# Preface

RNA molecules are increasingly recognized both for their regulatory roles and as potential therapeutic targets in a range of microbial and viral infections and human diseases. Such RNAs include bacterial riboswitches that control translation, viral RNA structures critical to replication, microRNAs that control mRNA levels and expression, and long noncoding RNAs (lncRNAs) that regulate complex gene expression networks.*

Central to these RNA functions, and any related efforts to target them, is the specific recognition of RNA molecules. The mysteries of RNA recognition are particularly fascinating but difficult to unlock given the increased dynamics and reduced chemical diversity of RNA relative to protein and the more complex structure of RNA relative to DNA. Indeed, our current understanding suggests that it is the combination of sequence, structure, and dynamics that ultimately leads to specific recognition. Given these complexities, it is not surprising that a diverse set of methods have been and are continuously being developed to create a deep understanding of RNA recognition.

This volume includes a sampling of both traditional and novel methods in the study of RNA recognition, including the lessons we learn from natural systems, from engineering biomolecules, and from studying how small molecule probes can selectively recognize RNA. In each case, the authors illustrate how the methods described reveal insights into RNA recognition.

Looking at natural systems, several chapters investigate riboswitches, both as important biological regulators and as excellent model systems to understand RNA recognition. Ren and coauthors detail riboswitch characterization by X-ray diffraction and NMR methods while Ferré-D'Amaré, Woodson, and coauthors gain insights from cotranscriptional folding of riboswitches by combining super-helicases with single molecule fluorescence measurements. Das and coauthors reveal methods to model and push towards riboswitch optimality in both natural and synthetic systems as well as methods to model 3D RNA structures, which have been benchmarked using riboswitch aptamers. At the same time, such detailed characterization of structure and folding is generally more difficult for long RNAs, and Pyle

*Given the many excellent reviews and primary literature reports on these topics, readers are referred to individual chapters for the most relevant and up-to-date respective references.

and coauthors outline how to combine a suite of probing methods for the accurate determination of the natively folded secondary structure of long RNA molecules, using the Hepatitis C virus genome as a model system. Importantly, the majority of RNAs are thought to be bound by proteins, and Hall, Goldstrohm, and coauthors illuminate how crystallization and structure determination of protein:RNA complexes can reveal intricate details of cooperativity in RNA recognition.

Insights gained from studying native RNA structure, folding, and interactions directly contribute to, and are complemented by, efforts to design ligands for RNA. Ligand approaches include the engineering of peptide and nucleic derivatives as well as natural products and synthetic small molecules. The power of peptide design is demonstrated by Santos and coauthors through the study of branched peptides with RNA-targeting modifications and by Varani and coauthors through the design of conformationally constrained cyclic peptides. Peptide nucleic acids offer a distinct approach by taking advantage of nucleobase hydrogen bonding patterns, as illustrated by Rozners and coauthors in the recognition of RNA through triplex formation and by Bong and coauthors who generate allosteric triggers of RNA-driven catalysis.

Finally, small-molecule targeting of RNA is an area of intense interest but with the need for improved methods to evaluate recognition and selectivity, the latter of which can be particularly challenging for these lower molecular weight molecules in comparison to peptide or oligonucleotide derivatives. In addition, small molecules are particularly suited for the recognition of RNA structure rather than sequence, and high-resolution structures remain a challenge for many RNAs. Naturally occurring small-molecule RNA-ligands such as aminoglycosides offer unique insights into RNA recognition and also serve as good model systems, as revealed by Chow and coauthors in the use of mass spectrometry to monitor RNA:aminoglycoside interactions and by Arya and coauthors who use fluorescently-labeled aminoglycosides to screen for other RNA ligands. Hargrove and coauthors describe a method using pattern recognition to understand determinants of RNA structural differentiation and selectivity via aminoglycoside binding.

These and other methods can be extended to a range of small molecule classes. Garner and coauthors present a high-throughput method to screen natural product extracts against multiple pre-microRNAs for both discovery and selectivity analysis, and Disney and coauthors depict a computational and experimental pipeline to identify and validate small molecule ligands

for specific RNA secondary structures. Yet another approach to the challenge of selectivity is the generation of multivalent small molecules, and Miller and coauthors detail the use of dynamic combinatorial chemistry to identify multivalent ligands without the need for high-resolution structure. In addition to screening techniques, new biophysical methods for determining small molecule binding properties are also critical to progress in the field. Schneekloth and coauthors describe ligand-observed NMR techniques to rapidly assess binding and selectivity of small molecules to RNA and Engelhart and coauthors report a method to easily assess stability of small molecule:RNA complexes via thermal denaturation with fluorogenic dyes.

We hope that this compilation of select methods will serve as a valuable resource to those entrenched in the study of RNA and an inspiration to those interested in starting their own RNA adventure.

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