METODE U FIZIOLOGIJI INDUSTRIJSKIH MIKROORGANIZAMA

- pomoću različitih metoda moguće je:
    - identificirati, okarakterizirati i izolirati (izdvojiti) pojedine mikroorganizme i proizvode u ciljane svrhe;
    - opisati rasprostranjenost i aktivnost određenih mikroorganizama;
        - (bio)kemijsko i fizikalno-kemijsko međudjelovanje mikroorganizama i njihovih staništa;
        - povezanost biokemijskih reakcija u stanici i događanja na nivou stanice i čitave populacije;
        - različite biokemijske puteve i mehanizme prijenosa informacije u stanici i izvan stanice;
        - mehaničke karakteristike stanice (npr. turgor, elastičnost, ...);
        - raspodjelu i “kretanje” određenih molekula u različitim odjeljcima stanice;
        - ...

neke metode koje se mogu primijeniti u istraživanju fiziologije stanice (2)

- "vizualizacija":
  - stanice (udjel vode u stanici!)
  - organela
  - molekula (!)

prefiks	simbol(i)	potencija baze 10	potencija baze 2
yocto–	y	10^{-24}	—
zepto–	z	10^{-21}	—
atto–	a	10^{-18}	—
femto–	f	10^{-15}	—
pico–	p	10^{-12}	—
nano–	n	10^{-9}	—
micro–	μ	10^{-6}	—
milli–	m	10^{-3}	—
centi–	c	10^{-2}	—
deci–	d	10^{-1}	—
(-)	—	10^{0}	2^{0}
deka–	D	10^{1}	—
hecto–	h	10^{2}	—
kilo–	k ili K *	10^{3}	2^{10}
mega–	M	10^{6}	2^{20}
giga–	G	10^{9}	2^{30}
tera–	T	10^{12}	2^{40}
peta–	P	10^{15}	2^{50}
exa–	E	10^{18}	2^{60}
zetta–	Z	10^{21}	2^{70}
yotta–	Y	10^{24}	2^{80}
*k = 10^3 a K = 2^{10}			

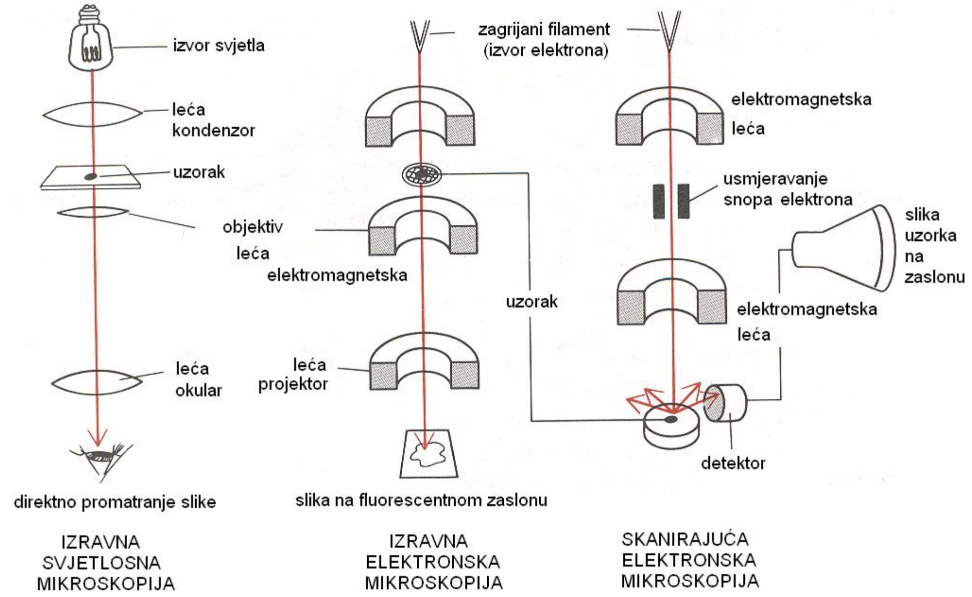
mikroskopija (1)

1. svjetlosna mikroskopija

λ = 0.4 - 0.7 μm (boje)

rezolucija 0.2 μm ili 200 nm (difrakcija)

- izravna (transmisijska)
- fazno-kontrastna
- diferencijalno-kontrastna (Nomarski)
- tamnog polja



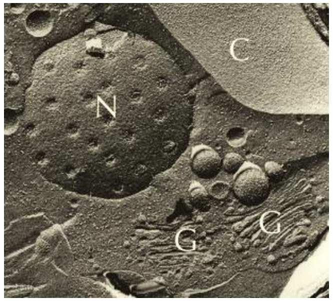
(TEM) (SEM)

2. elektronska mikroskopija

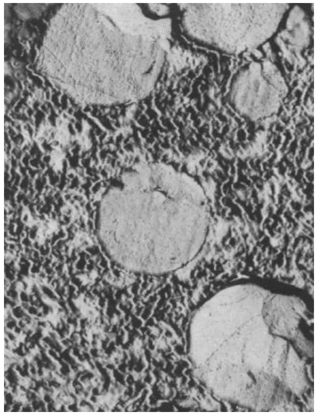
(rezolucija 1 Å, 100x bolja od svjetlosne)

- eng. Transmission Electron Microscope (TEM)
- eng. Scanning Electron Microscope (SEM)

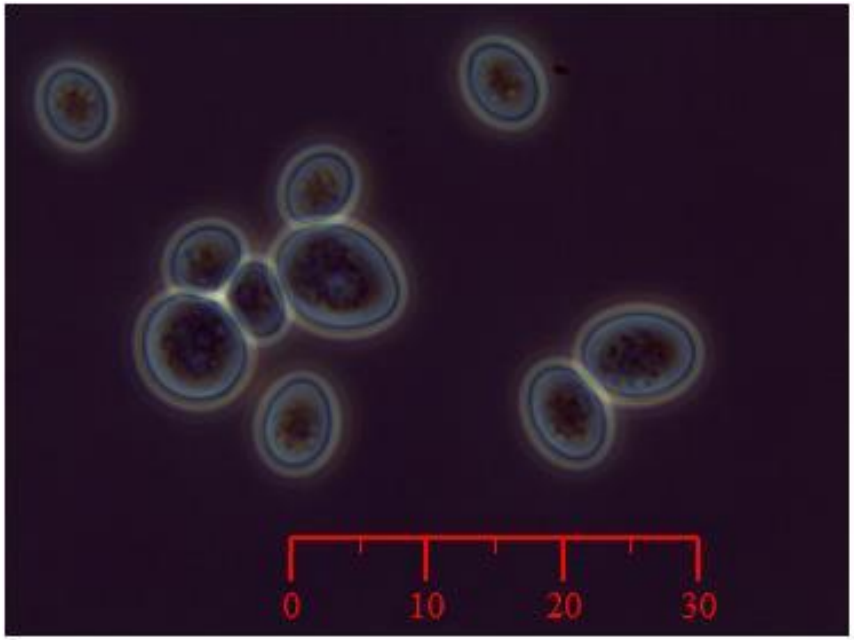
- tehnike: diferencijalno bojanje
  mikrotomski presjek
  eng. freeze-fracture
  eng. freeze-etch



freeze-fracture

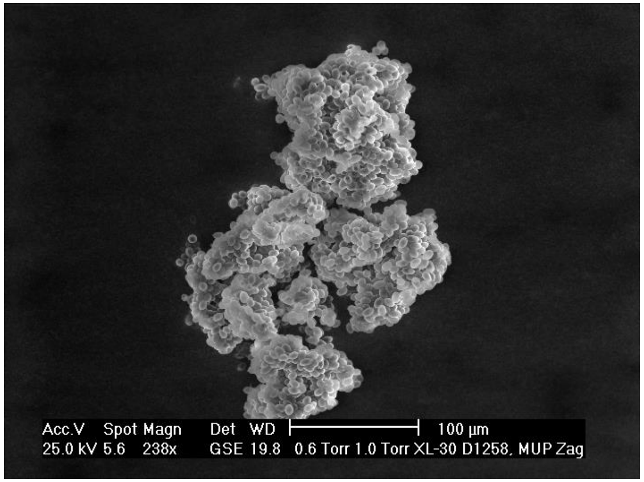


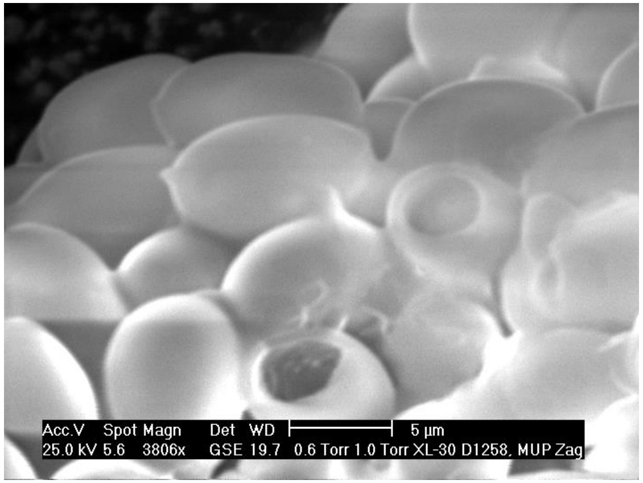
freeze-etch

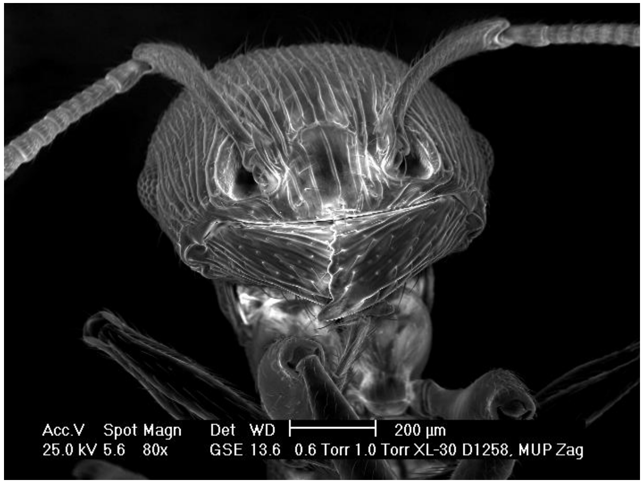


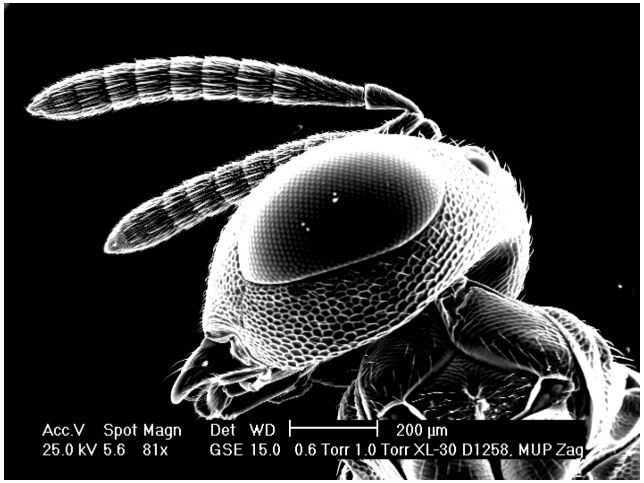
S. cerevisiae, reverse-phase microscopy (Leica E3 camera; Leica, Wetzlar, Germany) with respective size-scale in µm created by using Dia Diagram Editor version 0.97.2

2. elektronska
   mikroskopija

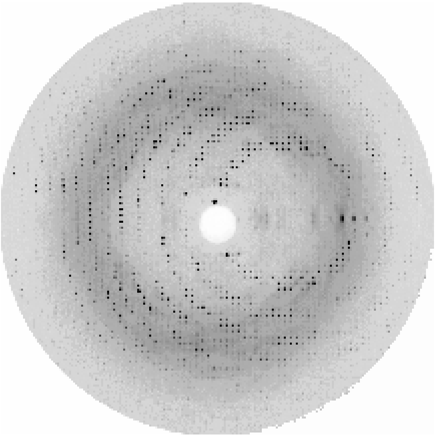




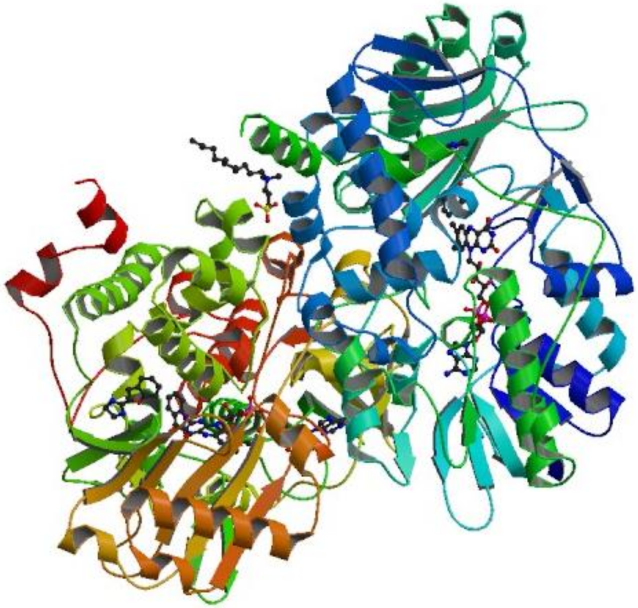




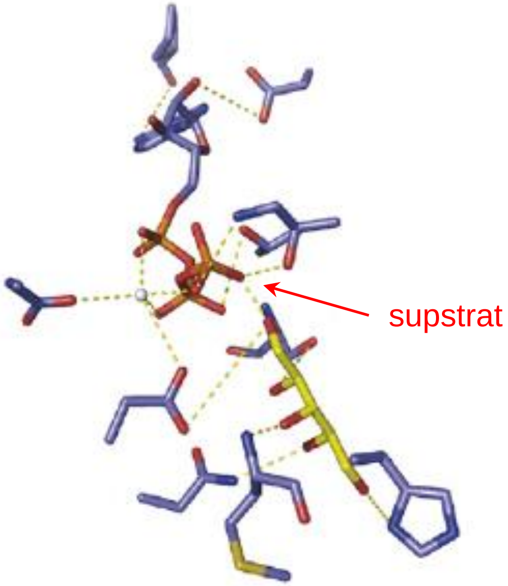
- λ = 10 - 0.01 nm
- proučavanje strukture makromolekula (Å, 0.1 nm, 10^{-10} m)



difrakcija X-zraka



struktura proteina / enzima



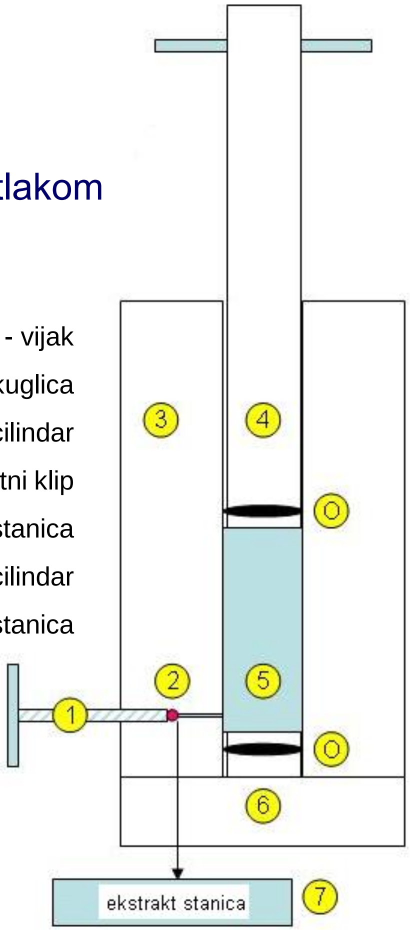
primjer: aktivno mjesto enzima

- dobivanje ekstrakta stanica: ultrazvukom
  osmotskim šokom
  u tarionicima i kugličnim mlinovima
  propuštanjem kroz mali otvor pod visokim tlakom

- upotreba ekstrakcijskih reagensa: liza stanice i
  ekstrakcija (rekombinantnih) proteina iz *E. coli* npr. B-PER
  (eng. Bacterial Protein Extraction Reagent)

- analiza: izolacija i pročišćavanje proteina i dr. makromolekula
  određivanje enzimske aktivnosti (kinetika)
  primjena inhibitora i analogona

1 - vijak
2 - teflonska kuglica
3 - cilindar
4 - pokretni klip
5 - suspenzija stanica
6 - donji cilindar
7 - ekstrakt stanica



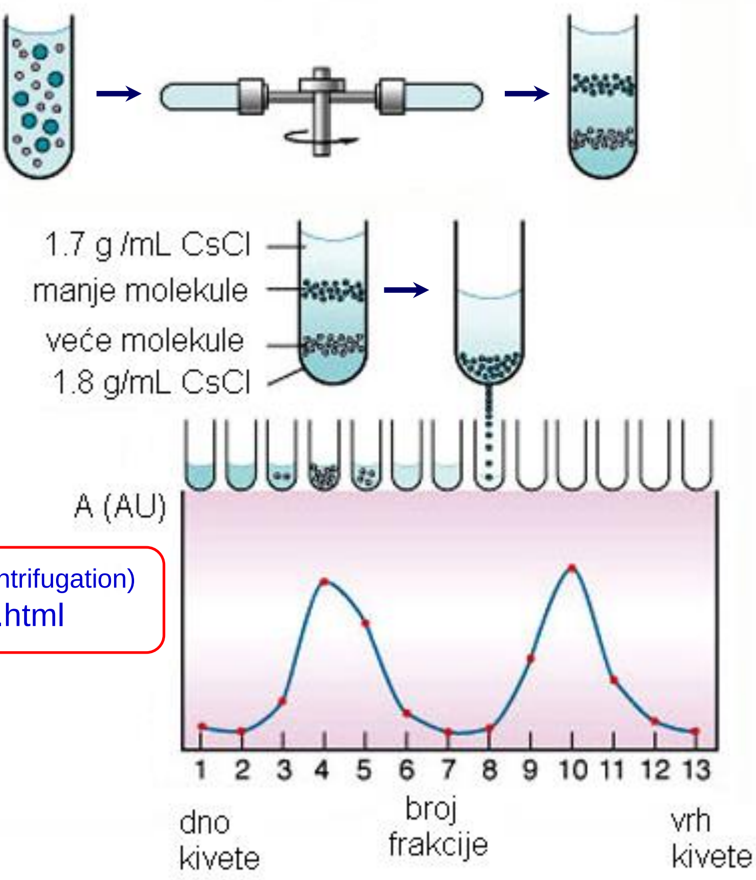
- primjena ultracentrifuga
- diferencijalno centrifugiranje

male brzine	npr.1,000 *g* / 10 min
srednje brzine	20,000 *g* / 20 min
velike brzine	80,000 *g* / 1 sat
vrlo velike brzine	150,000 *g* / 3 sata

g relativna centrifugalna sila ili centrifugalni učinak

- razdvajanje u gradijentu gustoće (saharoza, CsCl)

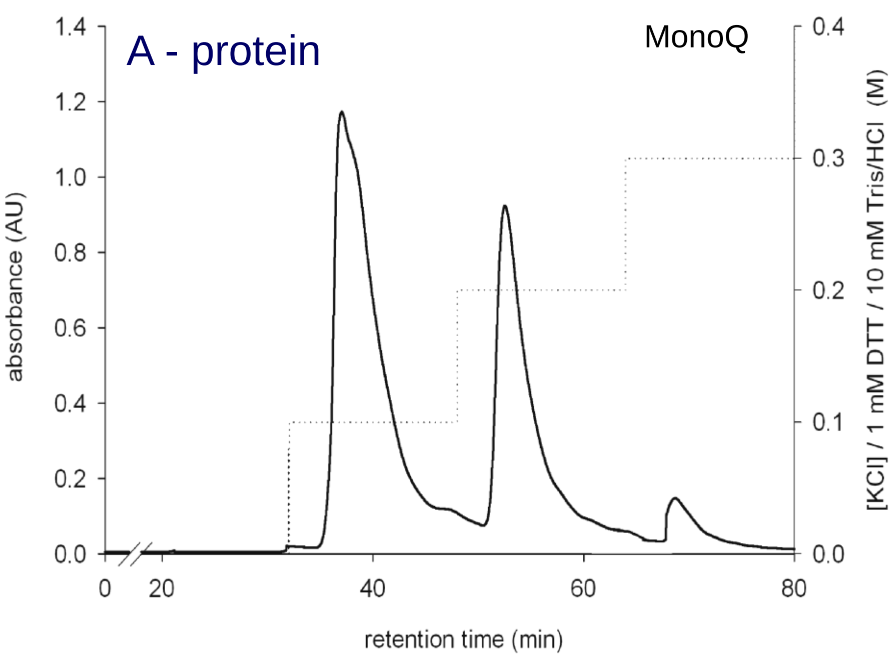
animacija: **Cell Fractionation** (Sonication, Differential and Buoyant Density Centrifugation)
www.sumanasinc.com/webcontent/animations/microbiology.html

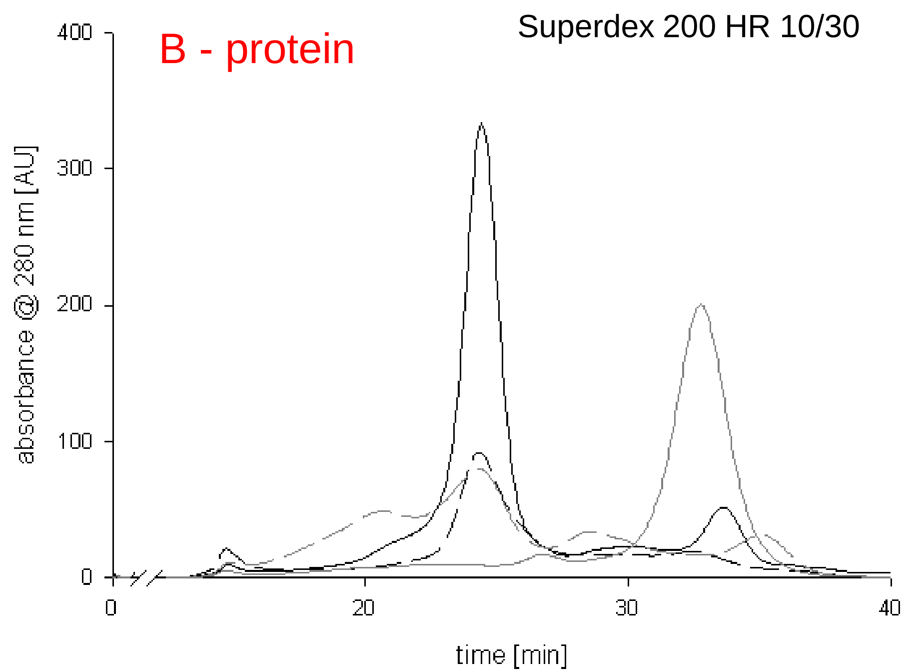


- razdvajanje na temelju gustoće plutanja
- mikroskopska, kemijska i/ili enzimska analiza različitih frakcija stanica

animacija: **The Meselson-Stahl Experiment** (CsCl gradient)
www.sumanasinc.com/webcontent/animations/biology.html

- kromatografija: razdjelna (tankoslojna, papirna)
  kromatografija u koloni: ionsko-izmjenjivačka (A)
  gel-filtracija (B)
  bio-specifična (afinitetna) kromatografija
  (npr. epoksi-aktivirana sefaroza za izdvajanje i pročišćavanje amilaza)

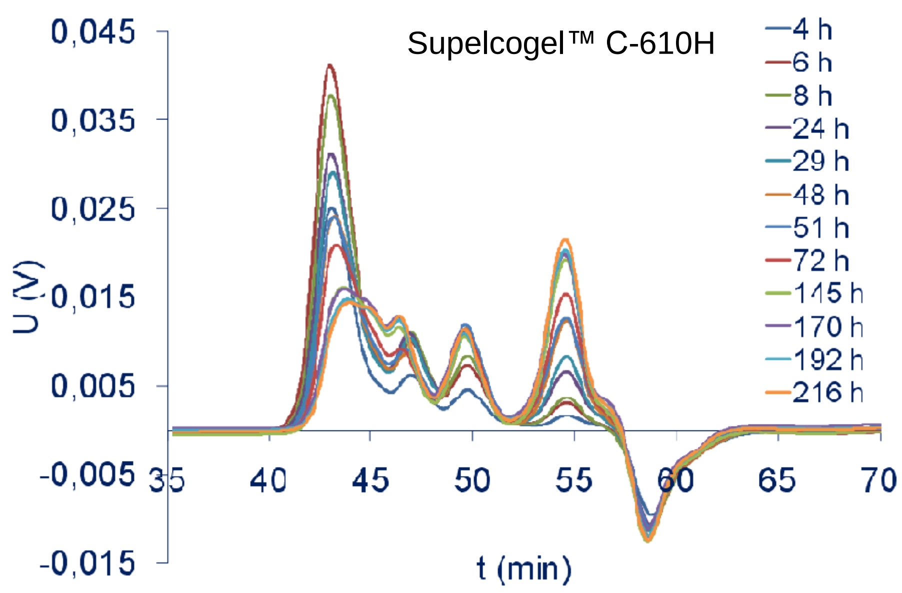


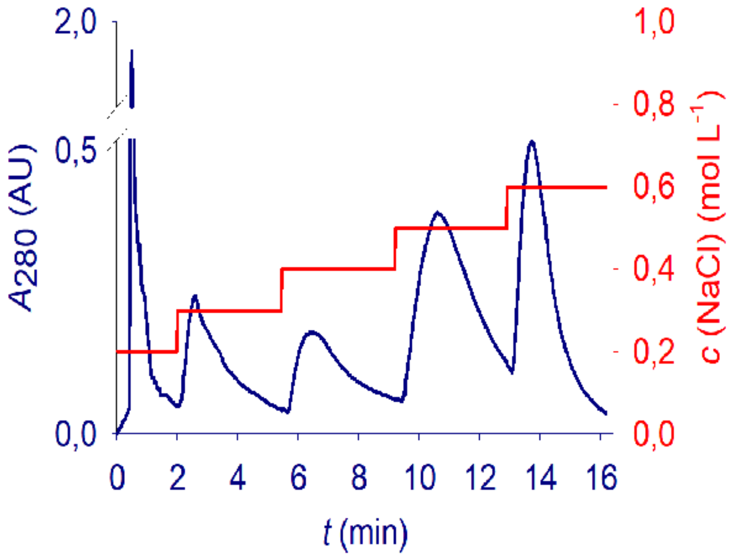


eng. Fast Protein Liquid Chromatography (FPLC)

- kromatografija: razdjelna (tankoslojna, papirna)
  kromatografija u koloni: ionsko-izmjenjivačka (C)
  gel-filtracija
  bio-specifična (afinitetna) kromatografija

C - oligosaharidi (G2 – G7) i proteini





eng. High Pressure Liquid Chromatography (HPLC)

- N- i C-terminalni *tag*-ovi:

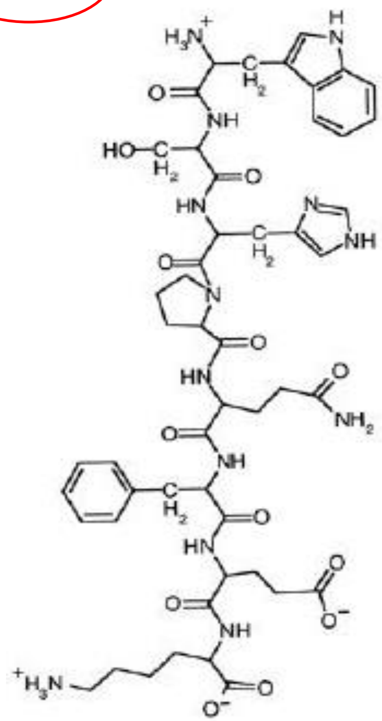
FPLC

Strep (NH_2-WSHPQFEK-COOH)

His (6 x His)

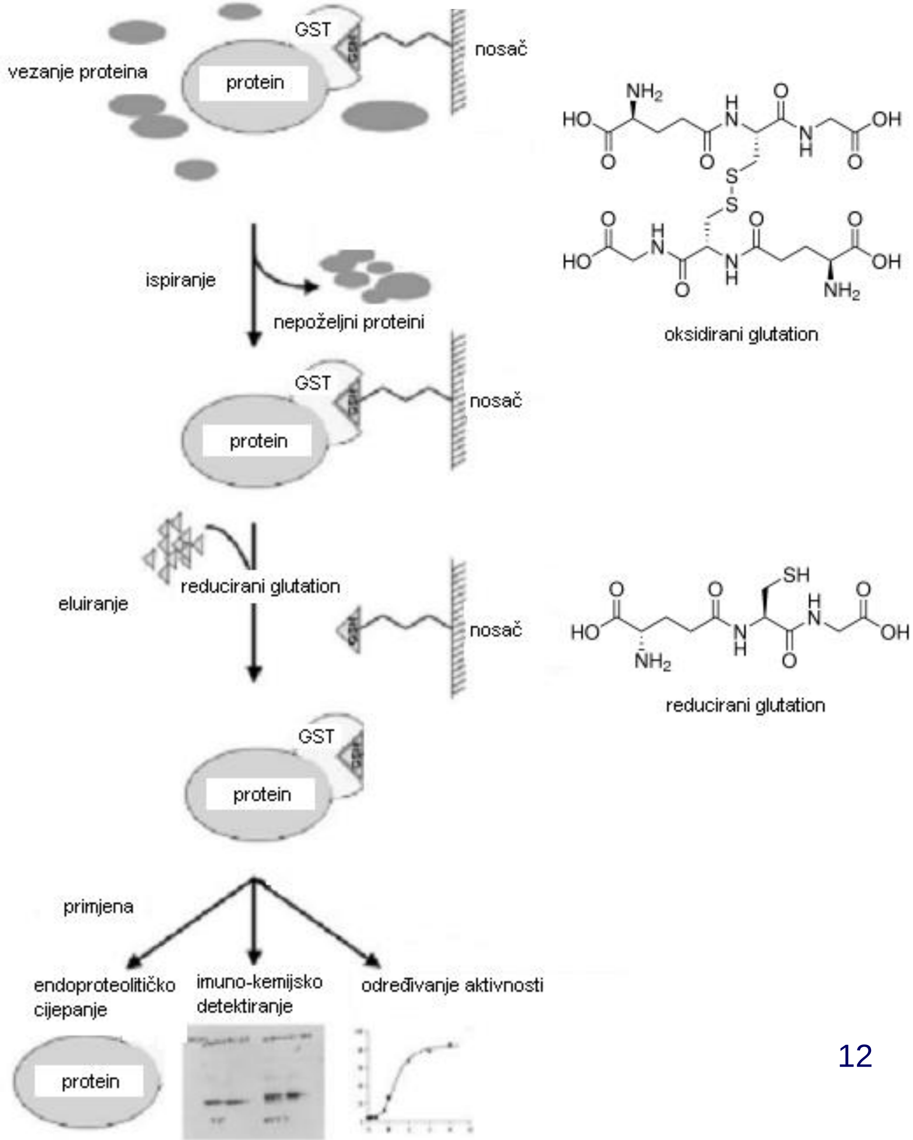
GST

Flag



Strep-tag

glutation-S-transferaza (GST), 35 kDa



Biocatalysis and Biotransformation, May–June/July–August 2005; 23(3/4): 261–269
Taylor & Francis
Taylor & Francis Group

- N- i C-terminalni *tag*-ovi

Integrated approach for production of recombinant acetylacetone dioxygenase from *Acinetobacter johnsonii*

GRIT D. STRAGANZ[1], ANITA SLAVICA[2], HANNES HOFER[1], ULRIKE MANDL[1], WALTER STEINER[1], & BERND NIDETZKY[1,2]

[1]*Institute of Biotechnology and Biochemical Engineering, and* [2]*Research Centre Applied Biocatalysis, Graz University of Technology, Petersgasse 12, A-8010 Graz, Austria*

Table I. Reverse oligonucleotide primers used for the construction of expression vectors used in this study.

Expression construct	Primer	Reverse primer sequence	Restriction sites
pTXB1-*dke1**	PDkBam	5′-AT**GGATCC**TCAGGCAGGCAGCCTCATTTTTG-3′	*Nde*I/*Bam*HI
pKYB1-*dke1*	PDkBam	5′-AT**GGATCC**TCAGGCAGGCAGCCTCATTTTTG-3′	*Nde*I/*Bam*HI
pKYB1-*dke1_Intein*	PDk*Int*	5′-GAGATA**GCTCTTC**CGCAGGCAGGCAGCCTCATTTTTGGTAGC-3′	*Nde*I/*Sap*I
pKYB1-*dke1_Strep*	PDk*Strep*	5′-GATAC**GGATCC**TTA*TTTTTCGAACTGCGGGTGGCTCC AAGCGCT*GGCAGCCTCATTTTTGGTAGC-3′	*Nde*I/*Bam*HI

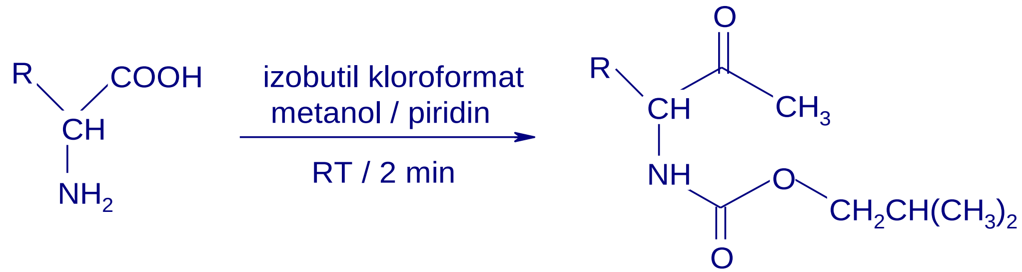
- nanoLC, hplc-chip-ms_384 hyperlink

- 2D tekućinska kromatografija (npr. IEX + RP)

izdvajanje, pročišćavanje i analiza aminokiselina: plinska kromatografija (5)

- GC i 2D GC

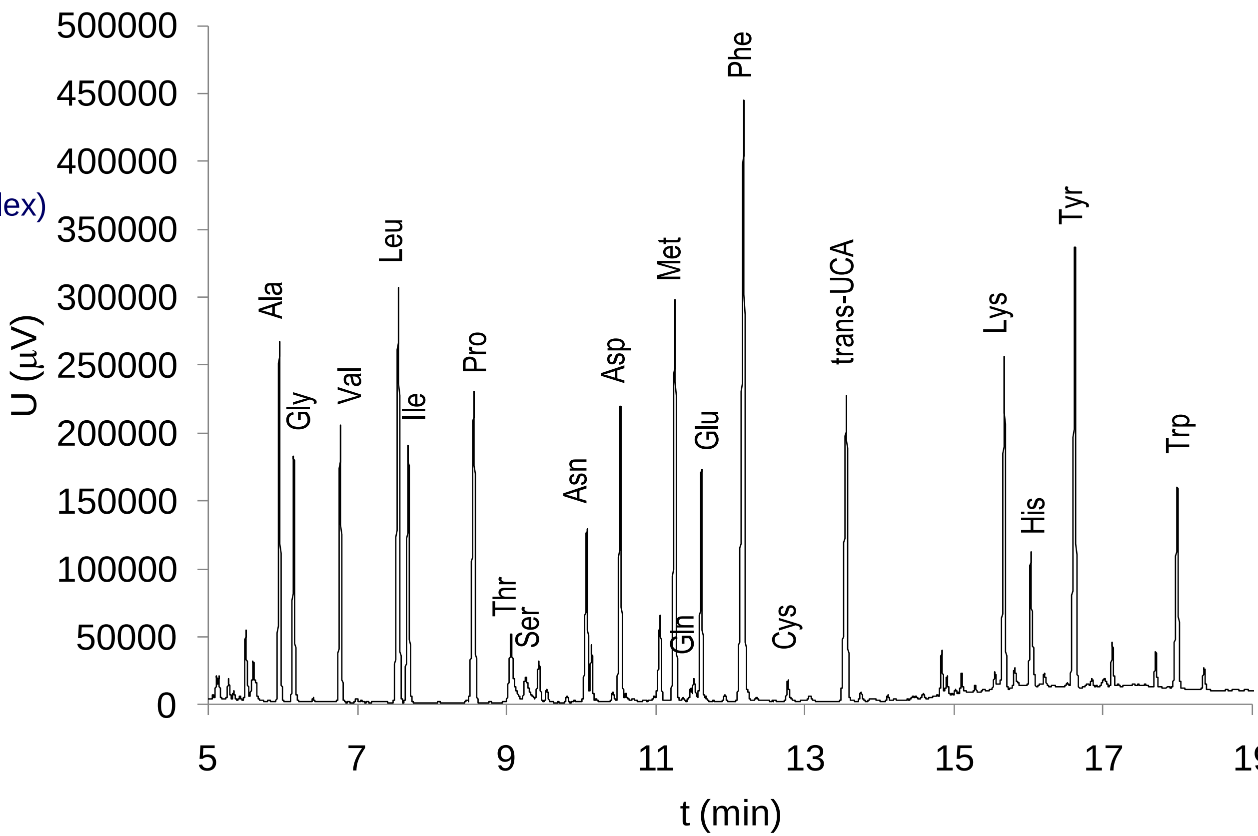
derivatizacija aminokiselina



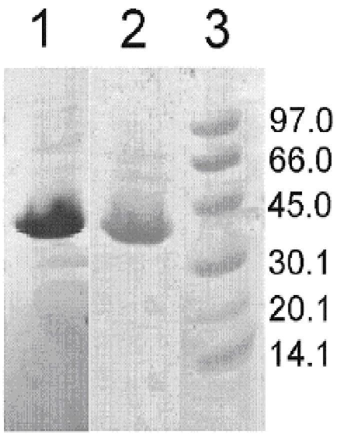
aminokiselina

N-alkoksikarbonil ester

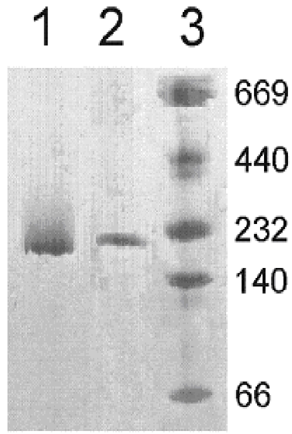
GC analiza
AT-1701 kolona (Alltech Heliflex)
(V = 2 µL, 1 : 6 split ratio)



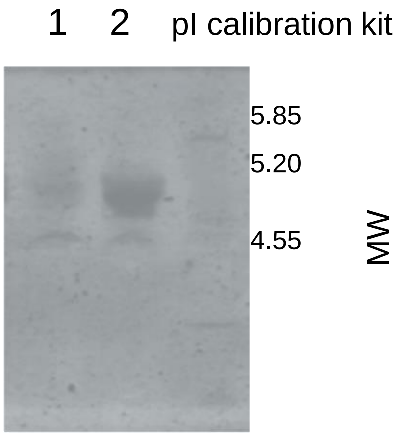
- gel elektroforeza (SDS-PAGE, LDS-PAGE, native PAGE, IEF, 2D)



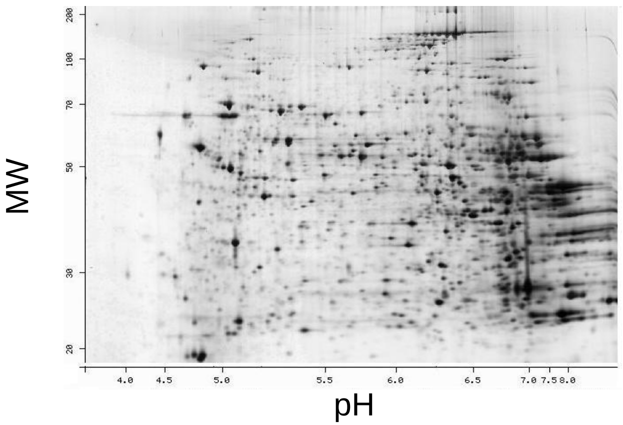
SDS-PAGE



native-PAGE

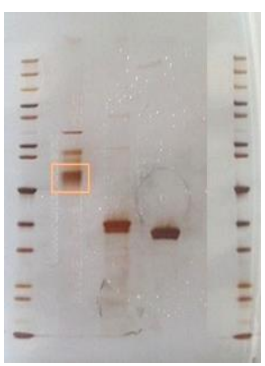


IEF



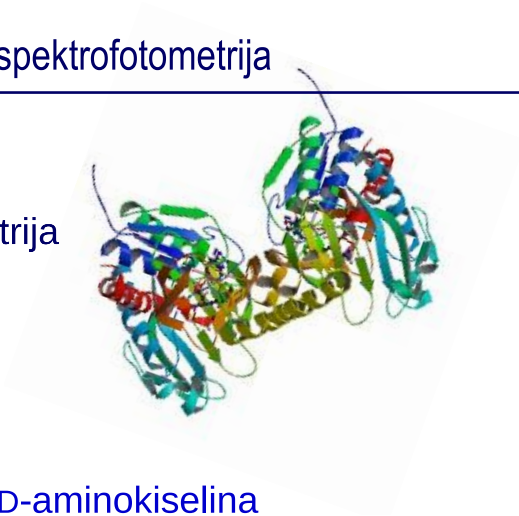
2D elektroforeza

LDS-PAGE

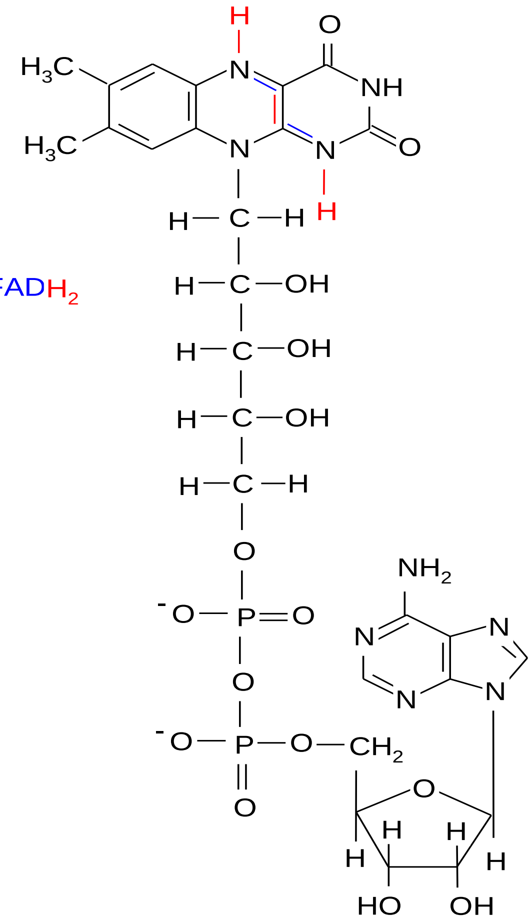


animacija: **Gel Electrophoresis** (DNA)
Heat Changes Protein Structure
www.sumanasinc.com/webcontent/animations/biology.html

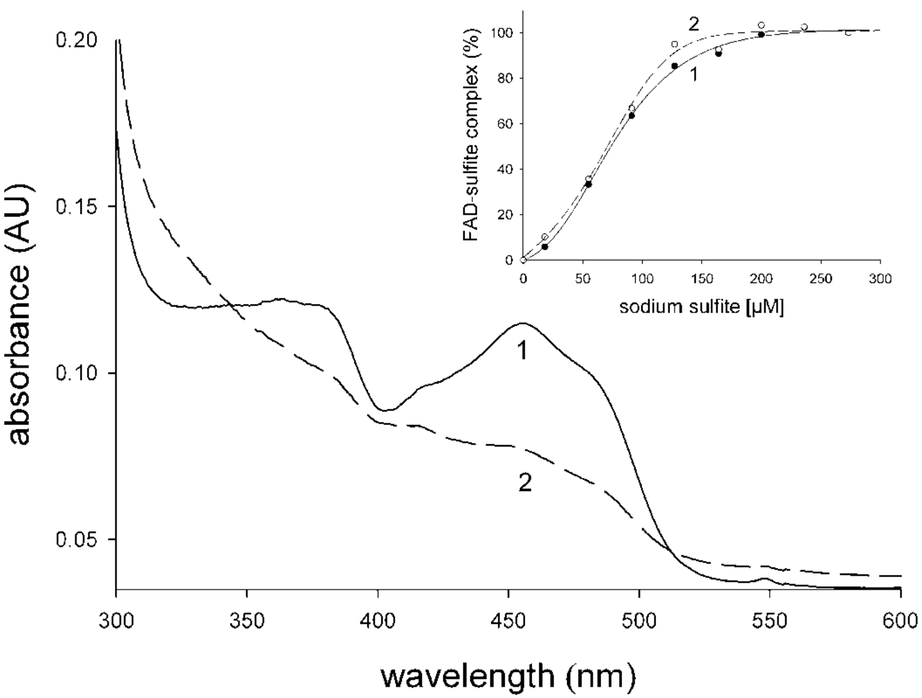
- UV/Vis spektrofotometrija

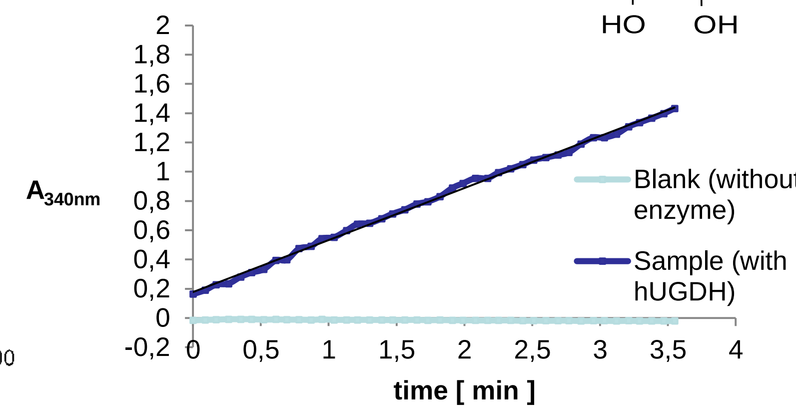


FADH$_2$

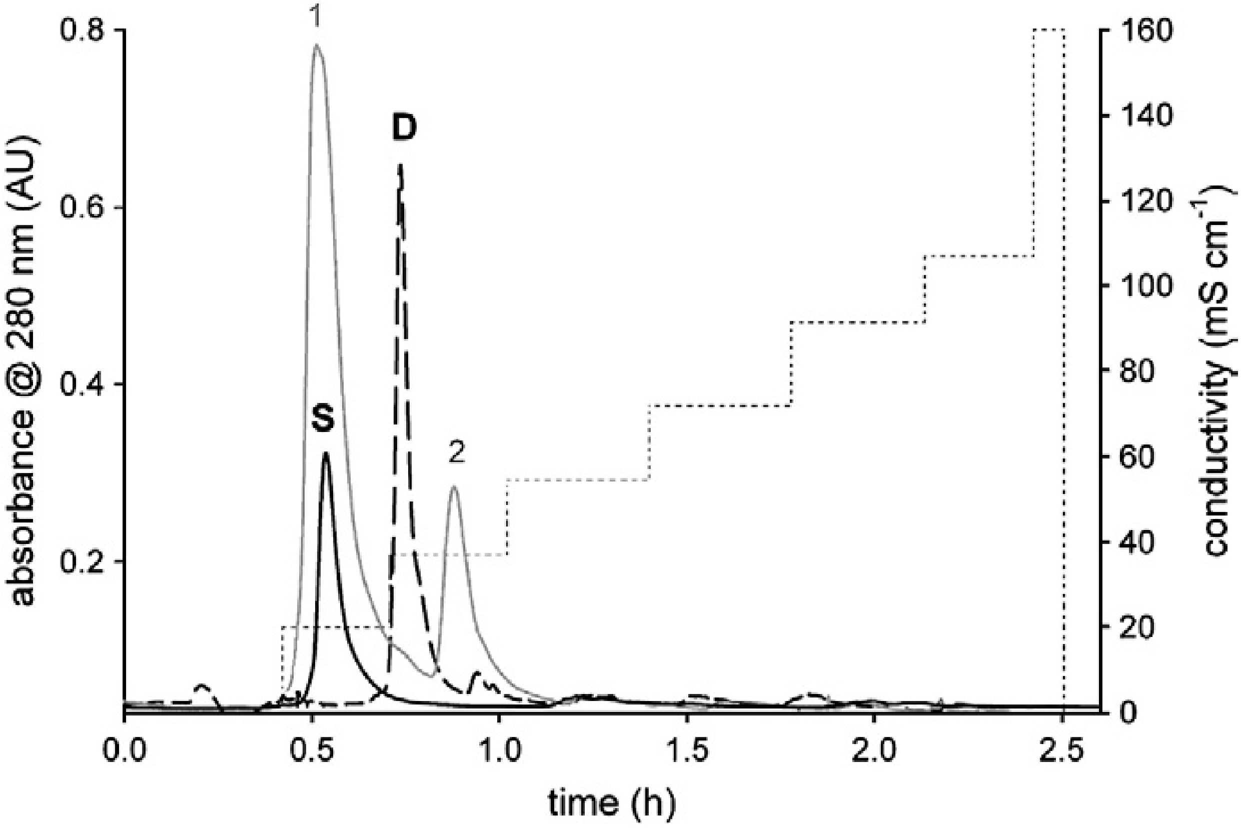


FAD-zavisna oksidaza D-aminokiselina



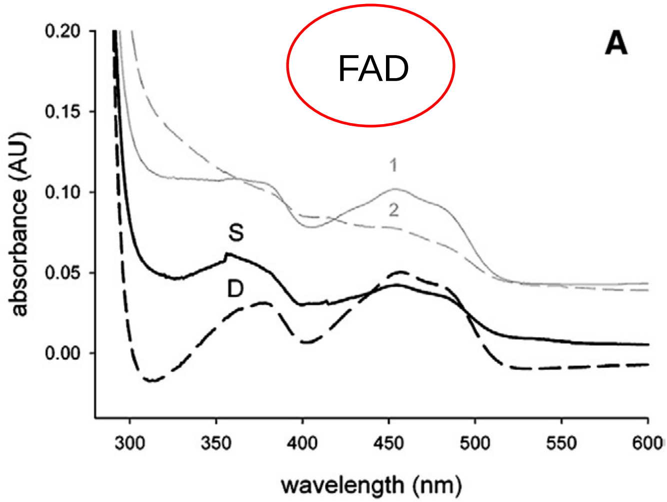


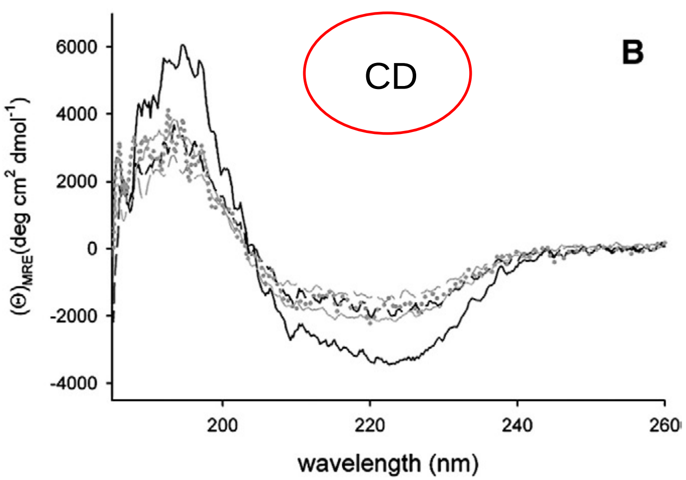
ionsko-izmjenjivačka kromatografija (FPLC) - Mono Q



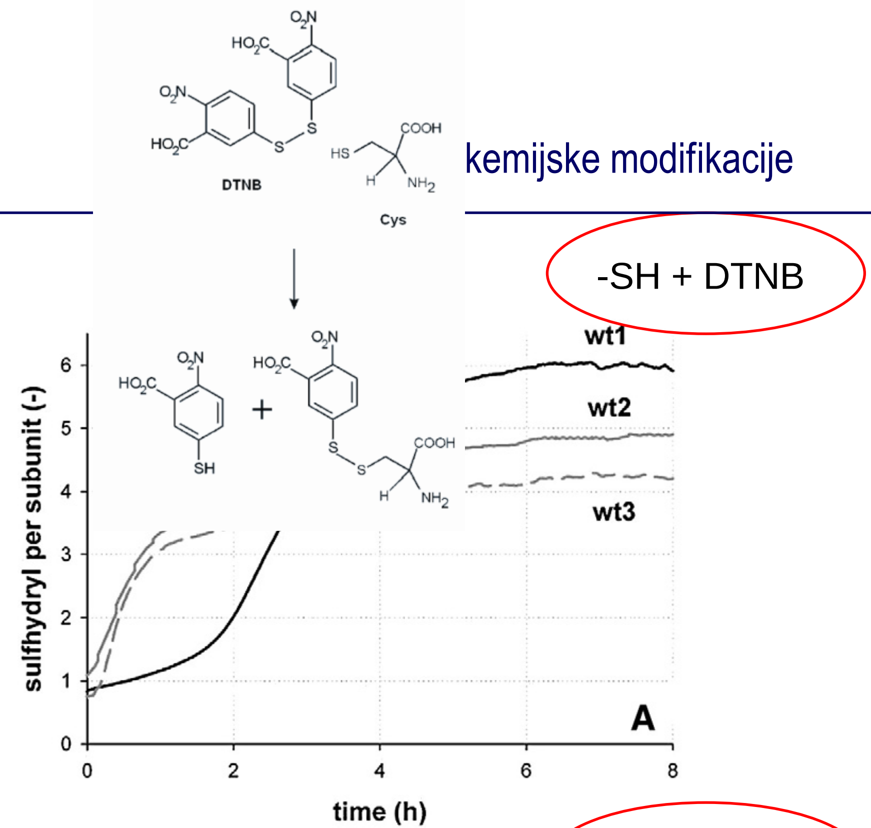
analiza proteina: specifične (ciljane) modifikacije (2)

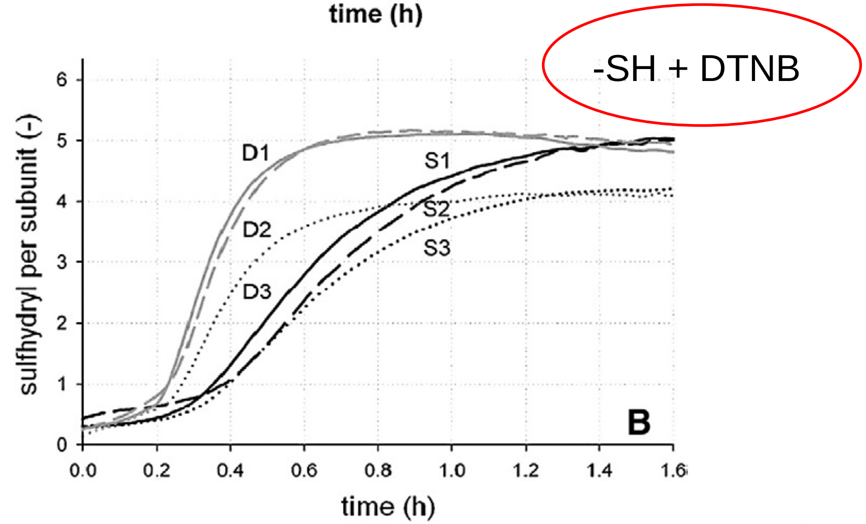
točkasta mutacija





kemijske modifikacije





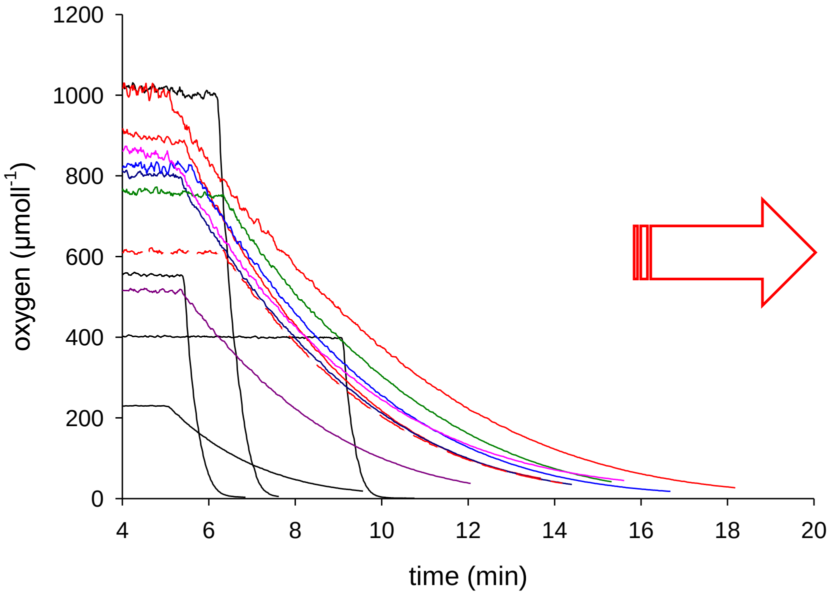
aktivnost enzima: primjena mikrosenzora (O_2) (1)

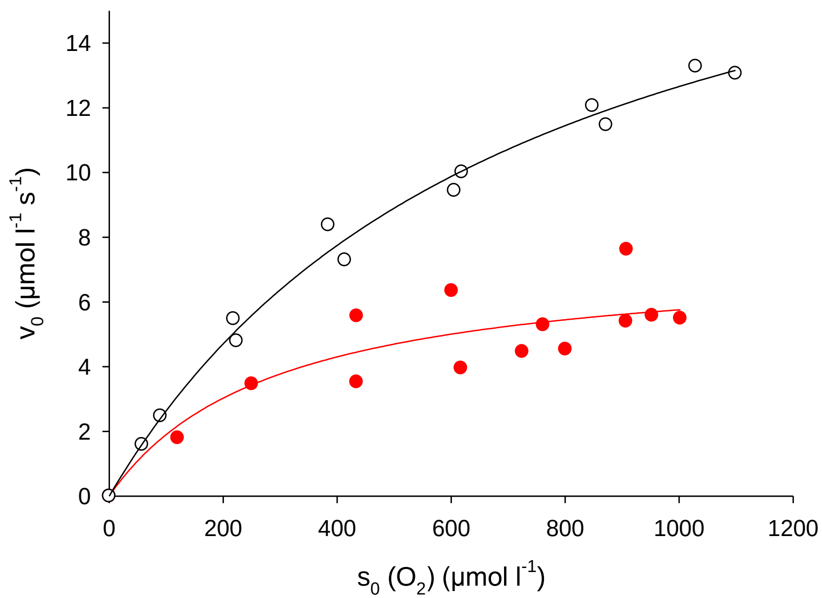
aktivnost enzima: primjena mikrosenzora (O_2) (2)

princip: smanjenje intenziteta fluorescencije

(eng. quenching) kisikom

Specifications	Gaseous & Dissolved Oxygen	Dissolved Oxygen
Measurement range	0 – 3 % O_2	0 – 1.1 mg/L
	0 – 28.8 hPa	0 – 34.5 μmol
Limit of detection	0.002 % oxygen	1 ppb
Resolution	± 0.0009 % O_2 at 0.002 % O_2	± 0.4 ppb at 10 ppb
	± 0.001 % O_2 at 0.02 % O_2	± 0.63 ppb at 200 ppb
	± 0.010 hPa at 0.23 hPa	± 0.013 μmol at 0.31 μmol
	± 0.015 hPa at 2.0 hPa	± 0.020 μmol at 6.20 μmol
Accuracy	± 1 ppb or ± 5 % of the respective concentration whichever is higher	
Drift at 0 % oxygen	< 10 ppb within 30 days (sampling interval of 1 min)	
Measurement temperature range	0 – 50 °C	
Response time TS* (t_{90})	not available	
Response time TF** (t_{90})	< 15 s (gas)	< 30 s (liquid)





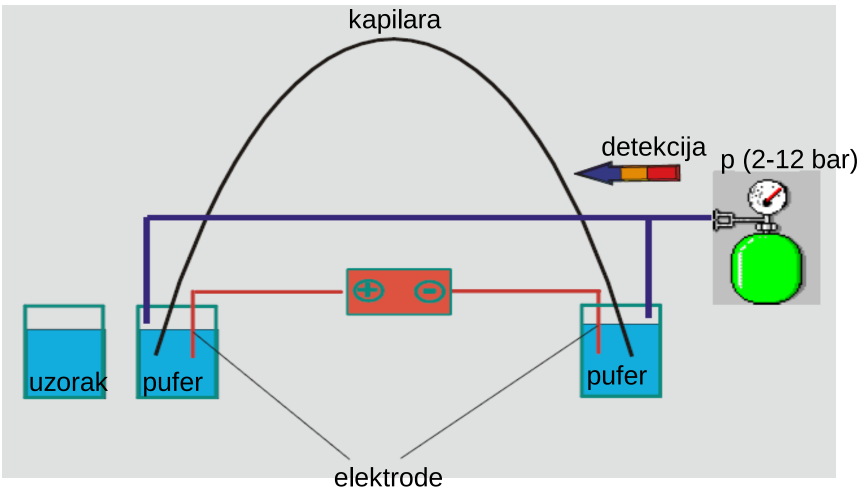
- kapilarna elektrokromatografija

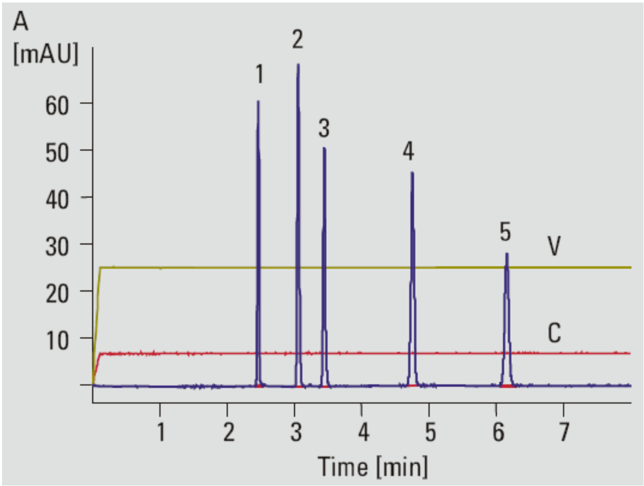
HPLC + CE (kapilarna elektroforeza),

elektrolit prolazi kroz kolonu (kapilaru) “tjeran” električnim poljem,

detekcija: UV/Vis, fluorecencija, MS i dr.

V = 25 kV
C – jakost (A)
t = 20°C
p = 10-12 bar
25 mM ACN/Tris.HCl, pH 8

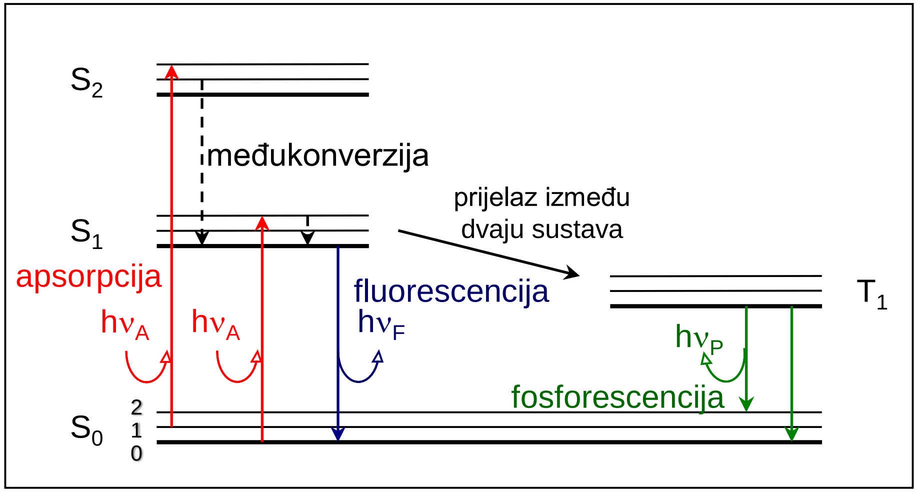




luminiscencija: molekule određene tvari u pobuđenom stanju emitiraju svjetlost određene valne duljine

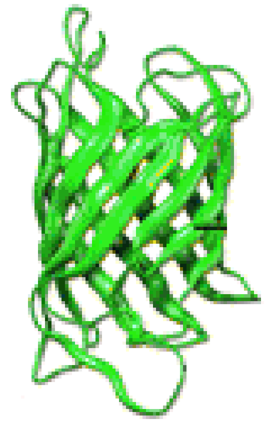
fosforescencija: e^- u orbitali višeg energetskog nivoa ima isti spin kao e^- u orbitali nižeg (osnovnog) energetskog nivoa, povratak e^- u orbitalu nije povoljan i odvija se relativno sporo (10^3-10^0 s^{-1}) uz emisiju fotona

fluorescencija: e^- u orbitali višeg energetskog nivoa ima suprotan spin s obzirom na e^- u orbitali nižeg energetskog nivoa, povratak u orbitalu je povoljan i odvija se brzo (10^8 s^{-1}) uz emisiju fotona



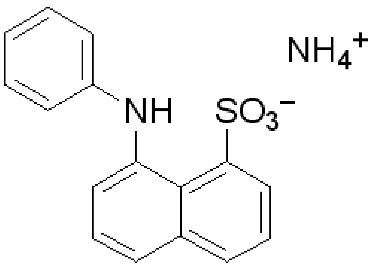
Jabłoński dijagram

fluorescentna spektrometrija: GFP i nekovalentno obilježavanje proteina



GFP (eng. <u>G</u>reen <u>F</u>luorescent <u>P</u>rotein)

- npr. *Aequorea victoria*, *Renilla reniformis*, itd.
- varijacije GFP (cijan, žuti, crveni,...)
- FRET (eng. <u>F</u>luorescence <u>R</u>esonance <u>E</u>nergy <u>T</u>ransfer)
- neke primjene GFP-a: obilježavanje stanica i organela, porijeklo stanica, marker kod fuzije, ekspresija gena, protein-protein međudjelovanje, lokalizacija proteina,...



amonijeva sol 8-anilino-1-naftalen sulfonske kiseline (ANS)

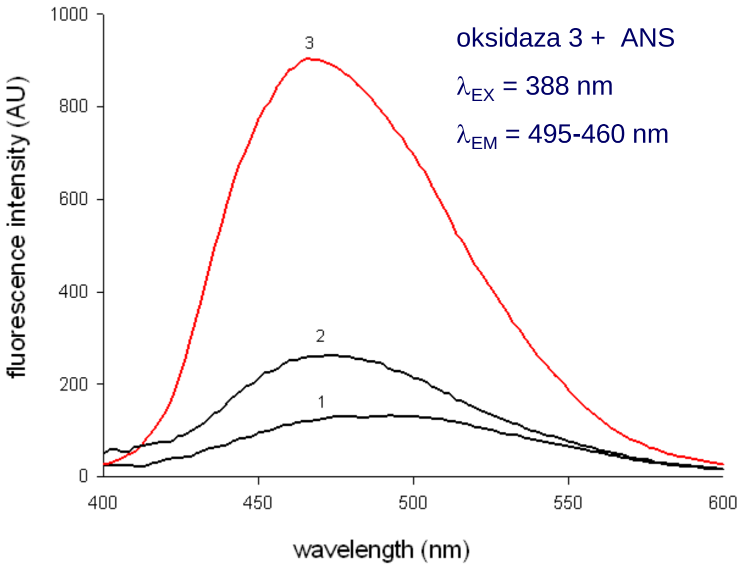
oksidaza 1 + ANS

oksidaza 2 + ANS

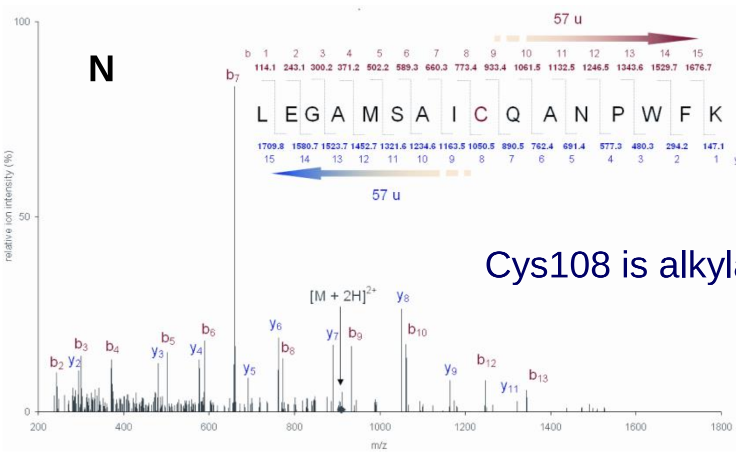
oksidaza 3 + ANS

λ_{EX} = 388 nm

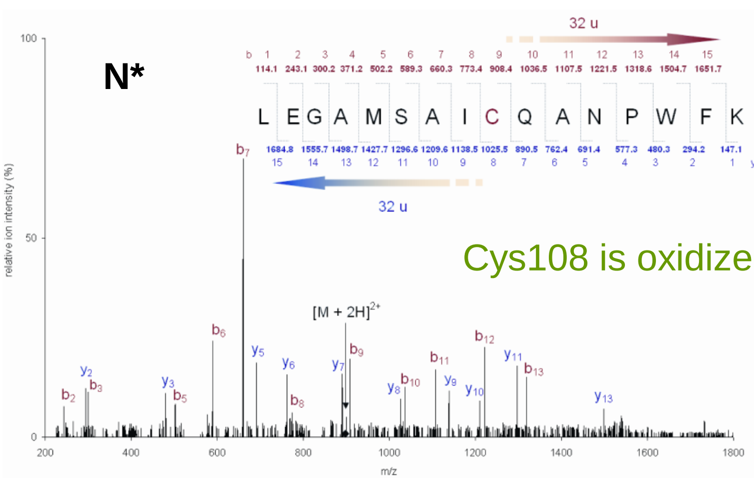
λ_{EM} = 495-460 nm



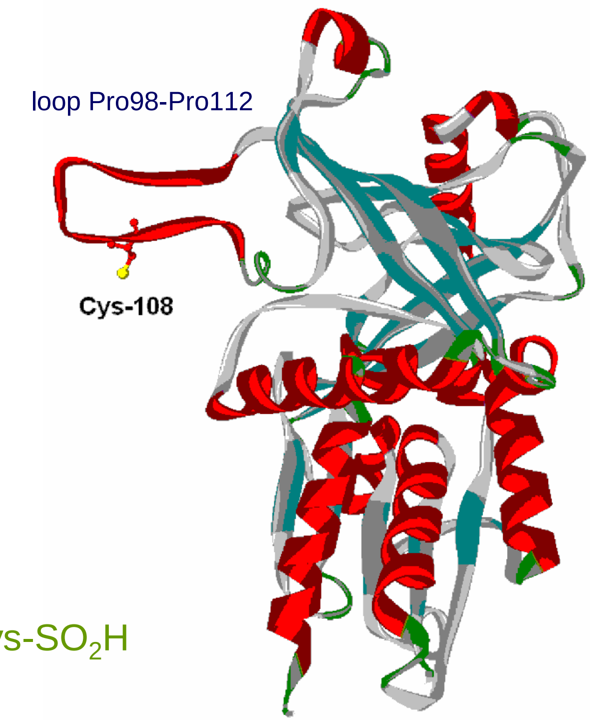
oksidativna posttranslacijska modifikacija: FAD-zavisna oksidaza D-aminokiselina (*Tv*DAO)



Cys108 is alkylated



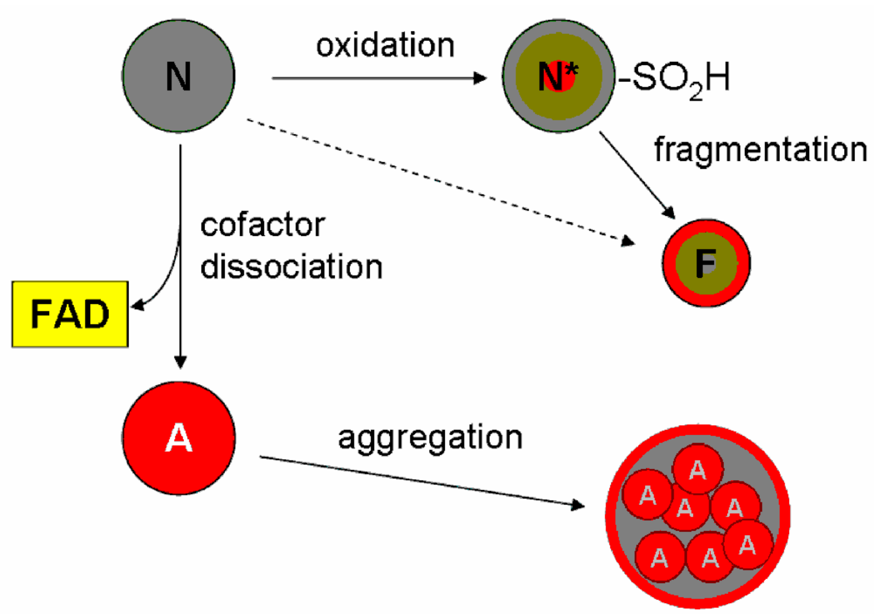
Cys108 is oxidized to Cys-SO_2H



Predviđena trodimenzionalna struktura *Tv*DAO

MS

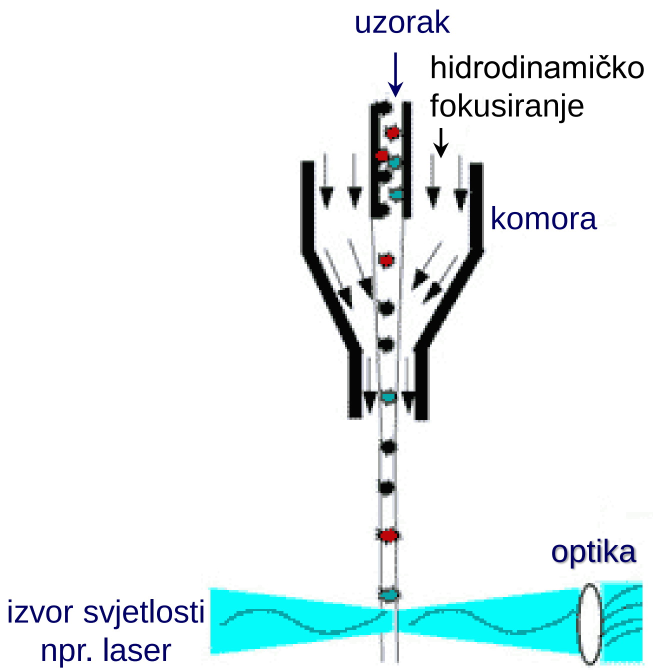
mehanizam inaktivacije: oksidacija (signalno cijepanje - fragmentacija) i agregacija *Tv*DAO

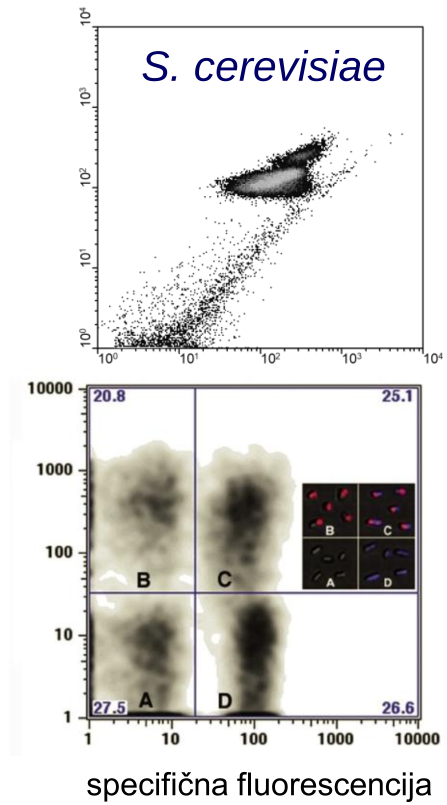


N...native
N*..oxidatively modified
F...fragmented
A...apo-protein form
green...active
red...inactive

animacija: **Life Cycle of a Protein**
www.sumanasinc.com/webcontent/animations/molecularbiology.html

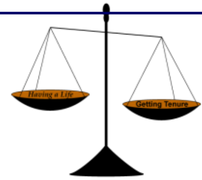
- vrlo precizna metoda koja koristi principe apsorpcije svjetlosti, rasapa svjetlosti (eng. light scattering), pobuđivanja svjetlošću (eng. light excitation) i emisije svjetlosti određene valne duljine
- tijekom analize uzorka (čestica, dijelova stanice ili cijelih stanica promjera 0.5-40 μm) nastaje set višeparametarskih podataka koji su karakteristični za uzorak





Professor Chris J. Hewitt
Ashton University, UK

spektrometrija masa (eng. mass spectrometry, MS)



- prvi spektrometar masa
- prvi MS stručnjak: Antoine Laurent de Lavoisier (1743-1794)

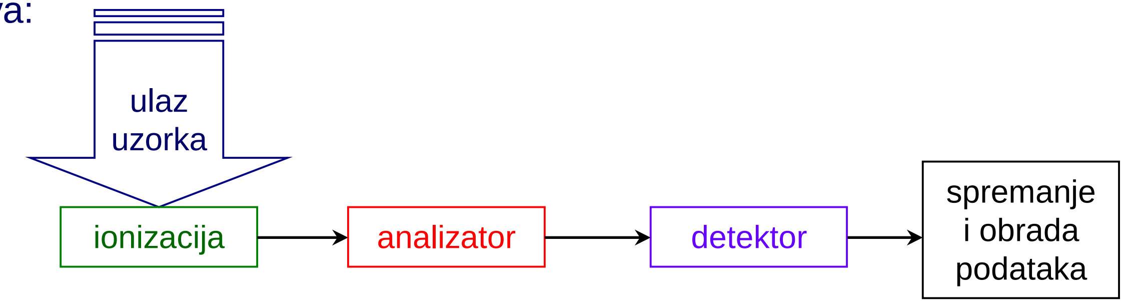
- MS analiza: prikupljanje, obrada i interpretacija spektara masa iona i njihovih fragmenata

J. B. Fenn, Nobelova nagrada za kemiju 2002. godine (1/4),

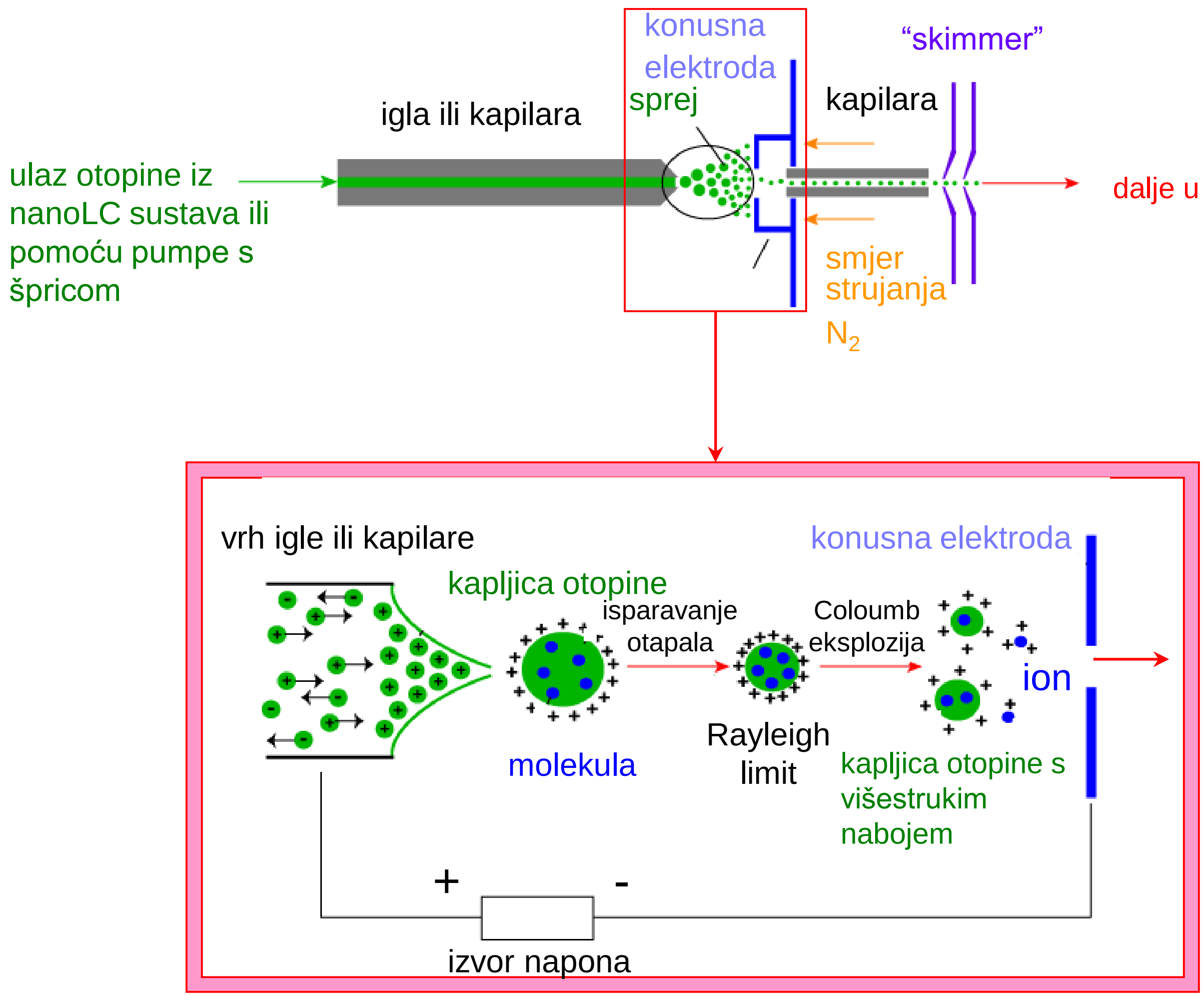
Fenn J.B., Mann M., Meng C.K., Wong S.F., Whitehouse C.M. (1989) Electrospray ionization for mass spectrometry of large biomolecules, *Science*, 246(4926):64-71.

Karas M., Hillenkamp F. (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons, *Anal. Chem.* 60(20):2299-301.

- naredna generacija MS sustava: MS putovnice (identifikacija proteoma)
- naredna generacija MS stručnjaka: carinski službenik
- “POVIJEST” I PRIPREMA UZORKA!(npr. denaturacija proteina, redukcija, alkilacija, tripsin, zaustavljanje reakcije)
- komponentne MS sustava:



spektrometrija masa (MS): ESI (eng. ElectroSpray Ionization) (“soft” ionization) (1)



$$p\,M + p\,e^-$$

$\downarrow$ ionizacija

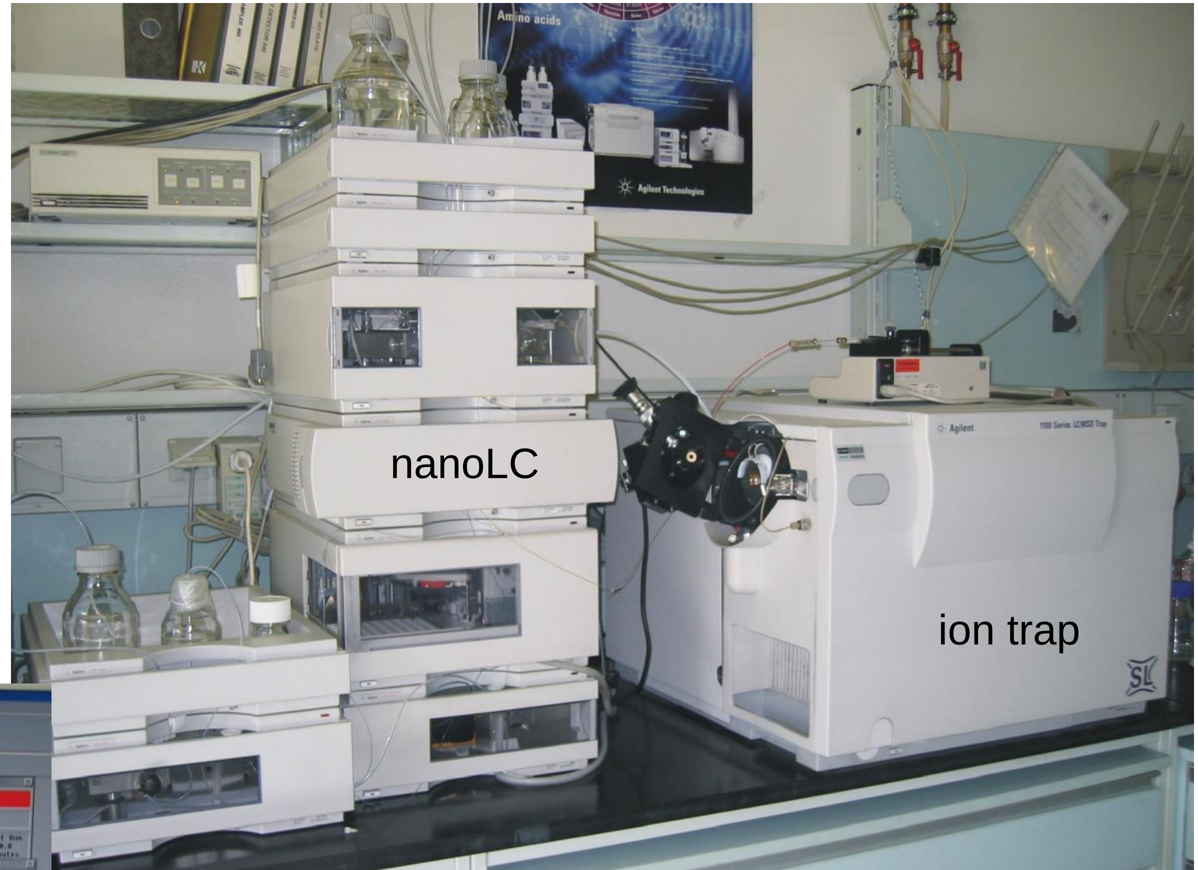
$$p\,M^{\bullet+} + 2p\,e^-$$

fragmentacija (CID)

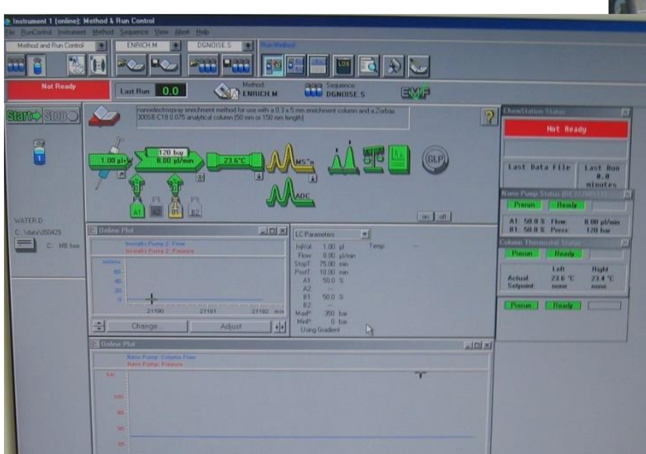
$$q\,M^{\bullet+} \quad r\,A^+ \quad s\,B^+$$

$$p = q + r + s$$

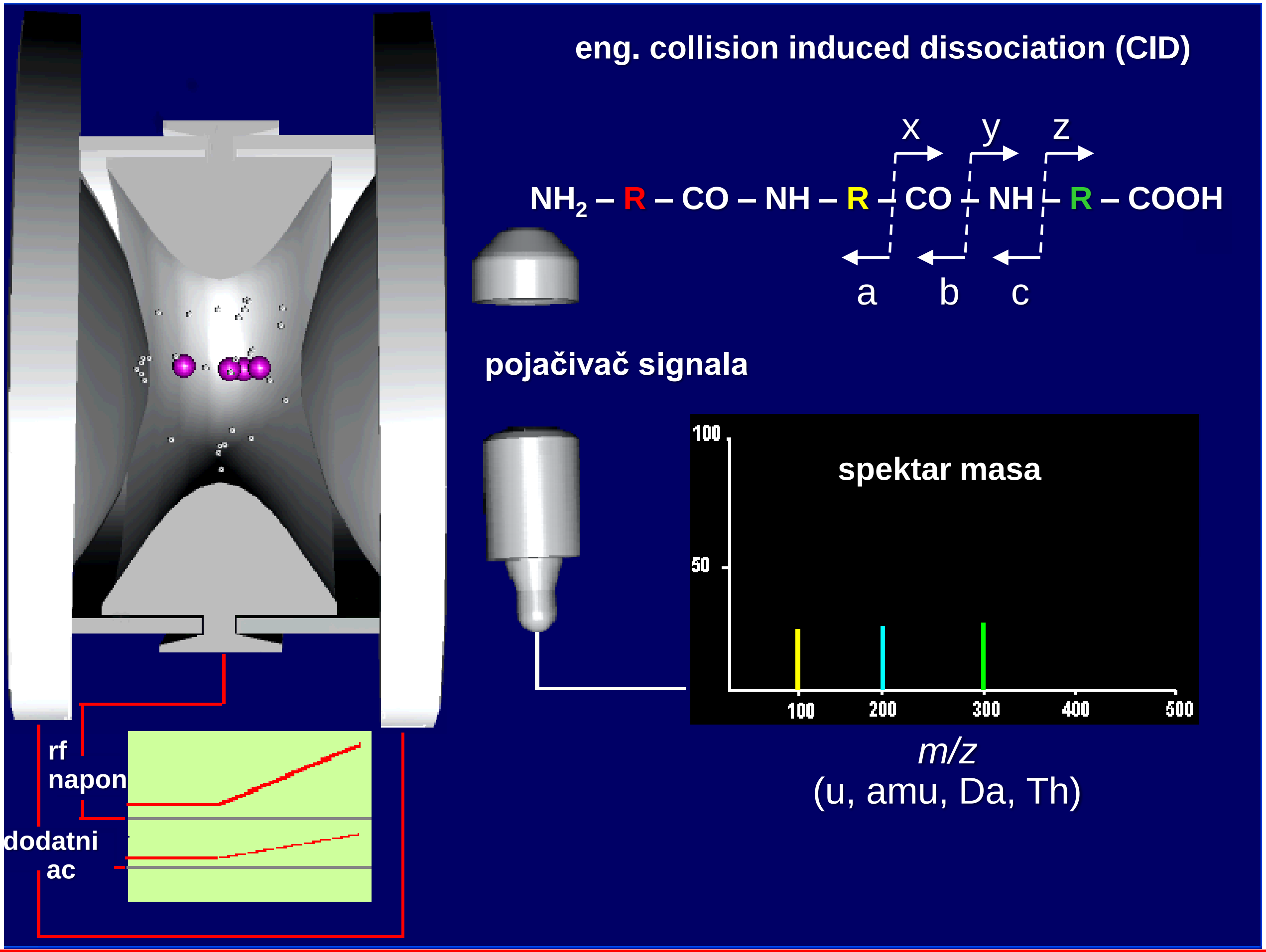
spektrometrija masa (MS): ESI (eng. ElectroSpray Ionization) (“soft” ionization) (2)



Software: ChemStation



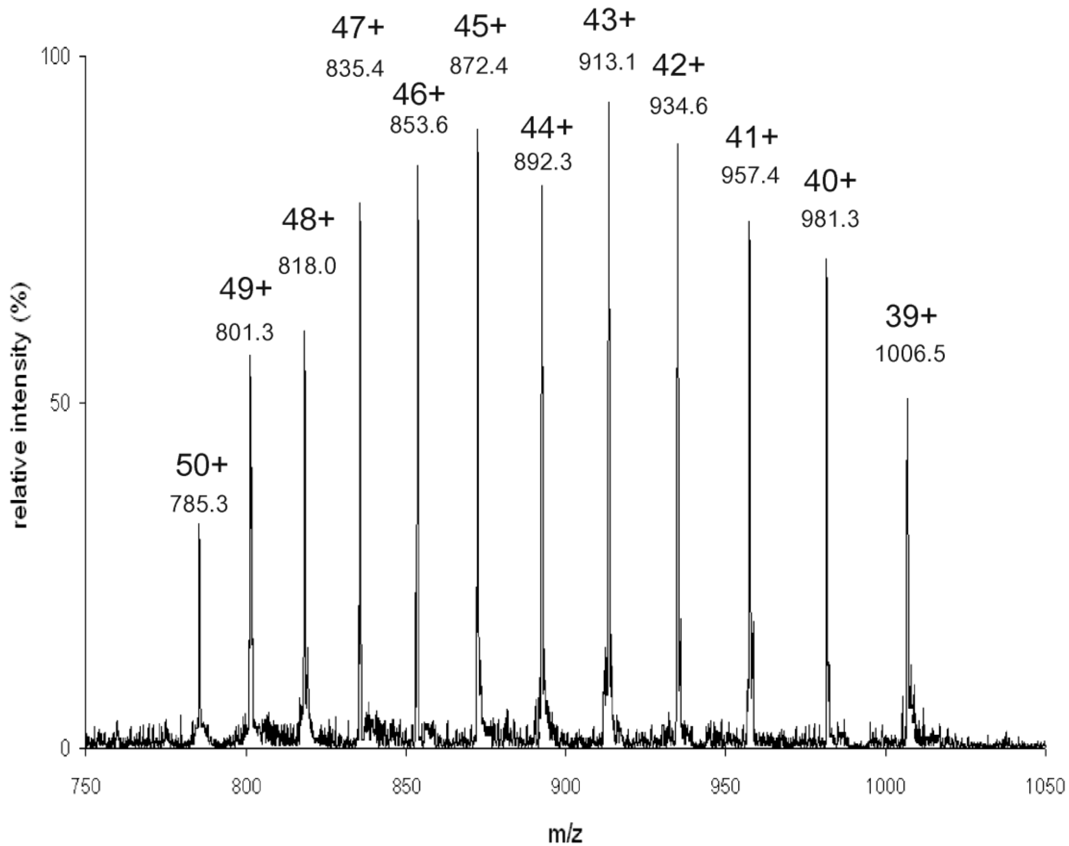
spektrometrija masa (MS): IT (eng. Ion Trap) spektrometar masa (3)



hyperlinks

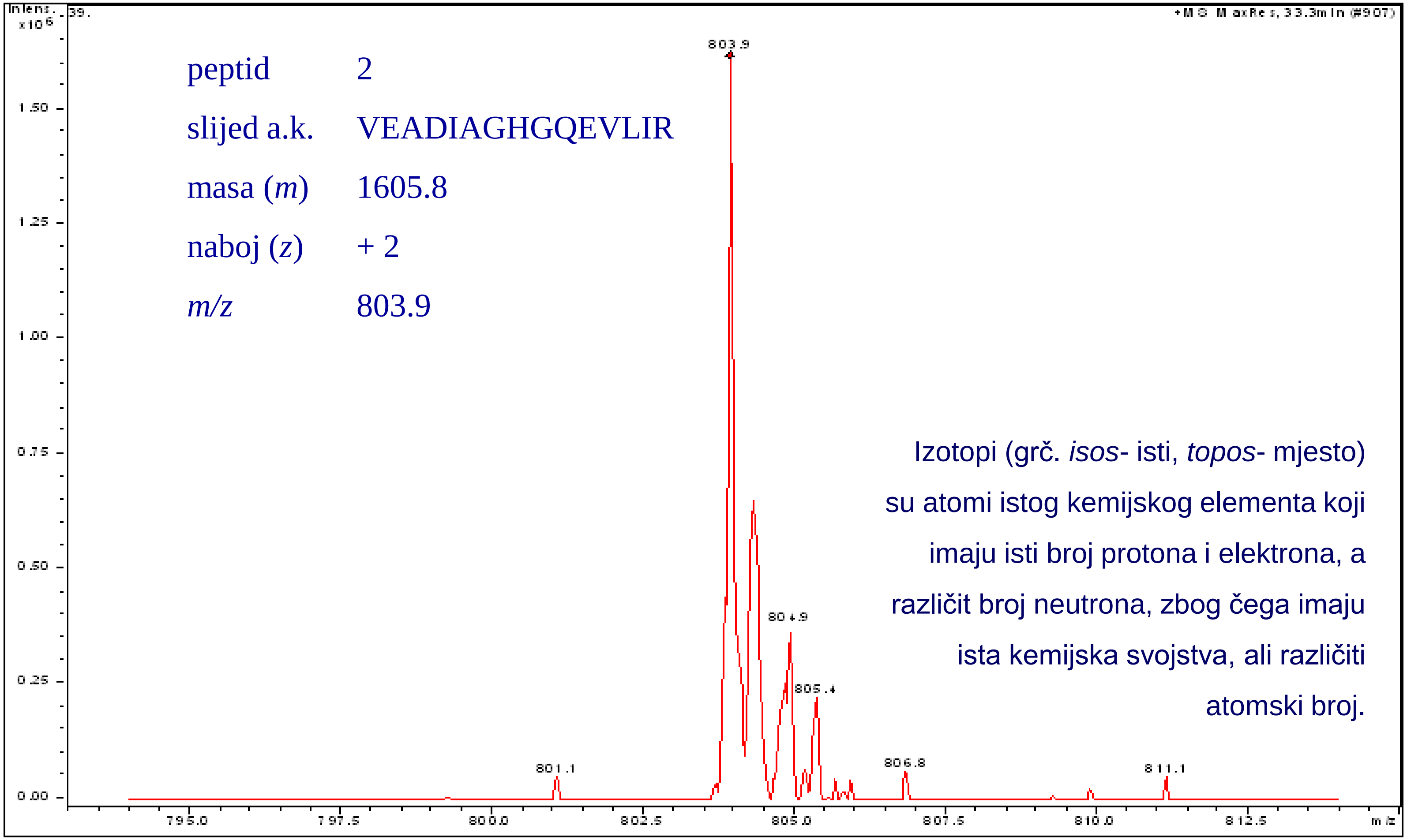
dampinggas capture scanning_ms detection_ms elimination collision trapping_msms scanning_msms

spektrometrija masa (MS): analiza FAD-zavisne oksidaze D-aminokiselina (ESI/MS)



DAO	*m/z*	*z*	deconvoluted mass*
F1			39214.1 ± 10
F2	836.0	47	
	854.2	46	
	873.0	45	
	893.0	44	
	913.8	43	
	935.4	42	
	958.3	41	
	982.1	40	39246.3 ± 10
F3	774.6	15	
	831.5	14	
	895.6	13	
	970.2	12	
	1058.6	11	
	1163.8	10	11630.2 ± 10

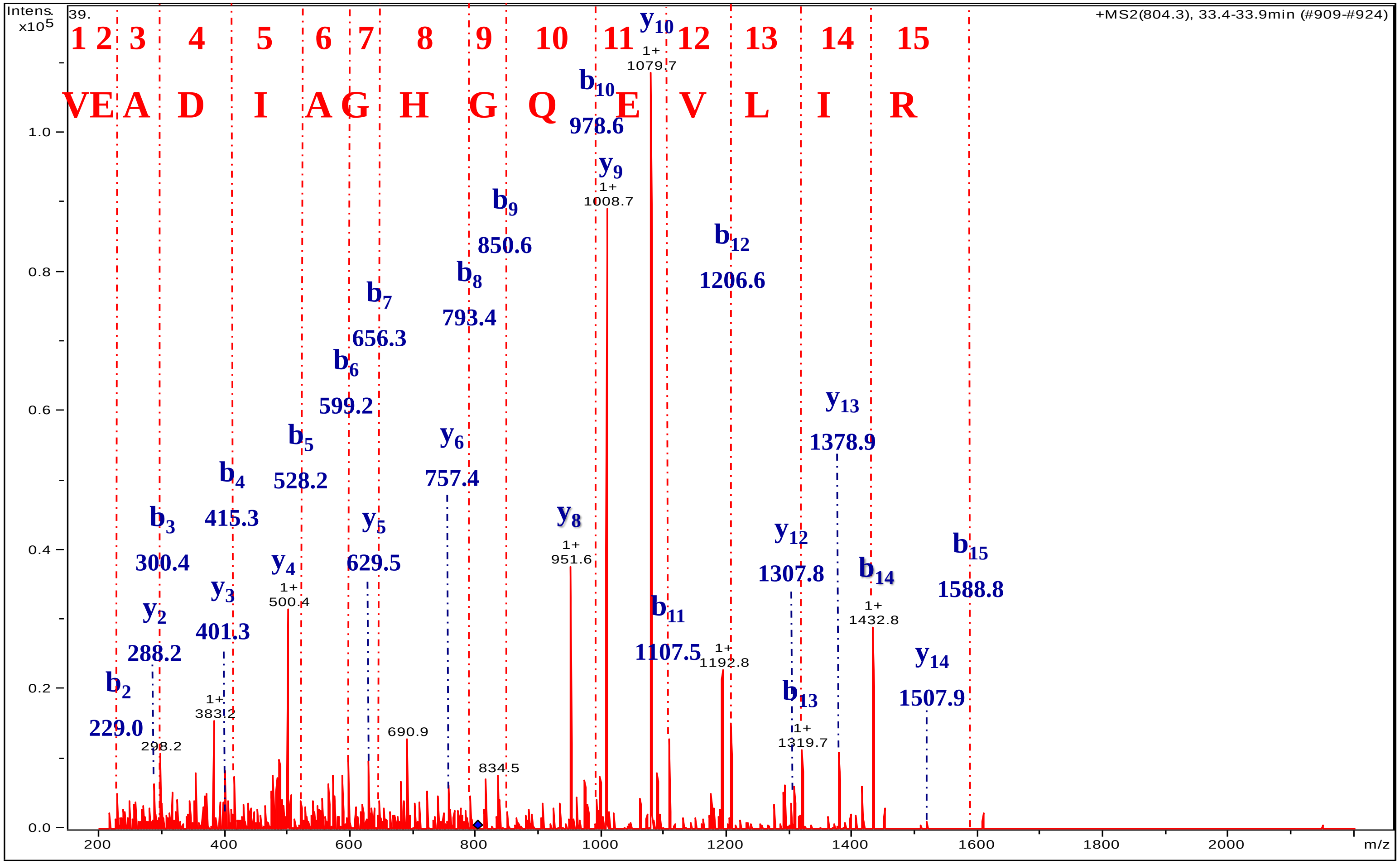
*amu



peptid	2
slijed a.k.	VEADIAGHGQEVLIR
masa (*m*)	1605.8
naboj (*z*)	+ 2
m/z	803.9

Izotopi (grč. *isos*- isti, *topos*- mjesto) su atomi istog kemijskog elementa koji imaju isti broj protona i elektrona, a različit broj neutrona, zbog čega imaju ista kemijska svojstva, ali različiti atomski broj.

spektrometrija masa (MS): određivanje primarne strukture mioglobina (nanoLC/ESI/MS/MS)



Peptide Mass Search: Matrix Science

User: Anita

Email: Slavica@biote.tu-graz.ac.at

Search title :

MS data file: C:\Dokumente und Einstellungen\Slavica\EigeneDateien\Data\DataAnalysis\Anita051202\MyoDenaturatedDigTry051202-2\Analysis.mgf

Database: SwissProt 40.39 (201563 sequences; 110352130 residues)

Timestamp: 24 Jan 2003 at 08:32:44 GMT

Significant hits:

P02188 **Myoglobin STANDARD VARSPLIC; STANDARD VARIANT; STANDARD CONFLICT FROM P02188**

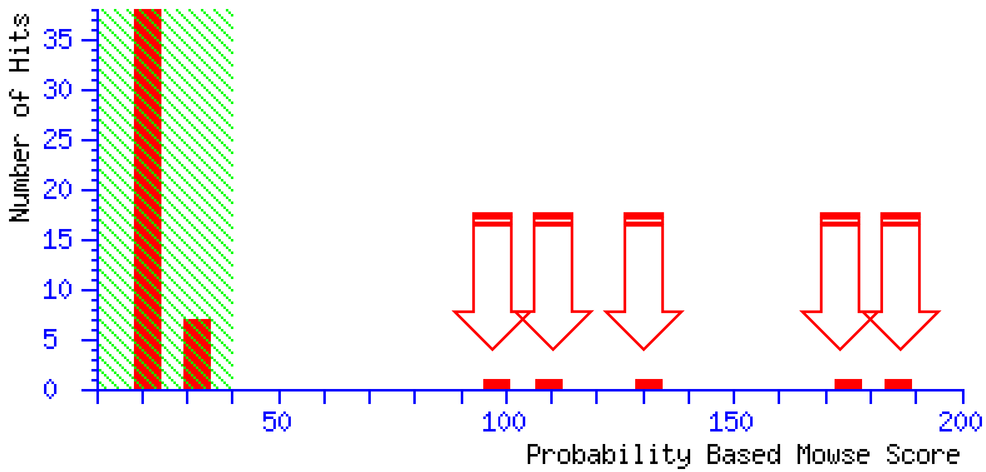
P02188-00-00-01 **Myoglobin STANDARD VARSPLIC; STANDARD VARIANT; REF. 1 FROM P02188**

P02170 **Myoglobin**

P02187 **Myoglobin**

P14396 **Myoglobin Probability Based Mowse Score**

Score is -10*Log(P), where P is the probability that the observed match is a random event. Individual ions scores > 40 indicate identity or extensive homology (p<0.05).

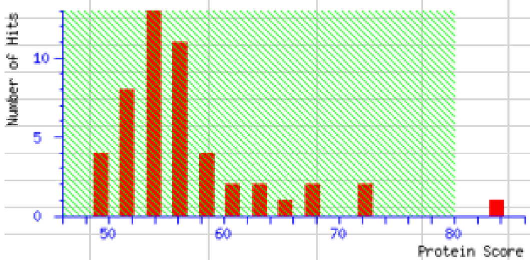


spektrometrija masa: *on-line* interpretacija i vrednovanje rezultata

Search title : Marios Project folder\bsa\MSMS 72\N1
Database : NCBInr 20140312 (37725837 sequences; 13391628756 residues)
Taxonomy : Firmicutes (gram-positive bacteria) (5487348 sequences)
Timestamp : 10 Jul 2017 at 18:11:03 GMT
Warning : A Peptide summary report will usually give a much clearer picture of MS/MS search results.
Top Score : 84 for gi|511434551, hypothetical protein Lpp126_18752, partial [Lactobacillus paracasei subsp. paracasei Lpp126]

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 80 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Index

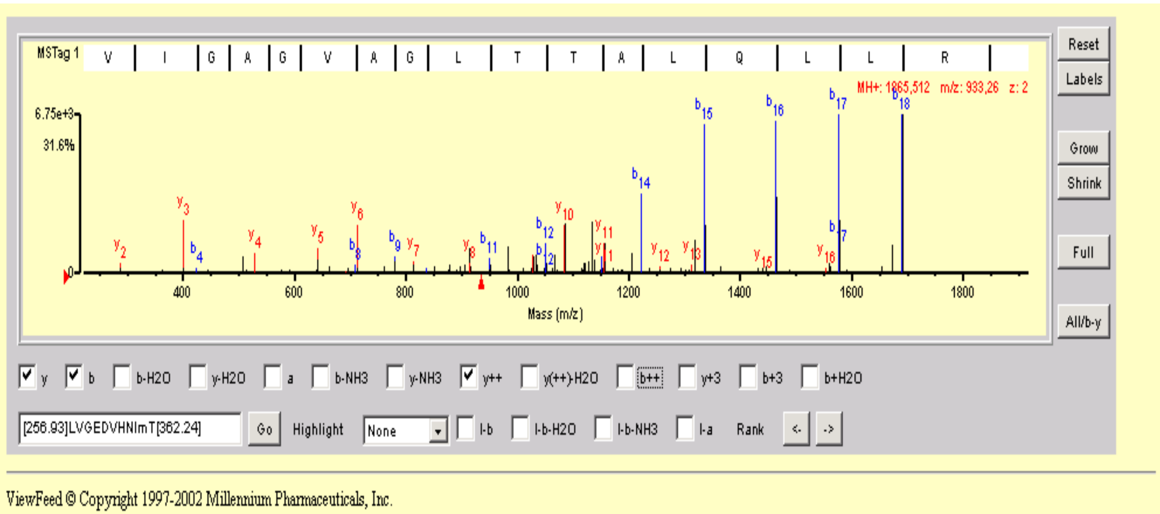
	Accession	Mass	Score	Description
1	gi|51143455	3381	84	hypothetical protein Lpp126_18752, partial [Lactobacillus paracasei subsp. paracasei Lpp126]
2	gi|48772733	3298	72	branched-chain amino acid ABC transporter, amino acid-binding protein, partial [Streptococcus pneumoniae]

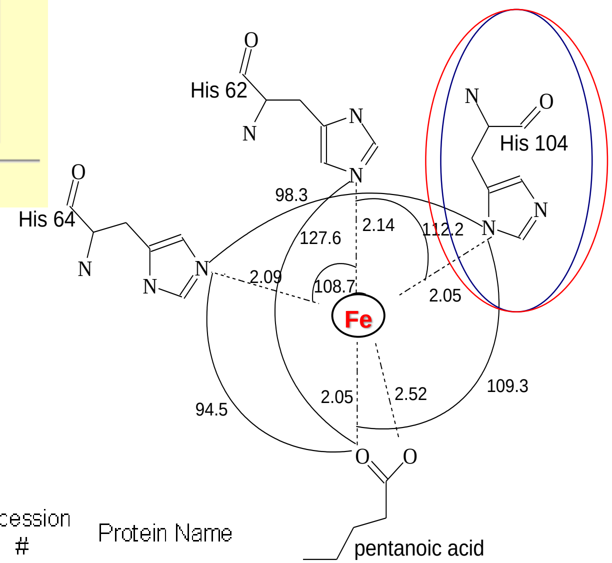
Results List

1. gi|511434551 Mass: 3381 Score: 84 Expect: 0.024 Matches: 80
hypothetical protein Lpp126_18752, partial [Lactobacillus paracasei subsp. paracasei Lpp126]

Observed	Mr(expt)	Mr(calc)	Delta	Start		End	Miss	Ions	Peptide
1006.4501	1005.4428	1005.3692	0.0736	17	-	24	0	---	L.NESFQKTA.T + 2 Deamidated (NQ); Phospho (ST)
1011.6142	1010.6069	1010.4920	0.1149	18	-	26	0	---	N.ESFQKTATV.S + Deamidated (NQ)
1023.4194	1022.4121	1022.4548	-0.0427	9	-	16	0	---	K.VYFIMTGL.N + Phospho (Y)
1027.5426	1026.5353	1026.4692	0.0661	12	-	20	0	---	F.IMTGLNESF.Q + Oxidation (M)
1044.5250	1043.5177	1043.4722	0.0455	21	-	29	0	---	F.QKTATVSVM.- + Phospho (ST)
1045.5370	1044.5297	1044.4563	0.0735	21	-	29	0	---	F.QKTATVSVM.- + Deamidated (NQ); Phospho (ST)

peptide sequencing (*Sherenga, Spectrum Mill v2.7*)
User: Anita
Email: Slavica@biote.tu-graz.ac.at





peptide 7 (analysis *via Spectrum Mill*)

GGEQEGGSTAYAPSYGFESSGAL$\mathbf{H}_{104}$GK

z	Score	SPI (%)	Spectrum Intensity	Sequence	Protein pI	Species	Accession #	Protein Name
3	18.72	93.9	1.36e+007	(R) GGEQEGGSTAYAPSYGFESSGALHGK(T)	4.96	Acinetobacter johnsonii	24078520	diketone-cleaving enzyme Dke1

preferential ionization of His residues

in silico analiza: *de novo* sequencing

peptide *de novo* sequencing (*Sherenga, Spectrum Mill v2.7*)
User: Anita
Email: Slavica@biote.tu-graz.ac.at

#	Filename	z	Precursor MH^+	Precursor m/z	# Detected Peaks	MS Precursor EIC Intensity	Max Tag Length	Longest Sequence Tag	
1	111103F3.0384.0384.0	2	3051.740	1526.370	25	1.30e+004	3	[951.58]CQE[1681.36]	Gln158 – Cys159 (opposite direction)
2	111103F3.0009.0040.0	2	933.530	467.270	25	2.91e+006	2	[237.46]AN[509.31]	
3	111103F3.0010.0039.0	2	845.170	423.090	25	2.16e+006	2	[1928.98]AV[-1255.56]	
4	111103F3.0014.0019.0	2	1021.540	511.270	23	6.38e+005	2	[1366.34]WP[-629.99]	
5	111103F3.0030.0030.0	2	2057.250	1029.130	25	4.68e+004	2	[523.69]LS[1331.68]	
6	111103F3.0050.0054.0	2	1021.070	511.040	23	3.71e+005	2	[1731.45]CL[-986.03]	Cys145 – Ile146
7	111103F3.0085.0085.0	2	2036.040	1018.530	16	1.21e+004	2	[1634.06]WW[27.82]	
8	111103F3.0100.0100.1	1	2102.210	2102.210	14	7.92e+003	2	[1063.87]DW[735.40]	
9	111103F3.0106.0106.0	2	3000.870	1500.940	25	8.86e+003	2	[1854.98]VL[931.84]	
10	111103F3.0134.0156.0	2	2993.980	1497.500	25	7.80e+003	2	[1258.32]CR[1416.01]	
11	111103F3.0191.0191.0	2	2134.380	1067.690	25	9.17e+003	2	[1838.09]CC[1036.88]	disulfide bond
12	111103F3.0212.0212.0	2	3449.370	1725.190	24	1.42e+004	2	[539.13]NC[2633.45]	Asn192 – Cys193
13	111103F3.0215.0215.0	2	1898.640	949.830	18	9.79e+003	2	[886.43]LE[768.38]	
14	111103F3.0221.0221.0	2	2521.750	1261.380	20	1.60e+004	2	[1672.23]DE[603.31]	
15	111103F3.0255.0255.0	2	1562.680	781.840	18	1.73e+004	2	[833.66]EW[411.63]	
16	111103F3.0261.0261.0	2	1089.120	545.060	17	1.27e+004	2	[205.83]AS[723.09]	
17	111103F3.0288.0288.0	2	3149.000	1575.000	17	1.02e+004	2	[3609.32]YL[833.15]	
18	111103F3.0297.0297.0	2	2754.540	1377.780	14	1.14e+004	2	[227.61]FL[2265.38]	
19	111103F3.0305.0305.0	2	3143.460	1572.230	23	1.05e+004	2	[145.00]VS[2810.75]	
20	111103F3.0308.0308.0	2	1335.530	668.270	15	2.15e+004	2	[532.65]VT[1266.25]	
21	111103F3.0342.0342.0	2	2126.330	1063.670	18	1.13e+004	2	[1959.33]TL[-49.72]	

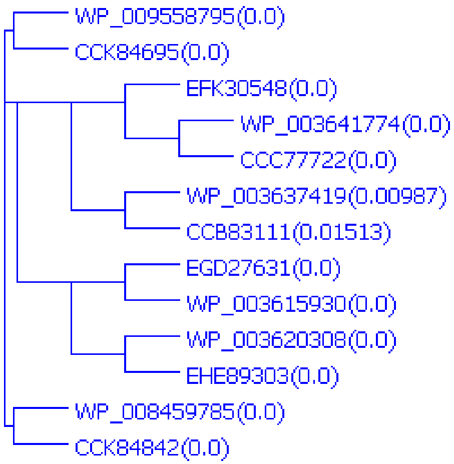
in silico analiza: multiple sequence alignment

cysteine residues are not conserved (< 30 sequences, search in TrEMBL)

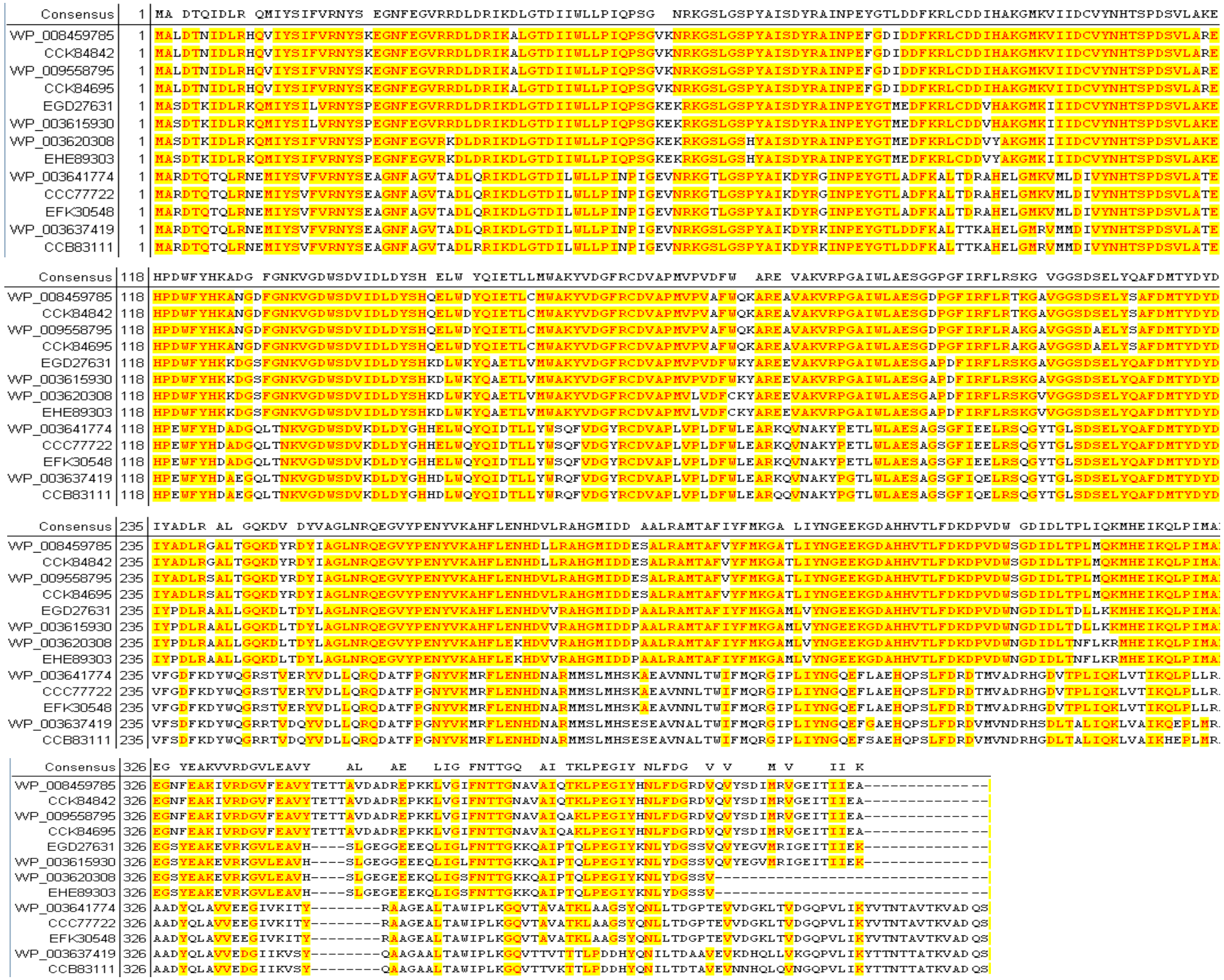
```
Trigonopsi   1 : ---MAKIVVIGAGVAGLTTALQLLR-KGHEVTIVSEFTPGDLSIGYTSPWAGANWLTFYDGGKLADYDAVSYPILRELARSSPEAGIRLISQRS--HVLK :  94
Fusarium_s   1 : --MSNTIVVVGAGVIGLTSALLLSKNKGNKITVVAKHMPGDYDVEYASPFAGANHSPMATEE-SSEWERRTWYEFKRLVEEVPEAGVHFQKSRIQRRNVD :  97
Rhodospori   1 : MHSQKRVVVLGSGVIGLSSALILARKGYSVHILARDLPEDVSSQTFASPWAGANWTPFMTLTDGPRQAKWEESTFKKWVELVPTG------------HAM :  88
Mus_muscul   1 : ----MRVAVIGAGVIGLSTALCIHERYHPTQP-LHMKIYADRFTPFTTSDVAAGLWQPYLSDPSN-PQEAEWSQQTFDYLLSCLHSPNAEK--------M :  86
Rattus_nor   1 : ----MRVAVIGAGVIGLSTALCIHERYHPAQP-LHMKIYADRFTPFTTSDVAAGLWQPYLSDPSN-PQEAEWNQQTFDHLQSCLHSPNAEK--------M :  86
Sus_scrofa   1 : ----MRVVVIGAGVIGLSTALCIHERYHSVLQPLDVKVYADRFTPFTTTDVAAGLWQPYTSEPSN-PQEANWNQQTFNYLLSHIGSPNAAN--------M :  87
homo_sapie   1 : ----MRVVVIGAGVIGLSTALCIHERYHSVLQPLDIKVYADRFTPLTTTDVAAGLWQPYLSDPNN-PQEADWSQQTFDYLLSHVHSPNAEN--------L :  87
Oryctolagu   1 : ----MRVVVIGAGVIGLSTALCIHELYHSALQPLDMTIYADRFTPLTNTDVAAGLWQPYLSDPSN-PQEADWSRQTFNHLLSHIHSPSAEK--------M :  87
Caenorhabd   1 : --MANIIPKIAIIGEGVIGCTSALQISKAIPNAKITVLHDKPFKKSCSAGPAGLFRIDYEENTEYGRASFAWFSHLYRTTK---GSETGVK--------L :  87

Trigonopsi  95 : RDLPKLEVAMSAICQRNPWFKNTVDSFEIIEDR--SRIVHDDVAYLVEFRSVCIHTGVYLNWLMSQCLSLGATVVKRRVNHIKDANLLHSSGSRPDVIVN : 192
Fusarium_s  98 : TEKAQRSGFPDALFSKEPWFKNMFEDFREQHPS--EVIPGYDSG--CEFTSVCINTAIYLPWLLGQCIKNGVIVKRAILNDISEAKKLSHAGKTPNIIVN : 193
Rhodospori  89 : WLKGTRRFAQNEDGLLGHWYKDITPNYRPLPSS--ECPPGAIGVT---YDTLSVHAPKYCQYLARELQKLGATFERRTVTSLEQAFDG------ADLVVN : 177
Mus_muscul  87 : GLALISGYNLFRDEVPDPFWKNAVLGFRKLTPS-EMDLFPDYG-YGWFNTSLLEGKSYLPWLTERLTERGVNLIHRKVESLEEVARGG-----VDVIIN : 179
Rattus_nor  87 : GLALISGYNLFRDEVPDPFWKSTVLGFRKLTPS-ELDMFPDYS-YGWFNTSLLEGKSYLSWLTERLTERGVKFIHRKVASFEEVVRGG-----VDVIIN : 179
Sus_scrofa  88 : GLTPVSGYNLFREAVPDPYWKDMVLGFRKLTPR-ELDMFPDYR-YGWFNTSLILEGRKYLQWLTERLTERGVKFFLRKVESFEEVARGG-----ADVIIN : 180
homo_sapie  88 : GLFLISGYNLFHEAIPDPSWKDTVLGFRKLTPR-ELDMFPDYG-YGWFHTSLILEGKNYLQWLTERLTERGVKFFQRKVESFEEVAREG-----ADVIVN : 180
Oryctolagu  88 : GLALISGYNLFRKAVPDPSWKDTVLGFRKLTLR-ELDMFPGYS-YGWFNTSLILDGRSYLQWLTKRLTERGVKLFQRKVESFDEVAGGG-----VDVIVN : 180
Caenorhabd  88 : VSGHIQSDNLESLKQQQRAYGDIVYNFRFLDDRERLDIFPEPSKHCIHYTAYASEGNKYVPYLKNLLLEQKIEFKQQEVTSLDAVADAG-----YDVIVN : 182

            Cys 193
Trigonopsi 193 : CSGLFARFLGGVEDKKMYPIRGQVVLVRNSLPFMASFSSTPEKENEDEALYIMTRFDG-TSIIGGCFQPNNWSSEPDPSLTHRILSRALDRFPELTKDG- : 290
Fusarium_s 194 : AIGLGSYKLGGVEDKTMAPARGQIVVVRNESSPMLLTSGVE--DGGADVMYLMQRAAGGGTILGGTYDVGNWESQPDPNIANRIMQRIVEVRPEIANGKG : 291
Rhodospori 178 : ATGLGAKSIAGIDDQAAEPIRGQTVLVKSPCKRCTMDSSDP-----ASPAYIIPRPGG-EVICGGTYGVGDWDLSVNPETVQRILKHCLRLDPTISSDGT : 271
Mus_muscul 180 : CTGVWAGALQADA--SLQPGRGQIIQVEAPWIKHFILTHDPSLGIYNSPYIIP---GSKTVTLGGIFQLGNWSGLNSVRDHNTIWKSCCKLEPTLKNARI : 274
Rattus_nor 180 : CTGVWAGALQADA--SLQPGRGQIIQVEAPWIKHFILTHDPSLGIYNSPYIIP---GSKTVTLGGVFQLGNWSELNSVHDHNTIWKSCCQLEPTLKNARI : 274
Sus_scrofa 181 : CTGVWAGVLQPDP--LLQPGRGQIIKVDAPWLKNFIITHDLERGIYNSPYIIP---GLQAVTLGGTFQVGNWNEINNIQDHNTIWEGCCRLEPTLKDAKI : 275
homo_sapie 181 : CTGVWAGALQRDP--LLQPGRGQIMKVDAPWMKHFILTHDPERGIYNSPYIIP---GTQTVTLGGIFQLGNWSELNNIQDHNTIWEGCCRLEPTLKNARI : 275
Oryctolagu 181 : CTGVWASALQPDP--LLQPGRGQIIKVDAPWVKHFIITHDPESGIYKSPYIIP---GVHAVTLGGIFQMGNWSEGNSTDDHNTIWKGCCSLEPTLKDARI : 275
Caenorhabd 183 : CAGLYGGKLAGDDD-TCYPIRGVILEVDAPWHKHFNYRD-------FTTFTIP---KEHSVVVGSTKQDNRWDLEITDEDRNDILKRYIALHPGMREPKI : 271

Trigonopsi 291 : --PLDIVRECVGHRPGREGGPRVELEKIPG----------------------VGFVVHNYGAAGAGYQSSYGMADEAVSYVERALTRPNL-------- : 356
Fusarium_s 292 : VKGLSVIRHAVGMRPWRKDGVRIEEEKLDD----------------------ETWIVHNYGHSGWGYQGSYGCAENVVQLVDKVGKAAKSKL------ : 361
Rhodospori 272 : IEGIEVLRHNVGLRPARRGGPRVEAERIVLPLDRTKSPLSLGRGSARAAKEKEVTLVHAYGFSSAGYQQSWGAAEDVAQLVDEAFQRYHGAARESKL- : 368
Mus_muscul 275 : VGELTGFRPVR--PQVRLEREWLRFGSS------------------------SAEVIHNYGHGGYGLTIHWGCAMEAANLFGKILEEKKLSRLPPSHL : 346
Rattus_nor 275 : MGELTGFRPVR--PQVRLERERLRFGSS------------------------SAEVIHNYGHGGYGLTIHWGCAMEAANLFGKILEEKNLSRMPPSHL : 346
Sus_scrofa 276 : VGEYTGFRPVR--PQVRLEREQLRFGSS------------------------NTEVIHNYGHGGYGLTIHWGCALEVAKLFGKVLEERNLLTMPPSHL : 347
homo_sapie 276 : IGERTGFRPVR--PQIRLEREQLRTGPS------------------------NTEVIHNYGHGGYGLTIHWGCALEAAKLFGRILEEKKLSRMPPSHL : 347
Oryctolagu 276 : VGEWTGFRPVR--PQIRLGREQLSAGPS------------------------KTEVIHNYGHGGYGLTIHWGCALEAAKLFGKILEEKKSSRMPPSHL : 347
Caenorhabd 272 : IKEWSALRPGR--KHVRIEAQKRTSVGNSK----------------------DYMVVHHYGHGSNGFTLGWGTAIEATKLVKTALGL---------- : 334
```

Pam250, ClustalW

Lactobacillus sp. alpha-amylases consisted of 412-440 amino acid residues (Vector NTI® Software)

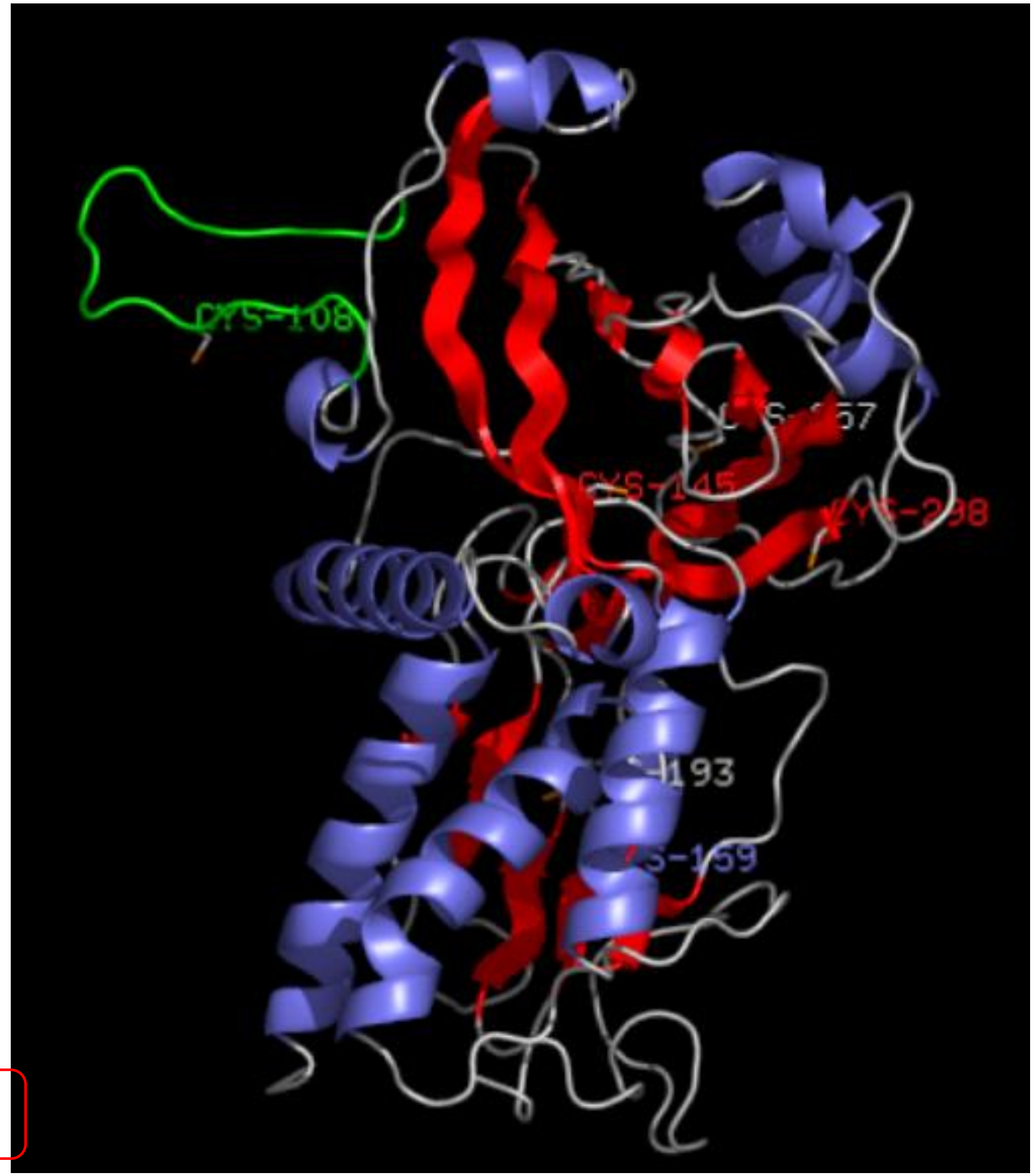


(alignment settings: ClustalW2, slow alignment, gap open 10, gap extension 0.20, gap distance 5, no end gaps, clustering NJ)



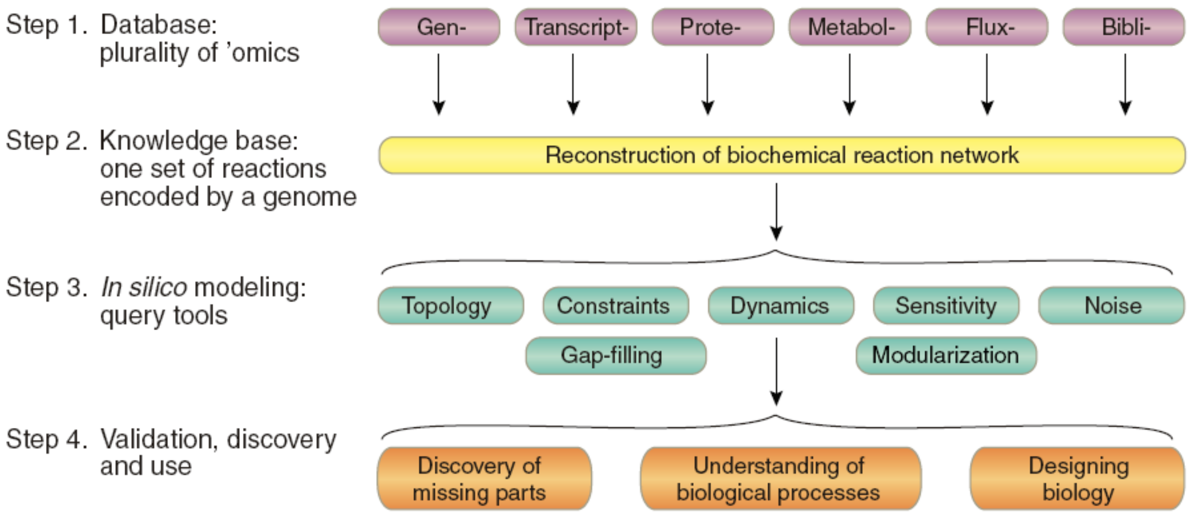
in silico analiza: 3D model proteina

http://swissmodel.expasy.org

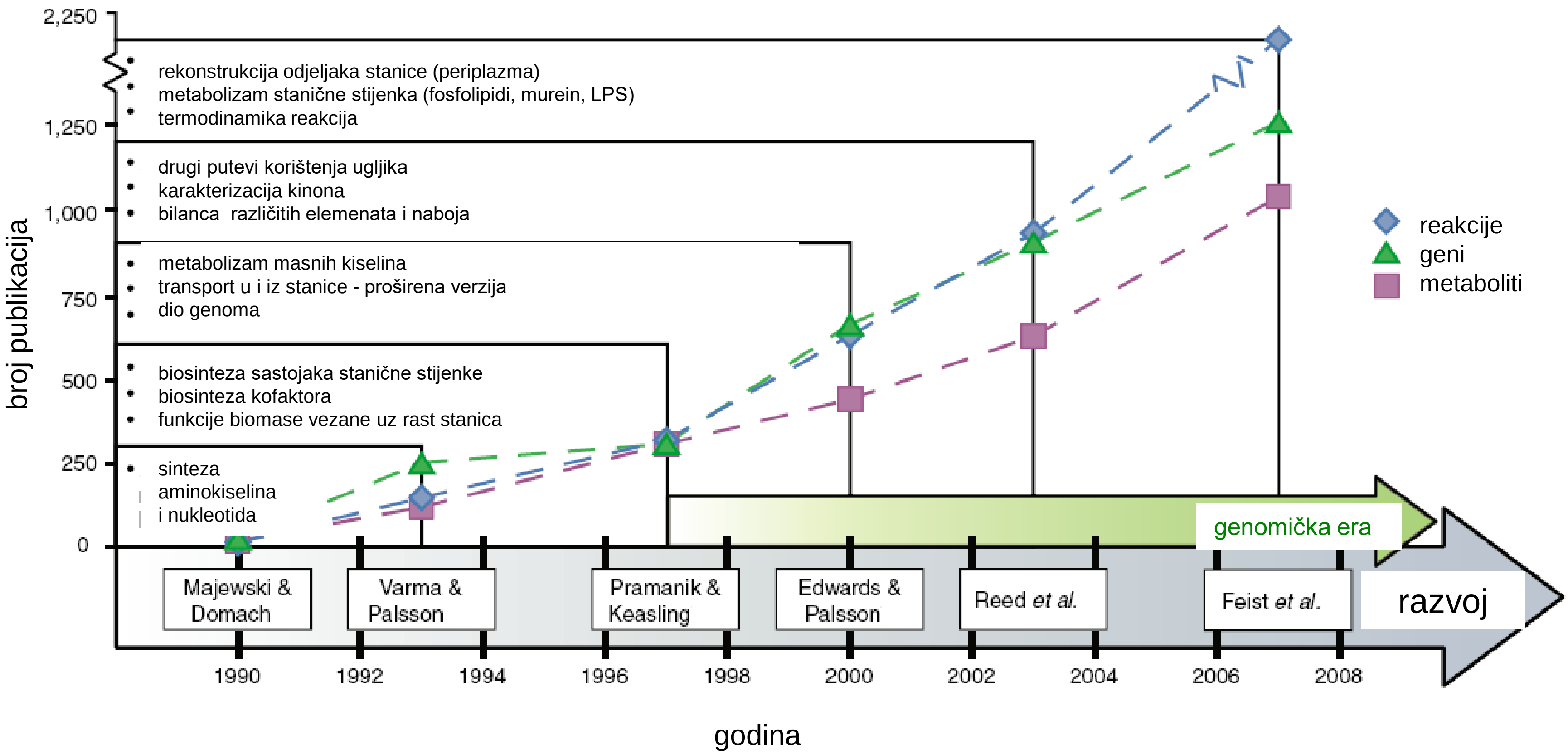


hyperlink

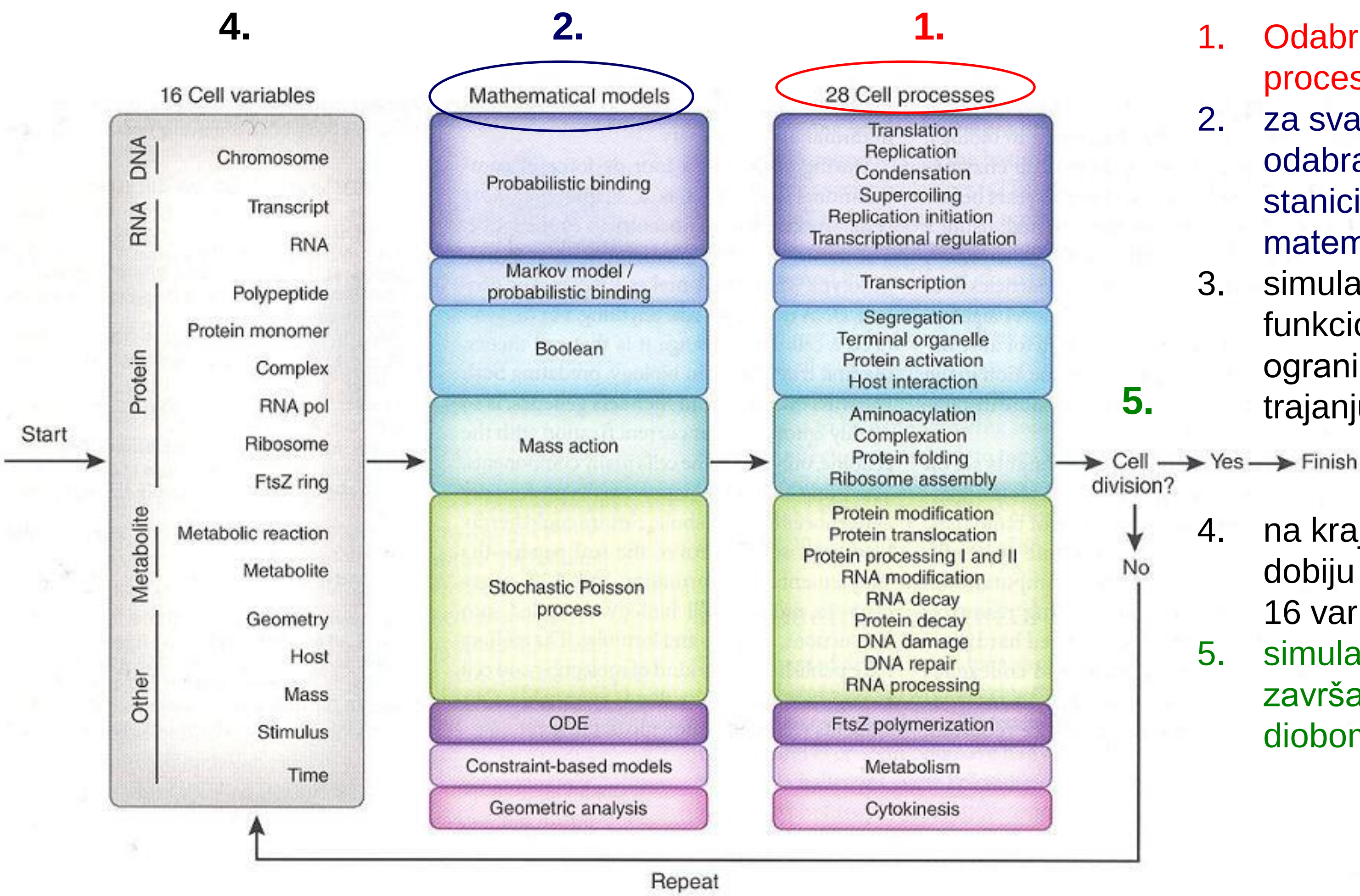
- set primarnih informacija koji se odnosi na određeni (mikro)organizam
- baza podataka načinjena od biokemijskih, genetičkih i drugih podataka čiji se međuodnos može opisati matematičkim jednadžbama



in silico modeli: razvoj metaboličkog modela *E. coli*



in silico model stanice patogena *Mycoplasma genitalium*

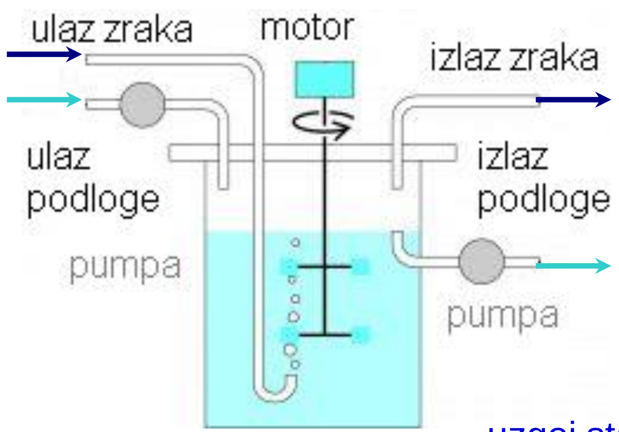


1. Odabrano 28 ključnih procesa u stanici;
2. za svaki od 28 odabranih procesa u stanici formiran matematički model;
3. simulacija funkcioniranja stanice ograničena na korake u trajanju od t = 1 s;
4. na kraju svakog koraka dobiju se vrijednosti za 16 varijabli;
5. simulacija završava/započinje diobom stanice.

FtsZ protein formira prsten gdje se kasnije formira septum (dioba bakt. stanice); ordinary differential equation, ODE.

- istraživanja uz korištenje kultura uzgojenih šaržnim postupkom npr. tijekom uzgoja uz stresanje:
  - hranjiva podloga nacijepljena relativno niskom koncentracijom stanica,
  - stanice rastu uz stalno smanjenje koncentracije supstrata,
  - stanice iz eksponencijalne faze rasta.

- kemostat



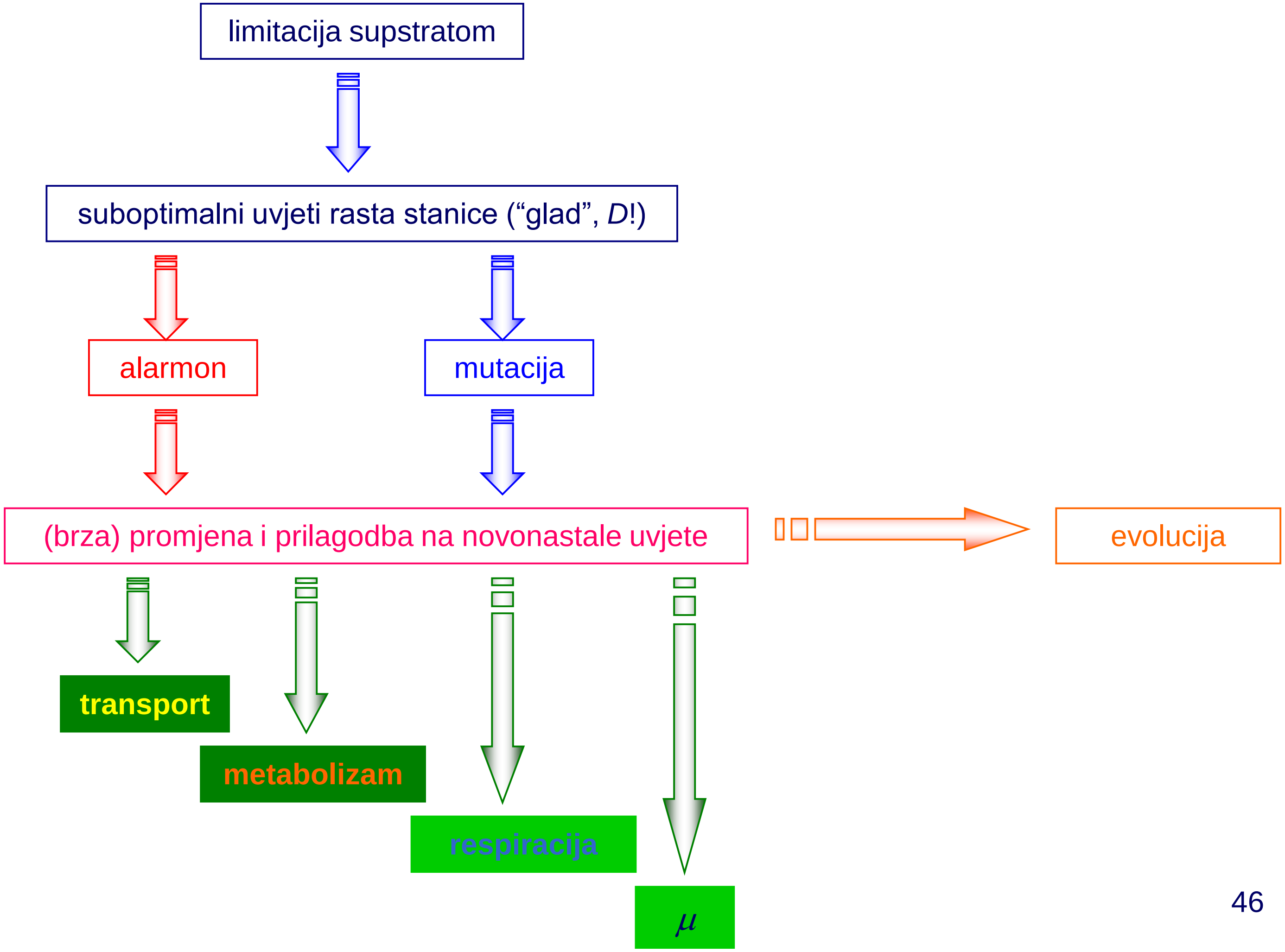
- konstantan volumen održava se preljevnom cijevi
- supstrat ulazi u bioreaktor i troši se za rast stanica pa je konc. supstrata u izlaznom toku manja od ulazne
- stanice u reaktoru rastu brzinom (μ) koja je određena dotokom svježe podloge (D), a istom se brzinom i razrjeđuju, pa je konc. stanica u reaktoru konstantna

$$D = \mu$$

- uzgoj stanica u (ne)kontroliranim (ustaljeno stanje) i ponovljivim uvjetima
- stanice kemostat kulture natječu se za limitirajući supstrat

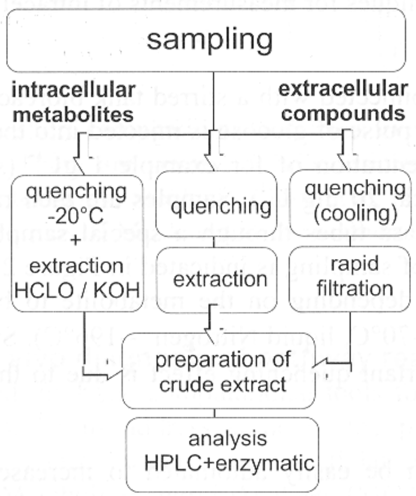
- problemi vezani za uspostavljanje ustaljenog stanja:
  - neidealno miješanje (koncentracijski gradijenti)
  - rast biomase na stijenkama
  - koncentriranje stanica u pjeni
  - vrijeme obrade uzorka prije analize

- istraživanje odziva staničnog metabolizma na promjene u okolini stanice, stupnjevite i pulsne

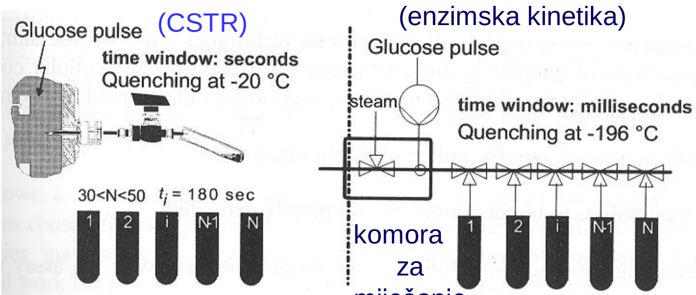


- uzorkovanje (A) i analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

A1



A2



(CSTR)

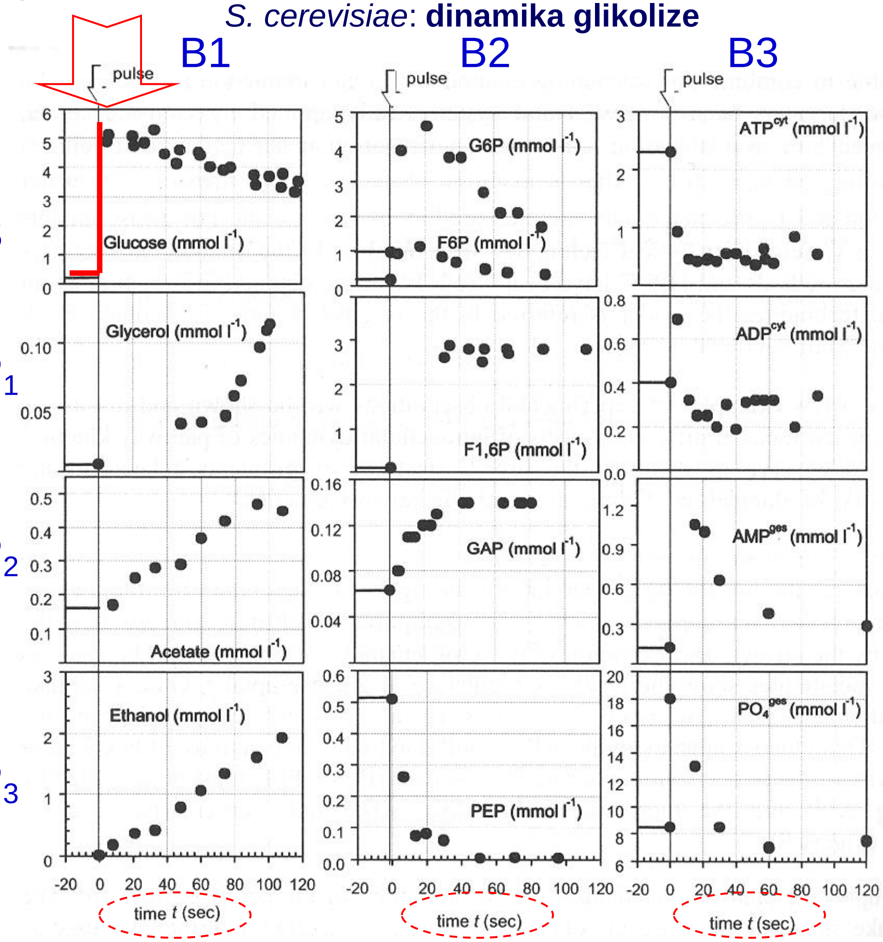
stopped-flow metod a (enzimska kinetika)

komora za miješanje

(vakumirane ohlađene epruvete koje sadrže odgovarajuću smjesu)

S. cerevisiae: **dinamika glikolize**

B



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- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

S. cerevisiae: **dinamika glikolize**

Metabolic Control Analysis (MCA)

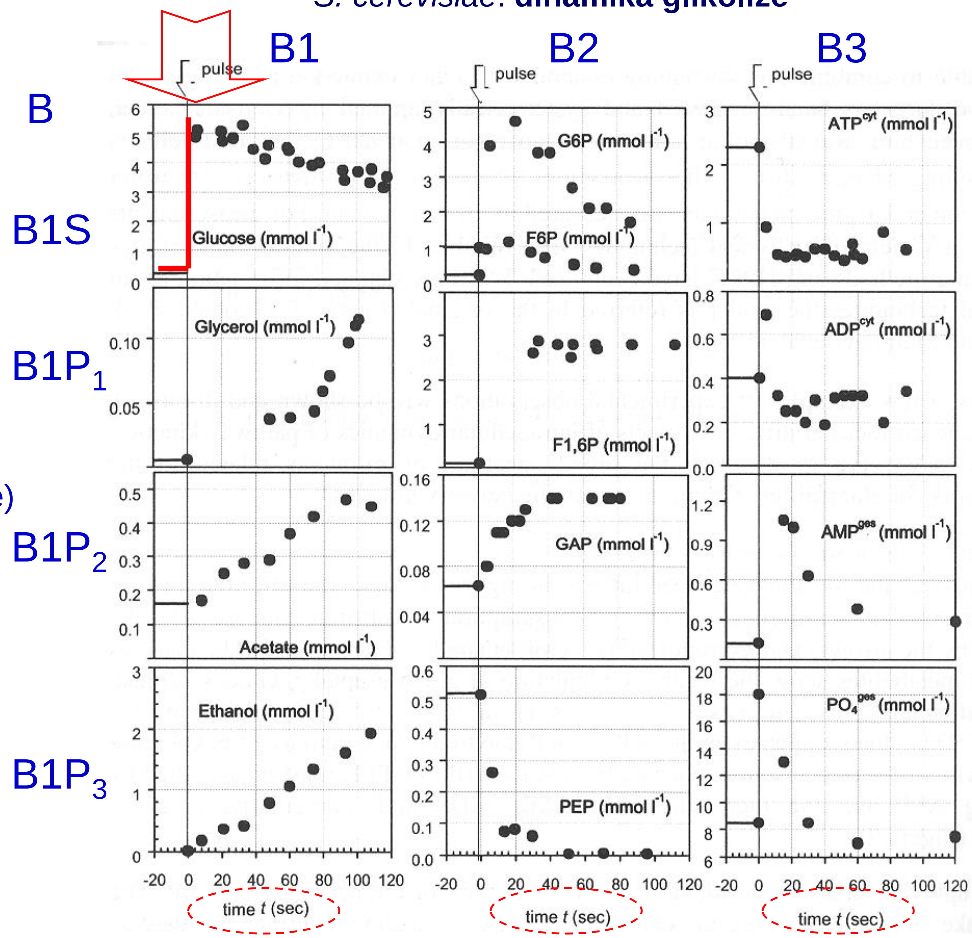
· kontinuirani uzgoj, puls glukoze

(npr. S = 20 mg L^{-1}, puls glukoze S = 1 g L^{-1})

· promjene u koncentraciji spojeva ($t \rightarrow$ s) zbog

regulacije metabolizma

(promjene konc. proteina zanemarive)



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- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

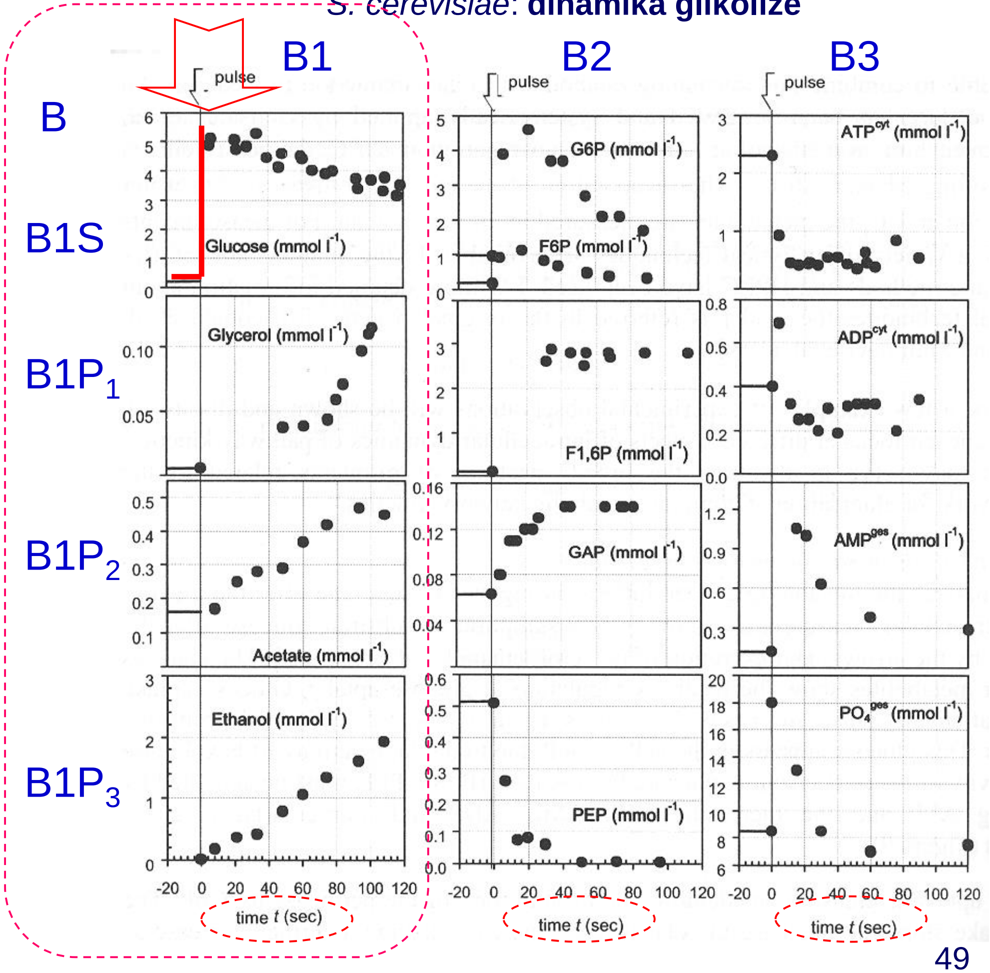
Metabolic Control Analysis (MCA)

glukoza → → glicerol ($B1P_1$)

acetat ($B1P_2$)

etanol ($B1P_3$)

S. cerevisiae: **dinamika glikolize**



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primjena kemostata kod *in vivo* istraživanja fiziologije mikroorganizama (4)

- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu *t* = 0 - 120 s

Metabolic Control Analysis (MCA)

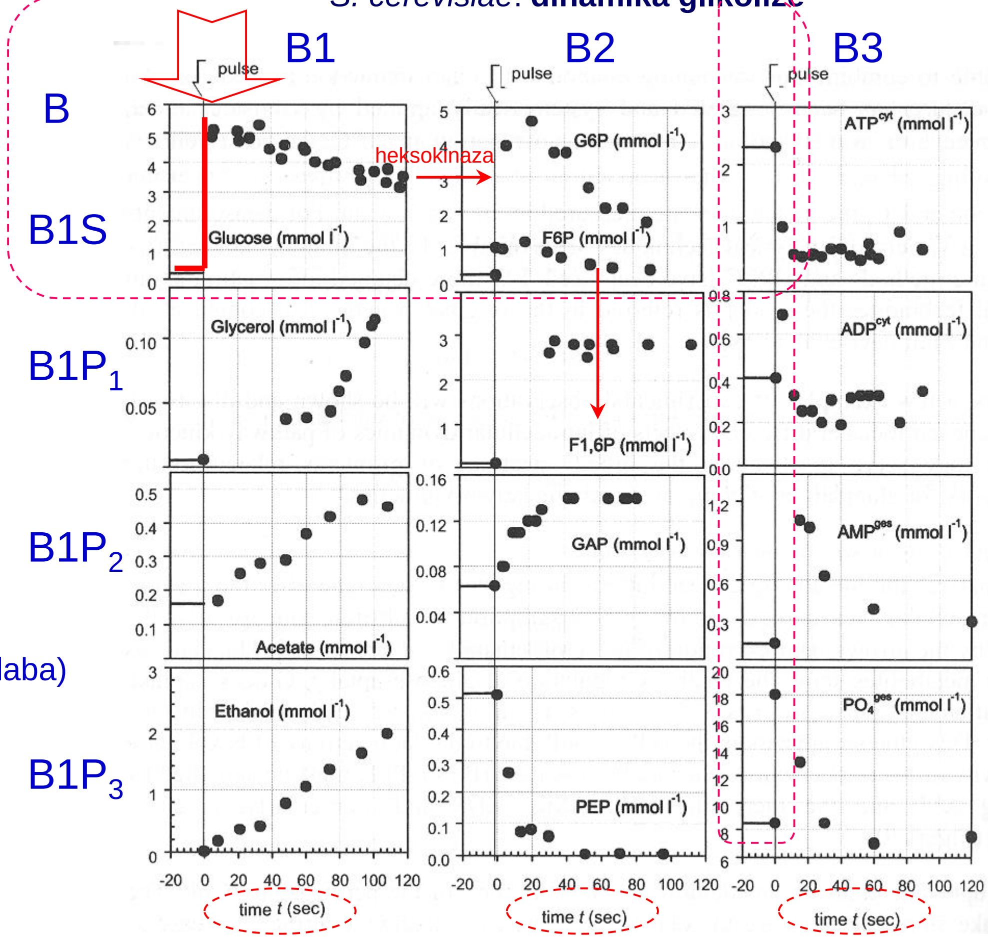
· promjena koncentracije unutarstaničnih međuspojeva pokazuje kontrolu metabolizma glukoze povratnom spregom

· nakon pulsa glukoze raste brzina metabolizma glukoze za ≈ 6 puta (B1S)

· "višak" glukoze se fosforilira (heksokinaza, HK) i tako se povećava unutarstanična koncentracija G6P (B2) (aktivnost fosfofruktokinaze,PFK, je slaba)

· PFK se aktivira zbog opadanja konc. ATP (B3) i zbog povećanja konc. F6P (B2), AMP i ADP (B3)

S. cerevisiae: **dinamika glikolize**



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primjena kemostata kod *in vivo* istraživanja fiziologije mikroorganizama (5)

- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

Metabolic Control Analysis (MCA)

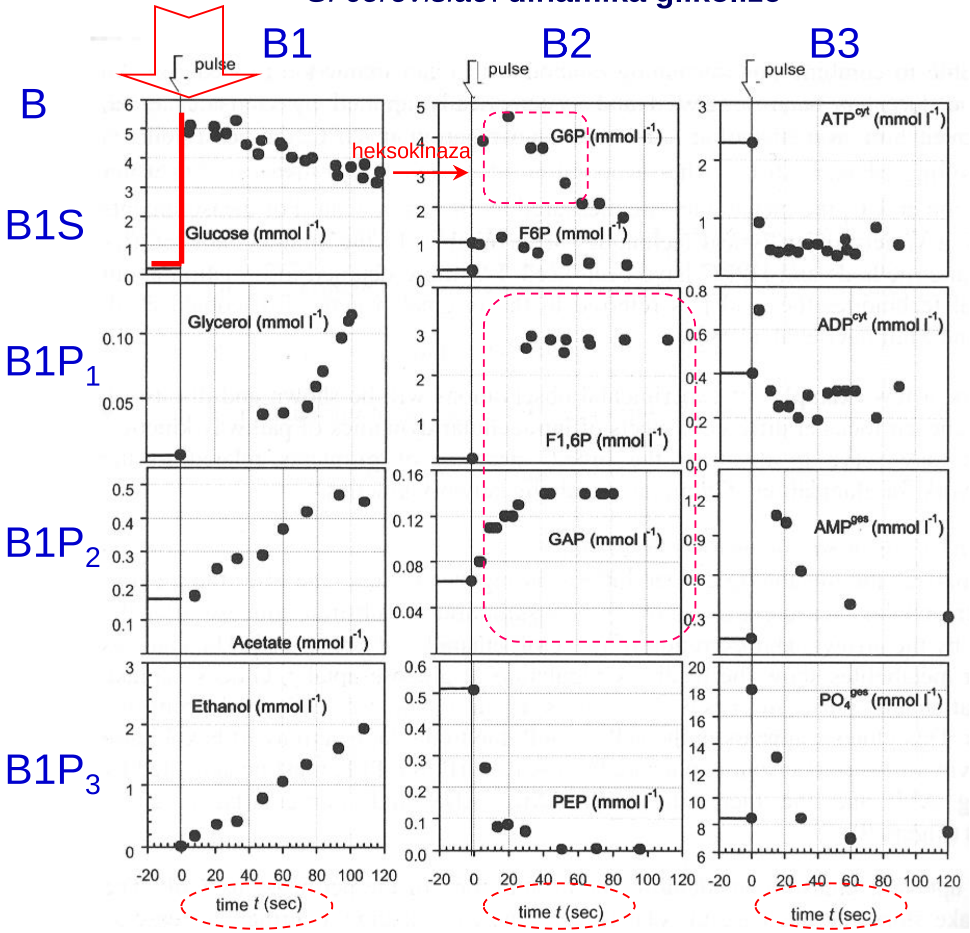
· opada potrošnja glukoze jer G6P (B2) inhibira njezin transport (transport permeazom)

· koncentracije F1,6P i GAP (B2) ostaju visoke kroz period od ≈ 100 s zbog regulacije glikolize i pentoza-P puta

· porast konc. G6P na početku povezan je s porastom konc. 6P-glukonata i porastom potrošnje glukoze kroz pentoza-P put

· zbog toga i konc. F1,6P i GAP ostaju relativno konstantne i visoke kroz ovaj period

S. cerevisiae: **dinamika glikolize**



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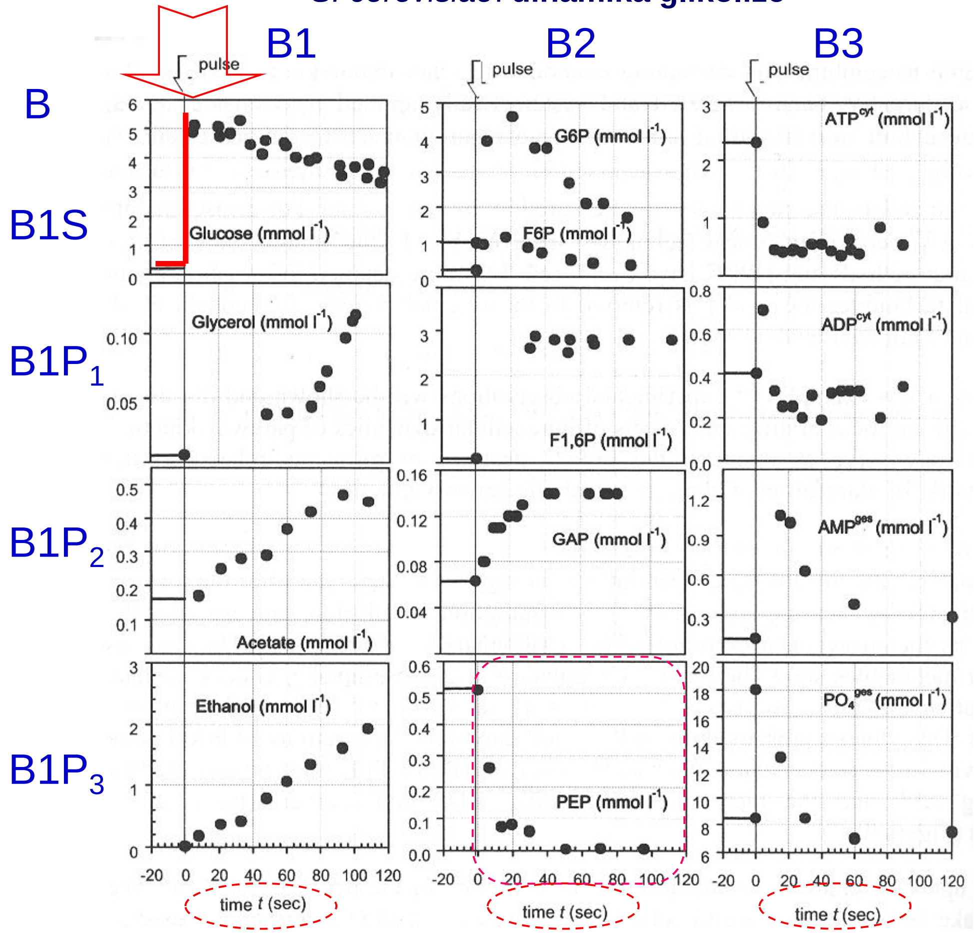
- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

S. cerevisiae: **dinamika glikolize**

Metabolic Control Analysis (MCA)

- koncentracija PEP (B2) opada zbog aktivacije piruvat kinaze (PK) i to visokom konc. F1,6P
- porast konc. F1,6P vodi ka brzom opadanju konc. PEP koji se prevodi u piruvat
- nakon pulsa glukoze "val" ugljika prenosi se "niz glikolizu"



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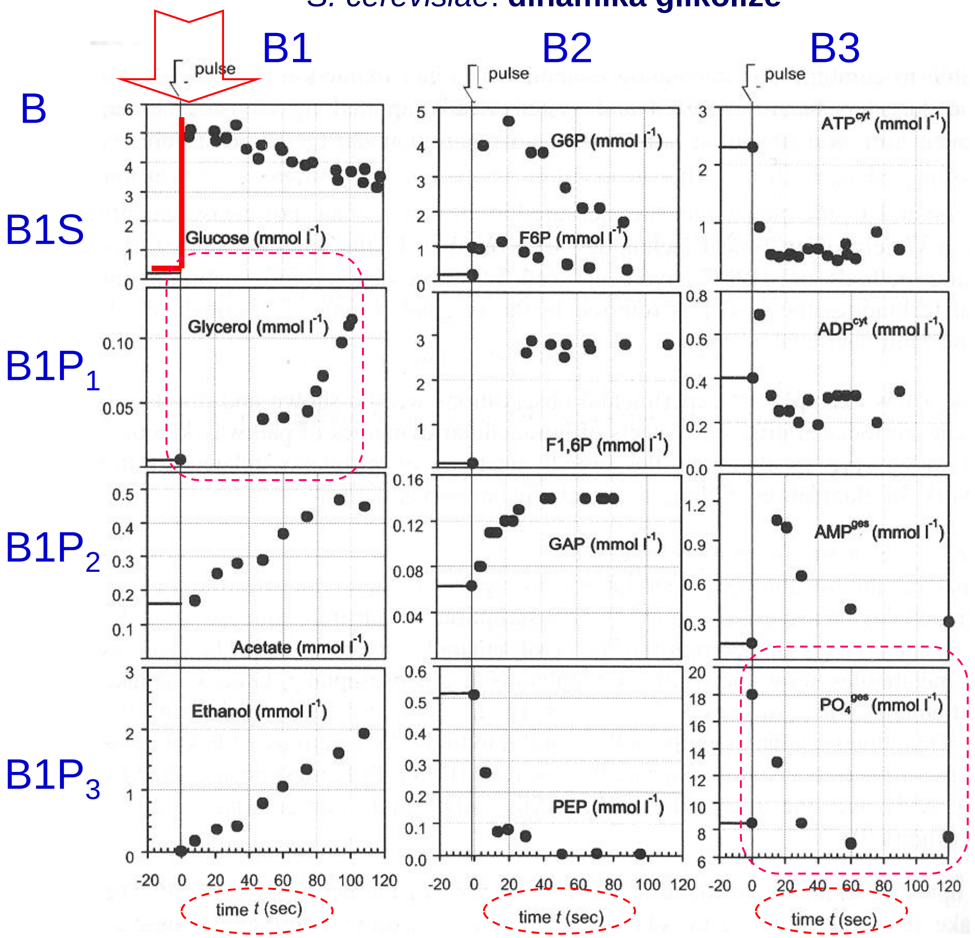
primjena kemostata kod *in vivo* istraživanja fiziologije mikroorganizama (7)

- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

S. cerevisiae: **dinamika glikolize**

Metabolic Control Analysis (MCA)

· raste konc. NADH u citoplazmi kao i konc. PO_4^{3-} (B3); PK "vuče" 1,3-DPG; odvija se transport glicerola iz stanice ($B1P_1$) i smanjuje se konc. NADH u stanici



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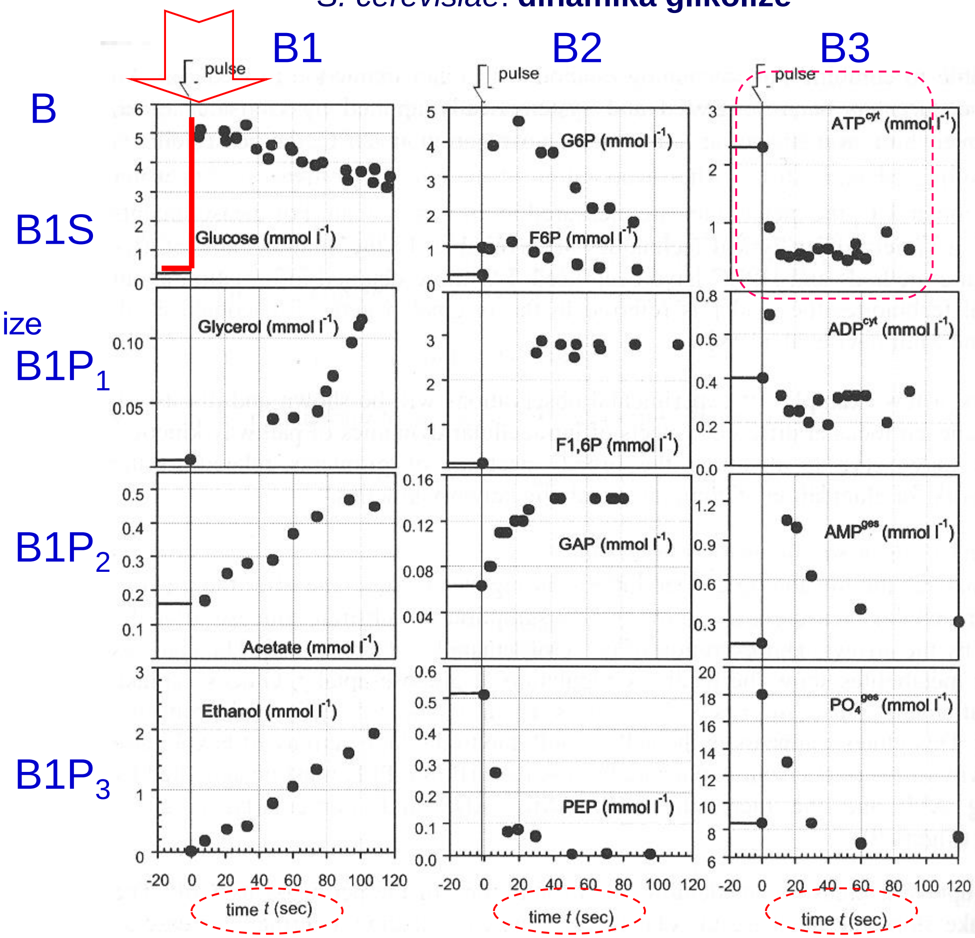
- analiza (B) supstrata i vanstaničnih proizvoda (B1), unutarstaničnih međuspojeva (B2) i energetskih spojeva ATP/ADP/AMP/Pi (B3) nakon pulsa glukoze u vremenu t = 0 - 120 s

S. cerevisiae: **dinamika glikolize**

Metabolic Control Analysis (MCA)

- konc. ATP opada brzo nakon pulsa glukoze
- ATP se troši za fosforilaciju (HK i PFK) brže nego što ATP nastaje u kasnijim reakcijama glikolize
- ne nastaje glikogen jer je za taj proces potrebna visoka konc. ATP



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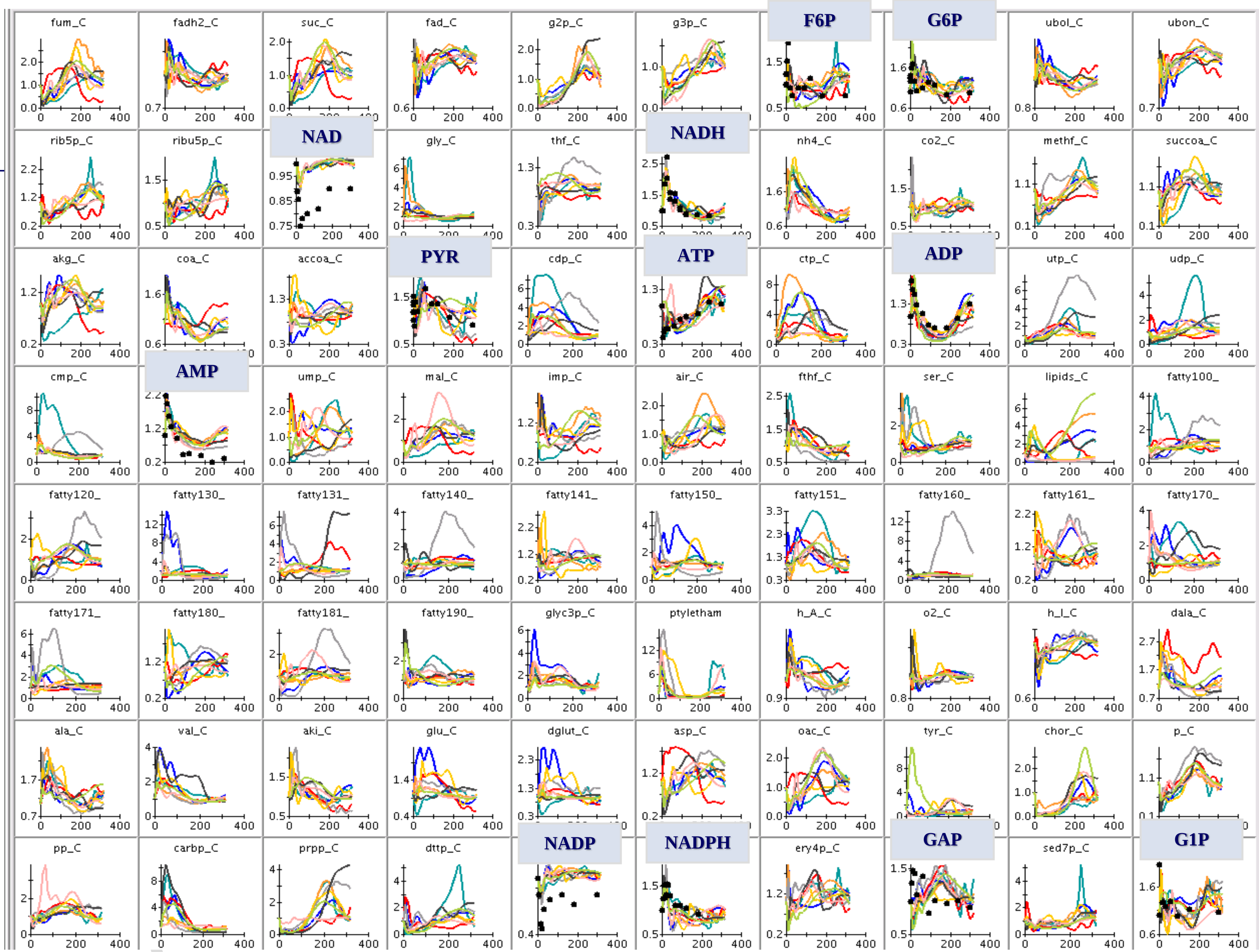


Escherichia coli

Prof. M. Reuss, BEC2016

(1) Regulacija metabolizma odvija se u vremenu od nekoliko sekundi ili kraćem.

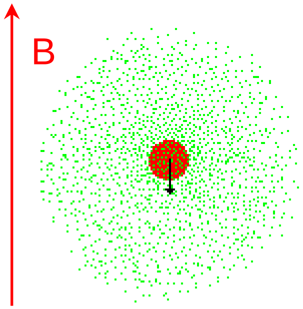
(2) U ovom vremenskom okviru promjene se događaju zbog regulacije metabolizma. Reakcije biosinteze (biosinteza enzima) smatra se „zamrznutom” (“frozen“ state).

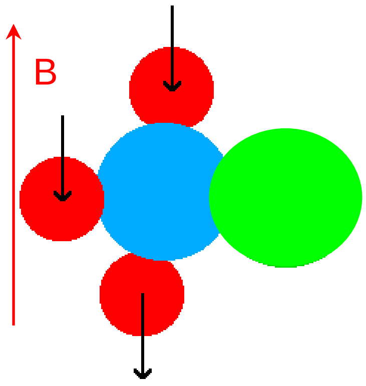
Prof. M. Reuss, BEC2016

NMR - nuklearna magnetna rezonancija

- *in vivo* NMR je neinvazivna metoda određivanja koncentracije metabolita u različitim odjeljcima stanice

- princip: u magnetnom polju elektroni određenog atoma kruže u smjeru ovog polja; kruženjem elektrona nastaje drugo, suprotno magnetno polje oko jezgre atoma; gustoća elektrona oko jezgre atoma ovisi o vrsti jezgre i vrsti veza kod određene molekule (npr. metanol)

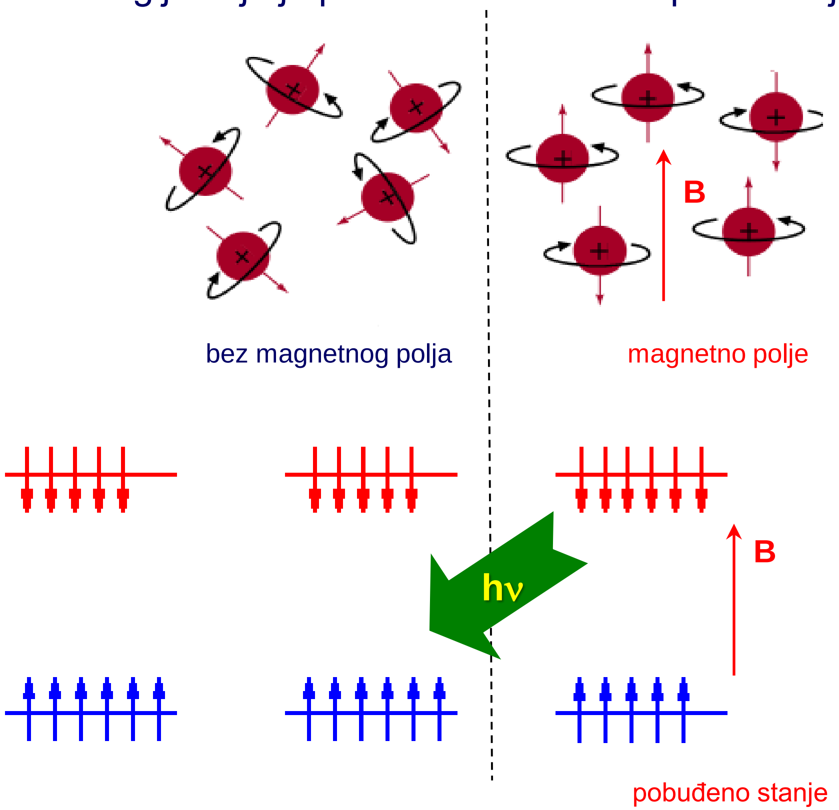




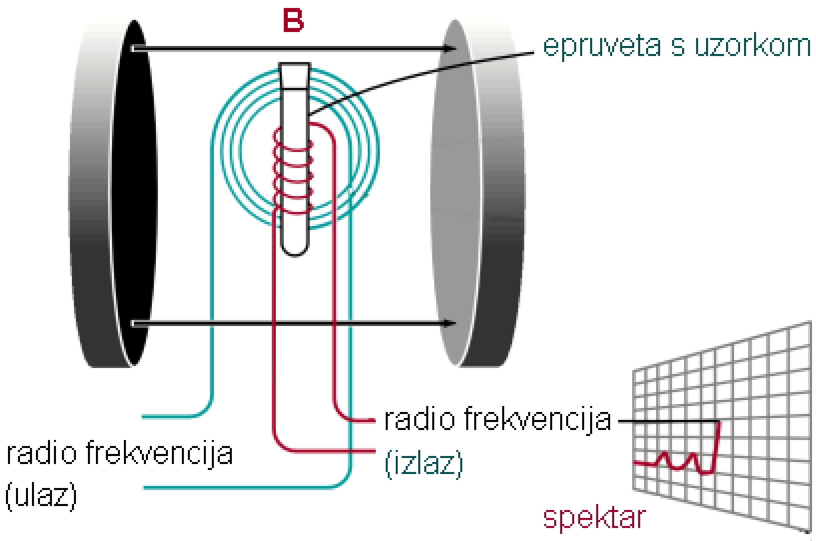
metanol

NMR analiza metaboličkih flukseva (2)

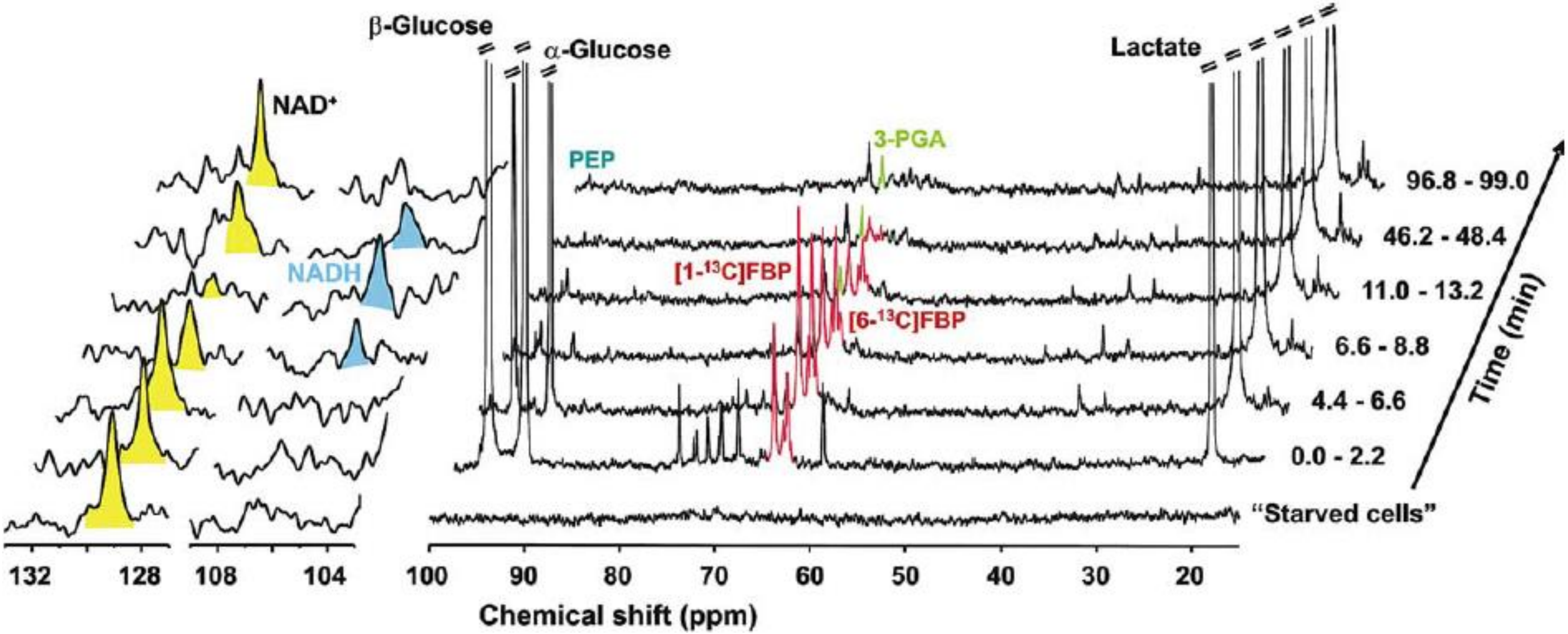
- princip: jezgre atoma koji imaju neparan atomski broj (npr. ^{1}H i ^{13}C) imaju spin (sličan spinu elektrona); jezgra predstavlja nabijenu česticu koja se kreće i samim time stvara magnetno polje; bez "vanjskog" magnetnog polja jezgre atoma imaju tzv. slučajnu orijentaciju, primjenom magnetnog polja jezgre ovih atoma orijentiraju se paralelno s poljem; EM se koristi za "okretanje" (eng. flip) spinova; količina energije koju je potrebno uložiti za "flip" ovisi o jačini magnetnog polja

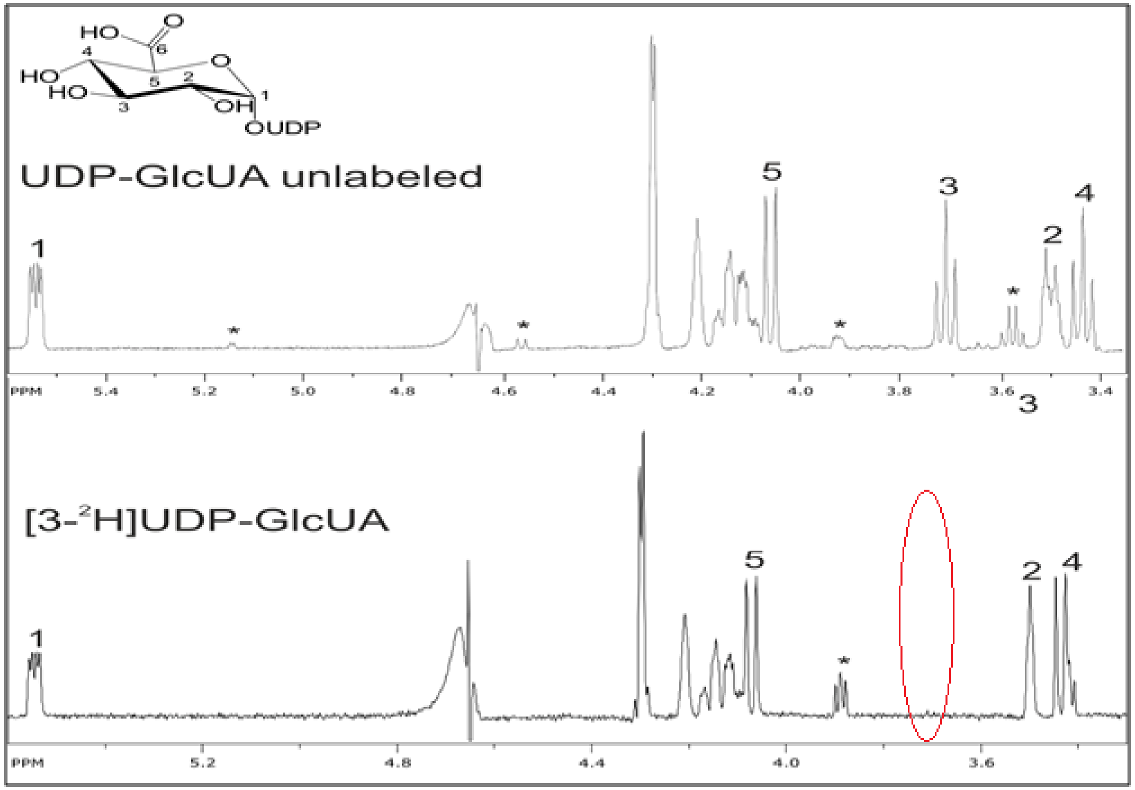


- osnovne komponente NMR sustava: uzorak smješten u magnetnom polju pobudi se pulsevima radio frekvencije, tada magnetno polje inducira radio signal koji se koristi za formiranje izlaznog signala, Fourier analiza kompleksnih izlaznih signala formira konačni spektar



- primjena: NMR analiza ^{31}P omogućava praćenje metabolizma ugljikohidrata jer se ovom metodom mogu razlikovati unutarstanični i van-stanični anorganski fosfat, zatim pirofosfat, ATP, ...;
NMR analiza ^{13}C zahtijeva korištenje spojeva bogatih na ovom izotopu;
metaboličko inženjerstvo


β-Glucose
α-Glucose
Lactate
NAD+
NADH
PEP
3-PGA
[1-13C]FBP
[6-13C]FBP
96.8 - 99.0
46.2 - 48.4
11.0 - 13.2
6.6 - 8.8
4.4 - 6.6
0.0 - 2.2
"Starved cells"
Time (min)
132
128
108
104
100
90
80
70
60
50
40
30
20
Chemical shift (ppm)


HO
O
HO
HO
OH
OUDP
UDP-GlcUA unlabeled
1
5
3
2
4
PPM
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
[3-²H]UDP-GlcUA
5
2
4
3.6

- Geiger i scintilacijski brojači
- primjena:

stupnjevita (eng. step) ili udarna (eng. pulse-chase) pobuda

(kratkotrajna pobuda radioaktivnim materijalom koji se ispire i zamjenjuje molekulama koje ne pokazuju radioaktivnost)

određivanje redoslijeda reakcija u biokemijskim putevima

praćenje ugradnje građevnih blokova u polimere

lokalizacija određenih funkcija u stanici

autoradiografija (istraživanje funkcija tkiva pod mikroskopom)

analiza frakcija stanica

proučavanje transporta u stanicu

transport glukoze u stanice kvasca *Saccharomyces cerevisiae*

- **scintilacijsko brojanje**: određivanje radioaktivnog raspada određene tvari tj. određivanje količine radioaktvnosti u uzorku.

  U "koktelu" se odvijaju fizikalno-kemijske reakcije tj. energija koja se oslobađa tijekom radioaktivnog raspada prevodi se u energiju koja se mjeri scintilacijskim brojačem.

  scintilacijski brojač: fotoćelija visoke rezolucije koja može detektirati α-čestice (jezgre He), β-čestice (elektroni), γ- i x-zrake, te pozitrone, Comptonove i Angerove elektrone.

  Najčešće su se koristile ^{14}C, ^{3}H i ^{32}P tj. detektirale β-čestice.

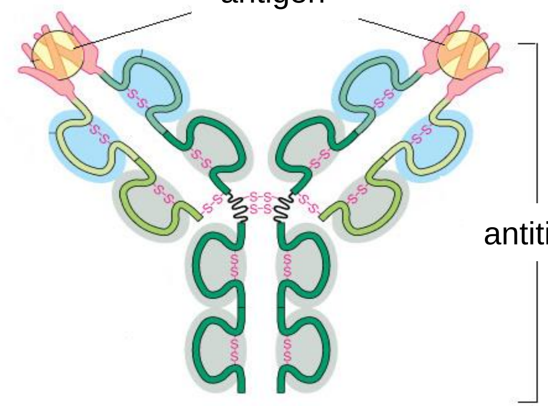
- protuijela su proteini koje proizvode kralješnjaci kao obranu nakon infekcije

- to su jedinstveni proteini koji “prepoznaju” specifičnu molekulu - antigen

- određeni antigen “izaziva” sintezu određenog proteina

- obilježavanje fluorecentnim spojevima (fluorescentna mikroskopija, FM)

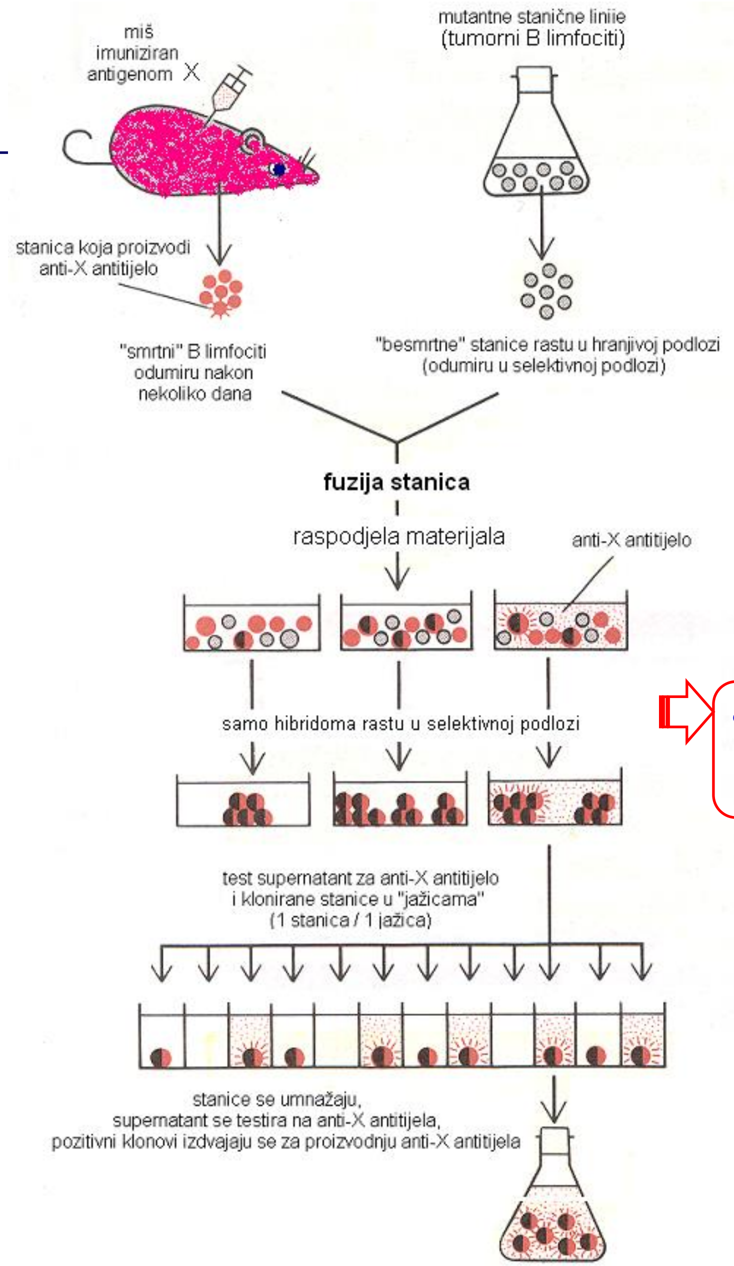
- visoka specifičnost reakcije antigen-protutijelo
- antigeni: proteini, DNA, RNA, polisaharidi, organeli, druga antitijela



- heteroklonska protutijela (B-limfociti, antiserum, životinje)

- monoklonska protutijela (tehnologija hibridoma)

primjena antitijela



- kloniranje B-limfocita koji proizvode jednu vrstu antitijela (homogena monoklonalna antitijela)
- B-limfociti iz imunizirane životinje fuzirani s “besmrtnim” tumornim B-limfocitima

animacija: **Producing Monoclonal Antibodies**
www.sumanasinc.com/webcontent/animations/molecularbiology.html

Primarne stanice

stanice oslobođene od ostalog dijela tkiva uzgojene u prikladnim uvjetima
rastu i razmnožavaju se tek nekoliko generacija

Besmrtne stanice

(kontinuirane stanične linije)
posebni izolati koji se razmnožavaju beskonačno

Hibridoma

hibrid između primarnih stanica B limfocita i mutiranih - tumornih stanica B limfocita,
besmrtne stanice sa značajkama primarnih stanica

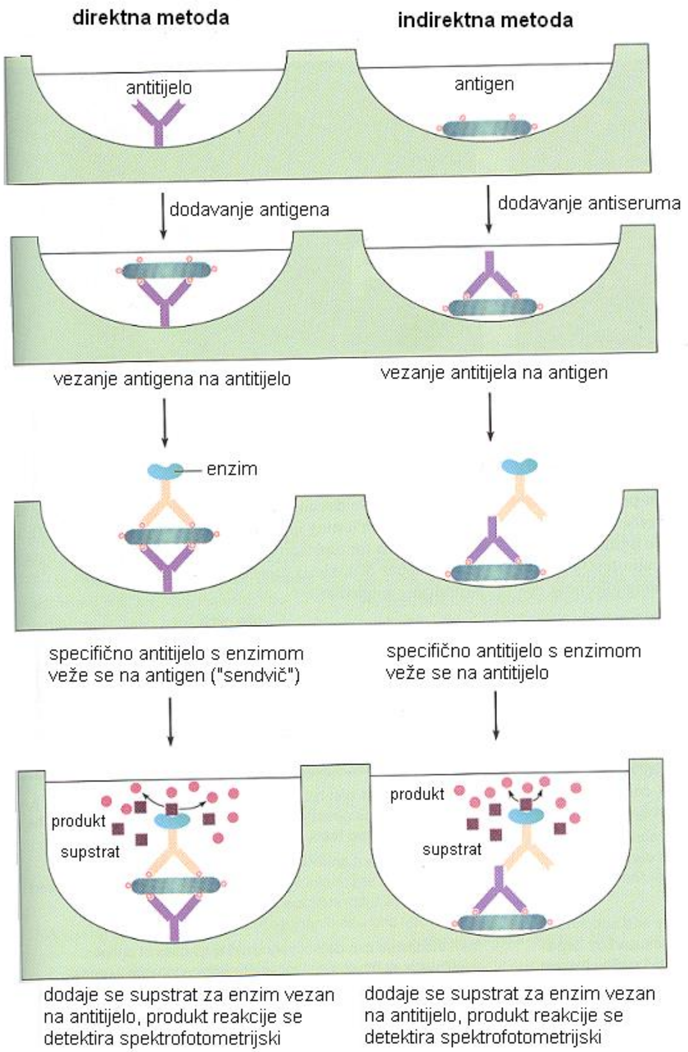
Uzgoj biljnih i životinjskih stanica omogućava industrijsku proizvodnju
vakcina, virusa, specifičnih proteina koji se ne mogu proizvesti drugim organizmima;
monoklonska antitijela u terapeutske, dijagnostičke i znanstvene svrhe;
specifičnih metabolita biljaka i životinja (prehrambeni aditivi, parfemi, agrokemikalije, insekticidi, ...)

animacija: **Enzyme-Linked Immunosorbent Assay** (Direct & Indirect ELISA Method)
www.sumanasinc.com/webcontent/animations/molecularbiology.html

- mogućnost kvalitativne i kvantitativne analize specifičnih antigena

- RIA (eng. RadioImmunoAssay; antitijela sa radioizotopom)

- ELISA (eng. Enzyme-Linked Immunosorbent Assay; antitijela s enzimom)

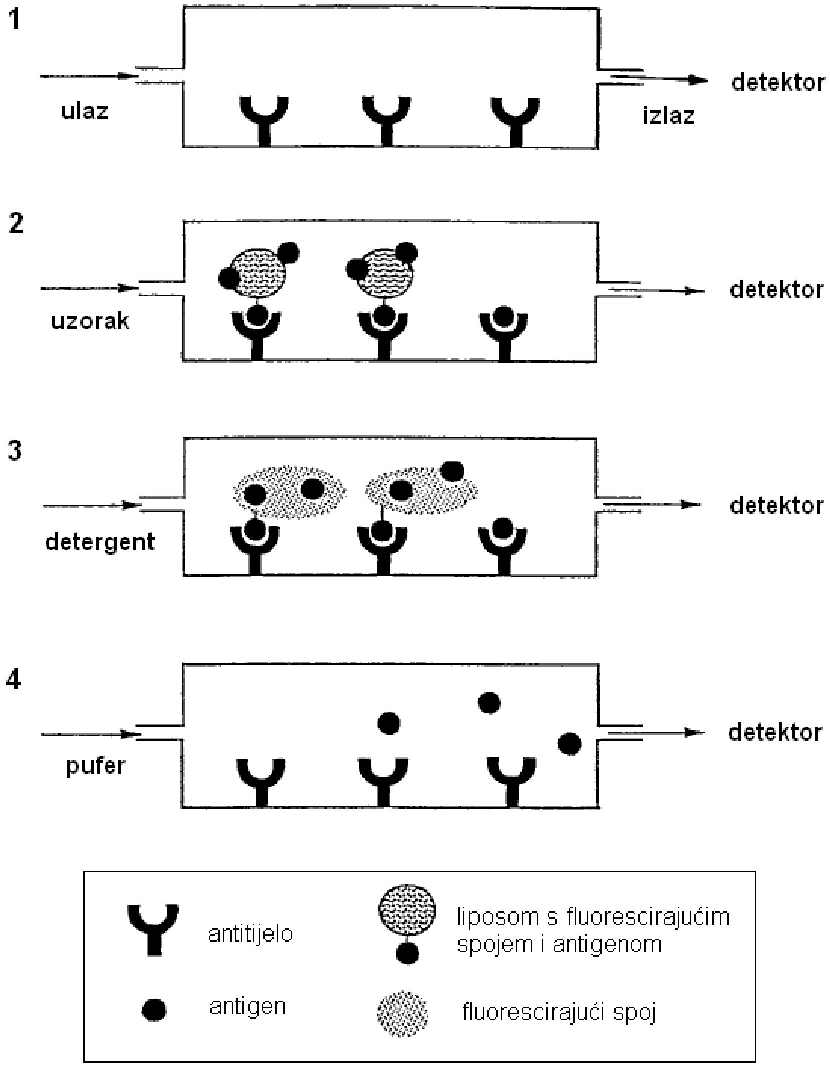


- imunocitokemija (antitijela s feritinom za TEM; fluorescentna antitijela za FM)

- afinitetna kromatografija (High-Performance Immuno Affinity Chromatography, HPIAC):

· izdvajanje i analiza specifičnih bioloških spojeva (RF, GC, CE, kiralni spojevi, tandem affinity)

· analiza teofilina pomoću HPIAC, kompetitivno vezanje teofilina na imuno-afinitetnu kolonu, teofilin povezan s liposomom koji sadrži karboksifluorescein kao marker



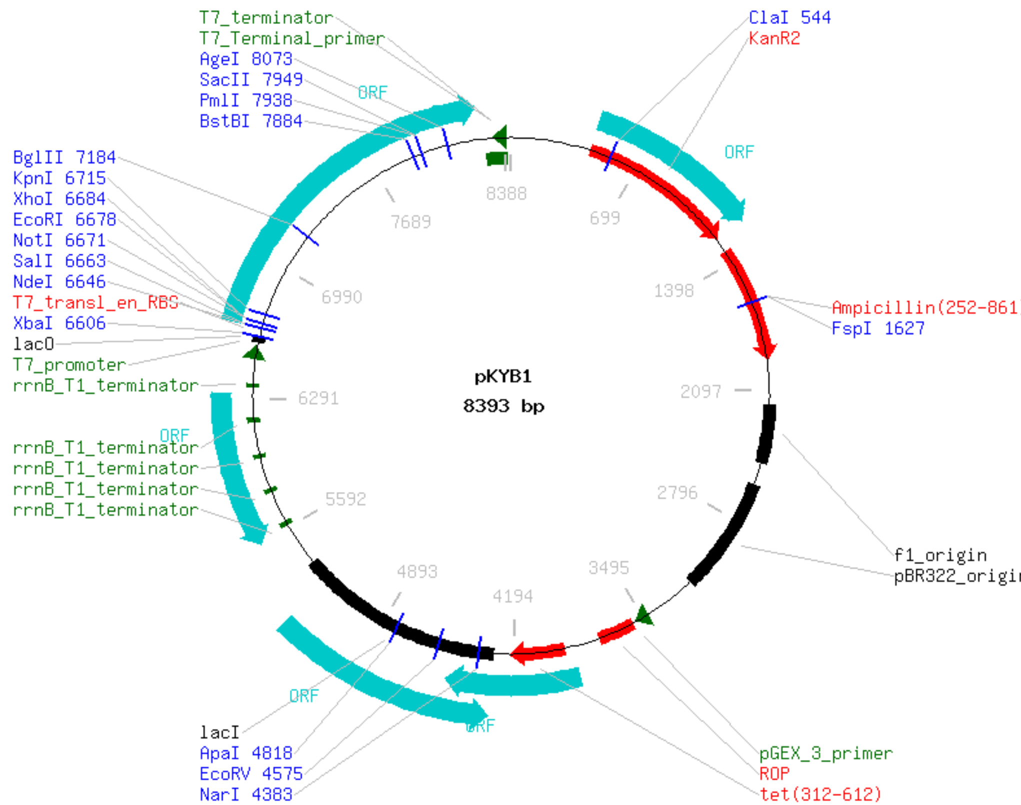
· proizvodni mikroorganizam može biti
- izolat iz prirode (nasumično)
- inducirani mutant (nasumično)
- konstruiran tehnologijom rDNA (selektivnost)

· povijesni pregled

1869	**Miescher** izolirao DNA
1944	**Avery** dokazao da je genetička informacija zapisana u DNA, a ne u proteinima
1953	**Watson** i **Crick** prikazali strukturu DNA modelom dvostruke uzvojnice na temelju rezultata **kristalografije X-zrakama** za koji su zaslužni **Franklin** i **Wilkins**
1961	**Marmur** i **Doty** otkrili renaturaciju DNA čime su omogućene reakcije hibridizacije DNA
1962	**Arber** otkrio restrikcijske endonukleaze, za njihovu purifikaciju i primjenu u genetičkom inženjerstvu zaslužni **Nathans** i **H. Smith**
1966	**Nirenberg**, **Ochoa** i **Khorana** objašnjavaju genetički kod
1967	**Gellert** otkrio DNA ligazu, enzim koji se koristi za spajanje fragmenata DNA
1972-1973	**Boyer**, **Cohen**, **Berg** (Stanford, UC San Francisco) razvili tehnike kloniranja
1975-1977	**Sanger** i **Barrel**, te **Maxam** i **Gilbert** razvili brzu metodu za sekvencioniranje DNA (određivanje redosljeda nukleotida)

· genetička informacija može biti prepisana iz
- DNA davatelja gena (niska selektivnost)
- banke gena davatelja (viši stupanj selektivnosti)
- mRNA davatelja gena (viši stupanj selektivnosti)

- restrikcijske endonukleaze
- restrikcijski fragmenti i restrikcijske mape
- DNA ligaza: kloniranje heterologne DNA u plazmide i viruse
- transformacija plazmidne ili virusne DNA u stanicu domaćina
- tehnike hibridizacije
- reverzna transkriptaza: kopiranje mRNA u cDNA (komplementarna, kopirana)
- (automatsko) određivanje redosljeda baza (eng. sequencing)
- određivanje redosljeda aminokiselina proteina iz redoslijeda baza
- genomika (sekvencioniranje ukupnog genoma; “omics”)
- proteinsko inženjerstvo



http://www.neb.com/nebecomm/products/productN6701.asp

- važnost genetičkog inženjerstva u biotehnologiji:

  - mogućnost dobivanja rijetkih proteina iz bilo kojeg izvora u dostatnim količinama za istraživanje,
  - određivanje redoslijeda aminokiselina u proteinima preko redoslijeda nukleotida u DNA,
  - određivanje redoslijeda nukleotida cijelog genoma,

  - proizvodnja specifičnih proteina u industrijskom mjerilu,
  - metaboličko inženjerstvo (dobivanje novih proizvoda metabolizma “ubacivanjem” gena za nove enzime),
  - razvitak vektora za “ubacivanje” heterolognih gena u životinjske i biljne stanice (transgenične životinje i biljke).

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- proteomics
- genomics
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- systems biology
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- population genetics
- transcriptomics
- biophysics
- imaging
- IT infrastructure
- drug design

Resources A..Z

Links/Documentation

NCBI Resources How To

NCBI
National Center for Biotechnology Information

- **NCBI Home**
- **Resource List (A-Z)**
- All Resources
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- Data & Software
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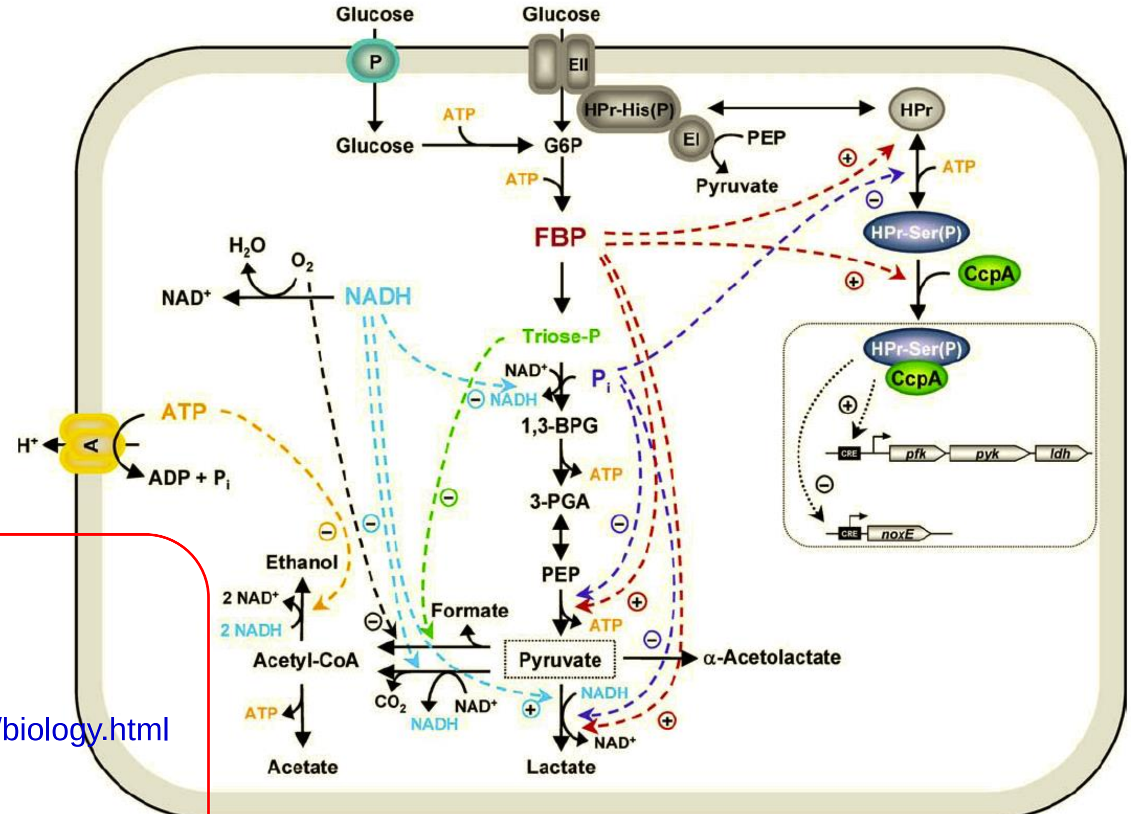
- Domains & Structures
- Genes & Expression
- Genetics & Medicine
- Genomes & Maps
- Homology
- Literature
- Proteins
- Sequence Analysis
- Taxonomy
- Training & Tutorials
- Variation

BRENDA
The Comprehensive Enzyme Information System
Technische Universität Braunschweig

EC-Number	Enzyme Name	Organism	Protein	Full text	Ligand	Advanced Search

- dobivanje i proučavanje mutanata
- mutanti: apsolutni i uvjetni (npr. temperaturni)
- određivanje redoslijeda reakcija kod biokemijskih puteva
- proučavanje regulacijskih mehanizama
- proučavanje transporta
- izrada genetičke mape
  (genetičke rekombinacije)

ccpA	catabolite control protein A
cre	catabolite responsive element
noxE	NADH oksidaza (H_2O)



animacije:
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Construction of a DNA Library
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Life Cycle of an mRNA
The Polymerase Chain Reaction (PCR)
Plasmid Cloning
www.sumanasinc.com/webcontent/animations/molecularbiology.html

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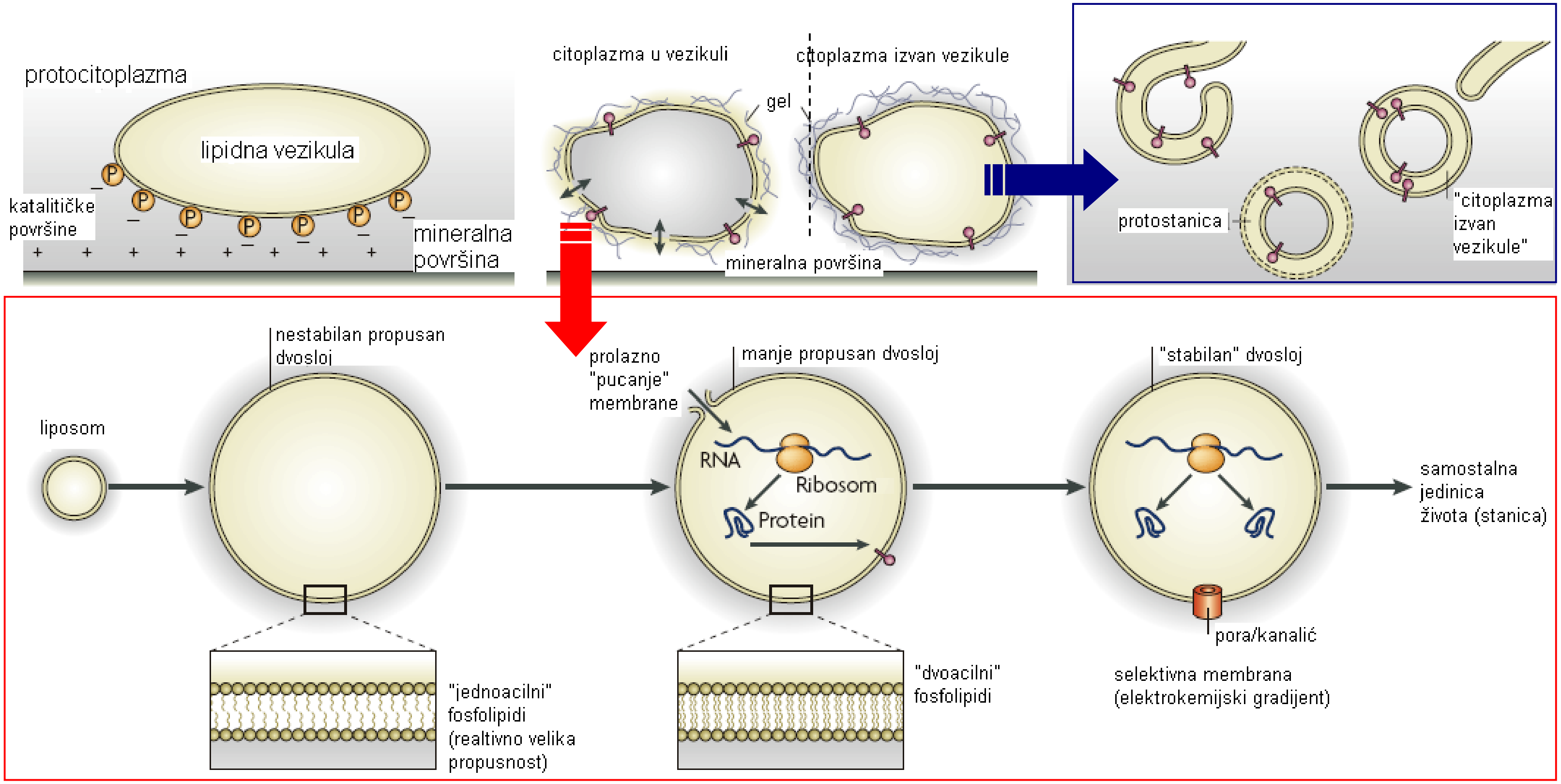
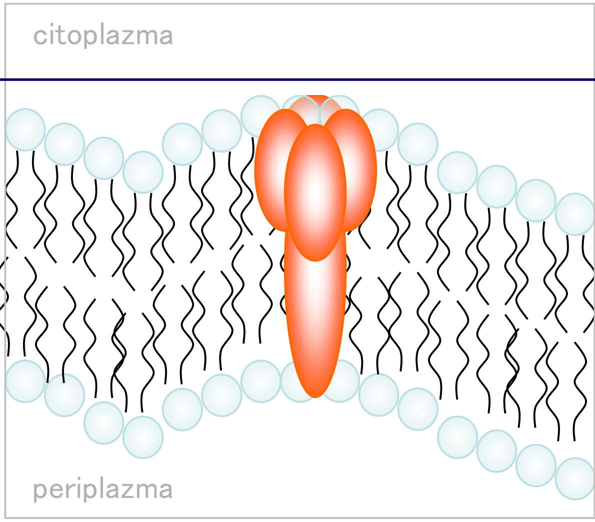
BIOMEMBRANE – GRAĐA I FUNKCIJA

- plazmina membrana: hidrofobni lipidni dvosloj širine 4-5 nm (30 Å-10 nm), dinamično organiziran okomito i lateralno, predstavlja granicu života i smrti za stanicu
- kako i gdje je nastala prva stanica ?

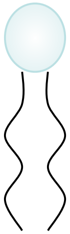
- (ko-)evolucija citoplazme i membrane: međudjelovanje površina i “problem” formiranja stanice



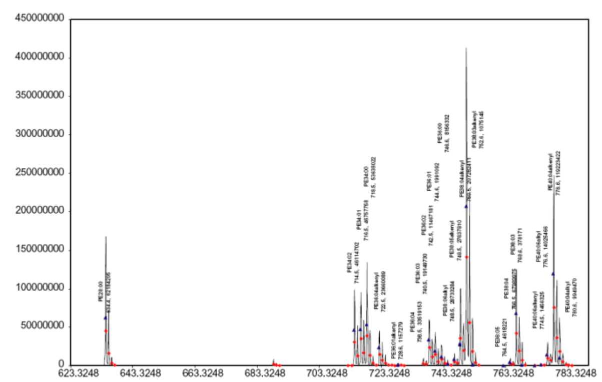
biomembrane - zajedničke karakteristike (2)



- sastoje se iz lipida (35-50%) i proteina (50-65%)
- molekule lipida poredane su u neprekinuti dvostruki sloj (lipidni dvosloj)
- lanci masnih kiselina su hidrofobni, dok je ostatak polaran
  polarna “glava”: glicerol + fosforna kiselina + alkoholamin

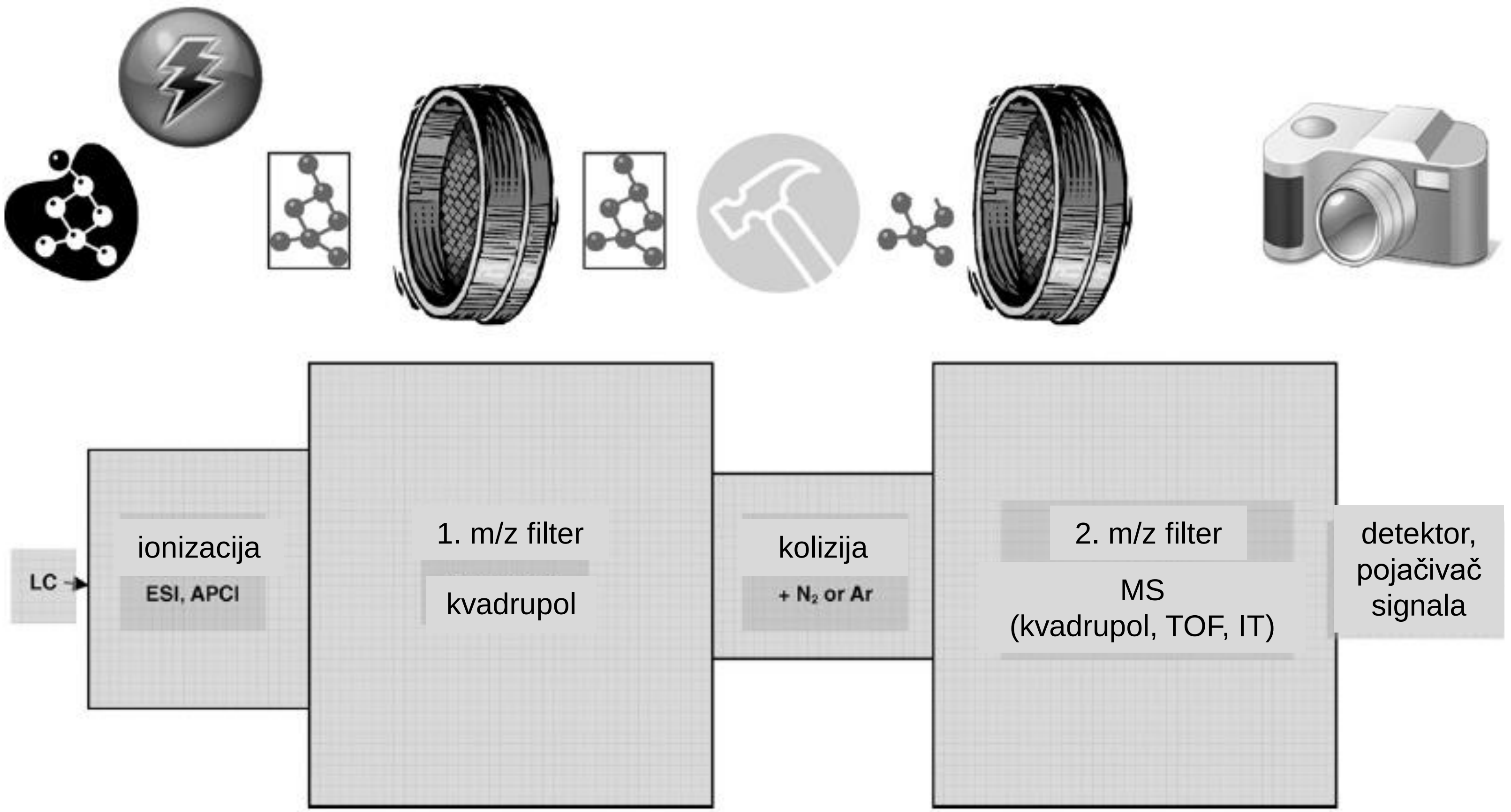


- molekula fosfolipida je amfifilna
- lipidni dvosloj predstavlja osmotsku barijeru (nepropustan je za većinu u vodi otopljenih molekula)
- proteini membrane (membranski proteini) “otopljeni” su u lipidnom dvosloju
- lipidni dvosloj je dvodimenzionalna tekućina, ima dinamičnu i fluidnu strukturu (velika horizontalna pokretljivost molekula lipida i proteina)



- lipidomics
- oko 5% gena eukariotske stanice kodira za sintezu lipida

lipidomics - MS/MS



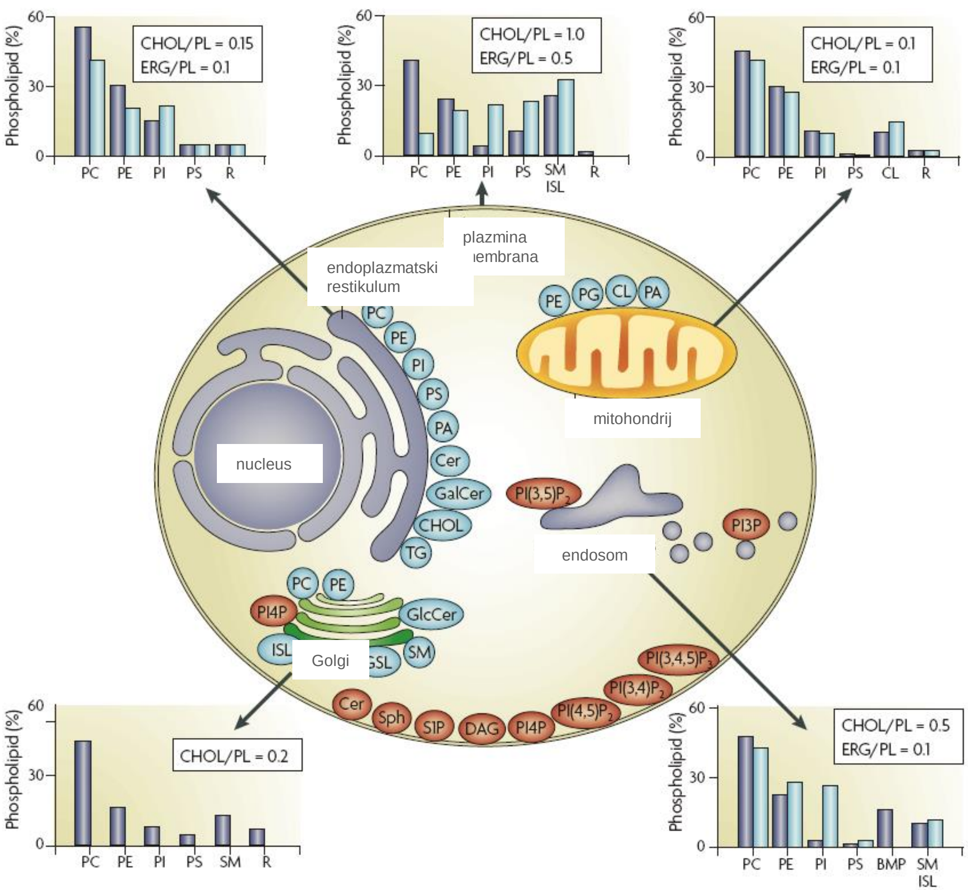
· sinteza lipida odvija se u

<u>endoplazmatskom retikulumu</u> (mrežici) i brzo se transportiraju do drugih organela;
<u>Golgi kompleksu</u> i transportiraju do endosoma, vakuola, ER, plazmine membrane;
<u>mitohondrijima</u> (kvasci i sisavci)

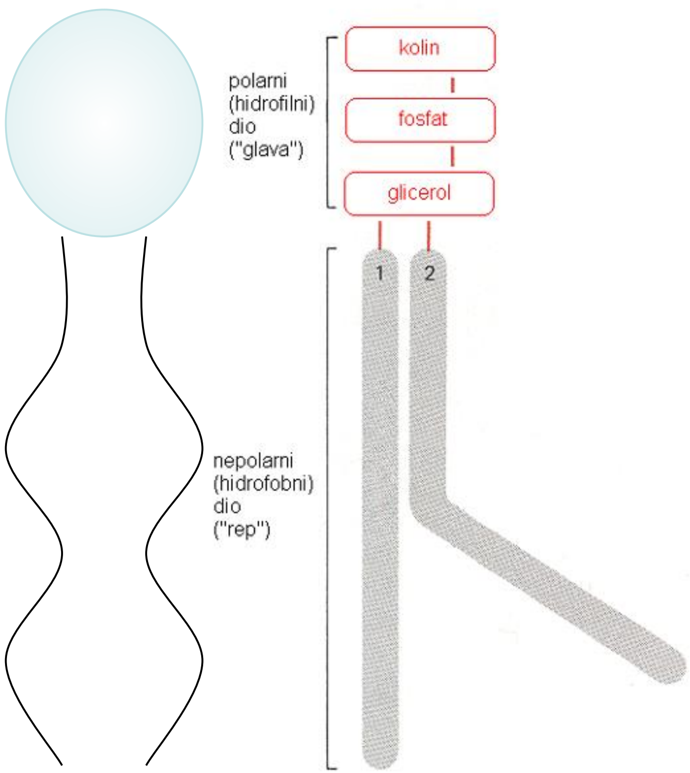
· plazmina membrana nije odjeljak gdje se odvija autonomna sinteza strukturnih lipida, ovdje se odvijaju specifične reakcije (protein-lipid reakcije) kao dio signalnih kaskada

· sve organele sadrže lipide koji su transportirani od odjeljka gdje su sintetizirani

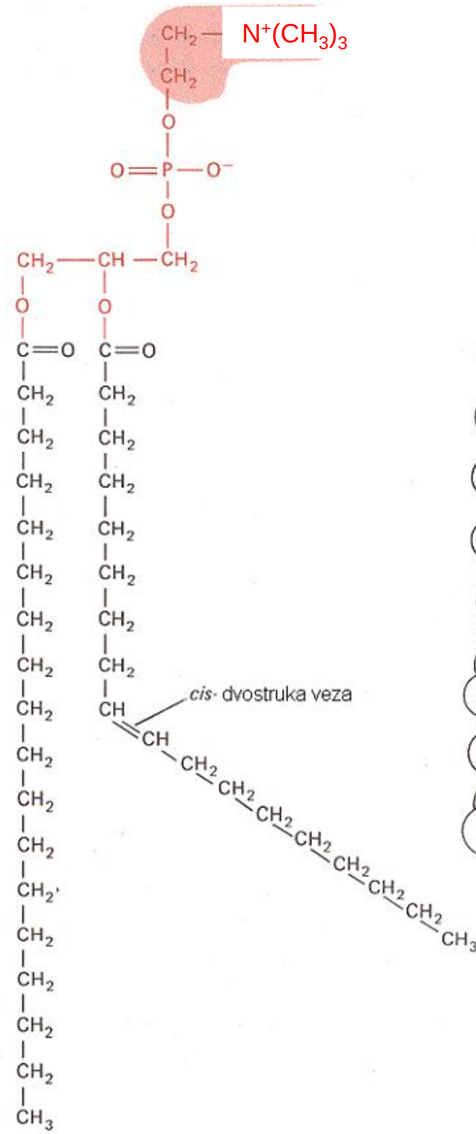
sisavci kvasci
PL - fosfolipid
plavi PL - struktura
crveni PL - “signaling
CHOL - kolesterol (si
ERG - ergosterol (kva
PC - fosfatidil-kolin
PE - fosfatidil-etanola
PI - fosfatidil-inozitol
PS - fosfatidil-serin
PA - fosfatidna kiselin
PG - fosfatidil-glicero
Cer - ceramid
TG - triacilglicerol
SM - sfingomijelin
GSL - glikosfingolipid
ISL - inozitol-sfingolip
DAG - diacil-glicerol
CL - kardiolipin
Sph - sfingozin

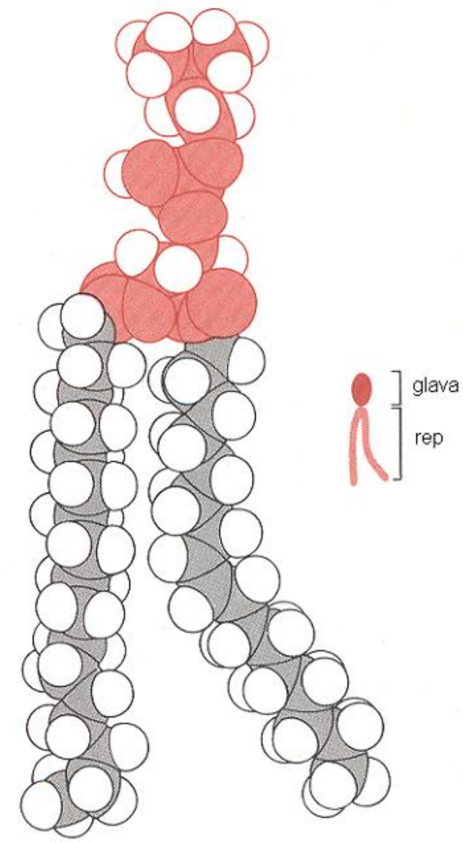


- struktura lipida membrane



1 i 2 - masne kiseline



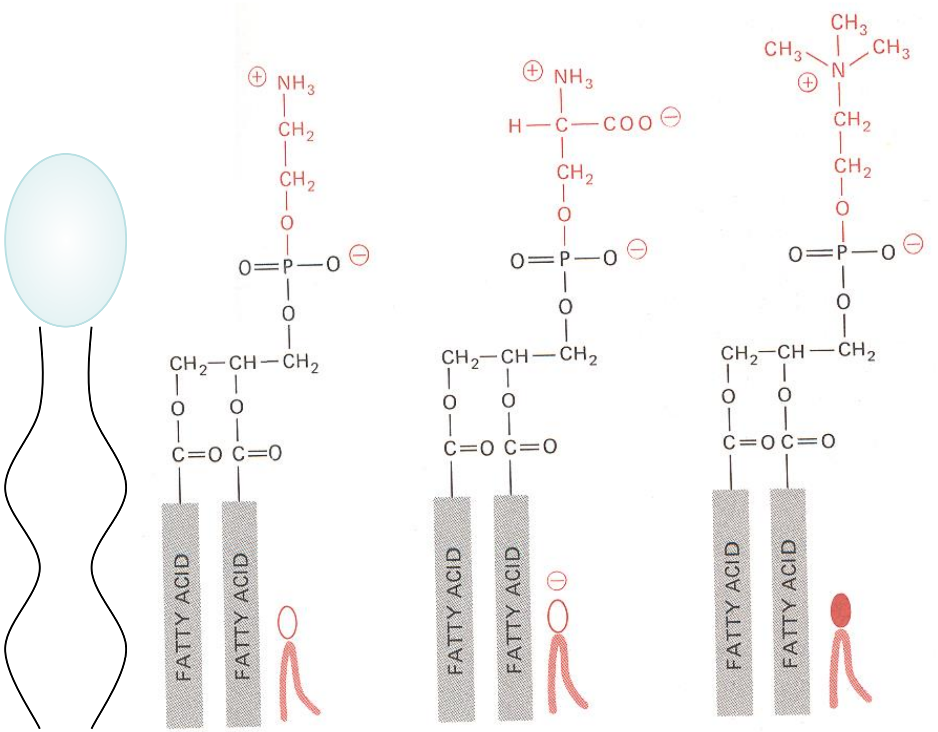


- struktura lipida membrane

arhebakterije: eteri glicerola i dugolančanih alkohola izoprenske strukture (C_{20}-C_{40})

prave bakterije i eukarioti: esteri glicerola tzv. glicerofosfolipidi (derivati fosfatidne kiseline)

najčešći: fosfatidil-kolin (eukarioti)
fosfatidil-etanolamin (eukarioti i eubakterije)
fosfatidil-serin (eukarioti i eubakterije)
fosfatidil-inozitol (eukarioti)

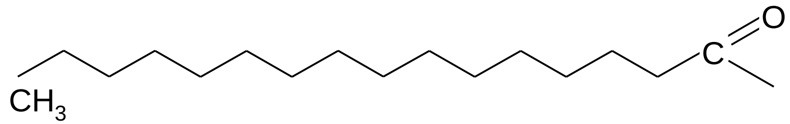


fosfatidil-etanolamin fosfatidil-serin fosfatidil-kolin

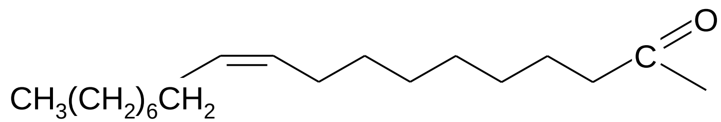
· struktura lipida membrane gliceroofosfolipidi fosfatidil-glicerol
bisfosfatidil-glicerol

generička imena za cijelu grupu spojeva ovisno o tome koji su radikali (acili) masnih kiselina vezani za molekulu glicerola; u pravilu su to masne kiseline s parnim brojem ugljikovih atoma (C_{10}-C_{20}); po strukturi to mogu biti:

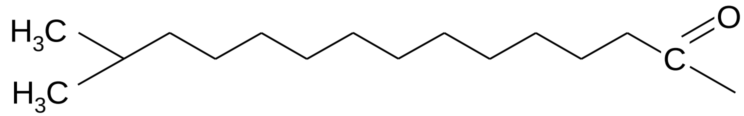
1. ravni zasićeni lanci: najčešće palmitinska (C_{16}) i stearinska kiselina (C_{18}; $CH_3(CH_2)_{14}COO^-$).



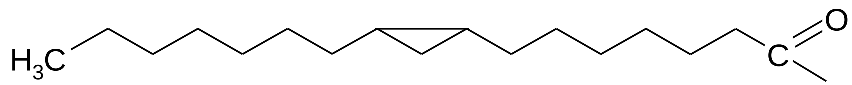
2. ravni nezasićeni lanci (*cis* dvostruka veza): oleinska kiselina ($C_{18:1}$; $CH_3(CH_2)_7CH{=}CH(CH_2)_7COO^-$).



3. razgranati lanci (prokarioti).



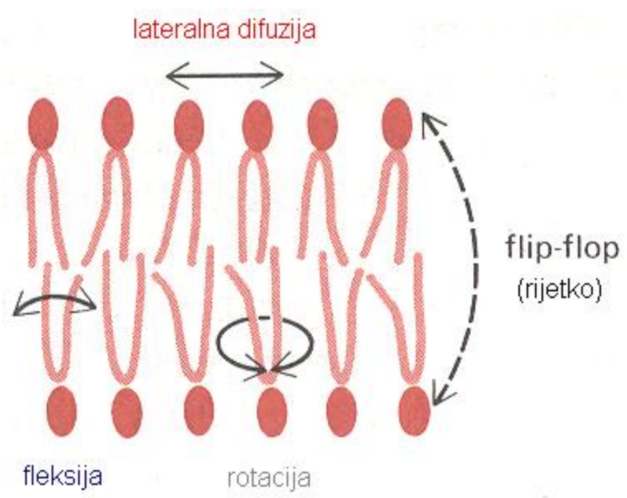
4. lanci sa ciklopropanom (prokarioti).

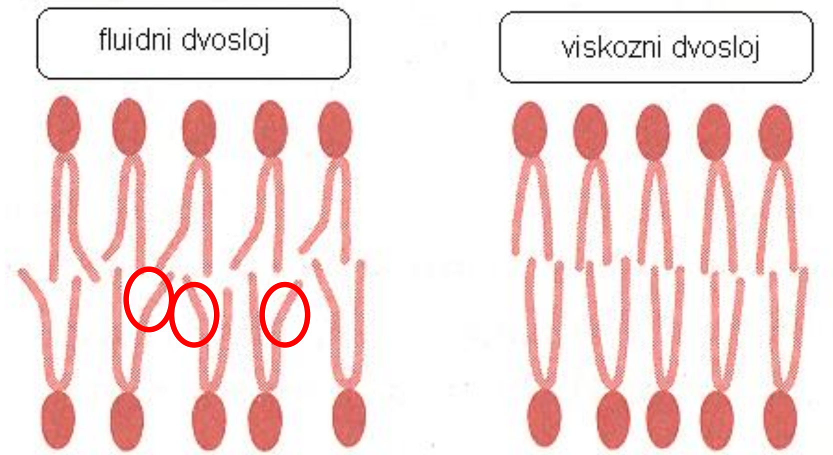


· pored glicerofosfolipida u građi lipida membrane sudjeluju i
glikolipidi (oligosaharidi vezani na glicerofosfolipid)
steroli (kolesterol, zimosterol, ergosterol)

· steroli stabiliziraju membranu i povećavaju fluidnost membrane; regulacija fluidnosti membrane od bitne je važnosti za stanične funkcije, naročito za funkcije proteina membrane.

· fluidnost lipidnog dvosloja ovisi o strukturi lipida membrane





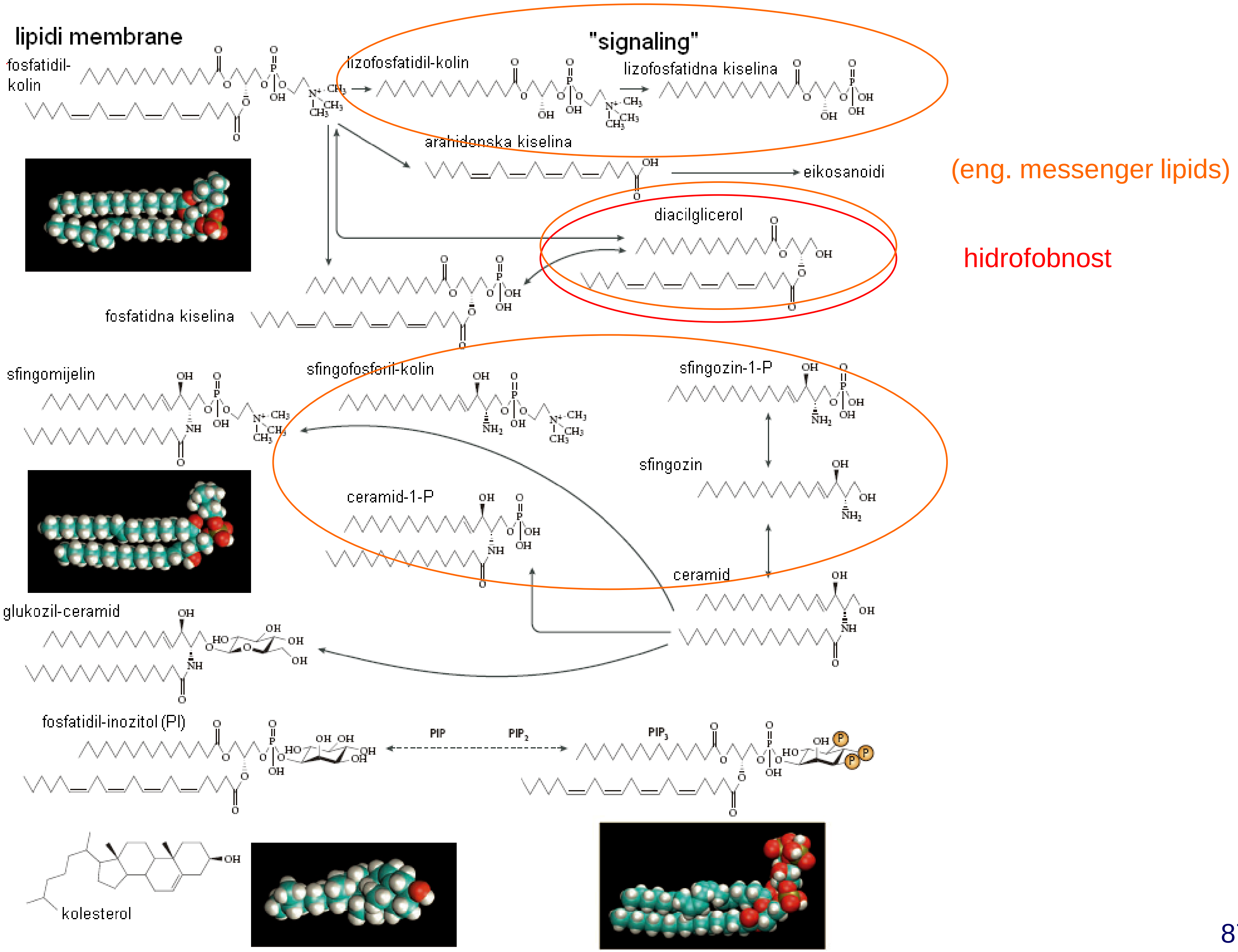
- osnovne funkcije lipida u stanici:

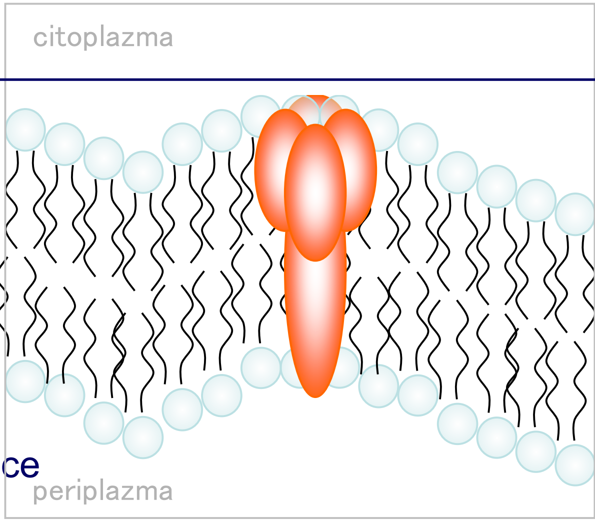
☺spremište energije i spojeva (m. kis. i steroli) za biogenezu membrana stanice

☺polarni lipidi dio su matriksa staničnih membrana (entropijski povoljna reakcija asocijacije hidrofobnih dijelova lipida, kao i njihovih hidrofilnih dijelova koji reagiraju jedni s drugima i s molekulama vode, osnova je spontanog formiranja membrana/organela)

☺osim barijere, lipidi omogućavaju pupanje, fuziju i druge promjene membrana koje su preduvjet za procese diobe stanice, reprodukcije i transporta

☺lipidi sudjeluju u procesima prijenosa signala/informacija u membrani (hidrofobni dio) i citosolu (hidrofilni dio)


lipidi membrane
"signaling"
fosfatidil-kolin
lizofosfatidil-kolin
lizofosfatidna kiselina
arahidonska kiselina
eikosanoidi
(eng. messenger lipids)
diacilglicerol
hidrofobnost
fosfatidna kiselina
sfingomijelin
sfingofosforil-kolin
sfingozin-1-P
sfingozin
ceramid-1-P
ceramid
glukozil-ceramid
fosfatidil-inozitol (PI)
PIP
PIP_2
PIP_3
kolesterol



· osnovne funkcije lipidnog dvosloja:

☺ osmotska barijera

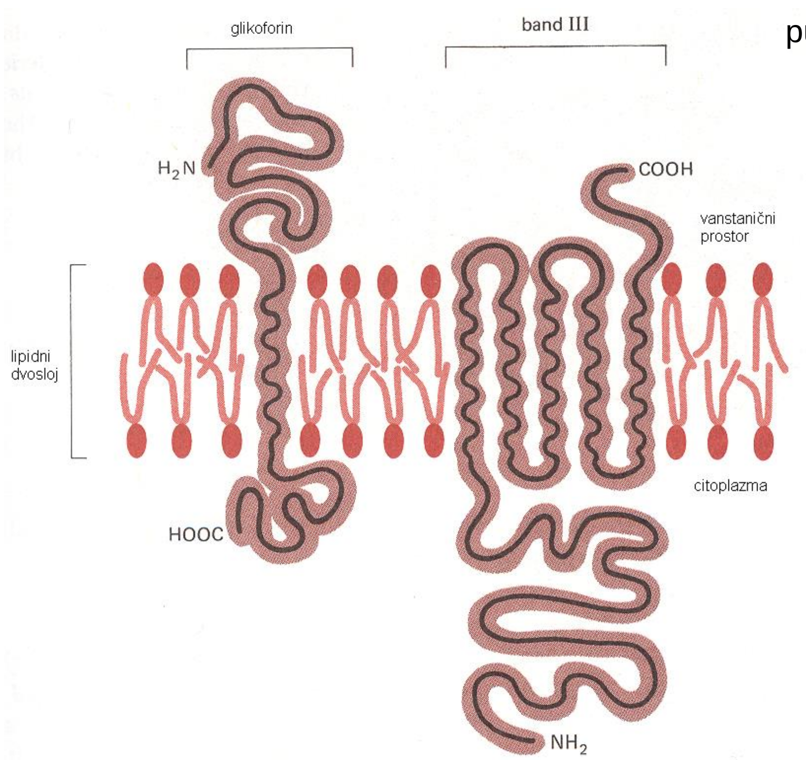
☺ smještaj proteina membrane koji obavljaju brojne funkcije membrane:

- transport određenih molekula u stanicu i iz stanice
- oksidoredukcijske reakcije (respir. lanac)
- fosforilacija (H^+-ATP-sintaze)
- fotofosforilacija
- biosinteza stanične stijenke
- primanje signala iz okoline stanice (proteini-receptori)
- povezivanje citoskeletona i vanjskog omotača (strukturni proteini)
- pretvorba energije potrebne organelima za proces pokretanja

· prokarioti: sve pobrojane funkcije odvijaju se u plazminoj membrani; ako ova površina nije dostatna za potrebe stanice, onda se obično površina membrane povećava invaginacijom u obliku mješinica, cjevčica, membranskih slojeva i tilakoida

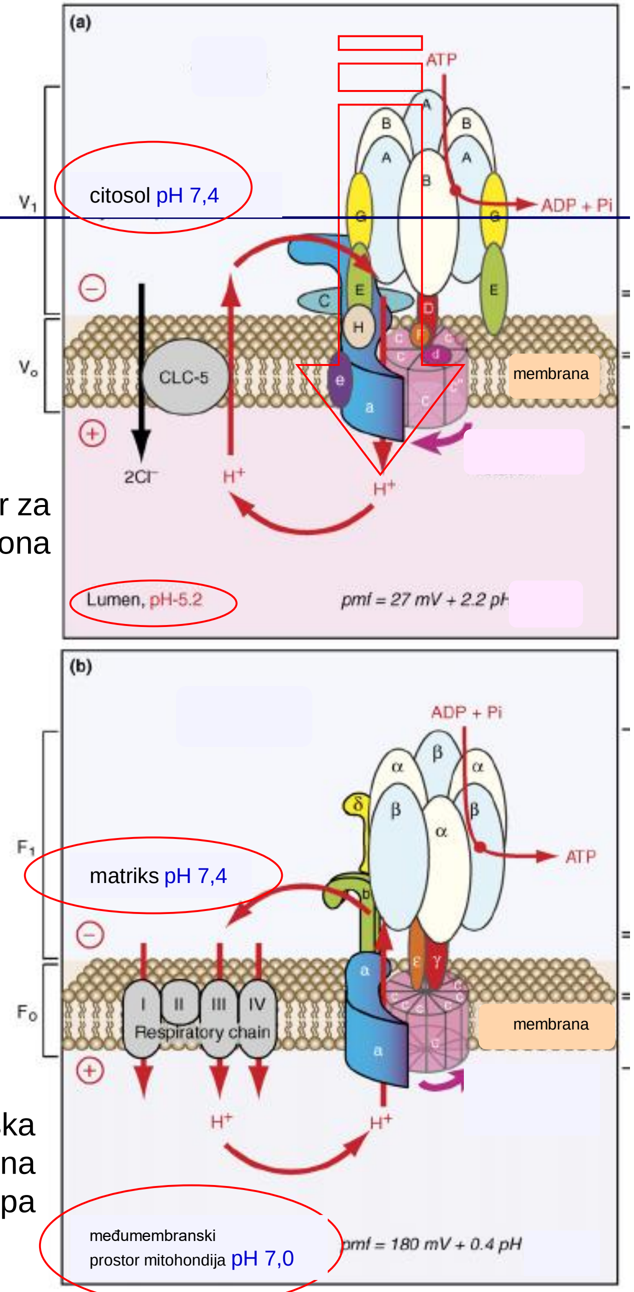
· eukarioti: stanice su znatno veće, pa je i omjer površine i volumena znatno manji; „manjak” membranske površine nadoknađuje se unutarstaničnim membranama koje formiraju odvojene odjeljke stanice - organele; kod životinjskih stanica plazmina membrana čini samo 2-5% ukupnih membrana stanice.

- većina proteina membrane ugrađena je u lipidni dvosloj hidrofobnim interakcijama sa lipidnim molekulama



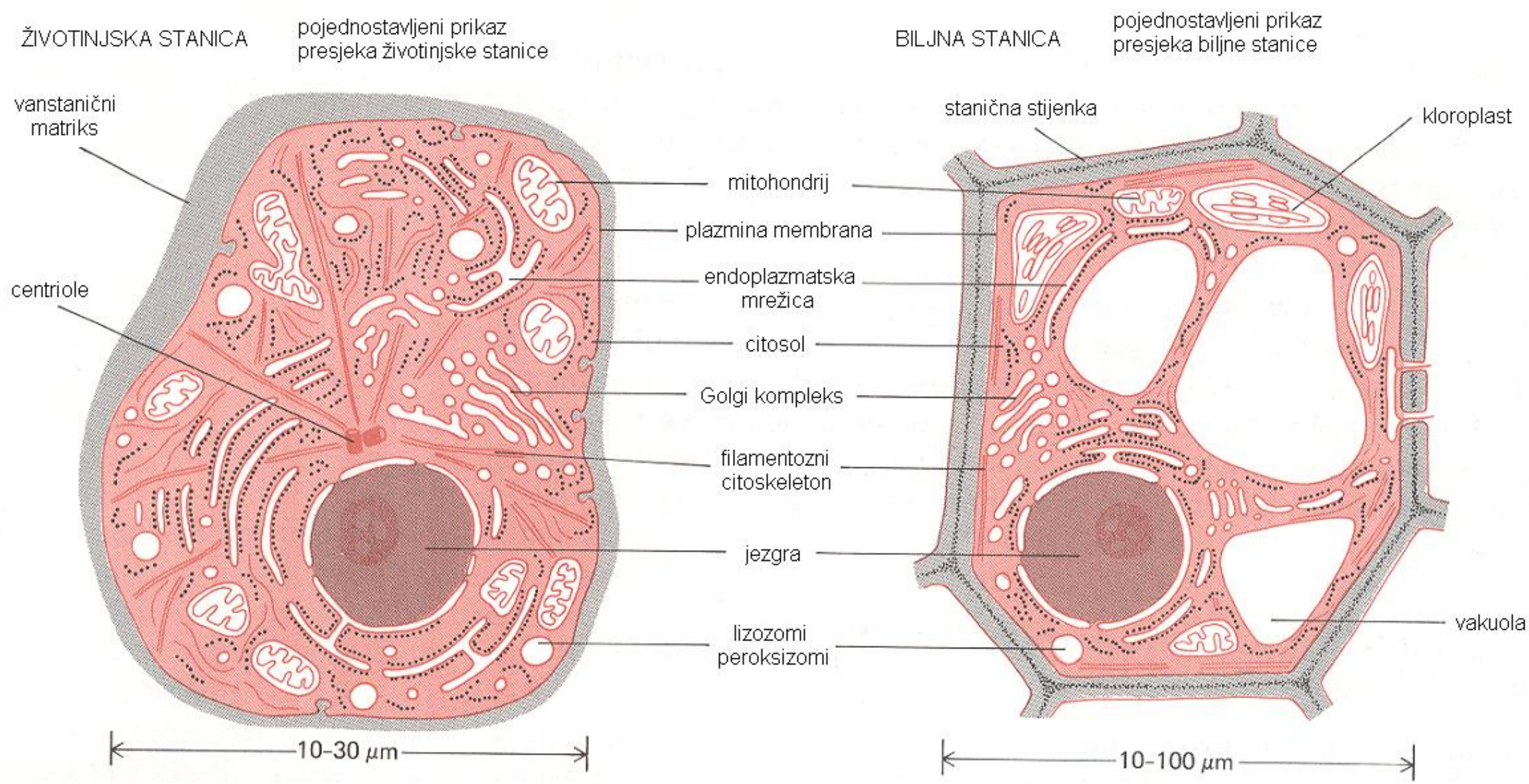
nano-motor za pumpanje protona

mitohondrijska sekundarna pumpa



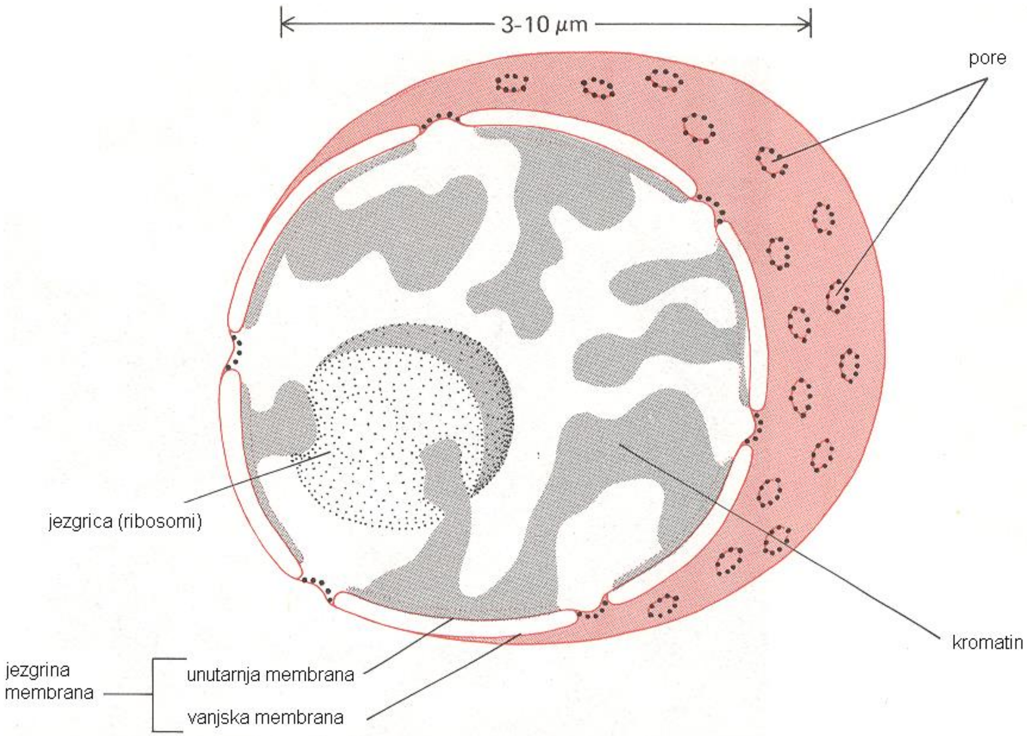
animacija: **ATP Synthase Mechanism**
www.sumanasinc.com/webcontent/animations/microbiology.html

biomembrane - odjeljci stanice (13)

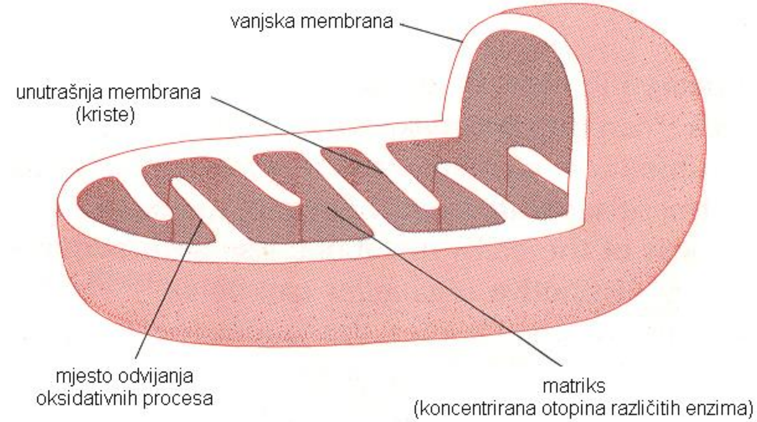


· organele s dvostrukom membranom

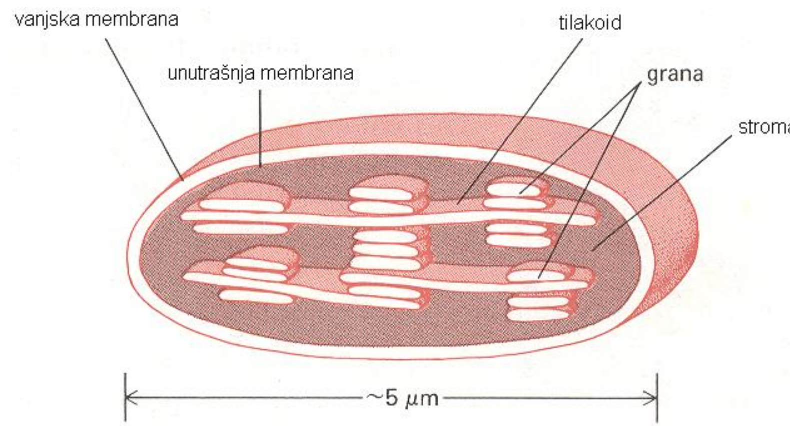
jezgra



mitohondrij



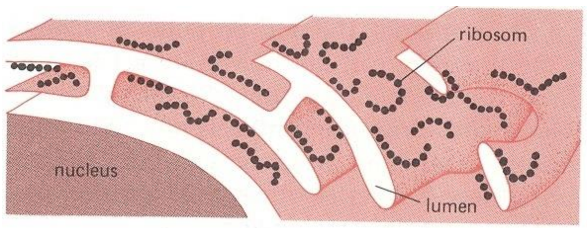
kloroplast

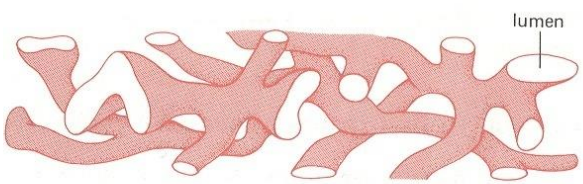


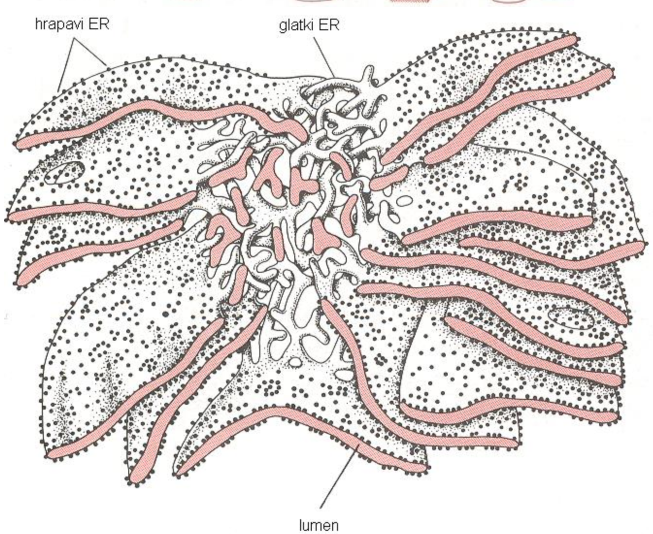
animacija: **Vesicle Budding and Fusing**
www.sumanasinc.com/webcontent/animations/biology.html

· organele s jednostrukom membranom

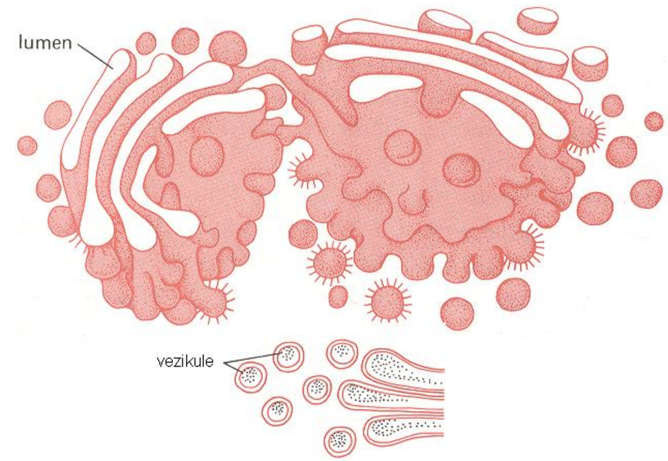
endoplazmatska mrežica (retikulum)



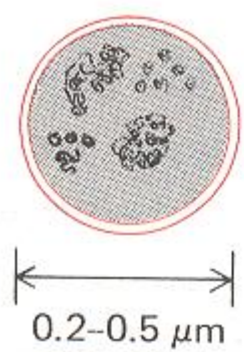


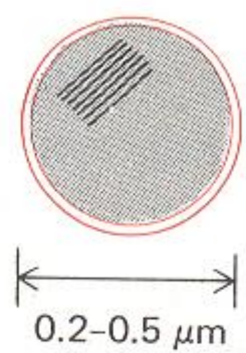


Golgijev kompleks (usmjeravanje proteina ka različitim odredištima)

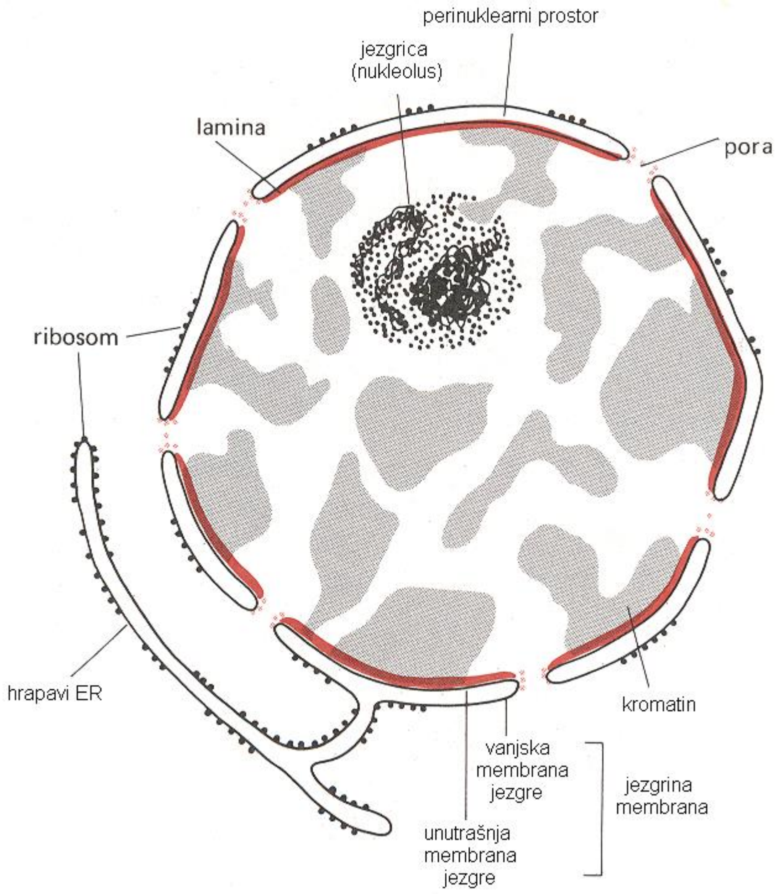


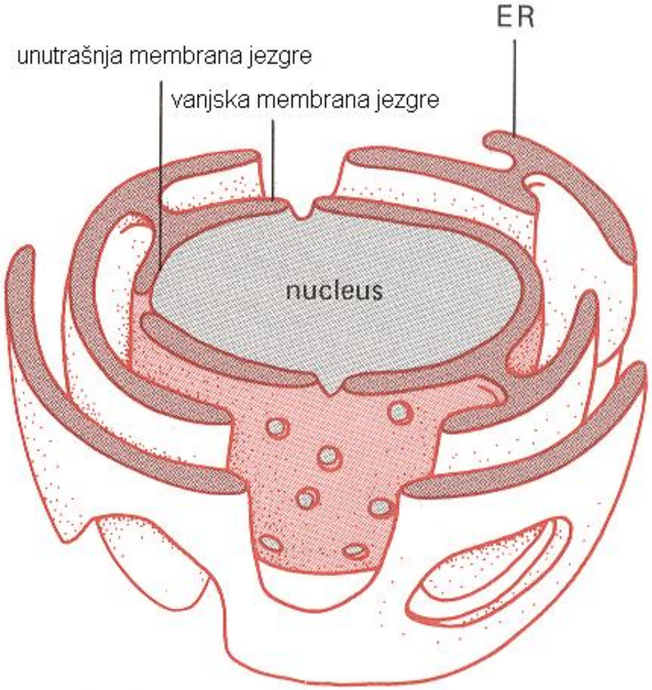
lizozom





peroksizom


perinuklearni prostor
jezgrica
(nukleolus)
lamina
pora
ribosom
hrapavi ER
kromatin
vanjska
membrana
jezgre
unutrašnja
membrana
jezgre
jezgrina
membrana

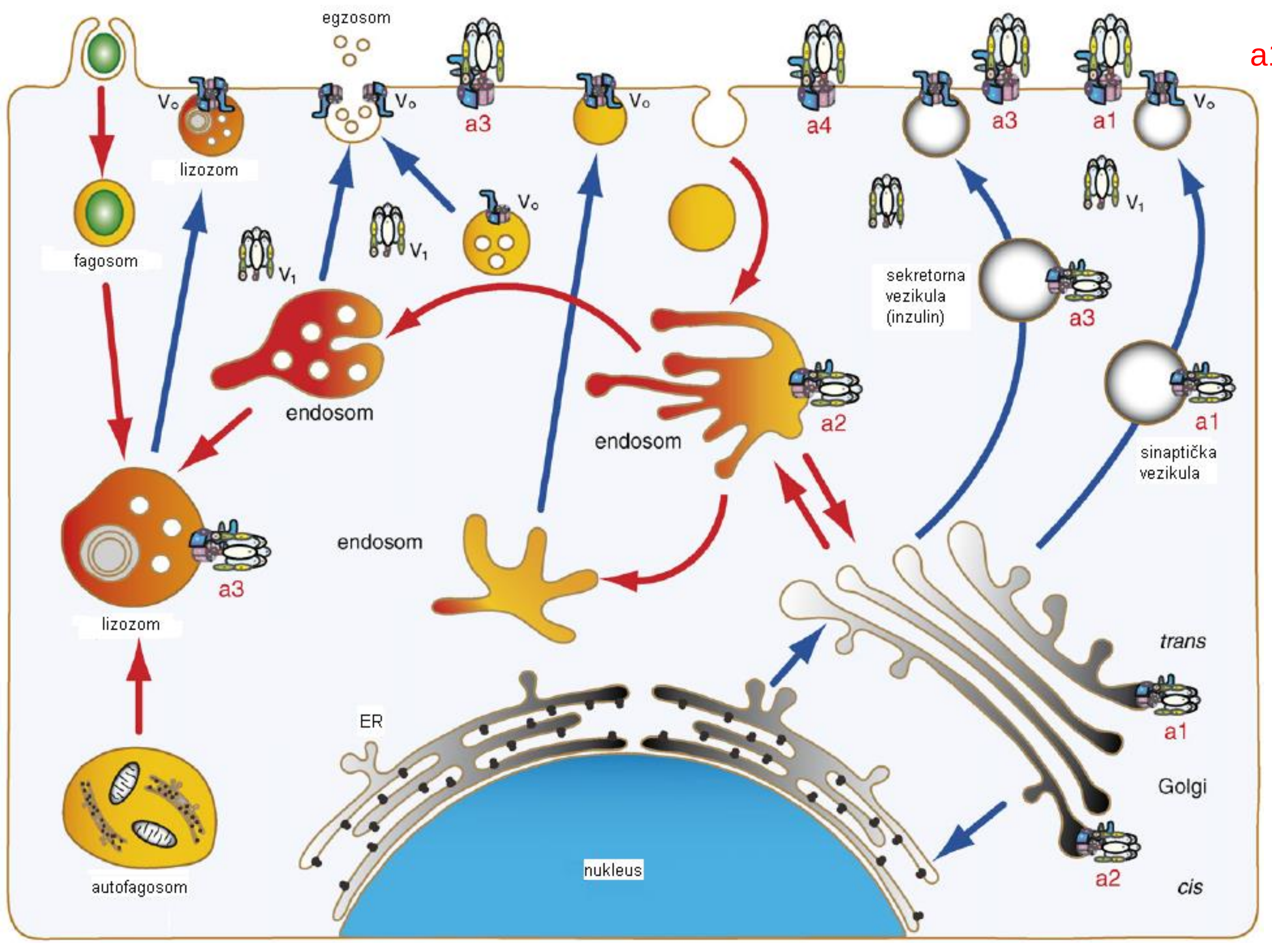

ER
unutrašnja membrana jezgre
vanjska membrana jezgre
nucleus

·povežimo prvo predavanje i biomembrane: proizvodnja aminokiselina s pomoću bakterija *C. glutamicum* i *E. coli* - transport molekula i iona u stanicu i iz stanice

Transporter	Gene(s)	Substrate(s)	Characteristics	Reference(s)
Corynebacterium glutamicum				
AroP	*aroP*	L-Trp, L-Tyr, L-Phe	Aromatic amino acids uptake system	Wehrmann et al. 1995
BrnQ	*brnQ*	L-Ile	Na^+-coupled uptake system	Tauch et al. 1998
GluABCD	*gluABCD*	L-Glu	Binding protein-dependent uptake system, expression glucose-repressed	Kronemeyer et al. 1995
Glutamate permease	?	L-Glu	Uptake active in complex medium	Burkovski et al. 1996
LysE	*lysE*	L-Lys, L-Arg	Exporter, expression regulated by LysG, coinducers L-citrulline and L-histidine	Vrljic et al. 1996; Bellmann et al. 2001
LysI	*lysI*	L-Lys, L-Ala, L-Val, L-Leu	Low capacity antiporter	Seep-Feldhaus et al. 1991
ThrE	*thrE*	L-Thr, L-Ser	Export carrier	Simic et al. 2001
Escherichia coli				
AroP	*aroP*	L-Trp, L-Tyr, L-Phe	General uptake system for aromatic amino acids	Brown 1970
Aspartate/glutamate carrier	?	L-Asp, L-Glu	Binding protein-dependent uptake system, inhibited by cysteate	Schellenberg and Furlong 1977
GltP	*gltP*	L-Asp, L-Glu	Na^+-independent uptake, inhibited by cysteate and β hydroxyaspartate	Deguchi et al. 1989; Wallace et al. 1990; Tolner et al. 1992
GltS	*gltS*	L-Glu	Na^+-dependent uptake, inhibited by α methylglutamate	Deguchi et al. 1990; Kalman et al. 1991
Glutamate excretion carrier	?	L-Glu	Stringent response-related export	Broda 1968; Burkovski et al. 1995
LIV1	*livGHJM*	L-Leu, L-Ile, L-Val, L-Ala, L-Thr, L-Hom	Binding-protein-dependent uptake system, expression repressed by LRP	Templeton and Savageau 1974
Orf299	*ydeD*	L-Cys and components of the cysteine pathway	Major facilitator protein involved in efflux	Daßler et al. 2000
PheP	*pheP*	L-Phe	High-affinity uptake system specific for phenylalanine	Pi et al. 1999
RhtA	*rhtA*		Confer resistance to high concentrations of homoserine and threonine, putative threonine excretion carriers	Zakataeva et al. 1997
RhtB	*rhtB*			Aleshin et al. 1999; Zakataeva et al. 1999
RhtC	*rhtC*			
SstT	*sstT*	L-Ser, L-Thr	Na^+-coupled serine/threonine importer	Ogawa et al. 1998
TdcC	*tdcC*	L-Leu, L-Ser, L-Thr, L-Hom	Importer active under anaerobic conditions	Sumantran et al. 1990
Threonine permease	?	L-Thr, L-Ser	Na^+-independent uptake system	Kruse et al. 2001

animacija: **Vesicle Budding and Fusing**
www.sumanasinc.com/webcontent/animations/biology.html
Protein Secretion
www.sumanasinc.com/webcontent/animations/molecularbiology.html

· transport vezikulama kod eukariota



a1, a2, a3, a4-izoforme ATPaze

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3. Grupa autora: *Molecular biology of the cell*, B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson (eds.), Garland Publishing, Inc., New York (1983).

4. Grupa autora: *Biology of the procaryotes*, J.W. Lengeler, G. Drews, H.G. Schlegel (eds.) Georg Thieme Verlag, Stuttgart, Germany (1999).

5. Grupa autora: *Applied Microbial Physiology*, P.M. Rhodes, P.F. Stanbury (eds.), IRL Press at Oxford University Press, Oxford, UK (1997).

6. V. Marshansky, M. Futai (2008) The V-type H^+-ATPase in vesicular trafficking: targeting, regulation and function, *Current Opinion in Cell Biology* **20**, 415-426.

7. S. Martens, H.T. McMahon (2008) Mechanisms of membrane fusion: disparate players and common principles, *Nature Reviews* **9**, 543-556.

8. A.G. Moat, J.W. Foster: *Microbial physiology*, 3rd edition, A.G. Moat, J.W. Foster (eds.), Wiley-Liss, New York, USA (1995).

9. G. van Meer, D.R. Voelker, G.W. Feigenson (2008) Membrane lipids: where they are and how they behave, *Nature* Reviews **9**, 112-124

10. C. Wolf, P.J.Quinn (2008) Lipidomics: practical aspects and applications, *Progress in Lipid Research* **47**,15-36.

BIOMEMBRANE I BIOENERGETIKA

bioenergetika (1)

· bioenergetika proučava molekulske mehanizme pretvorbe energije u živim stanicama

· sa gledišta termodinamike

živa stanica je NEIZOLIRANI, OTVORENI SUSTAV što znači da može obavljati izmjenu tvari i energije s okolinom;

živa stanica je vrlo uređena struktura (niske entropije) koja ima sposobnost samoreprodukcije pretvorbom jednostavnih kemijskih spojeva iz okoline;

(živa stanica naizgled prkosi II zakonu termodinamike; međutim,...)

ŽIVA JE STANICA KEMIJSKI SUSTAV KOJI DJELUJE U UVJETIMA KONSTANTNOG TLAKA, VOLUMENA I TEMPERATURE I PROVODI PRETVORBU ENERGIJE IZ JEDNOG OBLIKA U DRUGI, U SKLADU SA ZAKONIMA TERMODINAMIKE.

· STANICA NE MOŽE PROIZVODITI ENERGIJU IZ NIČEGA (I zakon termodinamike), NITI MOŽE TERMALNU ENERGIJU PRETVARATI U DRUGE OBLIKE SLOBODNE ENERGIJE (II zakon termodinamike)

· POVEĆANJE REDA (SMANJENJE ENTROPIJE) U STANICI POSLJEDICA JE POVEĆANJA NEREDA (ENTROPIJE) U OKOLINI STANICE

· živa stanica koristi ove izvore energije:

1. KEMIJSKA ENERGIJA (sva živa bića)

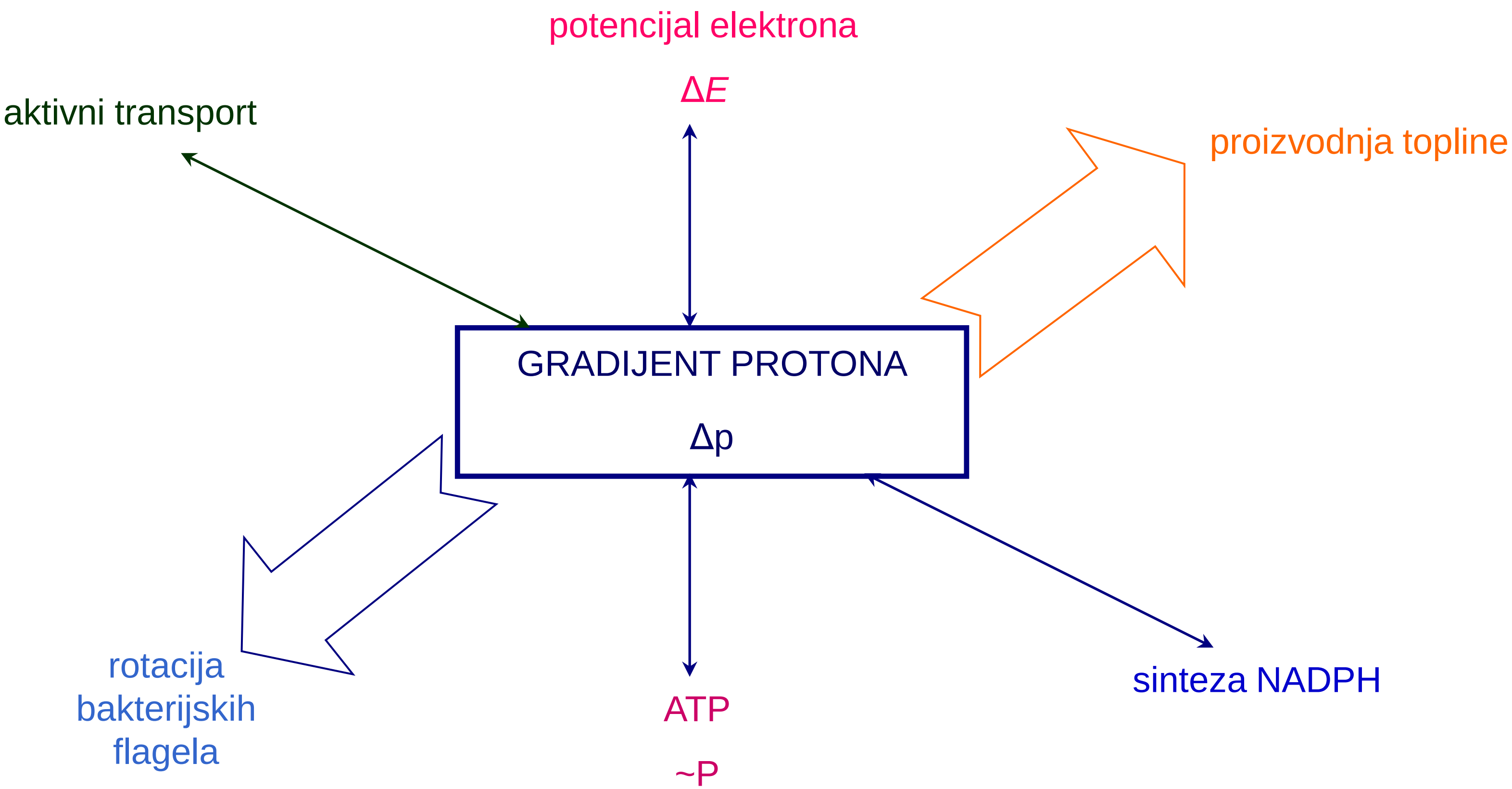
1.1. reducirani spojevi (SUPSTRATI: metan, alkani, alkoholi, organske kiseline, ugljikohidrati, H_2, H_2S, NH_3, itd.; KOENZIMI: NAD(P)H + H^+, FAD(H_2), FMN(H_2))

1.2. spojevi sa velikom energijom hidrolize (SUPSTRATI: 1,3-BPG, PEP, PP, acetil-CoA, acetil-fosfat; KOENZIMI: ATP, ADP, GTP i drugi trifosfonukleotidi)

1.3. gradijent protona (Δp)

2. ENERGIJA SVJETLOSTI ($h\nu$)

bioenergetika: transmembranski gradijent protona pokreće niz energetskih procesa u stanici (4)



· kemijske reakcije koje u ukupnosti čine metabolizam stanice dijele se na kataboličke i anaboličke

KATABOLIZAM	ANABOLIZAM
razgradnja	sinteza
dobivanje energije	trošenje energije
dobivanje ATP	trošenje ATP
dobivanje ekvivalenata redukcije	trošenje ekvivalenata redukcije
povećanje entropije	smanjenje entropije
energetski metabolizam	biosinteza staničnog materijala

· dobivanje ATP u kataboličkim reakcijama odvija se na tri načina:

1. fosforilacija u lancu supstrata (citoplazma, glikoliza)
2. oksidativna fosforilacija (biomembrane)
3. fotofosforilacija (biomembrane)

· oksidativna fosforilacija (stanično disanje ili respiracija)

prokarioti: plazmina membrana

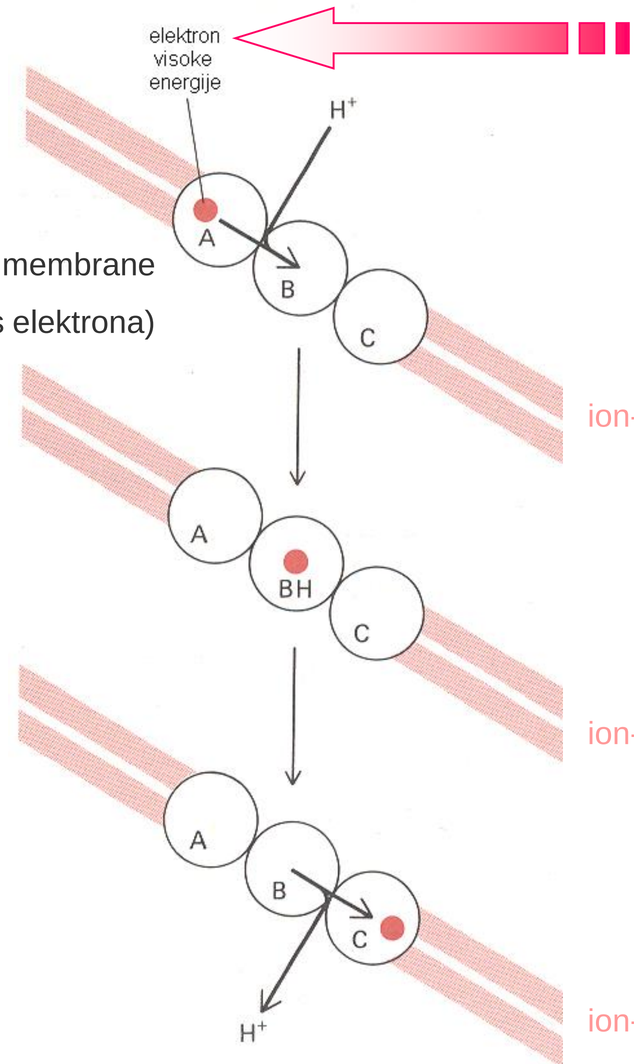
eukarioti: unutrašnja membrana mitohondrija

· povezivanje oksidativne fosforilacije i reakcije ATP-sintaze: oksido-redukcijske reakcije u membrani dovode do stvaranja transmembranskog protonskog gradijenta; tok protona niz gradijent koncentracije kroz protonski kanalić (podjedinica F_0 ATP-sintaze) dovodi do sinteze ATP iz ADP i fosfata na podjedinici F_1 ATP-sintaze

prijenos elektrona - kemiosmotski mehanizam

· prijenos elektrona i stvaranje gradijenta protona

npr. procesi oksidacije metabolita
ili hν



proteini membrane

(lanac za prijenos elektrona)

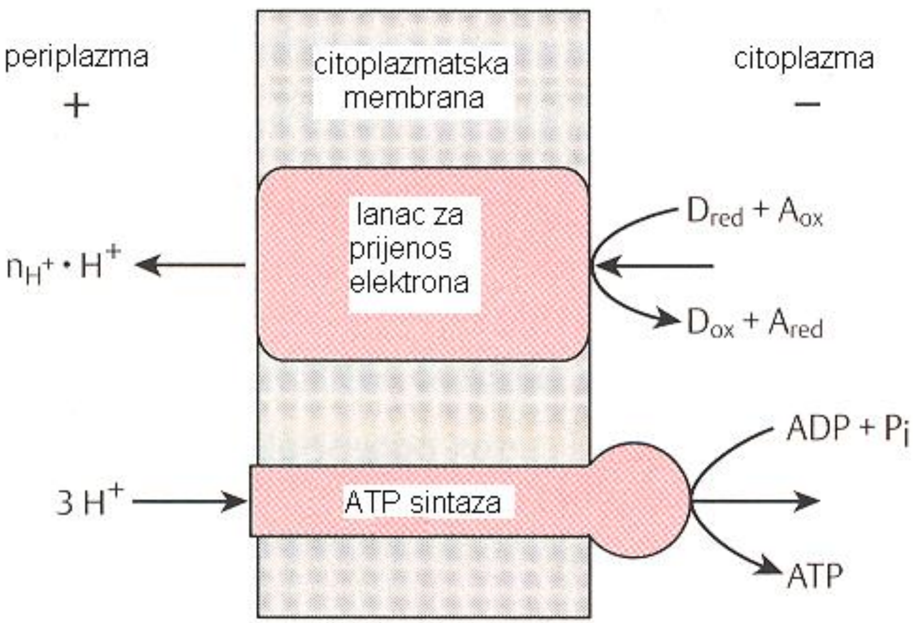
ion-nepropusna membrana

ion-nepropusna membrana

transmembranski gradijent protona
(Δp)

ion-nepropusna membrana

lanac za prijenos elektrona i ATP-sintaza u citoplazmatskoj membrani bakterija



D_{red} donor (npr. sukcinat)

A_{ox} akceptor (npr. O_2)

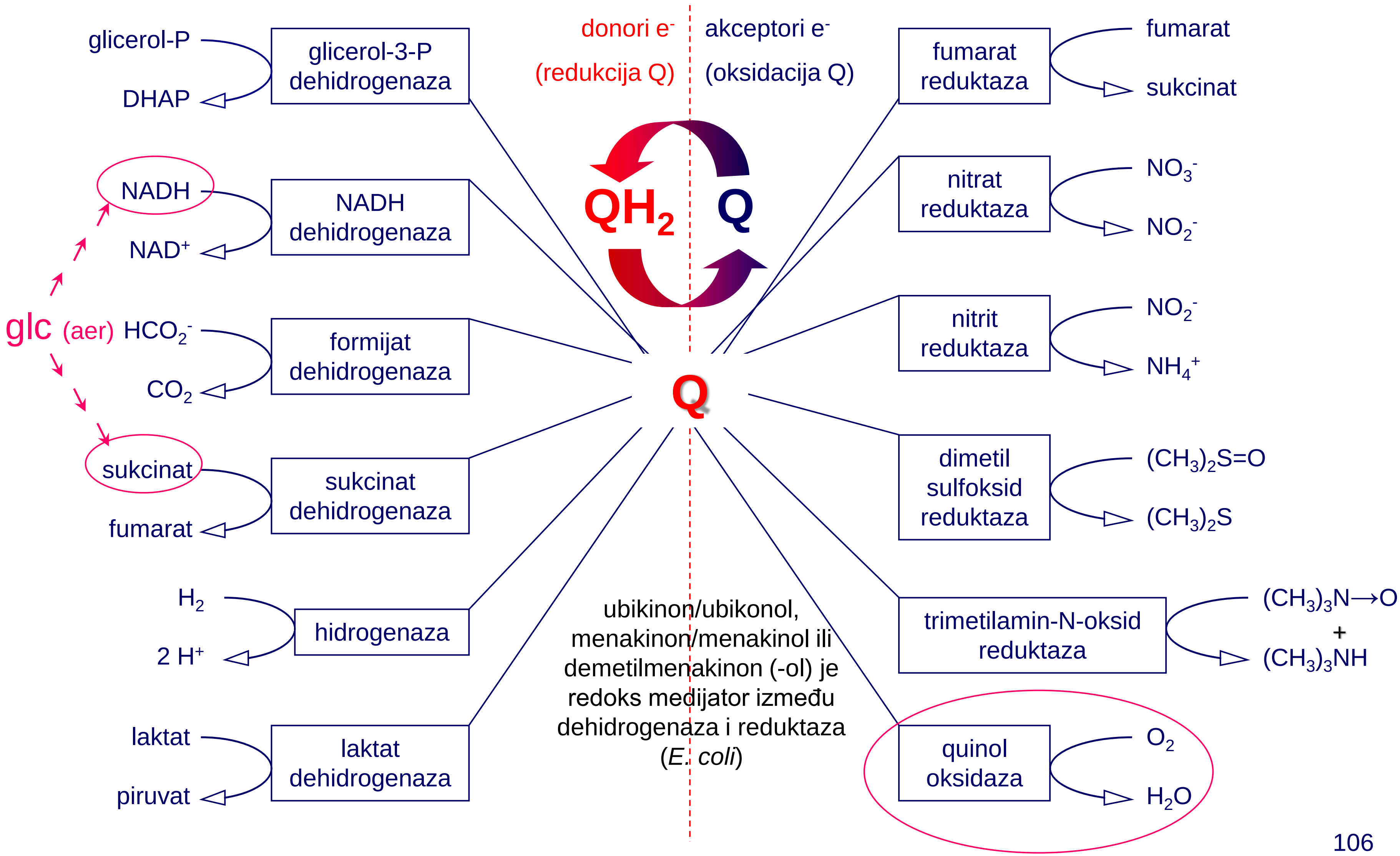
cilj: pridobivanje ATP

standardni redoks potencijal (E'_0) različitih donora i akceptora elektrona pri pH 7

redoks parovi	E'_0 (V)
HCO_3^-/HCO_2^-	−0.41
H^+/H_2	−0.42
HCO_3^-/acetate	−0.35
HCO_3^-/CH_4	−0.33
NAD/NADH	−0.32
S^0/HS^-	−0.27
Acetaldehid / etanol	−0.20
Piruvat / laktat	−0.19
DHAP / glicerol-P	−0.19
Oksaloacetat / malat	−0.17
HSO_3^-/HS^-	−0.12
SeO_4^{2-}/SeO_3^{2-}	+0.02
Fumarat / sukcinat	+0.03
NO_2^- / NH_4^+	+0.06
Trimetilamin oksid / trimetilamin	+0.13
Dimetilsulfoksid / dimetilsulfid	+0.16
NO_2^-/NH_4^+	+0.34
NO_2^-/NO	+0.35
NO_3^-/NO_2^-	+0.43
Fe^{3+}/Fe^{2+}	+0.77
O_2/H_2O (oxphos)	+0.82
NO/N_2O	+1.18
N_2O/N_2	+1.36

animacija: **Electron Transport: Aerobic and Anaerobic Conditions**
www.sumanasinc.com/webcontent/animations/biology.html

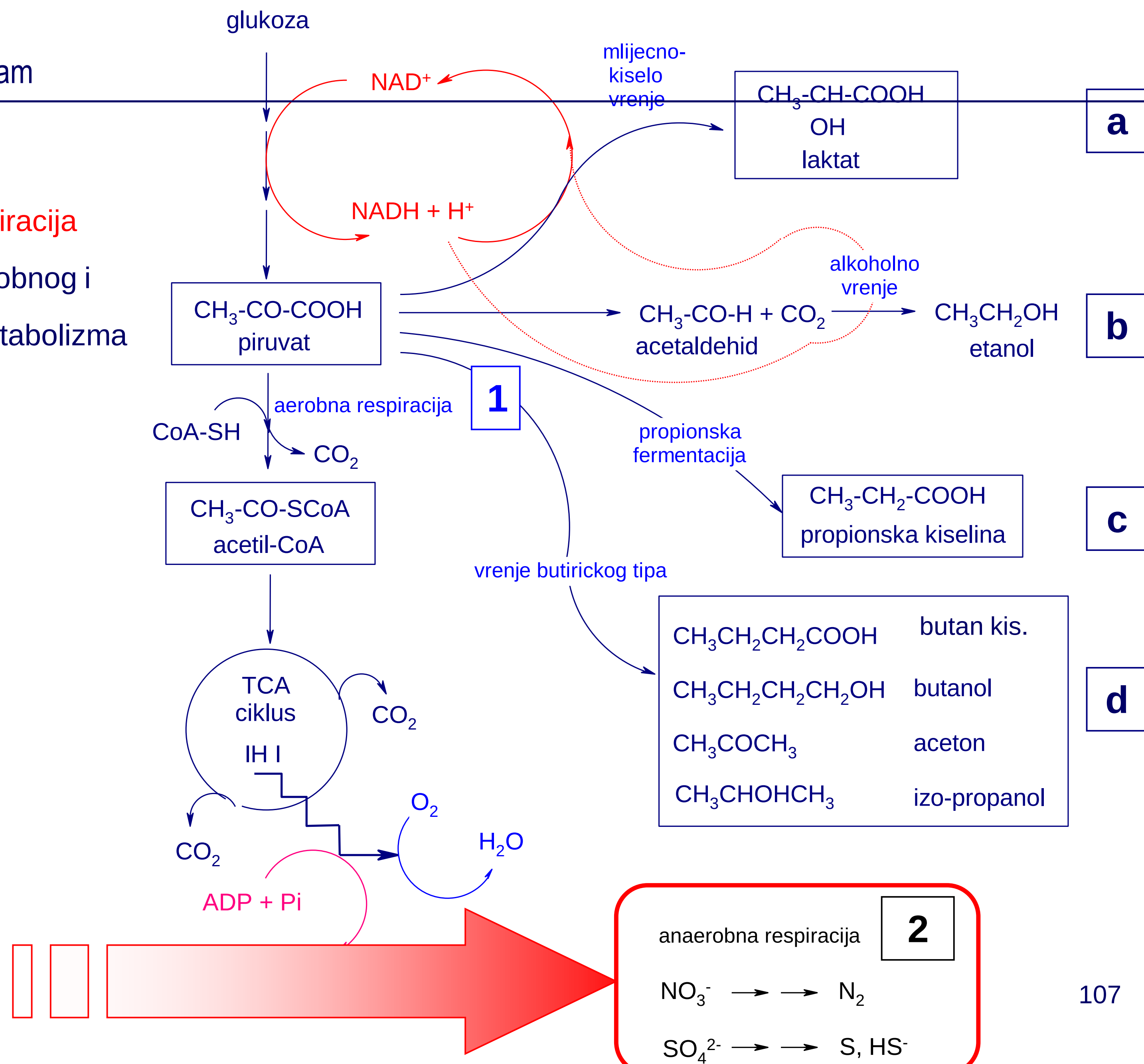
aerobna i anaerobna respiracija (disanje) kod *E. coli*



stanični metabolizam

· anaerobna respiracija

· razdvajanje aerobnog i anaerobnog metabolizma ugljikohidrata



· anaerobna respiracija

U anaerobnim respiracijama oksidirani su organski spojevi ili vodik, a primaoci elektrona (NO_3^-, NO_2^-, ioni željeza, dimetilsulfoksid, ugljični dioksid, sulfat ili organski spojevi klora) su reducirani.

DENITRIFIKACIJA (uzastopna redukcija počevši od nitrata) i AMONIFIKACIJA

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

$$NO_2^- \longrightarrow NH_4^+$$

$$NO_3^- + 2\,e^- + 2\,H^+ \longrightarrow NO_2^- + H_2O$$ nitrat redukataza

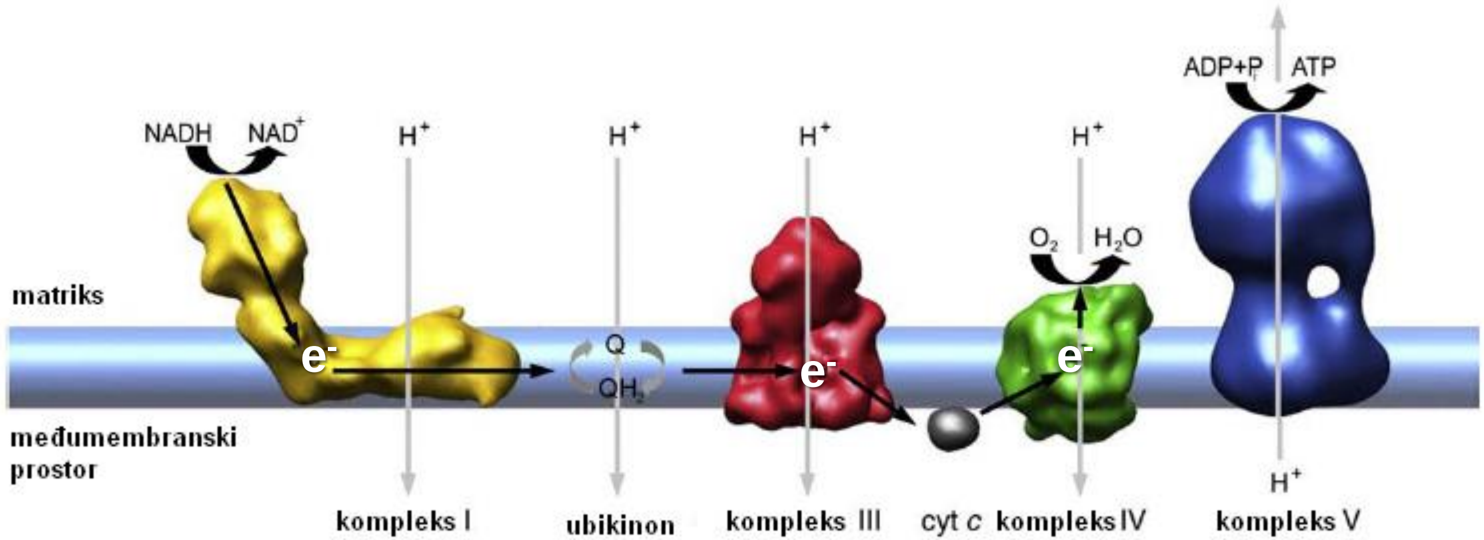
$$NO_2^- + e^- + 2\,H^+ \longrightarrow NO + H_2O$$ nitrit redukataza

$$2\,NO + 2\,e^- + 2\,H^+ \longrightarrow N_2O + H_2O$$

$$N_2O + 2\,e^- + 2\,H^+ \longrightarrow N_2 + H_2O$$

$$NO_2^- + 3\,NAD(P)H + 5\,H^+ \longrightarrow NH_4^+ + 3\,NAD(P)^+ + 2\,H_2O$$

RL – eukarioti (unutrašnja membrana mitohondrija)



animacija: **Electron Transport Chain**
www.sumanasinc.com/webcontent/animations/biology.html
→ Cellular Respiration → Electron Transport

kompleks I (protonska pumpa) NADH ubikinon oksidoreduktaza (L-oblik kod svih organizama)

kompleks II sukcinat dehidrogenaza (sukcinat → kompleks II → ubikinon)

kompleks III (protonska pumpa) ubikinol cytokrom *c* oksidoreduktaza (citokrom bc_1 kompleks)

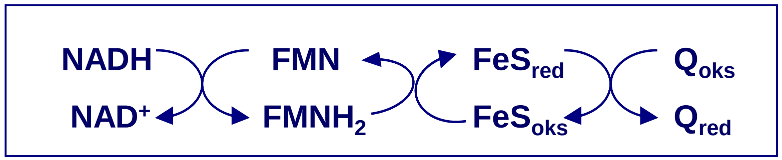
kompleks IV (protonska pumpa) citokrom *c* oksidaza

kompleks V F_1F_0-ATP sintaza (F_0 – pumpanje protona, F_1 – katalitička domena, sinteza ATP)

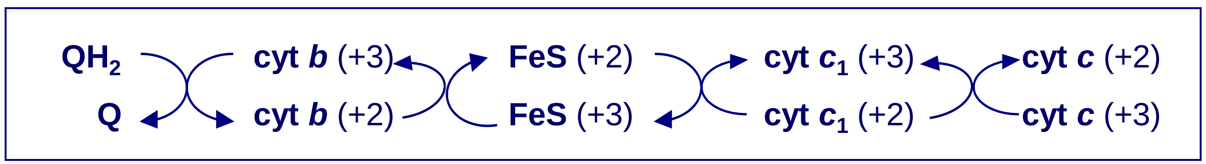
ATP **energetska moneta u stanici**

animacija: **ATP Synthase Mechanism**
www.sumanasinc.com/webcontent/animations/microbiology.html

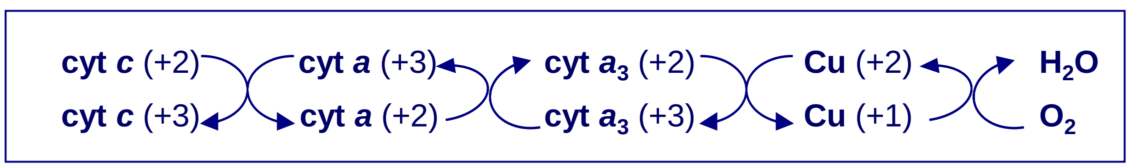
kompleks I (protonska pumpa) NADH ubikinon oksidoreduktaza



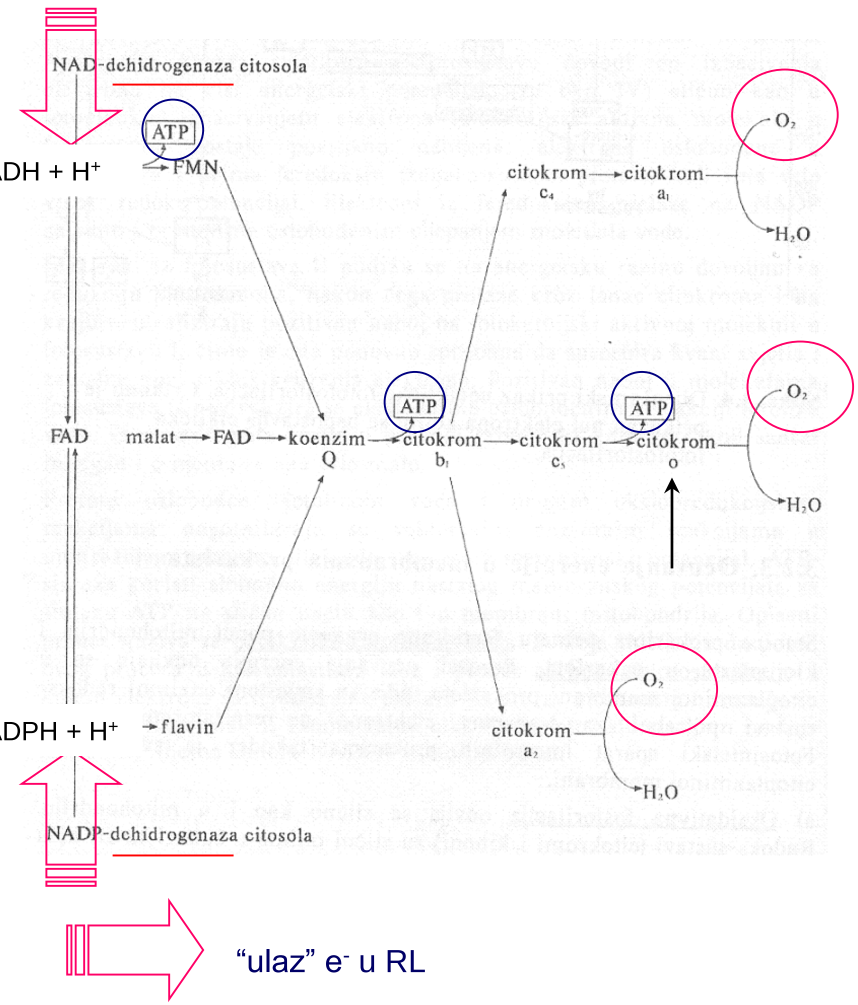
kompleks III (protonska pumpa) ubikinol cytokrom *c* oksidoreduktaza (citokrom bc_1 kompleks)

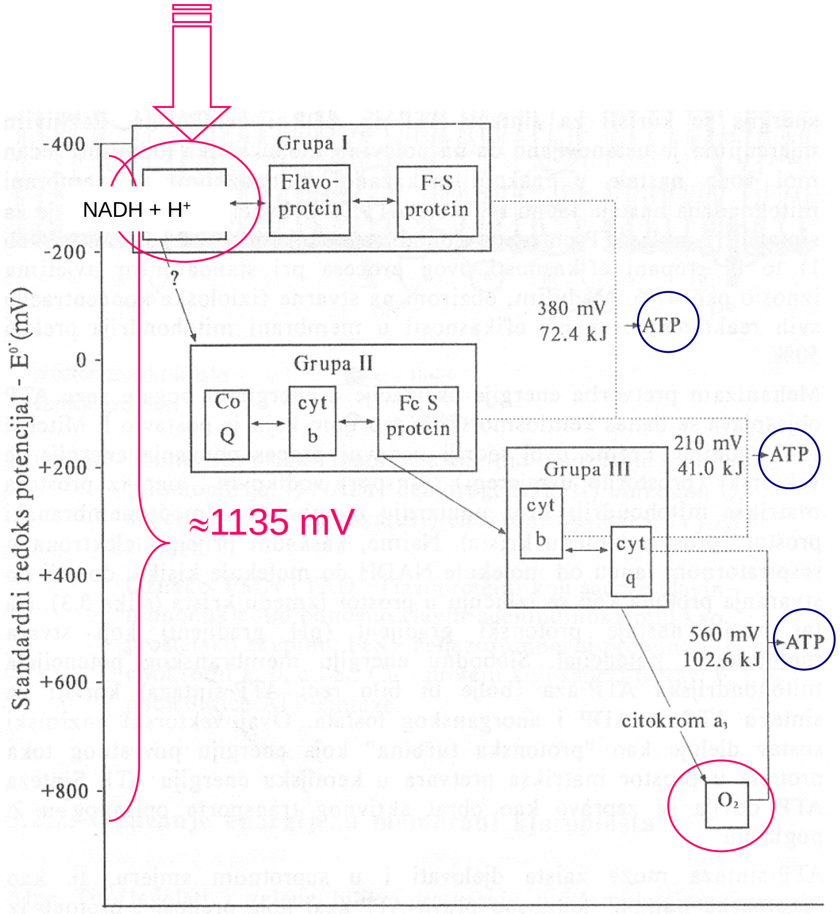


kompleks IV (protonska pumpa) citokrom *c* oksidaza



lanci za prijenos elektrona kod prokariota (*Azotobacter vielandii*) i eukariota





“ulaz” e- u RL

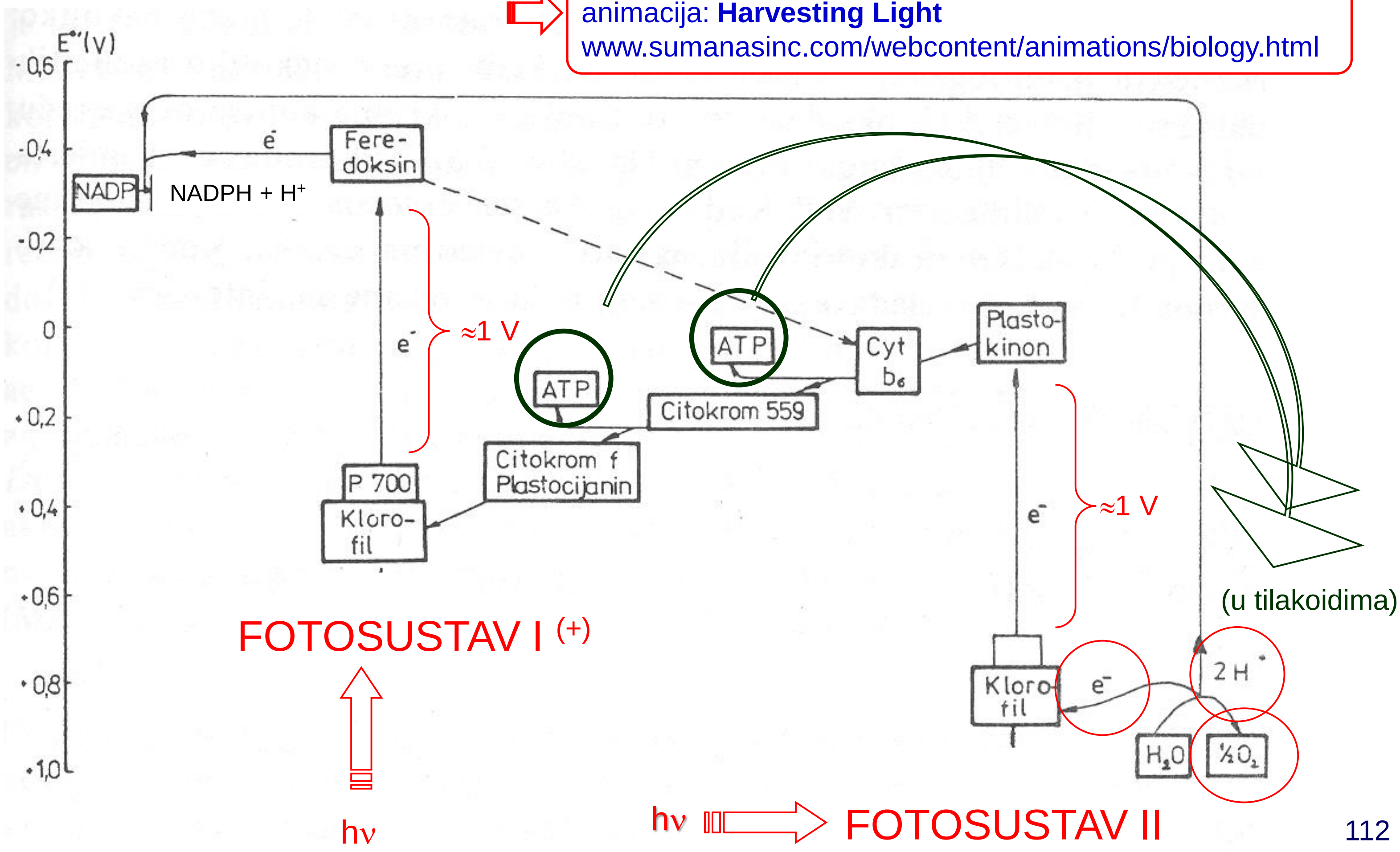
· razgranati lanci za transport elektrona

·alge, fitoflagelati, zelene biljke

·fotosinteza (fotoliza vode)

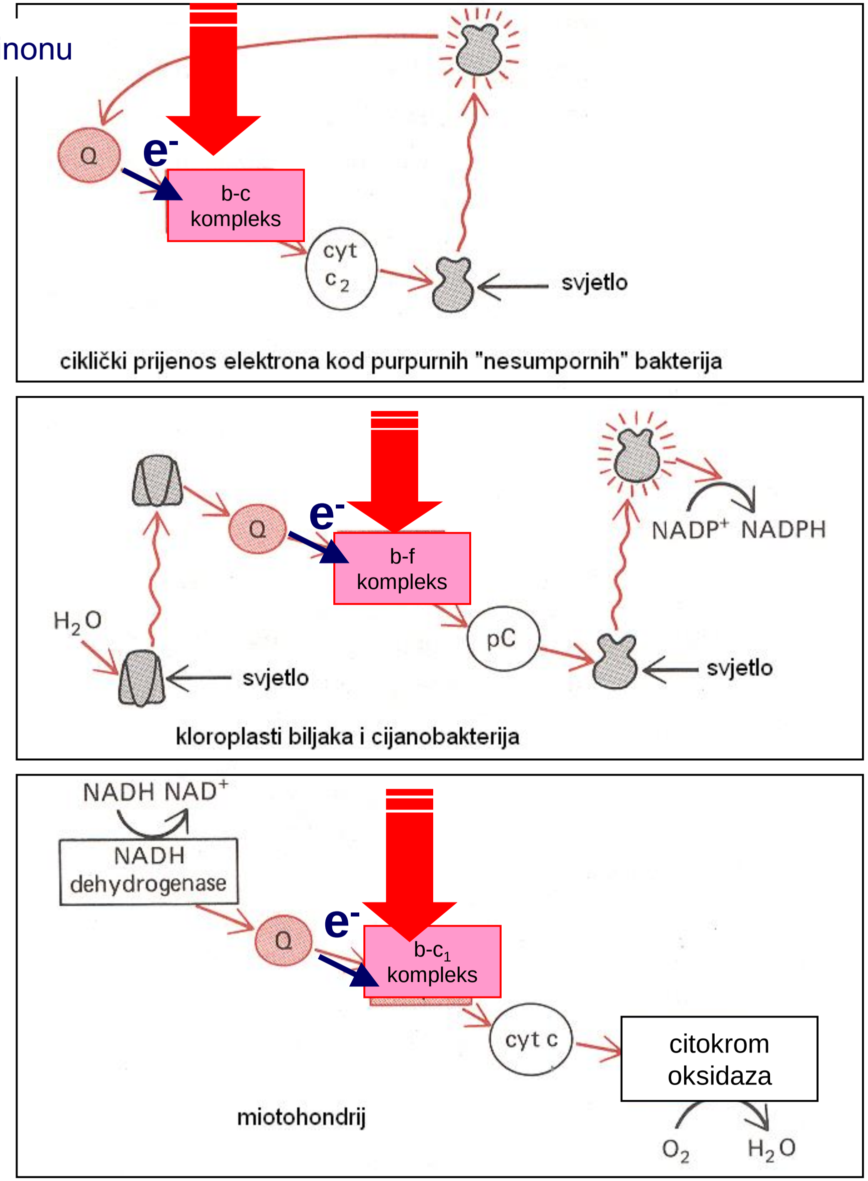
$$H_2O + NADP^+ \xrightarrow{h\nu} NADPH + H^+ + \frac{1}{2} O_2$$

animacija: **Harvesting Light**
www.sumanasinc.com/webcontent/animations/biology.html

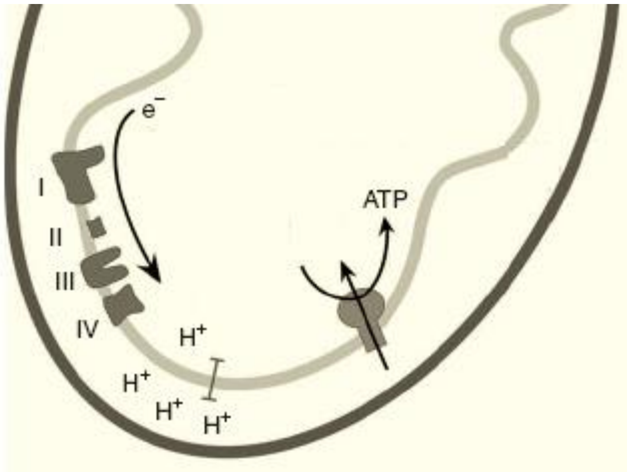


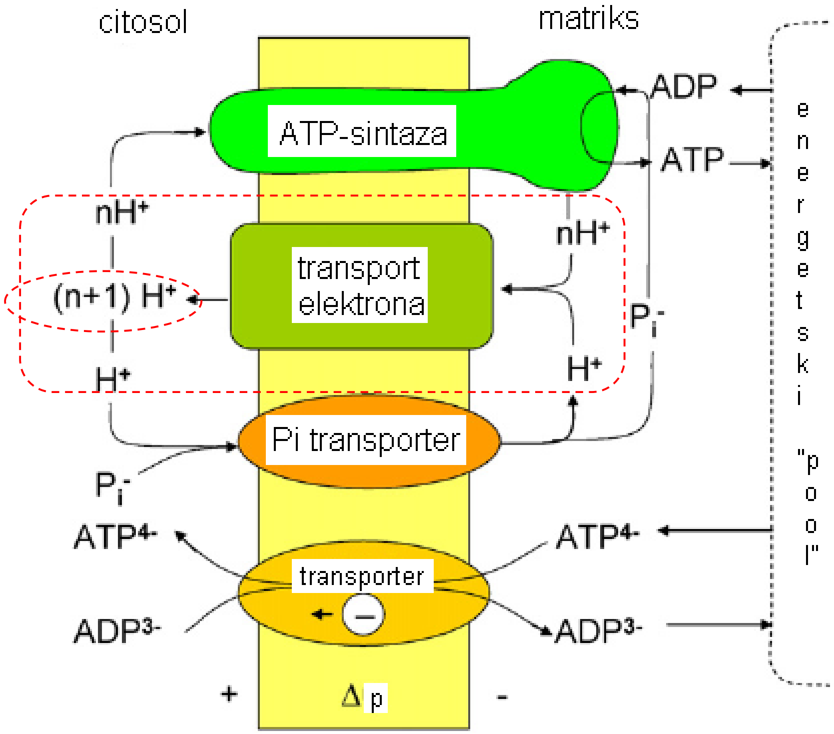
RL: usporedba fotofosforilacije i oksidativne fosforilacije

Q - prijenosnik e⁻ sličan ubikinonu

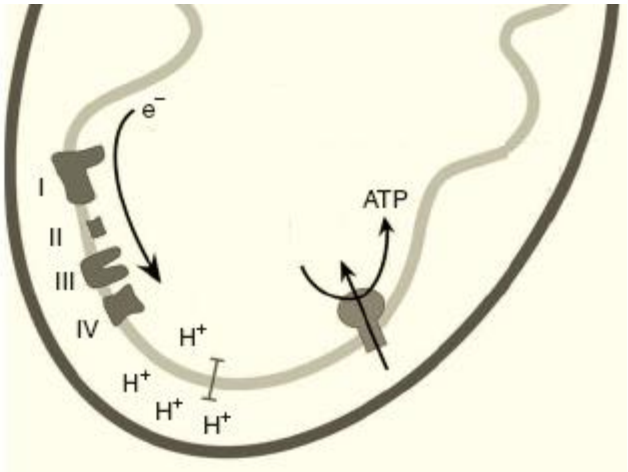


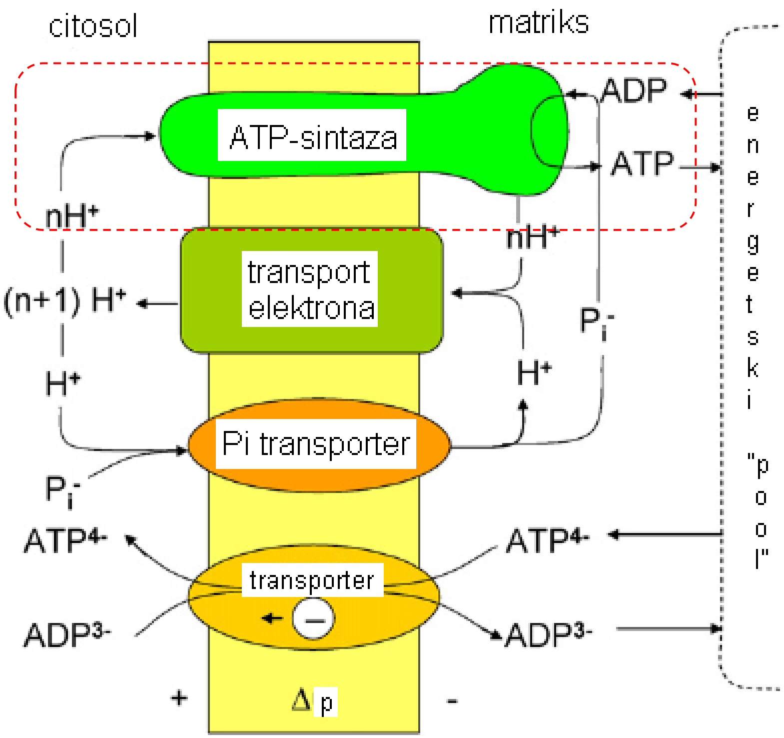
regulacija transporta H⁺ za sintezu citosolnog ATP (1)



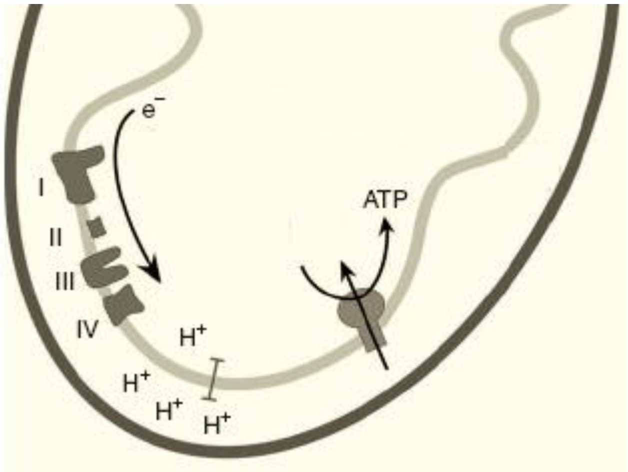


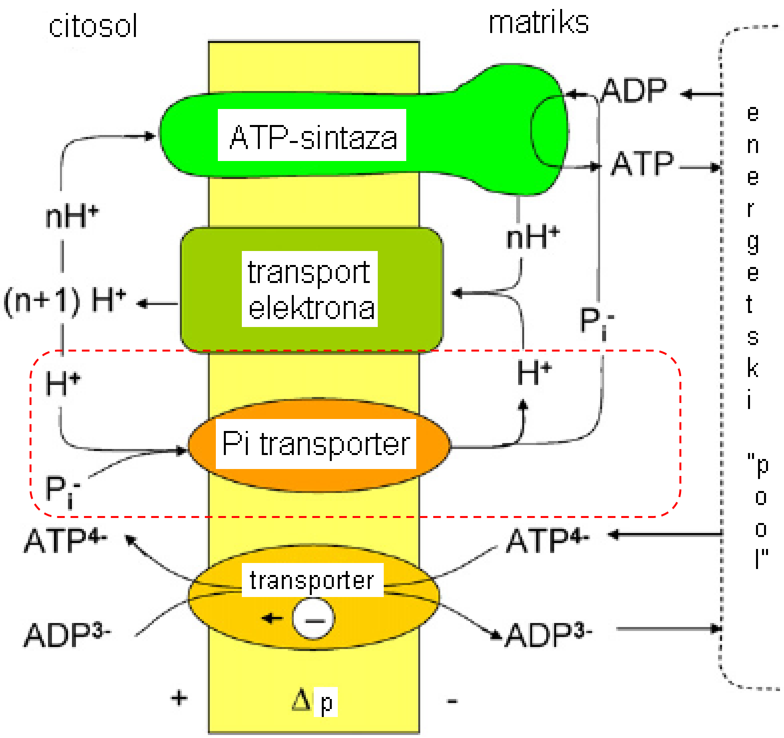
regulacija transporta H^+ za sintezu citosolnog ATP (2)



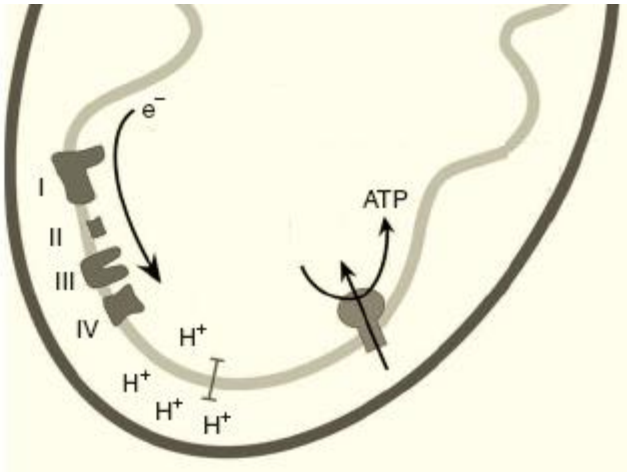


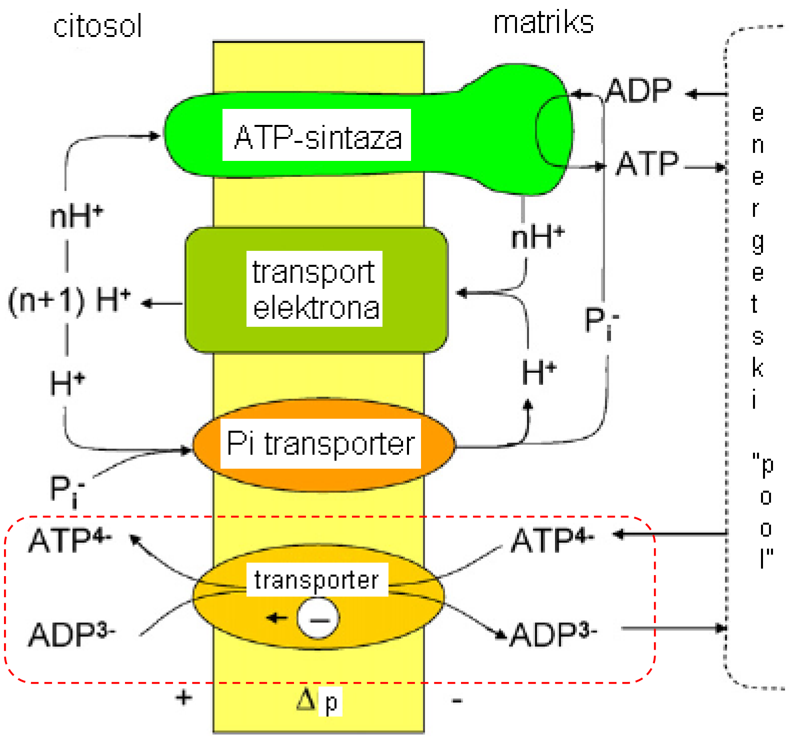
regulacija transporta H^+ za sintezu citosolnog ATP (3)

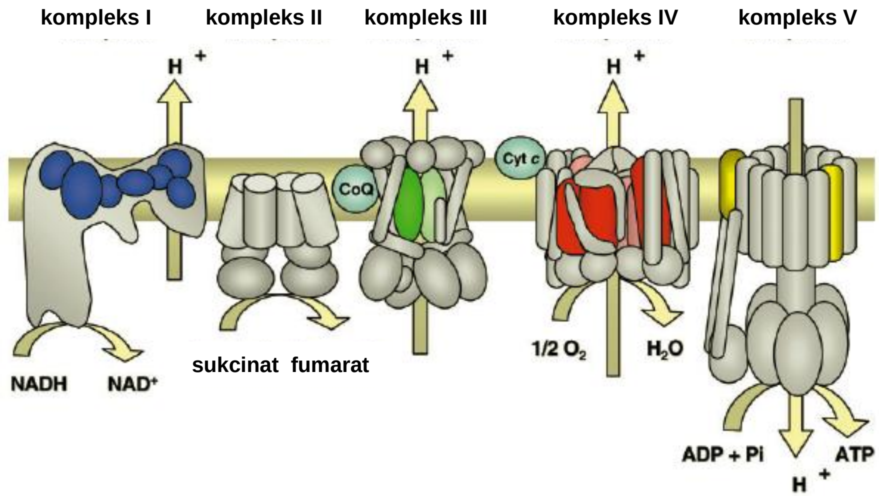


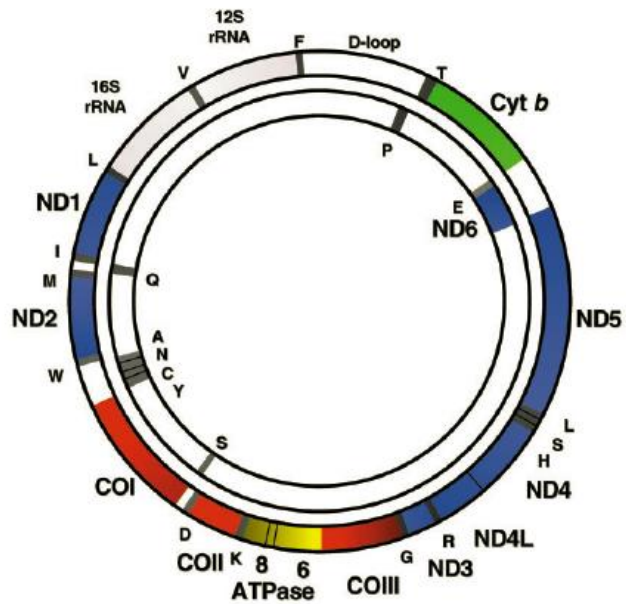


regulacija transporta H^+ za sintezu citosolnog ATP (4)



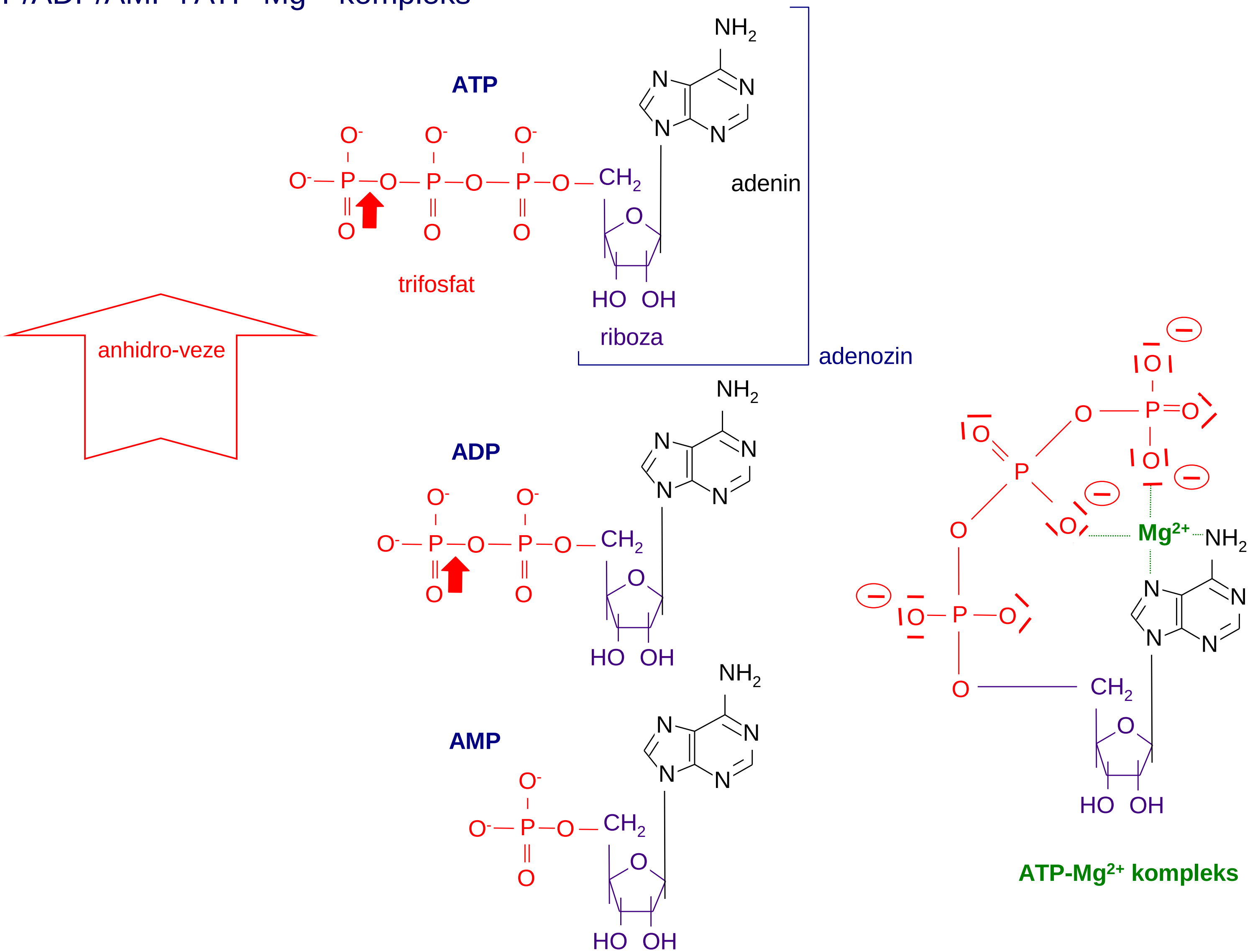



kompleks I
kompleks II
kompleks III
kompleks IV
kompleks V
H +
CoQ
Cyt c
NADH
NAD+
sukcinat fumarat
1/2 O2
H2O
ADP + Pi
ATP


12S rRNA
F
D-loop
T
Cyt b
V
16S rRNA
P
L
ND1
E
ND6
I
M
Q
ND2
ND5
A
N
C
Y
W
L
S
H
S
ND4
COI
D
R
ND4L
G
ND3
COII
K
8
6
ATPase
COIII

bioenergetika: neki važni energijom bogati spojevi

· ATP/ADP/AMP i ATP-Mg^{2+} kompleks



ATP-Mg^{2+} kompleks

ATP - energetska moneta stanice

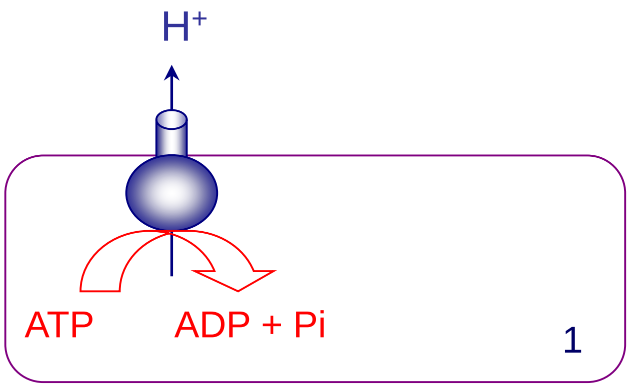
· promjena standardne slobodne energije hidrolize nekih energijom bogatih spojeva u fiziološkim uvjetima

reakcija		$G^{0'}$ (kJ mol^{-1})
PEP	piruvat + ortofosfat	-61.9
1,3-DPG	3-PG + ortofosfat	-54.5
acetil-P	acetat + ortofosfat	-42.3
ATP	AMP + difosfat	-37.4
acetil-CoA	acetat + koenzim A	-35.1
aminoacil-tRNA	aminokiselina + tRNA	-35.1
ATP	ADP + ortofosfat	-34.5
difosfat	2 ortofosfata	-33.4
glc-1-P	glukoza + ortofosfat	-20.9
alanin-glicin	alanin + glicin	-16.7
glc-P	glc + ortofosfat	-13.8

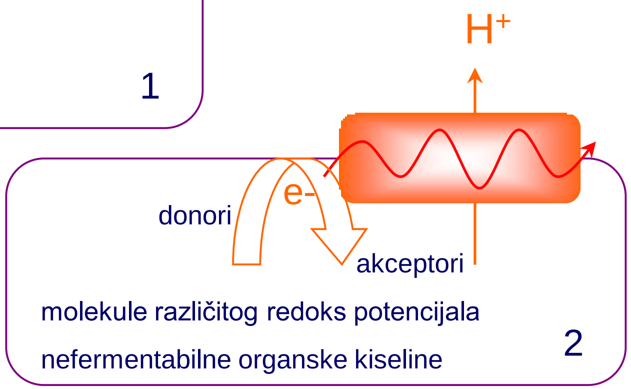
· ATP se po standardnoj slobodnoj energiji hidrolize nalazi između spojeva koji sudjeluju u energetskom metabolizmu prilikom izgradnje organske tvari i spojeva koji predstavljaju građevne blokove za izgradnju osnovnih sastojaka stanice (povezuje procese katabolizma i anabolizma)

animacija: **The Evolution of Cellular Organelles**
www.sumanasinc.com/webcontent/animations/biology.html

hipoteza: integracija organela za pridobivanje energije u eukariotsku stanicu ili evolucija oksidativne fosforilacije

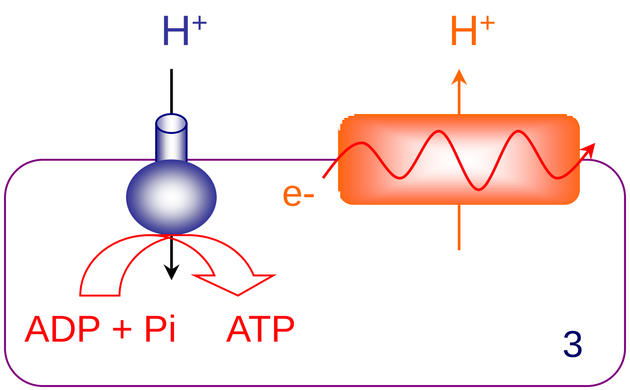


· fermentacija (proizvodnja kiselina), "unutarstanično zakiseljevanje"
"pumpanje" H^+ izvan stanice "na račun" energije hidrolize ATP



· "pumpanje" H^+ bez hidrolize ATP, ATP se čuva za druge procese u stanici, membranski proteini energiju dobivenu tijekom prijenosa e^- sa različitih molekula koriste za "pumpanje" H^+ izvan stanice

· elektrokemijski gradijent (*Δp*) nastao djelovanjem respiratornog (disajnog) lanca (RL) koristi se za "pumpanje" H^+ natrag u stanicu i na račun ovog transporta (pridobivene energije) fosforilira se ADP



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