Published in final edited form as:

Methods. 2016 July 01; 103: 188–195. doi:10.1016/j.ymeth.2016.04.022.

Microarray-based technologies for the discovery of selective, RNA-binding molecules

Fardokht A. Abulwerdi^{a,b} and John S. Schneckloth Jr.^{a,*}

^aChemical Biology Laboratory, National Cancer Institute, Frederick, MD, United States

^bBasic Research Laboratory, National Cancer Institute, Frederick, MD, United States

Abstract

The identification of small molecules that bind specifically to RNA is a challenge. However, the recent explosion in knowledge about the role RNA plays in a number of physiological processes apart from coding for protein sequences makes it a highly interesting target for chemical probes and therapeutics. One technology that has played an important role in the discovery of RNA-binding molecules is microarrays. Microarrays have been broadly employed to screen, profile, and quantify RNA interactions, and will likely play an important role in the discovery of new classes of ligands going forward. Here, we discuss the development of microarray technologies, including aminoglycoside, peptide, peptoid, and small molecule microarrays, and their use in studying RNA-interacting molecules.

Keywords

RNA; Microarrays; Aminoglycosides; Peptides; Peptoids; Small molecules

1. Introduction

The ubiquitous role of RNA in controlling normal physiology and disease makes it an important target for the development of small molecule probes as well as therapeutics. Therefore, new technologies to identify molecules that bind to RNA are in demand. Microarray-based ligand screening provides a fast, straightforward and high-throughput platform that is complementary to conventional phenotypic or target-based (solution phase) high-throughput screening. Historically, large-scale microarray-based screens were developed to identify ligands that bind “undruggable” protein targets [1–3]. More recently, this screening platform has been adapted to identify RNA binders as well. Microarrays are generally manufactured by printing small (typically nanoliter or subnanoliter) volumes of ligands onto a surface by a robotic microarrayer. The arrayed ligands generally have one of several functional handles for covalent attachment to the surface (Fig. 1). Next, the microarrays are incubated with an RNA of interest, commonly one that is fluorescently tagged. After incubation, microarrays are washed to remove unbound RNA and specific interactions are detected using a fluorescence scanner. Finally, signal quantification and

*Corresponding author. schnecklothjs@mail.nih.gov (J.S. Schneckloth Jr.).

statistical analysis is used to identify hits or to determine binding constants (K_d s). An important strength of microarray-based ligand screening is the simultaneous screening of a large number of immobilized ligands (often more than 10,000) with the biomolecule of interest, significantly more high-throughput than traditional RNA binding assays. Furthermore, the microarray approach requires a relatively small amount of RNA to be used, making it particularly powerful when studying RNA that is difficult to prepare in large quantities. Finally, microarray screens may be performed in parallel or multiplexed to generate selectivity information not available by traditional biochemical assays. While initial screening approaches to identify RNA binders focused on aminoglycosides, peptides, and peptoids on microarrays, recently small molecule chemical libraries have been arrayed and screened to identify small molecules that bind to RNA as well. In addition, oligonucleotide arrays such as RIPTide arrays [30] have been used to map or study RNA binding, and these approaches have been reviewed recently as well [31]. It is clear that microarray technology is a powerful tool to rapidly investigate the interactions of broad classes of molecules with RNA. This review will focus on recent advances in microarray screening platforms used to identify RNA binders and is organized based on the type of immobilized ligands printed onto the microarrays.

2. Aminoglycoside microarrays and the development of INFORNA

Aminoglycoside antibiotics are among the most studied classes of RNA binding small molecules. These polycationic sugar derivatives are commonly studied for their ability to bind to bacterial ribosomal RNA (rRNA) A-site and inhibit protein synthesis, among many other RNA structures [4]. The first proof of concept studies in adapting the microarray-based ligand screening to RNA were performed by Seeberger and coworkers [5] and Wong and coworkers [6] through the use of aminoglycoside microarrays. In both studies, a number of aminoglycosides were immobilized covalently onto a succinimidyl succinate functionalized glass slide or noncovalently onto a polystyrene microtiter plate. Both groups showed recognition of the aminoglycosides with a fluorescently labeled rRNA A-site mimic, highlighting that the microarray screening platform can be adapted to screen RNA. In the Seeberger study, the binding affinity of the aminoglycosides for the RNA was correlated to the fluorescence intensity of each spot. However, these results differed from the binding affinities previously reported for the aminoglycosides in solution-based assays. This difference was attributed to the different presentation of each aminoglycoside by its attachment. Thus immobilization methods have to be selected with care to minimize interference with the ligand RNA interaction.

Since aminoglycosides have long been considered privileged scaffolds that bind RNA, Disney and coworkers developed a two-dimensional combinatorial screening (2DCS) method (Fig. 2A) based on aminoglycoside interactions with RNA. In this method, both ligand and RNA motifs are probed simultaneously to identify selective interactions that can be used to target RNA [7]. Several azido-aminoglycosides were spatially arrayed and covalently anchored onto alkyne-functionalized agarose gel. Next, the aminoglycoside microarray was screened with a library of 5'-³²P-labeled internal loop RNAs in the presence of excess competitor nucleotides to ensure specificity. The spots with high ³²P signal were excised directly from the agarose. Bound RNAs were then amplified by RT-PCR and

identified by cloning and sequencing. Finally, privileged RNA motifs for a specific ligand were determined and a database was generated. From these experiments, it was shown that an alkynyl derivative of kanamycin A prefers to bind 2×2 nucleotide, pyrimidine-rich RNA internal loops [8]. These loops are prevalent in diseases of expanded RNA repeats such as myotonic muscular dystrophy type 1 and 2 (DM1 and DM2 respectively). DM is caused when the expanded repeat RNAs sequesters the RNA splicing regulator Muscleblind-like 1 protein (MBNL1), thus compromising its function. Therefore, a potential therapeutic strategy is to prevent MBNL1 from binding the toxic RNA repeats. Since DM2 expansion is an r(CCUG) quadruplet repeat which forms 2×2 nucleotide loop, the authors used their kanamycin A, **1**, and assembled it onto a peptoid backbone [9] After several iterations, changing the valency of the kanamycin modules as well as the distance between them, an optimal compound, **2**, was identified that displayed three kanamycin modules each separated by four spacing peptoid submonomers. This peptoid inhibited the RNA-MBNL1 interaction with an IC_{50} of 25 nM. A similar approach was also used to identify peptoids bearing aminoglycosides [10] or motifs derived from the Hoeschst dye [11] to target other diseases of expanded RNA repeats, such as DM1 and Spinocerebellar Ataxia type 3.

The authors later developed a statistical analysis method called structure–activity relationship through sequencing (StARTS) to score RNA motif–ligand partners selected by 2DCS [12]. This approach was initially developed using aminoglycoside microarrays. However, a more recent incarnation of this work has utilized microarray-based technology to generate a database of RNA–small molecule interactions. The integration of the microarray-based 2DCS and computational StARTS methods provided a powerful technology termed “Inforna” [13], which was applied to identify small molecules that bind to precursor miRNAs (pre-miRNAs) with extraordinarily high selectivity. MicroRNAs (miRNAs) are genetically encoded, short single-stranded RNAs that regulate gene expression, and are produced by processing of a short, pre-miRNA hairpin [14]. Through Inforna, all of the pre-miRNAs in the human transcriptome were mined for overlap with the RNA motif–small molecules interaction database. Lead small molecules were identified for several disease associated pre-miRNA precursors. StARTS analysis of hit compounds provided a “fitness” score for each small molecule interaction with the corresponding RNA motif. For example, benzimidazole **3** was identified to interact with pre-miR-96, a hairpin precursor to miR-96, which is upregulated in cancer and silences Forkhead box protein O1 (FOXO1) through translational repression. This compound gave an exceptionally good fitness score. **3** inhibited the biogenesis of miR-96 by binding to the pre-miR-96 hairpin. In addition, compound **3** treatment caused upregulation of FOXO1 protein, induced apoptosis in cancer cells and exhibited selectivity in targeting miR-96 comparable to an antagomir. In other examples, Inforna has been used to identify compounds that bind to hexanucleotide repeat expansion RNA [15], other miRs (miR-544) [16], and mutant *MAPT* pre-mRNA [17].

Historically, aminoglycosides have served as a useful class of molecules to study RNA–ligand interactions, and in the area of microarrays they are no exception. While aminoglycosides provided the first proof of concept for microarray screening of RNA, modern approaches generally focus on other classes of molecules with potentially increased selectivity and specificity. Importantly, the use of aminoglycoside microarrays provided the

basis to develop a microarray-based 2DCS method that was later coupled with higher-level statistical analysis to broadly map RNA/ligand interactions. This has in turn lead to the development of a powerful technology, Informa, which has been employed in the identification of small molecule RNA binders.

3. Peptoid and peptide microarrays

One of the most popular microarray-based methods to identify ligands for a particular RNA has been to use microarrays of oligomeric molecules. More specifically, microarrays of peptides and peptoids (Fig. 3A) have both been used with success to identify ligands for short RNA hairpins. Both peptides and peptoids are straightforward to synthesize by combinatorial approaches, and are derived from relatively simple, inexpensive, commercially available building blocks. The conjugation methods to attach peptides and peptoids to microarray slides include using cysteine residues that react with maleimide functionalized glass [18,19], hydrazide groups that react with epoxide-functionalized glass [20,21] or azide handles that undergo a cycloaddition reaction with an alkyne-functionalized agarose slide (Fig. 1) [22]. An early example of the use of microarray technology to identify peptoid ligands that bind to an oligonucleotide was reported by Luebke and coworkers [18]. Here, the authors used a microarray platform to screen a library of peptoids that bind selectively to a pre-miRNA and prevent its biogenesis.

The first step of miRNA biogenesis is the RNA polymerase II promoted production of primary miRNAs (pri-miRNAs) [23,24]. Nuclear cleavage of pri-miRNAs by the microprocessor, a complex of the endoribonuclease Drosha with the RNA binding protein DGCR8, produces shorter stem-loop RNAs known as pre-miRNAs. After being transported to the cytosol, pre-miRNAs are processed into ~22 base pair miRNA duplexes by removal of their apical loop by the endoribonuclease Dicer. One strand of the duplex becomes the mature miRNA, which functions as a gene regulator, while the other strand, termed passenger miRNA, is normally removed by degradation. Once the miRNA binds to a complementary sequence of the target mRNA, the mRNA is degraded by the RNA Induced Silencing Complex (RISC). Alternatively, binding of miRNA to a partially complementary mRNA leads to translational repression of target mRNAs.

Luebke and coworkers elected to target oncogenic miR-21 with a library of peptoids [18]. Peptoids (N-substituted oligoglycines) (Fig. 3A) are peptidomimetic polymers with improved proteolytic stability and ease of synthesis. In this case, a combinatorial library of 7680 peptoids was synthesized, each of which was functionalized with a cysteine residue at its C-terminus. Next, each peptoid was spatially arrayed and covalently linked to a maleimide functionalized glass surface. To screen the peptoid library, a 29-nt pre-miR21 hairpin (pre-miR-21 hp) was labeled with a Dy547 (a Cy3-like dye) at its 5'-end and screened at 5 μ M. The authors hypothesized that molecules capable of binding to pre-miR21 hp could interfere with miRNA maturation. In addition to the pre-miR21 hp, a second labeled hairpin with a closely related structure to pre-miR21 hp was screened as a control for specificity. Hits were identified after each array was washed to remove any unbound RNA and scanned with a fluorescence scanner. Spots with a fluorescent signal above background for pre-miR21 hp but not for the control hairpin were subjected to MS/MS analysis to

identify the hit peptoids. Two peptoids, **4** and **5**, were identified to bind to pre-miRNA with K_{ds} of 15 μ M and 10 μ M, respectively, as determined by a fluorescence assay using a 2-aminopurine variant of the hairpin. **4** and **5** also showed a 5-fold and a 2-fold selectivity over the control RNA, respectively. A Mg^{2+} -induced cleavage assay of the pre-miR21 hairpin showed that the presence of **4** induces a conformational change near the apical loop of pre-miR21 hp. Finally, several derivatives of **4** were synthesized to identify the structural determinants of affinity and specificity in the peptoid structure. From this study, peptoid **6** showed a 7-fold improvement in binding affinity over **4** and 20-fold selectivity over the control RNA. However, despite its improved potency, **6** did not show binding to pre-miR21 hp in the presence of Mg^{2+} at levels required for an in *vitro* Drosha assay.

In a follow-up study [19], the authors modified the peptoid library and screening conditions to identify more potent inhibitors with activity in their in *vitro* assays. A larger library of 14,024 peptoids was prepared, arrayed onto slides, and screened with a lower concentration of the labeled pre-miR-21 hp (417 nM) as well as a closely related control RNA hairpin. Peptoid **7** was the most potent out of eleven identified hits. It exhibited a higher binding affinity to pre-miR-21 hp compared to **6**, even in the presence of 5 mM $MgCl_2$. Additionally it had a greater than 15-fold selectivity over the control hairpin (pre-miR-21 hp $K_d = 12 \pm 11$ μ M and control RNA $K_d > 300$ μ M). Peptoid **7** also bound proximal to the pre-miR21 hp apical loop, similar to **6**. Additionally, **7** showed activity in an in *vitro* Drosha cleavage assay and inhibited Drosha cleavage with specificity for pri-miR21 over pri-miR-16. Currently, it is unclear how binding to the apical loop of miR-21 specifically interferes with Drosha processing of the miRNA, and further work will be needed to specifically address this question.

A related use of peptoid microarrays to identify RNA-binding ligands was reported by Disney and coworkers around the same time [25]. Here, a library of 109 arrayed peptoids was screened against *Candida albicans* group I ribozyme. *C. albicans* is a pathogenic fungus known to infect and kill immunocompromised hosts such as AIDS patients. The choice of an RNA target made it possible to evaluate identified peptoids from the screen in a self-splicing assay and determine their IC_{50} values. Here, the peptoid library was designed to include structural features biased toward RNA-binding such as benzimidazoles (present in Hoeschst 33258, **8**, an RNA binding dye) and phenylamidine (present in pentamidine **9**, an FDA-approved drug used for inhibition of group I intron ribozyme). In addition, each peptoid contained an azide handle to facilitate its covalent attachment to alkyne-functionalized agarose slide. Microarrays were screened with 10 nM of ^{32}P -labeled ribozyme in the presence of bovine serum albumin and excess bulk yeast tRNA to avoid nonspecific interaction of RNA with the peptoids. Spots with high levels of ^{32}P were identified as hits after the microarrays were washed to remove unbound RNA and subsequent radiography. Twelve peptoids were identified from the screen and evaluated in the splicing assay. The combined data from the screening, statistical analysis of peptoid binders and their potency identified the structural determinants of binding to RNA, which informed the design and synthesis of peptoid **10**. This peptoid, with an IC_{50} of 31 ± 6 μ M, is 5-fold more potent than the most potent peptoid identified from the microarray screen. Furthermore, it is 6-fold more potent than pentamidine itself ($IC_{50} = 200 \pm 35$ μ M). Thus, a combined approach of

screening with RNA-focused microarrays to identify RNA binders coupled with statistical analysis to identify privileged features for binding to their targets can inform the design of more potent RNA binders (Fig. 4).

In addition to peptoid microarrays, peptide microarrays have also been employed to identify and profile RNA-binding molecules. In 2012, Shin and coworkers utilized a peptide microarray to profile a library of 111 amphiphilic peptides against six RNA hairpins including IRES, RRE, TAR, 16S-rRNA, IRE and TS-mRNA [21]. Amphiphilic peptides that adopt a helical structure have been previously shown to bind tightly to hairpin RNAs. For this study, authors designed the peptide library based on a 16-mer peptide, **11**, which was de novo designed to bind to calmodulin and later shown to bind to hairpin RNAs with high affinity. The sequence of **11** includes a number of positively charged lysines. The peptide library was synthesized using a standard solid-phase peptide synthesis protocol that incorporated a C-terminal hydrazide group to enable the covalent attachment of each peptide to an epoxidecoated glass surface under mild acidic conditions. For the peptide microarray screening, RNAs were labeled on their 5'-terminus with a Cy3-like dye, Dy547, and incubated with the microarrays at a concentration of 0.25 μ M. Next, the fluorescence intensity of each spot was quantified. Impressively, this approach was then used to measure hundreds of dissociation constants (K_d) for RNA-peptide complexes in parallel. Peptides were then rank ordered based on their affinities. In general, modest selectivity was observed between different hairpins. However, most peptides did have submicromolar affinities for the hairpins and the more potent ones showed mid nanomolar affinities. Furthermore, the affinity numbers for all of the peptides showed that while both the hydrophobic and hydrophilic faces of the amphiphilic peptides are likely involved in the interaction with RNAs, the helical nature of peptide does not strongly correlate with the peptide affinity for the hairpin. Finally, peptide **11** was observed to have a K_d of 144 nM for the HIV TAR hairpin (Fig. 5B), and showed inhibition of the Tat-TAR interaction in a cell-based luciferase reporter assay. However, the same peptide had similar if not better K_d s for IRES and RRE and therefore did not show selectivity over different hairpin sequences.

A more recent study from Shin and coworkers used their peptide microarray platform to screen pre-miRNA-155 [20], building on their previous profiling approach [21]. MiRNA-155 is overexpressed in many cancers and has been shown to suppress apoptosis in human cancer. For screening purposes, pre-miRNA-155 was used with the goal that peptides which bind to the RNA can inhibit its processing into mature miRNA-155 by Dicer and therefore induce apoptosis in cancer cells. The peptide library was prepared similar to the previous study, and was based on 185 derivatives of the amphiphilic peptide **1**. Both full-length and truncated pre-miRNA-155 (Fig. 5B) were labeled with a Dy547 and were prepared and screened in parallel. After screening, two peptides, **12** and **13**, were shown to bind to pre-miRNA-155 and inhibit its cleavage by Dicer *in vitro*. Dissociation constants for the interaction between pre-miRNA and these two peptides were measured to be in the mid nanomolar range based on peptide microarrays and fluorescence anisotropy. Next, both **12** and **13** significantly suppressed miRNA-155 processing in MCF-7 cells while increasing the expression of miRNA-155 target genes such as Forkhead box protein O3a (FOXO3a). These peptide inhibitors also induced apoptosis via a caspase-dependent mechanism in MCF-7 and

MDA-MB-231 breast cancer cells (both of which overexpress miRNA-155). Finally, NMR and modeling studies indicate that peptide **12** binds to the upper bulge and apical stem-loop region of pre-miRNA-155, validating the observed inhibition of Dicer-mediated miRNA-155 processing.

In sum, the studies discussed here demonstrate that arrays of oligomeric molecules such as peptides and peptoids are useful tools to discover RNA-binding molecules, especially in targeting RNA hairpin structures. However, all of the identified peptoids and peptides described above include positively charged residues in their sequences to enable their binding to RNA through electrostatic interactions, which often cause nonspecific interactions with the anionic phosphate backbone of RNA. Therefore, peptoids and peptides often do not show much selectivity in binding to a specific RNA of interest despite having reasonable binding constants. In addition, these classes of molecules often suffer from limitations such as susceptibility to proteolytic cleavage, poor permeability/distribution, or poor physicochemical properties. While these limitations make it difficult to advance such molecules as therapeutics, they do not prevent peptides and peptoids from being useful models to study RNA-ligand interactions and establish a basis for small molecule binding.

4. Small molecule microarrays

Most microarray-based approaches to identify RNA binders incorporate an inherent bias toward known RNA-interacting motifs such as cationic peptides, aminoglycosides, or Hoescht dye-like compounds. Work from our laboratory has shown that microarray-based screening of diverse collections of libraries of small molecules that do not contain such functionalities is also an approach to identify novel RNA-binding structures. We assembled a library of 20,000 small molecules with nucleophilic functionalities such as primary alcohols, secondary alcohols, and primary amines, and coupled them to isocyanate-functionalized glass slides (Fig. 1) to assemble small molecule microarrays (SMMs) [26], based on a previous approach [27]. In this case, the library is composed of a diverse collection of small molecules predicted to comply with Lipinski's parameters such as low molecular weight or fewer than five hydrogen bond donors/acceptors [28]. Known RNA- and DNA-interacting motifs are not included in the library. This library can then be screened by incubation with a fluorescently tagged oligonucleotide (typically at a concentration of 500 nM) and the identification of highly fluorescent spots can reveal small molecule-RNA interactions. The first proof of concept study used the HIV transactivation response (TAR) hairpin [26]. TAR is a cis-acting regulatory element at the 5'-untranslated region (UTR) of HIV RNA genome responsible for HIV-1 gene expression and viral replication. In addition to the TAR hairpin, a fluorescently labeled miR-21 hairpin was also screened against the same library as a control for specificity. Several molecules were identified from the screen, and displayed selectivity in binding to HIV-1 TAR (Fig. 6B) over miR-21. A more soluble analog of one of these hits, compound **14**, was found to bind and stabilize the TAR hairpin with a K_d of 2.4 μ M. Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) analysis of **14** performed on a 365-nucleotide sequence derived from the 5'-UTR of the HIV-1 genome showed selective interaction of **14** with TAR over other RNA motifs. Finally, **14** rescued HIV-induced toxicity in T lymphocytes with an EC_{50} of 28 μ M. The same 20,000 member SMM library was next screened against the DNA G-quadruplex (G4)

present in the promoter region of the MYC gene [29]. MYC is an important transcription factor and growth signal, and is an oncogene that is misregulated in 70% of human cancers. The mechanism of transcriptional regulation by G4 formation is not clear, however small molecules able to stabilize G4 have been shown to silence MYC expression. A Cy5-labeled G4 DNA (Fig. 6B) derived from NHEIII₁ region in the MYC promoter was screened using the SMM platform. One of the small molecules identified from the screen, compound **15**, exhibited a K_d of 4.5 μ M to MYC G4. In addition, compound **15** inhibited MYC transcription *in vitro* in a G4-dependent fashion and exhibited a decrease in MYC transcription across a panel of multiple myeloma cell lines. Finally, compound **15** reduced the expression of MYC and MYC target genes while not targeting other genes controlled by G4s, indicating its selectivity. In sum, while our initial efforts using SMMs have been successful in identifying novel selective classes of small molecules against a hairpin RNA as well as a G4 DNA, more work needs to be done to indicate the generality of this platform in identifying small molecules targeting noncoding nucleic acids. It is anticipated that this platform will have broad utility in identifying new RNA-binding small molecule scaffolds in the future.

5. Conclusion and outlook

It is clear that microarray-based technologies hold substantial promise for the discovery of RNA-binding molecules. As illustrated in this review, the strengths of microarray-based ligand screening platforms include the small amount of ligand and RNA that are required for this approach as compared to other conventional screening methods. Through microarray-based screening, a large number of interactions can be simultaneously screened in parallel. Additionally, if the ligand loading is known and can be accurately quantified, binding constants can be obtained from the screening as well. Microarray-based ligand screening is also amenable to immobilization of oligomers as well as small molecules. In addition, as was discussed, a number of RNA structures (hairpins, loops, internal bulges, and oligonucleotide repeats) can be screened. Another powerful approach is parallel screening with multiple RNAs to identify selective ligands, which can be deconvoluted with sequencing or differentially tagged RNAs. Additionally, when coupled with complementary biochemical techniques such as dimethyl sulfate (DMS) or nuclease footprinting, SHAPE, or NMR, as well as biological evaluation, microarray-based approaches provide a unique mechanism to identify and study new types of RNA/ligand interactions. Finally, one limitation of this platform includes the requirement of selecting an appropriate immobilization chemistry and the potential effects of immobilization on ligand presentation and its' binding to RNA. However, it is clear that microarrays represent a unique and powerful approach to identify new classes of highly selective, structurally unique RNA-binding molecules and will have continued use in the development of RNA-targeting small molecule probes and therapeutics.

Acknowledgment

This work was supported by the Intramural Research Program of the National Institutes of Health, Center for Cancer Research, the National Cancer Institute (NCI), and the IATAP program, National Institutes of Health.

References

- [1]. Koehler AN, Shamji AF, Schreiber SL, J. Am. Chem. Soc 125 (2003) 8420–8421. [PubMed: 12848532]
- [2]. Barnes-Seeman D, Park SB, Koehler AN, Schreiber SL, Angew. Chem. Int. Ed 42 (2003) 2376–2379.
- [3]. Kawasumi M, Bradner J, Kim Y, Koehler A, Mazitschek R, Schreiber S, Nghiem P, J. Invest. Dermatol 124 (2005) A39.
- [4]. Thomas JR, Hergenrother PJ, Chem. Rev 108 (2008) 1171–1224. [PubMed: 18361529]
- [5]. Disney MD, Magnet S, Blanchard JS, Seeberger PH, Angew. Chem. Int. Ed. Engl 43 (2004) 1591–1594. [PubMed: 15022242]
- [6]. Bryan MC, Wong CH, Tetrahedron Lett 45 (2004) 3639–3642.
- [7]. Disney MD, Labuda LP, Paul DJ, Poplawski SG, Pushechnikov A, Tran T, Velagapudi SP, Wu M, Childs-Disney JL, J. Am. Chem. Soc 130 (2008) 11185–11194. [PubMed: 18652457]
- [8]. Childs-Disney JL, Wu ML, Pushechnikov A, Aminova O, Disney MD, ACS Chem. Biol 2 (2007) 745–754. [PubMed: 17975888]
- [9]. Lee MM, Pushechnikov A, Disney MD, ACS Chem. Biol 4 (2009) 345–355. [PubMed: 19348464]
- [10]. Lee MM, Childs-Disney JL, Pushechnikov A, French JM, Sobczak K, Thornton CA, Disney MD, J. Am. Chem. Soc 131 (2009) 17464–17472. [PubMed: 19904940]
- [11]. Pushechnikov A, Lee MM, Childs-Disney JL, Sobczak K, French JM, Thornton CA, Disney MD, J. Am. Chem. Soc 131 (2009) 9767–9779. [PubMed: 19552411]
- [12]. Velagapudi SP, Seedhouse SJ, Disney MD, Angew. Chem. Int. Ed 49 (2010) 3816–3818.
- [13]. Velagapudi SP, Gallo SM, Disney MD, Nat. Chem. Biol 10 (2014) 291–297. [PubMed: 24509821]
- [14]. Bartel DP, Cell 116 (2004) 281–297. [PubMed: 14744438]
- [15]. Su Z, Zhang Y, Gendron TF, Bauer PO, Chew J, Yang WY, Fostvedt E, Jansen-West K, Belzil VV, Desaro P, Johnston A, Overstreet K, Oh SY, Todd PK, Berry JD, Cudkowicz ME, Boeve BF, Dickson D, Floeter MK, Traynor BJ, Morelli C, Ratti A, Silani V, Rademakers R, Brown RH, Rothstein JD, Boylan KB, Petrucelli L, Disney MD, Neuron 83 (2014) 1043–1050. [PubMed: 25132468]
- [16]. Haga CL, Velagapudi SP, Strivelli JR, Yang WY, Disney MD, Phinney DG, ACS Chem. Biol 10 (2015) 2267–2276. [PubMed: 26181590]
- [17]. Luo Y, Disney MD, ChemBioChem 15 (2014) 2041–2044. [PubMed: 25115866]
- [18]. Chirayil S, Chirayil R, Luebke KJ, Nucleic Acids Res 37 (2009) 5486–5497. [PubMed: 19561197]
- [19]. Diaz JP, Chirayil R, Chirayil S, Tom M, Head KJ, Luebke KJ, RNA 20 (2014) 528–539. [PubMed: 24497550]
- [20]. Pai J, Hyun S, Hyun JY, Park SH, Kim WJ, Bae SH, Kim NK, Yu J, Shin I, J. Am. Chem. Soc 138 (2016) 857–867. [PubMed: 26771315]
- [21]. Pai J, Yoon T, Kim ND, Lee IS, Yu J, Shin I, J. Am. Chem. Soc 134 (2012) 19287–19296. [PubMed: 23110629]
- [22]. Velagapudi SP, Pushechnikov A, Labuda LP, French JM, Disney MD, ACS Chem. Biol 7 (2012) 1902–1909. [PubMed: 22958065]
- [23]. He L, Hannon GJ, Nat. Rev. Genet 5 (2004) 522–531. [PubMed: 15211354]
- [24]. Ameres SL, Zamore PD, Nat. Rev. Mol. Cell Biol 14 (2013) 475–488. [PubMed: 23800994]
- [25]. Labuda LP, Pushechnikov A, Disney MD, ACS Chem. Biol 4 (2009) 299–307. [PubMed: 19278238]
- [26]. Sztuba-Solinska J, Shenoy SR, Gareiss P, Krumpe LR, Le Grice SF, O’Keefe BR, Schneekloth JS, Jr., J. Am. Chem. Soc 136 (2014) 8402–8410. [PubMed: 24820959]
- [27]. Bradner JE, McPherson OM, Koehler AN, Nat. Protoc 1 (2006) 2344–2352. [PubMed: 17406478]
- [28]. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ, Adv. Drug Delivery Rev 23 (1997) 3–25.

- [29]. Felsenstein KM, Saunders LB, Simmons JK, Leon E, Calabrese DR, Zhang S, Michalowski A, Gareiss P, Mock BA, Schneekloth JS, Jr., ACS Chem. Biol (2015).
- [30]. Gude L, Berkovitch SS, Santos WL, Kutchukian PS, Pawloski AR, Kuimelis R, McGall G, Verdine GL, J. Biol. Chem 287 (2012) 18843–18853. [PubMed: 22451672]
- [31]. Kierzek R, Turner DH, Kierzek E, Nucleic Acids Res 43 (2015) 1–12. [PubMed: 25505162]

Fig. 1. Strategies for generating microarrays used to screen and profile RNA. Depicted are aminoglycoside, peptide, peptoid, and small molecule arrays as well as multiple immobilization chemistries.

Fig. 2.

A. Two-dimensional combinatorial screening (2DCS). Ligands are immobilized on a functionalized-agarose. Microarrays are screened with a library of ³²P-labeled RNA library and bound ³²P-labeled RNAs are visualized by radiography and excised from the gel. RNAs are then identified by cloning and sequencing, producing A database of privileged RNA motifs. B. Structure of kanamycin module **1** assembled onto a peptoid backbone **2**. Peptoid **2** targets r(CCUG) repeats and inhibit formation of DM2 RNA-MBNL1 complex. C. compound **3** was discovered by Infora to inhibit miRNA-96.

Fig. 3.

A. General structure of peptide and peptoid backbones. B. Secondary structure of the miR-21 hairpin used to screen a peptoid library. C. Structures of peptoids **4–7** that bind to miR-21 hairpin

Hoechst 33258

8

Pentamidine

9

10

 $IC_{50} = 31 \pm 6 \mu M$ **Fig. 4.**

Structures of the RNA binding dyes Hoechst 33258, an FDA-approved drug containing two phenylamidines (Pentamidine), and peptoid 10, which inhibits *C. albicans* group I ribozyme.

Fig. 5.

A. Sequences of peptides **11–13**. Positively charged lysine residues highlighted in blue. B. Secondary structures of HIV TAR hairpin, pre-miR-155, and truncated pre-miR-155 used to screen peptide microarrays.

Fig. 6.

A. Structures of hits identified from SMM screens that bind to the HIV TAR hairpin (**14**) and the MYC G-quadruplex (**15**). B. Secondary structure of HIV TAR hairpin and cartoon representation of MYC DNA G-quadruplex.