

De la biocatalyse à la catalyse hybride, les enzymes au cœur de procédés sélectifs et durables



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Les activités enzymes répertoriées

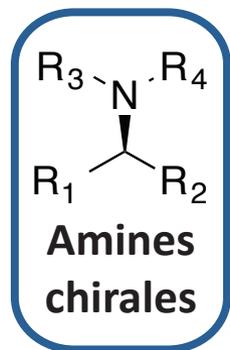
- Encore beaucoup à découvrir : « enzymes » dans Uniprot = **7,642,015 *unreviewed*** et **59,200 *reviewed*** soit **0,8%** (sur **230,328,648 protéines**)
- **8282 EC numbers dans BRENDA** (autant de réactions chimique différentes)

EC1 : Oxydoreductases *EC4 : Lyases*
EC2 : Transferases *EC5 : Isomerase*
EC3 : Hydrolases *EC6 : Ligases*



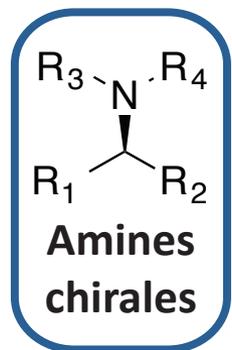
L'alanine racémase est une isomérase (EC 5), en particulier une racémase (EC 5.1) agissant sur l'acide aminé (EC 5.1.1) alanine (EC 5.1.1.1)

- **Seule une faible proportion est utilisée en synthèse :**
un fort potentiel pour la chimie verte à explorer !



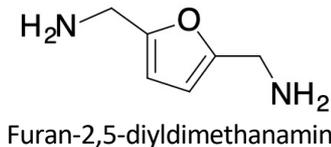
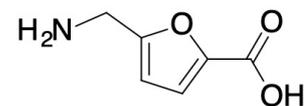
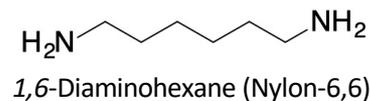
⇒ Interactions moléculaires

⇒ Diversité structurale
importante

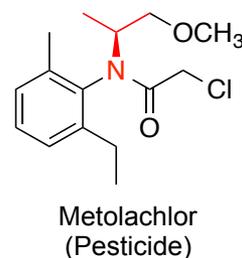
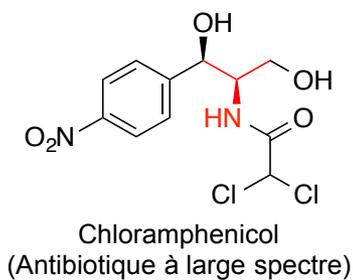
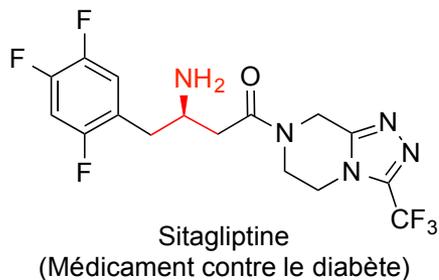


⇒ Interactions moléculaires

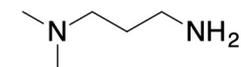
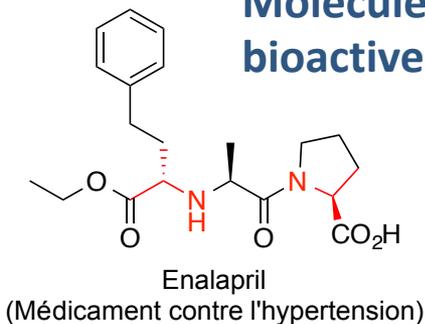
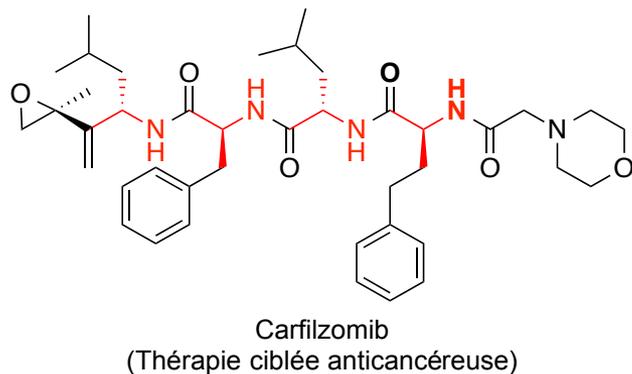
⇒ Diversité structurale importante



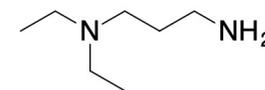
Précurseurs de polymères



Molécules bioactives



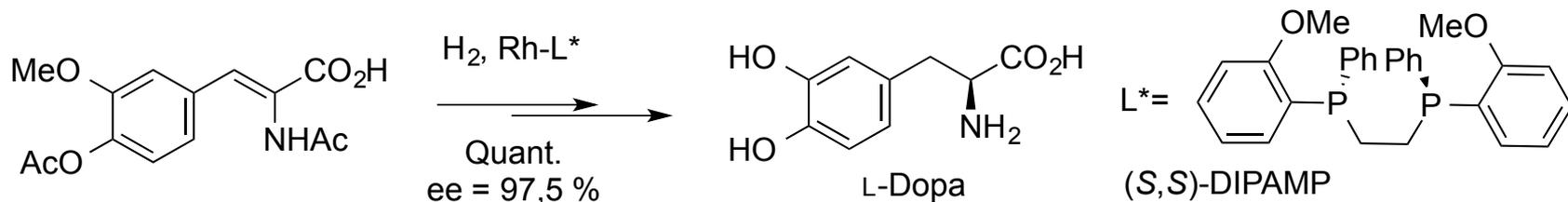
3-(Diéthylamino)propylamine (DEAPA – durcisseur résines époxy)



3-(Diméthylamino)propylamine (DMAPA – additif pour détergent, peinture)

Précurseurs divers

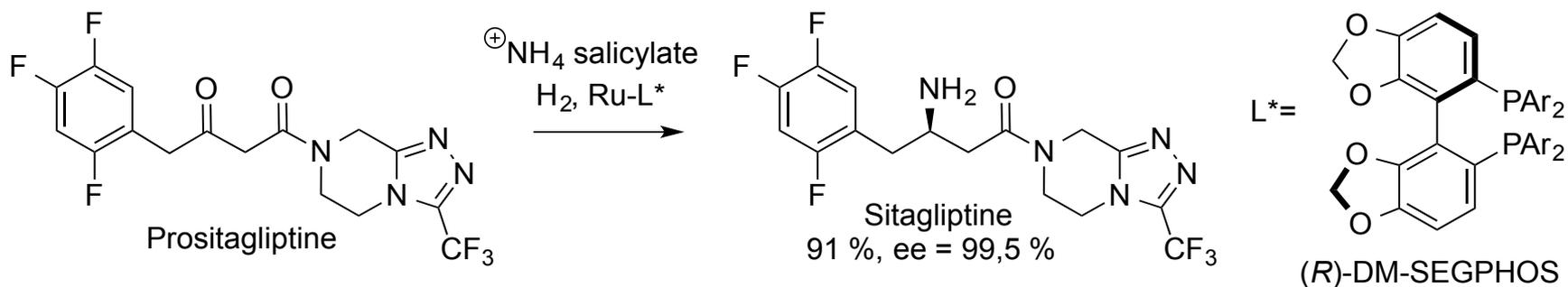
Hydrogénation



Traitement contre la maladie de Parkinson

Vineyard *et al.*, *J. Am. Chem. Soc.* 1977, 99 (18), 5946.

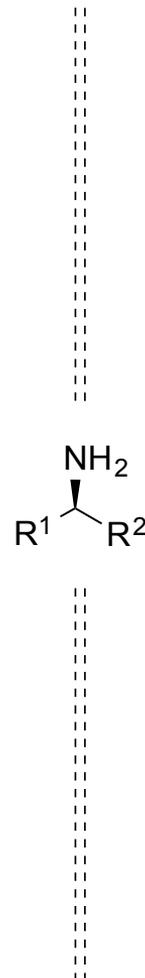
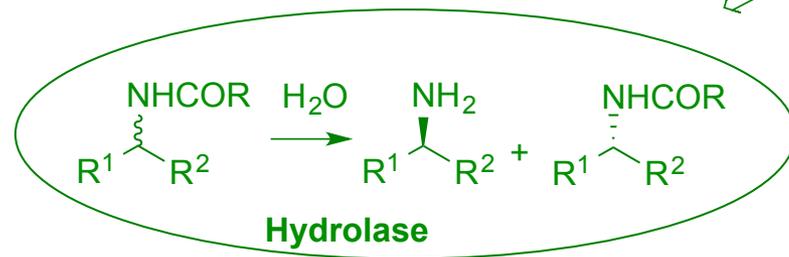
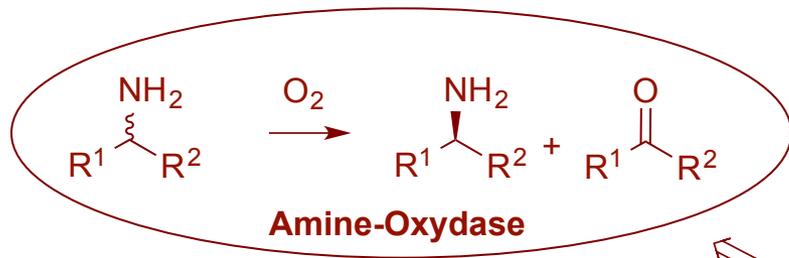
Amination réductrice



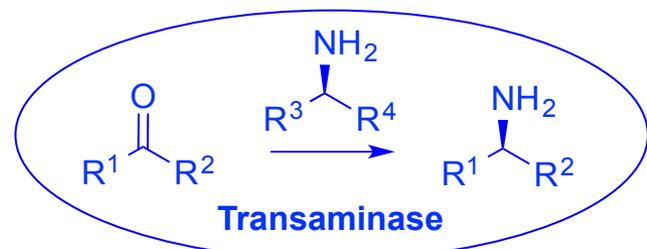
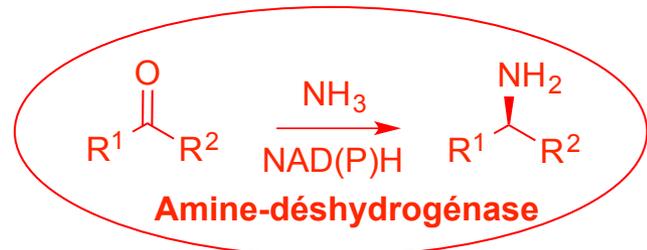
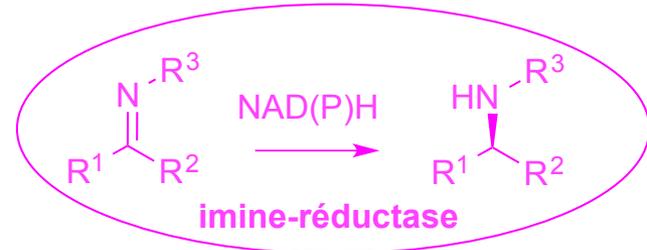
Médicament contre le diabète

Steinhuebel *et al.*, *J. Am. Chem. Soc.* 2009, 131 (32), 11316.

Dédoublément cinétique



Désymétrisation

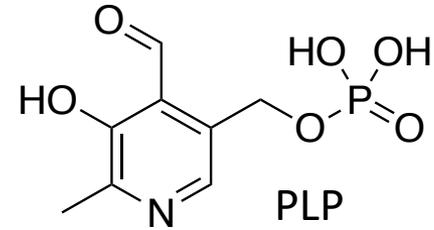


Les transaminases

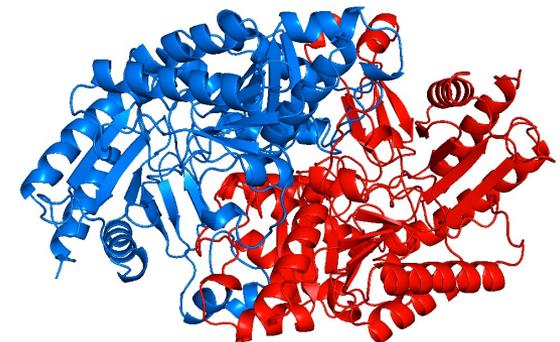
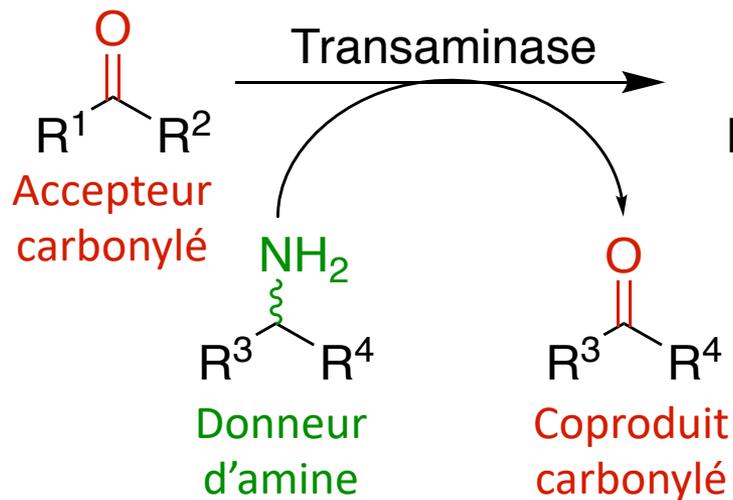
Transaminases

Les transaminases (TA)

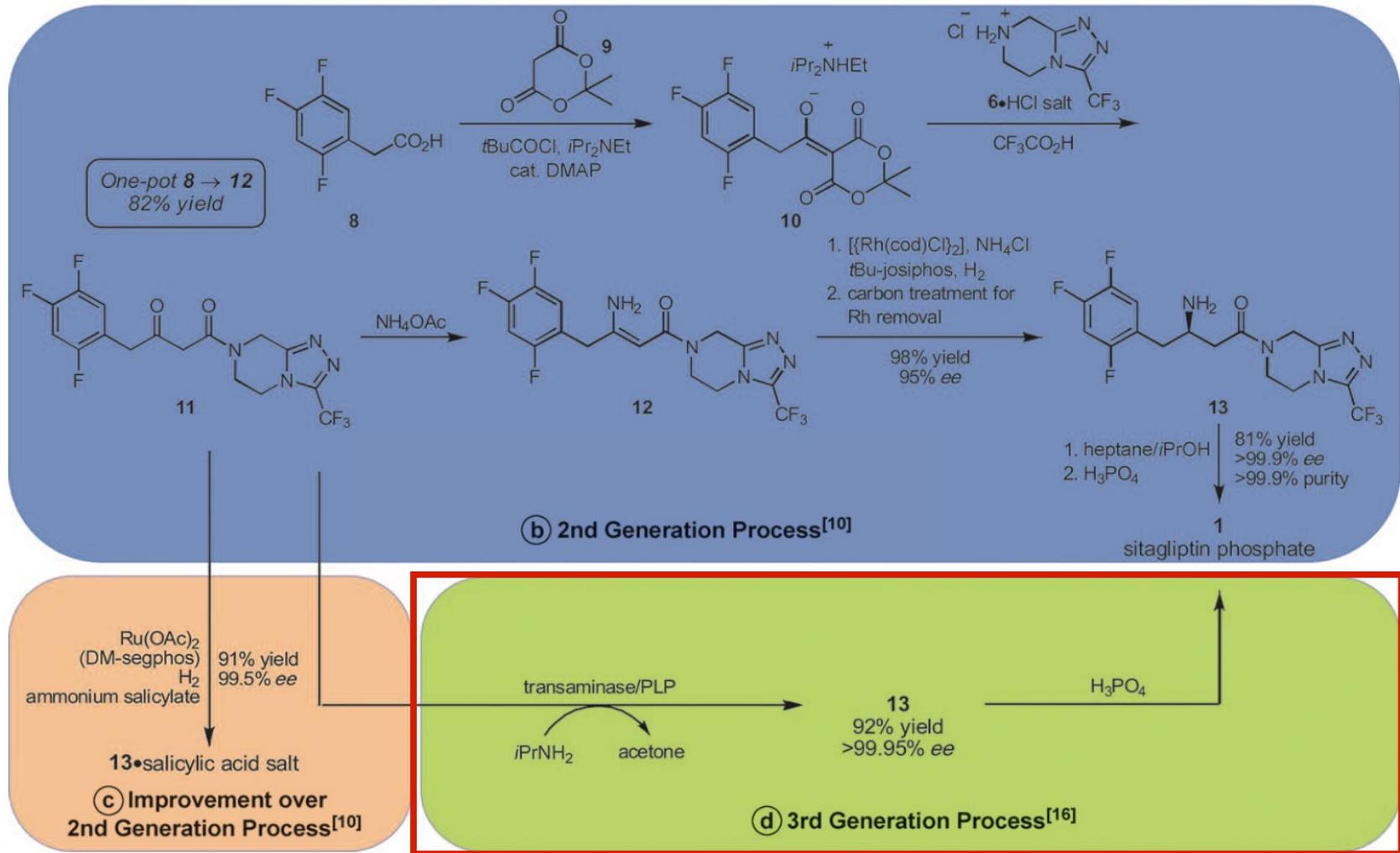
- Utilisent le Phosphate de Pyridoxal (PLP) comme cofacteur
- Mécanisme de type ping-pong bi-bi
- Subdivisées en 2 grandes familles :
 - α-TA : Alpha-aminoacides
 - Amine-TA : Tout type d'amines



Transaminases	Substrats	Substrat commun
α-TA	α-Aminoacides	L/D-Glutamate
Amine-TA	Tous types d'amines	L/D-Alanine



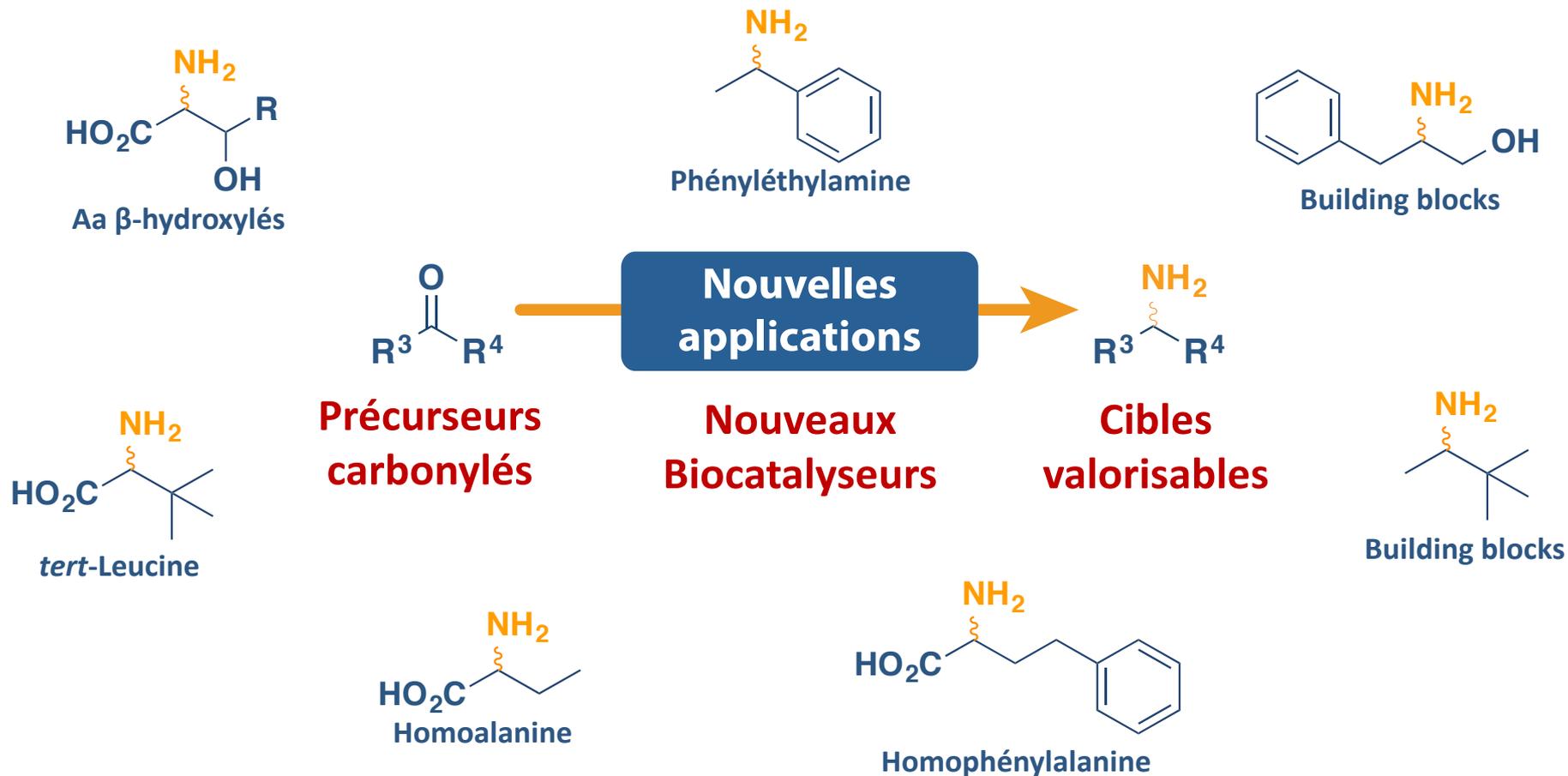
Synthèse enzymatique de la sitagliptine par MERCK



11 cycles de mutagenèse / 27 mutations

Performances accrues + meilleure tolérance aux solvants (DMSO, Acétone, etc.)

Recherche de nouvelles transaminases



⇒ Applications très variées (Boîte à outil)
⇒ Amélioration des connaissances sur les TA

RATIONAL DESIGN

1. Computer aided design



2. Site-directed mutagenesis



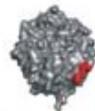
Individual mutated gene

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

Exploration génomique



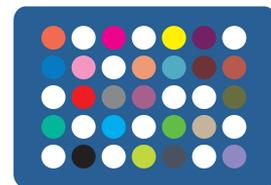
« **Transaminases** »

```

>P00509|Escherichia coli|Aspartate transaminase
ATGTTTGAGAACATTACCGCCGCTCTGCGCCGACCCGATTCTGGCCCTGGCCGATCT
GTTTCGTGCCGATGACACGTCCCGCAAATAACCTCGGGATTGGTGCTATAAAG
ATGAGACGGGCAAAACCCCG...
>Q9A671|Caulobacter crescentus|Histidinol-phosphate aminotransferase
ATGCCAAGCCCGGATCATGGACATCCACGCCTATGTCGGCGCAAGTCCAAGG
TCGAGGGCATCGCCATCCGGTGAAGCTGTCGAGCAACGAGAACATCTGGGCA
GCAGCGCAAGGCCAAGGAC...
>F2XBU9|Vibrio fluvialis|Omega transaminase
ATGAACAAACCGCAAAGCTGGGAAGCCCGGCCGAGACCTATTGCTCTATGGTT
TCACCGACATGCCTTCGCTGCATCAGCGCGGCACGGTCTGCTGTGACCATGGCGCA
GGGACCTATATCGTCGATGTG...
>P19938|Bacillus sp.|D-alanine transaminase
ATGGGATACACTTTATGGAATGACCAAATCGTGAAGATGAAGAAGTCAAATTG
ATAAAGAAGATCGCGGTTATCAATTCGGTGATGGCGTATGAAGTTGTGAAAGTAT
ATAACGGTGAATGTTACTGT...
> ...
    
```

1075000 séquences

Annotation semi-automatique



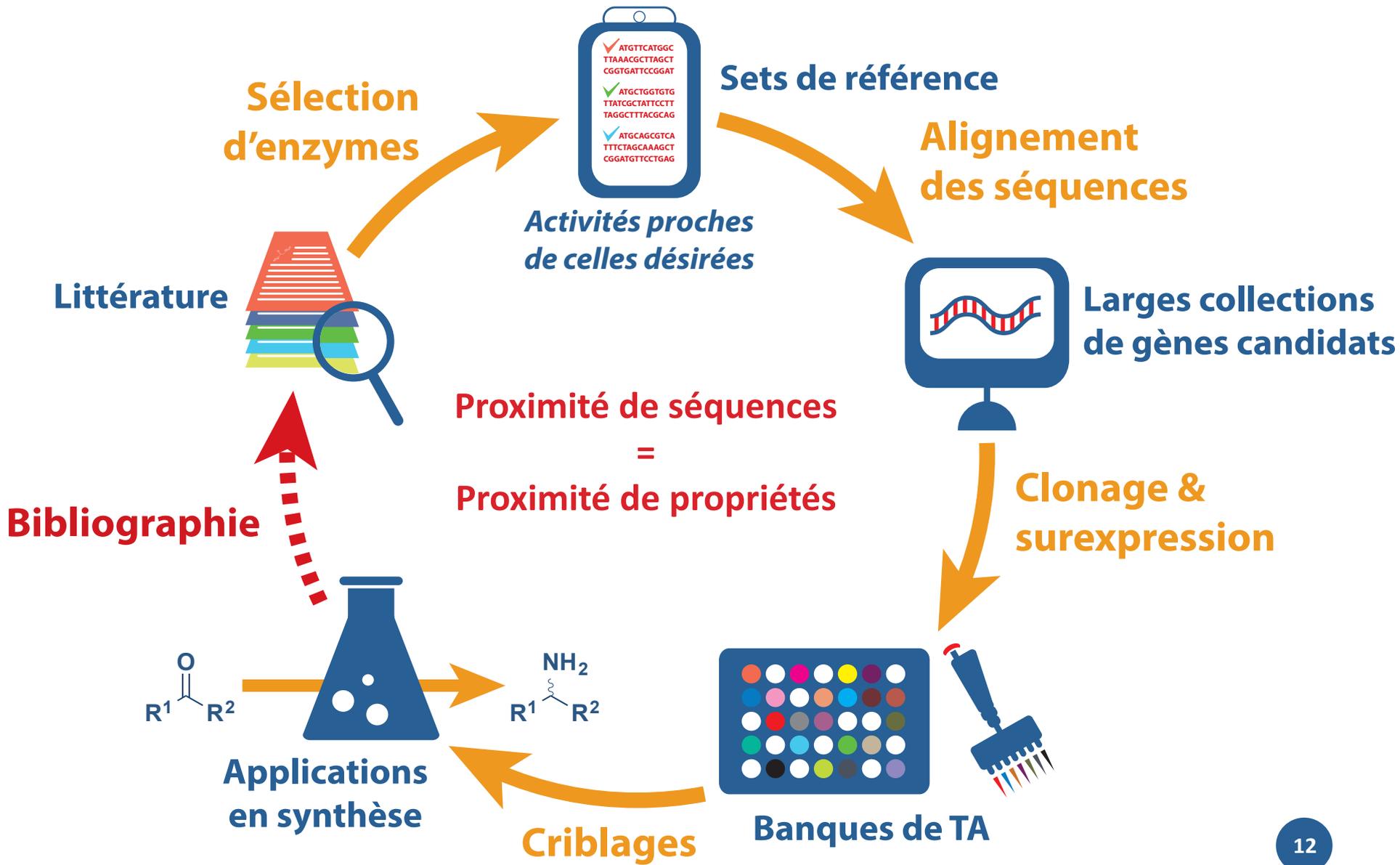
Banque de 500 ~ 1000 enzymes

Reviewed (3,119)
Swiss-Prot

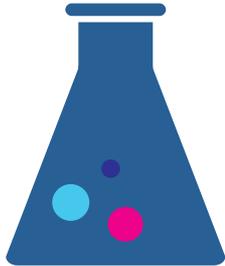
Unreviewed (1,092,944)
TrEMBL

Environ 0.3%

Stratégie de sélection des enzymes



Utilisation du clustering



- Grande variété d'applications
- Nouvelles propriétés

- ✓ Diversité de séquences
- ✓ Large spectre de substrats
- ✓ Thermostabilité, etc. ?

Set de référence (18 enzymes)

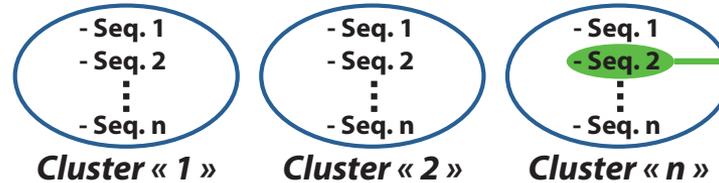


Uniprot
Metagenome

BLAST
> 30% d'identité

10000 Séquences

« Clustering »
(80% d'identité)



Disponibilité
dans la souchothèque

```

>P00509|Escherichia coli|Aspartate transaminase
ATGTTTGAGAACATTACCGCCGCTCTGCCGACCCGATTCTGGCCCTGGCCGATCT
GTTTCGTGCCGATGAACGTCCCGGCAAATAACCTCGGGATTGGTGTCTATAAAG
ATGAGACGGGCAAACCCCG...

>Q9A671|Caulobacter crescentus|Histidinol-phosphate aminotransferase
ATGCCAAAGCCCGGATCATGACATCCACGCCTATGTCGGCGGCAAGTCCAAGG
TCGAGGGCATCGCCATCCGGTGAAGCTGTGAGCAACGAGAACATCTGGGCA
GCAGCGACAAGGCCAAGGAC...

>F2XBU9|Vibrio fluvialis|Omega transaminase
ATGAACAAACCGCAAAGCTGGGAAGCCCGGCGAGACCTATTGCTCTATGGTT
TCACCGACATGCCTTCGCTGCATCAGCGCGCACGGTCTGTCGTGACCCATGGCGA
GGGACCCTATATCGTCGATGTG...
    
```

462 gènes à cloner

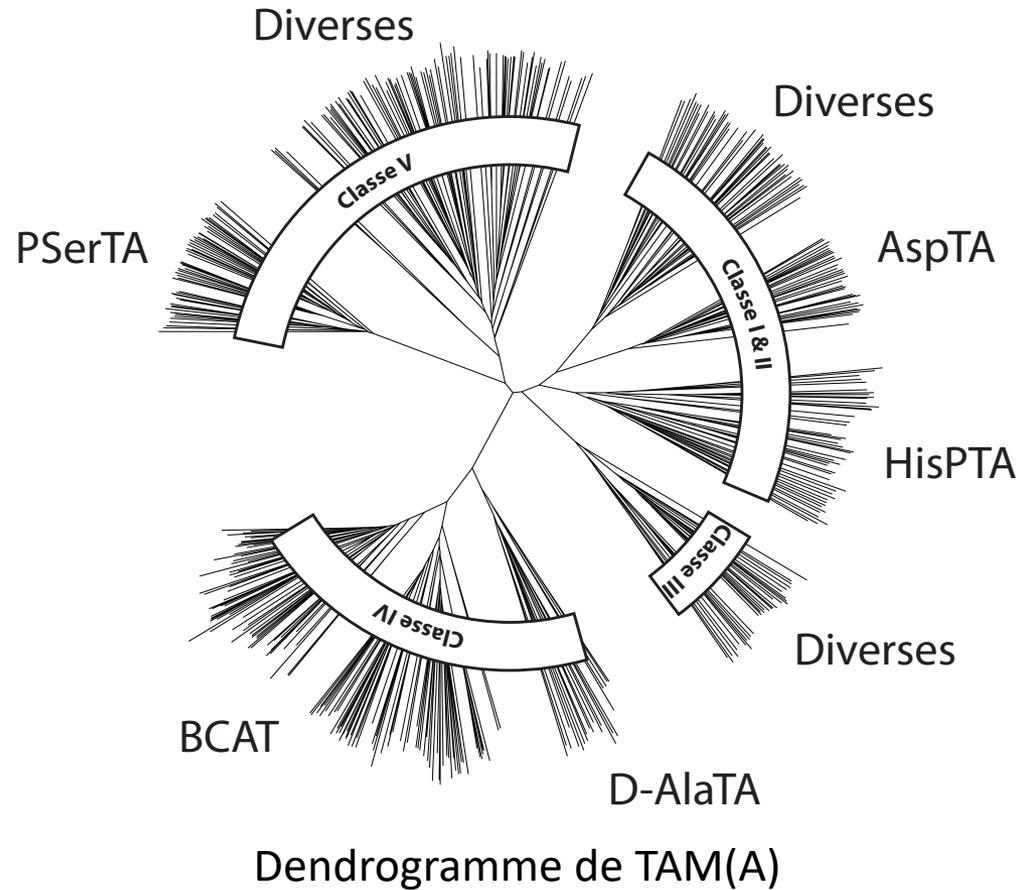
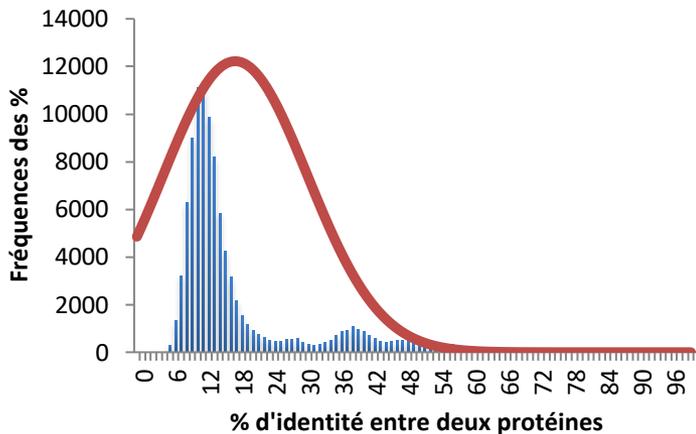
⇒ Set de référence : **18** Enzymes

⇒ **10000** séquences candidates

⇒ **462** gènes à cloner

⇒ **232** protéines confirmées

Diversité :



Criblages et résultats

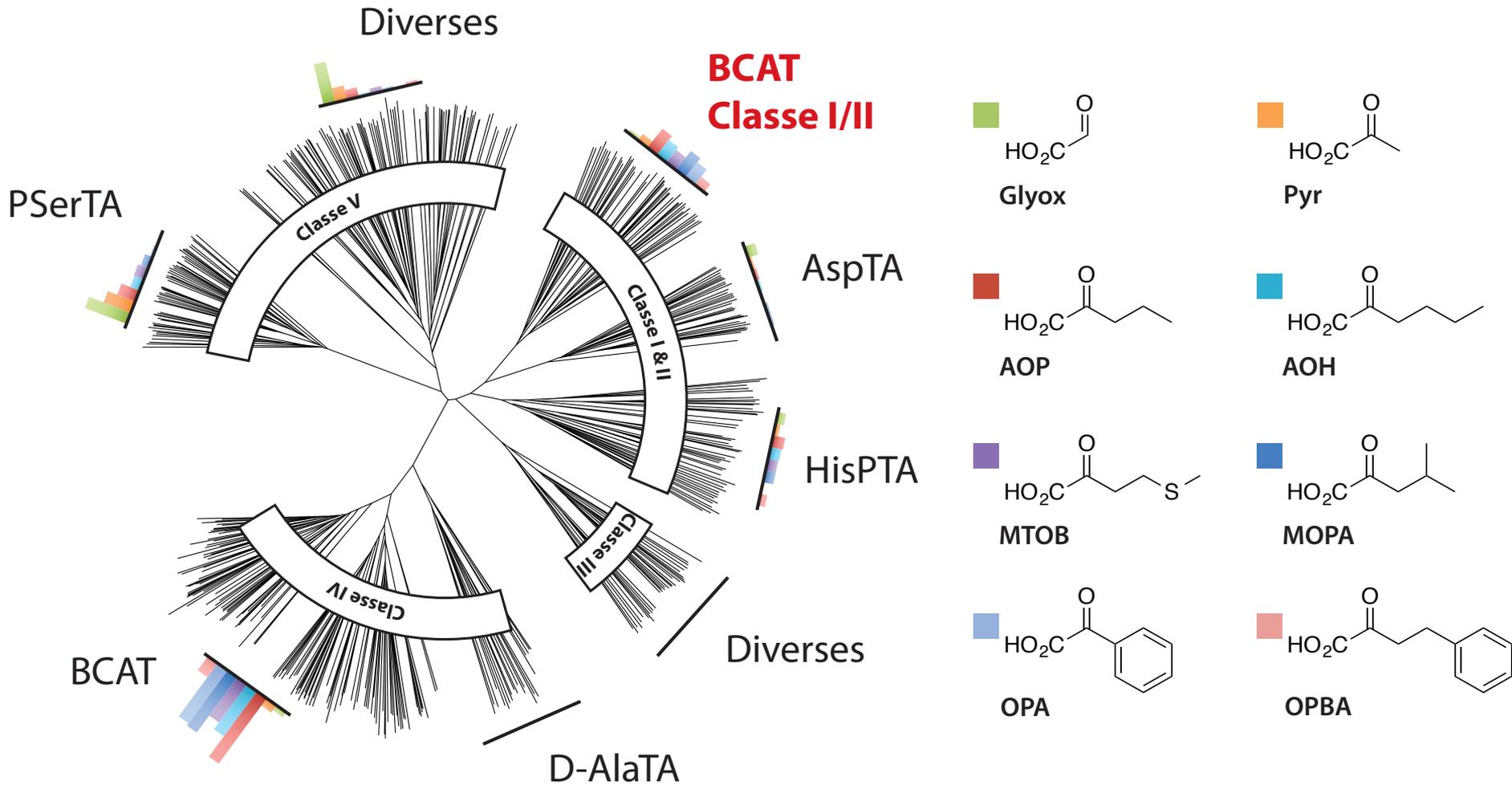
Banque	Classes de TA	Test	Donneur principal	Donneur secondaire	Enzyme Auxillaire	TA ciblées
TAM(A)	I - V	Direct - ACS	L-ACS	---	---	L- α -BASTA
			D-ACS	---	---	D- α -BASTA
		Couplé - ACS	L-Glu	L-ACS	AspTA	L- α -TA
TAM(C)	IV	Couplé - ACS	D-Glu	L-ACS	AspTA	D- α -TA
TAM(D)	III	Direct - HPT	HPT	---	---	HypoTA

3 banques avec **800 enzymes** / Criblage sur **19 accepteurs**

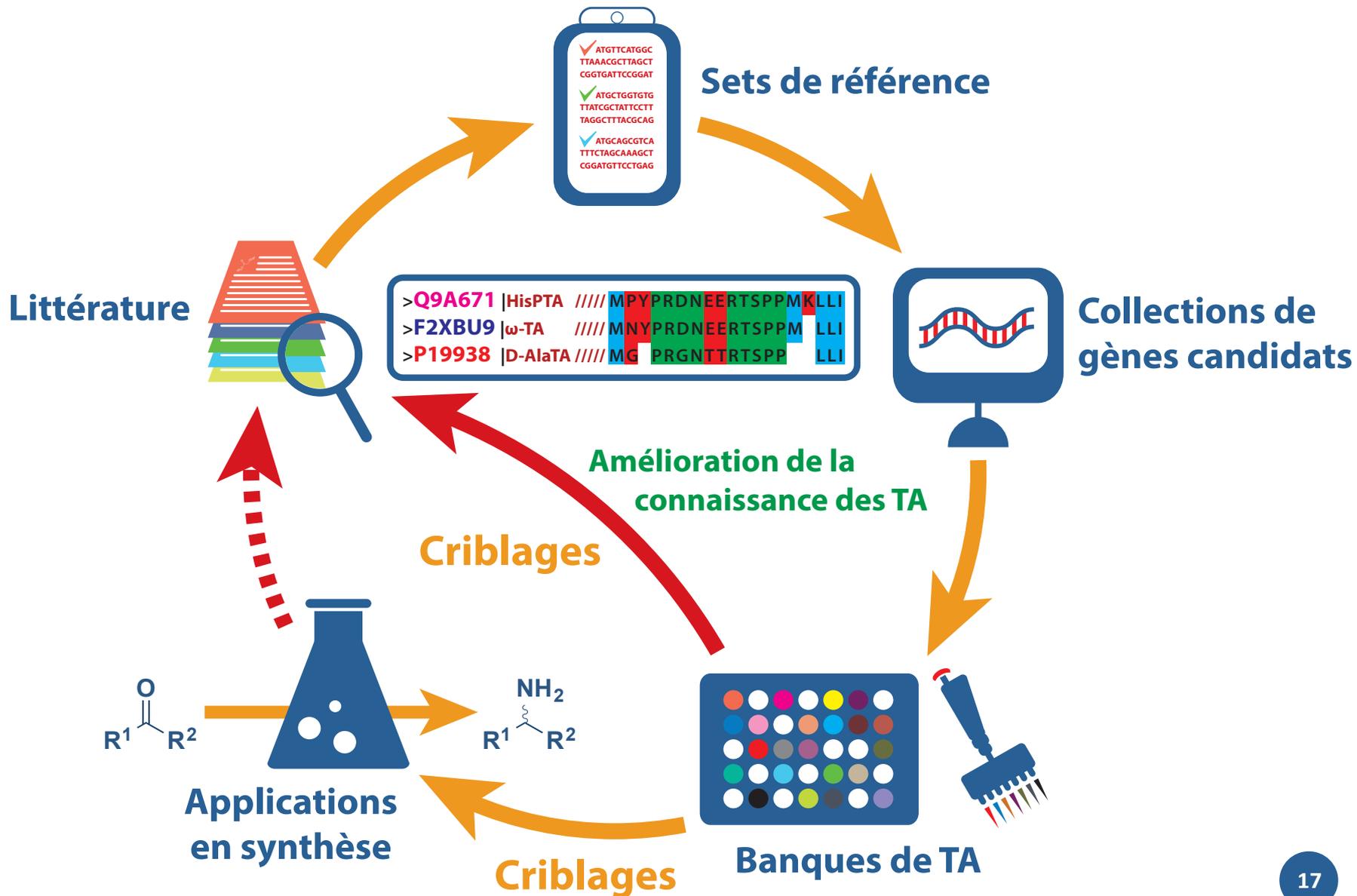


≈ **50000 tests enzymatiques**

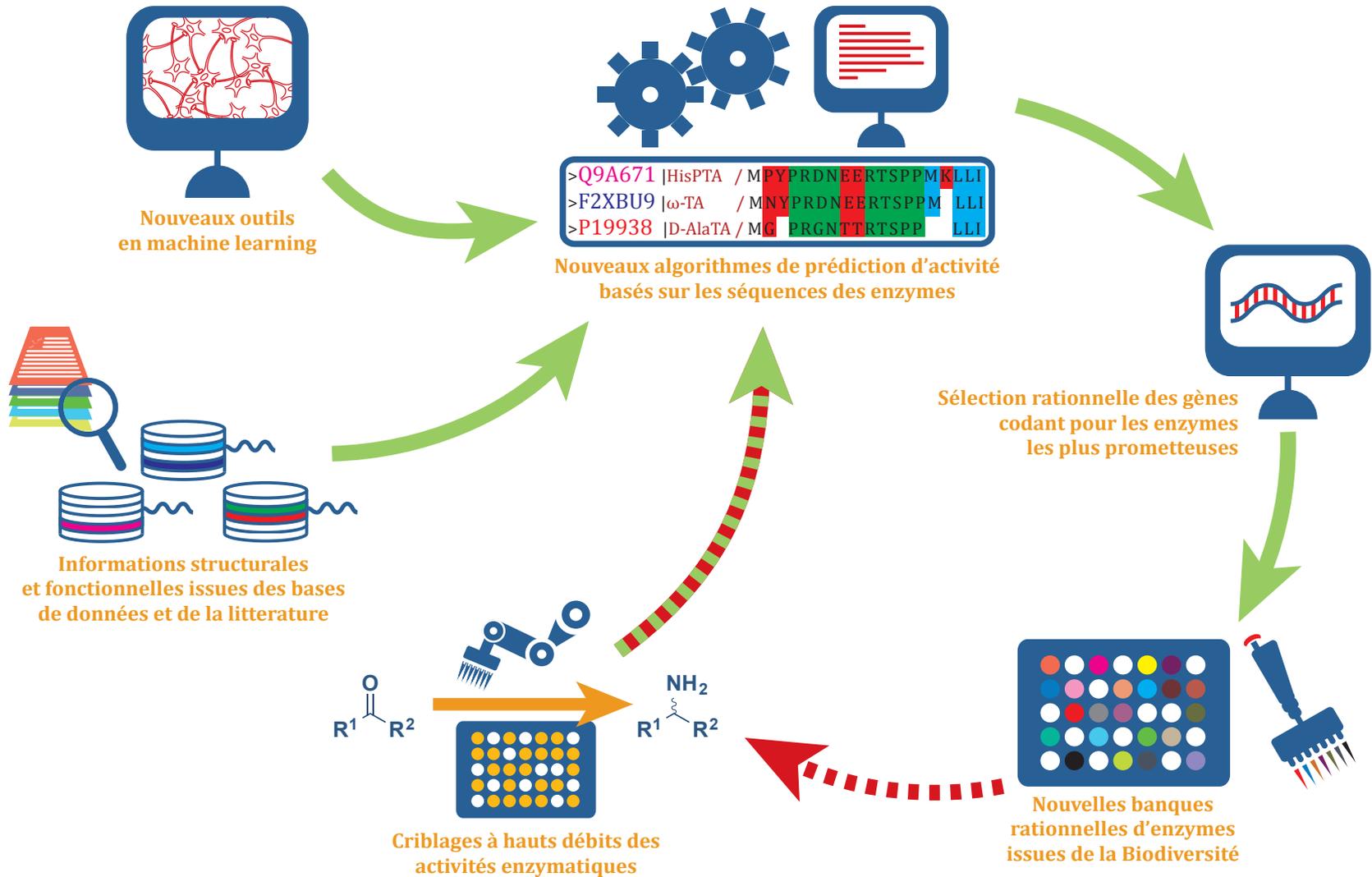
Résultats pour TAM(A)



Aller plus loin dans l'exploration ?



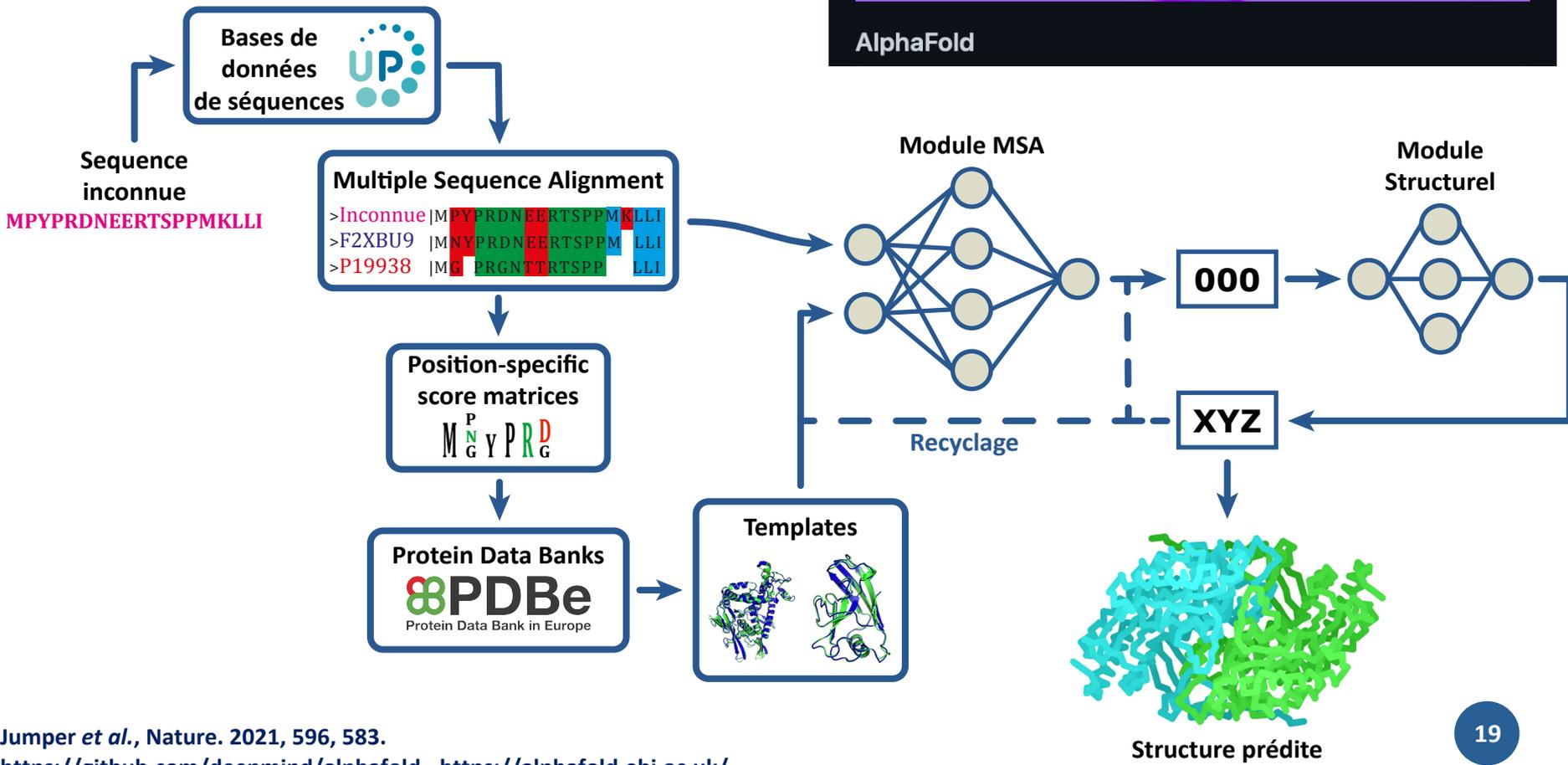
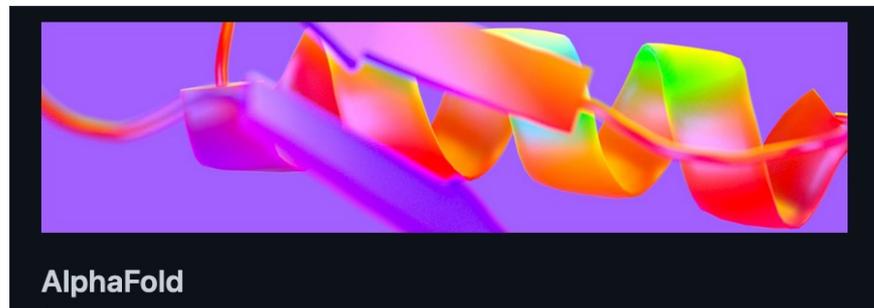
Utilisation du *Machine Learning*



La révolution AlphaFold?

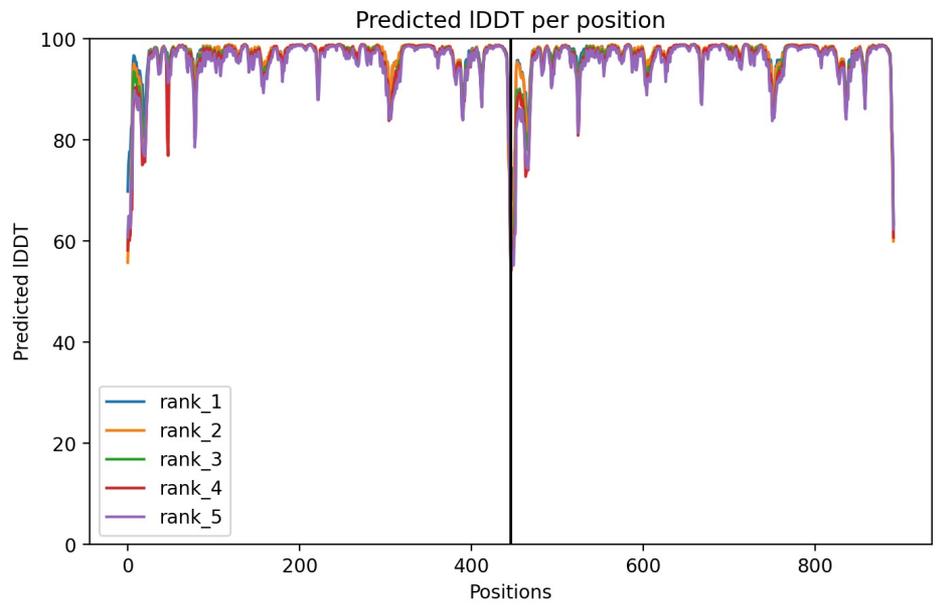
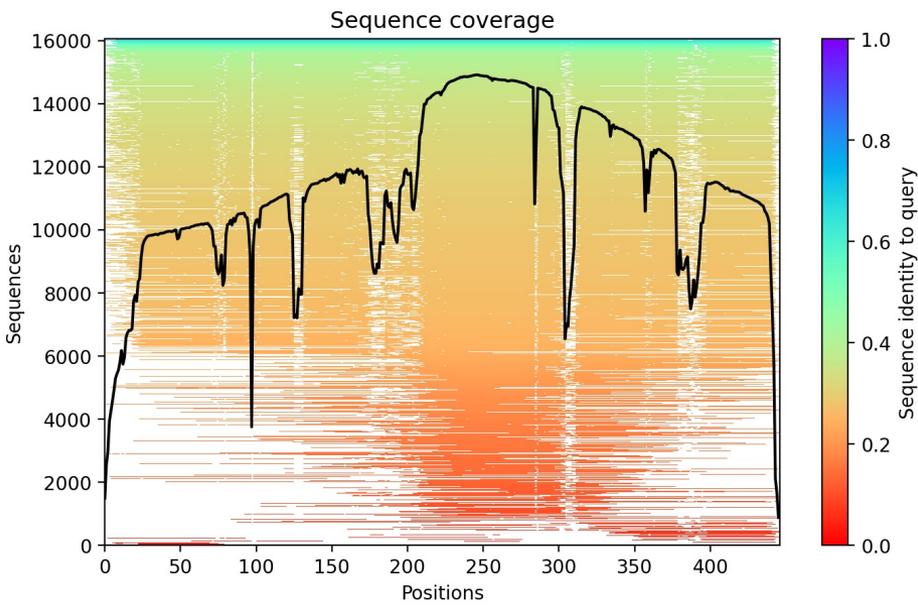
AlphaFold2 – Prédiction de structures

➤ Gagnant du CASP14 (2020)



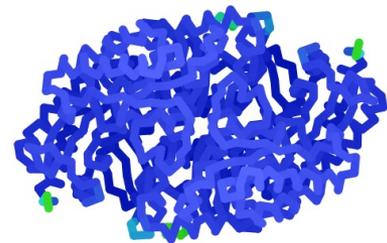
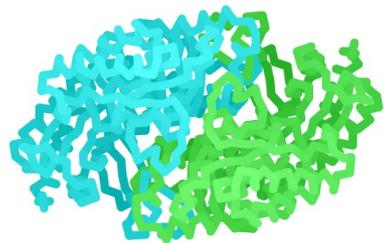
Prédiction de la structure de B9AZ94

```
>tr|B9AZ94|B9AZ94_9BURK Aminotransferase, class III superfamily OS=Burkholderia multivorans CGD1 OX=513051 GN=BURMUCGD1_4017 PE=3 SV=1
```



colored by chain

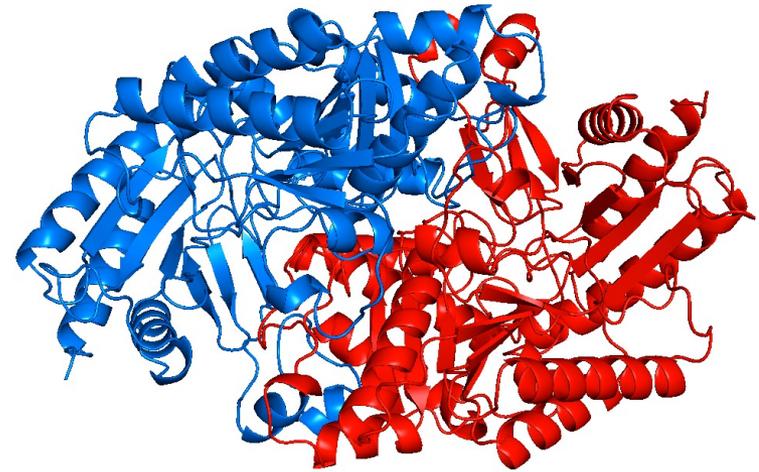
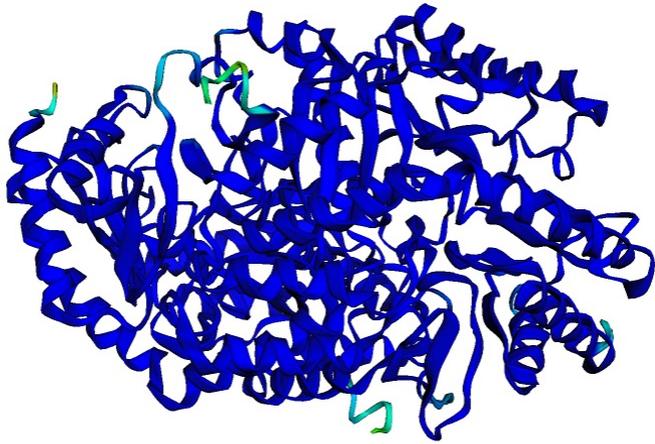
colored by pLDDT



pLDDT : 96,71
pTMscore : 0,96

Structure prédite

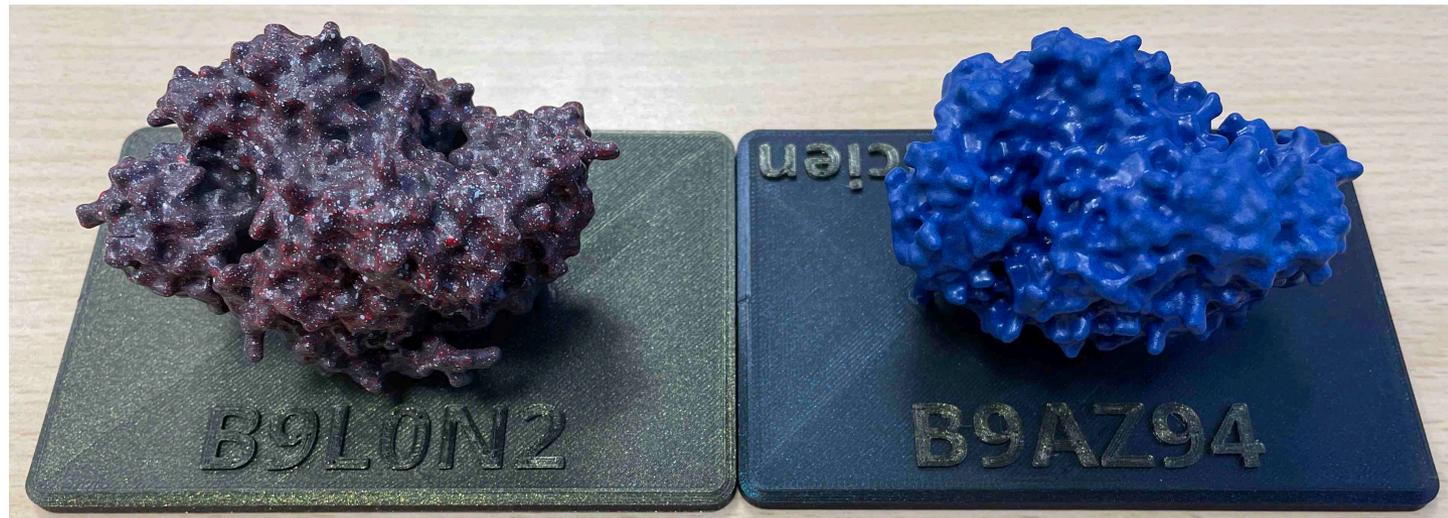
Aller plus loin sur la compréhension ?



pIcDT: ■ Very low (<50) ■ Low (60) ■ OK (70) ■ Confident (80) ■ Very high (>90)

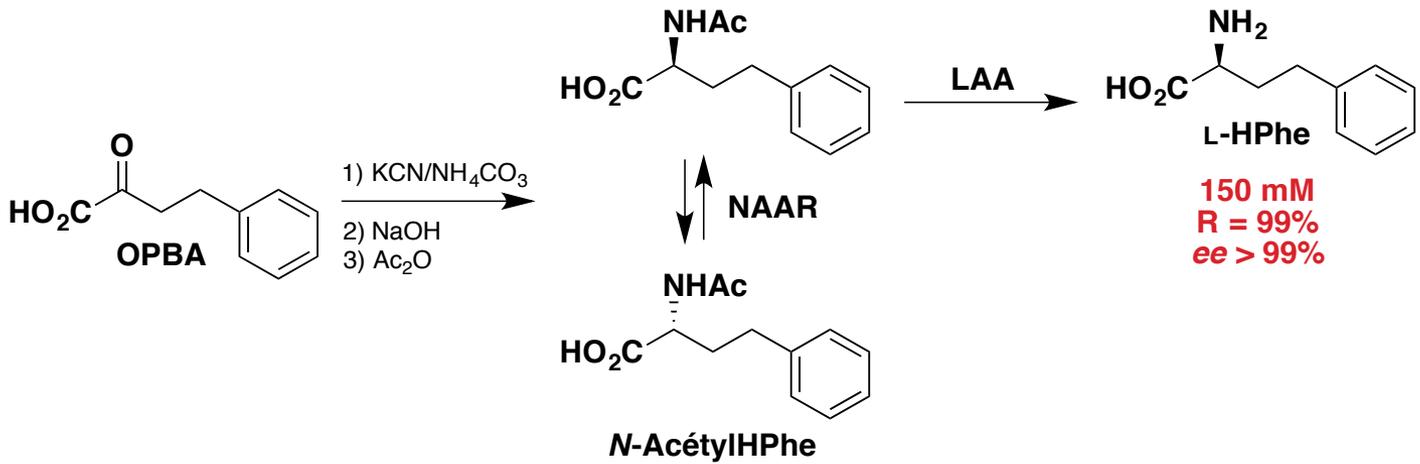
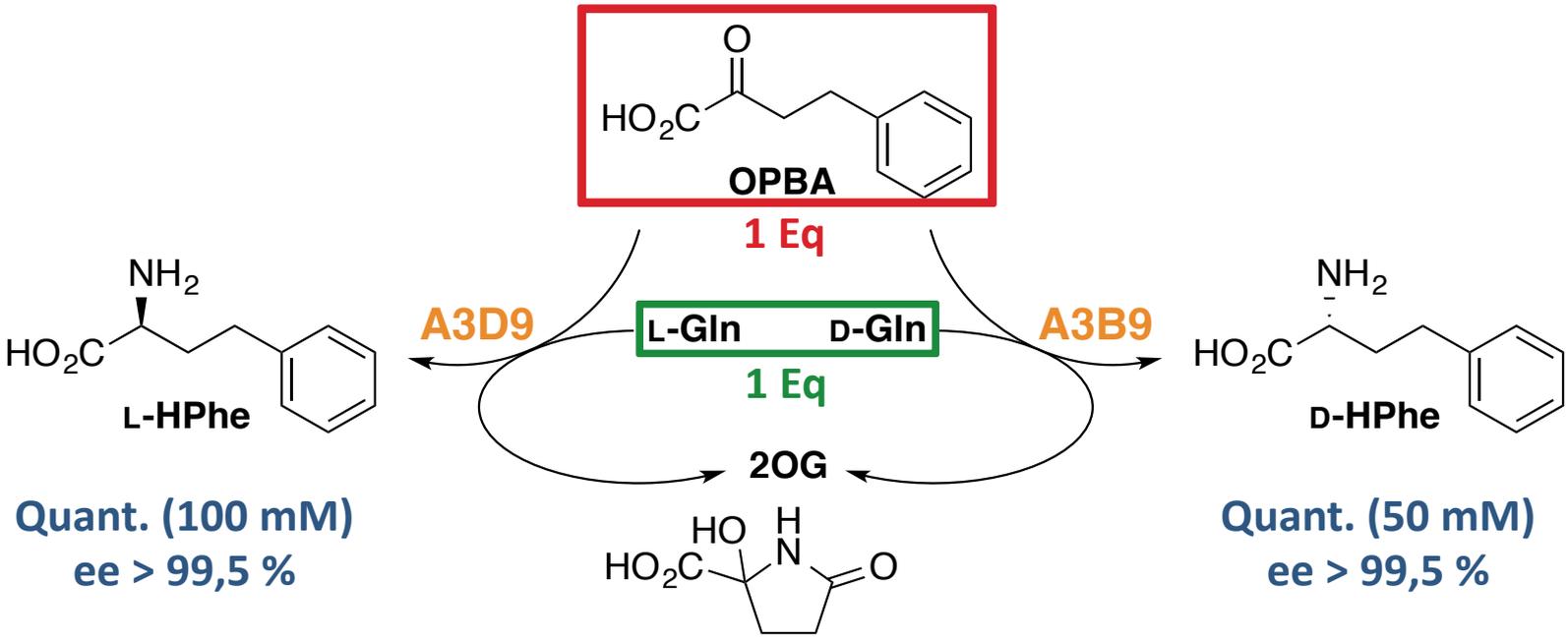


Impression 3D SLA



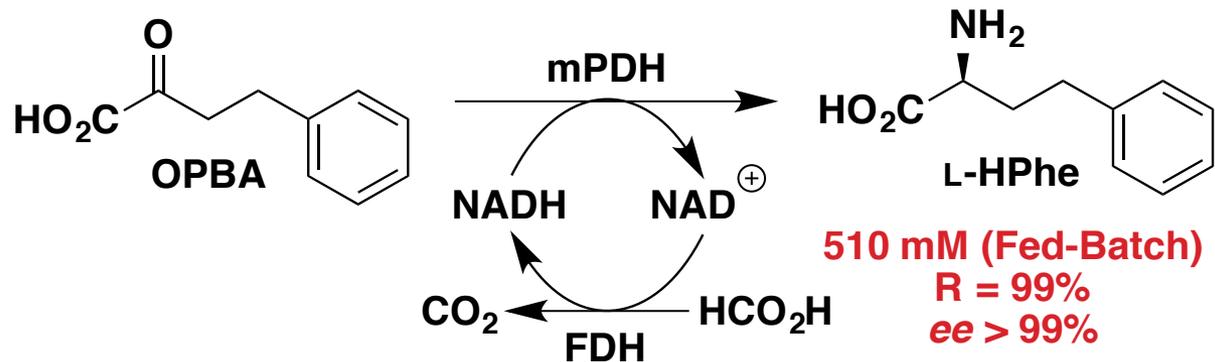
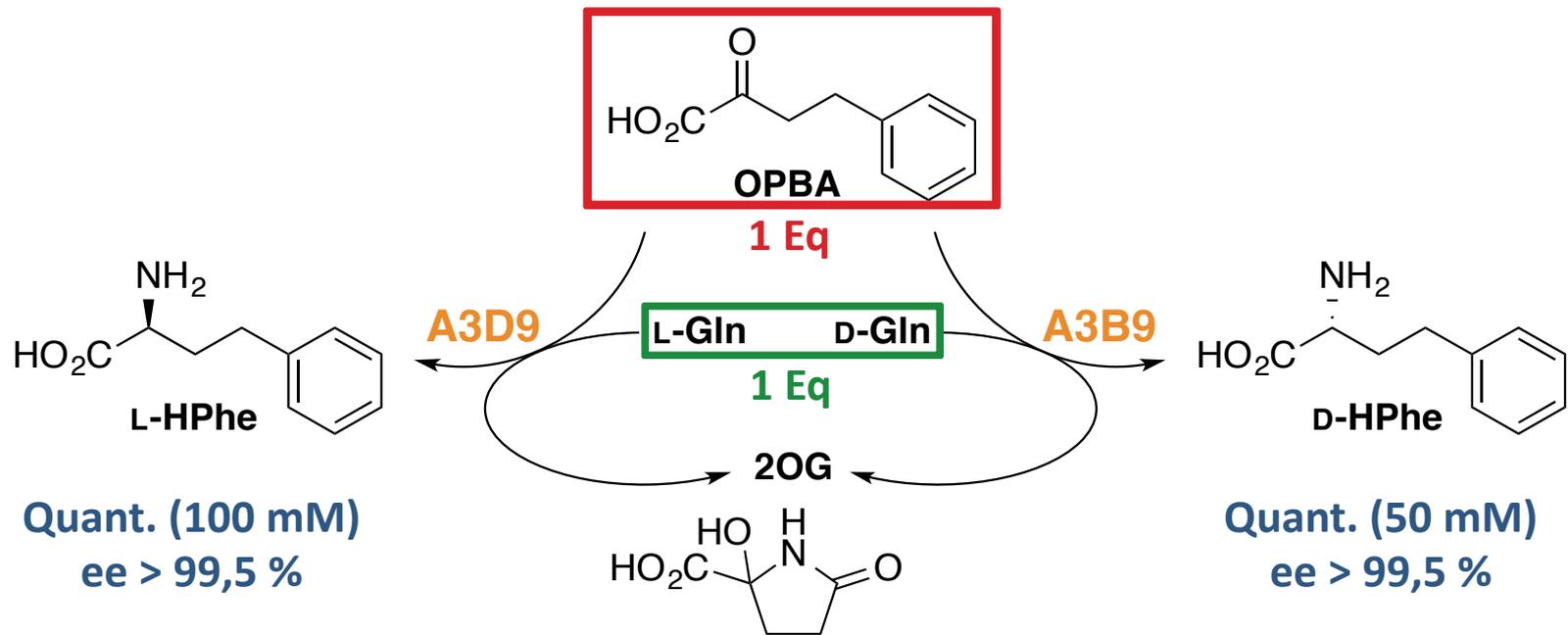
Application des nouvelles enzymes

Synthèse de l'Homophénylalanine



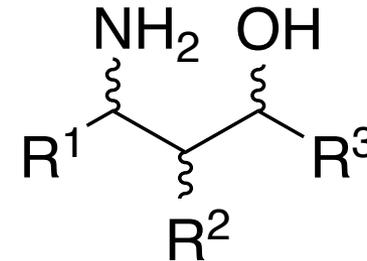
Heuson et al., Advanced Synthesis & Catalysis. 2019, 361 (4), 778. / Ahmad et. al., Biotechnol. Adv.. 2009, 29, 286.

Synthèse de l'Homophénylalanine

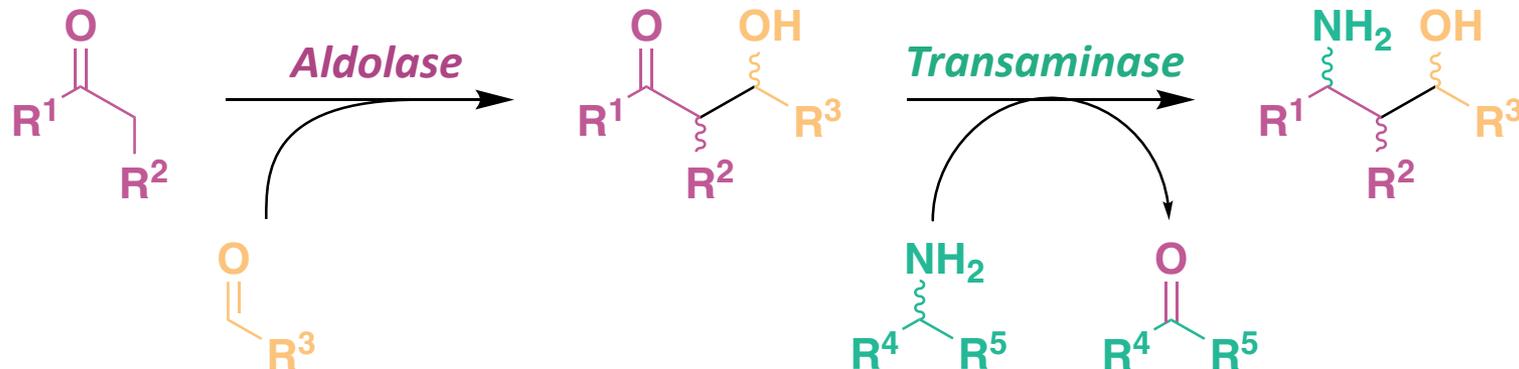


Synthèse de γ -aminoacides

- Précurseurs de composés à activité biologique
- Motifs complexes avec beaucoup de carbones asymétriques
- Synthèse en cascades avec :
 - **Aldolases** : création d'une liaison C-C (aldolisation)
 - **Transaminases** : Transfert d'une amine

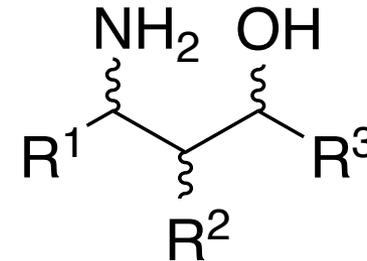


γ -aminoalcools

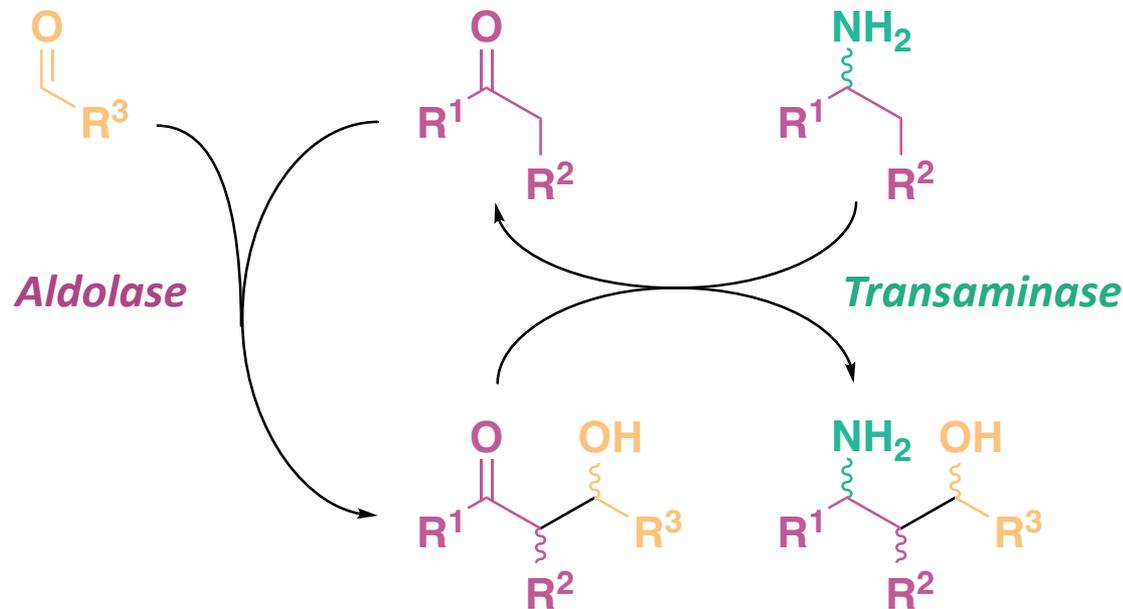


Utilisation de boucles enzymatiques

- Economie d'atomes
- Contrôle cinétique ou thermodynamique
- Rendements accrus
- Stéréosélectivité

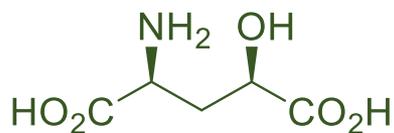
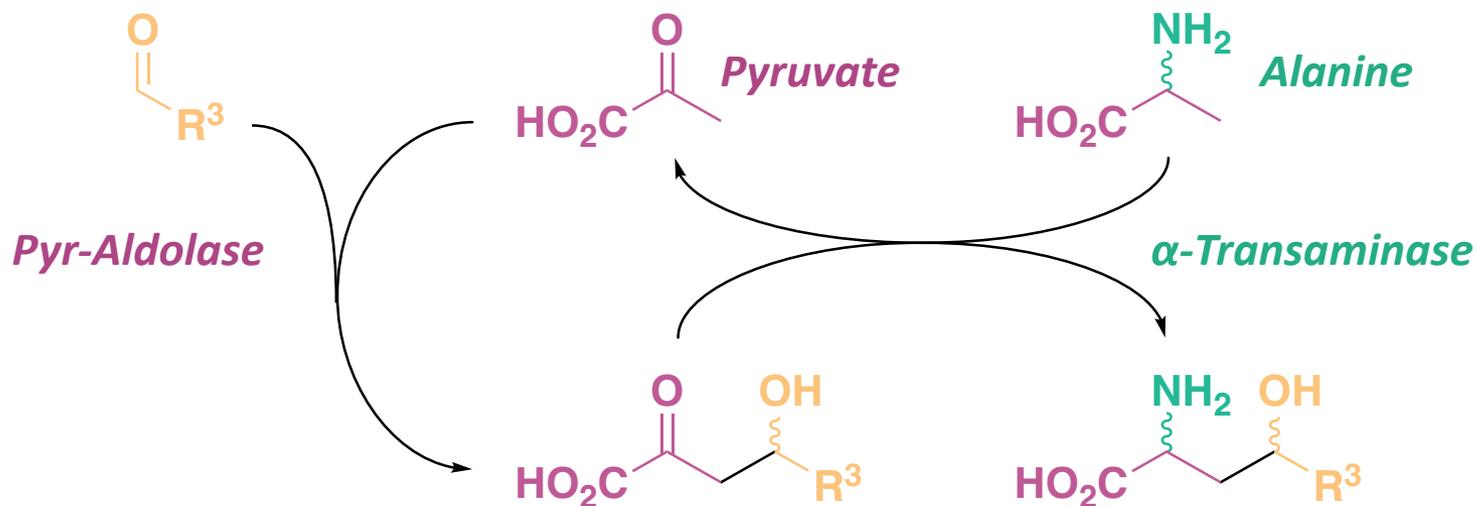


γ -aminoalcools



Enzymes avec
des sélectivités
complémentaires
(issues des
criblages)

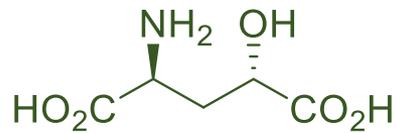
Boucles Aldolases-Transaminases



L-syn-4-HGlu

92 %

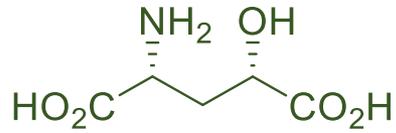
syn/anti : 98/2



L-anti-4-HGlu

95 %

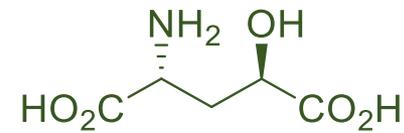
syn/anti > 2/98



D-syn-4-HGlu

65 %

syn/anti : 94/6



D-anti-4-HGlu

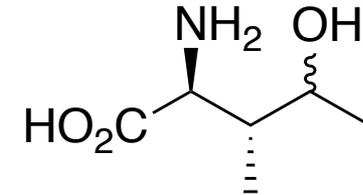
69 %

syn/anti : 6/94

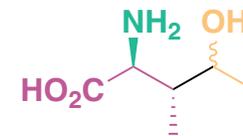
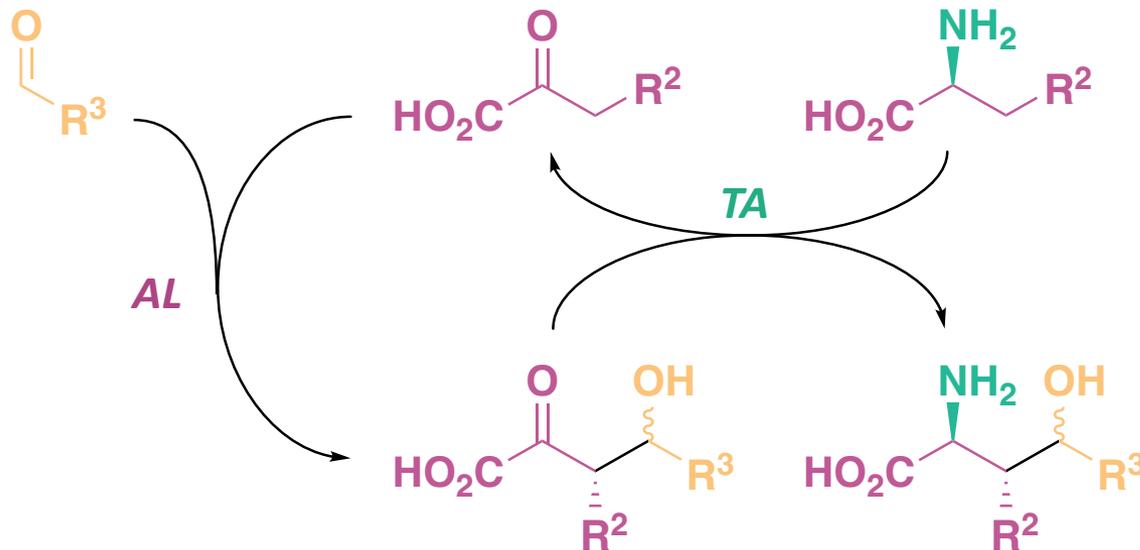
Analogue du glutamate hydroxylé : analogue de neurotransmetteur, peptidomimétiques, etc.

Synthèse de la 4-hydroxyisoleucine et dérivés

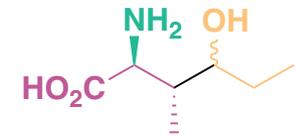
- Acide aminé potentialisateur de la sécrétion d'insuline
- Rendement atteint dans la littérature avec une cascade linéaire : 21%
- Stéréochimie à contrôler



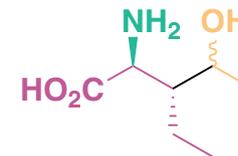
4-Hydroxyisoleucine (Hile)



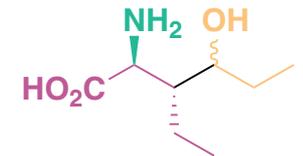
Conversion : 97 %
syn/anti : 4/96



Conversion : 85 %
syn/anti : 15/85

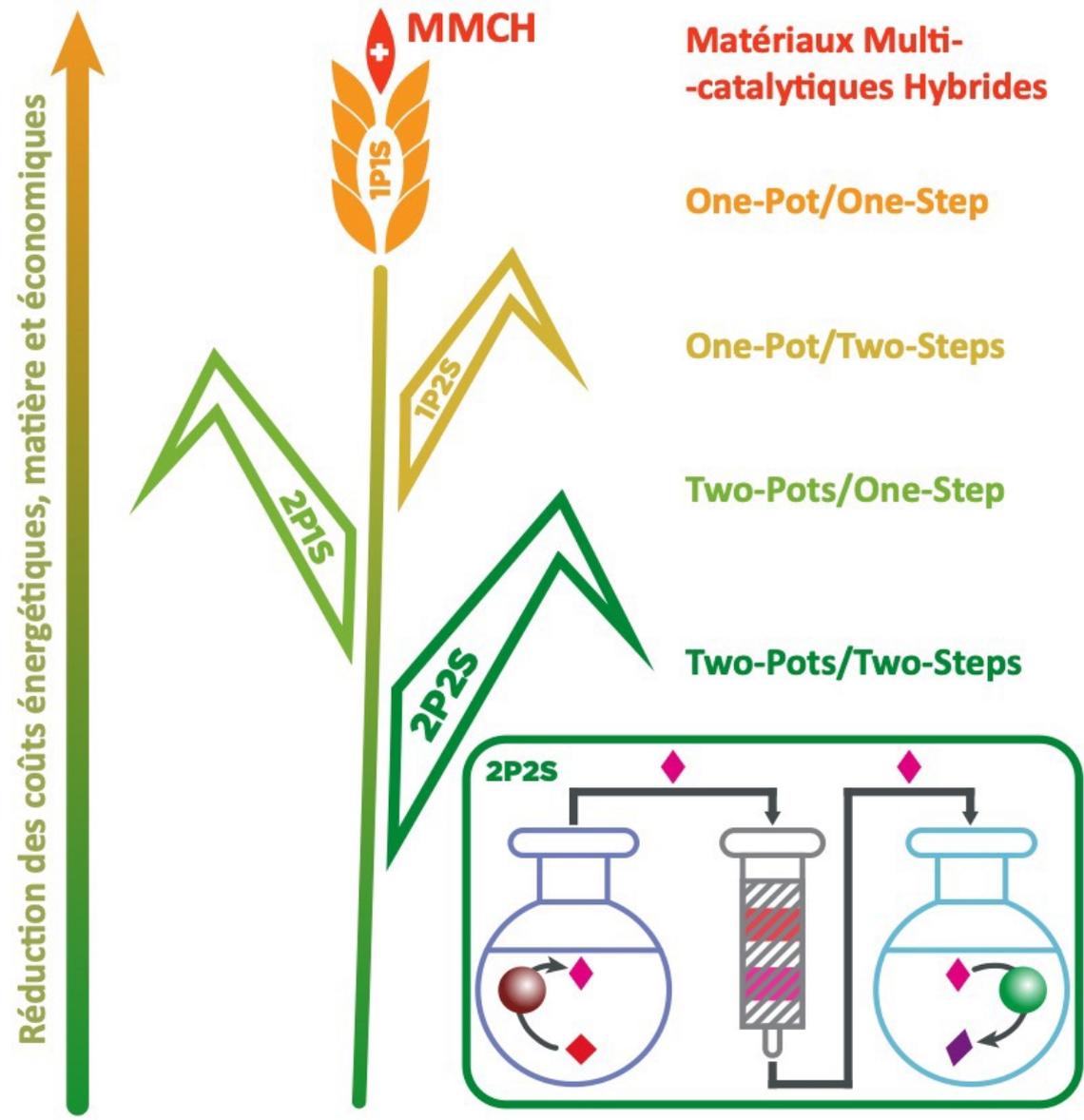


Conversion : 65 %
syn/anti : 18/82



Conversion : 46%
syn/anti : 17/83

Combinaison chimie-biologie : la catalyse hybride



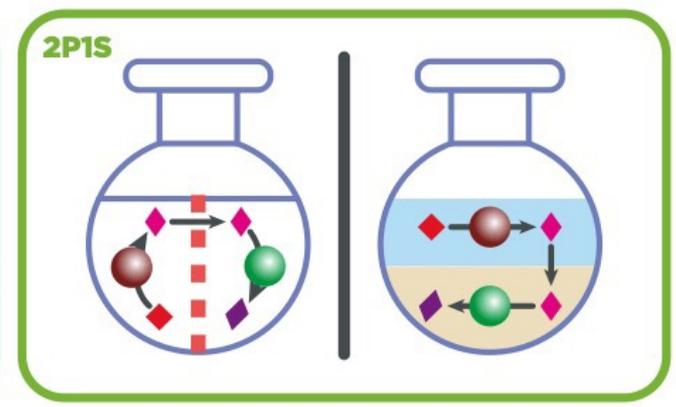
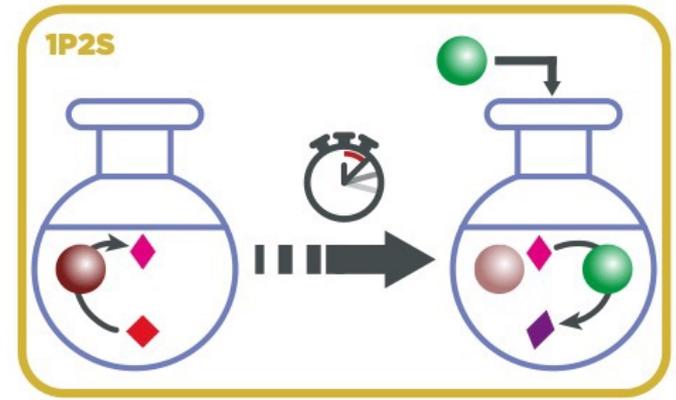
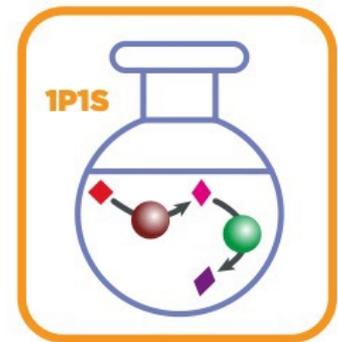
Matériaux Multi-catalytiques Hybrides

One-Pot/One-Step

One-Pot/Two-Steps

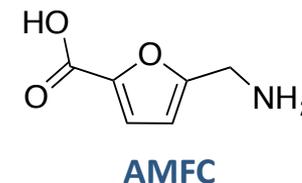
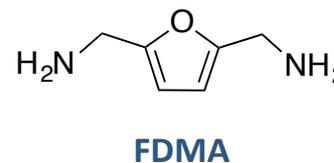
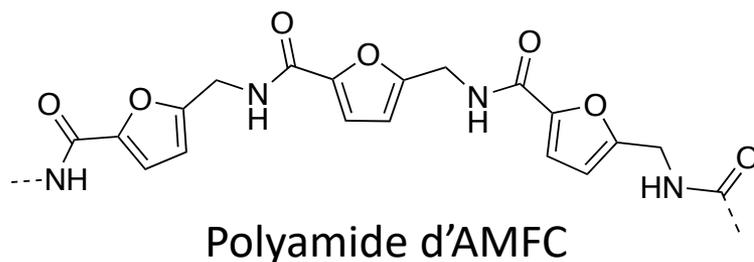
Two-Pots/One-Step

Two-Pots/Two-Steps



Applications

- Très peu d'applications décrites
- AMFC : Oligopeptide trimère cyclique
 - Production de nouveaux polyamides/polyimines et autres polymères



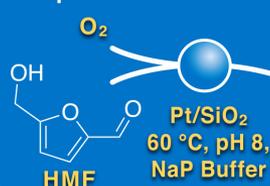
Voies de synthèse

- Amination réductrice (nécessite de nombreuses protections/déprotections)
 - Récente nouvelle méthodologie sans protection
- Biocatalytique (transamination)
 - Deux études seulement, aucune méthodologie pour l'AMFC, l'AMFA, et la FDMA

Combinaison de **Pt@SiO₂** et de **Cv-TA@EziG™ OPAL**

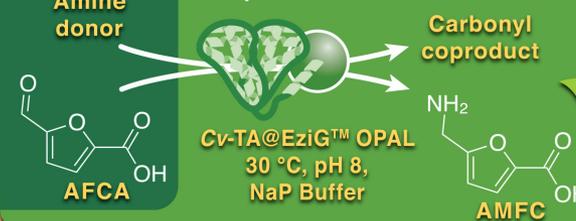
One-pot/two-steps hybrid catalysis

Step 1: Oxidation

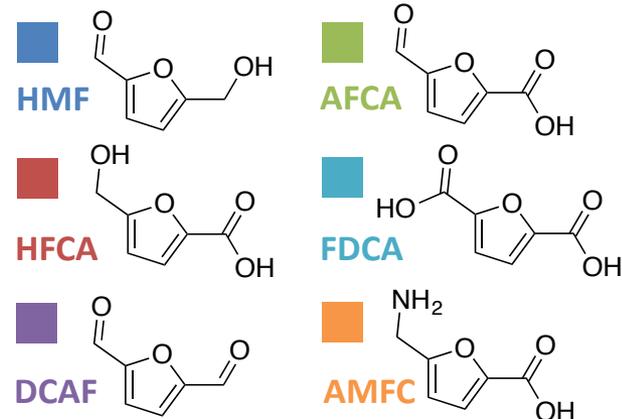


Amine donor

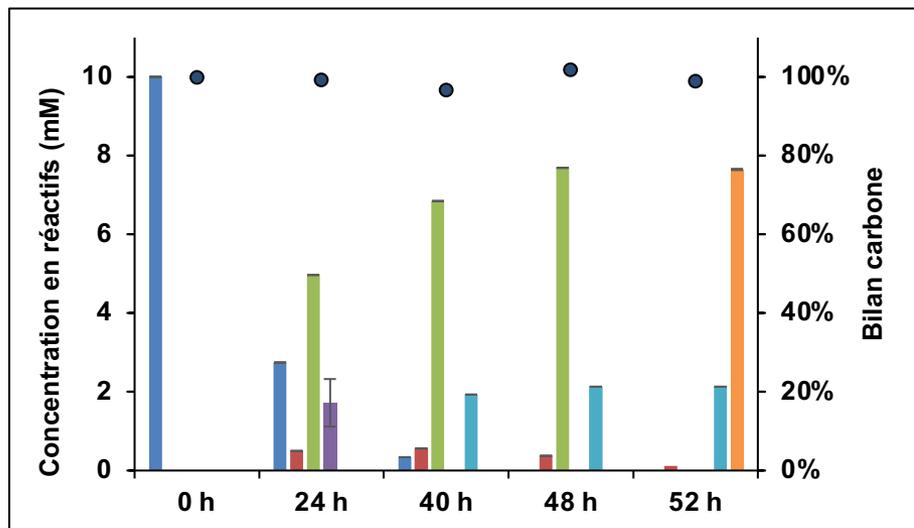
Step 2: Reductive amination



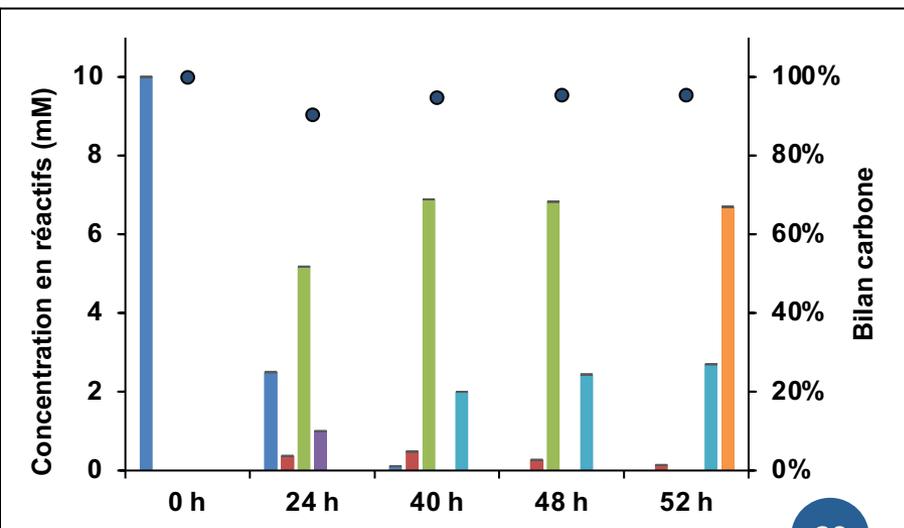
Carbonyl coproduct

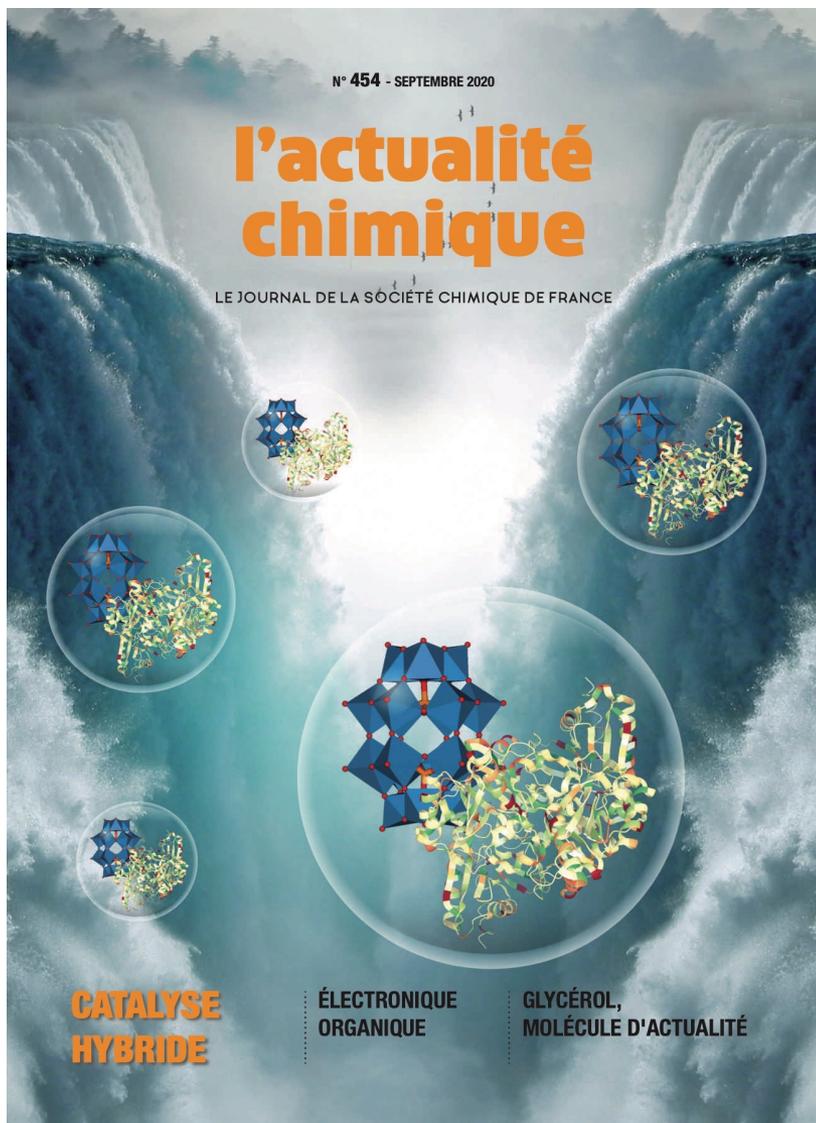


Donneur : (S)-Methylbenzylamine (1:1)

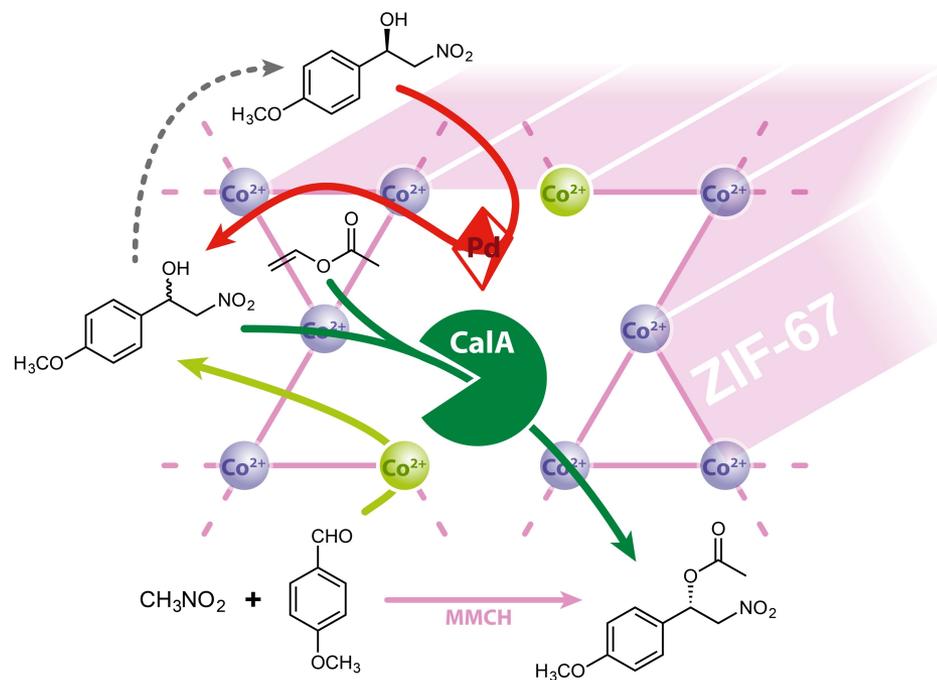


Donneur : Isopropylamine (10:1)



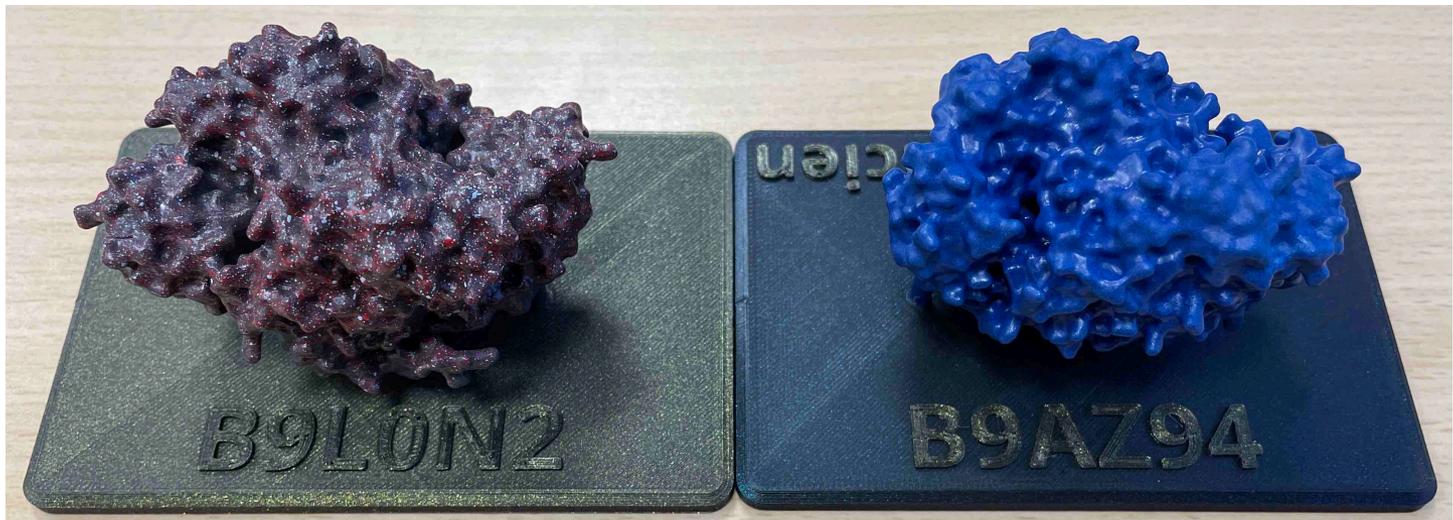


MMCH tri-catalytique à base de Metal-Organic Frameworks (MOF)



CalA + Pd-NPs@Co-ZIF-67

Merci pour votre attention!



... au Nord, c'étaient les Corons!