

# THE CELL

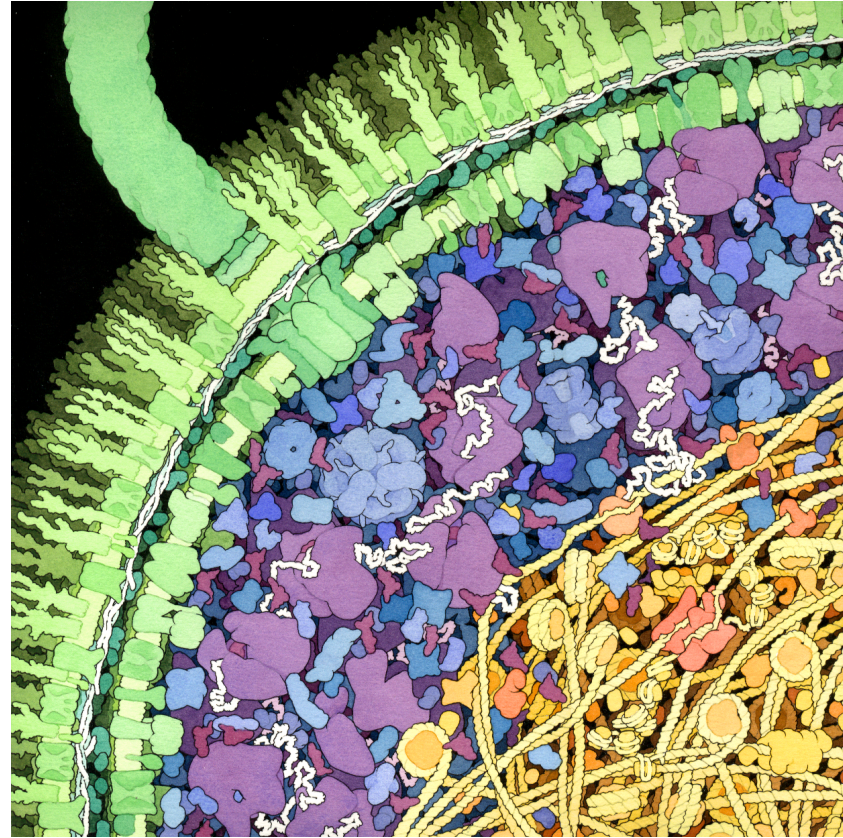
Water

DNA

RNA

Protein

Lipids, sugars and  
other small molecules

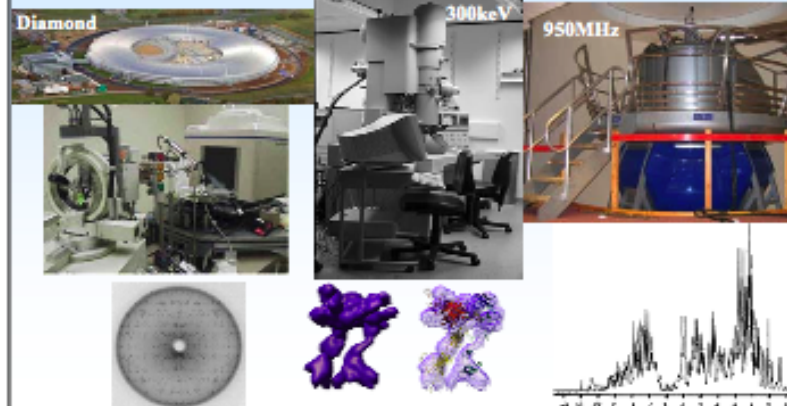


Molecular Art by David S. Goodsell

# Structural biology techniques

**Current situation**  
**High resolution (<1nm) structural tools:**

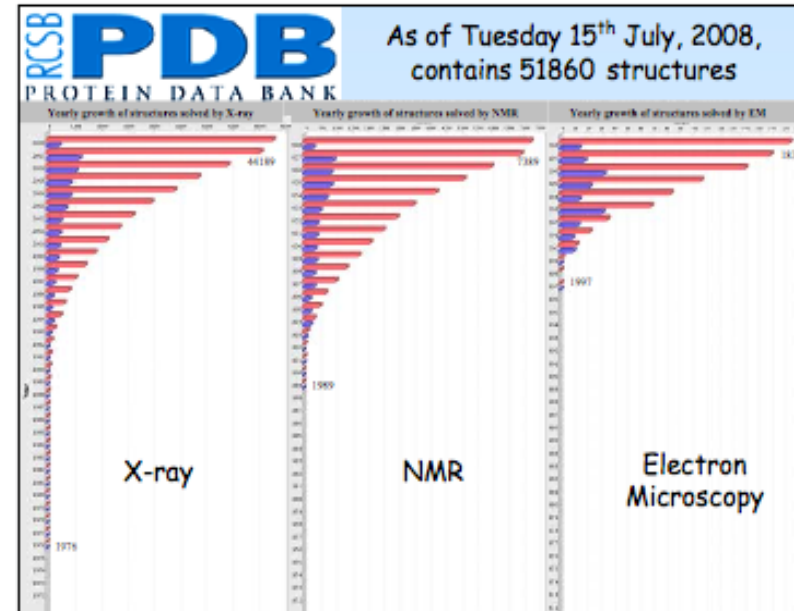
X-ray diffraction      electron microscopy      nuclear magnetic resonance



Diamond

300keV

950MHz



# Protein-RNA interactions

As seen by structural biology

# Gene expression program

DNA



Transcription

RNA Pol I  
RNA Pol II  
RNA Pol III

RNA (rRNA,mRNA,tRNA)



Translation

Protein

RNA biogenesis

RNA packaging, stability  
5' capping, RNA editing  
splicing, alternative splicing  
3' end processing  
(cleavage and polyadenylation)  
export

# THE CELL

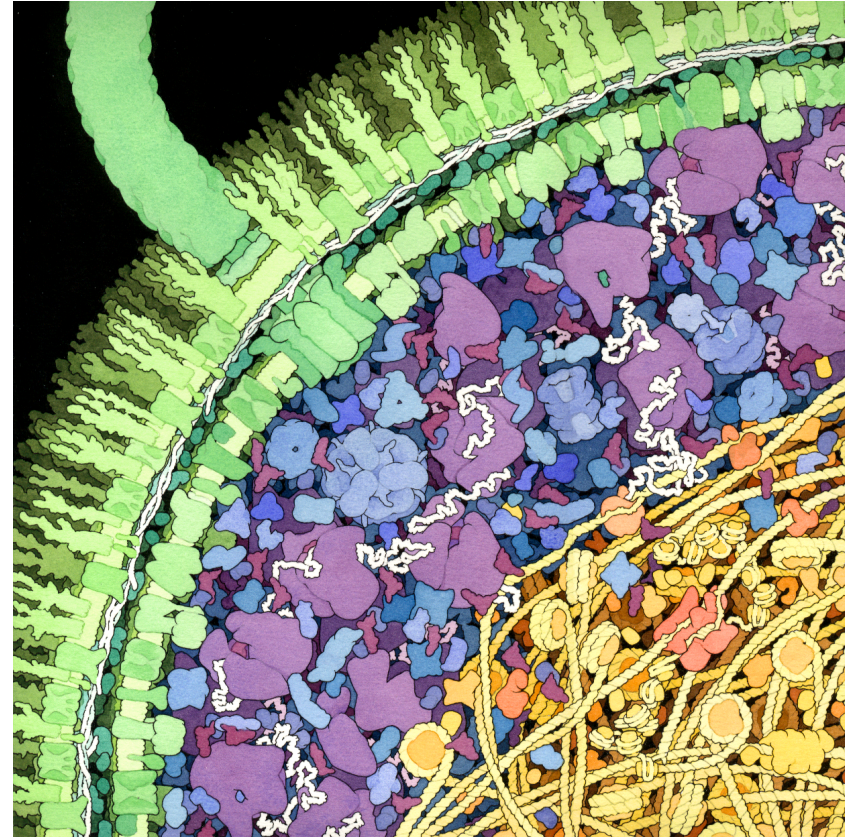
Water

DNA

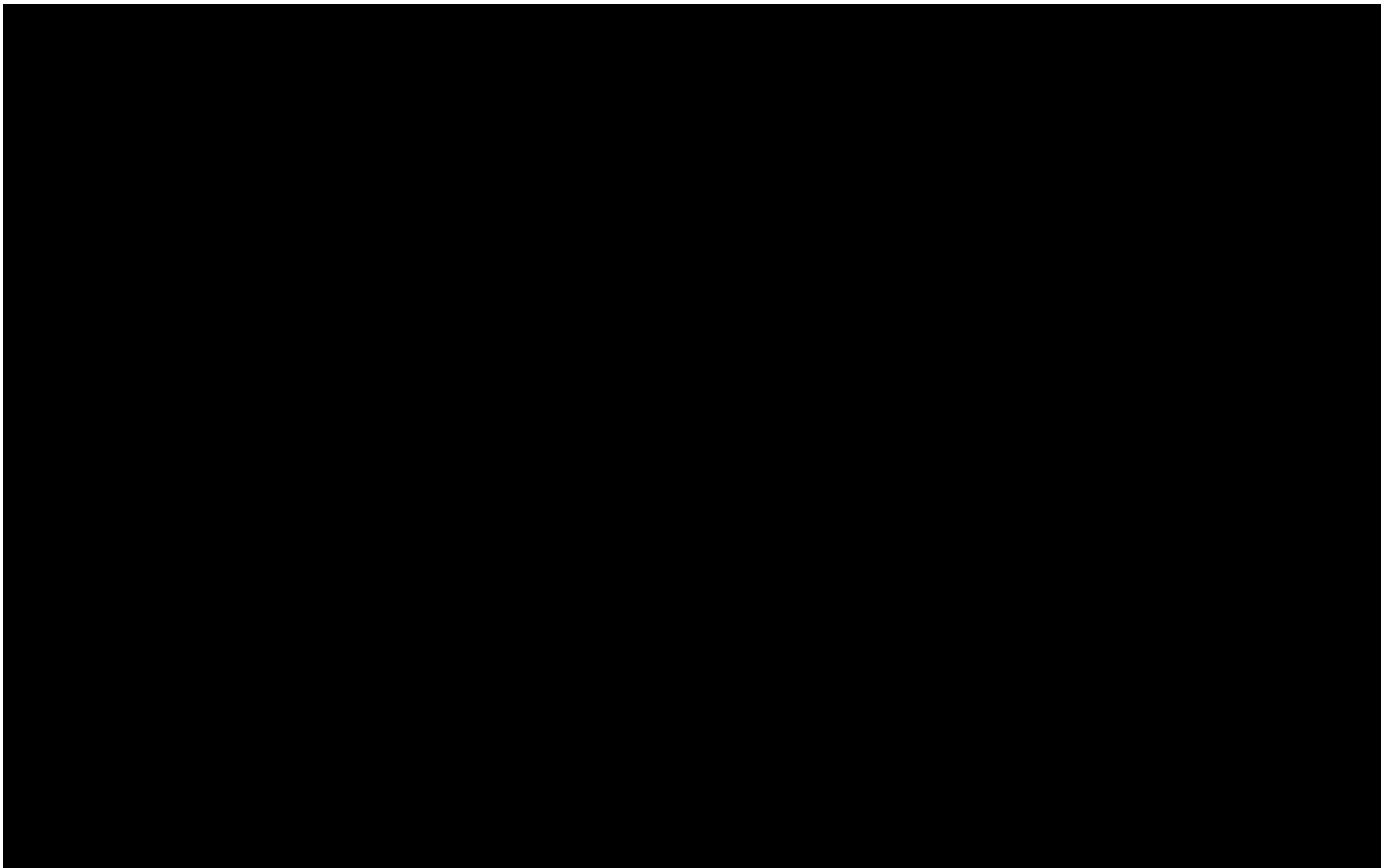
RNA

Protein

Lipids, sugars and  
other small molecules



Molecular Art by David S. Goodsell



# Gene expression program

DNA



Transcription

RNA Pol I  
RNA Pol II  
RNA Pol III

RNA (rRNA,mRNA,tRNA)



Translation

Protein

RNA biogenesis

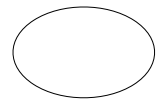
RNA packaging, stability  
5' capping, RNA editing  
splicing, alternative splicing  
3' end processing  
(cleavage and polyadenylation)  
export

## Disease-causing mutations in RNAs & RNPs

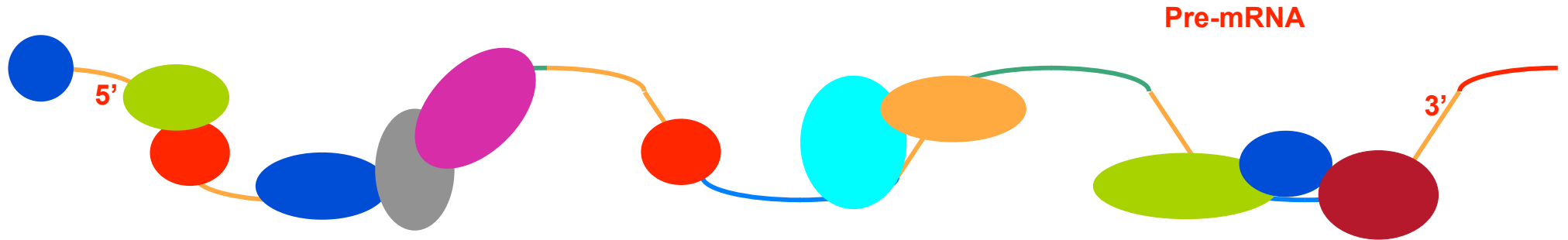
<b><i>Disease</i></b>	<b><i>Gene/Mutation</i></b>	<b><i>Function</i></b>
Prader Willi syndrome	SNORD116	ribosome biogenesis
Spinal muscular atrophy (SMA)	SMN2	splicing
Dyskeratosis congenita (X-linked)	DKC1	telomerase/translation
Diamond-Blackfan anemia	RPS19, RPS24	ribosome biogenesis
Prostate cancer	SNHG5	ribosome biogenesis
Myotonic dystrophy, type 2 (DM2)	ZNF9 (RNA gain of function)	RNA binding
Huntington's disease-like 2 (HDL2)	JPH3 (RNA gain of function)	ion channel function
Fragile X syndrome	FMR1	translation/mRNA localization
Retinitis pigmentosa	HPRP3	splicing
Autism	7q22-q33 locus breakpoint	noncoding RNA
Amyotrophic lateral sclerosis (ALS)	TARDBP	splicing, transcription
Deafness	MTRNR1	ribosome biogenesis (mitochondrial)
Cancer	SFRS1	splicing, translation, export
Cancer	miR-17-92 cluster RNA miR-372, miR-373	RNA interference

Cooper et al. Cell, 2009





**RNA binding proteins**



**RNA binding proteins control the fate of the pre-mRNA**

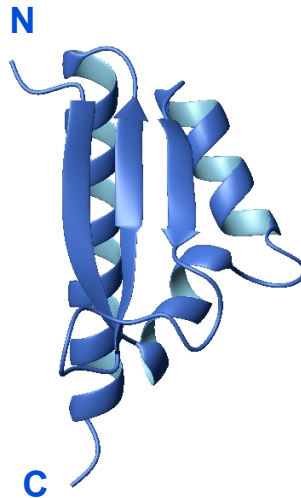
# RNA binding proteins



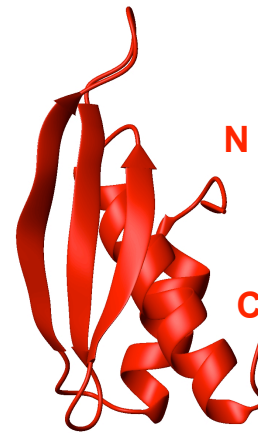
RNA Binding Domain



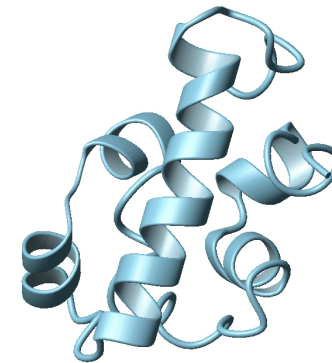
RRM/RBD/RNP



KH domain



dsRBM



SAM domain

## RNA binding proteins of two types:

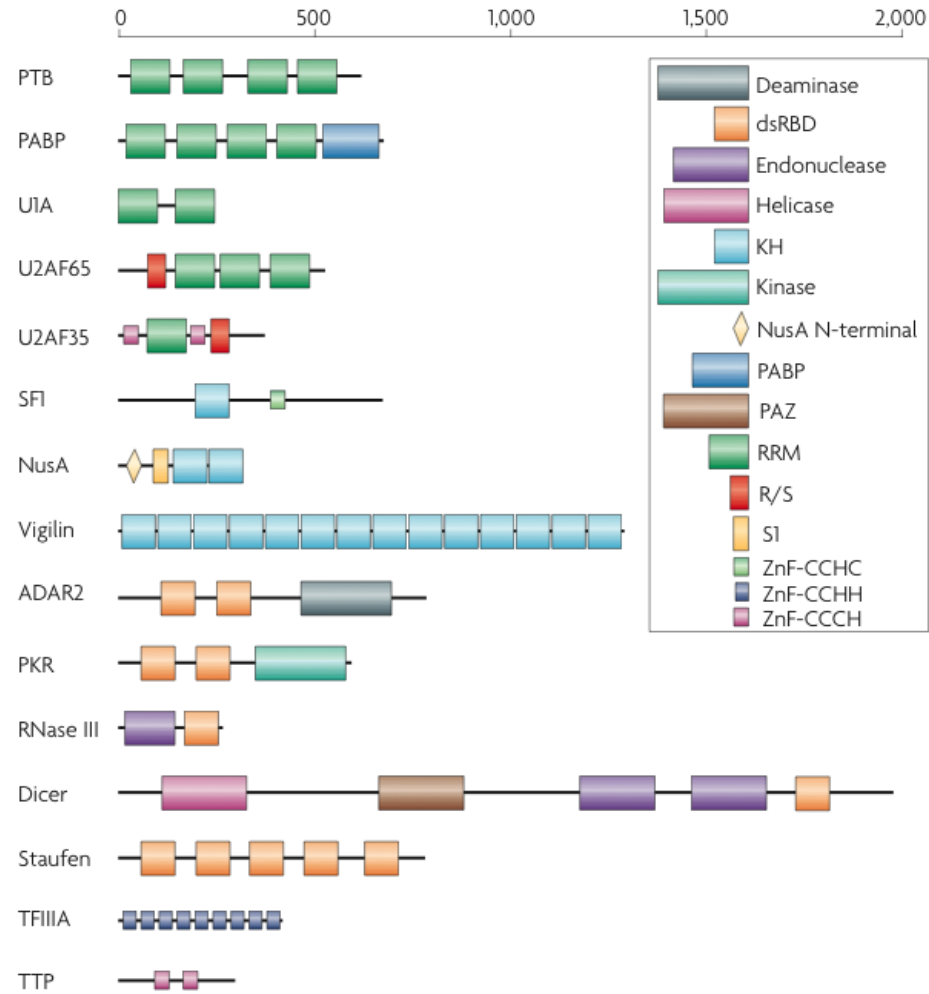
- **enzymes**

polymerase, nuclease, modifying enzymes

- **binding proteins**

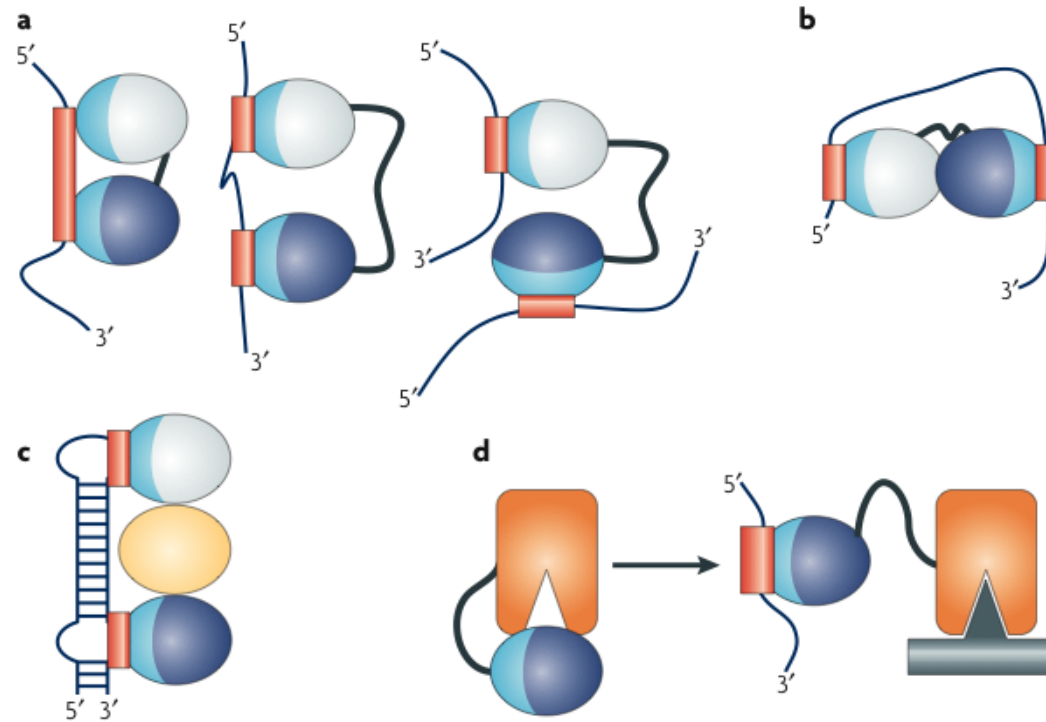
protection, folding (chaperone), gene regulation

# Modular architecture of RNA-binding proteins

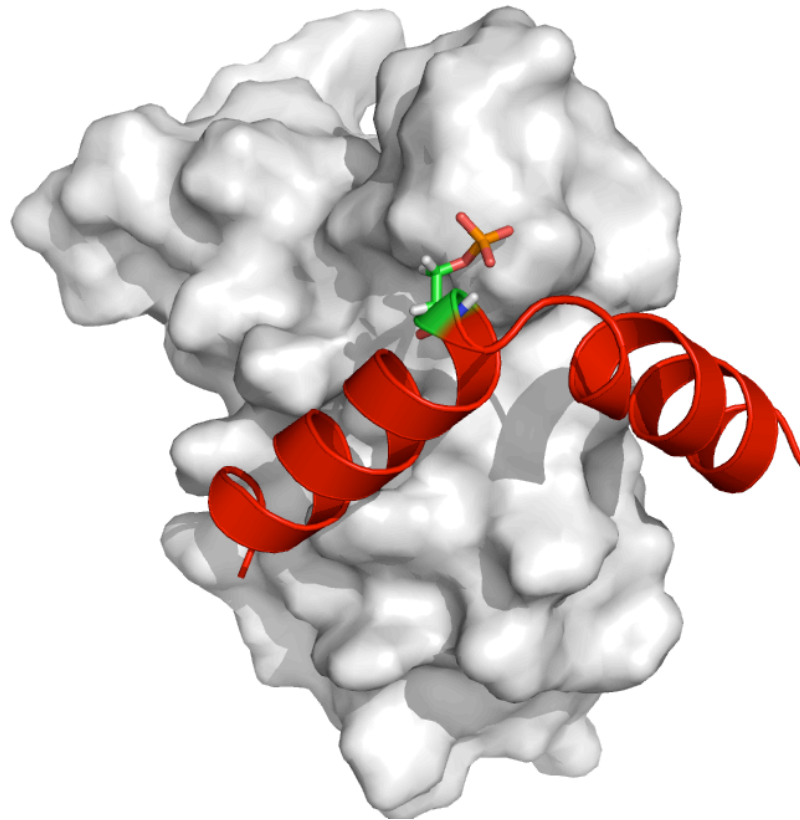


Taken from Lunde et al. Nat. Rev. Mol. Cell Biol 2007

# RNA-binding modules are often combined to perform multiple functional roles

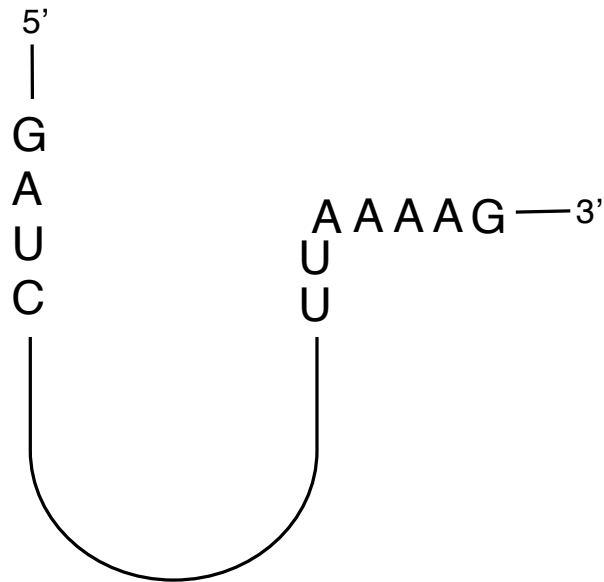


Coupled protein binding and folding  
(phosphorylation dependent)

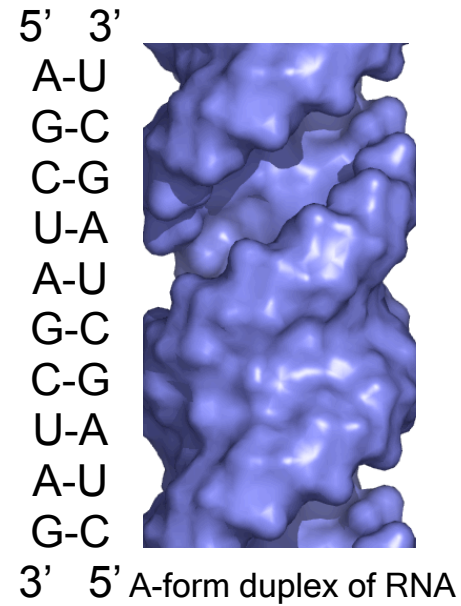


# What information is recognized by proteins:

Recognition of RNA sequence

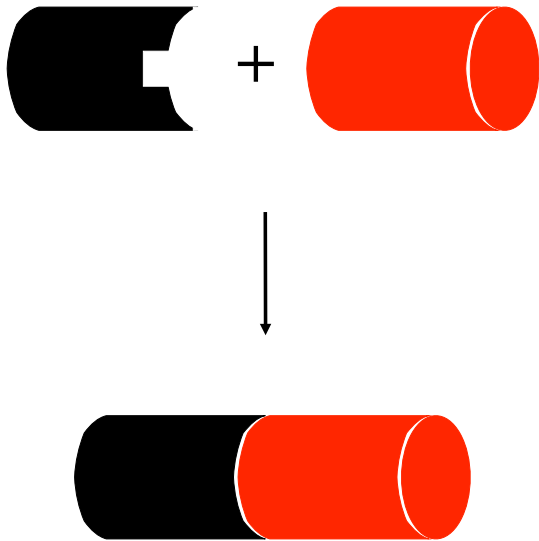


Recognition of RNA shape

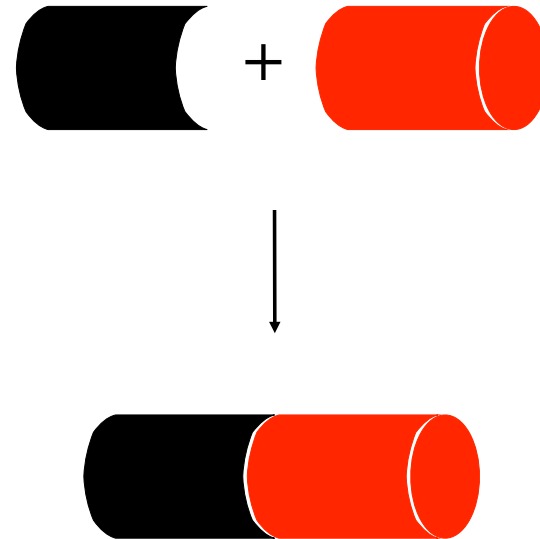


What recognition mode is used by proteins:

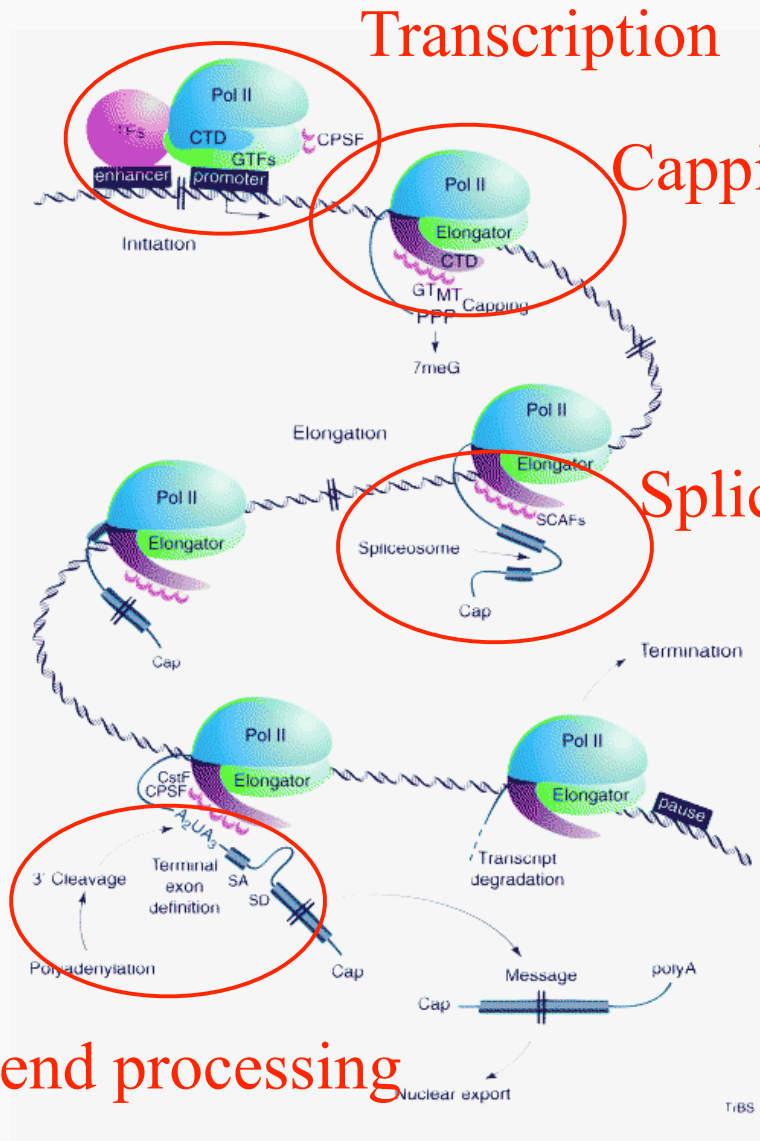
Induced fit



Rigid-body docking





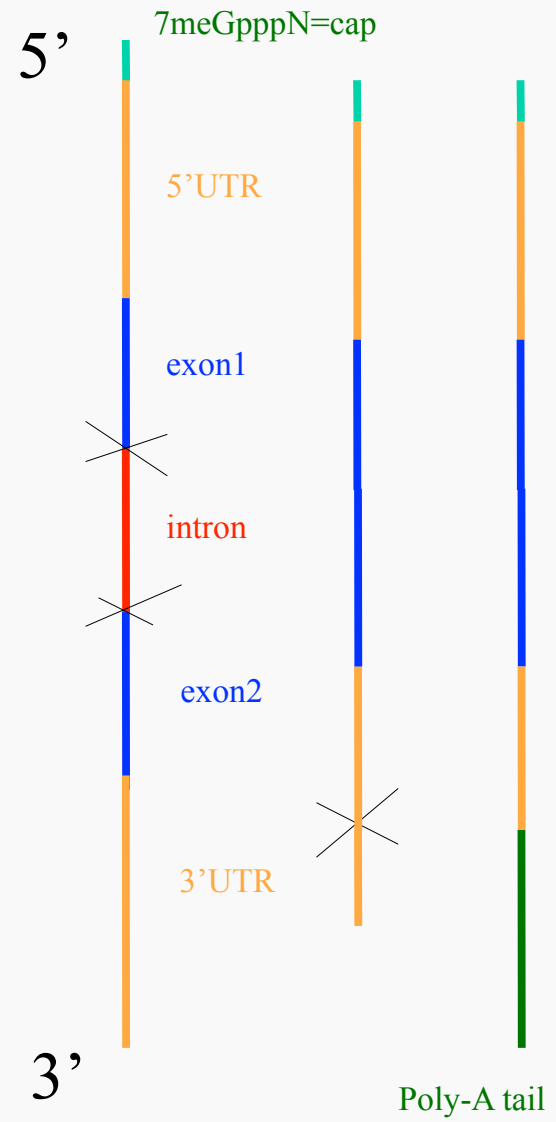


Transcription

Capping

Splicing

3' end processing



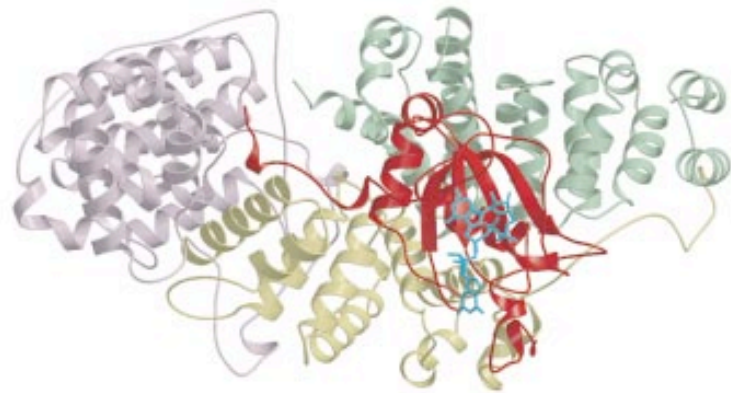
# Induced fit recognition

Examples

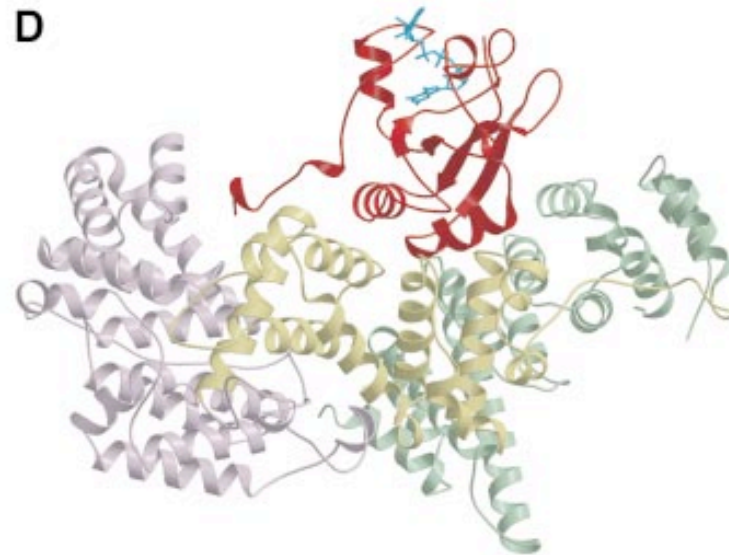
Induced fit binding

5'cap binding protein, **CBP20**-CBP80

C



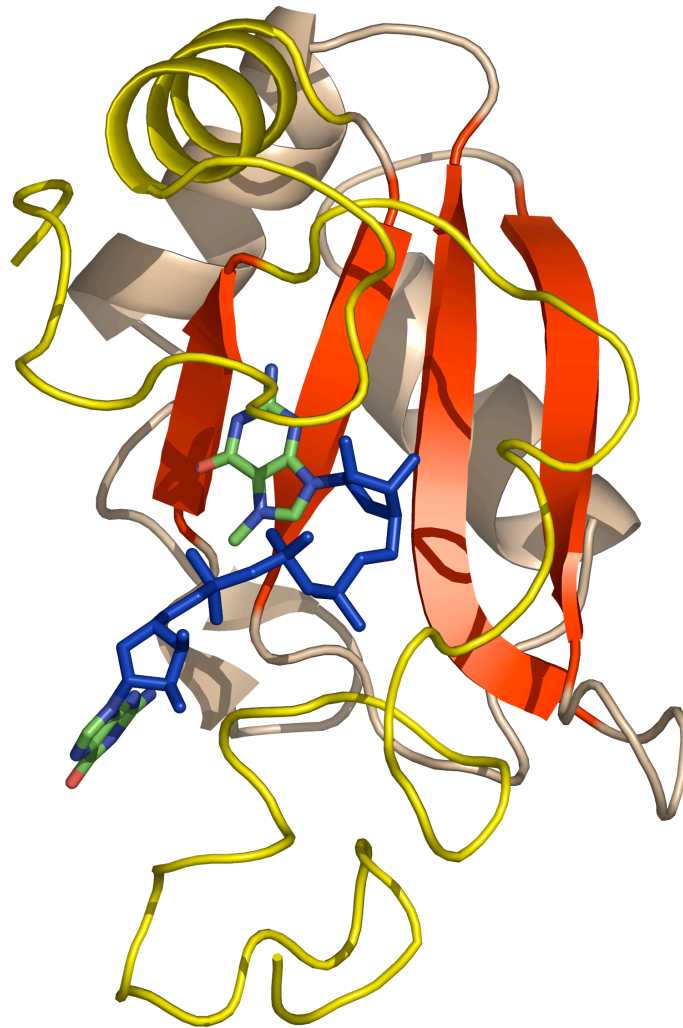
D



**CBP20**-CBP80

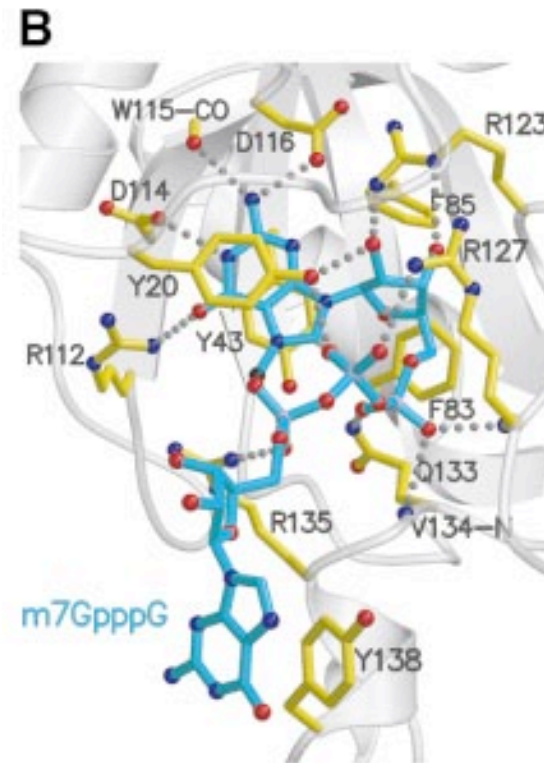
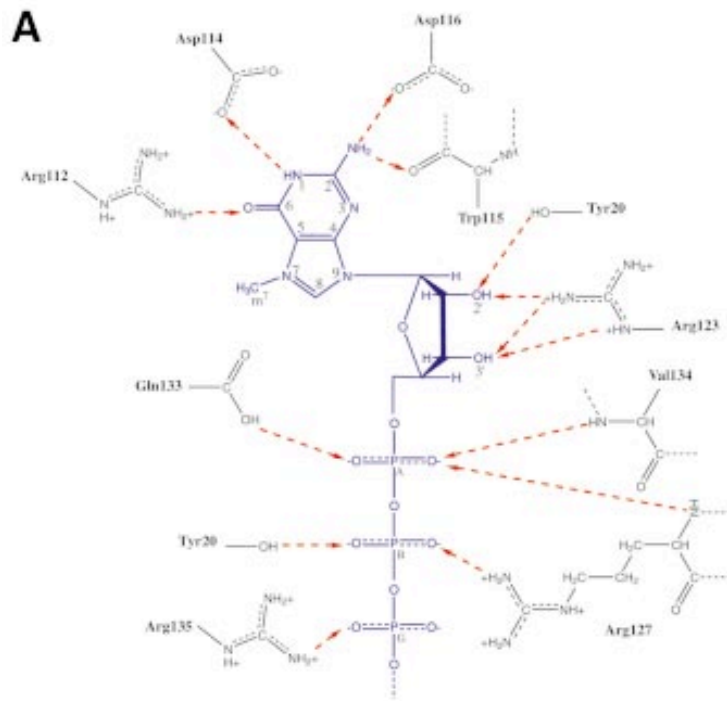
Mazza et al, EMBO J (2002)

Induced fit binding



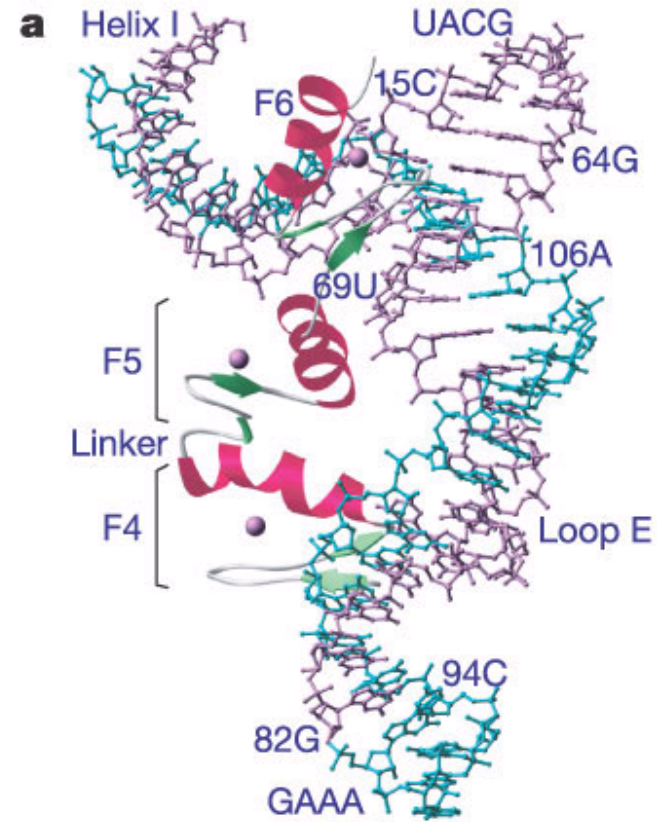
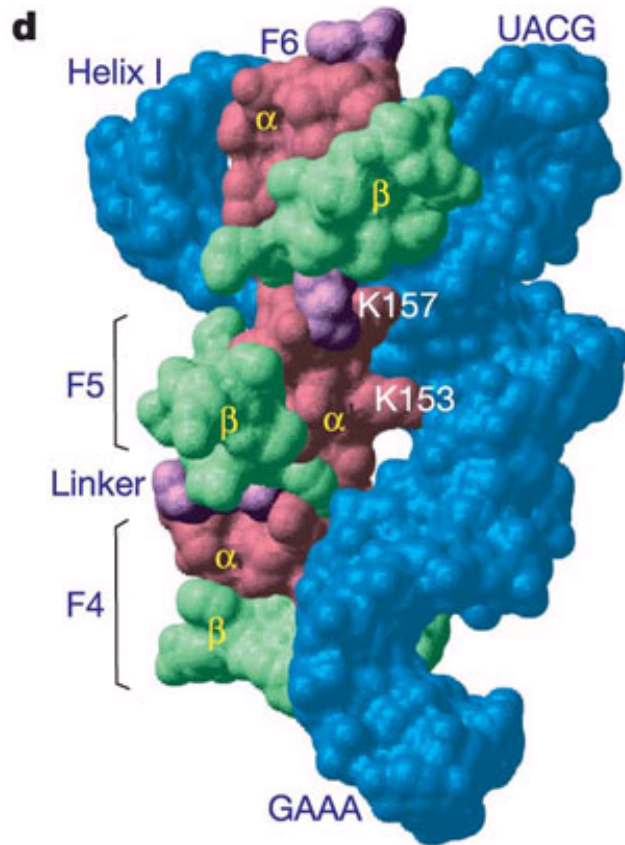
# Induced fit binding

## CBP20-m7GpppG contacts



Induced fit binding

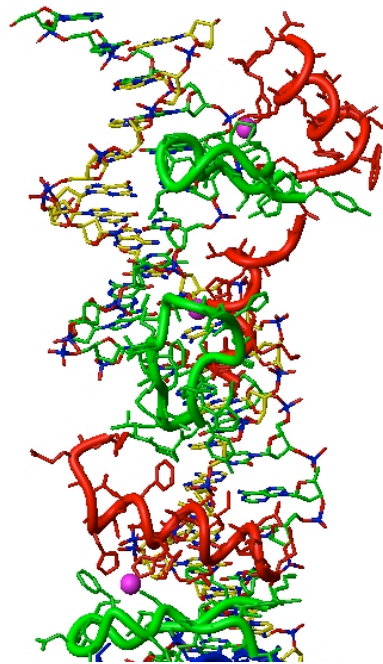
# Zinc finger-RNA



(Lu et al, Nature , 2003)

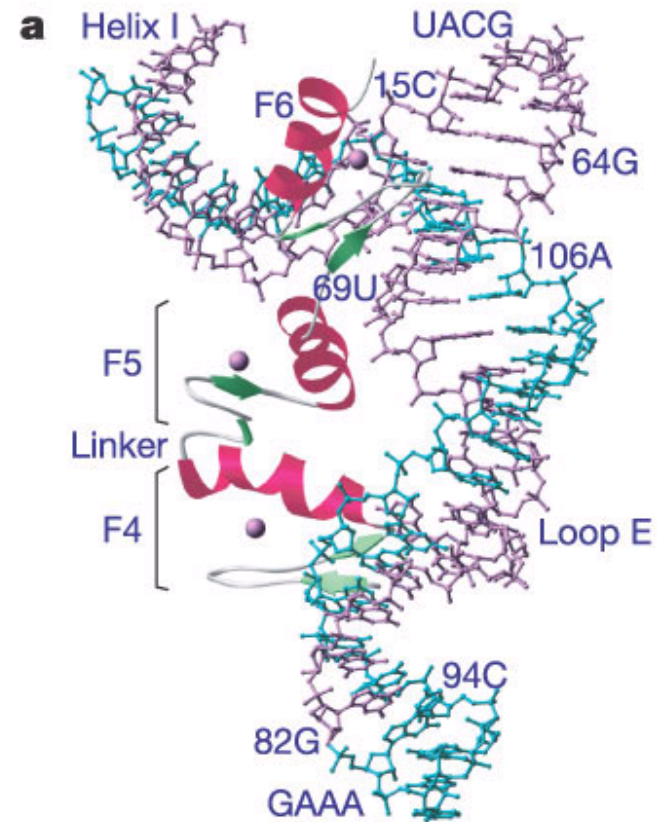
Induced fit binding

## Zinc finger-DNA



(Nolte et al, PNAS , 1998)

## Zinc finger-RNA

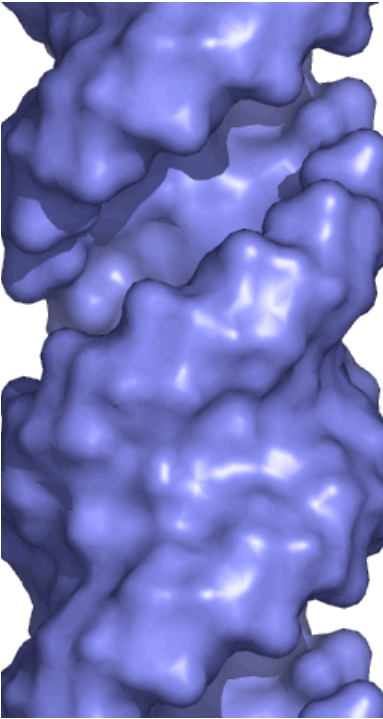


(Lu et al, Nature , 2003)

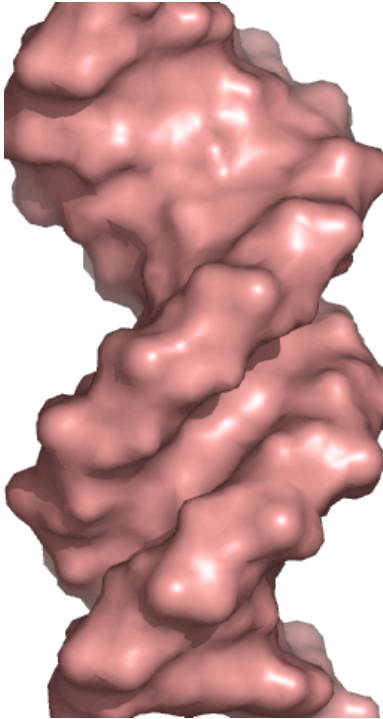
# A-form double-helix vs. B-form double-helix

---

5' 3'  
A-U  
G-C  
C-G  
U-A  
A-U  
G-C  
C-G  
U-A  
A-U  
G-C  
3' 5'



A-form duplex of RNA

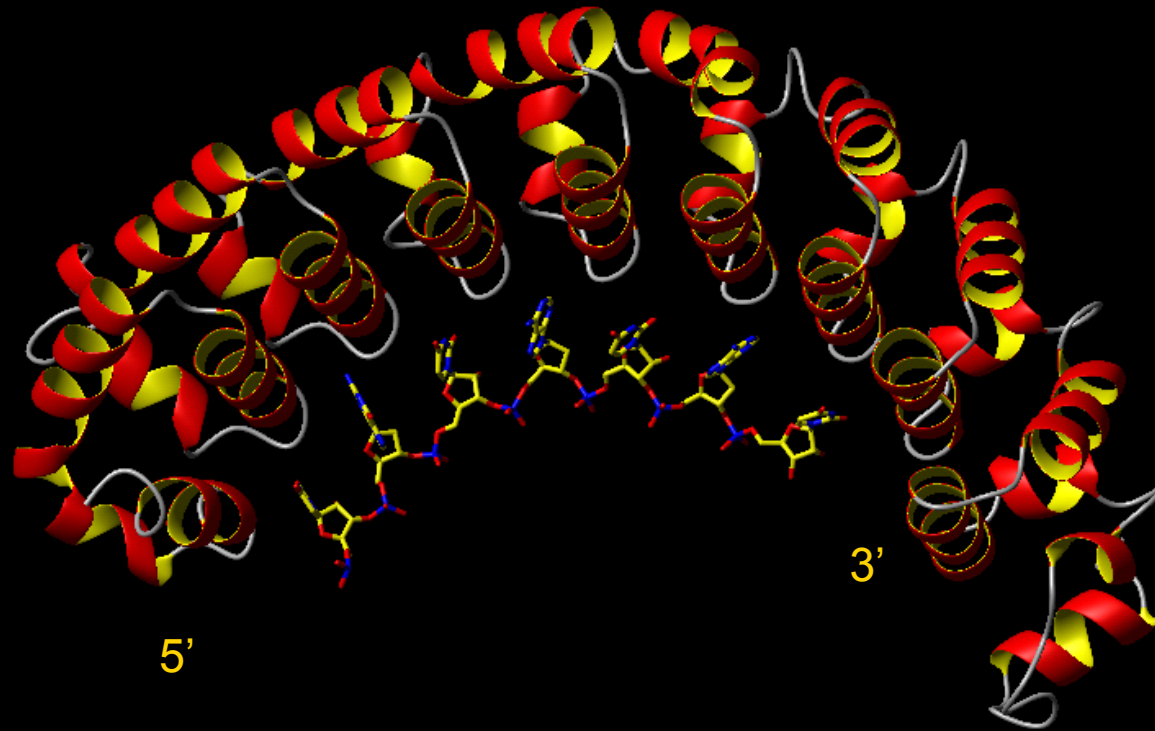


5' 3'  
A-T  
G-C  
C-G  
T-A  
A-T  
G-C  
C-G  
T-A  
A-T  
G-C  
3' 5'

B-form duplex of DNA



# Pumilio domain

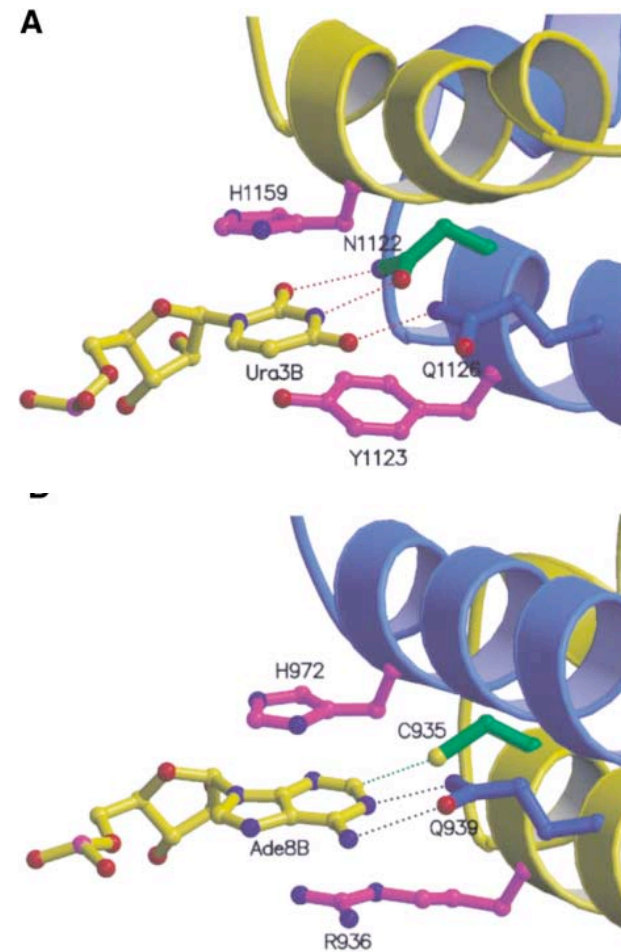
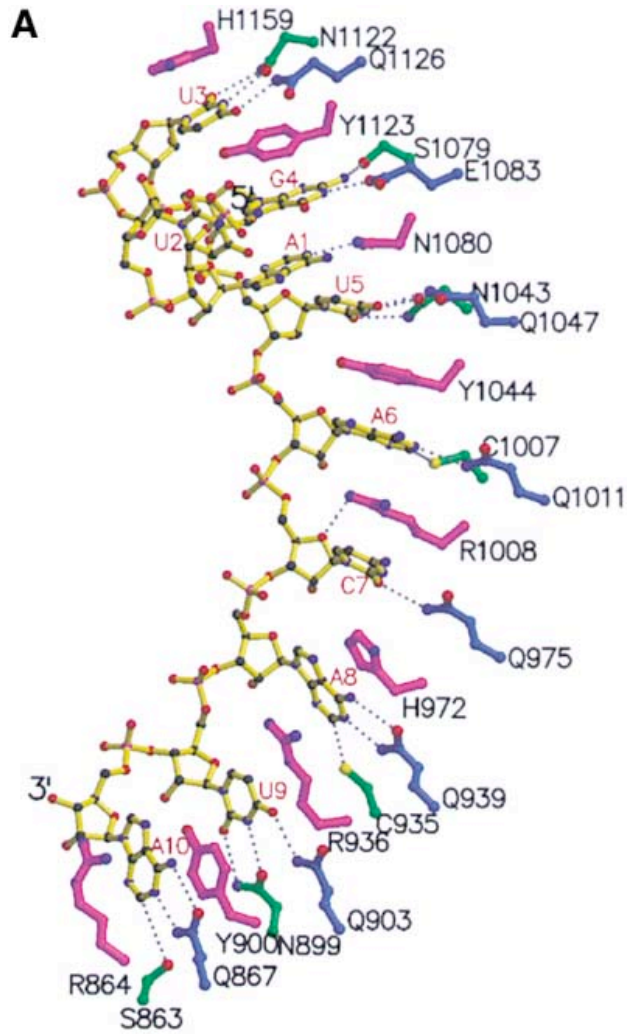


hPUM-UGUAUUAU

(Wang et al, Cell, 2002)

# Induced fit binding

## Pumilio domain

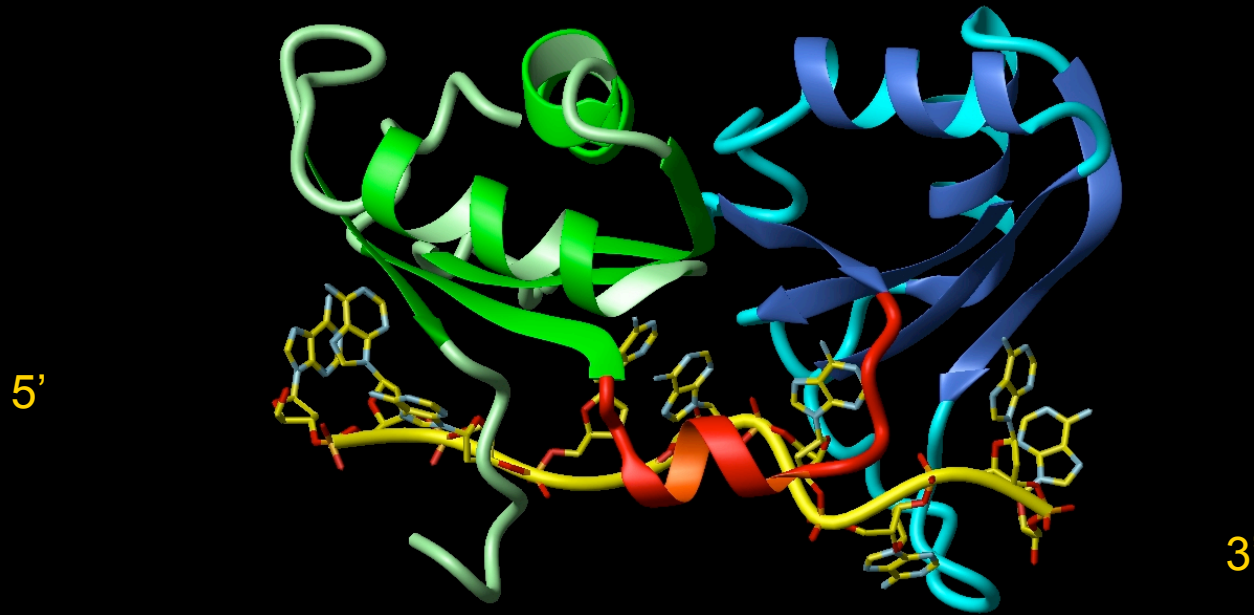


(Wang et al, Cell, 2002)

# Poly A binding protein

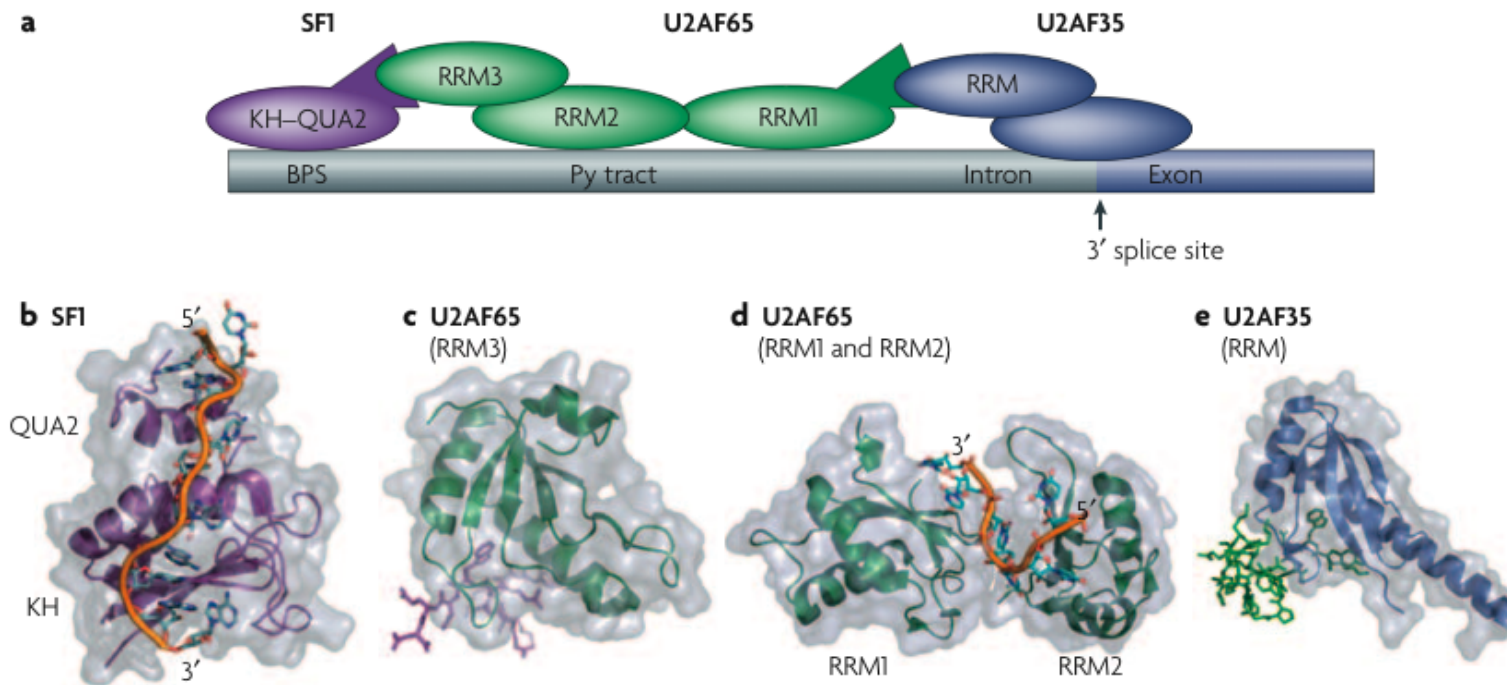
RBD2

RBD1



(Deo et al, Cell, v98 1999)

# Protein-protein interactions and protein-RNA interactions define the site of spliceosomal assembly



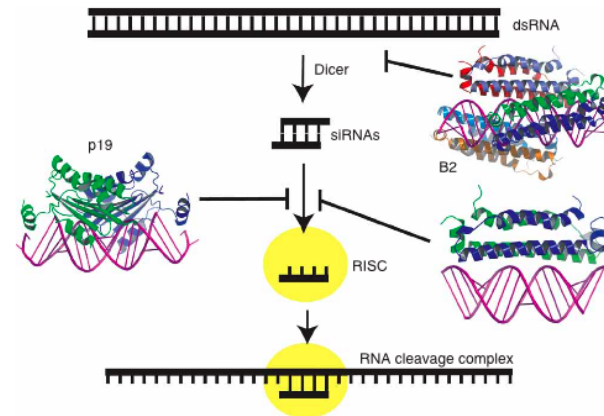
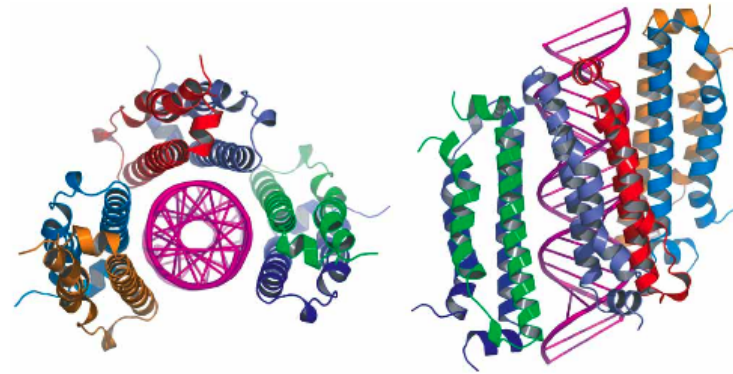
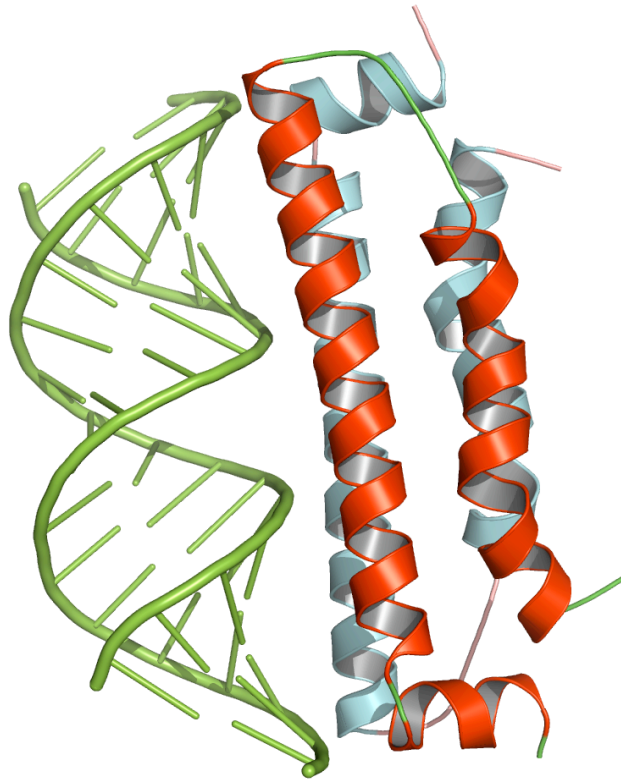
Taken from Lunde et al. Nat. Rev. Mol. Cell Biol 2007

# Rigid body docking

Examples

Viral B2 protein suppresses RNAi by masking dsRNA or siRNA.

## Rigid-body docking



Taken from Chao et al. Nat. Struct. Mol. Biol 2005

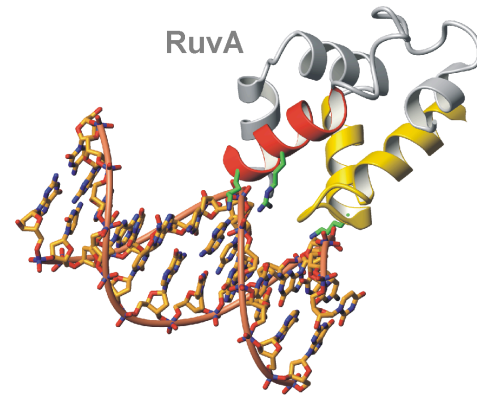
# SAM domain

Sterile Alpha Motif (SAM)

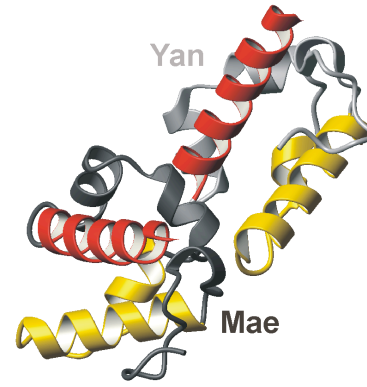
# SAM domains

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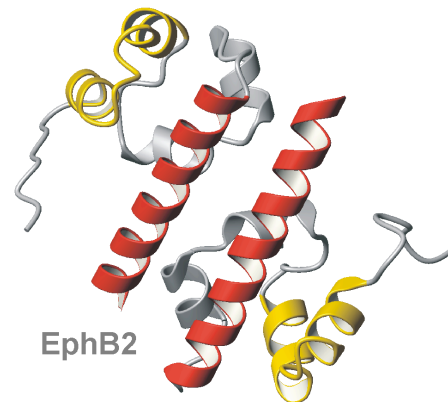
SAM-DNA



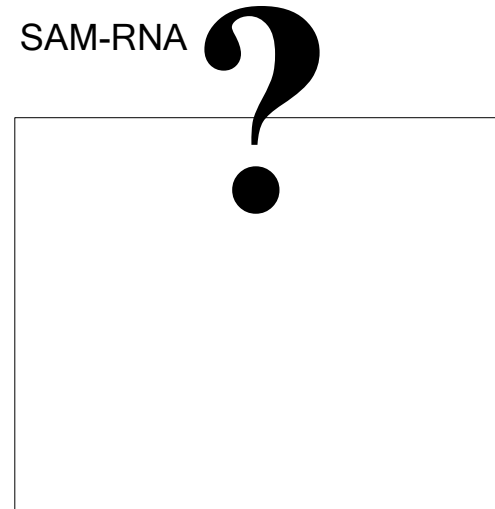
SAM-protein



SAM-SAM

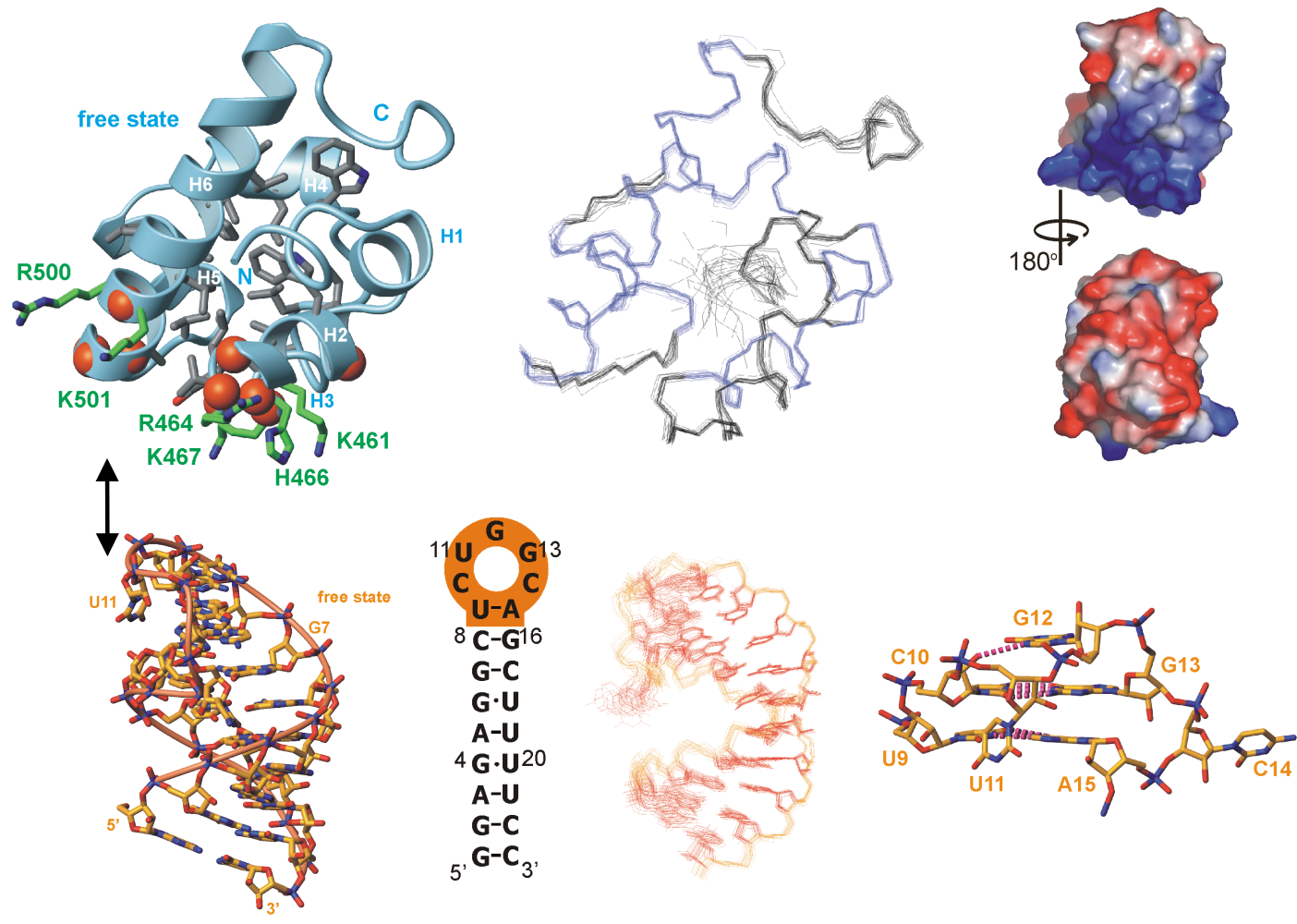


SAM-RNA

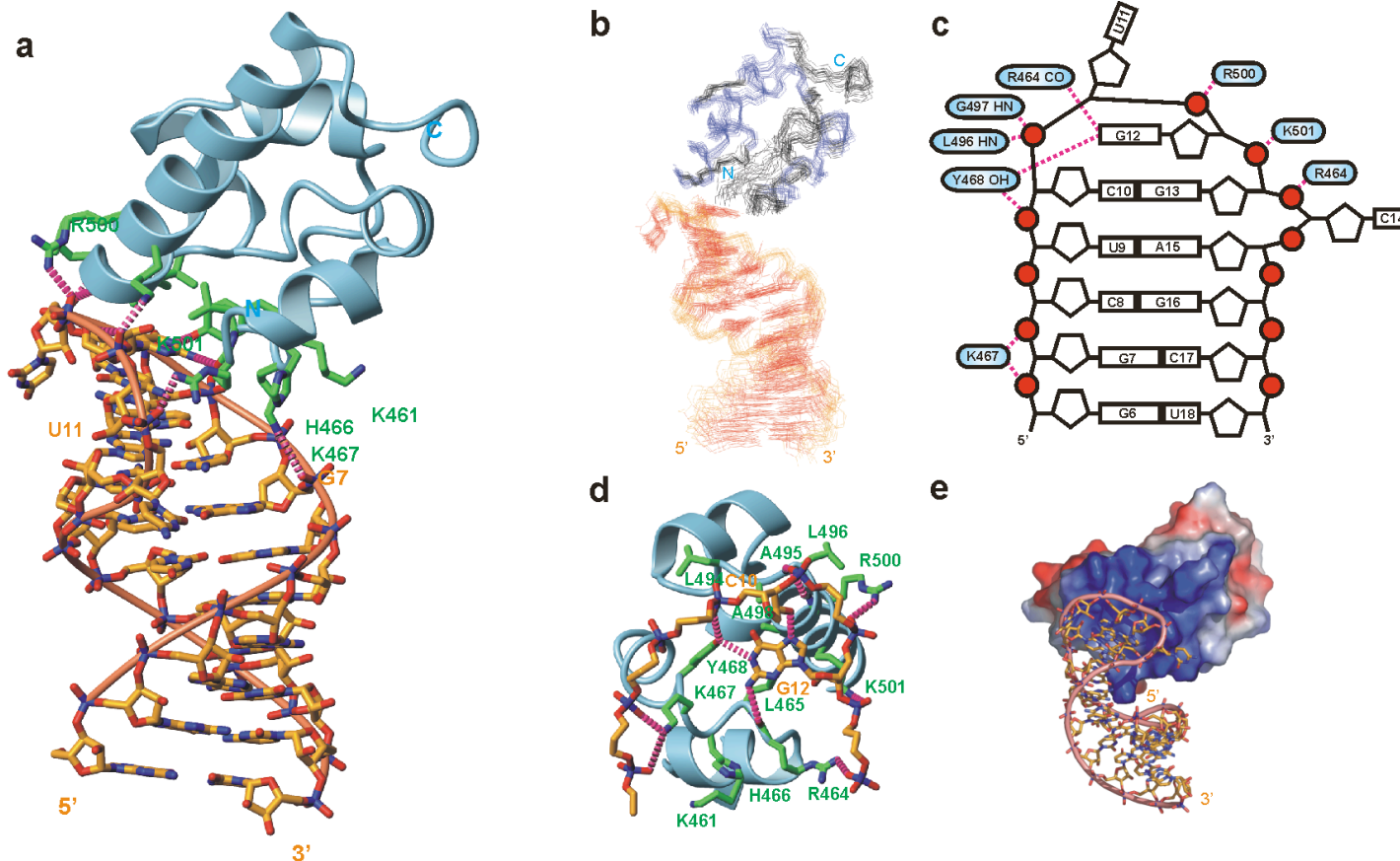




# structure of SAM<sup>Vts1p</sup> (yeast) and its RNA substrate (SRE; fruit fly)



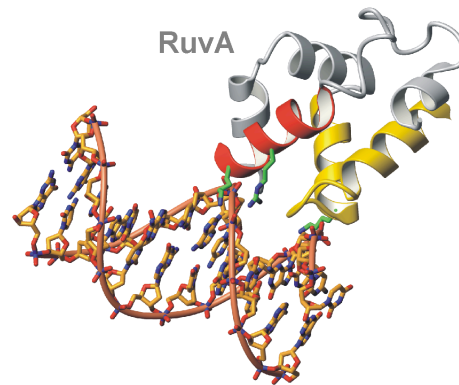
# structure of SAM<sup>Vts1p</sup> with RNA: a shape specific recognition



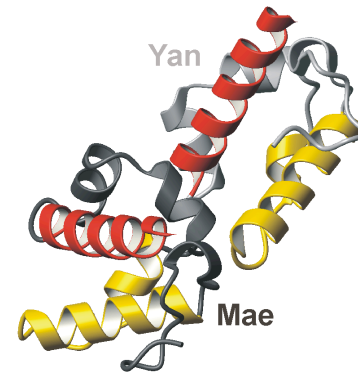
# structure of SAM<sup>Vts1p</sup> with RNA: a shape specific recognition

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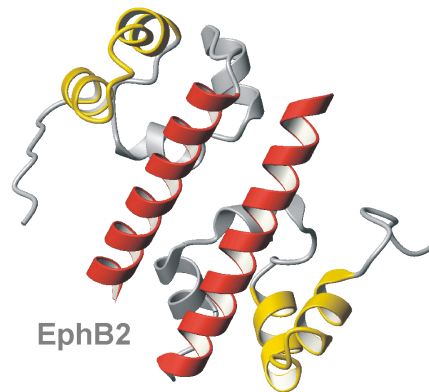
SAM-DNA



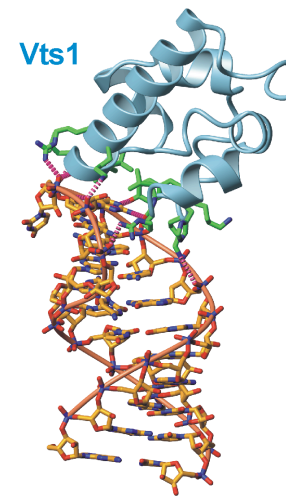
SAM-protein



SAM-SAM

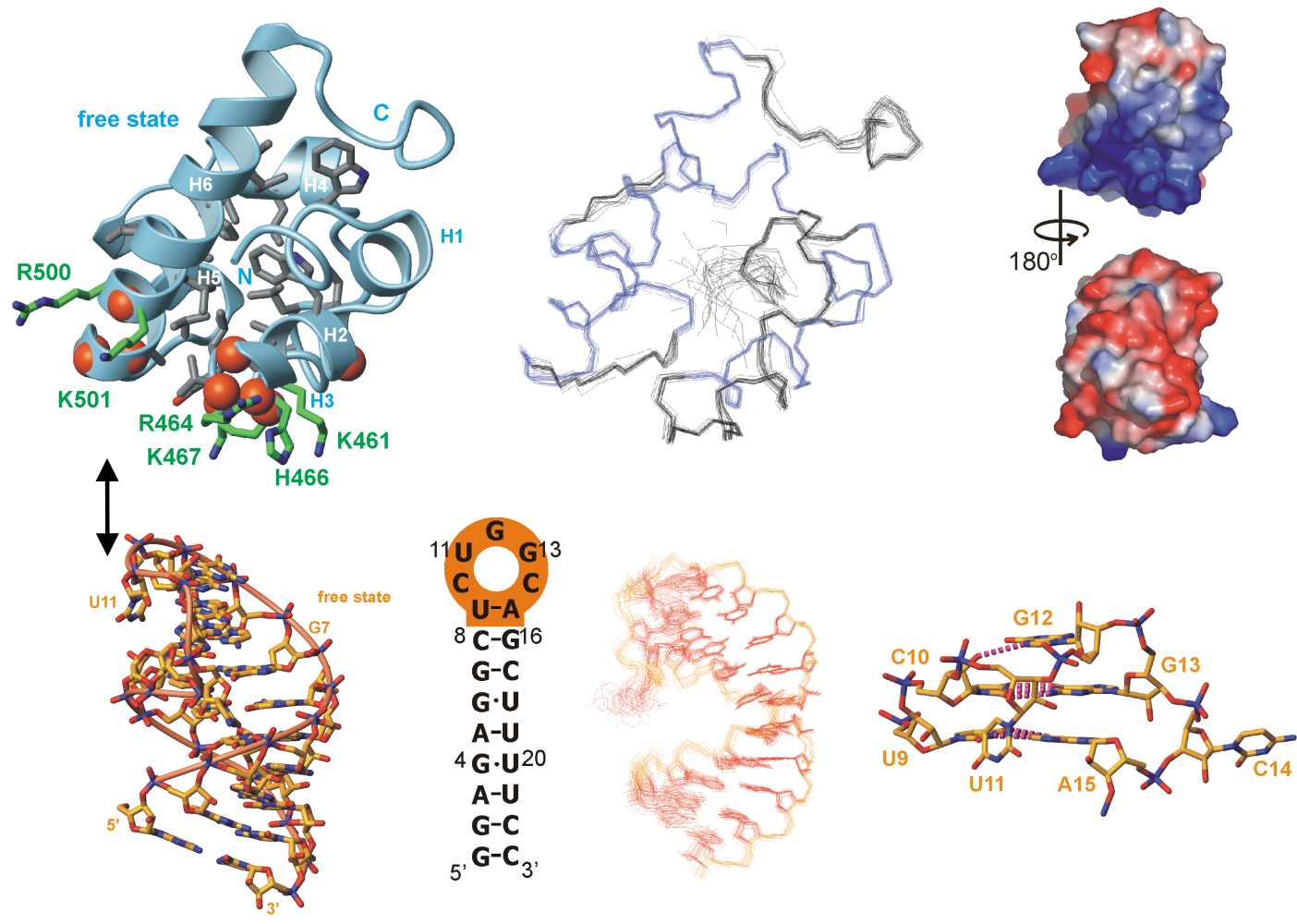


SAM-RNA

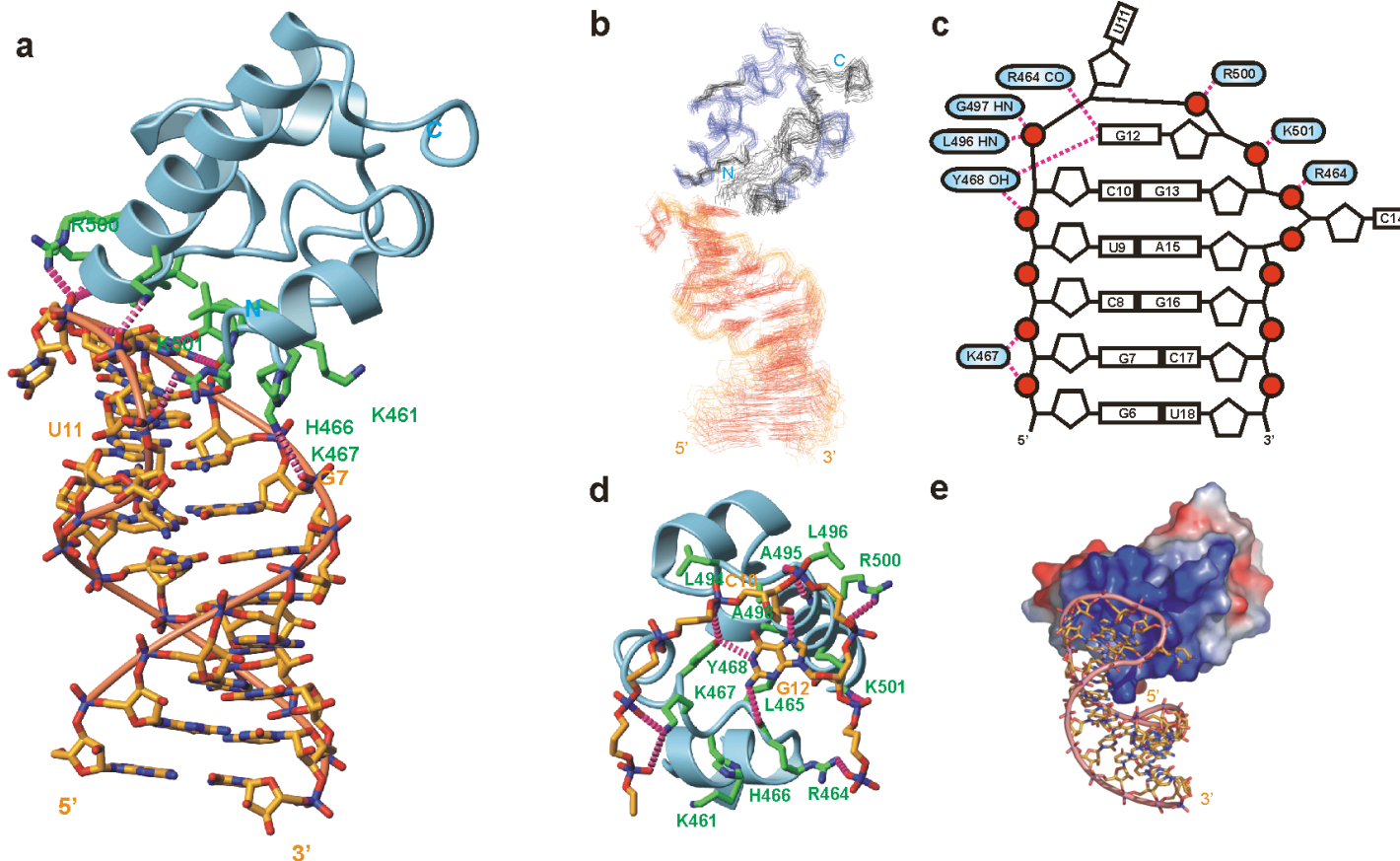


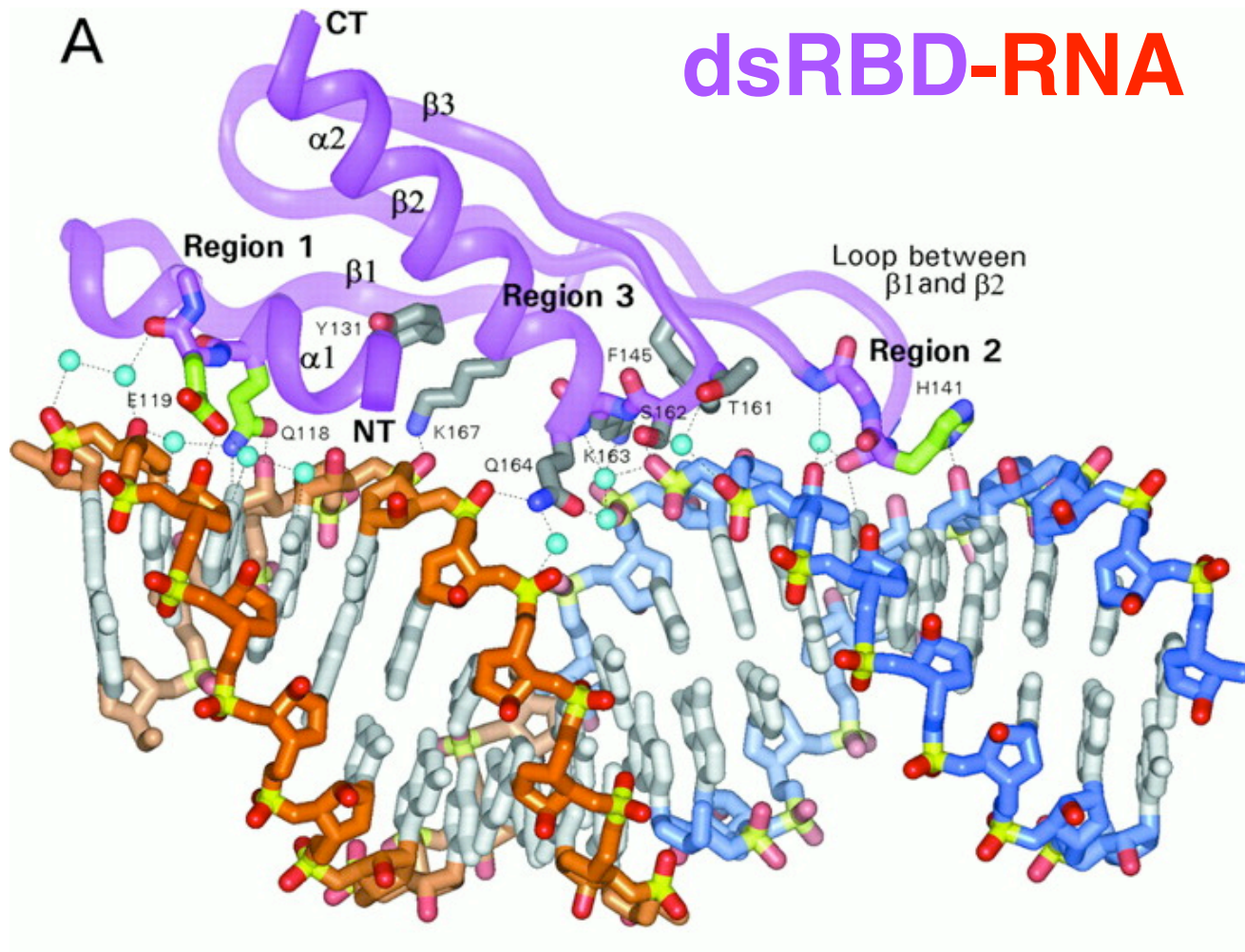


# structure of SAM<sup>Vts1p</sup> (yeast) and its RNA substrate (SRE; fruit fly)



# structure of SAM<sup>Vts1p</sup> with RNA: a shape specific recognition





Ryter and Schultz, *Embo J*, 1998

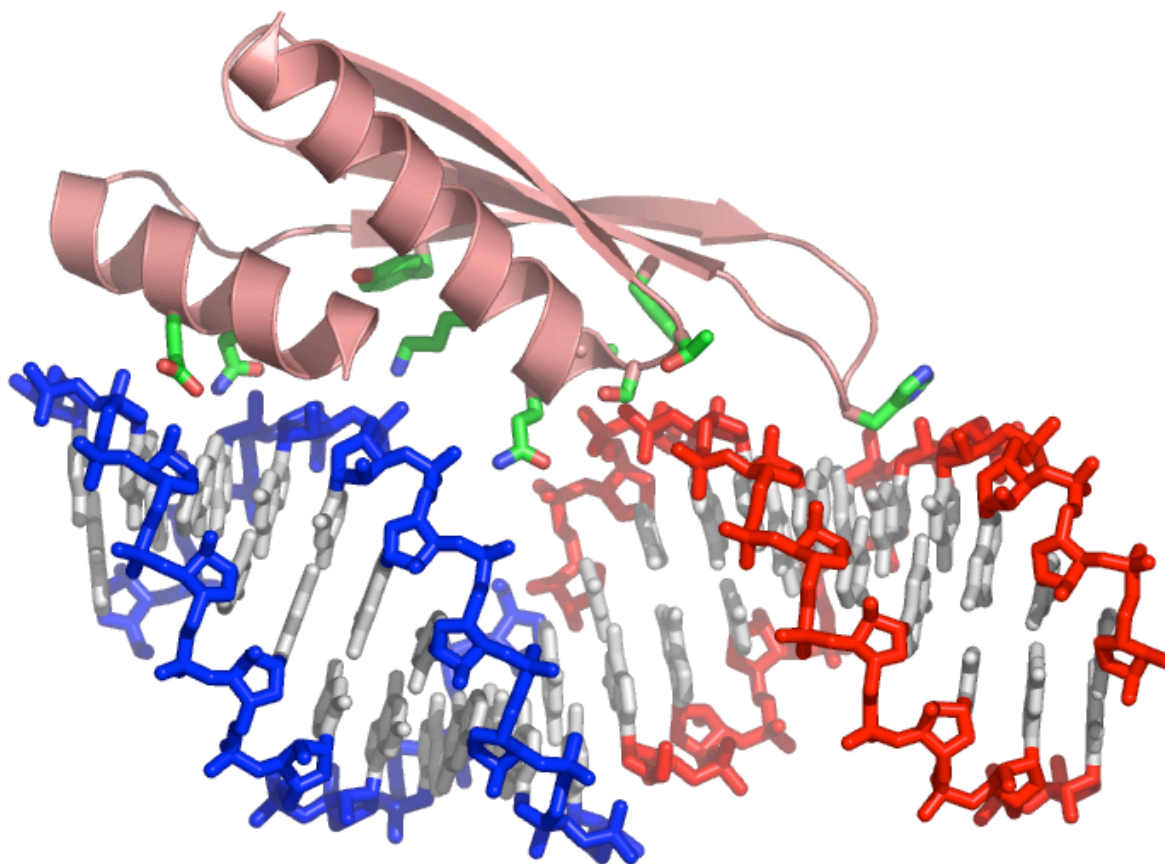
# dsRBMs

double-stranded RNA Binding Motif (dsRBM)



xlrpba dsRBM2 with 20 bp RNA duplex

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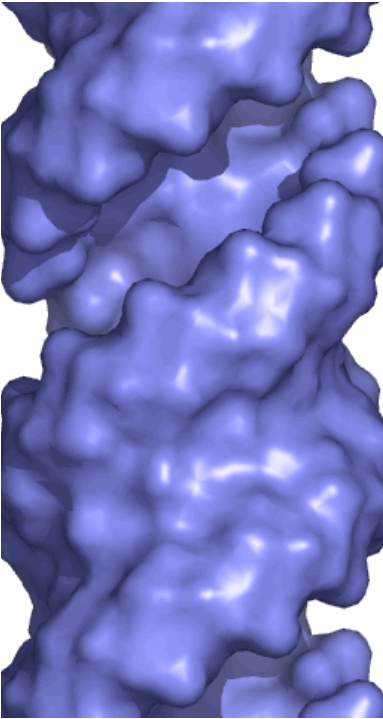


Ryter & Schultz EMBO J 1998

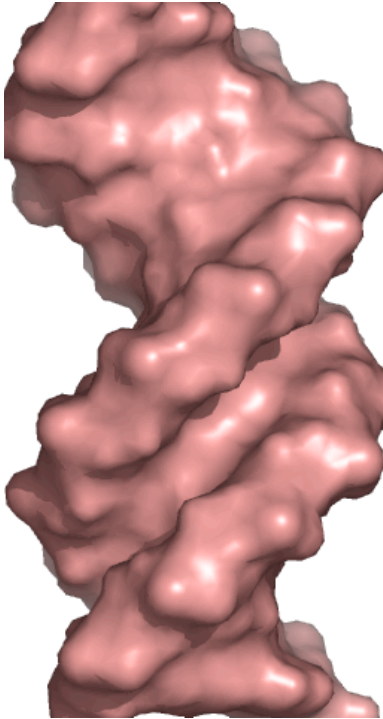
A-form double-helix vs. B-form double-helix

---

5' 3'  
A-U  
G-C  
C-G  
U-A  
A-U  
G-C  
C-G  
U-A  
A-U  
G-C  
3' 5'



A-form duplex of RNA

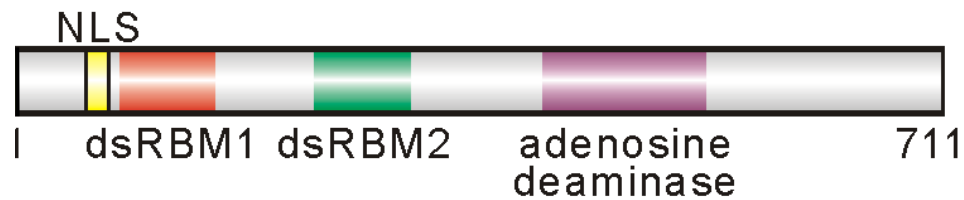


5' 3'  
A-T  
G-C  
C-G  
T-A  
A-T  
G-C  
C-G  
T-A  
A-T  
G-C  
3' 5'

B-form duplex of DNA

## mysterious ADAR (Adenosine deaminase acting on RNA)

---

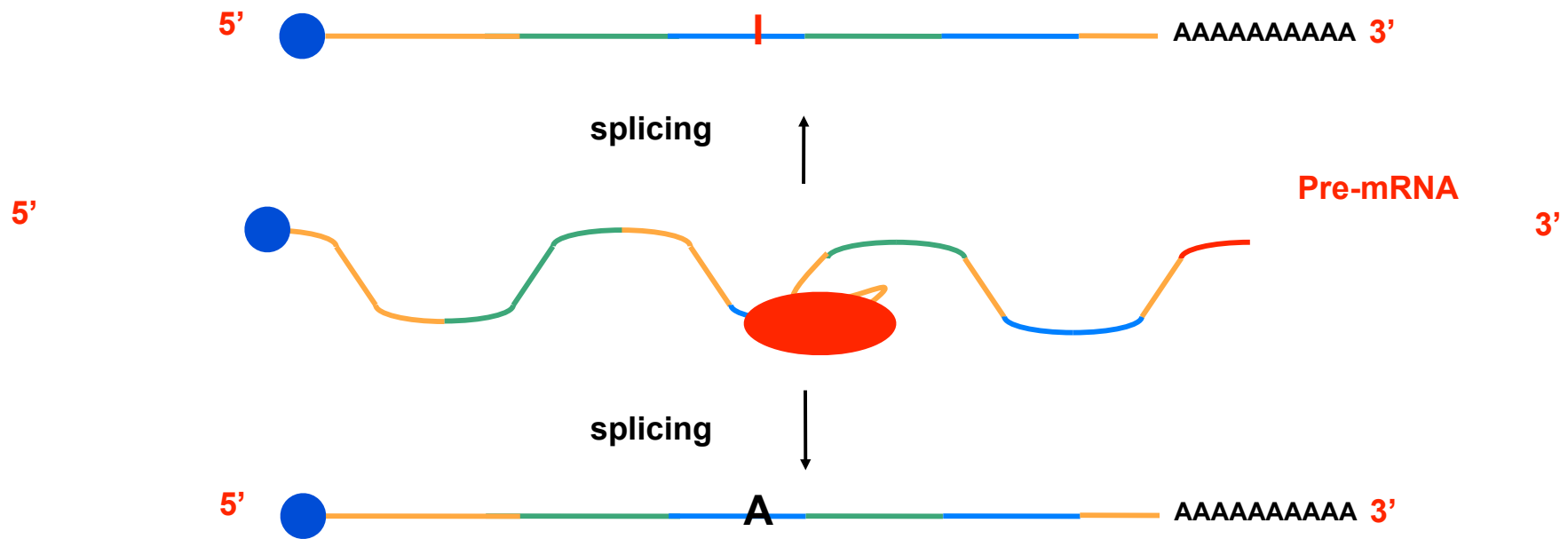
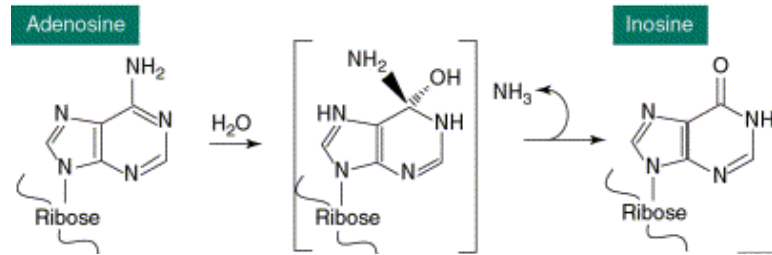


## functions of ADARs

---

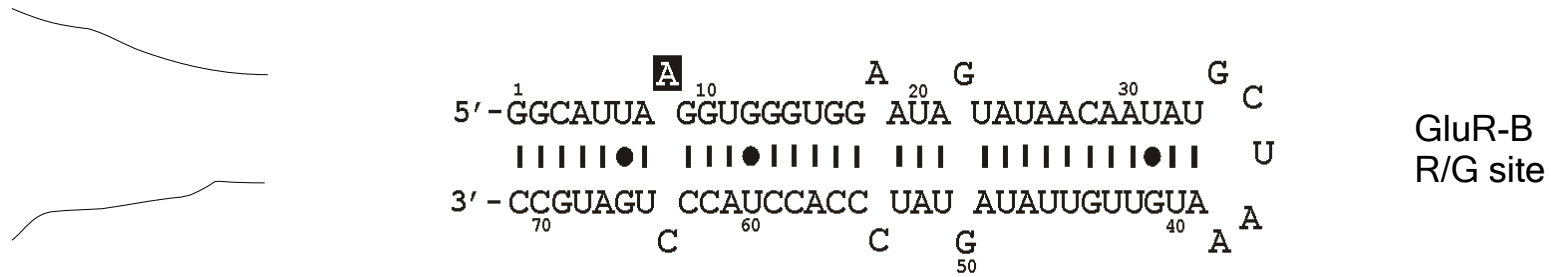
- to repair incorrect information in genome
- to diversify proteome
- regulation

# A to I RNA editing

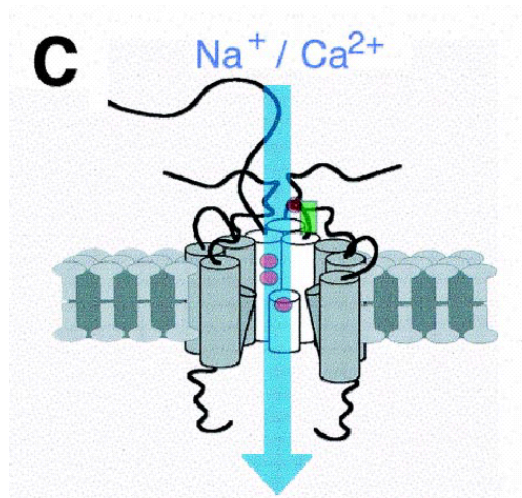


Adenosine deaminase acting on RNA (ADAR)

## GluR-B R/G editing site



## GluRs subunits of AMPA receptors in neurotransmission

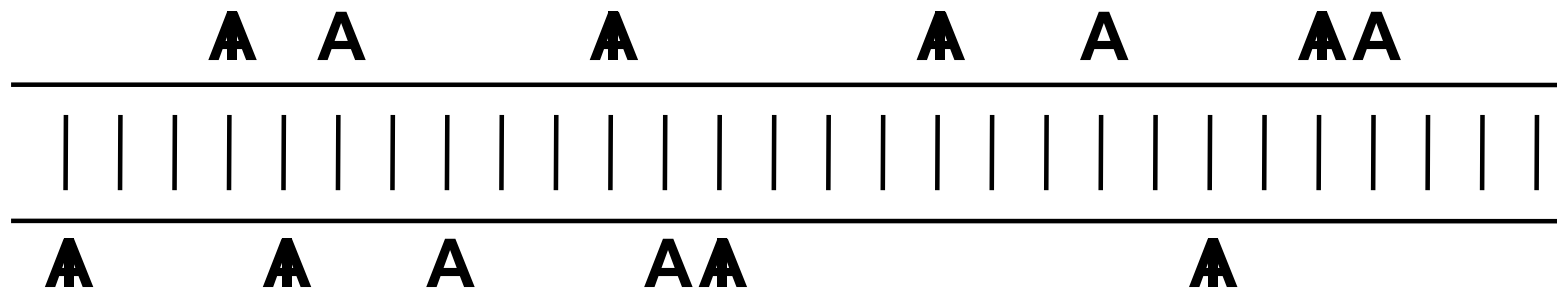


- Editing-created codon changes in GluRs affect aa positions of critical impact on biophysical properties of glutamate-activated cation channels. GluRs are subunits of AMPA receptors that mediate postsynaptic currents in CNS

hyper-editing by ADARs (nonspecific)

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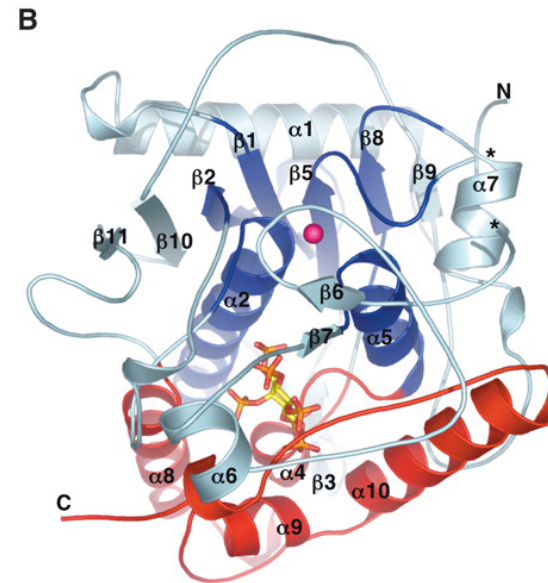
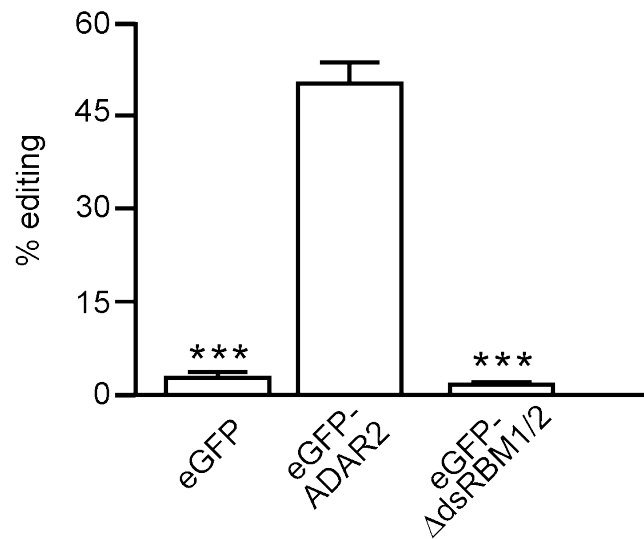
UTR's, introns



regulate gene silencing triggered by intramolecular structures in mRNA



what does the catalytic domain of ADAR2 do alone?

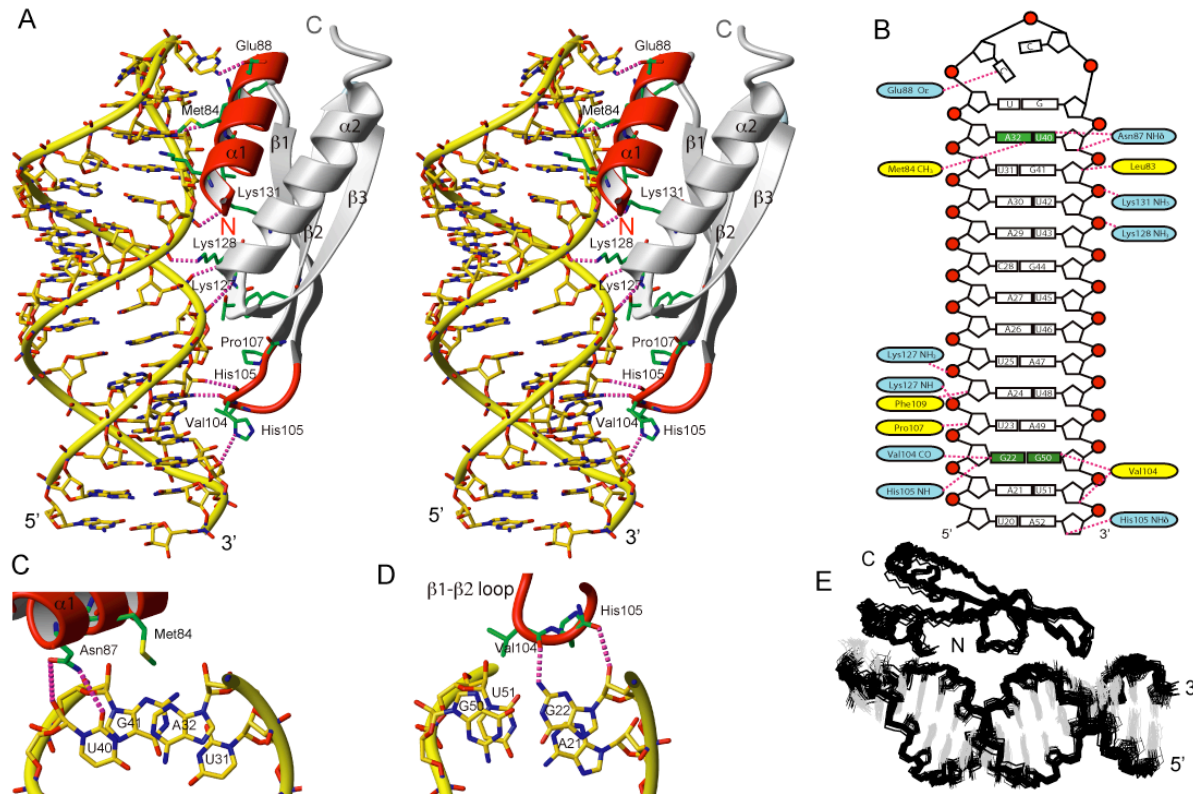


Macbeth et al. Science, 2005

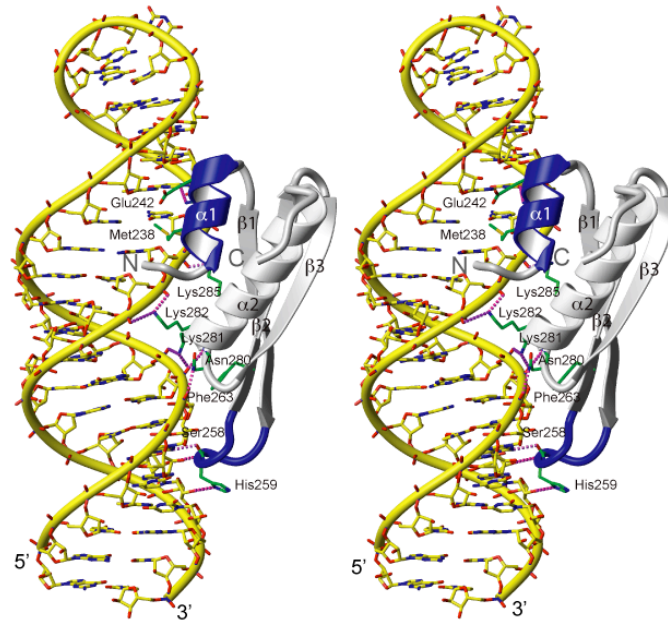




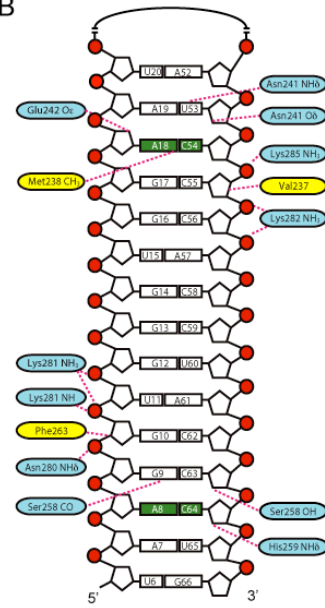
# Non-canonical elements of dsRNA change its shape - recognition signal



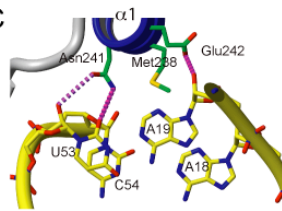
A



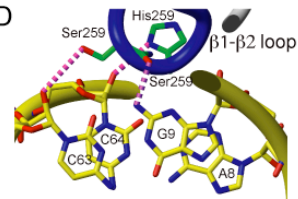
B



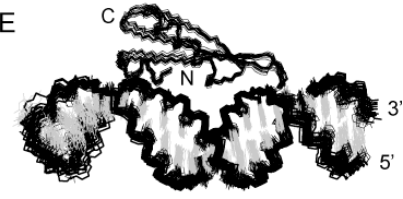
C

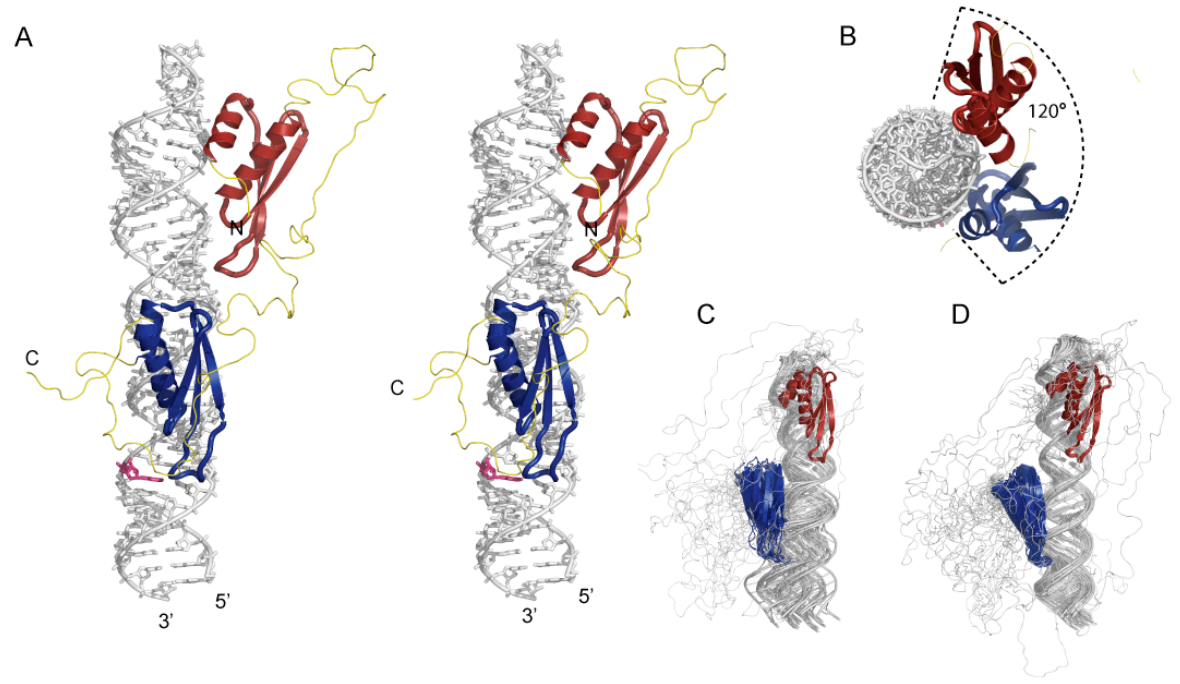


D



E

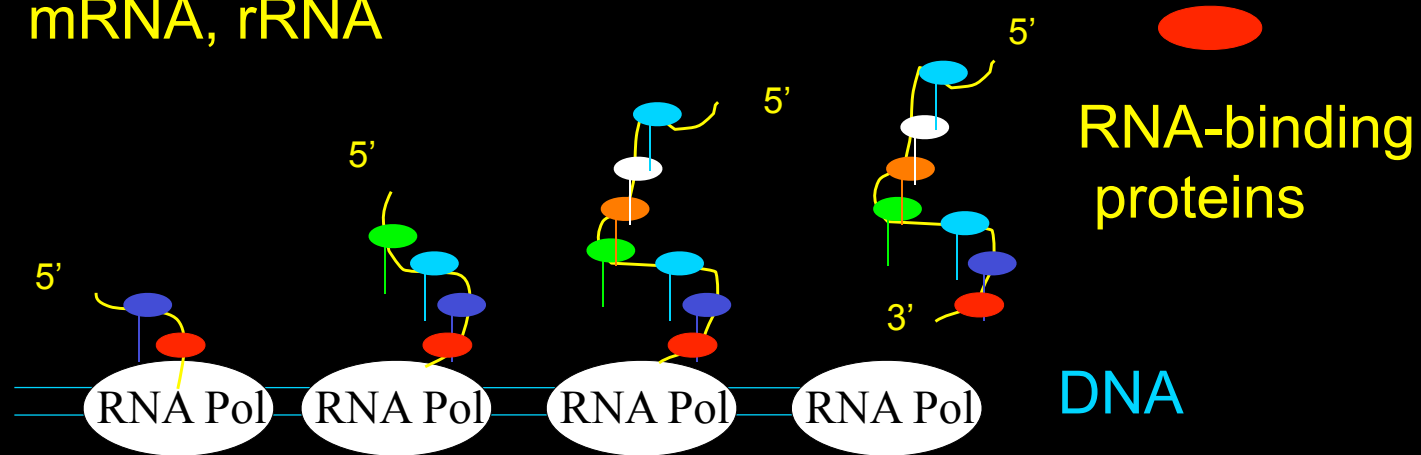






# Take Home Messages

mRNA, rRNA



protection, folding (chaperone), **gene regulation**

**RNA binding specificity**

# RNA binding proteins:

## multidomain protein



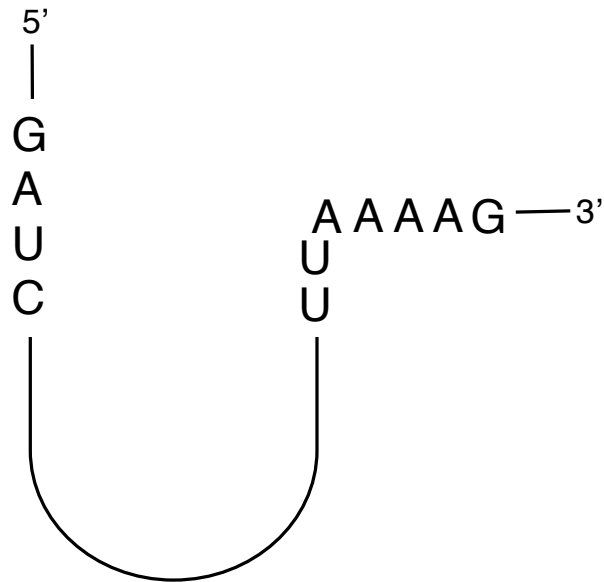
RGG  
(Gar)  
SR  
Dimerisation

RBD/RRM/RNP  
KH, Sam  
dsRBD  
Arg-rich  
Zinc knuckle  
Zinc finger

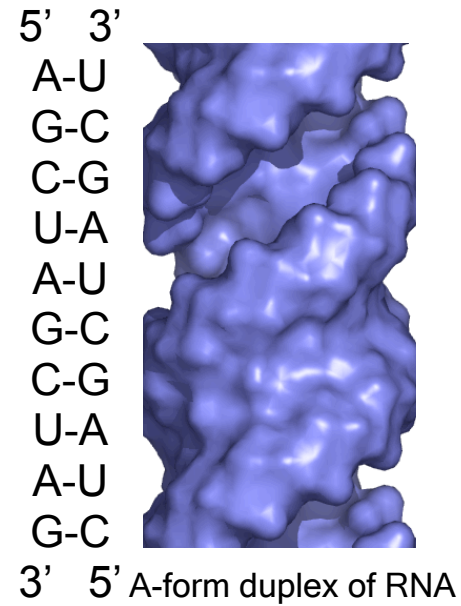


# What information is recognized by proteins:

Recognition of RNA sequence

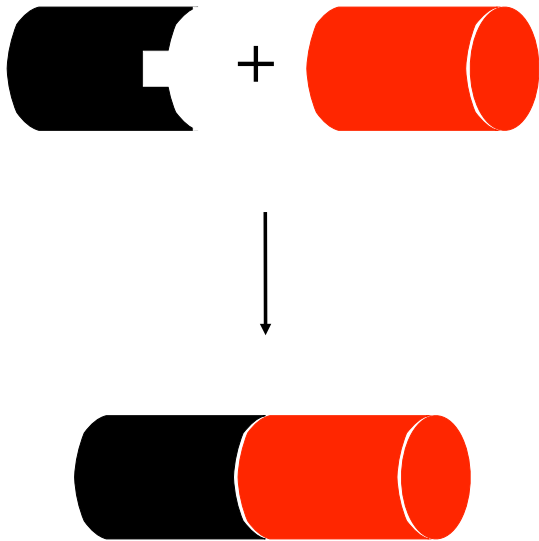


Recognition of RNA shape

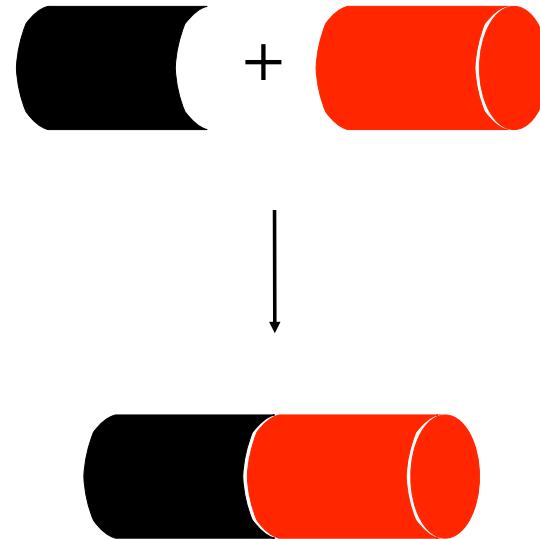


What recognition mode is used by proteins:

Induced fit

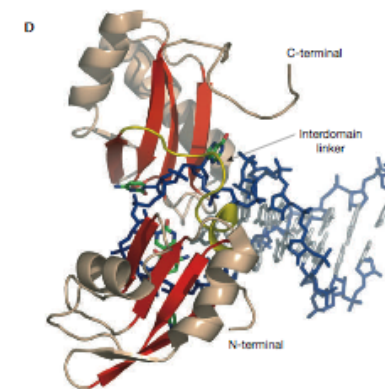
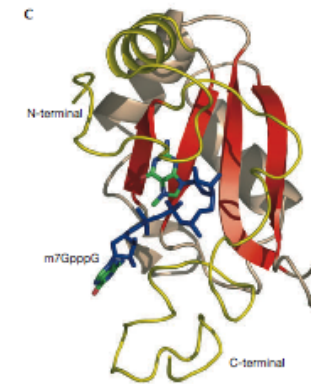
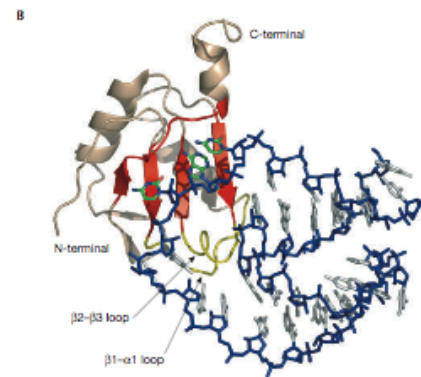
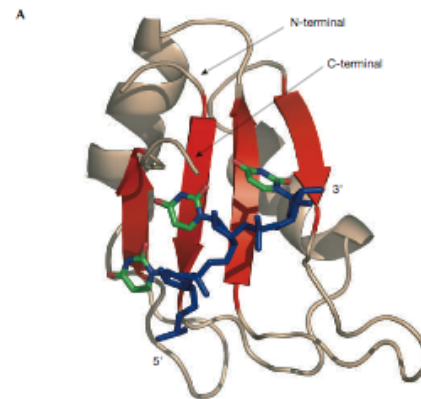
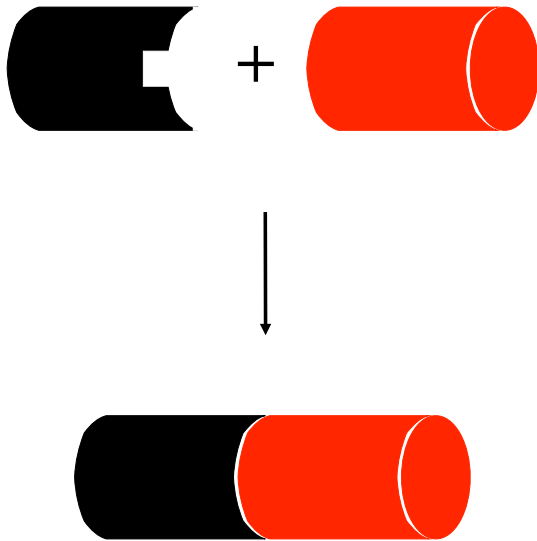


Rigid-body docking

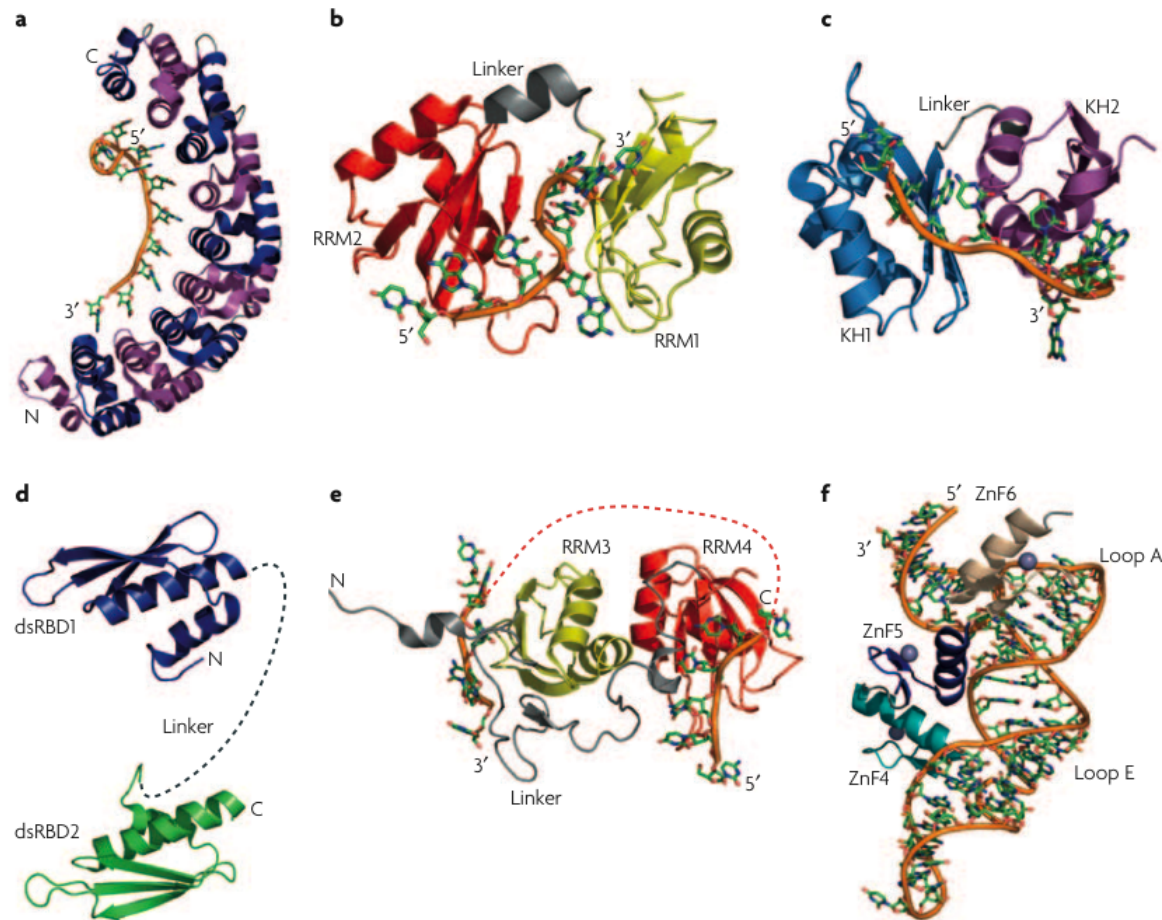


# What recognition mode is used by proteins:

Induced fit



# Modular architecture of RNA-binding proteins



Taken from Lunde et al. Nat. Rev Mol. Cell Biol 2007