Prediction of small RNA targets incorporating seed regions and target site accessibility

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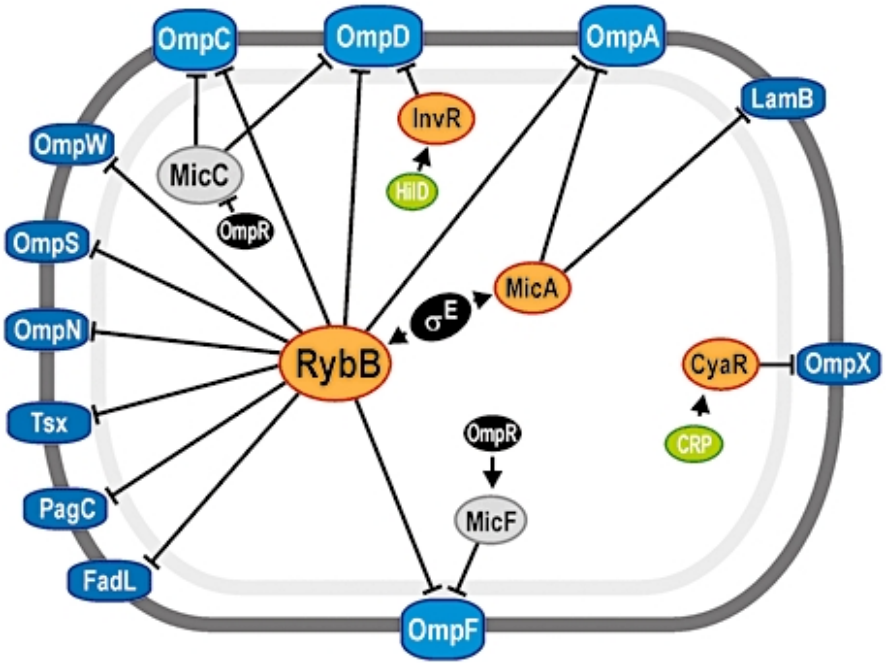
Bled, February 2009

Motivation



(Vogel, Mol. Microbiol. 2008)

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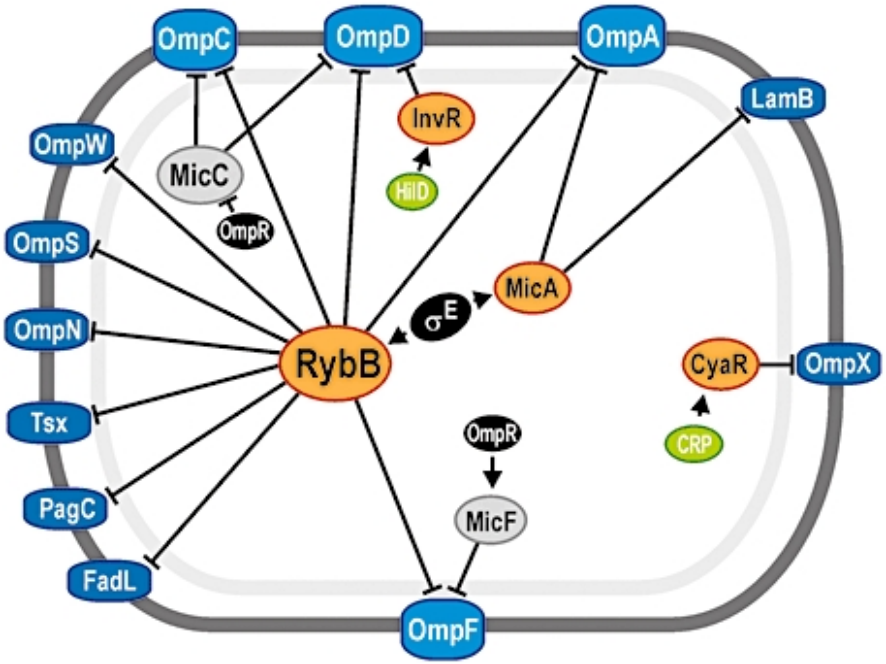
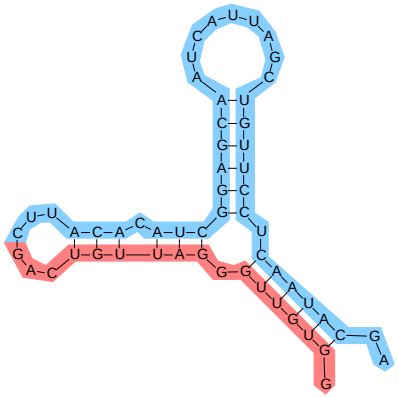


(Vogel, Mol. Microbiol. 2008)

- numerous non-coding RNAs regulate *trans*-encoded mRNAs by antisense base-pairing
- examples: eukaryotic miRNAs and siRNAs, bacterial sRNAs
- finding ncRNAs: biocomputational predictions, microarrays, deep sequencing
- one of many tasks during characterization of these ncRNAs: find putative targets

Approaches for predicting RNA-RNA interactions

- PairFold, RNAcofold:
  predict common secondary structure of 2 concatenated sequences



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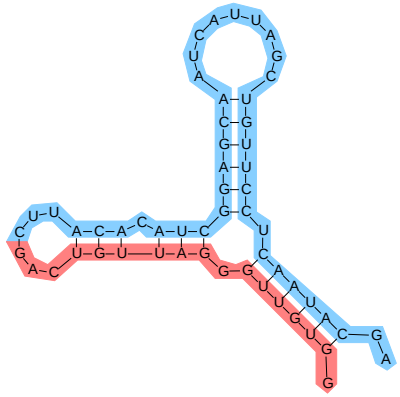
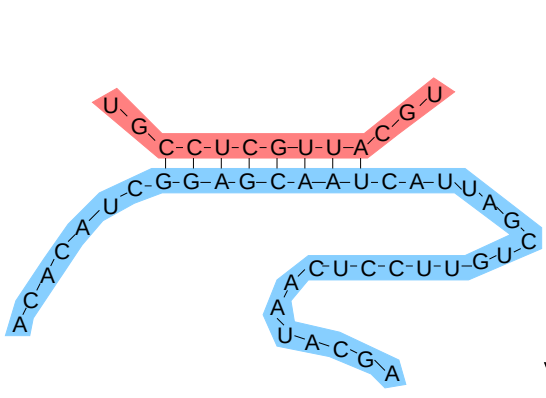
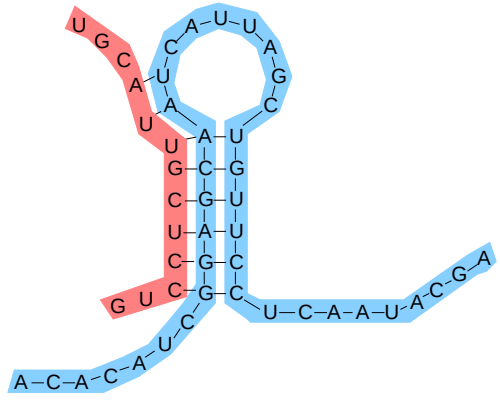


- RNAhybrid, RNAduplex, RNAplex, TargetRNA:
  optimize only hybridization energy between 2 sequences



vs.



Approaches for predicting RNA-RNA interactions

- RNAup:
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$$ED_{a,b} = E_{a,b}^{unpaired} - E^{all}$$

$$= \text{energy that is necessary to unfold the subsequence } S_a \ldots S_b$$

E^{all} ... ensemble free energy
$E_{a,b}^{unpaired}$ ... ensemble free energy, given that $S_a \ldots S_b$ is unpaired

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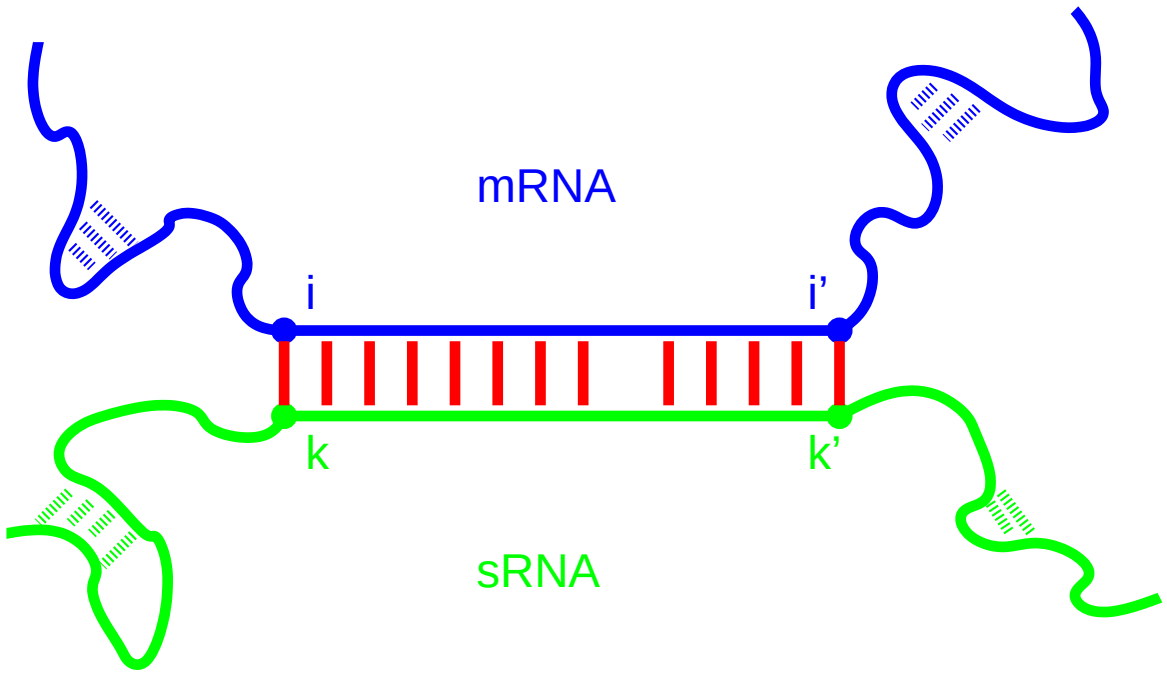
- but:
  - quite slow for genome-wide predictions
  - does not support seed regions

The Idea of IntaRNA



$$E = E^{hybrid} + ED^{mRNA}_{i,i'} + ED^{sRNA}_{k,k'}$$

$ED^{mRNA}_{i,i'}$ ↓ *RNAplfold*

$ED^{sRNA}_{k,k'}$ ↓ *RNAplfold / RNAup*

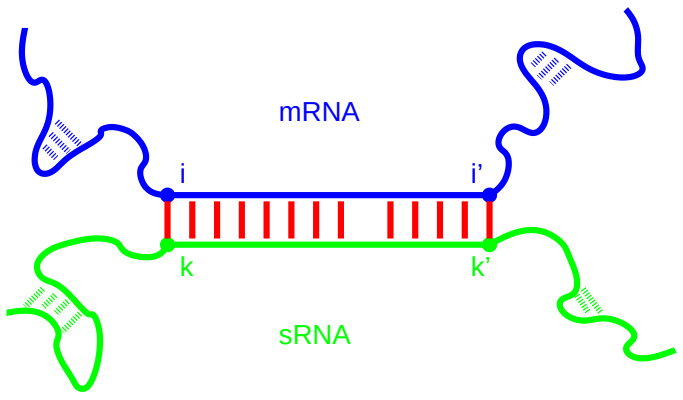
(Busch&Richter&Backofen, Bioinformatics 2008)

The Idea of IntaRNA

- use a matrix for all starts of hybridization (i', k')

$$C^{i',k'}(i,k) = \text{best energy score given that } (i,k) \text{ pair and hybridization } \textbf{starts at base pair } (i',k') \textbf{ and ends at } (i,k)$$

$$= E\left(\right.$$



$$\left. \right)$$

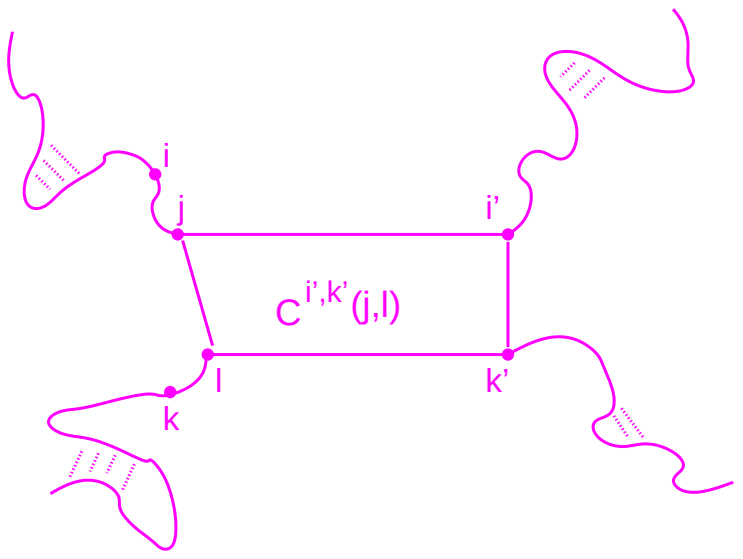
$$= Hybrid(i,k,i',k') + ED^{mRNA}_{i,i'} + ED^{sRNA}_{k,k'}$$

- can be calculated recursively using an RNAhybrid-like approach

The Recursion of IntaRNA

- for all $(i,k) \prec (i',k')$

$$C^{i',k'}(i,k) = \min_{\substack{i < j \leq i' \\ k < l \leq k'}} \left(\begin{array}{ll} & + C^{i',k'}(j,l) \\ - & - \\ + & + \end{array} \right)$$



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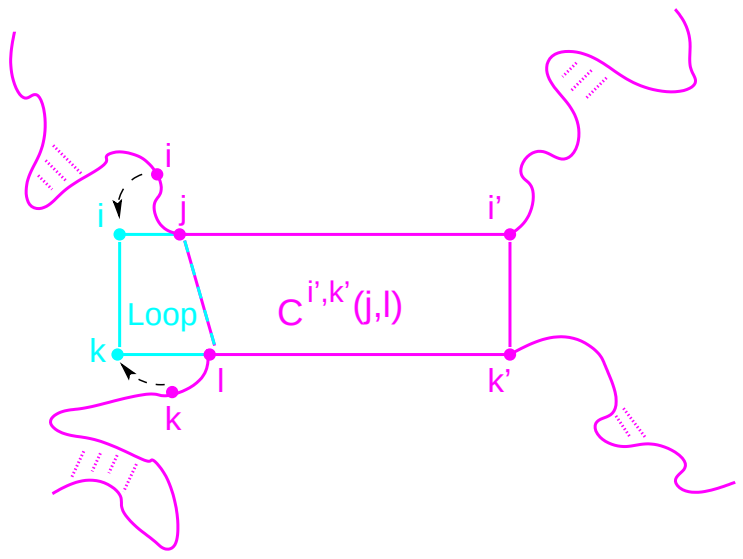

$$\left. \right)$$

$$= \min_{\substack{i < j \leq i' \\ k < l \leq k'}} \begin{pmatrix} Loop(i,k,j,l) + C^{i',k'}(j,l) \\ - \qquad\qquad - \\ + \qquad\qquad + \end{pmatrix}$$

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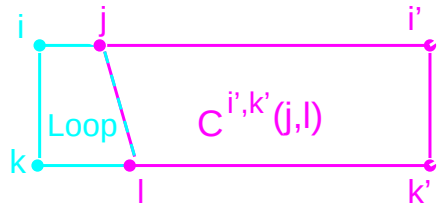

$$\left. \right)$$

$$= \min_{\substack{i < j \leq i' \\ k < l \leq k'}} \left(\begin{array}{ccc} Loop(i,k,j,l) + C^{i',k'}(j,l) \\ -ED^{mRNA}_{j,i'} - ED^{sRNA}_{l,k'} \\ + \qquad\qquad + \end{array} \right)$$

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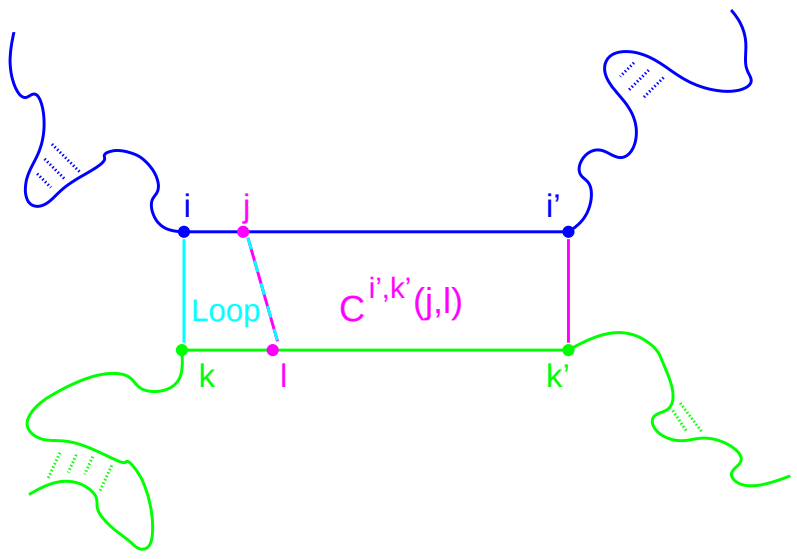

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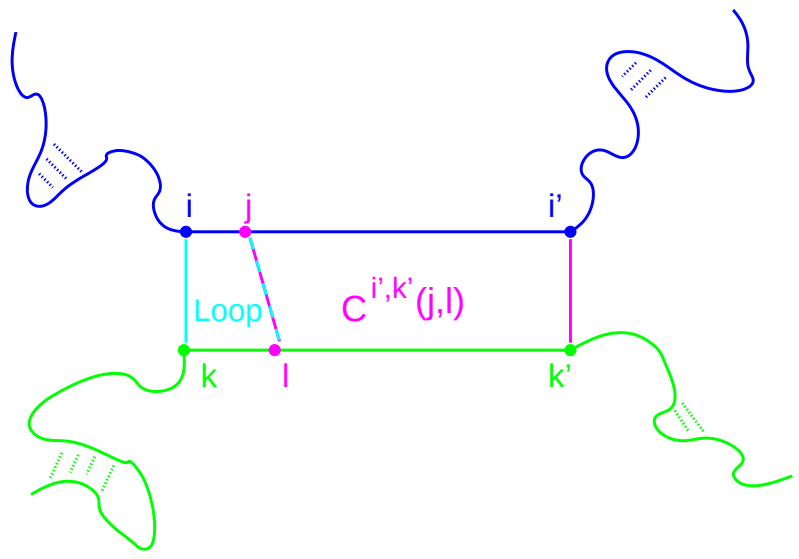

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$$= \min_{\substack{i<j\le i' \\ k<l\le k'}} \left(\begin{array}{l} Loop(i,k,j,l) + C^{i',k'}(j,l) \\ -ED^{mRNA}_{j,i'} - ED^{sRNA}_{l,k'} \\ +ED^{mRNA}_{i,i'} + ED^{sRNA}_{k,k'} \end{array} \right)$$

- heuristic simplification:
  - for all pairs (i,k) store only values for one hybridization start (i',k') instead of all possible starts
  - $O(mn)$ time and space for recursion

Incorporating Seeds

- **seed:** short subsequence of nearly perfect complementarity, often conserved
- found in miRNAs and siRNAs near 5'end, in sRNAs at variable positions

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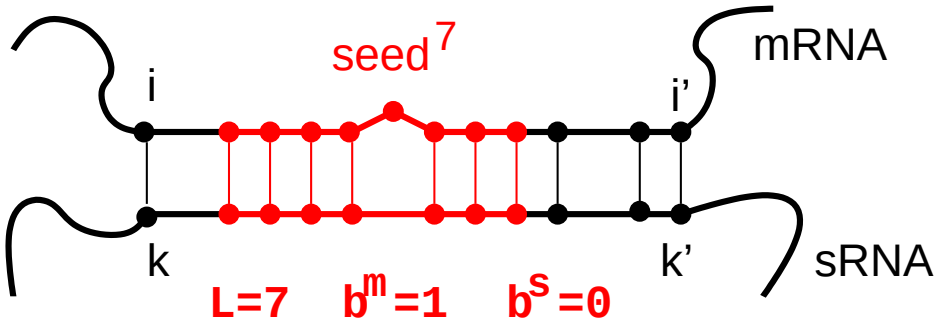
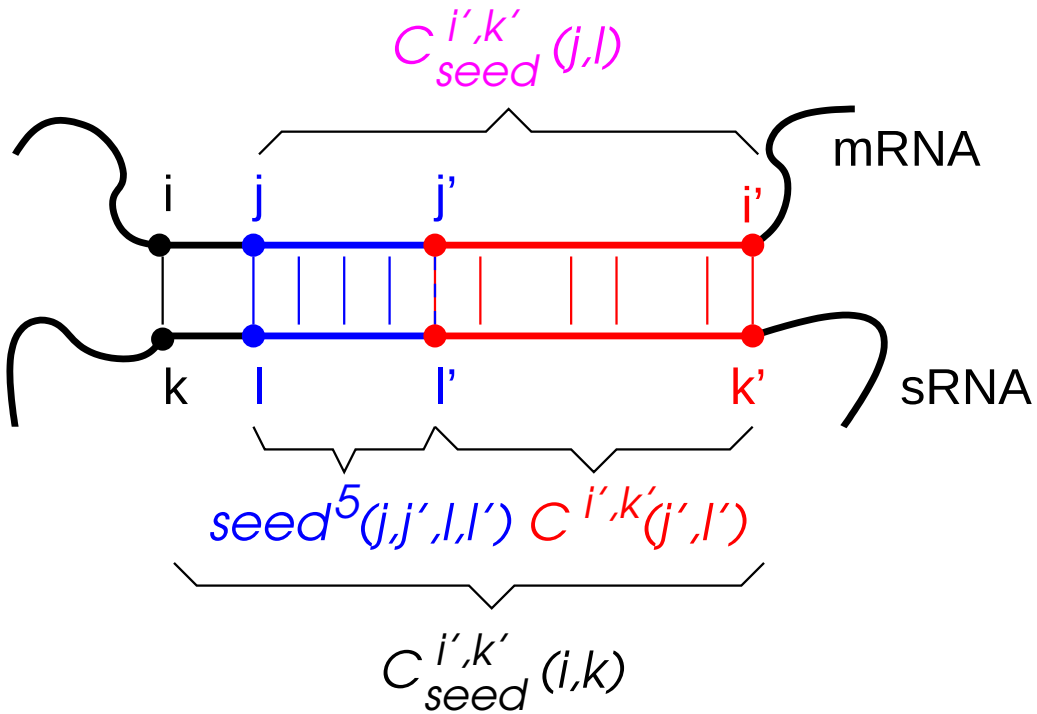
- **seed:** short subsequence of nearly perfect complementarity, often conserved
- found in miRNAs and siRNAs near 5'end, in sRNAs at variable positions
- incorporated into IntaRNA up to now with following features:
  - L ... number of bases perfectly paired in the seed region
  - b^m / b^s ... max. number of mismatches in seed region of mRNA/sRNA
  - position of seed region



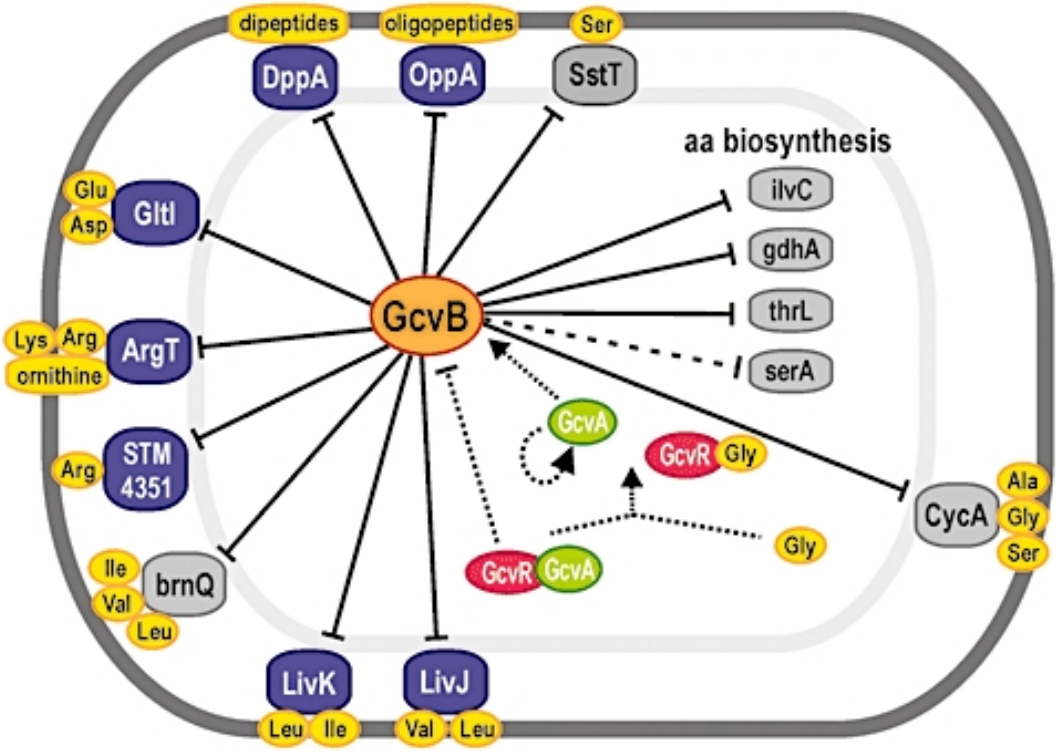
Incorporating Seeds

Incorporating a seed into the recursion to allow it at variable positions:

$C_{seed}^{i',k'}(i,k)$ = best energy score given that (i,k) pair and hybridization starts at base pair (i',k'), ends at (i,k) and **includes a seed region**



Salmonella sRNA GcvB and its targets



(Vogel, Mol. Microbiol. 2008)

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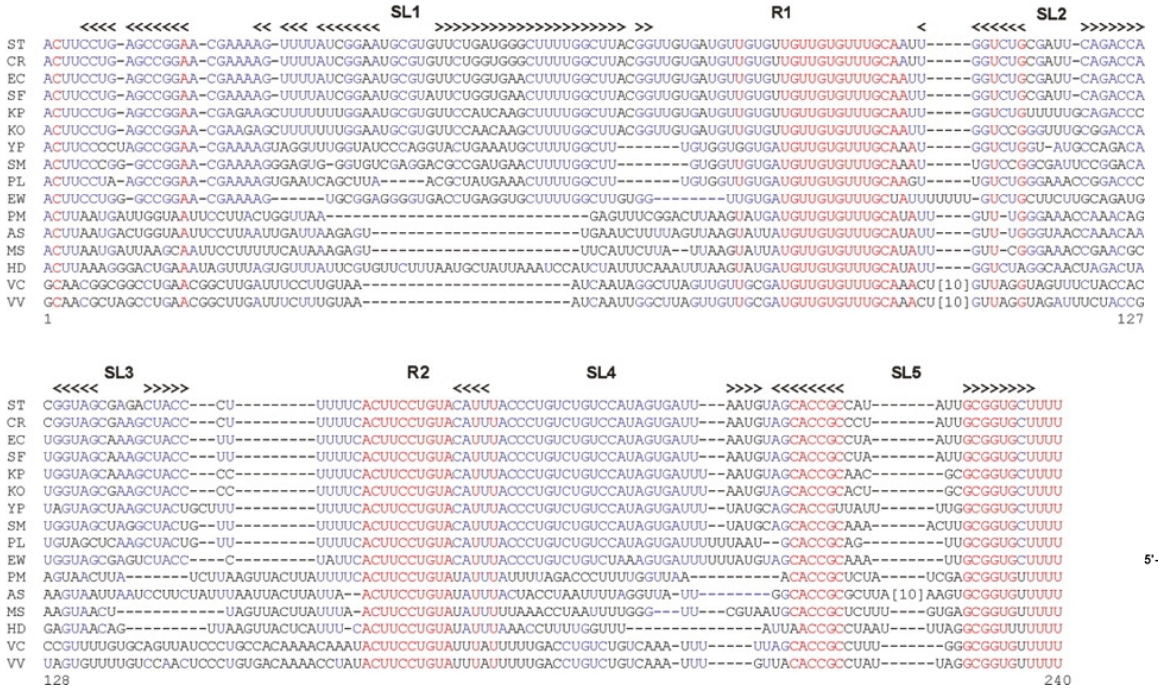
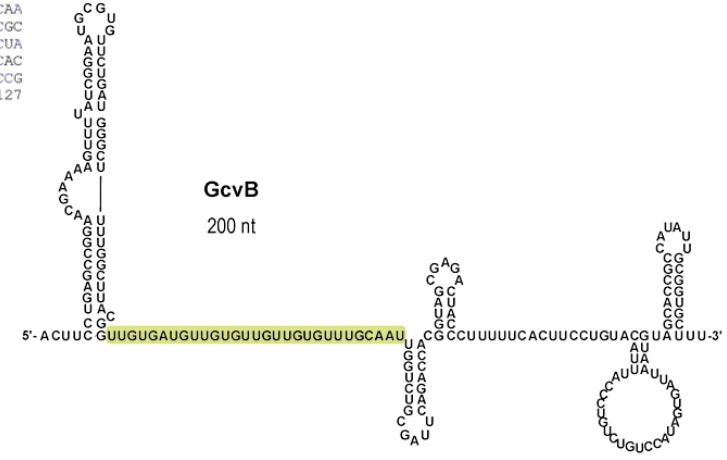
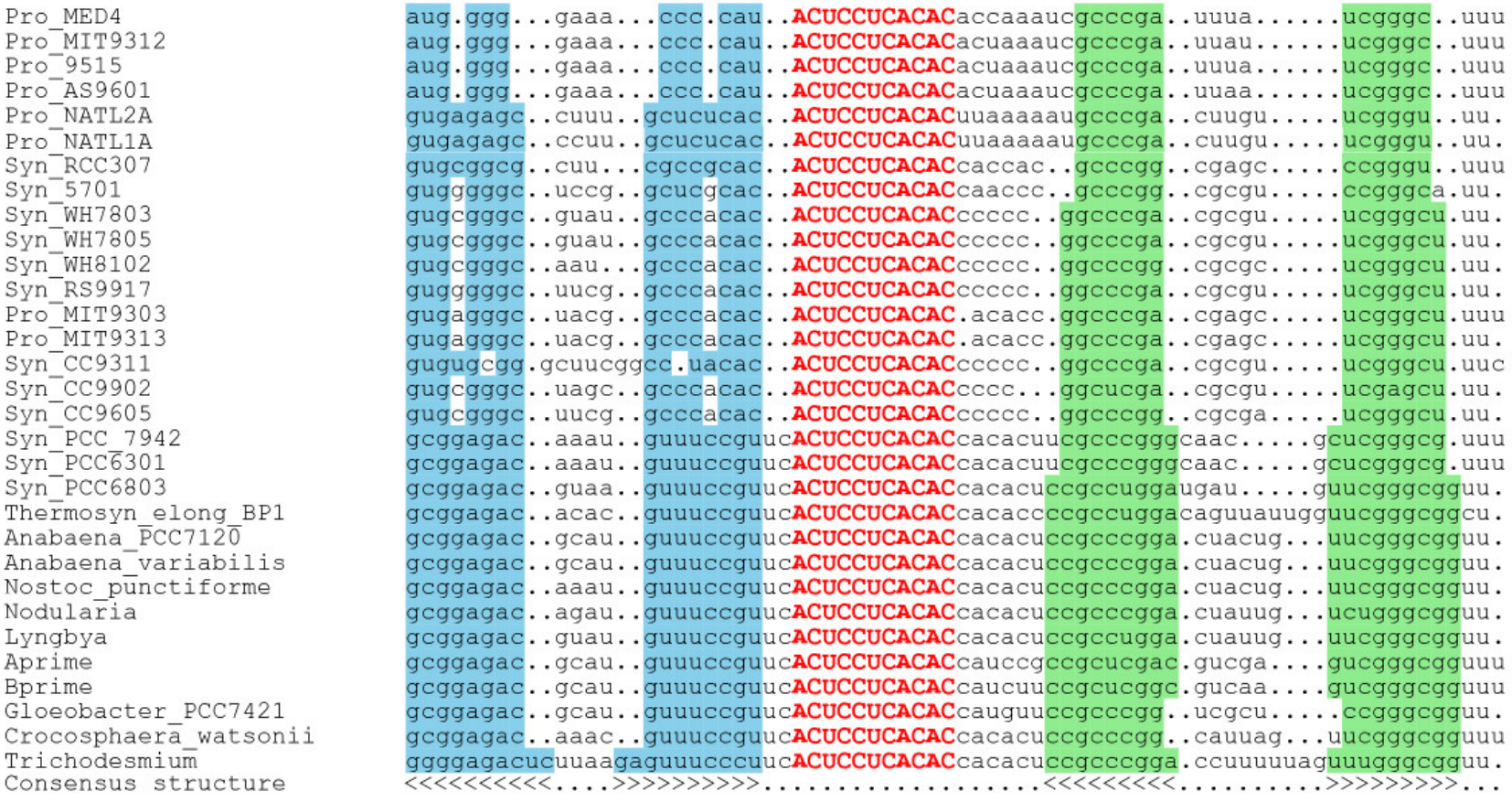




- $\sim$ 30nt G/U-rich region in GcvB interacts with all target mRNAs
- region partially ultra-conserved

(Sharma *et al.*, Genes & Dev. 2007)

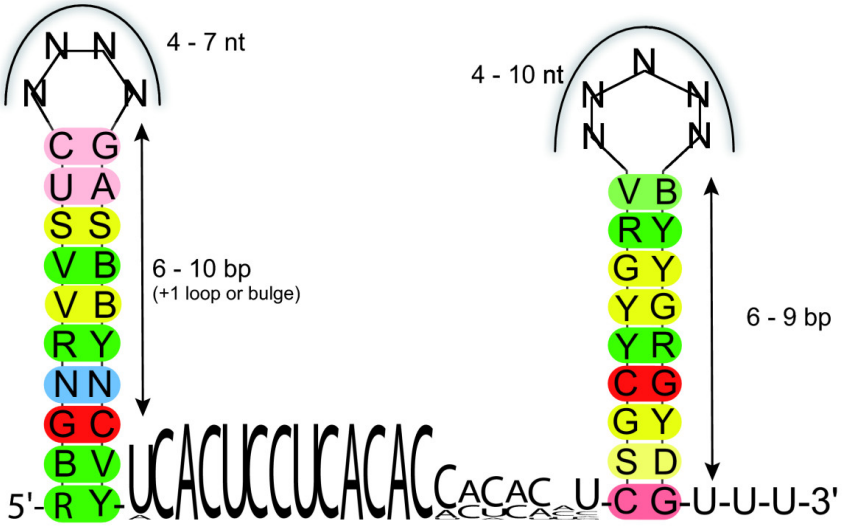
Cyanobacterial sRNA Yfr1



Multiple alignment of sRNA Yfr1 from 31 cyanobacteria

(Voß *et al.*, BMC Genomics 2007)

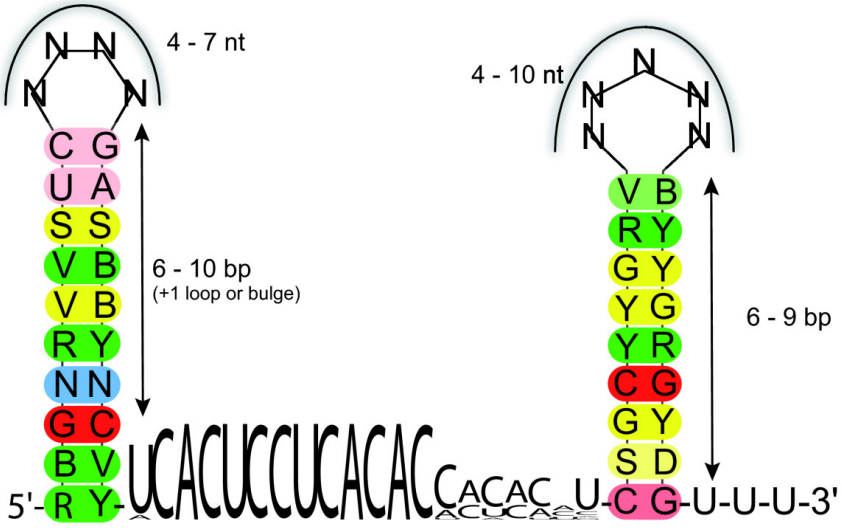
Cyanobacterial sRNA Yfr1



Sequence/structure model of Yfr1 RNA
(Voß *et al.*, BMC Genomics 2007)

sequence motif ACUCCUCACAC perfectly conserved in 31 cyanobacteria

Cyanobacterial sRNA Yfr1



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Scan for Yfr1 targets in *Prochlorococcus* MED4 5'-UTR and CDS (200 nt region):

variable seed (no fixed position): 725 putative targets
ultra-conserved sequence motif as seed: 47 putative targets
thereof: 4 high-scoring interactions at RBS

Predicted Yfr1 interactions for experimental validation

som (possible porin) -2 to +8

```
5'-...GAUUAUUUUUAAAUUACUCAAAU          UUUUUAUGAAGCUUU...-3'
                              UGUGUGAGGA
                              ACACACUCCU
3'-UUUCGGGCUAUUUAGCCCGCUAAACC          CAUACCCCAAAGGGGGUA-5'
```

ppa (putative inorganic pyrophosphatase) -35 to -23

```
5'-...AUUUACGUUUUAGAAUUUGAUUGA         U   GGAUAAAUAAAA...-3'
                               GUGUGAGGA GUA
                               CACACUCCU CAU
3'-UUUCGGGCUAUUUAGCCCGCUAAACCA             ACCCCAAAGGGGGUA-5'
```

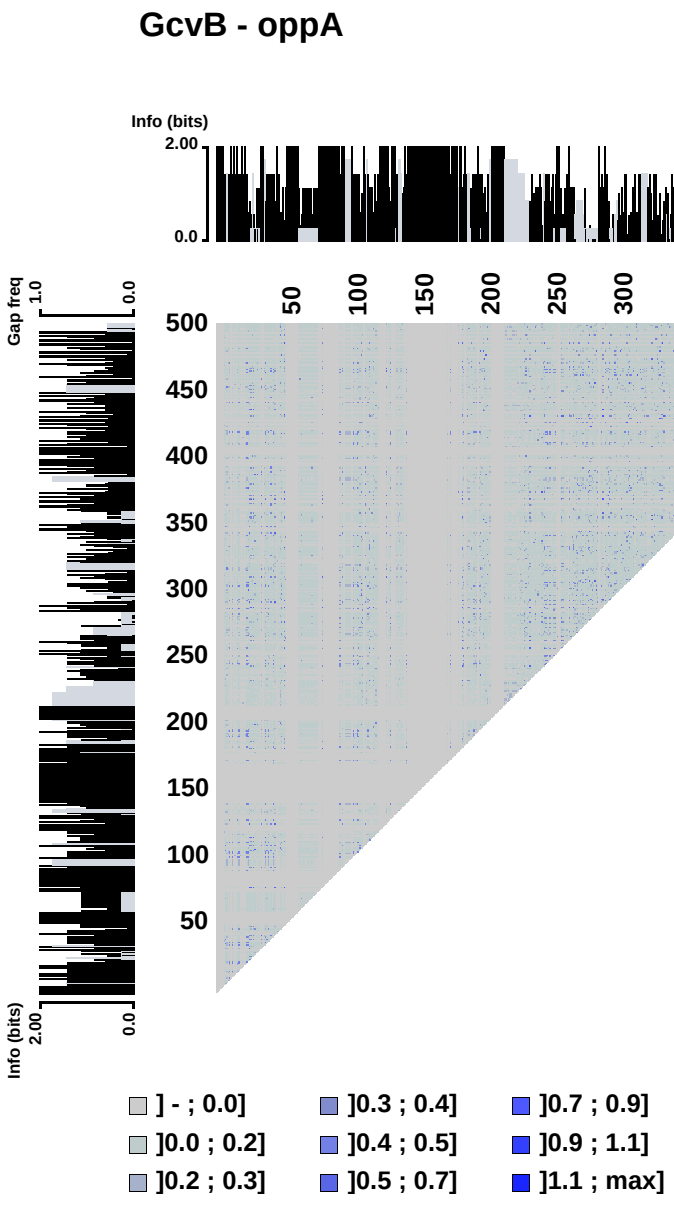
PMM1697 (Type II altern. RNA polymerase sigma factor, sigma-70 family) -4 to +8

```
5'-...AAAAUCCACUUAAAGAGGCCA      A      UCCUUCUGGAAUCUG...-3'
                            GG GUG UGGGGA
                            CC CAC ACUCCU
3'-UUUCGGGCUAUUUAGCCCGCUAAA  A          CAUACCCCAAAGGGGGUA-5'
```

som (possible porin) -2 to +9

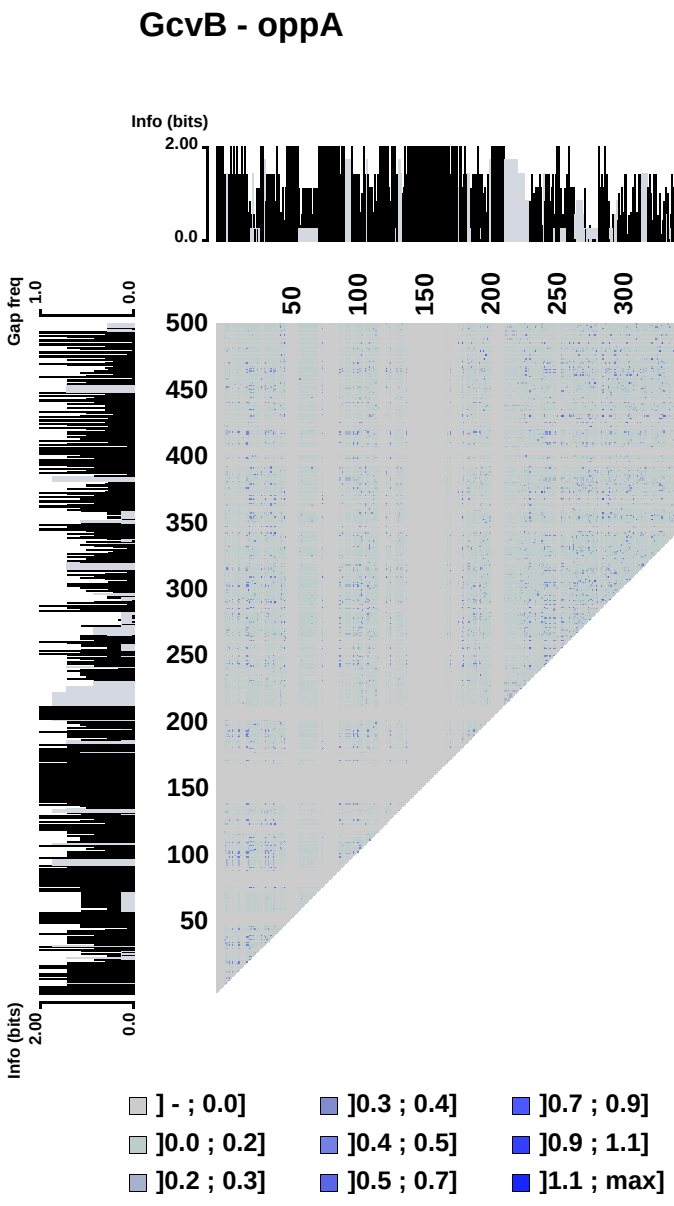
```
5'-...GUAUCUUAAGGUGUCCCUAAUAU         C AUUUAUGAAGCUUUU...-3'
                              UGUGUGAGG A
                              ACACACUCC U
3'-UUUCGGGCUAUUUAGCCCGCUAAACC           CAUACCCCAAAGGGGGUA-5'
```

Covariance at interaction sites



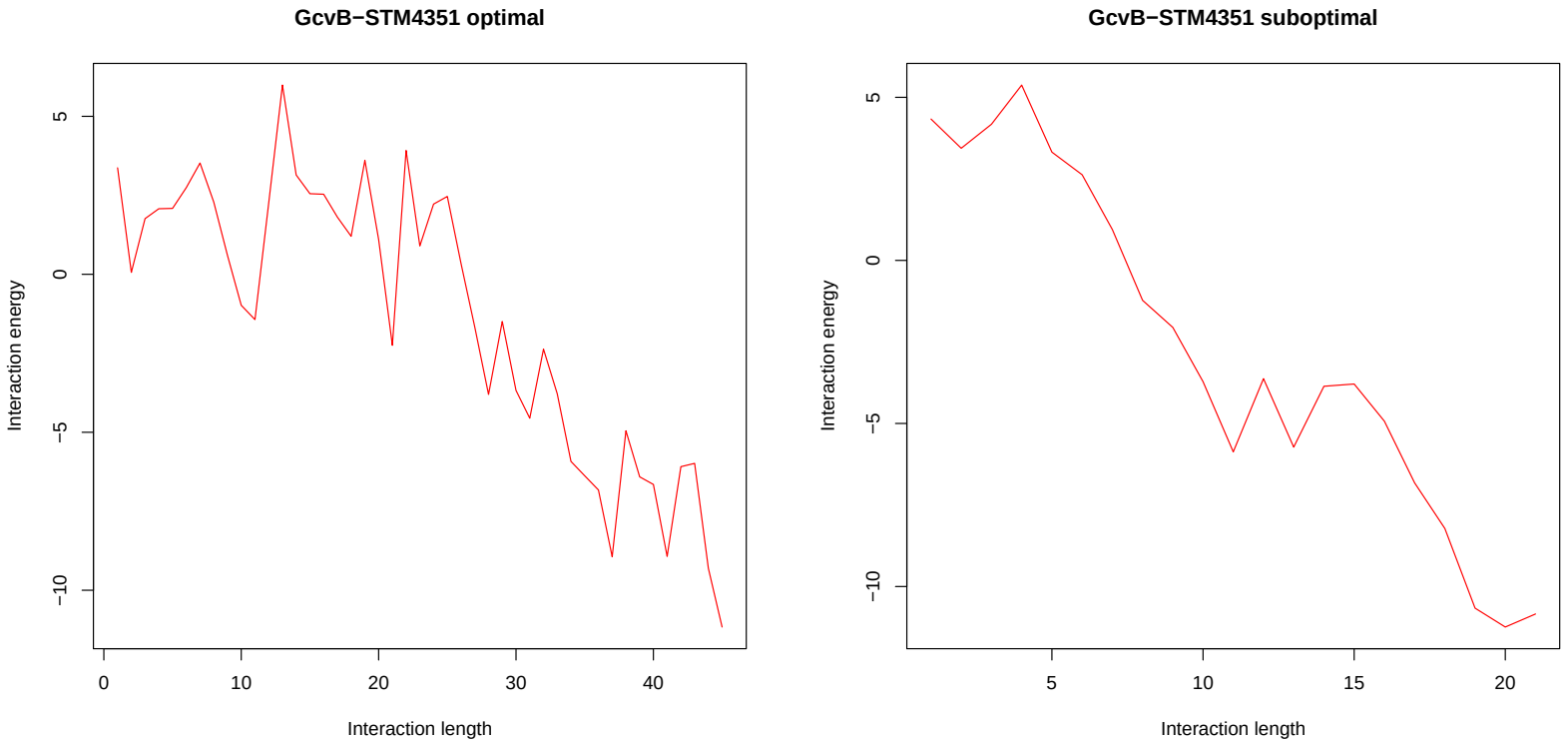
- region of interaction: (68,92), (391,414)

Covariance at interaction sites



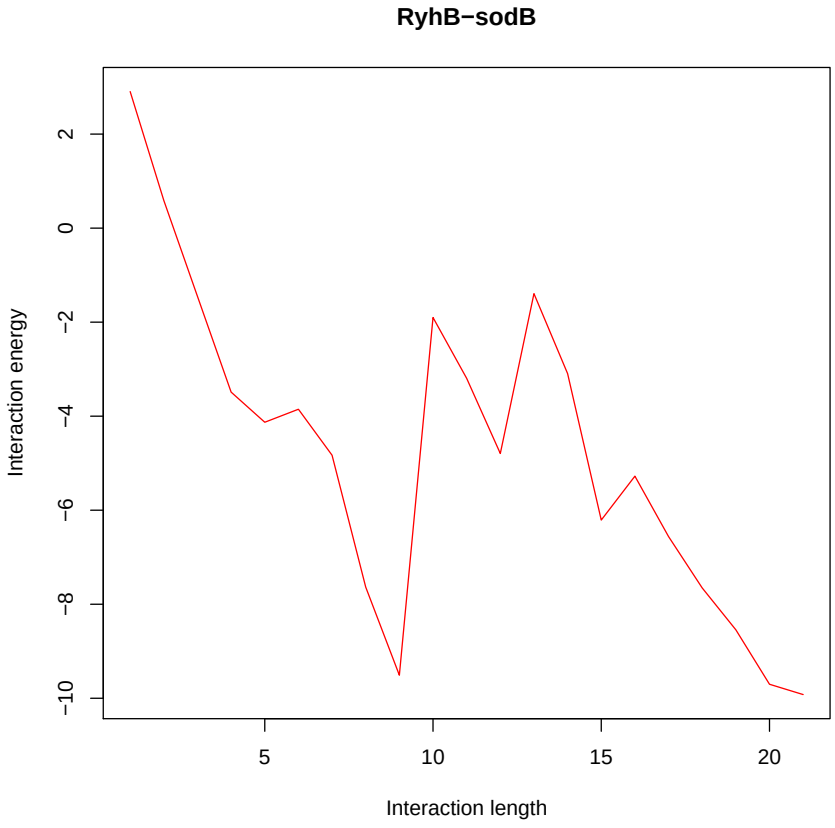
- region of interaction: (68,92), (391,414)
- high sequence conservation at interaction site, but no clear covariance signal
- consequences for target predictions:
  - find seed region with high sequence conservation in mRNA and sRNA
  - start interaction with seed and extend

IntaRNA energy score curves



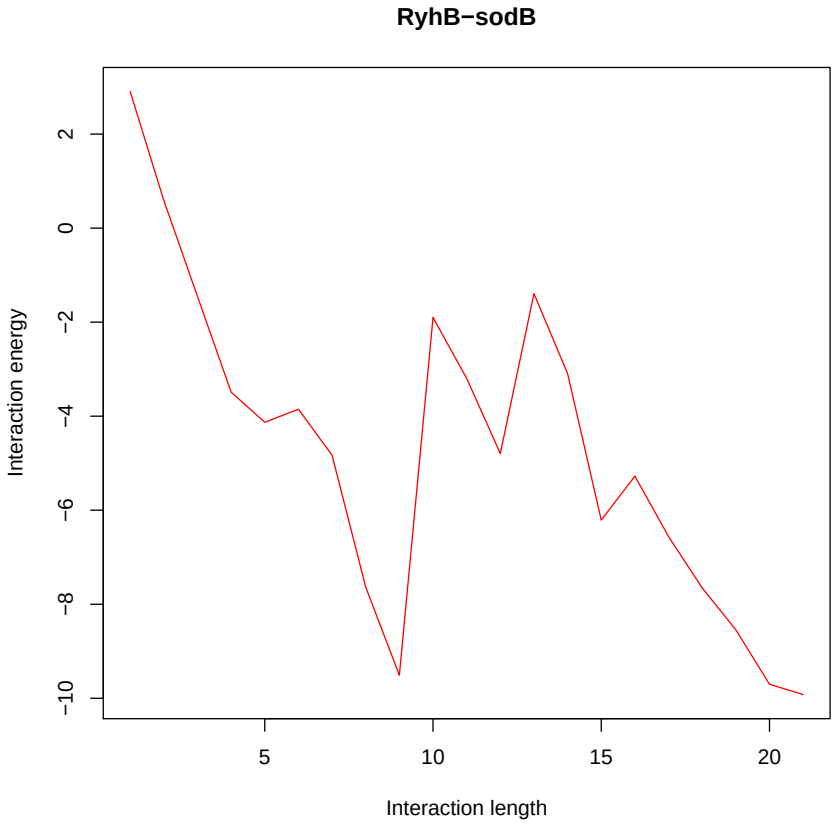
predicted optimal and suboptimal GcvB-STM4351 interactions in *Salmonella*

IntaRNA energy score curves



predicted RyhB-sodB interaction in *E. coli*

IntaRNA energy score curves



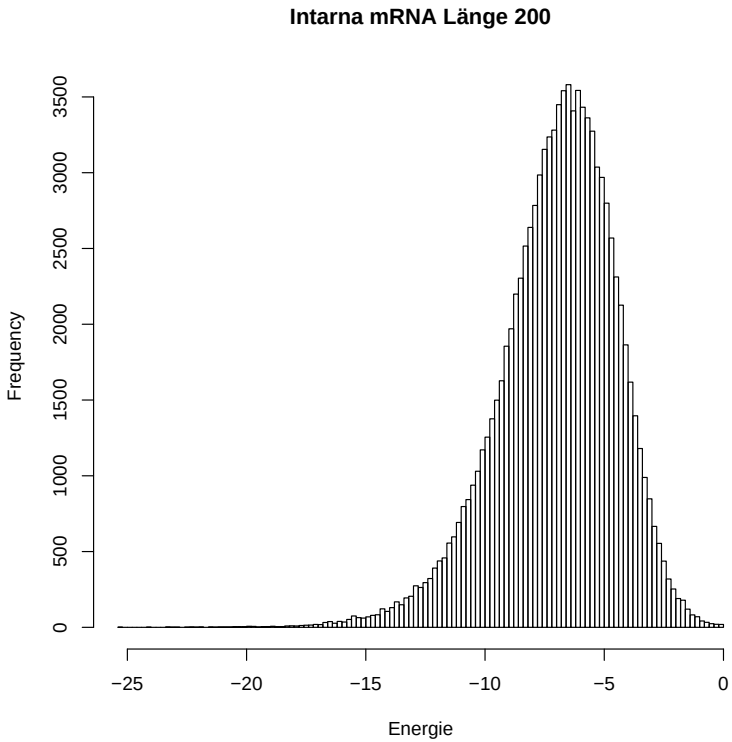
predicted RyhB-sodB interaction in *E. coli*

$\Rightarrow$ forbid distinct increases in energy score of an interaction

Significance of IntaRNA scores

- statistical significance of IntaRNA scores of interest, especially for genome-wide predictions
- straightforward way: estimate *P*-values for given scores by running simulations with shuffled sequences for the null model
- more desirable: analytic expressions giving dependence between sequence features and *P*-values to avoid simulations
- idea:
  - generate thousands of random sequences of various length and nucleotide compositions
  - try to find dependencies between parameters of IntaRNA score distribution and sequence features

Significance of IntaRNA scores



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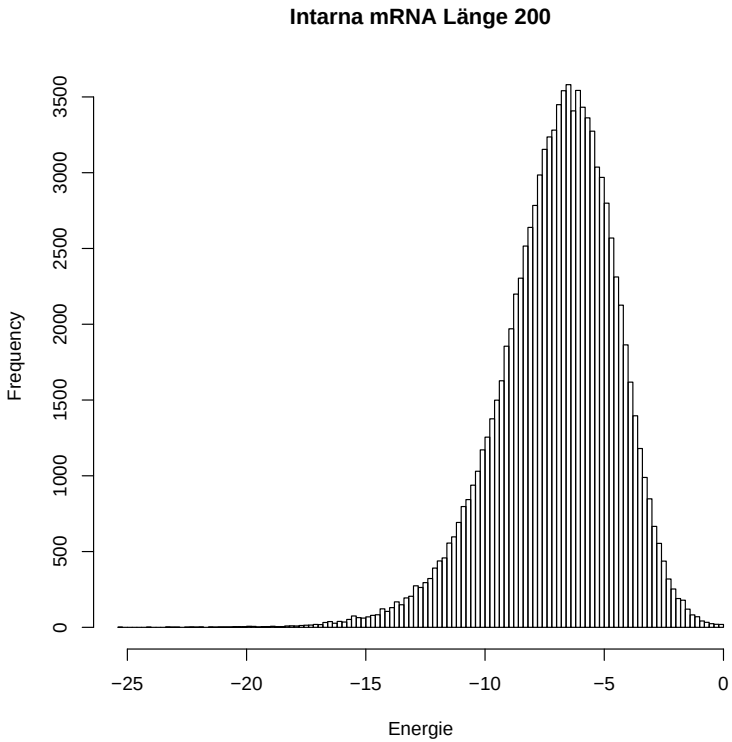
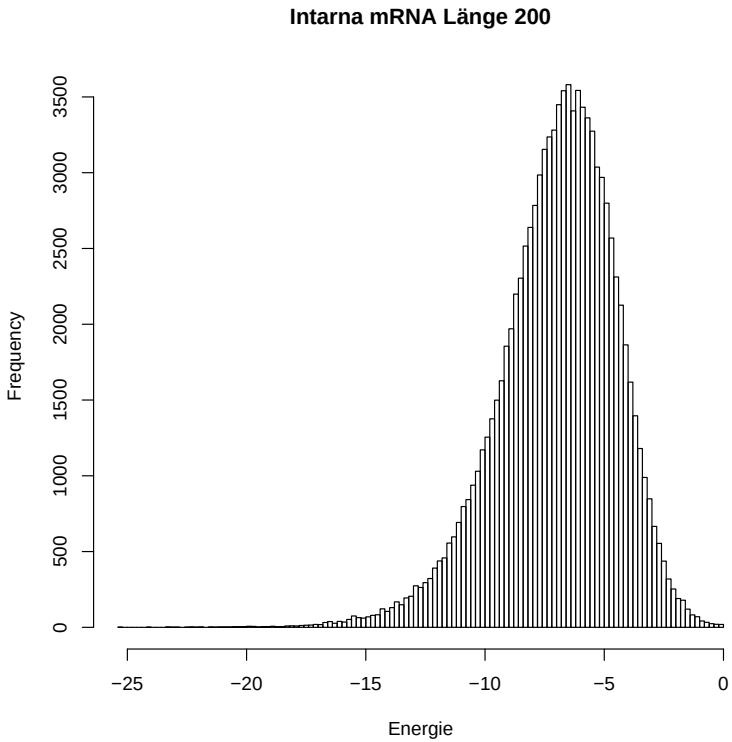
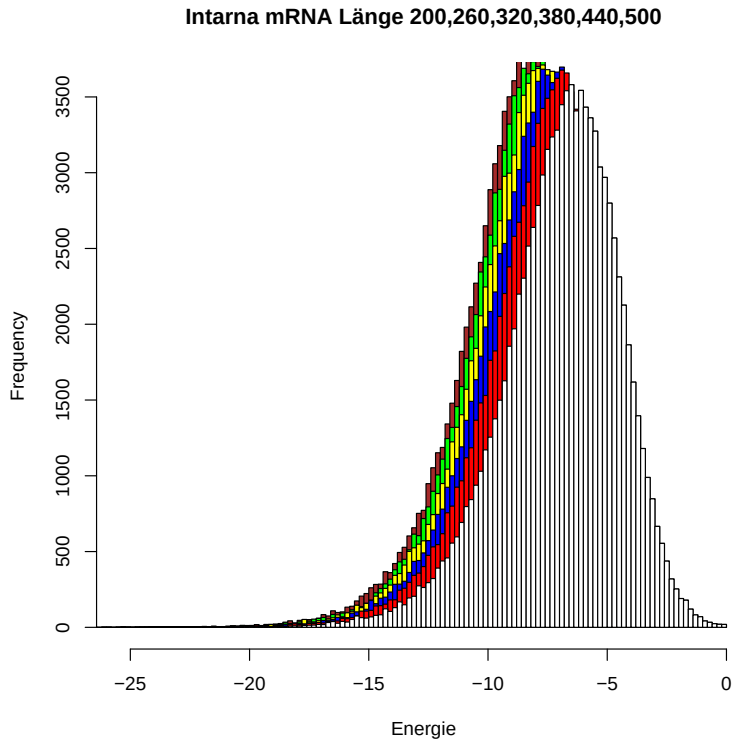


distribution of IntaRNA scores can be approximated by extreme value distribution ($F(x) = e^{-e^{-\frac{x-b}{a}}}$, b . . . location, a . . . scale)

Significance of IntaRNA scores





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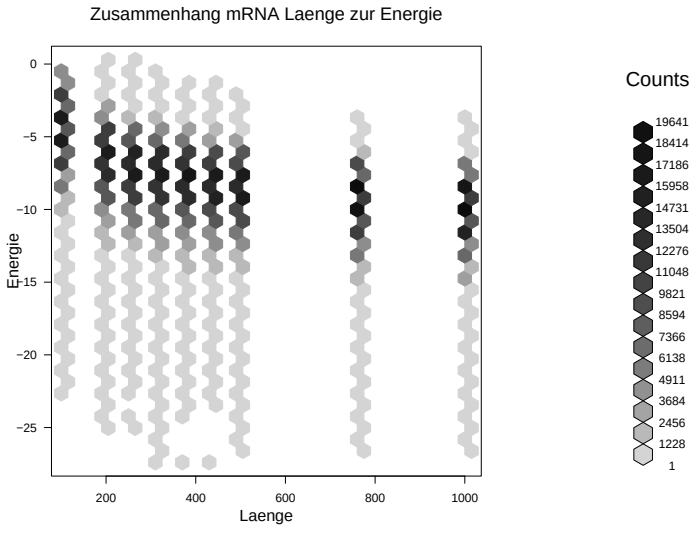

it seems reasonable to normalize IntaRNA scores with $\frac{1}{\log(nm)}$
(following Rehmsmeier *et al.*, Bioinformatics 2004)

Thanks

Thanks to:

Rolf Backofen
Anke Busch
Benjamin Schulz
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