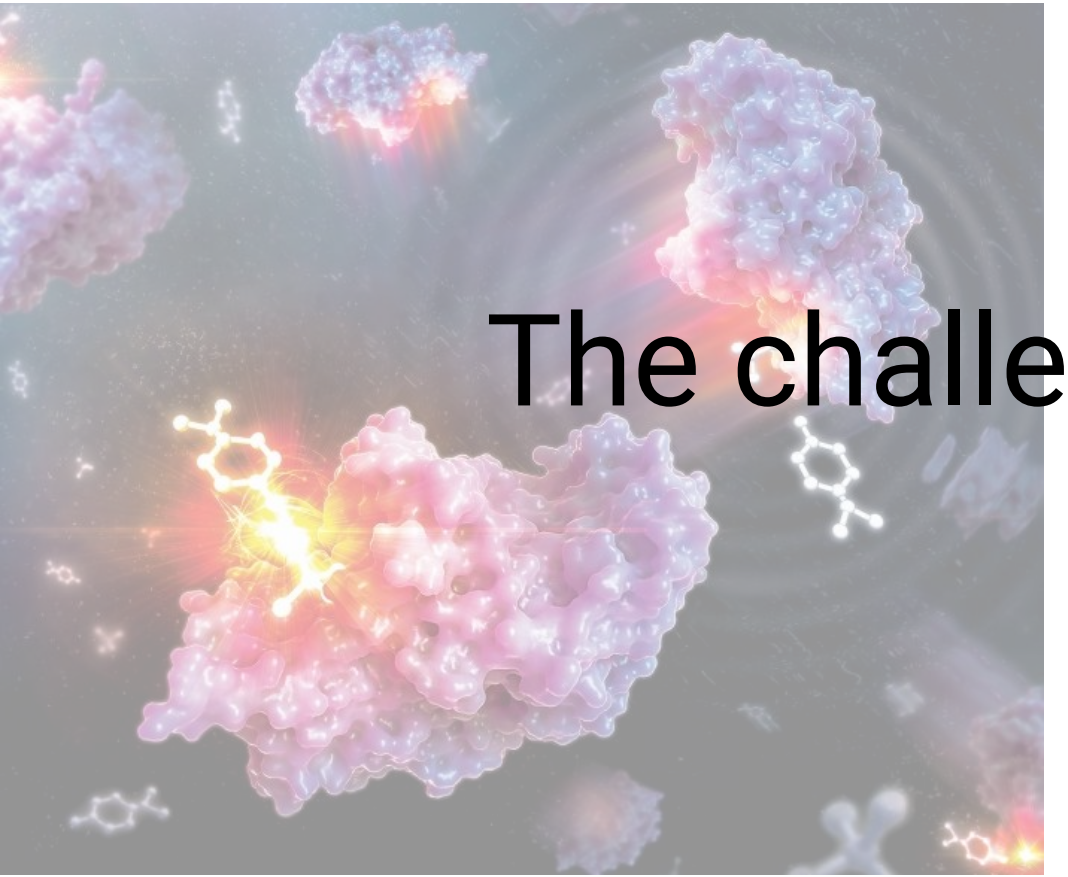
Class core values

1. Be **respect**ful to yourself and others
2. Be **confident** and believe in yourself
3. Always do your **best**
4. Be **cooperative**
5. Be **creative**
6. Have **fun**
7. Be **patient** with yourself while you learn
8. Don’t be shy to **ask “stupid” questions**



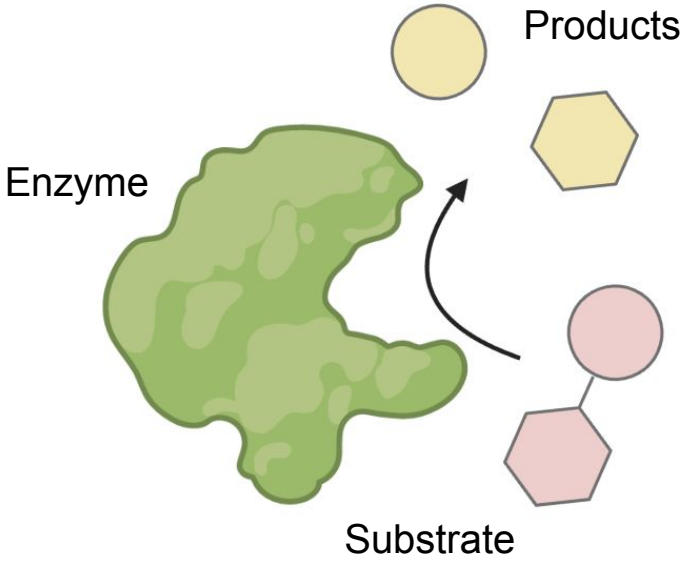
Week 5, Lecture 1

The challenging case of Enzymes

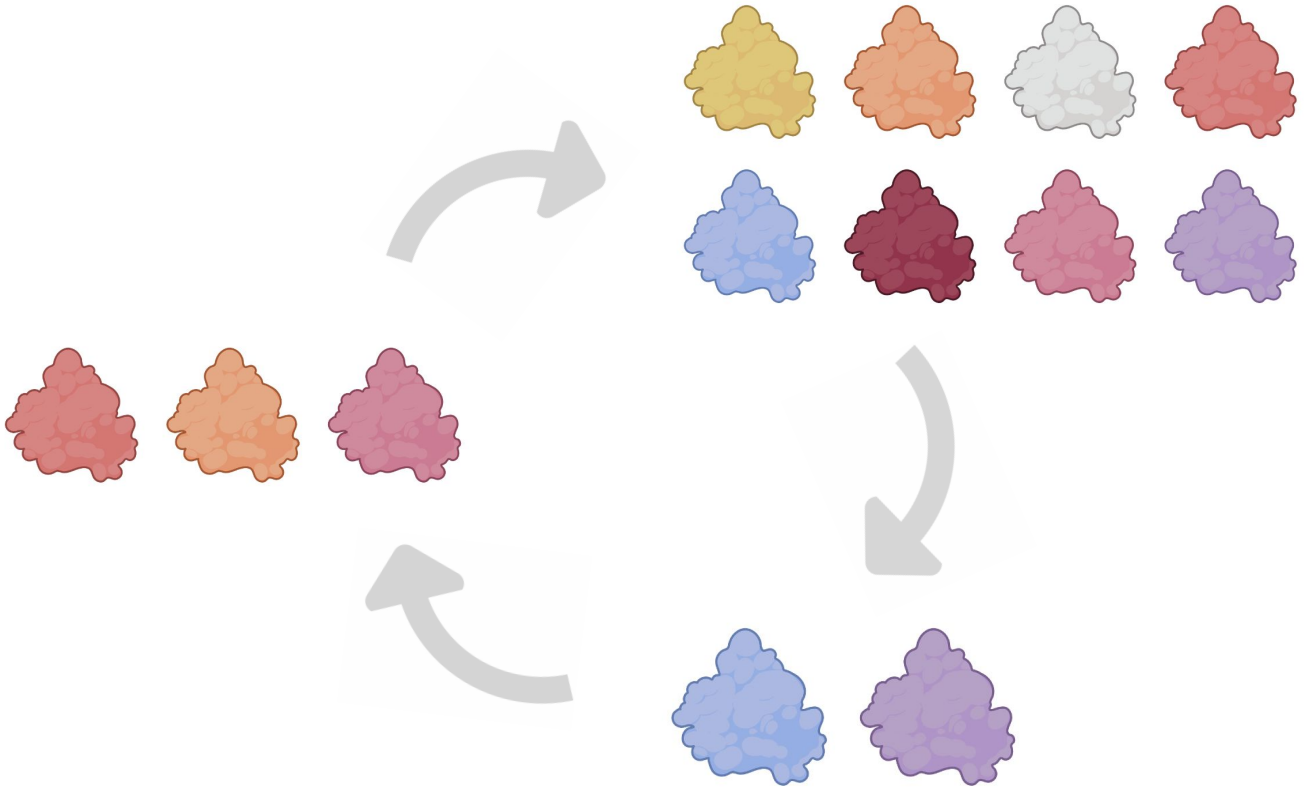
Learning Objectives

1. Identify different properties of enzymes that can be improved
2. Evaluate proper choice of scaffold for enzyme evolution
3. Identify the right assays for measuring and evolving enzymes
4. Critically evaluate literature on directed evolution

Enzymes catalyze the transformation of substrates to products



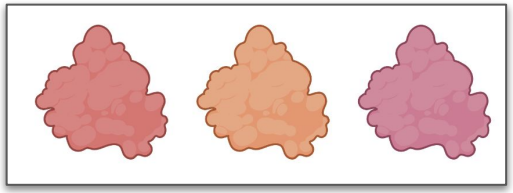
Schematic view of directed evolution pipeline



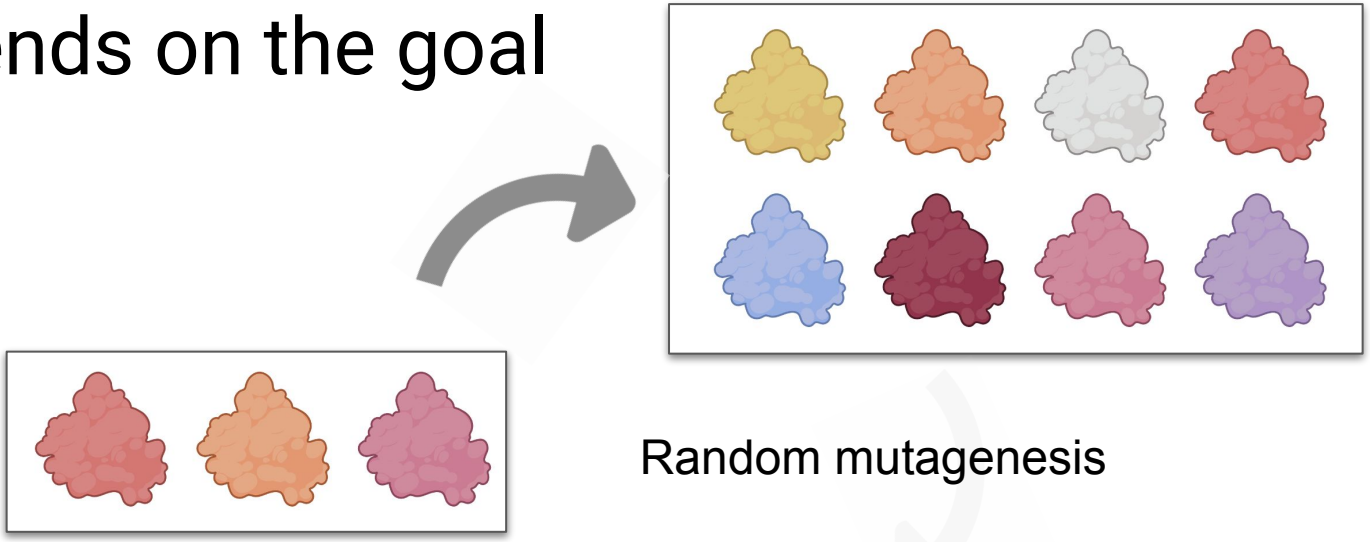
One of the most important steps is to pick the right scaffold

Stability vs Activity

Selectivity vs Promiscuity



The choice of diversification method heavily depends on the goal

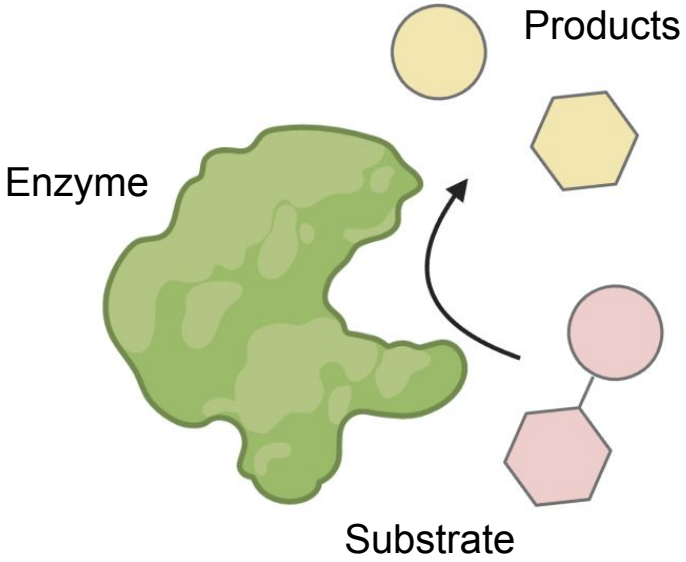


Random mutagenesis

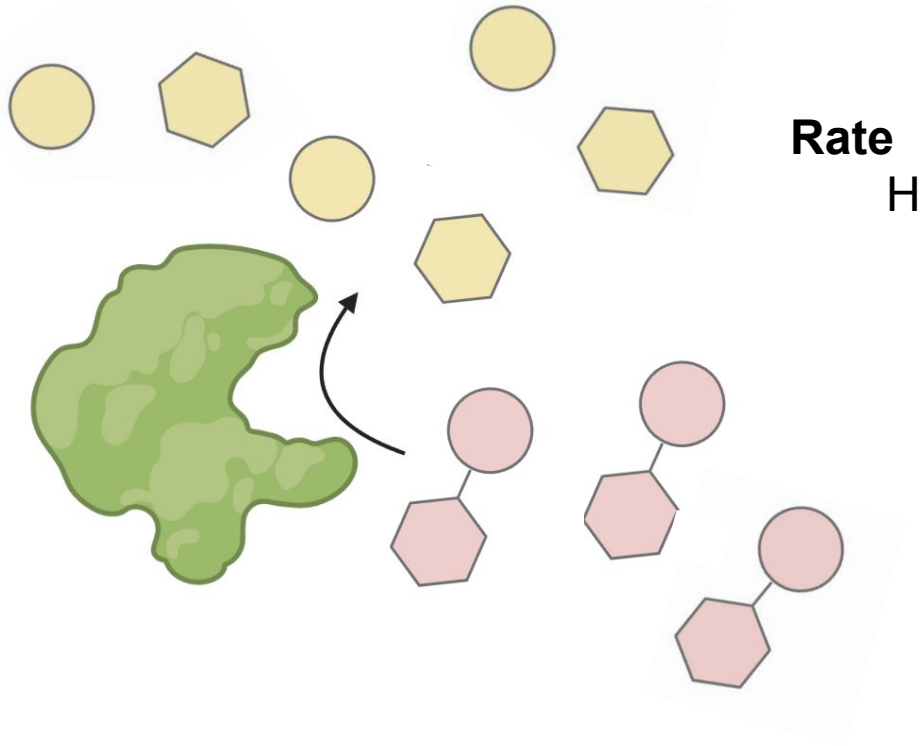
ISM

Shuffling

One of the goals of enzyme engineering is to **increase its activity**



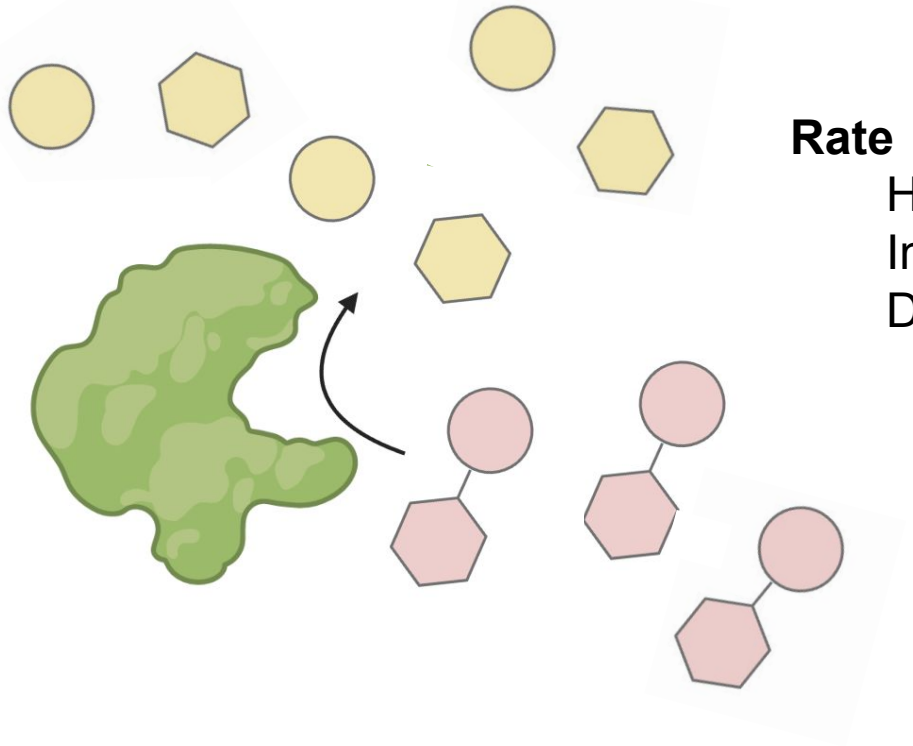
Enzymatic activity can be described by its rate



Rate

How fast the enzyme turns S → P

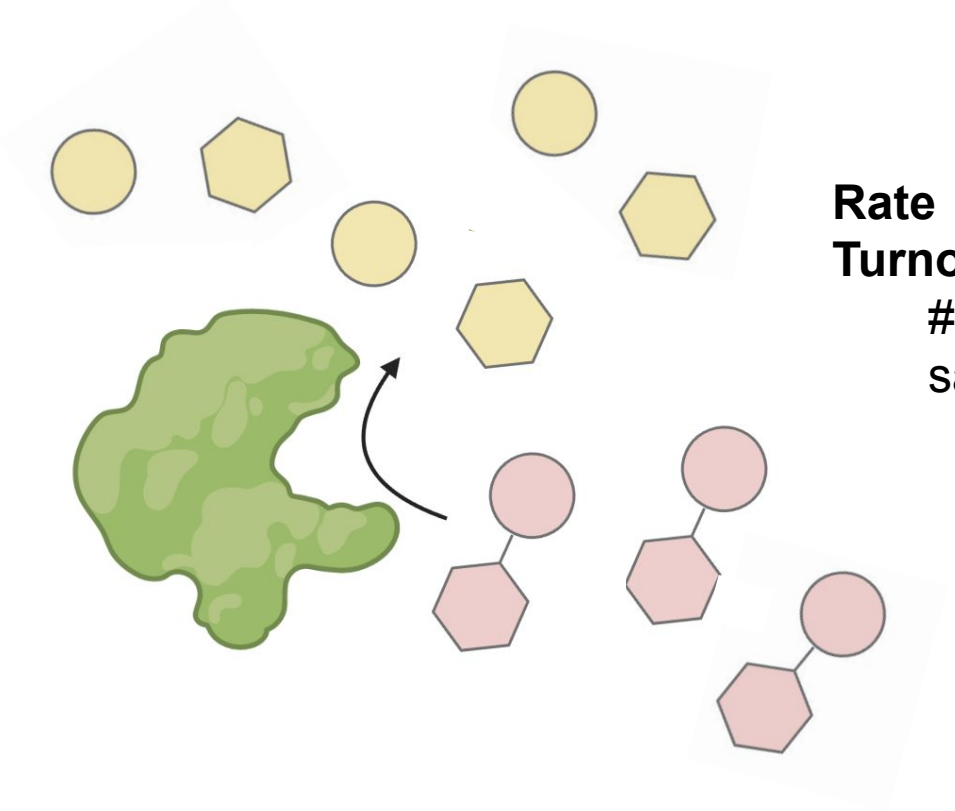
Enzymatic activity can be described by its rate



Rate

How fast the enzyme turns S → P
Increase by increase in T
Depends on [S]

Turnover number can describe the activity



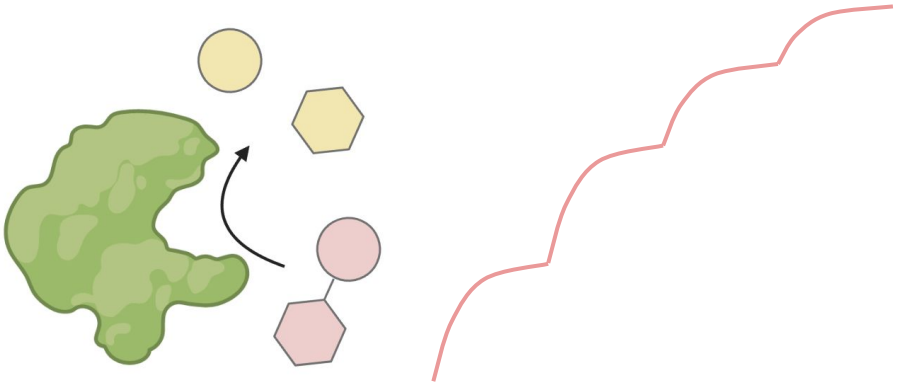
Rate

Turnover number

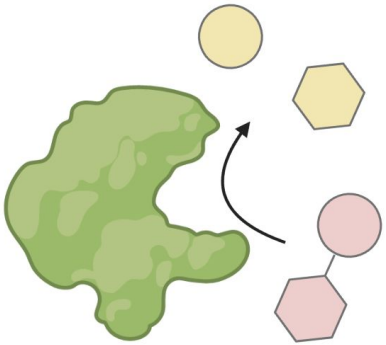
S → P / time when enzyme is fully saturated with substrate

Enzyme’s catalytic cycle has a half-life

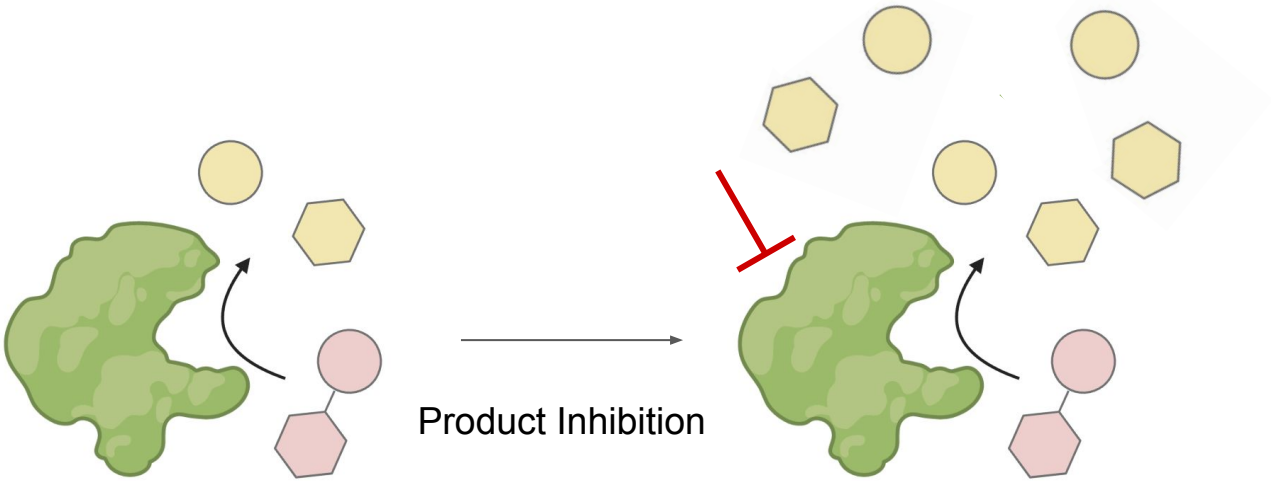
Rate
Turnover number
Half-life



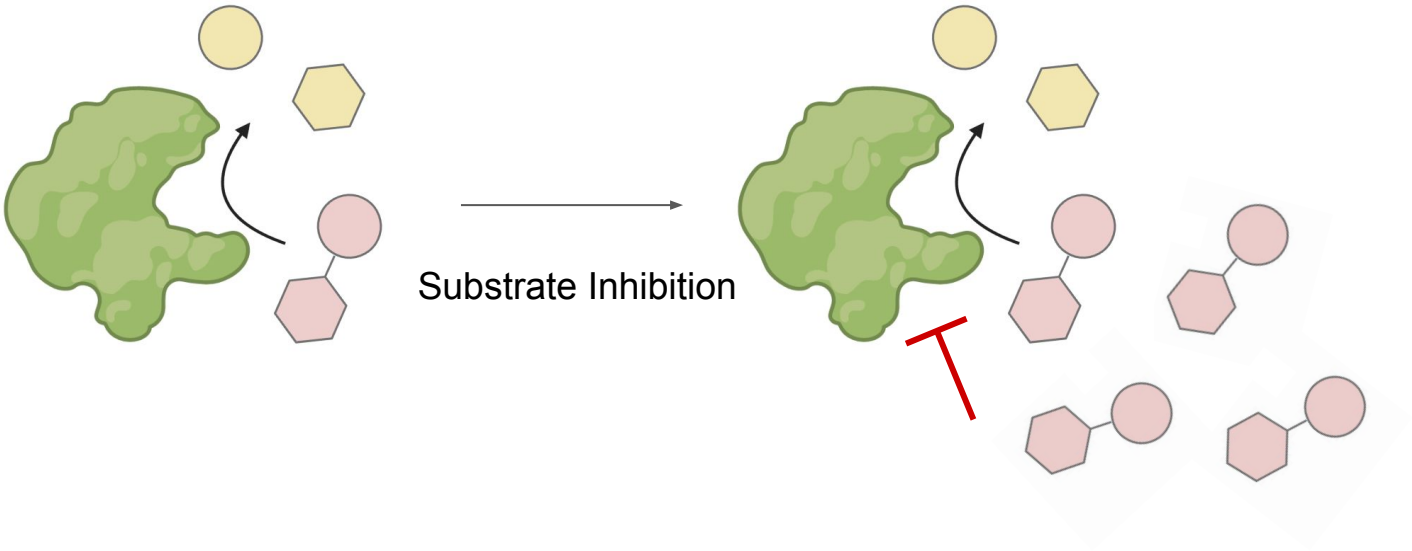
Altering enzyme’s feedback loop is another area of interest in enzyme engineering



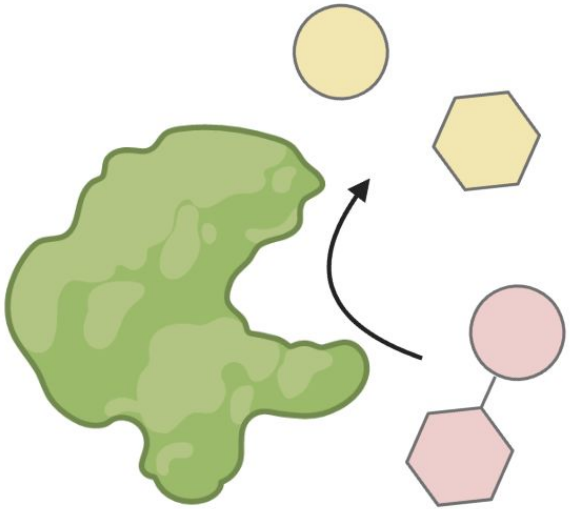
High concentration of product can have negative feedback loop



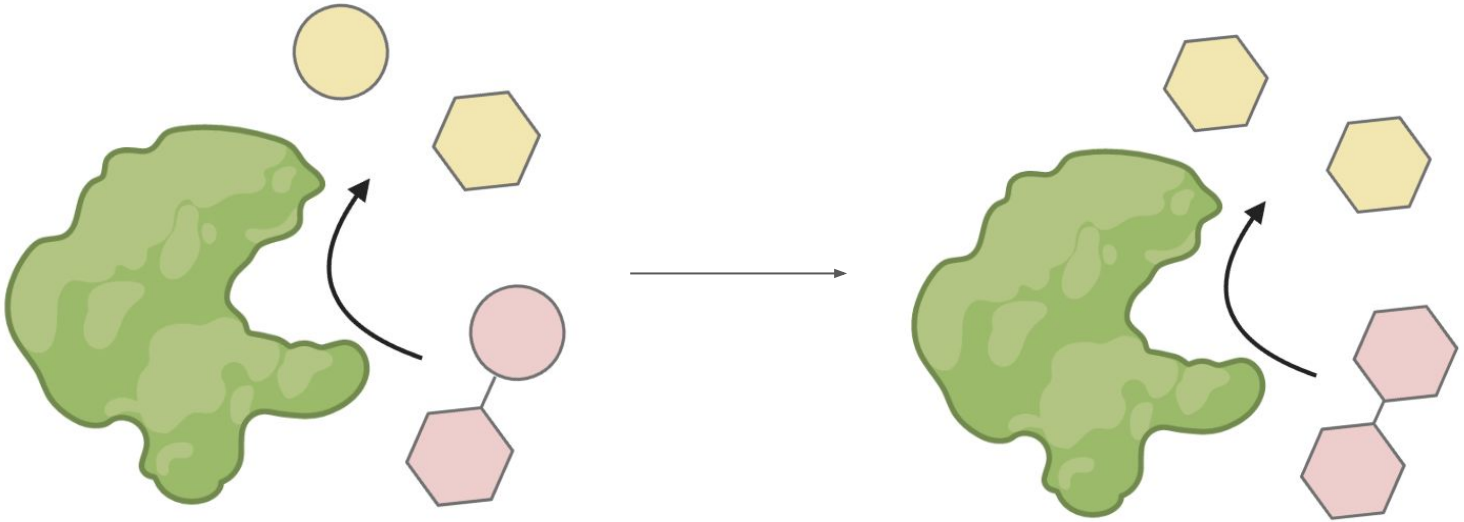
High substrate concentration can inhibit enzyme activity



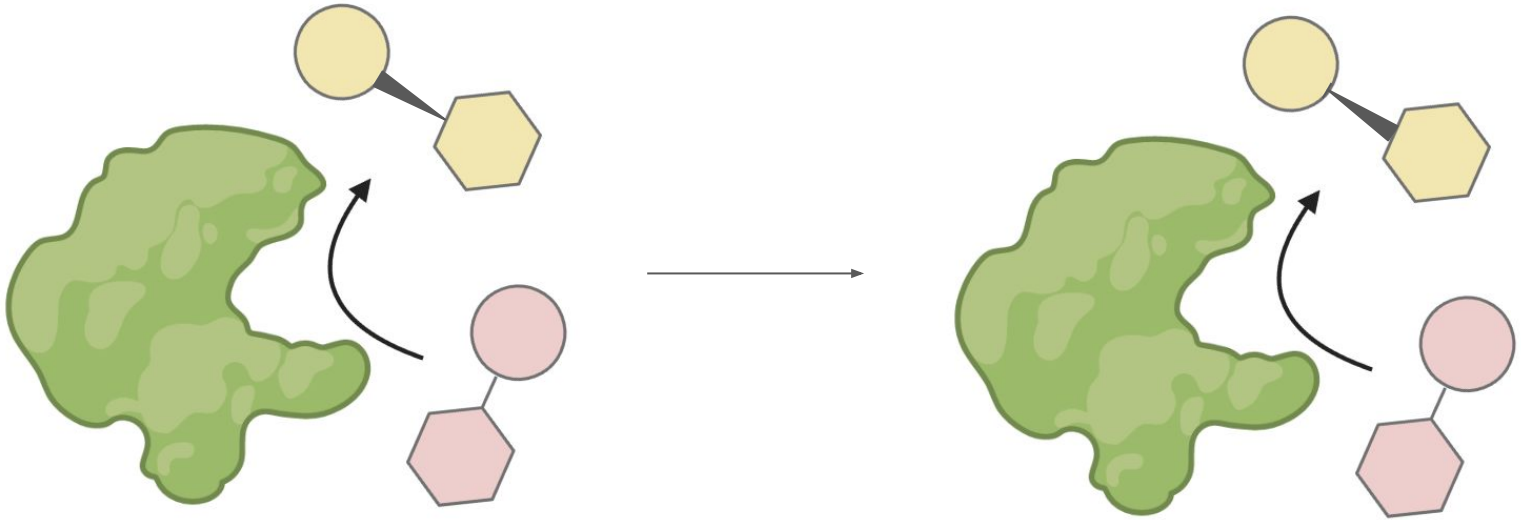
Known enzymes can be engineered for new function



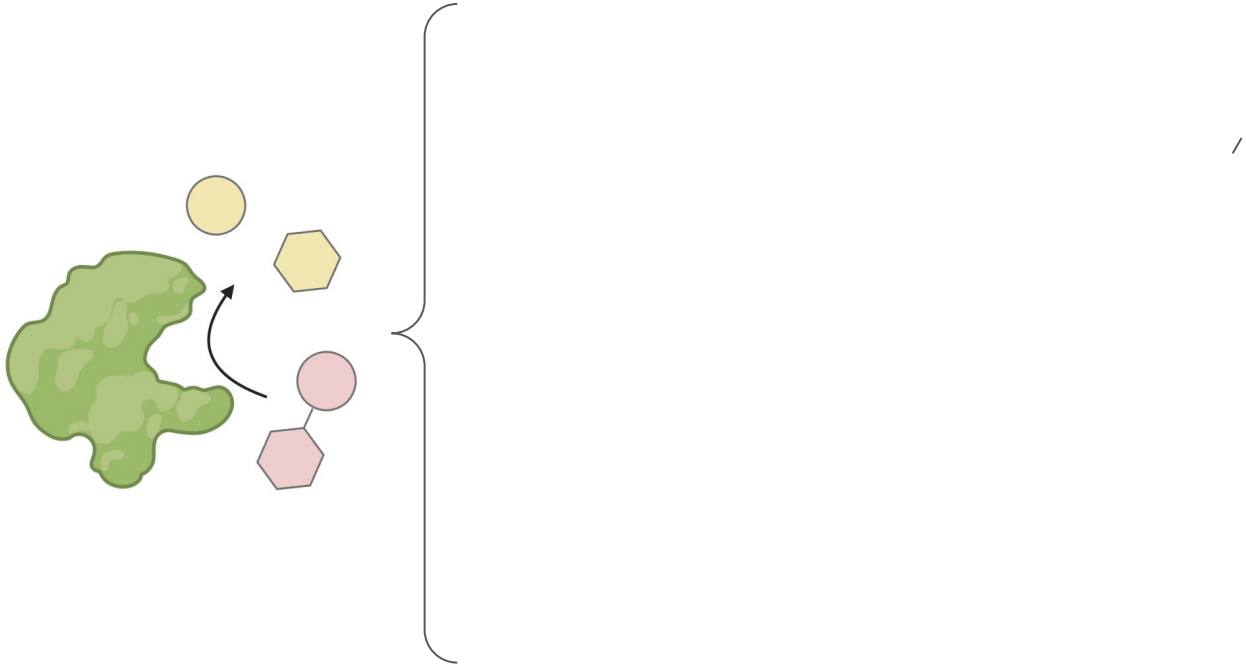
Easiest modification is to change substrate specificity



The features of generated product can be altered



Sometimes, non-catalytic features need to improve



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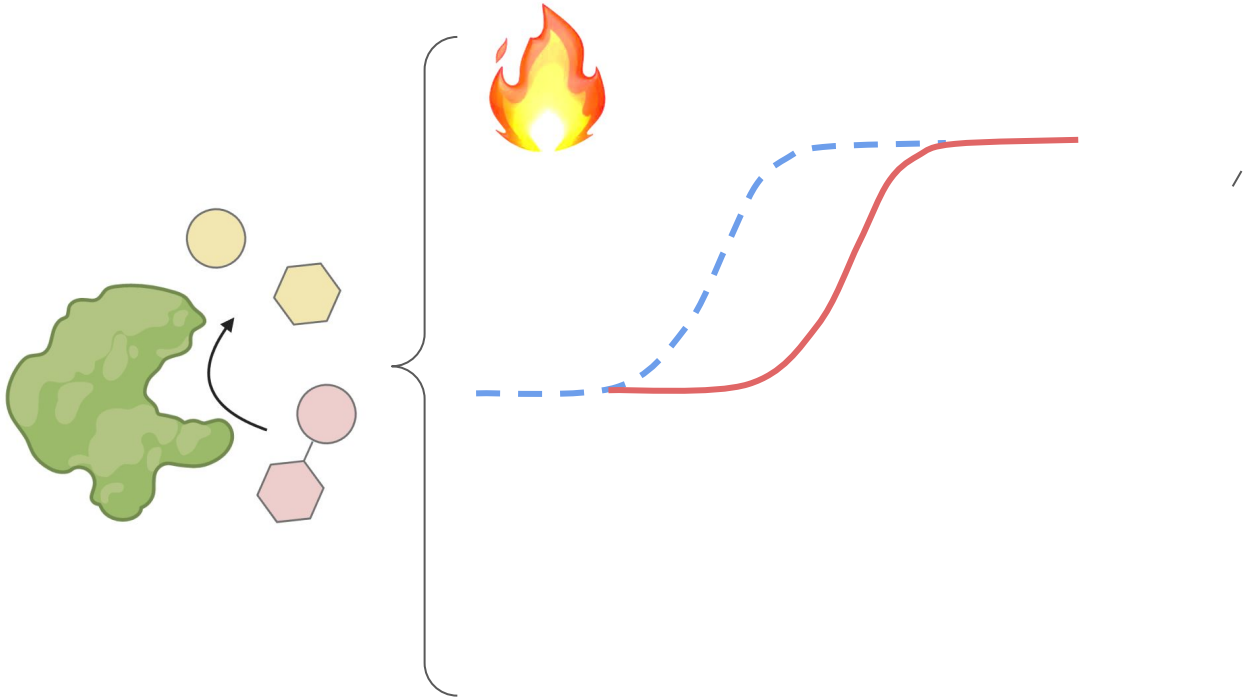

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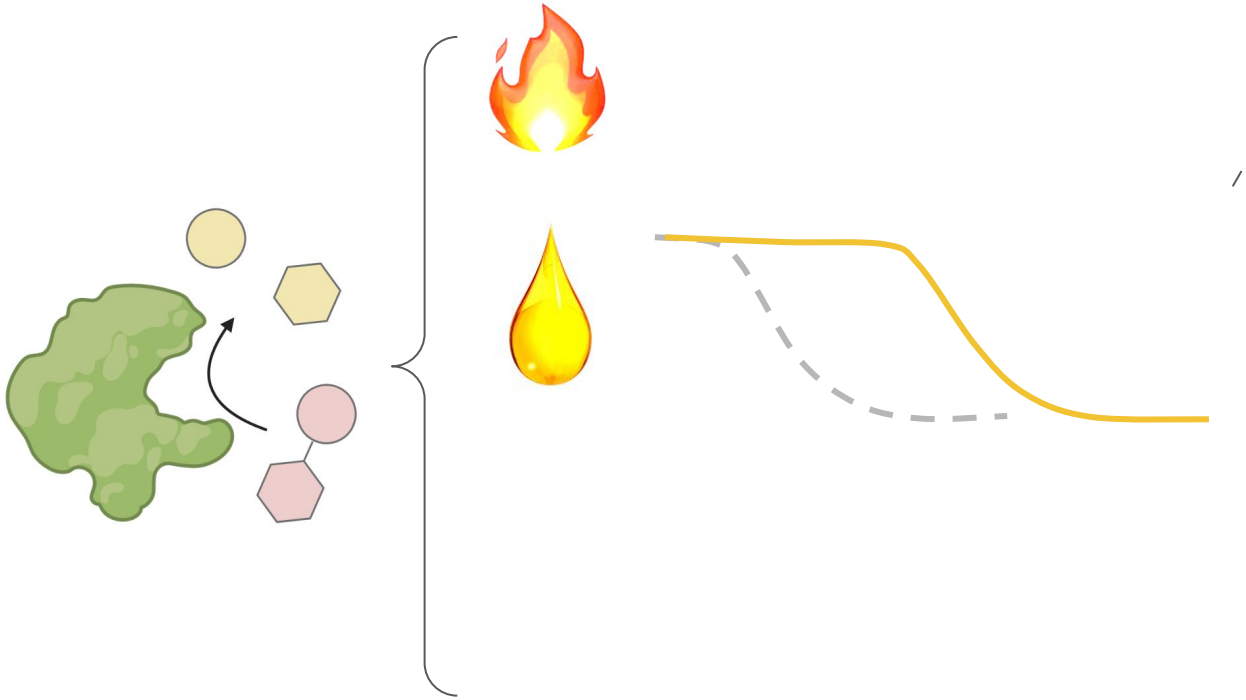

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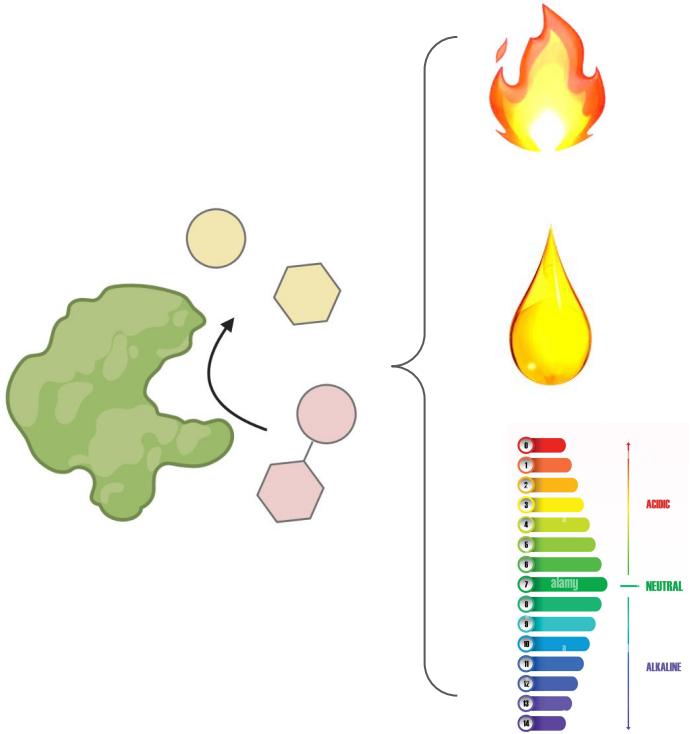
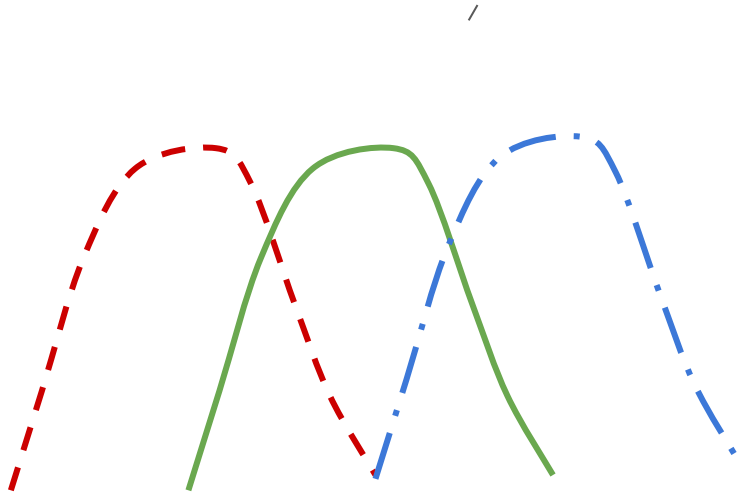
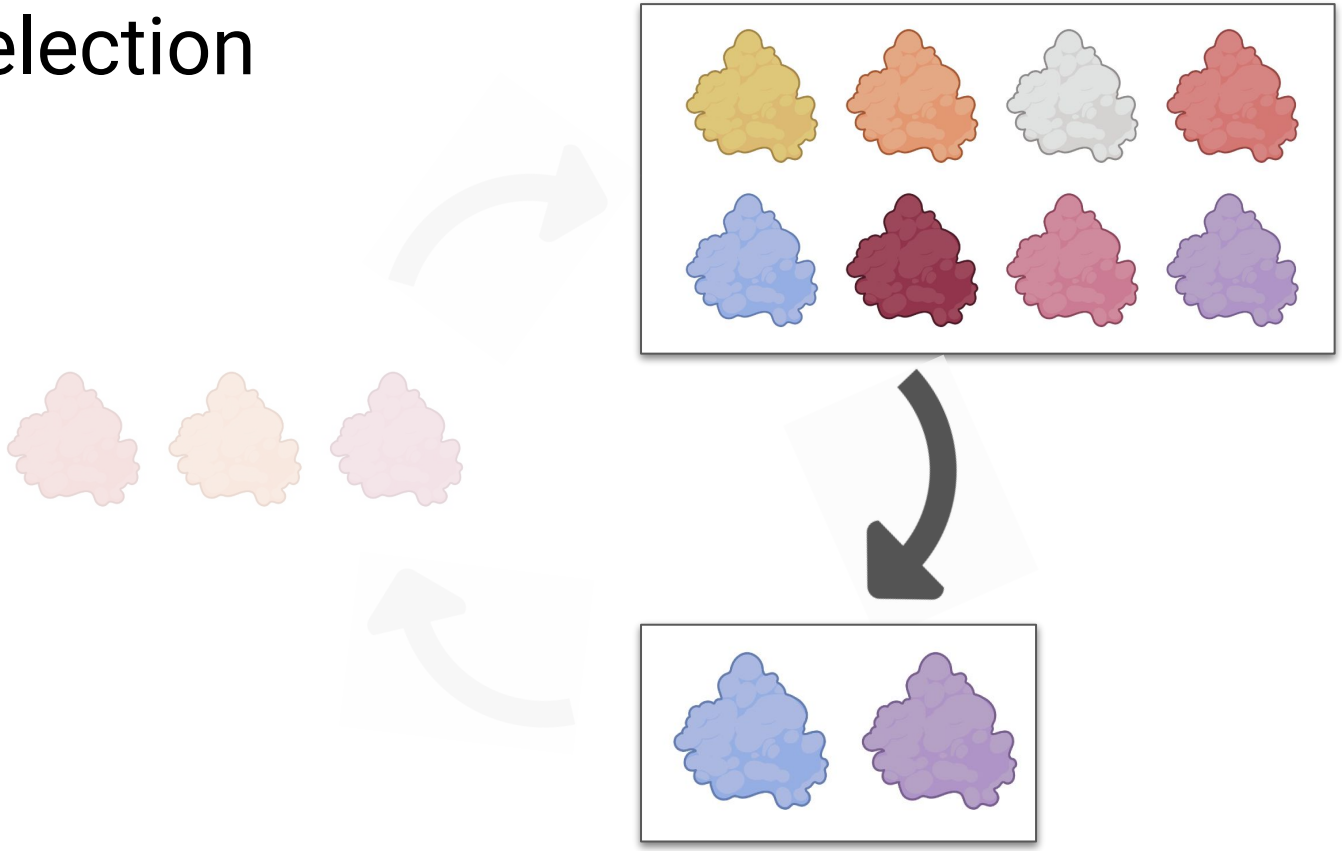




One of the key challenges in evolving enzymes is the selection



Enzyme-based life-death selection is a high throughput way to test enzyme activity

1. Antibiotic resistance
2.

Linking enzyme activity to fluorescence or luminescence signal is another HTP method

Requirements:

Linking enzyme activity to fluorescence or luminescence signal is another HTP method

Requirements:

- Generate signal
- Signal is contained within the cell
- Meaningful difference

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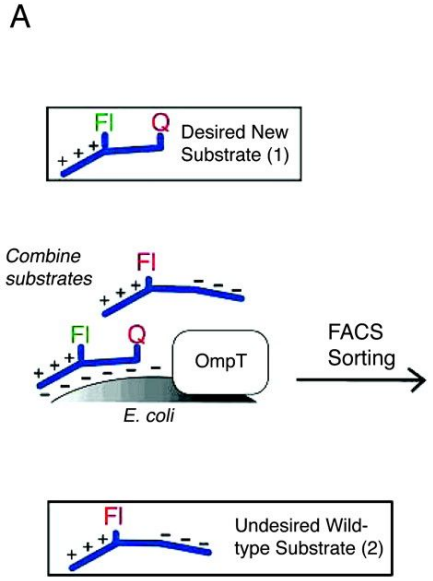

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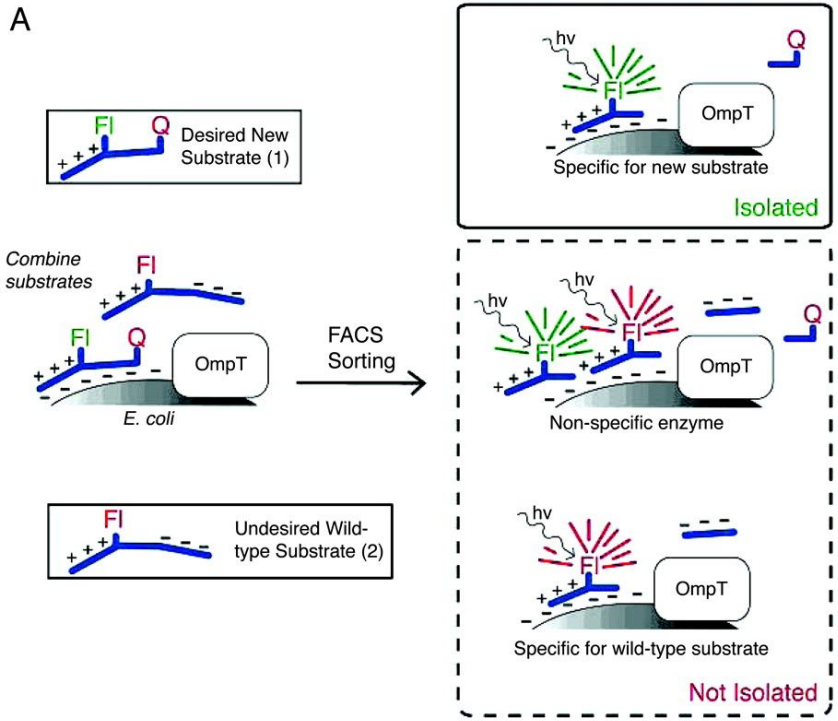


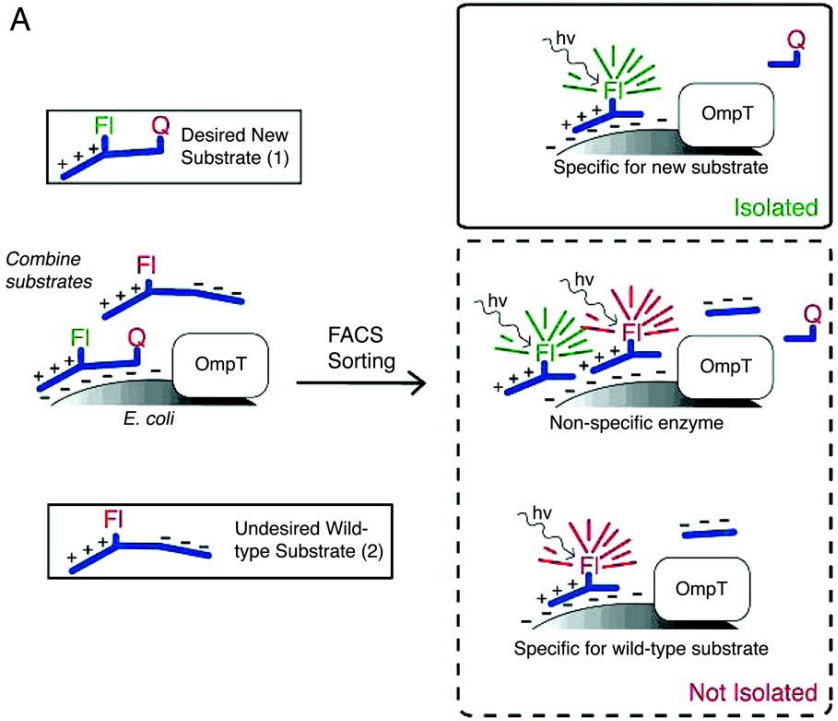
Linking enzyme activity to fluorescence or luminescence signal is another HTP method

Requirements:

- Generate signal
- Signal is contained within the cell
- Meaningful difference

Library generation:

- Error prone PCR
- Site directed mutagenesis



Linking enzyme activity to fluorescence or luminescence signal is another HTP method

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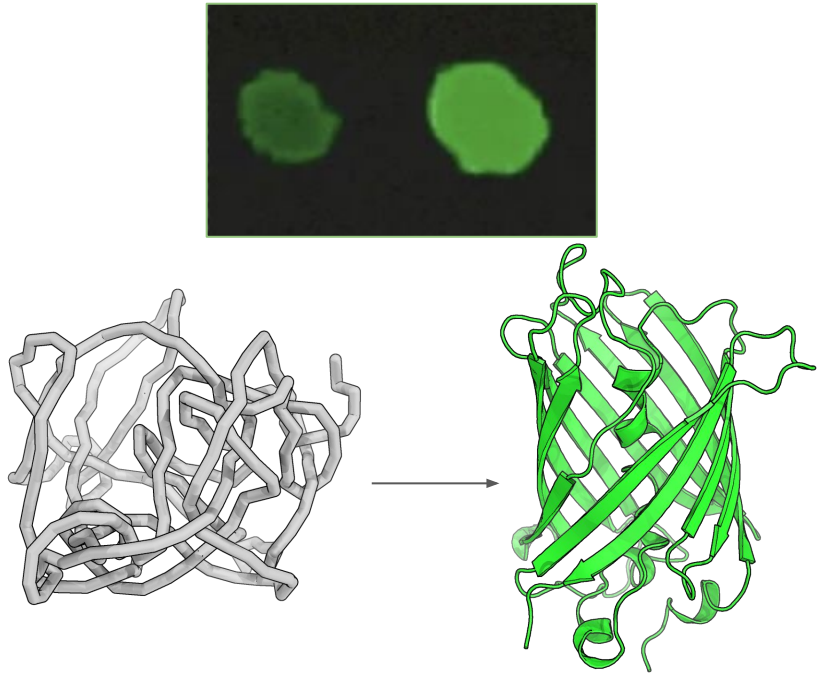


Nature Biotechnology 20, 927–932(2002)

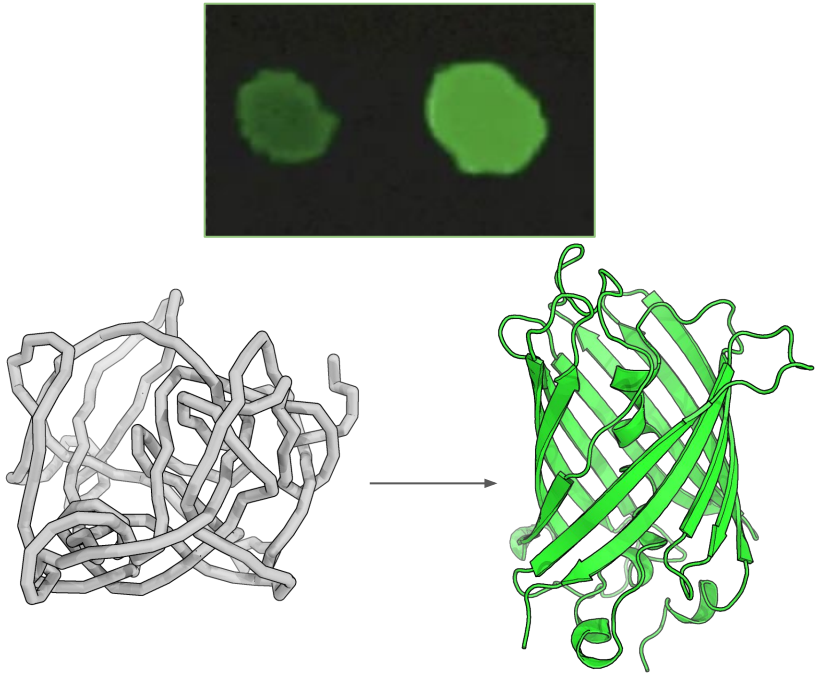
Linking enzyme activity to fluorescence or luminescence signal is another HTP method

Requirements:

- Generate signal
- Signal is contained within the cell
- Meaningful difference

Library generation:

- 4x forward evolution (shuffling)



Nature Biotechnology 20, 927–932(2002)

Colors can be helpful as a medium throughput screening method

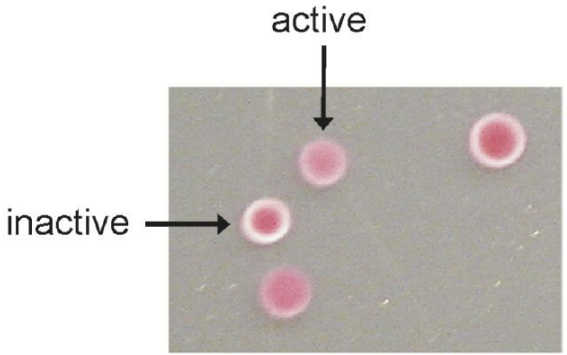
Requirements:

- Generate signal
- No need for containment
- Meaningful difference = easy to pick

Colors can be helpful as a medium throughput screening method

Requirements:

- Generate signal
- Signal is contained within the cell
- Meaningful difference = easy to pick



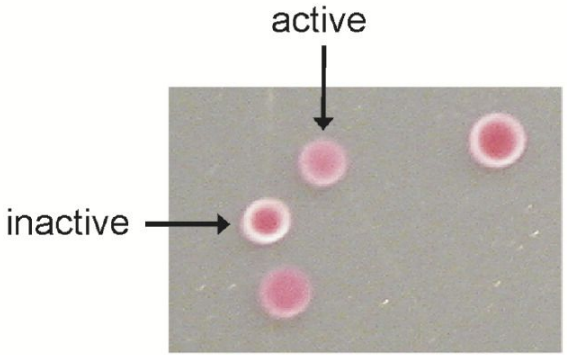
Colors can be helpful as a medium throughput screening method

Requirements:

- Generate signal
- Signal is contained within the cell
- Meaningful difference = easy to pick

Library generation:

- Error prone PCR (5-7 bp per gene)
- DNA shuffling



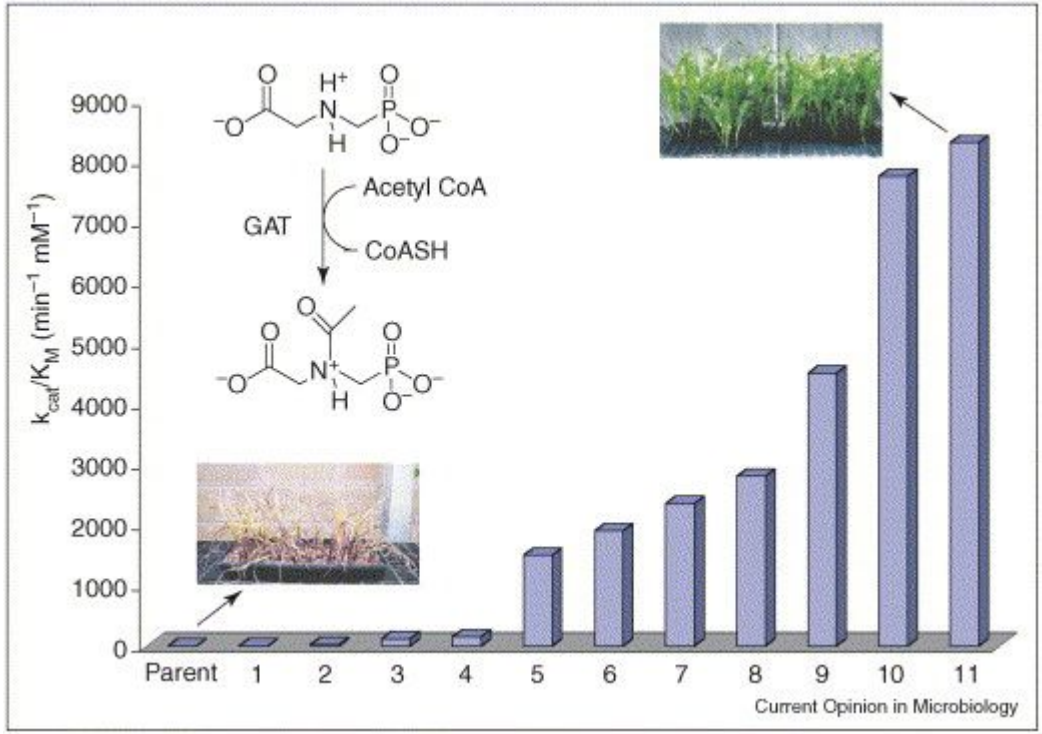
Running low throughput assays is sometimes the only way to screen

Library generation:

11 rounds of shuffling from 3 starting proteins

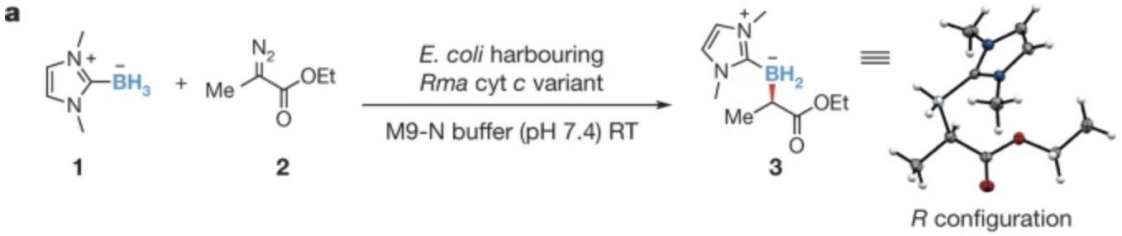
Screening:

Growth and measurement of product formation in media



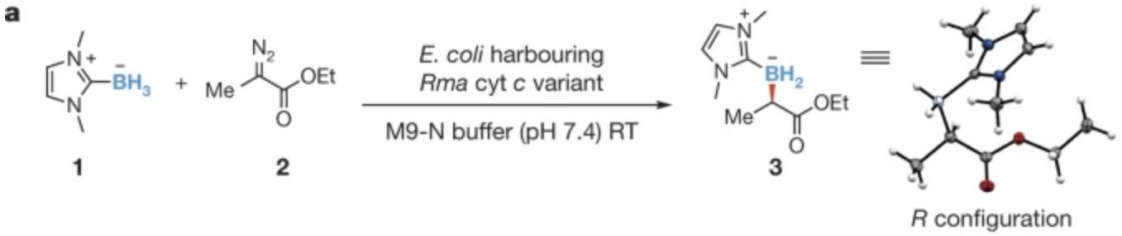
Creating completely new reactivities is a more challenging task

Creating completely new reactivities is a more challenging task – the case of C-X bond



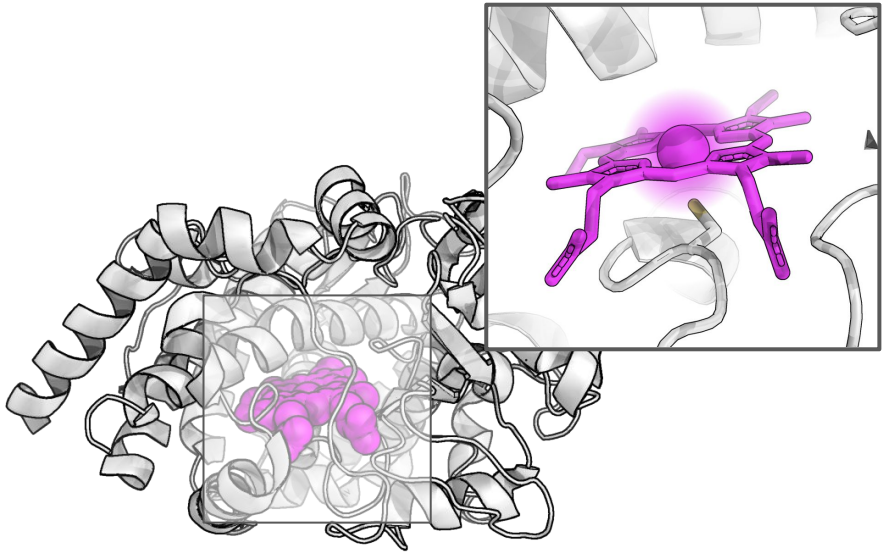
Nature 552, 132–136(2017)

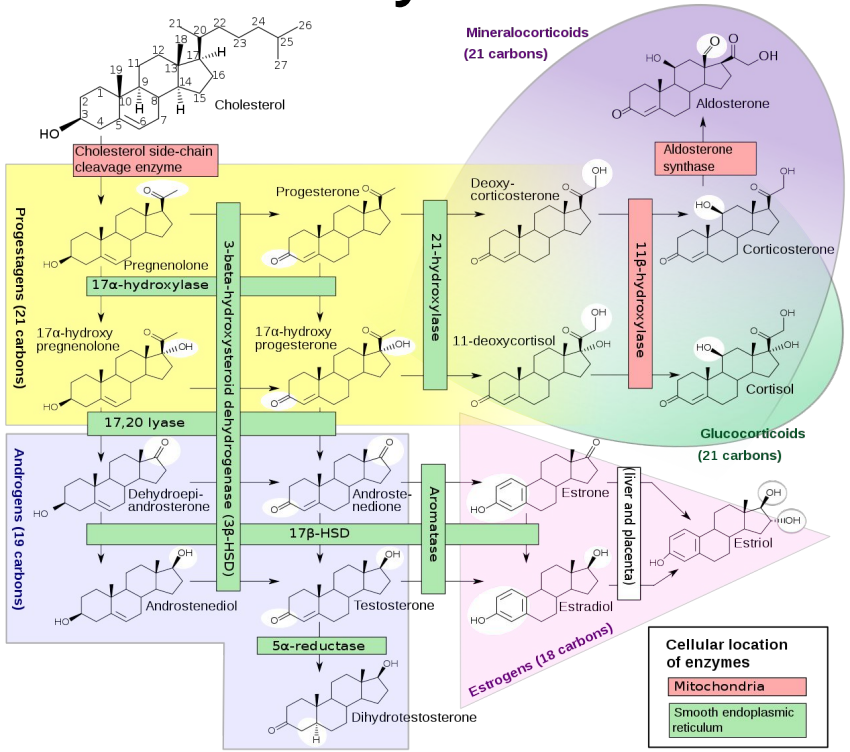
Creating completely new reactivities is a more challenging task – the case of C-X bond



C-X bond: In search of a proper scaffold

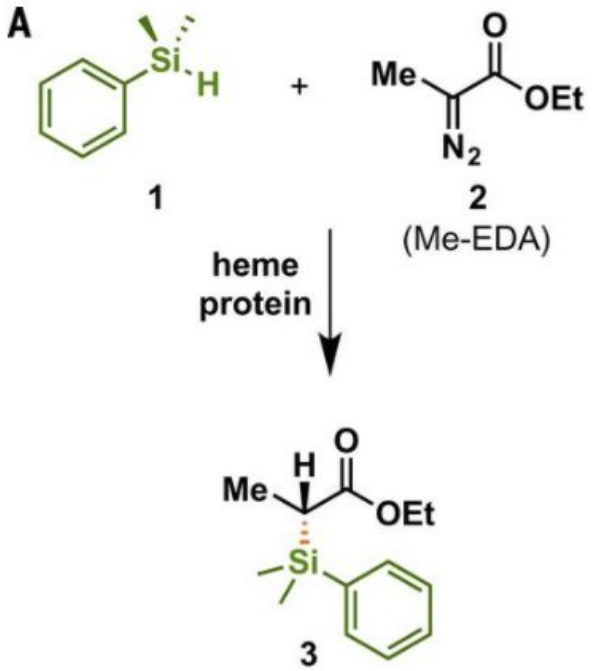
C-X bond: In search of a proper scaffold → Heme proteins offer reactivity and versatility

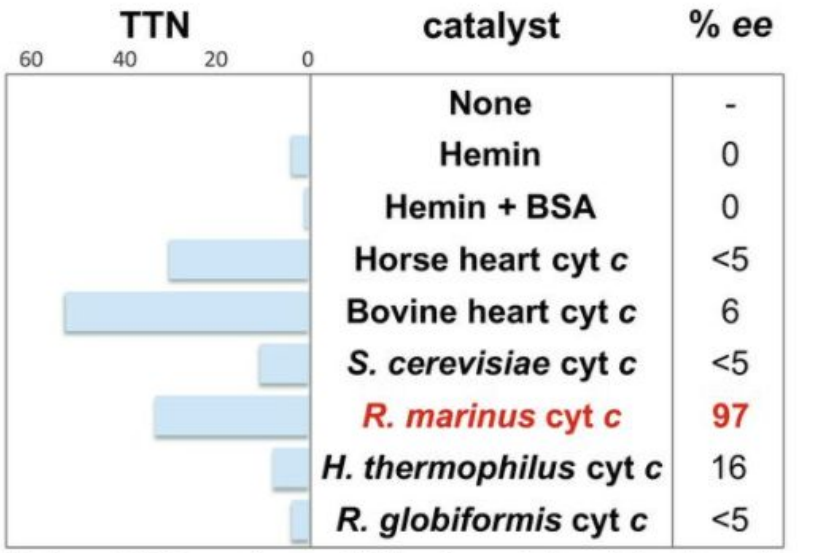




Mikael Hägström, Wikipedia

C-X bond: Just a little bit of initial activity …

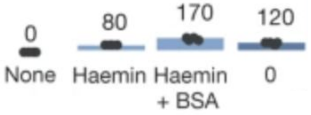




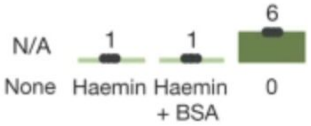
Various **P450s** and **myoglobin** also catalyzed the formation of carbon–silicon bonds, but the reactions were not enantioselective (see Supplementary Materials).

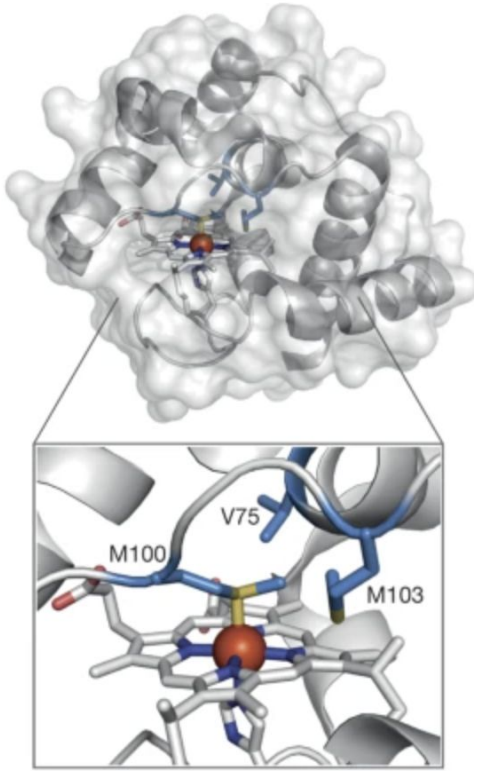
C-X bond: Evolving the scaffold

Turnover

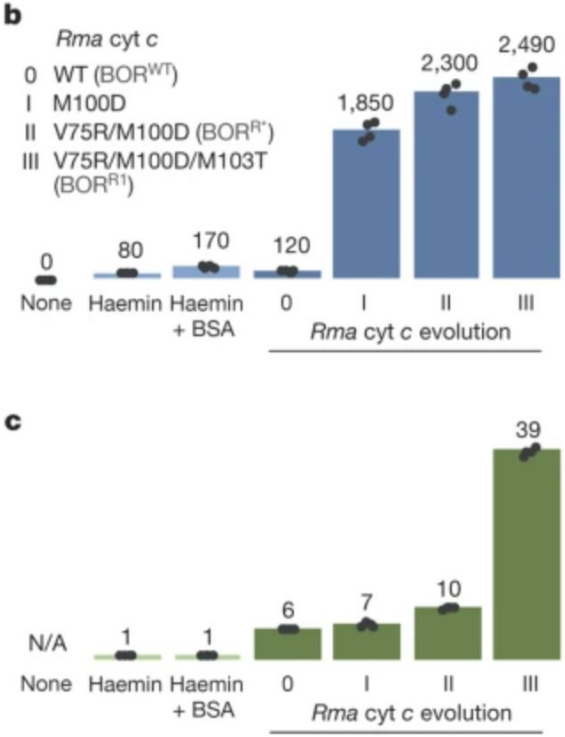


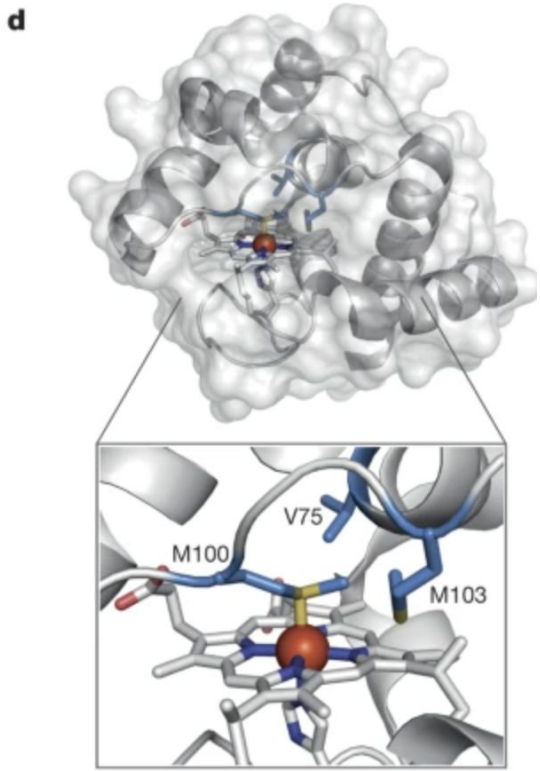
enantioselectivity





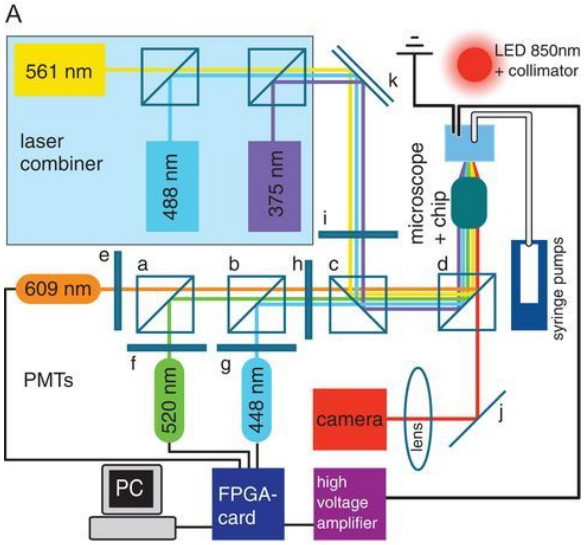
C-X bond: Evolving the scaffold



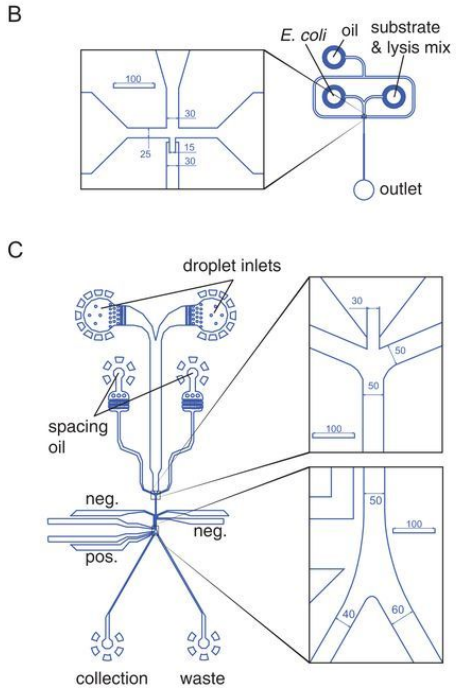


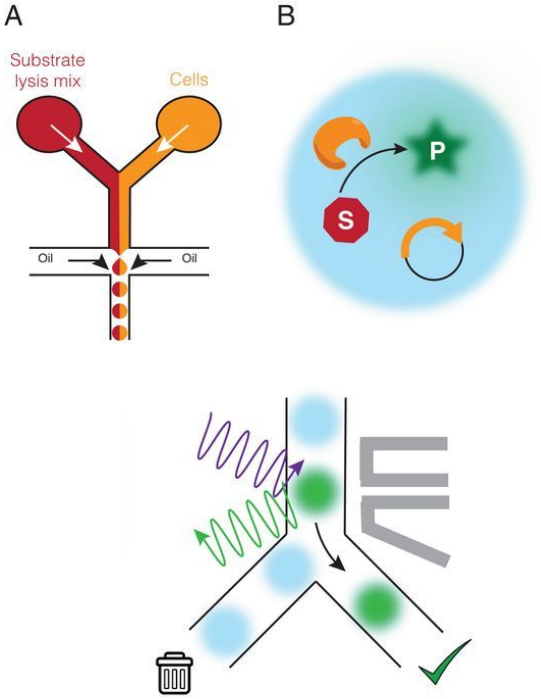
Science 25 Nov 2016:Vol. 354, Issue 6315, pp. 1048-1051

New technologies open up new avenues for evolving enzymes



(a) Di02-R561
(b) Di02-R488
(c) Di01-R405/488/561/635
(d) FF665-Di02
(e) FF01-609/57-25
(f) FF02-520/28-25
(g) FF01-448/20-25
(h) NF01-405/488/561/635
(i) ND 0/0.5/1
(j) broadband mirror
(k) persicope w/ broadband mirror





Protein Engineering, Design and Selection, Volume 29, Issue 9, September 2016, Pages 355–366

For the next lecture:

1. Be ready for our panel with lots of questions
2. Have your proposal in mind. We'll be walking through specific aims.

Next lecture:

Protein Engineering in Action

Per Jr. Greisen

Director for Computational Drug Discovery

Novo Nordisk

Manasi Pethe

Protein Engineering Data Scientist

Bayer Crop Science

Alex Carlin

Protein Engineer

Ginkgo Bioworks

Amandeep Sangha

Research Scientist

Arzeda