

Front Row Lecture

Accelerating Evolution to Create New Medicines

Christian S. Diercks

October 14th, 2026

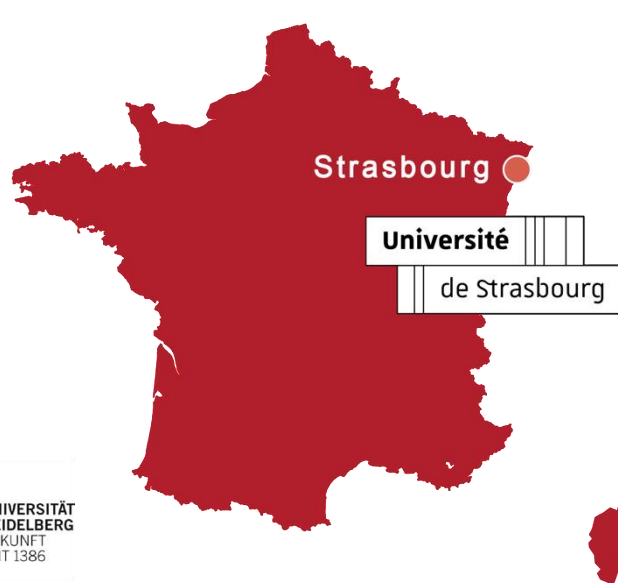


How I ended up here...

Germany



France



Illinois



California



Outline

- 1. The Speed of Natural Evolution**
- 2. Accelerated Evolution in Nature and in the Lab**
- 3. Accelerated Evolution of Enzyme Therapeutics for Refractory Gout**
- 4. Evolution of Enzyme Therapeutics as a Replacement for Opioids in the Treatment of Pain**

Outline

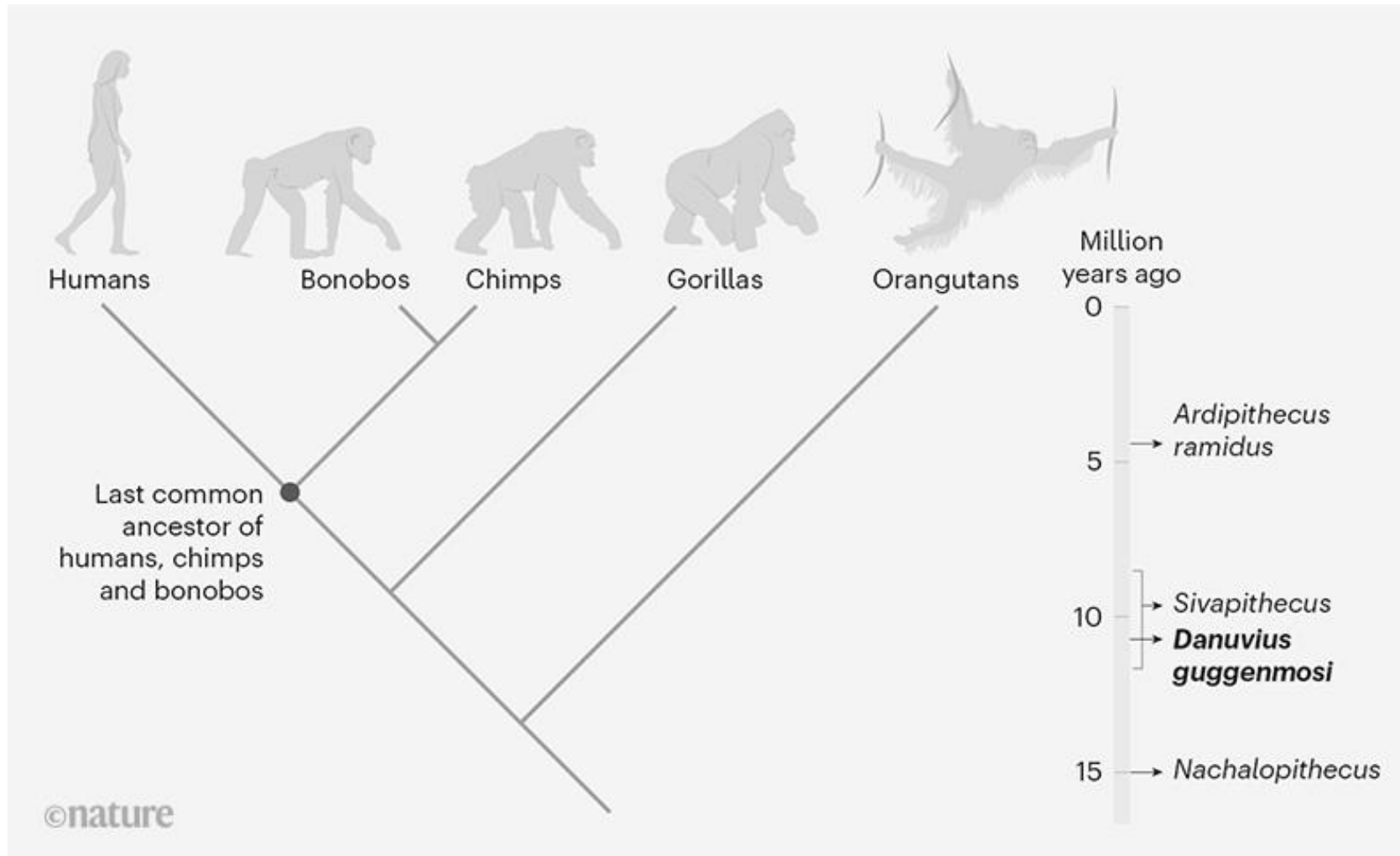
1. The Speed of Natural Evolution

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3. Accelerated Evolution of Enzyme Therapeutics for Refractory Gout

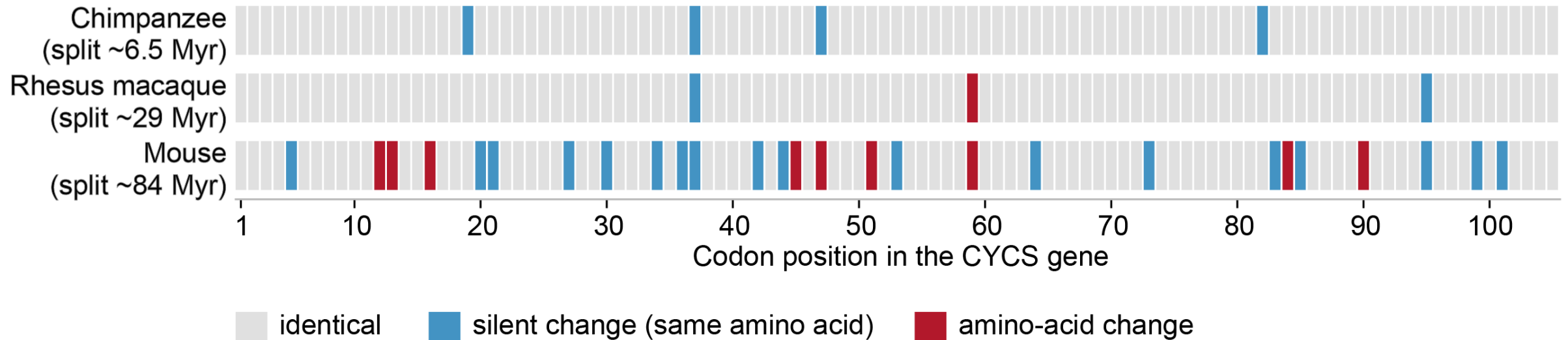
4. Evolution of Enzyme Therapeutics as a Replacement for Opioids in the Treatment of Pain

The Timescale of Evolution



The Speed of Evolution on the Molecular Level

Evolution of cytochrome c (CYCS) compared to the human gene



How does the immune system adapt to fight infections by pathogens when evolution requires millions of years for meaningful change of individual genes?

Outline

1. Timescales of evolution

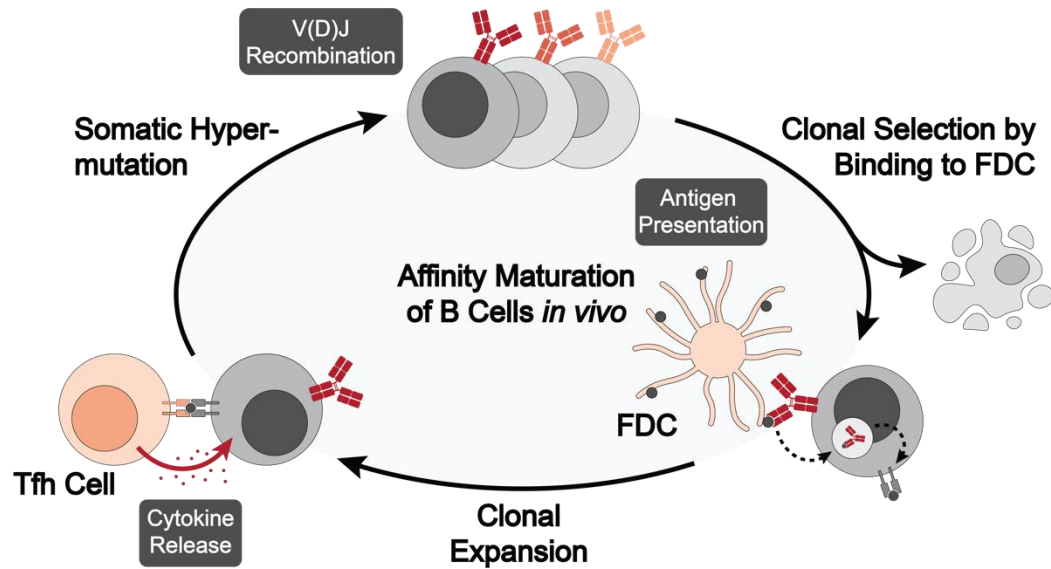
2. Accelerated Evolution in Nature and in the Lab

3. Accelerated Evolution of Enzymes for Treatment of Refractory Gout

4. Evolution of Enzyme Therapeutics as a Replacement of Opioids in Pain Treatment

Accelerated Evolution by the Immune System

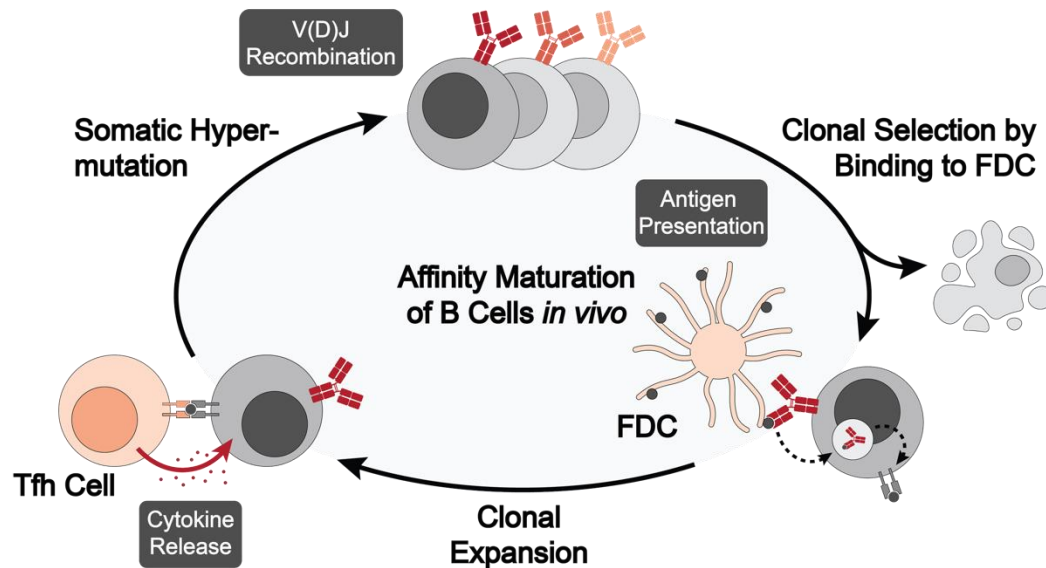
Affinity Maturation by the Immune System



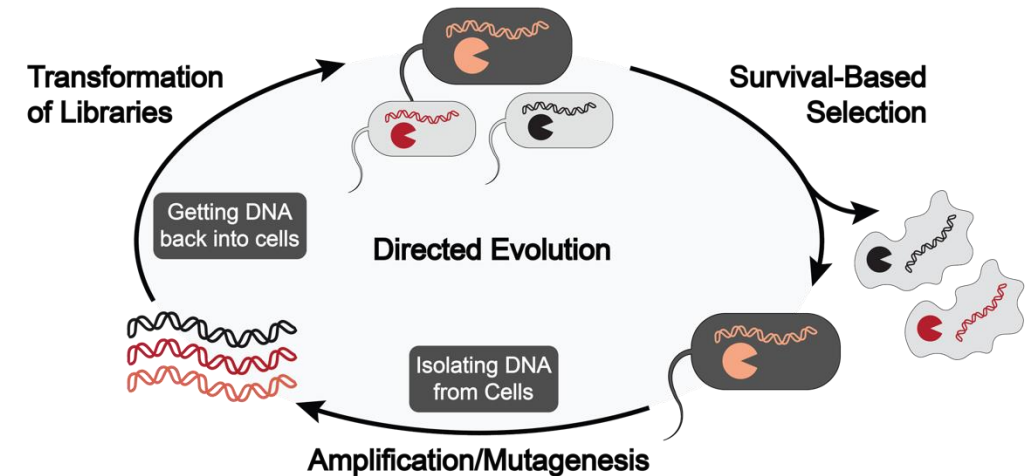
- ❑ Mutation rate of Somatic Hypermutation is up to a million-fold higher than germline mutation rate
- ❑ ~25% of approved drugs in recent years have been derived from accelerated evolution of antibodies in germinal centers
- ❑ **No comparable natural system for “activity maturation” of enzymes exists in nature**

Directed Evolution

Affinity Maturation by the Immune System



Directed Evolution of Biomacromolecules



Directed Evolution

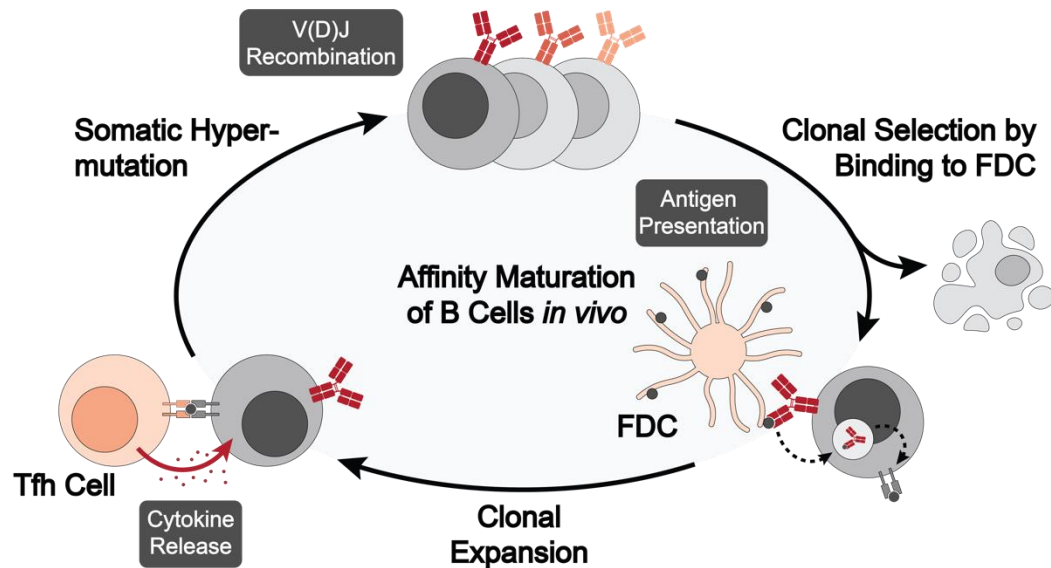
- Genomic mutation rates of microbial hosts are too low for directed evolution ($\sim 10^{-10}$ s.p.b.)
- *In vitro* mutagenesis can be used to create the required genetic diversity, *in vivo* selection/screening is used to identify improved mutants
- **Every round of directed evolution takes ~1 week, limiting the throughput of these experiments**

Bio Technology, **1991**, 9, 1073; *Science*, **2016**, 354, 1048–1051

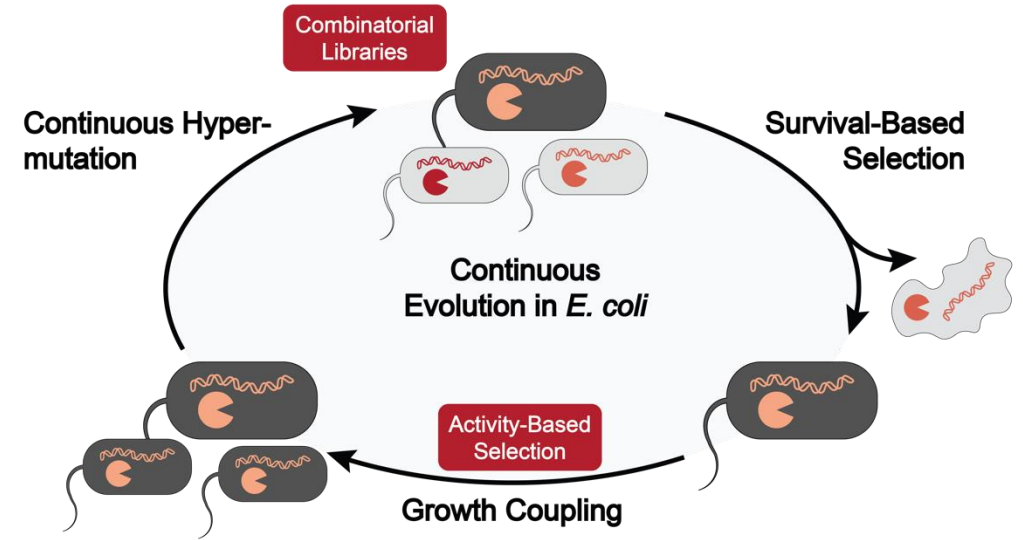


Continuous Evolution of Enzyme Therapeutics

Affinity Maturation by the Immune System



Accelerated “Activity Maturation” in Microbial Hosts

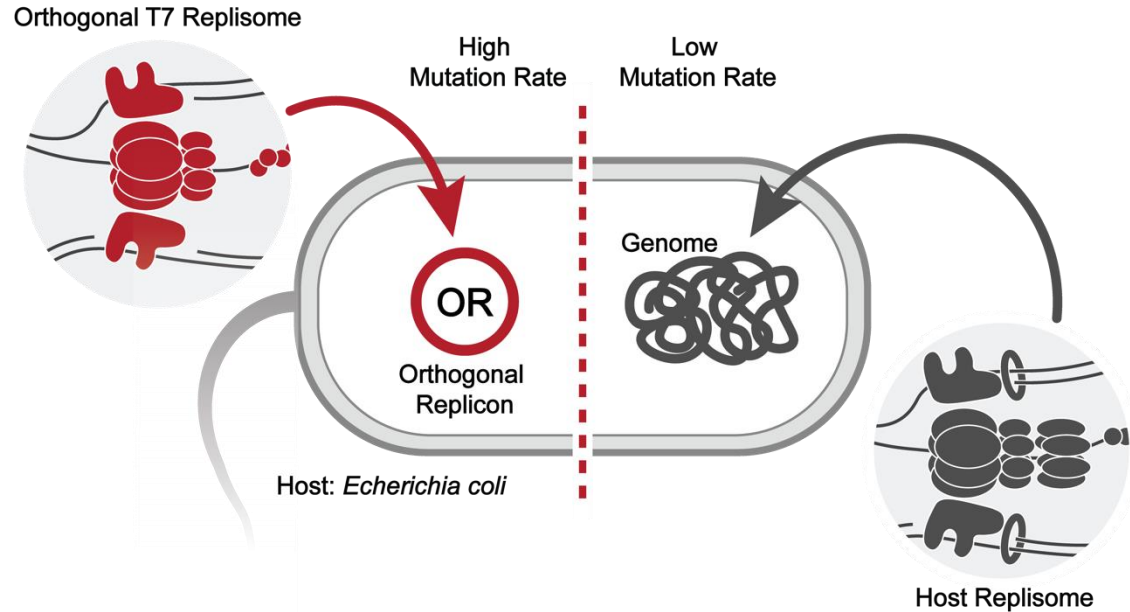


Accelerated Evolution for Activity Maturation of Enzymes

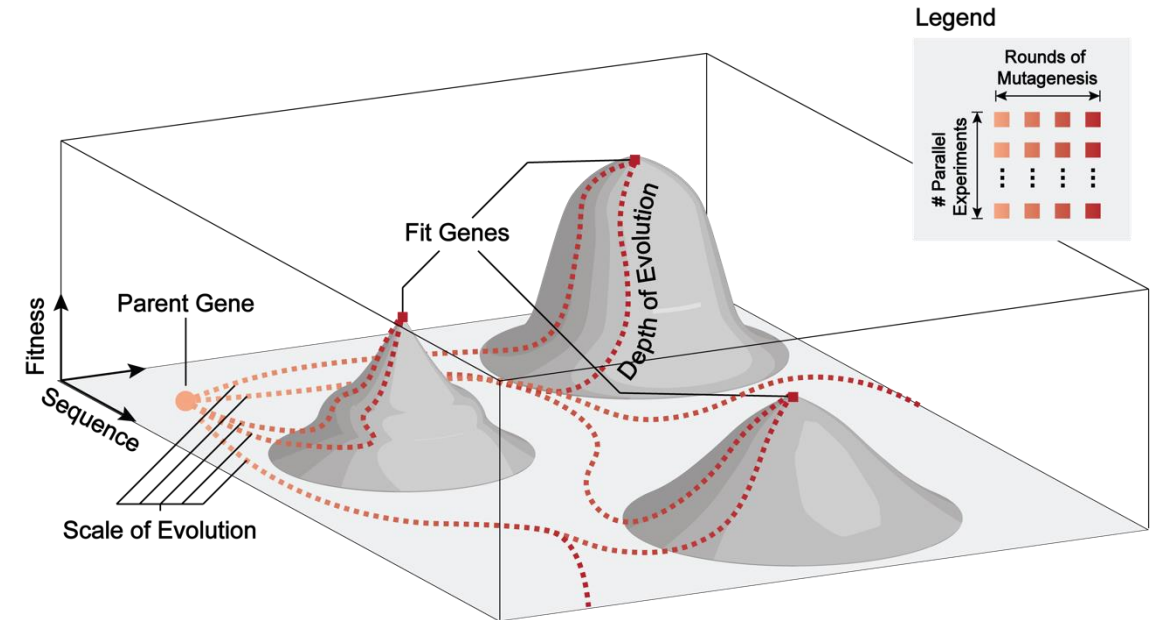
- We aim to develop systems that can integrate combinatorial libraries with continuous hypermutation and growth coupled selection to enable “activity maturation” of enzymes as therapeutics

An Orthogonal Replication System in *E. coli* for Continuous Evolution

Orthogonal Replication System



Fitness Landscape



In vivo Continuous Evolution

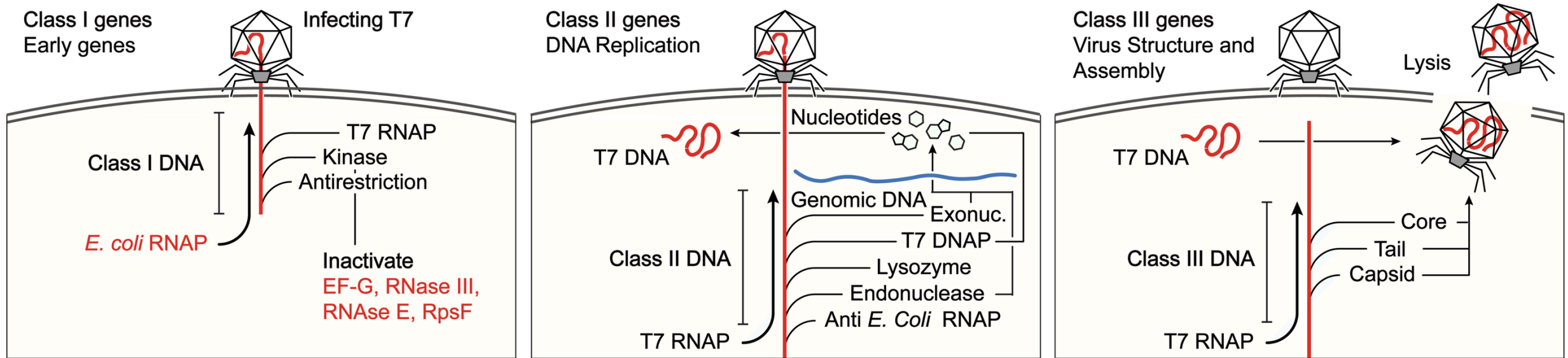
- Continuous evolution systems devise strategies to mutagenize specified genes without compromising host fitness
- This enables selection at every doubling of the cell (~20 min) and greatly facilitates evolution at depth and scale

Esvelt, Kevin M., Jacob C. Carlson, and David R. Liu. *Nature* 472.7344 (2011): 499-503.

Ravikumar, A., Arzumanyan, G. A., Obadi, M. K., Javanpour, A. A., & Liu, C. C. (2018). *Cell*, 175(7), 1946-1957.

Tian, Rongzhen, Fabian BH Rehm, Dariusz Czernecki, Yangqi Gu, Jérôme F. Zürcher, Kim C. Liu, and Jason W. Chin. *Science* 383, no. 6681 (2024): 421-426.

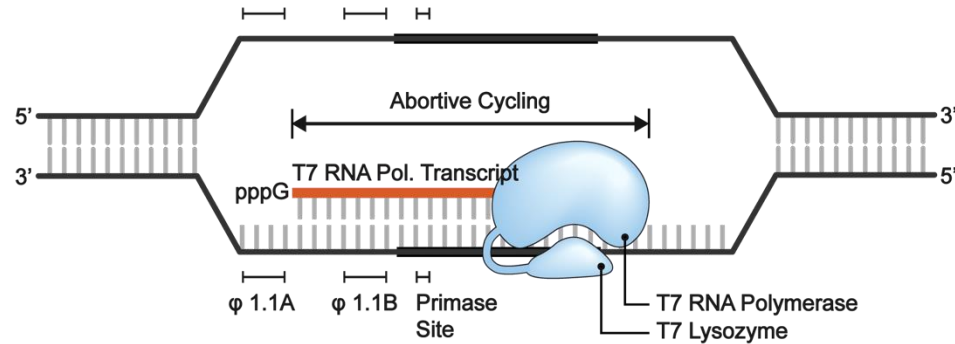
Bacteriophage T7 Life Cycle



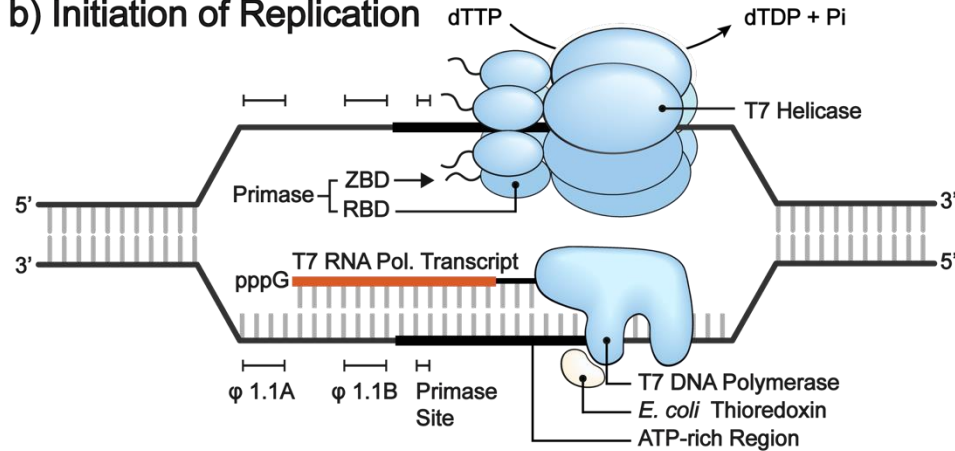
The lytic bacteriophage T7 expresses its own replisome to selectively replicate its own genetic material

An Orthogonal T7 Replisome in *E. coli*

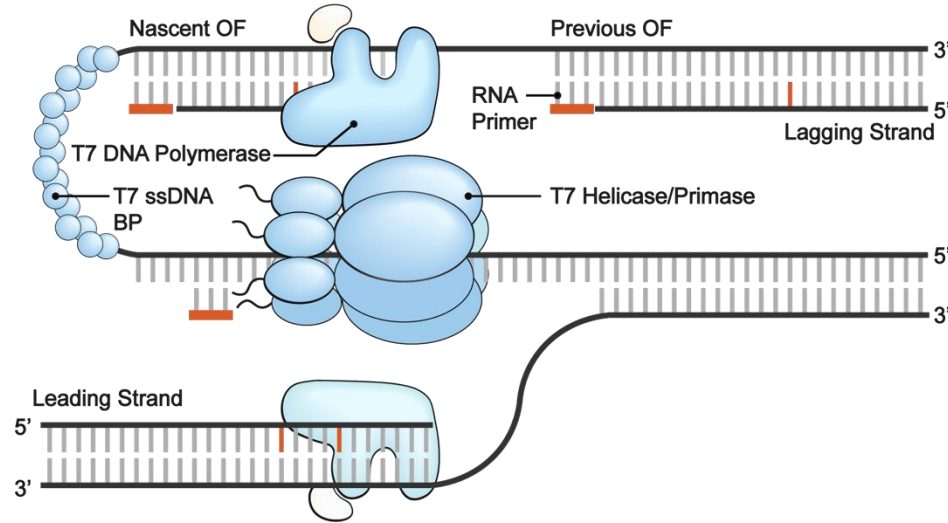
a) Origin of Replication



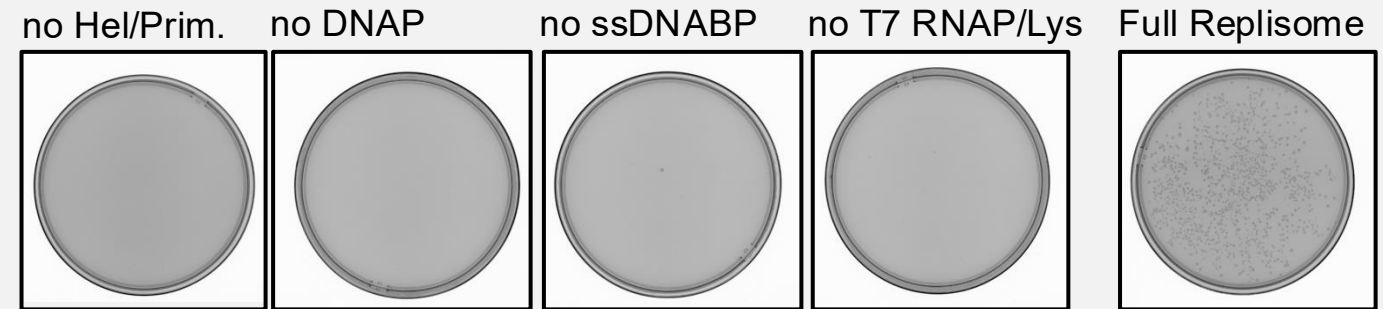
b) Initiation of Replication



c) Replisome Coordination



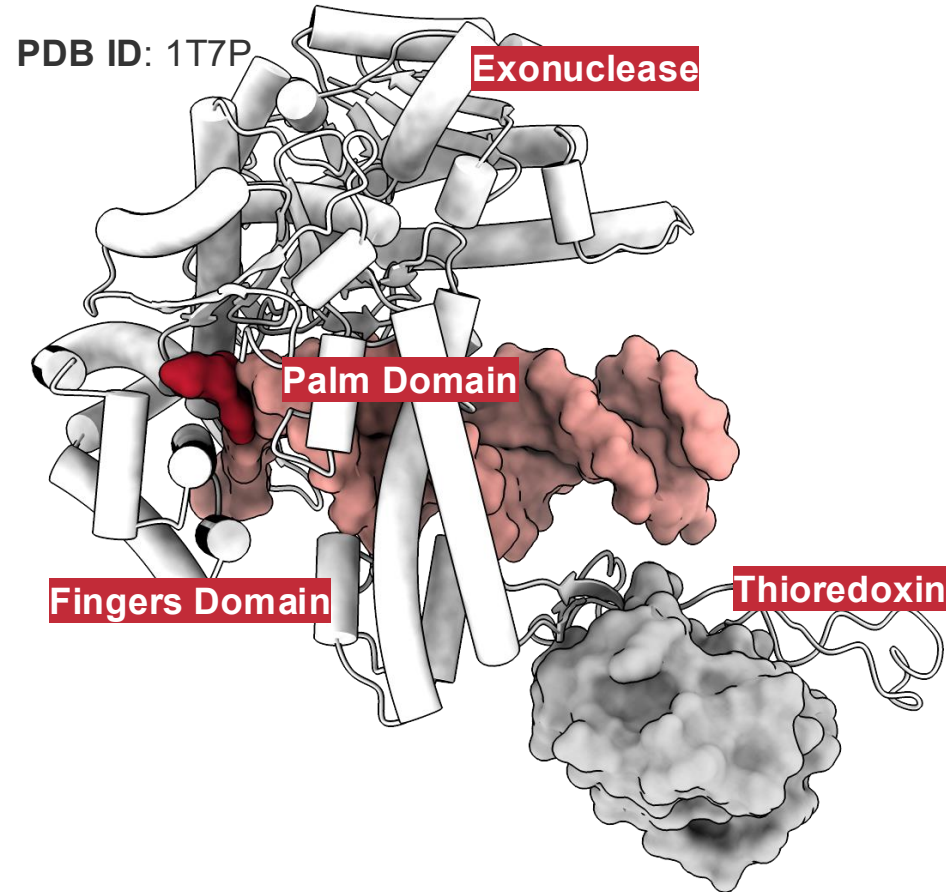
Maintenance of T7 origin plasmid



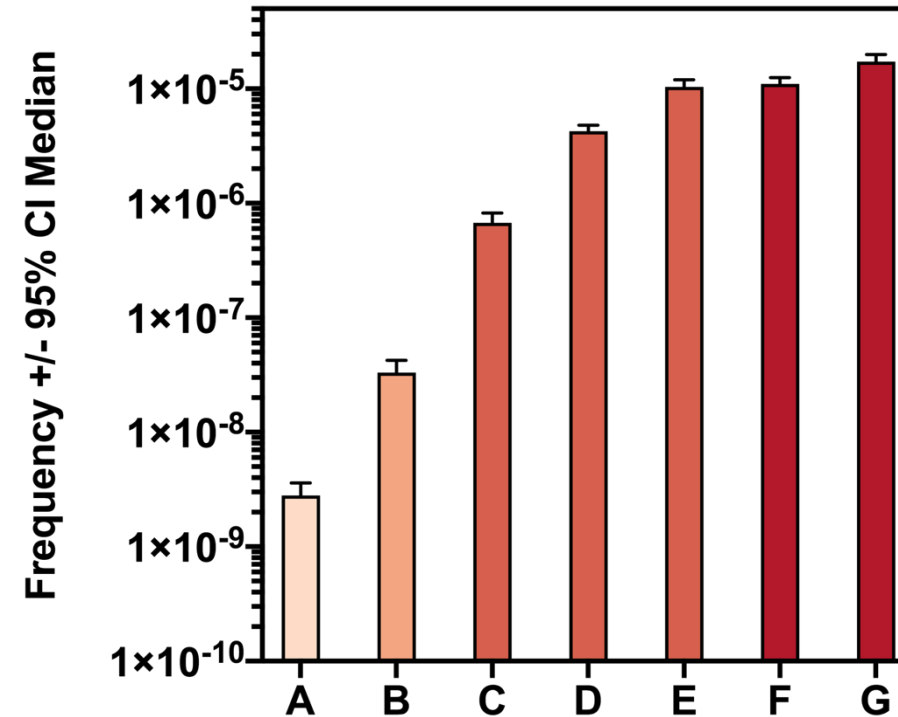
Replication of a plasmid carrying the T7 origin of replication is strictly dependent on all 6 exogenous proteins

Increasing the T7 DNA Polymerase Mutation Rate

Structure T7 DNA Polymerase



Mutation Rate of T7 Replisome

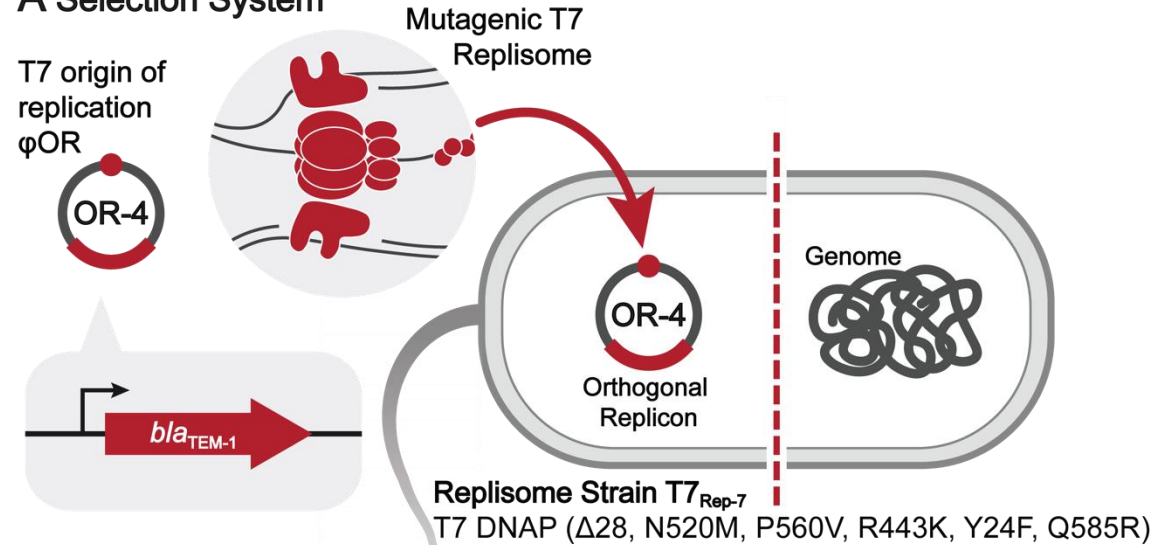


Clone	Mutations
A	WT
B	$\Delta 28$
C	$\Delta 28$, N520M
D	$\Delta 28$, N520M, P560V
E	$\Delta 28$, N520M, P560V, R443K
F	$\Delta 28$, N520M, P560V, R443K, Y24F
G	$\Delta 28$, N520M, P560V, R443K, Y24F, Q585R

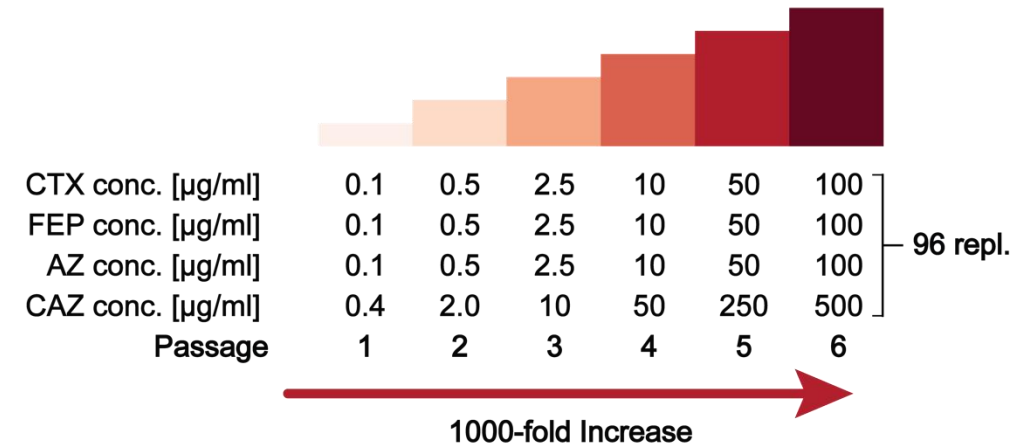
T7 DNA polymerase engineering makes the replisome mutagenize genes of interest 100,000-fold faster than genomic mutation rates

Proof of Concept: Continuous Evolution of TEM-1 β -Lactamase

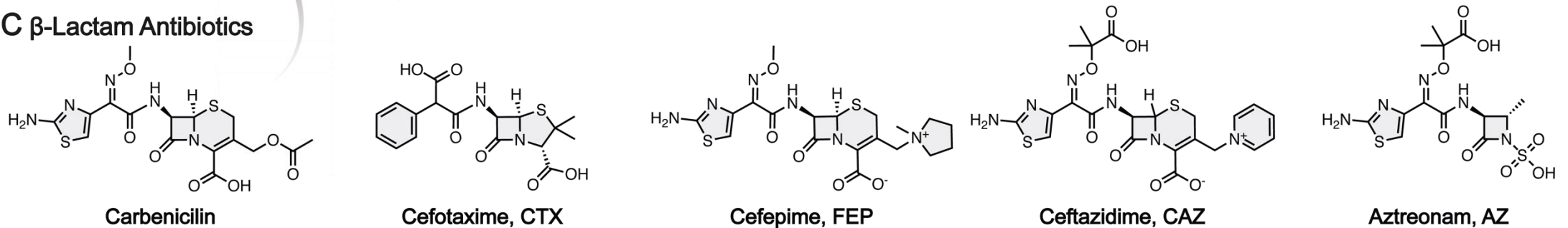
A Selection System



B Selection Stringency



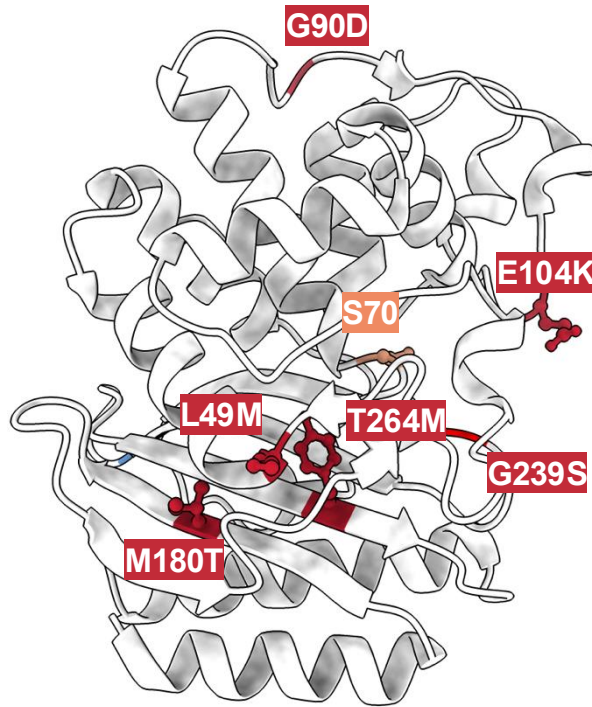
C β -Lactam Antibiotics



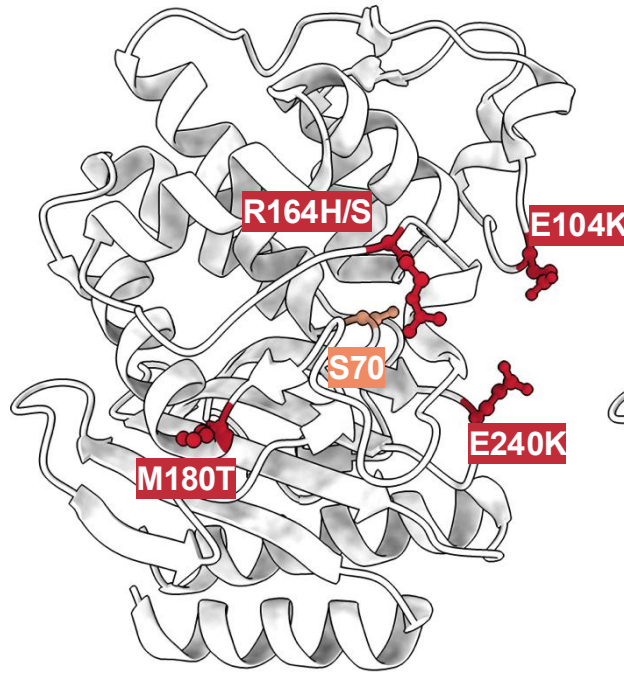
Evolution for TEM-1 β -Lactamase activity on next-generation β -Lactam antibiotics

Continuous Evolution of TEM-1 β -Lactamase

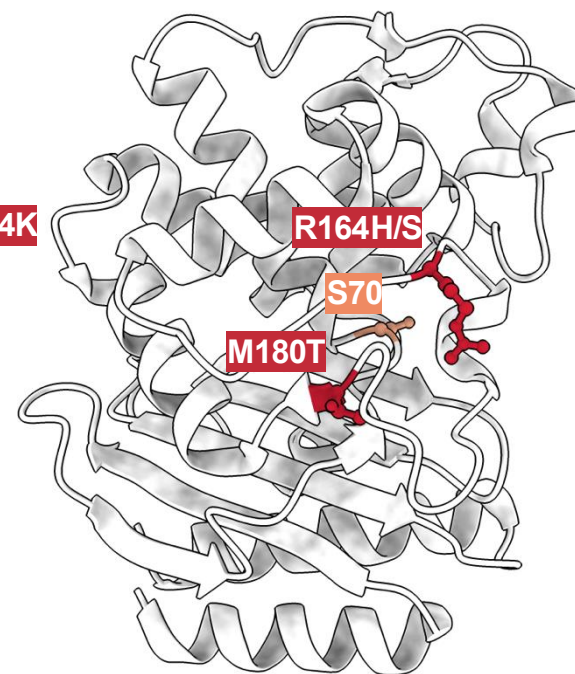
Cefotaxime Resistance



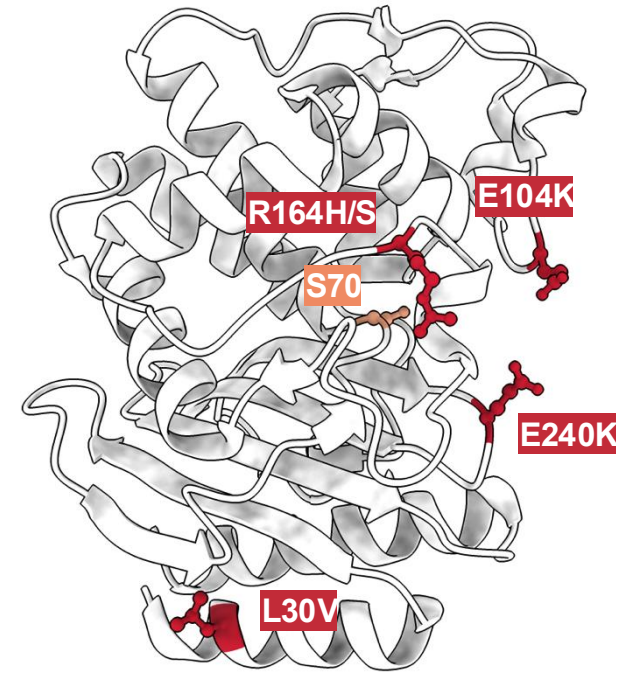
Ceftazidime Resistance



Cefepime Resistance



Aztreonam Resistance



PDB ID: 1BTL

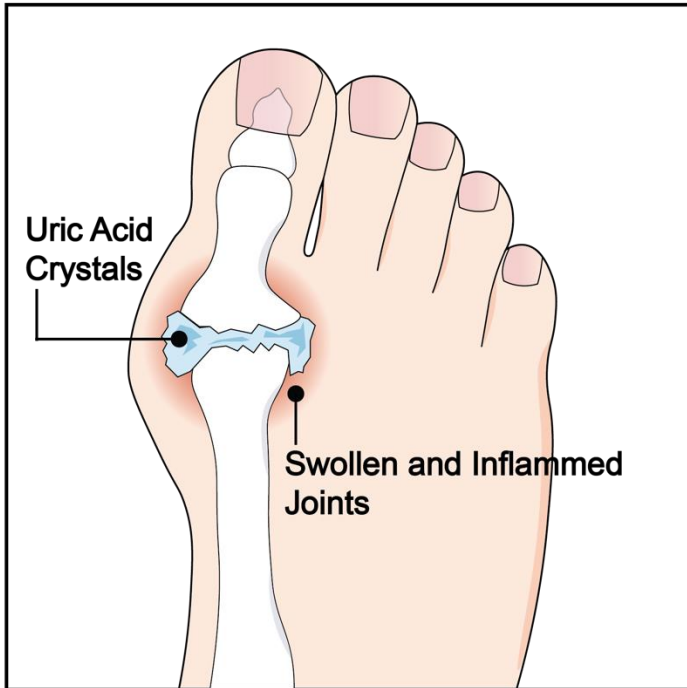
- Enrichment for different mutations and/or different combinations of mutations for the four antibiotics
- Most mutations observed in our experiment have been described for clinical isolates of multi-drug resistant bacteria or from lab evolution experiments
- Newly identified mutations localize to the active site pocket

Outline

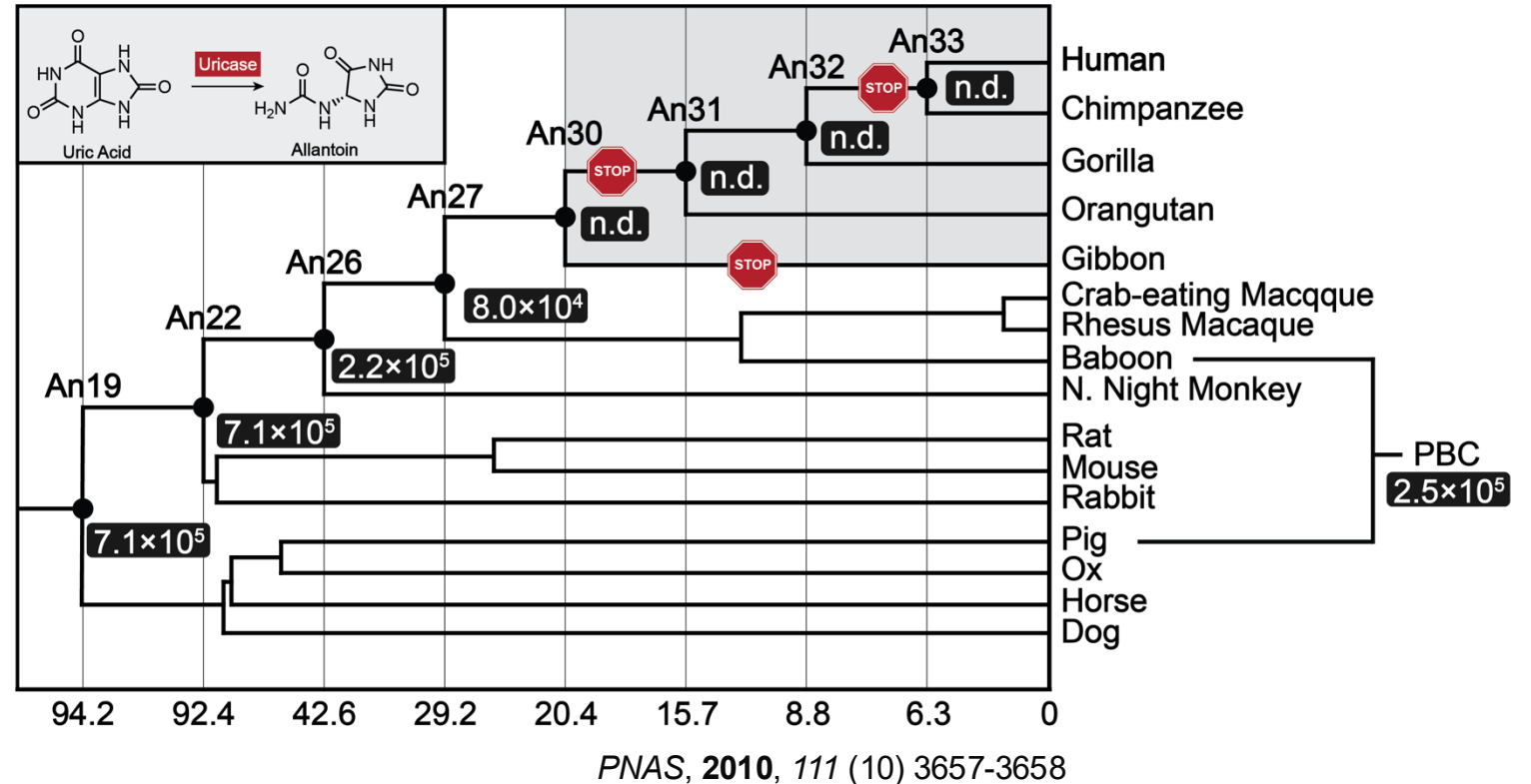
1. Timescales of evolution
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Directed Evolution of a Human-like Uricase

Refractory Gout



Loss of Human Uricase Gene in Evolutionary History

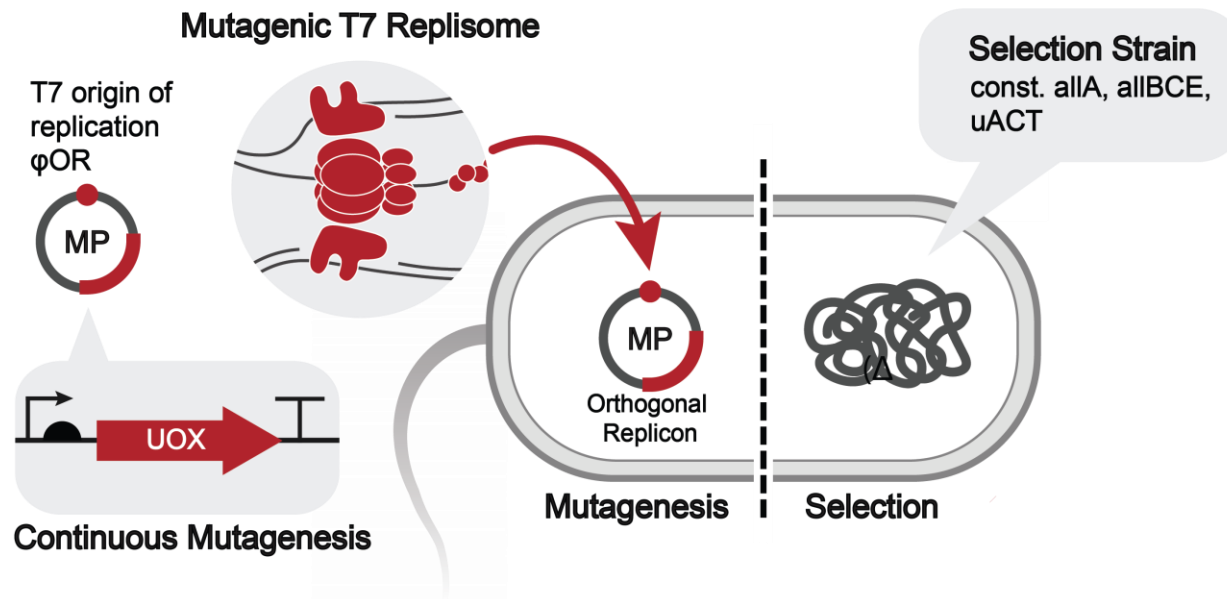


Evolution of Uricase Enzyme Therapeutics for Gout

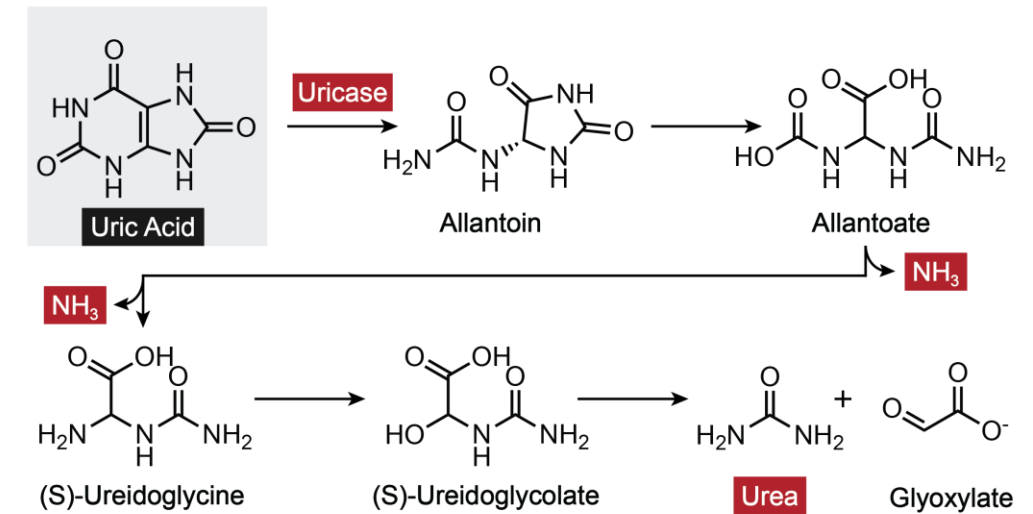
- In evolutionary history, humans have lost activity of their uricase gene. This causes build up and crystallization of uric acid crystals in joints
- Pig-baboon uricase chimera (PBC) is an FDA approved uricase therapeutics, but the therapeutic efficacy is limited by anti-drug antibodies due to low sequence similarity to the human pseudogene and hyper-PEGylation
- **We aim to evolve more human-like Uricases from ancestrally reconstructed genes**

Selection System For Uricase Evolution

Evolution of Ancestor27 UOX by T7 ORACLE



Metabolic Engineering of Aerobic Uric Acid Degradation Pathway

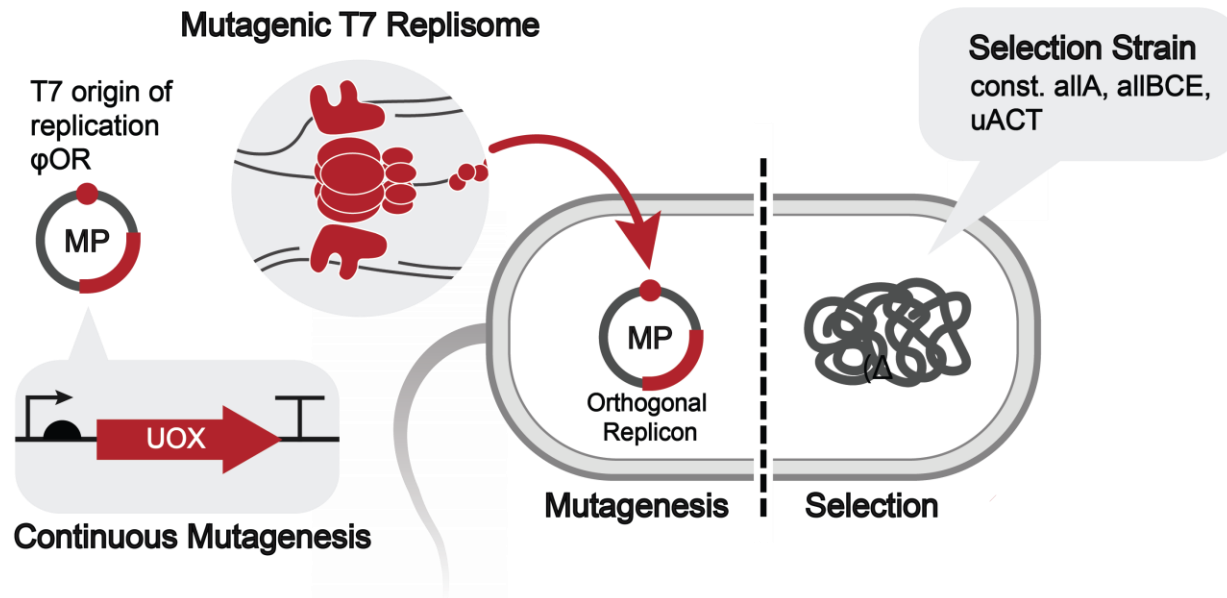


Growth-Coupled Continuous Evolution of Human-like Uricase

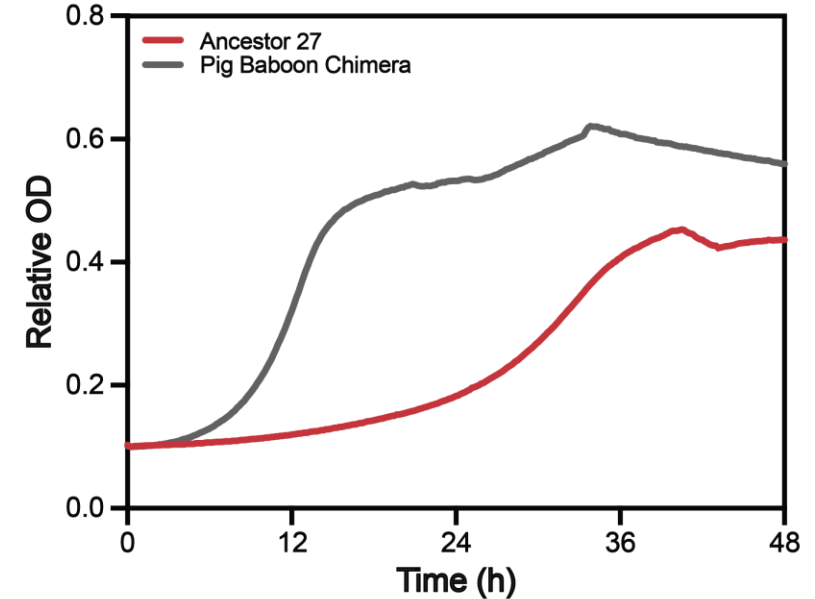
- We established a constitutive allantoin degradation pathway under aerobic conditions in *E. coli* to link Uricase activity to the formation of ammonia as an essential nitrogen source
- Growth-coupled continuous evolution of An27 Uricase using T7 ORACLE identified three mutants with comparable growth to PBC

Selection System For Uricase Evolution

Evolution of Ancestor27 UOX by T7 ORACLE



Growth-Coupled Selection

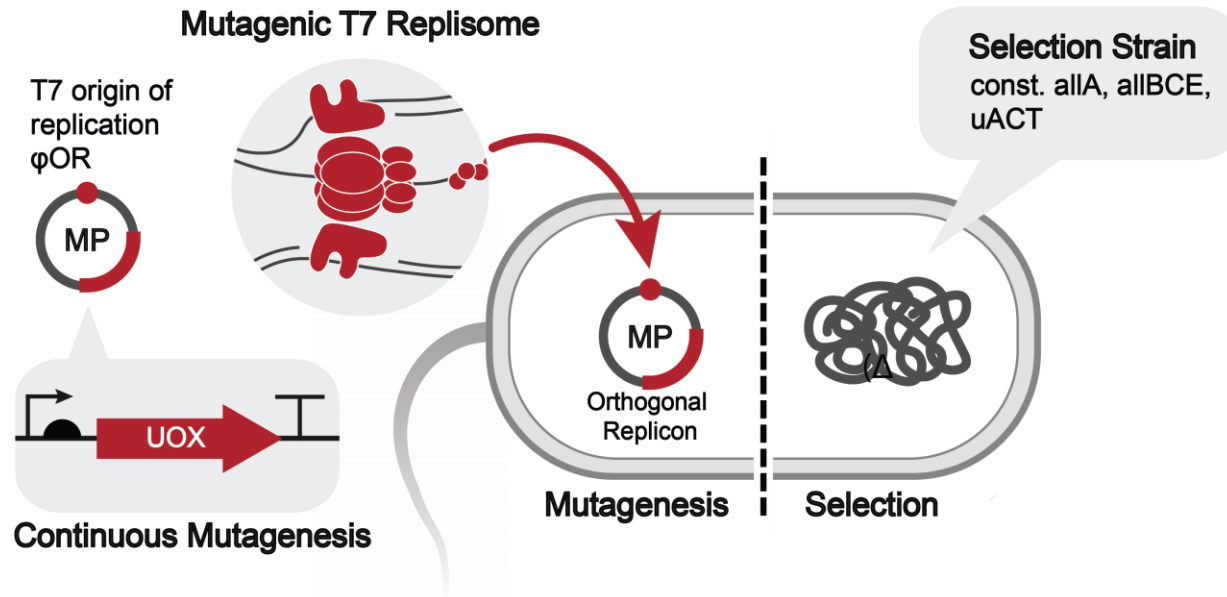


Continuous Evolution of Human-like Uricase

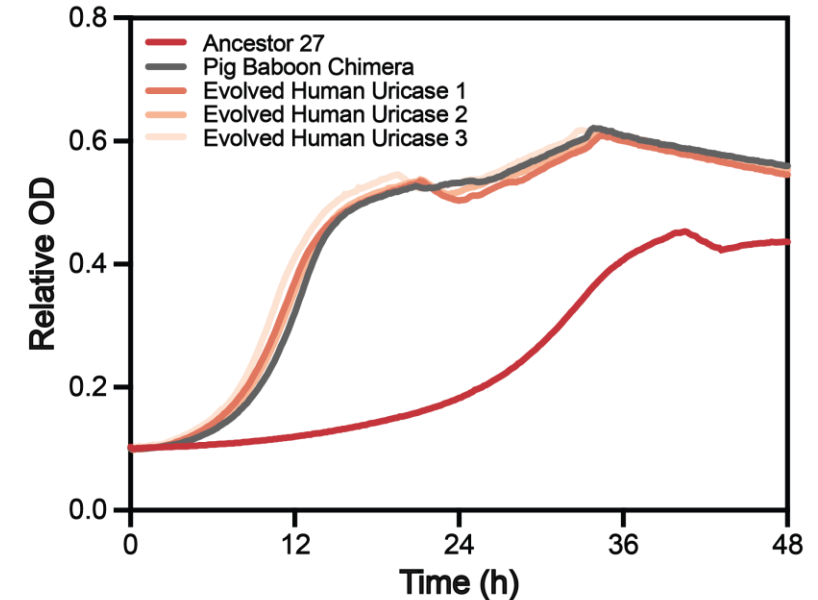
- Growth of the selection strain is limited by uricase activity when uric acid is the only available nitrogen source

Auxotrophic Selection System For Uricase Evolution

Evolution of Ancestor27 UOX by T7 ORACLE



Growth-Coupled Selection

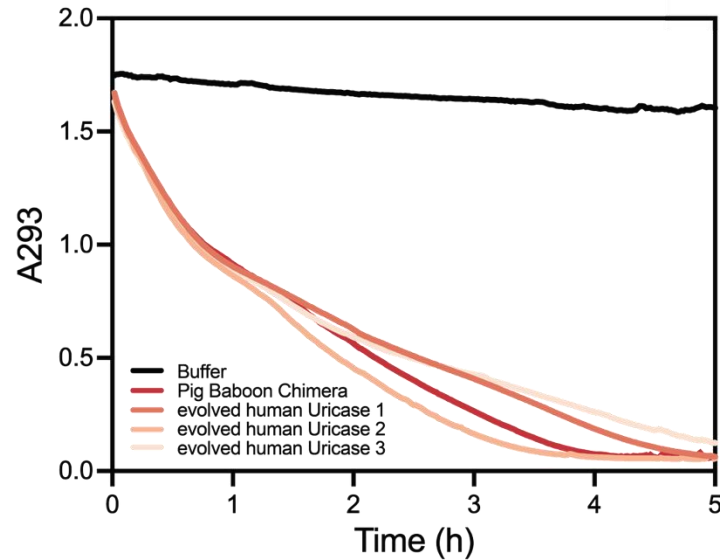


Continuous Evolution of Human-like Uricase

- Growth of the selection strain is limited by uricase activity when uric acid is the only available nitrogen source
- Passing under selection conditions yielded variants that restore growth comparable to strains that express the PBC UOX gene

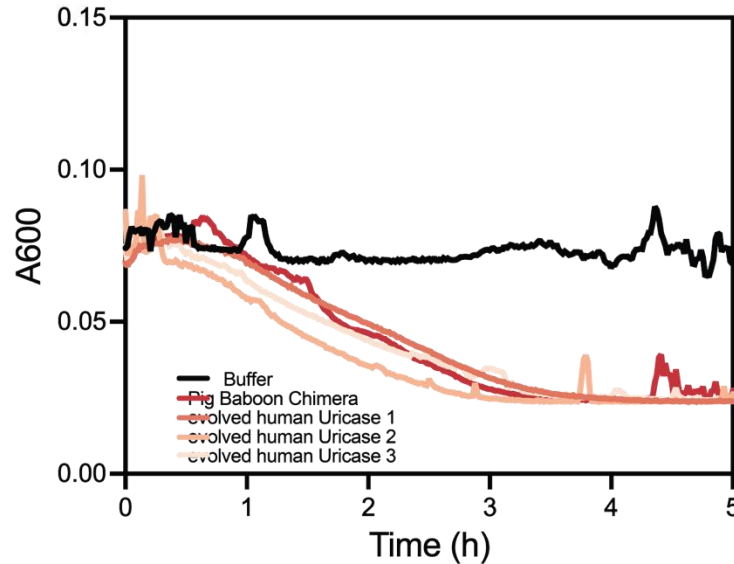
Directed Evolution of a Humanized Uricase

Free Uric Acid

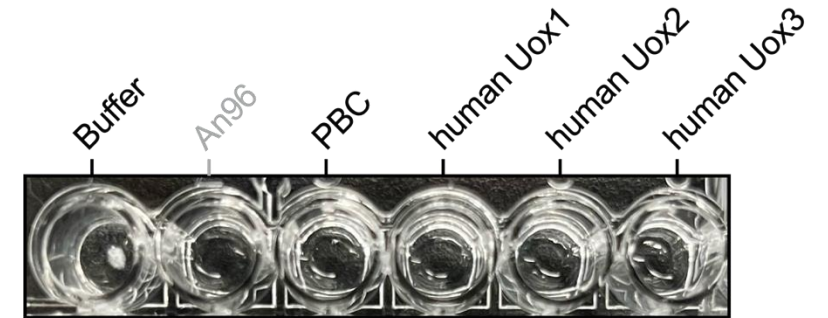


9 mg/dL sodium urate with 2.5 mg/L uricase

Uric Acid Crystals



Uric Crystal Degradation



Evolved Human-like Uricases show high activity on both free uric acid and on uric acid crystals in both buffer and mouse serum.

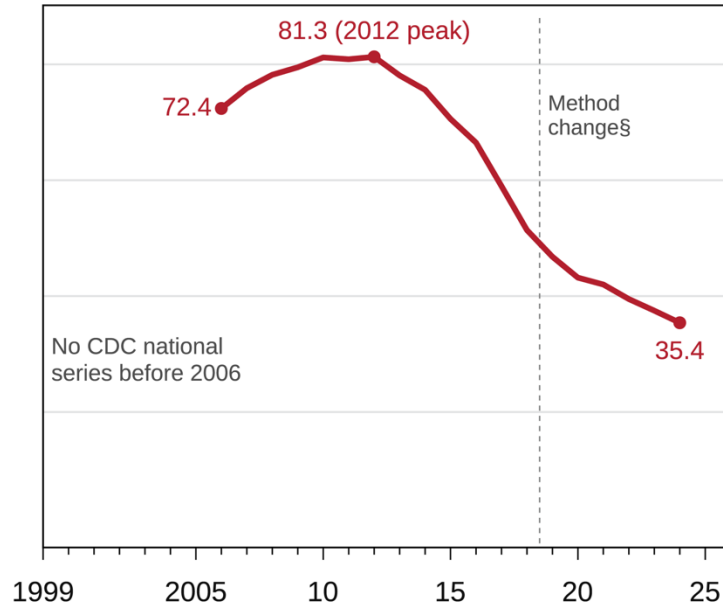
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Opioid Prescription and Drug Overdose Crisis

Opioid prescriptions dispensed‡

Per 100 people, through 2024¶



Source: CDC, National Center for Injury Prevention and Control; IQVIA Xponent

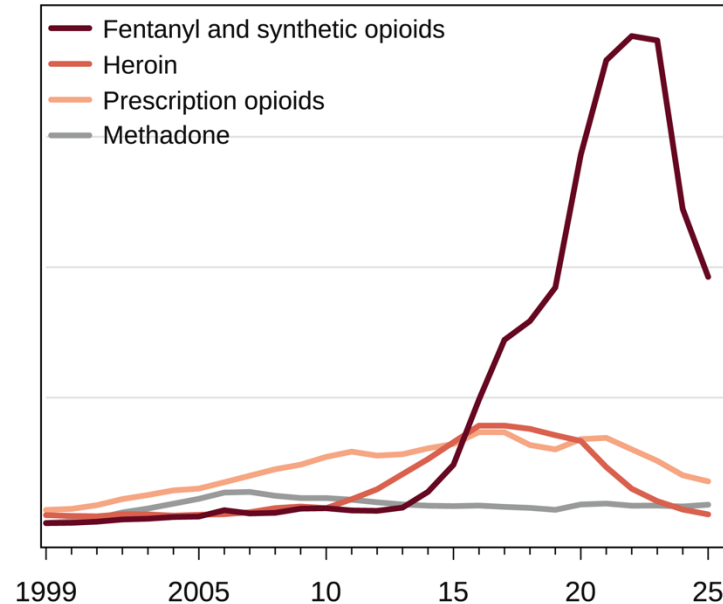
‡Retail pharmacies; excl. mail order and methadone programmes

§From 2019 assigned by prescriber, not pharmacy, location

¶Latest available year: 2024

Drug overdose deaths*

'000, 12 months to December

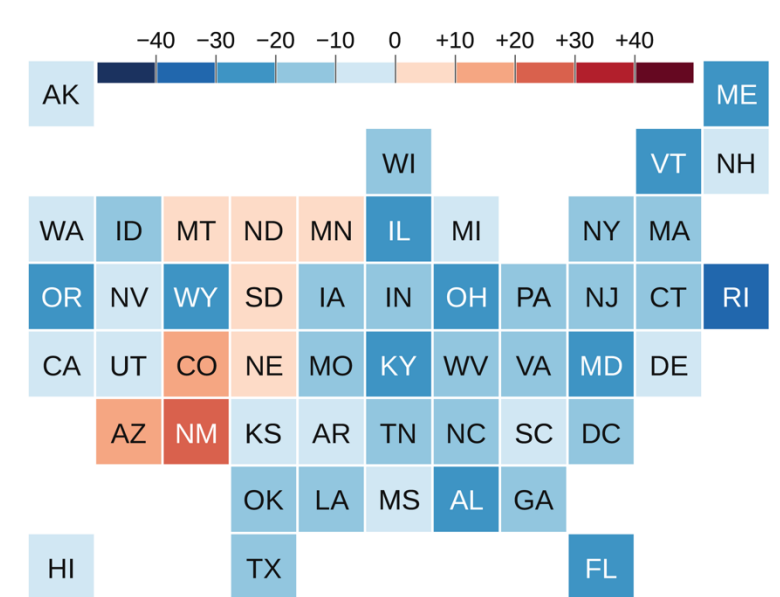


Source: CDC, National Center for Health Statistics

*Deaths involving multiple drugs counted in each category

Overdose deaths by state, 2025†

% change on a year earlier



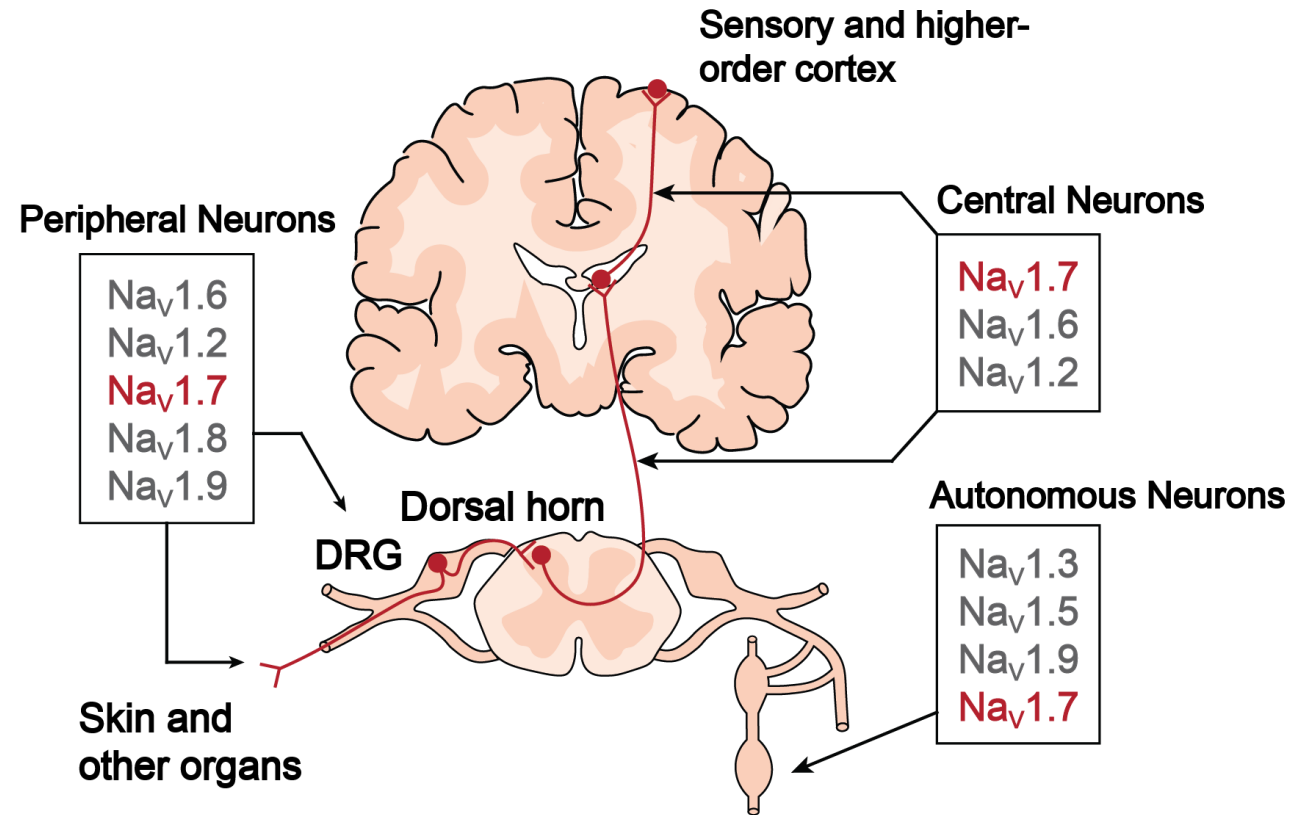
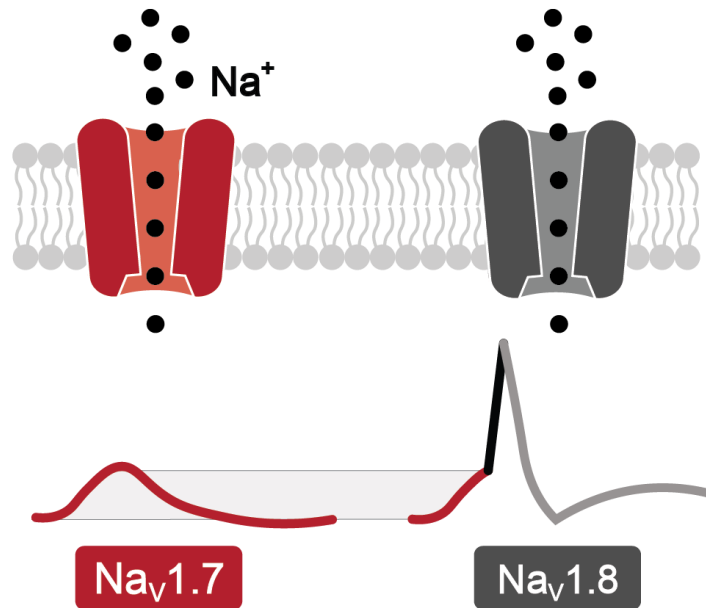
†12 months ending December 2025, predicted provisional counts

Problem: Opioids relieve pain by binding to specific receptors in the brain and spinal cord to block pain signals, but they also trigger the brain's reward centers, which drives addiction

Targeting Voltage-Gated Sodium Ion Channels in Pain

Expression of Na_v Isoforms in the Nervous System

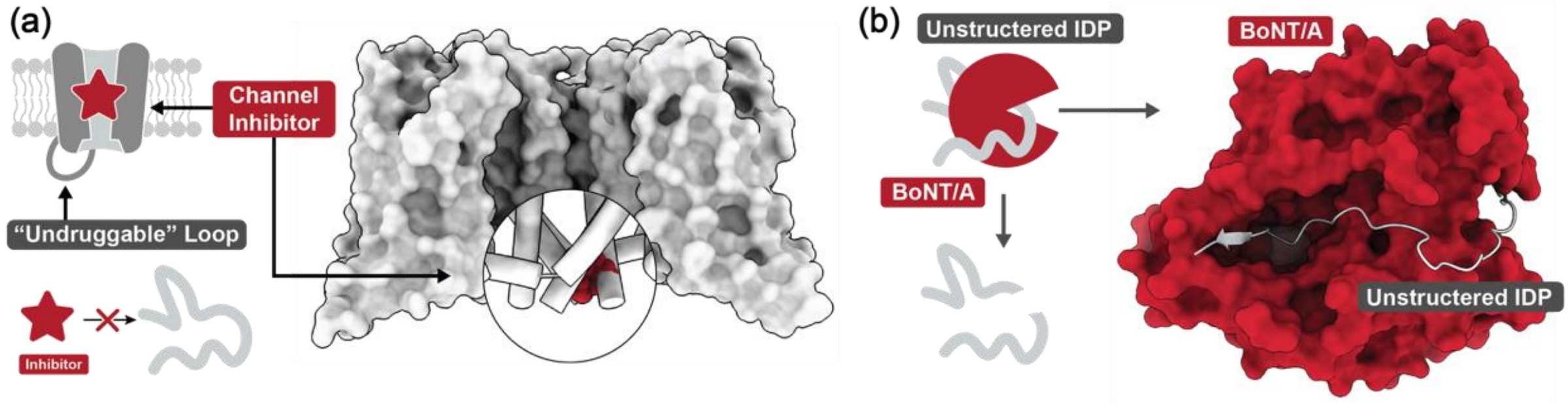
Voltage-gated Na_v Channels



Na_v1.7: Most genetically verified pain target. Mis- or nonsense mutations manifest as congenital insensitivity to pain without impacting other senses.

Challenges: Off-target toxicity of inhibitor drugs on other sodium ion channels, as well as on-target toxicity due to activity against Na_v1.7 in the autonomous/central nervous system

Evolved BoNT/A Protease for Targeted Degradation of Na_v1.7

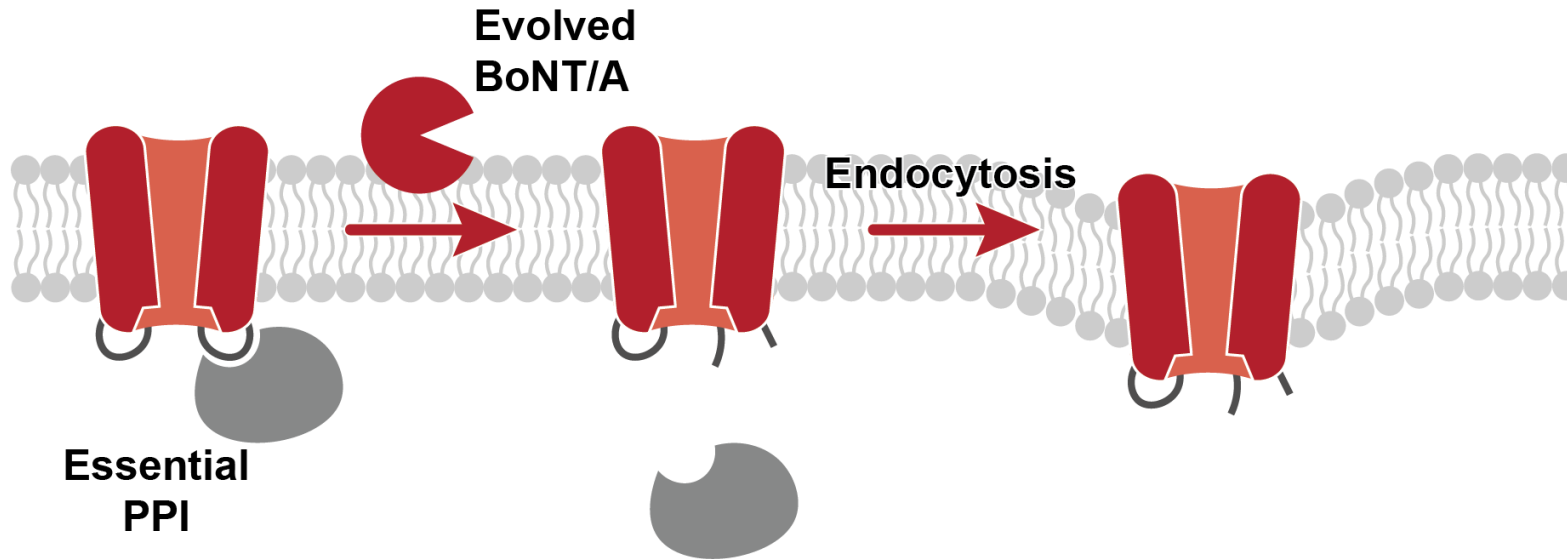


Directed Evolution of BoNT/A

- ❑ **Safety:** Millions of people are treated with BoNT/A proteases every year in the USA alone
- ❑ **Specificity:** BoNT protease has high specificity (>16 amino acids recognition sequence) for its IDP target SNAP25
- ❑ **Delivery:** wt-BoNT/A is injected locally (3.5 cm diffusion radius), with high efficiency and selectivity for peripheral neurons
- ❑ **Changing the sequence specificity of the BoNT/A protease enables degradation of “undruggable” targets such as unstructured intracellular loops in peripheral neurons**

Cleavage of Na_v1.7 Cytosolic Loops for Targeted Endocytosis

Targeting Essential Interaction Sites in Cytosolic Loops

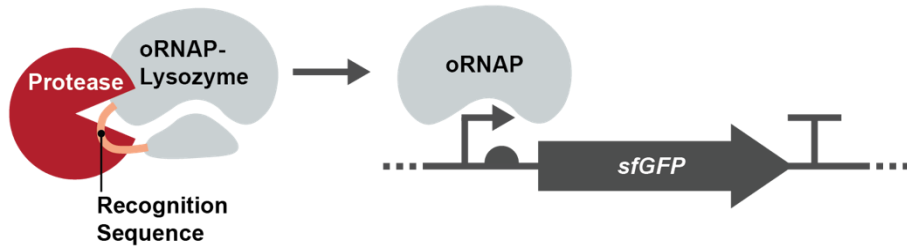


Sequence Similarity to other Na_v Channels

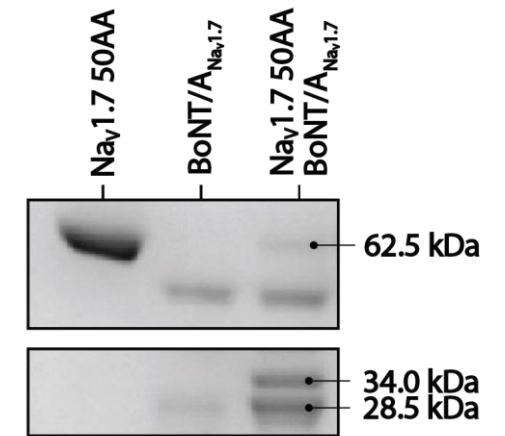
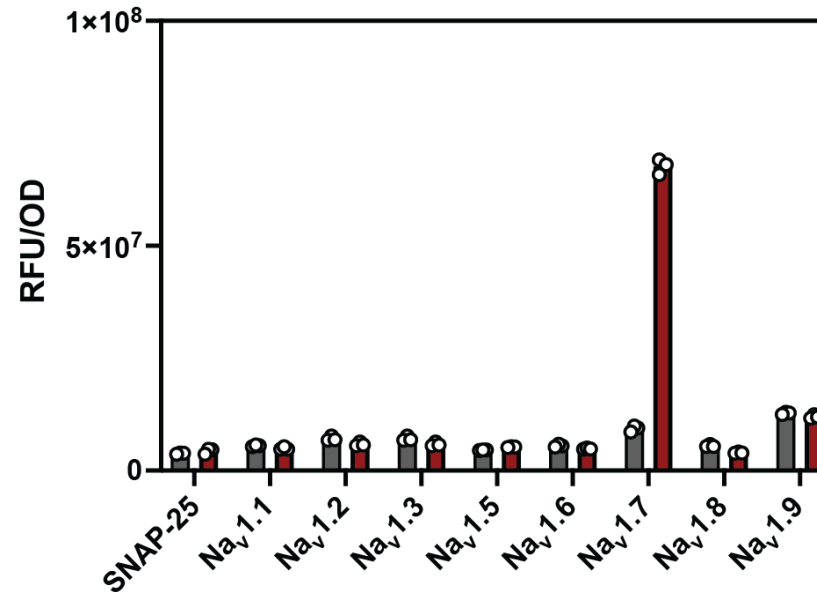


Evolved BoNT/A Protease Selectively Cleaves Na_v1.7

Evolution System for BoNT/A Evolution



Selectivity/Activity of Identified Mutants



Evolution of BoNT/A for Degradation of Na_v1.7 Loop 2

- "Substrate Walking" to change the substrate specificity from wt-SNAP25 to an essential and unique interaction site in a cytosolic loop of Na_v1.7
- High proteolytic activity on the target Na_v1.7 without any remaining activity against wt-SNAP25 or any of the other sodium ion channels expressed in peripheral nociceptors

Take Home Messages

- **We have developed a system for continuous hypermutation in E. coli to accelerate the evolution of biomacromolecules**
- **We use this technology to evolve therapeutics for the degradation of metabolites in diseases including gout, cancer, or for novel enzyme replacement therapies**
- **We are repurposing the cosmetic Botox into a therapeutic for pain by evolving proteolytic activity against ion channels involved in pain signaling to the brain**

Thanks for your Attention!

Diercks and Liu Lab



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