PART II.
Prediction of functional regions within disordered proteins

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Large-scale analysis of IDPs

Possible through prediction methods

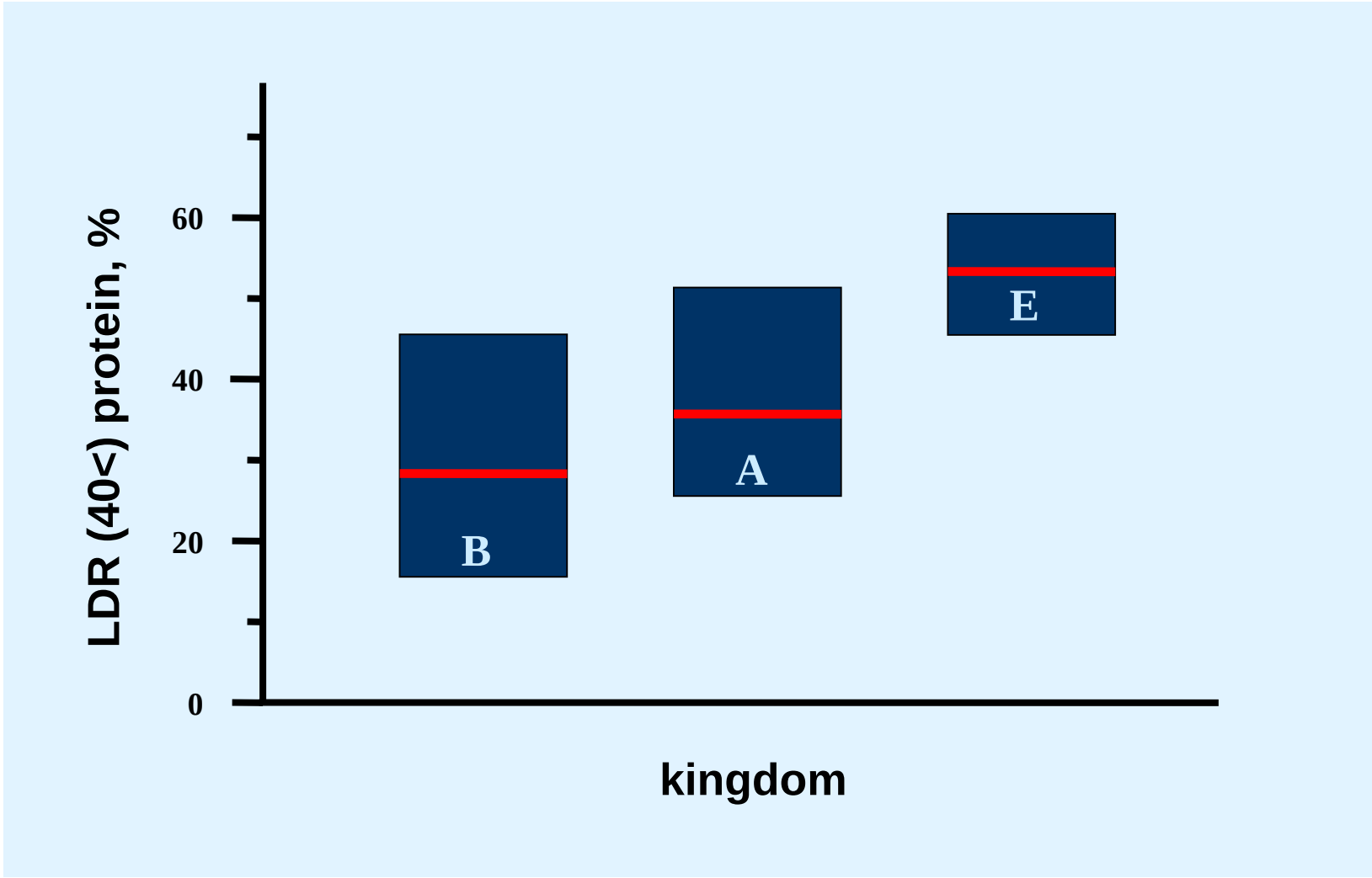
- Functional properties
- Evolutionary properties

- Disorder characterization
  - Percentage of properties with long (>30 or >40 aa)
  - Percentage of disordered residues

How common is protein disorder?

- Around 50% of human proteins have long disordered regions
- Around 30% of residues in the human proteome are predicted as disordered
- Disorder content increases with evolutionary complexity

Protein disorder is prevalent



Protein disorder complements the functional repertoire of globular proteins

Table 2. Correlation and anticorrelation of structural disorder with Swiss-Prot functional categories

Top functions that correlate with long disorder[a]	Top functions that anticorrelate with long disorder
Differentiation	GMP biosynthesis
Transcription	Amino acid biosynthesis
Transcription regulation	Transport
Spermatogenesis	Electron transport
DNA condensation	Lipid A biosynthesis
Cell cycle	Aromatic hydrocarbons catabolism
mRNA processing	Glycolysis
mRNA splicing	Purine biosynthesis
Mitosis	Pyrimidine biosynthesis
Apoptosis	Carbohydrate metabolism
Protein transport	Branched-chain amino acid biosynthesis
Meiosis	Lipopolysaccharide biosynthesis

Xie H et al. J Proteome Res. 2007, 6, 1882

Functions of intrinsically disordered proteins

I Entropic chains

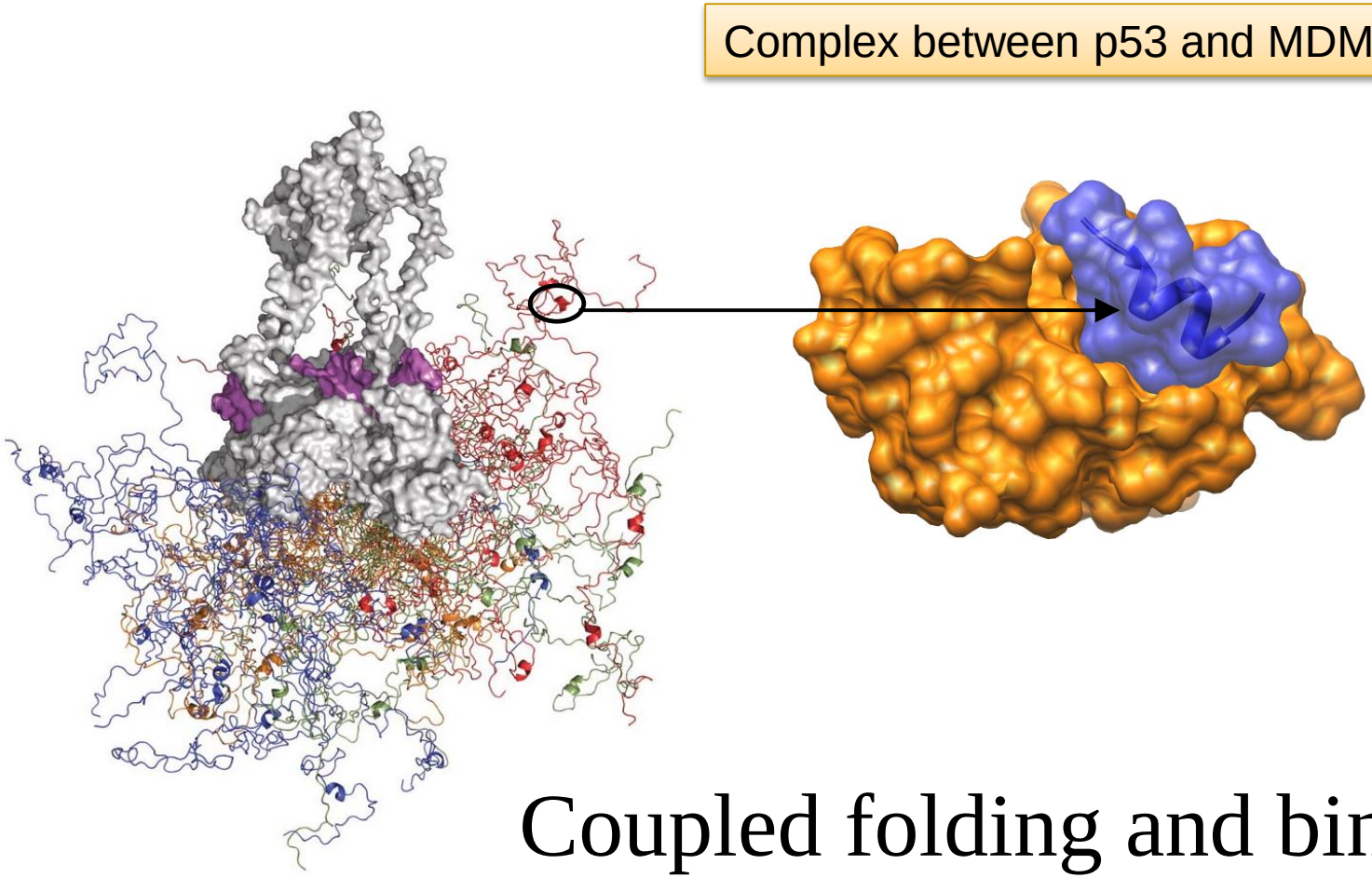
II Linkers

III Molecular recognition

IV Protein modifications (e.g. phosphorylation)

V Assembly of large multiprotein complexes

Interaction of IDPs

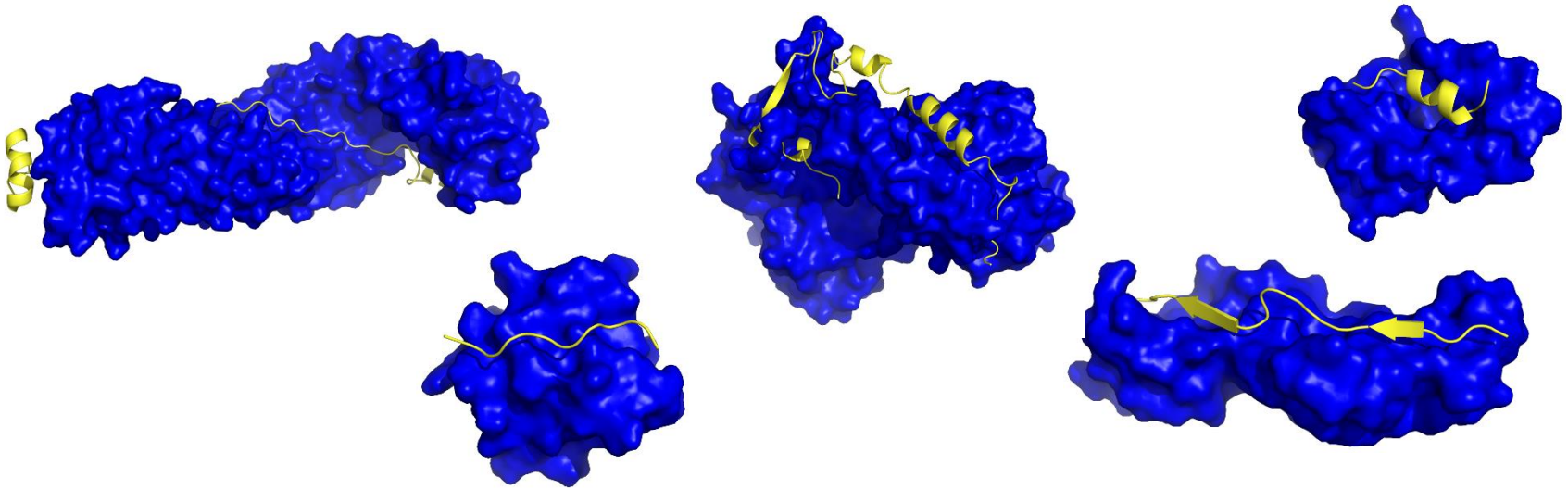


Coupled folding and binding

Coupled folding and binding

- Functional advantages
  - Weak transient, yet specific interactions
  - Post-translational modifications
  - Flexible binding regions that can overlap
  - Evolutionary plasticity

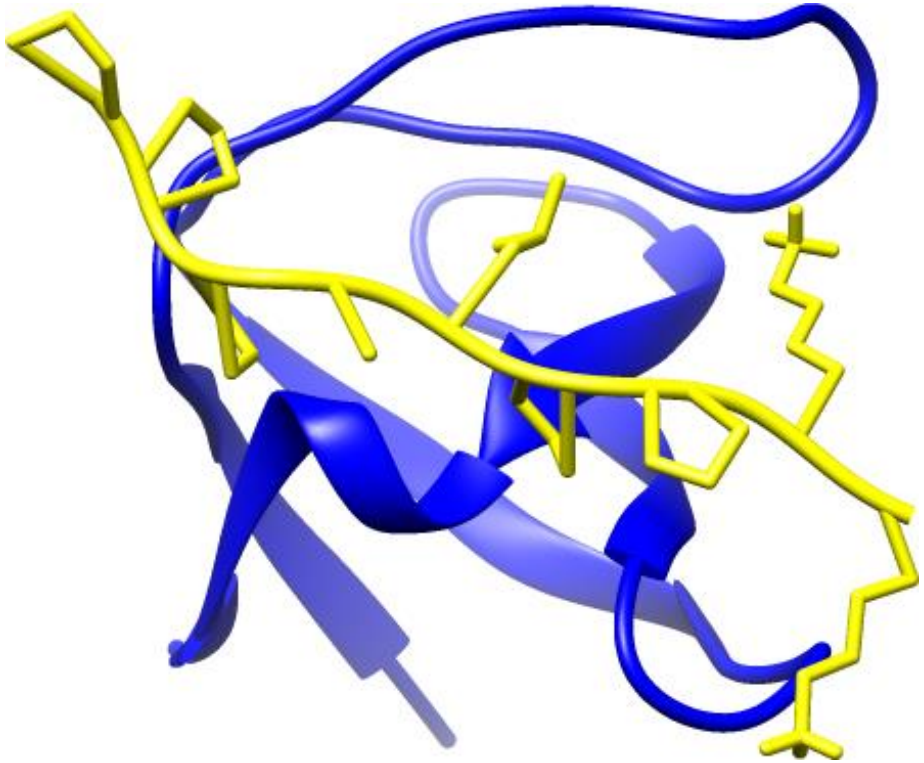
Various complexes of IDPs



- Can be grouped according the adopted secondary structure elements
  - alpha helical
  - beta strand
  - polyproline
  - irregular

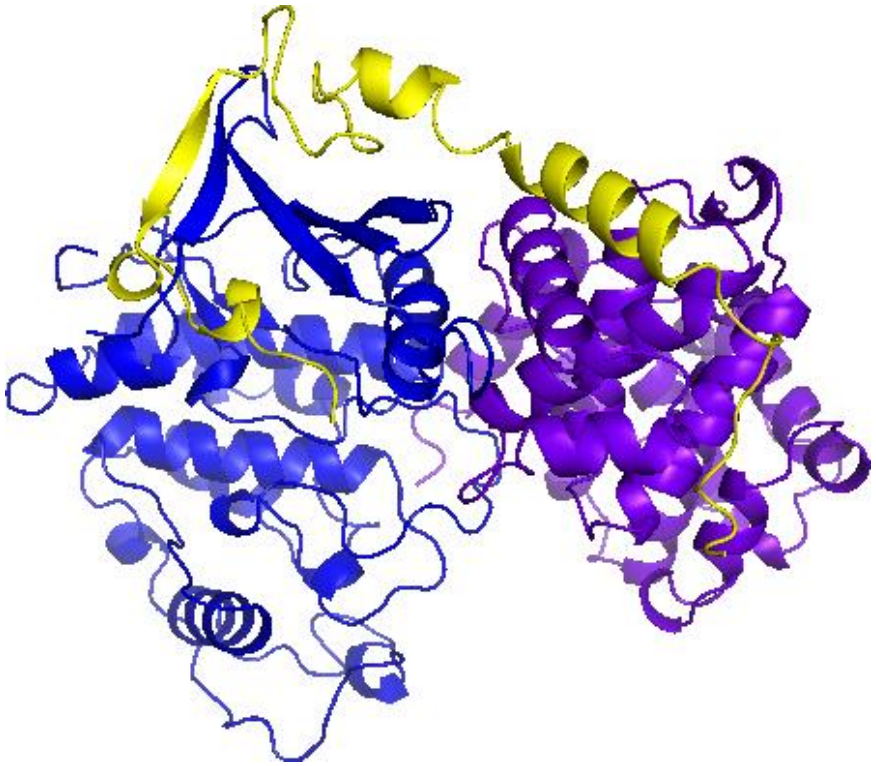
Small interfaces

SH3 domain

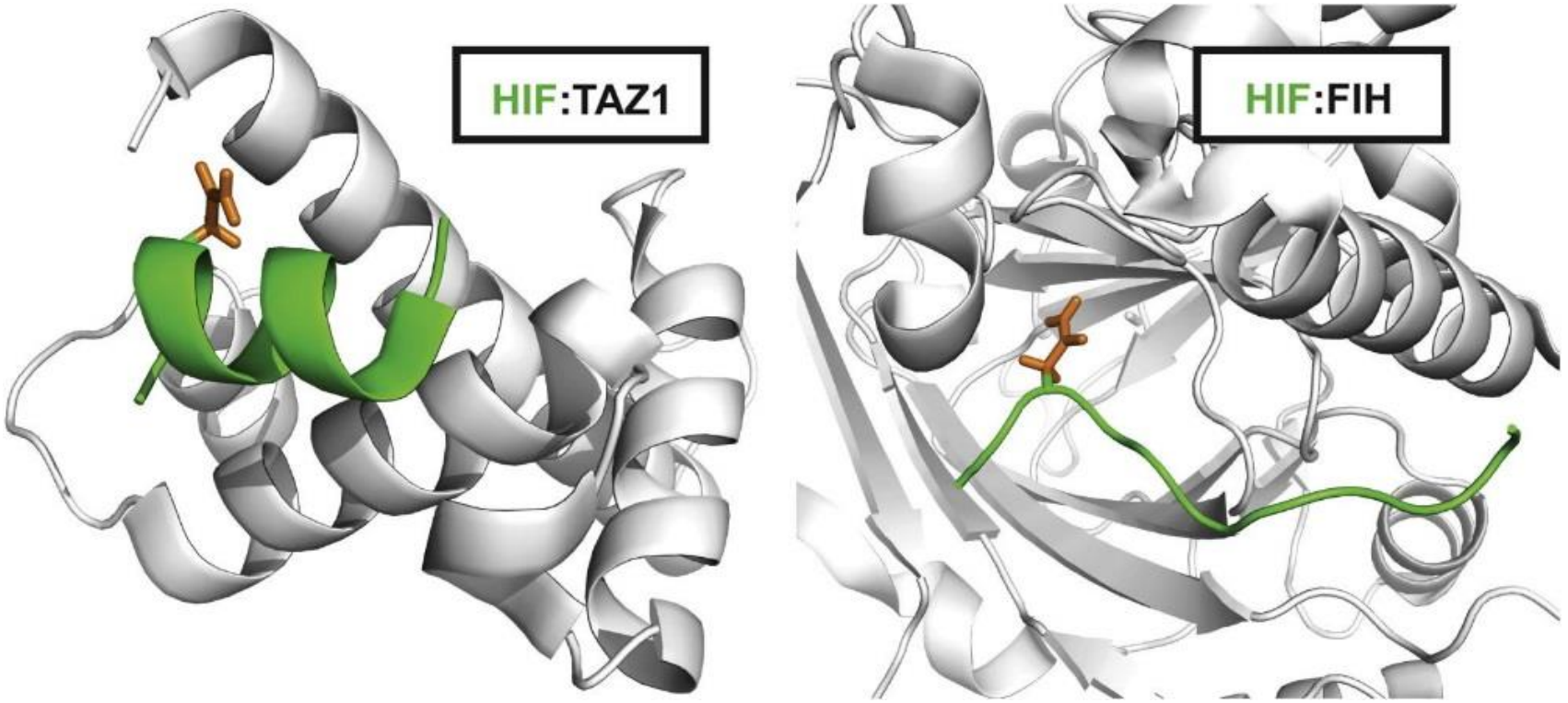


Large interfaces

- Cyclin-dependent kinase (Cdk) inhibitor, p27Kip1 (p27)
- Binds to cdk-cyclin komplex and inhibits their activity
- Fully disordered protein



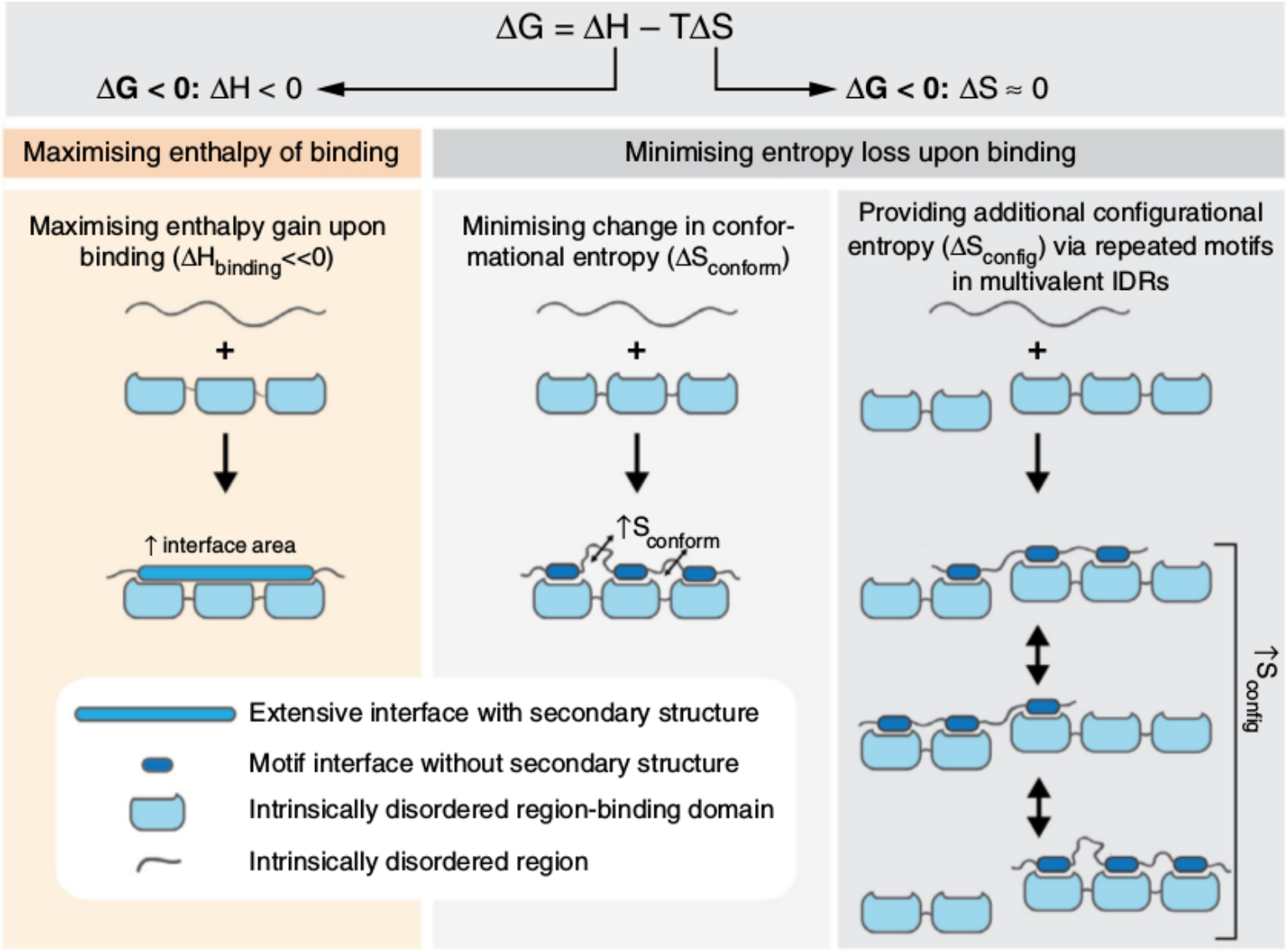
Conformational plasticity



C-terminal transactivation domain (CTAD) of the hypoxia inducible factor-1α

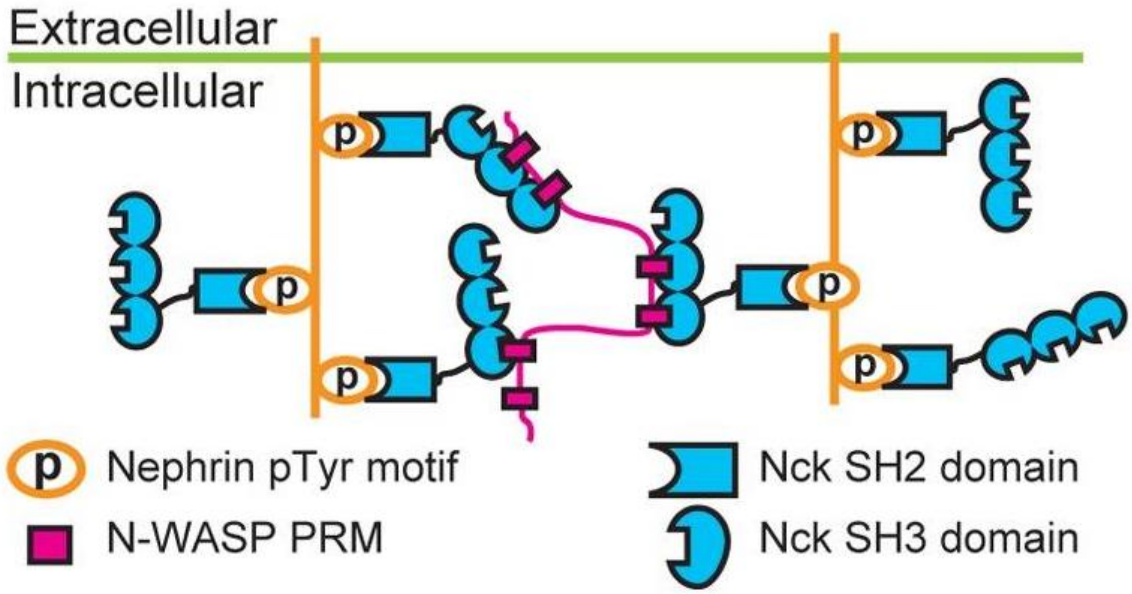
Berlow et al. FEBS Lett. 2015;589:2433

Fine tuning the entropic component



Flock et al Curr Opin Struct Biol. 2014; 26:62

Phase transitions



- Multivalency and weak interactions
- Regulated by phosphorylation
- Transition from small complexes and large, dynamic supramolecular polymers.

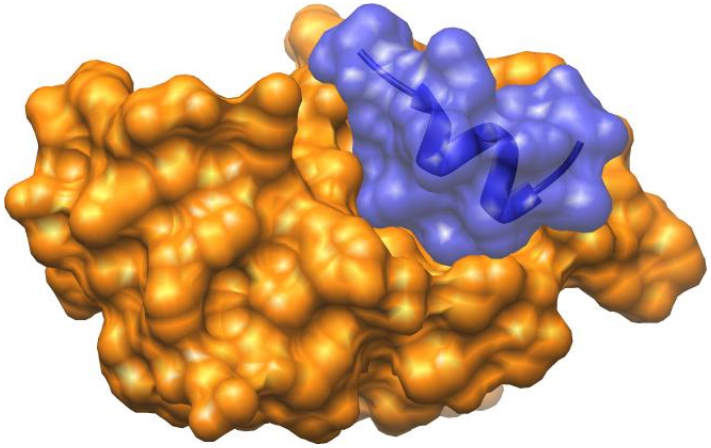
Li et al. Nature. 2012 483:336

Disordered binding regions

- Complexes of IDPs in the PDB: ~ 200
- Known instances: ~ 2 000
- Estimated number of such interactions in the human proteome: ~ 1 000 000

- Experimental characterization is very difficult
- Computational methods

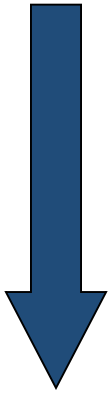
Disordered protein complexes



Complex between p53 and MDM2

- Interaction sites are usually ***linear*** (consist of only 1 part)
- enrichment of interaction prone amino acids

Sequence



Binding sites

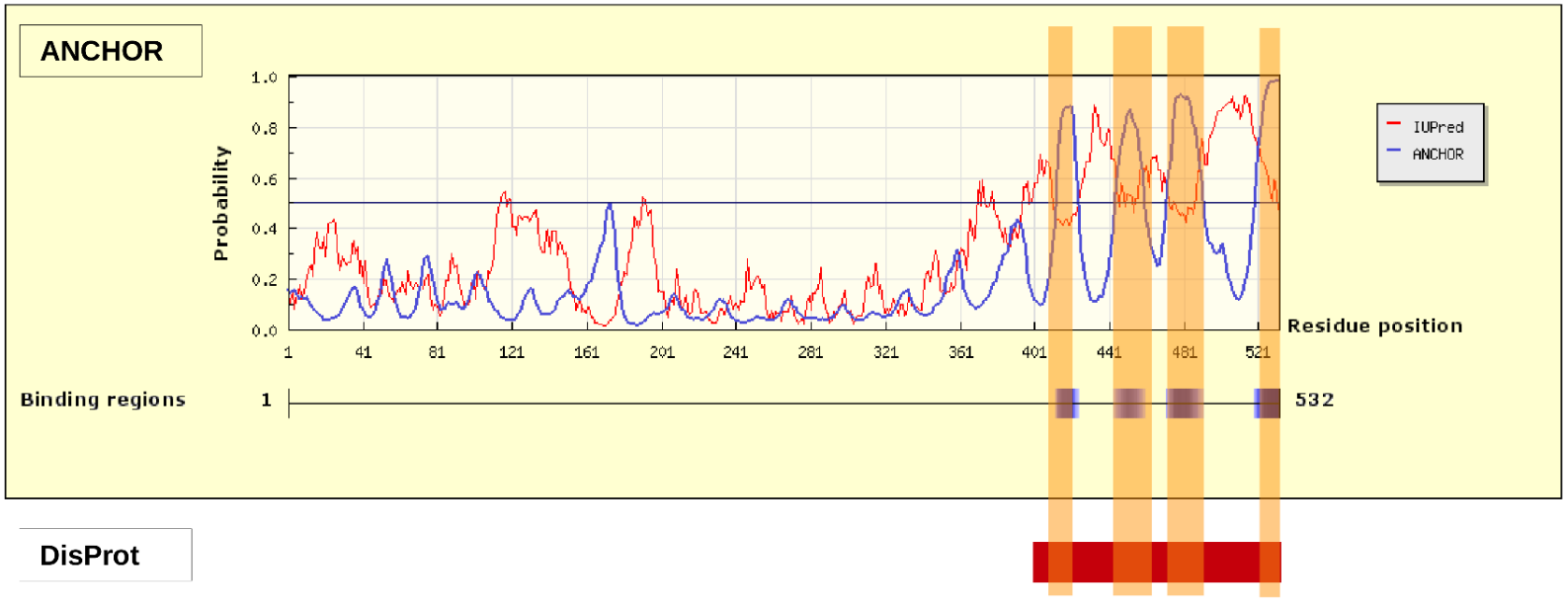
No need for structure, binding sites can be predicted from sequence alone

Prediction of disordered binding regions – ANCHOR

- What discriminates disordered binding regions?
  - A cannot form enough favorable interactions with their sequential environment
  - It is favorable for them to interact with a globular protein
- Based on simplified physical model
  - Based on an energy estimation method using statistical potentials
  - Captures sequential context

ANCHOR

nucleoprotein from Nipah virus (DP00697)



Dosztanyi et al. Bioinformatics. 2009;25:2745

Machine learning approaches

MORFchibi

- Uses two SVMs
  - One recognizes the different amino acid composition of flanking regions compared to the binding region
  - One recognizes the similarity to known binding regions
- trained on short chains in complex

Malhis N, Gsponer J. Bioinformatics. 2015; 31:1738

Machine learning approaches

DISOPRED3

- Uses three SVMs
  - Simple sequence profile
  - PSI-Blast profiles (very slow)
  - PSI-Blast profiles with global features
- trained on short chains in complex

Jones DT, Cozzetto D. Bioinformatics. 2015; 31:857

Amount of disordered binding regions

- What is the amount of disordered protein regions in the human proteome?
  - ANCHOR: 93429
  - MORFchibi: 275013
  - DISOPRED3: 63848
- We cannot tell what is the *false positive rate* of these methods

Conservation

The functionality of a protein segment is often approached by investigating the evolutionary history of its primary sequence

Can this approach used for disordered proteins?

Sometimes …

Constrained and flexible disorder

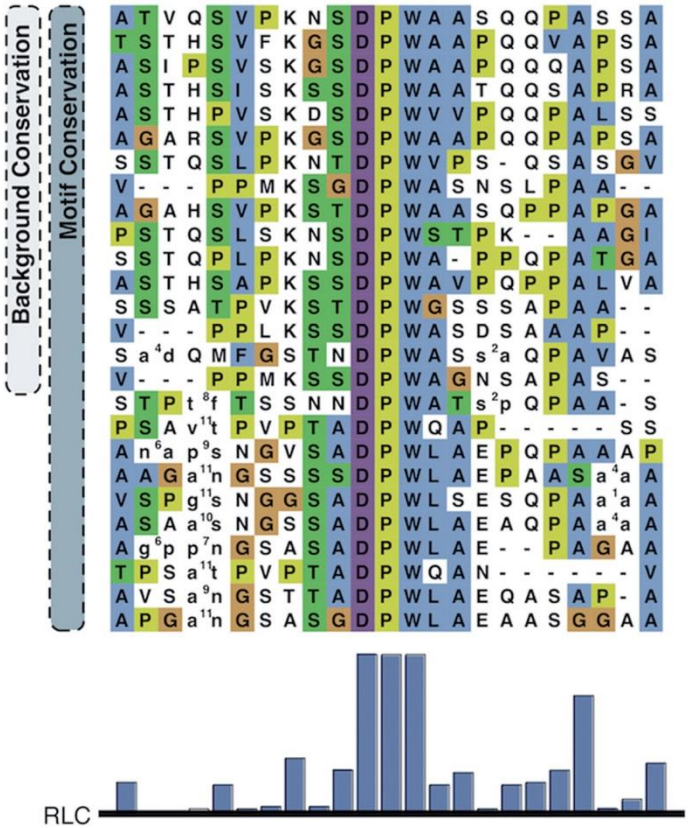
- ‘constrained’,
  - if both features (amino acid sequence and the property of disorder) are conserved
- ‘flexible’,
  - if only disorder is conserved
- ‘non-conserved’ positions
  - where disorder is not conserved

DISCONS novel server (preliminary)

Bellay et al. Genome Biol. 2011;12:R14
Varadi et al. BMC Bioinformatics. 2015;16:153

Conservation patterns of linear motifs

- No evolutionary constraints to keep the structure
- Strong constraints on functional site



C

Island-like conservation

Davey et al. Nucleic Acids Res. 2012; 40:10628

SlimPrints

- Generates sequence alignments of orthologous sequences
- Relative conservation score per position
- Filters out less reliable regions
- Fails if sequences are too divergent, or too similar
- http://bioware.ucd.ie/slimprints.html.

The next challenge:

- Characterizing the ensemble of conformations of IDPs
- And their relationship with function

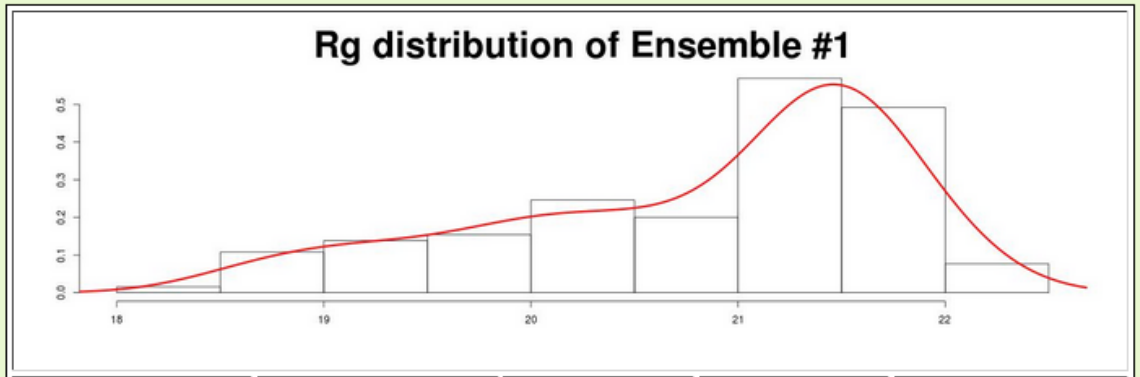
Ensemble characterization for IDPs

- Experimental methods cannot detect a single conformation, only time or ensemble averages

- Combination of methods are needed (NMR, SAXS)

- Methods are used to characterize
  - Radius of gyration
  - Transient secondary structure elements
  - Transient long range contacts

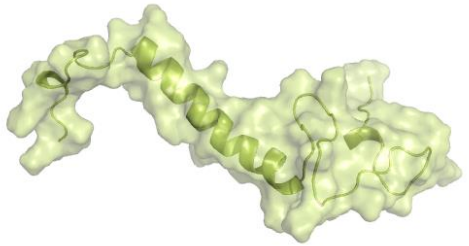
PED database

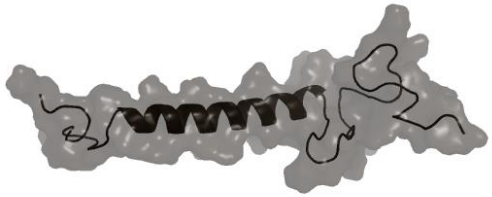


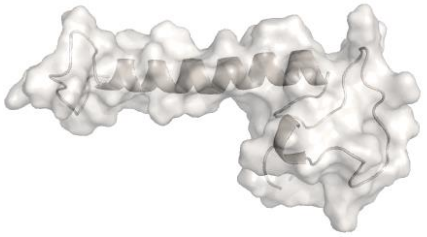
Ensemble index	# of conformers	Rg mean	Dmax mean	Show/Hide
1	130 conformers	20.81+-0.94 A	70.89+-4.49 A	Show List

Select a conformer from the list below to display it

Conformer	Rg	Dmax	Display
Conformation 1	Rg: 20.19	Dmax: 65.17	View conformer - Up
Conformation 2	Rg: 18.99	Dmax: 60.44	View conformer - Up
Conformation 3	Rg: 20.16	Dmax: 67.9	View conformer - Up







Varadi et al. Nucleic Acids Res. 2014 ;42:D326