

Part II - Applications of MultiSeq: Network Analysis of Dynamical Recognition in RNA:Protein Complexes

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NIH Center Macromolecular Modeling and Bioinformatics
LMU Workshop 2014

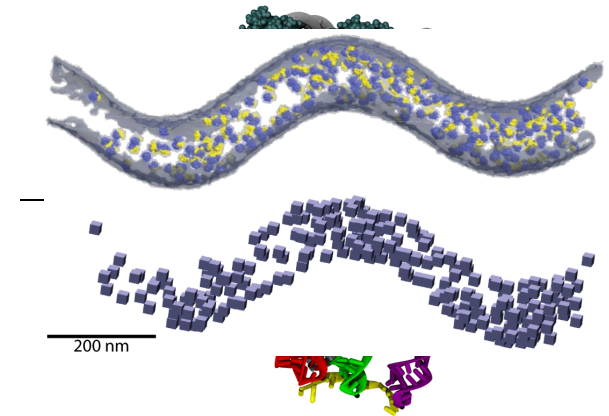
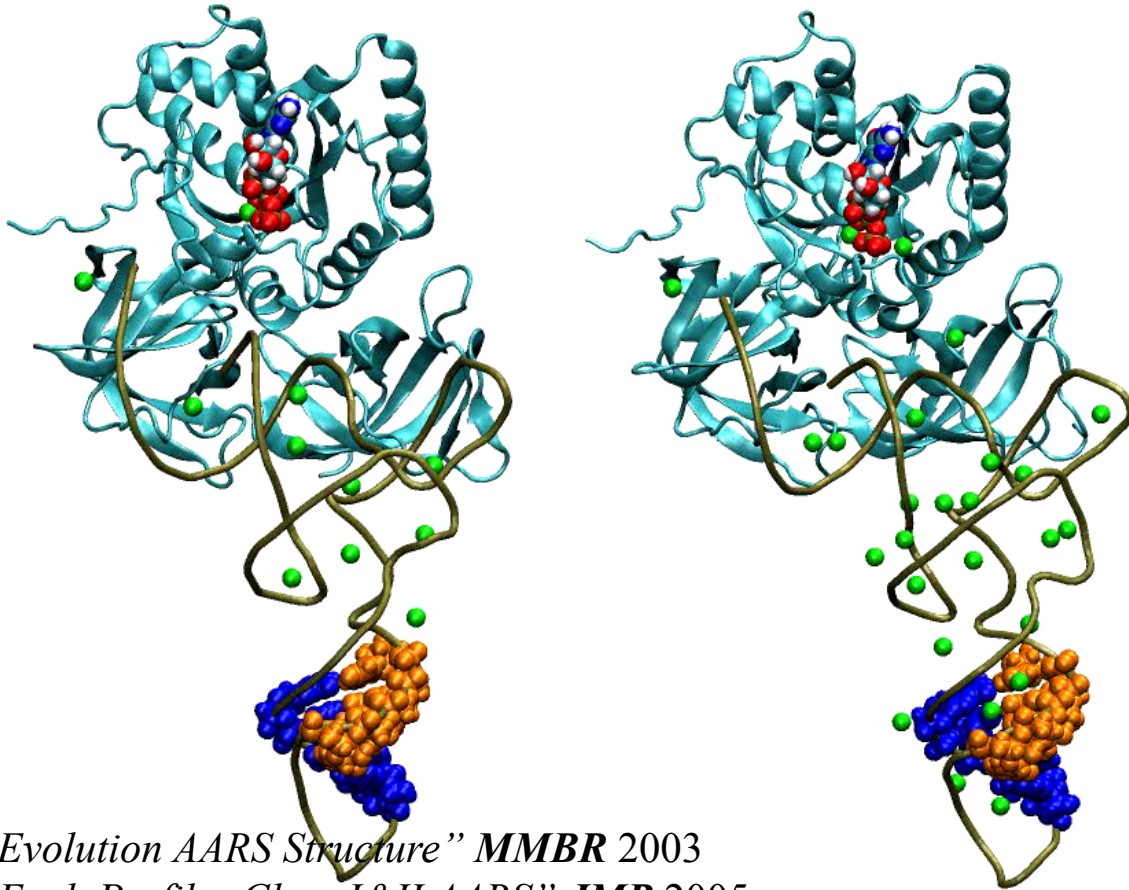


I L L I N O I S

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Protein:RNA Complexes in Translation

Evolutionary Analysis & Dynamics



“Evolution AARS Structure” *MMBR* 2003
 “Evol. Profiles Class I&II AARS” *JMB* 2005
 “Evolution SepRS/CysRS” *PNAS* 2005
 “Dynamic Signaling Network” *PNAS* 2009
 “Exit Strategy Charged tRNA” *JMB* 2010
 “Mistransl. in Mycoplasma” *PNAS* 2011
 “Capture & Selection of ATP” *JACS* 2013

“Dynamical Recognition & tRNA
 Dynamics” *JMB* 2008, *FEBS* 2010
 Network Viewer, *Bioinf.*, *JCTC* 2012

r-Proteins/r-RNA
Ribosome LSU
 “Signatures ribosomal evolution”
PNAS 2008, *BMC* 2009, *BJ* 2010
 “Motion L1 Stalk:tRNA” *JMB* 2010,
 “Ribosome Biogenesis” *JPC* 2012,3
 “Whole cell simulations on GPUs”
IEEE 2009, *Plos CB* 2011, *PRL* 2011,
JCC 2013, *PNAS* 2013,
PRL 2013, *CSB* 2013
Nature 2014

Molecular Dynamics Simulations

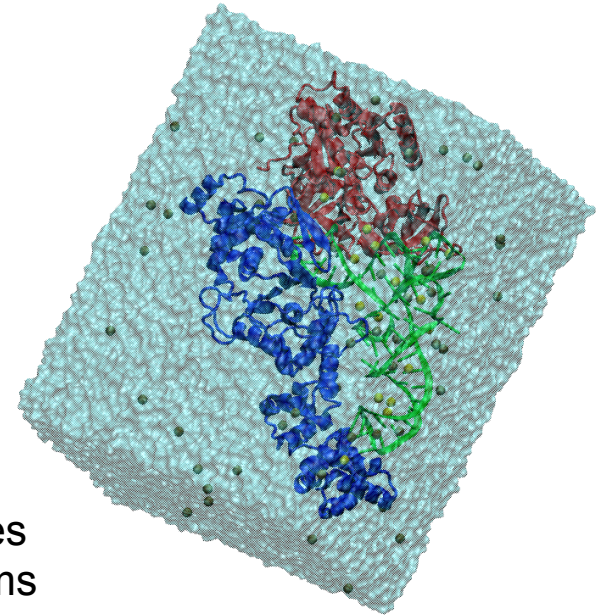
MD performed with NAMD2 (1) - System Setup

Simulation Parameters

Minimization: 290,000 steps
Production run: 108 ns
Forcefields: CHARMM27 (2),
AMBER (3)
Time step: 1 fs
VdW frequency: 2 fs
VdW cutoff: 12 Å
Switching distance: 10 Å
Pair list distance: 14 Å
Particle Mesh Ewald
Full electrostatic update: 4 fs
Ensemble: NPT
Langevin temperature: 298.15 K
Langevin pressure: 1 atm
Periodic boundary conditions

Contents of System(4)

GluRS
Glu-tRNA^{Glu}
EF-Tu
GTP
Ions: Mg²⁺, K⁺
H₂O: ~27,000 molecules
System: ~130,000 atoms

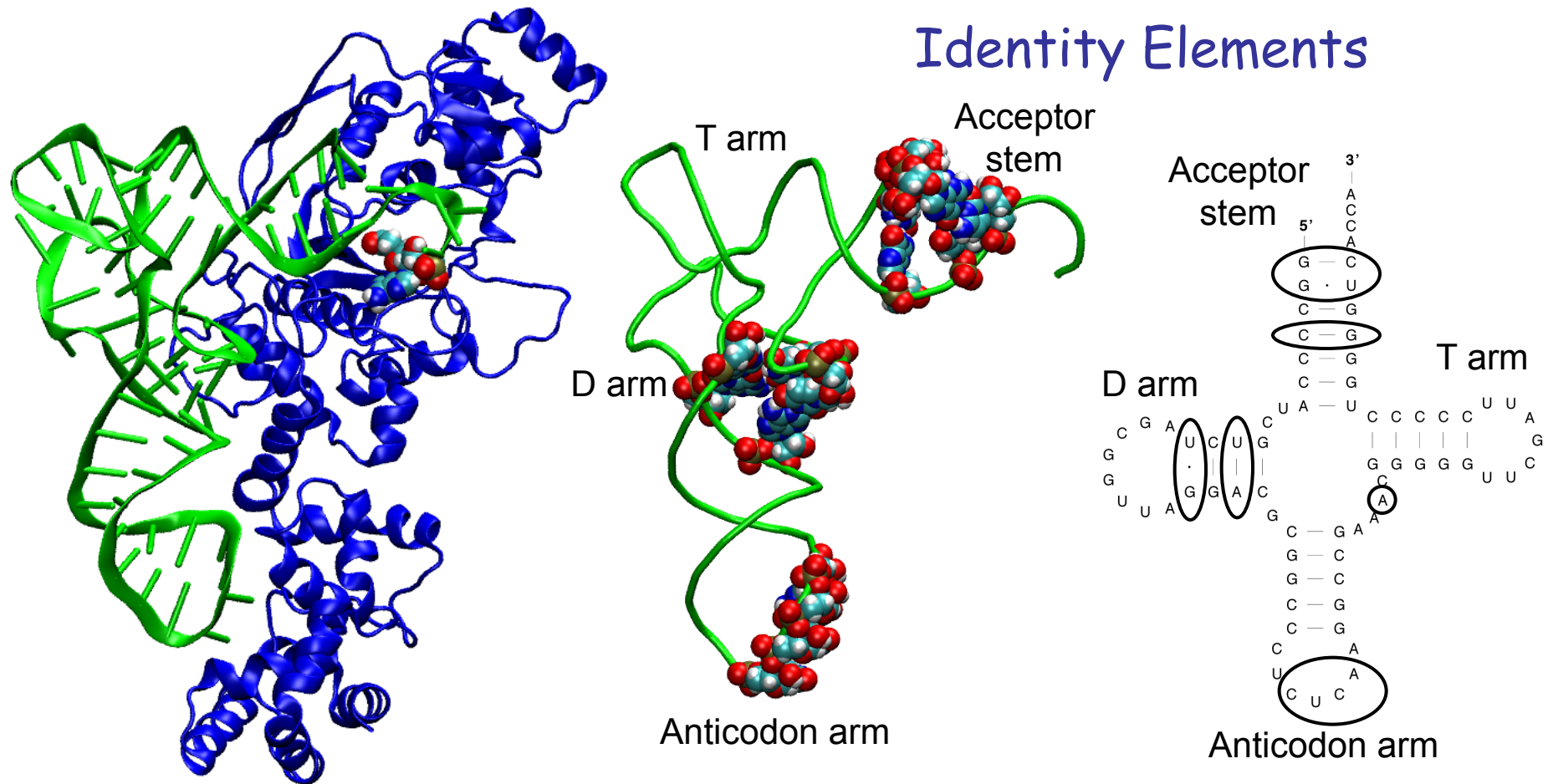


System Perturbations

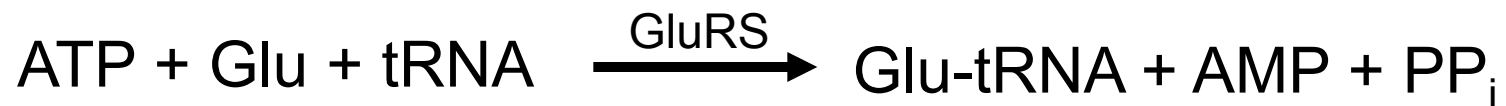
Deprotonation/protonation of reactants (aa,NTs)
Changing rotamer states of residues (5)
involved in long-lived salt bridges

- (1) Phillips, J.C. et al. *J. Comput Chem*, (2005); (2) MacKerell, A. et al. *Biopolymers* (2001);
(3) Case, D. et al. *J. Comput. Chem.* (2005); (4) Eargle, J. et al. *JMB* 2010, *FEBS Let.* 2010;
(5) Dunbrack Jr. and Cohen. *Protein Sci.* (1997)

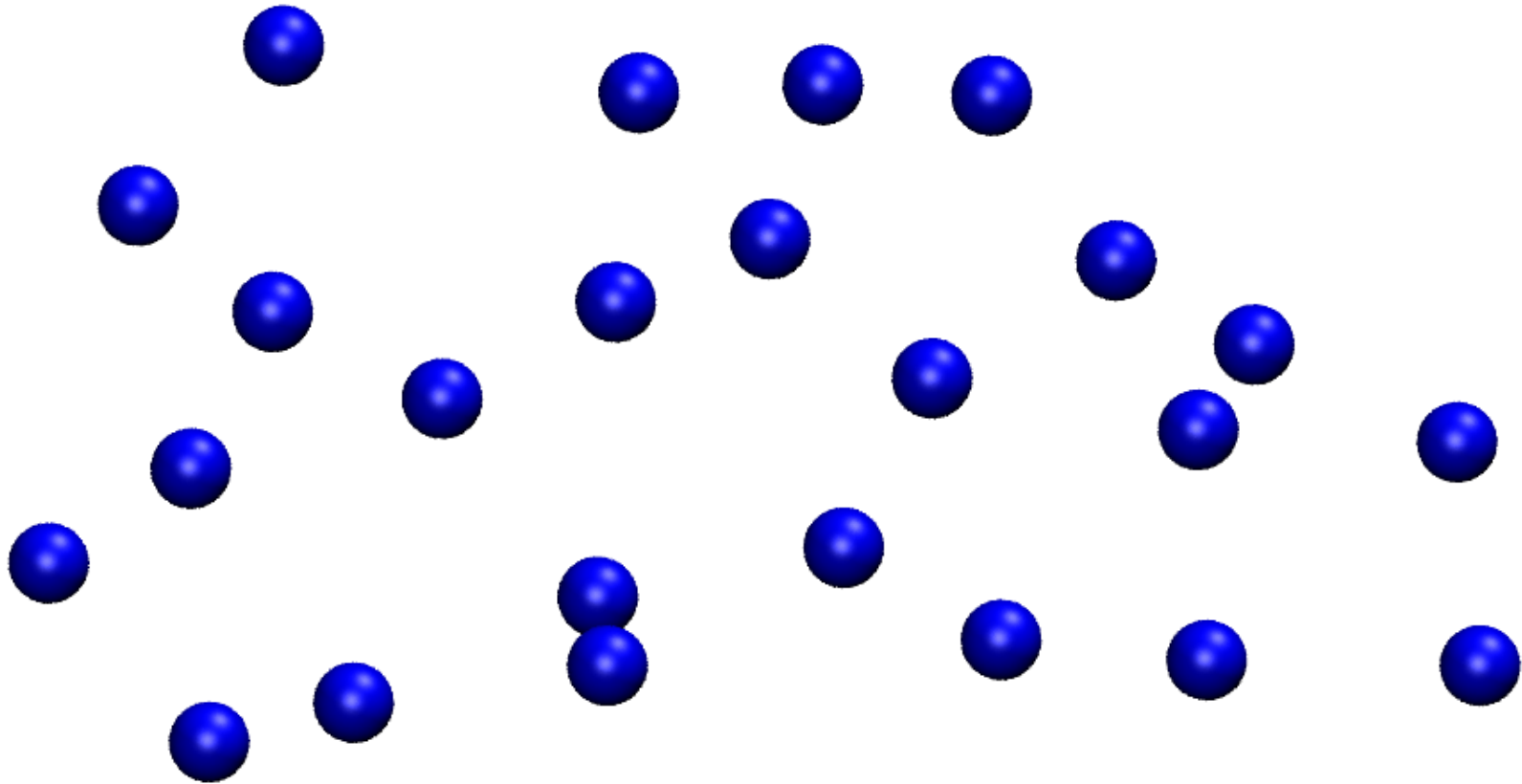
Charging tRNA through allosteric signaling



Overall Charging Reaction

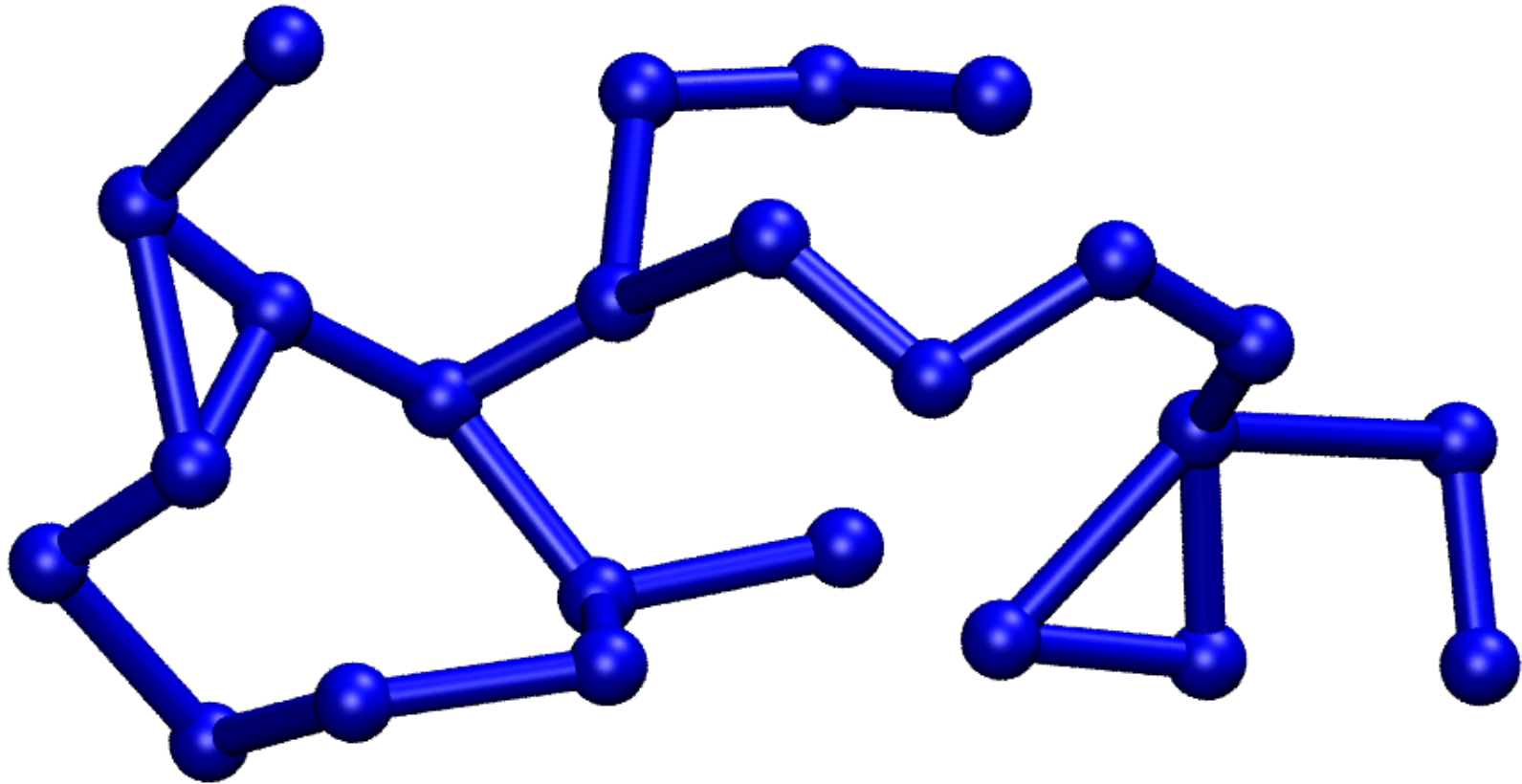


How to Construct a Network?



Nodes - defined at C _{α} (protein) and P (nucleotide) atoms

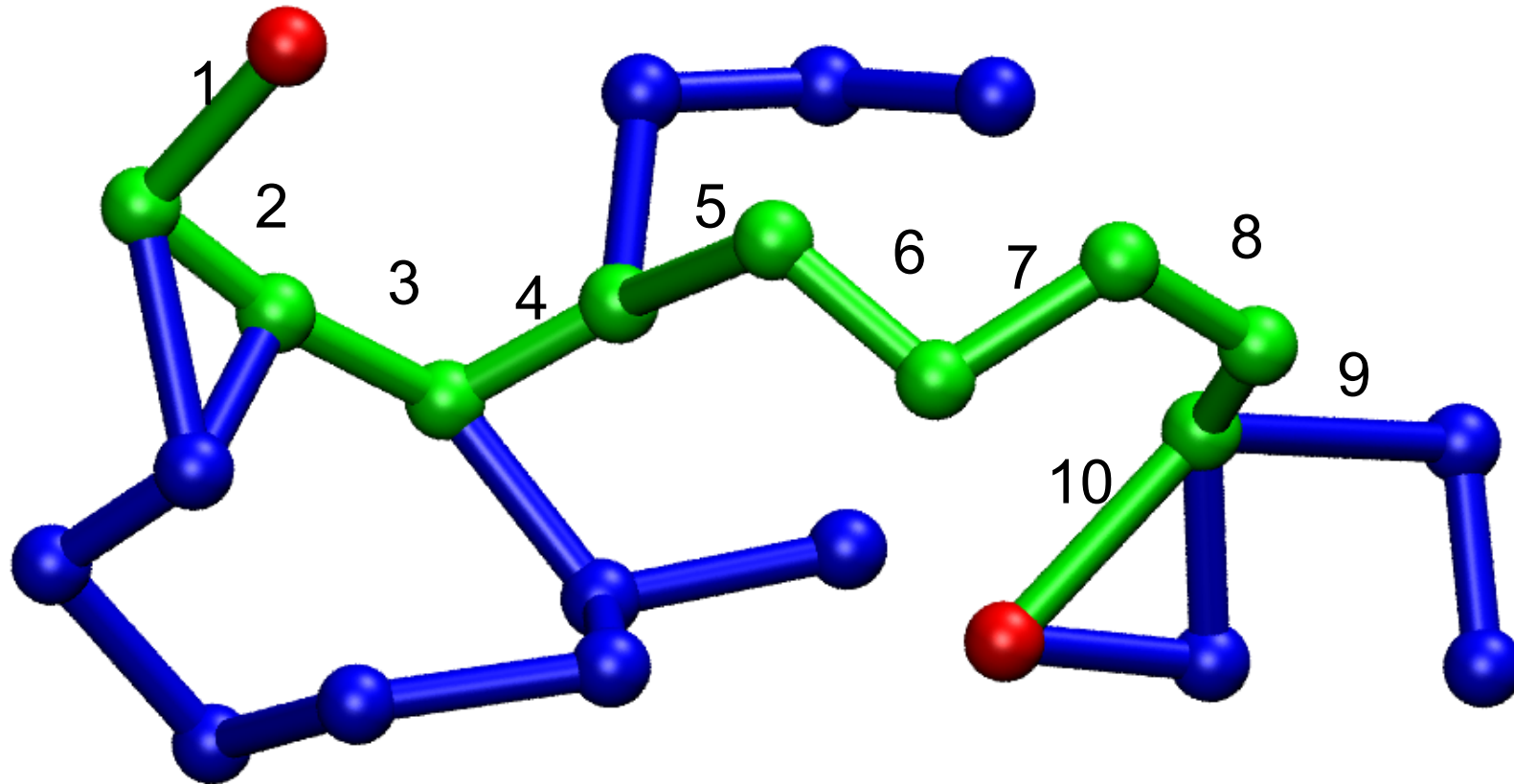
How to Construct a Network?



Edges - connect nodes that are within a contact distance threshold for more than 75% of an MD trajectory

Communication between Identity Elements and Site of Chemistry

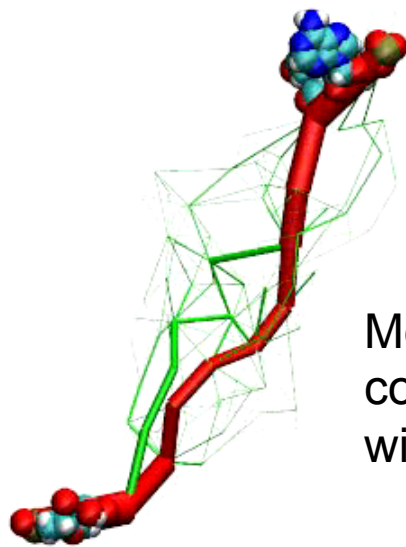
Different Paths can have Different Lengths



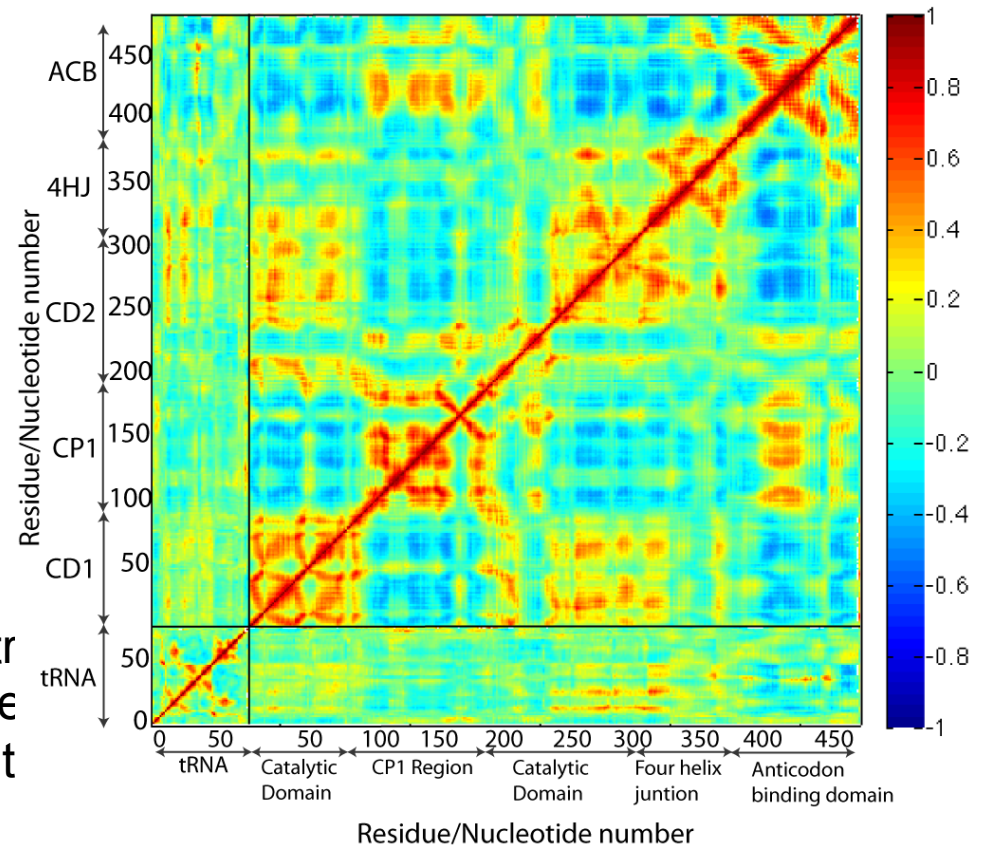
Path length = 10 (*unweighted*)

Information transfer? **Weight contacts/links by correlations !**

Correlations (C_{ij}) define signaling pathways in GluRS:tRNA

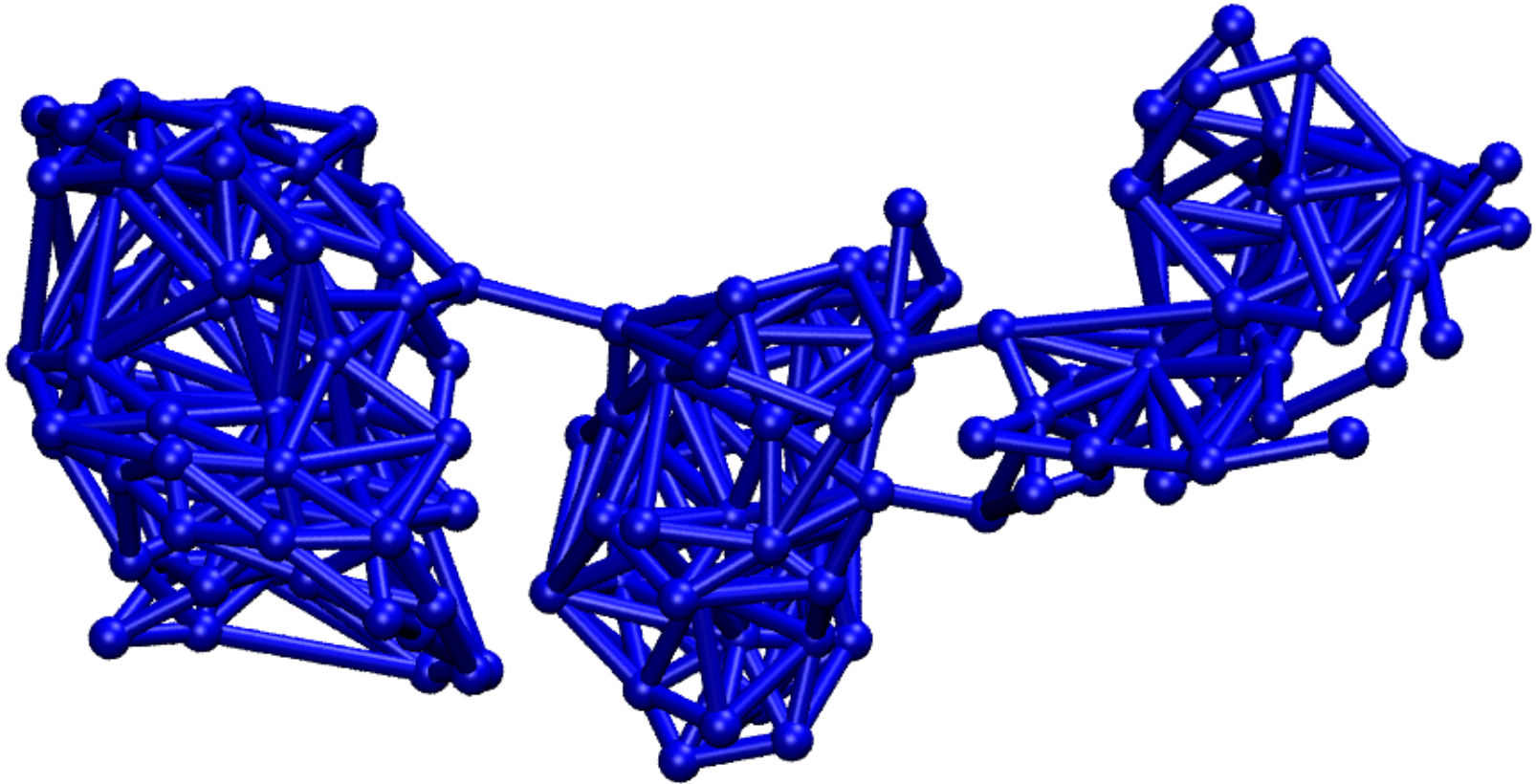


Modular str
communitie
with weight

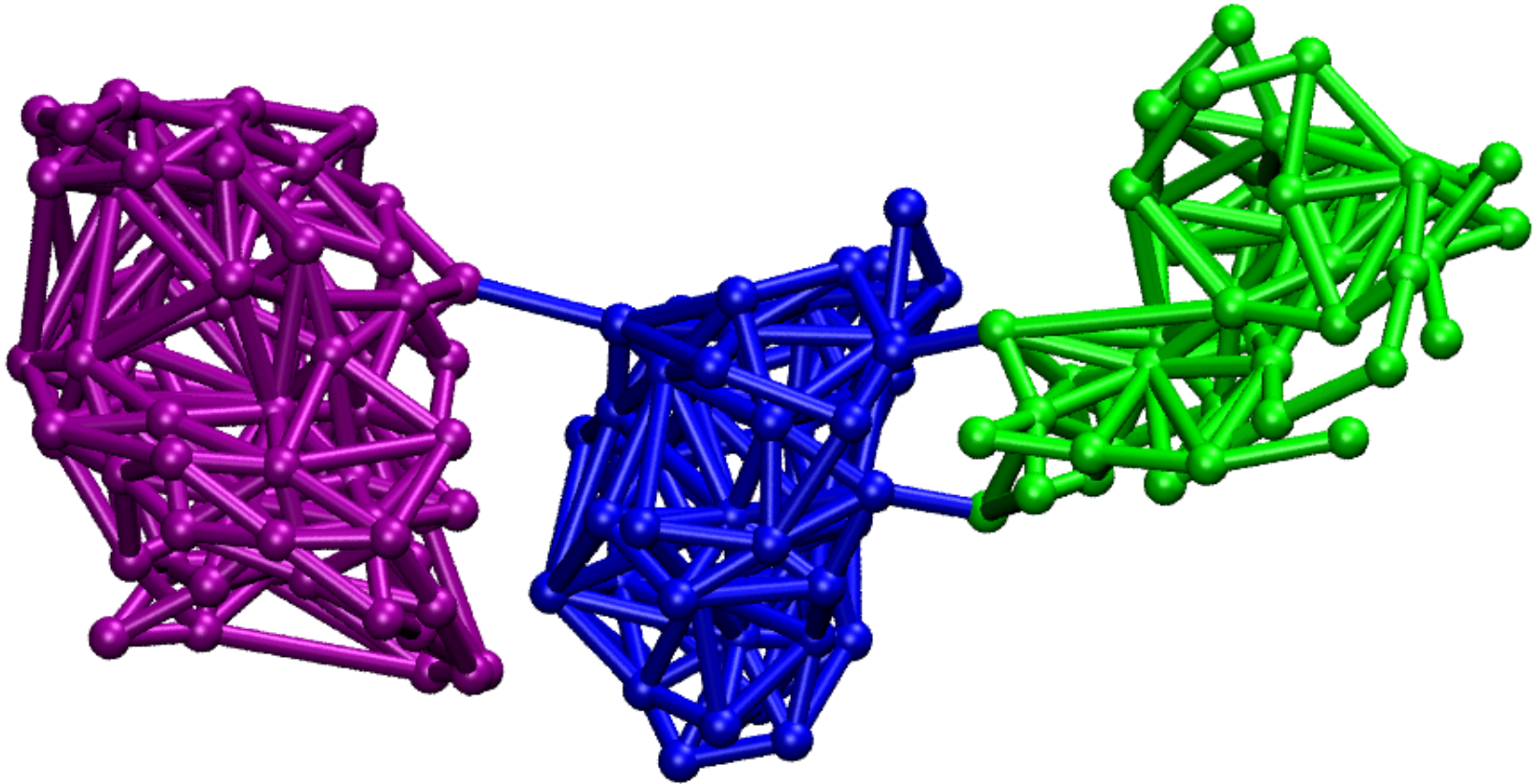


- MD simulations run with NAMD
- NTP ensemble
- Neutralized with Mg^{2+} and K^{+}
- C_{ij} values calculated over a 16-ns window

Nodes Cluster Together in Modules
called Communities

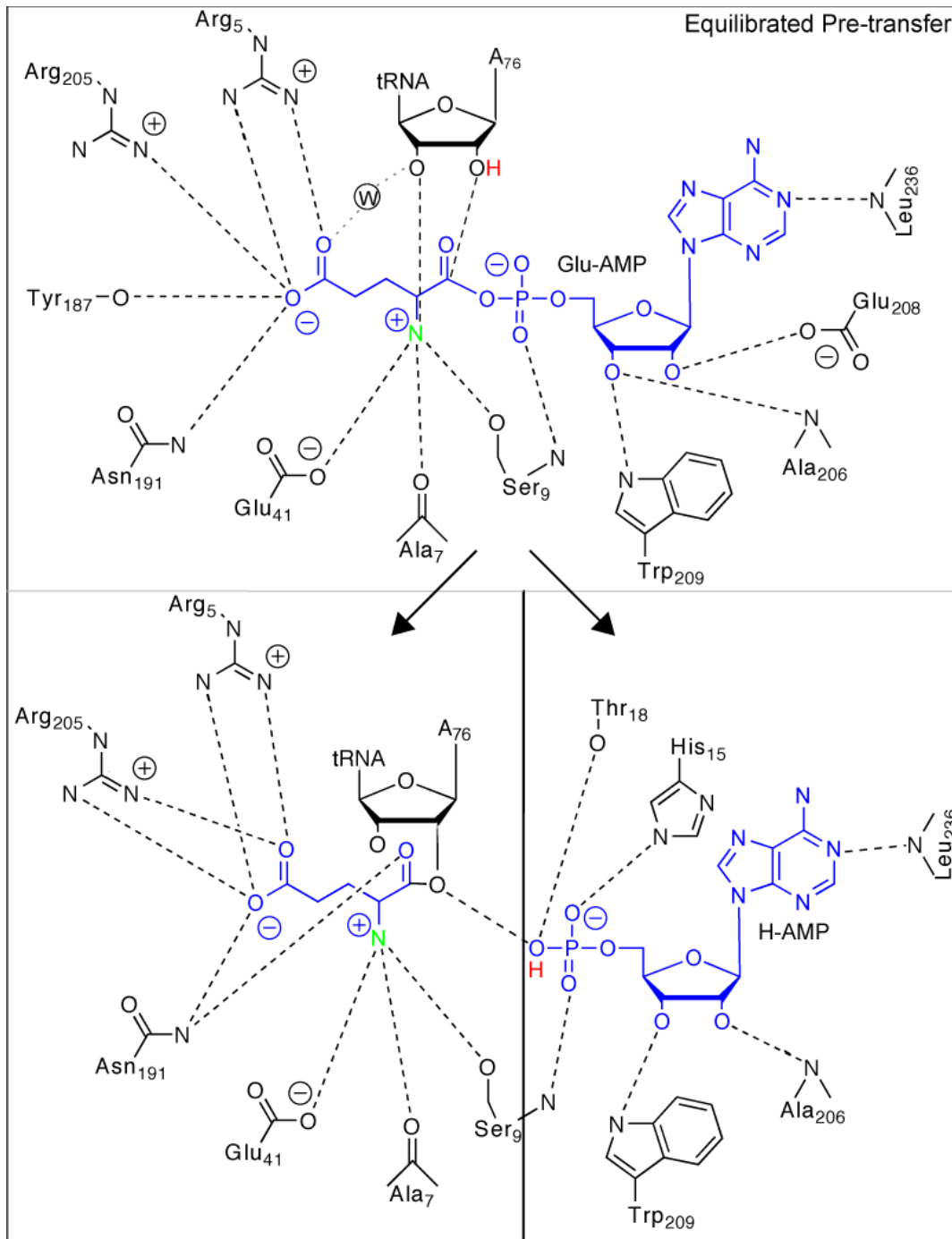


Nodes Cluster Together in Modules called Communities



Communities are subnetworks with many intracommunity edges but few intercommunity edges. (Girvan-Newman Algorithm)

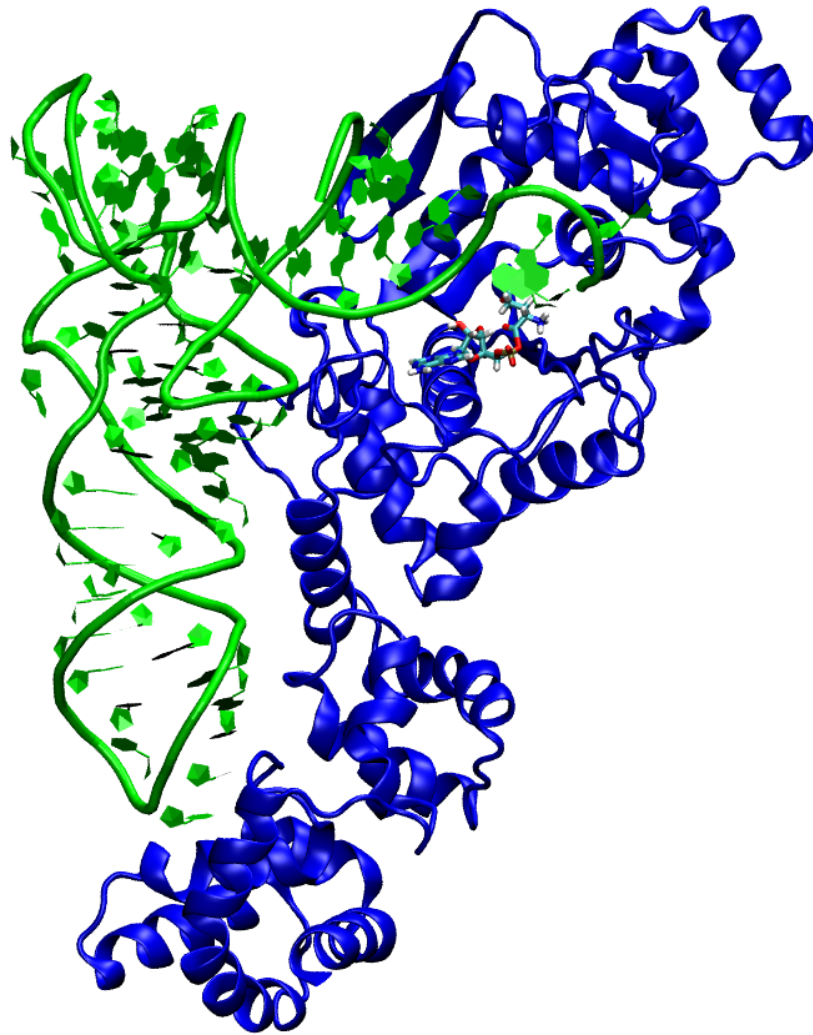
Reaction Mechanism for the Transfer of Glu to tRNA^{Glu}



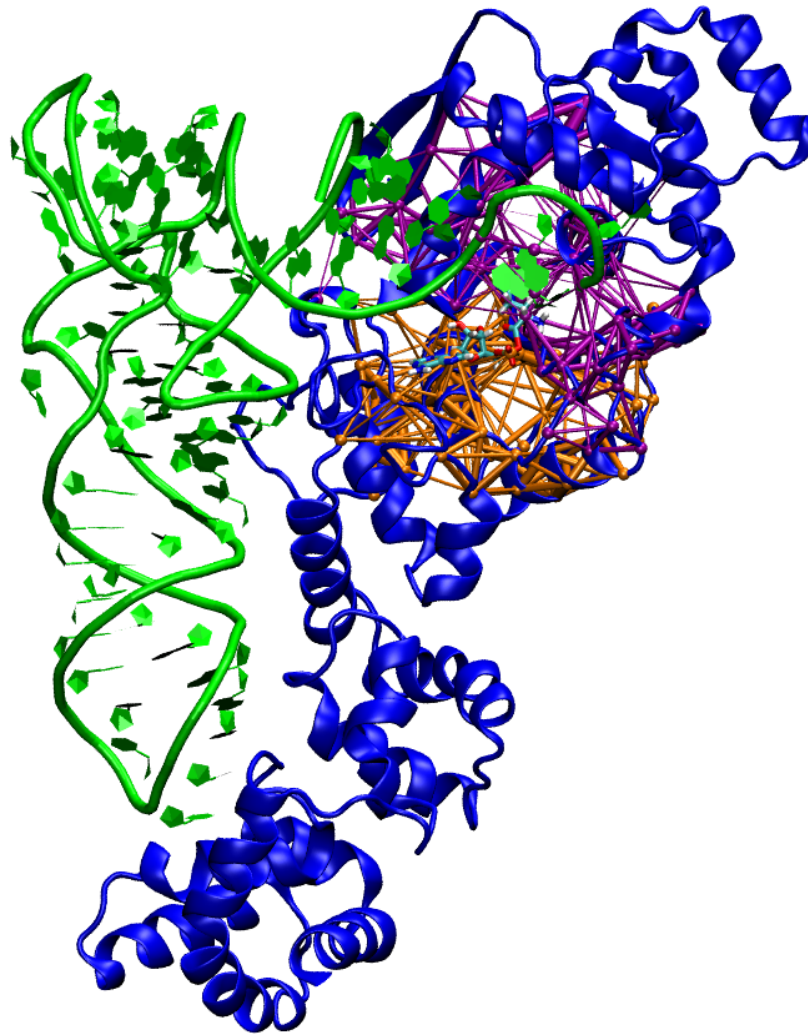
Perona JJ, Rould MA, Steitz TA
Biochemistry 1993

Black A, Eargle J, Sethi A,
Luthey-Schulten Z. *JMB* 2010
100s ns MD simulations

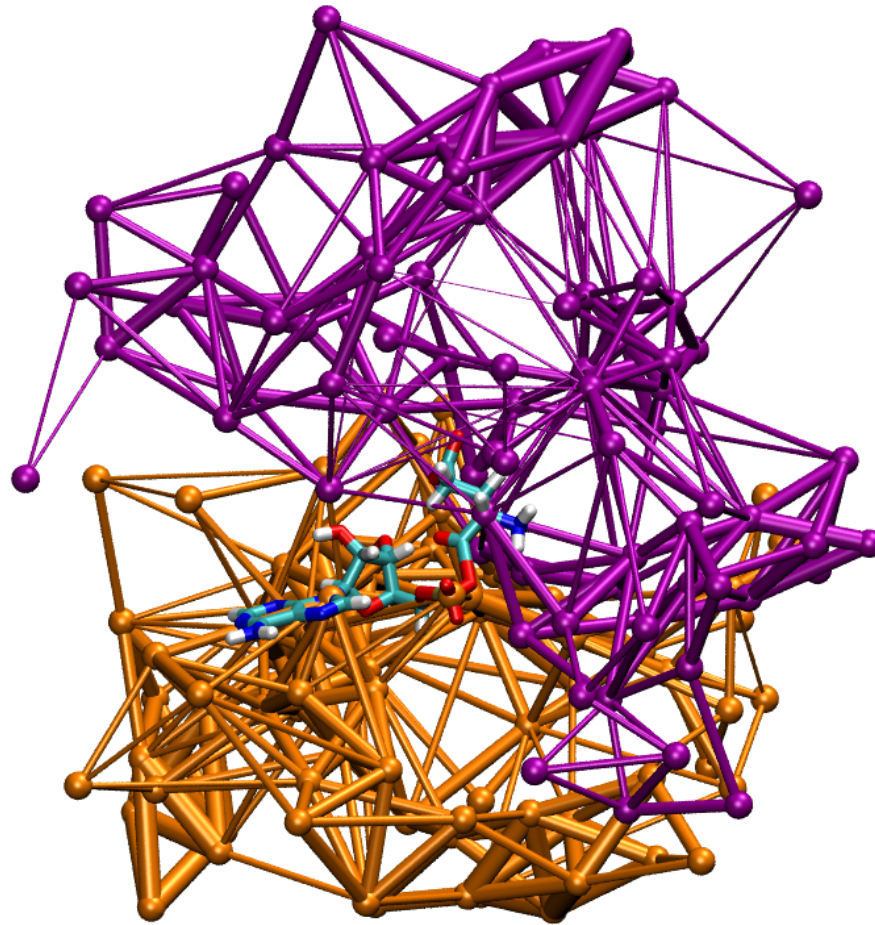
Community Structure Reveals Function in GluRS



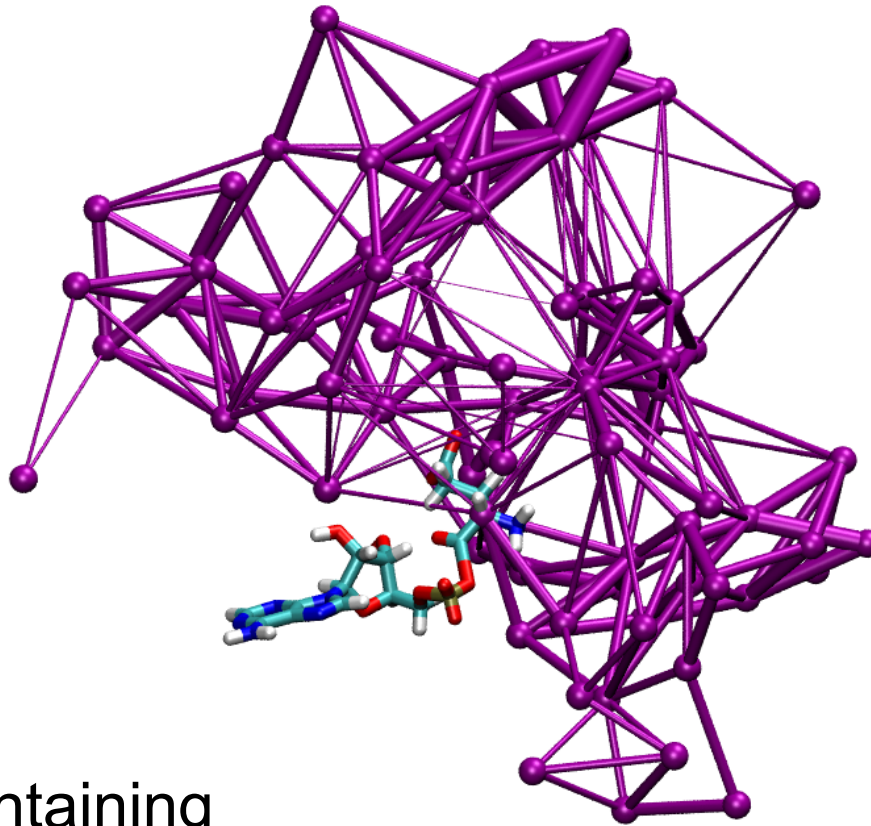
Community Structure Reveals Function in GluRS



Community Structure Reveals Function in GluRS



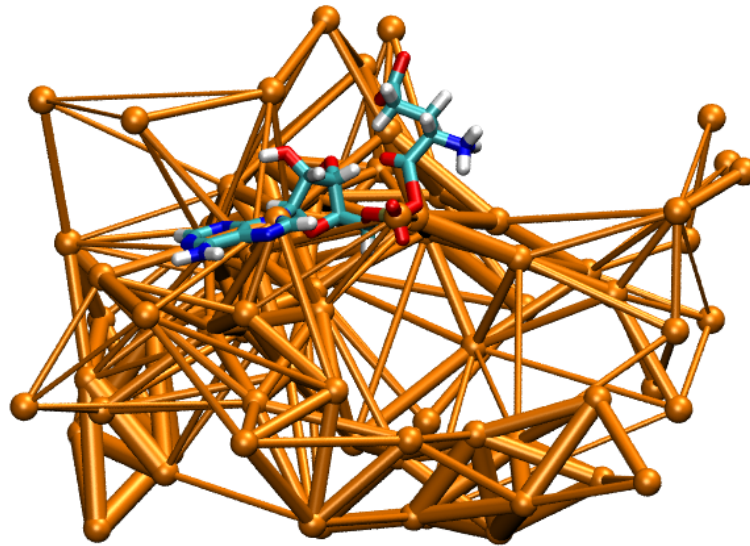
Community Structure Reveals Function in GluRS



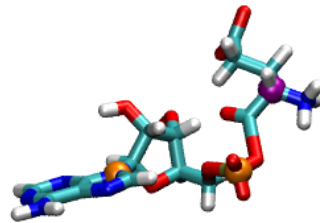
community containing
amino acid moiety

Community Structure Reveals Function in GluRS

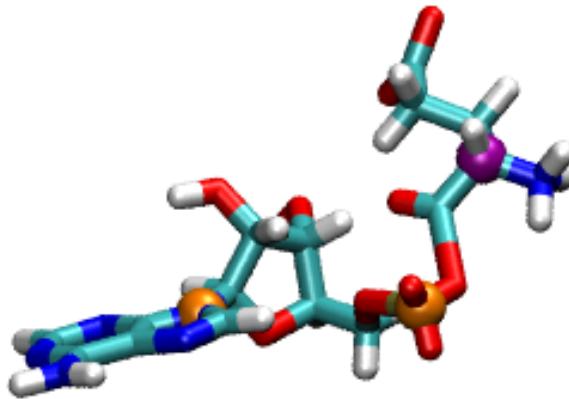
community containing
AMP moiety



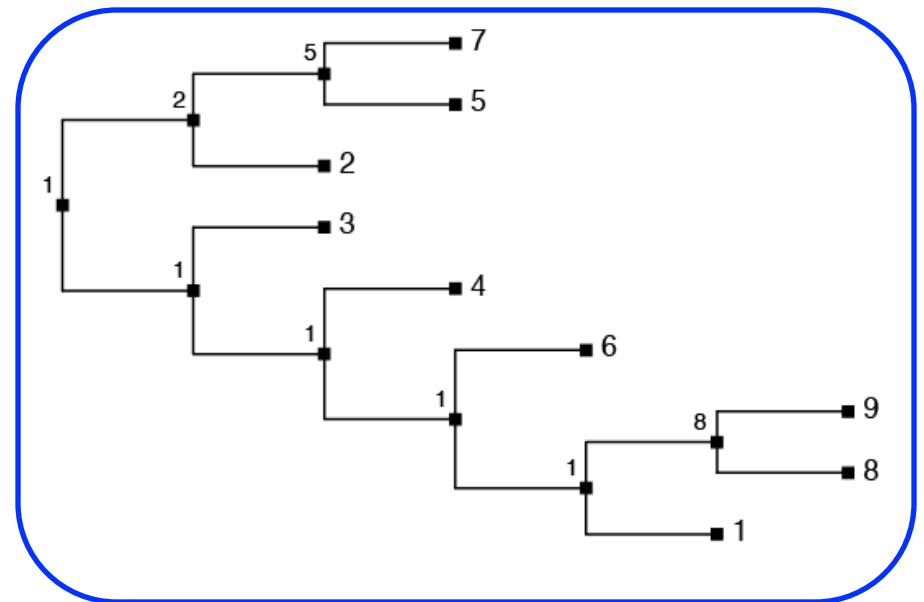
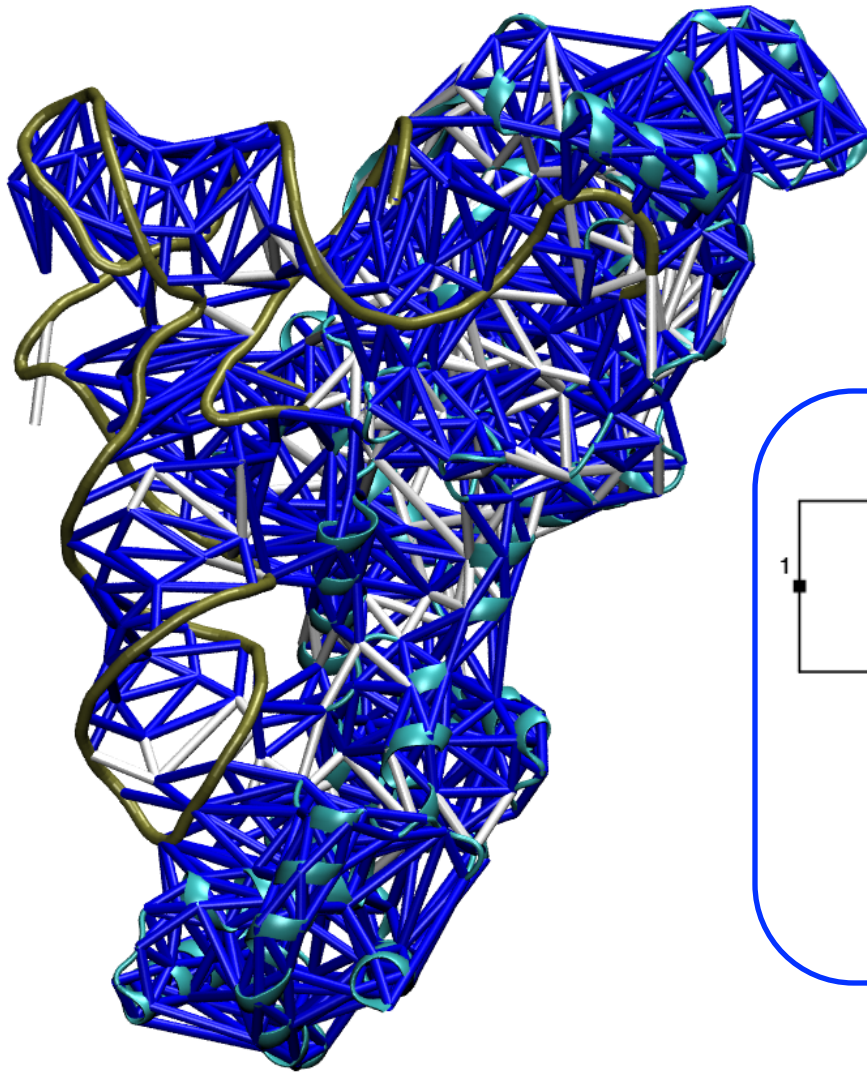
Community Structure Reveals Function in GluRS



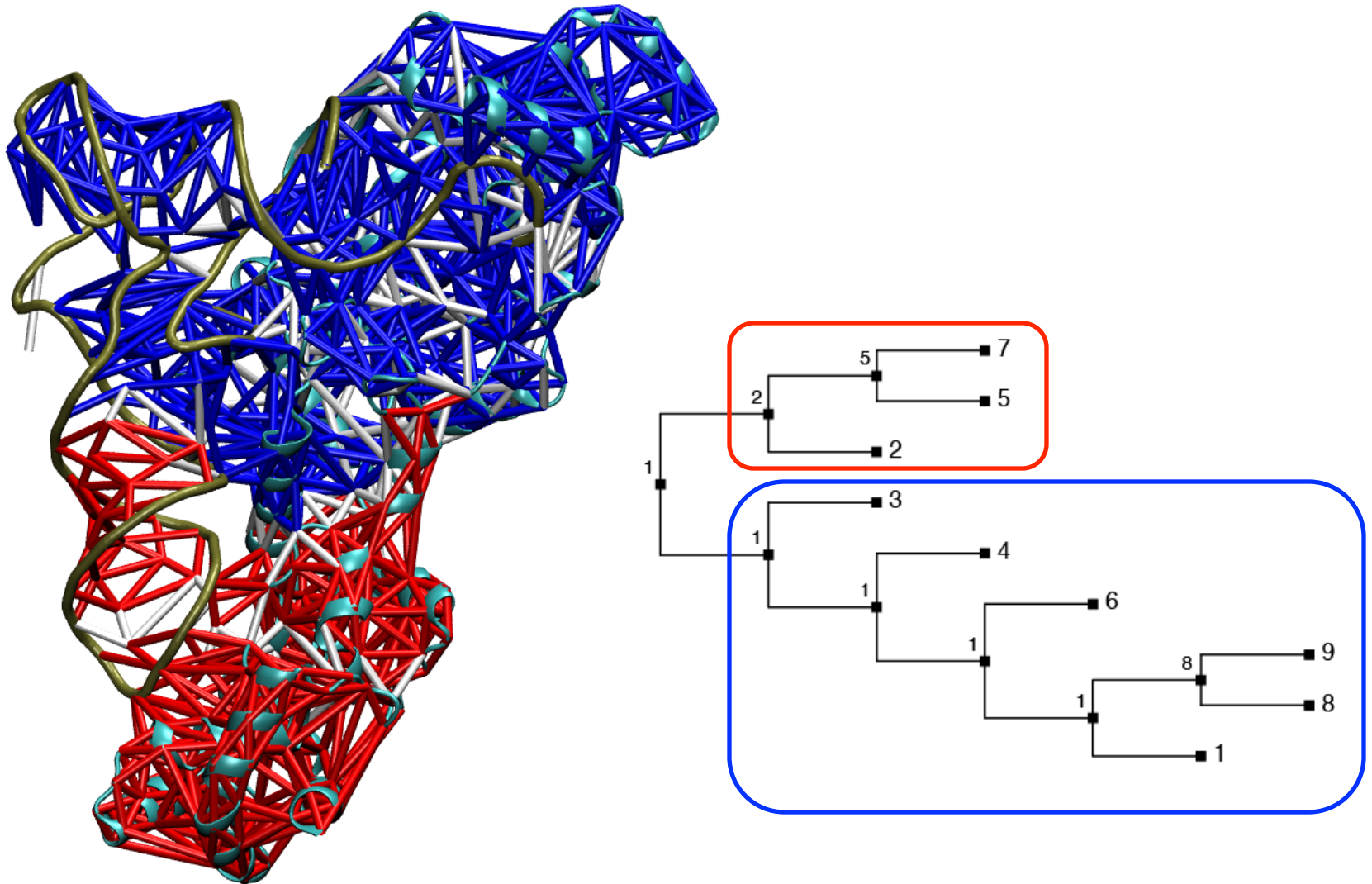
Community Structure Reveals Function in GluRS



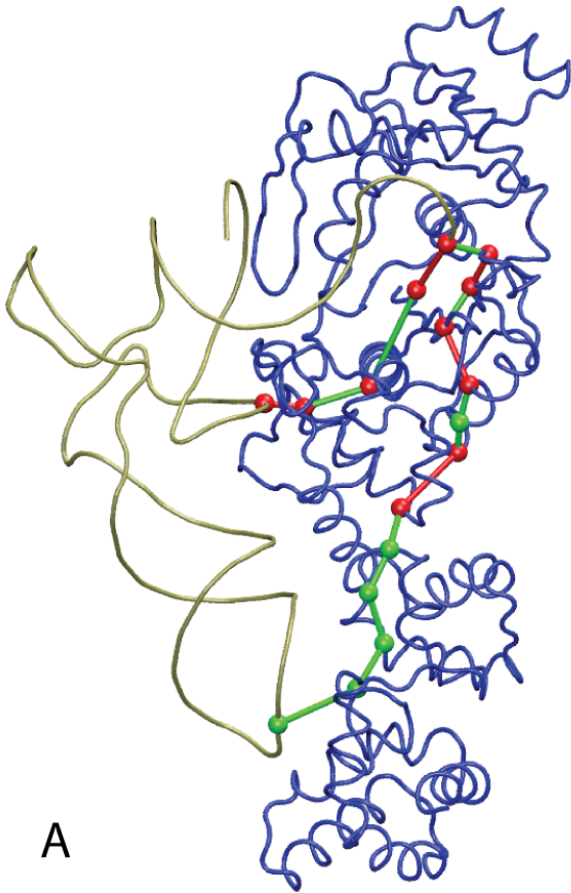
Communities Partition the Interaction Network Hierarchically



Communities Partition the Interaction Network Hierarchically



Dynamical Networks, Conservation, and Betweenness



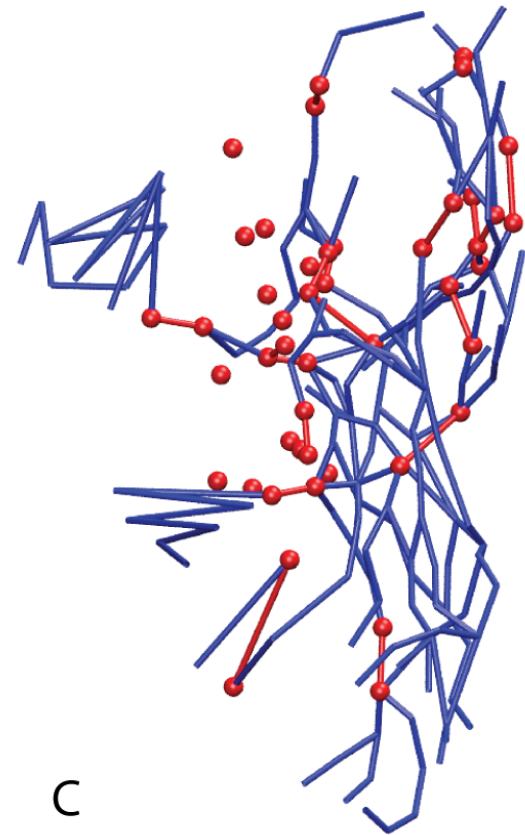
A

Optimal signal pathways:
U13, U35 to A76



B

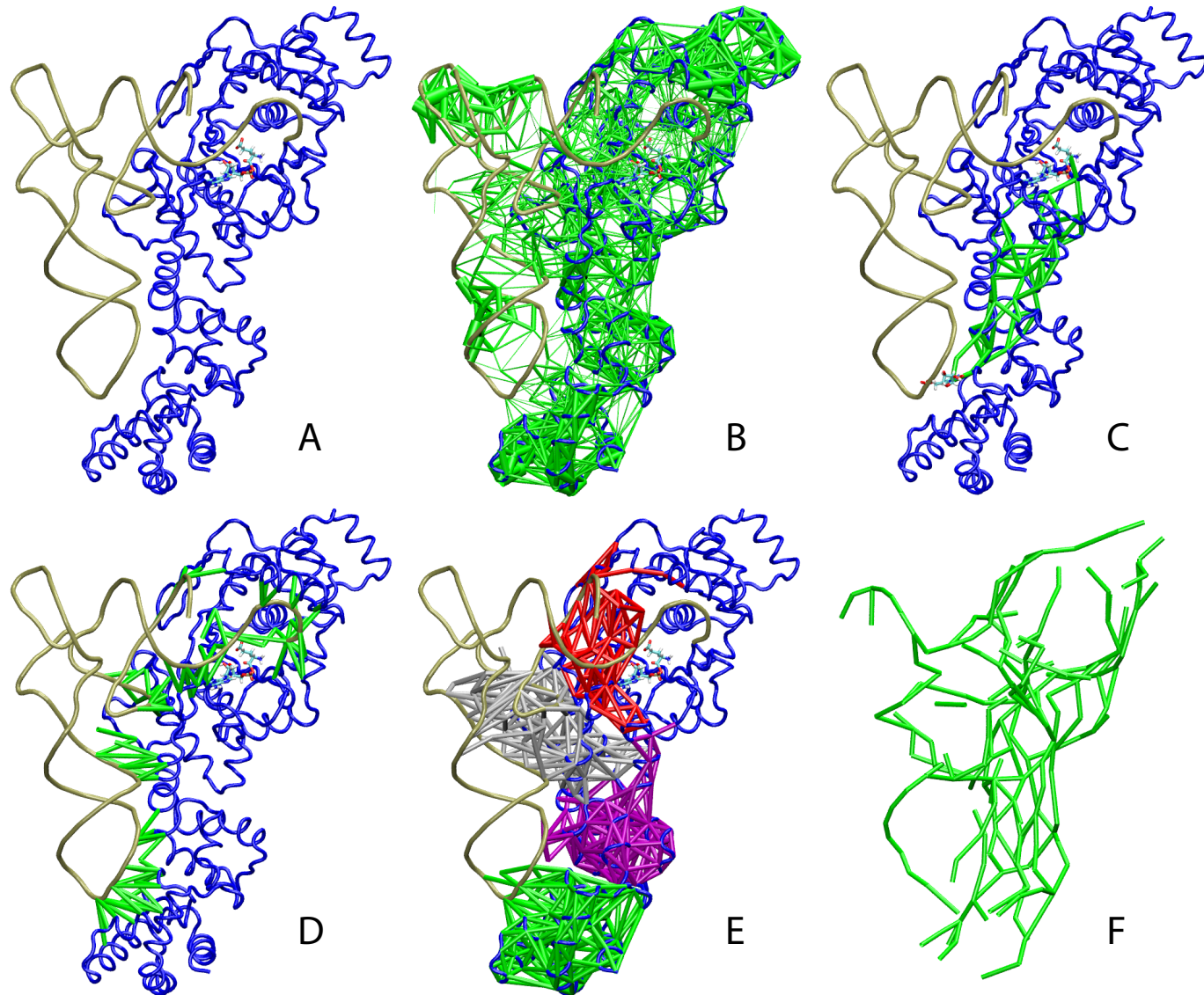
Critical(conserved) nodes
connecting communities



C

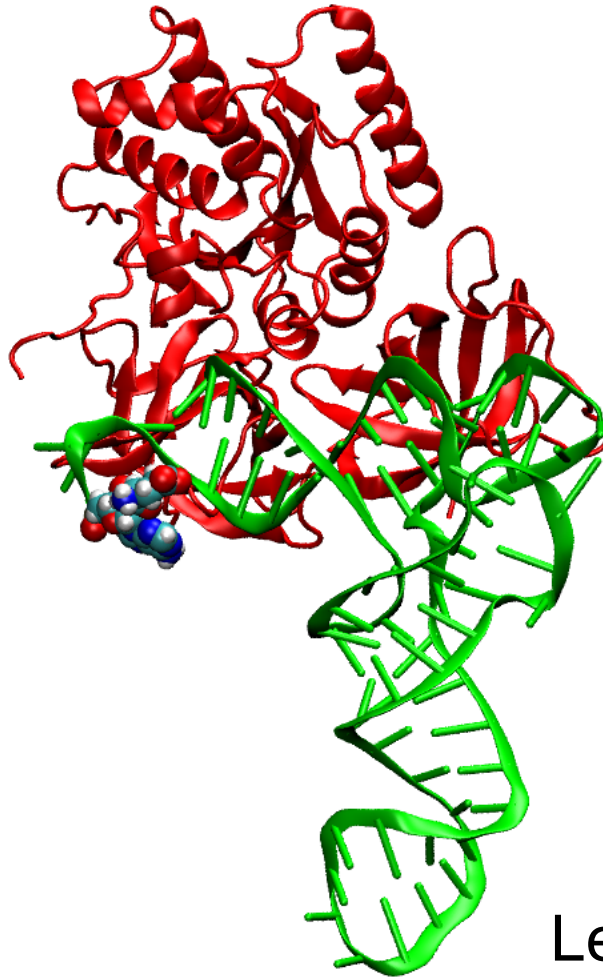
Betweenness routes - highest
density pair optimal paths

Network Viewer in VMD*



* J. Eargle and Z. Luthey-Schulten, Bioinformatics, 2012, **28**, 3000–3001

Changing Networks: tRNA Migration from GluRS to EF-Tu



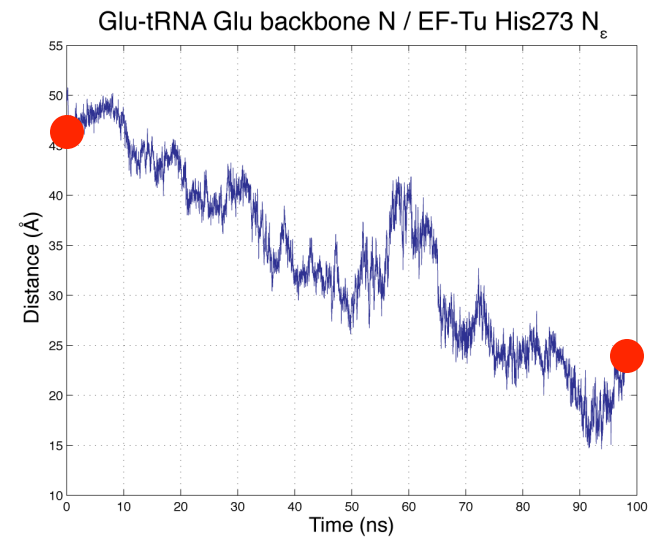
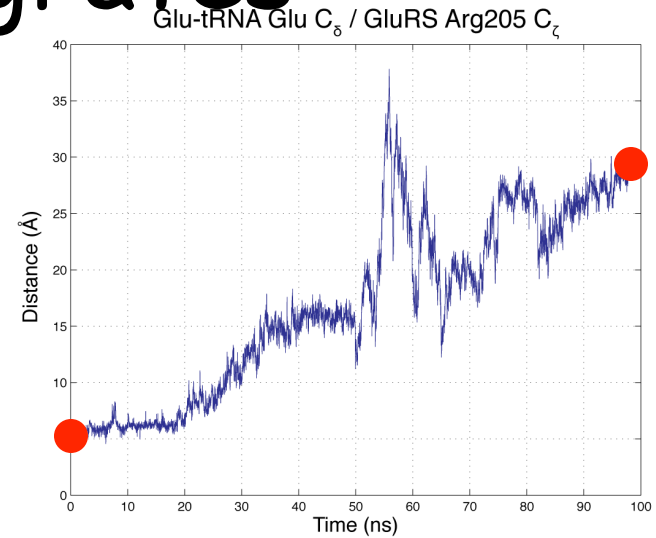
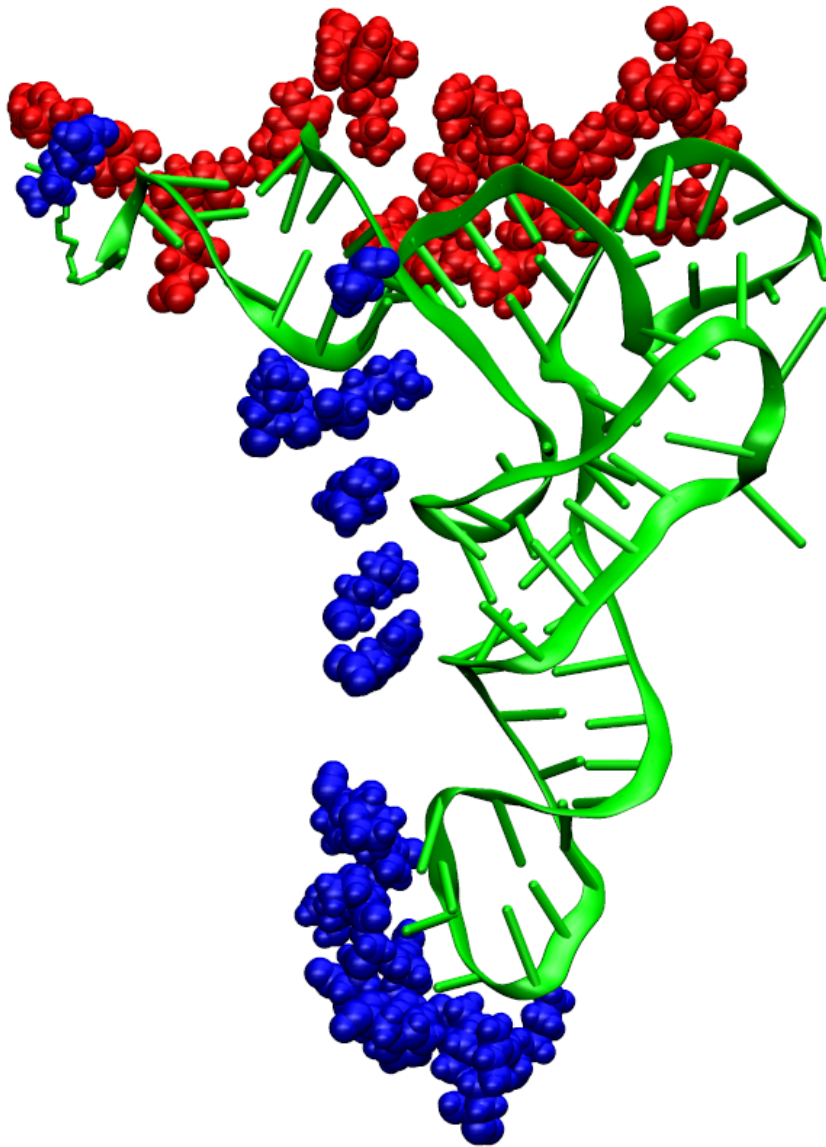
>2-fold increase
in k_{cat} for
CysRS:tRNA:EF-Tu

8-fold increase
in k_{cat} for
LeuRS:tRNA:EF-Tu

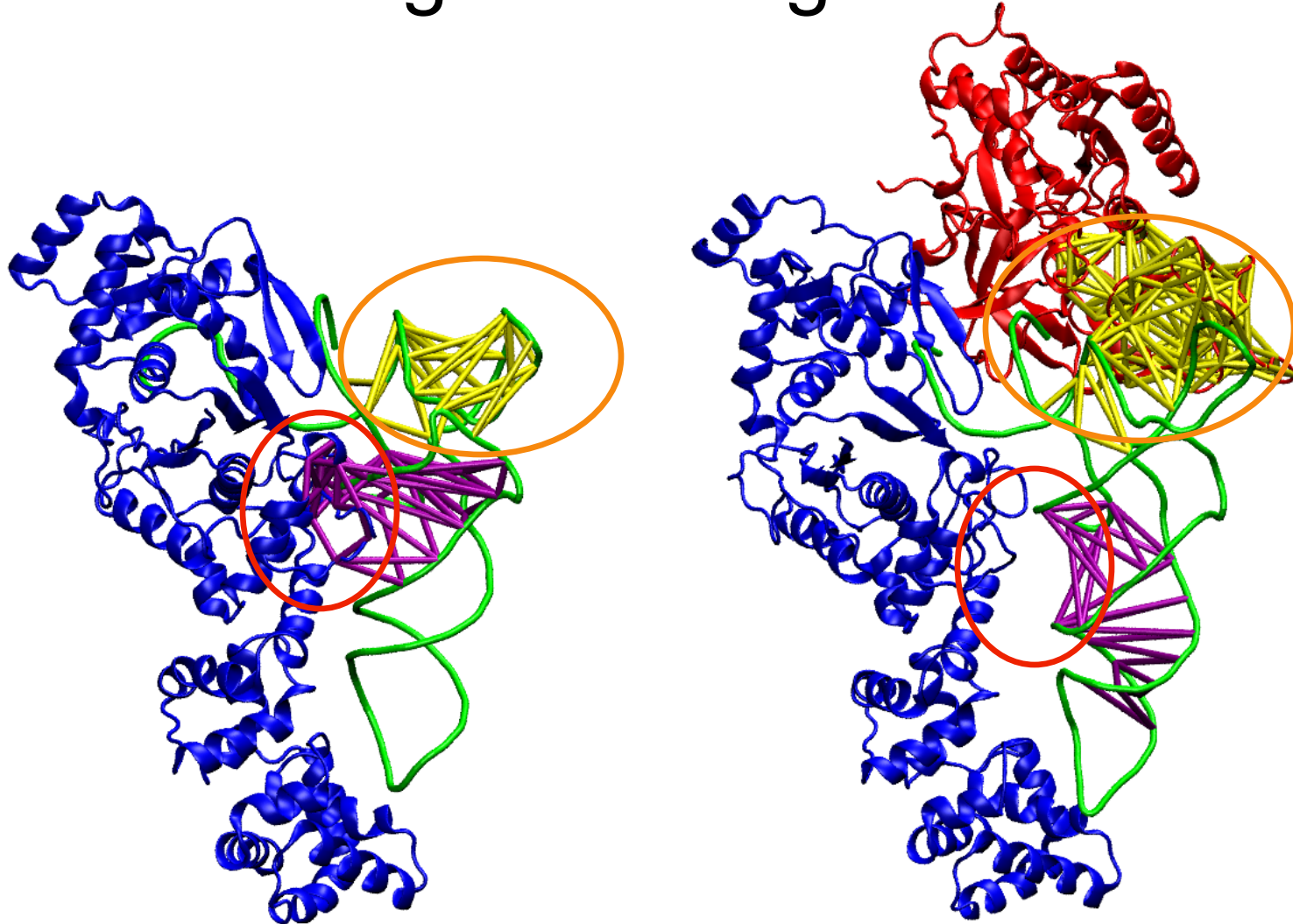
Zhang C, Perona J, Kang R,
Francklyn C, Hou Y. *JMB* 2006

Hausmann C, Praetorius-Ibba M,
Ibba M. *NAR* 2007

Change in Protein:RNA Contacts as tRNA Migrates



Change in Protein:RNA Contacts During tRNA Migration

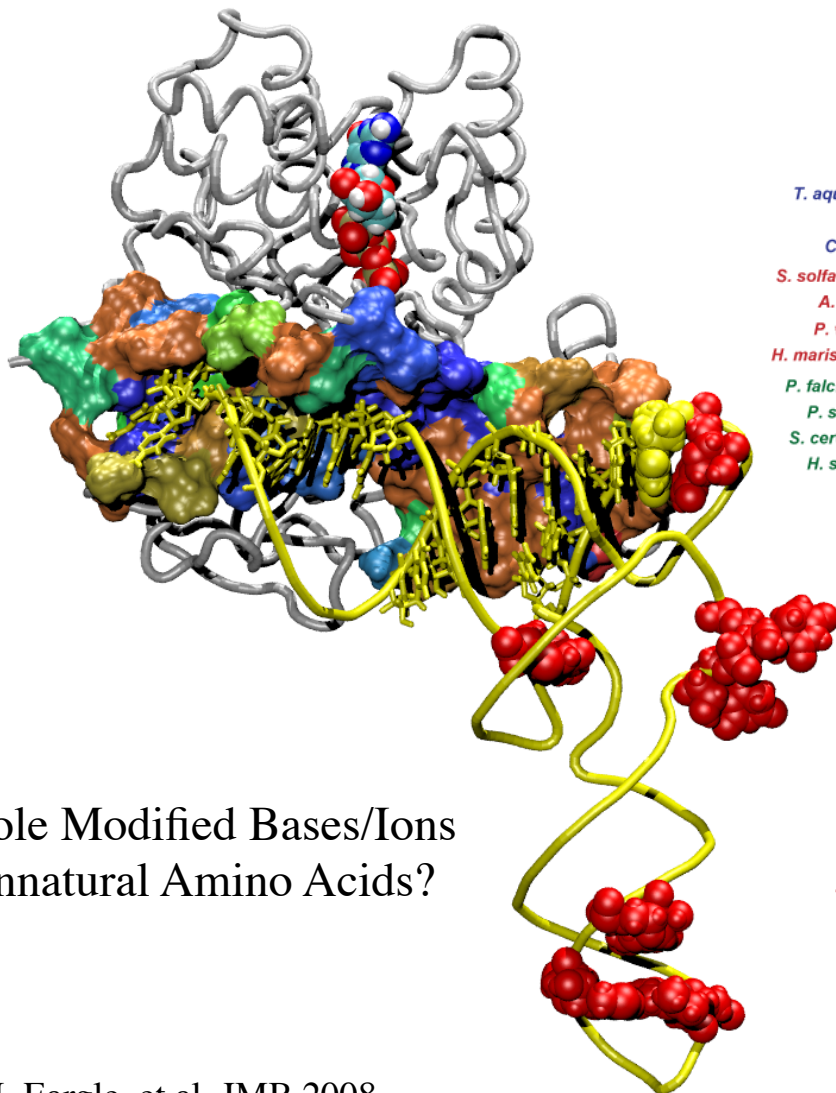


FEBS 2012

purple - D arm community

yellow - T arm community

Evolution of Protein/RNA Interfaces: Dynamics of EF-Tu/tRNA Recognition



	Switch I							Switch II							Domain II																			
	50	+	+	*	57			85	*	●	*	●	92		224	*																	240	
<i>T. aquaticus</i>	I	D	K	A	P	E	E	R	H	A	D	Y	I	K	N	M	P	V	E	D	V	F	T	I	T	G	R	G	T	V	A	T	G	B
<i>E. coli</i>	I	D	N	A	P	E	E	K	H	A	D	Y	V	K	N	M	P	I	E	D	V	F	S	I	S	G	R	G	T	V	V	T	G	
<i>C. jejuni</i>	I	D	N	A	P	E	E	K	H	A	D	Y	V	K	N	M	P	I	E	D	V	F	S	I	S	G	R	G	T	V	V	T	G	
<i>S. solfataricus</i>	L	D	R	L	K	E	E	R	H	R	D	F	V	K	N	M	P	I	Q	D	V	Y	S	I	S	G	V	G	T	V	P	V	G	A
<i>A. pernix</i>	L	D	K	M	K	E	E	R	H	R	D	F	V	K	N	M	P	V	Q	N	V	Y	S	I	P	G	A	G	T	V	P	V	G	
<i>P. woesei</i>	M	D	R	L	R	E	E	R	H	R	D	F	V	K	N	M	P	I	Q	D	V	Y	S	I	K	G	V	G	T	V	P	V	G	
<i>H. marismortui</i>	M	D	N	L	A	E	E	R	H	R	D	F	V	K	N	M	P	I	Q	D	V	Y	T	I	S	G	I	G	T	V	P	V	G	E
<i>P. falciparum</i>	L	D	K	L	K	A	E	R	H	K	D	F	I	K	N	M	P	L	Q	G	V	Y	K	I	G	G	I	G	T	V	P	V	G	
<i>P. sativum</i>	L	D	K	L	K	A	E	R	H	R	D	F	I	K	N	M	P	L	Q	D	V	Y	K	I	G	G	I	G	T	V	P	V	G	
<i>S. cerevisiae</i>	L	D	K	L	K	A	E	R	H	R	D	F	I	K	N	M	P	L	Q	D	V	Y	K	I	G	G	I	G	T	V	P	V	G	
<i>H. sapiens</i>	L	D	K	L	K	A	E	R	H	R	D	F	I	K	N	M	P	L	Q	D	V	Y	K	I	G	G	I	G	T	V	P	V	G	

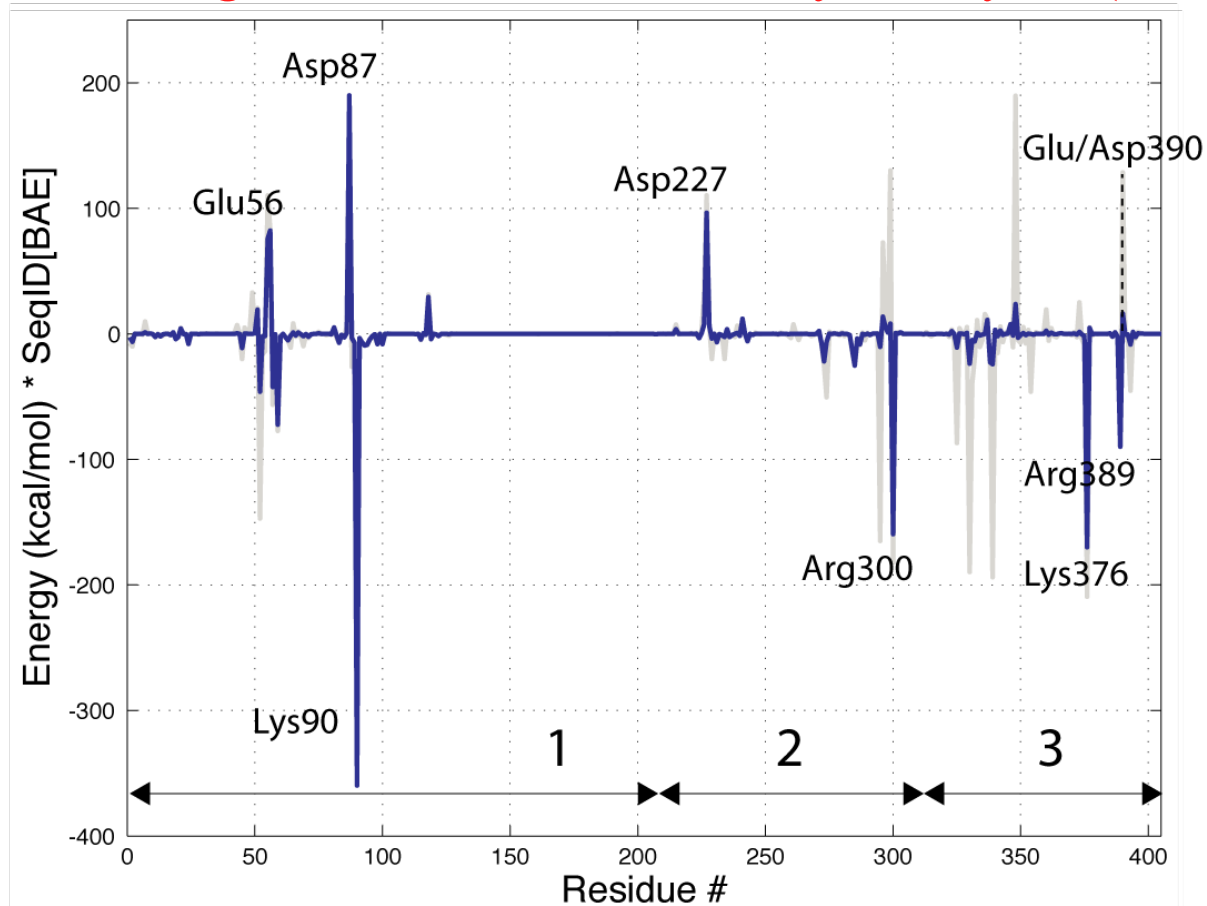
	Domain II																																
	271																													302			
<i>T. aquaticus</i>	E	M	H	R	K	T	L	Q	E	G	I	A	G	D	N	V	G	L	L	L	R	G	V	S	R	E	E	V	E	R	G	Q	B
<i>E. coli</i>	E	M	F	R	K	L	L	D	E	G	R	A	G	E	N	V	G	V	L	L	R	G	I	K	R	E	E	I	E	R	G	Q	
<i>C. jejuni</i>	E	M	F	R	K	E	M	D	Q	G	E	A	G	D	N	V	G	V	L	L	R	G	T	K	K	E	E	V	I	R	G	M	
<i>S. solfataricus</i>	E	T	H	H	T	K	M	D	K	A	E	P	G	D	N	I	G	F	N	V	R	G	V	E	K	K	D	I	K	R	G	D	A
<i>A. pernix</i>	E	M	H	Y	Q	Q	L	Q	A	E	P	G	D	N	I	G	F	A	V	R	G	V	S	K	S	D	I	K	R	G	D		
<i>P. woesei</i>	E	M	H	H	E	P	L	E	E	A	L	P	G	D	N	I	G	F	N	V	R	G	V	S	K	N	D	I	K	R	G	D	
<i>H. marismortui</i>	E	M	H	H	E	E	V	P	K	A	E	P	G	D	N	V	G	F	N	V	R	G	V	G	K	D	D	I	R	R	G	D	E
<i>P. falciparum</i>	E	M	H	K	E	V	L	E	E	A	R	P	G	D	N	I	G	F	N	V	K	N	V	S	V	K	E	I	K	R	G	Y	
<i>P. sativum</i>	E	M	H	H	E	A	L	T	E	A	L	P	G	D	N	V	R	F	N	V	K	N	V	A	V	K	D	L	K	H	G	L	
<i>S. cerevisiae</i>	E	M	H	H	E	Q	L	E	Q	G	V	P	G	D	N	V	G	F	N	V	K	N	V	S	V	K	E	I	R	R	G	N	E
<i>H. sapiens</i>	E	M	H	H	E	A	L	S	E	A	L	P	G	D	N	V	G	F	N	V	K	N	V	S	V	K	D	V	R	R	G	N	

Role Modified Bases/Ions
Unnatural Amino Acids?

Dynamical Recognition EF-Tu/tRNA (E.coli)

Combining MD with Evolutionary Analysis (MultiSeq)

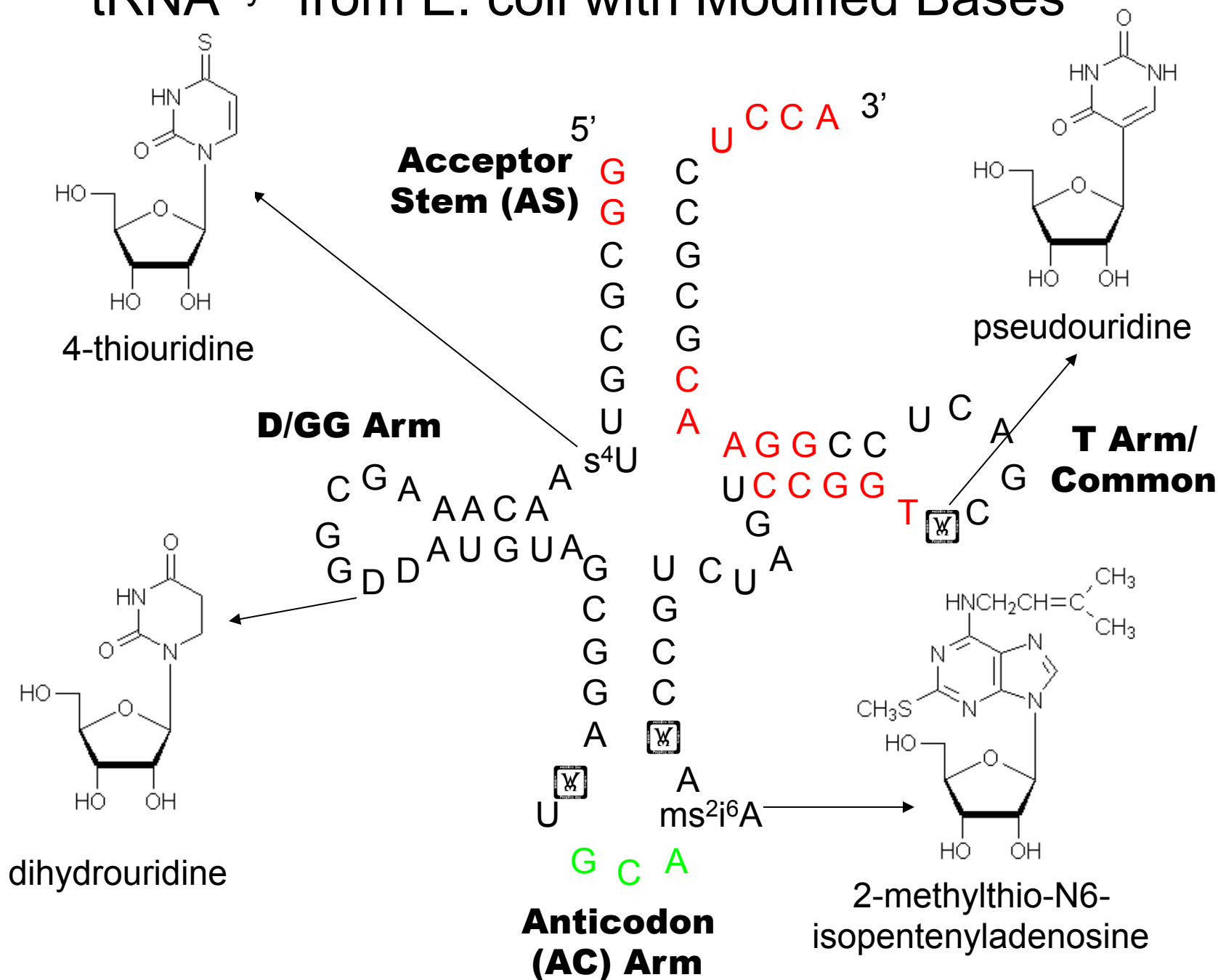
Non-bonded Interaction Energy



BAE

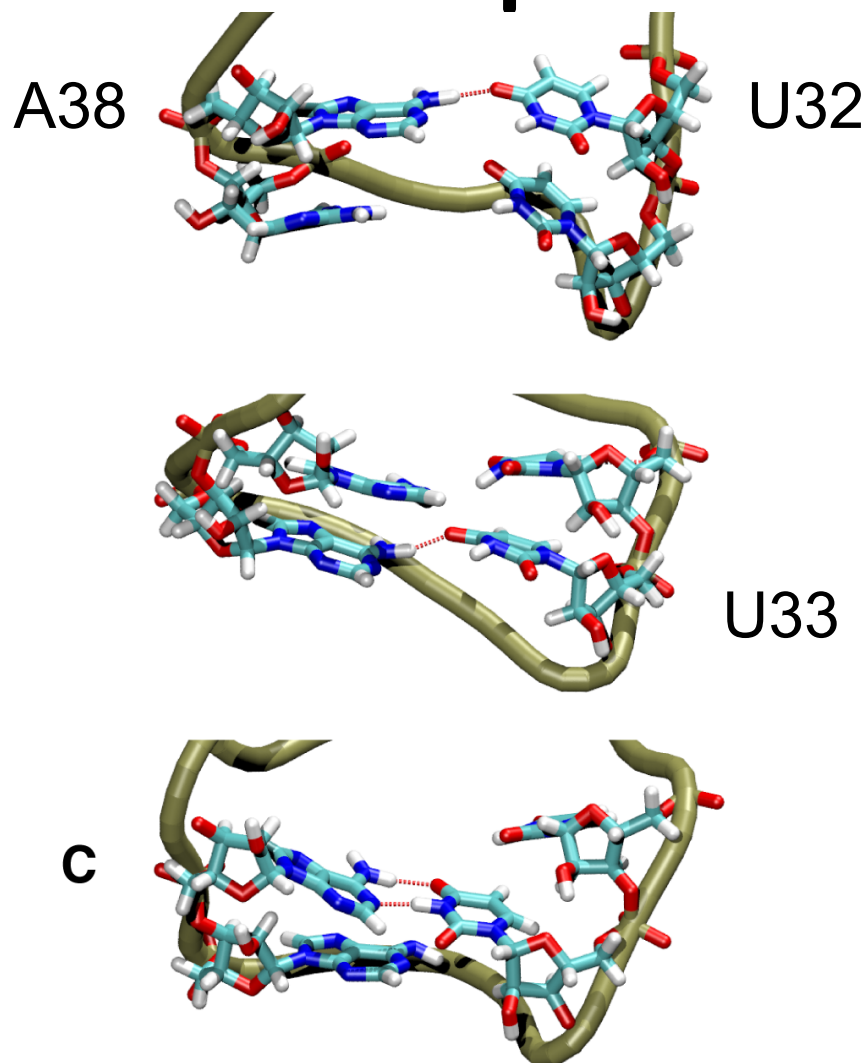
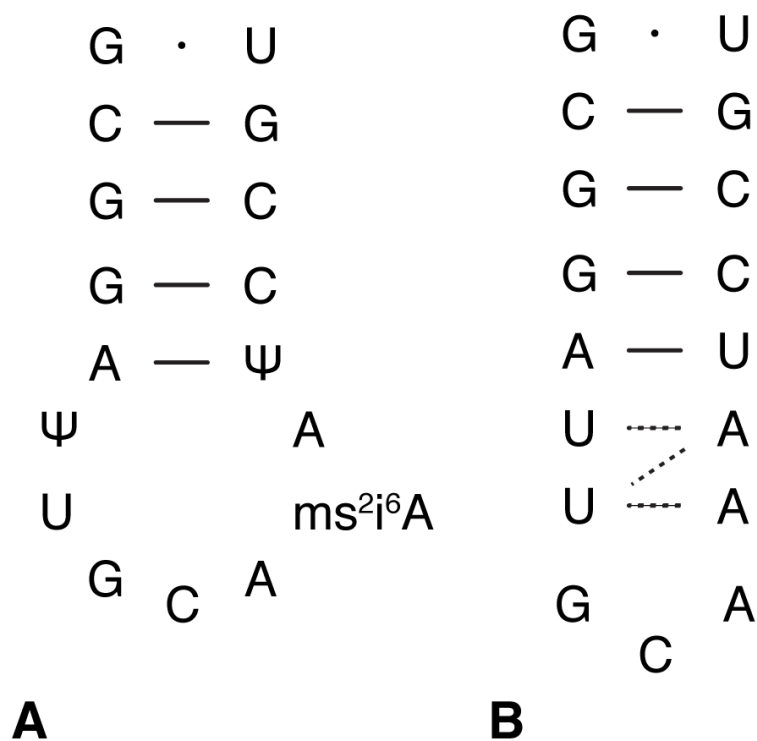
*J. Eargle, A. Sethi, A. Black, L. Trabuco & Z. Luthey-Schulten. JMB
“Dynamics of Recognition in EF-Tu/tRNA Complex” (2008)*

tRNA^{Cys} from E. coli with Modified Bases



Effects of Modified Bases on Dynamics of AC Stem/Loop

MD simulations of unmodified tRNA:Cys



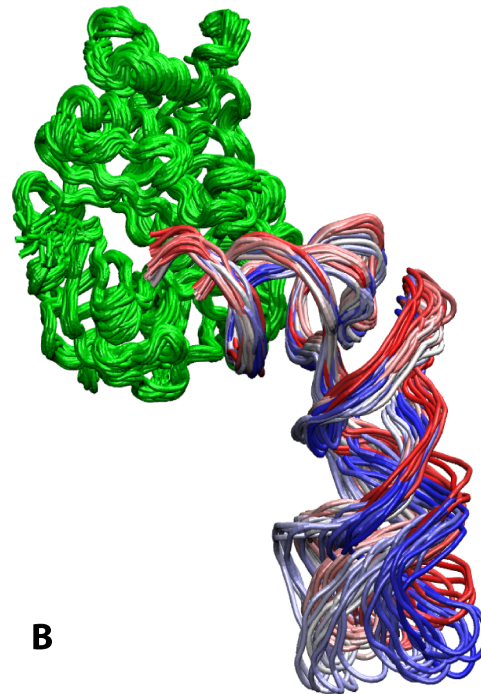
Similar NMR structures for unmodified tRNA-Phe
Nikonowicz, *JMB* 2002, *personal communication*

Eargle, et al. *JMB* (2008))

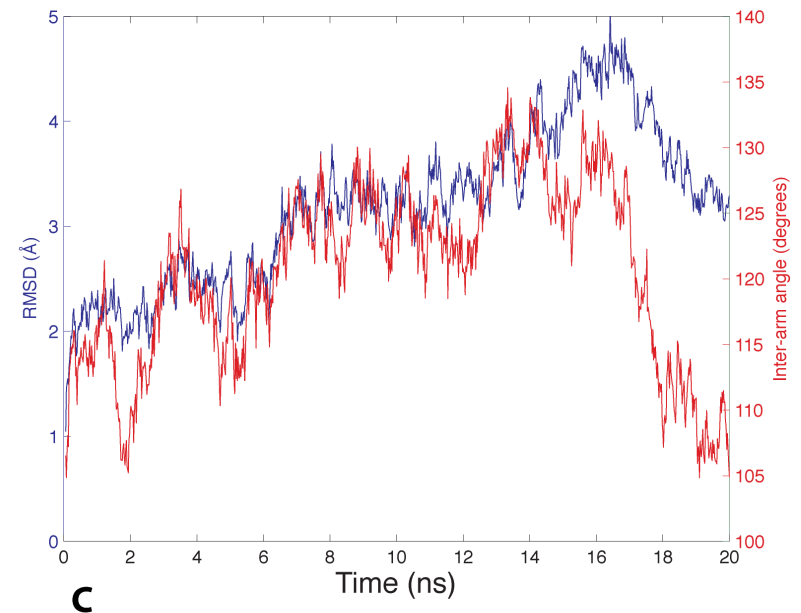
Flexibility in tRNA structures observed in crystallography and simulations



X-ray 5 tRNAs

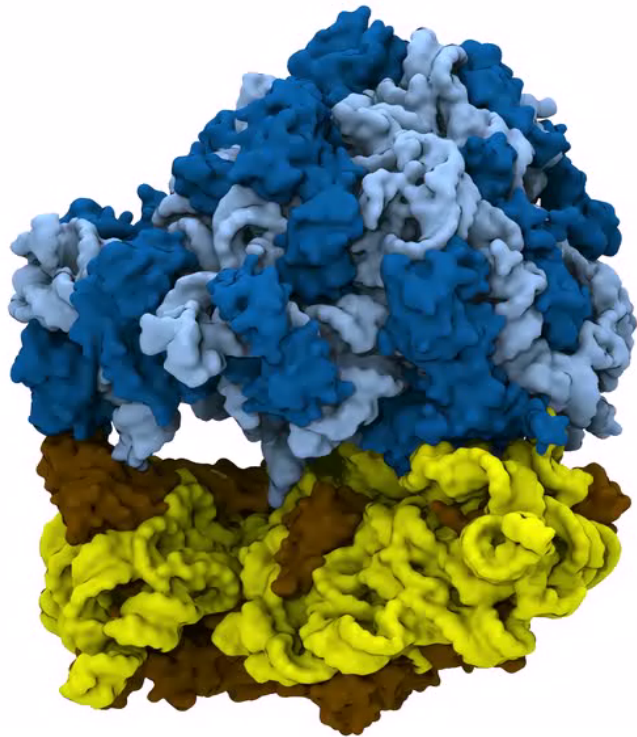


MD EF-Tu:tRNA:cys

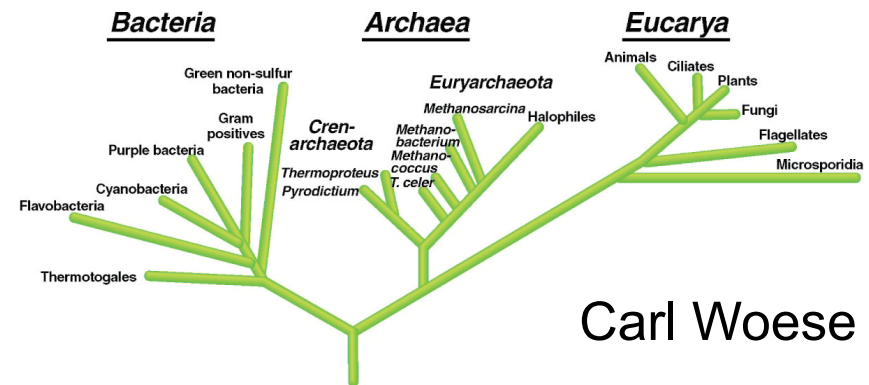


MD: RMSD and Interarm
Angle in tRNA:cys

Molecular Signatures in Evolution of Translation

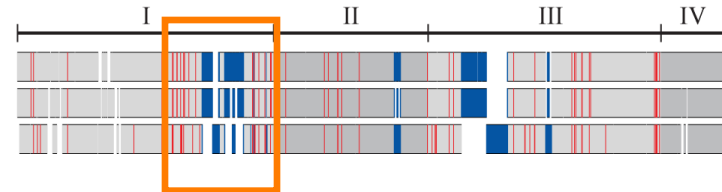


Universal Phylogenetic Tree



16S rRNA

E. coli
T. thermophilus
H. marismortui

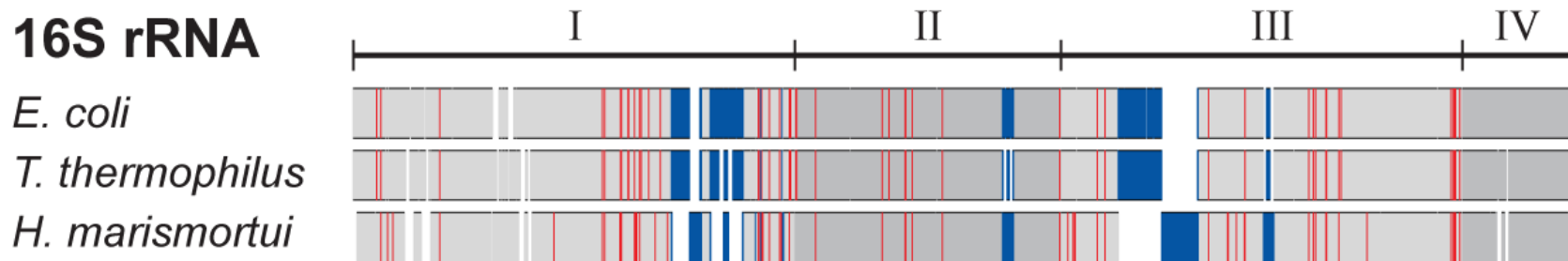


Dynamical function of ribosomal signatures: idiosyncrasies in ribosomal RNA and/or proteins characteristic of the domains of life

Roberts, ... Woese, Luthey-Schulten (2008) *PNAS*; Chen, ... Gruebele, Luthey-Schulten (2010) *BJ*
Chen, ... Ha, Woodson, Luthey-Schulten, (2012) *JPCB*; Lai, Chen, Luthey-Schulten (2013) *JPCB*
Kim, ... Luthey-Schulten Z., Ha, and Woodson (2014) *Nature* "Protein-guided RNA dynamics during early ribosome assembly"

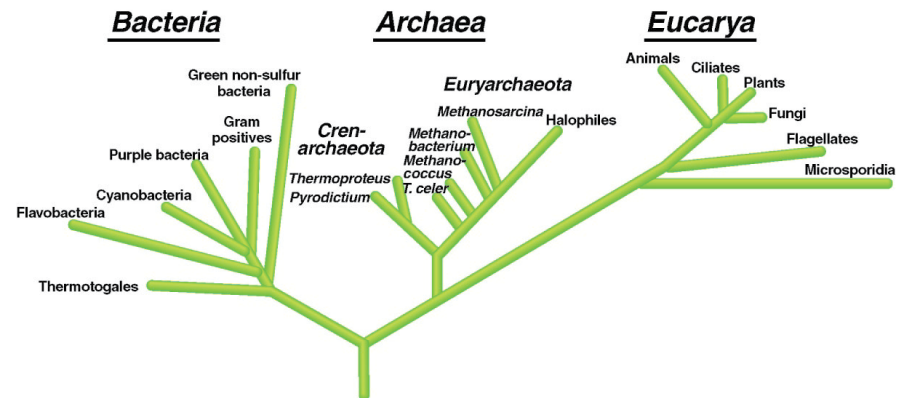
Sequence and Structure Signatures in Ribosomal RNA

16S rRNA

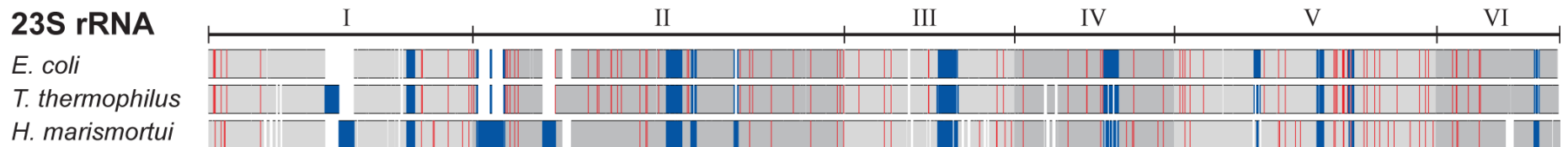


Ribosomal Signatures: Idiosyncrasies in rRNA and/or rproteins characteristic of the domains of life

69 (119) & 6 (14) in 16S (23S)



23S rRNA



Signature analysis

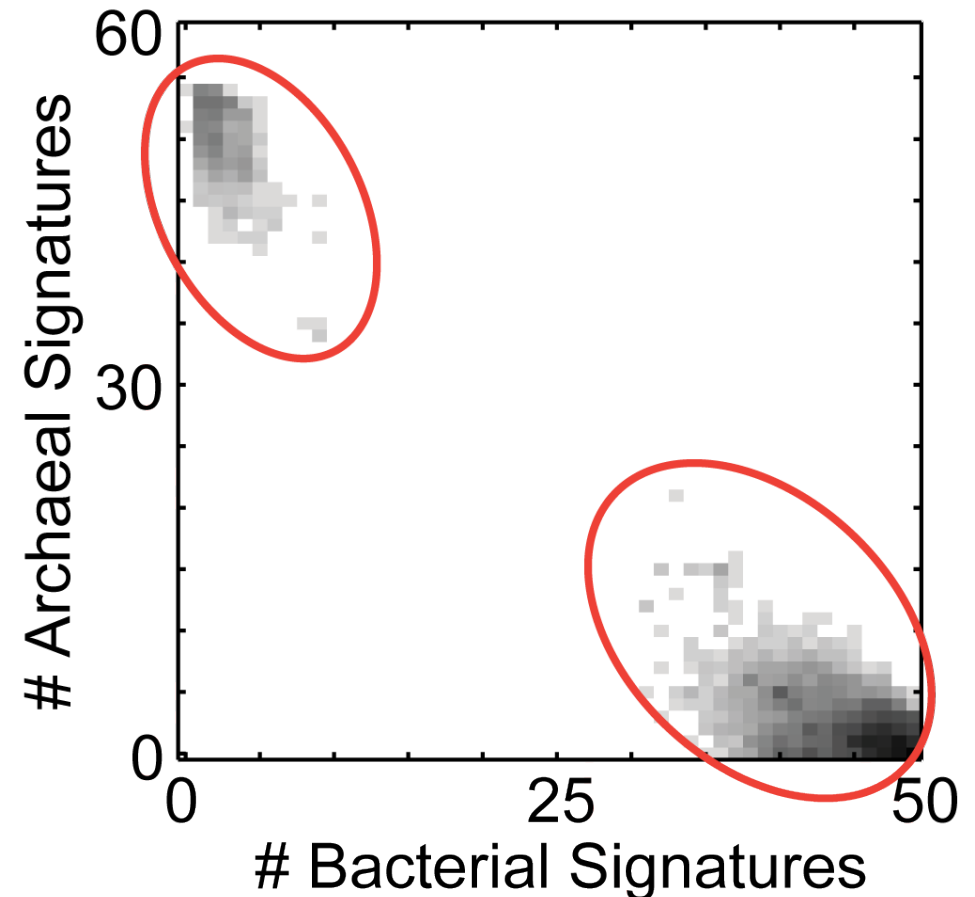
- Signatures are sequence and/or structural features that are characteristic of a domain of life*.
- Identify the sequence signatures in the 16S rRNA and proteins using MultiSeq
- MultiSeq has a coloring based on signatures, but use Scripts to collect quantitative data about the signatures: position, composition.
- Signature analysis can be applied to any set of sequences/groupings

* E. Roberts, A. Sethi, J. Montoya, C. Woese, Z Luthey-Schulten (2008) *PNAS*

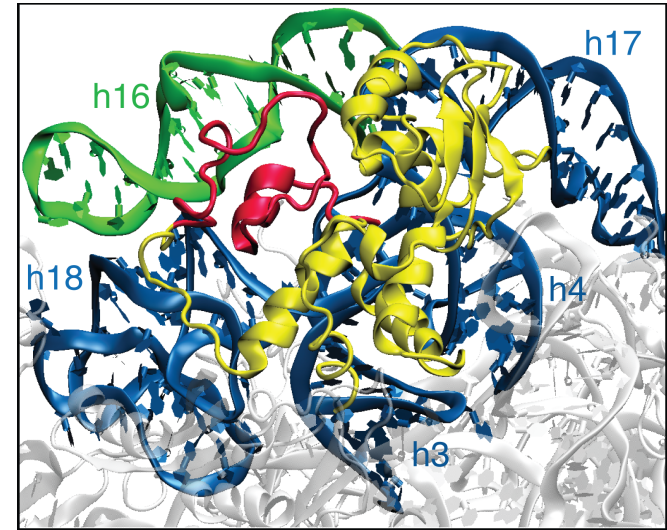
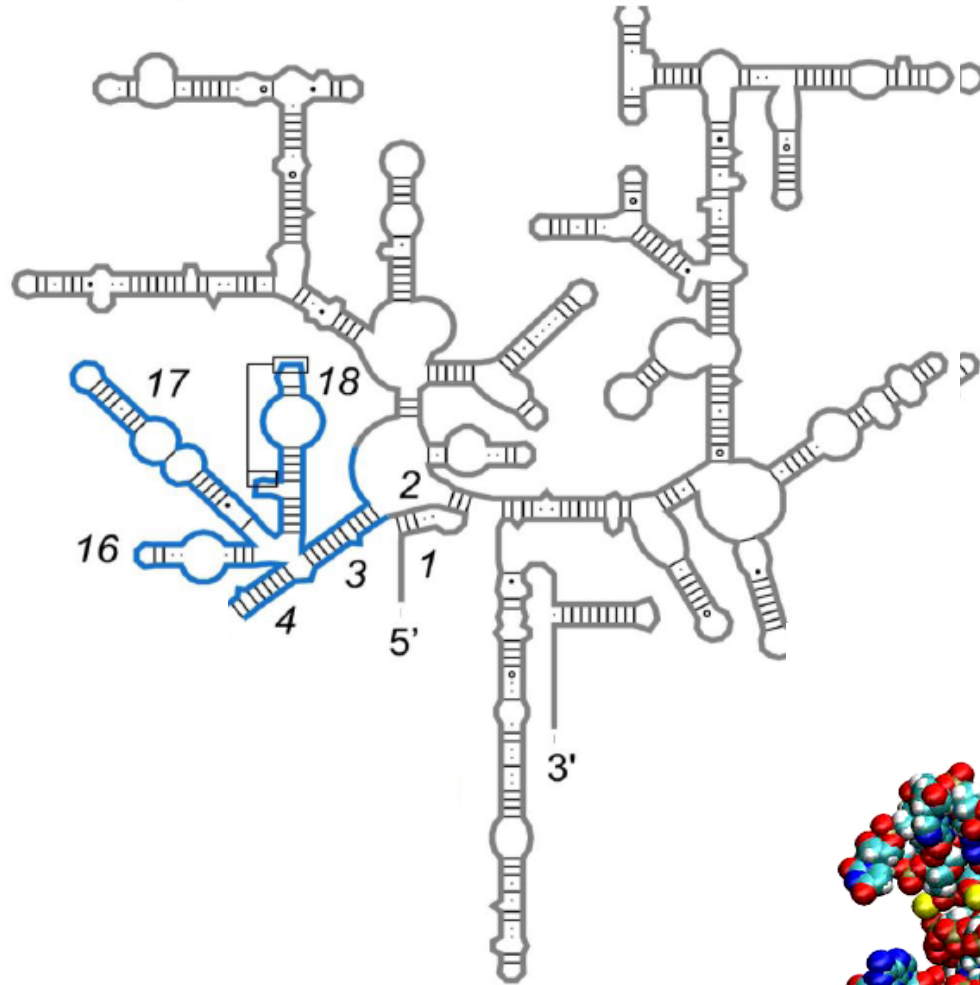
90,000 Environmental 16S rRNA

Distinct A & B Sequence Signatures

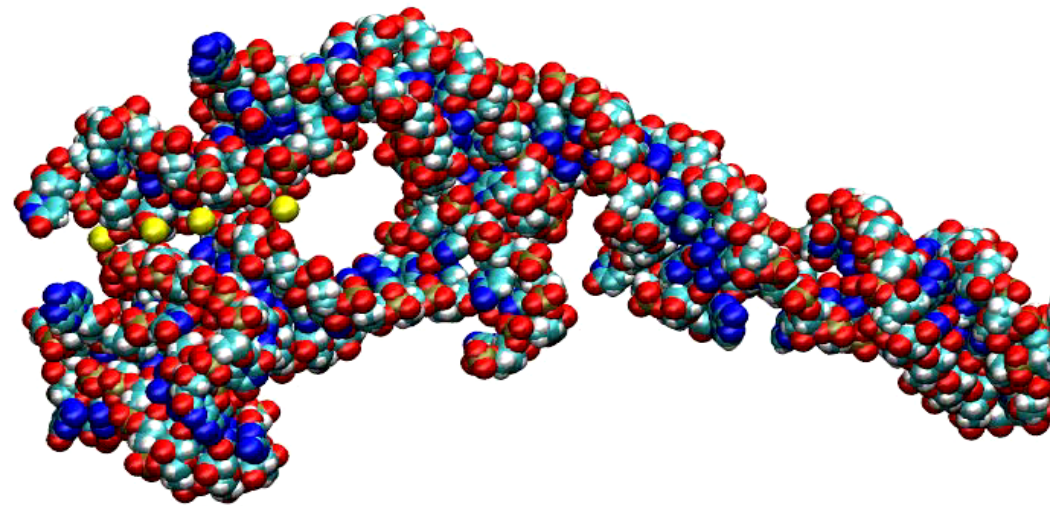
- Analysis of the ribosomal signatures in 90,000 new environmental samples shows that no “gray” area exists: a ribosome is either bacterial or archaeal in nature.
- Split across cluster: 10,000 sequence on each node



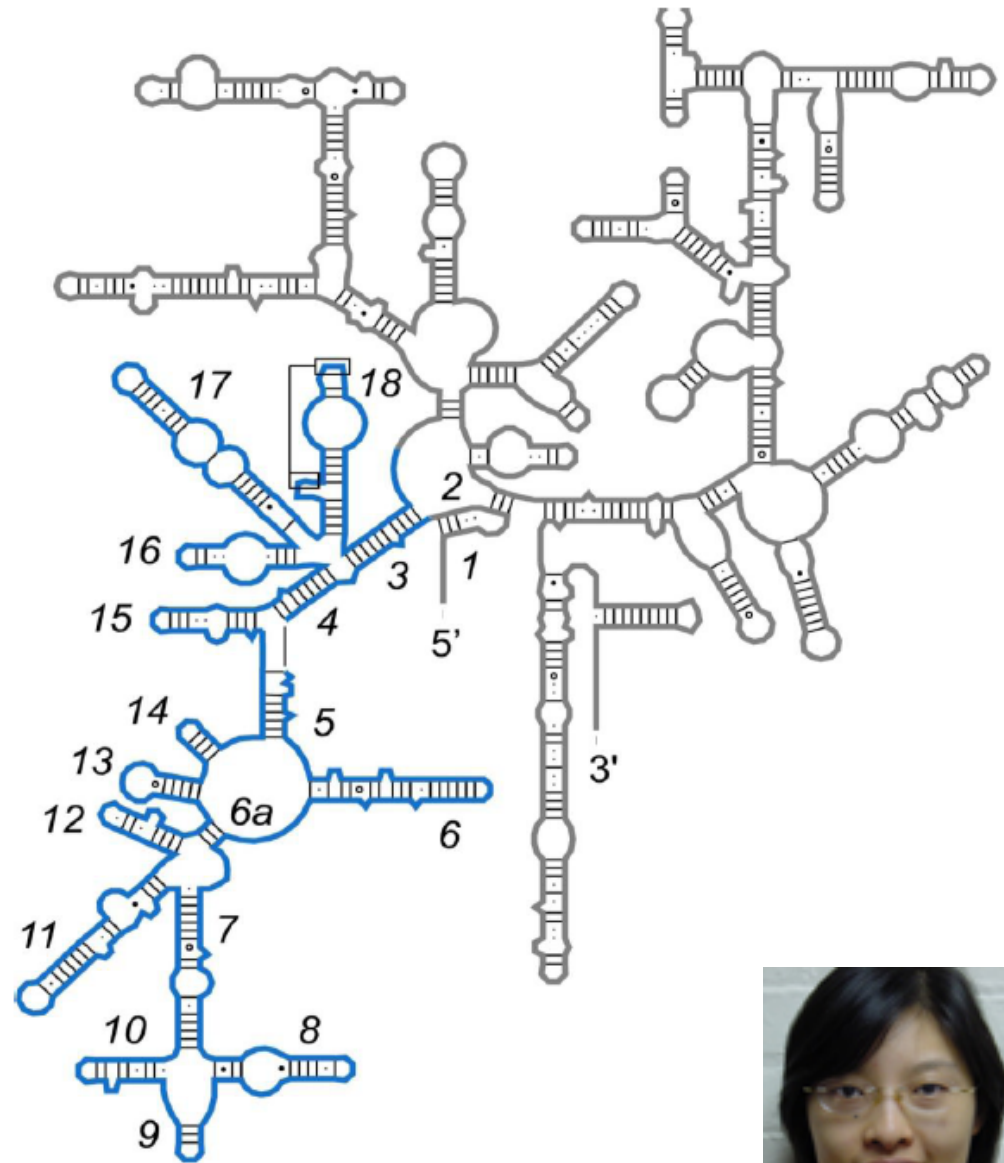
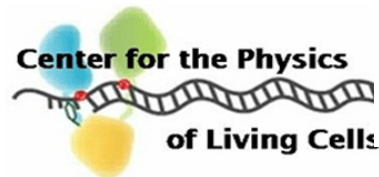
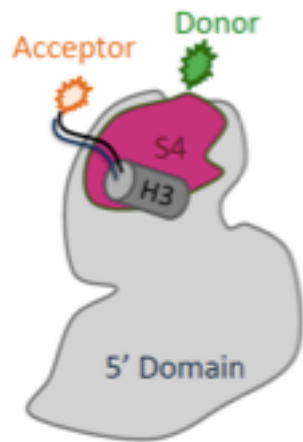
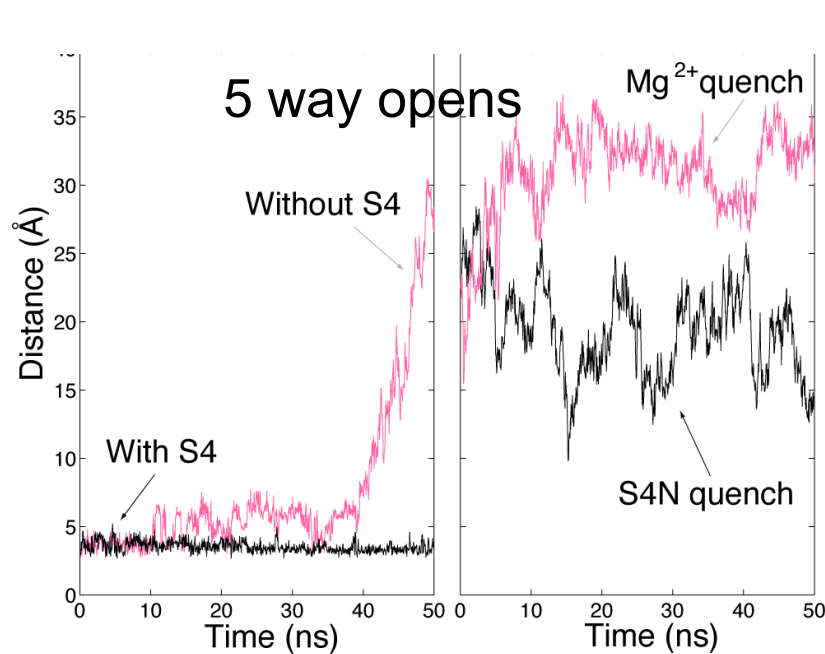
Largest signature region in the SSU



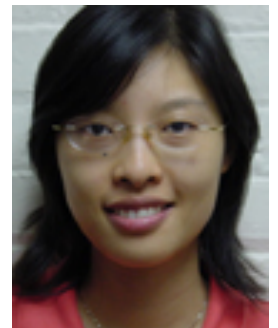
5-way helical junction rRNA with S4



Model for Ribosome Assembly – Structural Intermediates



Ke Chen

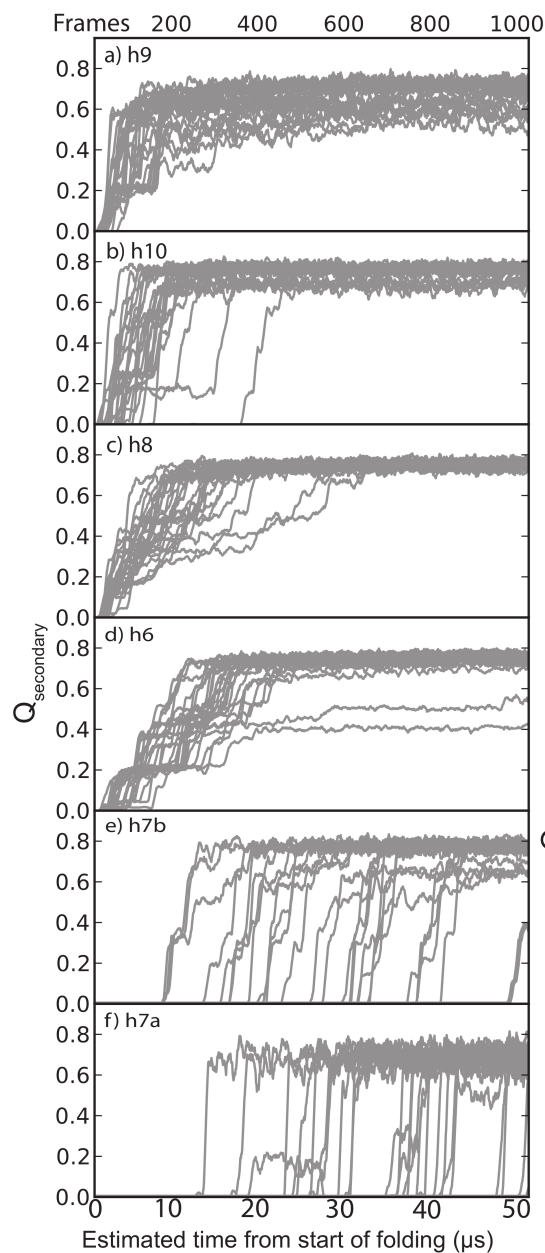


Collaboration with Gruebele, Ha, Woodson labs

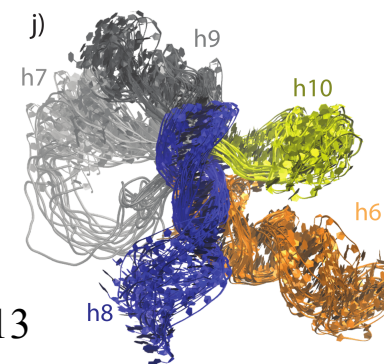
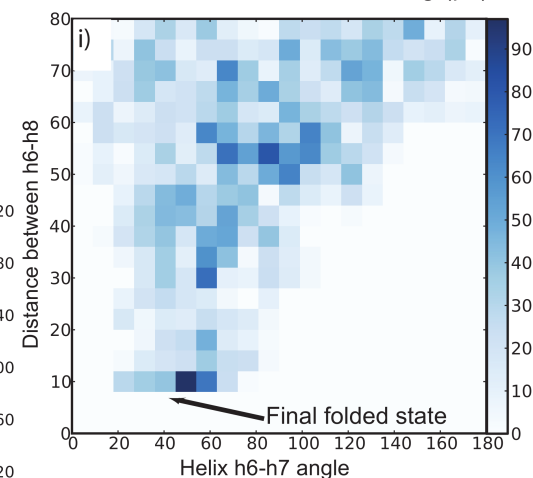
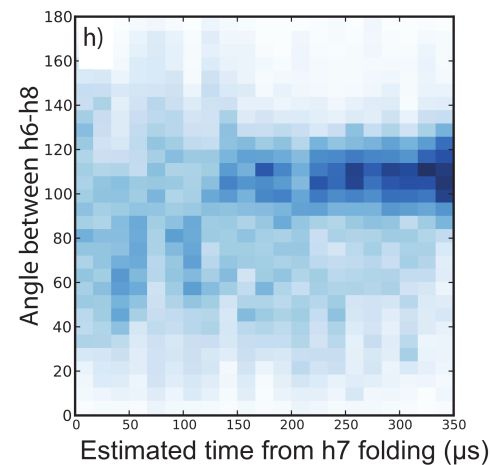
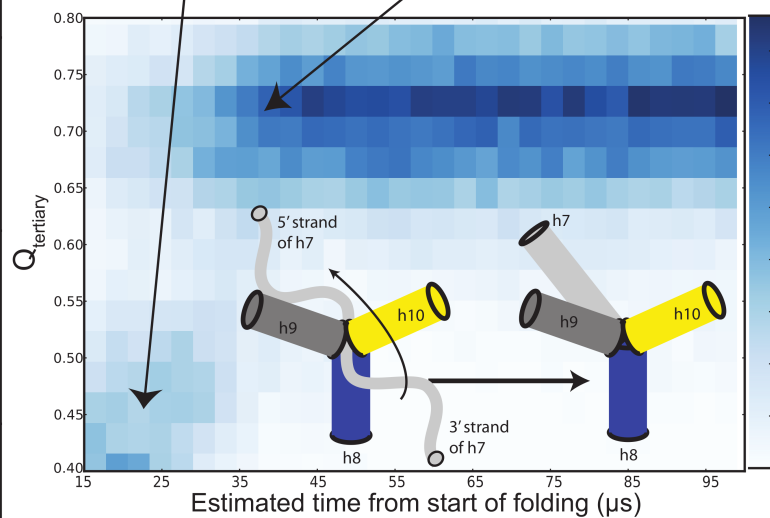
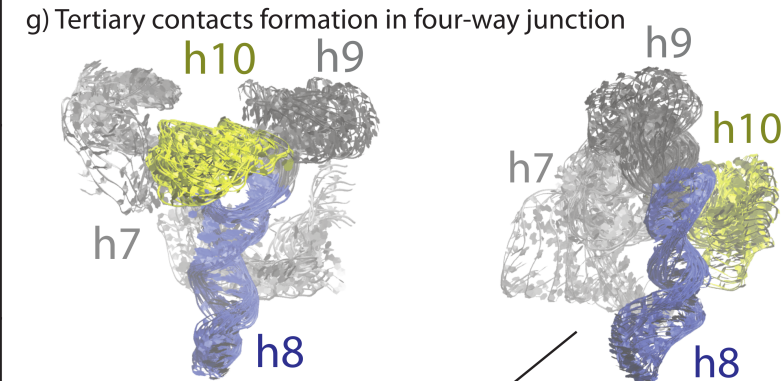
Secondary
structure
formation

Formation of
lower four-way
junction

Helix h6 and h8
tertiary formation



g) Tertiary contacts formation in four-way junction



Hybrid MD-GO, J. Lai, K. Chen, ZLS JCPB 2013

VMD/MultiSeq Tutorials

1. Evolution of Translation: AARS:tRNA
2. Evolution of Translation: EF-Tu:tRNA
3. Evolution of Translation: Ribosome
4. Dynamical Network Analysis
5. Hybrid MD-GO Folding of RNA