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TITLE OF YOUR THESIS OR DISSERTATION

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Chapel Hill

20XX

Approved by:

Supervisor

Committee chair

Committee member 1

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ABSTRACT

[ Author’s Name ]: [ Title of Thesis/Dissertation ]

(Under the direction of [ Advisor’s Name ])

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I dedicate this work to my mentor and friend. I couldn’t have done this without you.

Thank you for all your support along the way.

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ACKNOWLEDGEMENTS

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Disclosure

An optional disclosure statement of any conflicts of interest.

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preface

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# LIST OF ABBREVIATIONS AND SYMBOLS

|  |  |
| --- | --- |
| °C | degrees Celsius |
| 13C | NMR carbon-13 nuclear magnetic resonance |
| 1H | NMR proton nuclear magnetic resonance |
| Å | angstrom |
| DCM | dichloromethane |
| dd | doublet of doublets |
| EtOAc | ethyl acetate |
| FMN | flavin mononucleotide |
| HRMS | high resolution mass spectrometry |
| Hz | Hertz |
| kcal | kilocalorie |
| m/z | mass to charge ratio |
| Methanol-d4 | deuterated methanol |
| mg | milligram |
| MHz | megahertz |
| NMR | nuclear magnetic resonance |
| SAM | S-Adenosyl methionine |
| SASA | solvent accessible surface area |
| TLC | thin layer chromatography |
| Tris | tris(hydroxymethyl)aminomethane |
| δ | chemical shift |
| μg | microgram |
| μL | microliter |
| μM | micromolar |
| π-π | pi-pi interaction |

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# : PRINCIPLES OF RNA THERAPEUTICS

## RNA centrality

RNA is the primary conduit of genetic information transfer in all cells. Messenger RNAs (mRNAs) are essential intermediaries between DNA and proteins. RNAs also serve as multifaceted information carriers, regulators, and catalysts for nearly all cellular functions (1, 2). Strikingly, approximately 70% of the human genome is transcribed into non-coding RNAs (ncRNAs) (3). ncRNAs are not translated into proteins but can themselves catalyze biochemical reactions (4), silence chromosomes (5, 6), and fine-tune gene expression (7, 8). Critically, RNA operates upstream of nearly all cellular processes. No other macromolecules cover such a vast scope of biological functions, making RNA a unique and useful target for modulating biology (9).

## RNA structure and pockets

RNA is a biopolymer made of ribonucleic acid building blocks, almost identical to the deoxyribonucleic acid building blocks of DNA. The sequence of nucleic acids (A, U, G, C) is what allows RNA (and DNA) to carry genetic information (Figure 1.1**A**). In a cellular context, DNA is persistently accompanied by a complementary DNA molecule, largely limiting DNA to form simple and predictable double-helix structures via intermolecular Watson-Crick base pairs (10). RNA molecules are not typically accompanied by complementary pairs (or are “single-stranded”), allowing RNA to form intramolecular interactions that create diverse secondary structures. RNA secondary structures are formed by intramolecular interactions, including base-stacking, Watson-Crick base pairing, and prevalent “non-canonical” base pairing that pair nucleotides far away in the RNA sequence (FIG. 1A) (11). The secondary structure motifs discussed in this work include bulges, loops, consecutive bulges/loops, multi-helix junctions, and pseudoknots (Figure 1.1**B**). The base stacking patterns at junctions of unpaired nucleotides, non-canonical interactions, and long-range base pairing can further transform RNA secondary into an even more complex three-dimensional tertiary structure (12). Finally, RNAs can interact



**Figure 1.1. RNA structure**

(A) is comprised of a primary structure (sequence). An RNA can form intermolecular base pairs to bring nucleotides that are far away in sequence near to one another in secondary structure. The combination of all the intermolecular interactions in an RNA molecule can lead to a complex three-dimensional tertiary structure capable of forming cavities or pockets that can interact with small-molecule ligands such as S-adenosyl-L-methionine (SAM). (B) Common RNA secondary structural motifs and their abbreviations used in this work sorted by their complexity (information content).

with other RNAs and proteins to form quaternary complexes, often referred to as ribonucleoproteins (RNPs). The structural and catalytic properties of RNA are often central to the function of RNP complexes, as demonstrated in the ribosome, spliceosome, and telomerase (13, 14). All levels of RNA structure contribute to its versatile functionality and enable essential roles in storing genetic information, catalysis, ligand sensing, and macromolecular assembly.

## What to do when you adapt a figure from another author

Use a footnote to acknowledge figures that have been adapted from earlier works such as reviews or seminal papers. See the footnote below (Figure 1.2**A**) an(Figure 1.2**B** for an example of what this looks like.

A close-up of several images of a protein

AI-generated content may be incorrect.

**Figure 1.2. Protein and RNA bound to flavin mononucleotide (FMN)**[[1]](#footnote-2)**.**

(A) Riboflavin kinase protein (PDB 1nb9) and (B) FMN riboswitch (PDB 3f2q) can both form selective interactions with the FMN ligand. In both macromolecules, FMN has a similar pose and modes of interaction (H-bonding, stacking, and Van der Waals).

## Research overview

This section provides a high-level summary of the dissertation’s main research goals, innovations, and structure. It should describe the central question or problem being addressed, outline the key contributions or approaches used, and briefly summarize the content and purpose of each major chapter. The goal is to give readers a roadmap of the dissertation and establish the context and significance of the research.

## Perspective

This section reflects on the broader impact and implications of the research. It may discuss how the work advances the field, addresses a critical gap, or opens new directions for future investigation. The perspective is typically forward-looking, highlighting the relevance of the findings and suggesting how the research might influence future studies, applications, or technologies.

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# : What to do when your chapter has been previously published[[2]](#footnote-3)

## Introduction

Add a footnote to the title of chapters that have previously appeared in publications. See the right side of the Chapter 2 title and the bottom of this page for an example of what this looks like.

## Results

### Subheading that is soooooooo long that is wrapped onto the second line of the table of contents

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A diagram of a dna structure

AI-generated content may be incorrect.

**Figure 2.1. Geometry-based pocket finding approach and optimization for detecting sites in RNAs that bind ligands with favorable physicochemical properties.**

1. Blah Blah Blah. (B) If you keep talking that blah, blah, blah, blah, blah. (C) Kesha (feat. 3OH!3).

### How to reference tables and figures from your supplemental Information

You can incorporate your SI into your chapter, or you can incorporate your SI as an appendix chapter. Here is how to reference tables (**Table A.1**, **Table A.2**) and figures (**Figure A.1**, **Figure A.2**) in an appendix.

## Discussion

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## Materials and methods

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### Subheading 2

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## Data availability

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#### : SUPPLEMENTAL INFORMATION FOR CHAPTER 2

## Supplemental figures

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Figure .. Parameter space evaluated during fpocketR optimization.

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Figure .. Pockets detected in the SAM-III riboswitch using fpocketR.

(PDB: 3E5C) (15).

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## Supplementary Tables

Table .. Training set library used to optimize fpocketR.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PDB code** |  | | **length** | | **ligand name** | **QED** |
| 1F27 | |  | | 30 | Biotin | 0.49 |
| 8D2B | |  | | 33 | TAL2 | 0.83 |
| 1Q8N | |  | | 38 | Malachite green | 0.75 |
| 7ELR | |  | | 45 | Xanthine | 0.45 |
| 3E5C | |  | | 53 | SAM (III) | 0.34 |
| 6FZ0 | |  | | 53 | SAM (V) | 0.34 |
| 3NPQ | |  | | 54 | SAH | 0.35 |
| 6UBU | |  | | 67 | Guanine | 0.46 |
| 2GDI | |  | | 80 | TPP | 0.79 |
| 6LAS | |  | | 93 | SAM (VI) | 0.34 |
| 4B5R | |  | | 94 | SAM (I) | 0.34 |
| 4RZD | |  | | 101 | PreQ1 (III) | 0.46 |
| 5KX9 | |  | | 112 | FMN | 0.33 |
| **Average** | |  | | **66** | **-** | **0.48** |

Table .. Test set library used for evaluating fpocketR optimizations.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PDB code** |  | | **length** | | **ligand name** | **QED** |
| 7FJ0 | |  | 20 | KG022 | | 0.63 |
| 6VA4 | |  | 21 | MIP | | 0.70 |
| 1LVJ | |  | 31 | Acetylpromazine | | 0.76 |
| 3Q50 | |  | 33 | Pre-Q1 (I) | | 0.46 |
| 6YL5 | |  | 35 | SAH (SAM-SAH) | | 0.35 |
| 2KTZ | |  | 38 | Isis-11 | | 0.87 |
| 6UP0 | |  | 38 | YO3-biotin | | 0.66 |
| 6XB7 | |  | 41 | DMA-135 | | 0.40 |
| 6GZR | |  | 48 | 5-TAMRA | | 0.38 |
| 1YKV | |  | 49 | Ethanoanthracene | | 0.64 |
| 7EOH | |  | 49 | HBC | | 0.68 |
| 8EYU | |  | 49 | DFAME | | 0.68 |
| 2QWY | |  | 52 | SAM (II) | | 0.34 |
| 7OAW | |  | 52 | DMHBI+ | | 0.62 |
| 8HB3 | |  | 55 | Nicotinamide riboside | | 0.45 |
| 6XJQ | |  | 58 | 2,3-disubstituted epoxide | | 0.38 |
| 3SKI | |  | 68 | 2'-Deoxy-guanosine | | 0.51 |
| 5OB3 | |  | 69 | DFHBI | | 0.86 |
| 5KPY | |  | 71 | 5-Hydroxytryptophan | | 0.62 |
| 4LX5 | |  | 71 | PPDA | | 0.55 |
| 5BTP | |  | 75 | ZMP | | 0.31 |
| 4JF2 | |  | 77 | Pre-Q1 (II) | | 0.46 |
| 7KVT | |  | 83 | DFHBI-1T | | 0.67 |
| 7DWH | |  | 102 | SAM | | 0.34 |
| 3D0U | |  | 161 | Lysine | | 0.46 |
| **Average** | |  | **58** | **-** | | **0.55** |

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1. Figure adapted from K. D. Warner, C. E. Hajdin, K. M. Weeks, Principles for targeting RNA with drug-like small molecules. *Nat Rev Drug Discov* **17**, 547–558 (2018). [↑](#footnote-ref-2)
2. This chapter previously appeared as an article in *Proceedings of the National Academy of Sciences of the United States of America*. The original citation is as follows: S.D. Veenbaas,J.T. Koehn,P.S. Irving,N.N. Lama, & K.M. Weeks, Ligand-binding pockets in RNA and where to find them, Proc. Natl. Acad. Sci. U.S.A. 122, e2422346122 (2025). [↑](#footnote-ref-3)