Alignment of RNA with Structures of Unlimited Complexity

Alessandro Dal Palù, Mathias Möhl, Sebastian Will

WCB 2010, Edinburgh

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Solving the general problem of RNA sequence-structure alignment with CP

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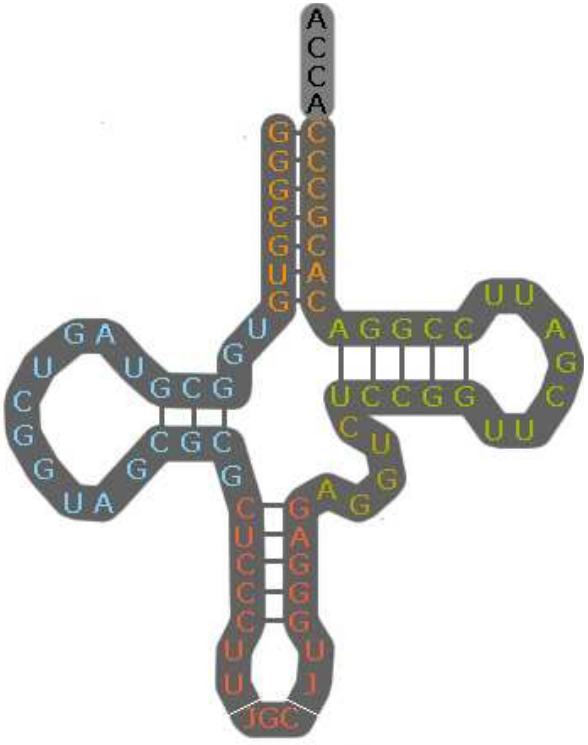
Solving the general problem of
RNA sequence-structure alignment with CP

RNA Structure

primary structure = sequence (of bases A,C,G,U)

GGGCGUGUGGCGUAGUCGGUA ... GUUCGAUUCCGGACACGCCCACCA

secondary structure



tertiary structure

RNA Structure

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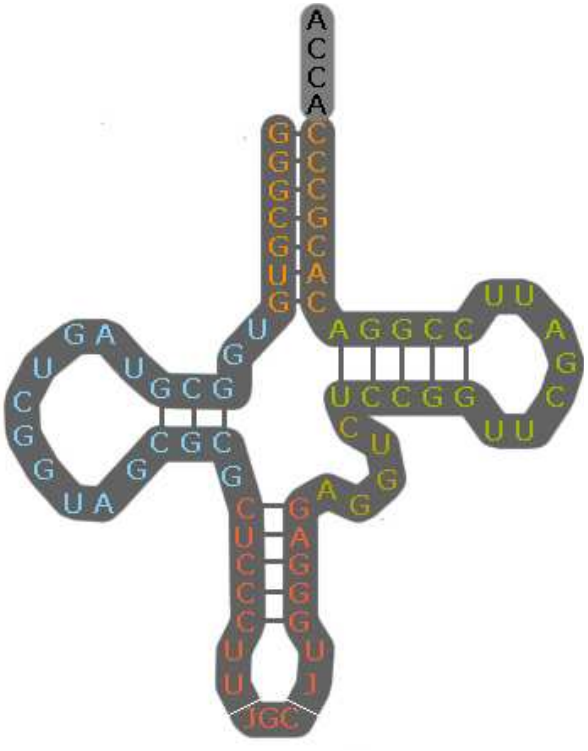
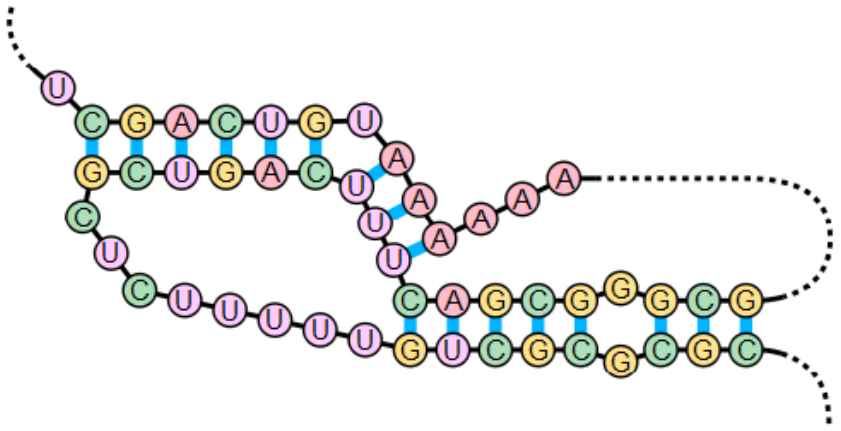


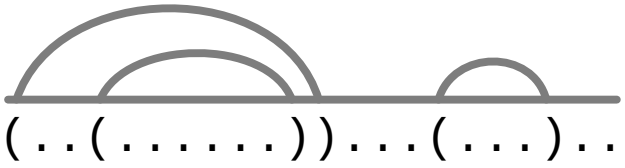
tertiary structure

secondary structure = set of base pairs (i, j)

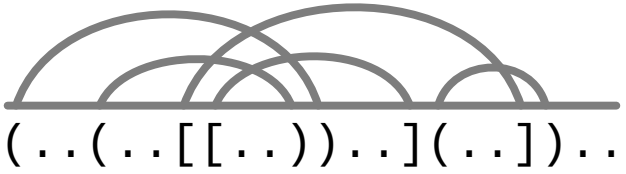
Pseudoknots



- RNA may contain pseudoknots



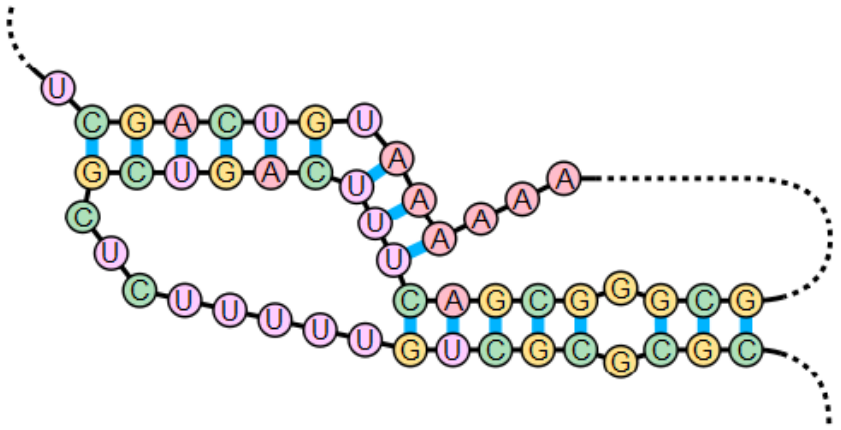
- no pseudoknots
- non−crossing
- well bracketed



- with pseudoknots
- crossing
- not well bracketed

- Pseudoknots make RNA problems NP-hard
  - Structure Prediction
  - Alignment

Pseudoknots



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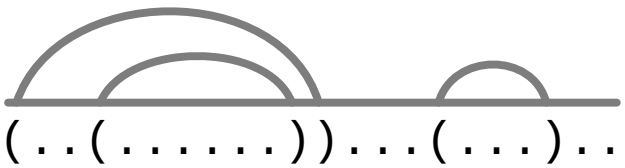

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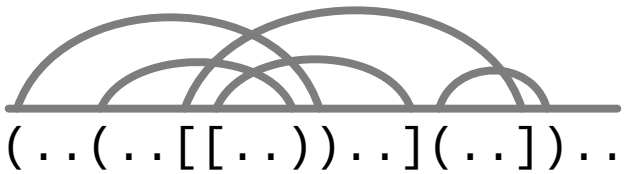


– with pseudoknots
– crossing
– not well bracketed

- Pseudoknots make RNA problems NP-hard
  - Structure Prediction
  - Alignment

Structure Classes

arbitray set of base pairs (i, j)

(N) Nested Structure

no pseudoknots, degree $<= 1$

efficient DP-algorithms

(C) Crossing Structure

degree $<= 1$ (pseudoknots allowed)

NP-hard

(U) Unlimited Structure

unrestricted, NP-hard

RNA Alignment Example: Antizyme FSE

IN:

```
>RNA1
UGAUGUCCCUCUCCCACCCCUGAAGAUCCCAGGUGGGCGAGGGAAUGAUCAGCGGGAUC
.....((((((.((((((......[[[[[[.))))))).))))))........]]]]]]
>RNA2
UGAUGCCCCUCACCCACUGUUGAAGACCCUCAGUGGGUGAGGGGGCGGCAAGGAUC
.....((((((.((((((......[[[[[[.))))))).))))))......]]]]]]
```

OUT:

```
.....((((((.((((((......[[[[[[.))))))).))))))........]]]]]]
UGAUGUCCCUCUCCCACCCCUGAAGAUCCCAGGUGGGCGAGGGAAUGAUCAGCGGGAUC
UGAUGCCCCUCACCCACUGUUGAAGACCCUCAGUGGGUGAGGGGGCG-GCA--AGGAUC
.....((((((.((((((......[[[[[[.))))))).))))))...-...--]]]]]]
```

RNA Alignment Toy Example

IN:

```
>RNA1
GCCAUACGGCAUAC
(((.[[.))).]].
>RNA2
GGUUGCCGCCAACAC
((([[..)))..]].
```

OUT:

```
     (((.[[-.)))-.]].
RNA1 GCCAUA-CGGC-AUAC
RNA2 GGU-UGCCGCCAACAC
     (((-[[..)))..]].
```

alignment = set of edges
between 'aligned' bases of RNA 1 and RNA 2

The RNA Alignment Problem

Given: sequence-structure pairs (A, PA) and (B, PB).

The *alignment problem* is

$$\underset{A \text{ alignment of } (A,PA) \text{ and } (B,PB)}{\operatorname{argmax}} \quad \textbf{score}(A).$$

$$\begin{aligned}
\textbf{score}(A_m \cup A_g) := & \sum_{(i,i') \in A_m} \sigma(i,i') && \text{(sequence)} \\
& + \sum_{\substack{(i,j) \in P_a, (i',j') \in PB, \\ (i,i') \in A_m, (j,j') \in A_m}} \tau(i,j,i',j') && \text{(structure)} \\
& + \quad \gamma |A_g| && \text{(gaps)}
\end{aligned}$$

Related Work

- Easy: alignment of nested RNA
  - $O(n^4)$ Jiang et al., General Edit Distance, JCB 2002.
  - $O(n^3)$ Demaine et al., Optimal Decomposition for Tree Edit Distance, TALG 2009.
- Hard: crossing/pseudoknots
  - Evans, Finding Common Pseudoknot Structures in Polynomial Time, CPM 2006. (restricted crossing, efficient)
  - Möhl et al., Lifting prediction to alignment of RNA pseudoknots, JCB 2010. (restricted crossing, efficient)
  - Möhl et al., FPT alignment of RNA structures including arbitrary pseudoknots, CPM 2008. (arbitrary crossing, FPT)

Related Work II: SA&F

Simultaneous Alignment and Folding (SA&F)

- aligns RNA with unlimited structure
- but selects ($\rightarrow$ F!) a nested or crossing structure

Approaches

selecting nested structure (dynamic programming)

- Sankoff, original SA&F, J Appl Math 1985.
- Mathews et al., Dynalign, JMB 2002.
- Havgaard et al., Foldalign, Plos Comp Biol 2007.
- Will et al., LocARNA, Plos Comp Biol, 2007.

selecting crossing structure (ILP)

Bauer et al., Lara, BMC Bioinformatics 2007.

Recall: here, we wont select a sub-structure for scoring,
but score all aligned structural elements

Constraint Model

For sequence-structure pairs (A, PA) and (B, PB),

define variables $MD_1, \ldots, MD_{|A|}$ and $M_1, \ldots, M_{|B|}$.

Semantics

- $MD_i = j$: "match or deletion of A_i to/after B_j"
- $M_i = 1$: "match of A_i"

For example, the alignment $\begin{matrix}\texttt{A--CUG}\\ \texttt{ACAC-G}\end{matrix}$,

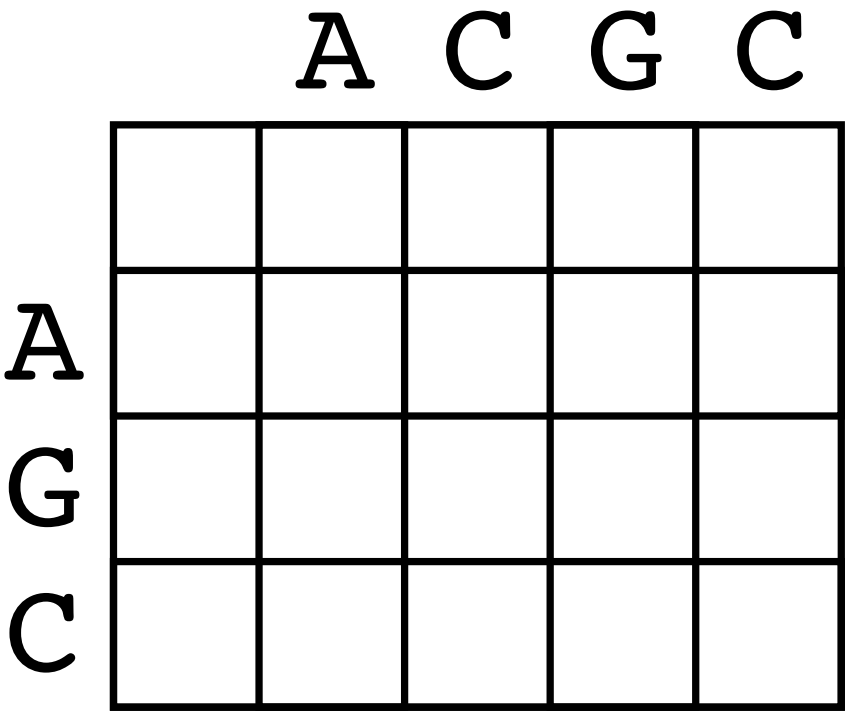
corresponds to variables and values

$(MD_1, \ldots, MD_4) = (1, 4, 4, 5)$ and $(M_1, \ldots, M_4) = (1, 1, 0, 1)$.

We don't need all details. Vars define traces in "alignment graph".

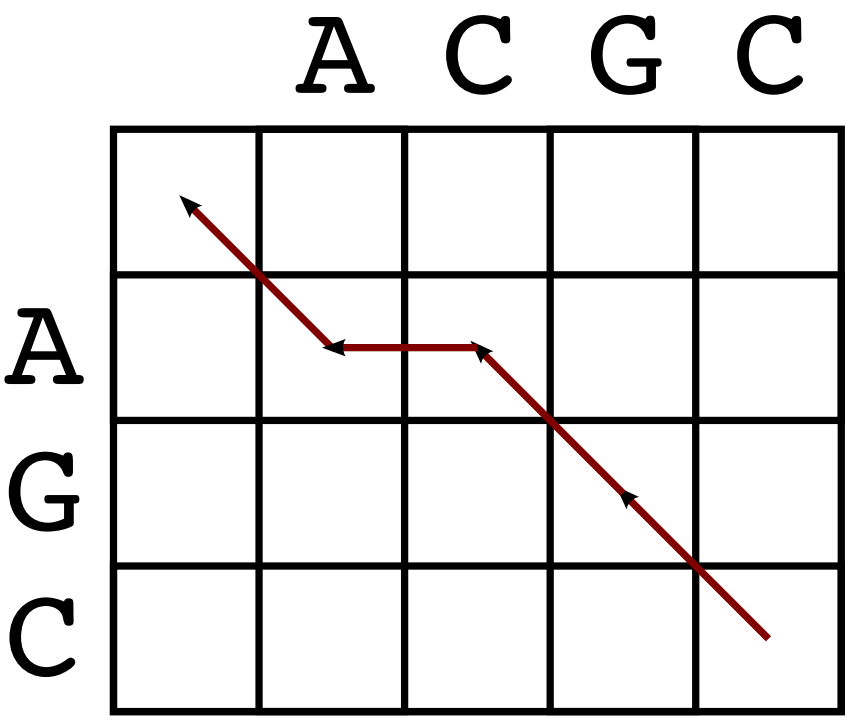
Alignment Graph

Sequences A =AGC, B =ACGC



Alignment Graph

Sequences A =AGC, B =ACGC

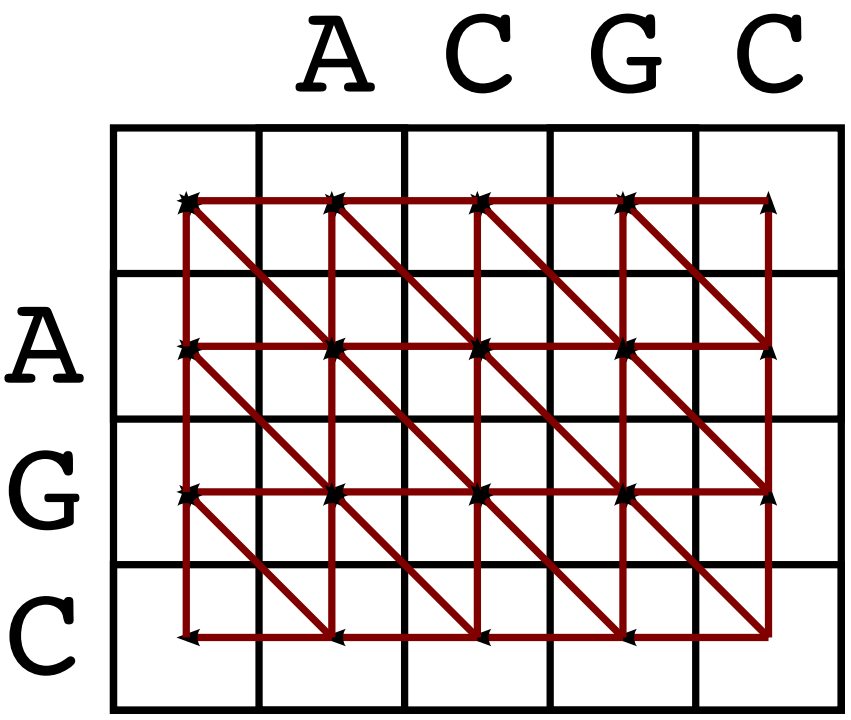


A-GC
ACGC

1 Trace $\equiv$ 1 Alignment

Alignment Graph

Sequences A =AGC, B =ACGC

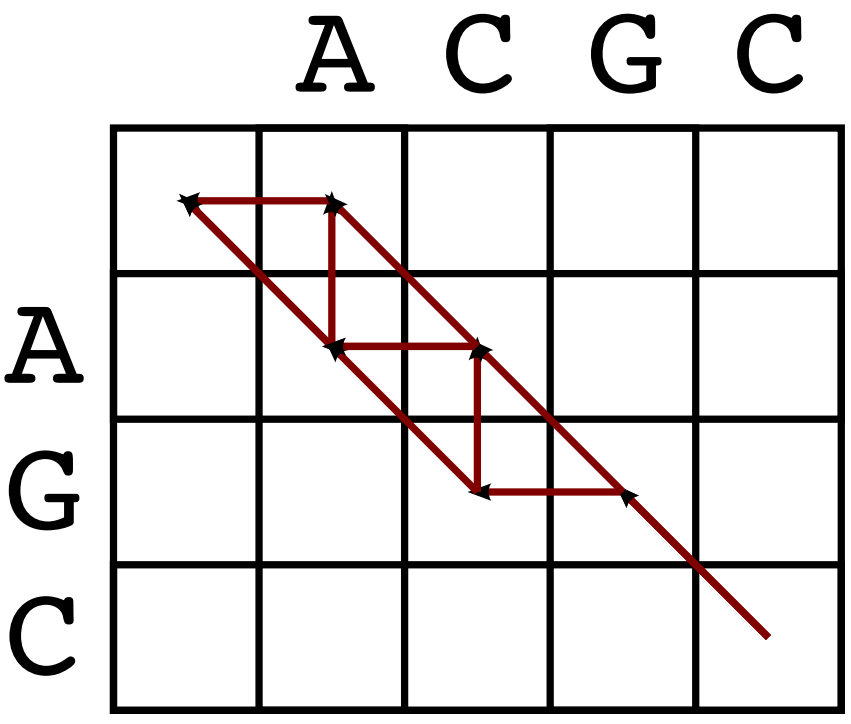


ALL

Graph of all Match/Delete/Insert edges $\equiv$ All alignments

Alignment Graph

Sequences A =AGC, B =ACGC




```
A-GC
ACGC

AG-C
ACGC

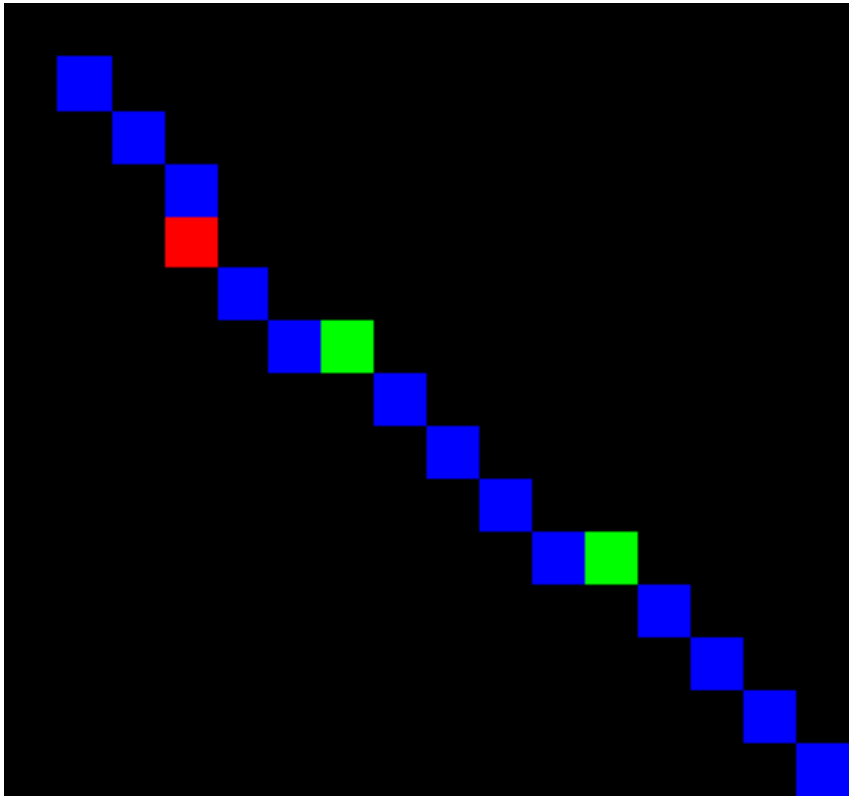
AG-C
ACGC

-AG-C
AC-GC

...
```

Graph of M/I/D edges ≡ Set of alignments
(specified by variable domain or "Constraint Store")

Alignment Graph Color Code



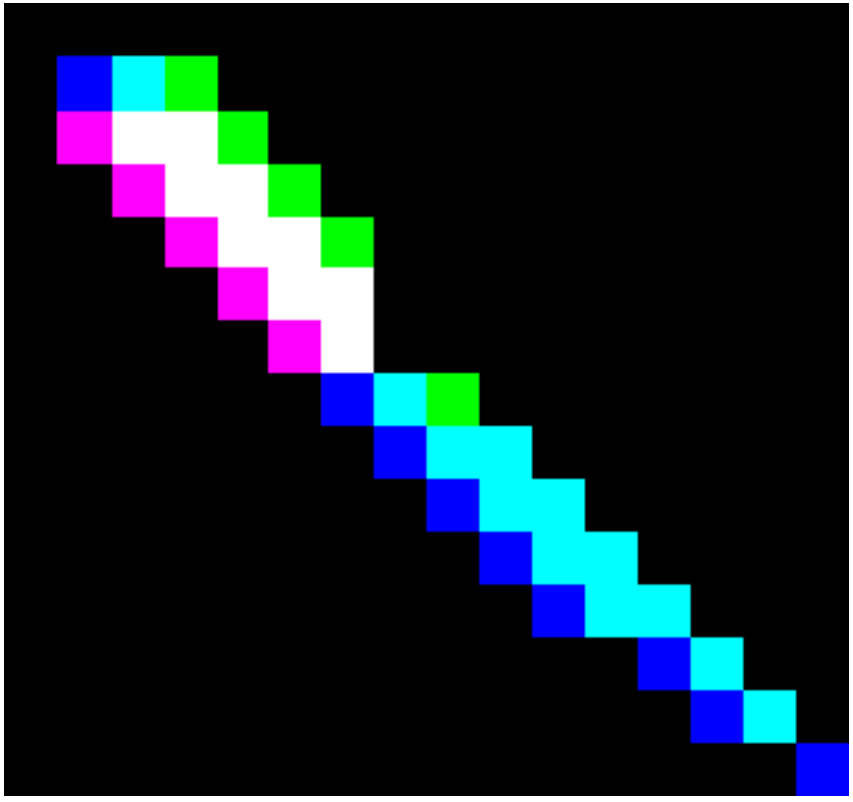
red = deletion

green = insertion

blue = match

Use to represent valuation of variables, i.e. one alignment

Alignment Graph Color Code

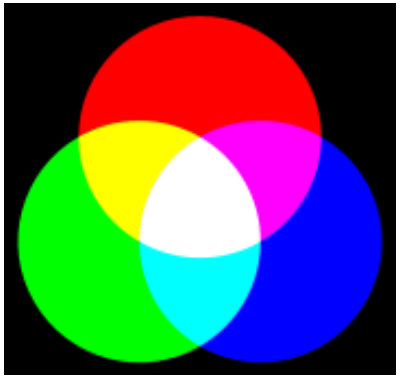


red = deletion

green = insertion

blue = match

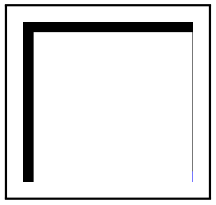
. . . and synthesize colors



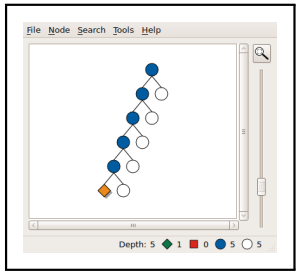
Use to represent set of valuations of variables, i.e. Constraint Store

Solving Strategy

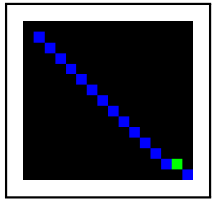
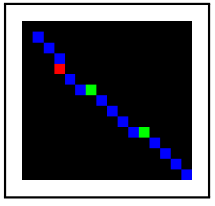
- Start with all possible alignments:



- Enumerate: binary intervall splitting

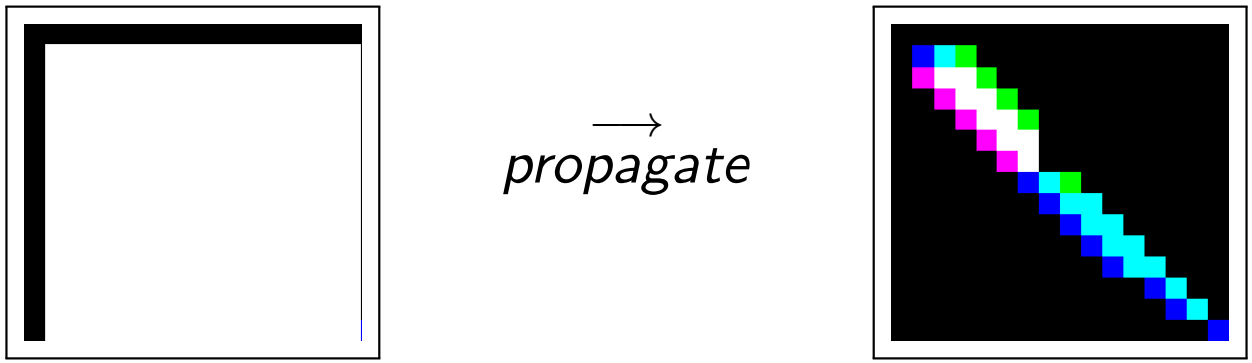


- Branch-and-Bound: search better and better alignments


, 
, . . .

- Crucial: new global constraint propagator

Constraint Propagator



Propagates two constraints:

- $\mathit{Alignment}(\mathbf{MD}, \mathbf{M})$

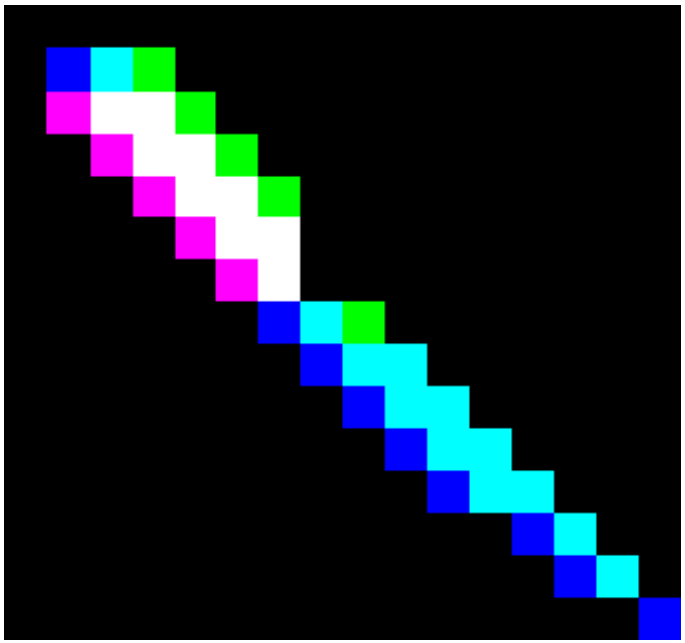
  "variables encode alignment"

- $\mathit{AlignmentScore}_{(A,PA),(B,PB)}(\mathbf{MD}, \mathbf{M}, \mathit{Score})$

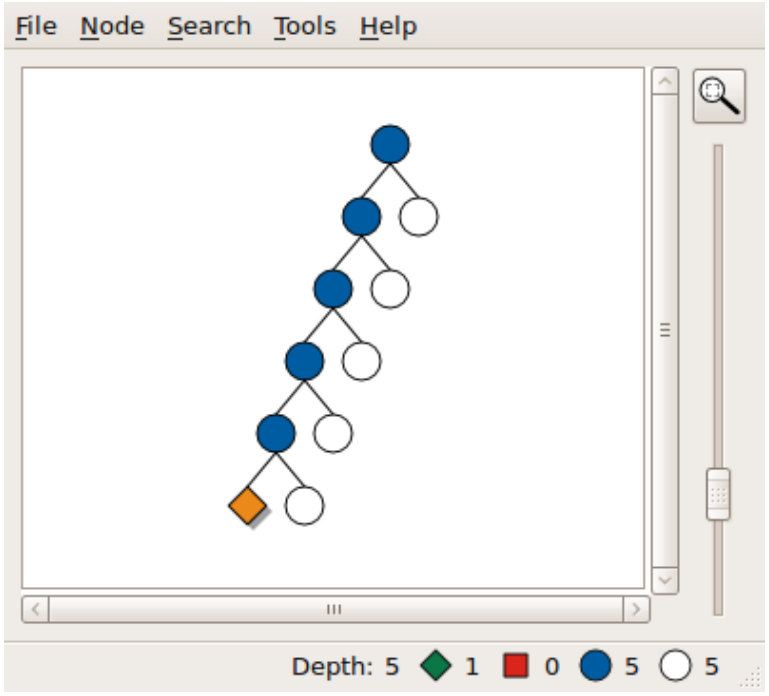
  "*Score* is score of alignment by $\mathbf{MD}, \mathbf{M}$"

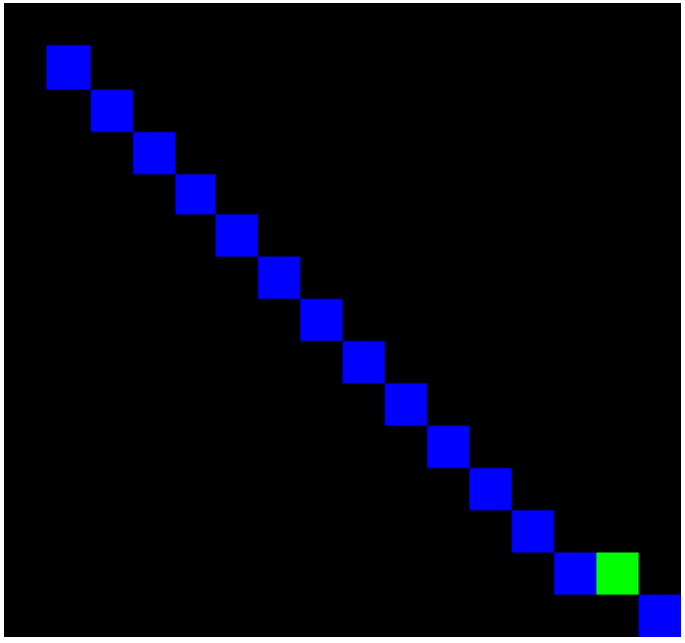
Example
(Run of Gecode Implementation on Toy Example)

```
>RNA1
GCCAUACGGCAUAC
(((.[[.))).]].
>RNA2
GGUUGCCGCCAACAC
((([[..)))..]].
```



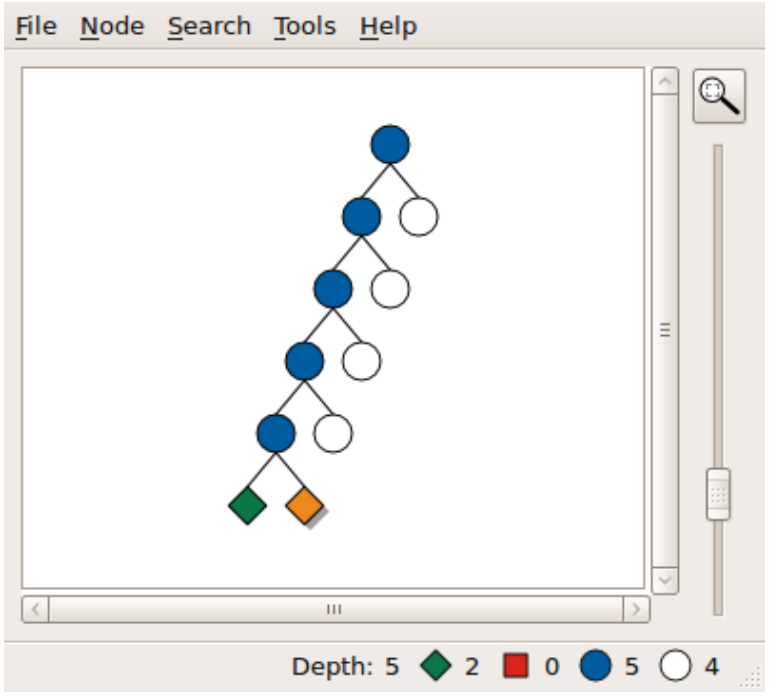
Example

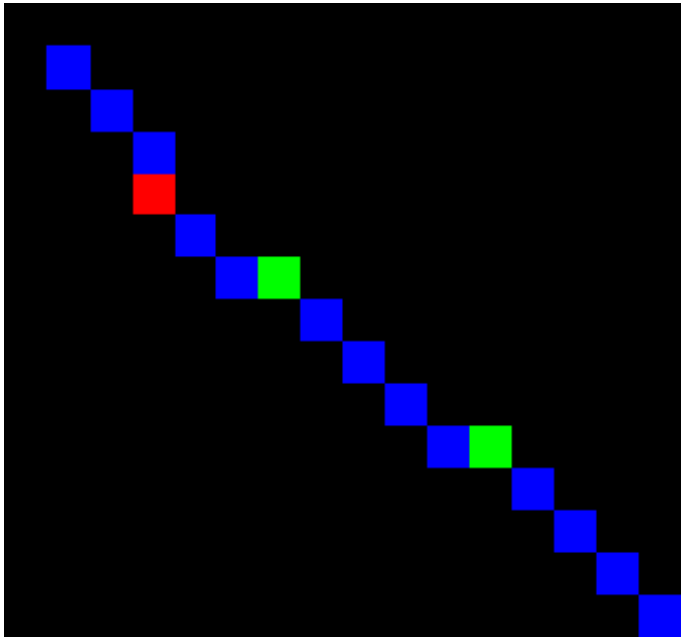




```
(((.[[.))).]]-.
GCCAUACGGCAUA-C
GGUUGCCGCCAACAC
((([[..)))..]].
```

Example





```
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Propagator: Upper Bound by Relaxation

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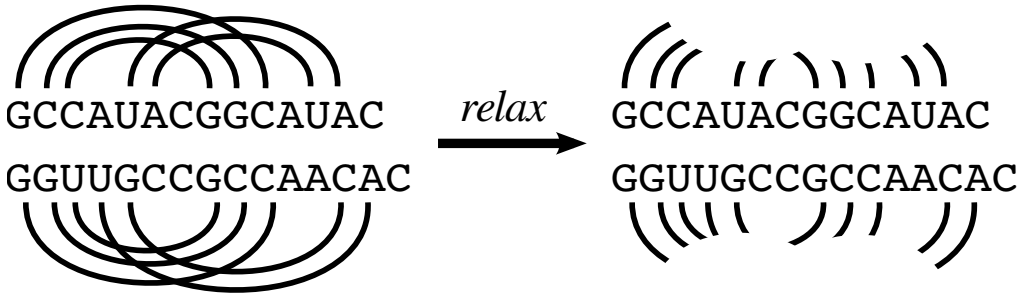
```



$$\textbf{score}_{\text{relaxed}}(A_m \cup A_g) := \sum_{(i,i') \in A_m} \left[\sigma(i,i') + \text{ub}(i,i')\right] \quad + \quad \gamma|A_g|,$$

$$\text{ub}(i,i') = \begin{cases} \frac{1}{2}\tau(i,j;i',j') & \text{iff match } (j,j') \text{ allowed by constraint store} \\ 0 & \text{otherwise} \end{cases}$$

$\textbf{score}_{\text{relaxed}}$ is upper bound of **score**. Computed in $O(n^2)$ by DP!

Given ub for crossing. For unlimited, sum over all (j,j'). Use structural sparsity to limit complexity!

Propagator: Upper Bound by Relaxation

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GCCAUACGGCAUAC
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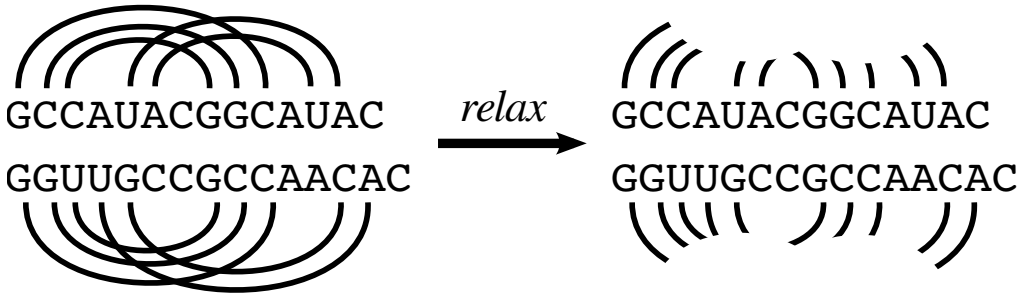
```



$$\textbf{score}_{\text{relaxed}}(A_m \cup A_g) := \sum_{(i,i') \in A_m} \left[\sigma(i,i') + \text{ub}(i,i')\right] \quad + \quad \gamma|A_g|,$$

$$\text{ub}(i,i') = \begin{cases} \frac{1}{2}\tau(i,j;i',j') & \text{iff match } (j,j') \text{ allowed by constraint store} \\ 0 & \text{otherwise} \end{cases}$$

score$_{\text{relaxed}}$ is upper bound of **score**. Computed in $O(n^2)$ by DP!

Given ub for crossing. For unlimited, sum over all (j,j'). Use structural sparsity to limit complexity!

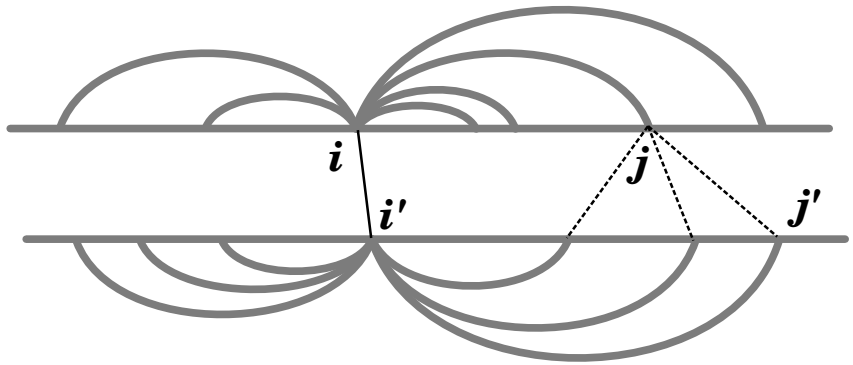
Upper Bound UB Revisited: Structural Sparsity

Full form of upper bound *ub*:

$$\mathrm{ub}_D(i,i') = \frac{1}{2} \max_{A \in \mathcal{A}(D)} \left\{ \begin{array}{c} \sum_{\substack{(i,j)\in PA,(i',j')\in PB \\ (i,i')\in A,(j,j')\in A}} \tau(i,j;i',j') \\ +\text{symm. term for } \tau(j,i;j',i') \end{array} \right\}$$

D domain

$\mathcal{A}(D)$ allowed alignment for domain D



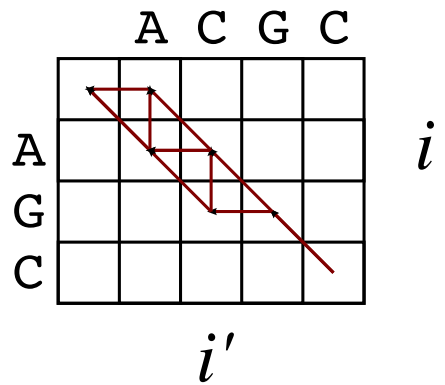
In many applications: structure is sparse, i.e. degree is limited by constant. Then: propagator complexity is $O(n^2)$. (E.g. aligning pair probability matrices, confer SA&F by Will et al., LocARNA)

Propagator: Bound for each match/deletion/insertion

- $\textbf{score}_{\text{relaxed}}$ is a "sequence alignment score"
- compute the relaxed scores of prefix alignments by DP (Smith-Waterman), filling a matrix: "forward algorithm"
- perform symmetric "backward algorithm", relaxed scores of suffix alignment
- putting together: e.g.

$$\text{Prefix}\,(i, i') + \text{Suffix}\,(i, i')$$

is upper bound for trace through (i, i').



- upper bound $<$ lower bound (from B&B) $\Longrightarrow$ PRUNE
- trace through DP-matrix yields alignment A_0; $\textbf{score}(A_0)$ is lower bound for the alignment problem

Problem Decomposition and Symmetry Breaking

- decomposition into independent subproblems speed up branch-and-bound search (AND/OR-search, Marinescu&Dechter,AI2009.)
- in general, overhead for detecting components during search
- here: problem suggests decomposition along sequence order
- cheap check by global propagator
- upper and lower bounds of independent components available
- full decomposition during search is Future Work
- currently: decompose in simple case
  - subsequences $ii'; jj'$ without structure; ii' matched, jj' matched
  - optimal solution of subproblem = optimal sequence alignment
  - fix vars of subproblem (= form of symmetry breaking)

Results

Implementation `Carna` (Constraint Alignment of RNA) in Gecode compared to `Lara` (ILP) on crossing structure

Benchmark set = pairwise alignments from 16 PK-families in Rfam

Family	Lengths		Run-time (s)		Carna Search Tree		
	A	B	Carna	Lara	Depth	Fails	Size
Entero_OriR	126	130	0.03	0.18	38	13	50
IRES_Cripavirus	202	199	0.2	0.04	157	127	296
RNaseP_arch	303	367	0.46	1.4	63	8	64
RNaseP_nuc	317	346	0.07	2.9	14	4	16
tmRNA	384	367	63	3.7	433	14347	28785
RNaseP_bact_b	408	401	3.0	2.3	370	677	1463
Intron_gpI	443	436	0.1	0.2	0	0	1
Telomerase-vert	448	451	0.47	2.3	146	32	161

Results for hard instances from the Rfam PK-families

8 easy instances: both progs align in ≤ 0.1 seconds.

Results (Unlimited structure)

Folding of bi-stable, riboswitch RNAs (100 instances per family)

Family	Length	Base Pairs	Time (s)	Limit
SAH_riboswitch	79	81	0.13	2%
SAM_alpha	79	96	0.03	0%
Purine	101	74	0.07	0%
Glycine	101	83	0.44	3%
SAM	106	74	0.06	0%
TPP	107	96	0.43	13%
SAM-IV	116	128	0.05	2%
MOCO_RNA_motif	141	111	0.24	10%
Lysine	181	210	60	60%
Cobalamin	204	237	60	71%

Strict time limit 1 min.

Conclusion

- Successful CP-application to general RNA alignment

  crossing structure: On par with ILP/Lara
  unlimited structure: NEW
- Extensible to affine gap cost: use Gotoh-like DP in propagator
- Extensible by (low-overhead) AND/OR search
- Applicable to alignment of
  - Pseudoknotted RNA
  - RNA switches / bi-stable RNA
  - RNA with conserved dynamics
- Open Problem: simultaneous folding and alignment in CP
  — stronger bounds, but select structure
- Read more details in conference paper: Dal Palù et al., A Propagator for Maximum Weight String Alignment with Arbitrary Pairwise Dependencies, CP 2010.