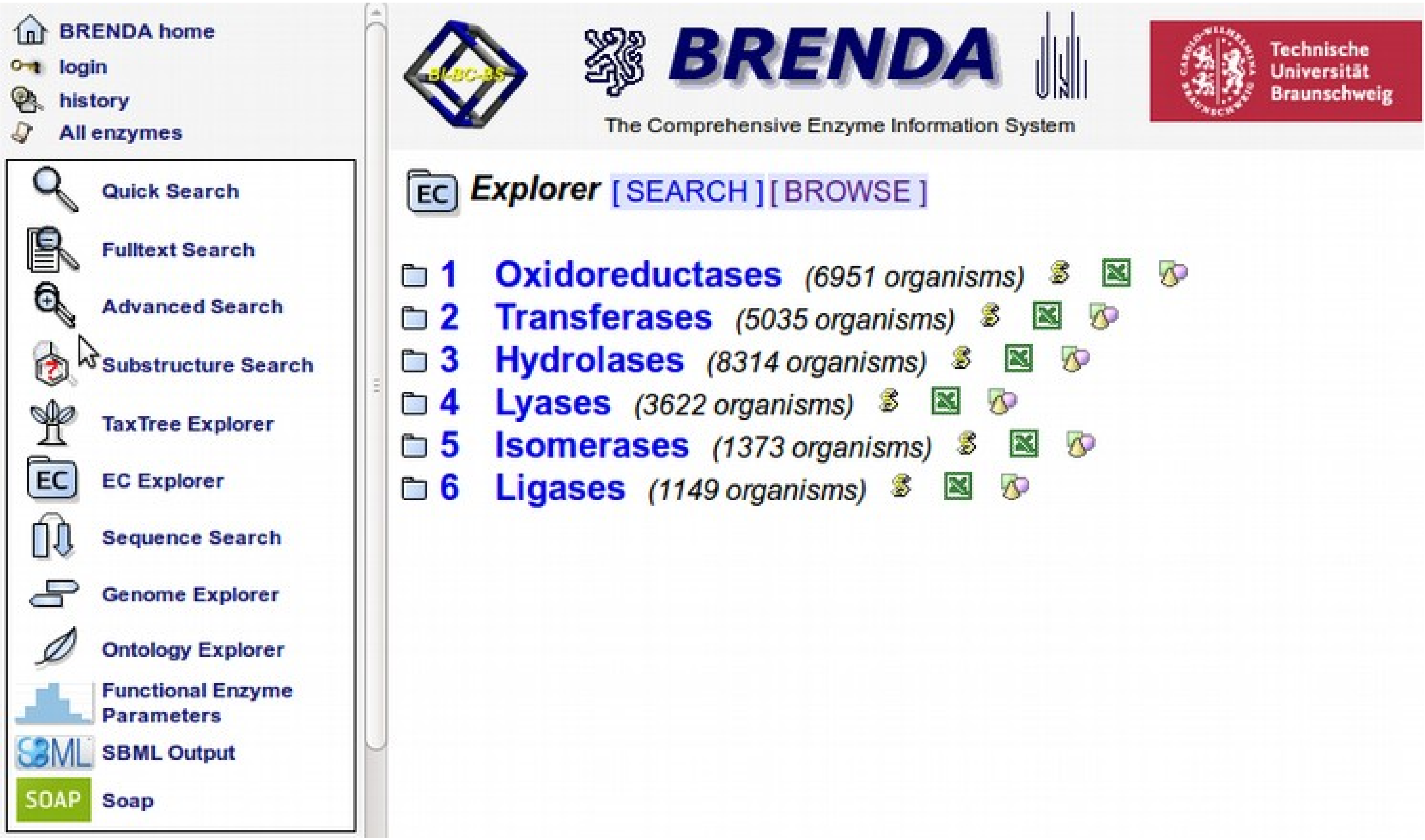
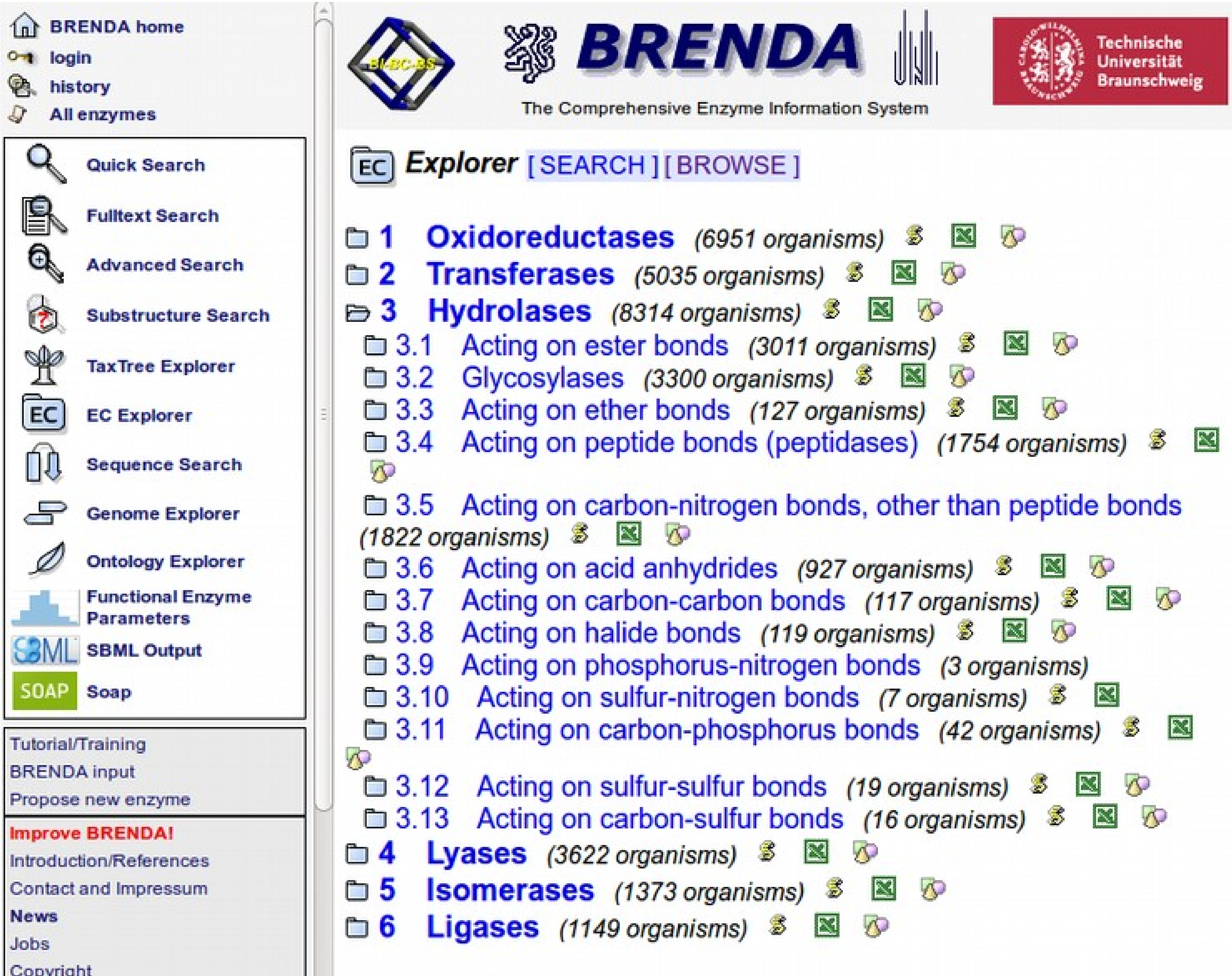
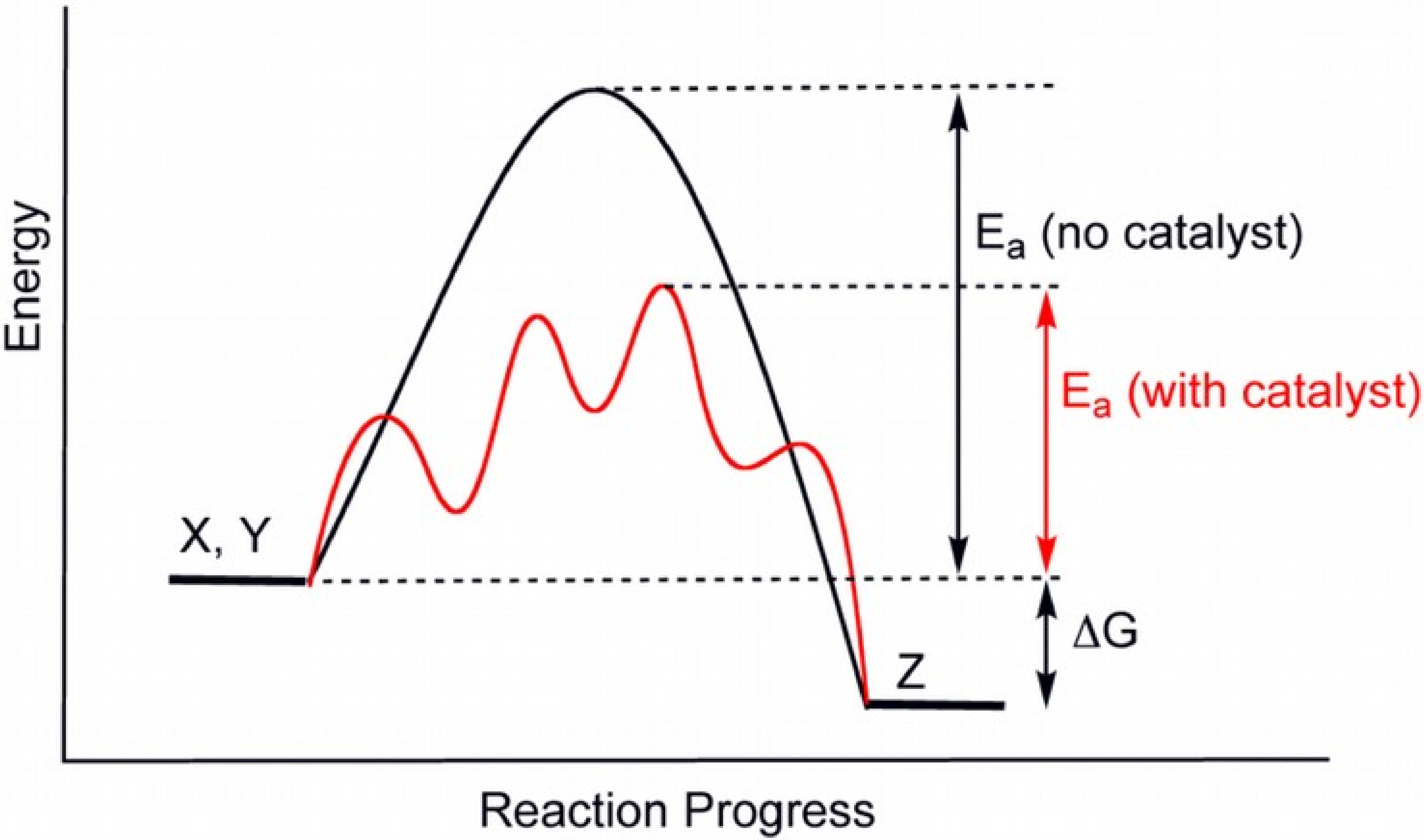
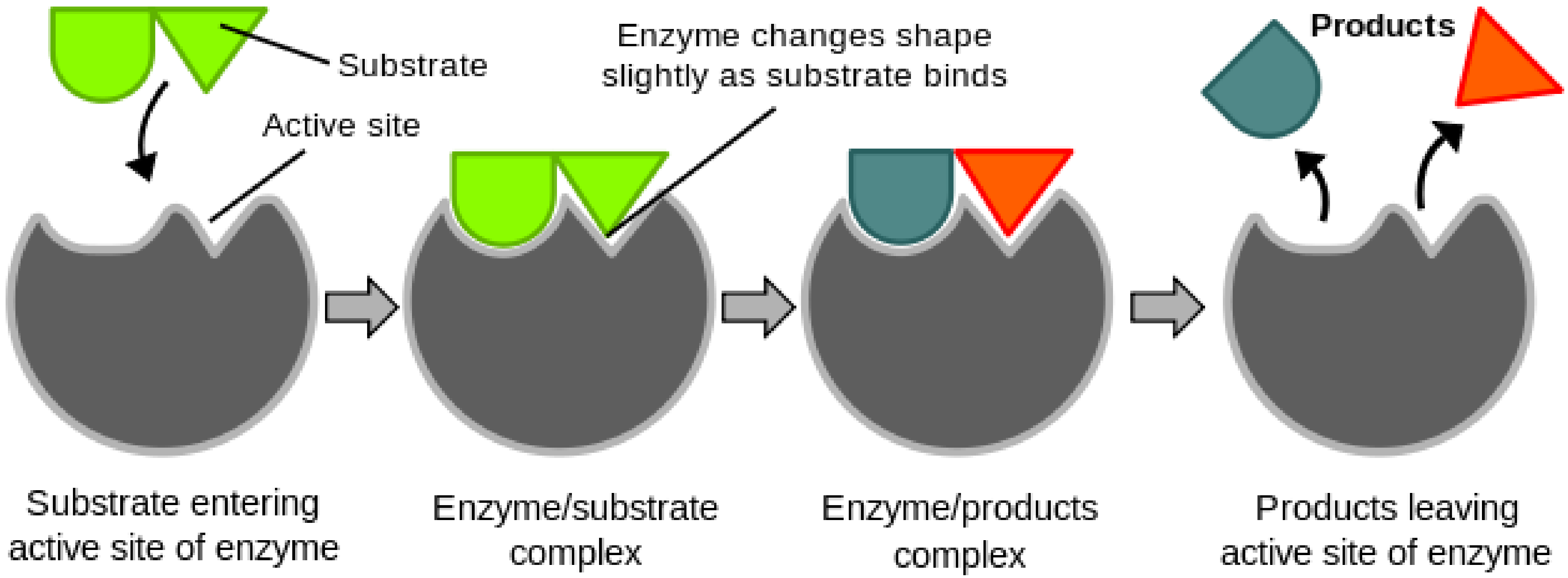
biyokatalistler
biyotransformasyon


BRENDA home
login
history
All enzymes
Quick Search
Fulltext Search
Advanced Search
Substructure Search
TaxTree Explorer
EC Explorer
Sequence Search
Genome Explorer
Ontology Explorer
Functional Enzyme Parameters
SBML Output
SOAP
Soap
BRENDA
The Comprehensive Enzyme Information System
Technische Universität Braunschweig
EC
Explorer [SEARCH] [BROWSE]
1 Oxidoreductases (6951 organisms)
2 Transferases (5035 organisms)
3 Hydrolases (8314 organisms)
4 Lyases (3622 organisms)
5 Isomerases (1373 organisms)
6 Ligases (1149 organisms)


BRENDA home
login
history
All enzymes
Quick Search
Fulltext Search
Advanced Search
Substructure Search
TaxTree Explorer
EC Explorer
Sequence Search
Genome Explorer
Ontology Explorer
Functional Enzyme Parameters
SBML Output
SOAP
Soap
Tutorial/Training
BRENDA input
Propose new enzyme
Improve BRENDA!
Introduction/References
Contact and Impressum
News
Jobs
Copyright
BI-EC-BS
BRENDA
The Comprehensive Enzyme Information System
Technische Universität Braunschweig
EC
Explorer [SEARCH] [BROWSE]
1 Oxidoreductases (6951 organisms)
2 Transferases (5035 organisms)
3 Hydrolases (8314 organisms)
3.1 Acting on ester bonds (3011 organisms)
3.2 Glycosylases (3300 organisms)
3.3 Acting on ether bonds (127 organisms)
3.4 Acting on peptide bonds (peptidases) (1754 organisms)
3.5 Acting on carbon-nitrogen bonds, other than peptide bonds (1822 organisms)
3.6 Acting on acid anhydrides (927 organisms)
3.7 Acting on carbon-carbon bonds (117 organisms)
3.8 Acting on halide bonds (119 organisms)
3.9 Acting on phosphorus-nitrogen bonds (3 organisms)
3.10 Acting on sulfur-nitrogen bonds (7 organisms)
3.11 Acting on carbon-phosphorus bonds (42 organisms)
3.12 Acting on sulfur-sulfur bonds (19 organisms)
3.13 Acting on carbon-sulfur bonds (16 organisms)
4 Lyases (3622 organisms)
5 Isomerases (1373 organisms)
6 Ligases (1149 organisms)



Energy
E_a (no catalyst)
E_a (with catalyst)
X, Y
ΔG
Z
Reaction Progress


Substrate
Active site
Enzyme changes shape
slightly as substrate binds
Products
Substrate entering
active site of enzyme
Enzyme/substrate
complex
Enzyme/products
complex
Products leaving
active site of enzyme

Alternative Reaction Route

Mechanisms:

- "over the barrier" -
- "through the barrier" -

Bond strain

Enzyme Affinity to transition State > Enzyme Affinity to substrate

Electrostatic catalysis

ionic bond formation w/ active site

Covalent catalysis

covalent bond formation w/ active site

Proximity & orientation

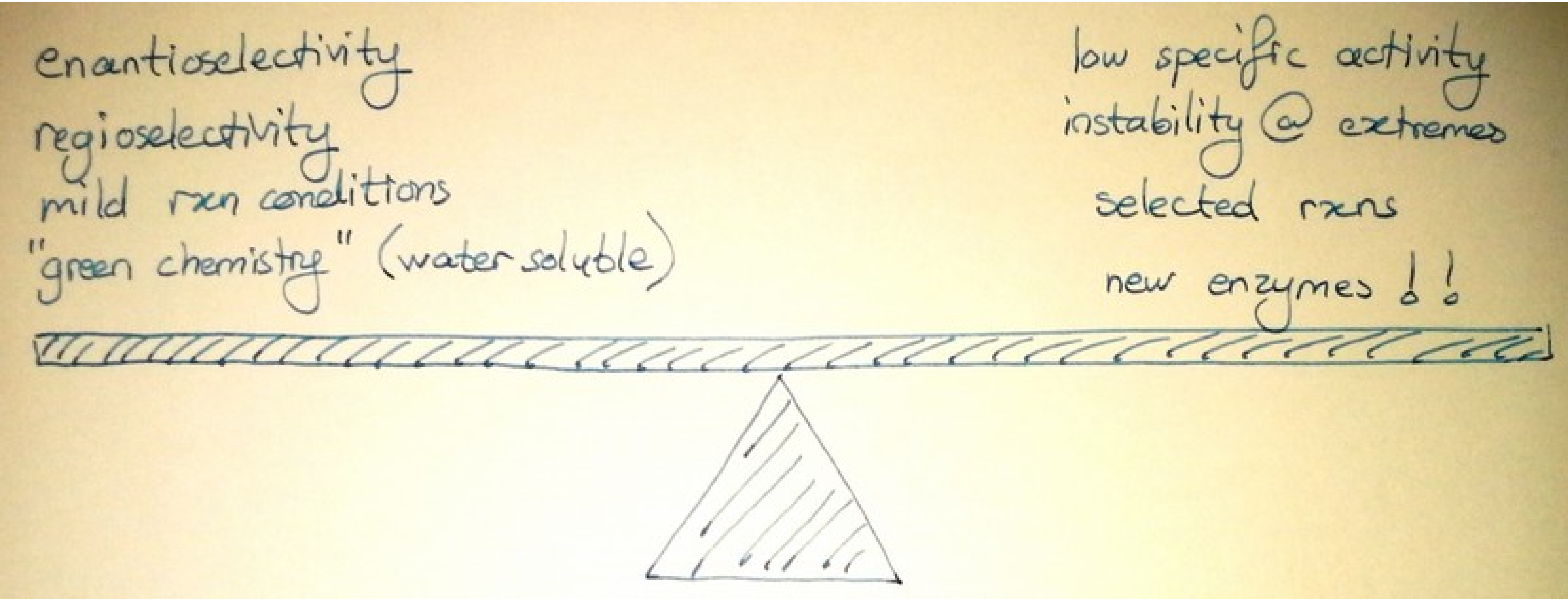
align reactive chemical groups together

Quantum tunelling

"through the barrier"
proton/e^- tunnel through activation barriers

Proton donors / acceptors

acid groups donate H^+
base groups accept

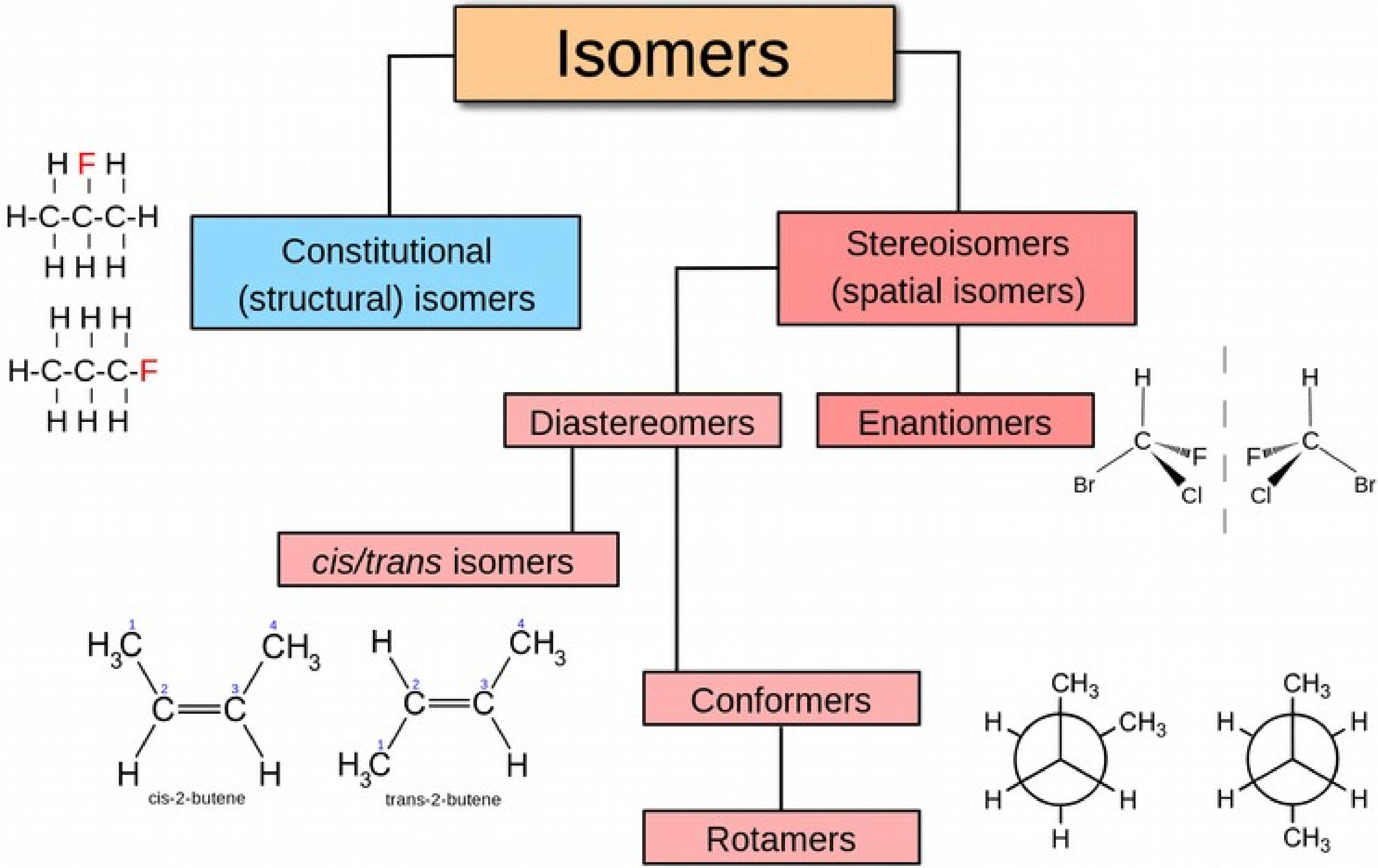


enantioselectivity
regioselectivity
mild rxn conditions
"green chemistry" (water soluble)
low specific activity
instability @ extremes
selected rxns
new enzymes ¡!

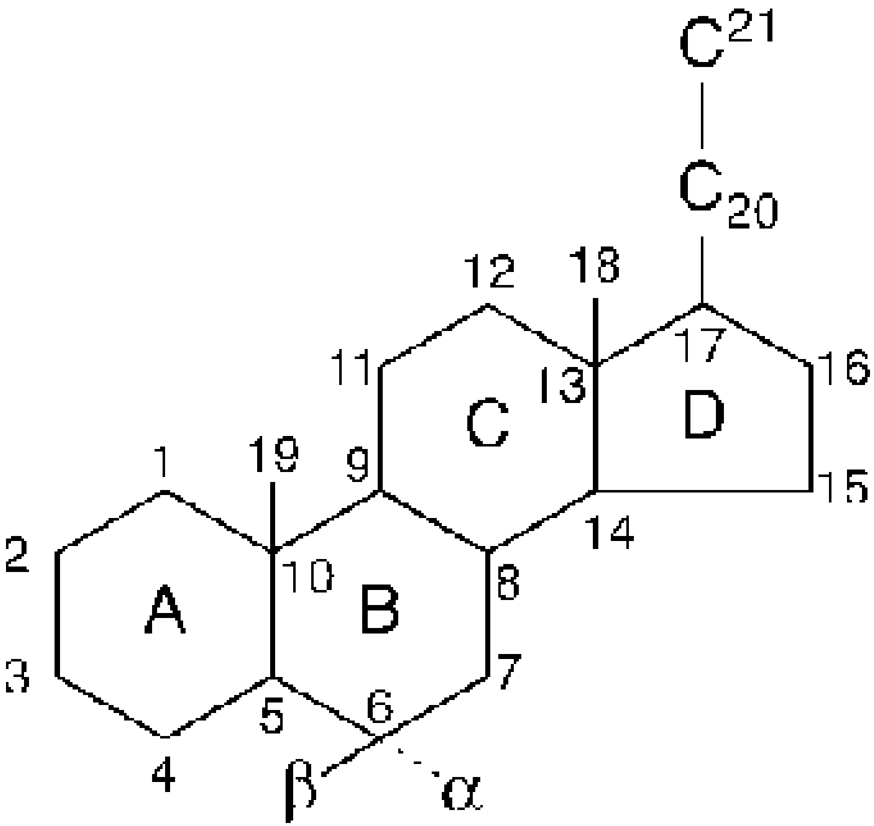
enzim pazarı

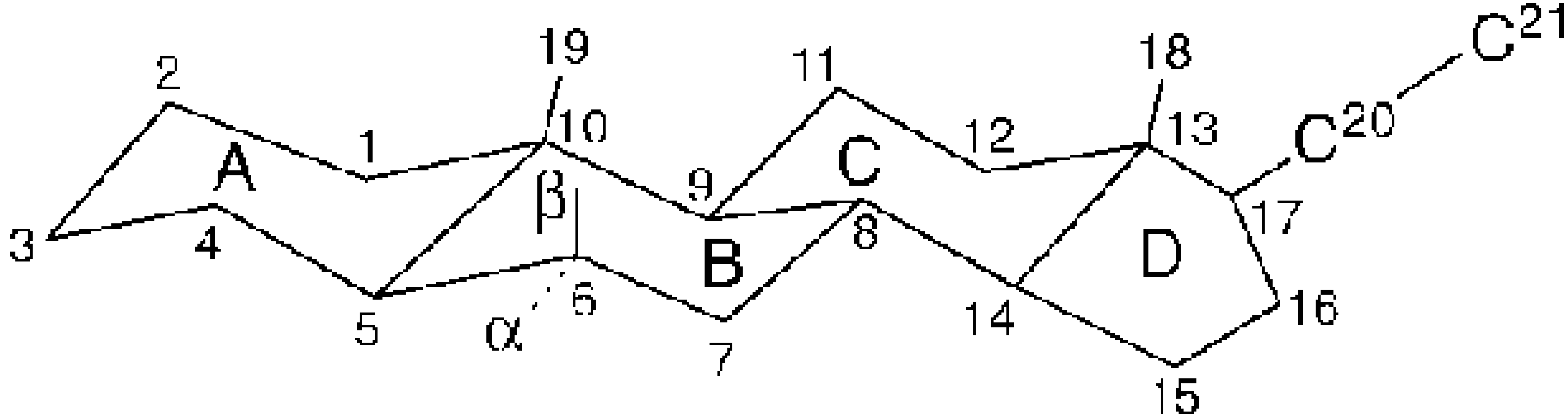
t/a	product	enzyme
> 1 000 000	high-fructose corn syrup	glucose isomerase
> 100 000	lactose-free milk	lactase
> 10 000	acrylamide	nitrilase
	cocoa butter	lipase
> 1 000	nicotinamide	nitrilase
	D-pantothenic acid	aldonolactonase
	(*S*)-chloropropionic acid	lipase
	6-aminopenillanic acid	penicillin amidase
	7-aminocephalosporanic acid	glutaryl amidase
	aspartame	thermolysin
	L-aspartate	aspartase
	D-phenylglycine	hydantoinase
	D-*p*-OH-phenylglycine	hydantoinase
> 100	ampicillin	penicillin amidase
	L-methionine, L-valine	aminoacylase
	L-carnitine	dehydrase/ hydroxylase
	L-DOPA	β-tyrosinase
	L-malic acid	fumarase
	(*S*)-methoxyisopropyl-amine	lipase
	(*R*)-mandelic acid	nitrilase
	L-alanine	L-aspartate-β-de-carboxylase

geeeel vatandaaaş...



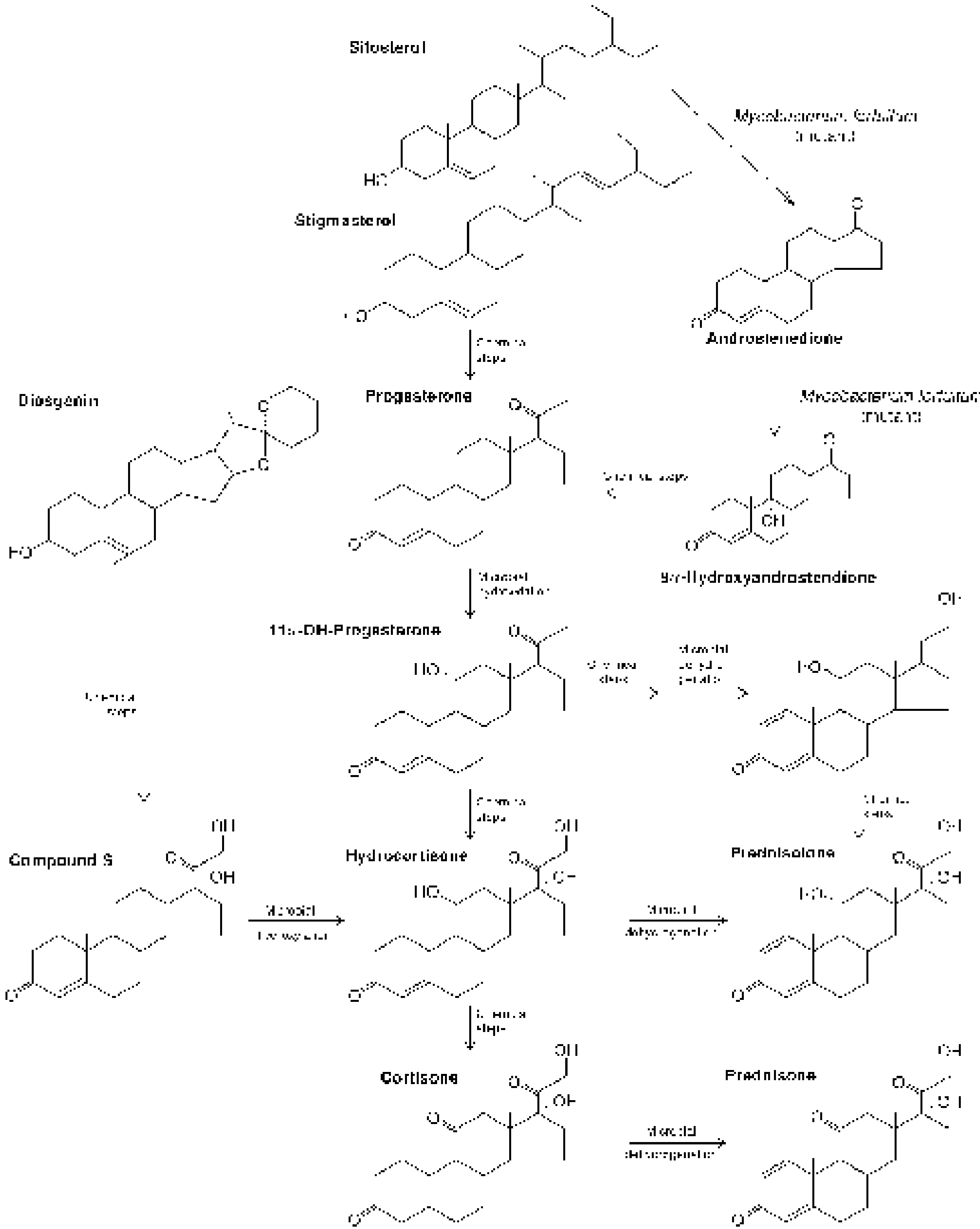
Isomers
Constitutional (structural) isomers
Stereoisomers (spatial isomers)
Diastereomers
Enantiomers
cis/trans isomers
Conformers
Rotamers
cis-2-butene
trans-2-butene


C21
C20
12
18
11
13
17
16
C
D
1
19
9
15
2
14
10
B
A
8
3
7
6
5
4
β
α


19
18
C21
11
2
10
12
13
C20
A
1
C
9
β
17
3
8
4
D
B
6
16
14
5
α
7
15

Position of hydroxylation	Stereochemistry of incoming hydroxyl group	Position of hydroxylation	Stereochemistry of incoming hydroxyl group
1	α	10	β
1	β	11	α
2	α	12	β
2	β	13	α
3	α	14	α
3	β	15	α
4	α	15	β
4	β	16	α
5	α	16	β
6	α	17	α
6	β	17	β
7	α		
7	β		
9	α		

Source: Davies, H. G., Green, R. H., Kelly, D. R., and Roberts, S. M. (1989). *Biotransformations in Preparative Organic Chemistry. The Use of Isolated Enzymes and Whole Cell Systems in Synthesis*, pp. 175–176, London: Academic Press.


Sitosterol
Stigmasterol
Androstenedione
Diosgenin
Progesterone
9α-Hydroxyandrostendione
11α-OH-Progesterone
Compound S
Hydrocortisone
Prednisolone
Cortisone
Prednisone

Hydroxylation position	Substrate	Product	Microorganism
1α	Androst-4-ene-3,17-dione	1α-Hydroxyandrost-4-ene-3,17-dione	*Penicillium* sp
1β	Androst-4-ene-3,17-dione	1β-Hydroxyandrost-4-ene-3,17-dione	*Xylaria* sp.
3α	Androstane-7,17-dione	3α-Hydroxyandrostane-7,17-dione	*Diaporthe celastrina*
3β	17β-Hydroxyandrostan-11-one	3β,17β-Dihydroxyandrostan-11-one	*Wojnowicia graminis*
11α	Progesterone	11α-Hydroxyprogesterone	*Rhizopus* sp.
11β	11-Deoxycortisone	Hydrocortisone	*Curvularia lunata*
12β	17β-Hydroxy-estr-4-ene-3-one	12β,17β-Dihydroxy-estr-4-ene-3-one	*Colletotrichum derridis*

Source: Neidleman, S. L. (1991). Industrial chemicals: fermentation and immobilized cells. In *Biotechnology. The Science and the Business*, V. Moses and R. E. Cape (eds.), pp. 306–307. Chur, Switzerland: Harwood Academic Publishers

işe uygun enzimi bulmak...

barofil

halofilik

hipertermofil

mikrobiyal çeşitlilik

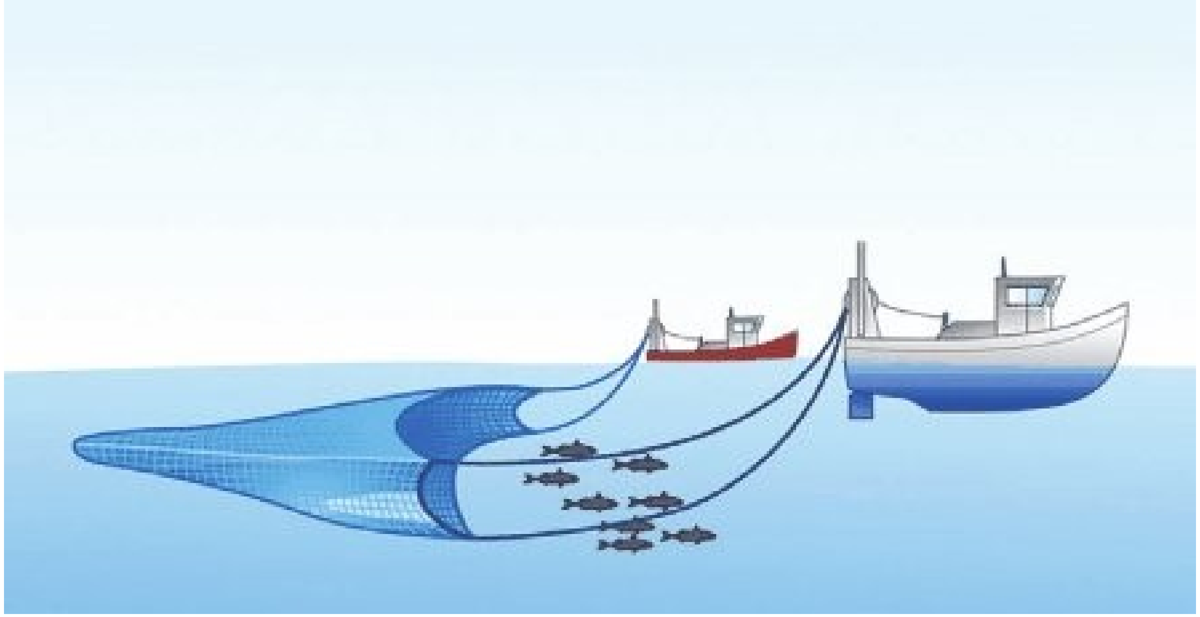
asidofil

mezofil

psikrofil

alkalifil

çevresel (metagenomik) DNA kütüphanesi...



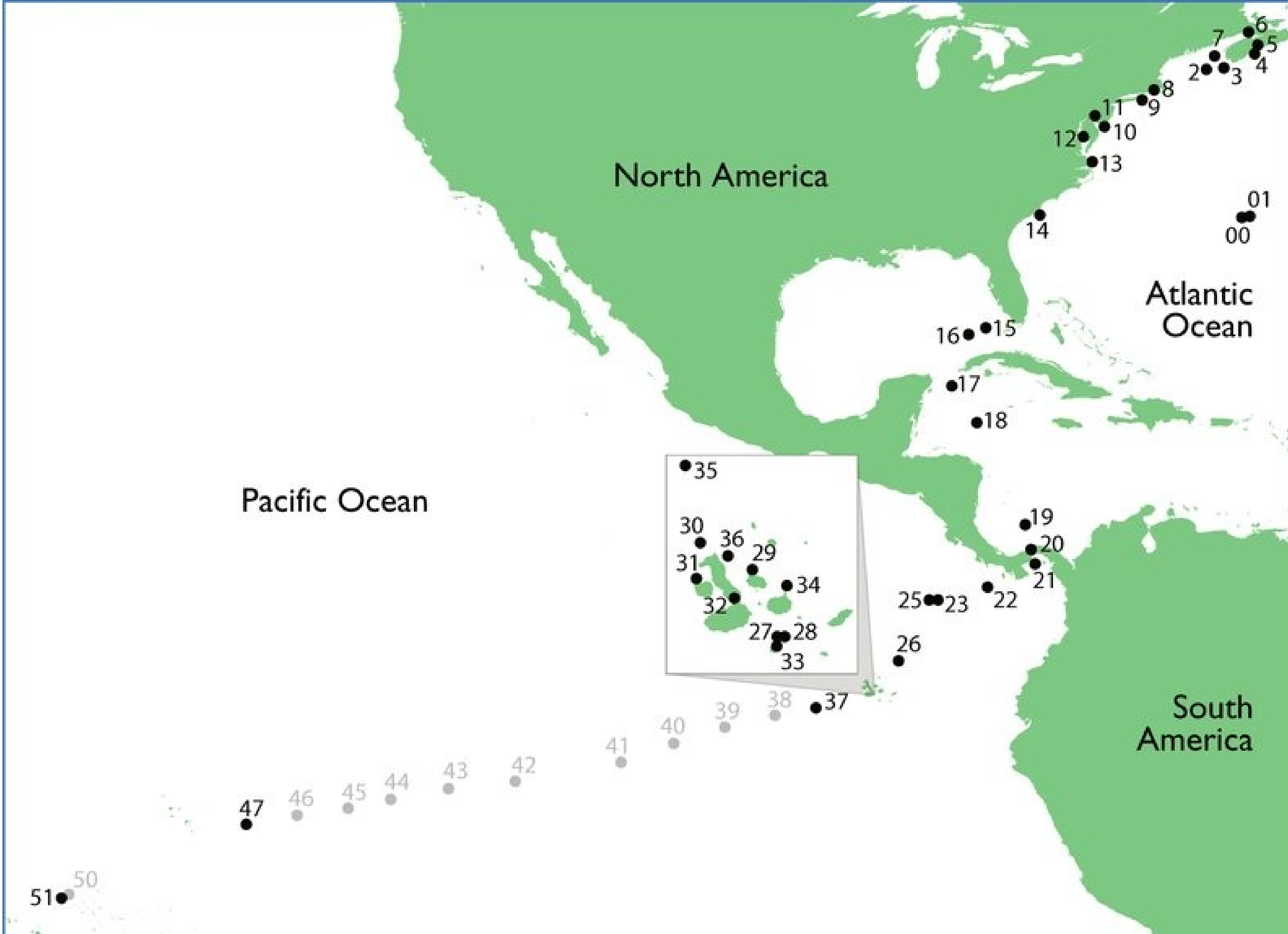
ekspresyon kütüphanesi oluşturulması

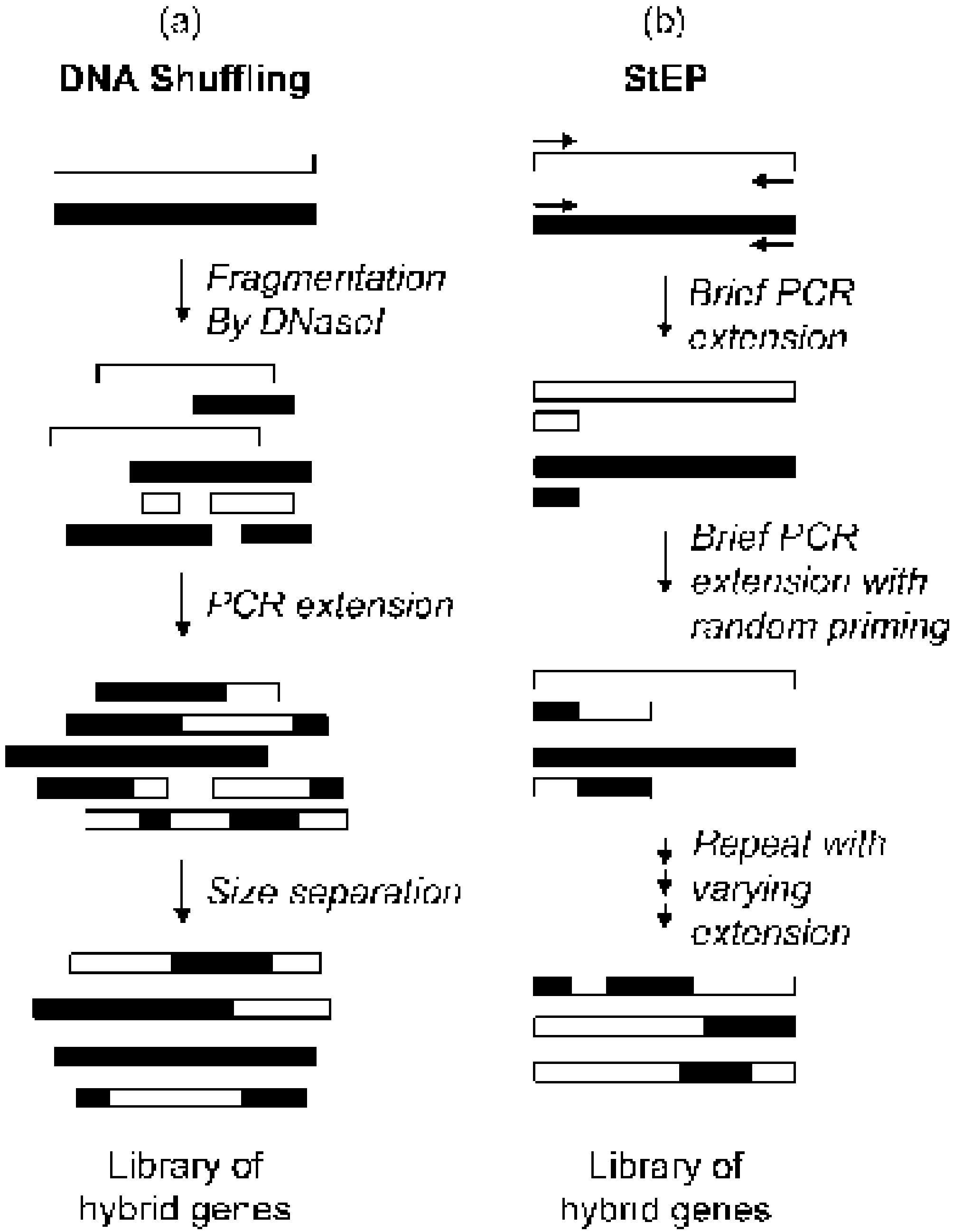
biyolüminesan substrat kullarak transformantların taranması

GLOBAL OCEAN SAMPLING EXPEDITION

The J. Robert Beyster and Life Technologies Foundation 2009-2010 Research Voyage of the Sorcerer II Expedition

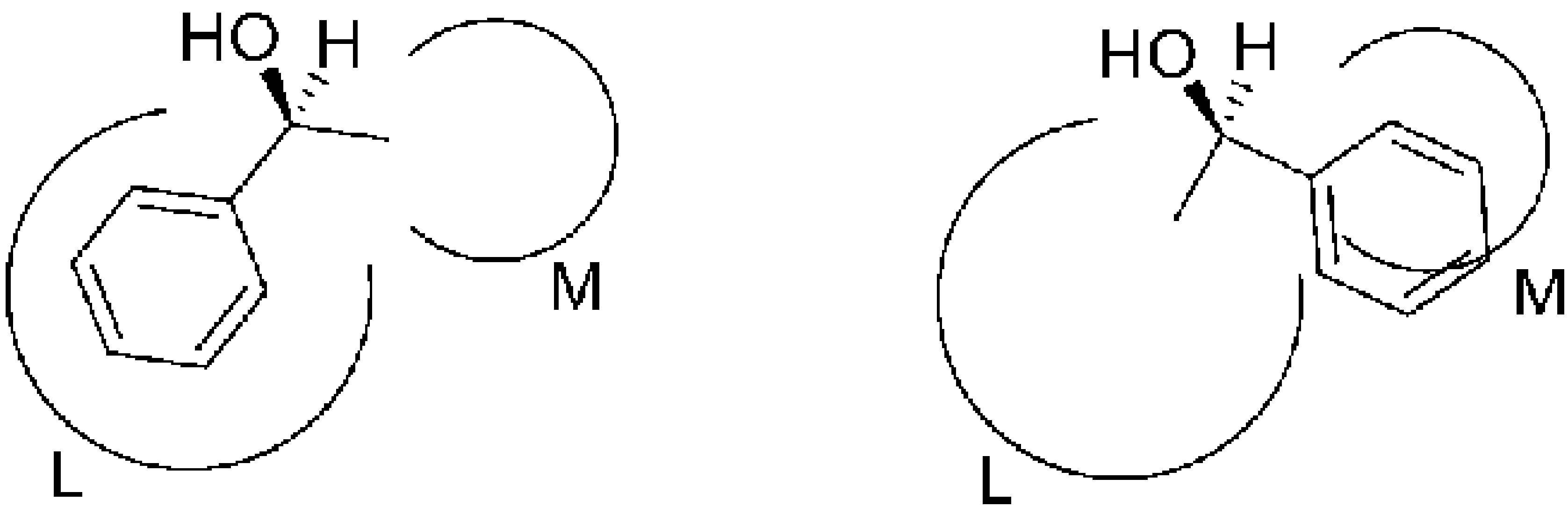
▼ SAMPLED SITES

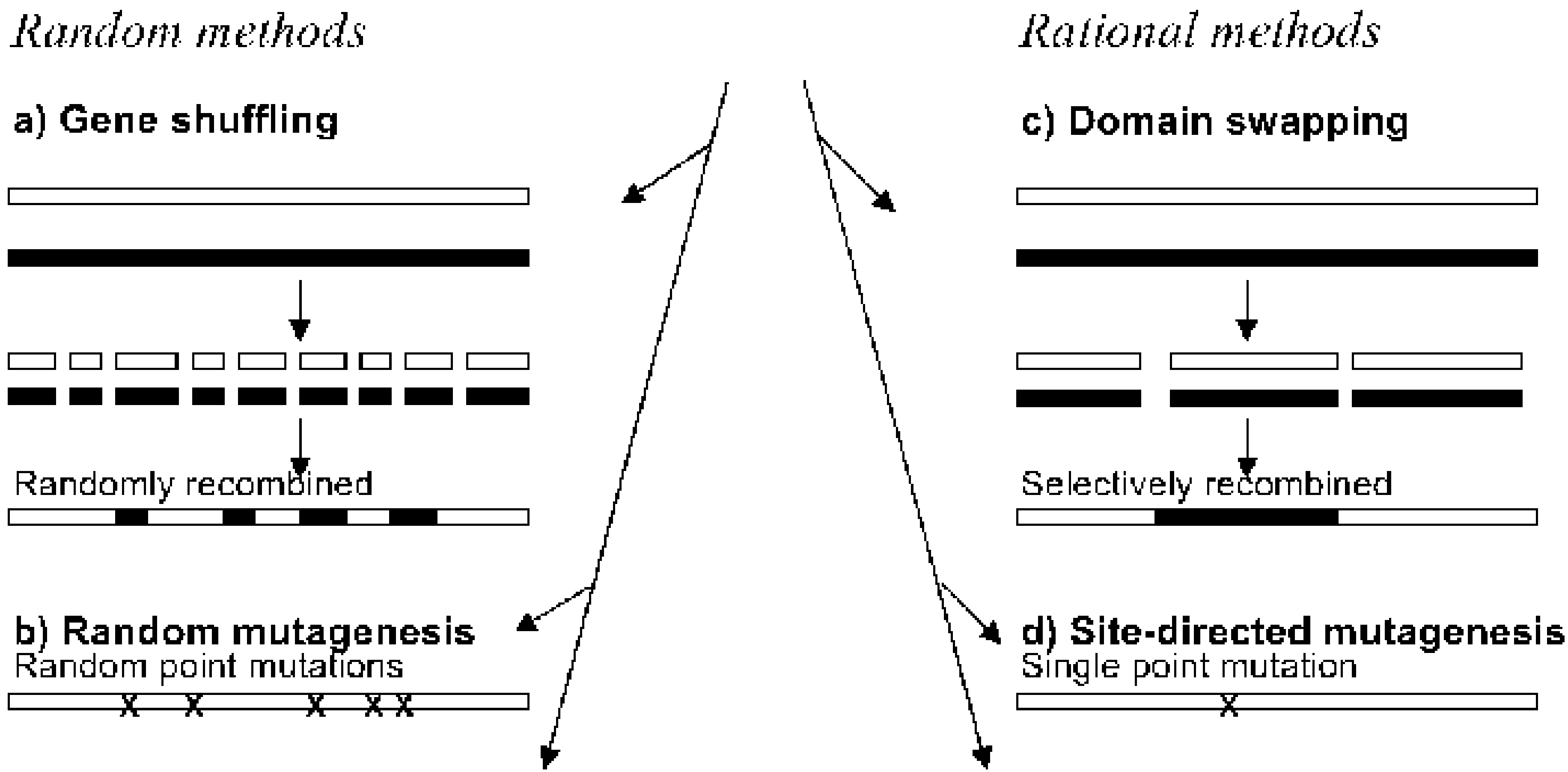




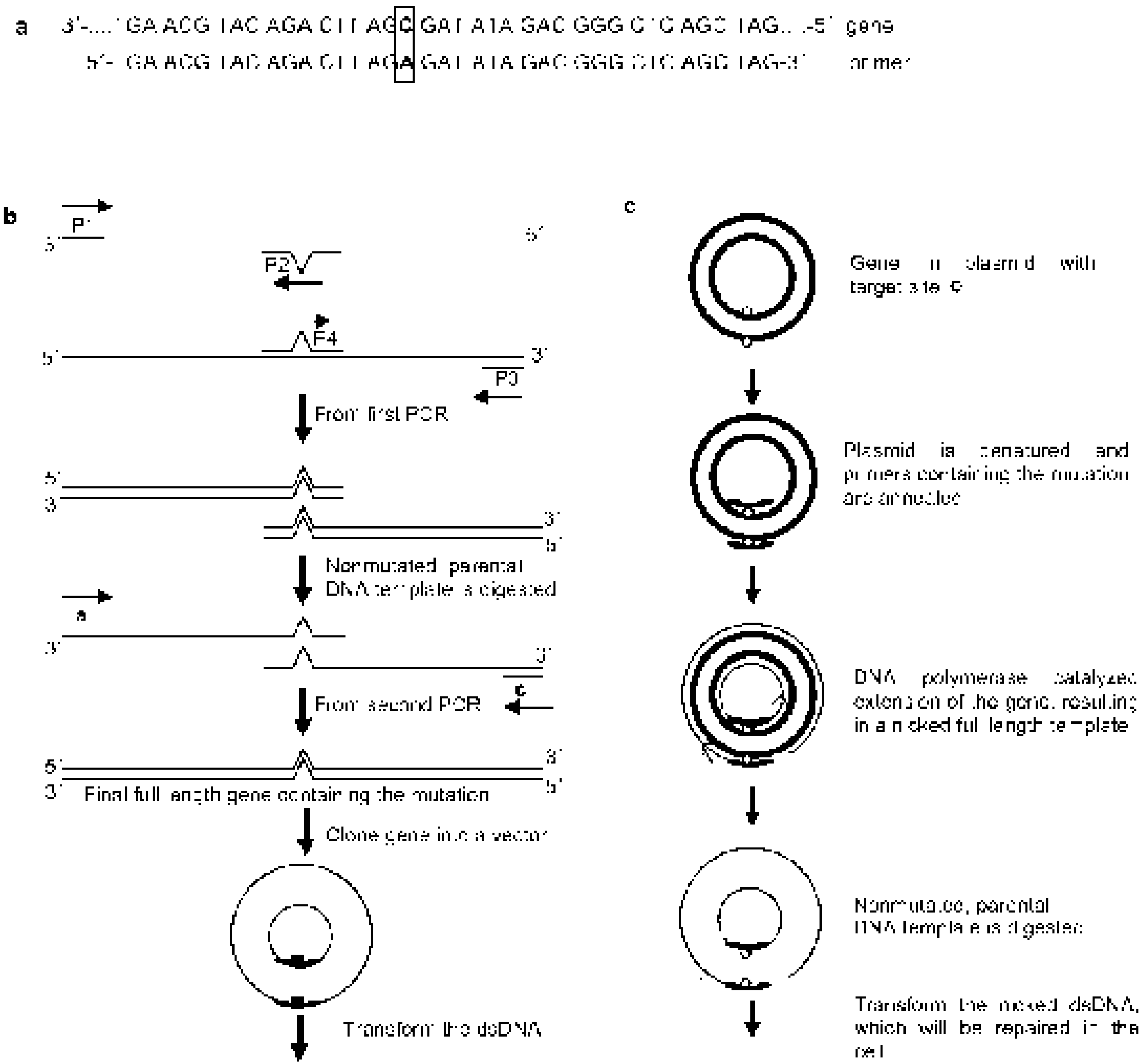
Directed Enzyme Evolution and High-Throughput Screening

Michael J. McLachlan, Ryan P. Sullivan and Huimin Zhao


HO
H
M
L
HO
H
M
L



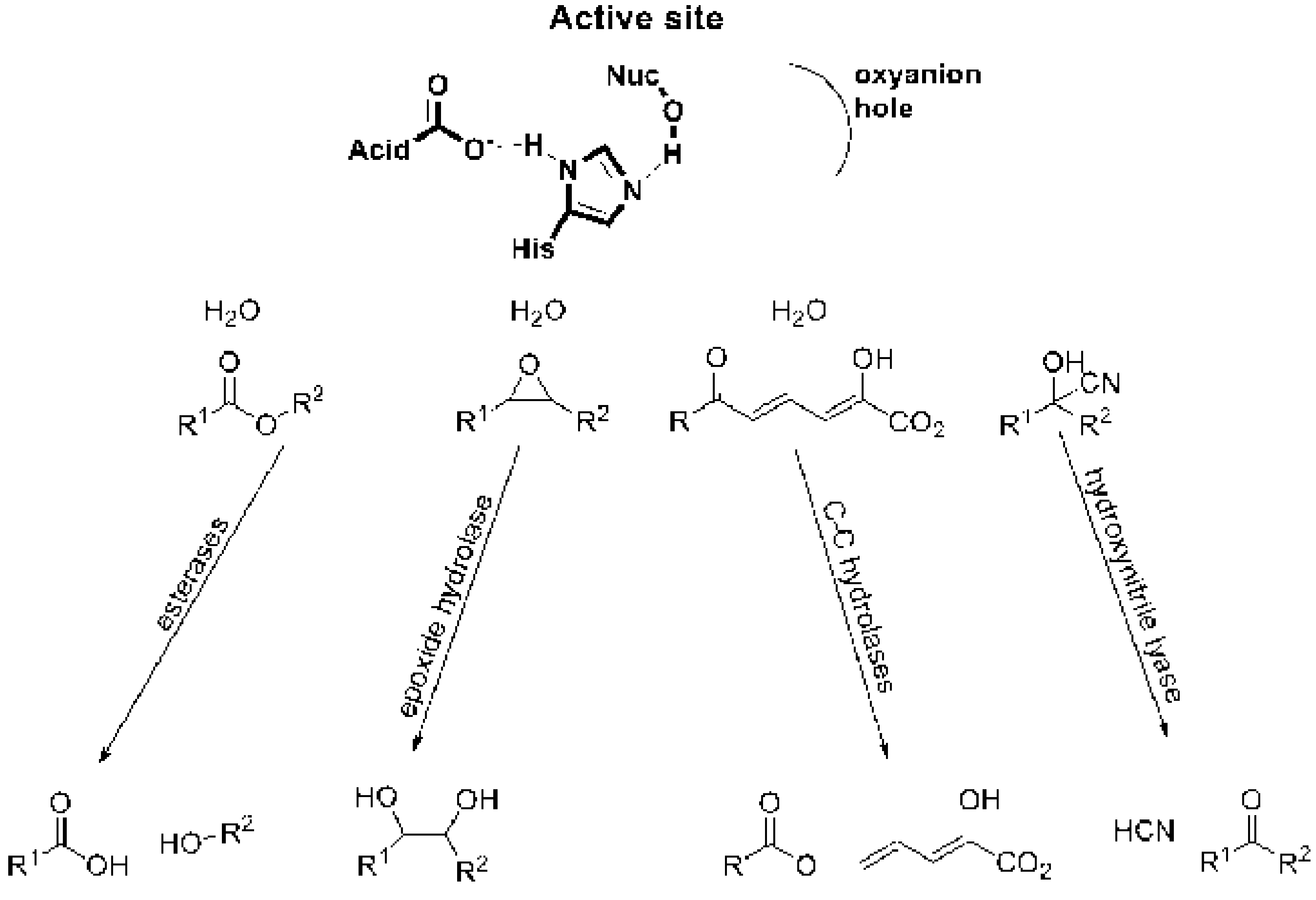
Exploiting Enzyme Promiscuity
for Rational Design
Cecilia Branneby



transformasyon

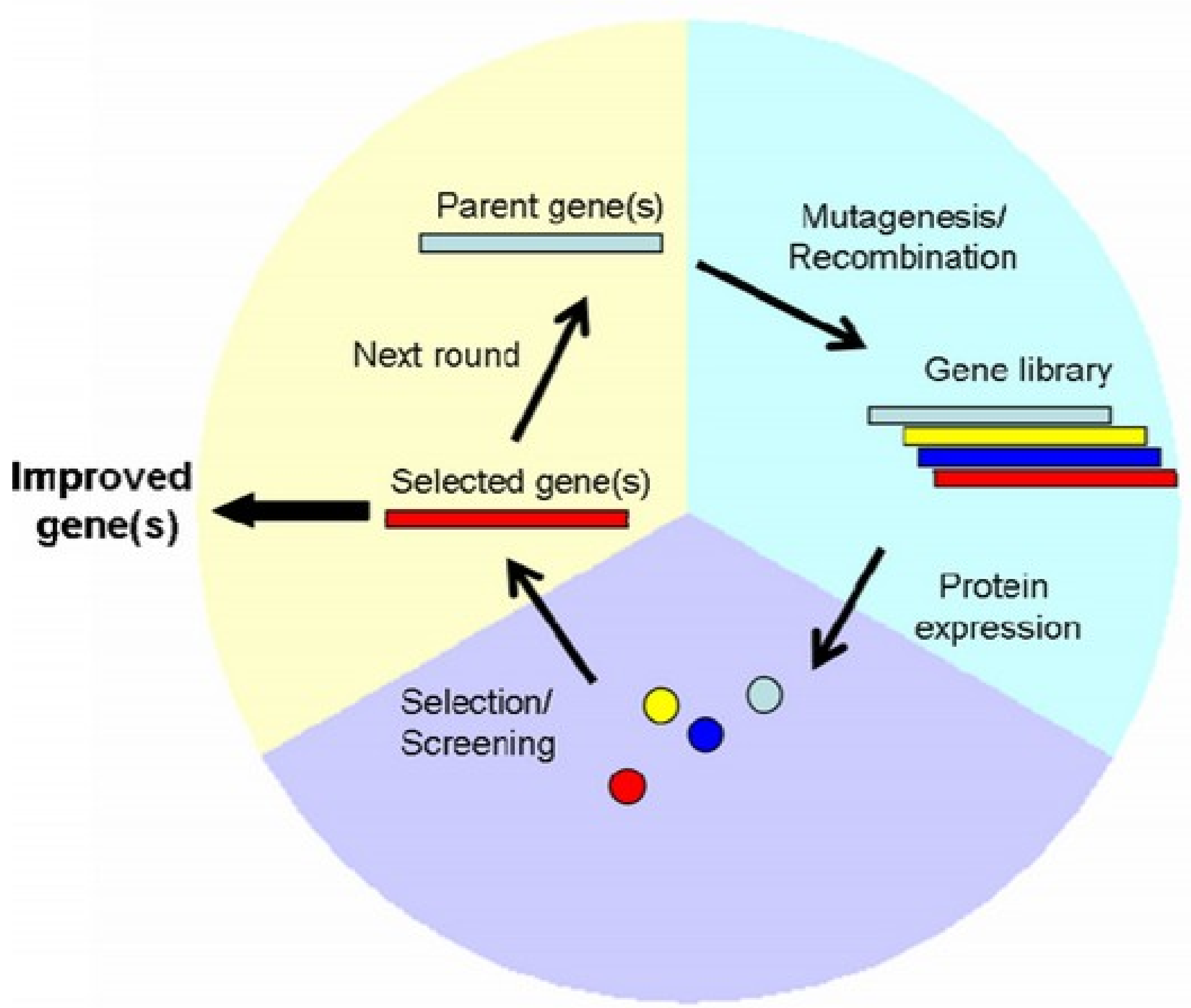
Exploiting Enzyme Promiscuity for Rational Design
Cecilia Branneby

enzimlerde “müsamahakarlık / gelişigüzellik”



Exploiting Enzyme Promiscuity for Rational Design

Cecilia Branneby


Parent gene(s)
Mutagenesis/
Recombination
Next round
Gene library
Improved
gene(s)
Selected gene(s)
Protein
expression
Selection/
Screening

> %90 endüstriyel enzim rekombinant olarak üretiliyor → saflık, aktivite
→ koşullara dayanıklılık!

① Rasyonel tasarım

işi şansa bırakma

Kritik noktalara hedeflenmiş mutasyonlar

② yönlendirilmiş evrim

azbilgi – çok şans

Darwinian optimizasyon

③ Orta Yol

Rassal ve yarı rasyonel mutagenez için:

"error prone PCR"

- baz analogları
- alkilleyici ajanlar
- $Mg^{2+} \rightarrow Mn^{2+}$
- dNTP dengesizliği
- - - - -

"Sequence Saturation Mutagenesis"

- alkaliye duyarlı α-fosfotiyonat
- TdT enzimi

Uzun oligolar

Çooook büyük kütüphanelerde bir çok anlamsız dizi...
samanlıkta iğne aramaktan farksız !!!

"Gene Shuffling"

- Homoloji bağımlı
- Homolojiden bağımsız

Bir ya da daha fazla kaynaktan gen segmentlerini içeren kimerik genler oluşturulur

Yönlendirilmiş evrim kütüphanesinin taranması

$$ istediğimiz mutantları nasıl seçmeliyiz?

in vivo → Genetik komplementasyon

! hedef enzime özel! ☹ her enzim için bir oksotrof gerekli

seçilmek istenen enzim yönünden oksotrof yaratmak

→ Kimyasal komplementasyon

genel bir "raporlayıcı sistem" geliştirmek

→ Gösterim sistemleri

mikroorganizma ya da faj partikülü yüzeyinde

FACS ile seçilim

Yönlendirilmiş evrim kütüphanesinin taranması

in vitro → Lizat deneyi

Enzim üreten hücrelerin parçalanması, enzimin saflaştırılması

→ Ribozomal gösterim

→ In vitro kompartmentalizasyon

translasyon yağ emülsiyonu içinde katalitik aktiviteye sahip partiküllerin seçilmesi

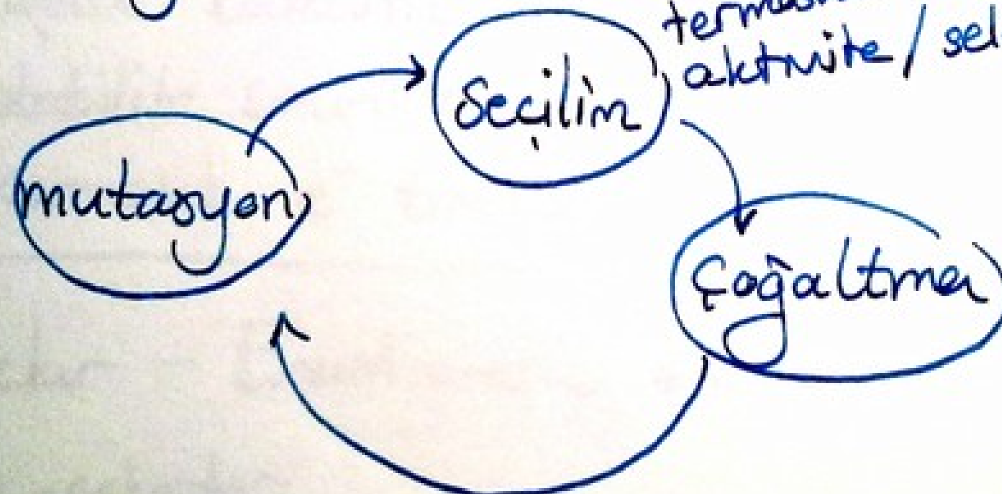
Hedefler

- Aktivite artışı
- Termostabilite
- Substrat özgüllüğü
- Ürün özgüllüğü
- Enantioselectivity

En başarılı yaklaşım #1

Halihazırda var olan bir biyokatalist, endüstriyel uygulama için yönlendirilmiş evrime uğratılır...

termostabilite/organik çözücü toleransı/aktivite/selektivite/...



Oksidoredüktazlar – Nonspesifik oksidazlar – lakkaz, peroksidaz

kağıt sanayi, fonksiyonalizasyon, biyoremediasyon, tekstil ... Termostabilite, pH dayanıklılığı, ...

P450 monooksigenazlar – Geniş spektrumlu enzimler :)

:(düşük katalitik etkinlik
stabilite sorunları
karmaşık e⁻ transport sistemi gerekli

} Düzeltilmesi gerek ...

Dehidrogenazlar – Enantiomeric saf alkoller ** → Farma $$ → Kimyasal → Tarım ...

– L-şekerler

Transferazlar

Glikokonjugatların sentezlenmesi – kimyasal sentez !

Birçok ardışık proteksiyon-deproteksiyon basamağı zooor !

– enzimatik glikozilasyon

yüksek özgüllük

Hidrolazlar – hidroliz / transesterifikasyon / esterifikasyon / alkolizis / asidolizis / aminolizis

---

Lipazlar – Önemli bir grup enzim

Yüksek sıcaklık ve organik solvent dayanımı

Stereoselectivity

Esterazlar – Tüm enzimlerin %8'i !

tersiyer alkollere enantioselektif enzimler ...

Epoksid hidrolazlar – Şiral epoksidler ve dioller → farma / kimyasal

_ Biyokütle degrade eden enzimler — THE HAVUÇ!

Lignosellülozik biyoyakıtlar

Sellülazlar ile: sellüloz → Glukoz → Etanol $$$

- ekzoglukanazlar
- β-glukosidazlar
- endoglukanazlar

} Süper *Saccharomyces cerevisiae* (Yamada, 2010)

Diğer enzimler:

Liyazlar
İzomerazlar
Ligazlar
Transport proteinleri

ENZİM KÜTÜPHANESİ

En büyük sorun, taramak ve "sihirli" enzim varyantını bulmak ...

Kütüphane boyutlarını KÜÇÜLT

"akıllı" kütüphane verimi artırmak!

Ultra-high througput tarama yöntemleri

- FACS
- mikrafludik sistemler

Bir başka olasılık: Sentetik biyoloji

Tamamen yeni enzimler oluşturmak


BIO CON SEPT
BIO-CONVERSION AND SEPARATION TECHNOLOGY
Type your keywords...
Search
Start
Project Overview »
Consortium »
Scientific Methodology »
Media Corner
Links
Contact
From Plants to Plastics
BioConSepT aims to develop processes to convert 2nd generation biomass into valuable chemicals which are 30% cheaper and 30% more sustainable than the conventional chemicals routes or the biotechnology processes starting from 1st generation feed-stocks.