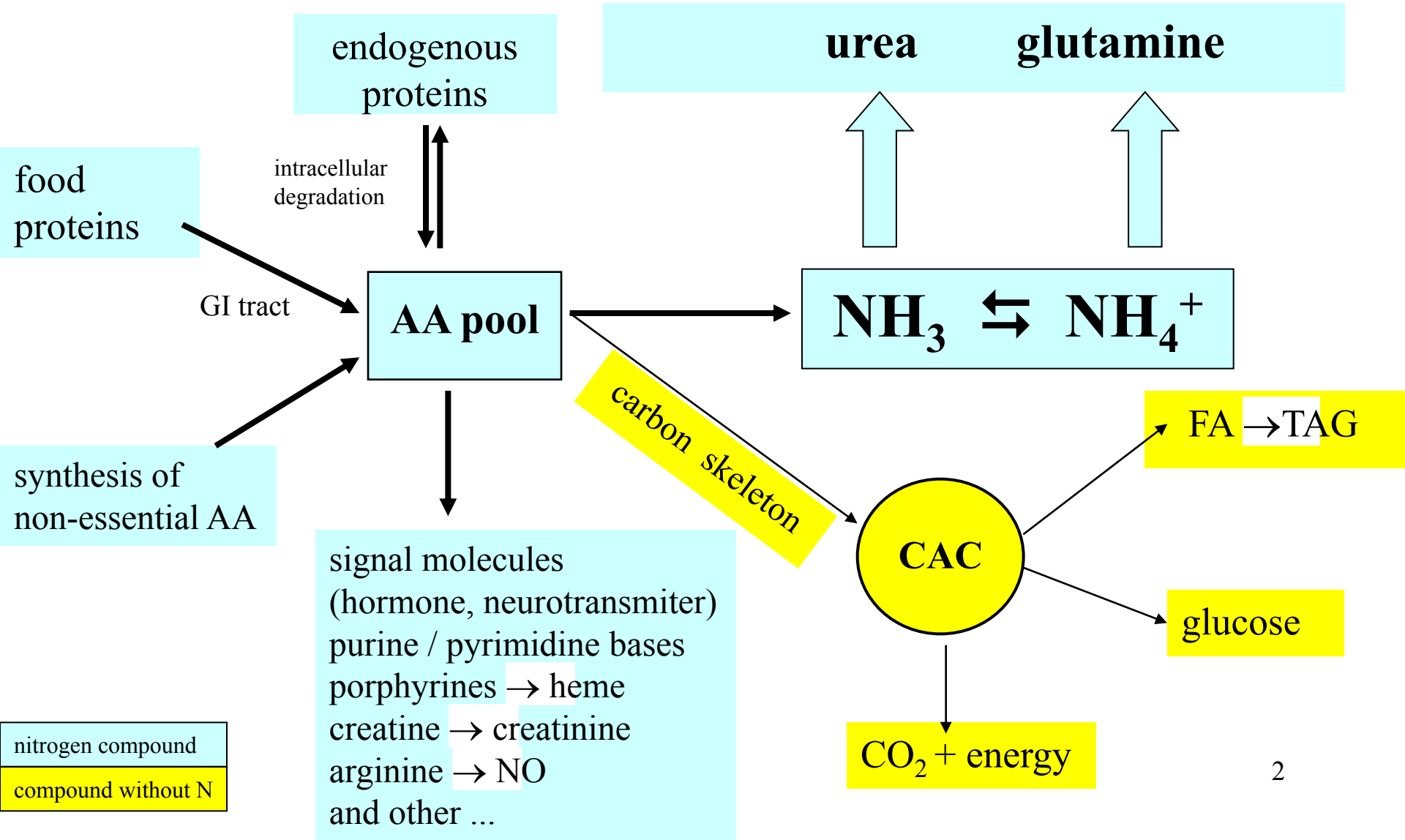
Catabolism of amino acids

Ammonia detoxification

Biosynthesis of non-essential amino acids

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.)

Endogenous proteins

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

more details in BCH II and physiology

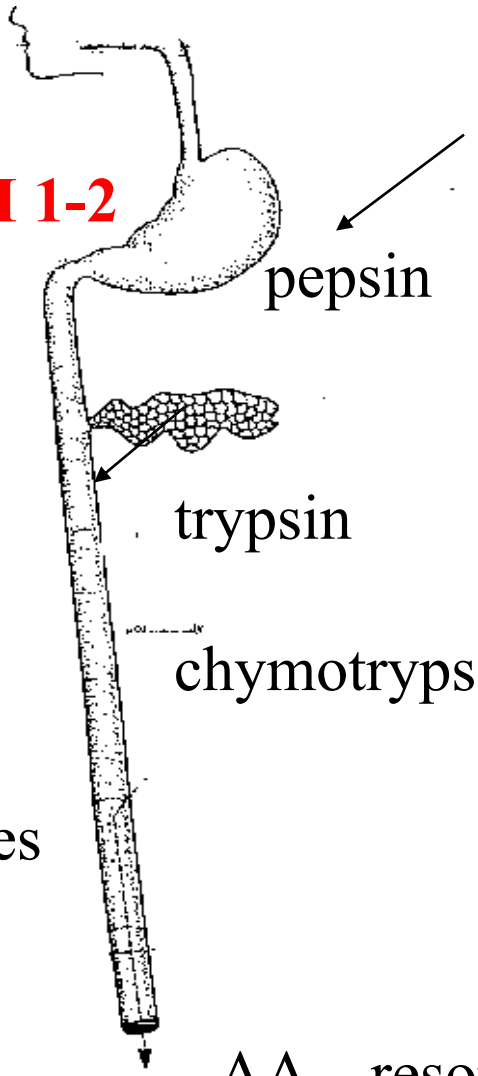
Digestion of dietary proteins

proteases are secreted

as **inactive proenzymes**

activated by the cleavage

of certain peptide sequence



pepsinogen
activated by HCl

pH 1-2

pepsin

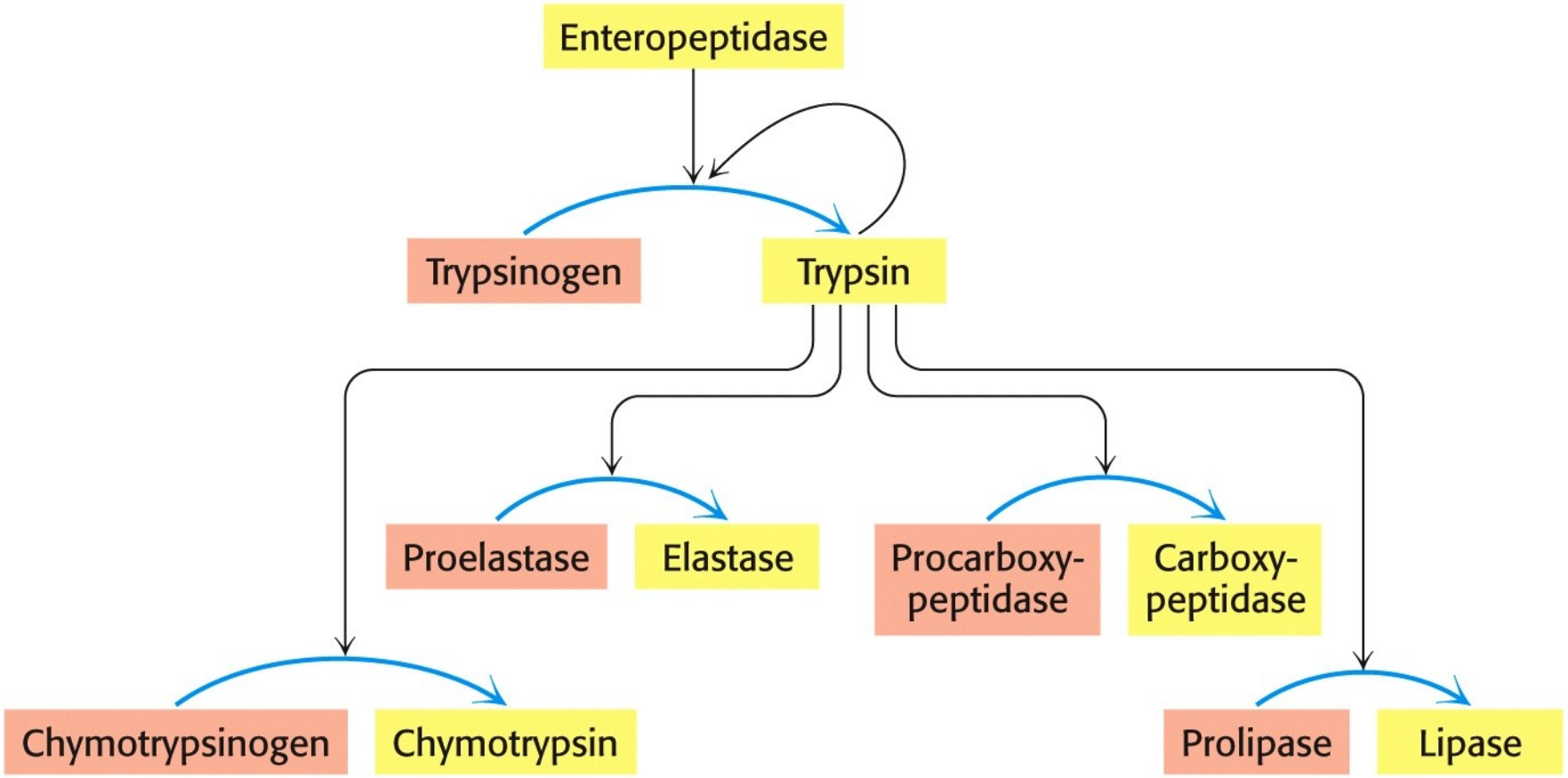
trypsin

chymotrypsin, elastase

carboxypeptidases
aminopeptidases

AA – resorption
into portal blood

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

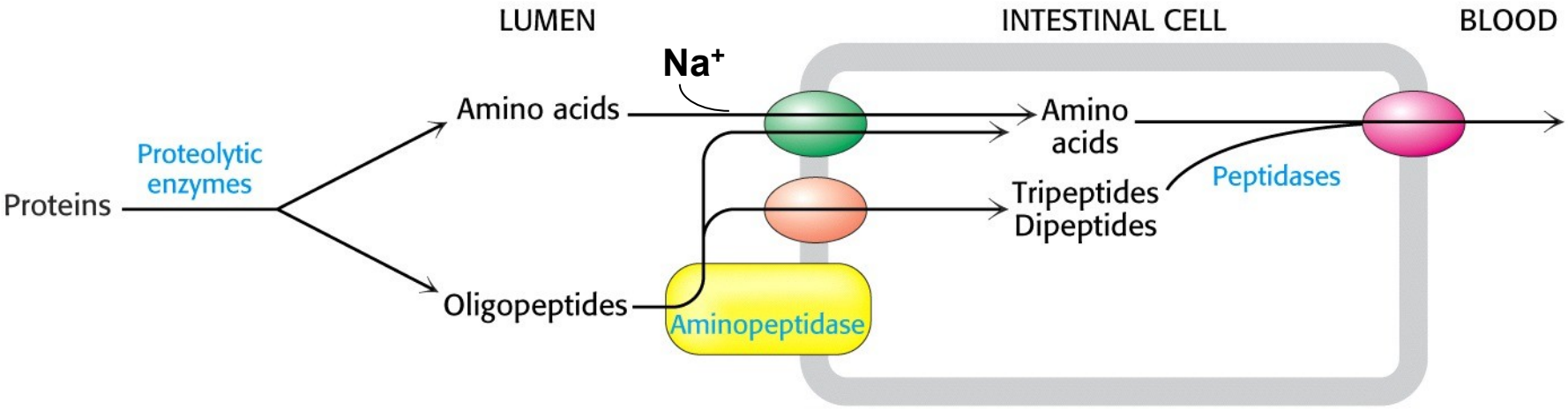
Proteinases preferentially attacks the bond after:

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:

Carboxypeptidase A	nearly all AA (not Arg and Lys)
Carboxypeptidase B	basic AA (Arg, Lys)
aminopeptidase	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 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 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
Cathepsin	**peptide**
Collagenase	**peptide**
Elastase	**peptide**
Ribonuclease	phosphodiester
Lipase	ester
Phosphatase	phosphoester
Ceramidase	amide

Ubiquitin (Ub) targets proteins for proteasome degradation

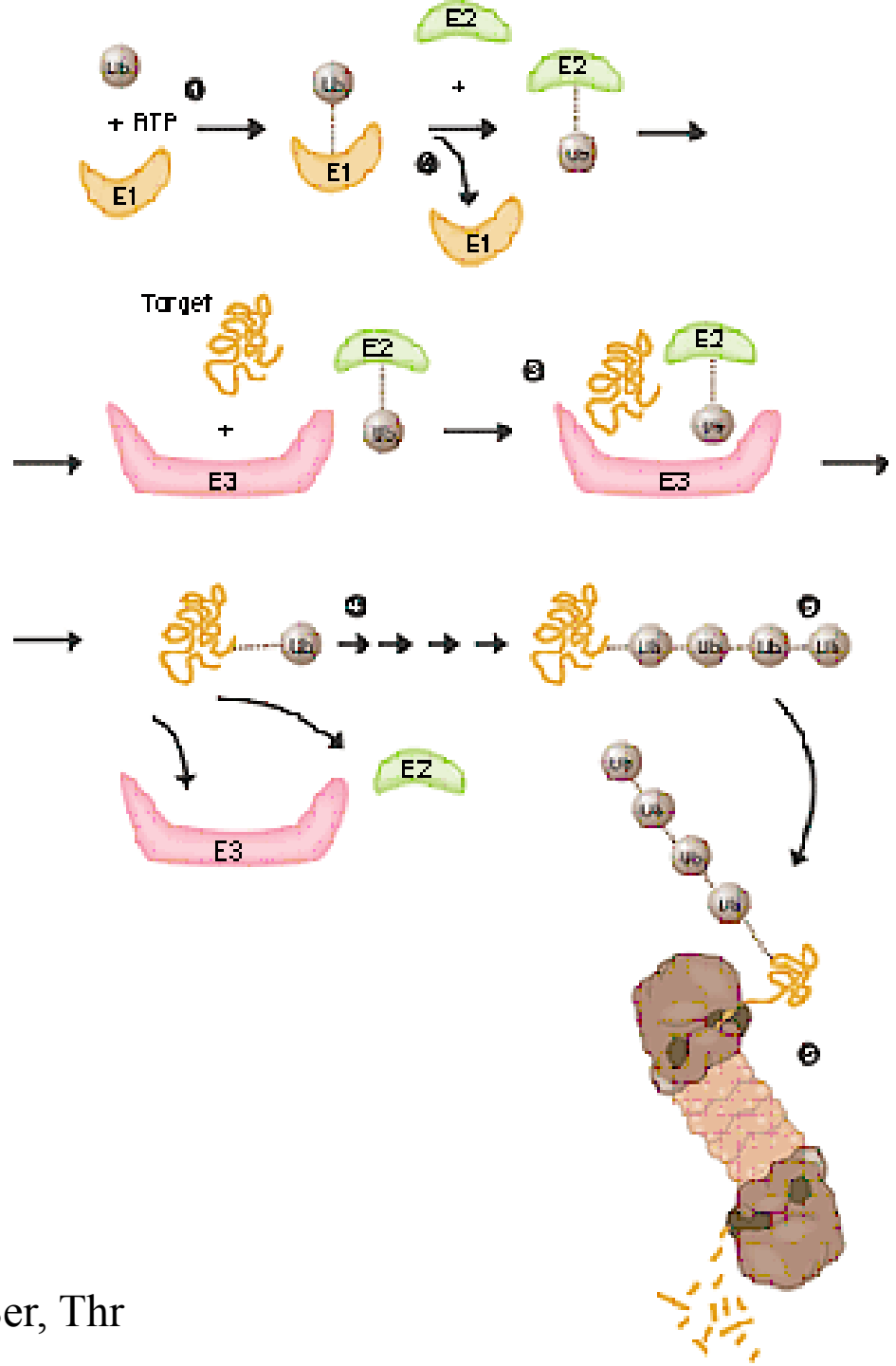
- small protein, in all cells - ubiquitous
- C-terminus binds Lys of proteins to be degraded (*kiss of death*)
- binding Ub to protein has three phases, with three enzymes E_1,E_2,E_3
- binding Ub to E_1-SH requires **ATP**
- more Ub molecules are attached - **polyubiquitination**
- Ub-tagged protein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

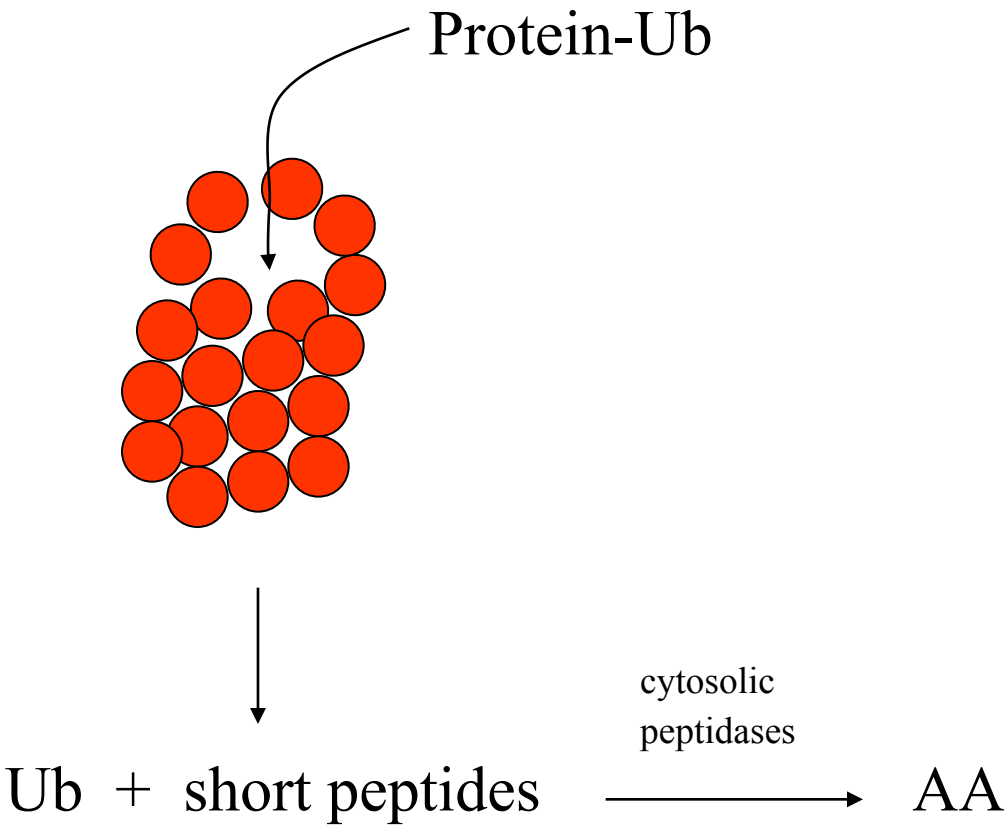
- Phe, Leu, Asp, Lys, Arg
- PEST proteins: segments rich in Pro, Glu, Ser, Thr

Proteasome

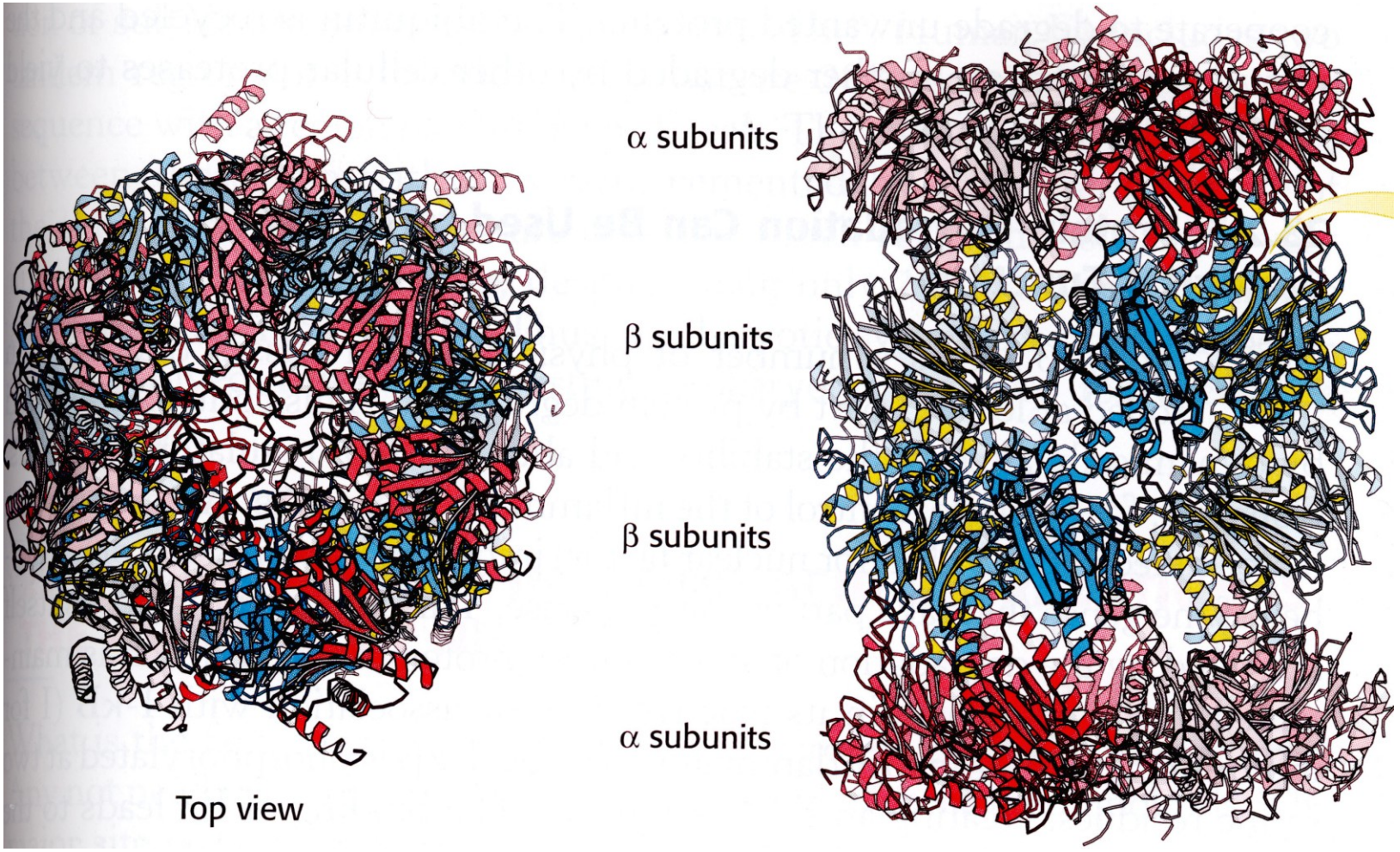
- hollow cylindric supramolecule, 28 polypeptides
- four cyclic heptamers ($4 \times 7 = 28$)
- the caps on the ends regulate the entry of proteins into destruction chamber, upon ATP hydrolysis
- inside the barrel, differently specific proteases hydrolyze target protein into short (8 AA) peptides
- ubiquitin 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



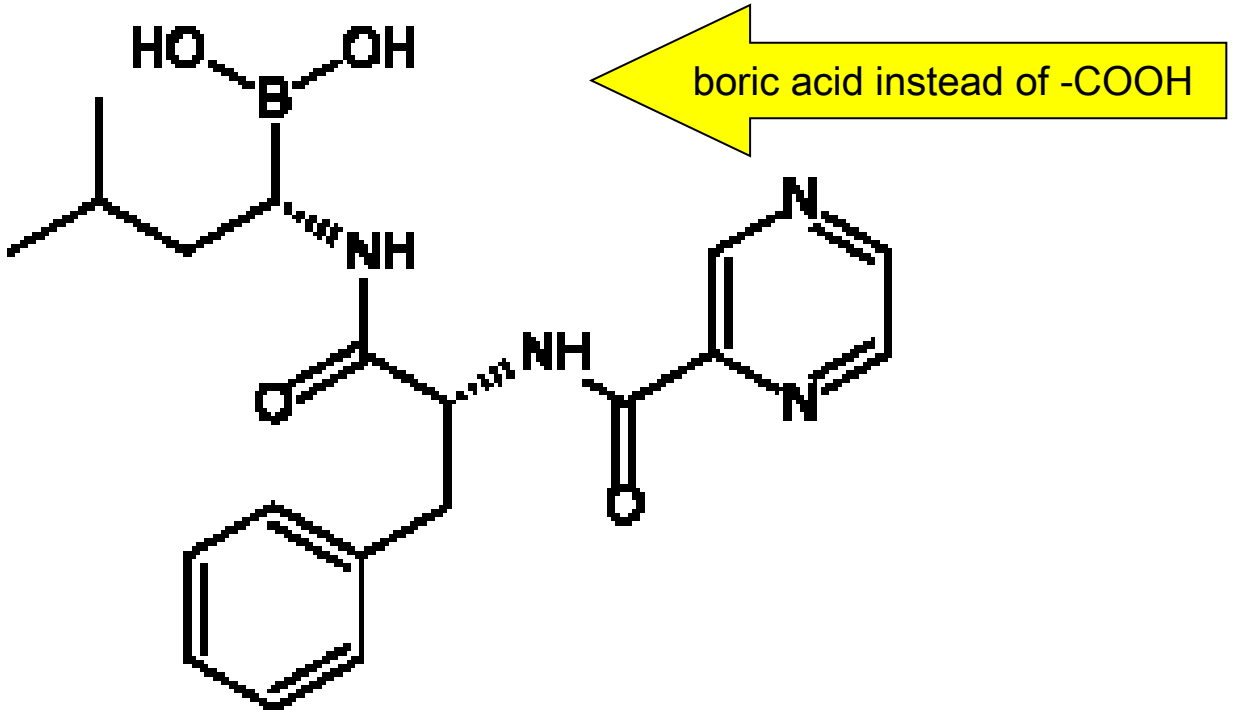
Spatial model of proteasome



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 (**c**ysteinyl **asp**artate-specific proteina**se**)
- 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 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

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

wheat – deficit in Lys, Trp, Thr, Met

legumes – deficit in Met, Cys

Daily intake of proteins: 0.8 g/kg

PDCAAS $= \frac{\text{amount of limiting AA in test protein}}{\text{amount of the same AA in reference protein}} \times$ true digestibility (%)

- **p**rotein **d**igestibility-**c**orrected **a**mino **a**cid **s**core
- 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_3)

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 chesse (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 %	**ro**stlinné **bí**lkoviny, 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 *Rhizopus oligosporus*

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 ($\rightarrow$ putrefaction in large intestine, correlates with some types of cancer)
- liver ($\rightarrow$ urea synthesis), kidneys (excretion of urea, NH_4^+, free AA)
- may be adulterated with anabolic steroids !!!

Catabolic pathway of amino acids

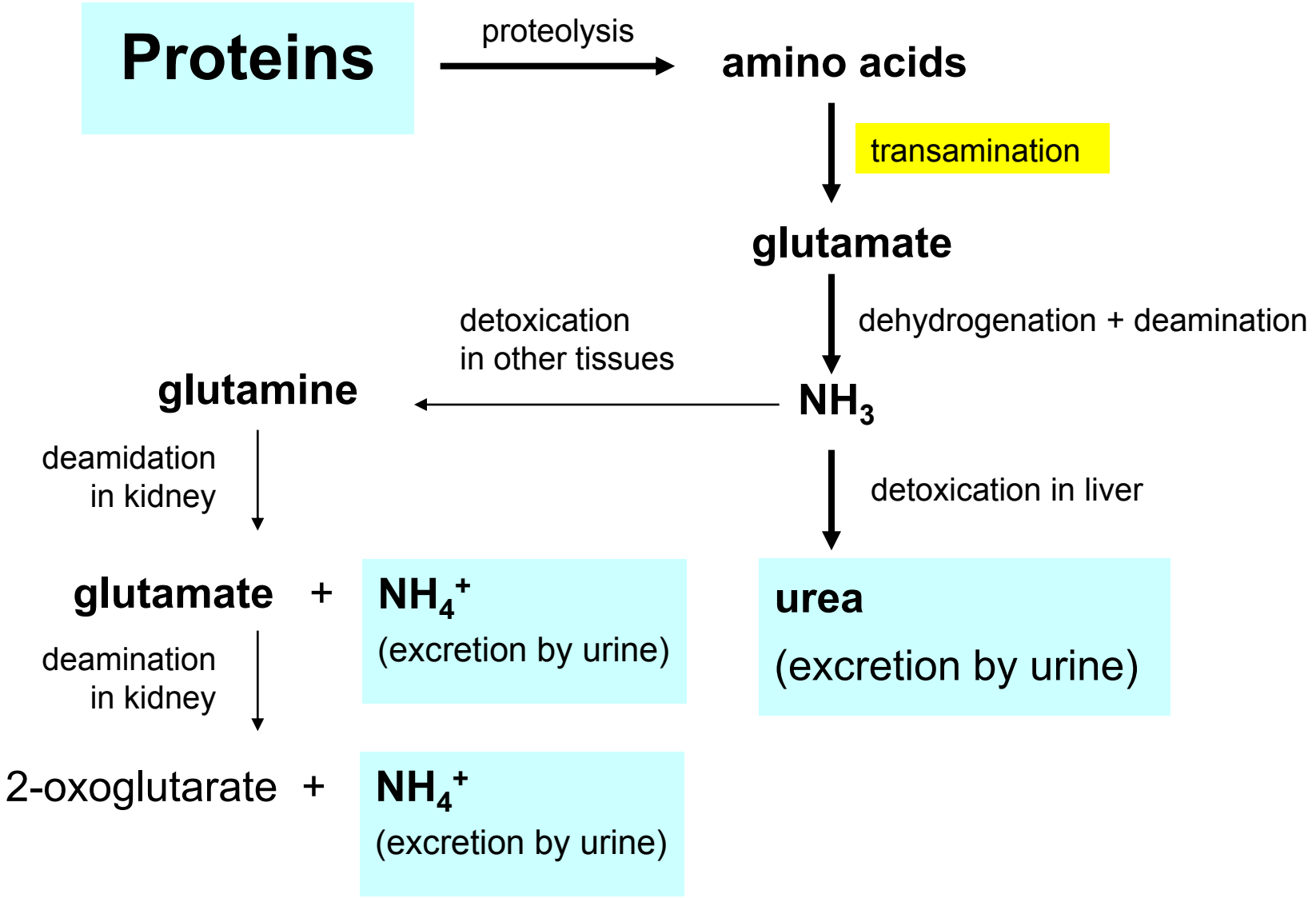
Transamination

Dehydrogenation + deamination of glutamate

Detoxication of ammonia

Excretion of nitrogen catabolites

Intake, catabolism, and excretion of nitrogen

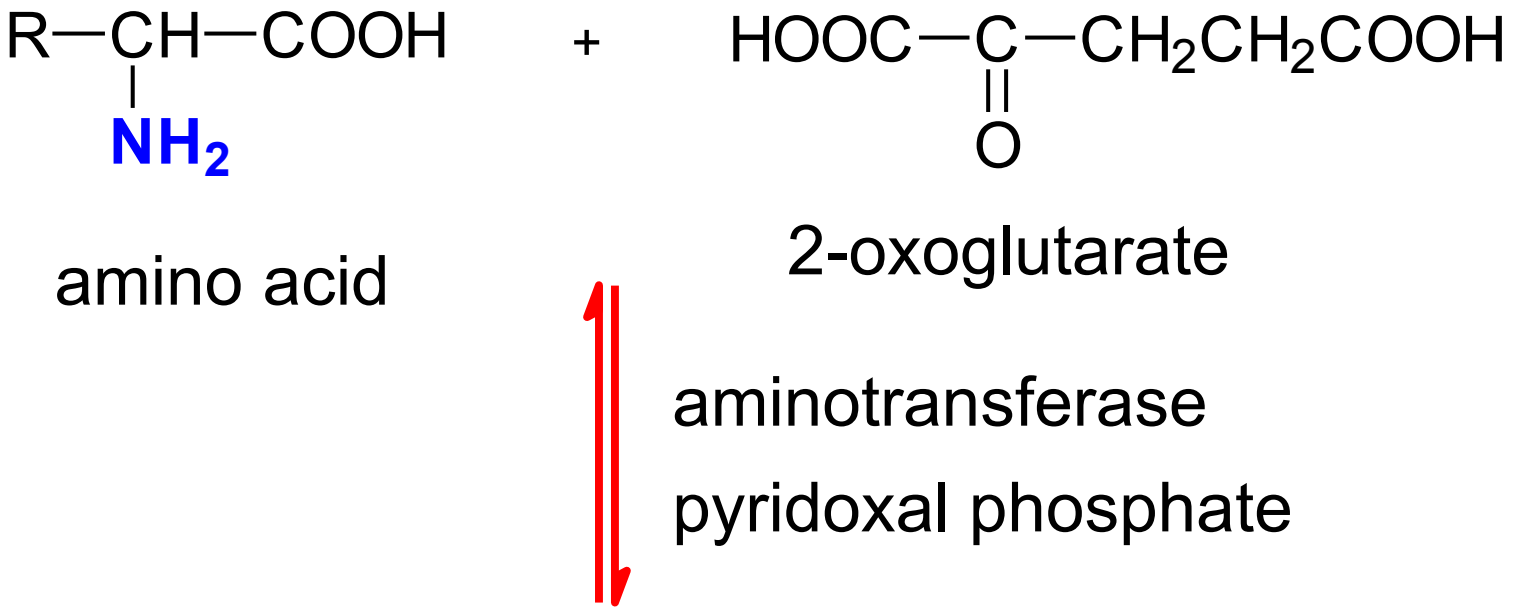


Transamination

transfer of -NH_2 group from one substrate to other

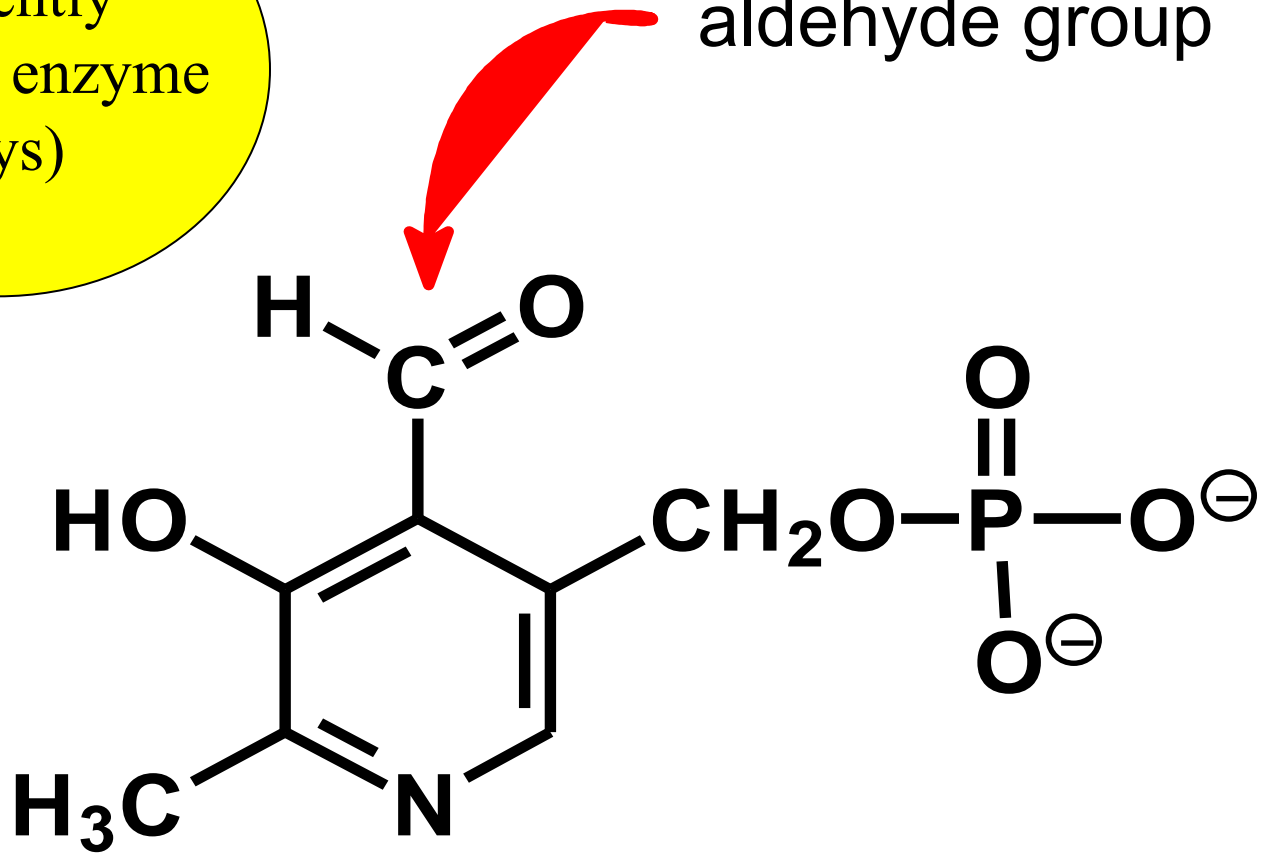
- most AA (not Lys, Thr, Pro, His, Trp, Arg, Met)
- amino group is transferred from AA to 2-oxoglutarate
- cofactor – **pyridoxal phosphate** ($\rightarrow$ Schiff bases)
- **reversible reaction** $\Rightarrow$ important for synthesis of AA

General scheme of transamination



Pyridoxal phosphate has a reactive aldehyde group

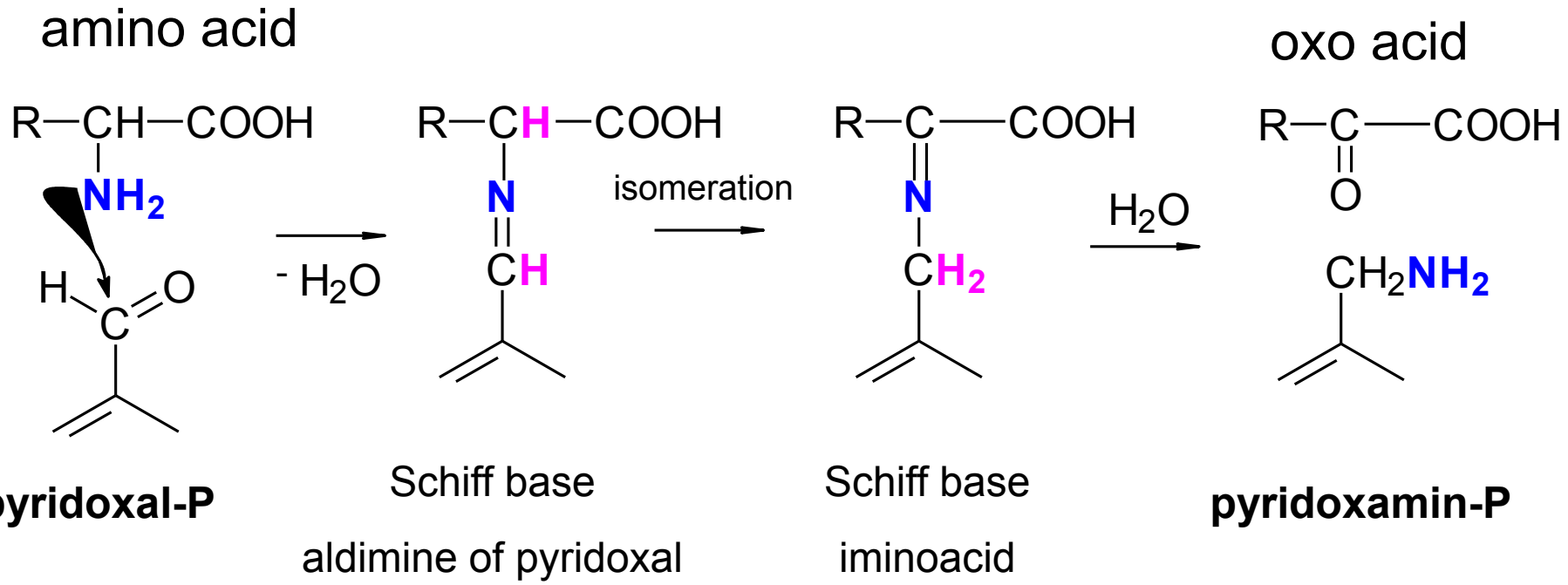
covalently linked to enzyme (Lys)



1. Phase of transamination

AA → oxoacid

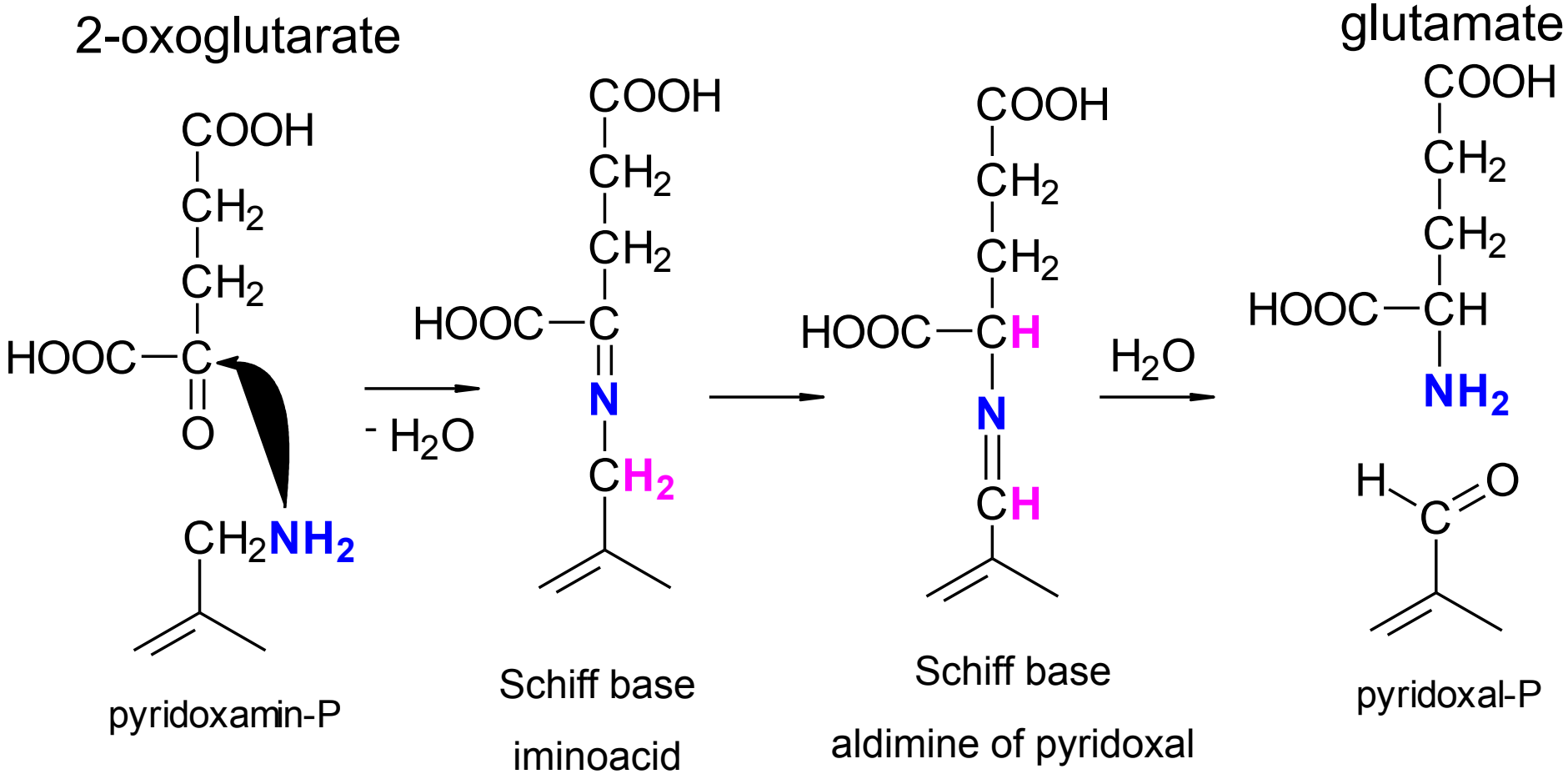
pyridoxal-P → pyridoxamine-P



2. Phase of transamination

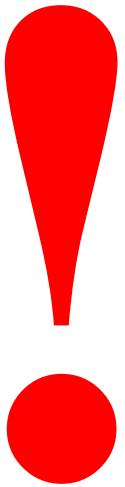
2-oxoglutarate → glutamate

pyridoxamine-P → pyridoxal-P

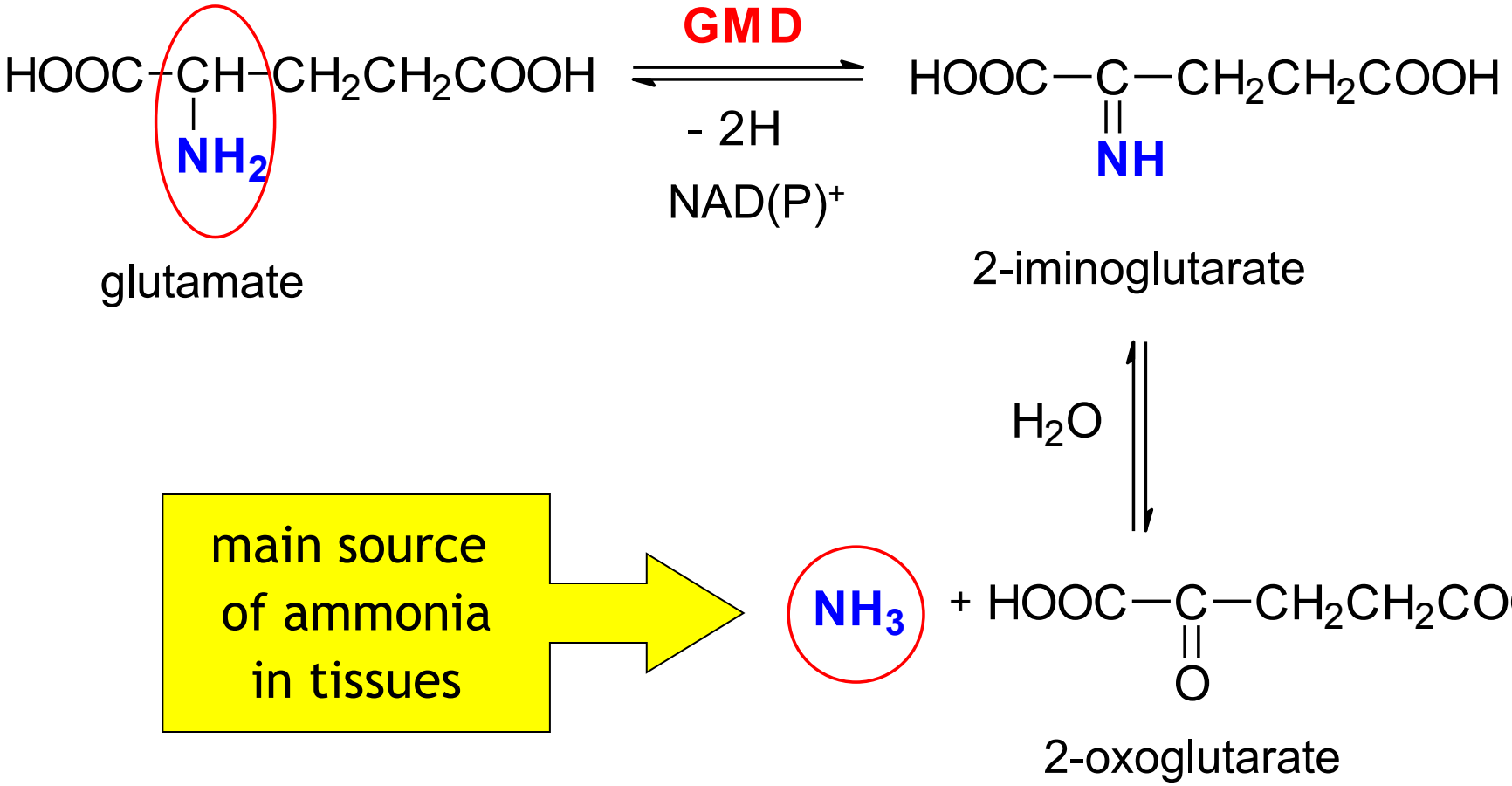


In transaminations, nitrogen of most AA is concentrated in glutamate

Glutamate then undergoes dehydrogenation + deamination and releases free ammonia NH_3



Dehydrogenation + deamination of glutamate is reversible reaction



Glutamate dehydrogenase (GMD, GD, GDH)

- requires pyridine cofactor $NAD(P)^+$
- GMD reaction is reversible: dehydrogenation with NAD^+, hydrogenation with $NADPH+H^+$
- two steps:
- **dehydrogenation** of $CH\text{-}NH_2$ to imino group 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 NH_4^+ $\Rightarrow$ eliminated by liver

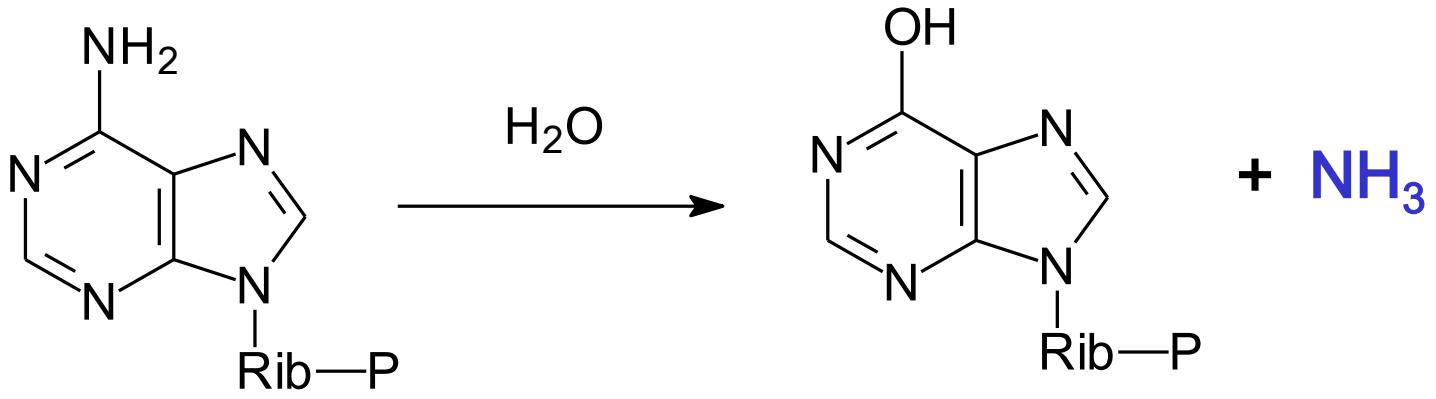
Other sources of ammonia: deaminations of various substrates

- deamination of adenine
- oxidative deamination of some AA ($\rightarrow H_2O_2$)
- desaturation deamination of histidine $\rightarrow$ urocanic acid + **NH_3**
- oxidative deamination of terminal $-NH_2$ in lysine

  lysyl oxidase(Cu^{2+}): Lys + O_2 $\rightarrow$ **NH_3** + allysine + H_2O
- dehydratation deamination of serine (see next lecture)
- oxidative deamination of biogenous amines, MAO monoamine oxidase

  ($\rightarrow H_2O_2$, see also Med. Chem. II, p. 60)

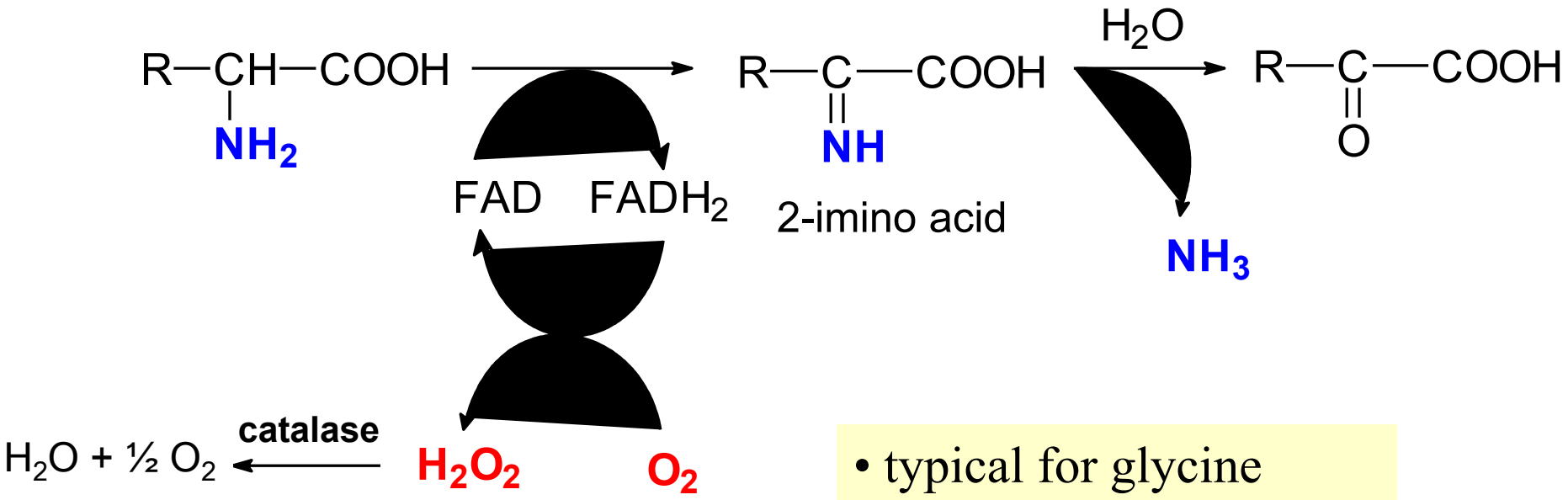
Deamination of adenine



adenosine monophosphate (AMP)

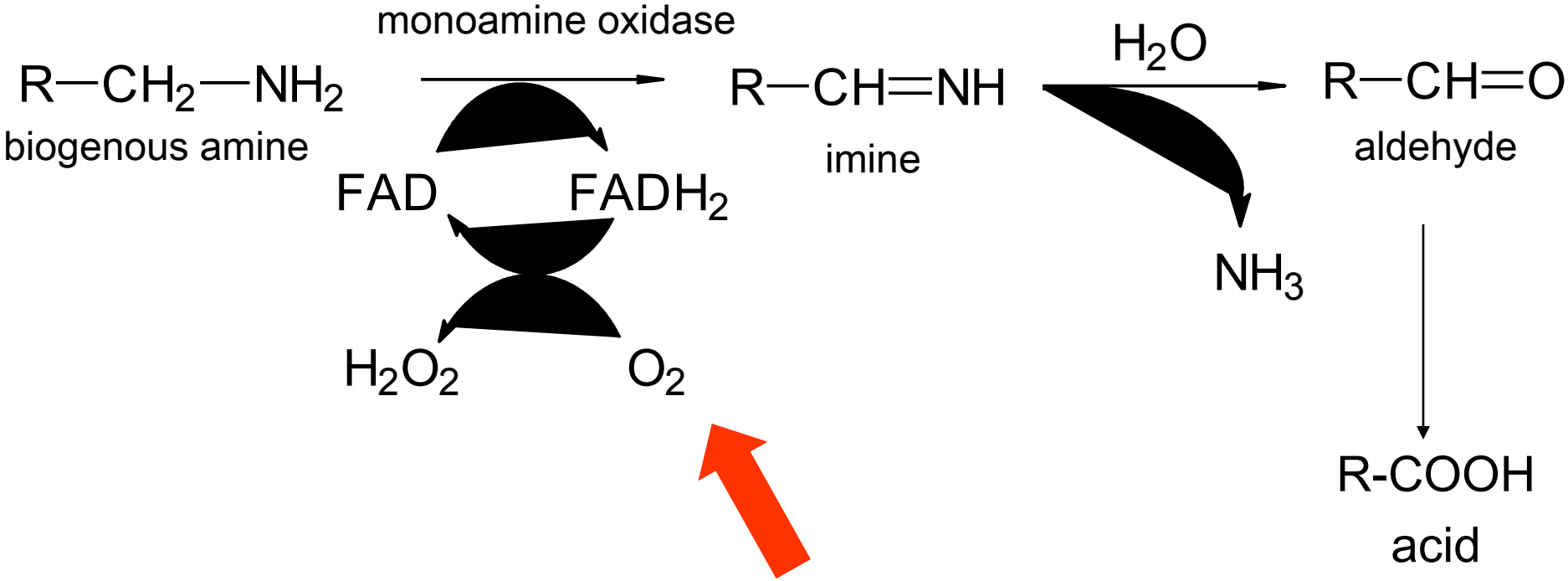
inosine monophosphate (IMP)

Oxidative deamination of some AA uses flavine cofactor and dioxygen

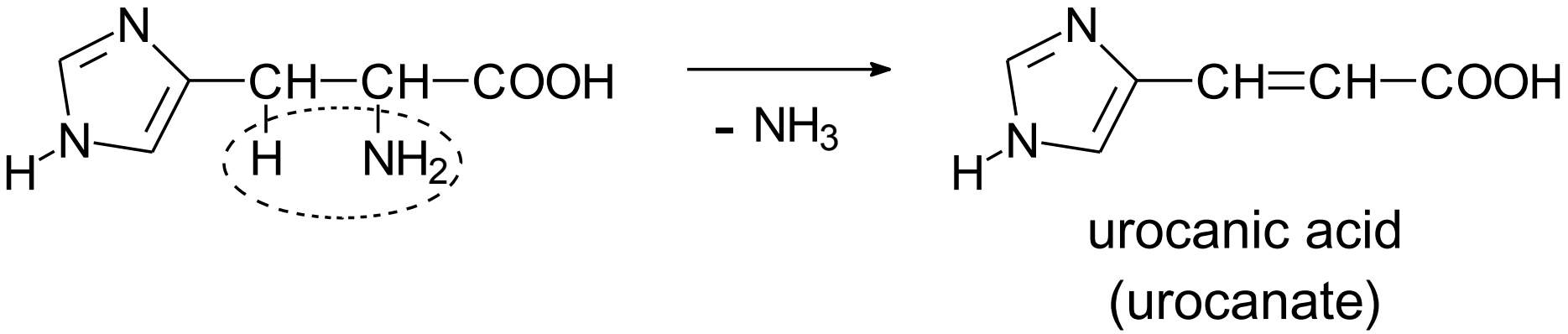


- typical for glycine
- D-amino acids
- side product is H_2O_2

Oxidative deamination of biogenous amines



Desaturation type of deamination in histidine



urocanic acid
(urocanate)

Other reactions producing ammonia

- **non-enzymatic carbamylation of proteins**

  Prot-NH_2 + NH_2-CO-NH_2 → **NH_3** + Prot-NH-CO-NH_2

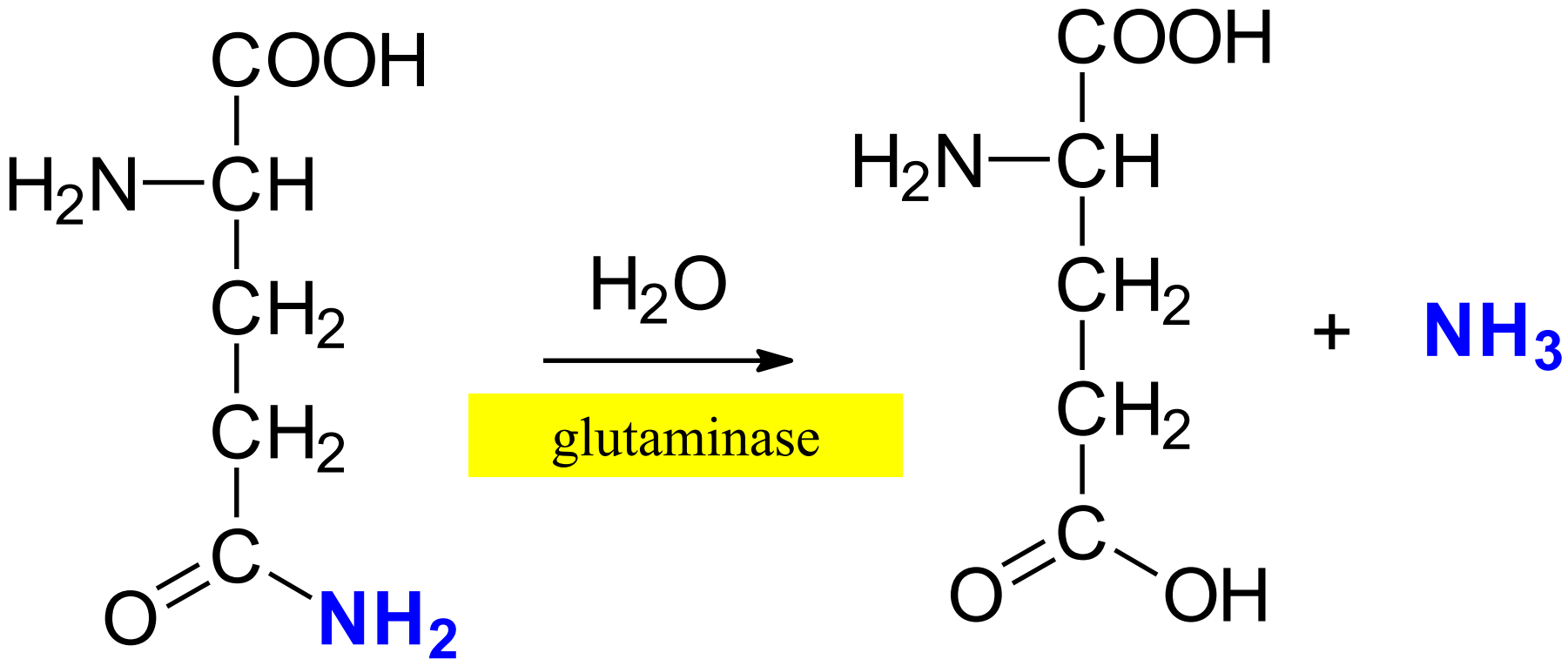
- **catabolism of pyrimidine bases**

  cytosine/uracil → **NH_3** + CO_2 + β-alanine

  thymine → **NH_3** + CO_2 + β-aminoisobutyrate

- **synthesis of heme** (4 porphobilinogen → **4 NH_3** + uroporphyrinogen)

Hydrolysis of amide group in glutamine releases ammonia (deamidation). In kidneys, NH_4^+ ions are released into urine.



glutamine

glutamate

Glutamine is non-toxic transport form of ammonia

Ammonia production under pathological conditions

- **bleeding in GIT** $\Rightarrow$ increased NH_3 in portal blood
- **uroinfections** – bacterial urease catalyzes the hydrolysis of urea

$$H_2N\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2\,\mathbf{NH_3} + CO_2$$

$$NH_3 + H_2O \leftrightarrows NH_4^+ + \mathbf{OH^-}$$

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

Acide-base properties of NH_3

$pK_B\,(NH_3) = 4.75$ (weak base)

$NH_3 + H_2O \leftrightarrows NH_4^+ + OH^-$

$pK_A\,(NH_4^+) = 14 - 4.75 = 9.25$ (very weak acid)

under physiological pH values in ICF and ECF (~ 7.40):

98 % NH_4^+

2 % NH_3

Compare: Ammonium ions in body fluids

Body fluid	Concentration of NH_4^+ ions	Metabolic origin of 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 μmol/l	protein putrefaction (GIT), Gln/Glu catabolism (enterocyte)
Venous blood	5 – 30 μmol/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_2CO_3 diamide	γ-amide of Glu	α-amino acid
Reaction(s)	urea cycle	Glu + NH_3	hydrog. amin. 2-OG
Enzyme	5 enzymes	Gln-synthetase	GMD
Energy needs	**4 ATP**	1 ATP	1 $NADPH+H^+$
Organelle(s)	mitoch. + cytosol	cytosol	mitochondria
Organ(s)	only liver	liver, **brain**, other	(brain)

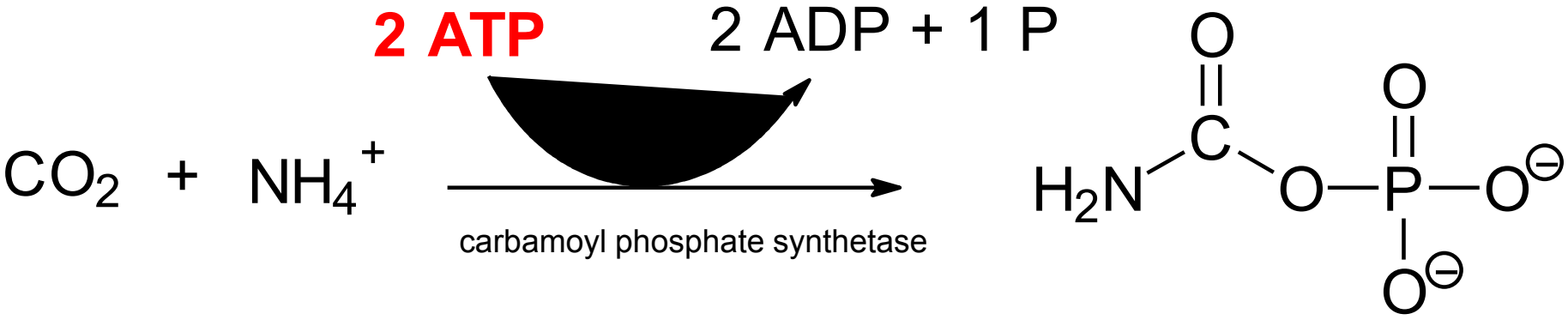
Ureasynthesis in liver

five reactions

1. and 2. in mitochondria

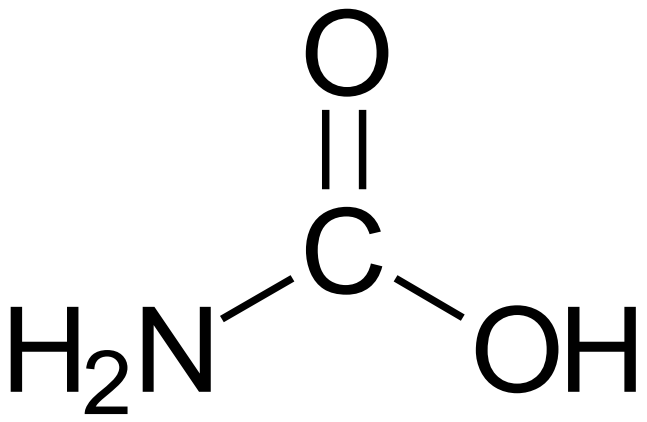
3. - 5. in cytosol

1. Carbamoyl phosphate (matrix)



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

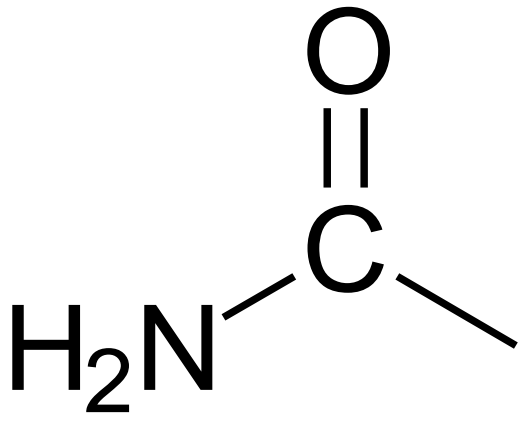
Carbamoyl is the acyl of carbamic acid



carbamic acid

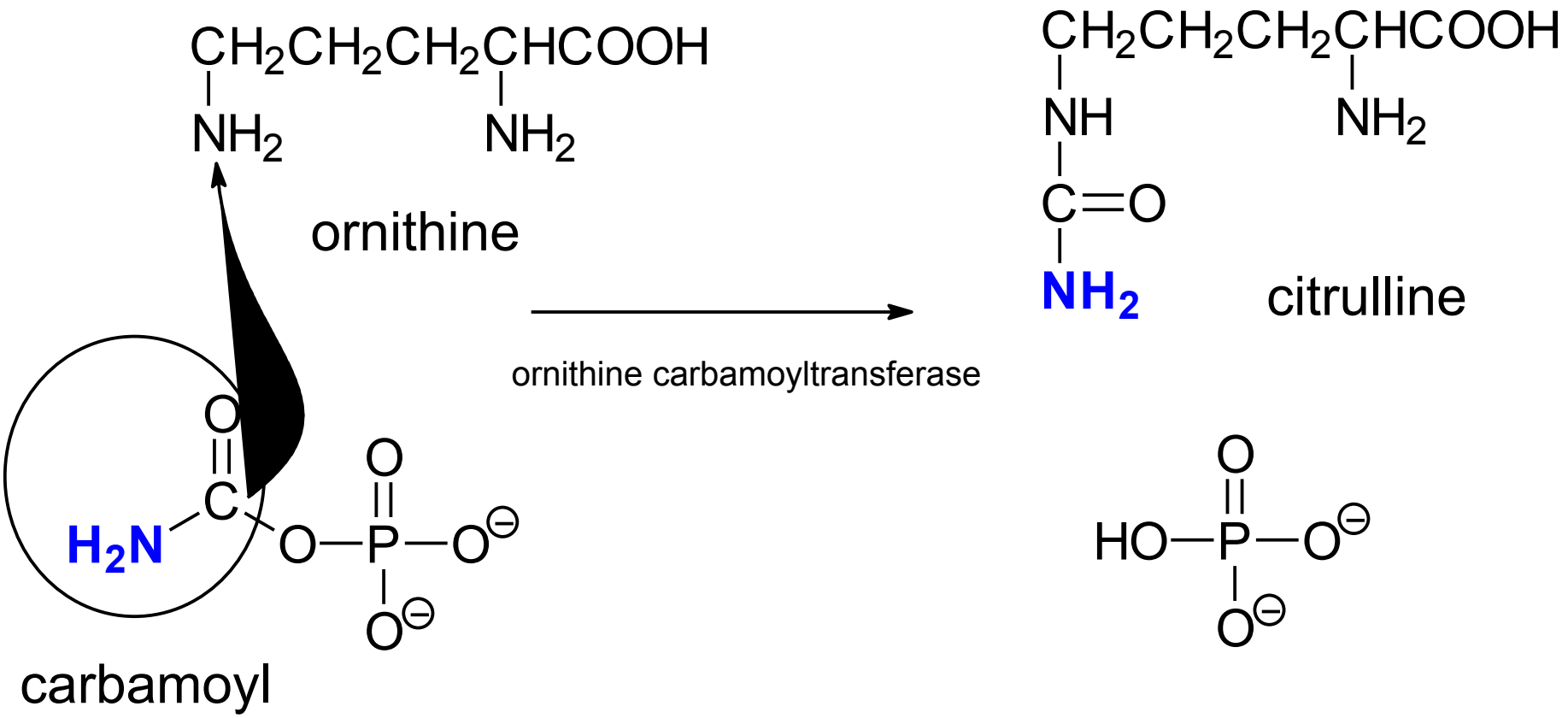
(carbonic acid monoamide)

does not exist

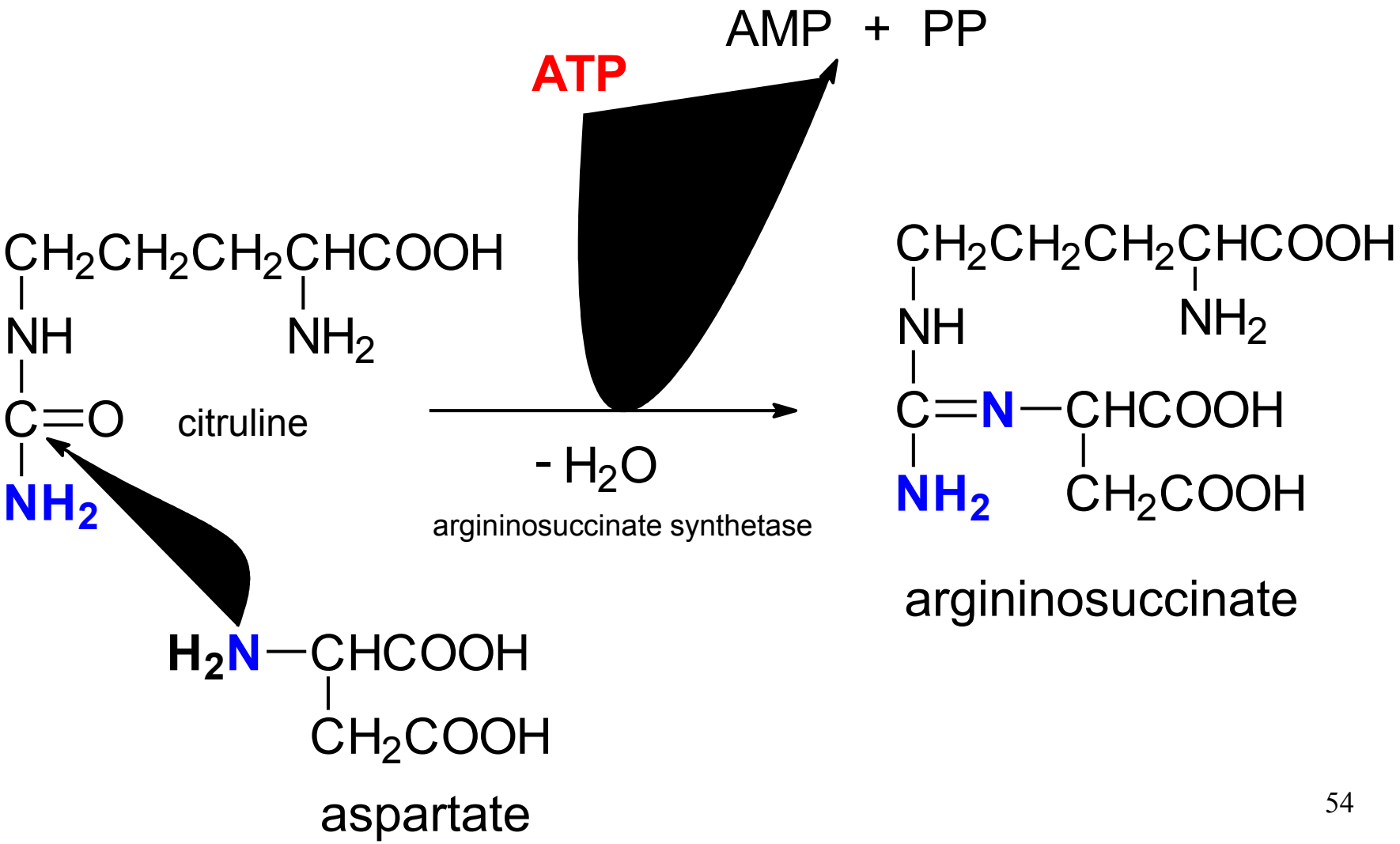


carbamoyl

2. Citrulline formation (matrix)



3. The second -NH_2 group comes from aspartate (cytosol)



Less usual hydrolysis of ATP means that two ATP are consumed

$ATP + H_2O \rightarrow AMP + PP_i$

$PP_i + H_2O \rightarrow 2\ P_i$ (diphosphatase, pyrophosphatase)

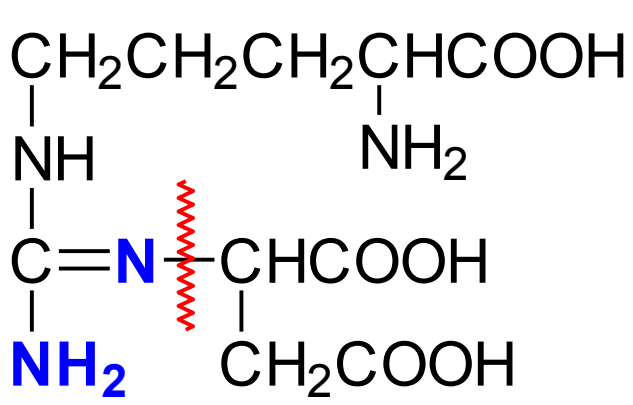
$AMP + ATP \rightarrow 2\ ADP$ (adenylate kinase)

---

summary:

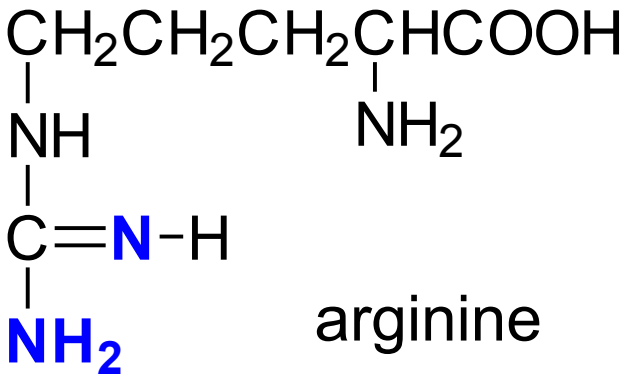
$2\ ATP + 2\ H_2O \rightarrow 2\ ADP + 2\ P_i$

4. The cleavage of argininosuccinate

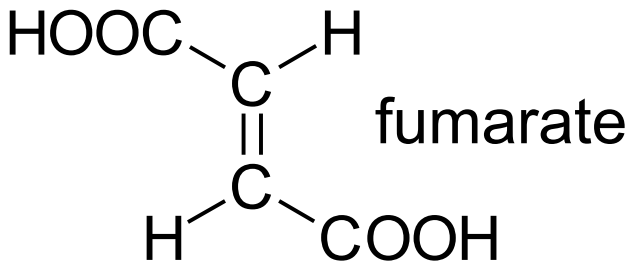


argininosuccinate

argininosuccinate lyase

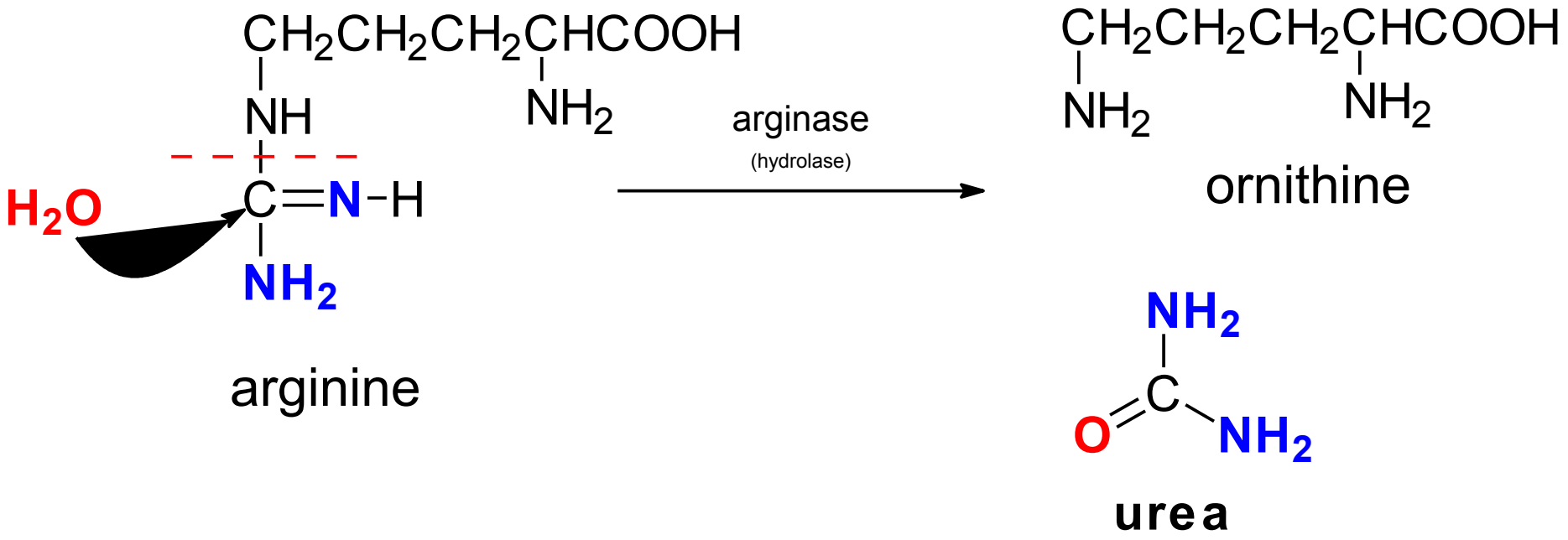


arginine

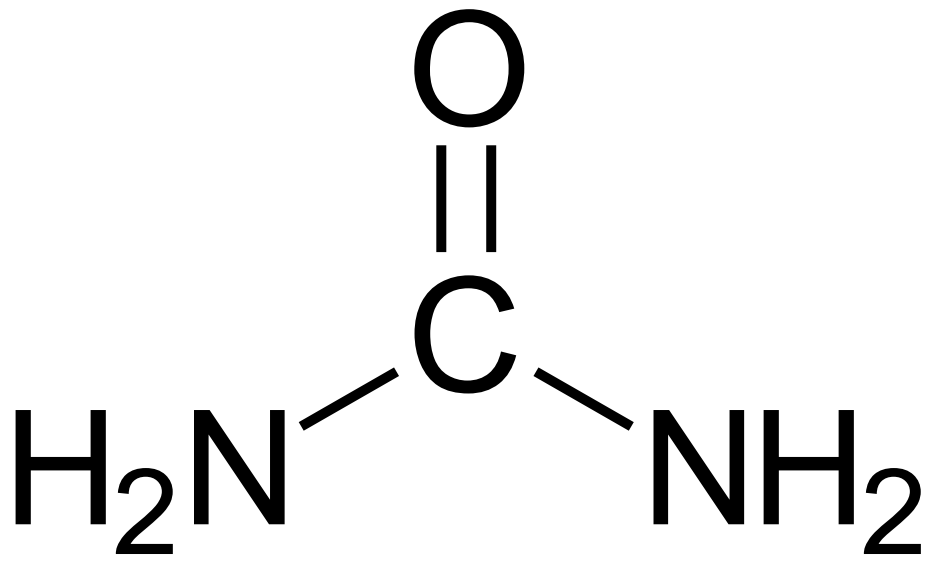


fumarate

5. Hydrolysis of arginine affords urea

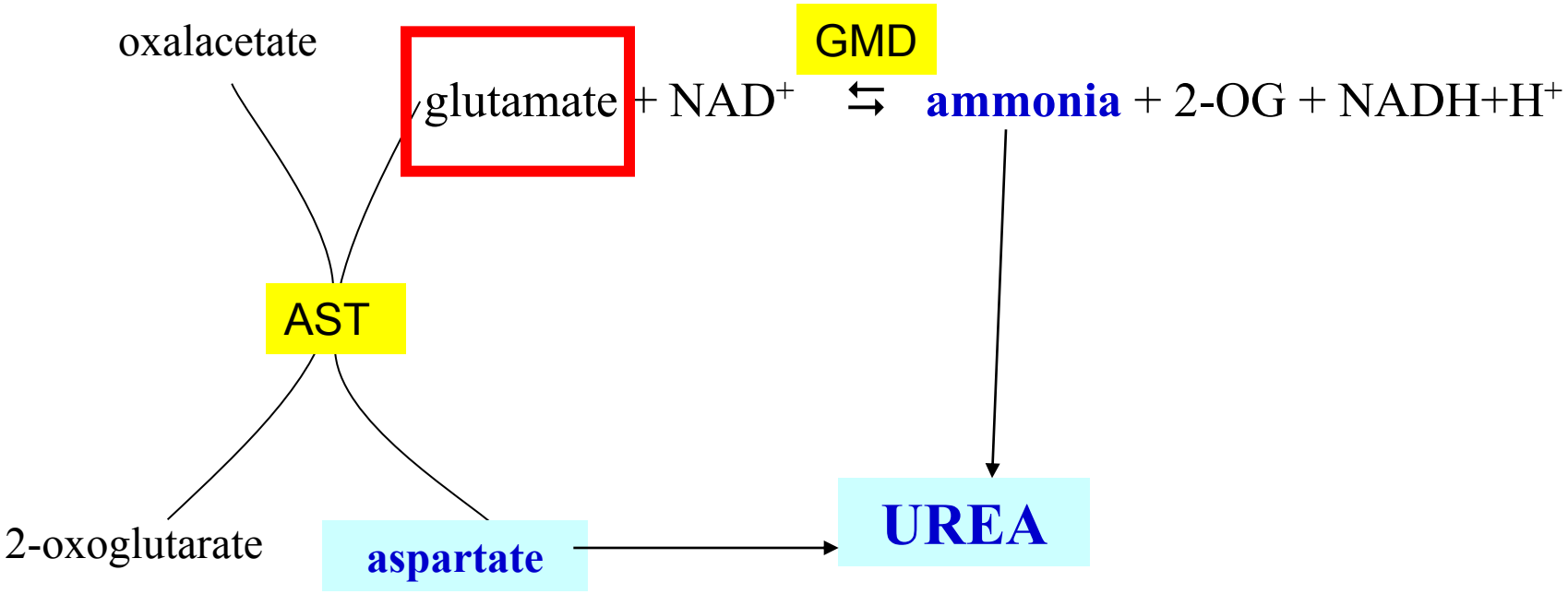


Metabolic origin of nitrogen in urea



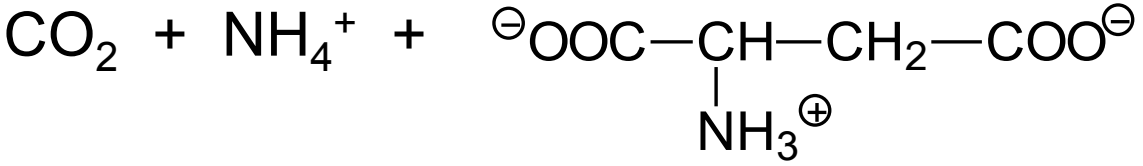
free ammonia **aspartate**

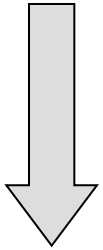
Dual function of glutamate in AA catabolism



Urea synthesis is proton-productive reaction

$$CO_2 + NH_4^+ + \text{aspartate} \rightarrow \text{urea} + \text{fumarate} + H_2O + 2\,H^+$$





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 $\approx 2\ [Na^+] + [\text{glucose}] + [\text{urea}]$ mmol/kg H_2O
- 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.)

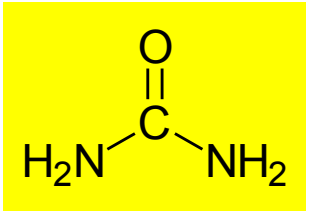
Decreased concentration

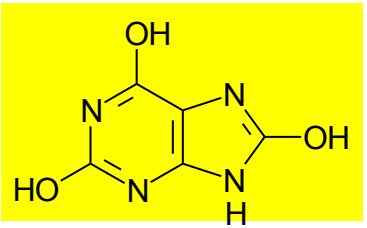
- lack of proteins in diet
- liver failure

Compare and distinguish

urea uric acid

Compare

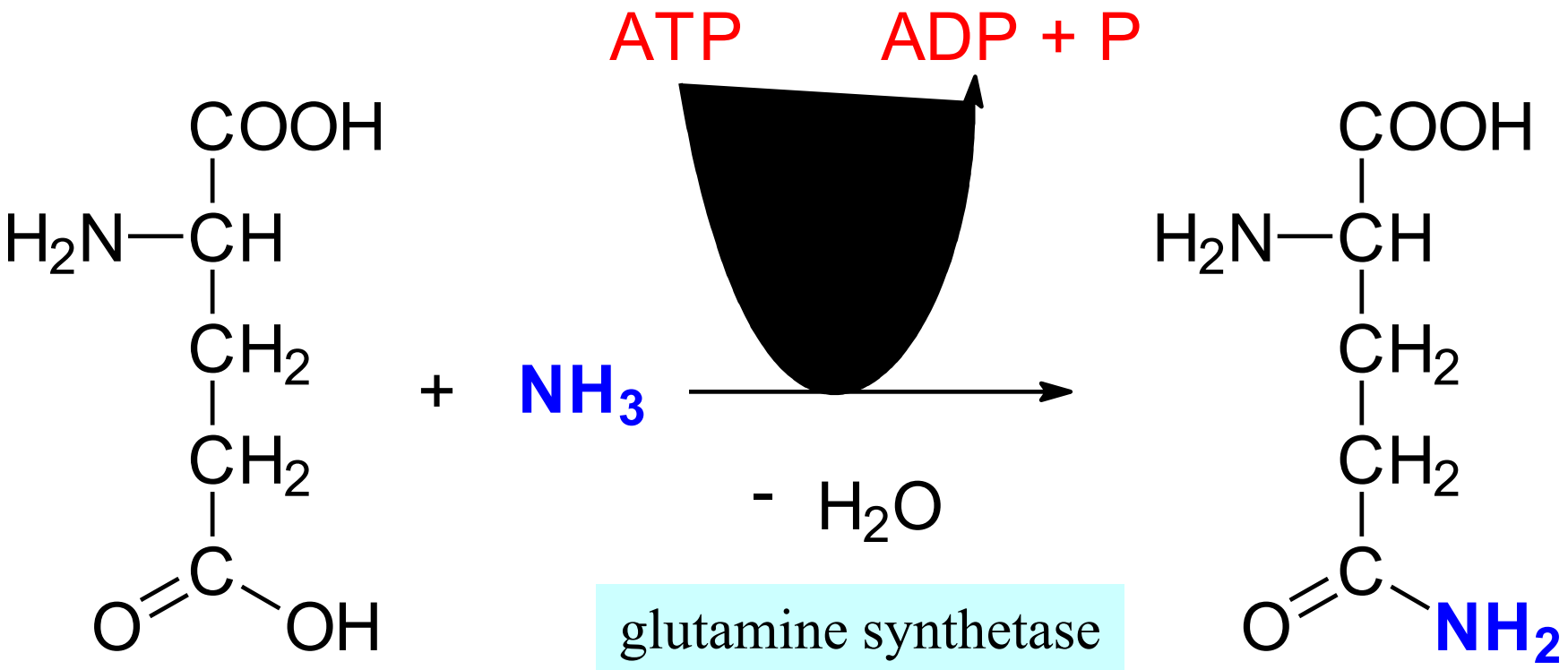




Feature	Urea	Uric acid
Chemical name	carbonic ac. diamide	2,6,8-trihydroxypurine
Latin name	urea	acidum uricum
In water	non-electrolyte	weak diprotic acid
Solubility in water	excelent	poor, depeds on pH
Reducing property	no	yes ⇒ antioxidant
Salt formation	no	yes (two types)
Catabolite of	amino acids	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

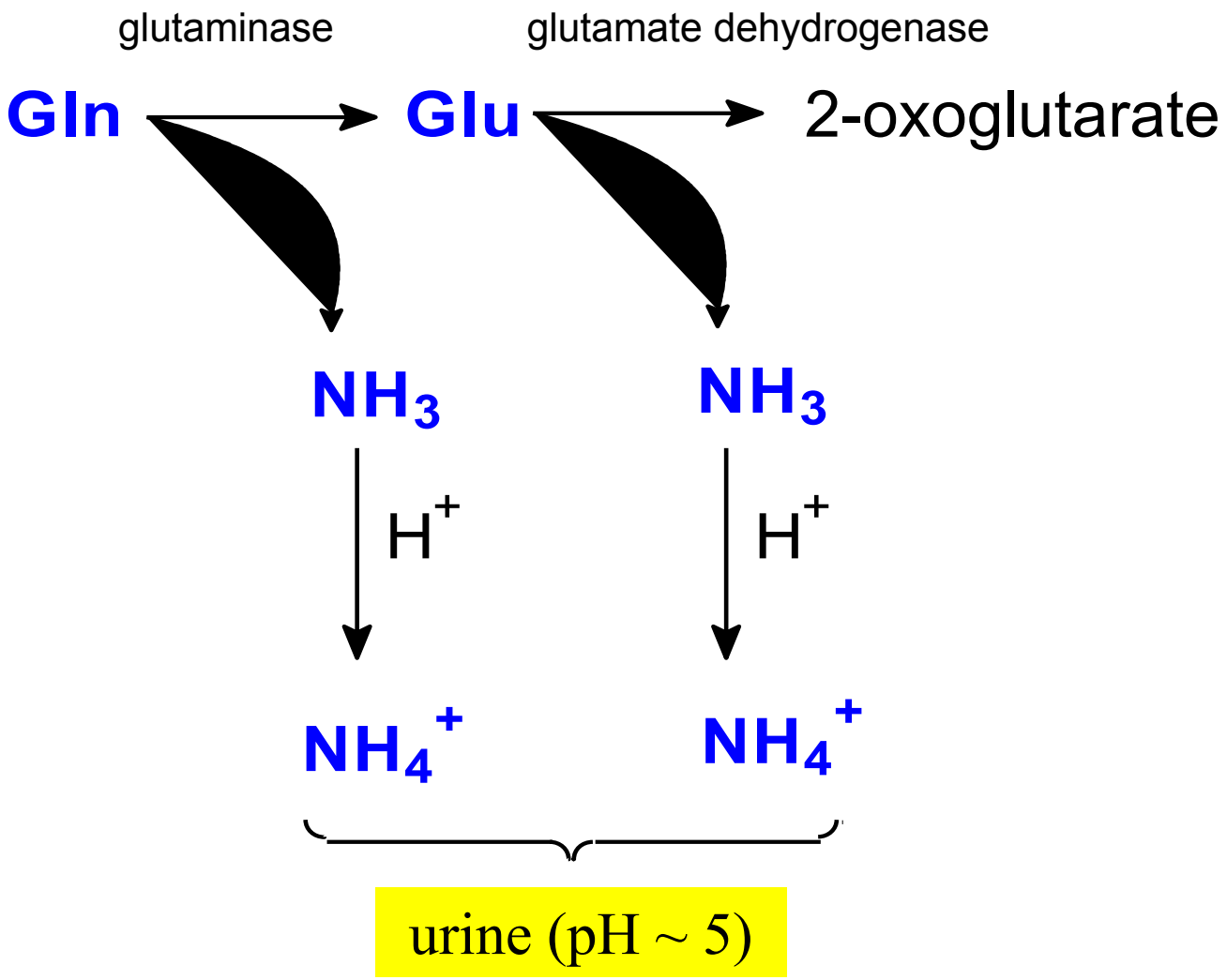
2. way of detoxification



glutamate

glutamine

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



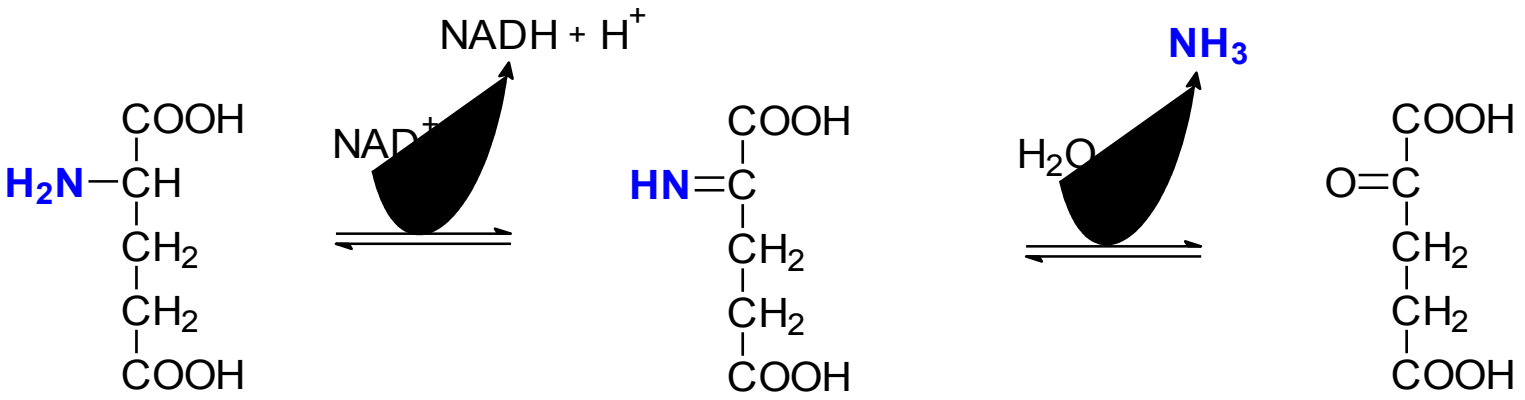
Multiple functions of glutamine

- Synthesis of proteins
- Metabolic fuel – for some tissues: enterocytes, lymphocytes, macrophages, fibroblasts, kidneys
- Source of nitrogen in synthesis – purine, pyrimidines, NAD^+, aminosugars
- Source of glutamate – glutathione (GSH), GABA,
  Glu $\rightarrow$ ornithine, Glu $\rightarrow$ 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

ammonia formation

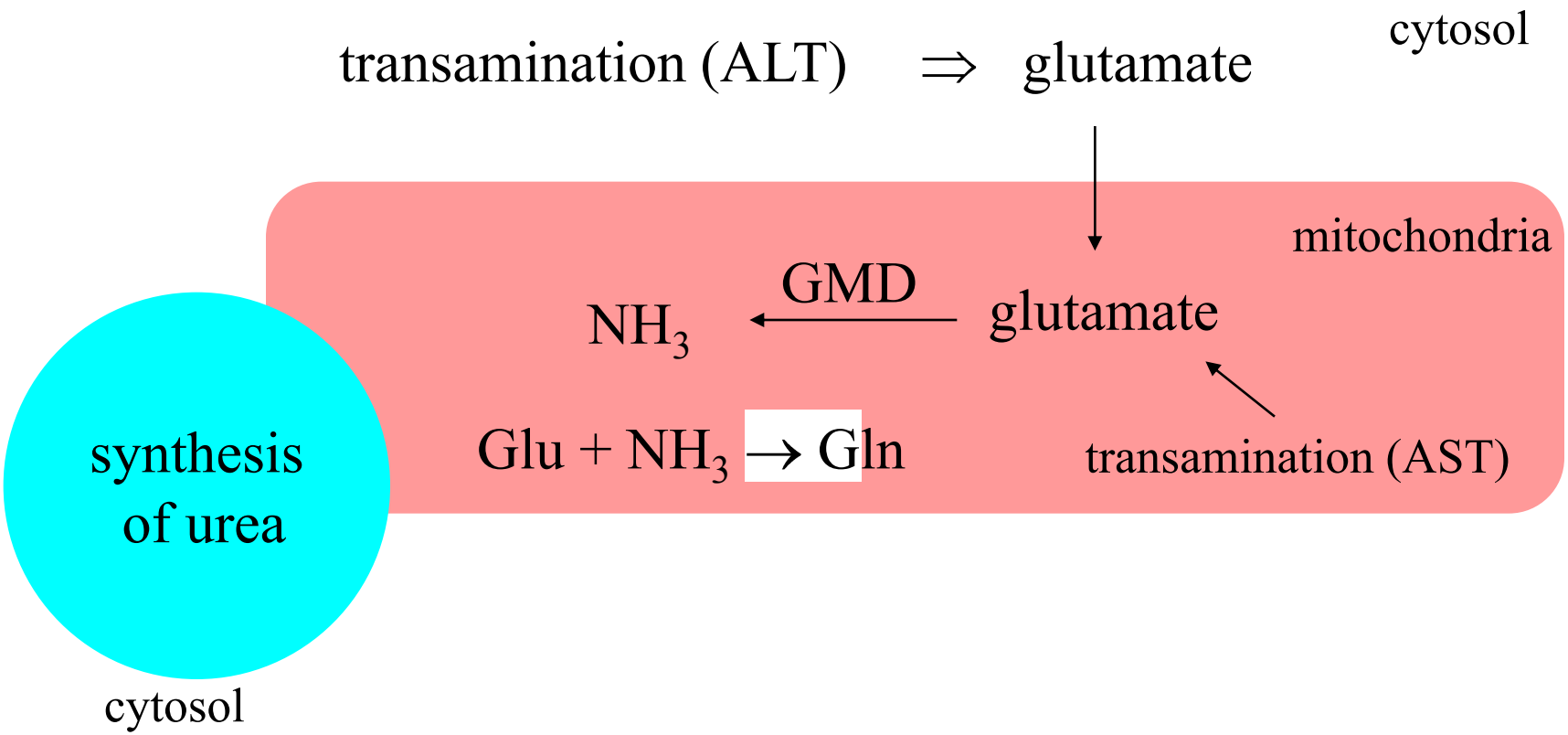
dehydrogenation **deamination** **of glutamate**



hydrogenation **amination** **of 2-oxoglutarate**

3. way of ammonia detoxication

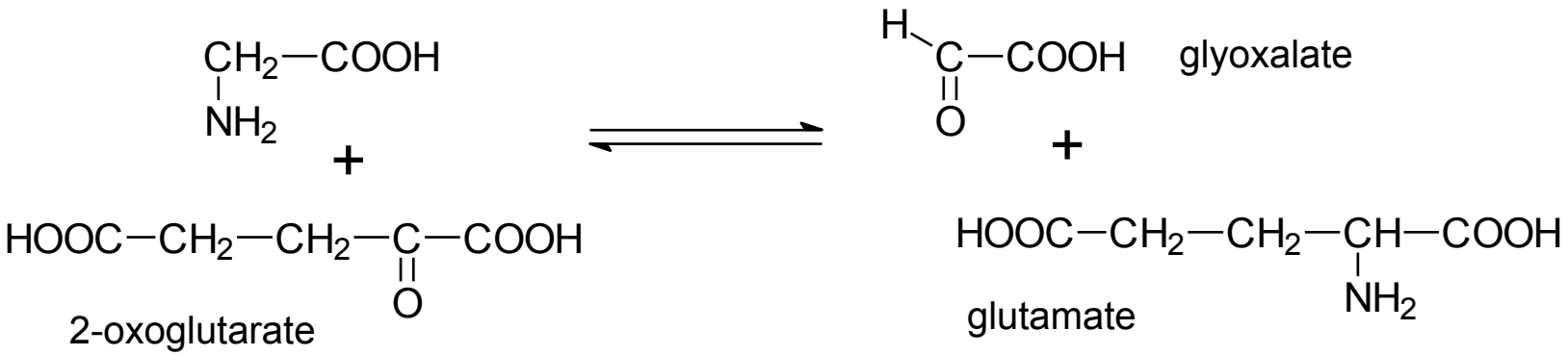
Subcellular location of AA conversions



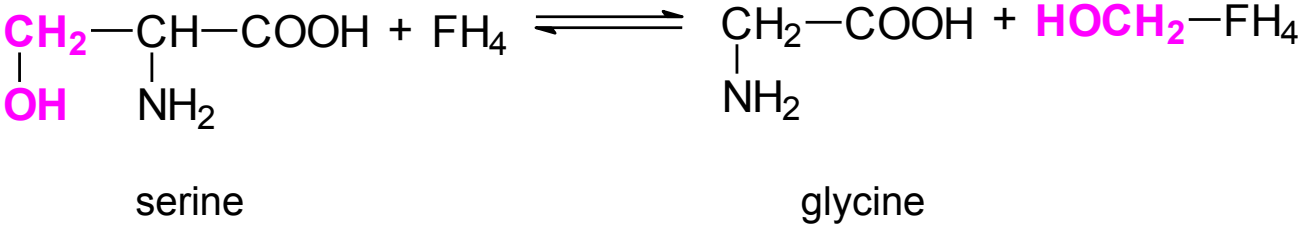
Synthesis of non-essencial amino acids

Synthesis of glycine

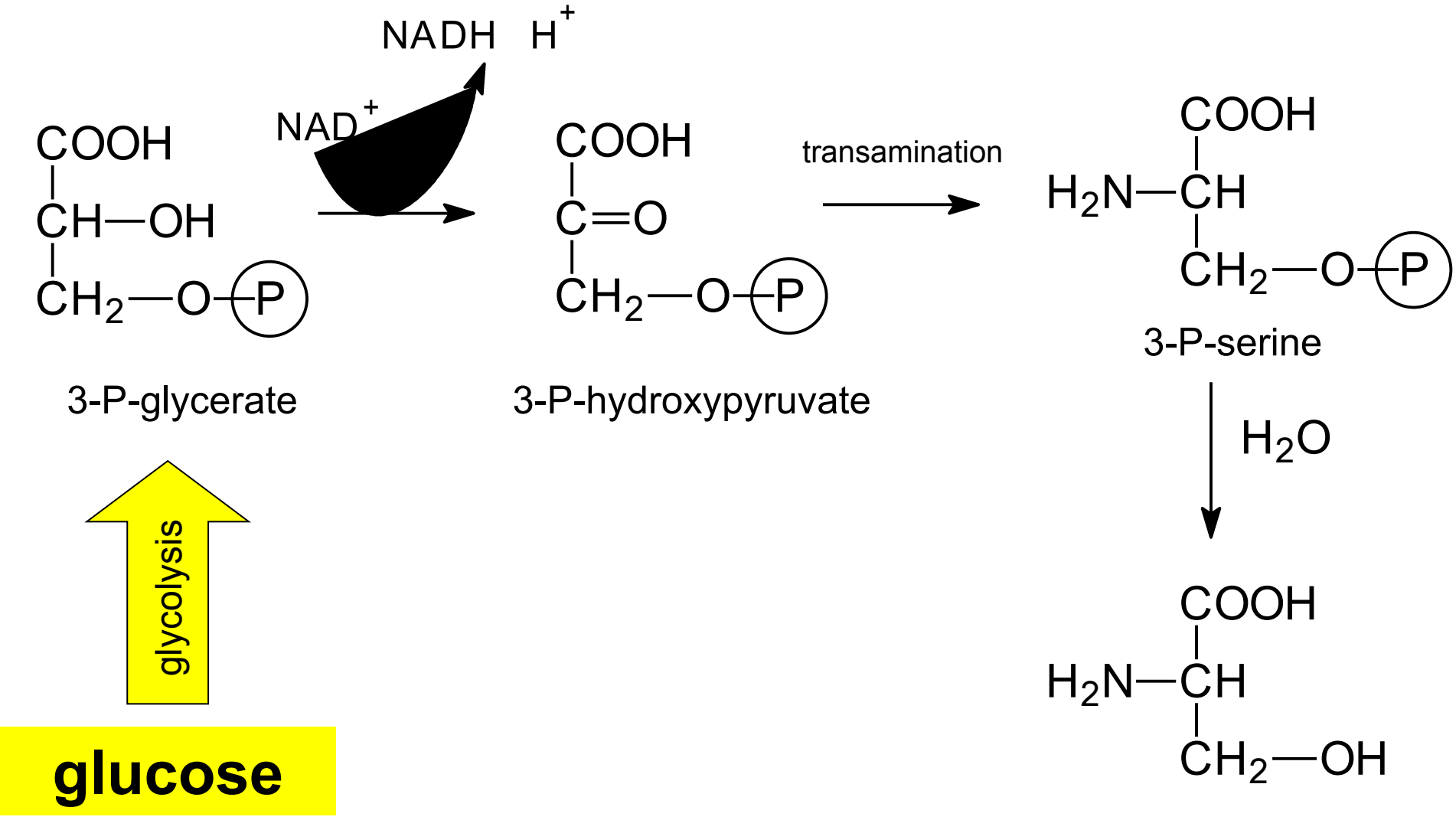
1. The reverse of transamination



2. From serine

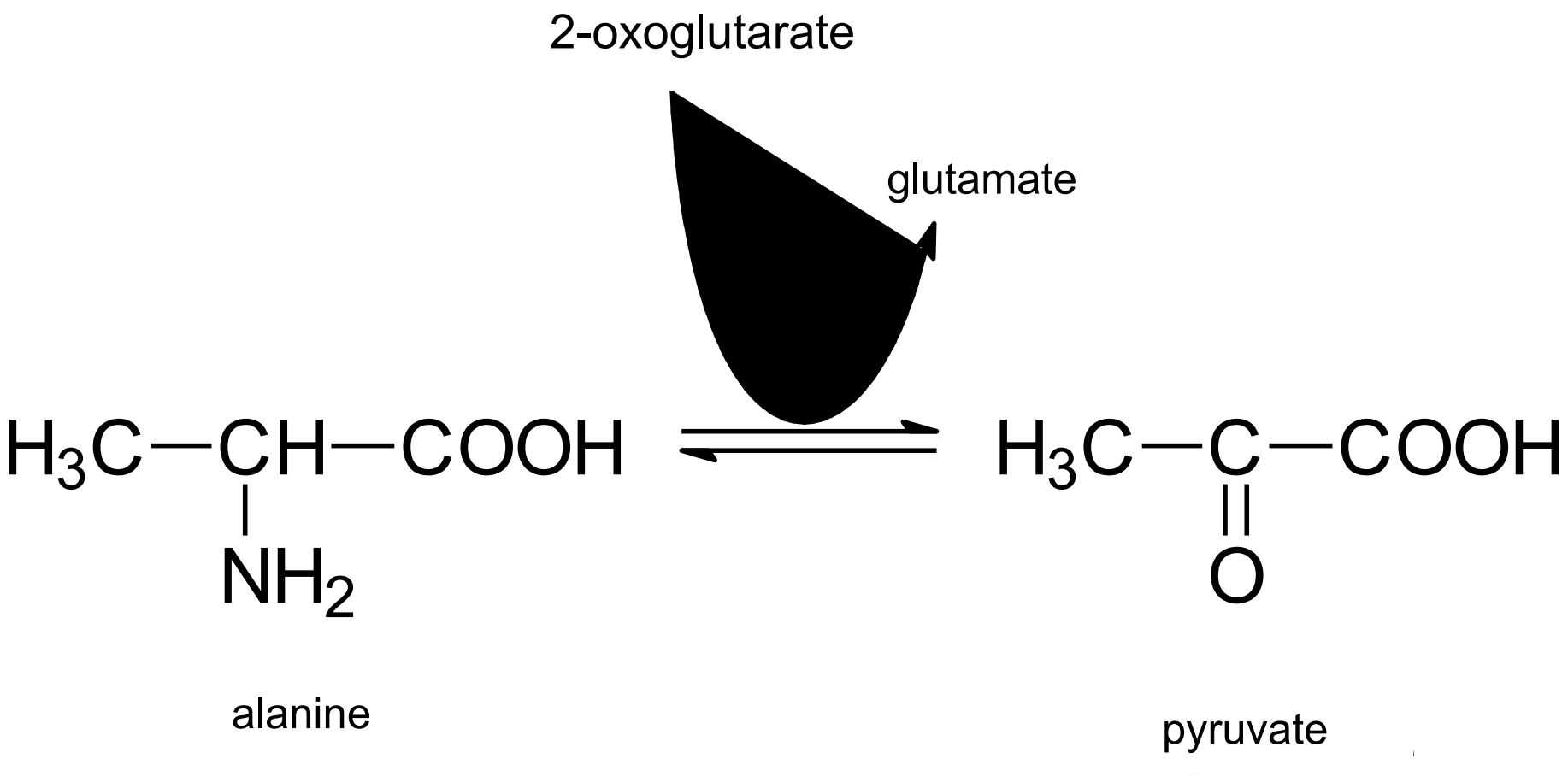


Formation of serine from the glycolysis intermediate



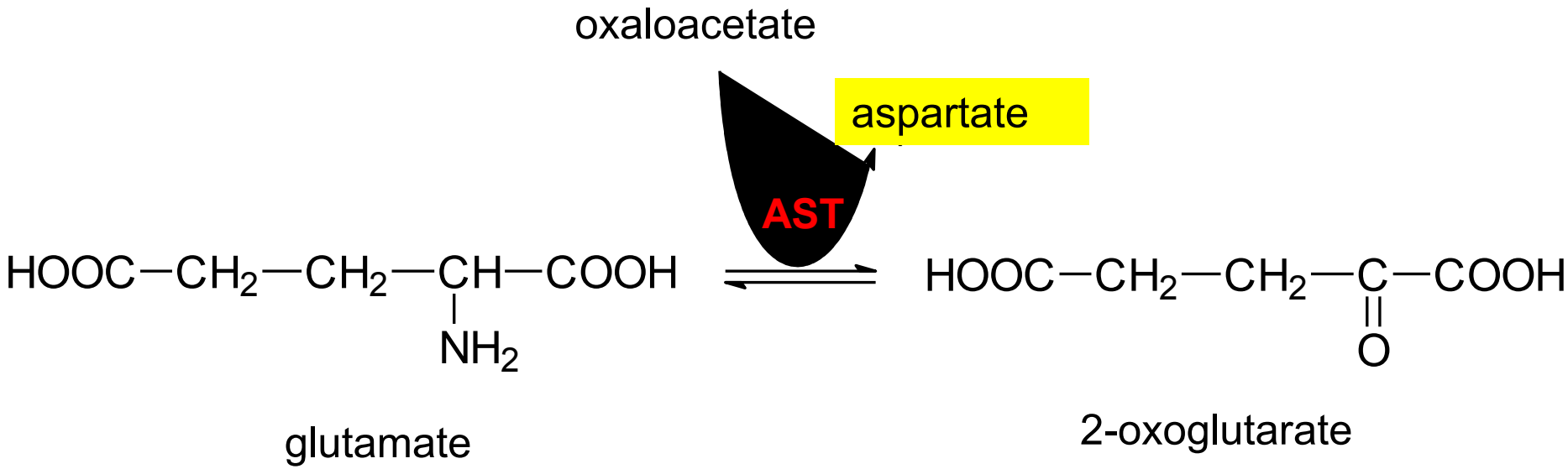
Synthesis of alanine from pyruvate and glutamate

(ALT = alanine aminotransferase)



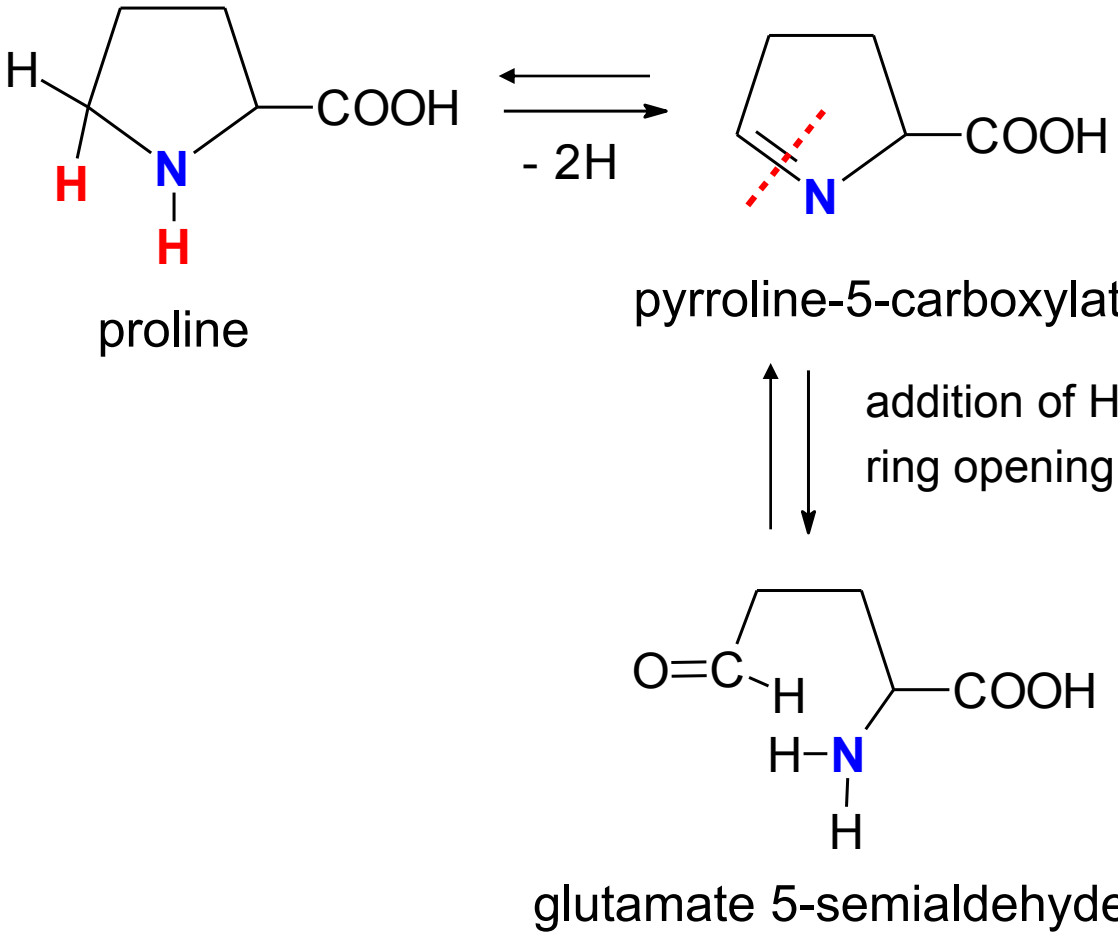
Aspartate from oxaloacetate and glutamate

(AST = aspartate aminotransferase)

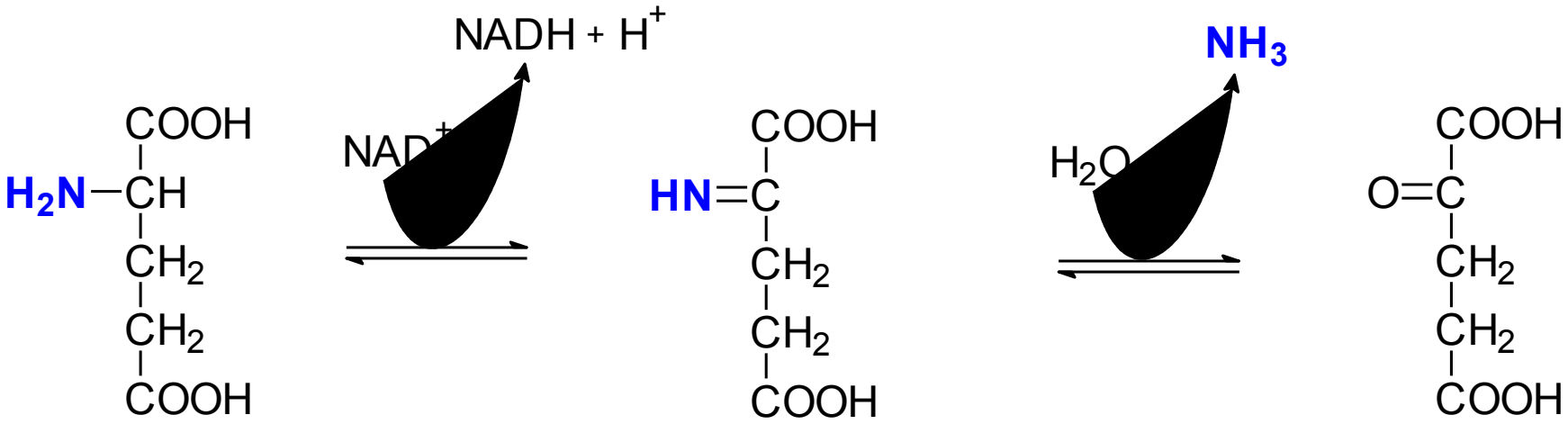


AST reaction produces aspartate for urea synthesis

Proline synthesis is the opposite of its catabolism

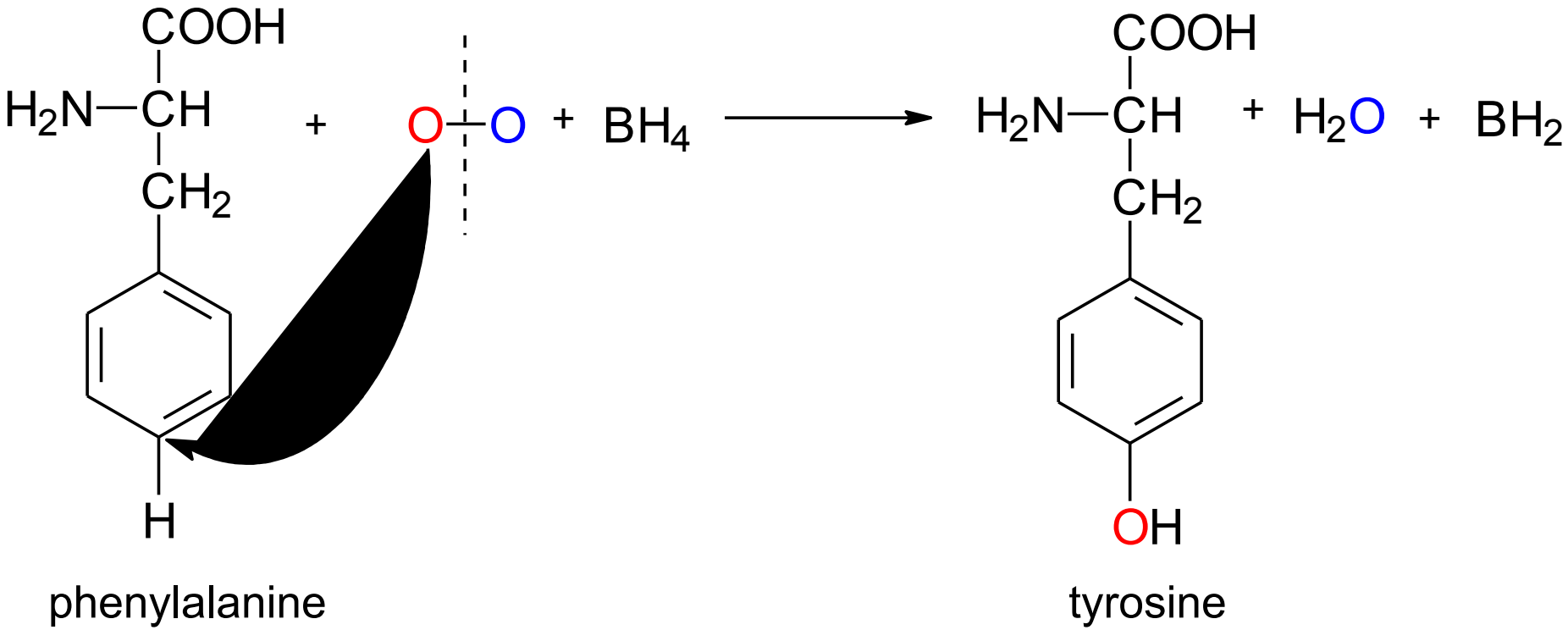


Glutamate is formed by the reductive amination of 2-oxoglutarate (GMD reaction)



L-glutamate 2-iminoglutarate 2-oxoglutarate

Hydroxylation of essential phenylalanine gives non-essential tyrosine

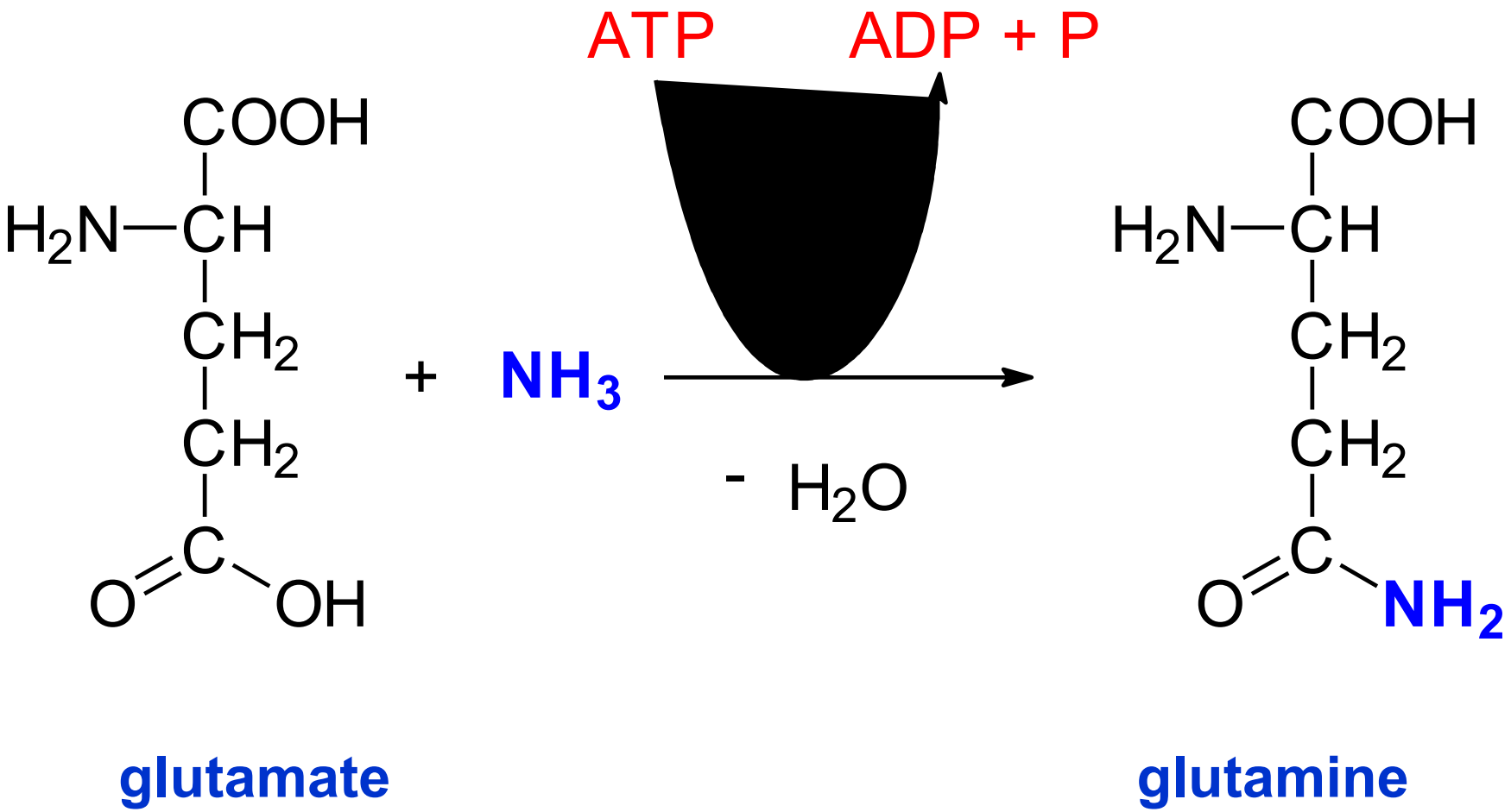


phenylalanine

tyrosine

tetrahydrobiopterine (BH_4) is a donor of 2H to form water from the second oxygen atom

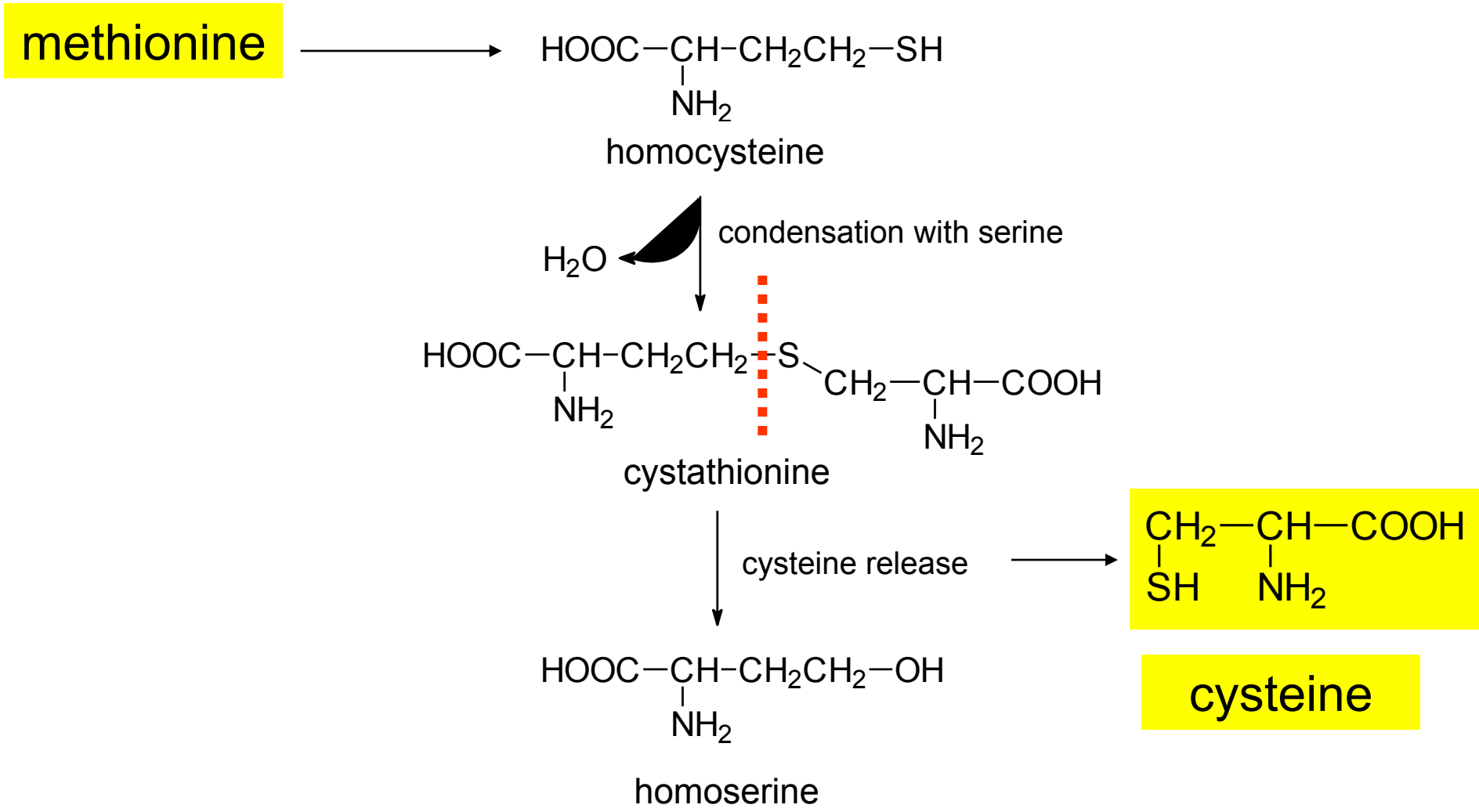
Glutamine from glutamate and ammonia



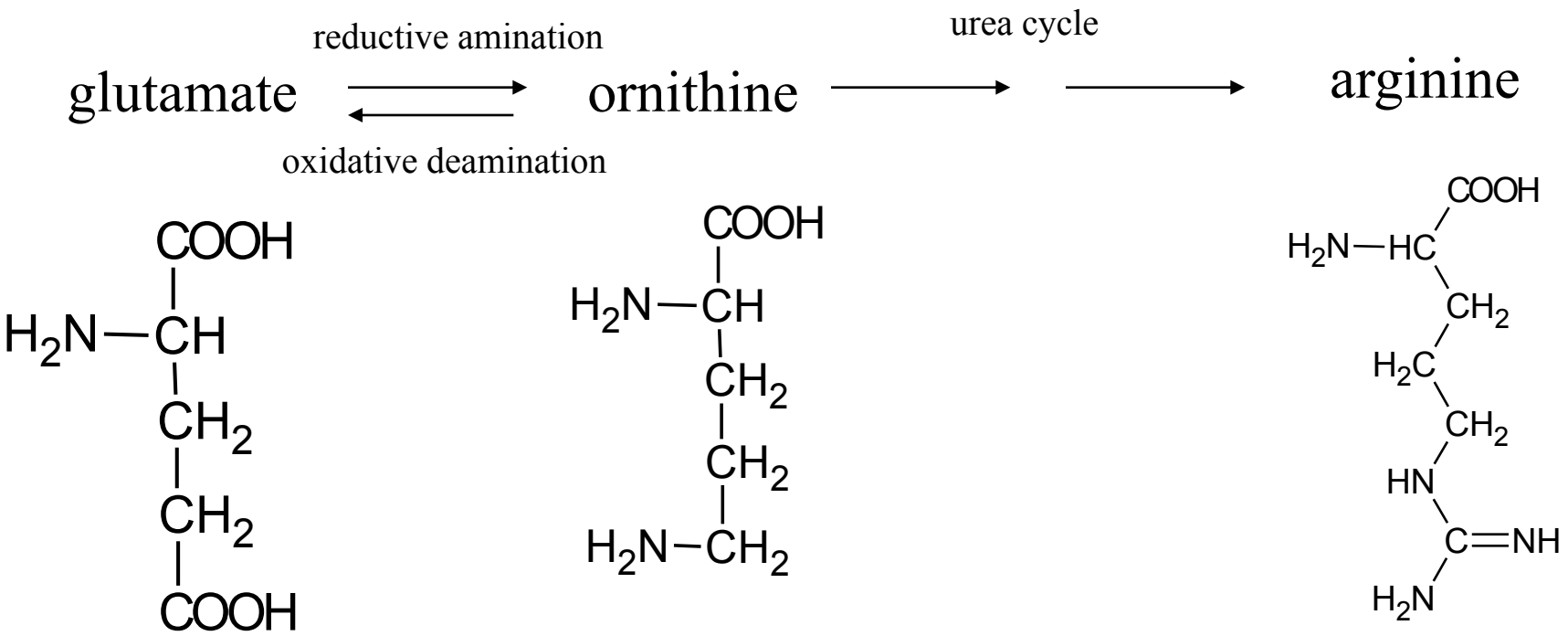
glutamate **glutamine**

Similarly: asparagine from aspartate

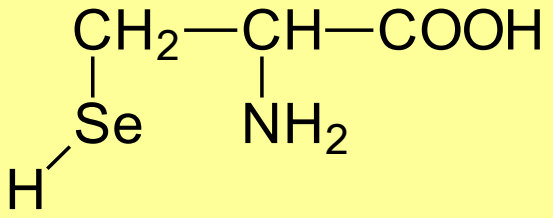
Cysteine is made from methionine



Arginine from glutamate via ornithine



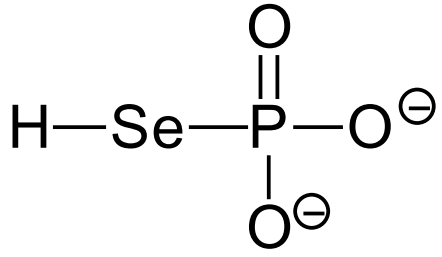
Selenocysteine arises co-translationally from serine and selenophosphate



Seryl-tRNA + selenophosphate → selenocysteyl-tRNA + phosphate

Selenophosphate is made from selenide (food) and ATP

$$Se^{2-} + ATP + H_2O \rightarrow AMP + P_i +$$



few enzymes (redox reactions) contain selenocysteine

Glutathione peroxidase (2 GSH + H_2O_2 → 2 H_2O + G-S-S-G)

Deiodase of thyronines (thyroxine T4 → triiodothyronine T3)

Thioredoxin reductase (ribose → 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_1 group)
Gly	serine (transfer of C_1 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)