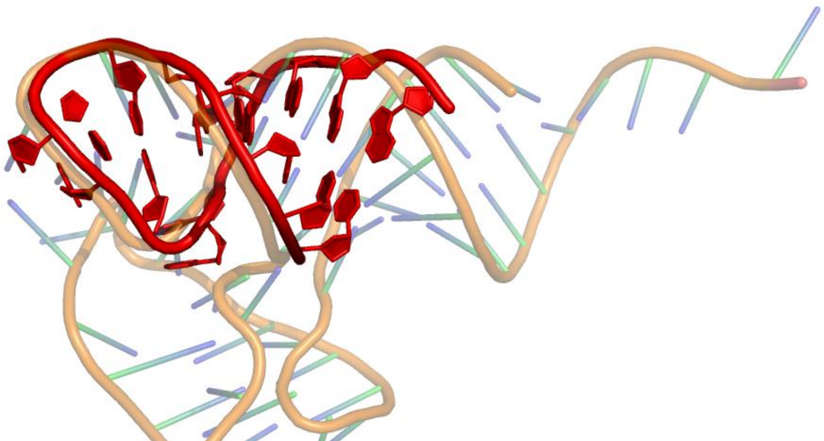
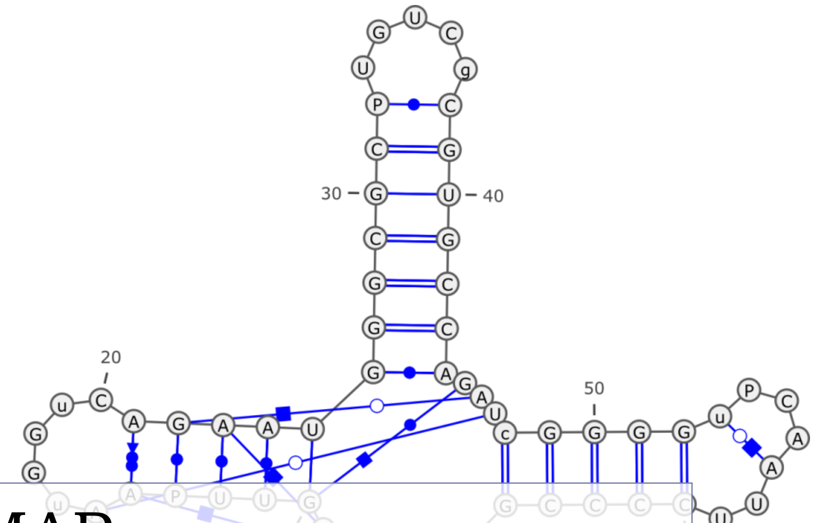




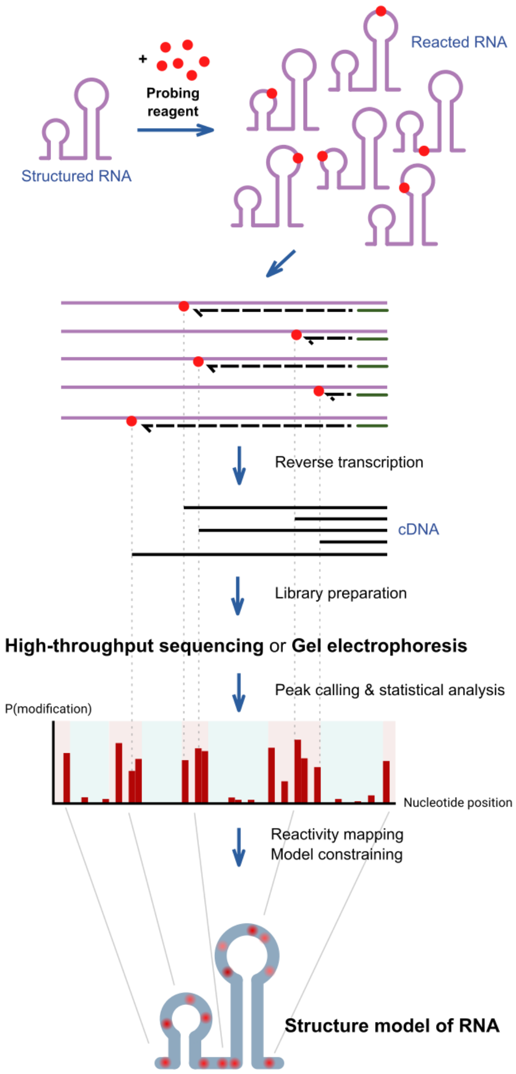
IPANEMAP

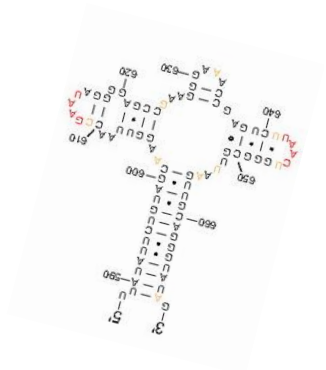
Integrative **P**robing **A**nalysis of **N**ucleic Acids **E**mpowered by **M**ultiple **A**ccessibility **P**rofiles

Afaf SAAIDI & Yann PONTY
CNRS/Ecole Polytechnique

Bruno SARGUEIL & Delphine ALLOUCHE
Univ. Paris Descartes

Structure probing in a nutshell





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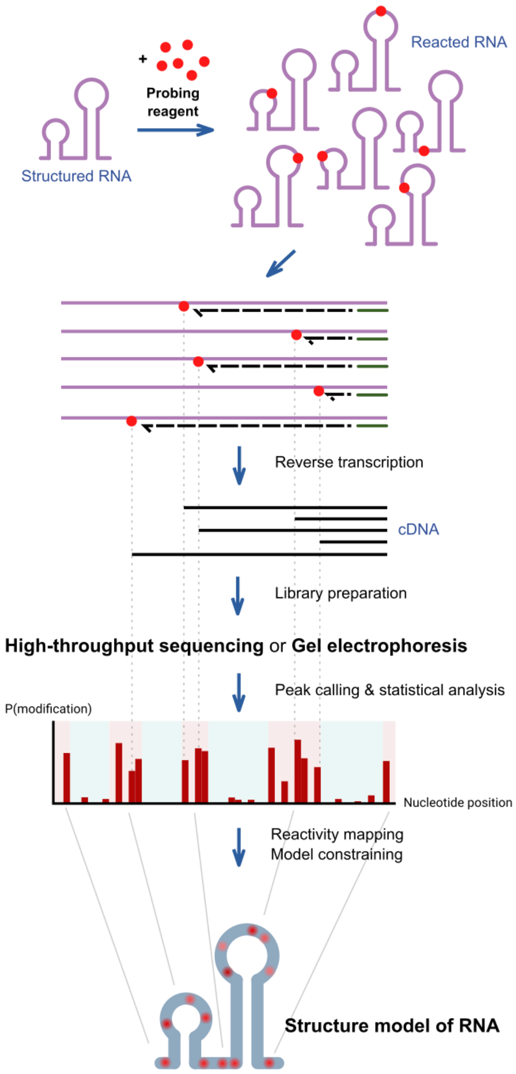

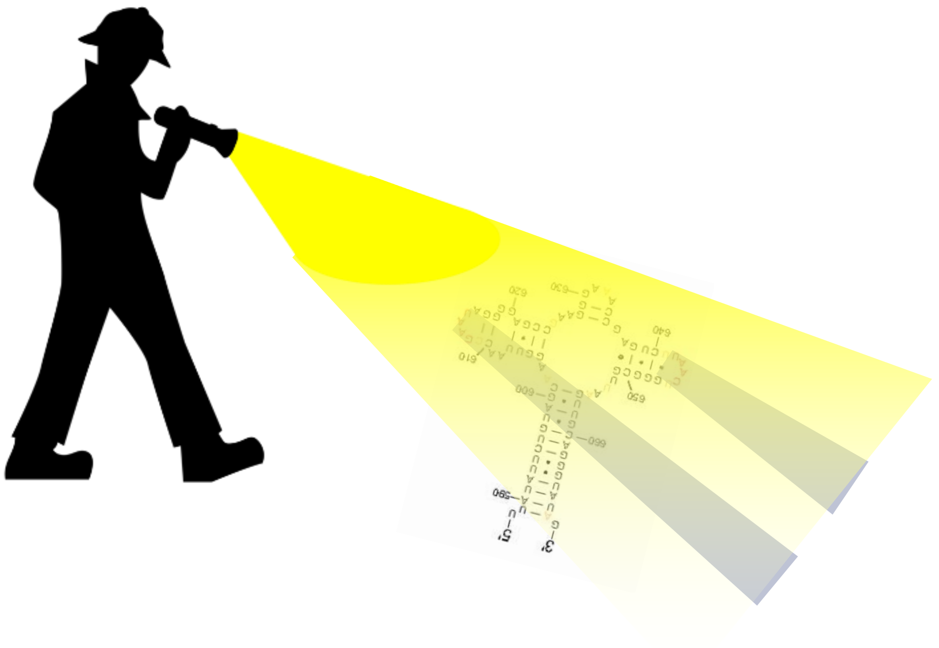

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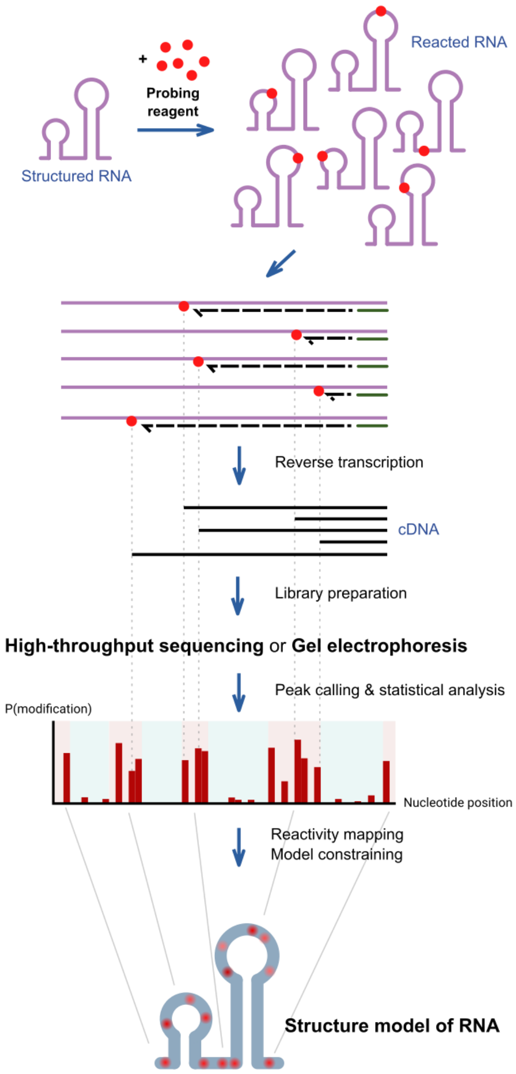

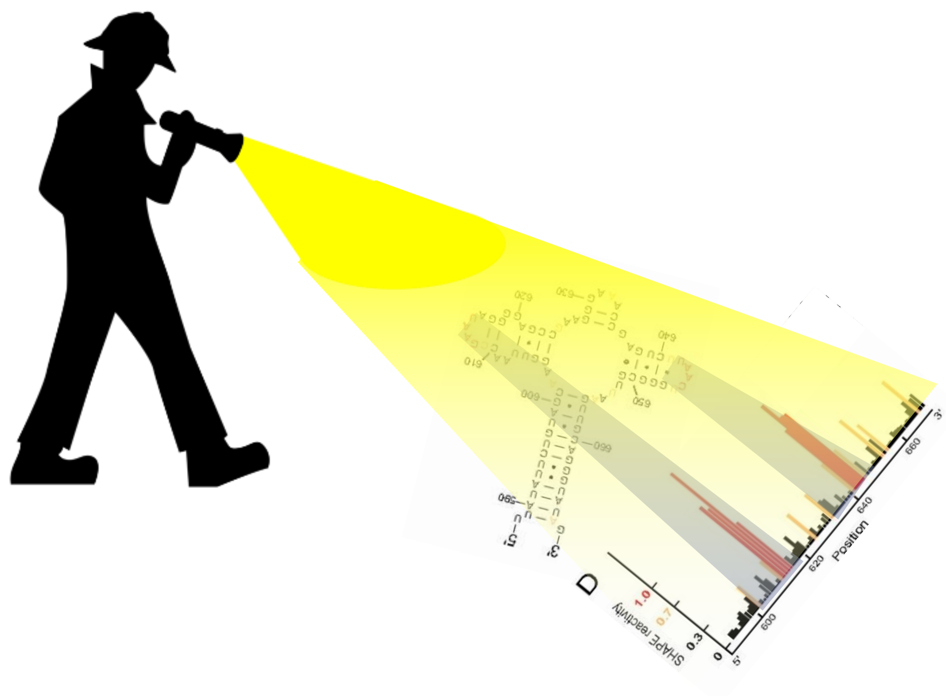

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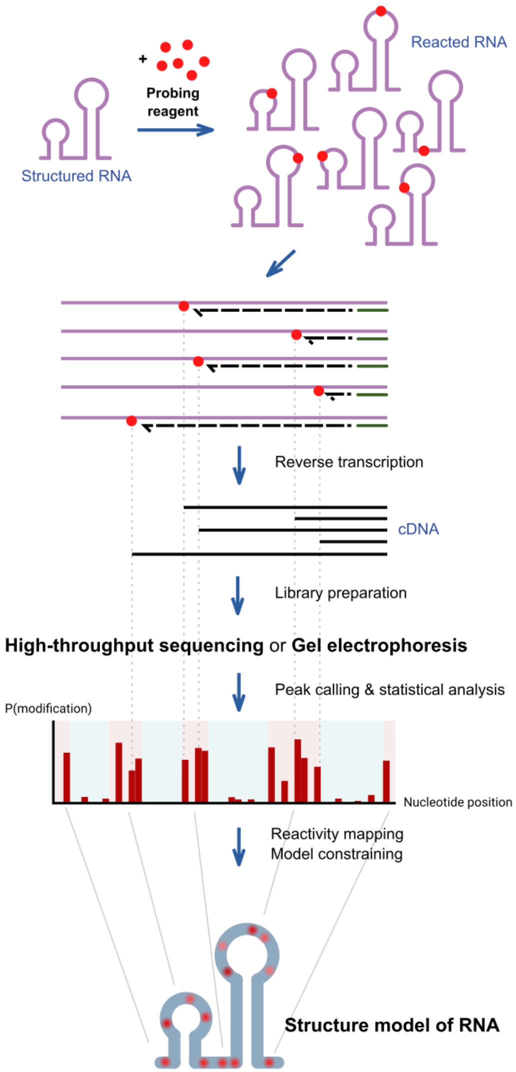
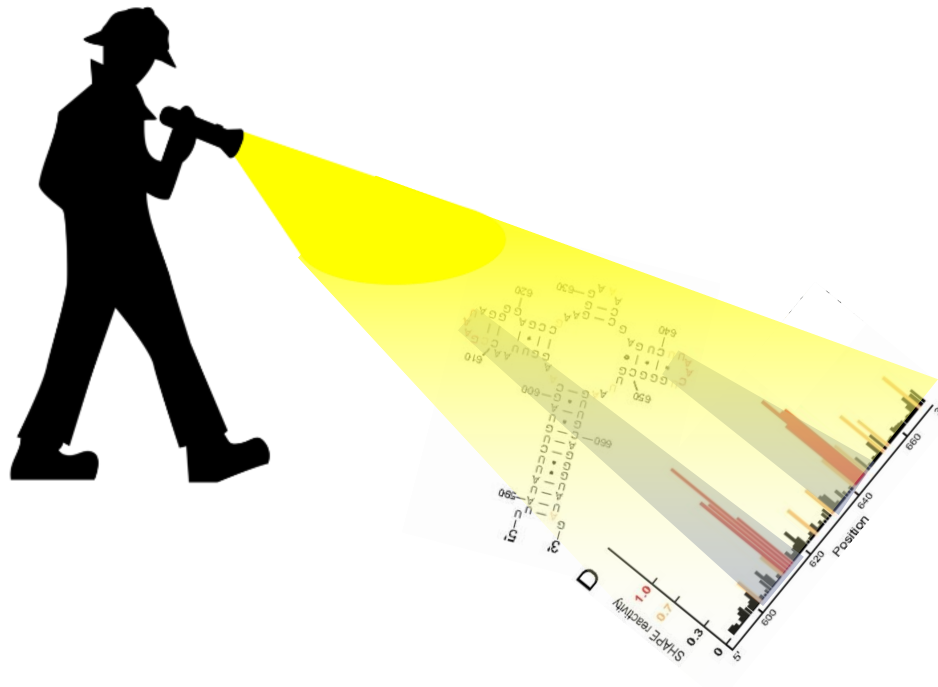
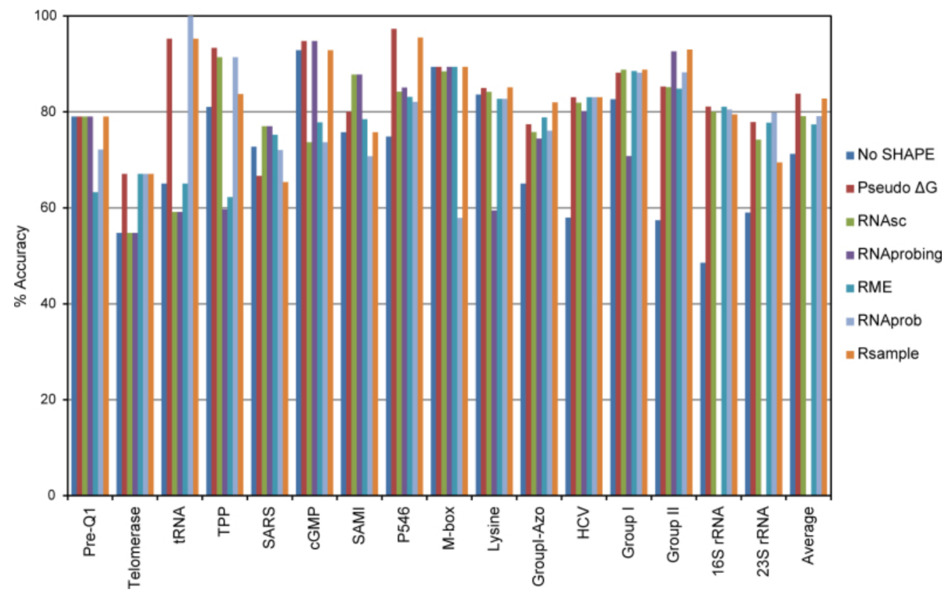




The SHAPE wars: 100% accuracy and beyond…

Now, wait a minute guys…!

David H Mathews



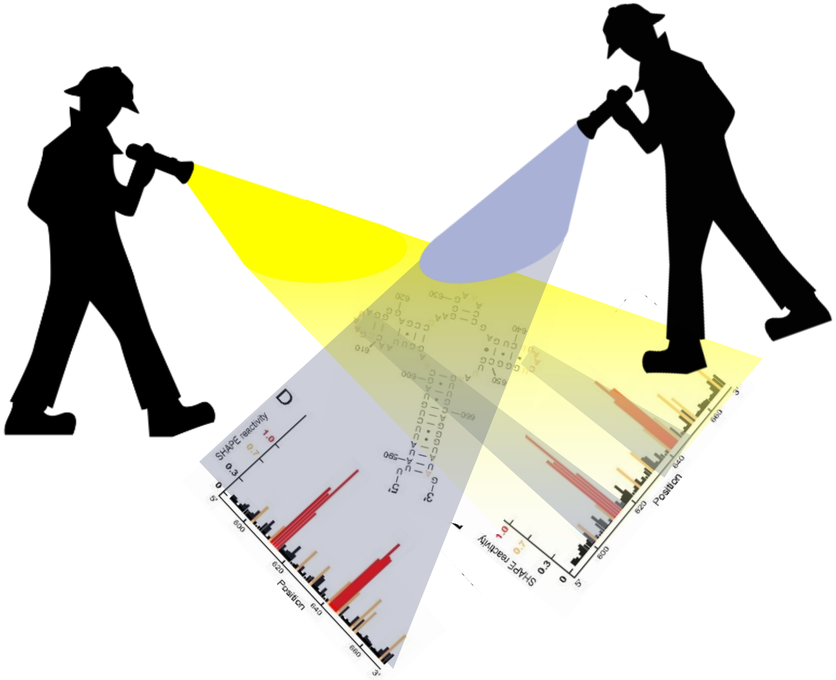
Spasic *et al*, NAR 2018

The practice of RNA modeling

Bruno Sargueil

Probing is tricky to perform and interpret.

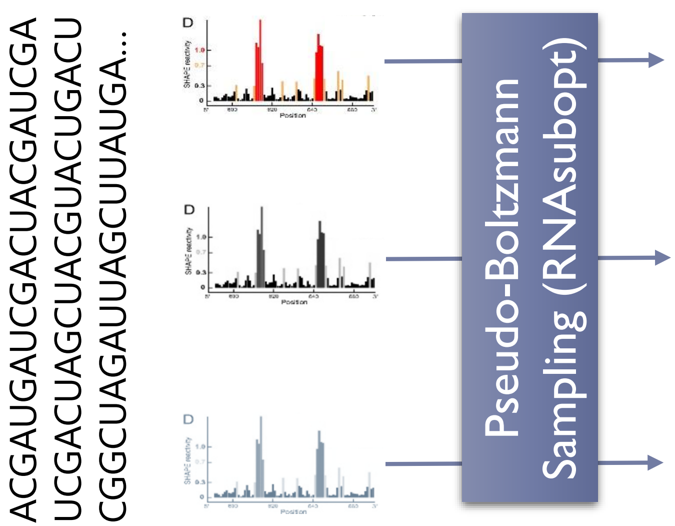
Beyond RNAPuzzle, modelers usually try different techniques and reagents, but integration is no gimme…



How to integrate multiple probing profiles?

A KISS approach

- Reactivity profiles uniformly captured as pseudo potentials in prediction methods (RNAsubopt + Soft constraints)
- Native structure should be represented in each of the pseudo-Boltzmann ensemble (maybe not at the top)



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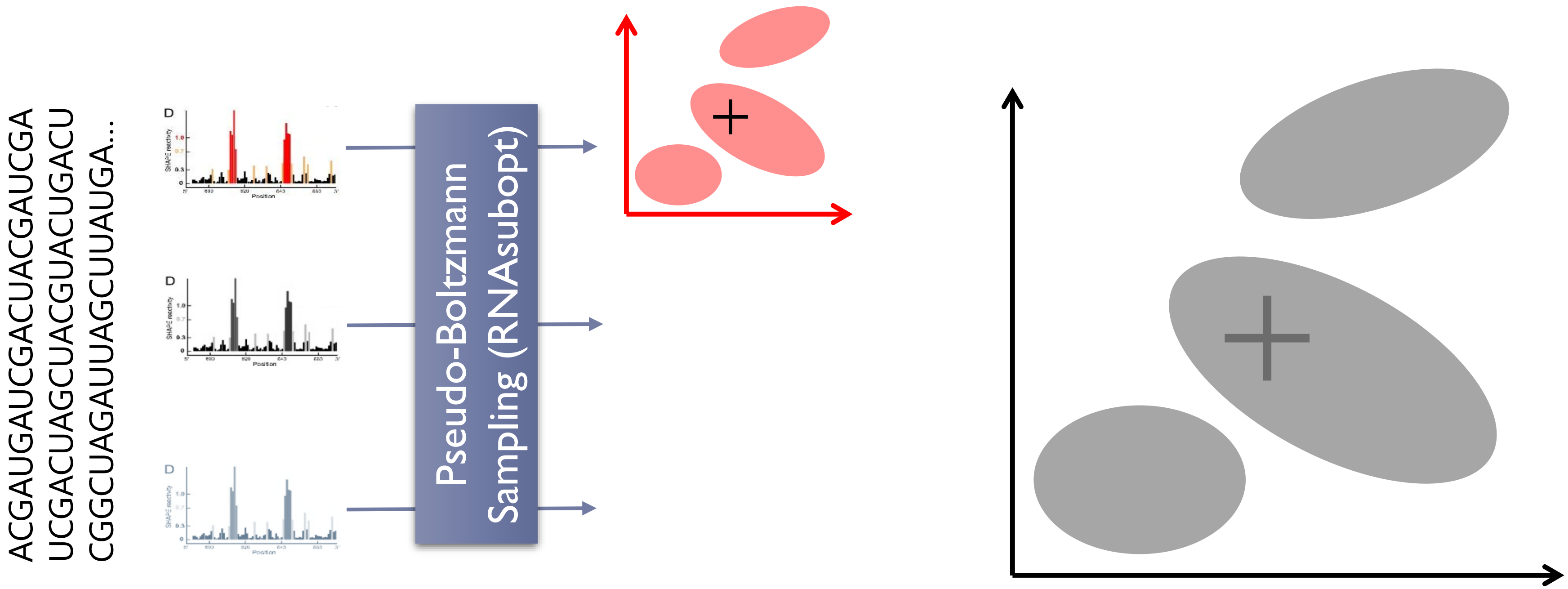

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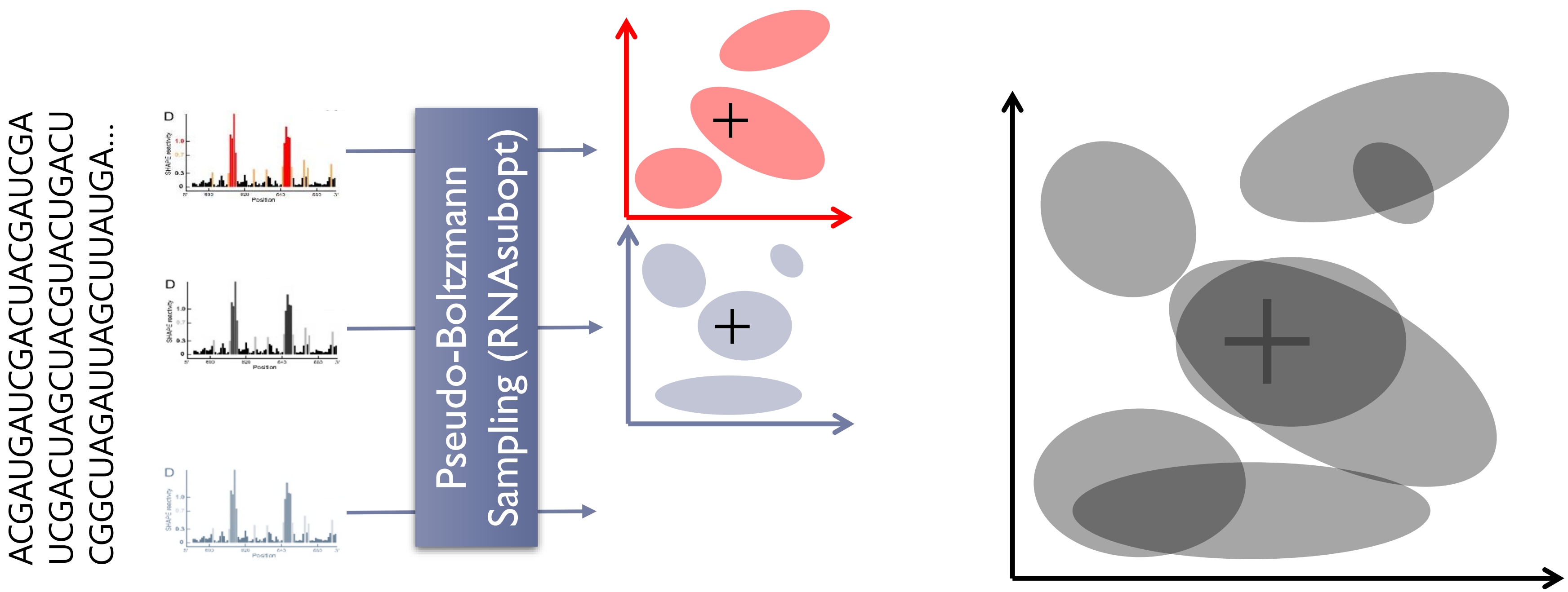

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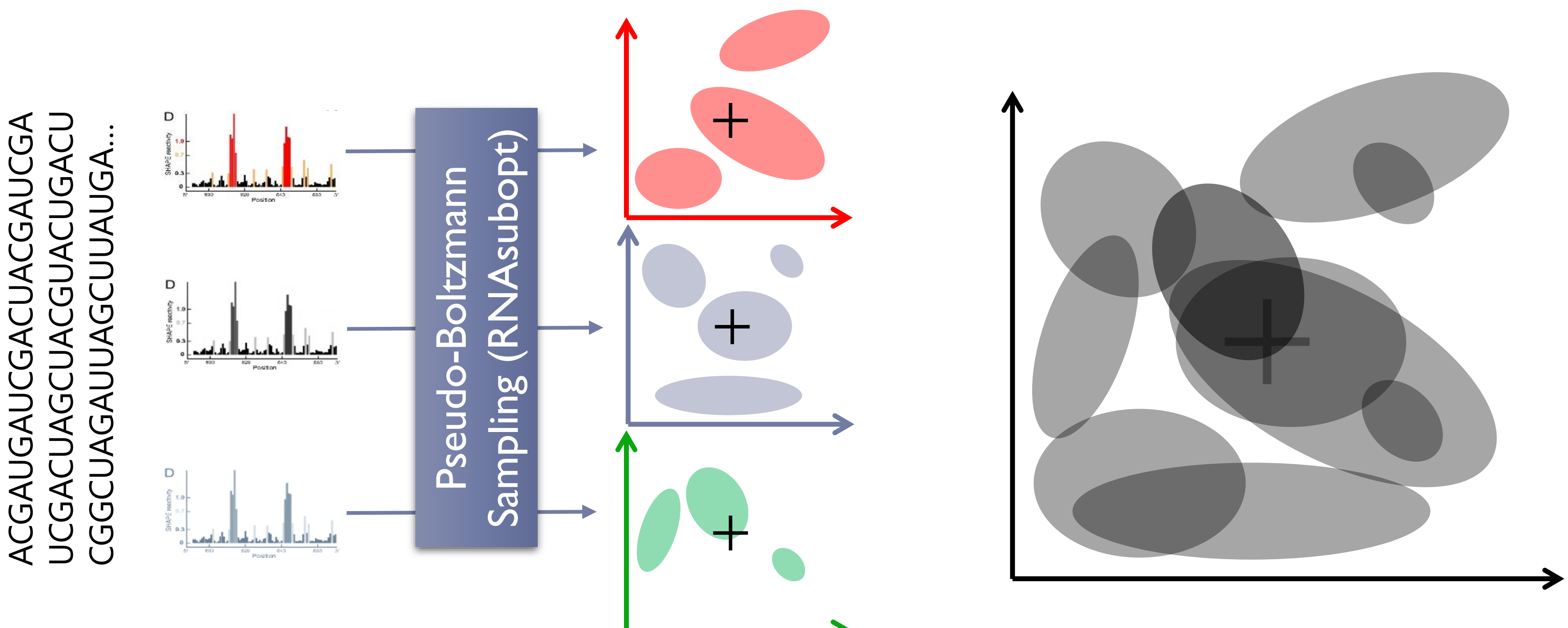
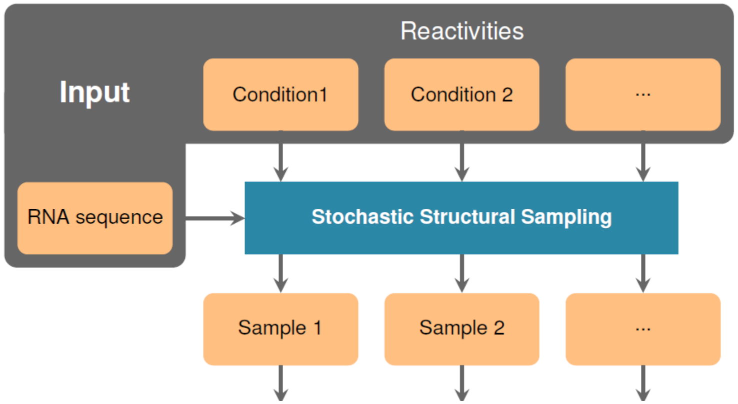
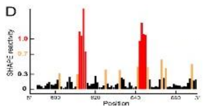
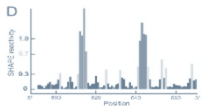
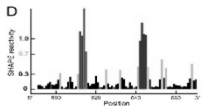


IPANEMAP Pipeline









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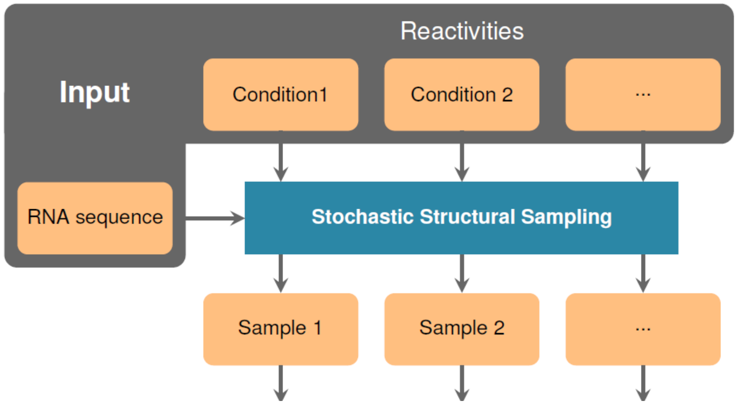

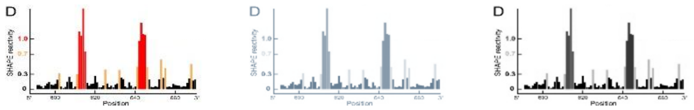

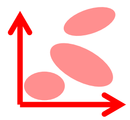

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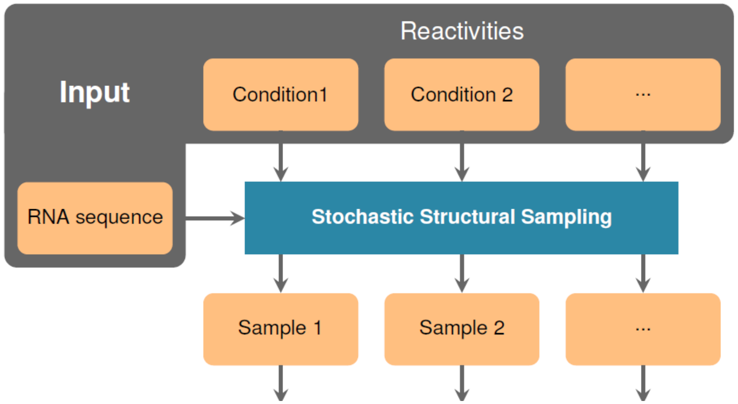

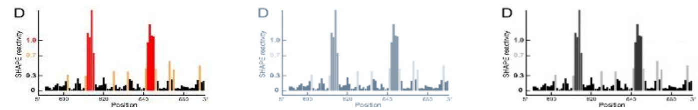

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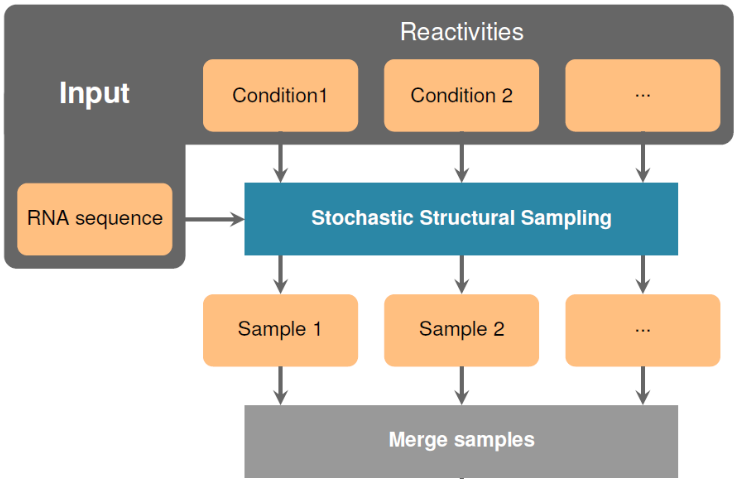

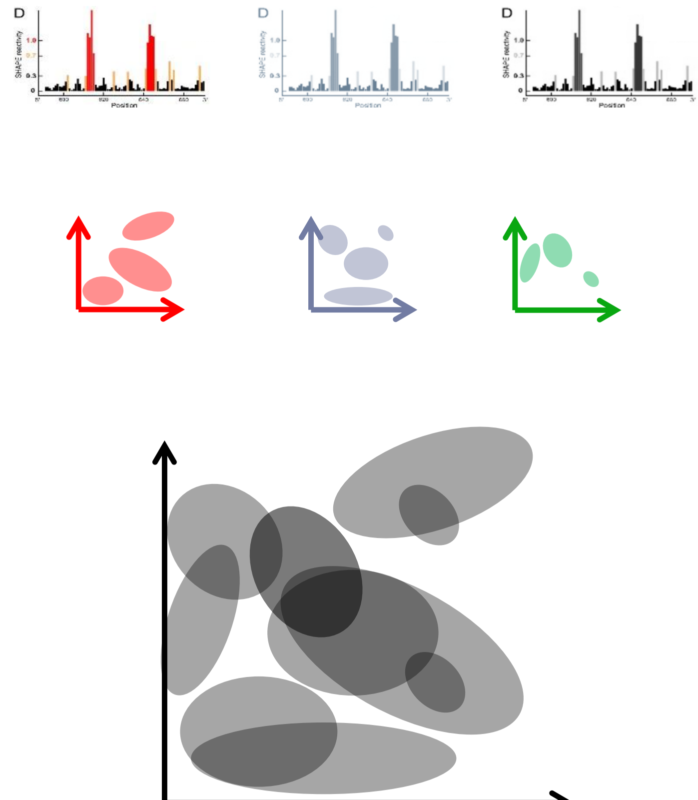

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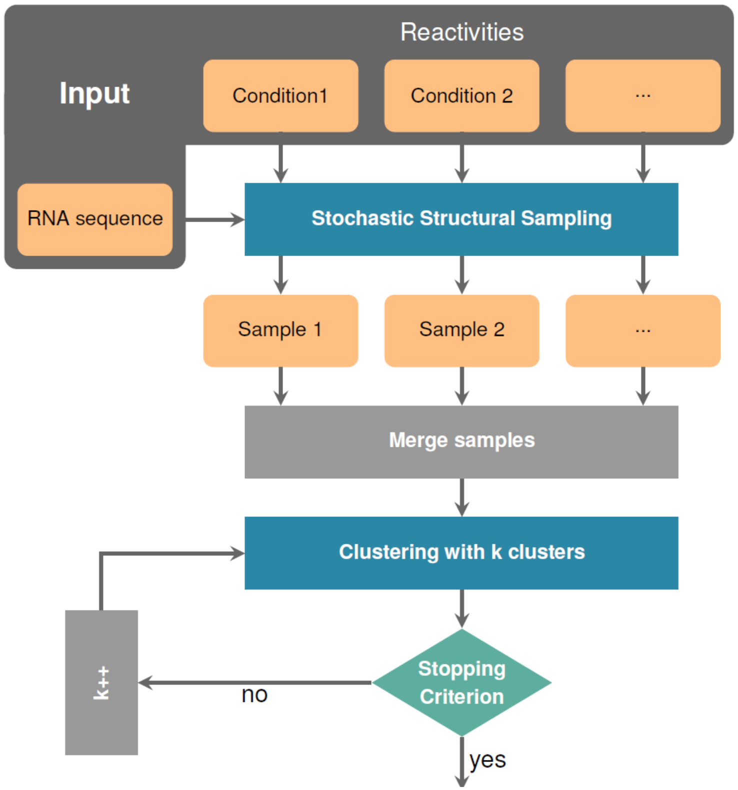

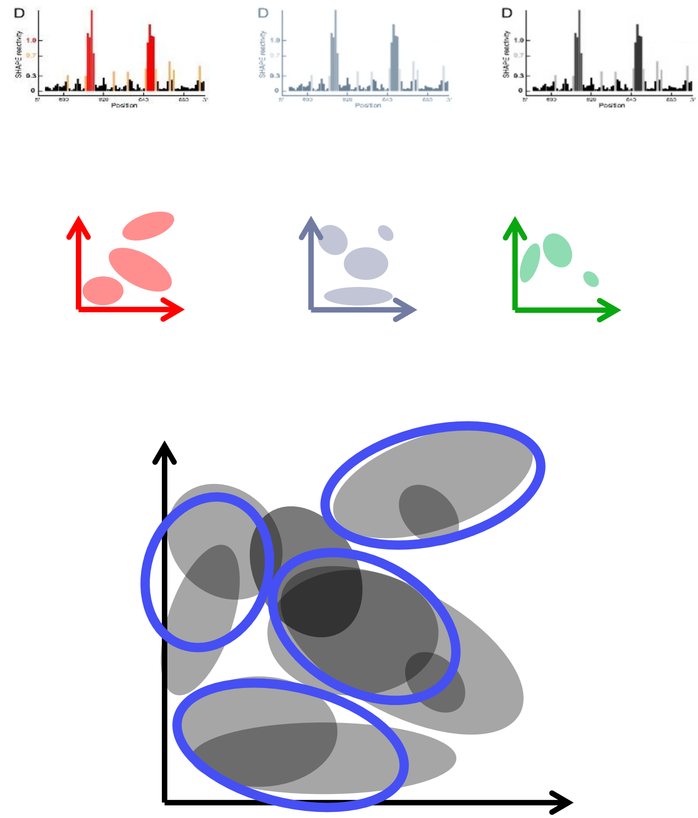

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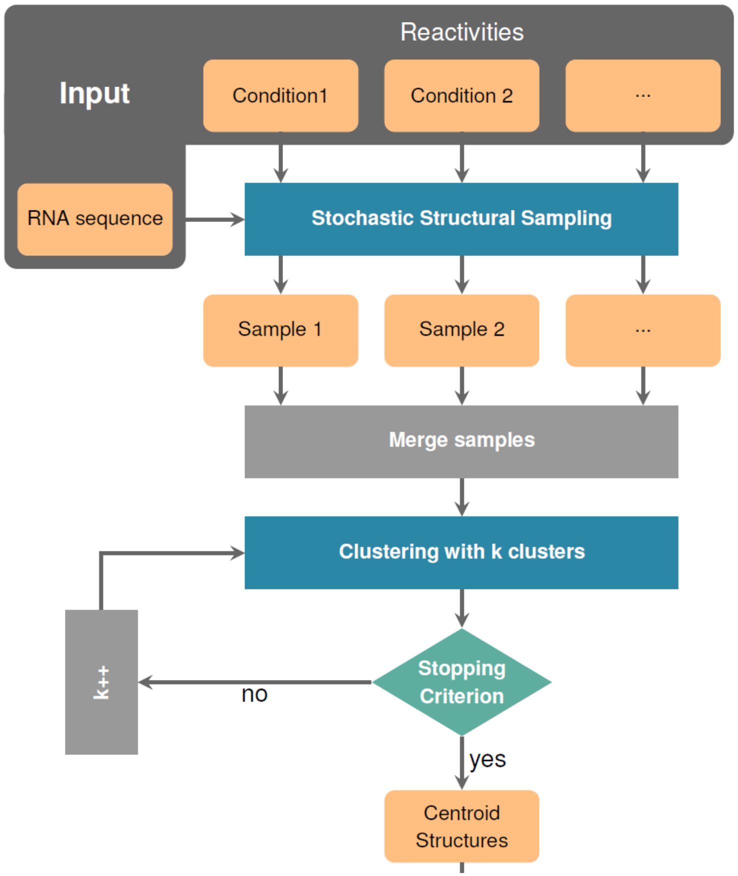

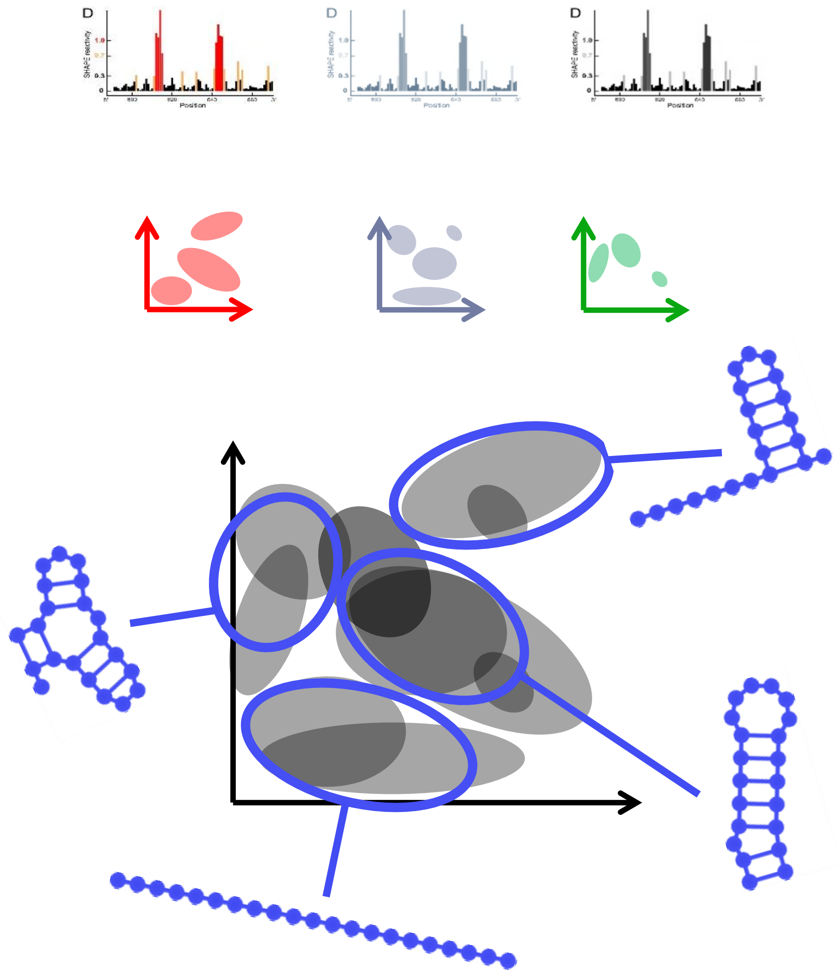

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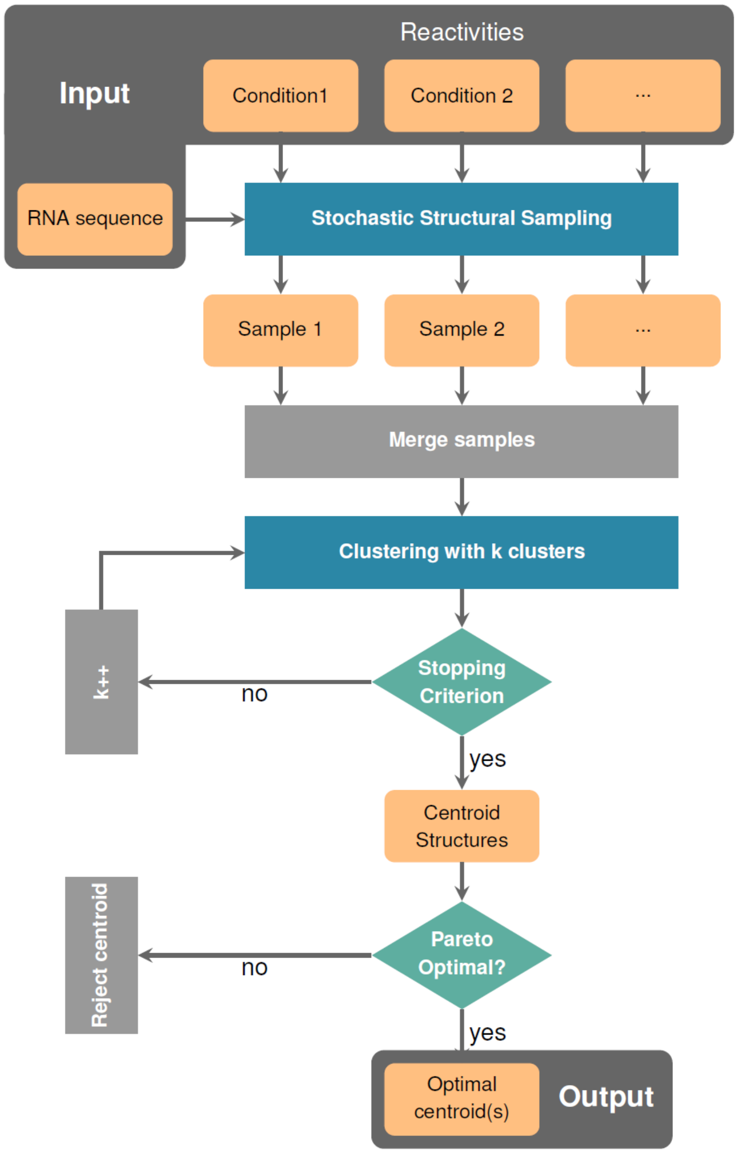
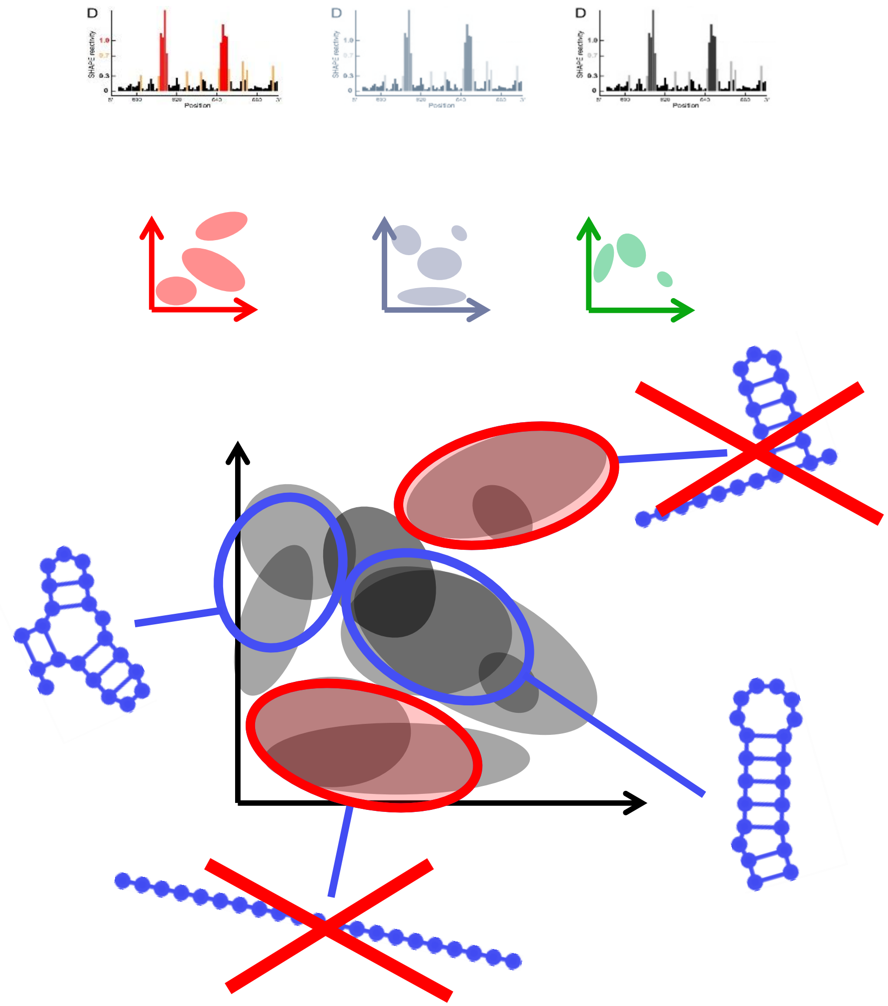
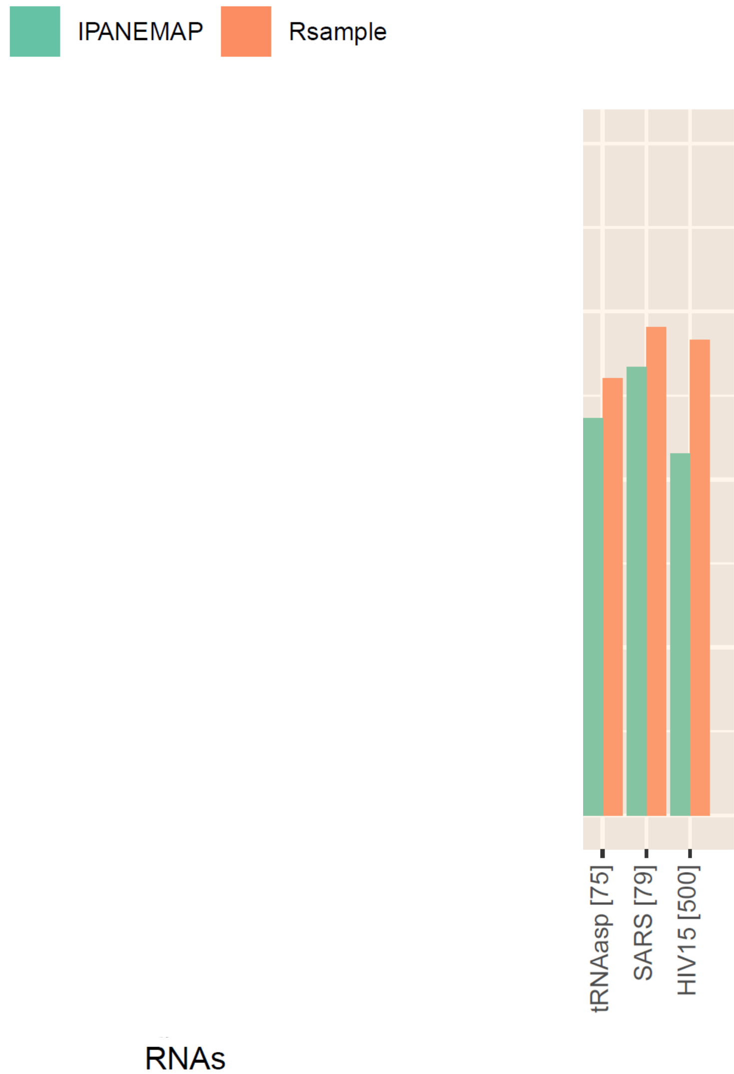




Single condition/single structure dataset



- IPANEMAP vs Rsample

  [Spasic *et al*, NAR 2018]

- Hajdin *et al* dataset
  SHAPE 1M7
  50-500nts RNAs

Geometric **M**ean $\mathrm{GM}(S \mid R) = \sqrt{\mathrm{Sens}(S \mid R) \times \mathrm{PPV}(S \mid R)}$

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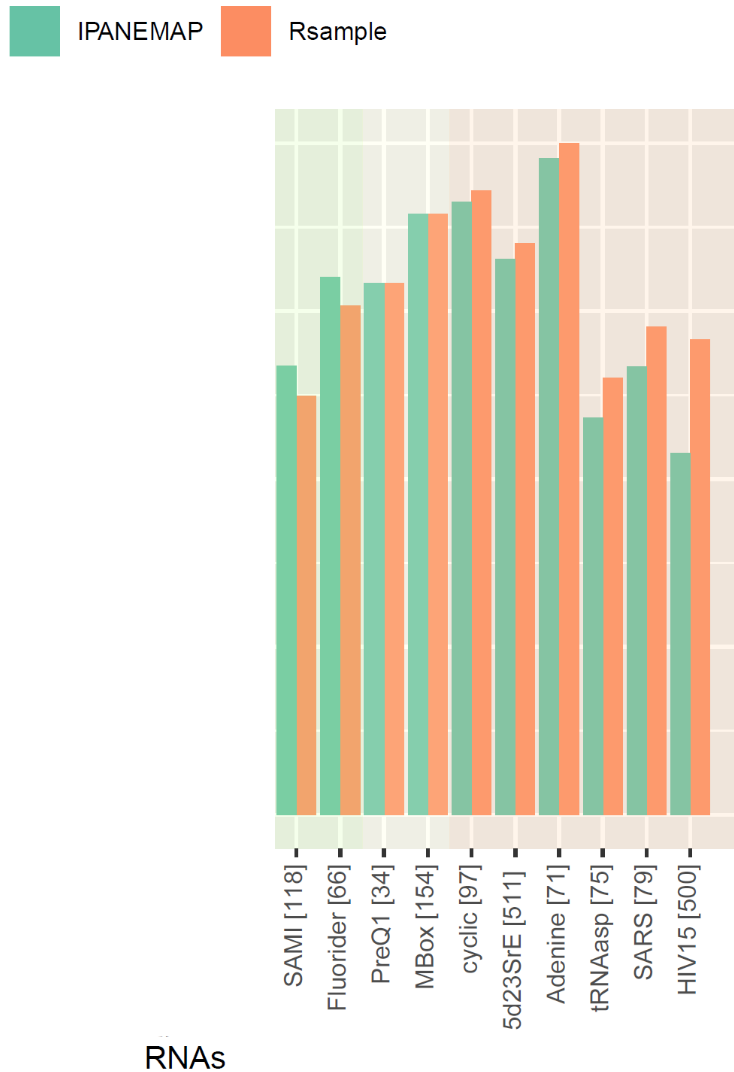

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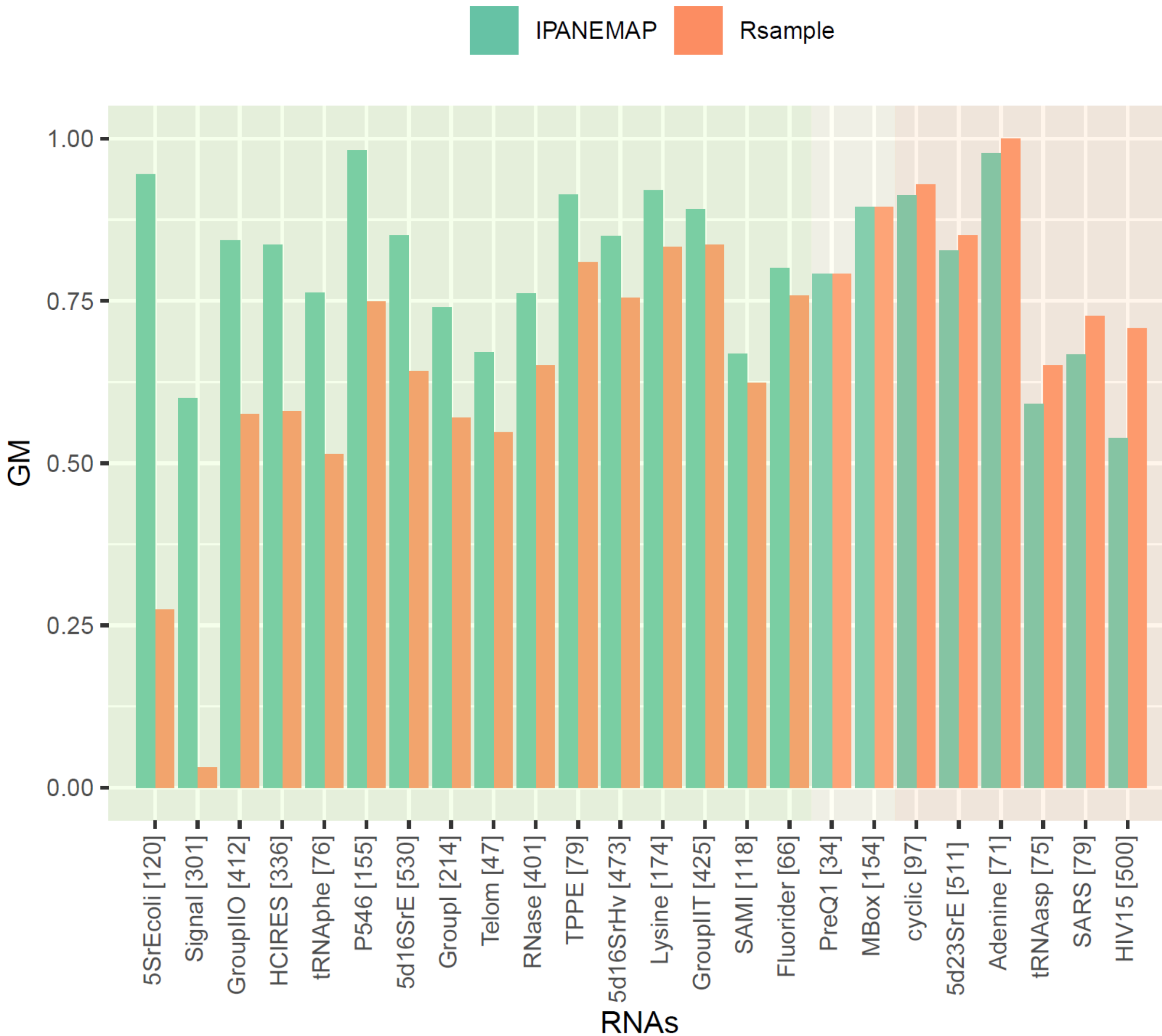

- IPANEMAP vs Rsample
  [Spasic *et al*, NAR 2018]
- Hajdin *et al* dataset
  SHAPE 1M7
  50-500nts RNAs
- 80% GM IPANEMAP
  vs 63% GM for Rsample
- Reason: Rsample ≈ MEA

Geometric **M**ean $\mathrm{GM}(S \mid R) = \sqrt{\mathrm{Sens}(S \mid R) \times \mathrm{PPV}(S \mid R)}$

Multiple conditions improve predictions?

- 6 RMDB RNAs
  - 5s RNA *E. coli*
  - Glycine Riboswitch *F. Nucleatum*
  - cidGMP riboswitch *V. Cholerae*
  - P4 - P6 domain Tetrahymena ribozyme
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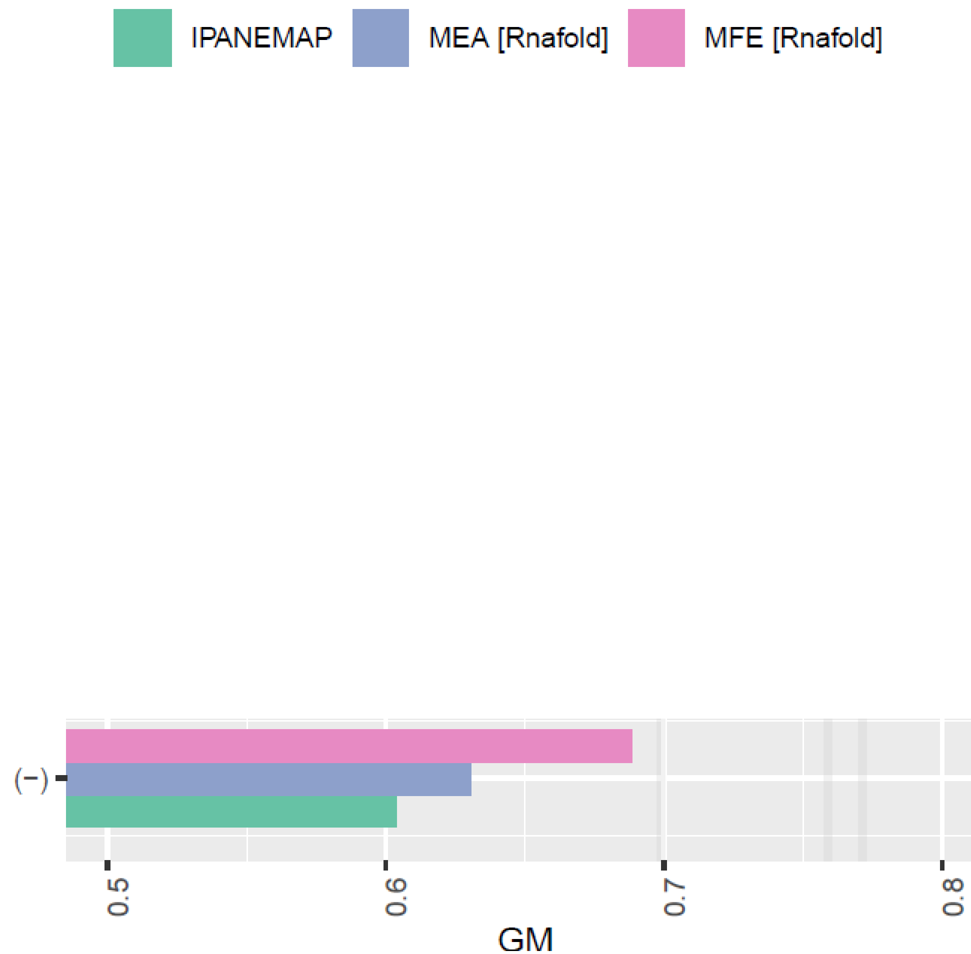
- 3 conditions
  - SHAPE (NMIA)
  - DMS
  - CMCT



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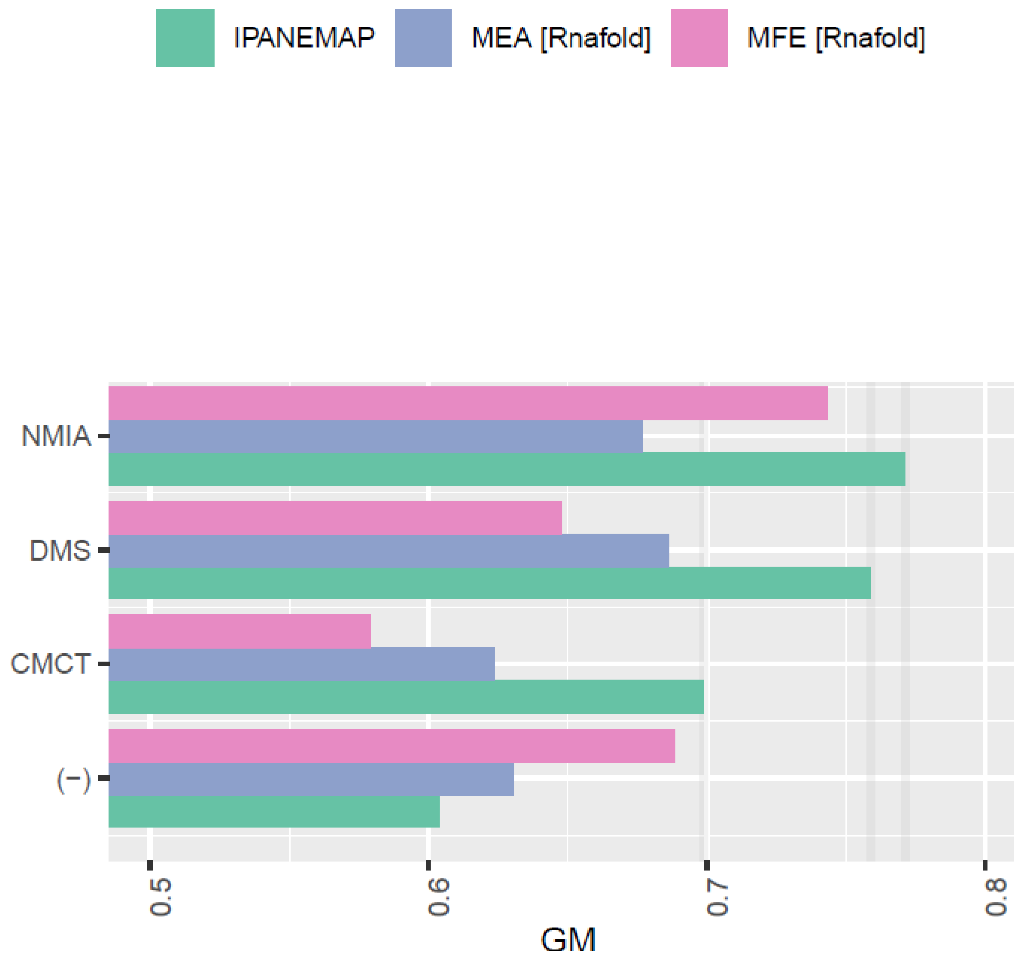

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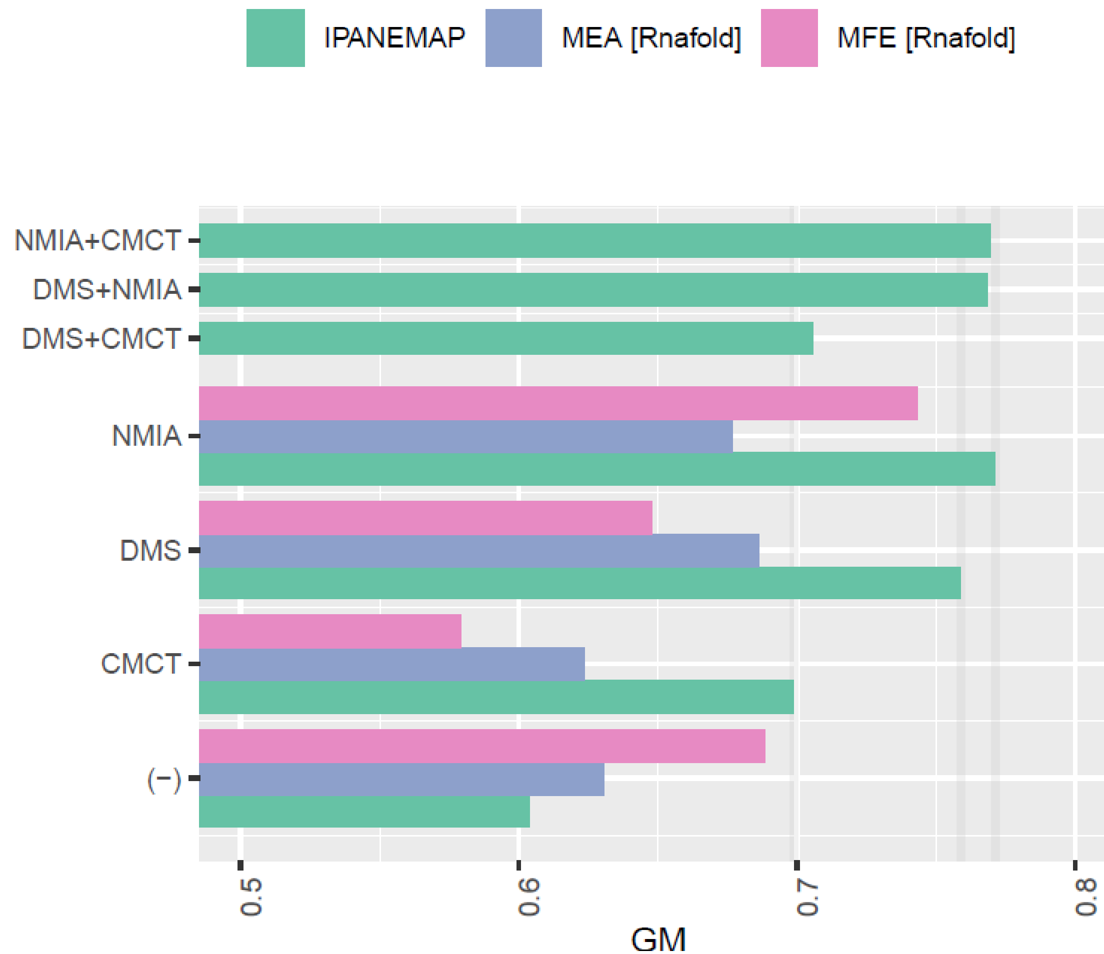

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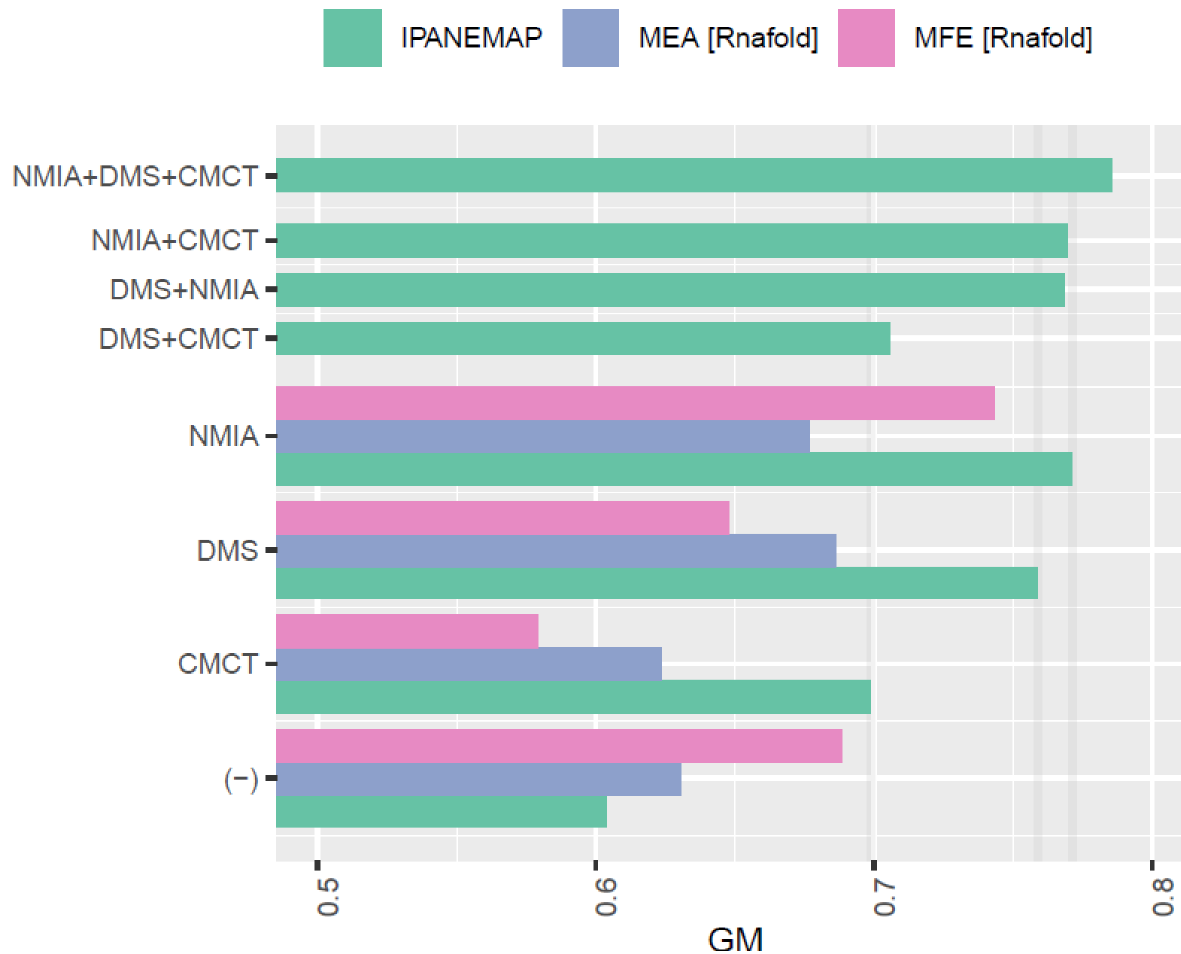

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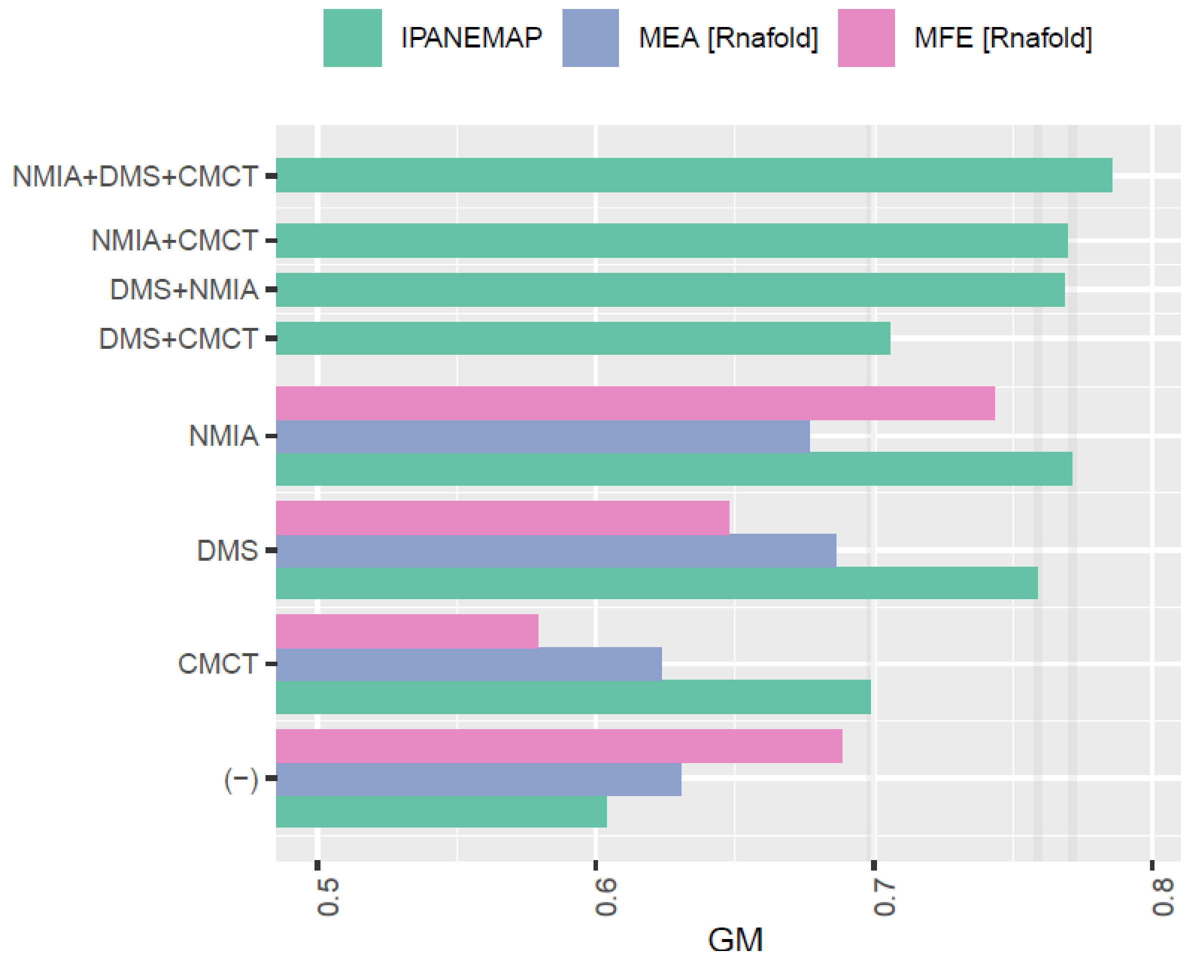
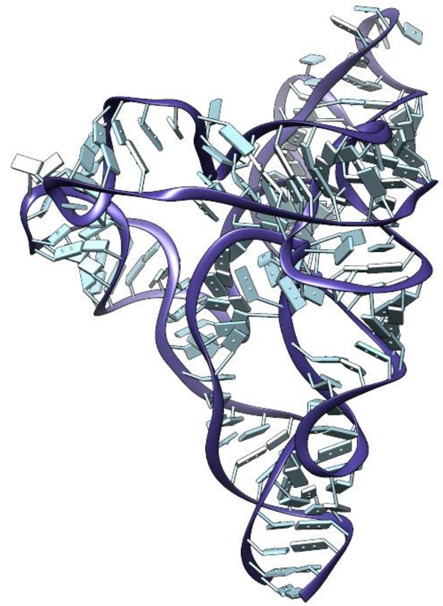
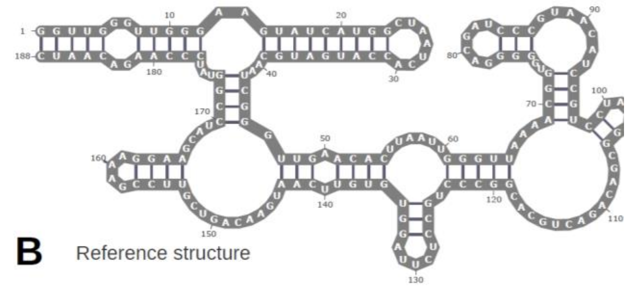


Back to the lab for even more data





B Reference structure

- Lariat Capping Ribozyme (PDB 4P8Z) from Didymium iridis

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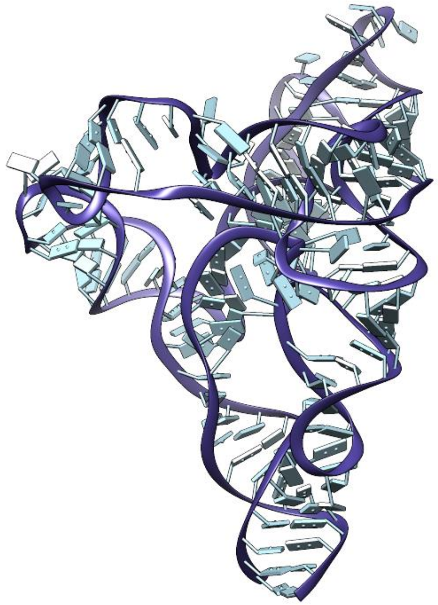

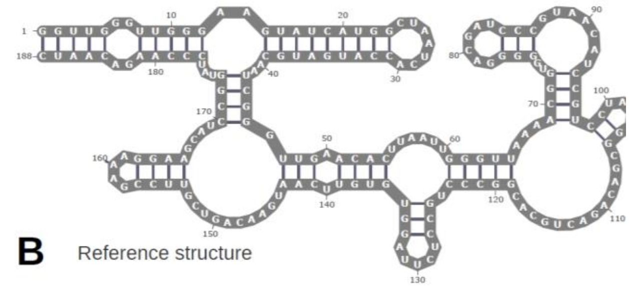

- Lariat Capping Ribozyme (PDB 4P8Z) from Didymium iridis
- Probed under 14 different conditions:
  - Technique: SHAPE, DMS, CMCT
  - Reagents: 1M7, NMIA, DMS, CMCT, NAI, BzCN
  - Stop based and Mutation based readouts
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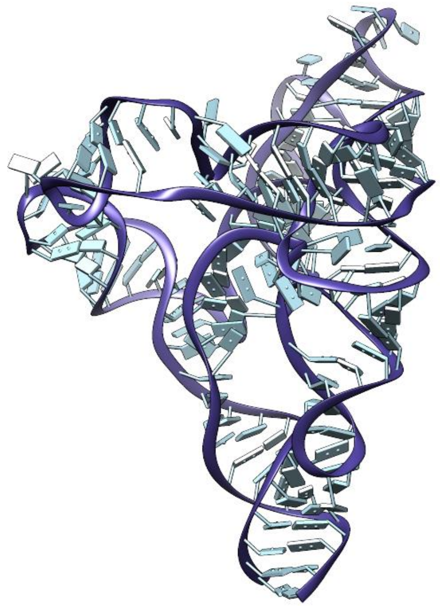

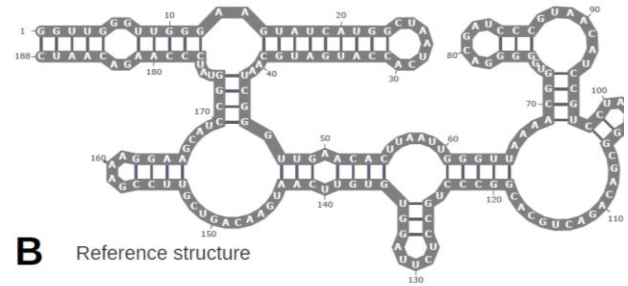

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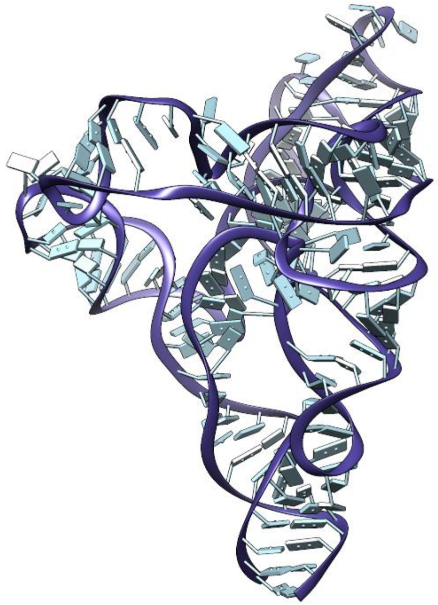

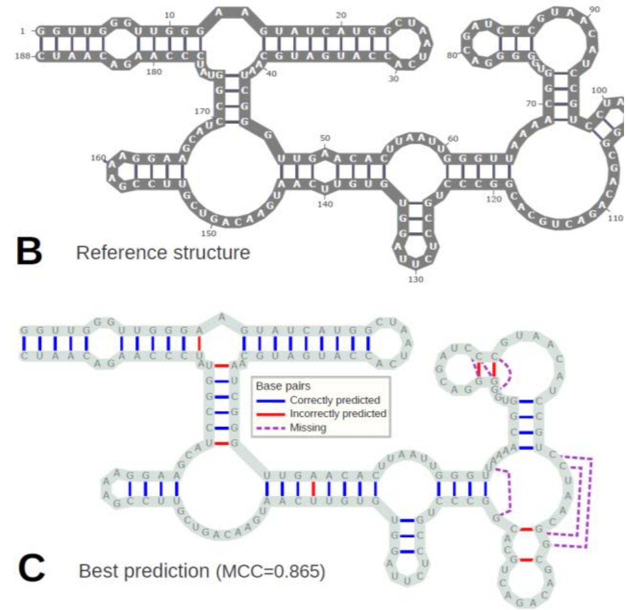

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Mono probing analysis

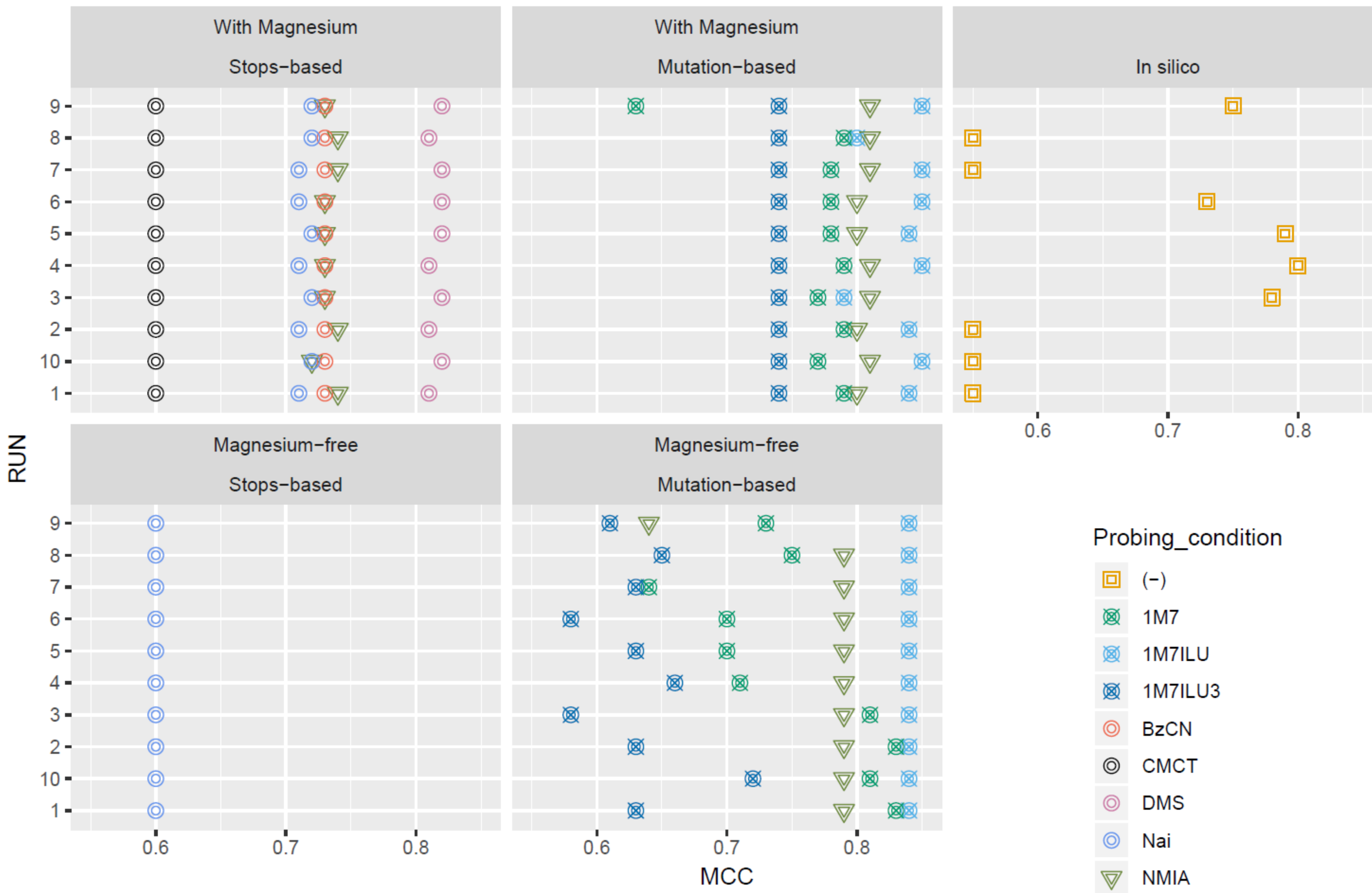
Condition name	Tech.	+/- Mg^{2+}	MCC IPANEMAP	MFE	MEA
1M7ILU	Mut	○	.84	.82	.85
1M7ILUMg	Mut	●	.83	.82	.8
DMSMg	Stop	●	.82	.41	.86
NMIAMg	Mut	●	.81	.8	.8
1M7ILU3Mg	Mut	●	.77	.75	.76
NMIAMgCE	Stop	●	.77	.675	.74
1M7Mg	Mut	●	.74	.735	.64
BzCNMg	Stop	●	.74	.72	.73
NMIA	Mut	○	.73	.69	.71
NaiMg	Stop	●	.73	.65	.71
1M7	Mut	○	.71	.51	.56
1M7ILU3	Mut	○	.62	.59	.67
CMCTMg	Stop	●	.6	.58	.59
Nai	Stop	○	.6	.59	.6
Avg Technology	Mut	–	.76	.71	.72
	Stop	–	.71	.61	.71
Avg +/- Mg^{2+}	–	○	.76	.68	.74
	–	●	.70	.64	.68
Average overall	–	–	.74	.67	.72

- Different performances
- No clear factor
- Some outliers (NAI & CMCTMg)

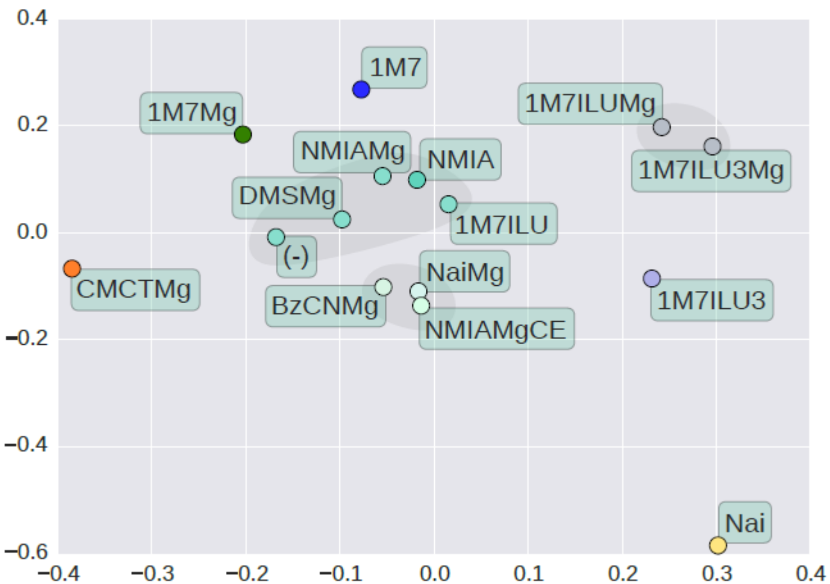
MCC:

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

Reproducibility



More mono probing analysis



(Squared) Euclidian distance
between conditions

$$\mathrm{Dist}(d, d') = \sum_{i=1}^{n} \sum_{j=x+1}^{n} \left(\mathbb{P}(i,j \mid d) - \mathbb{P}(i,j \mid d')\right)^2$$

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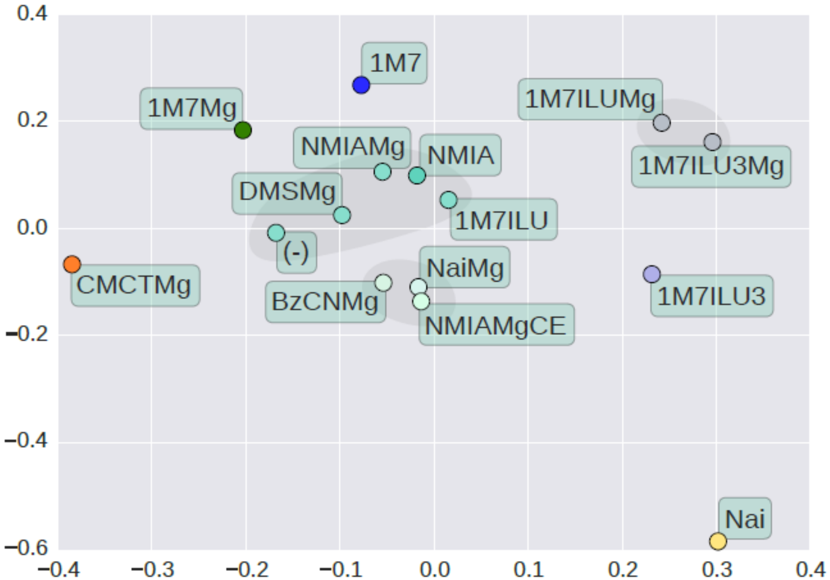

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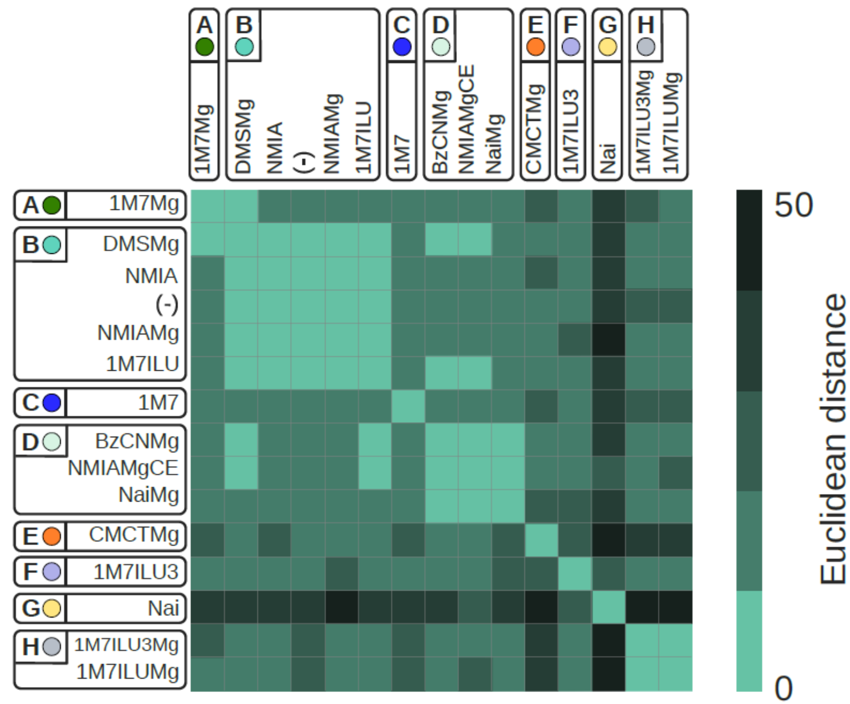
- 14 conditions + no-probing (-)



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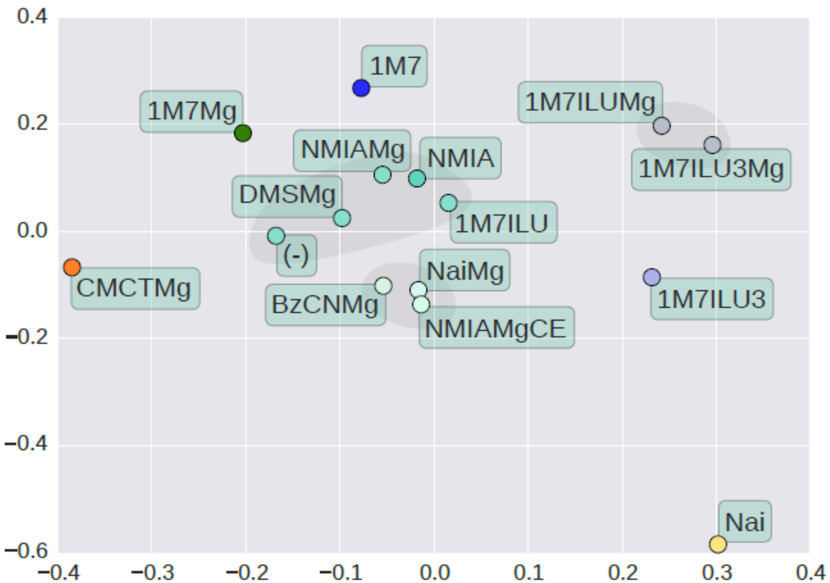

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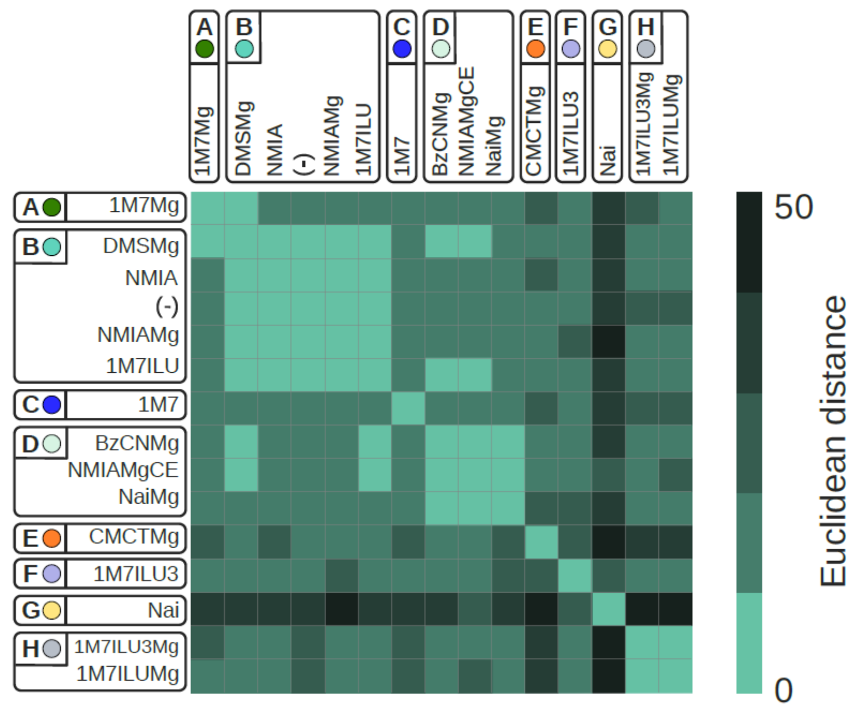
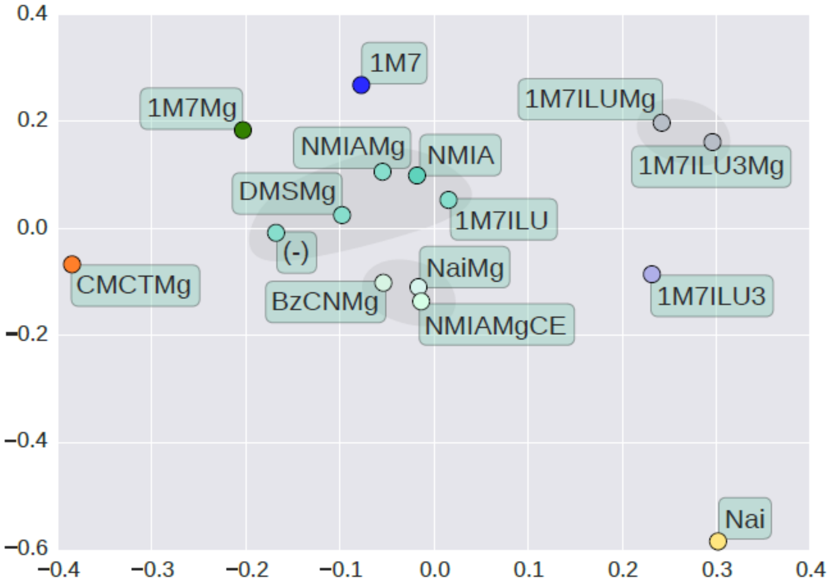


- 14 conditions + no-probing (-)
- Conditions cluster in 8 groups

More mono probing analysis



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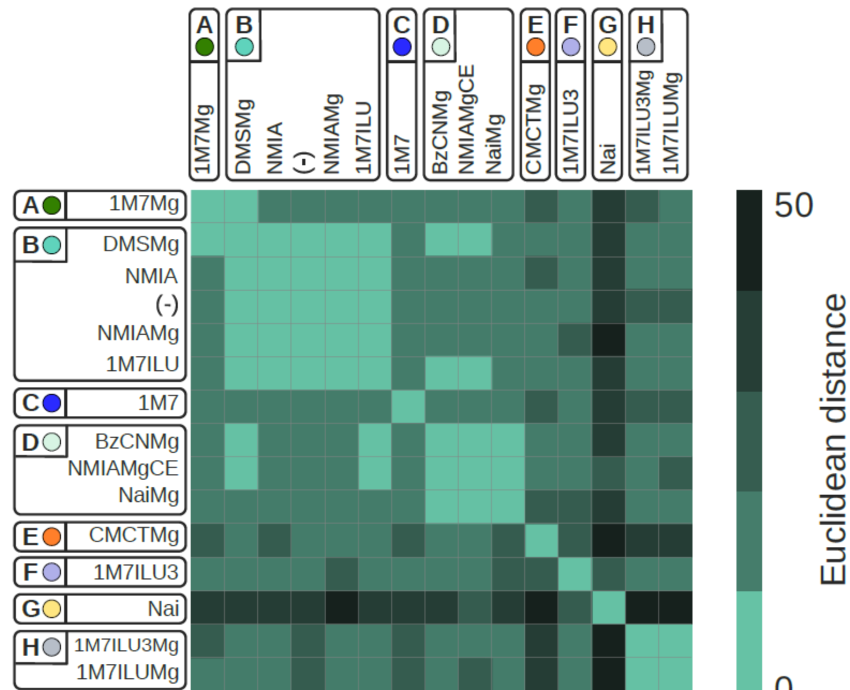

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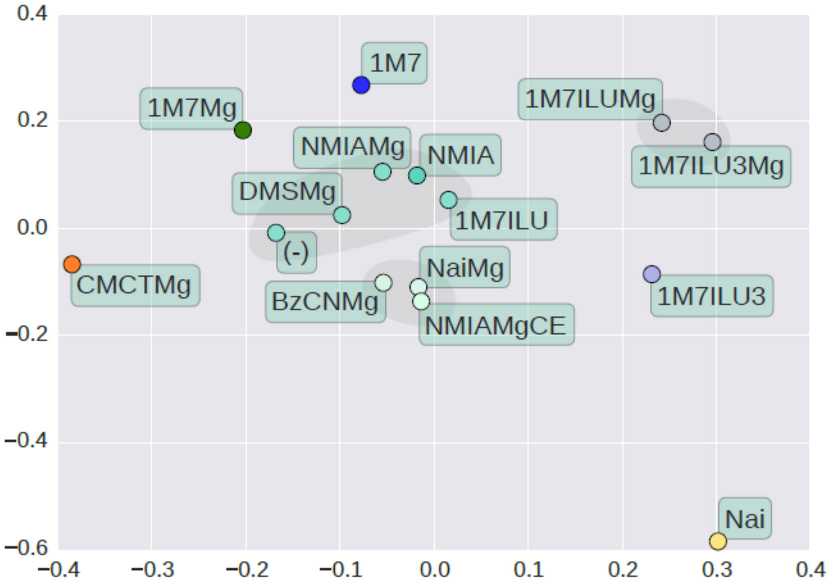

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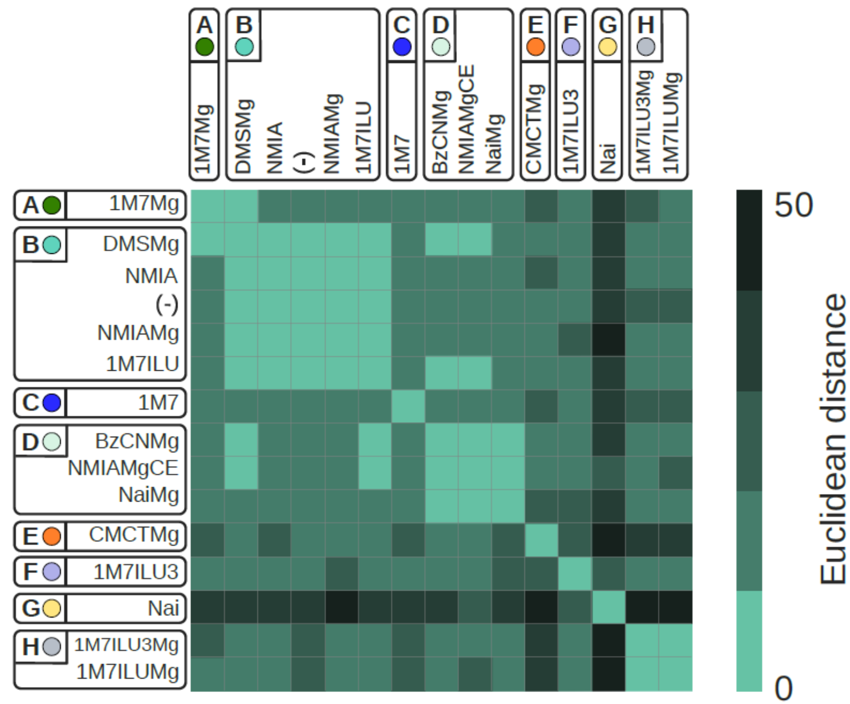

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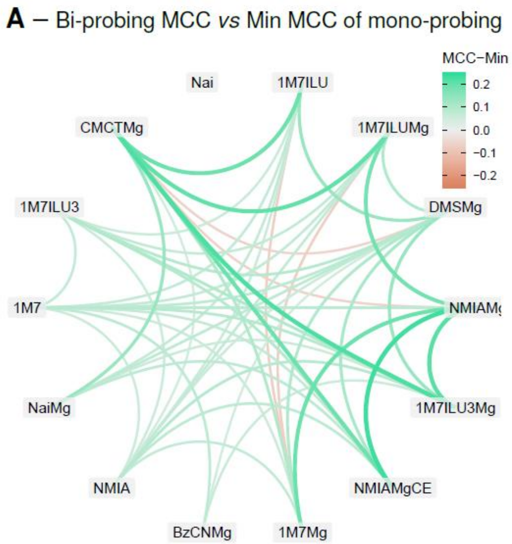
- 5 singletons
- Outliers confirmed: NAI & CMCTMg

Dual probing analysis



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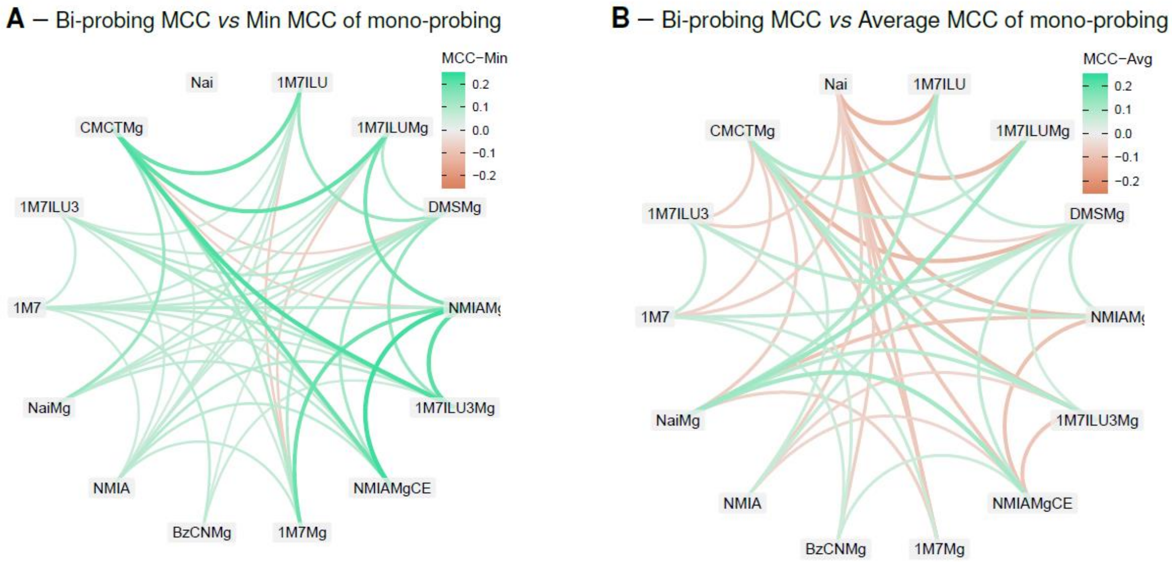

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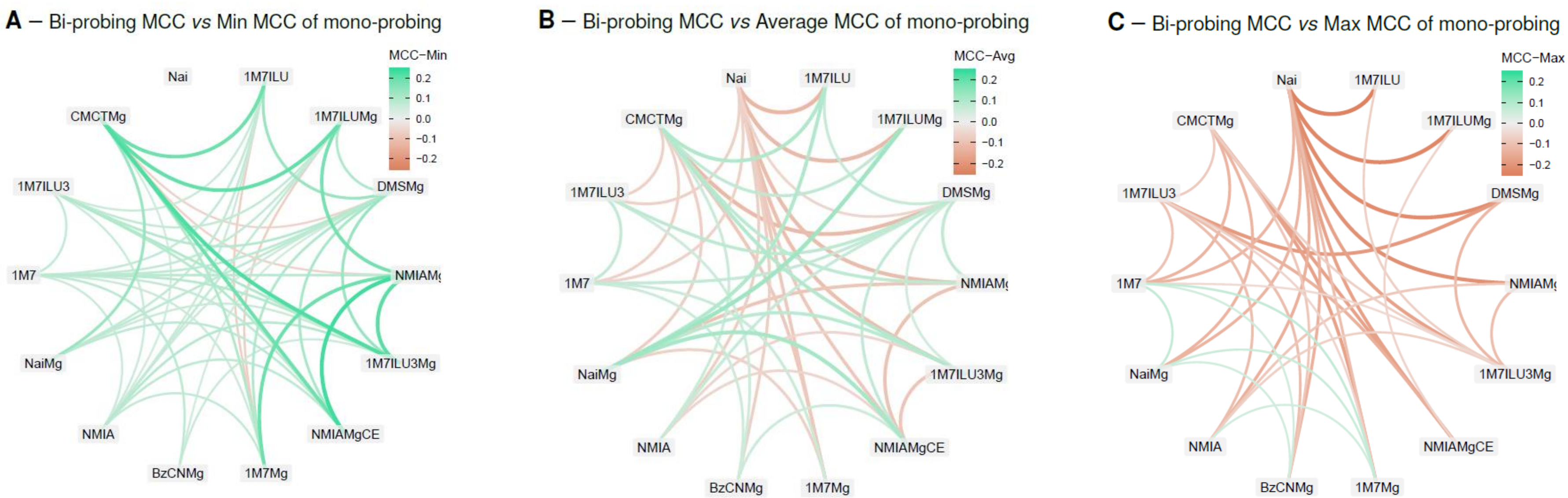

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- Sensitive to contamination:
  -4.4% against best, -1.5% w/o NAI & CMCTMg

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Considering triplets of conditions (364 runs) :

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- Some triplets improve overall best MCCs
  - 1M7ILUMg + NMIAMgCE + 1M7ILU3
  - 1M7ILUMg + 1M7 + BzCNMg
  - 1M7ILUMg + 1M7ILU3 + 1M7

→ 85.3%MCC

Beyond three conditions?

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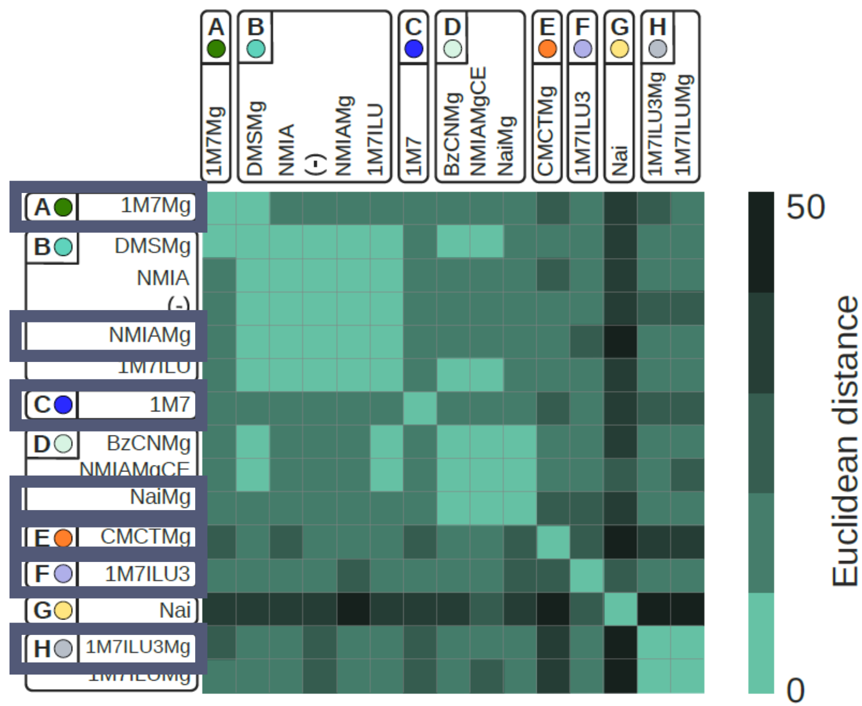

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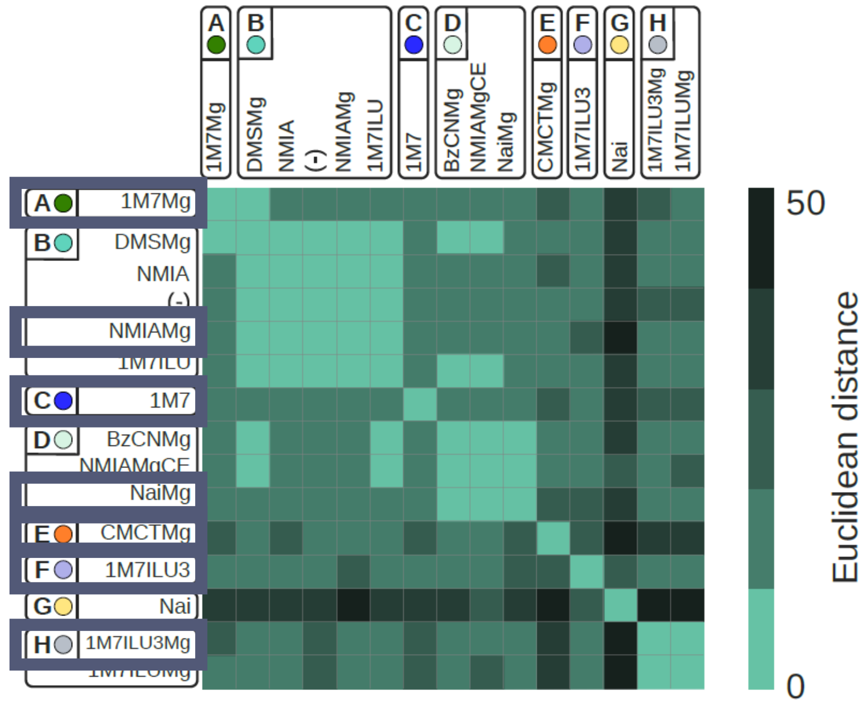
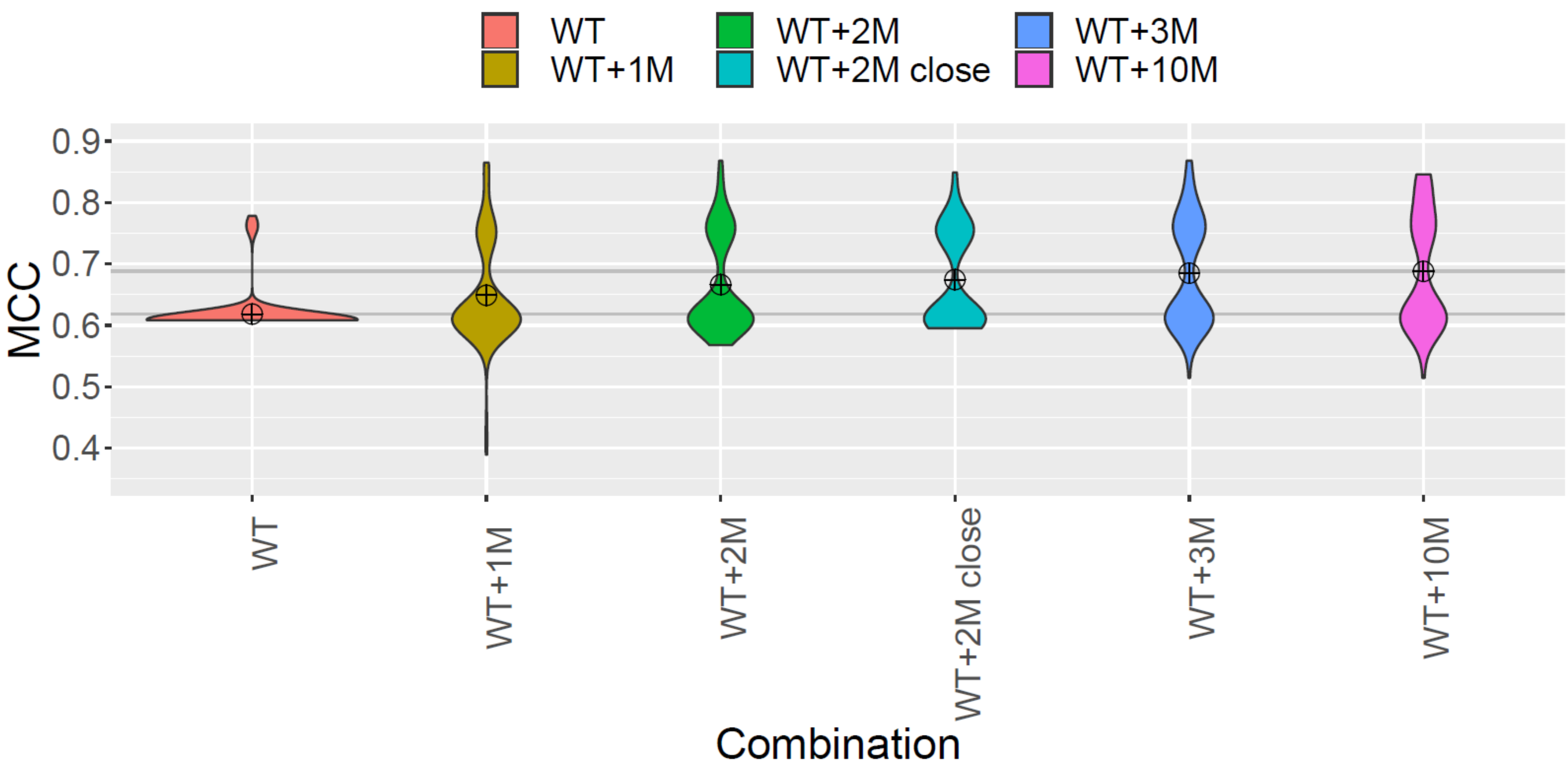
- Orthogonality?
- Complementarity?



Preliminary: Treating mutants as conditions

- Mutate-And-Map profiles produced by Das lab
- Systematic single-point mutants, usually similar structure



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- More data to build mechanical understanding of SHAPE

Thank you!

Afaf Saaidi
Postdoc?

Bruno Sargueil

Delphine Allouche

Ronny (*da main man*) for modular extensions to Vienna package

Soft constraints x Sampling = Awesomeness

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