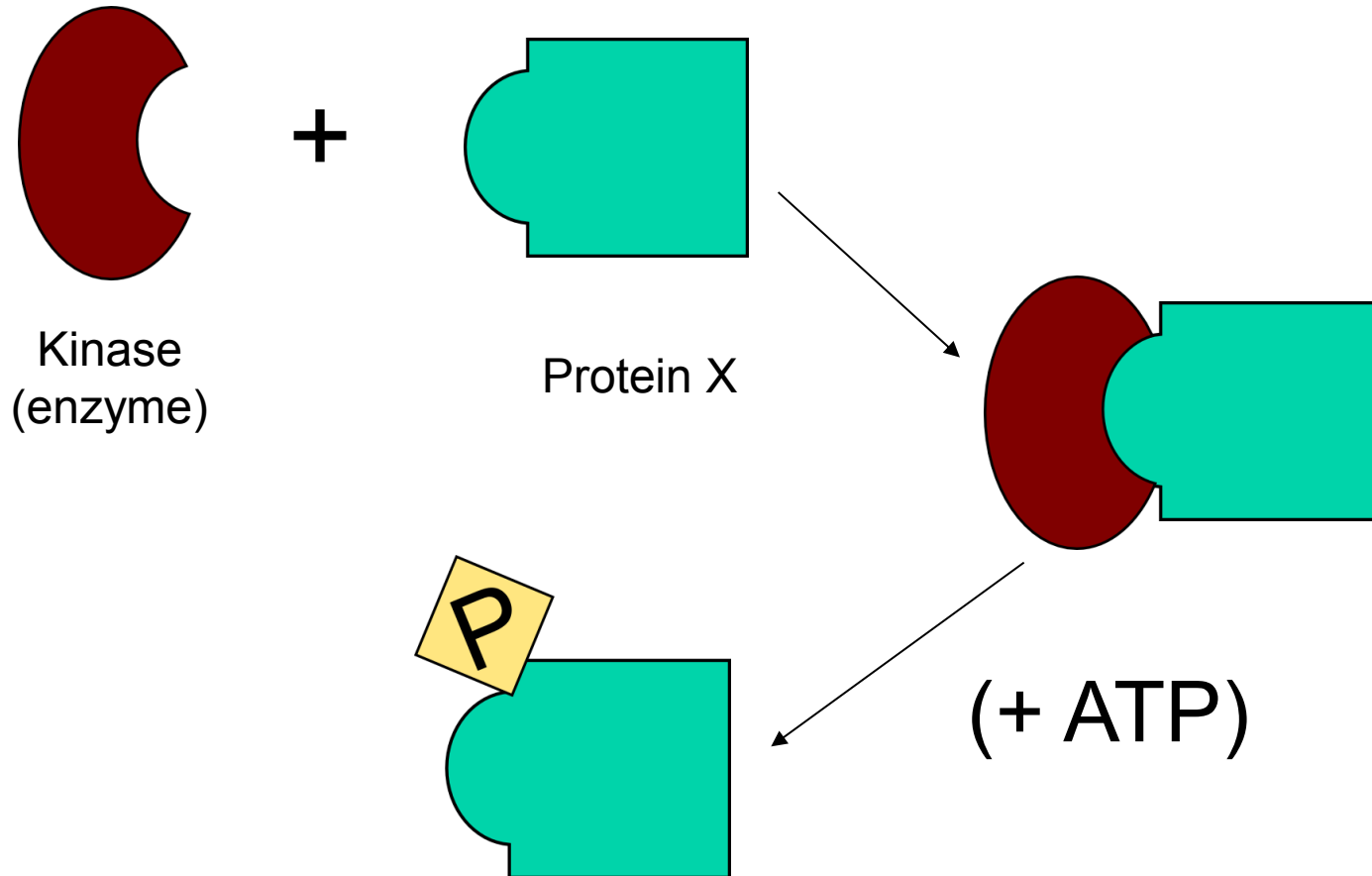
Finding new virulence effectors



Genetic mapping identifies rhoptry proteins as virulence factors

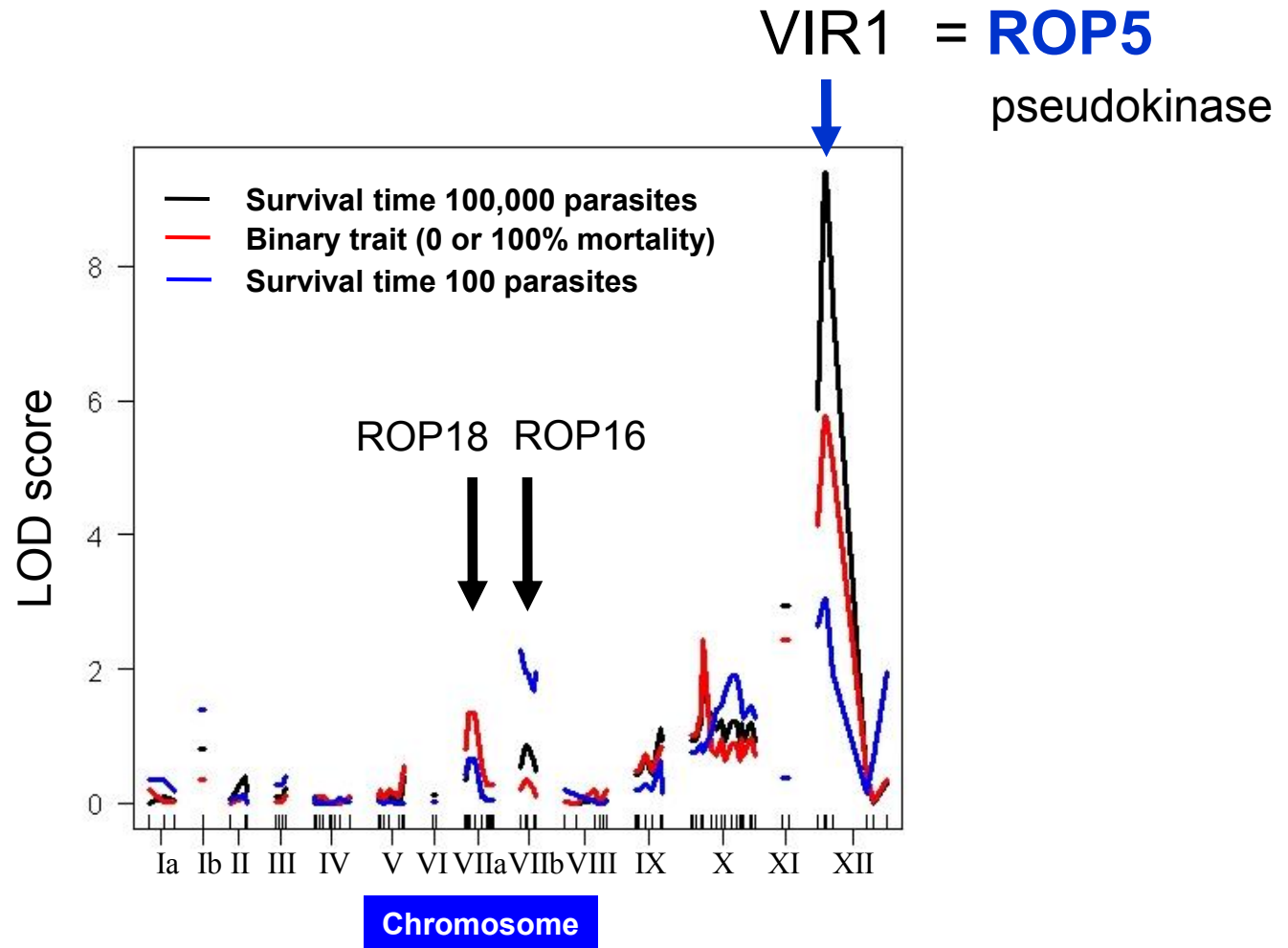


Kinases phosphorylate proteins to transmit signals



Addition of PO_4 : Alters the charge and shape of the target

Genetic mapping identifies rhoptry proteins as virulence factors



A pseudokinase?

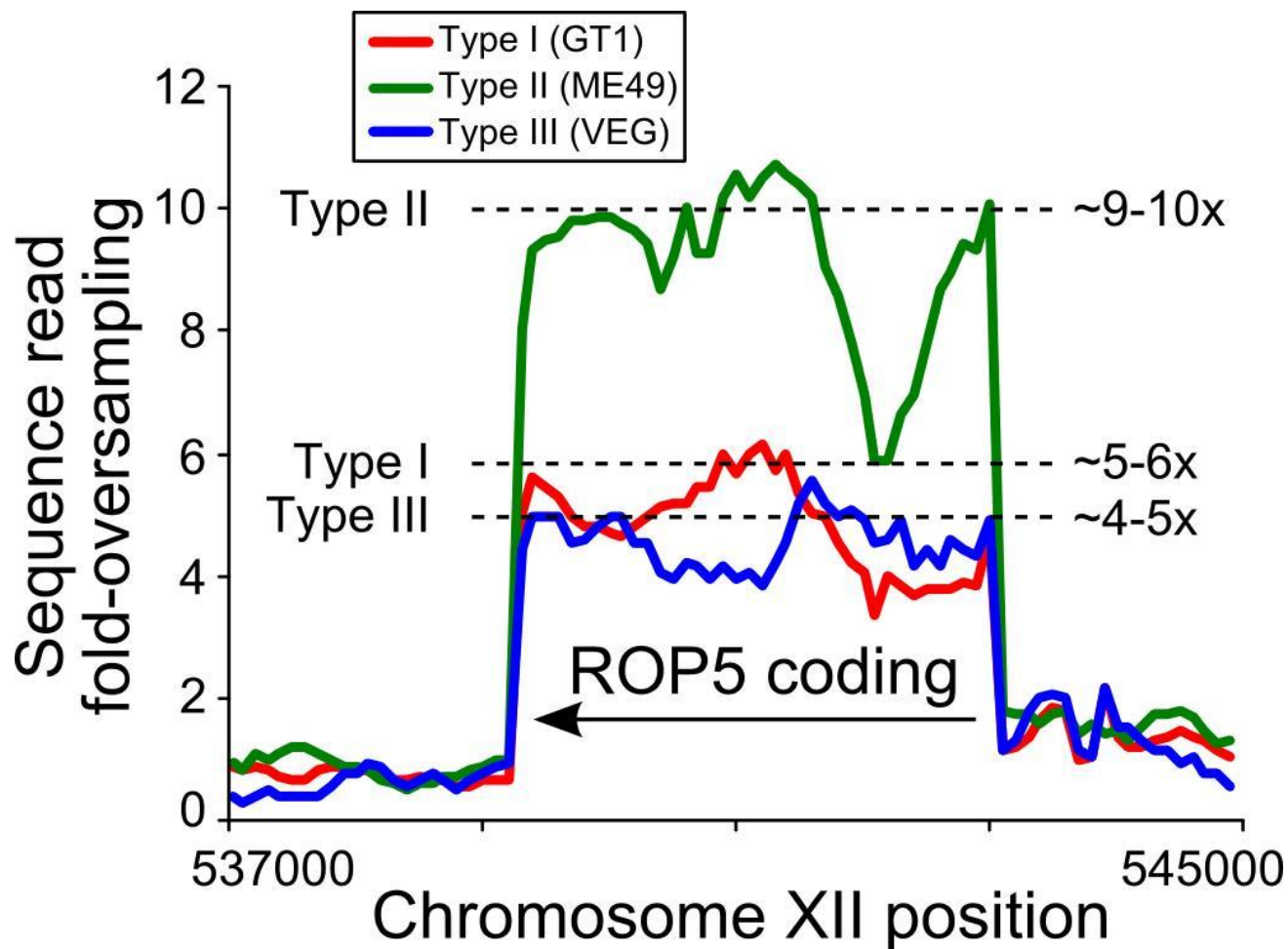


A kinase, machined to do its job

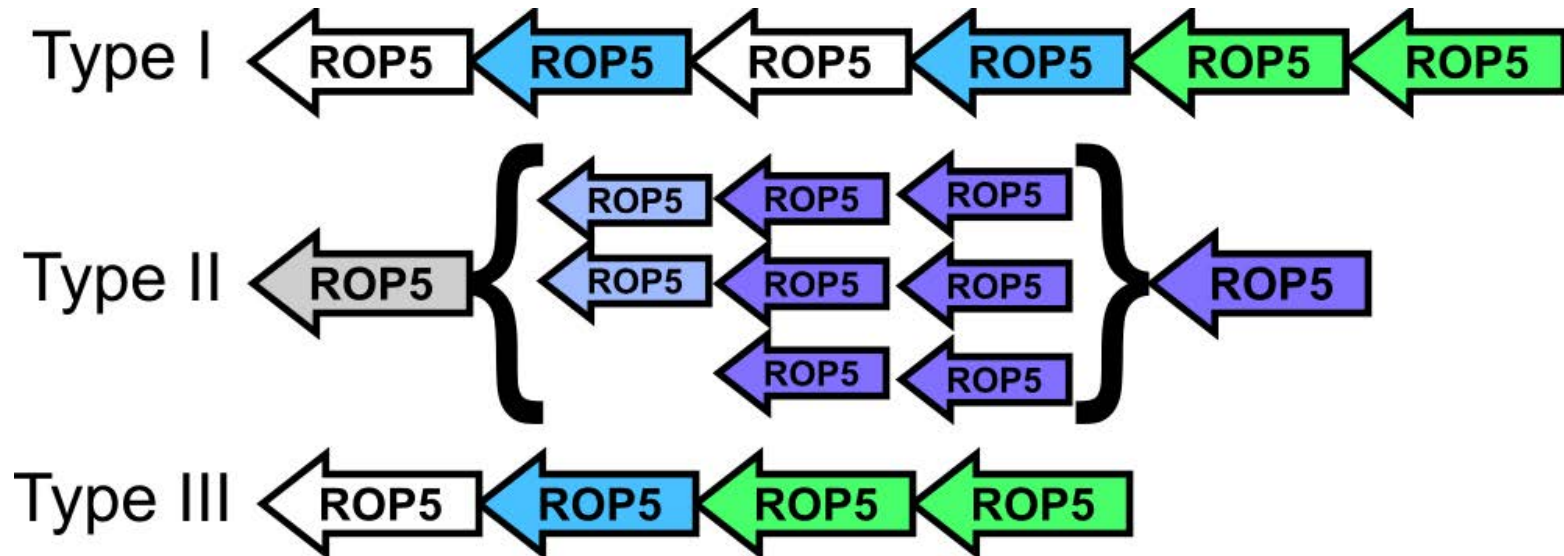


Pseudokinase: Like a truck without an engine

ROP5 locus sequencing reads are oversampled



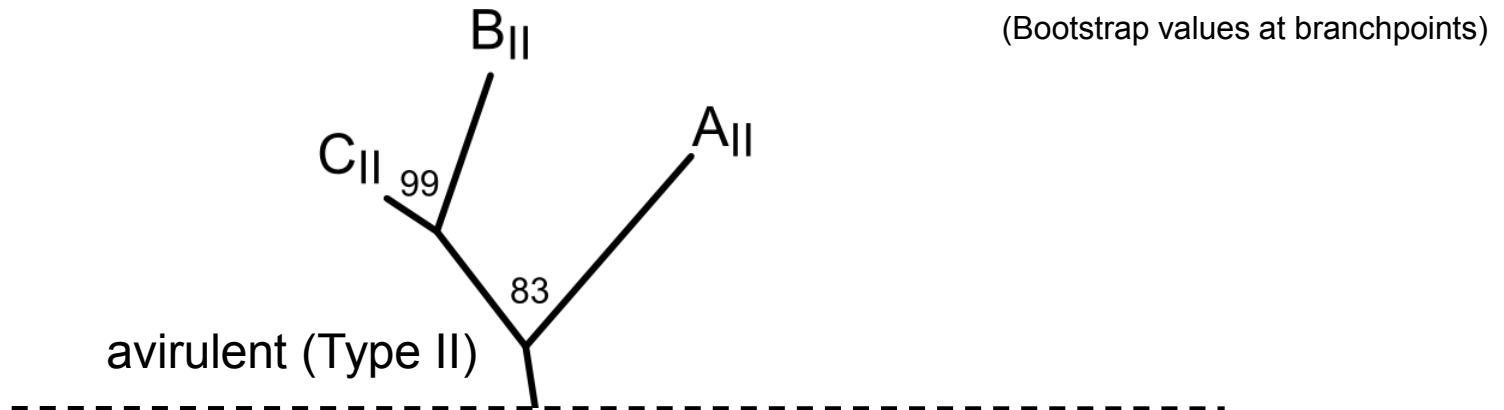
ROP5 locus encodes three divergent isoforms



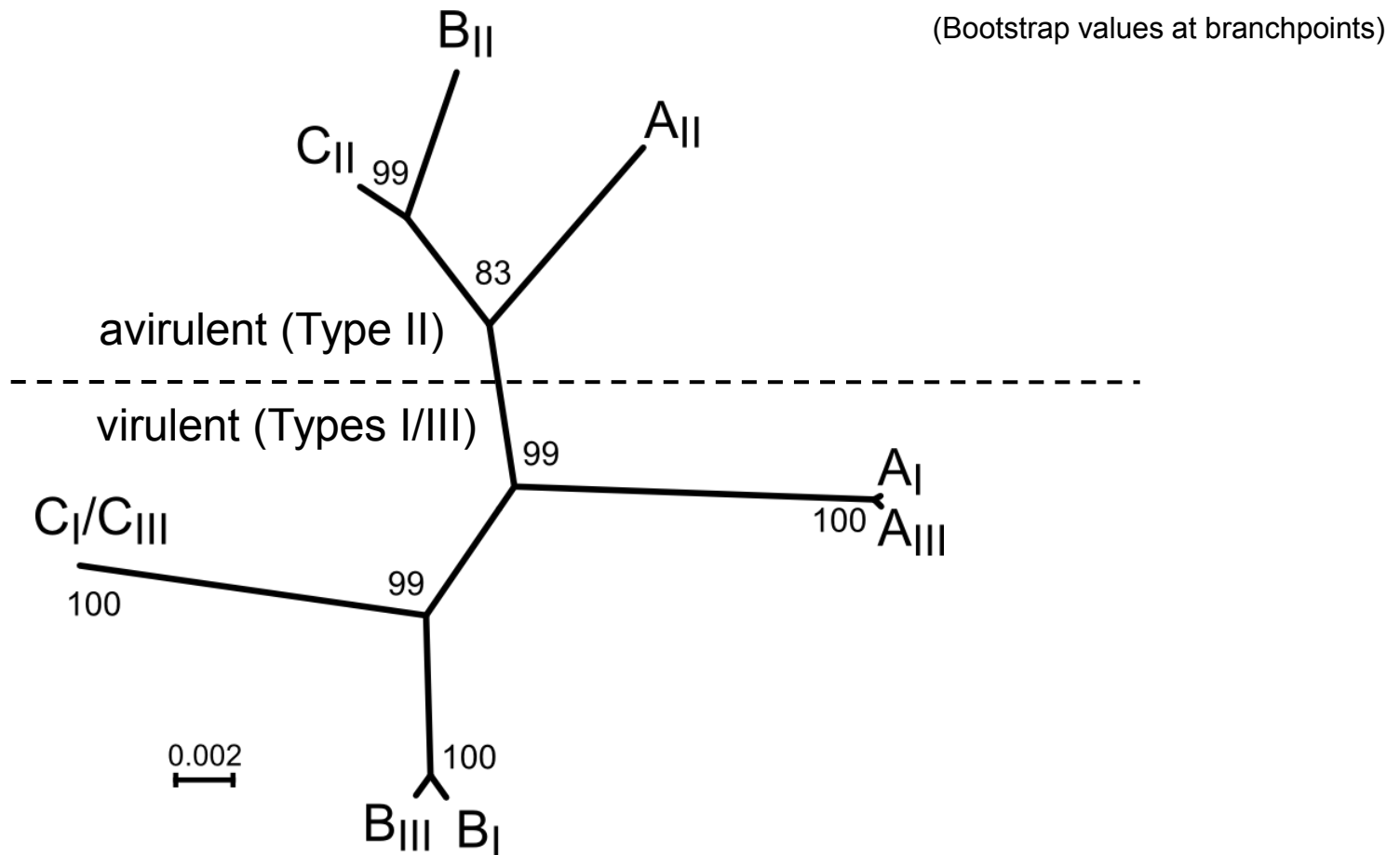
Virulent type I/III loci have same alleles, but different copy number (Isoforms are color-coded according to allele)

20-30 amino-acid polymorphisms between individual isoforms
– **Map to two surfaces on the pseudokinase structure**

ROP5 isoforms are quite divergent

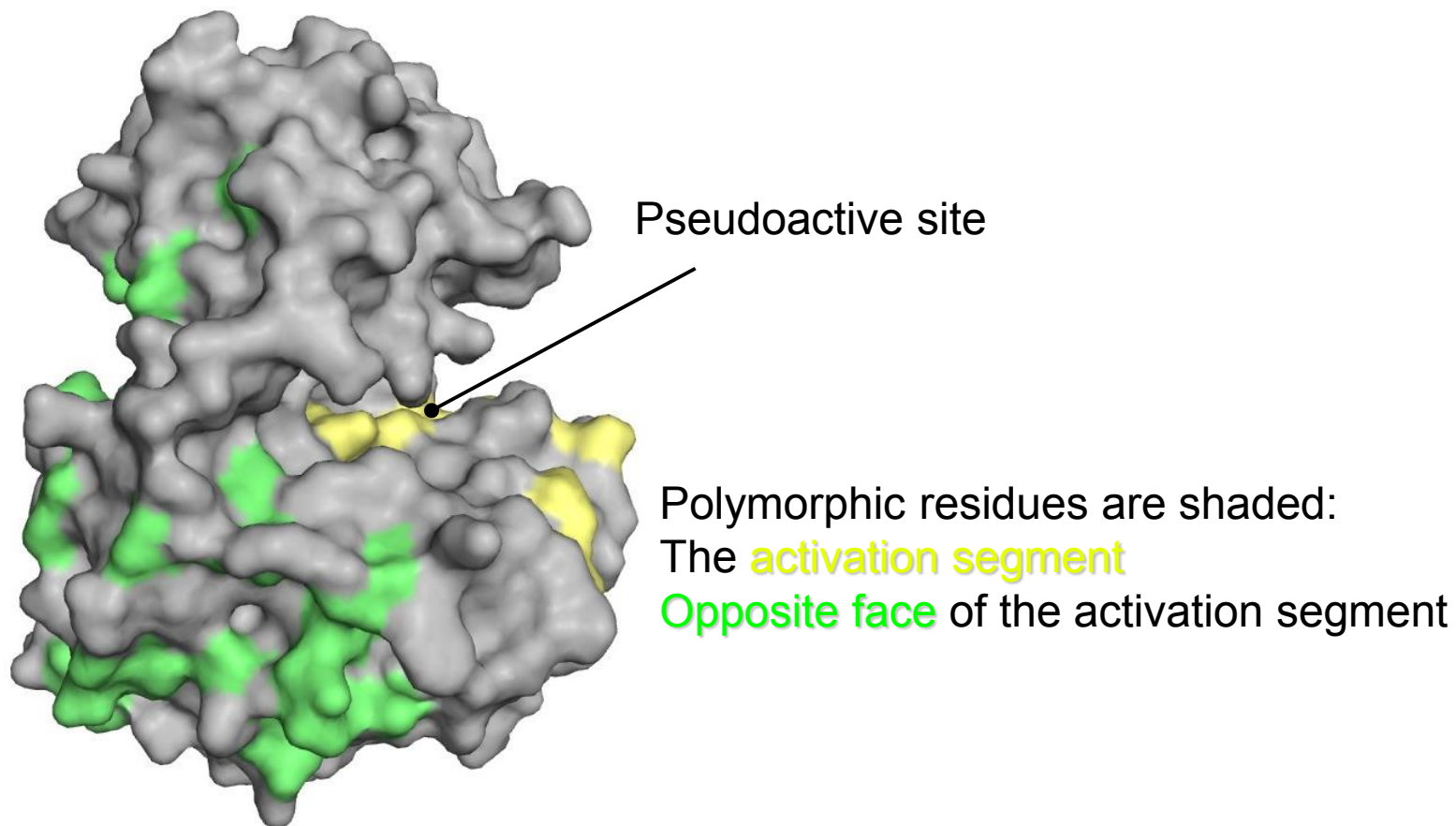


ROP5 isoforms are quite divergent

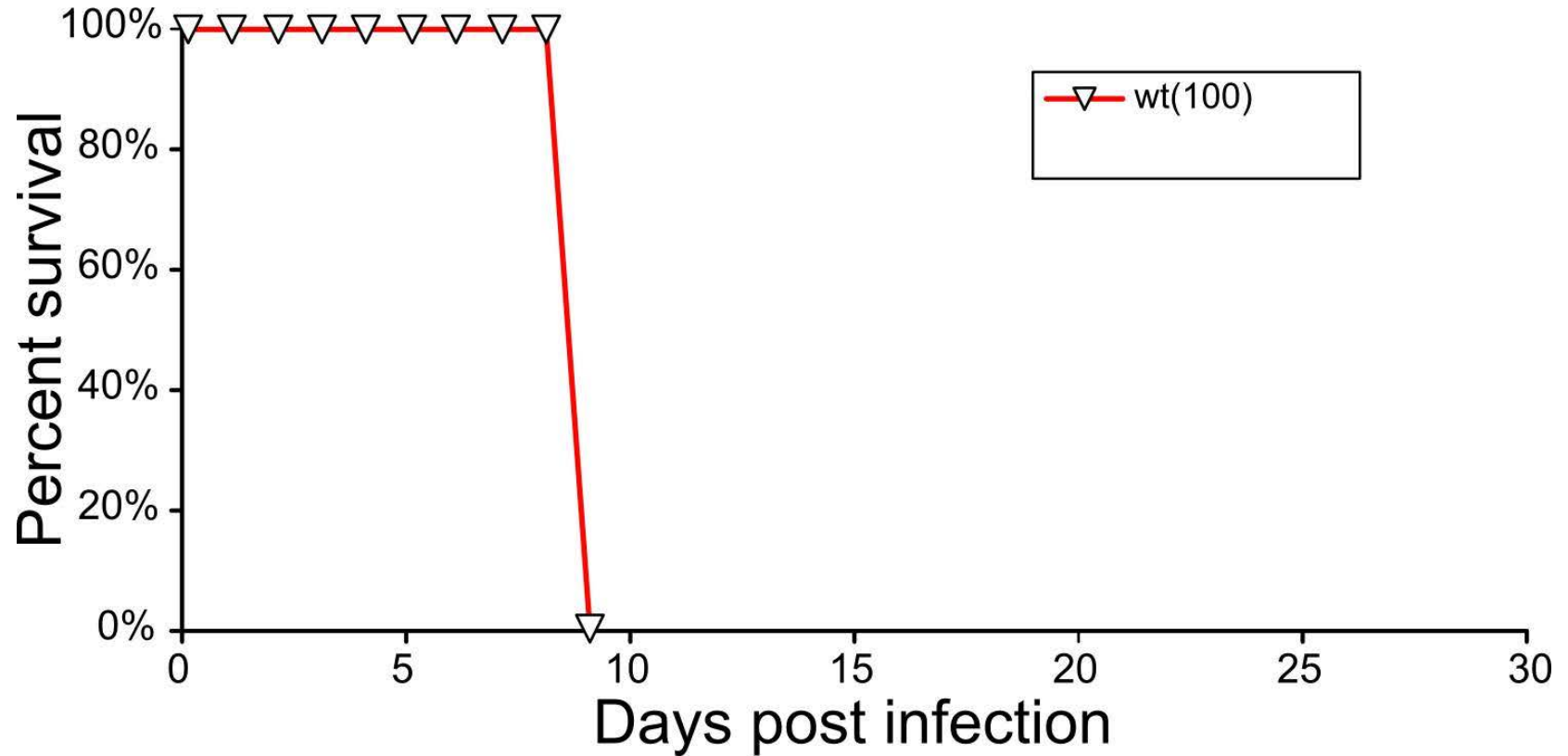


Isoforms from virulent loci are more divergent than avirulent

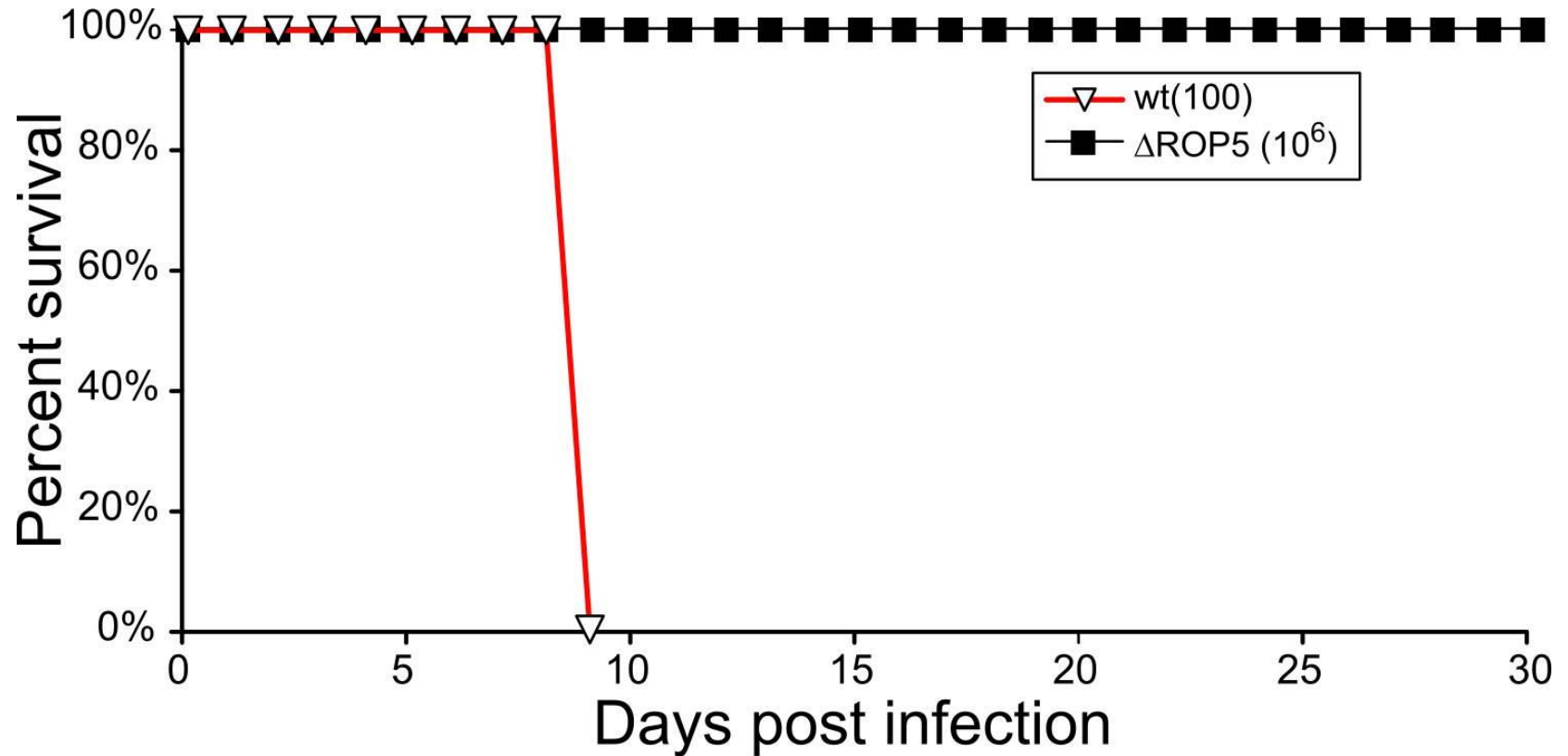
ROP5 polymorphisms form a surface on structure



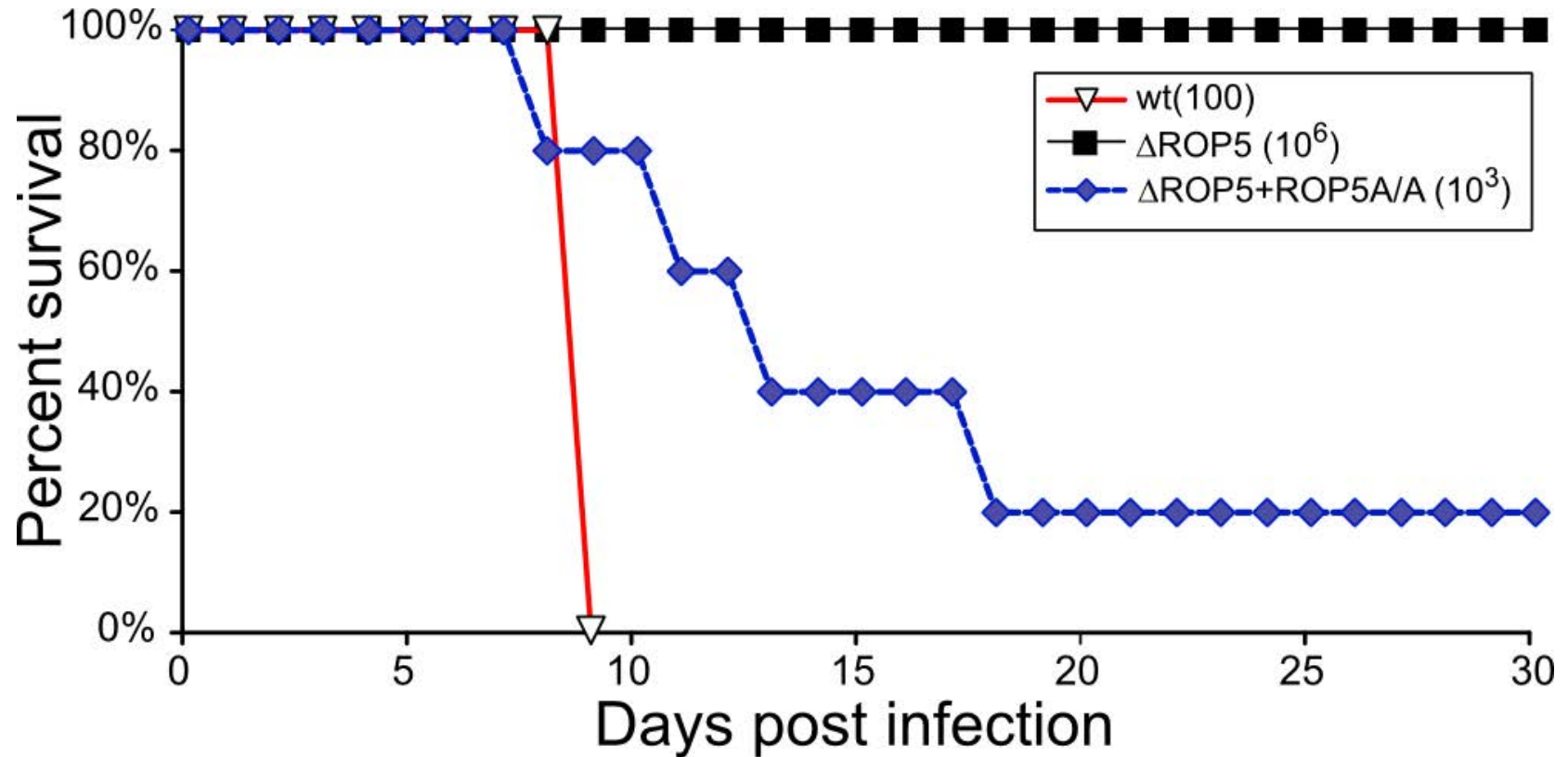
Δ ROP5 parasites do not kill mice



Δ ROP5 parasites do not kill mice

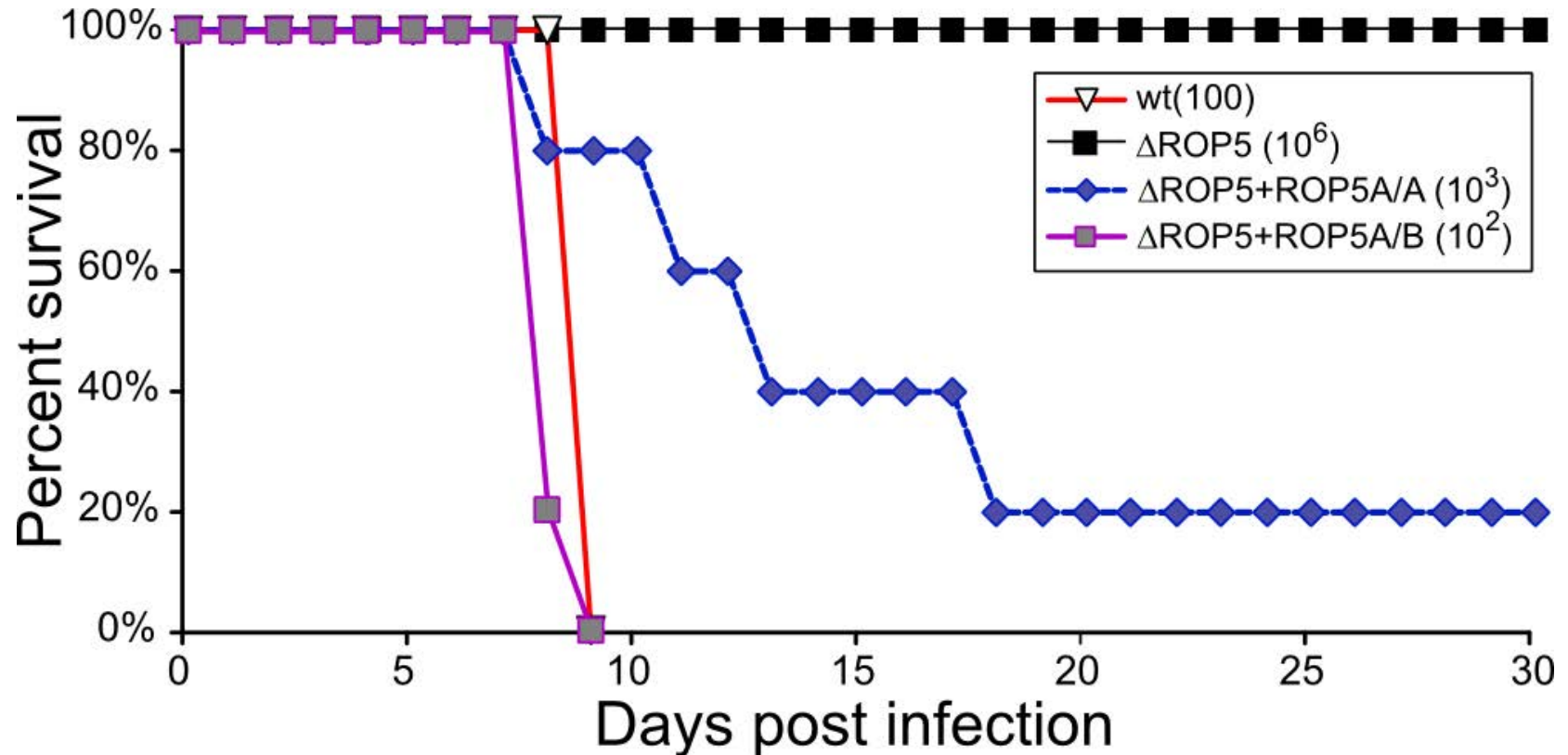


ROP5 isoforms have different potencies



ROP5A/A = 2 copies of the ROP5A_{III} isoform

ROP5 isoforms have different potencies



ROP5A/A = 2 copies of the ROP5A_{III} isoform

ROP5A/B = 1 copy each ROP5A_{III} and ROP5B_{III} isoforms

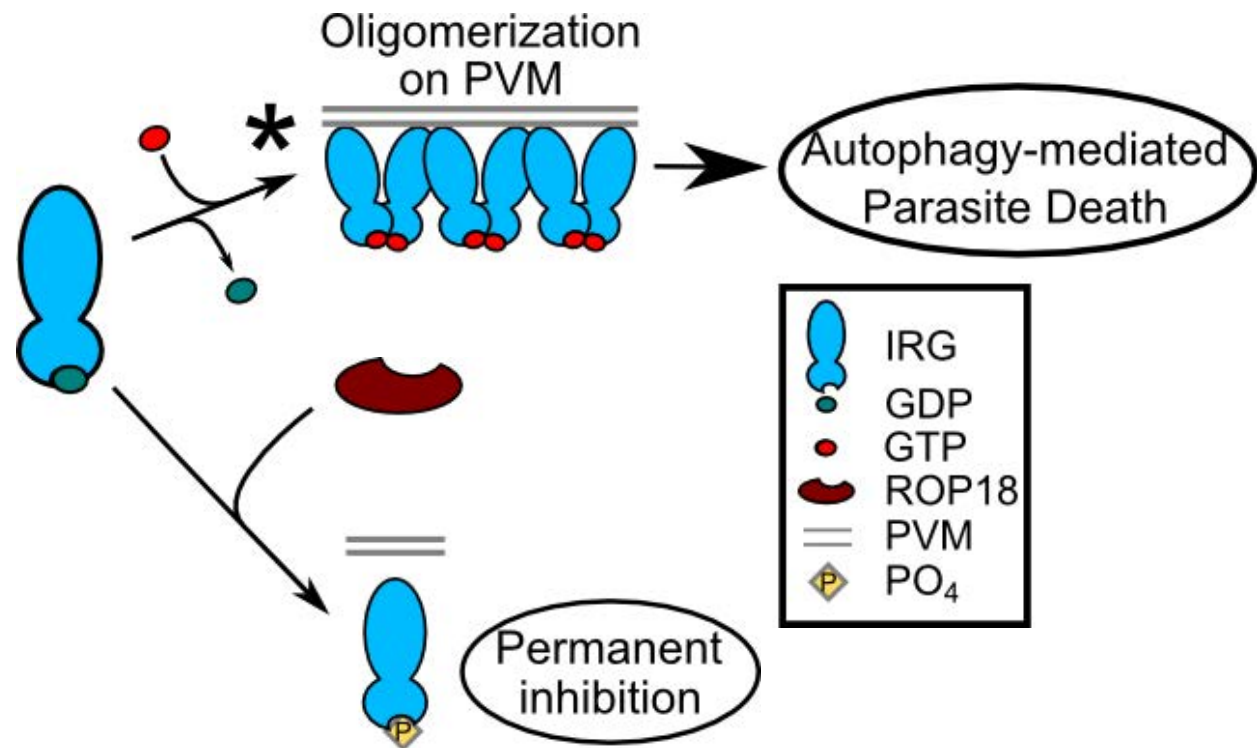
Implications of family expansion

Given that *Toxoplasma* has:

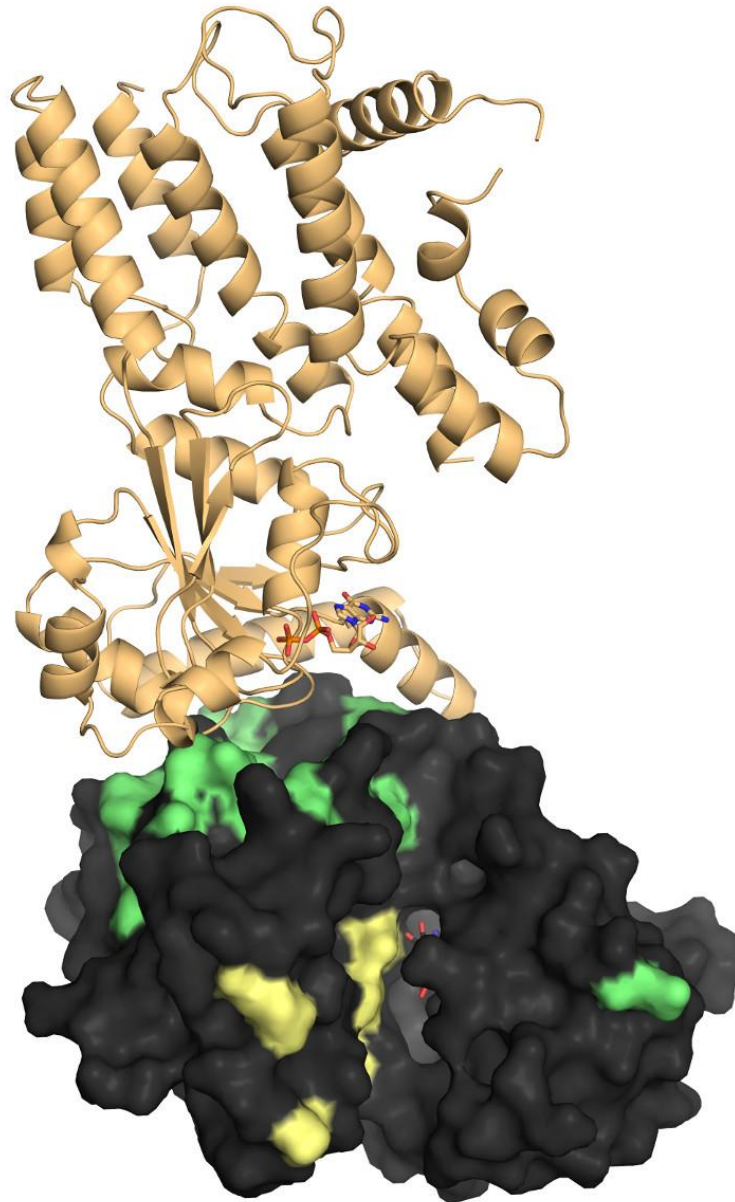
- The ability to forgo its sexual cycle
 - A low recombination frequency (100 kbp/cM)
 - A relatively “clonal lifestyle”
-
- Expansion of ROP5 may facilitate divergence of individual isoforms
 - ROP5 may represent a “swiss-army knife” method of adaptation
 - Adaptation to a greater host range?

Immunity related GTPases: the IRGs

- IFN γ -induced GTPases are >20% of protein abundance whose expression is induced by IFN γ
- Three families: **47kDa (“IRG”)**, 65kDa (“GBP”), ~280kDa (“very large”)
- Critical to control of intracellular pathogens (e.g., *Listeria*, *Toxoplasma*)

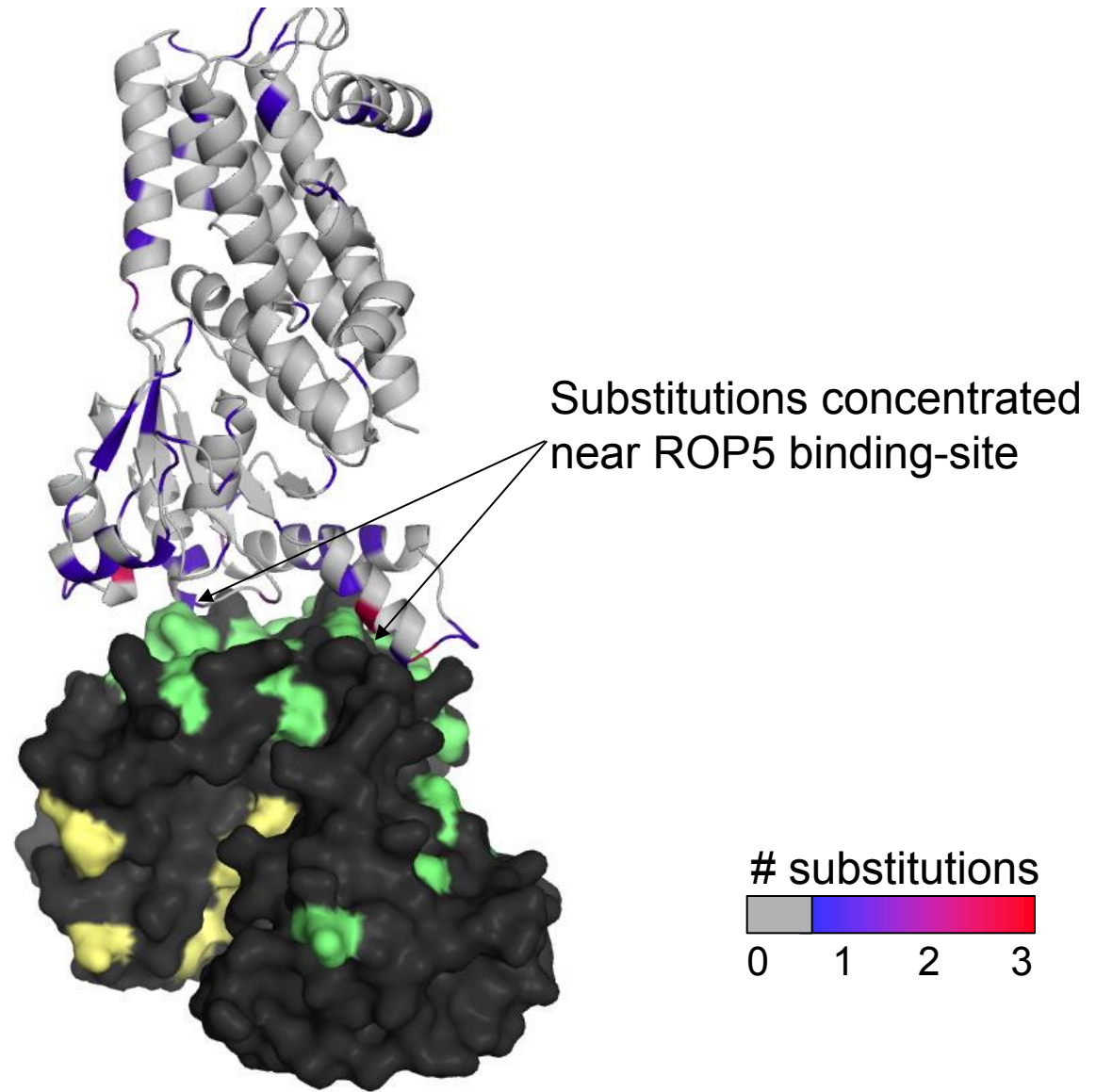


ROP5 uses polymorphic surface to recognize IRG

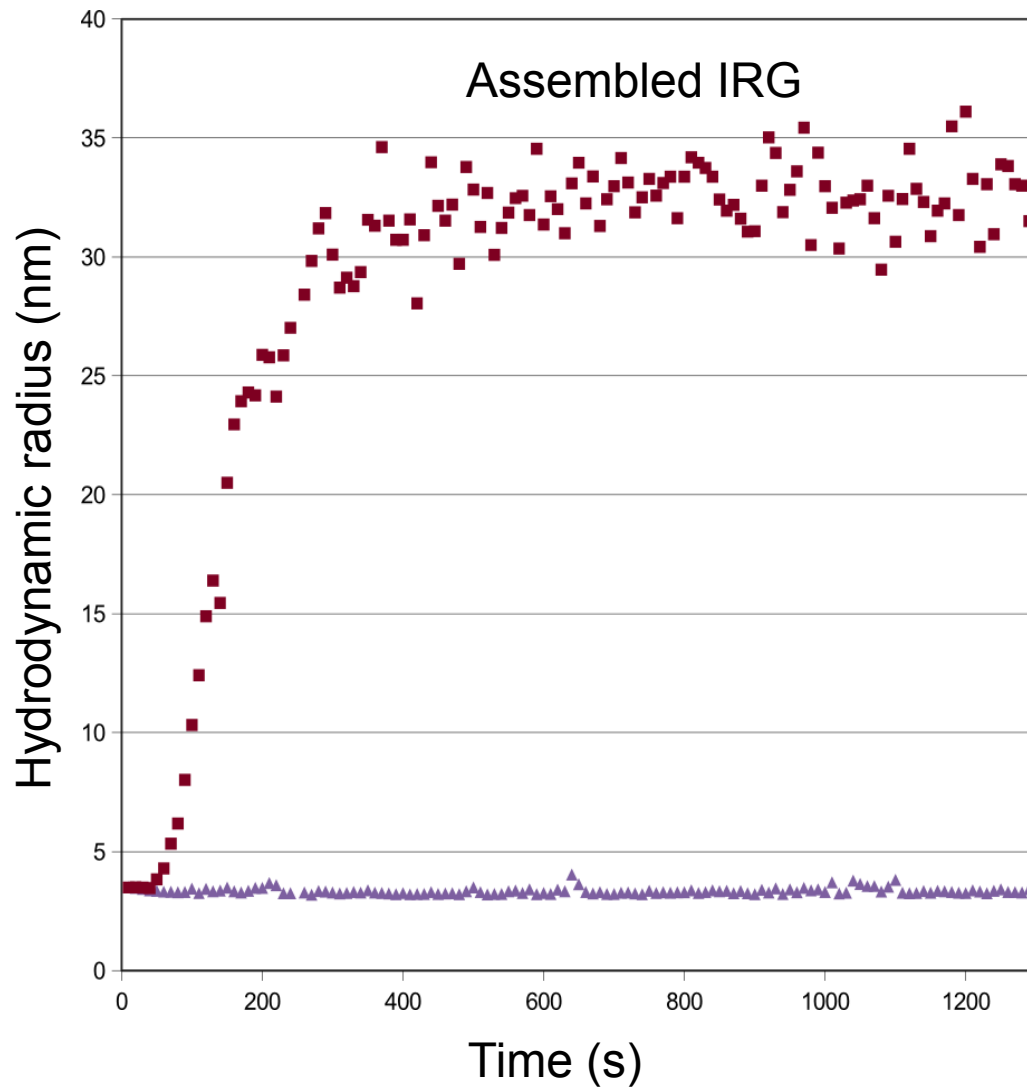


ROP5 – Polymorphic residues are green and yellow (active site)

IRGs are polymorphic at ROP5 binding site

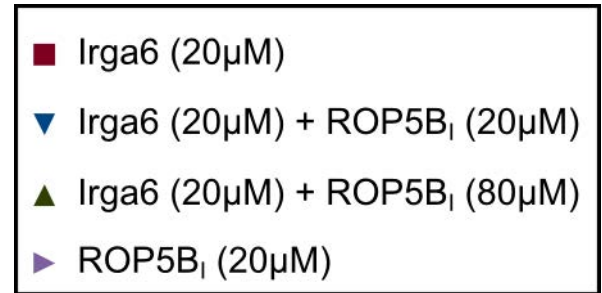
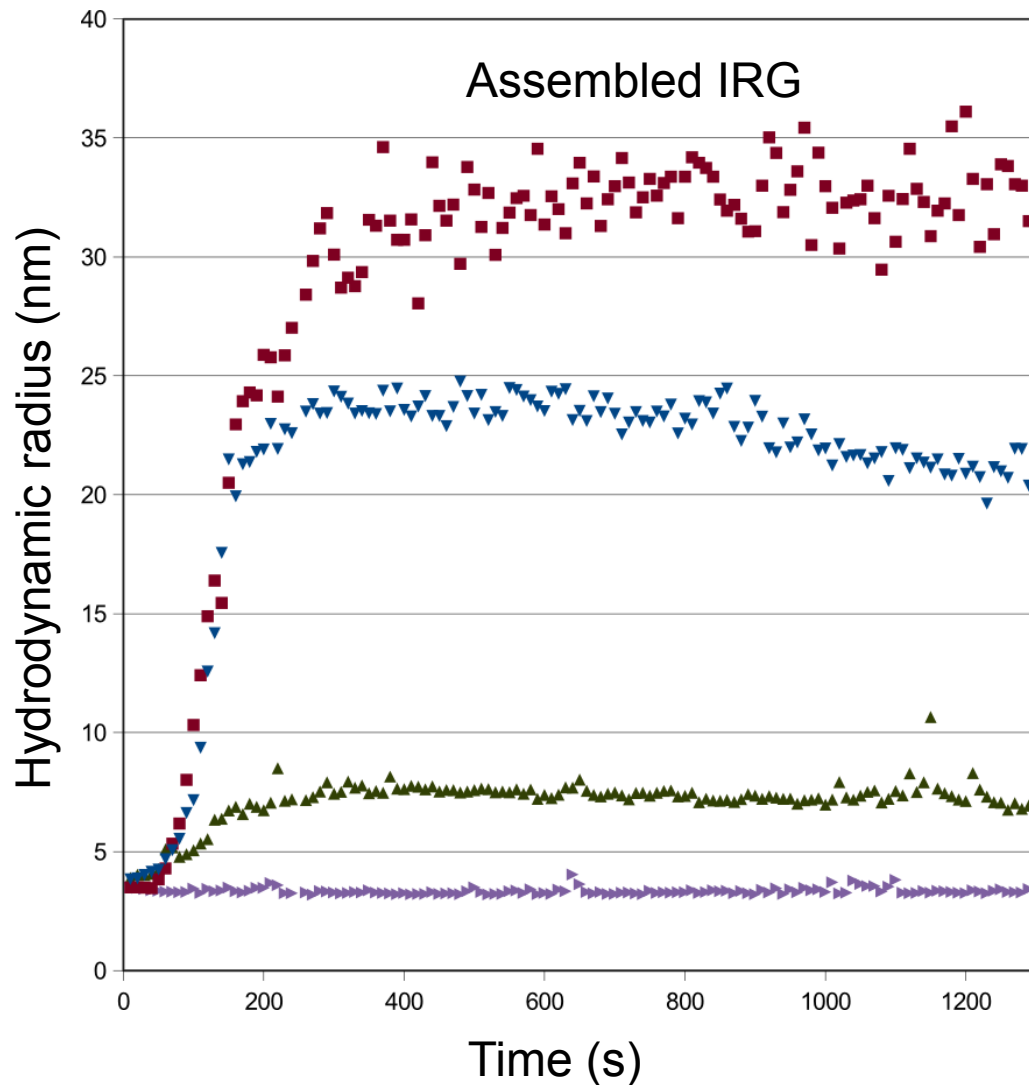


ROP5 inhibits IRG assembly *in vitro*



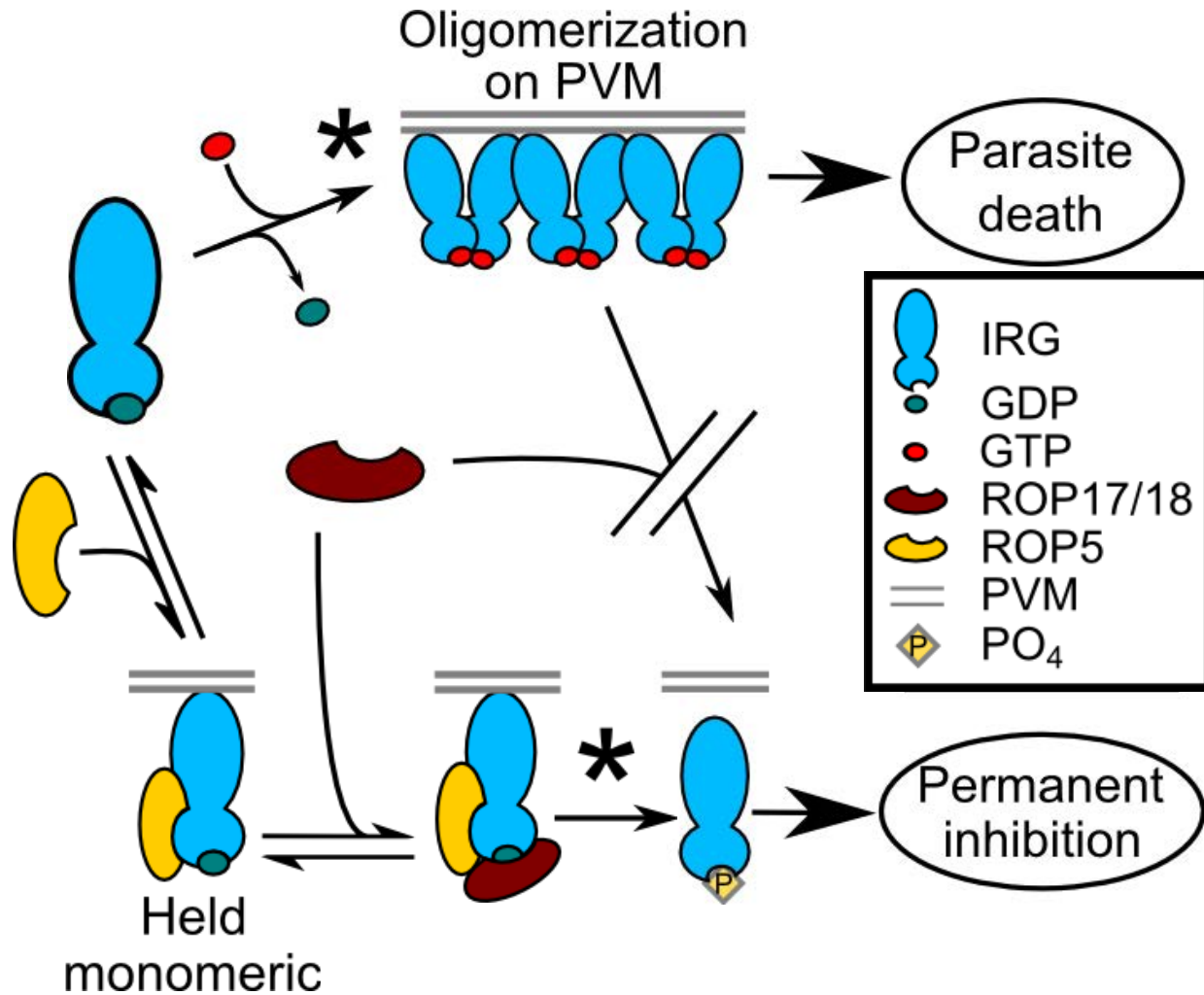
Dynamic Light Scattering

ROP5 inhibits IRG assembly *in vitro*



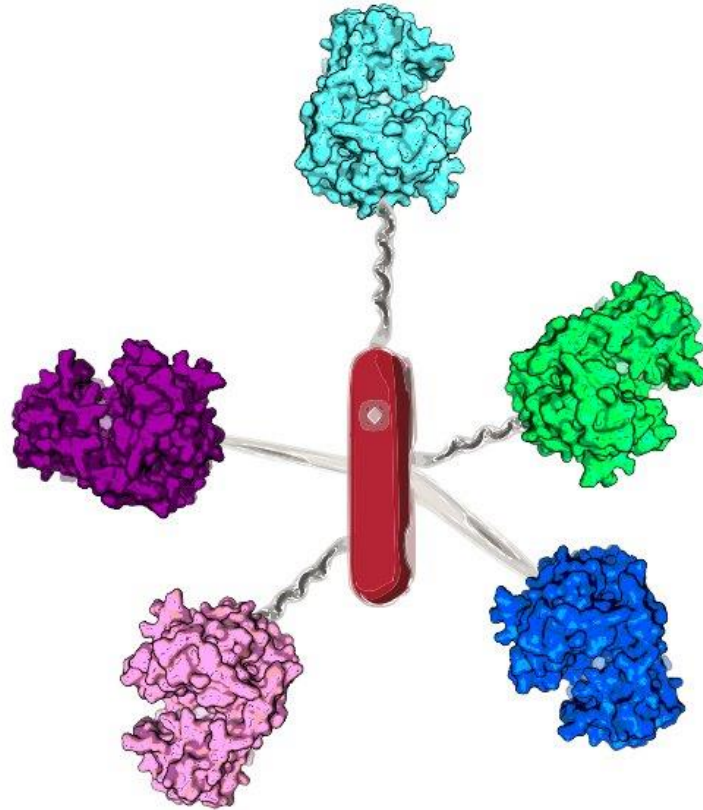
Dynamic Light Scattering

A model for ROP inhibition of the IRG family



* Non-equilibrium steps

Toxo's motto: Be prepared.



Different ROP5's for different hosts?

Different functions altogether?

Thanks



UT SOUTHWESTERN

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