Designing Exoribonuclease resistant Riboswitches

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39th Winterseminar Bled

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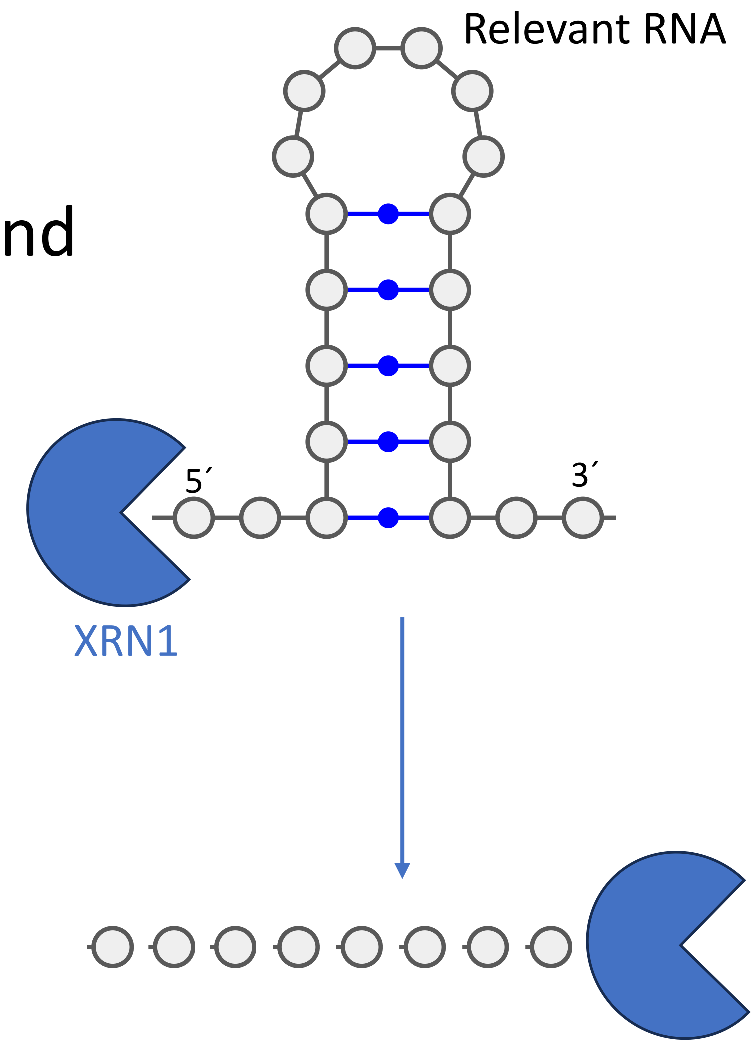
Introduction

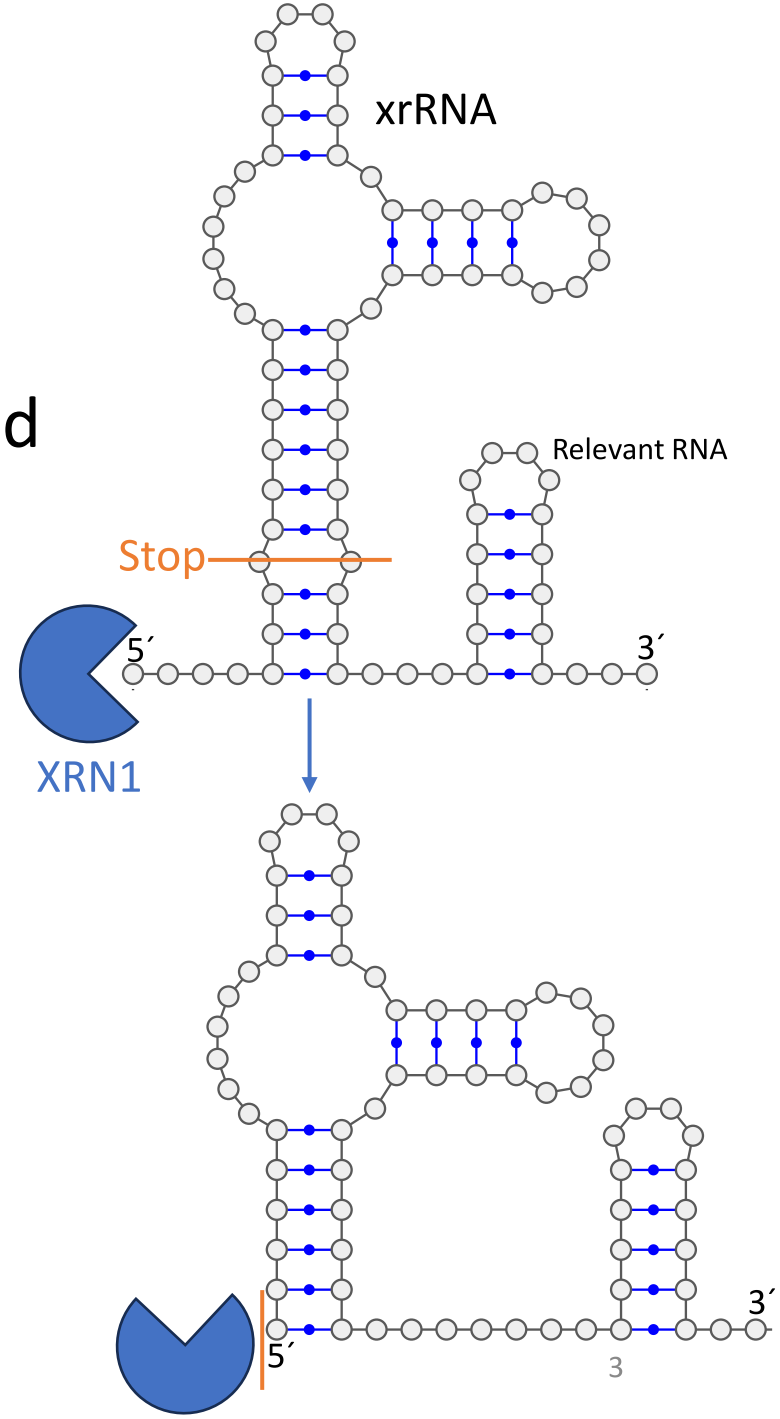
- Exoribonuclease (XRN1) degrades RNA from 5´ end



Introduction

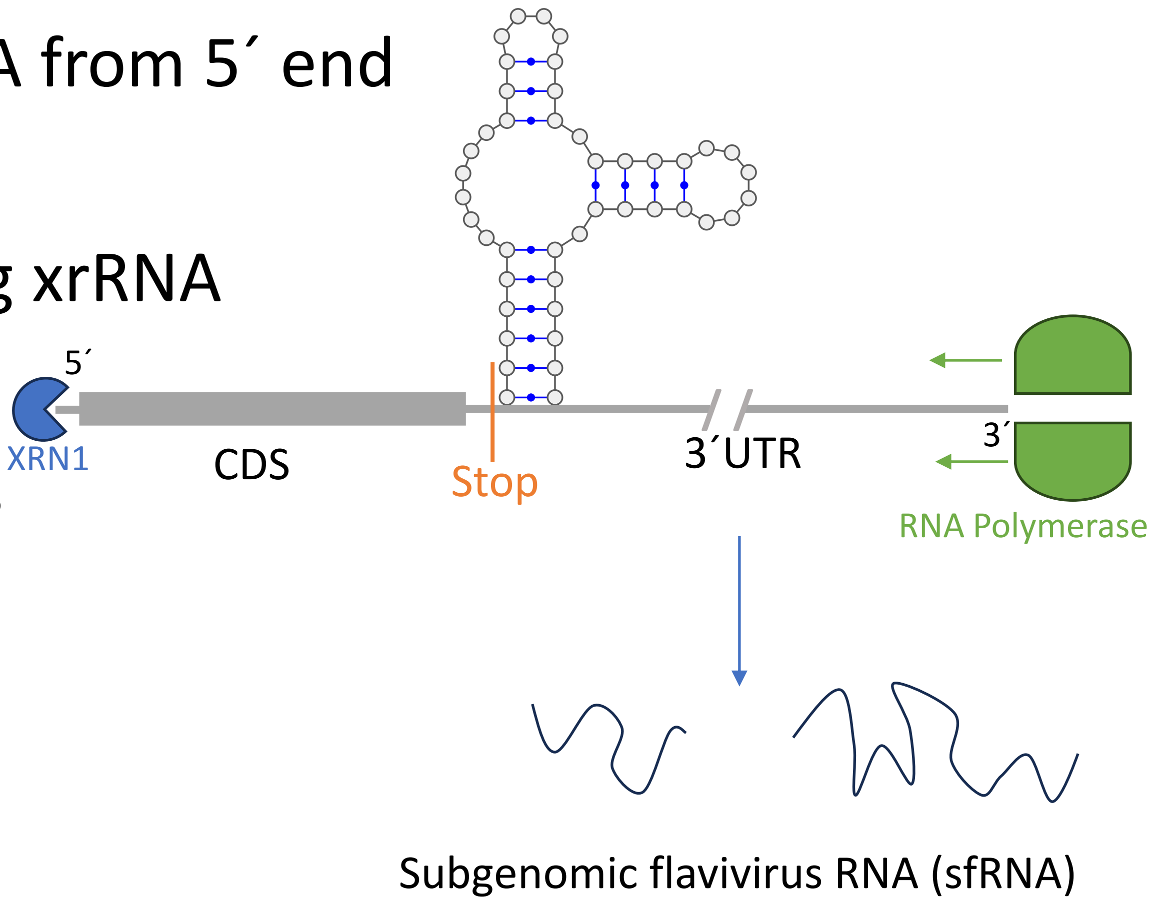
- Exoribonuclease (XRN1) degrades RNA from 5´ end

- Flaviviruses protect their 3‘ UTR using xrRNA



Introduction

- Exoribonuclease (XRN1) degrades RNA from 5´ end
- Flaviviruses protect their 3‘ UTR using xrRNA
- Essential for pathogenesis of the virus
- Must be replicatable from the 3`end



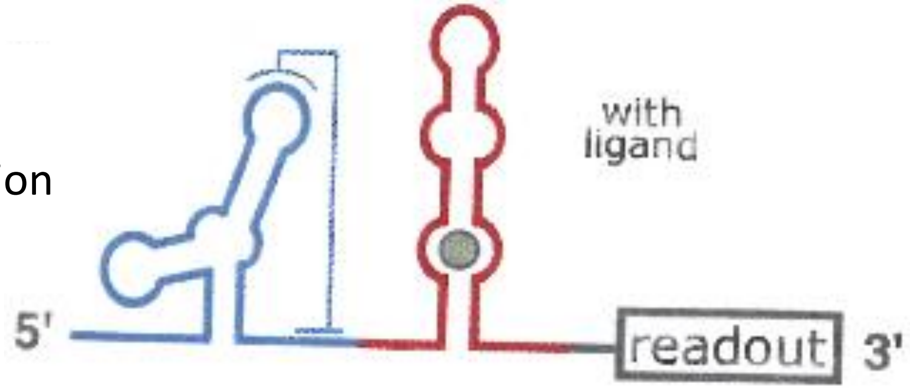
Project Goals

- Design synthetic xrRNA

- Final goal: Design a riboswitch with a synthetic xrRNA

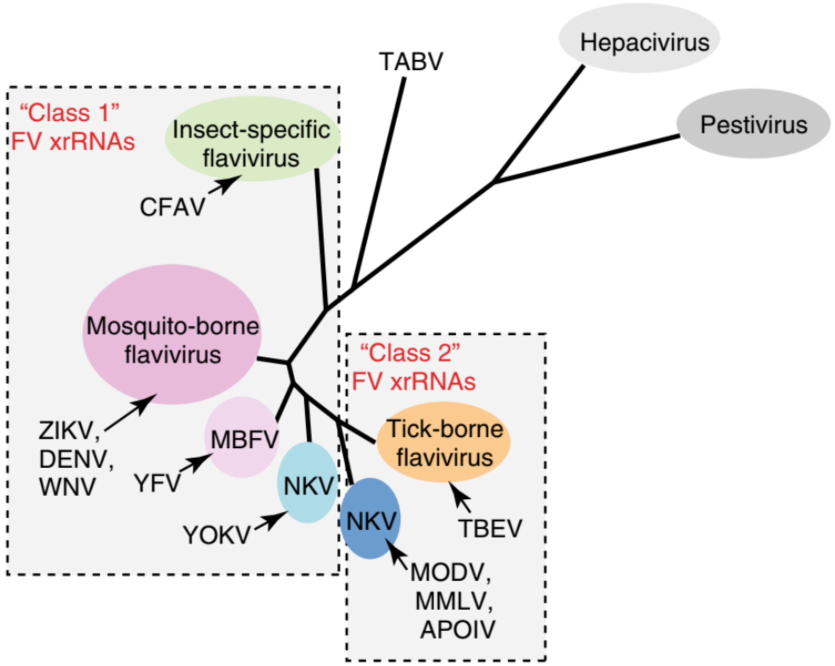
Off-configuration

On-configuration



First Task: Characterization

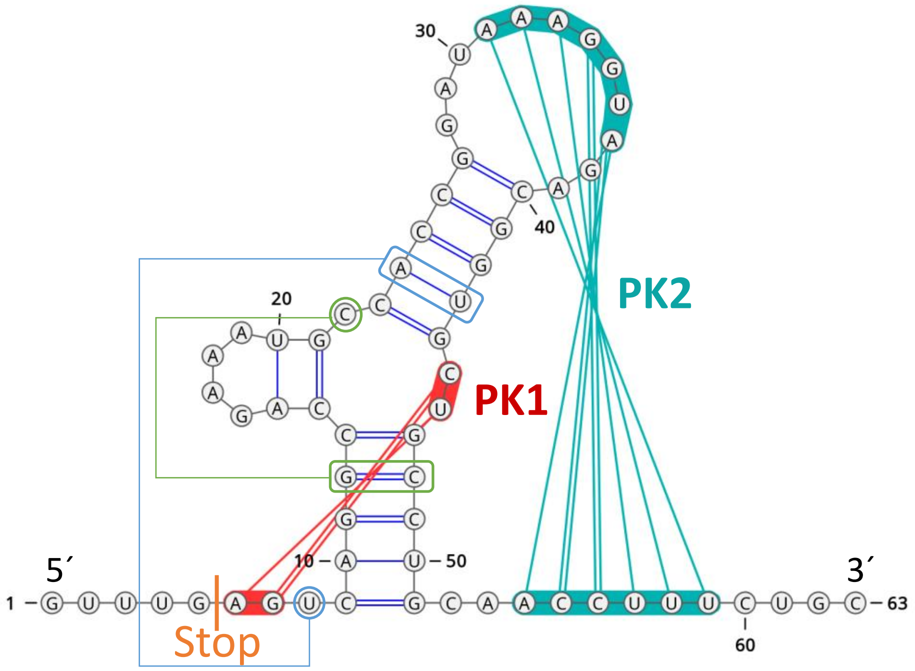
- Need to understand what we want to design
- Which features are important for function?
- Two classes of xrRNA with different structures
- We know more about the first class
- Very little experimental data on the second class



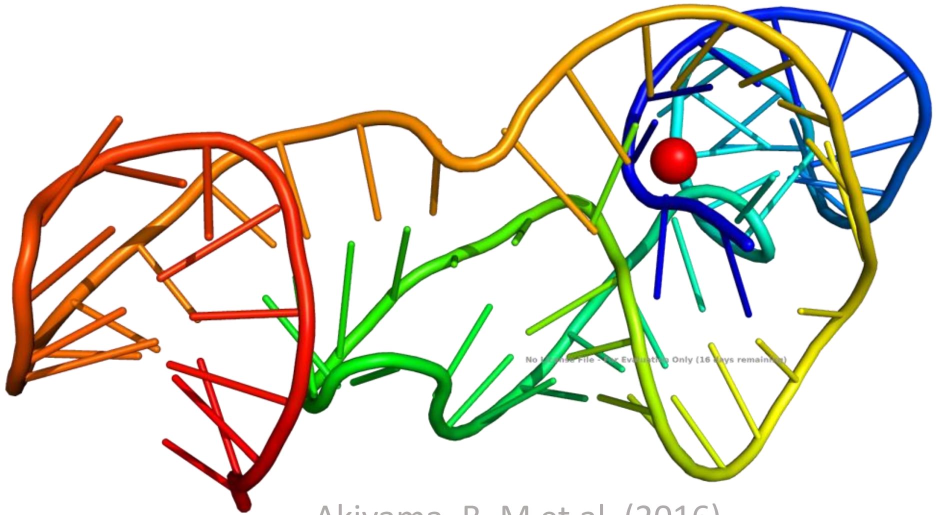
MacFadden, A. et al. (2018)

Class I

- Two important pseudoknots
- Two basetriples
- Functions via a ringlike Structure which sterically blocks the XRN1
- Can be unfolded from the 3´ end



Denis Skibinski



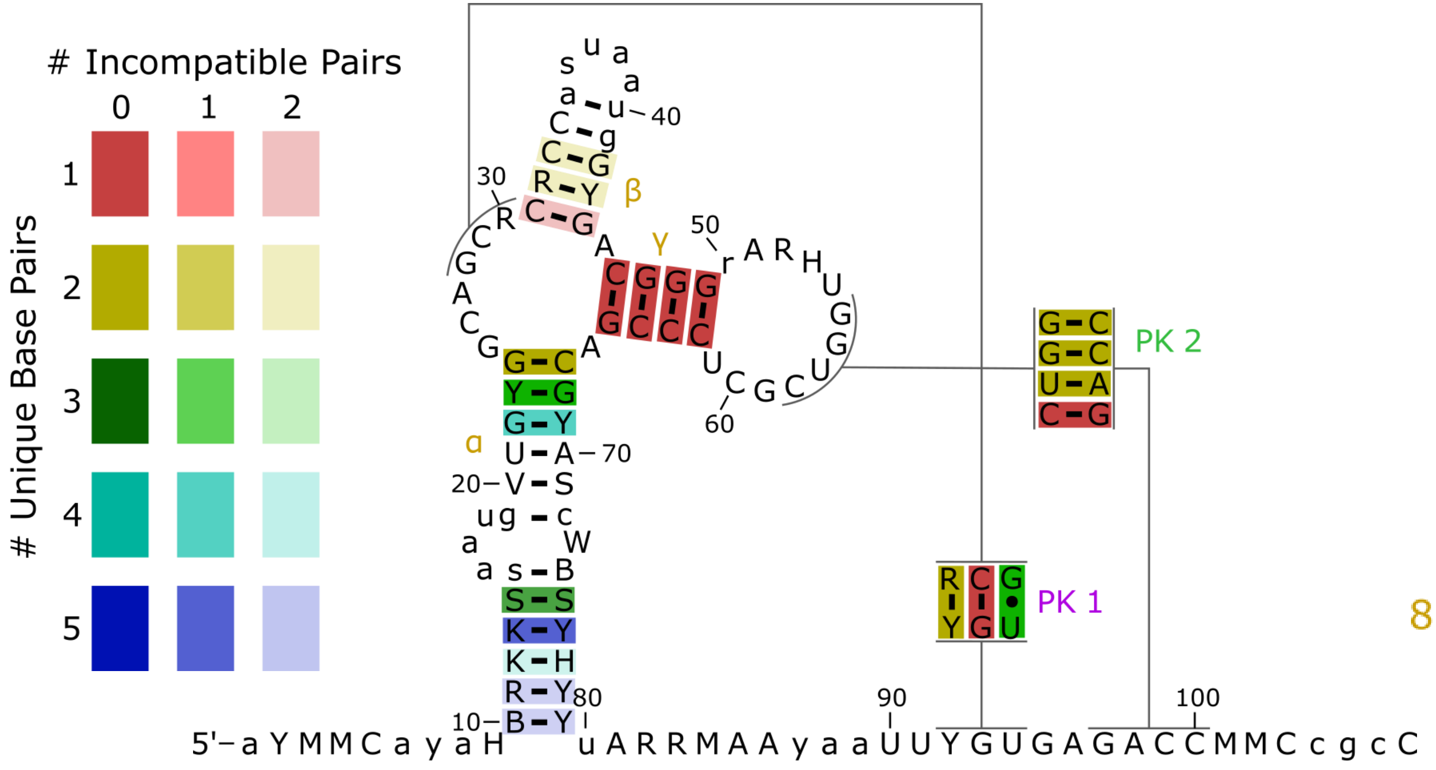
Akiyama, B. M et al. (2016)

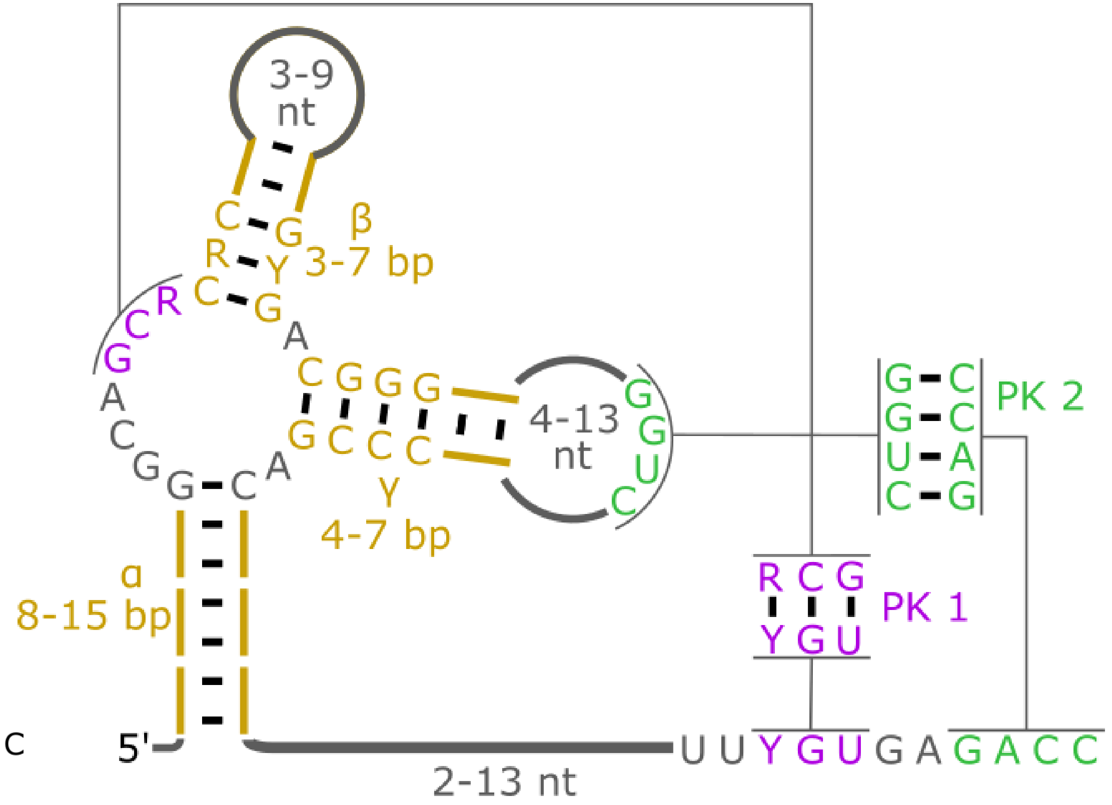
Class II – What we began with

- Sequences (1-100) from 14 different spezies that contain xrRNA

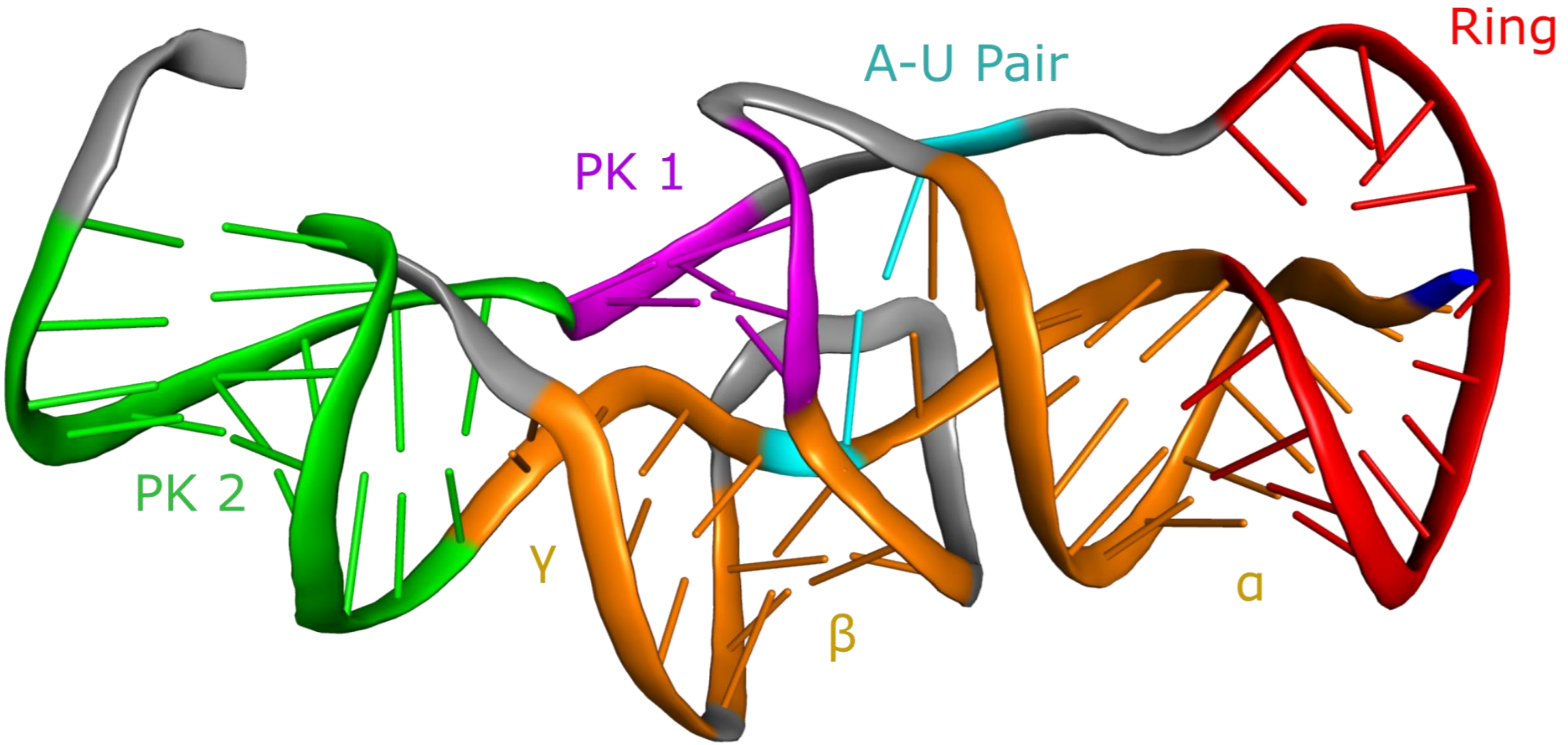
- The assumtion, that the mechanism is somewhat similar to Class I

Class II – Proposed Structure





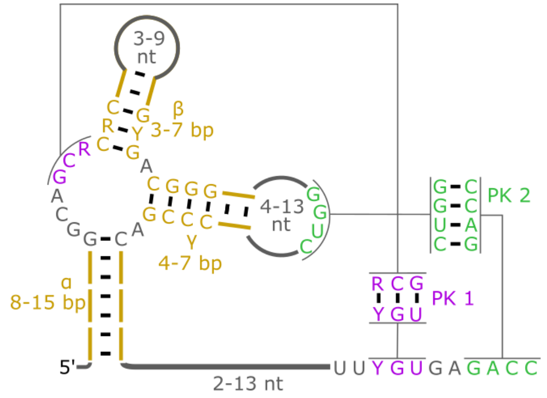
Class II – 3D Structure



Outlook – Design

- Encode known Information as Constraints in Infrared

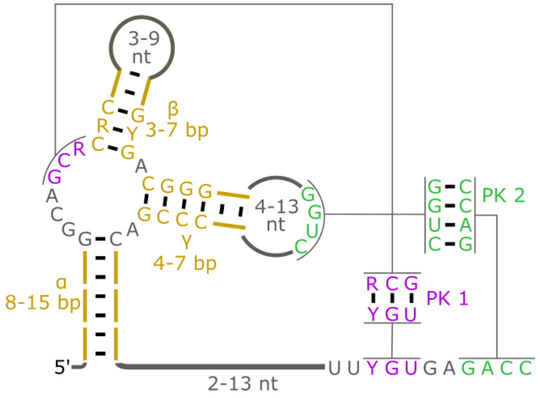
- Start with designing simple xrRNA



Yao, Hua-Ting, et al (2023)

Outlook – Design

- Encode known Information as Constraints in Infrared
- Start with designing simple xrRNA
- Design functional Riboswitches
- Continiuous experimental evaluation

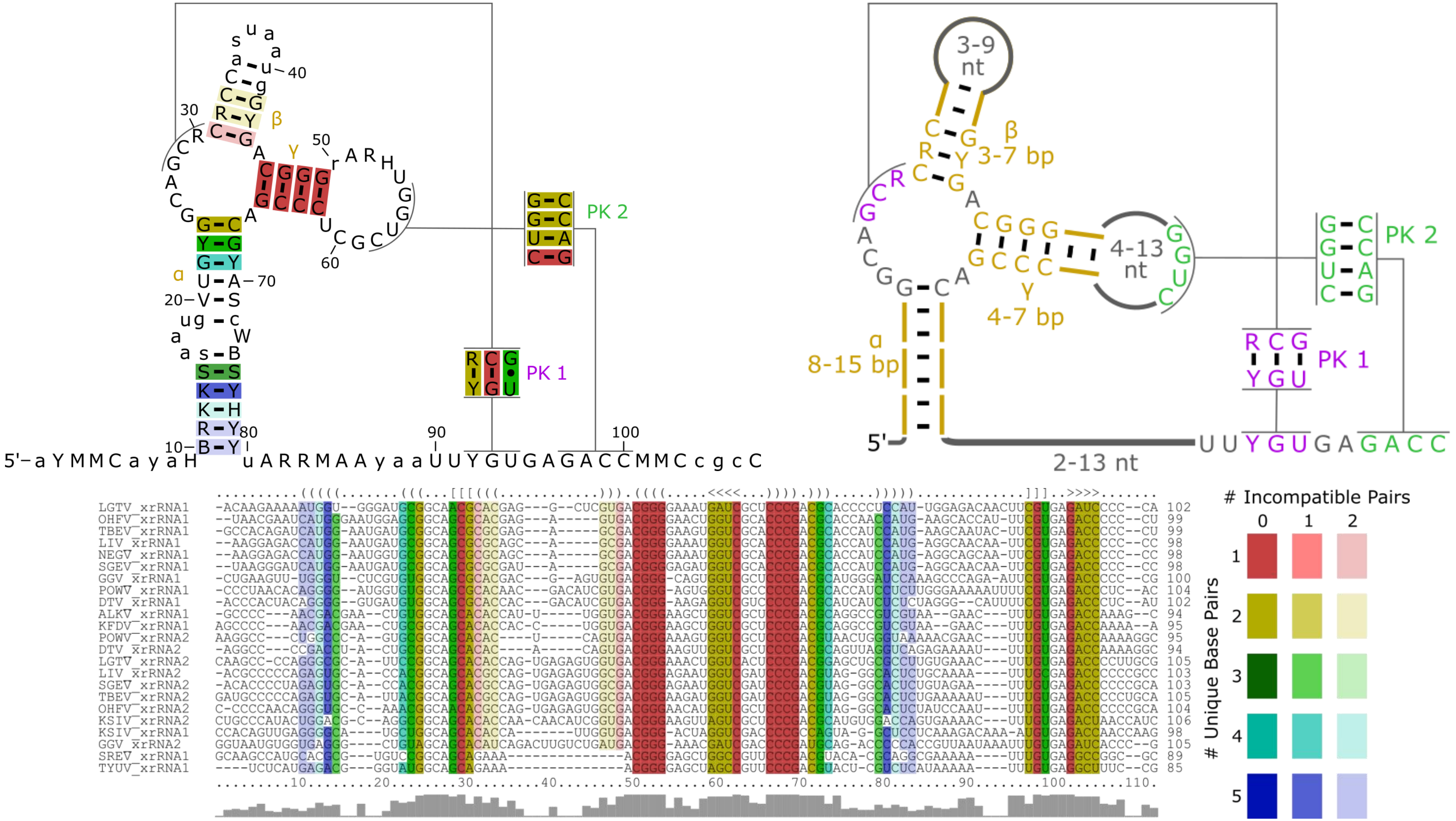


Yao, Hua-Ting, et al (2023)

Thank you for your attention

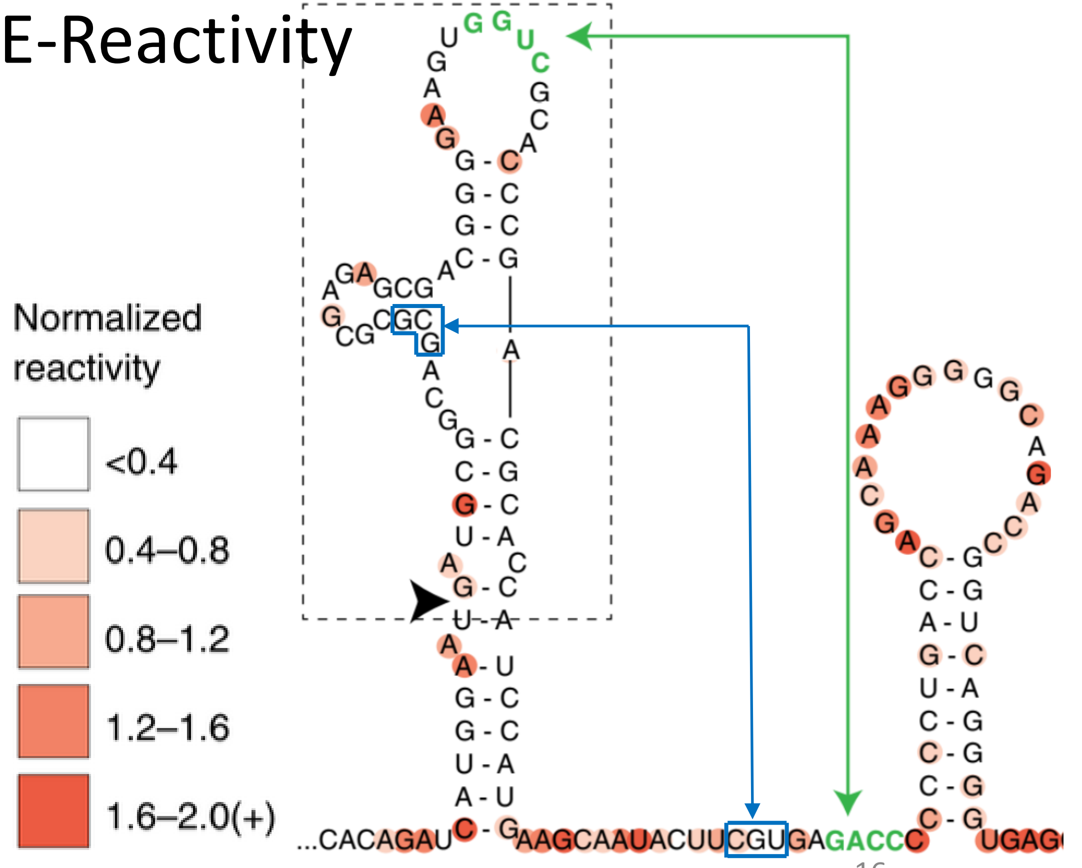
Backup

Class II – Proposed Structure



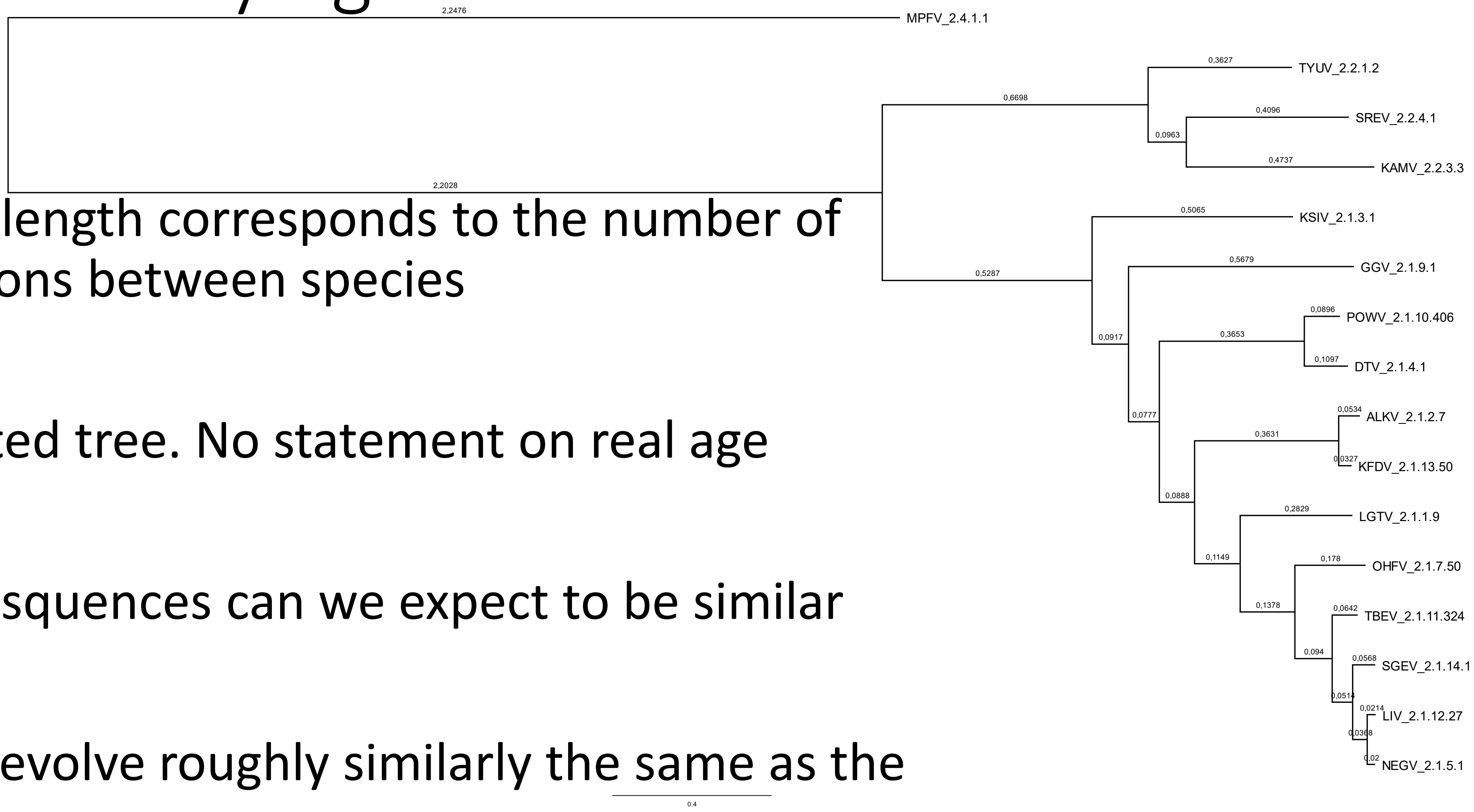
Class II – Reasons we belive in PK2

1. Possible in nearly every single sequence. Shows covariance
2. Consistent with the experimental SHAPE-Reactivity



McFadden 2018

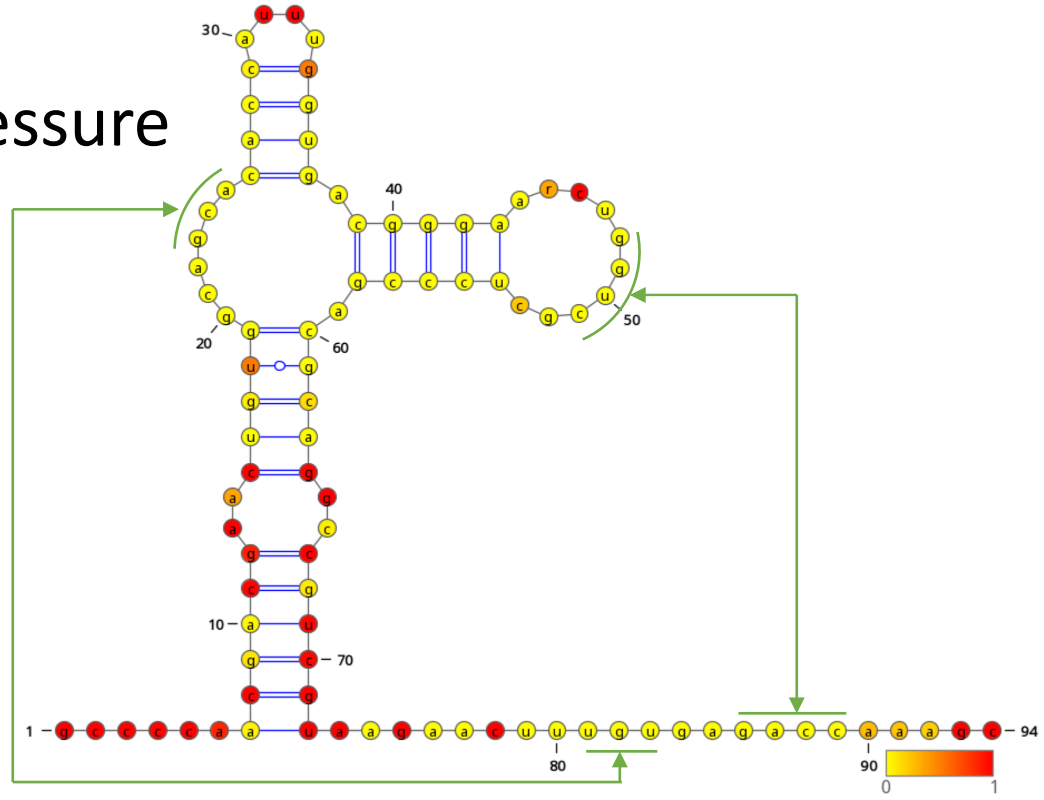
Class II – Phylogenetic Tree



- Branchlength corresponds to the number of mutations between species
- Unrooted tree. No statement on real age
- Which squences can we expect to be similar
- xrRNA evolve roughly similarly the same as the whole genome

Class II – Evolutionary Pressure

- Models that calculate a consensus tree assign each base position a change rate
- Can be interpreted as evolutioary pressure
- Consistent with both pseudoknots
- Threeway junction has low rate



Class II: Nr of Sequences

```
[sidl@coridan tbfv_stk]$ wc -l *.stk
    7 ALKV_xrRNA1.stk
    7 DTV_xrRNA1.stk
    7 DTV_xrRNA2.stk
    7 GGV_xrRNA1.stk
    7 GGV_xrRNA2.stk
    9 KFDV_xrRNA1.stk
    8 KSIV_xrRNA1.stk
    8 KSIV_xrRNA2.stk
    7 LGTV_xrRNA1.stk
    7 LGTV_xrRNA2.stk
   22 LIV_xrRNA1.stk
   21 LIV_xrRNA2.stk
    8 MPFV_xrRNA1.stk
    7 NEGV_xrRNA1.stk
    7 NEGV_xrRNA2.stk
   16 OHFV_xrRNA1.stk
   13 OHFV_xrRNA2.stk
   28 POWV_xrRNA1.stk
   52 POWV_xrRNA2.stk
    7 SGEV_xrRNA1.stk
    7 SGEV_xrRNA2.stk
   70 TBEV_xrRNA1.stk
  102 TBEV_xrRNA2.stk
    7 TYUV_xrRNA1.stk
   10 XiFV_xrRNA1.stk
  451 total
[sidl@coridan tbfv_stk]$
```

Riboswitch Ligand

- Thephyllin