Part II - Applications of MultiSeq Evolution of Translation: Dynamics of Recognition in RNA:Protein Complexes

Part III – Towards in silico Cells: Simulating processes in entire cells

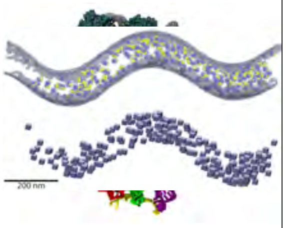
Zaida (Zan) Luthey-Schulten
Dept. Chemistry, Physics, Beckman Institute, Biophysics,
Institute of Genomics Biology

NIH Resource Macromolecular Modeling and Bioinformatics
Pittsburgh Workshop 2011

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Protein:RNA Complexes in Translation

Evolution, Dynamics, Analysis



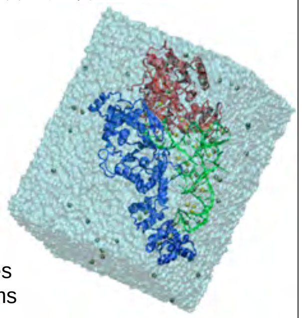
Proteins/RNA
Polyribosomes
Ribosome

"Evolution SepRS/CysRS", ***PNAS***,*2005*
"Dynamic Signaling Network", ***PNAS*** 2009
"Exit Strategy Charged tRNA" ***JMB*** 2010

"Dynamical Recognition Novel Amino Acids", ***JMB*** 2008

"Signatures ribosomal evolution" ***PNAS*** *2008*, ***BMC*** *2009*
"Whole cell simulations on GPUs" ***IEEE*** *2009*
"Dynamics of tRNA" ***FEBS*** *2010*

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: Mg^{2+}, K^+
- H_2O: ~27,000 molecules
- System: ~130,000 atoms

System Perturbations

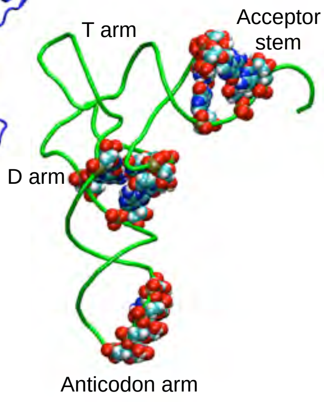
Deprotonation/protonation of reactants (aa,NTs)
Changing rotamer states of residues (5) involved in long-lived salt bridges

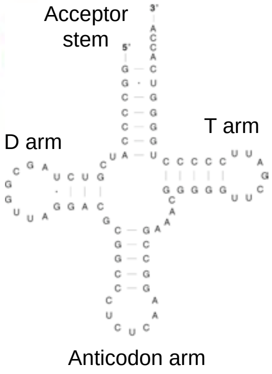
(1) Phillips, J.C. et al. *J. Comput Chem*, (2005); (2) MacKerell, A. et al. *Biopolymers* (2001);
(4) Case, D. et al. *J. Comput. Chem.* (2005); (4) Eargle, J. et al. JMB 2010, FEBS Let. 2010;

(5) Dunbrack Jr. and Cohen. *Protein Sci.* (1997)

Charging tRNA through allosteric signaling

Identity Elements

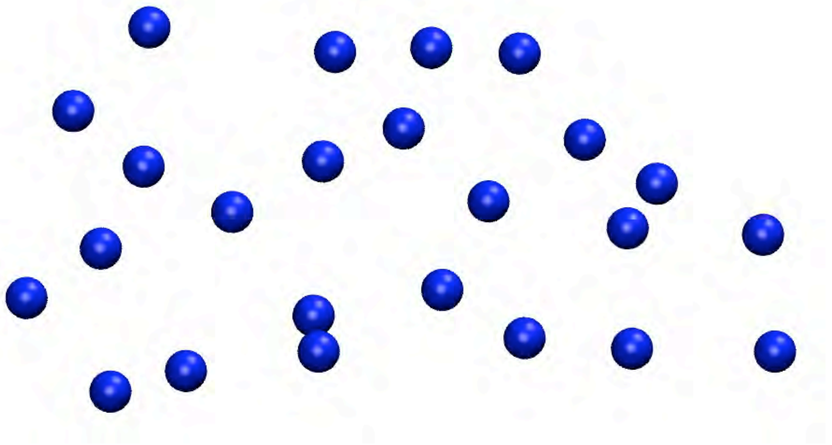




Overall Charging Reaction

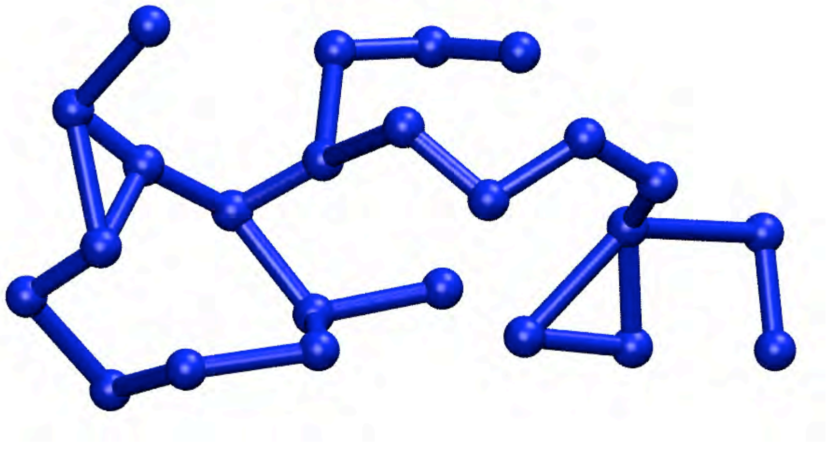
$$\text{ATP + Glu + tRNA} \xrightarrow{\text{GluRS}} \text{Glu-tRNA + AMP + PP}_i$$

How to Construct a Network?



Nodes - defined at C_a (protein) and P (nucleotide) atoms

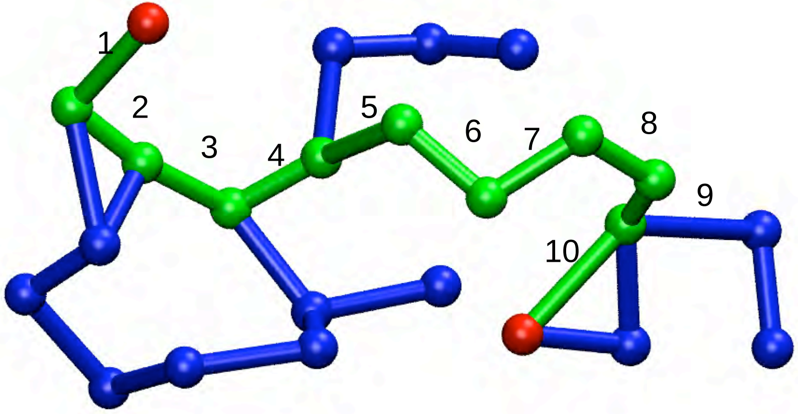
How to Construct a Network?



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

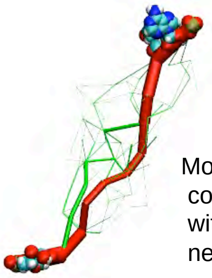
Different Paths can have Different Lengths



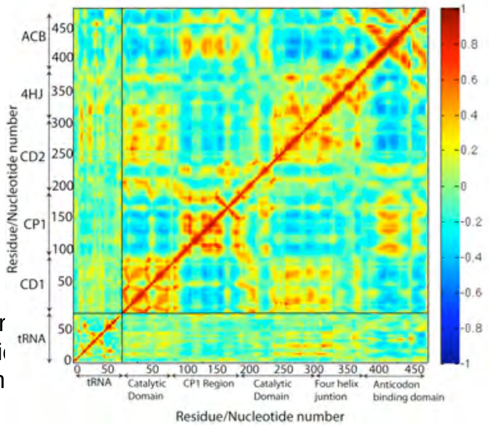
Path length = 10 (*unweighted*)

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

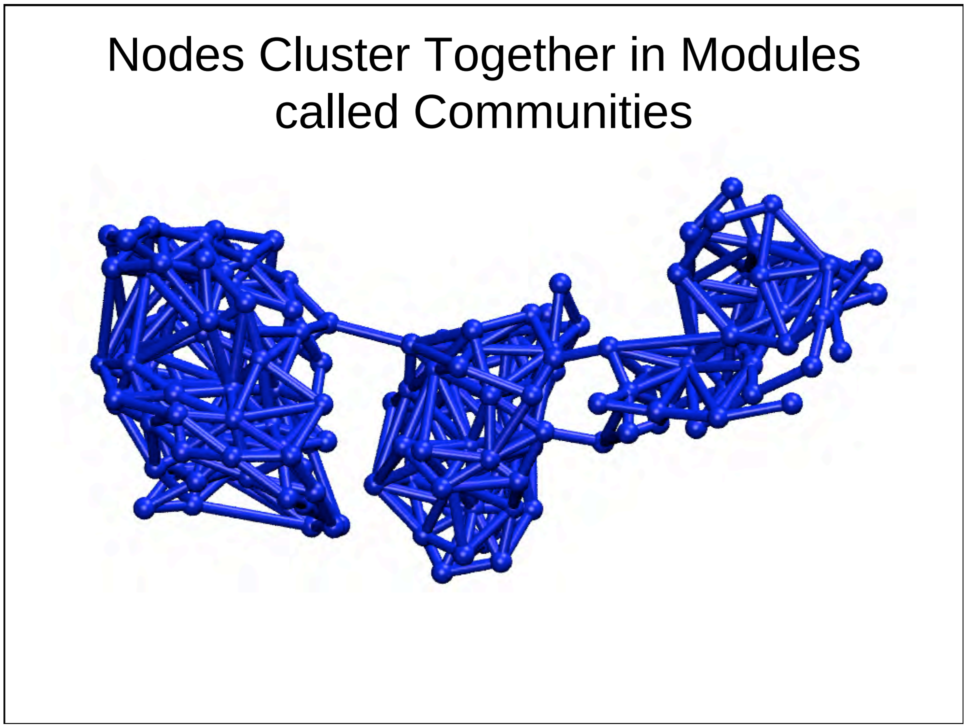
Correlations (C_{ij}) define signaling pathways in GluRS:tRNA



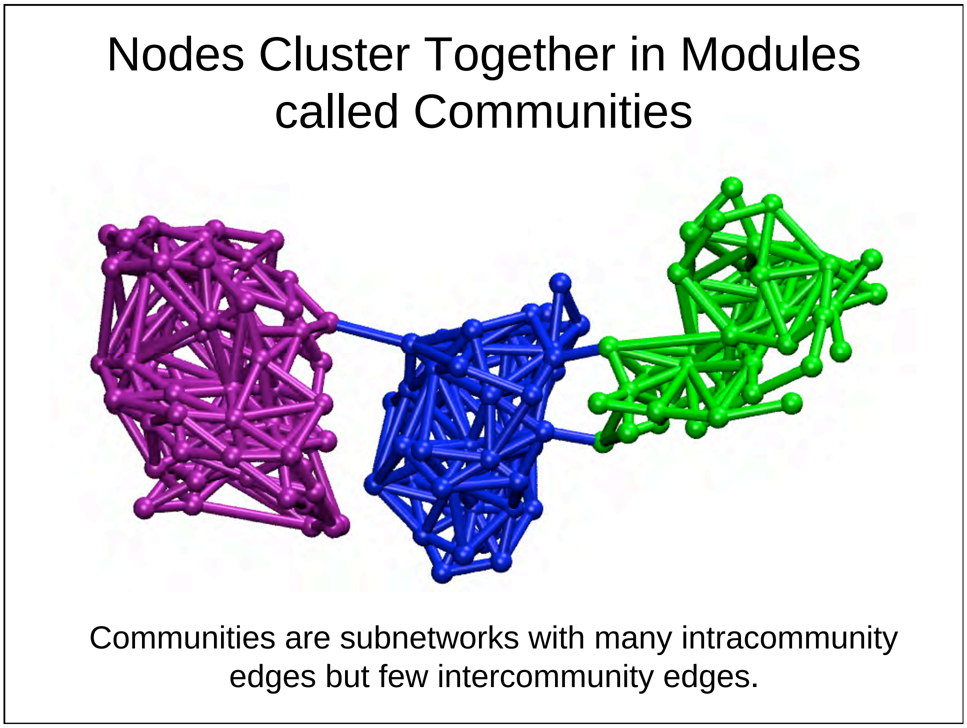
Modular str
communiti
with weigh
network



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

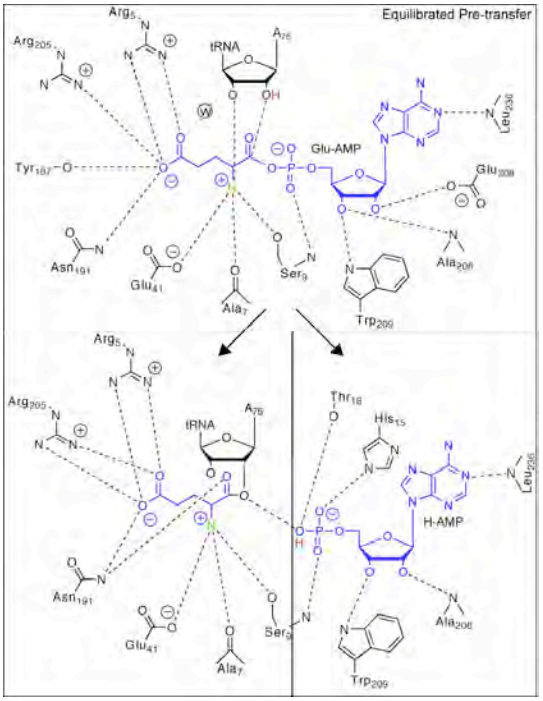


Nodes Cluster Together in Modules called Communities



Nodes Cluster Together in Modules called Communities

Communities are subnetworks with many intracommunity edges but few intercommunity edges.



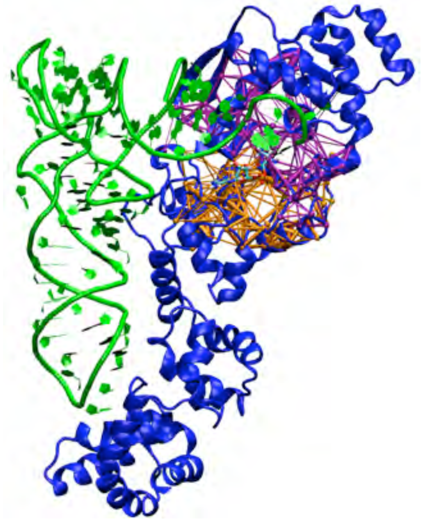
Reaction Mechanism for the Transfer of Glu to $tRNA^{Glu}$

Perona JJ, Rould MA, Steitz TA
Biochemistry 1993

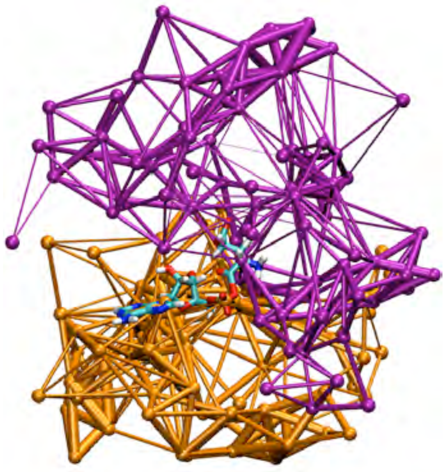
Black A, Eargle J, Sethi A,
Luthey-Schulten Z. *JMB* 2010
100s ns MD simulations

Community Structure Reveals Function in GluRS

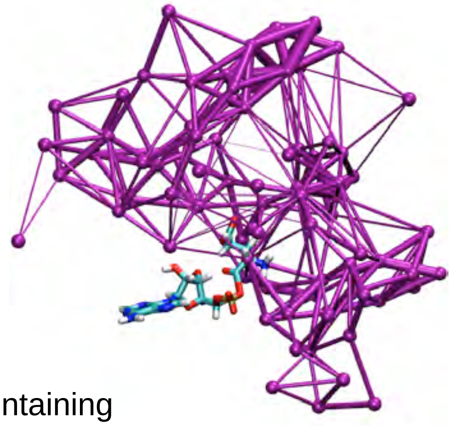
Community Structure Reveals Function in GluRS



Community Structure Reveals Function in GluRS



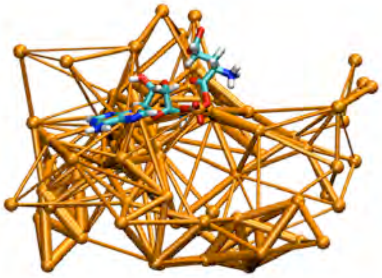
Community Structure Reveals Function in GluRS



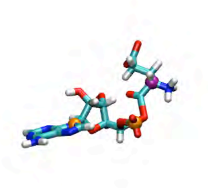
community containing amino acid moiety

Community Structure Reveals Function in GluRS

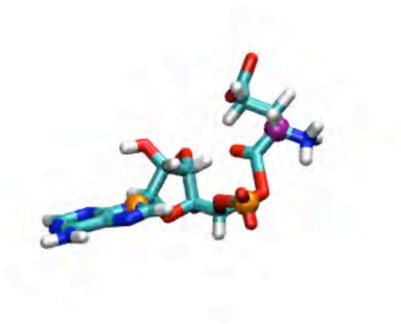
community containing AMP moiety



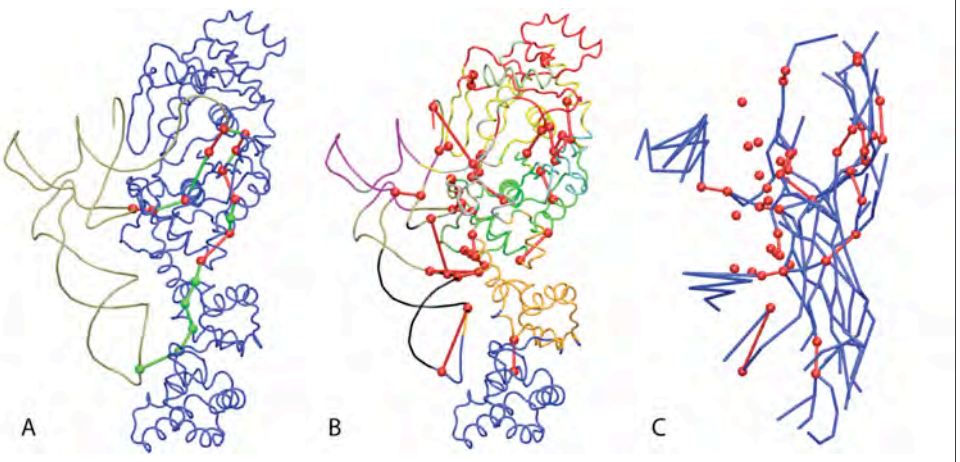
Community Structure Reveals Function in GluRS



Community Structure Reveals Function in GluRS



Dynamical Networks, Conservation, and Betweeness



Optimal signal pathways:
U13, U35 to A76

Critical(conserved) nodes
connecting communities

Betweeness routes - highest
density pair optimal paths

Changing Networks: tRNA Migration from GluRS to EF-Tu

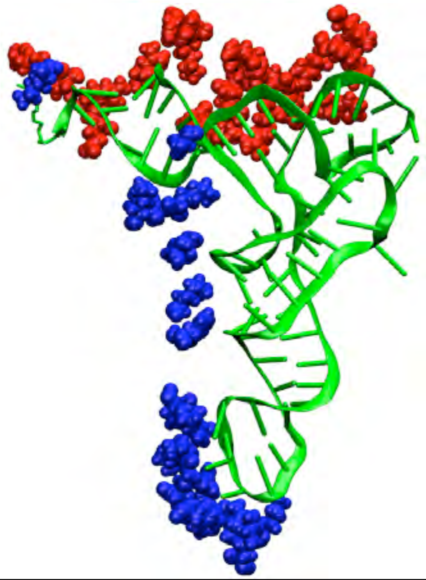
>2-fold increase
in k_{cat} for
CysRS:tRNA:EF-Tu

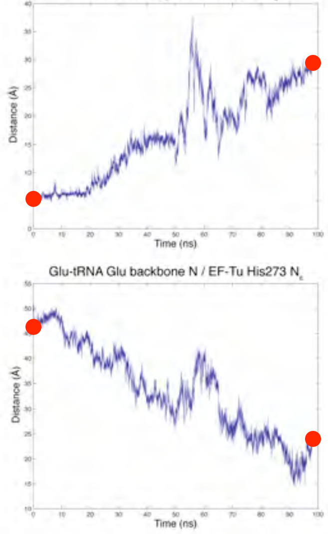
8-fold increase
in k_{cat} for
LeuRS:tRNA:EF-Tu

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

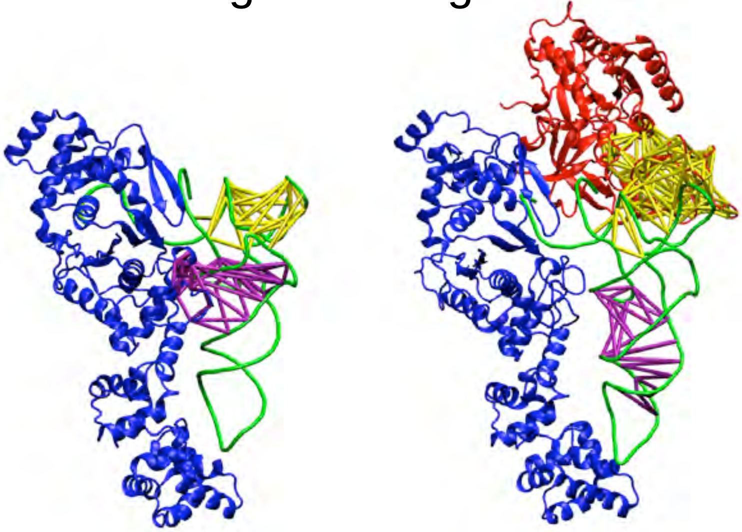
Hausmann C, Praetorius-Ibba M,
Ibba M. *NAR* 2007

Change in Protein:RNA Contacts as tRNA Migrates



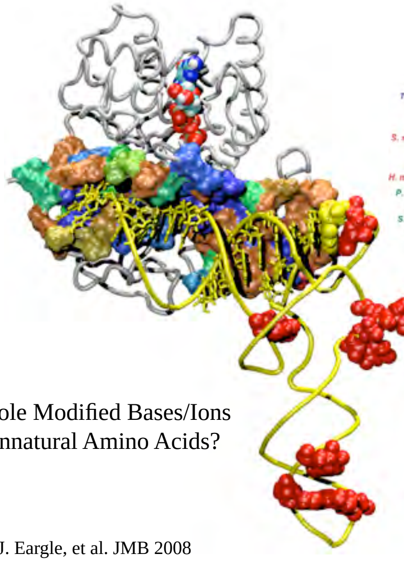


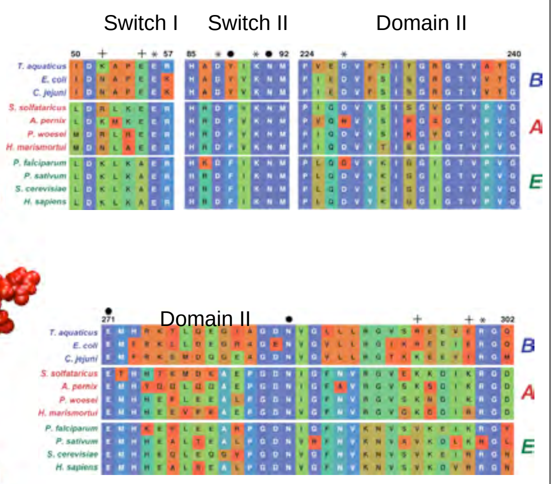
Change in Protein:RNA Contacts During tRNA Migration



purple - D arm community yellow - T arm community

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





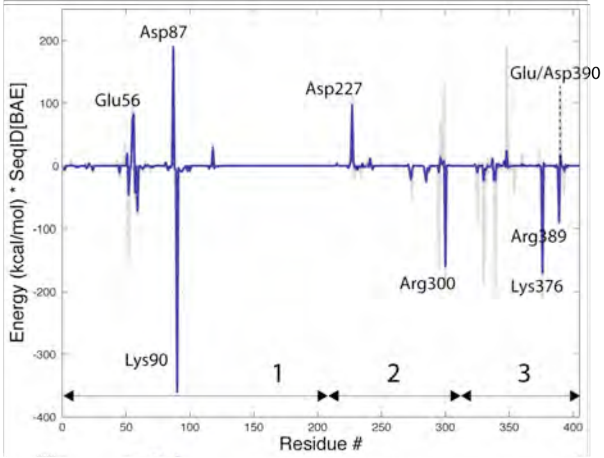
Role Modified Bases/Ions
Unnatural Amino Acids?

J. Eargle, et al. JMB 2008

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

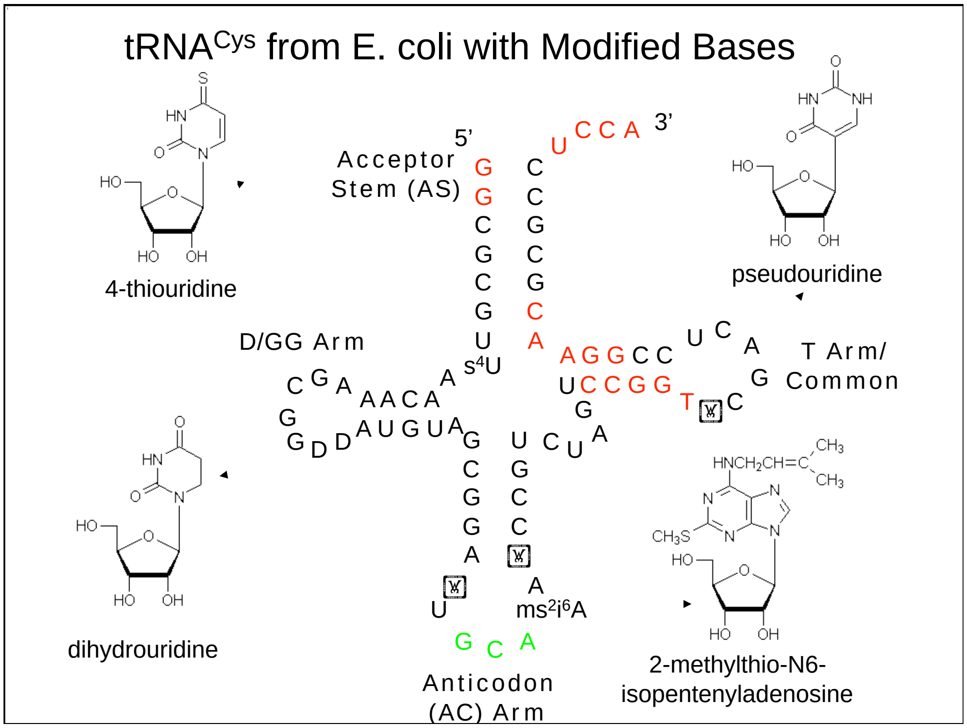
Combining MD with Evolutionary Analysis (MultiSeq)

Non-bonded Interaction Energy

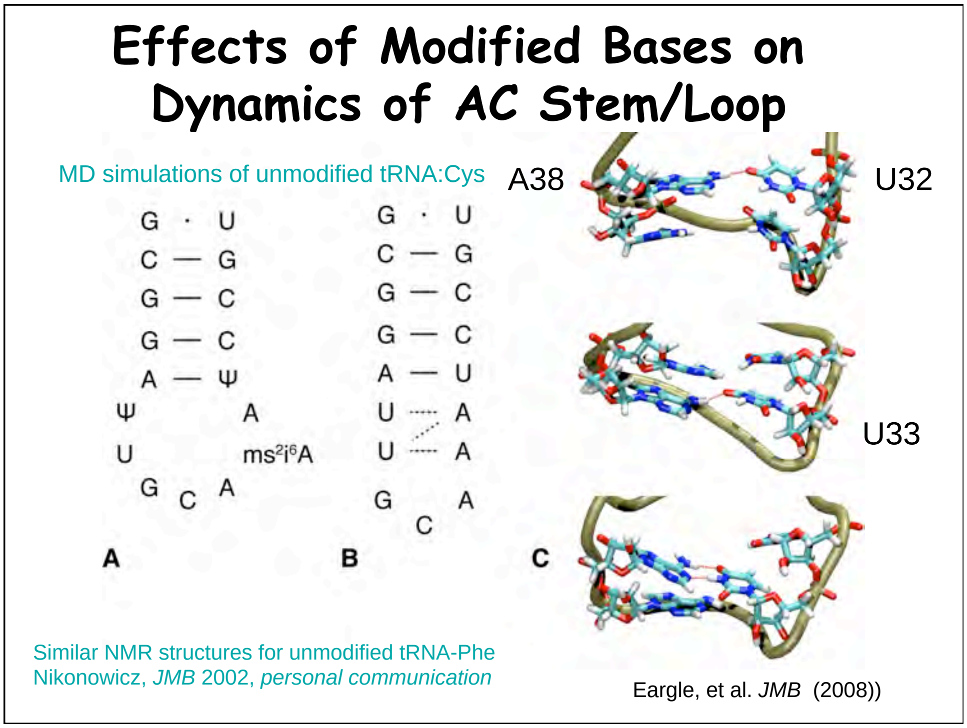


BAE

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



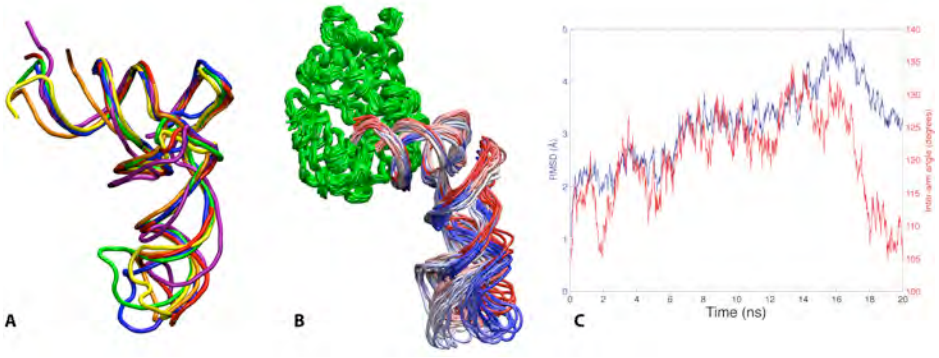
Effects of Modified Bases on Dynamics of AC Stem/Loop

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

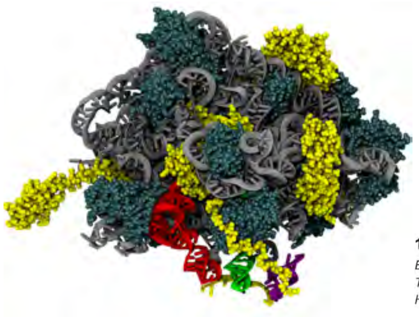
MD EF-Tu:tRNA:cys

MD: RMSD and Interarm Angle in tRNA:cys

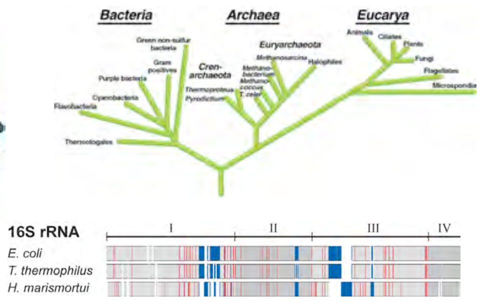
JMB 2008 & FEBS Let. 2010

Molecular Signatures in Ribosome Evolution

Archaeal



Universal Phylogenetic Tree

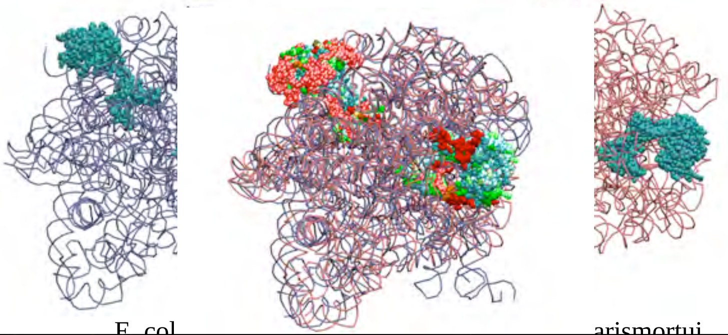


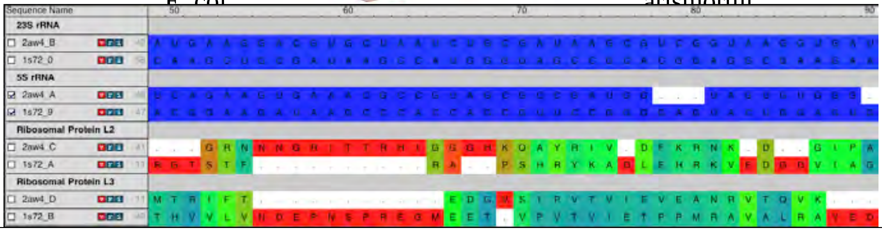
Dynamical function of ribosomal signatures: idiosyncrasies in ribosomal RNA and/or proteins characteristic of the domains of life

Roberts, Sethi, Montoya, Woese, Luthey-Schulten (2008) ***PNAS*** 105:13953
Chen, Eargle, Sarkar, Gruebele, Luthey-Schulten (2010) ***Biophysical Journal***

Structural Overlaps with STAMP

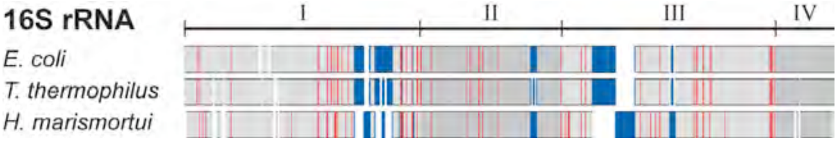
Comparing ribosomal large subunit with r-proteins L2 and L3
180,000 atoms in 4 rRNAs and 58 proteins



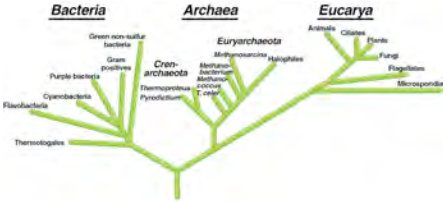


Sequence Name	50–90
Ribosomal Protein L2	
☐ 2aw4_C	. . . G R N N N G R I T T R H I G G G H K Q A Y R I V . D F K R N K . D . . G I P A
☐ 1s72_A	R G T S T F . . . . R A . . P S H R Y K A D L E H R K V E D G D V I A G
Ribosomal Protein L3	
☐ 2aw4_D	M T R I F T . . . . E D G V S I R V T V I E V E A N R V T Q V K . . .
☐ 1s72_B	T H V V L V N D E P N S P R E G M E E T . V P V T V I E T P P M R A V A L R A Y E D

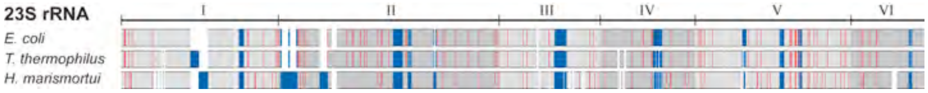
Sequence and Structure Signatures in Ribosomal RNA



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



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



Roberts, Montoya, Sethi, Woese, ZLS, *PNAS* 2008

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*

Signature analysis script

```
proc get_taxa_signatures {args} {

    initializeMultiSeqEnvironment

    # Load the alignment.
    set sequenceIDs [::SeqData::Fasta::loadSequences $alignmentFilename]

    # Find the reference species.
    set refSequenceIDs {}
    foreach species $referenceSpecies {
        set found 0
        foreach sequenceID $sequenceIDs {
            if {[string toupper [::SeqData::getScientificName $sequenceID]] == [string toupper $species]} {
                lappend refSequenceIDs $sequenceID
                set found 1
                break
            }
        }
    }

   # Group the sequence ids.
    array set groupData {names {}}
    foreach sequenceID $sequenceIDs {

        set group ""
        if {$rankName == "domain"} {
            set group [::SeqData::getDomainOfLife $sequenceID]
        } else {
            set group [::SeqData::getLineageRank $sequenceID $rankName]
        }
        if {![info exists groupData($group)]} {
            lappend groupData(names) $group
            set groupData($group) {}
        }
        lappend groupData($group) $sequenceID
    }
```

Signature analysis script (cont)

```
    # Calculate the signatures for the groups.
    set signatures [::SeqEdit::Metric::Signatures::calculateSignatures $sequenceGroups $groupConsensusCutoff \
                                $otherGroupMaxCutoff $otherGroupMaxGapFraction $maxConservedBlockDistance $minConse

    # Print the signatures.
    puts ""
    puts "Ordered Signatures"
    printSignatureHeader $groups $refSequenceIDs
    for {set position 0} {$position < [llength [lindex $signatures 0]]} {incr position} {

        # Get the signature for each of the groups.
        set isSignature 0
        for {set groupIndex 0} {$groupIndex < [llength $groups]} {incr groupIndex} {
            set groupSignature [lindex [lindex $signatures $groupIndex] $position]
            if {[string length $groupSignature] == 1} {
                set isSignature 1
            }
        }

        if {$isSignature} {
            printSignatureLine $position $groups $refSequenceIDs $signatures $sequenceGroups
        }
    }
}
```

Signature analysis script (cont)

```
proc getCompositionString {sequenceIDs position} {

    # Go through each group to count the elements.
    array set counts {values {}}
    foreach sequenceID $sequenceIDs {
        set element [::SeqData::getElement $sequenceID $position]
         if {![info exists counts($element)]} {
             lappend counts(values) $element
             set counts($element) 1
         } else {
             incr counts($element)
         }
    }

    set ret ""
    foreach element $counts(values) {
        set percentage [expr round(100.0*double($counts($element))/double([llength $sequenceIDs]))]
        if {$percentage > 5} {
            if {$ret != ""} {
                append ret "/"
            }
            append ret "${element}($percentage\%)"
        }
    }

    return $ret
}
```

Signature script output

```
Ordered Signatures
  Pos  E._coli T._therm H._maris   Archaea                        Bacteria
  --- -------- -------- -------- ------------------------------ ------------------------------
   35       33       33       27   U(28%)/C(72%)                  *A(96%)
   42       39       39       33   *U(100%)                       *G(95%)
   51       47       47       42   *G(97%)                        C(85%)/U(7%)/G(8%)
   59       53       53       49   *C(100%)                       A(83%)/G(14%)
  222      113      106       87   *C(100%)                       *G(95%)
  661      248      243      227   *G(97%)                        C(75%)/G(9%)/U(11%)
  749      314      309      293   *G(100%)                       *C(97%)
  753      317      312      296   *G(97%)                        *U(91%)/G(7%)
  777      338      333      317   *G(97%)                        A(89%)/N(9%)
  779      339      334      318   *G(97%)                        *C(98%)
  780      340      335      319   *C(94%)                        *U(92%)/A(6%)
  790      349      344      328   G(86%)/N(11%)                  *A(92%)
  791      350      345      329   C(67%)/U(19%)/N(11%)           *G(98%)
  800      358      353      337   *G(100%)                       *U(91%)/C(7%)
  802      359      354      338   *C(97%)                        G(59%)/U(9%)/C(9%)/A(20%)
  804      361      356      340   *C(94%)                        G(84%)/A(14%)
  809      365      360      344   *A(92%)/U(8%)                  *U(97%)
  811      367      362      346   C(83%)/G(14%)                  *U(97%)
  823      377      372      356   C(89%)/U(11%)                  *G(92%)
  833      386      381      365   *G(94%)                        C(89%)/U(6%)
  840      393      388      372   G(83%)/C(14%)                  *A(97%)
  851      403      398      382   *A(100%)                       C(89%)
  920      447      441      416   C(31%)/U(67%)                  *G(92%)
  990      487      471      424   G(89%)/A(8%)                   *A(91%)
 1083      508      491      446   *A(97%)                        *U(92%)
 1085      510      493      448   *G(97%)                        *A(97%)
 1087      512      495      450   *C(92%)/U(8%)                  *U(97%)
 1089      514      497      452   *G(92%)/N(6%)                  C(89%)/U(8%)
 1101      523      506      461   *C(94%)/A(6%)                  *A(96%)
 1119      537      520      475   *C(100%)                       *G(91%)/A(8%)
 1121      539      522      477   *G(92%)/A(6%)                  A(89%)/G(10%)
 1134      549      532      487   *U(94%)                        *C(99%)
```

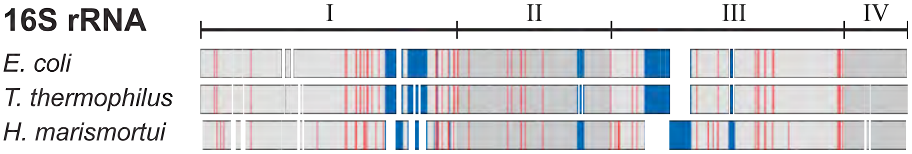
Draw signature figure

- Want to create a graphical representation of the signatures.
- Can use the TCL graphics object to draw figures.

```
proc draw_one_d_alignment {args} {

    # Load the alignment.
    set alignment [::SeqData::Fasta::loadSequences $alignmentFilename]
    set numberSequences [llength $alignment]
    set numberPositions [::SeqData::getSeqLength [lindex $alignment 0]]
    puts "Loaded $numberSequences sequences of length $numberPositions"

    # Create the drawing canvas.
    set canvasWidth [expr $draw(xBorder)+($numberPositions*$draw(positionWidth))+$draw(xBorder)]
    set canvasHeight [expr $draw(yBorder)+($numberSequences*$draw(sequenceHeight))+(($numberSequences-1)*\

                   $draw(sequenceSpacing))+$draw(yBorder)]
    set g [canvas .drawing -width $canvasWidth -height $canvasHeight]

    # Draw the alignment.
    for {set i 0} {$i < $numberSequences} {incr i} {
        set sequenceID [lindex $alignment $i]
        set y1 [expr $draw(yBorder)+($i*($draw(sequenceHeight)+$draw(sequenceSpacing)))]
        set y2 [expr $y1+$draw(sequenceHeight)]
        drawSequencePositions $g $draw(xBorder) $y1 $y2 $sequenceID 0 [expr $numberPositions-1] \
                                                              $draw(positionWidth) $draw(sequenceColor) $dra
    }

    # Save the canvas.
    $g postscript -x 0 -y 0 -width $canvasWidth -height $canvasHeight -file $outputFilename

    cleanupMultiSeqEnvironment
}
```

Draw signature figure (cont)

```
proc drawSequencePositions {g x y1 y2 sequenceID firstPosition lastPosition positionWidth sequenceColor borderColor
                                                                                                          borderWidth}
{
    set startPosition ""
    for {set j $firstPosition} {$j <= $lastPosition} {incr j} {
        set element [::SeqData::getElement $sequenceID $j]

        # If this is the beginning of a sequence block, record that one started.
        if {$element != "-" && $startPosition == ""} {
            set startPosition $j
        }

        # If this is the end of a sequence block, draw the block.
        if {($element == "-" || $j == $lastPosition) && $startPosition != ""} {
            if {$element == "-"} {
                set endPosition [expr $j-1]
            } else {
                set endPosition [expr $j]
            }
            set x1 [expr $x+($startPosition*$positionWidth)]
            set x2 [expr $x+(($endPosition+1)*$positionWidth)]
            $g create rectangle $x1 $y1 $x2 $y2 -fill $sequenceColor -outline $sequenceColor -width $borderWidth
            $g create line $x1 $y1 $x2 $y1 -fill $borderColor -width $borderWidth
            $g create line $x1 $y2 $x2 $y2 -fill $borderColor -width $borderWidth
            set startPosition ""
        }
    }
}
```

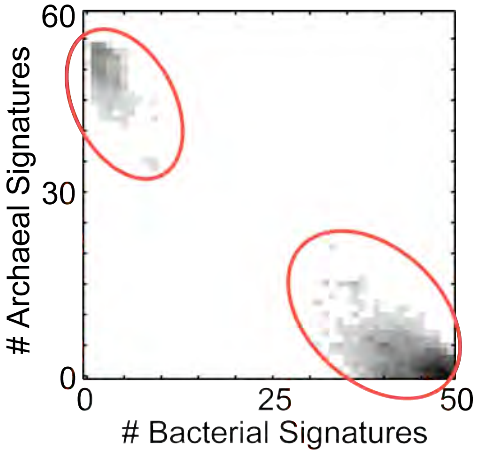


90,000 Environmental 16S rRNA

Distinct A & B Sequence Signatures

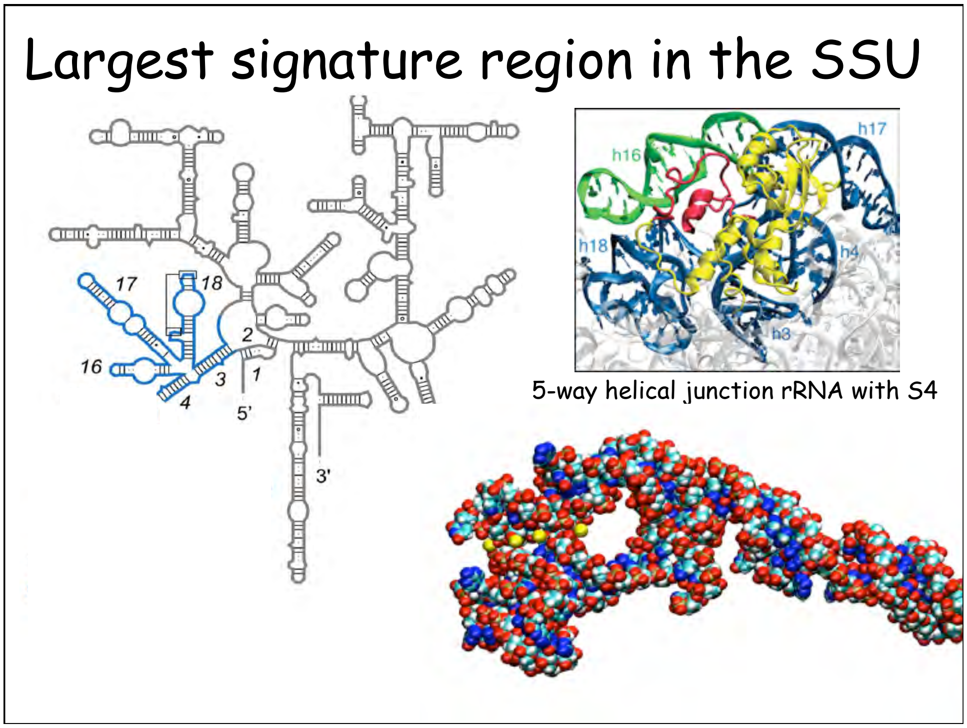
- Analysis of the ribosomal signatures in 90,000 new environmental samples shows that no “gray” area exists: a ribosome is either bacterial or archaeal in nature.

- Split across cluster: 10,000 sequence on each node



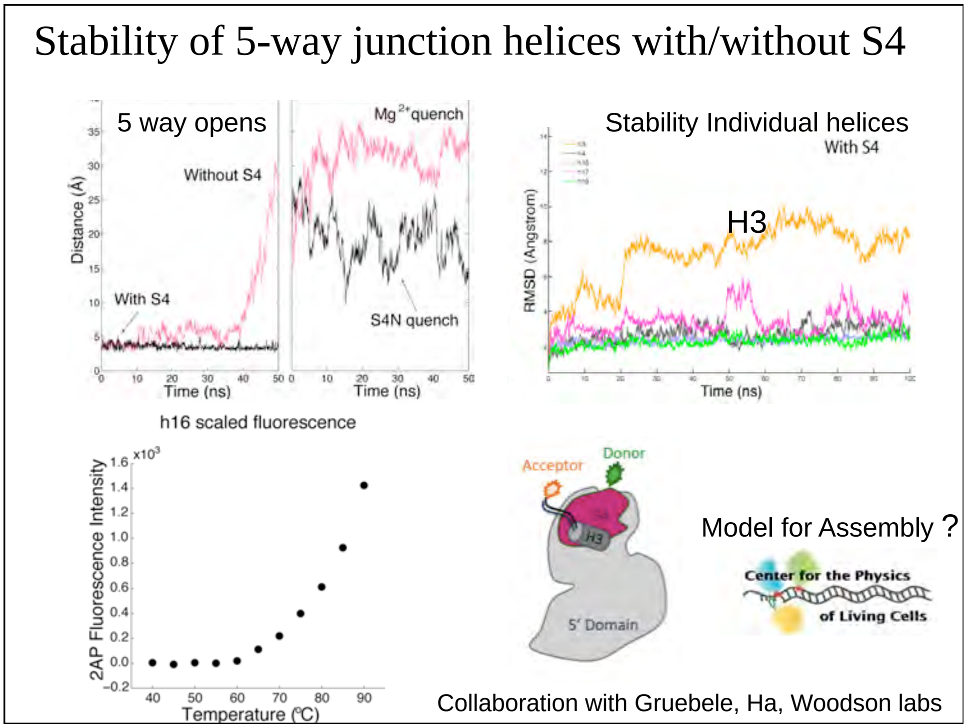
Data: “Greengenes”, Lawrence Berkeley

Roberts, et al. *PNAS* 2008



Largest signature region in the SSU

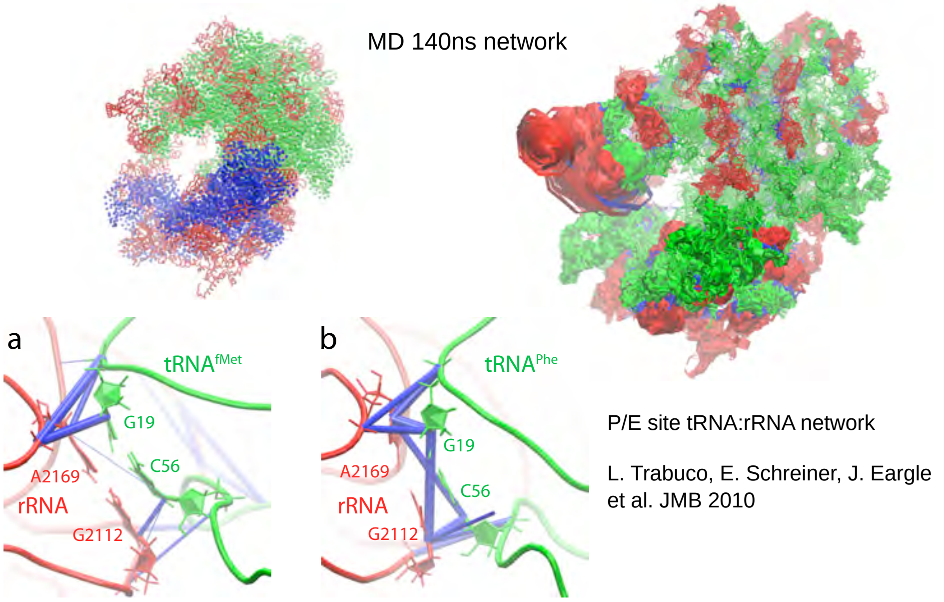
5-way helical junction rRNA with S4



Stability of 5-way junction helices with/without S4

Collaboration with Gruebele, Ha, Woodson labs

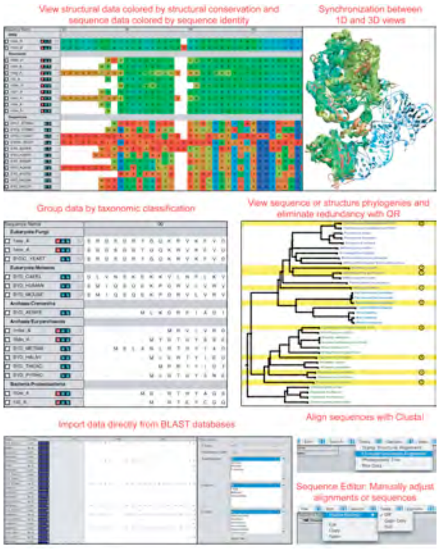
Signaling Networks in LSU &SSU



P/E site tRNA:rRNA network

L. Trabuco, E. Schreiner, J. Eargle et al. JMB 2010

New Tools in VMD/MultiSeq



Protein / RNA
Sequence Data

SwissProt DB (400K),
Greengenes RNA (100K)
Signatures, Zoom

Metadata Information,
Clustal &
Phylogenetic Trees

RAXml Trees,
Genomic Content,
Temperature DB

Blast & PsiBlast

Sequence Editor

Sequence /Structure
Alignment

Protein & RNA
secondary structure

QR non-redundant
seq / str sets

Cluster
analysis /
Bioinformatics
scripting

Tutorials MultiSeq/
AARS
EF-Tu/Ribosome

J. Eargle, D. Wright, Z. Luthey-Schulten, *Bioinformatics*, 22:504 (2006)
E. Roberts, J. Eargle, D. Wright, Z. Luthey-Schulten, *BMC Bioinformatics*, 7:382 (2006)

VMD/MultiSeq Tutorials

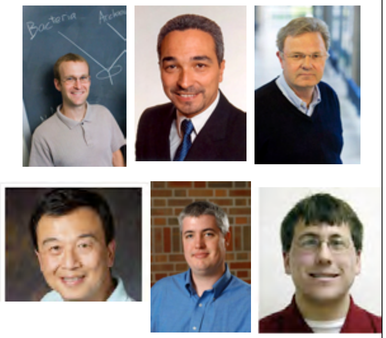
1. Evolution of Translation: AARS:tRNA
2. Evolution of Translation: EF-Tu:tRNA
3. Evolution of Translation: Ribosome
4. Dynamical Network Analysis
5. Participant's project

Acknowledgements

Collaborators
Carl Woese
Mike Heath

Martin Gruebele
Taekjip Ha
Sarah Woodson
Susan Martinis
Wen-Mei Hwu
John Stone
Julio Ortiz, W. Baumeister

Students/Postdocs
Alexis Black
Ke Chen
John Eargle
Li Li
Piyush Labhsetwar
Jonathan Montoya
Elijah Roberts
Anurag Sethi
Dan Wright
Mike Hallock



NIH, BI, NSF, DOE & Nvidia
Cuda Center of Excellence

NIH Resource for Macromolecular Modeling and Bioinformatics, Beckman Institute, UIUC – John Stone, Kirby Vandivort

Part III Towards in silico cells: Simulating processes in entire bacterial cells

See also Mcell

Fast Monte Carlo simulation methods for biological reaction-diffusion systems in solution and on surfaces

RA Kerr, TM Bartol, B Kaminsky, M Dittrich, JC Chang, SB Baden, TJ Sejnowski, and JR Stiles

SIAM J Sci Comput 30,3126 (2008)