Modified Nucleotides in RNA structure prediction

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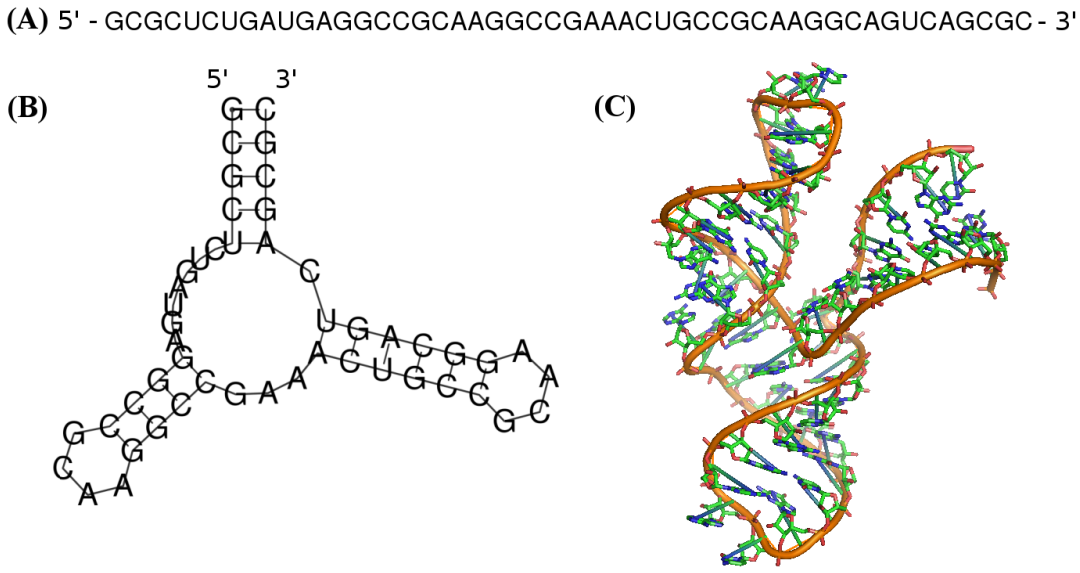
RNA Secondary Structures

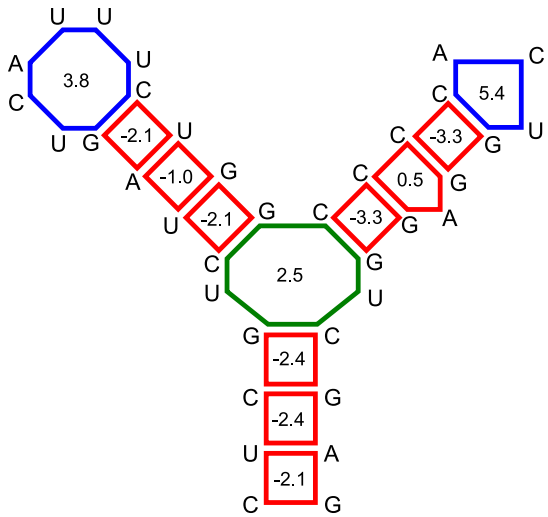
RNAs fold hierarchical (A) $\rightarrow$ (B) $\rightarrow$ (C)

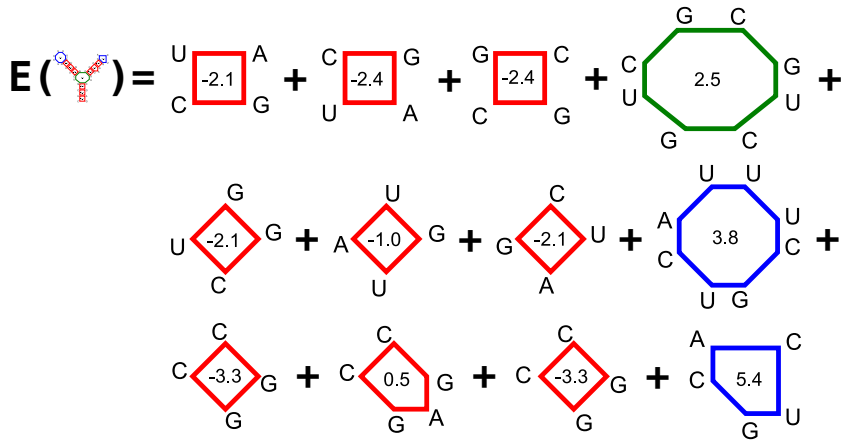


Secondary Structure (B):

- Set of nested base pairs
- Captures majority of stabilizing interactions
- Many thermodynamic properties can be predicted efficiently
- Very good prediction accuracy for small RNAs
- Starting point for 3D structure modeling
- Good abstraction to perform folding kinetics simulation

RNA Secondary Structures - Nearest Neighbor Energy Model





- Secondary structures s can be uniquely decomposed into loops L
- Contributions of a base pair only depends on neighboring pairs
- Each loop L is assigned a free energy contribution E_L[1]

$$E(s) \approx \sum_{L \in s} E_L$$

[1] Turner et al., “NNDB: The nearest neighbor parameter database for predicting stability of nucleic acid secondary structure.”, 2009, NAR 38, D280–D282

RNA Secondary Structures - Statistical Thermodynamics

$$
\begin{aligned}
p(F) &\propto e^{-\beta E(F)}, \quad \text{with} \quad \beta = \frac{1}{RT} \\
&= \frac{1}{Q} \sum_{s|F \in s} e^{-\beta E(s)}, \quad \text{with} \quad Q = \sum_{s} e^{-\beta E(s)}
\end{aligned}
$$

Efficient dynamic programming algorithms are available to compute

- Minimum free energy $MFE = \min_s E(s)$
- Partition function Q
- Probabilities p_{kl} for base pairs (k,l), i.e. $p_{kl} = p(F)$ with $F = (k,l)$
- Suboptimal and locally stable structures, ligand binding, ...

The `ViennaRNA Package`[2] provides fast implementations for

- single sequences
- (multiple) interacting sequences
- sequence alignments (consensus structure)

[2] Lorenz et al., "ViennaRNA Package 2.0.", 2011, ALMOB 6.1, 26

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Implementations are restricted to unmodified bases!

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RNA Secondary Structure - Modified Bases

Many RNAs require modified bases to fulfill their function!

- Modifications may change pairing partner preference
- Modifications may (de-)stabilize loop formation
- `Modomics` Database[3] lists 172 different modified bases
- Only a few energy parameters involving modified bases available, e.g. stacking with $\Psi - A$[4] and $I - U$[5] pairs

How to incorporate modified bases in predictions?

- Use constraints
  1. Mask modified bases, e.g. to stay unpaired using letter “N”
  2. Write wrapper (Python/Perl/C/C++) using the *constraints framework* of the `ViennaRNA Package`[6] to supplement energy parameters
- Re-implement algorithms with
  1. enhanced nucleotide alphabet
  2. additional pairing rules
  3. more energy parameters

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[3] Boccaletto et al., “MODOMICS: a database of RNA modification pathways. 2017 update.”, 2018, NAR 46.D1, D303–D307

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[5] Wright et al., “Nearest Neighbor Parameters for Inosine-Uridine Pairs in RNA Duplexes.”, 2007, Biochemistry 46, 4625–4634

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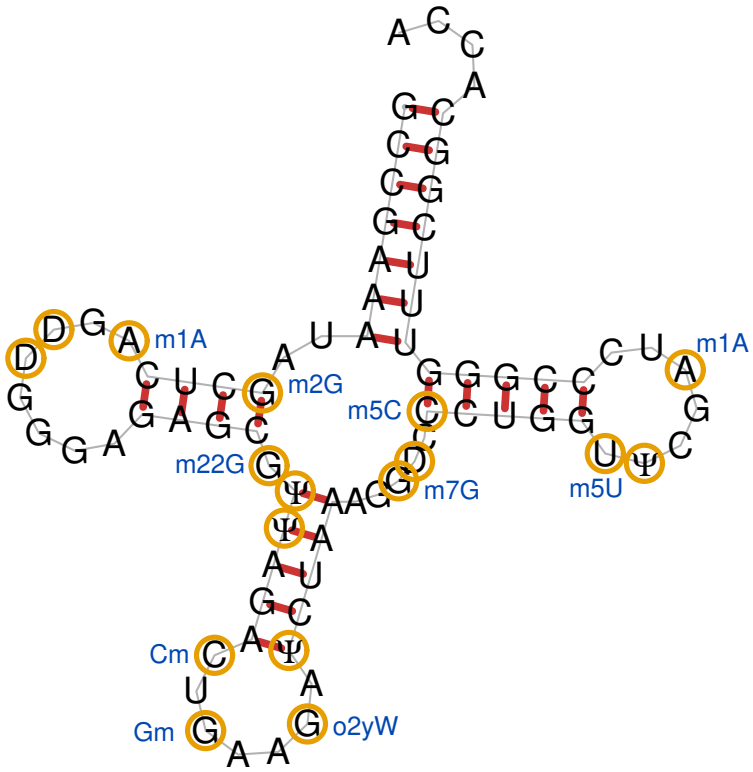
Example: human tRNA Phe (tdbR00000103[7])



- 17 out of 76 nucleotides are modified
- Predicted ground state (MFE) is **not** cloverleaf structure

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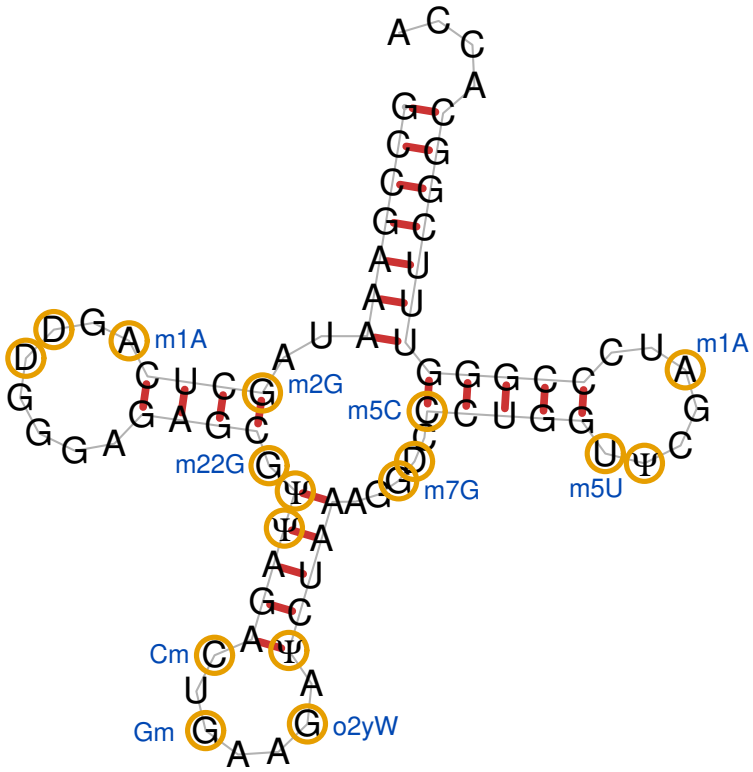

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What about suboptimal structures $S^{\delta} = \{s \in \Omega \mid E(s) \leq E_{\text{MFE}} + \delta\}$?

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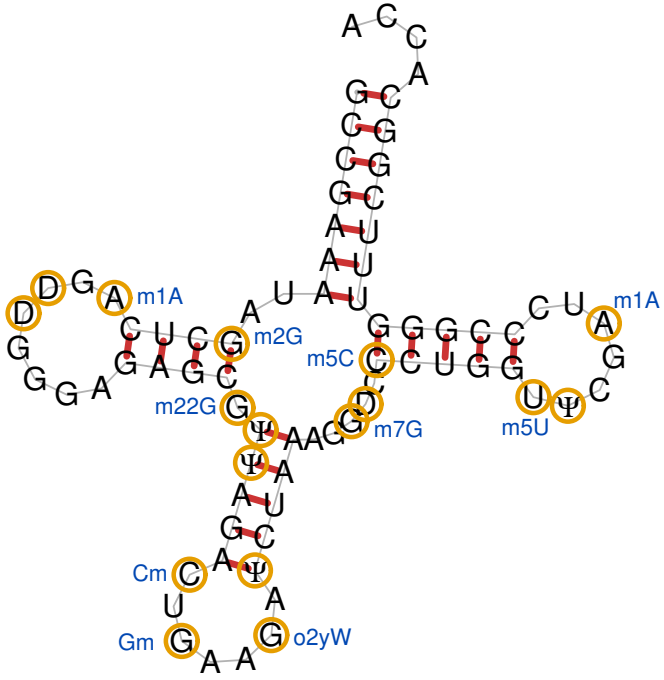
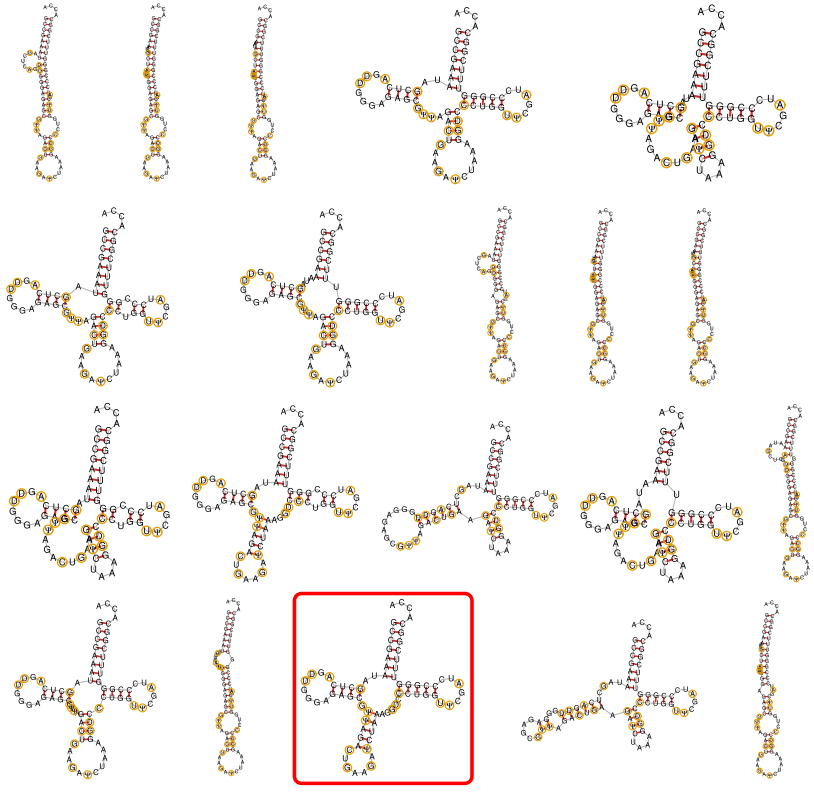
Example: human tRNA Phe (tdbR00000103)

1. Suboptimals for *unmodified sequence*





- Correct cloverleaf structure at position 18
- $\bar{d}^{3}_{\mathrm{BP}} = \frac{1}{|S^3|}\sum_{s\in S^3} d_{\mathrm{BP}}(s_{\mathrm{ref}}, s) = 21.55$ bp, $E_{\mathrm{ref}} - E_{\mathrm{MFE}} = 1.7$ kcal/mol
- Most predictions are not cloverleaf structure

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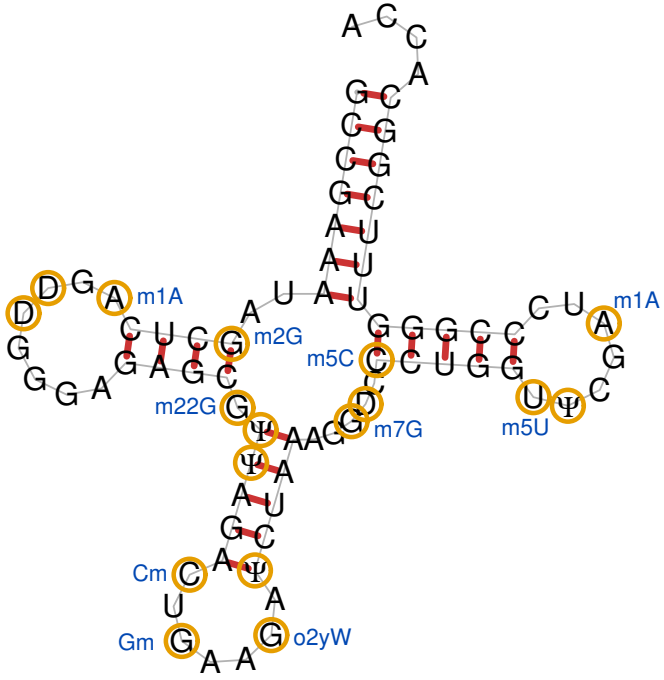

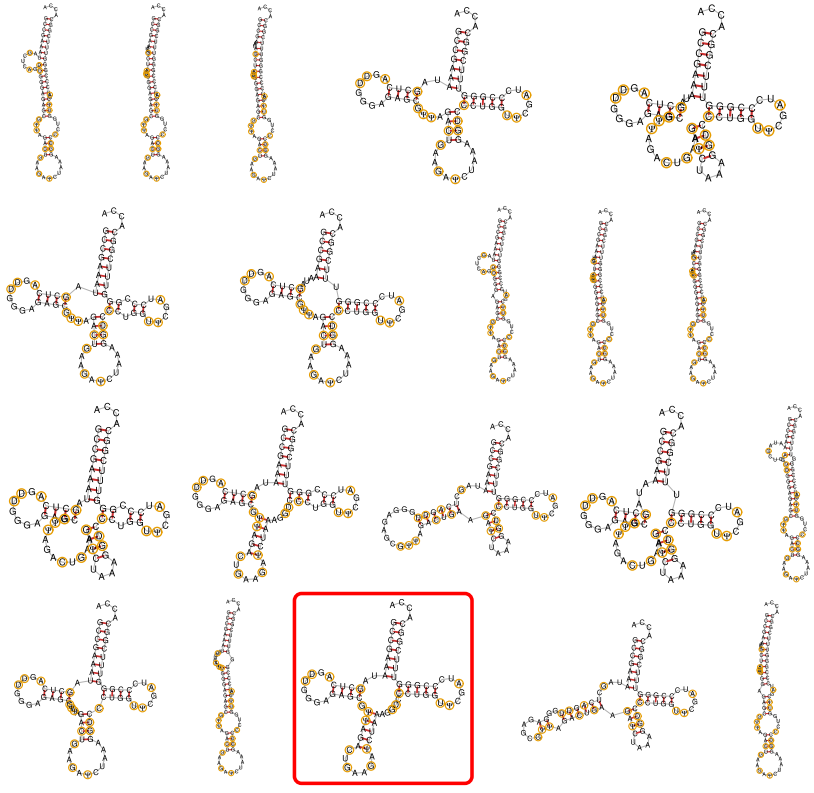

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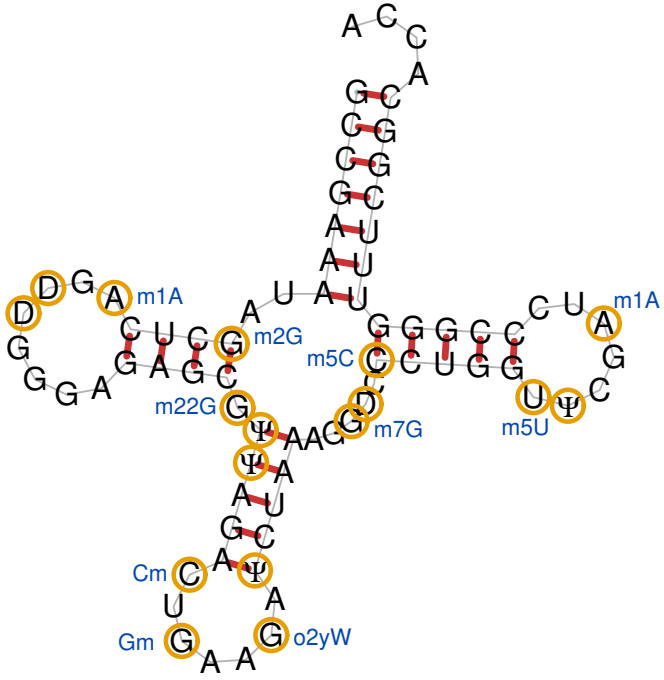
How about masking modified bases?

Example: human tRNA Phe (tdbR00000103)

2. Suboptimals for sequence with *all modified bases masked*



- Modified bases are excluded from pairing
- Correct cloverleaf structure not among the solution set
- $\bar{d}^{3}_{\mathrm{BP}} = 16.7$ bp, anticodon arm is missing

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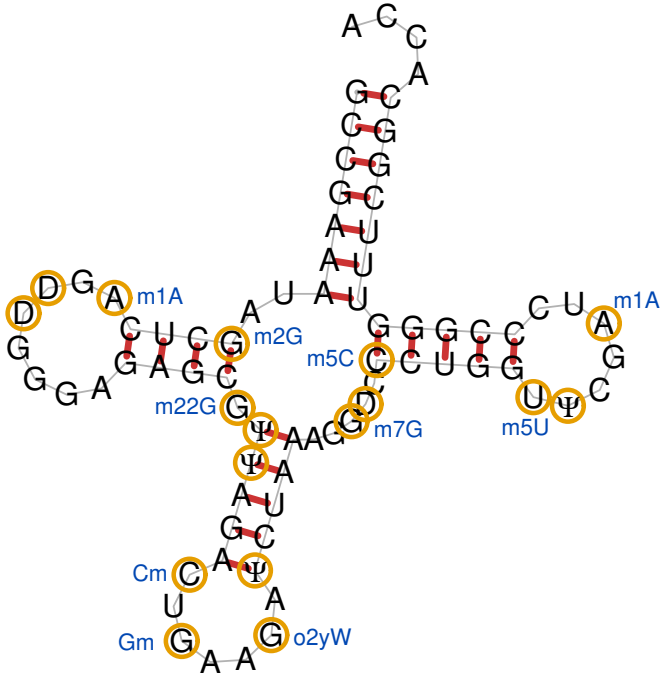

- Modified bases are excluded from pairing
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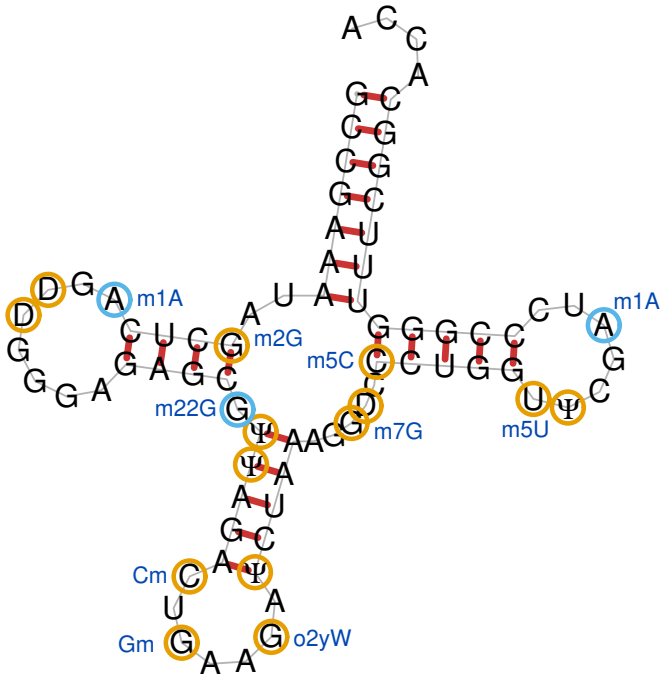
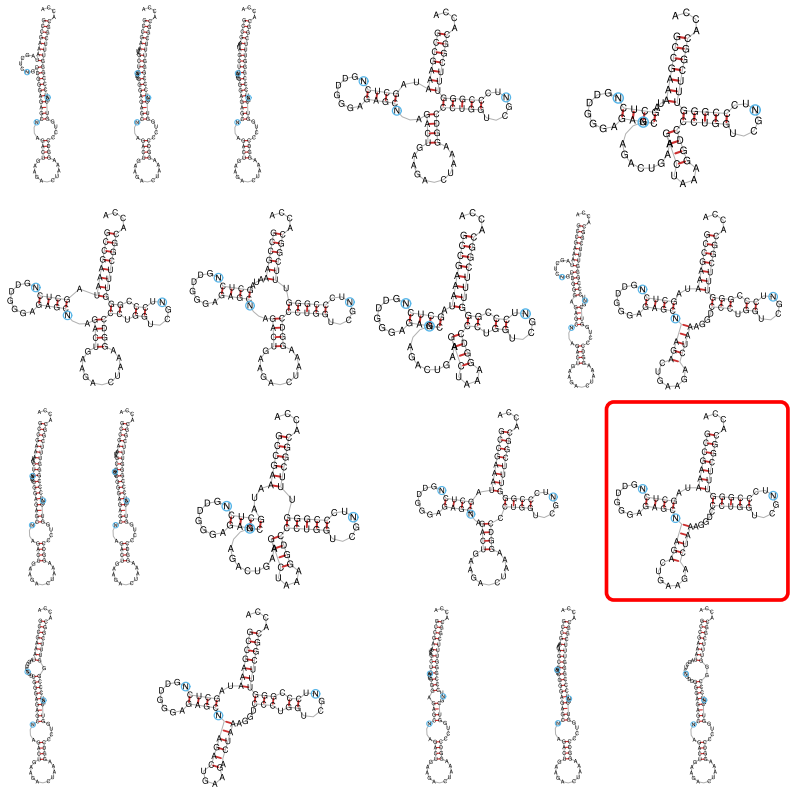
Some modified bases are required to pair!

Example: human tRNA Phe (tdbR00000103)

3. Suboptimals with *masked bases* for RT blocking modifications[8]





- Correct cloverleaf structure at position 15
- $\bar{d}^{3}_{\mathrm{BP}} = 19.7$ bp, $E_{\mathrm{ref}} - E_{\mathrm{MFE}} = 1.6$ kcal/mol
- Still, most predicted structure are not tRNA-like!

[8] Motorin et al., "Identification of modified residues in RNAs by reverse transcription-based methods.", 2007, Methods in Enzymology 425, 21–53

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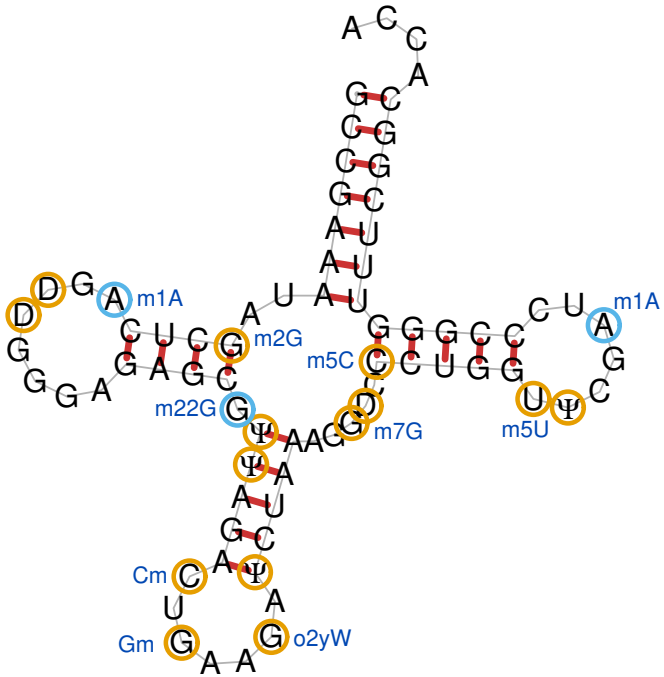

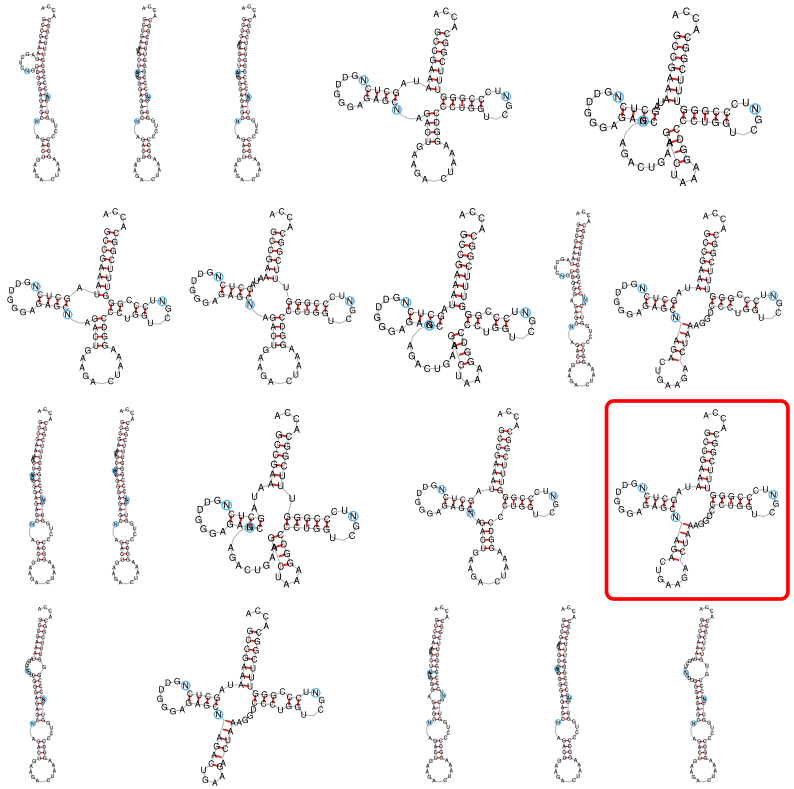

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- Still, most predicted structure are not tRNA-like!

Ψ-A stacking energies are available in literature!

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Example: human tRNA Phe (tdbR00000103)

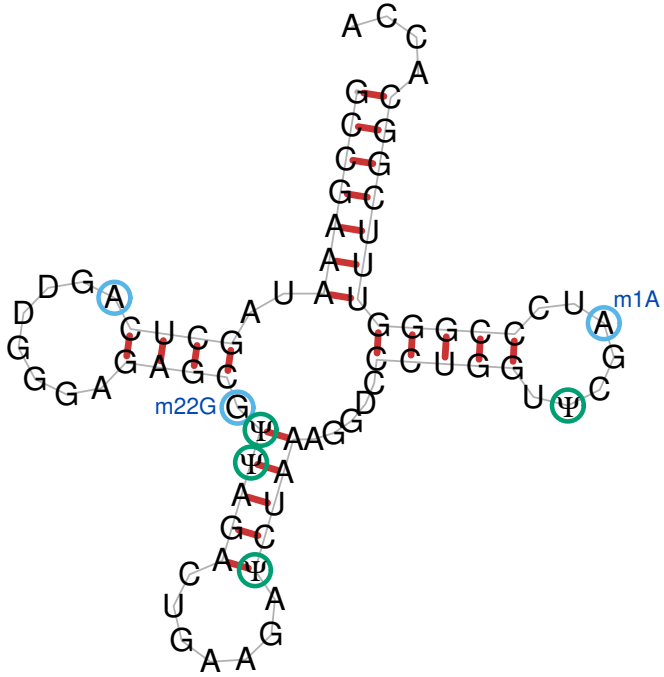
Python overhead to add energies for stacks with Ψ-A[9]:

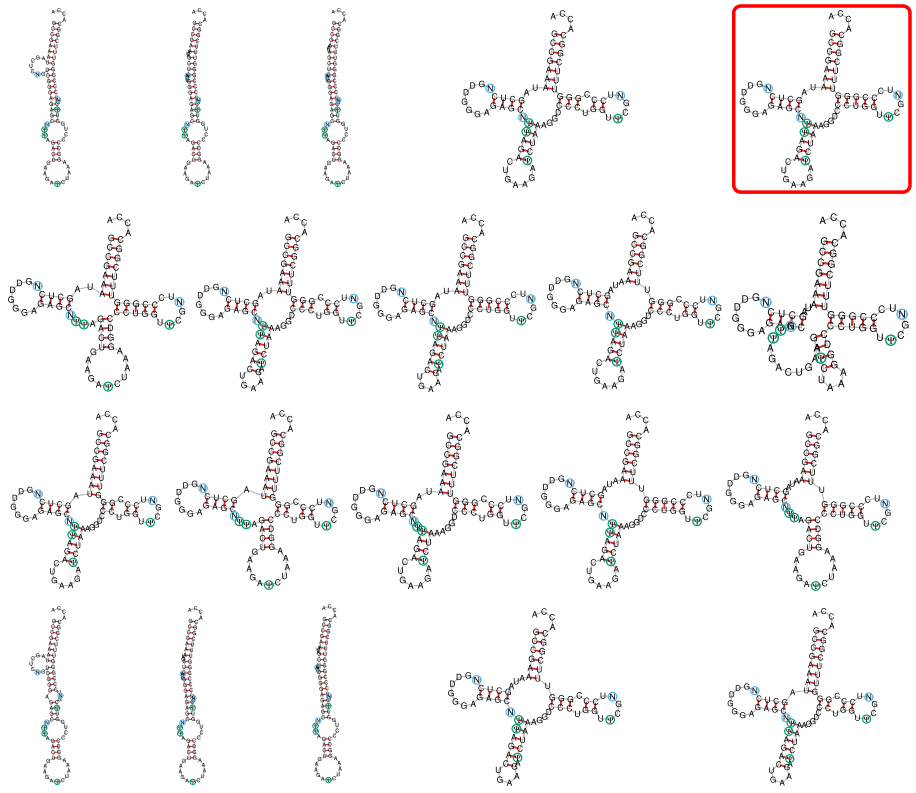
```
# Energy parameters from Hudson et al., 2013
stacking_psi_A = [
  # N   A     C   G     U     P
  [ 0,  0,    0,  0,    0,    0 ], # N
  [ 0,  0,    0,  0,    -77,  0 ], # A
  [ 0,  0,    0,  -14,  0,    0 ], # C
  [ 0,  0,    -9, 0,    0,    0 ], # G
  [ 0,  -181, 0,  0,    0,    0 ], # U
  [ 0,  0,    0,  0,    0,    0 ]  # P
]

stacking_A_psi = [
  # N   A     C     G     U     P
  [ 0,  0,    0,    0,    0,    0 ], # N
  [ 0,  0,    0,    0,    -69,  0 ], # A
  [ 0,  0,    0,    -105, 0,    0 ], # C
  [ 0,  0,    -69,  0,    0,    0 ], # G
  [ 0,  -170, 0,    0,    0,    0 ], # U
  [ 0,  0,    0,    0,    0,    0 ]  # P
]

# Energy correction callback passed to ViennaRNA library
def correction(i, j, k, l, step, encoding):
    if (step == RNA.DECOMP_PAIR_IL): # an internal loop is evaluated
        if  i + 1 == k and j - 1 == l:  # (i,j) and (k,l) are stacking
            if (encoding[i] == 5 and encoding[j] == 1):
                return stacking_psi_A[encoding[k]][encoding[l]]
            elif (encoding[k] == 1 and encoding[l] == 5):
                return stacking_psi_A[encoding[j]][encoding[i]]
            elif (encoding[i] == 1 and encoding[j] == 5):
                return stacking_A_psi[encoding[k]][encoding[l]]
            elif (encoding[k] == 5 and encoding[l] == 1):
                return stacking_A_psi[encoding[j]][encoding[i]]

    return 0
```

[9] Hudson et al., “Thermodynamic contribution and nearest-neighbor parameters of pseudouridine-adenosine base pairs in oligoribonucleotides.”, 2013, RNA 19.11, 1474–1482

Example: human tRNA Phe (tdbR00000103)

4. Suboptimals with RT masking and stacking energies for Ψ-A[10]





- Correct cloverleaf structure at position 5
- $\bar{d}^{3}_{\mathrm{BP}} = 15.5$ bp, $E_{\mathrm{ref}} - E_{\mathrm{MFE}} = 0.83$ kcal/mol
- Can we do better?

[10] Hudson et al., "Thermodynamic contribution and nearest-neighbor parameters of pseudouridine-adenosine base pairs in oligoribonucleotides.", 2013, RNA 19.11, 1474–1482

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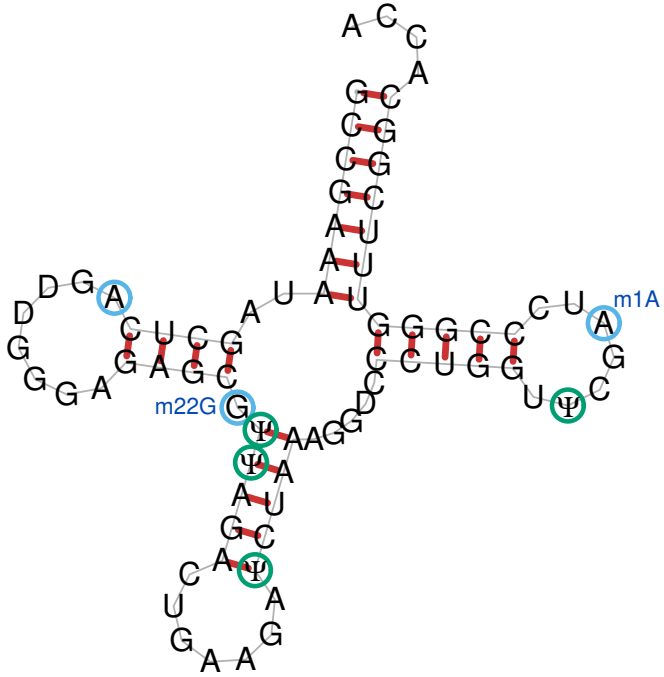

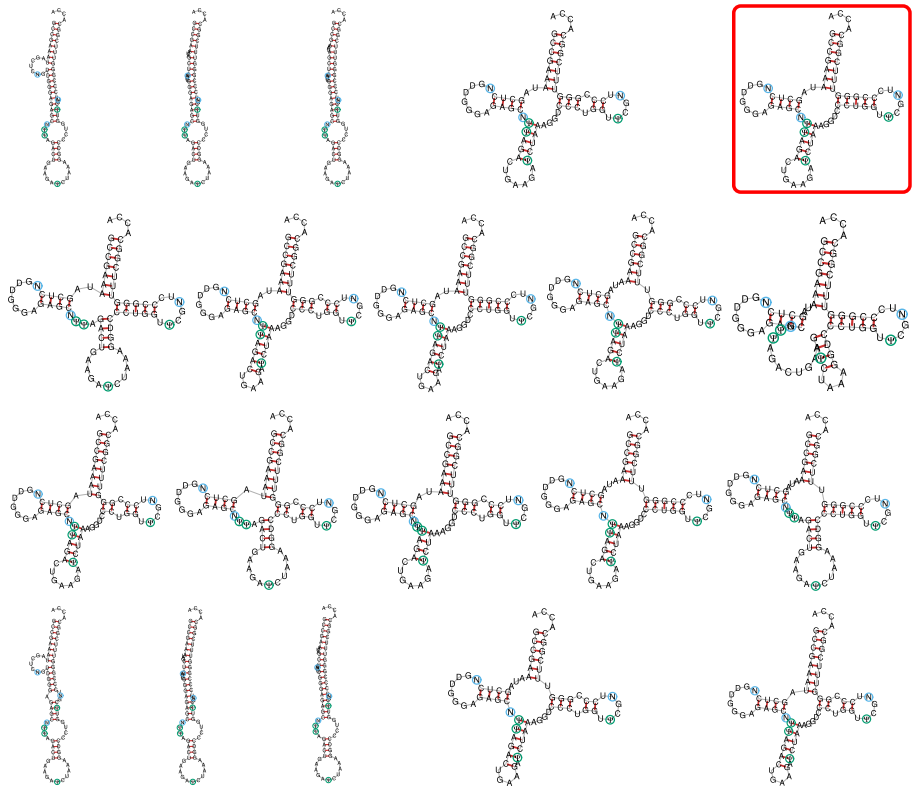

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Dihydrouridine (D) adds flexibility to the D-loop!

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No (stacking) energies with dihydrouridine (D) are available

Structural effects of dihydrouridine[11]

- C3'-endo sugar conformation is destabilized in favor of C2'-endo
- more flexibility
- promotes destacking
- destabilization of 1.5 kcal/mol (mono), up to 5.3 kcal/mol (oligo)

Required changes for `correction` function:

```
def correction(i, j, k, l, step, encoding):
    e = 0 # default correction

    if (step == RNA.DECOMP_PAIR_IL): # an internal loop is evaluated
        if  i + 1 == k and j - 1 == l:  # (i,j) and (k,l) are stacking
            if (encoding[i] == 5 and encoding[j] == 1):
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            elif (encoding[k] == 5 and encoding[l] == 1):
                e += stacking_A_psi[encoding[j]][encoding[i]]

            if  encoding[i] == 6 or \
                encoding[j] == 6 or \
                encoding[k] == 6 or \
                encoding[l] == 6:
                e += 150 # D destabilizes stacking by at least 1.5 kcal/mol

    return e
```
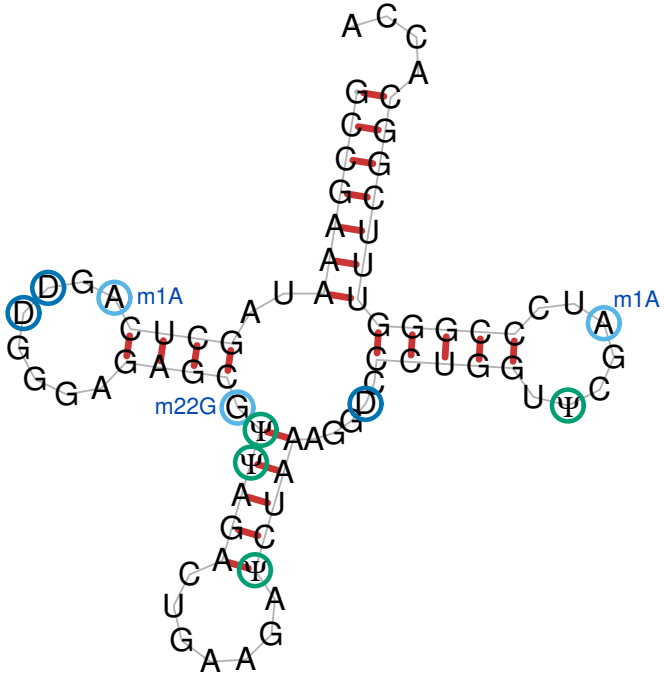
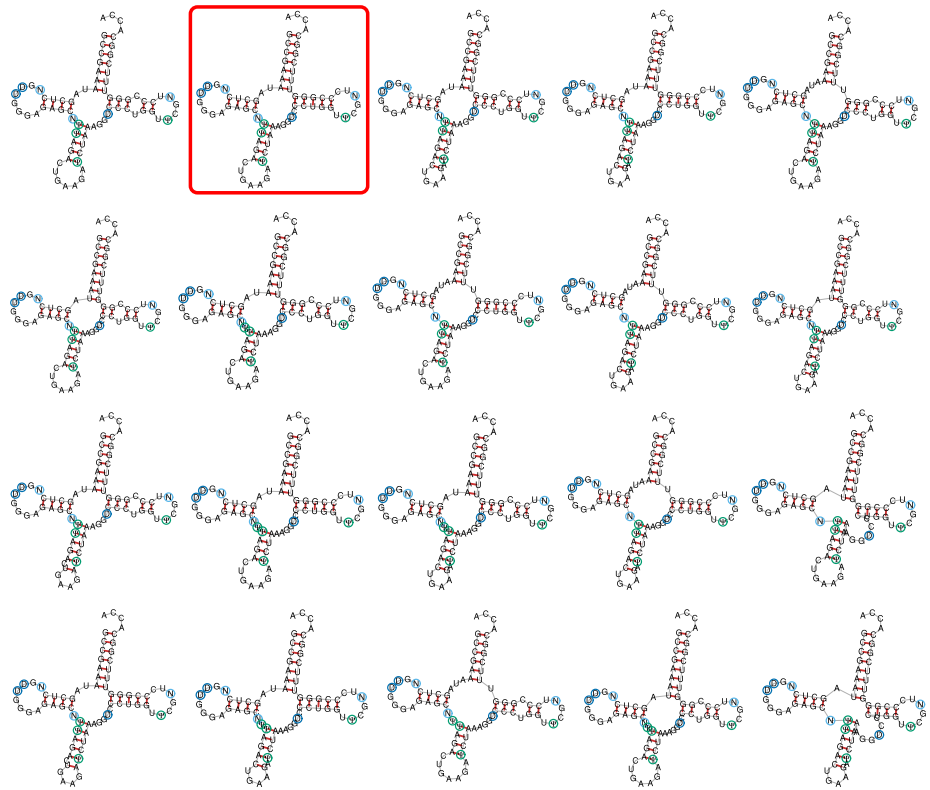

[11] Dalluge et al., "Conformational flexibility in RNA: the role of dihydrouridine.", 1996, NAR 24.6, 1073–1079

Example: human tRNA Phe (tdbR00000103)

5. Suboptimals with RT masking and energies for Ψ-A and D[12]





- Correct cloverleaf structure at position 2
- $\bar{d}^{3}_{\mathrm{BP}} = 6.92$ bp, $E_{\mathrm{ref}} - E_{\mathrm{MFE}} = 0.2$ kcal/mol

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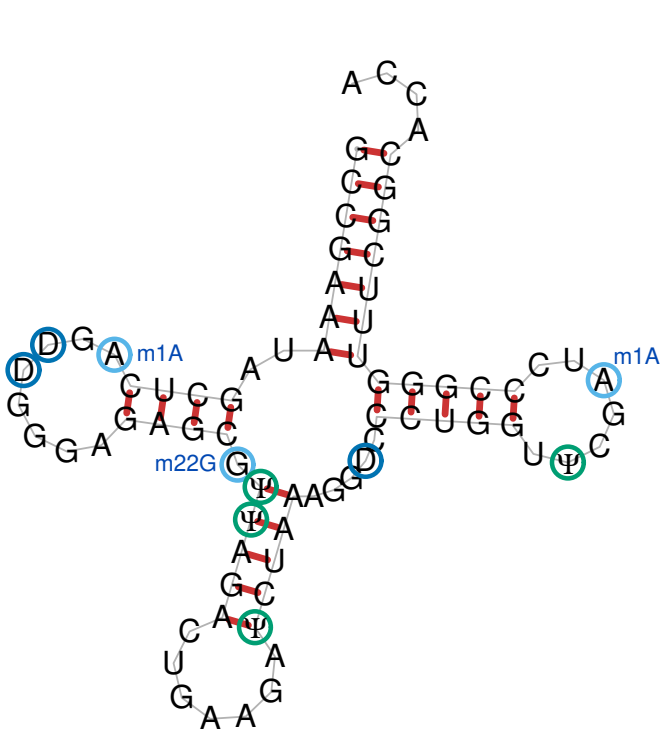

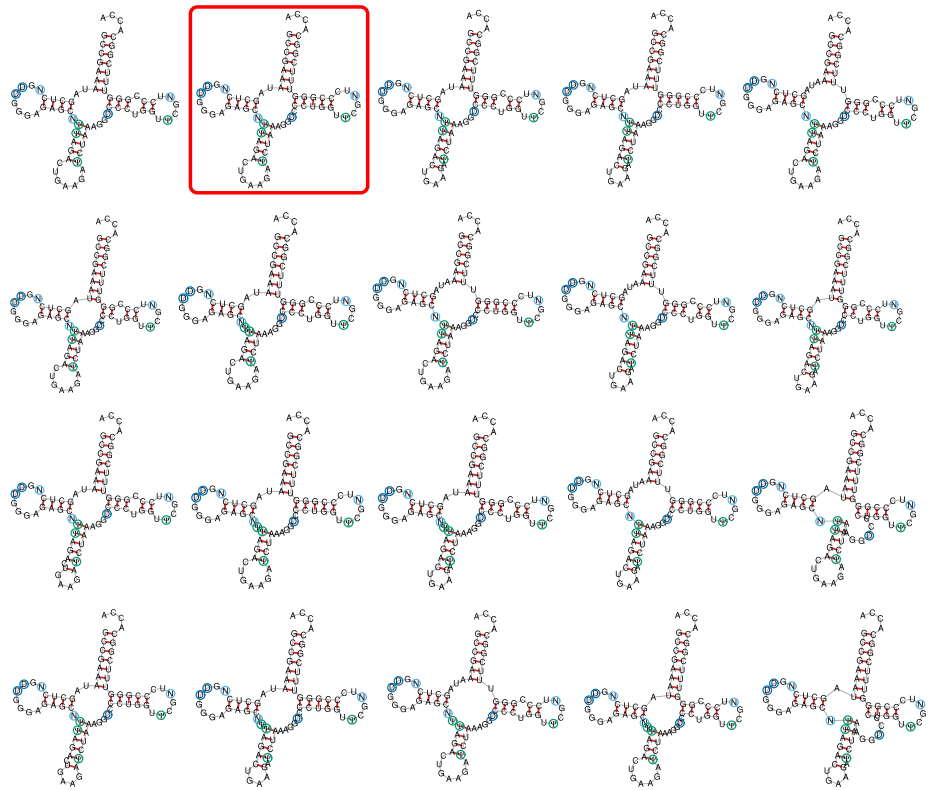

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How do these constraints affect prediction for other tRNAs?

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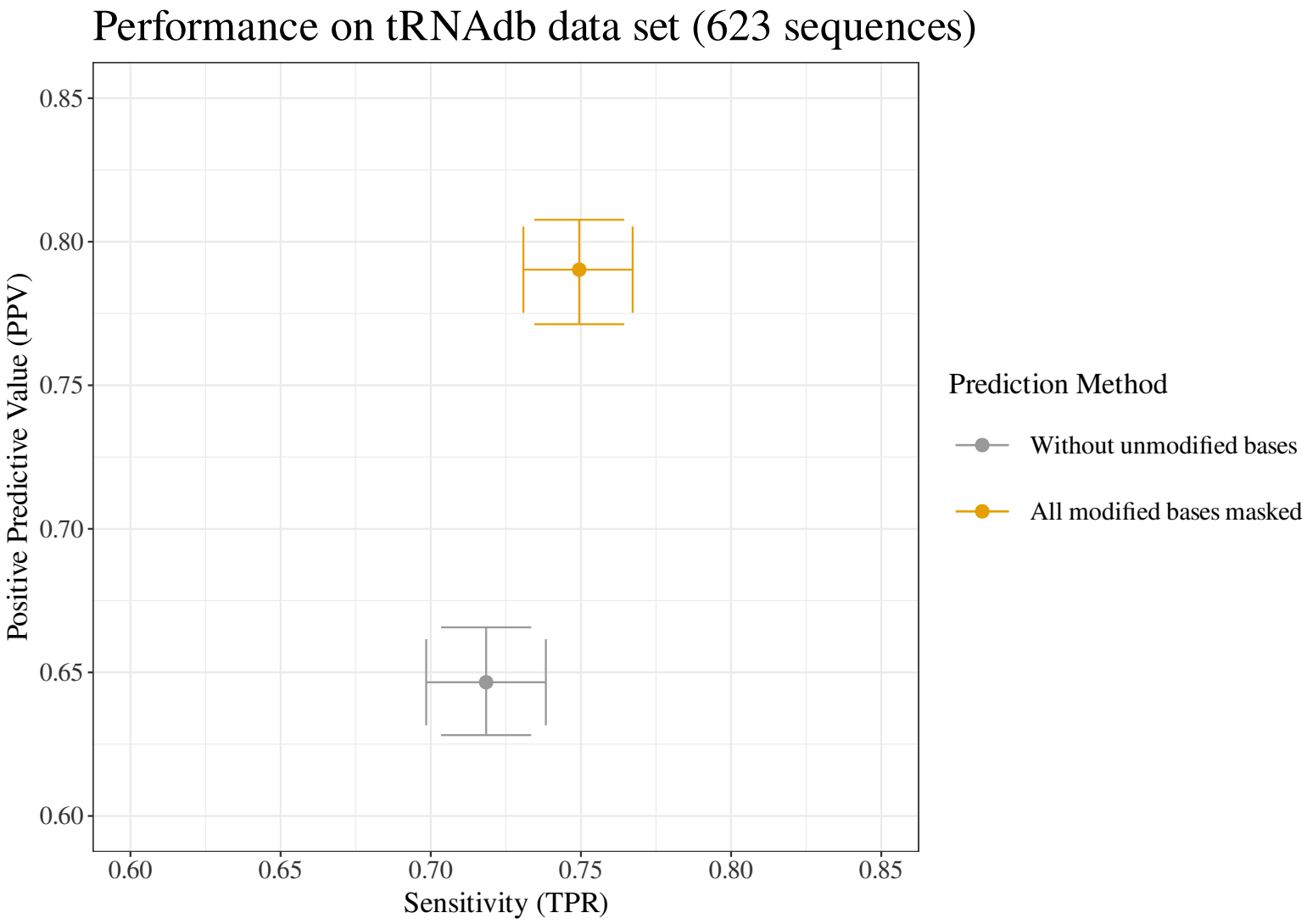
Overall prediction improvement



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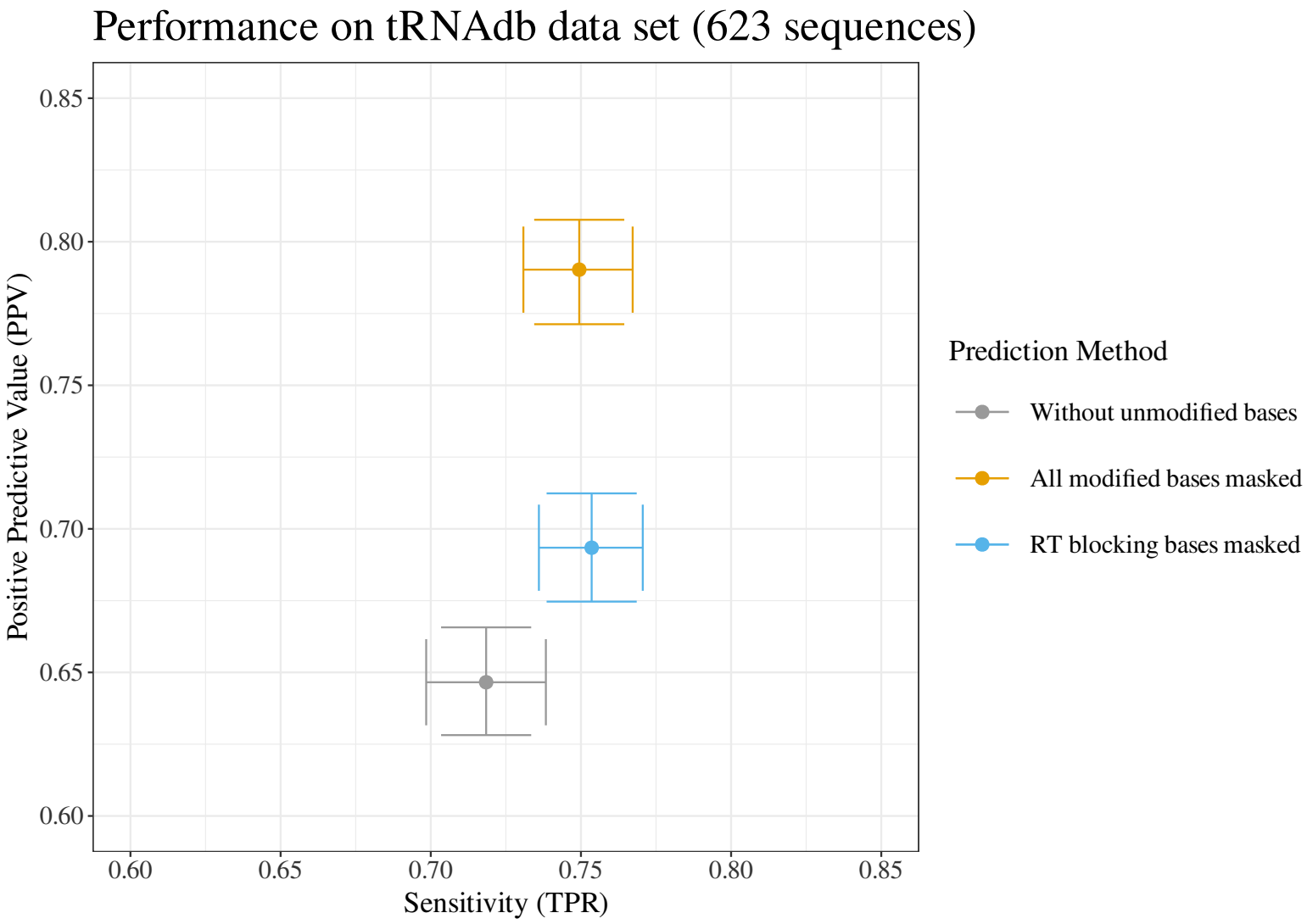

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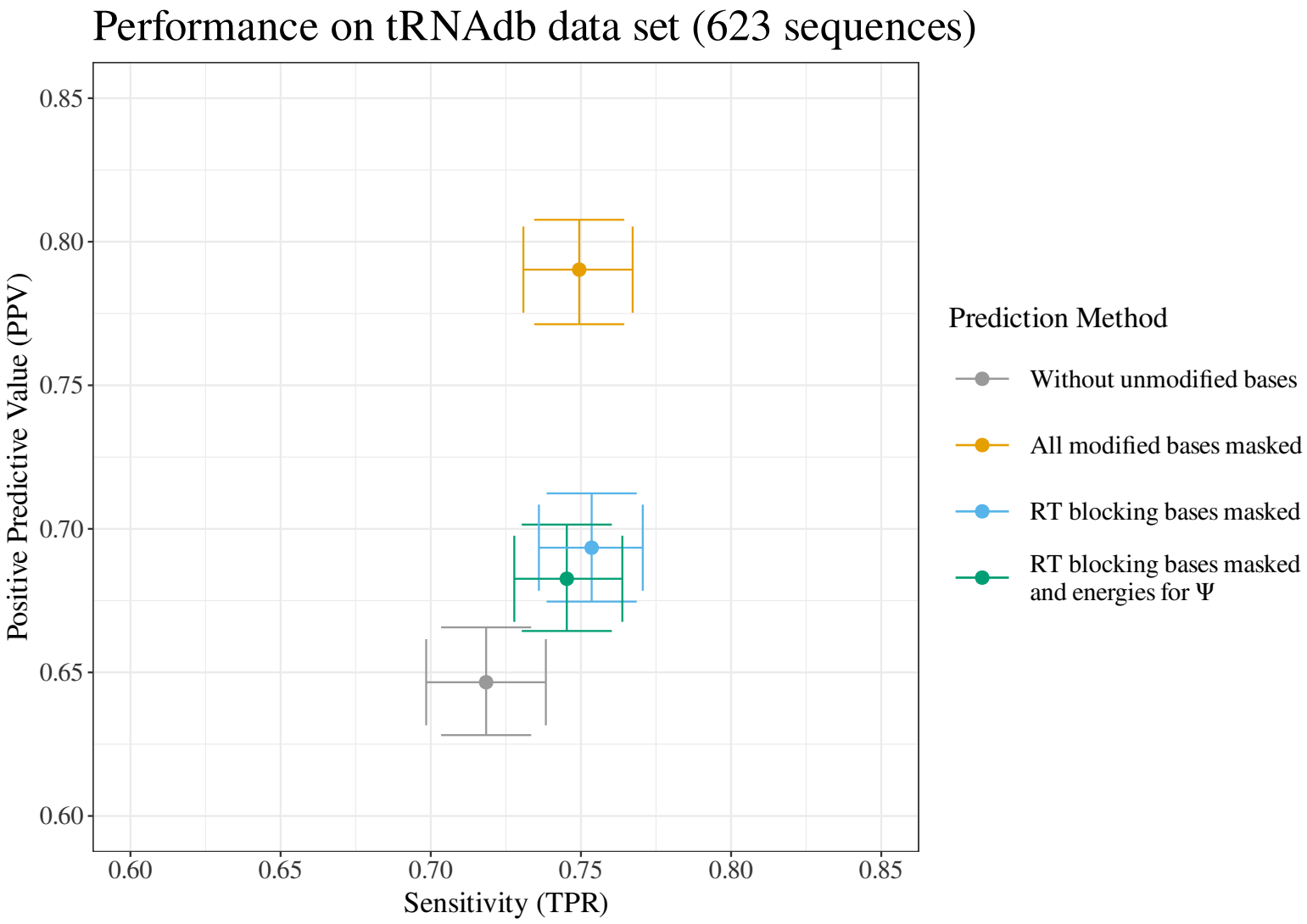

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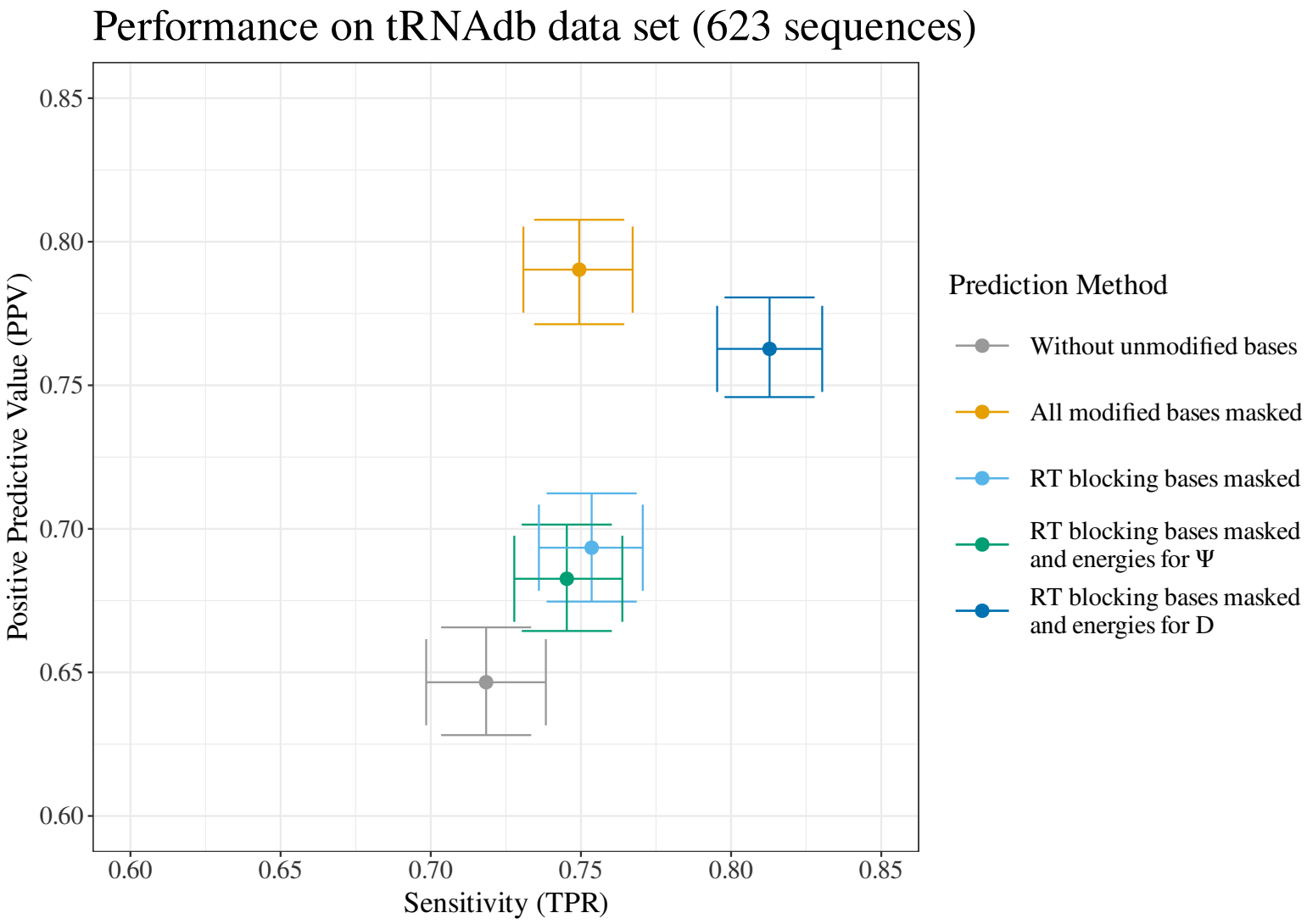

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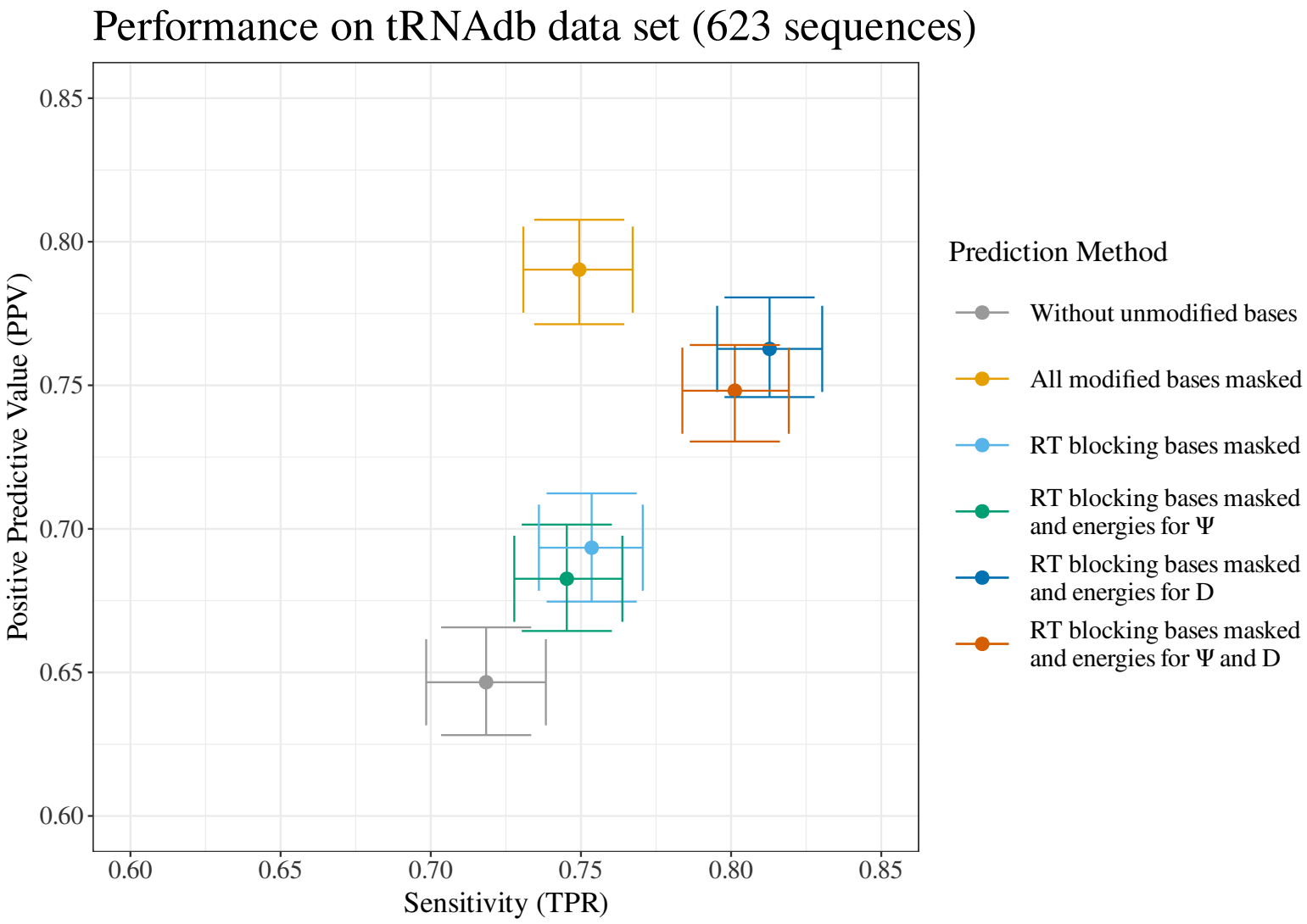

Conclusion

Modified bases may heavily influence structure space

The good news

- Some data on structural effects of modifications available
- Better prediction performance through constraints
- Constraints available for many prediction algorithms

The not so good news

- Additional parameters do not necessarily increase performance
- Constraints become complex for more modifications and contexts
- Unrealistic to include full parameters with many modified bases
- No unique base annotation (`tRNAdb`[13], `RNAmod`[14], `MODOMICS`[15])

Outlook

- Gather more data on structural effects of modified bases
- Rule and energy parameter set for pairs with modified bases
- Integration of modified bases in `ViennaRNA Package`

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[13] Jühling et al., "tRNAdb 2009: compilation of tRNA sequences and tRNA genes.", 2009, NAR 37, D159–D162

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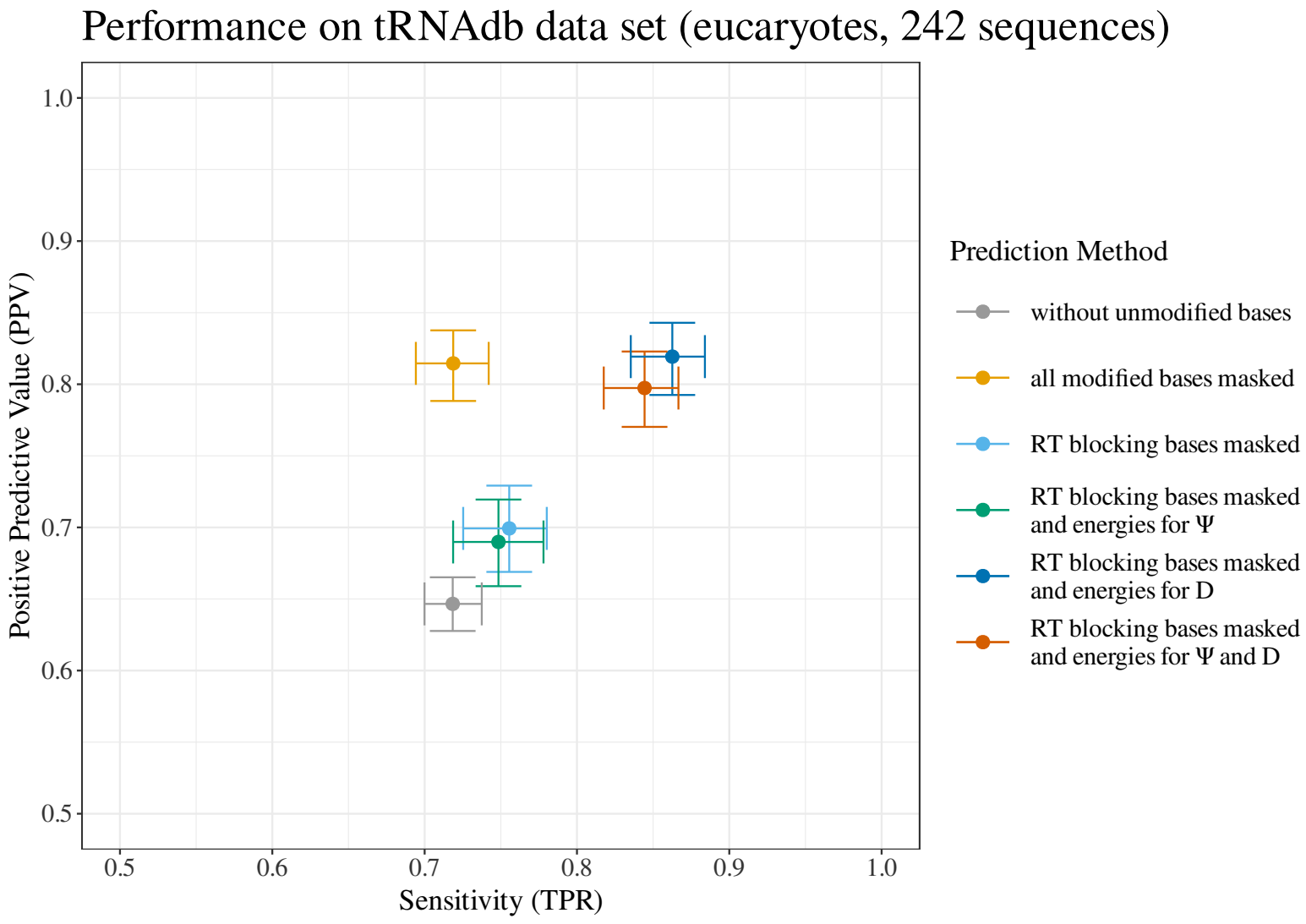
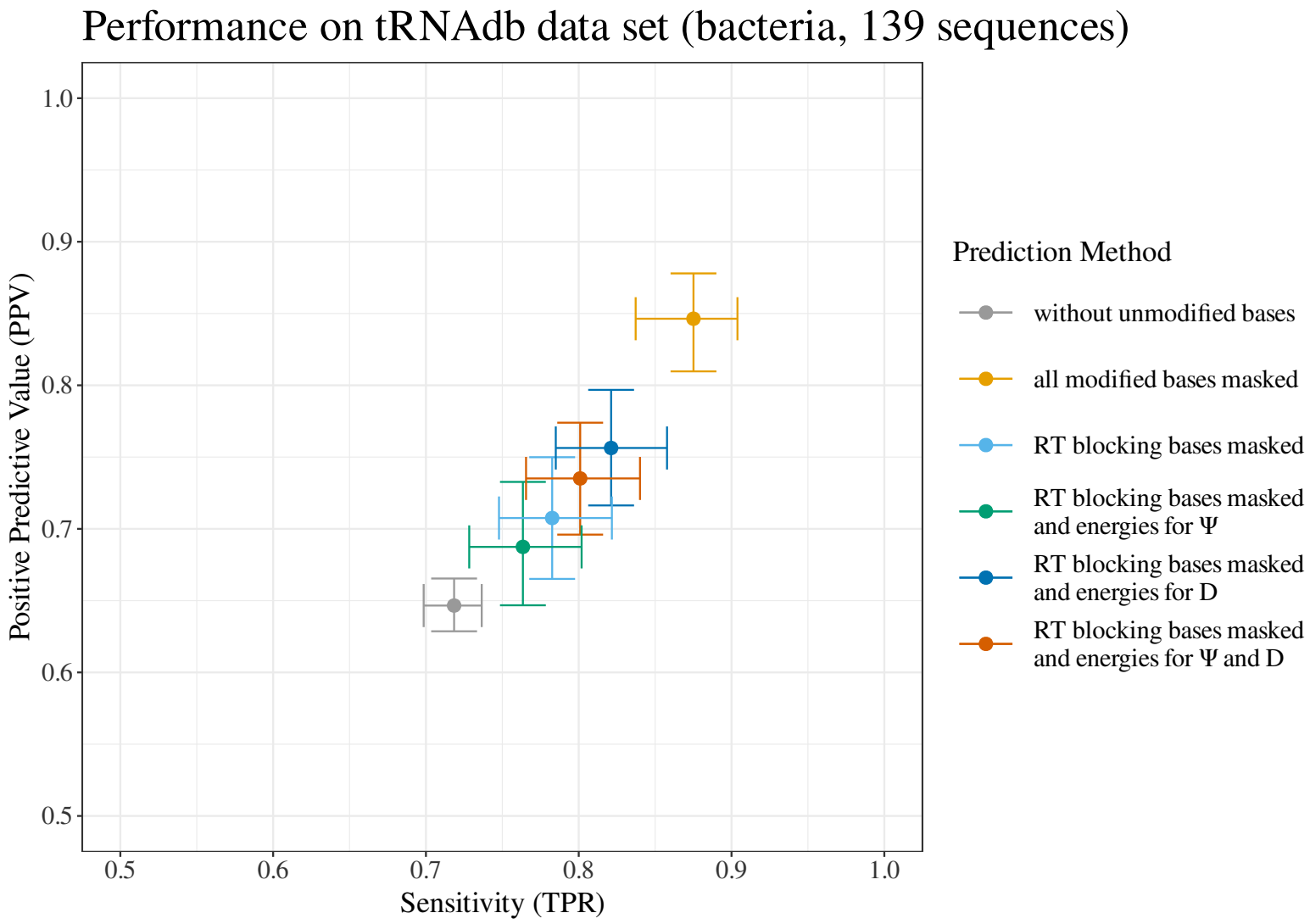
FWF Der Wissenschaftsfonds.

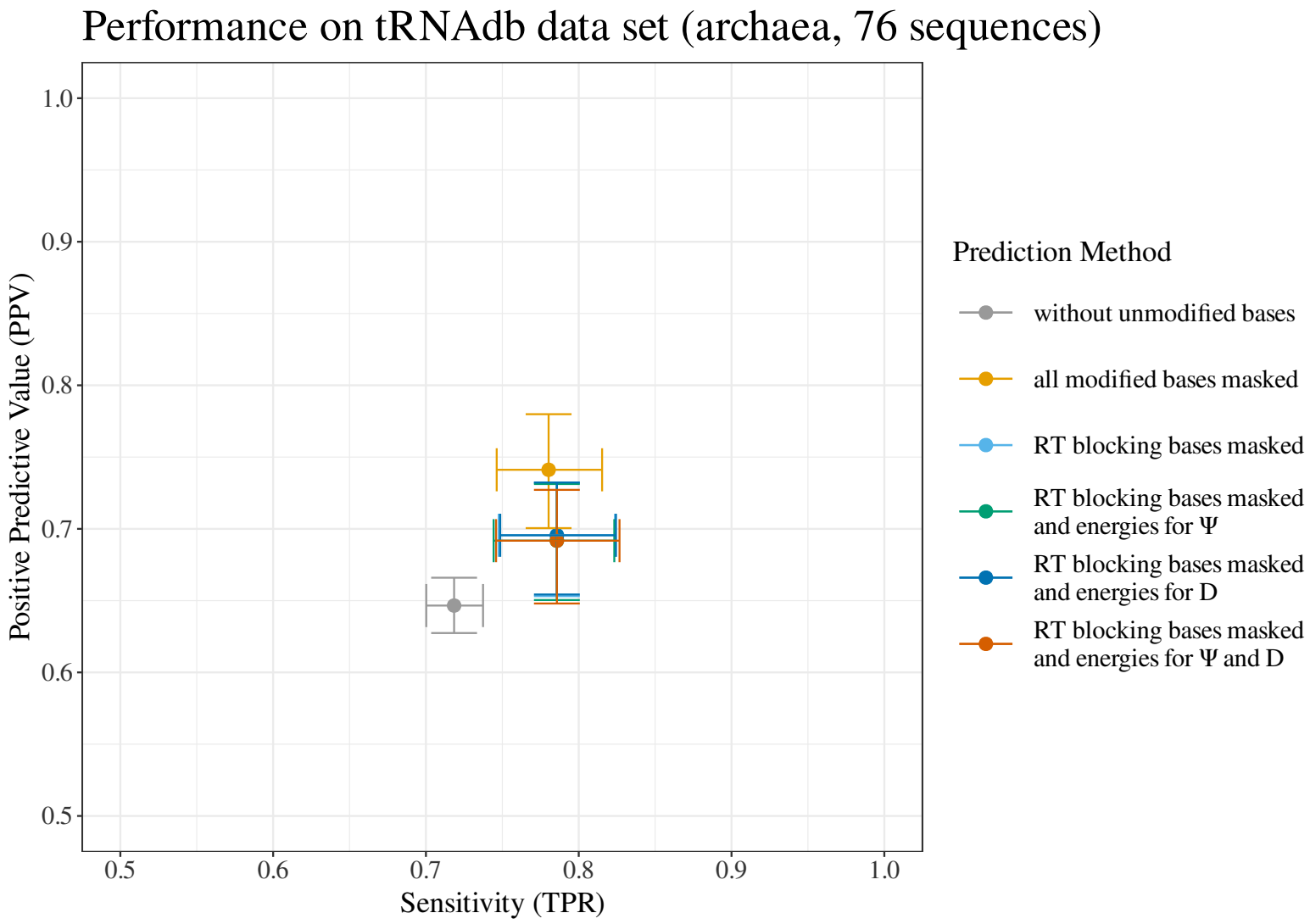
Overall prediction improvement



Overall prediction improvement



Overall prediction improvement



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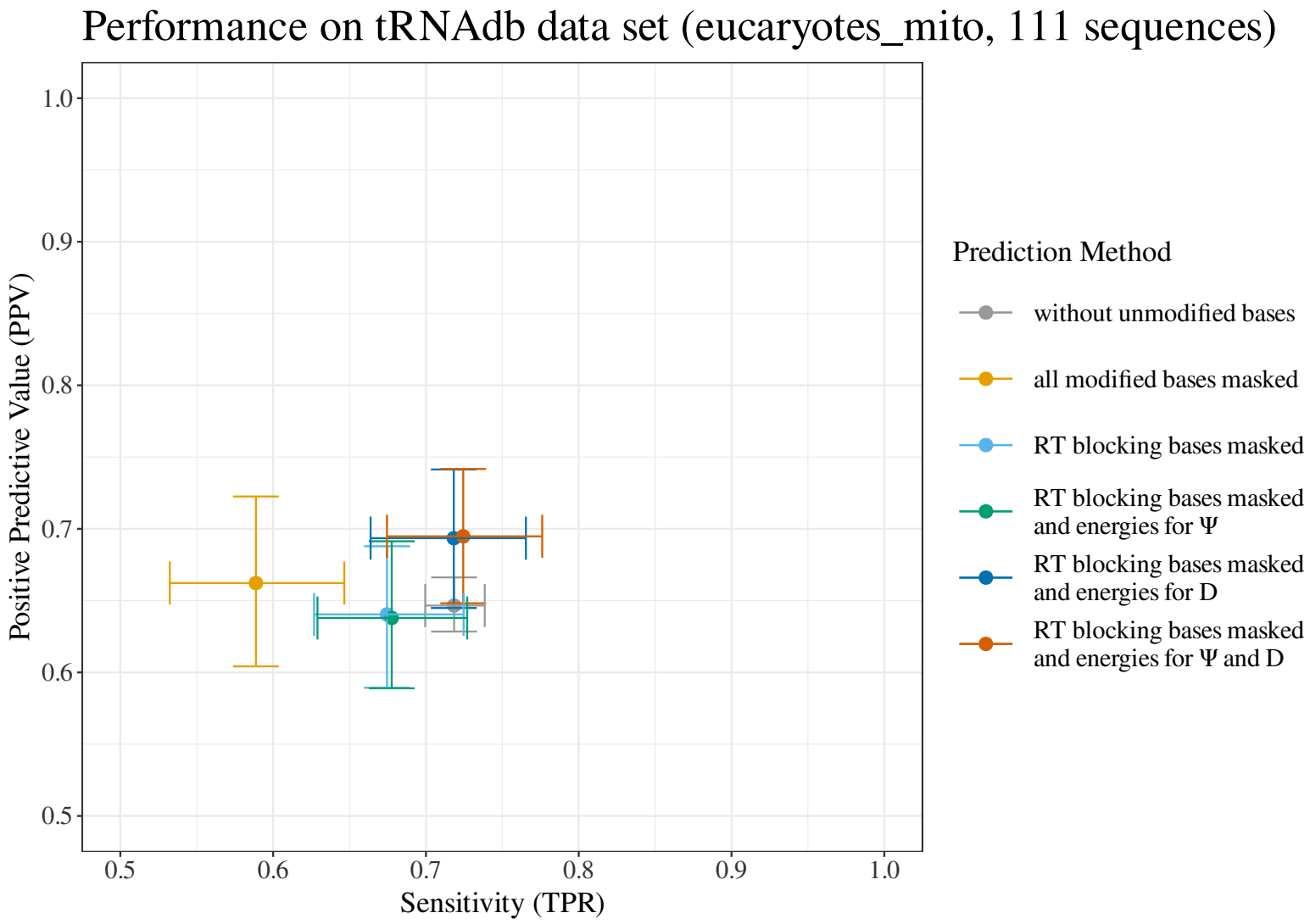
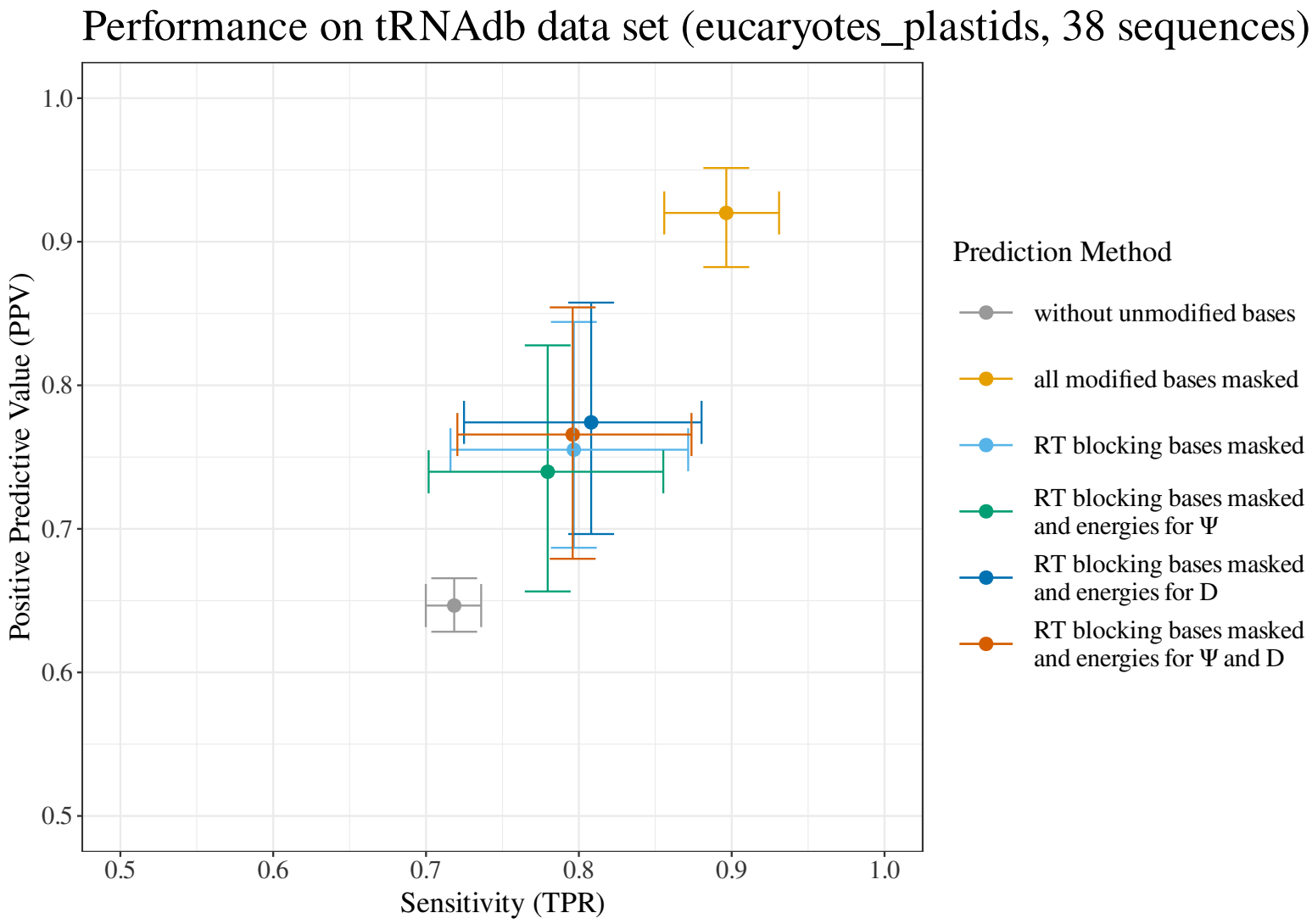


Overall prediction improvement



Overall prediction improvement