RNA structural ensembles from energy landscape explorations

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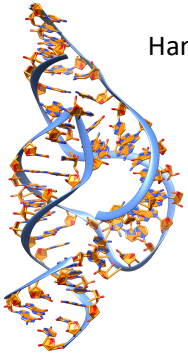
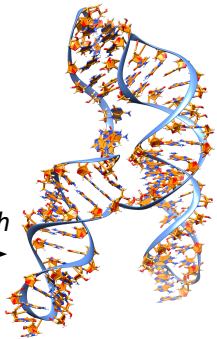
The importance of non-coding RNAs

Non-coding RNA **regulates** many **important processes**, e.g. in transcription or translation. Despite their centrality in regulation, ncRNAs are **underutilised** as **drug targets**.



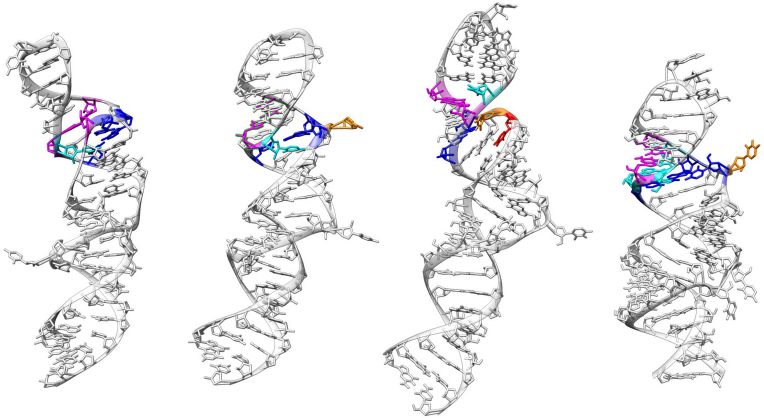
Hammerhead ribozyme
← *RNA cleavage*

RNA pseudoknot
from SARS CoV2
Regulatory switch →



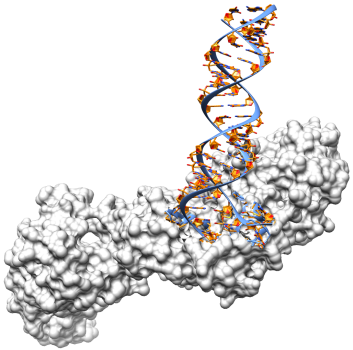
Structural polymorphism in RNAs

In contrast to proteins, RNAs exhibit **competing structural ensembles**. These may be characterisable in experiment, but their relative occupancy may be unobtainable.



External factors impacting RNA structural ensembles

- sequence changes
- epigenetic alterations
- environmental changes (pH, salt, ...)
- interaction partners
- ...



RNA-protein interaction in SARS-CoV2
Proofreading during RNA synthesis; critical for the viral life cycle

What insight can we gain from understanding RNA structures?

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- m6A methylation in a stem-loop within a RNA transcript leads to viral activation in Kaposi's sarcoma-associated herpesvirus.
  Why is single methyl group leading to viral activation and through which mechanism?

Exploring energy landscapes

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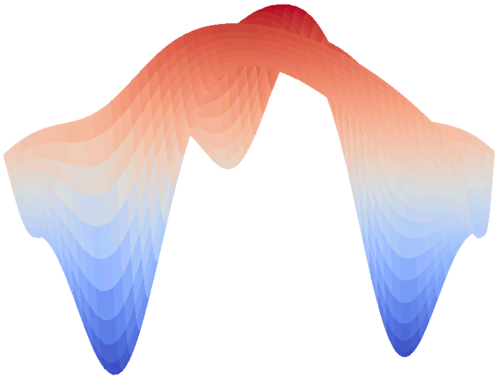
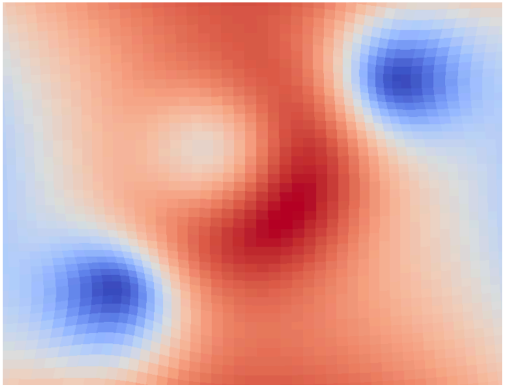
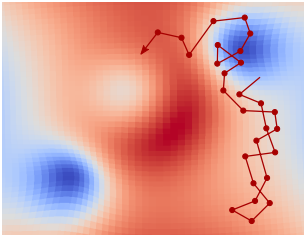


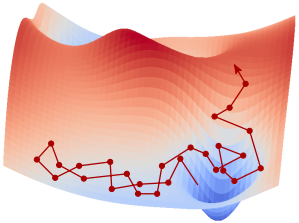


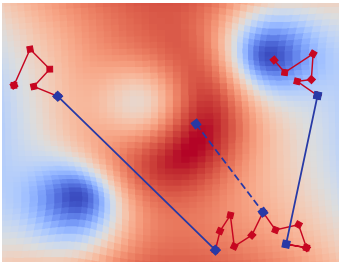
Classical explorations - MD and MC simulations

Molecular dynamics

- discrete time propagation using Newton's laws
- rare events remain rare
- need enhanced sampling methods
- challenge to simulate long time scales



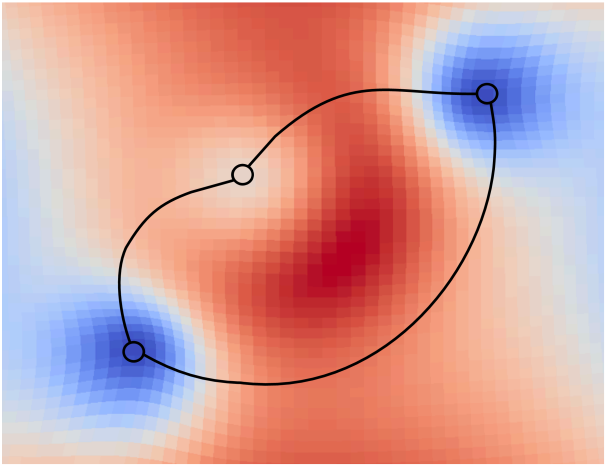




Monte Carlo sampling

- create "random" new configurations
- accept/reject based on their energy
- moves difficult to design

Changing the point of view: Minima and transition states



Stable configurations are **local minima** and transitions between them are characterised by Hessian-index 1 **saddle points** (*aka* transition states).

These stationary points **determine** structural, thermodynamic and kinetic **properties**.

Principles of the computational potential energy landscape framework

1. **Coarse-grain** the energy landscape as a set of minima and transition states.

For more details, see: Joseph *et al.*, *Chem. Commun.* 2017, Röder *et al.*, *Adv. Theory Simul.* 2019 and Röder and Wales, *Front. Mol. Biosci.* 2022

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6. **No projections** into lower dimensions or the use of reaction coordinates are required.

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Opening the bonnet - what we actually do

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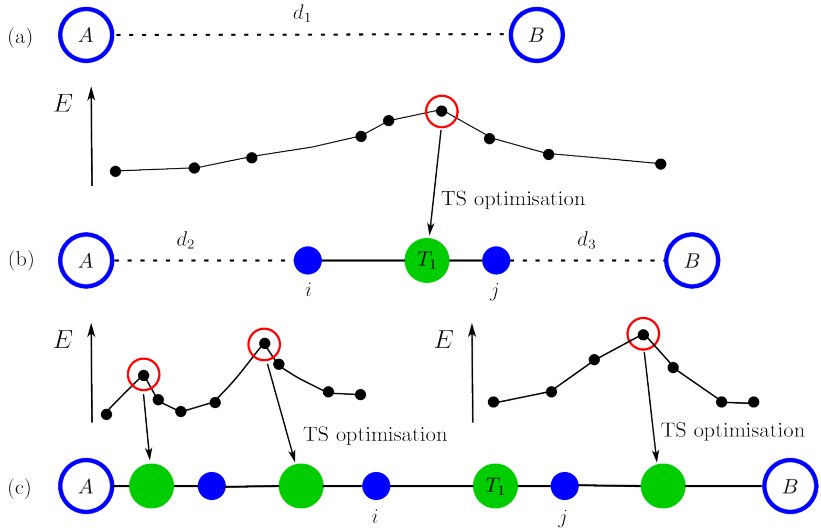

The regulatory role of 7SK RNA HP1

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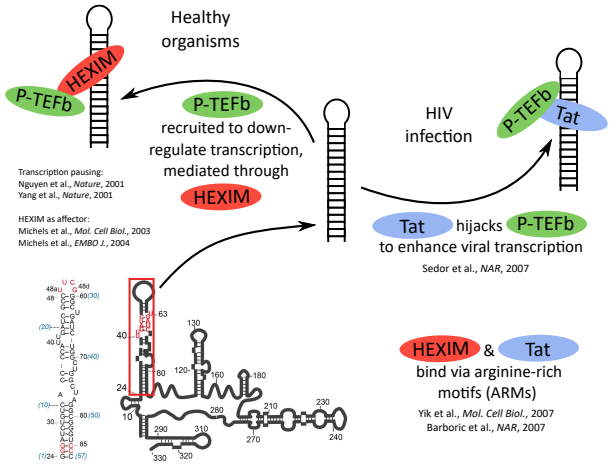

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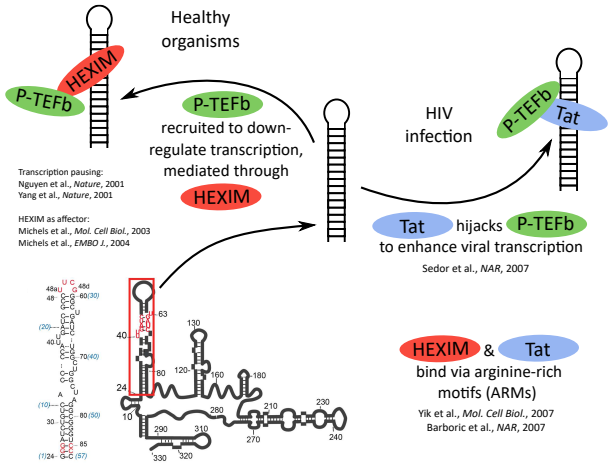

- HP1 hairpin contains binding motif

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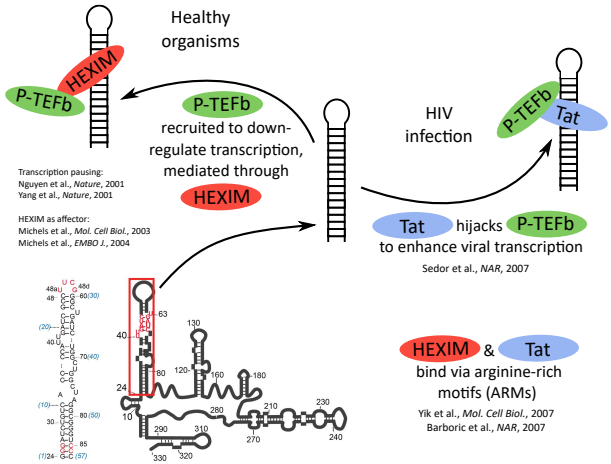

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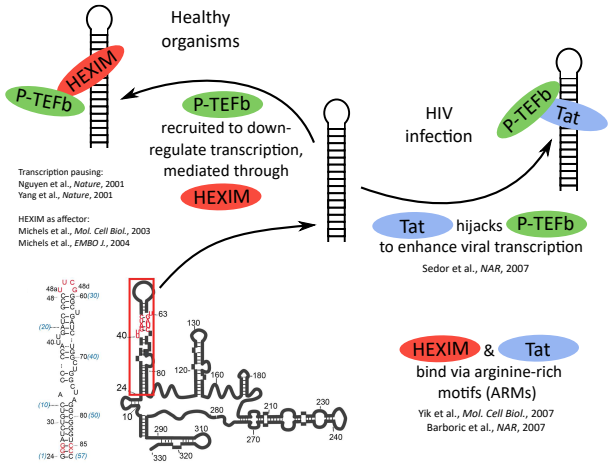

- HP1 hairpin contains binding motif
- use of stable, shortened model
- key motif: U(U)GAUC

Experimental structure - a puzzle with missing pieces

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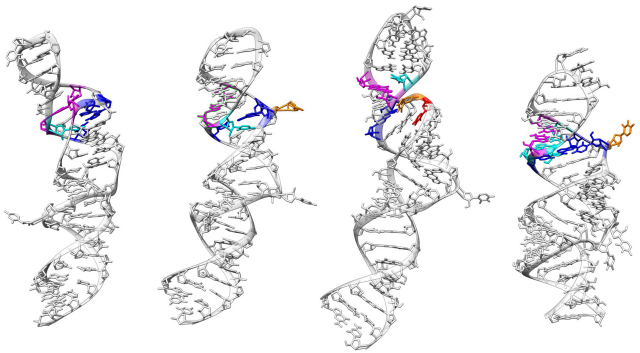

Two co-existing structures from crystallography and two structures from NMR

1,2 - Martinez-Zapien et al., *NAR*, 2017; 3 - Bourbigot et al., *RNA*, 2016; 4 - Pham et al., *Nat. Commun.*, 2018

How to explore the energy landscape

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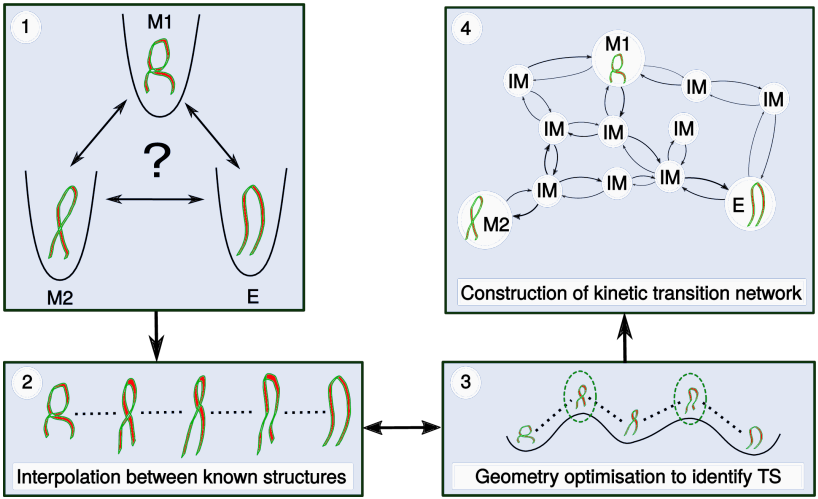

The energy landscape for the native sequence

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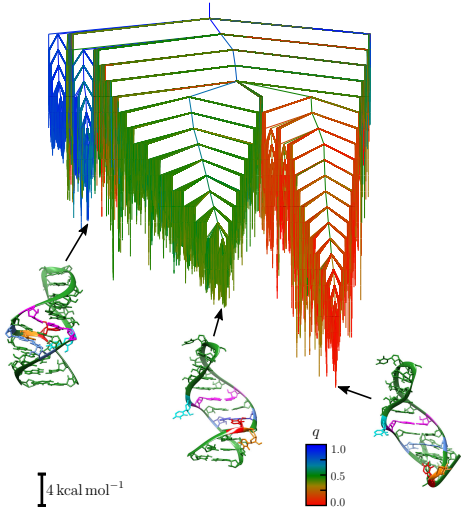

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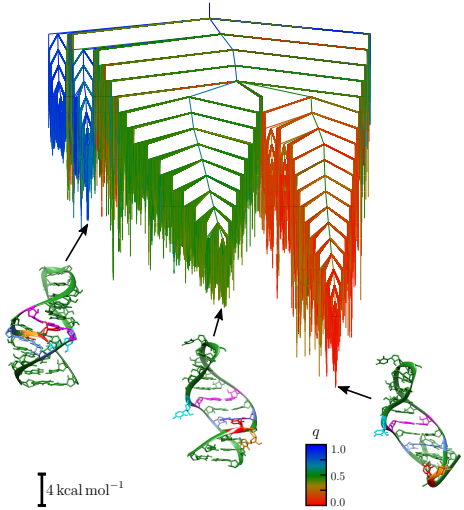
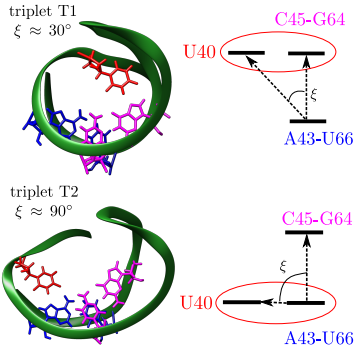


The configurations can be identified by an order parameter based on the angle ξ.



H-REX MD simulations in agreement

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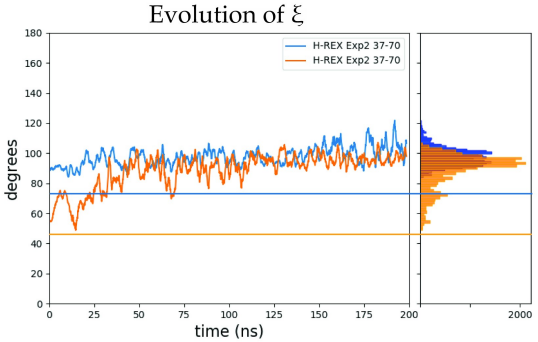

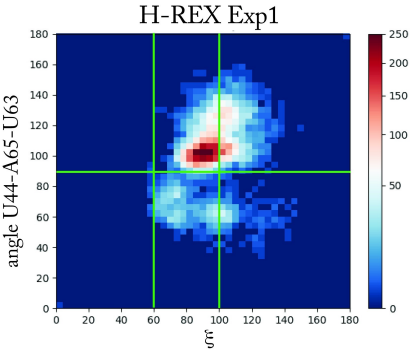

Binding of 7SK RNA HP1 and ARM

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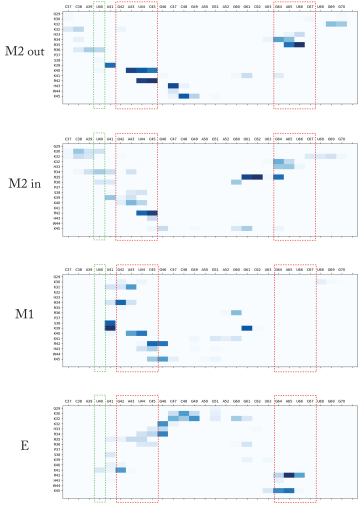
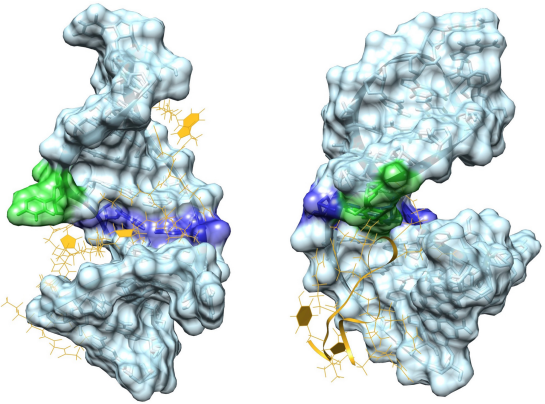
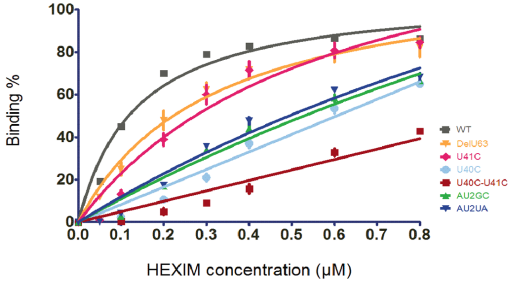
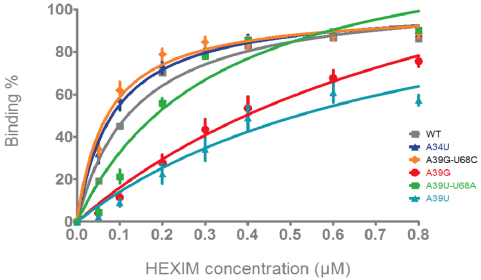




Mutations and their impact on peptide binding





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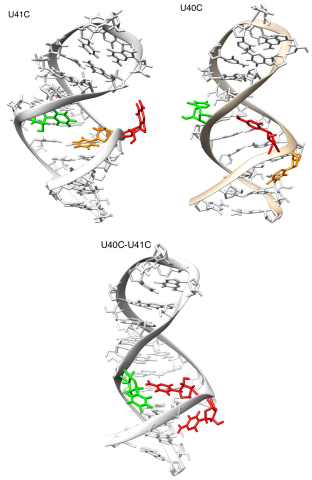

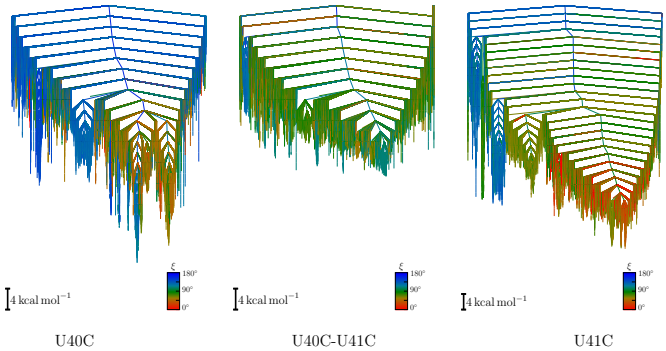

Side note: Evolutionary signatures in the energy landscapes

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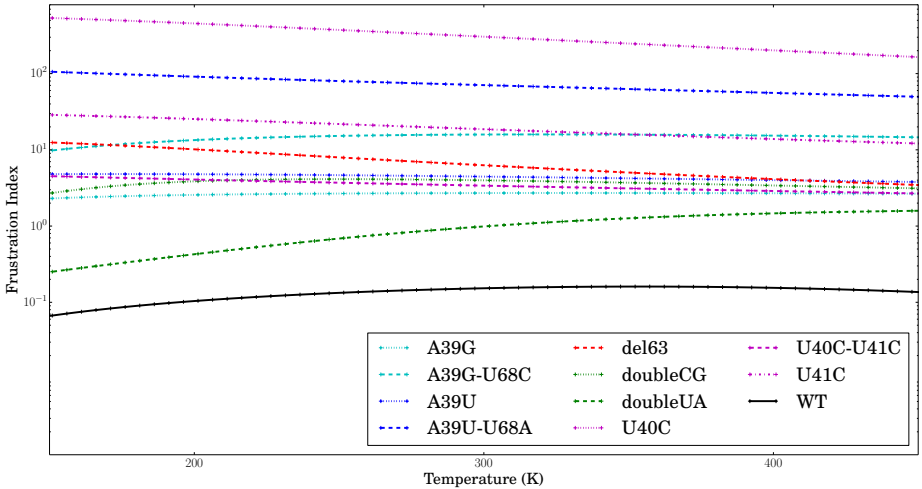

7SK RNA work - a summary

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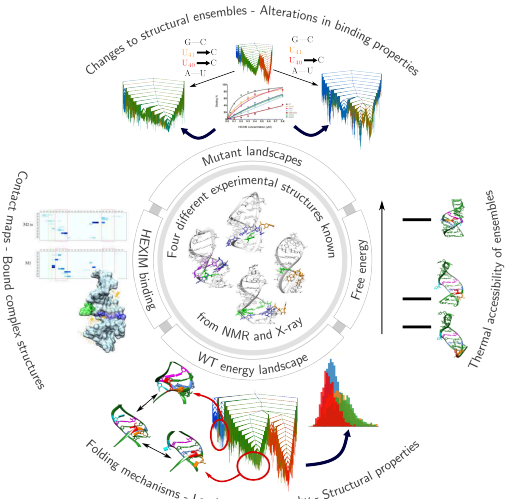

Röder et al., *Nucleic Acids Res.*, 2020

KSHV ORF50 transcript - viral reactivation

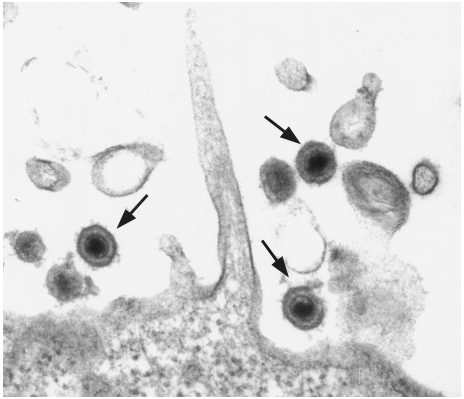

Image: Ablashi et al., *Clin. Microbiol. Rev.*, 2002

KSHV is a human **oncovirus**, and its transcription is relying on a viral protein encoded by **ORF-50**.

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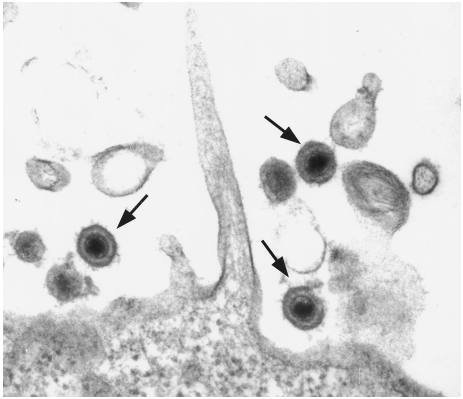

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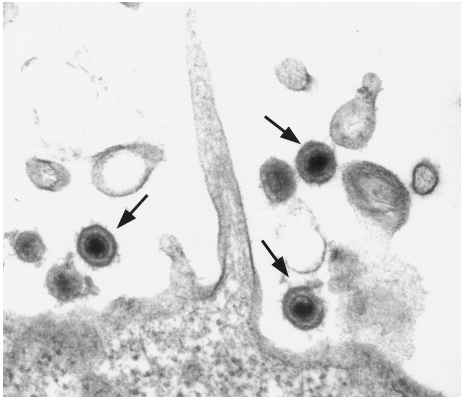

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m6A methylation of the ORF50 transcript enhances virus replication.

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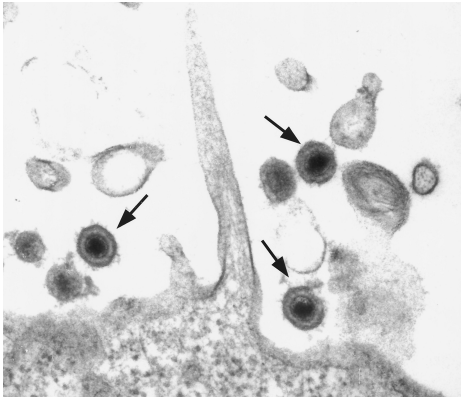

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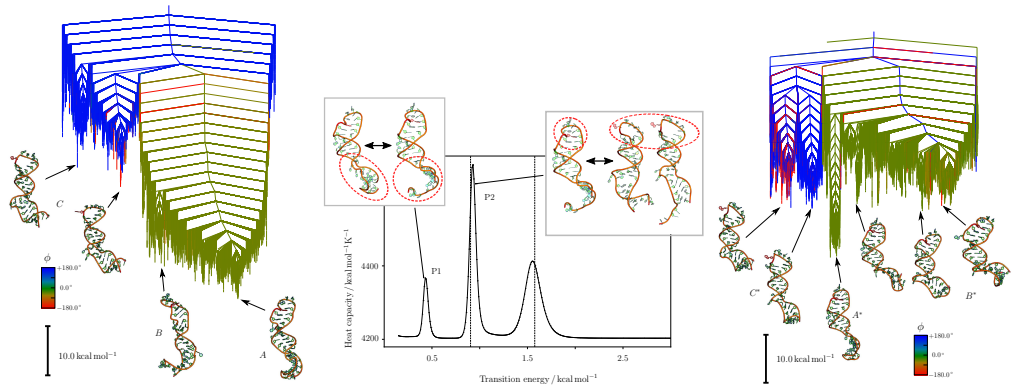
m6A modified site within the ORF50 transcript is a 43mer RNA stem-loop.

KSHV ORF50 transcript - m6A methylation changes everything



Röder et al., *PLoS Comput. Bio.*, 2022

Structure determines rates in tRNA-amino acyl transfer reactions

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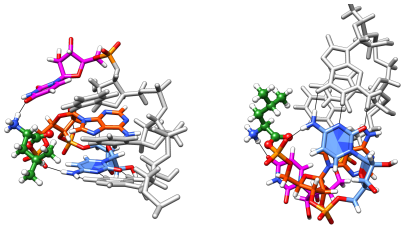

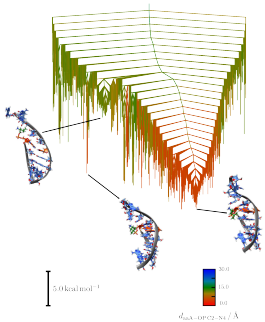

Amino acyl transfer in tRNA overhangs is dependent on the **tRNA stem** sequence.

Collaboration with Meng Su and John Sutherland, LMB, under review.

Rationalising bicyclic peptide design

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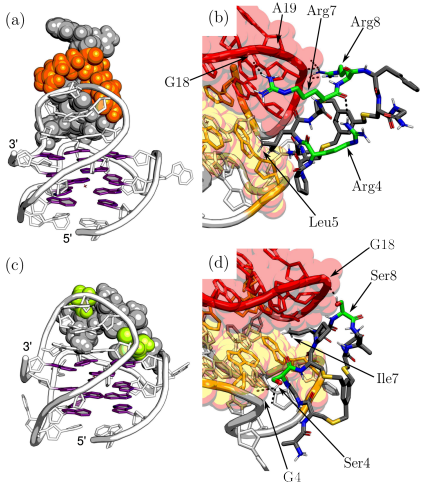
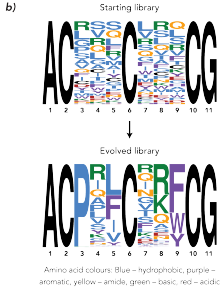
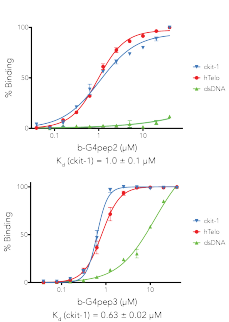


Bicyclic peptides mimic **protein-like binding** with G4s, allowing DNA **recognition by structure.**

Liu et al., *JACS*, 2020





(K)not complex ?!

SARS-CoV2 frameshifting RNA element employs **shifting to backtrack and pause** during translation. Graph-based computation and chemical probing show **multiple alternative structures**. Schlick et al., *JACS*, 2021

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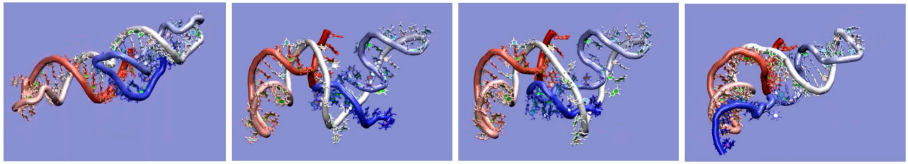

QCI - the magic ingredient

The Problem

- NEB and DNEB rely on **discrete** images.
- Even if neighbouring images are physical (correct cis-trans isomers, chirality, ...), the continuous path between the might be **unphysical**.
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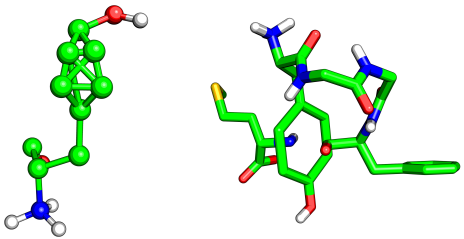

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Such unphysical structures **can be detected** with relative ease, and often (not always!) they leave high-energy signatures. Specifically designed **sampling algorithms** also help.

```
              E+        Ets - E+           Ets       Ets - E-           E-          S       D      gamma   ~N
  -10013.5448812  3.8330       -10009.7118548  3.8330       -10013.5448812   3.907   3.0731537.088   3.000
 chirality_check> Atom   996          inverted.
 chirality_check> Originally S chirality.
 chirality_check> Atom   996          inverted.
 chirality_check> Originally S chirality.
tryconnect> Chirality inversion detected in at least one of the carbon centres, rejecting
```

```
       E+        Ets - E+           Ets       Ets - E-           E-          S       D      gamma   ~N
  -9986.9012644  21.360        -9965.5408247  13.747        -9979.2879197  86.082  65.325 172.965  26.659
 cis_trans_check> Peptide bond    29    28    30    31 changed to cis.
 cis_trans_check> Originally trans.
 cis_trans_check> Peptide bond    29    28    30    31 changed to cis.
 cis_trans_check> Originally trans.
tryconnect> Cis-trans isomerisation of a peptide bond detected (wrt. the original structure), rejecting
tryconnect> Transition state with energy      -9965.540825     ignored, cis-trans isomerisation detected in one or more peptide bonds.
```

```
 tryconnect> 400-iteration DNEB run for minima 1_S and 2_F using 40 images  (DNEB attempt #1)  ...
 lbfgs> Cold fusion diagnosed - step discarded, energy, limit=    -2054448299.        -1000000.000
 lbfgs> Final DNEB force constant      10.00000000
 Time to go through NEB:   0.553106999999997
 Double-ended search iterations= 0 RMS= ******************** Dev= 0.00% S= 0.00 time= 0.55
```

QCI - the magic ingredient

The Solution - Quasi-continuous interpolation (QCI)

Auxiliary potential for interpolation

- Constraint term: chemical bonds and geometric constraints (planarity, cis-trans ...)
- Repulsion term: Coulombic repulsion for non-constraint atom at close proximity
- Quasi-continuous term: repulsion based on local minima in the distances between bonds in adjacent images

Wales and Carr, *JCTC*, 2012 and Röder and Wales, *JCTC*, 2018

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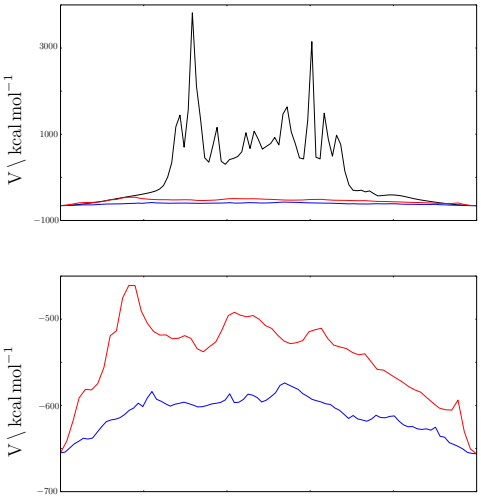

Simplifying things

Use of multi-scale simulations for further acceleration

Require a physical coarse-grained scheme with enough information to obtain good all-atom structures.

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Use of HiRE-RNA (High-resolution CG Energy Model for RNA)

- potential: Pasquali and Derreumaux, *JPCB*, 2010 and Cragnolini et al., *JCTC*, 2015
- 6/7 bead per nucleotide model
- physically motivated with focus on non-canonical interactions

A test case - PK1 pseudoknot

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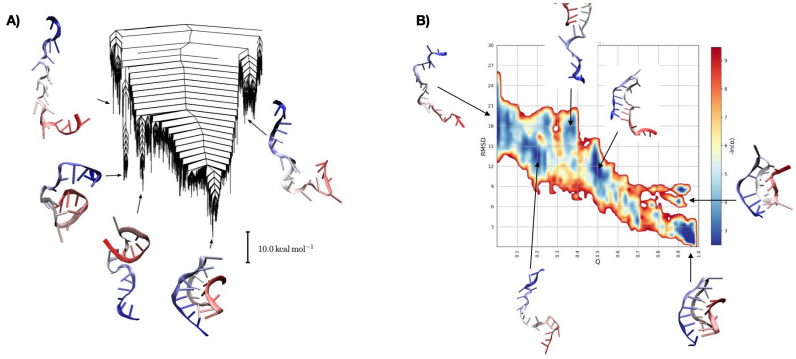
Pseudoknot1 (PK1) from the thermophilic bacterium *A.aeolicus* - predicted to be **smallest pseudoknot** (21 nucleotides) and NMR studies available.
All-atom ff with different methods: Correct folding/unfolding of PK1



Röder et al., *QRB Discovery*, 2022

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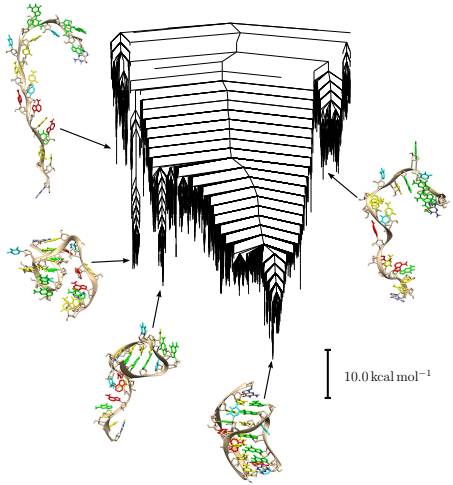

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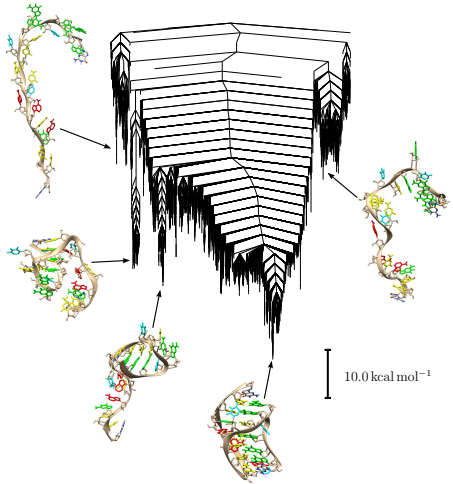

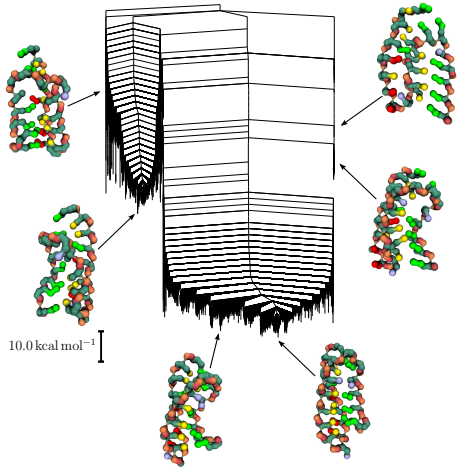

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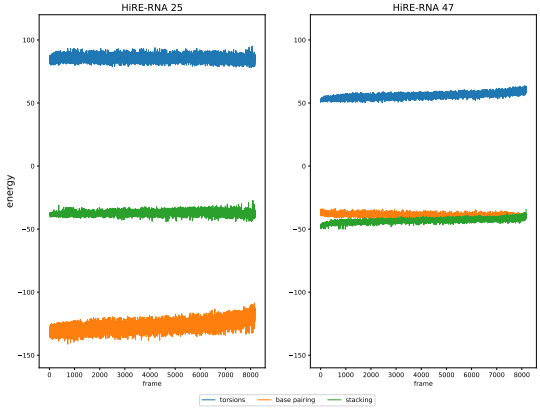

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Acknowledgements

Collaborations

Prof. Samuela Paquali (Université de Paris Cité, France)
Prof. David J. Wales (University of Cambridge, UK)
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Dr Meng Su and Prof. John Sutherland (LMB, Cambridge, UK)
Dr Kim C. Liu (University of Cambridge and LMB, UK)

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