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ADD3 -GAMMA-ADDUCTIN AND BILIARY ATRESIA

Mirela Lungu [1], Claudiu N. Lungu[2*]

1. Department of Pediatric Surgery, Emergency Clinical Hospital for Children Galati 800487, Romania.

2. Department of Surgery, Emergency Clinical Hospital Braila 810325, Romania.

ABSTRACT: Biliary atresia is a disease of the liver that affects children. Abnormally narrow, blocked, or absent bile ducts are observed. Association between the ADD3 gene and biliary atresia was notice. Gamma adduction, the product of the ADD3 gene, is express ubiquitary with peak expression in the brain (mean RPKM 57.483±10.391) and the smallest expression in the liver (mean RPKM 6.724±1.174). The study focuses on gene product – gamma adducin (ADD3) and hypothesizes on its mode of action (MOA) using computational methods. Results show that ADD3 is critical in forming the gamma adducin1/gamma adducin 2:gamma adducin 3: demantin complex (ADD1/ADD2:ADD3: DMNT), which is involved in abnormal development of the bile ducts. In conclusion, ADD3 is a crucial component in biliary atresia pathogenesis.

Keywords: gamma adducin, cytoskeleton, biliary atresia, ADD3 complex.

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Corresponding Author: Dr. Claudiu N. Lungu* Ph.D.

Department of Surgery, Emergency Clinical Hospital Braila 810325, Romania.

Email address: lunguclaudiu5555@gmail.com

1.INTRODUCTION

Biliary atresia, an illness of the liver and bile ducts, occurs in infants. The disease can be congenital or acquired. Incidence is 1:15000 live births in the United States, with a prevalence of 1/16.700 in the British Isles. Biliary atresia is frequent in East Asia, with a frequency of 1/ 5.000. The cause of biliary has been confirmed, in some cases, to be the aflatoxin induced cholangiopathy acquired prenatally in newborns who have M1 glutathione S transferase deficiency.[1] As an entity, Syndromic biliary atresia [2] (Biliary Atresia Splenic Malformation -BASM)) has been associated

with specific genes (Polycystic Kidney Disease 1 Like 1 - PKD1L1)[3], and newborns with isolated biliary atresia are noticed as a result of an autoimmune inflammatory response, possibly due to a viral infection of the liver soon after birth.[4] The only effective treatments are operations like the Kasai procedure and liver transplantation.[5] The symptoms of biliary atresia are identical to neonatal jaundice, a gentle condition seen in infants. Infants with biliary atresia evolve with gradual conjugated jaundice, white stools, and dark urine. Fail to thrive is present in some infants, and a degree of fat and fat-soluble vitamin malabsorption (e.g., Vitamin K) is present. The bleeding tendency may be noticed. Cirrhosis with portal hypertension will finally develop. Biliary atresia can lead to liver failure if left untreated [6]. In contrast with other forms of jaundice, biliary atresia related cholestasis does not result in kernicterus. This is because the liver, although diseased, is still able to conjugate bilirubin, and conjugated bilirubin is unable to cross the blood-brain barrier. Biliary atresia is classified as type I (atresia involving the common bile duct), type II (atresia implying the common hepatic duct), type III (atresia that involves the t proximal part of the bile ducts >95% of all cases). 10% of cases, other anomalies are correlated with biliary atresia. The most common of the syndrome is BASM and includes heart lesions, polysplenia, situs inversus, missing venae cavae, and a preduodenal portal vein.[7] Progressive cirrhosis is correlated with portal hypertension manifesting with esophagogastric varix bleeding, hypersplenism, hepatorenal syndrome, and hepato-pulmonary syndrome. Abnormally high levels of aflatoxin B1 and aflatoxin B2 were found in liver tissue and blood of infants with biliary atresia. Aflatoxins cause damage to the hepatocytes that lead to hepatitis and damage to bile ducts with inflammation, adhesions, and obstruction of bile ducts.[8] Infants have a genetic detoxification defect that prevents the detoxification of aflatoxins. The patients have a homozygous deficiency of glutathione S transferase (GST) M1.[9] Aflatoxin affects the liver. Other factors are also involved: CCL-2, tumor necrosis factor (TNF), interleukin-6 (IL-6), TGF-beta, endothelin (ET), and nitric oxide (NO). TGF-beta is the most important pro-fibrogenic cytokine identified as inactive chlorosis. In infants with biliary atresia, regeneration is deficient and results in cirrhosis, while these infants have disrupted p53 and disrupted GSTPi[10] ADD1 is a protein that is encoded by the same gene. It is a cytoskeleton protein[11]. The highest expression is found in the brain (mean RPKM 57.264±3.652) and moderate expression in the liver (mean RPKM 11.284±1.171), salivary glands(mean RPKM10.434 ±2.642), and pancreas (mean RPKM5.472 ±0.186), respectively. Hypertension is associated with ADD1 polymorphism DMTN plays a role in maintaining membrane and hypocellular skeleton integrity. It is ubiquitary[12]. ADD3 is implied in biliary atresia[13]. Being a constitutional and ubiquitary protein (according to gene expression), gamma adductin (ADD3) forms complexes with alpha adducin(ADD1) and dematin binding protein (DMTN). The complex ADD1:ADD3: DMNT coexists in the cytosol with ADD1:ADD2: DMNT complex. The molecular function of all proteins encoded by these genes is membrane- cytoskeleton-associated proteins. Their molecular functions

are actin-binding, actin filament binding, signaling receptor binding, and spectrin binding, respectively. Atresia as an extrahepatic disorder has three forms: common bile duct restricted (TypeI), atresia of the common hepatic duct(Type II), and atresia of the most proximal part of bile ducts (Type III), the last form being most common. ADD3, while being ubiquitary, has the smallest expression in the liver (mean RPKM 6.724±1.174) compared to all parenchyma organs [14,15]. Judging by the expression data, the specific anatomical localization of atresia, and the condition of three protein complexes, a hypothesis regarding misstep in 3 protein complex formation is feasible. In this study, the complex ADD1:ADD3: DMNT is characterized using computational methods: molecular dynamics, molecular base descriptor characterization, sequence analysis.

2. MATERIALS AND METHODS

In silico models for ADD1, ADD3 and DMNT were generated computationally. For the ADD1 UniProt sequence, P35611was used. ADD2 was generated using UniProt sequence P35612. For computing ADD3 UniProt sequence, Q9UEY8 was utilized. PDB crystallographic model 1QZP was employed for DMNT. Homology models were generated. The models were prepared for docking. In this respect, models were protonated at physiological conditions and minimized using the AMBER 99 force field[16]. In order to characterize the mode of action (MOA) of gene ADD3, a homology model of a gene product, namely gamma- adducin, was computationally generated using a template Uniprot sequence Q9UEY8-1. Their dimensional structure resulted was minimized and protonated at physiological pH and temperature. The ADD3 nucleotide sequence (**Table 1**) was further computed. ExPASy[17] software was used to translate the nucleotide sequence to a protein sequence both ways: 5'3' and 3'5', respectively. Open reading frames (ORF) detected were further used to predict by homology modeling the respective proteins. ADD1 complex with ADD2: DMNT and ADD3 DMNT were computed using the ZDOCK server. Properties of the resulted protein complexes were calculated using Schrodinger 2009[18] and MOE 2009 software packages.[19] Protein properties computed were protein mass[20], protein extinction coefficient[21], debye screening length[22], Henry's function[23], isoelectric point[24], percent helicity[25], the radius of gyration[26], hydrodynamic radius[27], eccentricity[28], VdW surface area[29], hydrophobic surface area[30], hydrophilic surface area[31], Vdw volume[32], sedimentation constant[33], frictional coefficient[34], diffusion coefficient[35], isoelectric point[36], average net charge[37], apparent charge[38], dipole moment[39], hydrophobicity moment[40], Zeta potential[41], the dipole moment of Zeta[42], quadruple moment of Zeta[43].

Table 1 ADD3 nucleotide sequence

1 agagcgcgag ccgccagccc gtaacggtcg

ccagtgtgag gggcgggagg gaaagaagag

61 gggtttaaat tagatttttt aaaacacaga gcaagcgcca

gaggcgtcgg catcccaggt

121 gtcgccgctt cctgctgcac agggctcggc

gtacaggtcc ctccctcctc aagccccctc

181 cccttctccc gccctaccct ctggggctct

gcggcgctta agaggcggcc gcagcggcgg

241 atccggcggc tgctgcagcc cgggcggctg

ccgagaagga gggaggggaa acacaaagcc

301 ggctacgcgc tgcgagataa caagagtaat

ccacagactt aaaacatgag ctcagatgcc

361 agccaaggcg tgattaccac tcctcctcct

cccagcatgc ctcacaaaga gagatatttt

421 gaccgcatca atgaaaatga cccagaatac

attagggaga ggaacatgtc tcctgatcta

481 cgacaagact tcaacatgat ggagcagagg

aaacgagtta ctcagatcct gcaaagtcct

541 gcctttcggg aagacttgga atgccttatt

caagaacaga tgaagaaagg ccacaaccca

601 actggattac tagcattaca gcagattgca

gattacatca tggccaattc tttctcgggt

661 ttttcttcac ctcctctcag tcttggcatg gtcacaccta

tcaatgacct tcctggtgca

721 gatacatcct catatgtgaa gggagaaaaa

cttactcgct gtaaacttgc cagcctgtac

781 agacttgtag acttgtttgg atgggcacac

ctggcaaata cctatatctc agtaagaata

841 agtaaggagc aagaccacat tataataatt

cccagaggcc tatctttttc tgaagctaca

901 gcctccaatt tggtgaaagt caatataata

ggagaagtgg ttgaccaggg aagtaccaat

961 ttgaaaattg accatacagg attcagtccc

catgctgcaa tctattcaac acgtcctgat

1021 gttaagtgtg tgatacacat ccataccctt

gcaacagcag ctgtatcctc catgaaatgt

1081 gggatccttc caatttctca agagtctctt

cttctgggag atgttgccta ttatgactac

1141 caagggtcac ttgaagaaca ggaggagaga

attcaactgc agaaggttct gggaccaagt

1201 tgtaaggtgc tggtactcag gaatcatggt

gtggttgcac ttggagaaac attagaggag

1261 gcttttcatt atatttttaa tgtgcaacta gcctgtgaga

ttcaggtgca ggccctagca

1321 ggtgcaggtg gagtagacaa tctccatgta

ctggactttc agaagtataa agctttcact

1381 tacactgtag cagcgtctgg tggaggaggt

gtgaatatgg gttcccatca aaaatggaag

1441 gttggcgaaa ttgagtttga agggcttatg

aggactctgg acaacttggg gtatagaaca

1501 ggctatgctt acaggcatcc tctcattcga

gagaagccta ggcacaagag tgatgtggaa

1561 atcccagcaa ctgtgactgc tttttccttt

gaagacgata cagtgccact ctctcctctc

1621 aaatacatgg cacagaggca acagcgtgaa

aaaacaagat ggctgaactc accaaatact

1681 tacatgaaag tgaatgtgcc tgaggagtct

cggaacggag aaaccagtcc ccgaaccaaa

1741 atcacgtgga tgaaagcaga agactcatct

aaagttagtg gtggaacacc tatcaaaatt

1801 gaagatccaa atcagtttgt tcctttaaac

acaaacccga atgaggtact agaaaagaga

1861 aataagattc gggaacaaaa tcgatatgac

ttgaaaacag caggaccaca atctcagttg

1921 cttgctggaa ttgttgtgga taagccacct

tctactatgc aatttgaaga tgatgatcat

1981 ggcccaccag ctcctcctaa cccatttagt

catctcacag aaggagaact tgaagagtat

2041 aagaggacaa tcgaacgtaa acaacaaggc

ctagaagatg ctgagcagga attactctca

2101 gatgacgctt catctgtttc acaaattcag

tctcaaactc agtcaccgca aaatgtccct

2161 gaaaaattag aagaaaacca tgagctgttt

tccaagagct tcatctccat ggaagtgcct

2221 gtcatggtag taaatggcaa ggatgatatg

catgatgttg aagatgagct tgctaagcga

2281 gtgagtaggt taagcacaag tacaaccata

gaaaacatcg agattactat taagtctcca

2341 gagaaaatcg aagaagtcct gtcacctgaa

ggctcccctt caaaatcgcc atccaagaaa

2401 aagaagaaat tccgcactcc ttcttttctg

aaaaagaaca aaaaaaagga gaaagttgag

2461 gcctaaataa agtcttttta taattattat tataacaatg

tgacattgca catctaaata

2521 ccacatttaa gttgatcatt aatatgcaat

ggtagatcag attgggggat gtagcaaact

2581 ggactttaag aactggaaag aggttttaca

aaagaaaaac tttcagattc atctctcatt

2641 ttatatgtcc agaaatggct ttgaatttta agcaattact

agttttaatt agctctgccc

2701 tcatgaagta ttattataat tcaccataaa

cagctatctg tctgaattac ttcaggcctt

2761 ctccataata tctgttagaa agaaattgcc

agtgagcaag tgagaatttt tatttctcaa

2821 tacctgcttc acttgataat catattataa ttttttatca

tgattattga ctatattttt

2881 ggagtcccat tgtttcagtg ggcattaaca

gaatgcttta aaaacttcta agacaagaat

2941 ctatagcatt agtatacact ggcacataat

tttttaaaaa gttttaagaa aagattcatt

3001 tggaatttta ttcacagtat aaaatttcct cacctgaagt

aactttgttt gccaaaaaag

3061 ttgttttaat aaactataat ttttgaaaac ttccttttt

attagtttag aaagcccctt

3121 atttttcaac aaaggggatt ttgtacacat

aacatgggtt atttagttta actctggcaa

3181 aaaaaaaaaa aaaaattttg tatgttgatg

tttgtatacc gttcagtata aaagtgtcct

3241 aagcatatta gccaatcttt tcacagtaga

gcatacttaa ggctgcttgg tactgagtat

3301 acttaaatat aactccagaa tccagggact

tggtgttaaa acaggattag agcatgtaaa

3361 ggtacatcta gattcatatt tgaatcttaa actgtatttt

tctcttagta ttgctaatga

3421 gtaaagaaaa gtctcataag gtagccaaat

gaaaaagaat gaaagggaaa gtgaaaaatt

3481 aaggggacaa aagatgggat gtgaaaagaa

gaattctagt ttgatggtga ctcatattca

3541 cgataggata caaagtgtga tttgttggaa

acatgtccca aatttctaaa attctgcttc

3601 tctgccaaaa gcaatgtctt tcttggttga tatttgagtt

ttaaaagggt caaatctttc

3661 taatttttg tatctttaga gggcagcact

agaagaaatc agcaggtcta atcccaccag

3721 taagaaaact accacttctt gatttttaca

gatttaaaaa aatcttttca gtgacctttc

3781 tttttaatgt aaatacaaat ttaaacctag gcttaatata

ggcgtttccc ctttcaccca

3841 agtgatgtca cagttcgatg caaaatcaat

gatccagaat gatcgtgggt aaaaataact

3901 caaagtgttt cttaagggtg agttggcatg

caaaaaatta cattgattac agtgtgtttt

3961 ggagctggct ctgtttgtgt gcatatgata

atgcagagtt gagccagagc ctggaaatgt

4021 cattctagat ctcactaact actggaatca gtgttttaat

ctcttggtgg aaactttcag

4081 ttgcttaact ctctattgga agatttttt aatgttctac

atcatttatg ttgtattaca

4141 atgtatgtag aaatagtaac ctgtgaacta

tgcttttcca taacttttta aaaatatata

4201 tatctaaatg aatgcaatgt gcataaatat tttttaaaca

taacagtgaa ctattgcacc

4261 ttttgctaat gcctctattt acttgctttg gcataaagaa

tgagccaatg aacctctgtg

4321 tcctgtggaa aaatgtataa atgttatctg atattgctct

tagatgtaat gctaattaat

4381 gttaaatcac aaataaacag tattttaaat ata

3. RESULTS AND DISCUSSION

Homology models of DDA1, DDA3, DMNT are represented below(**Figure1**)

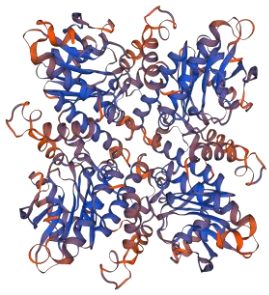
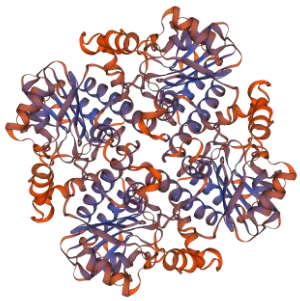
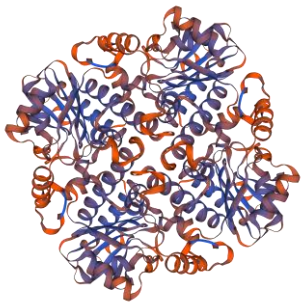
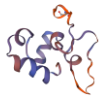
ADD1	**ADD2**	**ADD3**	**DMNT**
			
Class II aldolase/adducin domain protein	**Class II aldolase/adducin domain protein**	**Class II aldolase/adducin domain protein**	**Actin-binding LIM protein homolog**

Figure 1: Homology models of ADD1,ADD2, ADD3, DMNT proteins and their activity classes.

Table 2: Open reading frames of the ADD3 nucleotide sequence

5'3' Frame 1

RARAASP-RSPV-

GAGGKEEGFKLDFLKHRASARGVGIPGVAASCCTGLGVQVPPSSSPLPFSRPTLWGSAA

LKRRPQRRIRRLLQPGRLPRRREGKHKAGYALRDNKSNPQT-

NMSSDASQGVITTPPPPSMPHKERYFDRINENDPEYIRERNMSPDLRQDFNMMEQRKRV

TQILQSPAFREDLECLIQEQMKKGHNPTGLLALQQIADYIMANSFSGFSSPPLSLGMVTPI

NDLPGADTSSYVKGEKLTRCKLASLYRLVDLFGWAHLANTYISVRISKEQDHIIIIPRGLS

FSEATASNLVKVNIIGEVVDQGSTNLKIDHTGFSPHAAIYSTRPDVKCVIHIHTLATAAVS

SMKCGILPISQESLLLGDVAYYDYQGSLEEQEERIQLQKVLGPSCKVLVLRNHGVVALG

ETLEEAFHYIFNVQLACEIQVQALAGAGGVDNLHVLDFQKYKAFTYTVAASGGGGVN

MGSHQKWKVGEIEFEGLMRTLDNLGYRTGYAYRHPLIREKPRHKSDVEIPATVTAFSFE

DDTVPLSPLKYMAQRQQREKTRWLNSPNTYMKVNVPEESRNGETSPRTKITWMKAED
SSKVSGGTPIKIEDPNQFVPLNTNPNEVLEKRNKIREQNRYDLKTAGPQSQLLAGIVVDK
PPSTMQFEDDDHGPPAPPNPFSHLTEGELEEYKRTIERKQQGLEDAEQELLSDDASSVSQ
IQSQTQSPQNVPEKLEENHELFSKSFISMEVPVMVVNGKDDMHDVEDELAKRVSRLSTS
TTIENIEITIKSPEKIEEVLSPEGSPSKSPSKKKKKFRTPSFLKKNKKKEKVEA-
IKSFYNYYYNNVTLHI-IPHLS-SLICNGRSDWGM-QTGL-
ELERGFTKEKLSDSSLILYVQKWL-ILSNY-F-LALPS-SIIIIHHKQLSV-ITSGLLHNIC-
KEIASEQVRIFISQYLLHLIIIL-FFIMIIDYIFGVPLFQWALTECFKNF-DKNL-H-
YTLAHNFLKSFKKRFIWNFIHSIKFPHLK-LCLPKKLF--TIIFENFLFY-
FRKPLIFQQRGFCTHNMGYLV-LWQKKKKKFCMLMFVYRSV-
KCPKHISQSFHSRAYLRLLGTEYT-I-LQNPGTWC-NRIRACKGTSRFIFES-TVFFS-YC--
VKKSLIR-PNEKE-KGK-KIKGTKDGM-KEEF-FDGDSYSR-
DTKCDLLETCPKFLKFCFSAKSNVFLG-YLSFKRVKSF-FFVSLEGSTRRNQQV-SHQ-
ENYHFLIFTDLKKSFQ-PFFLM-IQI-T-A-YRRFPFHPSDVTVRCKINDPE-SWVKITQSVS-
G-VGMQKITLITVCFGAGSVCVHMIMQS-ARAWKCHSRSH-LLESVF-
SLGGNFQLLNSLLEDFFNVLHHLCCITMYVEIVTCELCFSITF-
KYIYLNECNVHKYFLNITVNYCTFC-CLYLLALA-RMSQ-
TSVSCGKMYKCYLILLLDVMLINVKSQINSILNI

5'3' Frame 2

EREPPARNGRQCEGREGKKRGLN-IF-
NTEQAPEASASQVSPLPAAQGSAYRSLPPQAPSPSPALPSGALRRLRGGRSGGSGGCCSP
GGCREGGRGNTKPATRCEITRVIHRLKT-AQMPAKA-
LPLLLLPACLTKRDILTASMKMTQNTLGRGTCLLIYDKTST-
WSRGNELLRSCKVLPFGKTWNALFKNR-RKATTQLDY-
HYSRLQITSWPILSRVFLHLLSVLAWSHLSMTFLVQIHPHM-REKNLLAVNLPACTDL-
TCLDGHTWQIPISQ-E-VRSKTTL--FPEAYLFLKLQPPIW-KSI--EKWLTREVPI-
KLTIQDSVPMLQSIQHVLMLSV-YTSIPLQQQLYPP-
NVGSFQFLKSLFFWEMLPIMTTKGHLKNRRREFNCRRFWDQVVRCWYSGIMVWLHLE
KH-RRLFIIFLMCN-PVRFRCRP-QVQVE-TISMYWTFRSIKLSLTL-QRLVEEV-
IWVPIKNGRLAKLSLKGL-GLWTTWGIEQAMLTGILSFERSLGTRVMWKSQQL-
LLFPLKTIQCHSLLSNTWHRGNSVKKQDG-THQILT-K-MCLRSLGTEKPVPEPKSRG-
KQKTHLKLVVEHLSKLKIQISLFL-TQTRMRY-KREIRFGNKIDMT-
KQQDHNLSCLLELLWISHLLLCNLKMMIMAHQLLLTHLVISQKENLKSIRGQSNVNNKA
-KMLSRNYSQMTLHLFHKFSLKLSHRKMSLKN-KKTMSCFPRASSPWKCLSW--
MARMICMMLKMSLLSE-VG-AQVQP-
KTSRLLLSLQRKSKKSCHLKAPLQNRHPRKRRNSALLLF-KRTKKRRKLRPK-

SLFIIIIITM-HCTSKYHI-VDH-
YAMVDQIGGCSKLDFKNWKEVLQKKNFQIHLSFYMSRNGFEF-AITSFN-LCPHEVLL-
FTINSYLSELLQAFSIISVRKKLPVSK-EFLFLNTCFT--SYYNFLS-LLTIFLESHCFSGH-
QNALKTSKTRIYSISIHWHIIF-KVLRKDSFGILFTV-NFLT-SNFVCQKSCFNKL-
FLKTSFFISLESPLFFNKGDFVHITWVI-FNSGKKKKKNFVC-
CLYTVQYKSVLSILANLFTVEHT-
GCLVLSILKYNSRIQGLGVKTGLEHVKVHLDSYLNLKLYFSLSIANE-RKVS-
GSQMKKNERESEKLRGQKMGCEKKNSSLMVTHIHDRIQSVICWKHVPNF-
NSASLPKAMSFLVDI-VLKGSNLSNFLYL-RAALEEISRSNPTSKKTTTS-FLQI-
KNLFSDLSF-CKYKFKPRLNIGVSPFTQVMSQFDAKSMIQNDRG-K-
LKVFLKGELACKKLH-LQCVLELALFVCI--
CRVEPEPGNVILDLTNYWNQCFNLLVETFSCLTLYWKIFLMFYIIYVVLQCM-K--
PVNYAFP-LFKNIYI-MNAMCINIF-T-Q-TIAPFANASIYLLWHKE-ANEPLCPVEKCINVI-
YCS-M-C-LMLNHK-TVF-I

5'3' Frame 3

SASRQPVTVASVRGGRERRGV-
IRFFKTQSKRQRRRHPRCRRFLLHRARRTGPSLLKPPPLLPPYPLGLCGA-
EAAAAADPAAAAARAAAEKEGGETQSRLRAAR-QE-
STDLKHELRCQPRRDYHSSSSQHASQREIF-PHQ-K-PRIH-GEEHVS-
STTRLQHDGAEETSYSDPAKSCLSGRLGMPYSRTDEERPQPNWITSITADCRLHHGQFFL
GFFFTSSQSWHGHTYQ-PSWCRYILICEGRKTYSL-
TCQPVQTCRLVWMGTPGKYLYLSKNK-GARPHYNNSQRPIFF-SYSLQFGESQYNRRSG-
PGKYQFEN-PYRIQSPCCNLFNTS-C-
VCDTHPYPCNSSCILHEMWDPSNFSRVSSSGRCCLL-LPRVT-RTGGENSTAEGSGTKL-
GAGTQESWCGCTWRNIRGGFSLYF-CATSL-DSGAGPSRCRWSRQSPCTGLSEV-
SFHLHCSSVWWRRCEYGFPSKMEGWRN-V-RAYEDSGQLGV-NRLCLQASSHSREA-
AQE-CGNPSNCDCFFL-RRYSATLSSQIHGTEATA-KNKMAELTKYLHESECA-
GVSERRNQSPNQNHVDESRRLI-S-WWNTYQN-RSKSVCSFKHKPE-GTRKEK-
DSGTKSI-LENSRTTISVACWNCCG-ATFYYAI-R--SWPTSSS-PI-SSHRRRT-RV-EDNRT-
TTRPRRC-AGITLR-RFICFTNSVSNSVTAKCP-KIRRKP-AVFQELHLHGSACHGSKWQG-
YA-C-R-AC-ASE-VKHKYNHRKHRDYY-VSRENRRSPVT-
RLPFKIAIQEKEEIPHSFFSEKEQKKGES-GLNKVFL-LLL-QCDIAHLNTTFKLIINMQW-
IRLGDVANWTLRTGKRFYKRKTFRFISHFICPEMALNFKQLLVLISSALMKYYYNSP-
TAICLNYFRPSP-YLLERNCQ-ASENFYFSIPASLDNHIIIFYHDY-LYFWSPIVSVGINRML-
KLLRQESIALVYTGT-FFKKF-EKIHLEFYSQYKISSPEVTLFAKKVVLINYNF-KLPFLLV-
KAPYFSTKGILYT-HGLFSLTLAKKKKKILYVDVCIPFSIKVS-AY-PIFSQ-SILKAAWY-

VYLNITPESRDLVLKQD-SM-RYI-IHI-ILNCIFLLVLLMSKEKSHKVAK-KRMKGKVKN-GDKRWDVKRRILV-W-LIFTIGYKV-FVGNMSQISKILLLCQKQCLSWLIFEF-KGQIFLIFCIFRGQH-KKSAGLIPPVRKLPLLDFYRFKKIFSVTFLFNVNTNLNLGLI-AFPLSPK-CHSSMQNQ-SRMIVGKNNSKCFLRVSWHAKNYIDYSVFWSWLCLCAYDNAELSQSLEMSF-ISLTTGISVLISWWKLSVA-LSIGRFF-CSTSFMLYYNVCRNSNL-TMLFHNFLKIYISK-MQCA-IFFKHNSELLHLLLMPLFTCFGIKNEPMNLCVLWKNV-MLSDIALRCNAN-C-ITNKQYFKY

3'5' Frame 1

YI-NTVYL-FNIN-HYI-EQYQITFIHFSTGHRGSLAHSLCQSK-IEALAKGAIVHCYV-KIFMHIAFI-IYIFLKSYGKA-FTGYYFYIHCNTT-MM-NIKKIFQ-RVKQLKVSTKRLKH-FQ-LVRSRMTFPGSGSTLHYHMHTNRASSKTHCNQCNFLHANSPLRNTLSYFYPRSFWIIDF ASNCDITWVKGETPILSLGLNLYLH-KERSLKRFF-ICKNQEVVVFLLVGLDLLISSSAAL-RYKKLERFDPFKTQISTKKDIAFGREAEF-KFGTCFQQITLCILS-I-VTIKLEFFFSHPIFCPLNFSLSLSFFFIWLPYETFLYSLAILREKYSLRFKYESRCTFTCSNP VLTPSPWILELYLSILSTKQP-VCSTVKRLANMLRTLLY-TVYKHQHTKFFFFFLPELN-ITHVMCTKSPLLKNKGLSKLIKKEVFKNYSLLKQLFWQTKLLQVRKFYTVNKIPNESFL KTF-KIMCQCILML-ILVLEVFKAFC-CPLKQWDSKNIVNNHDKKL-YDYQVKQVLRNKNSHLLTGNFFLTDIMEKA-SNSDR-LFMVNYNNTS-GQS-LKLVIA-NSKPFLDI-NER-I-KFFFCKTSFQFLKSSLLHPPI-STIAY--ST-MWYLDVQCHIVIIIIIKRLYLGLNFLLFFVLFQKRRSAEFLLFLGWRF-RGAFR-QDFFDFLWRLNSNLDVFYGCTCA-PTHSLSKLIFNIMHIILAIYYHDRHFHGDEALGKQLMVFF-FFRDILR-LSLRLNL-NR-SVI-E-FLLSIF-ALLFTFDCPLILFKFSFCEMTKWVRRSWWAMIIIFKLHSRRWLIHNNSSKQLRLWSCCFQ VISILFPNLISLF-YLIRVCV-RNKLIWIFNFDRCSTTNFR-VFCFHPRDFGSGTGFSVPRLLRHIHFHVSIW-VQPSCFFTLLPLCHVFERREWHCIVFKGKSSHSCWDFHITLVPRLLSNERMPVSIACSIPQ VVQSPHKPFKLNFANLPFLMGTHIHTSSTRRCYSVSESFILLKVQYMEIVYSTCTC-GLHLNLTG-LHIKNIMKSLL-CFSKCNHTMIPEYQHLTTWSQNLLQLNSLLLFFK-PLVVIIGNISQKKRLLRNWKDPTFHGGYSCCCKGMDVYHTLNIRTC-IDCSMGTESCMVNFQIGTSLVNHFSYYIDFHQIGGCSFRKR-ASGNYYNVVLLLTYSY-DIGICQVCPSKQVYKSVQAGKFTASKFFSLHI-GCICTRKVIDRCDHAKTERR-RKTRERIGHDVICNLL-C--SSWVVAFLHLFLNKAFQVFPKGRTLQDLSNSFPLLHHVEVLS-

IRRHVPLPNVFWVIFIDAVKISLFVRHAGRRRSGNHALAGI-
AHVLSLWITLVISQRVAGFVFPLPPSRQPPGLQQPPDPPLRPPLKRRRAPEGRAGEGEGA-
GGRDLYAEPCAAGSGDTWDADASGACSVF-KI-FKPLFFPSRPSHWRPLRAGGSRS

3'5' Frame 2

IFKILFICDLTLISITSKSNIR-HLYIFPQDTEVHWLILYAKASK-RH-QKVQ-
FTVMFKKYLCTLHSFRYIYF-KVMEKHSSQVTISTYIVIQHK-CRTLKKSSNRELSN-
KFPPRD-NTDSSS--DLE-HFQALAQLCIIICTQTEPAPKHTVINVIFCMPTHP-ETL-
VIFTHDHSGSLILHRTVTSLG-KGKRLY-A-V-ICIYIKKKGH-KDFFKSVKIKKW-
FSYWWD-TC-FLLVLPSKDTKN-
KDLTLLKLKYQPRKTLLLAEKQNFRNLGHVSNKSHFVSYREYESPSN-
NSSFHIPSFVPLIFHFPFHSFSFGYLMRLFFTH-QY-EKNTV-DSNMNLDVPLHALILF-
HQVPGFWSYI-VYSVPSSLKYALL-KDWLICLGHFYTERYTNINIQNFFFFFCQS-TK-
PMLCVQNPLC-KIRGFLN--KRKFSKIIVY-NNFFGKQSYFR-GNFIL-
IKFQMNLFLKLFKKLCASVY-CYRFLS-KFLKHSVNAH-NNGTPKI-SIIMIKNYNMIIK-
SRY-EIKILTCSLAISF-QILWRRPEVIQTDSCLW-IIIILHEGRAN-N--
LLKIQSHFWTYKMRDESESFSFVKPLSSS-SPVCYIPQSDLPLHINDQLKCGI-MCNVTLL-
--L-KDFI-
ASTFSFFLFFFRKEGVRNFFFFLDGDFEGEPSGDRTSSIFSGDLIVISMFSMVVLVLNLLTR
LASSSSTSCISSLPFTTMTGTSMEMKLLENSSWFSSNFSGTFCGD-V-D-
ICETDEASSESNSCSASSRPCCLRSIVLLYSSSSPSVR-LNGLGGAGGP-
SSSSNCIVEGGLSTTIPASN-DCGPAVFKSYRFCSRILFLFSSTSFGFVFKGTN-
FGSSILIGVPPLTLDESSAFIHVILVRGLVSPFRDSSGTFTFM-
VFGEFSHLVFSRCCLCAMYLRGESGTVSSSKEKAVTVAGISTSLLCLGFSRMRGCL-A-
PVLYPKLSRVLISPSNSISPTFHF-WEPIFTPPPPDAATV-VKALYF-
KSSTWRLSTPPAPARACT-ISQASCTLKI--KASSNVSPSATTP-FLSTSTLQLGPRTFCS-
ILSSCSSSDPW-S--ATSPRRRDS-EIGRIPHFMEDTAAVARVWMCITHLTSGRVE-
IAAWGLNPVWSIFKLVLPWSTTSPIILTFTKLEAVASEKDRPLGIIIMWSCSLLILTEI-
VFARCAHPNKSTSLYRLASLQRVSFSPFTYEDVSAPGRSLIGVTMPRLRGGEEKPEKELA
MM-SAICCNASNPVGLWPFFICS-IRHSKSSRKAGLCRI-
VTRFLCSIMLKSCRRSGDMFLSLMYSGSFSLMRSKYLSL-
GMLGGGGVVITPWLASELMF-VCGLLLLSRSA-
PALCFPSLLLGSRPGCSSRRIRRCGRLLSAAEPQRVGREKGRGLEEGGTCTPSPVQQEAA
TPGMPTPLALALCFKKSNLNPSSFPPAPHTGDRYGLAARA

3'5' Frame 3

YLKYCLFVI-H-
LALHLRAISDNIYTFFHRTQRFIGSFFMPKQVNRGISKRCNSSLLCLKNIYAHCIHLDIYIF

KKLWKSIVHRLLFLHTL-YNINDVEH-KNLPIES-ATESFHQEIKTLIPVVSEI-
NDISRLWLNSALSYAHKQSQLQNTL-SM-FFACQLTLKKHFELFLPTIILDH-FCIEL-
HHLGERGNAYIKPRFKFVFTLKRKVTEKIFLNL-KSRSGSFLTGGIRPADFF-
CCPLKIQKIRKI-PF-
NSNINQERHCFWQRSRILEIWDMFPTNHTLYPIVNMSHHQTRILLFTSHLLSP-
FFTFPFILFHLATL-DFSLLISNTKRKIQFKIQI-I-MYLYML-
SCFNTKSLDSGVIFKYTQYQAALSMLYCEKIG-YA-
DTFILNGIQTSTYKIFFFFFARVKLNNPCYVYKIPFVEK-GAF-TNKKGSFQKL-
FIKTTFLANKVTSGEEILYCE-NSK-IFS-NFLKNYVPVYTNAIDSCLRSF-
SILLMPTETMGLQKYSQ-S--KIII-LSSEAGIEK-KFSLAHWQFLSNRYYGEGLK-
FRQIAVYGEL--YFMRAELIKTSNCLKFKAISGHIK-EMNLKVFLL-
NLFPVLKVQFATSPNLIYHCILMINLNVVFRCAMSHCYNNNYKKTLFRPQLSPFFCSFSE
KKECGISSFSWMAILKGSLQVTGLLRFSLET---SRCFLWLYLCLTYSLA-
QAHLQHHAYHPCHLLP-QALPWR-
SSWKTAHGFLLIFQGHFAVTEFETEFVKQMKRHLRVIPAQHLLGLVVYVRLSSYTLQVL
LL-DD-MG-EELVGHDHHLQIA--
KVAYPQQFQQATEIVVLLFSSHIDFVPESYFSFLVPHSGLCLKEQTDLDLQF--VFHH-L-
MSLLLSST-FWFGDWFLRSETPQAHSLSCKYLVSSAILFFHAVASVPCI-
EERVALYRLQRKKQSQLLGFPHHSCA-ASLE-EDACKHSLFYTPSCPESS-
ALQTQFRQPSIFDGNPYSHLLHQTLLQCK-
KLYTSESPVHGDCLLHLHLLGPAPESHRLVAH-KYNEKPPLMFLQVQPHHDS-
VPAPYNLVPEPSAVEFSPPVLQVTLGSHNRQHLPEEETLEKLEGSHISWRIQLLLQGYGC
VSHT-HQDVLNRLQHGD-ILYGQFSNWYFPGQPLLLLY-LSPNWRL-LQKKIGLWELL-
CGLAPYLFLLRYRYLPGVPIQTSLQVCTGWQVYSE-VFLPSHMRMYLHQEGH--V-
PCQD-EEVKKNPRKNWP-CNLQSAVMLVIQLGCGLSSSVLE-GIPSLPERQDFAGSE-
LVSSAPSC-SLVVDQETCSSP-CILGHFH-CGQNISLCEACWEEEEW-
SRLGWHLSSCFKSVDYSCYLAARSRLCVSPPSFSAAARAAAAAGSAAAAAS-
APQSPRG-GGRRGGGLRREGPVRRALCSRKRRHLGCRRLWRLLCVLKNLI-
TPLLSLPPLTLATVTGWRLAL

Proteins resulted from using open sequences (form homology modeling) are represented in **Figure 3**. From frame 2, 5'3', no protein sequence were retrieved.

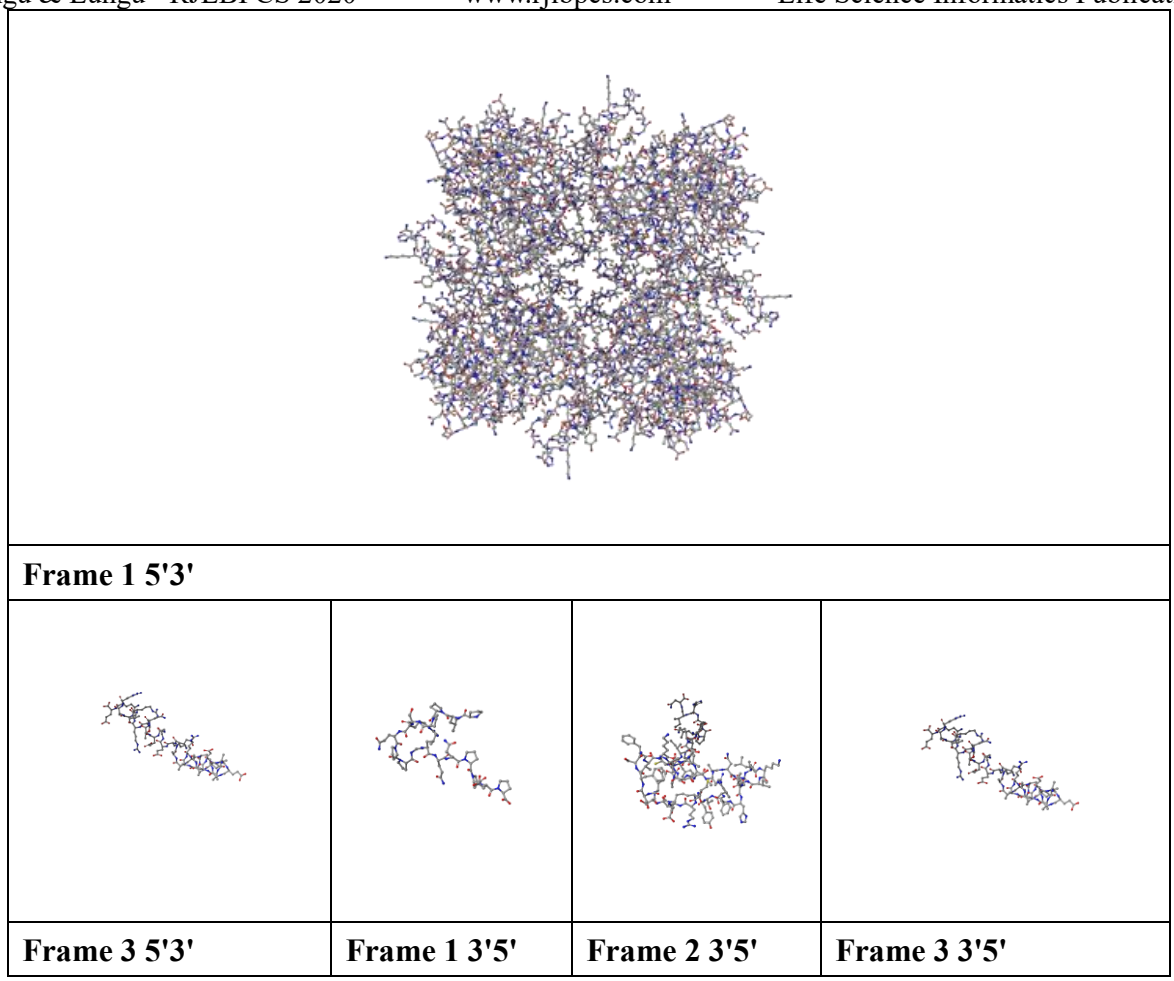


Figure 3: 3D protein models btained from frame 15'3', 35'3', 13'5', 23'5' and 33'5' repspectivelly.

Complexes between ADD1:ADD3: DMNT was simulated using protein-protein docking ADD1-ADD2-DNMT and ADD1-ADD3-DMNT complexes obtained are represented in **Figure 4.**

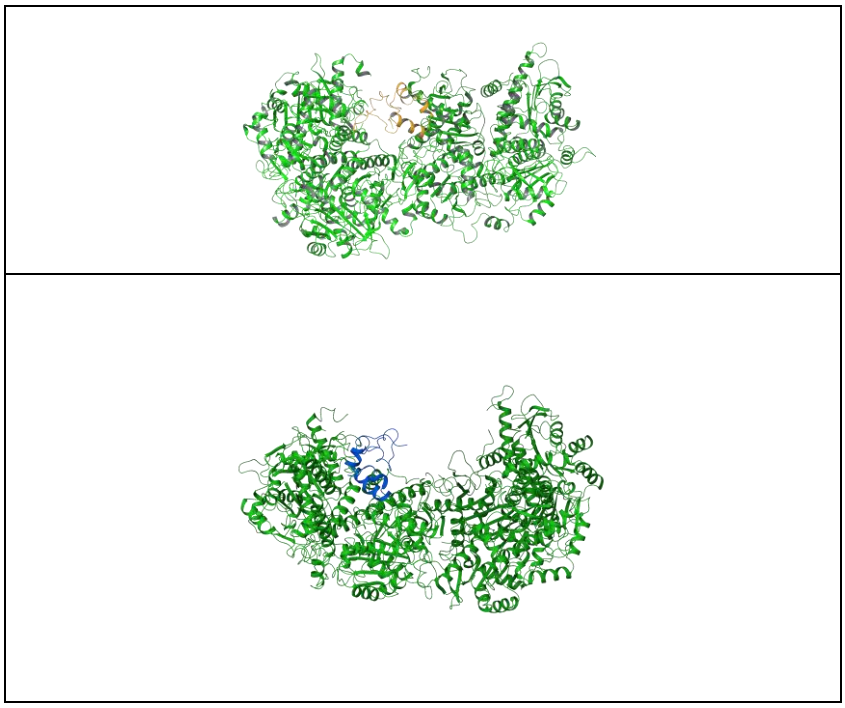
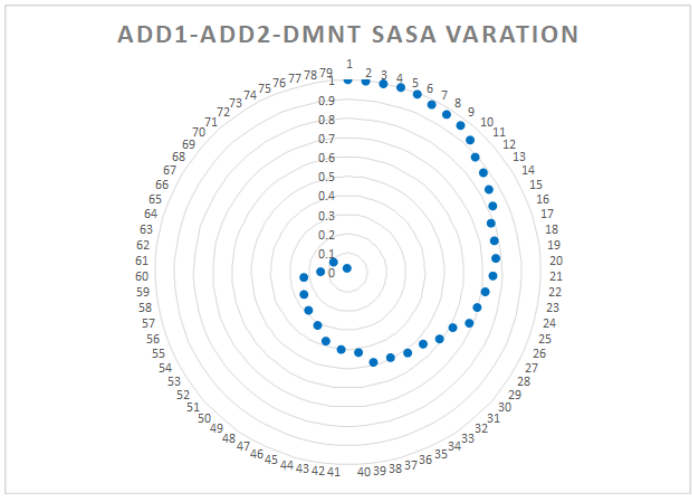
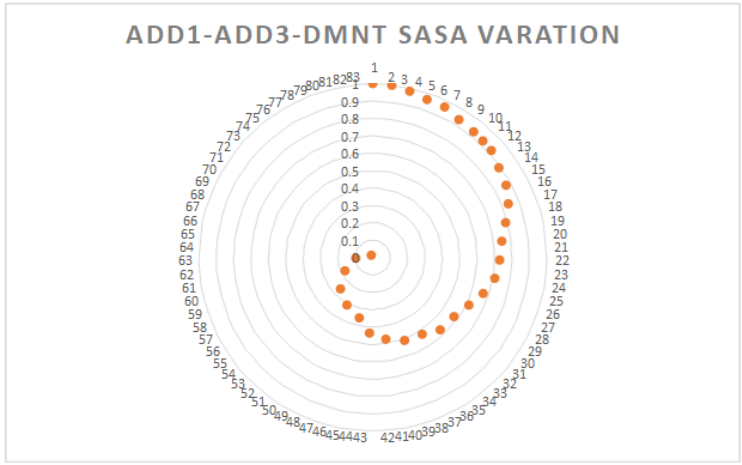


Figure 4: 3D models of ADD1-ADD2-DNMT and ADD1-ADD3-DMNT complexes

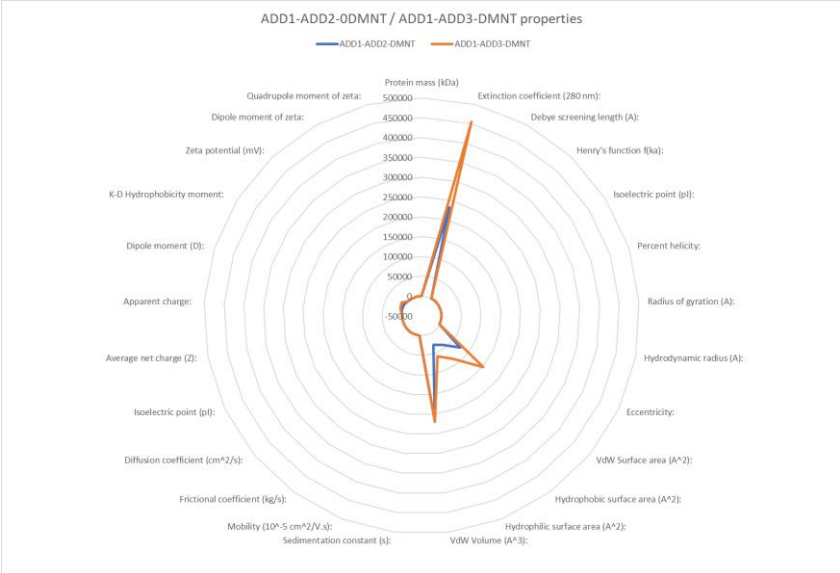


$y=-12x^6-0.9x^5-0.7x^4-0.5x^3-0.0015x^2+0.0042x+1.003, r^2=0.9973$



$y=-11x^6-0.8x^5-0.6x^4-04x^3-0.0024x^2+0.0114x+0.976, r^2=0.998$

Figure 5: SASA variation for ADD!-ADD2-DMNT and ADD1-ADD3-DMNT complexes, respectively.



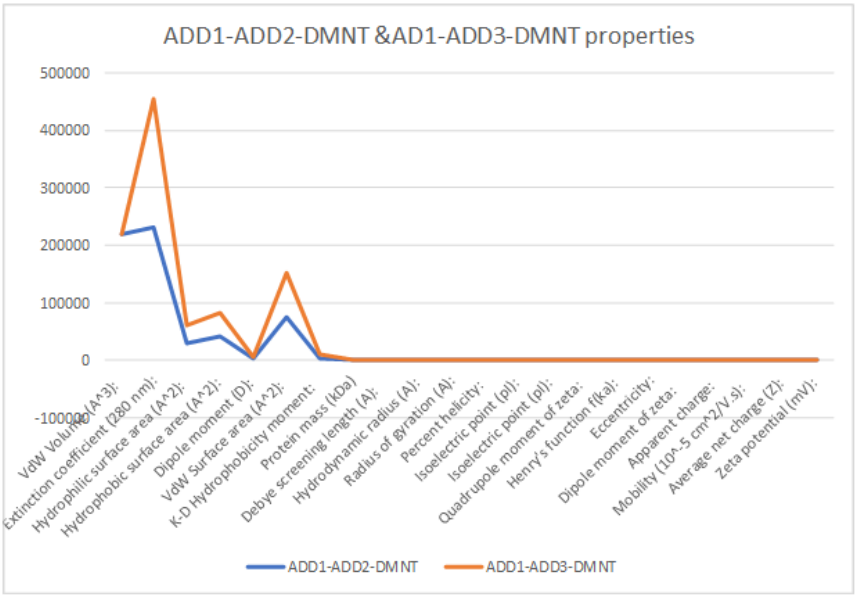


Figure 6: Property space of ADD1-ADD2-DMNT and ADD1-ADD3-DMNT complexes.

Homology modeling (represented in figure 2) shows that ADD2 and ADD3 are similar. However, biliary atresia occurs when a disfunction in the ADD3 3D structure is observed. DMNT is a relatively small molecule that forms a complex with ADD1, ADD2, and ADD3, respectively. Open reading frames show that it is relatively hard for a protein sequence to be misread, thus explaining the reduced frequency of biliary atresia. An open reading frame (ORF) has the property of being translated. ORF is a continuous string of codons that has a start codon (AUG) and stop codon (UAA, UAG, or UGA). ATG codon (AUG in terms of RNA) indicates where the process of translation begins. The termination signal is located after the ORF. If transcription ceases before the stop codon, an incomplete protein will result during translation. So these findings suggest that an inaccuracy in the transcription process may result in a dysfunctional ADD3 protein whose function is highly specific and can not be replaced with ADD2, which is morphologically similar to ADD3. As represented in table 2 only six transcription situations are available: 5'3' Frame 1, 5'3' Frame 2, 5'3' Frame 3, 3'5' Frame 1, 3'5' Frame 2, 3'5' Frame 2. Frome those Frame 15'3' retrieves fully functional ADD3 protein structures. If a transcription bias occurs, the rest of the frame retrieved the structures represented in Figure 3. As observed, those proteic structures do not match with ADD1 or DMNT and will not form an active complex. However, as stated before, ADD2 having a 99.9% structural similarity with ADD3 can replace it in forming ADD1: DMNT complex and thus correcting a possible transcription bias. Furthermore, as shown in Figure 5, where the same radar graph was obtained when computing the surface accessible solvent area (SASA), the trend line equations show that between ADD2 and ADD3 are small structural differences. Those contrasts are demonstrated by computed various protein properties for ADD2 and ADD3, respectively, and compare them. As shown in Figure 6, some properties demonstrate notable variations like VdW volume, extinction coefficient, hydrophilic and hydrophobic surface areas, dipole moment, VdW

surface area, and hydrophobicity moment, respectively. Additionally, those findings suggest that other than ADD3 bias transcription, there must be additional factors that lead to biliary atresia. If only ADD3 is malfunctioning" ADD2 can easily replace ADD3 function. So in biliary atresia, ADD3 defect must be synchronized with factors that incapacitate ADD2 to replace ADD3 in the embryogenesis cycle.

4. CONCLUSION

ADD1-ADD2-DMNT and ADD1-ADD3-DMNT complexes are similar to inherit architecture. ADD3 and ADD2 are crucial in biliary ducts embryogenesis. ADD2 and ADD3 are similar in their secondary, tertiary, and quaternary structures, respectively. Specific protein properties make ADD3 a crucial protein in biliary tract development.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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The authors contributed equally to this manuscript.

CONFLICT OF INTEREST

There is no conflict of interest

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