STRUCTURAL DYNAMICS AND FUNCTIONAL ROLE OF DOMAINS IN RNA-BINDING PROTEINS: A FOCUS ON DISORDERED REGIONS

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by

ABHINAY KUMAR PANDEY

Roll No.: 21BT3EP11

under the guidance of

Prof. Ranjit Prasad Bahadur

DEPARTMENT OF BIOSCIENCE & BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY KHARAGPUR

KHARAGPUR 721302

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Abhinay Kumar Pandey
Roll No.: 21BT3EP11

CONTENTS

LIST OF ABBREVIATIONS

RBP: RNA-Binding Protein

RBD: RNA-Binding Domain

RPC: RNA-Protein Complex

IDR: Intrinsically Disordered Region

PDB: Protein Data Bank

UniProt: Universal Protein Resource

FASTA: Fast Alignment Search Tool

IUPred3: Intrinsically Unordered Protein Regions Prediction

XML: Extensible Markup Language

RRM: RNA Recognition Motif

KH: K Homology

mRNA: Messenger RNA

SUMMARY

This project aimed to explore the intrinsically disordered regions (IDRs) within human RNA-binding proteins (RBPs) and understand their structural and functional significance. We started with the RPC_complete.xlsx dataset, which initially contained 1,433 UniProt IDs of RBPs. After filtering the dataset to focus only on proteins with solved 3D structures (those with associated PDB IDs), we narrowed it down to 658 unique UniProt IDs. These proteins were selected for further analysis due to their availability of experimentally resolved structures, which are essential for studying the relationship between sequence and structure.

Using Python scripting, we automated the retrieval of FASTA sequences for each protein, followed by the prediction of disordered regions using the IUPred3 tool. The tool provided long and short disorder scores, which helped identify regions of flexibility and disorder within each protein. These scores were then used to analyze the presence of IDRs, crucial for understanding the dynamic behavior of these proteins.

In addition to predicting disordered regions, we extracted domain information from XML files provided by UniProt for each protein. This allowed us to annotate the disordered regions with functional domain data. In total, we identified 246 unique domain files, shedding light on how disordered regions intersect with known protein domains. This analysis revealed the pervasive presence of disorder in RBPs and its potential functional roles.

Looking ahead, the project sets the stage for deeper exploration into how these disordered domains interact with RNA, especially in the context of disease mechanisms. By leveraging solved PDB structures, we aim to model these interactions and understand how the misregulation of protein-RNA interactions can lead to diseases like neurodegenerative disorders and cancers. Understanding these dynamics will not only enhance our knowledge of protein functionality but could also open doors for therapeutic strategies targeting misregulated RBPs.

INTRODUCTION

RNA-Binding Proteins (RBPs):

RNA-binding proteins (RBPs) are vital players in regulating RNA processes like splicing, transport, translation, and stability. They influence post-transcriptional gene regulation by interacting with various RNA forms, such as mRNA, non-coding RNA, and ribosomal RNA, thus ensuring proper cellular functioning and adaptability to environmental changes.

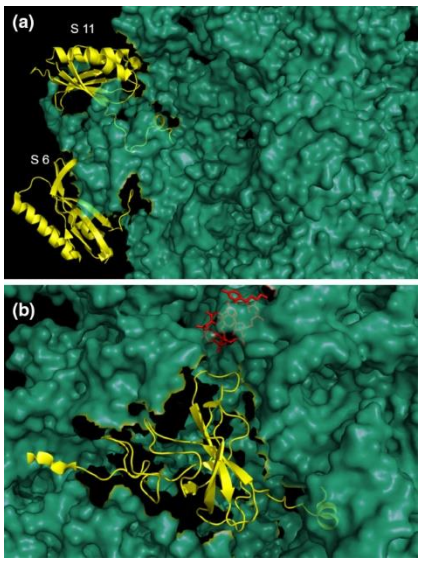


Fig.1:

Ribosomal proteins of 30S subunit (PDB ID: 1N34).

a. Small subunit proteins S6 and S11 are shown in *yellow cartoon* and other small subunit proteins are shown in *green surface*.

b. Small subunit protein S12 (shown in *yellow cartoon*) is interacting with mRNA (shown as a *red fragment*) through its disordered extension, the other small subunit proteins are shown in *green surface*.

Domains in RBPs:

RBPs typically contain RNA-binding domains (RBDs), specialized regions that allow for precise recognition of RNA sequences. Common RBDs, like RNA recognition motifs (RRMs) and K homology (KH) domains, are essential for the specific interactions RBPs have with RNA molecules, guiding RNA processing events.

Instrinsically Disordered Regions (IDRs) in RBPs:

In contrast to structured domains, RBPs can also feature intrinsically disordered regions (IDRs), which lack a fixed three-dimensional structure. These regions offer flexibility, enabling RBPs to engage with multiple RNA targets and other proteins. This adaptability is crucial for regulating cellular functions, but it can also lead to problems, especially in disease states.

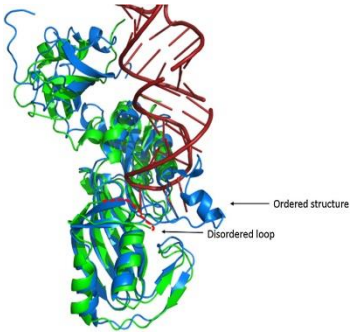


*Fig.2:*Superposed structure of TruB with its partner RNA in bound (*coloured in blue cartoon*, PDB ID: 1R3E) and unbound (*coloured in green cartoon*, PDB ID: 1R3F) conformations.The disordered thumb loop (*red dashed lines*) of TruB undergoes conformational transitions and become ordered upon binding with its partner RNA

Structure of Normal Domains and Disordered Regions: The structures of RNA-binding domains, like RRMs, have stable secondary structures like β-sheets and α-helices that enable specific RNA binding. IDRs, on the other hand, lack a defined structure, that allows dynamic interactions but increases susceptibility to instability.

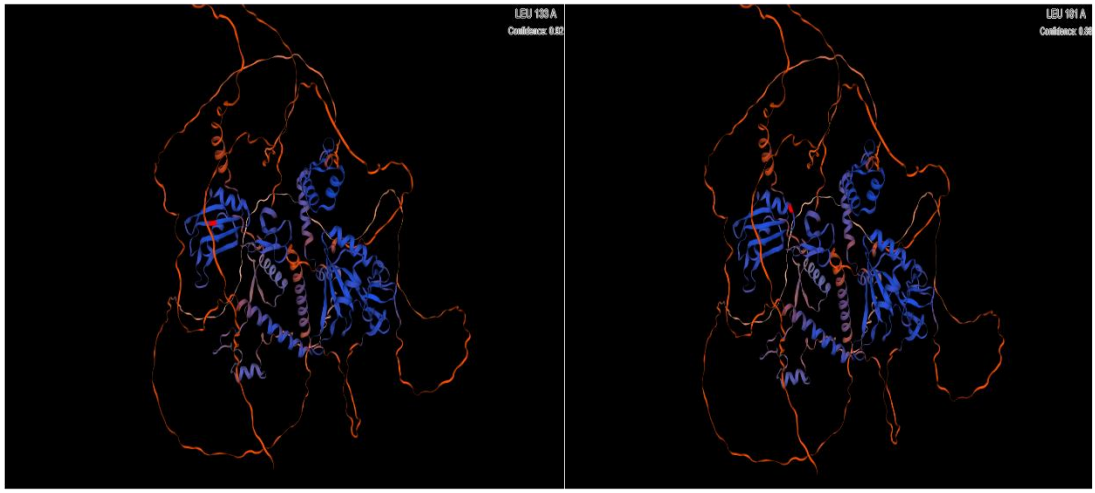


Fig.3 In RBP having uniport id A0A0A0MR66 which has been merged with P91875 at domain RRM 1 which is from residue 129-209, residue position 133 Leu(*red coloured marked in left image*) is disordered and position 181(*red coloured marked in right image*) is disordered.

Impact of IDRs on Cellular Function and Disease: Intrinsically disordered regions (IDRs) in RBPs provide flexibility but can also cause dysfunction. Mutations in these regions can impair protein-RNA interactions, leading to cellular stress and diseases like ALS, SMA, and fragile X syndrome. This highlights the fine balance between flexibility and stability in maintaining proper cellular function.

OBJECTIVE AND SCOPE OF STUDY

The objective of this project is to identify and analyze domain-disordered sequences within RNA-binding proteins (RBPs), with a focus on understanding how these intrinsically disordered regions (IDRs) contribute to RNA-protein interactions. These disordered sequences play critical roles in RNA regulation and processing, including splicing, transport, and translation. The project aims to uncover how disruptions in these IDRs can affect RNA interactions and contribute to diseases like neurodegenerative disorders and cancers.

The long-term goal is to identify how mutations or misfolding in these disordered regions lead to disease by disrupting RNA binding and regulatory functions, ultimately contributing to disease mechanisms. This insight could aid in developing targeted therapeutic strategies to restore proper RNA-protein interactions.

MATERIALS & TOOLS

Datasets:

RPC_complete.xlsx: A curated dataset listing human RNA-binding proteins (RBPs) with UniProt IDs and PDB IDs, ensuring selection of proteins with experimentally resolved structures. This served as the primary reference for analyzing intrinsic disorder.

UniProt Database: Provided FASTA sequences and XML files for each UniProt ID, including annotated sequence data and domain information for human RBPs.

Protein Data Bank (PDB): Source of 3D structural data. Only proteins with associated PDB IDs were considered, allowing insights into sequence-structure relationships within RBPs.

Tools and Software:

Python: Used for automating data processing, including filtering, downloading files, and integrating domain annotations. Libraries like wget streamlined downloads, while pandas and openpyxl managed Excel data.

IUPred3: IUPred3 predicts intrinsically disordered regions by estimating **residue-specific free energy (ΔG)** changes. It evaluates how amino acids interact within a structured protein environment, identifying regions with destabilized interactions as

disordered. This approach relies on pairwise interaction potentials derived from the sequence rather than full structural data, making it efficient for disorder prediction.

Long disorder scores: These scores are suited for detecting extended regions of disorder that might play roles in protein-protein or protein-nucleic acid interactions.

Short disorder scores: These scores provide a finer, more localized view of disorder, useful for identifying small flexible regions.

Microsoft Excel: Organized and stored domain-specific data into Excel sheets, aiding in analysis and visualization.

Annotation and Data Organization:

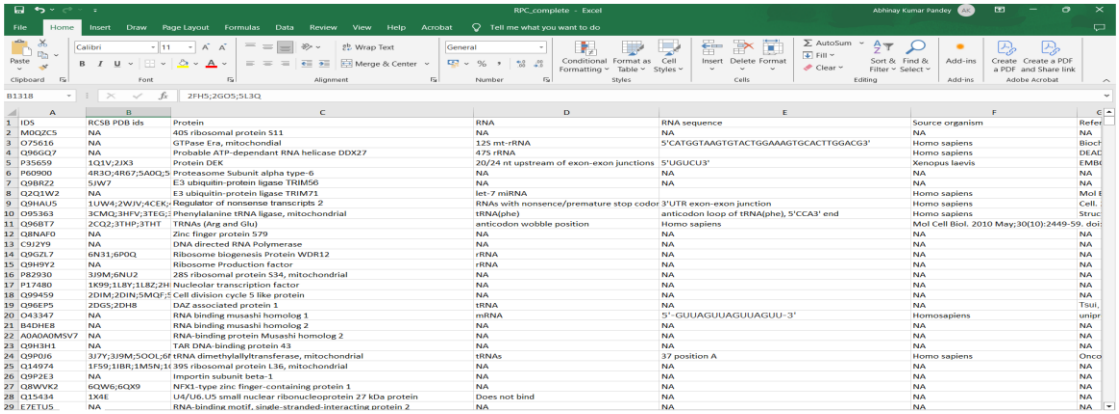
XML Files from UniProt: Provided domain annotations, including start/end positions and descriptions. These annotations were linked to disorder predictions.

Domain Information Excel Sheets: Generated for each unique domain, these sheets consolidated UniProt IDs, domain positions, sequences, and IUPred scores, enabling clear visualization of disorder patterns across proteins.

METHODS

1. Dataset Preparation and Filtering

We started with **RPC_complete.xlsx**, containing 1,433 UniProt IDs of RNA-binding proteins (RBPs). Using Python with the **pandas** and **openpyxl** libraries, we filtered the dataset to include only proteins with associated PDB IDs, focusing on those with solved structures for further analysis..



	A	B	C	D	E	F	G
1	ID5	RCSB PDB ids	Protein	RNA	RNA sequence	Source organism	Refer
2	M0Q2C5	NA	40S ribosomal protein S11	NA	NA	NA	NA
3	O75616	NA	GTPase Era, mitochondrial	12S mt-rRNA	5'CATGGTAAGTGTACTGGAAAGTGCACTTGGACG3'	Homo sapiens	Bioch
4	Q96GQ7	NA	Probable ATP-dependant RNA helicase DDX27	47S rRNA		Homo sapiens	DEAD
5	P35659	1Q1V;2JX3	Protein DEK	20/24 nt upstream of exon-exon junctions	5'UGUCU3'	Xenopus laevis	EMBO
6	P60900	4R3O;4R67;5A0Q;5	Proteasome Subunit alpha type-6	NA	NA	NA	NA
7	Q9BRZ2	5JW7	E3 ubiquitin-protein ligase TRIM56	NA	NA	NA	NA
8	Q2Q1W2	NA	E3 ubiquitin-protein ligase TRIM71	let-7 miRNA		Homo sapiens	Mol E
9	Q9HAU5	1UW4;2WJV;4CEK;	Regulator of nonsense transcripts 2	RNAs with nonsence/premature stop codor	3'UTR exon-exon junction	Homo sapiens	Cell.
10	O95363	3CMQ;3HFV;3TEG;	Phenylalanine tRNA ligase, mitochondrial	tRNA(phe)	anticodon loop of tRNA(phe), 5'CCA3' end	Homo sapiens	Struc
11	Q96BT7	2CQ2;3THP;3THT	TRNAs (Arg and Glu)	anticodon wobble position	Homo sapiens	Mol Cell Biol. 2010 May;30(10):2449-59. doi:	
12	Q8NAF0	NA	Zinc finger protein 579	NA	NA	NA	NA
13	C9J2Y9	NA	DNA directed RNA Polymerase	NA	NA	NA	NA
14	Q9GZL7	6N31;6P0Q	Ribosome biogenesis Protein WDR12	rRNA	NA	NA	NA
15	Q9H9Y2	NA	Ribosome Production factor	rRNA	NA	NA	NA
16	P82930	3J9M;6NU2	28S ribosomal protein S34, mitochondrial	NA	NA	NA	NA
17	P17480	1K99;1L8Y;1L8Z;2H	Nucleolar transcription factor	NA	NA	NA	NA
18	Q99459	2DIM;2DIN;5MQF;5	Cell division cycle 5 like protein	NA	NA	NA	NA
19	Q96EP5	2DG5;2DH8	DAZ associated protein 1	tRNA	NA	NA	Tsui,
20	O43347	NA	RNA binding musashi homolog 1	mRNA	5'-GUUAGUUAGUUAGUU-3'	Homosapiens	uniprot
21	B4DHE8	NA	RNA binding musashi homolog 2	NA	NA	NA	NA
22	A0A0A0MSV7	NA	RNA-binding protein Musashi homolog 2	NA	NA	NA	NA
23	Q9H3H1	NA	TAR DNA-binding protein 43	NA	NA	NA	NA
24	O9P0I6	3J7Y;3J9M;5OOL;6I	tRNA dimethylallyltransferase, mitochondrial	tRNAs	37 position A	Homo sapiens	Onco
25	Q14974	1F59;1IBR;1M5N;1(	39S ribosomal protein L36, mitochondrial	NA	NA	NA	NA
26	Q9P2E3	NA	Importin subunit beta-1	NA	NA	NA	NA
27	Q8WVK2	6QW6;6QX9	NFX1-type zinc finger-containing protein 1	NA	NA	NA	NA
28	Q15434	1X4E	U4/U6.U5 small nuclear ribonucleoprotein 27 kDa protein	Does not bind	NA	NA	NA
29	E7ETU5	NA	RNA-binding motif, single-stranded-interacting protein 2	NA	NA	NA	NA

Proteins with unique UniProt IDs and associated PDB IDs (indicating experimentally resolved structures) were selected, resulting in a refined dataset of 658 unique UniProt IDs. This filtering step ensured the dataset focused exclusively on proteins with reliable and experimentally validated structural data.

2. Retrieving Protein Sequences

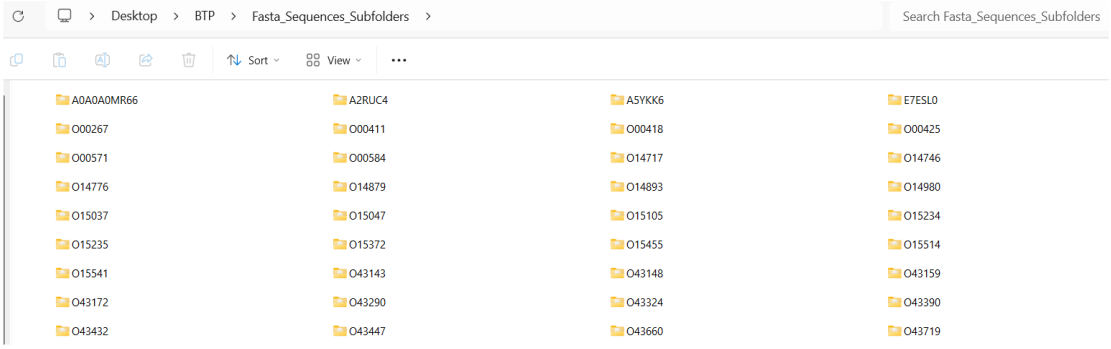
Using the filtered UniProt IDs, protein sequences were systematically downloaded:

UniProt fasta sequences: Fetched using the command:
wget https://rest.uniprot.org/uniprotkb/{uniprot_id}.fasta.

PDB fasta sequences: Retrieved using:
wget https://www.rcsb.org/fasta/entry/{pdb_id}
Below id the example of one fasta sequence for uniport id:

```
>sp|P98175|RBM10_HUMAN RNA-binding protein 10 OS=Homo sapiens OX=9606 GN=RBM10 PE=1 SV=3
MEYERRGGRGDRTGRYGATDRSQDDGGENRSRDHDYRDMDYRSYPREYGSQEGKHDYDDS
SEEQSAEDSYEASPGSETQRRRRRRHRHSPTGPPGFPRDGDYRDQDYRTEQGEEEEEEED
EEEEEKASNIVMLRMLPQAATEDDIRGQLQSHGVQAREVRLMRNKSSGQSRGFAFVEFSH
LQDATRWMEANQHSLNILGQKVSMHYSDPKPKINEDWLCNKCGVQNFKRREKCFKCGVPK
SEAEQKLPLGTRLDQQTLPLGGRELSQGLLPLPQPYQAQGVLASQALSQGSEPSSENAND
TIILRNLNPHSTMDSILGALAPYAVLSSSNVRVIKDKQTQLNRGFAFIQLSTIVEAAQLL
QILQALHPPLTIDGKTINVEFAKGSKRDMASNEGSRISAASVASTAIAAAQWAISQASQG
GEGTWATSEEPPVDYSYYQQDEGYGNSQGTESSLYAHGYLKGTKGPGITGTKGDPTGAGP
EASLEPGADSVSMQAFSRAQPGAAPGIYQQSAEASSSQGTAANSQSYTIMSPAVLKSELQ
SPTHPSSALPPATSPTAQESYSQYPVPDVSTYQYDETSGYYYDPQTGLYYDPNSQYYYNA
QSQQYLYWDGERRTYVPALEQSADGHKETGAPSKEGKEKKEKHKTKTAQQIAKDMERWAR
SLNKQKENFKNSFQPISSLRDDERRESATADAGYAILEKKGALAERQHTSMDLPKLASDD
RPSPPRGLVAAYSGESDSEEEQERGGPEREEKLTDWQKLACLLCRRQFPSKEALIRHQQL
SGLHKQNLEIHRRAHLSENELEALEKNDMEQMKYRDRAAERREKYGIPEPPEPKRRKYGG
ISTASVDFEQPTRDGLGSDNIGSRMLQAMGWKEGSGLGRKKQGIVTPIEAQTRVRGSGLG
ARGSSYGVTSTESYKETLHKTMVTRFNEAQ
```

A total of 658 unique UniProt IDs were identified. A dedicated folder was created for each uniport ID, containing its canonical protein sequence and corresponding structural sequences:



3. Intrinsic Disorder Prediction

IUPred3 was employed to predict regions of intrinsic disorder across all protein sequences.

Disorder scores were calculated using:

python3 iupred3.py {fasta_file} long/short.

Separate files for long and short disorder scores were generated for each sequence.

eg. For uniport id A0A0A0MR66 , for each amino acid we have short and long run iupred scores as:

```
# IUPred3 - improved prediction of protein disorder with a focus on specific user applications
# Gábor Erdős, Mátyás Pajkos, Zsuzsanna Dosztányi
# Nucleic Acids Research 2021, Submitted
#
# IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding
# Balint Meszaros, Gabor Erdos, Zsuzsanna Dosztanyi
# Nucleic Acids Research 2018;46(W1):W329-W337.
#
# Prediction type: short
# Smoothing used: medium
# Prediction output
# POS   RES     IUPRED2
1       M       0.9570
2       E       0.9567
3       Y       0.9362
4       E       0.9014
5       R       0.8582
6       R       0.8116
7       G       0.7659
8       G       0.7247
9       R       0.6910
10      G       0.6666
11      D       0.6541
12      R       0.6527
13      T       0.6600
14      G       0.6722
15      R       0.6843
16      Y       0.6969
17      G       0.7052
18      A       0.7116
19      T       0.7122
20      D       0.7073
21      R       0.7061
22      S       0.7049
23      Q       0.7074
24      D       0.7090
25      D       0.7090
26      G       0.7137
27      G       0.7133
28      E       0.7112
```

```
# IUPred3 - improved prediction of protein disorder with a focus on specific user applications
# Gábor Erdős, Mátyás Pajkos, Zsuzsanna Dosztányi
# Nucleic Acids Research 2021, Submitted
#
# IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding
# Balint Meszaros, Gabor Erdos, Zsuzsanna Dosztanyi
# Nucleic Acids Research 2018;46(W1):W329-W337.
#
# Prediction type: long
# Smoothing used: medium
# Prediction output
# POS   RES     IUPRED2
1       M       0.8139
2       E       0.8215
3       Y       0.8212
4       E       0.8165
5       R       0.8102
6       R       0.8047
7       G       0.8016
8       G       0.8020
9       R       0.8064
10      G       0.8151
11      D       0.8269
12      R       0.8446
13      T       0.8641
14      G       0.8818
15      R       0.8954
16      Y       0.9088
17      G       0.9171
18      A       0.9232
19      T       0.9253
20      D       0.9214
21      R       0.9186
22      S       0.9145
23      Q       0.9152
24      D       0.9156
25      D       0.9142
26      G       0.9181
27      G       0.9180
28      E       0.9170
```

Insights:

Scores > 0.5 indicate disordered residues.

Scores < 0.5 correspond to ordered residues.

This provided a detailed residue-level disorder profile.

4. Domain Annotation Extraction

UniProt XML files were downloaded using:

wget https://rest.uniprot.org/uniprotkb/{uniprot_id}.xml.

```
<?xml version="1.0" encoding="UTF-8"  standalone="no" ?>
<uniprot xmlns="http://uniprot.org/uniprot" xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance" xsi:schemaLocation="http://uniprot.org/uniprot
http://www.uniprot.org/docs/uniprot.xsd">
<entry dataset="Swiss-Prot" created="1996-10-01" modified="2024-10-02" version="223" xmlns="http://uniprot.org/uniprot">
  <accession>P98175</accession>
  <accession>A0A0A0MR66</accession>
  <accession>C4AM81</accession>
  <accession>Q14136</accession>
  <accession>Q5JRR2</accession>
  <accession>Q9BTE4</accession>
  <accession>Q9BTX0</accession>
  <accession>Q9NTB1</accession>
  <name>RBM10_HUMAN</name>
  <protein>
    <recommendedName>
      <fullName evidence="15">RNA-binding protein 10</fullName>
    </recommendedName>
    <alternativeName>
      <fullName>G patch domain-containing protein 9</fullName>
    </alternativeName>
    <alternativeName>
      <fullName>RNA-binding motif protein 10</fullName>
    </alternativeName>
    <alternativeName>
      <fullName evidence="1">RNA-binding protein S1-1</fullName>
      <shortName>S1-1</shortName>
    </alternativeName>
  </protein>
  <gene>
    <name evidence="16" type="primary">RBM10</name>
    <name type="synonym">DXS8237E</name>
    <name type="synonym">GPATC9</name>
    <name type="synonym">GPATCH9</name>
```

Domain Details:

To extract domain descriptions and boundaries from XML files, a Python script parses lines starting with <feature type="domain". It retrieves description, <begin position>, and <end position> details from the respective tags, providing essential information for identifying functional regions in RNA-binding proteins.

```
</feature>
<feature type="domain" description="RRM 1" evidence="4">
  <location>
    <begin position="129"/>
    <end position="209"/>
  </location>
</feature>
<feature type="domain" description="RRM 2" evidence="4">
  <location>
    <begin position="300"/>
    <end position="384"/>
  </location>
</feature>
<feature type="domain" description="G-patch" evidence="3">
  <location>
    <begin position="858"/>
    <end position="904"/>
  </location>
</feature>
```

For each UniProt ID, domain data (name, start, and end positions) were saved into domain-specific Excel sheets for easy reference.

A	B	C	D
domain	**description**	**begin position**	**end position**
domain	RRM 1	129	209
domain	RRM 2	300	384
domain	G-patch	858	904

5. Integrating Disorder and Domain Data:

The IUPred score files were enriched with domain information by appending a column that annotated residues within domain regions. For example:

123	E	0.8110	
124	E	0.7776	
125	E	0.7415	
126	K	0.7060	
127	A	0.6832	
128	S	0.6696	
129	N	0.6552	domain RRM 1
130	I	0.6350	domain RRM 1
131	V	0.6197	domain RRM 1
132	M	0.6049	domain RRM 1
133	L	0.5871	domain RRM 1
134	R	0.5647	domain RRM 1
135	M	0.5383	domain RRM 1
136	L	0.5239	domain RRM 1
137	P	0.5109	domain RRM 1
138	Q	0.5071	domain RRM 1
139	A	0.5051	domain RRM 1
140	A	0.5086	domain RRM 1
141	T	0.5249	domain RRM 1
142	E	0.5438	domain RRM 1
143	D	0.5628	domain RRM 1

123	E	0.8638	
124	E	0.8338	
125	E	0.8029	
126	K	0.7718	
127	A	0.7528	
128	S	0.7426	
129	N	0.7344	domain RRM 1
130	I	0.7203	domain RRM 1
131	V	0.7098	domain RRM 1
132	M	0.7008	domain RRM 1
133	L	0.6881	domain RRM 1
134	R	0.6655	domain RRM 1
135	M	0.6376	domain RRM 1
136	L	0.6196	domain RRM 1
137	P	0.6058	domain RRM 1
138	Q	0.6006	domain RRM 1
139	A	0.6015	domain RRM 1
140	A	0.6122	domain RRM 1
141	T	0.6404	domain RRM 1
142	E	0.6745	domain RRM 1
143	D	0.7120	domain RRM 1

This step connected structural disorder predictions to functional annotations.

6. Unique Domain Analysis

All unique domains across the dataset were identified. For each domain, a dedicated sheet was created summarizing:

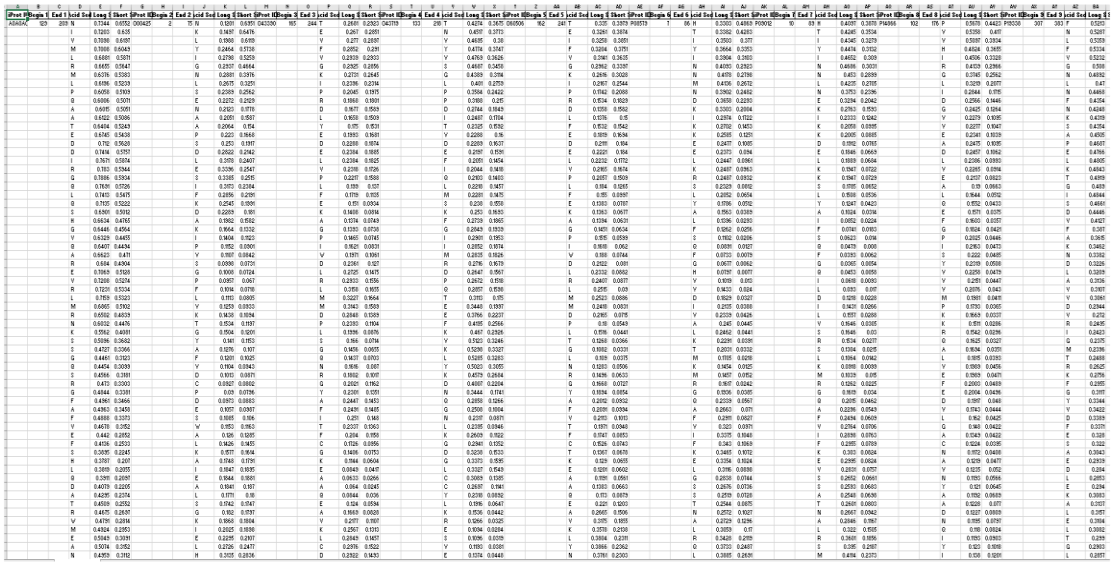
UniProt IDs where the domain appears.

Start and end positions of the domain in each protein.

Domain sequence.

Long and short IUPred disorder scores.

eg. RRM1 domain is found in multiple RBPs:



RESULTS & CONCLUSION

The project began by filtering 1,433 human RNA-binding proteins (RBPs) to focus on 658 UniProt IDs with experimentally solved structures, ensuring that our analysis was based on proteins with reliable 3D structural data. From this subset, we identified 246 unique domains that are likely involved in RNA interactions, a hallmark of RNA-binding proteins. These domains play critical roles in cellular processes, and disruptions in their function may contribute to cellular dysfunction and various diseases, particularly those involving the misregulation of RNA-binding proteins.

By linking intrinsically disordered regions (IDRs) to specific functional domains, we've established a valuable resource for understanding how these domains contribute to RNA-binding protein function. This foundational dataset can guide future studies aimed at elucidating the precise molecular mechanisms by which these domains interact with RNA and how their dysregulation leads to diseases. Our work lays the groundwork for exploring potential therapeutic targets, offering insights into the broader implications of RNA-binding protein dysfunction in cellular health and disease.

DISCUSSION

In this study, we investigated the relationship between intrinsically disordered regions (IDRs) and functional domains in RNA-binding proteins (RBPs). By analyzing domain annotations and disorder prediction scores, we observed that a substantial proportion of domain regions in RBPs exhibit disordered behavior. This highlights the flexibility and dynamic nature of these regions, crucial for their interactions with RNA and involvement in cellular processes.

Our findings suggest that disordered regions play a significant role in the functionality of RBPs, as they facilitate protein-RNA interactions, particularly in RNA processing and gene regulation. On average, a substantial portion of these domains demonstrates disordered behavior, supporting the hypothesis that IDRs are key drivers of molecular recognition in RBPs.

Looking ahead, we plan to leverage solved PDB structures to explore how these disordered domains interact with RNA. This will provide deeper insights into the molecular mechanisms of RNA metabolism and its regulation. Furthermore, this research could help us better understand the implications of IDR misregulation in human diseases, such as neurodegenerative disorders and cancers, where RBPs and their disordered regions are often implicated. By integrating structure-function relationships, we aim to contribute to the development of therapeutic strategies targeting these dynamic protein regions, offering potential avenues for intervention in diseases linked to RBP dysfunction.

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