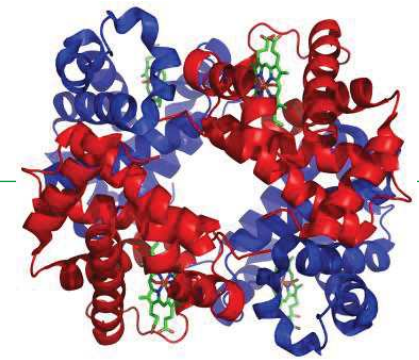


Determination of total protein concentration in body fluids

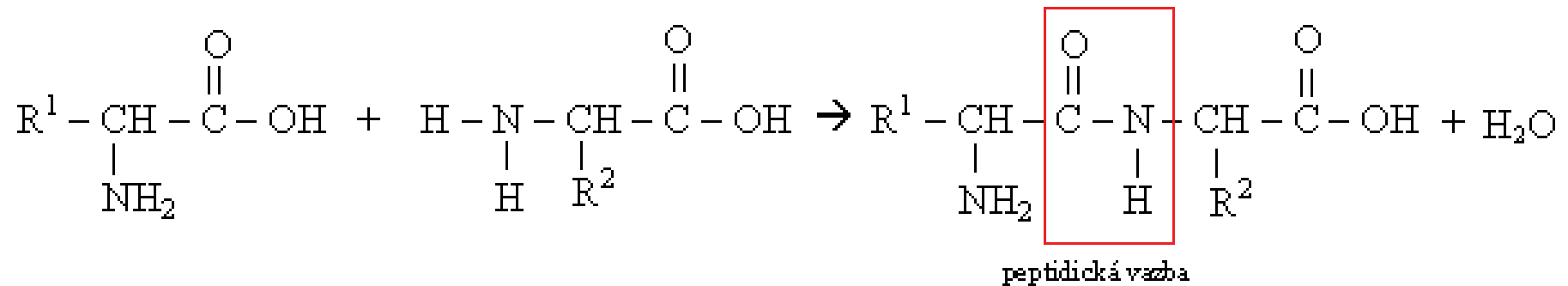


- proteins
- biuret reaction and its use in practice
- body fluids
- model organisms
- practical part: determination of protein concentration

Proteins



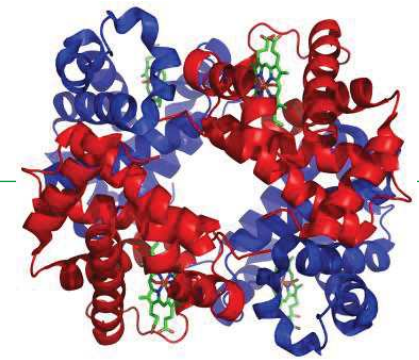
- Peptide-linked AMKs
- formation on ribosomes
- oligopeptides (2–10 AMK), polypeptides (11–50 / 100 AMK), own proteins (more than 50/100 AMK)



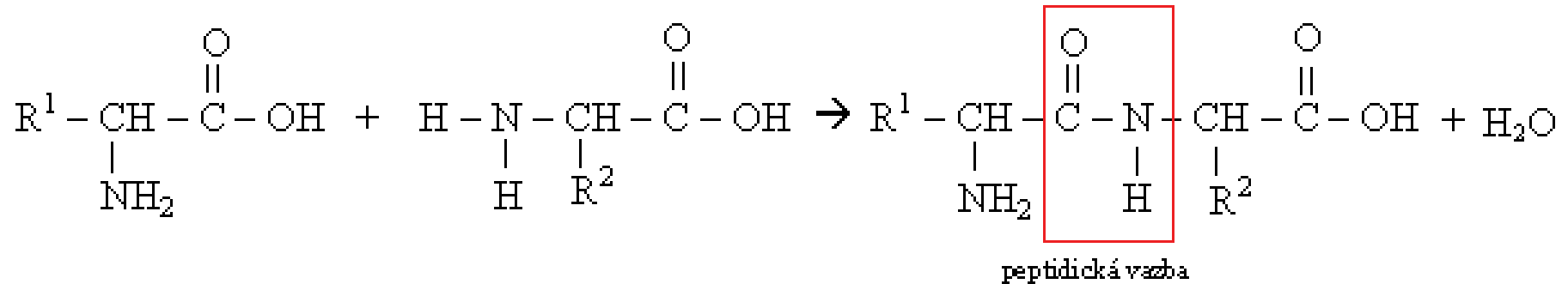
General protein function:

- construction
- transport and storage
- ensuring movement
- catalytic, control and regulation
- protective and defensive

Proteins



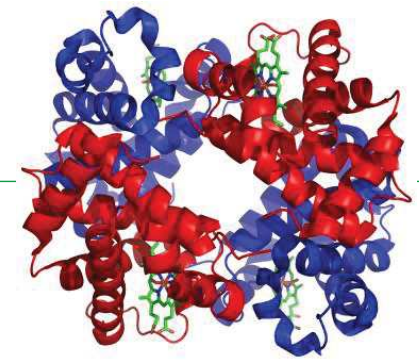
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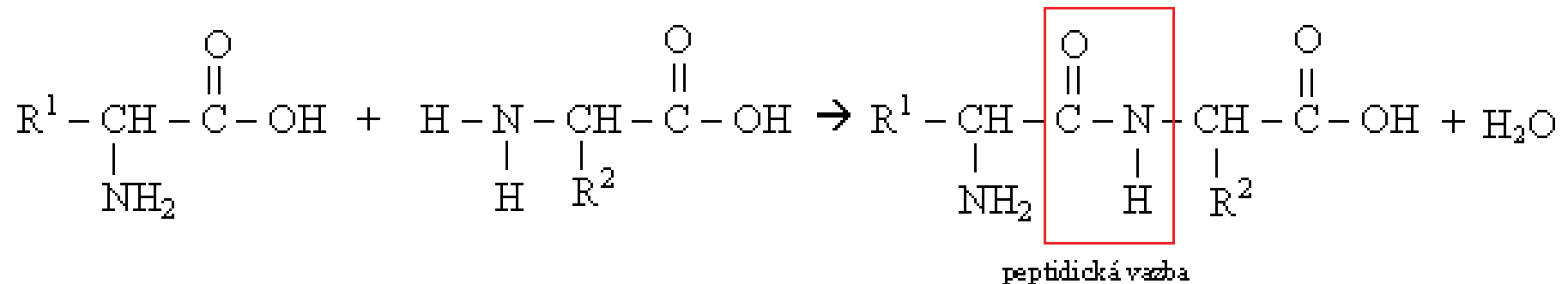
General protein function:

- building (collagen, elastin, keratin...)
- transport and storage (hemoglobin, transferrin...)
- ensuring movement (actin, myosin...)
- catalytic, controlling and regulatory (enzymes, hormones, receptors)
- protective and defensive (immunoglobulin, fibrin, fibrinogen...)

Proteins



- Peptide-linked AMKs
- formation on ribosomes
- oligopeptides (2–10 AMK), polypeptides (11–50 / 100 AMK), own proteins (more than 50/100 AMK)



Plasma protein function:

- immune (globulins)
- hemostatic (fibrinogen)
- transport (albumins - non-polar fats, cholesterol, steroid hormones)
- oncotic pressure maintaining function (albumins)
- pH maintaining (buffering) functions
- control and catalytic (hormones, enzymes)

Body fluids

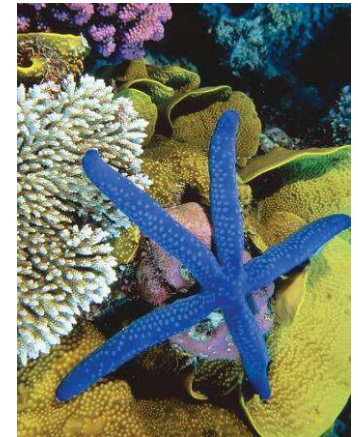
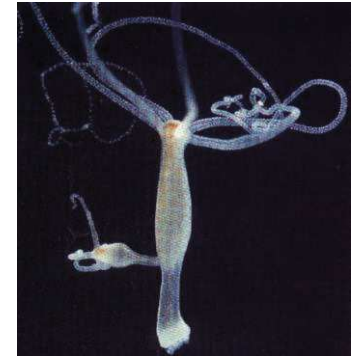
- transport, storage, support functions, etc.

Hydrolymph (lychees, echinoderms)

- solution of salts, low protein and free cells

Hemolymph (insects, crustaceans, molluscs)

- total protein about 6%
- dyes, free cells, proteins :
 - storage proteins (especially larval stages)
 - transport proteins (lipophorins, transferrin...)
 - hormones (adipokinetic, prothoracicotropic, bursikon...)
 - vitellogenins (female proteins that form the main part of the yolk sac)
 - immune proteins (lysozyme, coagulation proteins...)



Body fluids

Blood (vertebrates) = plasma + blood elements

- transport of cholesterol, glucose, fats, ions (Fe, Cl and others)
- blood protein (total protein 6-8%):
 - albumins (60% plasma proteins) - bind water, transport of Cu, Zn, fatty acids, hormones
 - globulins (40% of plasma proteins) - bind fat, hormones, immune reactions (Ig)
 - fibrinogen , etc. (<1%)

Tissue fluid (extracellular fluid; without plasma proteins)

Lymph (from tissue fluid; immune and transport functions)

Amniotic fluid, cerebrospinal fluid, perilymph and endolymph in the ear, ventricular water and more.

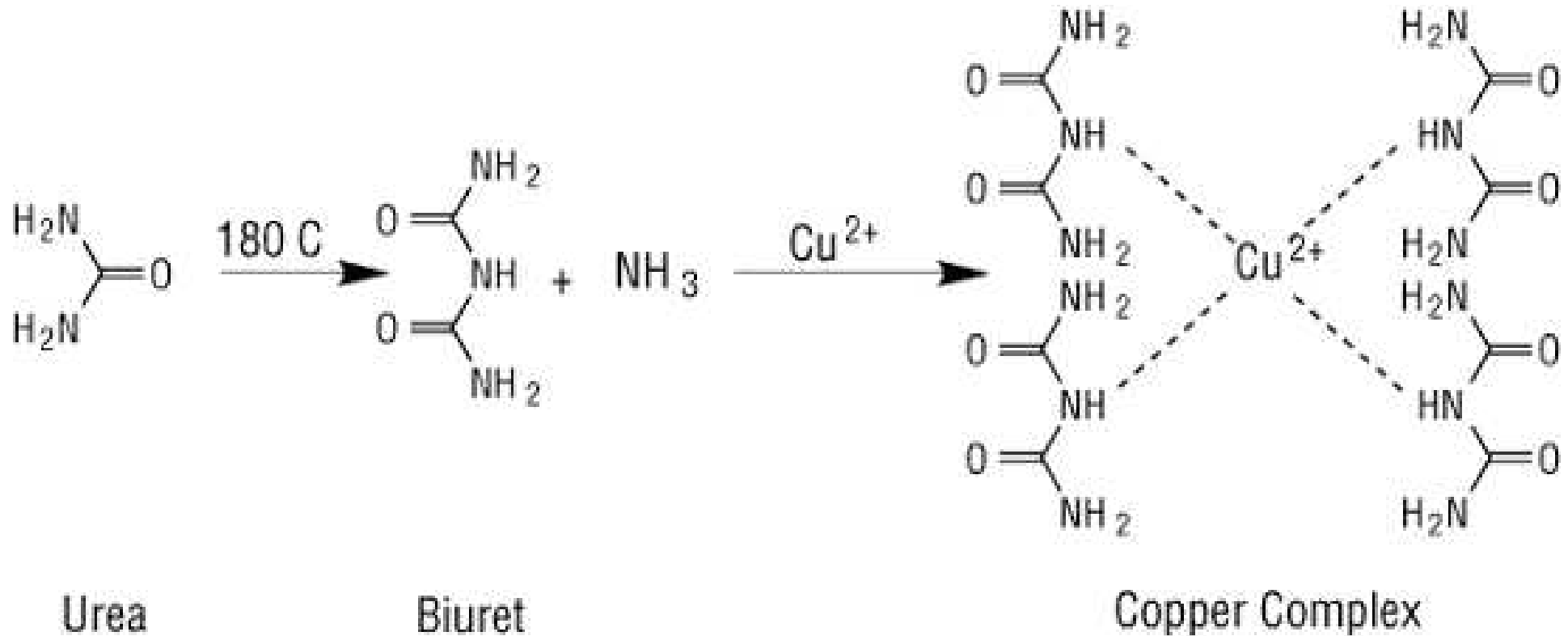
Total protein in vertebrate plasma

	Total protein [g / l]		Total protein [g / l]
žako	35-45	caiman	47
Amazon	29-51	grass snake	43
ara	26-43	viper	55
parakeet	14-36	crocodile	65
hawk	24-31	varan	69
pigeon	15-35		
duck	35-45	Scot	65-80
		pig	65-85
sturgeon	45	horse	46-70
carp	41.5	cat	60-80
trout	34.6	dog	60-80

Note: Indicative values (reptiles, fish selected from specific studies, relatively small *n*).

Determination of proteins - principle of biuret reaction

- in the alkaline environment, the protein chain forms a "matrix" around Cu^{2+} > purple color
- the intensity of the staining is proportional to the number of peptide bonds



Protein determination - laboratory practice

- commercial kits, eg Bio-Rad Protein Assay:
 - modification to microtiter plate
 - 5 μ l sample / standard + 25 μ l reagent solution A + 200 μ l reagent solution B
 - measured at 700 nm after 15 minutes of incubation
 - calibration curve required



Model organisms

silkworm - silk processing

- before the butterfly hatches, it is necessary to dry the cocoons with pupae
- cooking cocoons
- fiber (fibroins)
- silk proteins (sericins)



Model organisms



silkworm - interesting facts

- monophagy
- sexual dimorphism (larger female, targets)



- hybrids
 - monovoltic species ("monocotyledons") with a white cocoon
 - polyvoltine species having eg yellow cocoon (xanthophylls)
- the adult does not fly and does not eat
- bred in China (3000 BC); today SE Asia, Japan, Brazil
- fiber up to 1.5 km long
- 2 t leaves > 120 kg cocoons > 20 kg silk

Model organisms

house mouse (*Mus musculus*)

- traditional model organism
- blood (blood plasma / serum + blood cells)
- major proteins
 - 3-5% albumin
 - 1-3% globulin
 - 0.5% fibrinogen



domestic dog (*Canis lupus f. Familiaris*)

domestic cat (*Felis silvestris f. Catus*)

domestic tur (*Bos taurus*) common carp

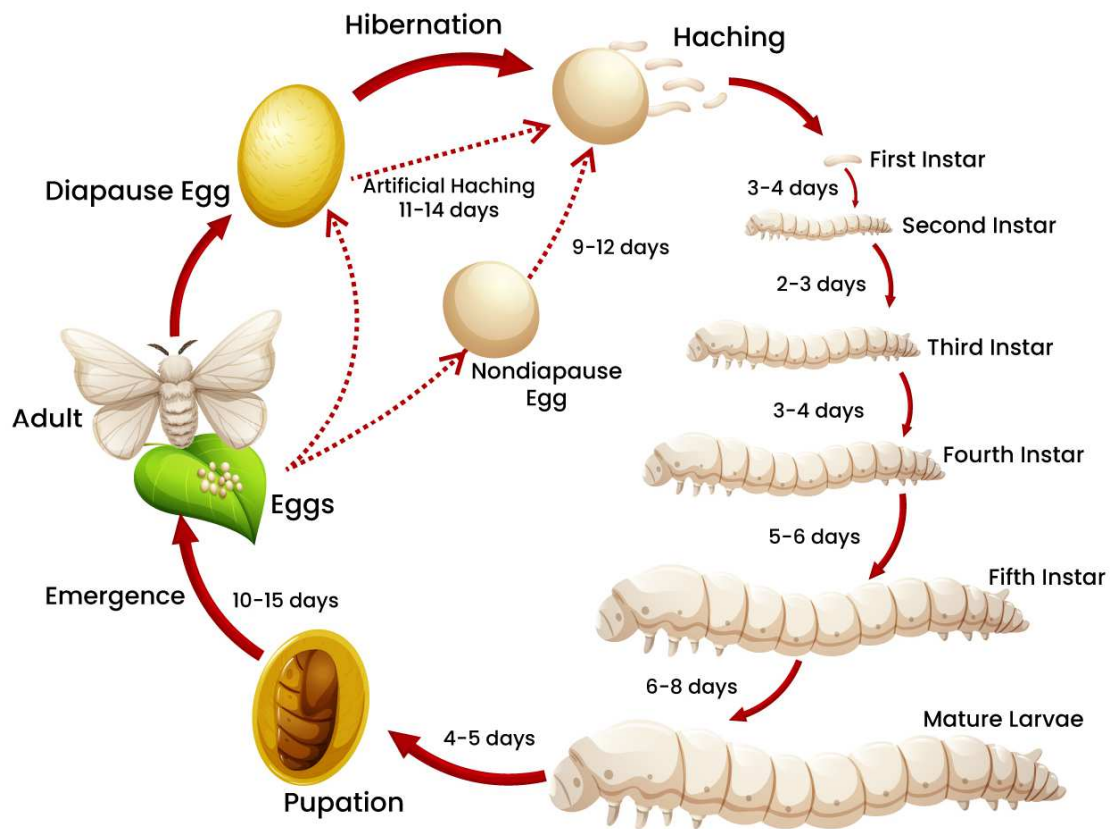
(*Cyprinus carpio*) tench (*Tinca tinca*)

crucian carp (*Carassius carassius*)



Silkworm - *Bombyx mori*

- Silkworm (*Lepidoptera, Bombycidae*)
- Silk production



Wax moth - *Galleria mellonella*

- Wax moth (*Lepidoptera*, *Pyralidae*, *Gallerinae*)
- Bee pests - feeds on wax, creates corridors in wax plates lined with fiber
- Excellent model organism - metamorphosis, immunity, metabolism, ...



	1	2	3	4	5	6
Biuret reagent	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Standard	20 µl					
Blank - H ₂ O		20 µl				
Hemolymph BM			20 µl			
Hemolymph BM				20 µl		
FBS					20 µl	
Carp / linseed serum						20 µl
...						

Total protein (g / l) = a.



70 (protein concentration in the standard)

A₁ ... Absorbance of the sample

A₂ ... Absorbance of the standard