

Dr. Xiao Li

now postdoc at Stanford

co-supervised w/
Howard Lipshitz



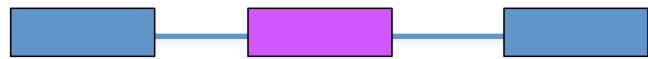
Dr. Hilal Kazan

now faculty at

Antalya International University,
Turkey

Post-transcriptional regulation is *ubiquitous* and *substantive*

transcription



VS



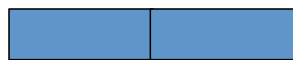
alternative poly-A sites

1000s of genes have alternative poly-A sites

splicing



VS



alternative splicing

80-95% of mammalian genes and 10-50% of exons are alternatively spliced

export



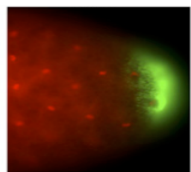
Nucleus

Cytoplasm

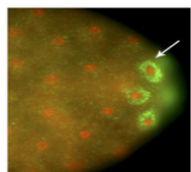
localization

(LeCuyer et al, Cell 2007)

70% fly oocyte mRNAs localized

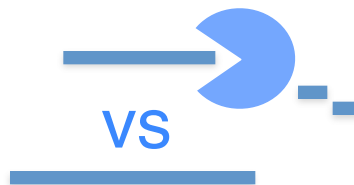


VS



mRNA stability

~50% of transcripts have conserved miRNA seed regions

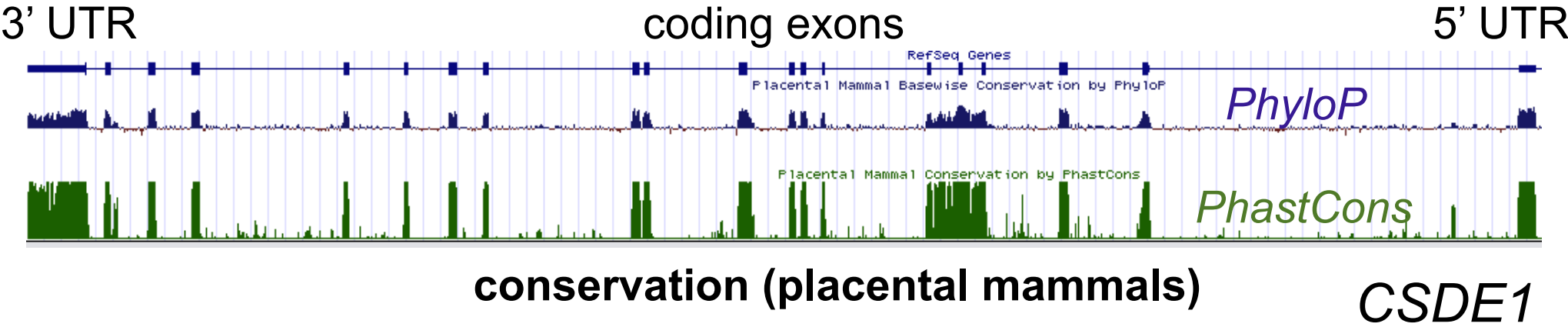
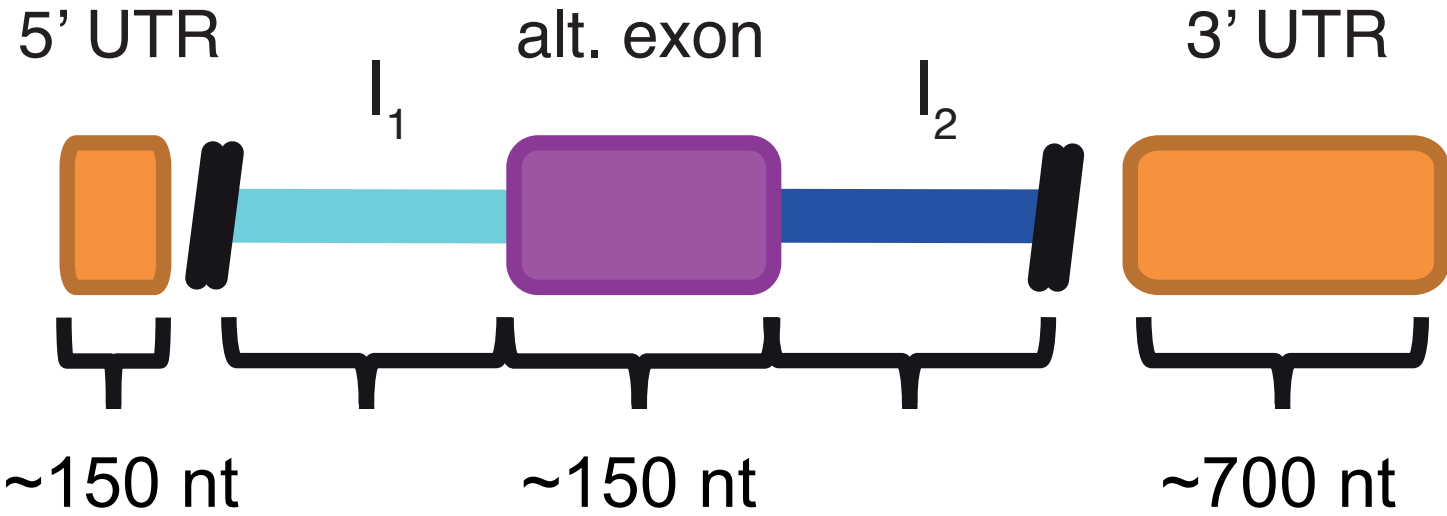


mRNA translation rate

(Schwanhäusser et al, Nature 2011)
explains 20-40% of protein abundance

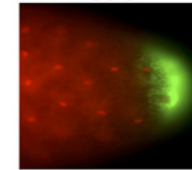
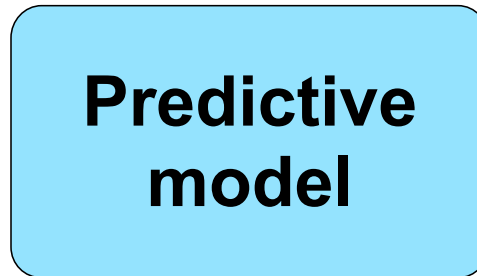


UTRs of human mRNAs are compact and information-rich

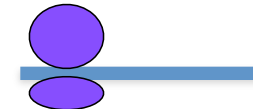
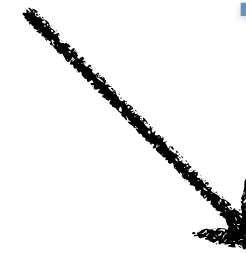
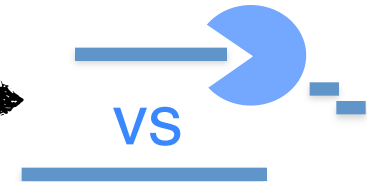
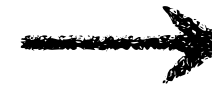
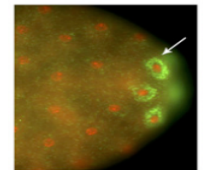


Predictive models of PTR

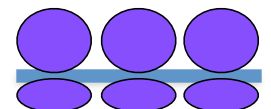
transcript sequence
...CGUUCGCCGCGCU...



VS



VS



Cellular context
(e.g. RBP and miRNA activities)



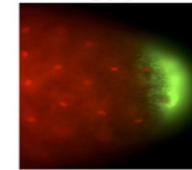
Predictive models of PTR

transcript sequence
...CGUUCGCCGCGCU...

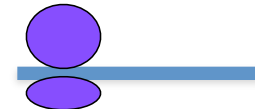
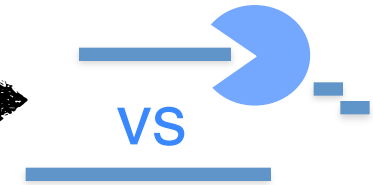
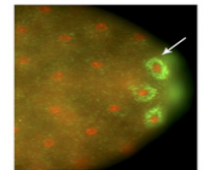
**What's
missing is
RNA-binding
protein (RBP)
motifs**

**Predictive
model**

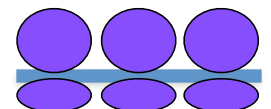
Cellular context
(e.g. RBP and miRNA activities)



vs



vs



Goals

1. Determining specificities of all eukaryotic RBPs
2. Developing models/methods to scan for RBP binding sites in mRNAs

Three types of RBPs

Sequence-specific

Shape-specific

Focus

1. RBPs with many targets; RBPs with a small number of targets are probably different.

2. Binding assays \rightarrow preferences; rather than from a handful of targets to preferences.

Estimate of prevalence:

70%

25%

5%

1) bir

(A stem

Pun

fly)

Puf3

human)

Pum1/



5'



3'

N



Three types of RBPs

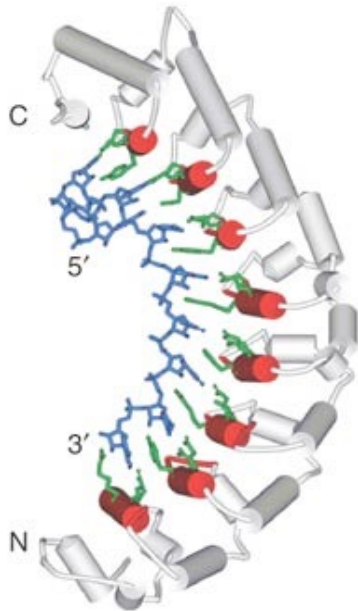
Sequence-specific

1) binds ssRNA

Pumilio (fly)

Puf3p (yeast)

Pum1/2 (human)



Estimate of prevalence:

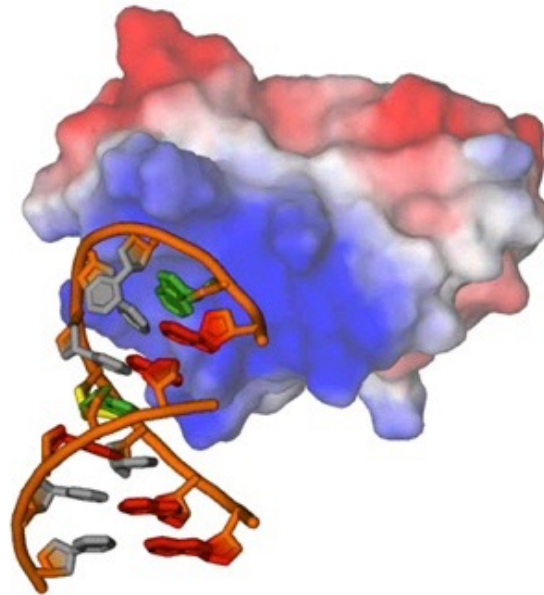
70%

2) binds ssRNA in a *structural context* (e.g. hairpin loop)

Smaug (fly)

Vts1 (yeast)

SAM4DA/B (human)



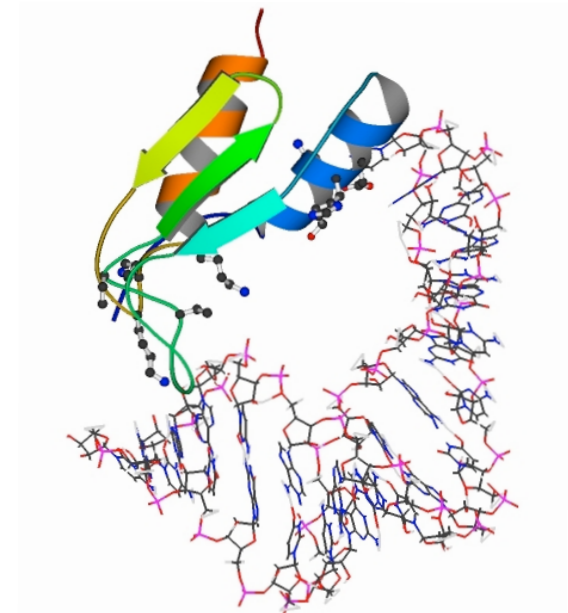
25%

Shape-specific

3) binds dsRNA stem

Staufen (fly)

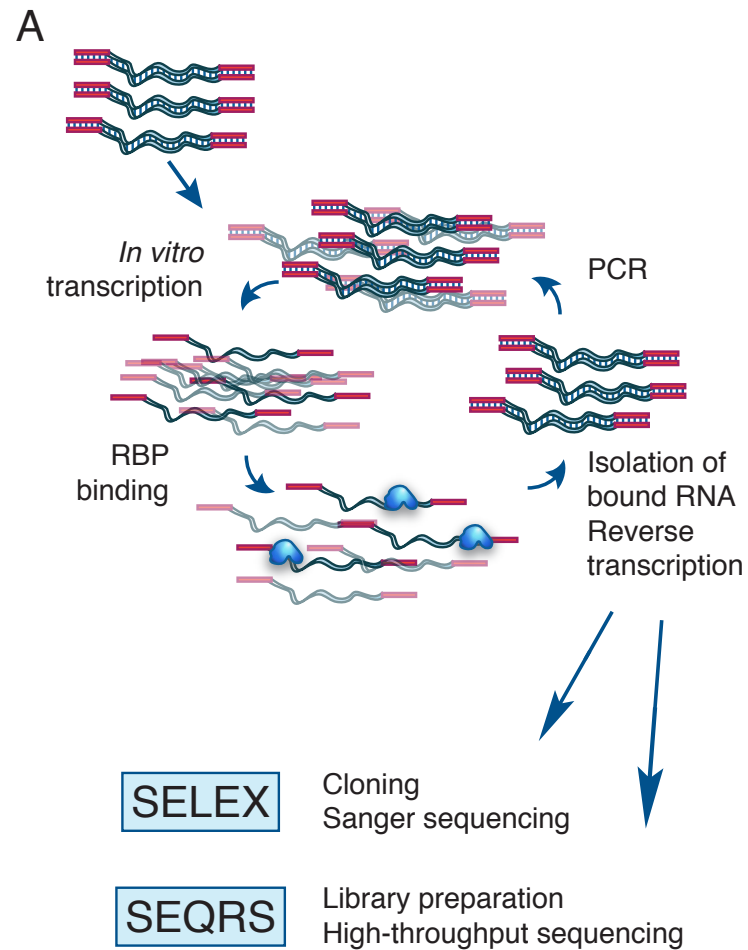
Staufen1/2 (human)



5%

In vitro RBP binding assays pre-2009

SELEX



~70 RBPs

(Cook et al, Brief Funct Genomics 2015)

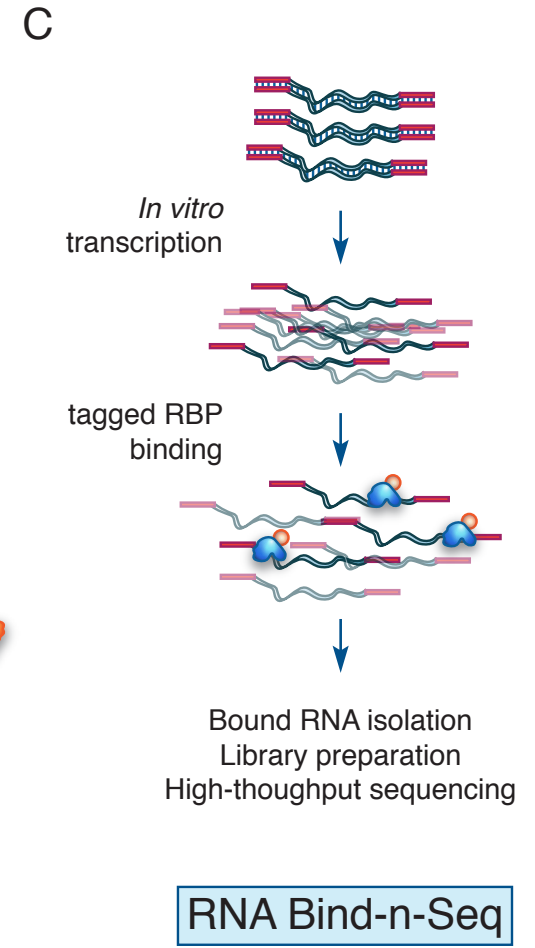
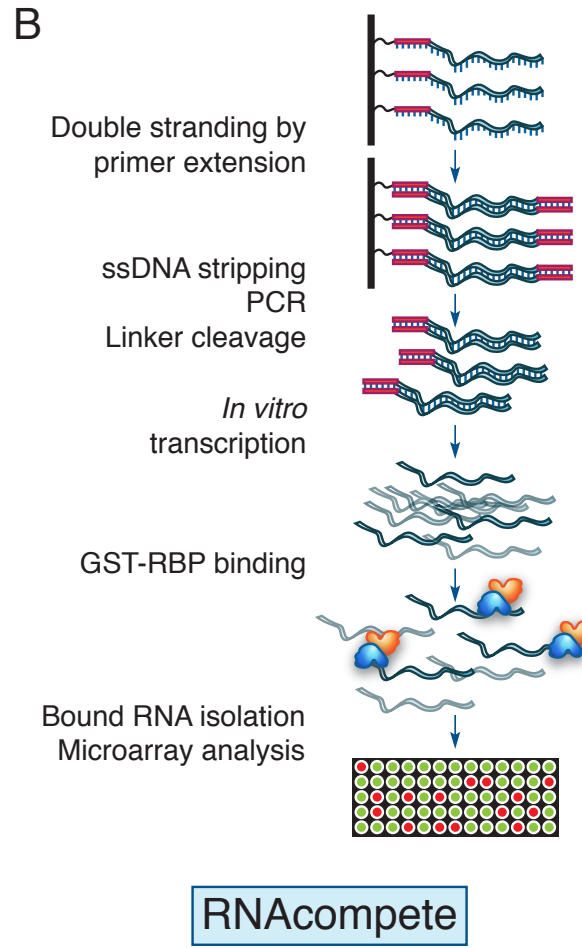
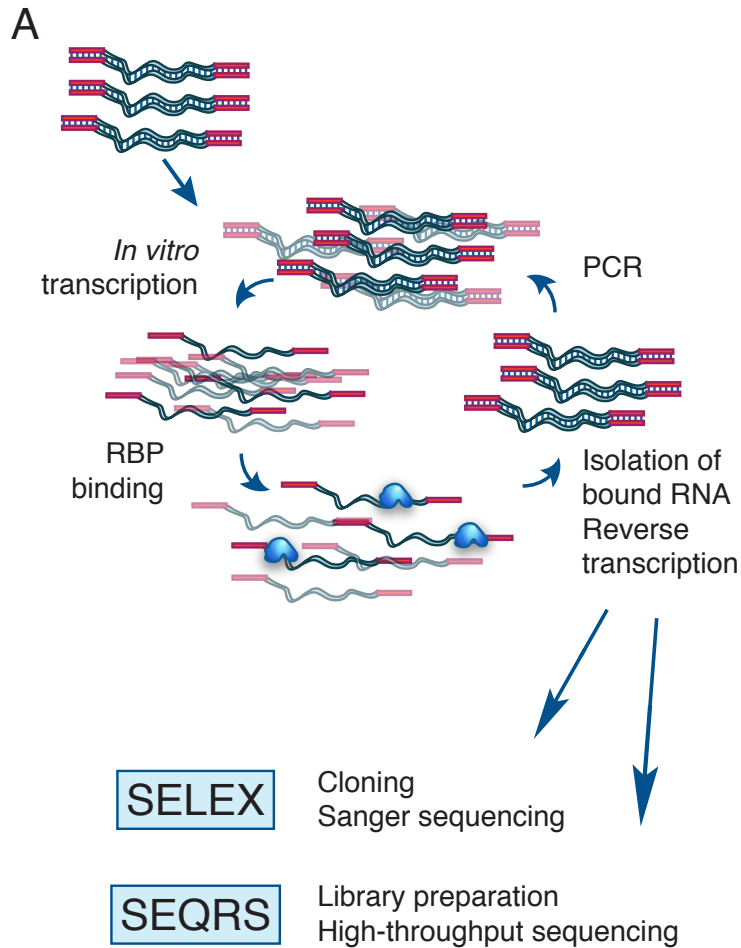


In vitro RBP binding assays

(HT-)SELEX

RNAcompete 2009, 2013

RNA Bind-n-seq 2014



~70 RBPs

~220 RBPs
(~100 unpublished)

3 RBPs
(6 unpublished)

(Cook et al, Brief Funct Genomics 2015)

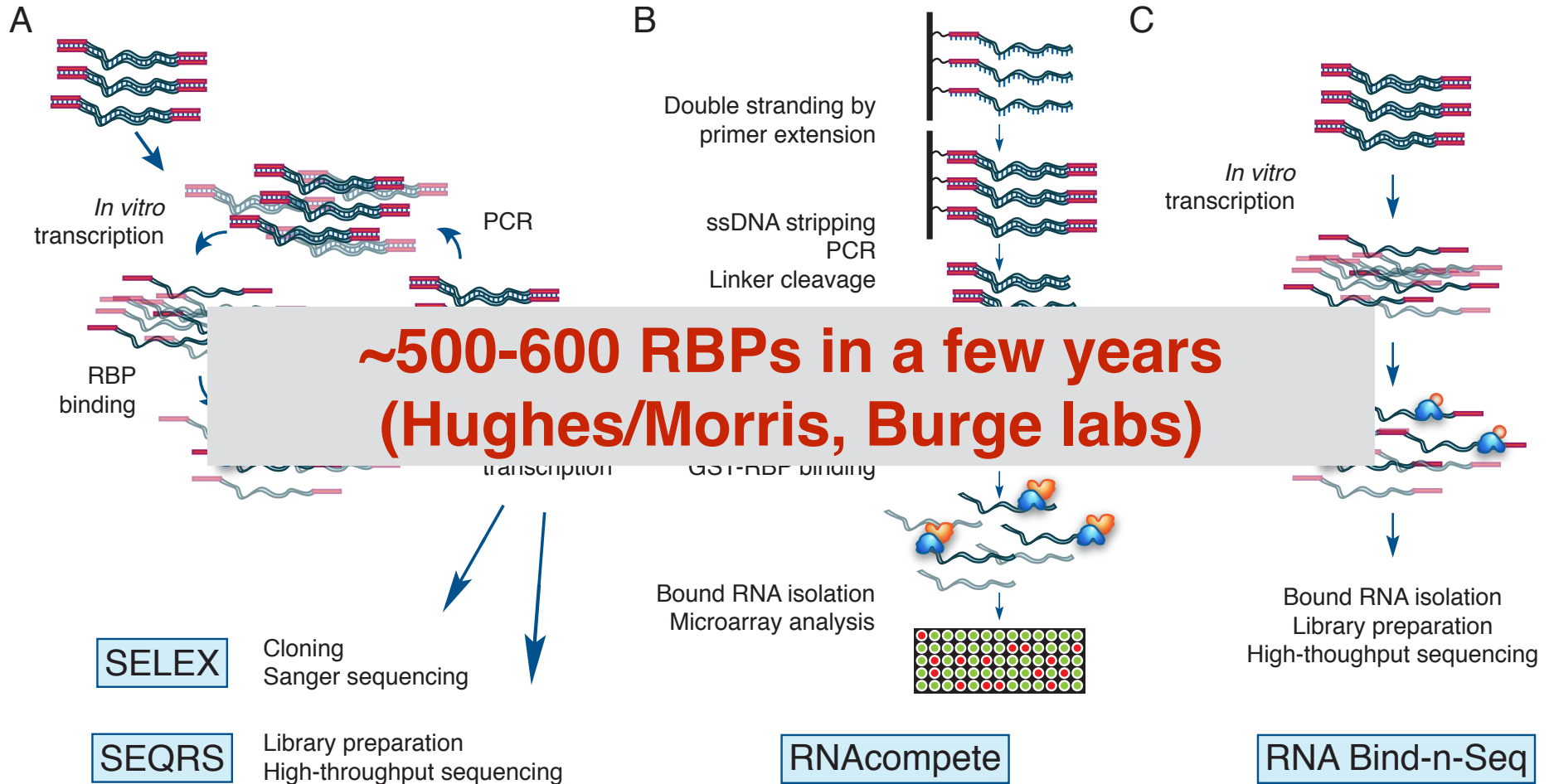


In vitro RBP binding assays

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~70 RBPs

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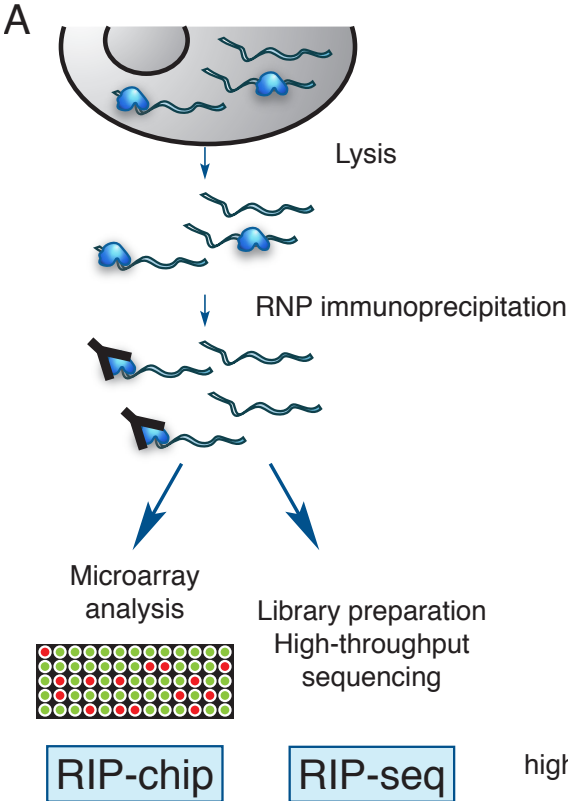
3 RBPs
(6 unpublished)

(Cook et al, Brief Funct Genomics 2015)

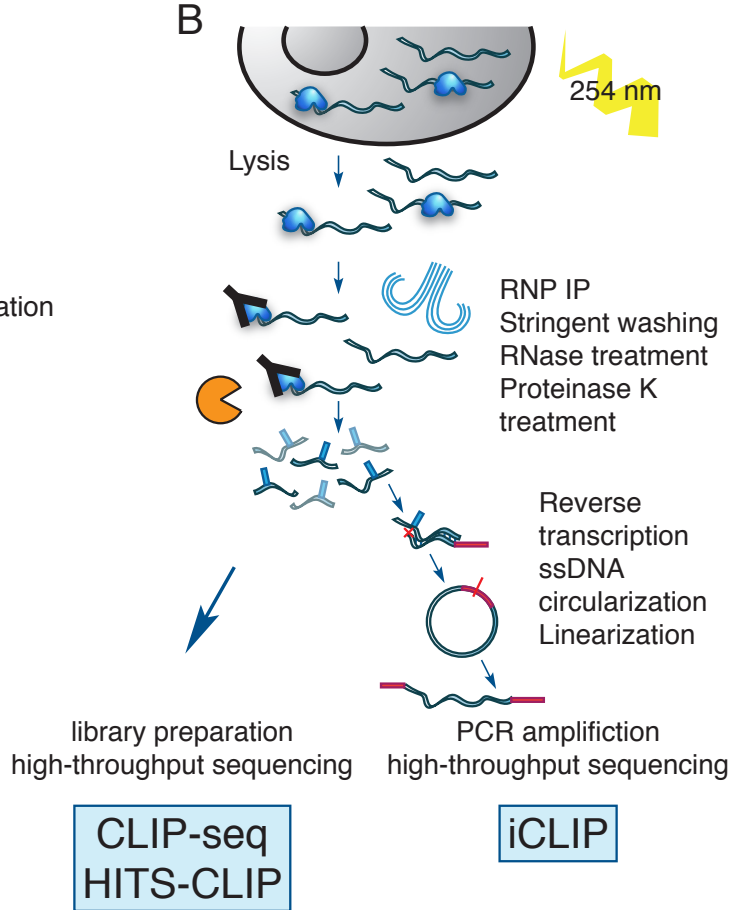


In vivo RBP binding assays

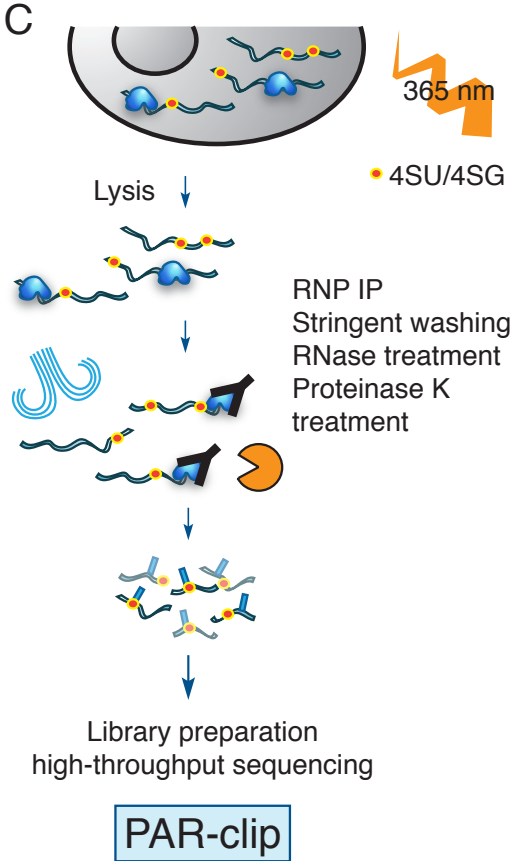
RIP



CLIP



PAR-CLIP

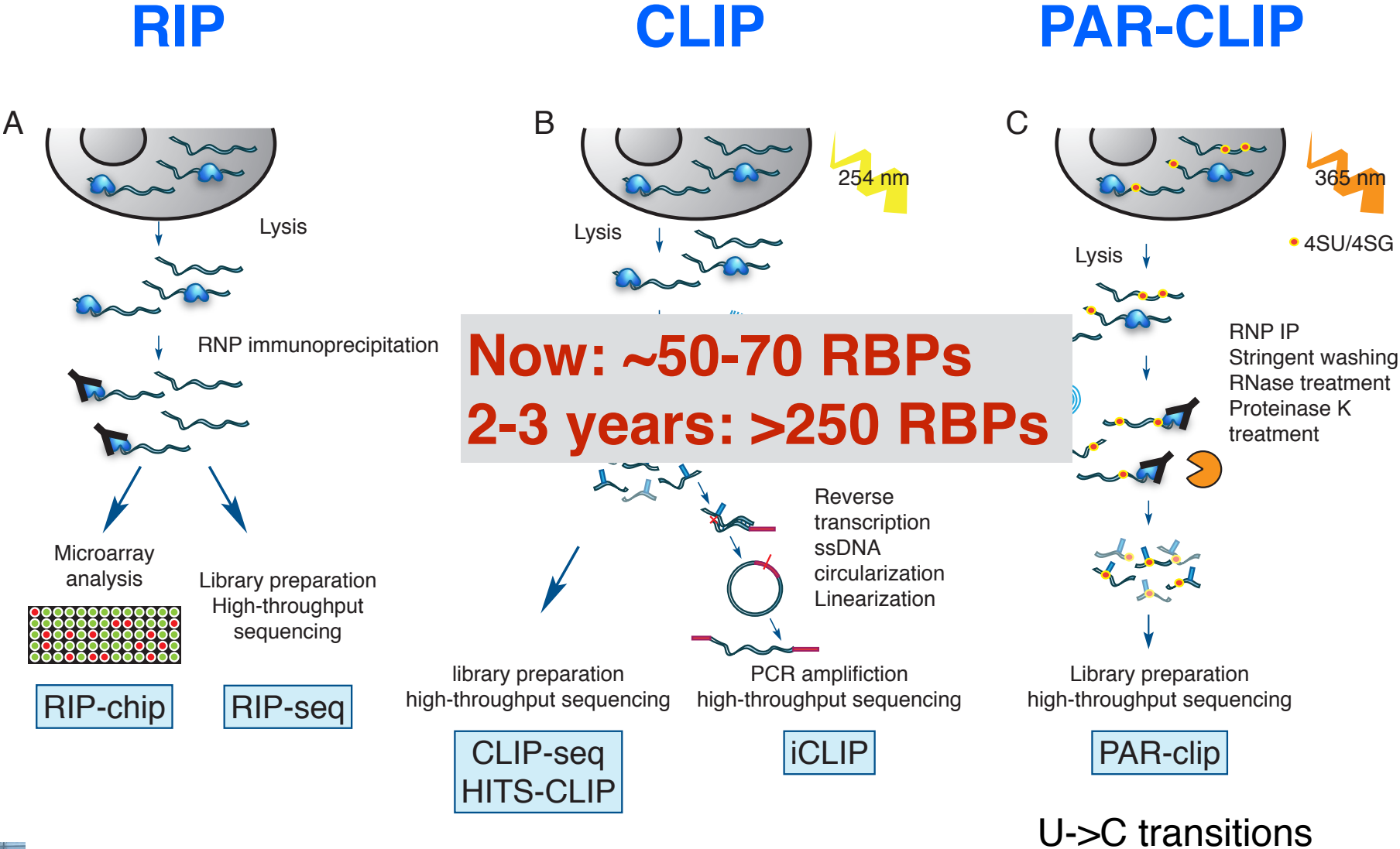


U->C transitions



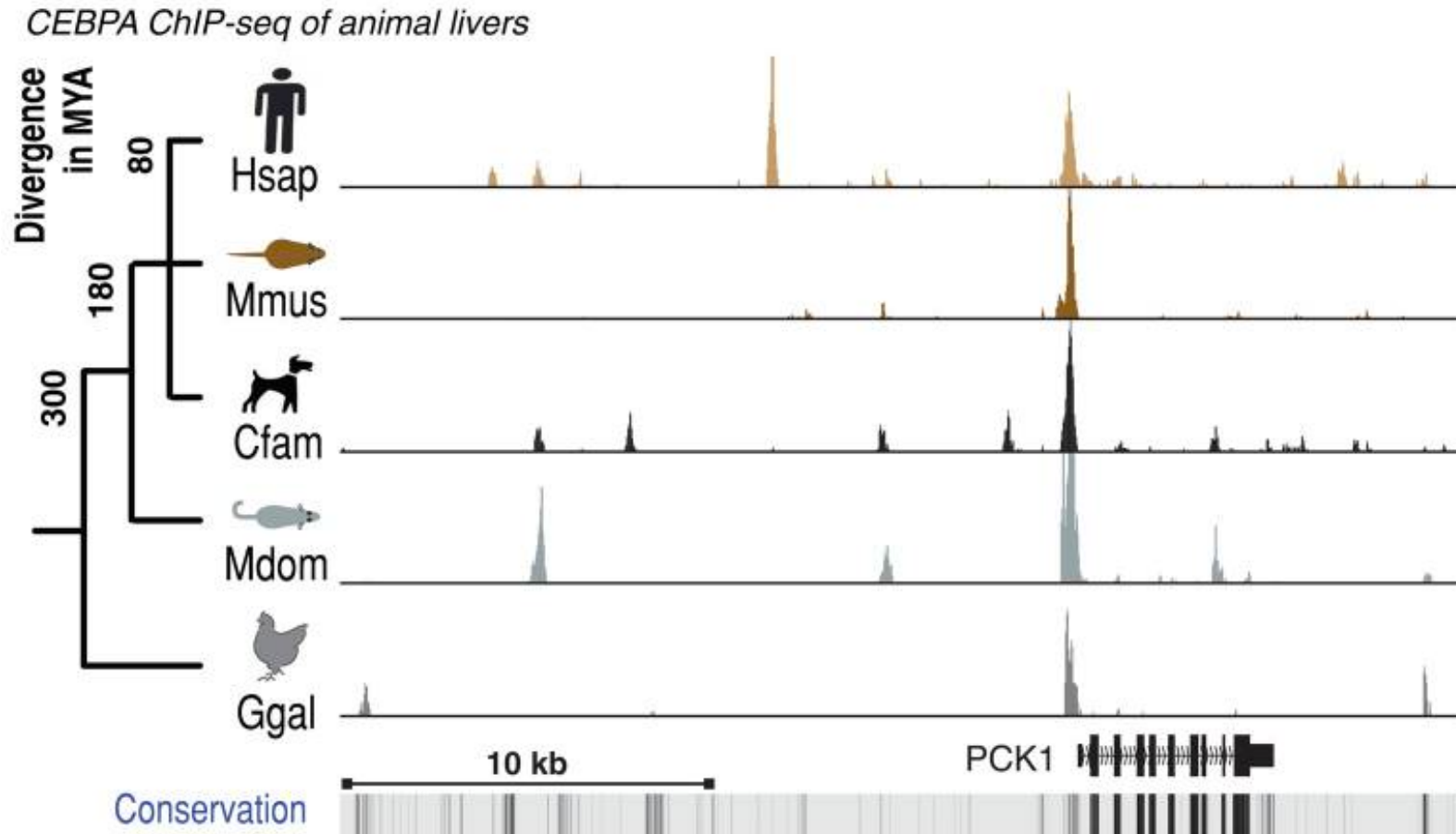
(Cook et al, Brief Funct Genomics 2015)

In vivo RBP binding assays



(Cook et al, Brief Funct Genomics 2015)

Need to consider binding site turnover



Similar turnover likely occurs for RBPs

Implications for RBP binding preferences

1. RBP binding sites need to be “evolutionarily easy” to generate, so either:

a) They have variable affinity, clustered sites

- Sequence-specific ssRBP

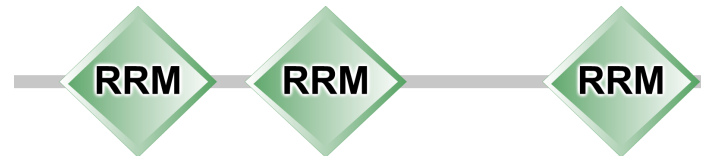
b) They are easy to generate via ‘copy-and-paste’ mechanisms,

- dsRNA binding proteins

Excludes very complex structures for RBPs with many targets?

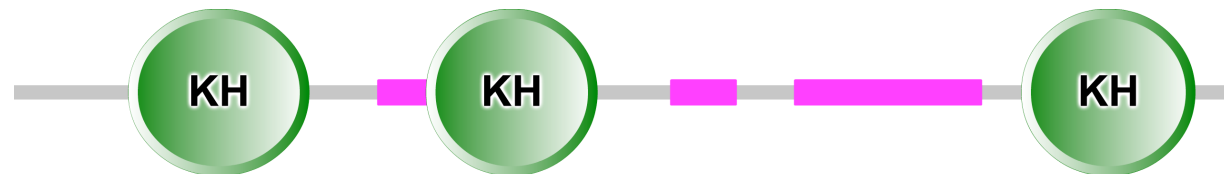
Canonical ssRNA-binding domains

RRM domain
binds 2-5nts
~250 RBPs in
mammals



HuR (also known as ELAVL1)
motif: UUNNNUU

KH domain
binds 2-4 nts
~50 RBPs in
mammals



Nova1
motif: YCAY

Other domains: CCCH Zinc finger, Pumilio/Puf, Cold Shock Domains, others (sometime C2H2)

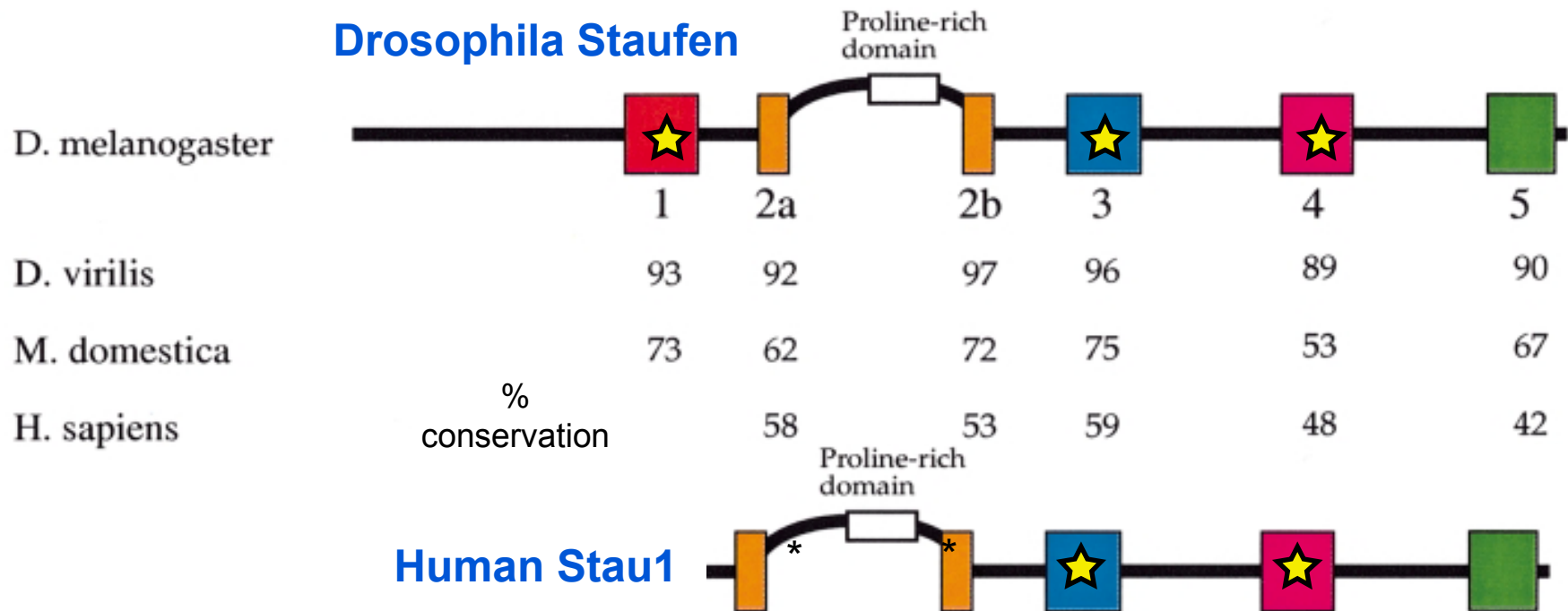
Auxiliary domains: CCHC Zinc fingers

Canonical dsRNA-binding domain

double-stranded RNA binding domain

binds dsRNA 10-12bp

~20 in RBPs in mammals



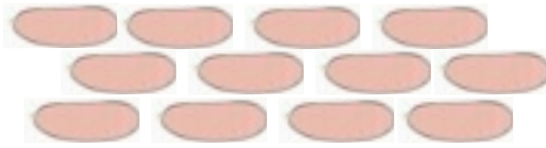
★ = binds dsRNA

Adapted from: Micklem, D. R. et al. *The EMBO journal*, 19(6), 1366-1377.

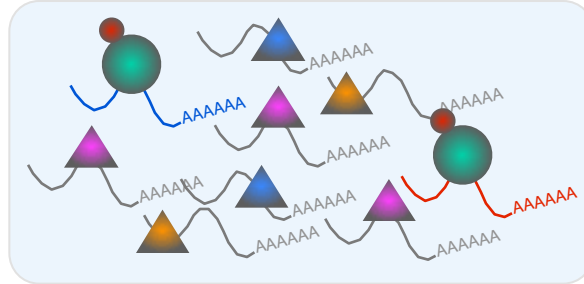
Modelling RBP binding preferences

1. What RNA structures are available for RBP to bind?
mRNA secondary structure prediction
2. What RNA structure / sequences does the RBP want to bind?
sequence/structure motif (SSM) finding
3. How does RBP binding affects the available RNA structures?
Ralf Bundschuh

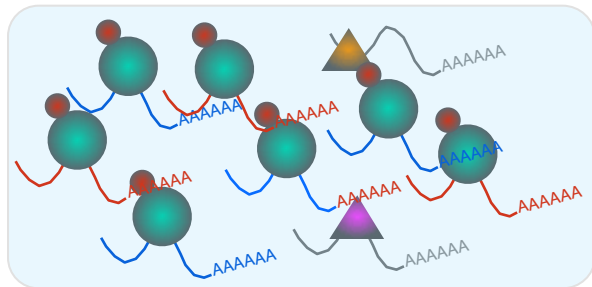
Defining positives and negatives for RBP binding



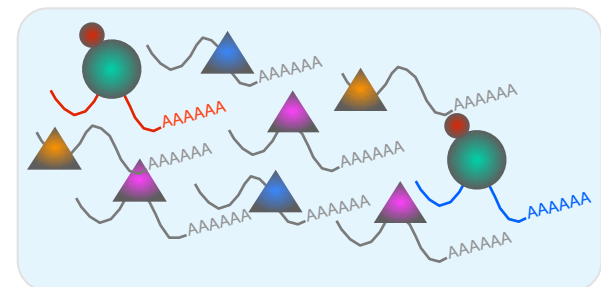
embryo or cell extract



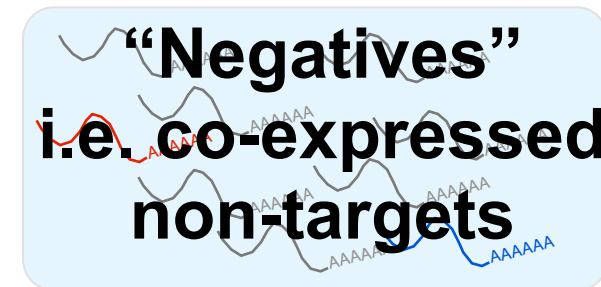
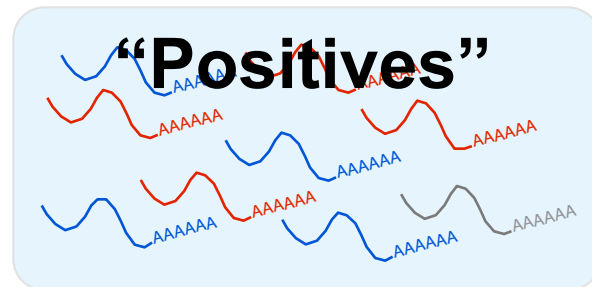
Specific RBP IP



“Mock” RBP IP



RNA purification

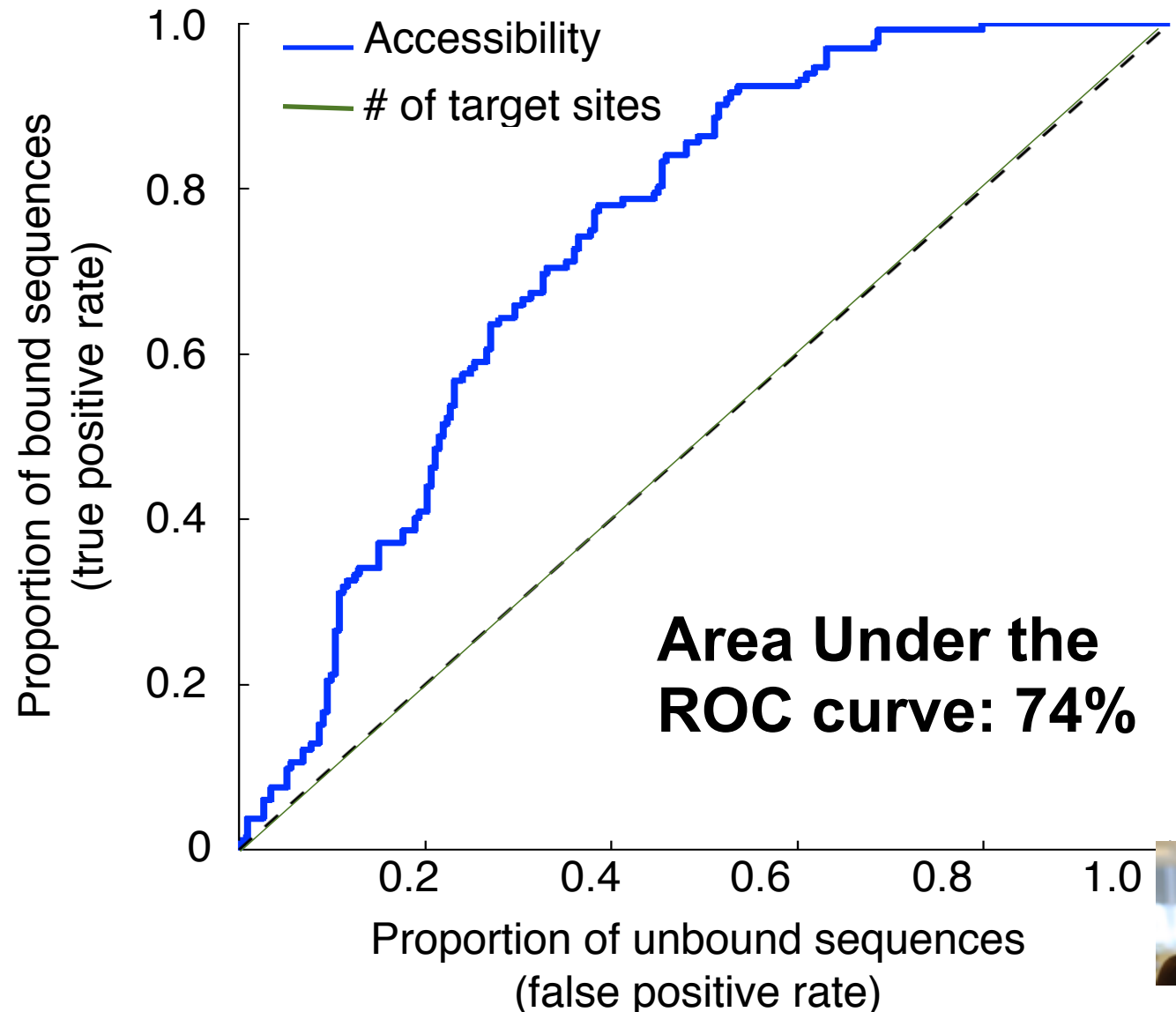
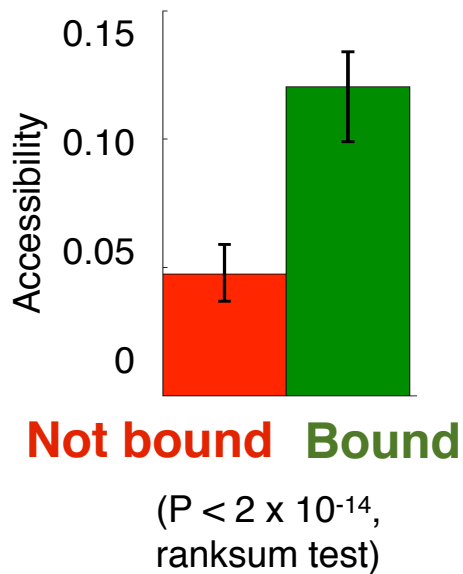


microarrays/RNA-seq

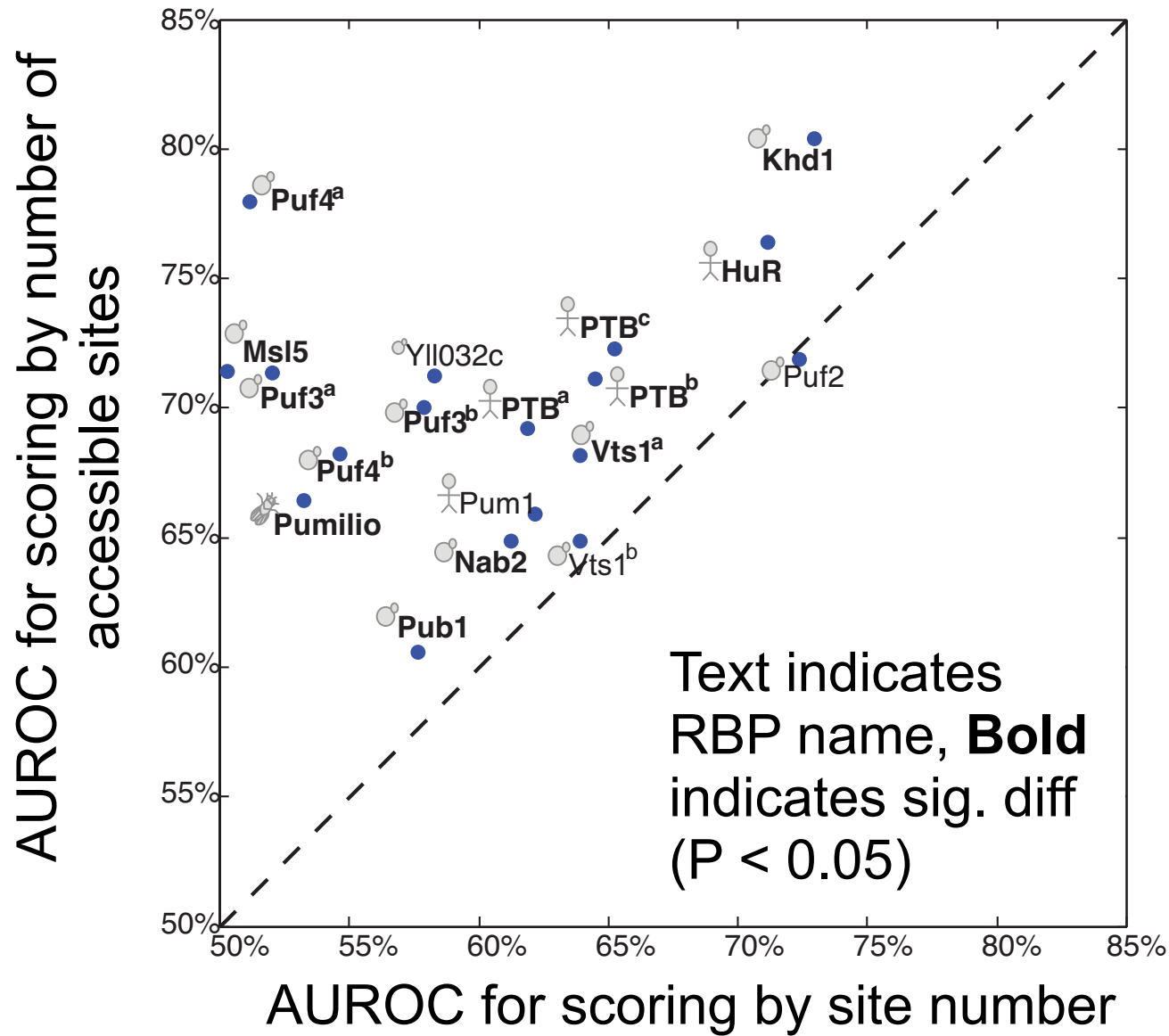
Slide courtesy of H. Lipshitz

Site accessibility predicts in vivo Puf3p binding

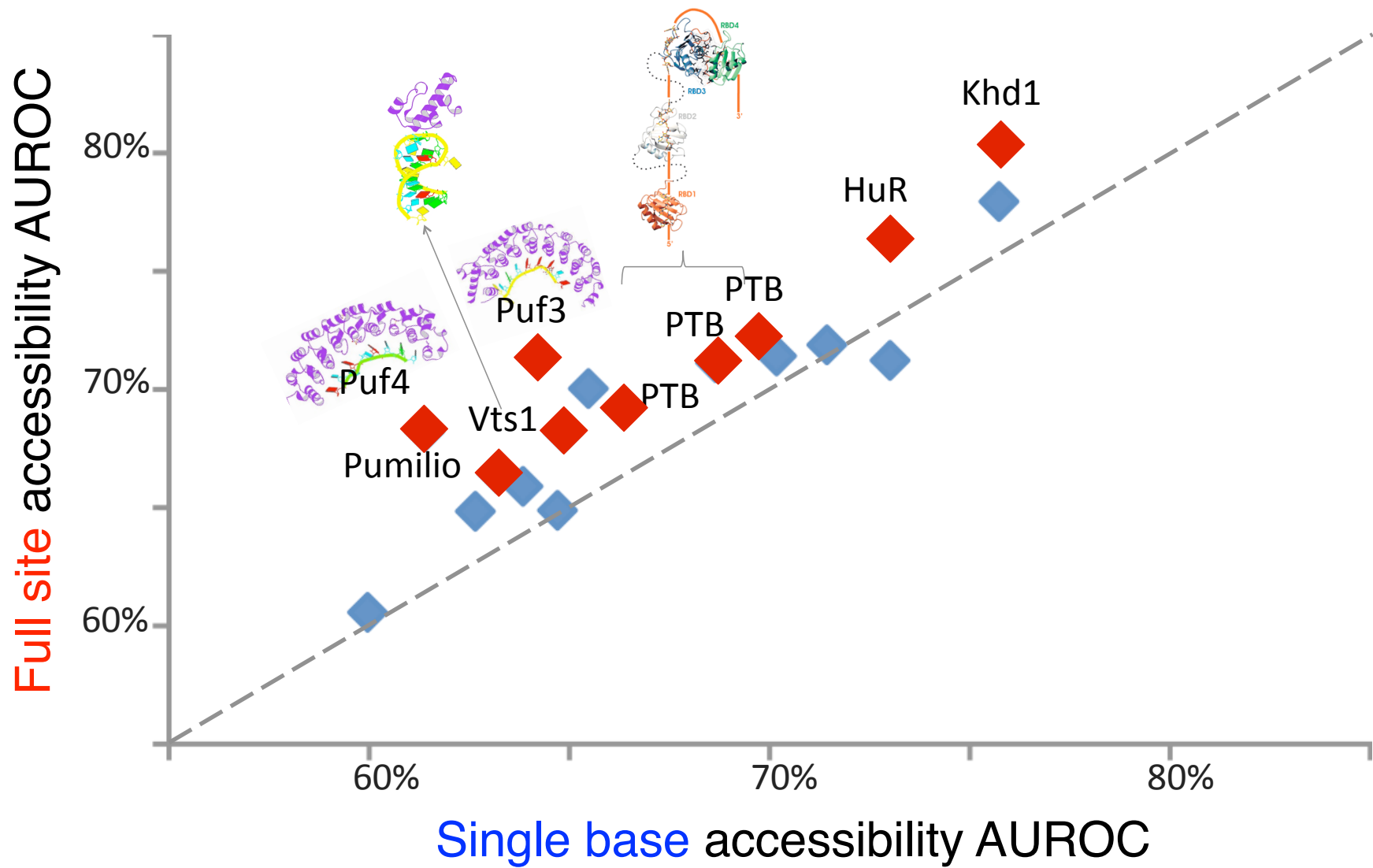
ROC curve for prediction of Puf3p binding



Site accessibility predicts general RBP binding



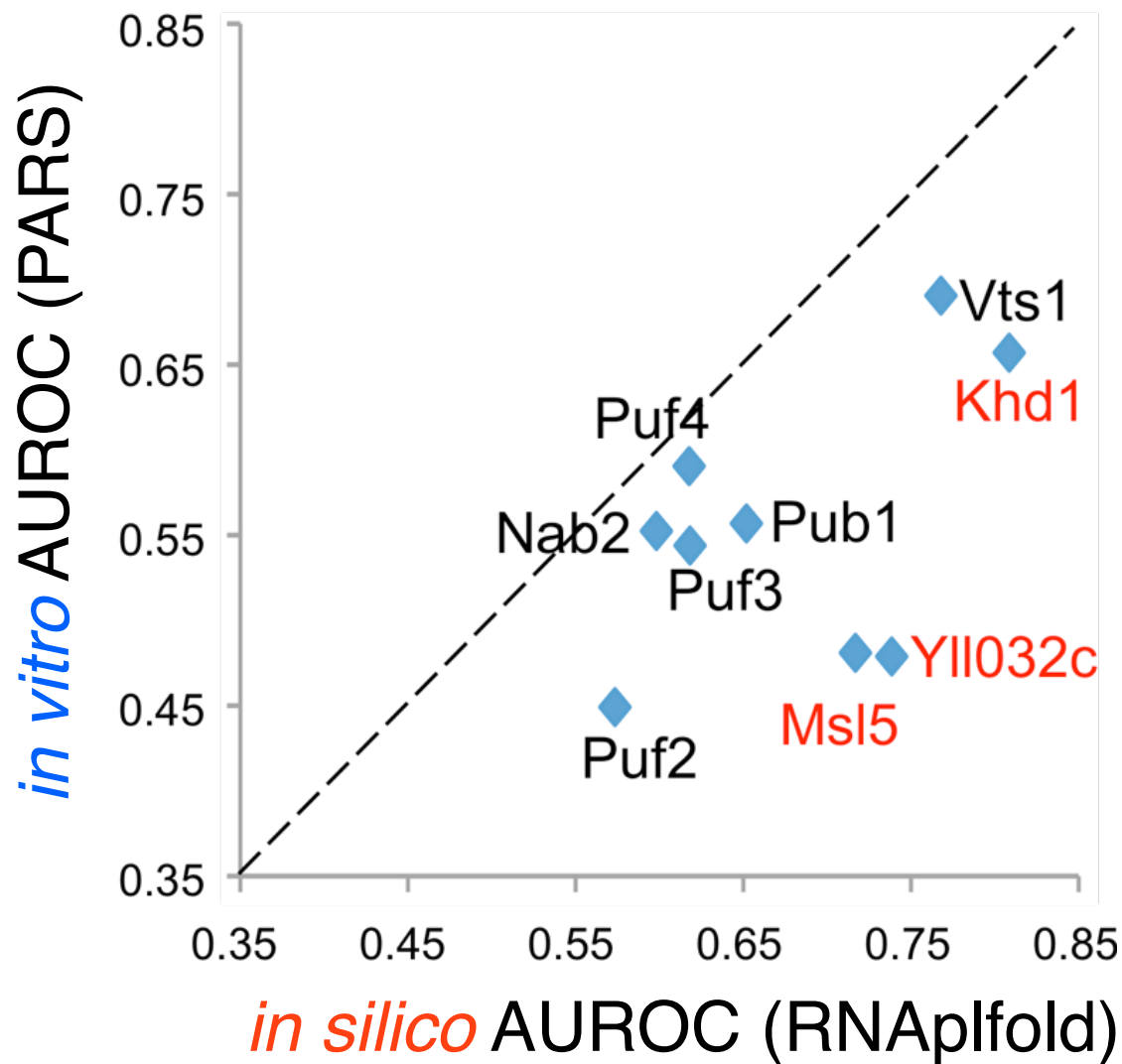
Strict is better than permissive accessibility



◆ indicates stat. sig. improvement (p < 0.05 DDCP test, Bonferroni)



in silico versus *in vitro* estimates of site accessibility

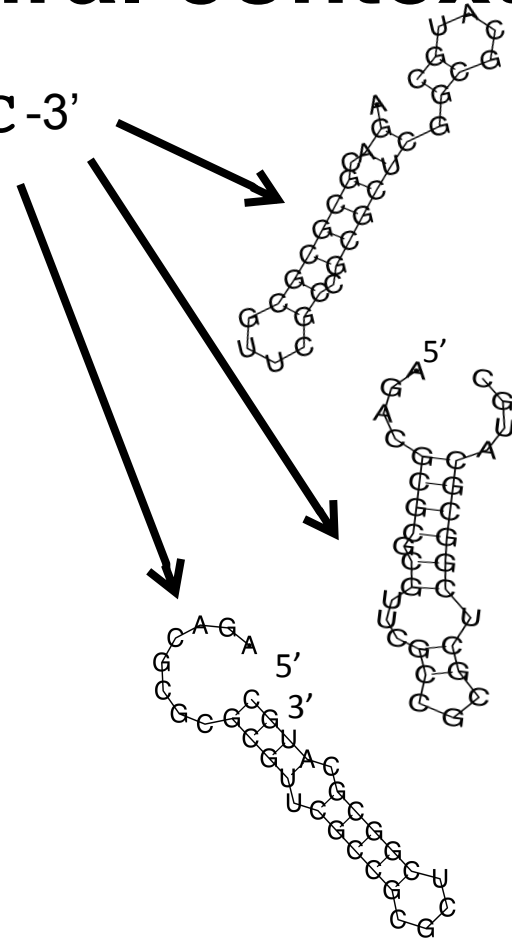


Red text indicates sig. differences (P < 0.05 DDCP)

PARS data from (Kestesz et al, Nature 2010)

Representing structural context

5'-AGACGCGCGCGUUCGCCGCGCUCGGCGCAUGC-3'



Representing structural context

5'-AGACGCGCGCGUUCGCCGCGCUCGGCGCAUGC-3'

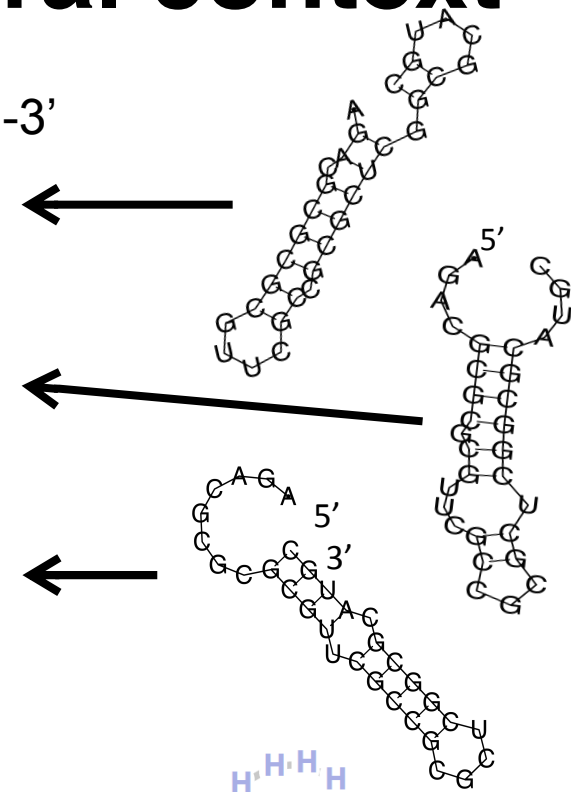
U P P I P P P P P H H H H P P I P P P P P P U P P H H H H P P

U U U P P I P P P P P H H H H H P P P P P I P P U U U U U U U U U

U U U U P P P P I P P I I I P P H H H P P I P P P P P P U U U U

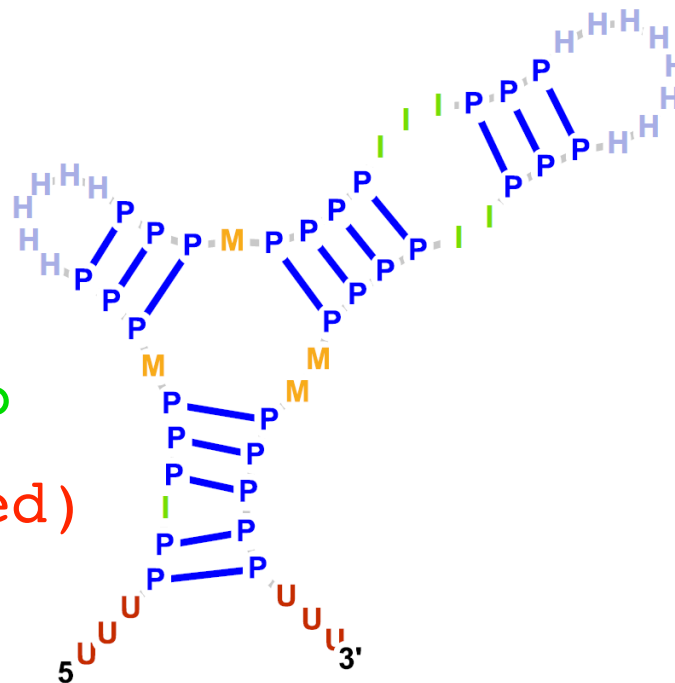
U P P P P H H H H P P P P I P P P P H H H H P P P P U U U U U U

U U U U U U U U P P P P I P P P P P H H H H P P P P P I P P P P



Legend

- P - paired
- H - hairpin loop
- I - internal / bulge loop
- M - multiloop
- U - external (unstructured)



Representing structural context

5'-AGACGCGCGCGUUCGCCGCGCUCGGCGCAUGC-3'

UPPIPP PPPPHHHHPPIPP PPPPUPPHHHHPP
 UUUPPIPP PPPPHHHHPPPPP IPPUUUUUUUU
 UUUUPPPP IPPPIIIPP HHHHPPIPP PPPP UUUU
 UPPP HHHHPPPPP IPPPP HHHHPPPPP UUUUUU
 UUUUUUUU PPPPIPP PPPPHHHHPPPPP IPPPPP

Proposed binding site

State Probability

P	45%
H	45%
I	10%
M	0%
U	0%

Single nucleotide context

Legend

- P - paired
- H - hairpin loop
- I - internal / bulge loop
- M - multiloop
- U - external (unstructured)



Structural context of a binding site

5'-AGACGCGCGCGUUCGCCGCGCUCGGCGCAUGC-3'

U P P I P P P P P H H H H P P I P P P P P P U P P H H H H P P
 U U U P P I P P P P P H H H H H P P P P P I P P U U U U U U U U U
 U U U U P P P P I P P I I I P P H H H P P I P P P P P P U U U U
 U P P P P H H H H P P P P I P P P P H H H H P P P P U U U U U U
 U U U U U U U U P P P P I P P P P P H H H H P P P P P I P P P P

Proposed binding site

Legend

- P - paired
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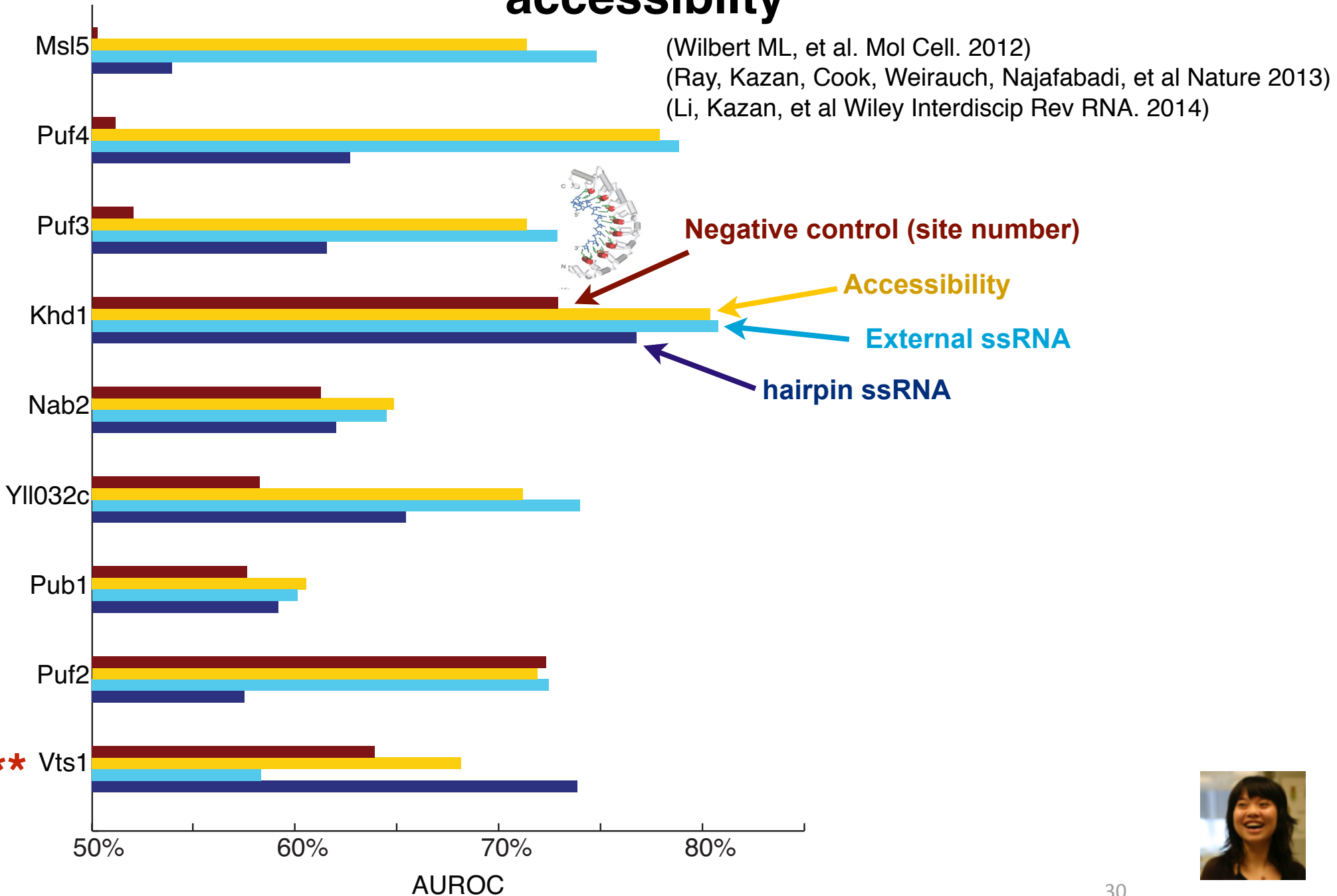
State Probability

PPPP	20%
HHHH	40%
IIII	0%
MMMM	0%
UUUU	0%
paired	40%

Full site context



Structure context predicts RBP binding better than site accessibility

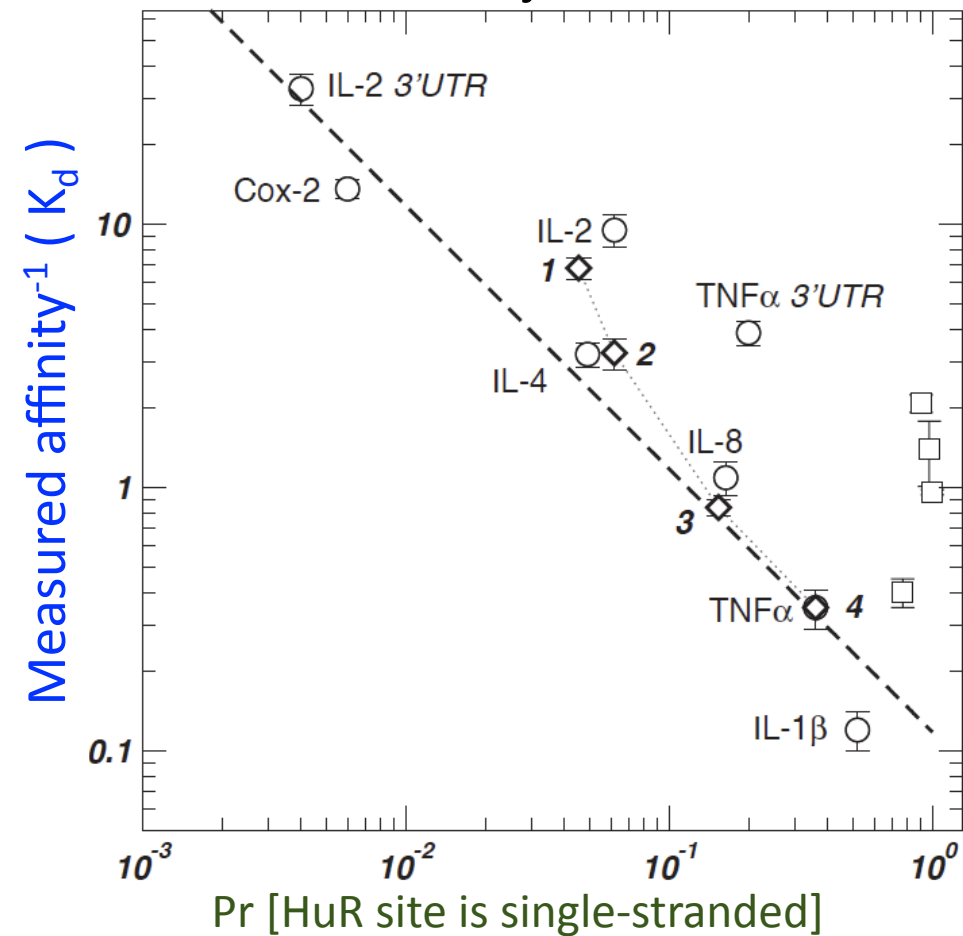


Summary

1. RNA secondary structure predictions helps identify *in vivo* RBP binding (>70% of RBPs),
2. *In silico* predictions better recover *in vivo* binding than circa 2010 *in vitro* experimental predictions,
3. Estimates of site 'structural context' often provide more information than site accessibility,

Models of structure binding preferences.

In vitro K_d of HuR for RNA sequences with exactly one HuR site



Hackermuller-Stadler model

$$K_a(\text{site}) = \text{Pr}[\text{site is in preferred context}] \times K_a(\text{site in preferred context})$$

MEMERIS*

MEME

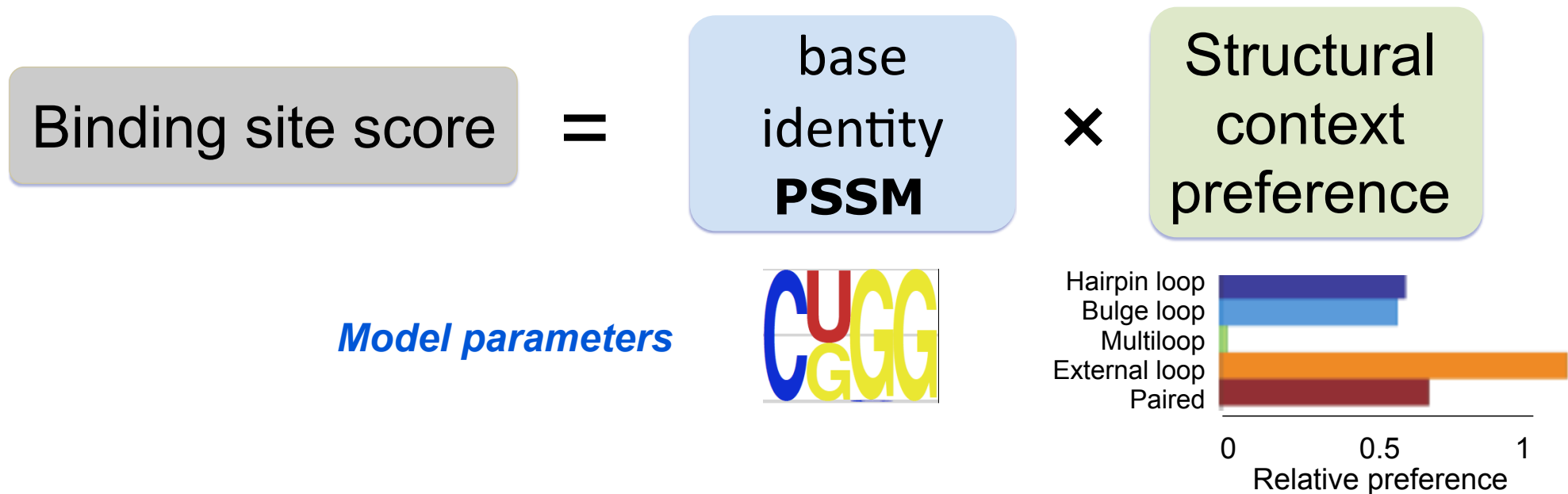


adapted from Hackermuller et al (2005) Gene 345:3.

*Hiller et al (2006) Nuc. Acids Res. 34:e117.

Motif models for structure preference

Model of RNA sequence preferences



See: RNAcontext & Malarkey

Single nucleotide structural context

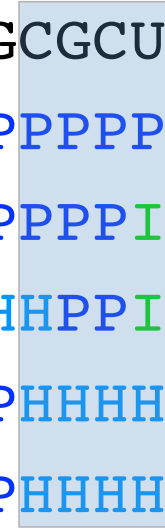
Proposed binding site

structural
feature
1



```

AGACGCGCGCGUUCGCCGCGCUCGGCGCAUGC
UPPIPPPPPHHHHPPIPPPPPPUPPHHHHP
UUUPIPPPPPHHHHPPIPPUUUUUUUU
UUUUPPPPIPIIIPPHHPPIPPPPPPUUUU
UPPPPHHHHPPIPPPPHHHHPPPPUUUUUU
UUUUUUUUUPPPPIPPPPPHHHHPPIPPPP
    
```

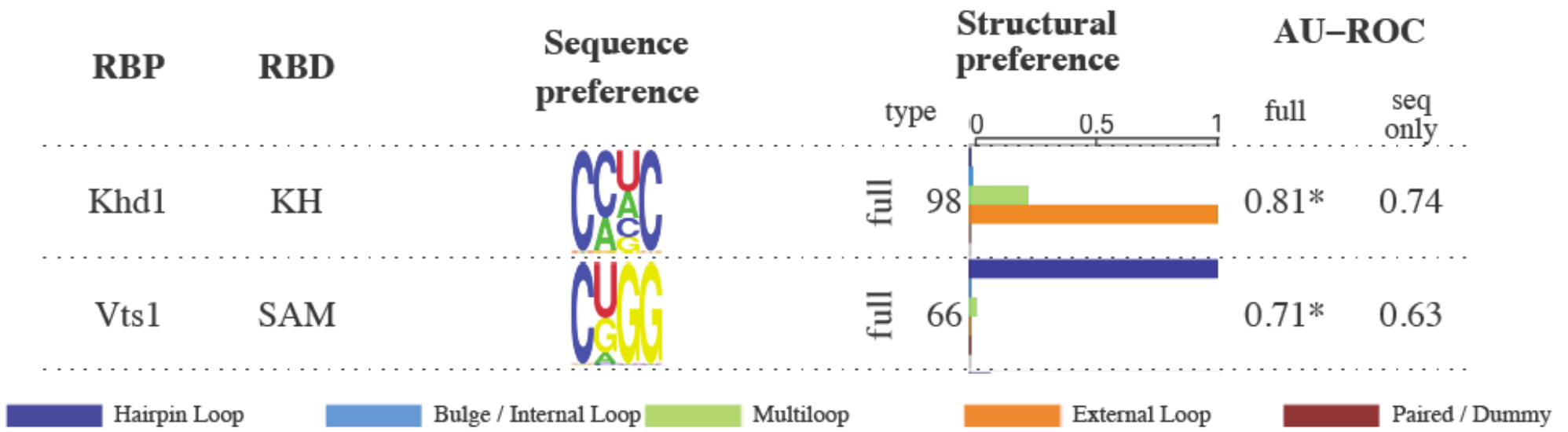


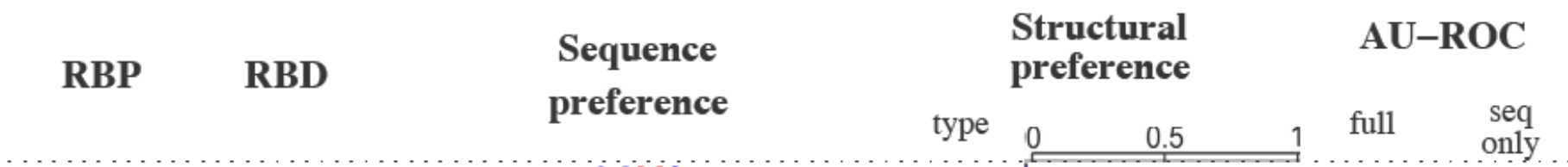
State	Value	Θ_1
-------	-------	------------

P	45% x	1
H	45% x	5
I	10% x	0
M	0% x	0
U	+ 0% x	0
		2.7

Legend

- P - paired
- H - hairpin loop
- I - internal / bulge loop
- M - multiloop
- U - external (unstructured)





RBP

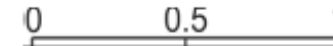
RBD

Sequence preference

Structural preference

AU-ROC

type



full

seq only

Hairpin Loop
 Bulge / Internal Loop
 Multiloop
 External Loop
 Paired / Dummy

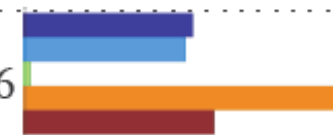
Puf3

Pum



full

66



0.9

0.9

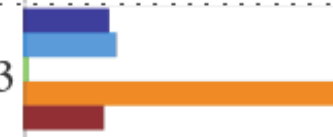
Puf4

Pum



full

73



0.87*

0.86

Puf5

Pum



full

50



0.7

0.7



RBP

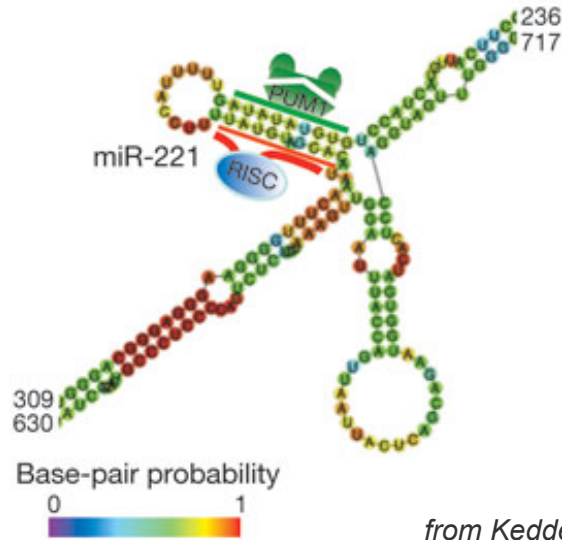
RBD

Sequence preference

Structural preference

type 0 0.5 1

AU-ROC full seq only



Evidence for functional binding of Pumilio to paired target sites

from Kedde et al, Nature Cell Biology 12, 1014–1020 (2010)

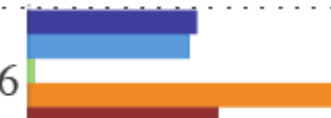
Puf3

Pum



full

66



0.9

0.9

Puf4

Pum



full

73



0.87*

0.86

Puf5

Pum



full

50



0.7

0.7

Hairpin Loop

Bulge / Internal Loop

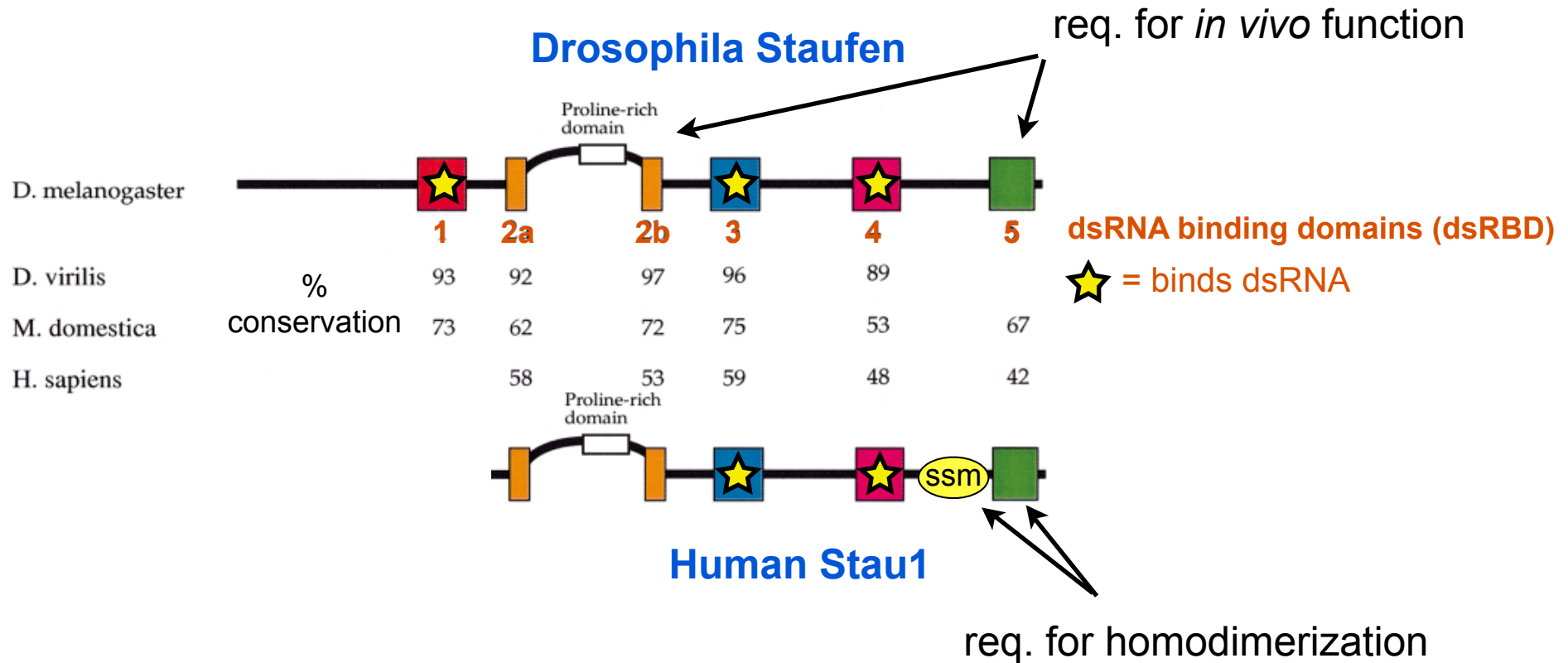
Multiloop

External Loop

Paired / Dummy

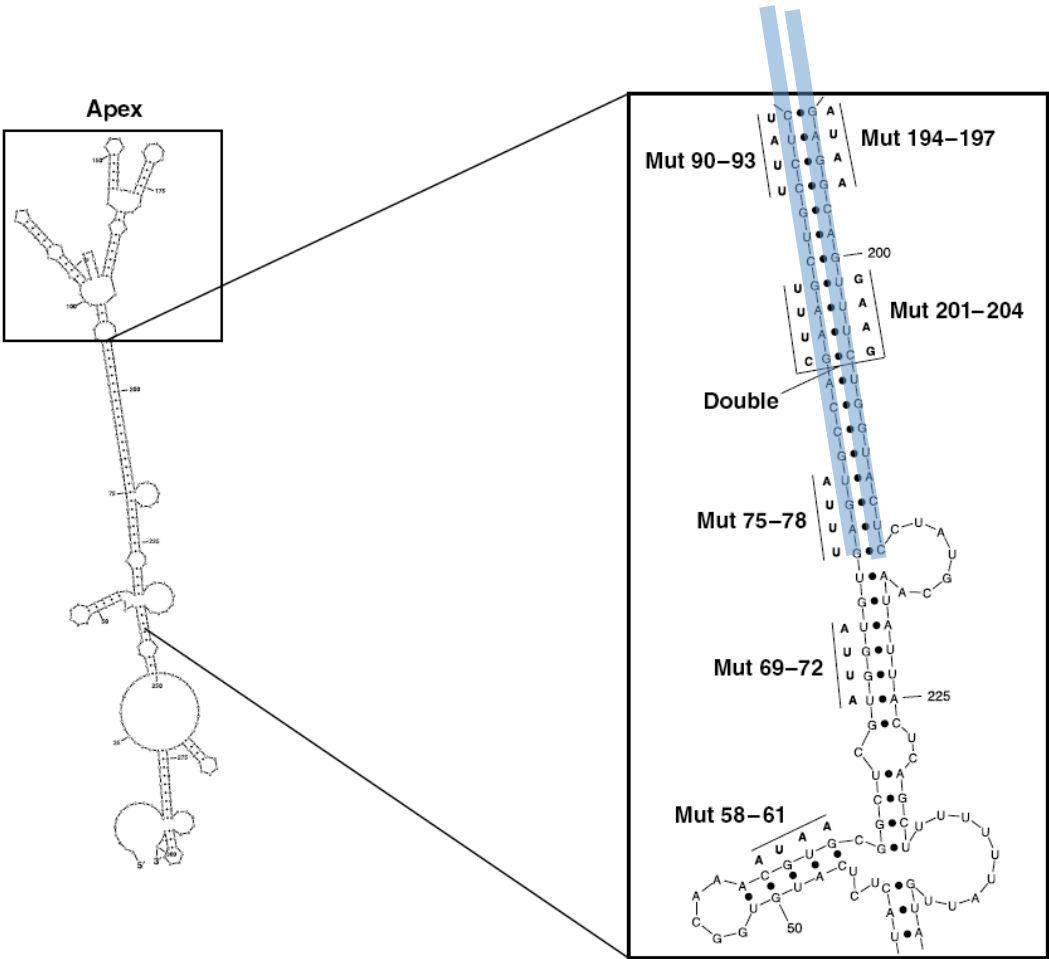


Human vs Drosophila Staufen domain structures

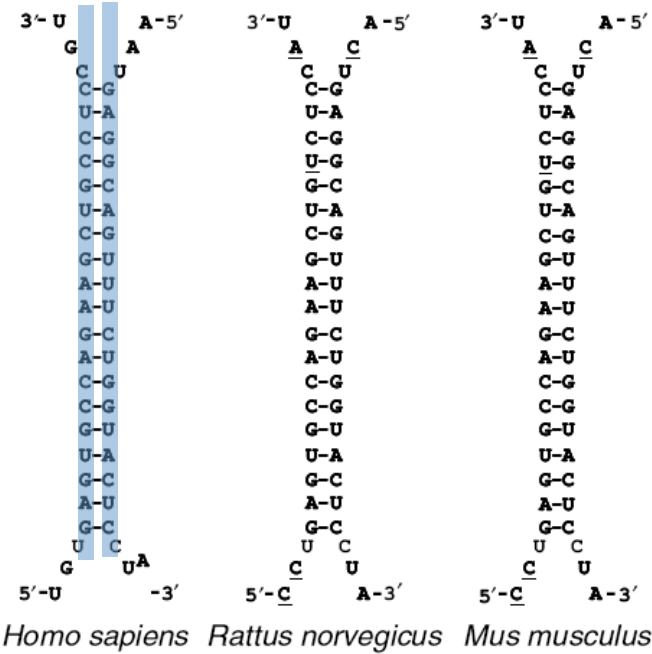


Adapted from: Micklem, D. R. et al. *The EMBO journal*, 19(6), 1366-1377.

Human Staufen binds a 19bp dsRNA in human



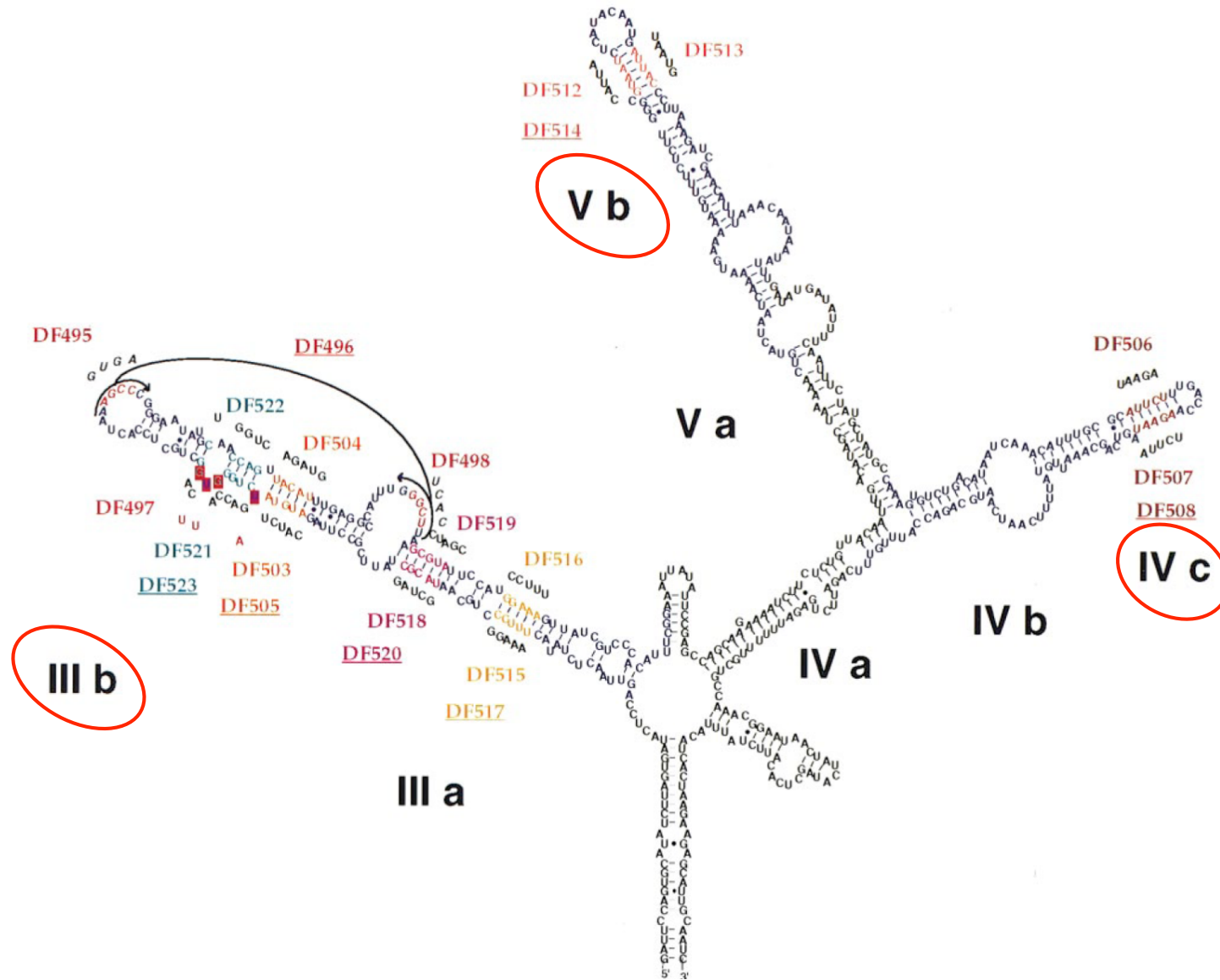
The 19-bp stem conserved



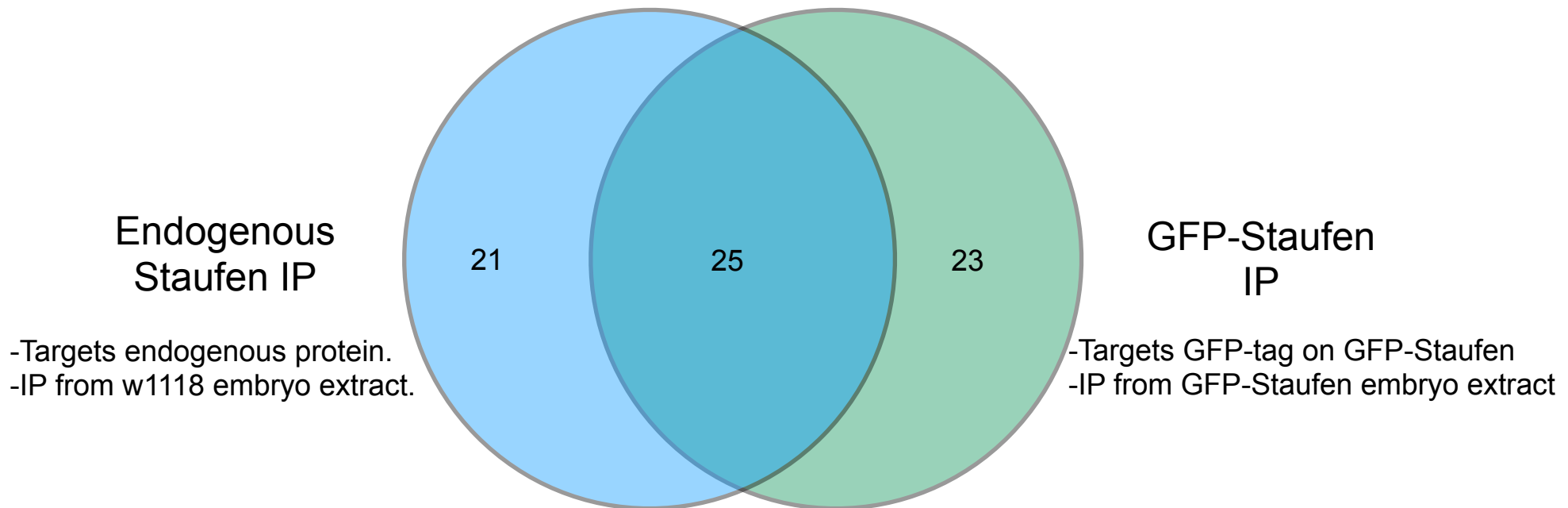
human ARF1 Staufen binding site (SBS) 3'UTR nt 1-300

The SBSs within c-JUN, SERPINE1, IL7R and GAP43 mRNAs do not contain an uninterrupted stem that is more than 12 bp

Fly Staufen binds bicoid 3'UTR in three locations



***Drosophila* Staufen targets in embryos were identified using two RIP-Chip experiments**



John Laver



Kristin Ancevicus

What does Staufen bind?

Paired region motifs



Stems



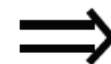
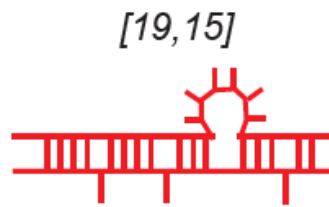
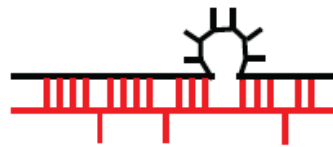
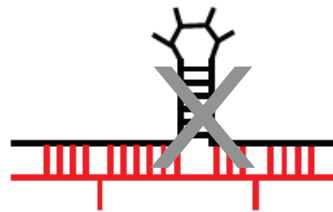
Refined stems

15 of 19; 10 of 12

[19,15]; [12,10]

[19,15,0]
[19,15,4]
[12,10,2]

e.g. 15 of 19



mismatches
 unpaired bases

[19,15,4]



Stems enriched in *Drosophila* Staufen targets

[19, 15]

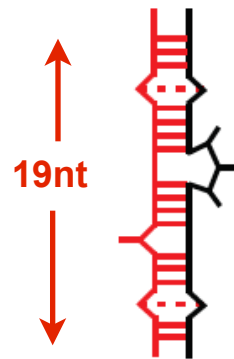
[12, 10]

Structure motif description:

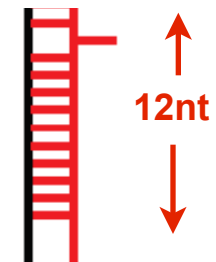
Stem spanning **19** bps with at least **15** Watson-Crick paired bases

Stem spanning **12** bps with at least **10** Watson-Crick paired bases

Representative structures:



↑ ↓ mismatches
↑ ↓ unpaired bases

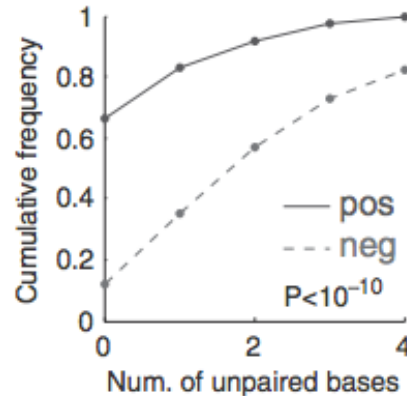


Distinguishing features of Staufen-bound stems

Distinguishing features:

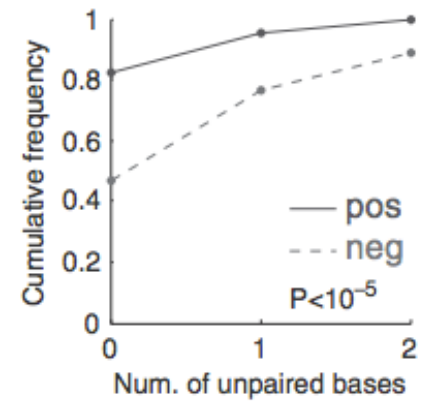
1) “Balanced” stems

[19,15]



⬆ mismatches
⊕ unpaired bases

[12,10]



pos: Staufen-bound 3'UTRs

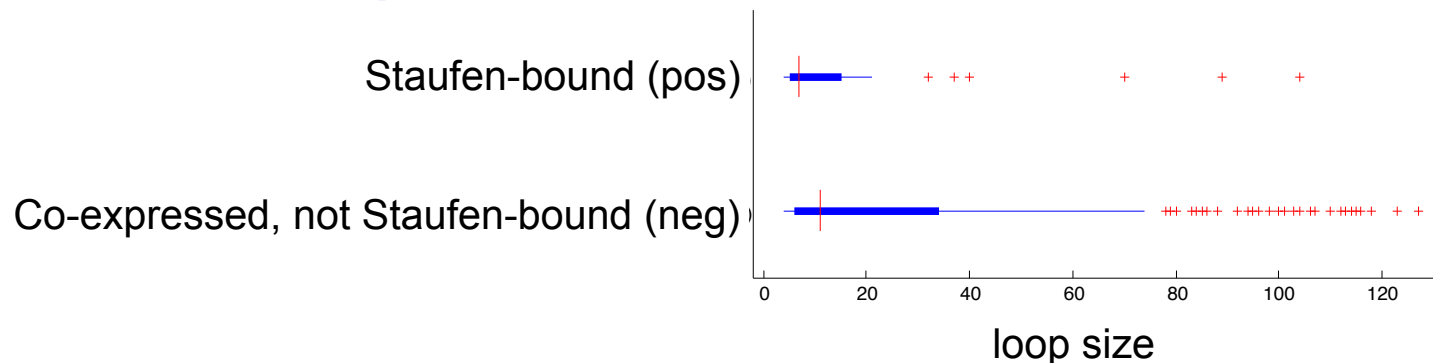
neg: Co-expressed 3'UTRs, not Staufen-bound

2) Short stems occur near end of stem-loops:

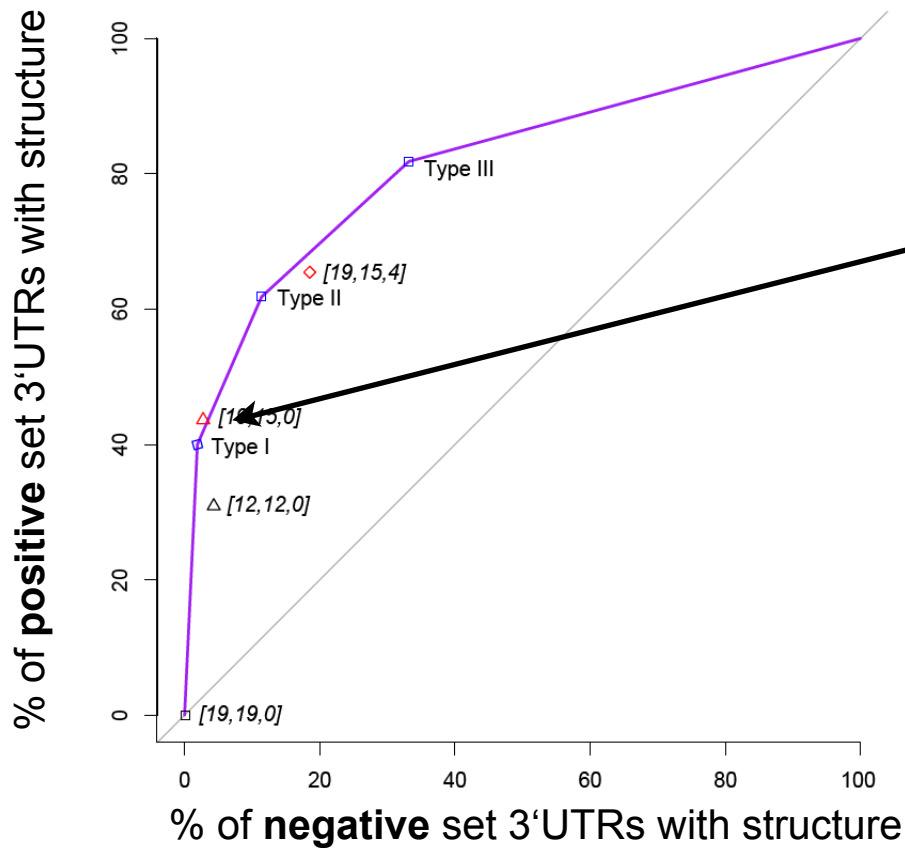
Median min distance between interacting sequences in [12,10]:

pos: 7nt

neg: 11nt



Staufen-recognized structures (SRSs)



'Best structure':

[19, 15, 0]

[19, 15] with **0** unpaired bases (i.e. all non-paired bases are 'mismatches')

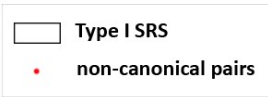


**20-fold enrichment
(40% vs 2%) in
Staufen-bound set**

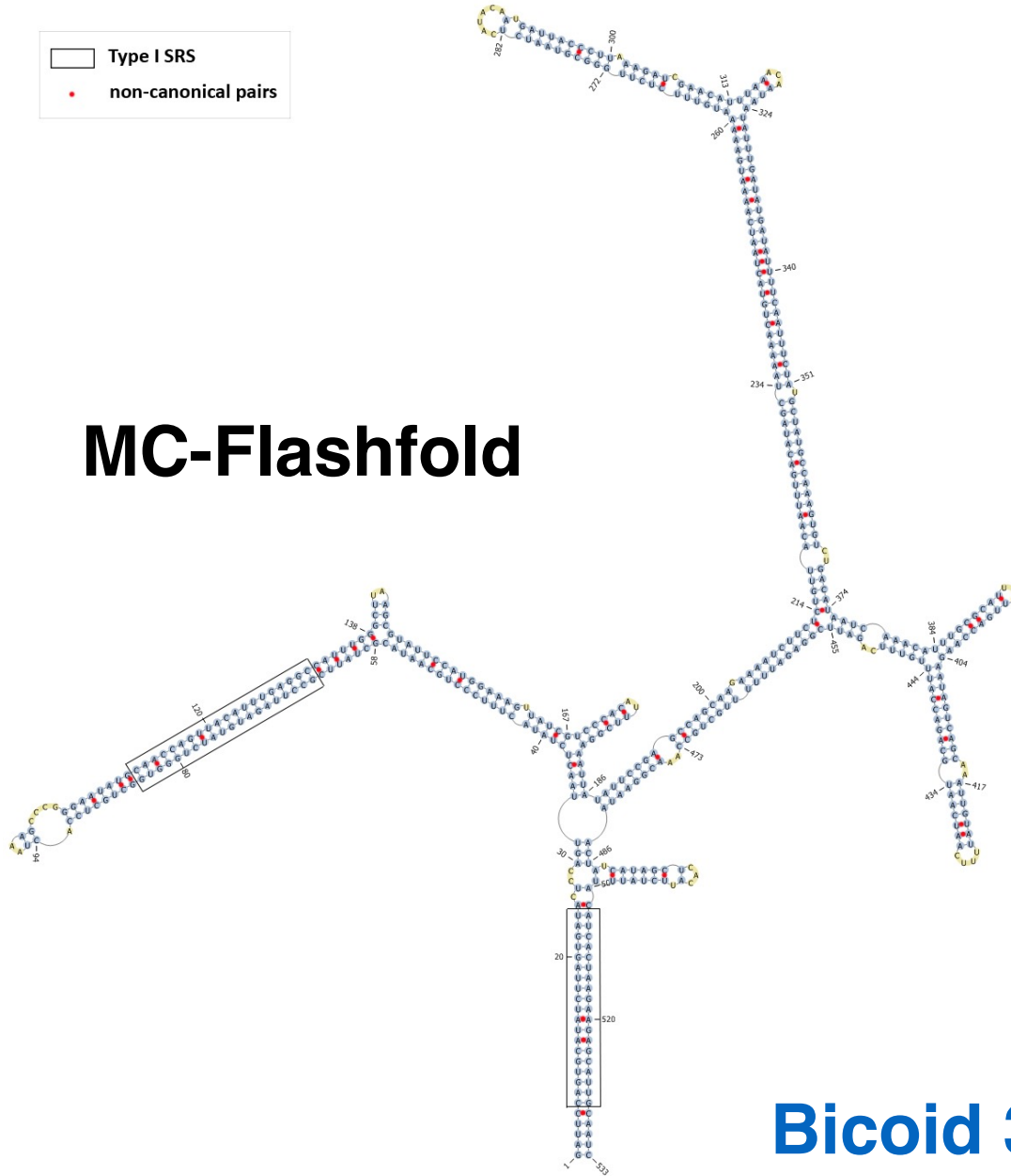
w/ Jerome Waldispühl lab:

- 1) no G-G mismatches in positive set!
- 2) Negative set structures have high entropy
- 3) Positive set structures are highly conserved

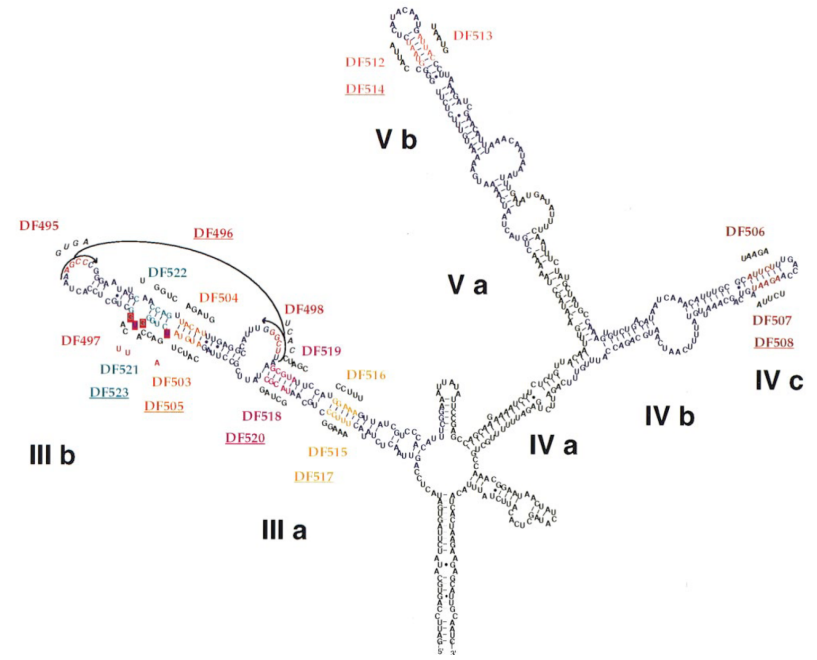
Non-canonical basepairing helps find Staufen sites



MC-Flashfold



Mfold(?)

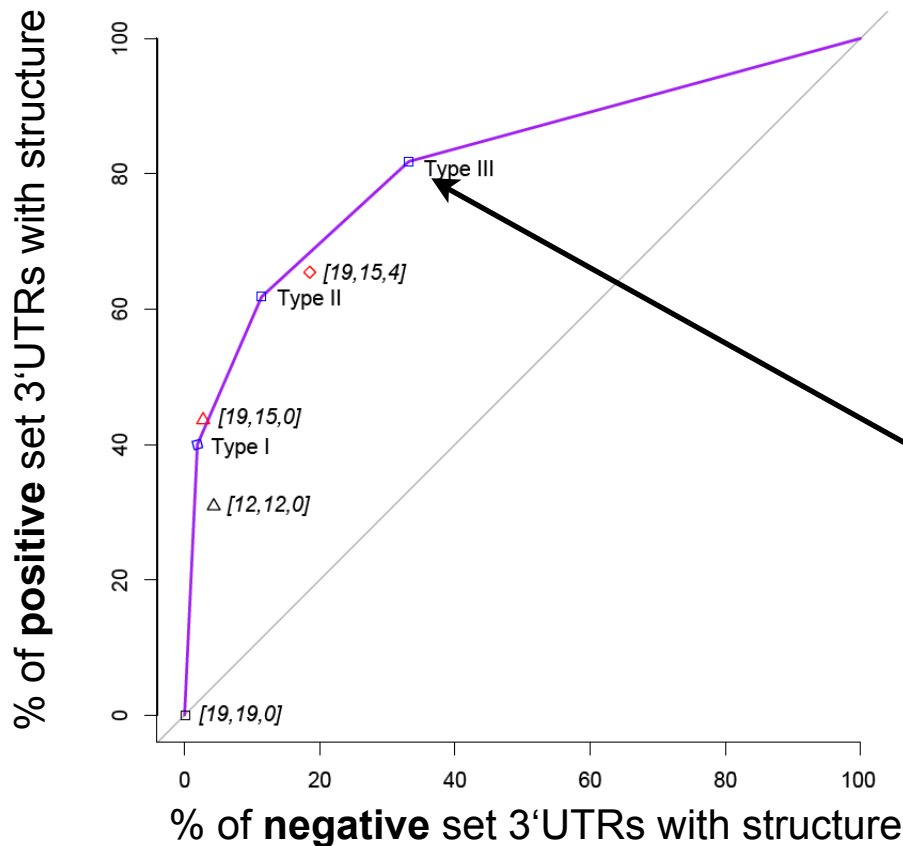


Bicoid 3'UTR

Kate Niu



Staufen-recognized structures (SRSs)



'Best structure':

[19,15,0]



[19,15] with **0** unpaired bases (i.e. all non-paired bases are 'mismatches')

'Minimum structure':

[12,10,2]



[12,10] with no more than **2** unpaired bases, occurs near end of stem-loop

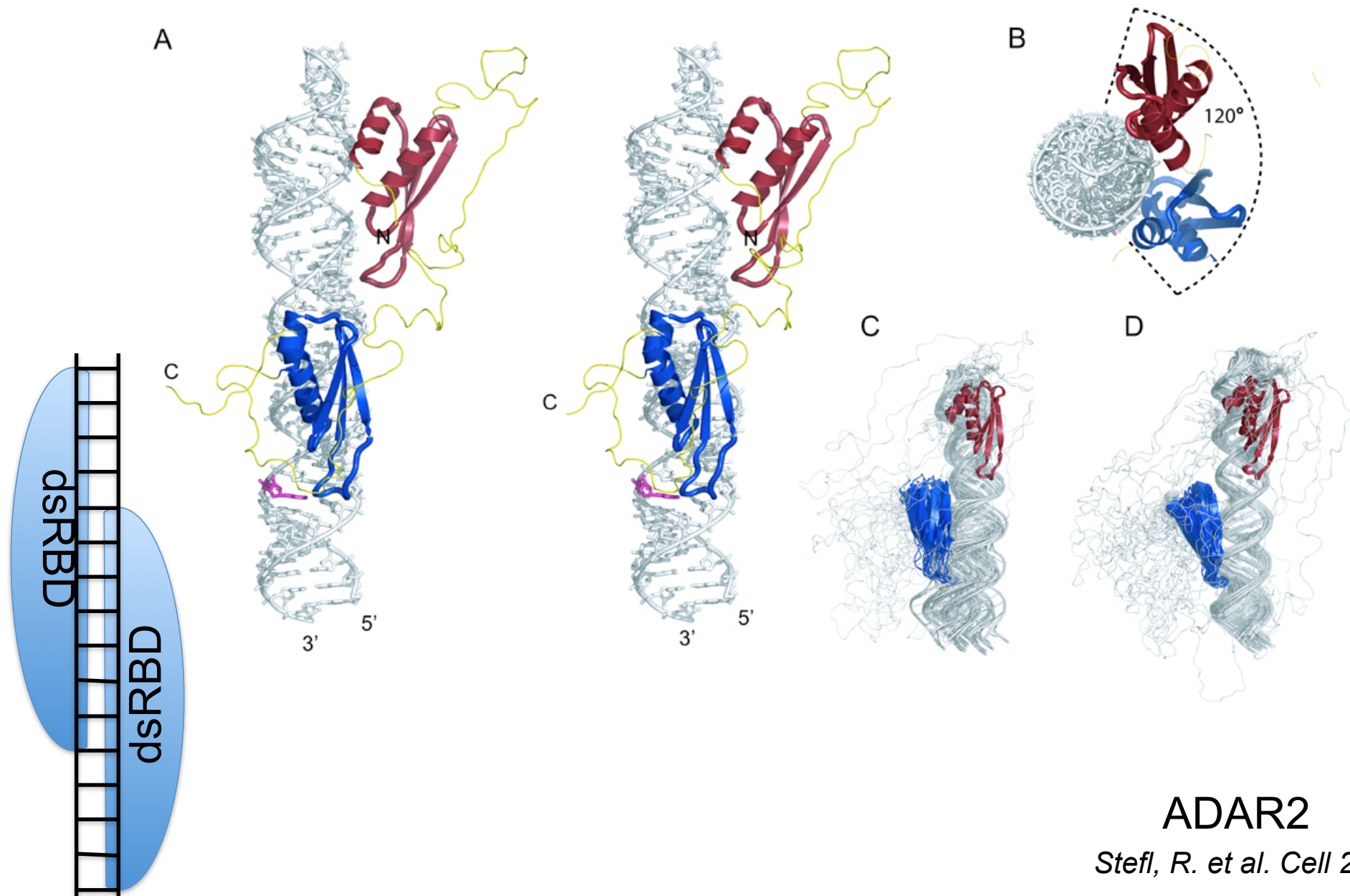
Genome-wide analysis of Staufen-associated mRNAs identifies secondary structures that confer target specificity

John D. Laver¹, Xiao Li¹, Kristin Ancevicus^{2,3}, J. Timothy Westwood^{2,3,*}, Craig A. Smibert^{1,4,*}, Quaid D. Morris^{1,5,*} and Howard D. Lipshitz^{1,*}

[Nucleic Acids Res.](https://doi.org/10.1093/nar/gkt702) 2013 Nov 1;41(20):9438-60. doi: 10.1093/nar/gkt702. Epub 2013 Aug 13.



ADAR2 has two dsRNA that bind a 16-bp stem



Re-analysis of Human Stau1 data

Staufen1 senses overall transcript secondary structure to regulate translation

Emiliano P Ricci¹⁻³, Alper Kucukural¹⁻³, Can Cenik¹⁻⁴, Blandine C Mercier¹⁻³, Guramrit Singh¹⁻³, Erin E Heyer¹⁻³, Ami Ashar-Patel¹⁻³, Lingtao Peng¹⁻³ & Melissa J Moore¹⁻³

Question #1: Are our Drosophila SRSs predictive of Human Stau1 binding?

- Yes!

Question #2: Does the same analysis applied to Stau1 data produce similar structures?

- **See next slide**

Question #3: Does Human Stau1 detect “overall transcript secondary structure” or the presence (and abundance) of specific secondary structures?

- *“Overall transcript secondary structure” and GC content are no longer predictive of Stau1 binding once you account for the abundance of two specific secondary structures.*

Re-analysis of Human Stau1 data

1: Find enriched 'paired regions'

2: Remove redundant enrichments

3: Filter for stems

4: Analyze stem properties

13of15; 16of19

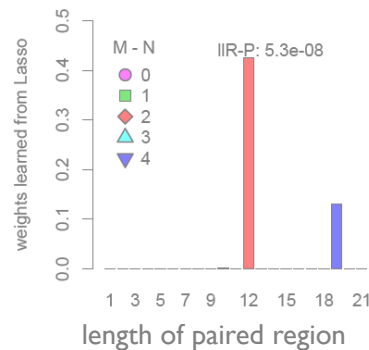
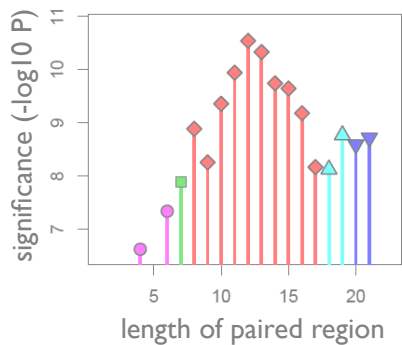
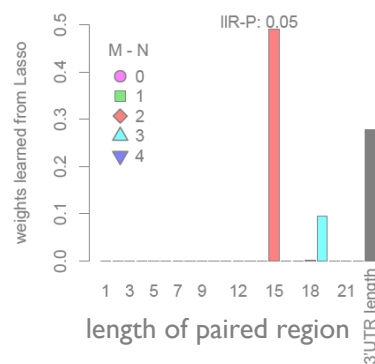
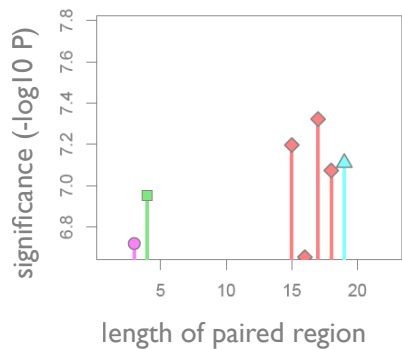
[15,13]; [16,19]

Stems in bound set are more balanced

Sig. larger % of paired regions in Staufen-bound set are actually stems.

[15,13], $p = 0.4$
[19,16], $p < 0.01$

[15,13] loops are smaller in Staufen-bound set,
 $p < 0.01$



10of12; 15of19

[12,10]; [19,15]

Stems in bound set are more balanced

Sig. larger % of paired regions in Staufen-bound set are actually stems.

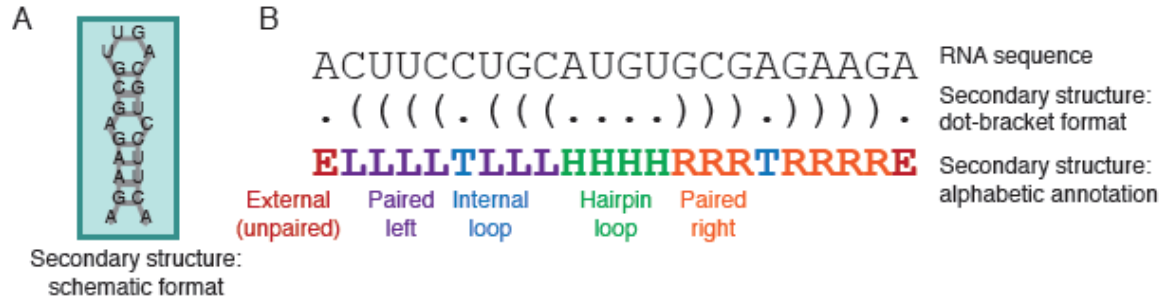
[12,10], $p < 10^{-5}$
[19,15], $p < 10^{-10}$

[12,10] loops are smaller in Staufen-bound set,
 $p < 10^{-5}$

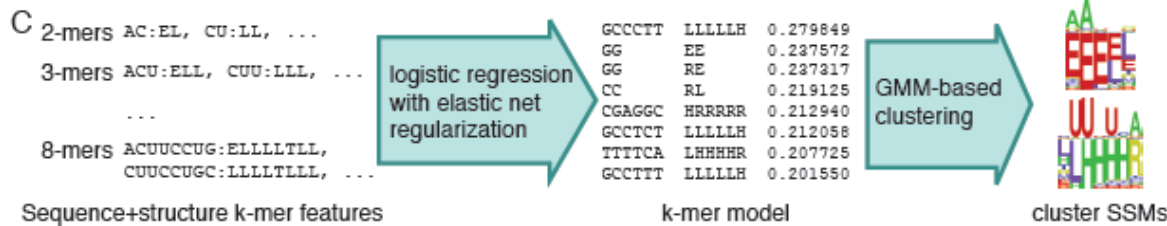
Summary

- 1:** Like *Drosophila* Staufen, human Stau1 has at least two binding modes
- 2:** Human Stau1 and *Drosophila* Staufen recognize a similar “best structure”, [19,16,0] vs [19,15,0], but may differ on the “minimum structure”, [15,13] vs [12,10].
- 3:** Differences may arise from lack of dsRBD1 in human Stau1 and lack of SSM in *Drosophila* Stau
- 4:** No evidence that human Stau1 recognizes ‘overall transcript structure’ except when it generates one of the two structural motifs that we found.

Computationally-derived motif for SLBP



SSMfinder

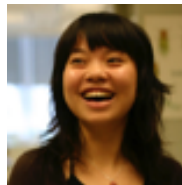
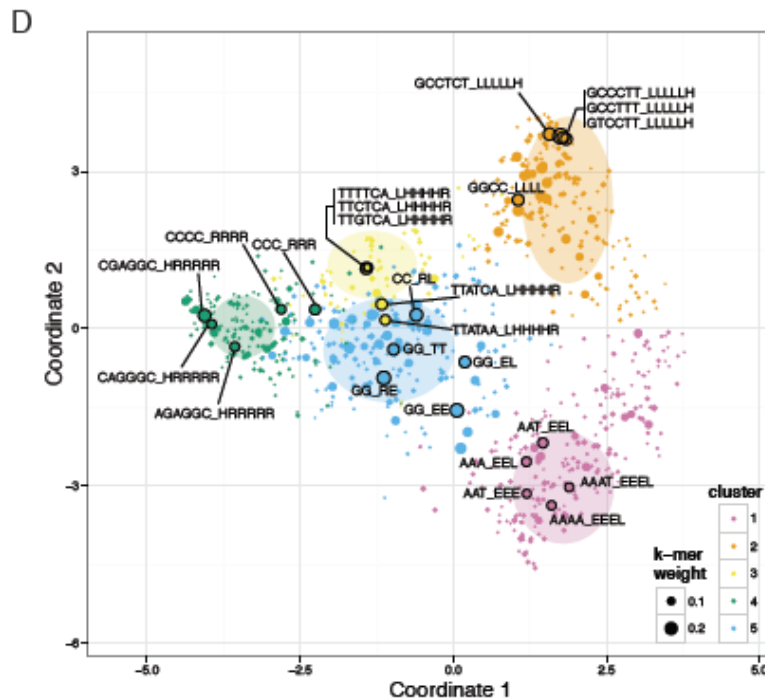


B. Fold sequences and annotate structural context***

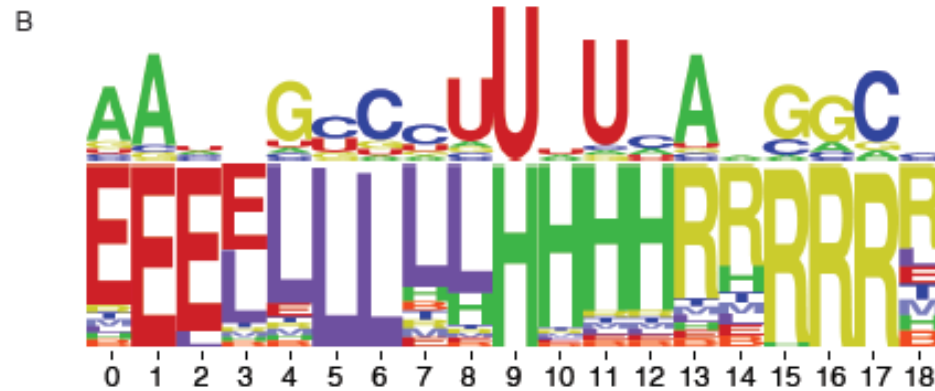
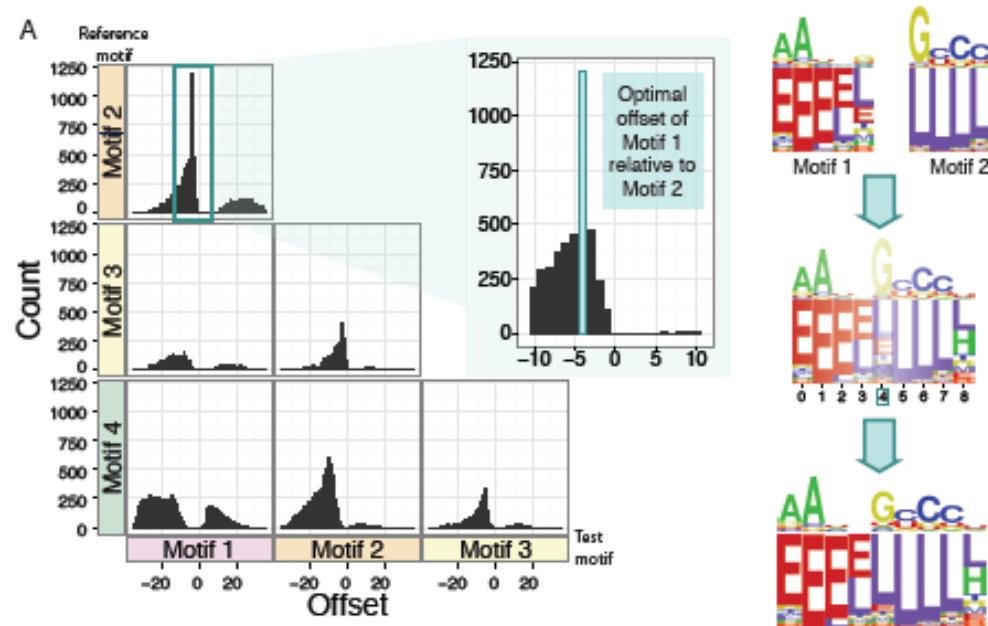
C. Find enriched k-mers (seq and struct)

D. Cluster k-mers

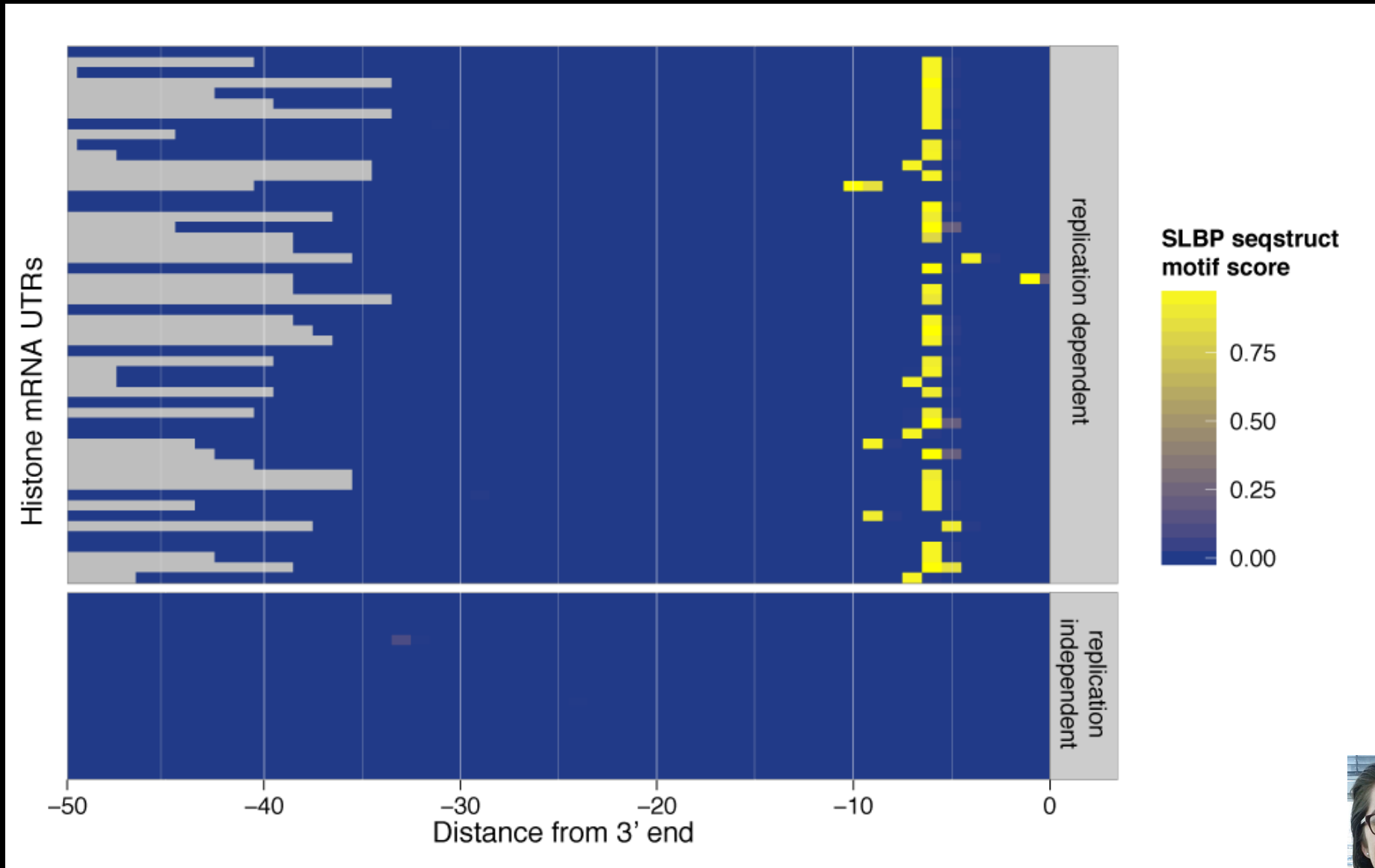
E. Order and overlap clusters (next slide)



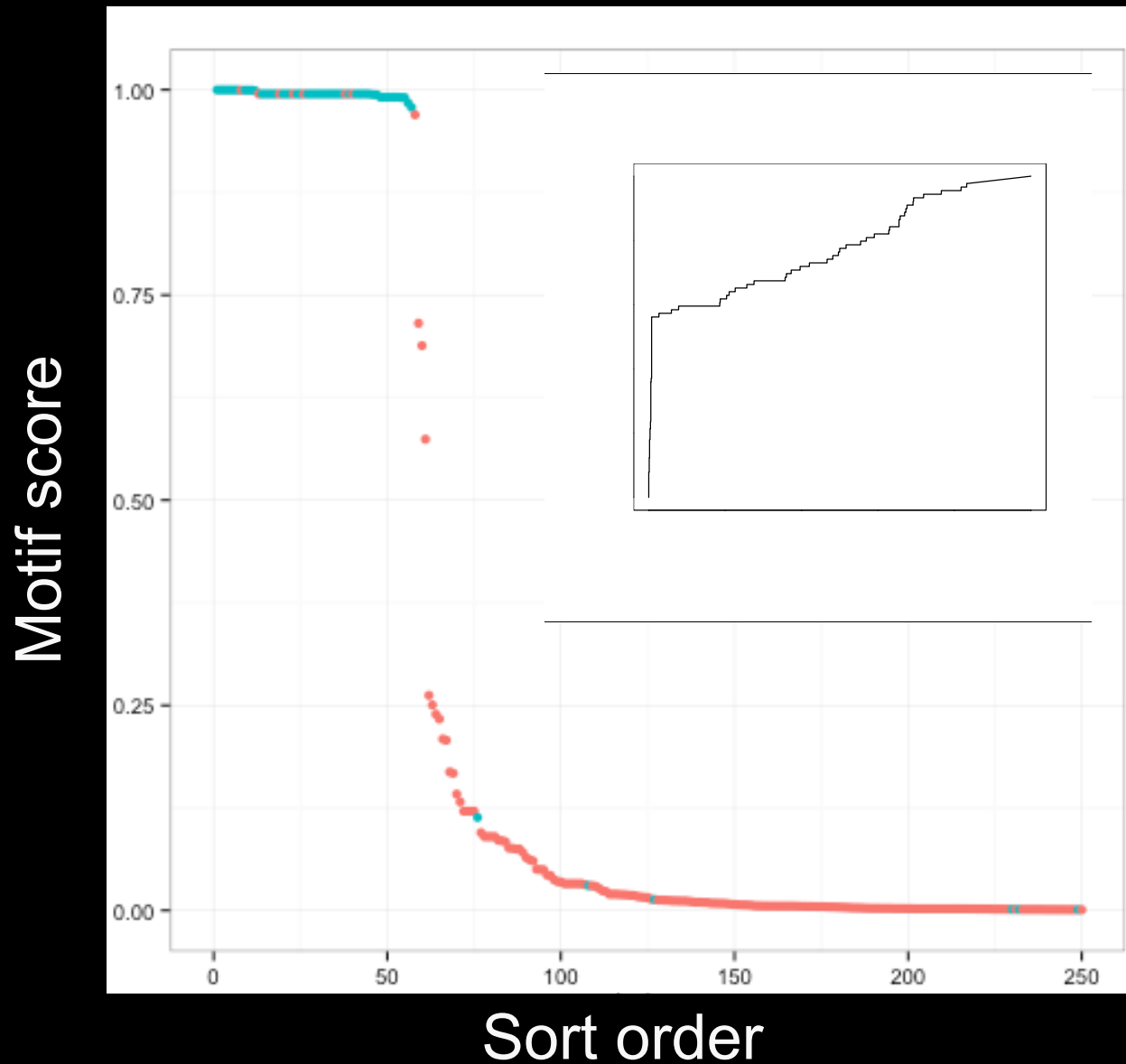
Ordering and overlapping SLBP clusters



RNAcompete-S derived SLBP motif is present predominantly at histone 3' ends



RNAcompete-S derived SLBP motif is present predominantly at histone 3' ends



Replication-dependent histones

Other genes



Eukaryote-wide mapping of RBP sequence binding preferences

Core RNAcompete Team

PIs

Prof. Tim Hughes @ Donnelly



me

Molecular biology

Dr. Deb Ray



Computational biology



Prof. Hilal Kazan



Kate Cook



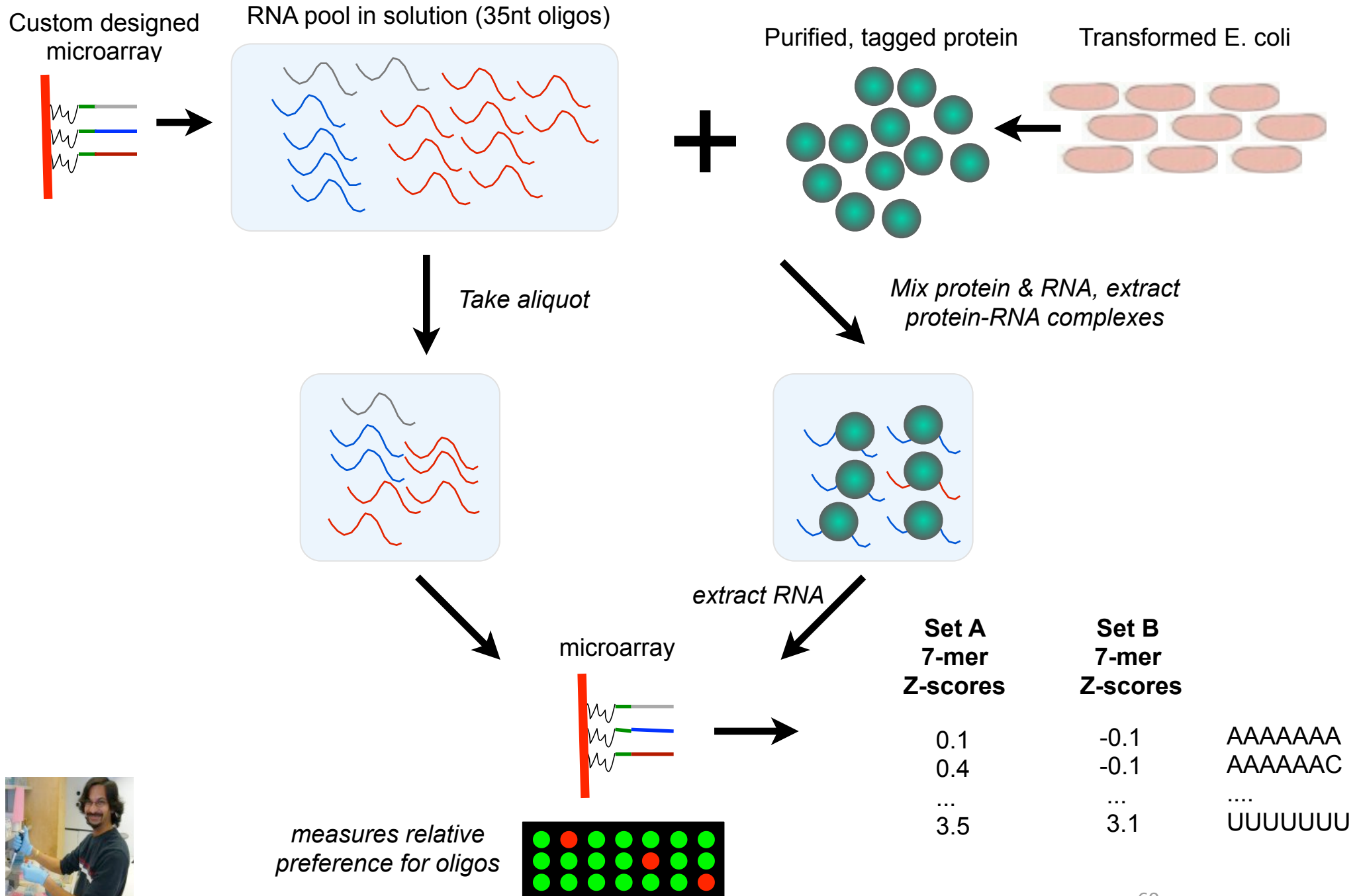
Prof. Matt Weirauch



Dr. Hamed Najafabadi

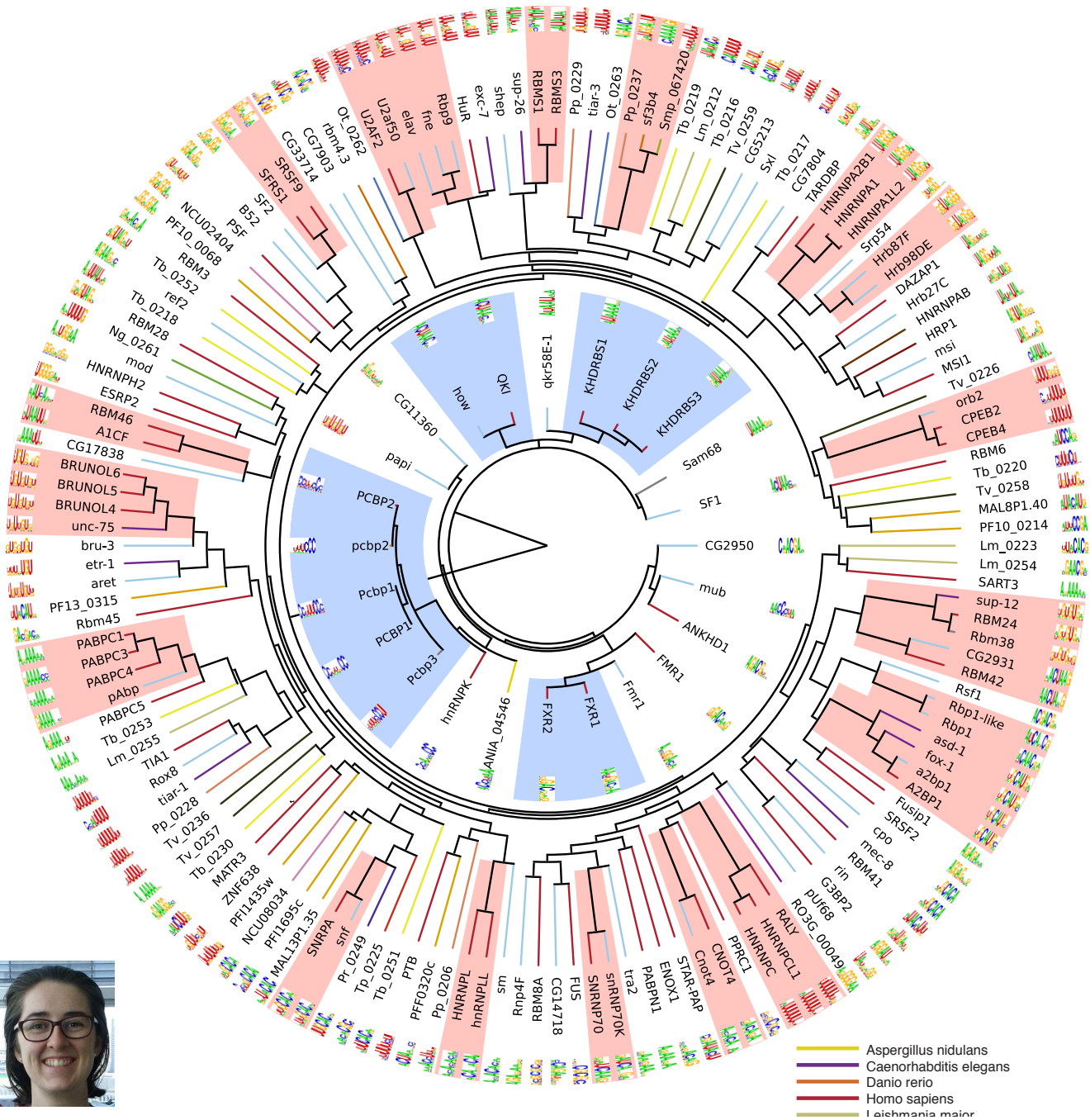
(Ray*, Kazan* et al, Nat Biotech 2009; Ray*, Kazan*, Cook*, Weirauch*, Najafabadi* et al, Nature 2013)

RNAcompete-based measurement of RBP RNA binding



In vitro sequence preferences of >200 RBPs

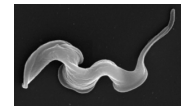
209 sequence-specific RBPs profiled



85 RBPs
31%* of genome



61 RBPs
36%* of genome



73 RBPs
<1% of eukaryote genomes



61

- *Aspergillus nidulans*
- *Caenorhabditis elegans*
- *Danio rerio*
- *Homo sapiens*
- *Leishmania major*



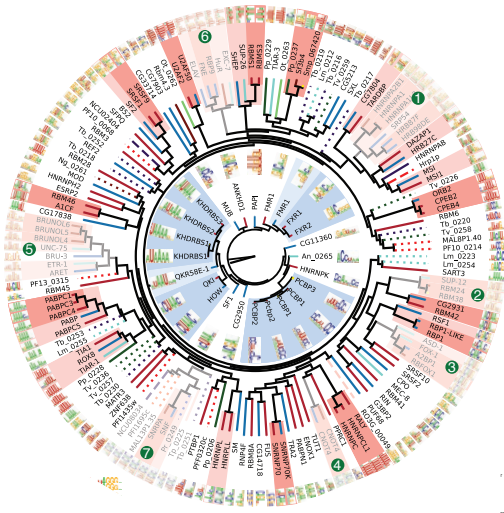
(Ray*, Kazan*, Cook*, Najafabadi*, Weirauch* et al, Nature 2013, in press)

Analysis of RBP secondary structure preferences

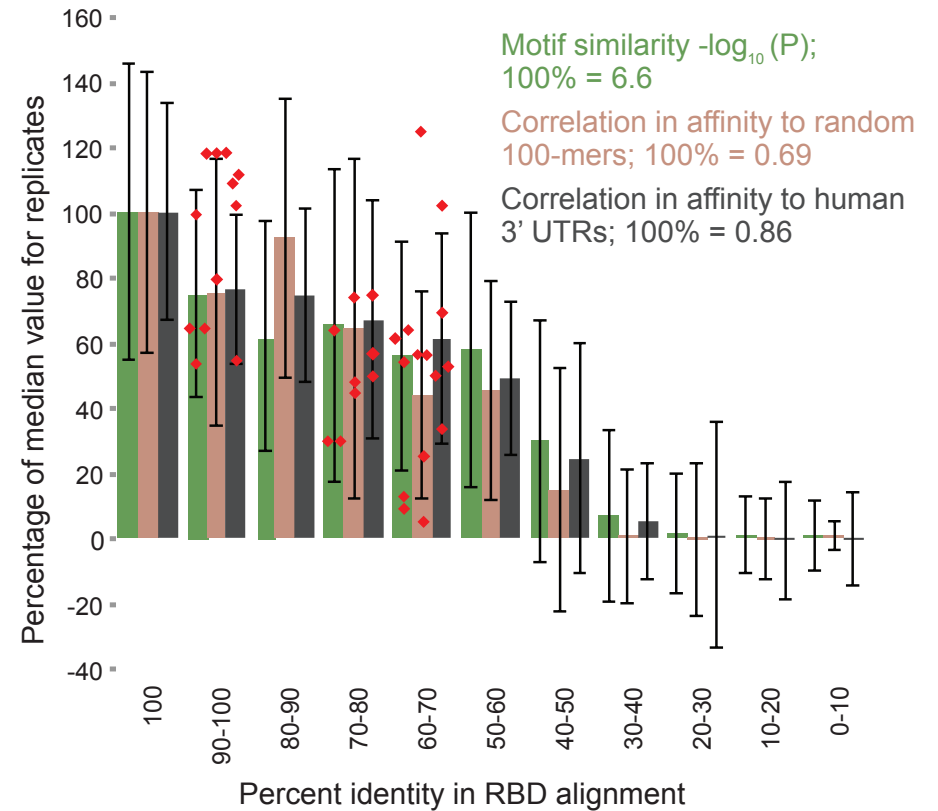
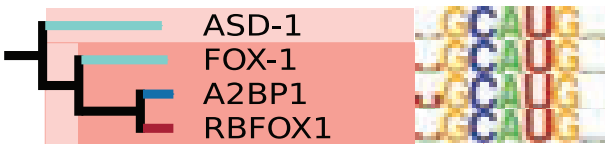
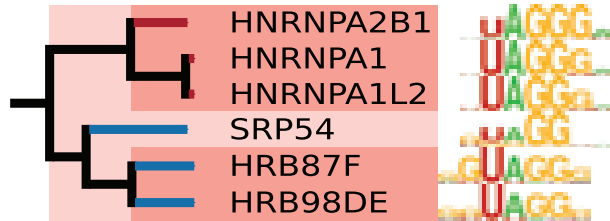
- RNA oligos in RNAcompete were designed to have no or weak secondary structure
- Nonetheless, we were able to detect a **significant preference for ssRNA for 55 of the RBPs (no RBPs preferred dsRNA)**
- 7 showed a preference **for** binding loops
- 15 showed a bias **against** binding loops



Similar protein sequence implies similar motifs

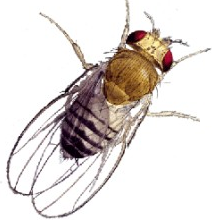


Zoom in

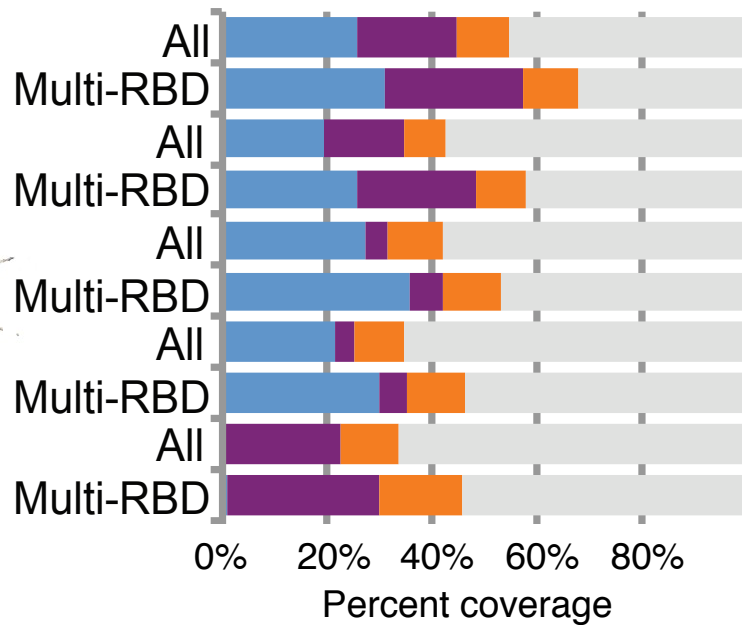


Inferring RBP motifs by protein sequence identity

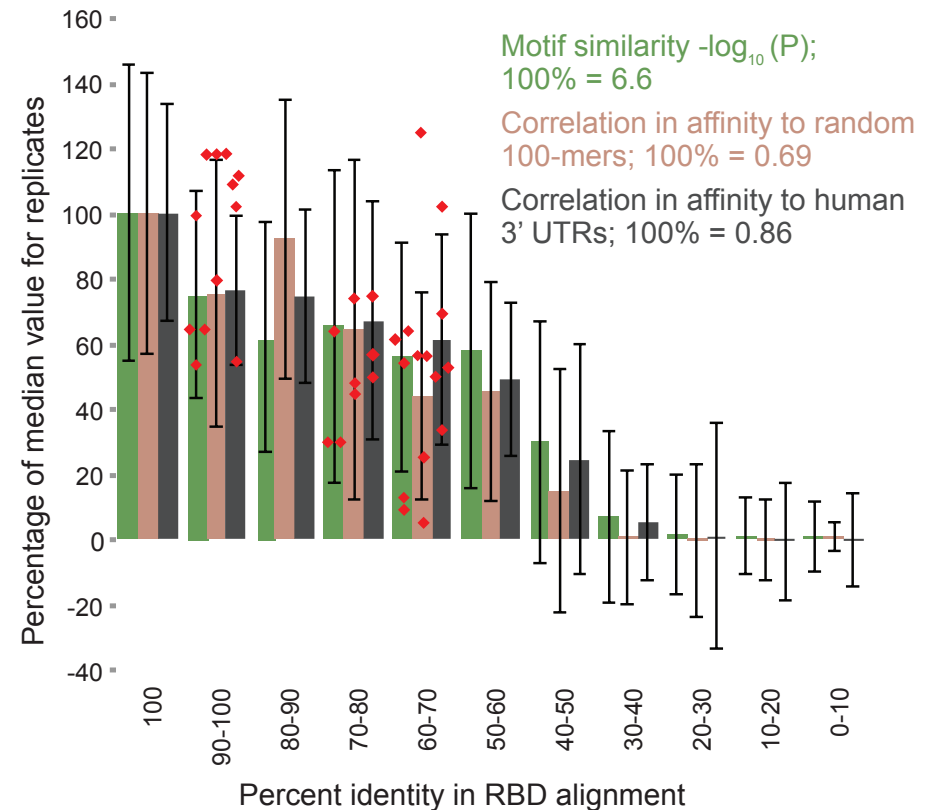
(domains)



Metazoa



— Directly measured (100%)
 70% similar —
 50% similar —



CISBP-RNA Database: Catalog of Inferred RNA Binding Proteins

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CISBP-RNA

Welcome to CIS-BP-RNA, the online library of RNA binding proteins and their motifs.

Search for a RBP

By Identifier

(e.g. Puf*, YGL122C, RNCMPT00046)

Browse RBPs / Restrict Search for RBPs

By Model Organism

By Any Species

By Domain Type

By Motif Evidence

By Evidence Type

By Study

Database Build

Latest build: 0.5

GO!

Last updated: 18-11-2012 Database Build 0.5

Current database contents: 7753 RBP binding motifs(238 from direct experiments), out of a total of 62587 Eukaryotic RBPs from 55 families in 289 species

CISBP-RNA Database: Catalog of Inferred RNA Binding Proteins

We are continuing with this project... please contact me (or Quaid) if you have a specific protein or species of interest!

a1cf (*Danio rerio*)
RRM


RBP Information

Pfam ID	Interpro ID	Gene ID	CISBP-RNA ID	Sequence source
PF00076 (RRM_1)	IPR000504	ENSDARG00000002968	T36035_0.5	Ensembl (2011-Oct-26)

Directly determined binding motifs

Name/Motif ID	Species	Sequence Logo	IUPAC	Type/Study/Study ID	RBD Identity
No direct experiments					

Motifs from related RBPs

Name/Motif ID	Species	Sequence Logo	IUPAC	Type/Study/Study ID	RBD Identity
A1CF M001_0.5	<i>Homo sapiens</i>		WUAAUUR	RNAcomplete Ray et al.(2012) RNCMPT00001	0.848

For this family, RBPs with RBD identity > 0.7 will likely have a similar motif
























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Summary

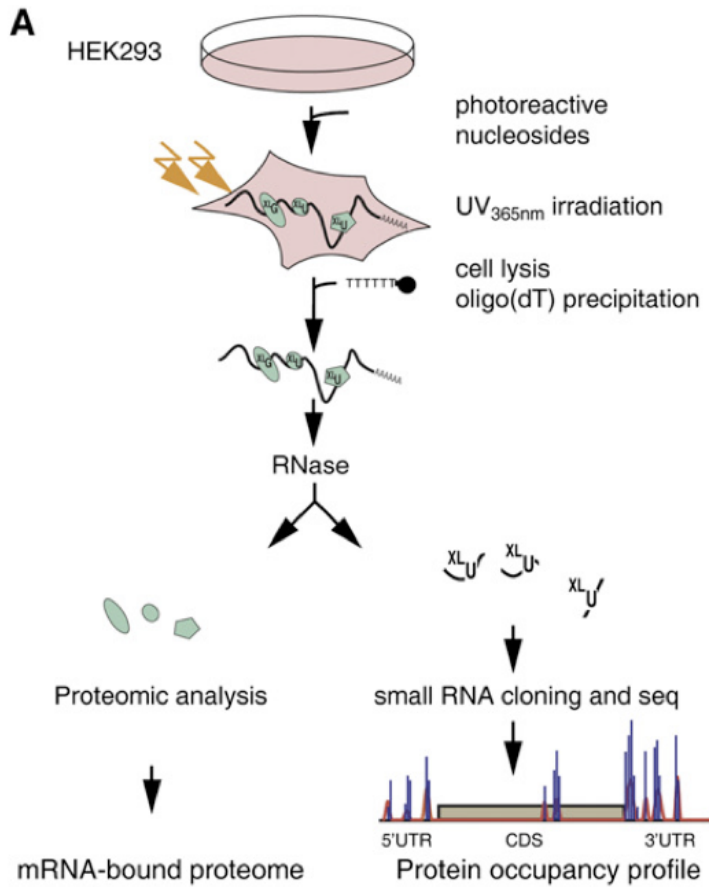
- We have RNA sequence motifs for 209 RBPs
- Can infer motifs for 1,000s more RBPs by homology, including 57% of human RBP complement and 30% of metazoan RBPs
- Motif scans allow the prediction of RBP function based on location of conserved motif hits and simple correlation analysis.

A bunch of different motifs from different species (selected from >100)

RRM	AT3G55460	Plantae	Arabidopsis_thaliana	
RRM	PK27672.1	Plantae	Cannabis_sativa	
RRM	PK26404.1	Plantae	Cannabis_sativa	
RRM	PK13173.1	Plantae	Cannabis_sativa	
RRM	PK15111.1	Plantae	Cannabis_sativa	
RRM	PK23225.1	Plantae	Cannabis_sativa	
RRM	PK23842.1	Plantae	Cannabis_sativa	
RRM	PFI1175c	Protista	Plasmodium_falciparum	
RRM	Smp_036270	Protista	Schistosoma_mansoni	
RRM	Smp_032060	Protista	Schistosoma_mansoni	
RRM x 2	DDB_G0286331	Amoebozoa	Dictyostelium_discoideum	
RRM x 2	DDB_G0288391	Amoebozoa	Dictyostelium_discoideum	
RRM x 2	CBG14639	Animalia	Caenorhabditis_briggsae	
RRM x 2	CBG13971	Animalia	Caenorhabditis_briggsae	
RRM x 2	CBG14639	Animalia	Caenorhabditis_briggsae	
RRM x 2	CBG04067	Animalia	Caenorhabditis_briggsae	
RRM x 2	CBG05471	Animalia	Caenorhabditis_briggsae	
RRM x 2	CBG03563	Animalia	Caenorhabditis_briggsae	
RRM x 2	rnp-2	Animalia	Caenorhabditis_elegans	
RRM x 2	Y111B2A.18	Animalia	Caenorhabditis_elegans	
RRM x 2	W02B12.2	Animalia	Caenorhabditis_elegans	
RRM x 2	K08D10.3	Animalia	Caenorhabditis_elegans	
RRM x 2	ENSDARG00000036161	Animalia	Danio_rerio	

RRM x 2	FBgn0031607	Animalia	Drosophila_melanogaster	
RRM x 2	PTBP2	Animalia	Homo_sapiens	
RRM x 2	CPEB1	Animalia	Homo_sapiens	
RRM x 2	ENSMGAG00000016128	Animalia	Meleagris_gallopavo	
RRM x 2	ENSMGAG00000006135	Animalia	Meleagris_gallopavo	
RRM x 2	ENSXETG00000027221	Animalia	Xenopus_tropicalis	
RRM x 2	ENSXETG00000026650	Animalia	Xenopus_tropicalis	
RRM x 2	PGTG_09691	Fungi	Puccinia_graminis	
RRM x 2	spo5	Fungi	Saccharomyces_cerevisiae	
RRM x 2	srp2	Fungi	Saccharomyces_cerevisiae	
RRM x 2	SPCC306.04c	Fungi	Schizosaccharomyces_pombe	
RRM x 2	AT2G46610	Plantae	Arabidopsis_thaliana	
RRM x 2	AT2G41060	Plantae	Arabidopsis_thaliana	
RRM x 2	PK03611.1	Plantae	Cannabis_sativa	
RRM x 2	PK03611.1	Plantae	Cannabis_sativa	
RRM x 2	PK15181.1	Plantae	Cannabis_sativa	
RRM x 2	PK04894.1	Plantae	Cannabis_sativa	
RRM x 2	PK14112.1	Plantae	Cannabis_sativa	
RRM x 2	PK11774.1	Plantae	Cannabis_sativa	
RRM x 2	PK25912.1	Plantae	Cannabis_sativa	
RRM x 2	PK00513.1	Plantae	Cannabis_sativa	
RRM x 3	DDB_G0270634	Amoebozoa	Dictyostelium_discoideum	
RRM x 3	CBG15837	Animalia	Caenorhabditis_briggsae	

New RBPs identified recently by mass spectrometry



The mRNA-Bound Proteome and Its Global Occupancy Profile on Protein-Coding Transcripts

Alexander G. Baltz,^{1,3} Mathias Munschauer,^{1,3} Björn Schwanhäusser,¹ Alexandra Vasile,¹ Yasuhiro Murakawa,¹ Markus Schueler,¹ Noah Youngs,² Duncan Penfold-Brown,² Kevin Drew,² Miha Milek,¹ Emanuel Wyler,¹ Richard Bonneau,² Matthias Selbach,¹ Christoph Dieterich,¹ and Markus Landthaler^{1,*}

¹Max Delbrück Center for Molecular Medicine, Berlin Institute for Medical Systems Biology, 13125 Berlin, Germany

²Center for Genomics and Systems Biology, Department of Biology, New York University, New York, NY 10003, USA

Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins

Alfredo Castello,^{1,4} Bernd Fischer,^{1,4} Katrin Eichelbaum,¹ Rastislav Horos,¹ Benedikt M. Beckmann,¹ Claudia Strein,¹ Norman E. Davey,¹ David T. Humphreys,² Thomas Preiss,^{2,3} Lars M. Steinmetz,¹ Jeroen Krijgsveld,^{1,*} and Matthias W. Hentze^{1,2,*}

¹European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, Heidelberg 69117, Germany

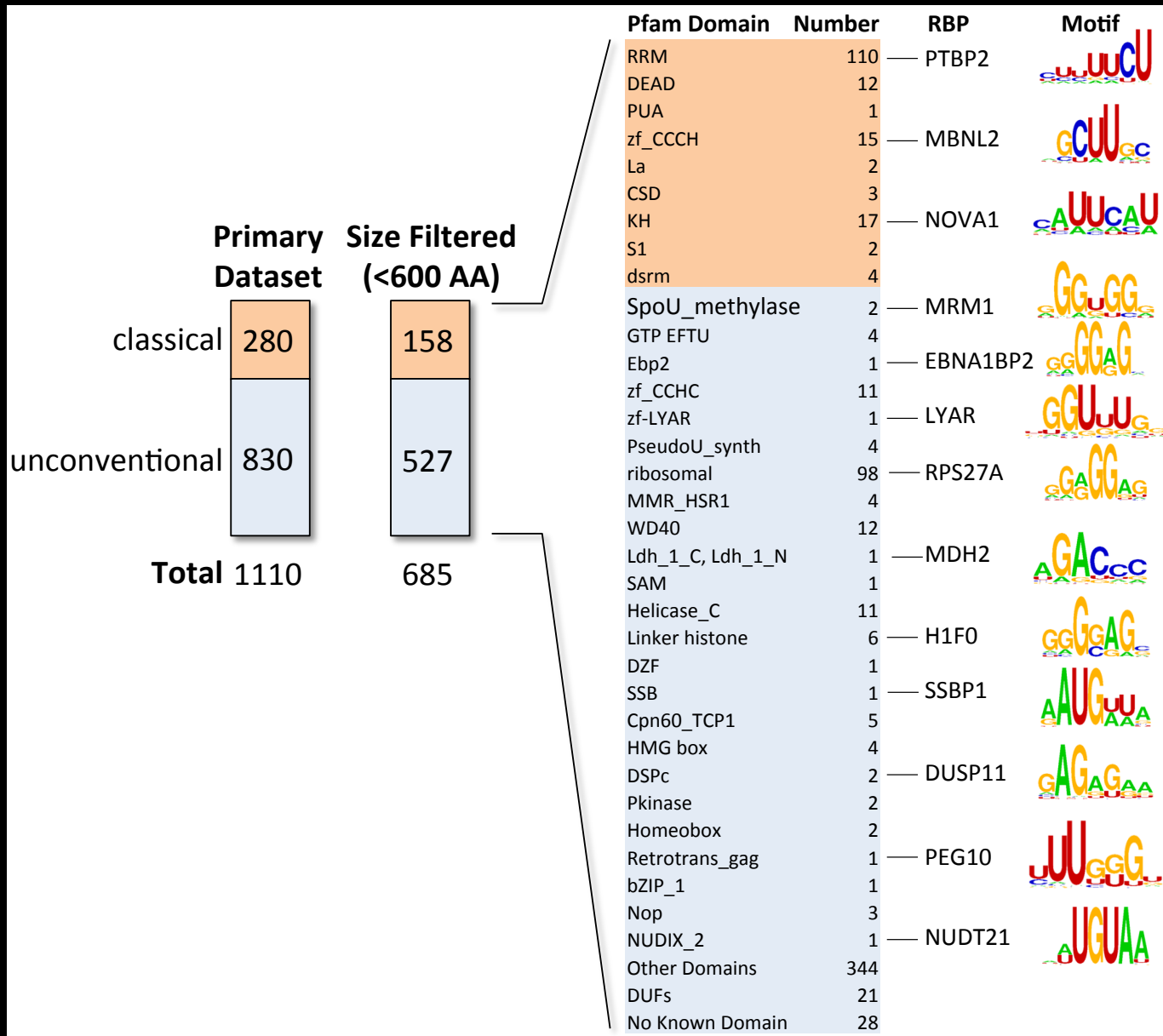
²Molecular Genetics Division, Victor Chang Cardiac Research Institute, Sydney NSW 2010, Australia

³Genome Biology Department, The John Curtin School of Medical Research, The Australian National University, Building 131, Garran Road, Acton ACT 0200, Australia

Pfam domains

233	Classical RBD
225	Non-classical RBD
402	RBD unknown

Human ucRBPs are often sequence specific (initial result: 9/37 = 24% yield motif)



Ribosomal protein

ssDNA binding protein,
mitochondrial biogenesis

(Positive control)

MORRIS LAB
XIAO LI (STANFORD)
HILAL KAZAN (AIU)
WEI JIAO (OICR)
GERALD QUON (MIT)
HOSSEIN RADFAR (OICR)
AMIT DESHWAR
SHANKAR VEMBU
KEVIN HA
SIMON ENG
KHALID ZUBERI
MAX FRANZ
JASON MONTOJO
HAROLD RODRIGUEZ
KATE NIU
JEFF WINTERSINGER
CHRIS CREMER

TIMOTHY HUGHES
DEB RAY
KATE COOK
MATT WEIRAUCH (CCH)
HAMED NAJAFABADI
MIHAI ALBU
HONG ZHENG
ALLY YANG

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MANUEL IRIMIA

ANDY FRASER
ARUN RAMANI

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