Mikrobna biosinteza

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M9 minimalni medij: *E. coli*

sestava

- $MgSO_4$
- $CaCl_2$
- Na_2HPO_4
- KH_2PO_4
- NaCl
- NH_4Cl
- glukoza

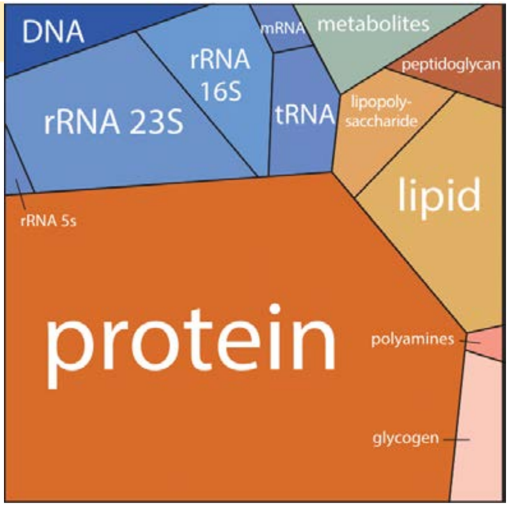
Elemental Breakdown	% dry mass of an *E. coli* cell
Major elements	
Carbon	50
Oxygen	20
Hydrogen	8
Nitrogen	14
Sulfur	1
Phosphorus	3
Minor elements	
Potassium	2
Calcium	0.05
Magnesium	0.05
Chlorine	0.05
Iron	0.2
Trace elements	
Manganese	All trace elements combined comprise 0.3% of dry weight of cell
Molybdenum	
Cobalt	
Copper	
Zinc	

Kemijska sestava *E. coli*

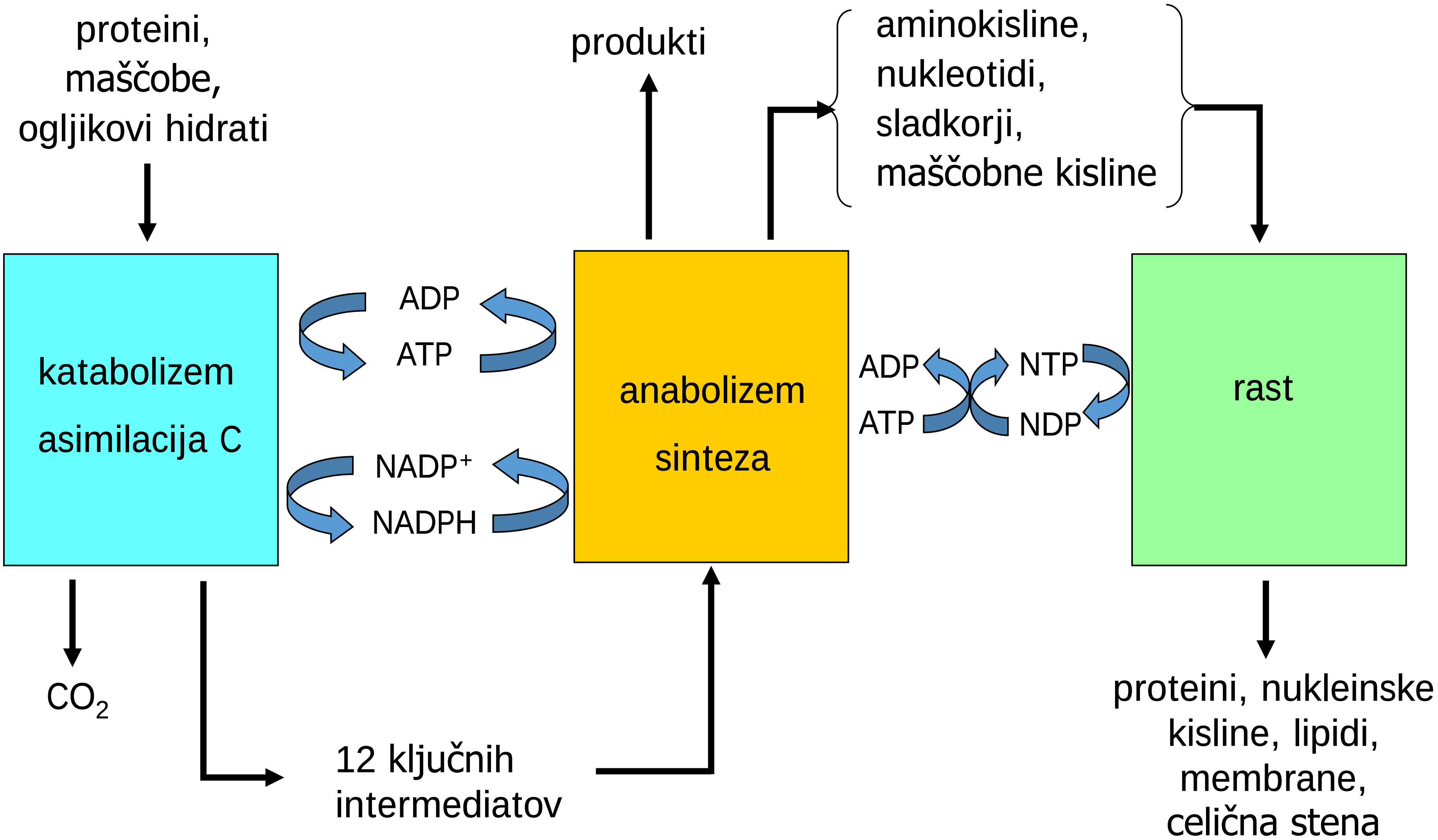
macromolecule	percentage of total dry weight	weight per cell (fg)	characteristic molecular weight (Da)	number of molecules per cell
protein	55	165	3×10^4	3,000,000
RNA	20	60		
23 S rRNA		32	1×10^6	20,000
16 S rRNA		16	5×10^5	20,000
5 S rRNA		1	4×10^4	20,000
transfer		9	2×10^4	200,000
messenger		2	1×10^6	1,400
DNA	3	9	3×10^9	2
lipid	9	27	800	20,000,000
lipopolysaccharide	3	9	8000	1,000,000
peptidoglycan	3	9	$(1000)_n$	1
glycogen	3	9	1×10^6	4,000
metabolites and cofactors pool	3	9		
inorganic ions	1	3		
total dry weight	100	300		
water (70% of cell)		700		
total cell weight		1000		

composition rules of thumb

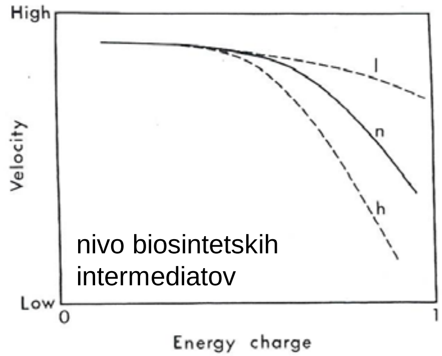
- carbon atoms ~10^{10}
- 1 molecule per cell gives ~1 nM conc.
- ATP required to build and maintain cell over a cell cycle ~10^{10}
- glucose molecules needed per cell cycle ~3×10^9 (2/3 of carbons used for biomass and 1/3 used for ATP)



Katabolizem, anabolizem in rast na ogljiku

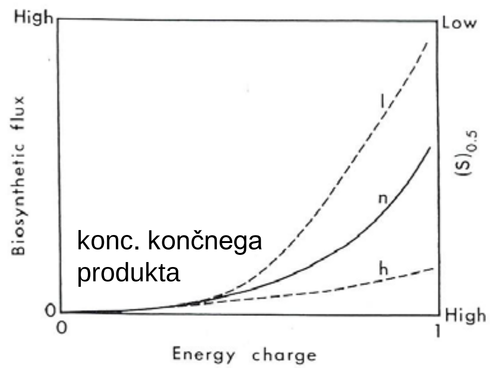


Osnovna regulacija katabolnih in anabolnih reakcij z energijskim nabojem celice

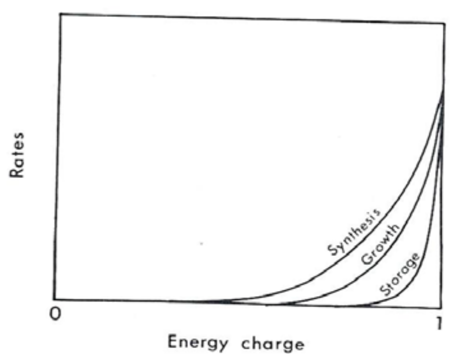


regulacija katabolnih poti, ki služijo energijskim in biosintetskim potrebam, z energijskim nabojem celice in koncentracijo intermediatov

$$\text{energijski naboj} = \frac{ATP + \frac{1}{2} ADP}{ATP + ADP + AMP}$$

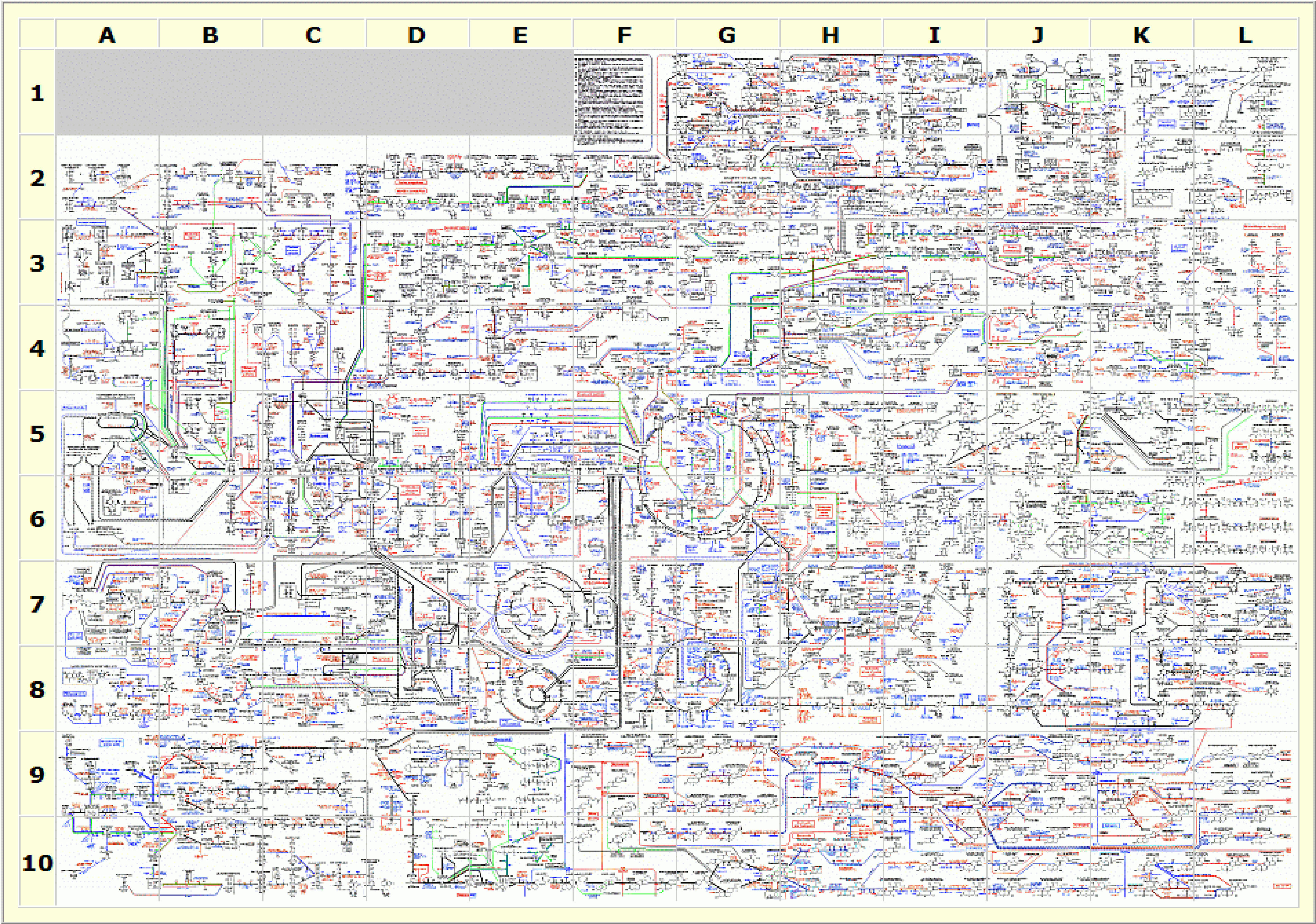


regulacija anabolnih poti preko zaznave koncentracije končnega produkta in energijskega naboja celice omogoča variiranje afinitete za substrat ($S_{0.5}$)



hierarhija regulacije na nivoju sinteze, rasti in sinteze rezervnih snovi v odvisnosti od energijskega naboja celice, preko master encima nukleozid difosfat kinaza

($ATP + NADP \rightleftarrows ADP + NTP$)


A
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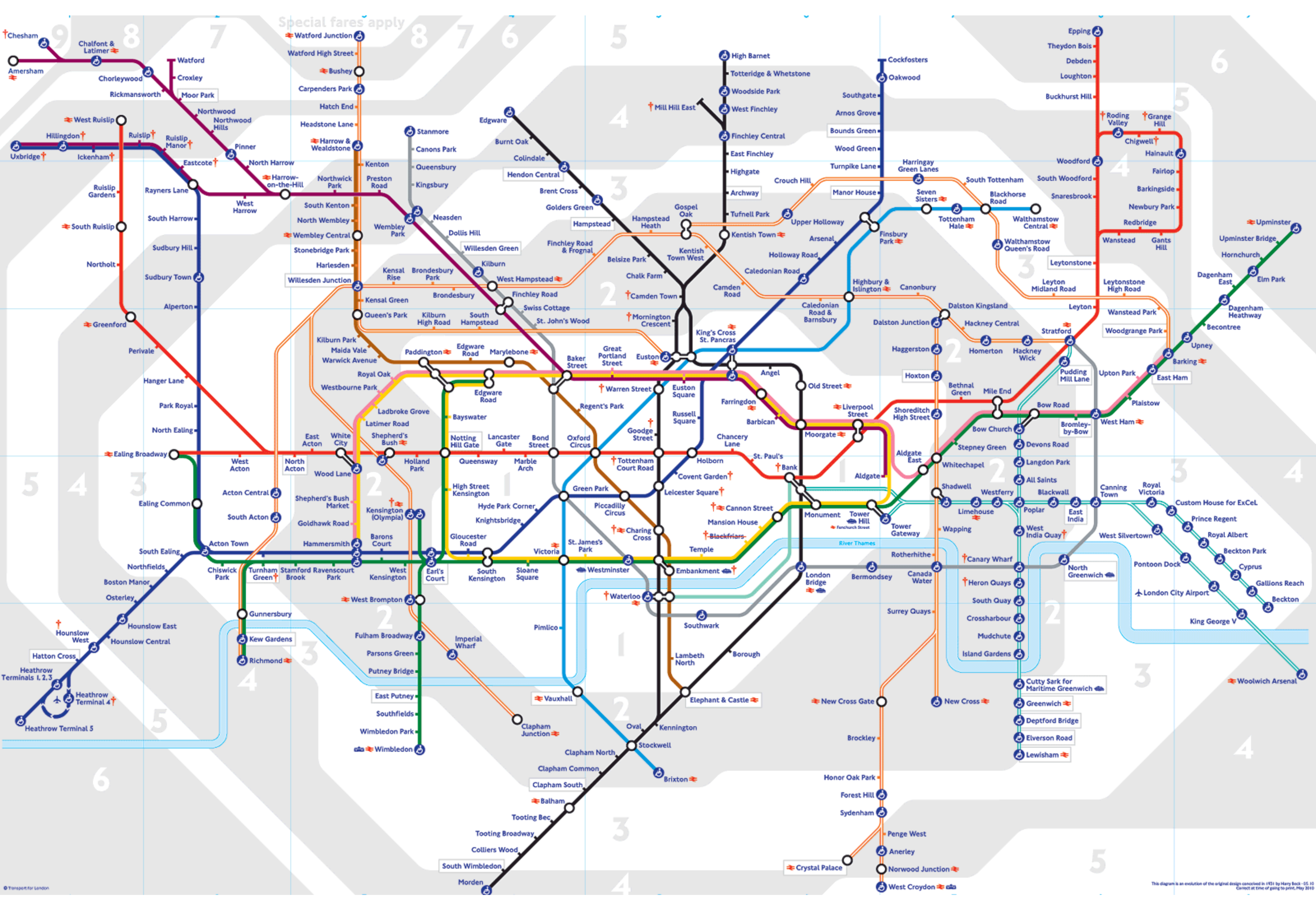
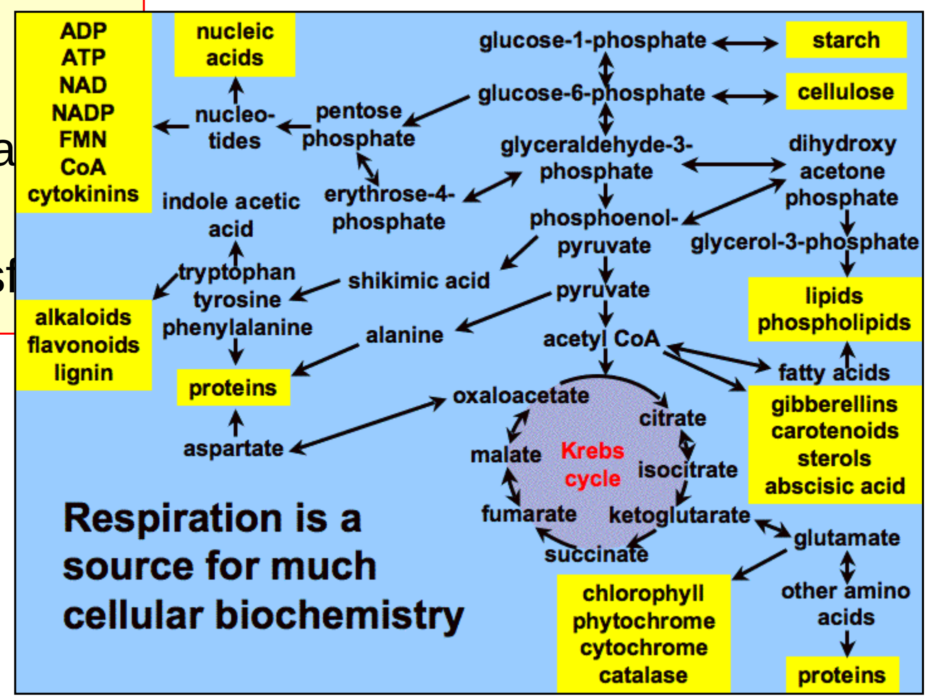


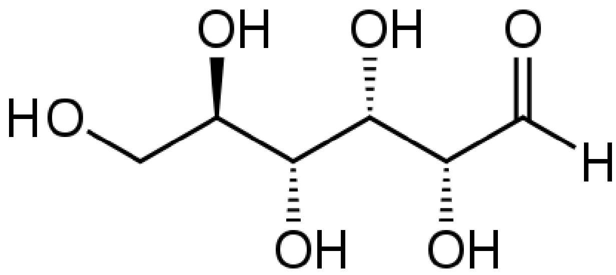
za potovanje od točke A do B so pomembna ključna križišča (ves čas se je sicer potrebno zavedati lokalnih podrobnosti in delujočih mehanizmov)

Ključni intermediati

glukoza-6-fosfat	acetil-CoA
fruktoza-6-fosfat	α-ketoglutarat
dihidroksiaceton fosfat	sukcinil-CoA
3-fosfoglicerat	oksalacetat
fosfoenolpiruvat	riboza-5-fosfa
piruvat	eritroza-4-fosf



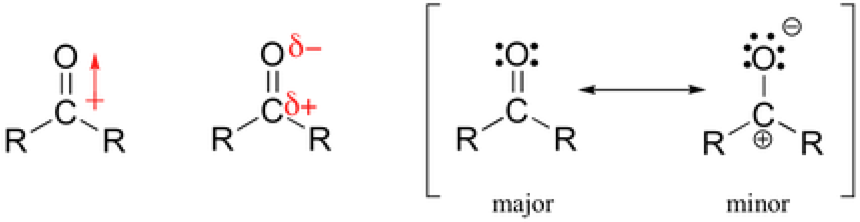
Glukoza



funkcionalni skupini:

- CHO

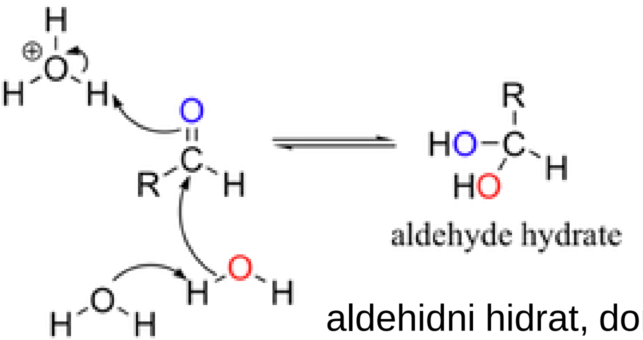
- OH



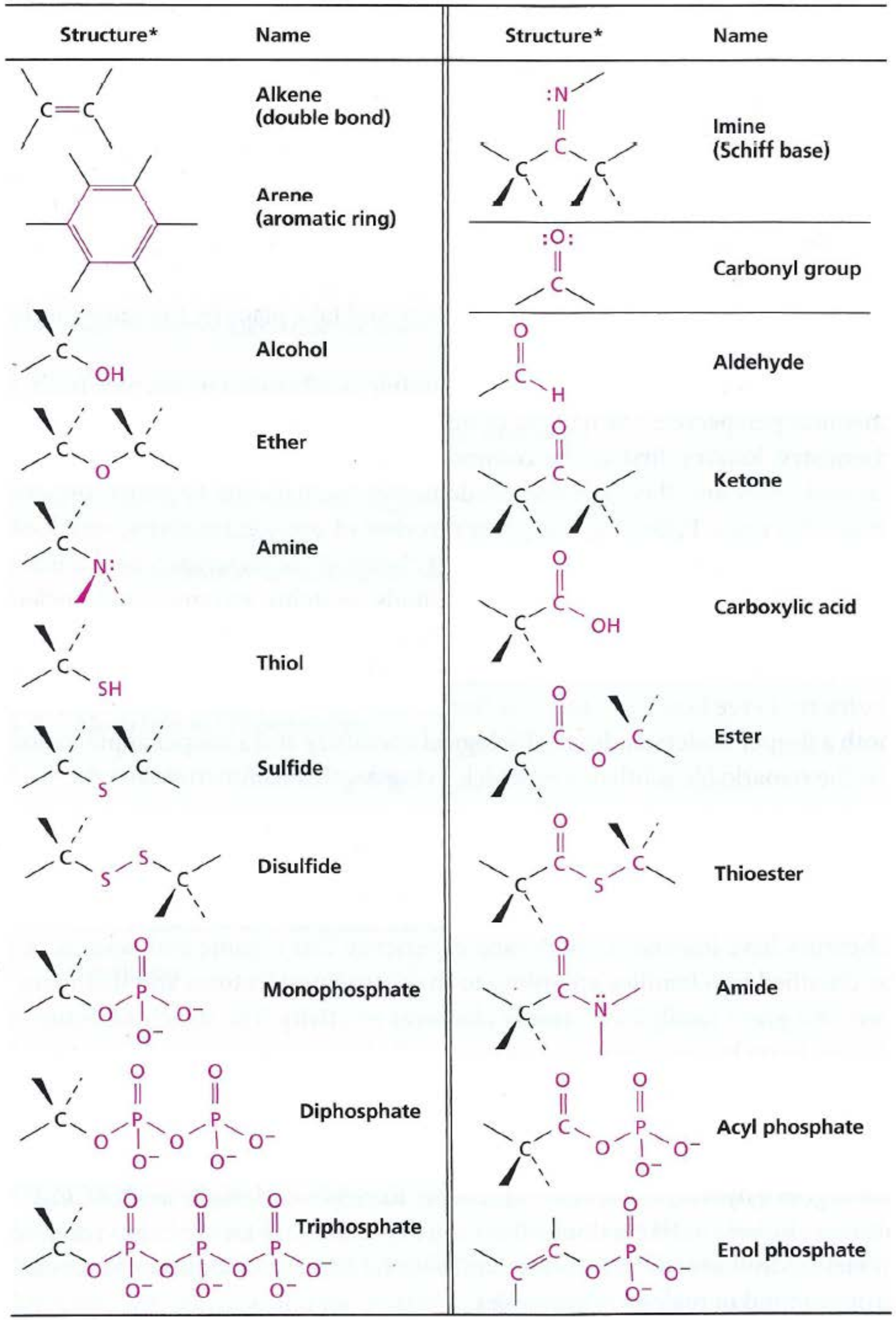
različni načini prikaza polarizirane karbonilne skupine

reakcije aldehidov

- nukleofilne adicije
- oksidacije, redukcije
- tvorba hemiacetalov
- izomerizacije
- aldolne kondenzacije



aldehidni hidrat, dominantna oblika aldehida v vodi, kot posledica prerazporejanja elektronske gostote



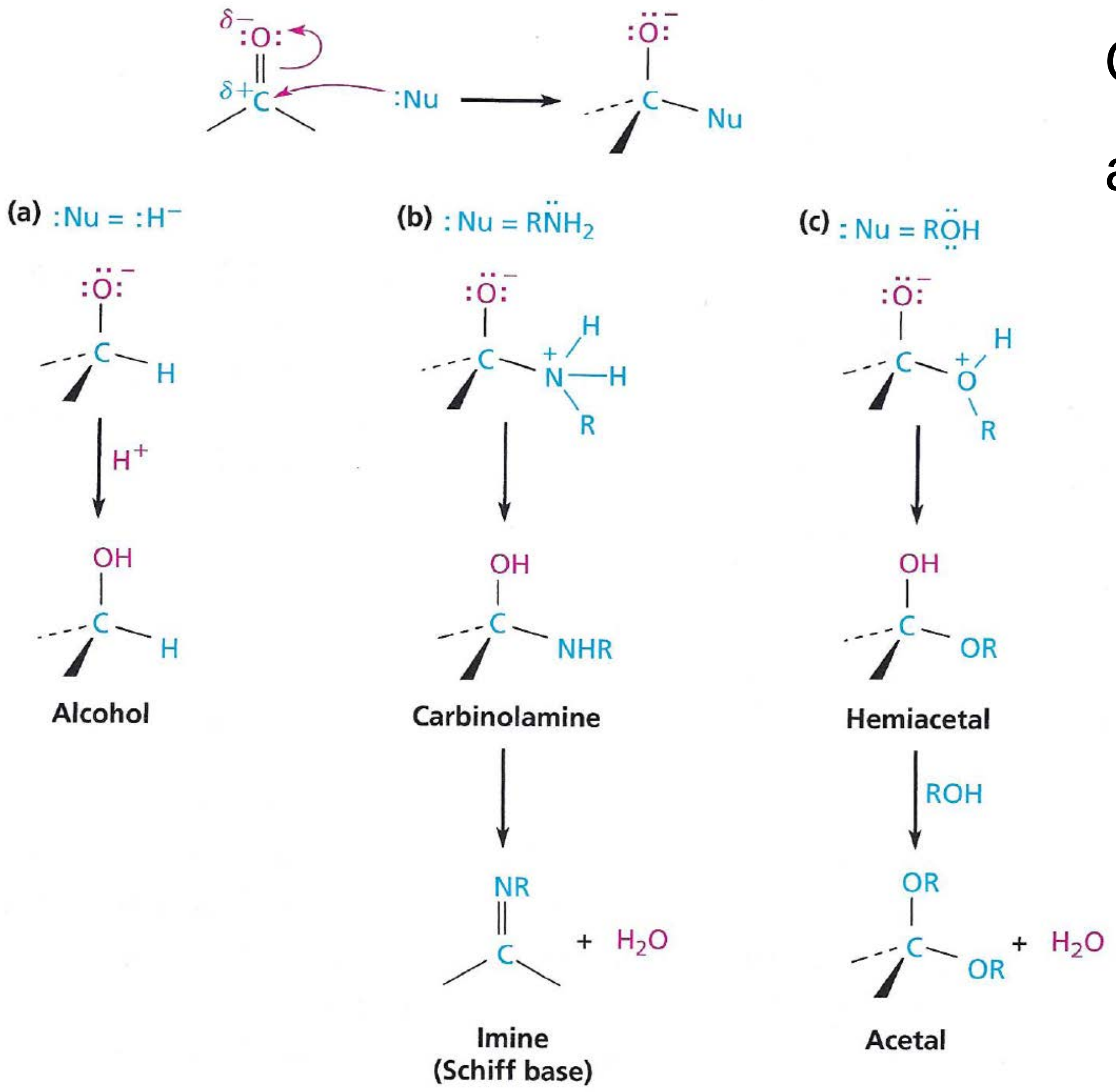
Običajne funkcionalne skupine bioloških molekul

Običajni mehanizmi reakcij bioloških molekul

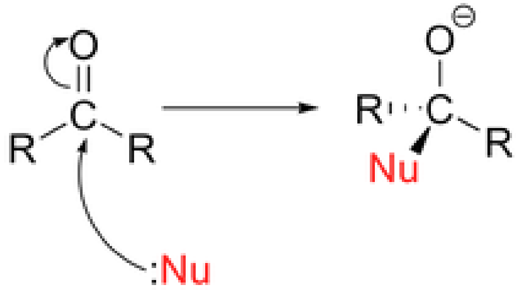
- nukleofilna adicija na karbonil (nastanek alkohola, Šifove baze, acetalov)
- nukleofilna alifatska substitucija (S_N1 (geraniol biosinteza) in S_N2 (SAM metilacije))
- elektrofilna adicija na alkene (sinteza sterolov in terpenov)
- elektrofilna aromatska substitucija (pogosta pri morskih org., sinteza tiroksina)
- konjugacijska (1,4) nukleofilna adicija karbonilov(npr. konverzija fumarata v malat)
- nukleofilna acilna substitucija (npr. peptidaze)
- kondenzacija karbonilov: - kondenzacija aldehidov ketonov - aldolne reakcije (sinteza fruktoze1,6P),

  - kondenzacija estrov – Claisnova kondenzacija (m. kisline, terpeni, stroli))
- eliminacije (E1, E2, E1cB, dehidratacije, sinteza maščobnih kilin)
- radikalske reakcije (abstrakcija vodika, biosinteza prostaglandinov)
- periciklične reakcije (Claisnova reorganizacija, konverzija korizmata v prepenat)
- oksidacije in redukcije (uporaba rekukcijskih ekvivalentov, NADH, $FADH_2$)

Običajne nukleofilne adicije na karbonile

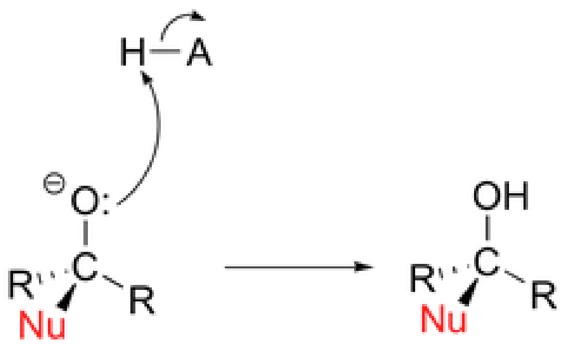


Nukleofilna adicija na karbonil

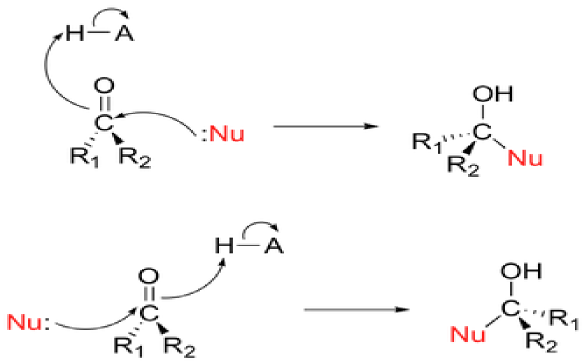


nukleofili, ki napadajo karbonil

- alkoholni kisik
- amino dušik
- tiolno žveplo
- rezonančno stabiliziran karboanion



po adiciji nukleofila na karbonil alkoksid lahko deluje kot nukleofil, bolj pogosto pa kot baza, ki abstrahira protone iz bližnjih kislin v topilu ali aktivnem mestu encima



zaradi spremembe hibridizacije iz sp^2 v sp^3 po nukleofilni adiciji pride do nastanka steroizomera (ni vseeno s katere strani pride nukleofil)

Nukleofil

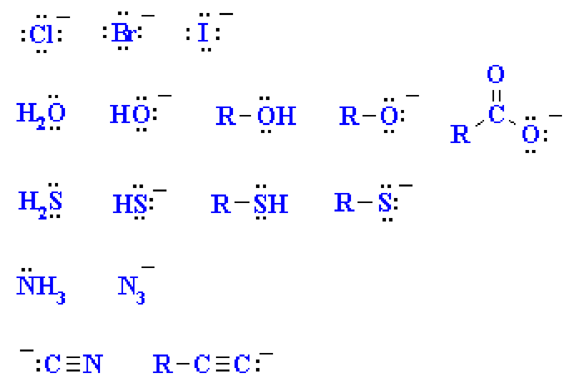
atom ali funkcionalna skupina bogata z elektroni (Lewisova baza), daje elektronski par

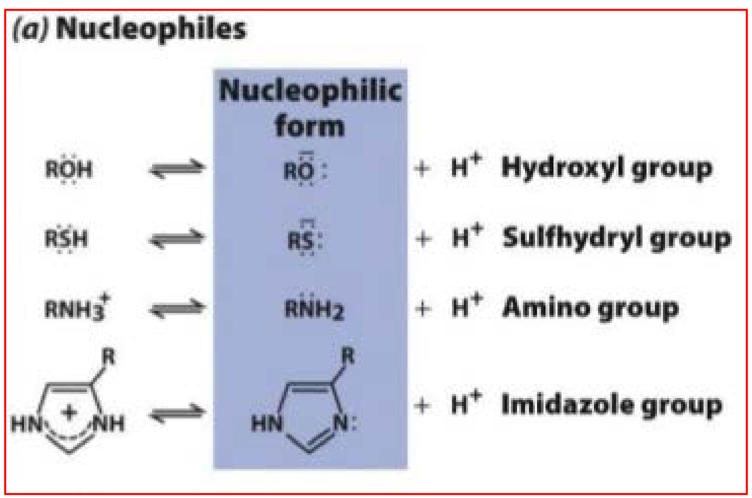
nukleofilni atomi: kisik, dušik, žveplo, halogeni elementi

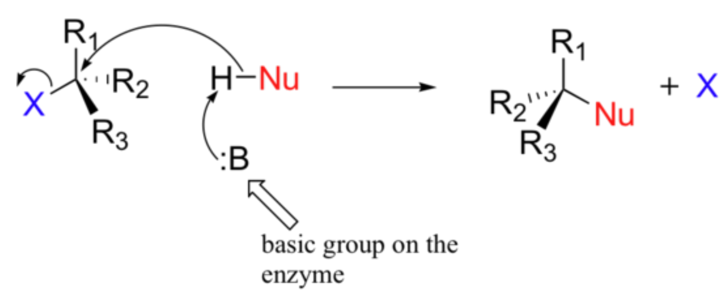
nukleofilne funkcionalne skupine:

voda, alkoholi, fenoli, amini, tioli,

enolati, občasno karboksilati





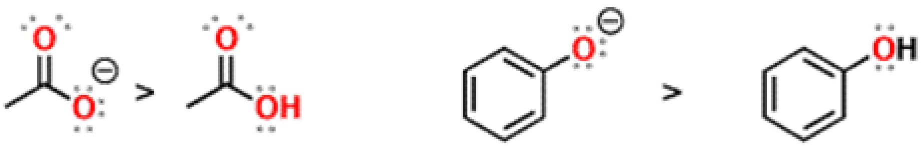


ker deprotonacija nukleofila poveča njegovo moč, je v aktivnem mestu encima zelo pogosto baza

Moč nukleofilov

1. Charge *The conjugate base is always a stronger nucleophile*

$HO^{-} > H_2O \qquad H_2N^{-} > NH_3 \qquad HS^{-} > H_2S$

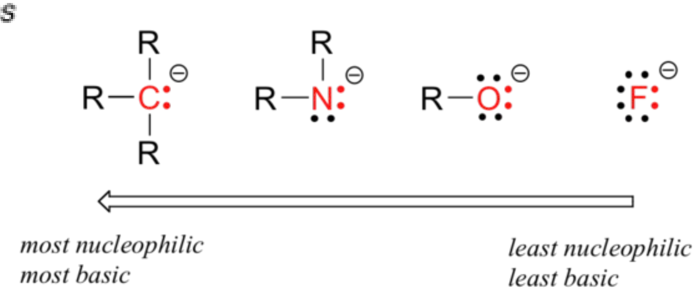


Reason: **Nucleophilicity increases with increasing electron density on an atom**

2. Electronegativity *Across the periodic table, nucleophilicity increases with decreasing electronegativity*

$H_3C^{-} > H_2N^{-} > HO^{-} > F^{-}$

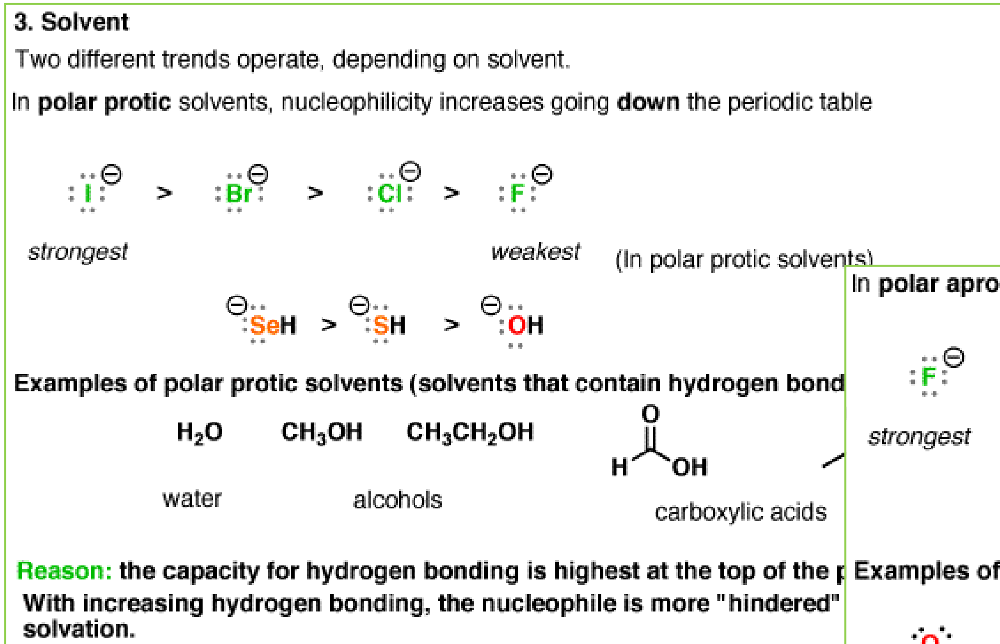
$H_3N > H_2O > HF$

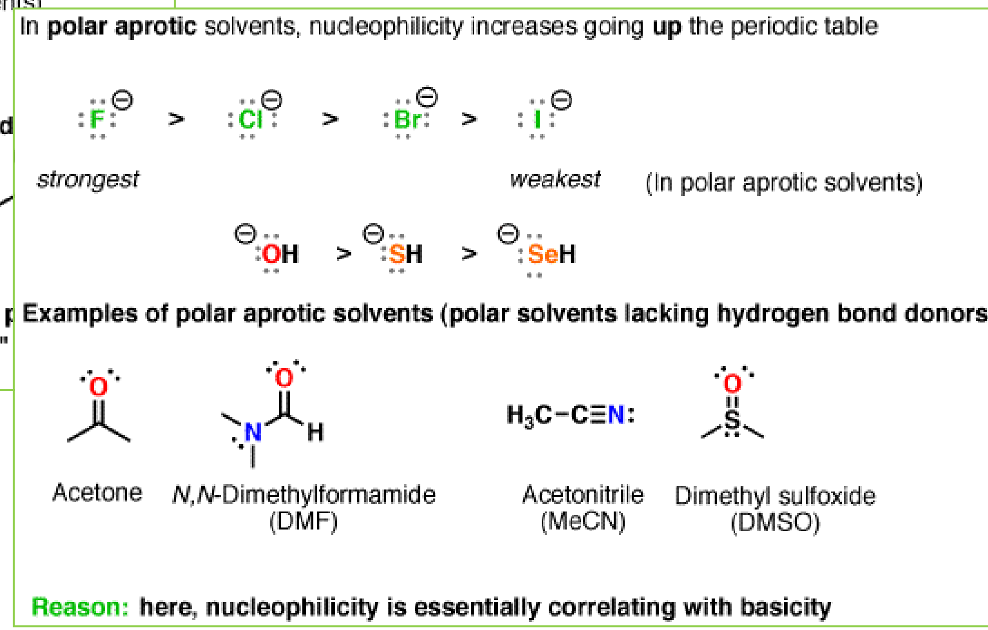


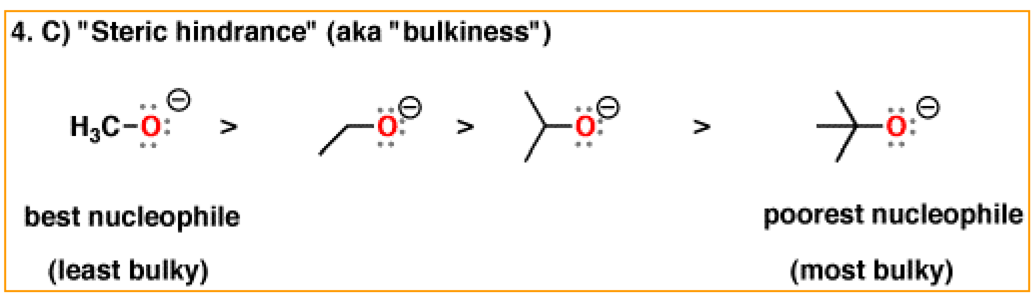
Reason: Nucleophilicity is the donation of an electron pair. The less electronegative the atom, the less "tightly held" those electrons will be.

These two factors correlate strongly with basicity.

Moč nukleofilov







Odhajajoča skupina

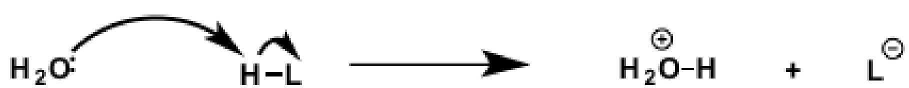
Good Leaving Groups:...

The more stable L is, the
more reactive R-L will be

L = leaving group

Nu = nucleophile

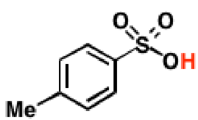
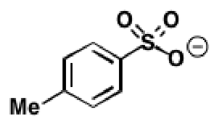
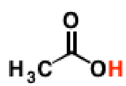
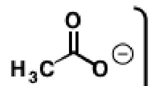
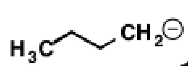
...are weak bases



**The more stable L is, the
more acidic H-L will be**

Odhajajoča skupina

A pKa table is a handy guide to leaving groups

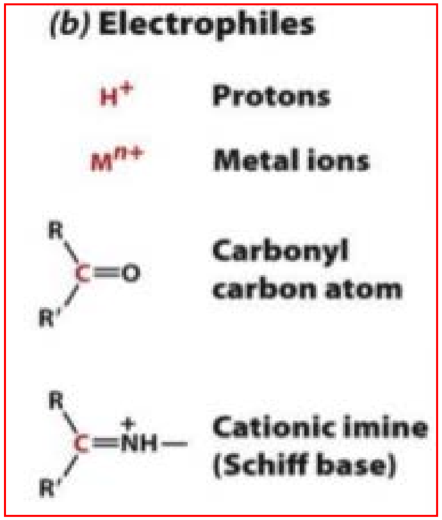
Functional group / Example		pKa	Conjugate base	
Hydroiodic acid	HI	–10	$I^{\ominus}$	*Excellent leaving groups (extremely weak bases)*
Hydrobromic acid	HBr	–9	$Br^{\ominus}$	
Hydrochloric acid	HCl	–6	$Cl^{\ominus}$	
Sulfuric acid	H_2SO_4	–3	$HSO_4^{\ominus}$	
Sulfonic acids	 (tosic acid)	–3		
Hydronium ion	$H_3O^{\oplus}$	–1.7	H_2O	
Hydrofluoric acid	H–F	3.2	$F^{\ominus}$	**Exception:** $F^{\ominus}$ is typically an extremely poor leaving group (forms strong bonds)
Carboxylic acids		4		*Moderate leaving groups (weak bases)*
Protonated amines	$NH_4^{\oplus}\ Cl^{\ominus}$	9-11	NH_3	
Water	HO–H	16	$HO^{\ominus}$	*Poor leaving groups (strong bases)*
Alcohols	CH_3O–H	16-18	$CH_3O^{\ominus}$	
Amine	NH_3	~35	$NH_2^{\ominus}$	*Extremely poor leaving groups (very strong bases)*
Hydrogen	H–H	42	$H^{\ominus}$	
Alkane		~50		

Elektrofil

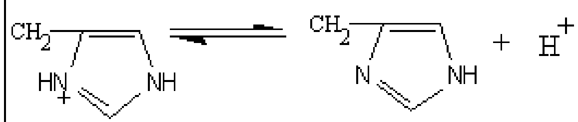
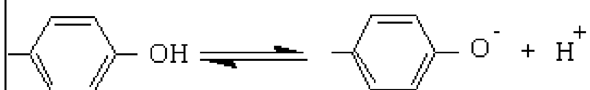
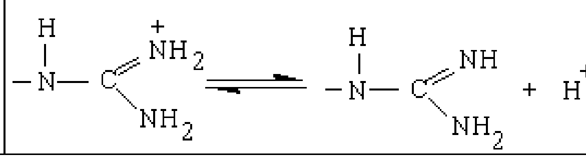
spojina ki ljubi elektrone, običajno pozitivno nabita ali nevtralna s prosto orbitalo, ki jo privlači z elektroni bogato mesto

sprejema elektronski par, Lewisova kislina

elektrofili: proton, kovinski ioni, ogljik v povezavi s kisikom, dušikom, žveplom ali halogeni



Aminokisline, ki pogosto sodelujejo v aktivnem mestu encimov kot Lewisove baze ali kisline (nukleofili in elektrofili)

Group	Acid ⇌ Base + H^+	pKa value
Aspartic/glutamic acid	$COOH \rightleftharpoons COO^- + H^+$	4.4
Histidine		6.0
Cysteine	$-SH \rightleftharpoons S^- + H^+$	8.5
Tyrosine		10.0
Lysine	$-NH_3^+ \rightleftharpoons NH_2 + H^+$	10.0
Arginine		12.0

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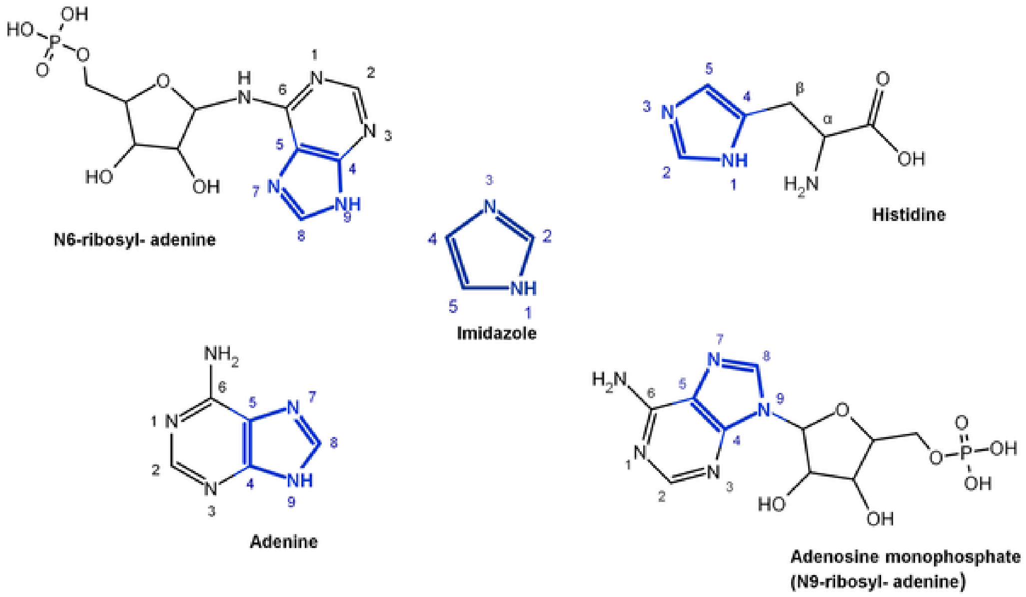
Imidazol – pomemben nukleofil in funkcionalna skupina



histidin:

- ionizirajoča skupina, pKa 6.0 – 6.5
- koordinacijski ligand za Ca^{2+} in Zn^{2+}
- donor in akceptor za vodikovo vez

Histidin lahko reagira s tvorbo:

(1) kation-π interakcij

(2) π-π prekrivanjem

(3) vodik-π interakcij

(4) koordinacijskih vezi

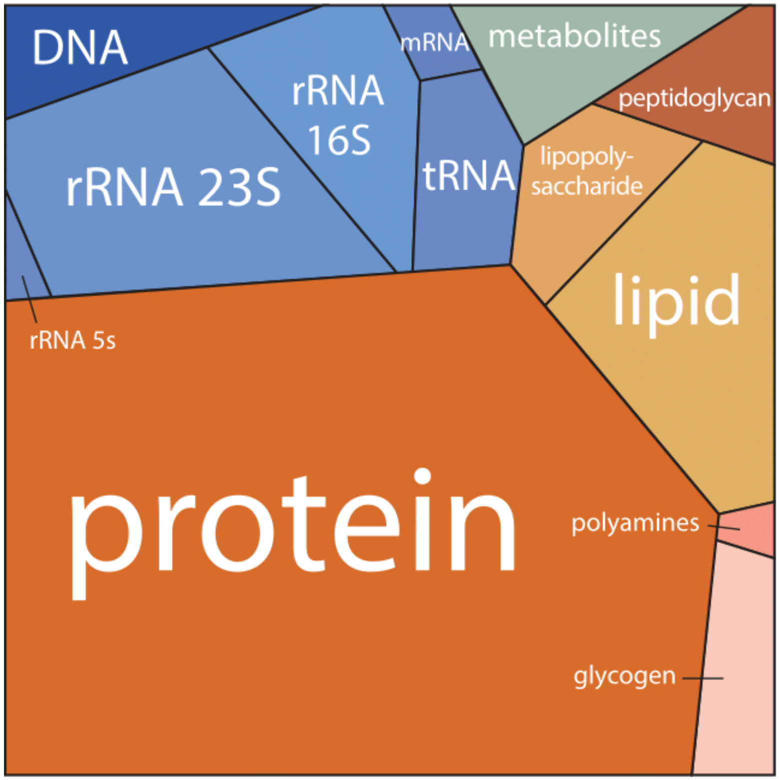
(5) vodikovih vezi

prisoten v ključnih bioloških molekulah: purini, histidin

v nesubstituiranem imidazolu je pirolov dušik na poziciji 1 šibka kislina pKa = 14.4. dušik na poziciji 3 ima pKa 6.9 (lahko sprejme proton)

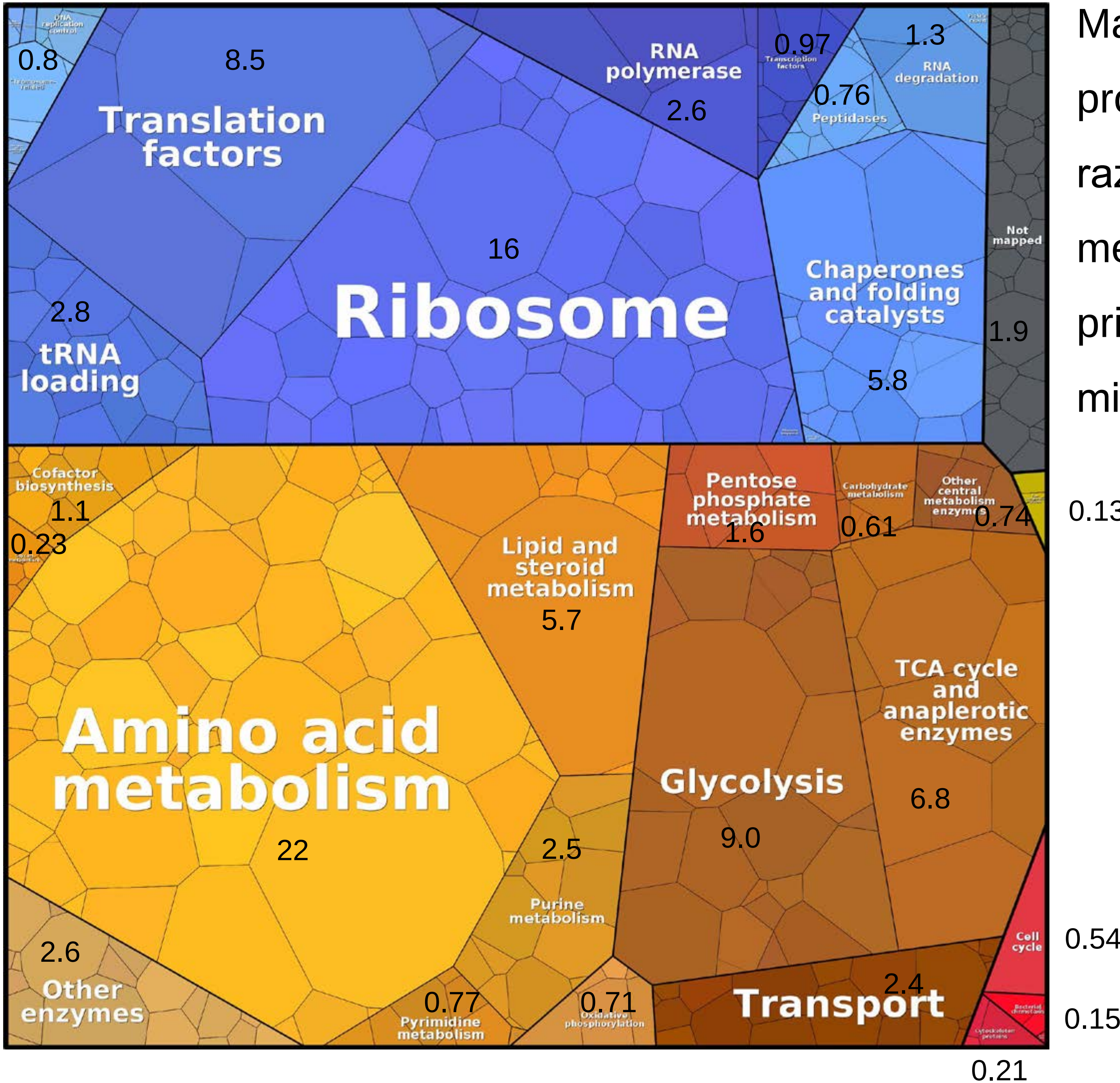
https://doi.org/10.1371/journal.pone.0196349

Proteinska mašinerija celice

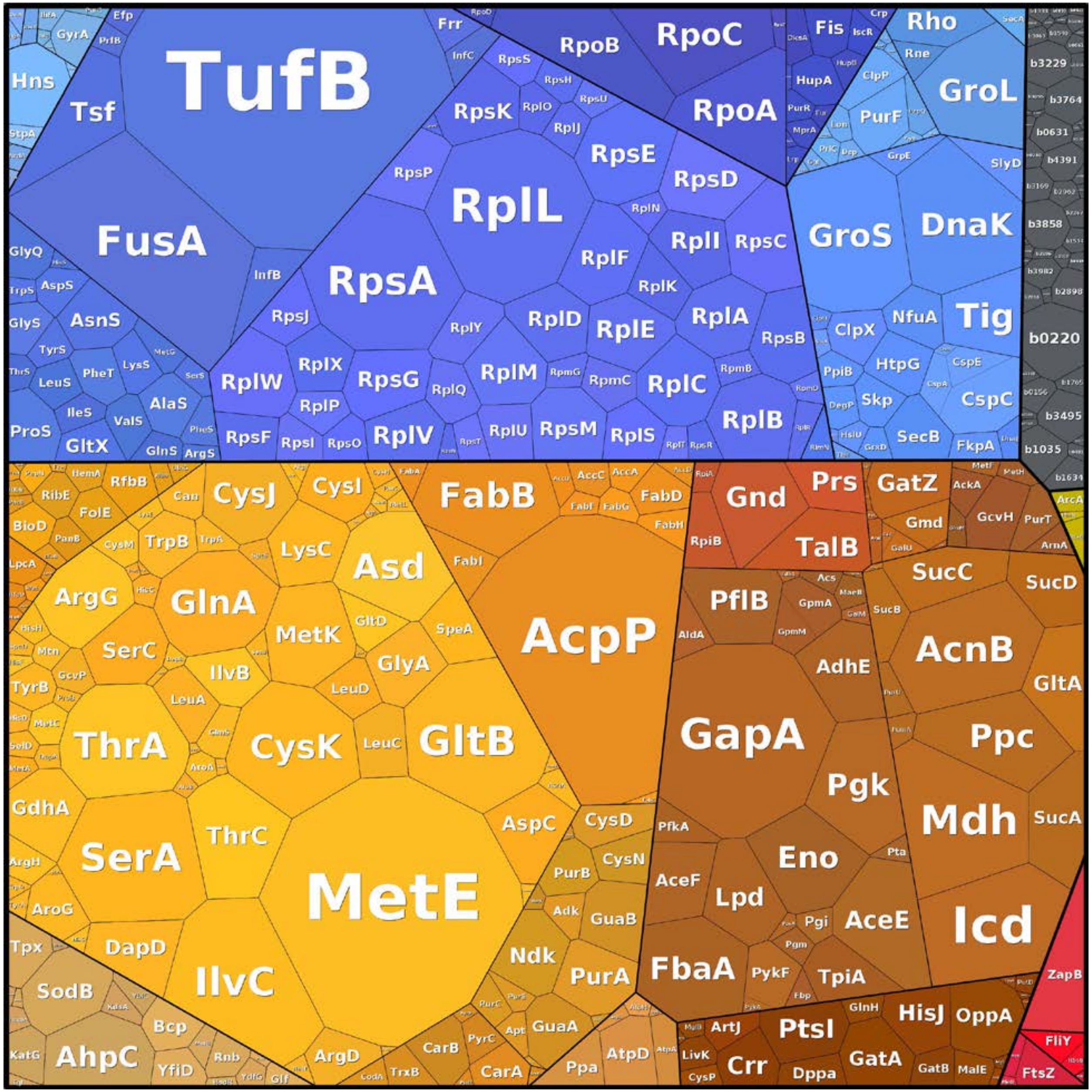


proteini predstavljajo ~ 50

% suhe mase bakterije



Masni delež proteinov (%) v različnih metabolnih poteh pri rasti *E. coli* na minimalnem gojišču



Najbolj pogosti proteini pri rasti *E. coli* na minimalnem gojišču

TufB = elongation factor Tu
MetE = methionine synthase
AcpP = acyl carrier protein
FusA = elongation factor G
RpsA = 30S ribosomal protein S1
RplL = 50S ribosomal protein L7/L12
RpoC = RNAP β‘ subunit
RpoA = RNAP α‘ subunit
GroL = 60 kDa chaperonin
GroS = 10 kDa chaperonin
DnaK = chaperon DnaK
Tig = trigger factor
GlnA = glutamin synthetase
Asd = aspartat semyaldehyde dehidrogenase
ThrA = aspartokinase/homoserine dehydro.
CysK = Cysteine synthase A
GltB = glutamt synthase
SerA = D-3-phosphoglycerate dehydrogenase
IlvC = Ketol-acid reductoisomerase (NADP(+))
Gnd = 6-phosphogluconate dehydrogenase
GapA = Glyceraldehyde-3P dehydrogenase A
AcnB = aconitate hydratase
Mdh = malate dehydogenase
Icd = isocitrate dehydrogenase
FabA = Enoyl-[ACP] reductase [NADH]
Fab B = 3-oxoacyl-[ACP] synthase 1
AcpP = acyl carrier protein
PtsI = PEP phosphotransferase

Dva cilja metabolizma

- vzdrževanje nizkih koncentracij intermediatov

  (preprečevanje ozmotskega stresa in nekontroliranih reakcij)

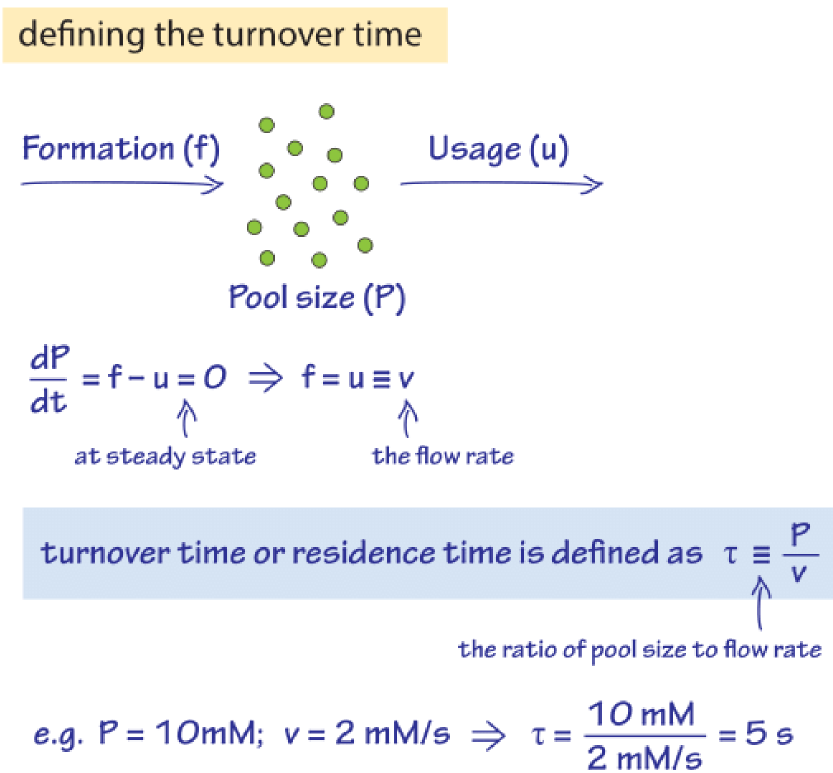
- visoki fluksi

oba cilja lahko zagotovimo s proteini

(encimi)

metabolite	mM	metabolite	mM
glutamate	96	S-adenosyl-L-methionine	0.18
glutathione	17	phosphoenolpyruvate	0.18
fructose-1,6-bisphosphate	15	threonine	0.18
ATP	9.6	FAD	0.17
UDP-N-acetyl-glucosamine	9.2	methionine	0.14
hexose-P	8.8	2,3-dihydroxybenzoicacid	0.14
UTP	8.3	NADPH	0.12
GTP	4.9	fumarate	0.11
dTTP	4.6	phenylpyruvate	0.090
aspartate	4.2	NADH	0.083
valine	4.0	N-acetyl-glucosamine-1P	0.082
glutamine	3.8	serine	0.068
6-phospho-D-gluconate	3.8	histidine	0.068
CTP	2.7	flavinmononucleotide	0.054
NAD	2.6	4-hydroxybenzoate	0.052
alanine	2.5	dGMP	0.051
UDP-glucose	2.5	glycerolphosphate	0.049
glutathionedisulfide	2.4	N-acetyl-ornithine	0.043
uridine	2.1	gluconate	0.042
citrate	2.0	malonyl-CoA	0.035
UDP	1.8	cyclic-AMP	0.035
malate	1.7	dCTP	0.034
3-phosphoglycerate	1.5	tyrosine	0.029
glycerate	1.4	inosine-diphosphate	0.024
coenzyme-A	1.4	GMP	0.024
citrulline	1.4	acetoacetyl-CoA	0.022
pentose-P	1.3	riboflavin	0.019
glucosamine-6_phosphate	1.2	phenylalanine	0.018
acetylphosphate	1.1	aconitate	0.016
gluconolactone	1.0	dATP	0.016
GDP	0.68	cytosine	0.014
acetyl-CoA	0.61	shikimate	0.014
carbamyl-aspartate	0.59	histidinol	0.013
succinate	0.57	tryptophan	0.012
arginine	0.57	dihydroorotate	0.012
UDP-glucaronate	0.57	quinolinate	0.012
ADP	0.55	ornithine	0.010
asparagine	0.51	dAMP	0.0088
2-ketoglutarate	0.44	adenosine-phosphosulfate	0.0066
lysine	0.40	myo-inositol	0.0057
proline	0.38	propionyl-CoA	0.0053
dTDP	0.38	ADP-glucose	0.0043
dihydroxyacetone-phosphate	0.37	anthranilate	0.0035
homocysteine	0.37	deoxyadenosine	0.0028
CMP	0.36	cytidine	0.0026
isoleucine+leucine	0.30	NADP+	0.0021
deoxyribose-5-P	0.30	guanosine	0.0016
AMP	0.28	adenine	0.0015
inosine-monophosphate	0.27	deoxyguanosine	0.00052
PRPP	0.26	adenosine	0.00013
succinyl-CoA	0.23		
inosine-triphosphate	0.20	Sum	231
guanine	0.19		

Veliki fluksi - koncept obratnega časa



metabolit	*E.coli* obratni čas(s)
AMP	9
ADP	0.8
ATP	2
3PGA	3
FBP	1.2
G6P	4
piruvat	1.5
glicerol-3P	13
TCA cikel (suc, fum, mal)	0.7 - 9

Veliki fluksi - encimi kot biokatalizatorji

povečanje hitrosti encimskih reakcij posledica:

- znižanja aktivacijske energije (stabilizacija prehodnega stanja, stereoelektronsko orientiranje in pozicioniranje, zagotovitev, kislin, baz, nukleofilov, kontra ionov)
- spremembe reakcijskega mehanizma (uporaba kofaktorjev)
- katalize preko visokoenergijskih intermediatov (sinteza lanosterola)

Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un}, s^{-1})	Catalyzed rate (k_{cat}, s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

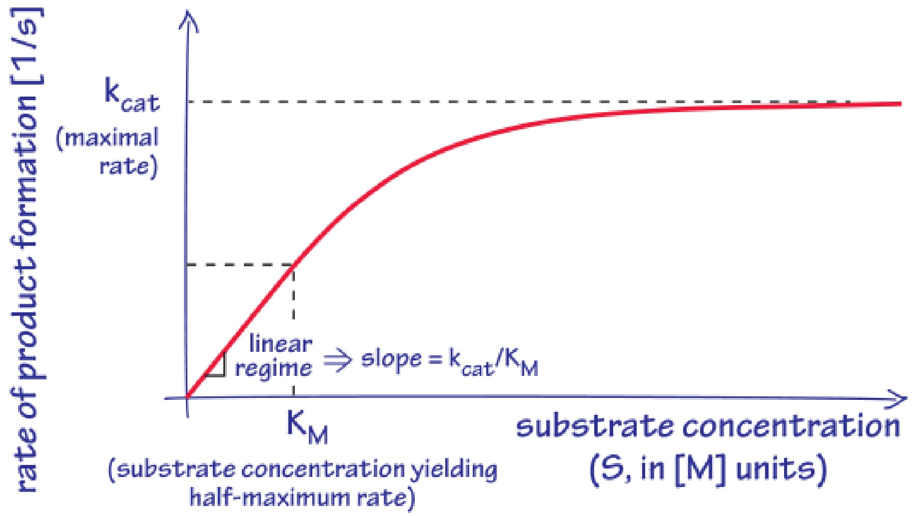
Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.

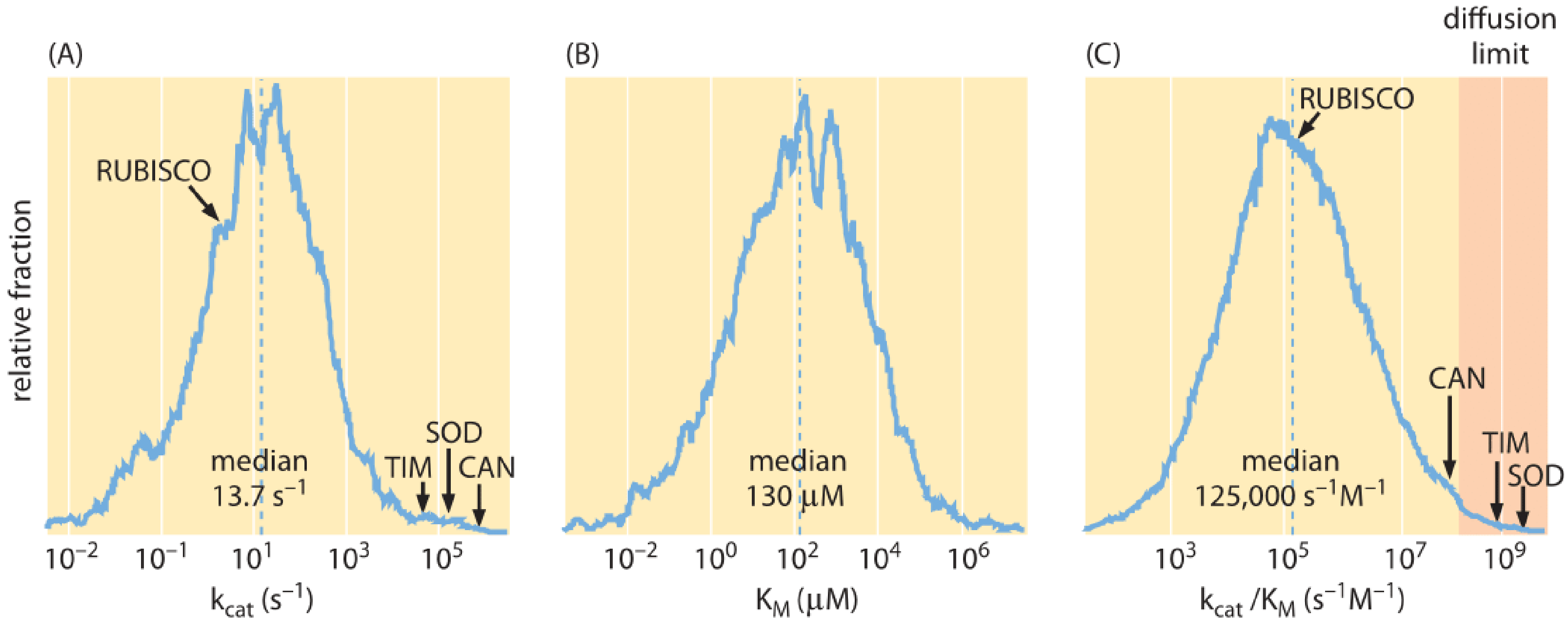
Source: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

Opis encimov

Michaelis-Menten

kinetika





število reakcij na sekundo

Michaelis-Menten konstanta

naklon, proporcionalna konstanta za trke med encimom in substratom

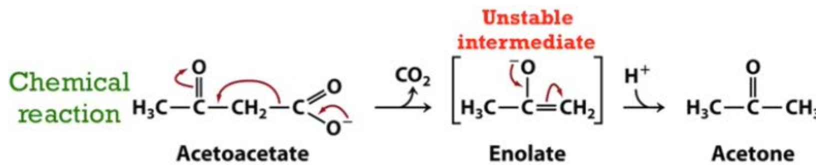
Tipi encimske katalize glede na mehanizem

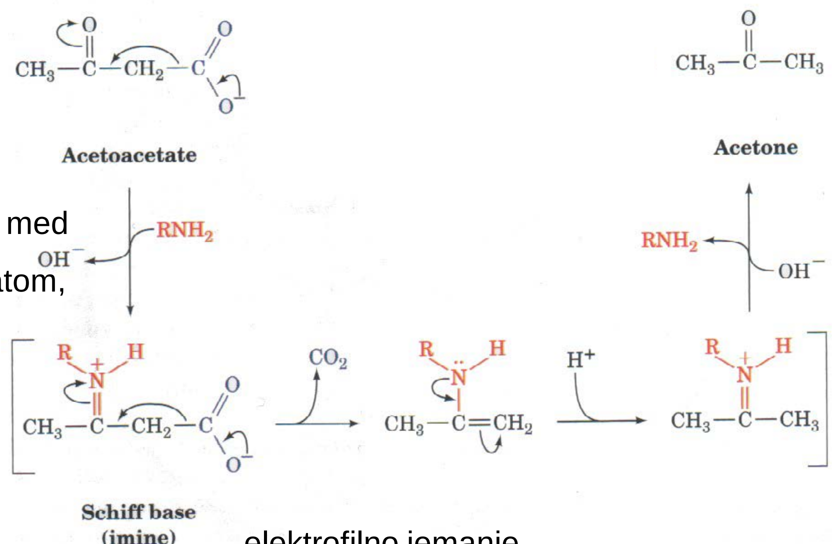
glede na to kako stabilizirajo prehodno stanje ločimo

- kovalentno katalizo
- kislinsko-bazno katalizo
- elektrostatsko katalizo
- orientacijsko katalizo

Kovalentna kataliza

začasno kovalentno vezan intermediat na encim stabilizira nestabilni intermediat, pri tem encim služi kot ponor za elektrone ali pa kot vir elektronov za reakcijo





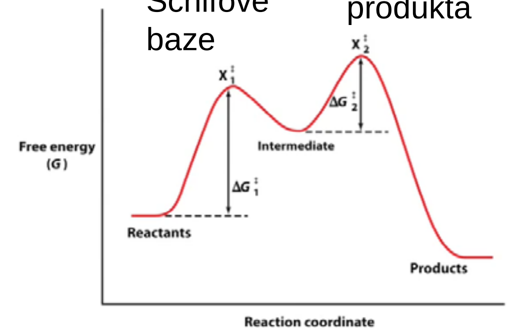
nukleofilna reakcija med encimom in substratom, vezava na substrat

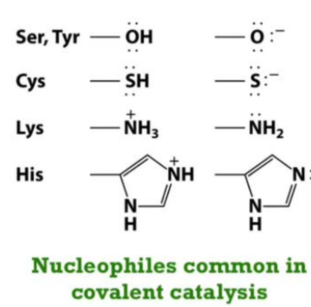
elektrofilno jemanje elektronov iz substrata

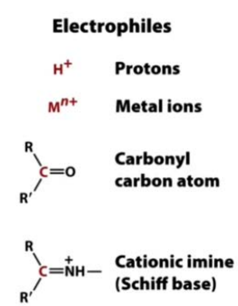
eliminacijska reakcija

formiranje Schifove baze

prekinitev vezi, nastanek produkta



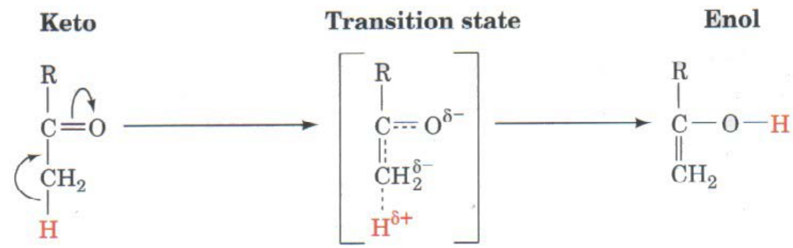




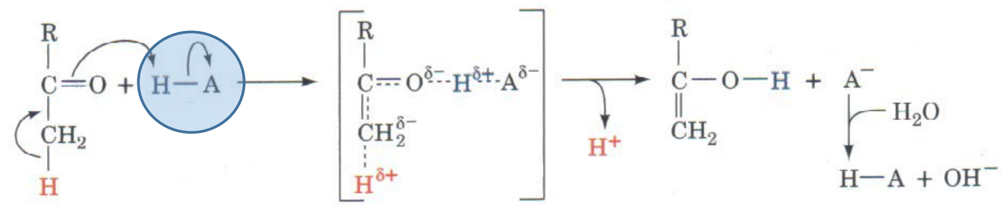
aminokisline, ki delujejo kot kovalentni katalizatorji

Kislinsko bazna kataliza

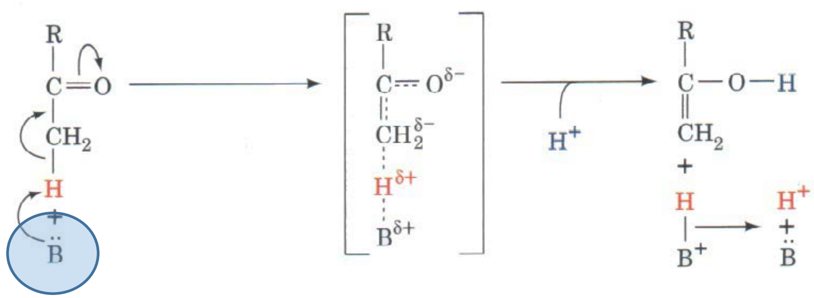
encim služi kot donor ali akceptor za proton ali pa ga prenaša med substrati



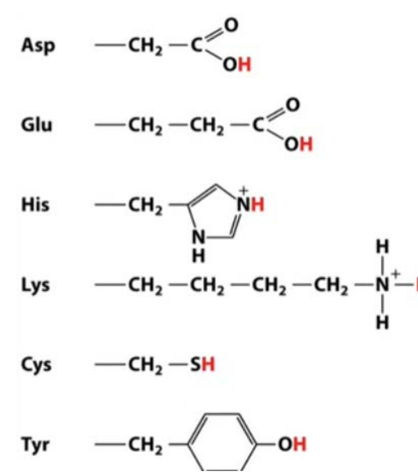
za hitrejšo reakcijo je potrebno zmanjšati razvoj naboja v prehodnem stanju



splošna kislinska kataliza, olajša keto-enol tavtomerizacijo



bazne katalize, olajša keto-enol tavtomerizacijo

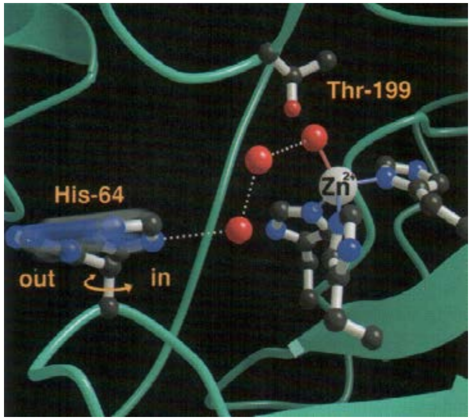


encimi uporabljajo ta mehanizem za:

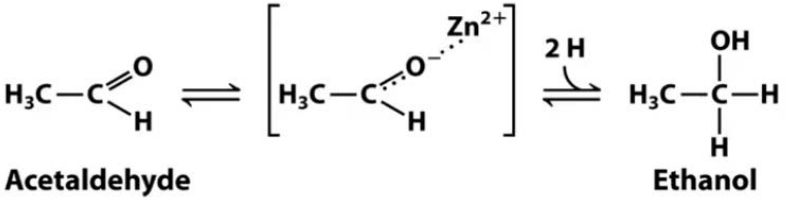
- hidroliza peptidov in estrov
- tautomerizacijo
- dodajanje fosfata na karbonil

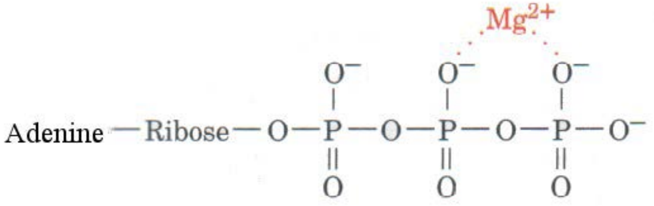
aminokisline, ki delujejo kot kislinsko-bazni katalizatorji

Elektrostatska kataliza z ioni kovin



- direktna mediacija redoks reakcije s pomočjo spremembe oksidacijskih stanj (npr. redukcija O_2 do H_2O s prenosom elektronov)
- elektrostatska stabilizacija in senčenje negativnih nabojev (npr. vezava Mg^{2+} na ATP)
- vezava in orientiranje substrata za reakcijo



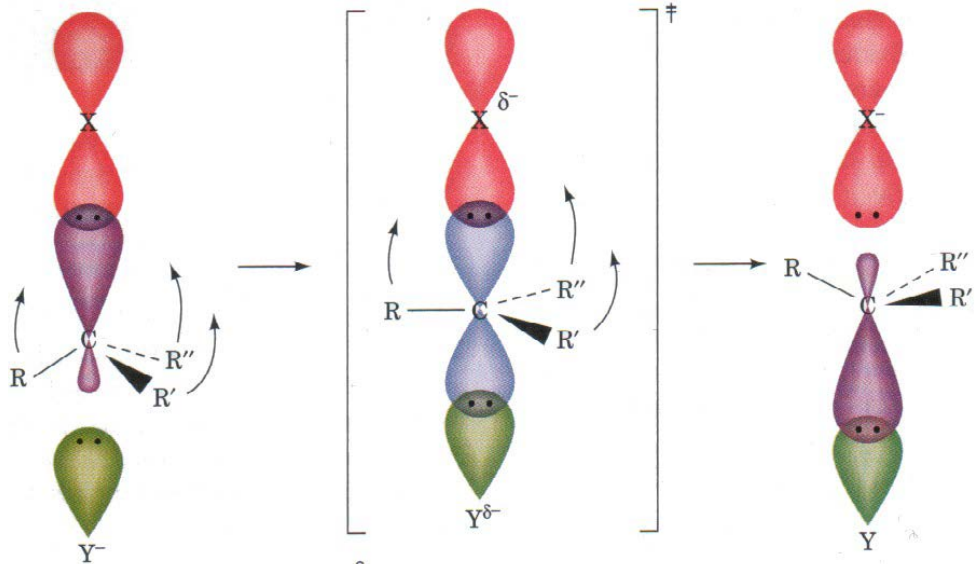


metaloencimi vsebujejo močno vezane ione (npr. Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{3+})

z metali aktivirani encimi vsebujejo šibko vezane ione (npr. Na^{+}, K^{+}, Mg^{2+} and Ca^{2+})

Bližina / orientacijski efekti in kataliza

encim povečuje uspešnost trkov med substrati



teoretično so molekule najbolj reaktivne takrat, ko so orbitale, orientirane tako, da je energija prehodna stanja najmanjša,

stereo elektronska asistenca

Vrste encimov glede na tip reakcije

OKSIDOREDUKTAZE
(oksidaze, dehidrogenaze, reduktaze)

A + B: → A: + B

TRANSFERAZE
(kinaze, fosfataze, metilaze, transacilaze, transaminaze)

A + BX → AX + B

HIDROLAZE
(lipaze, amilaze, peptidaze, nukleaze)

A + H_2O → B + C

LIAZE
(citrat liaza, dehidraze, dekarboksilaze)

A → B + C

LIGAZE
(DNA ligaza, sintetaze, karboksilaze)

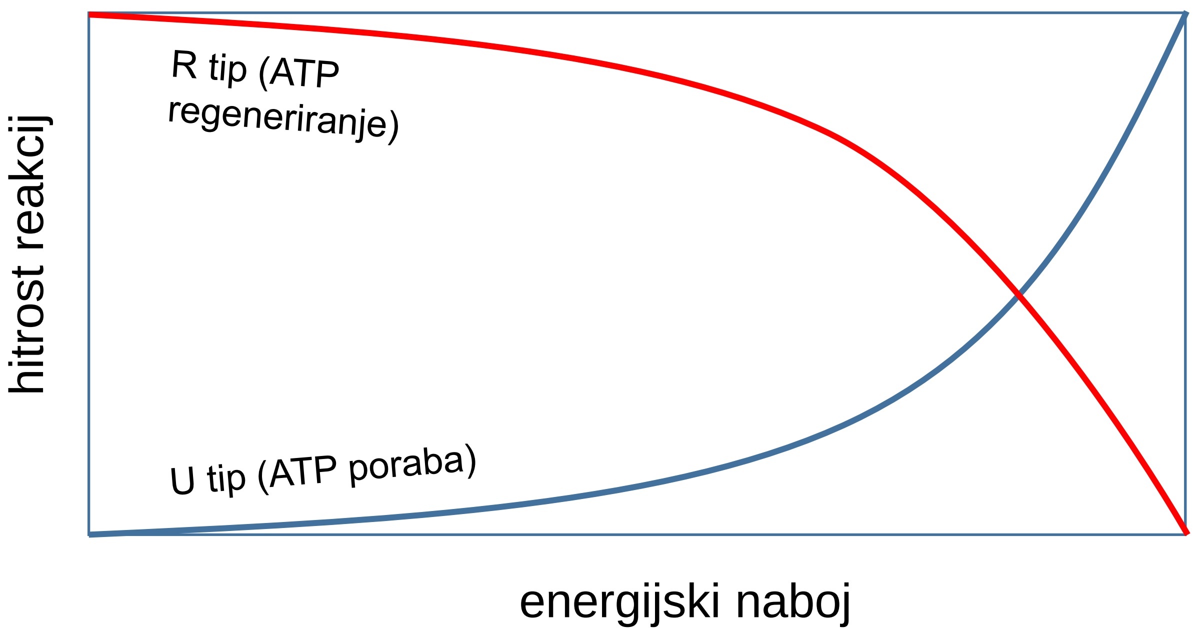
A + B → AB

IZOMERAZE
(fosfogluoza izomeraza, alanin racemaza, epimeraze)

A → B

Regulacije encimov glede na energijski naboj v celici

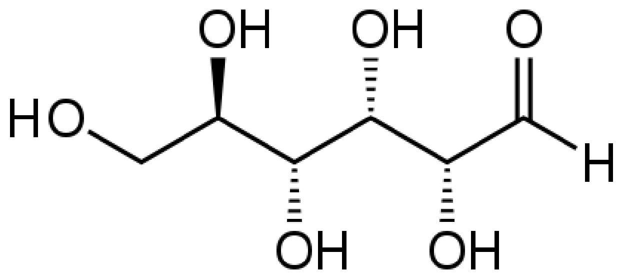
običajno je reguliran prvi encim vstopa v metabolno pot



$$\text{energijski naboj} = \frac{ATP + \frac{1}{2} ADP}{ATP + ADP + ADP}$$

Centralni metabolizem

Glukoza



funkcionalni skupini:

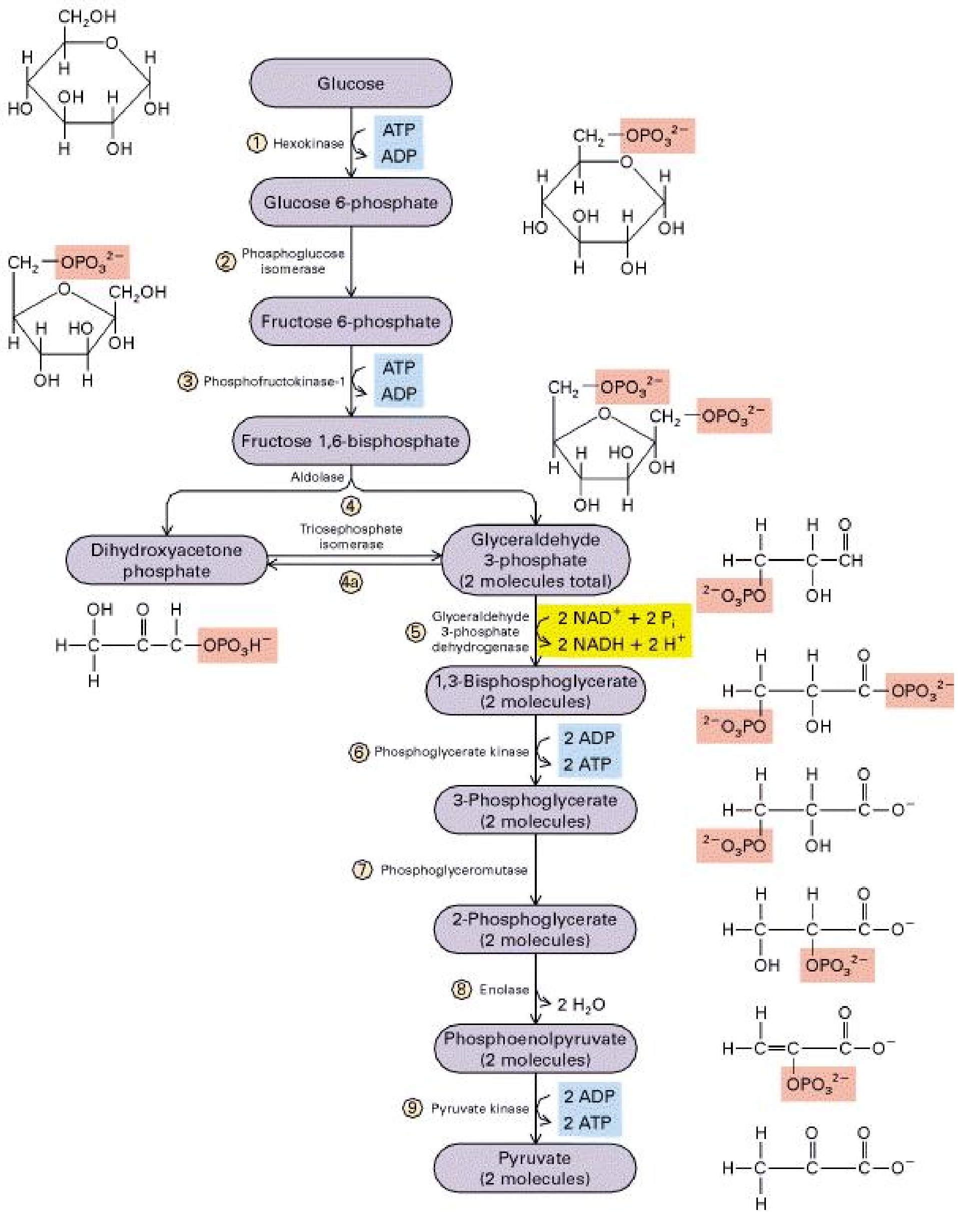
- CHO

- OH

reakcije alkoholov

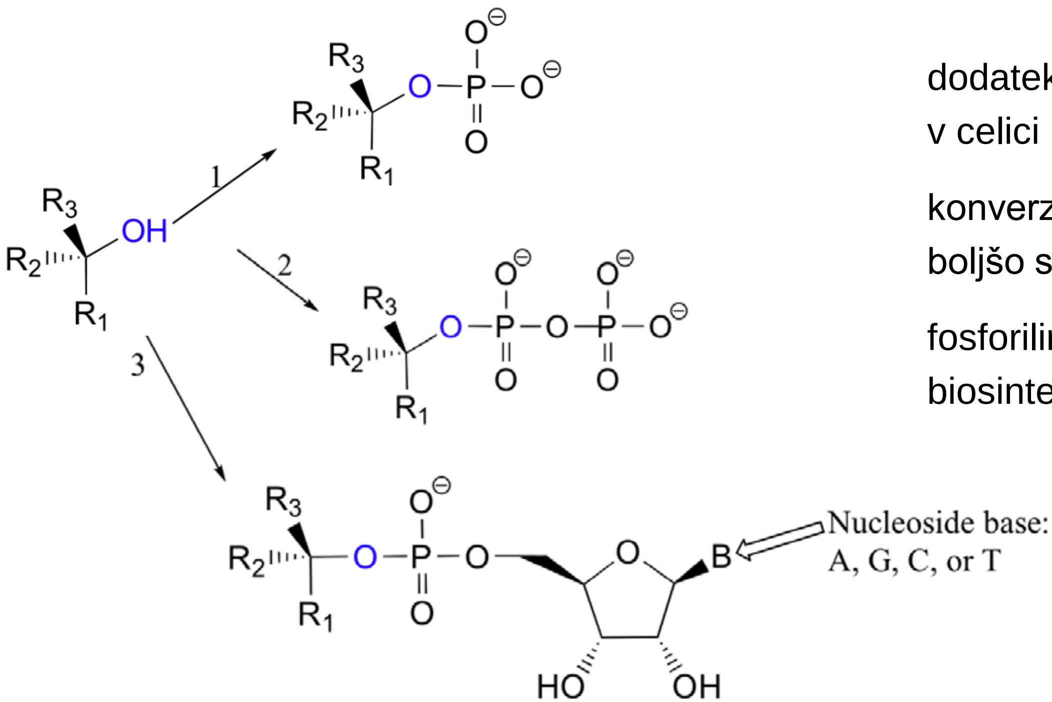
- oksidacije do aldehidov, ketonov
- dehidracije do alkenov
- deprotonacije
- nukleofilne substitucije
- esterifikacije (fosforilacije)

Glikoliza



- energijski vidik (oksidacija, sinteza ATP, NADH)
- biosintetski vidik (sinteza ključnih metabolnih intermediatov)
- regulacijski vidik (regulacijske točke: heksokinaza, fosfofruktokinaza, piruvat kinaza)

Fosforilacija alkoholov



dodatek naboja na molekulo, boljše zadrževanje v celici

konverzija alkohola v fosfatni ester omogoča boljšo skupino, ki zapušča reakcijo

fosforilirani intermediati vstopajo v različne biosintetske poti

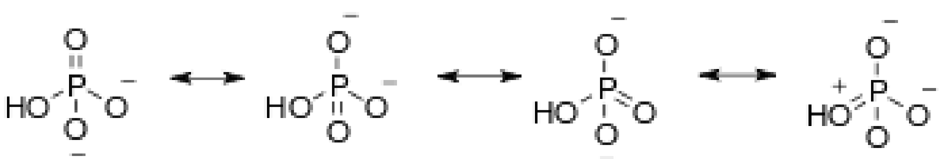
Biokemijske reakcije estrov:

hidroliza

transesterifikacija

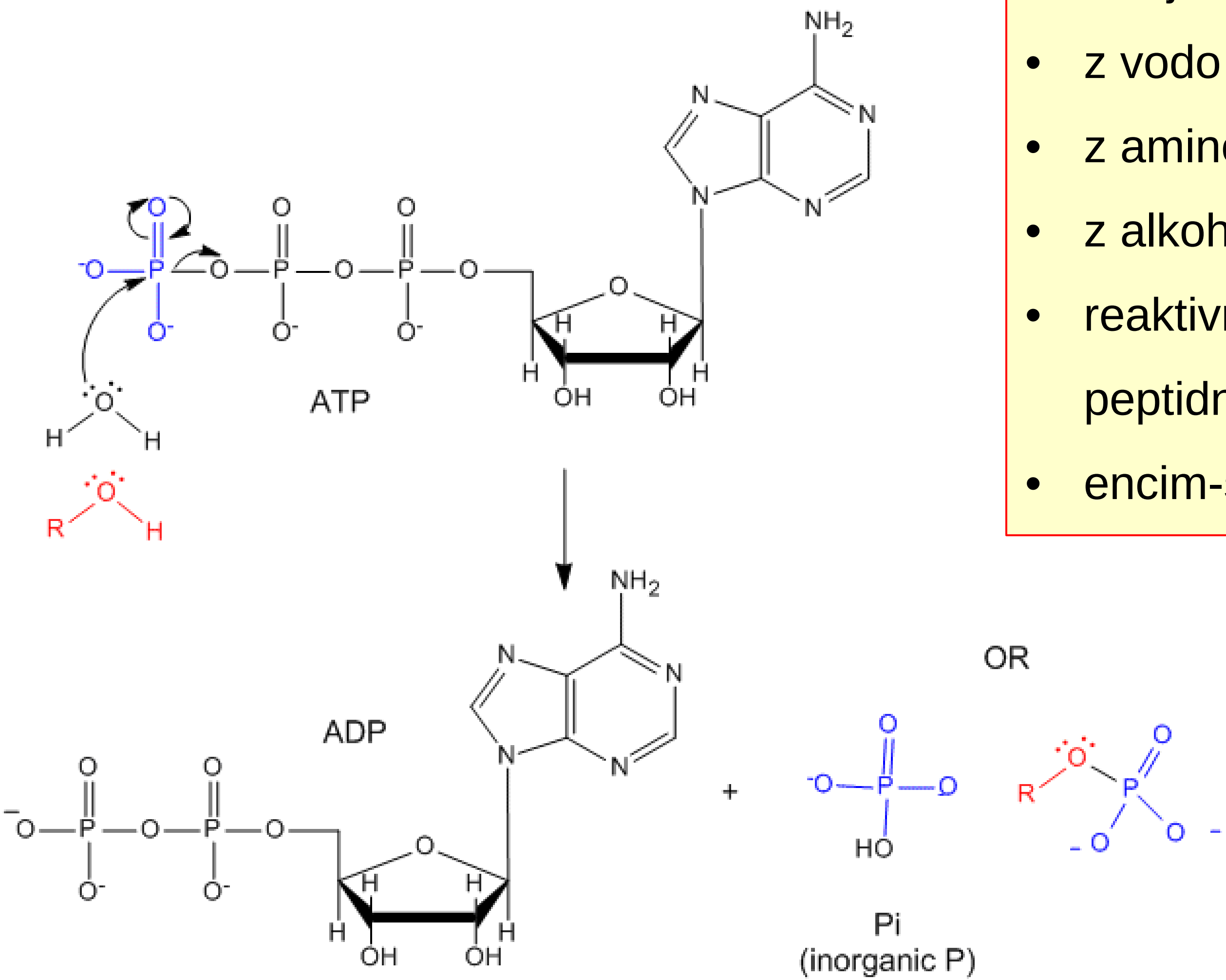
nastanek amidov

nastanek laktonov



- zaradi rezonančne delokalizacije so fosfati odlične odhajajoče skupine
- separacija naboja
- dobra topnost / stabilizacija v vodi

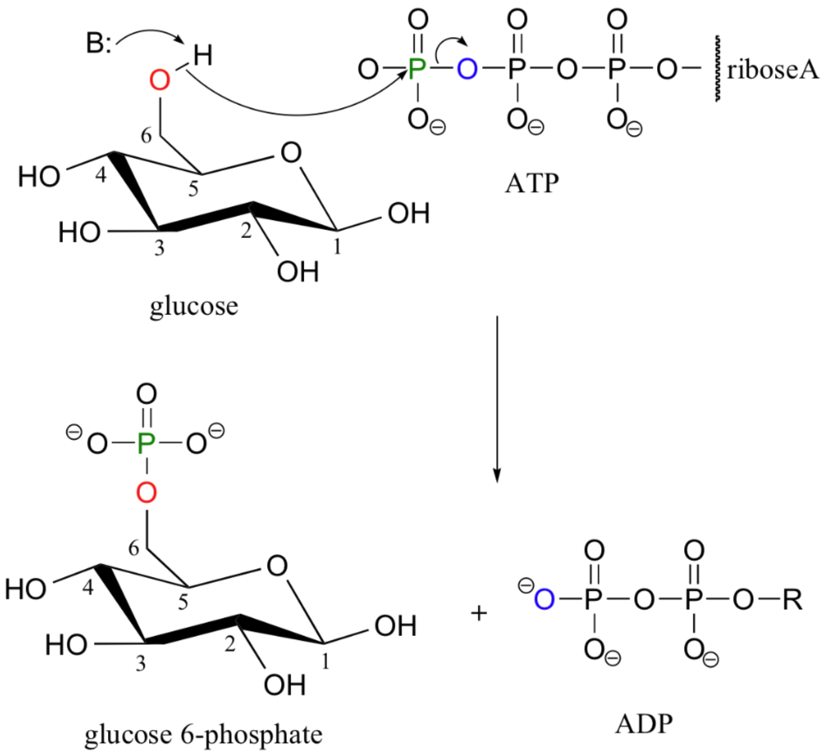
ATP hidroliza



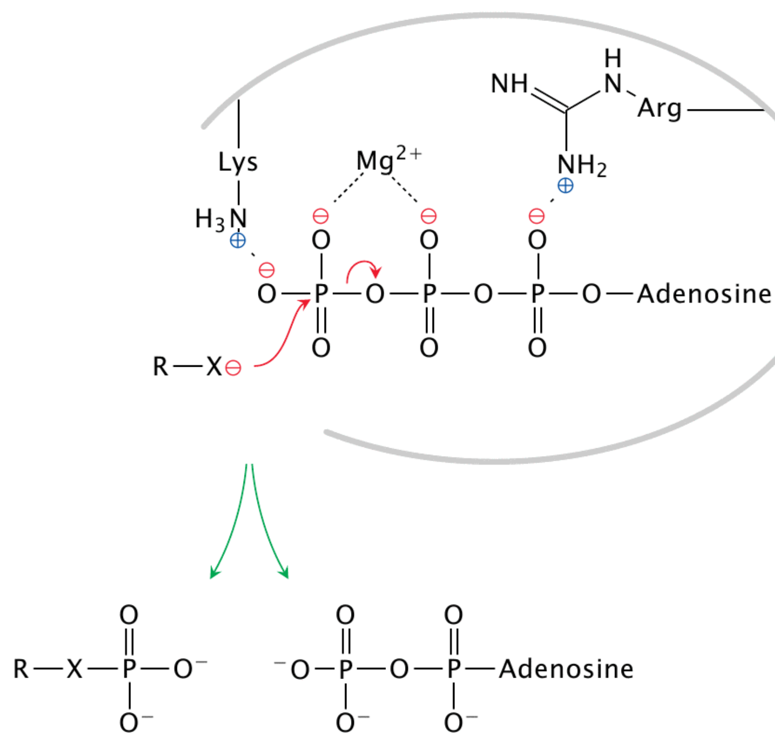
Reakcije kislih anhidridov:

- z vodo nastanejo kisline
- z amino skupinami amidi
- z alkoholi estri
- reaktivni intermediati pri peptidni sintezi
- encim-substrat intermediati

Heksokinaza

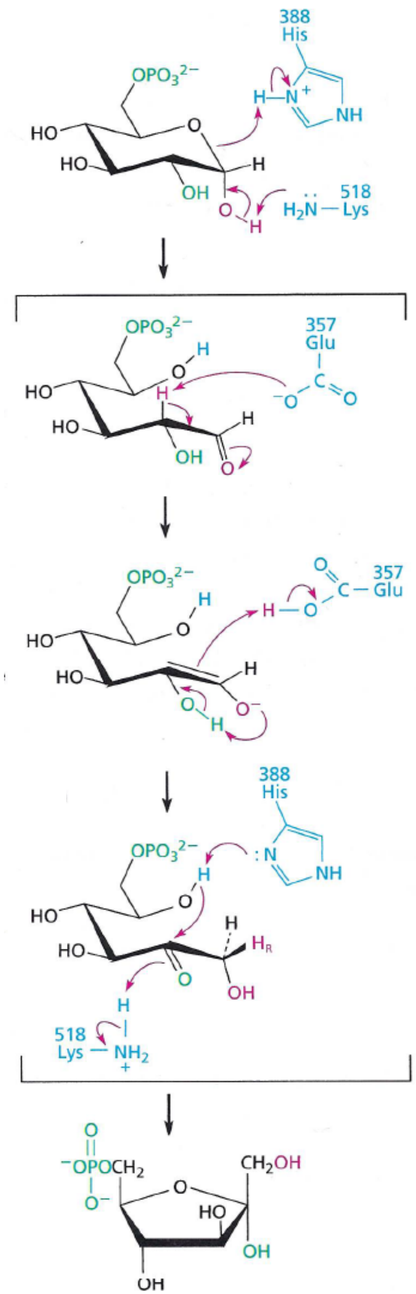


za uspešno fosforilacijo je potrebno nukleofilu (OH) omogočiti dostop do ATP (potrebna je neutralizacija nabojev v aktivnem mestu encima)

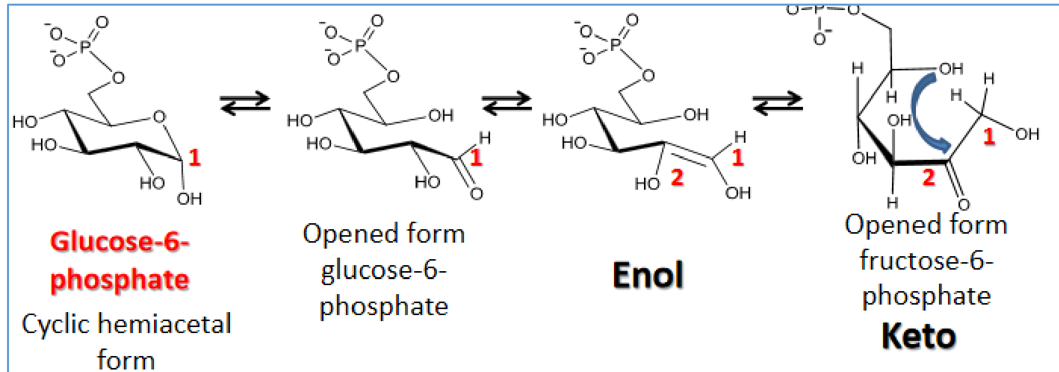


aktivacija molekul preprečuje nastanek visokih koncentracij intermediatov, ima lahko kinetično vlogo (pospešuje reakcijo) ali pa termodinamsko vlogo (poveča izplen produkta v ravnotežju)

Mehanizem delovanja fosfoglukoizomeraze

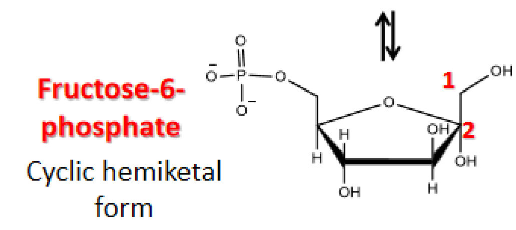


kislinsko-bazna kataliza (reverzibilna protonacija in deprotonacija substrata)

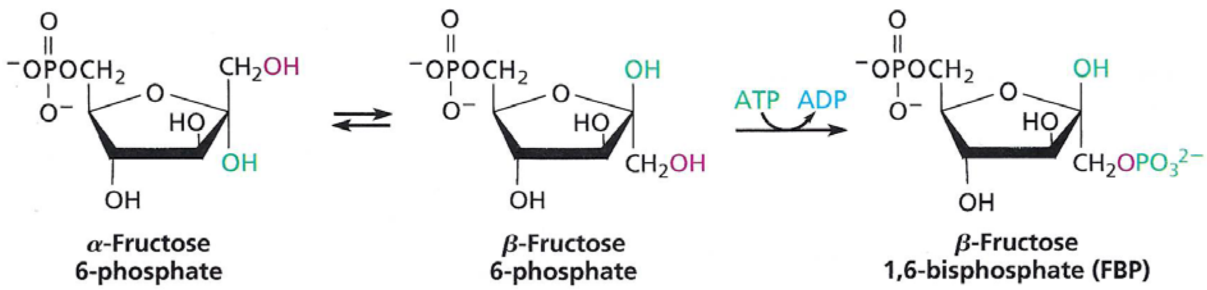


keto enolna tavtomerija,

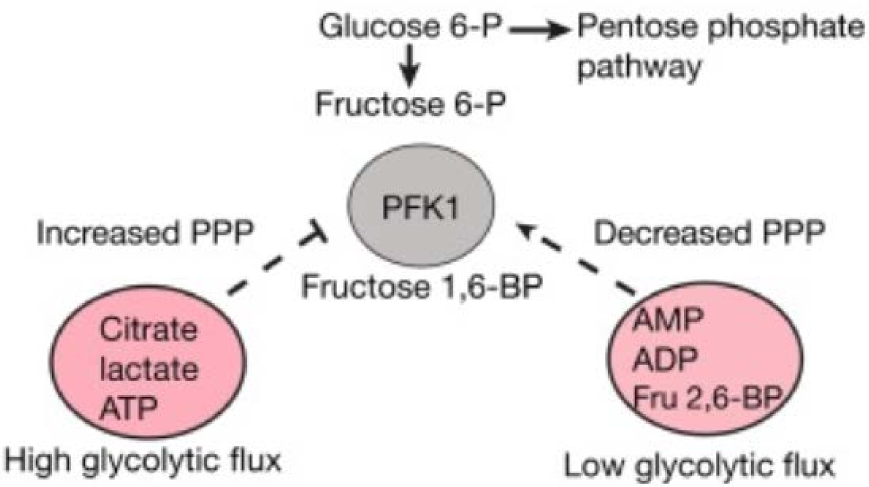
interni prenos protona



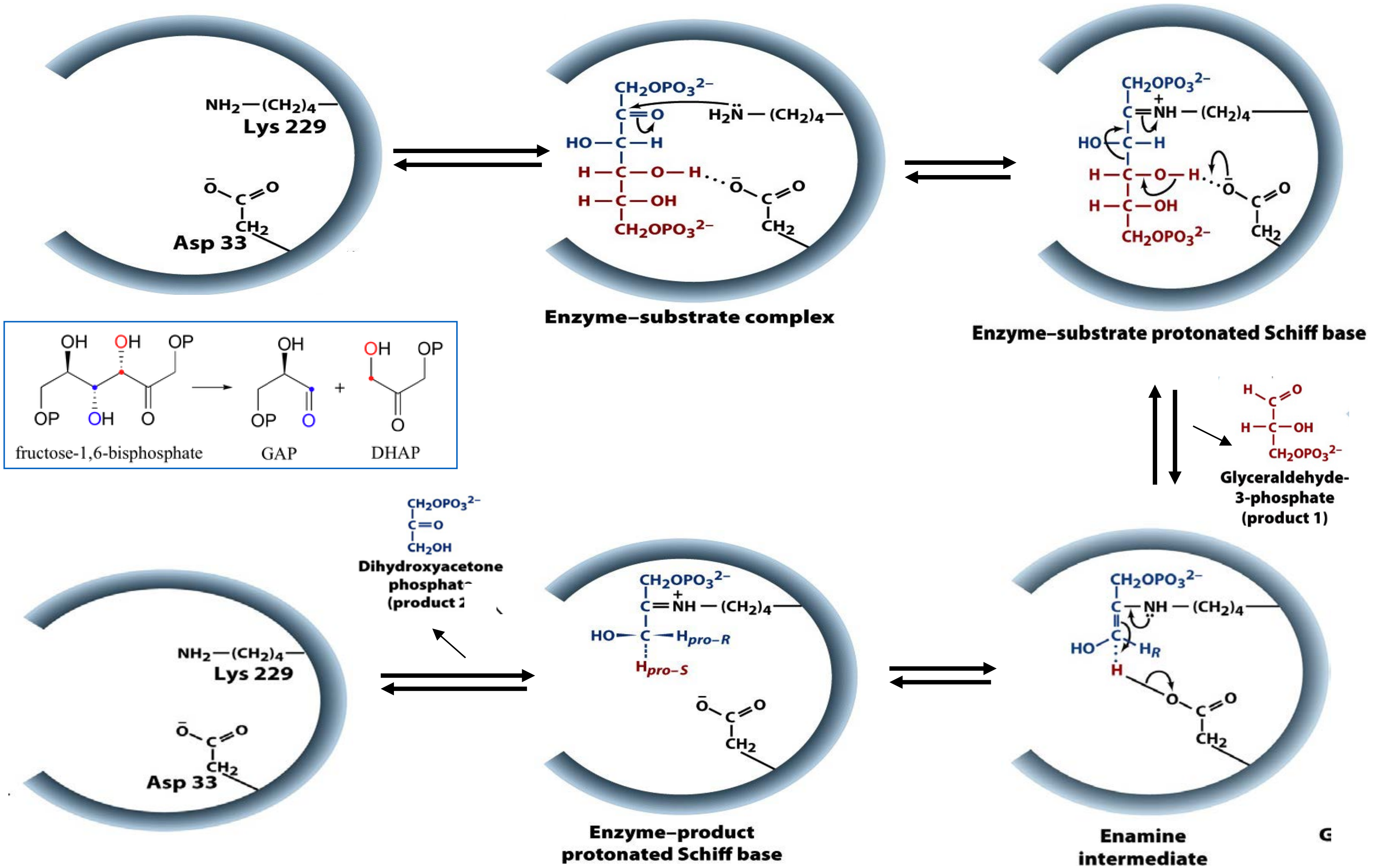
Fosfofruktokinaza



glavno regulacijsko mesto glikolize

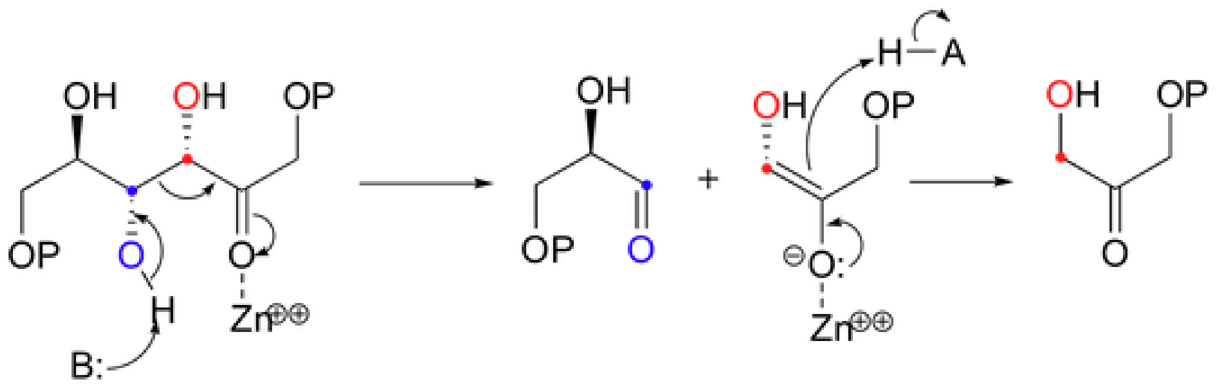


Mehanizem delovanja fruktoze bifosfat aldolaze (tip I)



Mehanizem delovanja fruktoze bifosfat aldolaze

(tip II) - *E. coli*

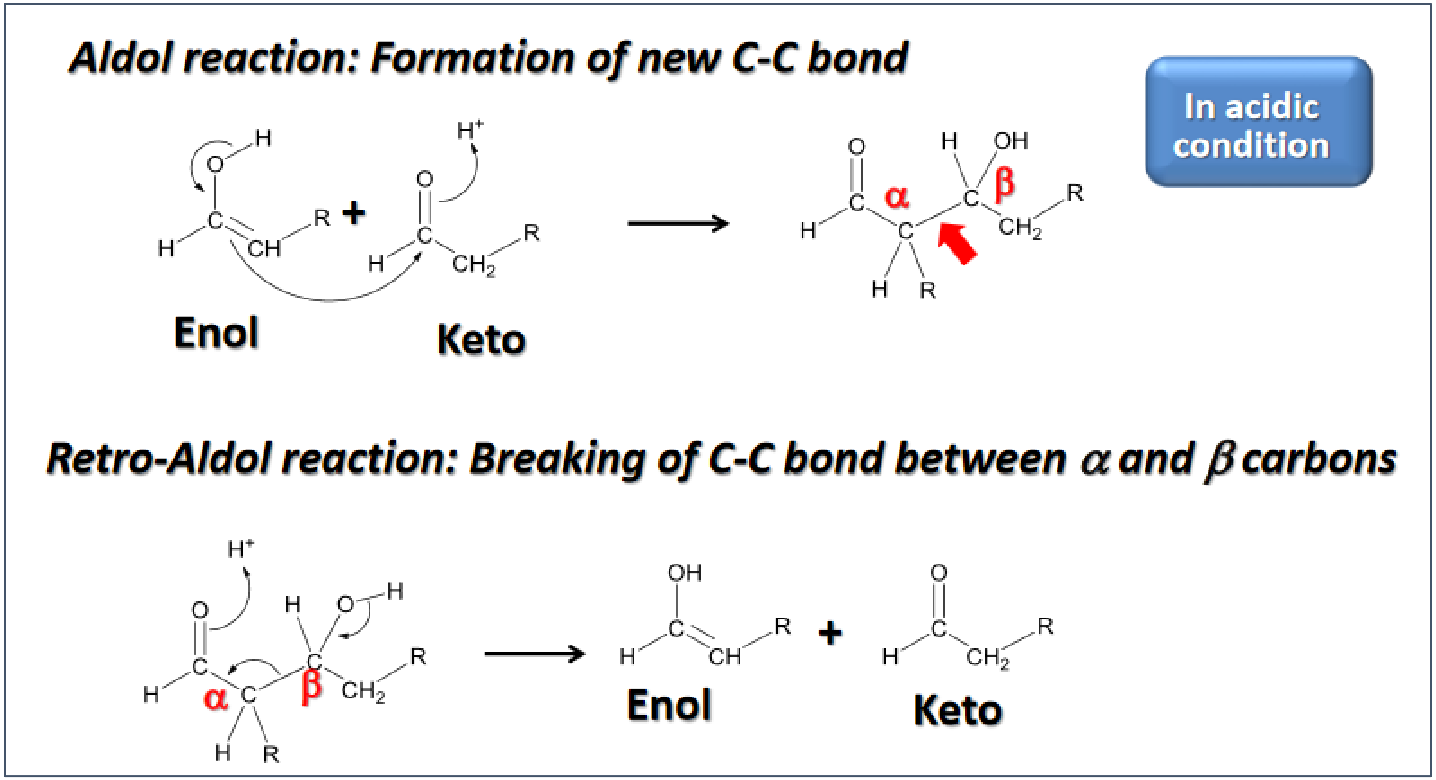


elektroni, ki ostanejo po cepitvi C-C vezi morajo biti nekam odloženi:

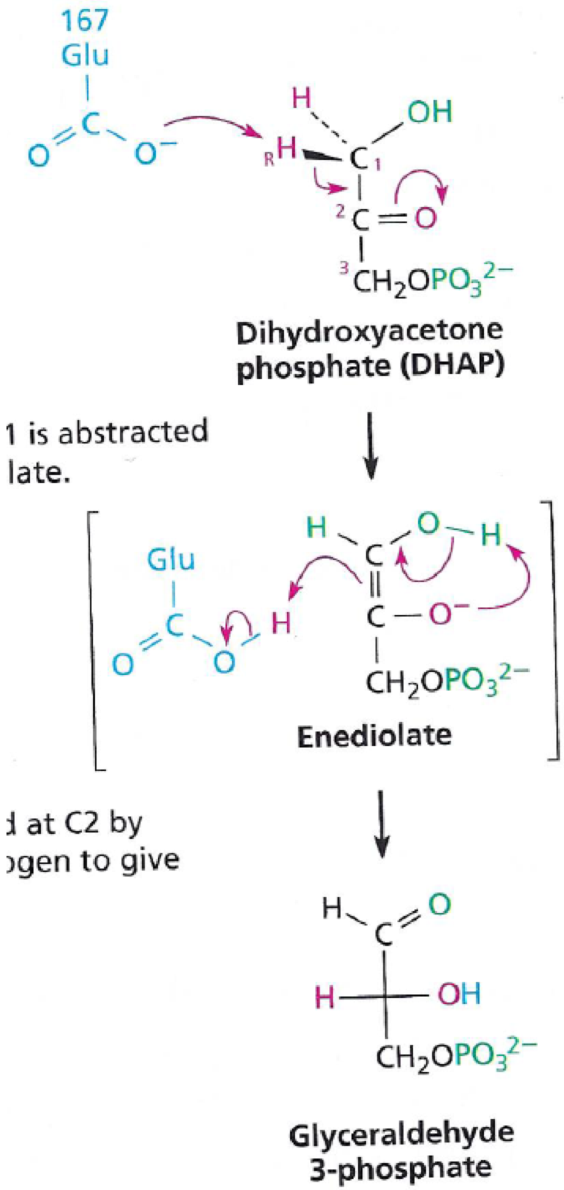
- pri aldolizi tipa I so odloženi na imin

- pri aldolazi tipa II so stabilizirani preko rezonančne strukture na karbonilu, ki jo pomaga vzdrževati Zn^{2+} v aktivnem mestu encima (neutralizira višek negativnega naboja na kisiku)

Aldolne in retro-aldolne reakcije

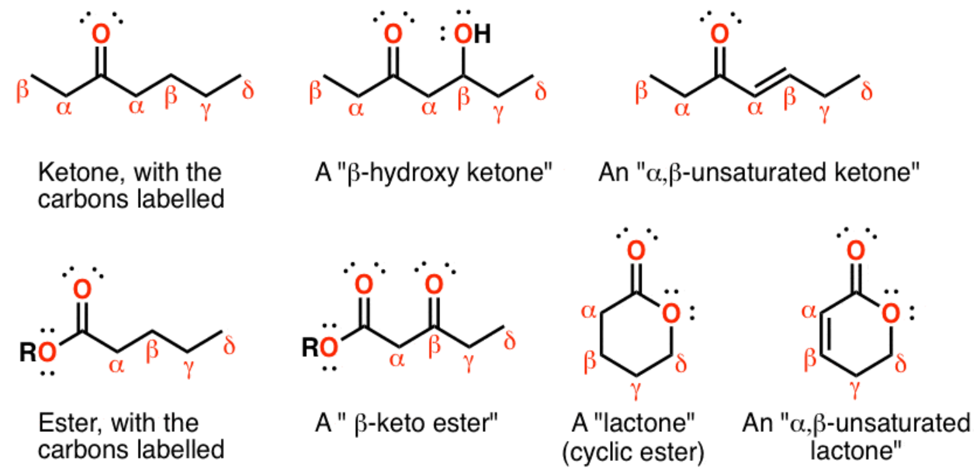


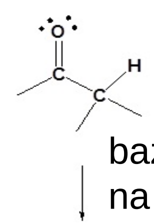
Mehanizem delovanja fosfat izomeraz - od ketona do aldehida



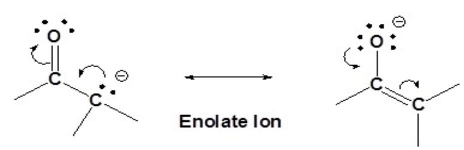
vodik na α ogljiku (H_R) je bolj kisel, kot ostali zaradi enolne stabilizacije po deprotonaciji (rezonančna stabilizacija)

poimenovanje ogljikov v karbonilih

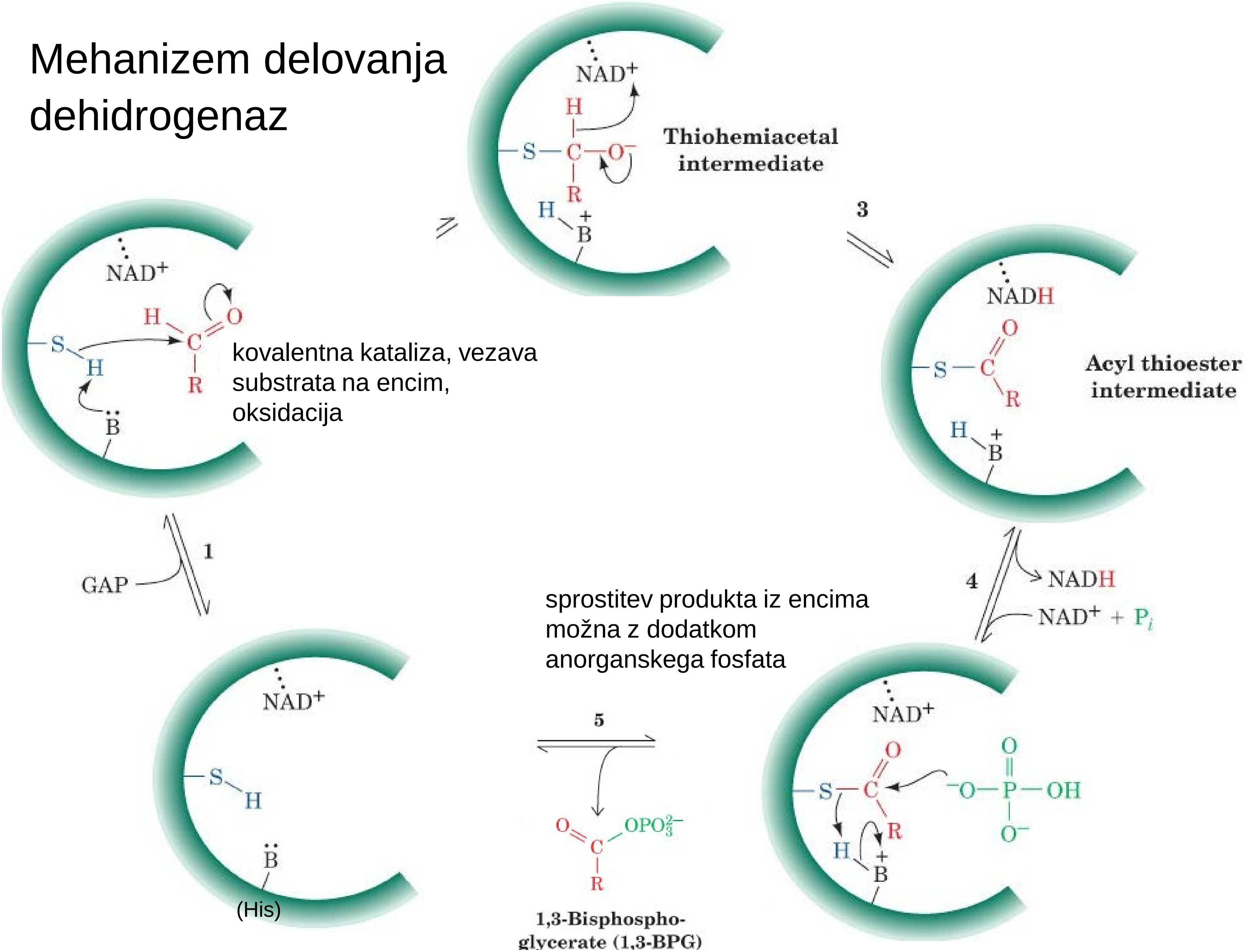




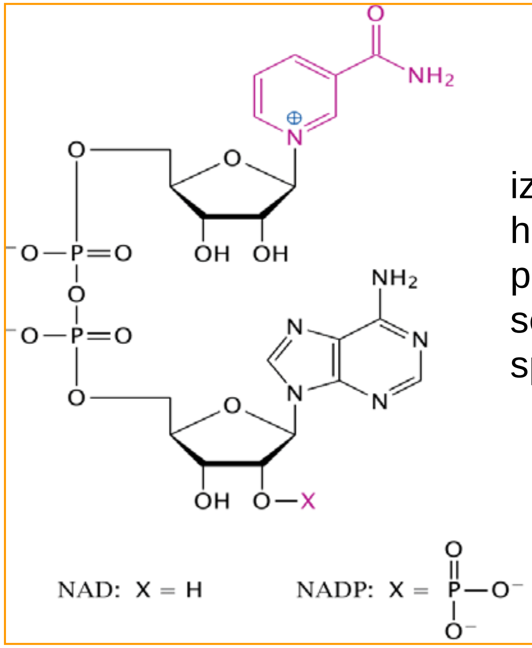
baza abstrahira proton
na α ogljiku



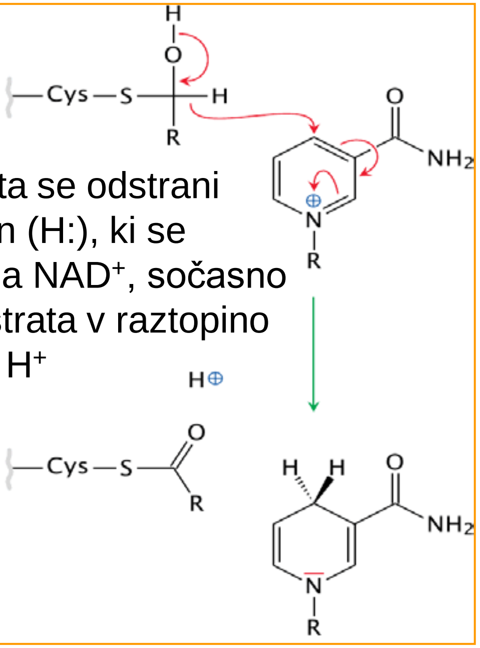
Mehanizem delovanja dehidrogenaz



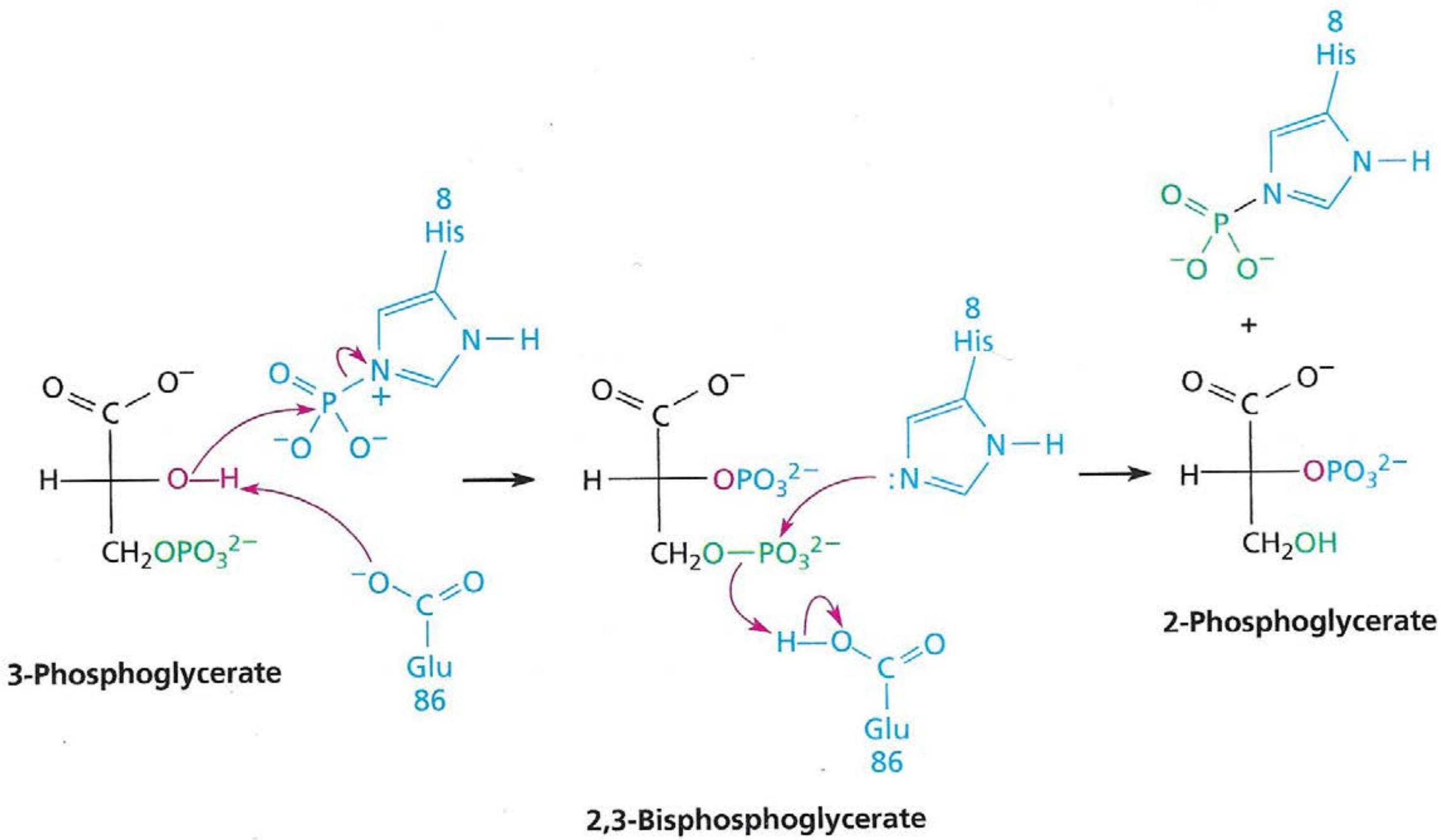
NAD



iz substrata se odstrani hidridni ion (H:), ki se prenese na NAD^+, sočasno se iz substrata v raztopino sprosti še H^+



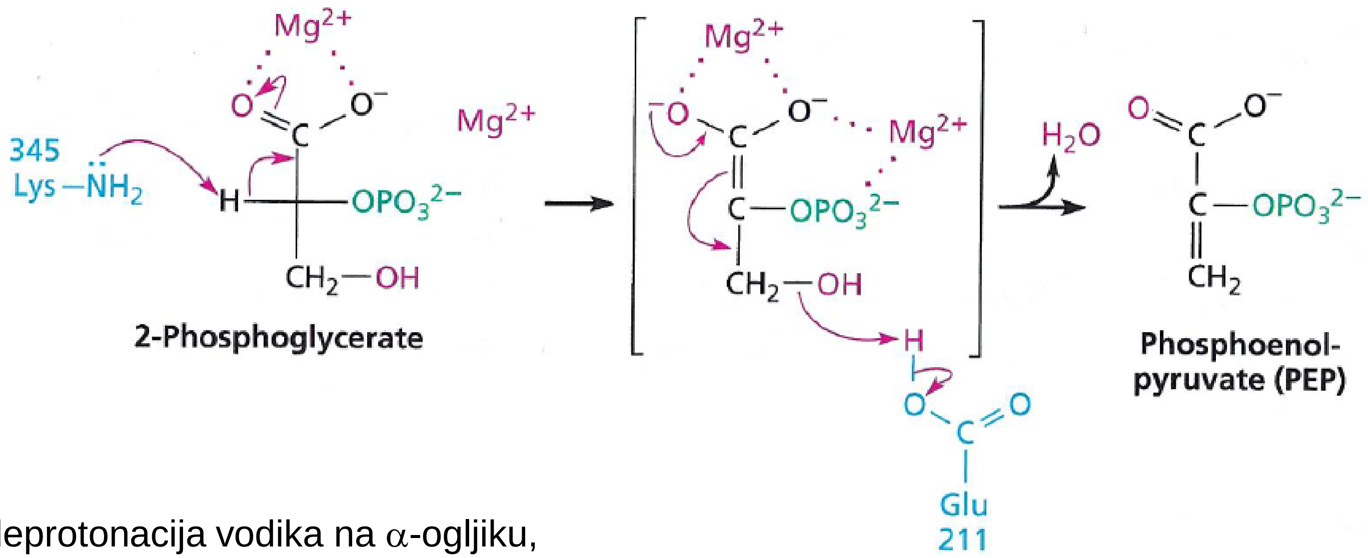
Mehanizem delovanja mutaz



fosforilacija in deprotonacija na

sosednjih oglikovih atomih

Mehanizem delovanja enolaz



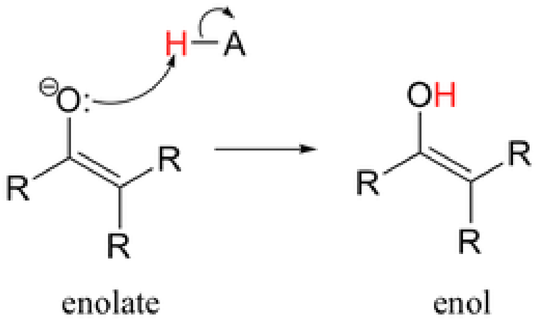
deprotonacija vodika na α-ogljiku,

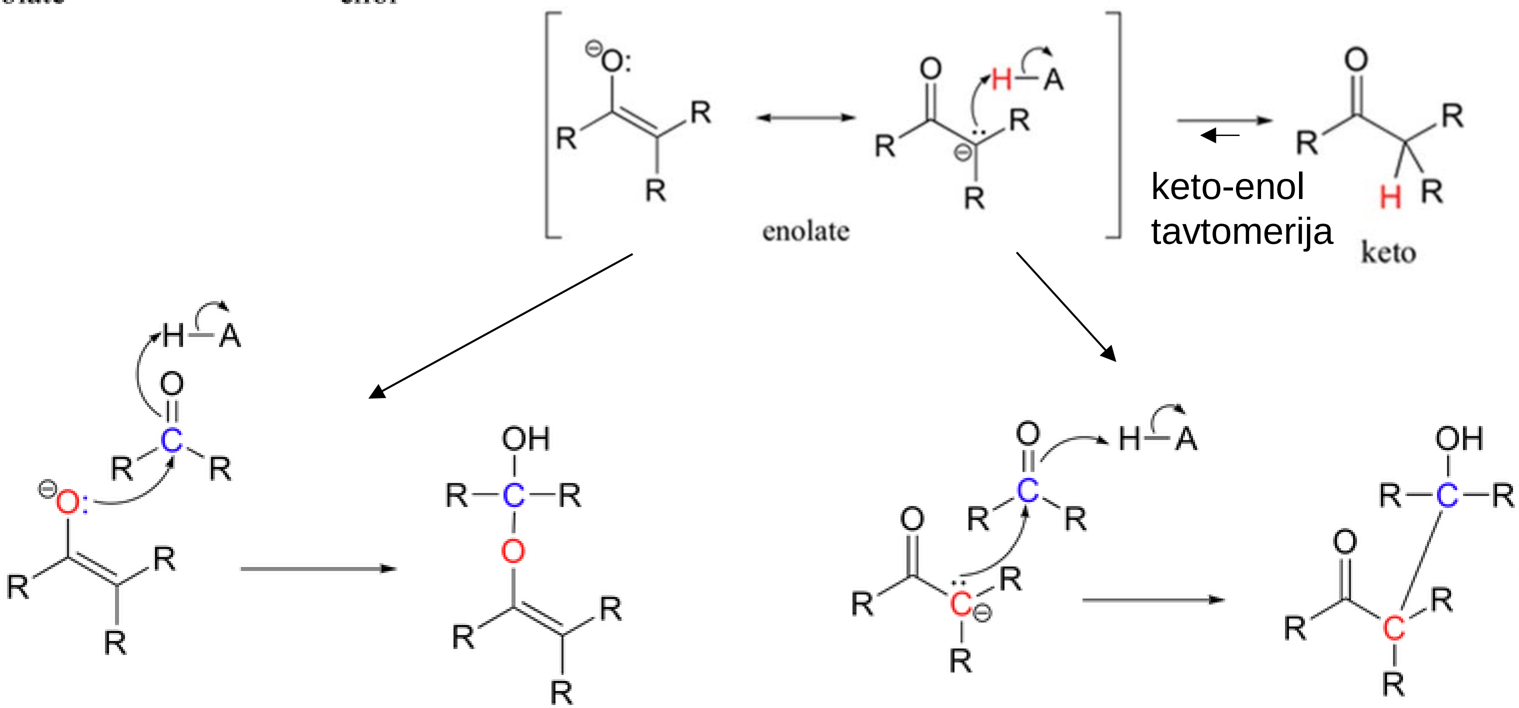
enolna stabilizacija

Enol / enolat

reakcije enolatov:

- močni nukleofili (rezonančno stabilizirani enolati)
- substitucije
- adicije na karbonile
  - aldolna kondenzacija,
  - Claisnova kondenzacija

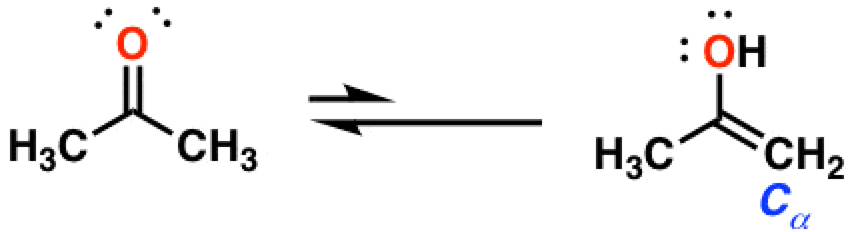




kisik enolata kot nukleofil, formiranje hemiketala

karboanion enolata kot nukleofil, formiranje C-C vezi

Keto-Enol Tautomerism



Keto | **Enol**

Electrophilic!

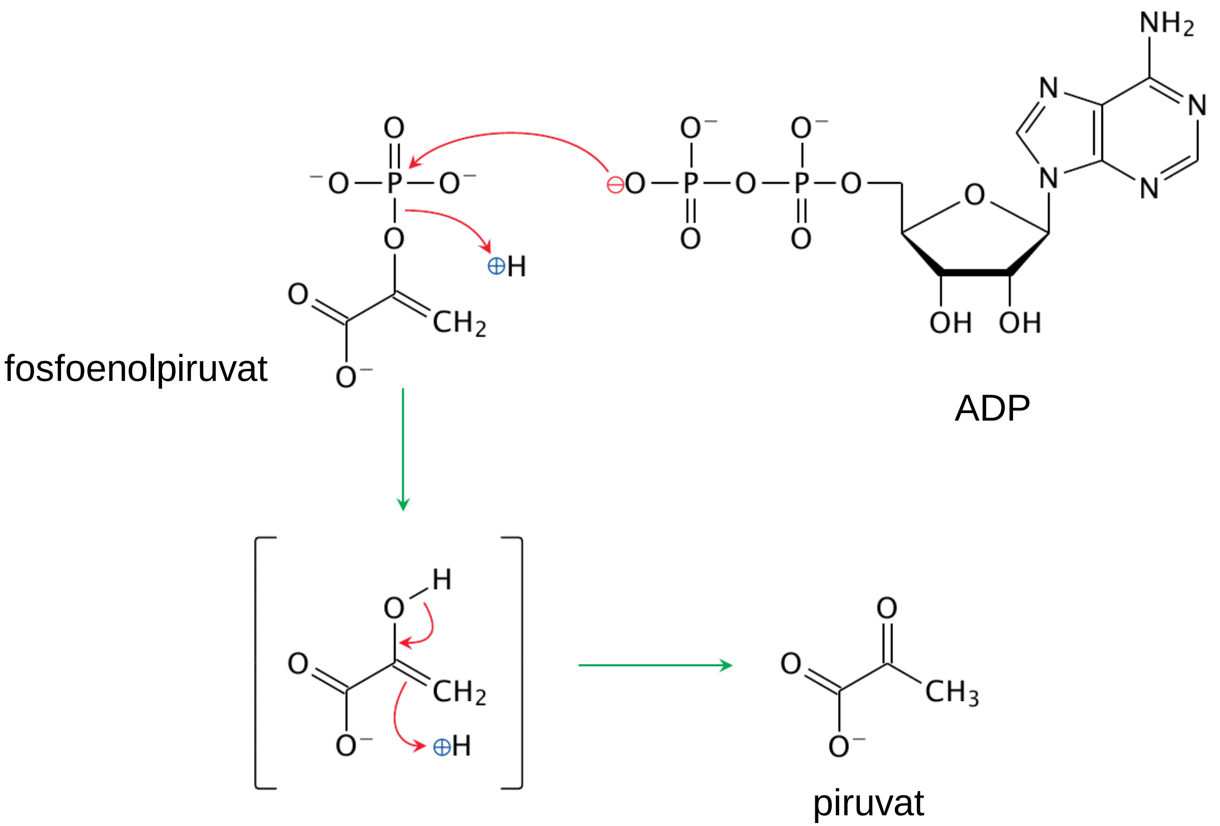
- *reacts with nucleophiles at carbonyl carbon*
- *acidic at α–carbon*
- *hydrogen bond acceptor*

Nucleophilic!

- *reacts with electrophiles at α–carbon*
- *acidic at oxygen (O–H)*
- *hydrogen bond donor **and** acceptor*

- *Equilibrium between isomers (not resonance)*
- *Under most conditions, keto form is favored (6600:1 for acetone)*
- *Important for aldehydes, ketones, but not so much for carboxylic acids, esters and amides under normal conditions (>10 million : 1 keto: enol)*

Mehanizem delovanja piruvat kinaze združen s enol-keto tavtomerijo



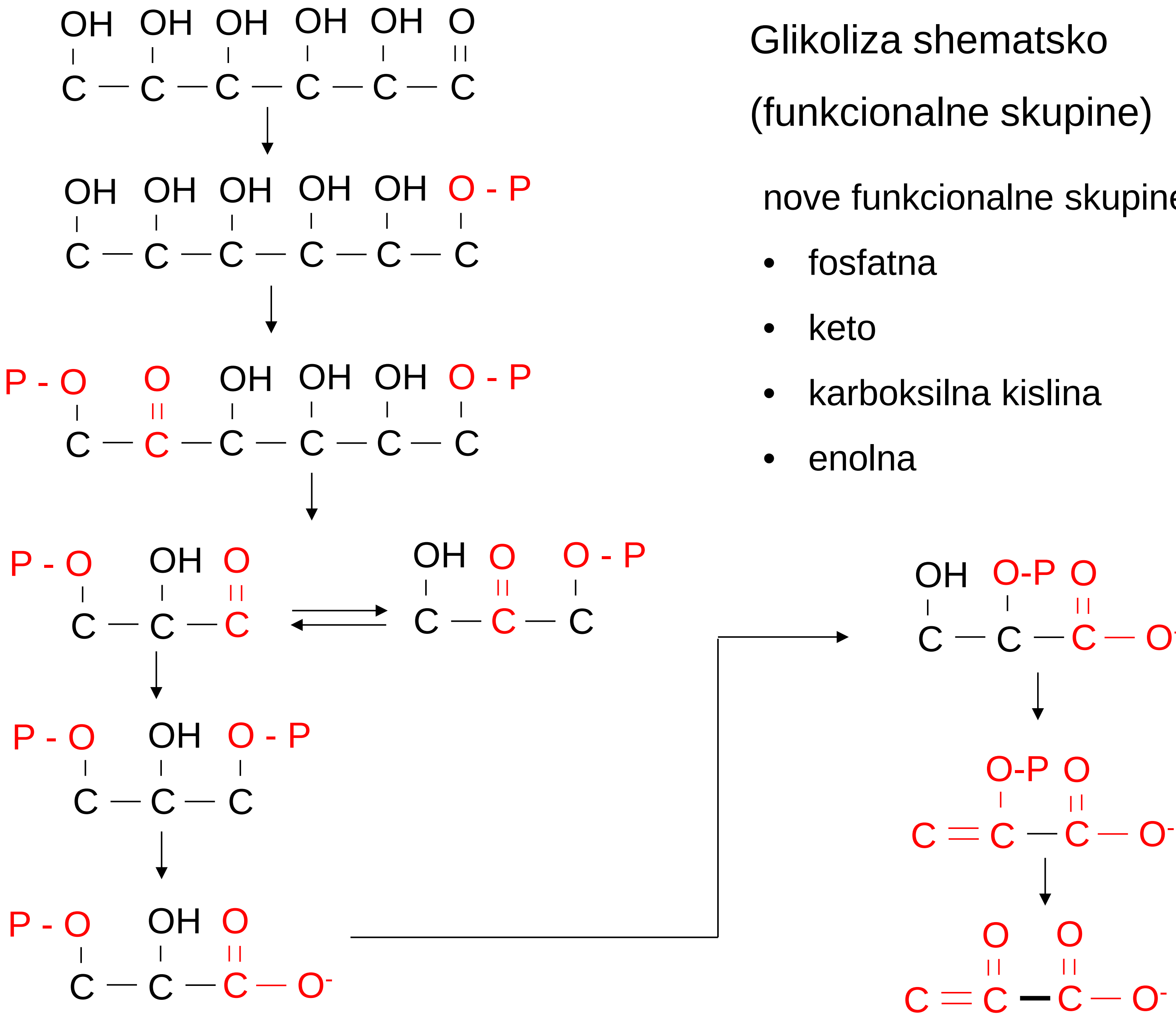
keto – enol tavtomerija omogoča nastanek ATP in ireverzibilnost reakcije (keto oblika je bolj stabilna)

Glikoliza shematsko

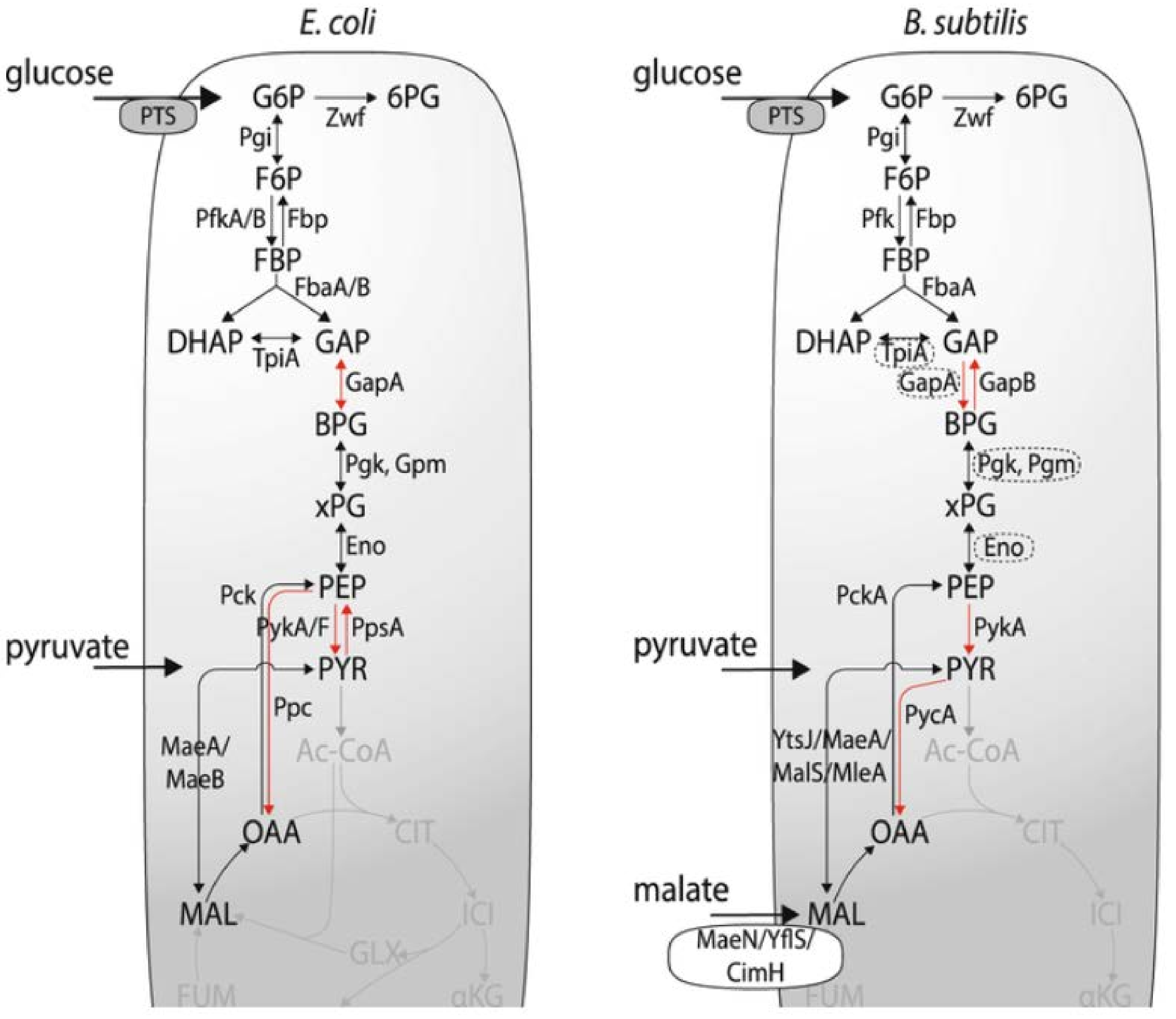
(funkcionalne skupine)

nove funkcionalne skupine:

- fosfatna
- keto
- karboksilna kislina
- enolna



Primerjalna glikoliza bakterij



razlike označene z rdečo,

pomembne razlike so:

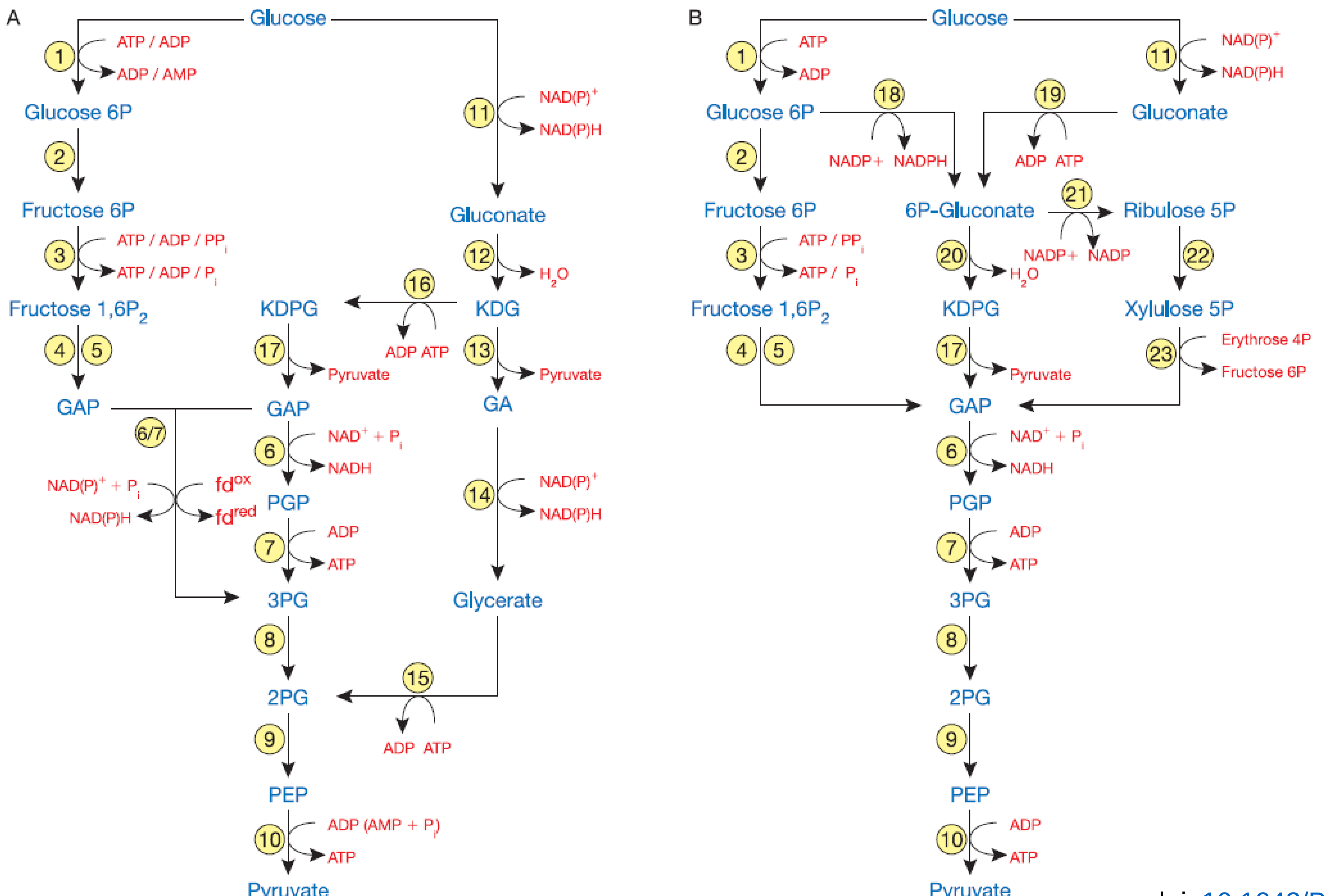
- PEP sintaza
- PEP karboksilaza
- PYR karboksilaza

doi.org/10.1038/s41598-018-30266-3

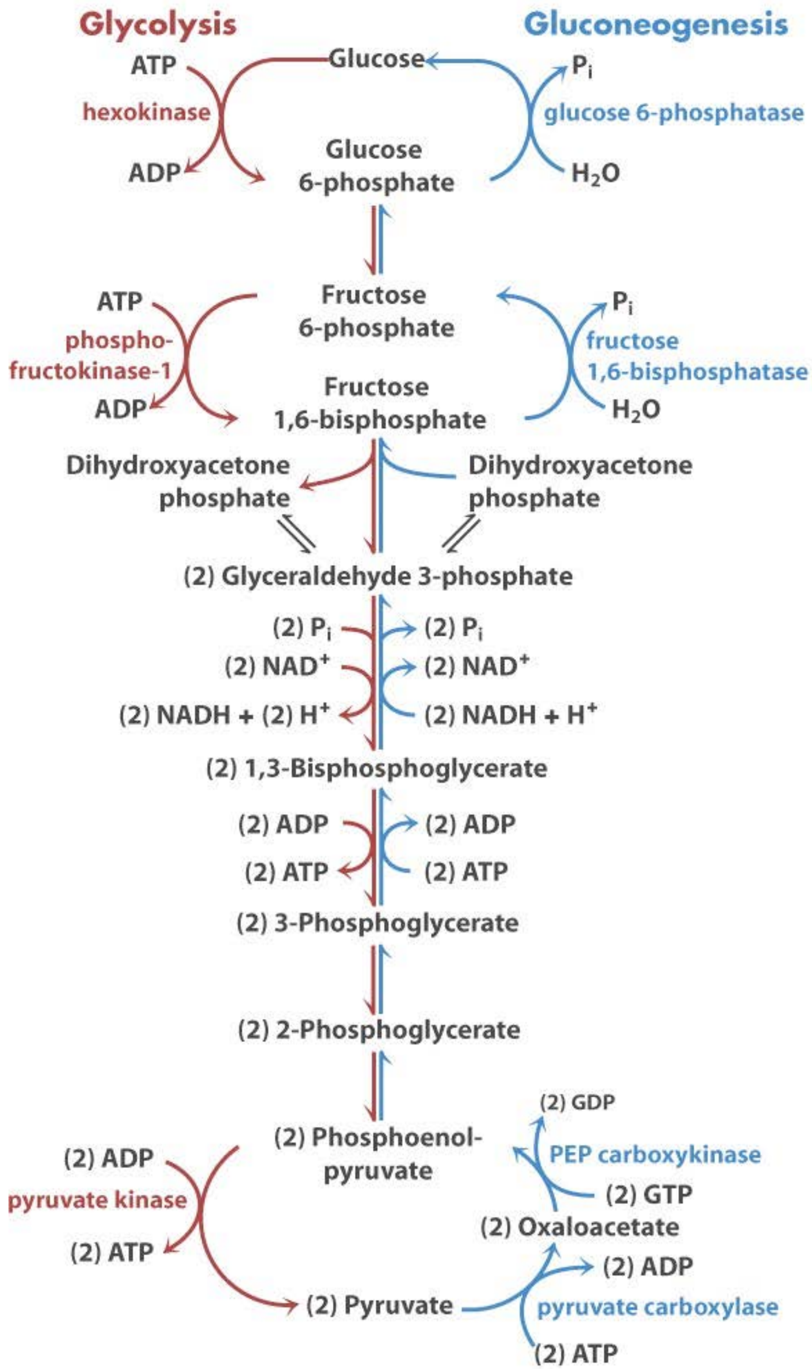
Primerjalna glikoliza arhej, bakterij, evkariontov

arheje

bakterije / evkarionti



doi: 10.1042/BJ20021472



Glikoliza in glukoneogenezo

povečanje glukoneogeneze:

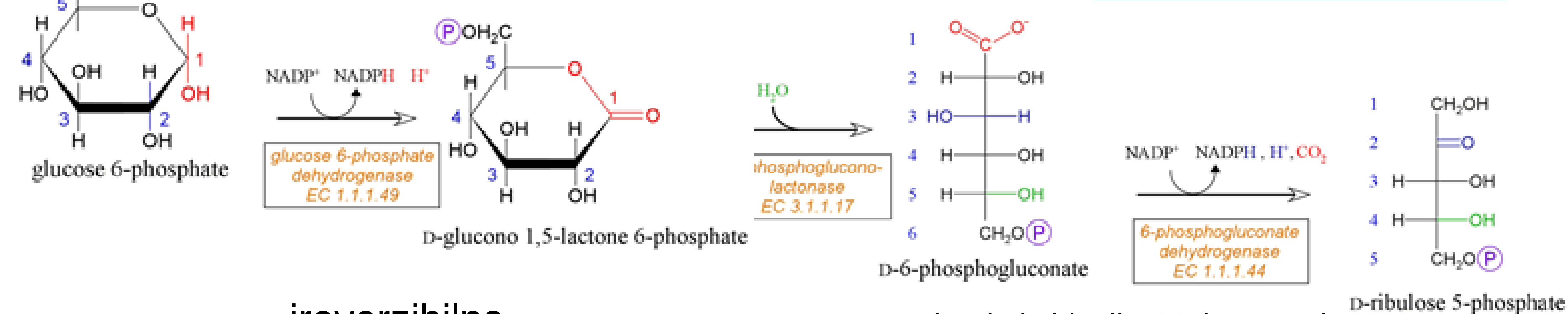
- stimulacija glukoza-6-fosfataze, fruktoza 1-6-bifosfataze, fosfoenolpiruvat karboksikinaze in piruvat karboksilaze, sinteza aminotransferaze (izraba alanina), stimulacija lipaze
- inhibicija piruvat kinaze, inhibicija izocitrat dehidrogenaze

Pentoza fosfat metabolna pot

oksidativni del

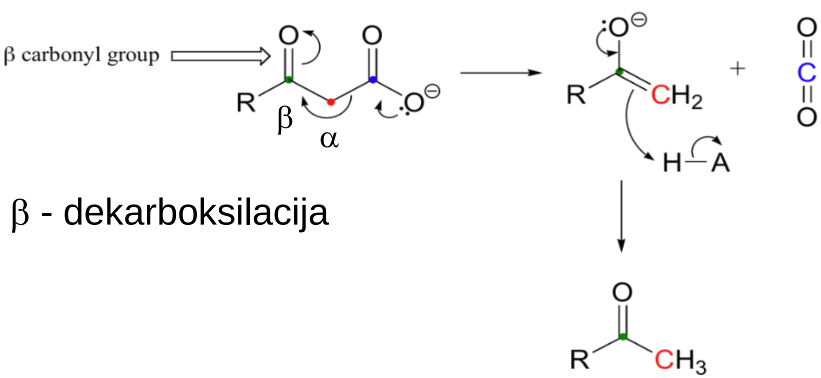
reakcije laktonov

- hidroliza
- redukcija
- aminoliza
- polimerizacija



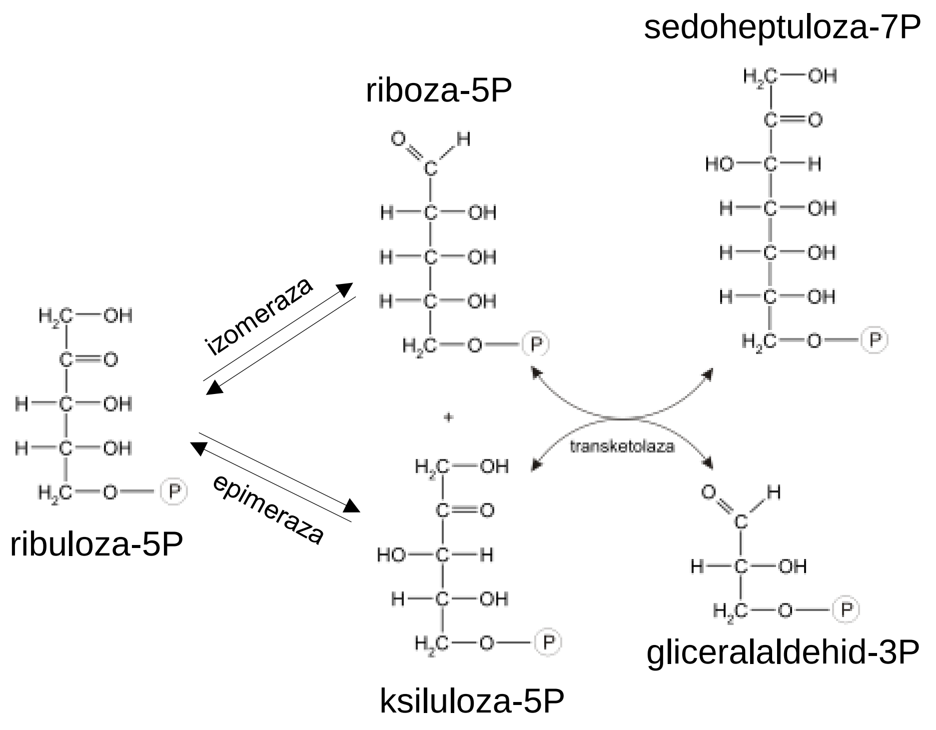
ireverzibilna pot

najprej oksidacija C3 (nastanek ketona) nato β-dekarboksilacija



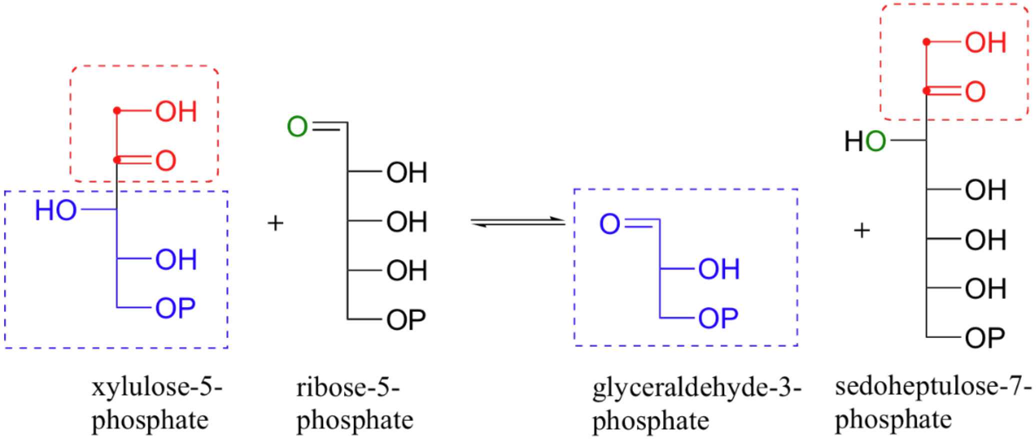
β - dekarboksilacija

Neoksidativni del pentoza fostat metabolne poti



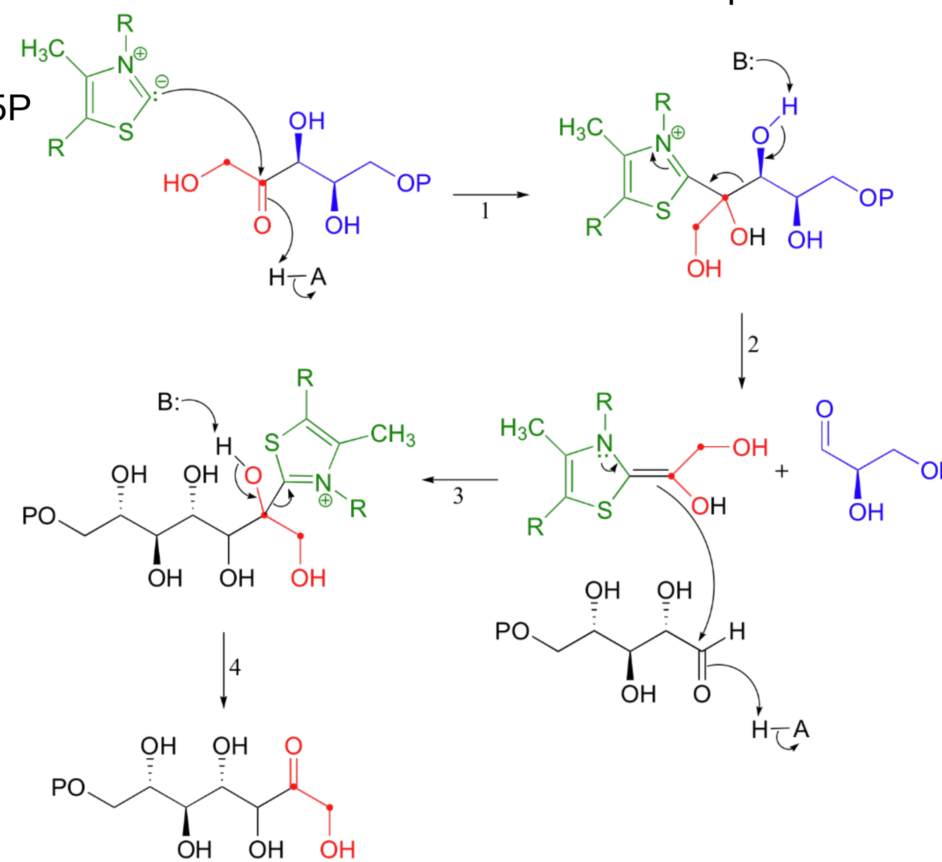
reverzibilna pot

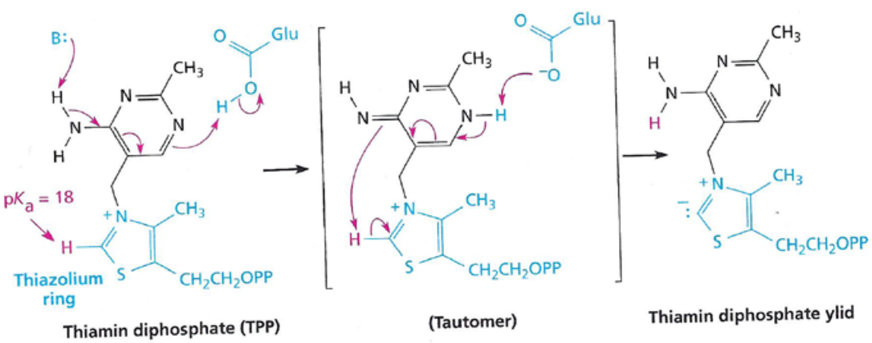
Transketolaza



karboanion tiamin difosfata (TPP) napade ksilulozo-5P

retro-aldolna cepitev



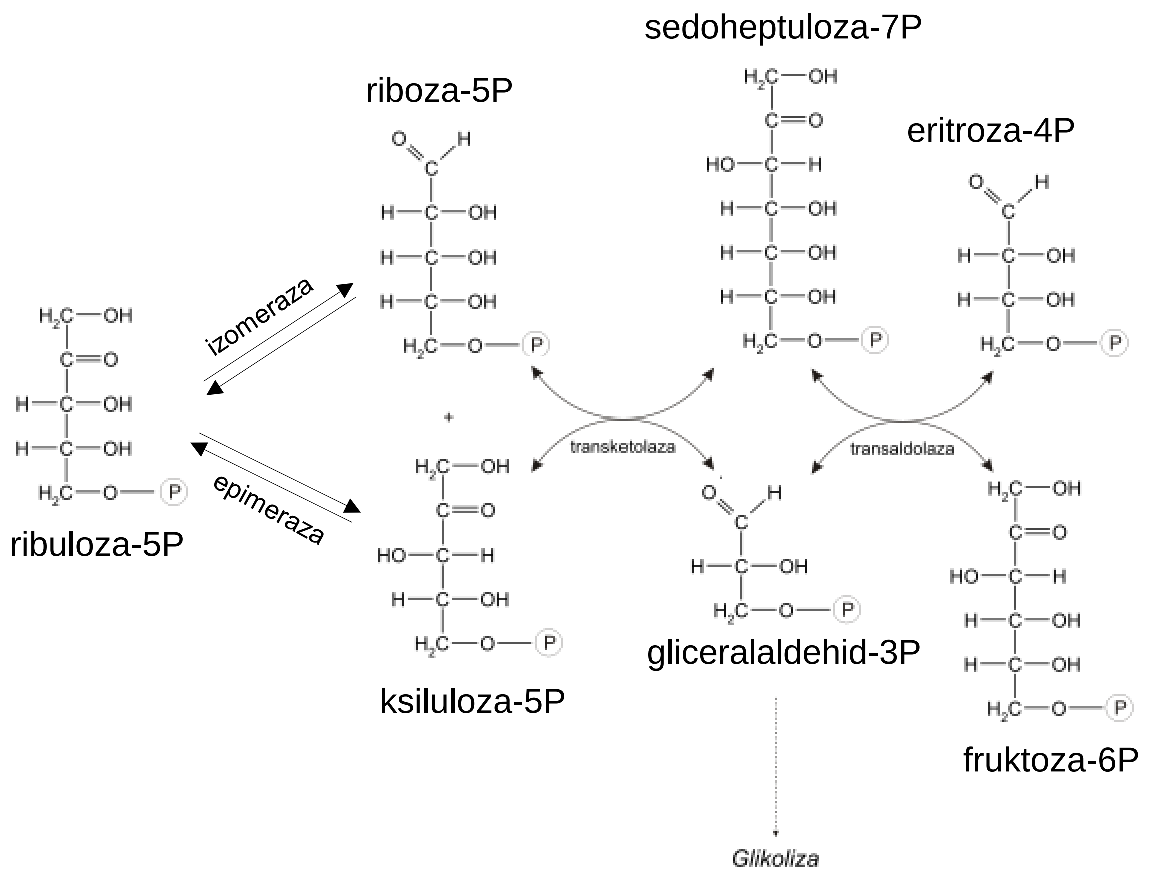


tiamin difosfat TPP (derivat vitamina B1),

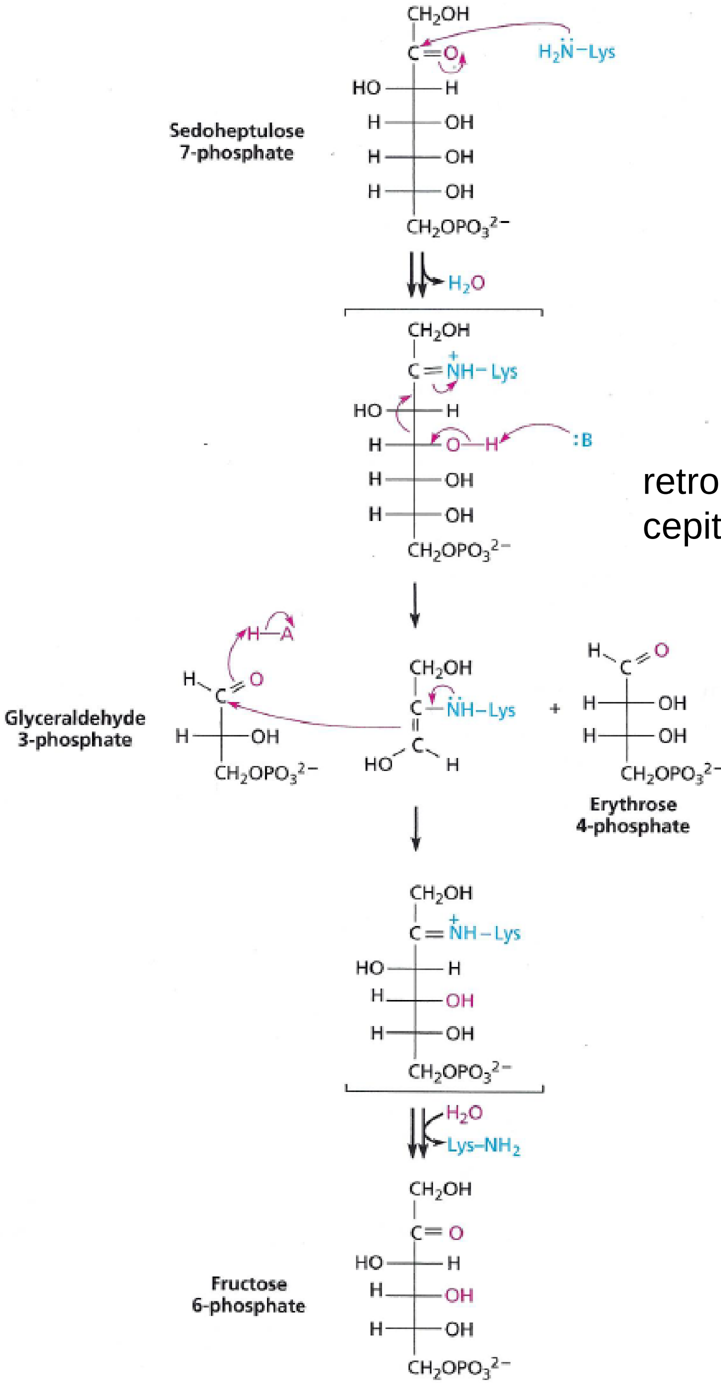
interni prenos protona, nastanek

karboaniona, močnega nukleofila

Neoksidativni del pentoza fostat metabolne poti

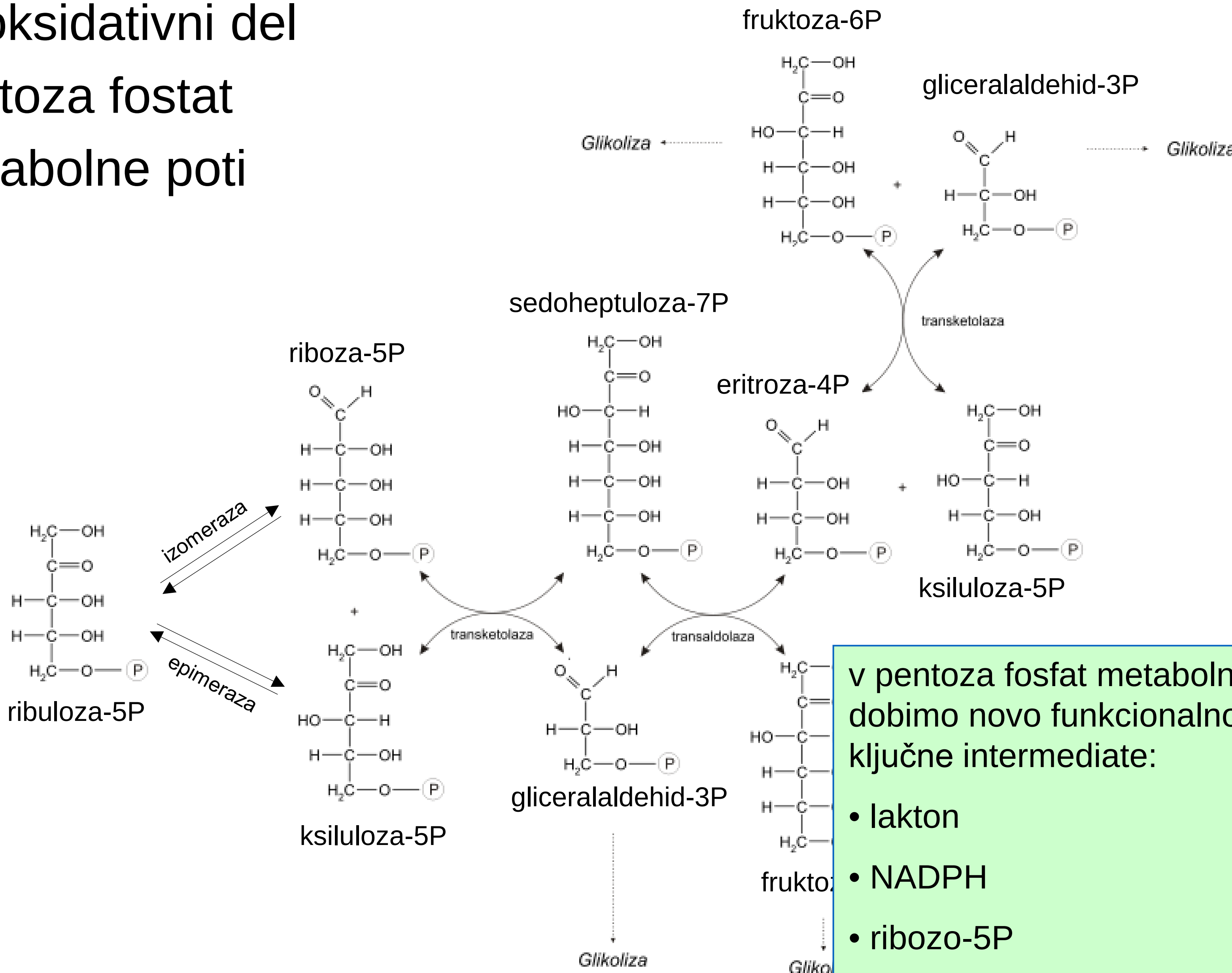


Transaldolaza



retro-aldolna cepitev

Neoksidativni del pentoza fostat metabolne poti



v pentoza fosfat metabolni poti dobimo novo funkcionalnost in ključne intermediate:

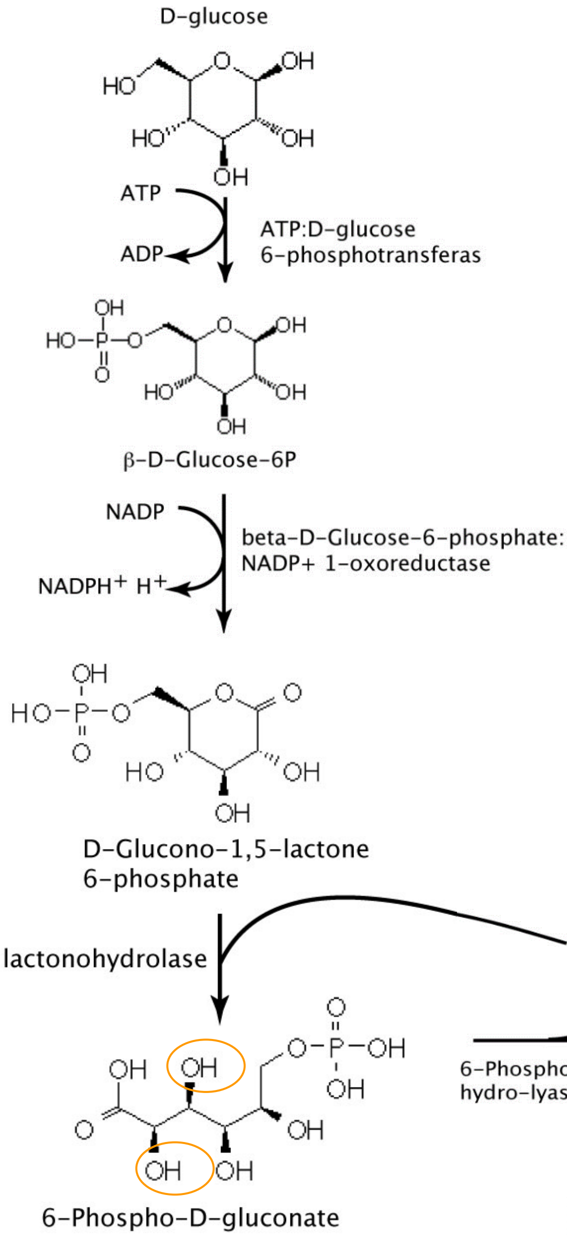
- lakton
- NADPH
- ribozo-5P
- eritrozo-4P

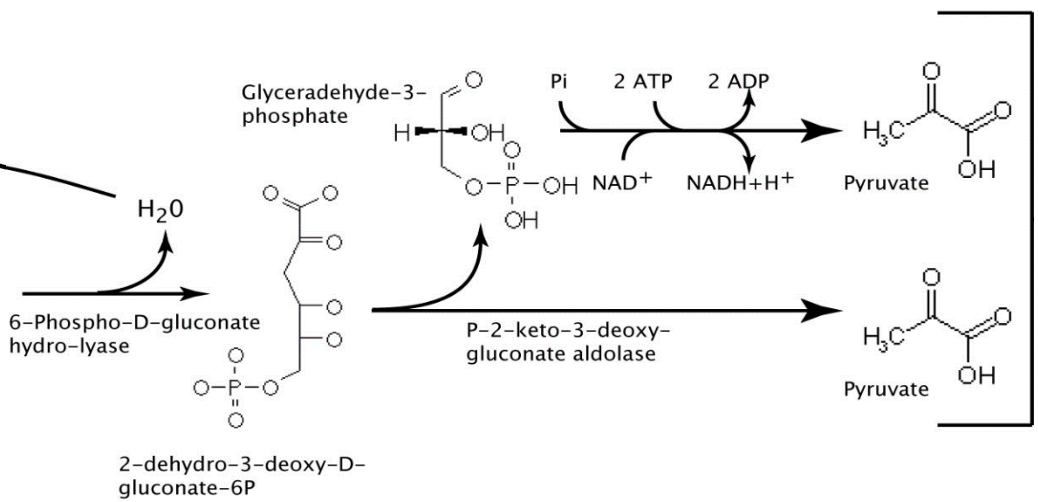
Entner-Doudoroff (ED) metabolna pot

to metabolno pot najdemo predvsem pri G-bakterijah (npr. pseudomonade), kompetitivna prednost je rast na glukonatih

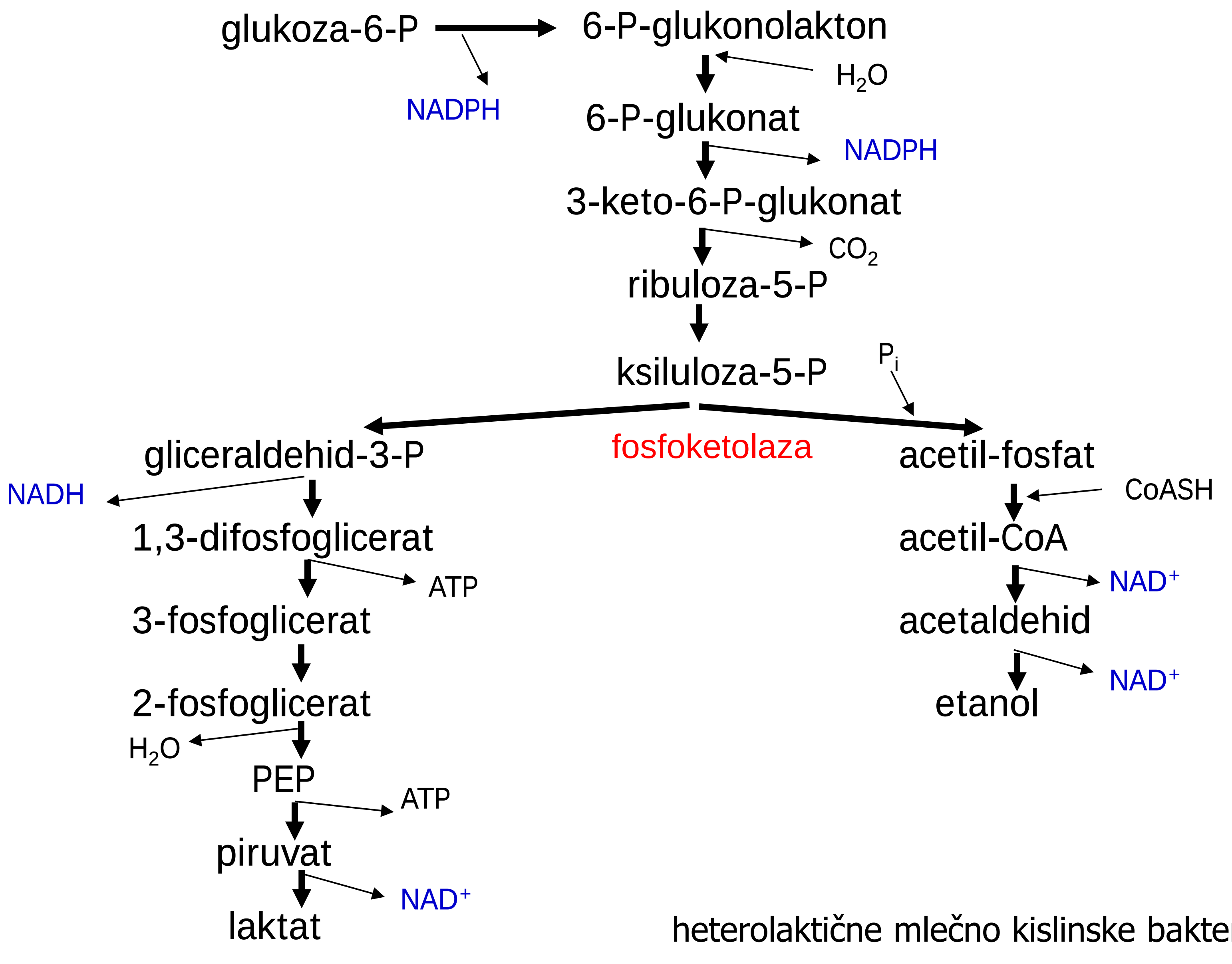
dehidracija alkoholov

- eliminacija (nastanek alkena, tert>sec>prim)
- dehidracija vicinalnih diolov



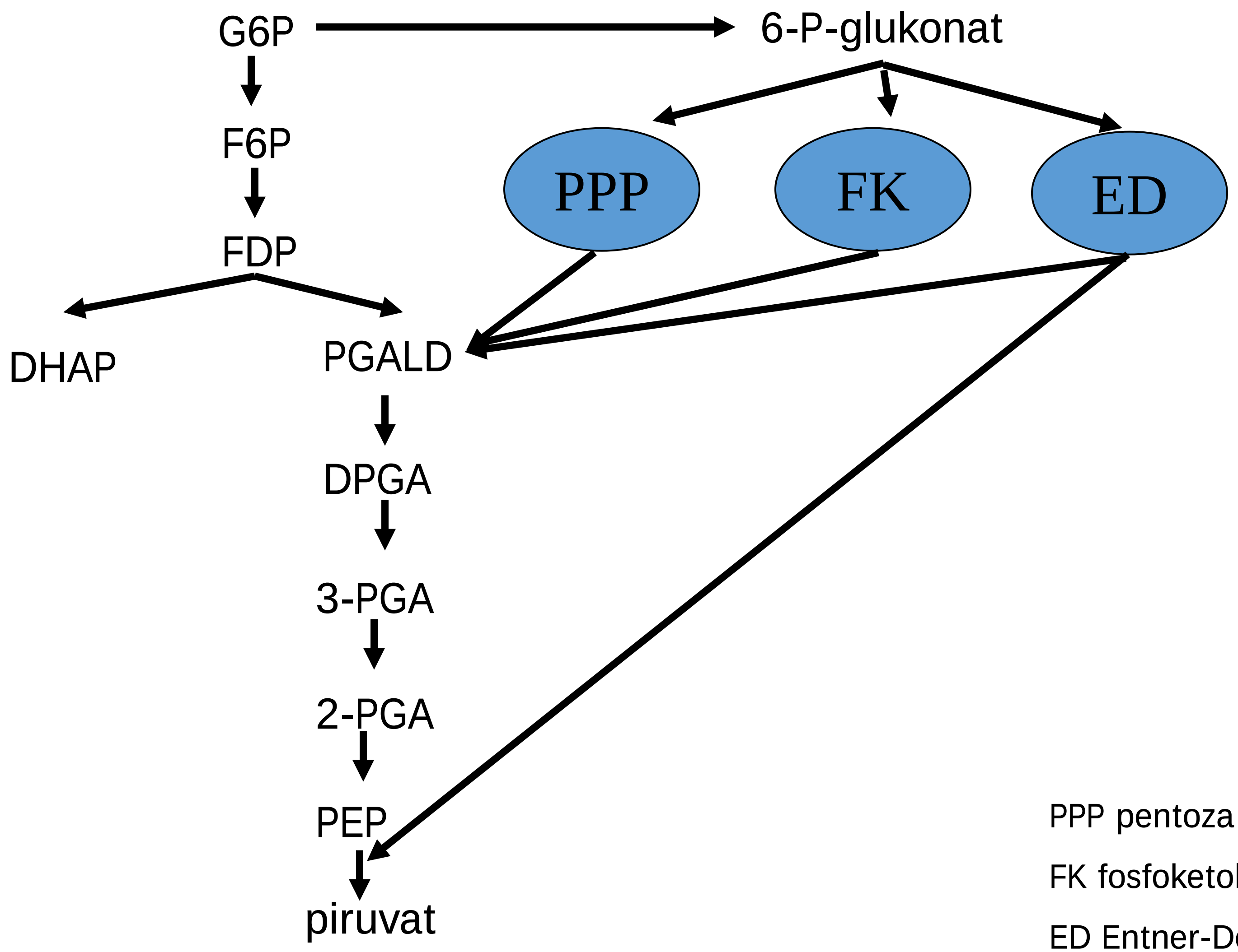


Fosfoketolazna (FK) metabolna pot



heterolaktične mlečno kislinske bakterije

Povezave med različnimi metabolnimi potmi

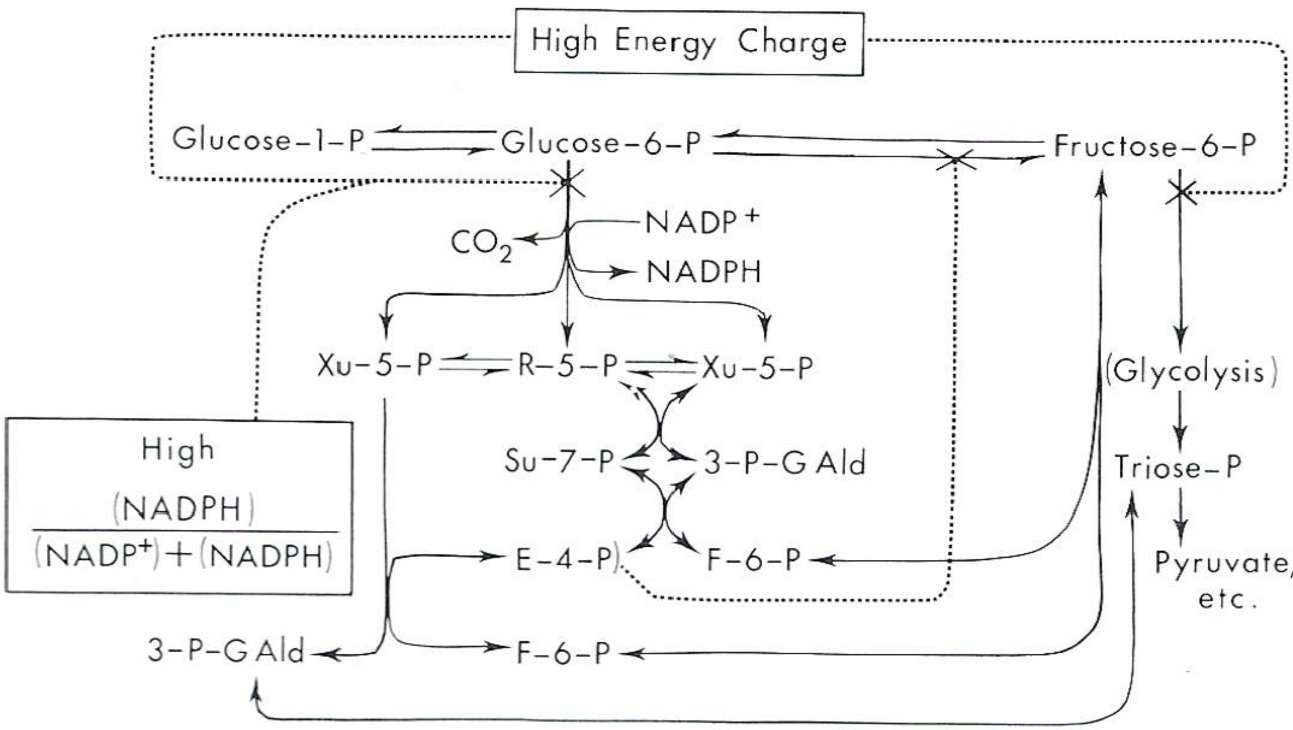


PPP pentoza fosfatna pot

FK fosfoketolazna pot

ED Entner-Doudoroff pot

Regulacija vstopa v pentoza fosfat metabolno pot z energijskim nabojem in NADPH/NADP$^+$



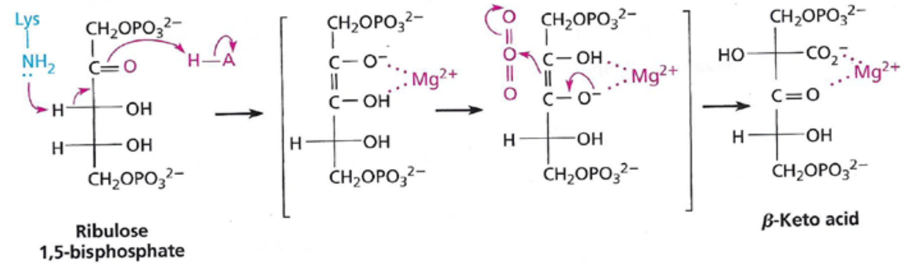
$$3\ G\text{-}6\text{-}P + 6\ NADP^+ \longrightarrow 2\ F\text{-}6\text{-}P + 3\ CO_2 + 3\ PGAld + 6\ NADPH + 6\ H^+ + 3\ H_2O$$

energijski naboj

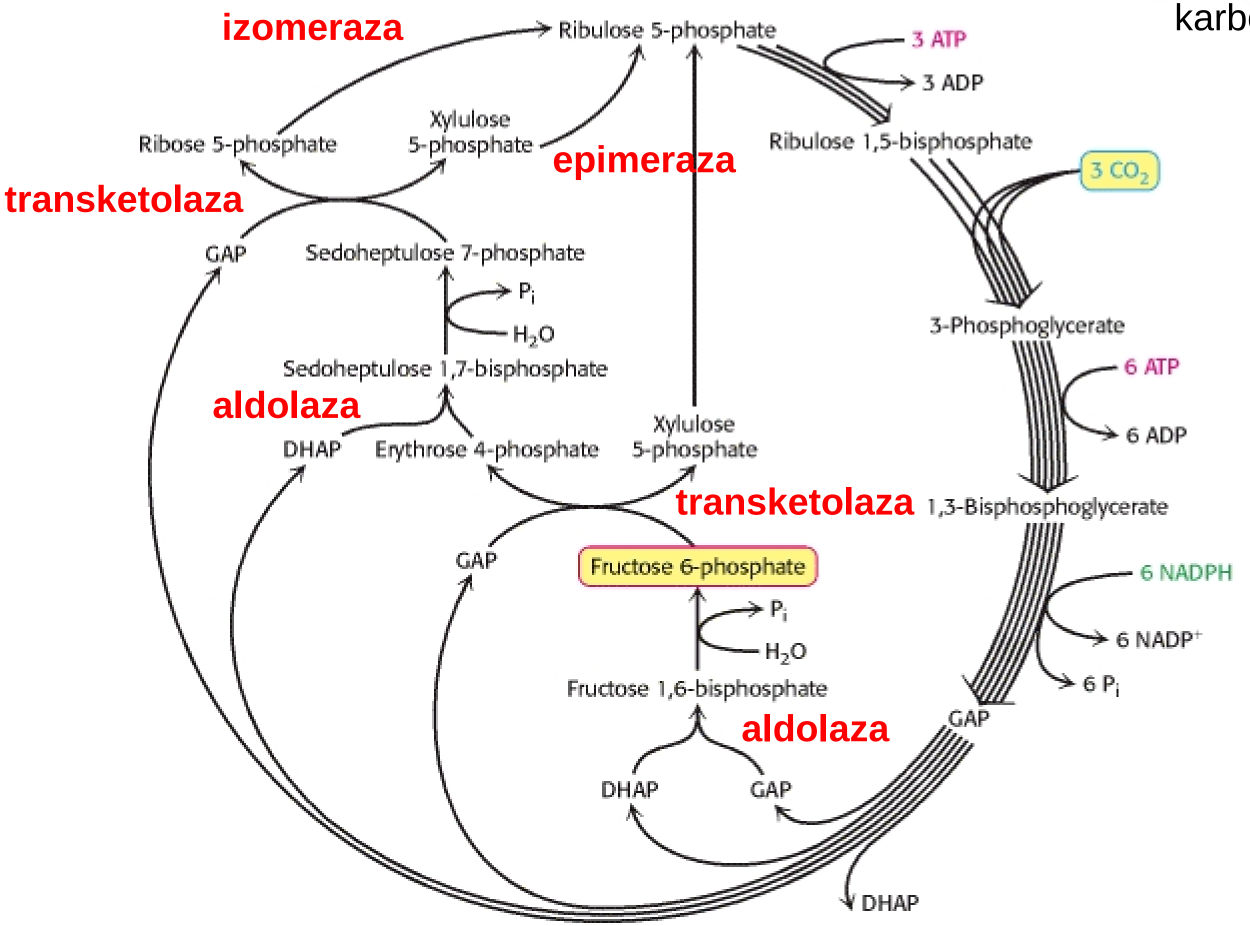
$$\frac{ATP + \frac{1}{2}\ ADP}{ATP + ADP + ADP}$$

vstop v pentoza fosfat metabolno pot blokira visok energijski naboj in visok delež NADPH

Calvinov cikel in fruktoza 6P

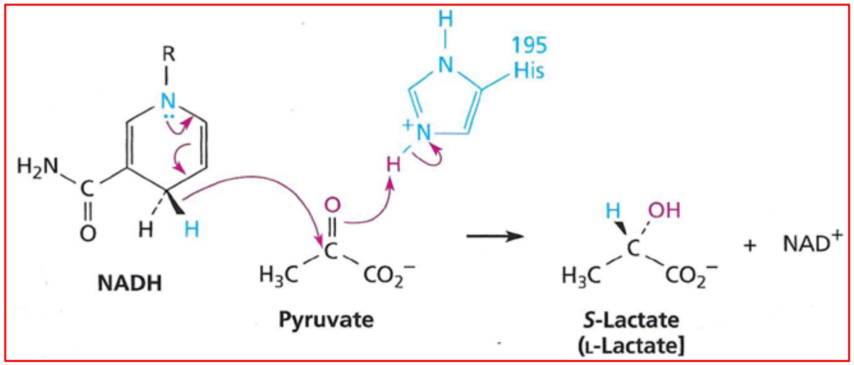


karboksilacija 1,5-ribulozefosfat



Transformacije piruvata

konverzija v laktat

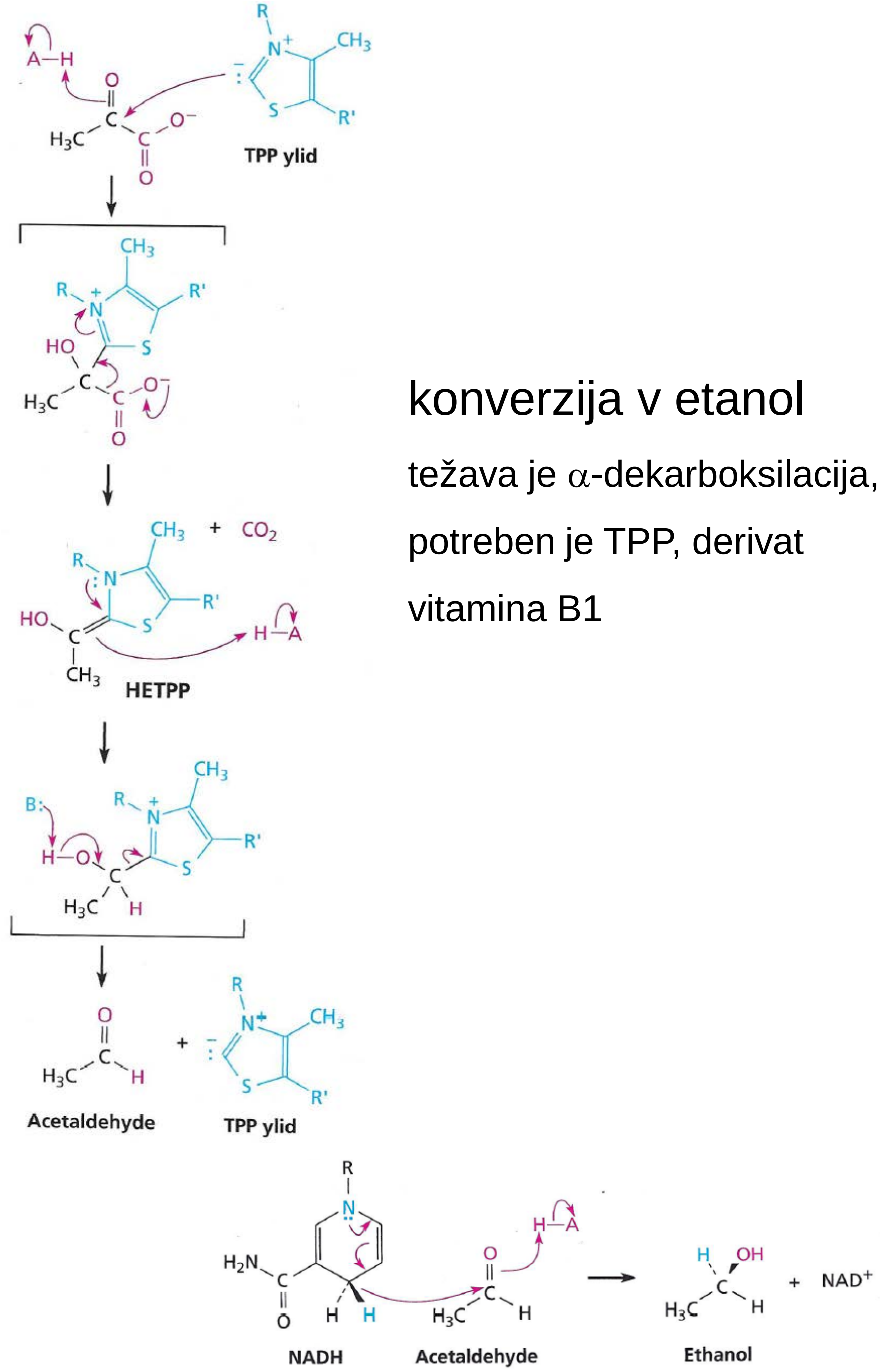


konverzija v etanol

težava je α-dekarboksilacija,

potreben je TPP, derivat

vitamina B1



TCA cikel

- oksidacija acetil-CoA, ketonskih teles, maščobnih kislin, amino kislin

- vezni člen za glukoneogenezo, lipigenezo in metabolizem aminokislin

regulacija na nivoju:

- piruvat dehidrogenaze
- izocitrat dehidrogenaze

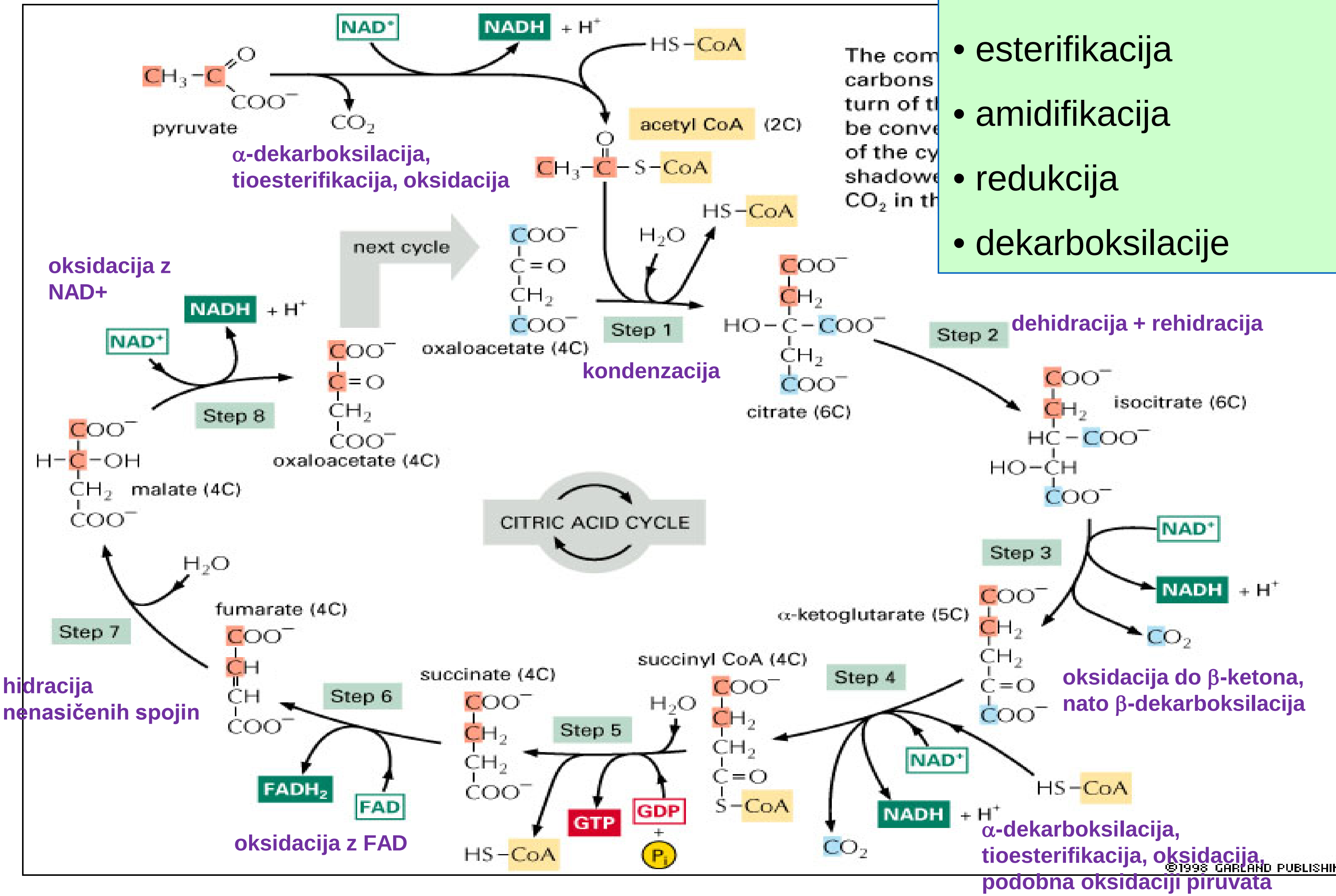
- zaviralci TCA cikla: visoke koncentracije ATP, NADH, acetil-CoA

- pospeševalci: piruvat, Ca^{2+}

Transformacija piruvata - TCA cikel

Reakcije karboksilnih skupin:

- tvorba soli
- esterifikacija
- amidifikacija
- redukcija
- dekarboksilacije



Mehanizem piruvat dehidrogenaze: α-dekarboksilacija

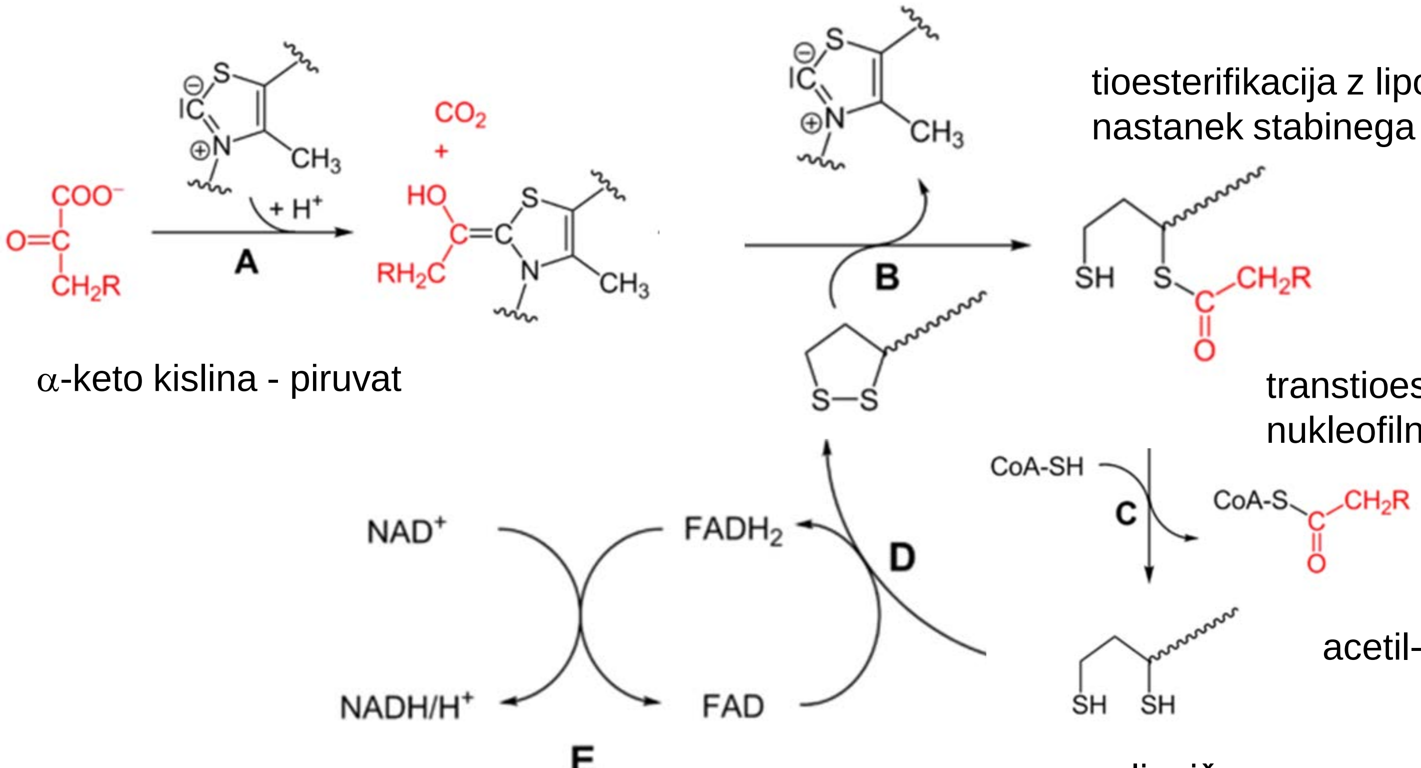
α - dekarboksilacija,

mnogo bolj zahtevna od

β-dekarboksilacije

tiamin difosfat (TPP, derivat vitamina B1)

stabilizira intermediat aldehidni karboanion



tioesterifikacija z lipoatom, prepreči nastanek stabinega acetata

α-keto kislina - piruvat

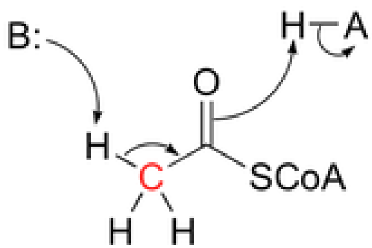
transtioesterifikacija, nukleofilna acilna substitucija

acetil-CoA

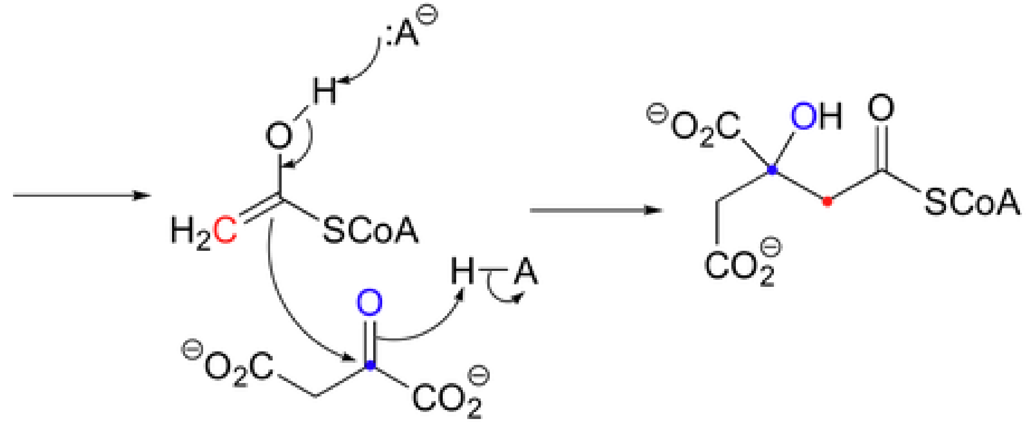
lipoična kislina

regeneracija lipoata, oksidacija in prenos e^- na NAD^+

Mehanizem citrat sintaze

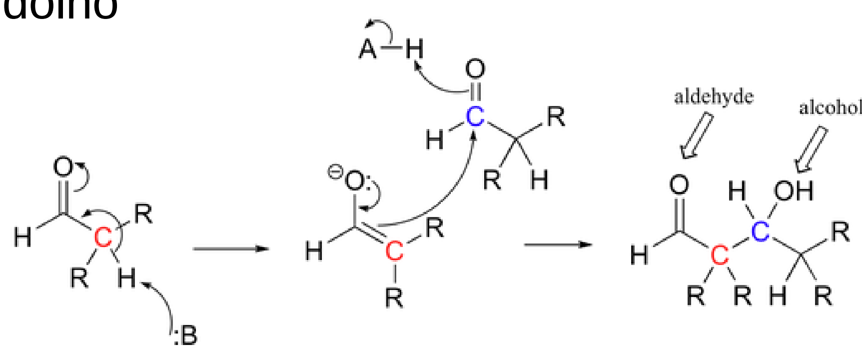


nastanek enola iz
acetil-CoA



nukleofilni napad karboaniona enola
na karbonil oksalacetata

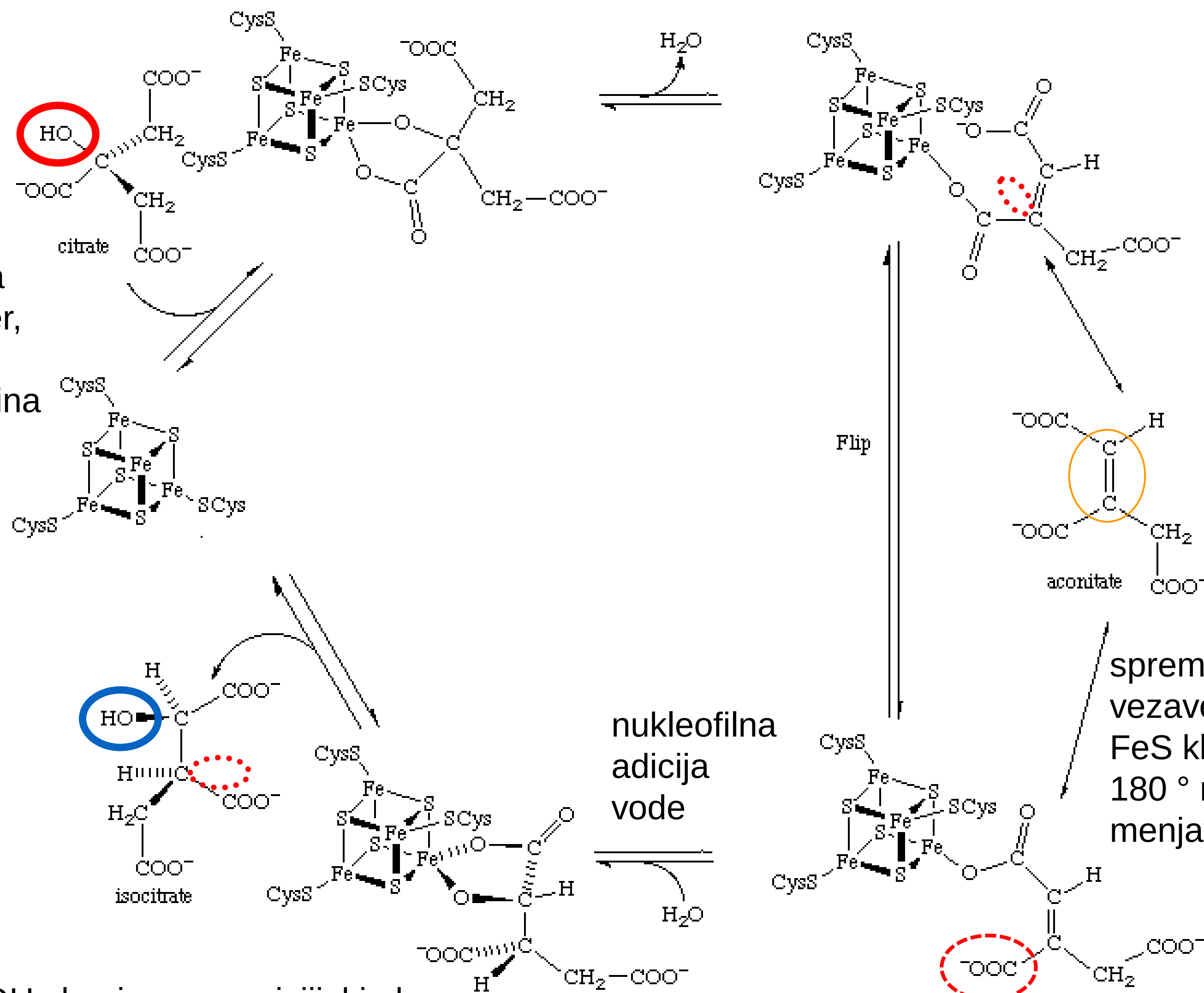
podobnost z aldolno
kondenzacijo



Mehanizem akonitat hidrataze

dehidracija, nastanek dvojne vezi, izguba OH

vezava citrata na FeS klaster, deluje kot Lewisova kislina

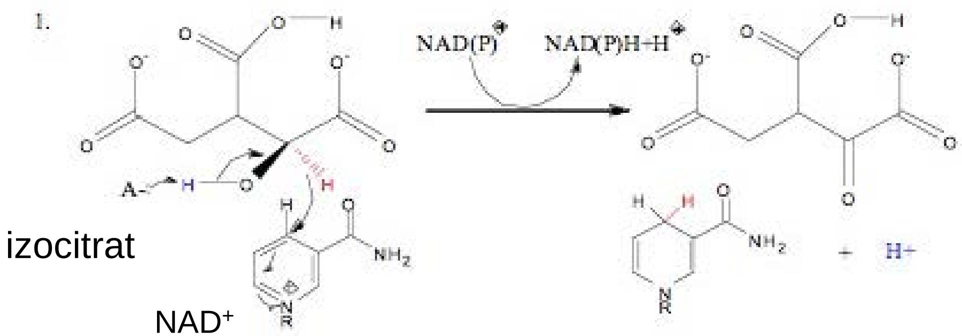


nukleofilna adicija vode

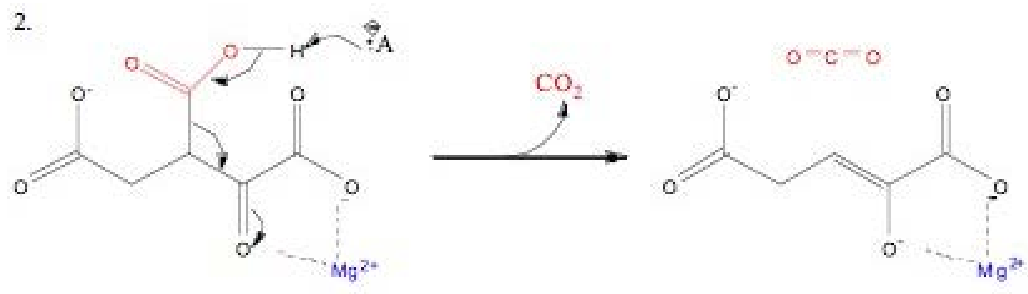
sprememba mesta vezave akonitata na FeS klaster, rotacija za 180 ° možnost za menjavo steroizomerije

pridobili smo OH skupino na poziciji, ki ob nadaljnji oksidaciji omogoča β-dekarboksilacijo sredinske karboksilne skupine

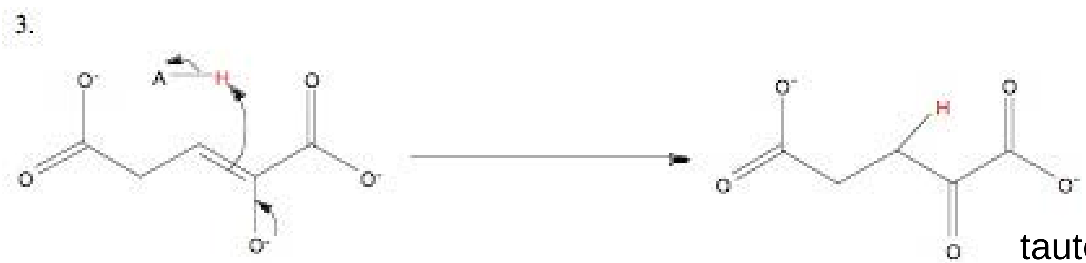
Mehanizem izocitrat dehidrogenaze



oksidacija sekundarnega alkohola, dobimo mesto za odlaganje elektronov pri β-dekarboksilaciji izocitrata

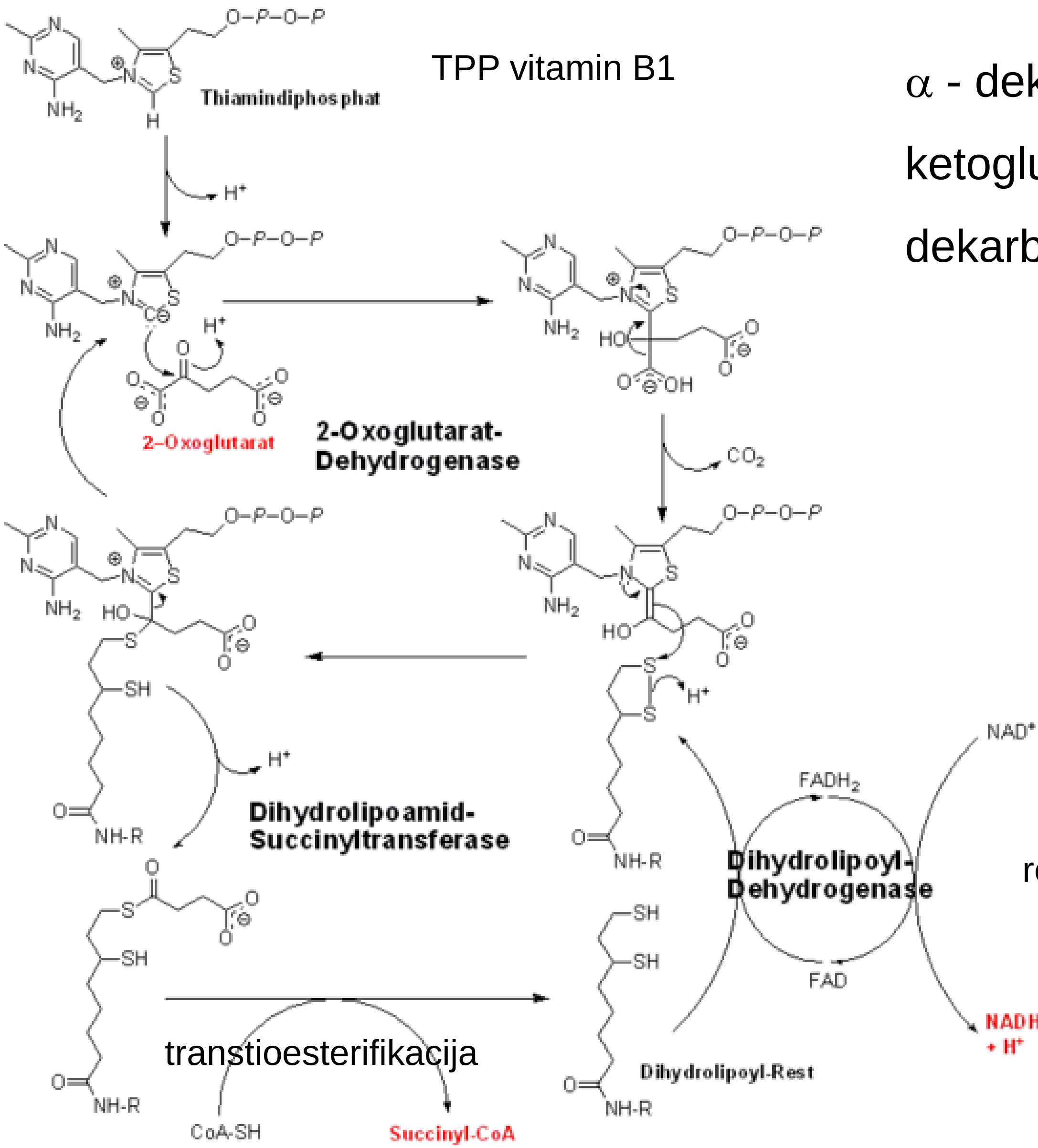


β-dekarboksilacija izocitrata



tautomerizacija in nastanek stabilnega α-ketoglutarata

Mehanizem α-ketoglutarat dehidrogenaze

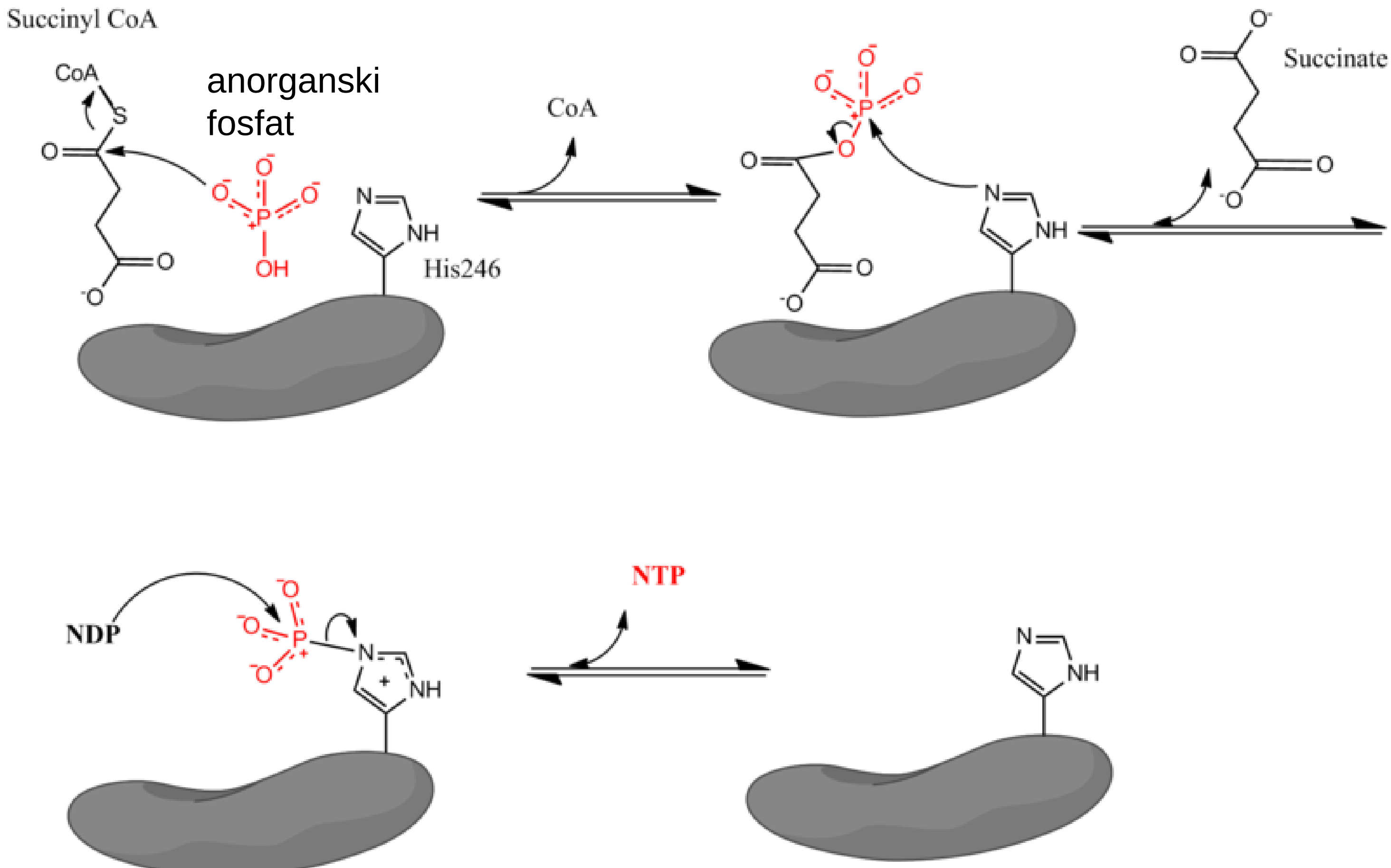


α - dekarboksilacija α-ketoglutarata, podobna α-dekarboksilaciji piruvata

regeneracija lipolata

Mehanizem sukcinil-CoA sintetaze: substratna fosforilacija

GTP s pomočjo anorganskega fosfata



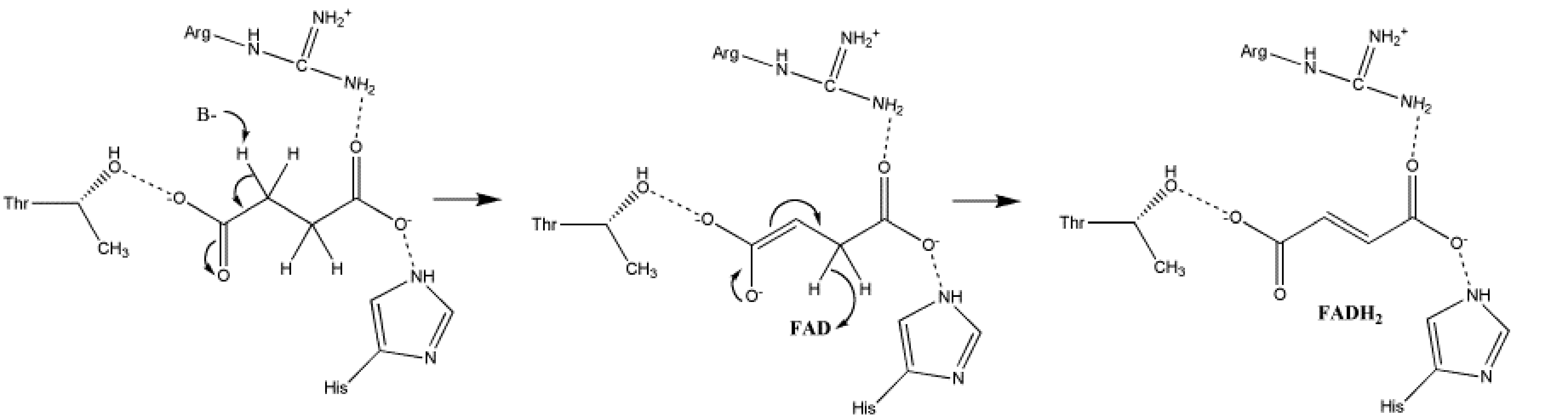
substratna fosforilacija,

nastanek GTP

Mehanizem sukcinat dehidrogenaze

sukcinat

fumarat



$NAD^+/NADH$ $E_h°$= -0.32 V

$FAD/FADH_2$ $E_h°$=-0.22 V

fumarat/sukcinat $E_h°$= 0.03 V

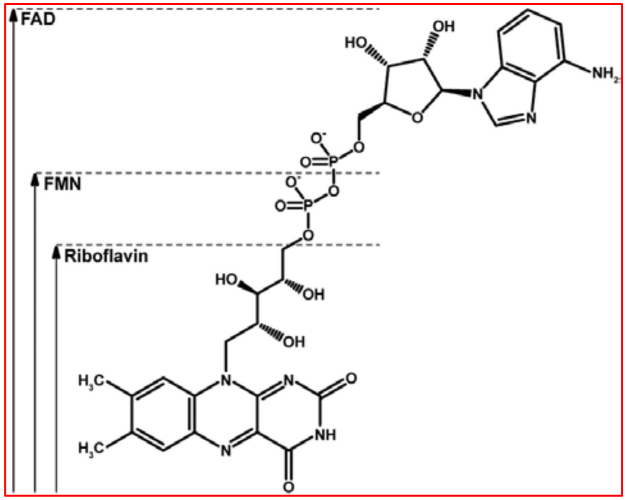
oksidacija sukcinata s FAD

kot zunanjim prevzemnikom

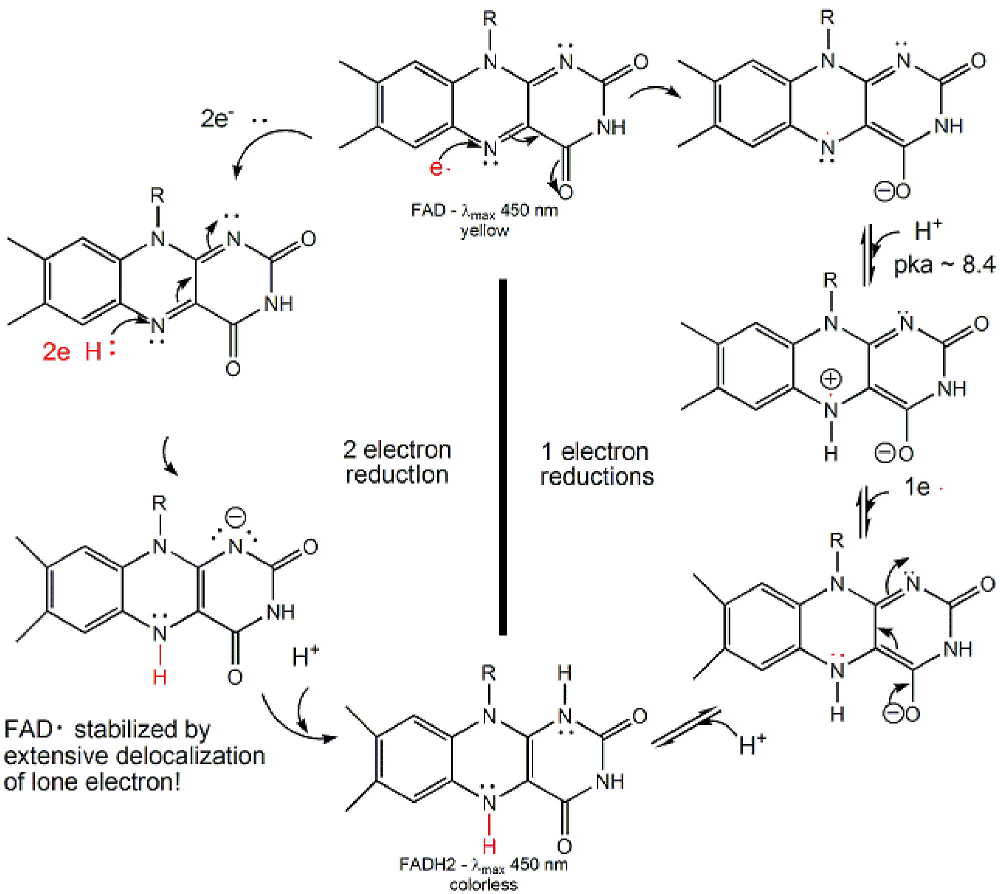
hidridnega aniona (:H), ni

dovolj energije za redukcijo

NAD+



FAD / FMN (flavin adenin dinukleotid / flavin mononukleotid)



s FAD je možen prenos enega ali dveh elektronov, vodika ali protona

močna vezave FAD na encim prepreči oksidacijo s kisikom (dobro, sicer bi nastajal ROS)

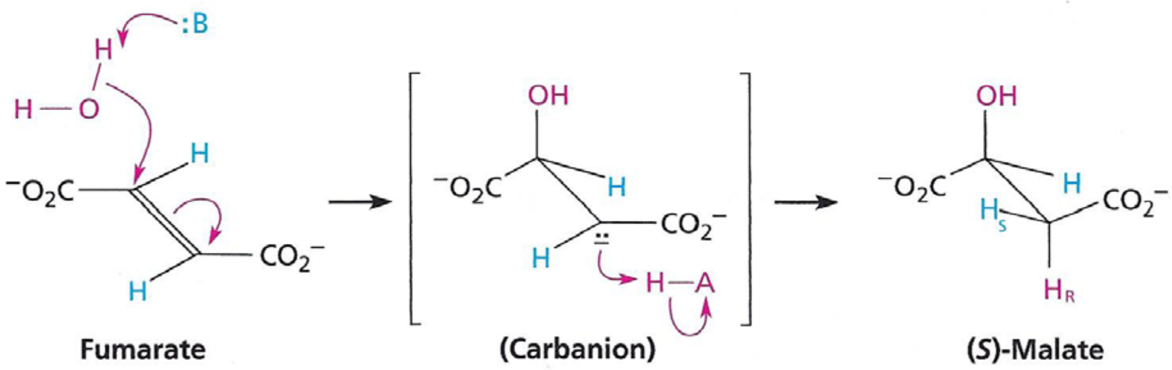
ker ni reoksidacije s kisikom je potreben drug reoksidacijski agens (potreben NAD^+)

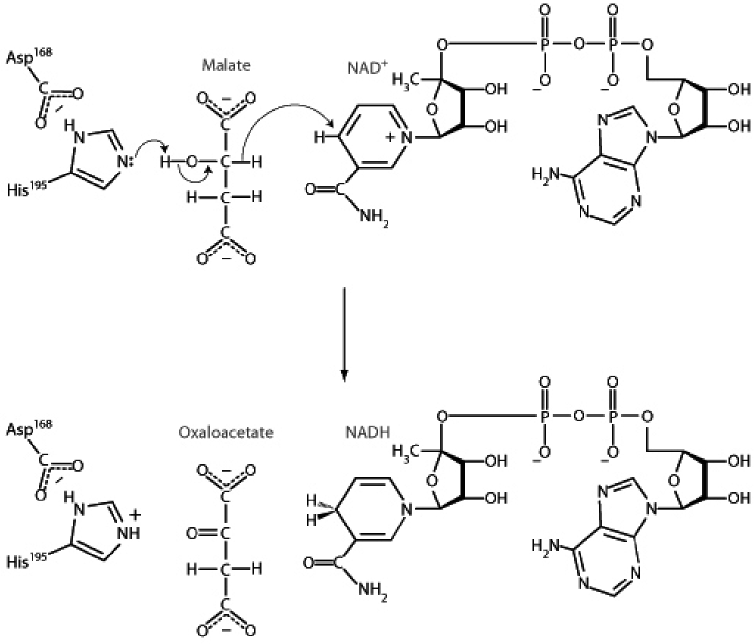
biosintetska selektivnost:

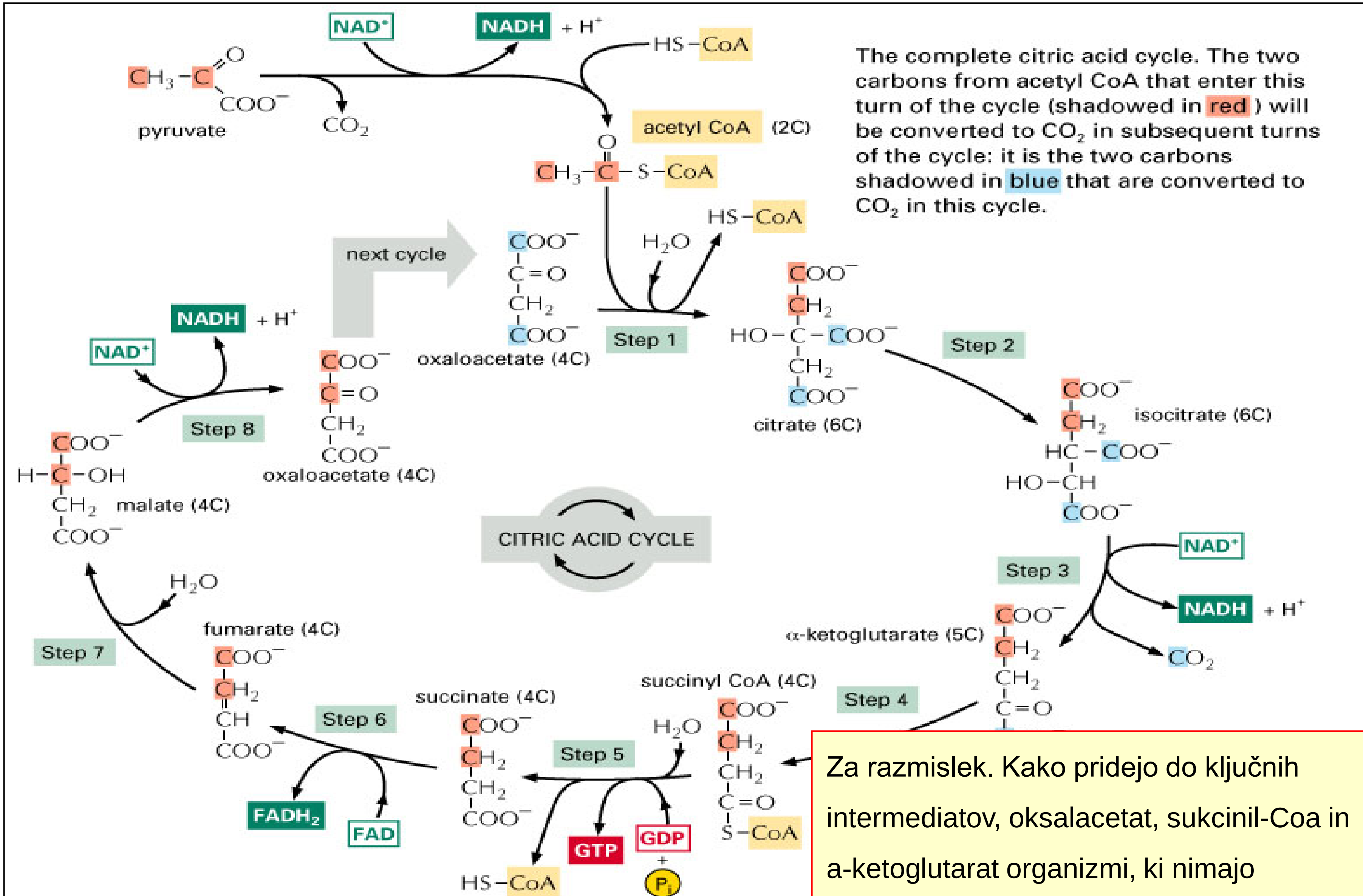
NADPH: biosinteza lipidov, DNA, ROS

FAD: nukleotidna biosinteza, sinteza CoA, hema

Hidracija fumarata in oksidacija do oksalacetata



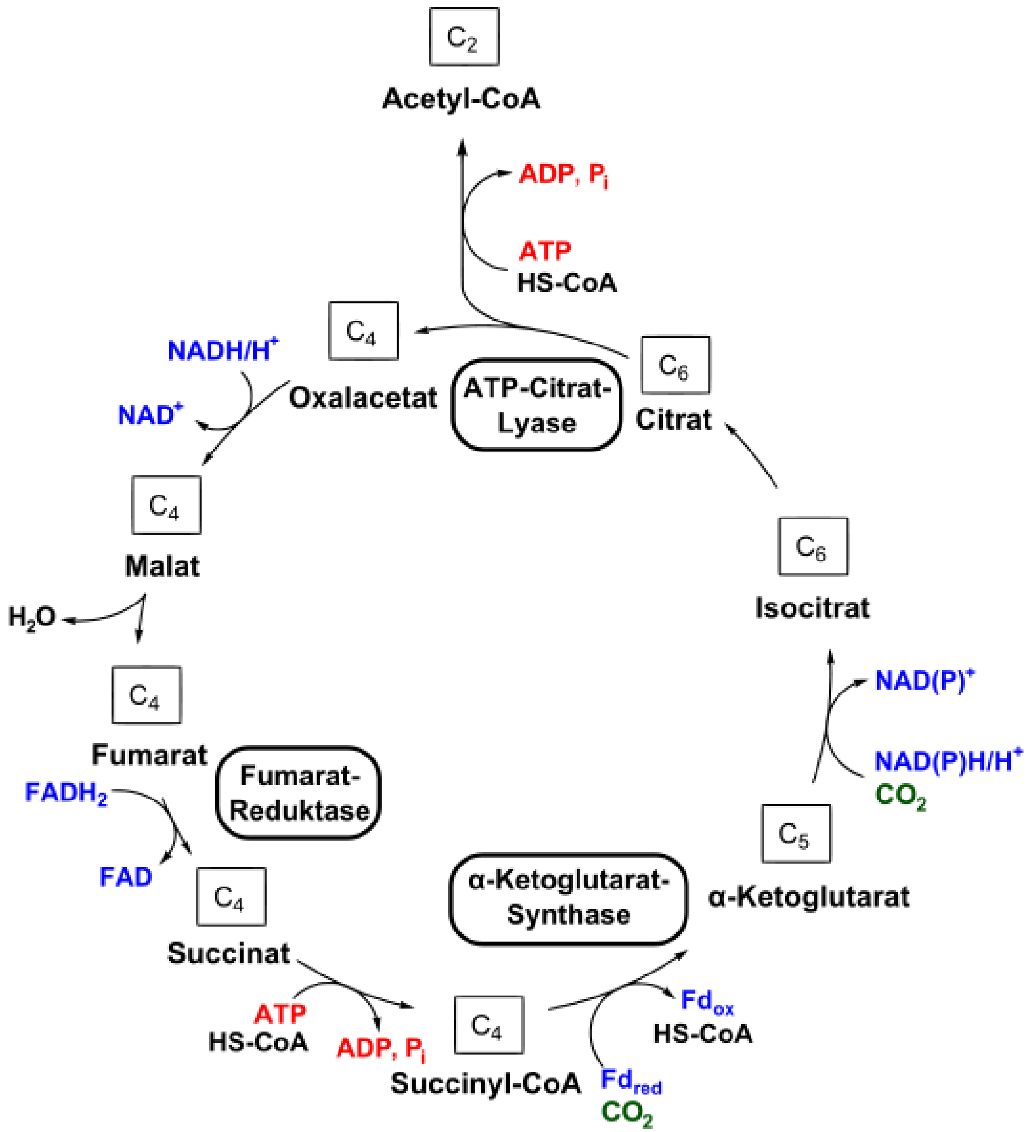




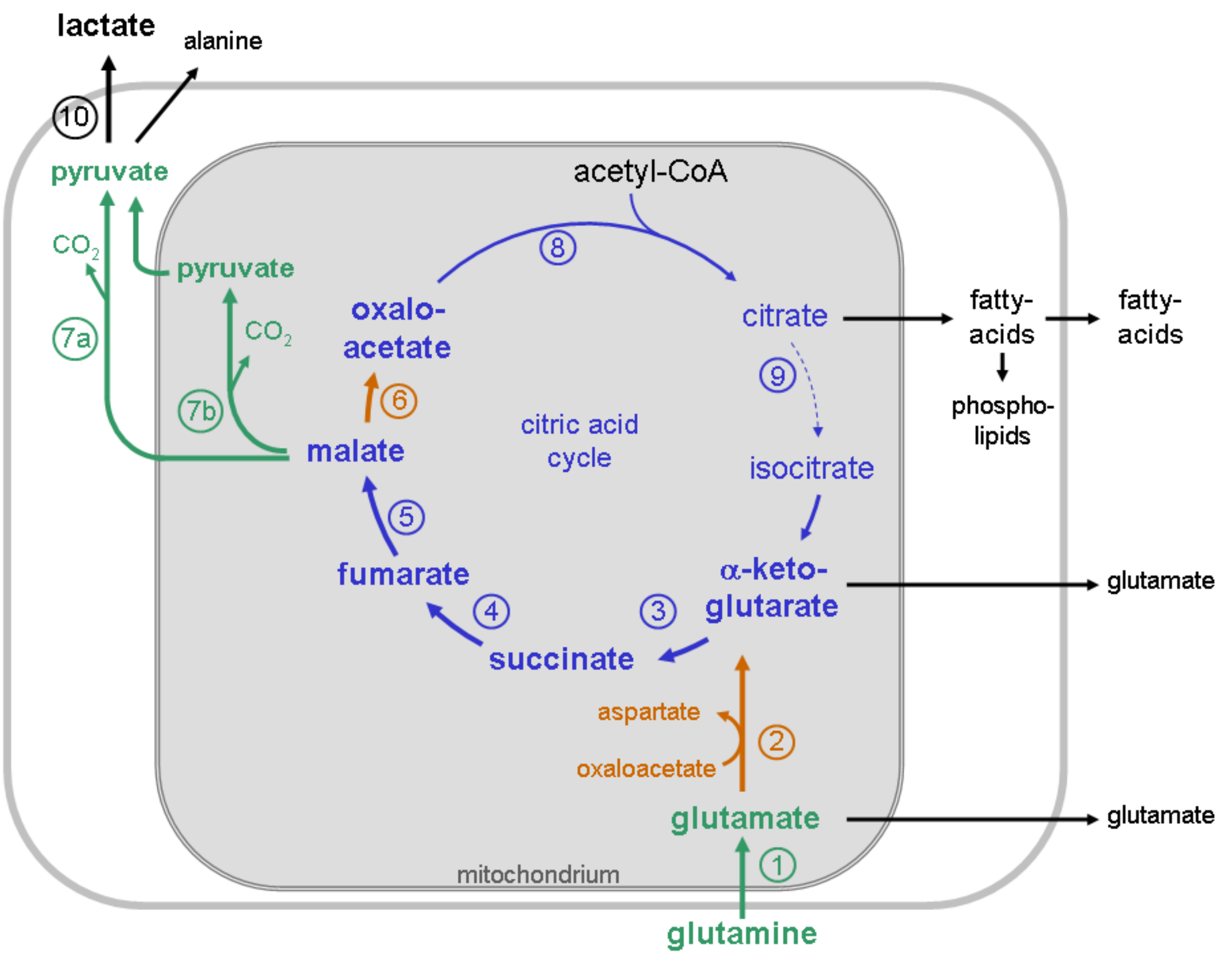
The complete citric acid cycle. The two carbons from acetyl CoA that enter this turn of the cycle (shadowed in red) will be converted to CO_2 in subsequent turns of the cycle: it is the two carbons shadowed in blue that are converted to CO_2 in this cycle.

Za razmislek. Kako pridejo do ključnih intermediatov, oksalacetat, sukcinil-Coa in a-ketoglutarat organizmi, ki nimajo Krebsovega cikla?

Modifikacije Krebsovega cikla - reverzni TCA cikel

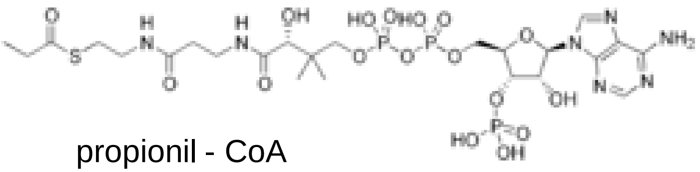


Modifikacije Krebsovega cikla - glutaminoliza

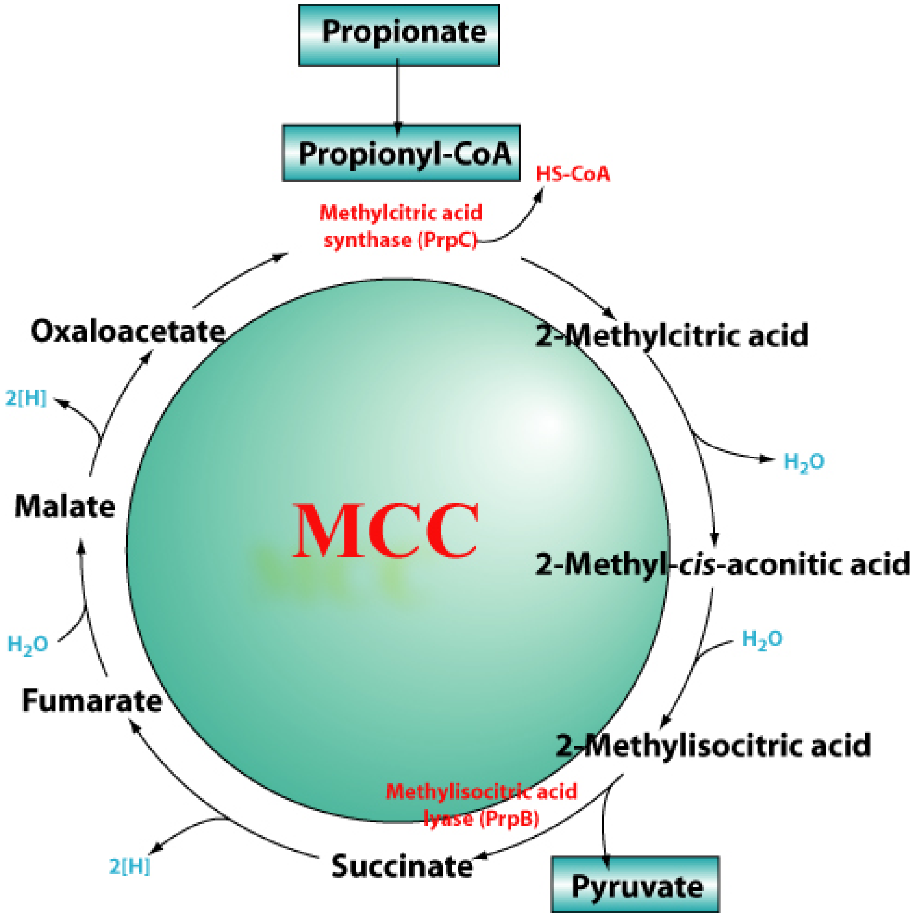


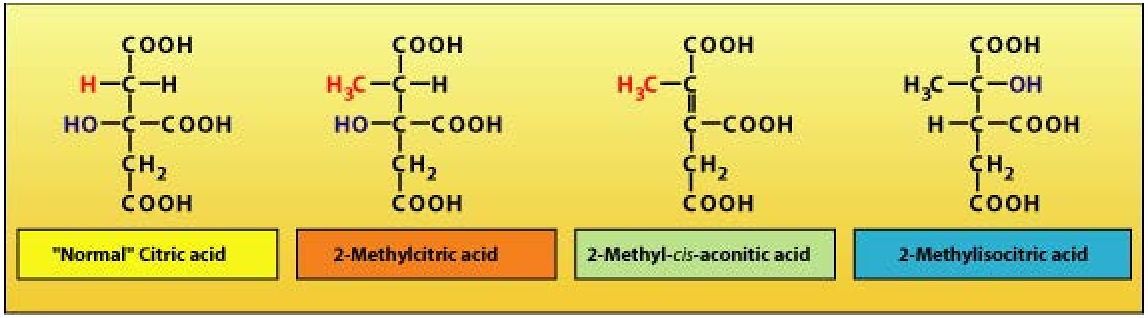
pri evkariontih (npr. rakastih celicah) omogoča rast na glutaminu kot edinemu viru ogljika in dušika

Modifikacije Krebsovega cikla- alternativni TCA cikel

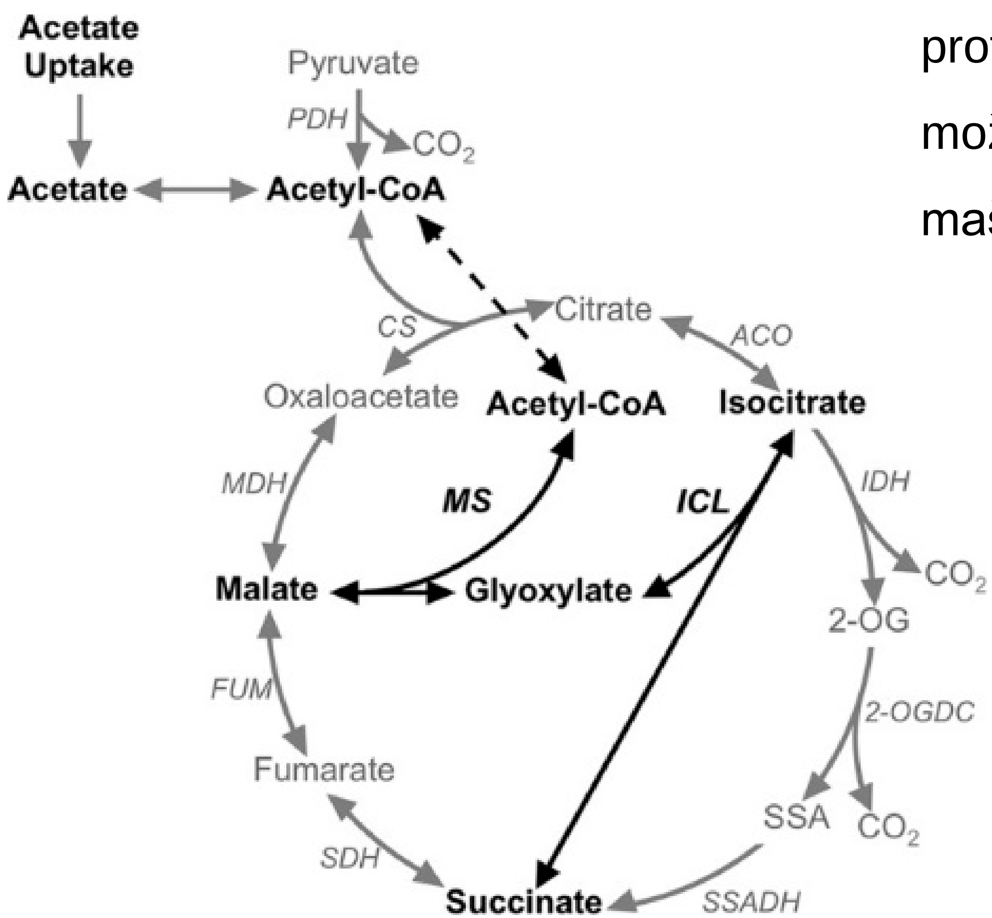


propionil - CoA





Modifikacije Krebsovega cikla - glioksilatni cikel

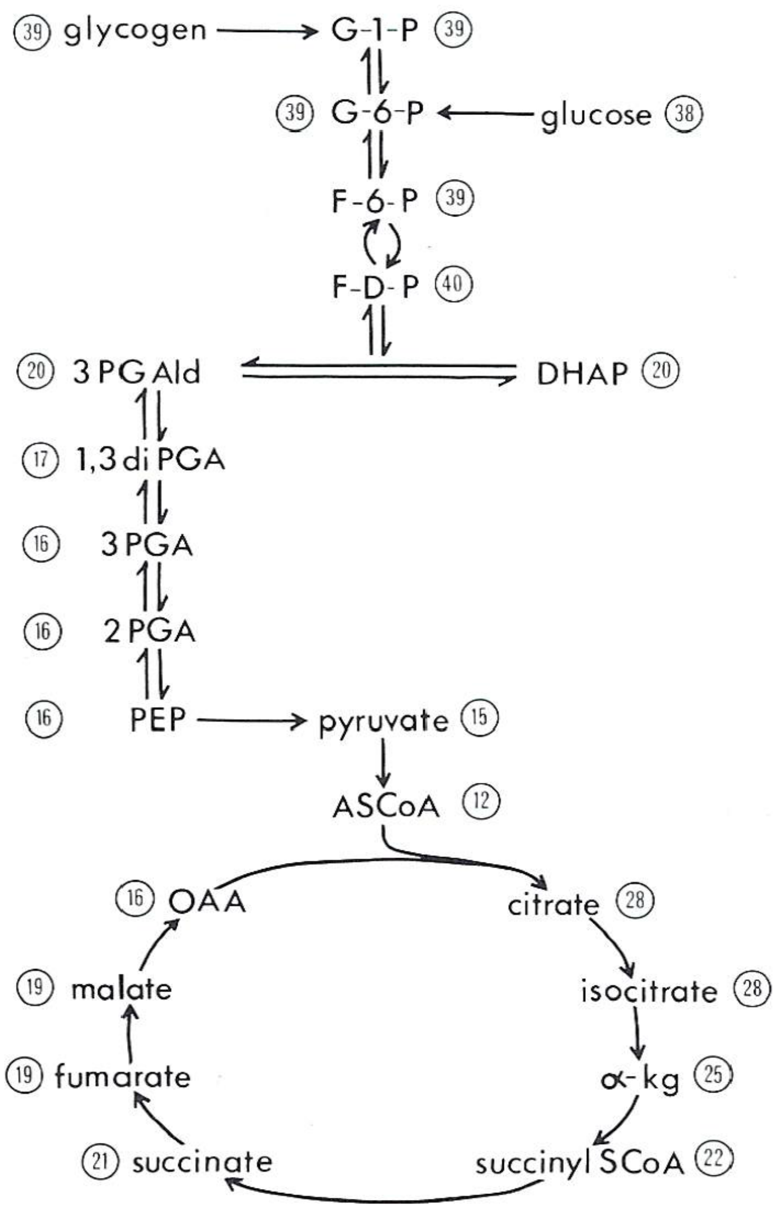


prisoten pri bakterijah, arhejah, protozojih, glivah in rastlinah, možnost uporabe acetata in maščob za biosintetske potrebe

Metabolna cena sklopitvenih agensov (ATP ekvivalenti)

sklopitveni agens	ATP ekvivalent
ATP → ADP	1
ATP → AMP	2
NADPH → $NADP^+$	4*
NADH → NAD^+	3
$FADH_2$ → FAD	2

*NADH + $NADP^+$ + ATP + H_2O → NAD^+ + NADPH + ADP + P_i
v primeru da nastane v pentoza fosfat metabolni poti ali z malat dekarboksilazo je vreden 3 ATP



Metabolna cena intermediatov (ATP ekvivalenti)

cena vezana na število ATP ekvivalentov pri oksidaciji intermediata (štartamo iz acetil-CoA, ki je v enem ciklu popolnoma oksidiran do CO_2, pri tem se sprosti 12 mol ATP na mol oksidiranega acetata)

Metabolna cena intermediatov (ATP ekvivalenti)

Cost, in ATP Equivalents, of Synthesizing Nucleotides

Product[a]	Starting materials	Conversion requirements	Cost
[IMP][b]	PRPP (35); glycine (12)	2 Glutamine → 2 glutamate (2); 4 ATP → 4 ADP (4); 2 formyl H_4-folate → 2 H_4-folate (4); aspartate → fumarate (2)	59
AMP	IMP (59)	GTP → GDP (1); aspartate → fumarate (2)	62
GMP	IMP (59)	NAD^+ → NADH (−3); ATP → AMP (2); glutamine → glutamate (1)	59
[OMP]	Aspartate (21); carbamyl-P (2) PRPP (35)	NAD^+ → NADH (−3)	55
CTP	OMP (55)	Glutamine → glutamate (1); 3 ATP → 3 ADP (3)	59
UMP	OMP (55)		55
dADP	AMP (62)	NADPH → $NADP^+$ (4); ATP → ADP (1)	67
dGDP	GMP (59)	NADPH → NAD^+ (4); ATP → ADP (1)	64
dCDP	CTP (59)	NADPH → NAD^+ (4); ADP → ATP (−1)	62
dTDP	UMP (55)	NADPH → $NADP^+$ (4); ATP → ADP (1); $HOCH_2 \cdot H_4$-folate → H_2-folate (6)	66

[a] This table lists the ribonucleoside monophosphates and the deoxyribonucleoside diphosphates because these are the first products, except that CTP appears to be the first cytosine nucleotide produced. Triphosphates obviously cost 2 ATP equivalents more than monophosphates and 1 equivalent more than diphosphates.

[b] Products in brackets are intermediates for which the synthesis cost must be calculated.

Cost, in ATP Equivalents, of Synthesizing Amino Acids[a,b]

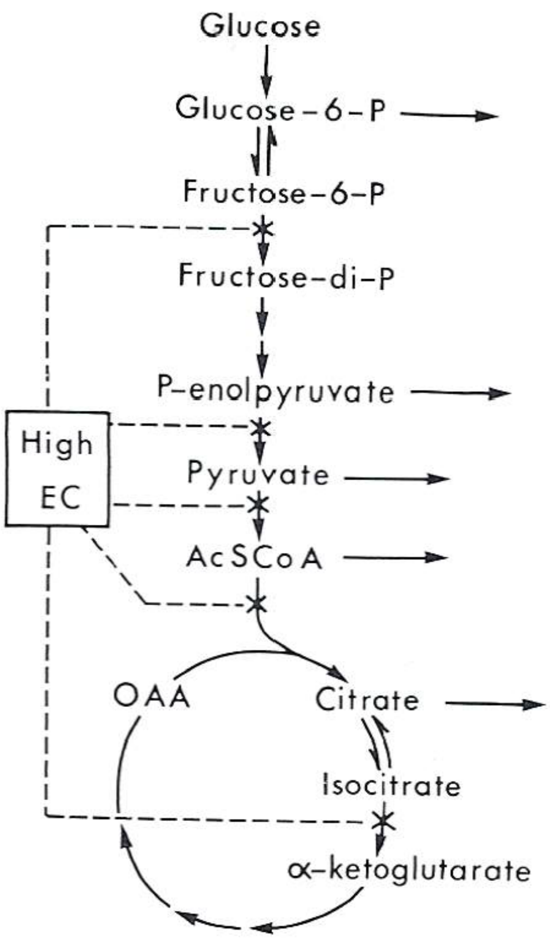
Amino acid product	Starting materials	Conversion requirements	Cost
Glutamate	α-Ketoglutarate (25)[c]	NADPH (4)[c]; ATP (1)	30
Aspartate	Oxaloacetate (16)	trans-NH_2 (5)[d]	21
Glutamine	Glutamate (30)	ATP (1)	31
Asparagine	Aspartate (21)	ATP (1)	22
Alanine	Pyruvate (15)	trans-NH_2 (5)	20
Serine	3-P-glycerate (16)	NAD^+ → NADH (−3); trans-NH_2 (5)	18
Glycine	Serine (18)	H_4-folate → methylene H_4-folate (−6)	12
Cysteine	Serine (18)	AcSCoA → AcOH (1)	19
Threonine	Aspartate (21)	2 NADPH (8); 2 ATP (2)	31
Isoleucine	Threonine (31); pyruvate (15)	NADPH (4); trans-NH_2 (5)	55
Valine	2 Pyruvate (30)	NADPH (4); trans-NH_2 (5)	39
Leucine	2 Pyruvate (30); AcSCoA (12)	trans-NH_2 (5)	47
Proline	Glutamate (30)	2 NADPH (8); ATP (1)	39
Arginine	Glutamate (30); carbamyl-P (2)	ATP (1); NADPH (4); trans-NH_2 (5); aspartate → fumarate (2)	44
Histidine	P-ribosyl-PP (35)	ATP → AICAR (7); trans-NH_2 (5); 2 NAD^+ → 2 NADH (−6); glutamine → glutamate (1)	42
[Chorismate][e]	2 P-enolpyruvate (32); erythrose 4-P (26)	NAD^+ → NADH (−3); ATP (1); NADPH (4)	60
Phenylalanine	Chorismate (60)	trans-NH_2 (5)	65
Tyrosine	Chorismate (60)	NAD^+ → NADH (−3); trans-NH_2 (5)	62
Tryptophan	Chorismate (60); P-ribosyl-PP (35) −Pyruvate (−15)	Glutamine → glutamate (1); serine → 3 P GAld (−3)	78
[Homocysteine]	Aspartate (21)	Cysteine → pyruvate (4); 2 NADPH (8); SuccSCoA → succinate (1); ATP (1)	35
Methionine	Homocysteine (35)	Me H_4-folate → H_4-folate (9)	44
Lysine (diaminopimelate pathway)	Pyruvate (15); aspartate (21)	ATP (1); 2 NADPH (8); SuccSCoA → succ (1); trans-NH_2 (5)	51
Lysine (aminoadipate pathway)	α-Ketoglutarate (25); AcSCoA (12)	2 NAD^+ → 2 NADH (−6); 2 NADPH (8); 2 trans-NH_2 (10); ATP (1)	50

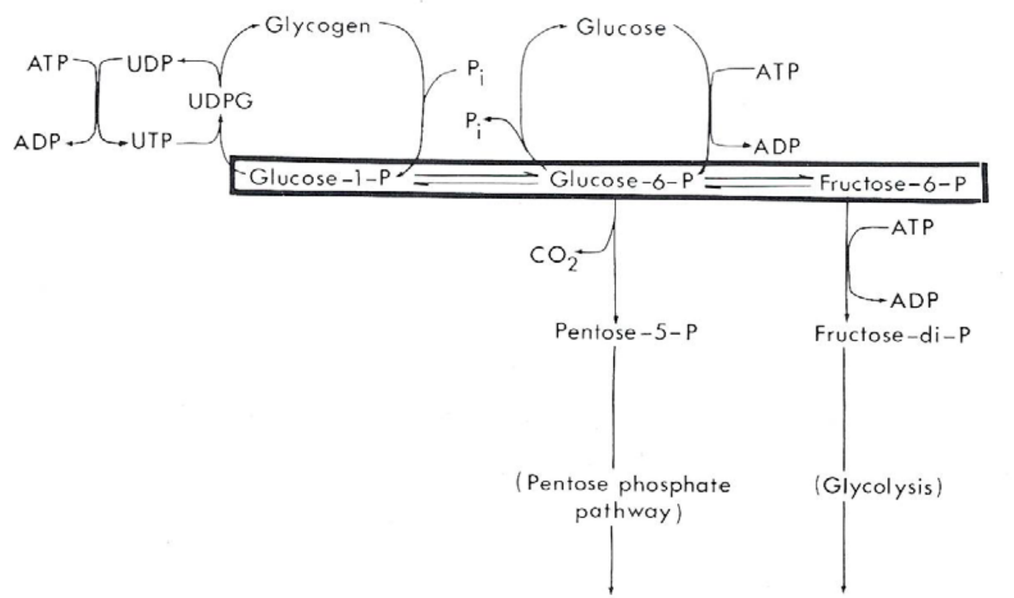
[a] Calculations are based on metabolic relationships in typical aerobic eukaryotic cells.
[b] A similar table, with some differences in assignments, was presented in Atkinson (*10*).
[c] Numbers in parentheses are the costs of starting materials or the costs of regenerating the coupling agents used in the conversion.
[d] Transamination involves the conversion of glutamate to α-ketoglutarate; therefore, the cost of transamination is taken as 5 ATP equivalents (see text).
[e] Products in brackets are intermediates for which the synthetic cost must be calculated.

Metabolna cena intermediatov

(ATP ekvivalenti)

Metabolne razvejitve in regulacije



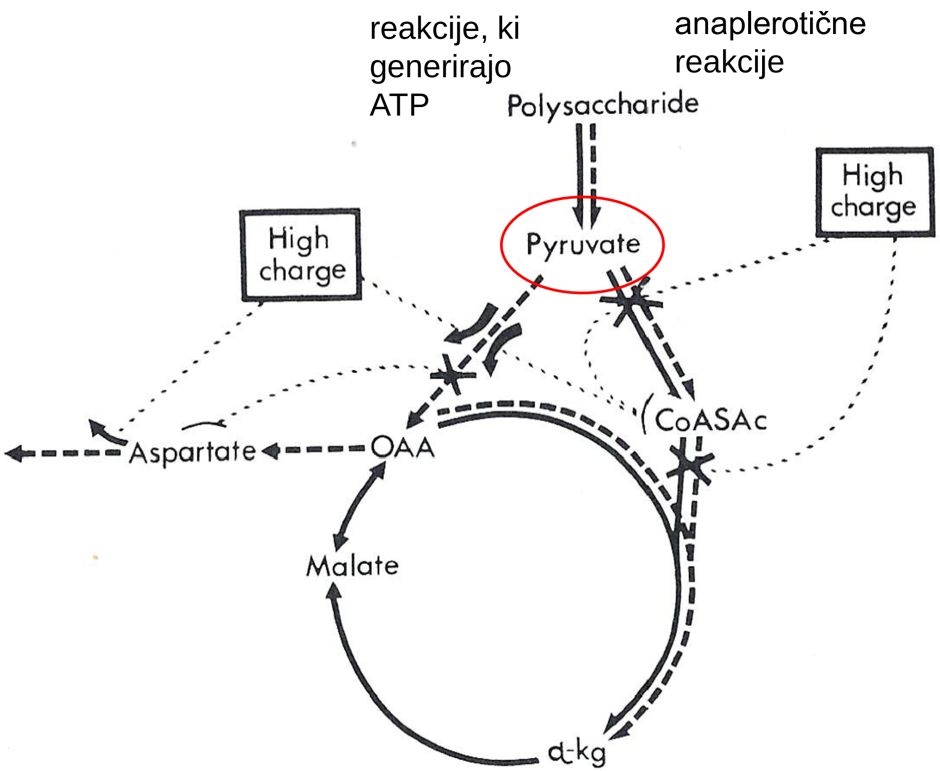


po metabolni razvejitviji običajno pride do encimske kontrole

pri prvi reakciji nove metabolne sekvence, običajno preko

adenilatnega bazena (energijski naboj)

Metabolne razvejitve in regulacije (evkarionti)



metabolna razvejitev (katabolno/anabolna)

na nivoju 3C molekule (piruvat), ki lahko gre

v C2 (acetil-CoA – pridobivanje energije) ali

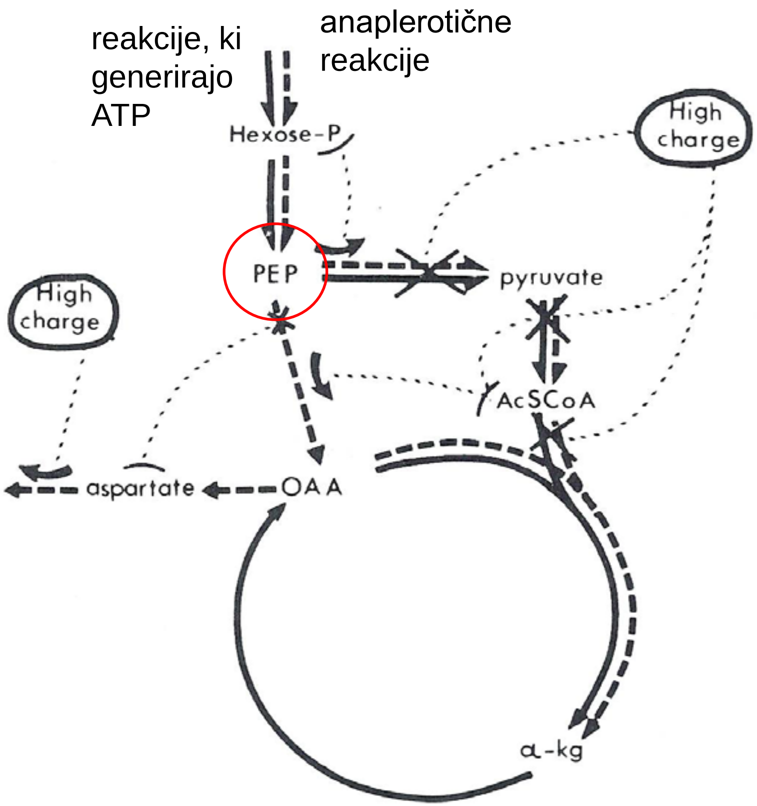
C4 molekulo (oksalacetat - biosinteza),

encima, ki tekmujeta sta piruvat

dehidrogenaza in piruvat karboksilaza,

regulacija je vezna na energijski naboj celice

Metabolne razvejitve in regulacije (prokarionti)



metabolna razvejitev (katabolno/anabolna)

na nivoju 3C molekule (fosfoenolpiruvat), ki

lahko gre preko piruvata v C2 (acetil-CoA)

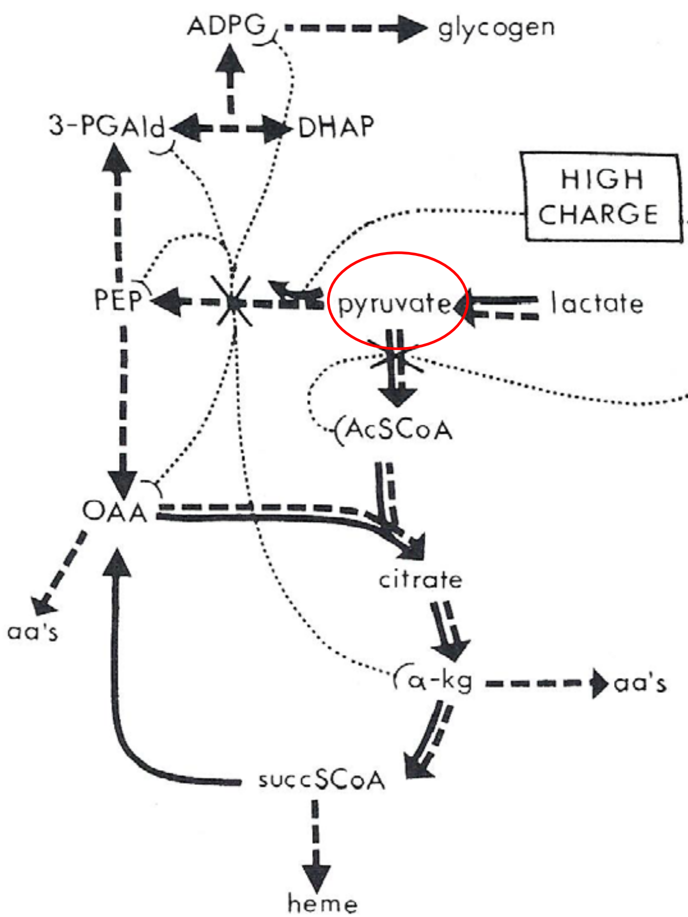
ali C4 (oksalacetat), encima, ki tekmujeta sta

piruvat kinaza in fosfoenolpiruvat

karboksilaza, regulacija je vezana na

energijski naboj celice

Metabolne razvejitve in regulacija rasti na laktatu (prokarionti)



metabolna razvejitev (anabolno/anabolna) na nivoju 3C molekule (piruvat), ki lahko gre v C2 (acetil-CoA) ali direktno v fosfoenolpiruvat za potrebe anabolizma, encima, ki tekmujeta sta piruvat dehidrogenaza in fosfoenolpiruvat sintaza, regulacije je vezana na energijski naboj celice

Research Article | Molecular Biology and Physiology

Metabolic Remodeling during Biofilm Development of *Bacillus subtilis*

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Jean-Marc Ghigo, Invited Editor, E. Peter Greenberg, Editor

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ABSTRACT

Biofilms are structured communities of tightly associated cells that constitute the predominant state of bacterial growth in natural and human-made environments. Although the core genetic circuitry that controls biofilm formation in model bacteria such as *Bacillus subtilis* has been well characterized, little is known about the role that metabolism plays in this complex developmental process. Here, we performed a time-resolved analysis of the metabolic changes associated with pellicle biofilm formation and development in *B. subtilis* by combining metabolomic, transcriptomic, and proteomic analyses. We report surprisingly widespread and dynamic remodeling of metabolism affecting central carbon metabolism, primary biosynthetic pathways, fermentation pathways, and secondary metabolism. Most of these metabolic alterations were hitherto unrecognized as biofilm associated. For example, we observed increased activity of the tricarboxylic acid (TCA) cycle during early biofilm growth, a shift from fatty acid biosynthesis to fatty acid degradation, reorganization of iron metabolism and transport, and a switch from acetate to acetoin fermentation. Close agreement between metabolomic, transcriptomic, and proteomic measurements indicated that remodeling of metabolism during biofilm development was largely controlled at the transcriptional level. Our results also provide insights into the transcription factors and regulatory networks involved in this complex metabolic remodeling. Following upon these results, we demonstrated that acetoin production via acetolactate synthase is essential for robust