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Structural Intricacy of Disordered Regions in Transcription Factors Imparting Colon Cancer

Animesh Mondal [a], Uttam Pal [b], Anupam Roy [a], Nakul C Maiti [a]*

[a] *Structural Biology and Bioinformatics Division, Indian Institute of Chemical Biology, Council of Scientific and Industrial Research, 4, Raja S.C. Mullick Road, Kolkata 700032, India*

[b] *Chemical Sciences Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata, India*

***Corresponding author:** Nakul C Maiti. Tel : +91-33-2499-5940; Fax: +91-33-2473-5197; E-mail: ncmaiti@iicb.res.in.

Running Title: Insight into the Colon Cancer Transcription Factors.

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Abstract

Transcription factors (TFs) linked to cancer contains a significant amount of disordered segments and the dynamics as well as coupling of it with partner molecules are keys in the processes of recognization and many other cellular activities. However, the sequence distribution, composition and the structural adaptability of TFs in the presence of other interacting small and large macromolecules such as nucleic acids, lipids and other proteins are not well understood. In a recent article (*Specific DNA Sequences Allosterically Enhance Protein–Protein Interaction in a Transcription Factor, Phys. Chem. Chem. Phys.*) we showed that a small fluctuation in energetic and related conformational adaptation is a key in the recognition of multiple DNA sequences by TFs. In the current investigation we determined and showed the amino acid residue distribution, intrinsic characteristics, conformational adaptibility of the disorder regions and their similarity among the TFs which are linked to colon cancer. About 38% of the residues in TFs in average found to belong in the disordered region (DoR) and the abundance of these DoRs follows a poisson distribution pattern with expectancy value of 12. Interestingly, the size (length) distribution of the individual DoR also follows a similar statistical pattern. Computational analysis further establish the tertiary structure of many of the TFs by homology modeling and, it was observed that the TFs with disordered content less than 70% can attain different kind of tertiary folds. However, a significant segments preferred to remain as disordered (coiled coil) and found to localize preferentially on the surface of the proteins. This surface localization suggested interactive and functional links of the disordered regions with proteins and genomic materials involves in cell function and other biological activities. The presence and their localization may thus aid in understanding of eukaryotic gene transcription machinery as well as developing new drug targeting the disorder prone regions in the TFs to cure cancers and related human diseases.

Keywords : TFs; Secondary structure; DoR; Functional link; PSIPRED

Note – Colored figures and Supplementary information are available on the journal website

Introduction

Recent investigations found that human proteome are enriched with intrinsically disorder regions (IDRs) in their sequence which in turn reflect their indispensable role in cellular signalling and various other metabolic pathways. The number of disorder proteins known to be involved in cell signalling and regulation are growing rapidly. Various well-characterized examples of individual disorder proteins involved in transcriptional regulation have been illustrated in literature (Dyson and Wright, 2002; Iakoucheva et al., 2002). However, the functional role of IDRs in crucial areas such as transcriptional regulation, translation and other cellular signal transduction has only been organized as a consequence of the use of new paradigms in biological methodology (DeForte and Uversky, 2017; Du and Uversky, 2017; Dyson and Wright, 2005). Intrinsically disorder region of the protein often involves in this recognization event. Several DNA binding proteins, including transcription factors (TFs) bind to DNA sequences however, with different affinity and the conformational adaptability (Banerjee and Chakraborty, 2017; Guo et al., 2017; Mazumder et al., 2017; Naiya et al., 2016; Uversky et al., 2014) of the protein-binding domain plays a key role in this protein-DNA interaction

Intrinsically disorder proteins (IDPs) or regions (IDRs) are characterized by the unique combination of high specificity and low affinity in their interaction with functional partners, which are very crucial for transient protein-protein, and protein-nucleic acid interactions, such as those that frequently occur during signal transduction, recognition and other cellular events. The propensity of disorder proteins or regions to form large interaction surfaces allowing them to wrap up or surround their binding partners (Liu et al., 2006). Disorder regions are essential for the function of transcriptional activators and numerous others signalling and regulatory proteins (Dyson and Wright, 2005). These IDRs are the site of many chromosomal translocations that are associated with the disease; for example translocation, that fuse region or CBP (CREB binding protein) or p300 to segments of MOZ (monocytic zinc finger leukemia protein) or MLL are associated with human leukemias (Goodman and Smolik, 2000; Yang, 2004). This phenomenon probably reflects the structural organization of proteins. Computational studies and experimental investigation further verified that binding regions in IDPs/IDRs are exposed and often considered as a primary contact site for the interaction and binding (Pal et al., 2016). In the present investigation, we aimed to procure the composition and conformational adaptability of

the disorder regions in the transcription factors associated with colon cancer and derived some statistical correlation. Illustrating disorder regions and associated statistical knowledge is crucial to address functional and binding roles of proteins.

We have selected the transcription factors that are responsible for colon cancer from the extensive literature survey and 35 transcription factors were taken for our analysis purposes. Colon cancer is the third most common type cancer in people. For deriving a new approach towards drug target analysis of DoR with in the TFs responsible for this cancer would be a novel stratagem.Various statistical models were enjoined to divulge the distribution of disorder region, hydrophobicity, and the structural preferences. Abundance (number) of disordered regions in the domain followed a Poisson distribution pattern with expectancy value of 12. Interestingly, the size (length) distribution of the individual DoR also followed a Poisson distribution. Conformational preferences of the disordered segments and the whole proteins were made by ab-initio method. It helped to trace the location of the disordered regions on the modeled structure; whether it is located on the inner surface or outer surface of the proteins. Interestingly, it was found that most of the disordered regions located on the outer surface of the proteins.

Materials and Methods

Dataset Formation :

Transcription Factors (TFs) that are responsible for colon cancer were obtained from an extensive literature search and 35 TFs were chosen for our analysis purpose (Ballian et al., 2008; Bruun et al., 2014; Brzozowa et al., 2015; Cajuso et al., 2014; Cathomas, 2014; Caunt et al., 2015; Dawson et al., 2014; Francipane and Lagasse, 2013; Gerlach et al., 2012; Hackl et al., 2010; Hibi et al., 2009; Hu et al., 2010; Irby and Yeatman, 2000; Kobayashi et al., 1999; Li et al., 2014; Morishita et al., 2010; Naccarati et al., 2012; Network, 2012; Petrova et al., 2008; Slattery et al., 2010; Smith et al., 1993; Spano et al., 2005; Yang et al., 2018; Yao et al., 2013; Zhu et al., 2014). Sequences of these TFs were retrieved from UniProt. Sequences obtained from Uniprot in FASTA format were converted to strings of single letter amino acid codes for the further analysis purpose (Table 2).

Ab initio Modelling :

Structural model of TFs were rendered using I-TASSER (Iterative Threading ASSEmbly Refinement) services of Zhang Lab. It first identifies structural templates from the PDB by multiple threading approach LOMETS with full-length atomic model constructed by iterative template fragment assembly simulations.Further function insights of the target were then retrieved by threading 3D model through protein function database BioLiP (Roy et al., 2010).

Calculation of Disorder Regions :

Disorder residues and regions were identified using IUPred web server. It recognizes disorder region from the amino acid sequences of protein based on the estimated paired energy content. The assumption behind is that globular protein are composed of amino acids which have the tendency to form a large number of interactions. On the other hand, IDPs do not adopt any suitable structure because of their amino acid composition which creates hindrance in case of their interaction. This web server takes single amino acid sequences as an input and calculates the pairwise energy profile along the sequence. The energy value is then transformed into a probabilistic score ranging from 0 (complete order) to 1 (complete disorder) (Dosztányi et al., 2005). Residues above 0.5 regarded as disorder and the regions are counted by finding a stretch

where is no ordered residue between the two consecutive disordered residues.

Analysis of Sequence :

The composition of amino acids, length, charged residues, total charge and molecular weight were calculated from the sequence of proteins. Gravy value of proteins and disorder regions were calculated as a result of hydropathy value of amino acids. Isoelectric points were calculated from ExPASy bioinformatics portal using ProtParam tool (Gasteiger et al., 2005). The secondary structural propensity of each protein for their amino acids were predicted using PSIPRED algorithm (Jones, 1999). Preference of a particular conformation of protein was measured by taking the ratio of total number of resiudes preferring particular conformation. Similarly, the conformation propensity of disorder residues and regions were derived from the mother protein using position and length parameter.

Functional Link :

Functional protein-protein interaction was determined by deriving the names of the associated proteins with our TFs.Proteins that are linked with TFs were fetched from String DataBase (Szklarczyk et al., 2015). Finally, all the proteins were compiled in Wolfram Mathematica 10. Interactions between them were determined by the number of their nodes i.e more nodes indicate more interactions.

Statistical Analysis :

All the statistical analysis was performed in Wolfram-Mathematica 10. Significance of the mean differences was established by performing Student's t-Test and the null hypothesis was rejected at the 5% level of significance.Poisson distribution was fitted to the Disorder region frequency (DoR) and length if the data.The Probability Mass Function is calculated by -

$$f(x;\mu) = \frac{e^{-\mu}\mu^{x}}{x!} \quad \text{..... (1)}$$

Where *e* is Euler's number and μ is the expected value of the random variable *x.*

The Cumulative Function is calculated by –

$$g(x;\mu) = e^{-\mu}\sum_{i=0}^{[x]}\frac{\mu^i}{i!} \quad \text{.....(2)}$$

Where $[x]$ is the floor function.

Generalised Poisson distribution function is calculated by the equation –

$$f(x;\mu) = \frac{e^{-x\lambda-\mu}(x\lambda+\mu)^{-1+x}}{x!} \quad \text{.....(3)}$$

Where λ is a real number between 0 and 1.

Normal distribution with mean (μ) and standard deviation (σ) was fitted to the Normally distributed data.The probability distribution function is formulated through –

$$P(x) = \frac{1}{\sqrt{2\pi}\sigma}e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad \text{.....(4)}$$

The Cumulative Distribution Function is calculated by –

$$D(x) = \frac{1}{2}\left[1 + \text{erf}\left[\frac{x-\mu}{\sqrt{2}\sigma}\right]\right] \quad \text{.....(5)}$$

Where *erf* is error function.

Result and Discussion

Transcription factors (TFs) are involved in different cell regulatory pathways. To understand their regulatory diversification in the purview of the structural aspect we analyzed the TFs sequences of the dataset. We used IUPred algorithm to determine disorder residues and regions. The analysis showed variation in TFs degree of disorderness and functions as given in (Table 1). The disorder regions (DoRs) were selected from the protein sequence based on the estimated paired energy content. Most of the proteins contained disorder regions. (Table 2) gives the details of the disorder region and the parent proteins. IUPred detected total 428 disorder regions with varying degree of disorderness and they were distributed with in 35 number of TFs.

The occurrence (frequency distribution) pattern of the DoRs present in the TFs showed a poisson distribution pattern (Fig. 1) and indicated that the occurrence of the regions was a stochastic in nature and it satisfied the Markov Property (Durbin et al., 1998). The poisson distribution always provides the expectation value (λ) and, the expected occurrence (λ , statistically obtained value) of disordered regions was found to be 12. The sizes (length) of the diorder regions also followed a poisson distribution pattern (Fig. 1) and the expectation value for the length was close to 22. Fig. 2 shows the regression analysis to derive a correlation between protein length and the content of residues in the DoRs. It was found that the percentage of the disordered residues and the length of the protein was poorly correlated to each other with a very low R^2 value of 0.15. This conferred that the protein disordereness was independent of protein length.

Analysis was also made to detail the content of different amino acid residues in the DoRs and in total TFs. The content of different amino acid residues in the composition of the DoRs and TFs are shown in (Fig. 3). Panel A in the figure compares the sequence composition of DoRs with the Total TFs. Although the distribution pattern of amino acids for both the two was similar, an apparent difference in the sequence population of amino acid in TFs was observed Panel B in (Fig.3). Residues S, Q, G and P were at least 1% higher in number than in the total protein sequence. It was expected that the disordered segments will be rich with G, P residues. However, also we observed higher amount of E,T, N and A in the disordered regions. Hydrophobic amino acids such as G and A are also abundant in the disorder prone region of TFs. Glycine is a unique amino acid because it contains hydrogen as its side chain other than carbon containing group in case of other amino acids. This confers much more flexibility when TFs interact with other partner. Moreover, it can reside in part of protein structures when it forms turn where all other amino acids are disallowed. Alanine is a non polar amino acid and its side chain is non reactive but it can play role in substrate recognition. These residues are with strong helical propensity (Koehl and Levitt, 1999) and may helps in attaining ordered structure upon binding with a suitable partner. It was also observed that V, I, L, F residues were specifically decreased in the disorder region in comparison to the total protein sequences. Interestingly the abundance of C residues was less; being TFs the proteins needs more pliability and evolutionary such selection was required. From our previous report (Das et al., 2014), it was well established that low complexity region which is also responsible for disorderness, promoted by P, G, E, S, Q and

A residues. While analyzing the distribution pattern of the previously mentioned amino acids we have found them in high number within the disordered regions of TFs (Fig. 3B). Additionally, the residues that are responsible for promoting compact structure like C and W increased and P decreased, but the rest of the residues remain almost unchanged in the three classes. In case of MDP and LDP, there was a distinct difference in the number of G residues. LDPs are highly flexible in nature and for satisfying that feature high number of G and a low number of M and V is highly justifiable. The whole TFs showed amino acid distribution pattern similar to the total human disorder proteins found in DisProt database (Fig. S1). Athough the abundance of K and V amino acids were higher in TFs compared to the DisProt proteins but P and S were lower in case of TFs.

From various investigation, it has been well documented that tight regulation of IDP is very necessary for response to specific stimuli (Babu, 2016). In our study we have predicted the possible link up among the TFs by deriving its interacting partner from String Database (Fig. 4). In the predicted link these TFs showed maximum interspecific interaction between the TFs. However,CASP1 and PROX1 preferred maximum intraspecific interaction. Functionally CASP1 is very important because it is the key player in apoptosis pathway and PROX1 is a specific target of the β-Catenin/TCF pathway. From the prospect of disorderness CASP1 and PROX fall under PDP and MDP group respectively.

As interaction and functionality of the DoRs are of significant importance the hydrophobicity of the regions were judged by measuring the hydropathy indexes. Hydropathy index is the most preferable tool (Wu et al., 2006) that provide much intricate results about the hydrophobicity. By using hydropathy indexes of each amino acids the grand average hydropathy (GRAVY) values of DoR and the parent TFs were determined (Table 3). The calculated GRAVY indexes of majority of the TFs were predominantly negative and varied between 0 to -2. However, in case of the DoRs it varied between -4.5 to +4.5 (Fig.5 and 6).Generally, if the hydropathy score lies below zero it confers the proteins most likely to be globular , where as hydropathy score above zero indicates membranous nature of the proteins.

Revealing disorder regions and its structural preference in TFs could unveil the functional peculiarity of TFs and the secondary structural preferences of the residues were calculated by IUPred algorithm. The amino acid residues, both in the TFs and DoRs within them prefer to adopt all the three major conformations such as helix, strand and coil which are depicted in the (Fig. 7). IUPred analysis is independent of secondary structure (Dosztányi et al., 2005) and therefore the predicted structure may not be largely biased. The analysis revealed less strand/beta sheet conformation in the DoRs (Fig. 8). Preferences for coiled conformation was found to be most abundant in the DoRs and some of the residues prefer to adopt helix conformation. Also, as expected, the structural propensity of DoR towards coil conformation was reasonably higher than the parental TFs. The overall structural content of TFs sequence was ~22% helix,7% strand/sheet and 70% coil indicating the flexible nature of the TFs. However, in the DoR, the conformational preference was ~11% helix, 2% strand and 86% coil conformation. This indicated that a good number of the DoR residues potentially can be converted into alpha helical structural domain under suitable solution condition or in the presence of other molecule.

We further determined the molecular structure (2D and 3D) of the TFs by homology modeling (Fig. 9 and Fig. S2) and investigated the status of the DoRs in the modeled 3D representation. As expected, the TFs with lesser (less than 70%) disordered sequences yield 3D structures rich in alpha helical folds. Secondary structural propensity of the DoRs quite matches with the Psipred predicted secondary structural propensity of the residues in the region. For instance, ATF3 (30 % of residues belong to disordered regions) the longest disordered region, residues from 76 to 102 showed structural propensity mainly to helix and coiled conformation that largely contribute to the disorderness. In the homology modeling structure also it showed similar structural propensity. Another important observation was that the disordered segments obtained by homology modeling remained mostly on the surface (Fig. S3) or stayed as flanks and extended to the solvent. However, large disordered regions, particularly in the middle of the protein sequence disallow the protein to adopt any compact and big folded structure. For instance, the disordered segment comprising of residues 70-89 in NFAC2 showed an extended structure in its 3D modeled structure (Fig. S2). ERBB2, also in its tertiary model reveals elongated conformation due to the presence of a long disorder segment. Amusingly, in case of CDX2 although it carries a long DoR of residues (70-90), its tertiary structure was not much elongated and contain significant amount of globular folded structure.

Conclusion :

In the current manuscript we discussed the sequence aspects and the distribution pattern of disordered regions in TFs associated with colon cancer. It is reported that transcription factors in general contain intrinsically disordered regions in their sequence and contribute to their transactivation activity. It was reported that more than 80% of all transcription factors contain regions that are intrinsically disordered. Highly disorder proteins do not adopt any stable structure because of their amino acid composition which mostly disfavor hydrophobic collapse. In addition favorable solvation energy creates hindrance in formation of ordered and compact structure. The presence of disorderness in protein sequences reflects their greater need for cooperation, coordination and liaisons in diverse signalling pathway. Here, we investigated the disorder in transcription factors that are associated with colon cancer. Through a critical examination of disorder region by combining statistical and physiochemical information we determined the occurrence of disorder region in TFs and showed the distribution of disorder region length and its frequency in the parent TFs. This disorder region showed amphipathic nature. In the modeled 3D structure the disordered region found to prefer localization on the surface area of the protein. Such localization may significantly enhance useful in the initial stages of target selection for drug molecule. It is well known that the presence of well defined three-dimensional structure is the prerequisite of protein function (Tompa, 2003). Majority of proteins used to adopt a defined three-dimensional structure to carry out their function. Therefore, the drug targets are often chosen in this structured regions. The development of a new advanced way to discover drug molecule would be interesting if this disorder region in TFs are taken into account.

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Conflict of Interest

The authors declare no competiting financial interests.

Abbreviations

TFs: Transcription Factors; DoR: Disorder Region; IDPs: Intrinsically disorder proteins; IDRs: Intrinsically disorder regions.

Supporting information

Figure - S1 Comparison of distribution of amino acid with in the TFs and total human protein found in DisProt Data base; Figure - S2 Prdicted 3D conformation Of Transcription factors and Figure - S3 Predicted model structure of TFs exhibiting DoRs.

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Figure legends

Fig. 1 : Frequency and length distribution of the DoR. (A) Probability of the occurence of DoR in TFs.Probability mass function (PMF) of the fitted Poisson distribution is shown. (B) Cumulative distribution function of the DoR frequency. (C) PMF of individual DoR length in TFs.(D) CDF of the DoR length distribution.

Fig. 2 : Correlation between the Protein length (TFs) and disorder % in TFs. (A) Distribution of the protein disorderness across its length. (B) Fitted linear model of the disorder % and length distribution.

Fig. 3 : (A) Comparison of Amino Acid distribution between Whole TFs and DoR. Here the difference in distribution of amino acids were considered significant at P value <0.05.

(B) Difference of amino acid between disorder region and total protein.

Fig. 4 : Predicted functional link among TFs.TFs acts via different other factors which are also inter linked with each other. Here probable link between these TFs are indicated.

Fig.5 : GRAVY distribution of the Whole TFs and DoR. Row1: GRAVY of the whole TFs.Row2: GRAVY of the DoR. Column 1&2: Histogram PDF & CDF. Column3&4: Fitted Normal distribution of the PDF & CDF.

Fig. 6 : Comparison of GRAVY between Whole TFs and DoR. (A) PDF,(B) CDF. Color Key: Light Blue – Whole TFs, Grey- DoR.

Fig. 7 : Distribution of Secondary structure in TFs and DoR.Here Coil conformation(86%) in highly preferred among the disorder regions, followed by helix and strand conformation .

Fig. 8 : Comparison of Secondary Structutre conformation in TFs and with in the disorder region (DoR).

Fig. 9 : Predicted model of TFs along with its disorder region (DoR) of CDX2 and NFAC2 . Predicted 3D conformation Of Transcription factors.

Color Key-

- Largely extended disordered segments.
- Comparatively less extended disordered segments and large disordered loop.
- More compact disordered globule.
- Moderate disordered globule.
- Swollen coil or molten disordered globule.
- Folded secondary structures.

Table 1: Poisson distribution parameters (λ) for DoR frequency and length of DoR.

Variable	**λ**
DoR Frequency	12.22
DoR length	22.26

Table 2: Details of TFs and their disorder percentage.

Sl No.	Protein Name	Uniprot ID	Localization	Function	Length	Disorder Seq.	Disorder %	PI	Hydropathy
1	Activin receptor type-1B isoform1	UniProtKB - P36896 (ACV1B_HUMAN)	Membrane	Forming an activin receptor complex with activin receptor type 2 and then interact with Smad. Helps in neuronal differentiation extracellular matrix production	505	0	0	6.6	-0.094
2	Activin receptor type-2A isoform1	UniProtKB - P27037 (AVR2A_HUMAN)	Membrane	On ligand binding form a receptor complex and activate SMAD	513	7	1.36	5.61	-0.202
3	AMER1	UniProtKB - Q5JTC6 (AMER1_HUMAN)	Cytoplasm/ Nucleus	Regulator of canonical Wnt signalling pathway	1135	846	74.53	4.77	-0.867
4	Adenomatous polyposis coli protein	UniProtKB - P25054 (APC_HUMAN)	Cytoplasm/cell membrane	Tumor suppressor	2843	2173	76.43	7.92	-0.89
5	ARID1A	UniProtKB - O14497 (ARI1A_HUMAN)	Nucleus	Involves in chromatin remodelling	2285	1755	76.80	6.24	-0.778
6	ATF3	UniProtKB - P18847 (ATF3_HUMAN)	Nucleus	Binds to cAMP response element	181	57	31.49	8.8	-0.671
7	Serine/threonine-protein kinase B-raf	UniProtKB - P15056 (BRAF_HUMAN)	Cell membrane/cytoplasm/Nucleus	Contribute to MAP Kinase signalling pathway	766	219	28.59	7.29	-0.36
8	CDH3	UniProtKB - P22223 (CADH3_HUMAN)	Cell adherent junction,Cytosol	It Regulates beta catenin	829	276	33.29	4.6	-0.404
9	Caspase-1	UniProtKB -	Cytoplasm	Promote apoptosis	404	40	9.90	5.63	-0.333

		P29466 (CASP1_HUMAN)							
10	CDX2	UniProtKB - Q99626 (CDX2_HUMAN)	Nucleus	Involved in transcriptional regulation of genes in intestinal epithelium	313	205	65.49	9.65	-0.674
11	CTHRC1 isoform 1	UniProtKB - Q96CG8 (CTHR1_HUMAN)	Extra cellular matrix	Over expression causes invasion of CRC cells	243	40	16.46	8.31	-0.282
12	Receptor tyrosine-protein kinase erbB-2	UniProtKB - P04626 (ERBB2_HUMAN)	Cytoplasm/ Nucleus	Plays role in RTK pathway	1255	244	19.44	5.58	-0.247
13	Receptor tyrosine-protein kinase erbB-3	UniProtKB - P21860 (ERBB3_HUMAN)	Plasma membrane	Transmembrane signalling molecule may interact with ERBB2	1342	353	26.30	6.11	-0.387
14	MEK 1	UniProtKB - Q02750 (MP2K1_HUMAN)	Cytoplasm/ Nucleus	Regulation of Map kinase signal Transduction pathway	393	63	16.03	6.18	-0.305
15	Serine/threonine-protein kinase mTOR	UniProtKB - P42345 (MTOR_HUMAN)	Cytoplam/Nucleus/ER/ Mitochondria	Activated mTORC control protein synthesis by phospho regulating key factor	2549	554	21.73	6.73	-0.193
16	Myc proto-oncogene protein(MYC)	UniProtKB - P01106 (MYC_HUMAN)	Nucleus	TF activates growth related genes	439	208	47.38	5.33	-0.772
17	NFE2L3	UniProtKB - Q9Y4A8 (NF2L3_HUMAN)	Nucleus	Activates erythroid specific globin gene expression	694	333	47.98	5.21	-0.627
18	NFATc2	UniProtKB - Q13469 (NFAC2_HUMAN)	Cytoplasm/ Nucleus	Involves in expression of cytokine gene in T Cells and promotes invasive migration through GPC6 expression and Wnt signalling	925	513	55.45	6.87	-0.544
19	P53	UniProtKB - P04637 (P53_HUMAN)	Cytolplasm/ Nucleus	Tumor suppressor	393	210	53.43	6.33	-0.756
20	PDX1	UniProtKB -	Nucleus/Cyt	Glucose dependent transcription	283	96	33.92	7.1	-0.671

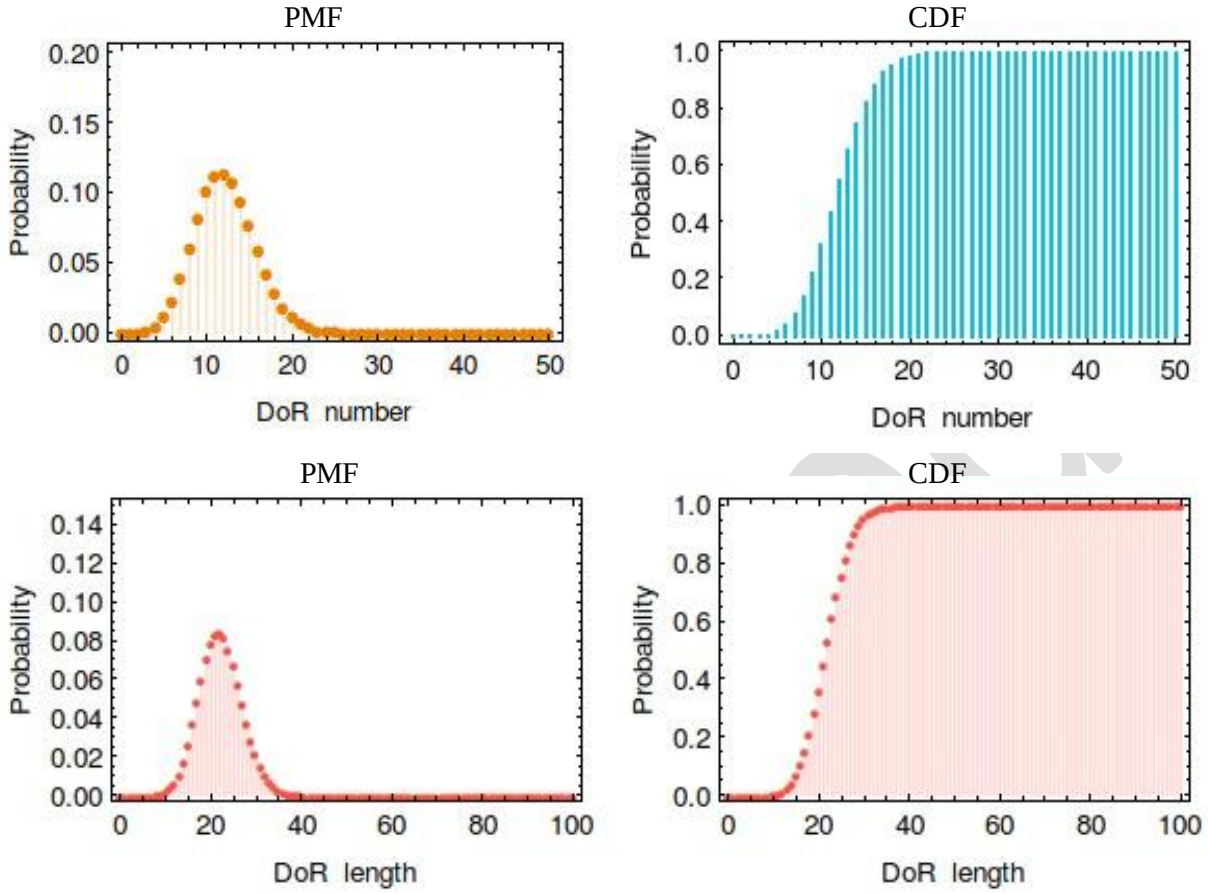
		P52945 (PDX1_HUMAN)	oplasm	regulation					
21	PIK3CA	UniProtKB - P42336 (PK3CA_HUMAN)	Cytosol	Involved in AKT activation and cell proliferation,growth	1068	17	1.59	6.88	-0.306
22	PLAC8(PLACENTA SPECIFIC 8)	UniProtKB - Q96EJ4 (Q96EJ4_HUMAN)	Cytosol(Intestine)	It regulates DUSP6 which in turn controls p-ERK2	115	0	0	7.34	0.125
23	PROX1	UniProtKB - Q92786 (PROX1_HUMAN)	Nucleus	Plays a role in embryonic development	737	432	58.61	6.74	-0.757
24	RAC-alpha serine/threonine-protein kinase	UniProtKB - P31749 (AKT1_HUMAN)	Cytoplasm/ Nucleus/Cell membrane	It is a kinase regulates cell growth ,metabolism,apoptosis and MAP3K5	480	90	18.75	5.75	-0.575
25	Mothers against decapentaplegic homolog 2 isoform-Long	UniProtKB - Q15796 (SMAD2_HUMAN)	Cytoplasm/ Nucleus	Intracellular signal transducer & transcriptional modulator activated by TGF-Beta	467	88	18.84	6.13	-0.444
26	Mothers against decapentaplegic homolog 3 isoform1	UniProtKB - P84022 (SMAD3_HUMAN)	Cyoplasm/Nucleus	Component of heteromeric complex required for Tgf-Beta signalling	425	83	19.52	6.73	-0.447
27	Mothers against decapentaplegic homolog 7	UniProtKB - O15105 (SMAD7_HUMAN)	Cyoplasm/Nucleus	Antagonist of signalling by TGF-Beta	426	90	21.12	8.63	-0.398
28	SNAI1	UniProtKB - O95863 (SNAI1_HUMAN)	Nucleus/Cytoplasm	Induction of EMT	264	74	28.03	8.97	-0.516
29	SOX9	UniProtKB - P48436 (SOX9_HUMAN)	Nucleus	Facilitate beta catenin degradation	509	449	88.21	6.31	-1.007
30	Protooncogene	UniProtKB -	Cell	Primary kinase plays role in	536	82	15.29	7.1	-0.473

	tyrosine protein kinase SRC(SRC)	P12931 (SRC_HUMAN)	membrane/ Nucleus/Cytoplasm	cytoskeletal reorganization					
31	Stimulator of interferon genes protein(STING)	UniProtKB - Q86WV6 (STING_HUMAN)	Cytoplasmic vesicle membrane/Endoplasmic reticulum/Goldi/Mitochondria	Innate immunity	379	45	11.87	6.6	-0.054
32	TGF-beta receptor type-2 isoform1	UniProtKB - P37173 (TGFR2_HUMAN)	Cytosol/Cell membrane	Receptor of Tgf-beta	567	17	2.99	5.6	-0.354
33	TGF-beta receptor type-1	UniProtKB - P36897 (TGFR1_HUMAN)	Cell membrane/Tight junction	Receptor of Tgf-beta	503	2	0.39	7.51	-0.055
34	Transforming growth factor beta-1	UniProtKB - P01137 (TGFB1_HUMAN)	Extra cellular matrix/Space	Controls cell proliferation, differentiation, invasion and also connected with SMAD	390	21	5.38	8.83	-0.311
35	Protein Wnt-5a	UniProtKB - P41221 (WNT5A_HUMAN)	Extra cellular matrix	Ligands for members of the frizzeled family	380	0	0	8.83	-0.29

Table 3: Fitted Normal distribution parameters for GRAVY.

Group	**μ**	**σ**
Protein(TFs)	-0.45	0.26
DoR	-0.615	1.85

Fig. 1 :



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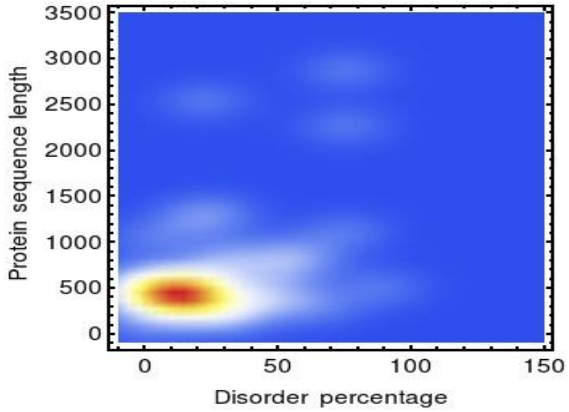

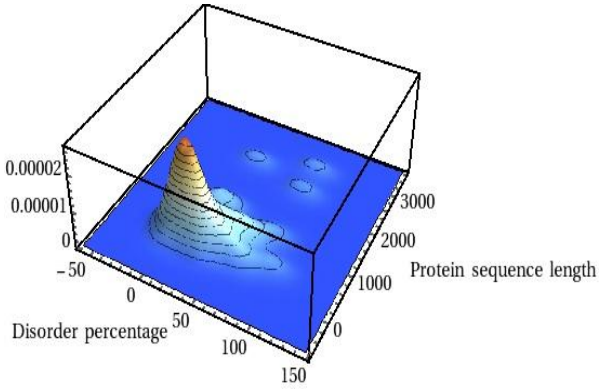

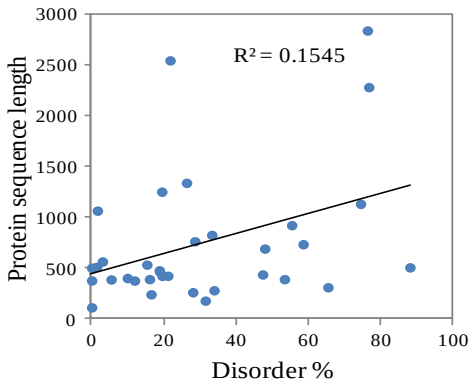

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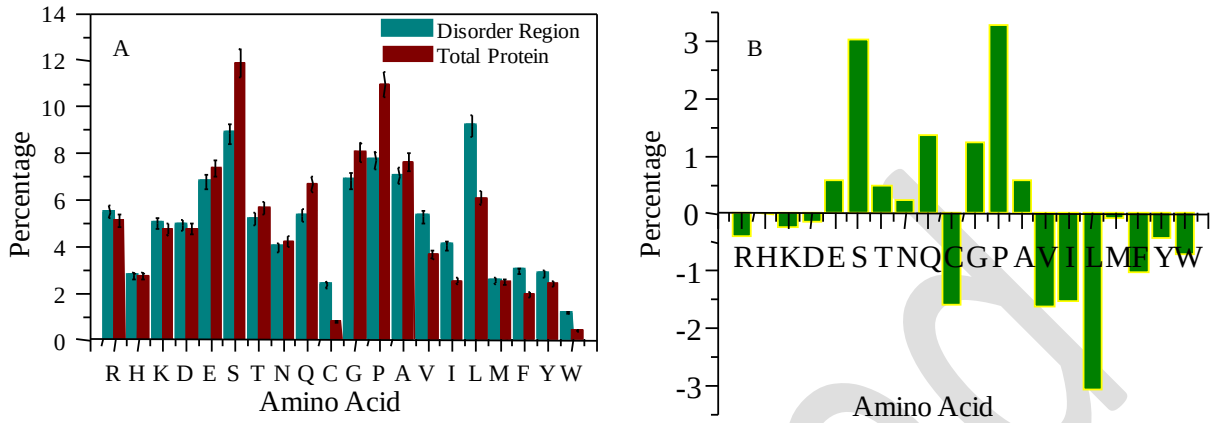

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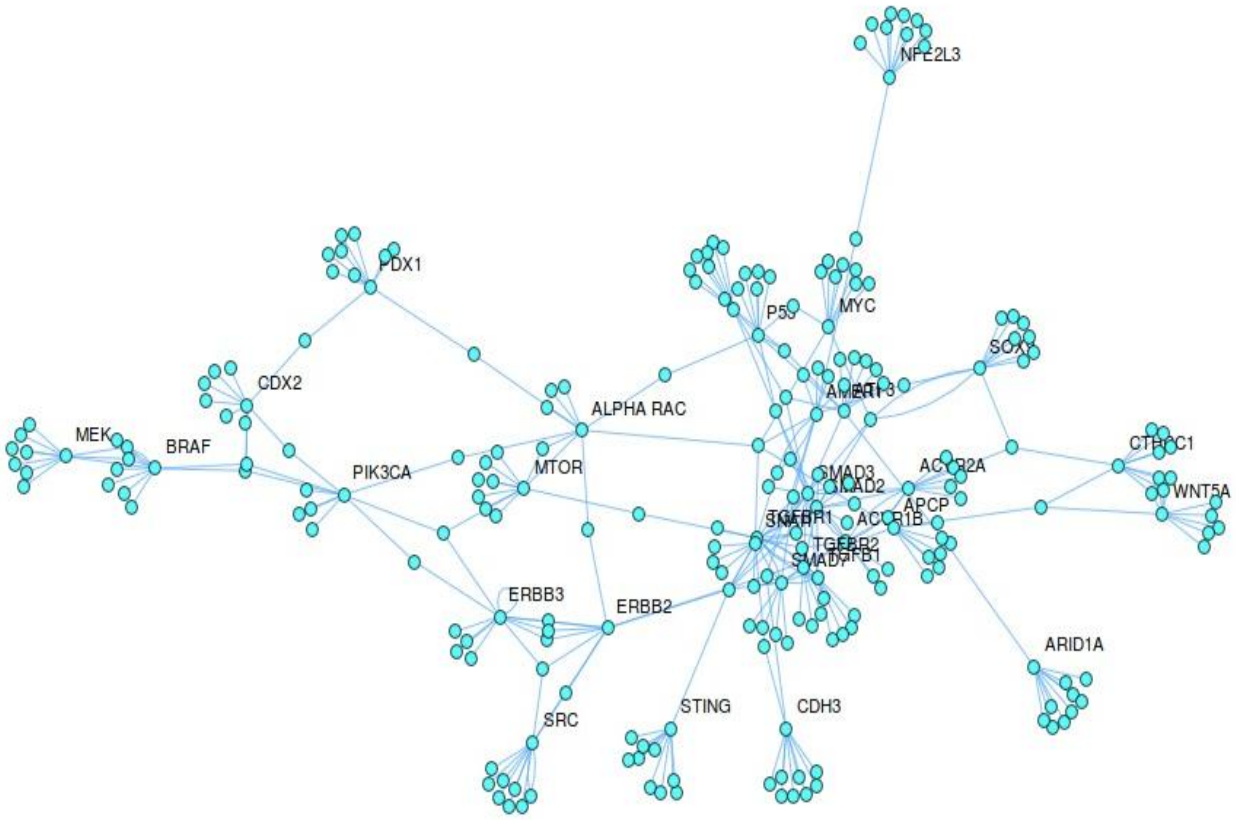
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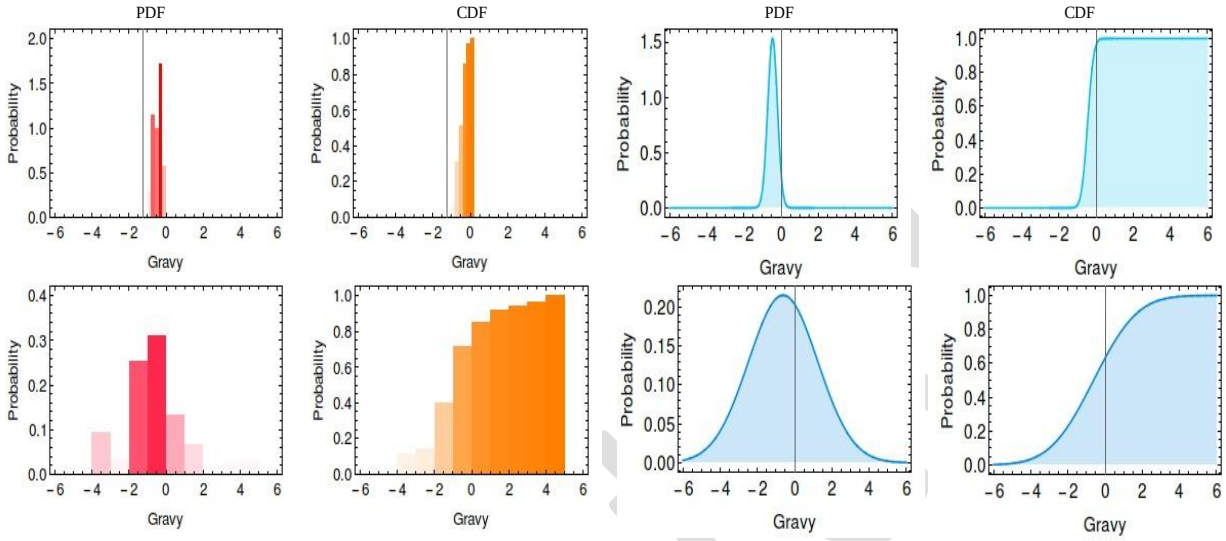
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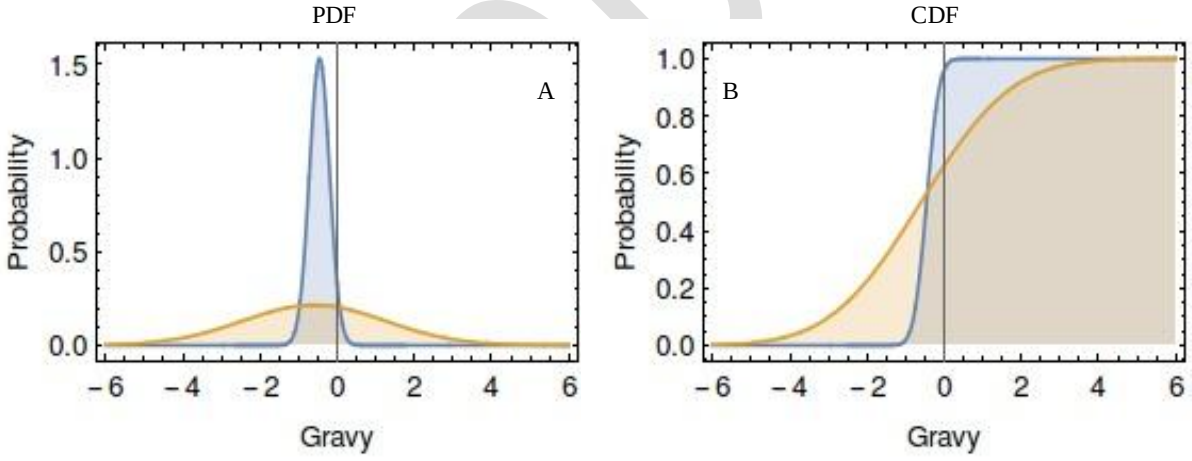
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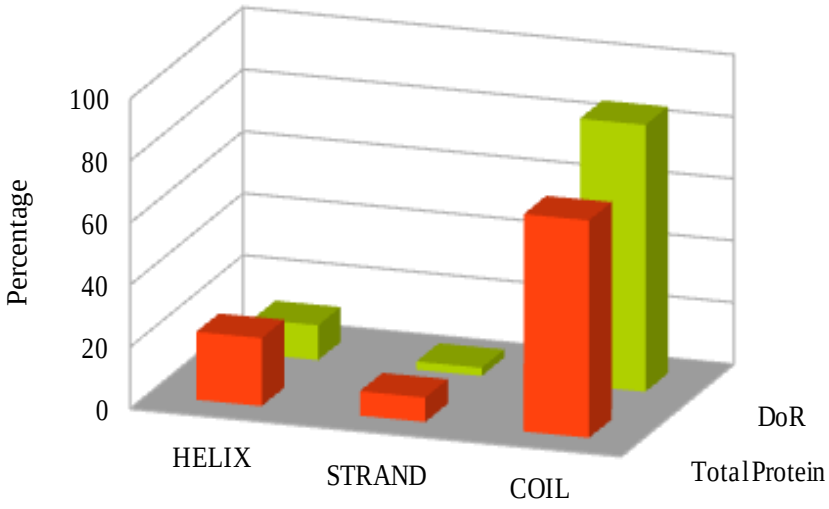

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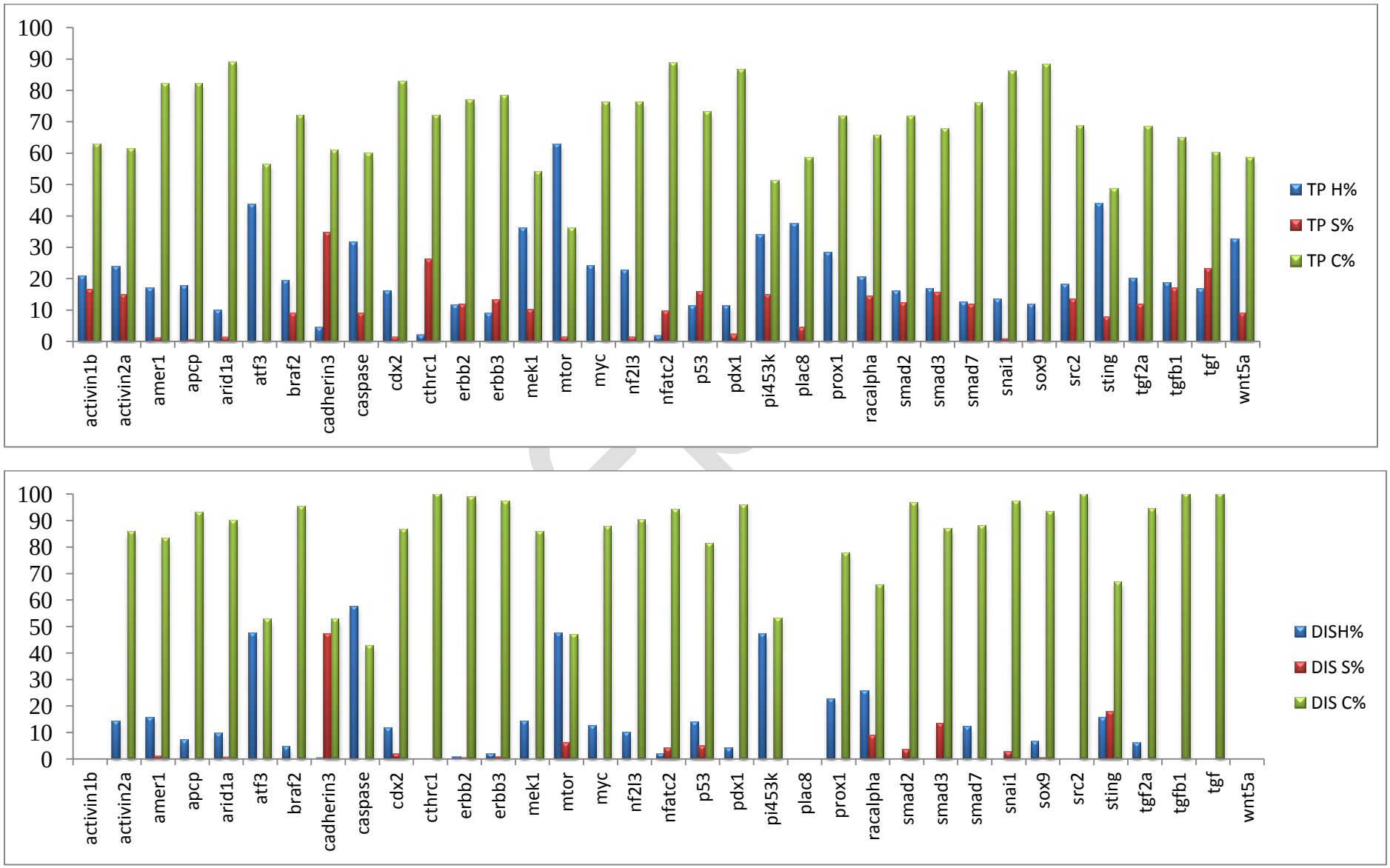
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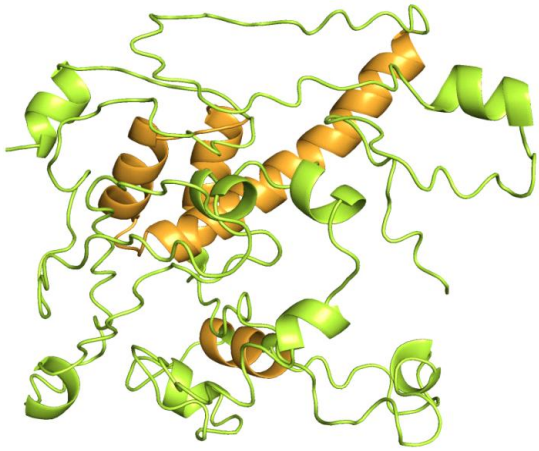
Fig.9-



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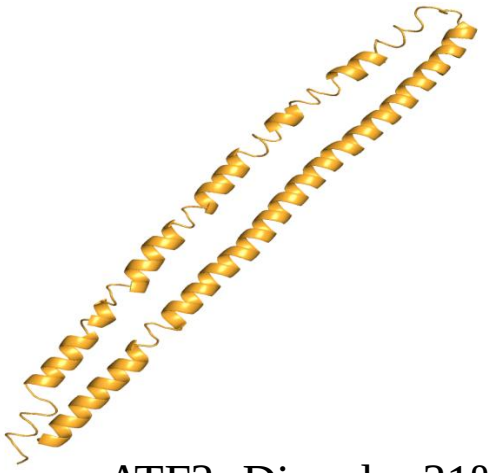
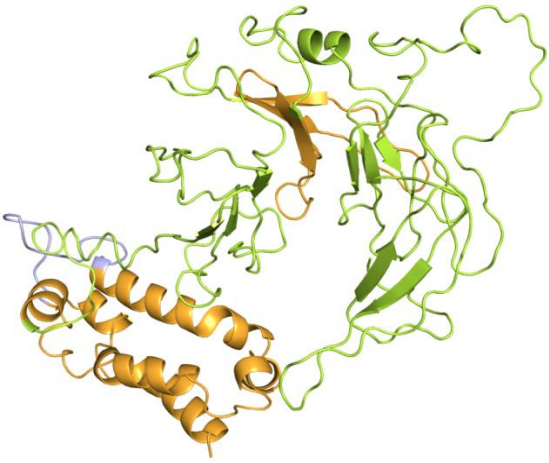


ATF3- Disorder 31%



MYC- Disorder 47%