

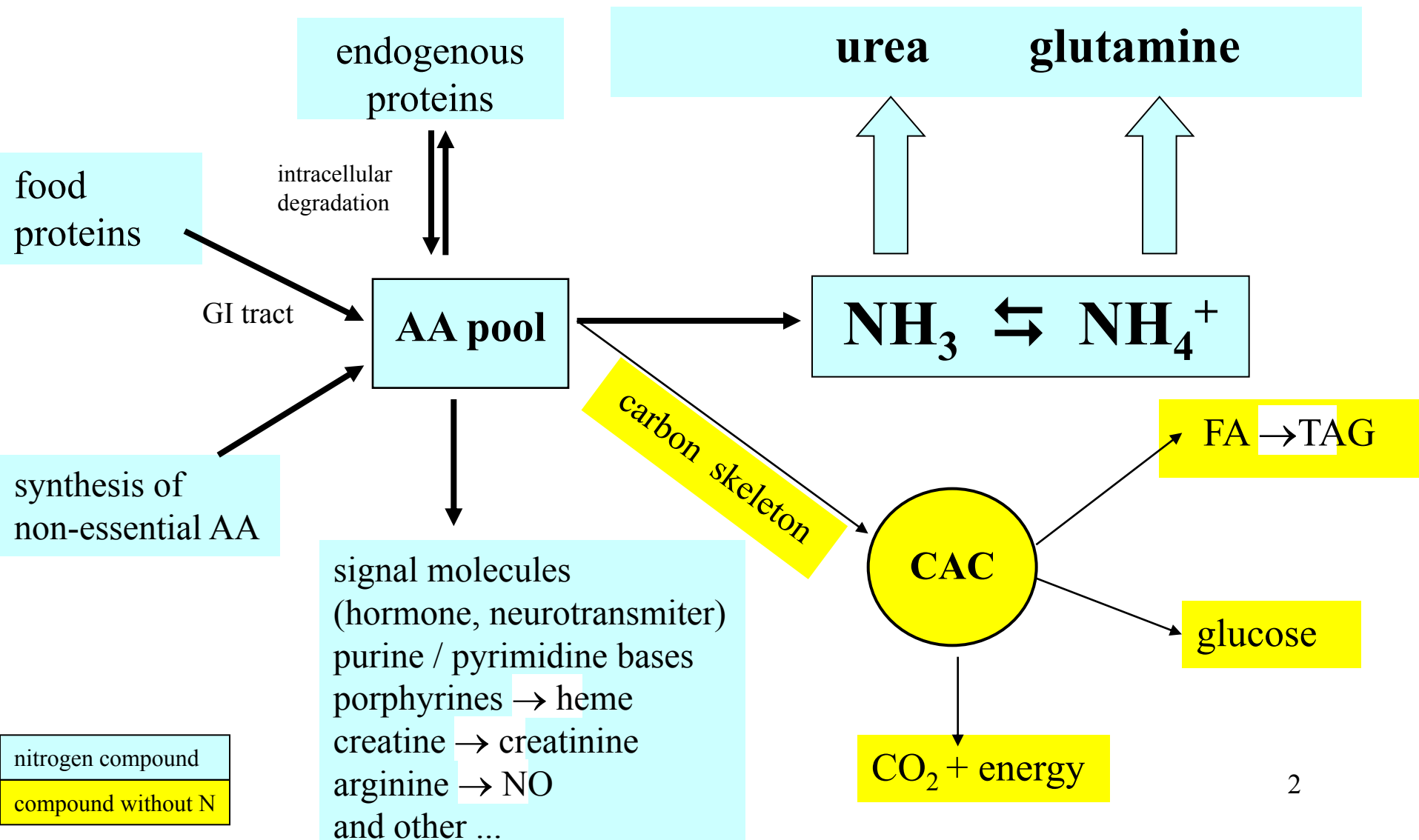
**Catabolism of amino acids**

**Ammonia detoxification**

**Biosynthesis of non-essential amino acids**

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# Anabolic and catabolic conversions of AA



# Amino acid pool

## Three sources of AA pool:

- 1) Proteolysis of dietary proteins (food)
- 2) Proteolysis of tissue proteins (physiological turnover, more in starvation)
- 3) Synthesis of non-essential AA (11)

## Three utilizations of AA pool:

- 1) Synthesis of tissue and blood plasma proteins (liver)
- 2) Synthesis of low-molecular nitrogen compounds (with specific functions)
- 3) Catabolism: deamination + utilization of carbon skeleton

## Three utilizations of AA carbon skeleton

- 1) Gluconeogenesis (in starvation, most AA are glucogenic)
- 2) Synthesis of FA and TAG (in AA excess)
- 3) Metabolic fuel = gain of energy (minor utilization)

# Degradation of proteins

## Exogenous proteins

- the lumen of GI tract
- stomach – pepsin
- intestine – pancreatic proteases (trypsin, chymotrypsin etc.)

more details in BCH II and physiology

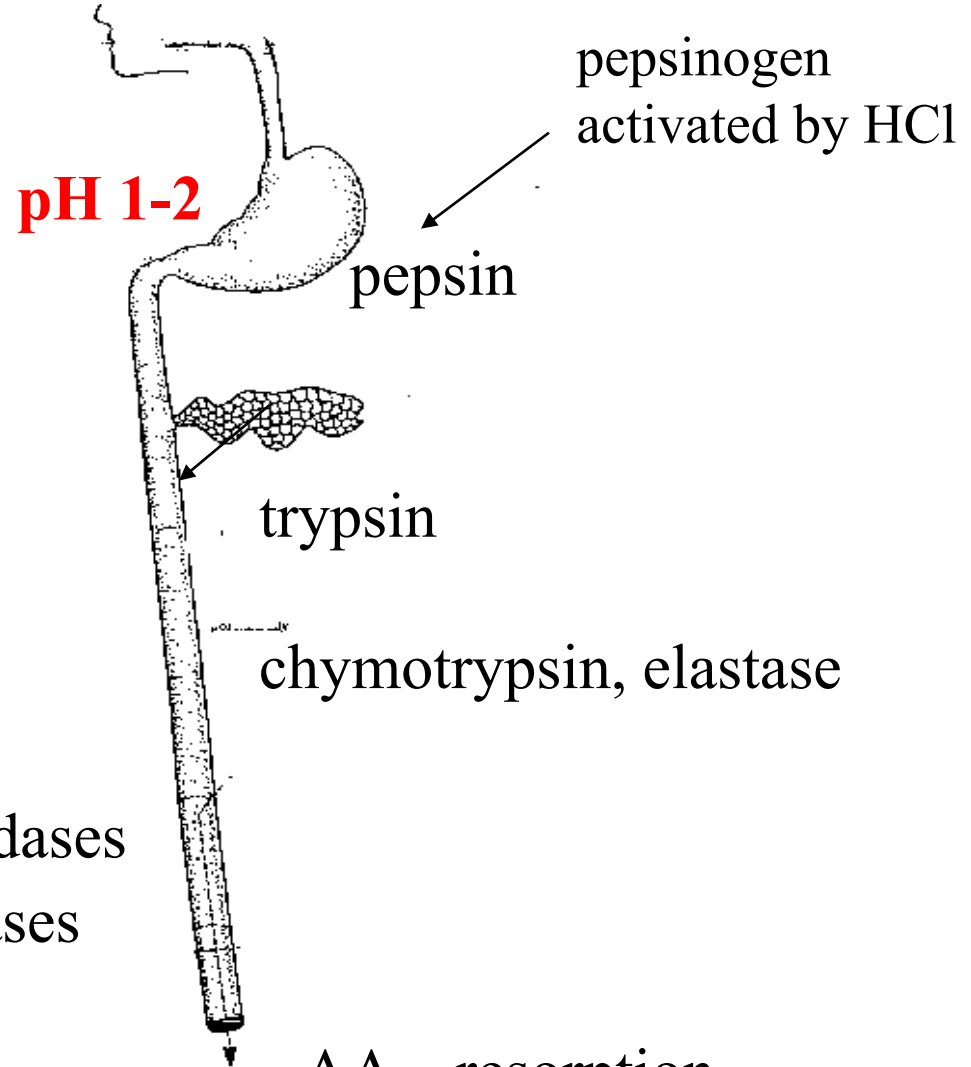
## Endogenous proteins

- intracellular proteases
- lysosome
- ubiquitin-proteasome
- caspases in apoptosis
- calpains and others ...

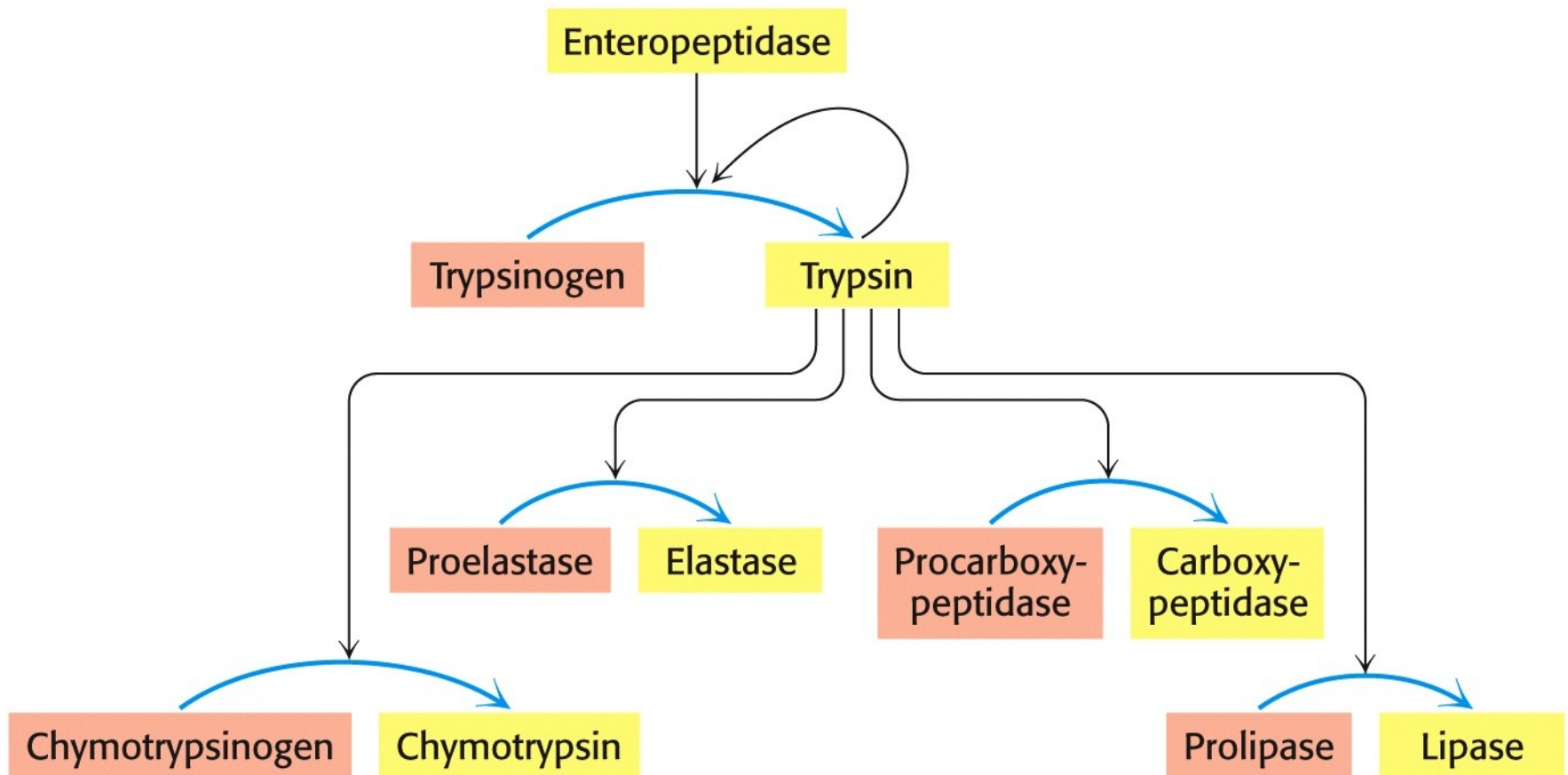
# Digestion of dietary proteins

proteases are secreted  
as **inactive proenzymes**  
activated by the cleavage  
of certain peptide sequence

carboxypeptidases  
aminopeptidases



Enteropeptidase secreted by the mucosa of duodenum initiates the activation of the pancreatic proenzymes



## Proteolytic enzymes exhibit the preference for particular types of peptide bonds

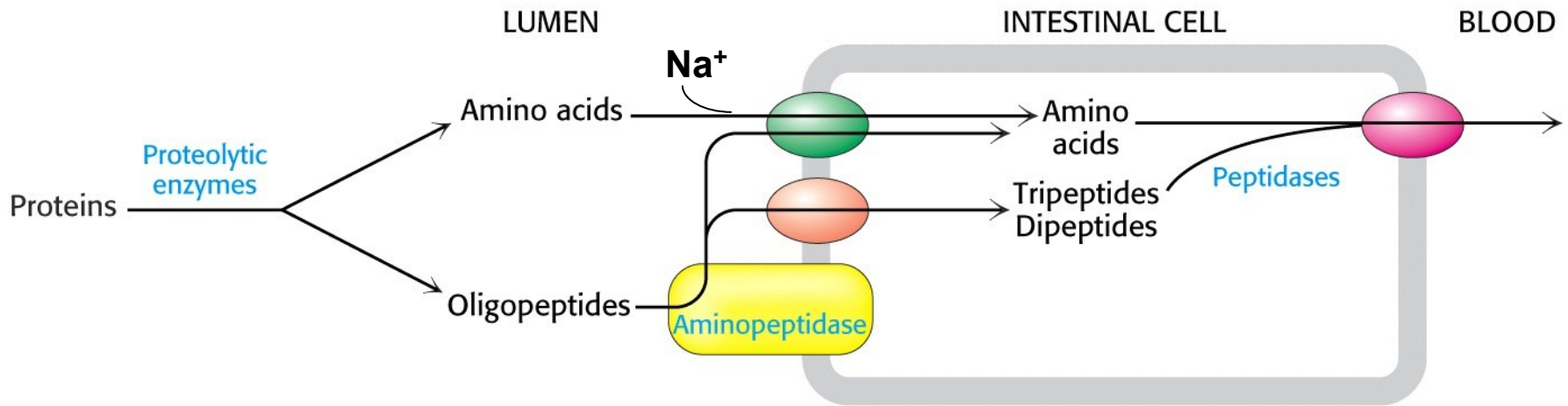
### Proteinases preferentially attacks the bond after:

Pepsin	aromatic (Phe, Tyr) and acidic AA (Glu, Asp)
Trypsin	basic AA (Arg, Lys)
Chymotrypsin	hydrophobic (Phe, Tyr, Trp, Leu) and acidic AA (Glu, Asp)
Elastase	AA with a small side chain (Gly, Ala, Ser)

### Peptidases:

<u>Carboxypeptidase A</u>	nearly all AA ( <u>not</u> Arg and Lys)
<u>Carboxypeptidase B</u>	basic AA (Arg, Lys)
<u>aminopeptidase</u>	nearly all AA
Prolidase	proline
Dipeptidase	only dipeptides

# Transcellular transport of AA from intestine to portal blood



## L-amino acids:

about seven **specific** transporters, symport with  $\text{Na}^+$

## D-amino acids (trace amounts):

**nonspecific** diffusion, hydrophilic pores in membranes,

D-AA cannot be utilized in the body, they are only catabolized to gain energy

Also small **oligopeptides** (symport with  $\text{H}^+$ )



## Endogenous proteins have different biological half-lives

Protein	Half-life
Ornithine decarboxylase	12 min
RNA polymerase I	1 hour
AST	12 hour
Prealbumin	2 days
Lactate dehydrogenase	4 days
Transferrin	10 days
Albumin	19 days
IgG	23 days
Collagen	several years
Elastin	whole life (?)

# Degradation of proteins in lysosomes

- does not require ATP, non-specific
- extracellular and membrane proteins
- long-lived intracellular proteins
- extracellular glycoproteins – sialic acid at terminal position on oligosaccharide chain is removed = asialoglycoproteins – they are recognized by liver receptors → degradation in liver lysosomes

# Examples of lysosomal hydrolases

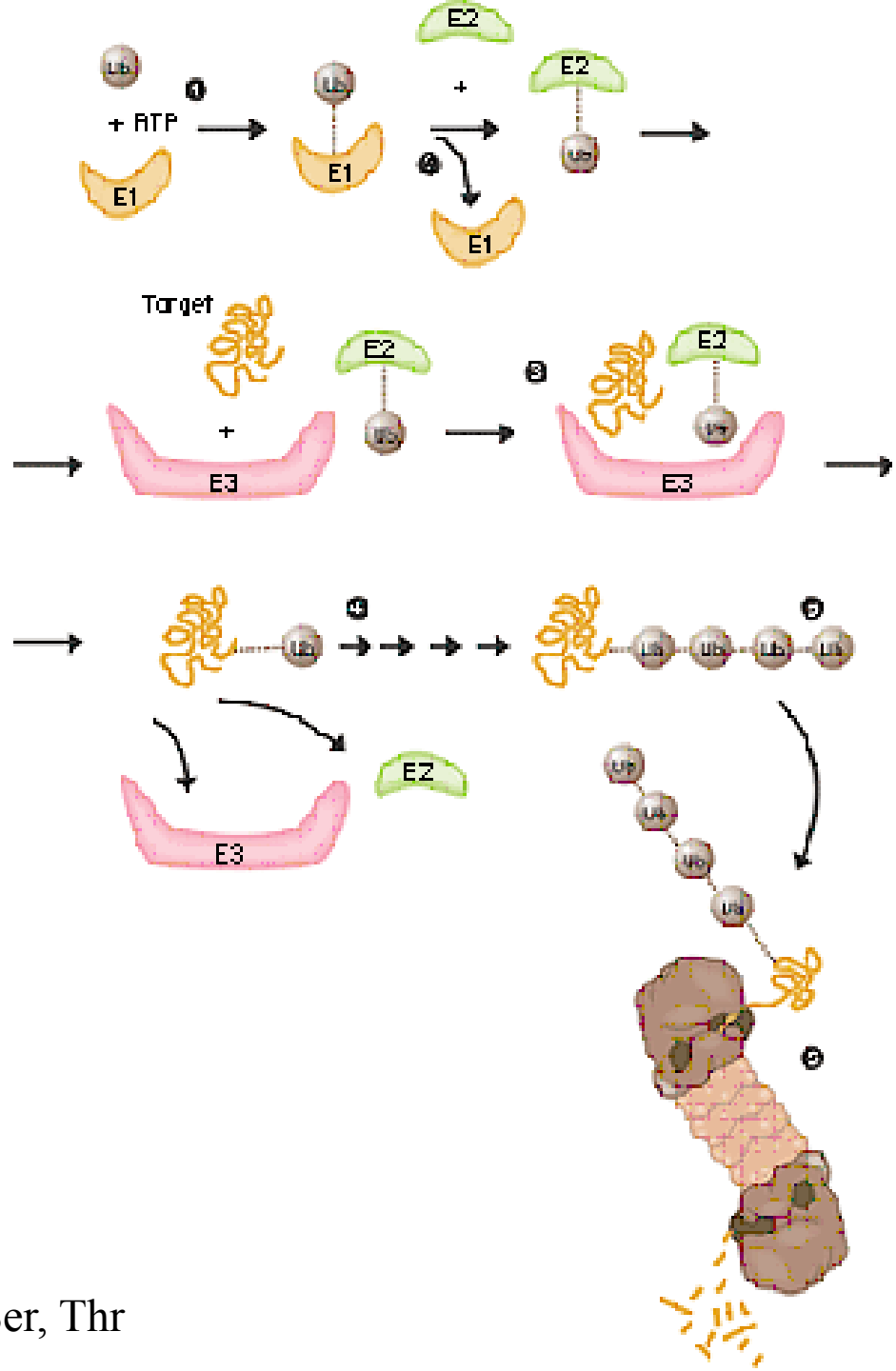
Hydrolase	Type of bond
Glucosidase	glycoside
Galactosidase	glycoside
Hyaluronidase	glycoside
Arylsulfatase	sulfoester
Lysozyme	glycoside
<b>Cathepsin</b>	<b>peptide</b>
<b>Collagenase</b>	<b>peptide</b>
<b>Elastase</b>	<b>peptide</b>
Ribonuclease	phosphodiester
Lipase	ester
Phosphatase	phosphoester
Ceramidase	amide

# Ubiquitin (Ub) targets proteins for proteasome degradation

- small protein, in all cells - ubiquitous
- C-terminus binds Lys of proteins to be degraded (*kiss of death*)
- binding Ub to protein has three phases, with three enzymes  $E_1, E_2, E_3$
- binding Ub to  $E_1$ -SH requires **ATP**
- more Ub molecules are attached - **polyubiquitination**
- Ub-tagged protein is directed to proteasome

# The targeting of proteins

- E1** ubiquitin-activating enzyme (ATP)
- E2** ubiquitin-conjugating enzyme
- E3** ubiquitin-protein ligase



## The N-terminal rule

### Stabilizing AA (long life):

- Met, Ser, Ala, Thr, Val, Gly, Cys

### Destabilizing AA (short life):

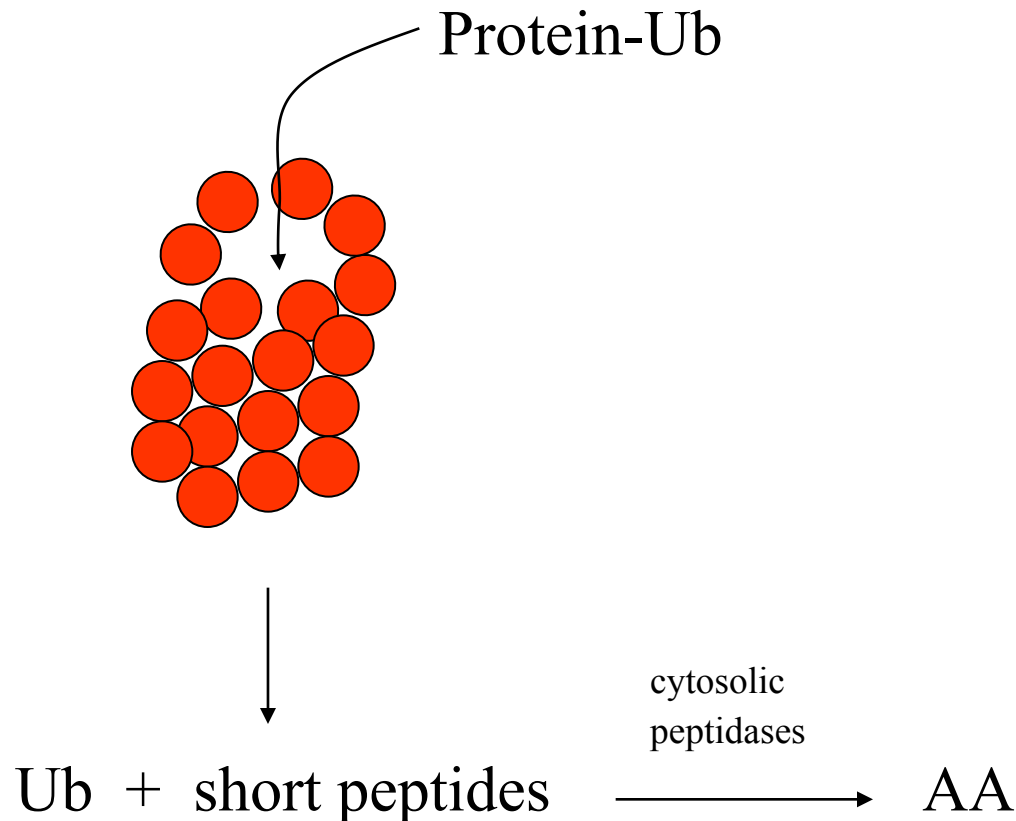
- Phe, Leu, Asp, Lys, Arg
- PEST proteins: segments rich in Pro, Glu, Ser, Thr

# Proteasome

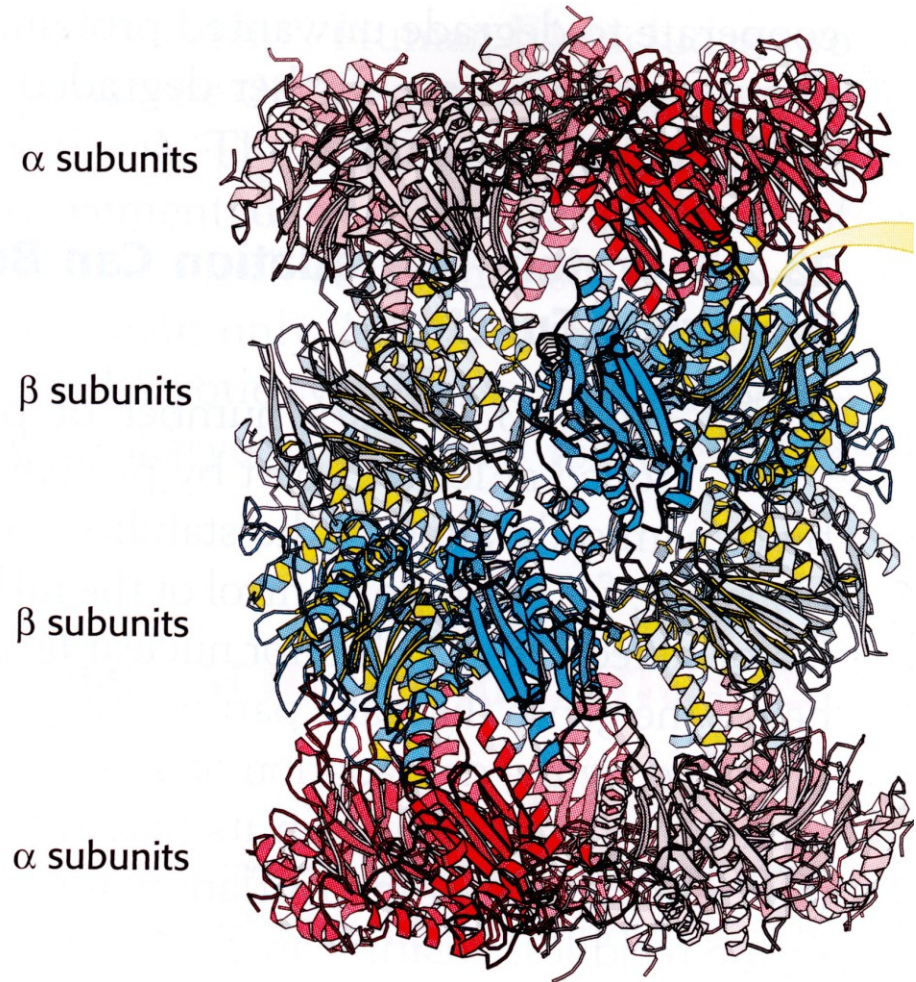
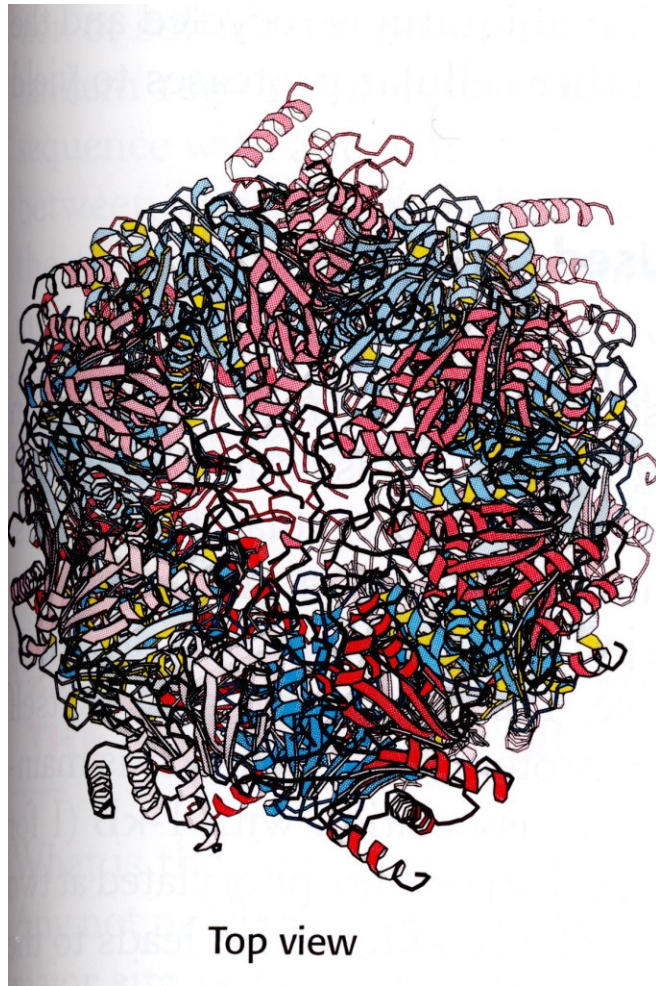
- hollow cylindrical supramolecule, 28 polypeptides
- four cyclic heptamers ( $4 \times 7 = 28$ )
- the caps on the ends regulate the entry of proteins into destruction chamber, upon **ATP hydrolysis**
- inside the barrel, differently specific proteases hydrolyze target protein into short (8 AA) peptides
- ubiquitin is not degraded, it is released intact

# Proteasomes degrade regulatory proteins (short half-life) and abnormal or misfolded proteins

important in regulation of cell cycle, growth, differentiation, apoptosis



# Spatial model of proteasome

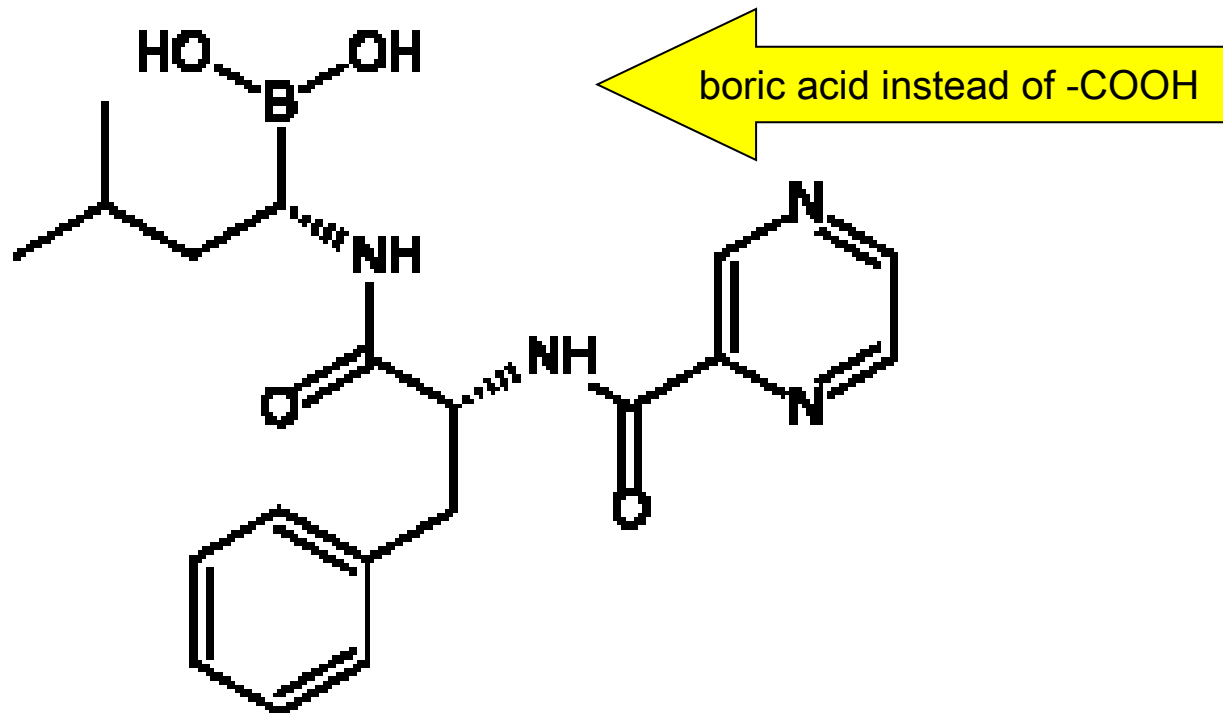


28 subunits in 4 rings (4 x 7),  
yellow chains in beta subunits contain proteolytic active sites on N-terminals



# Bortezomib is inhibitor of proteasome - boron atom binds to active site (Thr)

(inhibited proteasome does not degrade pro-apoptotic factors – it leads to apoptosis of myeloma cells, treatment of multiple myeloma)



Synthetic tripeptide: pyrazinoic acid-Phe-boroLeu

# Caspases trigger apoptosis

- caspase (cysteiny aspartate-specific proteinase)
- hydrolyse proteins near aspartate
- degradation of cellular proteins during apoptosis
- formed as inactive precursors (procaspases), activated by the actions of other caspase
- **initiator caspases** start the apoptic pathway
- after receiving stimulus they activate **effector caspases** – cascade of caspases (amplification of the process)
- accidentally acivated caspases are neutralized by specific inhibitors

**Calpains** – cytosolic proteases activated by  $\text{Ca}^{2+}$  ions. They occur in all cells, participate in many cell processes, e.g. the metabolism of cytoskeletal proteins, cell cycle progression etc.

## Proteins in nutrition:

**Biological value (BV) of proteins refers to how well the body can utilize the proteins we consume**

Relative amount of nitrogen (%) used to synthesis of endogenous proteins from total protein nitrogen absorbed from food.

BV depends on:

- total content of essential AA
- mutual ratios of essential AA
- protein digestibility

$$BV_{\text{animal prot}} > BV_{\text{plant prot}}$$

wheat – deficit in Lys, Trp, Thr, Met

legumes – deficit in Met, Cys

Daily intake of proteins: 0.8 g/kg

# PDCAAS

amount of limiting AA in test protein

-----  
amount of the same AA in reference protein

true digestibility (%)

- **protein digestibility-corrected amino acid score**
- a recent method based on essential AA requirement and protein digestibility
- reference protein = ideal protein with optimal ratio of all essential AA (often whey or egg white)
- true digestibility (%):  
amount of nitrogen absorbed from food per total food nitrogen

# Essential (9) and semiessential (3) amino acids

- valine, leucine, isoleucine (BCAA)
- threonine (two C\*)
- lysine, histidine (basic)
- phenylalanine, tryptophan (aromatic)
- methionine (-S-CH<sub>3</sub>)

## Semiessential AA

- arginine – in childhood
- alanine, glutamine – in metabolic stress (Ala-gluconeogenesis, Gln – ammonia detoxification)

- 
- about 30 % of methionine need can be substituted by cysteine
  - about 50 % of phenylalanine need can be substituted by tyrosine

# Quality of some proteins

Protein	BV (%)	PDCAAS (%)
Egg white	100	100
Whey	100	100
Milk casein	80	100
Beef	80	92
Beans	49	68
Wheat flour	54	40
Gelatin	25	8

# Egg white, whey, and gluten

**Egg white** is a viscous solution of globular proteins (ovalbumin, ovotransferrin, ovomucoid, ovomucin, ovoglobulins, avidine etc.)

**Whey** is a by-product in cottage cheese (curd) production a yellowish liquid (riboflavin), after precipitation of casein contains high quality proteins (lactoalbumin, lactoglobulins), B-vitamins, and lactose

**Gluten** is protein fraction in wheat and other cereals, containing mainly gliadin (high content of Pro and Gln). In genetically predisposed people, it may cause autoimmune celiac disease.

GF (gluten free)

BL (bezlepkový)



## Quantity of proteins in foodstuffs (%)

Parmesan cheese	40
Emmental cheese	30
Curd	25
Beans	25
Meat	20
Eggs	13
Yeast	11
Cereals, rice	8
Milk	4
Potatoes	2
Fruits, vegetables	1



# Alternative protein sources

Food	Protein content	Commentary
Šmakoun	13 %	processed egg white, Czech product
Robi	22 %	<u>r</u> ostlinné <u>b</u> ílkoviny, cereal + rice proteins
Seitan	25 %	isolated wheat proteins
Hemp seeds	30 %	good content of essential AA
Tofu	16 %	coagulated soy milk proteins
Tempeh	20 %	fermented soybeans by <i>Rhizopus oligosporus</i>

# Protein supplements

- high content of proteins (20 – 90%)
- mainly derived from dried whey
- and/or free AA (BCAA = Val, Leu, Ile)
  
- it is a metabolic load for:
- digestion (→ putrefaction in large intestine, correlates with some types of cancer)
- liver (→ urea synthesis), kidneys (excretion of urea,  $\text{NH}_4^+$ , free AA)
- may be adulterated with anabolic steroids !!!

# Catabolic pathway of amino acids

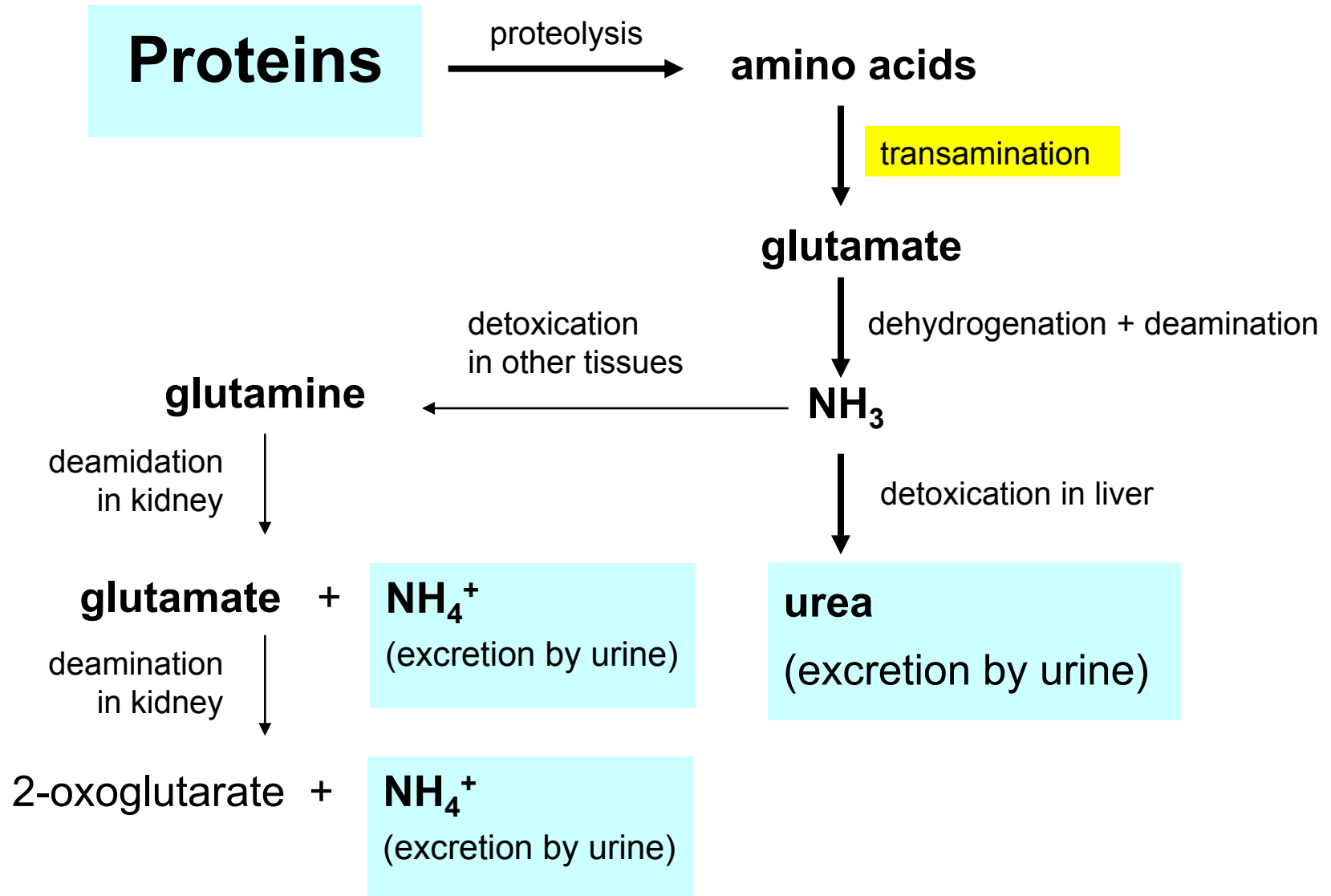
Transamination

Dehydrogenation + deamination of glutamate

Detoxication of ammonia

Excretion of nitrogen catabolites

# Intake, catabolism, and excretion of nitrogen

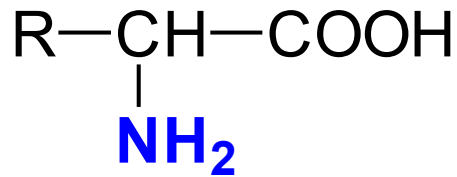


# Transamination

transfer of  $\text{-NH}_2$  group from one substrate to other

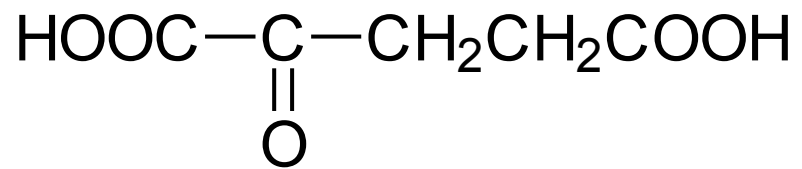
- most AA (not Lys, Thr, Pro, His, Trp, Arg, Met)
- amino group is transferred from AA to 2-oxoglutarate
- cofactor – **pyridoxal phosphate** ( $\rightarrow$  Schiff bases)
- **reversible reaction**  $\Rightarrow$  important for synthesis of AA

# General scheme of transamination



amino acid

+

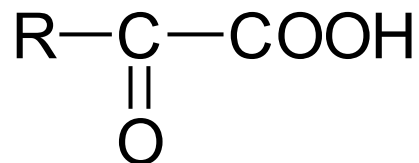


2-oxoglutarate



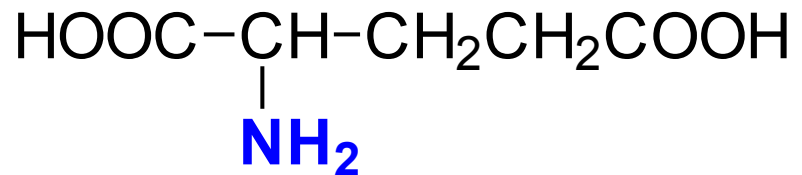
aminotransferase

pyridoxal phosphate



2-oxo acid

+

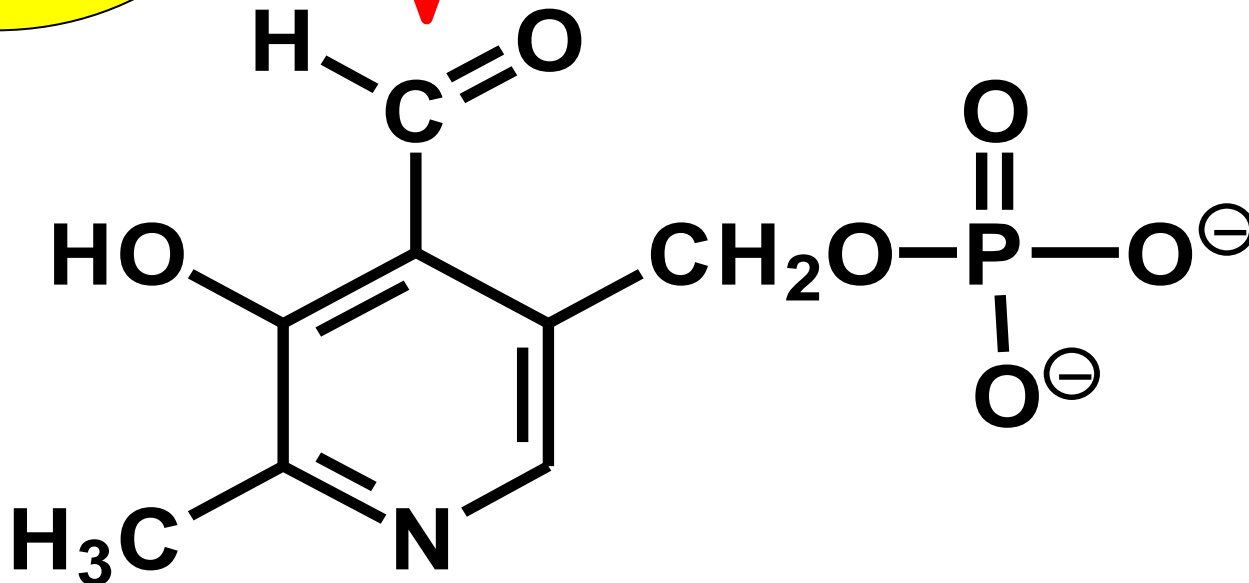


glutamate

# Pyridoxal phosphate has a reactive aldehyde group

covalently  
linked to enzyme  
(Lys)

aldehyde group

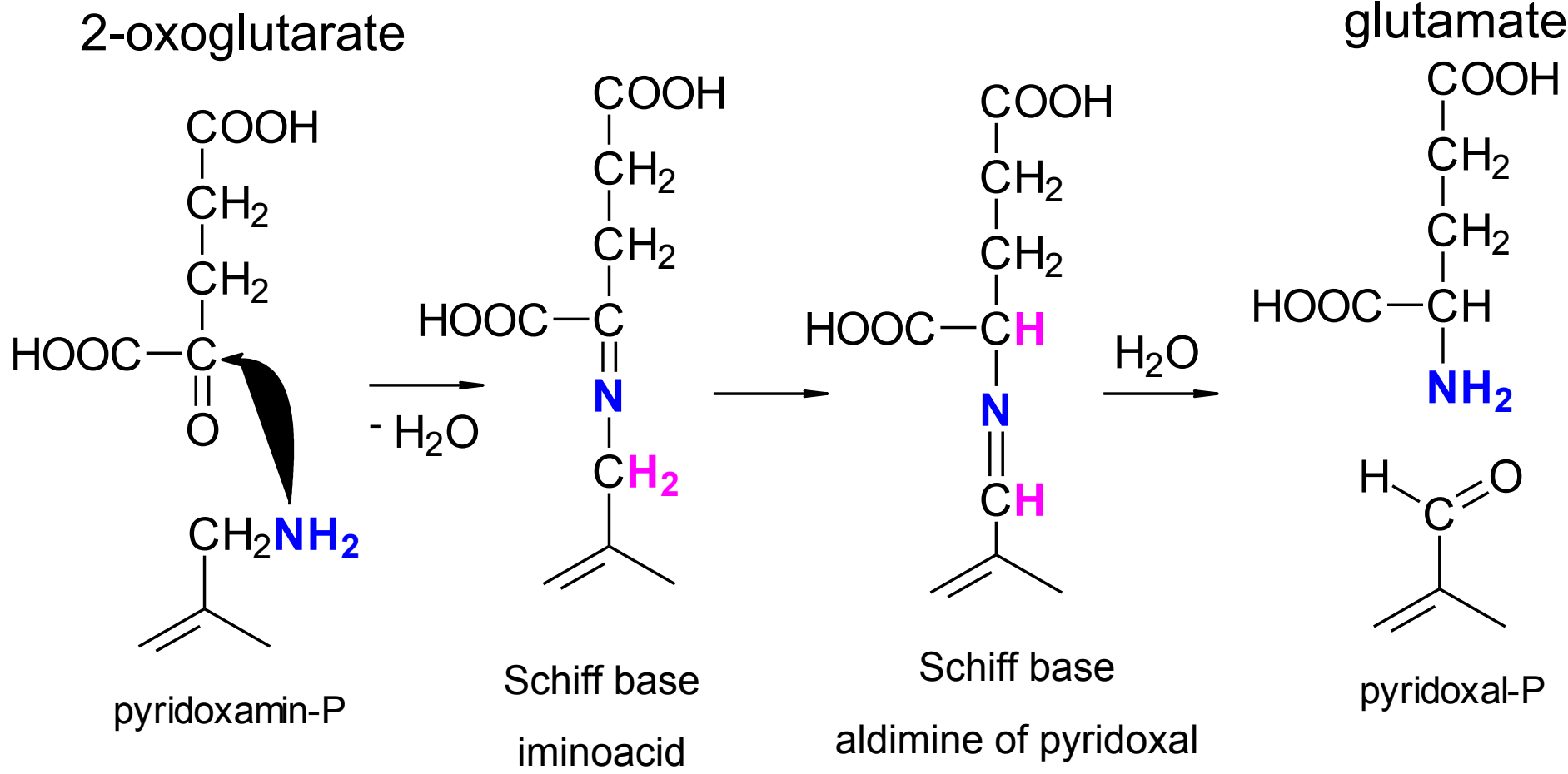






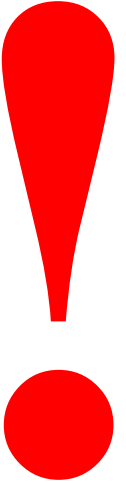
# 2. Phase of transamination

2-oxoglutarate → glutamate  
 pyridoxamine-P → pyridoxal-P

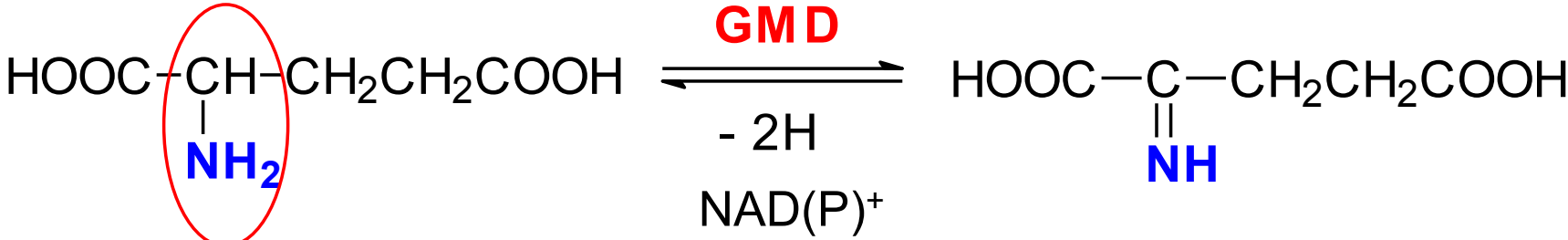


In transaminations, nitrogen of most AA is concentrated in glutamate

Glutamate then undergoes dehydrogenation + deamination and releases free ammonia  $\text{NH}_3$



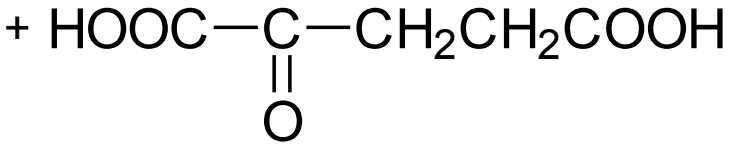
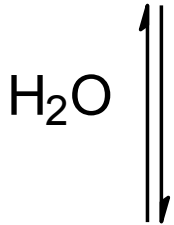
# Dehydrogenation + deamination of glutamate is reversible reaction



glutamate

2-iminoglutarate

main source of ammonia in tissues



2-oxoglutarate

# Glutamate dehydrogenase (GMD, GD, GDH)

- requires pyridine cofactor  $\text{NAD(P)}^+$
- GMD reaction is reversible: dehydrogenation with  $\text{NAD}^+$ , hydrogenation with  $\text{NADPH} + \text{H}^+$
- two steps:
- **dehydrogenation** of  $\text{CH-NH}_2$  to imino group  $\text{C=NH}$
- **hydrolysis** of imino group to oxo group and ammonia

# Two main sources of ammonia in organism

1. **Deamination of glutamate (GD reaction) in tissues**
2. **Bacterial putrefaction of proteins in the large intestine**

produces nitrogen catabolites (e.g. biogenic amines + ammonia),

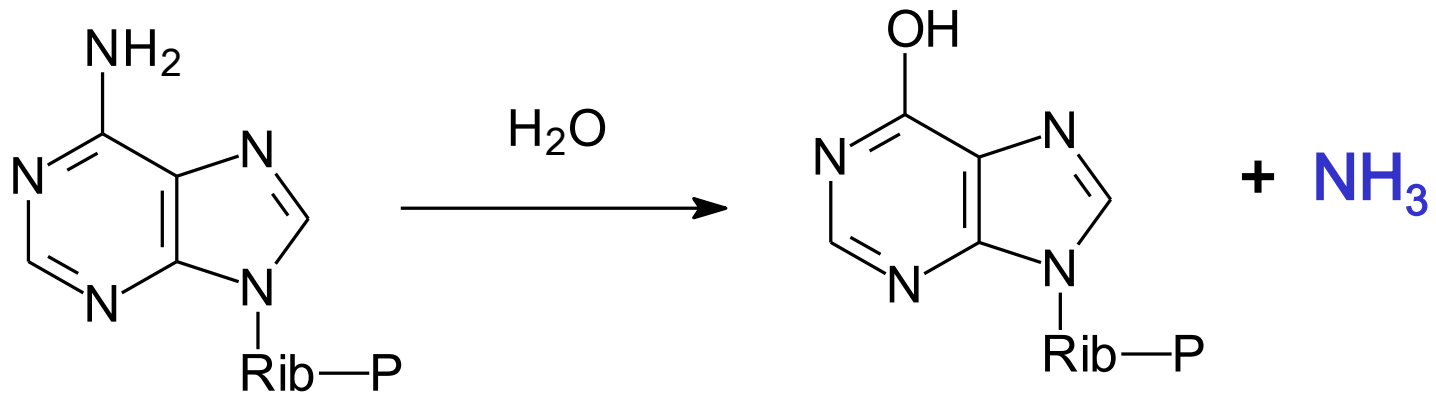
ammonia diffuses freely into portal blood  $\Rightarrow$  portal blood has high

concentration of  $\text{NH}_4^+$   $\Rightarrow$  eliminated by liver

## Other sources of ammonia: deaminations of various substrates

- deamination of adenine
- oxidative deamination of some AA ( $\rightarrow \text{H}_2\text{O}_2$ )
- desaturation deamination of histidine  $\rightarrow$  urocanic acid +  **$\text{NH}_3$**
- oxidative deamination of terminal  $-\text{NH}_2$  in lysine  
lysyl oxidase( $\text{Cu}^{2+}$ ):  $\text{Lys} + \text{O}_2 \rightarrow \text{NH}_3 + \text{allysine} + \text{H}_2\text{O}$
- dehydration deamination of serine (see next lecture)
- oxidative deamination of biogenous amines, MAO monoamine oxidase  
( $\rightarrow \text{H}_2\text{O}_2$ , see also Med. Chem. II, p. 60)

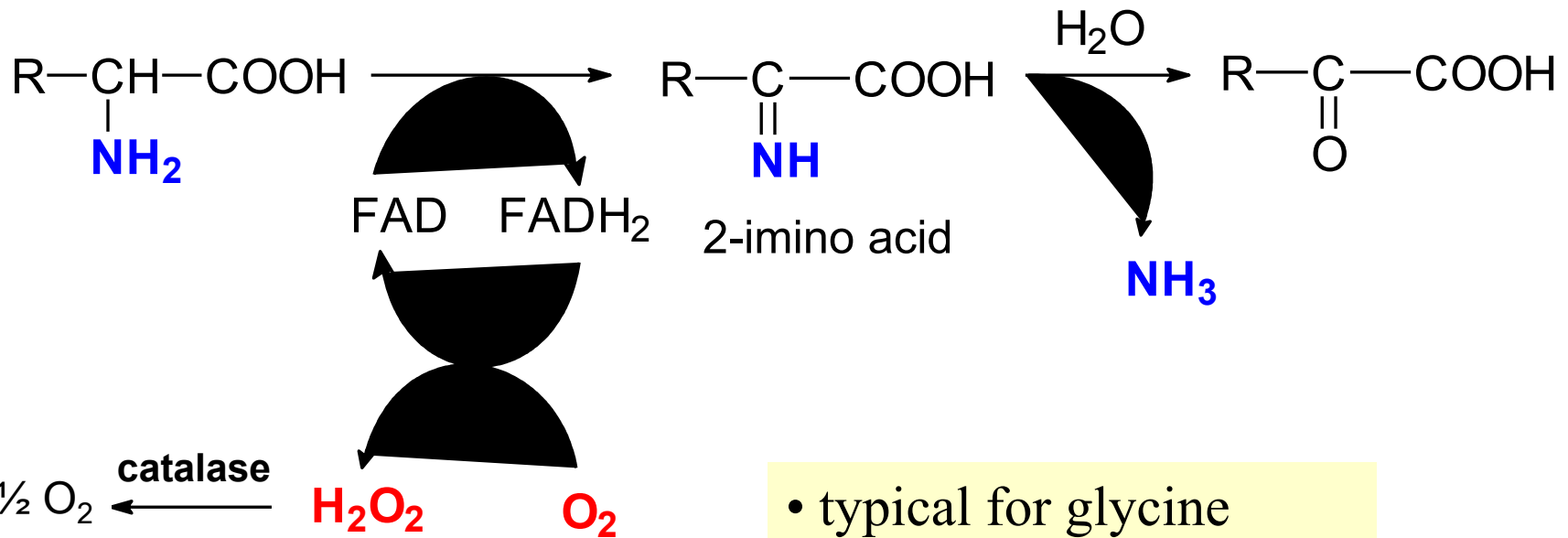
# Deamination of adenine



adenosine monophosphate (AMP)

inosine monophosphate (IMP)

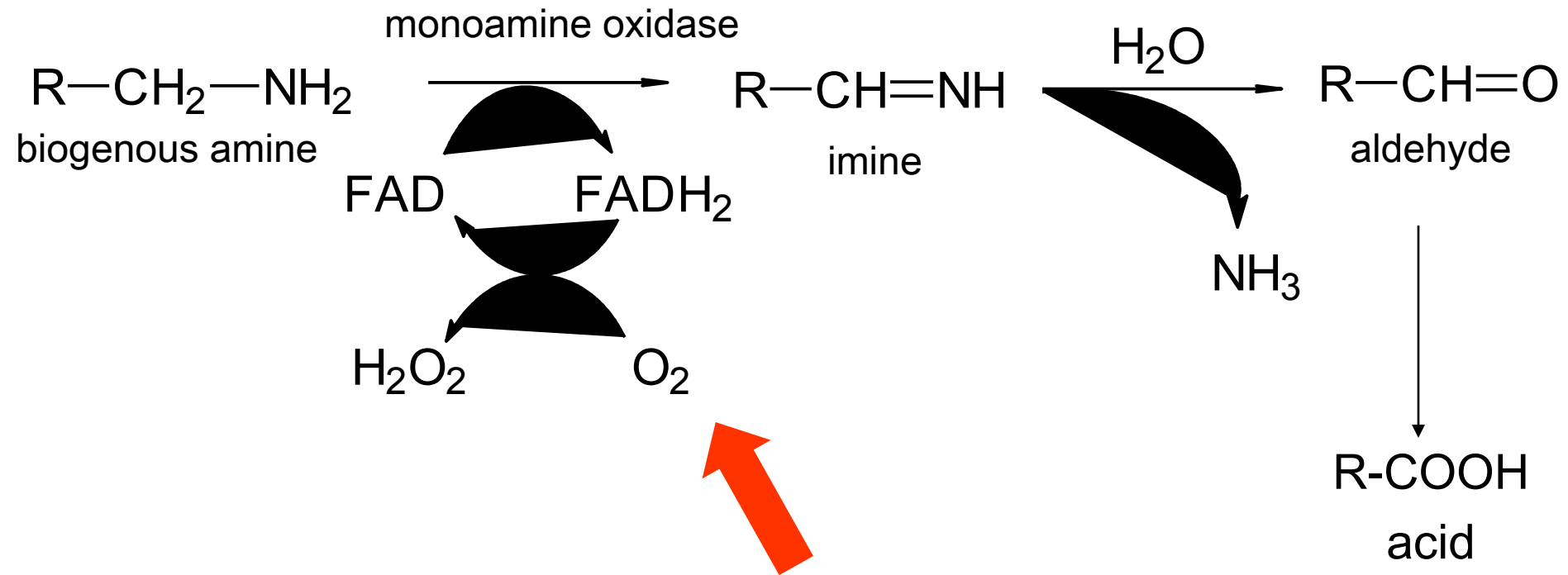
# Oxidative deamination of some AA uses flavine cofactor and dioxygen



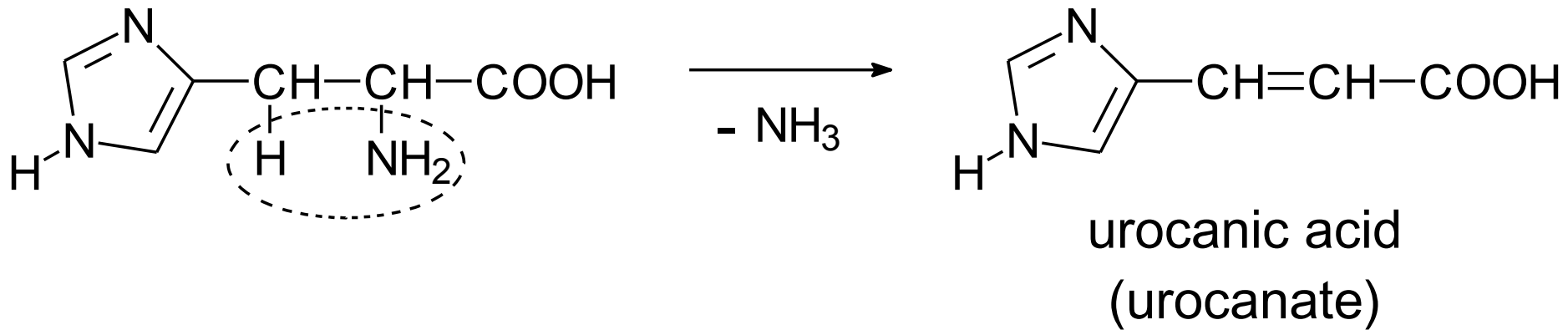
- typical for glycine
- D-amino acids
- side product is  $\text{H}_2\text{O}_2$



# Oxidative deamination of biogenous amines



## Desaturation type of deamination in histidine

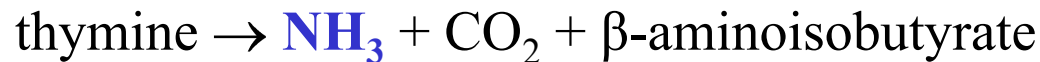


# Other reactions producing ammonia

- **non-enzymatic carbamylation of proteins**

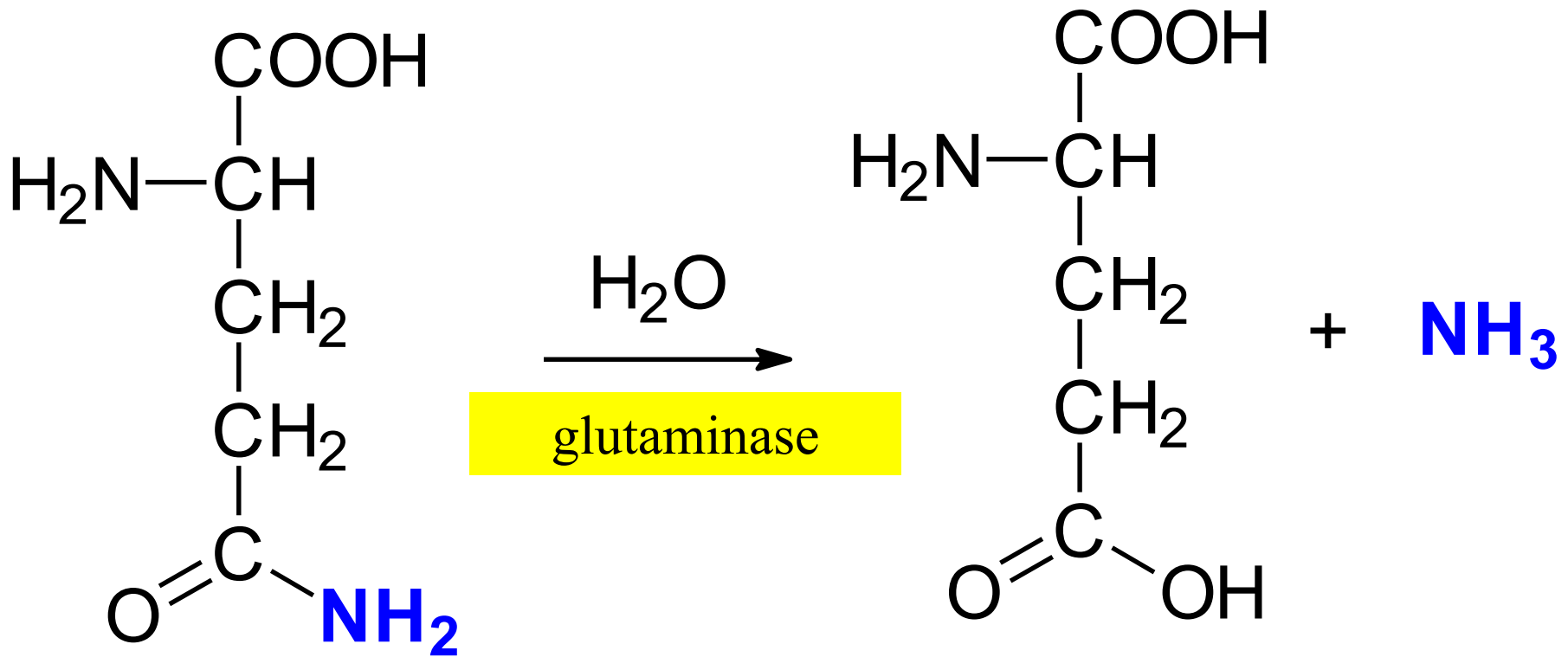


- **catabolism of pyrimidine bases**



- **synthesis of heme** (4 porphobilinogen  $\rightarrow$  4  $\text{NH}_3$  + uroporphyrinogen)

Hydrolysis of amide group in glutamine releases ammonia (deamidation). In kidneys,  $\text{NH}_4^+$  ions are released into urine.



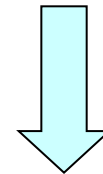
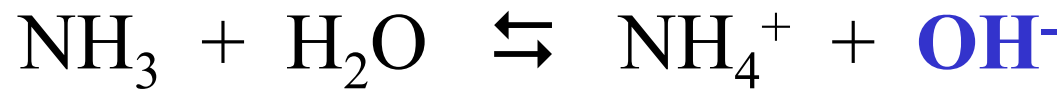
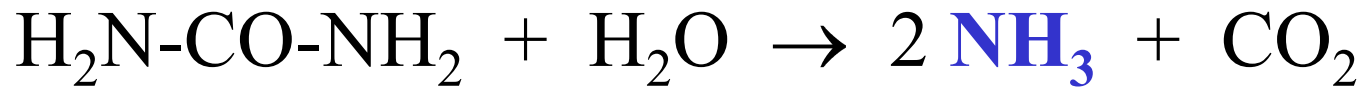
glutamine

glutamate +  $\text{NH}_3$

Glutamine is non-toxic transport form of ammonia

# Ammonia production under pathological conditions

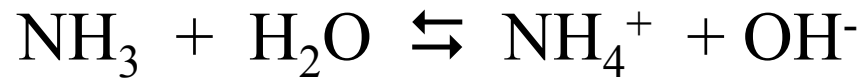
- **bleeding in GIT**  $\Rightarrow$  increased  $\text{NH}_3$  in portal blood
- **uroinfections** – bacterial urease catalyzes the hydrolysis of urea



alkaline urine (pH ~ 8)  $\Rightarrow$  phosphate stones

# Acide-base properties of $\text{NH}_3$

$$pK_B(\text{NH}_3) = 4.75 \text{ (weak base)}$$



$$pK_A(\text{NH}_4^+) = 14 - 4.75 = 9.25 \text{ (very weak acid)}$$

under physiological pH values in ICF and ECF ( $\sim 7.40$ ):

**98 %  $\text{NH}_4^+$**

**2 %  $\text{NH}_3$**

# Compare: Ammonium ions in body fluids

Body fluid	Concentration of $\text{NH}_4^+$ ions	Metabolic origin of $\text{NH}_4^+$
Urine	10 – 40 mmol/l	hydrolysis of Gln, deamination of Glu (tubules)
Saliva	2 – 3 mmol/l	hydrolysis of urea by oral microflora
Portal blood	100 – 300 $\mu\text{mol/l}$	protein putrefaction (GIT), Gln/Glu catabolism (enterocyte)
Venous blood	5 – 30 $\mu\text{mol/l}$	catabolism of AA in tissues

# How to decrease ammonia production in body?

1. **Low-protein diet** (especially important in liver diseases)
2. **Alteration of colon microflora by the ingestion of:**
  - **Probiotics** – live bacteria stimulating saccharolytic (fermentative) processes in large intestine instead of putrefactive ones (*Lactobacillus*, *Bifidobacterium*) – yoghurt, kefir milk, sauerkraut etc.
  - **Prebiotics** – non-digestible food ingredients (polysaccharides) that stimulate the growth probiotics in the colon (dietary fibre, inulin)



# Three ways of ammonia detoxification

Feature	Urea	Glutamine (Gln)	Glutamate (Glu)
Relevance	★ ★ ★ ★ ★ ★	★ ★ ★ ★	★
Compound type	H <sub>2</sub> CO <sub>3</sub> diamide	γ-amide of Glu	α-amino acid
Reaction(s)	urea cycle	Glu + NH <sub>3</sub>	hydrog. amin. 2-OG
Enzyme	5 enzymes	Gln-synthetase	GMD
Energy needs	<b>4 ATP</b>	1 ATP	1 NADPH+H <sup>+</sup>
Organelle(s)	mitoch. + cytosol	cytosol	mitochondria
Organ(s)	only liver	liver, <b>brain</b> , other	(brain)

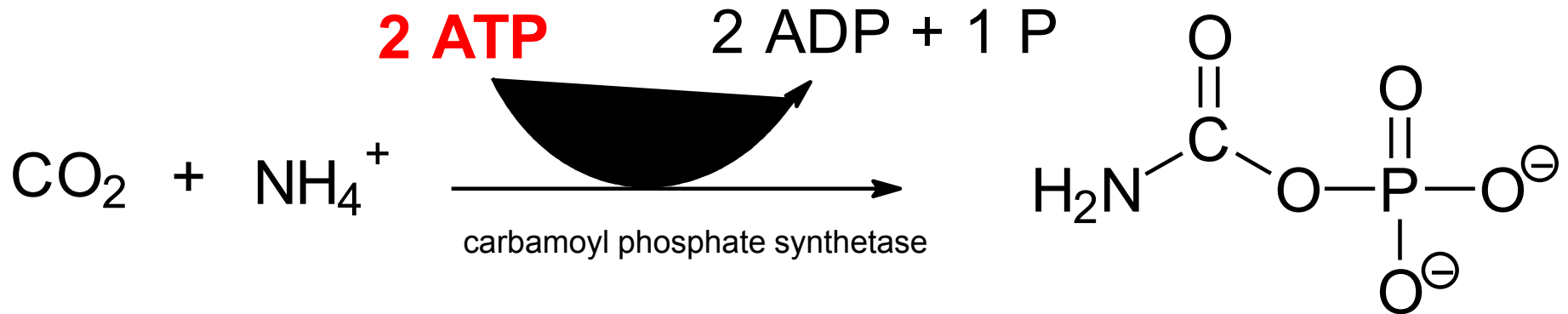
# Ureasyntesis in liver

five reactions

1. and 2. in mitochondria

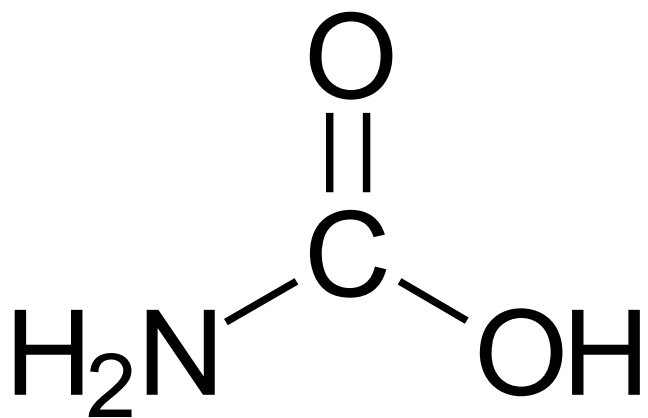
3. - 5. in cytosol

# 1. Carbamoyl phosphate (matrix)



- carbamoyl phosphate synthetase (activated by *N*-acetylglutamate)
- matrix of mitochondria
- **two moles of ATP**
- amide bond + mixed anhydride
- **macroergic compound**

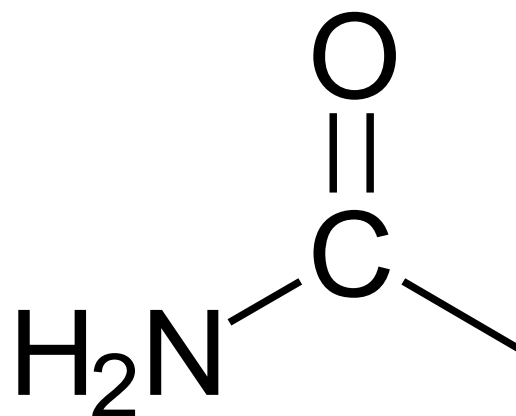
# Carbamoyl is the acyl of carbamic acid



carbamic acid

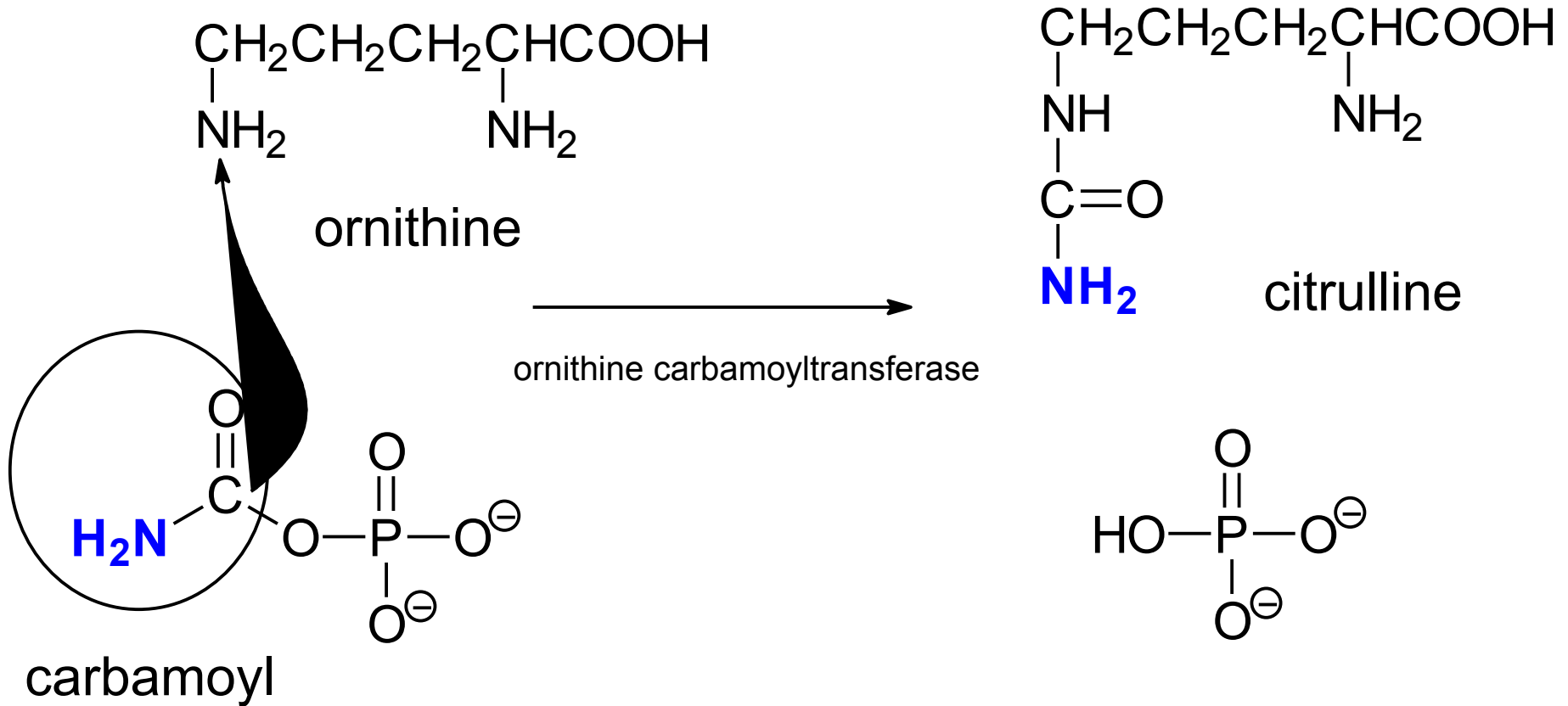
(carbonic acid monoamide)

does not exist

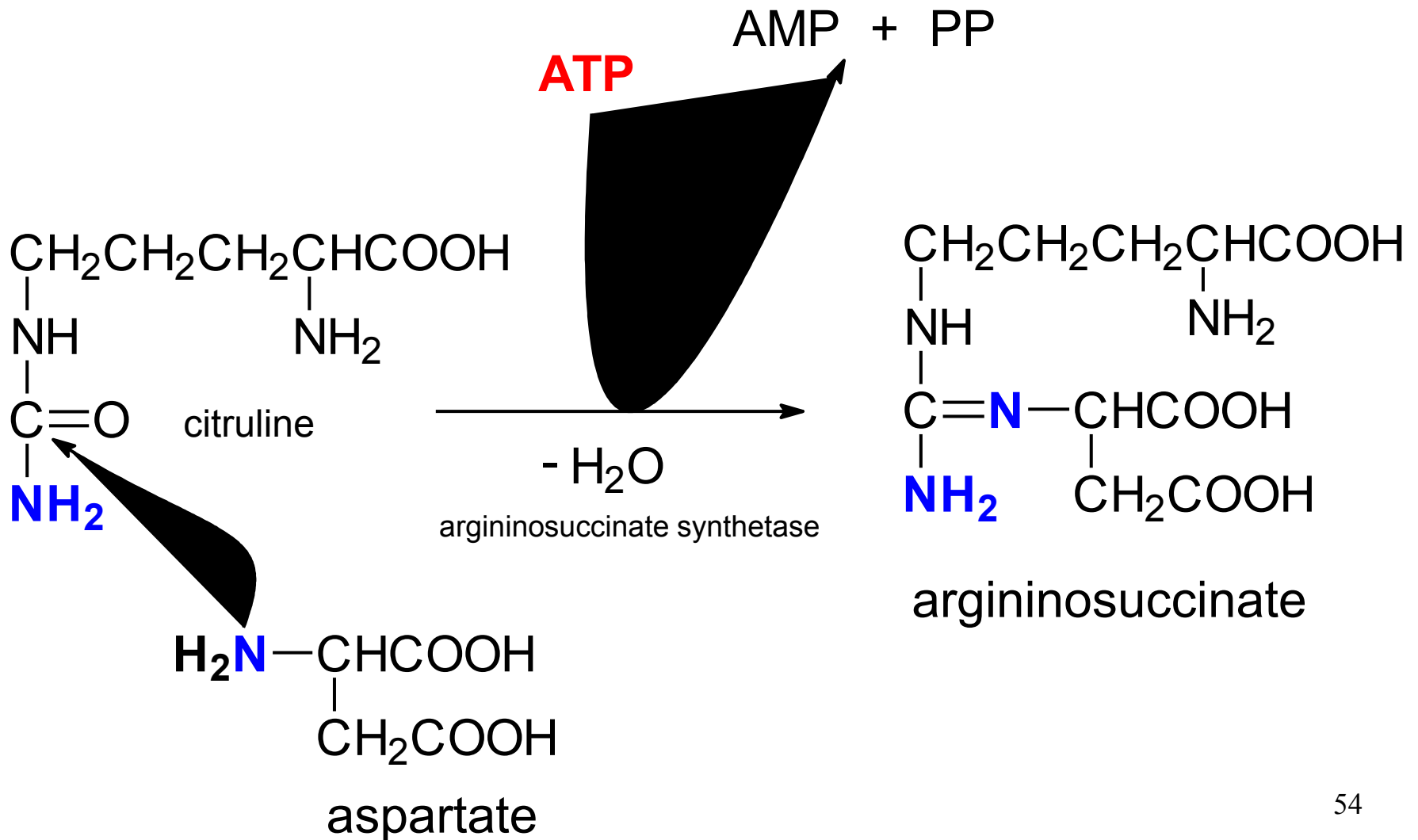


carbamoyl

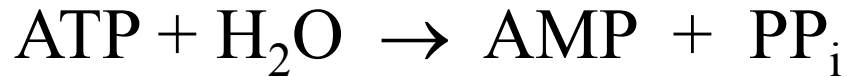
## 2. Citrulline formation (matrix)



### 3. The second -NH<sub>2</sub> group comes from aspartate (cytosol)



# Less usual hydrolysis of ATP means that two ATP are consumed

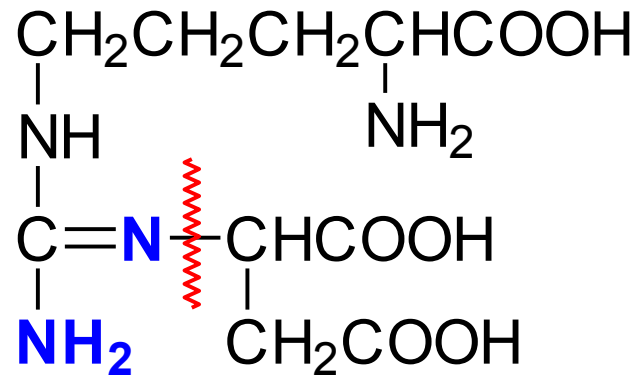


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summary:

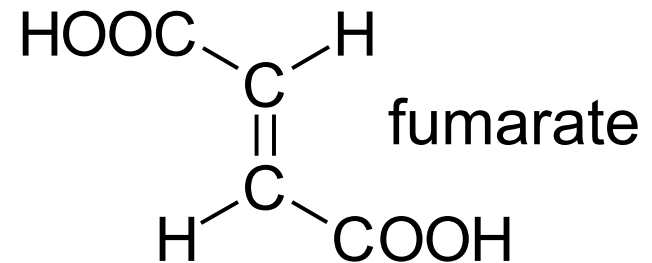
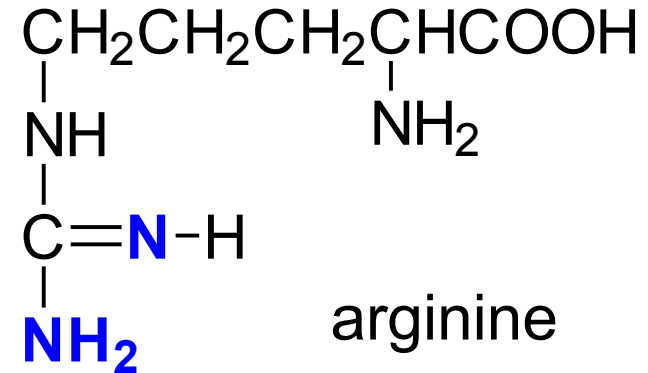


## 4. The cleavage of argininosuccinate



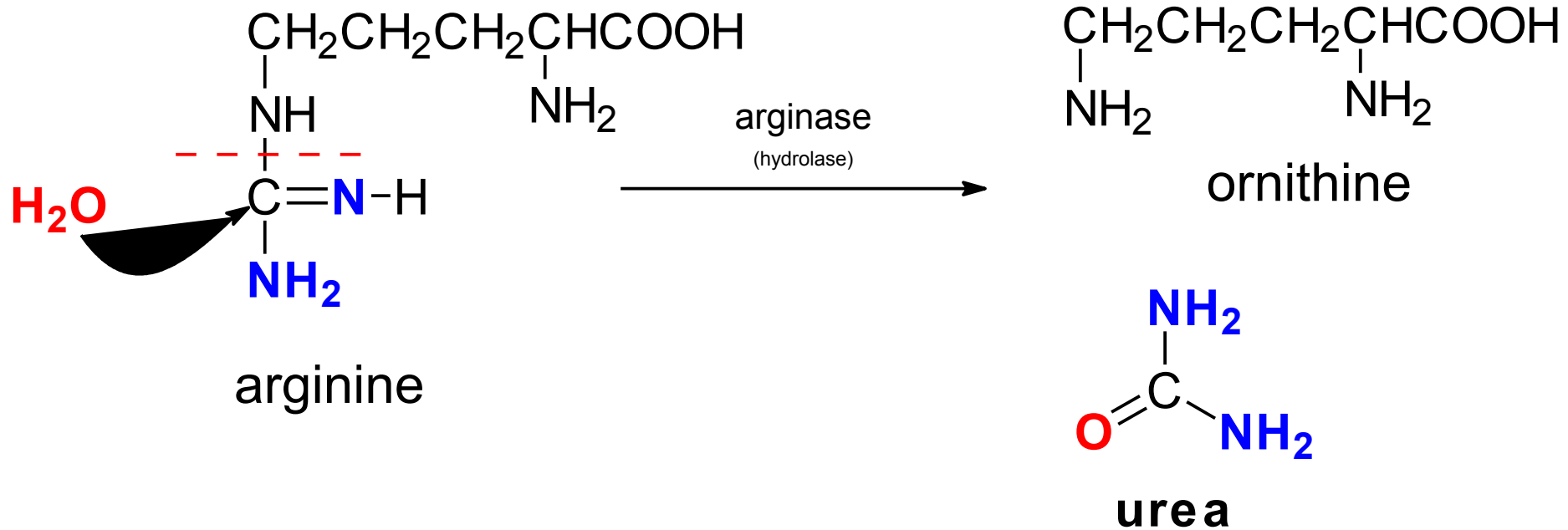
argininosuccinate

argininosuccinate lyase

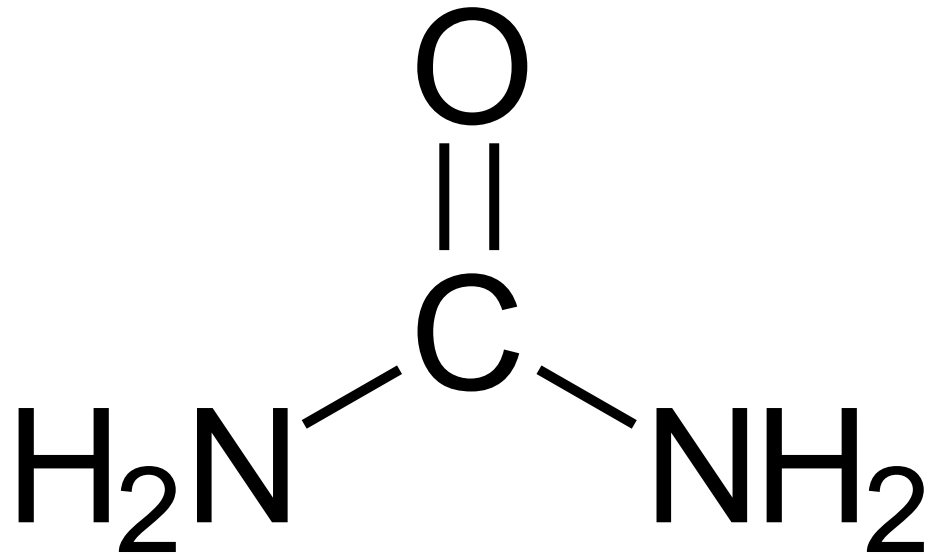




## 5. Hydrolysis of arginine affords urea



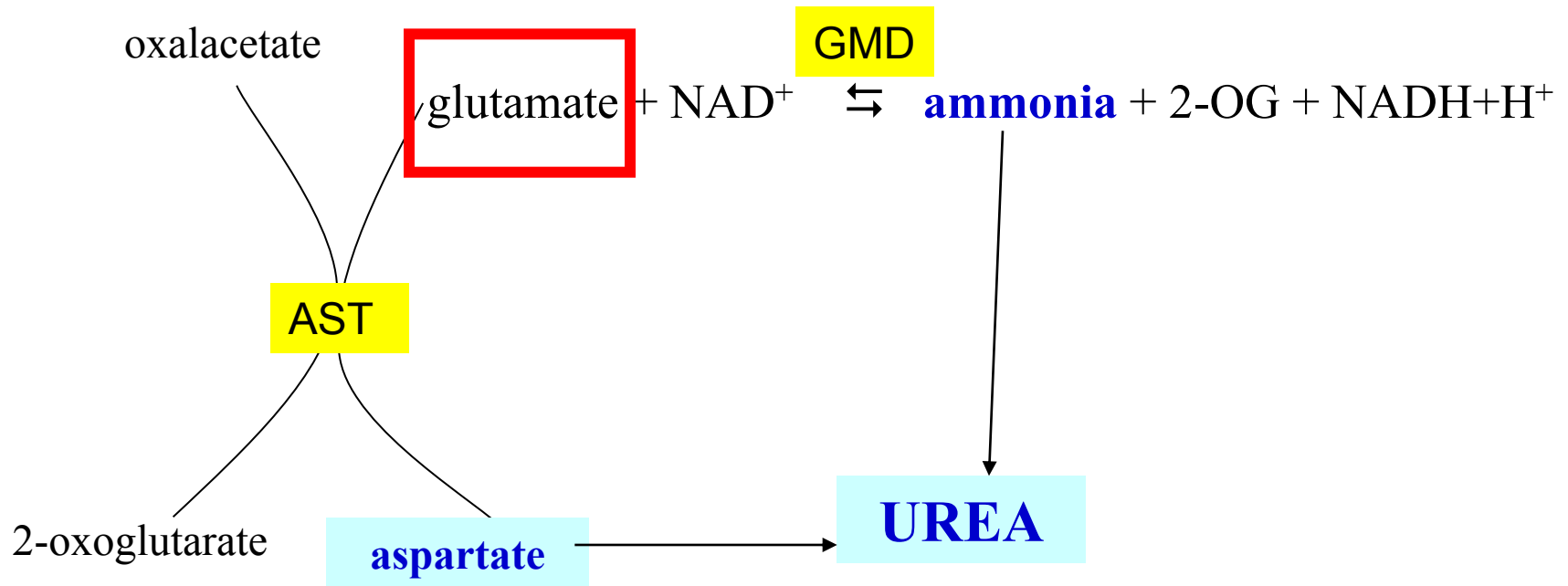
# Metabolic origin of nitrogen in urea



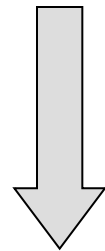
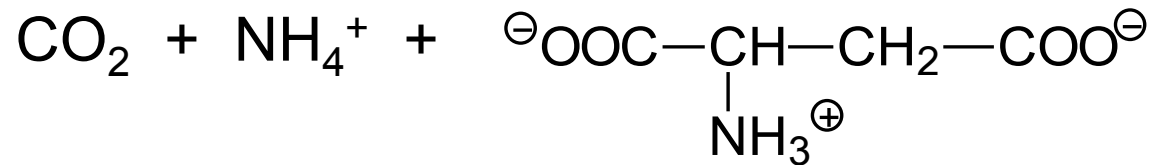
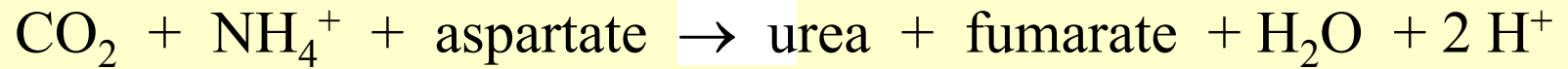
**free ammonia**

**aspartate**

# Dual function of glutamate in AA catabolism



# Urea synthesis is proton-productive reaction



# Urea is non-electrolyte

- carbonic acid diamide
- polar compound (dipole)  $\Rightarrow$  well soluble in water
- diffuses easily through cell membranes (hydrophilic pores)
- contributes to blood plasma osmolality

osmolality  $\approx 2 [\text{Na}^+] + [\text{glucose}] + [\text{urea}] \text{ mmol/kg H}_2\text{O}$

- synthesis only in liver
- excretion by urine depends on the amount of food proteins

**330-600 mmol/day (20-35 g/day)**

# Urea in blood serum (2-8 mmol/l)

## Increased concentration

- renal failure
- increased protein catabolism (sepsis, burns, polytrauma, fever etc.)

## Decreased concentration

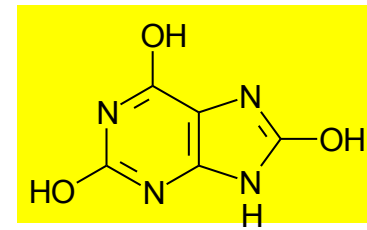
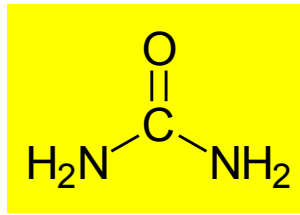
- lack of proteins in diet
- liver failure

# Compare and distinguish

urea      uric acid



# Compare

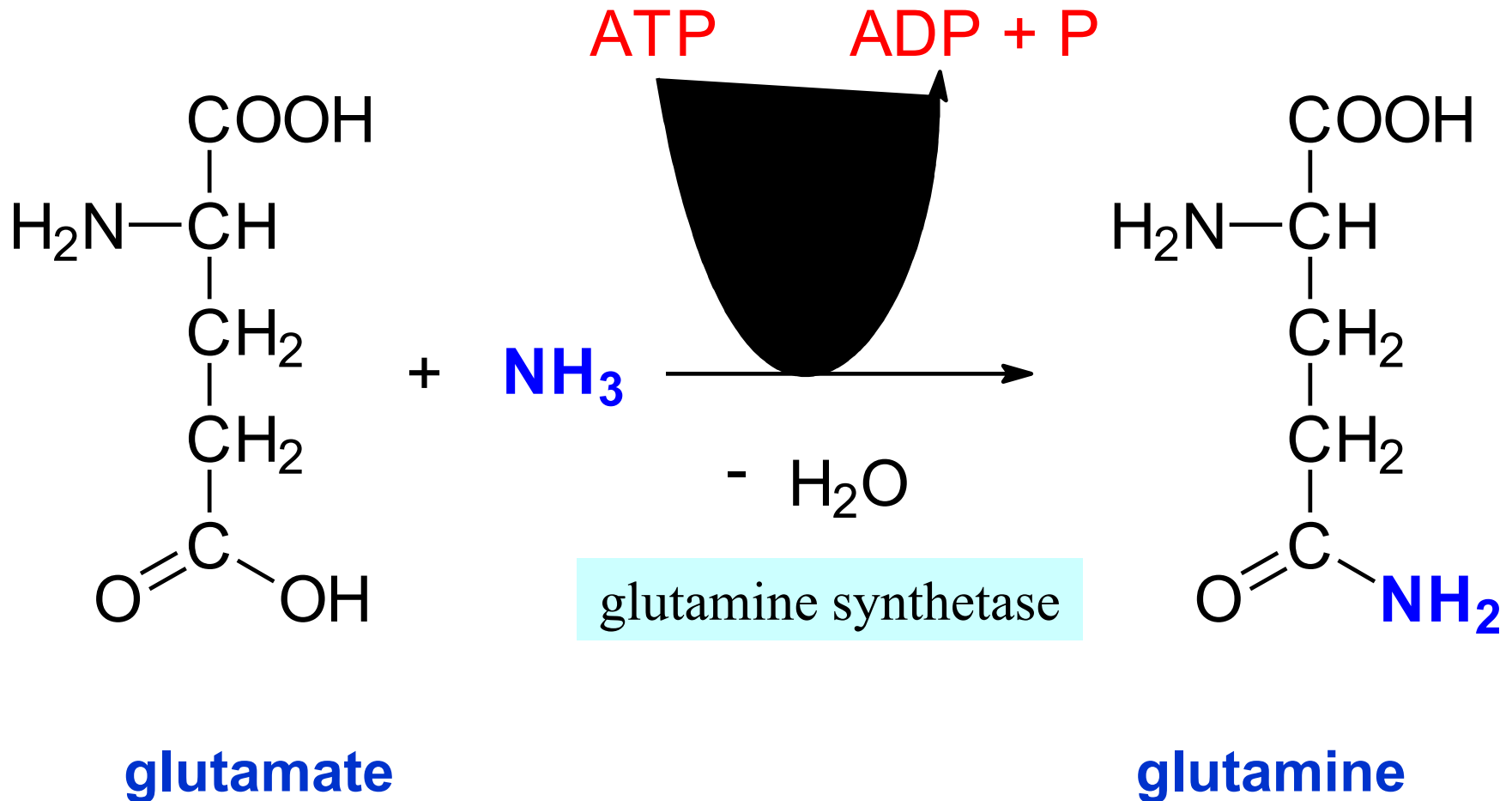


Feature	Urea	Uric acid
Chemical name	carbonic ac. diamide	2,6,8-trihoxypurine
Latin name	urea	acidum uricum
In water	non-electrolyte	weak diprotic acid
Solubility in water	excelent	poor, depeds on pH
Reducing property	no	yes ⇒ antioxidant
Salt formation	no	yes (two types)
Catabolite of	amino acids	adenine and guanine
Organe location	liver only	liver and other tissues
Subcellular location	mitochondria + cytosol	cytosol
Serum concentration	2 - 8 mmol/l	150 - 400 µmol/l
Urine excretion	20 - 35 g/day	0.5 - 1 g/day
Catabolic nitrogen	80 - 90 %	1- 2 %

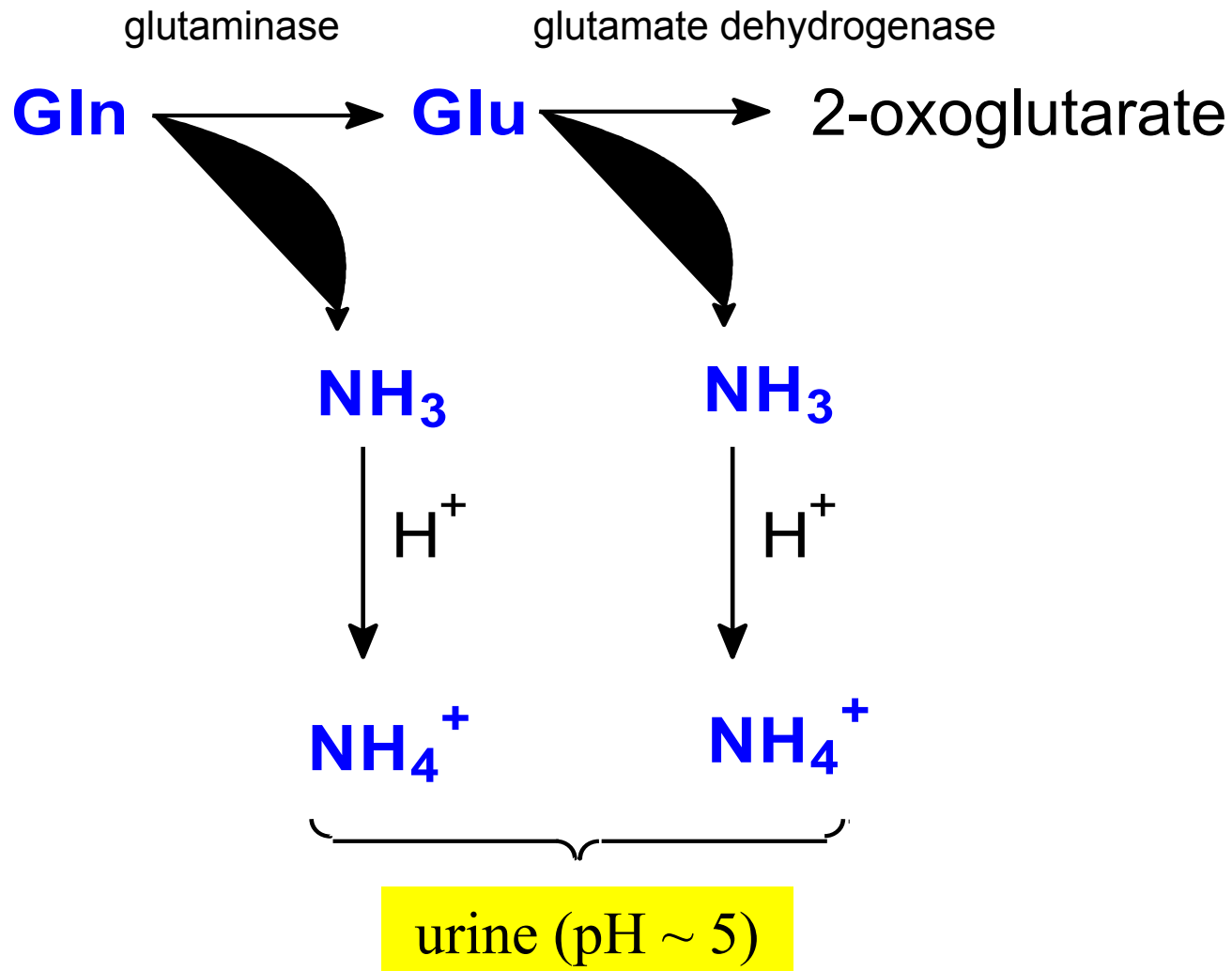


# Glutamine synthesis

2. way of detoxification



In kidneys, ammonia is released from glutamine and glutamate.  
Ammonium cation is excreted by mildly acidic urine

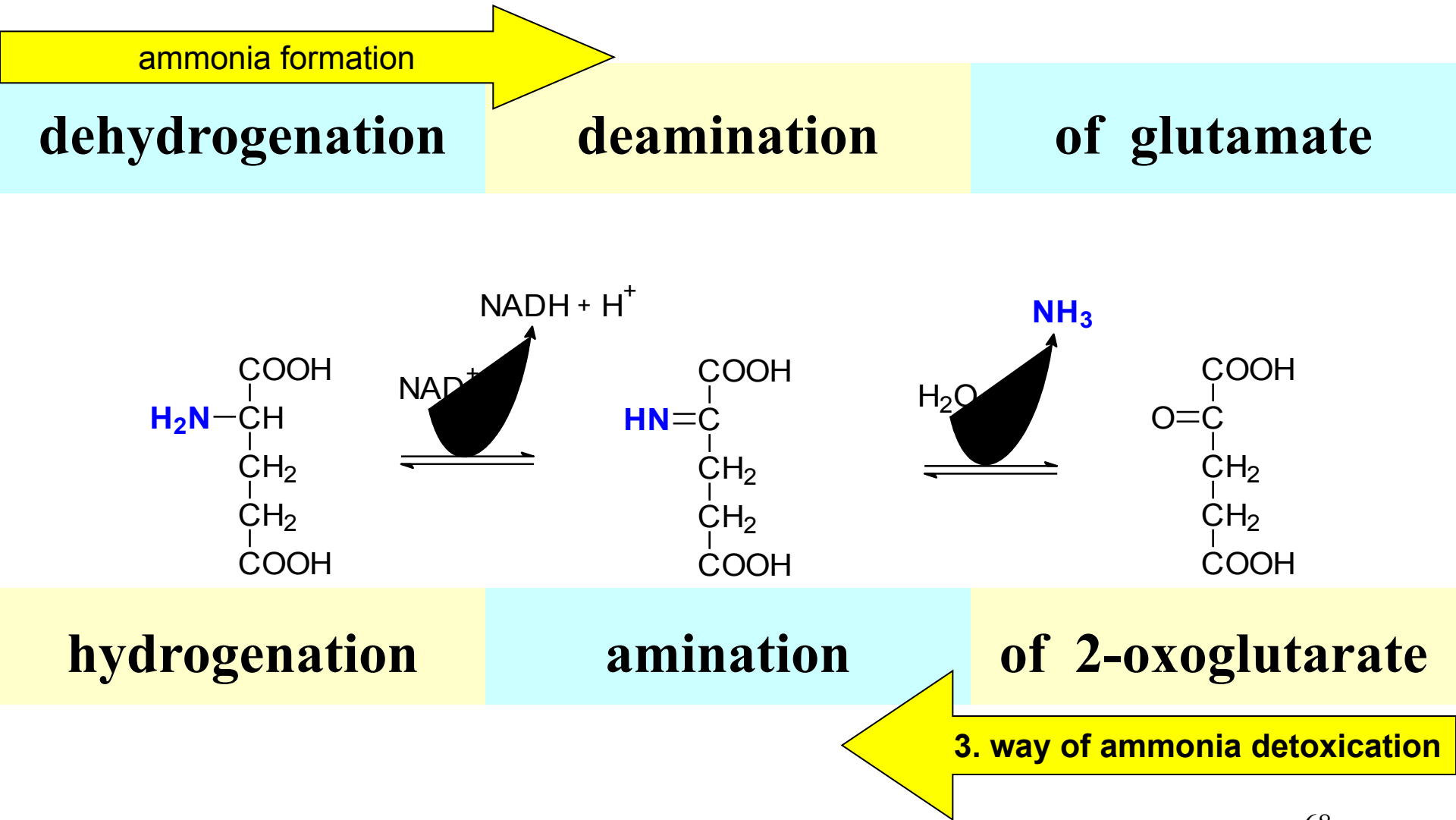


# Multiple functions of glutamine



- Synthesis of proteins
- Metabolic fuel – for some tissues: enterocytes, lymphocytes, macrophages, fibroblasts, kidneys
- Source of nitrogen in synthesis – purine, pyrimidines, NAD<sup>+</sup>, aminosugars
- Source of glutamate – glutathione (GSH), GABA,  
Glu → ornithine, Glu → proline
- Source of ammonium ions in urine
- detoxification of ammonia in tissues and non-toxic transport form of ammonia from tissues to liver

# Glutamate dehydrogenase reaction is reversible

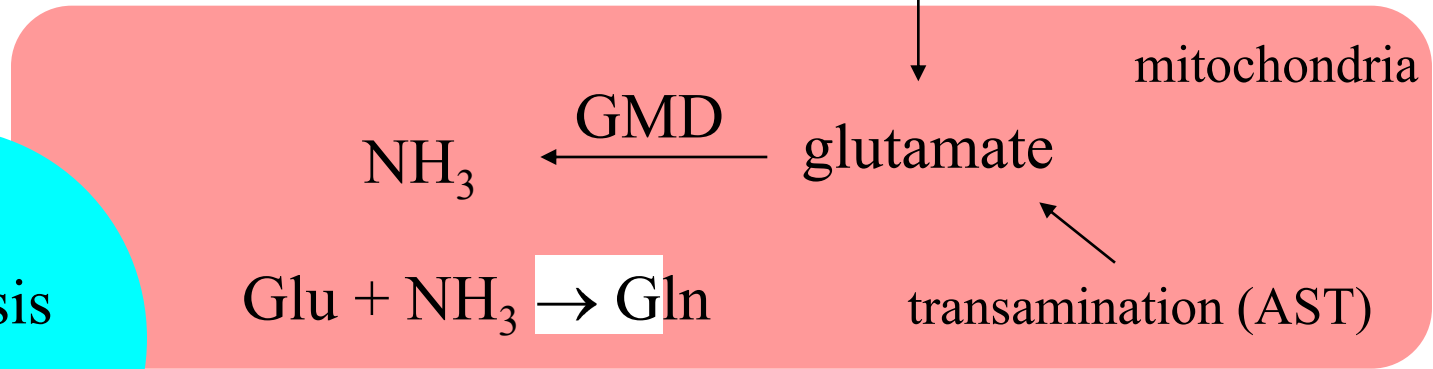


# Subcellular location of AA conversions

transamination (ALT)  $\Rightarrow$  glutamate cytosol

synthesis  
of urea

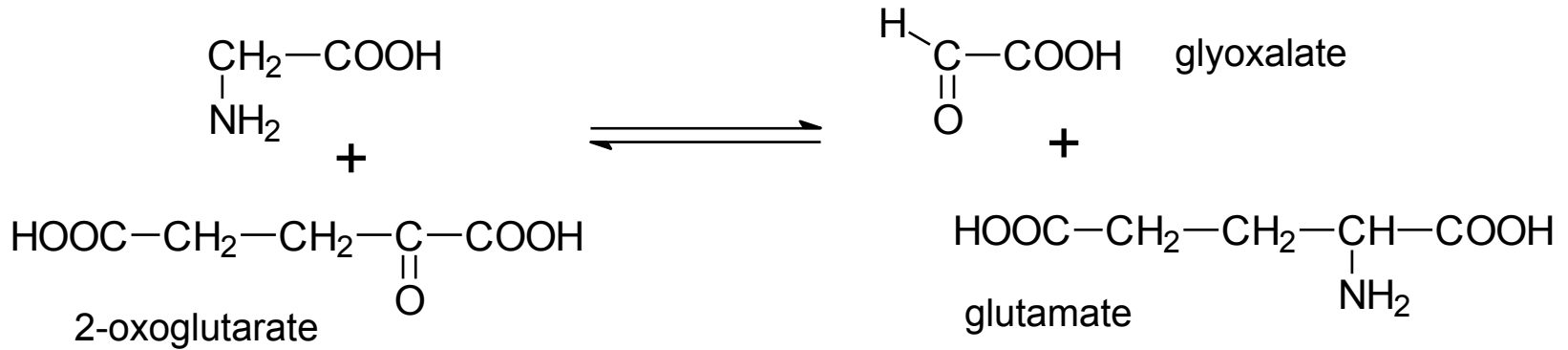
cytosol



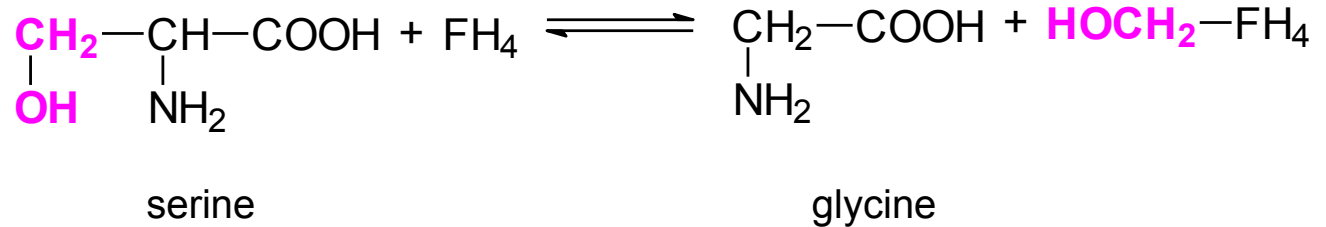
# **Synthesis of non-essential amino acids**

# Synthesis of glycine

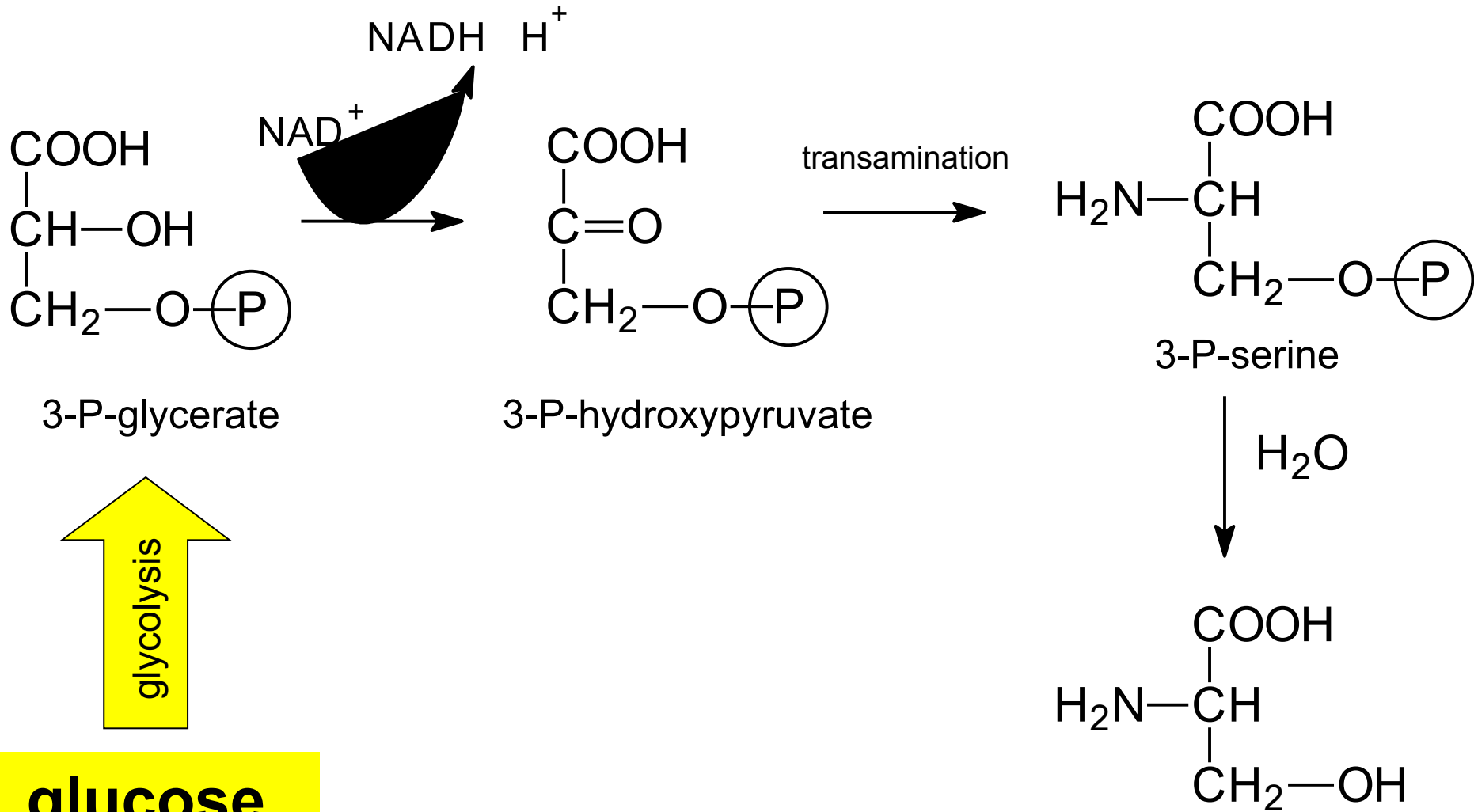
## 1. The reverse of transamination



## 2. From serine



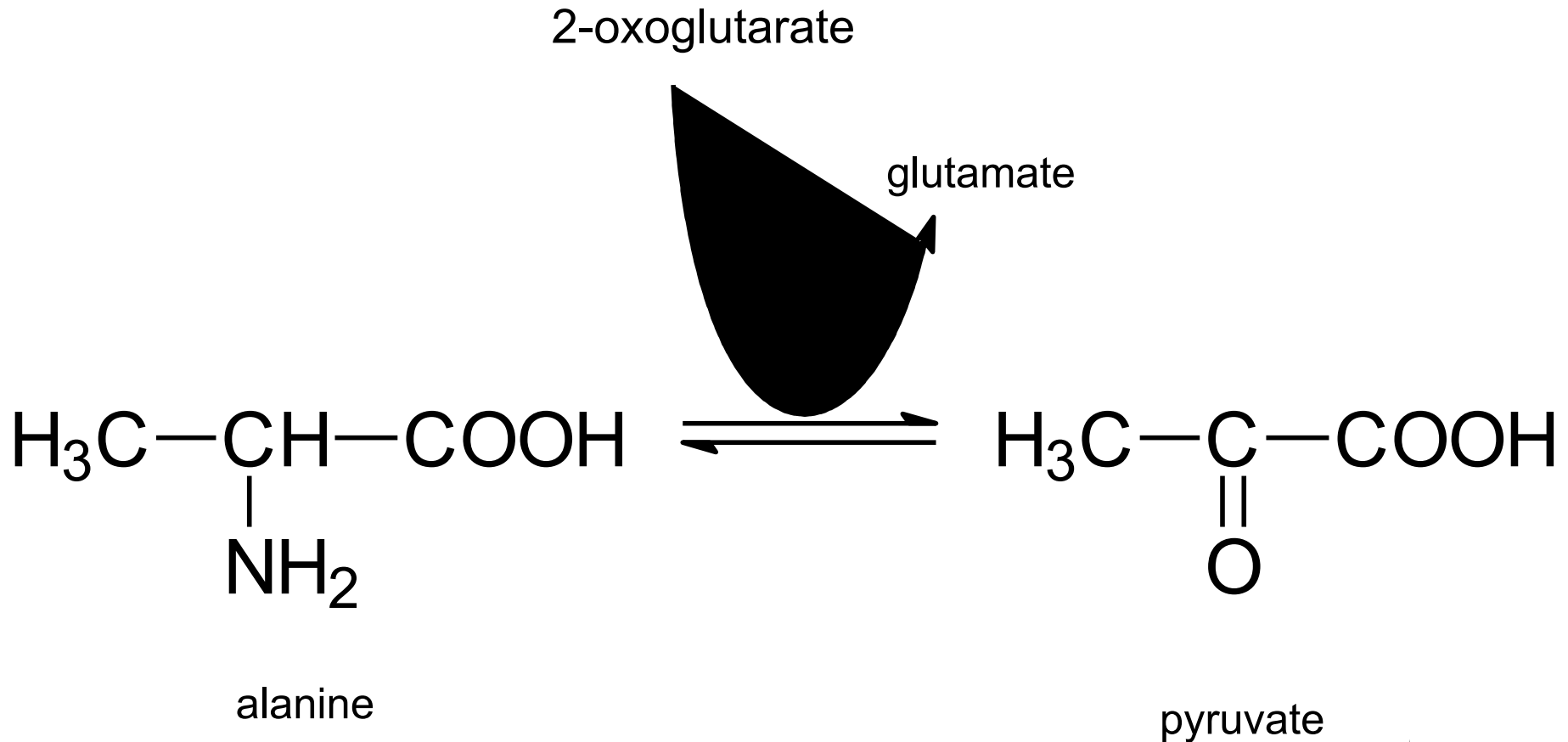
# Formation of serine from the glycolysis intermediate





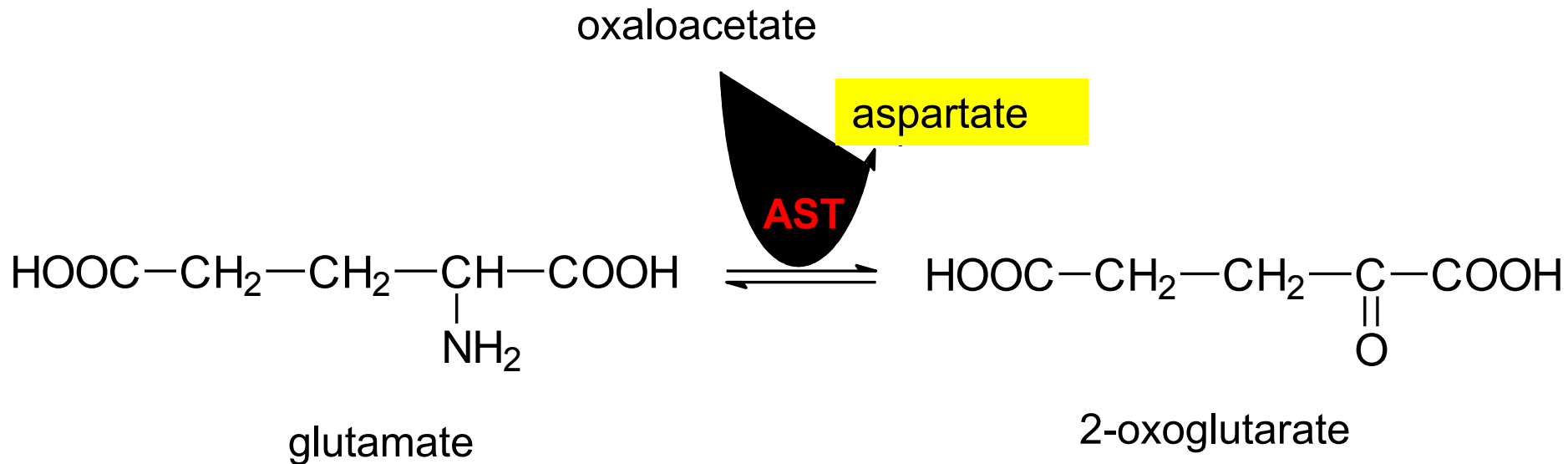
# Synthesis of alanine from pyruvate and glutamate

(ALT = alanine aminotransferase)



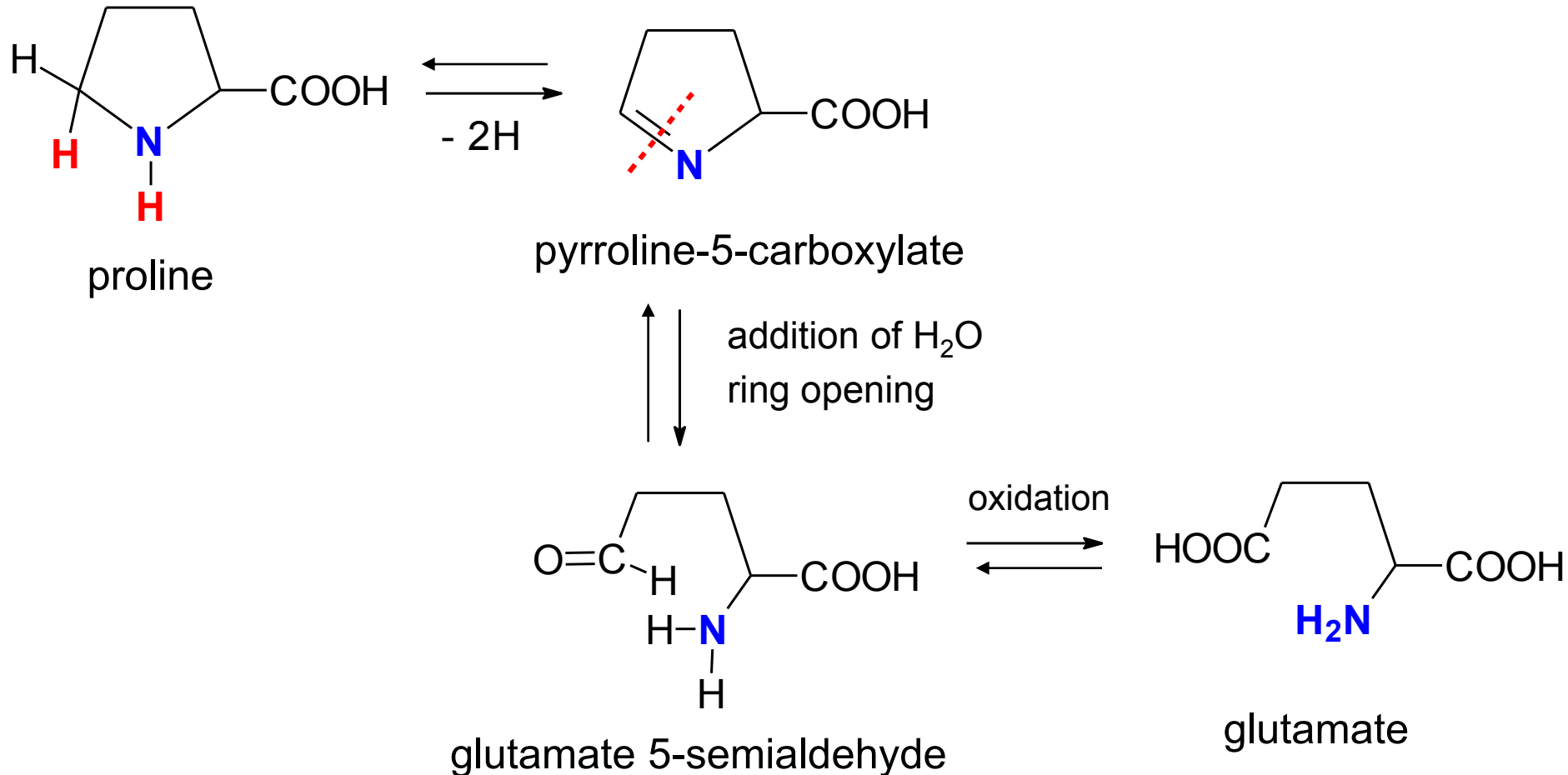
# Aspartate from oxaloacetate and glutamate

(AST = aspartate aminotransferase)

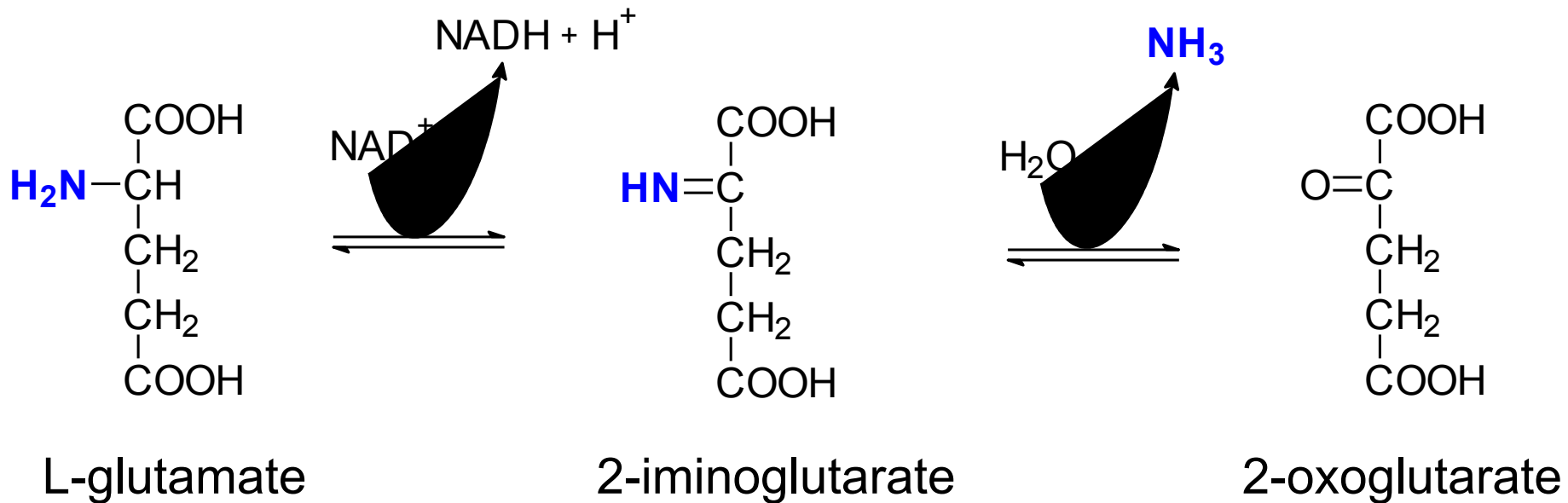


AST reaction produces aspartate for urea synthesis

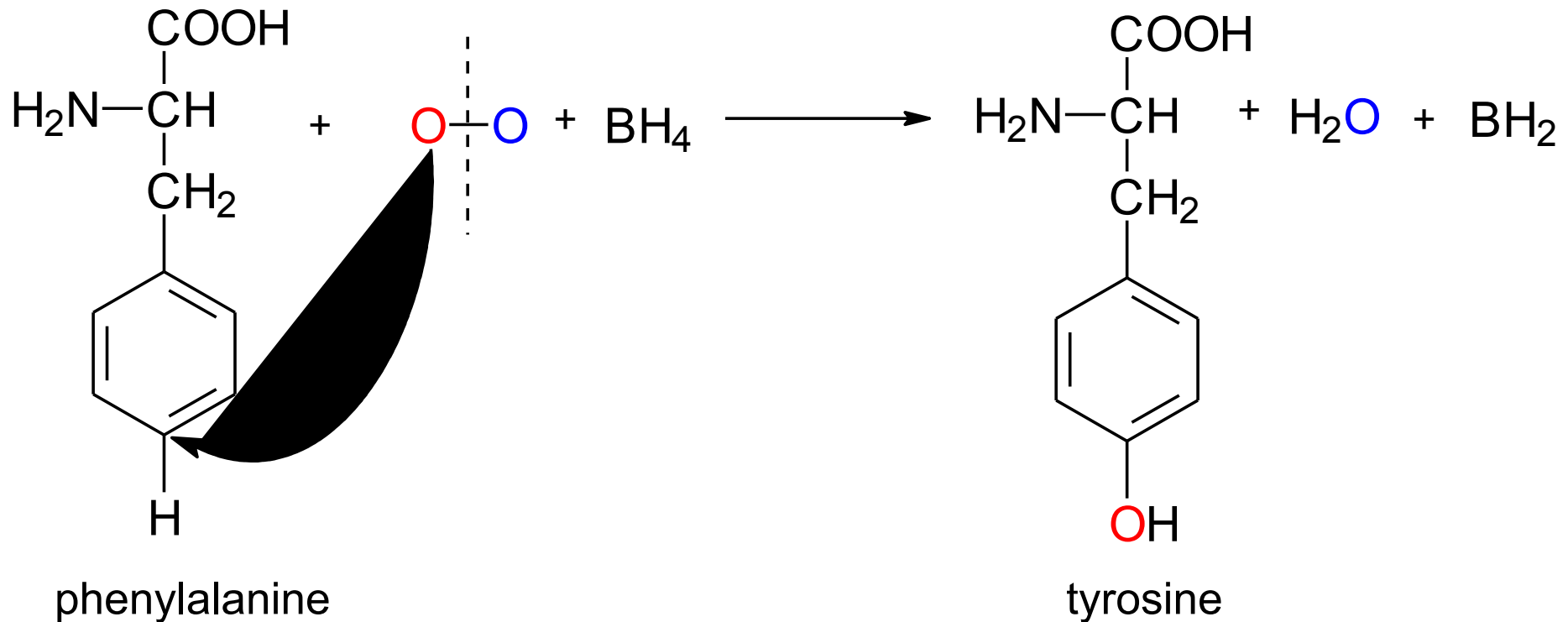
# Proline synthesis is the opposite of its catabolism



# Glutamate is formed by the reductive amination of 2-oxoglutarate (GMD reaction)



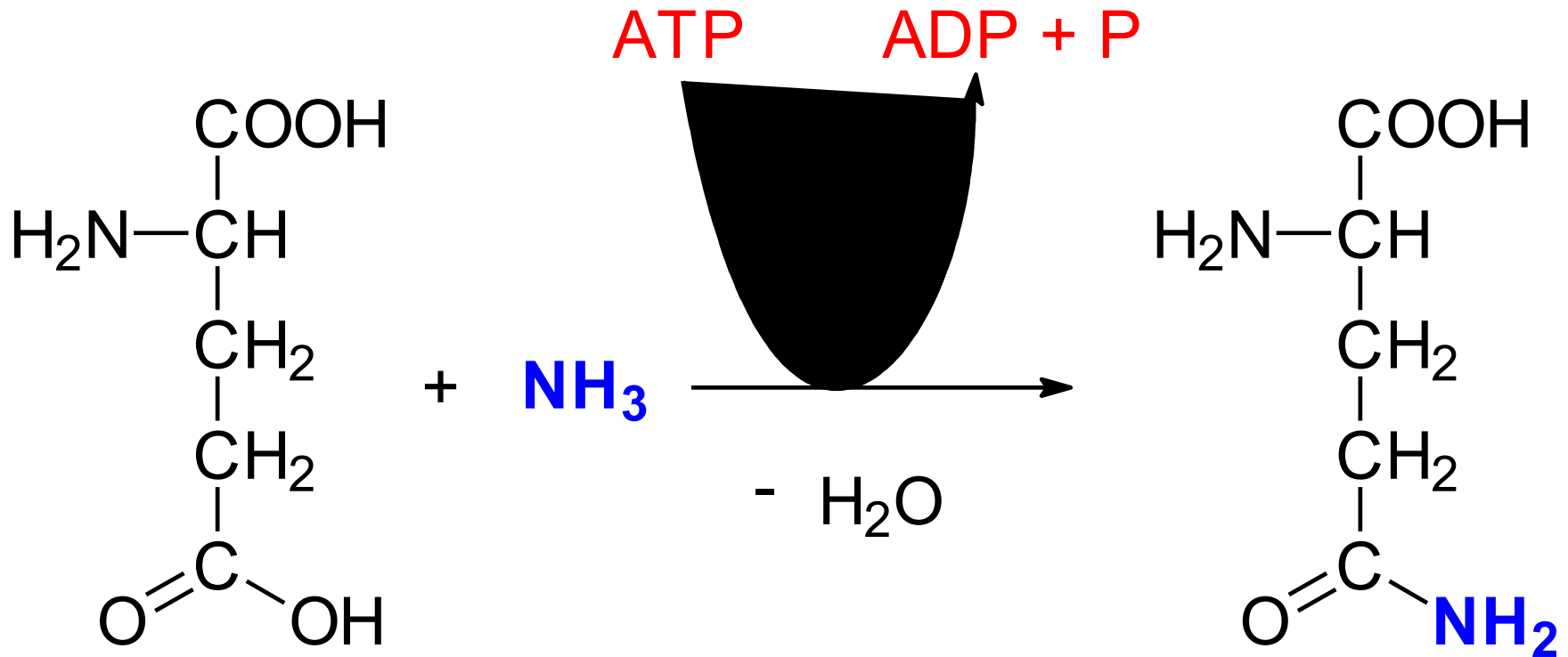
# Hydroxylation of essential phenylalanine gives non-essential tyrosine



tetrahydrobiopterine ( $\text{BH}_4$ ) is a donor of 2H to form water from the second oxygen atom

# Glutamine from glutamate and ammonia

glutamine synthetase



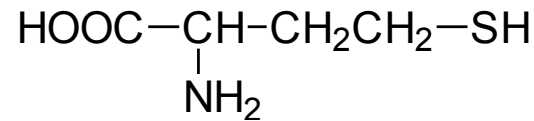
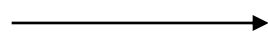
glutamate

glutamine

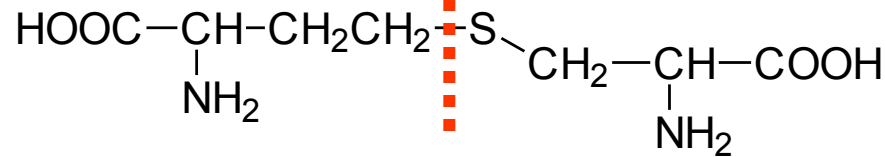
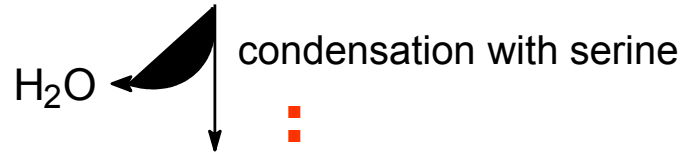
Similarly: asparagine from aspartate

# Cysteine is made from methionine

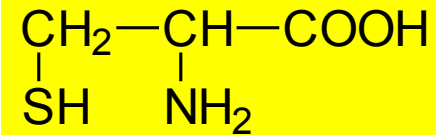
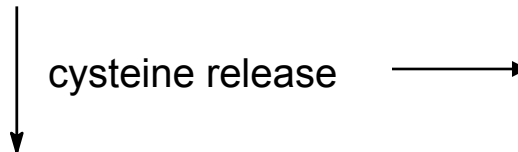
methionine



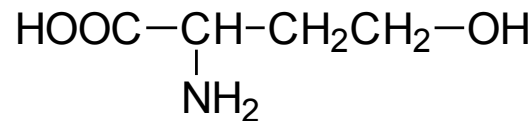
homocysteine



cystathionine

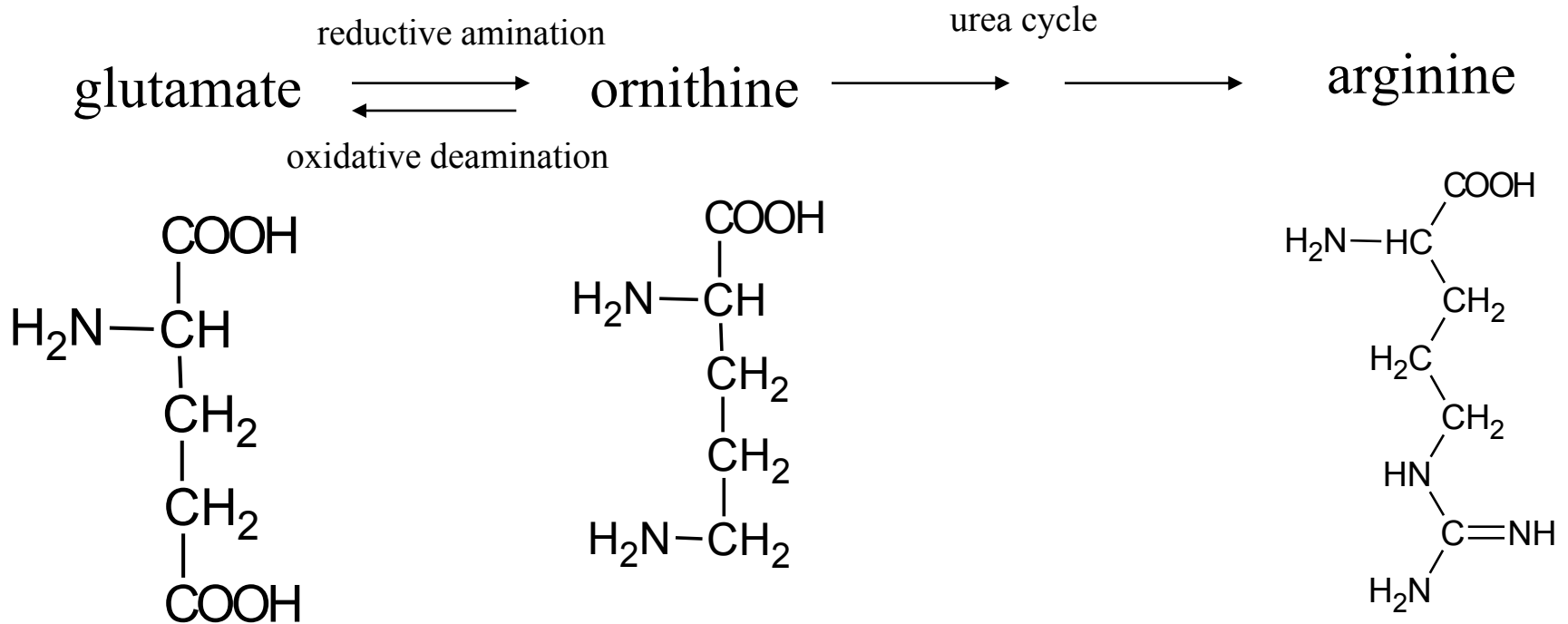


cysteine



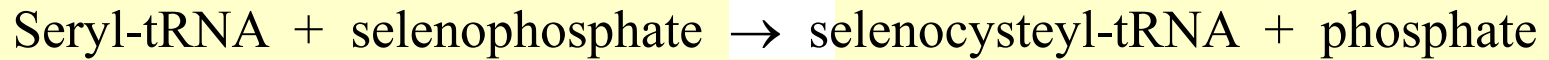
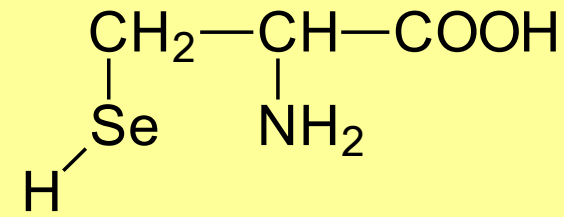
homoserine

# Arginine from glutamate via ornithine

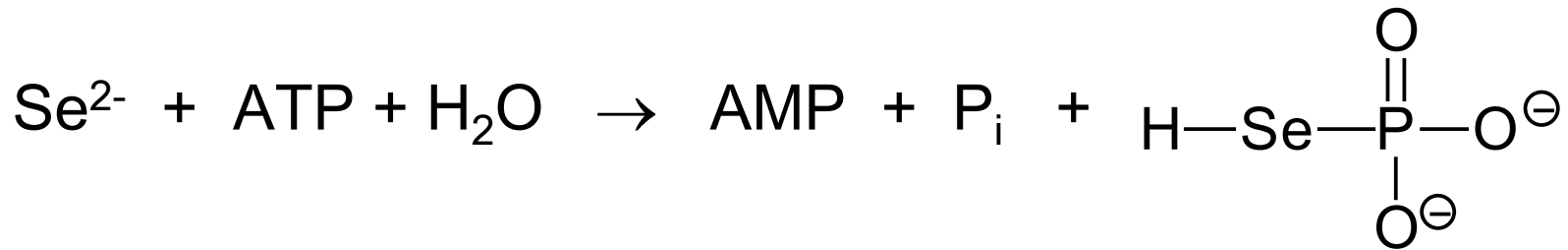




# Selenocysteine arises co-translationally from serine and selenophosphate



Selenophosphate is made from selenide (food) and ATP



few enzymes (redox reactions) contain selenocysteine

**Glutathione peroxidase** ( $2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{G-S-S-G}$ )

**Deiodase of thyronines** (thyroxine T4  $\rightarrow$  triiodothyronine T3)

**Thioredoxin reductase** (ribose  $\rightarrow$  deoxyribose)

# Synthesis of non-essential amino acids

AA	Precursor and reactions
Ala	pyruvate (transamination, ALT)
Glu	glutamine (deamidation), 2-OG (reductive amination), proline (catabolism), histidine (catabolism), ornithine (oxidative deamination)
Gln	glutamate (amidation – synthesis of amide group from ammonia)
Asp	oxaloacetate (transamination, AST), asparagine (demidation)
Asn	aspartate (amidation from ammonia)
Ser	3-P-glycerate (dehydrogenation, transamination, hydrolysis), glycine (transfer of C <sub>1</sub> group)
Gly	serine (transfer of C <sub>1</sub> group), glyoxalate (transamination)
Cys	methionine (demethylation to homocysteine, condensation with serine, cleavage)
Tyr	phenylalanine (hydroxylation)
Pro	glutamate (the reverse of proline catabolism)
Arg	glutamate (reductive amination to ornithine, urea cycle reactions)