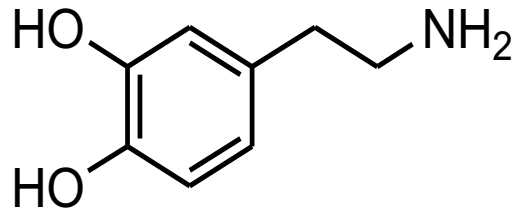
University of Crete
Department of Chemistry

Postgraduate Course: Protein Engineering

Metabolic pathway of dopamine



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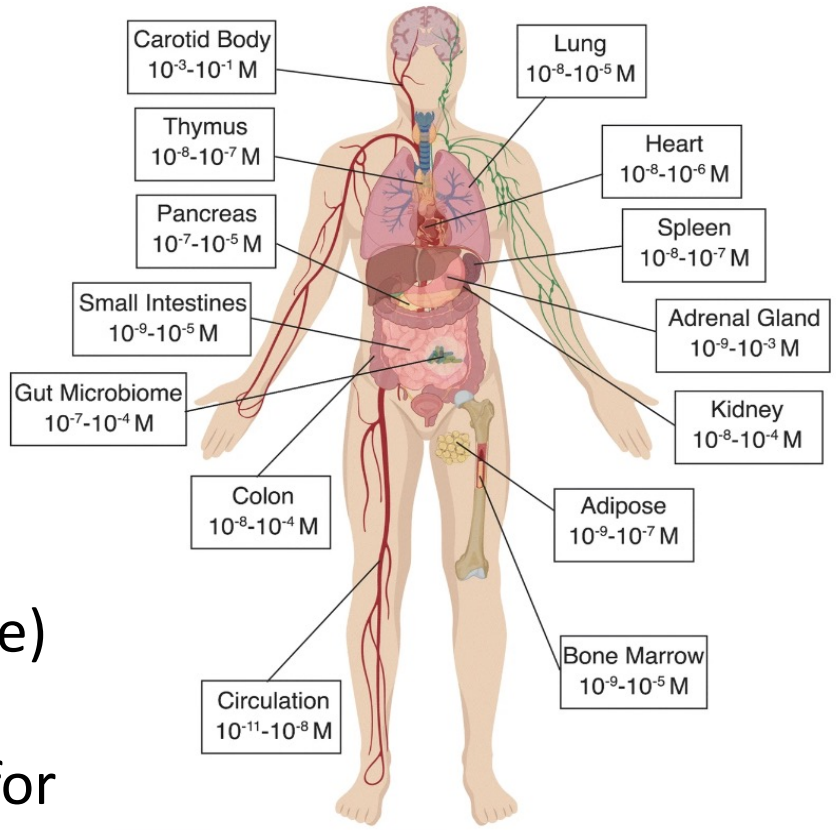
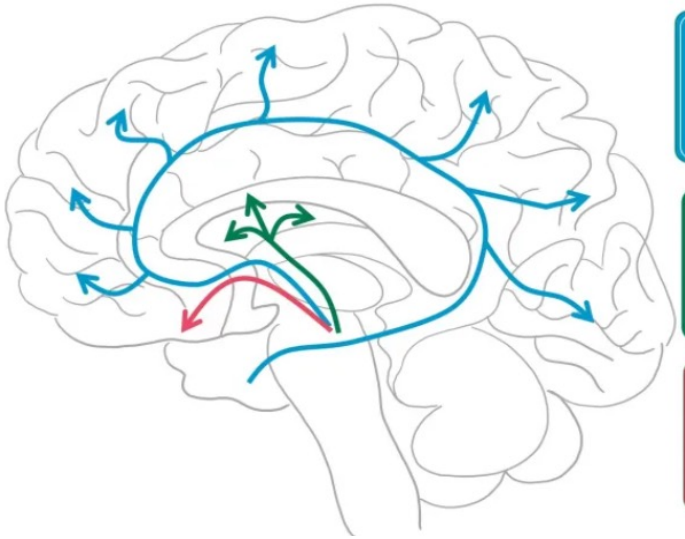
Dopamine

- **Neurotransmitter** produced in the substantia nigra, ventral tegmental area, and hypothalamus of the brain.
- **Regulates** cardiac, vascular, endocrine **function**
- Synthesized from **phenylalanine** or **tyrosine**
- **Precursor** in the biosynthesis of other **catecholamines** (norepinephrine and epinephrine)
- Arvid Carlsson was awarded in **2000 Nobel prize** for **proving dopamine as neurotransmitter**



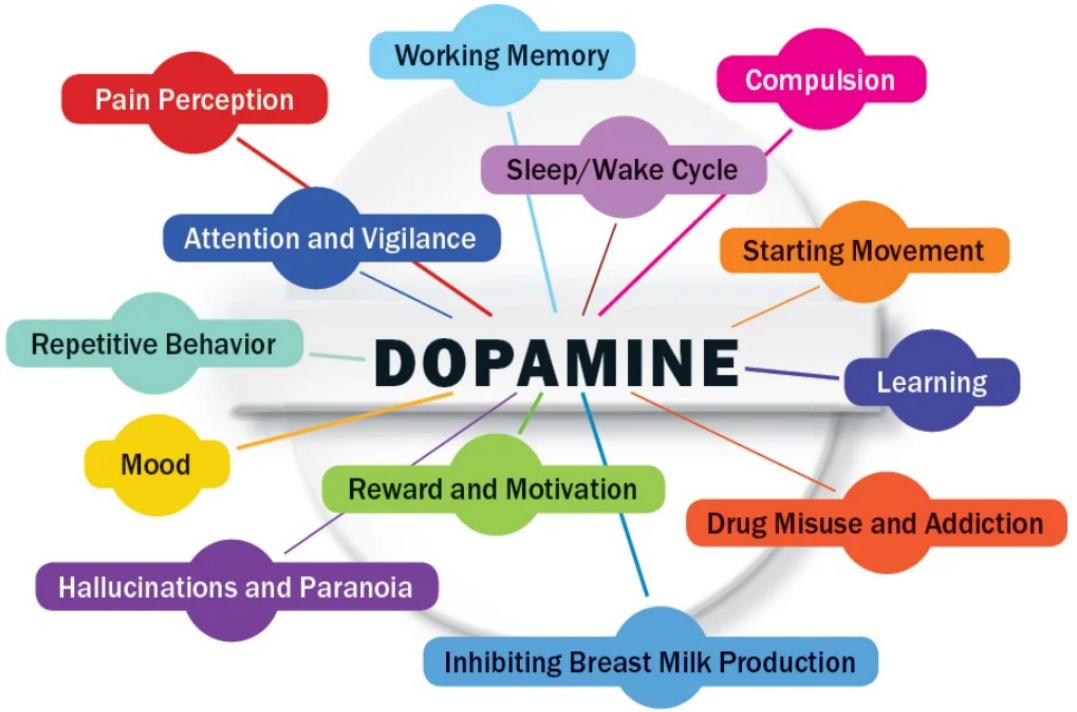
Effects of dopamine in humans



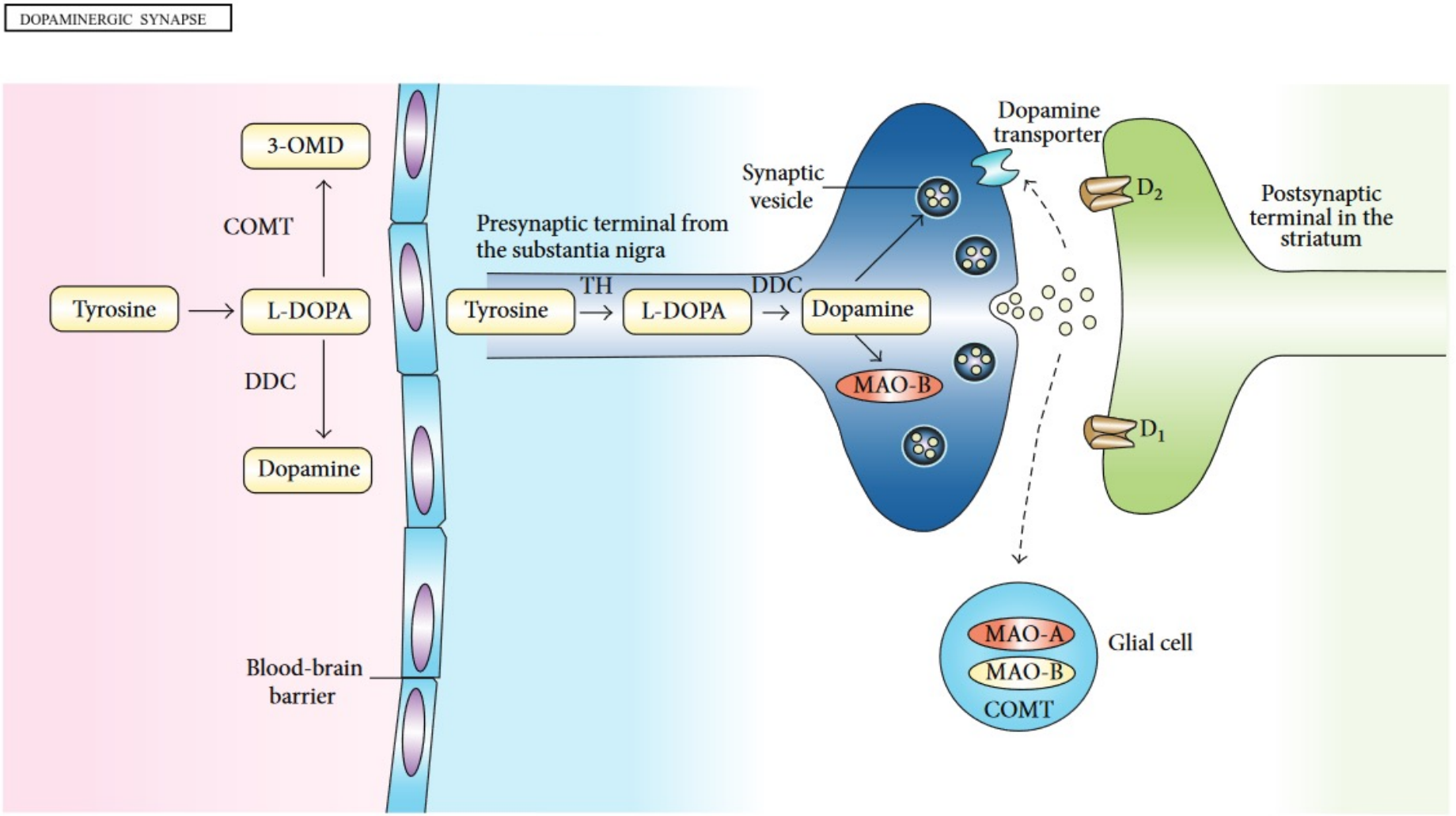
MESOCORTICAL
Cognition, Memory, Attention, Emotional Behavior, & Learning

NIGROSTRIATAL
Movement & Sensory Stimuli

MESOLIMBIC
Pleasure & Reward Seeking Behaviors; Addiction, Emotion, Perception



Dopamine metabolism pathways



Pathologies associated with dopamine (DA)

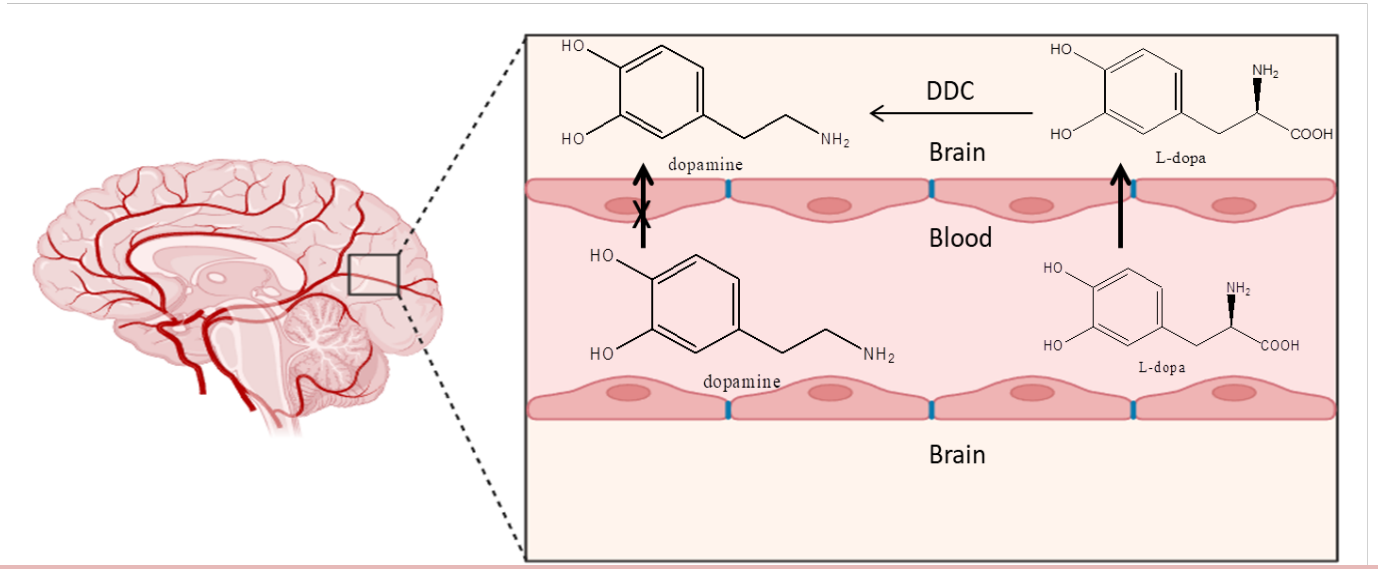
- **Dopamine deficiency: neurological, physiological, psychological symptoms**

Desease/disorder	Cause	Drugs
*PARKINSON'S DISEASE 8.5 m	Death of dopaminergic neuron/Decreased DA	Increase DA (L-dopa) Block DA reuptake, Inhibitors for MAO
*SCHIZOPHRENIA 24 m	Increased DA	Block DA receptors
DEPRESSION	Decreased DA	Prevent DA degradation, inhibitors for MAO
DRUG ADDICTION	Block the reuptake of DA (stays longer in synaptic cleft)	
ADHD	Decreased DA	Amphetamines: DA reuptake inhibitors

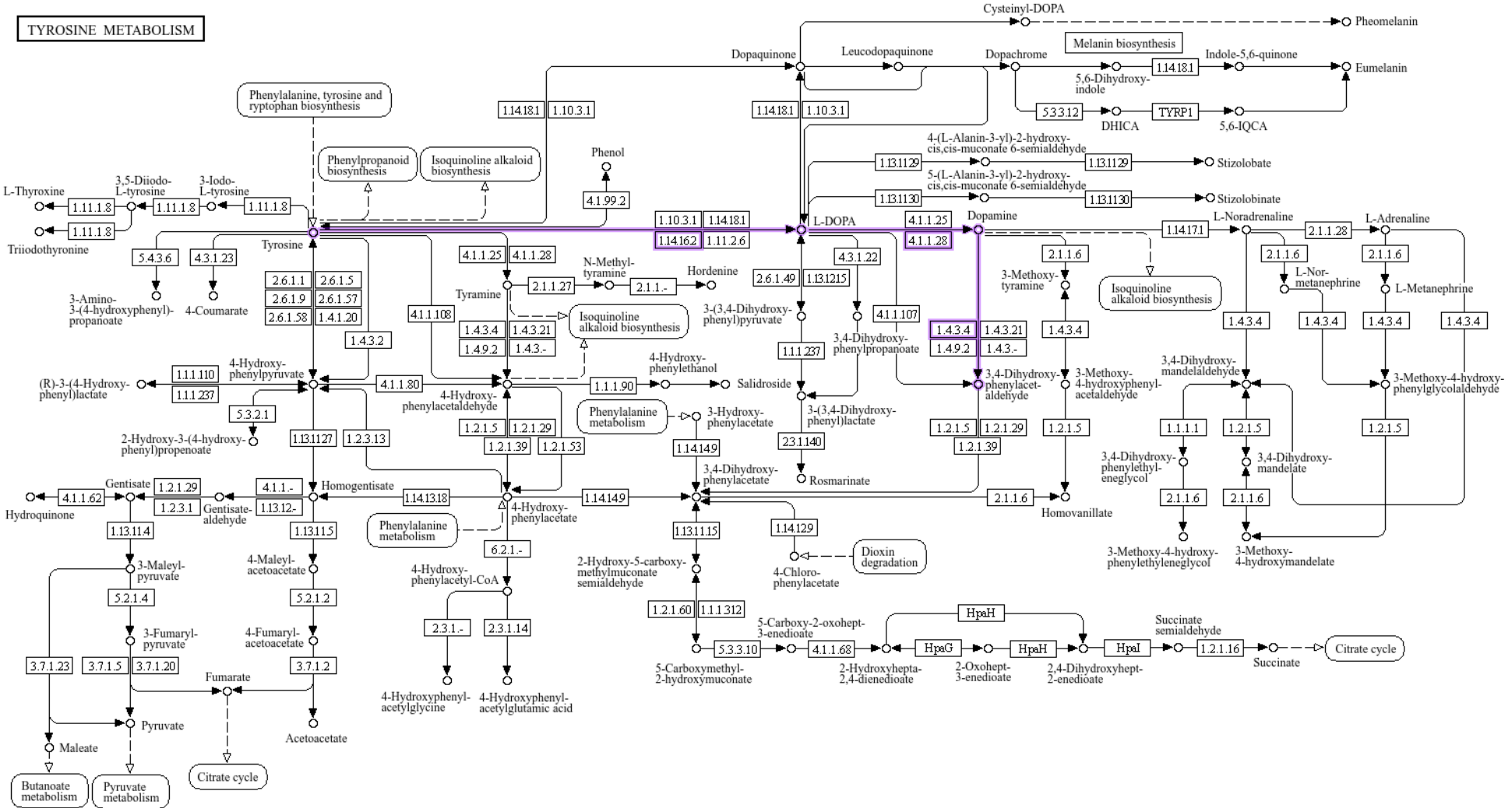
*https://www.who.int/news-room/fact-sheets/detail/schizophrenia
*https://www.who.int/news-room/fact-sheets/detail/parkinson-disease

Levodopa and blood-brain barrier

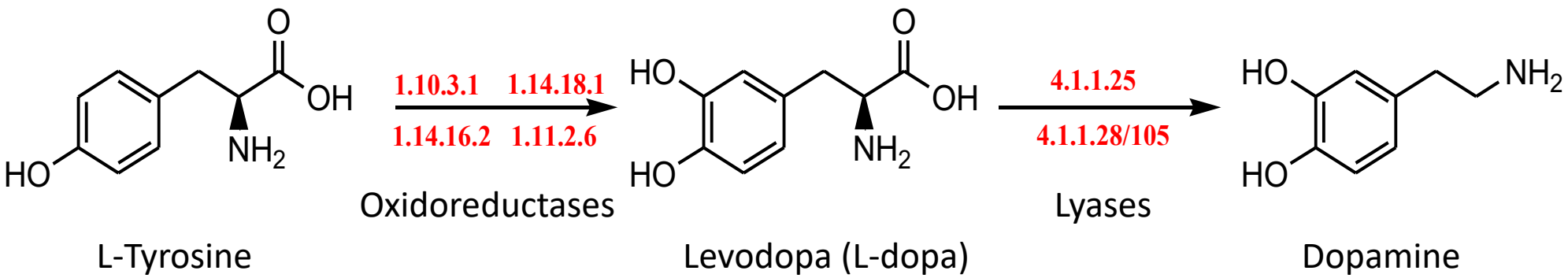
- ✓ Levodopa: utilizes a receptor on the BBB known as **Large amino acid transporter** (LAT)
- ✓ Dopamine: too polar of a molecule to move through the lipid membrane
- ✓ Use of L-dopa as medicine for dopamine production



Tyrosine metabolism



Dopamine synthesis



L-Tyrosine | Levodopa (L-dopa) | Dopamine

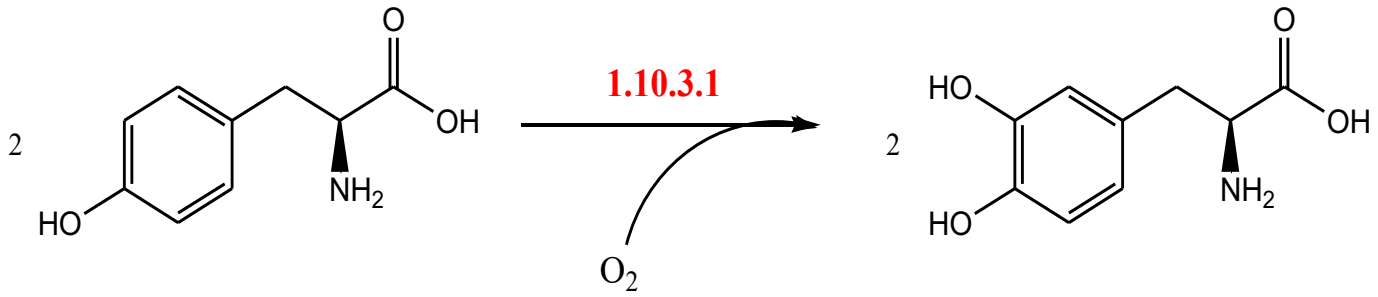
- **EC.1.10.3.1 Polyphenol Oxidase**
- **EC.1.14.18.1 Tyrosinase**
- **EC.1.14.16.2 Tyrosinase 3 – Monooxygenase**
- **EC.1.11.2.6 L -Tyrosinase Peroxygenase**

- **EC.4.1.1.25 Tyrosine decarboxylase**
- **EC.4.1.1.28/105 Aromatic L – Amino acid / L – Tryptophan decarboxylase**

EC.1.10.3.1 Polyphenol Oxidase

EC.1.10.3.1

- Oxidoreductases
- Acting on diphenols and related substances as donors
- With O_2 as an acceptor
- Catechol Oxidase (Polyphenol Oxidase)

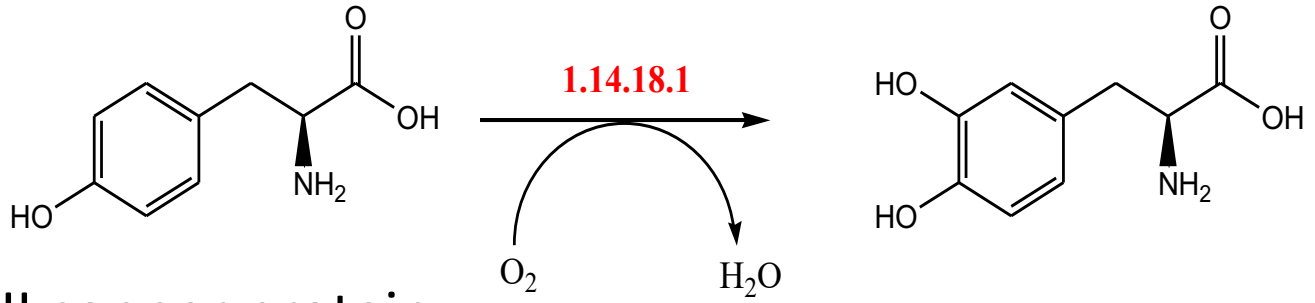


- Type III copper protein

EC.1.14.18.1 Tyrosinase

EC.1.14.18.1

- Oxidoreductases
- Acting on paired donors with incorporation or reduction of O_2
- With another compound as one donor and incorporation of one atom of O_2 into the other donor
- Tyrosinase

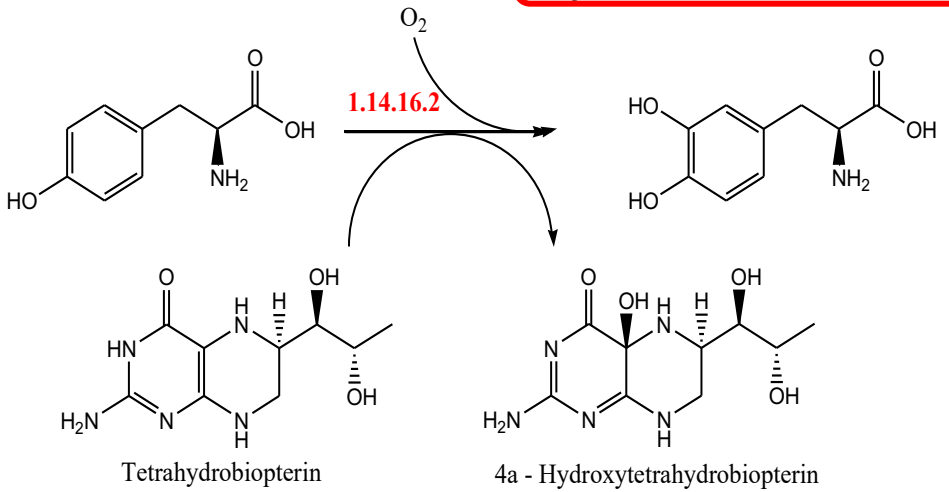


- Type III copper protein

EC.1.14.16.2 Tyrosinase 3 – Monooxygenase

EC.1.14.16.2

- Oxidoreductases
- Acting on paired donors with incorporation or reduction of O_2
- With reduced pteridine as one donor and incorporation of one atom of O_2 into the other donor
- Tyrosinase 3 - Monooxygenase

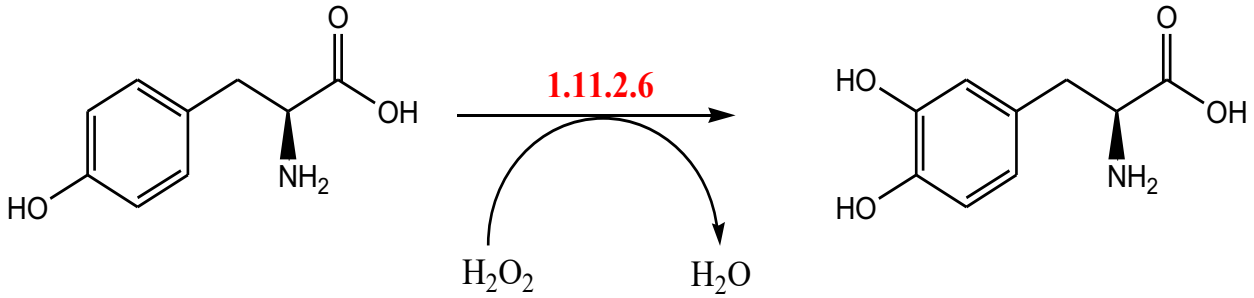


- The active center contains mononuclear iron(II)
- The enzyme is activated by phosphorylation

EC.1.11.2.6 L -Tyrosinase Peroxygenase

EC.1.11.2.6

- Oxidoreductases
- Acting on a peroxide as an acceptor
- Peroxygenases
- L – Tyrosine Peroxygenase

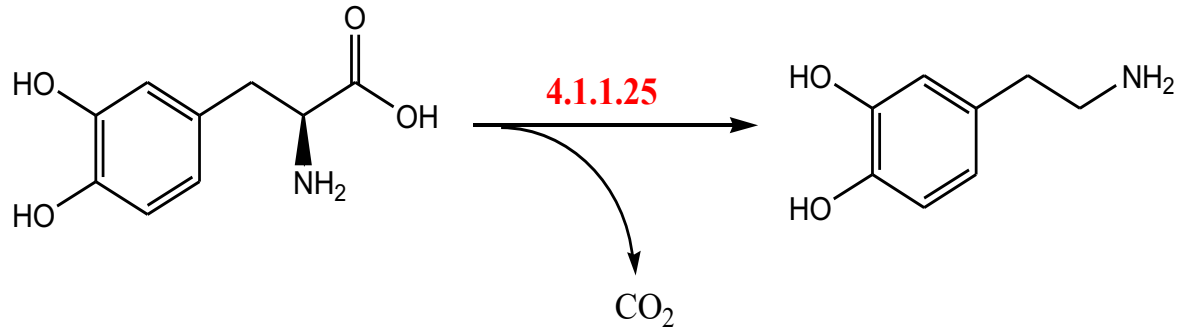


- Co - factor: heme *b*

EC.4.1.1.25 Tyrosine decarboxylase

EC.4.1.1.25

- Lyases
- Carbon – Carbon lyases
- Carboxy - lyases
- Tyrosine decarboxylase

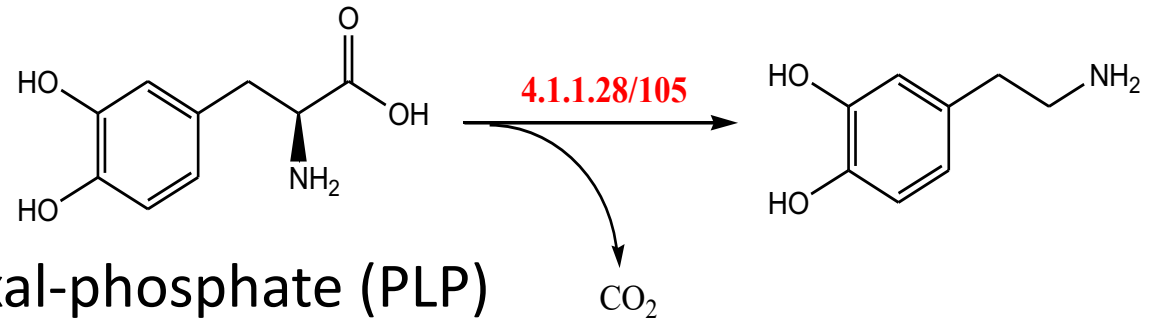


- Co - factor: Pyridoxal-phosphate (PLP)

EC.4.1.1.28/105 Aromatic L – Amino acid / L – Tryptophan decarboxylase

EC.4.1.1.28/105

- Lyases
- Carbon – Carbon lyases
- Carboxy - lyases
- Aromatic L – Amino acid decarboxylase
- L – Tryptophan decarboxylase

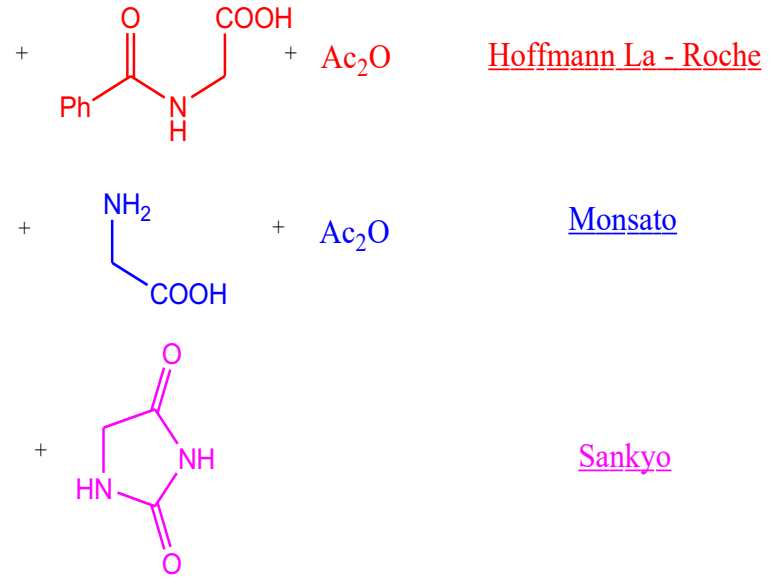


- Co - factor: Pyridoxal-phosphate (PLP)
- 4.1.1.**28 :** acts on a variety of aromatic L-amino acids

Chemical ways of L – DOPA synthesis

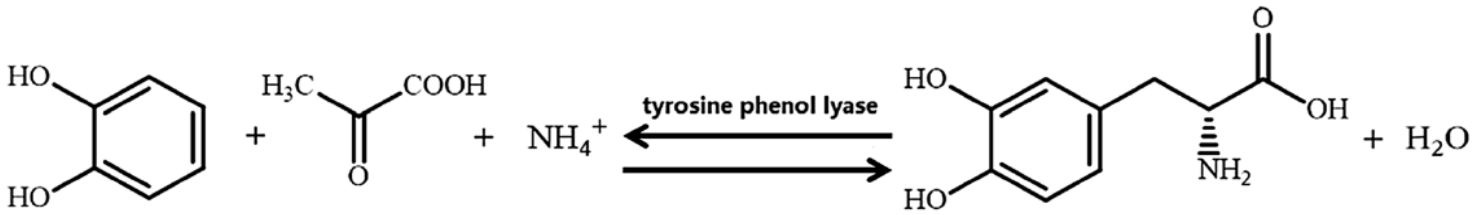
- ✓ 3 different chemically synthetic ways
- ✓ Synthetic goal of pharmaceutical companies
- ✓ Demanding synthesis – limited yields

Biocatalysis



Characteristics	Sankyo	Roche	Monsanto	Ajinomoto
Number of steps	7	4	4	1
Atom Economy	0.158	0.123	0.294	0.719
Synthetic ideality	29%	50%	50%	100%

L-DOPA production using a bacterial enzyme
Tyrosine phenol-lyase (Tpl)

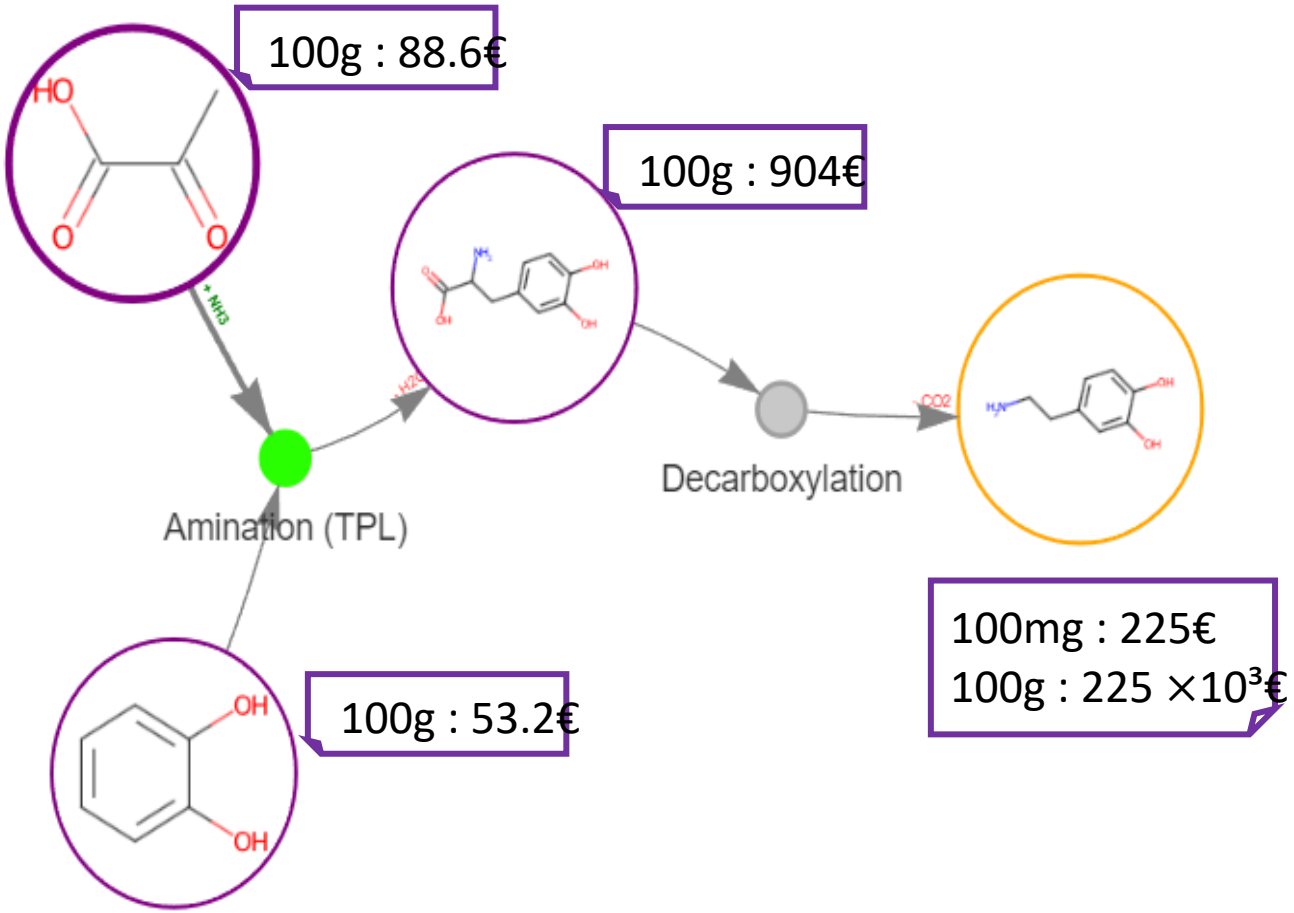


- ✓ Recombinant E. coli cells
- ✓ Optimum T: 15 °C and pH 8.0
- ✓ [L-DOPA] reached 120 g/L with pyrocatechol conversion higher than 96% (15 L reaction)

	Chemical method	Enzymatic method
Main raw materials	Vanillin, hydantoin, hydrogen gas, acetic anhydride	Pyruvate, ammonia, pyrocatechol
Reaction units	8	2
Side products	Ammonia, acetic acid, CO_2	H_2O
Optical resolution	Required	Not required
Working days	15 days	3 days
Impurities	Tyrosine, 3-methoxytyrosine, 3,4,6-trihydroxy-phenylalanine	L-tyrosine

Searching for another synthetic way

- ✓ 2 - Step reaction
- ✓ Need of 2 well characterized enzymes
- ✓ Cheapest way
- ✓ No side products

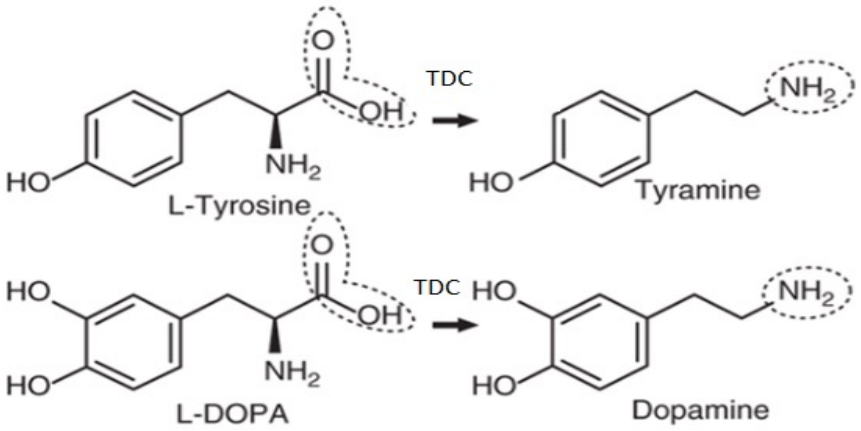


Enzyme of interest

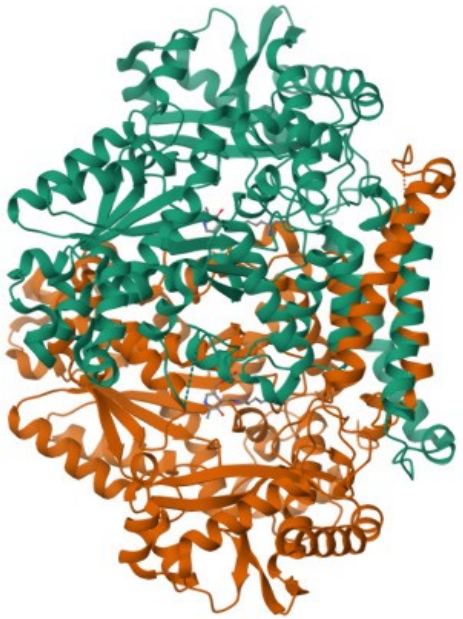
- Amination step is well – defined and widely used (L – dopa synthesis)
- Studying the carboxylation step
- Enzyme with a bacterial origin – more manageable in a biochemical lab
- Heterologous Expression in *E.Coli*
- Researching interest (Pharmaceutical)

EC.4.1.1.25 Tyrosine decarboxylase (TDC) Enzyme

Tyrosine decarboxylase (TDC) is a pyridoxal 5-phosphate (PLP)-dependent enzyme and is mainly responsible for the synthesis of tyramine, an important biogenic amine

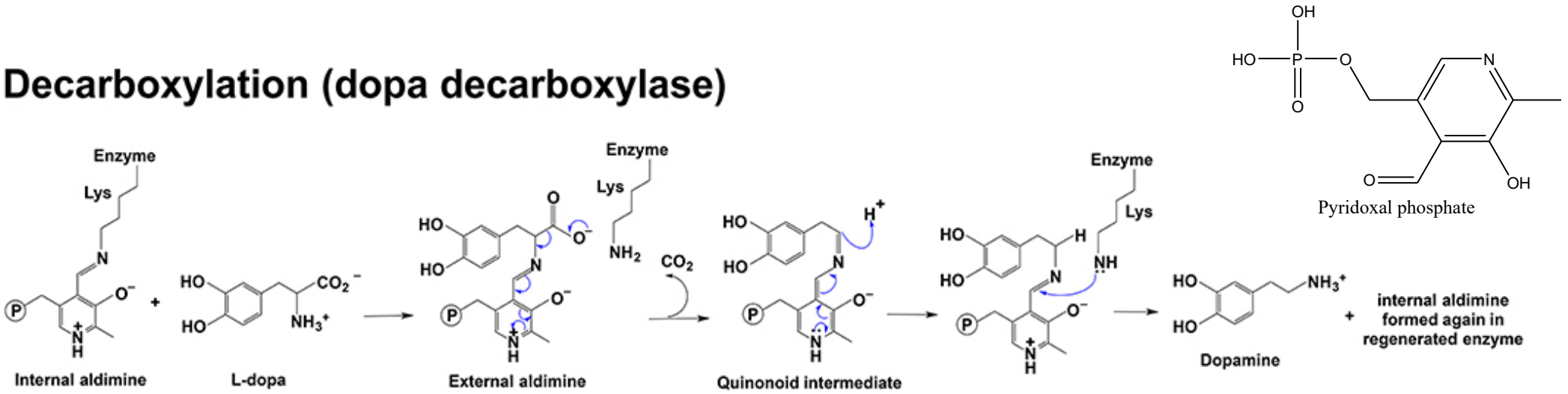


- ✓ Heterologous Expression in *E.Coli*
- ✓ pH stability
- ✓ Temperature stability
- ✓ Many Citations



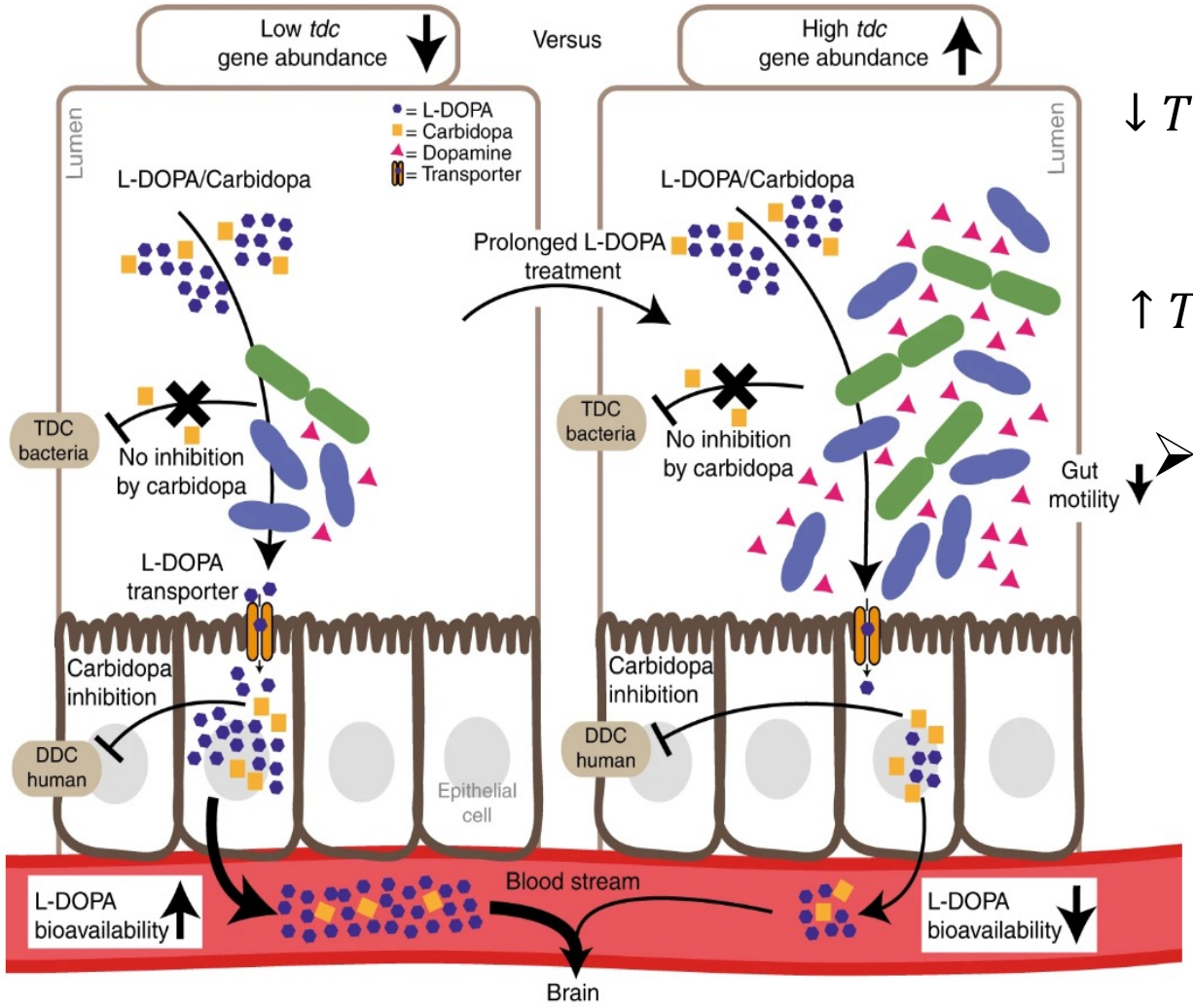
Structure of tyrosine decarboxylase complex with PLP at 1.9 Angstroms resolution

Decarboxylation mechanism



1. Formation of internal aldimine between PLP and lysine residue
2. Formation of PLP – levodopa intermediate (external aldimine)
3. PLP: stabilizes the carbanions generated adjacent to the Schiff base in the external aldimine intermediate by delocalization of the developed negative charge into the extended p bonded system:leaving of the CO2 group Protonation of the imine bond – formation other imine bond through electron / bond delocalization
4. Regeneration of internal aldimine and formation of the decarboxylated product (dopamine)

TDC activity



$$\downarrow TDC \rightarrow \uparrow L-dopa \rightarrow \uparrow Dopamine\ production$$

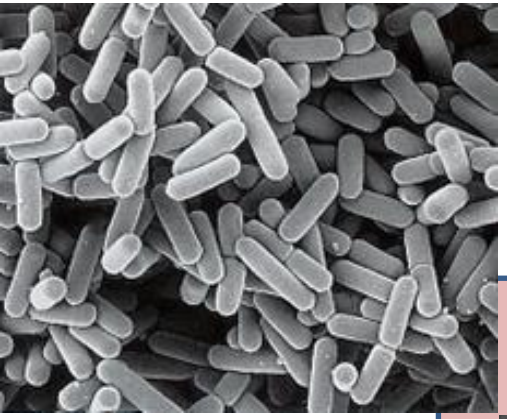
$$\uparrow TDC \rightarrow \downarrow L-dopa \rightarrow \downarrow Dopamine\ production$$

- Abundance of TDC in the small intestine contributes to interindividual variation in drug efficacy and explains the requirement for an increased dosage regimen of L-dopa treatment in Parkinson's disease patients

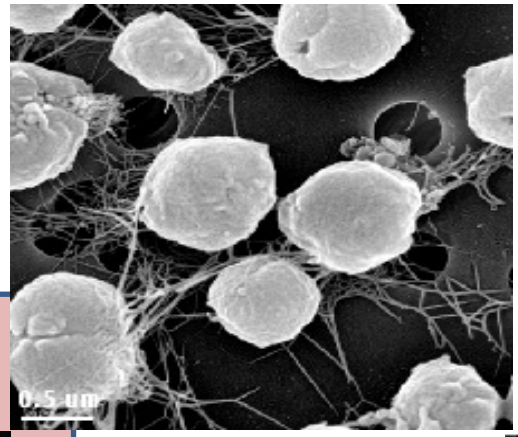
Van Kessel, El Aidy, S. Et al.(2019). Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nature communications*, *10*(1), 310.

Organisms with TDC

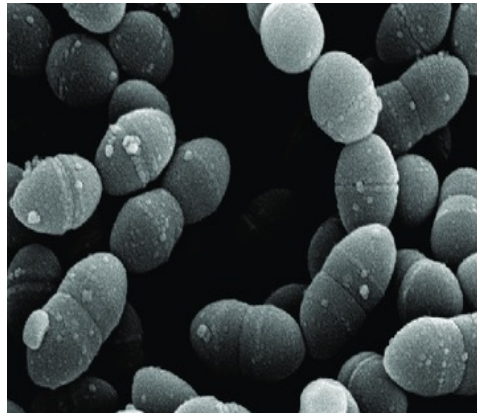
TDCs have been identified from different organisms, such as plants, insects and different microorganisms including :



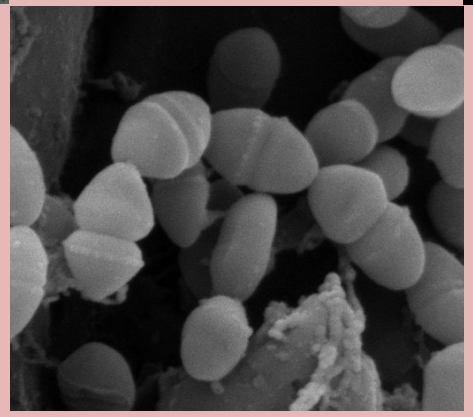
Enterococcus faecalis



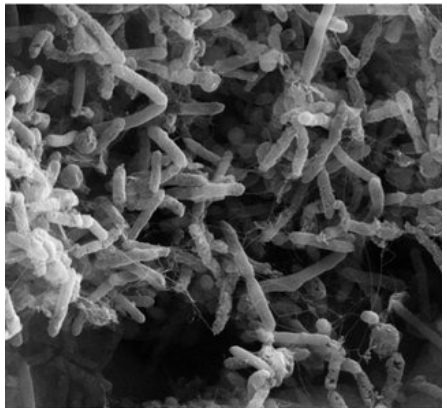
Sporolactobacillus sp.



Lactobacillus brevis



Methanocaldococcus jannaschii



Lactococcus lactis

Choosing the right gene

- Organism where the protein is naturally expressed
- Choosing the right strain (citations)
  - Vmax, K_M, Kcat
  - Exclusive substrates – enzyme preference
  - Available X ray structure
- Annotation score 5/5
- Necessary condition: expression in *E.coli*

Gene of interest

>ASWP01000005.1:31334-33196 Enterococcus faecalis V583 acyDH-supercont2.4.C5, whole genome shotgun sequence

ATGAAAAACGAAAAATTAGCAAAAGGCGAAATGAACCTTAATGCACTATTCATTGGGGACAAAGCCGAAA
ACGGACAATTATATAAAGACTTGTTGATCGACTTAGTAGATGAACATTTAGGATGGCGTCAAAACTACAT
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AAATATTTACAACAACGAATTCATTACTTCACATACTGACTTTGCTATTCCAGATTATGGCAACAGCCCA
TTAAAATTTGTAAACAGCTTGGGATTTAGTGACGAAGAATGGAACCGTGCAGGTAAAGTAACTGTGTTAC
GTGCTGCTGTAATGACTCCATACATGAACGATAAAGAAGAATTTGATGTTTACGCTCCTAAGATTCAAGC
TGCTTTACAAGAAAAATTAGAACAAATTTACGACGTAAAATAA

- ✓ In gut microbiota this enzyme is in fact exclusively responsible for the decarboxylation of levodopa, and thus reduces in situ levels of levodopa in the treatment of Parkinson's disease.

- ✓ **Activity regulation**

Levodopa decarboxylation is not inhibited by carbidopa, benserazide, and methyldopa, that are three human L-dopa decarboxylase inhibitors

- ✓ **Kinetics**

Km 3mM, Kcat 3531 min^{-1} (at pH 5.0)

- ✓ **Annotation score:** 5/5

Amino acid Sequence and Protein Characteristics

>AAO80459.1:17-636 decarboxylase, putative [Enterococcus faecalisV583]

MKNEKLAKGEMNLNALFIGDKAENGQLYKDLLIDLVDEHLGWRQNYMPQDMPVISS
QERTSESYEKTVNHMKDVLNEISSRMRTHSVPWHTAGRYWGHMNSETLMPSLLAYN
FAMLWNGNNVAYESSPATSQMEEEVGHEFAHLMSYKNGWGHIVADGSLANLEGLWY
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GKHLQAIGKWLVPQTKHYSWLKAADIIGIGLDQVIPVPVDHNYRMDINELEKIVRG
LAEEQIPVLGVVGVVGSTEEGAVDSIDKIIALRDELMKDGIYYYVHVDAAYGGYGR
AIFLDEDNNFIPYEDLQDVHEEYGVFKEKKEHISREVYDAYKAIELAESVTIDPHK
MGYIPYSAGGIVIQDIRMRDVISYFATYVFEKGADIPALLGAYILEGSKAGATAAS
VWAAHHVLPLNVAGYGKLIGASIEGSHHFYNFLNDLTFKVGDKEIEVHTLTHPDFN
MVDYVFKEKGNDDLVAMNKLNHDVYDYASYVKGNIYNNEFITSHTDFAIPDYGNSP
LKFVNSLGFSDEEWNRAGKVTVLRAAVMTPYMNDKEEFDVYAPKIQAALQEKLEQI
YDVK

Expasy

- ✓ **Number of amino acids:** 620
  Molecular weight: 70053.18
  Theoretical pI: 4.99

- ✓ **Extinction coefficients:** Extinction coefficients: (M^{-1} cm^{-1})at 280 nm measured in water.
- ✓ Ext. coefficient 112650 Abs 0.1% (=1 g/l) **1.608**
  - ➢ Phe (F) 19 3.1%
  - ➢ Trp (W) 11 1.8%
  - ➢ Tyr (Y) 35 5.6%

- ✓ **Instability index:** The instability index (II) is computed to be 35.61 This classifies the protein as stable.

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- Drugs Used in Parkinsonism and Other Movement Disorders - Katzung & Trevor's Pharmacology Examination and Board Review, 9th Edition. https://doctorlib.info/pharmacology/pharmacology-examination-board-review/28.html.
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- *https://web.expasy.org/protparam/*
- *https://loschmidt.chemi.muni.cz/soluprot/*
- *https://www.biorender.com/*
- *https://revvitysignals.com/products/research/chemdraw*
- *https://www.microbiologyinpictures.com/bacteria-photos/enterococcus-faecalis-images/enterococcus-faecalis-sem.html*
- *https://www.researchgate.net/figure/a-Scanning-electron-microscopy-image-of-L-lactis-subsp-lactis-CAB701-the-scale-bar_fig1_372032144*
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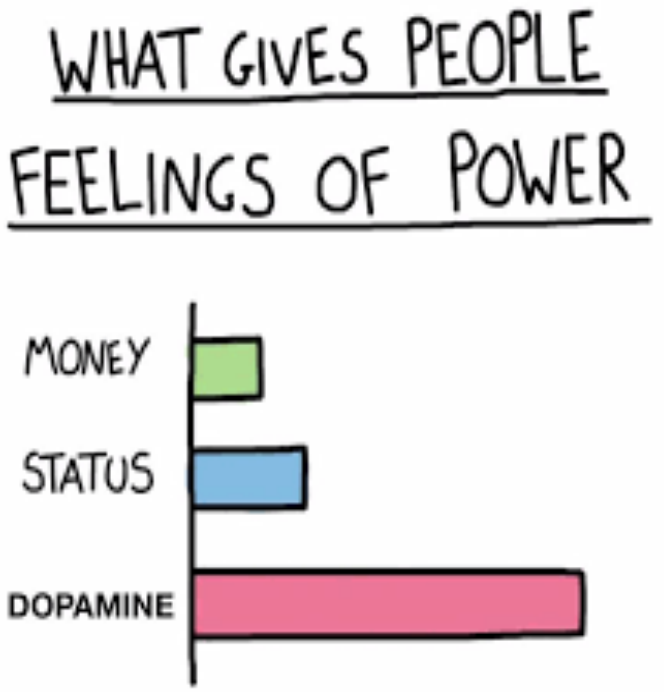
- *https://patentimages.storage.googleapis.com/04/da/a8/8e96c1522f8bec/US3714242.pdf*
- *https://microbewiki.kenyon.edu/index.php/Methanococcus_jannaschii*

Thank you for your attention

That one molecule of Dopamine trying to keep me going throughout the day



My last dopamine receptor not giving up on me be like: