Example BLAST

CH370

10/2/2011

Sequence 30) RDNMSVILIC FPNAPKVSPE

>gi|343960733|dbj|BAK61956.1| protein phosphatase 2C isoform alpha [Pan troglodytes]
MGNEELCDFVRSRLEVTDDLEKVCNEVVDTCLYKGSRDNMSVILICFPNAPKVSPEAVKKEAELDKYLEC
RVEGGSFNKK

(a) My protein is phosphatase 2C isoform alpha from the chimpanzee (*Pan troglodytes*). The complete amino acid sequence in FASTA format is:

>gi|343960733|dbj|BAK61956.1| protein phosphatase 2C isoform alpha [Pan troglodytes]
MGNEELCDFVRSRLEVTDDLEKVCNEVVDTCLYKGSRDNMSVILICFPNAPKVSPEAVKKEAELDKYLECRVEGGSFNKK

(b)

Table 1.1 - CLUSTAL 2.1 Multiple Sequence Alignment with Highlighted Conserved Sequences

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gi|55926082| [Zebrafish]            ------------------------------------------------M 1
gi|148237920| [African clawed frog] ------------------------------------------------M 1
gi|71895445| [Red jungle fowl]      ------------------------------------------------M 1
gi|148706643| [House rat]           ASVLHVVRTTGCQKQSAVDLPWASKFRKLRTNKACWRRFLGGPFYSLLNM 50
gi|284793885| [Human]              ------------------------------------------------M 1
gi|90961593| [Lactobac salivarius] ------------------------------------------------MEI 3
gi|289704854| [Micrococcus luteus] ------------------------------------------------MDPR 4


gi|55926082| [Zebrafish]            GAFLDKPKTEKHNAHGEGNGLRFGLSSMQGWRVEMEDAHTAAVGLPHGLD 51
gi|148237920| [African clawed frog] GAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAAVGIPRGLD 51
gi|71895445| [Red jungle fowl]      GAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGIPHGLE 51
gi|148706643| [House rat]           GAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGIPHGLD 100
gi|284793885| [Human]              GAFLDKPKMEKHNAQGQGNGLRYGLSSMQGWRVEMEDAHTAVIGLPSGLE 51
gi|90961593| [Lactobac salivarius] AYLSDLGKMRKNNEDYVG--------------KFVNKAGAVMVIVADGLG 39
gi|289704854| [Micrococcus luteus] PETPSAPRPQAAGATHVG-----AVRDLNEDAWTVVGEPLVLAAVADGMG 49
                                       .  : .  .    *               :     .   :. *:

gi|55926082| [Zebrafish]            DWSFFGVYDGHAGSRVANYCSKHLLEHIVAAGSADELRKAGAP-APETPA 100
gi|148237920| [African clawed frog] DWSFFAVYDGHAGSRVANYCSSHLLEHITDN---DDFRATEAPGSALEPT 98
gi|71895445| [Red jungle fowl]      DWSFFAVYDGHAGSRVANYCSTHLLEHITNN---EDFRAAEKPGSALEPS 98
gi|148706643| [House rat]           NWSFFAVYDGHAGSRVANYCSTHLLEHITTN---EDFRAADKSGSALEPS 147
gi|284793885| [Human]              SWSFFAVYDGHAGSQVAKYCCEHLLDHITNN--------QDFKGSAGAPS 93
gi|90961593| [Lactobac salivarius] GHNGGEVASEMAVSHLGYYFSETSFFSISQAS------------TWLSKK 77
gi|289704854| [Micrococcus luteus] GHEAGEVASAAAIETLRRRAPEVLAPGTAHTP------------QDLGHL 87
                                    . .   * .  * . :
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gi|55926082| [Zebrafish]            IEAVKRGIRAGFLRIDEHMRSFTDLRNGMDRSGSTAVAVLLSPEH---LY 147
gi|148237920| [African clawed frog] VENVKSGIRTGFLKIDEYMRNFADLRNGMDRSGSTAVAVLLSPNH---VY 145
gi|71895445| [Red jungle fowl]      VENVKSGIRTGFLKIDEYMRNFSDLRNGMDRSGSTAVGVMISPEH---VY 145
gi|148706643| [House rat]           VESVKTGIRTGFLKIDEYMRNFSDLRNGMDRSGSTAVGVMVSPTH---MY 194
gi|284793885| [Human]              VENVKNGIRTGFLEIDEHMRVMSEKKHGADRSGSTAVGVLISPQH---TY 140
gi|90961593| [Lactobac salivarius]  VDEENS-------LILKNSLHYKDLRG----MGTTLVVAIIFEQE---FL 113
gi|289704854| [Micrococcus luteus]  IHEADA----------------AVVEAGLGRSGTTLTVLACLDTERGRWA 121
                                    :.  .                    .     *:* .       .

gi|55926082| [Zebrafish]            FINCGDSRALLCRSGH--VCFSTMDHKPCDPREKERIQNAGGSVMIQRVN 195
gi|148237920| [African clawed frog] FINCGDSRSVLYRSGQ--VCFSTQDHKPSNPREKERIQNAGGSVMIQRVN 193
gi|71895445| [Red jungle fowl]      FINCGDSRAVLYRNGQ--VCFSTQDHKPCNPREKERIQNAGGSVMIQRVN 193
gi|148706643| [House rat]           FINCGDSRAVLCRNGQ--VCFSTQDHKPCNPVEKERIQNAGGSVMIQRVN 242
gi|284793885| [Human]              FINCGDSRGLLCRNRK--VHFFTQDHKPSNPLEKERIQNAGGSVMIQRVN 188
gi|90961593| [Lactobac salivarius]  LAHIGDSRGYILEKGN--LTRVTEDHSLVNELIKEGELSPEEAEHHPKKN 161
gi|289704854| [Micrococcus luteus]  VANVGDSRVYLRPVGSPVLQQLTVDHSAVQMLVDAGELTPAEARVHPRRN 171
                                    . : ****  :       :   * **.  :   .    ..  :    : *

gi|55926082| [Zebrafish]            GSLAVSRALGDYDYKCVEGKGPTEQLVSPEPEVFEIARSDAEDEFVVLAC 245
gi|148237920| [African clawed frog] GSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVYEIVRAD-EDEFIILAC 242
gi|71895445| [Red jungle fowl]      GSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVCEILRAE-EDEFIILAC 242
gi|148706643| [House rat]           GSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVYEIVRAE-EDEFVVLAC 291
gi|284793885| [Human]              GSLAVSRALGDFDYKCVHGKGPTEQLVSPEPEVHDIERSEEDDQFIILAC 238
gi|90961593| [Lactobac salivarius]  ---IITRSLG----------------ISKNSELDEEFFQITKNSILMLCT 192
gi|289704854| [Micrococcus luteus]  ---VITRALG----------------SREALTVDVAEVPLASGDLVLLCS 202
                                        ::*:**                    :       ...:::*.

gi|55926082| [Zebrafish]            DGIWDVMTNEDLCAFVRSRLEVTDDLERVCNEVVDTSLHKGSRDNMSIVL 295
gi|148237920| [African clawed frog] DGIWDVMSNEELCEFVKYRLELTDDLEKVCNSVVDTCLHKGSRDNMSIVL 292
gi|71895445| [Red jungle fowl]      DGIWDVMSNEELCEFVKSRLEVSDDLEKVCNWVVDTCLHKGSRDNMSIVL 292
gi|148706643| [House rat]           DGIWDVMSNEELCEFVKSRLEVSDDLENVCNWVVDTCLHKGSRDNMSVVL 341
gi|284793885| [Human]              DGIWDVMGNEELCDFVRSRLEVTDDLEKVCNEVVDTCLYKGSRDNMSVIL 288
gi|90961593| [Lactobac salivarius]  DGLTNMVEDTKLERLLN----ISESLPDKCQRLVDEANRAGGNDNISVLL 238
gi|289704854| [Micrococcus luteus]  DGLTGELLDEEIRALIDD----ADTLEDALEKLIGAALWRGGRDNVTAVL 248
                                    **: . : : .:  ::      :: *    : ::. .   *..**:: :*
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gi|55926082| [Zebrafish]            VCLPNAPQVSEDAVKRDAELDKYLESRVEELIEKAGEDGVPELAHVMSSL 345
gi|148237920| [African clawed frog] VCFHNAPKVSEEAIKKDADLDKHLESRVEEIMTNAGEEGMPDLAHVMRIL 342
gi|71895445| [Red jungle fowl]      VCLSNAPKVSDEAVKKDAELDKHLESRVEEIMEKSGEEGMPDLAHVIRIL 342
gi|148706643| [House rat]           VCFSNAPKVSEEAVKRDSELDKHLESRVEEIMQKSGEEGMPDLAHVMRIL 391
gi|284793885| [Human]              ICFPNAPKVSPEAVKKEAELDKYLECRVEEIIKKQGE-GVPDLVHVMRTL 337
gi|90961593| [Lactobac salivarius]  VSFDEEVKY---------------------------------------- 247
gi|289704854| [Micrococcus luteus]  VRIP------------------------------------------- 252
                                     : :

gi|55926082| [Zebrafish]            SQENIPNLPPGGGLASKHSVIETVYNRLNPQREEDG----SGADLEDPW- 390
gi|148237920| [African clawed frog] AAENIPHLPPGGGLAAKRSVIEEVYNRLNPHRESDG----DPTGTEEKST 388
gi|71895445| [Red jungle fowl]      SAENIPNLPPGGGLAGKRNIIEAVYSRLNPHRENEG----GAGDLEDPW- 387
gi|148706643| [House rat]           SAENIPNLPPGGGLAGKRHVIEAVYSRLNPHKDNDGFYQPSIAYSDNVFL 441
gi|284793885| [Human]              ASENIPSLPPGGELASKRNVIEAVYNRLNPYKNDDT----DSTSTDDMWL 383
gi|90961593| [Lactobac salivarius]  ------------------------------------------------
gi|289704854| [Micrococcus luteus]  ------------------------------------------------


gi|55926082| [Zebrafish]            ------------------------------------------------
gi|148237920| [African clawed frog] HGKLVEAFRELRINHRGNYRQLLEEMLCSYRLVKVQGEESTSGPSVPSSS 438
gi|71895445| [Red jungle fowl]      ------------------------------------------------
gi|148706643| [House rat]           L----------------------------------------------- 442
gi|284793885| [Human]              EHHHHHH---------------------------------------- 390
gi|90961593| [Lactobac salivarius]  ------------------------------------------------
gi|289704854| [Micrococcus luteus]  ------------------------------------------------


gi|55926082| [Zebrafish]            ----------------
gi|148237920| [African clawed frog] ISKPVDDPITRSHPEME 455
gi|71895445| [Red jungle fowl]      ----------------
gi|148706643| [House rat]           ----------------
gi|284793885| [Human]              ----------------
gi|90961593| [Lactobac salivarius]  ----------------
gi|289704854| [Micrococcus luteus]  ----------------
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(c) Important amino acids for preserving the structure of the protein are ones involved with polar or hydrophobic interactions and allowing sharp folds. Gly-19 and Gly-49 are both found between two antiparallel beta sheets. Since the R group of glycine is so small (H), it is less restricted and thus has a lot more acceptable phi/psi angle combinations. Thus, it can be twisted around a sharp turn as seen between two antiparallel beta sheets. Similarly, Ala-63 is found between a beta sheet and an alpha helix. Alanine also has a relatively small R group (-CH3), so it can also fit in tight turns like glycine residues can. Leu-264 and Gly-279 are also found at tight curves at the ends of an alpha helix. Hydrophobic interactions include those between Val-58 and Leu-236; both residues have hydrophobic R groups that are excluded from the polar environment. Such hydrophobic exclusions ensure that the protein is folded into the correct conformation. There are also polar interactions between residues, such as those between Gly-145, Asp-146, Ser-147, Arg-148, Asp-163, His-164, Thr-128. Each of those residues has a polar R group (glycine's H can be either hydrophobic or hydrophilic) that interact with each other and creates a polar pocket on the surface of the protein. Some of these residues may also lend a hand in the catalytic activity of the protein. Ser-147 and Asp-239 are probably the most important residues in terms of catalysis; they are highly conserved and are found within the polar pocket of the protein. Their side chains are polar (negatively charged for aspartate) and interact with the Mn^{2+} cofactor of the protein. These residues could be involved with initiating the breakage of the ester bond, possibly acting as a nucleophile and attack the phosphorus atom of the phosphate group.

(d) The PDB code for the human phosphatase 1a (Ppm1a) is 3FXJ.

(e)

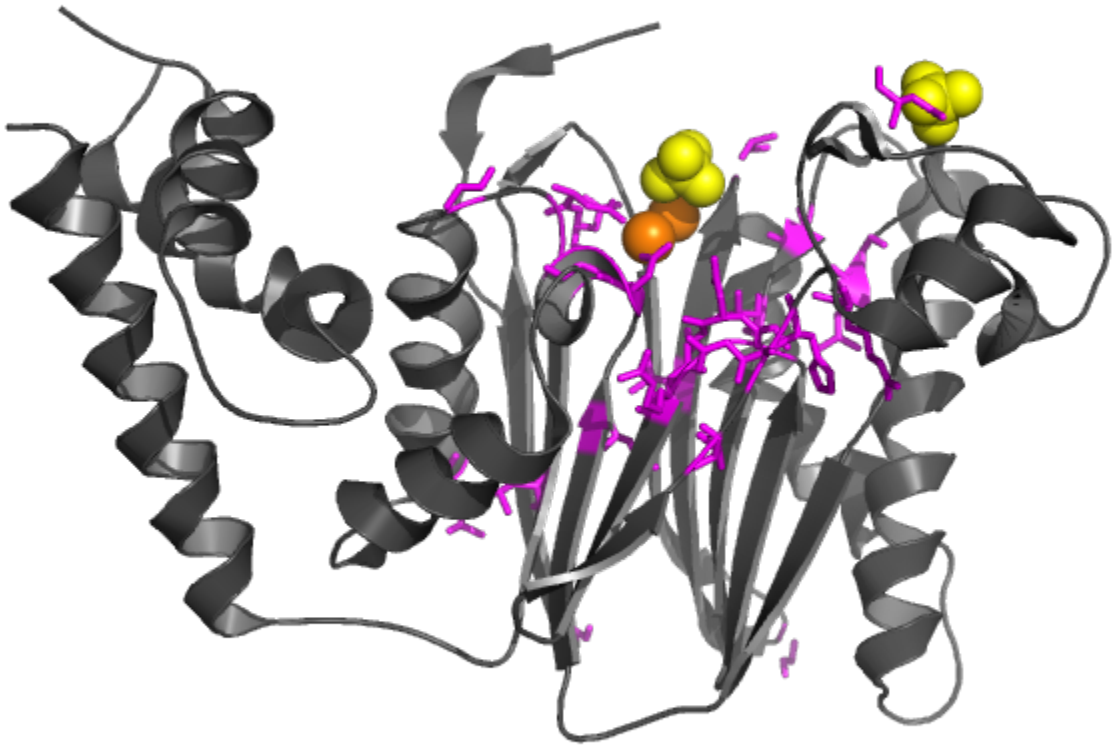

Figure 1.2 - Crystal Structure of human protein phosphatase 1a (Ppm1a) bound with phosphate at 3 Mm of Mn^{2+}. Conserved residues have side chains shown in magenta. Phosphate ligands are shown in yellow while Mn^{2+} cofactors are displayed in orange.

(f) The protein phosphatase 1A serves as a catalyst to break ester bonds between the phosphorus atom and an oxygen on the substrate. Dephosphorylation of molecules can cause a plethora of different changes within the body, depending on what molecule was modified. For example, protein

phosphatase 1A is involved with the signaling pathway involving bone morphogenetic proteins. Bone morphogenetic proteins are growth factors that regulate very important processes in the body, including the synthesis of various tissues like bone. Protein phosphatase 1A has been found to be in charge of the dephosphorylation of Smads, proteins that are crucial in the BMP signaling pathway, decreasing the effects of bone morphogenic proteins (1). Protein phosphatase 1A is also involved in the regulation of trophoblast invasion, a step that creates the foundation for pregnancy within females. Trophoblasts are specialized cells that eventually come together to create a thin layer that covers the placenta. Protein phosphatase 1A increases the ability for trophoblasts to invade and take root in the uterus, generally increasing tissue formation (2). The protein is also involved in the insulin pathway. Insulin is a hormone used by the body to regulate blood glucose levels. In response to high blood glucose levels, insulin is released to the body to cause a number of effects. Dephosphorylation by the protein is linked to an increased effect of insulin in the body, indicating why there are higher levels of the protein found in adipocytes (3). Alternatively, protein phosphatase 1A also has effects on consumed substances like antidepressants. Antidepressants are drugs developed to treat or lessen the effects of depression on a patient. Protein phosphatase 1A was seen to improve the response of nortriptyline, an antidepressant that prevents the reuptake of norepinephrine in neurons (4).

References

(1) Duan X, Liang YY, Feng XH, Lin X. (2006). J Biol Chem. 2006 Dec 1;281(48):36526-32.

(2) Zhang B, Zhou Z, Lin H, Lv X, Fu J, Lin P, Zhu C, Wang H. (2009). *Histochem Cell Biol.* 2009 Aug;132(2):169-79.

(3) Yoshizaki T, Maegawa H, Egawa K, Ugi S, Nishio Y, Imamura T, Kobayashi T, Tamura S, Olefsky JM, Kashiwagi A., (2004). *J Biol Chem.* 2004 May 21;279(21):22715-26.

(4) Malki K, Uher R, Paya-Cano J, Binder E, Rietschel M, Zobel A, Mors O, Hauser J, Henigsberg N, Jerman B, Souery D, Placentino A, Ng MY, Cohen-Woods S, Sluyter F, Farmer A, Aitchison KJ, Craig IW, Lewis CM, McGuffin P, Schalkwyk LC. (2011). *Biology Psychiatry.* 2011 Feb 15;69(4):360-5.