The effect of modified nucleotides on secondary structure prediction in tRNA

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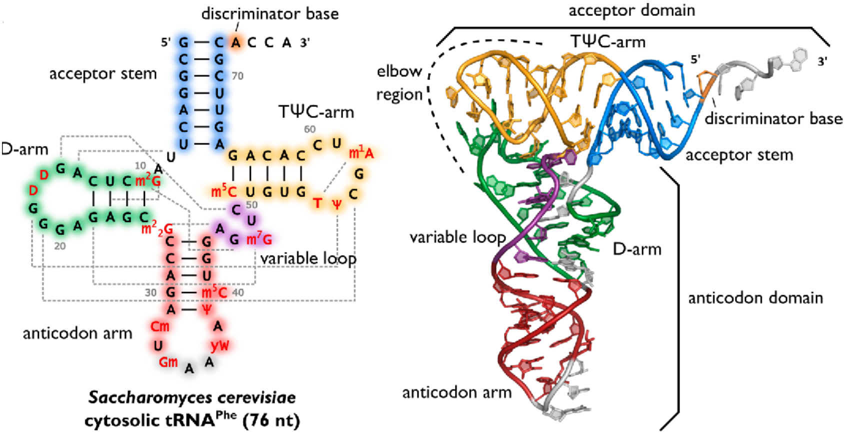
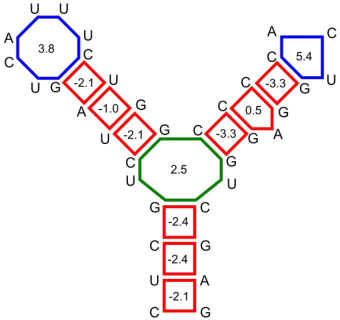
Modifications in tRNA sequences

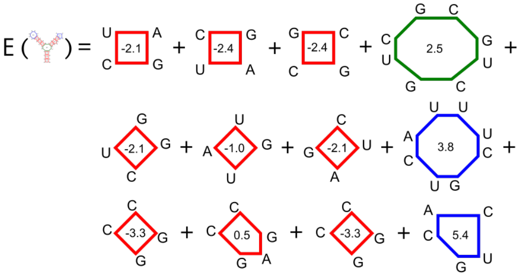


[1]

[1] Lorenz, C., Lünse, C.E., & Mörl, M. (2017). tRNA Modifications: Impact on Structure and Thermal Adaptation. Biomolecules, FIG1

RNA secondary structure prediction - Nearest Neighbor energy model





= -6.50 kcal/mol

- Secondary structures can be uniquely decomposed into loops.
- Contributions of a base pair only depends on neighboring pairs.
- Each loop is assigned a free energy contribution[2].

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

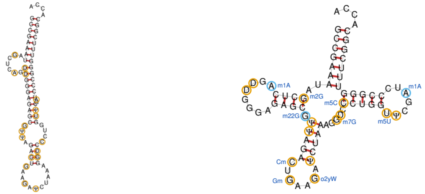
Images by Ronny Lorenz

RNA modifications & secondary structure prediction

Traditional methods lack the means for handling modifications in RNA structure prediction.

- Implementations are limited to the standard RNA nucleotide alphabet AUGC, no complete NN energy parameter set for other nucleotides is available .
- `ViennaRNA Package`[3] provides a plug-in mechanism to adjust free energy parameters and to influence the candidate structure space.

Example: human tRNA Phe (tdbR00000103). 17 out of 76 nucleotides are modified.



Structure prediction as unmodified sequence

Structure prediction including modifications

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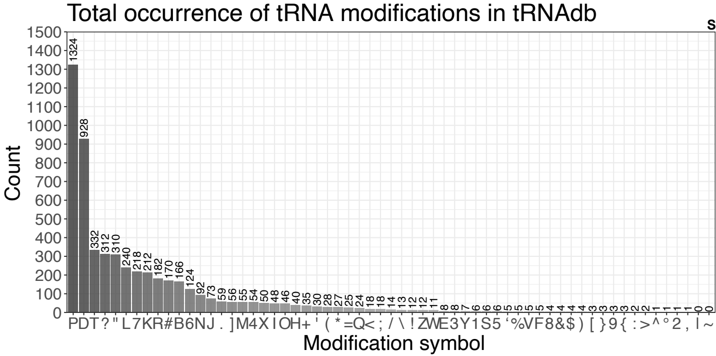
[3] Lorenz, R., Bernhart, S.H., Höner zu Siederdissen, C. et al. (2011). ViennaRNA Package 2.0. Algorithms Mol Biol

Databases of RNA modifications

- `MODOMICS` [Dunin-Horkawicz et al., 2006, Boccaletto et al., 2018]
- **Transfer RNA database (`tRNAdb`) [Jühling et al., 2009]**
- RNA Modification Database (`RNAMDB`) [Cantara et al., 2011]
- Small Subunit rRNA Modification Database (`SSUmods`) [McCloskeyand Rozenski, 2005]

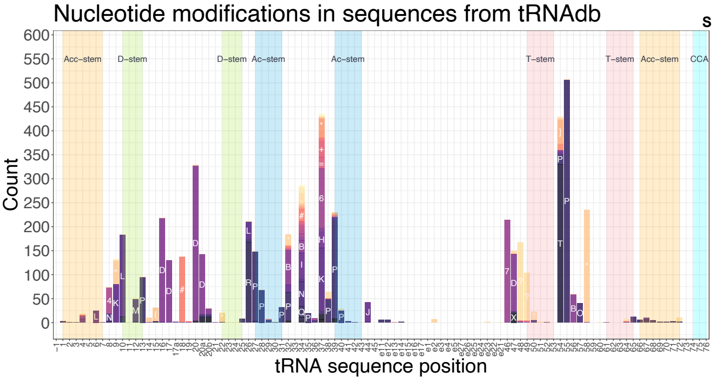
Modifications in `tRNAdb`



Symbol	Modification
P	pseudouridine
D	dihydrouridine
T	ribosylthymine
?	5-methylcytidine
”	1-methyladenosine
L	N2-methylguanosine
7	7-methylguanosine
K	1-methylguanosine
R	N2,N2-dimethylguanosine
#	2’-O-methylguanosine
B	2’-O-methylcytidine
6	N6-threonylcarbamoyladenosine
N	modified uridine
J	2’-O-methyluridine
.	unknown nucleotide
]	1-methylpseudouridine
M	N4-acetylcytidine
4	4-thiouridine
X	3-(3-amino-3-carboxypropyl)uridine

Location of modified bases in tRNA sequences (`tRNAdb`)

Nucleotide modifications in sequences from tRNAdb



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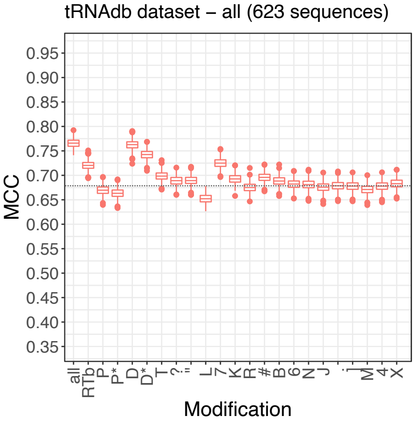
Evaluation of RNA secondary structure prediction (MFE)

- Matthews Correlation Coefficient

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}}$$

- Assessment of influence of each individual modification on the prediction results:
  - ◇ apply constraints for individual modification;
  - ◇ compare reference structure and prediction result with modification constraints (MCC);
  - ◇ bootstrap sample of tRNA dataset (1000 repeats);
  - ◇ analyze obtained sampling distribution.

Structure prediction of sequences from `tRNAdb`


tRNAdb dataset – all (623 sequences)

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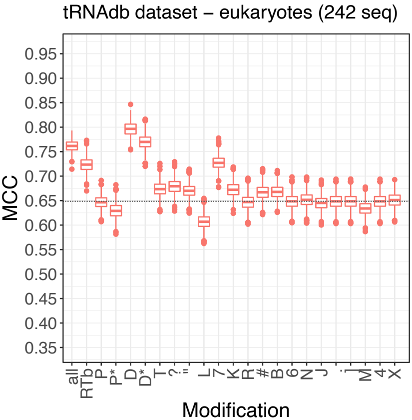
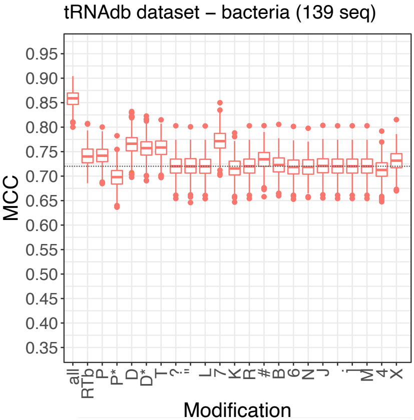
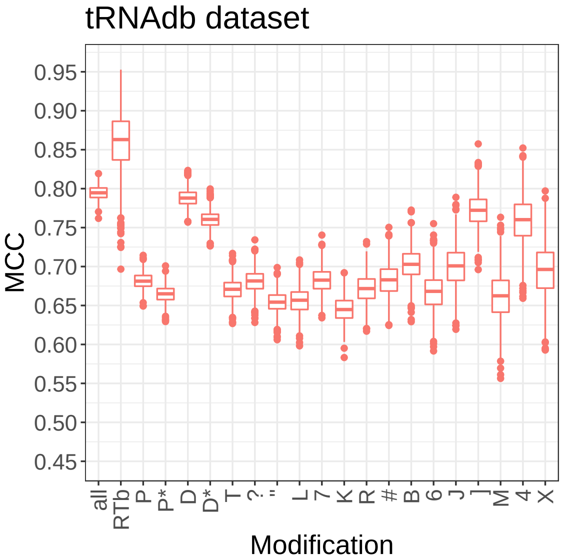
- **all** - mask all modified nucleotides to stay unpaired
- **RTb** - mask nucleotides to stay unpaired if their modification is known to block RT
- **symbol** - mask certain specified nucleotide to stay unpaired
- **symbol*** - apply energy parameter corrections

Structure prediction of sequences from `tRNAdb`

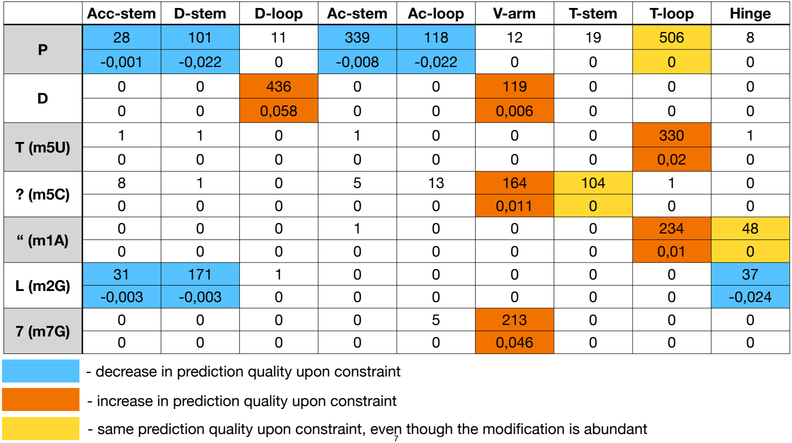

tRNAdb dataset – eukaryotes (242 seq)


tRNAdb dataset – bacteria (139 seq)

Structure prediction of sequences from `tRNAdb` - only sequences that contain the modification included



Prediction quality upon position-dependent constraints



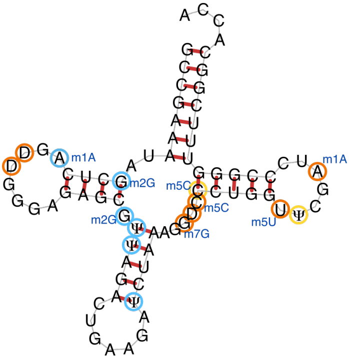
	Acc-stem	D-stem	D-loop	Ac-stem	Ac-loop	V-arm	T-stem	T-loop	Hinge
P	28	101	11	339	118	12	19	506	8
	-0,001	-0,022	0	-0,008	-0,022	0	0	0	0
D	0	0	436	0	0	119	0	0	0
	0	0	0,058	0	0	0,006	0	0	0
T (m5U)	1	1	0	1	0	0	0	330	1
	0	0	0	0	0	0	0	0,02	0
? (m5C)	8	1	0	5	13	164	104	1	0
	0	0	0	0	0	0,011	0	0	0
“ (m1A)	0	0	0	1	0	0	0	234	48
	0	0	0	0	0	0	0	0,01	0
L (m2G)	31	171	1	0	0	0	0	0	37
	-0,003	-0,003	0	0	0	0	0	0	-0,024
7 (m7G)	0	0	0	0	5	213	0	0	0
	0	0	0	0	0	0,046	0	0	0

🟦 - decrease in prediction quality upon constraint

🟧 - increase in prediction quality upon constraint

🟨 - same prediction quality upon constraint, even though the modification is abundant

Prediction quality upon position-dependent constraints



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8

Conclusions

Observations

- Identification of modifications of potential interest (e.g. 7-methylguanosine in eukaryotes and bacteria).
- Prediction improvement using constraints.

Outlook

- Further investigation of the structural effects of modifications positionwise.
- Investigation of modification effects in rRNA, mRNA.
- Assembly of database of structural effects of nucleotide modifications from available literature, partner groups of RNA DECO.

Thank you!

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Ronny Lorenz

Thomas Spicher

TBI group

RNA Deco

Bled Winterseminar