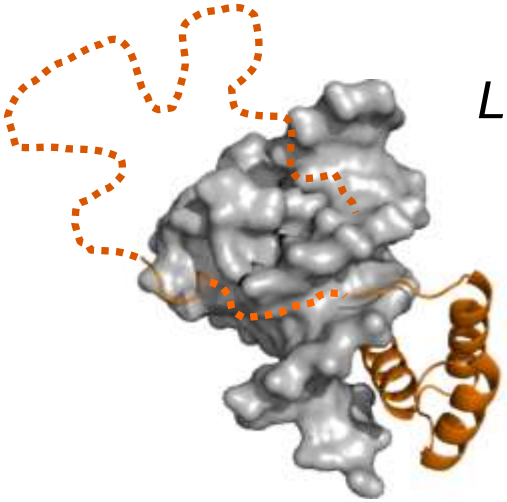
Fuzzy complexes:
ambiguity
in protein - protein and protein-DNA interactions

Monika Fuxreiter

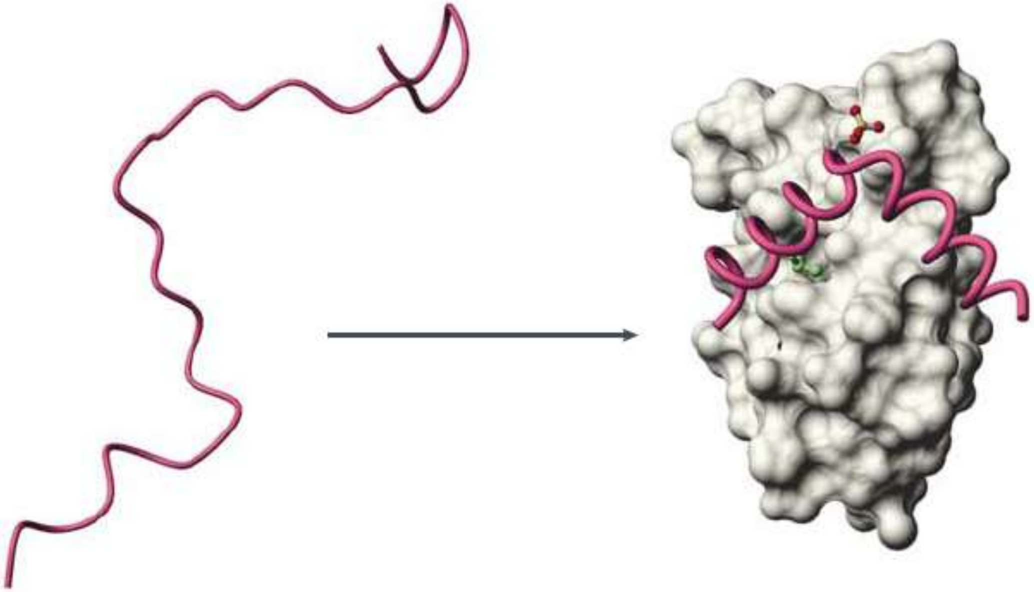
Institute of Enzymology, Budapest
Laboratory of Molecular Biology, Cambridge



Cambridge – 27.10.11

Classical view of complexes

Folding coupled to binding



KID (CREB)

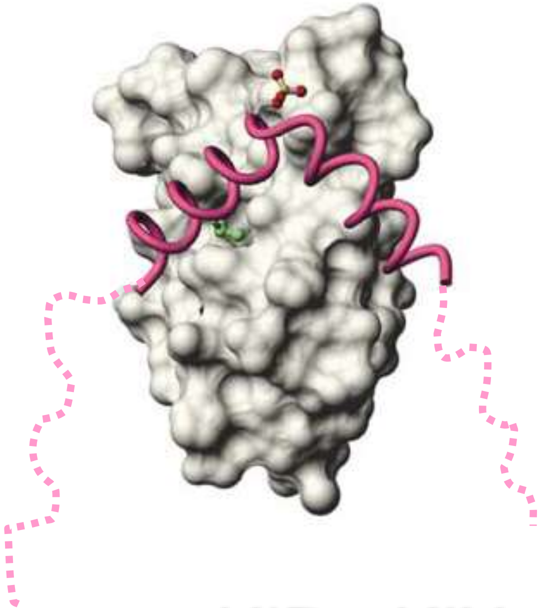
pKID - KIX (CBP)

Radhakrisnan (1997) Cell 91, 741

Dyson (2002) Curr Opin Sruct Biol 12, 54

Sugase (2007) Nature 447, 1021

Beyond what can be seen

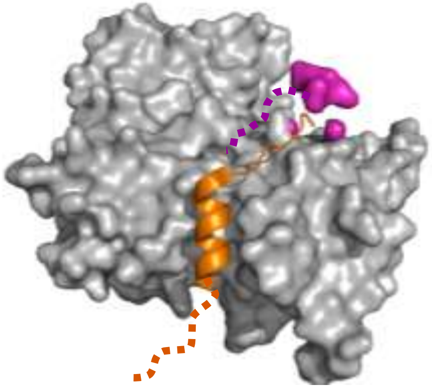


pKID - KIX

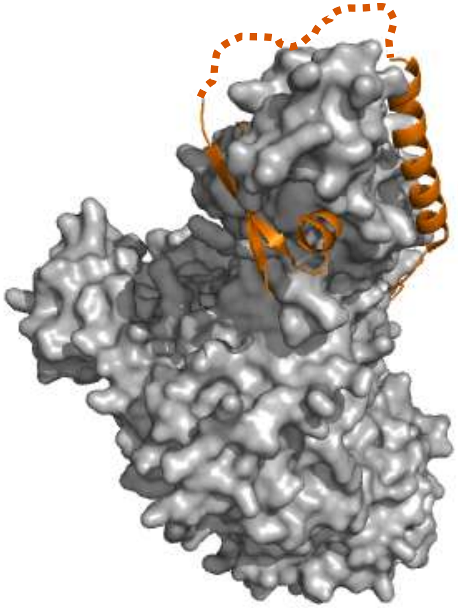
	K_d
KID 21 aa	80 μM
KID 29 aa	3.1 μM
KID 60 aa	0.7 μM

Zor (2002) J Biol Chem 277, 42241

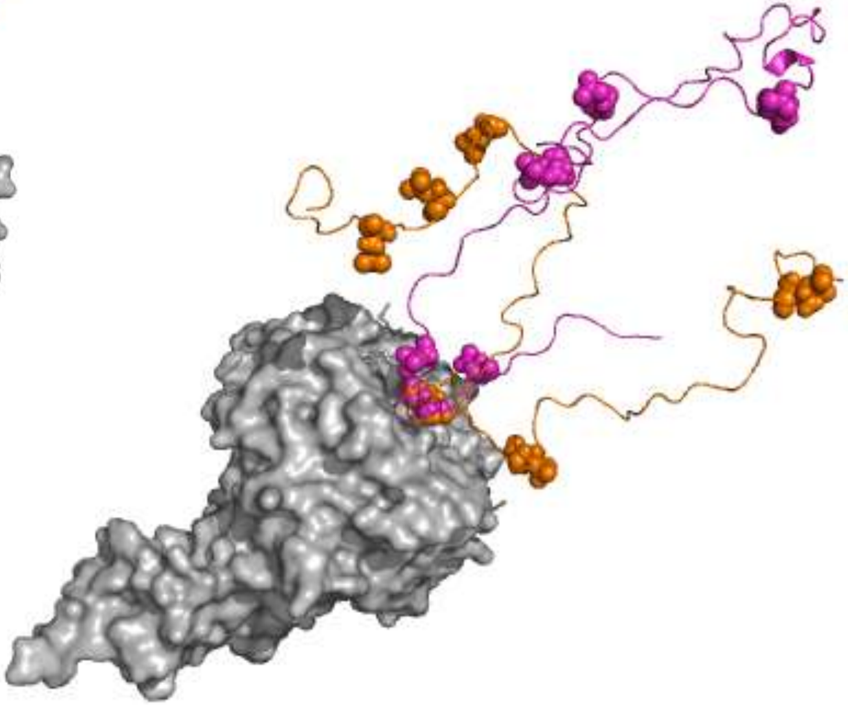
Ambiguity in complexes



WH2-actin



UPF2 - UPF1



Sic1 - Cdc4

Clerici (2009) EMBO J 28, 2293

Chereeau (2005) PNAS 102, 16644

Mittag (2008) PNAS 105, 17772

“**fuzz•y**”

(adj. fuzz•i•er, fuzz•i•est)

Wikipedia

Fuzzy logic is a form of many-valued logic; it deals with reasoning that is approximate rather than fixed and exact. In contrast with traditional logic theory, where binary sets have two-valued logic: true or false, fuzzy logic variables may have a truth value that ranges in degree between 0 and 1.

Fuzzy logic has been extended to handle the concept of partial truth, where the truth value may range between completely true and completely false.

Fuzziness:

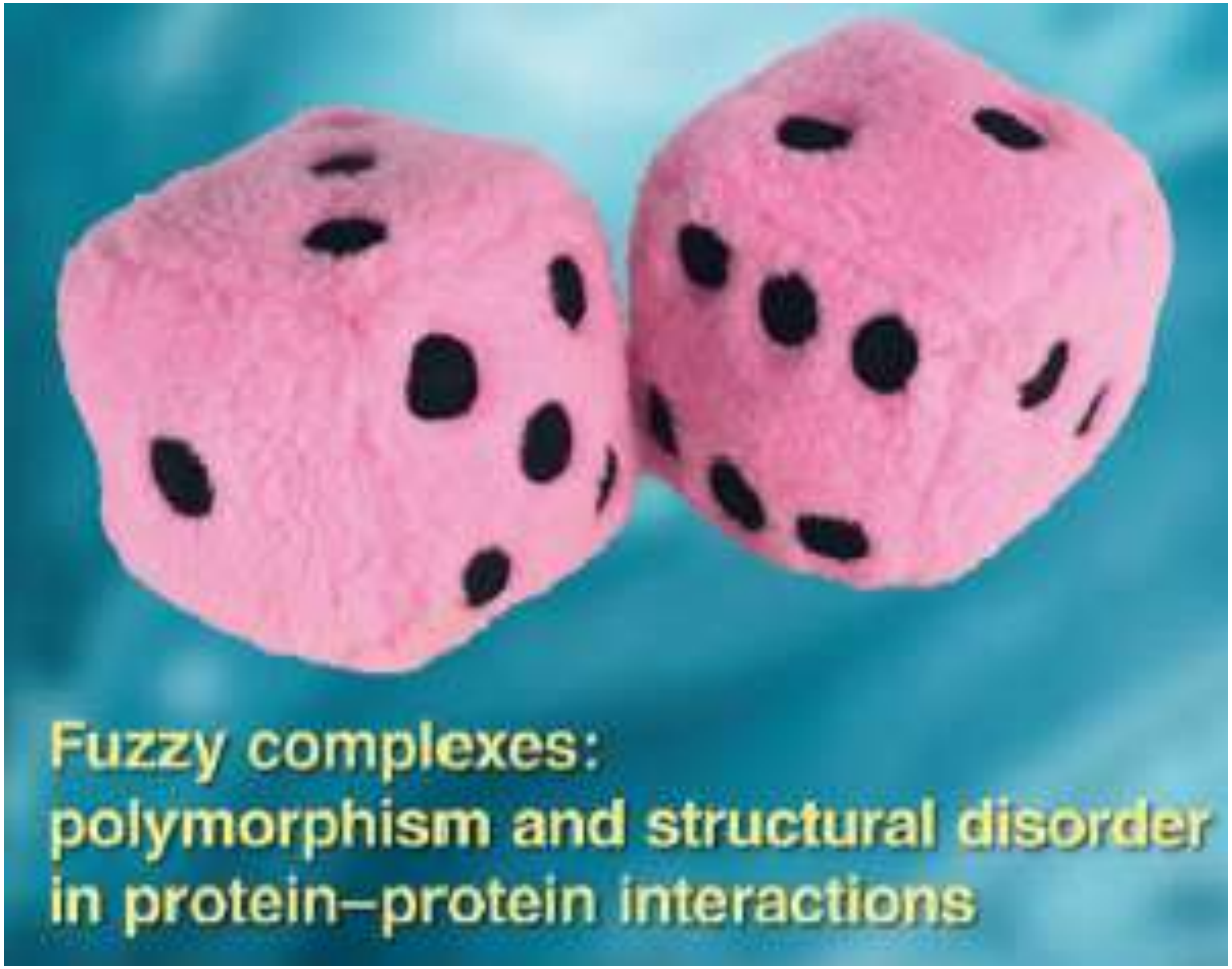
functional structural ambiguity in bound form

QuickTime™ and a
decompressor
are needed to see this picture.

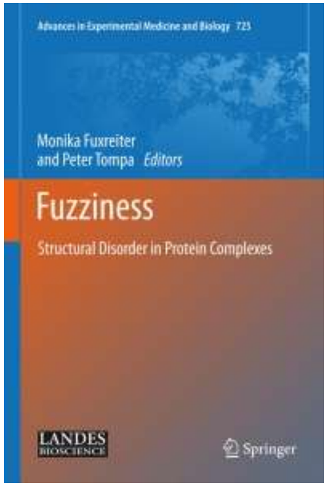
fuzzy complexes

Tompa and Fuxreiter (2008) Trends in Biochem Sci 33, 2-8

New view


Fuzzy complexes:
polymorphism and structural disorder
in protein–protein interactions

Fuzzy complexes



Advances in Experimental Medicine and Biology 725

Monika Fuxreiter
and Peter Tompa *Editors*

Fuzziness

Structural Disorder in Protein Complexes

LANDES BIOSCIENCE

Springer

Protein-protein complexes

- functional promiscuity
- ultrasensitivity
- sequence variability

Fuzziness: Structural disorder in protein complexes
Landes/Springer 2011

QuickTime™ and a
decompressor
are needed to see this picture.

Jensen (2011) *PNAS* 108, 9839

Fuzziness - opposite functions

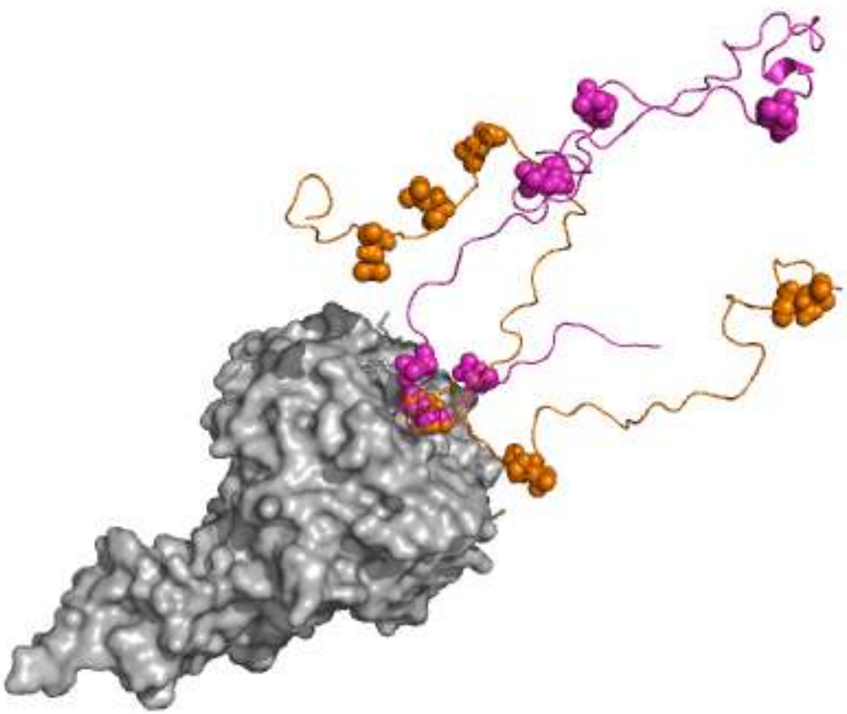
$p27^{Kip}$ - Cdk2-Cyclin

- inhibition G1→S transition
- Y88 dissociation
- T187 (Cterm) phosphorylation
- SCF/Skp2 binding
- polyubiquitination
- p27 degradation

Galea (2008) *J Mol Biol* 376, 827

Fuzziness- ultrasensitivity

Regulation by gradual post-translational modification



Sic1 - Cdc4

Sic1: 9 phosphorylation sites

Cdc4: 1 binding site

Optimal binding: > 6 P

Nash (2001) Nature 414, 514

Borg (2007) PNAS 104, 9650
Mittag (2008) PNAS 105, 17772

Fuzziness- variable sequences

scramble sequence -

same binding function

QuickTime™ and a
decompressor
are needed to see this picture.

H1 histone C-tail, H4 histone N-tail

conserved amino acid composition

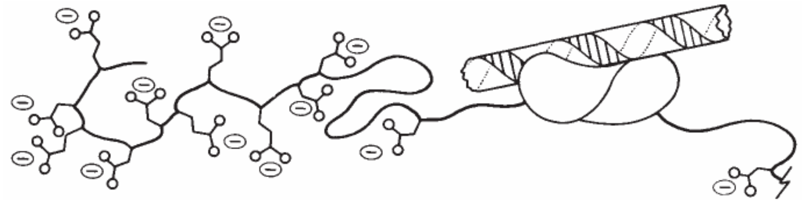
Hansen et al. (2006) JBC 281, 1853
McBryant et al. (2009) JBC 284,16716
Lu et al. (2009) Biochemistry 48, 164

Fuzziness- variable sequences

Resistance to sequence scrambling

- GCN4, EWS
- histone tails
- Ure2, Sup35

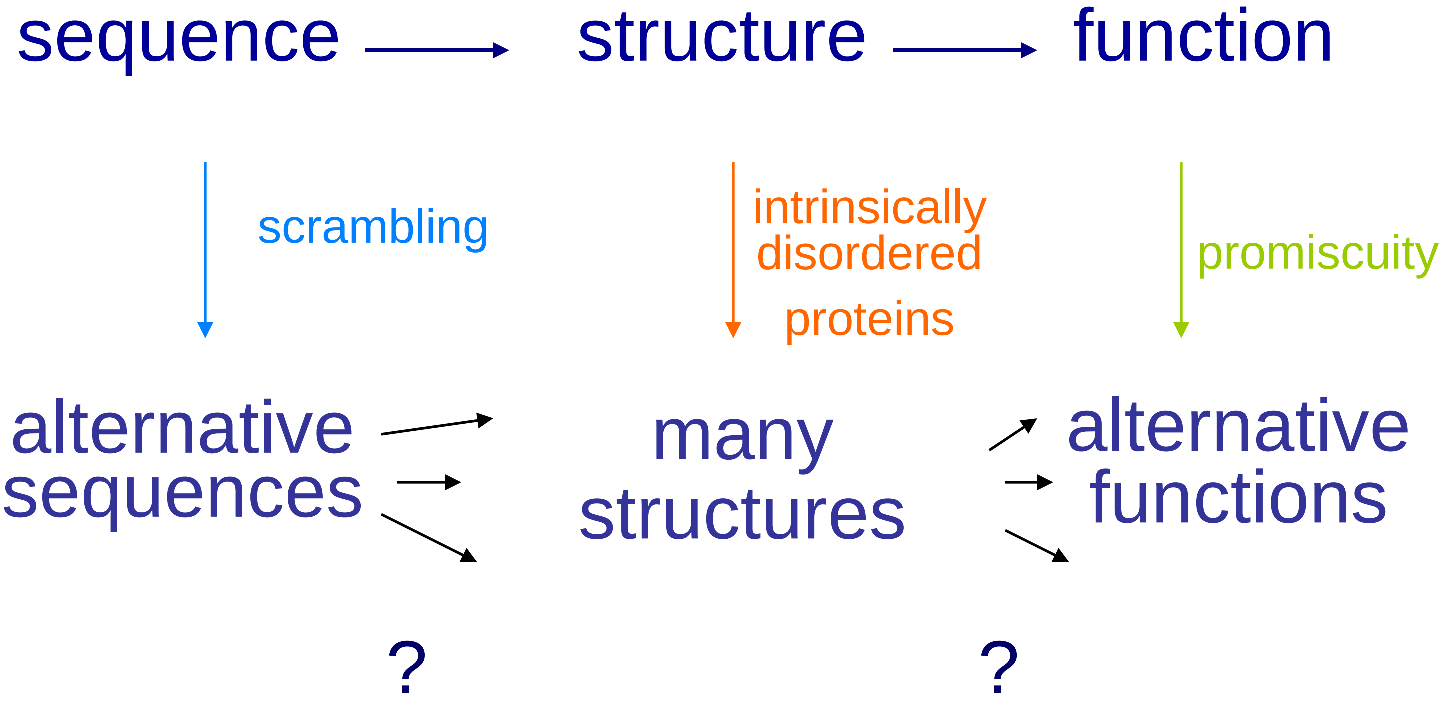
QuickTime™ and a
decompressor
are needed to see this picture.



Sigler et al. (1988) *Nature* 333, 210
Hope et al. (1988) *Nature* 333, 635

Ng et al. (2007) *PNAS* 104, 479
Ross et al. (2005) PNAS 102, 12825

New view - stochastic relationships



sequence → structure → function

scrambling

intrinsically disordered proteins

promiscuity

alternative sequences

many structures

alternative functions

?

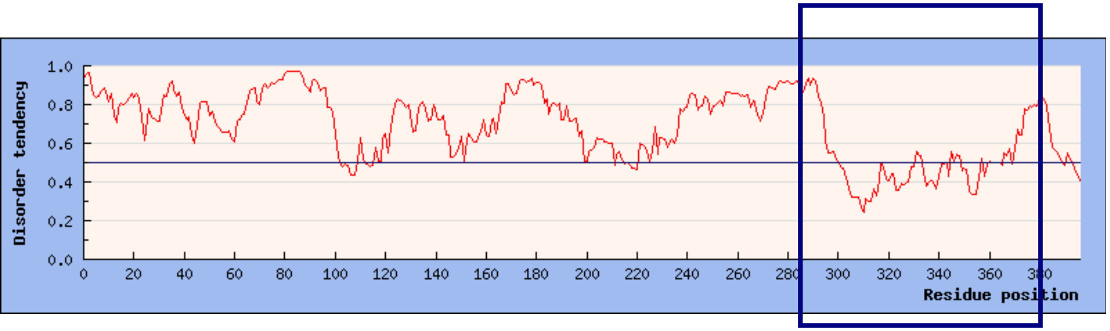
?

Specific DNA recognition

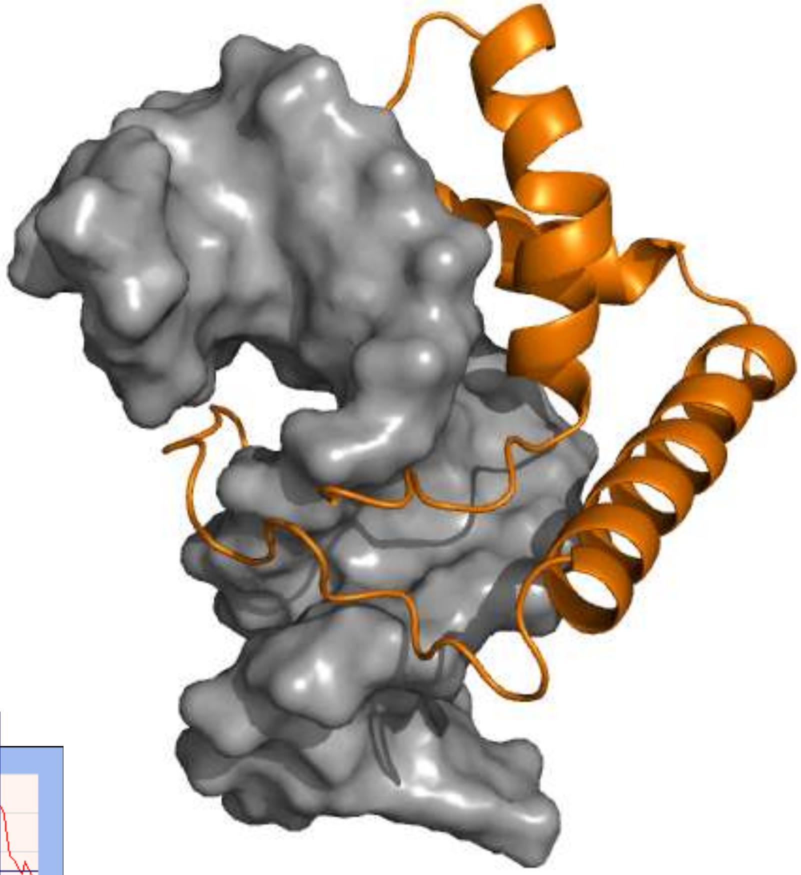
Classical theme:

Folding upon binding

What about the rest of
the protein?



Spolar (1994) *Science* 263, 777

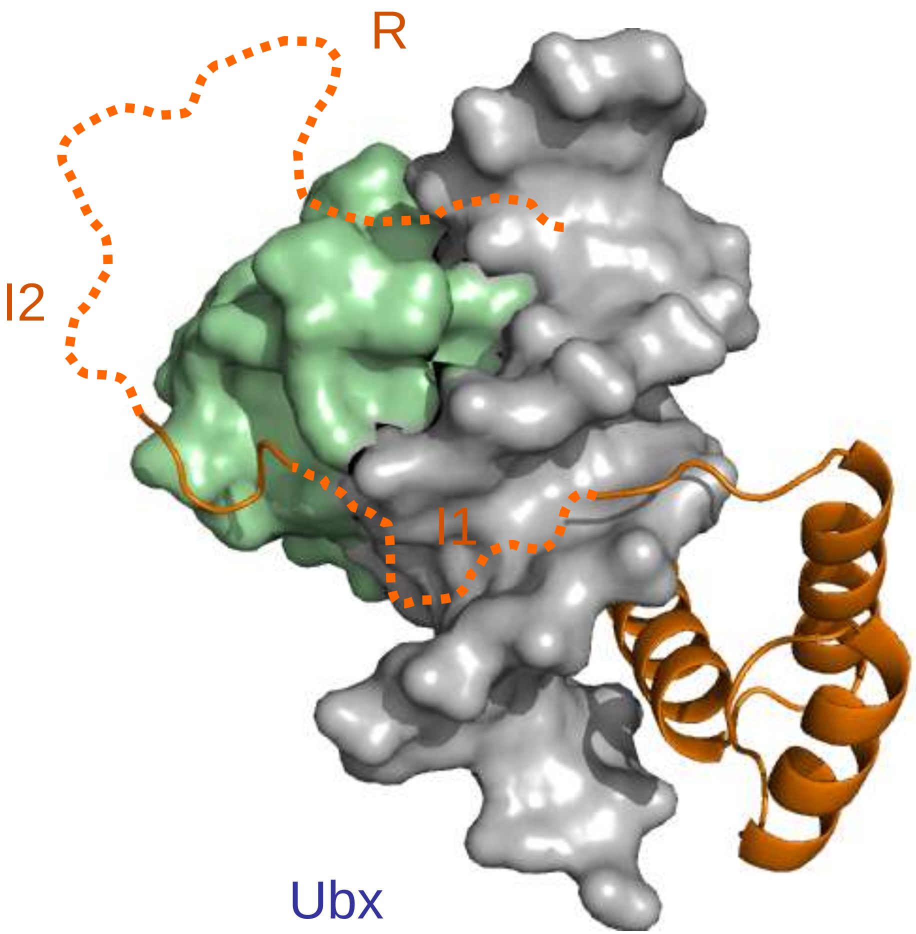


LEF-1

Love(1995) *Nature* 376, 791

Toth-Petroczy (2009) *J Am Chem Soc*, 131, 15084

DNA recognition - beyond what can be seen



Selectivity

in vitro ~3 fold

in vivo ~50 fold

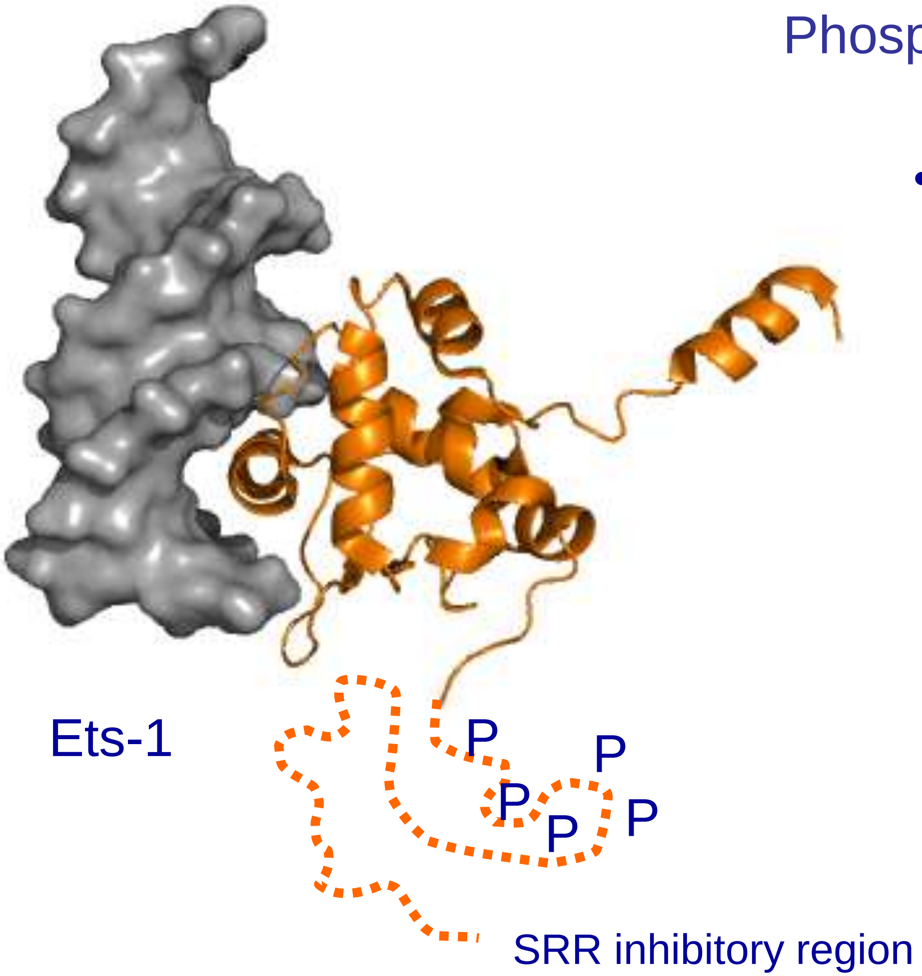
I1, I2, - decrease affinity

R - increases affinity

interplay between ID regions

Liu (2008) *J Biol Chem* 283, 20874

DNA recognition - beyond what can be seen



Phosphorylation

- 100-1000 fold affinity decrease
- no impact on secondary str.

in complex

DISORDERED

Pufall (2005) *Science* 309, 142
Lee (2005) *J Mol Biol* 382, 1014

Model	**IDP**	ID region	Conservation	Posttranslational modification
Conformational selection				
	Max	NTD CTD	*	phosphorylation
	MeCP2	NTD ID CTD		
	TDG	CTD		acetylation
	Neurogenin	Basic motif		
	ApLLP	NTD, CTD	*	
Flexibility modulation				
	Ets-1	SRR		phosphorylation
	SSB	CTD	*	
Competitive binding				
	PC4	NTD		phosphorylation, acetylation
	FACT	NTD CTD	*	phosphorylation
	HMGB1	CTD	*	phosphorylation
	Ubx	I1, I2, R	*	
	DSS1/Brh2	1-70		
	NKX3.1	AD, SI	*	phosphorylation
	PPAR-g	NTD		
	UvrD	CTD	*	
	b-telomere	CTD		phosphorylation
Tethering				
	Oct-1	Linker	*	
	RPA	IULD	*	phosphorylation
	KorB	NTD, linker		

bound ambiguous region affects affinity/specificity

structural + biochemical evidence

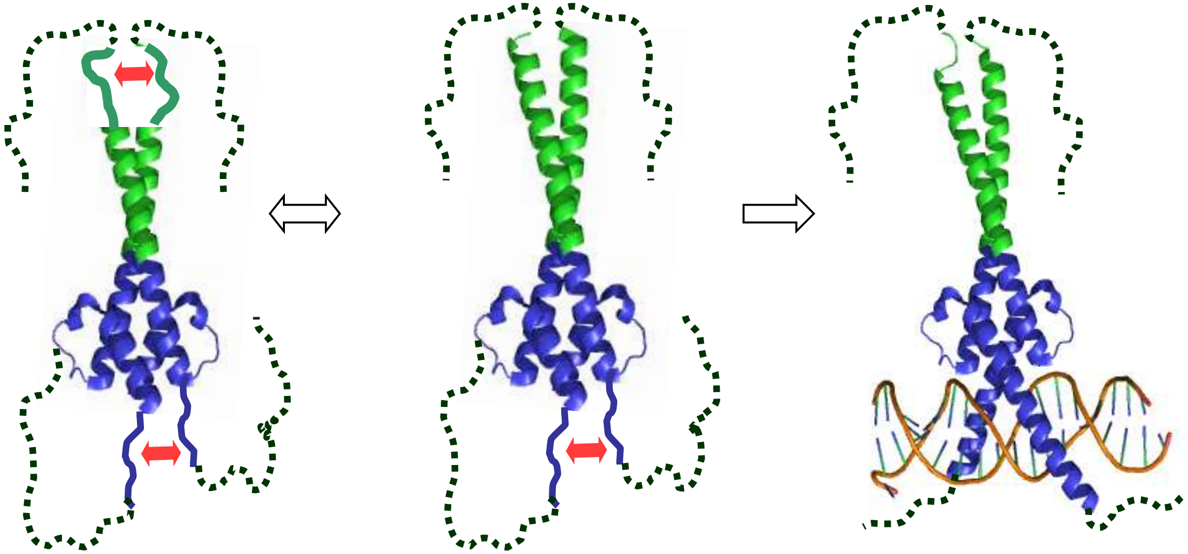
↓

fuzzy protein-DNA complexes

Fuxreiter (2011) *TiBS 36,* 415-423

Fuzzy protein-DNA complexes

Conformational selection



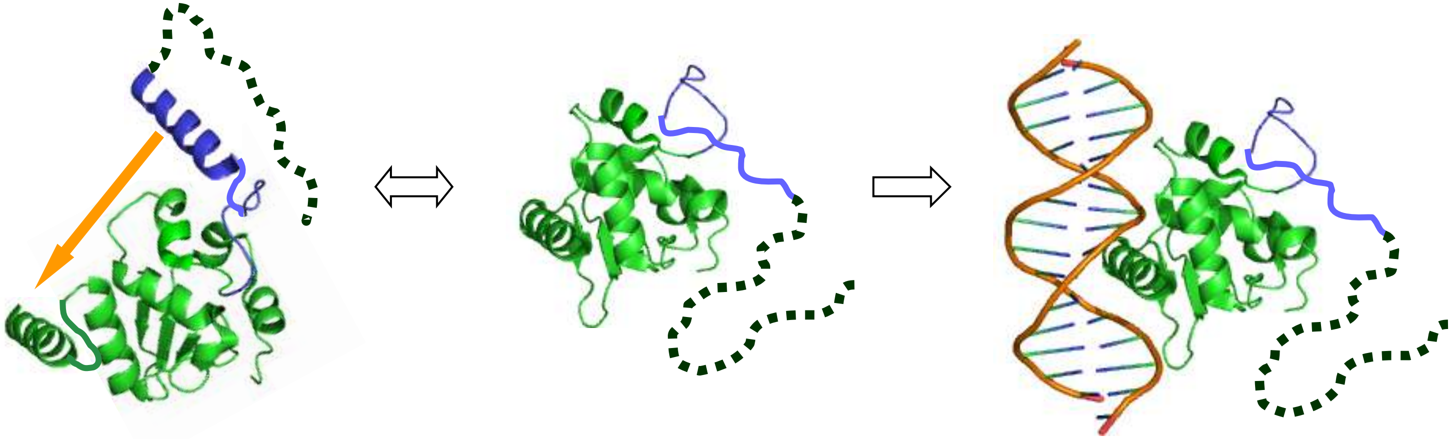
Max transcription factor, K_d ~100 fold decrease

ID NTD, CTD: promote formation of recognition helices

Naud (2005) *Biochemistry 44,* 12746

Fuzzy protein-DNA complexes

Flexibility modulation



Ets-1 K_d ~100-1000 fold increase

ID region: tunes dynamic properties of the recognition region

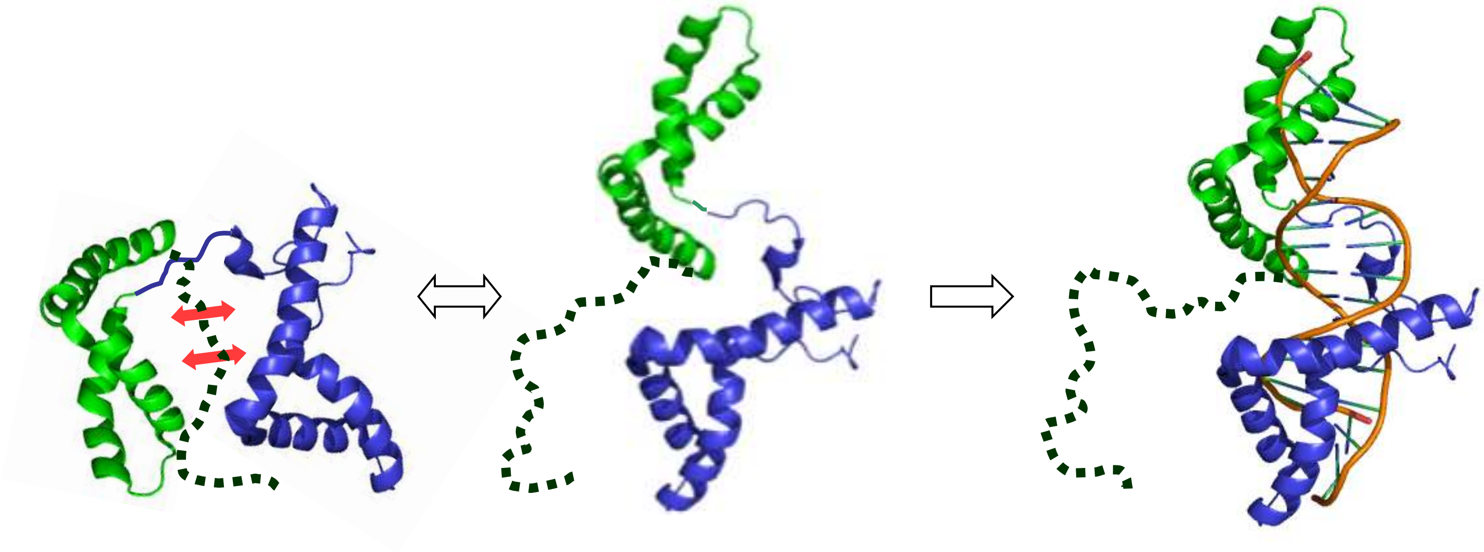
QuickTime™ and a
decompressor
are needed to see this picture.

Pufall (2005) *Science* 309, 142

→ decreasing flexibility

Fuzzy protein-DNA complexes

Competitive binding



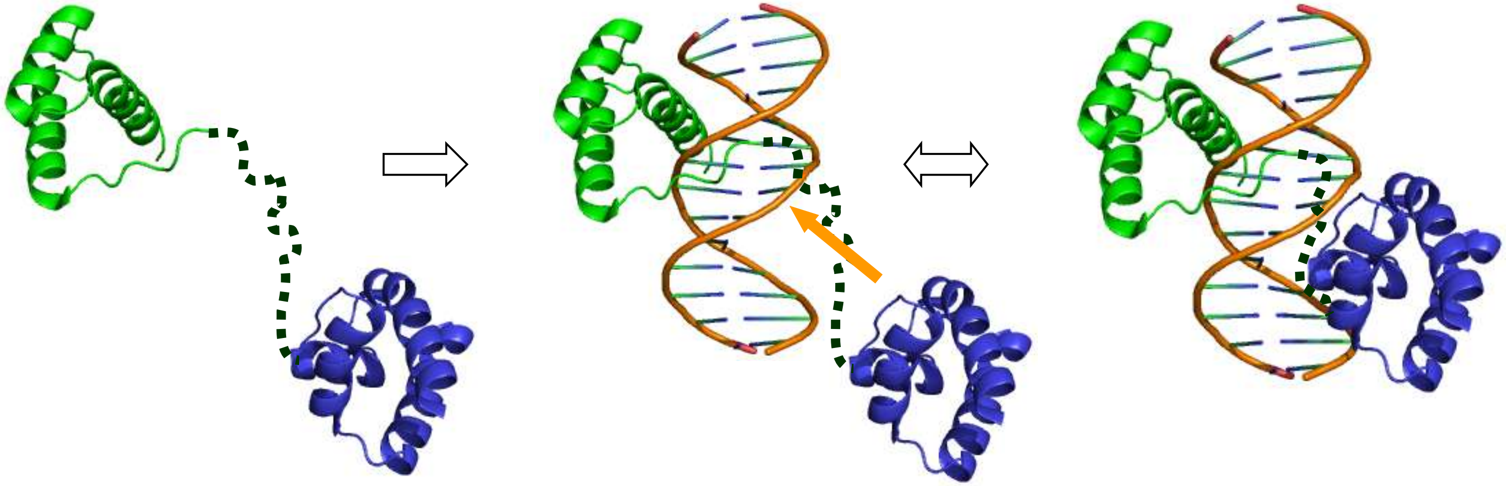
HMG-B: decreasing tail length increases affinity

ID region: competition between protein-DNA and intramolecular protein-protein interactions

Stott (2010) *J Mol Biol* 403, 706

Fuzzy protein-DNA complexes

Tethering



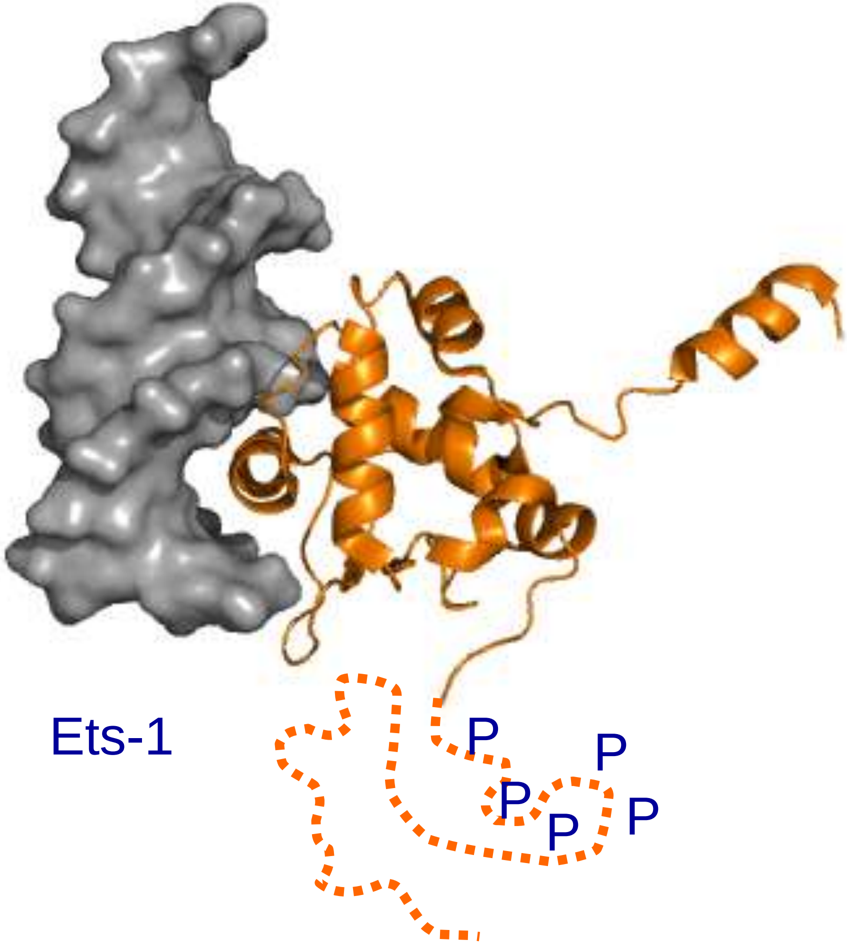
Oct-1: 2-6 aa shorter linker, 4-fold decrease in affinity

ID linker: tethers globular domains to DNA

van Leeuwen (1997) *EMBO J* 16, 2043

Vise (2005) *Nucl. Acids Res* 33, 2061

Dynamic DNA readout



- not only the interface
- global property
- modulation of dynamics
- transient interactions
- no regular structures

Fuxreiter (2011) *TiBS 36*, 415-423

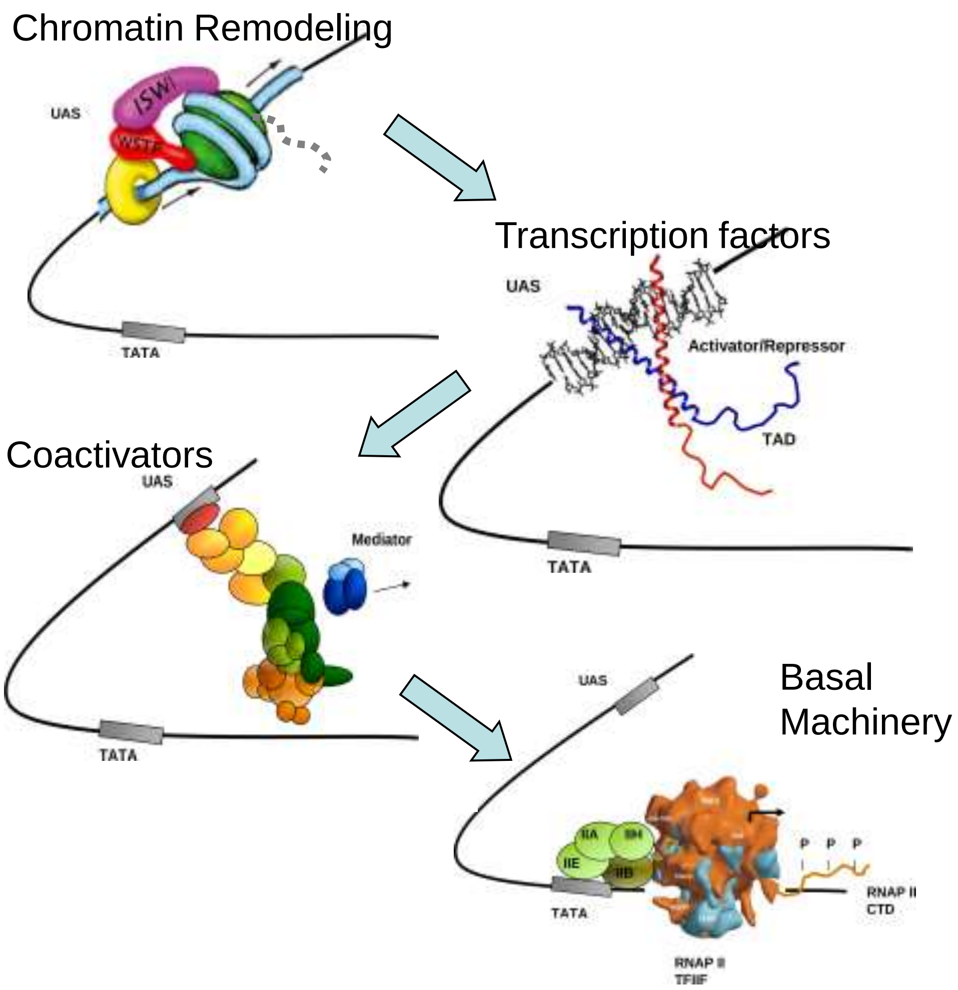
Tzeng and Kalodimos (2009) *Nature 462*, 368-372

Why fuzzy ...?

QuickTime™ and a
decompressor
are needed to see this picture.

QuickTime™ and a
decompressor
are needed to see this picture.

Transcription machinery - regulation


Chromatin Remodeling

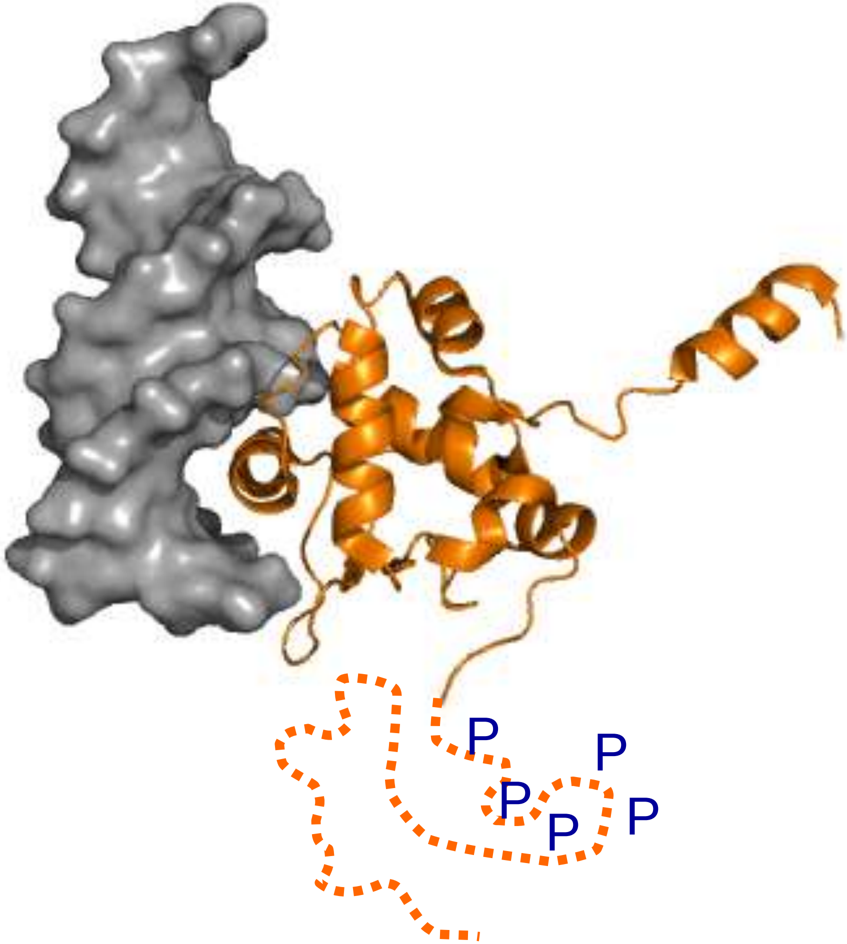
Transcription factors

Coactivators

Basal Machinery

Fuxreiter (2008) *Nat Chem Biol* 4 728

Regulation via fuzzy regions



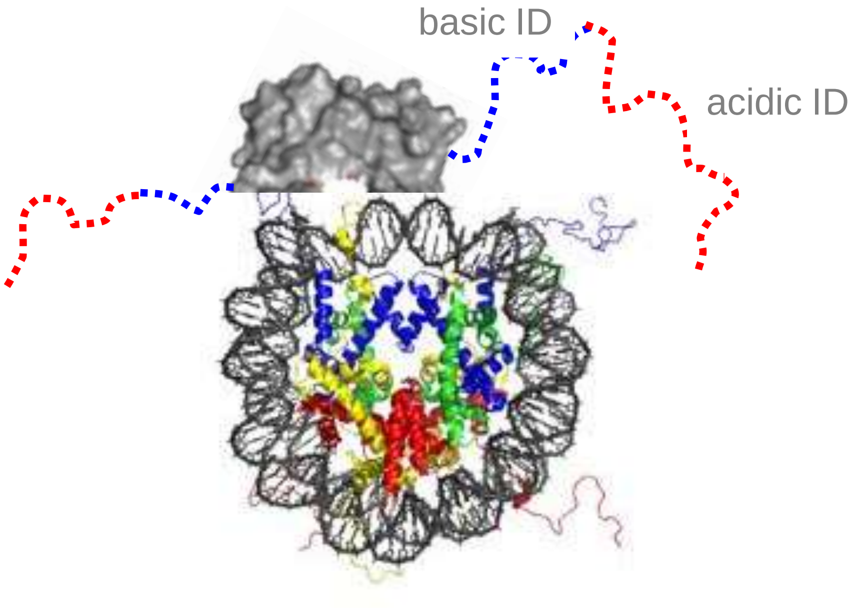
- protein-protein interaction
- post-translational modifications
- alternative splicing

Fuxreiter (2011) *TiBS 36*, 415-423

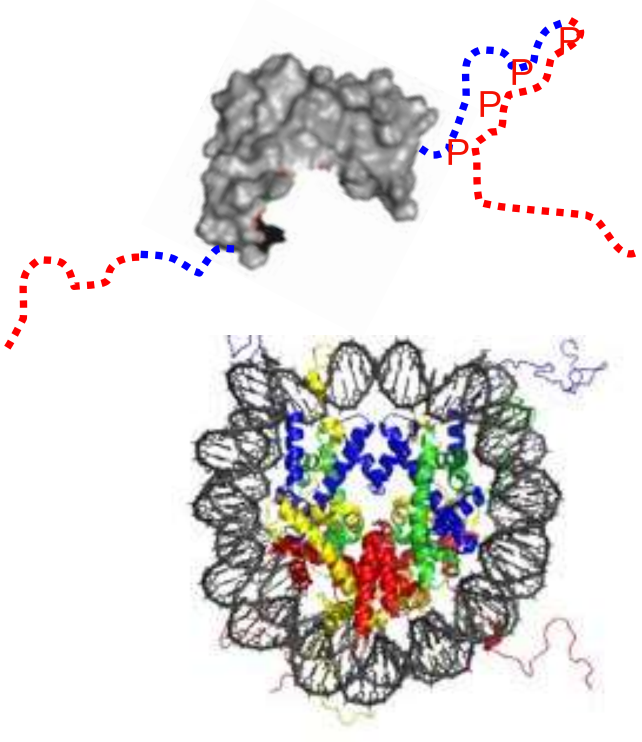
Regulation via fuzzy complexes

Post-translational modifications

FACT: facilitates chromatin transcription



unmodified form



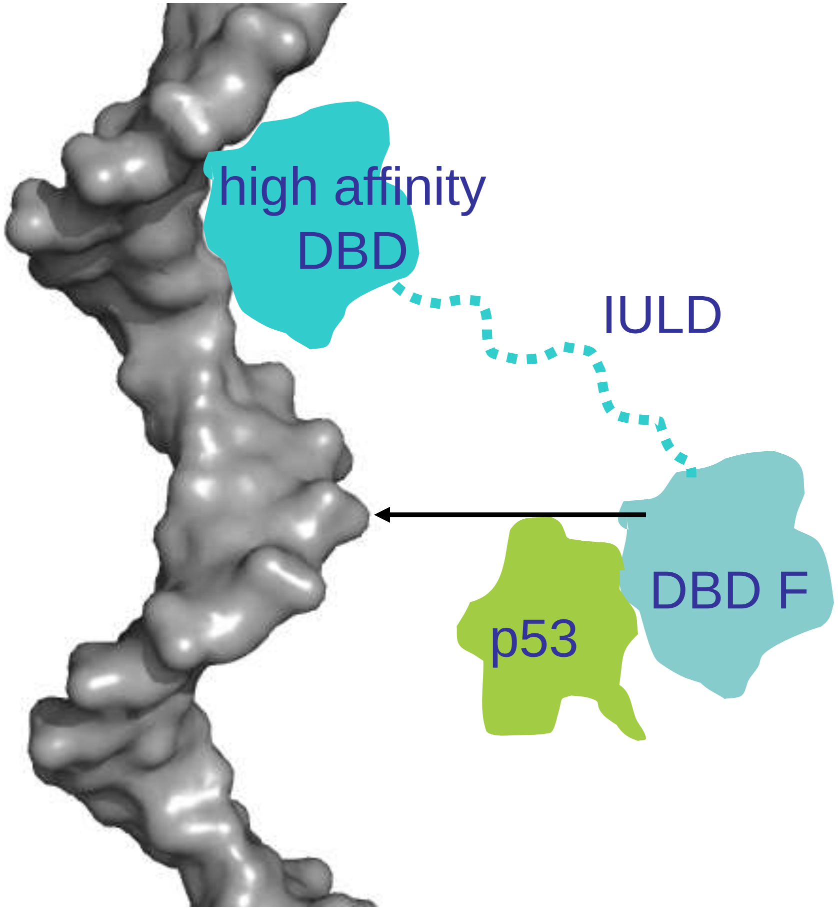
phosphorylated form

Tsunaka (2009) *J Biol Chem 284,* 24610

Regulation via fuzzy complexes

Protein-protein interactions

hRPA70: human replication protein A



QuickTime™ and a
decompressor
are needed to see this picture.

conservation of dynamics

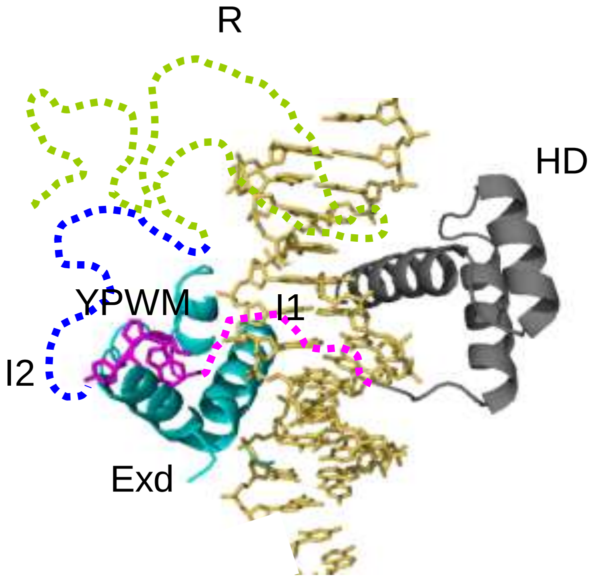
parameters by NMR

Vise (2005) *Nucl. Acids Res* 33, 2061

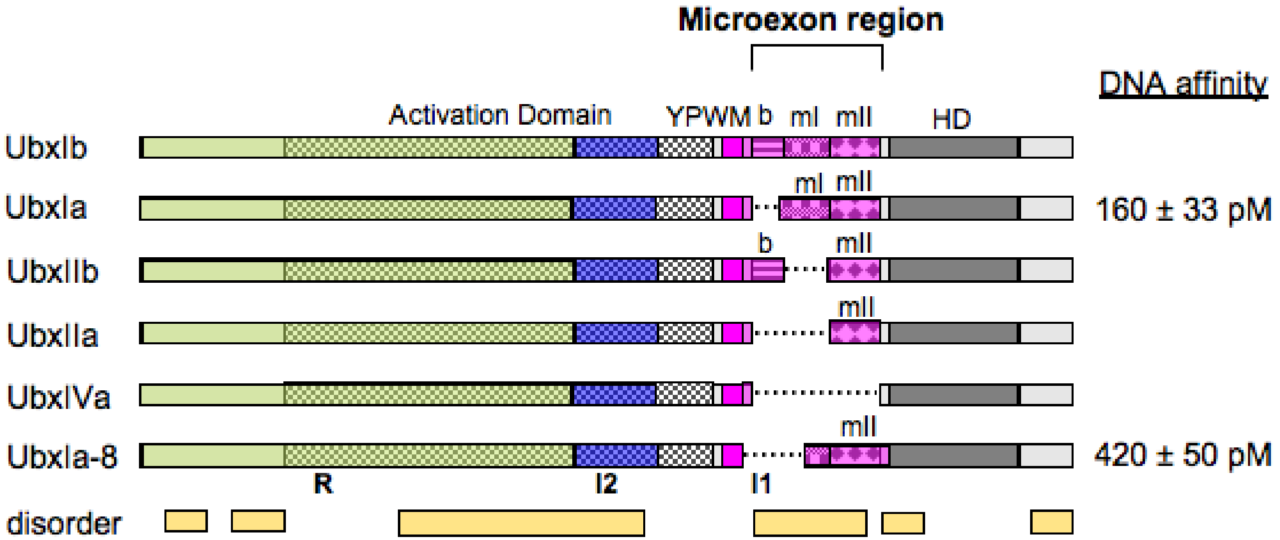
Daughdrill (2007) *J Mol Evol* 65, 277

Regulation via fuzzy complexes

Alternative splicing



Ultrabithorax homeotic
transcription factor



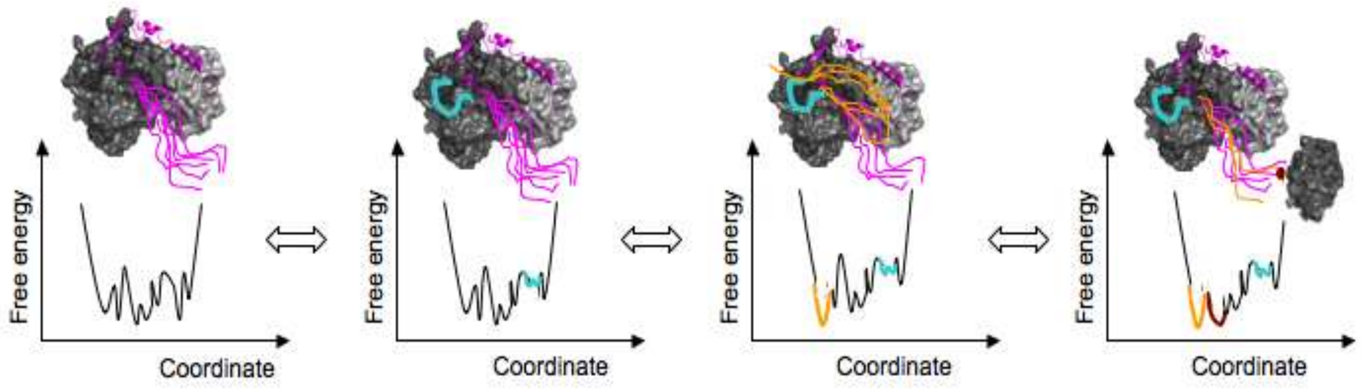
context-specific regulation

Liu (2009) *J Mol Biol* 390, 760

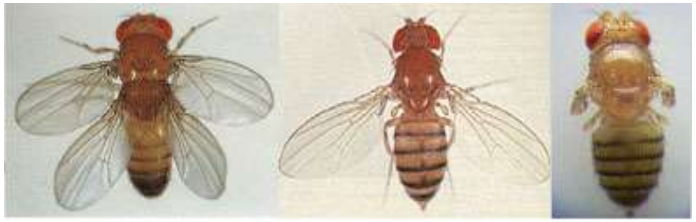
Fuzzy protein-DNA complexes

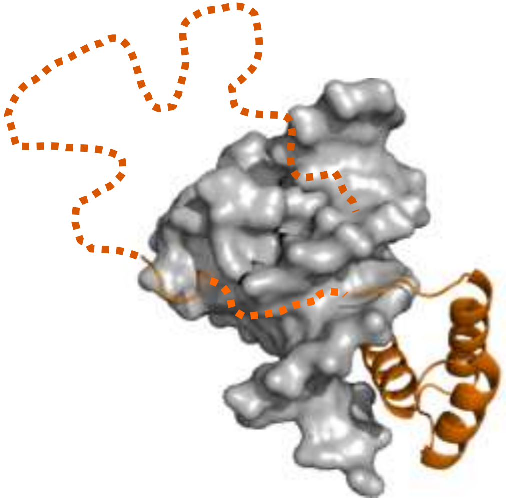
- dynamic protein-DNA recognition
- structurally ambigous regions as regulatory targets
- isoform-specific regulation of DNA binding

Allostery in fuzzy complexes



changes in regulatory ID regions of Ubx





Ma and Nussinov (2011) *Structure*

Fuxreiter (2011) *Mol Syst.Biol*

Fuzziness - perspectives

♦ functional complexity

♠ ultrasensitivity

♠ sequence robustness

♥ evolution of new functions

thanks

Peter Tompa

Sarah Bondos

Agnes Toth-Petroczy

Dan Tawfik

Madan Babu

Regulation via fuzzy complexes

Monod-Wyman-Chageux model

Koshland-Nemethy-Filmer model

QuickTime™ and a
decompressor
are needed to see this picture.

James & Tawfik (2003) *TiBS 28, 361*

Dynamic DNA readout

Dynamic activation in flexible, folded proteins

CAP

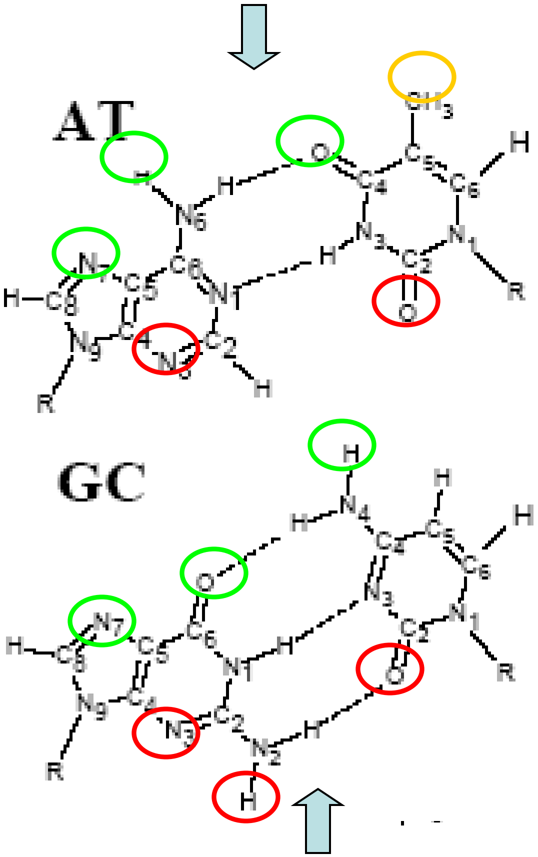
- not only the interface
- global property
- modulation of dynamics
- transient interactions

QuickTime™ and a
decompressor
are needed to see this picture.

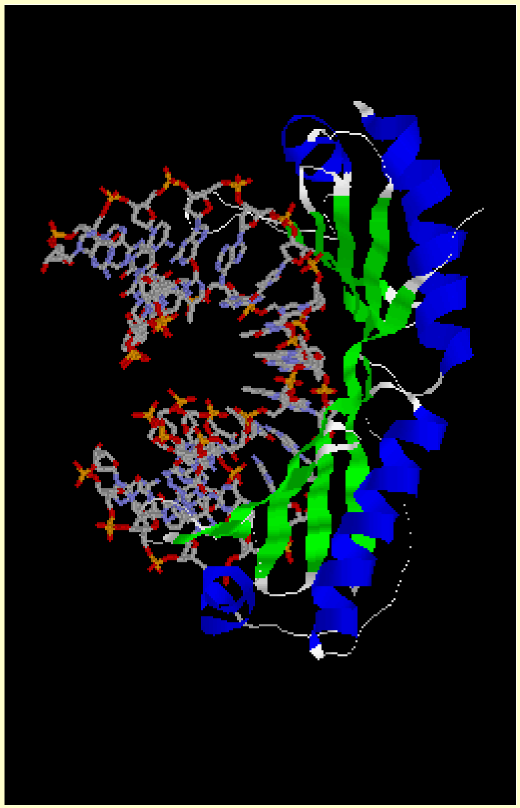
QuickTime™ and a
decompressor
are needed to see this picture.

Specific DNA recognition

specific H-bonds

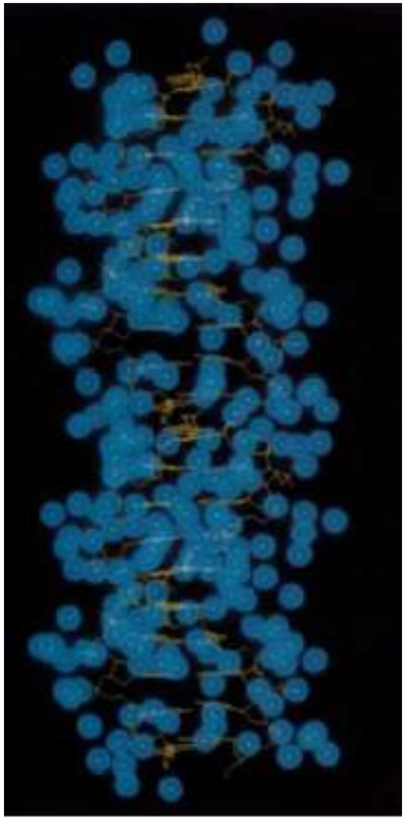


phosphate contacts



TBP -DNA

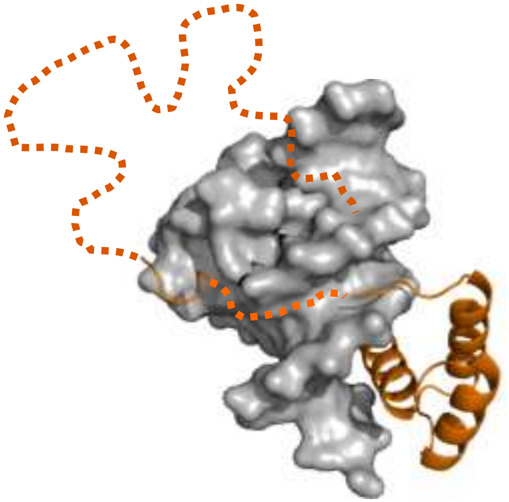
water



Trp repressor -DNA

Conservation of dynamic character - not sequence

Ubx: conservation of regions with predicted disorder characteristics



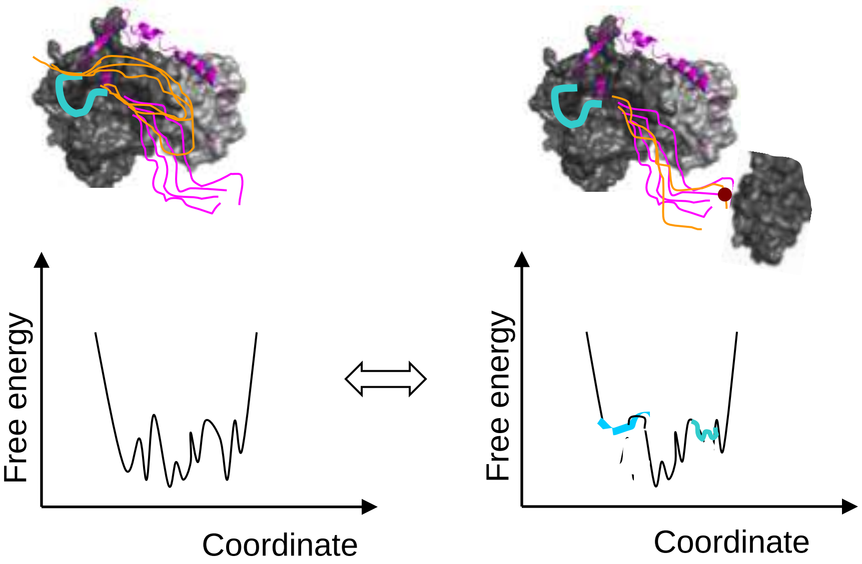
Liu (2008) *J Biol Chem* 283, 20874

Regulation via fuzzy complexes

Monod-Wyman-Chageux model

Koshland-Nemethy-Filmer model

QuickTime™ and a
decompressor
are needed to see this picture.



QuickTime™ and a
decompressor
are needed to see this picture.

Dynamic? allostery

James & Tawfik (2003) *TiBS 28, 361*

Fuxreiter (2011) *Mol Syst.Biol*

Evolutionary aspects

Interaction of HoxA11 and Foxo1a

resurrected ancestors

- adaptive changes only in HoxA11
- binding HD is invariant

Foxo1a

ancestral HoxA11

HoxA11/Foxo1a
complex

Brayer (2011) *PNAS*

Fuzzy complexes

Fuzziness: Structural disorder in protein complexes
Landes/Springer 2011


Advances in Experimental Medicine and Biology 725

Monika Fuxreiter
and Peter Tompa *Editors*

Fuzziness

Structural Disorder in Protein Complexes

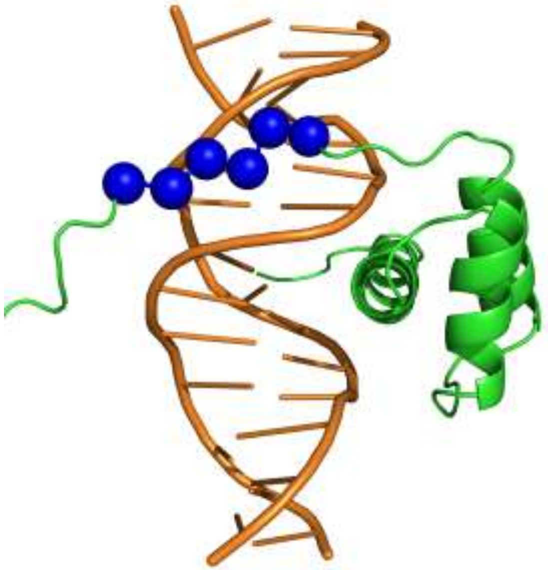
LANDES BIOSCIENCE

Springer


Table of Contents

ID in DNA binding - direct role

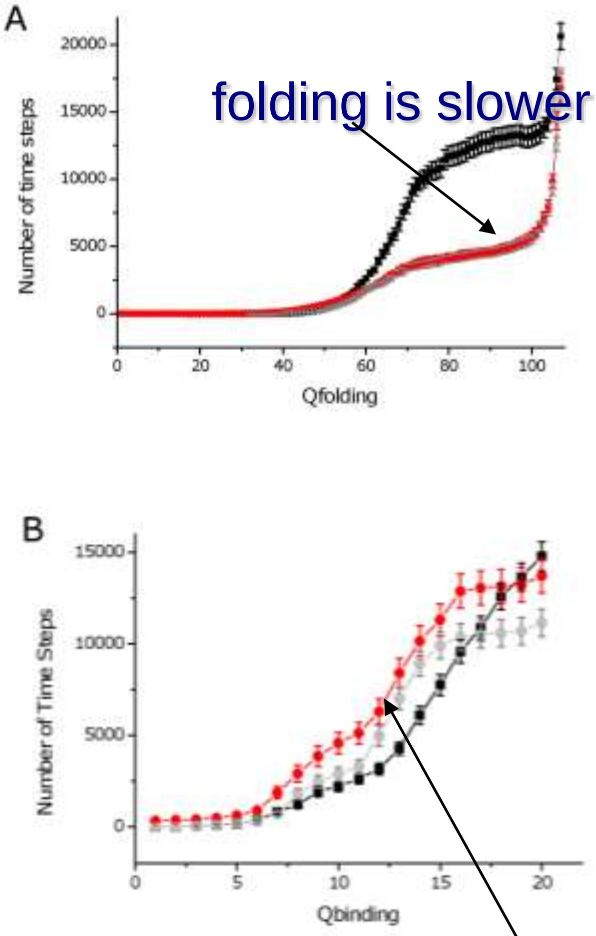
Homeodomain tails



NK2 - DNA

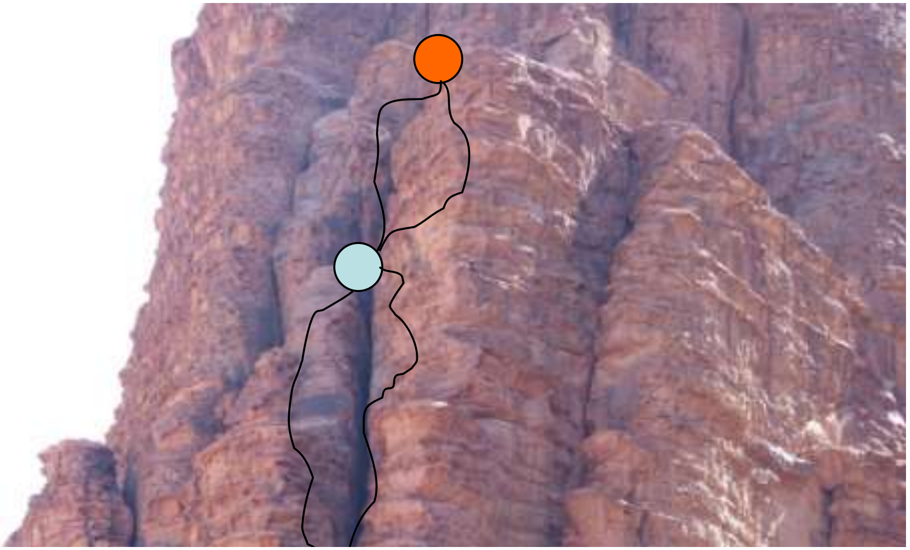
- coarse-grained simulations

Toth-Petroczy (2009) *J Am Chem Soc*, 131, 15084

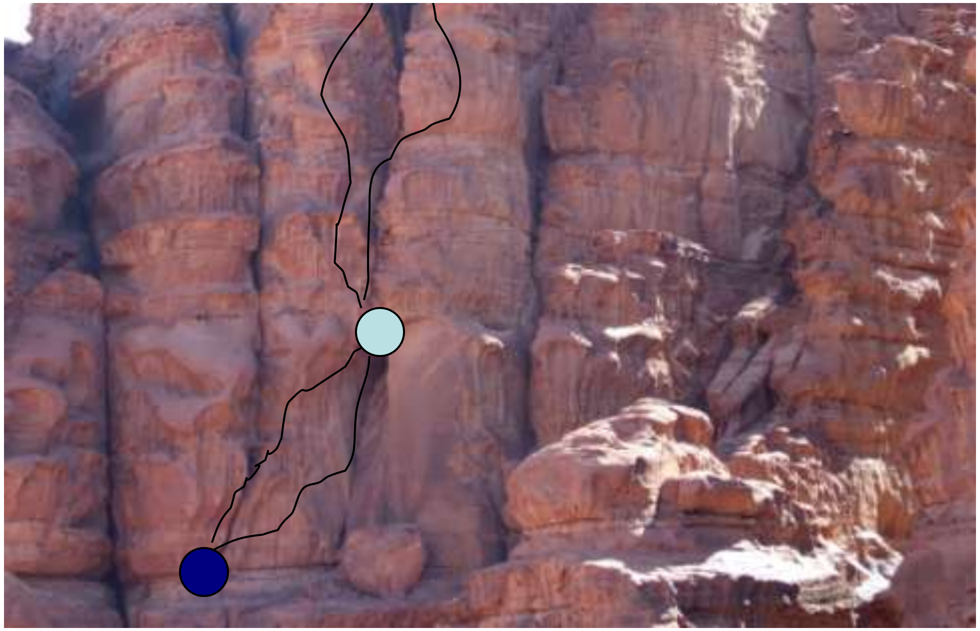


folding is slower

binding is faster

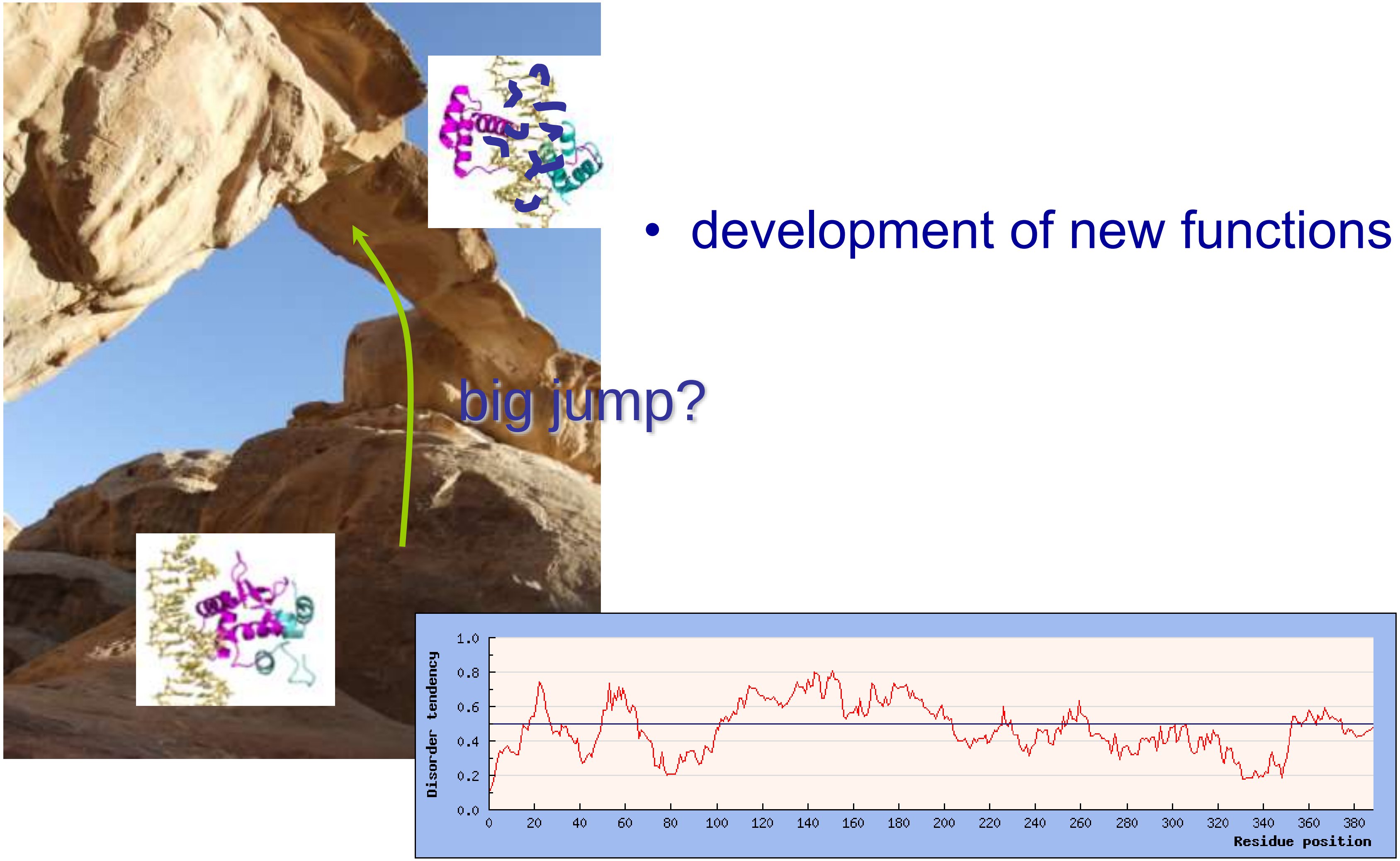


Function and evolution



IDPs - from an evolutionary perspective

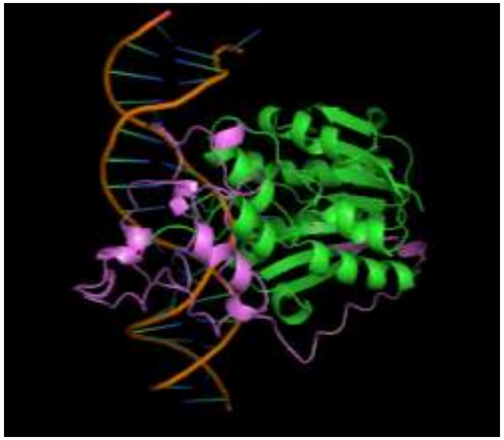
- development of new functions



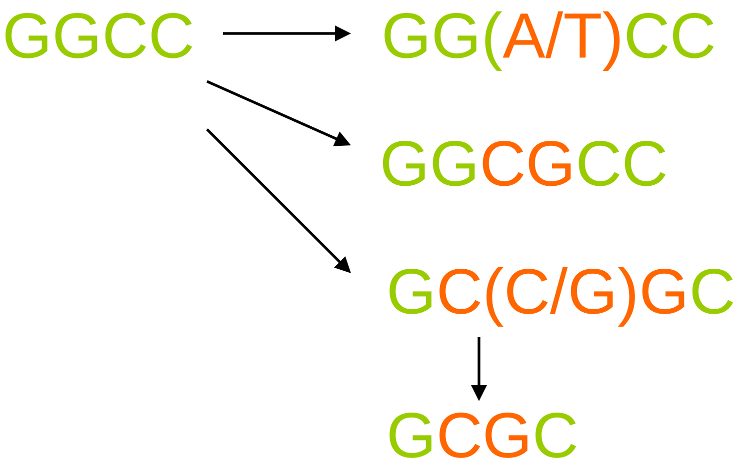
IDPs - from an evolutionary perspective

development of new functions

alter methyltransferase specificity



MHaeIII



GGCC

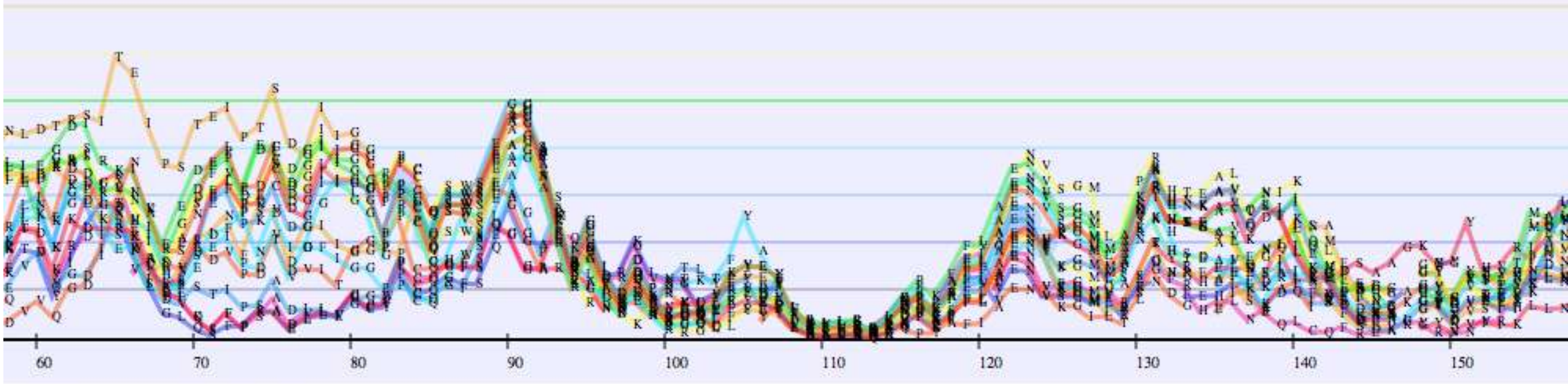
GG(A/T)CC

GGCGCC

GC(C/G)GC

GCGC

POD alignments - GGCC MTases



```
coloring scale is absolute.
........70........80........90.......100.......110.......120.......130.......140.......150.......160.......170.......180....
--VID--VREIQSFPSADVLVGCYPCQGFSQGGARQSSRGVNYLYR-EFVRALKRIKPKAFIVENVSGMQRSDFRHLLNSQLCQFRLAGYRVNYRVID-VS-DFGVPQERKRLIFVGVRSDLGEV
DYILGDISGLQSFPSAELLIGCYPCQGFSQGGARKADRKINTLYL-EFARALSKIKPKAFIVENVSGMVRRN-FEHLLKDQFKVFEEA-GY-TVSSQILNASHYGVSQDRKRIFIVGIRKDYG-
DYRLGSVADIKSFPKAELLVGCYPCQGFSQGGAREANRNINYLYL-EFLRALQQIQPKAFIVENVSGMIRSTYRHLLDDQISKFSAAGKYGYHVRPAVMNAQDFGVAQERKRIIIVGIRKDLGV
VQGDISKIDVSTIPSADILLGGWPCQGFSLAGPRQLDDSRNTLYK-YYVKYLEEKKPLAFVGENVKGMLTLGNG-EIFKAIISDMDKKGYT-LFYKLLNAKDYSVPQNRERVIIVGFRNDLGIT
VQGDIAKIDYSDVPDTDVITGGFPCQGFSLAGPRKINDERNKLYR-YFVKLVELKQPYAFIAENVKGILTLGDG-EIIEAIIEDFASKGYD-VYPNLVNAADYGVPQDRWRVILYGFRKDLEVK
NKKPIEWLKPSEIPDVVGFIGGPPCQSWSLAGSMCGADDPRGKTFYAYVDLVKEKDPLFFLAENVPGIVSRTHL-PEFKRLVNSFIDIGYN-VEYKVLNAKDY-GVPQDRKRVFIVGYREDLNL
DTRSITEIPSTEIPTSDGIIGGPPCQSWSEAGALRGINDHRGQLFFEYIRILKDKQPSFFLAENVSGILFERHREAFSEIIKQFNEI-GYNVSYYLLNAHD-YSVPQDRKRVIIVGYHVKT-EL
EGDIRKIKEEDFPEEIDGIIGGPPCQSWSEAGALRGIDDARGQLFFDYIRILKSKQPKFFLAENVSGMLANRHNGAVQNLLKMFDGC-GYDVTLTMANAKD-YGVAQERKRVFYIGFRKDL-EI
DRRSIVNIHESEIPDCDGIIGGPPCQSWSEA-CSLRGIKDKRGQLFFDFIRILKAKQPSFLAENVSGMLAPRHTEALTNIKQMFEDA-GYNLSFK-LLNS-SDFKVPQDRQRVFFVGIRKDLGF
DKRSIVNIPADEVPECDGIIGGPPCQSWSEAGAARGIKDKRGQLFYDFIRILEAKQPKFFLAENVSGMLISKHTEALEGIKELFRNA-GIGYELSFQMLNASDYNVPQDRKRVFFIGIRKDLNF
KGDIREIASGDFPDDCDGIIGGPPCQSWSEAGALRGIEDSRGQLFYEYIRLLRDKQPKFFVAENVSGMMAKRHSEAVQNIISHFAGA-GRGYNVFIQTLNANDFQVPQDRKRVFYIGFRKDLGI
EKDIKNLQTNEINFSVDGIIGGPPCQSWSEAGNLKGIDDARGQLFYEYLRLLKELKPKFFLAENVRGMLAQRHEKSVKNILNAFKEC-GYEVNTHLVNAK-DYGVAQERLRVFYIGFRRDLKIN
IEGDIRHILEEDFPNDIDGIIGGPPCHWSEAGALRGINDARGQLFYDYIRILKNKQPKVFLAENVSGMLANRHSDAVKSILNMFDDC-GYDVTVNMVNAK-NYGVAQERKRVFYIGFRKDLAIN
IKDDITNVRSNELPDCDGLIGGPPCQSWSEAGSLKGIEDPRGQLFYEYIRILRDKSPKFFLAENVKGMMAKRHNKAVQSIISQFNKA-GYDVFIHLLNAS-DYGVAQDRKRIFYVGFRKDLNIC
IKGDISKISSDEFPKCDGIIGGPPCQSWSEGGSLRGIDDPRGKLFYEYIRILKQKKPIFFLAENVKGMMAQRHNKAVQEFIQEFDNA-GYDVHIILLNAN-DYGVAQDRKRVFYIGFRKELNIN
IKKDIREILSEELPKSDGIIGGPPCQSWSEAGSLRGINDPRGKLFYEYIRILKDIQPKFFLAENVKGMLSKRNTEAVKDIIKEFEEA-GYNVFIKLLNAF-DYGVAQDRERVFYVGFRKDLNIS
........70........80........90.......100.......110.......120.......130.......140.......150.......160.......170.......180....
coloring scale is absolute.
```

Fuzziness - perspectives

♦ functional complexity

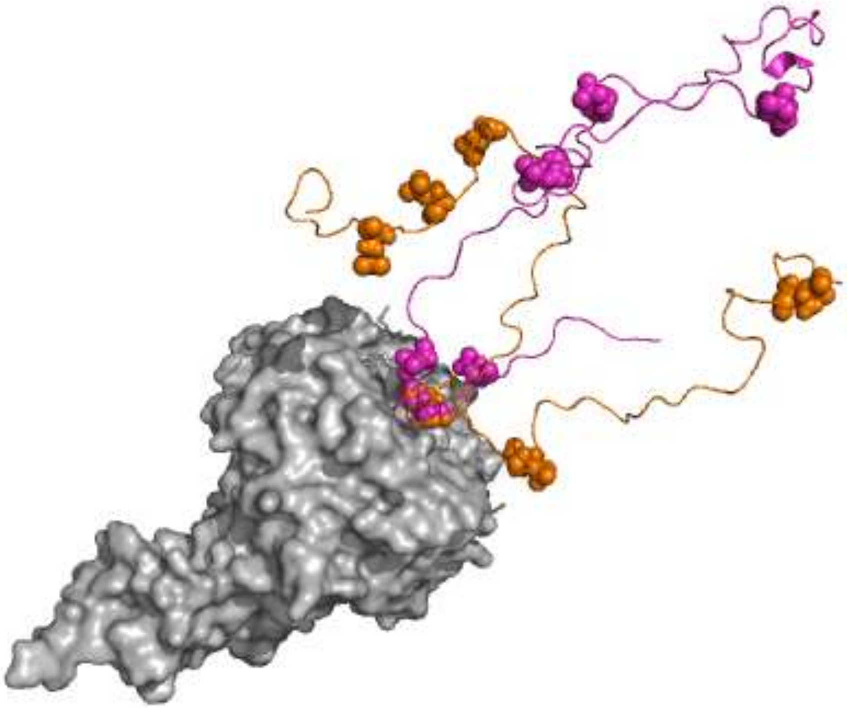
♠ multiple binding sites

♠ sequence insensitivity

♥ evolution of new functions

Disorder in IDP complexes

exchanging phosphorylation sites



Sic1 - Cdc4

Mittag (2008) PNAS 105, 17772

Fuzziness - some perspectives are challenging, but less dangerous

Fuzziness in DNA recognition

- likely to be ubiquitous within the transcription machinery
- new approach to fine-tune DNA binding
- PT modifications
- multimerization

QuickTime™ and a
decompressor
are needed to see this picture.

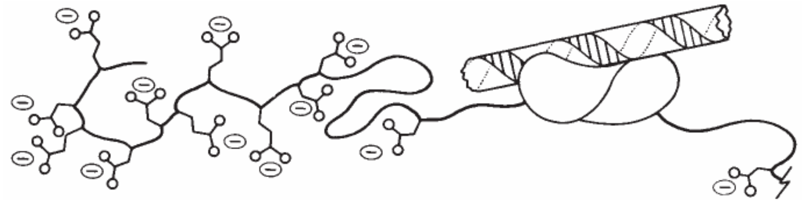
Biochemistry (2006) 45, 6873

Fuzziness- variable sequences

Resistance to sequence scrambling

- GCN4, EWS
- histone tails
- Ure2, Sup35

QuickTime™ and a
decompressor
are needed to see this picture.



Sigler et al. (1988) *Nature* 333, 210
Hope et al. (1988) *Nature* 333, 635

Ng et al. (2007) *PNAS* 104, 479
Ross et al. (2005) PNAS 102, 12825

Fuzziness - opposite functions

- inhibition G1→S transition
- Y88 dissociation

Disorder in bound state

- SCF/Skp2 binding
- polyubiquitination
- p27 degradation

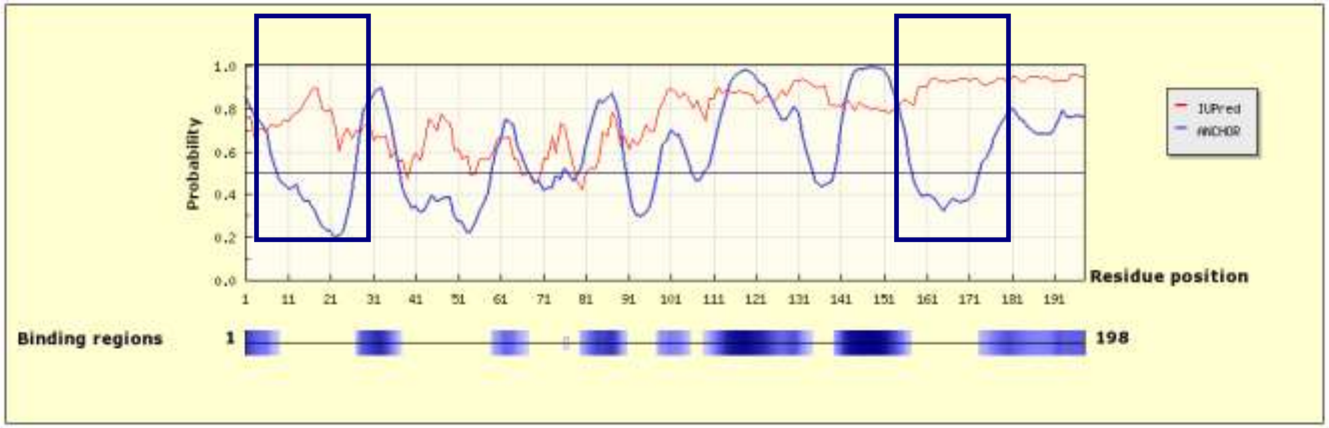
$p27^{Kip}$ - Cdk2-Cyclin

Galea (2008) *J Mol Biol* 376, 827

Fuzziness - multiple functions

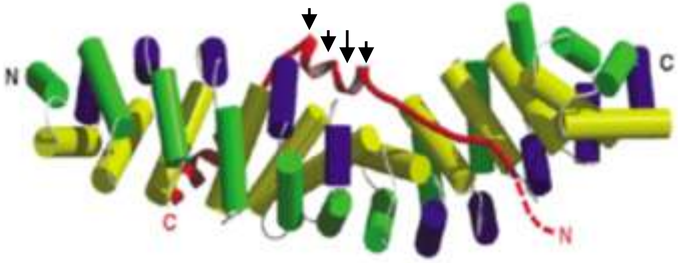
p27^{Kip} - Cdk2-Cyclin

QuickTime™ and a
decompressor
are needed to see this picture.

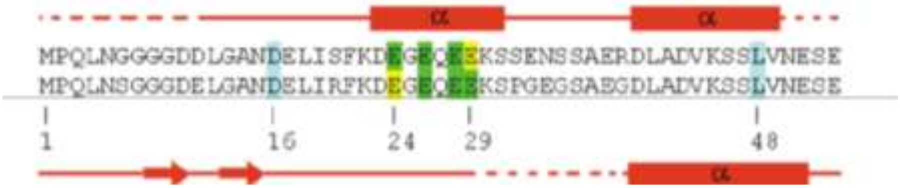


Meszaros (2009) *PLoS Comp Biol* 5, e1000376

Really unambiguous?



Tcf4 - catenin



Tcf4 α-helix

Tcf3 β -strand

mutagenesis

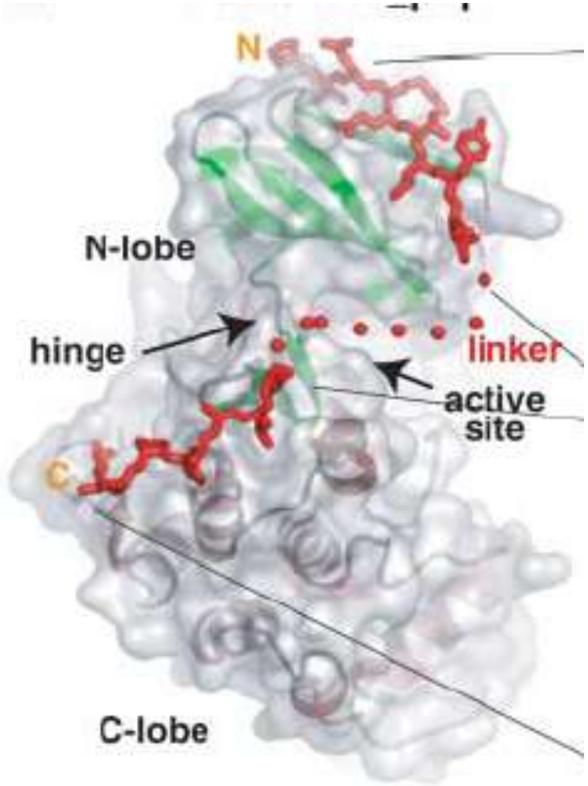
Glu-24, Glu-26, Glu-28, Glu-29

all eliminate binding

Structural polymorphism

Graham et al. (2001) *Nat Struct. Biol.* 8, 1048

Really unambiguous?



Ste5 - Fus3

absence of linker

- no measurable K_d

presence of linker

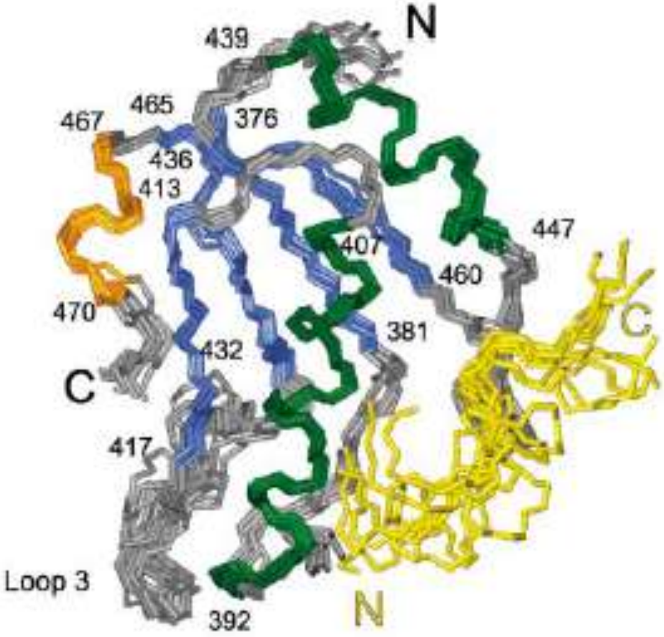
- $K_d = 4\mu M$

linker - functionally important

Dynamic complex

Bhattacharyya et al. (2006) *Science.* 311, 822

Really unambiguous?



SF2 - UA2F^{65}

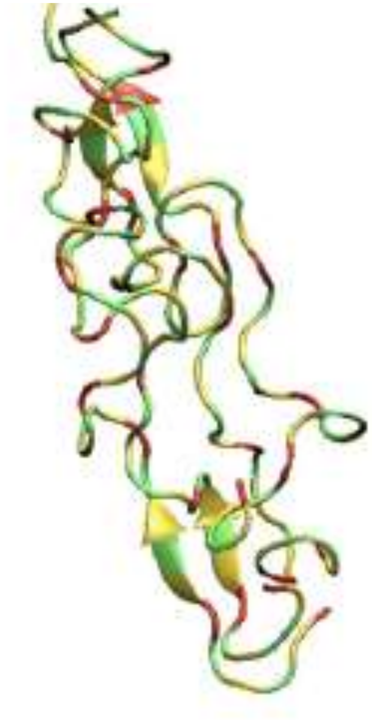
	K_d
full protein	11.8 nM
bound segment	23.8 nM
physically contacting residues	55.6 nM

critical, transient interactions

Selenko et al. (2003) *Mol Cell* 11, 965

Really unambiguous?

$(GVPGV)_7$



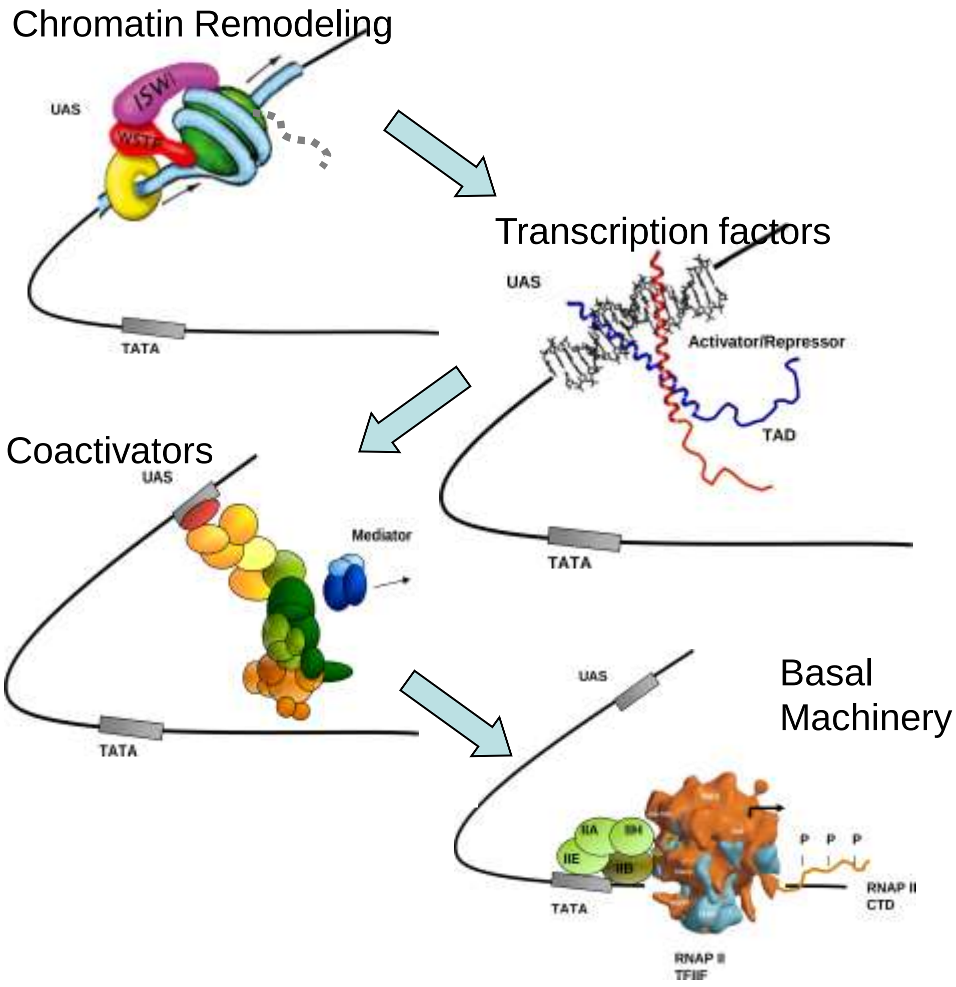
elastin

NMR: random coil-like secondary chemical shift for backbone

disorder in the bound state

Pometun et al. (2004) *J Biol Chem* 279, 7982

Communication via ID



Chromatin Remodeling

Transcription factors

Coactivators

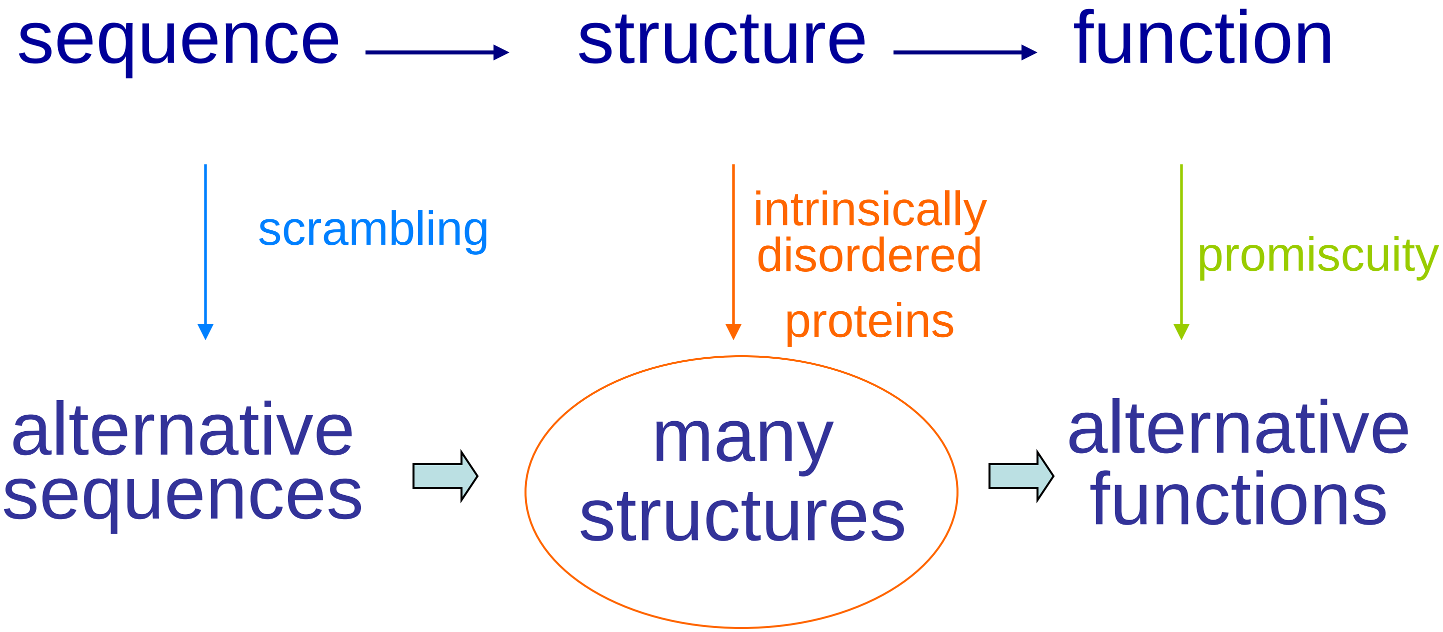
Basal
Machinery

Regulation

- protein-protein interactions
- post-translational modifications
- alternative splicing

Fuxreiter (2008) *Nat Chem Biol* 4 728

New perspective



sequence → structure → function

scrambling

intrinsically disordered proteins

promiscuity

alternative sequences

many structures

alternative functions

IDP recognition

folding coupled binding

restore structure-function paradigm

NO folding coupled to binding – disorder in bound form

disorder-function paradigm

Why fuzzy …?

Regulation

QuickTime™ and a
decompressor
are needed to see this picture.

- protein-protein interactions
- post-translational modifications
- alternative splicing

Par B

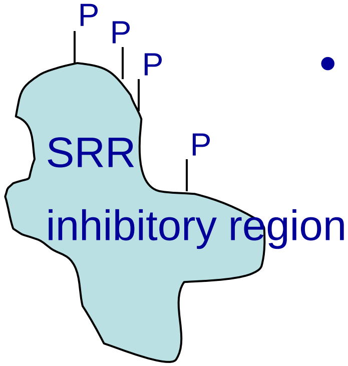
QuickTime™ and a
decompressor
are needed to see this picture.

Fuxreiter (2011) *TiBS 36*, 415-423

Fuzziness in DNA recognition

Ets-1 transcription factor

QuickTime™ and a
decompressor
are needed to see this picture.



- 100-1000 fold affinity decrease

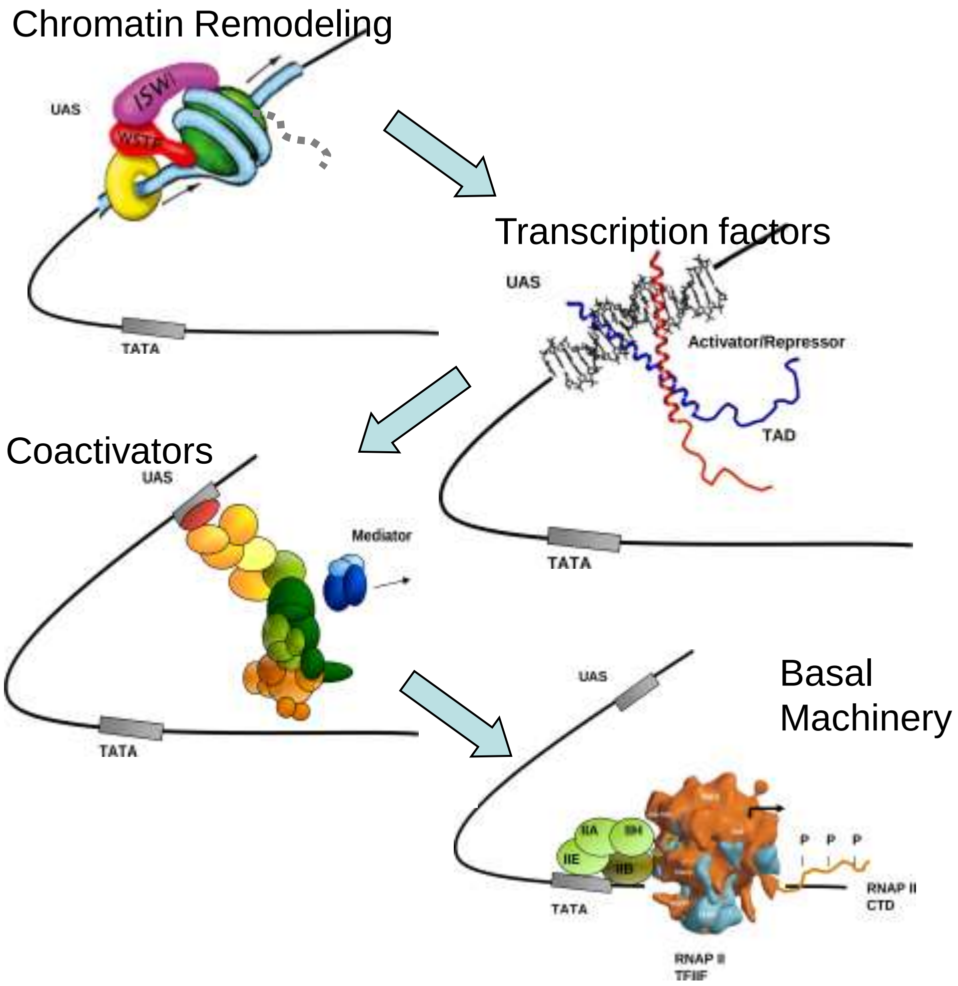
QuickTime™ and a
decompressor
are needed to see this picture.

- does not gain structure in complex
- no sec. str. increase upon phosphorylation

decreasing flexibility

Pufall (2005) *Science* 309, 142
Lee (2005) *J Mol Biol* 382, 1014

ID in the transcription machinery


Chromatin Remodeling

Transcription factors

Coactivators

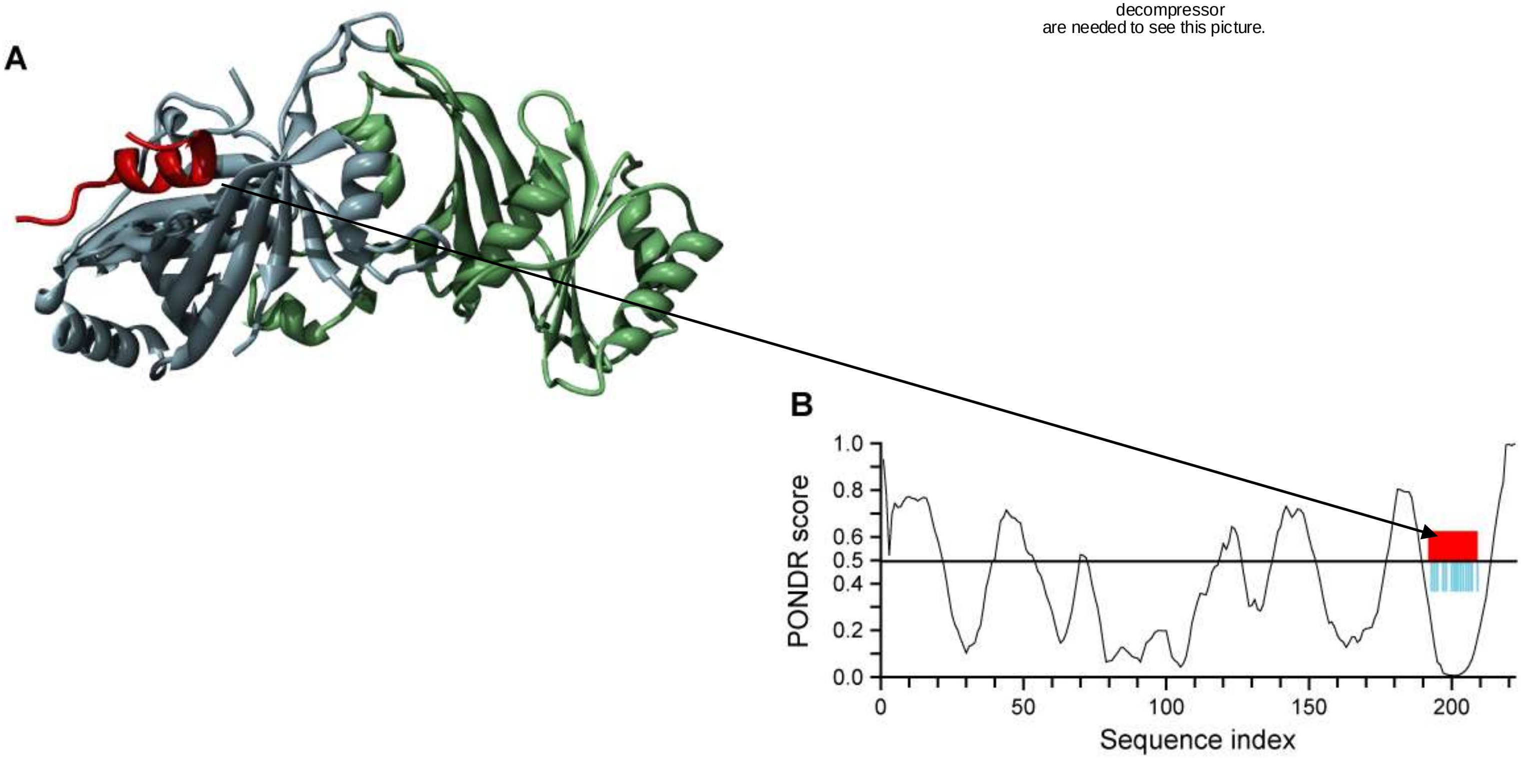
Basal Machinery

Fuxreiter (2008) *Nat Chem Biol* 4 728

Mediator

Med8/Med18/Med20

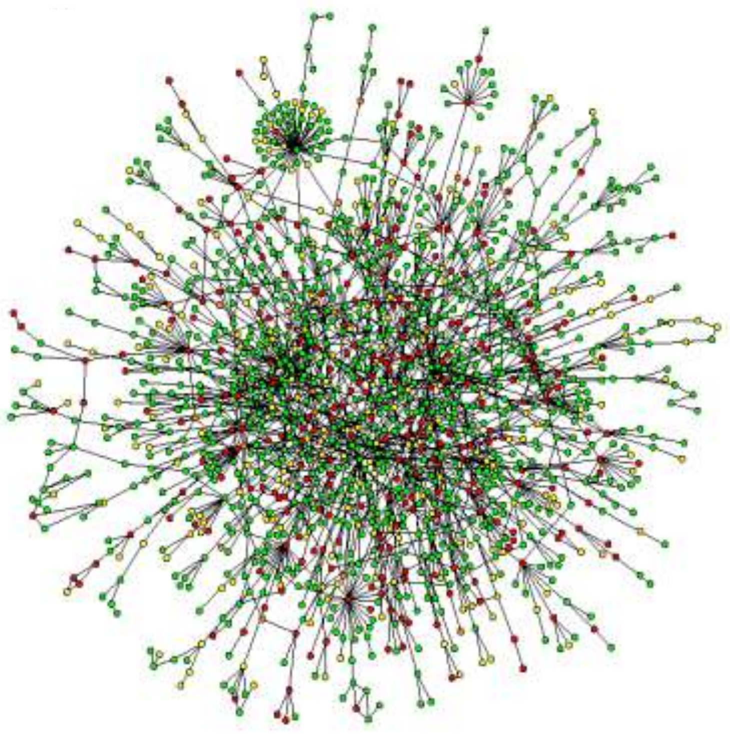
QuickTime™ and a
decompressor
are needed to see this picture.



Laviere (2006) *Nat Struct Biol 13, 895*

Toth-Petroczy (2008) *PLoS Comp Biol* 4, e1000243

Fuzziness - perspectives

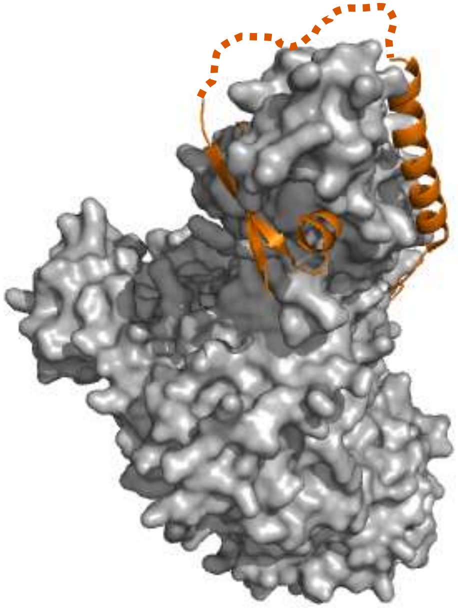


- complex functions
- fuzzy interactome

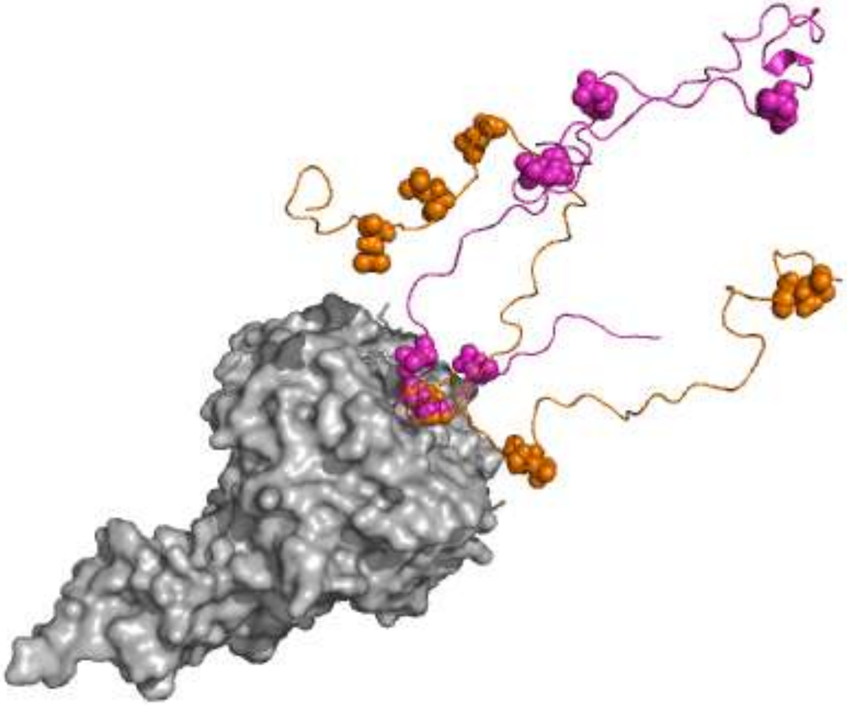
- stochastic models
- predictability

Welch (2009) *TiBS* 34, 1-2.

Disorder in IDP complexes



UPF2 - UPF1



Clerici (2009) EMBO J 28, 2293