Evaluation of the Structural disorder of the protein FMR1 with Carbon Composition

Abstract: Ever since the disorder of proteins is the main cause for many diseases. As compared with other disorders, the major reason that causes disease is of structural inability of many proteins. The potentially imminent availability of recent datasets helps one to discover the protein disorders, however in majority of cases, the stability of proteins depend on the carbon content. Addressing this distinct feature, it is possible to hit upon the carbon distribution along the sequence and can easily recognize the stable nature of protein. There are certain reported mental disorders which fall in to this category. Regardless, such kind of disorder prone protein FMR1p (Fragile X mental retardation 1 protein) is identified as the main cause for the disease Fragile X syndrome. This paper deals with the identification of defects in the FMR1 protein sequence considering the carbon contents along the sequence. This attempt is to evaluate the stability of proteins, accordingly the protein disorders in order to improvised the certain Biological functions of proteins to prevent disease. The transition of the disorder to order protein involves careful considerations and can be achieved by detecting the unstable region that lacks hydrophobicity. This work focuses the low carbon content in the FMR1 protein so as to attain the stable status in future to reduce the morbidity rate caused by Fragile X syndrome for the society.

Keywords: Disorder protein, FMR1 protein, Carbon composition, Fragile X syndrome

I. INTRODUCTION:

Fragile X syndrome evolves its greatest threat to human kind with severe mental retardation and in general known as genomic syndrome due to the expansion of trinucleotide gene sequence (CGG) within X chromosome [1, 2, 3, 4]. What if the gene expands? And what could be the effects? To address these issues, it is been identified that the CGG repeat get amplified just before the coding region and intensely disrupts the final end product FMR1 protein, thus the expression of FMR1 protein is hindered [5, 6, 7]. This results in macroorchidism (enlargement of the testicles), large ears, prominent jaw, and high-pitched, jocular speech [8, 9, 10, 11, 12, 13, 14, and 15]. People with fragile X syndrome is believed to have 55 to 200 CGG repeats whereas the normal individuals have 6-54 CGG sequences [16]. Ultimately it results in methylation of FMR gene and the biological function of the FMR1 protein like translational efficiency and/or trafficking of certain mRNAs is disturbed and remains unstable [17, 18]. It is intended that the unstable protein lost its nature to fold itself to involve in biological process. Current research shows that carbon content along the sequence plays important role in maintaining the stability of the proteins and it has been proved 31.45% carbon content prefers to promote the stable nature of protein [19]. With this analysis, the disordered region of the protein sequences can be identified for further research programs. Henceforth the development of robust scientifically informed guidance on how best to improve the stable nature of protein need to be implemented for better function of FMR1 protein to depose the Down syndrome. In this work we attempt to show the nonstable region of the FMR1 protein based on carbon composition that is responsible for the misfolding of proteins.

II. RESEARCH BACKGROUND:

Many research works are based on the folded confirmation of the proteins so as to retrieve the tertiary structure and to carry out specific biological function. Nonetheless, most of the proteins remain unfolded and thus reported as disordered proteins. As a result, it involves complex scientific, ethical and political considerations such as whether and how best to deal the protein disorder [20]. The role of FMR1 protein results in many functions as stated above. Kwon et al., 2001 in his work says the expression of FMR1 protein inherently increases the activation of parietal lobe and also involved in early brain development. It also accelerates the regulation of protein synthesis in synapses [17]. The estimation of FMR1 protein expression within neurons is been done by Yu cui Chen et al., 2010. All these works adds weight to the importance of FMR1 protein.

All previous studies have shown that FMR1 gene alone is considered unlikely to pose unique risks and also FMR1p found to accelerate all normal neural functions [22, 23]. Such gene expansion will disrupts the expression of FMR1p and ends up in synaptic abnormalities. As a result many functions of synaptic proteins are up or down regulated and ultimately affect the neurotransmission and ends up in some neuronal disorders [24]. Such defect in FMR1p expression is found likely to be reporting some adverse events like dendritic abnormalities [25]. Finally the main cause of Fragile X syndrome is found to be associated with the defects in FMR1 protein expression.
III. METHODOLOGY:

The goal is to understand the molecular level of FMR1 protein. In general the folding properties of proteins depend on non covalent interactions. The rich and scarce distribution of carbon along the sequence contributes the folding nature of proteins. Accordingly, it confers the stability of proteins [26]. It is clear that considerable inherent variability in the carbon content affects the structure of proteins. For this reason, it is important to calculate the carbon level to encounter the disorder protein. The ideology is that to predict the carbon content with applicable C program called CARBANA [19]

The carbon prediction may provide valuable insights in Medical research and the initial dataset for this analysis can be accessed through online available databases. Here the disorder protein of FMR1 is accessed with DisProt (www.disprot.org). Similar databases are Disopred2 [27], On-D-CRF [28], FoldIndex [29], GeneSilico Metadisoreder [30], MDFp [31]. The disorder FMR1 protein was retrieved and its disport ID is DP00134 and the Unprot ID is Q06787.

Fasta Sequence of FMR1 protein:

>DisProt|DP00134|uniprot|Q06787|unigene|Hs.103183|sp|
MELVVEVGSNGAFYKVPDKVDHESTDIFVENNYQPDQPIFDHVFRPFPVGVYNQNDSEDEVYLYSRANEKEPCVWMLAKVRMIKGYFVIEYACDA
TYNEIVETLRSLVPNPKATDKTPHIKIDVPELDRLQMCHEAHAHKDFK
AVGFASYTDYSGYNVLQILSINEVTSKRAMLIDHHRFSLRTKLISLMREE
ASKQLESSRQLSRFHEQFIVREDLMGLAGTHGANIQAQARVPGVYTAIDL
EDCTFHUYGEDQAVKARSPLFAEDVDPVQVRLVEKVIYGNKLGJIEV
DKGSVVRVIBEAEENENKPVPOJEEMMPNSLPSNRSVPNPACHEKKLIDE
NTHSFSQPSTNKTQVRLAVSSVSAYAGESQPKAWGMVFFVFPVGTK3DA
NATVLDDYHLNLYKEVDQLRLERLQDEQVRIGASRSSPPNRTDECKSYT
DDQGMGRSRRPRYRNYGRGPGYGTSQTNSEAAGNASTESDHDLSDLW
SLAPTEERERESFLRQGRGRGGGHRGGGGRRGGGRGGGGKNDDRHSDSNRP
RNPREAKGRTTDGSLQIRVDCNNERSVHTKLTQNSSESGRLTRGDKNQK
KEKPSVDGQLQPLVNGP

CARBANA (www.rajasekaran.net.in/tools/carbana.html) has window size limit and the chosen window size is 700 as its sequence length is 632. The DisProt shows 41% disorder in FMR1 Protein. It is analyzed with its carbon composition and has been visualized (Fig 1).

IV. RESULTS AND DISCUSSION:

FMR1 gene and FMR1 protein intricately get involved in Fragile X syndrome. The initiative of this work focused on disorder portion of the FMR protein sequence. It gives the analysis to certain extent with which the unstable portion of the protein is predicted with the help of C program CARBANA. It tried to cover the disorder portion to the maximum by estimating the carbon distribution along the sequence. The misfolding and unfolding region of protein depends on carbon content and protein acquires its stable form with 31.45% carbon (Sneha et al., 2011). If any change in the carbon content happens, it leads to the disorder. The prediction of disorder portion helps to figure out the root cause for fragile X syndrome.

Figure 1 represents the disorder region of the protein and at the molecule level; we tried to show the carbon distribution with the provided input. As a result the amino acid position and its carbon composition are predicted and visualized (Table 1).

V. CONCLUSION:

Overall analysis provides an evidence of disorder portion of the protein FMR1. This striking observation of this analysis helps to identify the disorder in FMR1 protein. Our finding of protein sequence below the normal carbon content (31.45%) indicates the protein can misregulate and that approximates the reported gene disruption. Our work greatly expands to detect the possible cause of malfunctioning of the protein in causing fragile X syndrome. It provides potential insights in to underlying mechanism such as the failure of FMR1 protein expression due to disorder nature and unfit to fold itself to attain certain conformations to perform its biological function. Further studies using recent technologies can explore the translation of disorder to order protein.

VI. REFERENCES

[1] M Peretti, FP Zhang, YH Fu, ST Warren, BA Oostra, CT Caskey, DL Nelson . Absence of expression of the FMR1 gene in fragile X syndrome.1991. Cell 66:817– 822.
[2] EE Eichler, JJ Holden, BW Popovich, AL Reiss, K Snow, SN Thibodeau, CS Richards, PA Ward, DL Nelson. Length of uninterrupted CGGrepeats determines instability in the FMR1 gene. 1994. Nat Genet 8:88–94.
[3] H Siomi, M Choi, MC Siomi, RL Nussbaum, G Dreyfuss Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. 1994. Cell 77:33–39.
[4] Y Feng, F Zhang, LK Lokey, JL Chastain, L Lakkis, D Eberhart, ST Warren . Translational suppression by trinucleotide repeat expansion at FMR1. 1995. Science 268:731–734.
[5] I Oberle, F Rousseau, D Heitz, C Kretz, D Devys, A Hanauer, J Boue, MF Berthes, JL Mandel . Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. 1991. Science 252:1097–1102.
[6] AJ Verkerk, M Peretti, JS Sutcliffe, YH Fu, DP Kuhl, A Pizzuti, O Reiner, S Richards, MF Victoria, FP Zhang, BE Eussen, G-J van Ommen, LAJ Blonden, GJ Riggins, JL Chastain, CB Kunst, H Galjaard, CT Caskey, DL Nelson, BA Oostra, et al. Identification of a gene (FMR1) containing a CGG repeat coincident with a breakpoint cluster region...
exhibiting length variation in fragile X syndrome. 1991. Cell 65:905–914.

[7] RF Kooey. Of mice and the fragile X syndrome. 2003. Trends Genet 19:148–154.

[8] H Siomi, MC Siomi, RL Nussbaum, G Dreyfuss. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. 1993. Cell 74:291–298. PubMed: 7688265.

[9] D Devys, Y Lutz, N Rouyer, JP Bellocq, JL Mandel. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation.1993. Nat. Genet. 4:335–340. PubMed: 8401578.

[10] R Valverde, I Poznyakova, T Kajander, J Venkatraman, L Regan. Fragile X mental retardation syndrome: structure of the KH1-KH2 domains of fragile X mental retardation protein. 2007. Structure 15:1090–1098. PubMed: 17850748.

[11] K de Boule, AJMH Verkerk, E Reynolds, L Vits, J Hendrickx, B van Roy, F van den Bos, E de Graaf, BA Oostra, PJ Willems. A point mutation in the FMR1 gene associated with fragile X mental retardation. 1993. Nat. Genet. 3:31–35. PubMed: 8490650.

[12] C Verheij, E de Graaf, CE Bakker, R Willemsen, PJ Willems, N Meijer, H Galjaard, AJJ Reuser, BA Oostra, AT Hoevegeen. Characterization of FMR1 proteins isolated from different tissues. 1995. Hum. Mol. Genet. 4:895–901. PubMed: 7633450.

[13] Y Feng, D Absher, DE Eberhart, V Brown, HE Malter, ST Warren. FMRP associates with polyribosomes as an mRNP, and the I04N mutation of severe fragile X syndrome abolishes this association.1997. Mol. Cell 1:109–118. PubMed: 9659908.

[14] JC Darnell, CE Fraser, O Mostovetsky, G Stefani, TA Jones, SR Eddy, RB Darnell. Kissing complex RNAs mediate interaction between the Fragile-X mental retardation protein KH2 domain and brain polyribosomes. 2005. Genes Dev. 19:903–918. PubMed: 15805463.

[15] B Linder, O Ploettner, M Kroiss, E Hartmann, B Laggerbauer, G Meister, E Keidel, U Fischer. Tdrd3 is a novel stress granule-associated protein interacting with the Fragile-X syndrome protein FMRP. 2008. Hum. Mol. Genet. 17:3236–3246. PubMed: 18664458.

[16] B Donald Bailey, Jr Debra Skinner and L Karen Sparkman. Discovering Fragile X Syndrome: Family Experiences and Perceptions. 2003. Pediatrics 2003;111:407. DOI: 10.1542/peds.111.2.407.

[17] P Jin, ST Warren. New insights into fragile X syndrome: from molecules to neurobehaviors. 2003. Trends Biochem Sci 28:152–158.

[18] R Willemsen, BA Oostra, GJ Bassell, J Dictenberg . The fragile X syndrome: from molecular genetics to neurobiology. 2004. Ment Retard Dev Disabil Res Rev 10:60–67.

[19] E Rajasekaran & M Vijayasarith. 2011. Bioinformatics S: 455 [PMID: 21423892]

[20] R Charles Kissinger, A Keith Dunker, Eugene Shakhnovich. Disorder in protein structure and function. 1999. Pacific Symposium on Biocomputing 4:517–519.

[21] H Kwon, V Menon, S Eliez, IS Warsofsky, CD White, J Dyer-Friedman,et al. Functional neuroanatomy of visuospatial working memory in fragileX syndrome: relation to behavioral and molecular measures. 2001. Am J Psychiatry; 158: 1040±51.

[22] Yucui Chen, Flora Tassone, Robert Berman, Paul Hagerman, Randi Hagerman, Rob Willemsen and Isaac Pessah, Murine hippocampal neurons expressing Fmr1 gene premutations show early developmental deficits and late degeneration. 2010. Human Molecular Genetics, Vol. 19, No. 1 196–208 doi:10.1093/hmg/ddp479.

[23] Verkerk, Pizzi, Sutcliffe, Fu, Kuhl, Pizzuti, Reiner, Richards, Victoria, Zhang et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. 1991. Cell, 65, 905–914.

[24] SA Irwin, R Galvez and WT Greenough. Dendritic spine structural abnormalities in fragile-X mental retardation syndrome. 2000. Cereb. Cortex, 10, 1038–1044.

[25] Lujuan Liao, Sung Kyo Park, Tao Xu, Peter Vanderklish, and John Yates. Quantitative proteomic analysis of primary neurons reveals diverse changes in synaptic protein content in fmrl knockout mice. 2008. PNAS, vol. 105, no. 40 , 15281–15286 www.pnas.org/cgi/doi/10.1073/pnas.0804678105.

[26] SA Irwin, B Patel, M Idupulapati, JB Harris, RA Crisostomo, BP Larsen, F Kooy, PJ Willems, P Cras, PB Kozlowski. RA Swain, JJ Wefer, WT Greenough. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. 2001. Am J Med Genet 98:161–167.

[27] NJ Smeha et al. CMBB-2010 IEEE. 2011 231–232.

[28] JJ Ward, JS Sudhi, LJ Mcguinn, BF Buxton, DT Jones. Prediction and functional analysis of native disorder in proteins using conditional random fields. 2008. Bioinformatics 24 (11): 1401–2. doi:10.1093/bioinformatics/btn312.

[29] J Prilusky, CE Felder, T Zeve-Ben-Mordeheu et al. FoldIndex: a simple tool to predict whether a given protein sequence is intrinsically unfolded. 2005. Bioinformatics 21 (16): 3435–8. doi:10.1093/bioinformatics/bti537. PMID 15955783.http://bioinformatics.oxfordjournals.org/cgi/pmidlookup?view=long&pmid=15955783.

[30] A Schlessinger, M Punta, G Yachdav, L Kajam, B Rost, Orgel, Joseph P, R.J4 O, ed. Improved disorder prediction by combination of orthogonal approaches. 2009. PLoS ONE 4 (2): e4433. doi:10.1371/journal.pone.0004433. PMC 2635965. PMID 19209228.

[31] MJ Mziani, W Stach, K Chen, KD Kedarisetti, FM Disfani, L Kurgan. Improved sequence-based prediction of disordered regions with multilayer fusion of multiple information sources. 2010. Bioinformatics 26 (18): 4489–96. doi:10.1093/bioinformatics/btp437. PMC 2935446. PMID 20823312.
### Table 1: Carbon Distribution in FMR1 protein

| Aminoacid | Carbon composition (in %) | Aminoacid | Carbon composition (in %) | Aminoacid | Carbon composition (in %) |
|-----------|--------------------------|-----------|--------------------------|-----------|--------------------------|
| 23        | 32.76                    | 221       | 31.62                    | 429       | 31.34                    |
| 24        | 32.76                    | 222       | 31.91                    | 430       | 31.91                    |
| 25        | 33.62                    | 223       | 32.19                    | 431       | 30.77                    |
| 26        | 33.9                     | 224       | 32.19                    | 432       | 30.77                    |
| 28        | 33.33                    | 225       | 31.05                    | 433       | 30.48                    |
| 28        | 34.19                    | 226       | 31.05                    | 434       | 30.48                    |
| 30        | 34.19                    | 227       | 31.05                    | 435       | 30.48                    |
| 31        | 34.19                    | 228       | 30.77                    | 436       | 31.05                    |
| 32        | 33.62                    | 229       | 29.91                    | 437       | 30.77                    |
| 33        | 33.05                    | 230       | 29.91                    | 438       | 30.77                    |
| 34        | 34.47                    | 231       | 30.48                    | 438       | 30.77                    |
| 35        | 34.47                    | 232       | 29.91                    | 439       | 29.63                    |
| 36        | 33.05                    | 233       | 30.48                    | 440       | 29.91                    |
| 37        | 32.76                    | 234       | 29.91                    | 441       | 29.34                    |
| 38        | 33.05                    | 235       | 29.91                    | 443       | 29.63                    |
| 39        | 33.05                    | 236       | 30.2                     | 444       | 29.63                    |
| 40        | 32.76                    | 237       | 30.48                    | 444       | 29.63                    |
| 41        | 33.33                    | 238       | 29.34                    | 445       | 29.91                    |
| 42        | 32.76                    | 239       | 30.2                     | 446       | 30.2                     |
| 43        | 32.48                    | 240       | 29.91                    | 447       | 29.06                    |
| 44        | 31.91                    | 241       | 30.2                     | 448       | 28.77                    |
| 45        | 31.91                    | 242       | 29.91                    | 449       | 30.77                    |
| 46        | 32.76                    | 243       | 29.91                    | 450       | 29.91                    |
| 47        | 33.05                    | 244       | 30.2                     | 451       | 30.48                    |
| 48        | 32.76                    | 245       | 30.77                    | 451       | 29.63                    |
| 49        | 33.62                    | 246       | 31.34                    | 452       | 29.63                    |
| 50        | 32.19                    | 247       | 31.34                    | 454       | 29.06                    |
| 51        | 31.62                    | 248       | 32.48                    | 455       | 29.34                    |
| 52        | 31.34                    | 249       | 32.48                    | 456       | 30.2                     |
| 53        | 31.62                    | 250       | 31.91                    | 456       | 30.48                    |
| 54        | 31.34                    | 251       | 31.91                    | 457       | 31.05                    |
| 55        | 31.34                    | 252       | 32.48                    | 458       | 29.91                    |
| 56        | 30.77                    | 253       | 33.33                    | 460       | 29.91                    |
| 57        | 32.48                    | 254       | 33.62                    | 462       | 31.34                    |
| 59        | 31.62                    | 255       | 31.91                    | 463       | 29.34                    |
| 60        | 33.33                    | 256       | 32.19                    | 463       | 30.77                    |
| 61        | 33.05                    | 257       | 32.76                    | 465       | 29.91                    |
| 62        | 32.76                    | 258       | 32.19                    | 466       | 29.06                    |
| 63        | 33.05                    | 259       | 31.34                    | 467       | 30.48                    |
| 65        | 33.33                    | 260       | 32.19                    | 468       | 30.48                    |
| 66        | 32.76                    | 261       | 31.91                    | 469       | 30.48                    |
| 67        | 32.76                    | 262       | 32.19                    | 470       | 31.05                    |
| 68        | 32.76                    | 263       | 33.62                    | 471       | 29.34                    |
| 69        | 32.48                    | 264       | 33.33                    | 472       | 29.63                    |
| 70        | 30.77                    | 265       | 32.48                    | 473       | 29.63                    |
| 71        | 31.91                    | 266       | 32.19                    | 474       | 30.77                    |
| 72        | 32.76                    | 267       | 32.48                    | 475       | 28.49                    |
| 73        | 33.9                     | 268       | 32.76                    | 476       | 28.77                    |
| 75        | 34.19                    | 269       | 32.48                    | 477       | 28.49                    |
| 76        | 33.9                     | 270       | 31.91                    | 478       | 29.06                    |
| 76        | 33.9                     | 271       | 32.48                    | 480       | 29.91                    |
| 77        | 34.47                    | 272       | 32.19                    | 481       | 29.63                    |
| 79        | 33.9                     | 273       | 31.91                    | 483       | 29.06                    |
| 80        | 34.47                    | 274       | 31.91                    | 484       | 29.63                    |
| 81        | 33.05                    | 275       | 31.05                    | 485       | 29.34                    |
| 82        | 32.19                    | 276       | 30.77                    | 487       | 30.2                     |
|   |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|
| 83| 32.48| 283| 31.62| 488| 29.06|
| 83| 33.05| 284| 31.34| 489| 30.77|
| 85| 31.91| 285| 32.76| 490| 29.34|
| 86| 33.62| 286| 30.48| 491| 29.91|
| 87| 33.05| 287| 30.77| 491| 29.63|
| 87| 33.33| 288| 31.05| 492| 29.91|
| 88| 33.9| 289| 30.2| 493| 29.06|
| 89| 33.33| 290| 29.63| 494| 31.05|
| 90| 33.62| 291| 29.63| 497| 30.77|
| 91| 33.62| 292| 30.2| 498| 29.91|
| 92| 33.9| 294| 30.2| 499| 29.63|
| 93| 33.05| 294| 29.91| 500| 30.48|
| 94| 31.34| 295| 30.2| 500| 31.05|
| 95| 31.34| 296| 30.2| 502| 30.48|
| 96| 30.77| 298| 29.34| 504| 32.19|
| 97| 31.05| 298| 29.91| 504| 31.34|
| 98| 30.48| 299| 29.63| 506| 31.34|
| 99| 32.2| 300| 29.91| 508| 31.05|
|101| 30.48| 301| 29.91| 509| 31.34|
|102| 31.62| 303| 29.34| 510| 32.19|
|104| 30.48| 304| 30.2| 511| 32.19|
|105| 29.91| 305| 30.2| 513| 31.91|
|106| 29.91| 306| 29.06| 514| 32.48|
|107| 29.63| 307| 28.77| 516| 32.19|
|108| 29.91| 308| 29.06| 517| 31.62|
|109| 30.2| 309| 30.2| 518| 33.05|
|110| 30.48| 310| 30.2| 520| 33.33|
|111| 30.48| 312| 29.34| 521| 33.05|
|112| 30.77| 313| 30.2| 522| 31.62|
|113| 30.77| 314| 30.2| 523| 31.34|
|114| 31.34| 314| 30.2| 524| 32.19|
|115| 31.34| 315| 30.2| 525| 31.62|
|115| 31.62| 317| 29.91| 526| 31.05|
|117| 32.19| 317| 29.91| 527| 31.91|
|118| 31.91| 319| 30.48| 529| 31.62|
|119| 31.62| 320| 29.91| 529| 29.34|
|120| 31.34| 321| 30.77| 531| 29.63|
|121| 31.34| 322| 30.77| 531| 30.2|
|122| 31.34| 324| 31.62| 533| 28.49|
|123| 31.34| 325| 31.05| 534| 28.49|
|124| 30.77| 325| 30.48| 535| 29.06|
|126| 31.34| 326| 30.77| 536| 28.77|
|127| 29.91| 327| 30.77| 538| 28.77|
|128| 31.34| 328| 31.05| 538| 27.92|
|129| 30.48| 329| 31.05| 539| 28.21|
|130| 29.63| 330| 31.05| 541| 27.92|
|131| 29.63| 331| 30.77| 541| 27.92|
|132| 29.06| 333| 31.05| 542| 27.92|
|133| 31.34| 334| 31.05| 543| 27.35|
|133| 31.62| 334| 31.05| 544| 27.35|
|134| 31.62| 336| 31.34| 545| 28.49|
|135| 30.77| 337| 31.91| 546| 28.77|
|136| 31.05| 338| 31.05| 548| 27.92|
|137| 31.05| 339| 31.34| 549| 29.63|
|139| 31.91| 340| 30.77| 549| 28.49|
|140| 30.77| 341| 29.91| 550| 28.77|
|141| 30.2| 342| 29.91| 551| 29.34|
|142| 29.91| 343| 30.2| 554| 29.91|
|143| 32.19| 344| 30.2| 555| 29.06|
|143| 32.48| 345| 30.2| 556| 30.2|
|144| 33.33| 347| 30.77| 558| 29.34|
|146| 32.76| 348| 29.63| 559| 29.91|
|147| 33.62| 349| 29.91| 562| 30.2|
|148| 33.62| 350| 30.2| 562| 29.06|
|149| 34.47| 351| 31.05| 564| 29.06|
|151| 33.05| 353| 30.48| 564| 30.77|
|152| 33.33| 354| 30.2| 567| 29.91|
|153| 34.19| 355| 32.48| 568| 28.77|
