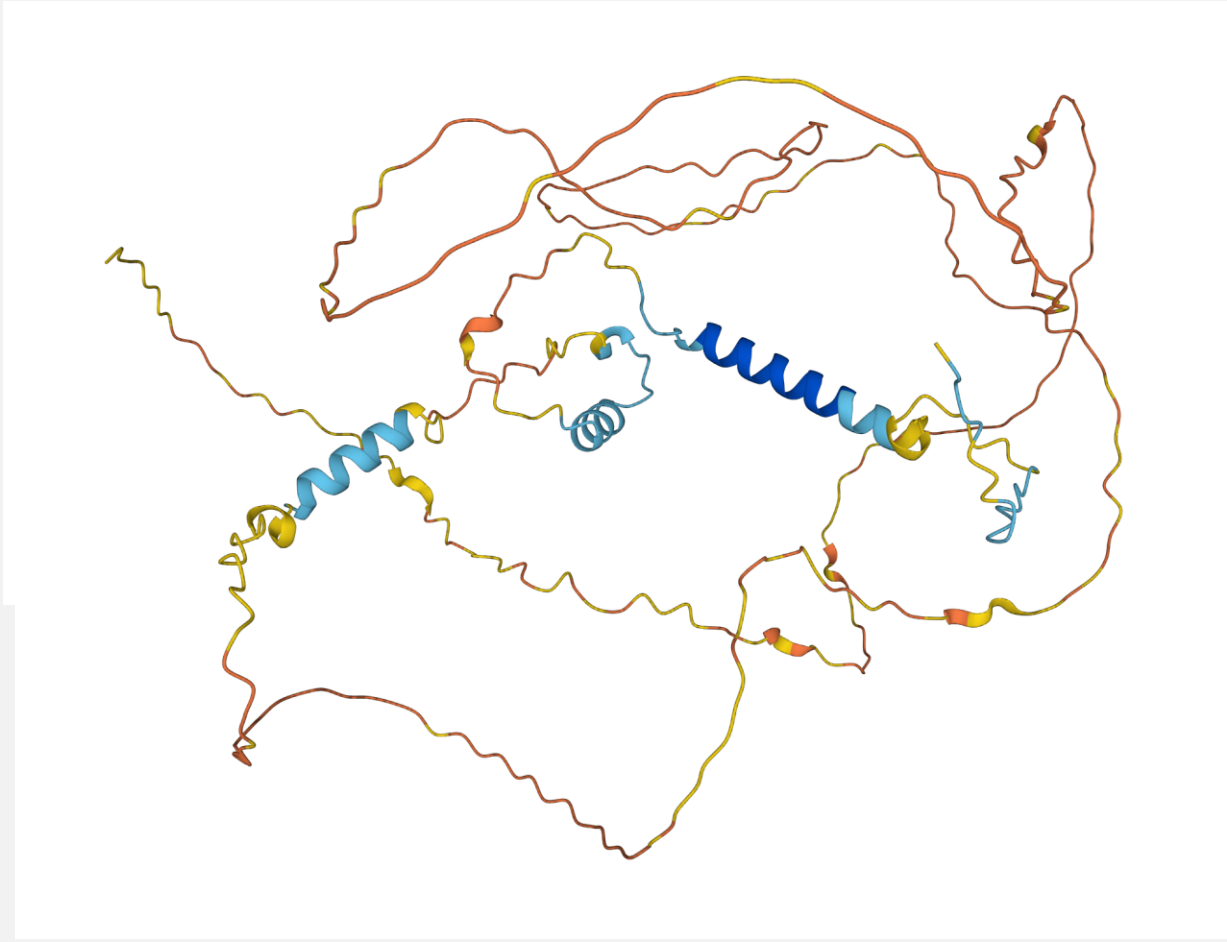
Everything you need to know about proteins with intrinsically disordered regions (IDRs)



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Memorial Sloan Kettering Cancer Center

November 2025

Topics to discuss

1. **What are intrinsically disordered regions (IDRs)?**
2. **What kinds of proteins contain IDRs?**

   Transactivation domains of transcription factors, histone tails, regulatory domains of enzymes, C-terminal tails of receptors, RNA-binding proteins
3. **Functions of proteins with IDRs**

   Important components of condensates, structural diversity allows interaction with different partners, transient structures
4. **Classification**

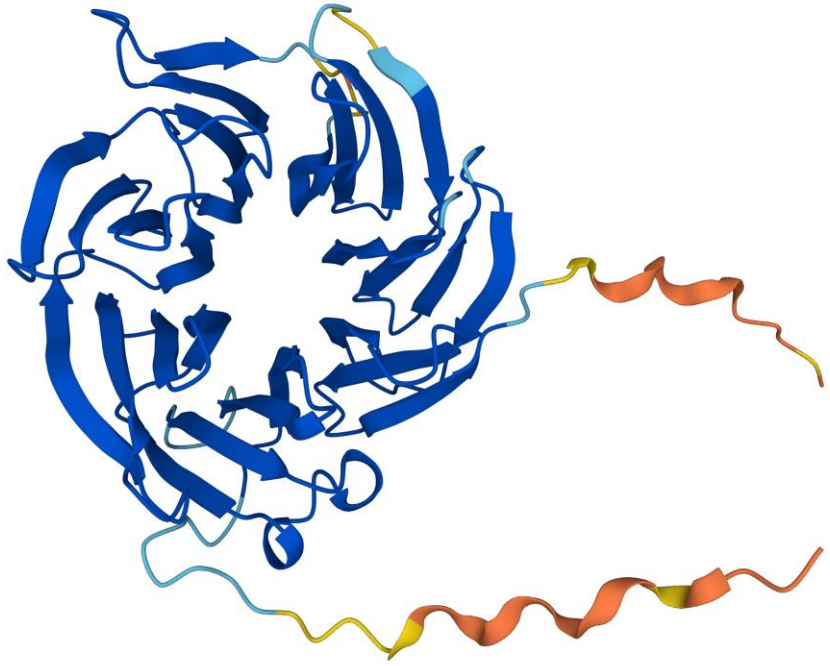
   Sequence features, biophysical and chemical features
5. **RNA as regulator of IDRs**

What are intrinsically disordered regions (IDRs)?

Only 37% of proteins are fully folded

GAPDH

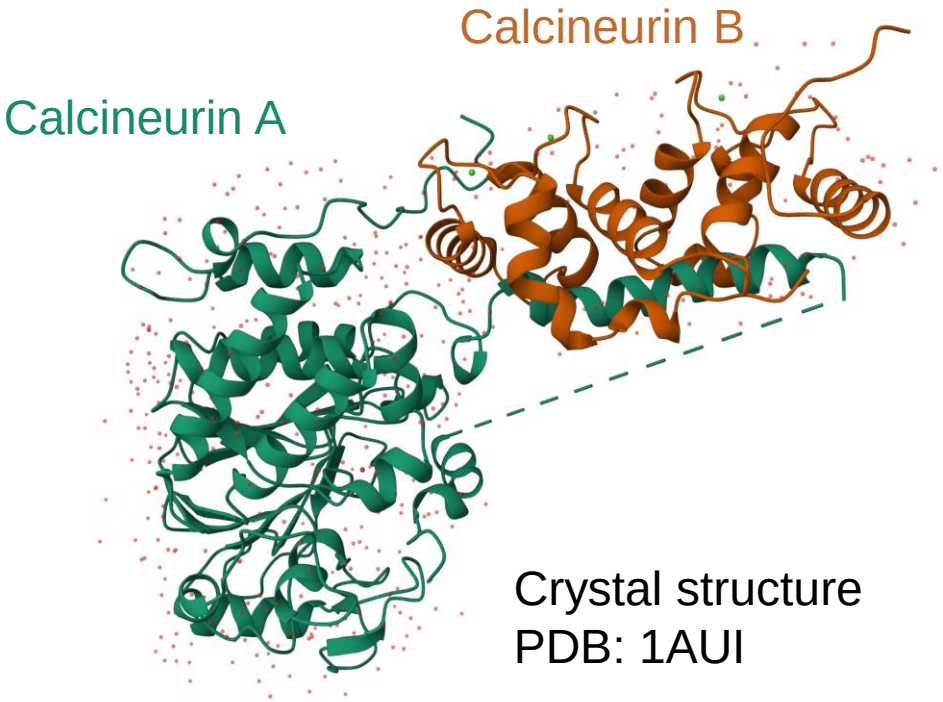
WDR77



AlphaFold prediction

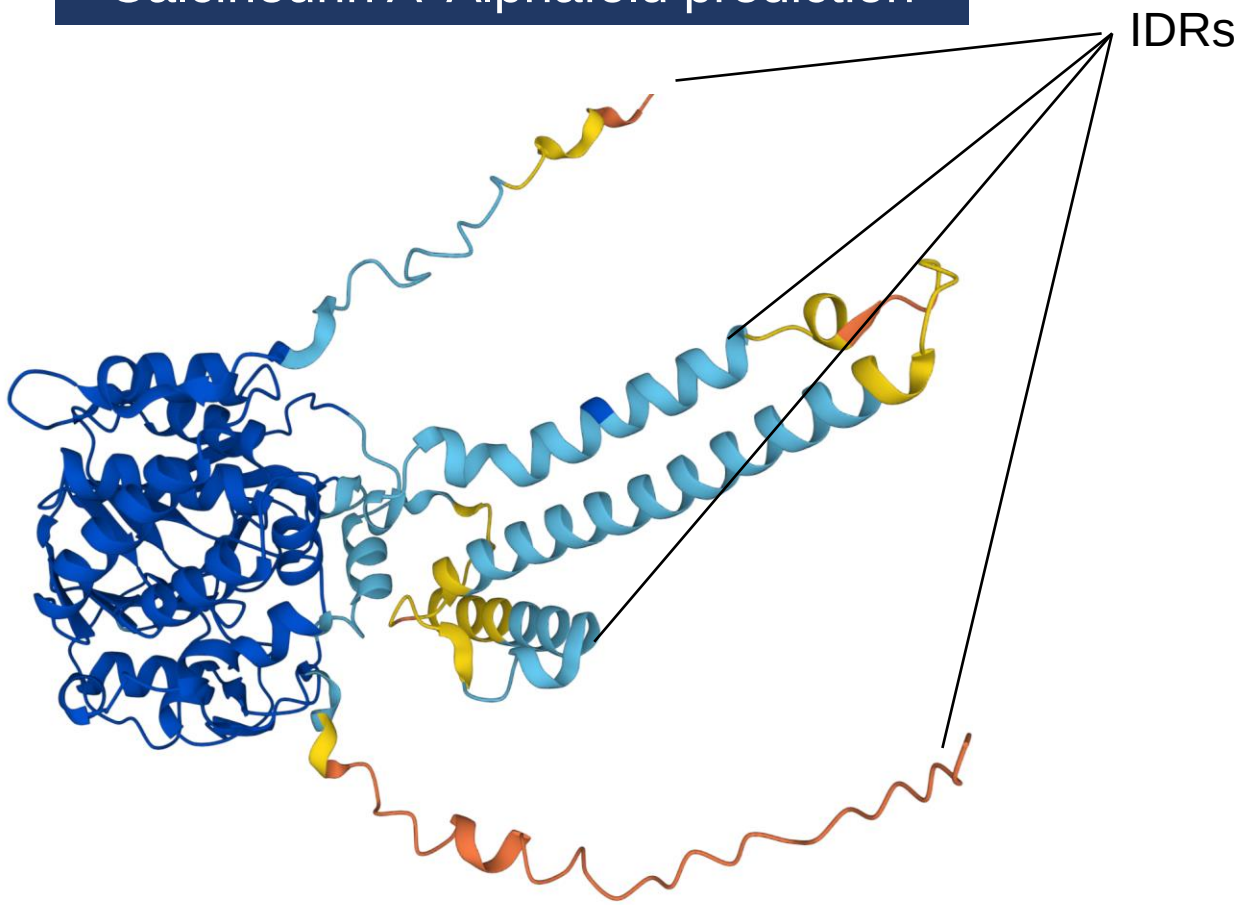
Discovery of IDRs: As these regions do not take on one single conformation, they were missing from crystal structures

Calcineurin crystal structure



Crystal structure
PDB: 1AUI

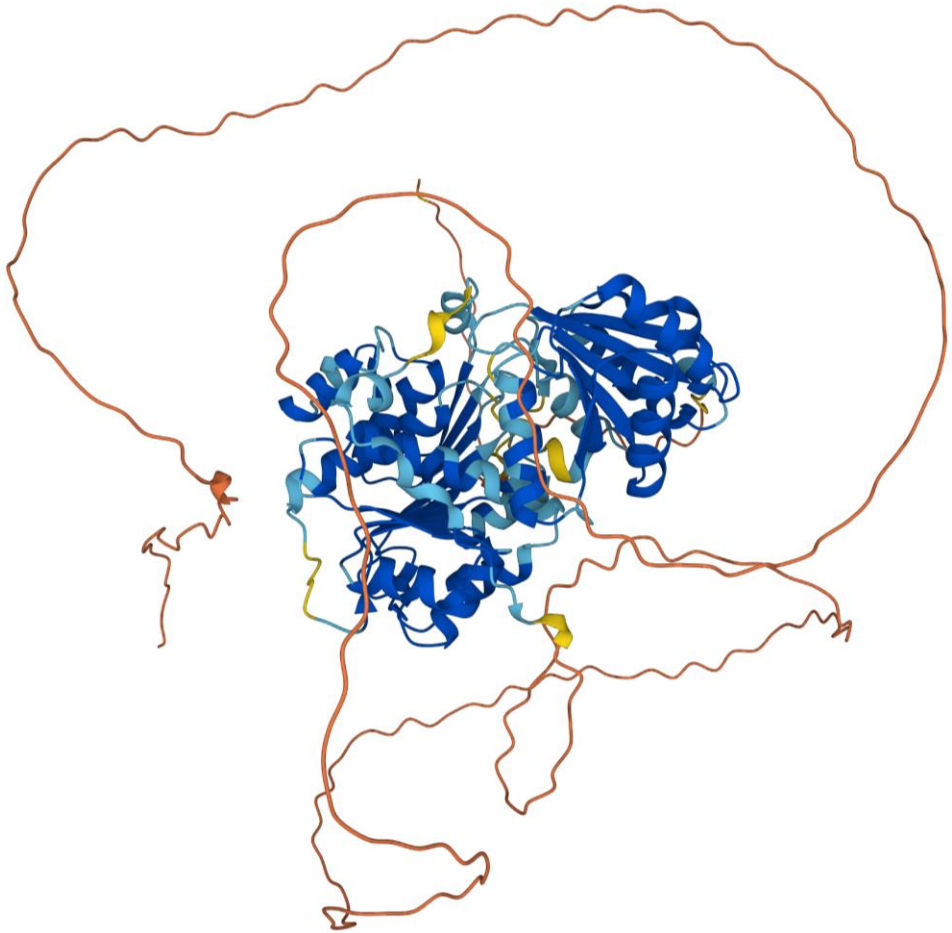
Calcineurin A Alphafold prediction



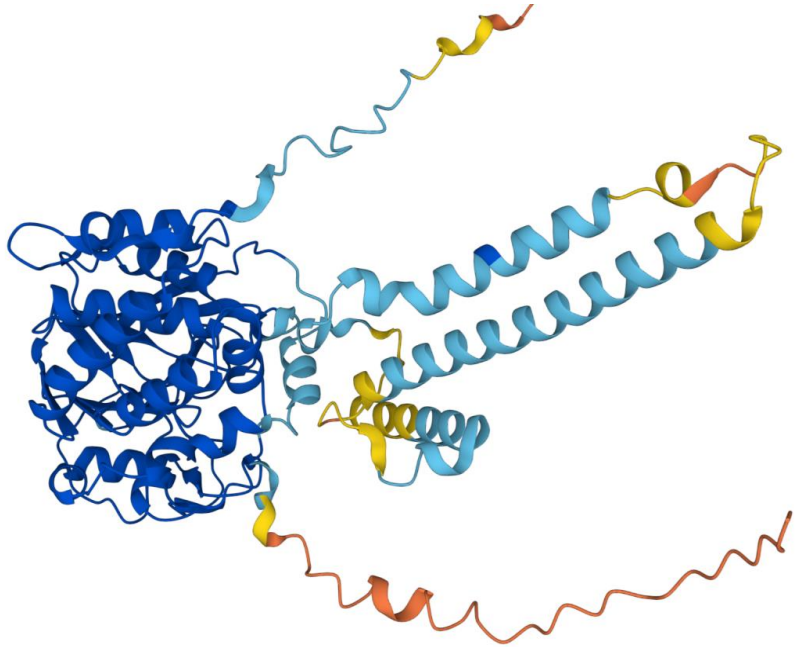
Because of their sequence composition, IDRs do not adopt a stable 3D conformation

Two types of IDRs

DDX4



Calcineurin A (PPP3CA)



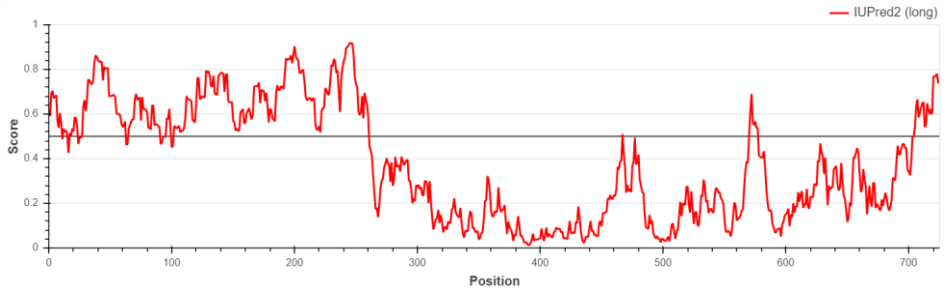
AlphaFold prediction

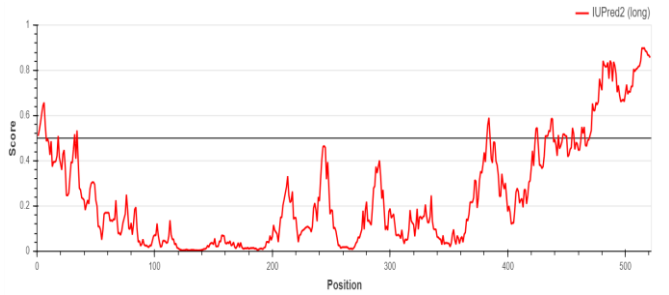
IDRs can be predicted from sequence

DDX4

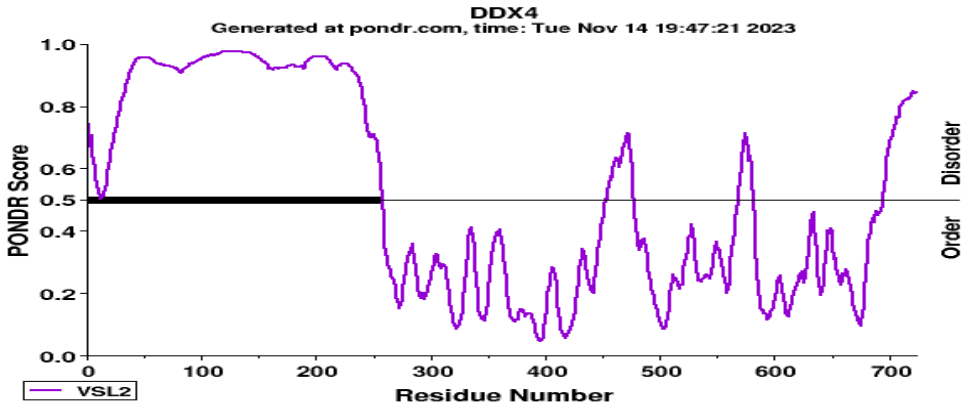
Calcineurin (PPP3CA)

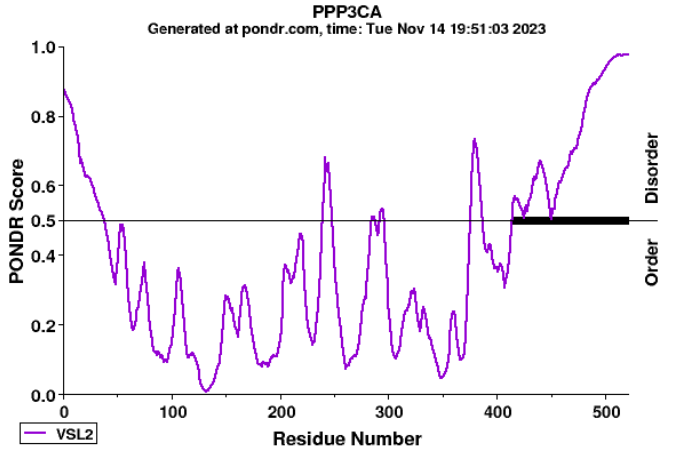
IUPred2A



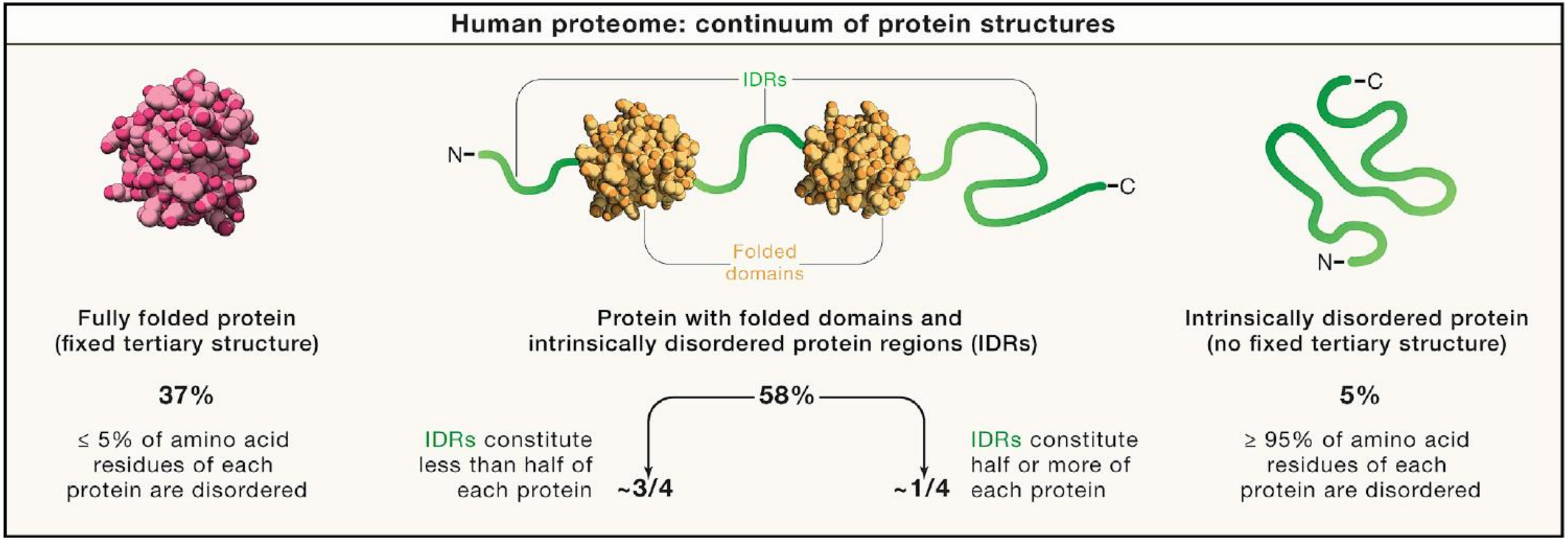


PONDR





63% of proteins have folded domains and IDRs



Tsang et al., Cell 2020

What protein classes contain IDRs?

Most proteins with regulatory function contain IDRs

Most proteins with regulatory function contain IDRs

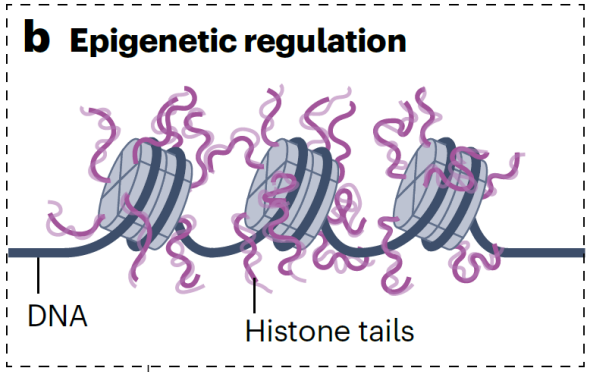
-Histone tails

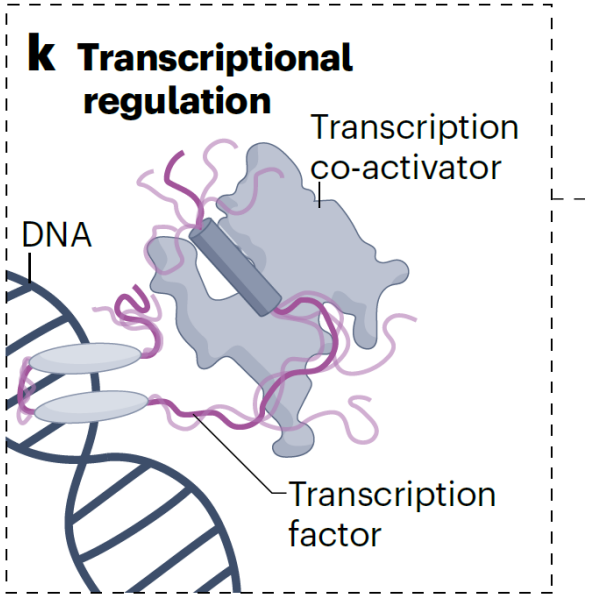
-Transactivation domains of transcription factors

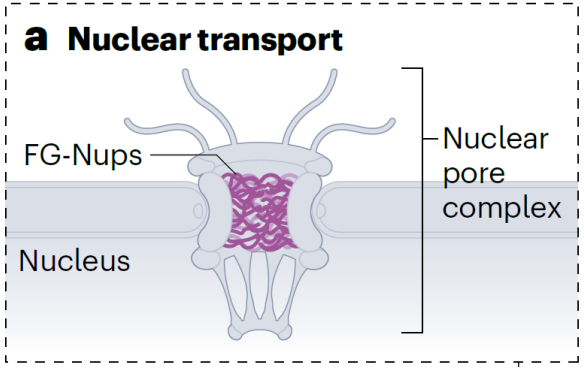
-RNA-binding proteins

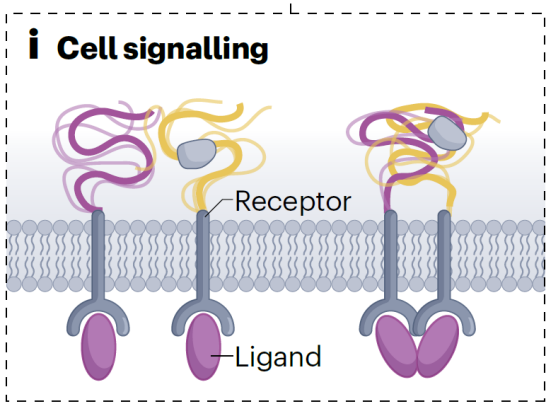
-Auto-inhibitory domains of enzymes

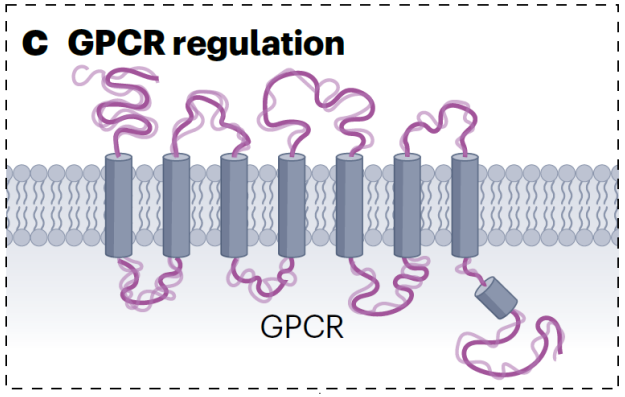
-Tails of membrane receptors











Holehouse & Kragelund Nat rev MCB 2023

The features of IDRs explain why they are present in regulatory proteins

-IDRs contain the majority of sites for post-translational modifications

-IDRs can take on different conformations and bind to structurally diverse binding partners

-They contribute to the formation of condensates, thus changing their interaction environment

→ Increased regulatory potential and diversification of protein function

Expansion of IDRs during evolution (5-10-fold)

One protein with one function → One protein, many functions in different contexts

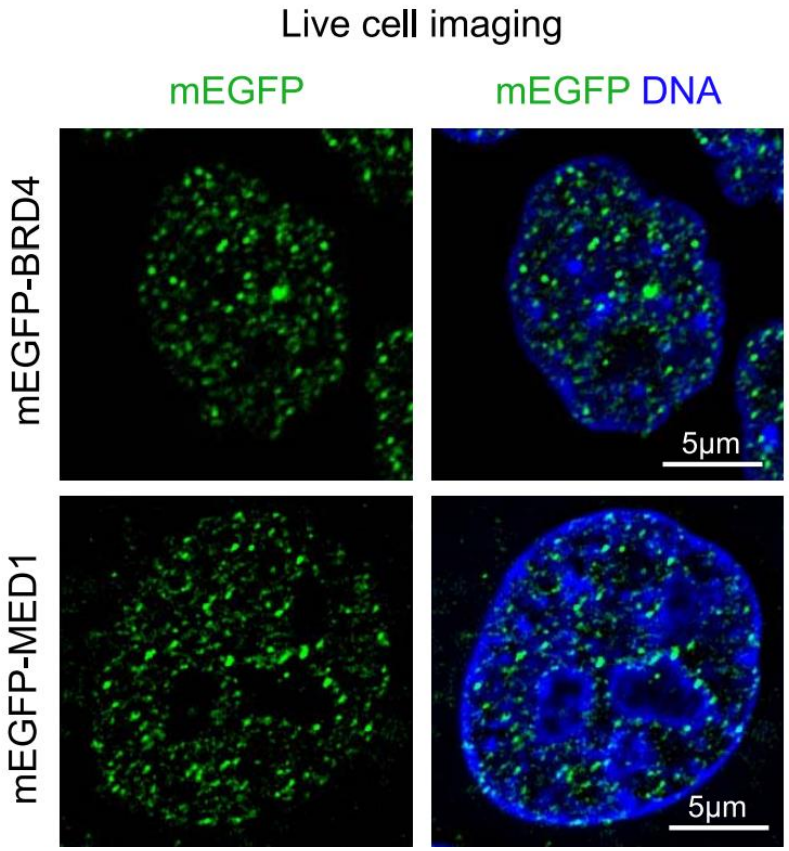
One protein with one regulation → One protein, many types of regulation in different contexts

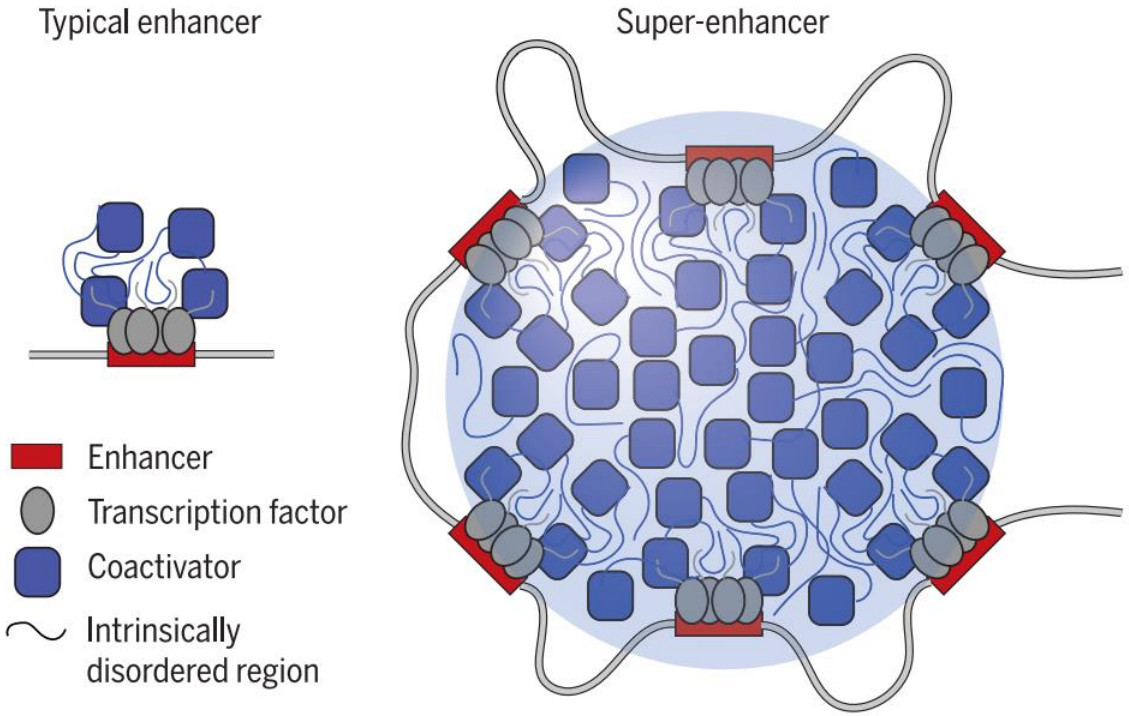
But it is largely unknown how IDRs function and how they are regulated

Tompa et al., Mol Cell 2014
Wright & Dyson, Nature Revies MCB 2015
Wang et al, Proteomics 2016

Functions of IDRs

The IDRs of Mediator and BRD4 help the formation of transcriptional condensates

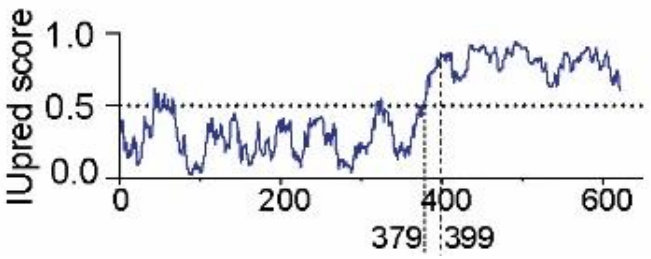


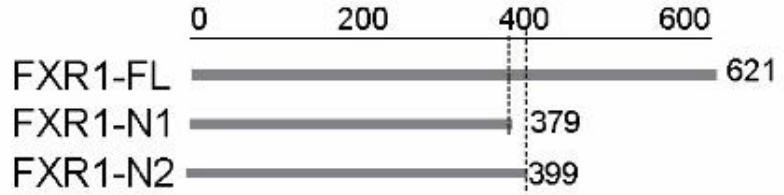


Proteins with IDRs can form condensates, which can compartmentalize and concentrate molecules to facilitate reactions

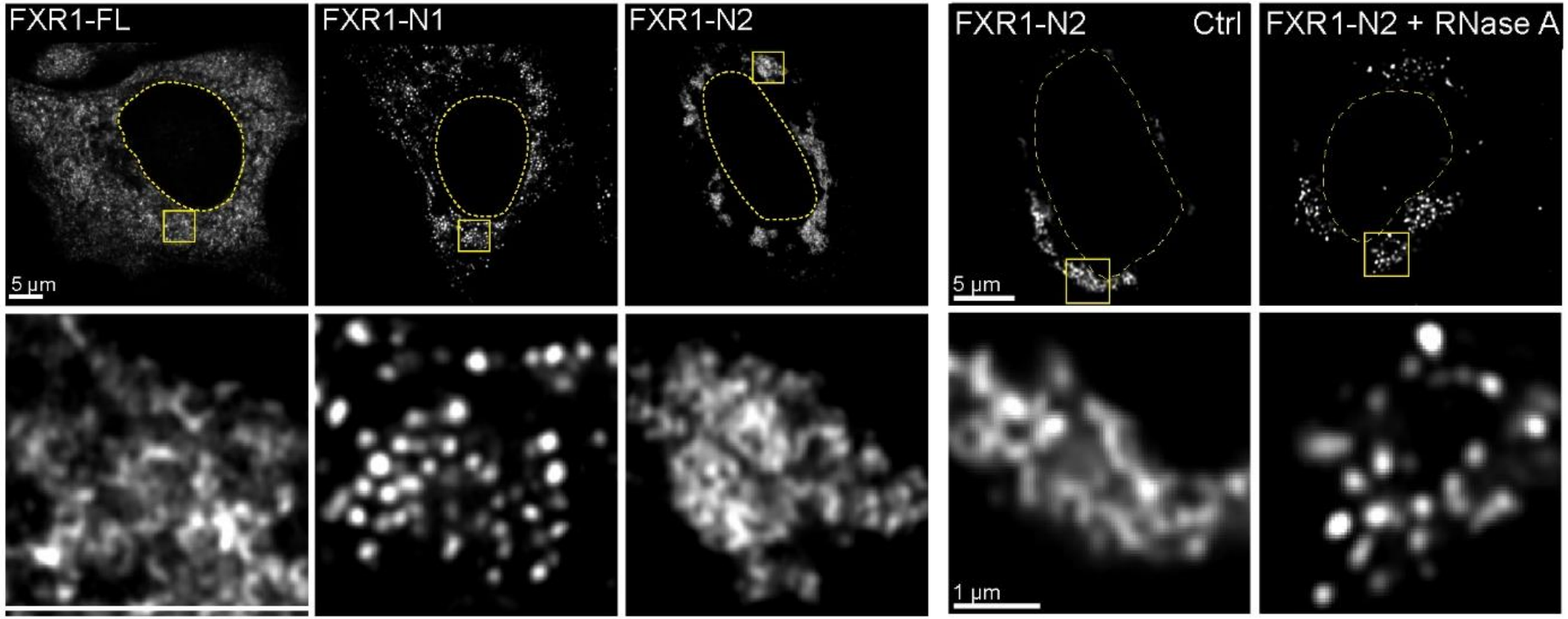
Sabari et al., Science 2018

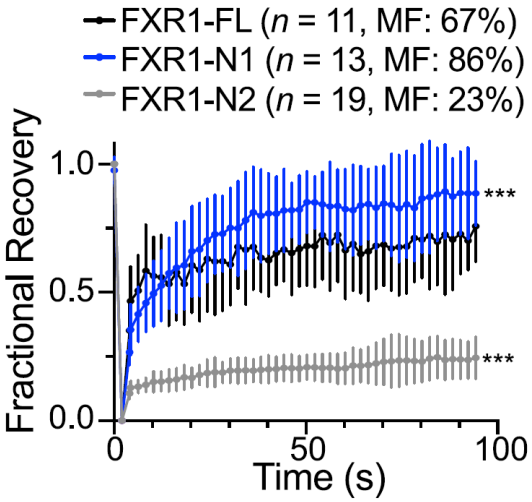
The IDRs of RNA-binding proteins can connect droplets into a condensate network and keep cytoplasmic condensates dynamic





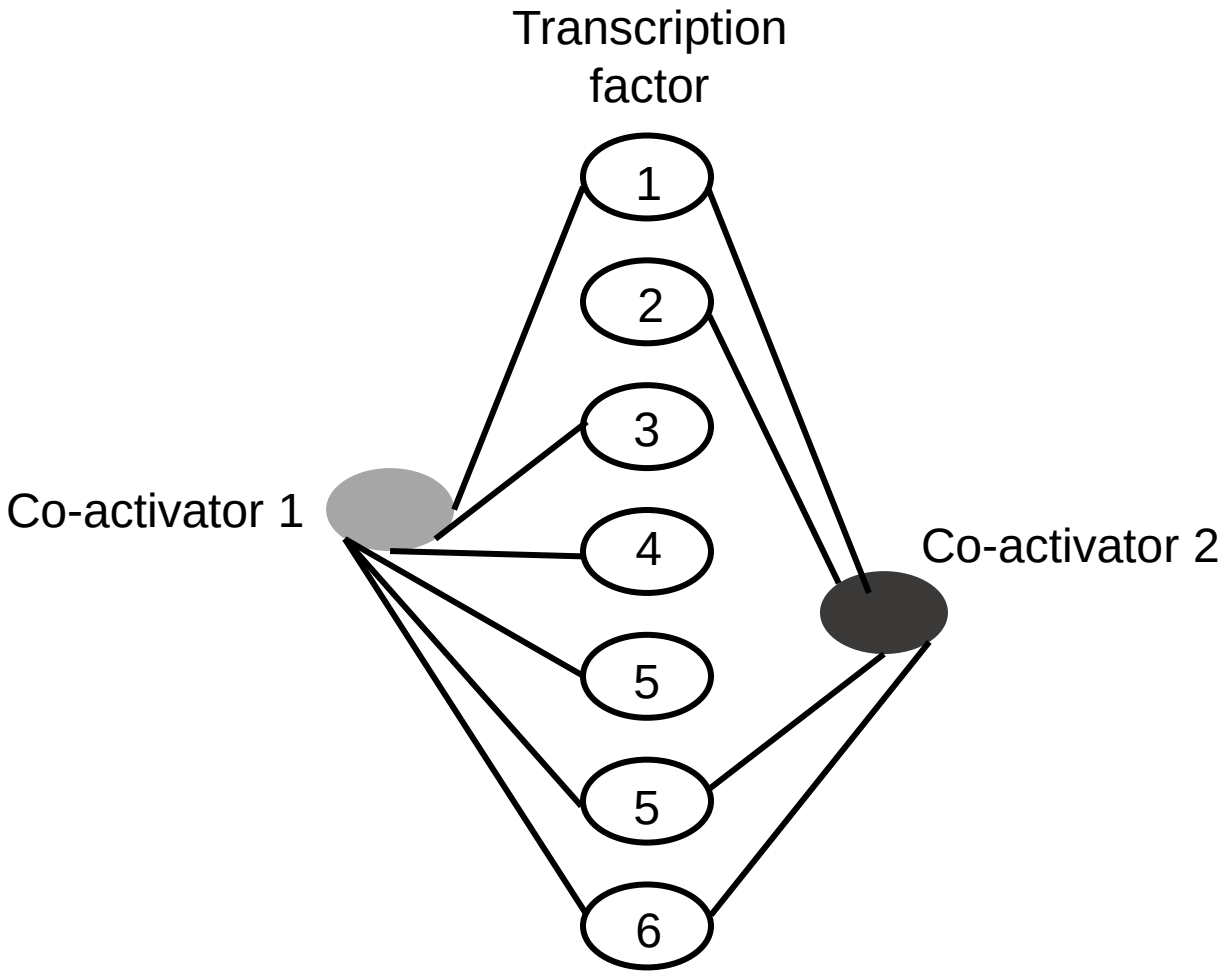
380-SRSYSGRGRGRRGPNYTSGY-399



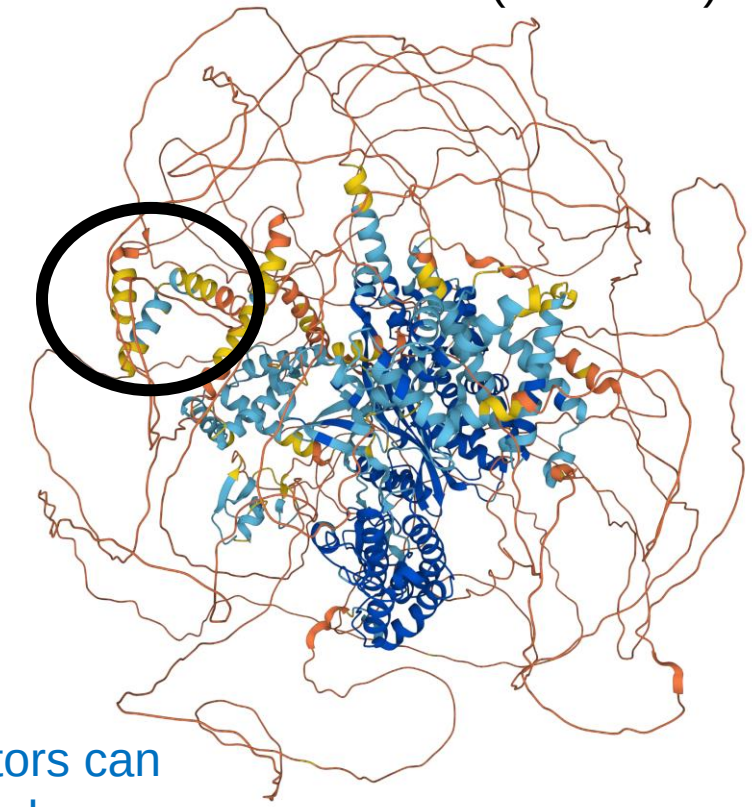


Chen et al., Cell 2024

Many different transcription factors bind to a limited set of coactivators

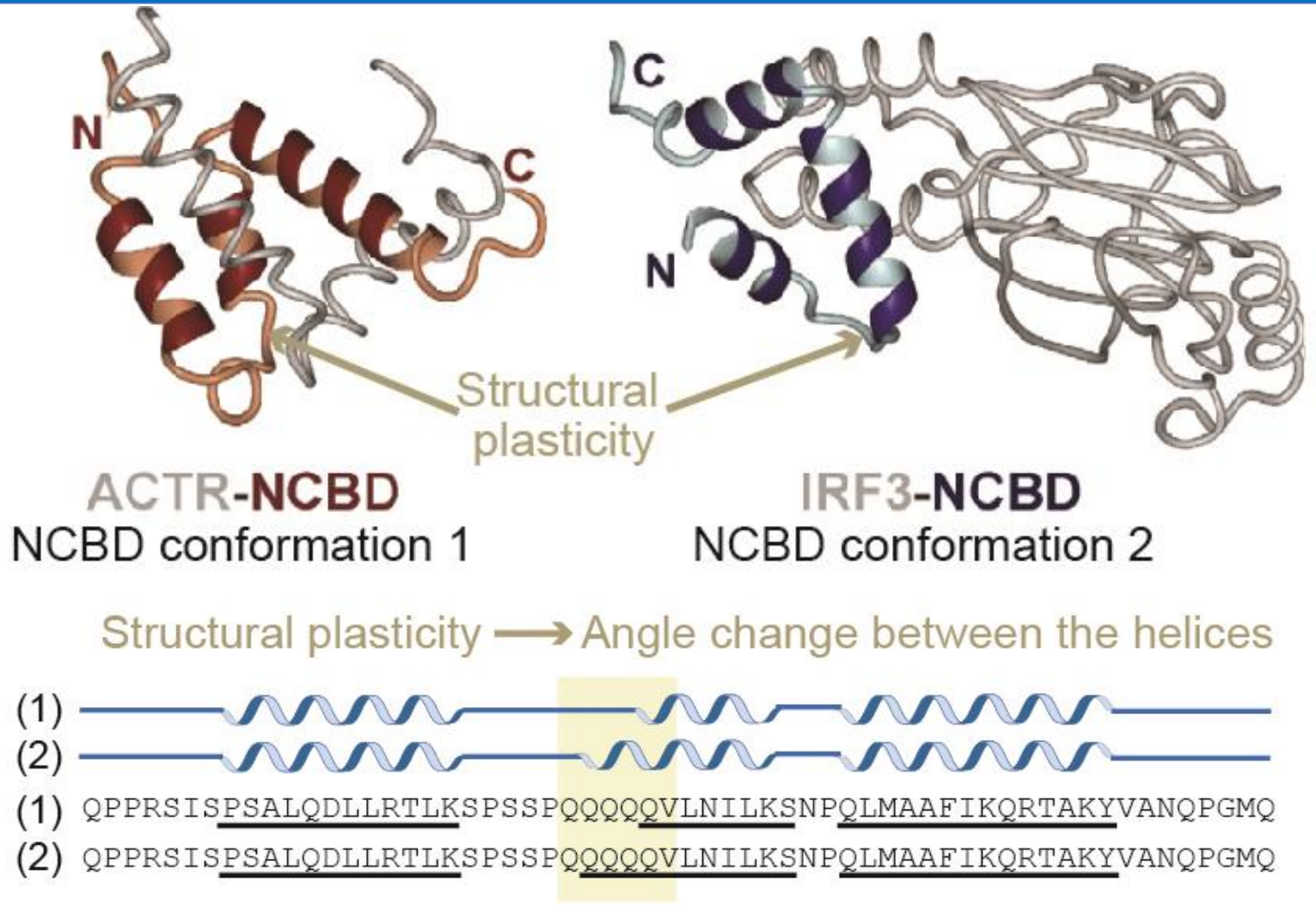


CBP coactivator (CREBBP)



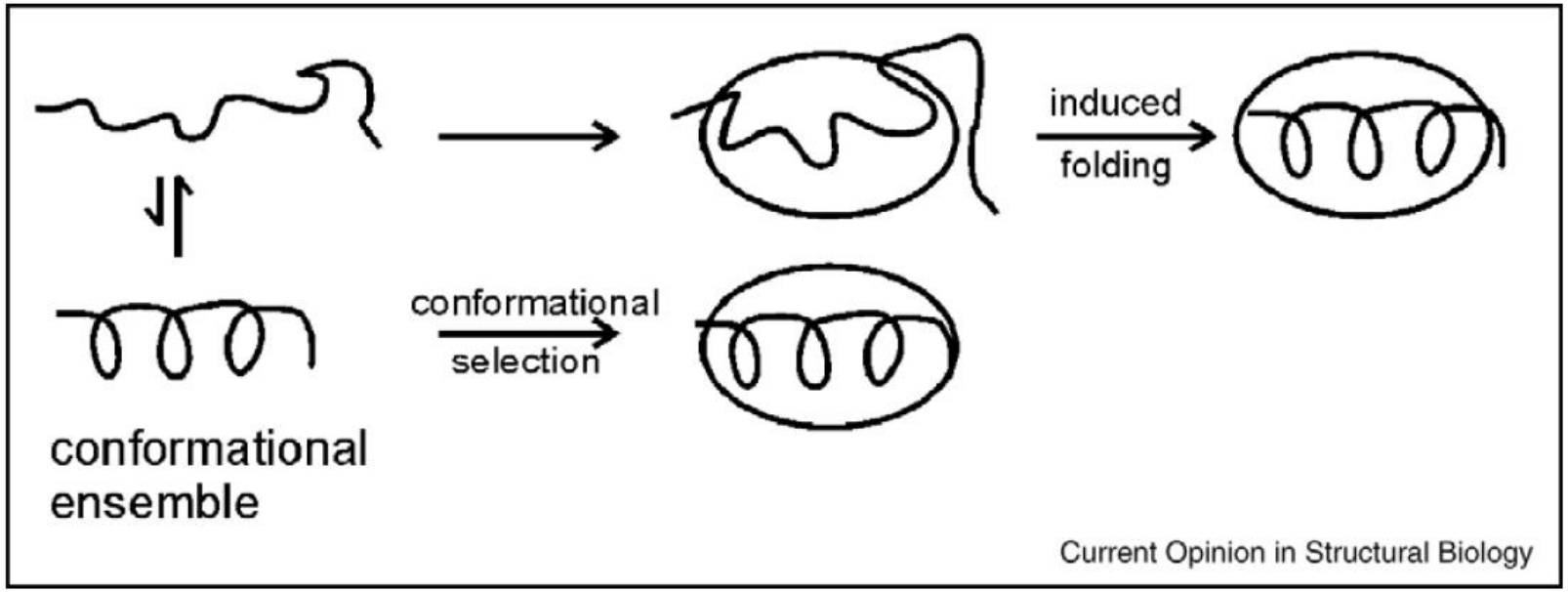
Coactivators can
-move nucleosomes
-modify chromatin-associated proteins
-change genome architecture
-recruit the transcription apparatus
-change transcription initiation/elongation rates

Structural plasticity of IDRs enables different protein conformations
The same domain can bind to structurally-diverse binding partners



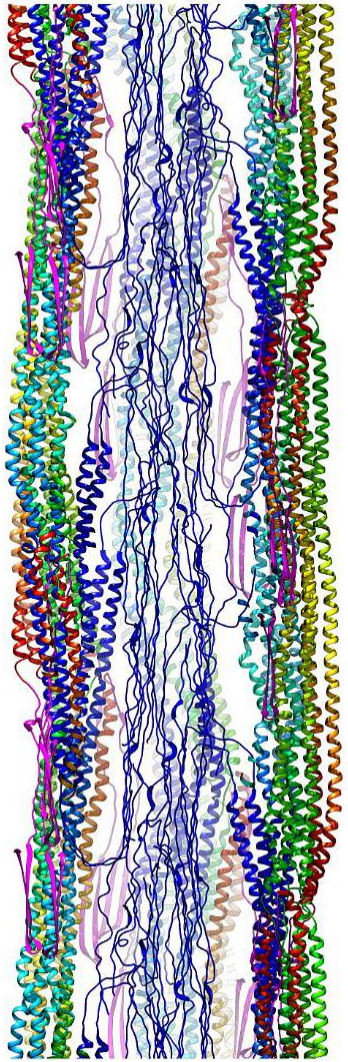
Qin et al., Structure 2005
Wright & Dyson, Curr opin struct biol 2009

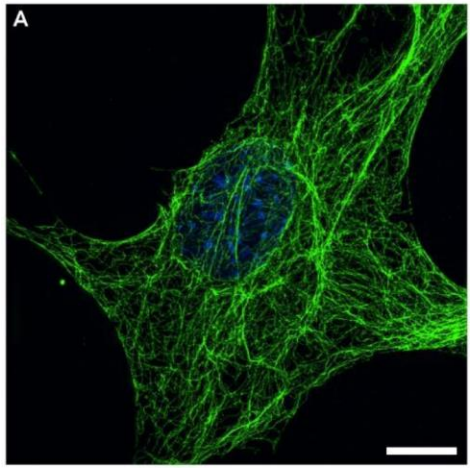
Folding upon binding (induced fit) or conformational selection?

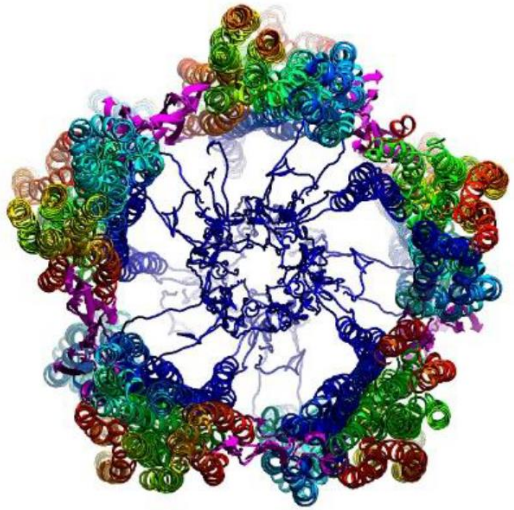


Wright & Dyson, Curr opin struct biol 2009

Intermediate filaments (one type of cytoskeleton fiber) use cross-beta interactions of their IDRs to assemble into large filaments



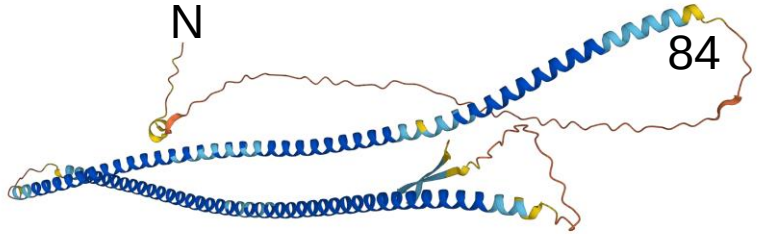




VIM 1-84

MSTRSVSSSSYRRMFGGPGTASRPSSSRSYVTTSTRTYSLGSALRPSTSR

SLYASSPGGVYATRSSAVRLRSSVPGVRLLQDSV



-5 alpha helix bundles

-Each alpha helix bundle contains 8 vimentin monomers

-The IDRs of the vimentin monomers (aa 1-84) form cross-beta interactions in the lumen

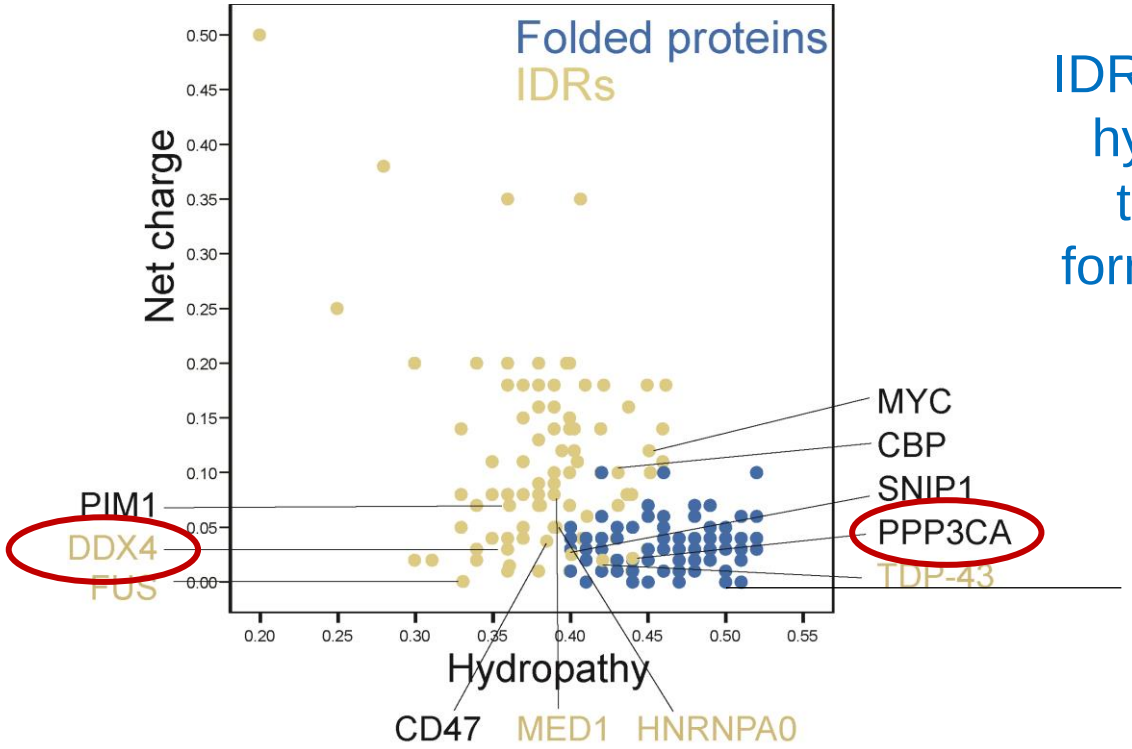
-The filaments disassemble during mitosis through phosphorylation

IDRs can assemble into stable structures, whose stability is controlled by post-translational modifications

Zhou et al., Science 2022,
Zhou, Kato, McKnight, Curr Opin Cell Bio 2023
Eibauer et al., bioRxiv 2023

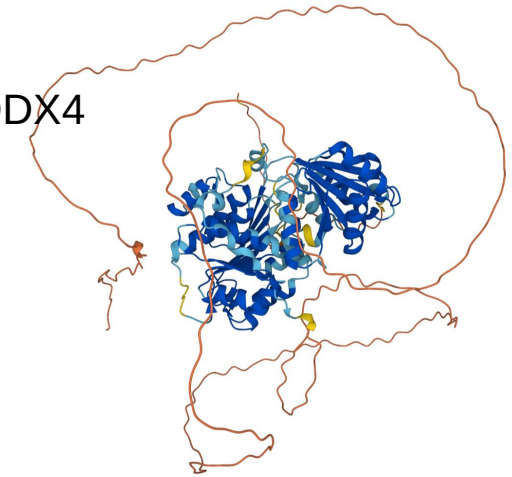
Classification of IDRs

The degree of unstructuredness is determined by the fraction of hydrophobic amino acids in the IDR

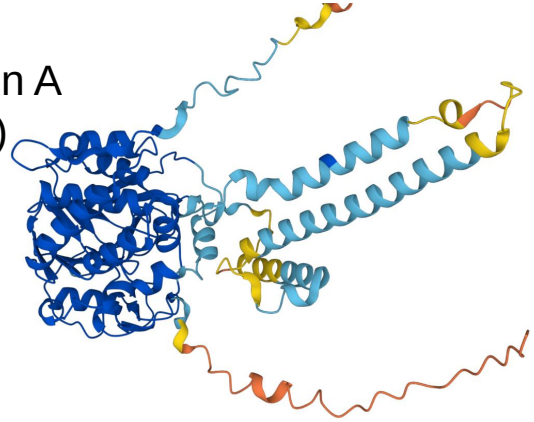


IDRs have a lower fraction of hydrophobic amino acids; this prevents them from forming stable 3D structures

DDX4



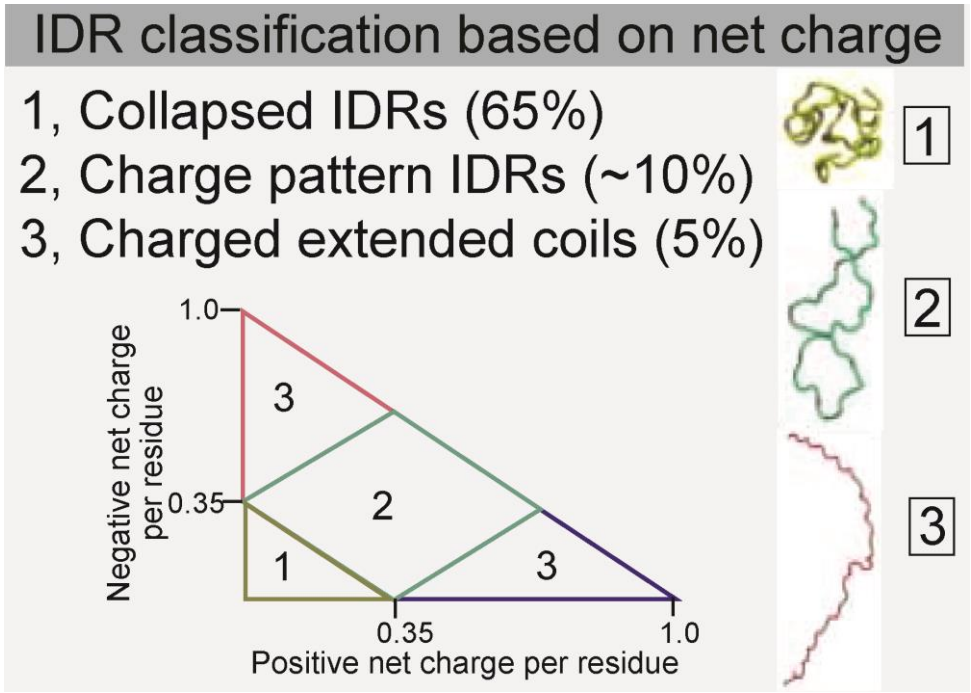
Calcineurin A (PPP3CA)

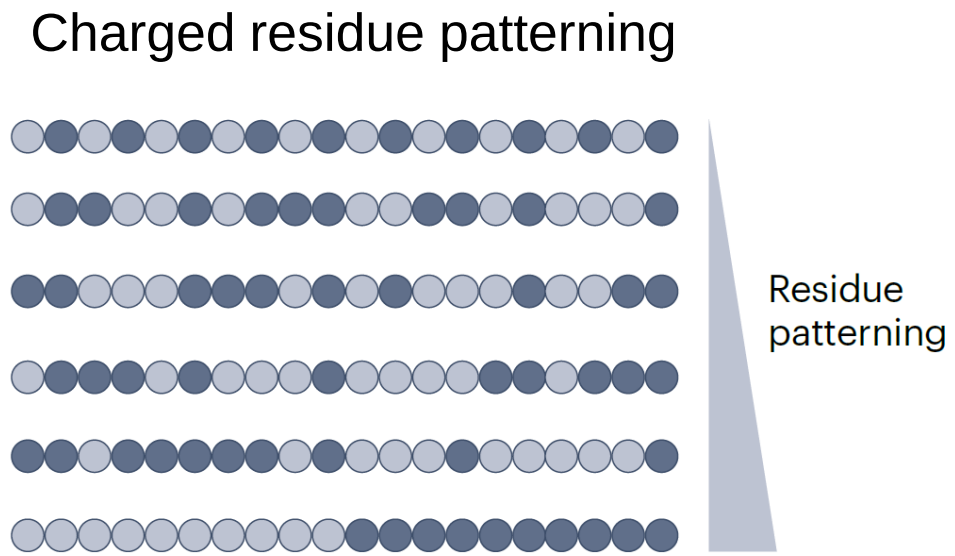


GAPDH

Uversky, Gillespie & Fink, Proteins 2000
Calculated using PONDR

The charge pattern of an IDR can determine if it is expanded or collapsed

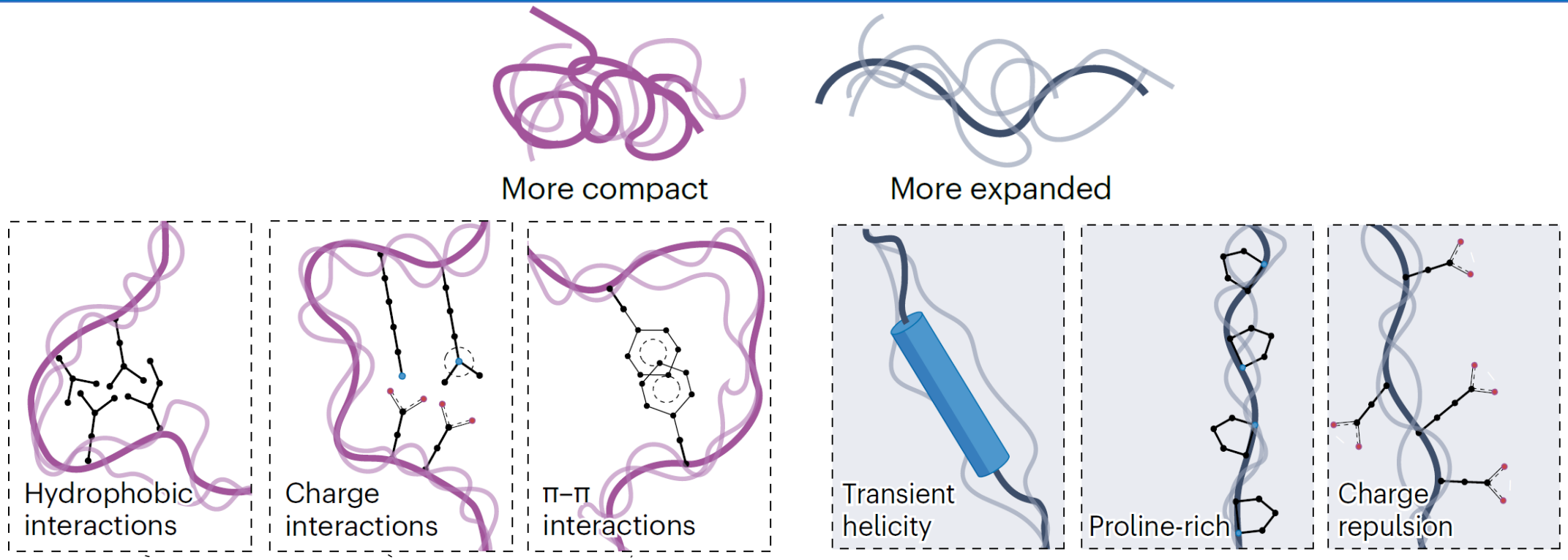




CIDER (on the Pappu lab webpage) calculates the charge pattern of a sequence

Das & Pappu, PNAS 2013

IDRs are further classified into compact or expanded IDRs
This feature is determined by repulsive and attractive interactions

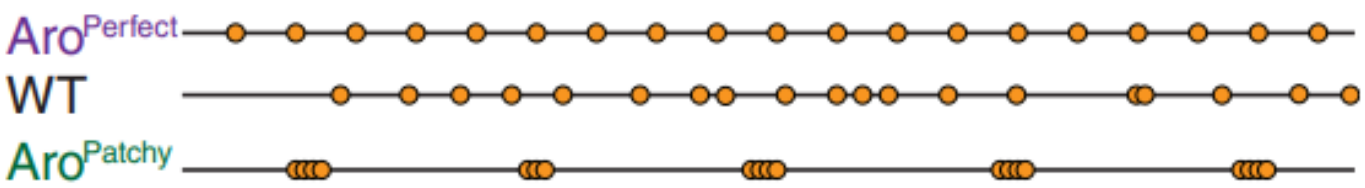


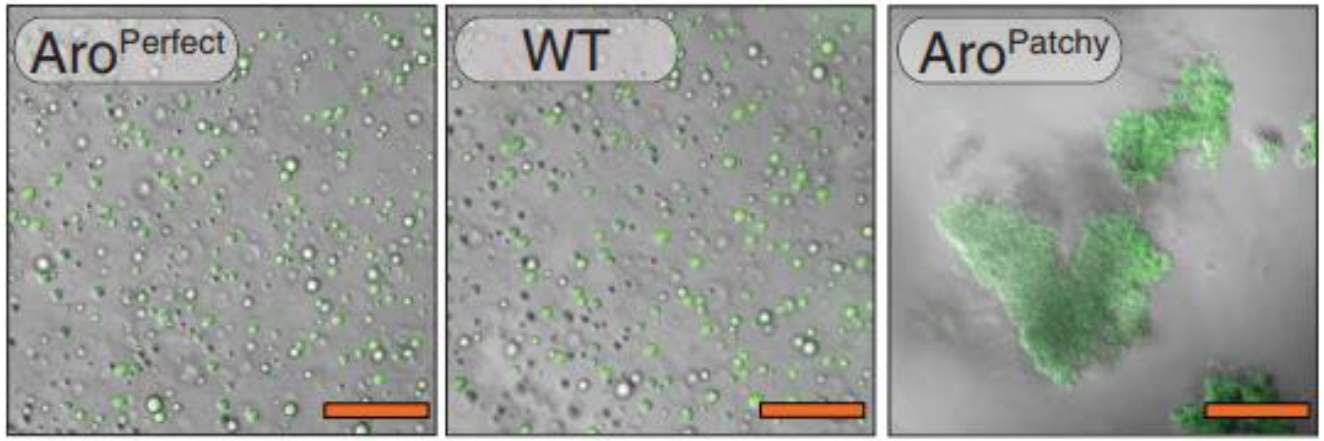
Holehouse & Kragelund Nat rev MCB 2023

Weak interactions allow dynamic assemblies, but there is a fine balance, which is often achieved by post-translational modifications

The pattern of aromatic residues in an IDR (HNRNPA1) determines its liquid-like or aggregation properties

MASASSSQRG RSGSGNFGGG RGGGFGGNDN FGRGGNFSGR GGFGGSRGGG
GYGGSGDGYN GFGNDGSNFG GGGSYNDFGN YNNQSSNFGP MKGGNFGGRS
SGPYGGGGQY FAKPRNQGGY GGSSSSSSYG SGRRF





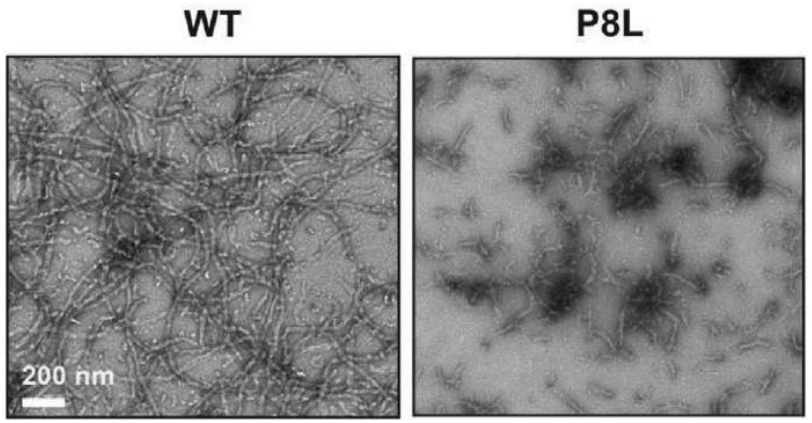
In addition to the amino acid content, the amino acid distribution pattern can influence the flexibility or degree of compactness (or ‘misfolding’) of an IDR

Martin et al., Science 2020

Mutations in IDRs can result in neurodegenerative diseases and aggregate formation

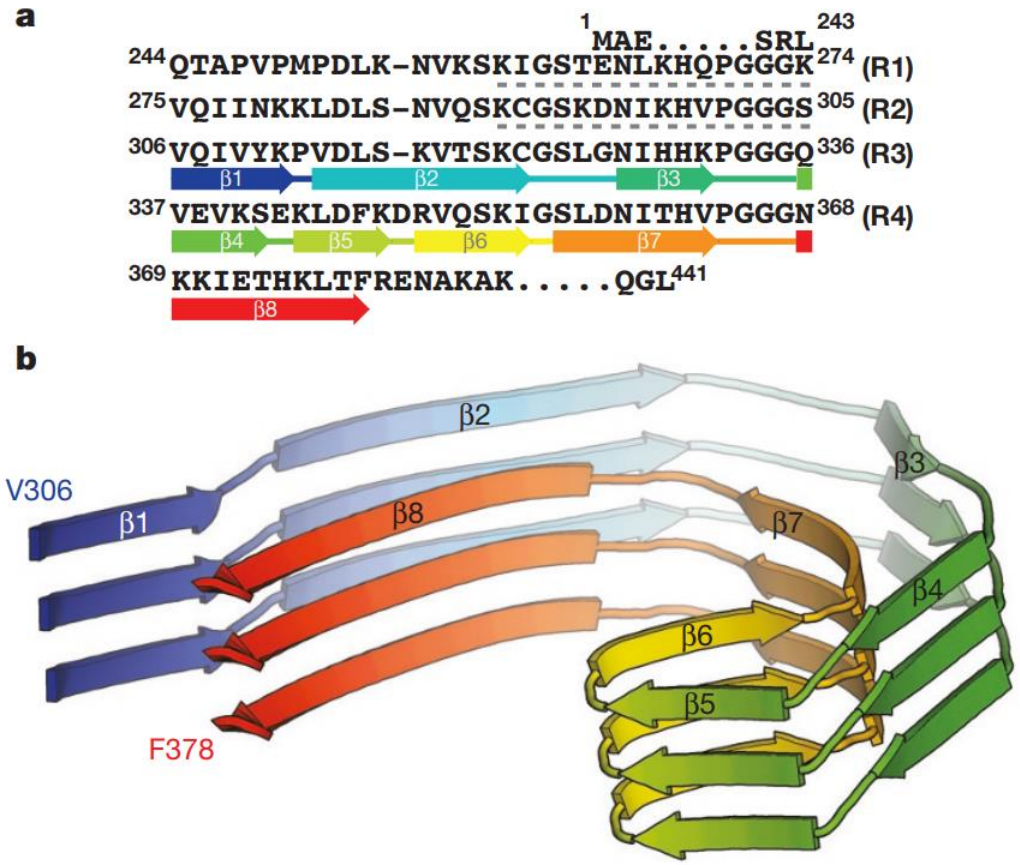
Mutations of prolines in IDRs of intermediate filaments (NEFL), actin-binding proteins (tau), RNA-binding proteins (hnRNPA2) cause neurogegenerative diseases and dementia

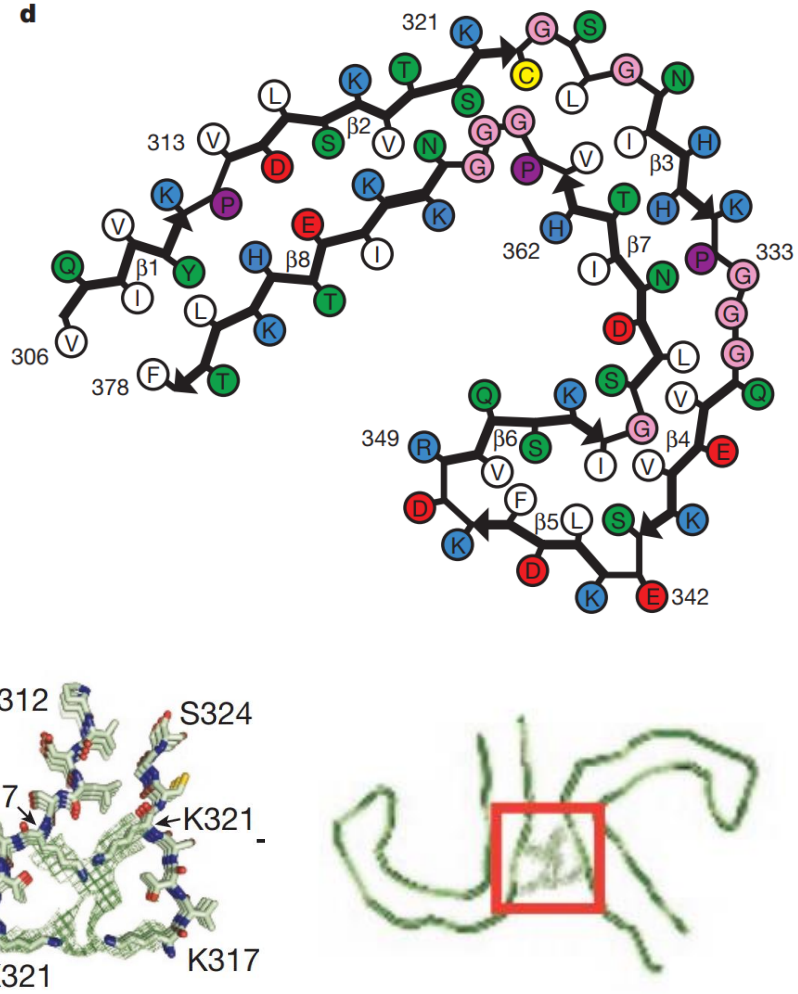
NEFL 1-74
MSSFSYEPYYSTSYKRRYVETPRVHISSVRSGYSTARSAYSS
YSAPVSSSLSVRRSYSSSSGSLMPSLENLDLS



Zhou et al., Science 2022
Zhou, Kato, McKnight, Curr Opin Cell Bio 2023

Tau filaments are characteristic for Alzheimer's disease
They are generated through cross-beta interactions

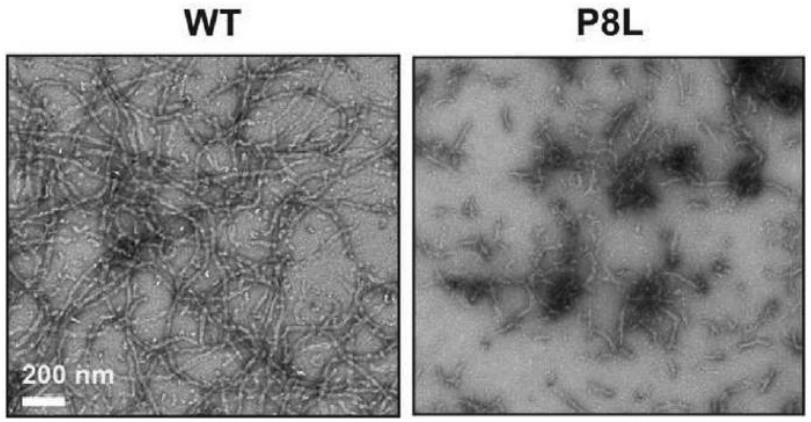




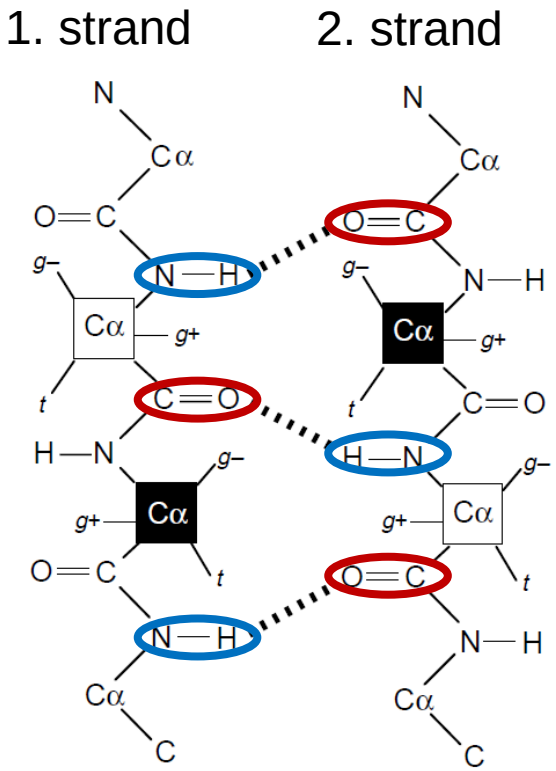
Fitzpatrick et al., Nature 2017

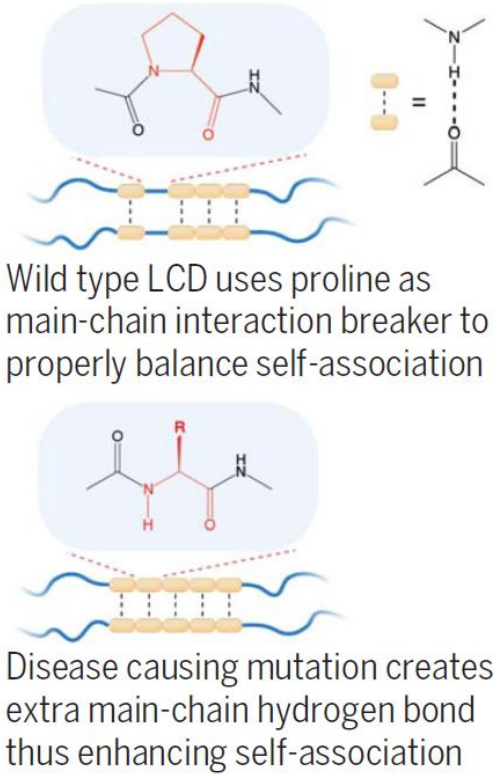
Mutations in prolines make intermolecular IDR interactions too strong, resulting in neurodegenerative diseases and aggregate formation

NEFL 1-74
MSSFSYEPYYSTSYKRRYVETPRVHIS
SVRSGYSTARSAYSSYSAPVSSSLSVR
RSYSSSSGSLMPSLENLDLS



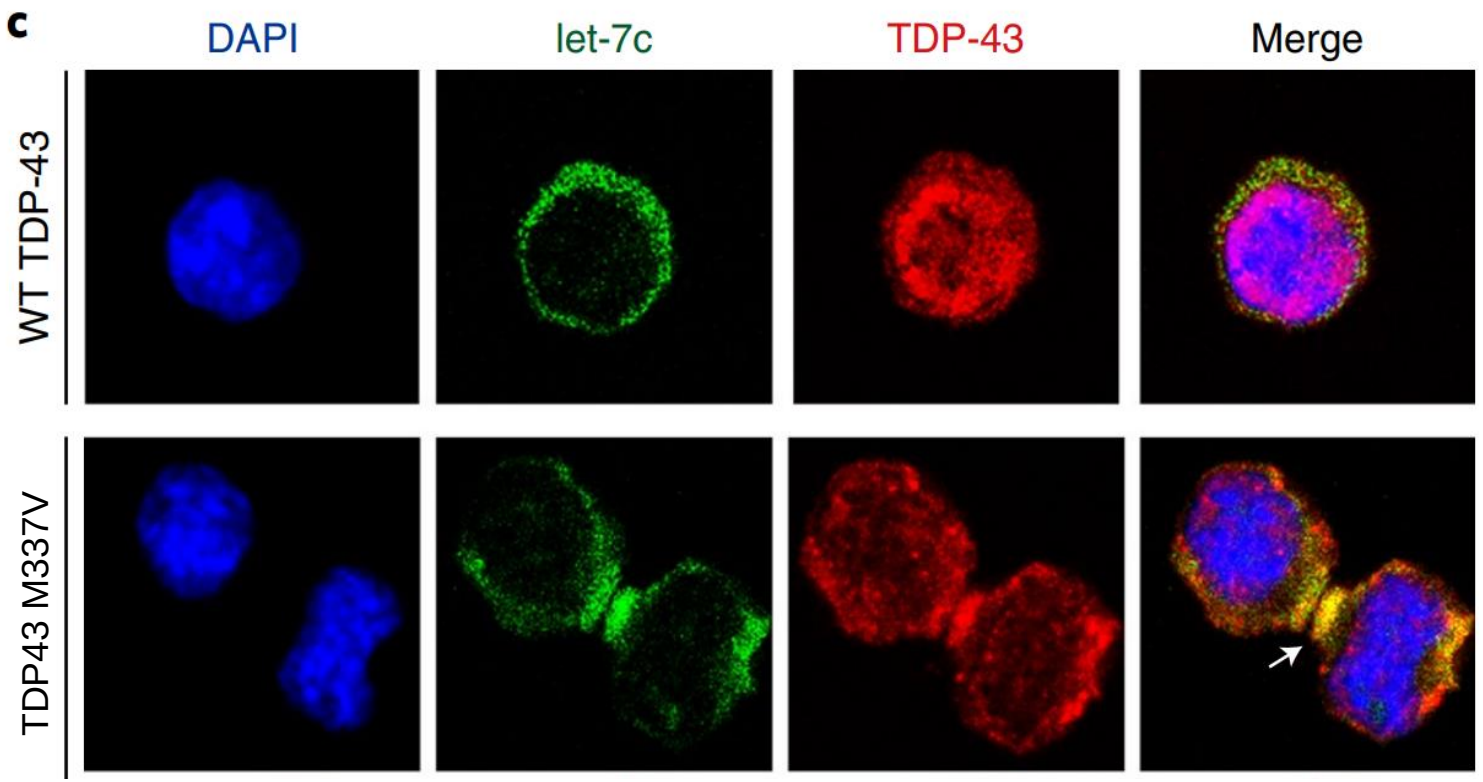
Proline acts as an insulator to prevent spreading of the cross-beta interactions





Zhou et al., Science 2022
Zhou, Kato, McKnight, Curr Opin Cell Bio 2023

97% of all ALS patients have aggregated TDP-43 in the cytoplasm



B

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                                                                                                  337
                                287                307 311          322 323              336 339
Homo                    GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Pongo                   GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Rattus                  GGFGNSRGGGAGLGNNQGGNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Mus                     GGFGNSRGGGAGLGNNQGGNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Bos                     GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Monodelphis             GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Oryctolagus             GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Canis                   GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Odobenus                GGFGNSRGGGAGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Gallus                  GGFGNSRGGGGGLGNNQGSNMGGGMNFGAFSINPAMMAAAQAALQSSWGMMGMLASQ
Danio                   G-FAGSRSNMGGGGGGSSSSLG---NFGNFNLNPAMMAAAQAALQSSWGMMGMLA-Q
                        * *..**.. .* *......:*   *** *.:**********************  *
```

O
||
WGMMGML → No aggregation

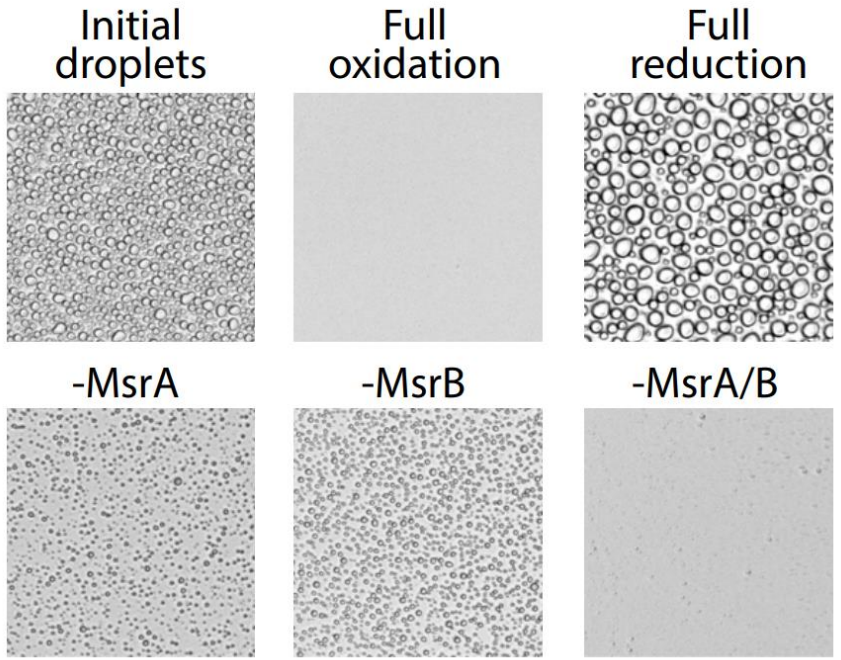
WGMLGML → Aggregation

M337V mutation is sufficient for aggregation

Zhuo et al., NSBM 2021
Lin et al., PNAS 2020

Post-translational modifications, such as methionine oxidation, can influence the phase separation behavior of TDP43

TDP43 IDR



O
||
WGMMGML

Phase separation assays can be used to interrogate IDR characteristics

MsrA/B =
Methionine sulfoxide reductase

Lin et al., PNAS 2020

Functions of IDRs

-Through their multivalent properties, they are often important components of biomolecular condensates

- -This allows a local concentration increase of a transcription factor (transcriptional condensates)
- -This allows different local translation environments (TIS granules/FXR1 network)

-They can take on different conformations, which allows them to interact with structurally-diverse interactors

- -They act as regulatory hubs for transcription and signaling

-They can take on transient structures, which allows them to generate cytoskeletal structures or work as sensors for the environment

- -IDRs can assemble into stable structures that are reversible
- -The stability of the structures can be controlled by post-translational modifications
- -A switch in environment can change the assembly state of a protein from diffusive to assembled (condensate) or from unstructured to structured

RNA is an important regulator of IDRs

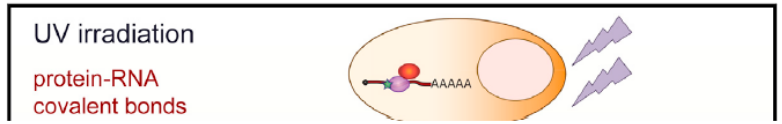
Half of all RNA-binding events in HeLa cells occur in IDRs

Resource

Molecular Cell

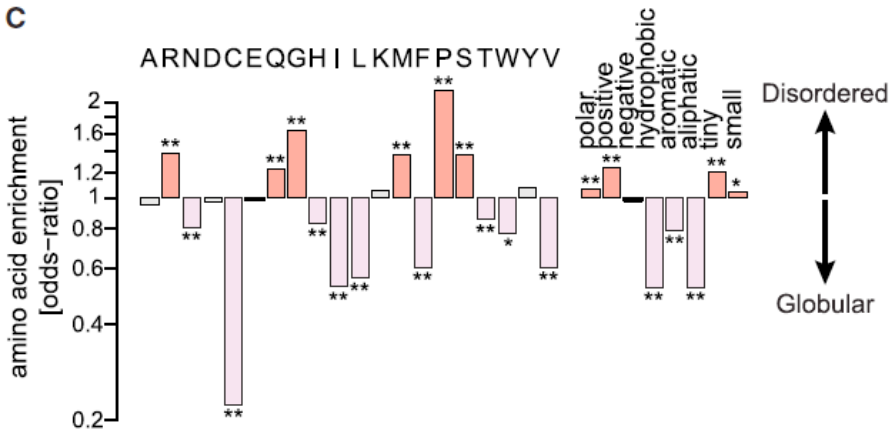
Comprehensive Identification of RNA-Binding Domains in Human Cells

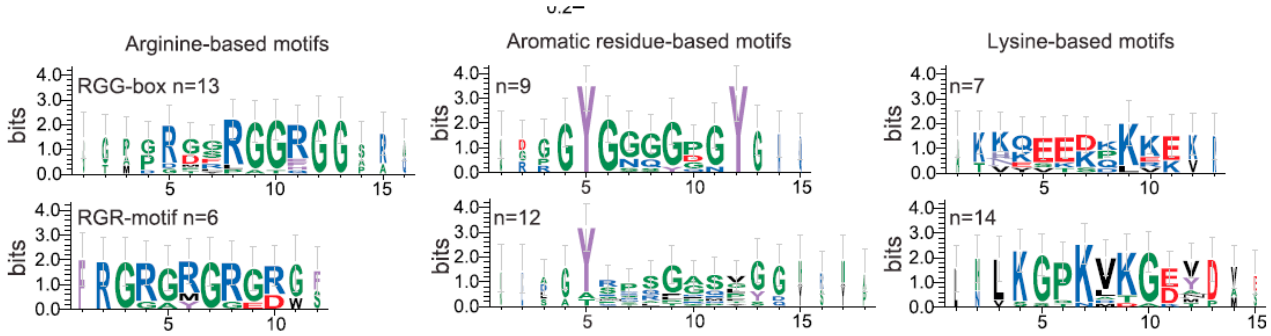
Graphical Abstract



Authors

Alfredo Castello, Bernd Fischer, Christian K. Frese, ..., Tomaz Curk, Jeroen Krijgsveld, Matthias W. Hentze





Castello et al., Mol Cell 2016

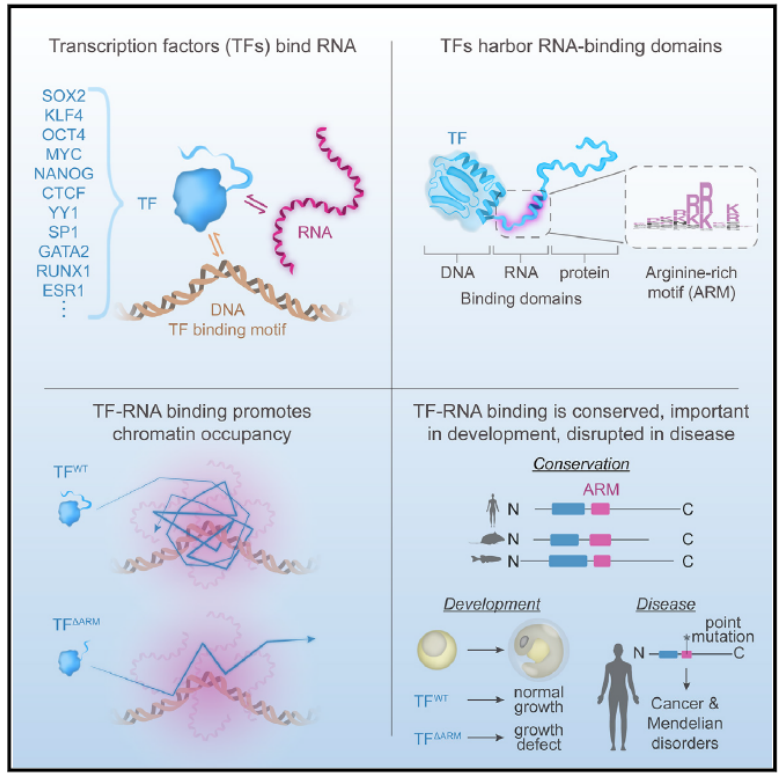
Transcription factors widely bind to RNA

Molecular Cell

Article

Transcription factors interact with RNA to regulate genes

Graphical abstract



Authors

Ozgur Oksuz, Jonathan E. Henninger, Robert Warneford-Thomson, ..., Leonard I. Zon, Roberto Bonasio, Richard A. Young

Correspondence

young@wi.mit.edu

In brief

Oksuz et al. provide evidence that transcription factors frequently bind RNA at active loci, doing so with a conserved domain resembling the arginine-rich motif of the HIV Tat protein. TF-RNA binding constrains TF mobility in chromatin, contributes to gene regulation, is important for normal development, and, when defective, is involved in disease pathogenesis.

Oksuz et al., Mol Cell 2023

What are the functional consequences of widespread RNA-IDR interactions?

RNA binding to RNA-binding proteins changes the conformation of the RNA and the protein in most cases

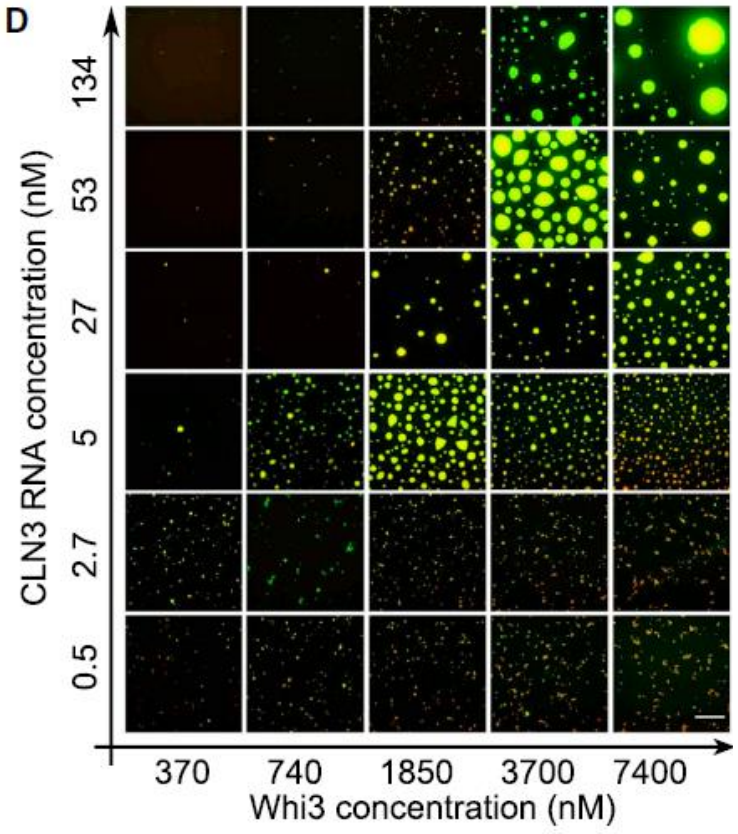
Cse3 plus RNA

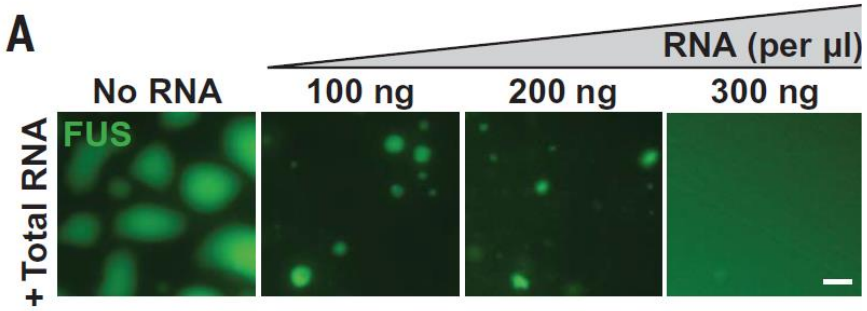
Grey: no RNA
Green: with RNA

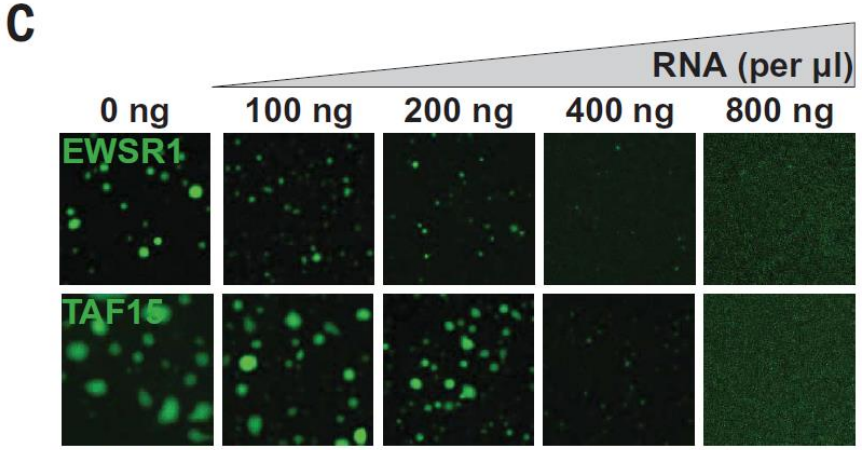
Sashital, Jinek, Doudna, NSMB 2011

RNA can solubilize assemblies

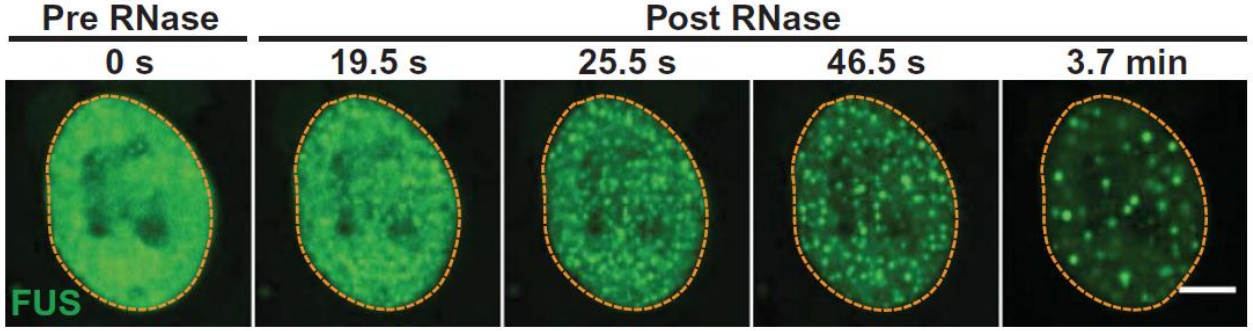
At high RNA concentrations, RNA inhibits phase separation.







RNA also keeps proteins soluble in cells.

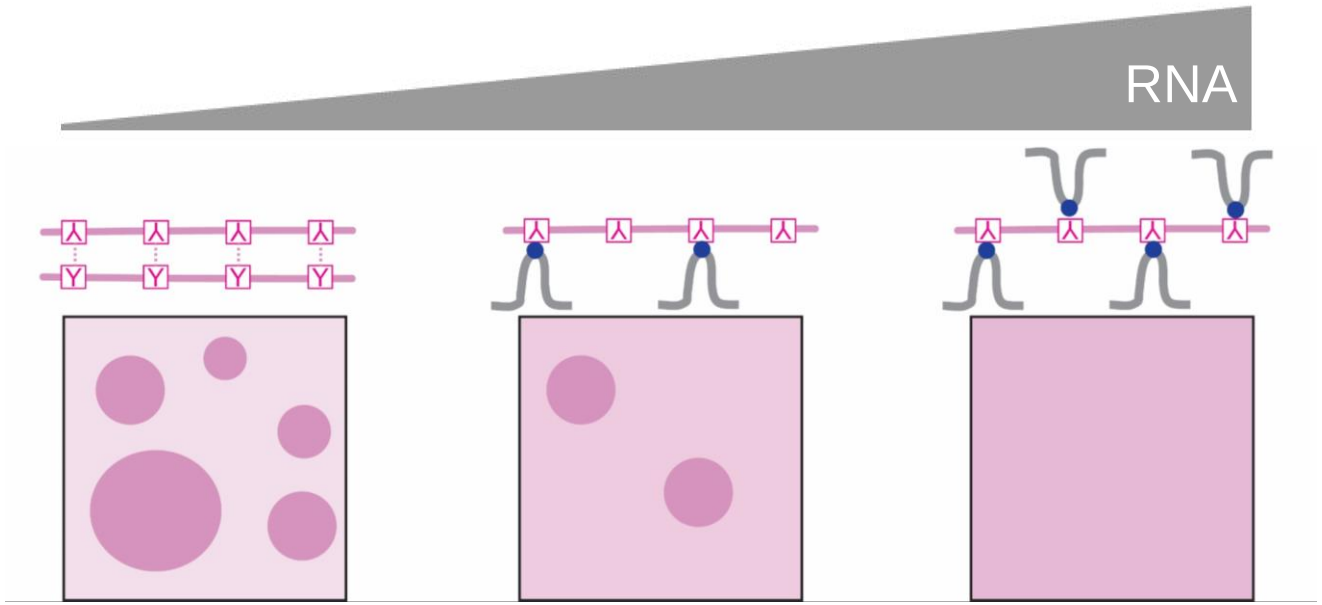


Zhang et al, Mol Cell 2015
Maharana et al, Science 2018

High RNA concentration can overcome phase separation of the FUS IDR, which is caused by weak interactions between aromatic residues (stickers)

FUS IDR
(1-214 aa)

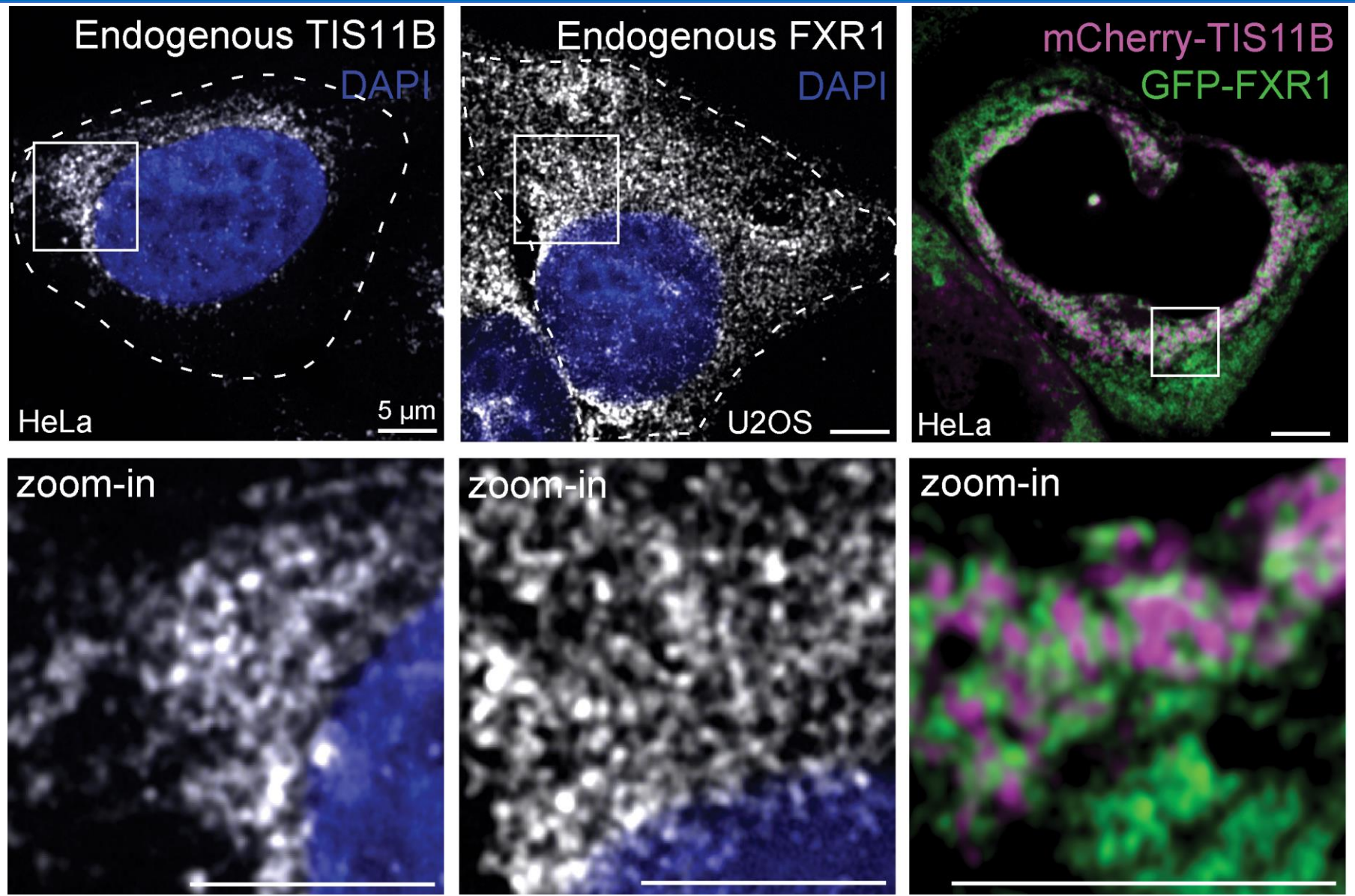
MASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSSYGQSQNTGYGTQSTPQGYGSTGG
YGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQSYNPPQGYGQQNQ
YNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRG



RNA-binding keeps IDRs in a dynamic state

Co-translational RNA-IDR interactions allow RNA to act as chaperone to control the folding of proteins with long IDRs

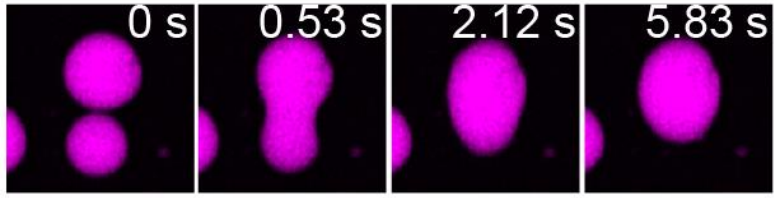
Spatial organization of biochemical activity by cytoplasmic mRNP networks such as TIS granules and the FXR1 network

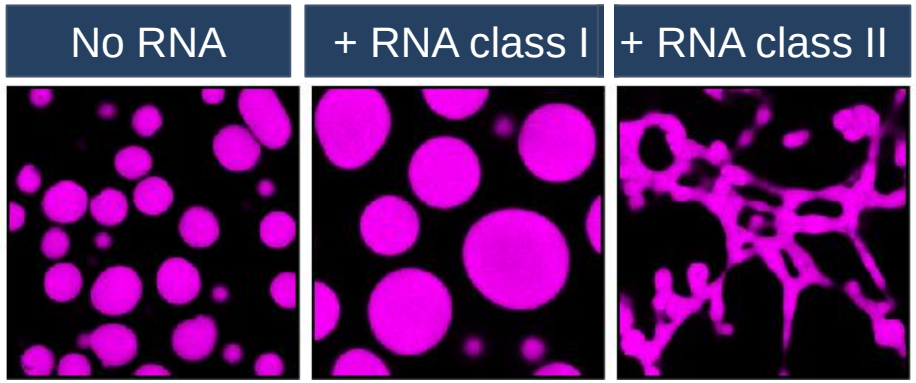


Ma & Mayr, Cell 2018
Chen et al., Cell 2024
Xiuzhen Chen, unpublished

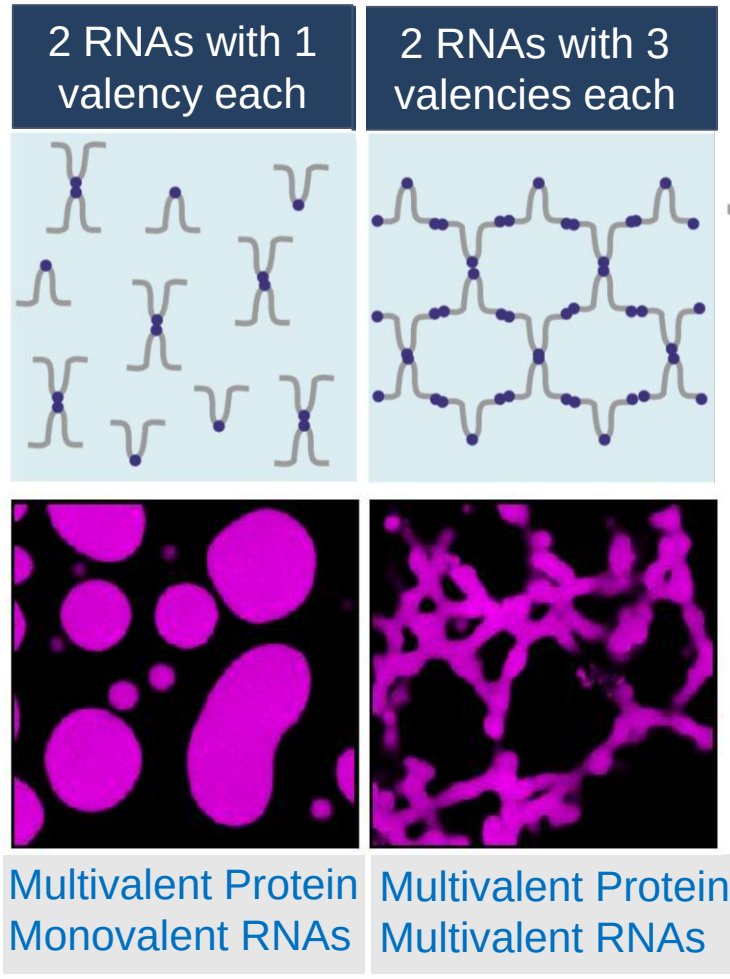
Reconstitute network-like condensates in vitro with a multivalent protein and multivalent RNAs

TIS condensates are liquid-like





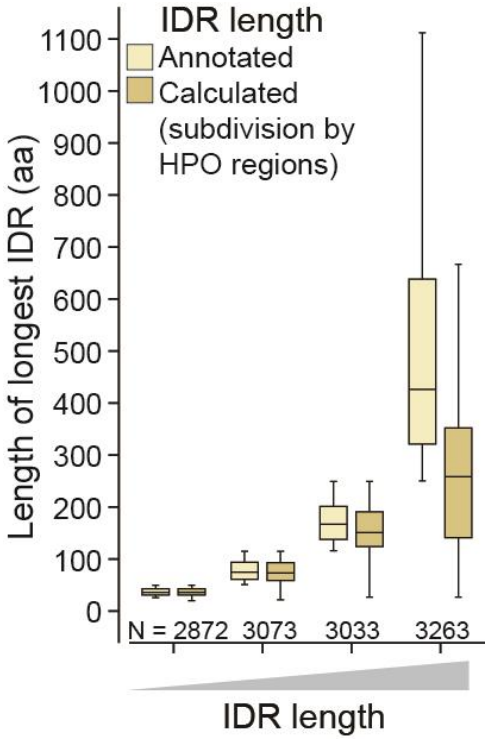
Reconstitution of network-like condensates in vitro



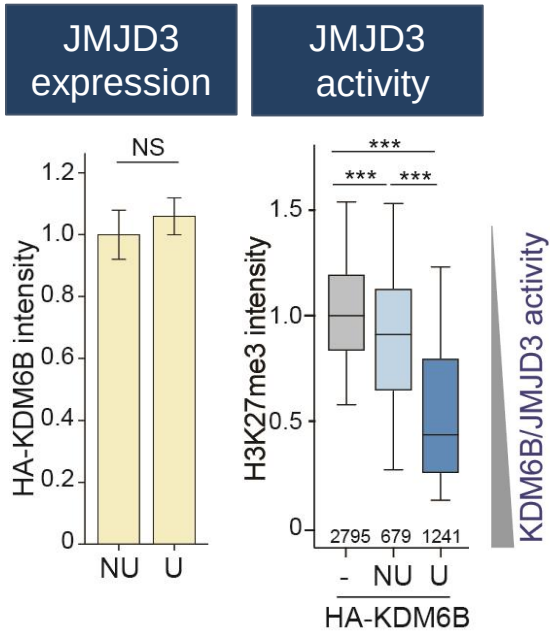
Ma et al., eLife 2021

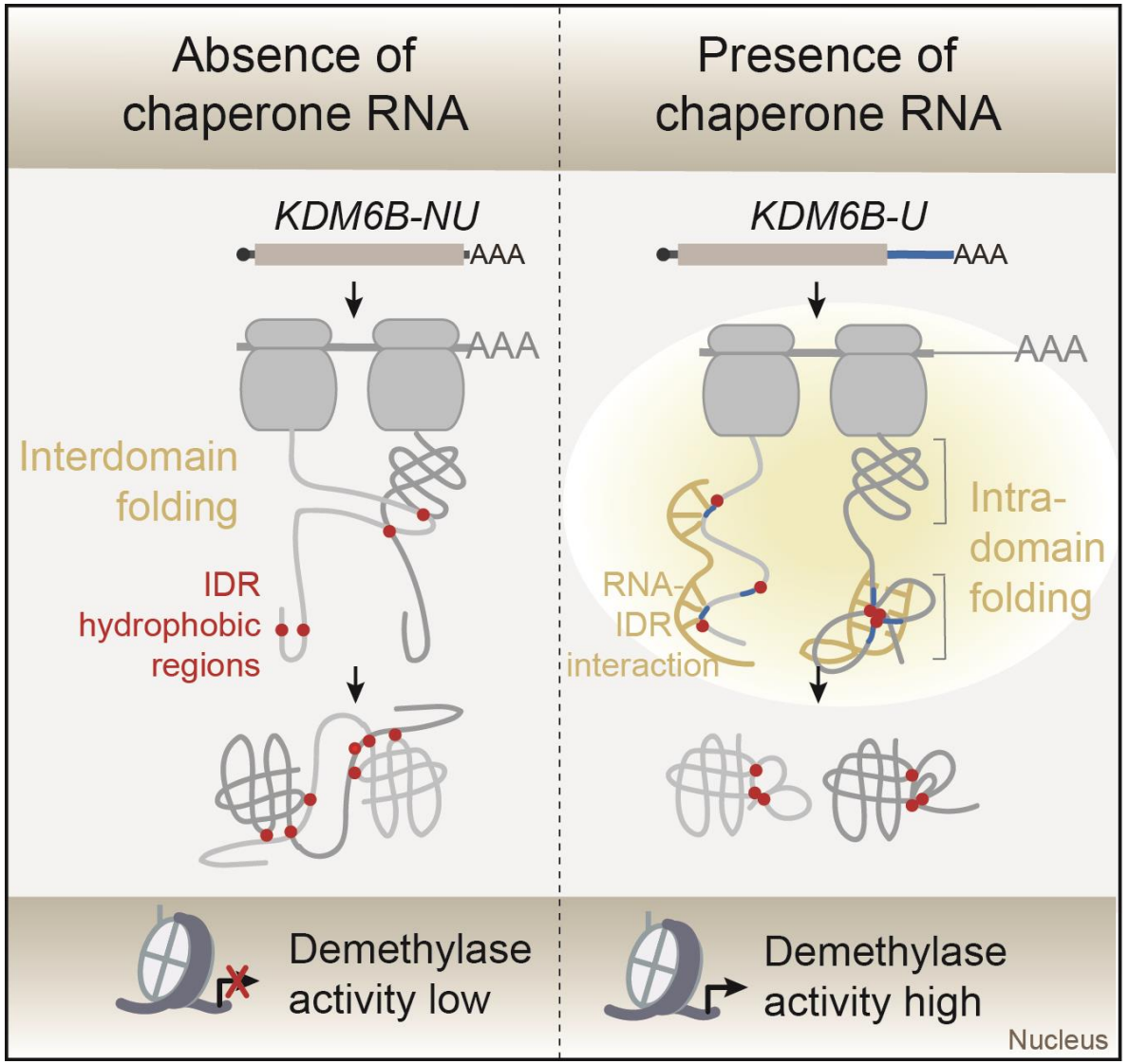
Meshlike condensates act as folding environments that allow co-translational RNA-IDR interactions; RNA acts as chaperone to control the folding of proteins with very long IDRs

Exceptionally long IDRs contain hydrophobic regions to make smaller IDR segments



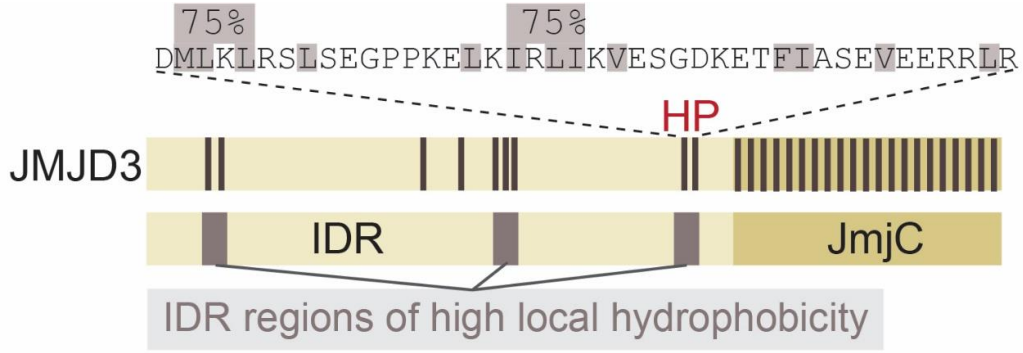
3′UTR-dependent activity regulation of proteins with very long IDRs





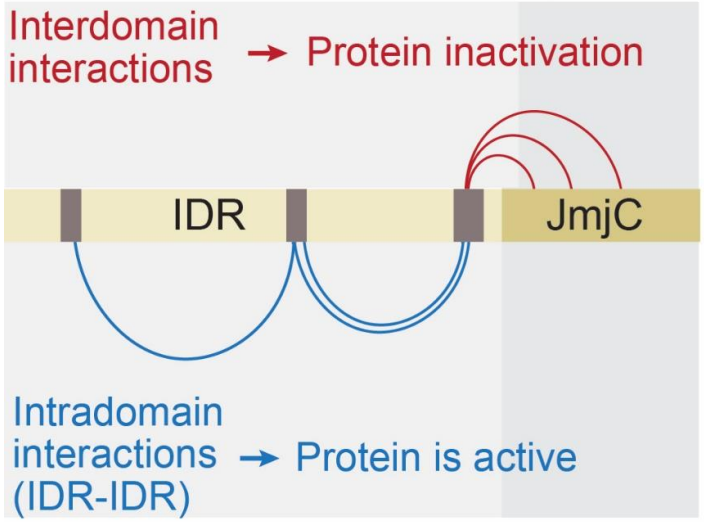
Luo, Zhong, Basu, Mayr, bioRxiv 2025

Model: Hydrophobic regions in long IDRs participate in co-translational protein folding; IDR-RNA binding promotes IDR-IDR interactions and prevents interdomain interactions

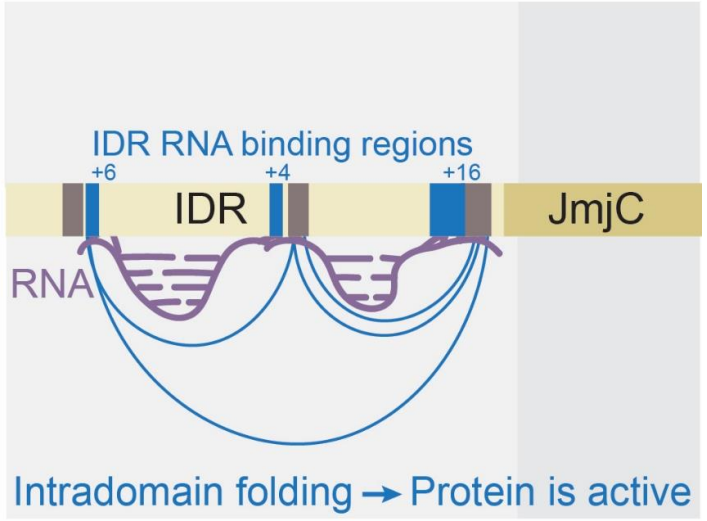


Hydrophobic regions need to be buried during protein folding

Without RNA-IDR binding



RNA-IDR binding scaffolds the IDR and promotes IDR-IDR interactions



Summary: IDRs and RNA

- Half of all RNA-binding events in HeLa cells occur not in canonical RNA-binding domains, but in IDRs
- Co-translational RNA-IDR interactions allow RNA to act as chaperones for the folding of proteins with long IDRs

Co-translational regulation of IDRs: Long IDRs contain hydrophobic regions for subdivision. These folding contacts participate in protein folding. They do not change their conformation post-translationally (KDM6B/JMJD3)

Post-translational IDRs: These IDR regions are unstructured when the protein is examined in isolation, but they usually form transient and diverse structures within their cellular environment (such as intermediate filaments)

Questions?

1. **What are intrinsically disordered regions (IDRs)?**
2. **What kinds of proteins contain IDRs?**

   Transactivation domains of transcription factors, histone tails, regulatory domains of enzymes, C-terminal tails of receptors, RNA-binding proteins

3. **Functions of proteins with IDRs**

   Important components of condensates, structural diversity allows interaction with different partners, transient structures

4. **Classification**

   Sequence features, biophysical and chemical features

5. **RNA as regulator of IDRs**