

Rational design of exoribonuclease-resistant RNAs

Leonhard Sidl

Computational Approaches to RNA Structure and Function – Benasque

5.7.2026 – 17.7.2026

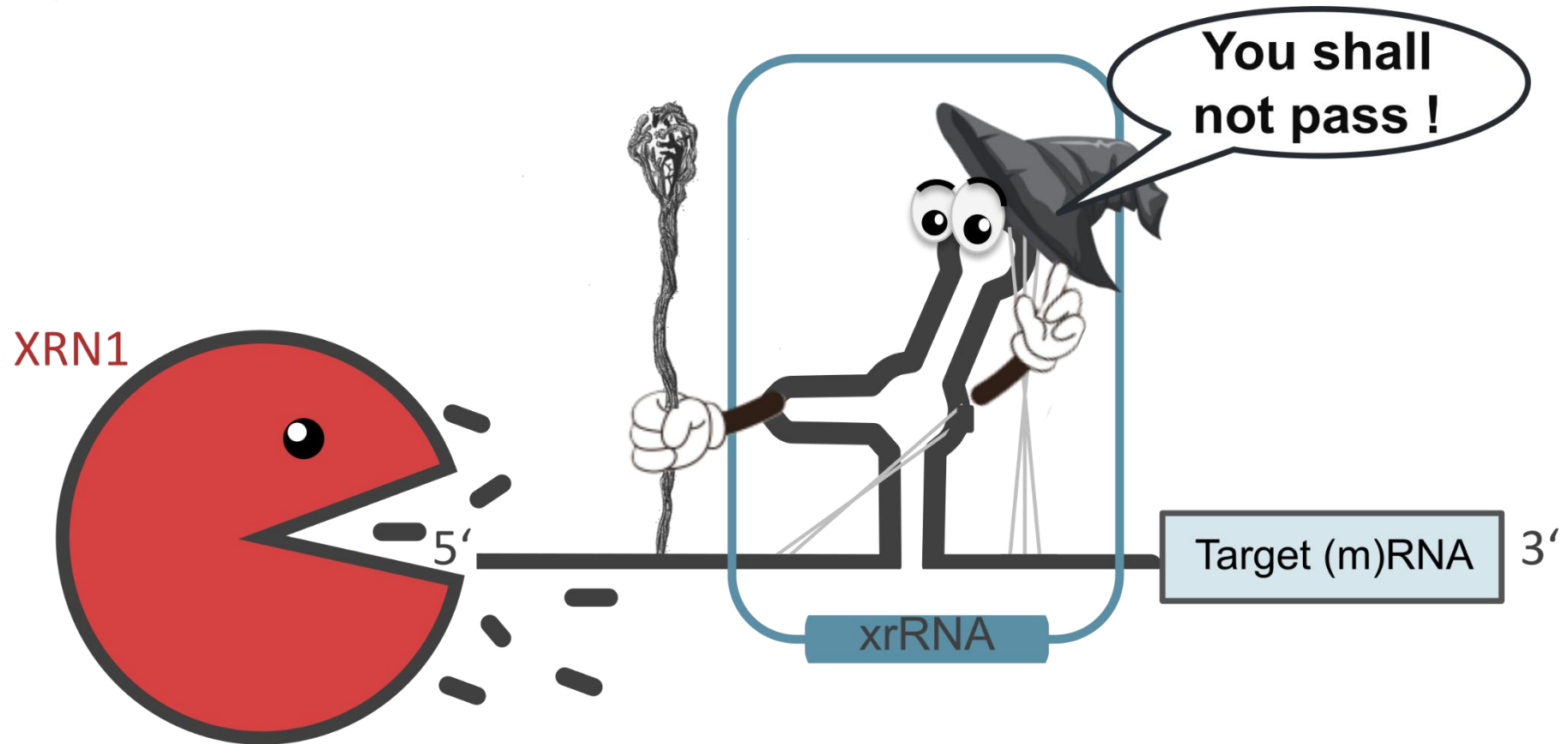


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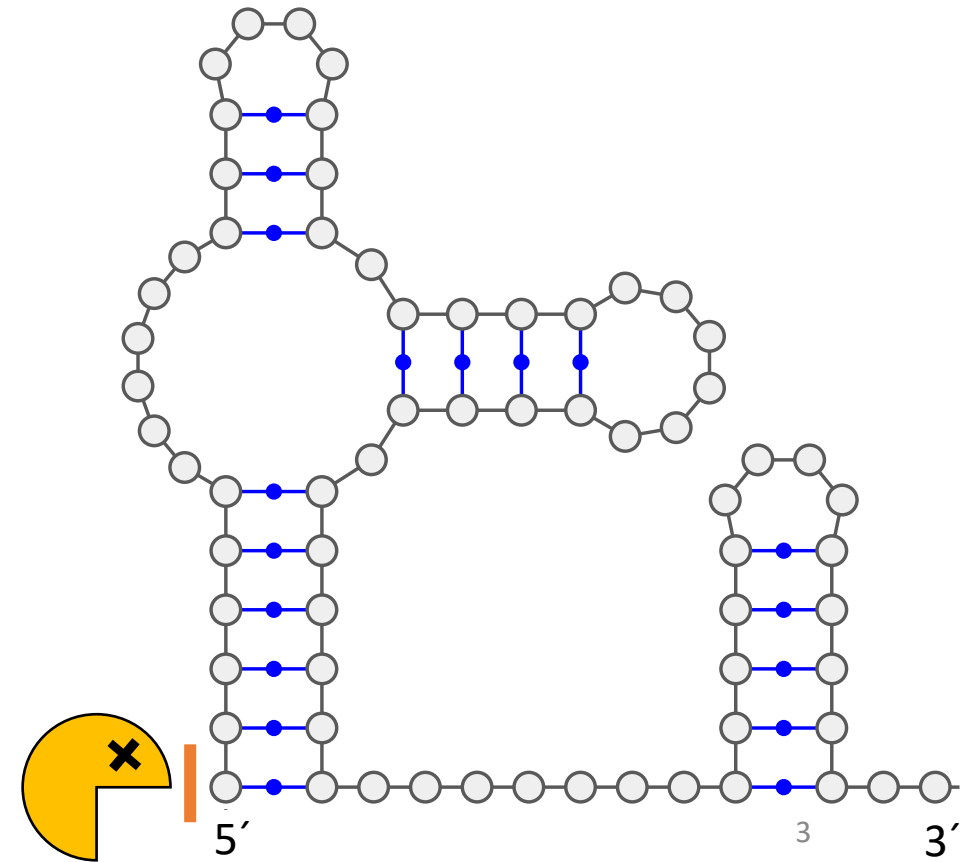
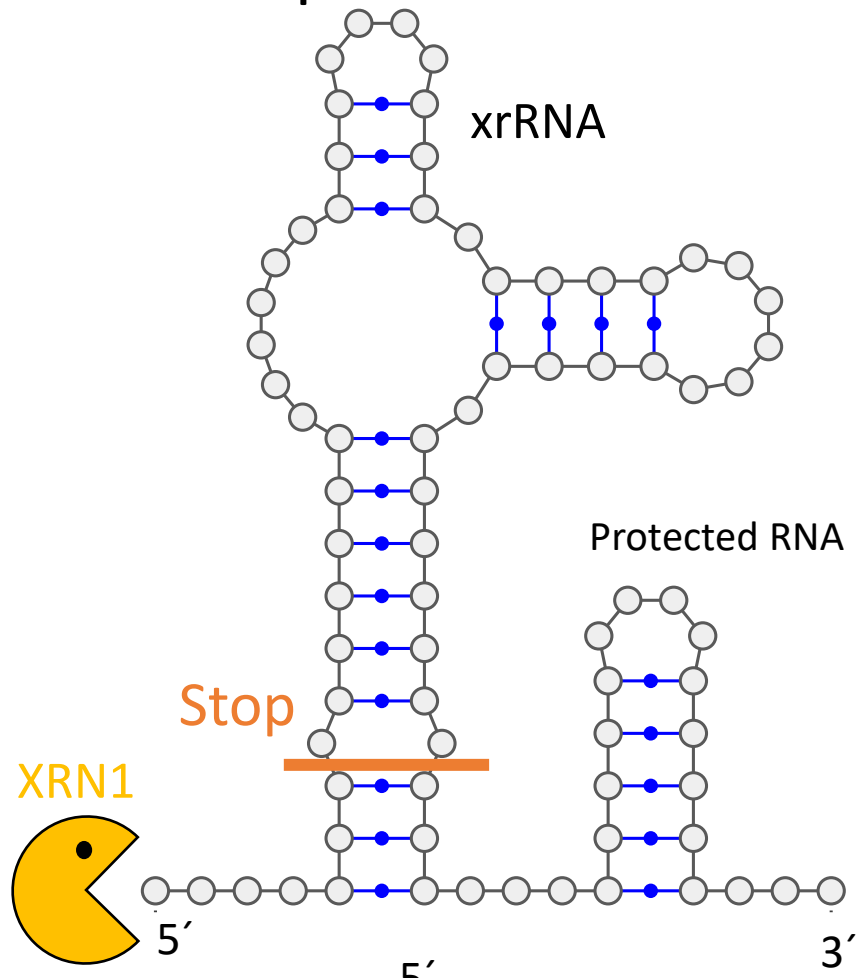
Introduction to Flavivirus xrRNA

- xrRNA stops exoribonuclease XRN1



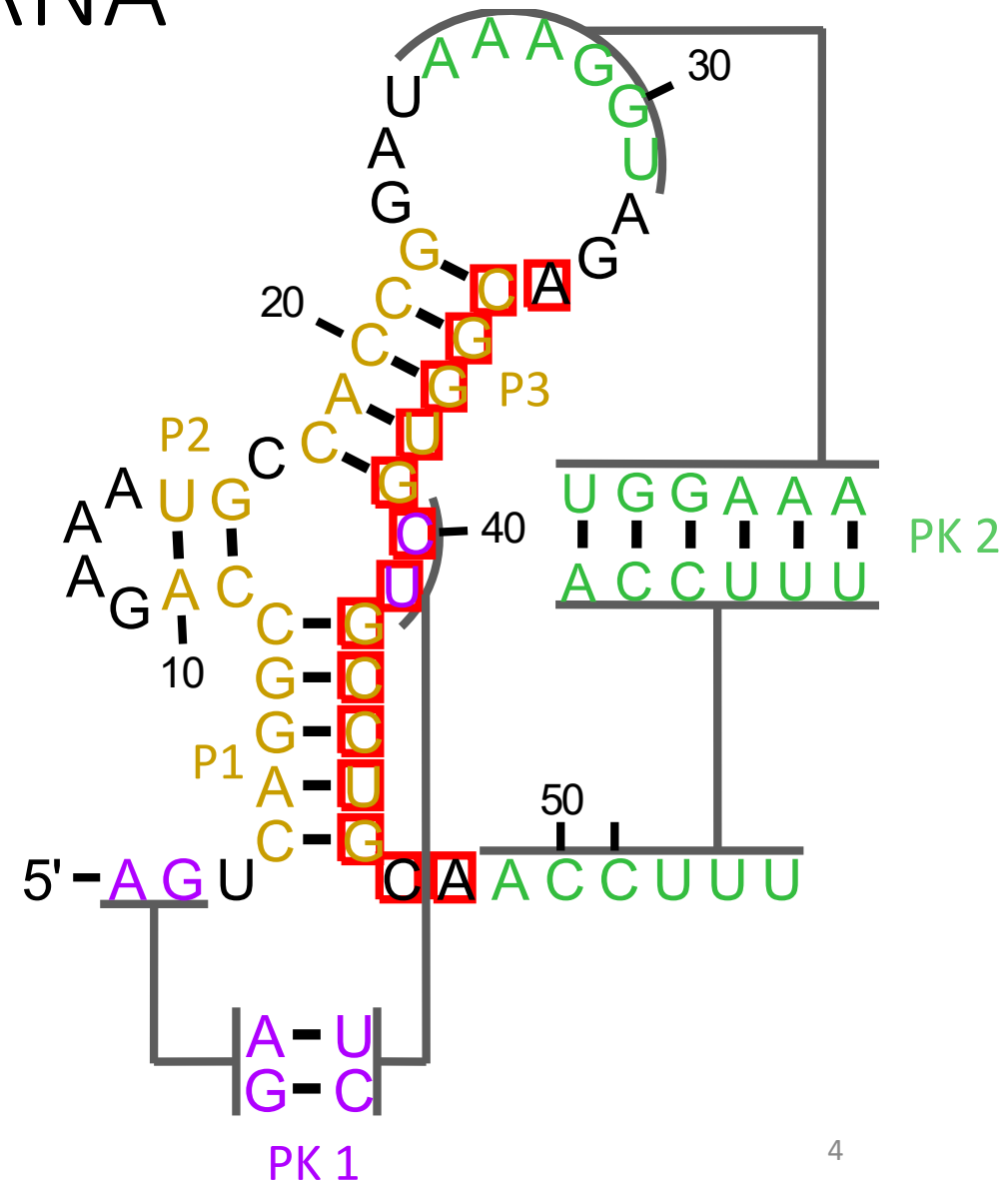
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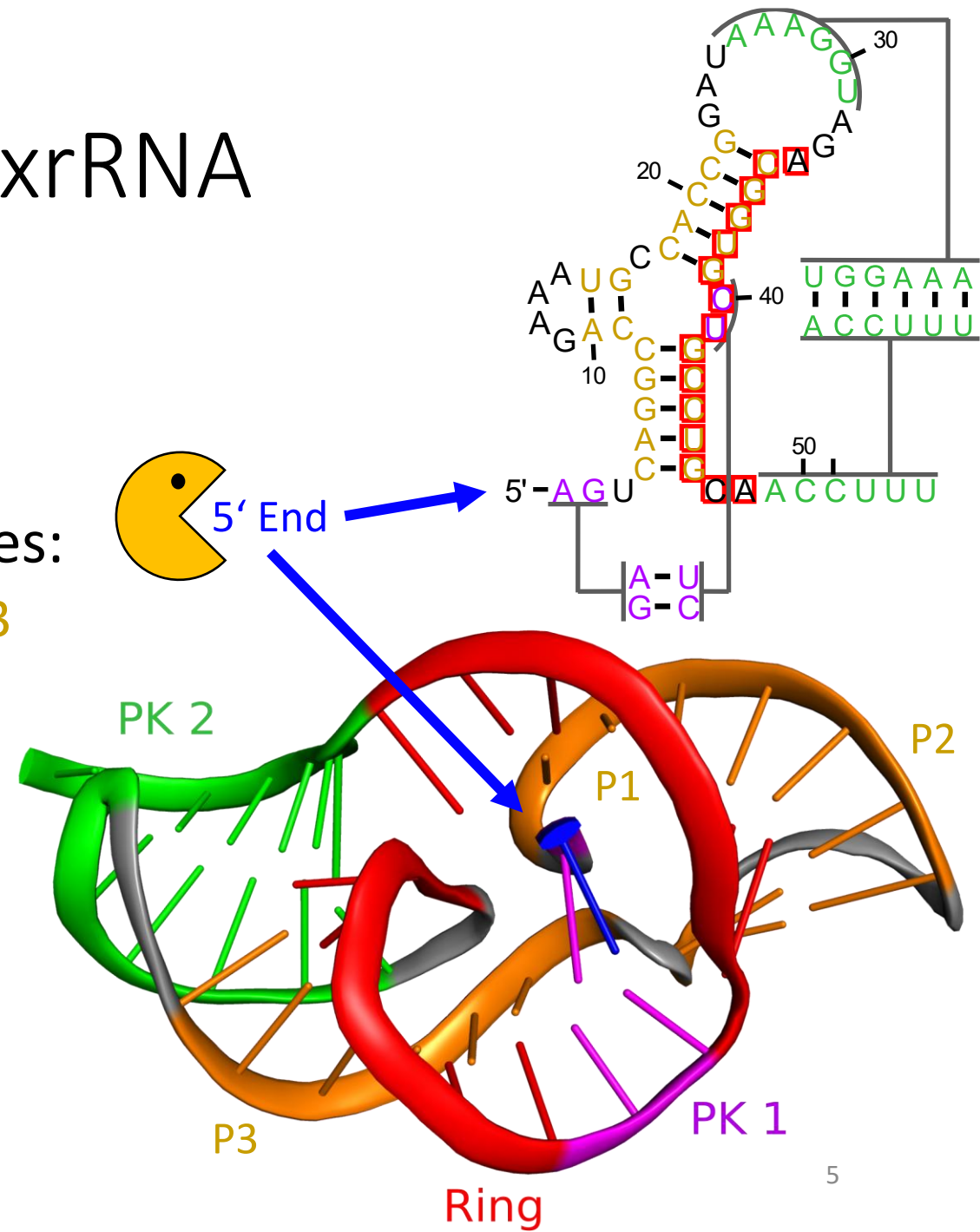
Introduction to Flavivirus xrRNA

- xrRNA stops exoribonuclease XRN1
- Conserved secondary structure features:
 - Central 3-way junction with helices P1-P3
 - Two pseudoknots PK1 and PK2



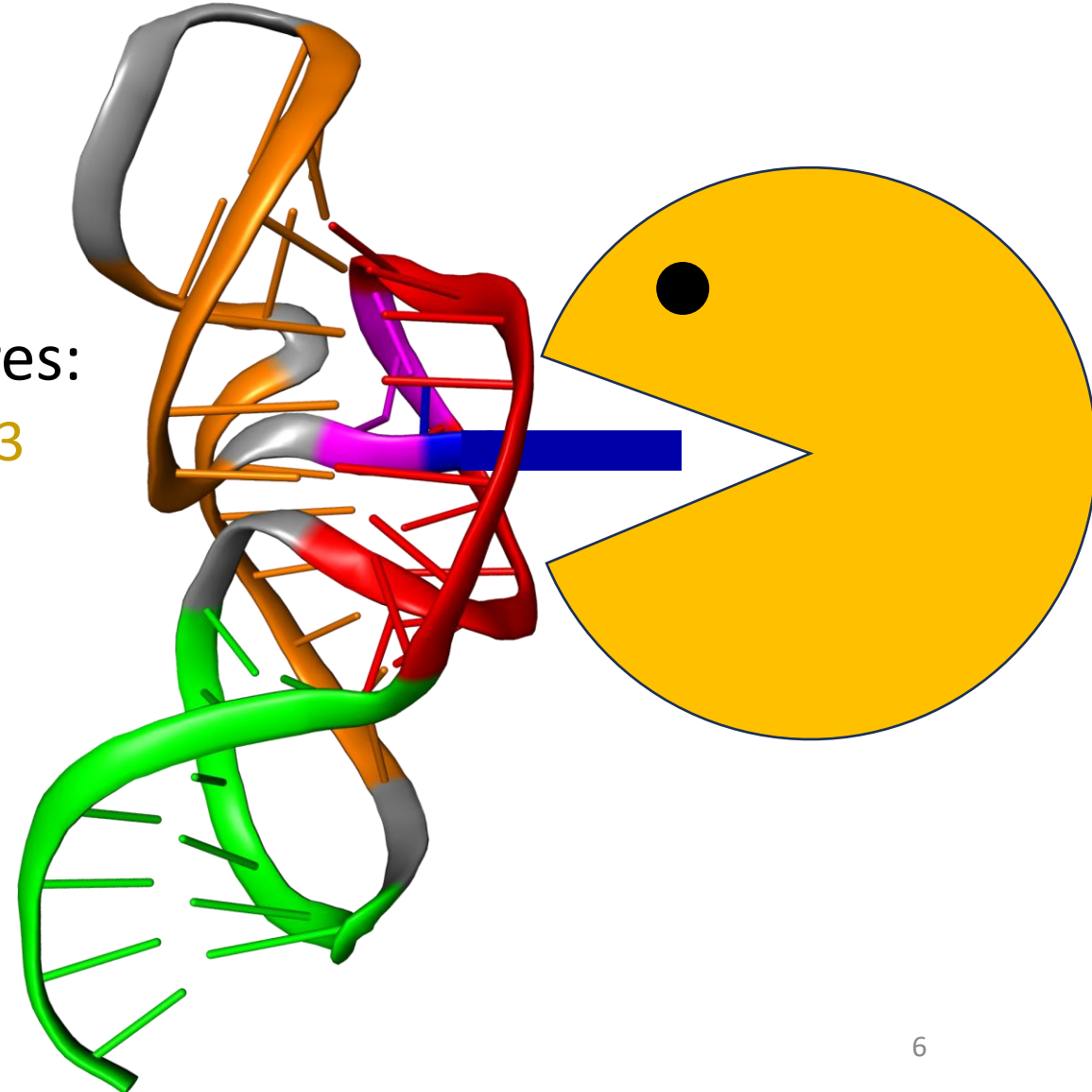
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 - **Ringlike** structure around the 5' end



Introduction to Flavivirus xrRNA

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- Mechanical block against XRN1

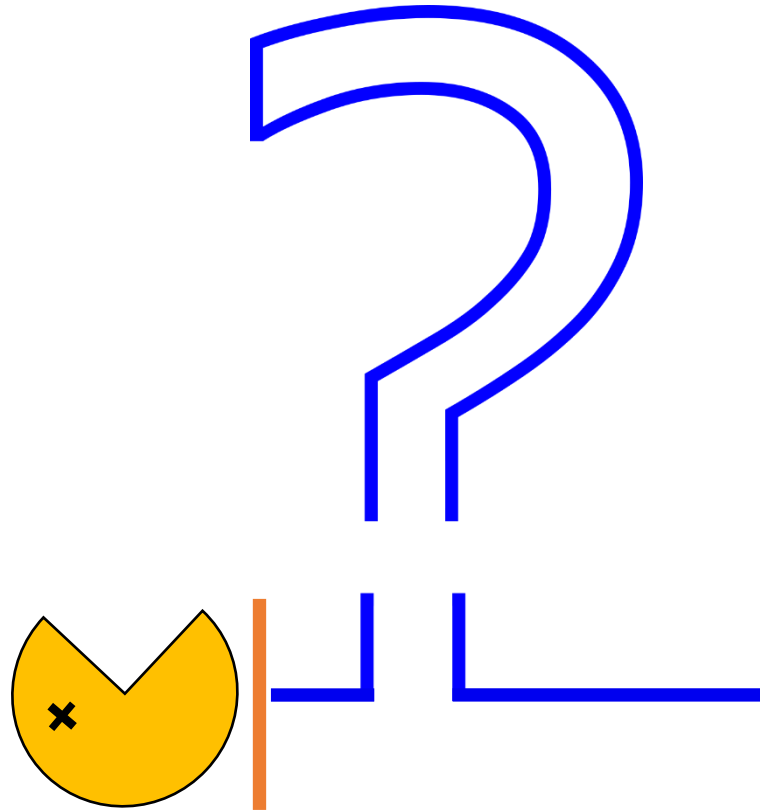


Levels of Design Novelty / Feasibility

- Function

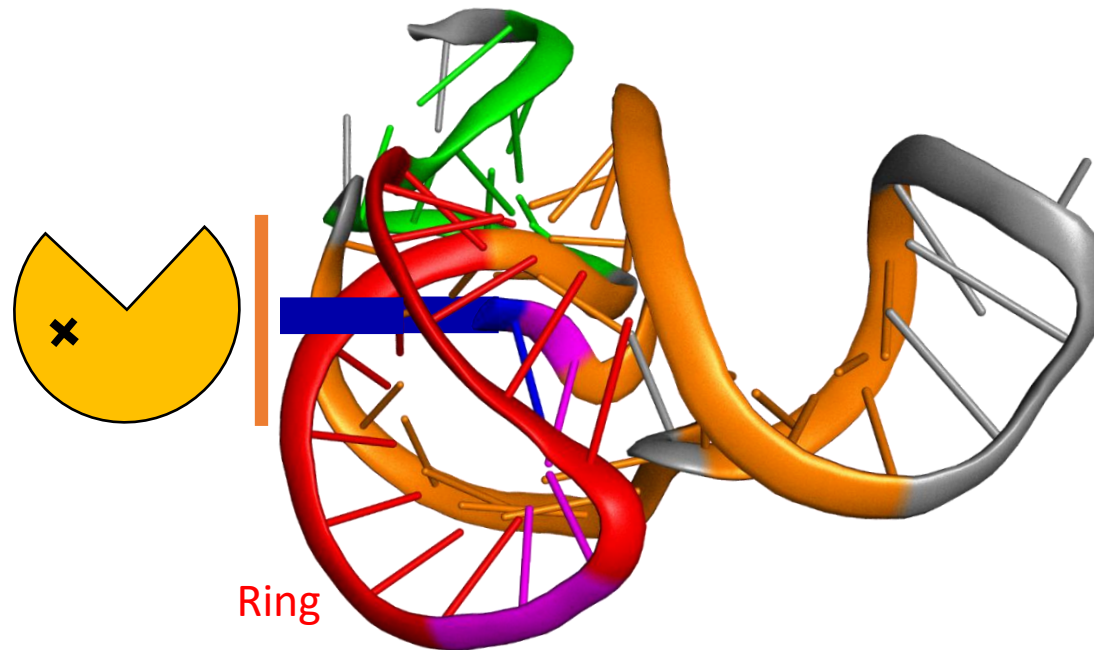
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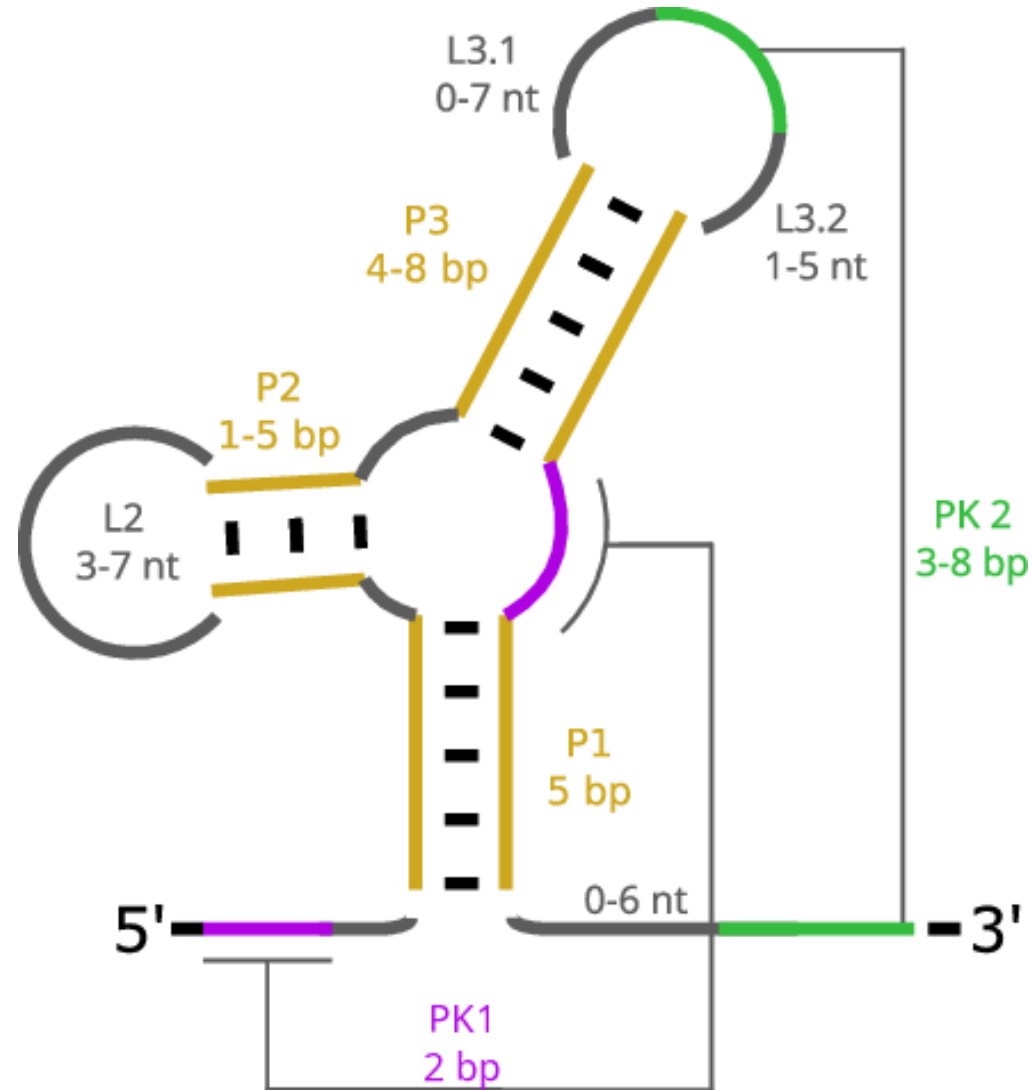
Levels of Design Novelty / Feasibility

- Function
- 3D Structure / Mechanism



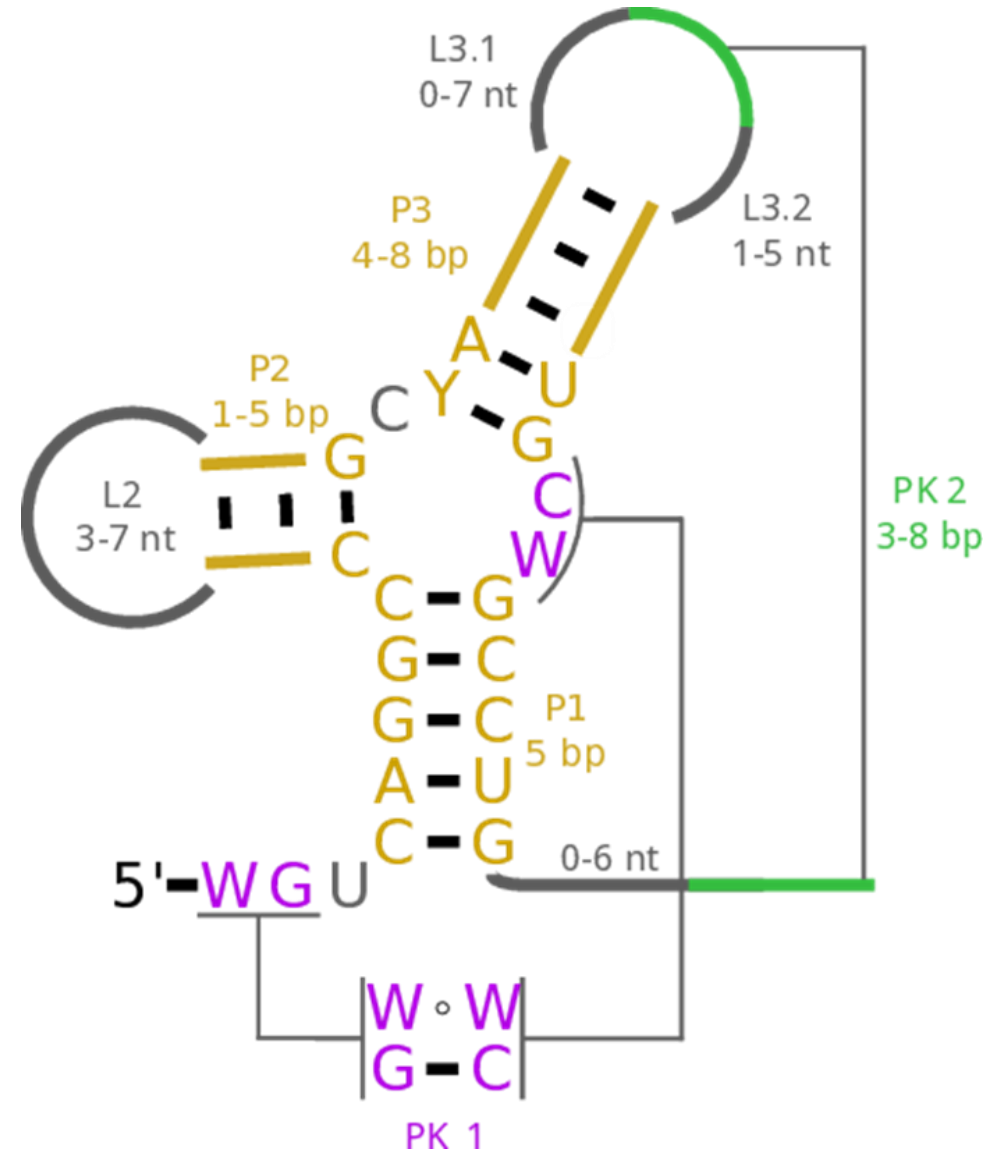
Levels of Design Novelty / Feasibility

- Function
- 3D Structure / Mechanism
- 2D Structure



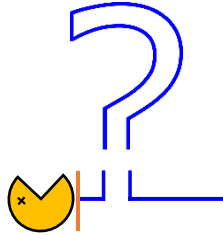
Levels of Design Novelty / Feasibility

- Function
- 3D Structure / Mechanism
- 2D Structure
- Sequence

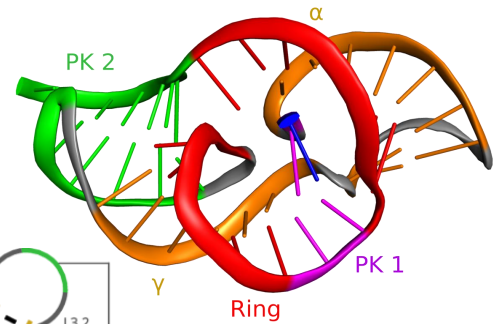


Levels of Design Novelty / Feasibility

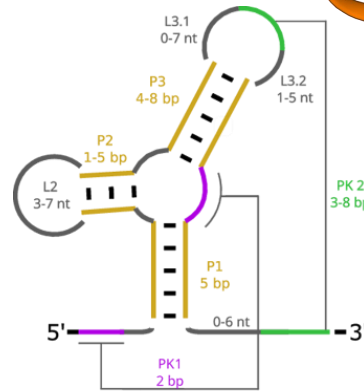
- Function



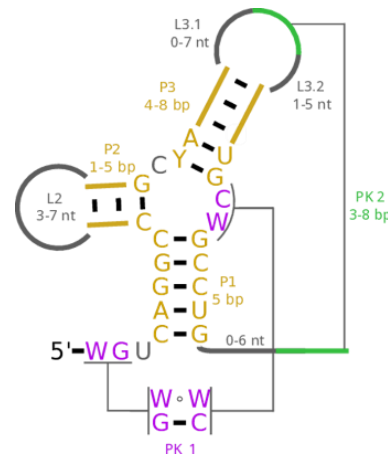
- 3D Structure / Mechanism



- 2D Structure

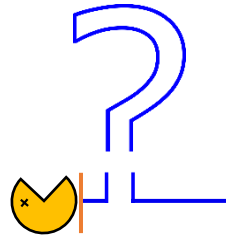


- Sequence

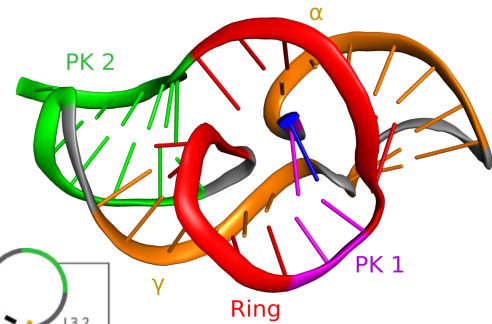


Levels of Design Novelty / Feasibility

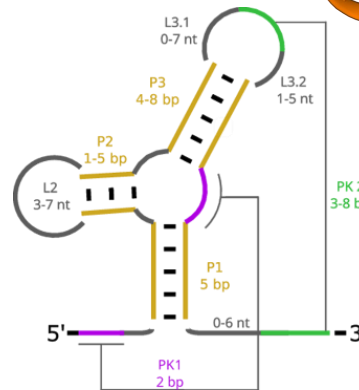
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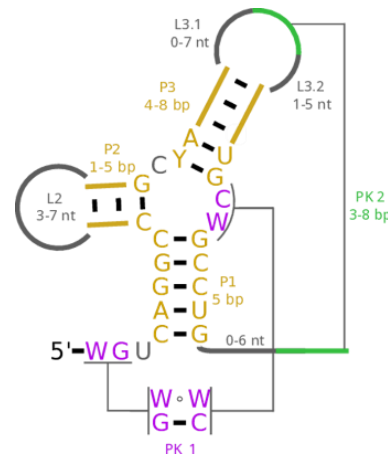
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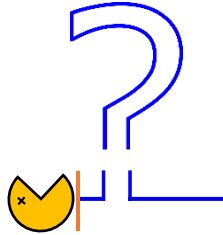
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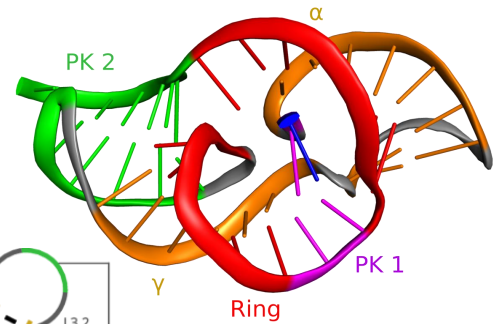
Design Novelty
Design Space

Levels of Design Novelty / Feasibility

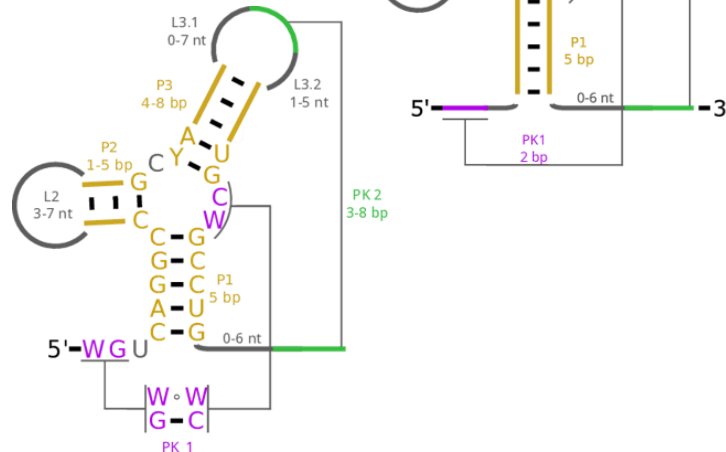
- Function



- 3D Structure / Mechanism



- 2D Structure



- Sequence

Design Novelty
Design Space

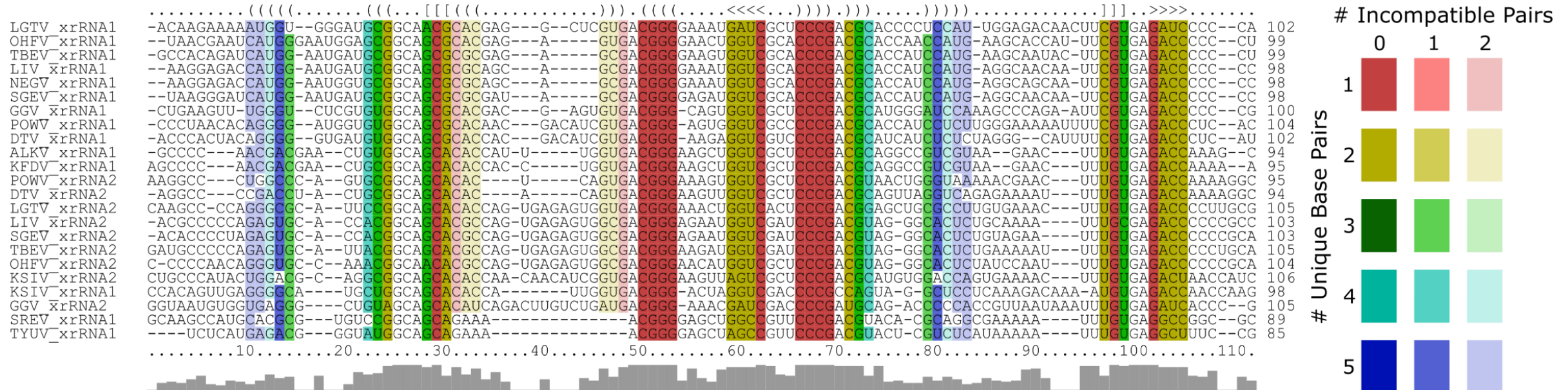
Design Feasibility

Design Pipeline – Analyzing Nature

- Extract information from biological sequences into a design model

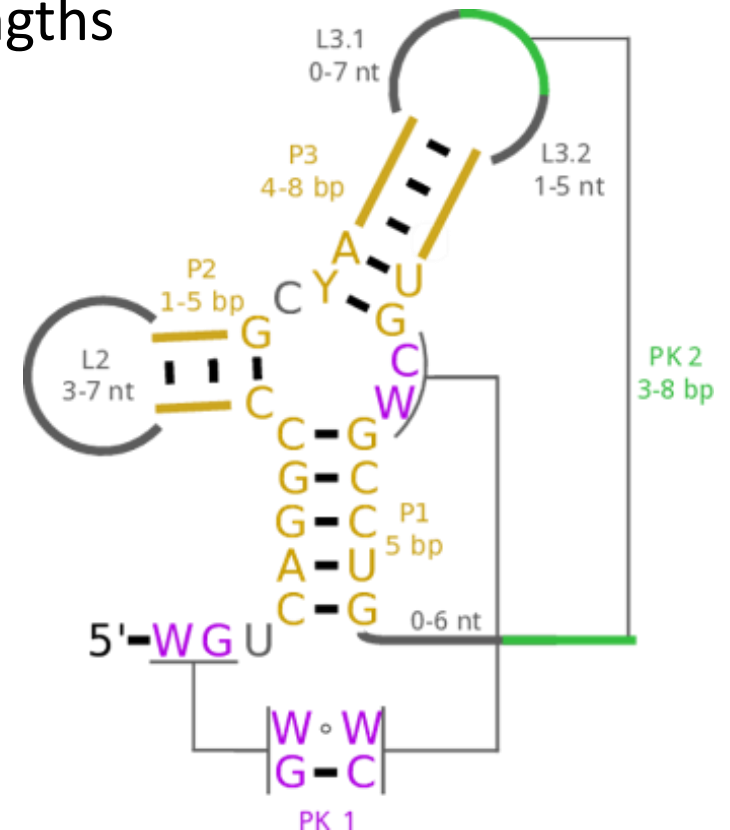
Design Pipeline – Analyzing Nature

- Extract information from biological sequences into a design model
 - Find biological xrRNAs using a covariance model and align results



Design Pipeline – Analyzing Nature

- Extract information from biological sequences into a design model
 - Find biological xrRNAs using a covariance model and align results
 - Analyze sequence conservation and structure lengths



Design Pipeline – 2D Structure



Yao, Hua-Ting, et al (2024)

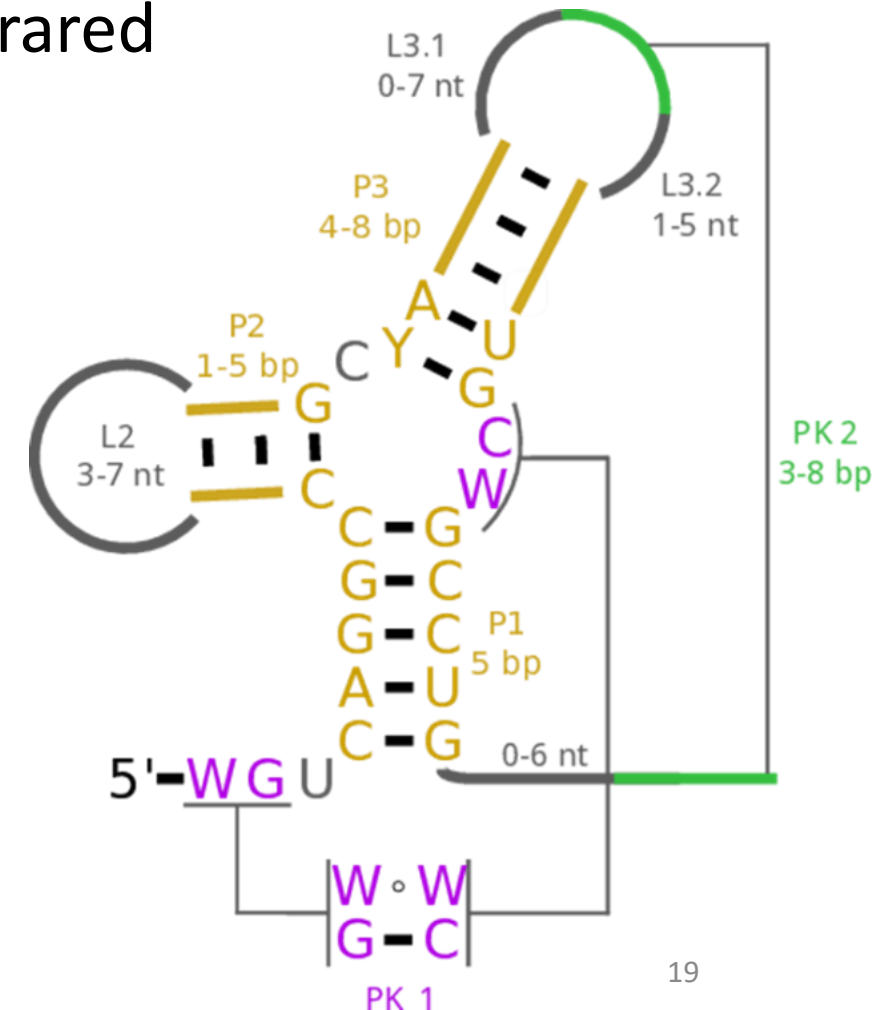
- Design sequences with those features using Infrared

Design Pipeline – 2D Structure



Yao, Hua-Ting, et al (2024)

- Design sequences with those features using Infrared
- Constraints to describe the design model

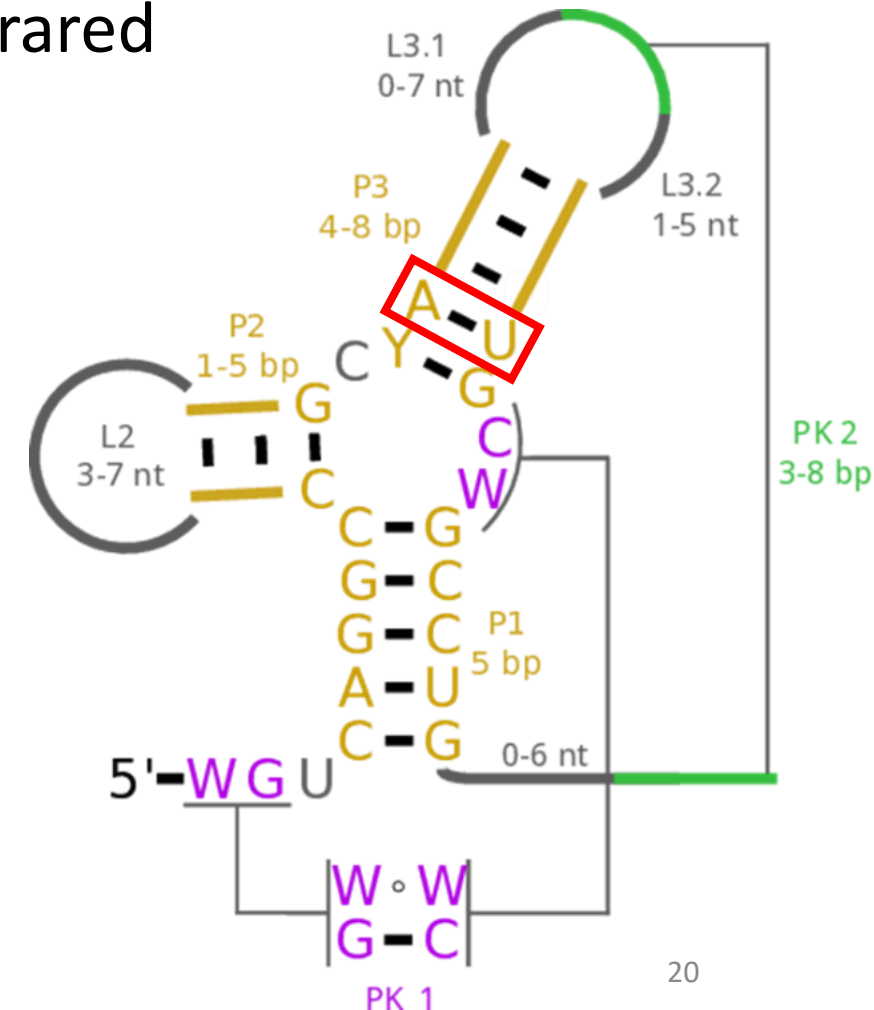


Design Pipeline – 2D Structure



Yao, Hua-Ting, et al (2024)

- Design sequences with those features using Infrared
- Constraints to describe the design model
 - Basepair complementarity

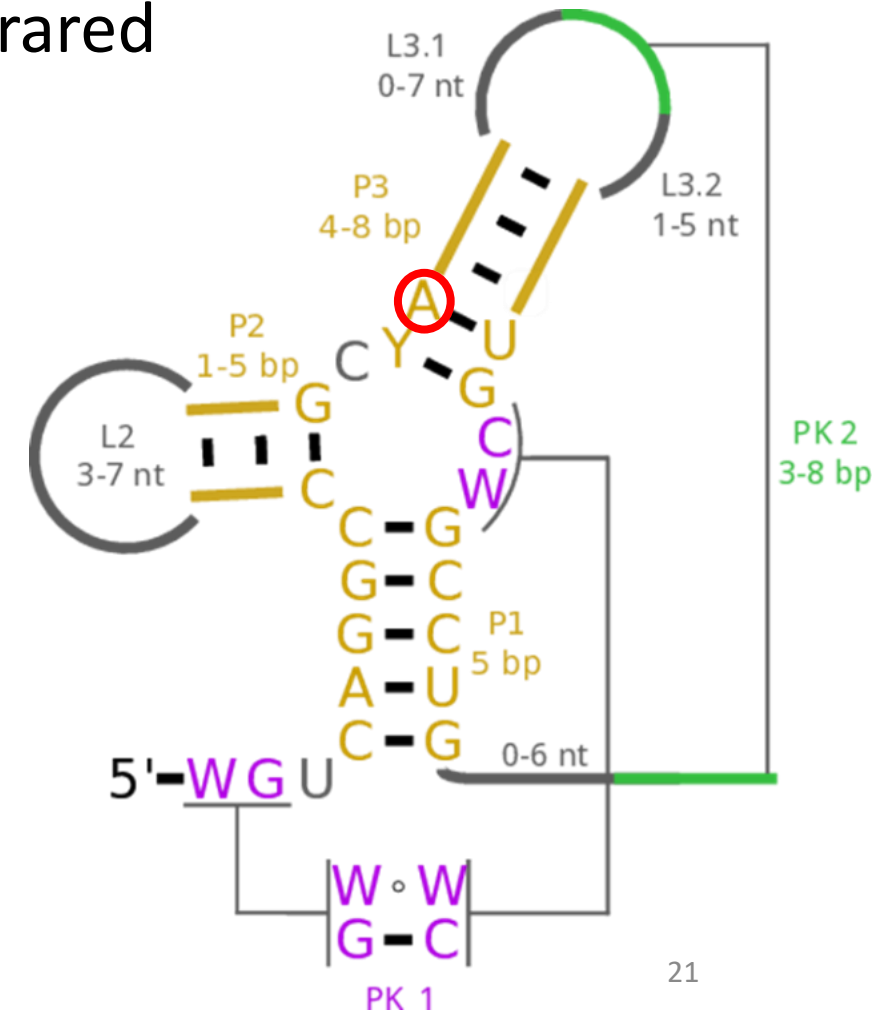


Design Pipeline – 2D Structure



Yao, Hua-Ting, et al (2024)

- Design sequences with those features using Infrared
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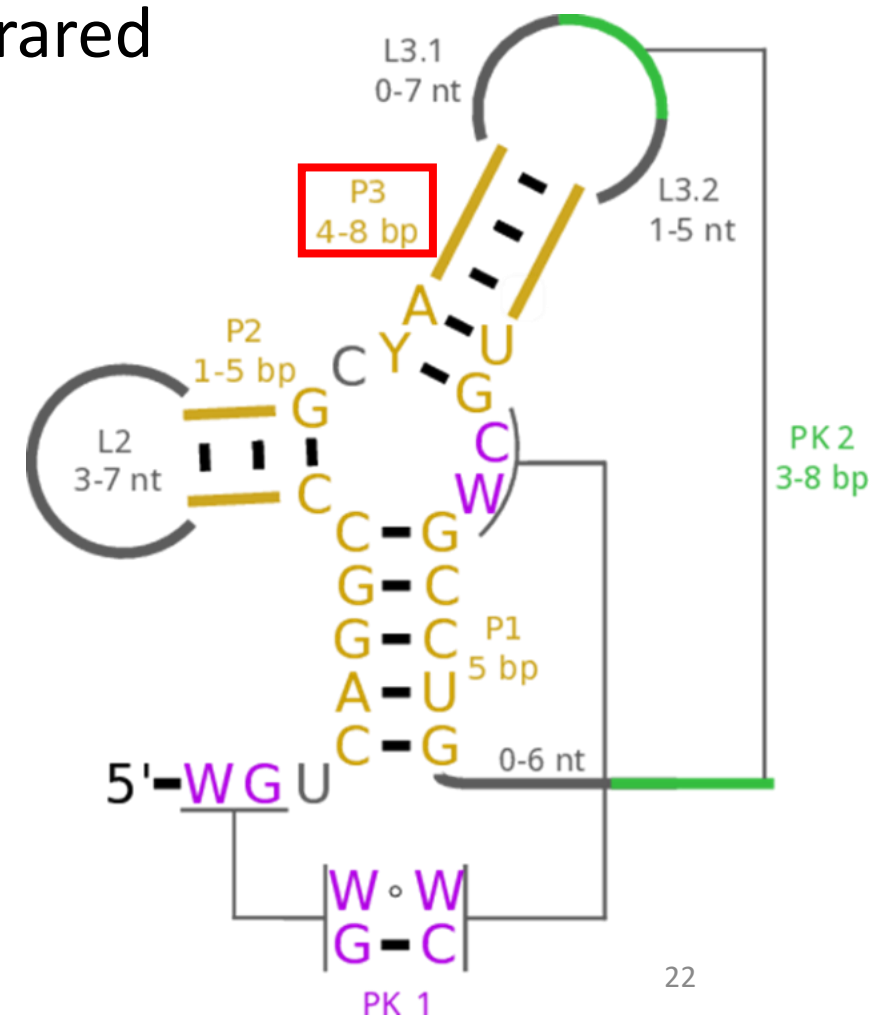




Yao, Hua-Ting, et al (2024)

Design Pipeline – 2D Structure

- Design sequences with those features using Infrared
- Constraints to describe the design model
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 - Feature length and total length

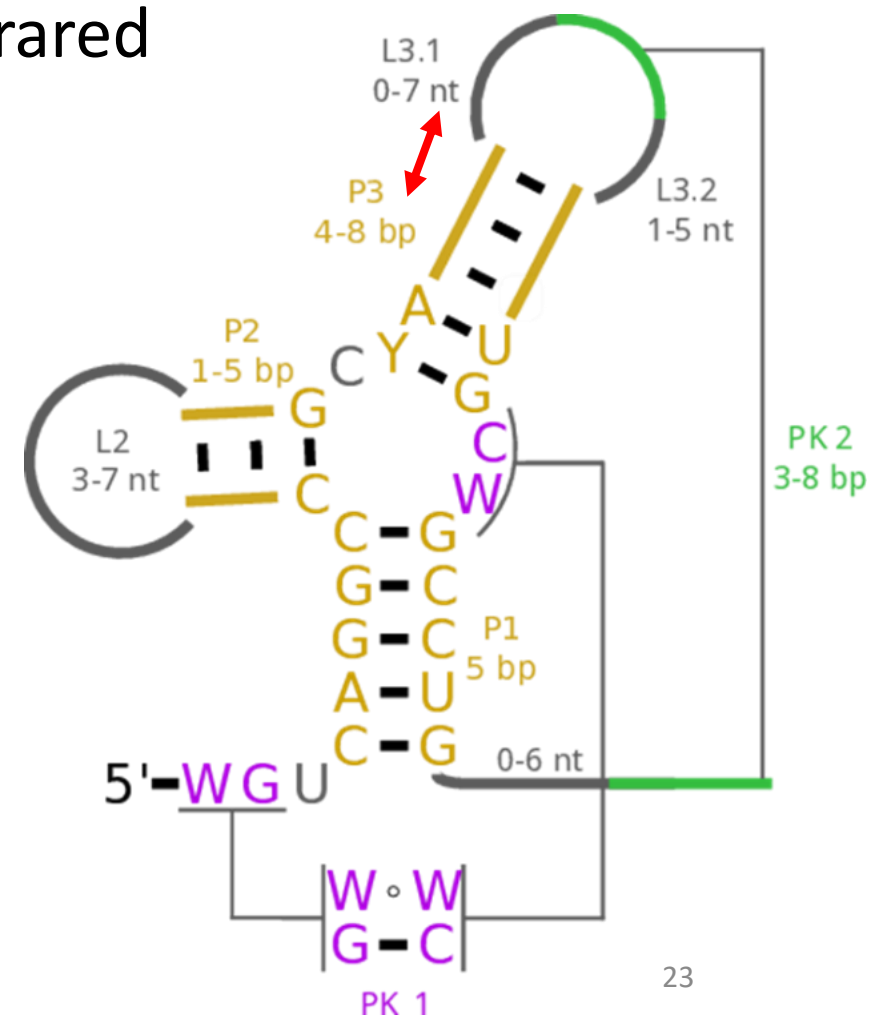




Yao, Hua-Ting, et al (2024)

Design Pipeline – 2D Structure

- Design sequences with those features using Infrared
- Constraints to describe the design model
 - Basepair complementarity
 - Sequence conservation
 - Feature length and total length
 - Length correlation

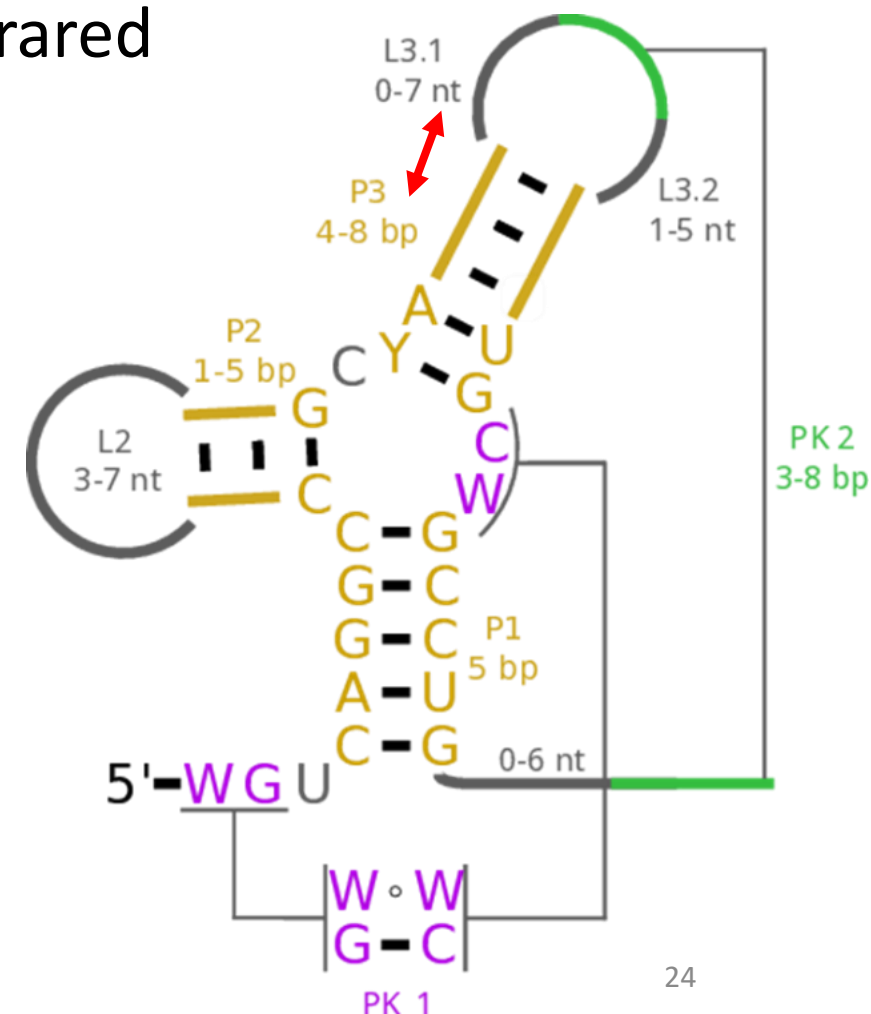




Yao, Hua-Ting, et al (2024)

Design Pipeline – 2D Structure

- Design sequences with those features using Infrared
- Constraints to describe the design model
 - Basepair complementarity
 - Sequence conservation
 - Feature length and total length
 - Length correlation
 - Design sequences can vary in length

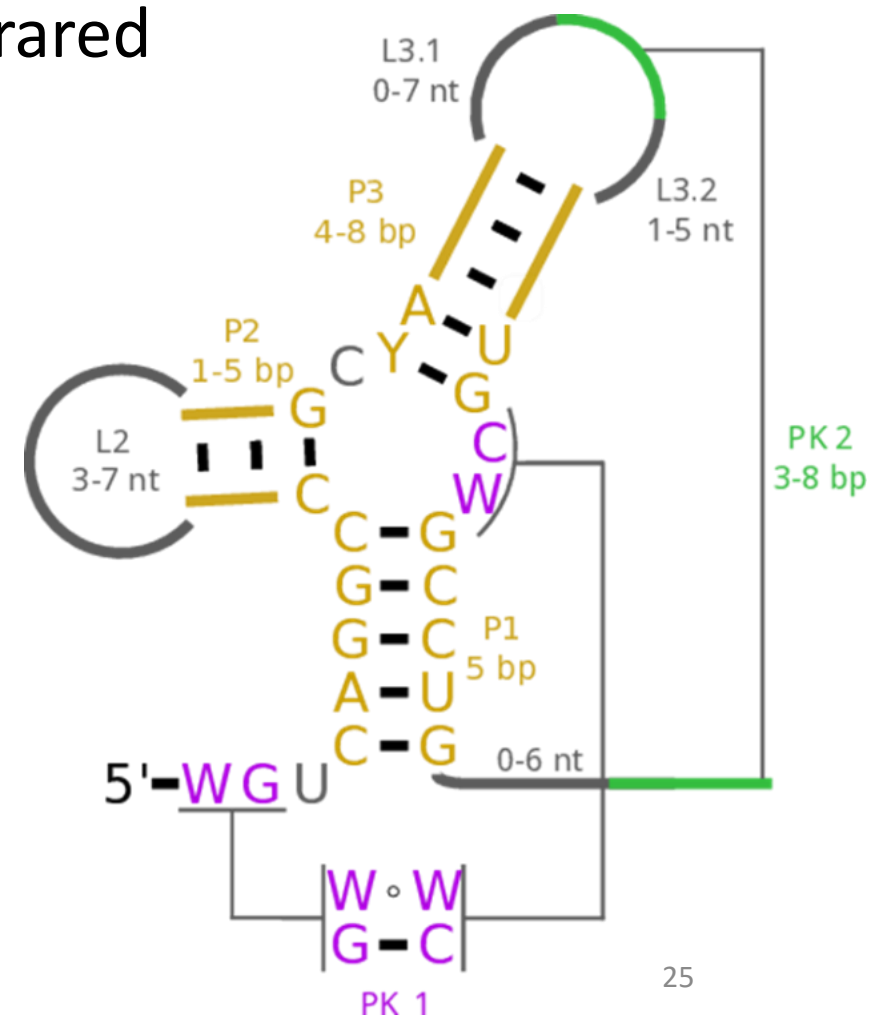




Design Pipeline – 2D Structure

Yao, Hua-Ting, et al (2024)

- Design sequences with those features using Infrared
- Constraints to describe the design model
- Sample sequences that fulfill the design model

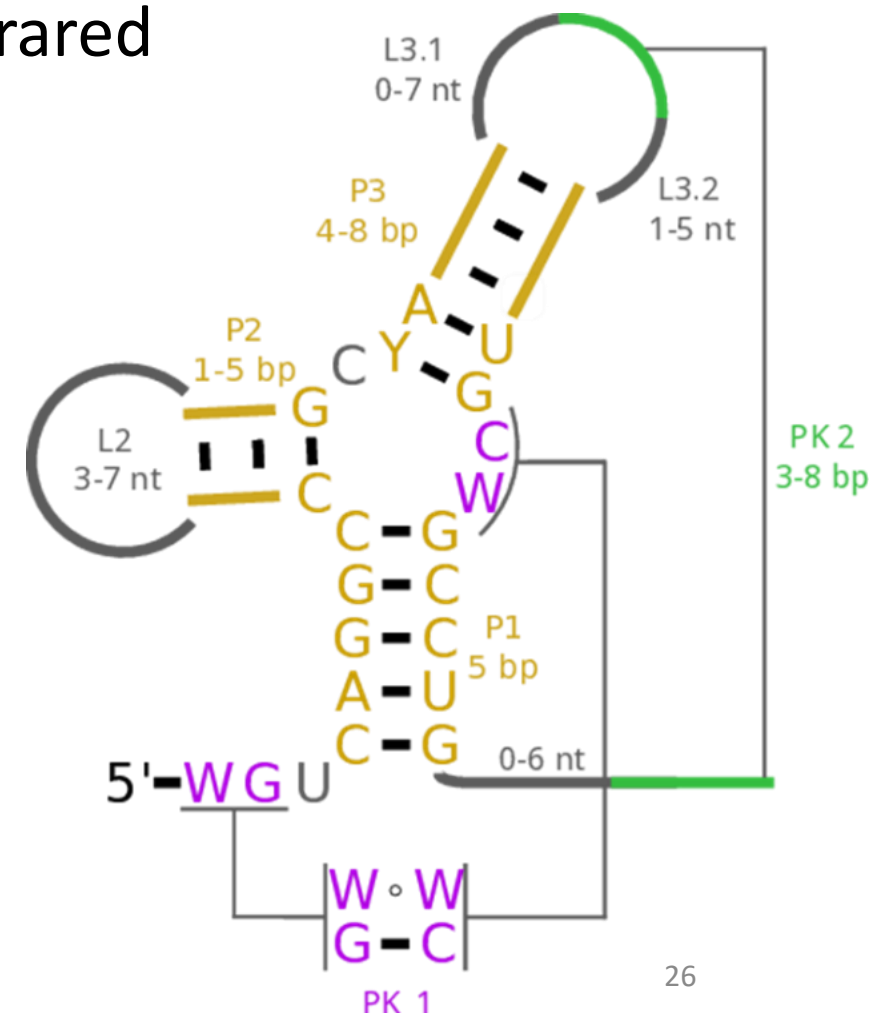
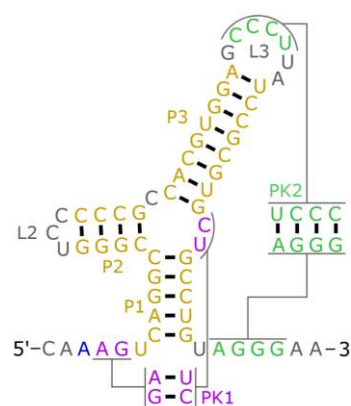
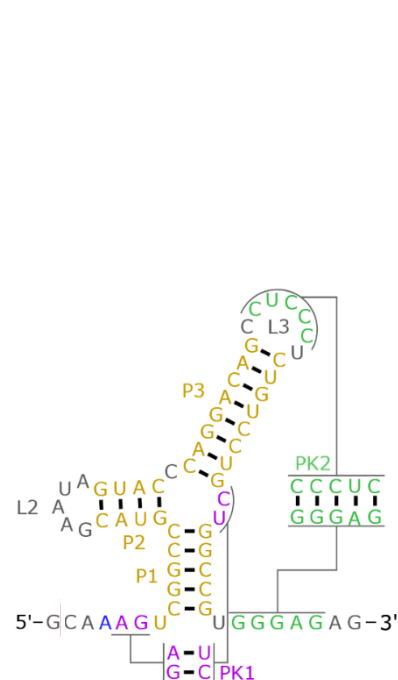




Yao, Hua-Ting, et al (2024)

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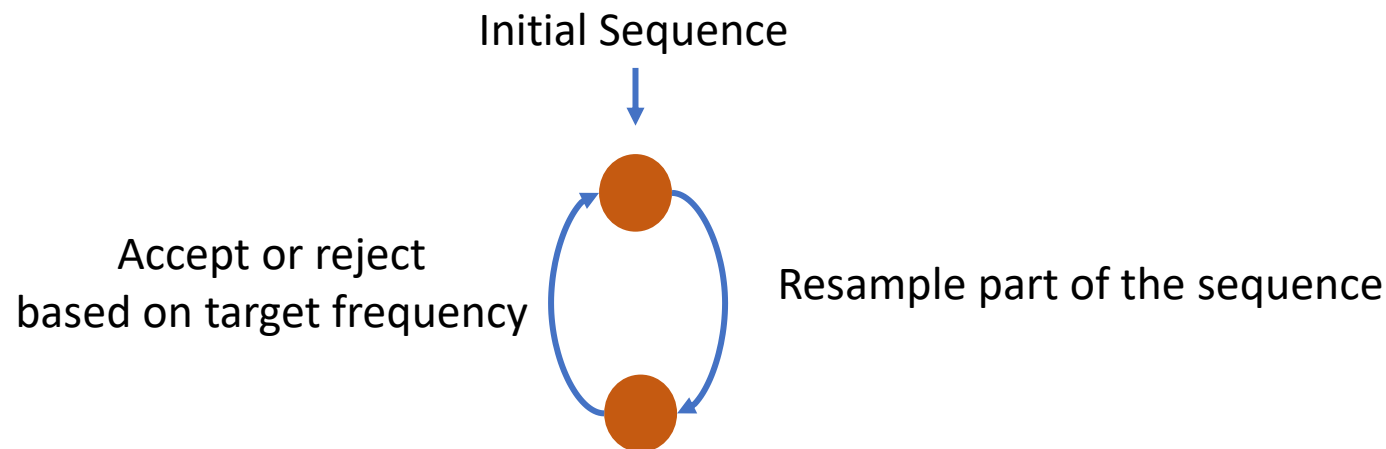




Yao, Hua-Ting, et al (2024)

Design Pipeline – 2D Structure

- Design sequences with those features using Infrared
- Constraints to describe the design model
- Sample sequences that fulfill the design model
- Optimize the target frequency using a Monte-Carlo Strategy



Design Pipeline – 3D Structure / Mechanism

- Validate 3D structure

Design Pipeline – 3D Structure / Mechanism

- Validate 3D structure
 - Structure prediction using SimRNA

SimRNA

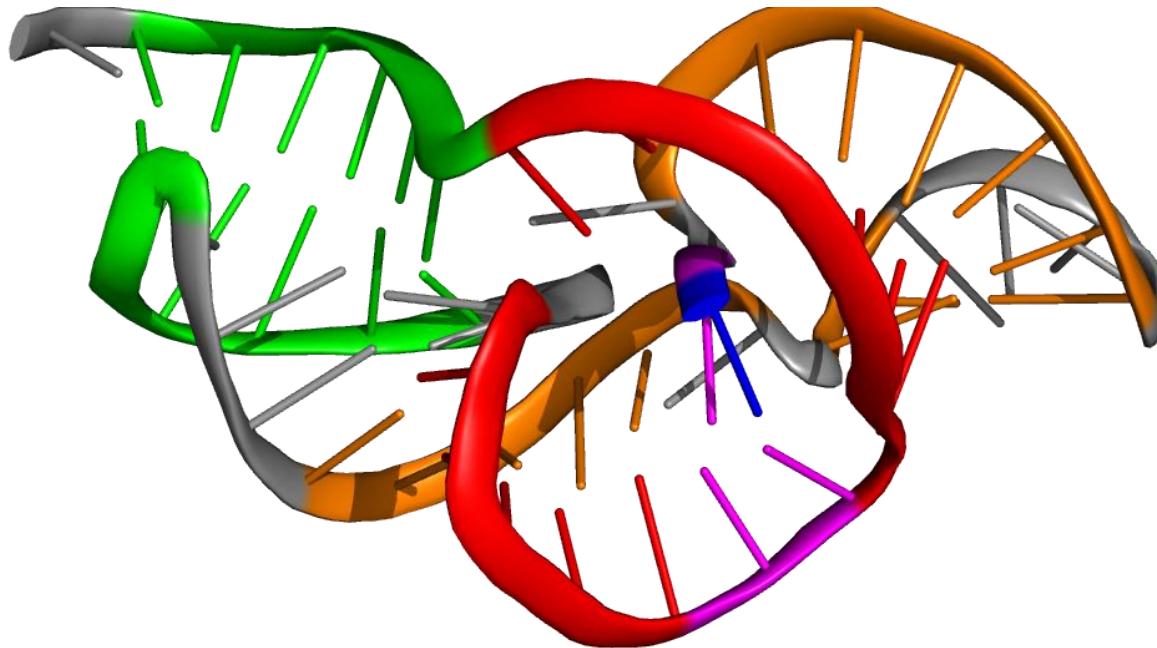
Boniecki, Michal J. et. al (2016)

Design Pipeline – 3D Structure / Mechanism

- Validate 3D structure
 - Structure prediction using SimRNA
 - Check for a ring and compare to wild-type

SimRNA

Boniewicz, Michal J. et. al (2016)

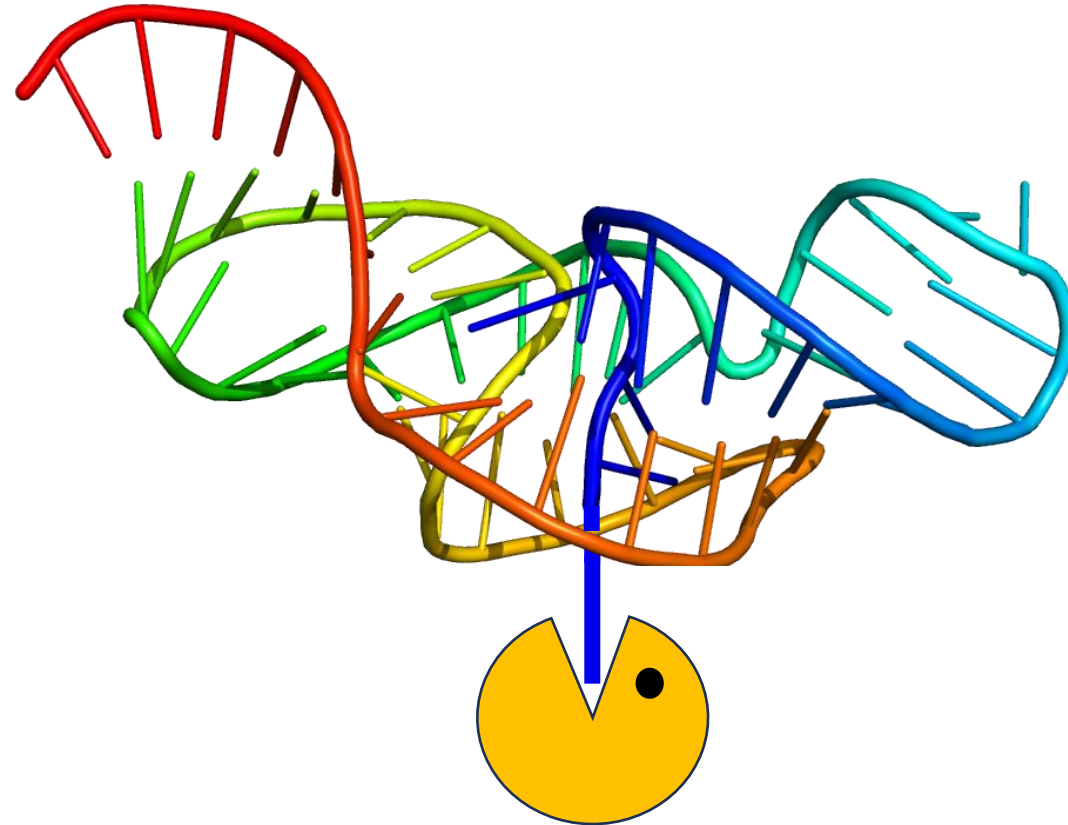


Design Pipeline – *In Silico* Function Validation

- Test function

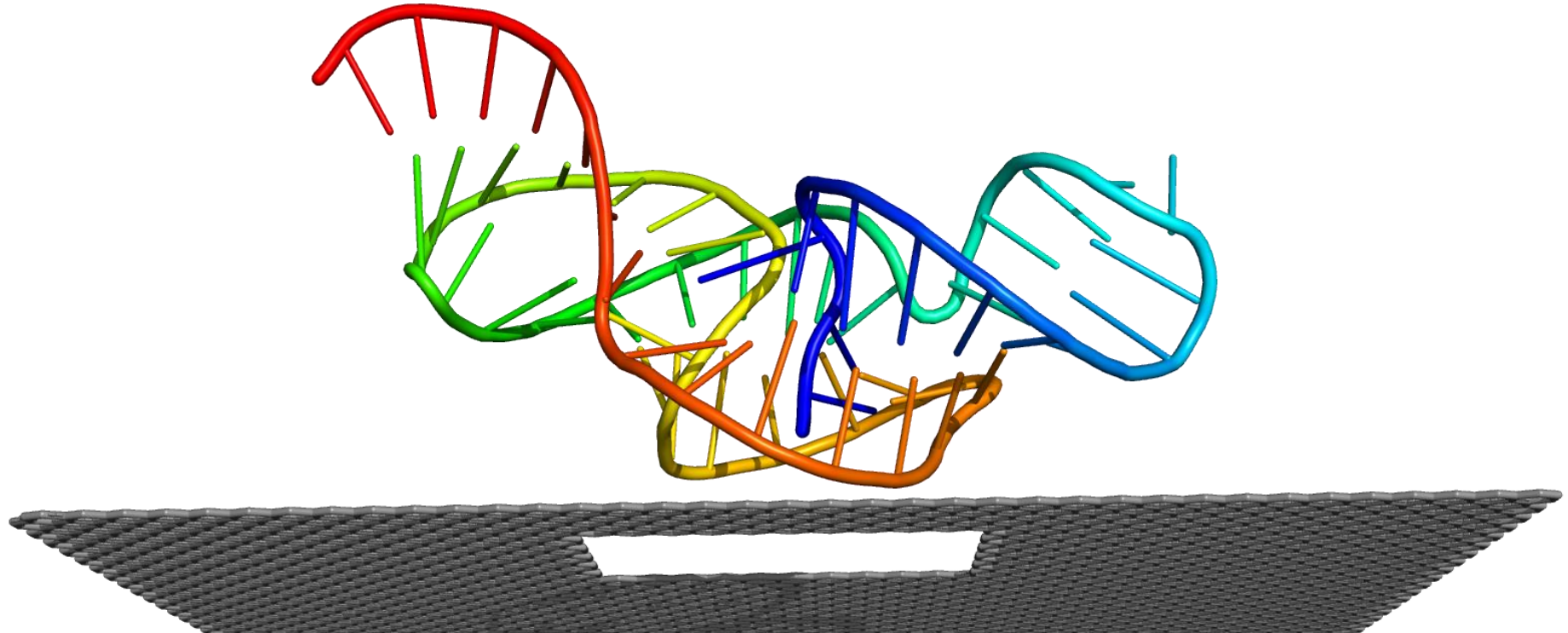
Design Pipeline – *In Silico* Function Validation

- Test function
 - Determine unfolding force using MD



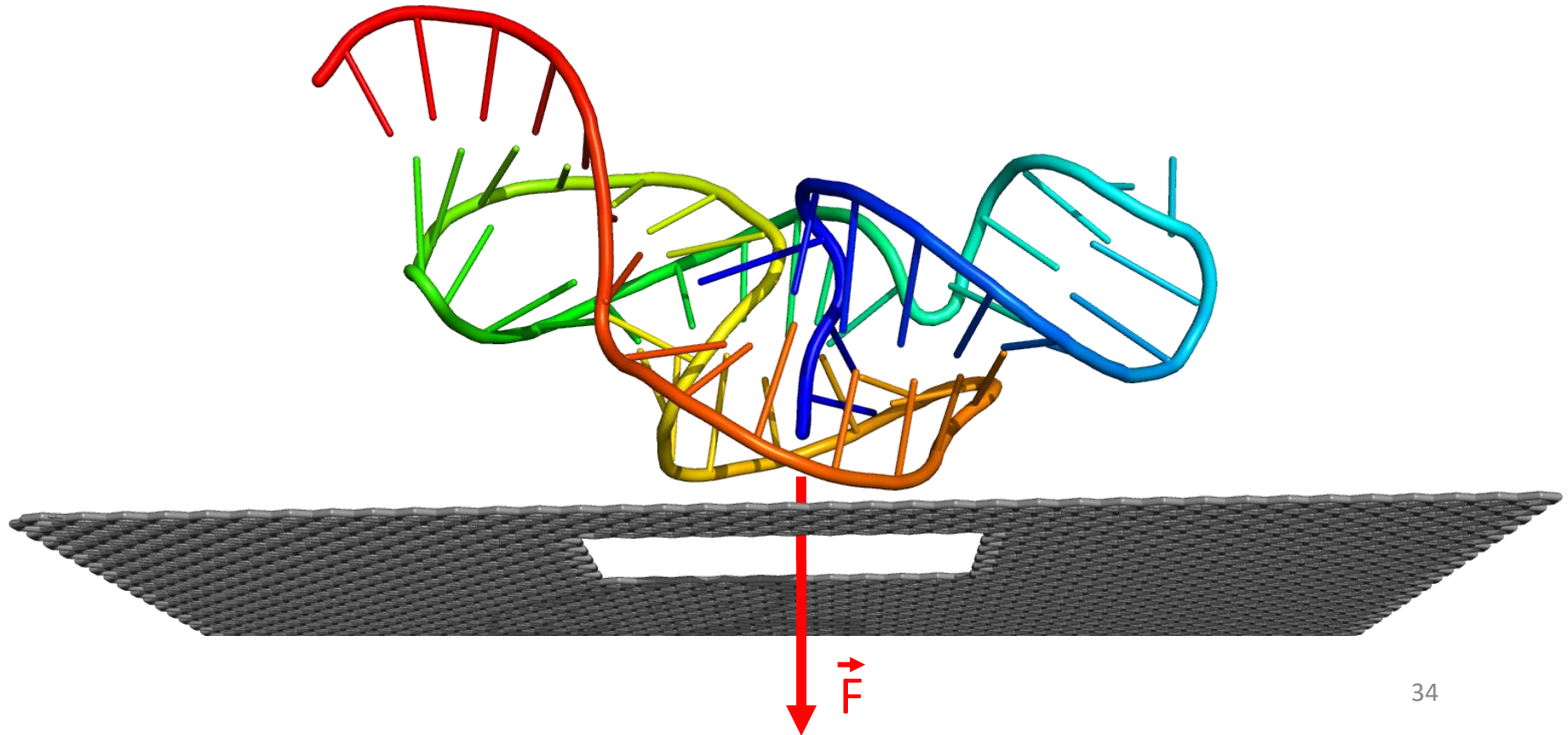
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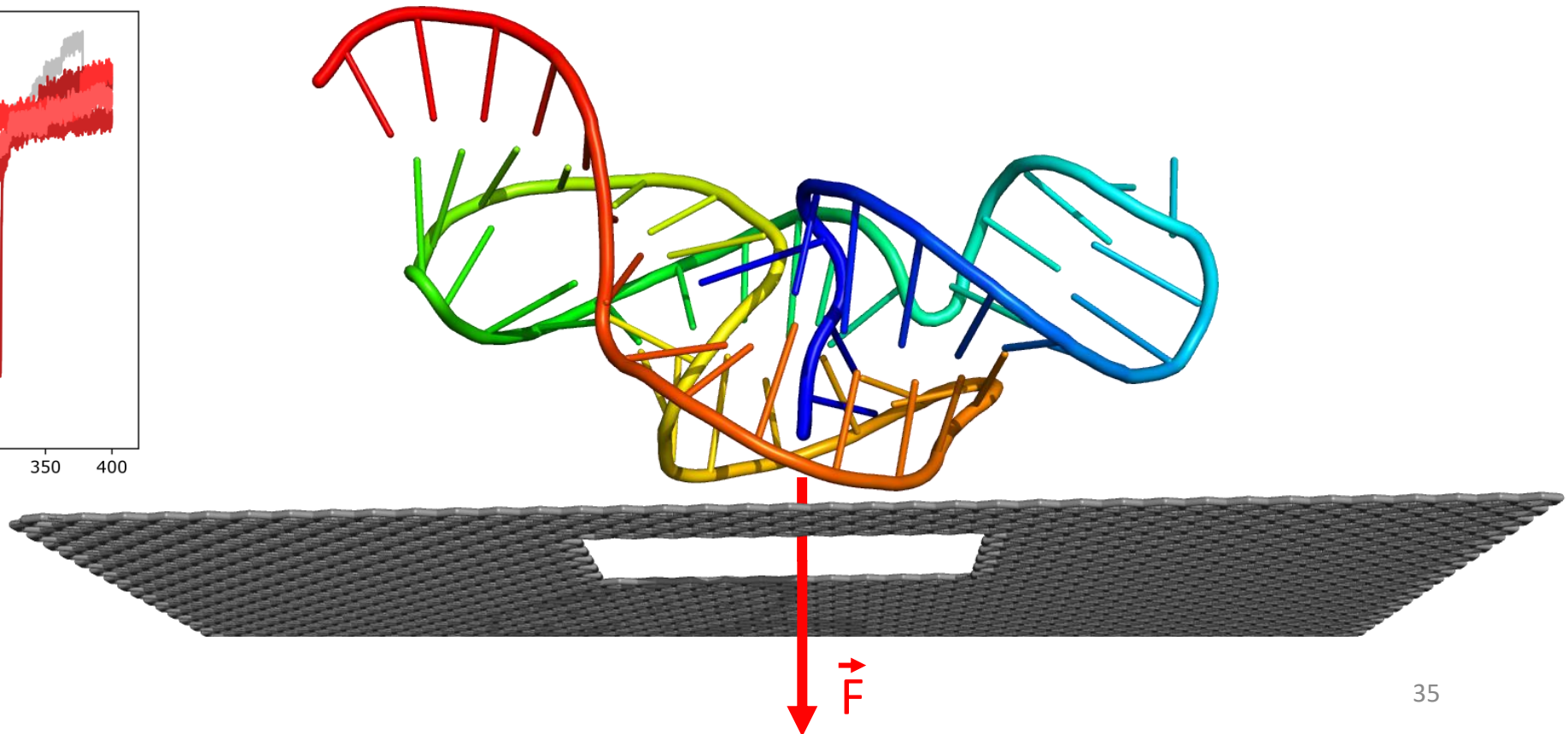
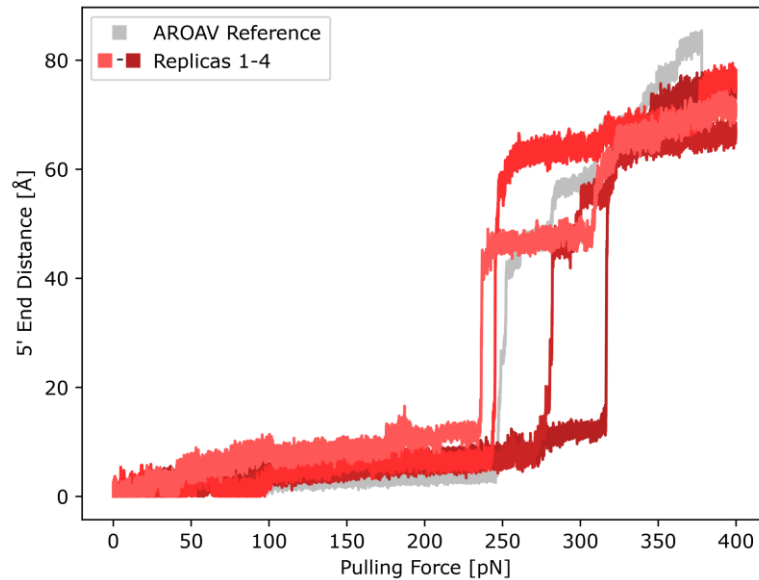
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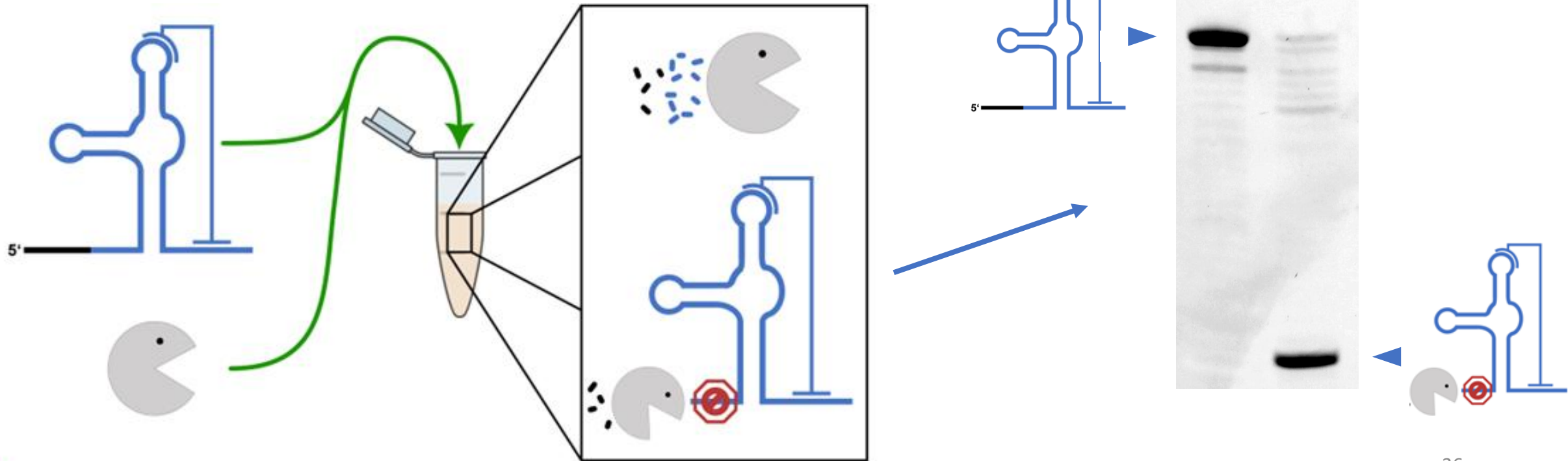
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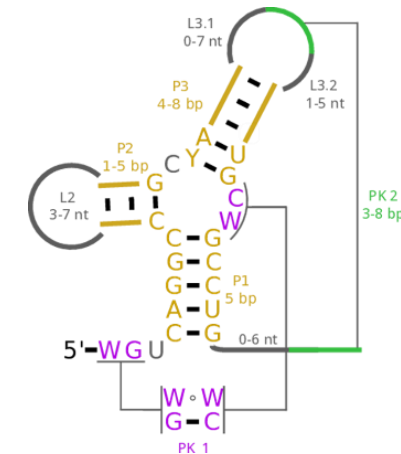
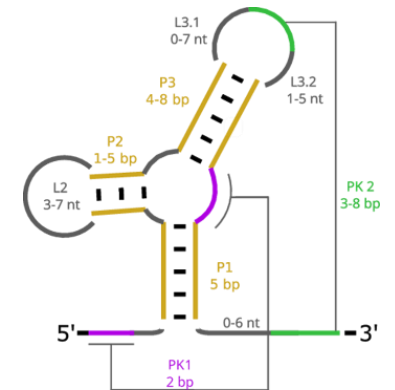
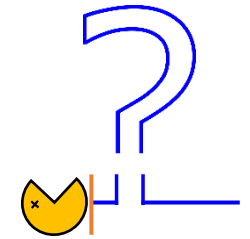
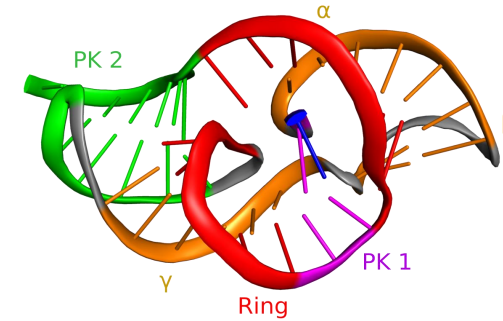
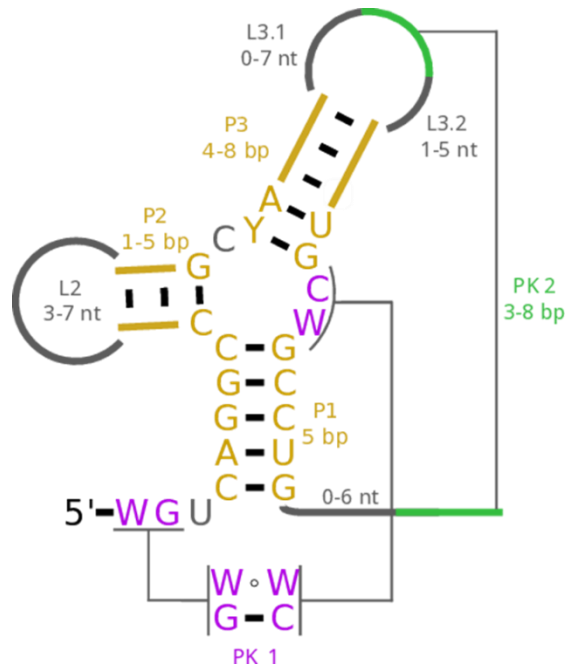
Design Pipeline – *In Vitro* Function Validation

- Test function
 - Determine unfolding force using MD
 - Experimental characterization



Rational xrRNA Designs

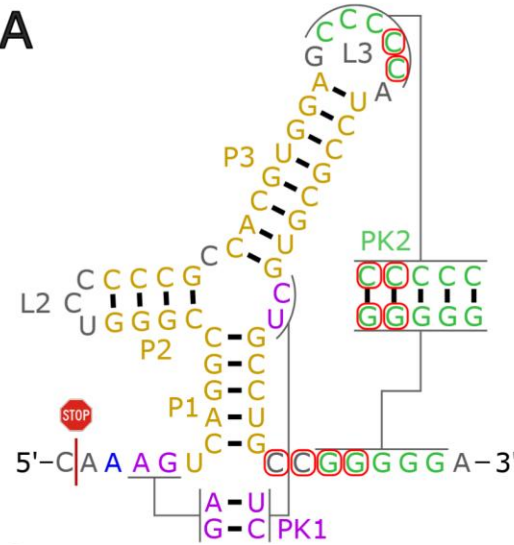
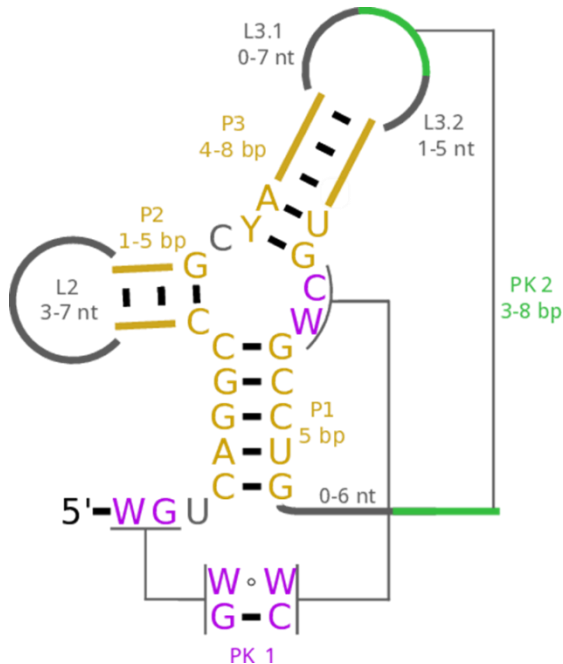
- First Design
 - High sequence similarity



Rational xrRNA Designs

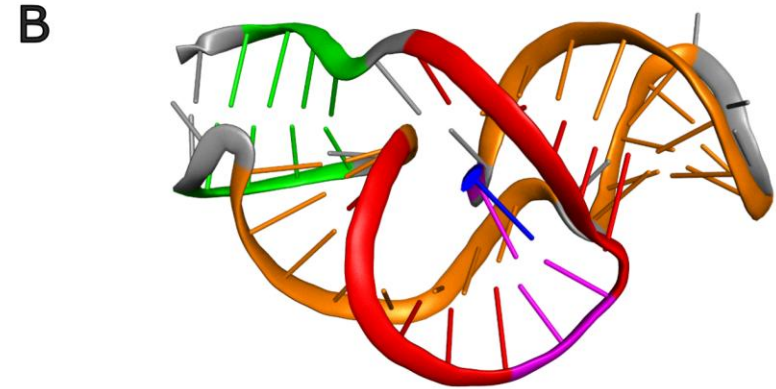
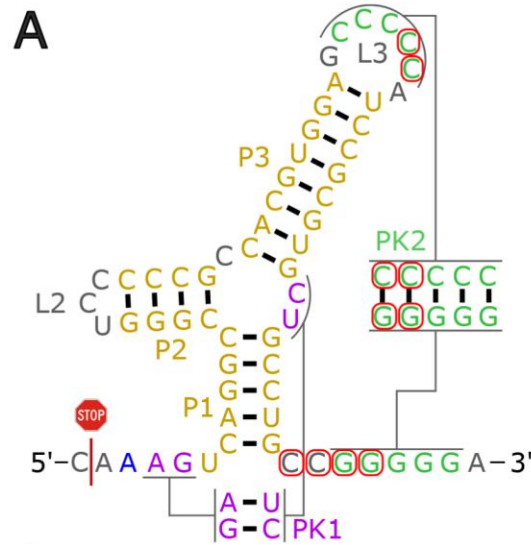
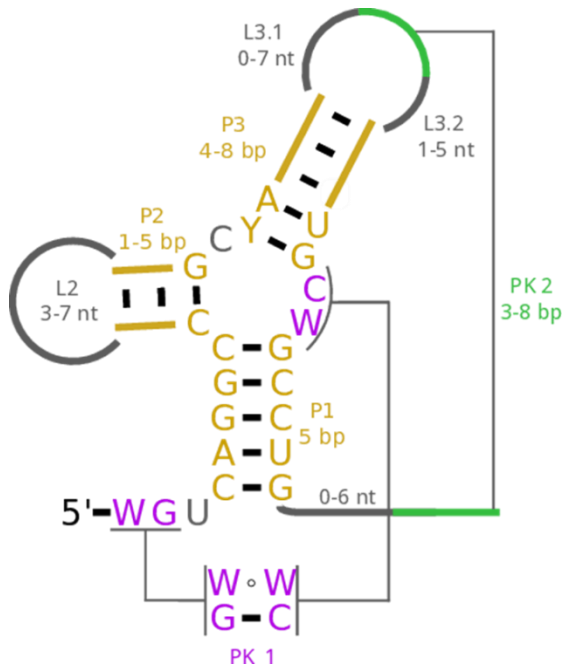
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- First Design
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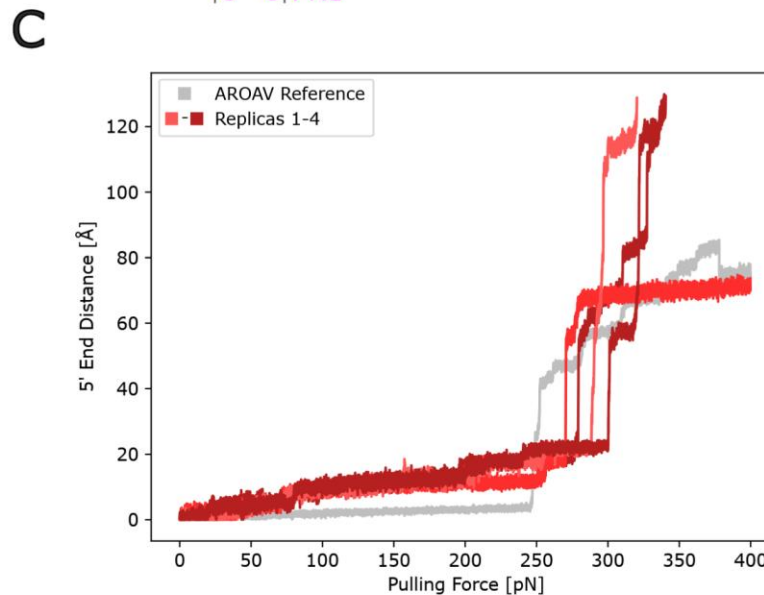
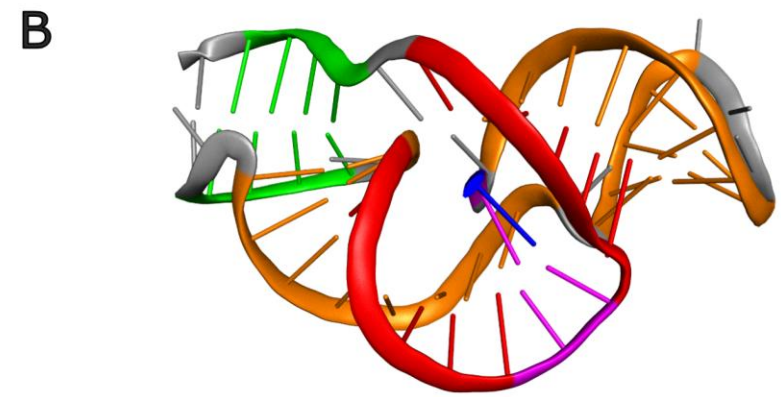
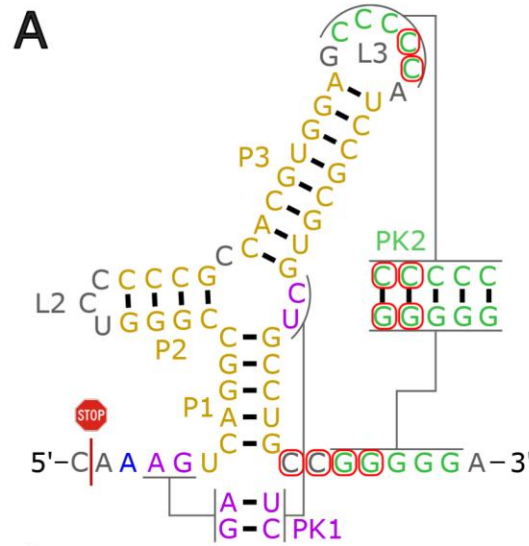
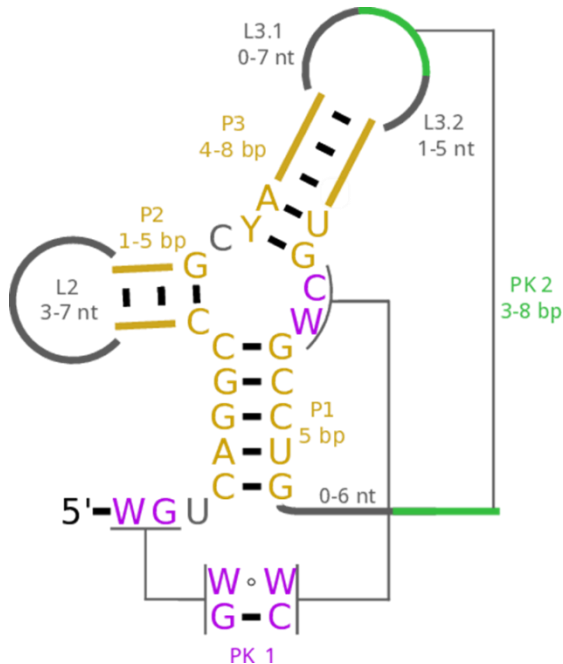
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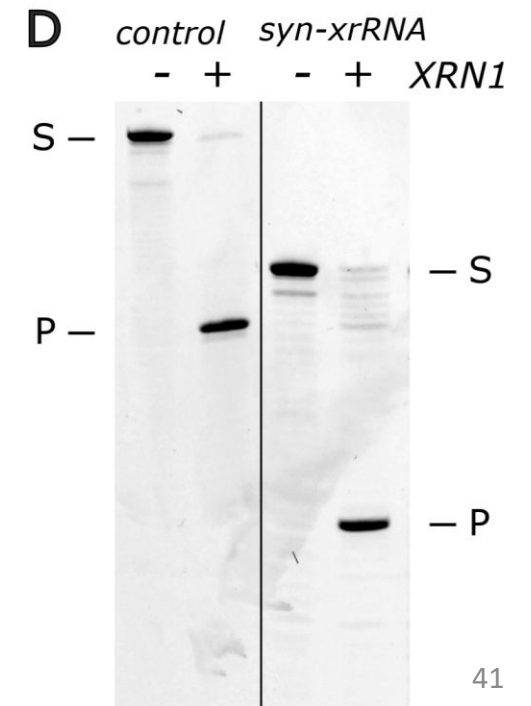
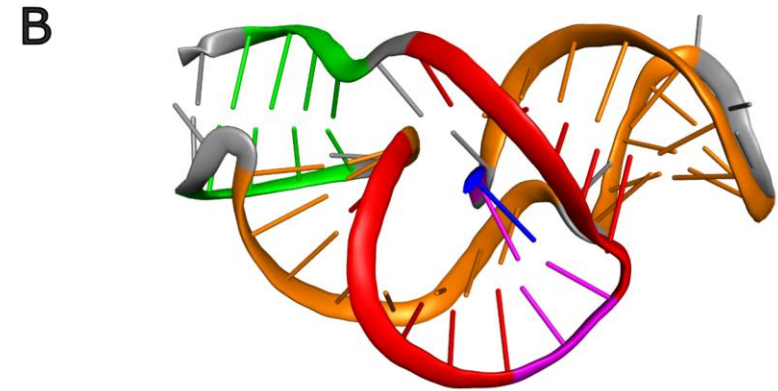
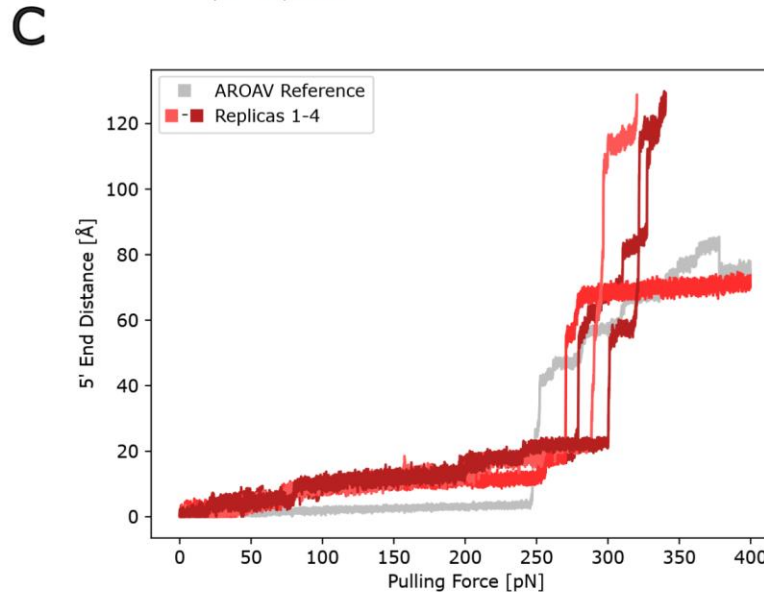
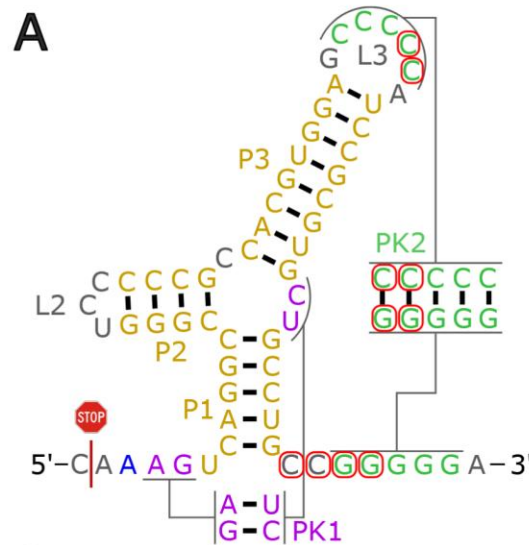
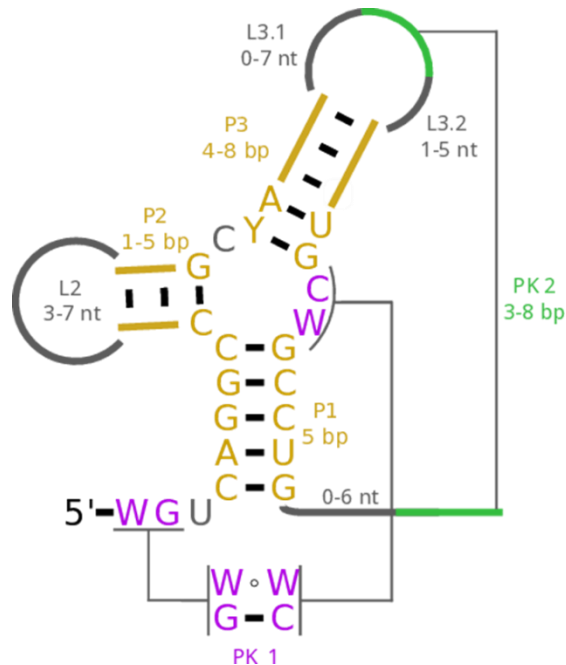
Rational xrRNA Designs

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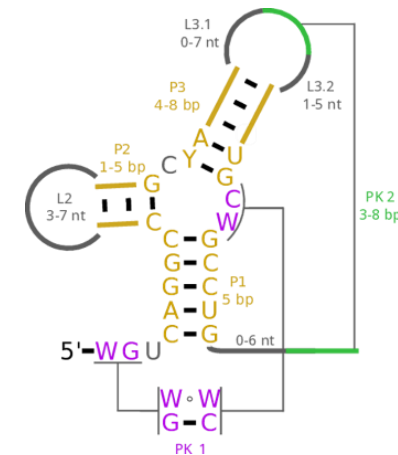
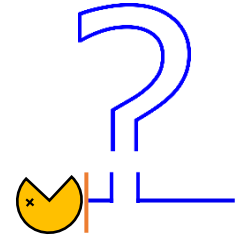
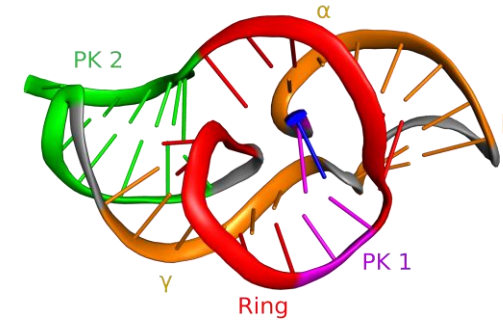
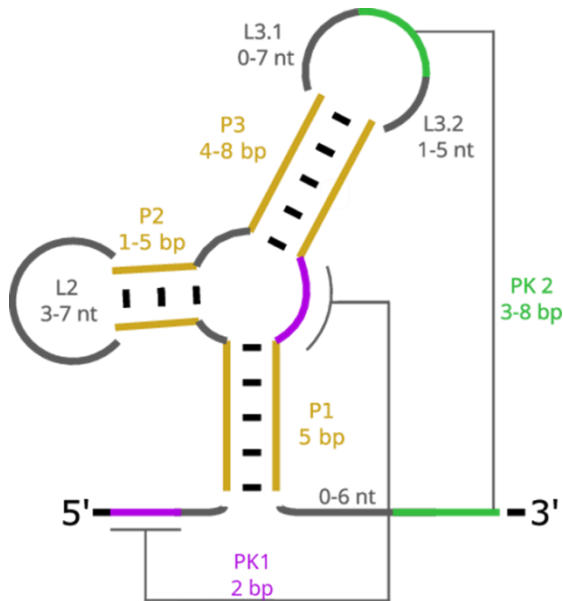
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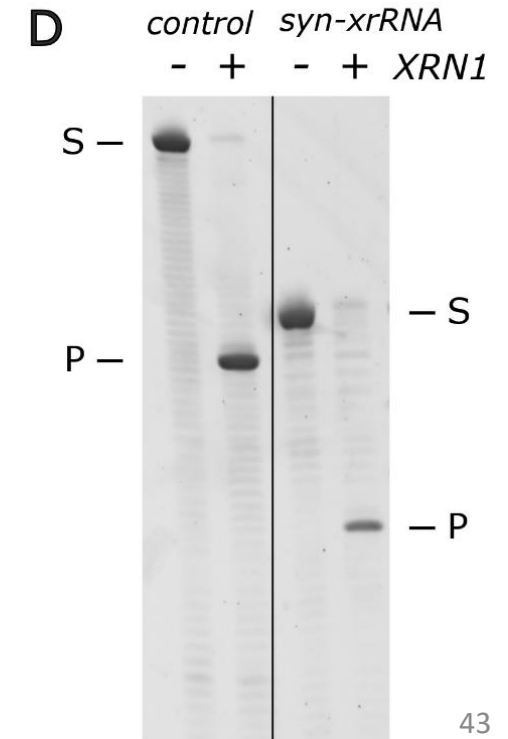
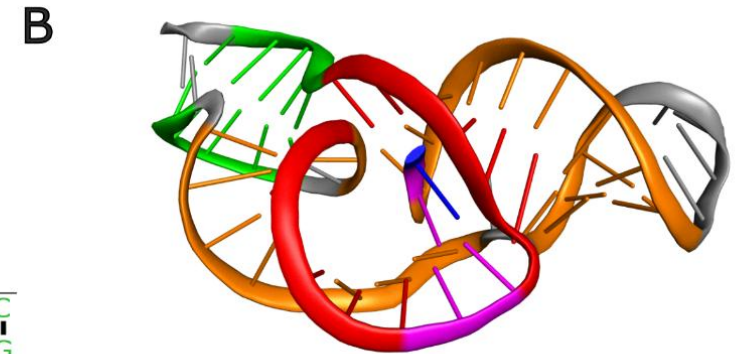
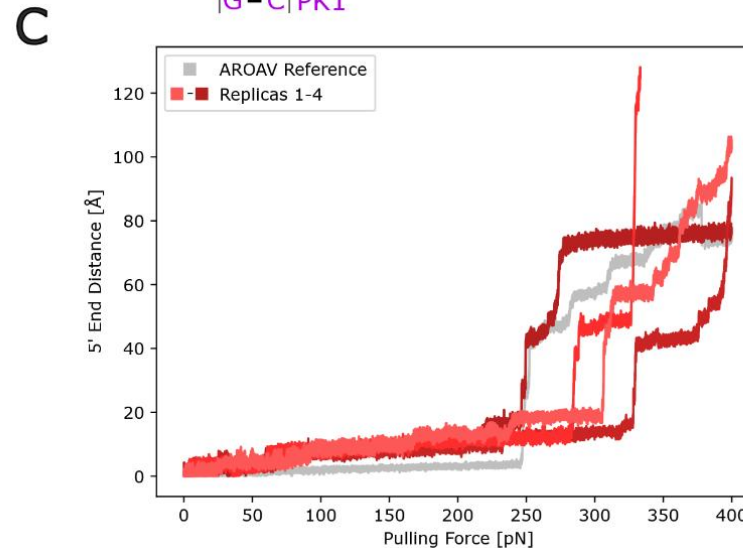
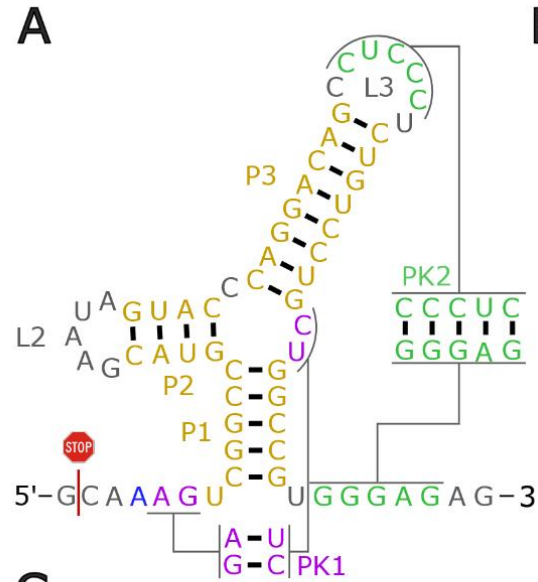
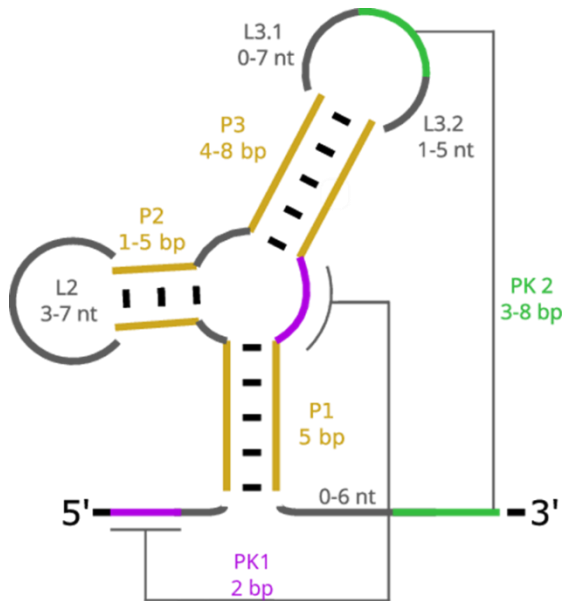
Rational xrRNA Designs

- First Design
 - High sequence similarity
- Second Design:
 - Mainly structure similarity

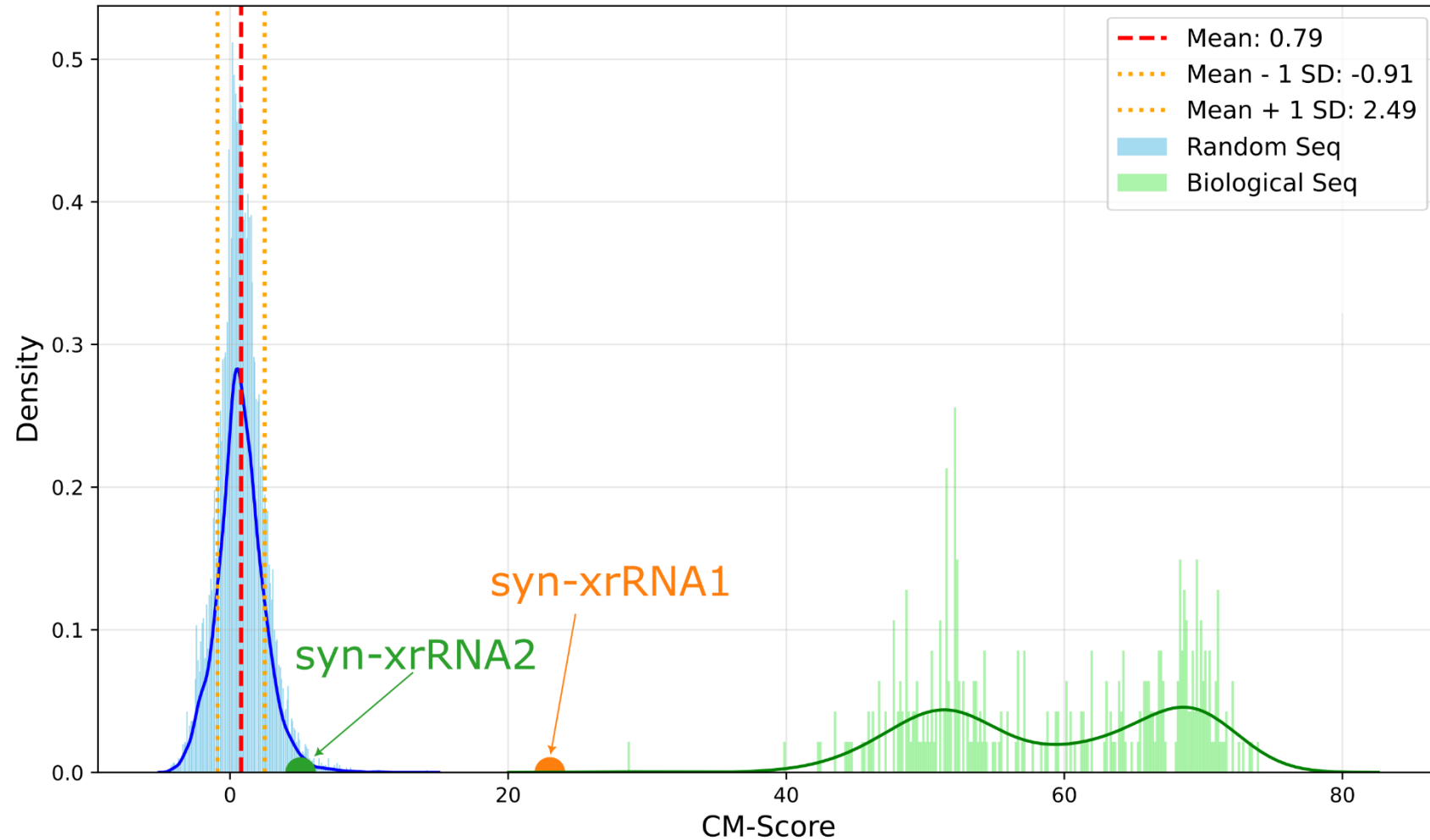


Rational xrRNA Designs

- First Design
 - High sequence similarity
- Second Design:
 - Mainly structure similarity



Novelty of xrRNA Designs



Sequences that only fulfill
basepair-complementarity

Biological sequences

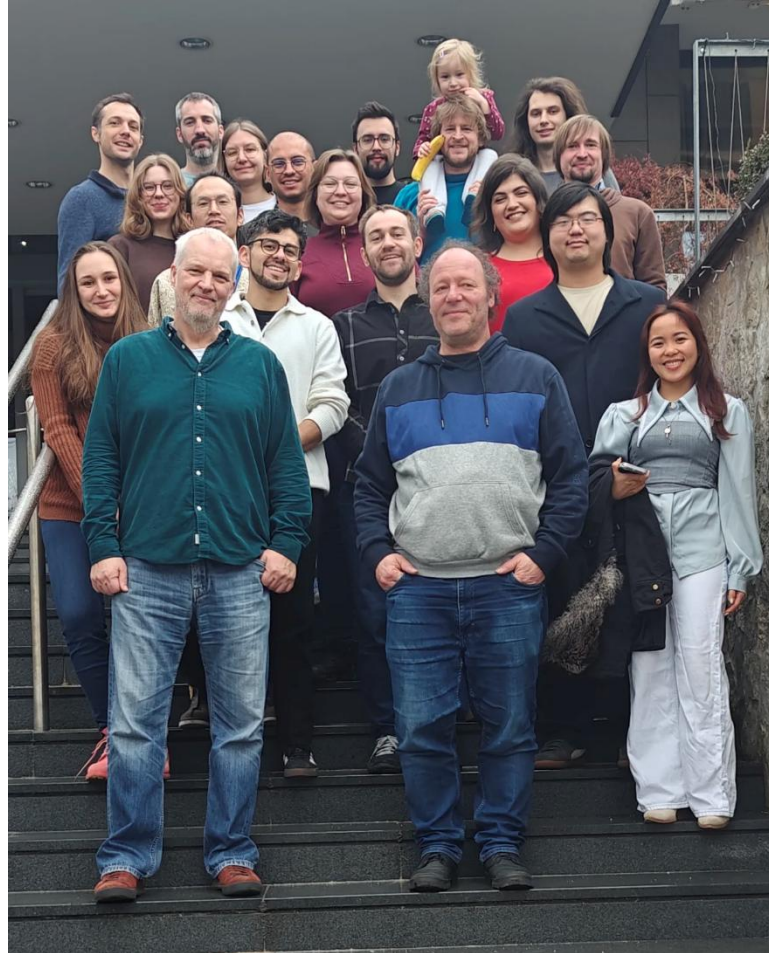
Conclusion

- Rational and novel design of RNA is possible
- Function can be decoupled from evolutionary sequence ancestry
- Potential improvements through more details such as 2.5D motifs

Acknowledgments

Ivo L. Hofacker
Michael T. Wolfinger
Hua-Ting Yao
Denis Skibinski
Katrin Gutenbrunner

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Mario Mörl
Jule Walter
Tim Kolberg



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Thank you for your Attention