Biological functions of IDRs

Bio5469 (Washington University in St. Louis)

Sept 16 2022

Alex Holehouse
alex.holehouse@wustl.edu

Protein function is driven by conformational behaviour

Protein function is driven by conformational behaviour

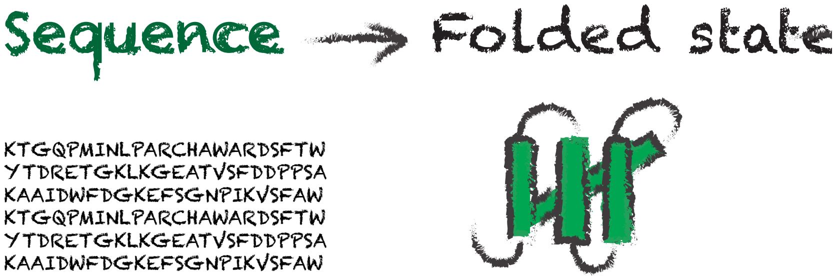
Folded domains

Sequence

KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW
KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW

Protein function is driven by conformational behaviour

Folded domains

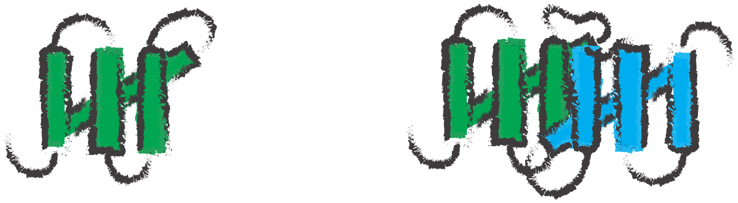


Protein function is driven by conformational behaviour

Folded domains

Sequence → Folded state → Function

KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW
KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW



Structure & dynamics

Protein function is driven by conformational behaviour

Folded domains

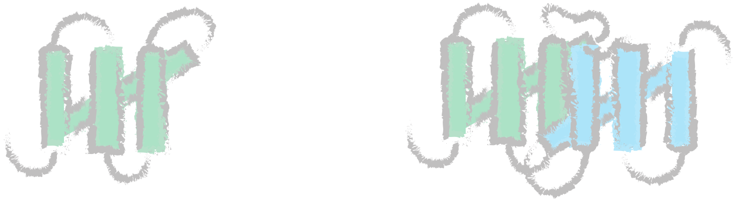
Intrinsically disordered regions

Protein function is driven by conformational behaviour

Folded domains

Sequence → Folded state → Function

KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW
KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW



Intrinsically disordered regions

Sequence

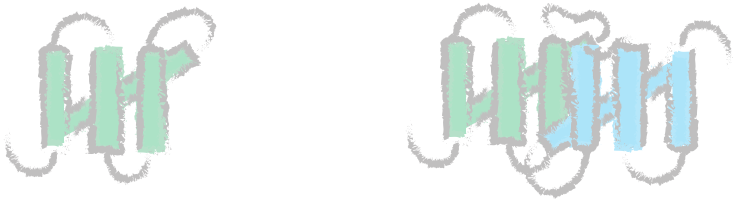
NECNQCKAPKPDGPGGGPGGSH
MGGNYGDDRRGGRGGYDRGG
YRGRGGDRGGFRGGRGGGDR
GGFGPGKMDSRGEHRQDRRERPY
NECNQCKAPKPDGPGGGPGGSHM
GGNYGDDRRGGRGGYDRGGY

Protein function is driven by conformational behaviour

Folded domains

Sequence → Folded state → Function

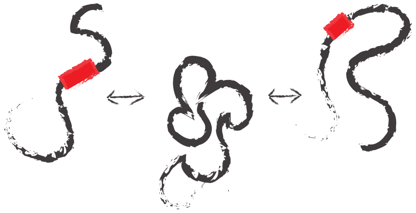
KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW
KTGQPMINLPARCHAWARDSFTW
YTDRETGKLKGEATVSFDDPPSA
KAAIDWFDGKEFSGNPIKVSFAW



Intrinsically disordered regions

Sequence → Ensemble

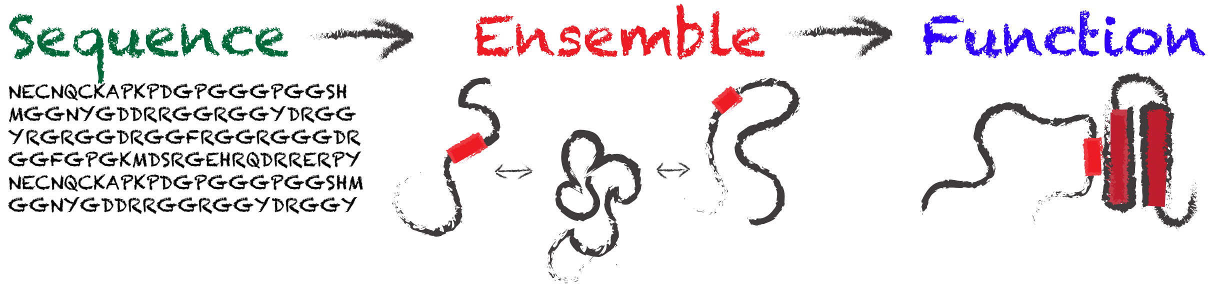
NECNQCKAPKPDGPGGGPGGSH
MGGNYGDDRRGGRGGYDRGG
YRGRGGDRGGFRGGRGGGDR
GGFGPGKMDSRGEHRQDRRERPY
NECNQCKAPKPDGPGGGPGGSHM
GGNYGDDRRGGRGGYDRGGY



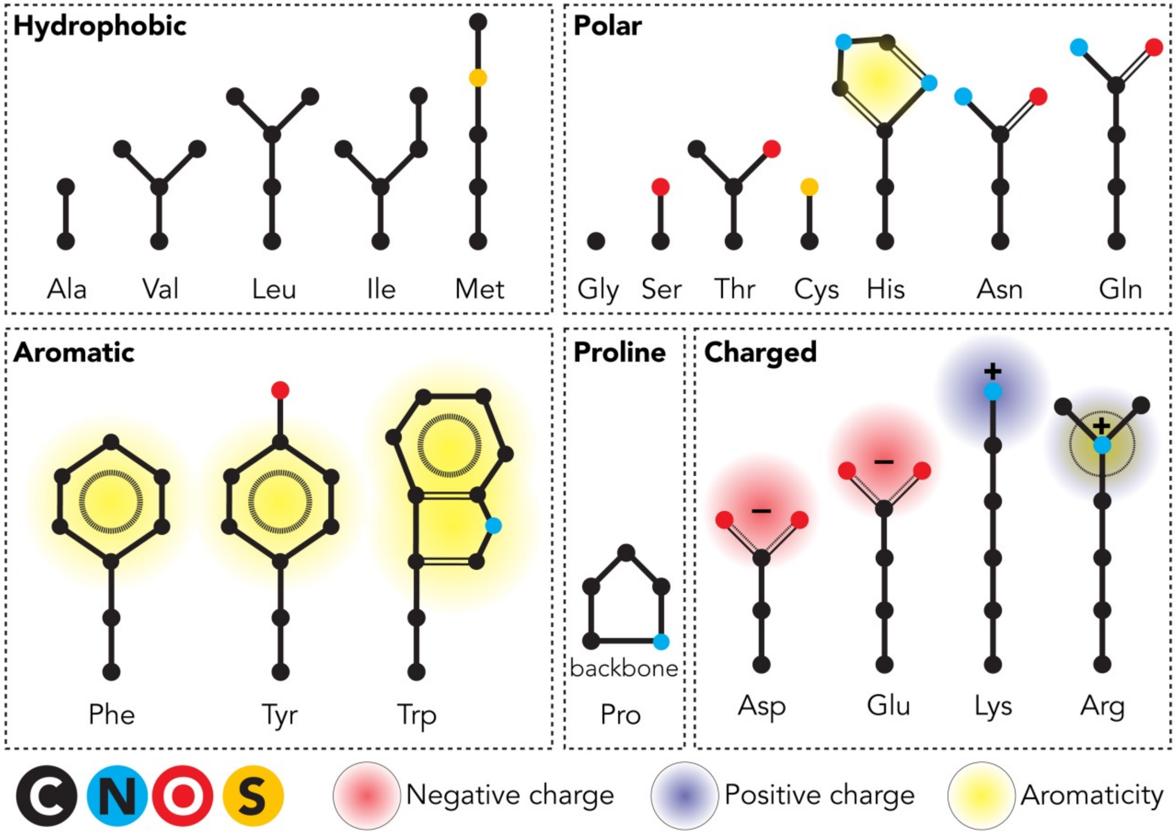
Protein function is driven by conformational behaviour

Folded domains

Intrinsically disordered regions

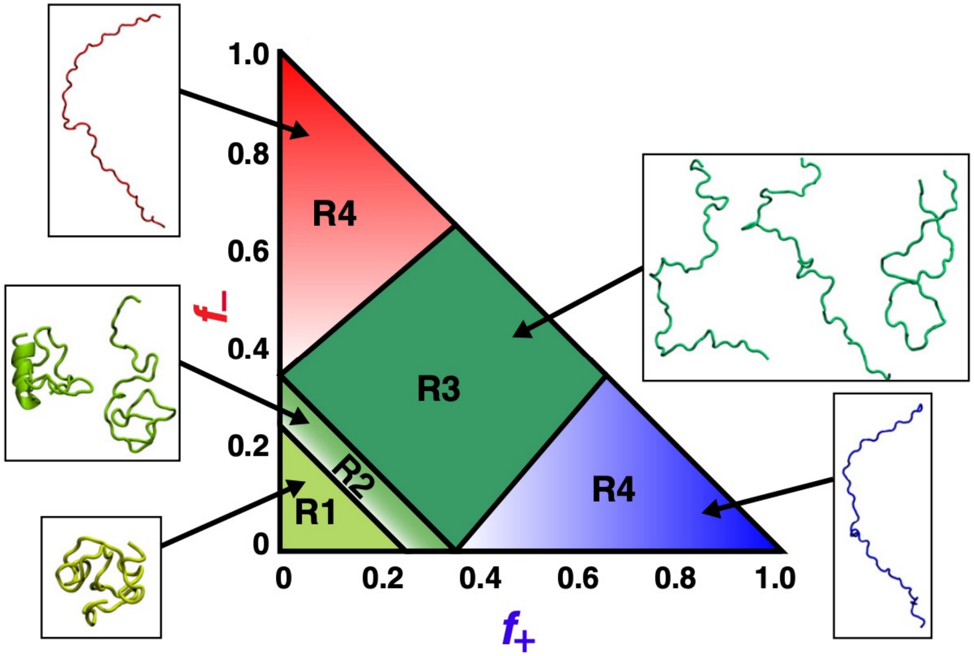


IDR amino acid chemistry



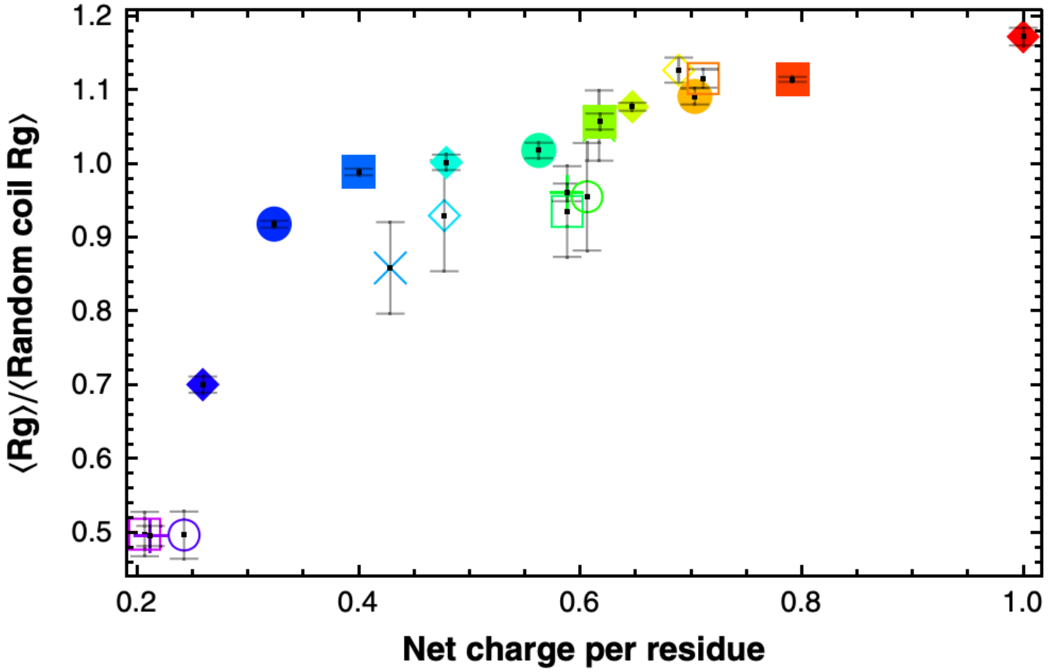
Martin & Holehouse **Emerg. Top. Life. Sci.** (2020)

Charge residues can dominate IDR dimensions



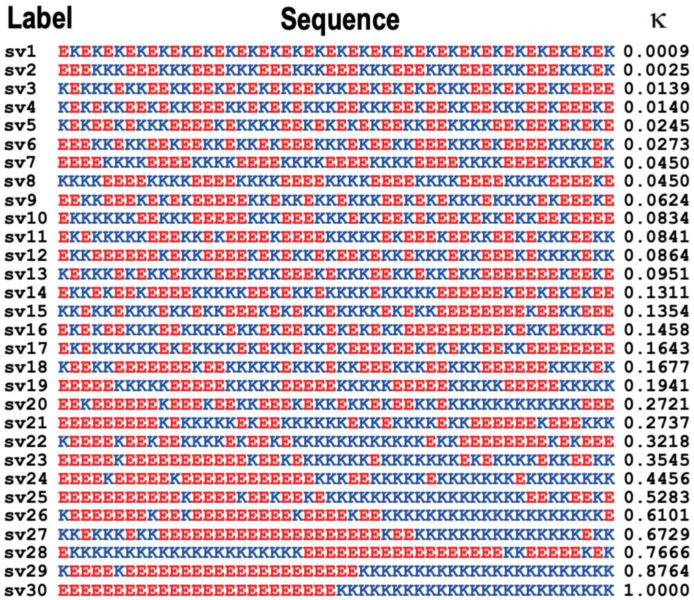
Das et al. ***COSB*** (2015)

Charge residues can dominate IDR dimensions

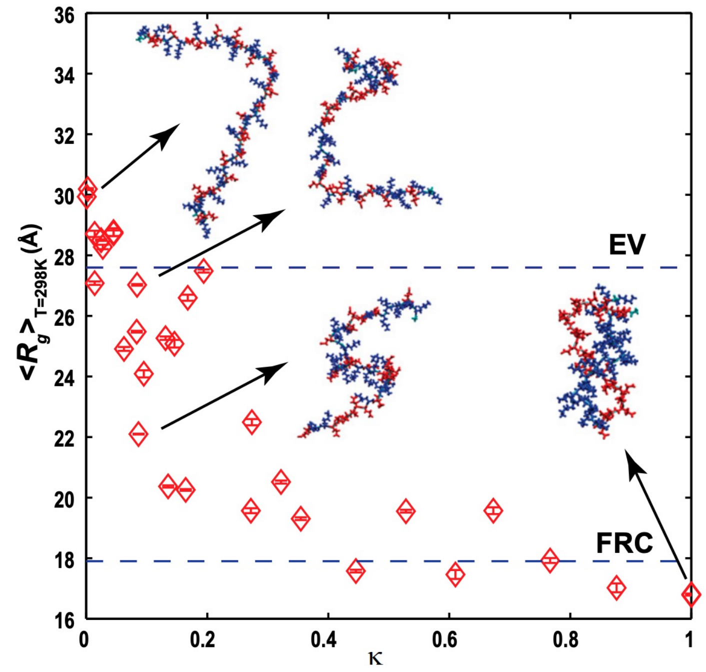


Mao et al. ***PNAS*** (2010)

Charge patterning can influence chain dimensions



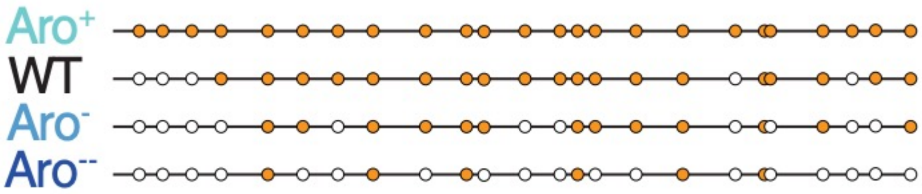
Label	Sequence	κ
sv1	EKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEKEK	0.0009
sv2	EEEKKKEEEKKKEEEKKKEEEKKKEEEKKKEEEKKKEEEKKKEEEKKKEK	0.0025
sv3	KEKKKEKKEEKKEEKEKEKEKEEKKKEEKEKEKEKKKEEKEKEEKKEEEE	0.0139
sv4	KEKEKKEEKEKKEEEKKEKEKEKKKEEKKKEEKEEKKEEKKKEEKEEEKE	0.0140
sv5	KEKEEKEKKKEEEEKEKKKKEEKEKEKEKEEKKEEKKKKEEKEEKEKEKE	0.0245
sv6	EEEKKEKKEEKEEKKEKKEKEEEKKKEKEEKKEEEKKKEKEEEEKKKKEK	0.0273
sv7	EEEEKKKKEEEEKKKKEEEEKKKKEEEEKKKKEEEEKKKKEEEEKKKKEK	0.0450
sv8	KKKKEEEEKKKKEEEEKKKKEEEEKKKKEEEEKKKKEEEEKKKKEEEEKE	0.0450
sv9	EEKKEEEKEKEKEEEEEKKEKKEKKEKKKEEKEKEKKKEKKKKEKEEEKE	0.0624
sv10	EKKKKKKEEKKKEEEEEKKKEEEKKKEKKEEKEKEEKEKKEKKEEKEEEE	0.0834
sv11	EKEKKKKKEEEKKEKEEEEKEEEEKKKKKEKEEEKEEKKEEKEKKKEEKK	0.0841
sv12	EKKEEEEEEKEKKEEEEKEKEKKEKEEKEKKEKKKEKKEEEKEKKKKEKK	0.0864
sv13	KEKKKEKEKKEKKKEEEKKKEEEKEKKKEEKKEKKEKKEEEEEEKEEEKE	0.0951
sv14	EKKEKEEKEEEEKKKKKEEKEKKEKKKKEKKKKKEEEEEEKEEKEKEKEE	0.1311
sv15	KKEKKEKKKEKKEKKEEEKEKEKKEKKKKEKEKKEEEEEEEEKEEKKEEE	0.1354
sv16	EKEKEEKKKEEKKKKEKKEKEEKKEKEKEKKEEEEEEEEEKEKKEKKKKE	0.1458
sv17	EKEKKKKKKEKEKKKKEKEKKEKKEKEEEKEEKEKEKKEEKKEEEEEEEE	0.1643
sv18	KEEKKEEEEEEEKEEKKKKKEKKKEKKEEEKKKEEKKKEEEEEEKKKKEK	0.1677
sv19	EEEEEKKKKKEEEEEKKKKKEEEEEKKKKKEEEEEKKKKKEEEEEKKKKK	0.1941
sv20	EEKEEEEEEKEEEKEEKKEEEKEKKEKKEKEEKKEKKKKKKKKKKKKEEE	0.2721
sv21	EEEEEEEEEKEKKKKKEKEEKKKKKKEKKEKKKKEKKEEEEEEKEEEKKK	0.2737
sv22	KEEEEKEEKEEKKKKEKEEKEKKKKKKKKKKKKEKKEEEEEEEEKEKEEE	0.3218
sv23	EEEEEKEEEEEEEEEEEKEEKEKKKKKKEKKKKKKKEKEKKKKEKKEEKK	0.3545
sv24	EEEEKEEEEEKEEEEEEEEEEEEKKKEEKKKKKEKKKKKKKEKKKKKKKK	0.4456
sv25	EEEEEEEEEEEKEEEEKEEKEEKEKKKKKKKKKKKKKKKKKKEEKKEEKE	0.5283
sv26	KEEEEEEEKEEKEEEEEEEEEKEEEEKEEKKKKKKKKKKKKKKKKKKKKE	0.6101
sv27	KKEKKKEKKEEEEEEEEEEEEEEEEEEEEKEEKKKKKKKKKKKKKKKEKK	0.6729
sv28	EKKKKKKKKKKKKKKKKKKKKKEEEEEEEEEEEEEEEEEEKKEEEEEKEK	0.7666
sv29	KEEEEKEEEEEEEEEEEEEEEEEEEEEKKKKKKKKKKKKKKKKKKKKKKK	0.8764
sv30	EEEEEEEEEEEEEEEEEEEEEEEEEKKKKKKKKKKKKKKKKKKKKKKKKK	1.0000



Das et al. ***PNAS*** (2013)

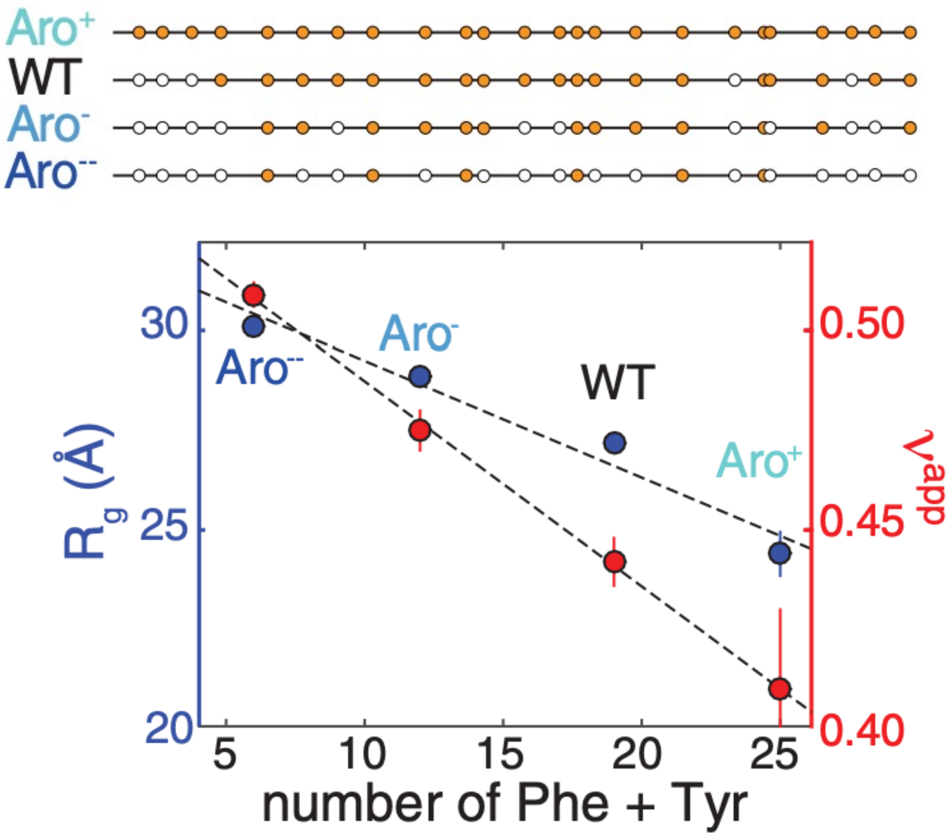
Hydrophobic/aromatic residues are “sticky”

Hydrophobic/aromatic residues are “sticky”



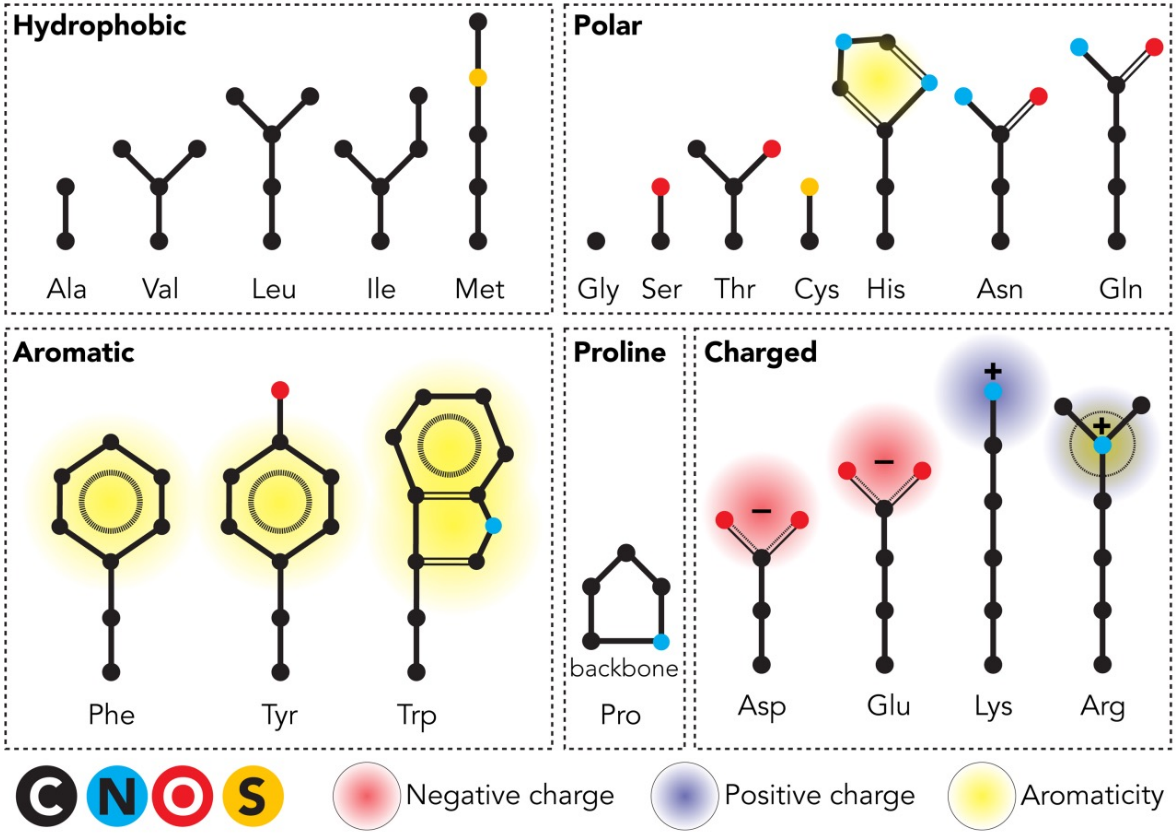
Martin/Holehouse/Peran
et al. ***Science*** (2020)

Hydrophobic/aromatic residues are “sticky”



Martin/Holehouse/Peran
et al. ***Science*** (2020)

Proline residues drive chain expansion

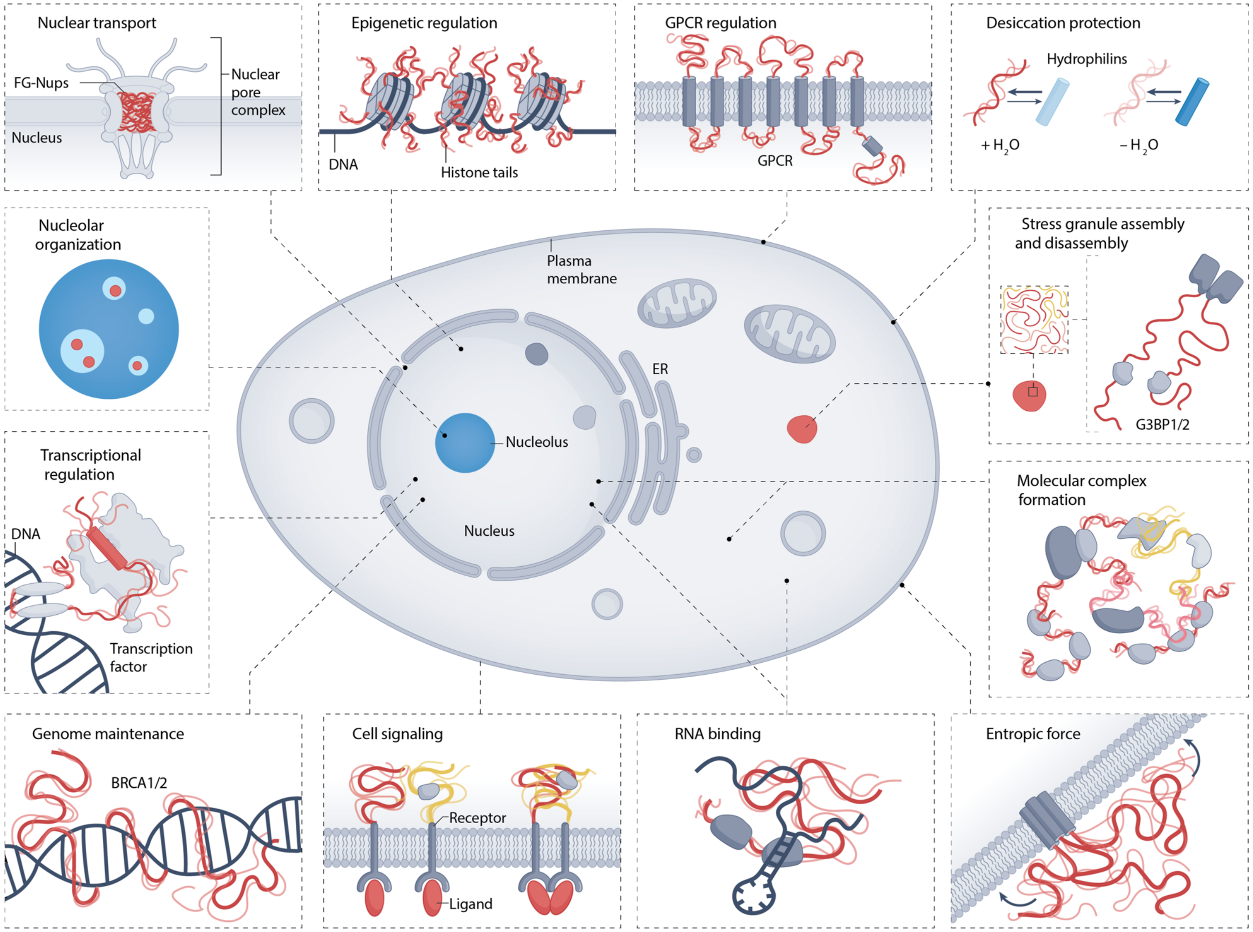


Proline is Inflexible

(imino acid)

Molecular functions of IDRs

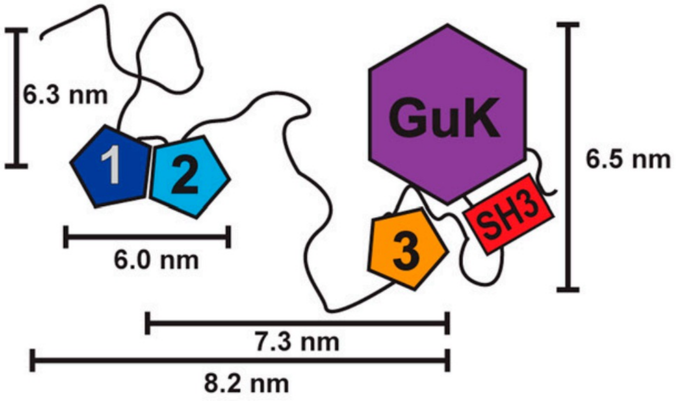
Molecular functions of IDRs



Holehouse & Kragelund ***Nat. Rev. Mol. Cell. Biol*** (2023)

1. Protein organization and structure

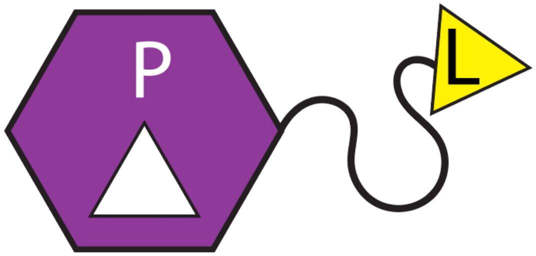
1. Protein organization and structure



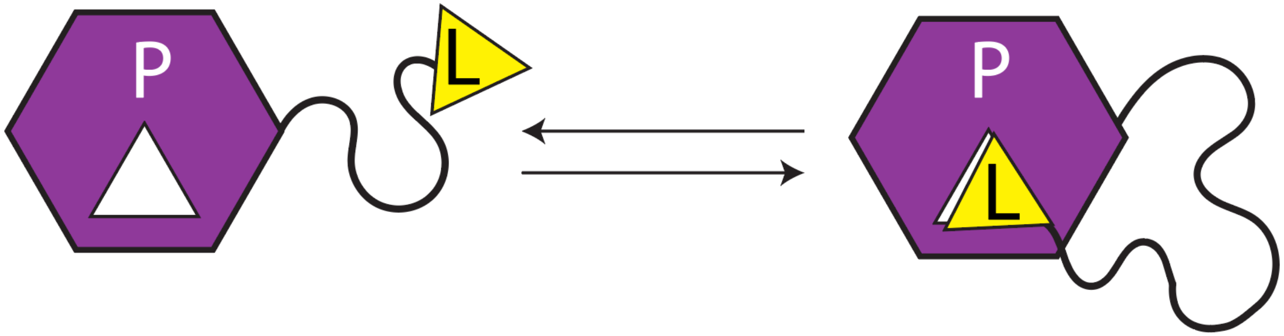
IDRs can determine relative position and flexibility of folded domains

McCann et al. ***PNAS*** (2012)

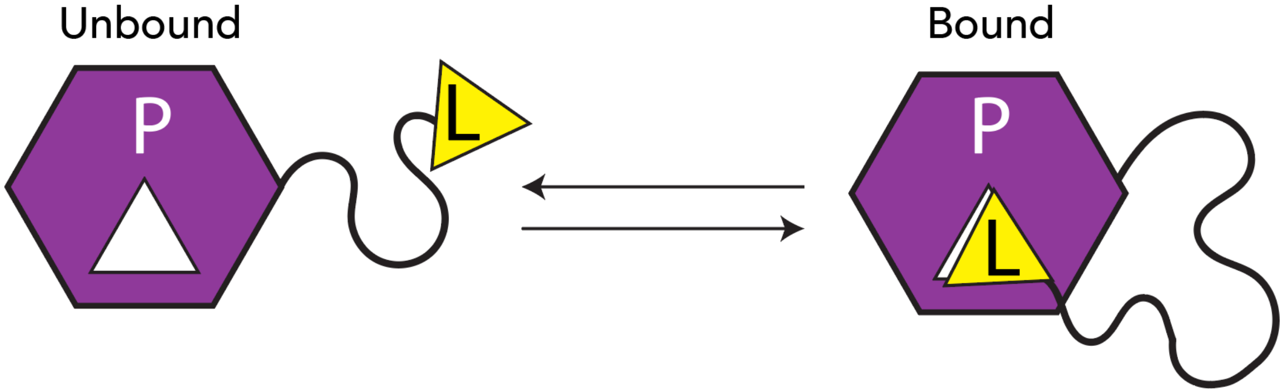
1. Protein organization and structure



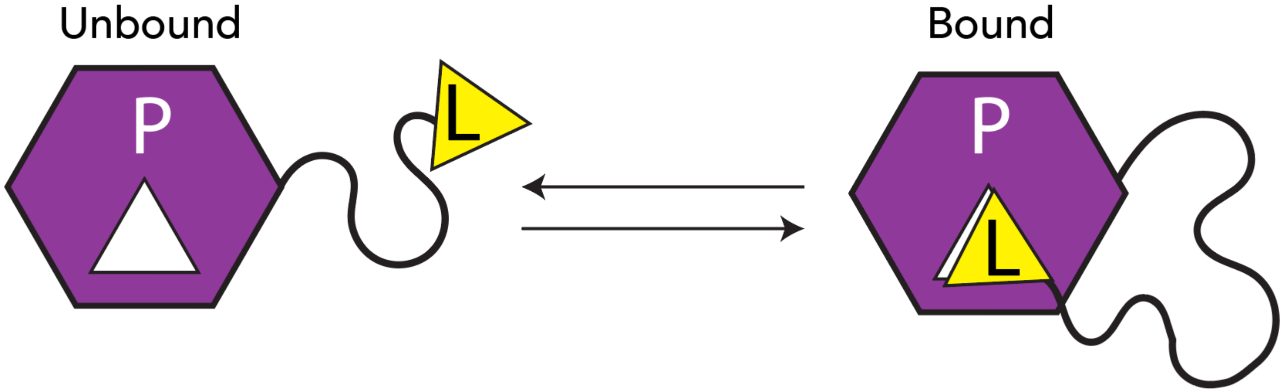
1. Protein organization and structure



1. Protein organization and structure

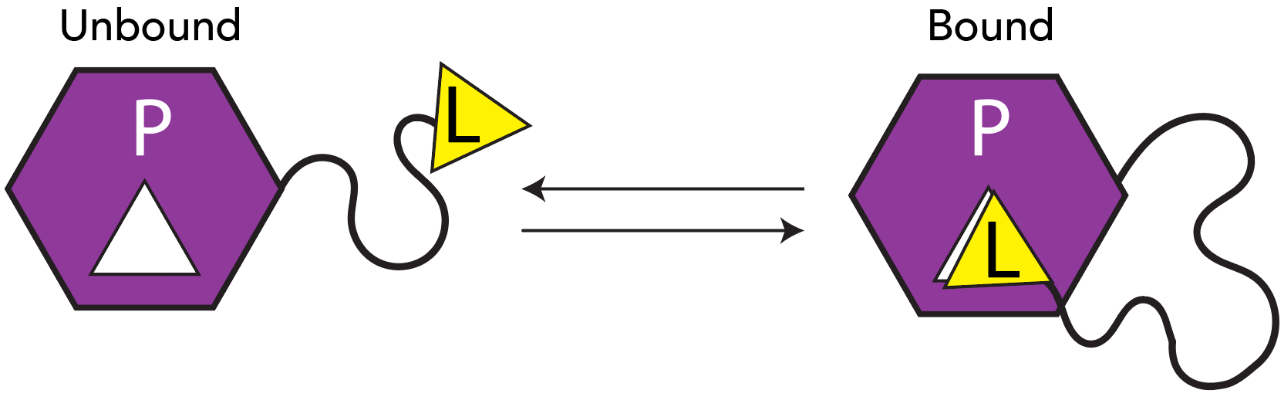


1. Protein organization and structure



P + L ← → PL

1. Protein organization and structure

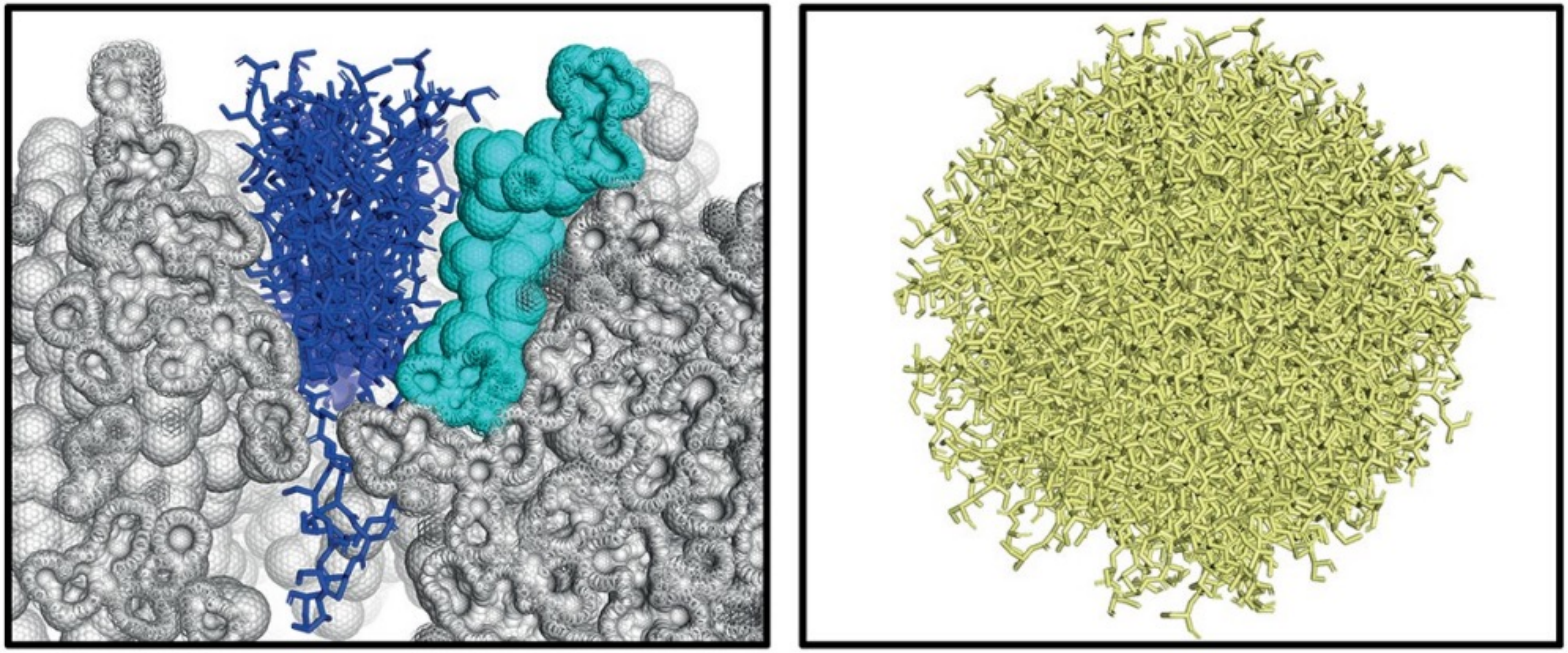


P + L ← → PL

IDRs can tune the **effective concentration** of inter-domain interactions

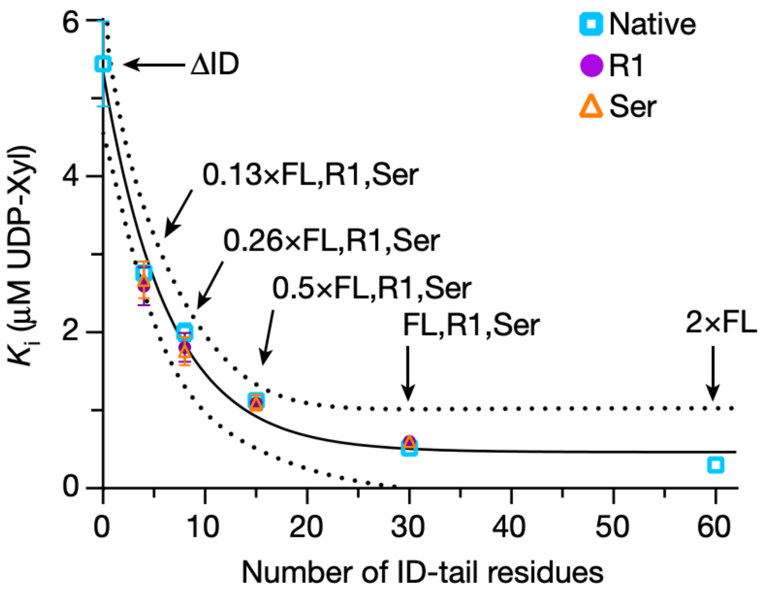
2. Entropic force generation

2. Entropic force generation



Keul et al. ***Nature*** 2018

2. Entropic force generation

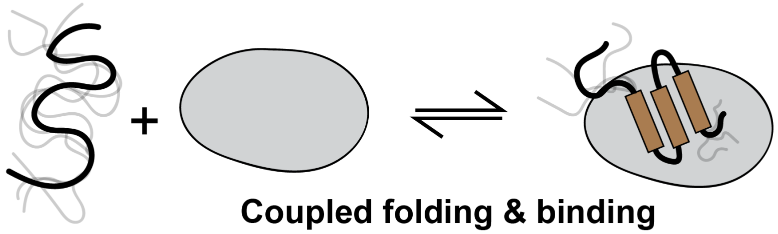


Keul et al. ***Nature*** 2018

3. IDRs in molecular recognition

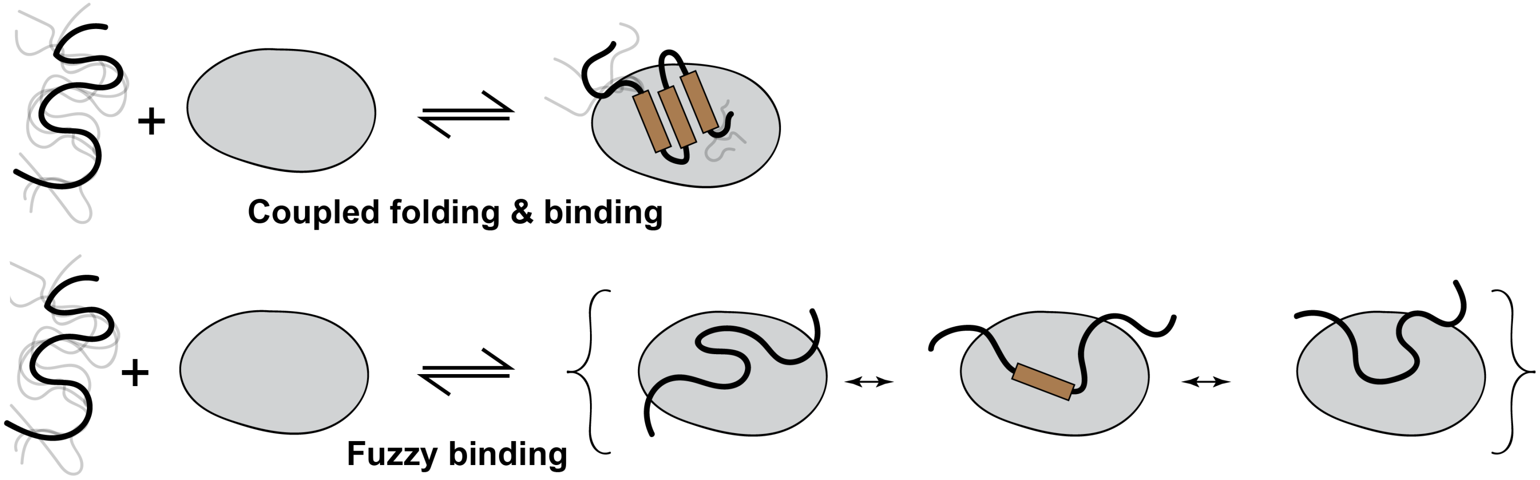
IDR-mediated interact via three “distinct” modes of binding

IDR-mediated interact via three “distinct” modes of binding



Coupled folding & binding

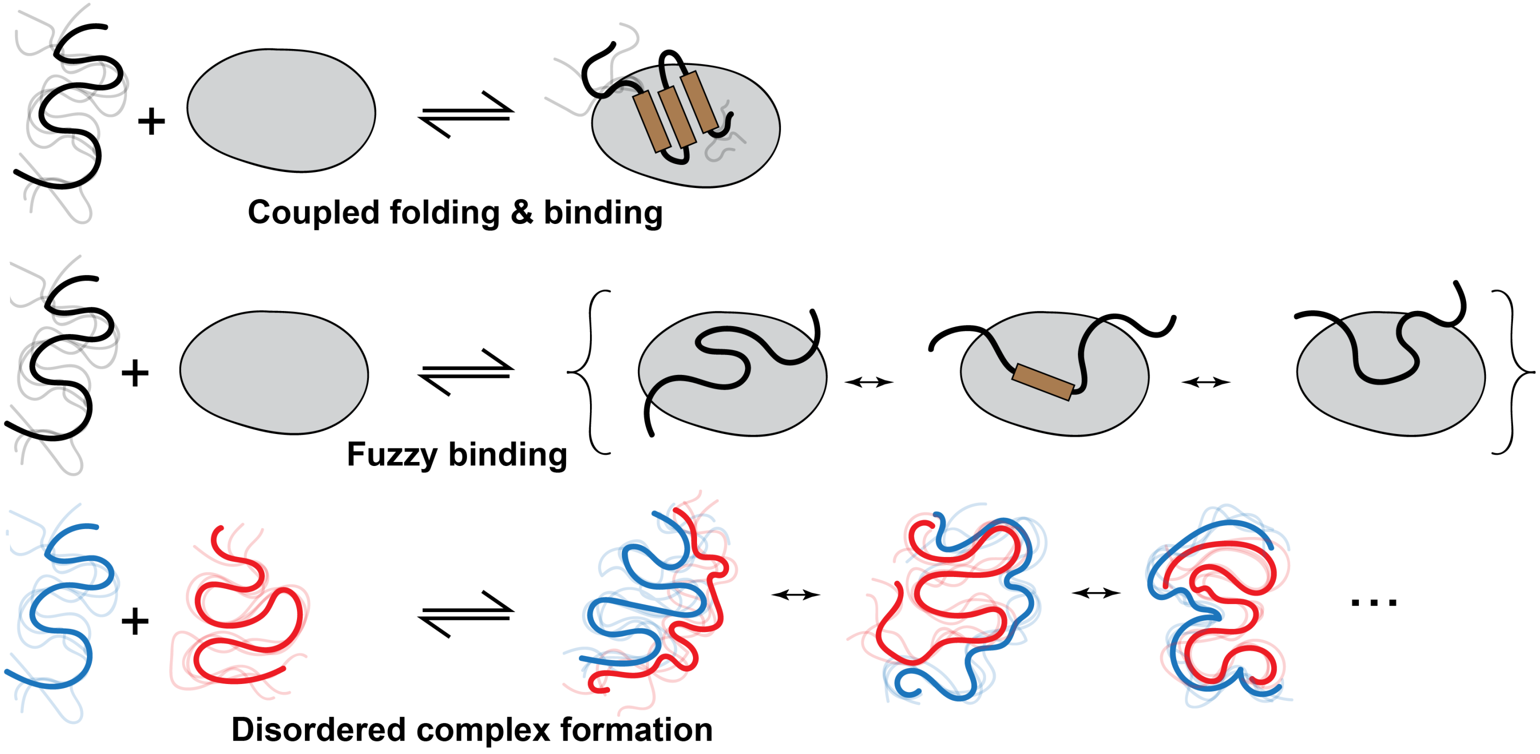
IDR-mediated interact via three “distinct” modes of binding



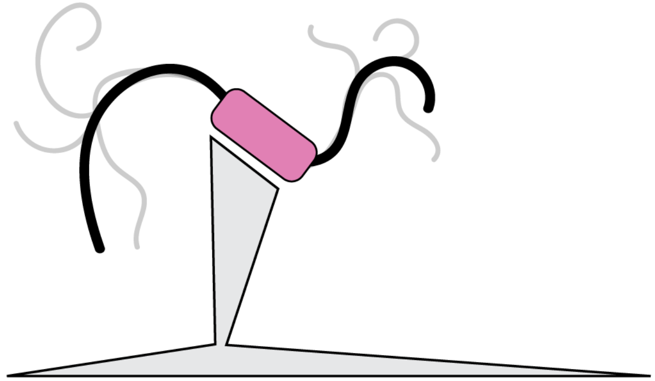
Coupled folding & binding

Fuzzy binding

IDR-mediated interact via three “distinct” modes of binding



IDRs often bind via Short Linear Motifs (SLiM)s



LPENND**LYCYE**QLNVAPAQ

RegEx: **L**x**C**x**E**

Actual examples

Eg. 1 D**L**Y**C**Y**E**QLN

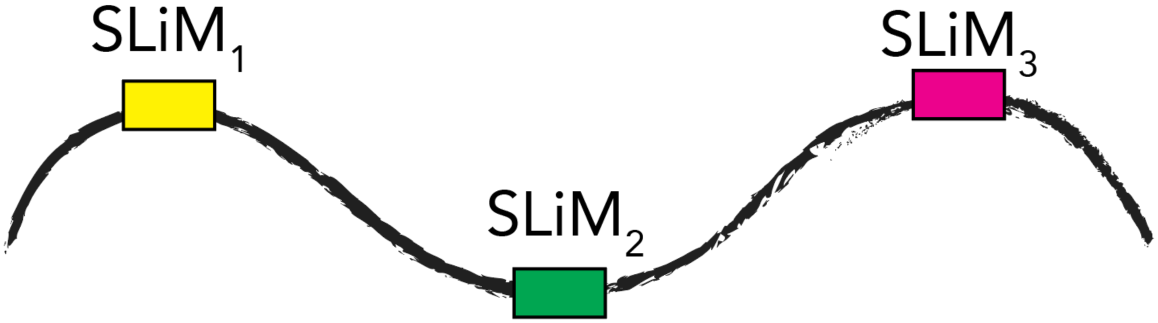
Eg. 2 T**L**V**C**H**E**VDL

Eg. 3 E**L**G**C**D**E**IID

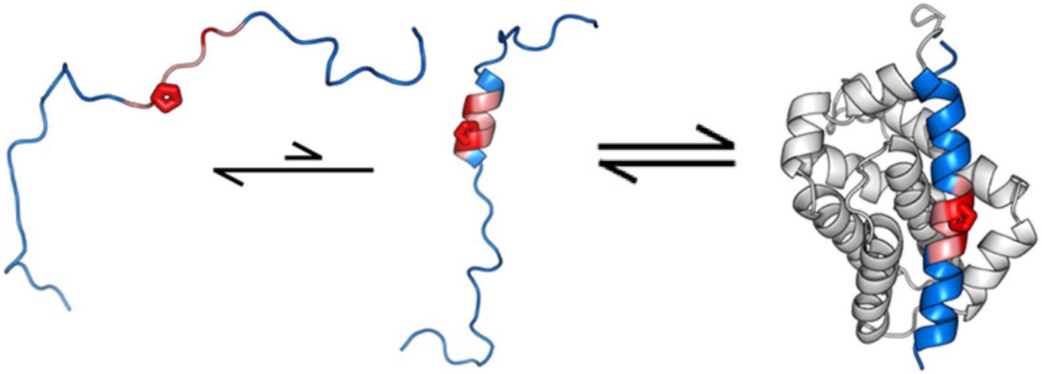
Eg. 4 R**I**A**C**E**E**EFS

SLiM

Multiple SLIMs can be placed on a single IDR



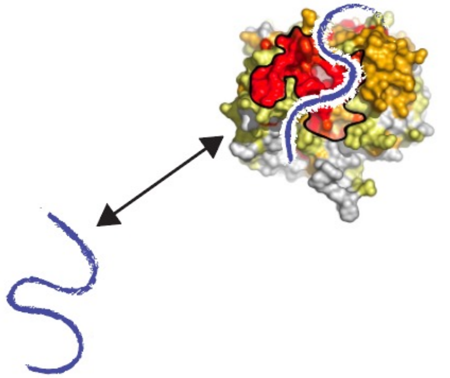
IDRs can bind through coupled folding & binding



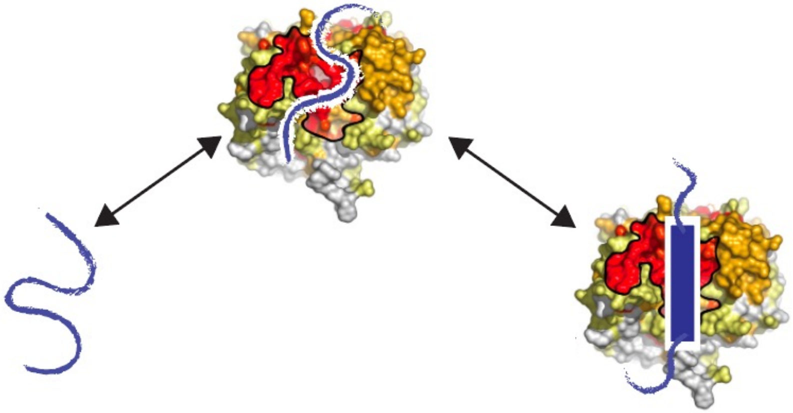
Rogers et al. ***JACS*** (2014)

Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism

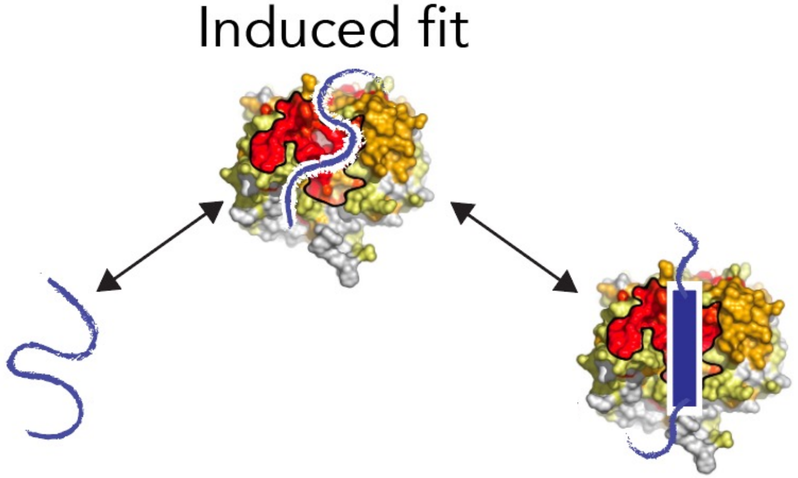
Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism



Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism

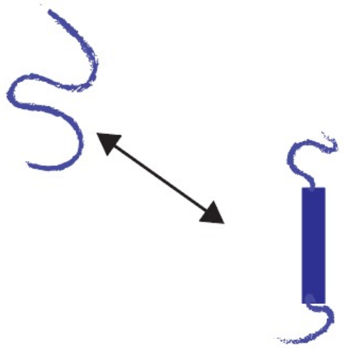


Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism

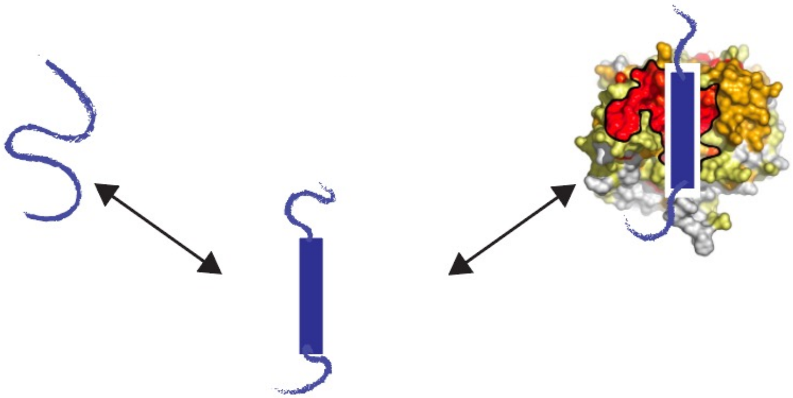


Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism

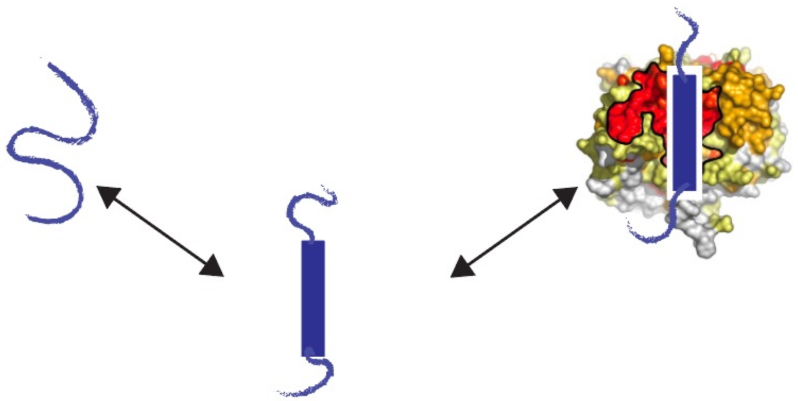
Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism



Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism

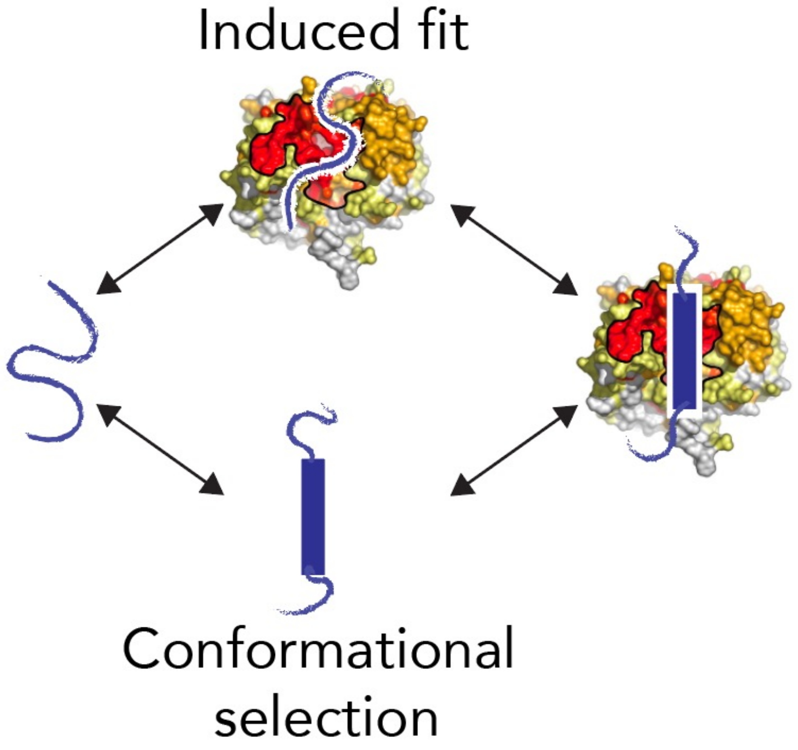


Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism



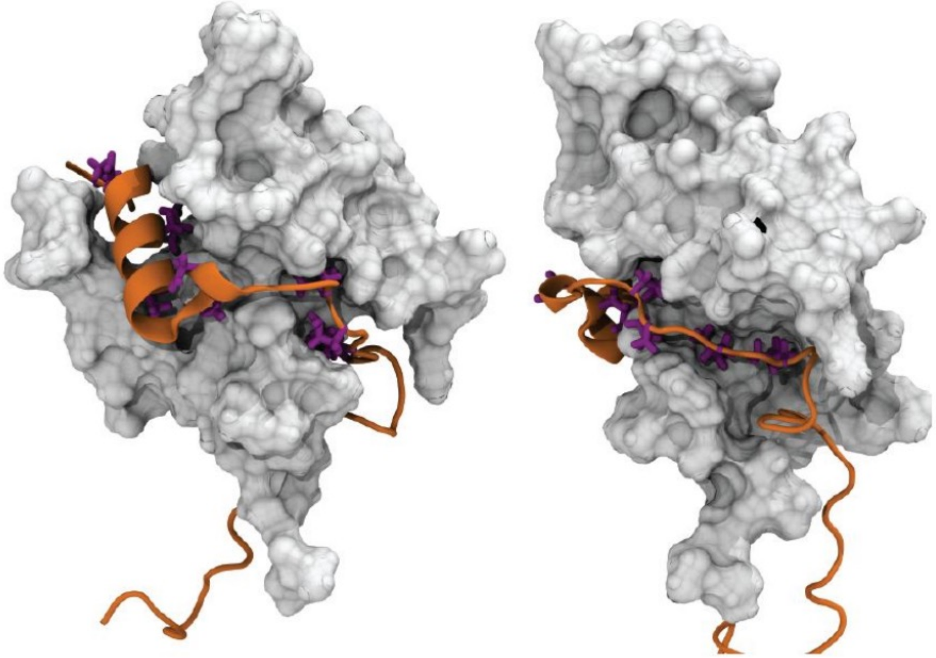
Conformational selection

Coupled folding and binding occurs via two possible (non-mutually exclusive) mechanism



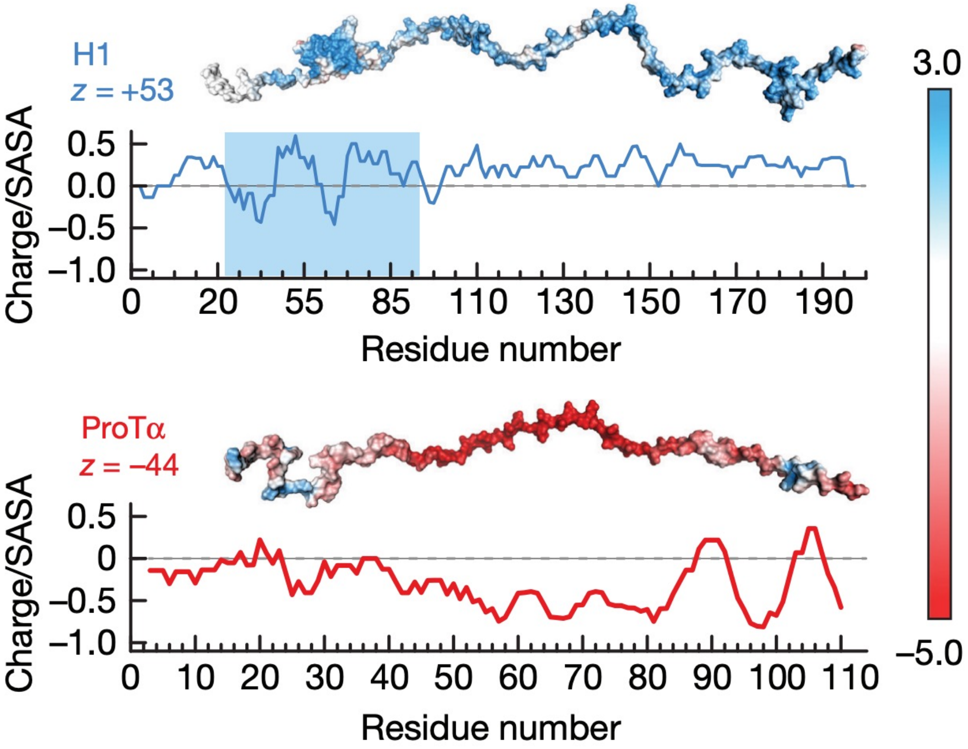
In many cases both pathways are relevant

Specific molecular recognition via folded and fuzzy complexes



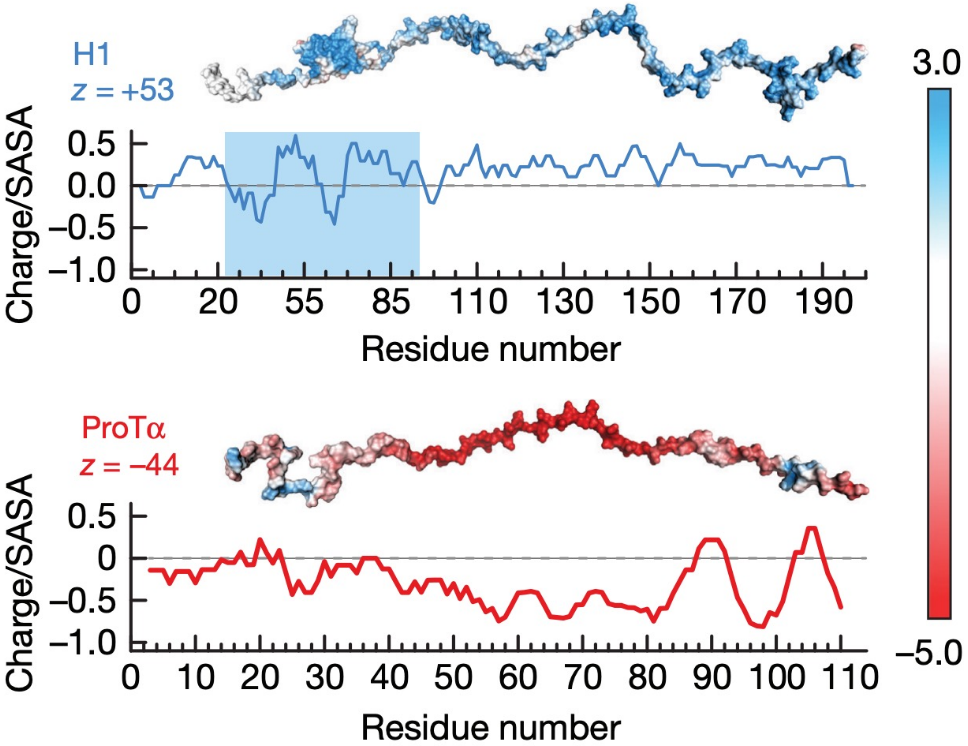
Staller et al. ***Cell Systems*** (2022)

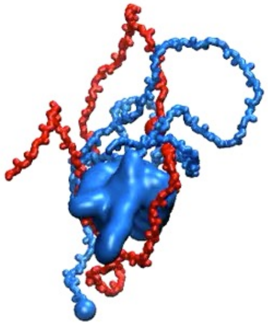
IDRs can also form high-affinity dynamic complexes



Borgia et al. ***Nature*** (2018)

IDRs can also form high-affinity dynamic complexes

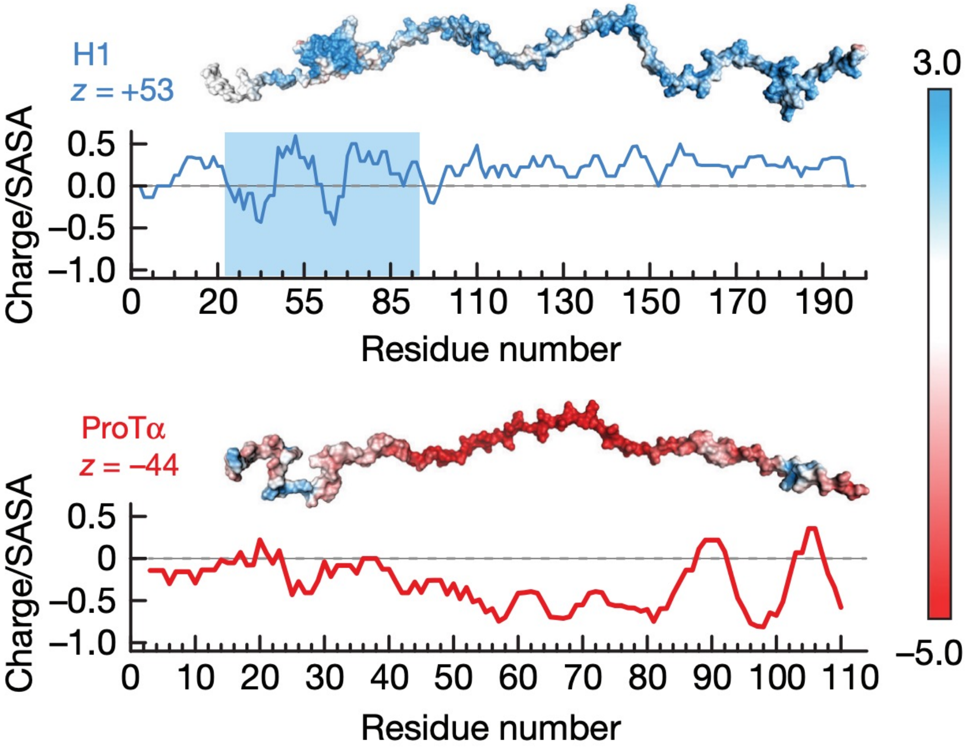


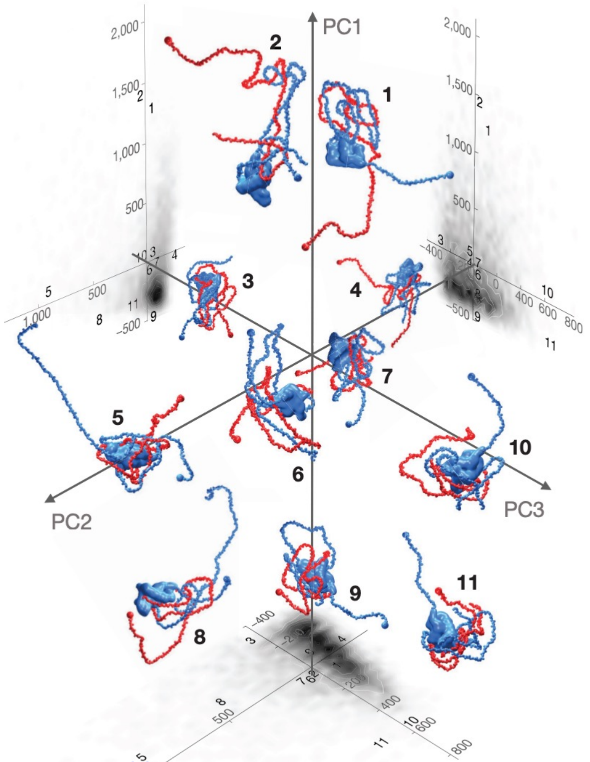


Fully disordered complexes can engage in high affinity interactions

Borgia et al. ***Nature*** (2018)

IDRs can also form high-affinity dynamic complexes



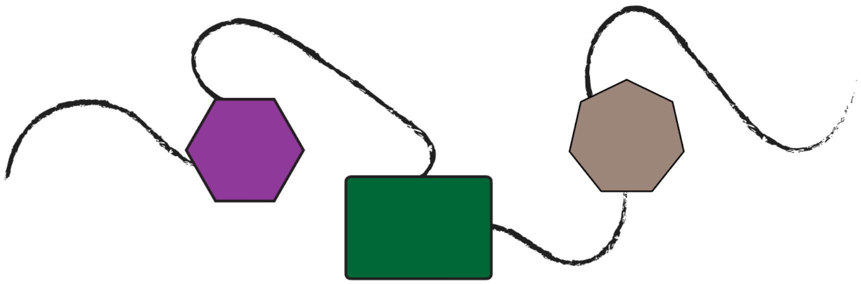


Fully disordered complexes can engage in high affinity interactions

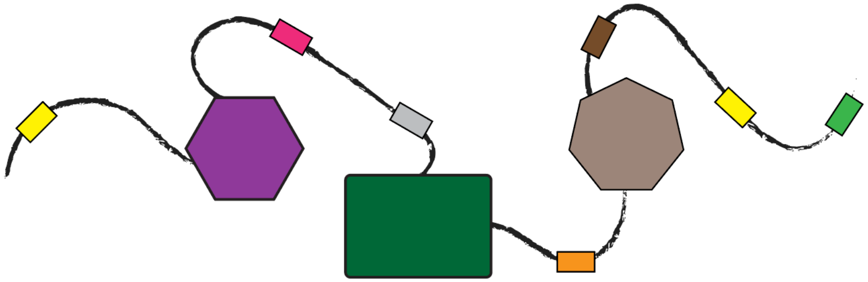
Borgia et al. ***Nature*** (2018)

4. IDRs as platforms for multivalent interactions

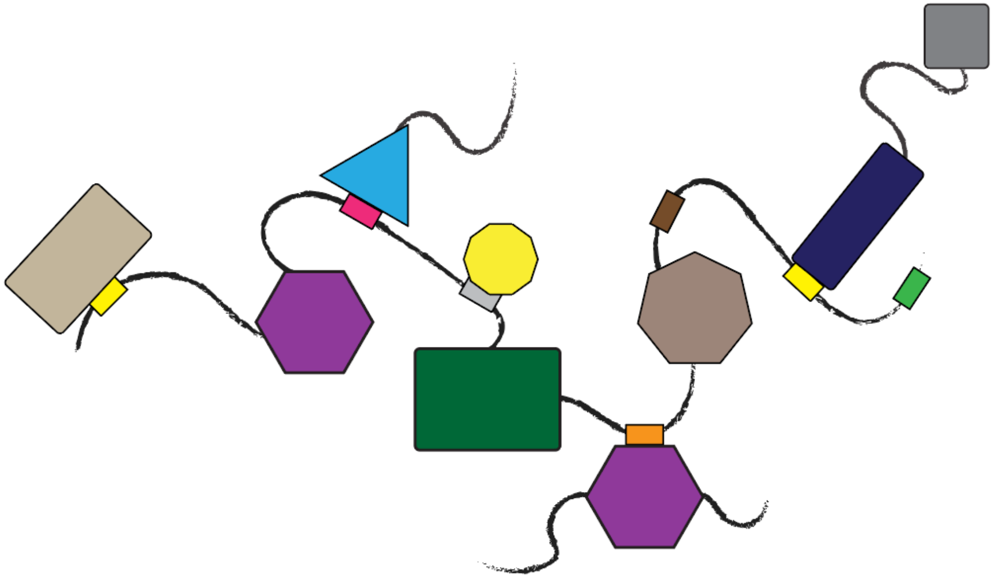
IDRs often connect folded domains but themselves can ALSO mediate binding



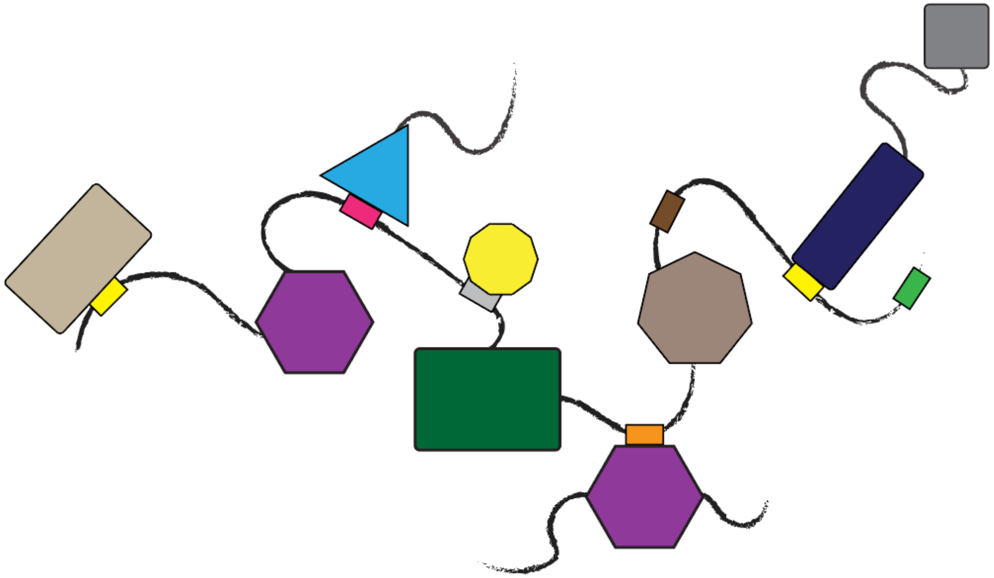
IDRs often connect folded domains but themselves can ALSO mediate binding



As a result – large IDR-containing proteins often function as molecular "hubs"



As a result – large IDR-containing proteins often function as molecular "hubs"

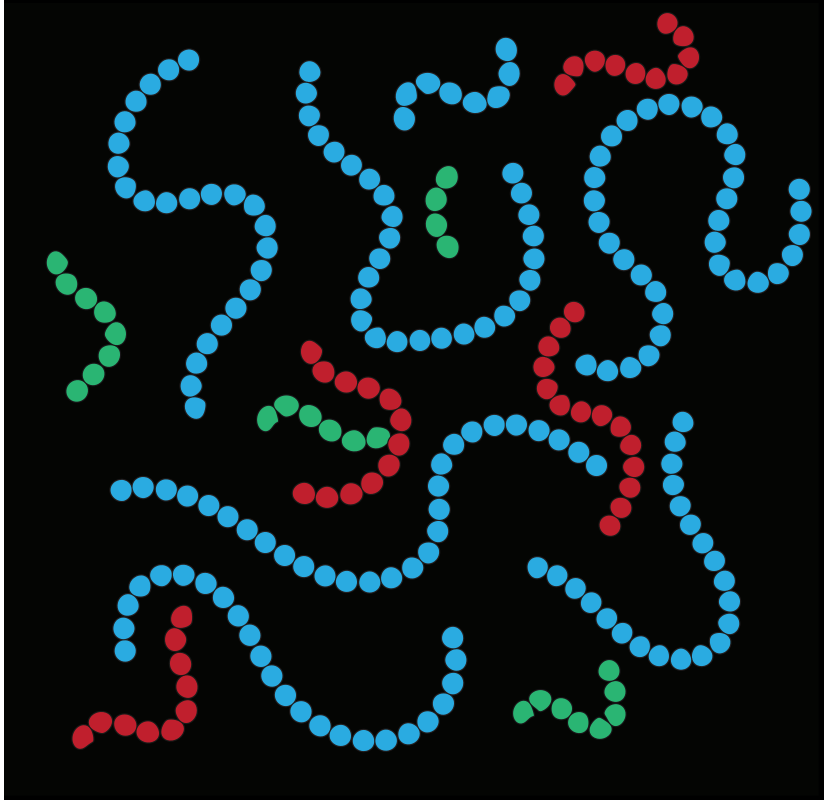


- Signaling
- Chromatin organization
- Transcription

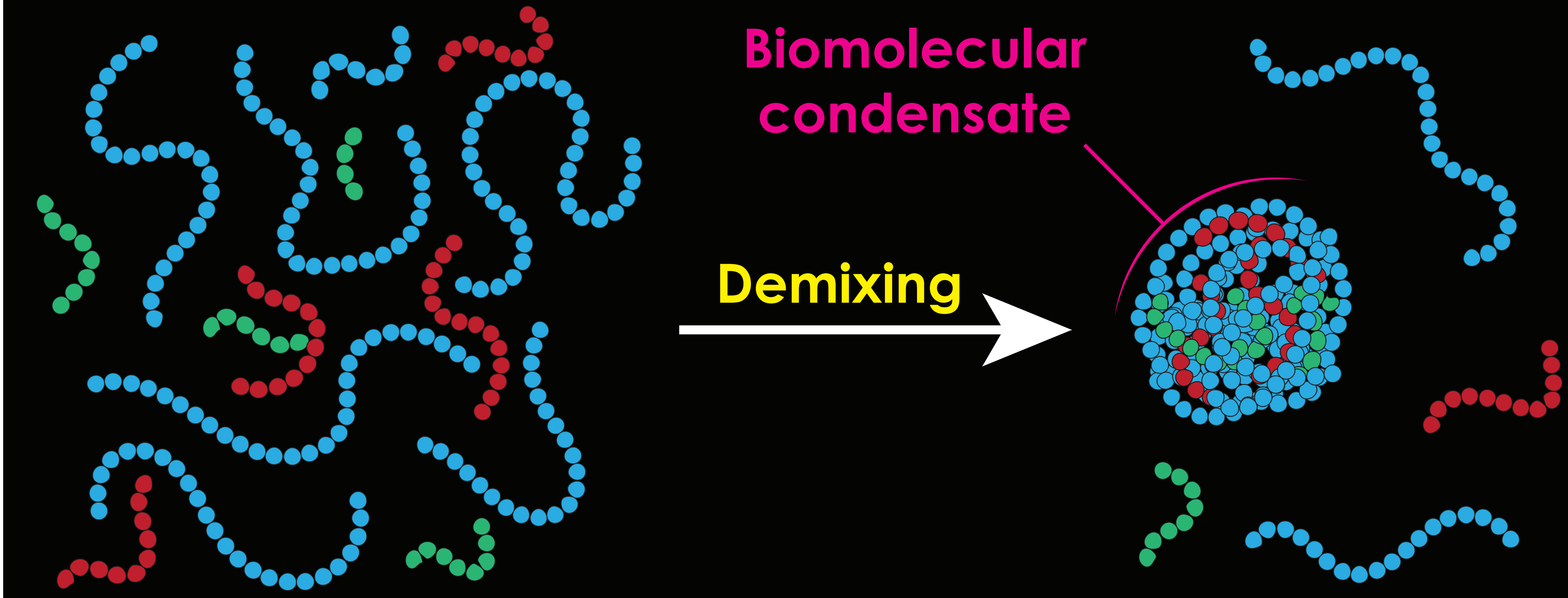
5. IDRs as drivers of higher-order cellular assemblies

IDRs can drive the formation of biomolecular condensates

IDRs can drive the formation of biomolecular condensates

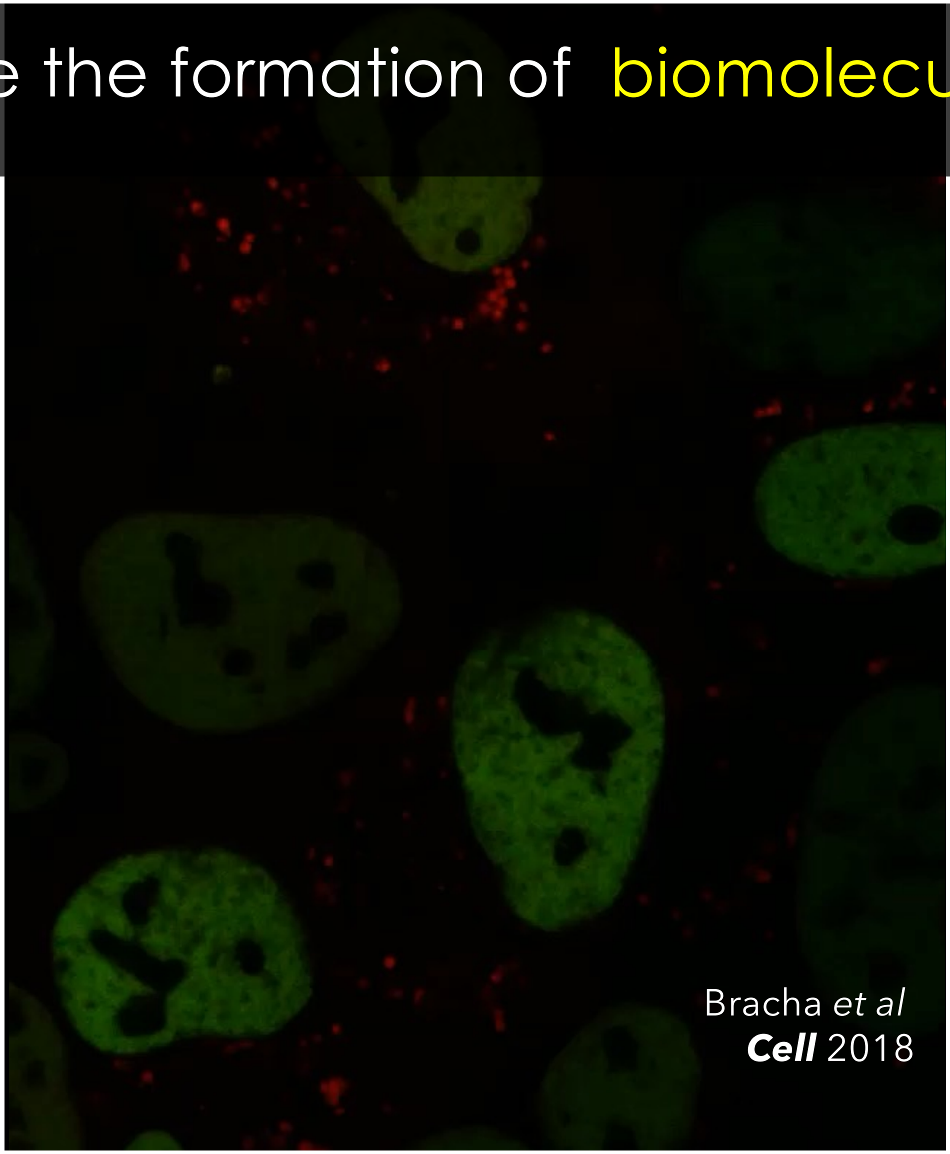


IDRs can drive the formation of biomolecular condensates

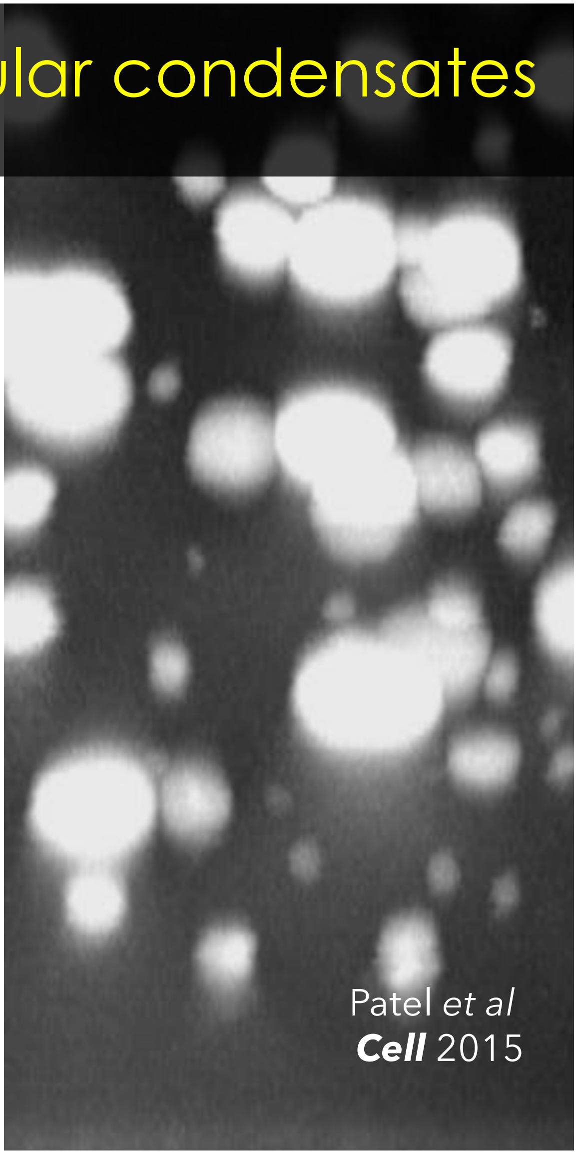


IDRs can drive the formation of biomolecular condensates

Guillén *et al*
Cell 2020

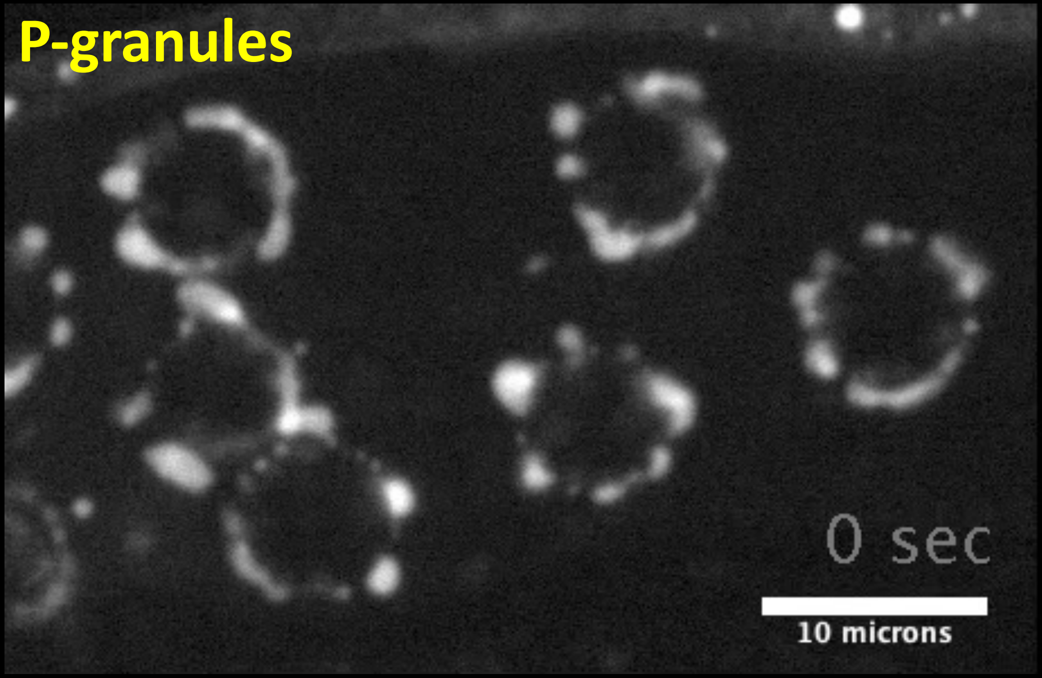


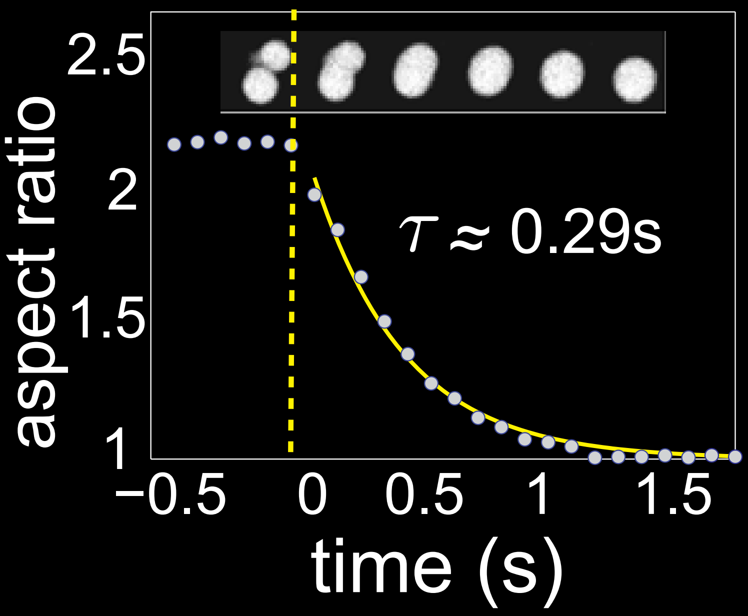
Bracha *et al*
Cell 2018



Patel *et al*
Cell 2015

Many condensates have liquid-like properties

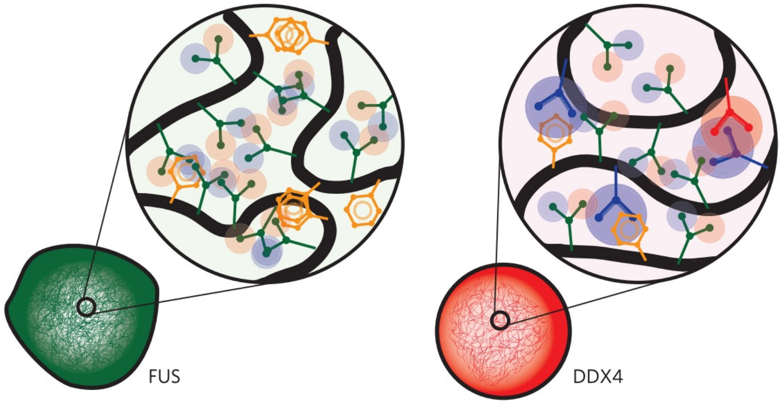


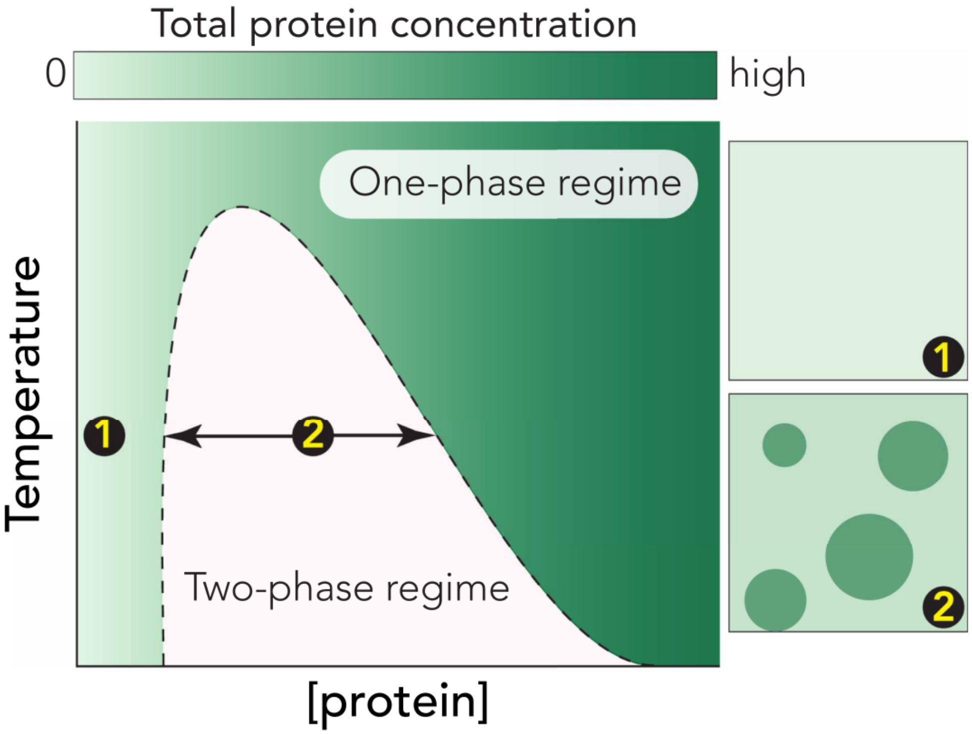


Brangwynne *et al.*
Science (2009)

Elbaum-Garfinkle *et al.*
PNAS (2015)

In many cases, IDRs are critical for the formation of these assemblies (although IDRs are NOT required)





READING:

Paper to read for discussion section:

Borgia, A., Borgia, M. B., Bugge, K., Kissling, V. M., Heidarsson, P. O., Fernandes, C. B., Sottini, A., Soranno, A., Buholzer, K. J., Nettels, D., Kragelund, B. B., Best, R. B., & Schuler, B. (2018). Extreme disorder in an ultrahigh-affinity protein complex. *Nature*, *555*(7694), 61–66.

Recommended reading for exam:

Holehouse & Kragelund (2023) The molecular basis for cellular function of intrinsically disordered regions. ***Nature Reviews Molecular Cell Biology*** *(in press)*

This review (up to line 674 [no condensate questions!]).

The answer to the questions on the exam will be out of the first 674 lines of this review.

Bonus reading if you want MORE DISORDER:

Martin, E. W., & Holehouse, A. S. (2020). **Intrinsically disordered protein regions and phase separation: sequence determinants of assembly or lack thereof**. *Emerging Topics in Life Sciences*, *4*(3), 307–329.