Enzymes as therapeutics

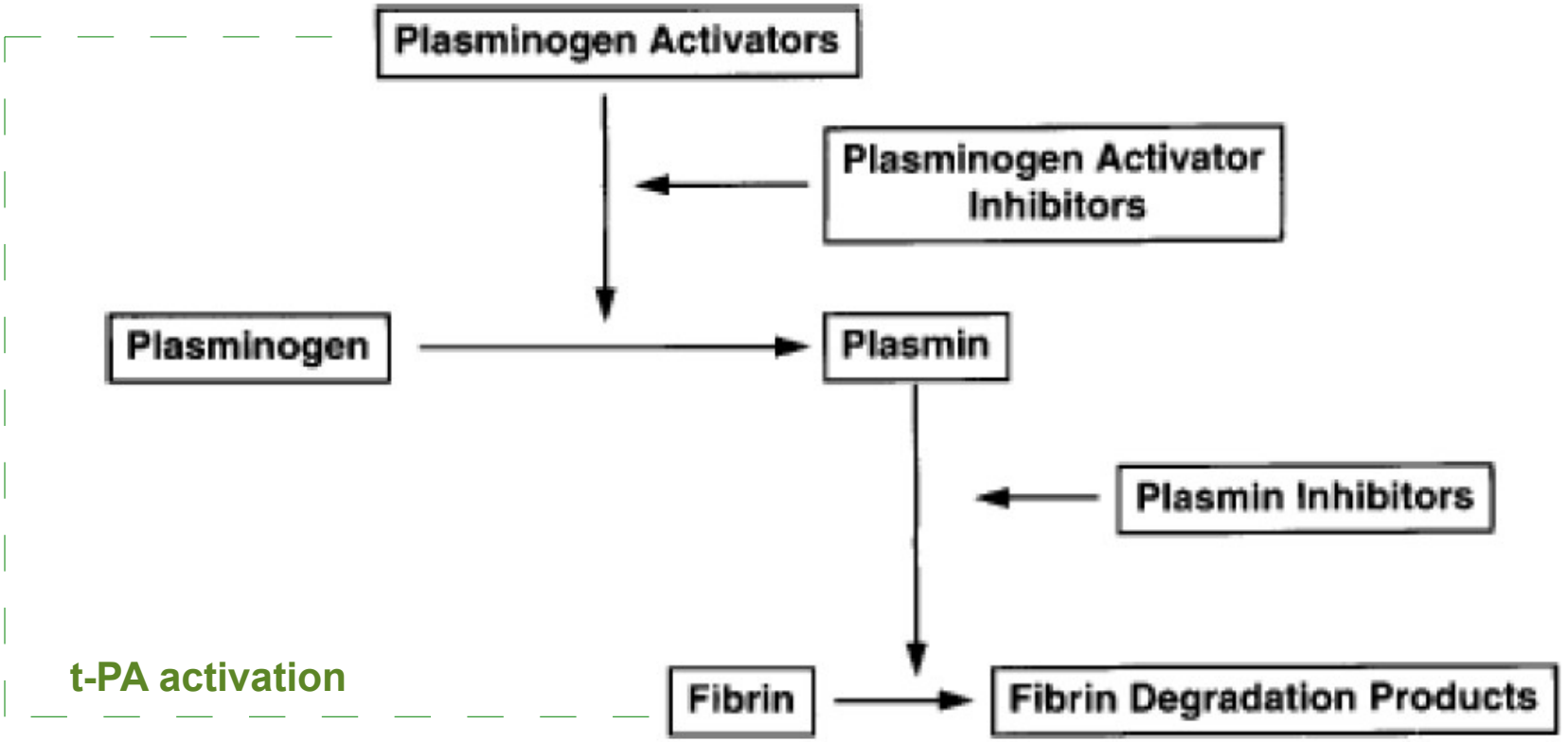
Hydrolases

Petidases

Fibrinolytics

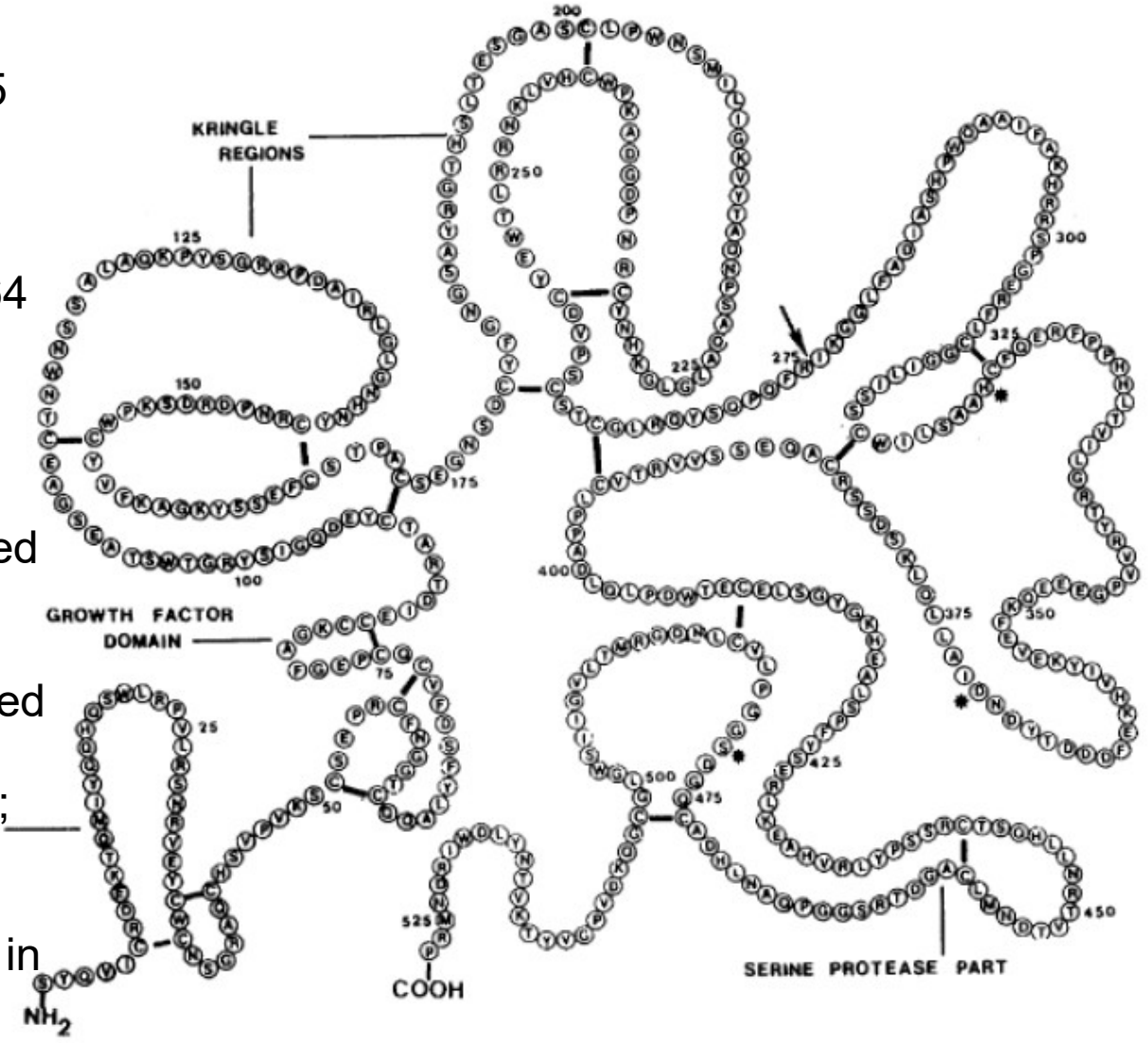
Plasminogenogen activators

•catalyze cleavage of the endogenous plasminogen under formation of plasmin, a non-specific proteinase, which degrades the fibrine matter of the thrombus


Plasminogen Activators
Plasminogen Activator Inhibitors
Plasminogen
Plasmin
Plasmin Inhibitors
t-PA activation
Fibrin
Fibrin Degradation Products

Alteplase

•fibrinolytic – (natural) tissue plasminogen activator (tPA)
•serine protease released from endothelium cells as one- or two-stranded molecule
•binds to fibrin fibers by means of binding sites on Lys and, being bound, activates plasminogen to fibrin dissolving several hundert times faster than its free form in the blood circulation
•572 AA
•glycoprotein
•M_r: 59 050 without sugars attached to Asn 117, 184 a 448, with sugars cca 65 000
•cleft by plasmin between AA 275 and 276 into chains A and B; they remain linked by -S-S- bridge between Cys 264 and Cys 395
•both one- and two-stranded have comparable fibrinolytic activity *in vitro*
•*Alteplasum ad iniectabile EP* must contain at least 60 % of the two-stranded form
•pI = 7 – 8
•preparation/production:originally isolated from a human melanoma cell line; produced by a recombinant technology; enzyme produced on mammalian cells has an optimal glycosylation; the one-stranded form is predominantly formed in the Chinese hamster ovaries cells



Therapeutic usage

Actilyse® lyophilizate for preparation of the infusion solution

- $T_{1/2}$ = 5 min
- trombolytic treatment of acute myocardial infarction (heart attack)
- thrombolytic treatment of acute massive lung embolism accompanied with a haemodynamic instability
- fibrinolytic treatment of acute ischemic events (brain strokes)

Reteplase

•recombinant, produced in *E. coli*
•sequence consists of two shorter fragments of t-PA with 355 AA together (1 – 3 and 176 – 527)
•M_r ~ 39 000
•removed 3 important domains: N-terminal „finger“, which is partly responsible for high affinity to, EGF (epidermal growth factor) homologous domain and „kringle* 1“ domain
•this + glycosylaton decrese ⇒ different (more suitable) both pharmacodymamic and pharmacokinetic properties were expected
•$T_{1/2}$ = 14 min
•greater fibrinolytic activity
•effectivities in coronary vessels passabling (reopening) and in lowering of mortality due to heart attack are, however, comparable to those of alteplase

Rapilysin® powder for preparation of solution or pre-filled syringe

Therapeutic indications

•thrombolytic therapy of a suspected acute myocardial infarction with persistent

ST elevation or recent left Bundle Branch Block within 12 hours after the onset of acute

myocardial infarction symptoms

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* shipmaster´s rope loop

Tenecteplase

•glycoprotein produced by a recombinant technology in Chinese hamster ovaries cell lines

•(T = Thr, N = Asn, K = Lys; an analogue of alteplase, in which Thr103 was changed with Asn, which gave a new glycosylation site, Asn117 was replaced with Gln, which removed the mannose glycosylation site, the sequence Lys296-His-Arg-Arg299 was replaced with 4 Ala, which increased both activity and resistance against plasmin activator inhibitor 1 (PAI-1)

•$T_{1/2}$ = 20 – 24 min

Metalyse® powder for preparation of injection solutions of 6, 8, 10 $.10^3$ i. u.

Therapeutic indications

•in adults for the thrombolytic treatment of suspected myocardial infarction with persistent ST elevation or recent left Bundle Branch Block within 6 hours after the onset of acute myocardial infarction (AMI) symptoms

•administration as a a single intravenous bolus over approximately 10 seconds

Desmoteplase

- syn. bat PA, vPA, DSPA$_{\alpha 1}$
- saliva of the vampyre *Desmodus rotundus* contain contain 4 different plasminogen activators (desmodus salivary plasminogen activators = DPSAs)
- the most important DSPA$_{\alpha 1}$ = desmoteplase; the longest, the greatest (85%) homology with human t-PA
- 477 AA
- vPA has the finger domain, EGF domain and one kringle domain, lacks the second kringle as well as the cleavage site needed two-stranded PA formation $\Rightarrow$ the only PA, which exists exclusively as one-stranded molecule with full catalytic activity
- $T_{1/2}$ = 2,8 h
- prepared by recombinant technology
- clinical tests of phases 2 and 3 for acute ischemic (brain) stroke in USA, DE, JP ...

Streptokinase

Streptokinasi solutio concentrata EP

- originally isolated from the filtrate of the culture of β-haemolytic streptococci of the group C
- one-stranded polypeptide, 414 AA, $M_r \sim 47\,000$
- after binding to human plasminogen (complex 1 : 1), forms a plasminogen activator
- $T_{1/2}$ = 23 min
- recombinant streptokinase produced in *E. coli* is currently predominantly used
- maximum activity at pH 7.5; pI = 4.7
- dissolves the fibrinous portion of exsudate also
- the most frequently used PA in Europe

Therapeutic indications

Streptase®

Systemic administration

- acute transmural myocardium infarction (not older than 12 hours) with persistent ST-elevation or simultaneous left bundle block
- deep vein thrombosis not older than 14 days
- acute masive lung embolism
- acute or subacute peripheral arteries thrombosis
- chronic occlusive arterial diseases (not older than 6 weeks)

Local administration

- acute myocardial infarction for re-canalisation of coronary vessels (not older than 12 hours)
- acute, subacute and chronic thrombosis; embolisms of peripheral veins and arteries

Anistreplase

- syn. APSAC (Acylated plasminogen – streptokinase activator complex)
- a complex of streptokinase and the active site of human lysine plasminogen, in which, the active proteolytic sites were inactivated by acylation – *p*-anisoic acid residues introduction
- $M_r \sim 131\ 000$
- does not need circulating plasminogen for its activity
- after injection, the acyl group is slowly hydrolyzed, producing an activator that converts plasminogen to plasmin, thereby initiating fibrinolysis
- $T_{1/2}$ = 40 - 90 min
- if the treatment is initiated within 3 hours of onset of symptoms for acute myocardial infarction, the drug preserves myocardial tissue and left ventricular function and increases coronary artery patency (throughput)

Urokinase

EC 3.4.21.73

Urokinasum EP

•an enzyme isolated from human urine, which activates plasminogen

•a mixture of low molecular (M_r about 33 000) and high molecular (M_r about 54 000) forms: the high molecular one predominates in urokinase isolated directly from kidneys, while the low molecular form in enzyme produced in kidneys cell lines

•recombinant, fully glycosylated form derived from murine cancer cells is also used

•activity at least 70 000 i.u./mg of protein

•$T_{1/2}$ = 16 min

Rheotromb® lyophilizate for preparation of the infusion solution, 500 000 i.u. (CZ)

Kinlytic ® up to 250 000 i.u./vial (USA)

✗both discontinued

Therapeutic indications

Systemic fibrinolysis:
- arterial thrombosis
- massive embolism of pulmonary arteries
- deep venous thrombosis

Local fibrinolysis
- arterial thrombosis
- arterio-venous fistula closure

- Clogged cathteters reopening

Ancrod

EC 3.4.21.74, formerly EC 3.4.21.28

syn. venombin A

Viprinex ®

- protease isolated from the venom of Malaysian snake *Agkistrodon rhodostoma*
- glycoprotein
- selectively cleves bonds from/to Arg
- 258 AA, M_r = 29 145
- capable to activate the change of fibrinogen to fibrin (pro-clotting effect), but also fibrin degradation (fibrinolytic), resulting effect depends on the species of recipient
- phase 3 clinical studies have not shown a significant difference in effect to improve survival after acute myocardial infarction between ancrod and placebo (STAT study:USA; ESTAT: F, DE...)
- more recently tested for treatment of sudden sensorineural hearing loss (*s.c.,* phase I and II; CZ, DE)

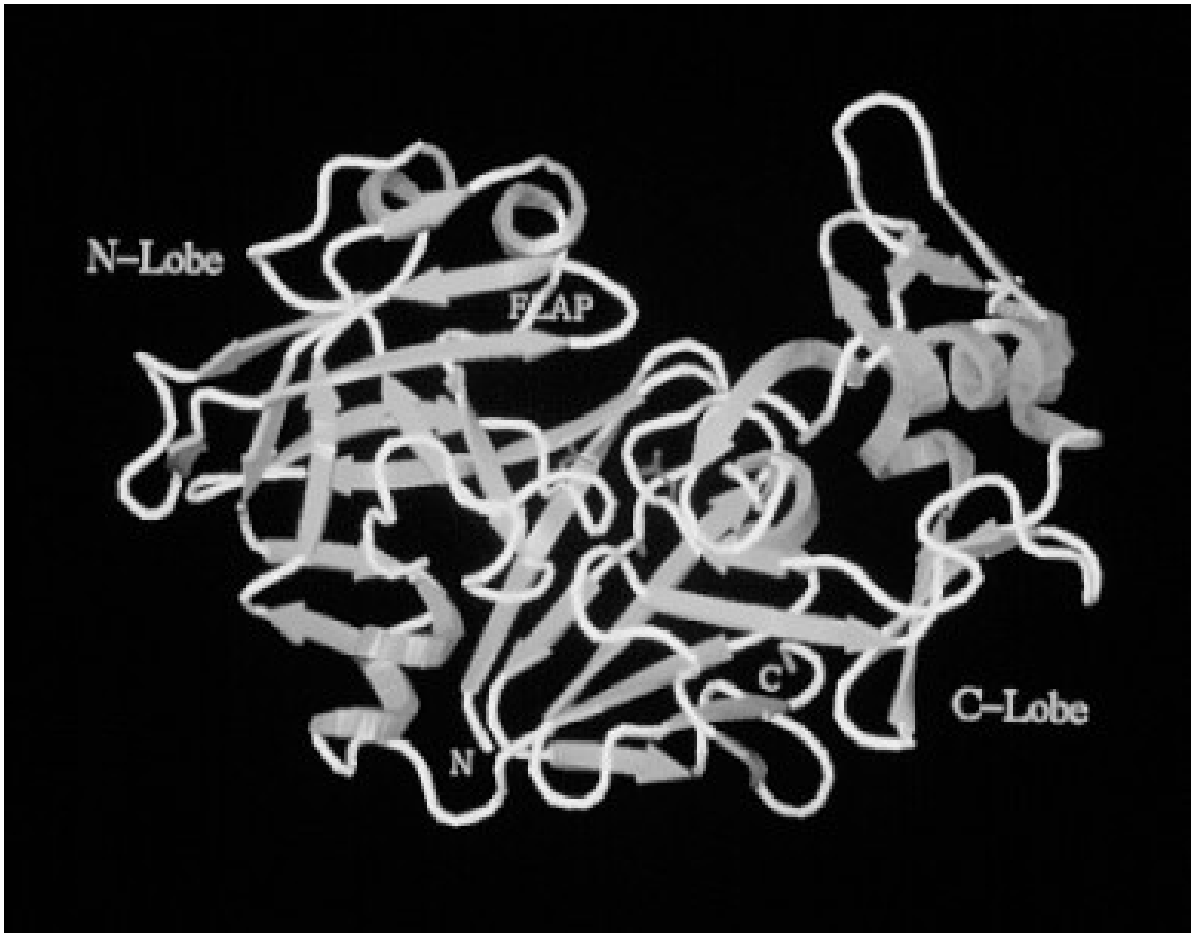
Alimentary tract peptidases

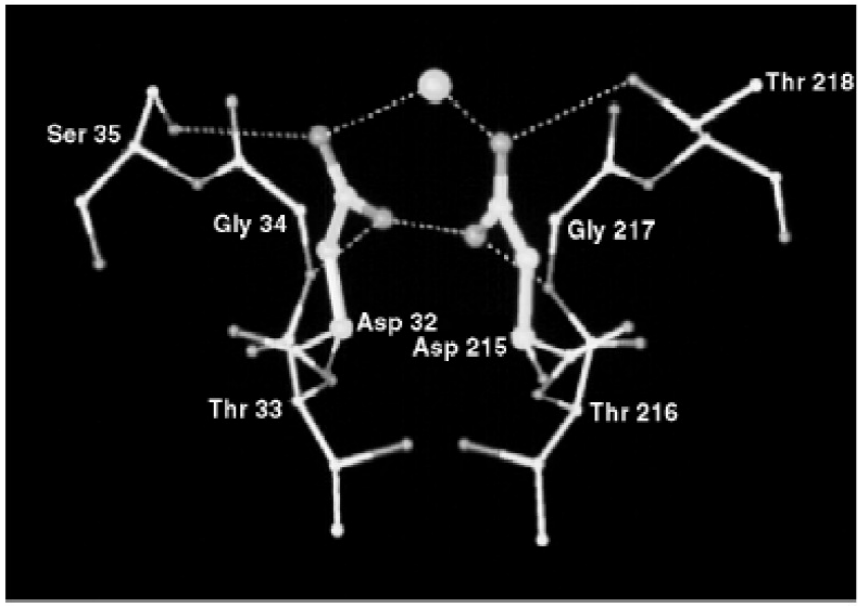
Pepsin

EC 3.4.23.1

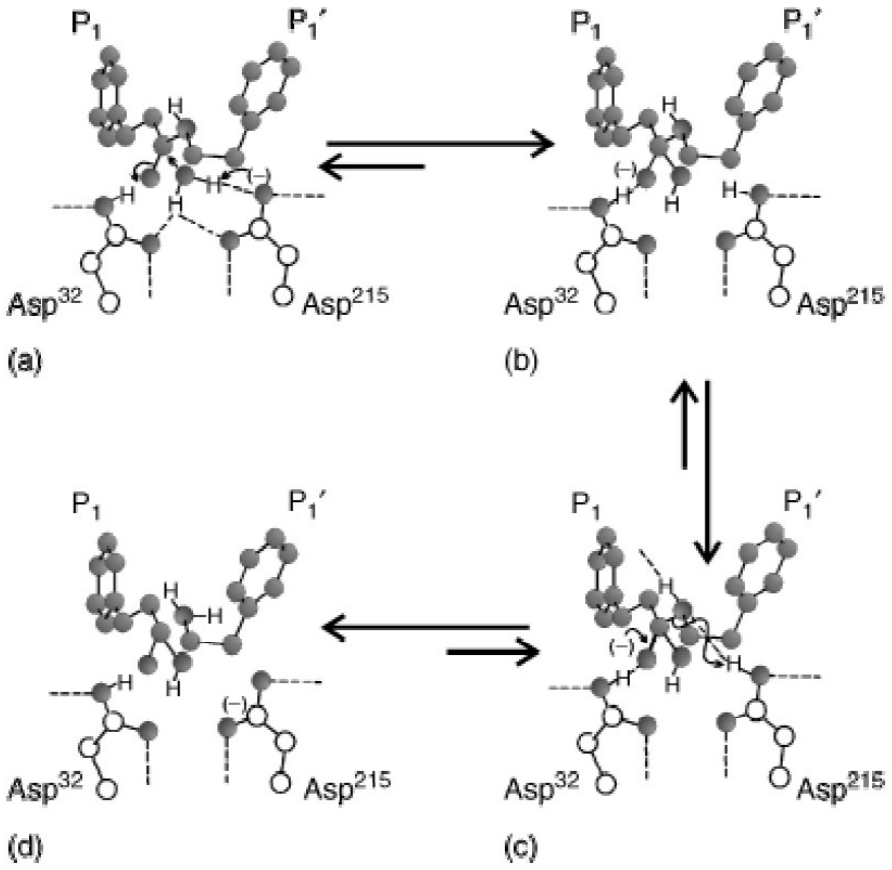
- fundamental acid protease of stomach
- endopeptidase with wide substrate specifity
- 1st discovered enzym at all – 18th century, name given by T. Schwann 1825; 2nd enzym isolated in crystalline form (after urease – Northtrop 1930)
- for distinguishing from minority gastric proteases: **pepsin A** (× pepsin B; pepsin C = gastricsin)
- 1 chain, 326 AA, $M_r \sim 34\,600$, active sites formed by Asp32 and Asp215 (porcine)
- catalyses hydrolysis in pH range 1 – 6, optimum activity at pH about 3.5
- pK_a values in the active site 1.57 and 5.02

Quarternary structure of porcine pepsin with marked N- and C-terminal lobes; „random coils“ are drawn by thin line, a substrate beeing cleft is marked between lobes





A



A (top): arrangement of the active site of pepsin: O atoms are represented by bigger balls, H-bridges are dashed, the biggest ball represents water molecule taking part in the nucleophilic attack (or its O in particular)

B (bottom): proposed catalytic mechanism of pepsin and other aspartate proteases: substrate (top molecule) is oriented by the pair Phe-Phe to the active site.
(a) -C**O**OH of Asp32 is partially protonated, -COOH of Asp215 is partially dissociated
(b) Molecule of H_2O is bound to (initially) C=O of P_1 – intermediate of hydrolysis with sp^3 carbon
(c) Asp215 supplies H^+ to N of substrate
(d) cleavage of the peptide bond

•pepsin is capable to cleave also Phe-Pro bond (in contrast to other peptidases)
•classical assay (of content/pepsine activity in given protein – Anson 1938): hydrolysis of bovine haemoglobin at pH 2, addition of trichloroacetic acid, absorbance measurement at 280 nm (in *EP* modified)
•roundworm *Ascaris suum* produces a specific peptide pepsin inhibitor (149 AA)
•*Pepsini pulvis EP*: from gastric wall of pigs, cattle or sheep

Pancreatic peptidase

•peptidases mixture used in drugs for support of digestion as a substitute for endogenous peptidases : Gastrix ® , Kreon ® , Panzytrat ® ...

•a component of *Pancreatinum* = pancreatine [USAN] – a mixture of digestion enzymes acquired from porcine pancreases (+ lipase, amylase)

Trypsin

EC 3.4.21.4

•1st reported 1876 by Kühne as a proteolytic activity of pancreatic secretion; he distinguished its activity from that of pepsin by higher optimal pH value

•name trypsin was later associated with cleavage of bonds LysCO-NH- , ArgCO-NH-.

•isolated in crystalline form 1931

•released as trypsinogen, activated

•bonds to Lys and Arg are cleft 10^5times more readily than to other natural AA, to Arg 2 – 5times readily than to Lys

•some AA in specific positions can inhibit its activity: Arg, Leu, Ile, Lys, Phe in P2 inhibits the activity 2 – 16times, Pro in P3 lowers the activity 3 – 9times

•stable as the lyophilized powder or in a solution of pH 3, where it is inactive

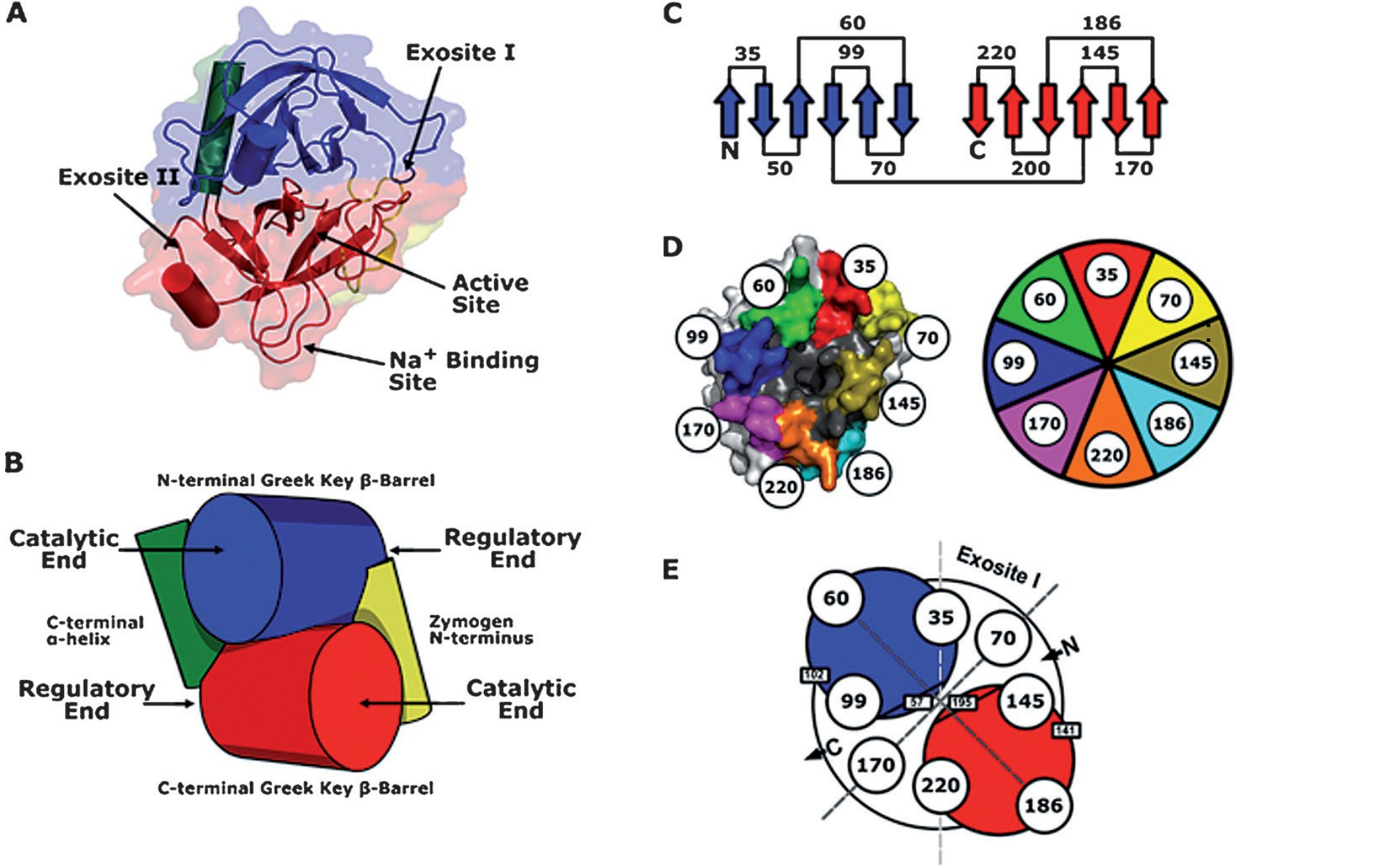
•primary structures differ markedly from species to species, tertiary ones are very similar

•one-chain peptide, in which AA residues with catalytic activity are linked with β-helix („β-barrel“) sequences.

Chymotrypsin

EC 3.4.21.1

- minority constituent of pancreatic peptidase
- in < 50 % homologous with trypsin
- different substrate specifity: hydrophobic AA residues: PheCO-NH-, TrpCO-NH-, TyrCO-NH-
- „catalytic triad“: His57, Asp102 a Ser195
- released as chymotrypsinogen, activated
- A and B, 80% homology
- preparation: acid extraction, precipitation by $(NH_4)_2SO_4$, crystallization (1955); precipitation with acetone, chromatography on ion exchangers – possibility of separation of A and B (1970); affinity chromatography (1996); gene transfer into a yeast cell, cloning, recombinant chymotrypsinogen production (1996)



Tertiary structure of chymotrypsin. A: N-terminal β-helix (blue) is situated above C-terminal β-helix (red), between them the active site. Zymogen-activating peptide (yellow) and C-terminal α– helix (green) stabilize the entire structure and active sites. Outer sites, active sites and Na^+ binding site are spread on the whole surface and are not in direct touch. B: Schematic of tertiary structure. C: Topology of chymotrypsin structure. D: On an enzyme model oriented by the active site to a viewer, 8 loops, potential sites of interactions with macromolecular substrates and inhibitors, are visible. E: Tertiary structure symmetry. The terminus of every o β-helix forms 3 loops on the side of the active site, and bents the back side of the molecule by the additional two ones.

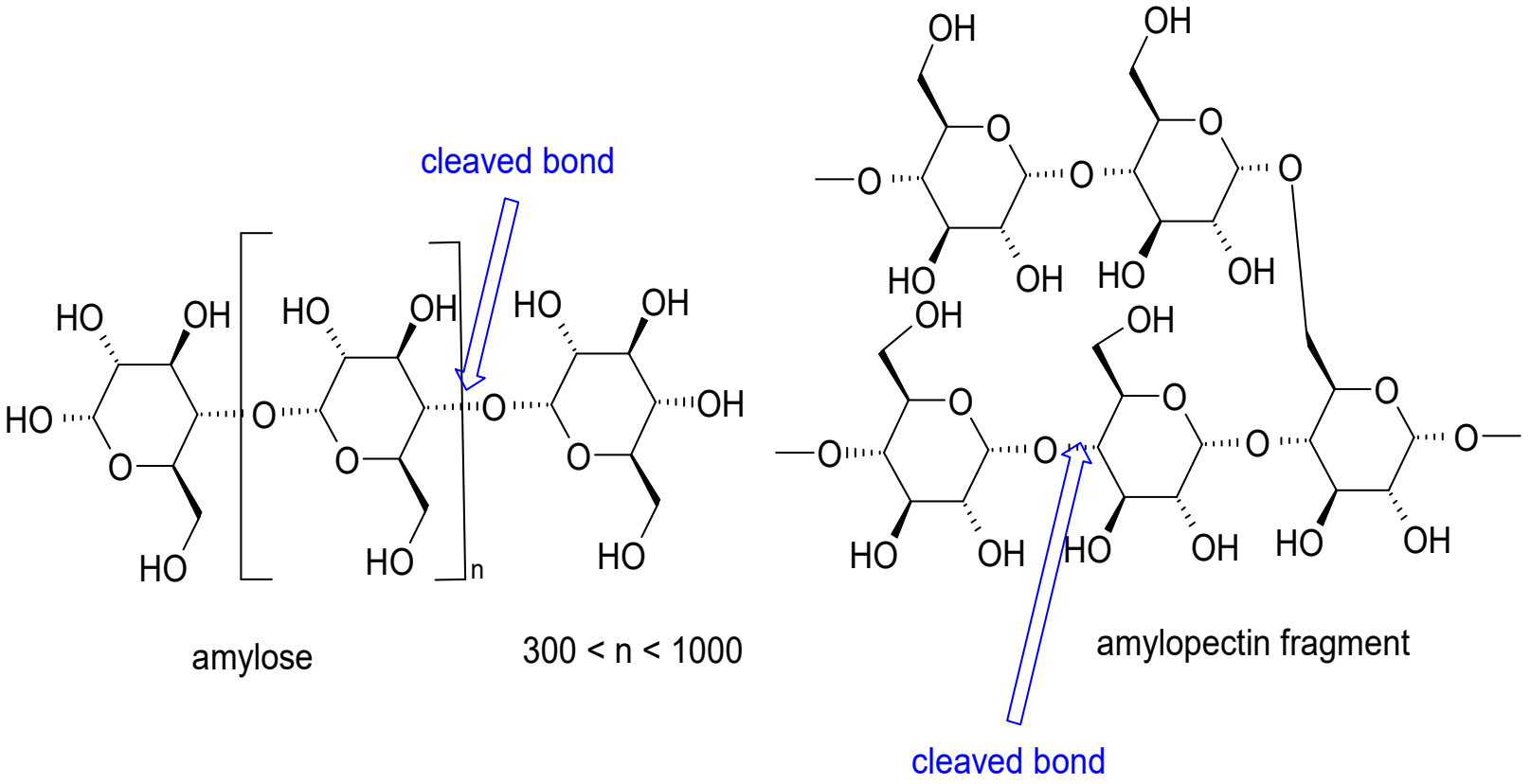
Glycosylases

Glycosidases, ie. enzymes hydrolyzing O- or S-glycosides

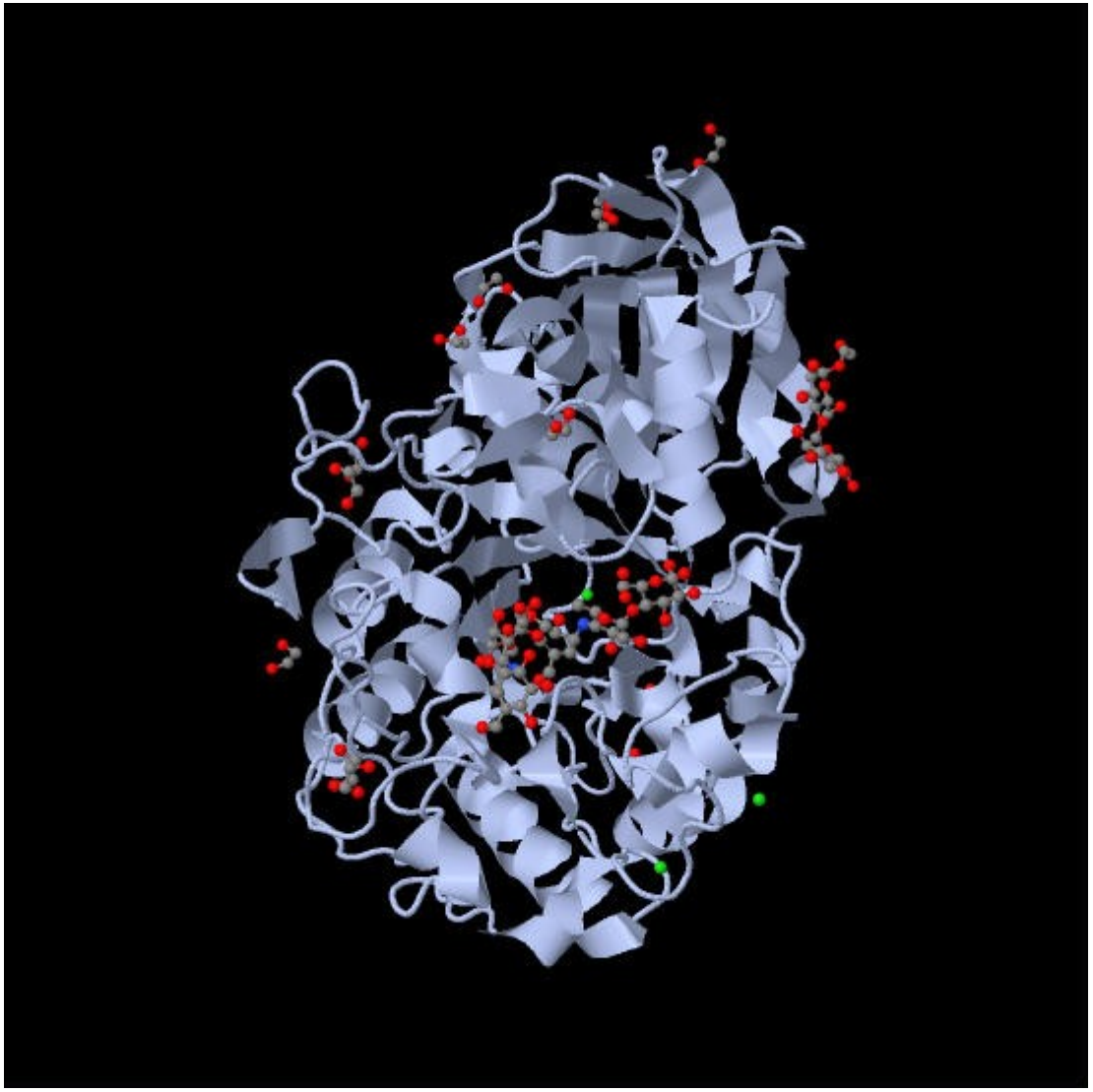
α-Amylase

syn. α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1

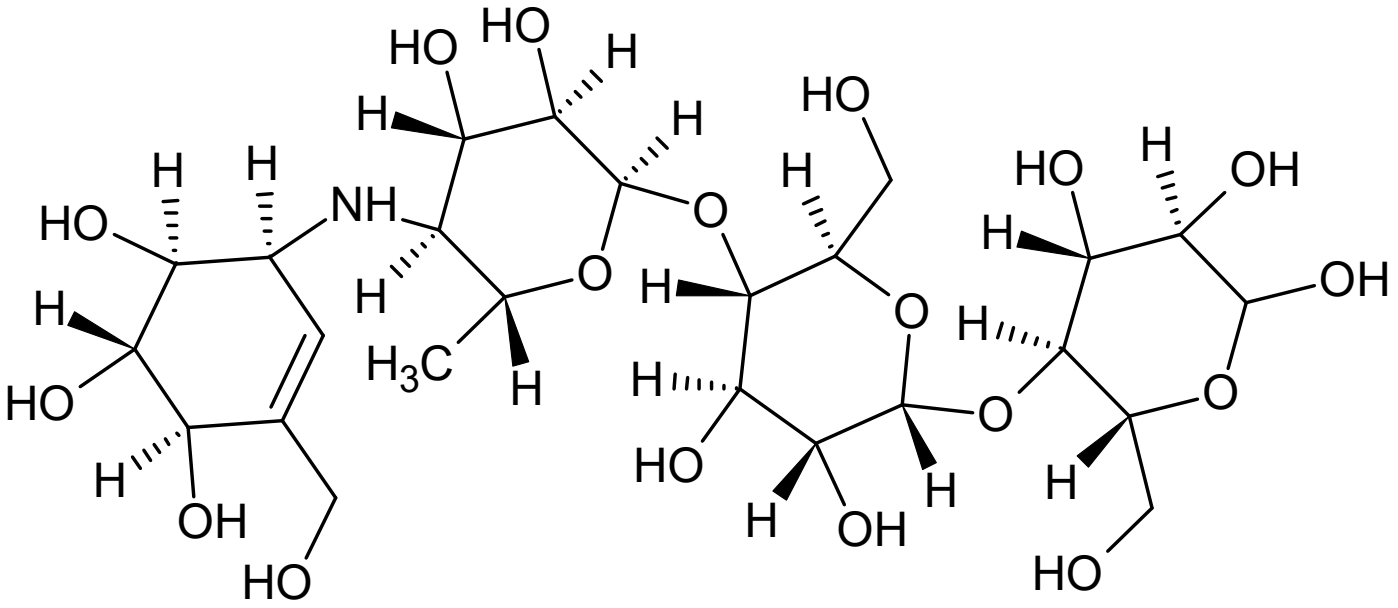
- catalyzes hydrolysis of α-(1,4)-glycoside bonds of components of starch, glycogen and various oligosaccharides
- contained in salivary glands and pancreas secretions of mammals
- tertiary structure and ability of interactions with saccharides and inhibitors of protein type are very similar in human and porcine amylase
- *endo*-amylase: hydrolyzes internal α-(1,4)-glycoside bonds of amylose and amylopectin, which leads to stepwise degradation of the substrate up to non-reducing terminus
- tertiary structure contains 3 domains: the catalytic „heart“ domain consisted from $(\beta/\alpha)_8$ helix, which contains an expanded loop inserted between the third β-chain and the third α-helix (called B domain, residues 100 – 169) and C-terminal eight-helical domain with β-helical arrangement (domain C, residues 405 – 496)
- parts of A and B domains are linked into the architecture are involved in the architecture of the three sites with the most important functions: the active site, Ca^{2+} binding site and Cl^- binding site



α–(1,4)-glycoside bond cleaved by α–amylase



Tertiary structure of α-amylase with bound „pseudotetrasachariде“ inhibitor acarbose



O-4,6-Dideoxy-4-(((1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl)amino)-α-D-glucopyranosyl-(1-4)-O-α-D-glucopyranosyl-(1-4)-D-glucose

acarbose

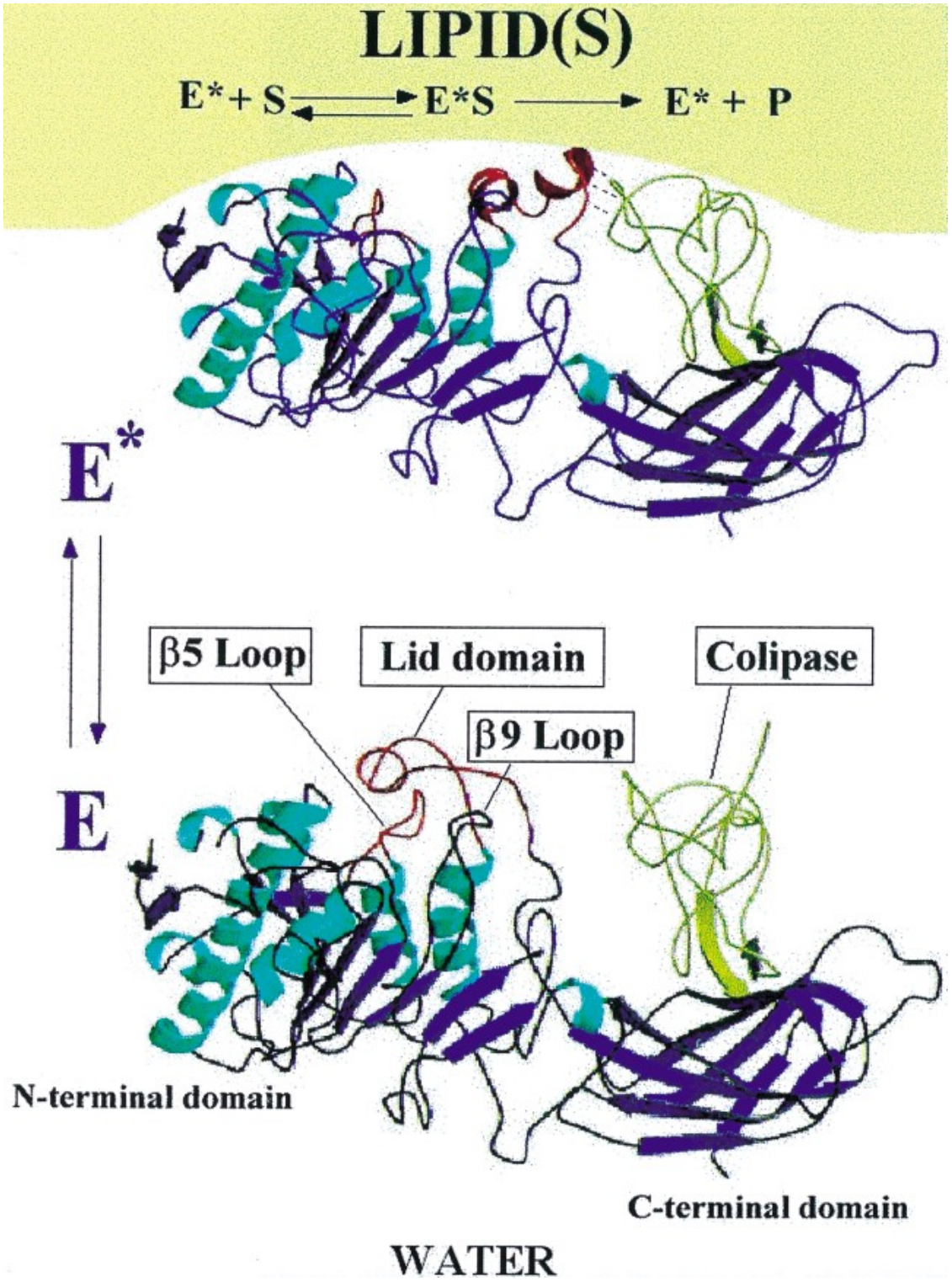
Glucobay®

•inhibitor of α-amylase – a drug for diabetes

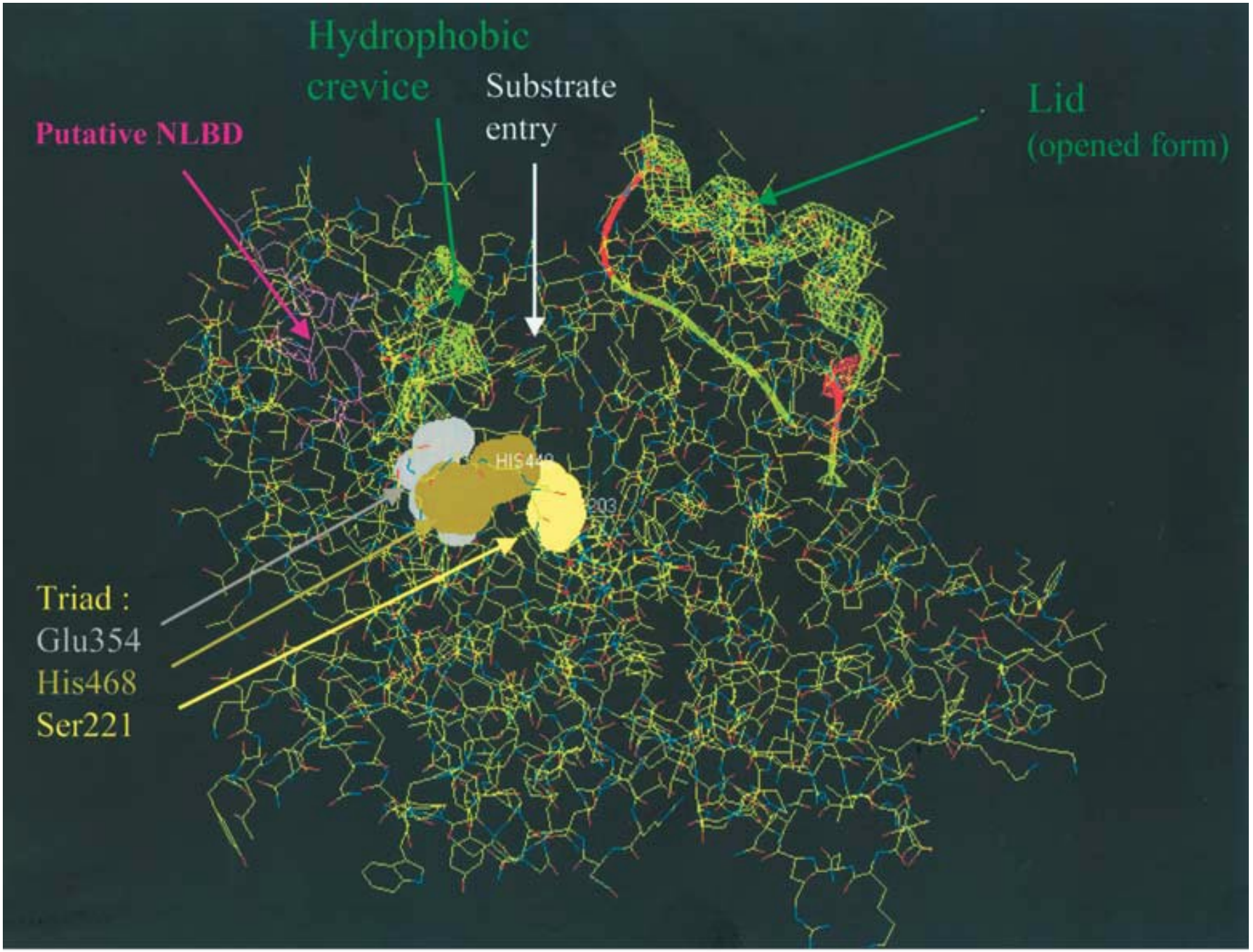
Esterhydrolases

Triacylglycerollipase, syn. triacylglycerolhydrolase, EC 3.1.1.3

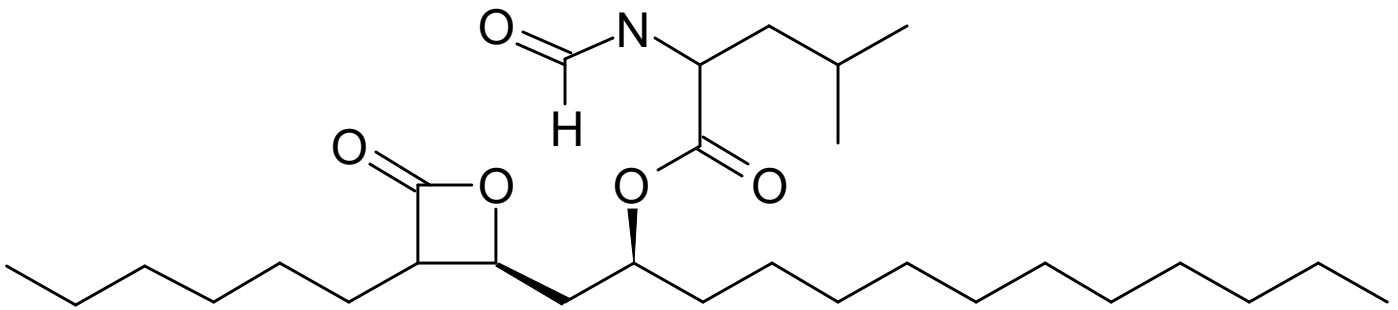
- human gastric lipase: 379 AA rests, $M_r \sim$ 45 000
- **human pancreatic lipase**: 449 AA, $M_r \sim 50\ 000$
- Asn166 has attached an oligosaccharide chain with high occurrence of mannose, or some more complex
- inter-species variability, but high homology: murine lipase sequence is in 92 % and the rat one in 93 % identical with the sequence of the human enzyme
- released in „ready-made“ form, not as a pro-enzyme
- functional at pH 4.5 – 9, optimal pH 7.0
- the active site is not available in a solution $\Rightarrow$ hydrolysis runs on interface water-lipid phase
- function needs binding of co-lipase, a protein of $M_r \sim 10\ 000$, released from pancreas; lipids are emulsified by bile acids salts, which avoid adsorption of the enzyme to their surface, co-lipase binding facilitates adsorption to the interface water-lipids covered with bile acids salts (released as procolipase, a peptid from 95 AA, activated by trypsin in the intestine)
- structure: 2 basic domains: the big N-terminal (1 - 336) and the small C-terminal (337 – 449); the big one belongs among α/β-hydrolase structures and contains the active site; superficial loop „lid“ domain covers the active site in „closed“ lipase conformation; colipase binds to the C-terminal domain and pushes hydrophobic tips of its „fingers“ to the opposite side of the domain, which leads to lid opening; co-lipase simultaneously forms a large hydrophobic plane, which interacts with phase interfaces water-lipid
- „catalytic triad“: Glu354, His468, Ser221
- porcine: M_r also $\sim$ 50 000 (50 084 exactly), 450 AA rests, glycosylation



„Closed“ (E) and „open“ (E*) lipase conformation



Quaternary structure of the lipase. Putative NLBD = proposed domain of binding of neutral lipids.



1-[(3S,4S)-3-hexyl-2-oxooxethan-4-yl]-2-(2S)tridecyl-N-formyl-2-amino-4-methylbutanoate

orlistat

Xenical®, Alli®

pancreatic lipase inhibitor – peripherally acting anobesic