

# Molecular simulations: a tool to infer structure/function- evolution relationships in macromolecules assembly

*sRNP H/ACA from Pyrococcus*

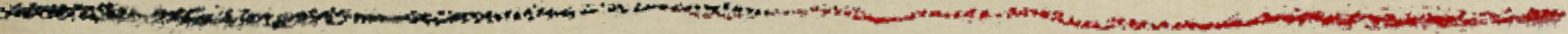
AREMS (ARN-RNP maturation-structure-fonction, Enzymologie Moléculaire et Structurale)

*Sébastien MULLER, Christiane BRANLANT, Fabrice LECLERC*



Nancy-Université  
  
Université  
Henri Poincaré

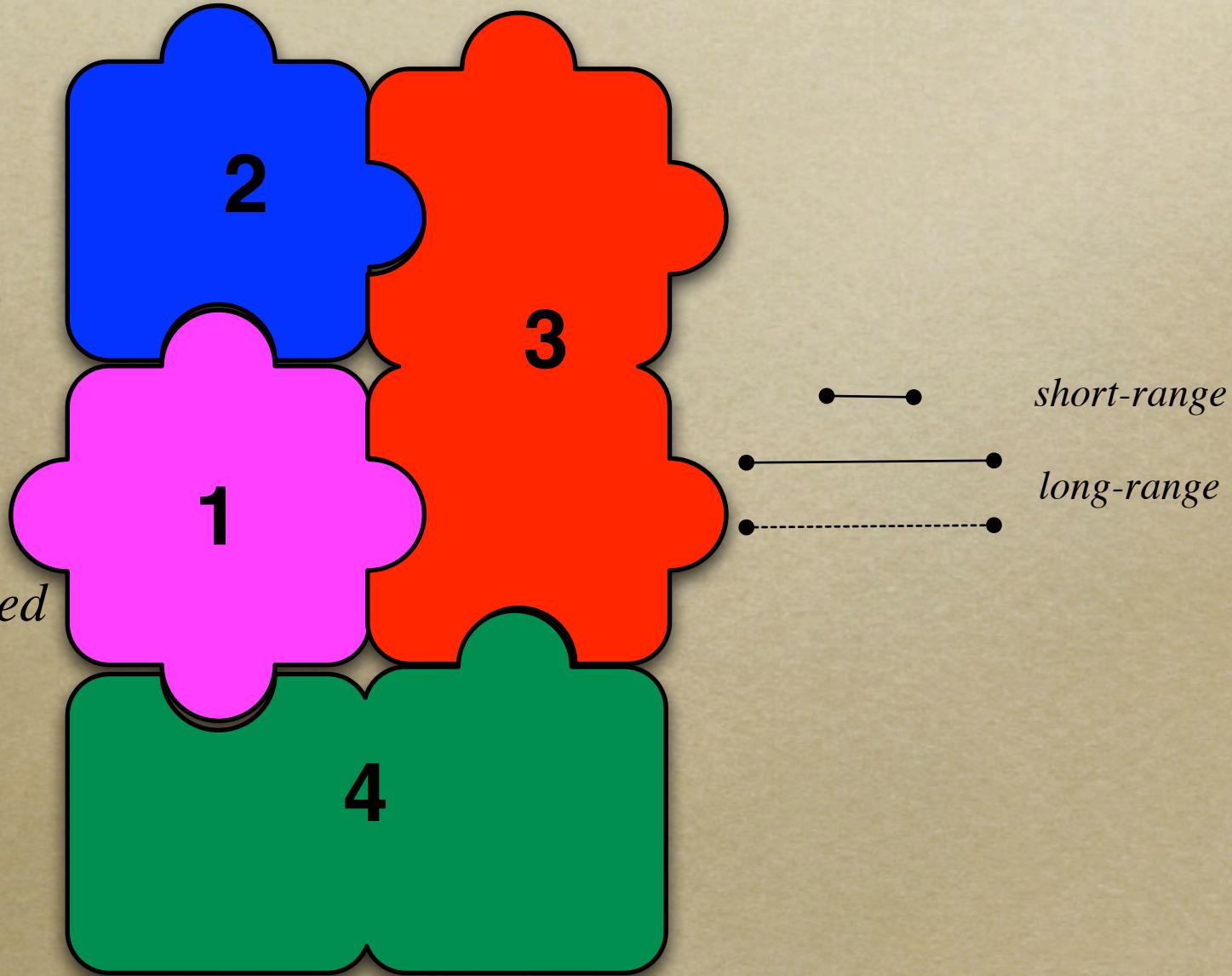
# Structure/function relationships: understanding macromolecules interactions



- *covariations and correlated mutation analysis to understand interactions at protein/protein or protein/RNA interfaces*
- *dynamics & flexibility at protein/protein or protein/RNA interfaces*

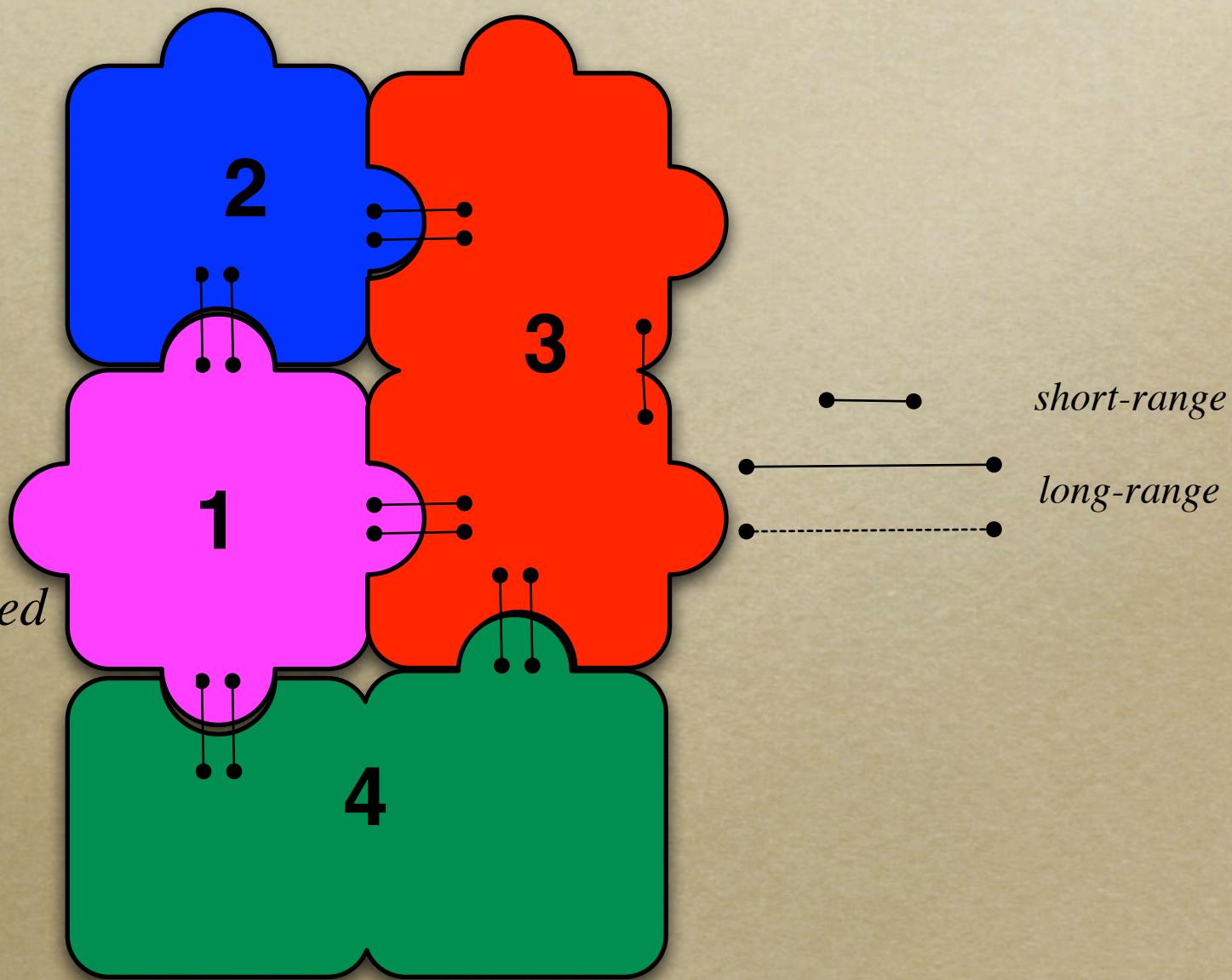
# Co-evolution dependencies within and between proteins

*correlated mutation  
analysis (CMA):  
sequence-based,  
sequence & structure-based*



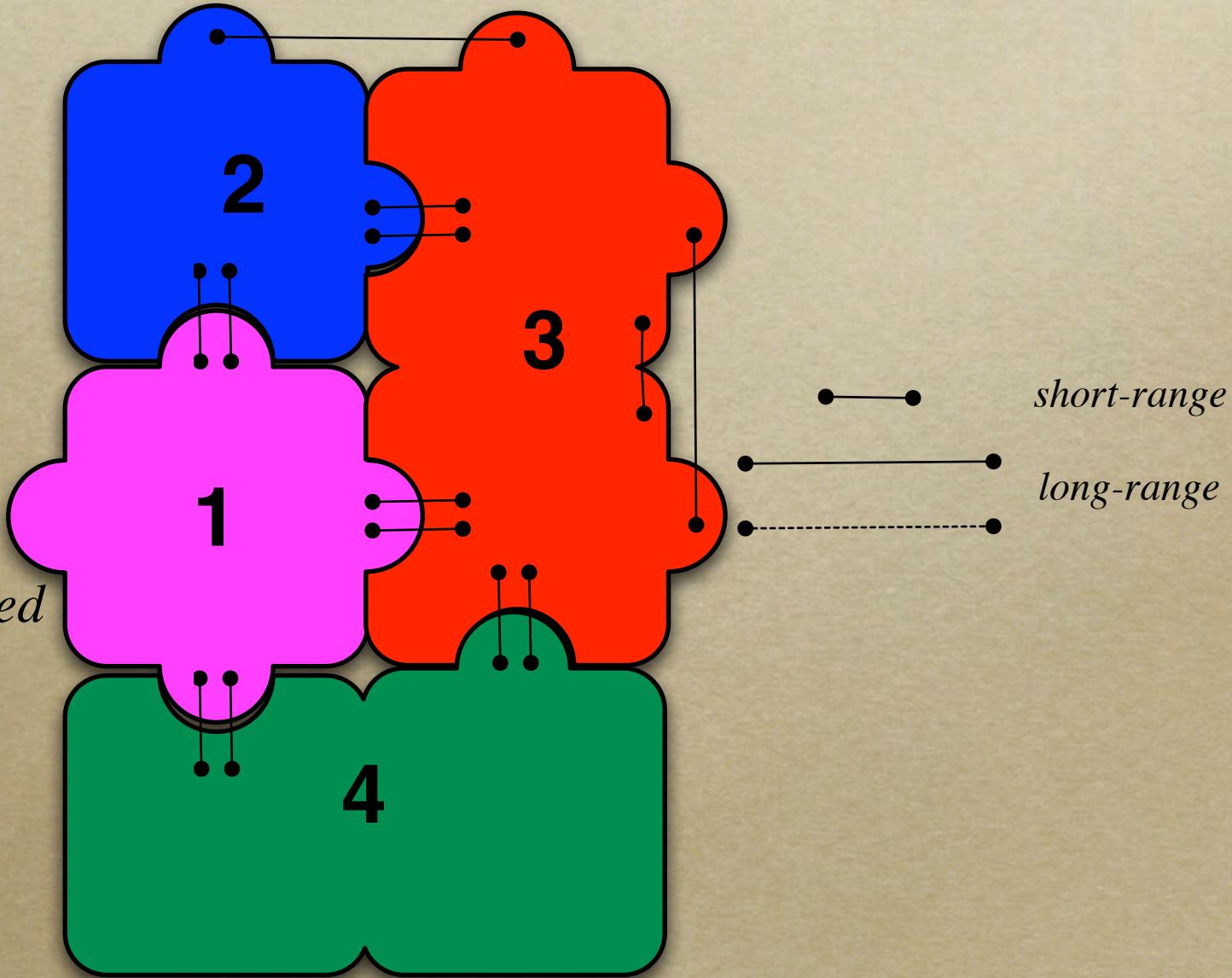
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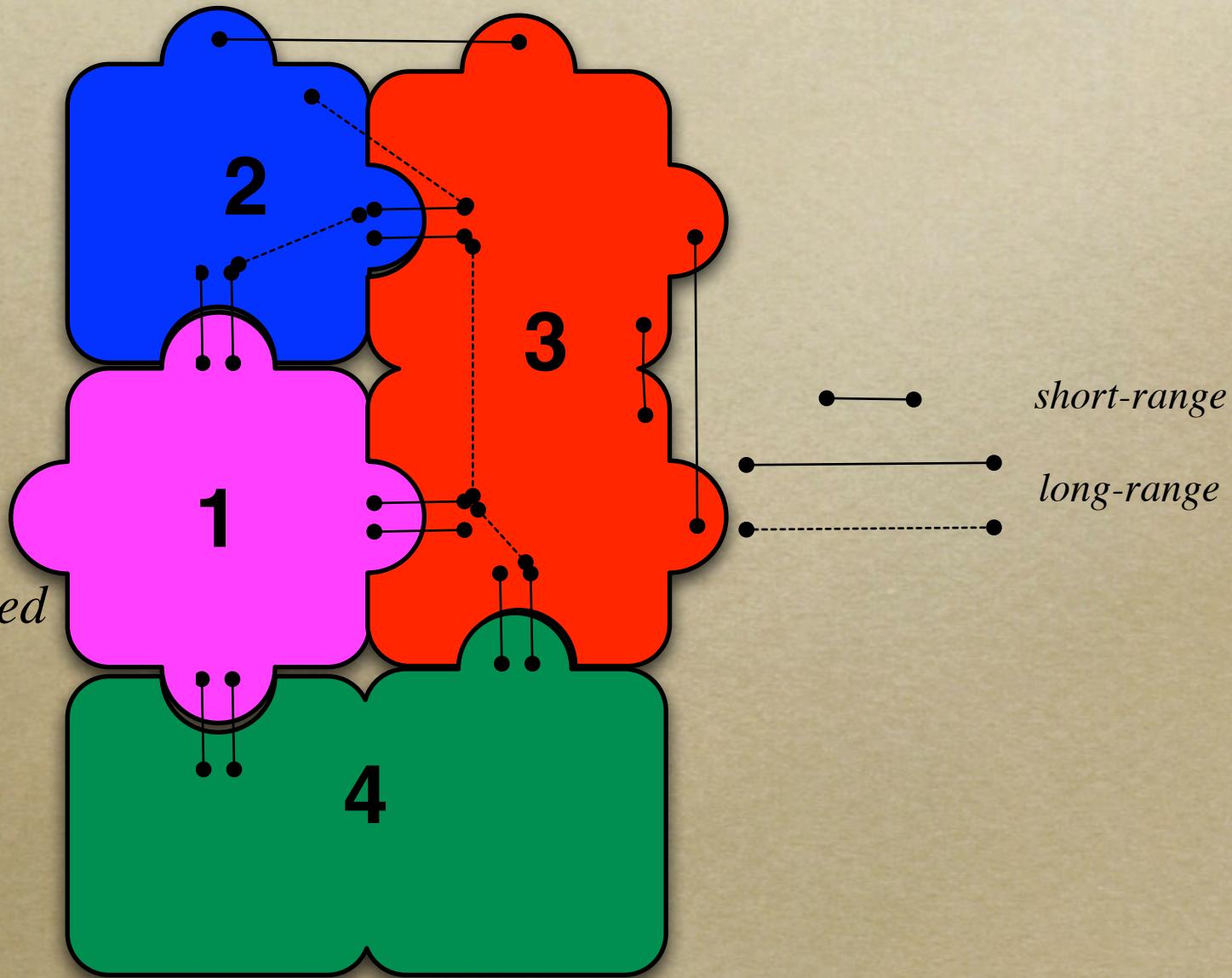
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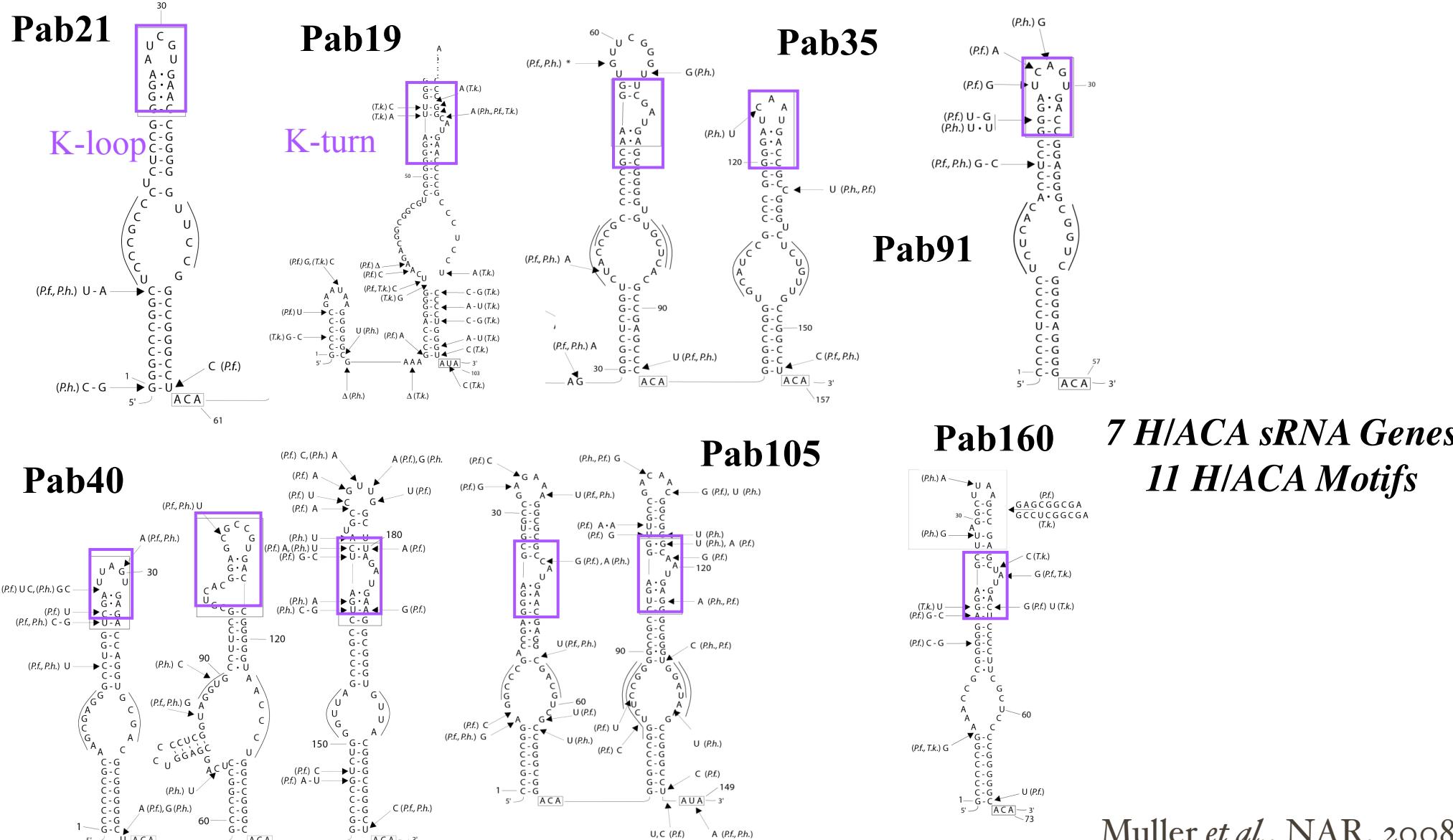


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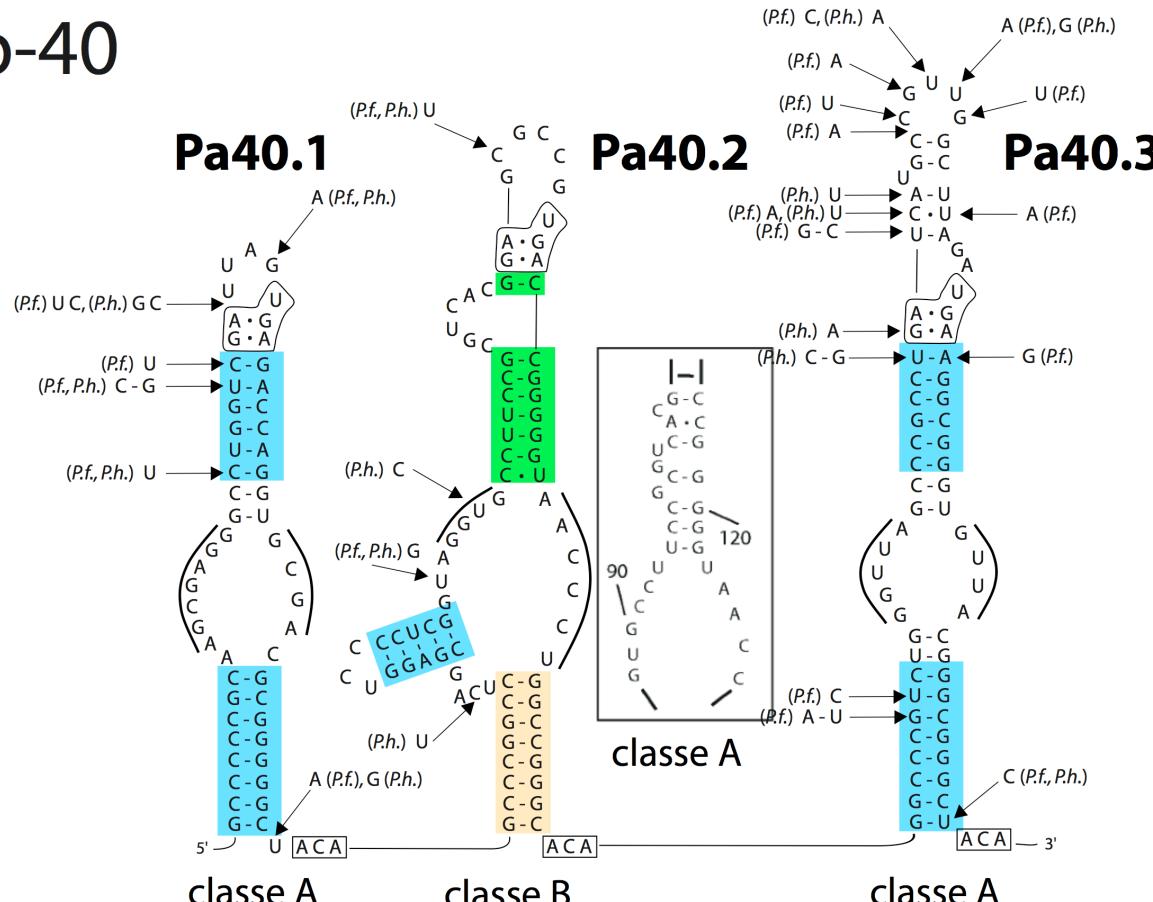
# RNomics on H/ACA sRNAs: comparative genomics & experimental validations



Muller *et al.*, NAR, 2008

# Two Alternative folds: sRNA H/ACA: class A & class B

Pab-40



# STOCKHOLM 1.0

Ph40.2-2673-2678

Pa40.2-2678

Pf7.2-2701-2678

# STOCKHOLM 1.0

Pa40.2-1932

Ph40.2-2673-78

Pf7.2-2701-2678

#=GC SS cons

```
GCCCCGGCc.ttagcgaggc.....CCCTcgG..GAggcgcttccgcgtacggagtgccGTGA.ccGGGGG....taaccct.gGCCGGGC..ACA
GCCCCGGCc.tcagcgaggc.....CCCTcgG..TAggtgccctccgcgtacggagcgccGTGA.ccGGGGG....taaccct.gGCCGGGC..ACA
GCCCCGGCc.tcagcgaggc.....CCCTcgG..GAggtgccctccgcgtacggagtgccGTGA.ccGGGGG....taaccct.gGCCGGGC..ACA
```

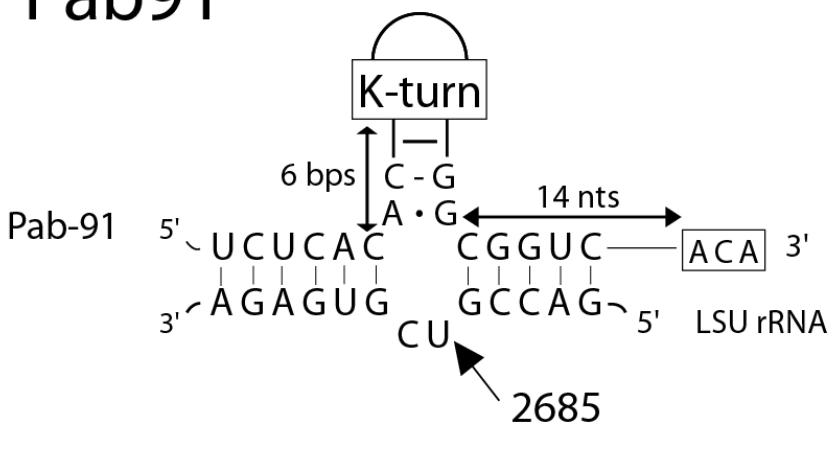
```
GCCCCGGCucagcgaggguCCCCUcgGUAggugcciuuccgcguacacgGAgcgccGUGAccGGGGGuacccugGCCGGGCACA
GCCCCGGCcuuagcgaggguCCCCUcgGGAggcgcuuccgcguacacggagugccGUGAccGGGGGuacccugGCCGGGCACA
GCCCCGGCucagcgaggguCCCCUcgGGAggugcciuuccgcguacacggagugccGUGAccGGGGGuacccugGCCGGGCACA
<<<<<<....<<<<....>>>>>>.....<<<....<<<....>>>>>>>.....>>>>>>....
```

# Structure/fonction Models: guide-target pairing model

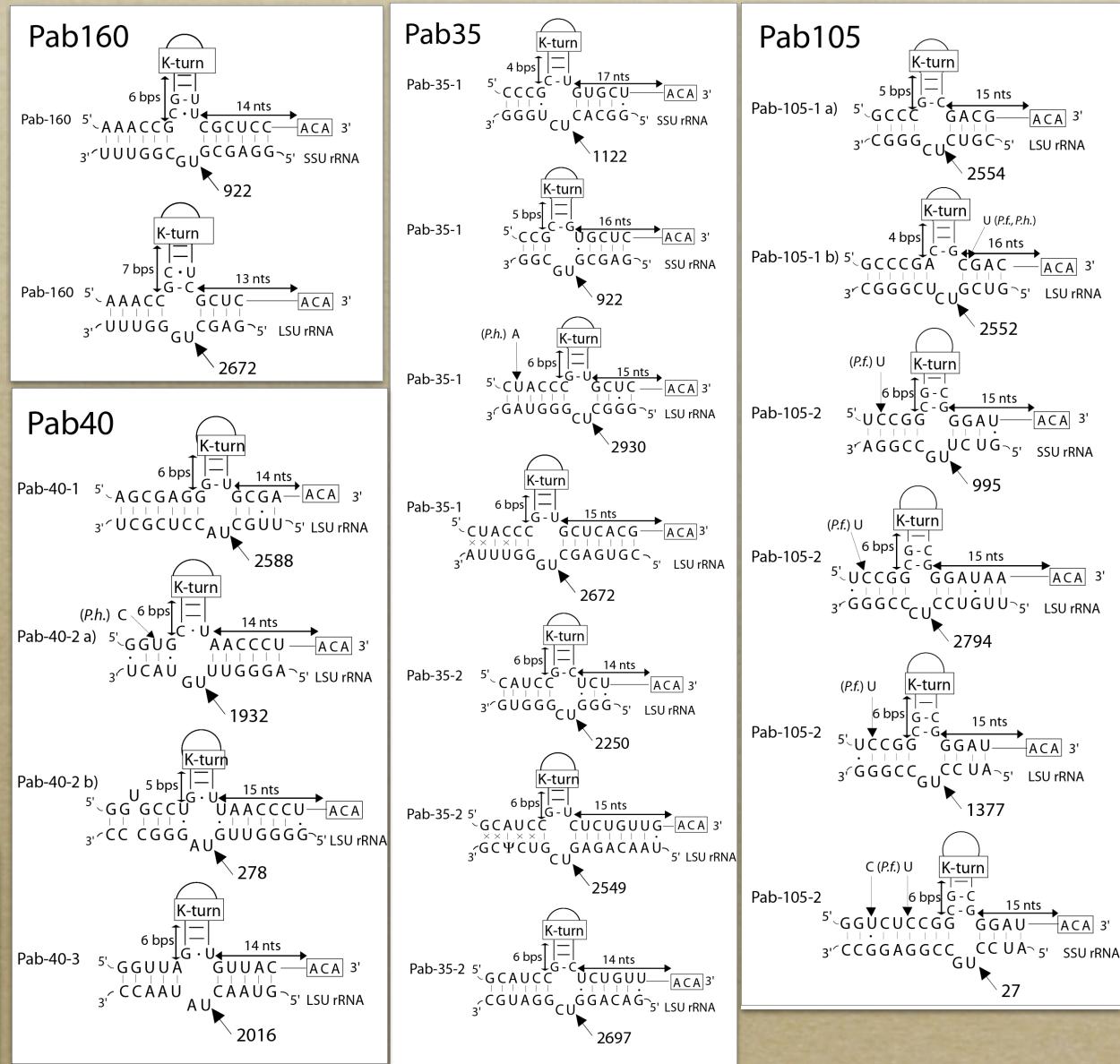
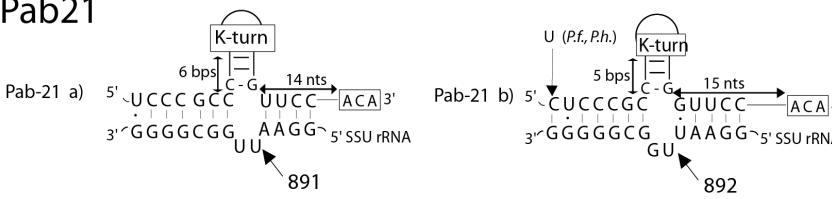
## *inferred rules of association:*

### *1. distance constraints*

Pab91



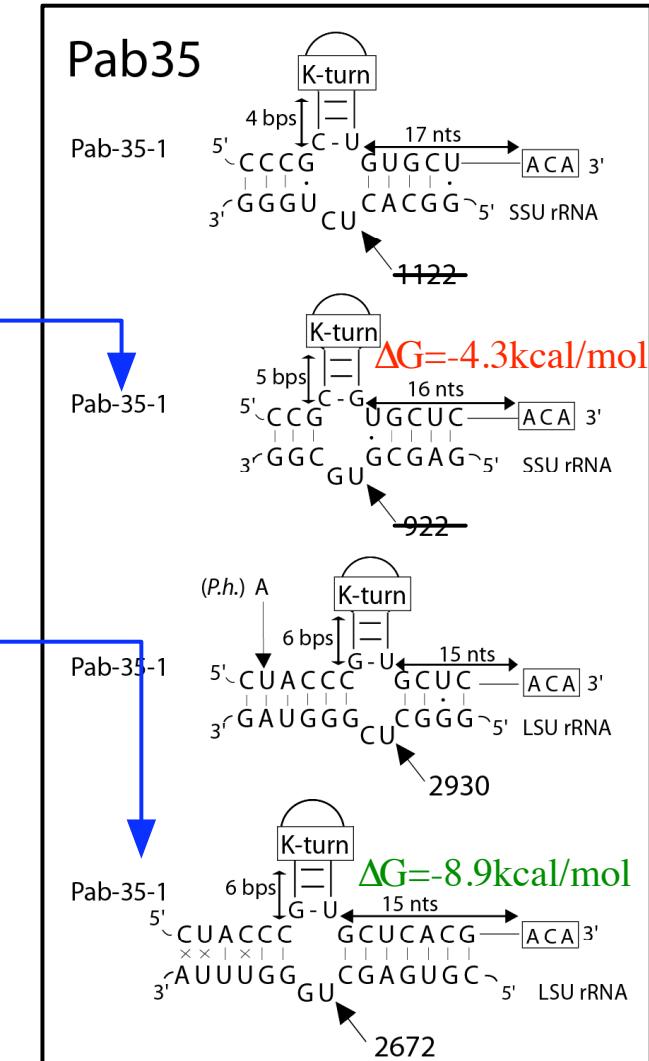
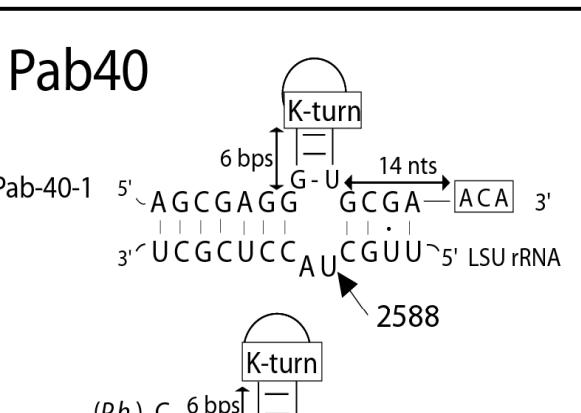
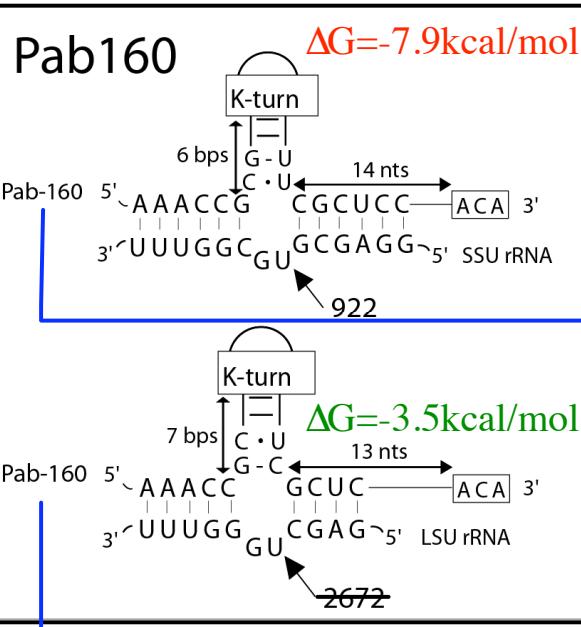
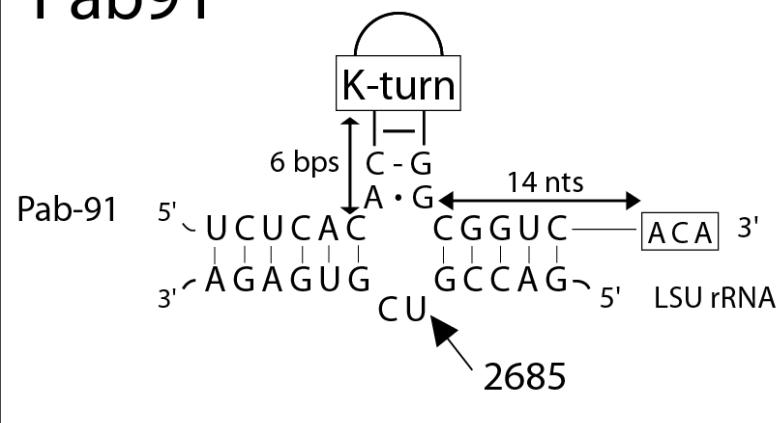
Pab21



# Structure/fonction Models: guide-target pairing model

*inferred rules of association:  
2. thermodynamic constraints*

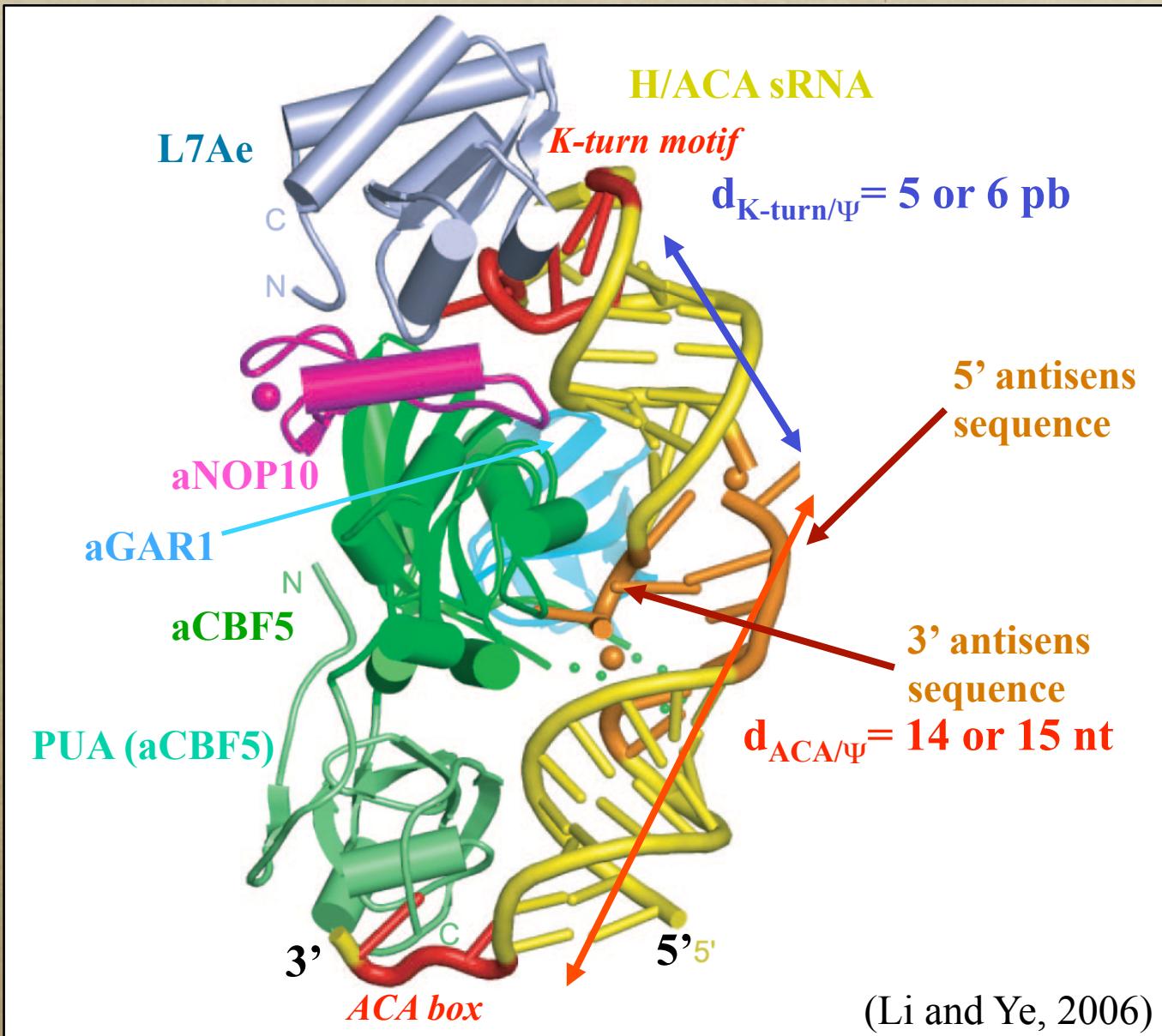
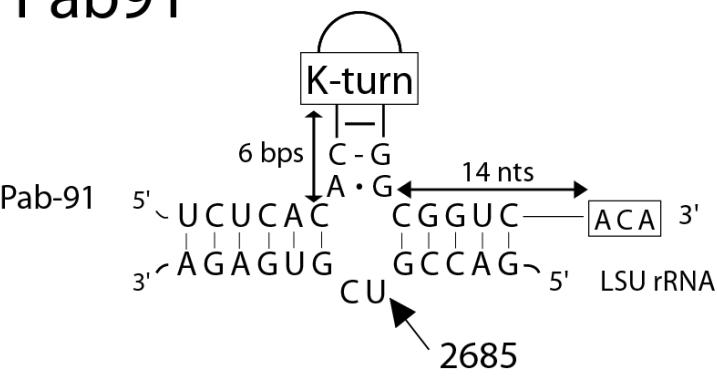
Pab91



# Structure/fonction Models from X-ray 3D structures

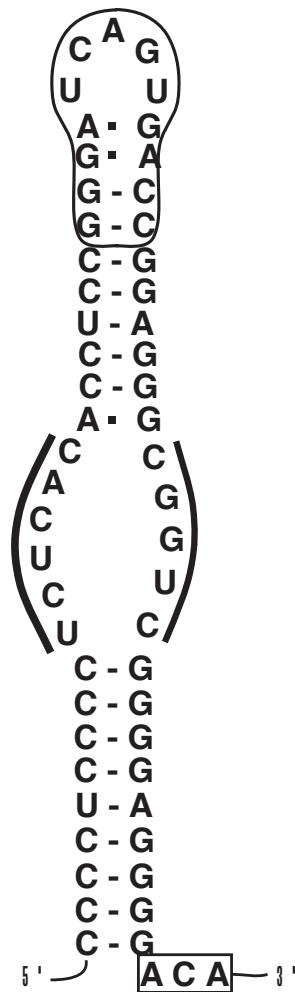
*rules of association:  
distance constraints  
vs  
positions of proteins in the  
assembly*

Pab91

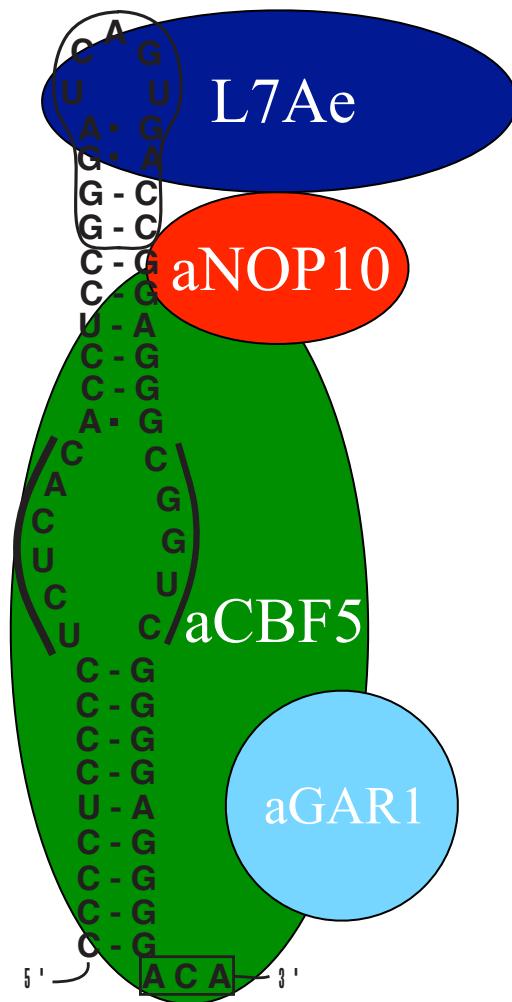


(Li and Ye, 2006)

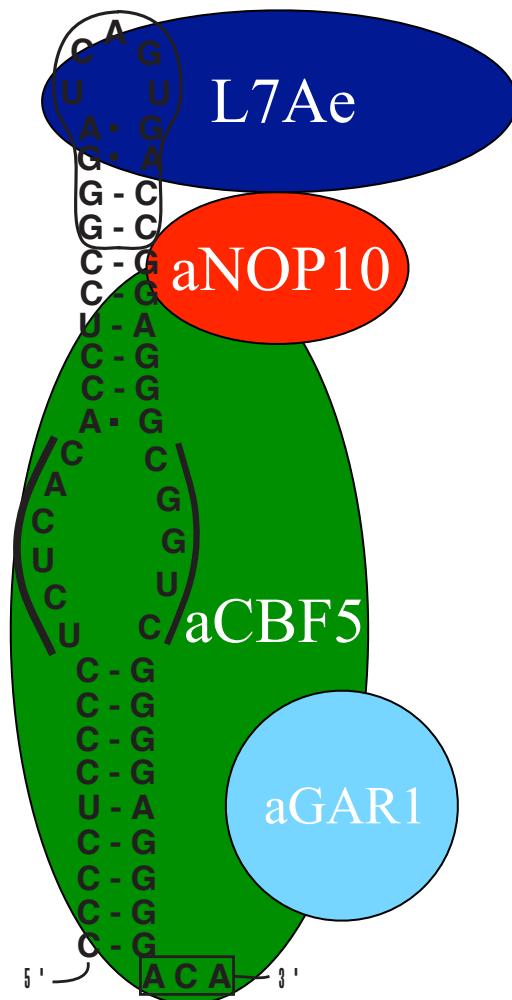
# Structure/function relationships: determinants in sRNP H/ACA assembly



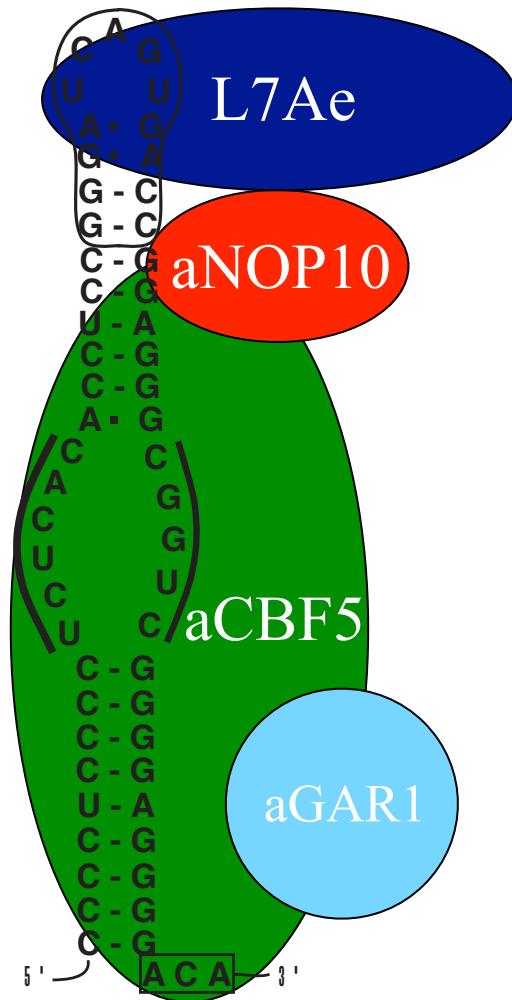
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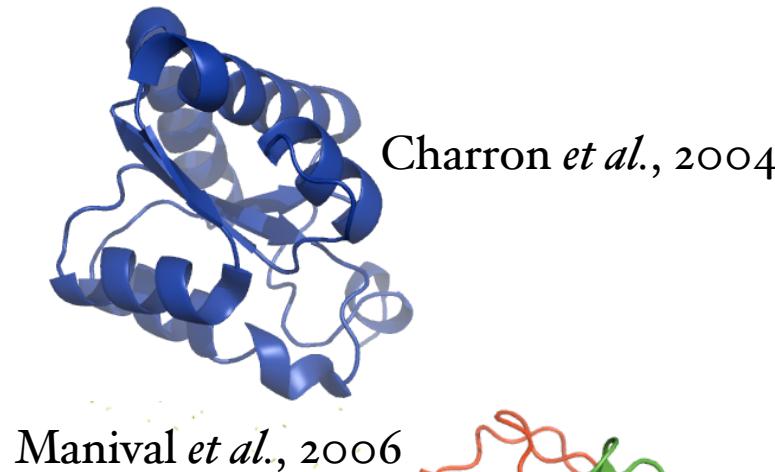
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# Structure/function relationships: determinants in sRNP H/ACA assembly



Charpentier *et al.*, 2005

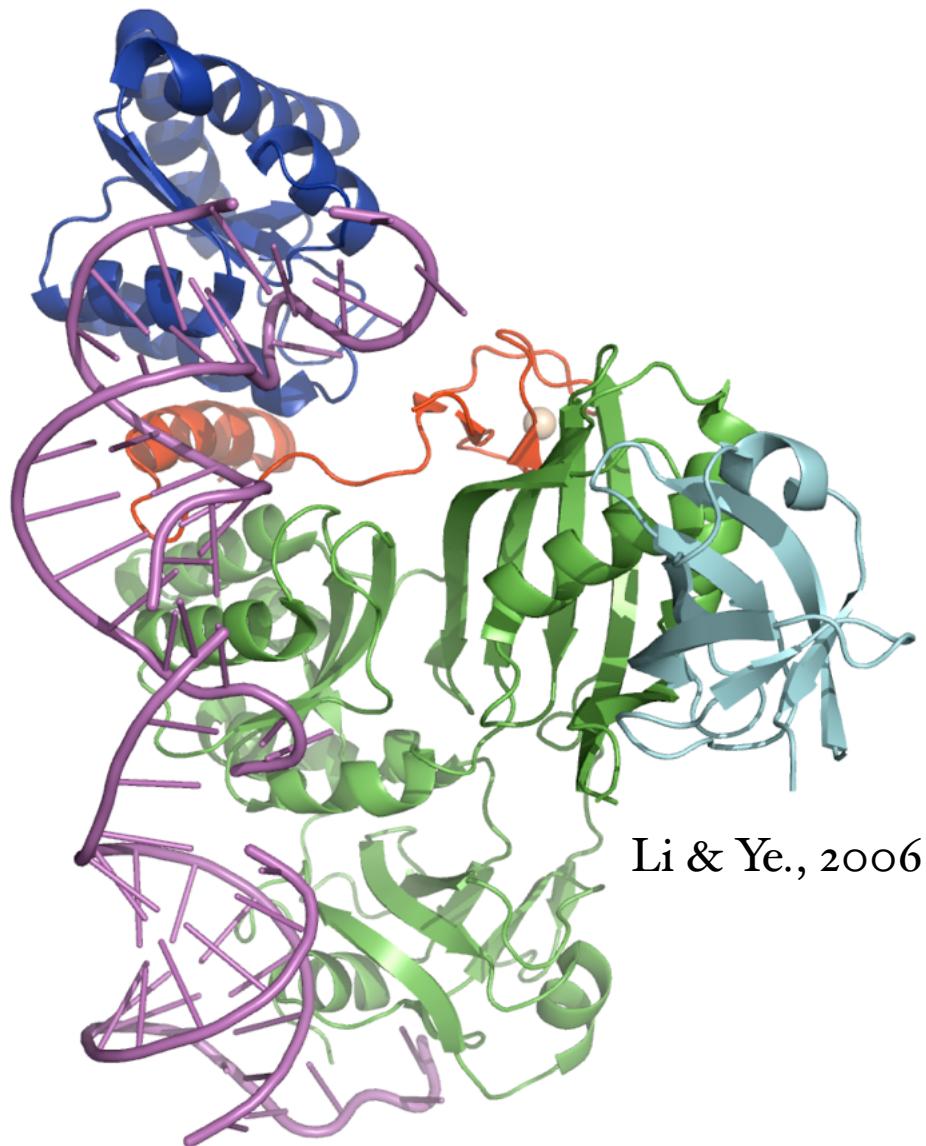
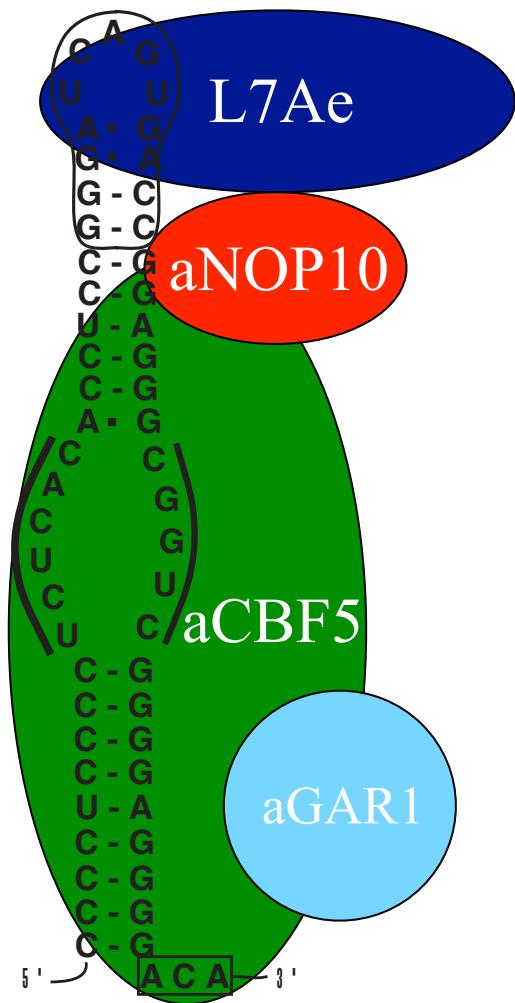


Charron *et al.*, 2004



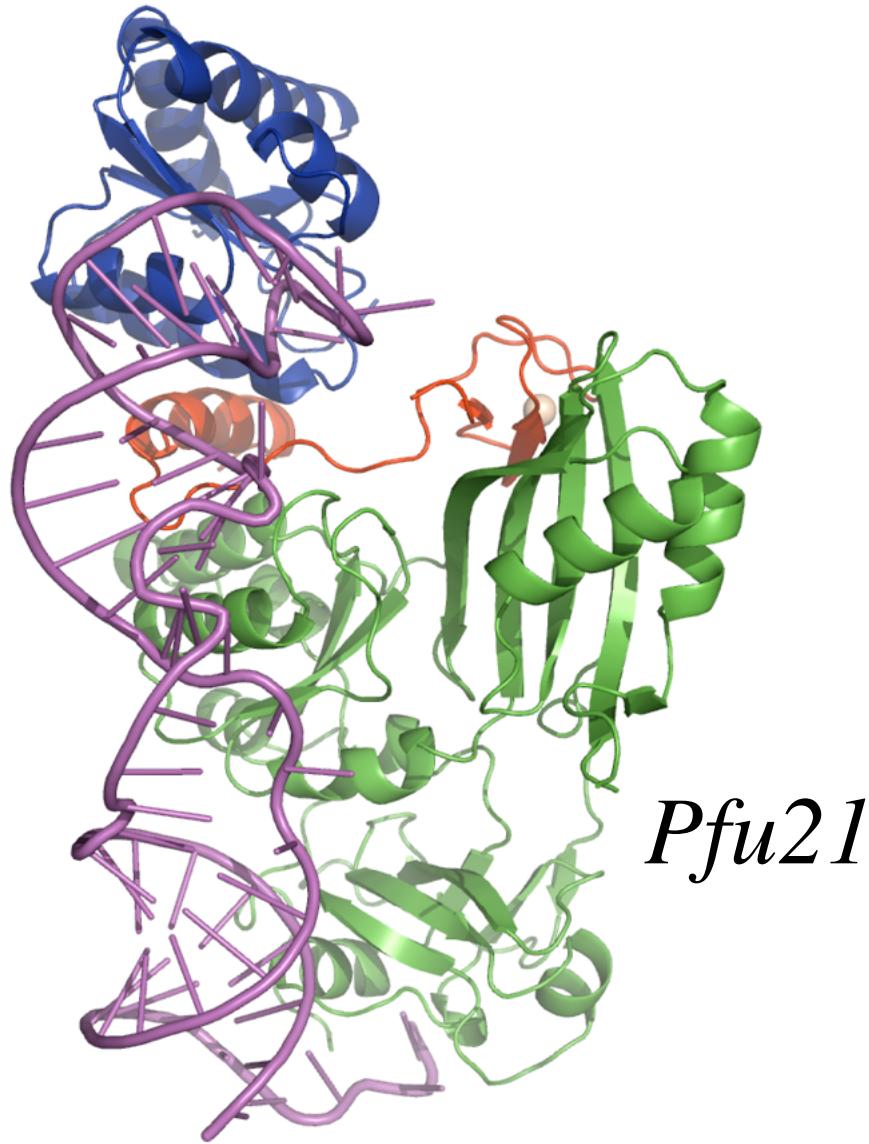
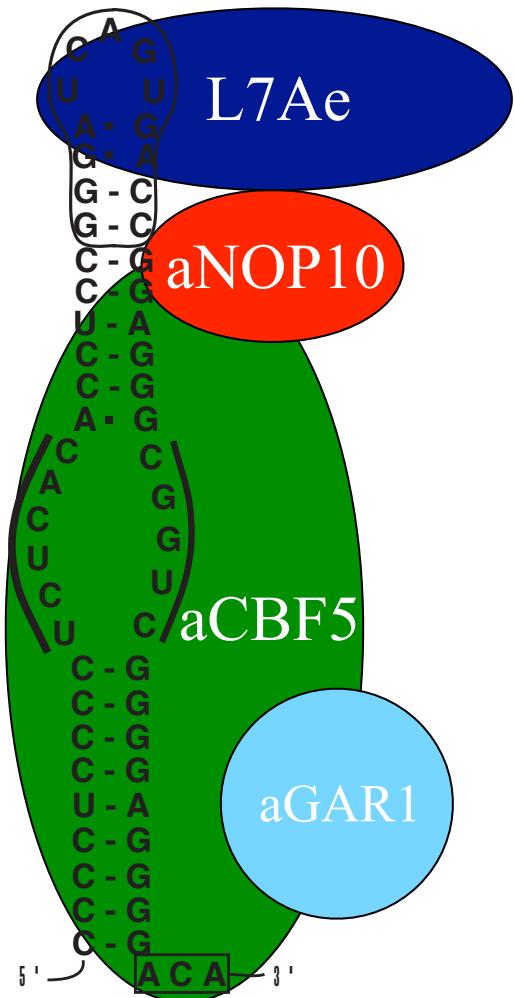
Manival *et al.*, 2006

# Structure/function relationships: determinants in sRNP H/ACA assembly



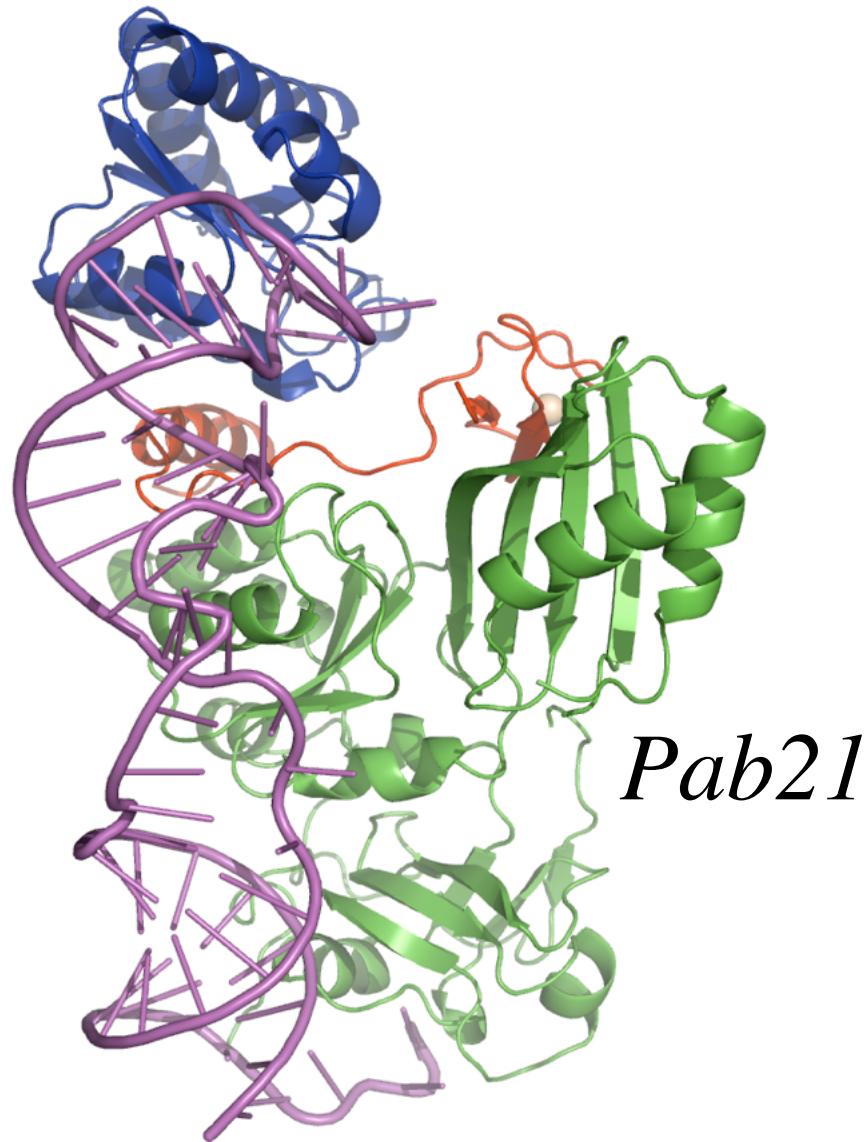
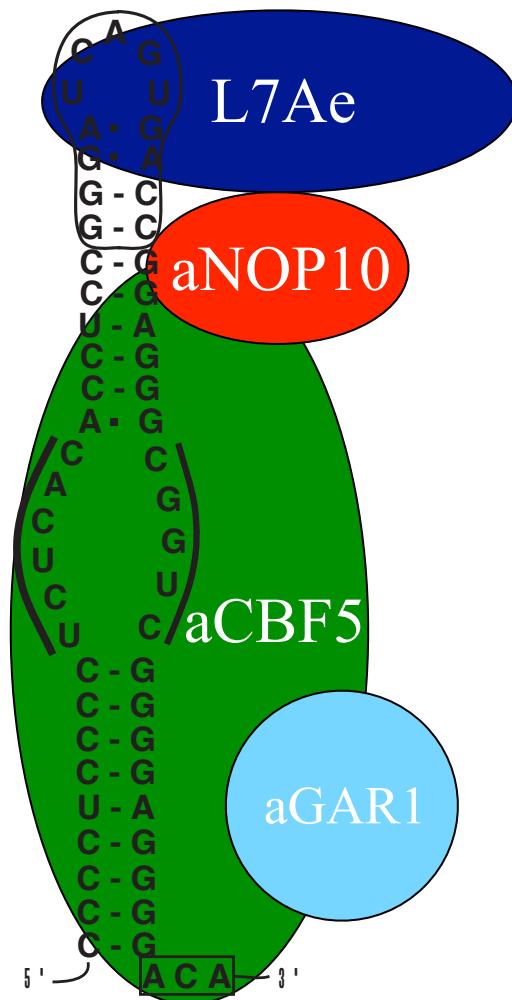
Charpentier *et al.*, 2005

# Structure/function relationships: determinants in sRNP H/ACA assembly

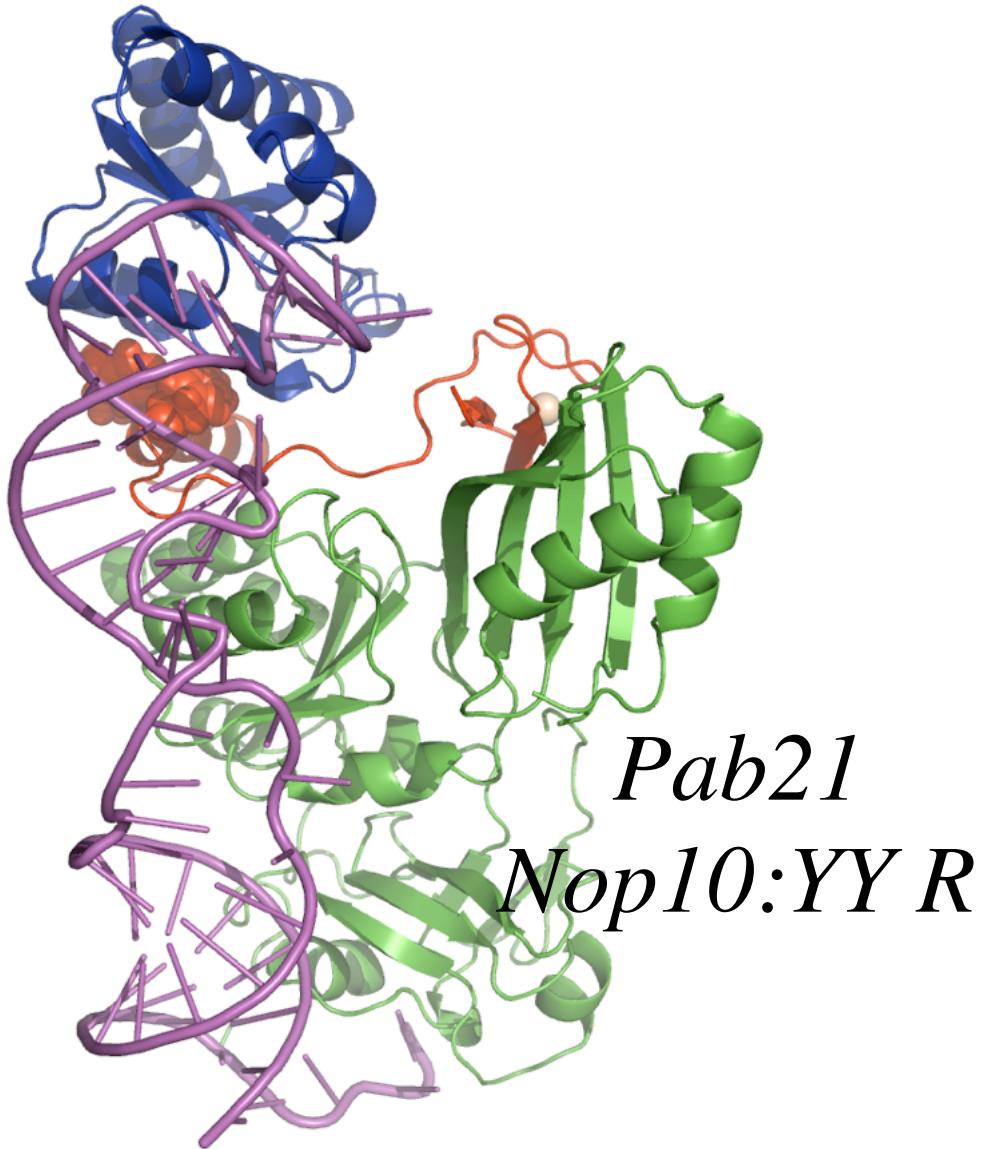
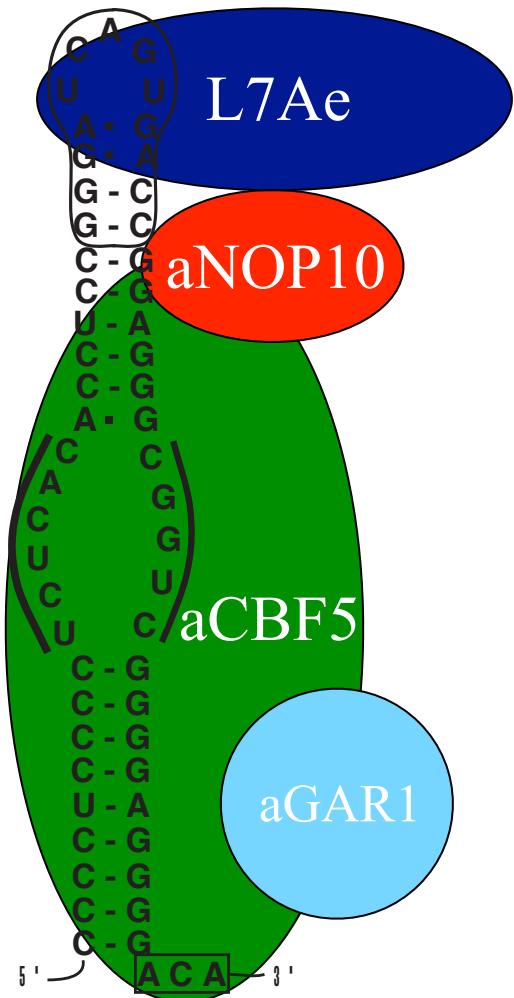


Charpentier *et al.*, 2005

# Structure/function relationships: determinants in sRNP H/ACA assembly

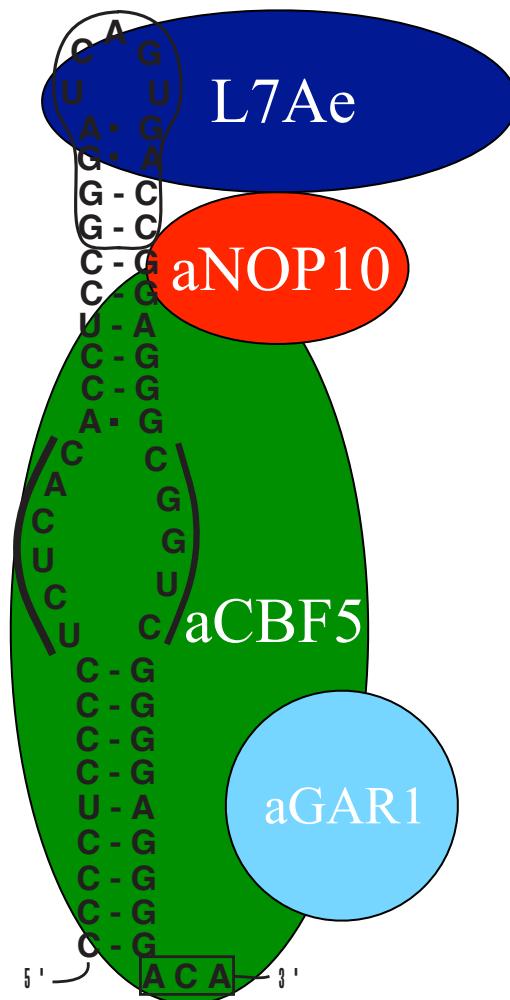


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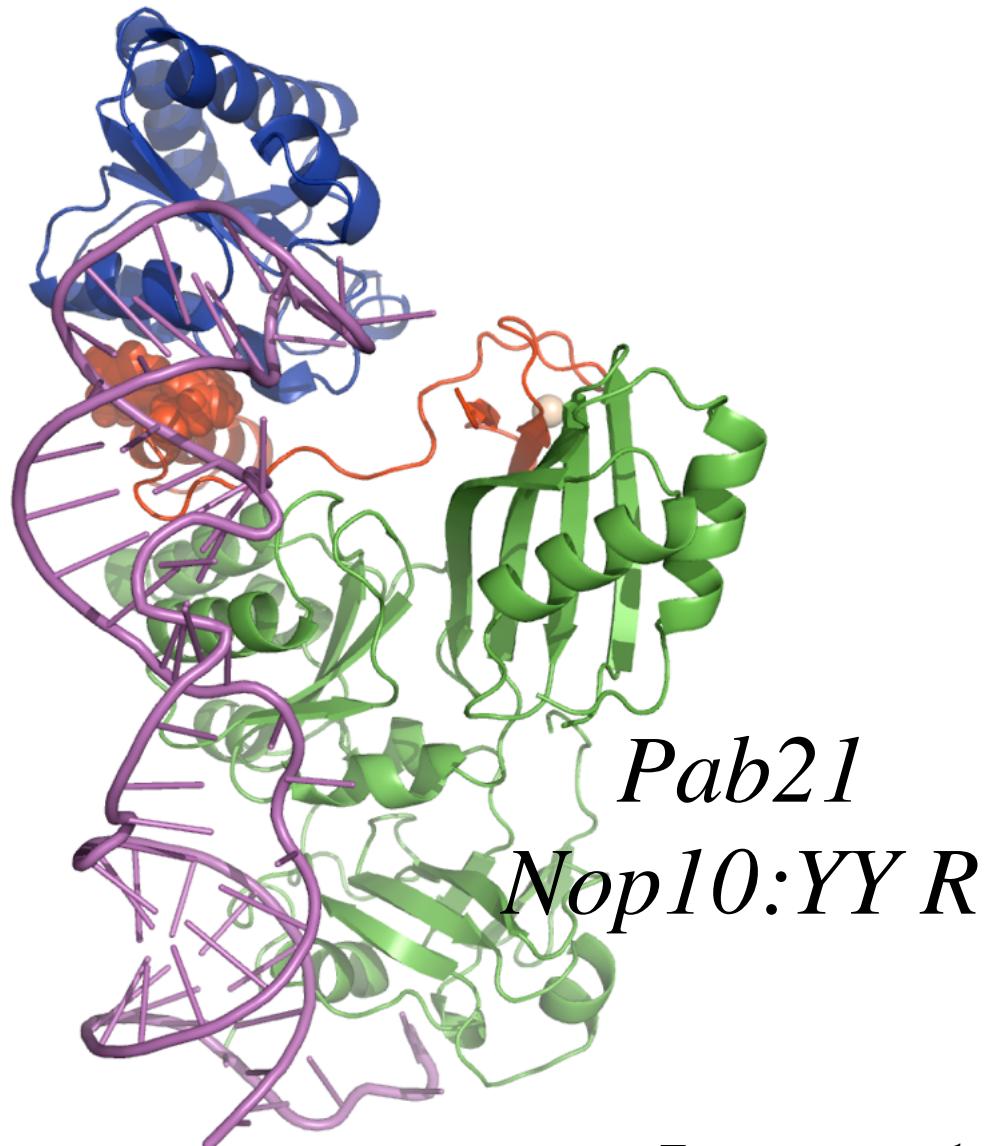


Charpentier *et al.*, 2005

# Structure/function relationships: determinants in sRNP H/ACA assembly

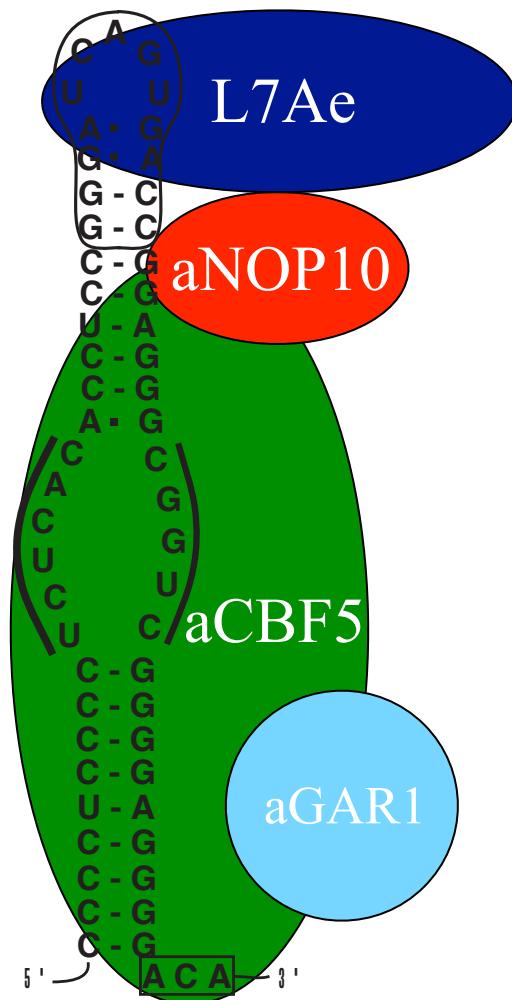


Charpentier *et al.*, 2005

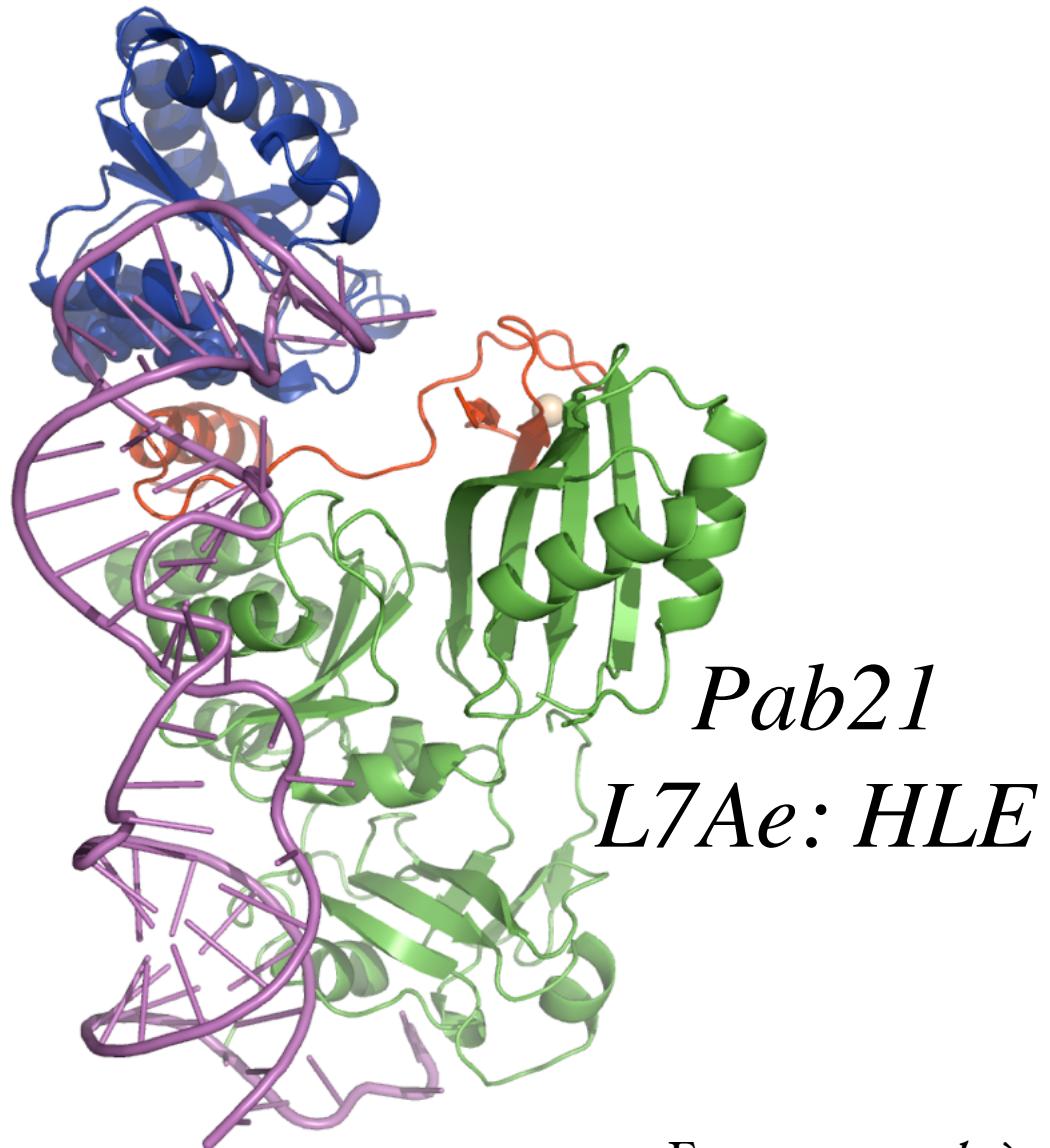


Fourmann *et al.*, à soumettre

# Structure/function relationships: determinants in sRNP H/ACA assembly

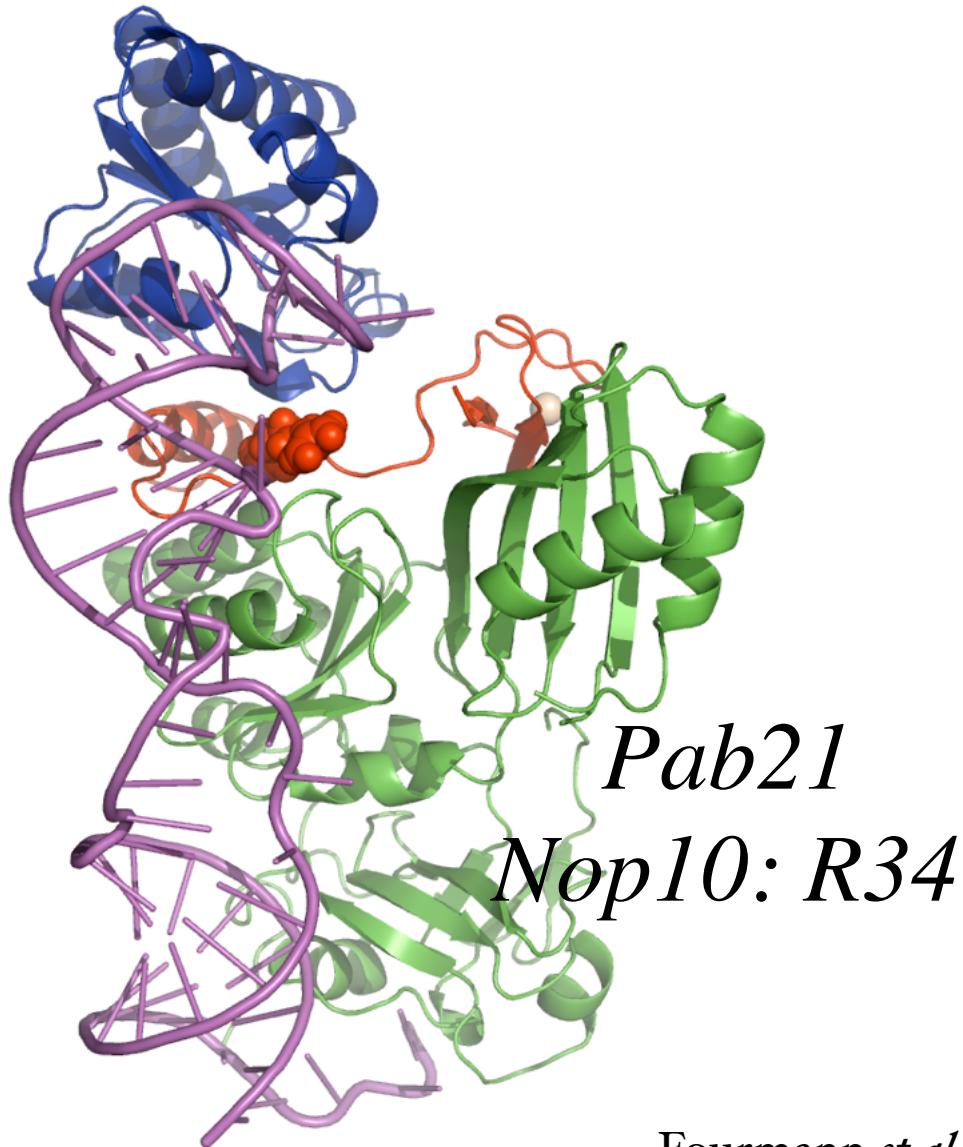
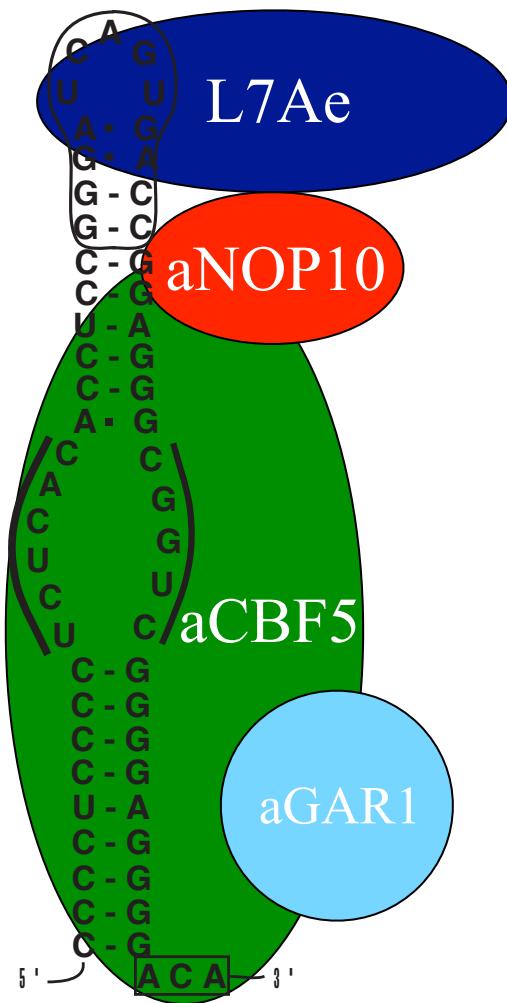


Charpentier *et al.*, 2005



Fourmann *et al.*, à soumettre

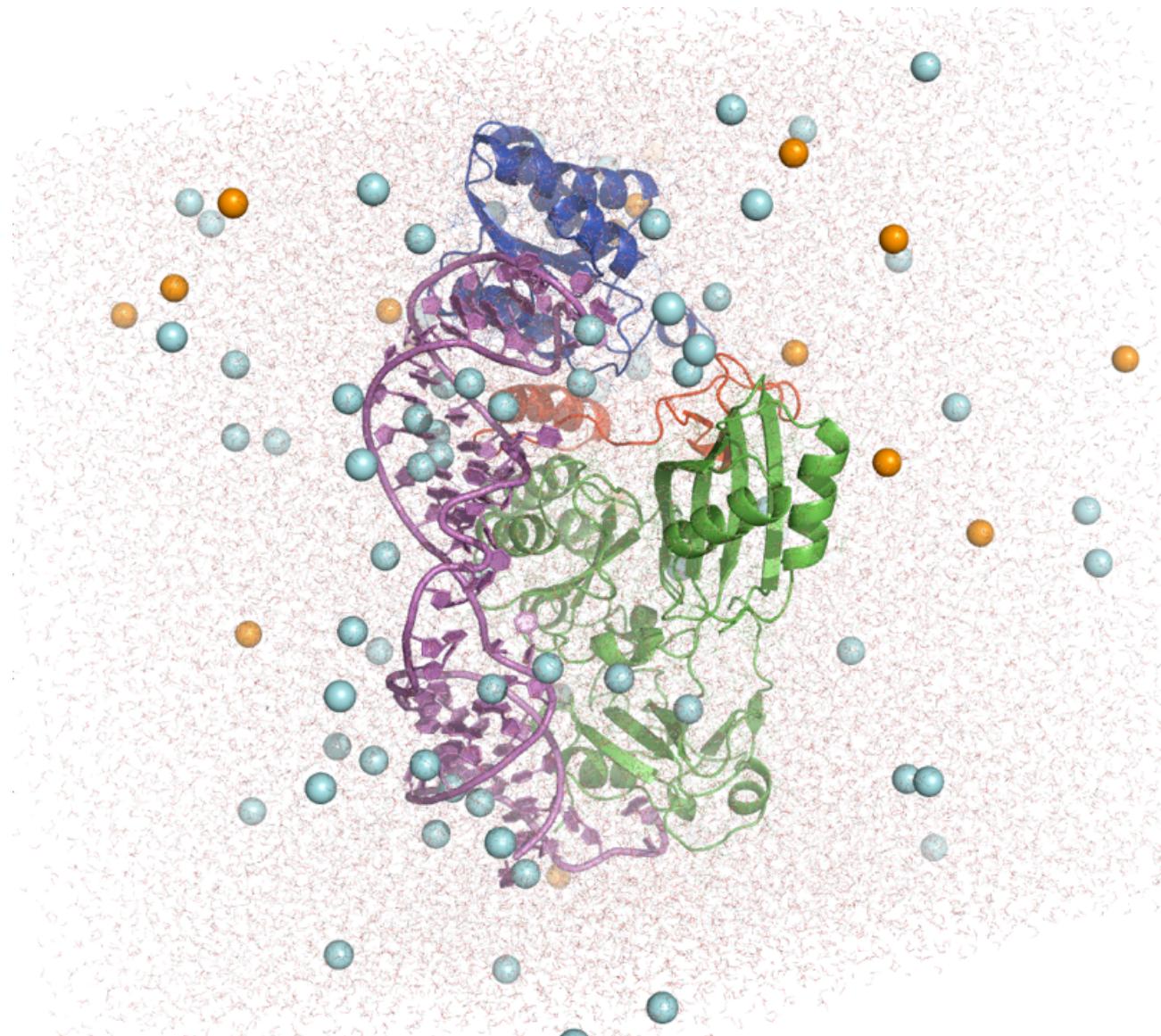
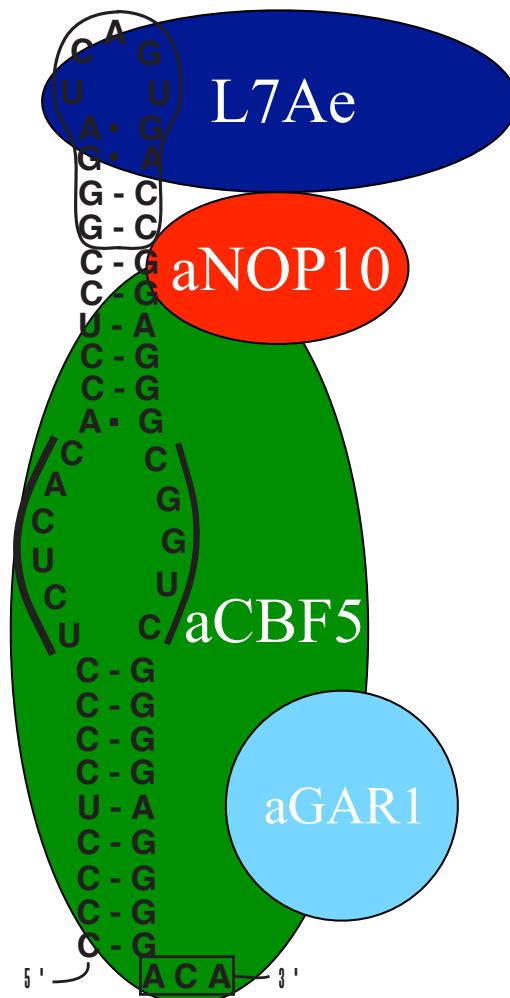
# Structure/function relationships: determinants in sRNP H/ACA assembly



Charpentier *et al.*, 2005

Fourmann *et al.*, à soumettre

# Structure/function relationships: determinants in sRNP H/ACA assembly



# Interaction L7Ae/Nop10: triads of essential residues HLE & YYR

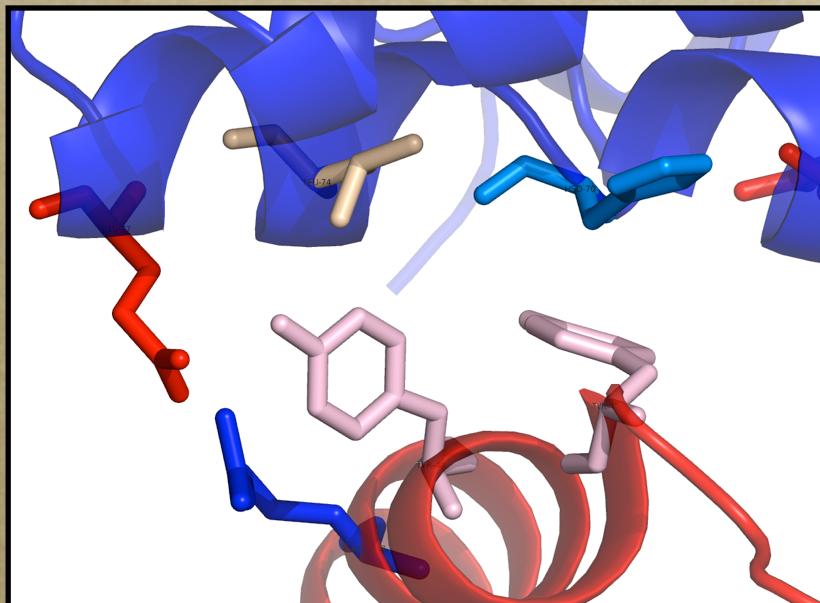
*Nop10*

P\_abyssi  
P\_horikosh  
P\_furiosus

-	M R F   R I   R K C   P K	-	C	R Y T L   K E T   C P M C	E K T K   V A H P P R F S P E D P Y	E Y R R R L   K R E L	- L G I   G R K E K -
-	M R F   R I   R K C   P R	-	C	R Y T L   K E   C P M C	E K T K   V A H P P R F S P E D P Y	E Y R R R L   K R E L	- L G I   G R K E K -
-	M R F   R I   R K C   P K	-	C	R Y T L   K E   V C P M C	E K T K   V A H P P R F S P E D P Y	E Y R R R W   K R E V	- L G I   G R K E K -

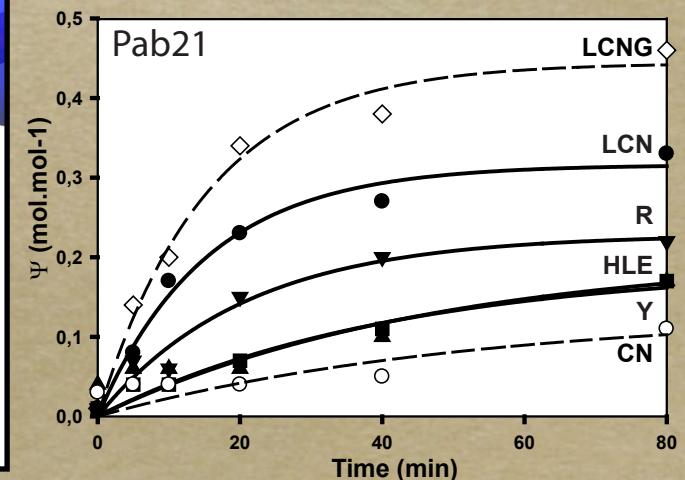
*L7Ae*

- M E G W M M A   K P S   Y V K   F E V   P K E L A E K A L Q   A V E   I A R D T   K I   R K   T N E   T T   K A V E R   Q A K L V I I   A E D   V D   P   E E   I   V A   H L   P   P   C   E E   K   E   I   P   Y   I   Y   V
- - - M M A   K P S   Y V K   F E V   P K E L A E K A L Q   A V E   I A R D T   K I   R K   T N E   T T   K A V E R   Q A K L V I I   A E D   V D   P   E E   I   V A   H L   P   P   C   E E   K   E   I   P   Y   I   Y   V
- - - M M A   K P S   Y V K   F E V   P K E L A E K A L Q   A V E   I A R D T   K I   R K   T N E   T T   K A V E R   Q A K L V I I   A E D   V D   P   E E   I   V A   H L   P   P   C   E E   K   E   I   P   Y   I   Y   V

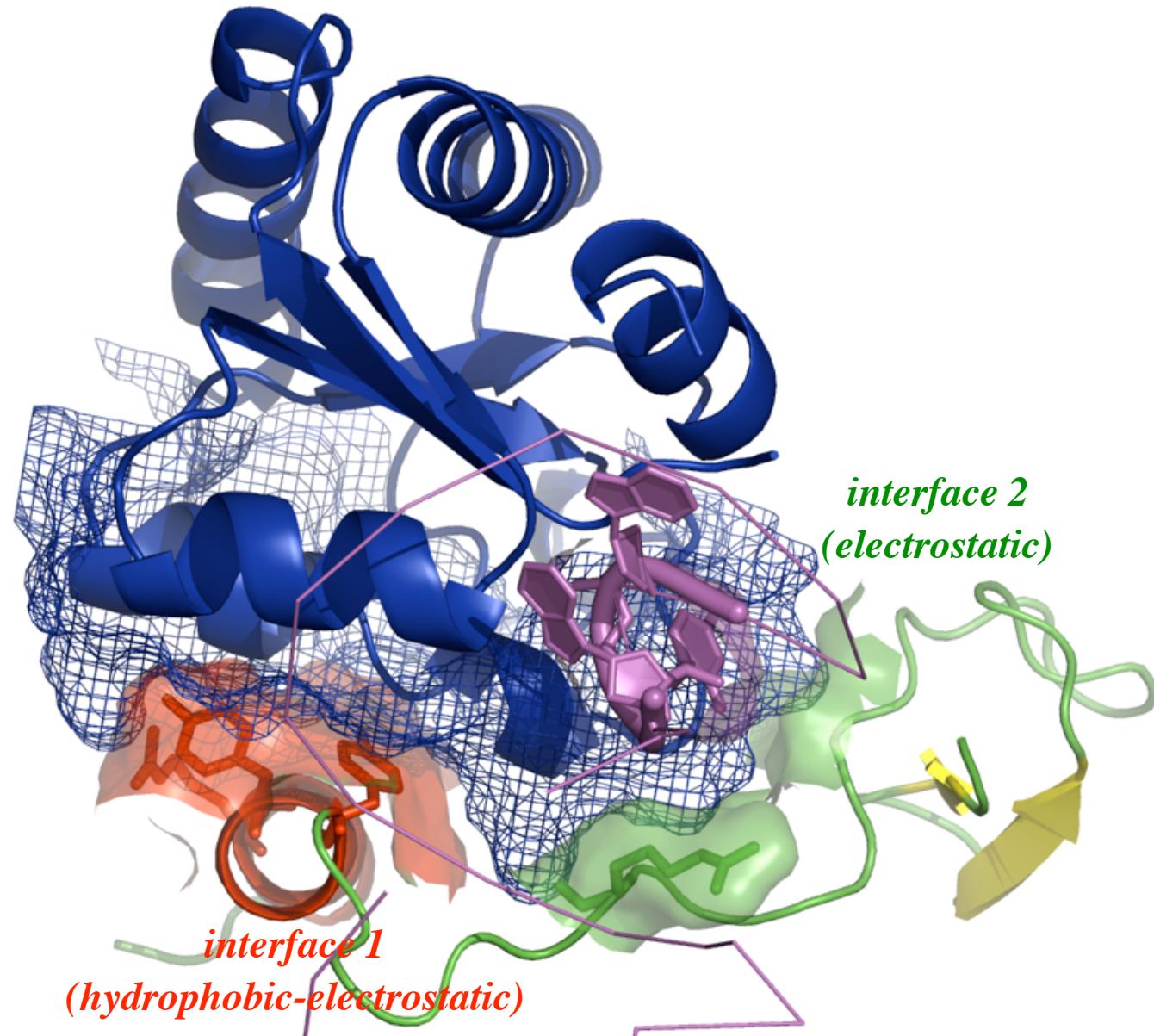


Y41 Y44 R47 YYR

HLE H70 L74 E77

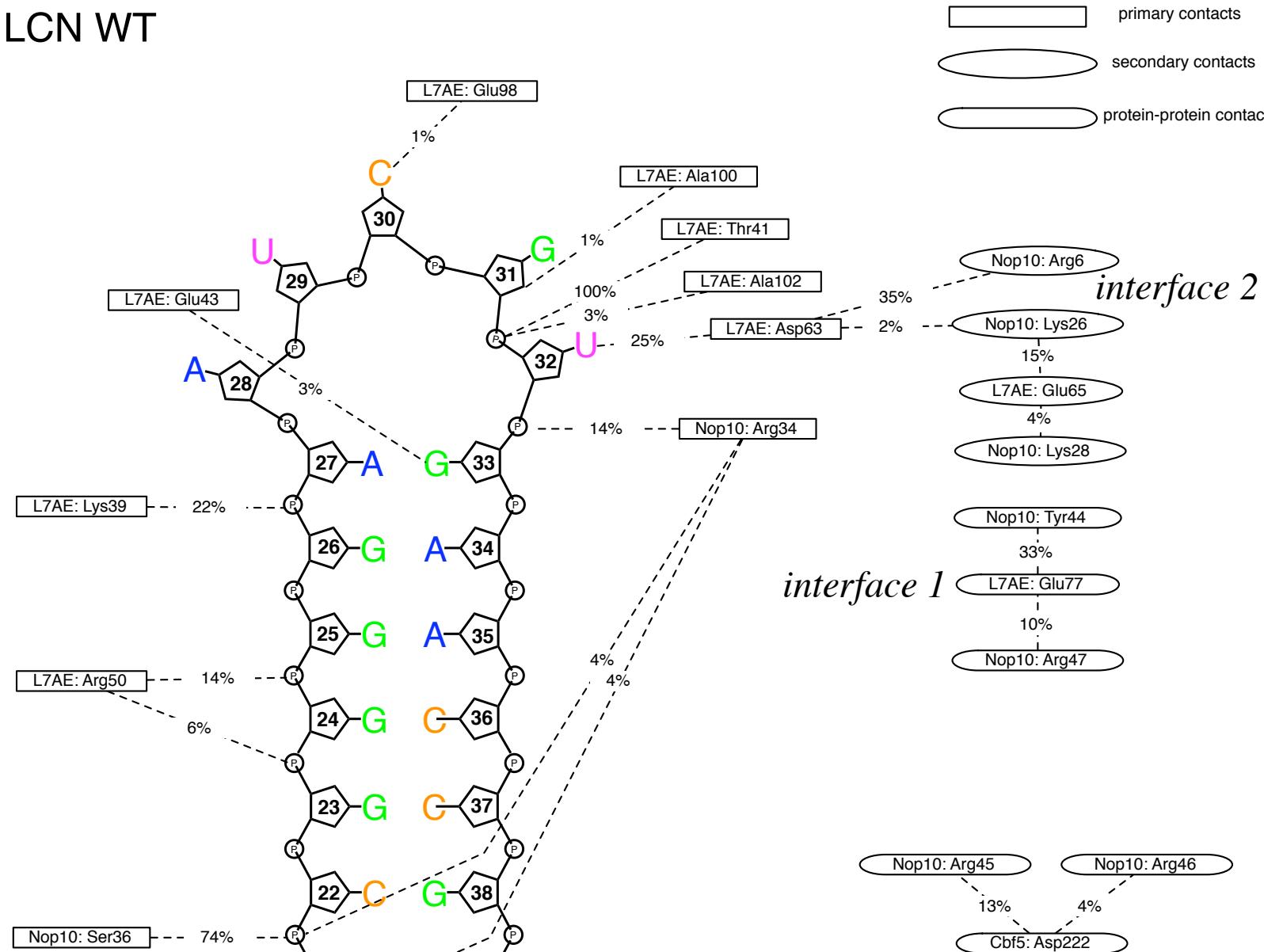


# Contacts at Nop10/L7Ae interface: from MD simulations on sRNP H/ACA



# Contacts at Nop10/L7Ae interface: from MD simulations on sRNP H/ACA

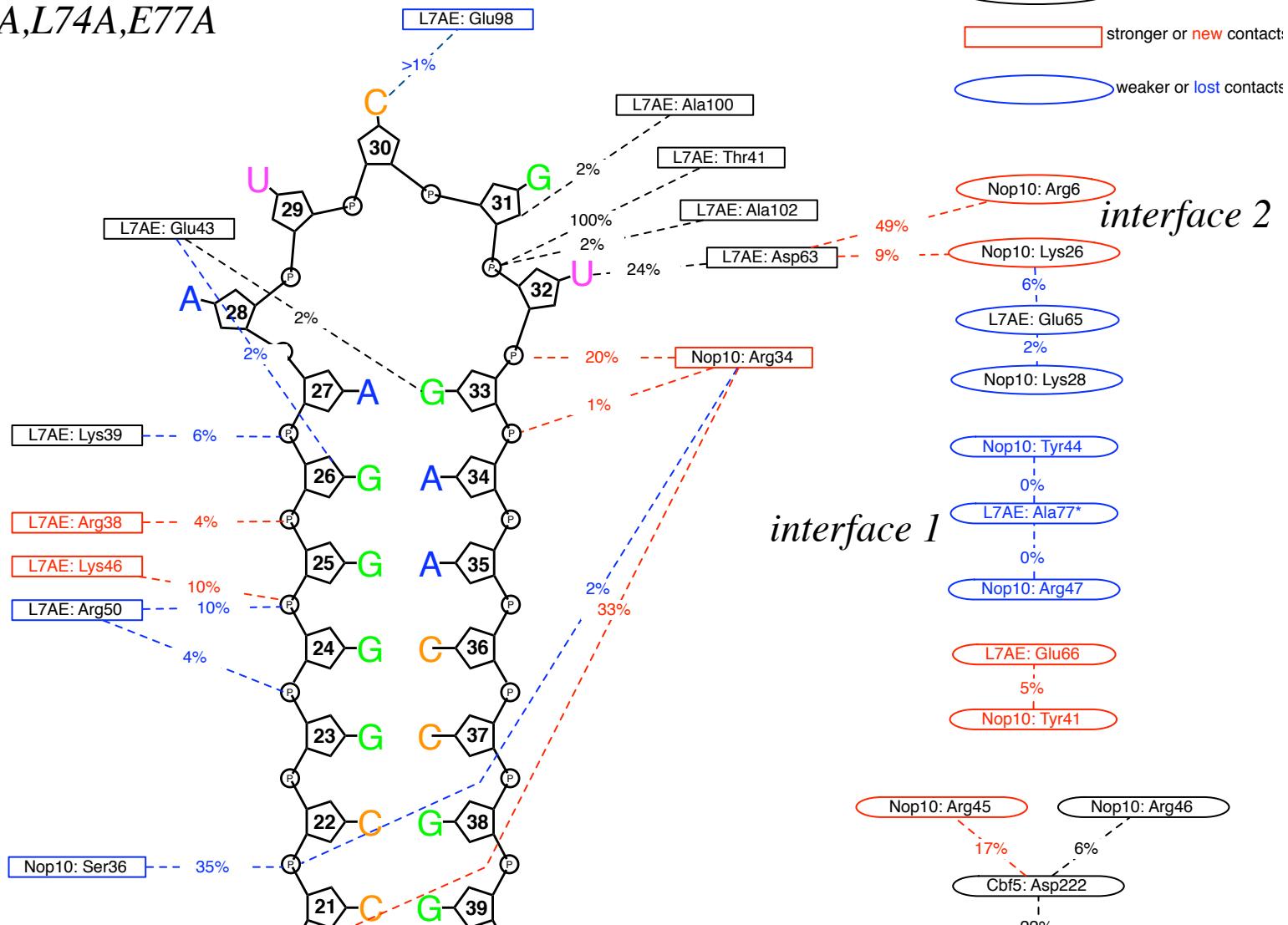
LCN WT



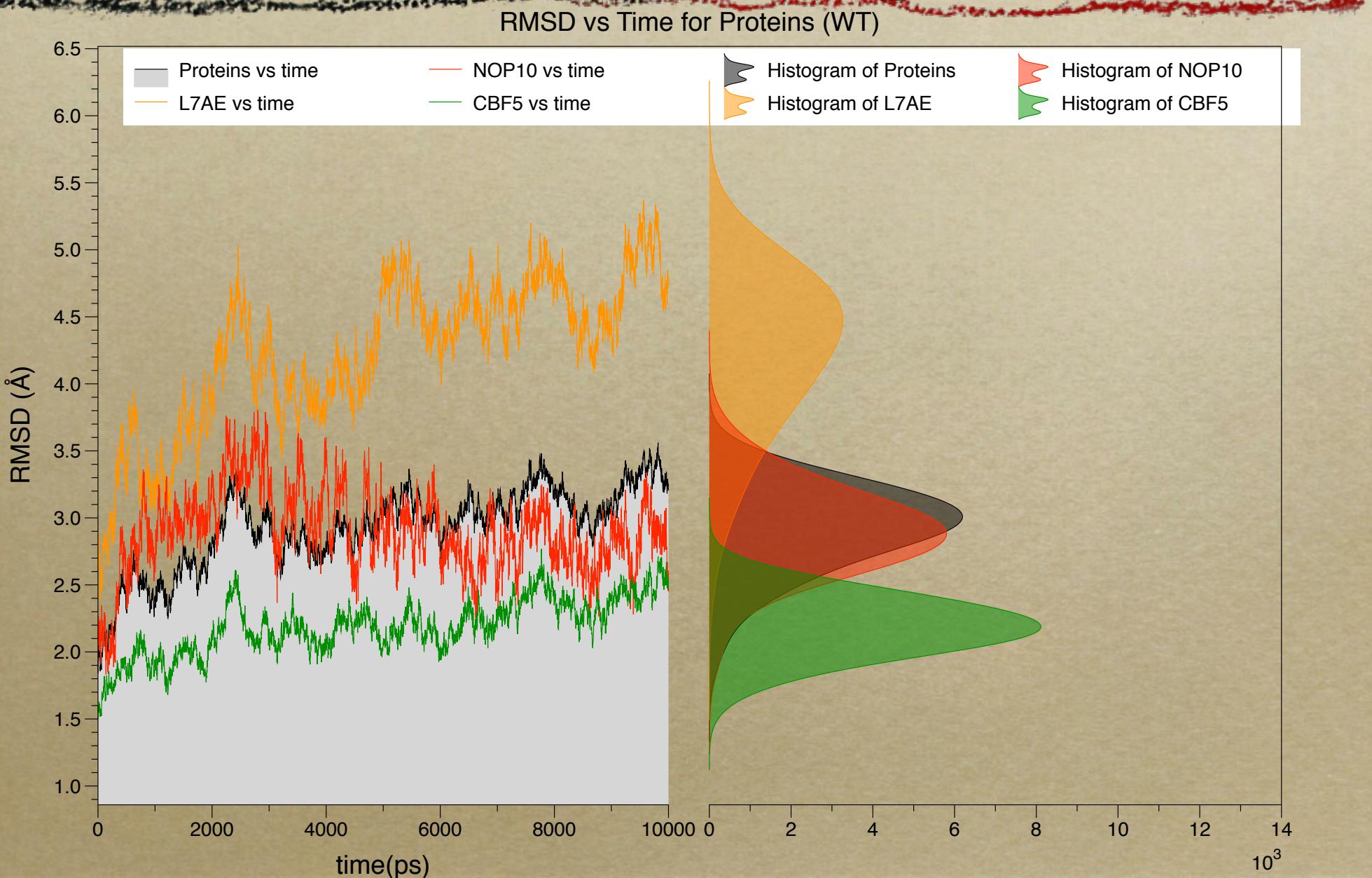
# Contacts at Nop10/L7Ae interface: from MD simulations on sRNP H/ACA

LCN L7AE-HLE

*H70A,L74A,E77A*

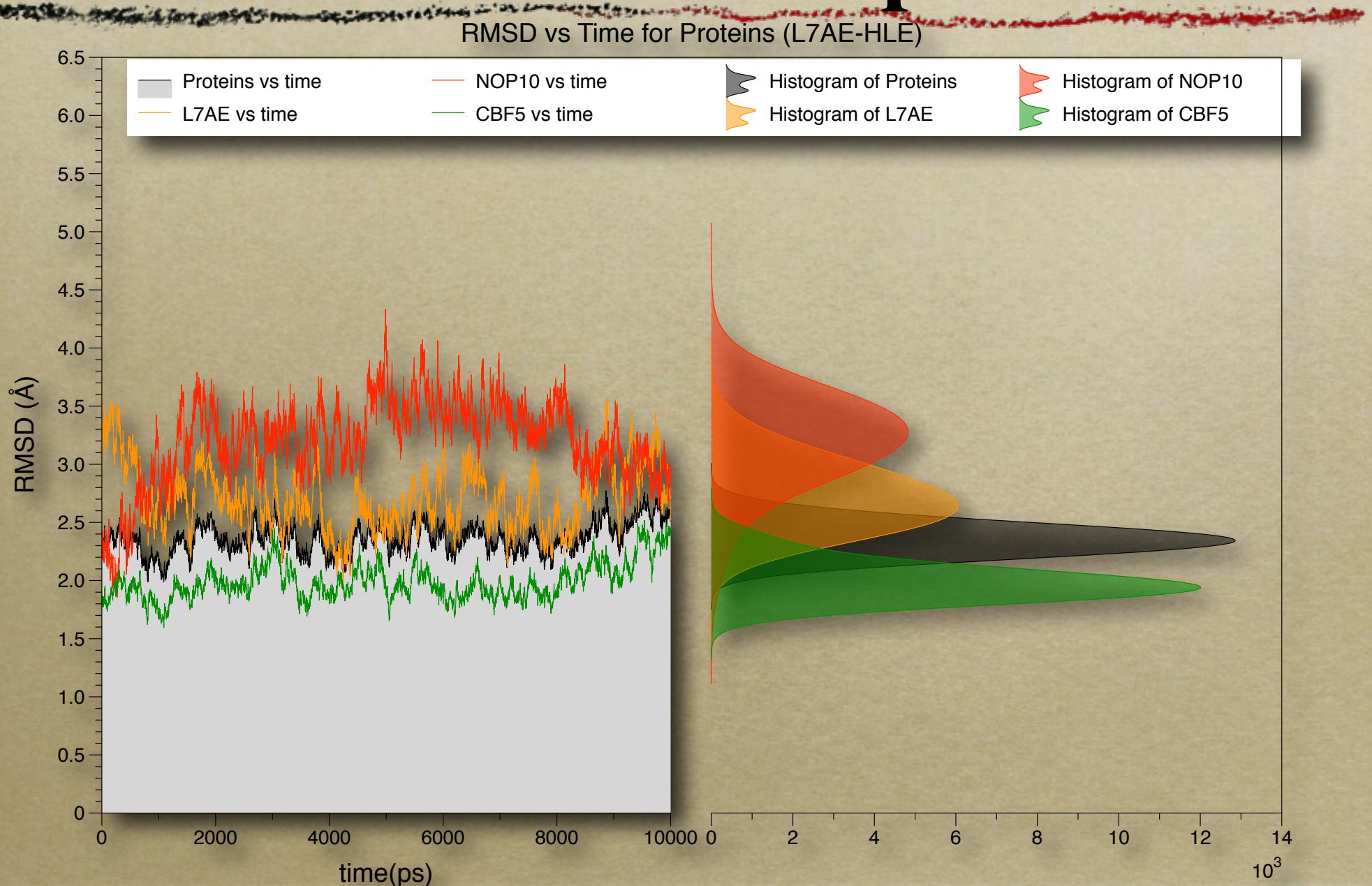


# Flexibilities of proteins within the sRNP H/ACA particles

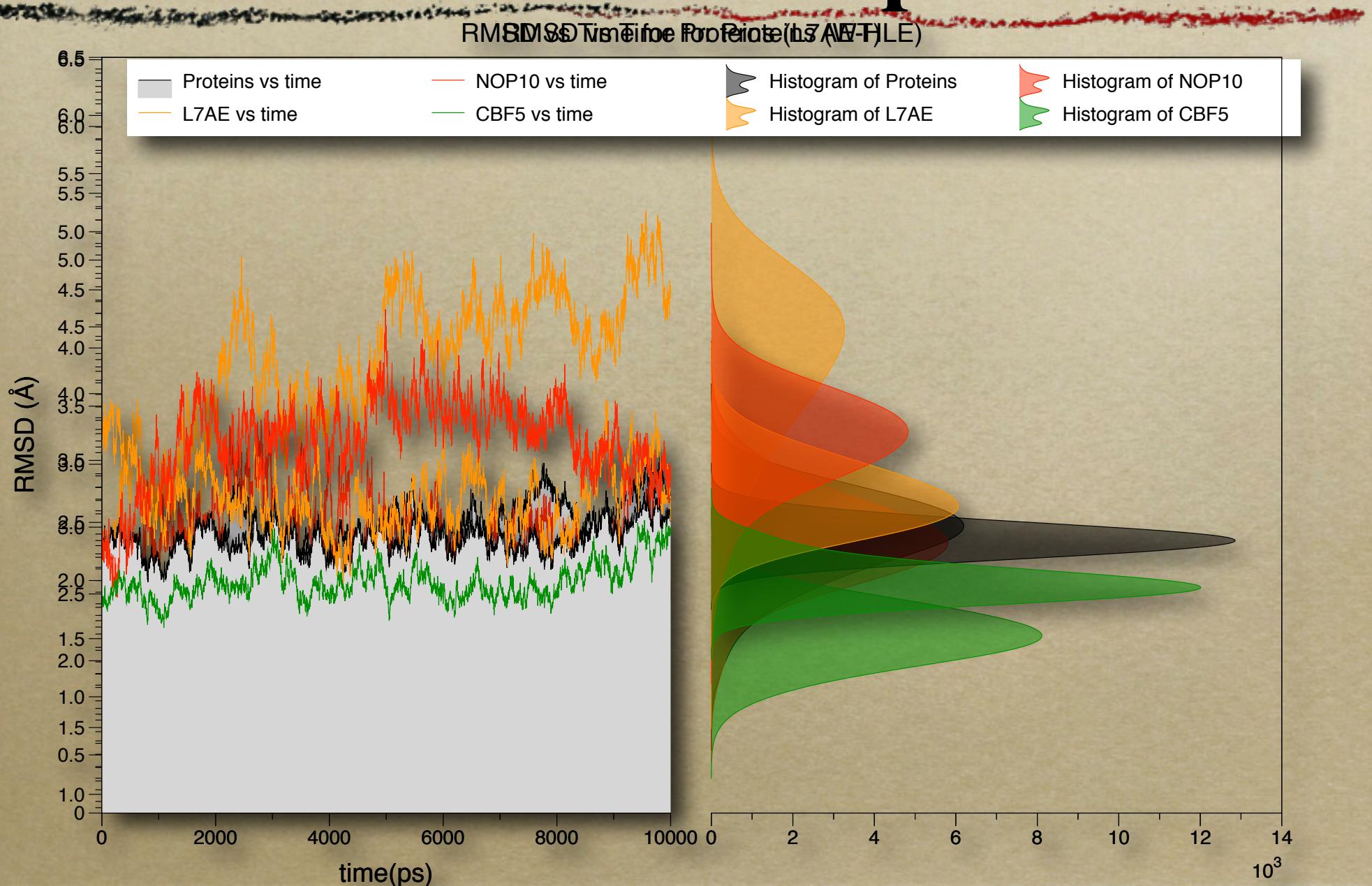


# Flexibilities of proteins within the sRNP H/ACA particles

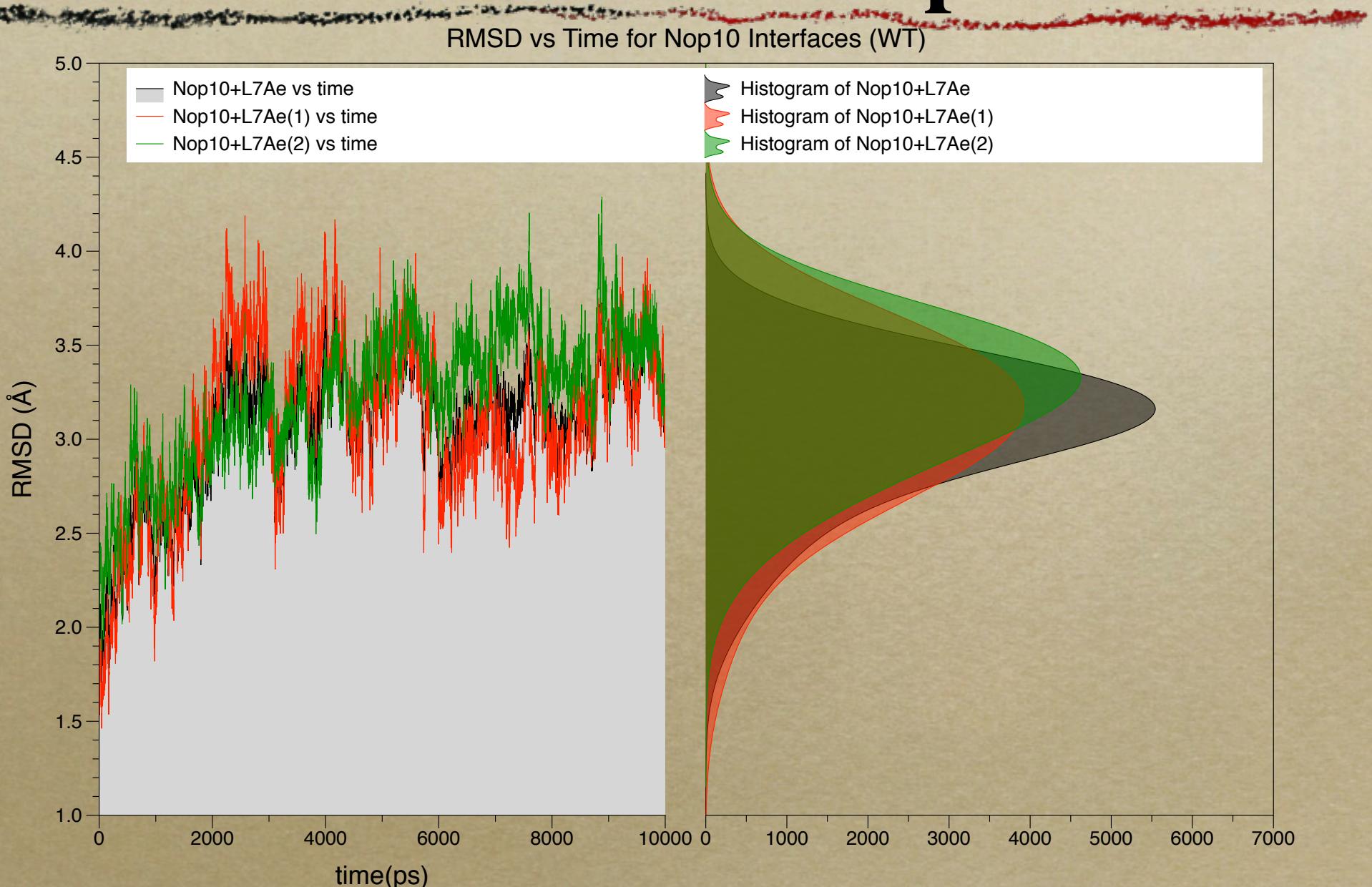
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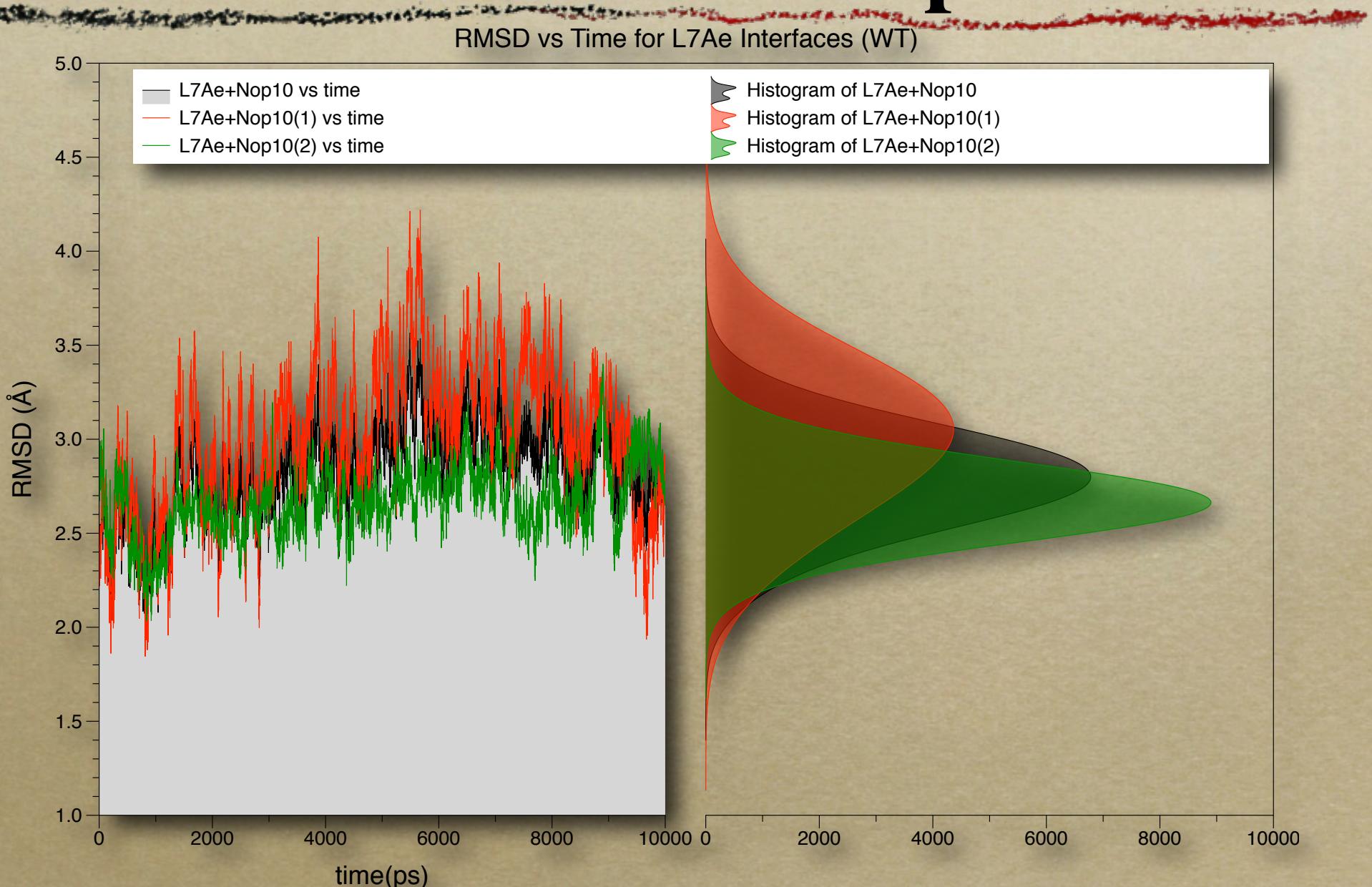


# Flexibilities at L7Ae/Nop10 interface in the sRNP H/ACA particles

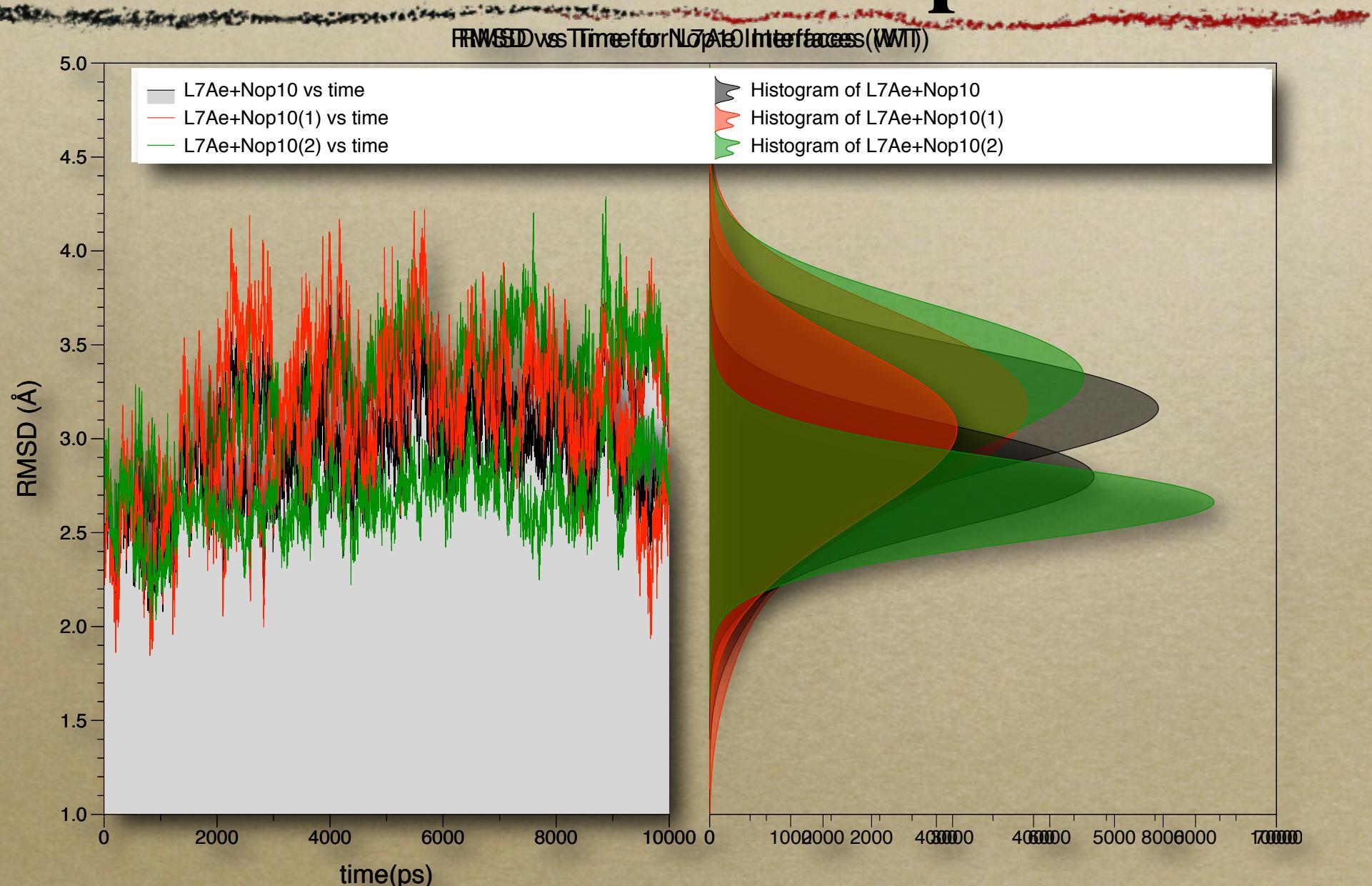


# **Flexibilities at L7Ae/Nop10 interface in the sRNP H/ACA particles**

# Flexibilities at L7Ae/Nop10 interface in the sRNP H/ACA particles



# Flexibilities at L7Ae/Nop10 interface in the sRNP H/ACA particles



# Flexibility at Nop10/L7Ae interface: a model for key contacts

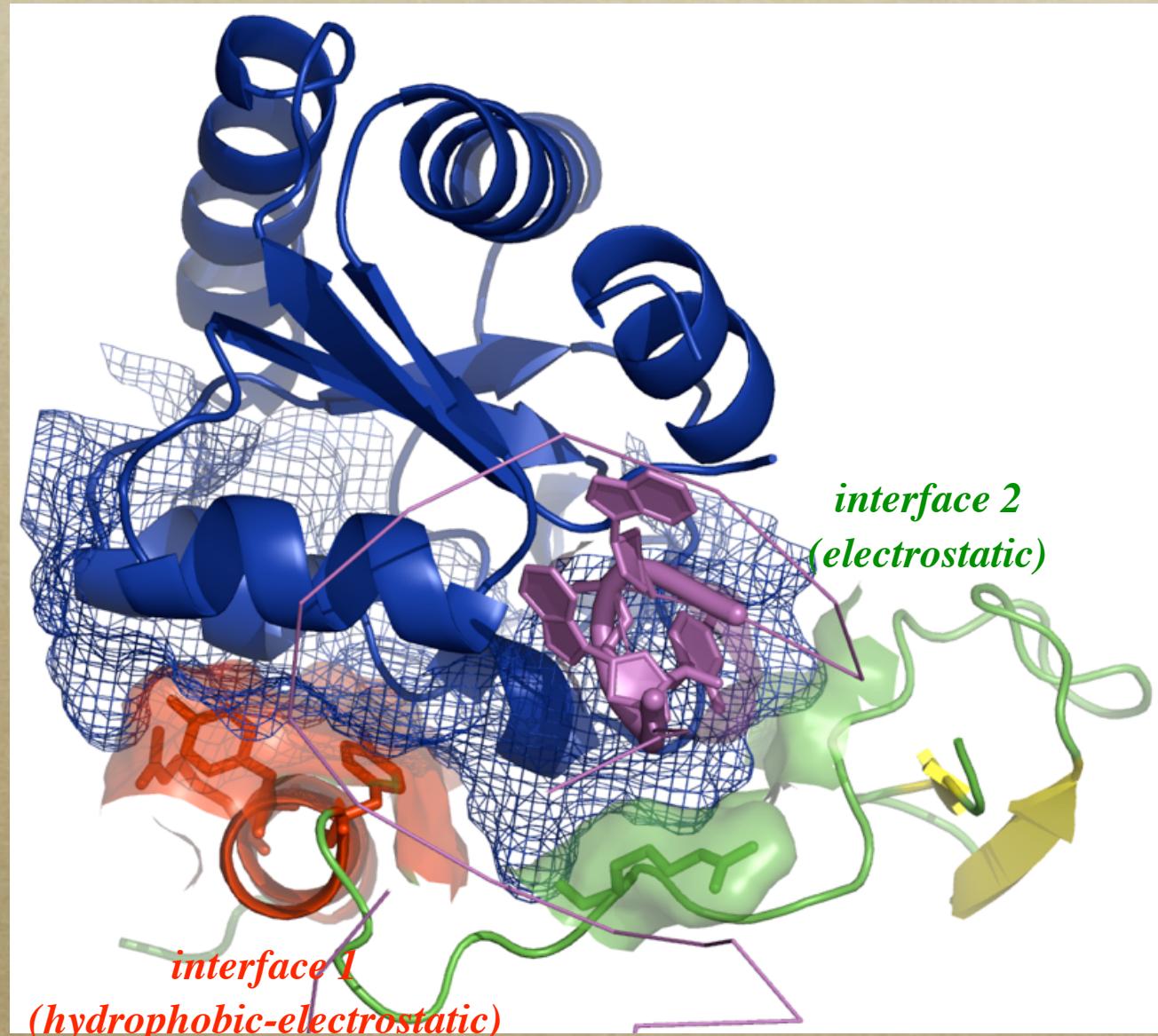
WT:  $L7Ae > Nop10$

$Nop10 + L7Ae \text{ (2)} \sim Nop10 + L7Ae \text{ (1)}$   
 $L7Ae \text{ (2)} > Nop10 \text{ (1)} \sim L7Ae \text{ (1)} >$   
 $Nop10 \text{ (2)}$

$WT > L7Ae\text{-HLE}$

**L7Ae-HLE:**  $Nop10 > L7Ae$

$Nop10 + L7Ae \text{ (1)} > Nop10 + L7Ae \text{ (2)}$   
 $Nop10 \text{ (1)} > L7Ae \text{ (1)} \sim L7Ae \text{ (2)} >$   
 $Nop10 \text{ (2)}$



# Flexibility at Nop10/L7Ae interface: a model for key contacts

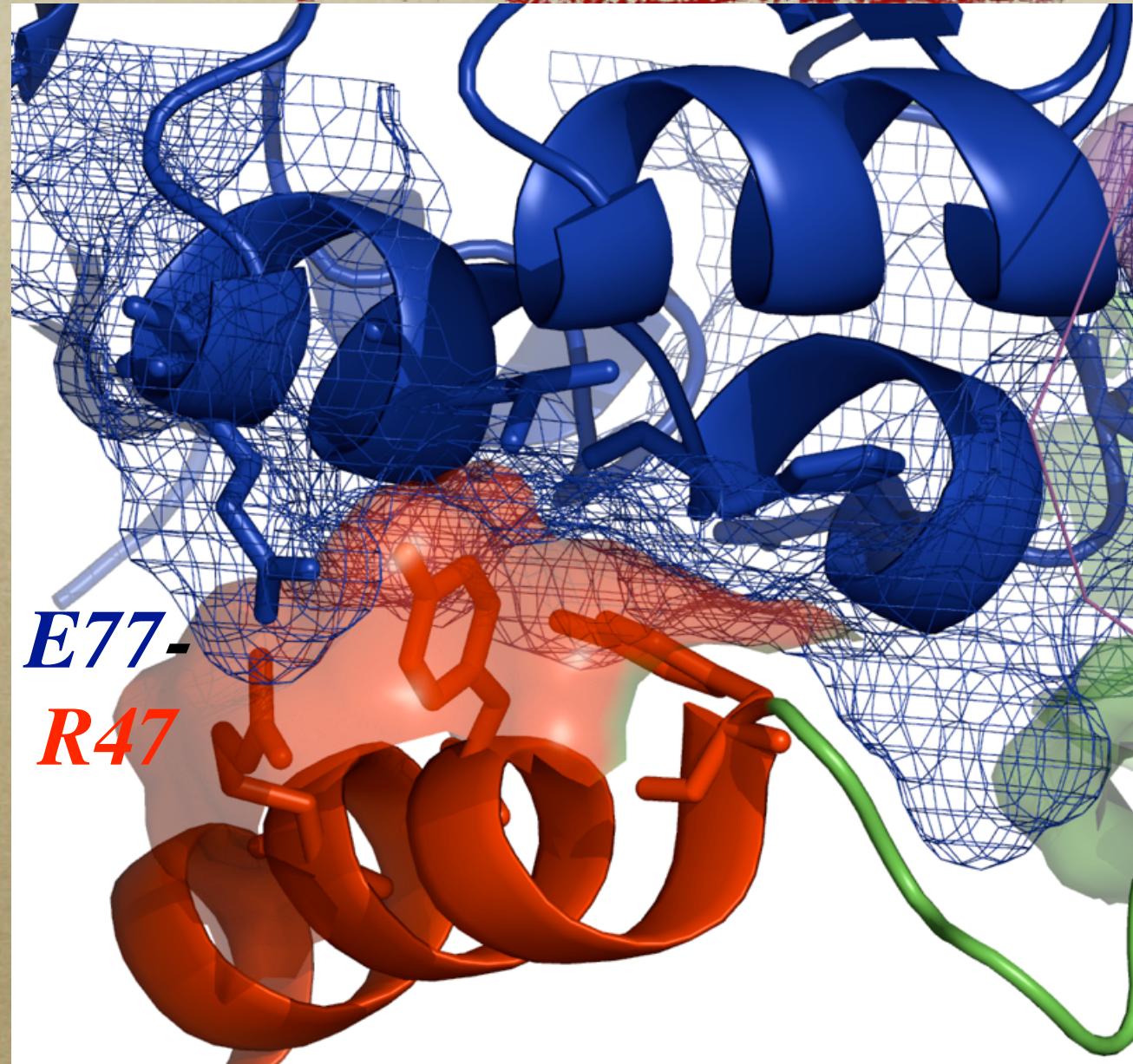
*WT: L7Ae > Nop10*

*Nop10+L7Ae (2) ~ Nop10+L7Ae (1)*  
*L7Ae (2) > Nop10 (1) ~ L7Ae (1) >*  
*Nop10 (2)*

*WT > L7Ae-HLE*

*L7Ae-HLE: Nop10 > L7Ae*

*Nop10+L7Ae (1) > Nop10+L7Ae (2)*  
*Nop10 (1) > L7Ae (1) ~ L7Ae (2) >*  
*Nop10 (2)*



# Flexibility at Nop10/L7Ae interface: a model for key contacts

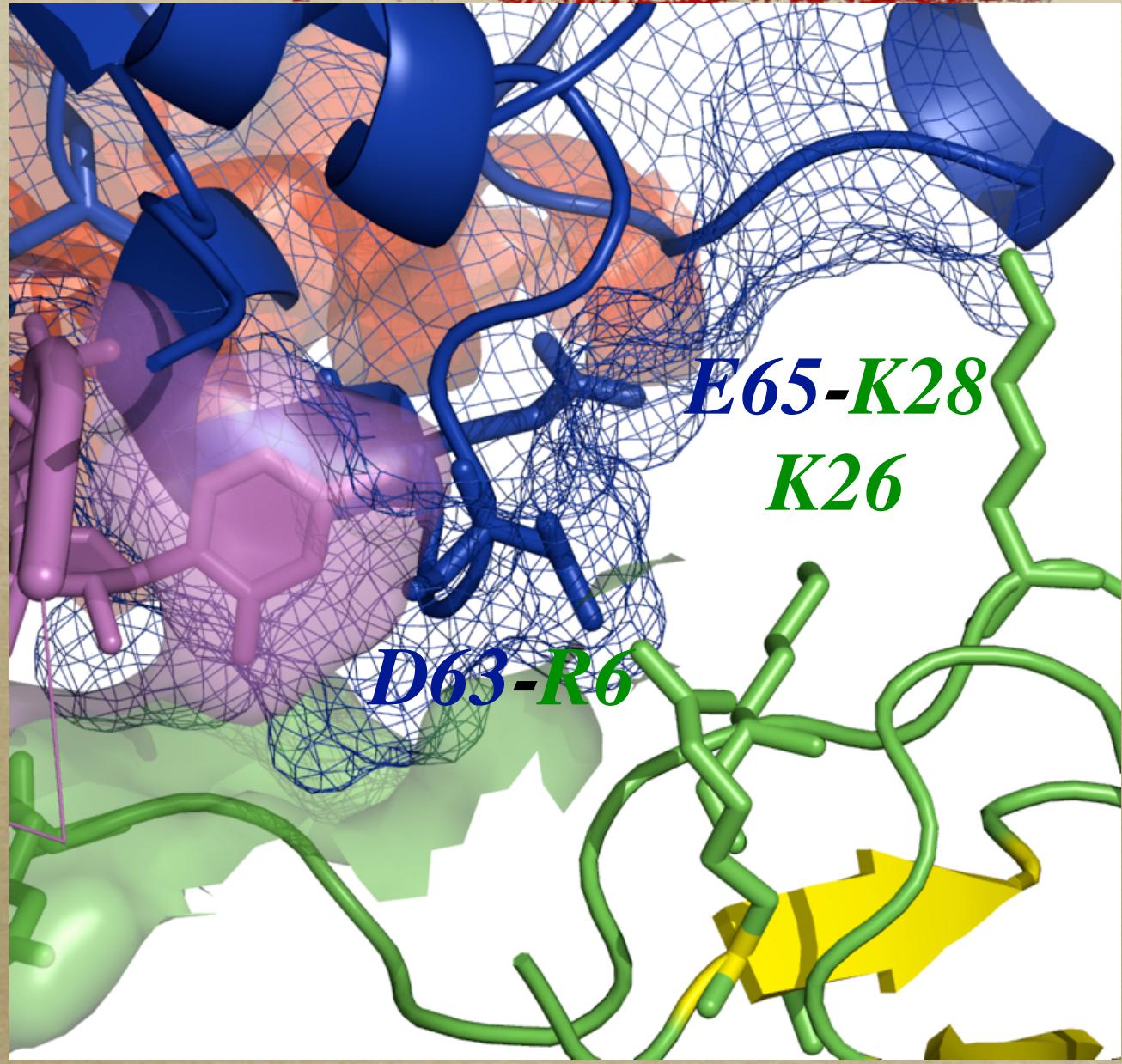
*WT: L7Ae > Nop10*

*Nop10+L7Ae (2) ~ Nop10+L7Ae (1)*  
*L7Ae (2) > Nop10 (1) ~ L7Ae (1) >*  
*Nop10 (2)*

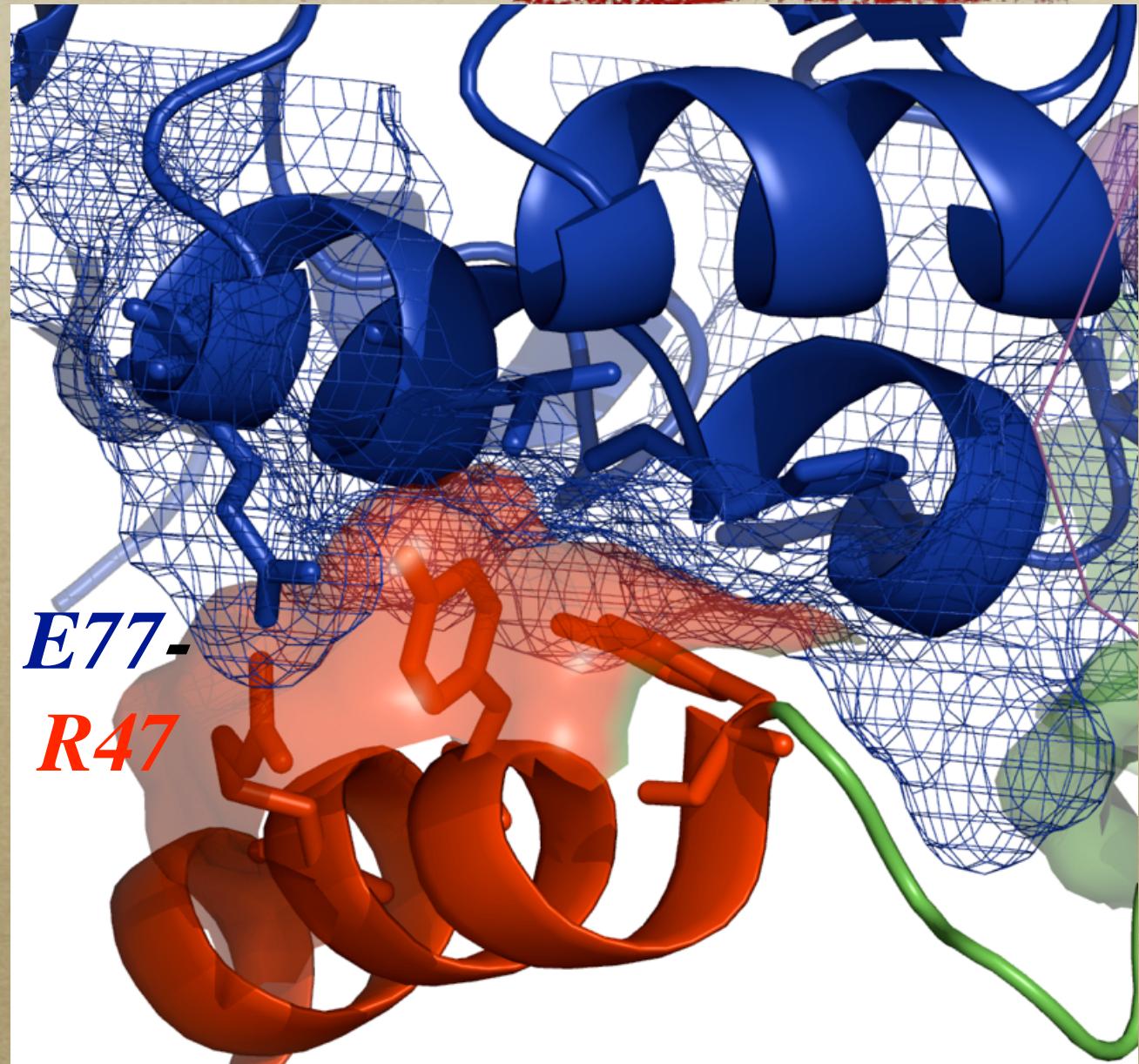
*WT > L7Ae-HLE*

*L7Ae-HLE: Nop10 > L7Ae*

*Nop10+L7Ae (1) > Nop10+L7Ae (2)*  
*Nop10 (1) > L7Ae (1) ~ L7Ae (2) >*  
*Nop10 (2)*



# Correlated mutation analysis: L7Ae & Nop10 residues at interface 1



# Correlated mutation analysis:

## Nop10:28/Nop10:47



<i>Pyrococcus</i>		R47				
<i>I<sub>2</sub></i>	<i>I<sup>1</sup></i>	<i>RKH</i>	<i>GAPVLIM</i>	<i>FYW</i>	<i>STCNQ</i>	<i>DE</i>
<i>RKH</i>		9	6			
<i>GAPVLIM</i>	1		12		1	7
<i>FYW</i>	1		1			2
<i>STCNQ</i>			2			
<i>DE</i>	1		2			

*K28*

# Correlated mutation analysis:

## Nop10:28/Nop10:47

*Pyrococcus*

R47

$I_2$	$I^1$	<i>RKH</i>	<i>GAPVLIM</i>	<i>FYW</i>	<i>STCNQ</i>	<i>DE</i>
<i>RKH</i>		20%	13%			
<i>GAPVLIM</i>	2%		27%		2%	16%
<i>FYW</i>	2%		2%			4%
<i>STCNQ</i>			4%			
<i>DE</i>	2%		4%			

K28

# Correlated mutation analysis:

## Nop10:26/Nop10:47

*Pyrococcus* R47

$I_2$	$RKH$	$GAPVLIM$	$FYW$	$STCNQ$	$DE$
$RKH$	10	10			3
$GAPVLIM$		7			2
$FYW$					
$STCNQ$		3			1
$DE$	2	3		1	3

*K26*

# Correlated mutation analysis:

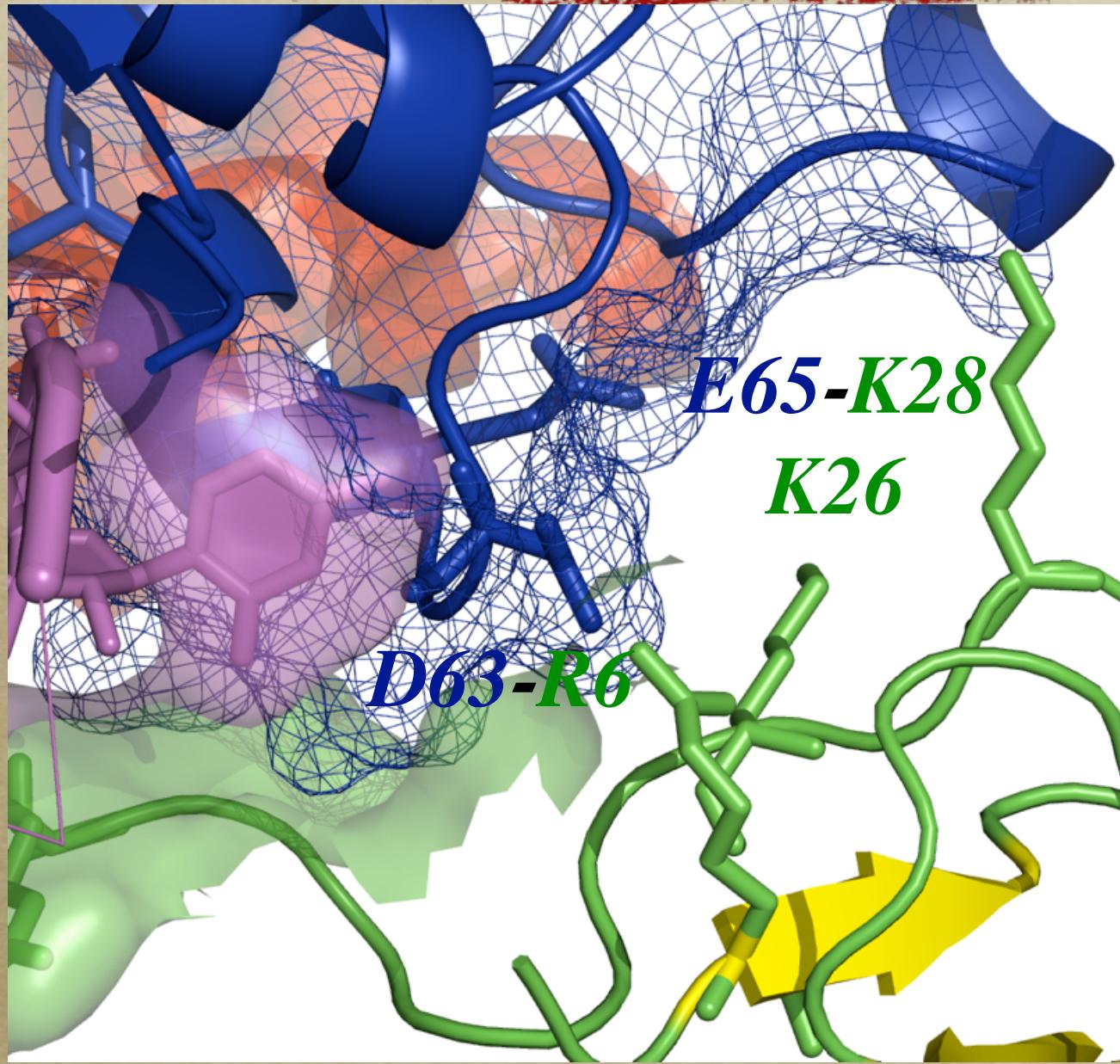
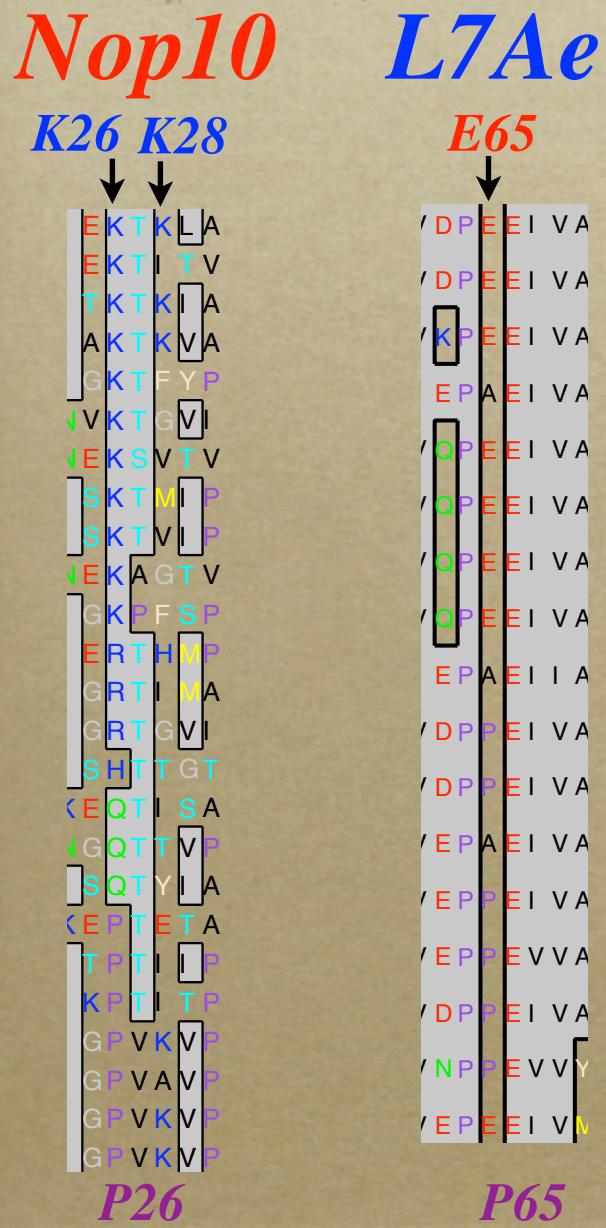
## Nop10:26/Nop10:47

*Pyrococcus* R47

$I_2$	$RKH$	$GAPVLIM$	$FYW$	$STCNQ$	$DE$
$RKH$	22%	22%			7%
$GAPVLIM$		16%			4%
$FYW$					
$STCNQ$		7%			2%
$DE$	4%	7%		2%	7%

K26

# Correlated mutation analysis: L7Ae & Nop10 residues at interface 2



# Correlated mutation analysis:

## Nop10:26/L7Ae:65



<i>Pyrococcus</i>		E65				
<i>Nop10</i>	<i>L7Ae</i>	<i>RKH</i>	<i>GAPVLIM</i>	<i>FYW</i>	<i>STCNQ</i>	<i>DE</i>
<i>RKH</i>			7			17
<i>GAPVLIM</i>			7			2
<i>FYW</i>						
<i>STCNQ</i>			3		1	
<i>DE</i>			4			4
<i>K26</i>						

# Correlated mutation analysis:

## Nop10:26/L7Ae:65

<i>Pyrococcus</i>		E65				
<i>Nop10</i>	<i>L7Ae</i>	<i>RKH</i>	<i>GAPVLIM</i>	<i>FYW</i>	<i>STCNQ</i>	<i>DE</i>
<i>RKH</i>			16%			38%
<i>GAPVLIM</i>			16%			4%
<i>FYW</i>						
<i>STCNQ</i>			7%		2%	
<i>DE</i>			9%			9%

*K26*

# Correlated mutation analysis:

## Nop10:28/L7Ae:65



<i>Pyrococcus</i>		E65			
<i>I<sub>2</sub></i>	<i>RKH</i>	<i>GAPVLIM</i>	<i>FYW</i>	<i>STCNQ</i>	<i>DE</i>
<i>RKH</i>		7			16
<i>GAPVLIM</i>		7			2
<i>FYW</i>					
<i>STCNQ</i>		3		1	
<i>DE</i>		5			4
<i>K28</i>					

# Correlated mutation analysis:

## Nop10:28/L7Ae:65



<i>Pyrococcus</i>		E65				
<i>I<sub>2</sub></i>	<i>I<sup>1</sup></i>	<i>RKH</i>	<i>GAPVLIM</i>	<i>FYW</i>	<i>STCNQ</i>	<i>DE</i>
<i>RKH</i>			16%			36%
<i>GAPVLIM</i>			16%			4%
<i>FYW</i>						
<i>STCNQ</i>			7%		2%	
<i>DE</i>			11%			9%

*K28*

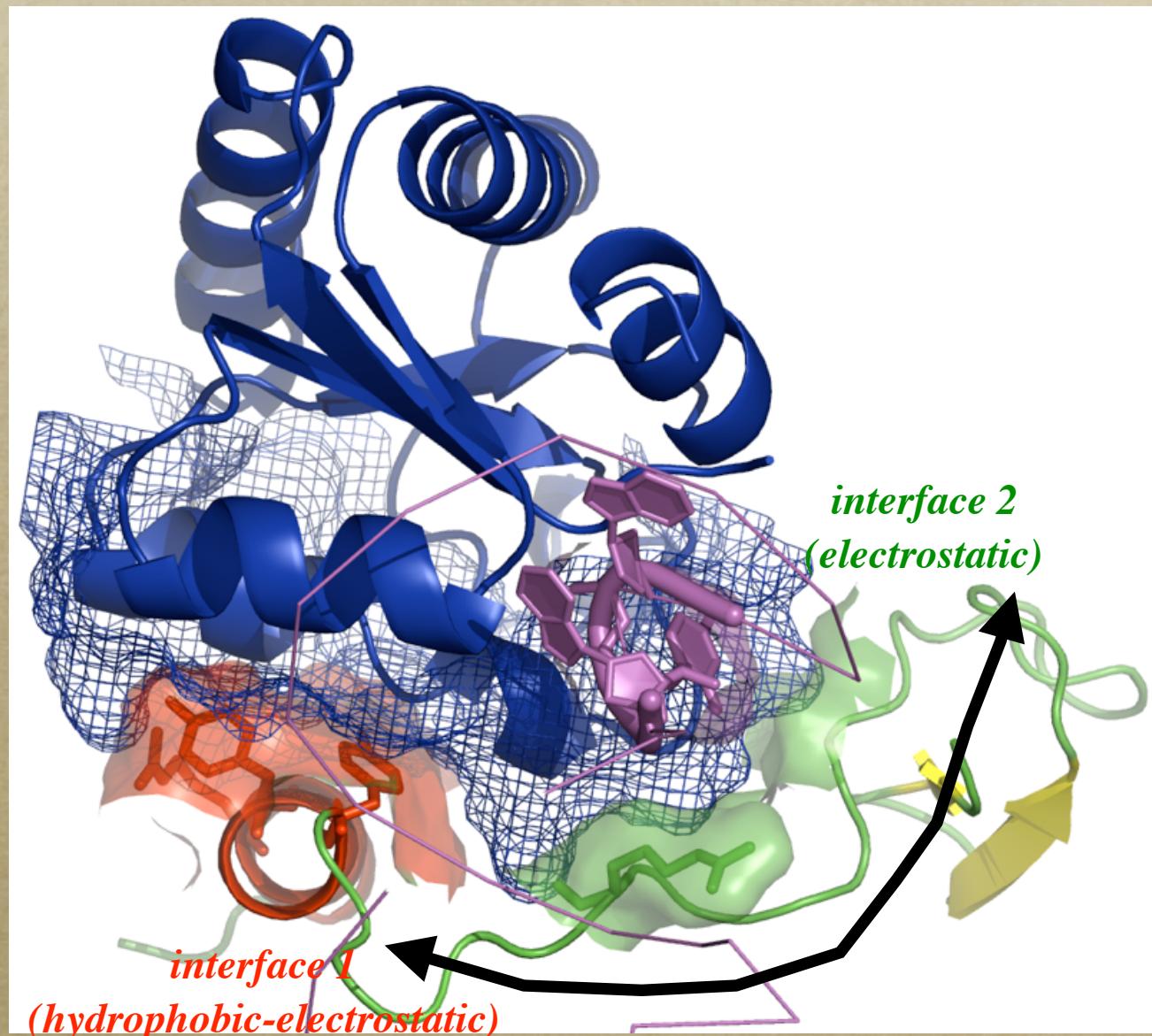
# Structure/function model: Nop10/L7Ae interface in sRNP H/ACA assembly

*Nop10*      *L7Ae*

K26 K28      E65

E	K	T	K	L	A
E	K	T	T	V	
T	K	T	K	A	
A	K	T	K	V	A
G	K	T	F	Y	P
V	V	K	T	G	V
V	E	K	S	V	T
S	K	T	M	I	P
S	K	T	M	I	P
V	E	K	A	G	T
G	K	P	F	S	P
E	R	T	H	M	P
G	R	T	I	M	A
G	R	T	G	V	I
S	H	T	T	G	T
C	E	Q	T	I	S
V	G	Q	T	T	V
S	S	Q	T	Y	I
C	E	P	T	E	T
T	T	P	T	E	A
K	P	T	T	P	
G	P	V	K	M	P
G	P	V	A	V	P
G	P	V	K	V	P
G	P	V	K	M	P

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# Conclusions & Perspectives



- *MD simulations: understand the role of particular residues and the perturbations induced by mutations;*
- *CMA: identify covariant positions corresponding to intra- or inter-molecular contacts*

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