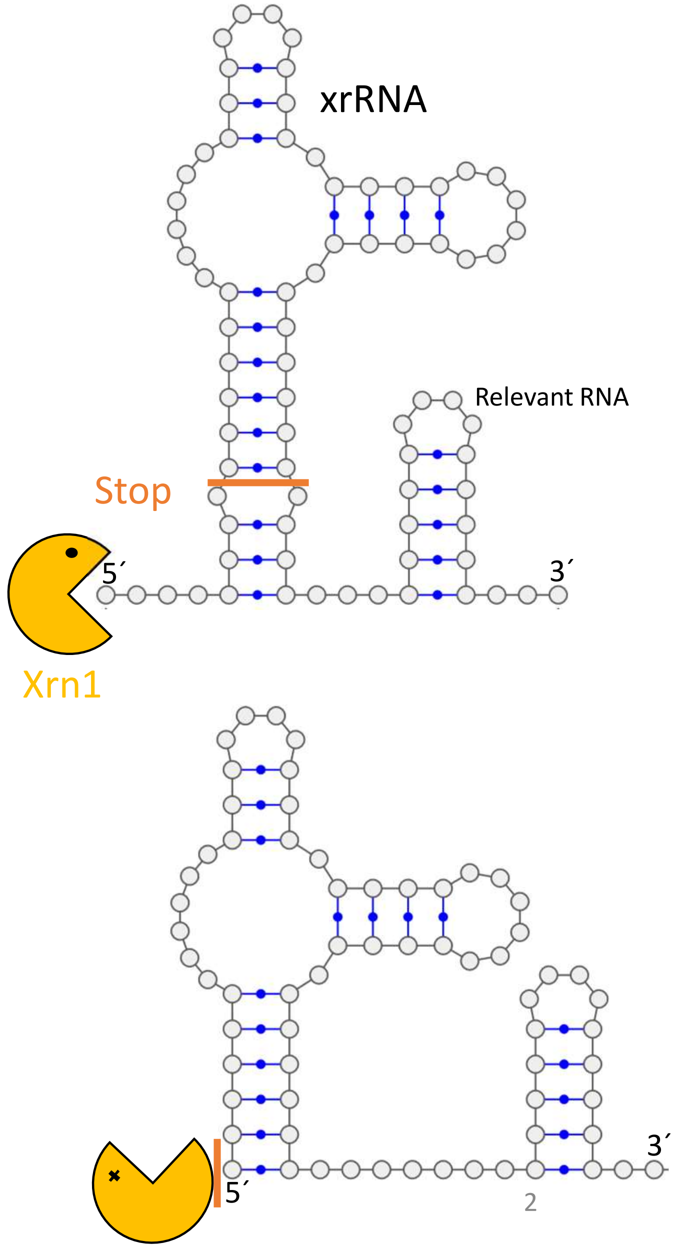
Designing Artificial xrRNA

Leonhard Sidl

Introduction to xrRNA

- xrRNA stops exoribonuclease Xrn1



Introduction to xrRNA

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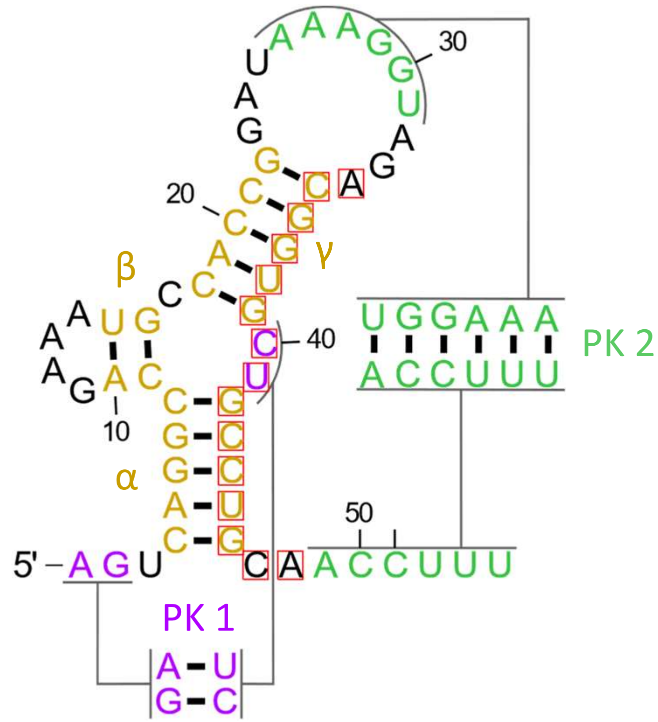
- Conserved secondary structure features:
  - Central 3-way junction with helices α-γ
  - Two pseudoknots PK1 and PK2



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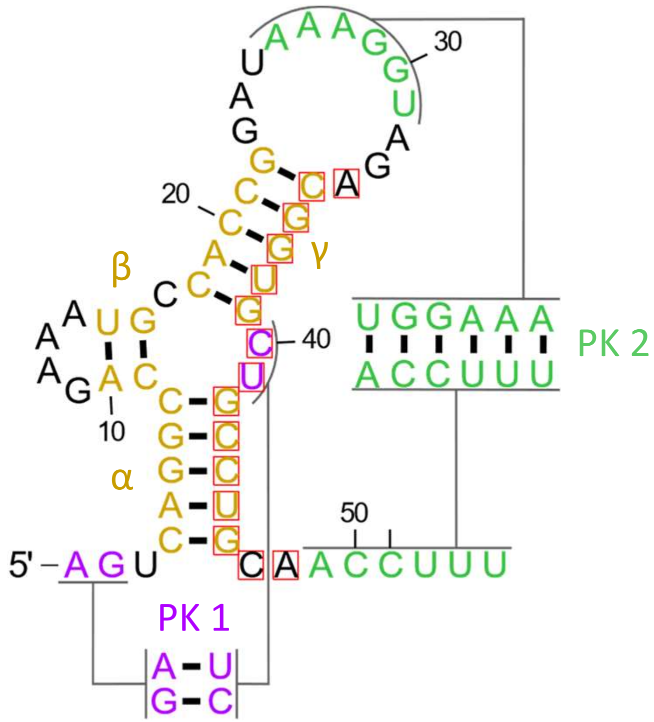
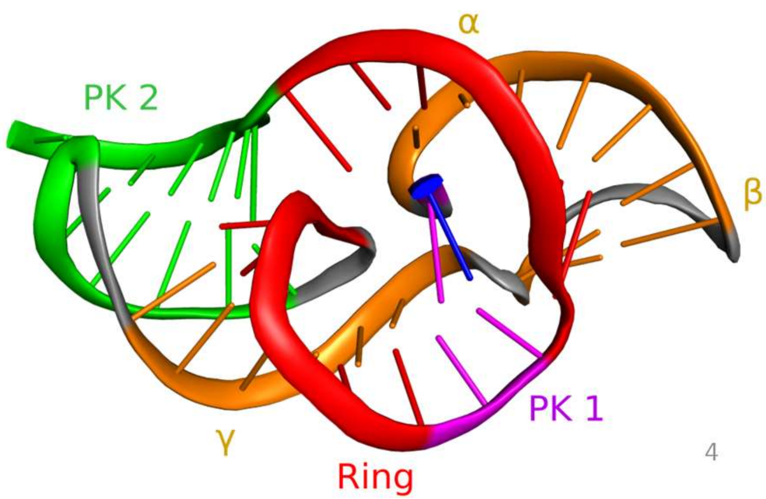
  - Ringlike structure around the 5’ end





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- Conserved secondary structure features:
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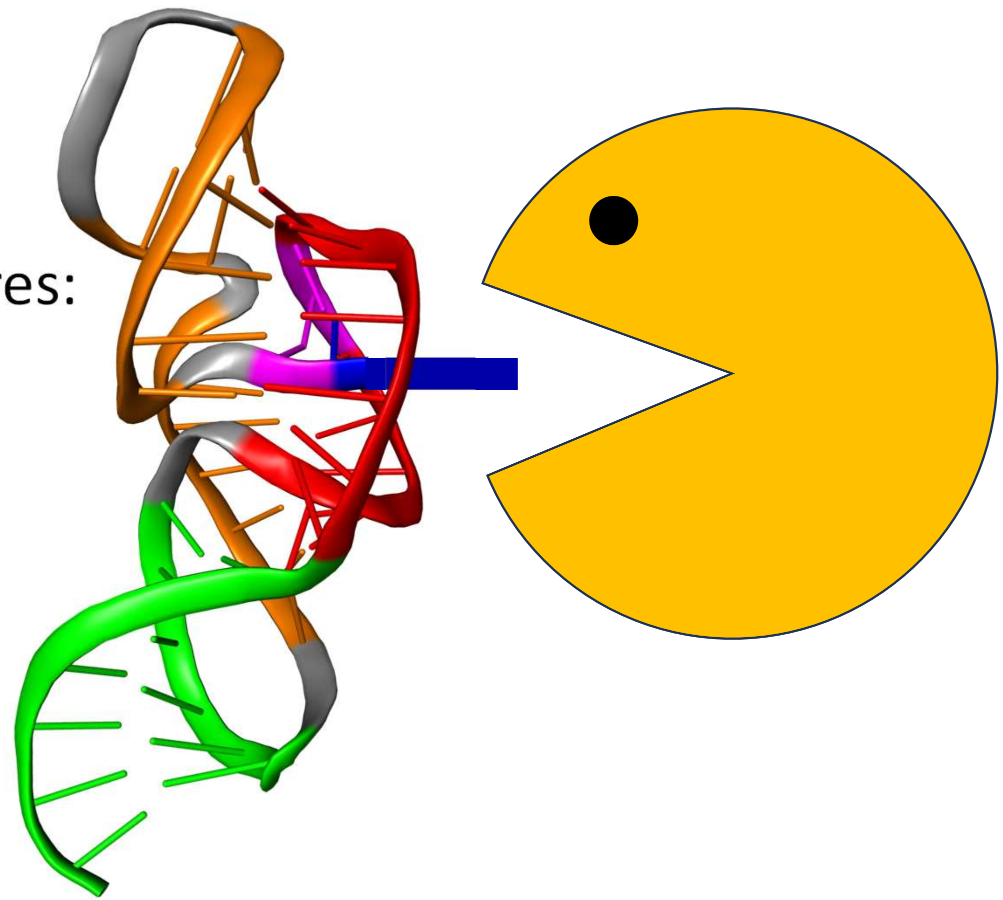
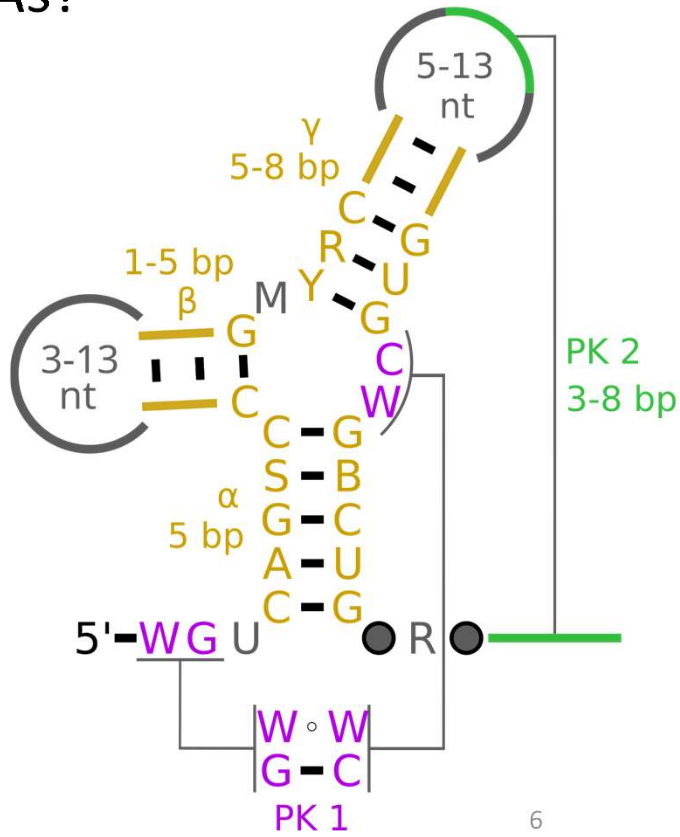
- Mechanical block against Xrn1



Designing xrRNAs

1. Which features are essential for biological xrRNAs?

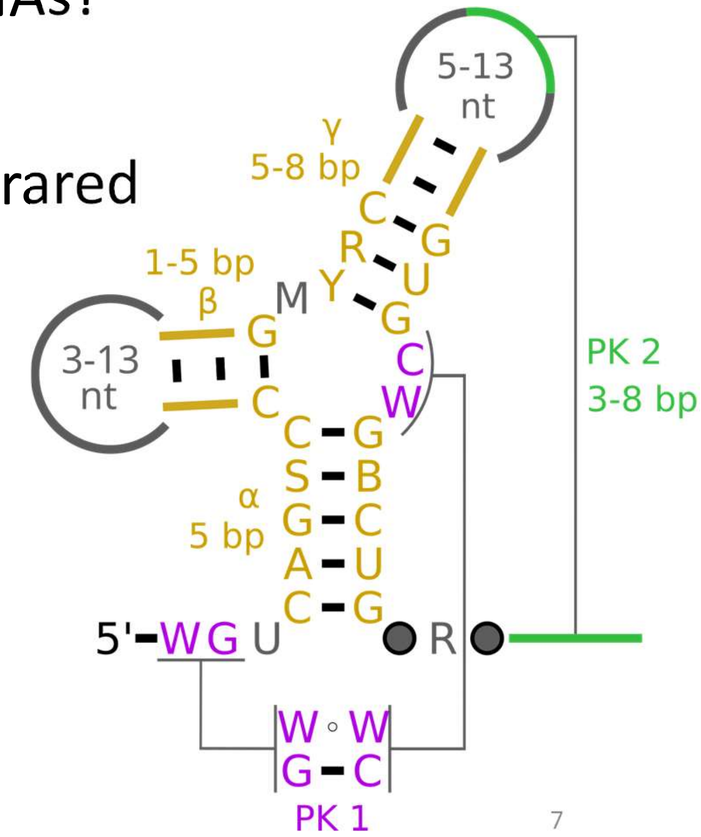


Designing xrRNAs

Yao, Hua-Ting, et al (2024)

1. Which features are essential for biological xrRNAs?

2. Design sequences with those features using Infrared



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- Sample sequences under a set of constraints and functions

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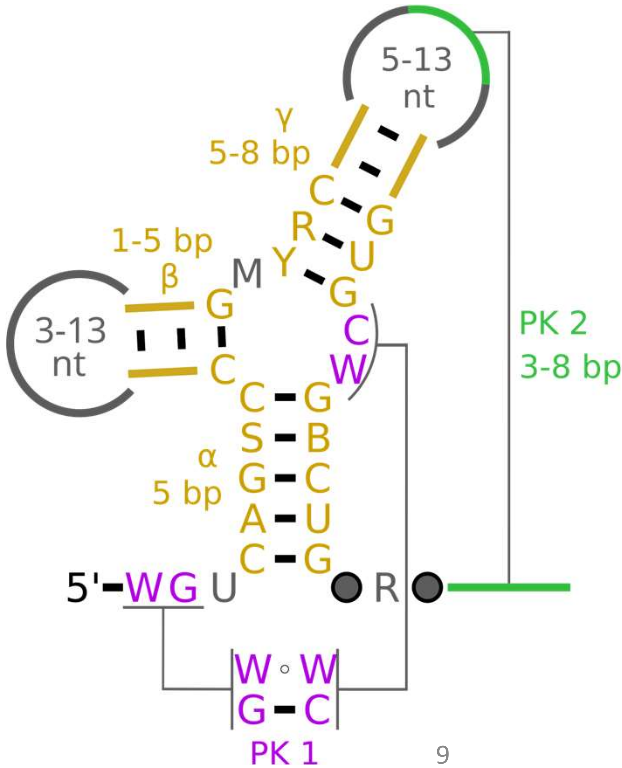

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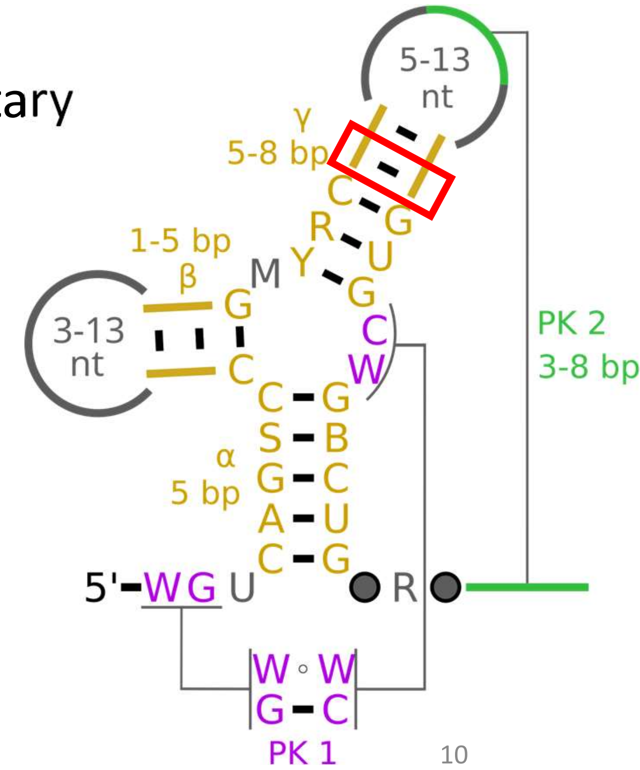

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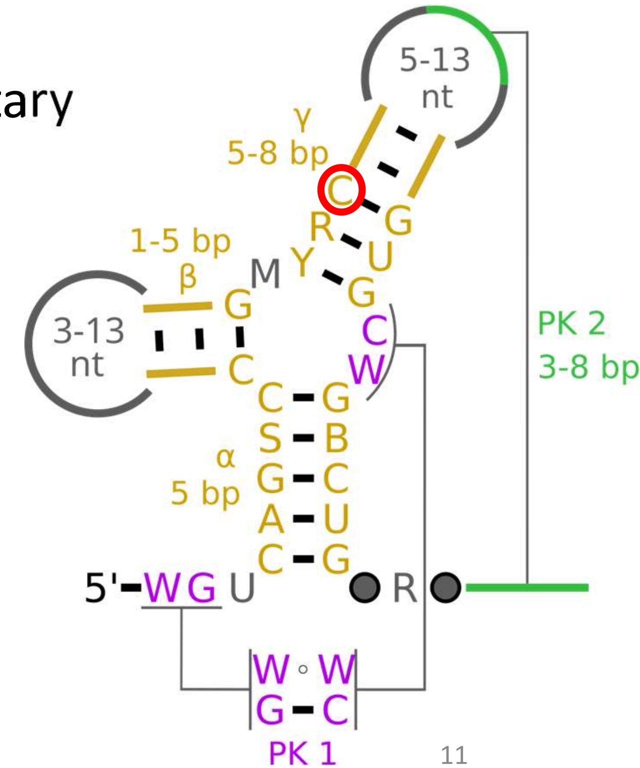

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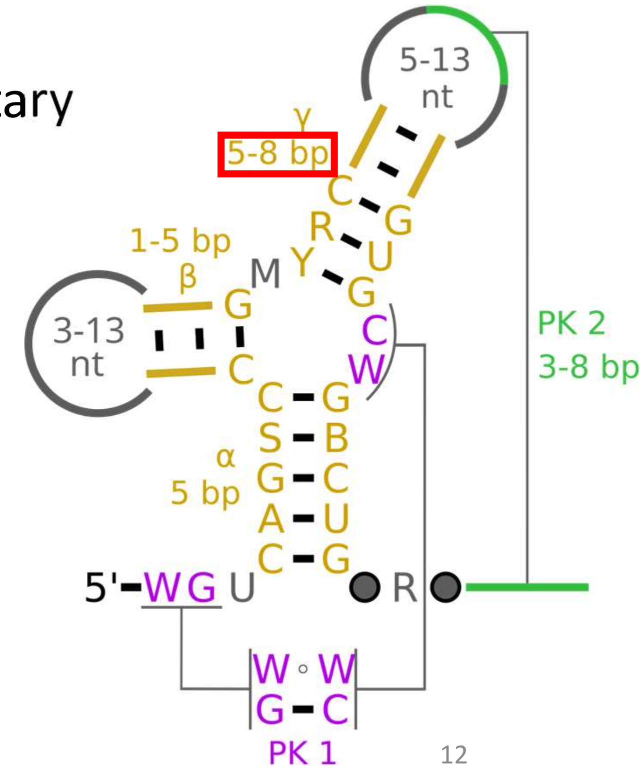

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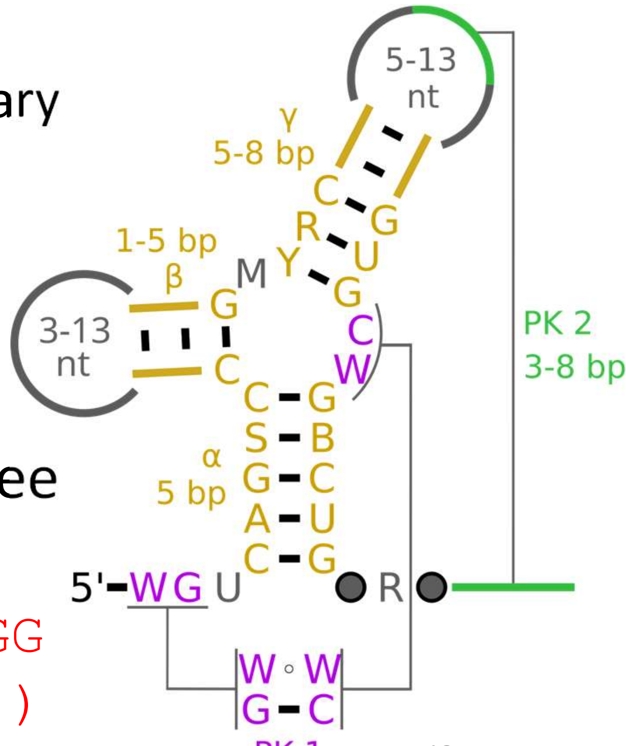

- Gaps only on one side of the helix/hairpin to guarantee uniform sampling

```
CC--AAAGG  CC-A-AAGG  CCA--AAGG
((.....))  ((.....))  ((.....))
```

2. Design Optimization

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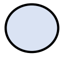

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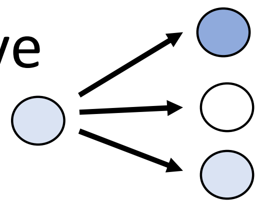
- Move around the local neighborhood and improve the objective function



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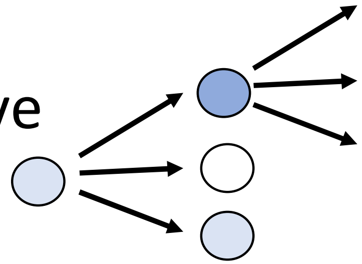

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```
Target Structure:
     ((...))
```

Initial Sample

```
AGACGUU
((...))
```

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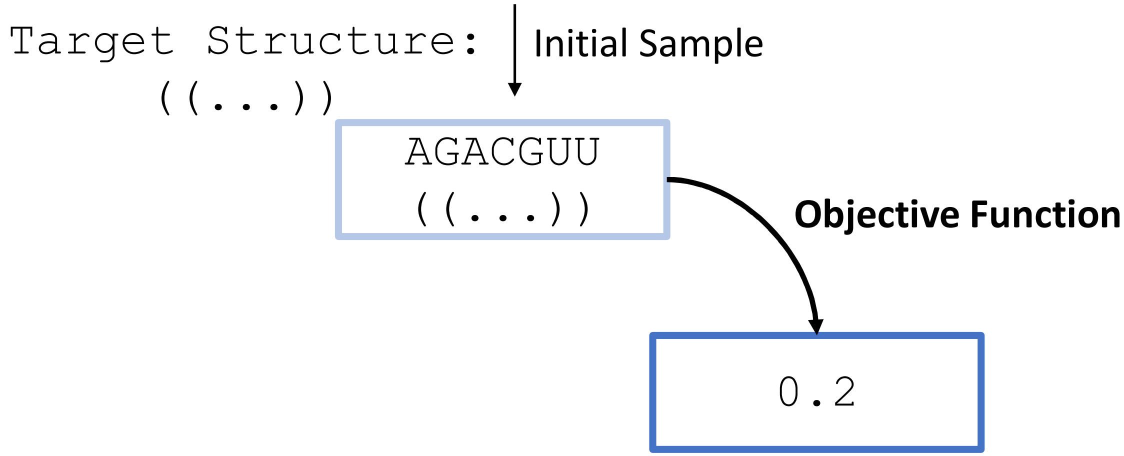

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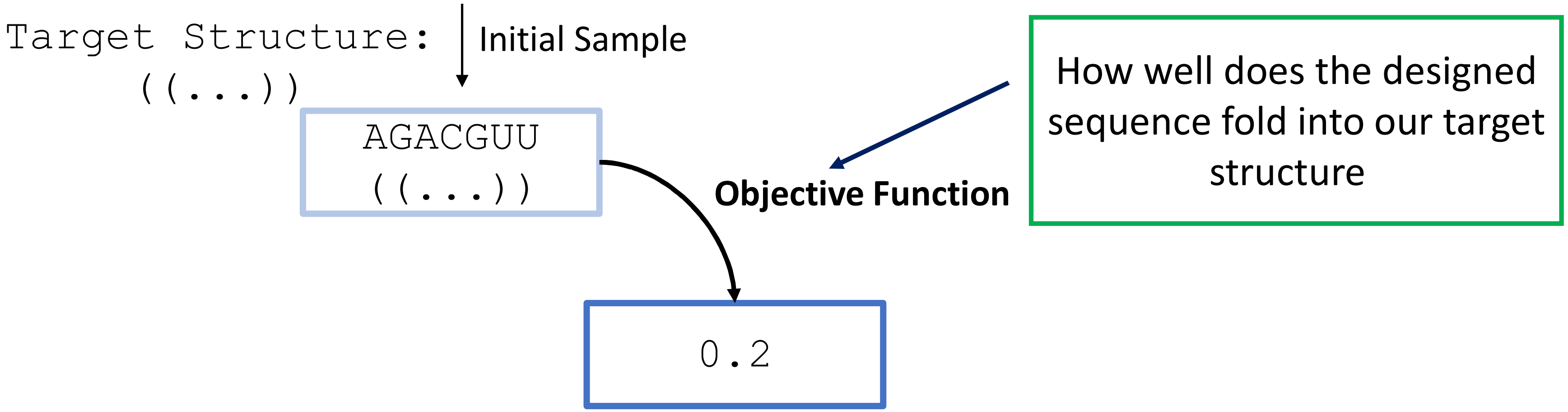

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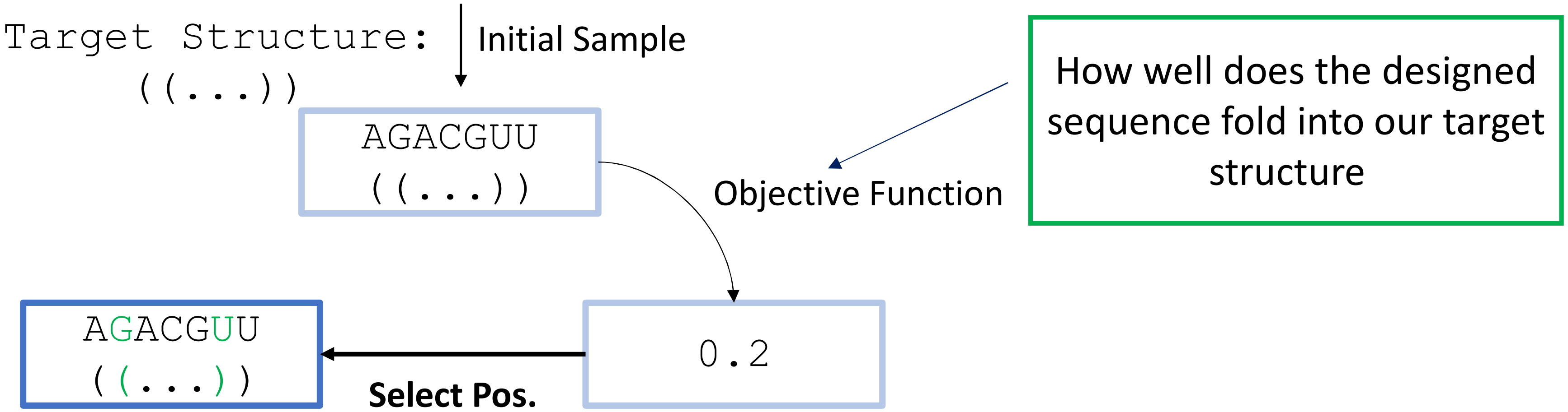

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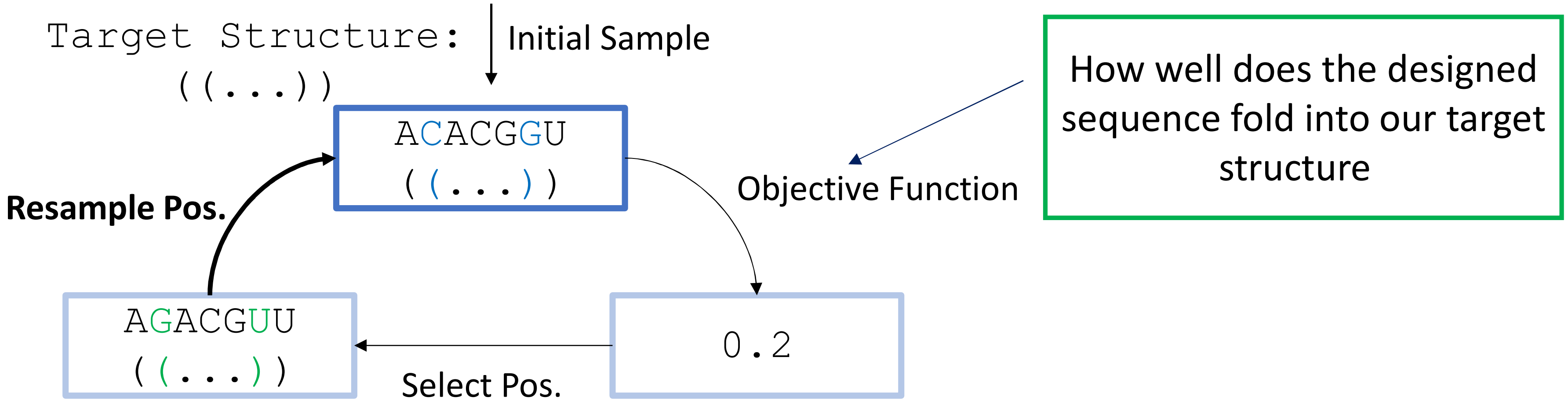

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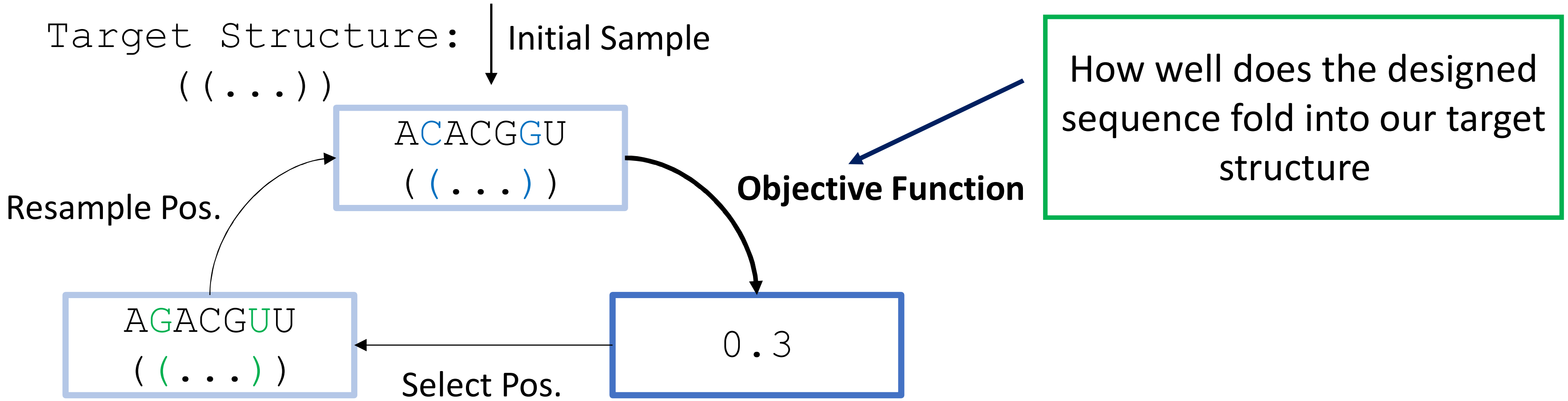

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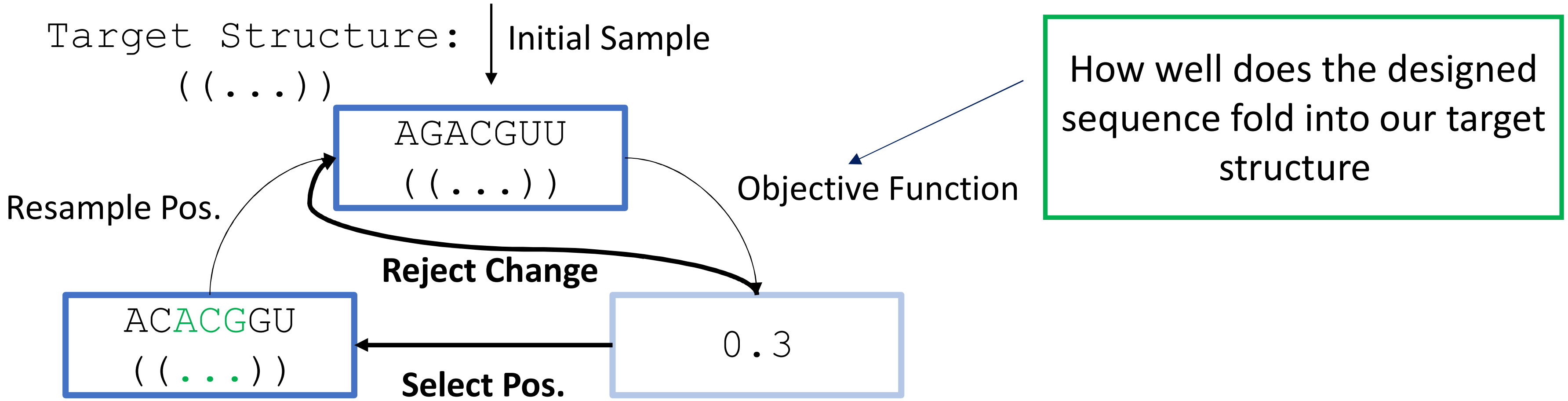

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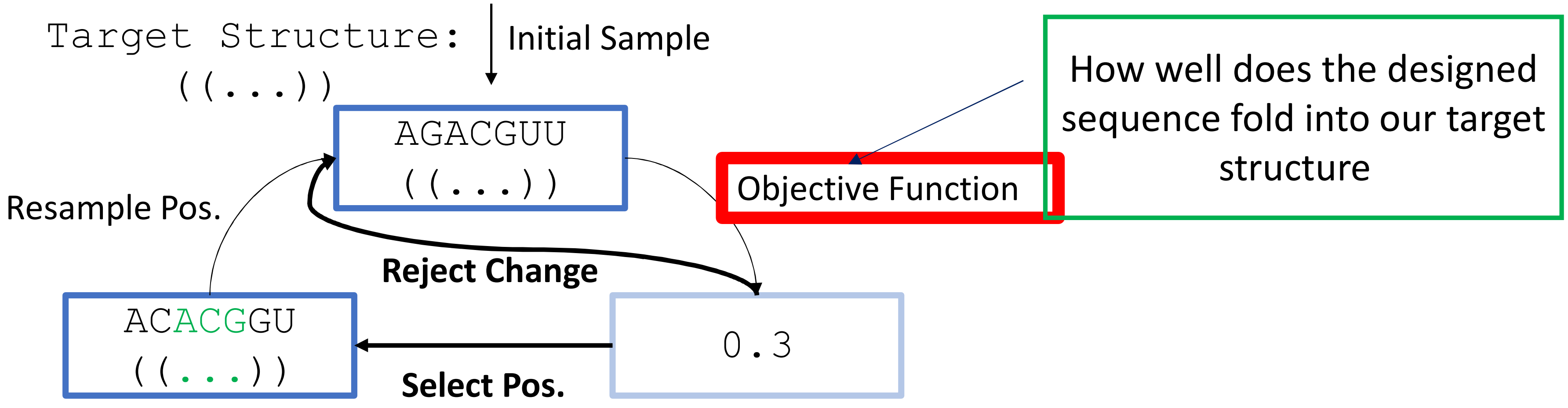

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- Probability that sequence *s* folds into structure *p*

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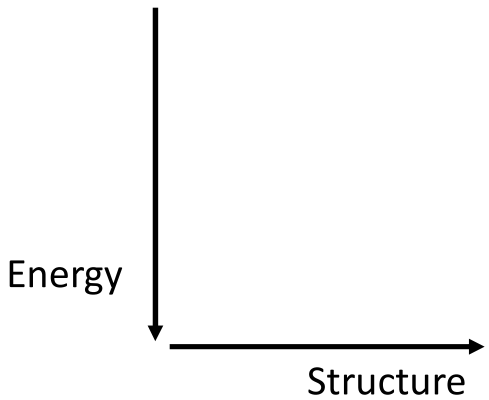

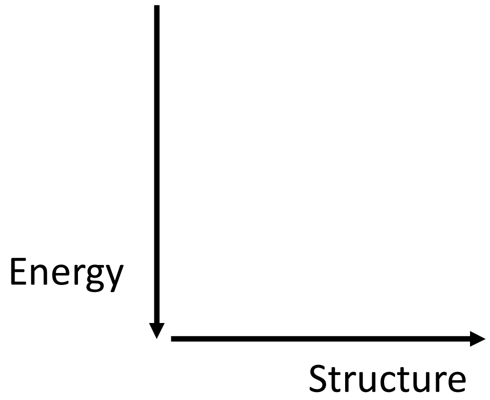

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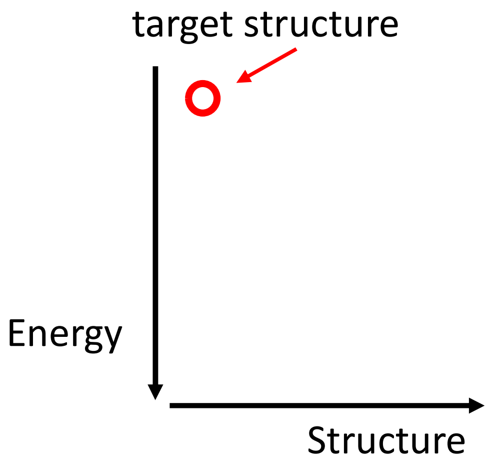

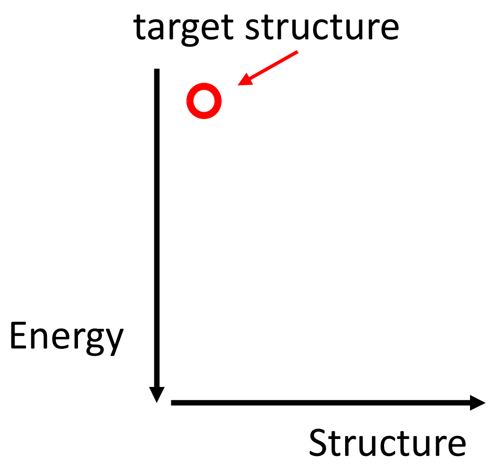

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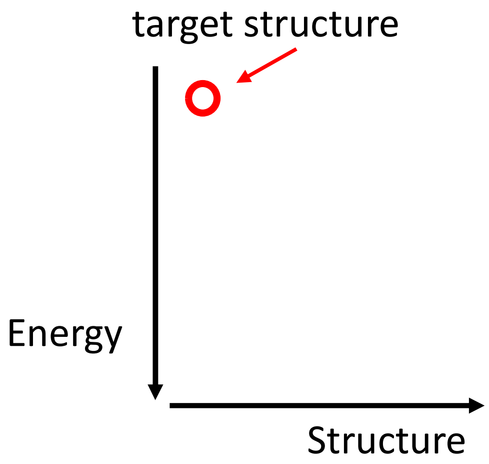

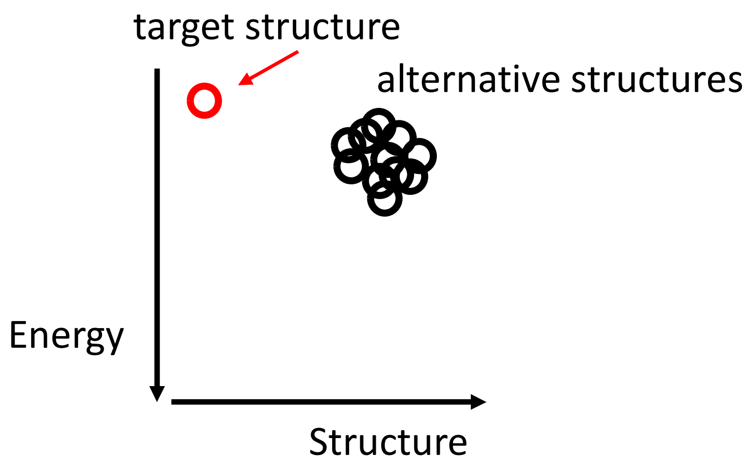

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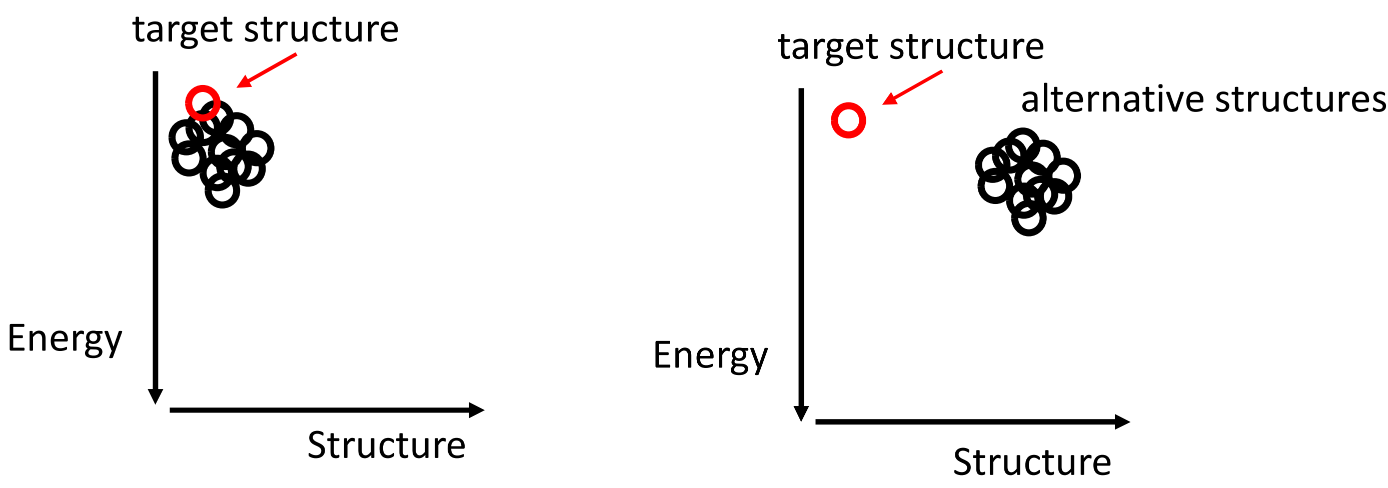
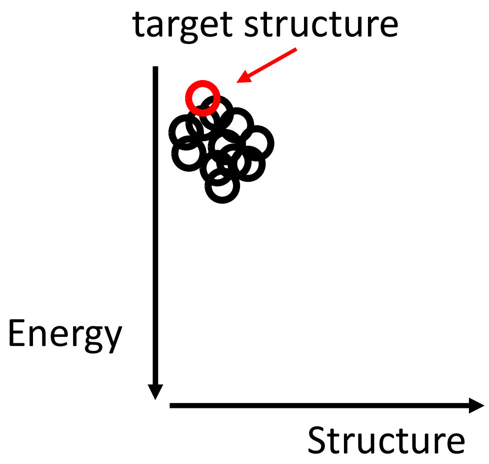
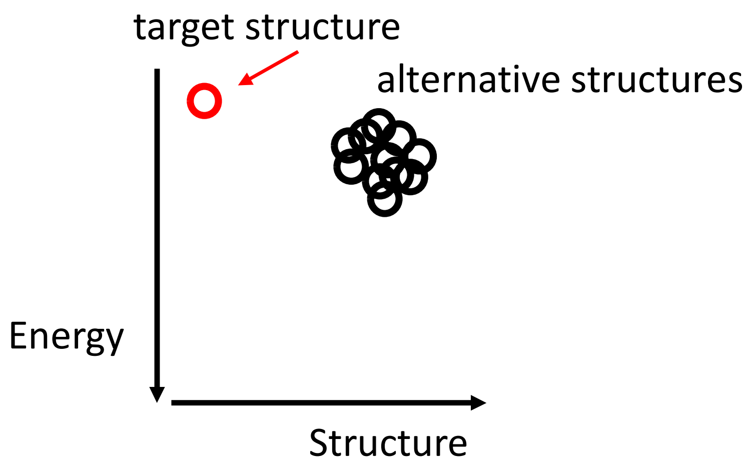


2. Objective Functions - Target Probability

- Probability that sequence *s* folds into structure *p*
- Sounds good, but RNAs don't fold into a single structure
- Same target probability





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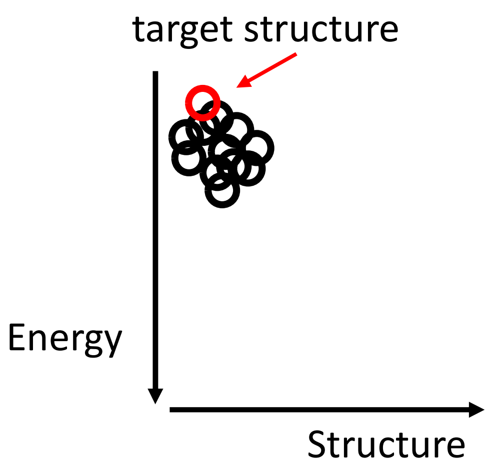
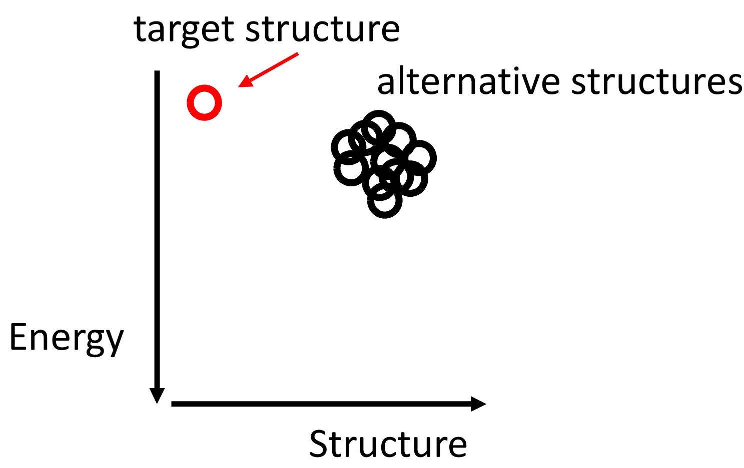
- But one is a significantly better design





2. Objective Functions - Target Probability

- Probability that sequence *s* folds into structure *p*
- Sounds good, but RNAs don't fold into a single structure
- Same target probability
- But one is a significantly better design
- Also not a good optimization function if far away from the optimum

2. Objective Functions - Ensemble Defect

- Average number of incorrect nucleotides

```
candidate design:    CCCAACCCAAAGGG
target structure:    .....(((...)))
```

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```
candidate design:    CCCAACCCAAAGGG
target structure:    .....(((...)))
```

ensemble

```
.((.........))
.....((.....))
......((...)).
.((........)).
.....(((...)))
(((........)))
```

E

2. Objective Functions - Ensemble Defect

- Average number of incorrect nucleotides

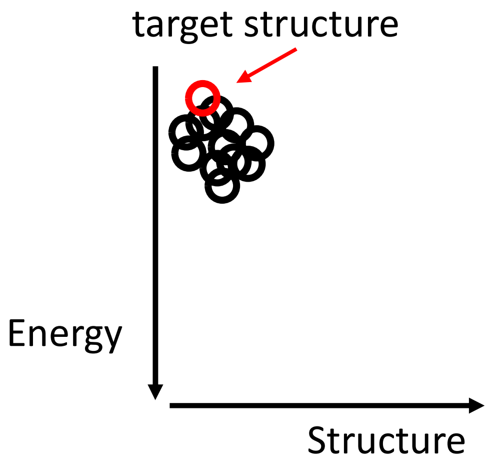
weighted average distance

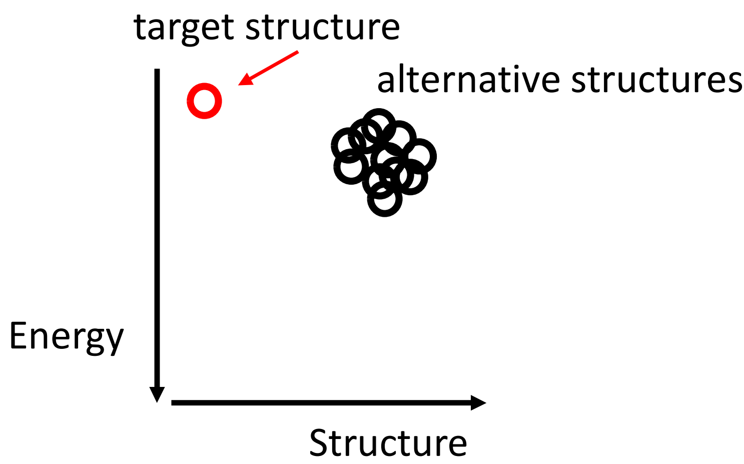
```
candidate design:   CCCAACCCAAAGGG
target structure:   .....(((...)))

.((.........))
.....((.....))
......((...)).
.((........)).
.....(((...)))
(((........)))
```

E

2. Objective Functions - Ensemble Defect

- Average number of incorrect nucleotides
- A sequence that can fold into many structures similar to the target structure has a good ED





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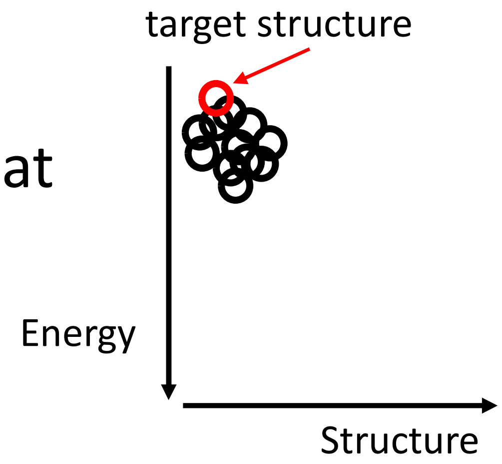
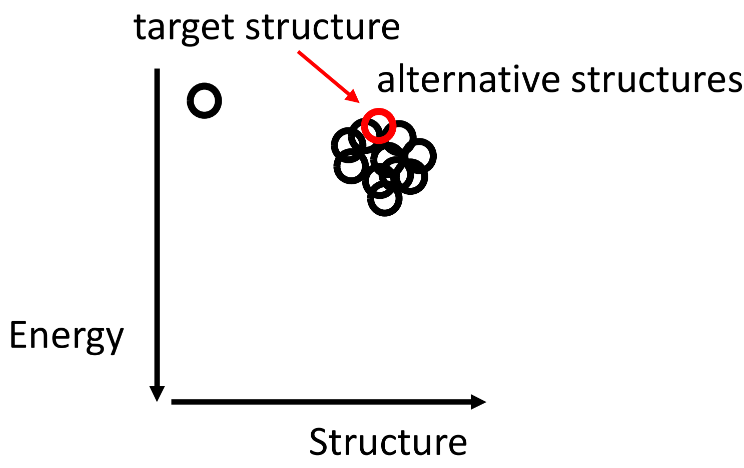
- A sequence that can fold into many structures similar to the target structure has a good ED

- Harder to find design that has the target as MFE





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- Harder to find design that

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- Works if far away from the optimum

- Start with ED; Use probability to make fine-grained optimizations

Designing xrRNAs

1. Characterize essential features conserved in biological xrRNAs

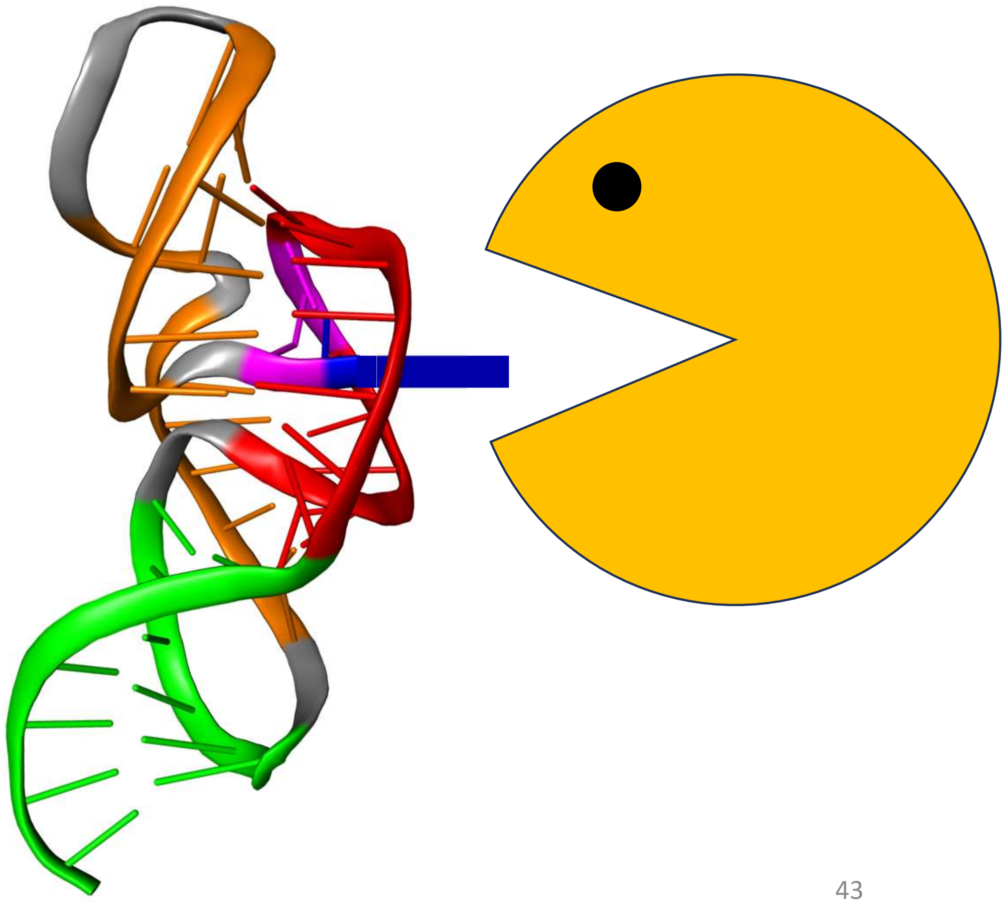
2. Sample sequences with those features using Infrared

3. Validate candidate designs in-silico

SimRNA

3. In-Silico Validation Using Steered MD

- Xrn1 braces against the ring of the xrRNA and can‘t pull the 5‘ end out

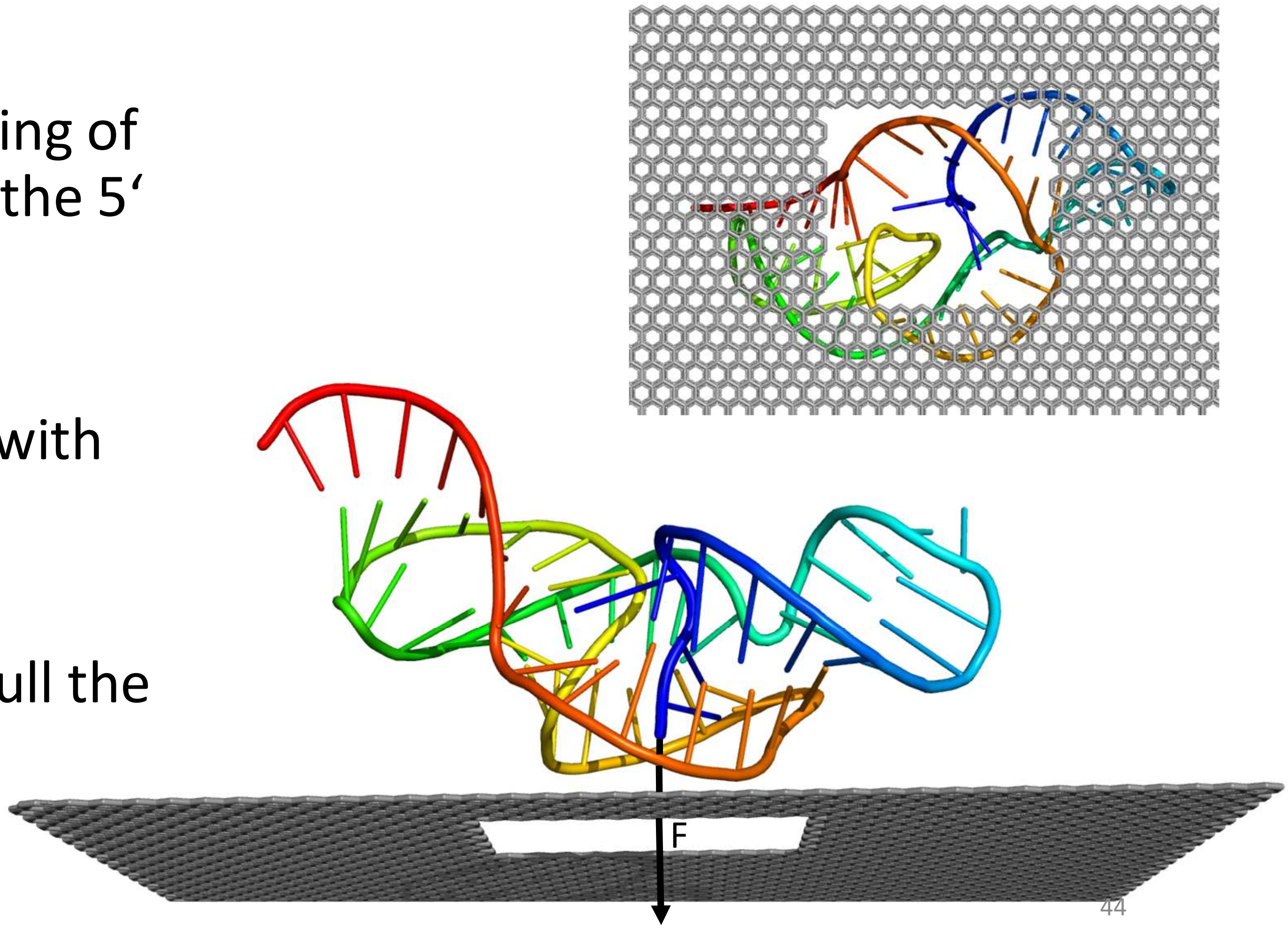


3. In-Silico Validation Using Steered MD

- Xrn1 braces against the ring of the xrRNA and can‘t pull the 5‘ end out

- Possible to approximate with steered MD

- Use a force gradient to pull the 5‘ end through a pore



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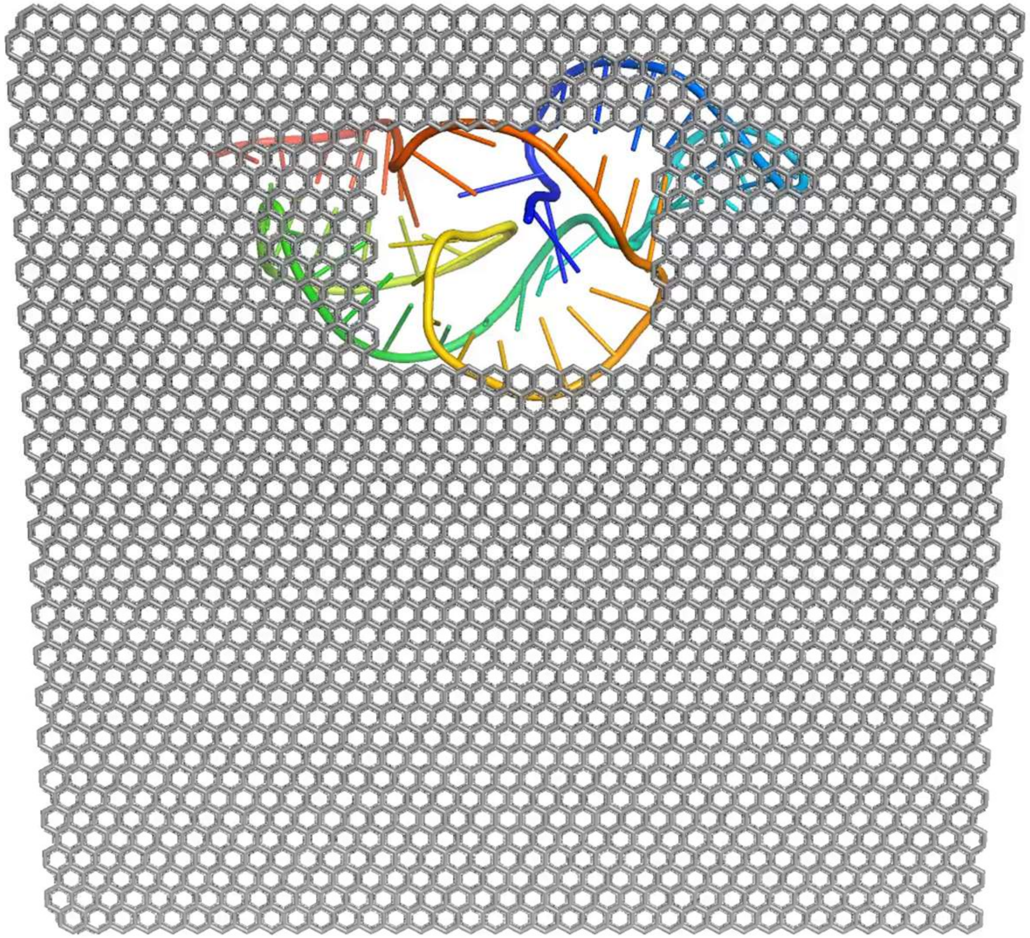

MD Simulation, 400 pN external force, 2fs timestep, Amber OL3 RNA FF, 500 ns, implicit solvent GBn2, 0.15 mol/L Salt Conc.

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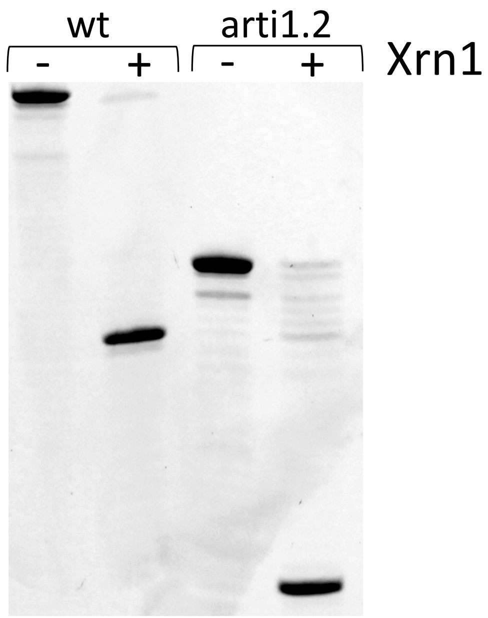
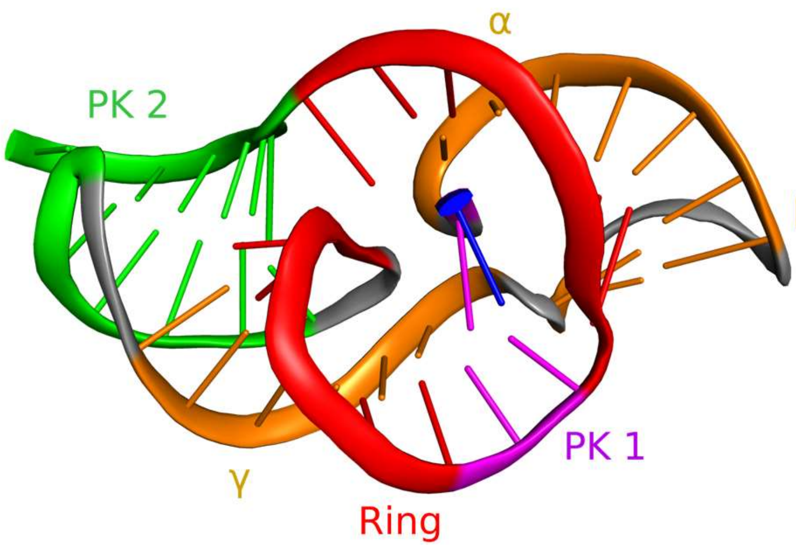
4. Test designs in-vitro



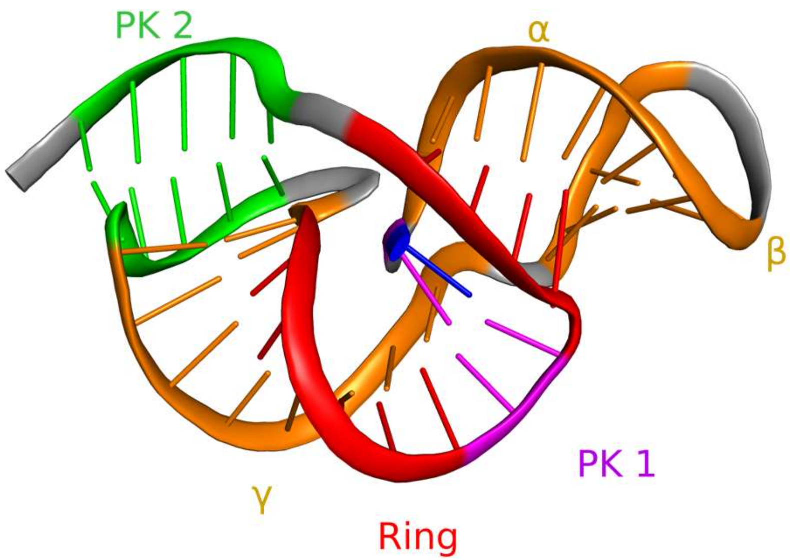
4. In-Vitro Experiments Validate Designs

- In-vitro experiments show our artificial xrRNA exhibits resistance to Xrn1 like biological examples

biological xrRNA



our design



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The whole TBI Team

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