Article

Distal Mutations Shape Substrate-Binding Sites during Evolution of a Metallo-Oxidase into a Laccase

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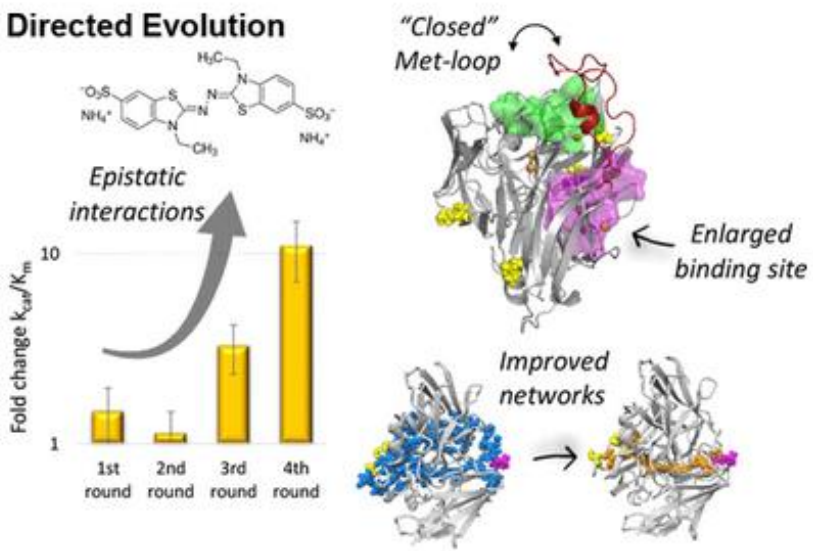

ACS Catalysis

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ABSTRACT

Biocatalysis for Industrial Biotechnology

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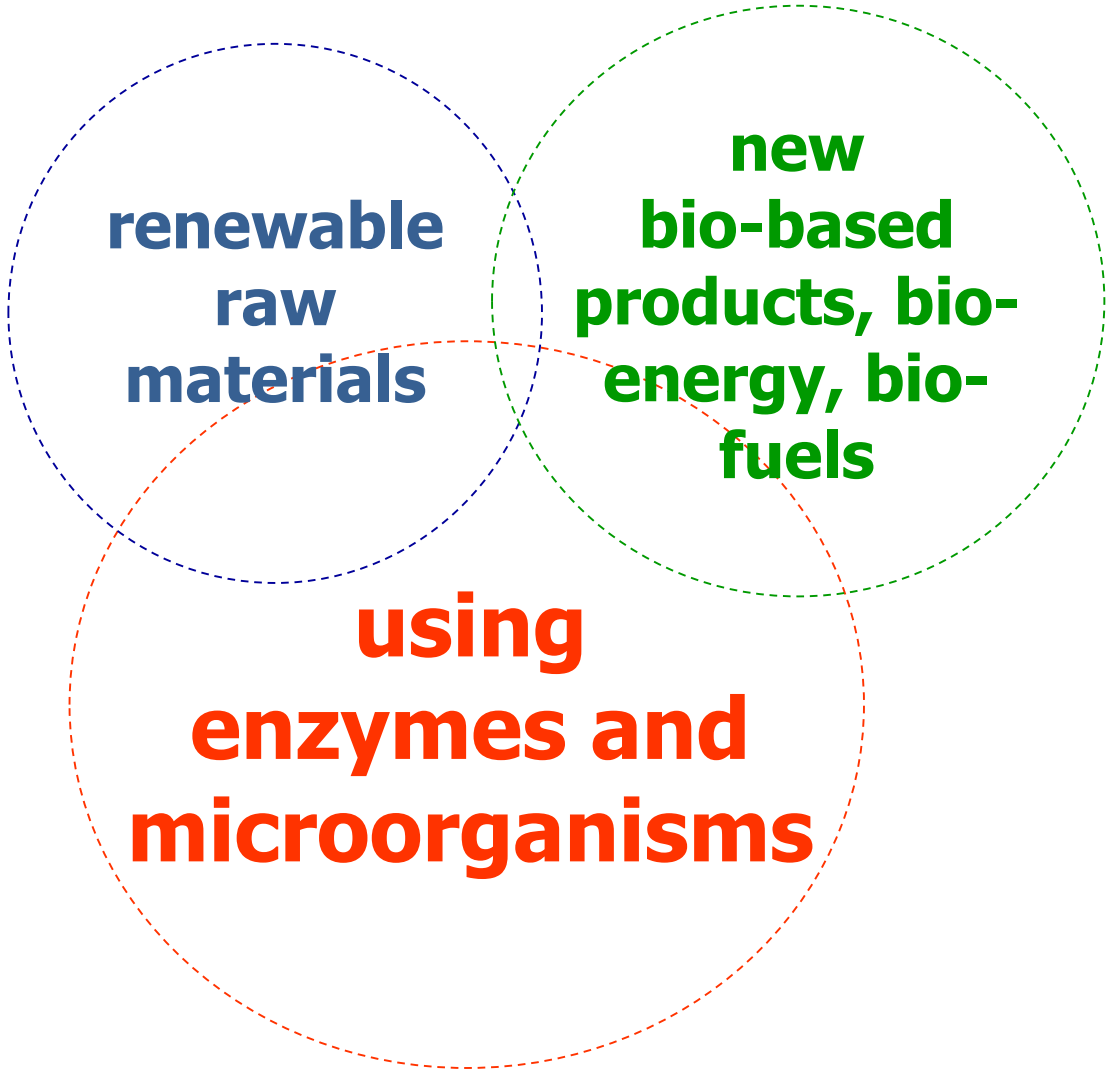
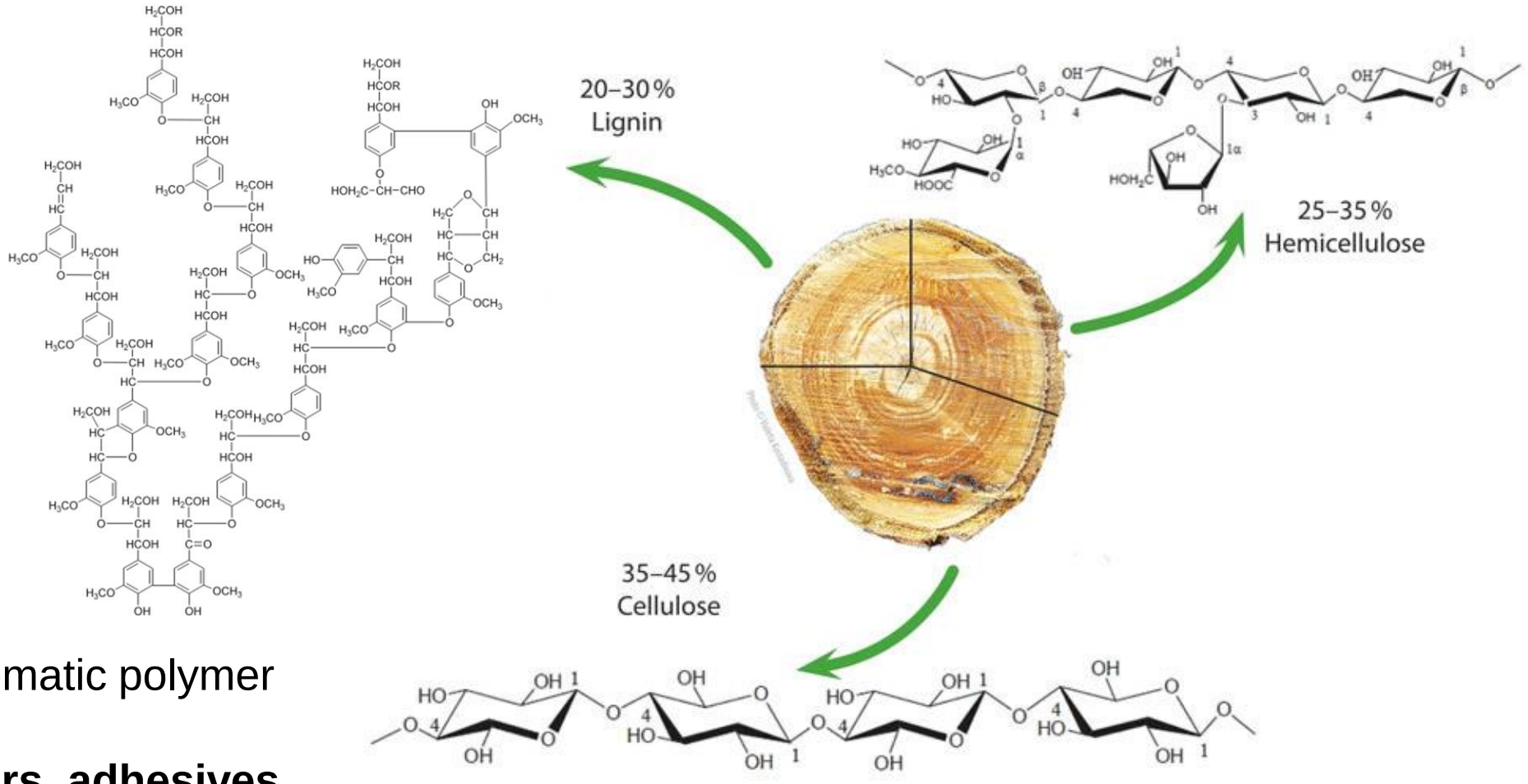
microbial & enzyme technology lab

white or industrial biotechnology for a bio-based society



transition for the post-petroleum society

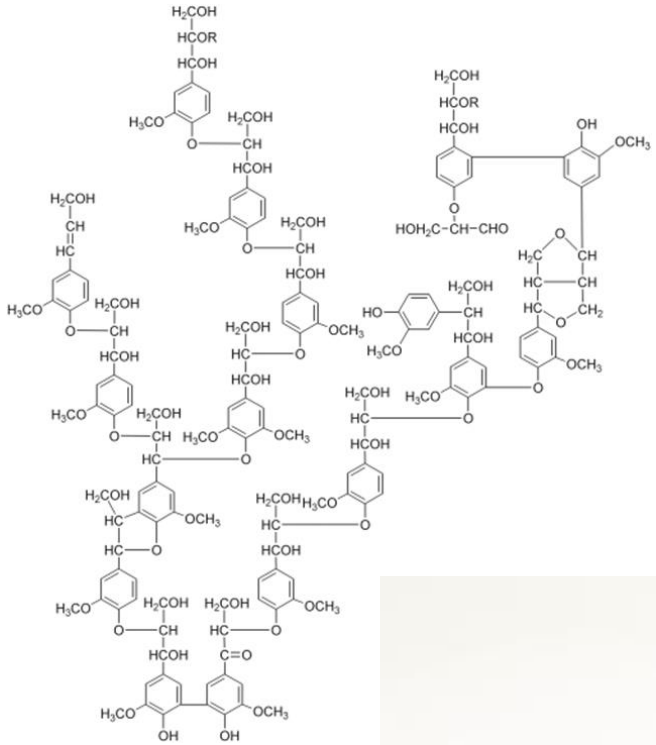
LIGNOCELLULOSE BIOREFINERIES



- Most abuntant aromatic polymer on Earth
- Source of **polymers**, **adhesives**, **coatings**, **resins**, **surfactants**, **anti-oxidants**, **cosmetics**

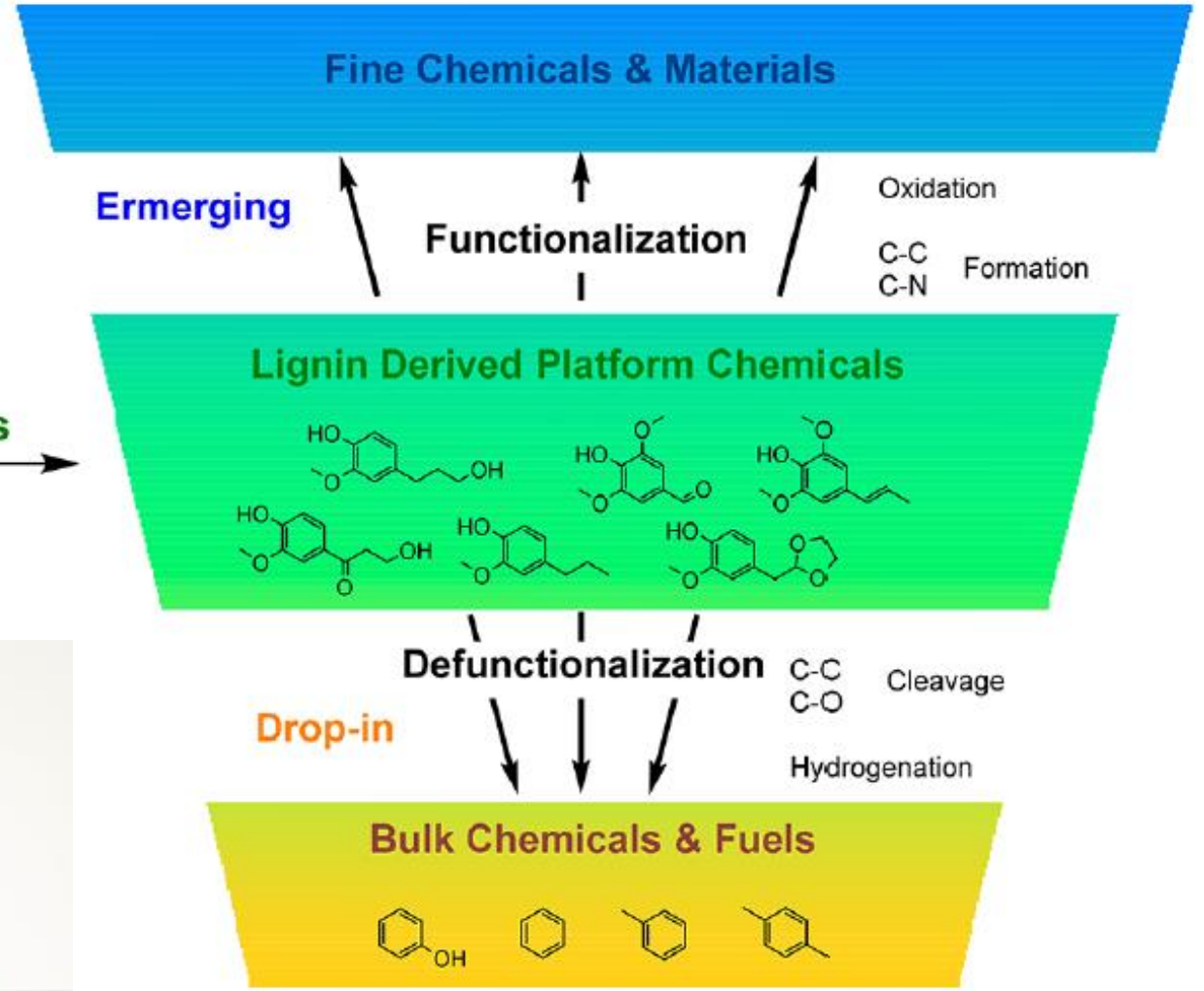
Lignin: from waste to value-added products

Pharmaceutical drugs, food additives, resins, cosmetics



Catalytic Methods

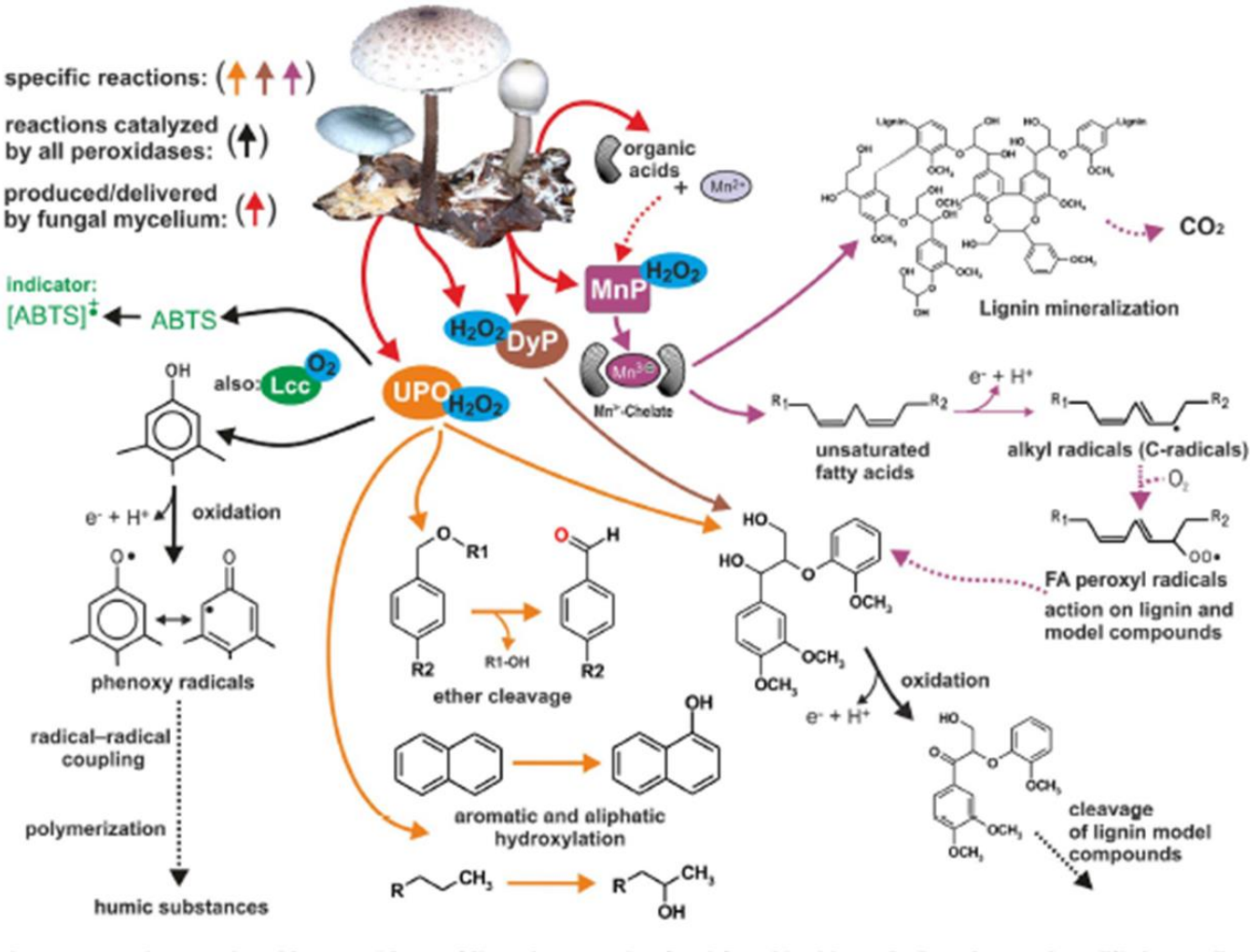
β-O-4 Cleavage



Gasoline-range aromatics, benzene, toluene, xylene

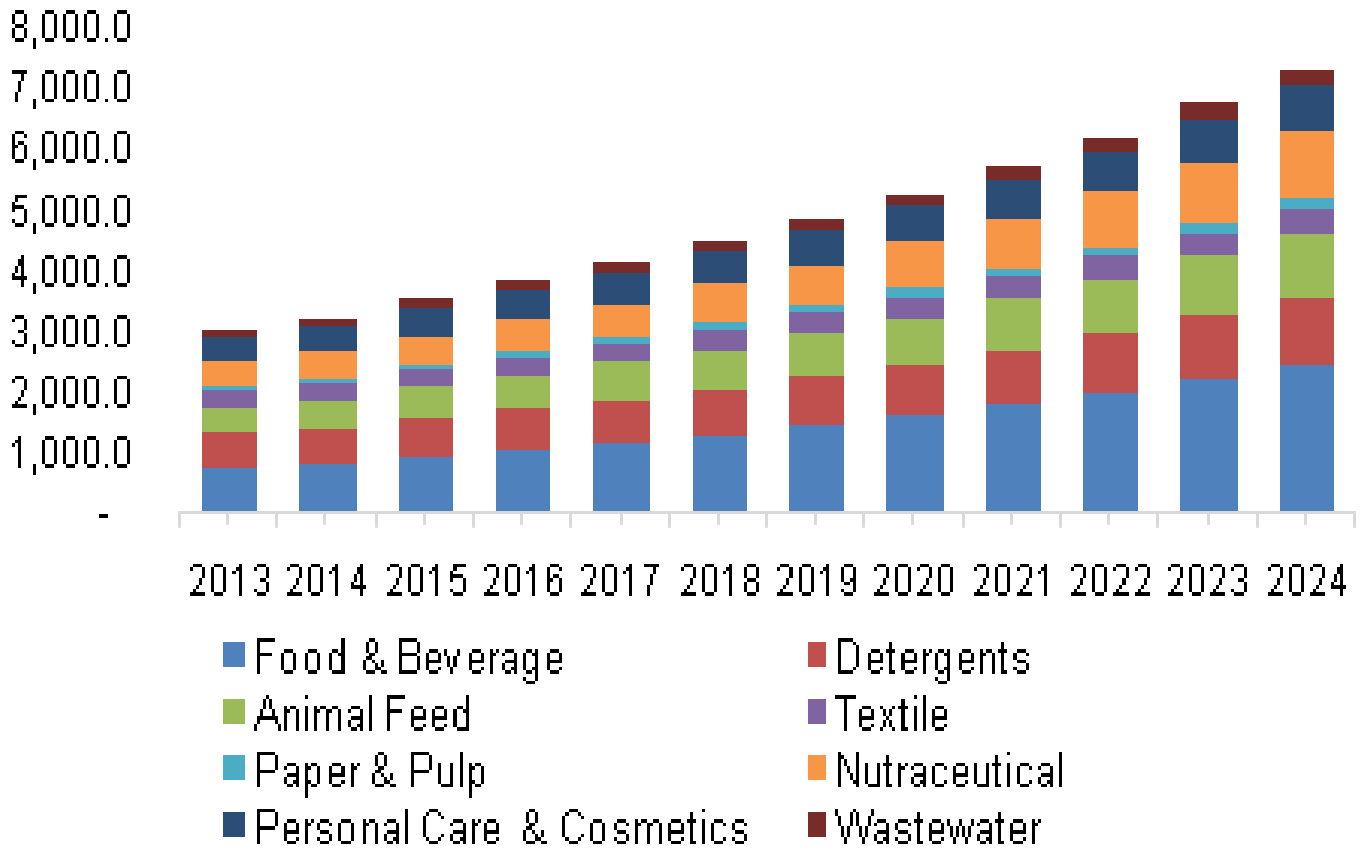
Sun et al 2018 Chem Rev 118: 614

Ligninolytic enzymes: laccases and peroxidases



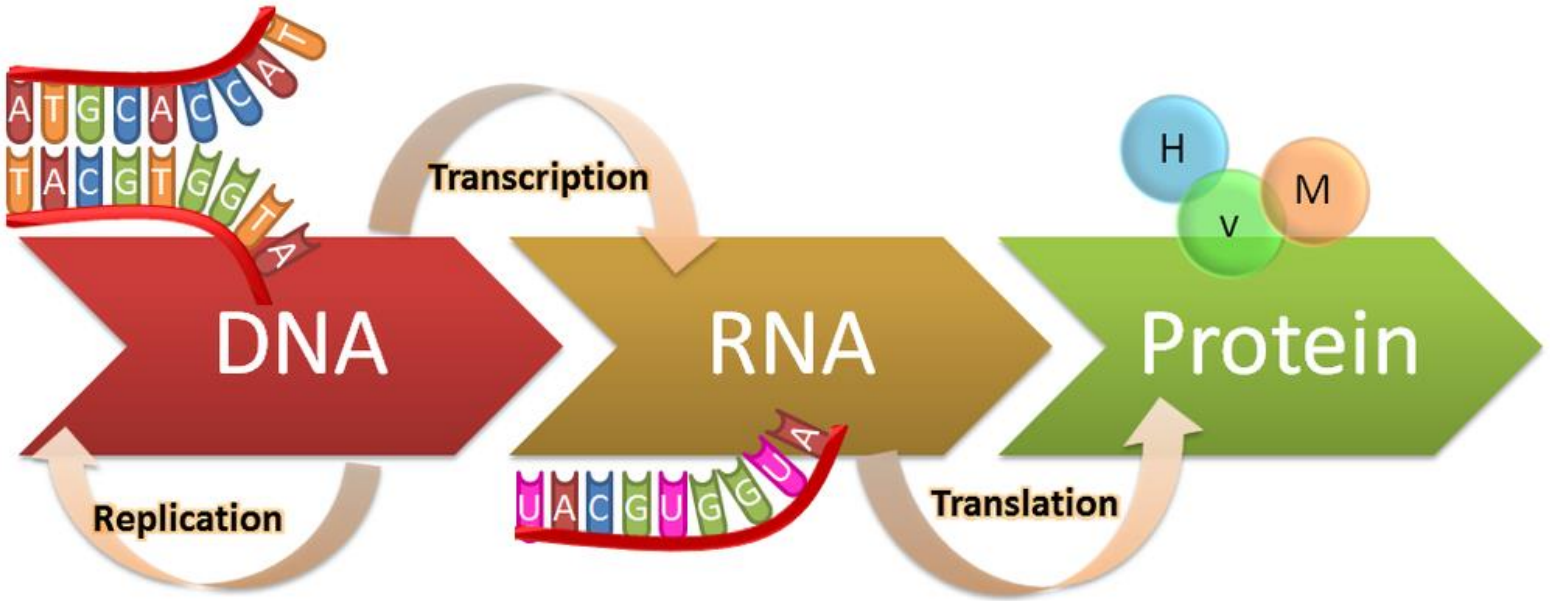
Kellner et al. 2014 PLOS ONE e95557

Industrial enzyme market

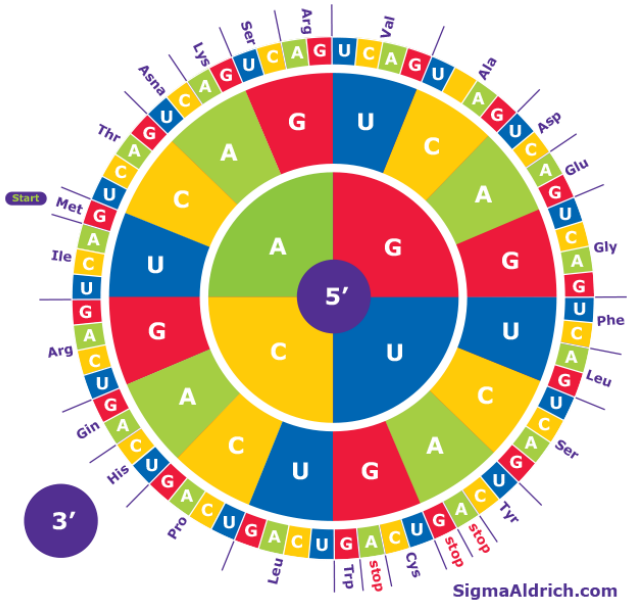


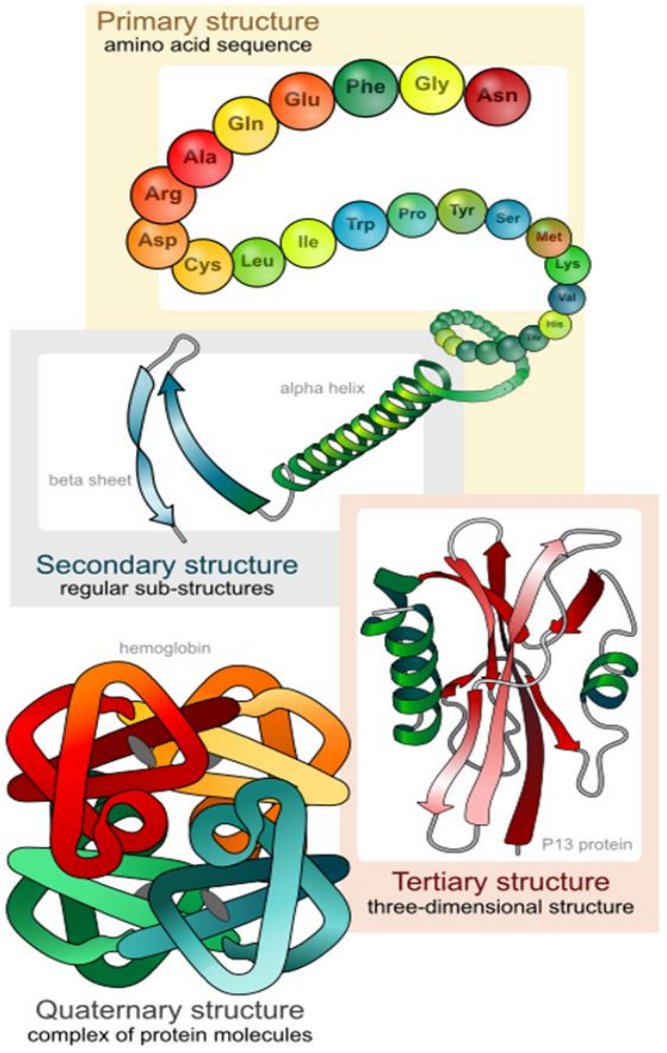
Enzymes evolved for the survival benefit of an organism usually do not exhibit features essential for *in vitro* application

- **60% of such enzymes are recombinant products**
- **most have been engineered to enhanced functional properties (k_{cat}, k_{cat}/K_m, stability)**



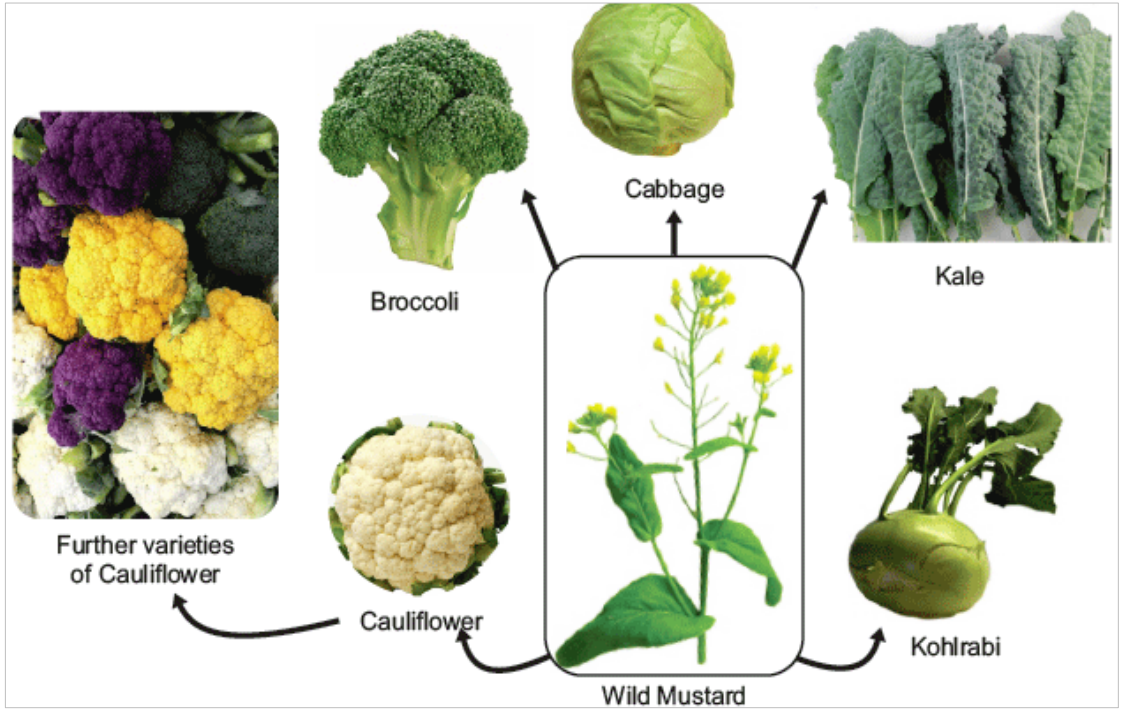
amino acid codon wheel

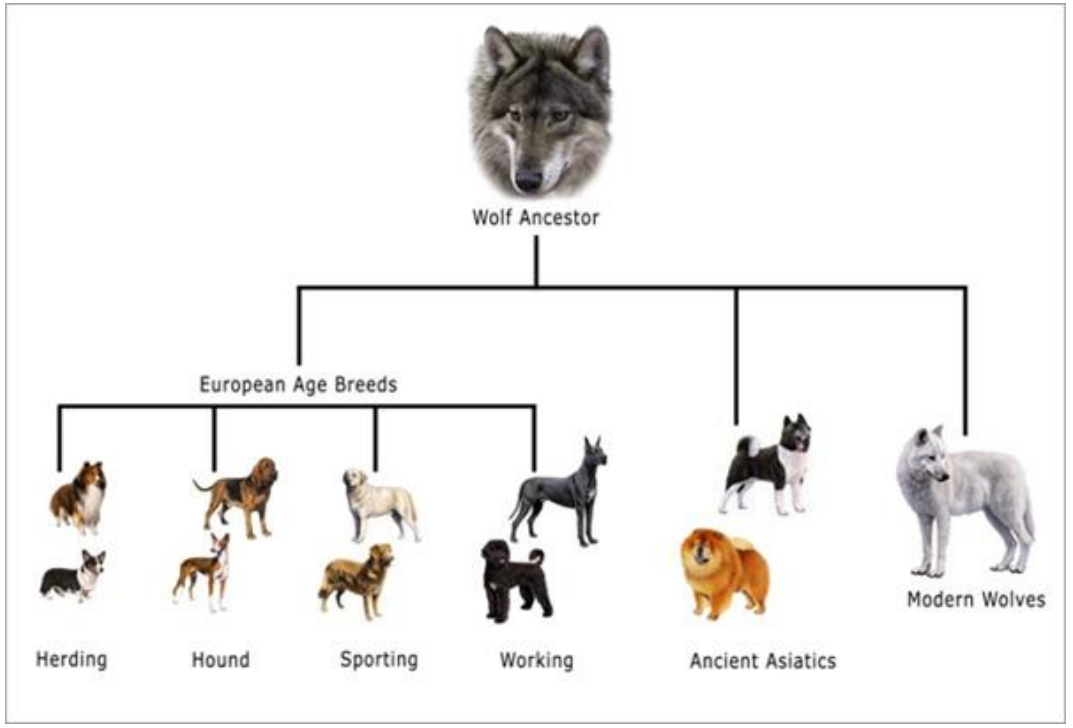




Artificial evolution has been used for thousands of years to solve human problems

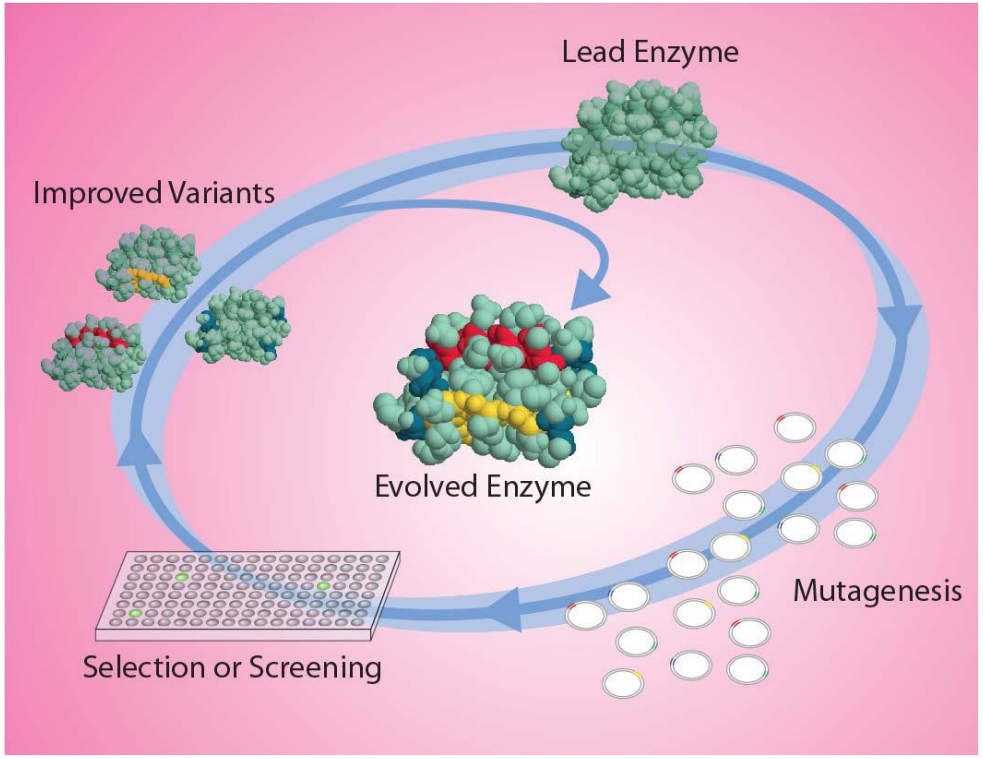
only the plants and animals with desirable characteristics to reproduce, causing the evolution of farm stock





Frances H Arnold
Nobel Prize of Chemistry 2018

DIRECTED EVOLUTION



RATIONAL DESIGN

1. Computer aided design

2. Site-directed mutagenesis

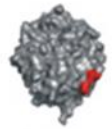
Individual mutated gene

3. Transformation

4. Protein expression

5. Protein purification

6. *not applied*



Constructed mutant enzyme

DIRECTED EVOLUTION

1. *not applied*

2. Random mutagenesis

Library of mutated genes
(>10,000 clones)

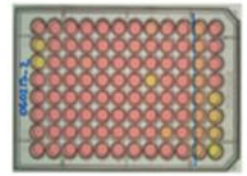
3. Transformation

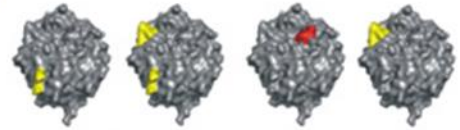
4. Protein expression

5. *not applied*

6. Screening and selection

- stability
- selectivity
- affinity
- activity



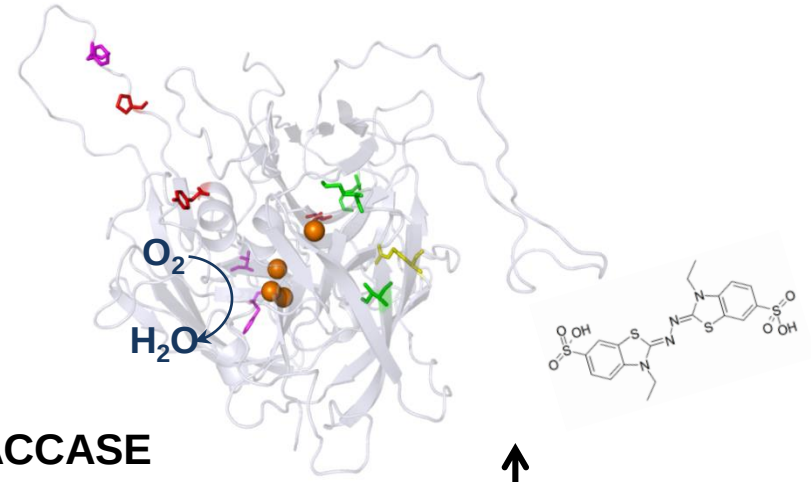


Selected mutant enzymes

IMPROVED ENZYME

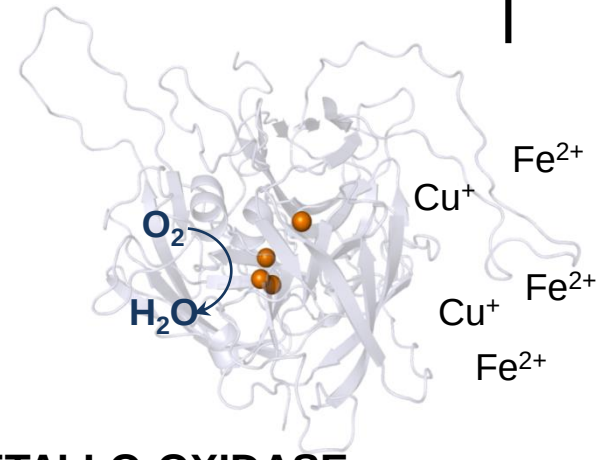
7. Biochemical testing

Turning an hyperthermophilic metalloxidase into a laccase



LACCASE

epPCR
>90,000 clones



METALLO-OXIDASE

2B3
F17S
V19A
F55S
P58S
Y172C
I199T
R471G
K246R
I441L
M449T

1D11
P58S
Y172C
I199T
R471G
K246R
I441L
M449T

2G6
R471G
K246R
I441L
M449T

1B7
K246R
I441L
M449T

WILD-TYPE

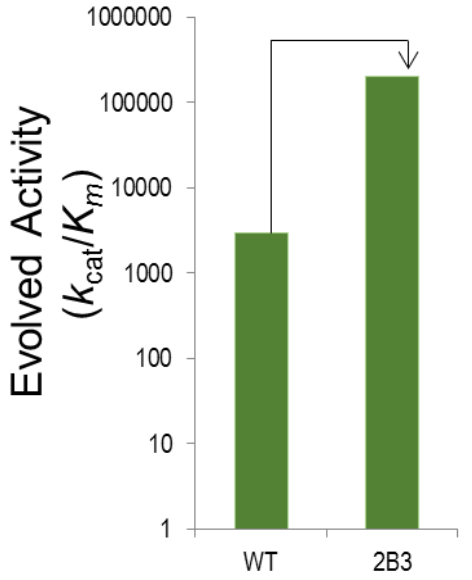
4th
2 mM ABTS
Ai = 37x

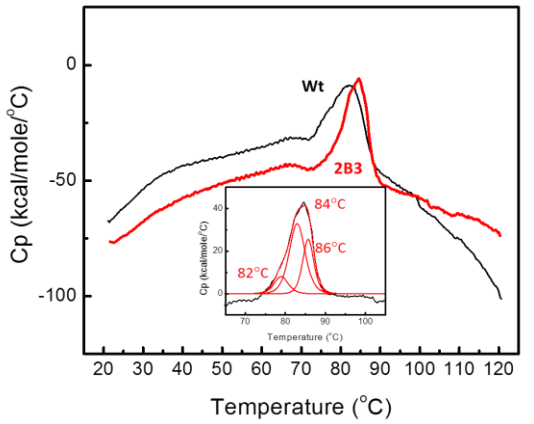
3rd
2.5 mM ABTS
Ai = 9x

2nd
4 mM ABTS
Ai = 2.6x

1st
10 mM ABTS
Ai = 2x

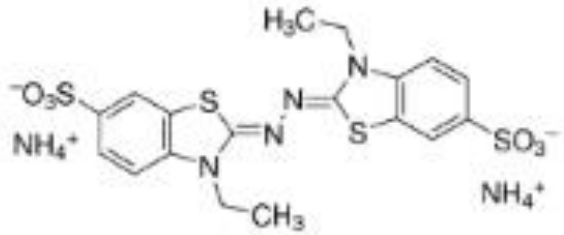
100-fold improvement for the aromatic ABTS

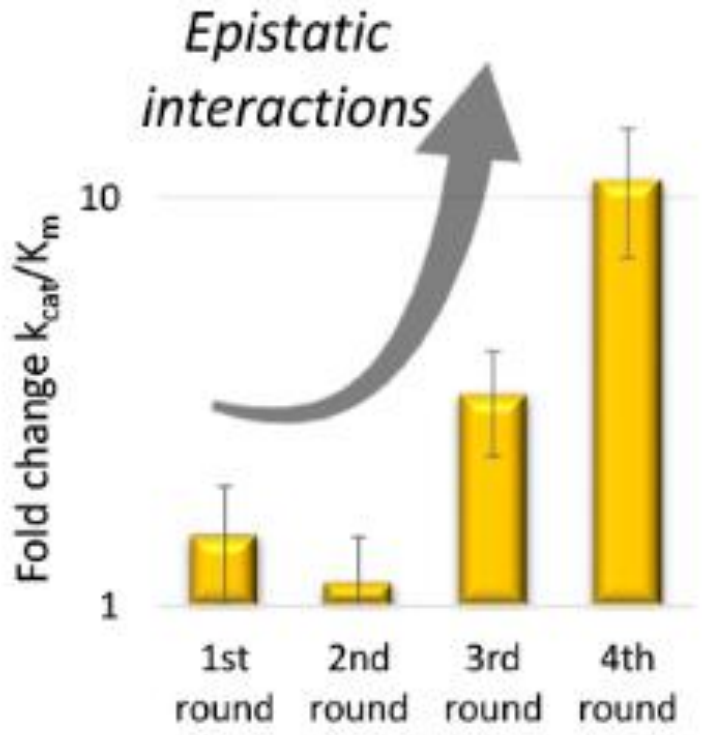


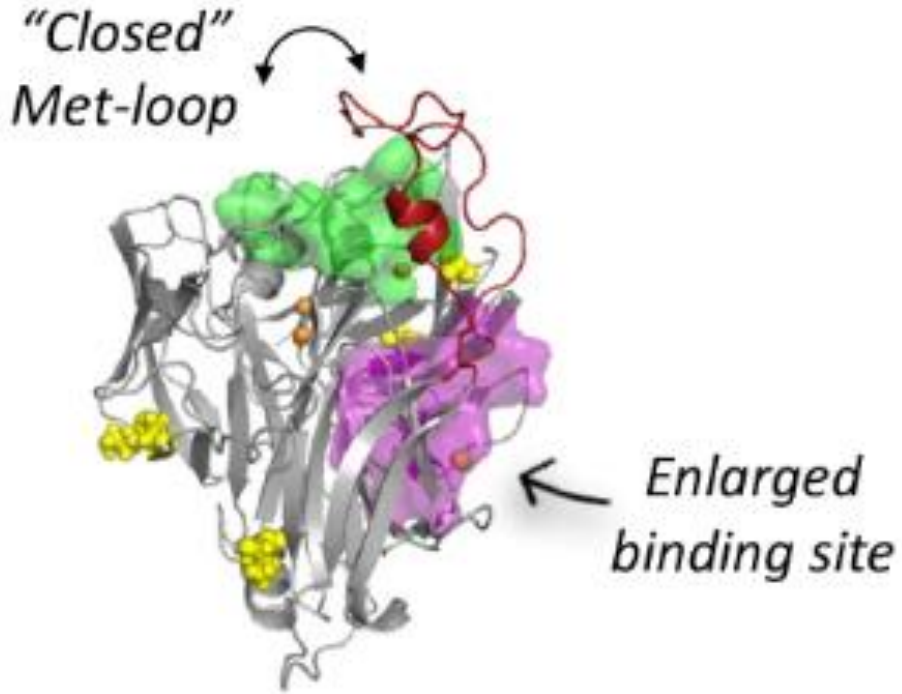


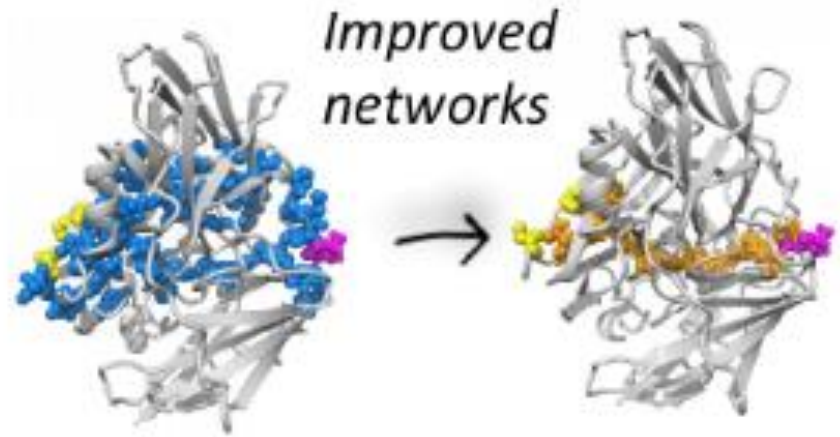
Brissos V. et al. (2015), ACS Catalysis, 5:4932-4941

Directed Evolution









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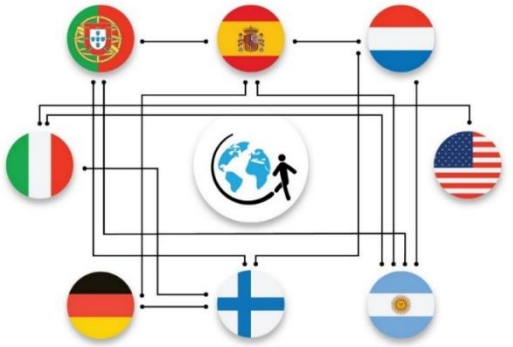

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