November 2023

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

Intrinsically Disordered Proteins

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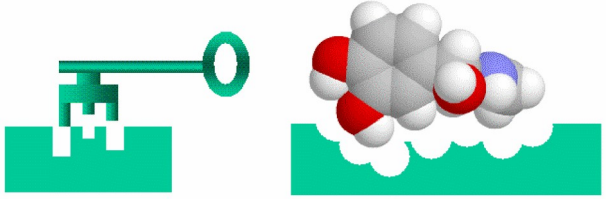
Protein structures

Representation of the 3D structure of a protein

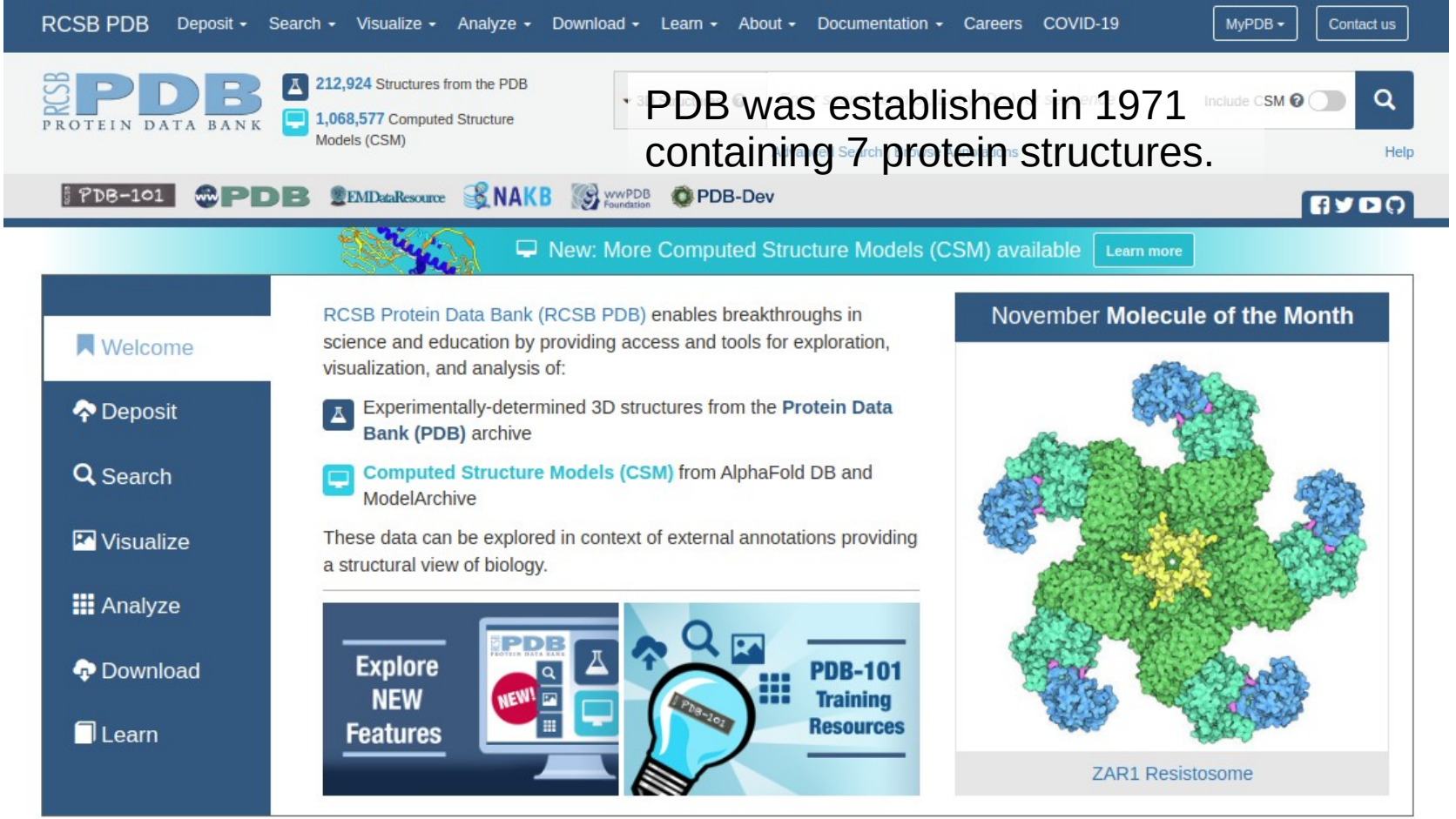
Structure-function paradigm:

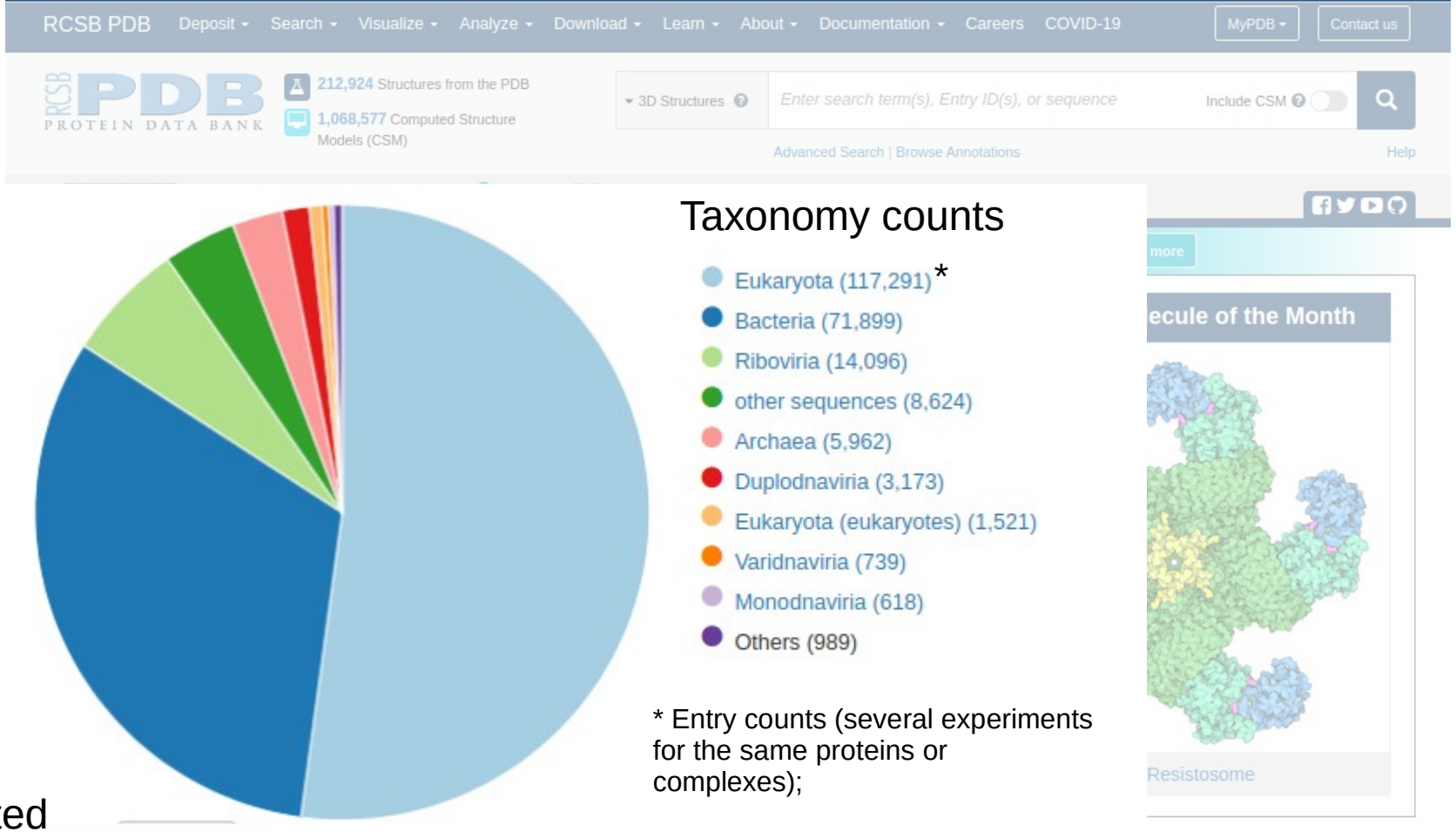
Structure → function

Emil Fisher’s key-lock model (1894)



PDB was established in 1971 containing 7 protein structures.



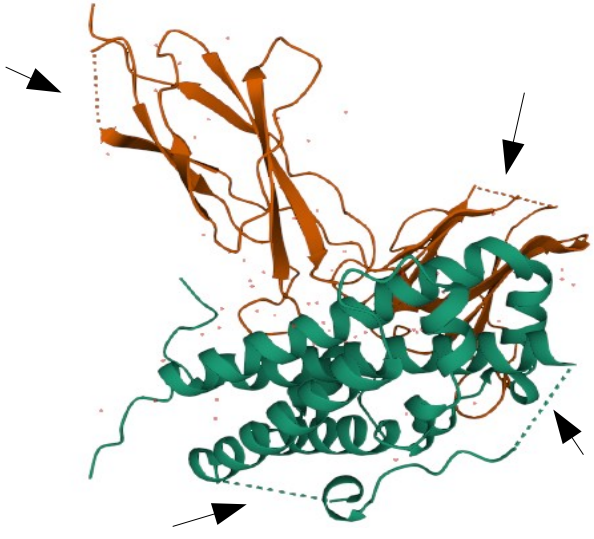


Expected structure

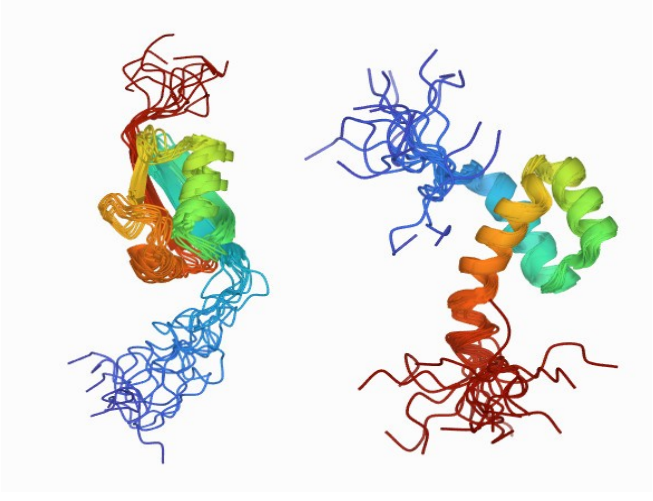
What is protein disorder?

Intrinsically disordered proteins (IDPs): proteins with regions that lack a single well-defined 3D structure in native conditions.

Missing electron densities in X-ray crystallography from PDB

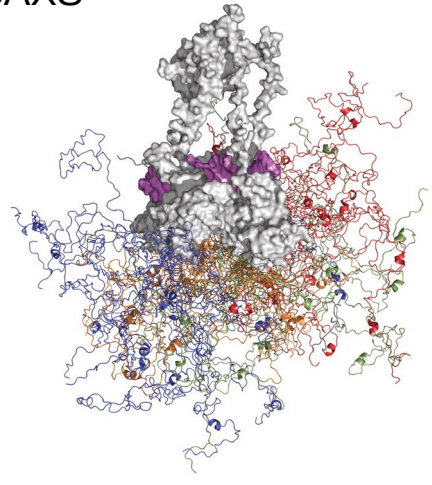


Human Growth hormone bound to receptor



NMR (nuclear magnetic resonance) ensembles of SUMO-1 and antennapedia from PDB

Model of tumor suppressor p53 using X-ray, NMR and SAXS



Disordered in 40% of its length!

Wells et al 2008

~30% of PDB structures have such regions!

Article No. jmbi.1999.3110 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (1999) 293, 321–331

JMB

Intrinsically Unstructured Proteins: Re-assessing the Protein Structure-Function Paradigm

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A major challenge in the post-genome era will be determination of the functions of the encoded protein sequences. Since it is generally assumed that the function of a protein is closely linked to its three-dimensional structure, prediction or experimental determination of the library of protein structures is a matter of high priority. However, a large proportion of gene sequences appear to code not for folded, globular proteins, but for long stretches of amino acids that are likely to be either unfolded in solution or adopt non-globular structures of unknown conformation. Characterization of the conformational propensities and function of the non-globular protein sequences represents a major challenge. The high proportion of these sequences in the genomes of all organisms studied to date argues for important, as yet unknown functions, since there could be no other reason for their persistence throughout evolution. Clearly the assumption that a folded three-dimensional structure is necessary for function needs to be re-examined. Although the functions of many pro-

→ Argues that disordered regions in themselves have important functions!

Disordered region functions

- Flexible linkers/spacers between domains
- Entropic chains (contribute to the structure energy)
- Molecular recognition:
  - binding to proteins, nucleic acid polymers, membrane, metal ions
  - As enzymes that undergo disorder-to-order transitions
  - Formation of multiprotein complexes
- Protein modifications and regulation (e.g. phosphorylation)

See Dunker et al 2002 for more details

Why study IDRs?

- Their mutation is involved in diseases:
  - Cancer
  - Neurodegenerative diseases (Parkinson’s, Dementia, Alzheimer’s, Down’s syndrome, SCA1)
  - Diabetes
  - Cardiovascular diseases
- Can be used in drug delivery (synthetic IDRs);
- Understanding of protein complexes interactions.

Revised lock-and-key model for IDPs

Disordered proteins can bind to many structured partners!

Disorder databases

Organism	Proteins	Regions
Viruses	217	848
Eukaryota	2,055	5,779
Bacteria	356	905
Archaea	21	54

- Disprot: 

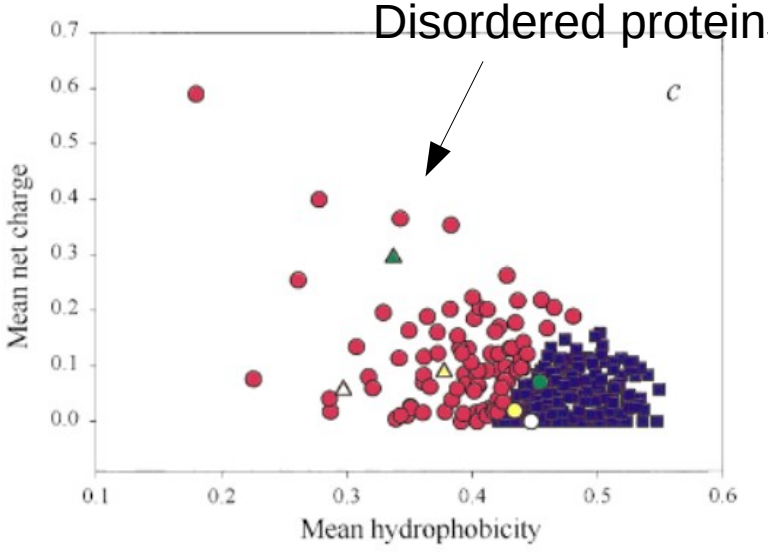
  database of experimentally verified IDPs
- IDEAL: database of experimentally verified 1110 IDPs
- MobiDB: 

  centralized resource that combines experimental and predicted data into a consensus annotation
- DIBS: Disordered Binding Sites (DIBS) with 1,576 complexes with curated interactions on the IDR region.

Disordered sequences

- Low content of bulky hydrophobic amino acids (Val, Leu, Ile, Phe, Trp, Tyr, Met)
- High content of charged and polar Ser, Pro, Glu, Lys)

Disordered proteins



Used in disorder predictor FoldIndex

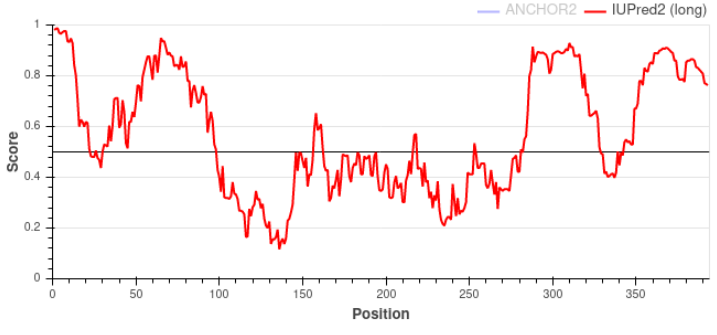
Uversky et al 2000

Disorder prediction

Prediction methods can be based on:

- Physical/chemical features (FoldIndex)
- Machine learning algorithms (DISOPRED2, Spritz, PONDR)
- Energy estimation (***IUPred2***):

  Globular proteins form many favorable interactions to ensure the stability of the structure.



IUPred output for p53

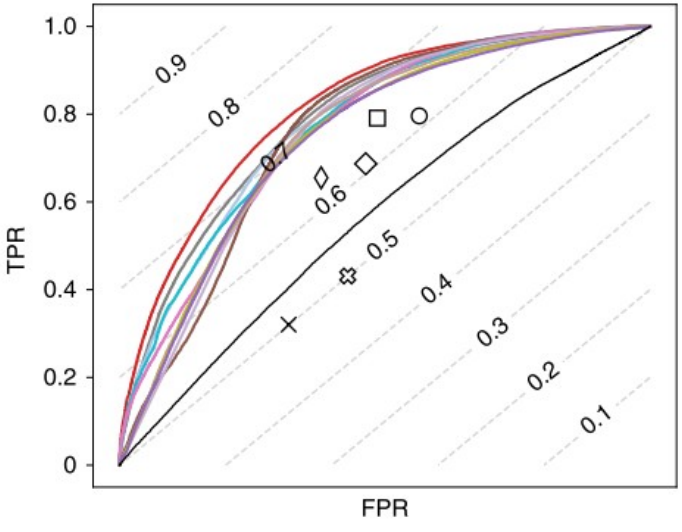
Disorder prediction

Critical Assessment of protein Intrinsic Disorder prediction (CAID)

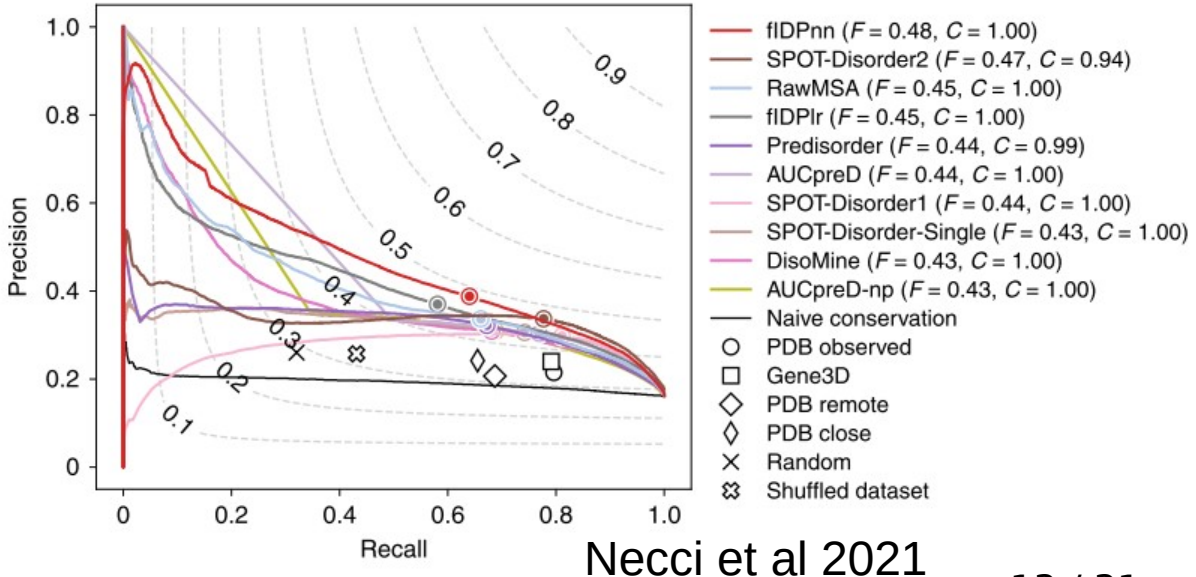
CAID

Bienal competition designed to access the quality of new IDR/IDP and and binding predictors.

AUC curve



Precision-Recall curve



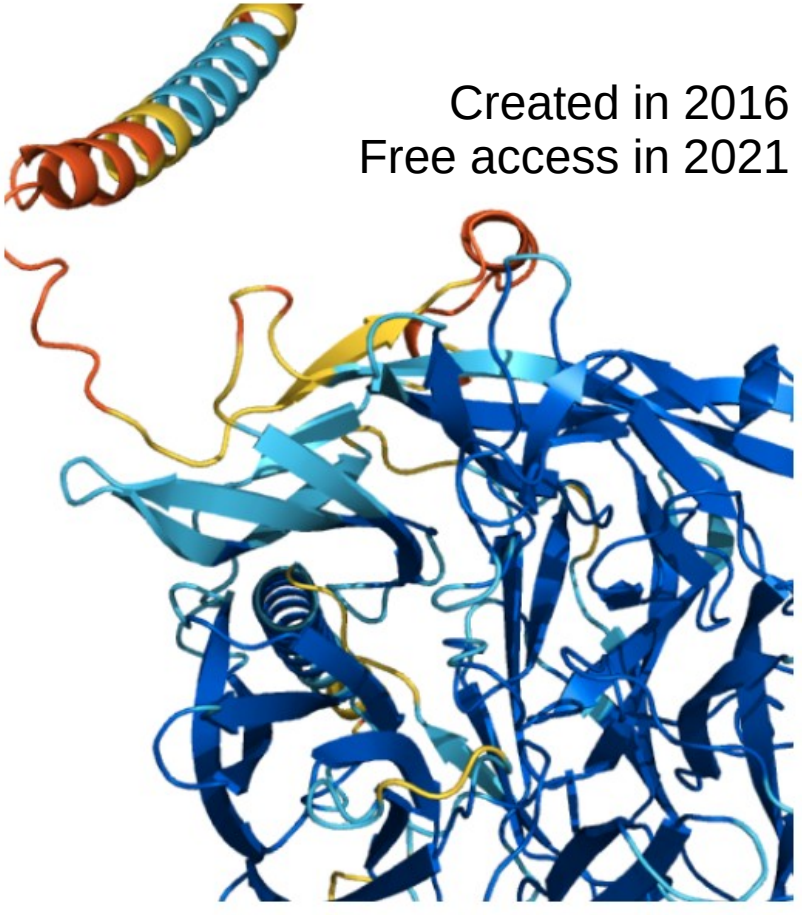
Necci et al 2021

What about order prediction?

AlphaFold is an AI system developed by DeepMind that predicts a protein's 3D structure from its amino acid sequence. It regularly achieves accuracy competitive with experiment.

DeepMind and EMBL's European Bioinformatics Institute (EMBL-EBI) have partnered to create AlphaFold DB to make these predictions freely available to the scientific community. The latest database release contains over 200 million entries, providing broad coverage of UniProt (the standard repository of protein sequences and annotations). We provide individual downloads for the human proteome and for the proteomes of 47 other key organisms important in research and global health. We also provide a download for the manually curated subset of UniProt (Swiss-Prot).

Created in 2016
Free access in 2021

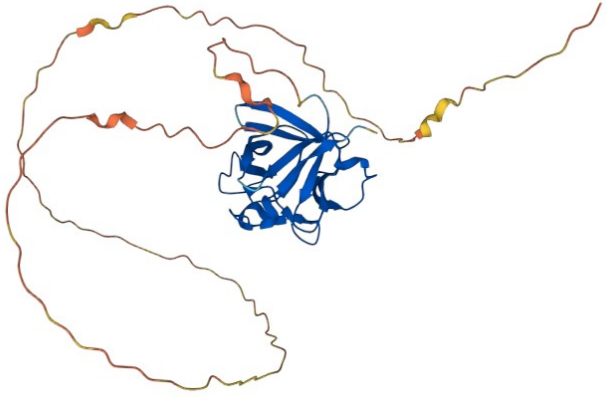


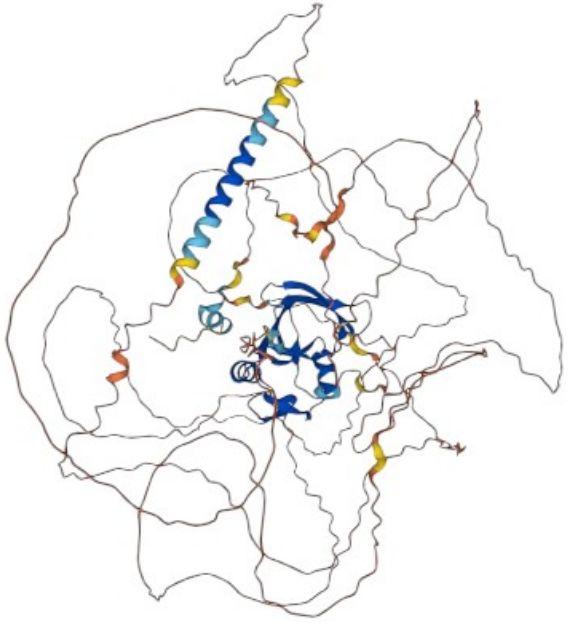
Q8I3H7: May protect the malaria parasite against attack by the immune system.
Mean pLDDT 85.57.

View protein

What about order prediction?
AlphaFold also “predicts” disorder

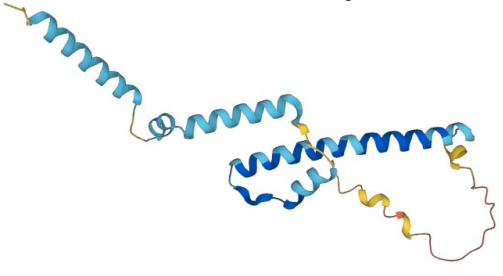
P09038 - Fibroblast growth Factor 2 (Human)



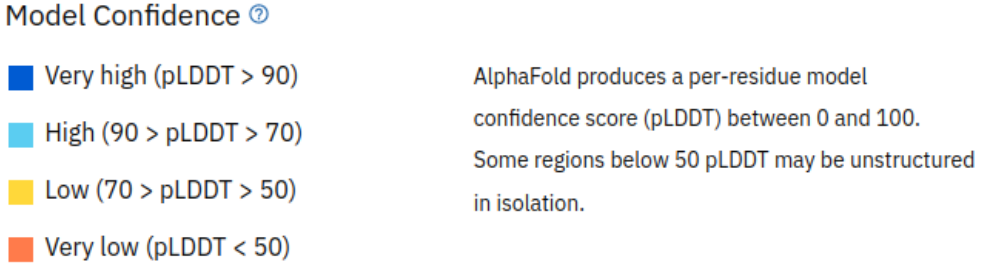


P54253 - Ataxin-1 (Human)

Q8WVH0 – Complexin-3 (Human)



Low pLDDT scores can be used as an indication of disorder.



Research examples
Structure in disordered regions

PolyXYs - QS, GS, RG...

- Filtered PolyX and PolyXYs within IDRs of the Human proteome;
- Extracted 100 residues surrounding the repeated region to analyse its structural content.

Goncalves-Kulik et al 2022: Based on sequences of homologous PDB structures

Goncalves-Kulik et al 2023: Analises based on AlphaFold predictions

```
>sp|P35637|FUS_HUMAN RNA-binding protein
MASNDYTQQATQSYGAYPTQPGQGYSQQSSQPYGQQSYSGYSQSTDTSGYGQSSYSSYGQSQNTGYGTQSTPQG
YGSTGGYGSSQSSQSSYGQQSSYPGYGQQPAPSSTSGSYGSSSQSSSYGQPQSGSYSQQPSYGGQQQSYGQQQS
YNPPQGYGQQNQYNSSSGGGGGGGGGGNYGQDQSSMSSGGGSGGGYGNQDQSGGGGSGGYGQQDRGGRGRGGSG
GGGGGGGGGYNRSSGGYEPRGRGGGRGGRGGMGGSDRGGFNKFGGPRDQGSRHDSEQDNSDNNTIFVQGLGENV
TIESVADYFKQIGIIKTNKKTGQPMINLYTDRETGKLKGEATVSFDDPPSAKAAIDWFDGKEFSGNPIKVSFAT
RRADFNRGGGNGRGGRGRGGPMGRGGYGGGGSGGGGRGGFPSGGGGGGGQQRAGDWKCPNPTCENMNFSWRNEC
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QDRRERPY
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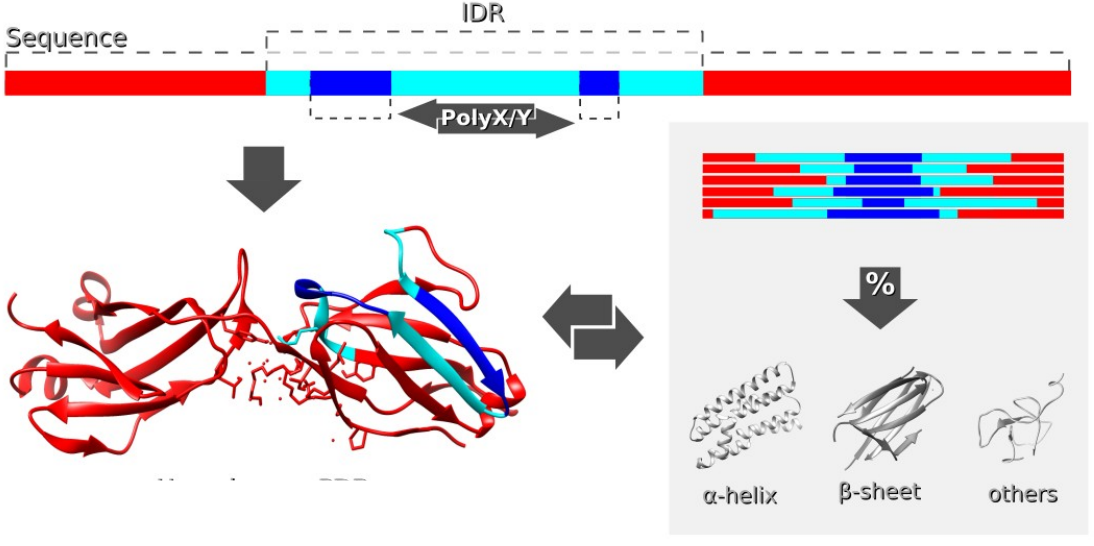
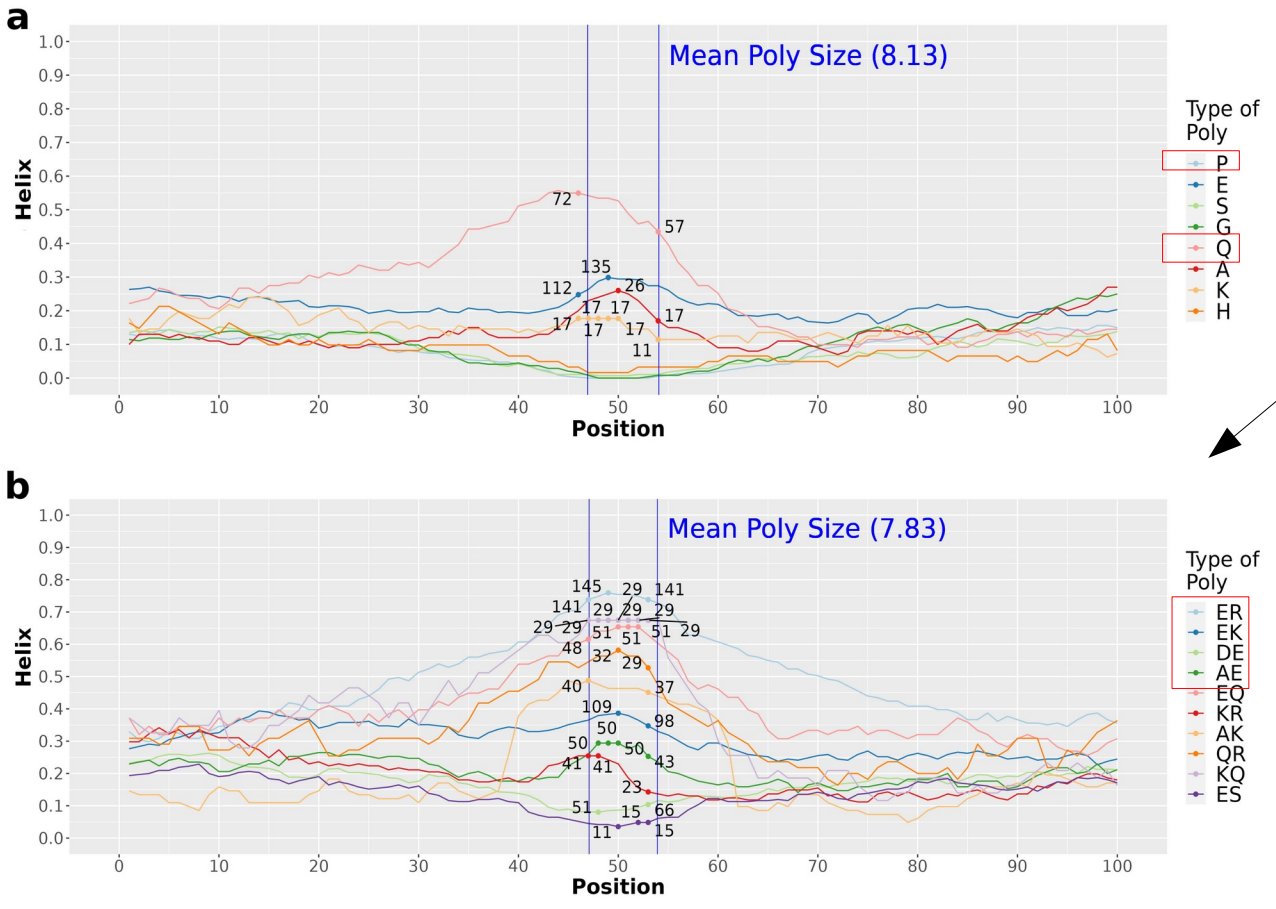


Research examples
Structure in disordered regions

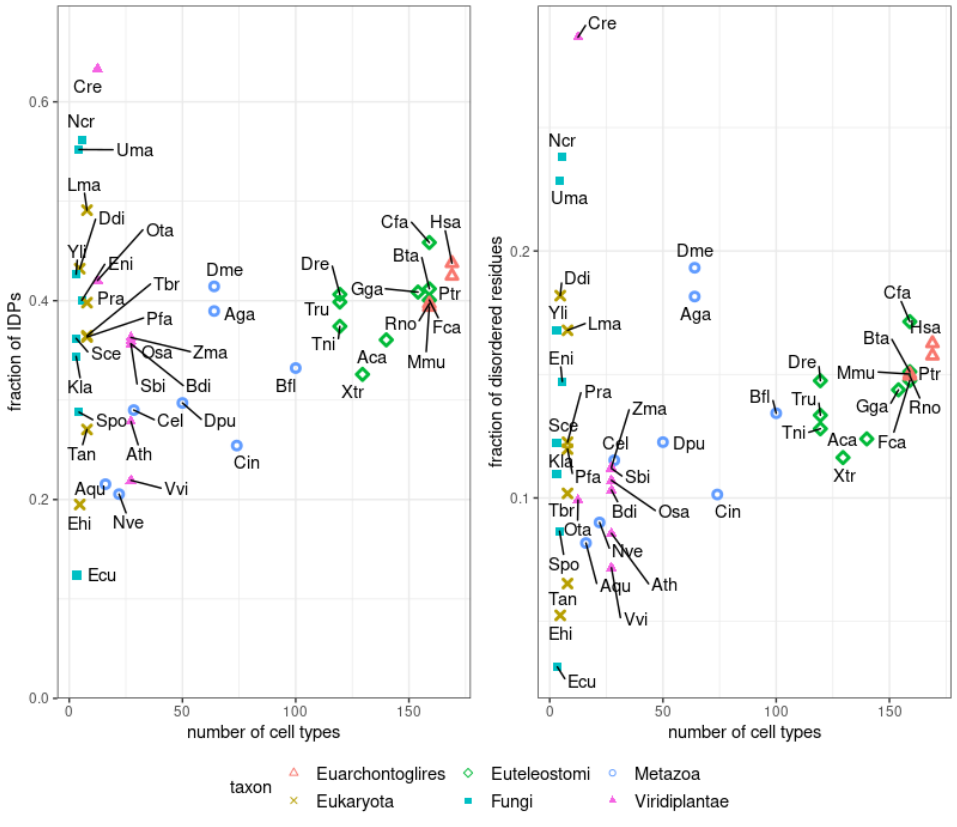


Some polyX (Q, P) and polyXYs (ER, EK, DE, AE) show preference to adopt helical structures in the repeated regions.

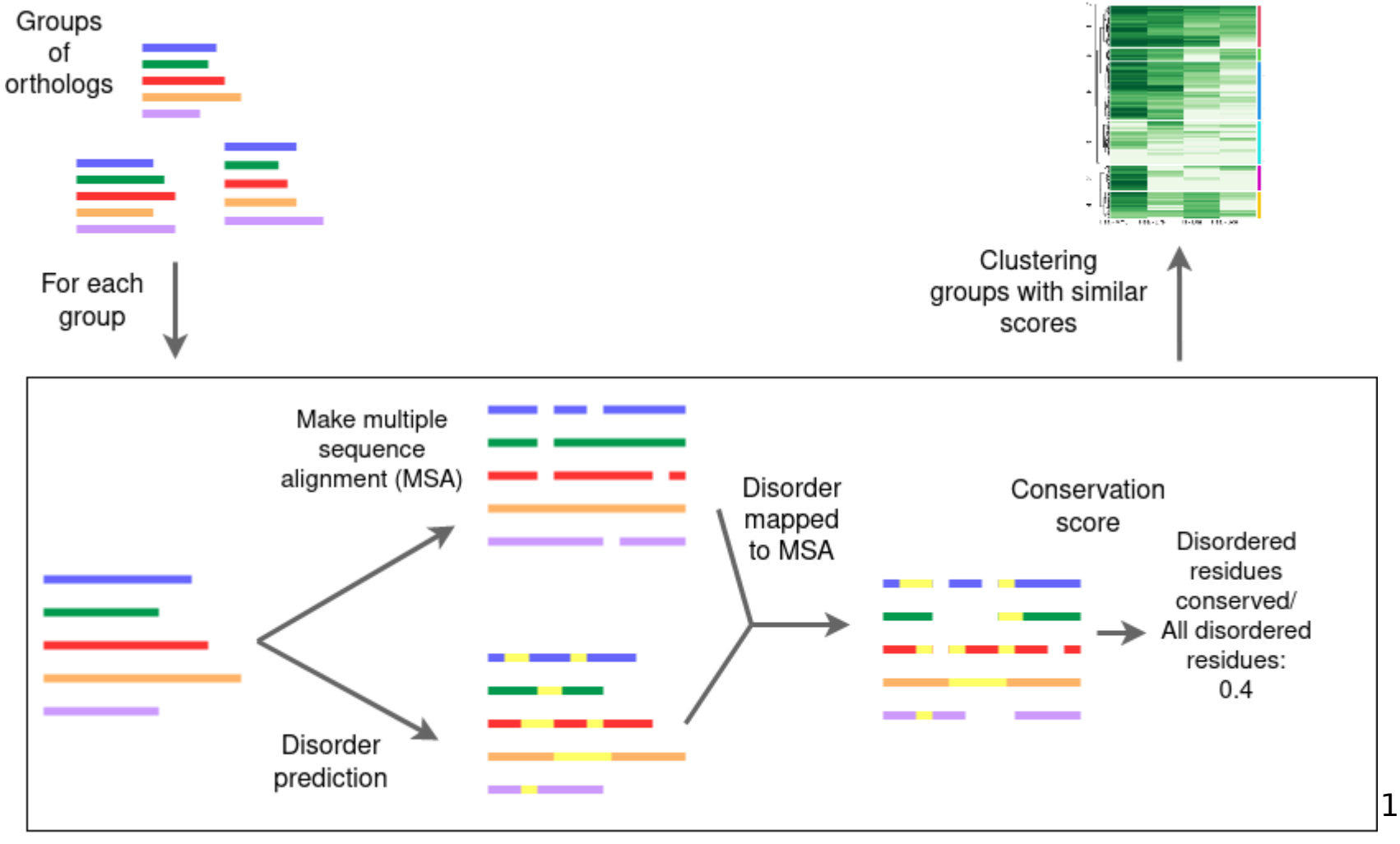
Research examples
Evolutionary study of disorder

Natural abundance and phylogenetic distribution

- ~40% of human proteins predicted to be IDPs
- ~30% of Eukaryotic proteins predicted to be IDPs

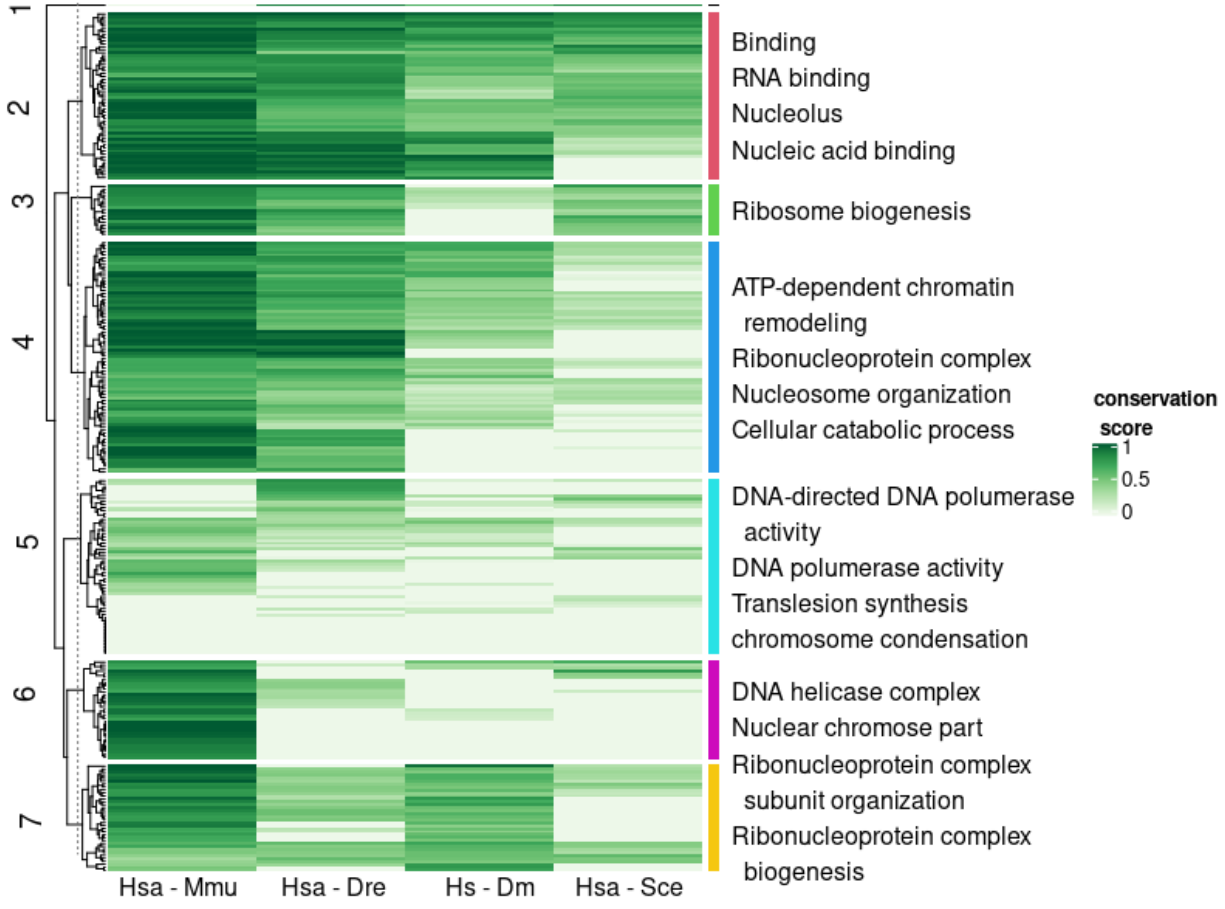


Research examples
Evolutionary study of disorder



Research examples

Evolutionary study of disorder



Correlating
similar disorder
conservation patterns
with protein functions

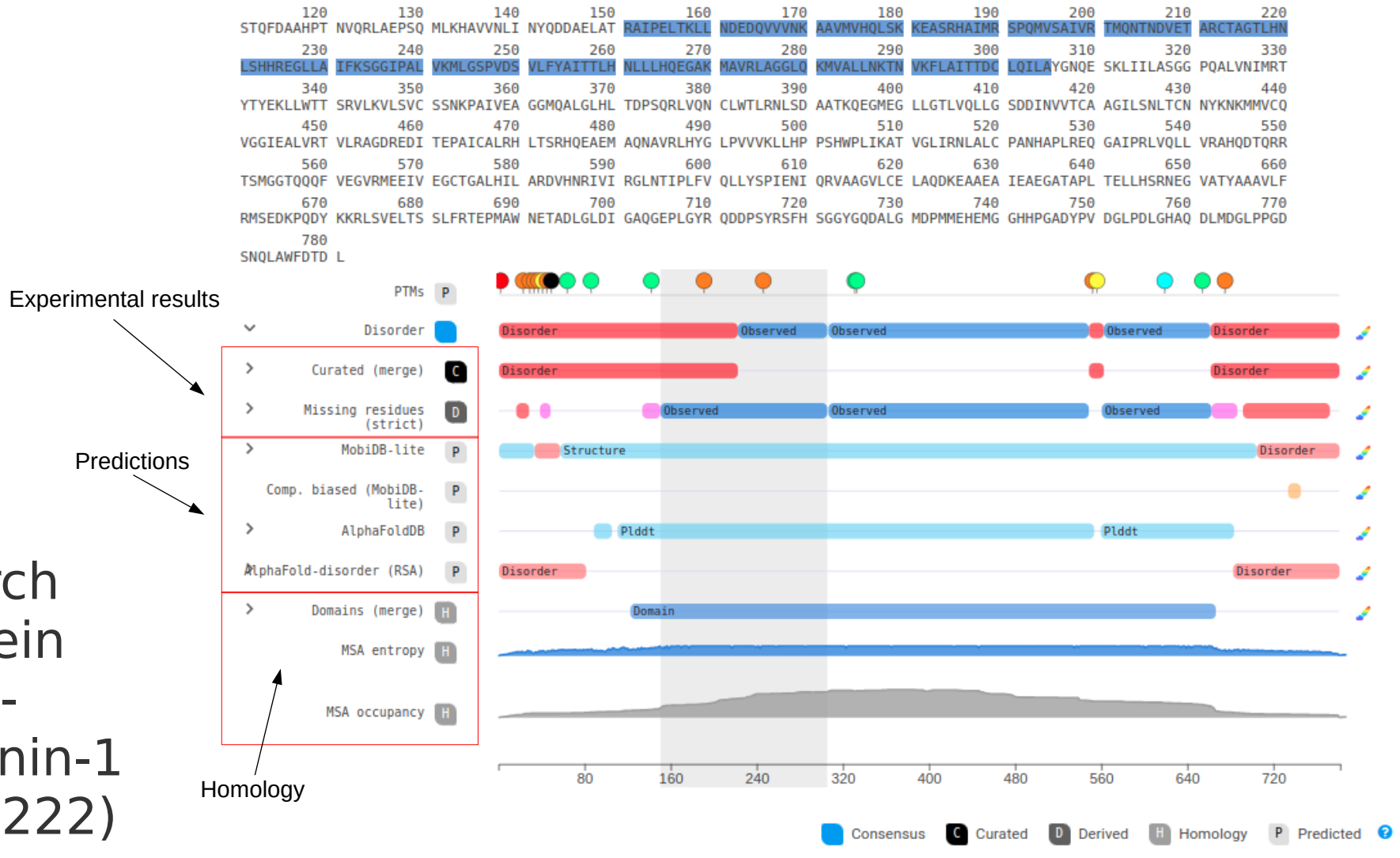
Kastano et al 2020

Exercises

Exercise time!

https://mobidb.bio.unipd.it/

Exercise: MobiDB and DisProt



Search protein beta-catenin-1 (P35222)

Exercise: MobiDB and DisProt

- Search beta-catenin-1 (P35222) on MobiDB. **On top of the protein structure, click on the Disorder tab.**
  - 1. What kind of disorder annotations are there for this protein?
  - 2. The different annotations don’t give exactly the same results. Which annotation gives the longest IDRs (Intrinsically Disordered Regions)?
- Go to the Disprot entry linked on top box with cross references.
  - 3. How many different Experimental techniques (indicated as “Evidence”) were used to identify the presence of disorder?

https://take.quiz-maker.com/QJRLDKS59

Exercise: MobiDB and DisProt

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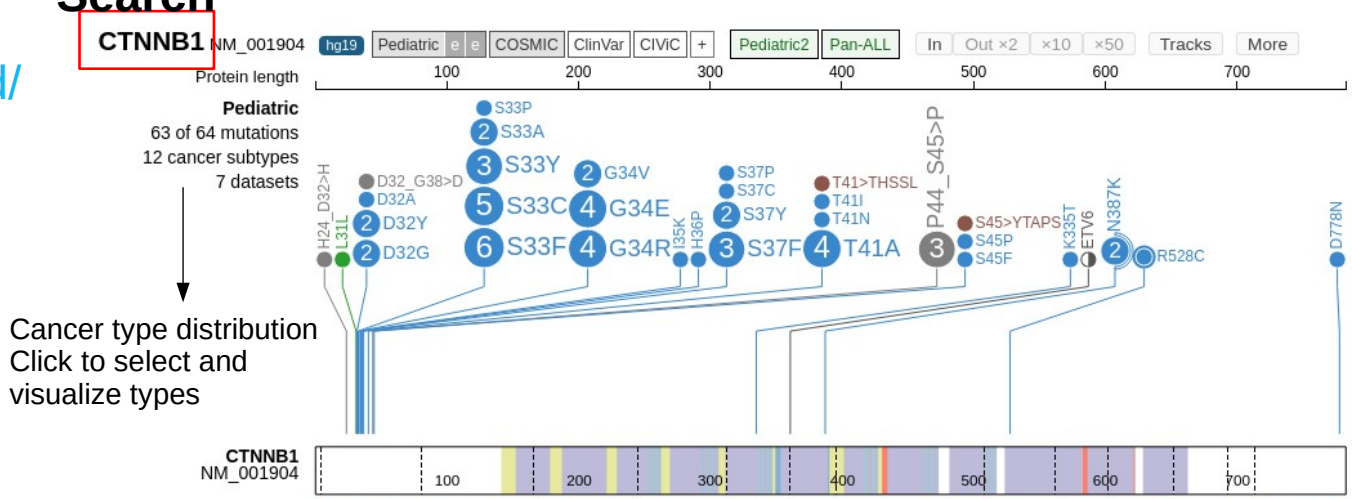
Exercise: IDPs and cancer mutations

https://pecan.stjude.cloud/variants/proteinpaint

Or https://pecan.stjude.cloud/
> Click in Variants (bottom-left);
> Click in “Explore Variants”;
> Then you search the tab “PROTEINPAINT”.

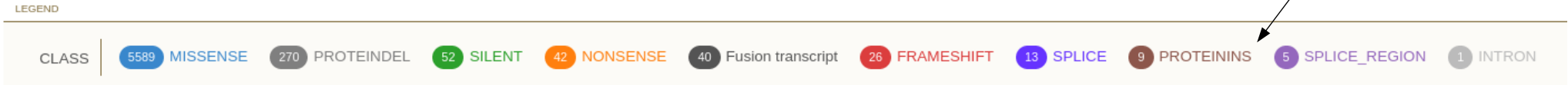
Search



Cancer type distribution
Click to select and visualize types

Proteinpaint a webtool to explore pediatric and adult cancer mutations.

You can click on these to filter the results



Exercise: IDPs and cancer mutations

- 4. Go on PECAN at https://pecan.stjude.cloud/variants/proteinpaint and search for beta-catenin (CTNNB1).Where in the sequence (residue number) are most of the mutations located in pediatric cancers?
- 5. In what type of cancer are the mutations common?
- 6. Turn on COSMIC (Catalogue Of Somatic Mutations In Cancer) mutations. In what location are mutations most common?
- 7. Go on DIBS (Database of Disordered Binding Sites) at http://dibs.enzim.ttk.mta.hu/search.php and search for beta-catenin (P35222). Which entry contains the mutated region?
- 8. Highlight the most mutated positions in the structure. Are they structured? What evidence is there for their status (ordered/disordered)?
- 9. What is the binding partner?

https://take.quiz-maker.com/QJRLDKS59

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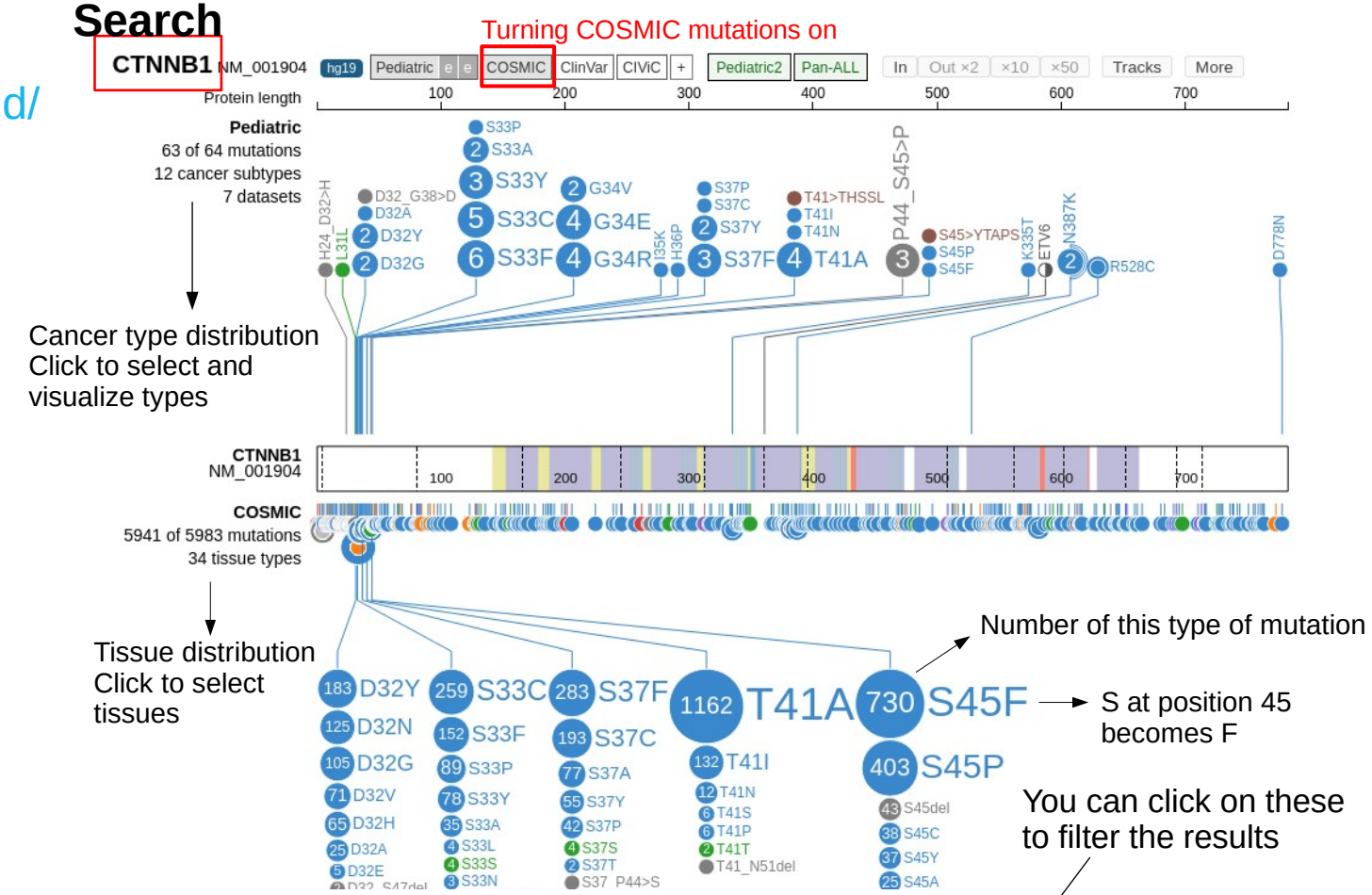

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- 9. Click in Evidence in the left menu. Can you identify which is the binding partner? (If you visualize the PDB structure on the top right, you will see that only one of the partners actually interact with beta-catenin – “orange ribbon”)

https://take.quiz-maker.com/QJRLDKS59

Exercise: IDPs and cancer mutations

DIBs: Database of Disordered Binding Sites

http://dibs.enzim.ttk.mta.hu/search.php

>Go to PDB 1p22
>Find the chain corresponding to P35222.

General Information

Function and Biology

Structure Summary

- Chain A
- Chain B

Evidence

Related Structure(s)

Click to go to evidence for structural status

Domain Type:
Bromodomain

Structure Summary

Entry contents: 2 distinct polypeptide molecules

Chains: A, B

Notes: *No modifications of the original PDB file.*

Chain A

Structural status

Name: Cellular tumor antigen p53 **Disordered** **Confirmed**

Source organism: *Homo sapiens*

Length: 20 residues

Sequence: SHLKSKKGQSTSRHKKLMFK

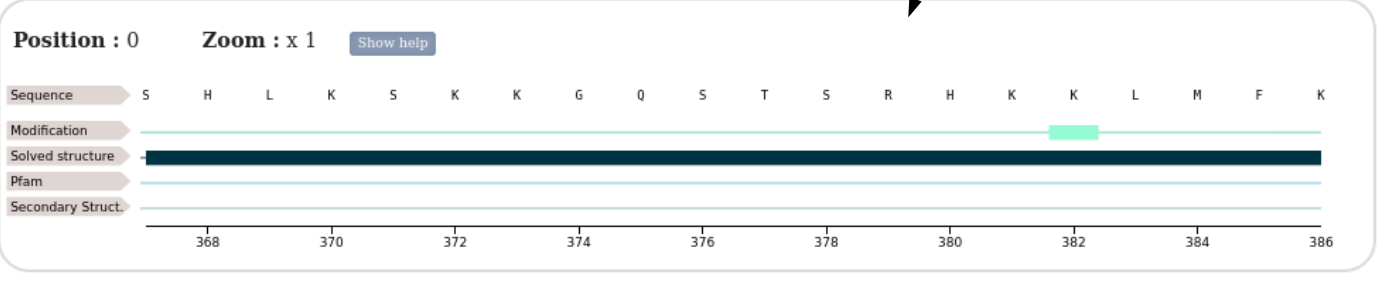
The sequence contains the following modified/non-standard residues:

- N(6)-acetyllysine (K) at position 382 (PDB position: 382)

UniProtKB AC: P04637 (positions: 367-386) **UniProt** Coverage: 5.1%

UniRef90 AC: UniRef90_P04637 (positions: 367-386) **UniRef90**

Residues of this chain involved in binding



Chain B

Name: CREB-binding protein **Ordered**

Source organism: *Homo sapiens*

Length: 121 residues

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References

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