Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Innovations in targeting RNA by fragment-based ligand discovery

Jordan T. Koehn[1], Simon Felder[1] and Kevin M. Weeks

Abstract

A subset of functional regions within large RNAs fold into complex structures able to bind small-molecule ligands with high affinity and specificity. Fragment-based ligand discovery (FBLD) offers notable opportunities for discovery and design of potent small molecules that bind pockets in RNA. Here we share an integrated analysis of recent innovations in FBLD, emphasizing opportunities resulting from fragment elaboration *via* both linking and growing. Analysis of elaborated fragments emphasizes that high−quality interactions form with complex tertiary structures in RNA. FBLD-inspired small molecules have been shown to modulate RNA functions by competitively inhibiting protein binding and by selectively stabilizing dynamic RNA states. FBLD is creating a foundation to interrogate the relatively unknown structural space for RNA ligands and for discovery of RNA-targeted therapeutics.

Addresses

Department of Chemistry, University of North Carolina, Chapel Hill NC 27599-3290, USA

Corresponding author: Weeks, Kevin M. (weeks@unc.edu)
[1] Contributed equally.

Current Opinion in Structural Biology 2023, **79**:102550

This review comes from a themed issue on **New Concepts in Drug Discovery**

Edited by **Andrew R. Leach** and **Alison E. Ondrus**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.sbi.2023.102550

Overview

RNA lies upstream of nearly all biology. In principle, it is therefore possible to modulate diverse downstream cellular functions by targeting RNA. Certain regions in large RNAs fold to form well-defined pockets capable of specific and high-affinity recognition of small-molecule ligands, supporting an enormous potential to manipulate RNA function [1,2]. Small-molecule-mediated modulation of RNA function could make possible targeting of difficult-to-drug, disease-implicated proteins and is now an intense focus of ongoing discovery efforts by pharmaceutical and academic groups [1,3,4−9]. Notable challenges must be overcome, however, before scalable, specific, and functional targeting of RNA with small-molecule ligands is routinely successful. Fragment-based ligand discovery (FBLD), well validated for clinical drugs that target proteins [10], is an attractive strategy to overcome these challenges in the design of small molecules that engage functional RNAs [11,12]. Here, we focus on recent innovations in RNA-targeted FBLD with an emphasis on elaborated fragment hits and high-quality fragment-inspired and fragment-related small molecule-RNA interactions.

Disclaimer: RNA-targeted FBLD is in its infancy

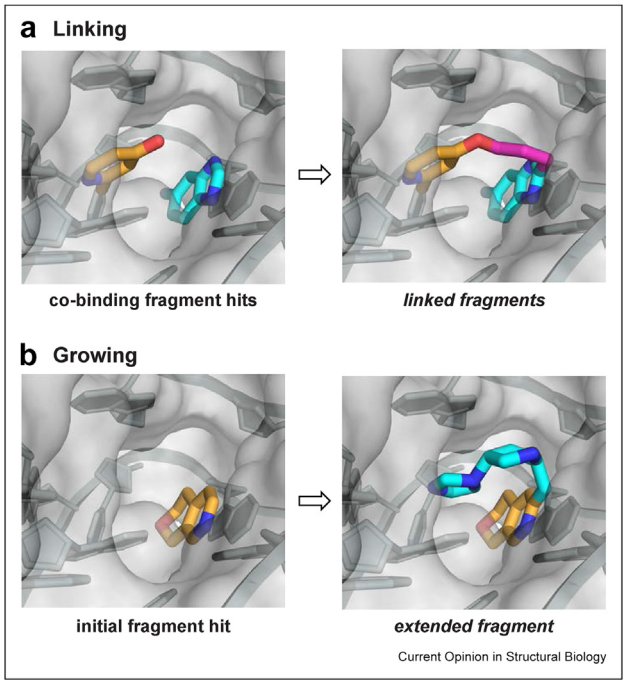
Although the promise of RNA-targeted drug discovery is expansive, the number of human designed or discovered classes of small molecules that bind RNA and alter biology is modest. Known RNA targeting molecules include a diverse set of natural products (many of which are too toxic for human use), the linezolid class of (highly successful) antibiotics, several splicing modulators (which likely function as molecular glues linking suboptimal splice sites to the spliceosome), and a handful of preclinical human-designed molecules. In this challenging scenario, FBLD has strong potential for creating novel chemical matter targeting RNA. FBLD leverages small libraries comprised of simple, chemically diverse molecules (<300 molecular weight) that carry functional groups capable of forming high−quality interactions with a target molecule [10,12]. Our current understanding of FBLD as applied to RNA is limited. First, FBLD has been applied to RNA but only a handful of elaborated molecules, based on initial fragment hits, have been reported [13−21]. Second, no fragment-inspired lead molecule has yielded a highly potent near-clinical molecule. Third, the druggable landscape of cellular RNA structures is poorly defined, as transcriptome-wide screening technologies are at intriguing, but very early, stages [22,23]. As a result, FBLD is far from creating a clinical drug that targets RNA. In this review, we will focus on what can be learned from critical analyses of published fragment-focused studies and will also extend our analysis to include a few "honorary" fragment-like small molecules. We highlight impactful examples where the initial

potency of a molecule was enhanced through fragment elaboration, showcase high-quality fragment-inspired interactions with complex RNA structures, and outline therapeutic mechanisms for modulating RNA function with small-molecules ligands.

Brief introduction to FBLD

FBLD of RNA is, broadly, a two-step process: first, initial (low affinity) fragments that form high-quality contacts with RNA are identified, and second, these fragment hits are elaborated to yield more complex, potent, and specific small molecules that modulate RNA function. Robust and sensitive biophysical methods are required to detect low-affinity fragments that engage RNA. Many screening strategies have proven successful in RNA-targeted FBLD and have been expertly reviewed [12]. Identified fragment hits can be elaborated into more potent RNA binders by either linking or growing. If two fragments are identified that occupy the same RNA pocket, or pockets that are close in space, these fragments can be linked synthetically using a flexible or rigid chemical linker (Figure 1a). Alternatively, a single fragment found to bind an RNA pocket can be synthetically extended into adjacent pocket space by adding functional groups or other fragment moieties (Fig. 1b). To date, there have been many examples of initial fragment hits that engage RNA [12], and two very recent studies show that it is possible to obtain high-affinity fragment-like hits for RNA [24,25]. Going forward, FBLD — with a focus on fragment elaboration — is well suited to fill current knowledge gaps in small molecule-RNA interactions, as FBLD efficiently and simultaneously interrogates both small molecule chemical space and RNA structural space.

Figure 1



Strategies for elaboration of fragments that bind RNA. (**a**) Linking of co-binding fragments *via* a synthetic bridging group. (**b**) Growing a fragment into neighboring space of an RNA pocket by adding functional groups.

Fragment linking

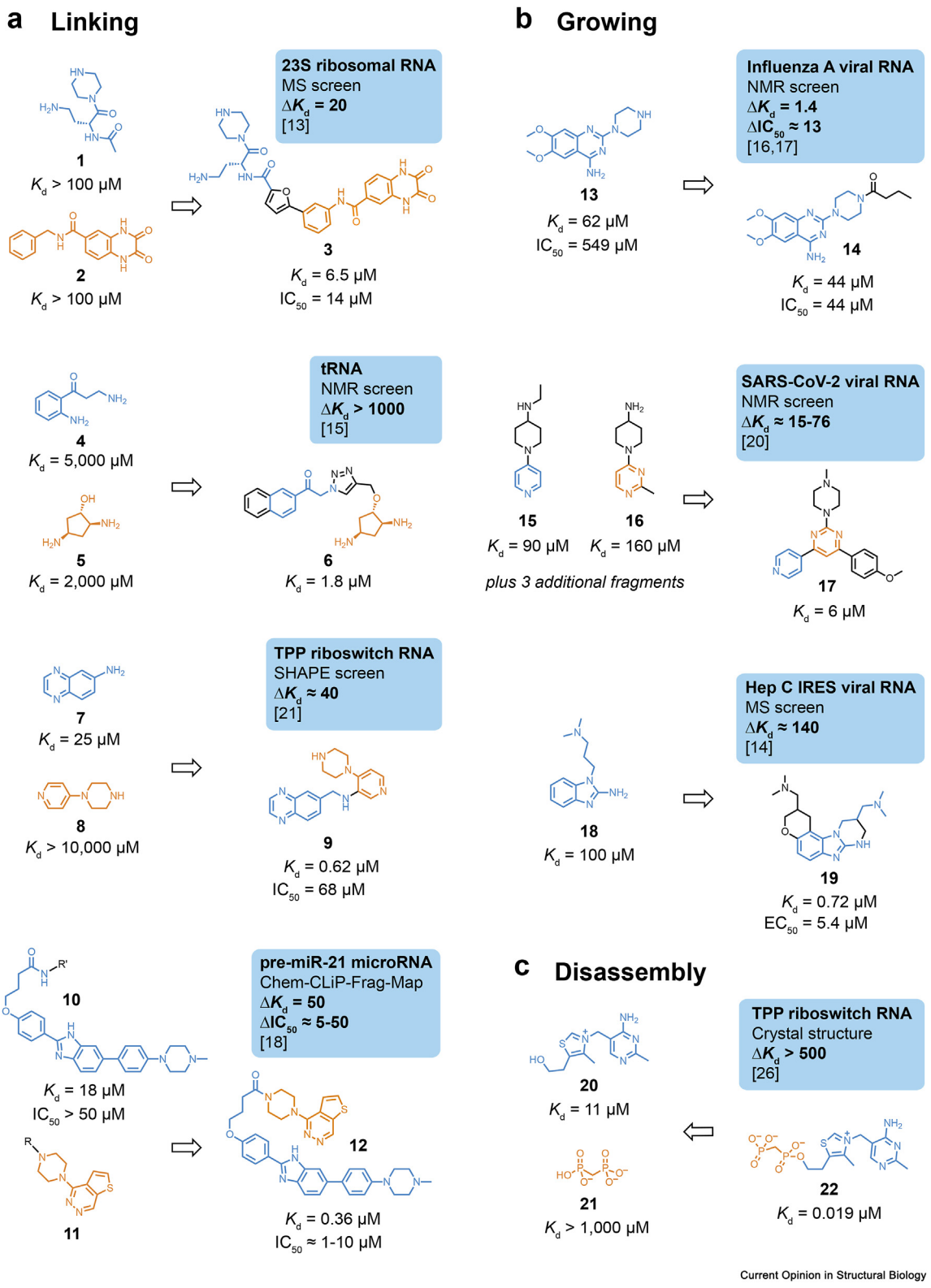
The earliest reported example of fragment elaboration and optimization toward an RNA target used a strategy called structure—activity relationships by mass spectroscopy (SAR by MS) to identify two fragments, **1** and **2**, that bound with low affinity to a segment of the bacterial 23S ribosomal RNA (Figure 2a) [13]. These fragments were linked using a rigid furan (a flexible linker resulted in weaker binding). The linked fragments yielded compound **3** with low micromolar affinity and functional activity in cells (Fig. 2a). Binding measurements for fragment analogs informed linking of the fragment motifs, demonstrating that high-resolution structural information is not a requirement for successful FBLD toward RNA. A ligand that binds the D-arm of $tRNA^{Lys3}$ was created by linking two independently binding fragments (**4** and **5**, with mM affinities), identified by NMR, creating low micromolar affinity compound **6**, and corresponding to an impressive >1000-fold improvement in K_d upon fragment linking (Figure 2a) [15].

Two recent studies further emphasize that elaboration can be successful in the absence of high-resolution structural information. Two fragments were identified by SHAPE chemical probing (**7,** K_d 25 μM, and **8**, $K_d > 10$ mM) as cooperative co-binders to the thiamine pyrophosphate (TPP) riboswitch, and were linked to yield a compound with high nanomolar affinity (compound **9**, Figure 2a) [21]. This work emphasized that per-nucleotide chemical probing information can guide compound elaboration. Compound **9** is one of the most druglike compounds identified as an RNA binder and induces conformational switching of the TPP riboswitch in a transcriptional assay (IC_{50} = 68 μM). Photocrosslinking with diazirine-linked fragments was used to identify compound **11** as a binder to a pre-miR-21 RNA [18]. This compound, when appended to known binder **10**, furnished **12** as a mid-nanomolar binder to the pre-micro-RNA (Figure 2a). Compound **12** has an IC_{50} of 1—10 μM in cellular assays of microRNA function. Here, the linkage to a photoreactive group also defined an accessible handle (R in Figure 2a), facilitating efficient fragment linking to form **12**.

Fragment growing and disassembly

Elaborations of initial fragments that broadly resemble fragment growing have been reported. A weak binding fragment to an RNA promoter element in the influenza

Figure 2



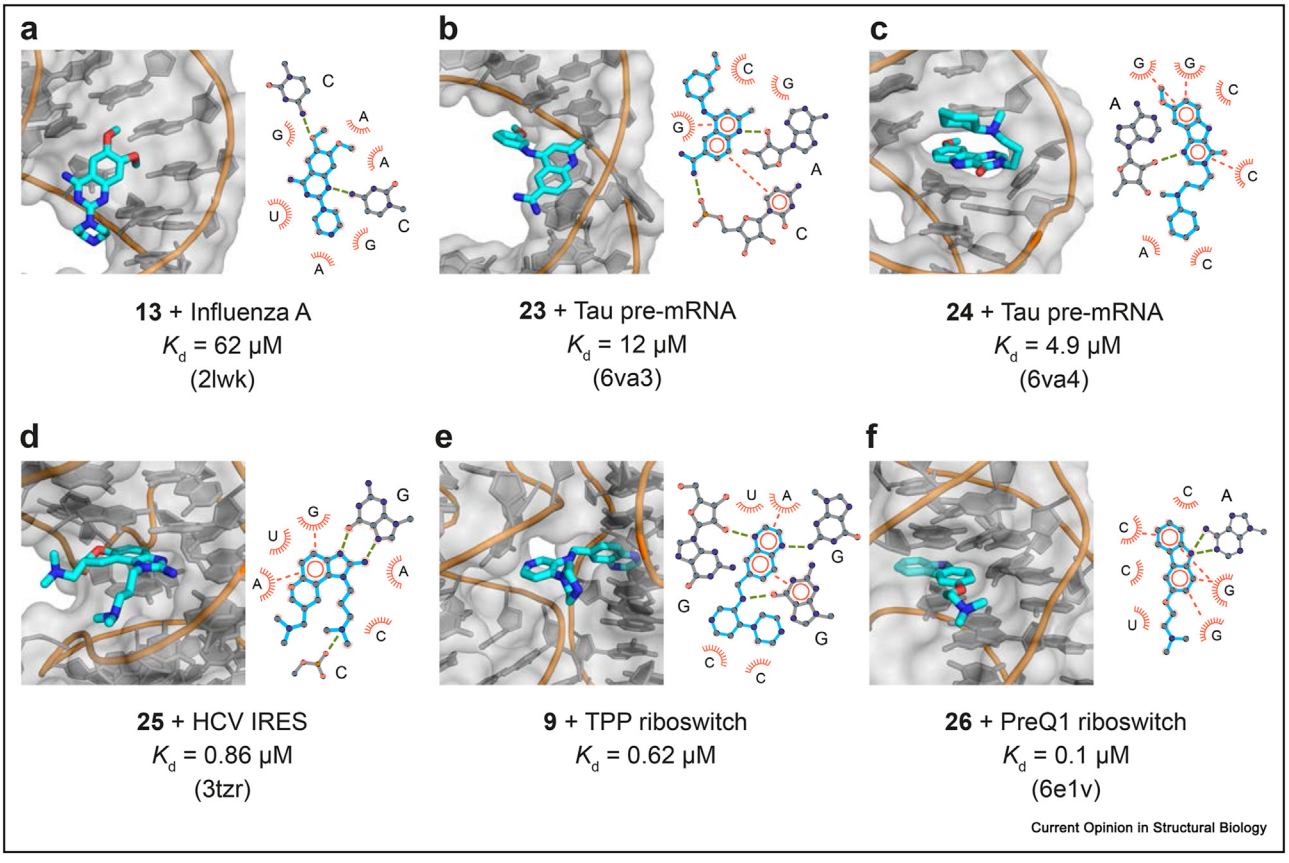
Examples of elaboration strategies for fragments targeting RNA. (**a**) Linking of co-binding fragments. (**b**) Growing an initially identified fragment. (**c**) Use of fragment disassembly to illustrate energetics of elaboration. Within a category, compounds are listed in order of increasing reported affinity. Boxes highlight the target, screening method, change in affinity upon elaboration, and reference; ΔK_d and ΔIC_{50} are *ratios*, corresponding to fold-change. R, sites of photocrosslinking groups.

A viral RNA (compound **13**) was initially identified by NMR (Figure 2b) [16,17]. The secondary amine on the piperazine moiety in **13** was subsequently modified to increase interactions with RNA. Compound **14**, with a butyl amide at this position, showed a small effect on affinity but is a 12-fold better replication inhibitor, providing a reminder that biophysically-measured K_d should not be the sole focus for modulating RNA function with small molecules. In a second example, an NMR screen of a pseudoknot structure in the frame-shifting element of SARS-CoV-2 viral RNA identified a family of five fragments with similar chemotypes, including compounds **15** and **16** (Figure 2b) [20]. Compound **17** was selected using SAR by catalog as an "elaborated" molecule. The 6 μM K_d of compound **17** is a 15-fold improvement in affinity compared to initially selected fragment **15**. Finally, MS was used to identify fragment hit **18**, which binds weakly to the hepatitis C virus IRES element (Figure 2b) [14]. Based on SAR by MS information from additional analogs, the compound was elaborated into **19**, which has high nanomolar affinity for the RNA target and an EC_{50} of 5.4 μM in a cellular replicon assay.

Many natural (often metabolite) ligands bind RNA in pockets consisting of distinct subsites, including for the TPP riboswitch. TPP was disassembled into thiamine and a soluble (methylene-bridged) analog of pyrophosphate that independently engage distinct subsites (Figure 2c) [26]. The "honorary" fragments **20** and **21** bind with micromolar to millimolar affinities and their re-linking yields **22**, which binds with 20 nM affinity. This deconstruction experiment emphasizes that potent ligands can be created from relatively weakly binding starting compounds that bind subsites in a well-defined RNA pocket.

In sum, these examples highlight the ability of FBLD linking and growing strategies to deliver intriguing small-molecule leads against functional motifs in RNA.

Figure 3



Three-dimensional structures (left) and interaction maps (right) for ligands that interact (**a**–**c**) with simple RNA motifs and (**d**–**f**) with complex RNA structures. In the interaction maps, hydrogen bonds are shown with green dashed lines; π-stacking is illustrated with orange circles and dashes. For NMR structures (panels **a-c**), interpretations of average structures are shown. Structures and interaction maps were composed with PyMOL and LigPlot+, respectively.

Visualization of fragment and fragment-like interactions with RNA

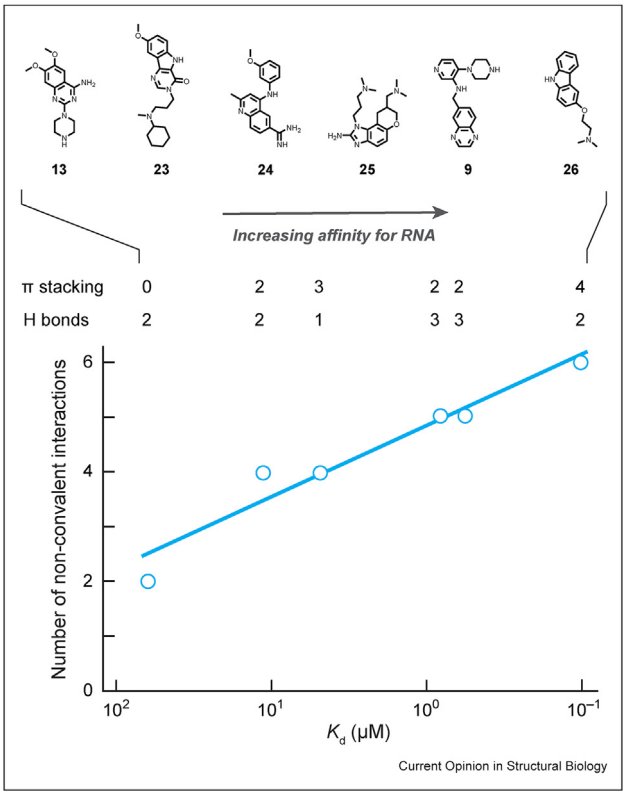
Non-covalent interactions between RNA and small-molecule ligands consist primarily of π-stacking and hydrogen bonding [27]. We analyzed available high-resolution models of fragment-based and fragment-like small-molecules bound with their target RNA. One group of these compounds binds simple stem-loop motifs in RNA (Figure 3a–c). Compound **13** binds to a viral stem-loop structure, widens the major groove of the helix, and disrupts base pairing by forming two hydrogen bonds with neighboring cytosine bases (Figure 3a) [16,17]. Fragment-like ligands **23** and **24** bind a hairpin that contains a bulged adenosine near a splice junction in the pre-mRNA encoding the Tau protein. Compound **23** binds in the major groove and forms two hydrogen bonds with the RNA backbone, involving non-bridging oxygen and 2′-hydroxyl groups; **24** intercalates into the helix and forms a direct hydrogen bond with a nearby adenosine (Figure 3b and c) [28].

Other ligands interact with complex RNA structures (Figure 3d–f). Compound **25** (closely related to **19**, see Figure 2b) induces a conformational switch in the HCV IRES by intercalating in a hydrophobic pocket. The benzimidazole scaffold forms hydrogen bonds with the Hoogsteen face of a nearby guanosine nucleotide (Figure 3d) [29]. The quinoxaline moiety of elaborated fragment **9** binds in the native thiamine-binding pocket of the TPP riboswitch, forming multiple hydrogen bonds, and the piperazine moiety appears to protrude into an electrostatic pocket normally occupied by a metal ion (Figure 3e) [21]. Finally, the fragment-like ligand **26** binds to the PreQ1 riboswitch in the same overall site as the native ligand *via* multiple π-stacking and hydrogen-bonding interactions. A change in heteroatom in the central 5-membered ring, from oxygen to nitrogen, results in a 1 Å shift inside the pocket and formation of two hydrogen bonds (Figure 3f) [30].

Potency reflects high-quality fragment contacts with RNA

For the RNA ligands with known structures discussed here, there is a roughly linear correlation between the total number of non-covalent stacking and hydrogen-bonding interactions formed with the RNA target and the free energy change of binding (Figure 4). The three ligands that form the fewest interactions (and also have the weakest affinities) interact with simpler base-paired RNA structures. In these structures of **13**, **23** and **24** with their RNA targets, canonical base pairing is prevalent, and the nucleobases are only partially accessible in the grooves of the helices. In contrast, the three ligands that interact with more complex structures (in increasing order of affinity: **25**, **9** and **26**) bind with sub-micromolar affinity to their RNA targets, and each forms extensive stacking interactions and multiple hydrogen bonds with well-structured but non-base-paired nucleotides. These features are likely to be core requirements for specific and potent recognition of RNA by small molecules [1,2,24].

Figure 4

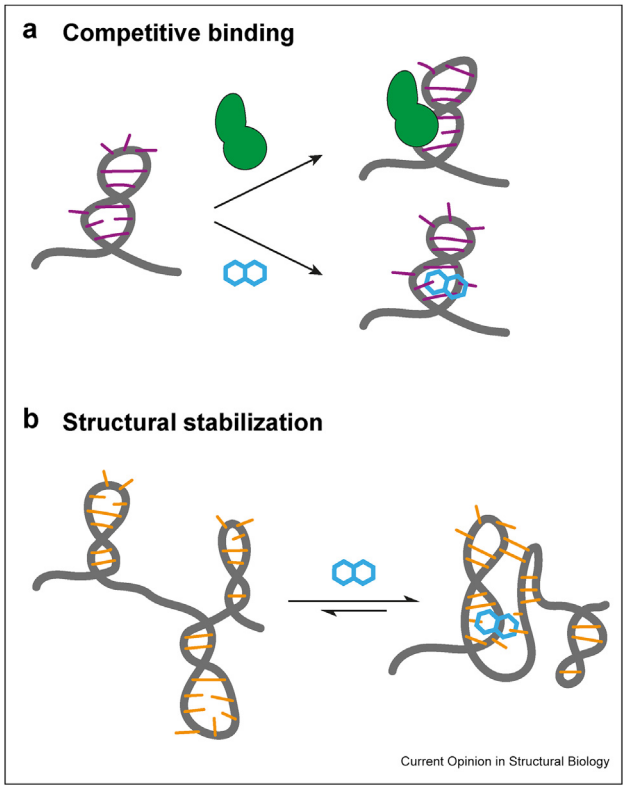


Qualitative correlation between binding affinity (K_d) and intermolecular hydrogen bonding and π-stacking interactions for fragment-like ligands that engage RNA. Note: plotting K_d values on a logarithmic scale yields a relationship proportional to ΔG. Complexes are the same as shown in Fig. 3.

Mechanisms for modulating RNA function

Fragment-derived compounds have been shown to modulate RNA-based functions *via* two primary mechanisms: through competitive binding to a protein-recognition site or through stabilization of an RNA structural motif (Figure 5) The former mechanism has been exploited to disrupt viral replication (compound **14**) [16,17] and to prevent microRNA maturation (compound **12**) [18]. For compounds that act by structural stabilization, ligand binding induces a change in the relative populations of RNA conformational states. This mechanism has been exploited to stabilize specific states for viral frameshift and IRES elements (compounds **17**, **19**, **25**) [20,29], to alter splicing (compounds **23**, **24**) [28], and to stabilize the bound states of riboswitches (compounds **9**, **26**) [11,21,30]. Ultimately, fragment-based ligands should be capable of modulating RNA-mediated

Figure 5



Mechanisms by which known fragment-based small molecules modulate RNA function. (**a**) Competition with RNA-binding proteins. (**b**) Stabilization of an RNA structure.

function by any extant mechanism [3,6,7,9], including many not (quite yet) reduced to practice.

Summary

FBLD for RNA targets is at an early stage but holds enormous promise. We infer three key lessons from studies reported to date.

First, obtaining a fragment hit is just a first step. Fragment elaboration, either by linking or growing, is essential. Using conventional medicinal chemistry strategies, it appears relatively straightforward to elaborate fragments into sub-micromolar small-molecule ligands that engage RNA with good specificity. Intriguingly, multiple examples show elaboration can be successful in the absence of initial high-resolution structural information.

Second, the quality of the RNA target matters. Complex RNA tertiary structures form more and higher-quality interactions with fragment hits and their elaborated versions than do simple structures such as RNA stem-loop motifs. Target selection should focus on both RNA structural complexity and the potential mechanism by which ligand binding will influence RNA-based cellular function.

Finally, FBLD provides an opportunity to understand fundamental principles underlying the chemical space of elaborated ligands, the physicochemical RNA space they bind, and their mechanisms for modulating biological processes. The major obstacle to the realization of FBLD targeted toward RNA lies in the efficient identification of clinically validated RNA structures whose cellular function can be altered by ligand binding.

Conflict of interest statement

K.M.W. is an advisor to and holds equity in Ribometrix. Other authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

Acknowledgements

Work in our laboratory is supported by the US National Institutes of Health, the National Science Foundation, and industry partners. J.T.K. is a UNC Lineberger Integrated Training in Cancer Model Systems Fellow (T32CA009156) and an NIH Kirschstein Postdoctoral Fellow (F32GM143863).

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Warner KD, Hajdin CE, Weeks KM: **Principles for targeting RNA with drug-like small molecules**. *Nat Rev Drug Discov* 2018, **17**:547–558.
2. Hewitt WM, Calabrese DR, Schneekloth JS: **Evidence for ligandable sites in structured RNA throughout the protein data bank**. *Bioorg Med Chem* 2019, **27**:2253–2260.
3. Connelly CM, Moon MH, Schneekloth JS: **The emerging role of RNA as a therapeutic target for small molecules**. *Cell Chemical Biology* 2016, **23**:1077–1090.
4. Umuhire Juru A, Patwardhan NN, Hargrove AE: **Understanding the contributions of conformational changes, thermodynamics, and kinetics of RNA–small molecule interactions**. *ACS Chem Biol* 2019, **14**:824–838.
5. Giorgio AD, Duca M: **Synthetic small-molecule RNA ligands: future prospects as therapeutic agents**. *Med Chem Commun* 2019, **10**:1242–1255.
6. Sztuba-Solinska J, Chavez-Calvillo G, Cline SE: **Unveiling the druggable RNA targets and small molecule therapeutics**. *Bioorg Med Chem* 2019, **27**:2149–2165.
7. Yu A-M, Choi YH, Tu M-J: **RNA drugs and RNA targets for small molecules: principles, progress, and challenges**. *Pharmacol Rev* 2020, **72**:862–898.
8. Zamani F, Suzuki T: **Synthetic RNA modulators in drug discovery**. *J Med Chem* 2021, **64**:7110–7155.
9. Childs-Disney JL, Yang X, Gibaut QMR, Tong Y, Batey RT, Disney MD: **Targeting RNA structures with small molecules**. *Nat Rev Drug Discov* 2022, **21**:736–762.
10. Erlanson DA, Fesik SW, Hubbard RE, Jahnke W, Jhoti H: **Twenty years on: the impact of fragments on drug discovery**. *Nat Rev Drug Discov* 2016, **15**:605–619.
11. Warner KD, Homan P, Weeks KM, Smith AG, Abell C, Ferré-D'Amaré AR: **Validating fragment-based drug discovery for biological RNAs: lead fragments bind and remodel the TPP riboswitch specifically**. *Chem Biol* 2014, **21**:591–595.

12. Lundquist KP, Panchal V, Gotfredsen CH, Brenk R, Clausen MH: **Fragment-based drug discovery for RNA targets**. *ChemMedChem* 2021, **16**:2588–2603.

13. Swayze EE, Jefferson EA, Sannes-Lowery KA, Blyn LB, Risen LM, Arakawa S, Osgood SA, Hofstadler SA, Griffey RH: **SAR by MS: a ligand based technique for drug lead discovery against structured RNA targets**. *J Med Chem* 2002, **45**:3816–3819.

14. Seth PP, Miyaji A, Jefferson EA, Sannes-Lowery KA, Osgood SA, Propp SS, Ranken R, Massire C, Sampath R, Ecker DJ, *et al.*: **SAR by MS: discovery of a new class of RNA-binding small molecules for the hepatitis C virus: internal ribosome entry site IIA subdomain**. *J Med Chem* 2005, **48**:7099–7102.

15. Chung F, Tisné C, Lecourt T, Dardel F, Micouin L: **NMR-guided fragment-based approach for the design of tRNALys3 ligands**. *Angew Chem Int Ed* 2007, **46**:4489–4491.

16. Lee M-K, Bottini A, Kim M, Bardaro MF, Zhang Z, Pellecchia M, Choi B-S, Varani G: **A novel small-molecule binds to the influenza A virus RNA promoter and inhibits viral replication**. *Chem Commun* 2013, **50**:368–370.

17. Bottini A, De SK, Wu B, Tang C, Varani G, Pellecchia M: **Targeting influenza A virus RNA promoter**. *Chem Biol Drug Des* 2015, **86**:663–673.

18. • Suresh BM, Li W, Zhang P, Wang KW, Yildirim I, Parker CG, Disney MD: **A general fragment-based approach to identify and optimize bioactive ligands targeting RNA**. *Proc Natl Acad Sci USA* 2020, **117**:33197–33203.
Functionalized, photoreactive fragments enabled capture of ligand binding to a microRNA and provided guidance for fragment linking. Linking to a known binder created a compound with mid nanomolar affinity and low micromolar biological activity.

19. Binas O, de Jesus V, Landgraf T, Völklein AE, Martins J, Hymon D, Kaur Bains J, Berg H, Biedenbänder T, Fürtig B, *et al.*: **19F NMR-based fragment screening for 14 different biologically active RNAs and 10 DNA and protein counter-screens**. *Chembiochem* 2021, **22**:423–433.

20. •• Sreeramulu S, Richter C, Berg H, Wirtz Martin MA, Ceylan B, Matzel T, Adam J, Altincekic N, Azzaoui K, Bains JK, *et al.*: **Exploring the druggability of conserved RNA regulatory elements in the SARS-CoV-2 genome**. *Angew Chem Int Ed* 2021, **60**:19191–19200.
A high-impact resource and tour de force for the FBLD field. Fifteen simple structures and 5 larger RNAs, derived from motifs in the SARS-CoV-2 viral RNA, were screened against a 768-member fragment library. Specific, promiscuous, and non-binding fragments were identified and characterized in the context of diverse RNA targets.

21. •• Zeller MJ, Favorov O, Li K, Nuthanakanti A, Hussein D, Michaud A, Lafontaine DA, Busan S, Serganov A, Aubé J, *et al.*: **SHAPE-enabled fragment-based ligand discovery for RNA**. *Proc Natl Acad Sci USA* 2022, **119**, e2122660119.
SHAPE RNA structure probing is used to identify co-binding fragment hits and inform elaboration *via* linking to create a fully non-native ligand that engages the TPP riboswitch with high nanomolar affinity. The resulting molecule has high ligand efficiency and druglikeness, and modulates RNA conformation during co-transcriptional folding.

22. Mukherjee H, Blain JC, Vandivier LE, Chin DN, Friedman JE, Liu F, Maillet A, Fang C, Kaplan JB, Li J, *et al.*: **PEARL-seq: a photoaffinity platform for the analysis of small molecule-RNA interactions**. *ACS Chem Biol* 2020, **15**:2374–2381.

23. Tong Y, Gibaut QMR, Rouse W, Childs-Disney JL, Suresh BM, Abegg D, Choudhary S, Akahori Y, Adibekian A, Moss WN, *et al.*: **Transcriptome-wide mapping of small-molecule RNA-binding sites in cells informs an isoform-specific degrader of QSOX1 mRNA**. *J Am Chem Soc* 2022, https://doi.org/10.1021/jacs.2c01929.

24. Menichelli E, Lam BJ, Wang Y, Wang VS, Shaffer J, Tjhung KF, Bursulaya B, Nguyen TN, Vo T, Alper PB, *et al.*: **Discovery of small molecules that target a tertiary-structured RNA**. *Proc Natl Acad Sci USA* 2022, **119**, e2213117119.

25. Suresh BM, Akahori Y, Taghavi A, Crynen G, Gibaut QMR, Li Y, Disney MD: **Low-molecular weight small molecules can potently bind RNA and affect oncogenic pathways in cells**. *J Am Chem Soc* 2022, **144**:20815–20824.

26. • Zeller MJ, Nuthanakanti A, Li K, Aubé J, Serganov A, Weeks KM: **Subsite ligand recognition and cooperativity in the TPP riboswitch: implications for fragment-linking in RNA ligand discovery**. *ACS Chem Biol* 2022, **17**:438–448.
Proof-of-concept analysis of fragment binding, linking, and cooperativity by disassembling a known riboswitch-binding ligand into "honorary", weak-binding fragments. Emphasizes the role of subsite binding in pockets of complex RNAs.

27. Padroni G N, Patwardhan N, Schapira M, Hargrove A E: **Systematic analysis of the interactions driving small molecule–RNA recognition**. *RSC Medicinal Chemistry* 2020, **11**:802–813.

28. Chen JL, Zhang P, Abe M, Aikawa H, Zhang L, Frank AJ, Zembryski T, Hubbs C, Park H, Withka J, *et al.*: **Design, optimization, and study of small molecules that target Tau pre-mRNA and affect splicing**. *J Am Chem Soc* 2020, **142**:8706–8727.

29. Dibrov SM, Ding K, Brunn ND, Parker MA, Bergdahl BM, Wyles DL, Hermann T: **Structure of a hepatitis C virus RNA domain in complex with a translation inhibitor reveals a binding mode reminiscent of riboswitches**. *Proc Natl Acad Sci USA* 2012, **109**:5223–5228.

30. • Connelly CM, Numata T, Boer RE, Moon MH, Sinniah RS, Barchi JJ, Ferré-D'Amaré AR, Schneekloth JS: **Synthetic ligands for PreQ1 riboswitches provide structural and mechanistic insights into targeting RNA tertiary structure**. *Nat Commun* 2019, **10**:1501.
Impactful example of structural characterization of a fragment-like molecule, applied to a riboswitch RNA. Changing a single heteroatom from O to N, induces a shift in binding pose and increases binding affinity.