Name Surname :_________________________________ **Group Nº** ___________**2023**

Co**Enzymes** vitamin **B3** NAD+(Ox), NADH(Red). **A.** Task descriptions: for studies research of: **ADH**

Alcohol Dehydrogenase ChemScape MDLi **RasMol** MAGE FireFox application.

B. Task Lunch the molecular tutorial prepared by Aris Kaksis 2023 Riga Stradin's University look at: http://aris.gusc.lv/ChemFiles/AlhoDeHydrogenase/NadDehydrogenase.htm the **CPK** color scheme 1965

1. What N- and C-terminus amino acids have **ADH** IV? Menu Backbone, Termini Display option starting amino acid is SER…..and finishing PHE........? What total number (see 2nd page in 1AGN.pdb) .... and amino acids on **1JU9.pdb** crystallized structure chain..........?

2. What Enzyme Class (of seven Classes 1.,2.,3.,4.,5.,6.,7.) present **ADH**? ....

3. What particle in **ADH** transfer two reducing equivalents **(2e-)** from alcohol to **NAD+**? ....

4. Summary Red-Ox reaction studies (endoergic or exoergic) of **ADH** by reduced form ethanol and oxidised NAD+ solutions **4.1 – 4.17** ! **Absolute** potential standard values **E°** by David Harris, Kortly Shucha: at standard conditions of **absolute** scale temperature …... K degree according to the Celsius scale 25° C .

Alcohol dehydrogenase alcohol oxidation to aldehyde (aerobic).

4 Oks $NAD^++H^-(2e^-)=NADH$; $-E^{\circ 1}$**=0,4095 V absolute inverse** standard potential David Harris.

4 Red $CH_3CH_2OH+H_2O=CH_3HC{=}O+H_3O^++H^-(2e^-)$; **absolute** standard potential $E^{\circ 2}$**=-0.0550 V**

4.3 Half reactions sum: $NAD^+ + CH_3CH_2OH+H_2O$=>.......................................

4.4 $\Delta E^\circ=E^{\circ 2}_{H_2O}+E^{\circ 1}$**=-0.0550+0,4095=** .... **V**, half reactions sum standard potencial ΔE°.

4.5 $\Delta G_{eqStandard}=\Delta E^\circ \bullet F \bullet n$**=0,3545*2*96485/1000=**......... kJ/mol standard free energy change.

$$1> K_{eqstandard}=\frac{[NADH]\cdot[CH_3CHO]\cdot[H_3O^+]}{[NAD^+]\cdot[CH_3CH_2OH]\cdot[H_2O]}=e^{-\frac{\Delta G_{eqAerobi}}{R\bullet T}}=EXP(-68400/8{,}314/298{,}15)=\mathbf{1{,}038\bullet 10^{-12}}=............$$

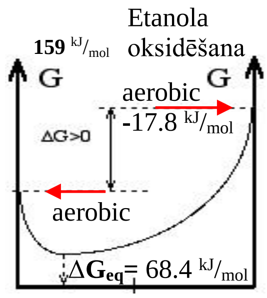
4.6 Is favored or unfavored aerobic reaction : .... page 8; ;

$$\Delta G_{Hess}=\Delta G^\circ_{H_3O}-\Delta G^\circ_{CH_3CHO}-\Delta G^\circ_{NADH}-(\Delta G^\circ_{CH_3CH_2OH}+\Delta G^\circ_{H_2O}+\Delta G^\circ_{NAD^+})=$$

$$=\mathbf{32{,}2824+1175{,}5732-151{,}549-(75{,}2864}+1059{,}11-237{,}191)=...........\ ^{kJ}/_{mol}\ ....$$

Unfavored **equilibrium** constant $K_{eqAerobic}=10^{-12}$ value shows stability in mixture.

Endothermic and endoergic etanol H_3CCH_2OH **oxidation** $H_3CCH{=}O$



A+B+C50%D+E+F

$NAD^++H_3CCH_2OH+H_2O$

$NADH+H_3CCHO+H_3O^+$

4.7 Hess free energy change positive $\Delta G_{Hess\_oxidation}$=........kJ/mol , but

4.8 minimizes $\Delta G_{min}=\Delta G_{eq}$=....... kJ/mol reaching aerobic equilibrium mixture:

4.9 Aerobic oxidation with $[NAD^+]/[NADH]=10^6$ homeostasis pH=7,36 is favored .

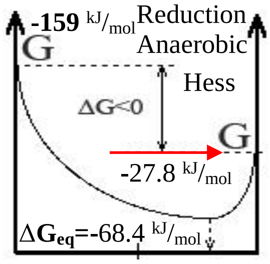
$\Delta G_{AerobicOx}$=**68,4**+8,3144*298,15*ln(1/10^6*1/1*10^(-7,36)/55,3457)/1000=-......kJ/mol ;

Inverse symmetry: aerobic oxidation is **inverse** symmetric anaerobic reduction :

$$10^{-12}=\frac{[NADH]\cdot[CH_3CHO]\cdot[H_3O^+]}{[NAD^+]\cdot[CH_3CH_2OH]\cdot[H_2O]}=K_{eqAerobic}<1<K_{eqAnaerobic}=\frac{[NAD^+]\cdot[CH_3CH_2OH]\cdot[H_2O]}{[NADH]\cdot[CH_3CHO]\cdot[H_3O^+]}=10^{12}$$

same number $|\Delta G_{Hess\_oxidation}|$=|....... | kJ/mol = $|\Delta G_{Hesa}|$=|-..... |.kJ/mol of opposite sign

Inverse exothermic and exoergic ethanal $H_3CCH{=}O$ **reduction** H_3CCH_2OH



D+E+F50%A+B+C

$NADH+H_3CCHO+H_3O^+$

$NAD^++H_3CCH_2OH+H_2O$

4.10 hypoxic anaerobic ethanal reduction is **inverse** negative: ΔG_{Hesa}=-......kJ/mol , but

4.11 minimized about $\Delta G_{eq}=\Delta E^\circ \bullet F \bullet n$=-0,3545 V•2 mol•96485 C/mol=-....**kJ/mol.**

4.12 Ethanal reduction about **ethanol** with anaerobic ratio $[NAD^+]/[NADH]$=1/10 and pH=7,36 is favored, negative, exoergic free energy change l:

$$\Delta G_{anaerobic}=\mathbf{-68{,}4}+8{,}3144*298{,}15*\mathbf{ln}\left(\frac{1}{10}\,\frac{1}{10}\,\frac{55{,}3}{10^{-7{,}36}}\right)=\mathbf{-......\ kJ/mol}\ ;$$

$\Delta G_{AnaerobicRed}$=-68,4+8,3144*298,15*ln(1/10*1/10*55,3457/10^(-7,36))/1000==-......kJ/mol ;

5.0 What **ADH IV** isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 ? To determine water solution pH with **ADH IV** concentration C=$10^{-7,05339}$ M ($^{mol}/_{Litre}$)!

Alcohol dehydrogenase **ADH** E.1.1.1.1. oxidoreductase

```
Sequence of 386 AA amino acids for human ADH IV molecule 1AGN.pdb:
MFAEIQIQDKDRMGTAGKVIKCKAAVLWEQKQPFSIEEIEVAPPKTKEVRIKILATGICRTDDHVIKGTMVSKFPVIVGH
EATGIVESIGEGVTTVKPGDKVIPLFLPQCRECNACRNPDGNLCIRSDITGRGVLADGTTRFTCKGKPVHHFMNTSTFTE
YTVVDESSVAKIDDAAPPEKVCLIGCGFSTGYGAAVKTGKVKPGSTCVVFGLGGVGLSVIMGCKSAGASRIIGIDLNKDK
FEKAMAVGATECISPKDSTKPISEVLSEMTGNNVGYTFEVIGHLETMIDALASCHMNYGTSVVVGVPPSAKMLTYDPMLL
FTGRTWKGCVFGGLKSRDDVPKLVTEFLAKKFDLDQLITHVLPFKKISEGFELLNSGQSIRTVLTF
```

AA	pKa_{COO^-}	$pKa_{NH_3^+}$	pK_{RR}	Nr	AA	pKa_{COO^-}	$pKa_{NH_3^+}$	pK_{RR}	Nr
M	9,21		M	1	D		3,65	D	59
E		4,25	E	2	E		4,25	E	60
D		3,65	D	3	K		10,53	K	61
K		10,53	K	4	C		8,18	C	62
D		3,65	D	5	C		8,18	C	63
R		12,48	R	6	Y		10,07	Y	64
K		10,53	K	7	K		10,53	K	65
K		10,53	K	8	K		10,53	K	66
C		8,18	C	9	K		10,53	K	67
K		10,53	K	10	C		8,18	C	68
E		4,25	E	11	C		8,18	C	69
K		10,53	K	12	K		10,53	K	70
E		4,25	E	13	R		12,48	R	71
E		4,25	E	14	D		3,65	D	72
E		4,25	E	15	K		10,53	K	73
K		10,53	K	16	D		3,65	D	74
K		10,53	K	17	K		10,53	K	75
E		4,25	E	18	E		4,25	E	76
R		12,48	R	19	K		10,53	K	77
K		10,53	K	20	E		4,25	E	78
C		8,18	C	21	C		8,18	C	79
R		12,48	R	22	K		10,53	K	80
D		3,65	D	23	D		3,65	D	81
D		3,65	D	24	K		10,53	K	82
H		6	H	25	E		4,25	E	83
K		10,53	K	26	E		4,25	E	84
K		10,53	K	27	Y		10,07	Y	85
H		6	H	28	E		4,25	E	86
E		4,25	E	29	H		6	H	87
E		4,25	E	30	E		4,25	E	88
E		4,25	E	31	D		3,65	D	89
K		10,53	K	32	C		8,18	C	90
D		3,65	D	33	H		6	H	91
K		10,53	K	34	Y		10,07	Y	92
C		8,18	C	35	K		10,53	K	93
R		12,48	R	36	Y		10,07	Y	94
E		4,25	E	37	D		3,65	D	95
C		8,18	C	38	R		12,48	R	96
C		8,18	C	39	K		10,53	K	97
R		12,48	R	40	C		8,18	C	98
D		3,65	D	41	K		10,53	K	99
C		8,18	C	42	R		12,48	R	100
R		12,48	R	43	D		3,65	D	101
D		3,65	D	44	D		3,65	D	102
R		12,48	R	45	K		10,53	K	103
D		3,65	D	46	E		4,25	E	104
R		12,48	R	47	K		10,53	K	105
C		8,18	C	48	K		10,53	K	106
K		10,53	K	49	D		3,65	D	107
K		10,53	K	50	D		3,65	D	108
H		6	H	51	H		6	H	109
H		6	H	52	K		10,53	K	110
E		4,25	E	53	K		10,53	K	111
Y		10,07	Y	54	E		4,25	E	112
D		3,65	D	55	E		4,25	E	113
E		4,25	E	56	R		12,48	R	114
K		10,53	K	57	F	1,83		F	115
D		3,65	D	58		.			

115 of 386 amino acids active values pKa

Sum = 881,66...............

= $\Sigma pK_{aRside\ group}+pK_{aNterminal}+pK_{aCterminal}$ =

pKa_{mean} =($\Sigma pK_{aRside\ group}+pK_{aNterminal}+pK_{aCterminal}$)/NpKa

IEP= pKa_{mean} =881,66 / 115 =**7.6666**.............

Calculation tasks for human ADH IV molecule 1AGN.pdb

Protolytic constant, isoelectric point IEP= pKa_{mean} calculate of side chains $\Sigma pK_{aRside\ group}$.. $pK_{aNterminal}NH_3$

and $pK_{aCterminalCOO-}$ constants sum divide with number of acid groups NpK_a:

$$IEP = pKa_{mean} = (\Sigma pK_{aRside\ group} + pK_{aNterminal} + pK_{aCterminal})/NpKa$$

1 Acid groups number in sum NpKa=113.......+2.........=.....

386 amino acids of them protolytic constants pK_a for side groups 113+2 terminus N and C,

N-terminal metionine M $pK_{aNterminal}$=9.21 and C-termināl phenilalanin F $pKa_{Cterminal}$=1,83

Sum are calculating as $\Sigma pK_{aRside\ group} + pK_{aNterminal} + pK_{aCterminal}$ = ...

2 Average acid group constant pKa_{mean} = IEP **ISOELEKTRIC POINT**

IEP = pKa_{mean} =881,66 / 115 =.............

At pH value of amino acid and protein on isoelectric point pH**=IEP total charge** is zero „**0**”

0——— plus (+) acidic————zero charge „**0**” **IEP**=pH———— minus (-) basic——→ 14 pH scale

-COOH & **-NH$_3^+$** positive charge ....**-COO$^-$** & **-NH$_3^+$**..........................charge is negative **-COO$^-$** & **-NH$_2$**

Underline and determine existing: positive (+) or negative (-) or zero !

3 Determine ADH IV molecule charge sign (+). zero „**0**” or (-) at physiologic pH=7.36

Underline existing:

-COOH & **-NH$_3^+$** positive (+) charge ……. pH=7.36 < IEP=**7.67** .... charge negative(-) **-COO$^-$** & **-NH$_2$**.

4 Determine ADH IV molecule charge sign (+). zero „**0**” or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH & **-NH$_3^+$** positive (+) charge ……. IEP=**7.67**< pH=8.8 .... charge negative(-) **-COO$^-$** & **-NH$_2$**.

5 Calculate ADH IV solution pH at concentration C=$10^{-7,05339}$ M (^{mol} / $_{Litre}$)

by *Ostwald dilution law* concentration M in logarithm:

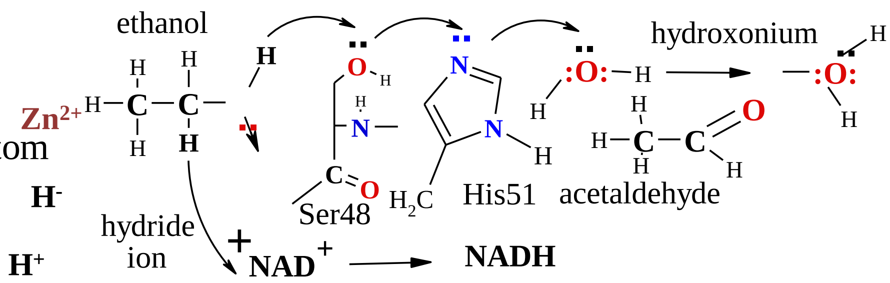
$$pH=\frac{pKa_{mean} - \log C}{2} = \frac{7.6666087 - \log 10^{-7,0533913}}{2} = \frac{7.6666087 + 7,0533913}{2} = \mathbf{14,72} / 2 = ...$$

7,36 Attractor ADH IV concentration is C=...............M .

5. . Place catalytic **Zn^{2+}**ion, ethanol oxygen atom **O** coordinate with donor acceptor bond and four jumping dissociated proton **H$^+$** pathway from alcohol group **-CH$_2$-O-H** to Ser48 to His51 and resulting bound proton **H$^+$** to water molecule **H$_2$O** forming hydronium ion **H$_3$O$^+$**.

Place hydride ion **H**⁻ in to tunneling pathway from alcohol group carbon atom-CH_2- to NAD^+ cyclic carbon atom –HC^+– producing NADH

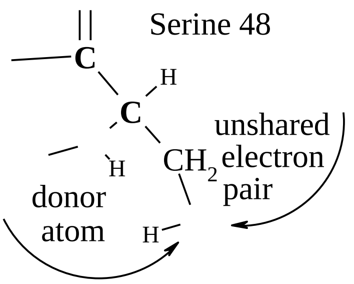
ADH during alcohol oxidation in water medium ,



O H⁺ H⁺ H⁺ H⁺

that H_2O water molecule forming hydroxonium ion H_3O^+ and aldehyde

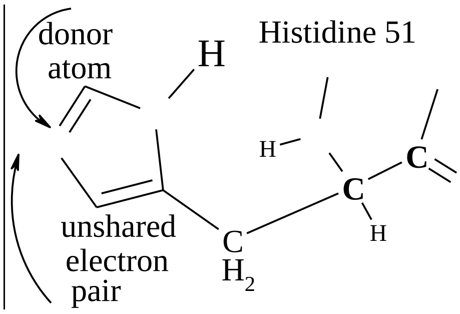
6. Place in **Ser-48, His-51** structures **O**, **N** atoms and electrons pair donor atoms **O:**, **:N**!



O

N

O:



N

N

:N

O

7. Place **O** , **Zn^{2+}**, **S**, **N** atoms and charge zero 0 of complex [**Zn^{2+}**(**S**⁻Cys)₂(**O**-*spirts*)(**N**His)]⁰

for tetragonal geometry,

like trigonaāl pyramid!

1HLD.pdb **Zn^{2+}** ion coordinates:

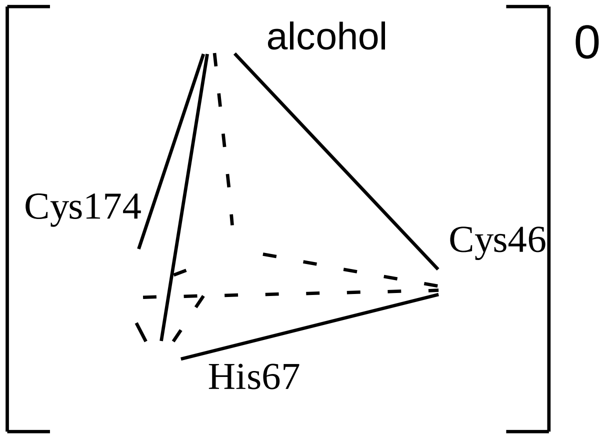
Cys46-Cys174-His67-**O** alcohol

O 0

S⁻

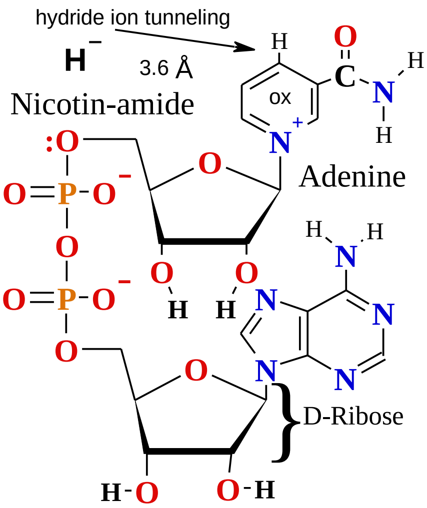
S⁻

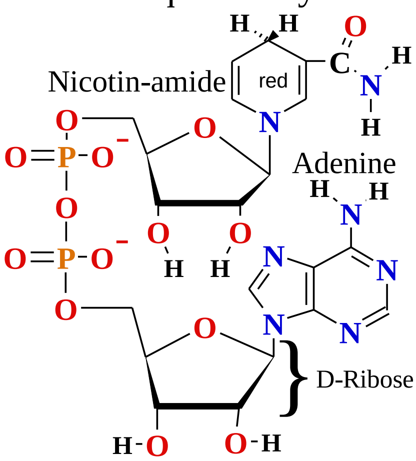
Zn^{2+}



8. What vitamin-cofactor oxidizes alcohols in **ADH dimer** quaternary structure?.......

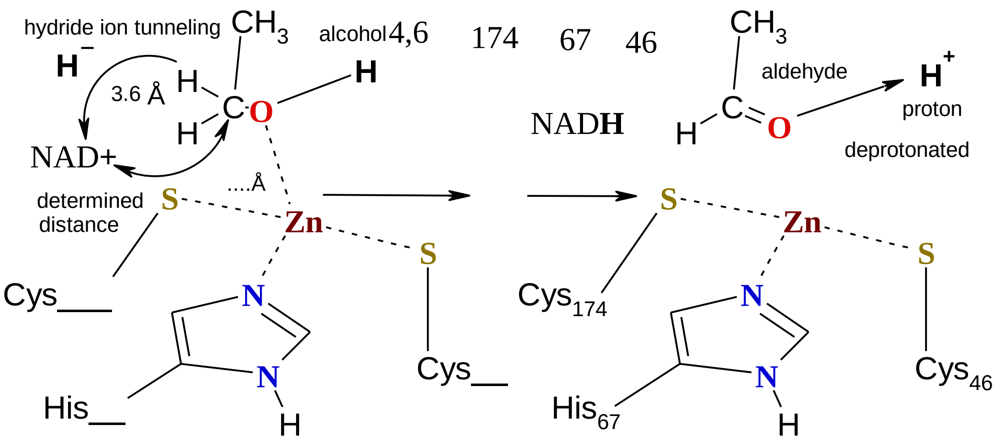
9. Oxidised **NAD⁺** hydride tunneling **nicotine adenine dinucleotide**: Nicotin-amide, Adenine, two riboses, two phosphates with anhydride bond between phosphates





10. NADH hydride **nicotine adenine dinucleotide** reduced form: Nicotin-amide, Adenine, two riboses, two phosphates with anhydride bond between phosphates

ADH 1HLD.pdb **Zn^{2+}** coordinates Cys46-Cys174-His67-**O** : Tunnel distance 3,6 Å for hydride



ion **H**$^-$ to NAD$^+$ nicotine amide positive charged cycle carbon atom –CH–. Measure distance ...... Å from alcohol –CH_2- carbon atom to NAD+ aromatic cycle –CH–in 1HLD.pdb molecule. With right button click in menu choose „Distance” from „Select Mouse Click Action” measure distance from alcohol carbon atom –CH_2- ......Å to NAD$^+$ nicotine amide cyclic carbon atom –CH–!

11. Place amino acid numbers for coordination sphere and measure distance in angstroms units.

12. Secondary structures in **ADH** are............helixes and................ sheets.

13. Count **alpha-helixes** on **ADH** polypeptide molecule? .... **alpha-helixes**.......................

14. Count **beta strands - sheets** in **ADH** molecule?.......**beta strands in-sheet**

.... **beta strands in-sheet** and ....**beta strand**....................

15. Count quaternary 4° structure components of 3° subunits in **ADH** molecule **1JU9zn.pdb** and **1HLDznNAD.pdb**? identical **ADH** molecules ...., each bind ....

each in domain binds **substrate alcohol group like** ....,......................

16. What physiological functions in human body have ADH against ethanol?.......................

.... remove two hydrogen atoms and so oxidize ....

18. What toxic physiological functions in human body have ethanol molecules at long time abused? CSDD Car transport certification limited concentration in blood 0.5 promilles and more? a) slow down the transport through membrane aquaporin-channels of ...+...

b).... long time abused in body leads in tissues to ....and ....

c).... ethanol to prevent formation and compete with ....

19. What toxic functions in human body have **ADH** against methanol?

to poison human body with ....

remove two hydrogen atoms of alcohol so to oxidize ....

20. Complete the oxidation reaction for methanol: **H_3C-OH+NAD$^+$** in water.

H_3C-OH+NAD$^+$+H_2O(His51)<=>......

Methanol B$_3$ vitamin Formaldehyde B$_3$ vitamin reduced

21. How compete ethanol with methanol? What ist the anti dot against methanol misuse in human body? high ethanol concentration oppress ....

....methanol oxidation silencing lets throw aquaporins .....

22. To call six crystalline shapes for ADH subunits designation by Greek alphabet letters!

1. alpha ...., 2. beta .....,. 3. gamma........, 4. pee...., 5. chi...., 6. sigma......

23. What kind human alcohol dehydrogenase crystallization failed? ....

To depict what kind of human alcohol dehydrogenases seven types - proteins identified in organism from data bank of Uni-Prot KB files:

1. ...._HUMAN, subunits designated ....
2. ...._HUMAN, subunits designated ....
3. ...._HUMAN, subunits designated ....
4. ...._HUMAN, subunits designated ....
5. ...._HUMAN, subunits designated ....
6. ...._HUMAN, subunits designated ....
7. ...._HUMAN, ...

http://aris.gusc.lv/ChemFiles/AlhoDeHydrogenase/4DXH5VJ5hOhBioChem1718/5VJ5hOhBioChem17.pdf

The Class	System	Protein gene	Uni-Prot KB	Gene New	Gene Old
Class I 1HSO		ADH1A	.	ADH1	ADH1A
Class I 1DEH		ADH1B	.	ADH2	ADH1B
Class I 1HT0		ADH1C	.	ADH3	ADH1C
Class II		ADH2	.	ADH4	ADH4
Class III 1MP0		ADH3	.	ADH5	ADH5
Class IV 1AGN		ADH4	.	ADH7	ADH7
Class V		ADH5		ADH6	ADH6

Table 1: Nomenclature for Human Alcohol Dehydrogenase

Abstract Background

All known attempts to isolate and characterize mammalian class V alcohol dehydrogenase (class V ADH), a member of the large ADH protein family, at the protein level have failed. This indicates that the class V ADH according Uni-Prot KB ADH6_HUMAN protein is not stable in a non-cellular environment, which is in contrast to all other human ADH enzymes. In this report we present evidence, supported with results from computational analyses performed in combination with earlier in vitro studies, why this ADH behaves in an atypical way.

Arch Biochem Biophys. 2018;653:97-106.4**DXHa**

Biochemistry, 2017, 56 (28), pp 3632-3646.

5ENV,8ADH,1QLH,4DWV,1N92,1N8K,1P1R,4DXH,1N92,1N8K,1LDE,1LDY,1MGO,5VKR,
1HEU,2JHF,1HET,2JHG,1H2B,1MAO,1PL6,1PL6,1YKF,**1YE3**,**4XD2**,**5VJ5**,**5VJG**,**5VKR**,**5VL0**,**5VN1**,,, 6