Post-translacijske modifikacije proteinov (PTM)

PTM

- post-translacijske modifikacije so kovalentne kemijske modifikacije proteinov, do katerih pride po translaciji
- s temi modifikacijami se močno poveča raznolikost proteinov: iz ~23 000 genov -> tudi do 1 000 000 različnih proteinov
- okrog 5% genoma višjih evkariontov kodira encime, ki opravljajo PTM (500 kinaz, 150 fosfataz, 500 proteaz, ...)

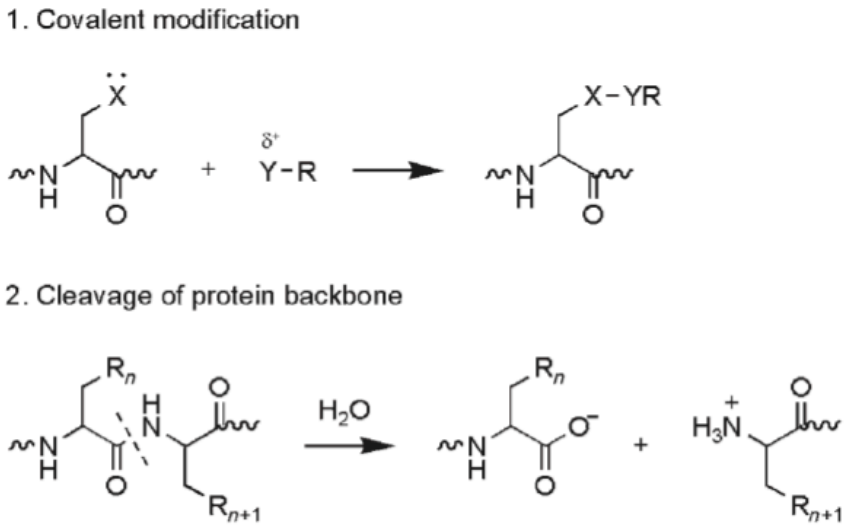
Biološke funkcije PTM

- z uvedbo novih kemijskih skupin se razširi repertoar funkcij nekega proteina
- aktivacija in inaktivacija encimov
- sprememba hidrofobnosti proteinov
- regulacija in prenos signalov
- razgradnja proteinov
- epigenetske spremembe DNA in organizacija kromosomov

...

Razdelitev PTM

- encimsko katalizirane kovalentne adicije različnih kemijskih spojin
  - razdelitev glede na aminokislino, ki se modificira
  - glede na kemijsko naravo skupine, ki se doda
  - glede na spremenjeno funkcijo PTM proteina
- proteolitična razgradnja proteinov, ki vpliva na lokalizacijo, aktivnost in življenjsko dobo proteina



PTM po aminokislinah

Residue	Reaction	Example
Asp	phosphorylation	protein tyrosine phosphatases; response regulators in two-component systems
	isomerization to isoAsp	
Glu	methylation	chemotaxis receptor proteins
	carboxylation	Gla residues in blood coagulation
	polyglycination	tubulin
	polyglutamylation	tubulin
Ser	phosphorylation	protein serine kinases and phosphatases
	O-glycosylation	notch O-glycosylation
	phosphopantetheinylation	fatty acid synthase
	autocleavages	pyruvamidyl enzyme formation
Thr	phosphorylation	protein threonine kinases/phos-phatases
	O-glycosylation	

PTM po aminokislinah

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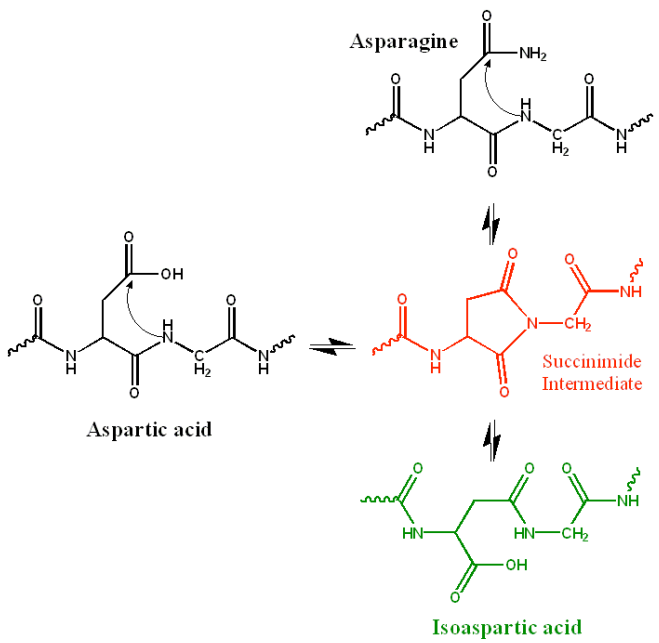
	O-glycosylation	


Asparagine
Aspartic acid
Succinimide
Intermediate
Isoaspartic acid

PTM po aminokislinah

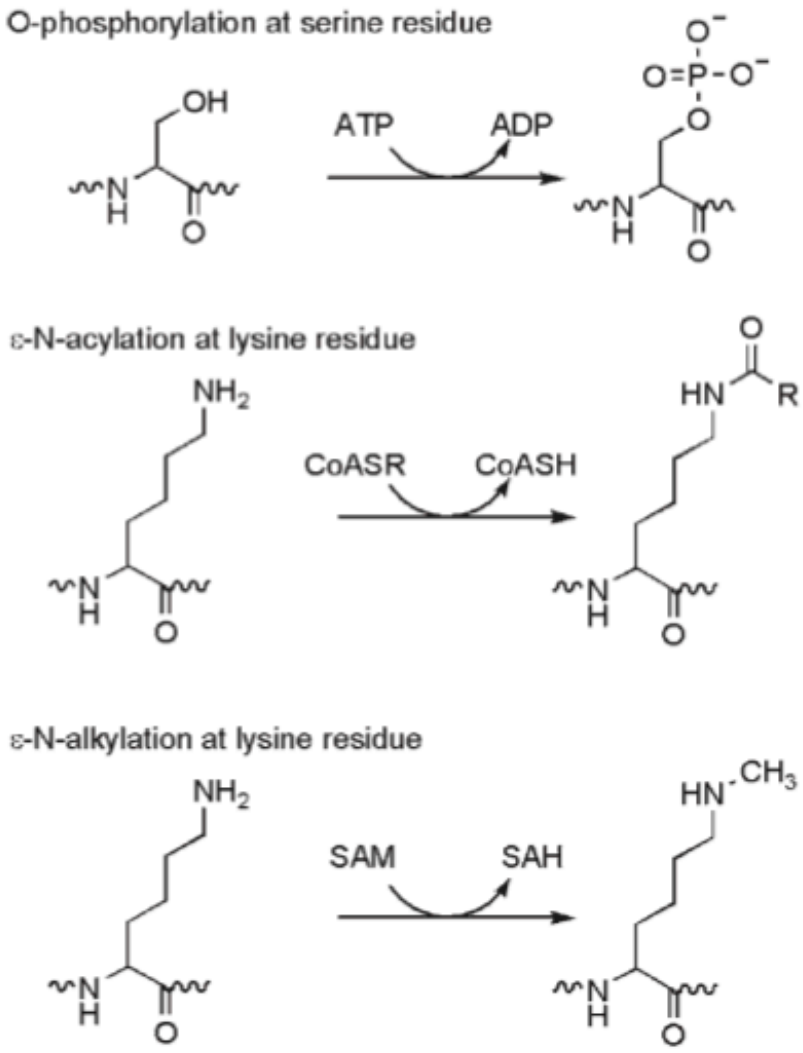
Tyr	phosphorylation	tyrosine kinases/phosphatases
	sulfation	CCR5 receptor maturation
	ortho-nitration	inflammatory responses
	TOPA quinone	amine oxidase maturation
His	phosphorylation	sensor protein kinases in two-component regulatory systems
	aminocarboxypropylation	diphthamide formation
	N-methylation	methyl CoM reductase
Lys	N-methylation	histone methylation
	N-acylation by acetyl, biotinyl, lipoyl, ubiquityl groups	histone acetylation; swinging-arm prosthetic groups; ubiquitin; SUMO (small ubiquitin-like modifier) tagging of proteins
	C-hydroxylation	collagen maturation
Cys	S-hydroxylation (S-OH)	sulfenate intermediates
	disulfide bond formation	protein in oxidizing environments
	phosphorylation	PTPases
	S-acylation	Ras
	S-prenylation	Ras
	protein splicing	intein excisions

PTM po aminokislinah

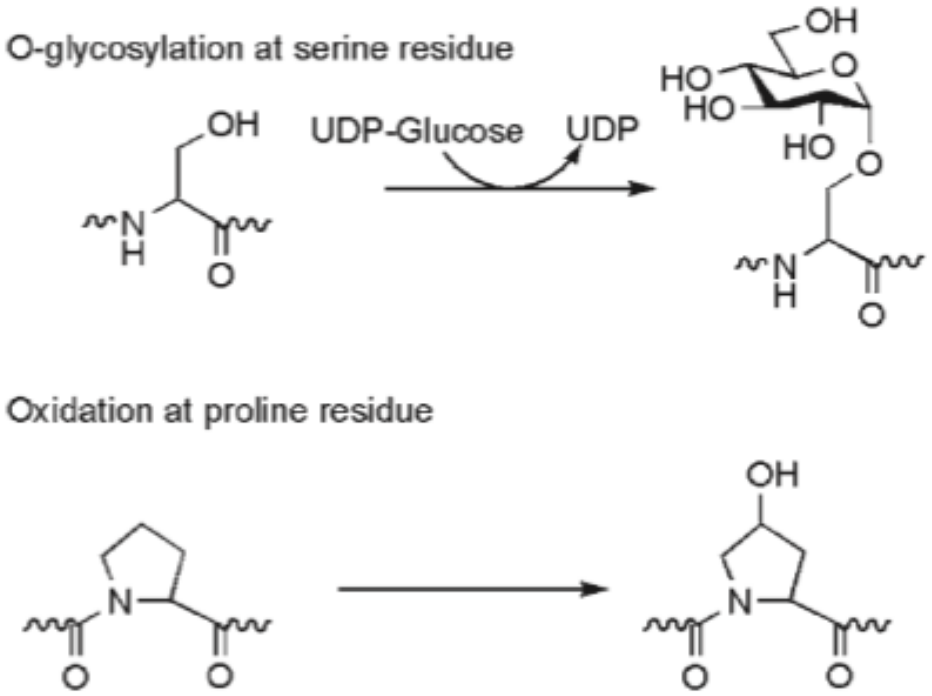
Met	oxidation to sulfoxide	Met sulfoxide reductase
Arg	N-methylation	histones
	N-ADP-ribosylation	$G_{s\alpha}$
Asn	N-glycosylation	N-glycoproteins
	N-ADP-ribosylation	eEF-2
	protein splicing	intein excision step
Gln	transglutamination	protein cross-linking
Trp	C-mannosylation	plasma-membrane proteins
Pro	C-hydroxylation	collagen; HIF-1α
Gly	C-hydroxylation	C-terminal amide formation

*Ni znanih modifikacij Leu, Ile, Val, Ala, Phe

Najpogostejše kovalentne adicije

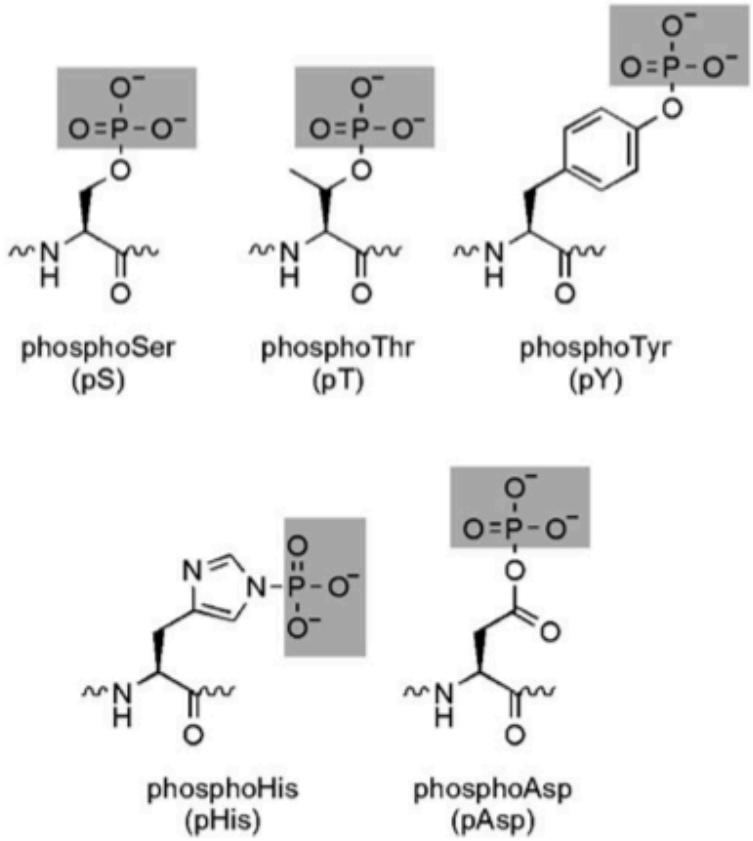


Najpogostejše kovalentne adicije



Fosforilacija

- sesalci pS, pT, pY
- bakterije in glive še pH in pD



Fosforilacija vpliva na aktivnost encimov

- fosforilacijo proteinov katalizirajo proteinske kinaze
- defosforilacija je spontana ali pa jo katalizirajo proteinske fosfataze
- Največkrat se fosforilirajo hidroksilne skupine Ser, Thr ali Tyr

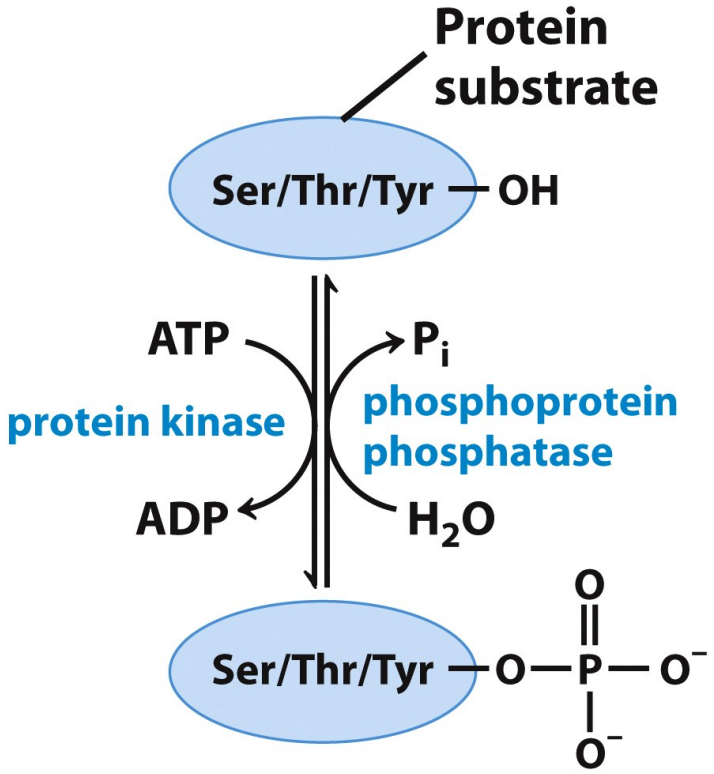
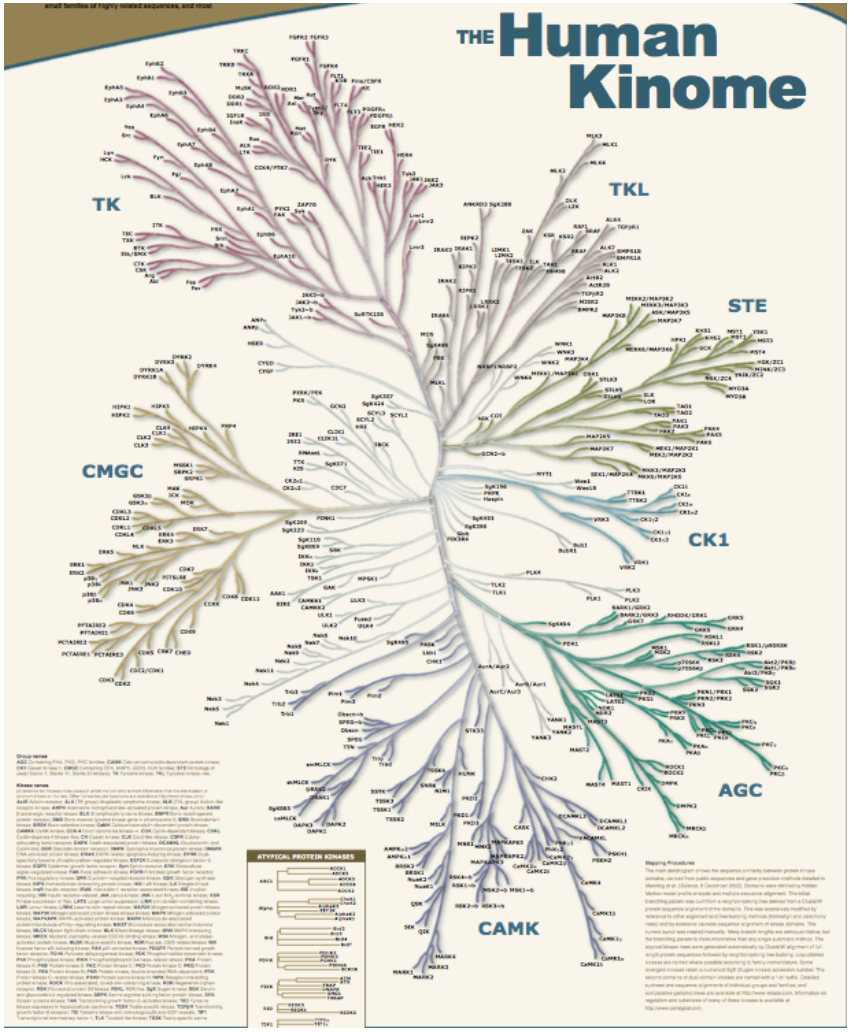
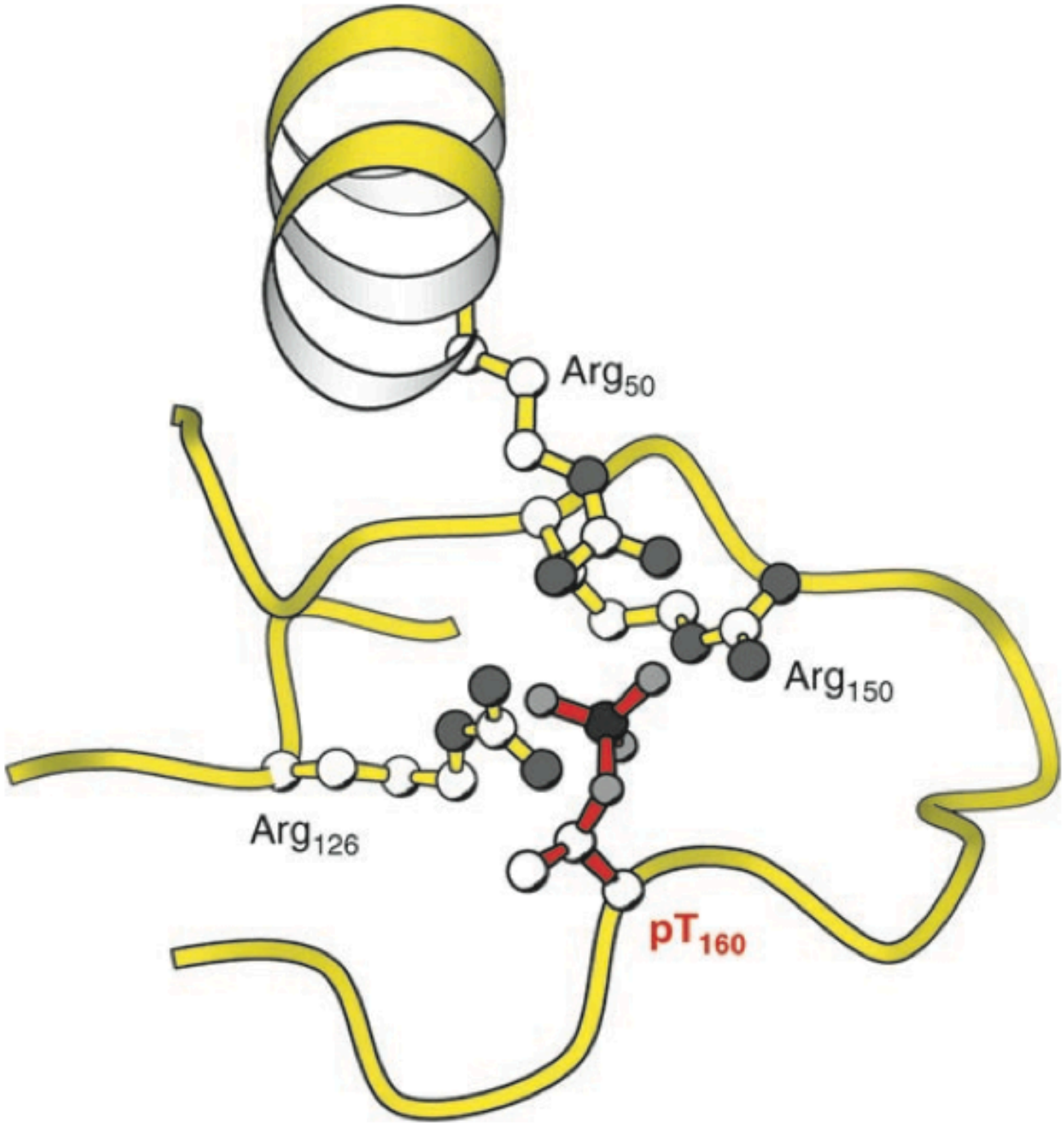


Figure 15-3
Lehninger Principles of Biochemistry, Fifth Edition

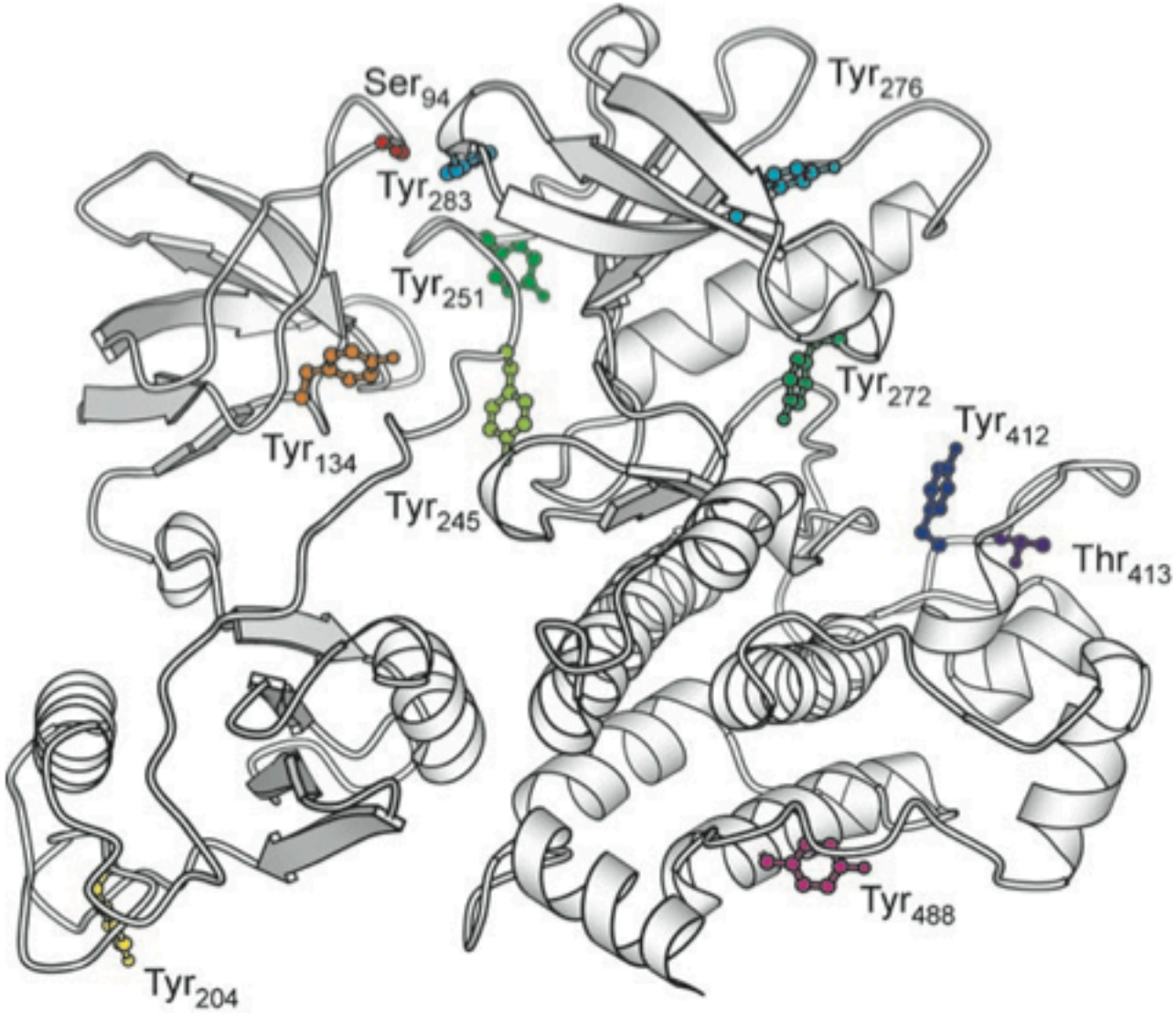
Človeški kinom

- približno 500 različnih kinaz
- veliko različnih fosforiliranih proteinov!!!
- substrati so lahko fosforilirani tudi na več mestih (npr. Abl protein kinaza ima fosforiliranih 11 različnih aminokislin)




Arg50
Arg150
Arg126
pT160

Abl-kinaza


Ser94
Tyr276
Tyr283
Tyr251
Tyr272
Tyr412
Tyr134
Tyr245
Thr413
Tyr488
Tyr204

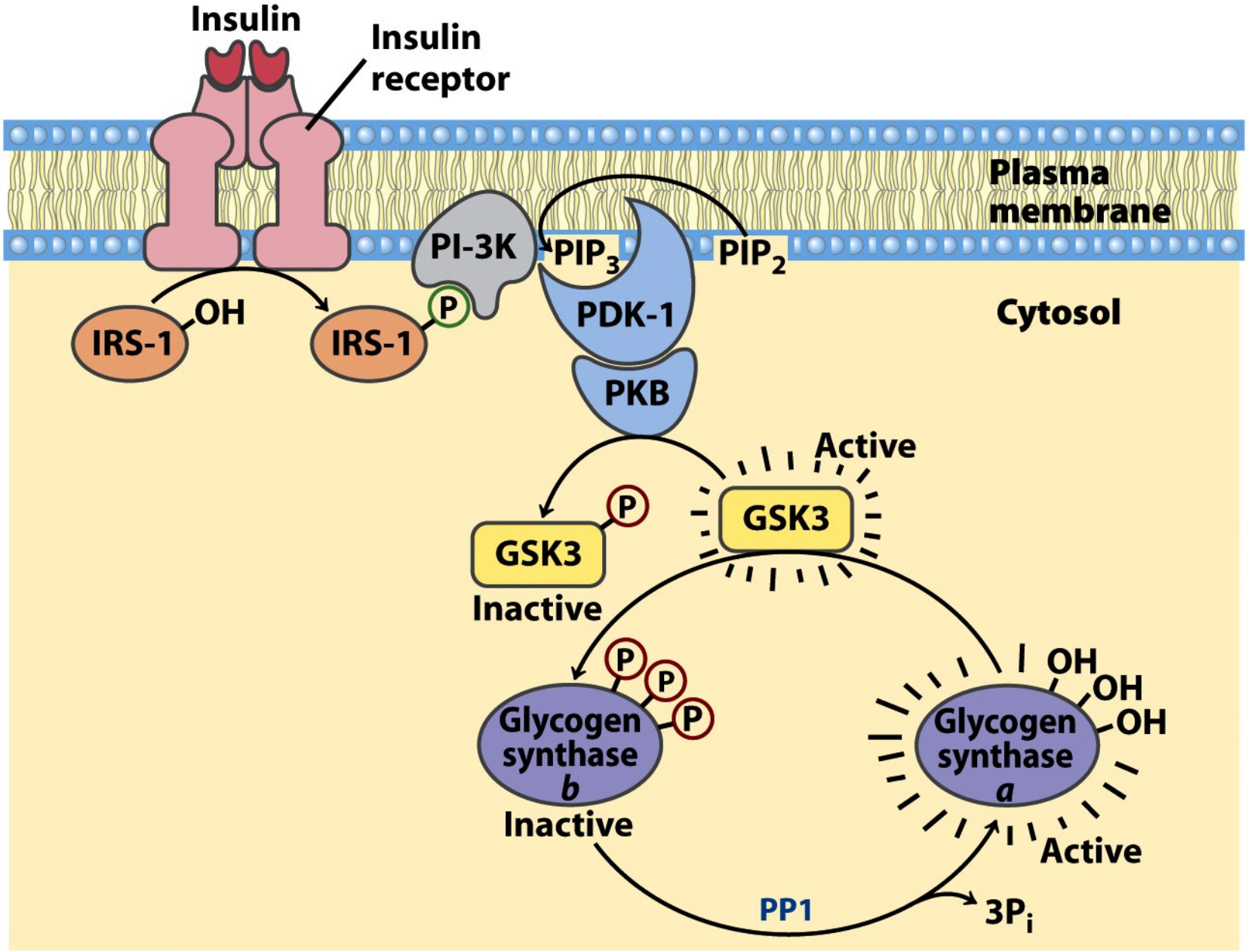
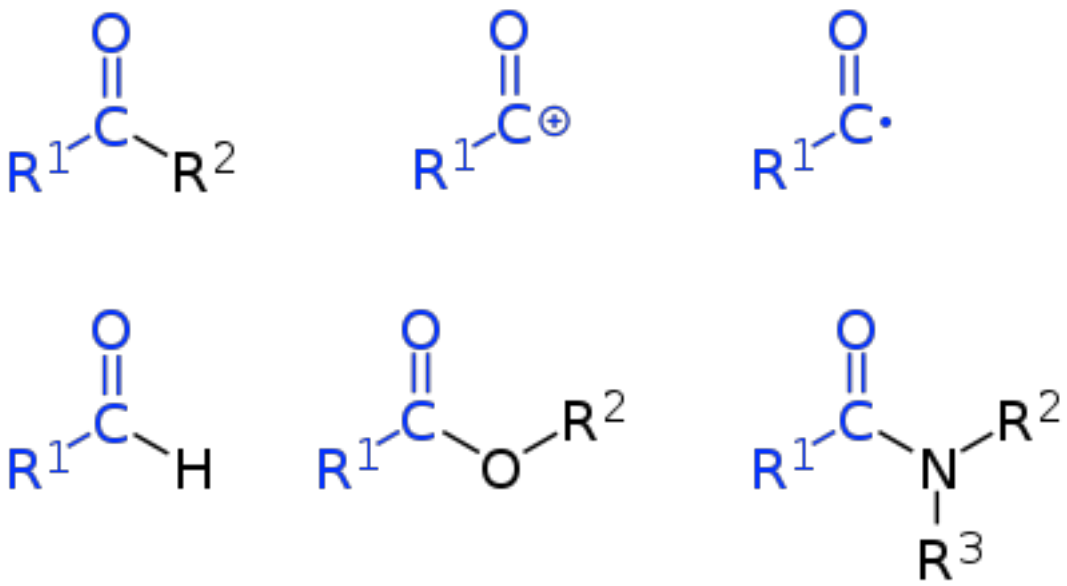


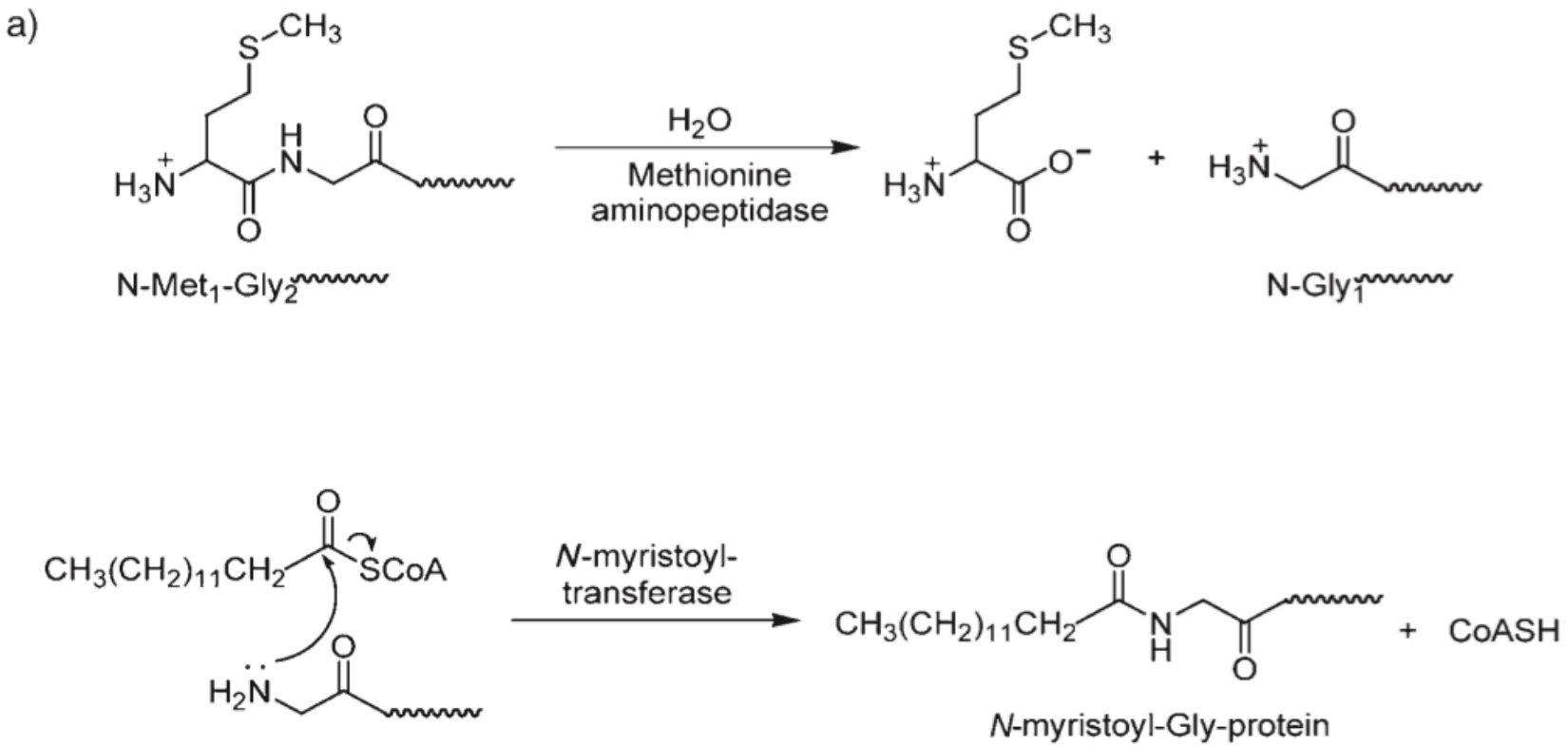
Figure 15-39
Lehninger Principles of Biochemistry, Fifth Edition

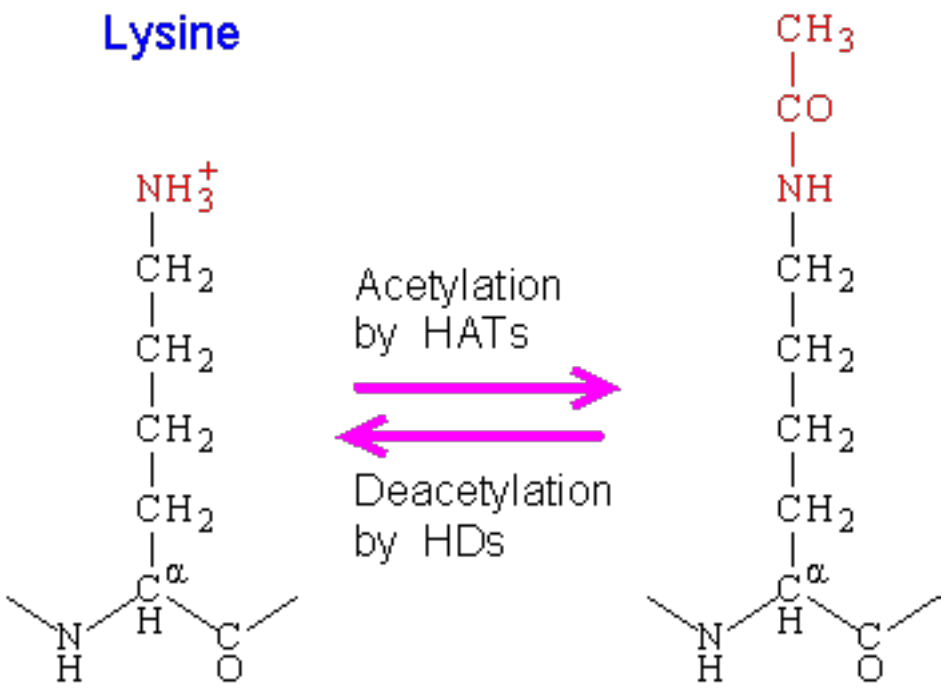
fosfoprotein fosfataza 1 (3x)

Acilacija proteinov

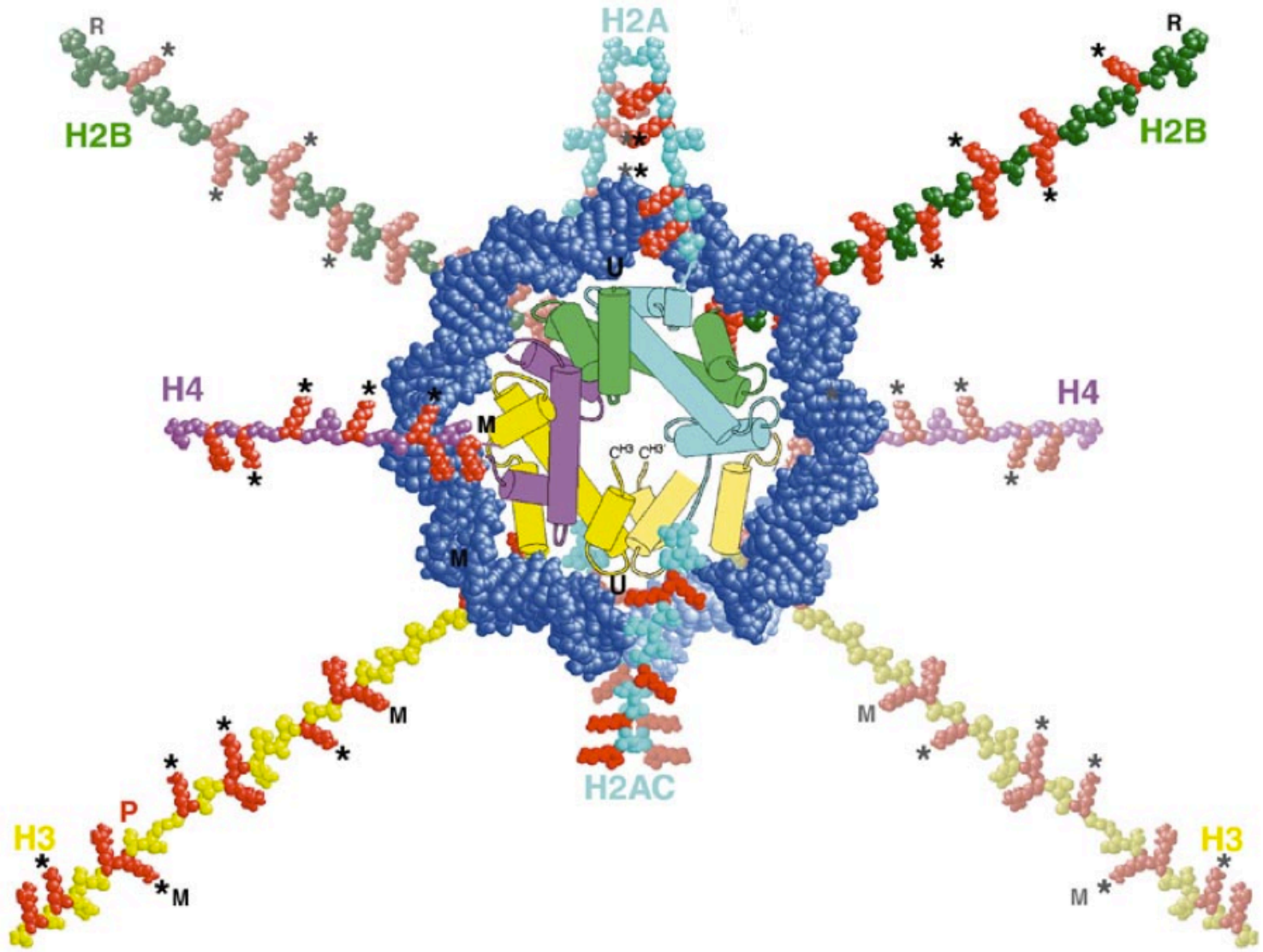
- najpogosteje acetilacija (C2), npr. acetilacija histonov
- miristoilacija (C14) glicinskih N-začetkov
- palmitoilacija (C16) cisteinov (-S)




a)
S
CH3
H3N+
H
N
O
O
N-Met1-Gly2
H2O
Methionine
aminopeptidase
O-
+
N-Gly1
CH3(CH2)11CH2
SCoA
H2N
N-myristoyl-
transferase
CH3(CH2)11CH2
N
H
+ CoASH
N-myristoyl-Gly-protein
b)
CH3(CH2)14
SCoA
S-
N
H
S-palmitoyl-
transferase
(CH2)14CH3
+ CoASH


Lysine
Acetylation
by HATs
Deacetylation
by HDs

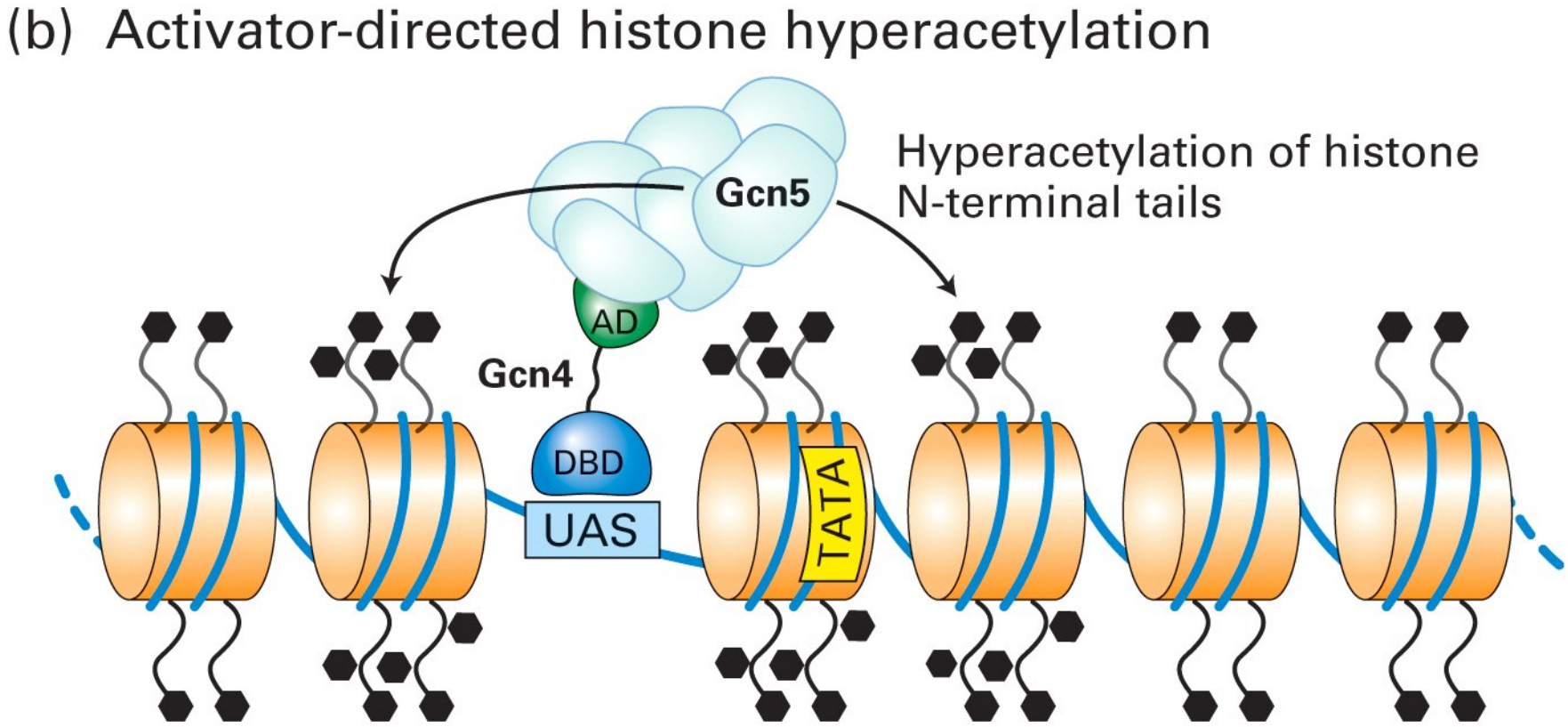
Acetilacija histonskih repov



14x2=28 potencialnih mest/oktamerno jedro

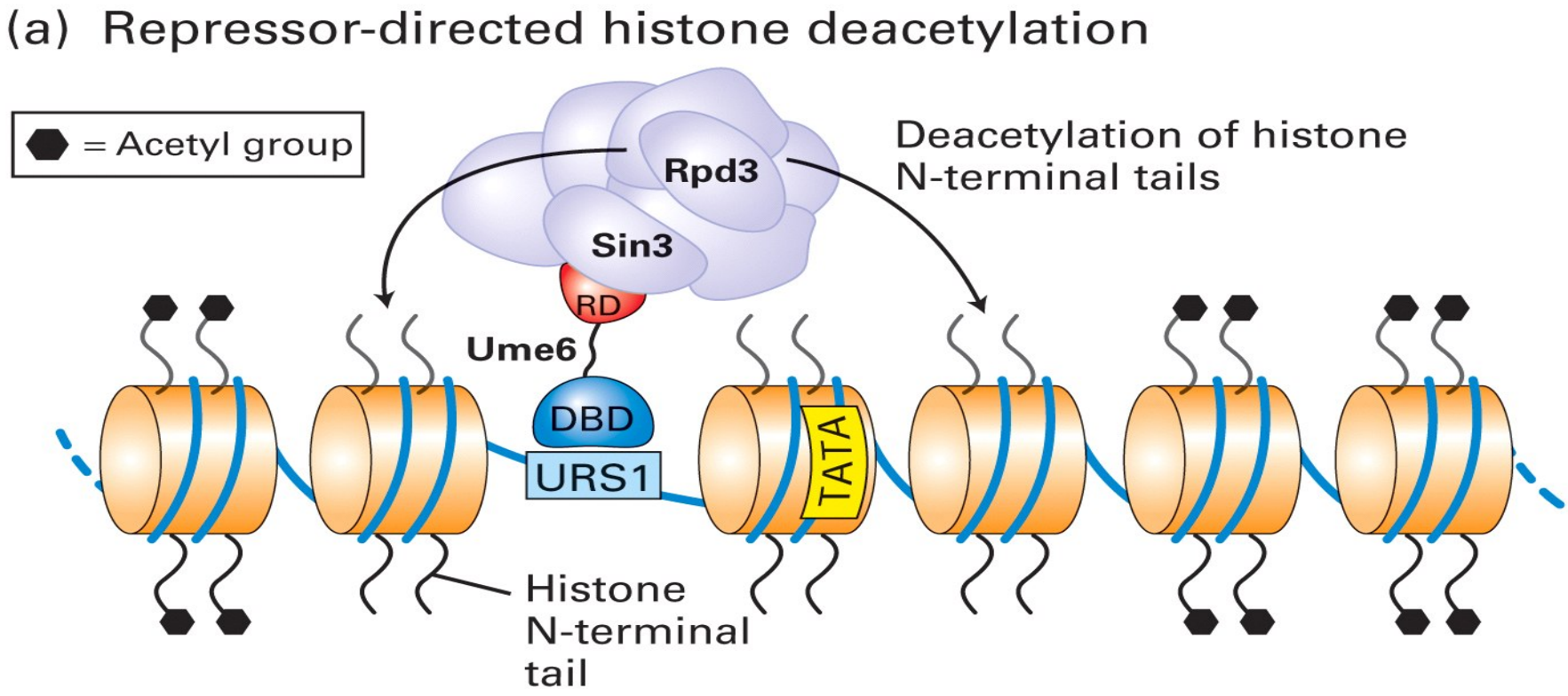
Wolffe and Hayes, NAR 27, 1999

Aktivatorji regulirajo izražanje genov z acetilacijo histonov (kromatin se "odpre"- omogoči aktivacijo transkripcije)



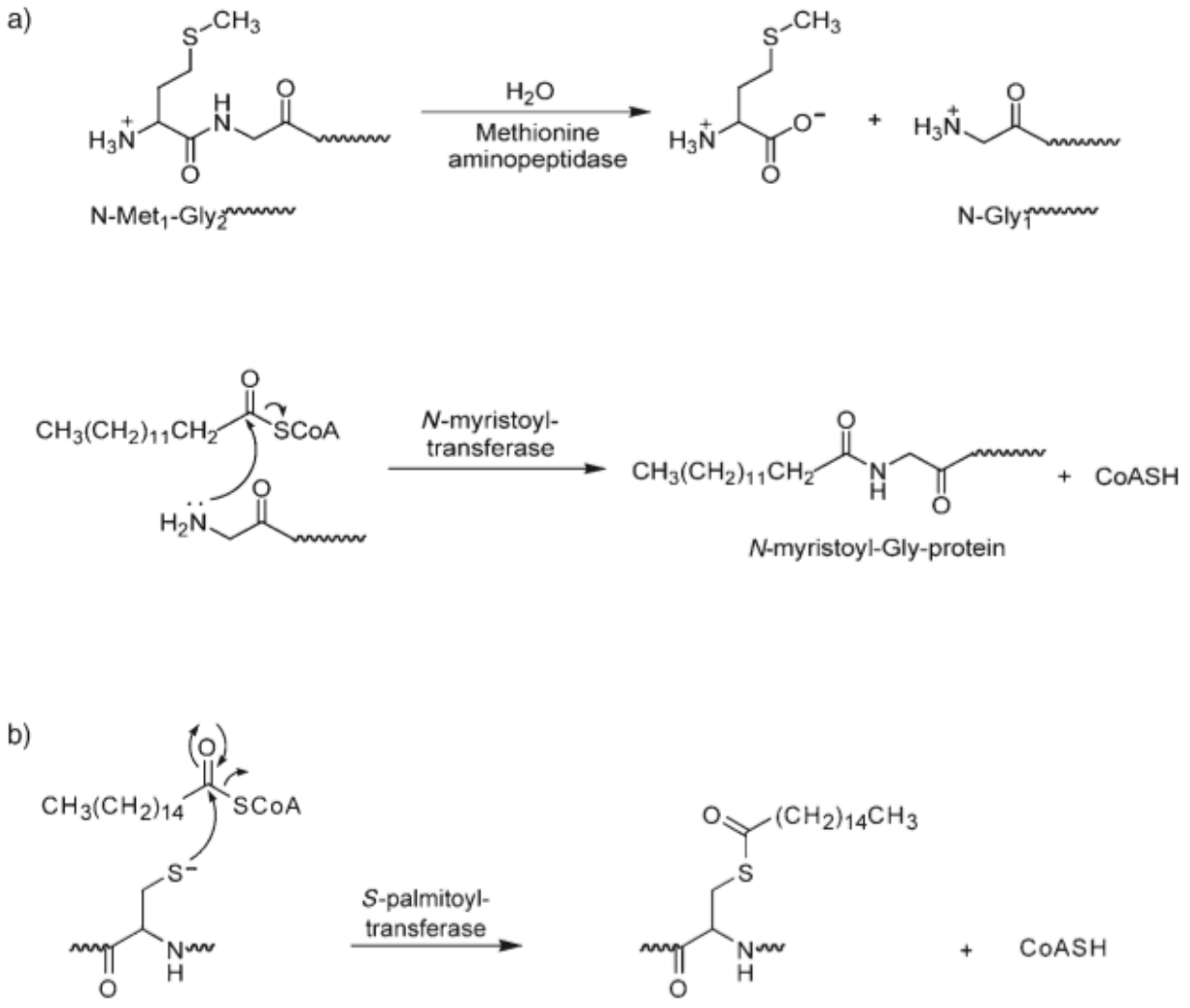
-The DBD of Activators like Gcn4 bind their Upstream Activating Sequence (UAS).
-Activation Domain (AD) attracts protein complexes containing histone acetylases (Gcn5)
-Subsequent acetylation of histone tails serve to open up chromatin.
-Thus HDACs and HATs are important global regulators of transcription

Represorji regulirajo izražanje genov z deacetilacijo histonov (kromatin se "zapre")



-The DBD of repressors (like Ume6) bind a DNA element (URS1) and the Repression Domain (RD) recruits a protein complex containing a histone deacetylase like Rpd 3.
-The subsequent deacetylation of histone N-terminal tails results in chromatin condensation which promotes gene repression.

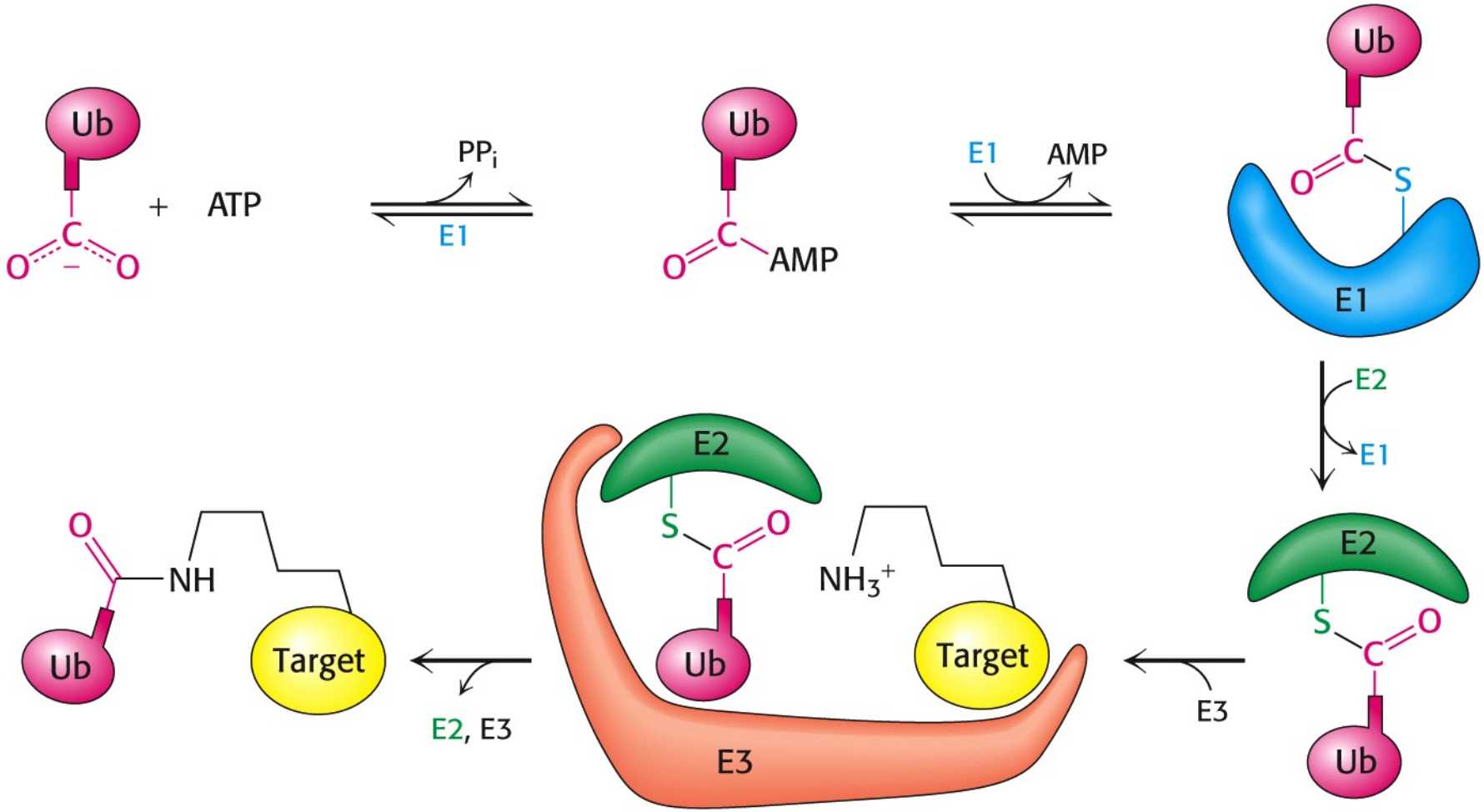
N-miristoilacija in S-palmitoilacija

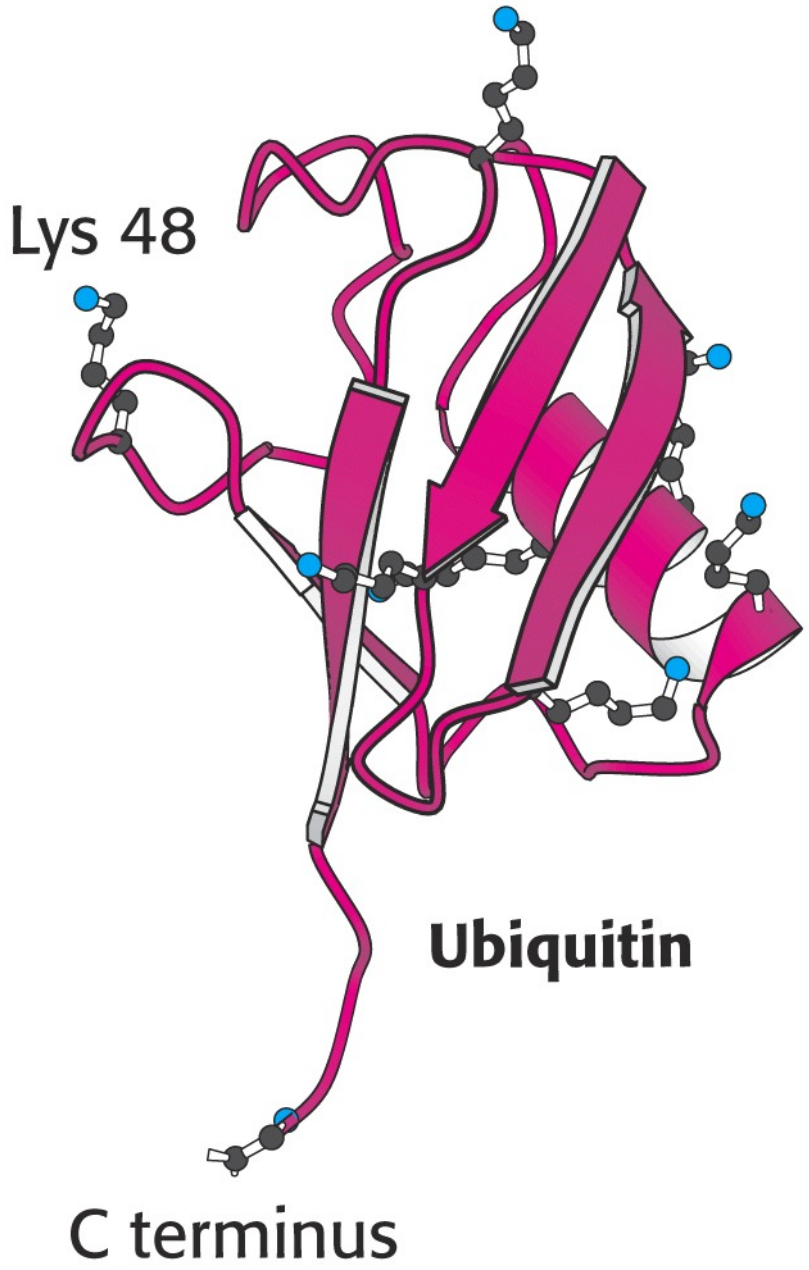


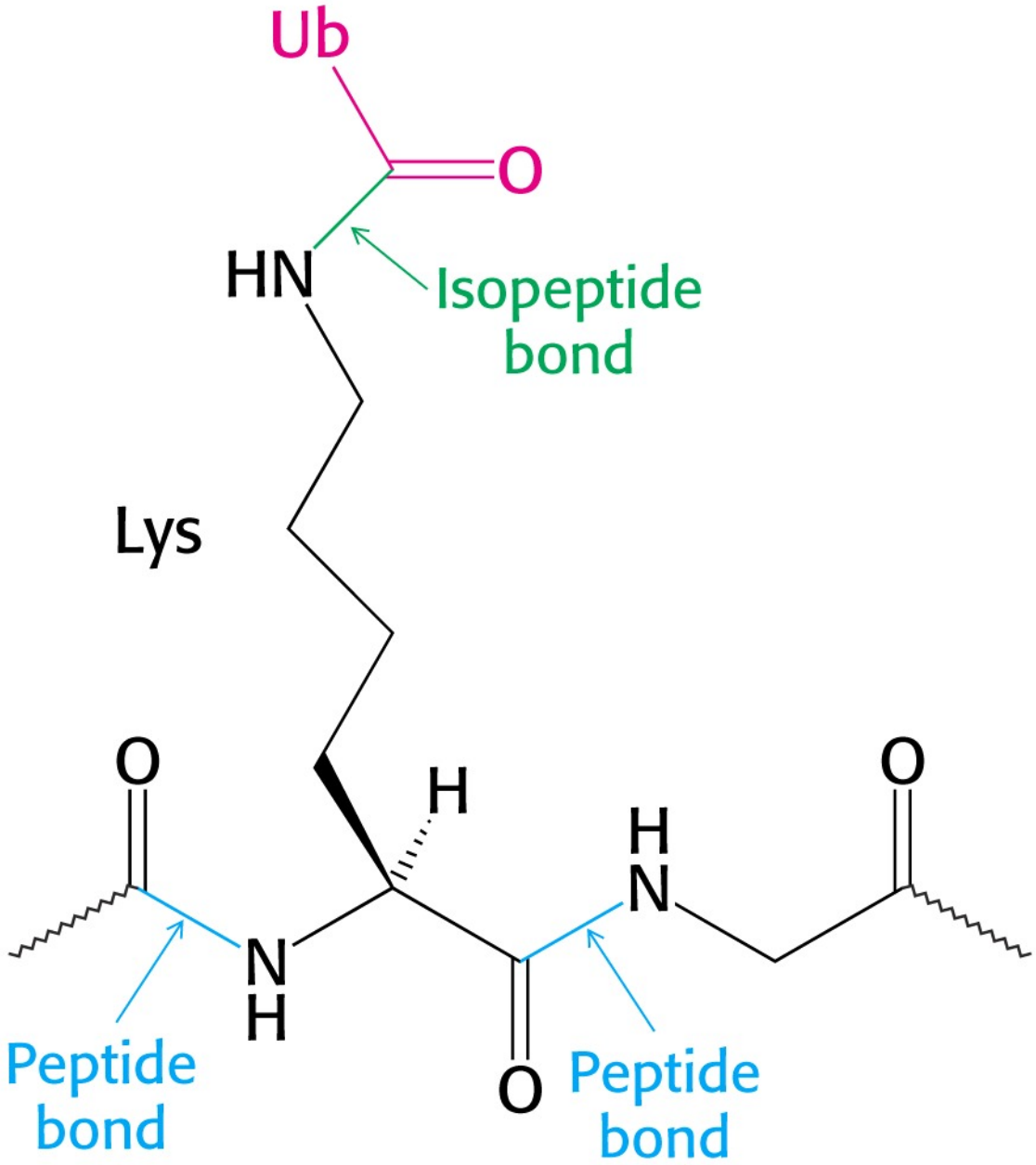
sidro za membrano

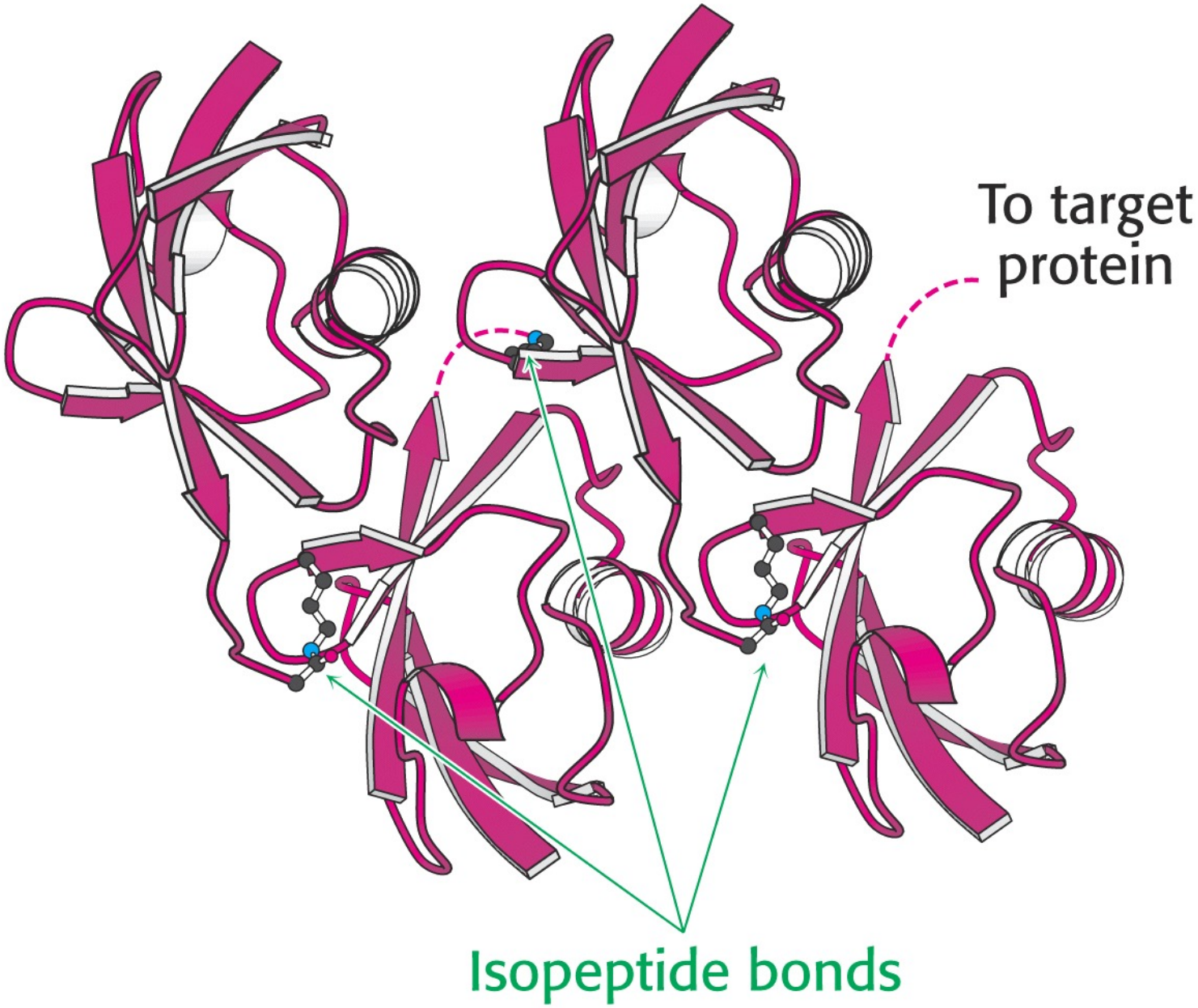
Ubikvitinacija

Pripenjanje 76-AK dolgega ubikvitina preko njegovega C-konca na Lys



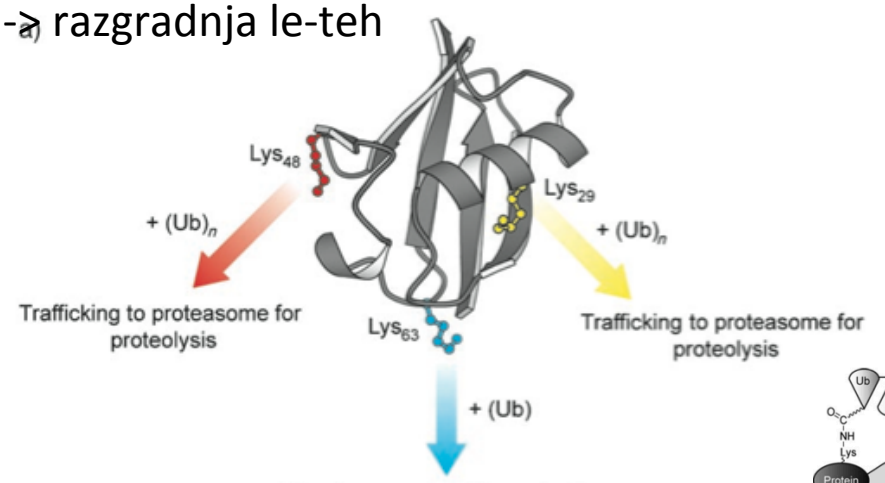

Lys 48
Ubiquitin
C terminus

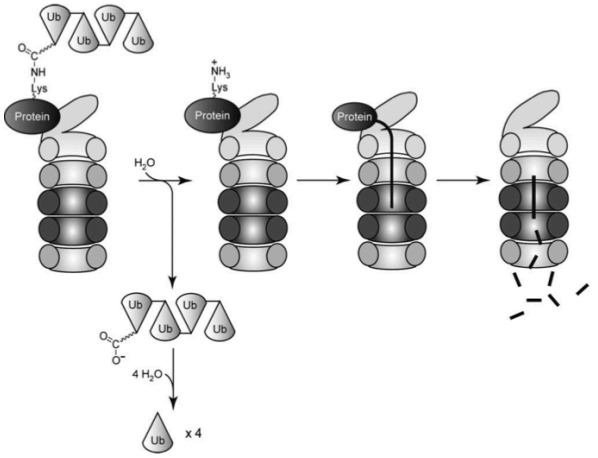

Ub
O
HN
Isopeptide bond
Lys
O
H
H
N
O
N
H
Peptide bond
O
Peptide bond

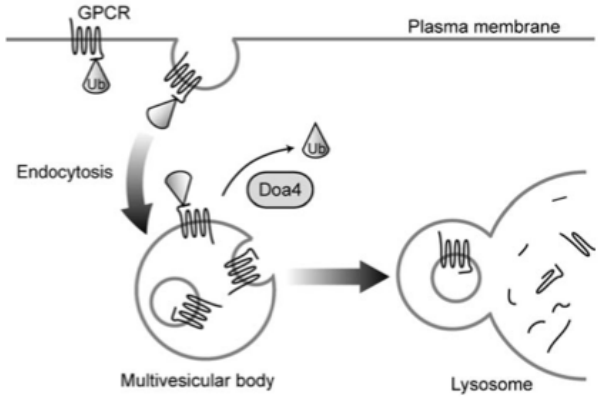

To target protein
Isopeptide bonds

Ubikvitinacija

Ubikvitinacija proteinov -> razgradnja le-teh

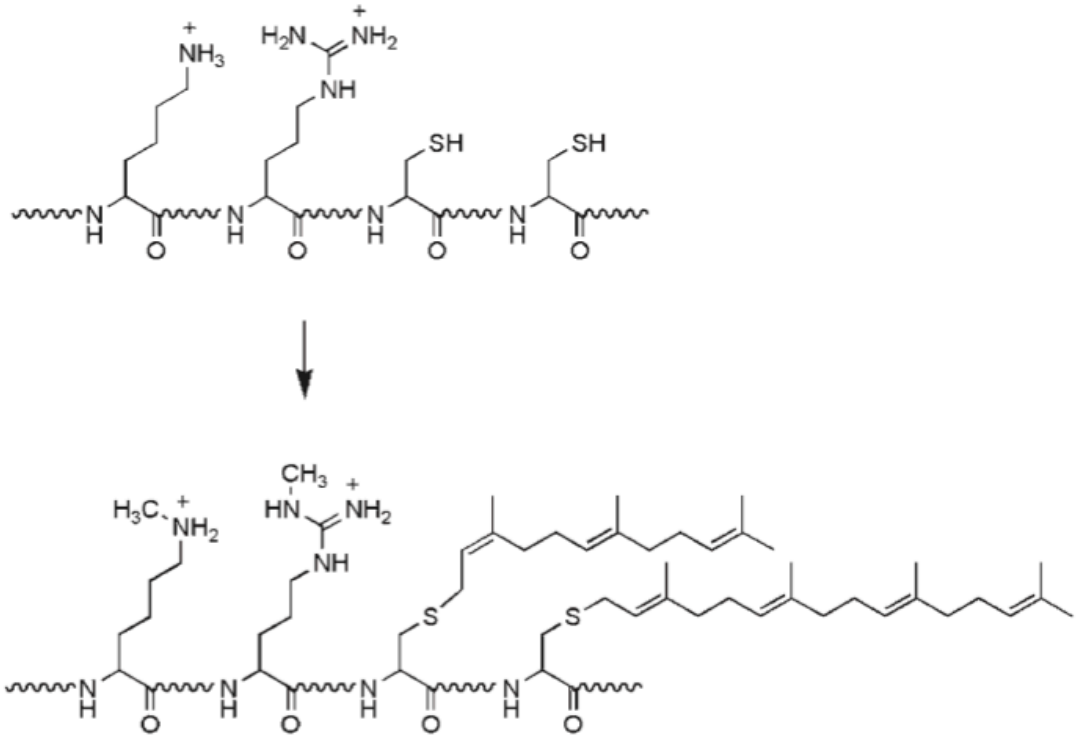






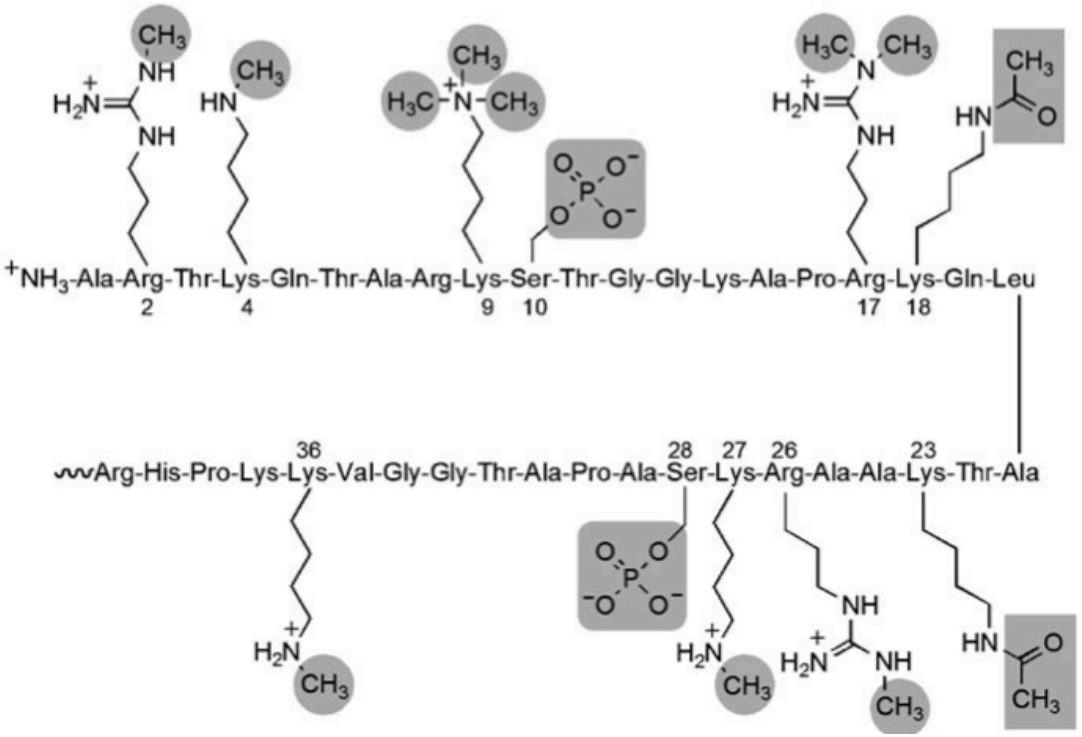
Alkilacija proteinov

- najpogosteje se prenesejo C1 ter C15 in C20 izoprenske skupine
- poveča se hidrofobnost proteina



Metilacija

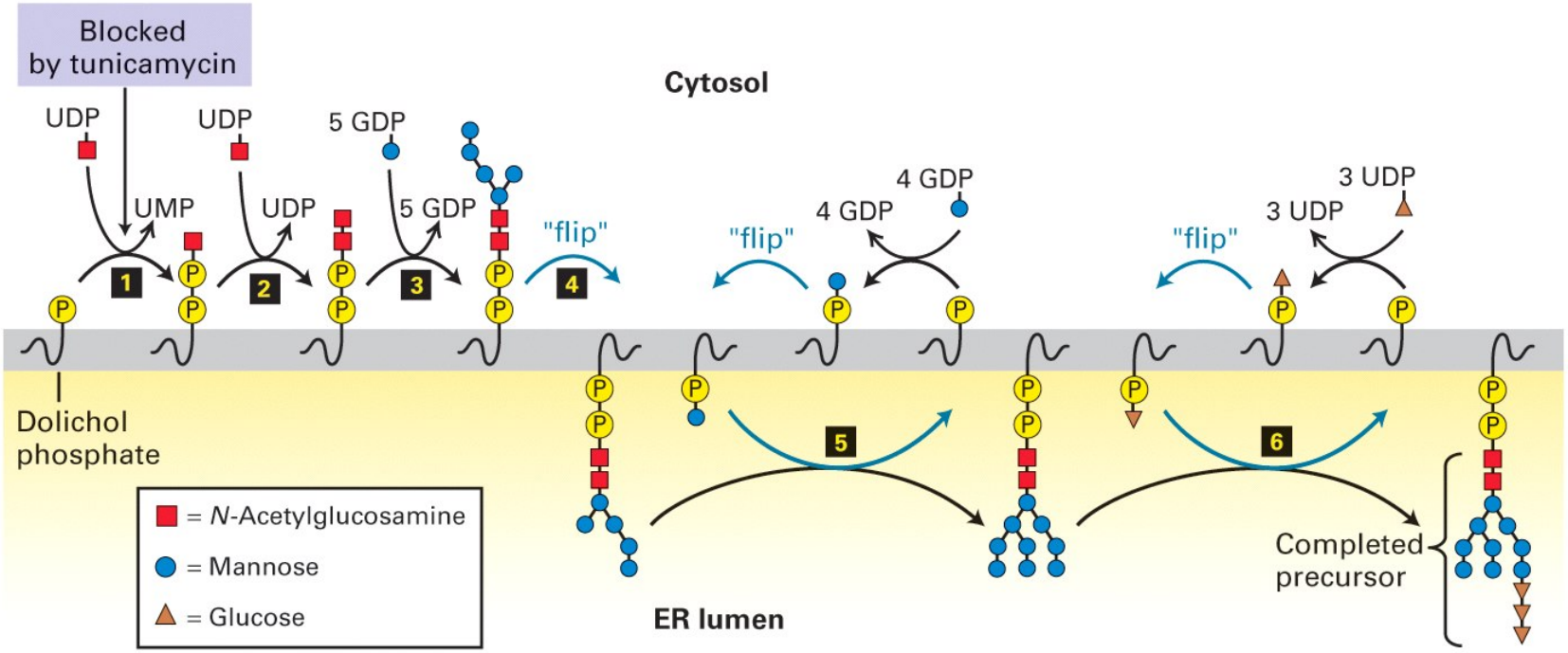
- N-metilacija Lys in Arg je najpogostejša, redkeje srečamo še C-, O- in S-metilacijo
- N-metilacija histonov Lys (mono-, di-, tri-metiliran), Arg (mono-, di-metiliran)- veliko kombinacij

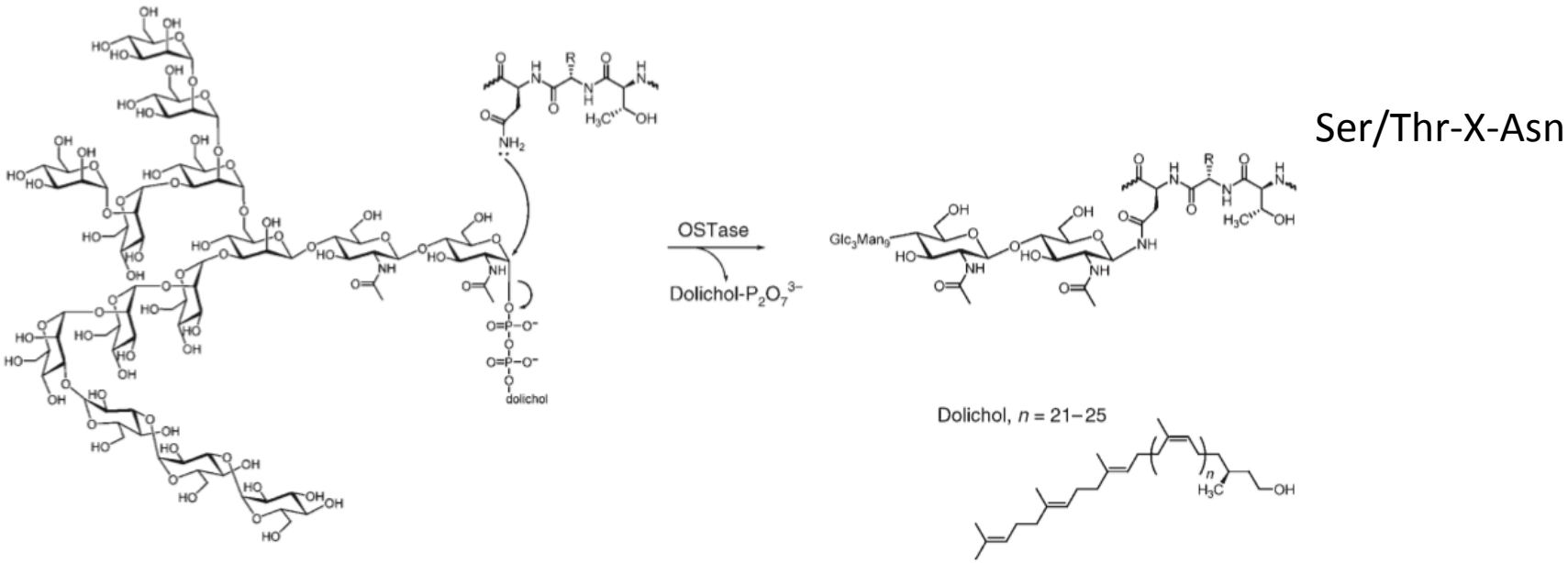


Glikozilacija proteinov

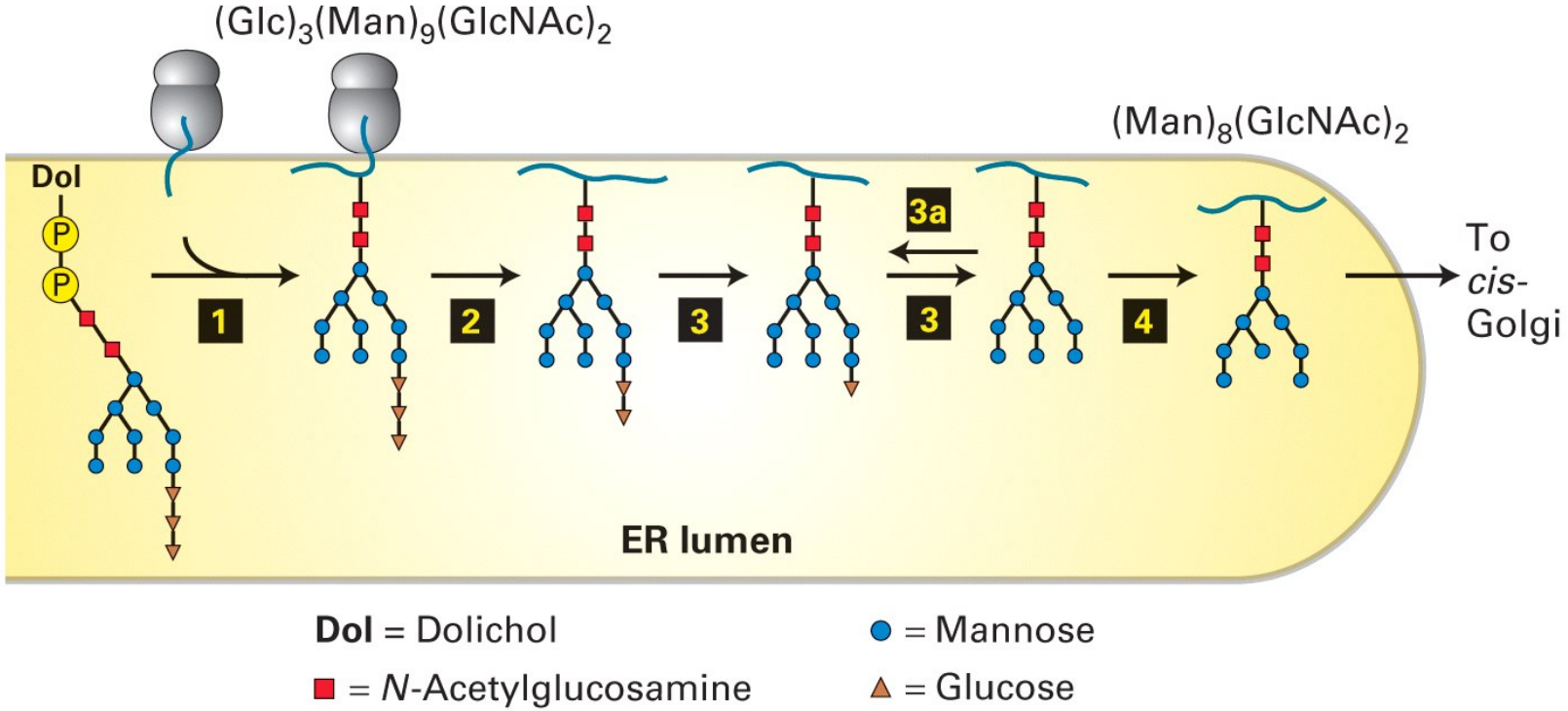
- redko pri prokariontih, zelo pogosto pri evkariontih
- N-glikozilacija asparagina, skoraj vedno v sekvenci Ser/Thr-X-Asn, lahko pa tudi na Arg
- O-glikozilacija- na aminokisline z –OH skupino
- redko C-glikozilacija na Trp

N-glikozilacija

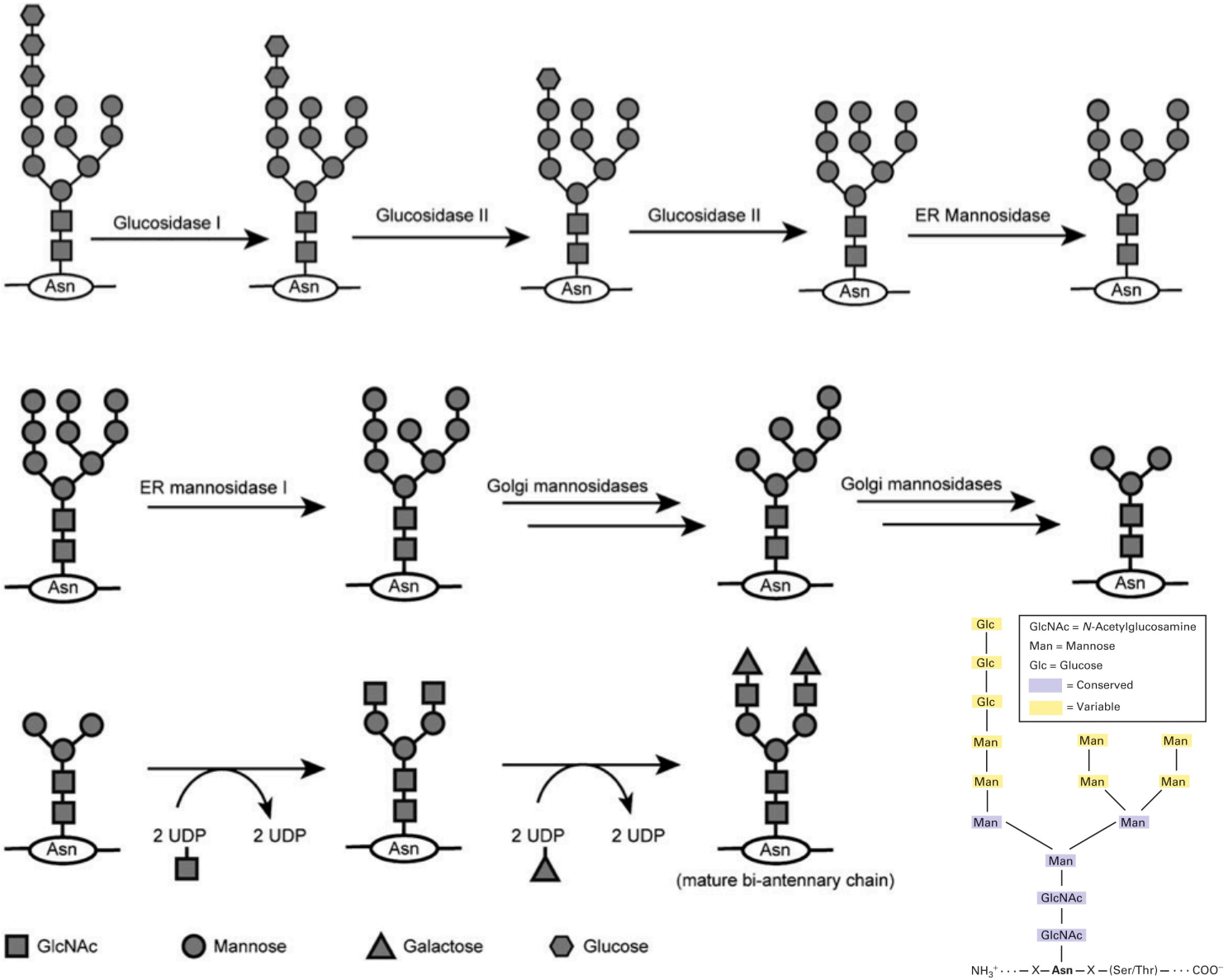



OSTase
Dolichol-$P_2O_7^{3-}$
dolichol
Glc_3Man_9
Dolichol, n = 21–25

Ser/Thr-X-Asn

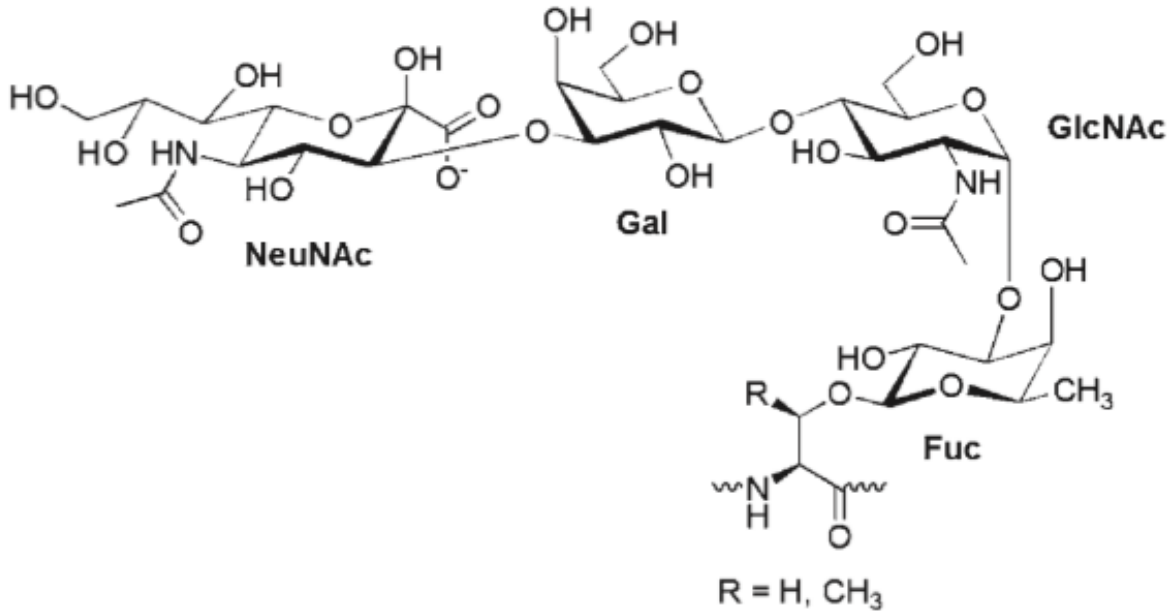

$(Glc)_3(Man)_9(GlcNAc)_2$
$(Man)_8(GlcNAc)_2$
Dol
P
P
1
2
3
3a
3
4
To *cis*-Golgi
ER lumen
Dol = Dolichol
= Mannose
= *N*-Acetylglucosamine
= Glucose

N-glikozilacija

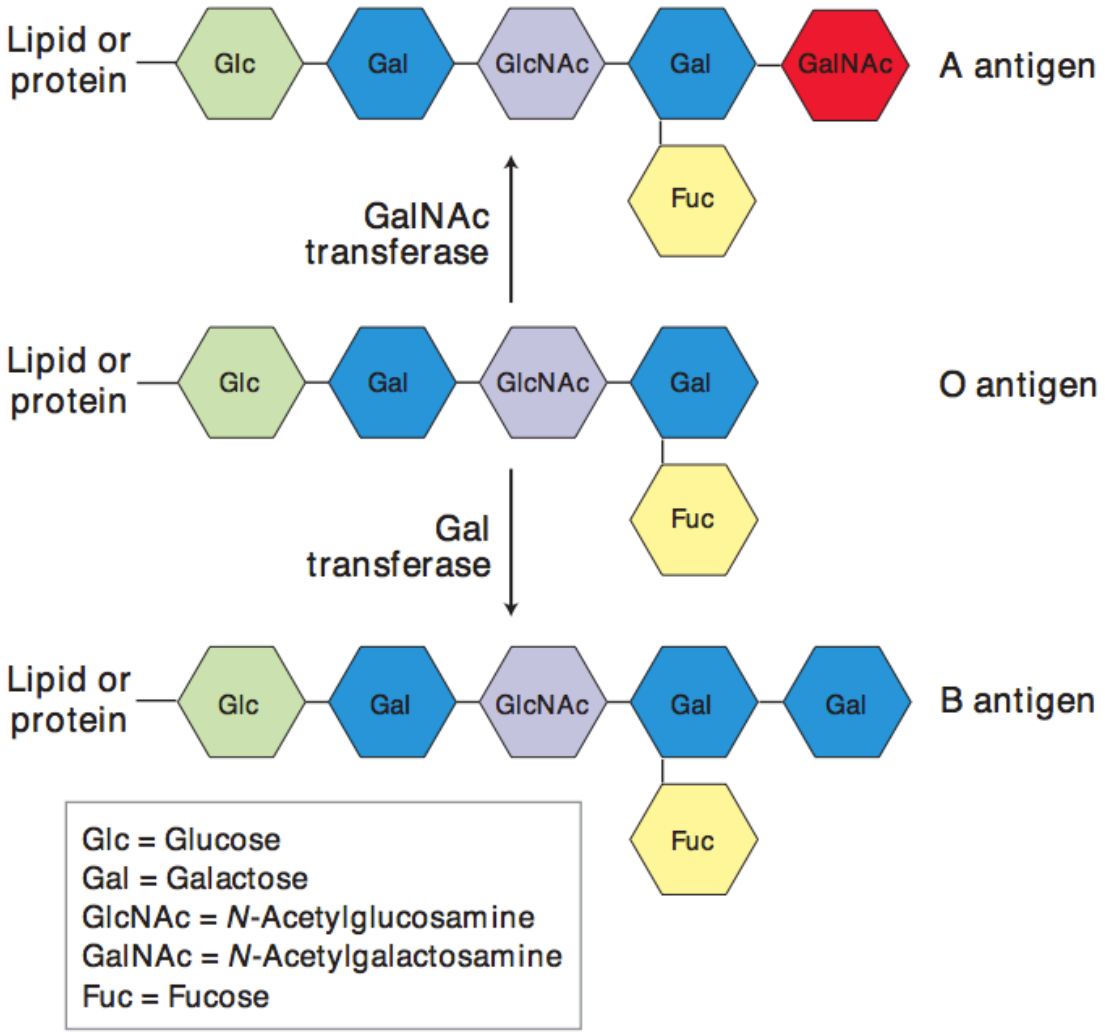


O-glikozilacija

- največkrat krajše verige in manj kompleksne od N-glikozilacije, primer so antigeni krvnih skupin



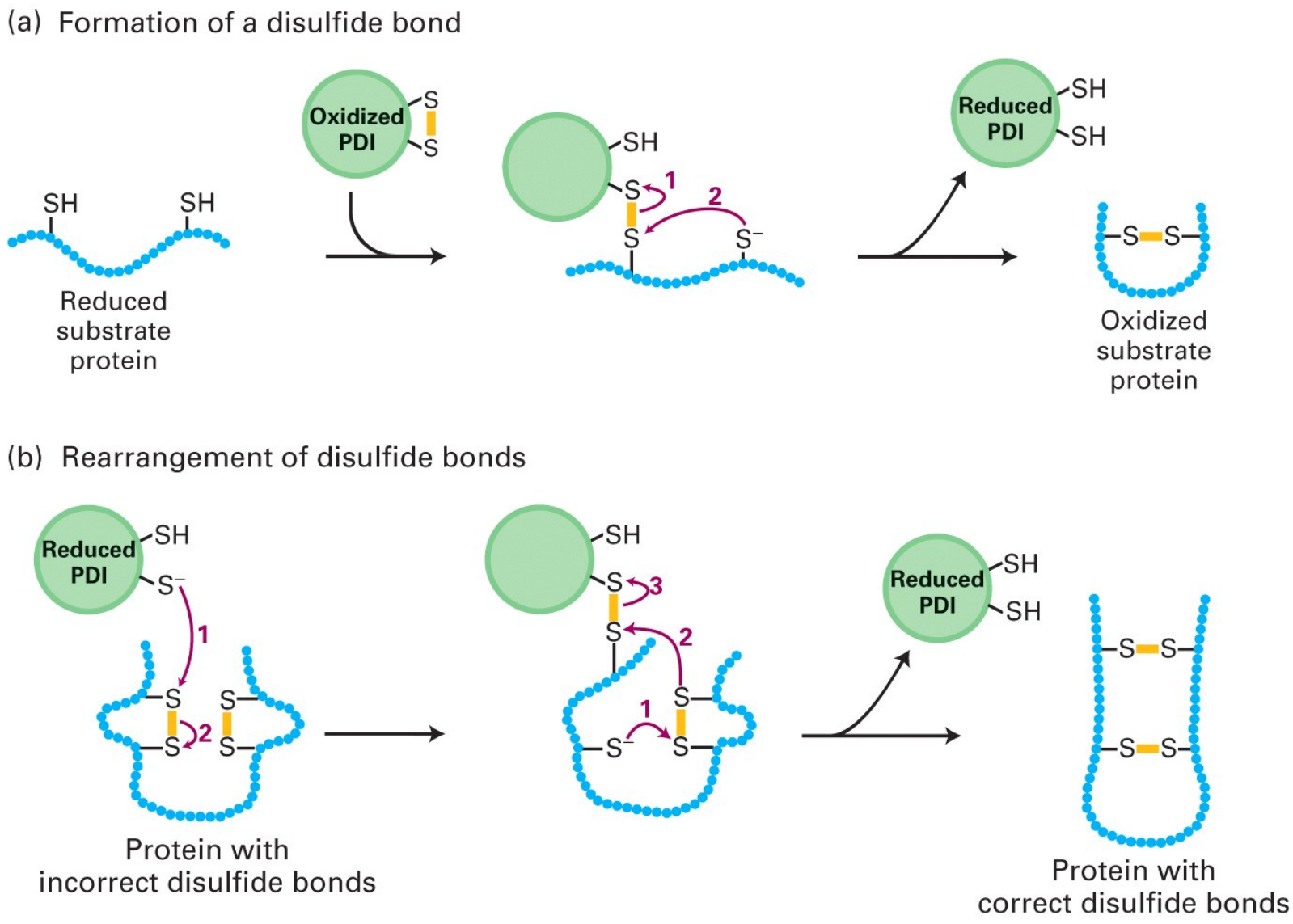
ABO antigeni



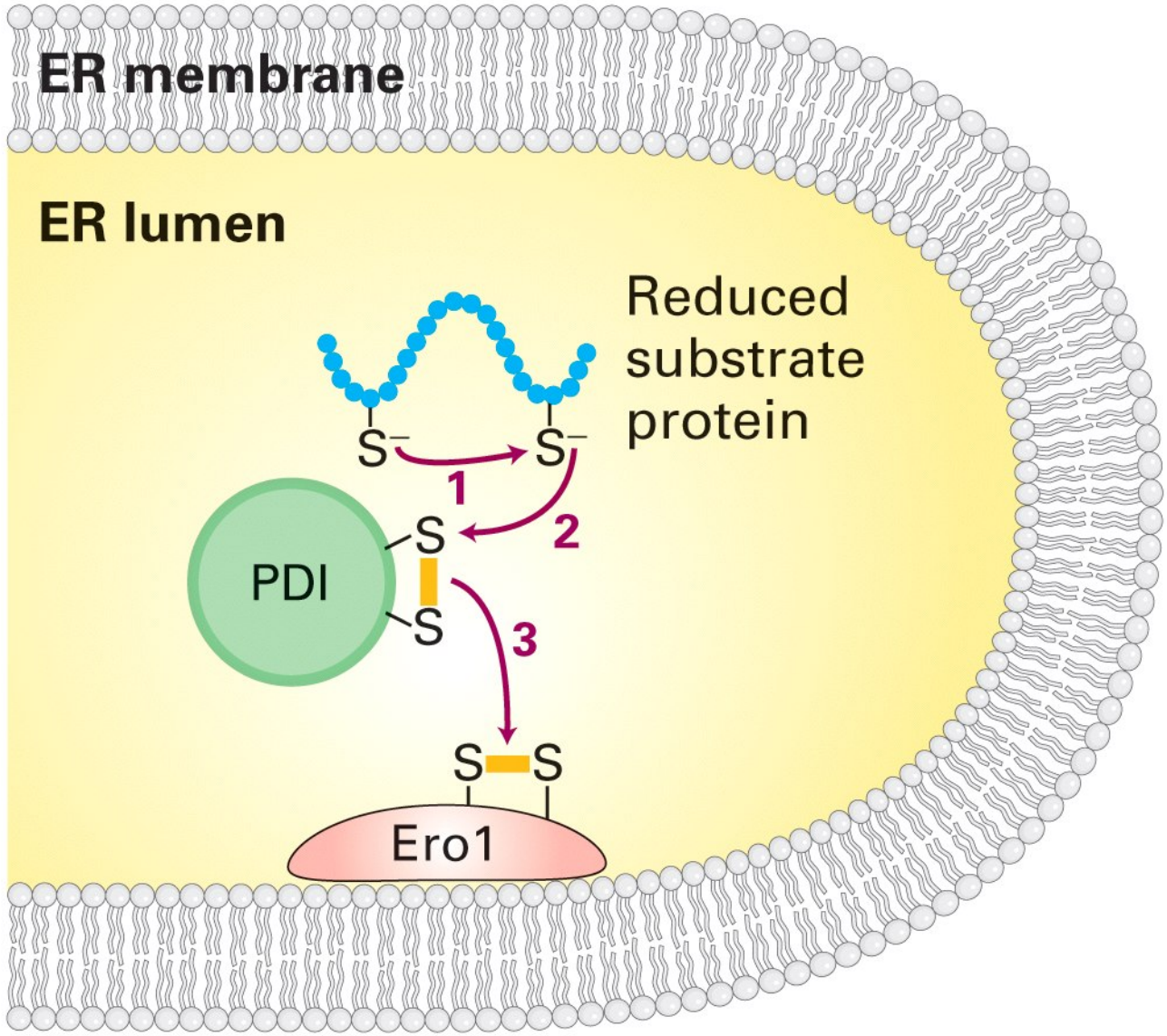
Tvorba disulfidnih vezi

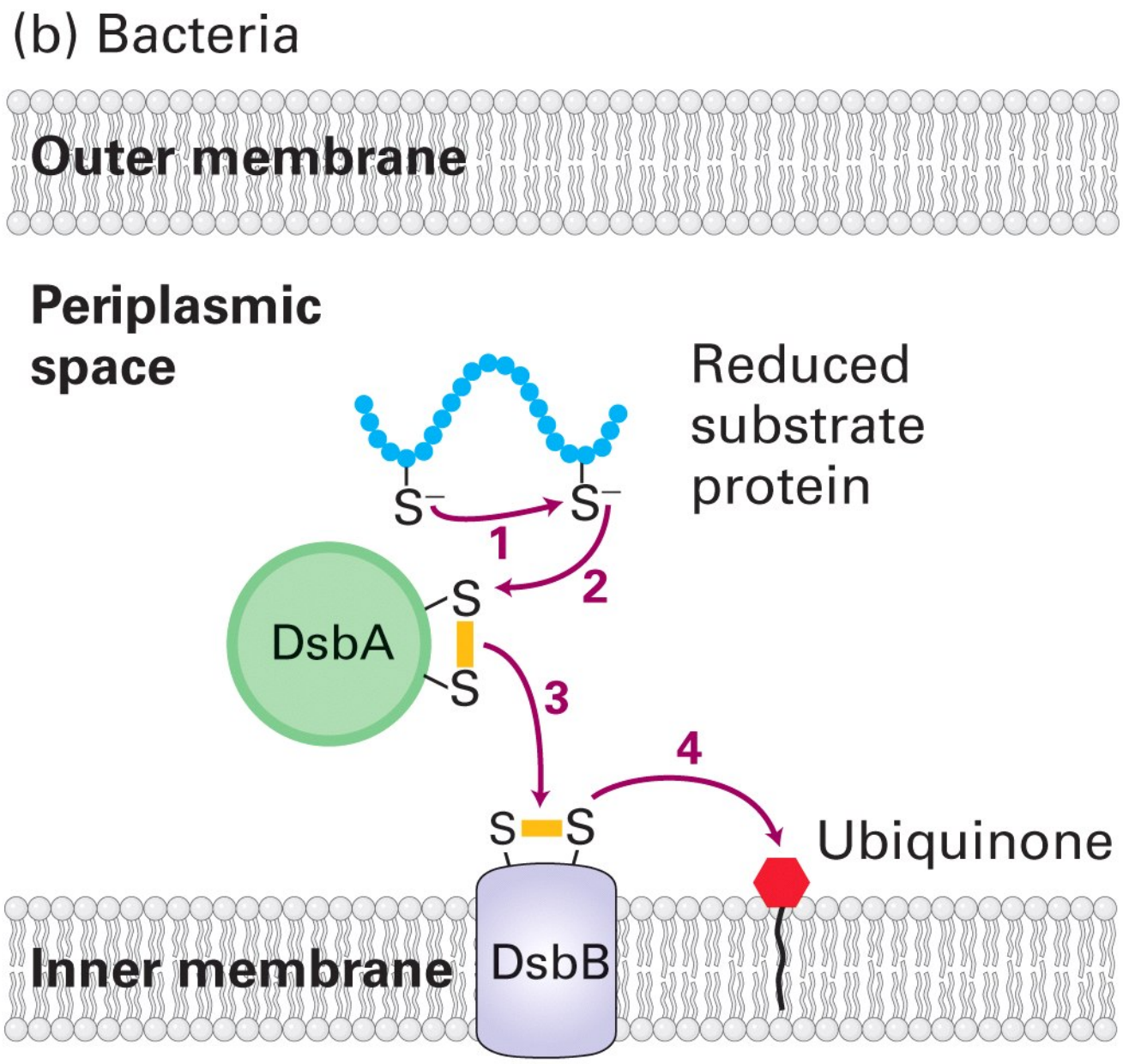
- v eukariontih je v citoplazmi in jedru redukcijsko okolje (GSH:GSSG=100:1, veliko tudi NAD(P)H
- disulfidi stabilizirajo strukturo proteinov

Tvorba disulfidnih vezi



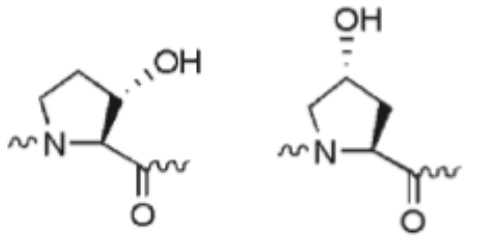
(a) Eukaryotes




(b) Bacteria
Outer membrane
Periplasmic space
Reduced substrate protein
S⁻
S⁻
1
2
DsbA
S
S
3
4
S
S
Ubiquinone
Inner membrane
DsbB

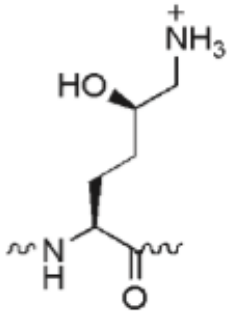
Hidroksilacija proteinov

- hidroksilacijo katalizirajo od železa odvisne monooksigenaze
- hidroksilacija kolagena (Gly-Pro-X/Gly-X-Hyp), ~30% vseh proteinov v telesu je kolagen
- HIF, Pro-hidroksilaza, raven O_2, ubikvitinil ligaza,

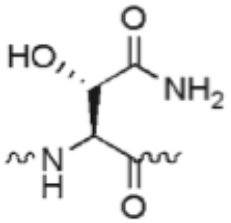


3-OH-Pro

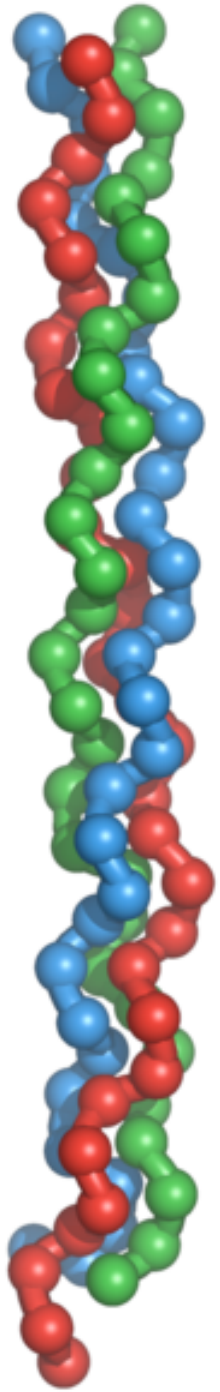
4-OH-Pro



5-OH-Lys

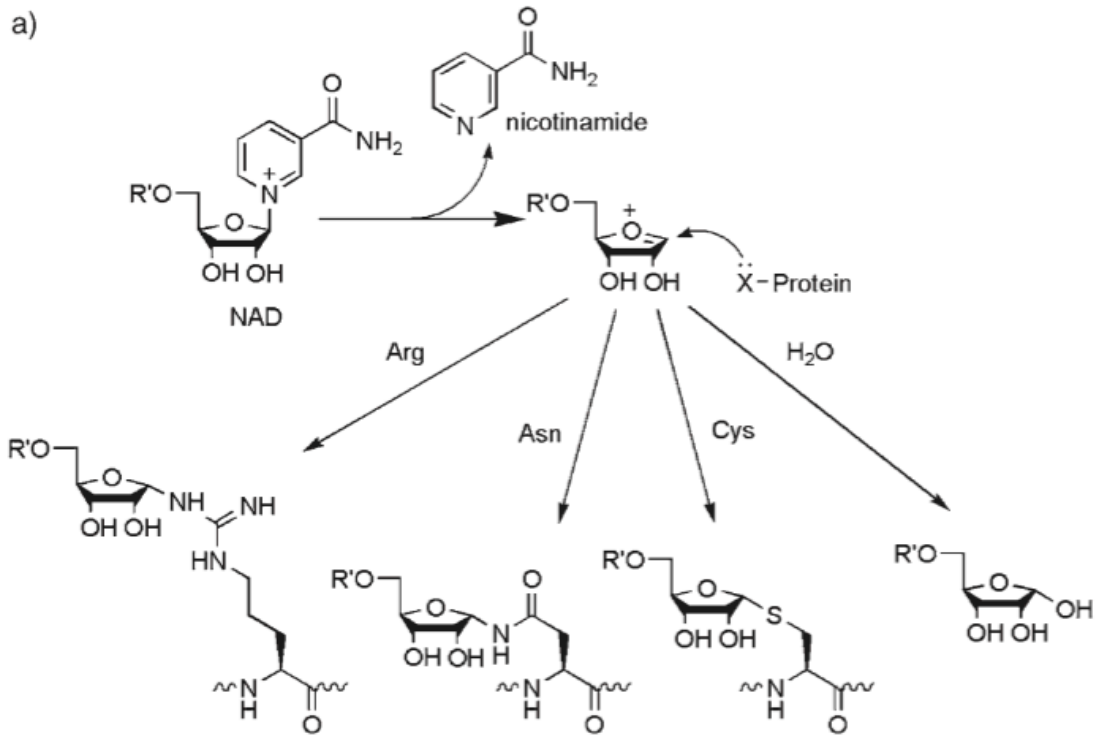


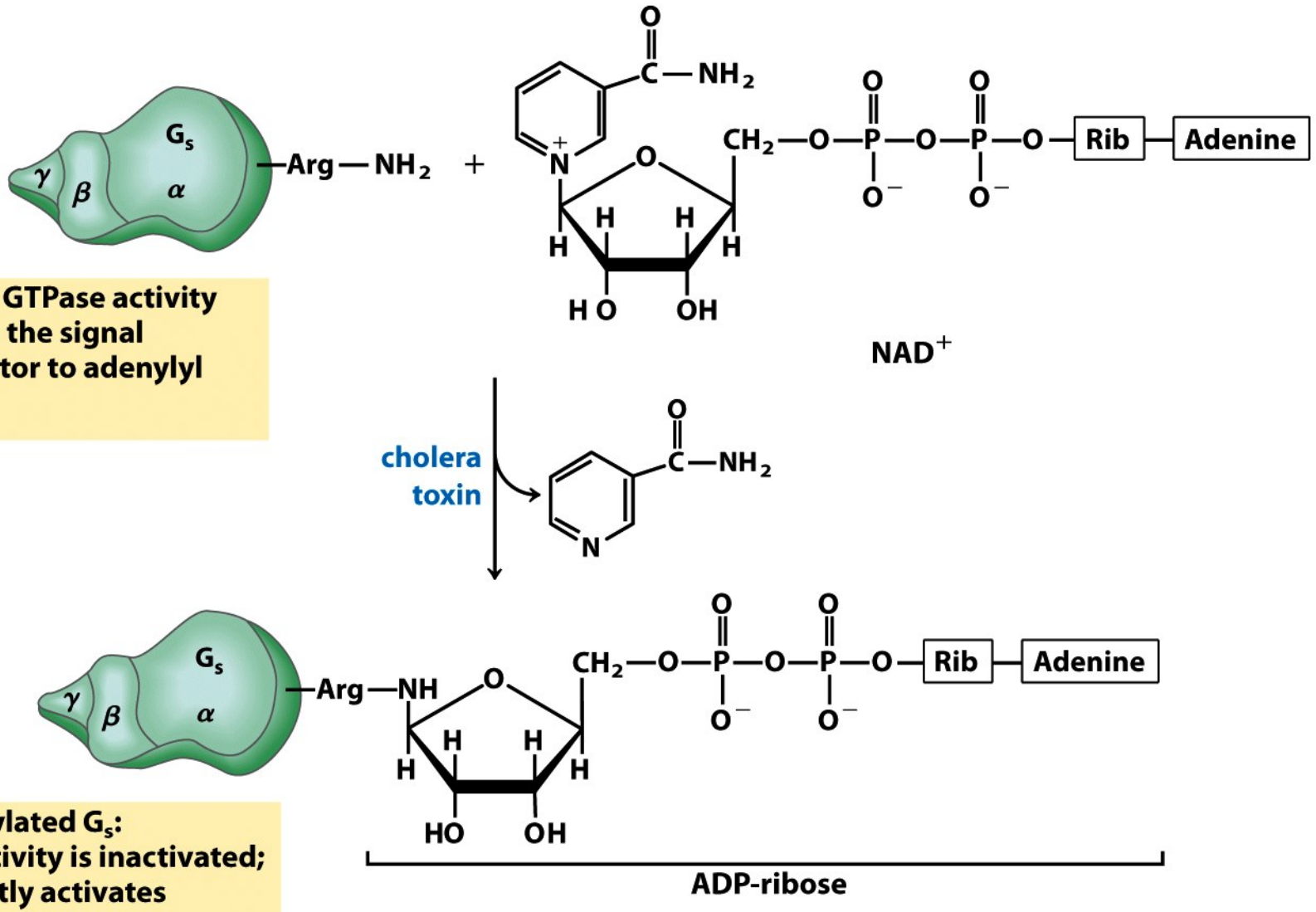
3-OH-Asn



Modifikacije proteinov z bakterijskimi toksini

- cilj je onemogočiti obrambne mehanizme gostitelja
- ADP-ribozilacija, glukozilacija, deaminacija





Box 12-2 figure 5

Lehninger Principles of Biochemistry, Fifth Edition

Modifikacije s prostetičnimi skupinami

npr.

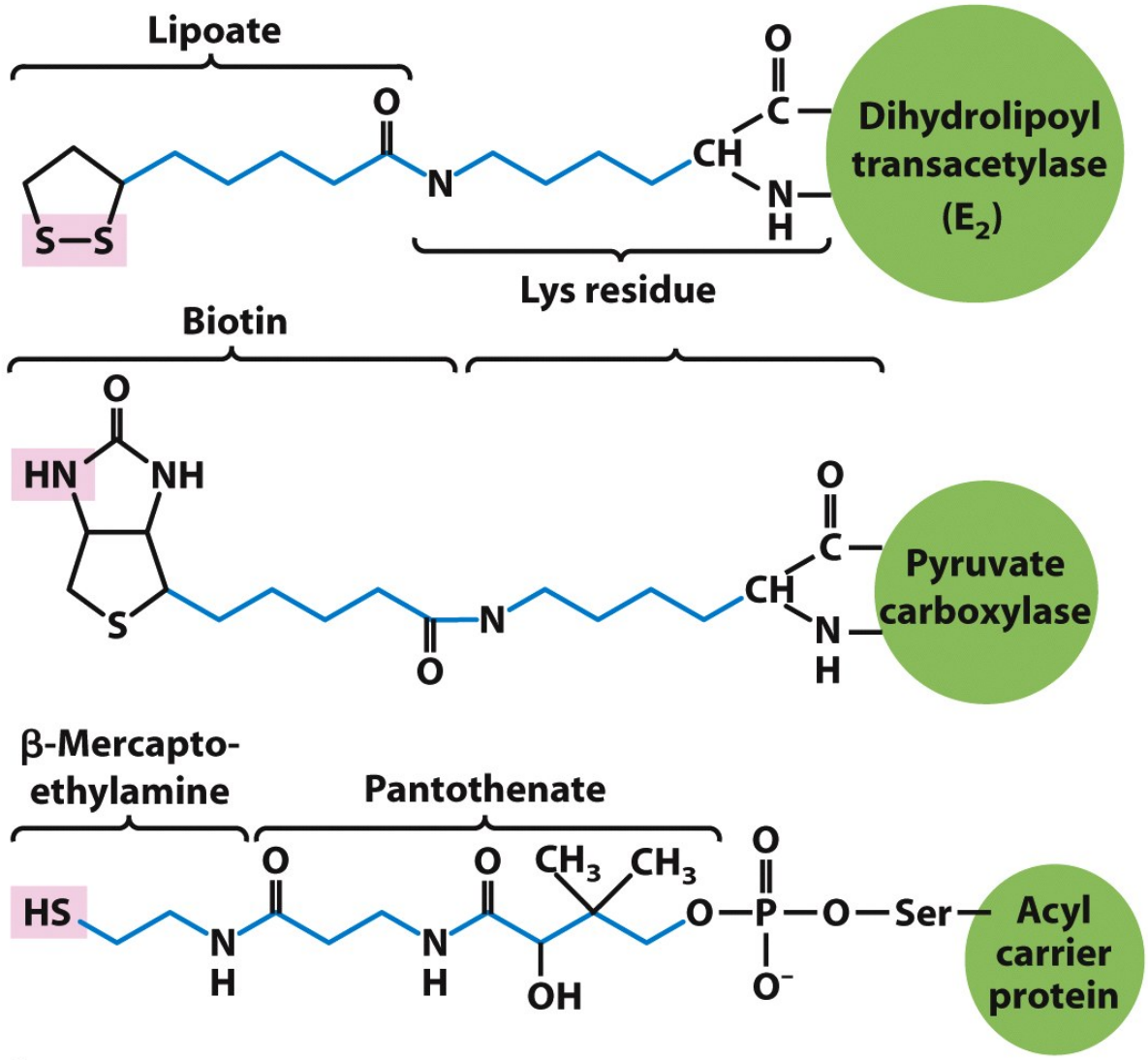
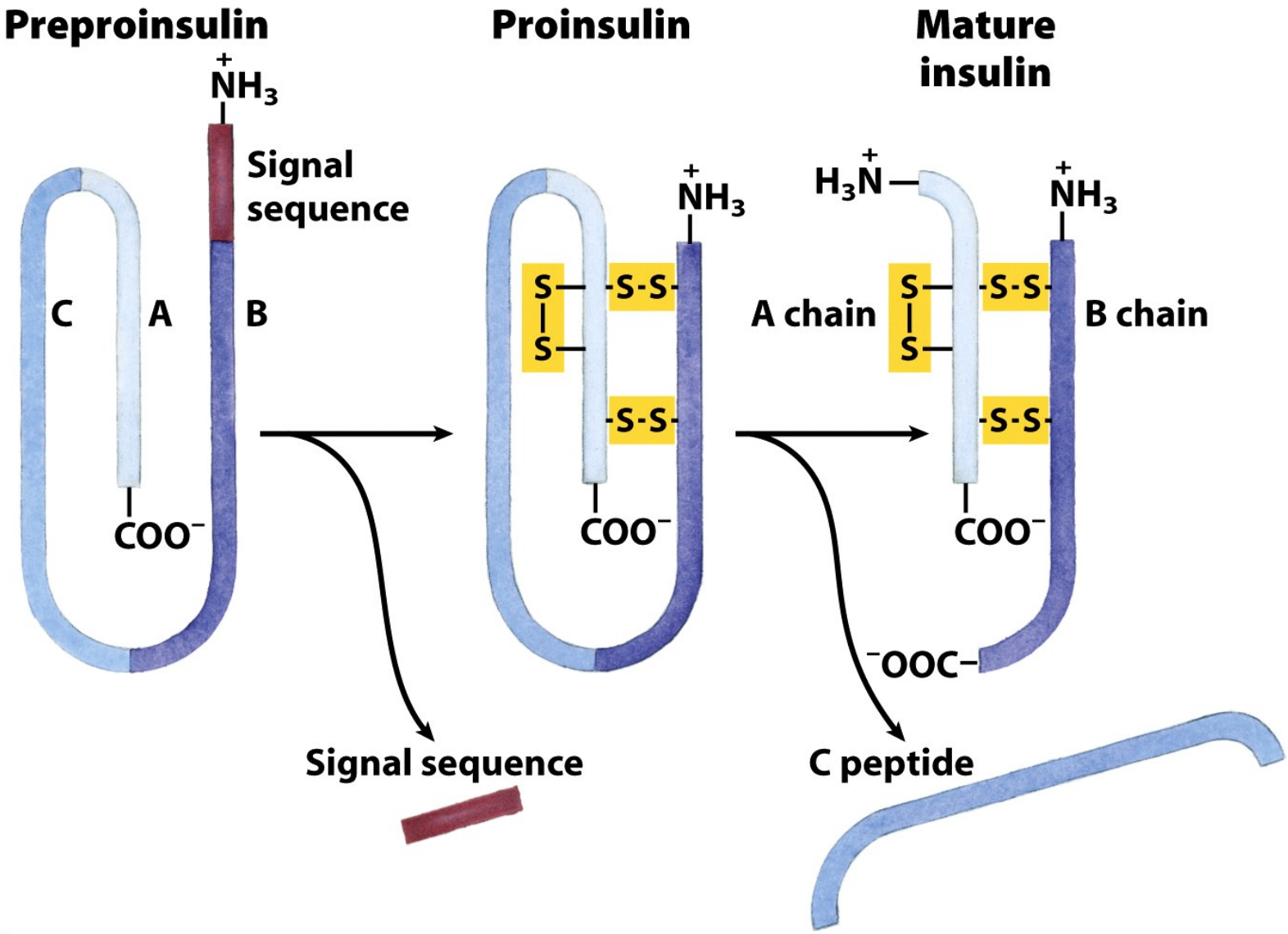


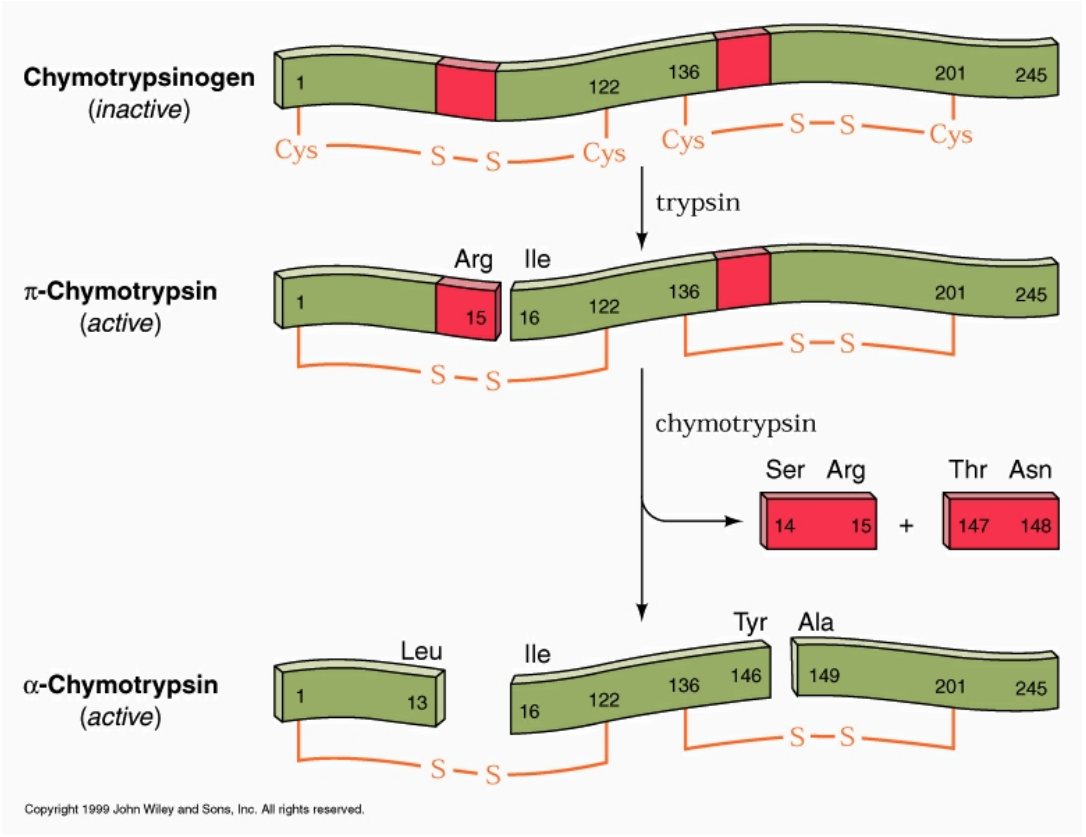
Figure 16-17
Lehninger Principles of Biochemistry, Fifth Edition

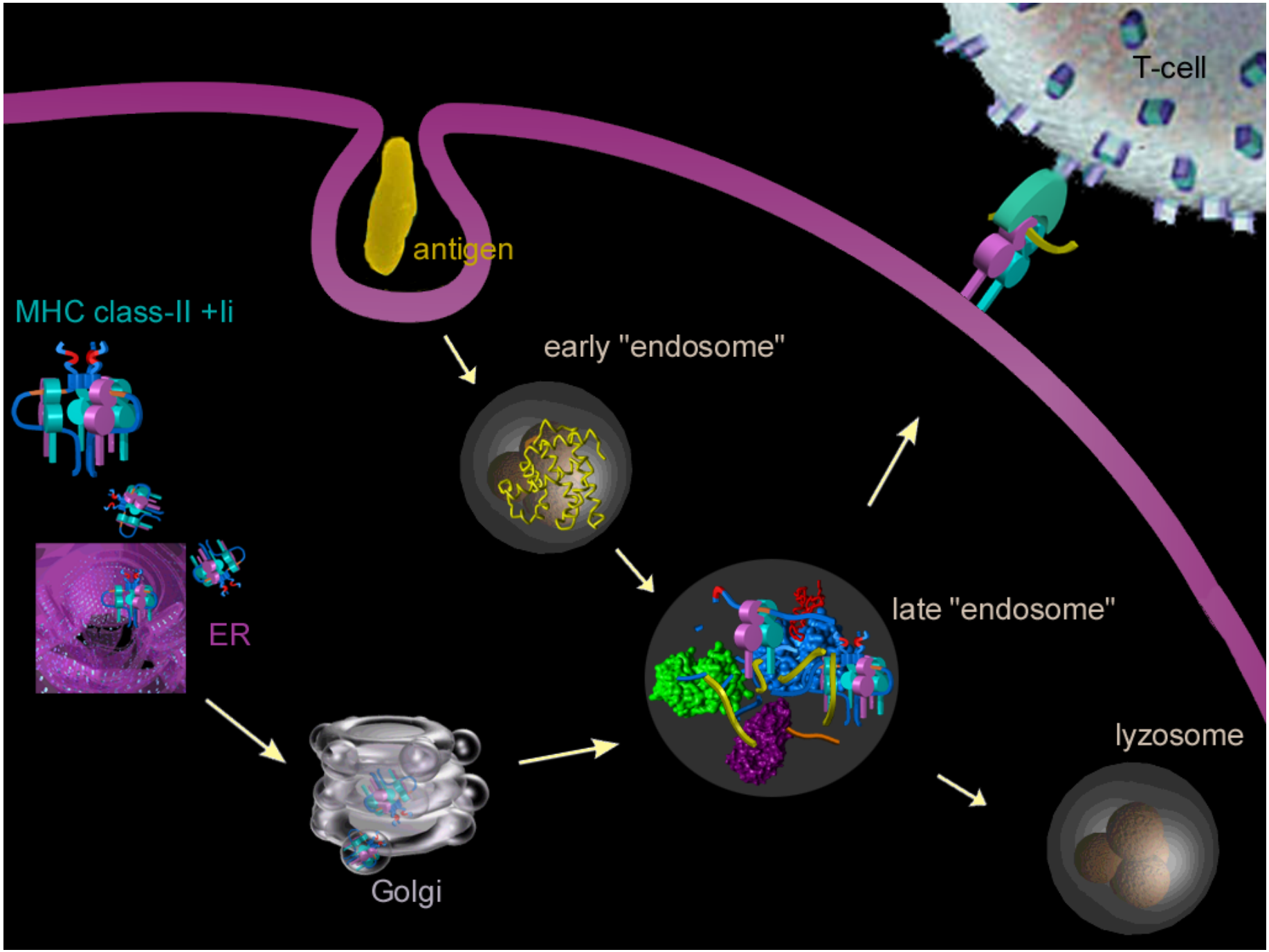
Proteolitično procesiranje



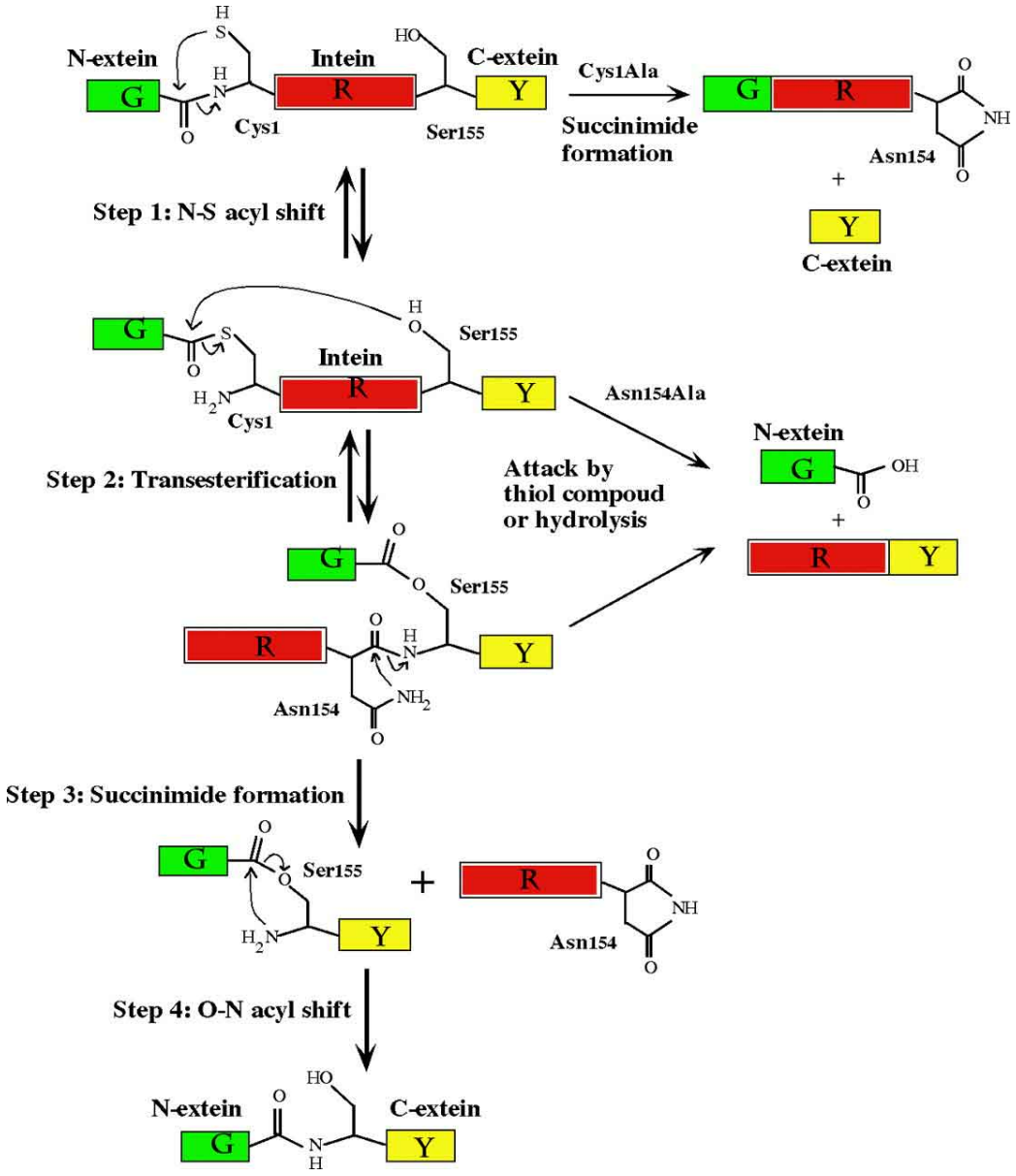
Aktivacija proencimov

Proteinaze cepijo proencim na 1 ali več mestih.




T-cell
antigen
MHC class-II +Ii
early "endosome"
late "endosome"
ER
Golgi
lyzosome

Proteinsko izrezovanje



- N-extein represents the N-terminal polypeptide segment that is retained
- C-extein represents the C-terminal segment that is retained
- the Intein is what is spliced out (much as a genomic DNA intron)
- Cys1, Asn154 and Ser155 represent conserved residues involved in the splicing reaction

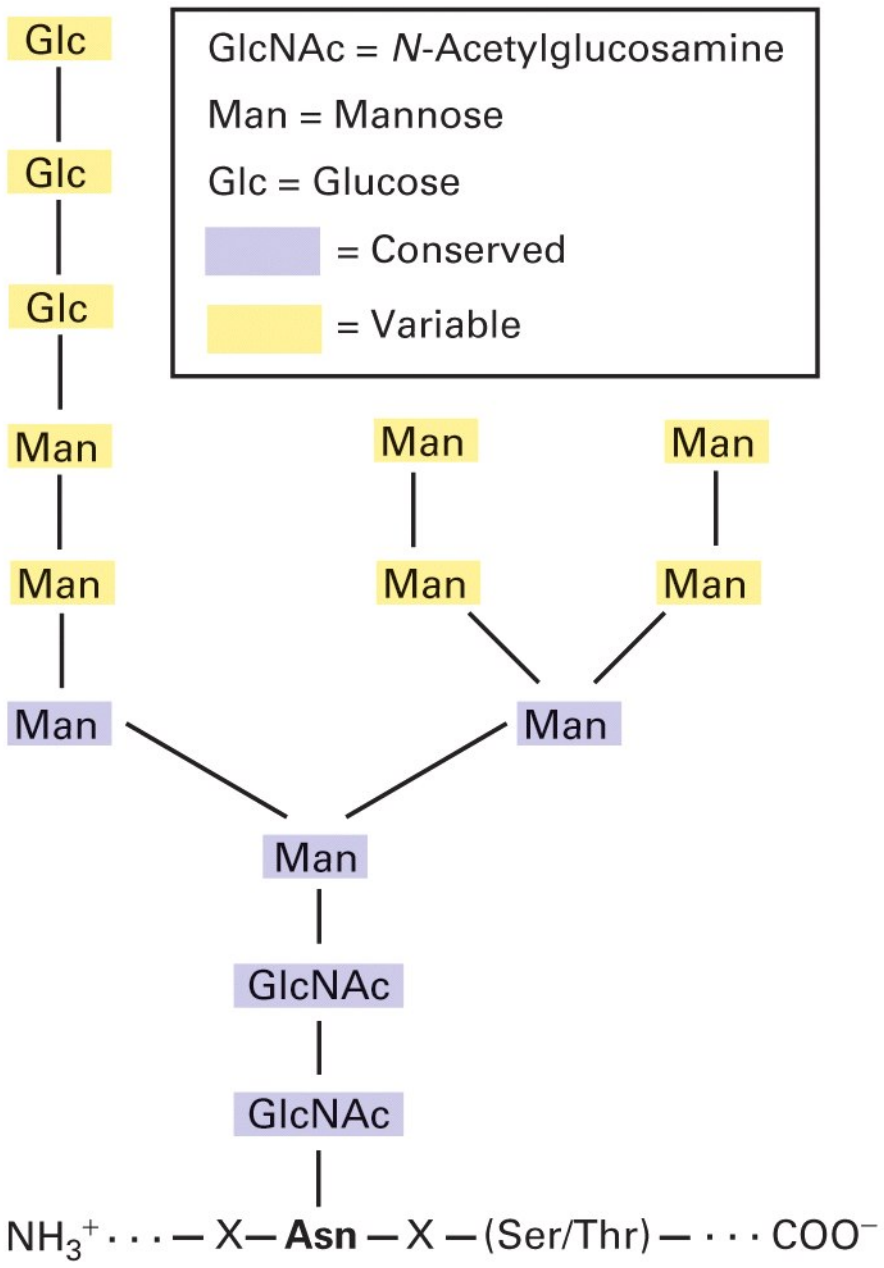
Protein Chemistry

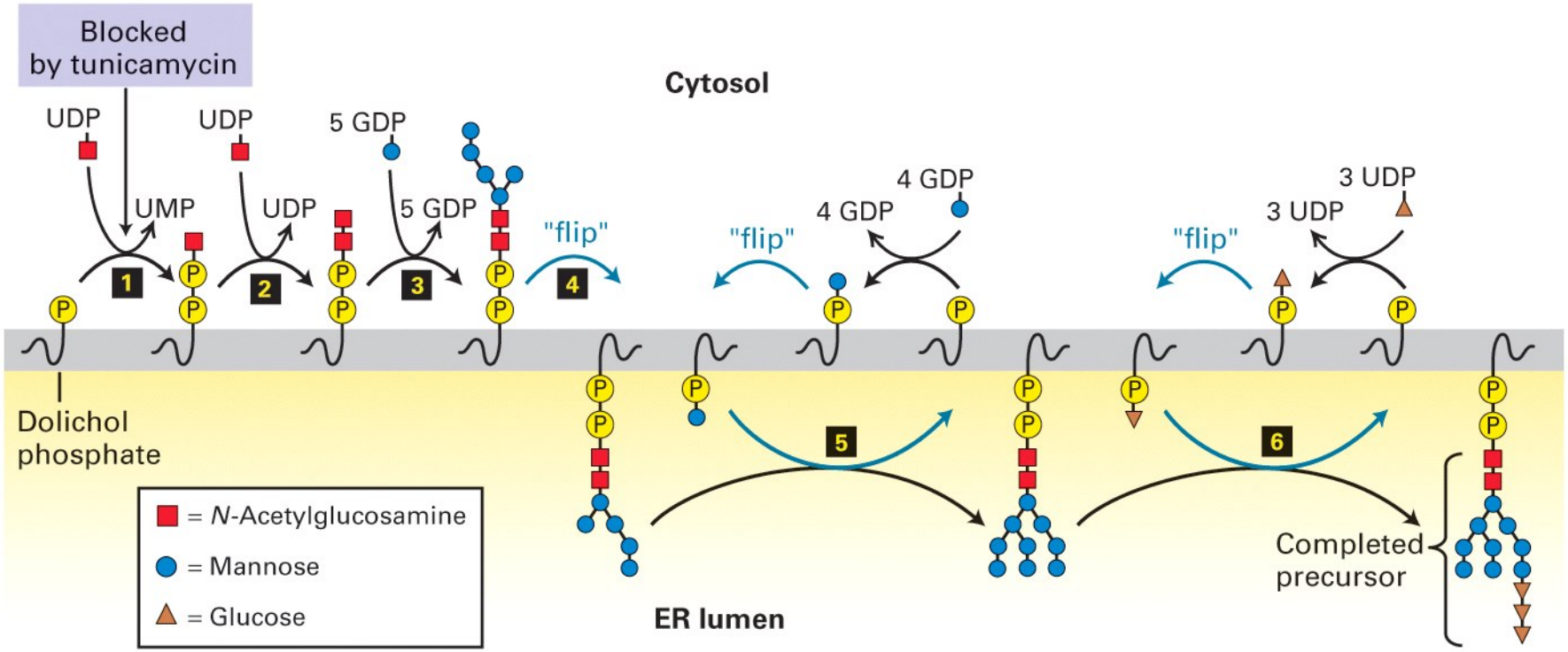
DOI: 10.1002/anie.200501023

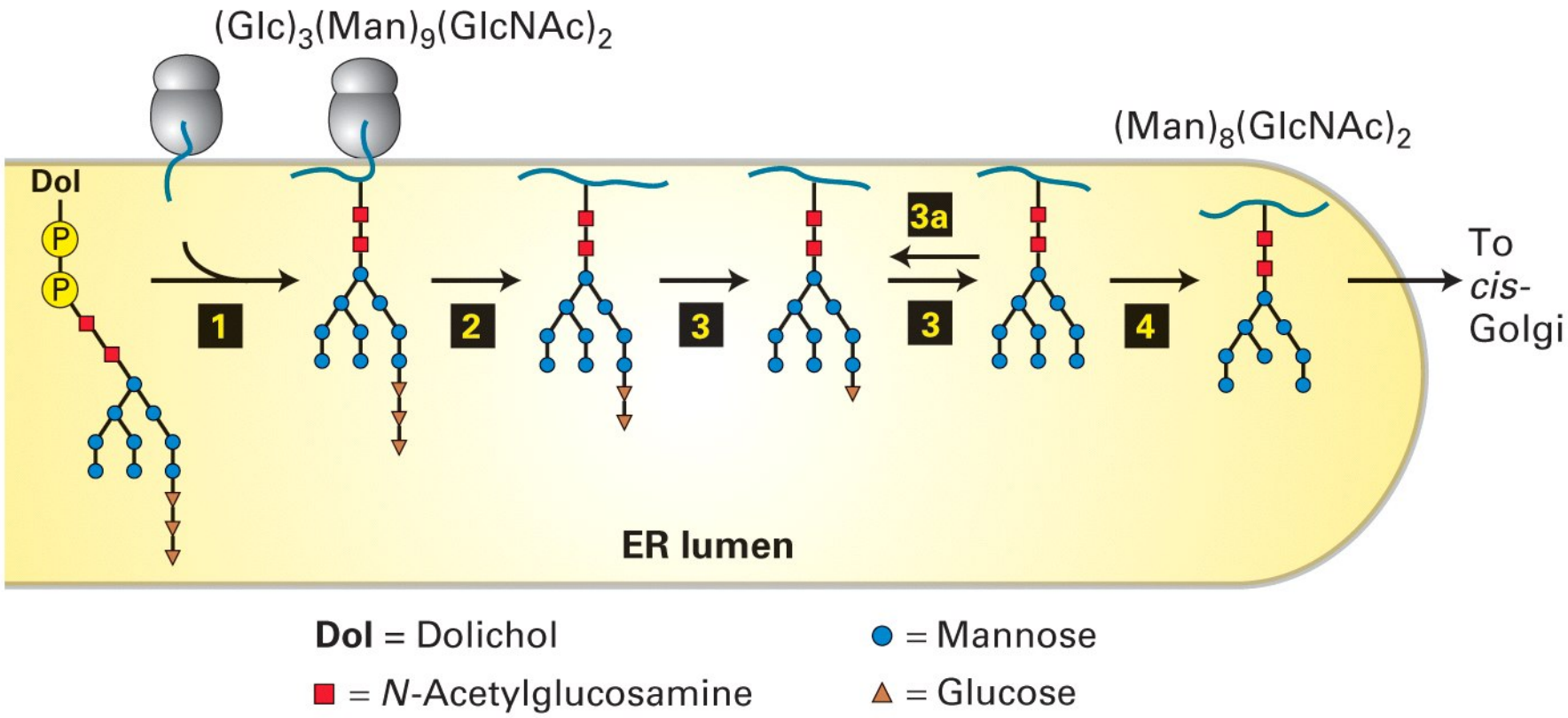
Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications

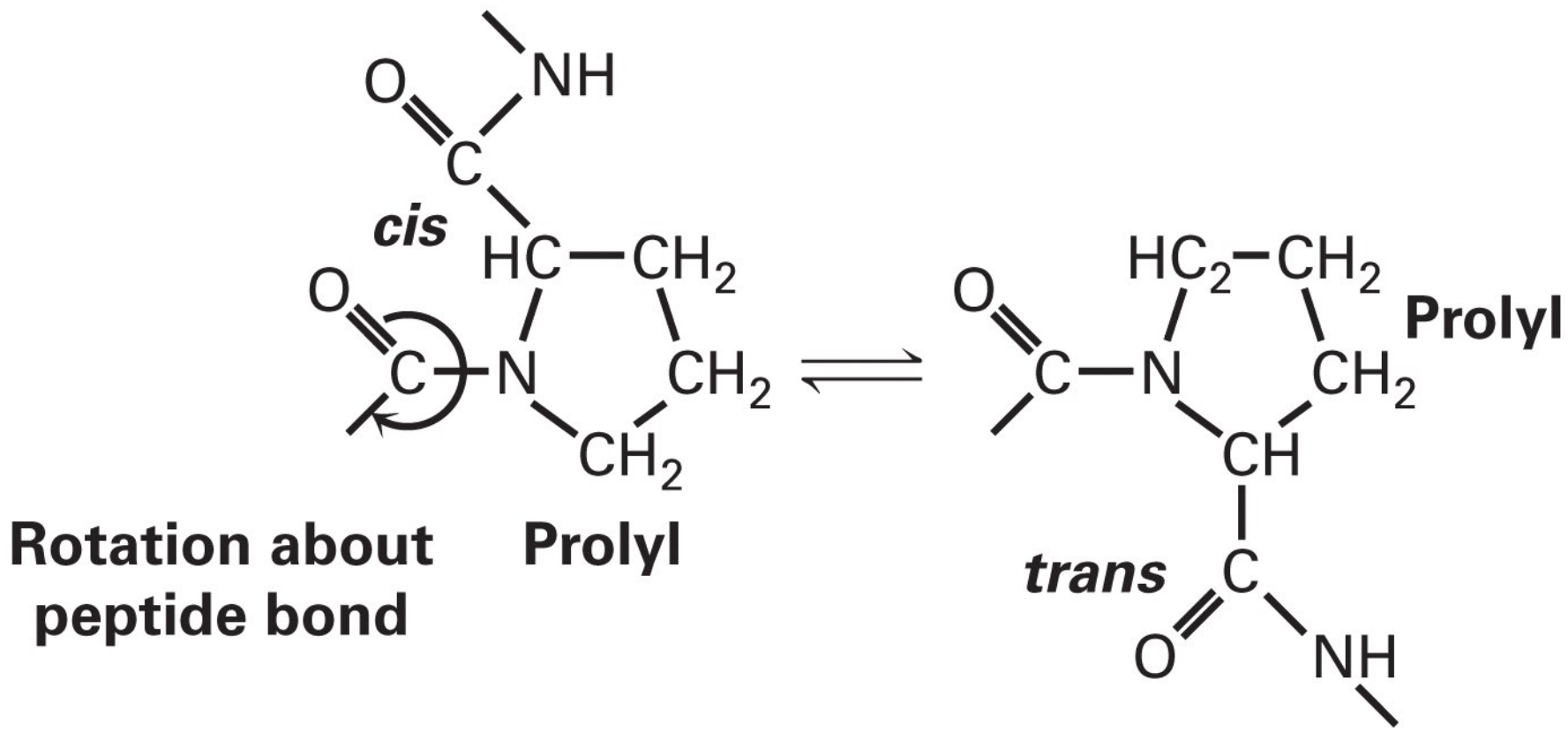
Christopher T. Walsh, Sylvie Garneau-Tsodikova, and Gregory J. Gatto, Jr.*

http://bit.ly/JcrQQO
http://bit.ly/K1lZfY


GlcNAc = N-Acetylglucosamine
Man = Mannose
Glc = Glucose
= Conserved
= Variable
Glc
Glc
Glc
Man
Man
Man
Man
Man
Man
Man
Man
Man
GlcNAc
GlcNAc
NH3+ · · · — X— Asn — X — (Ser/Thr) — · · · COO−


Blocked by tunicamycin
Cytosol
UDP
UDP
5 GDP
UMP
UDP
5 GDP
"flip"
4 GDP
4 GDP
"flip"
3 UDP
3 UDP
"flip"
1
2
3
4
5
6
Dolichol phosphate
= N-Acetylglucosamine
= Mannose
= Glucose
ER lumen
Completed precursor


$(Glc)_3(Man)_9(GlcNAc)_2$
$(Man)_8(GlcNAc)_2$
Dol
P
P
1
2
3
3a
3
4
To *cis*-Golgi
ER lumen
Dol = Dolichol
= Mannose
= *N*-Acetylglucosamine
= Glucose


O
NH
C
cis
HC
CH2
O
C
N
CH2
CH2
HC2
CH2
Prolyl
C
N
CH2
CH
trans
C
O
NH
Rotation about peptide bond
Prolyl