Published in final edited form as:

Methods Enzymol. 2019 ; 623: 85–99. doi:10.1016/bs.mie.2019.04.020.

A click chemistry assay to identify natural product ligands for pre-microRNAs

Amanda L. Garner^{a,b,*}, Daniel A. Lorenz^b, and Erin E. Gallagher^a

^aDepartment of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI, United States

^bProgram in Chemical Biology, University of Michigan, Ann Arbor, MI, United States

Abstract

Despite the great diversity of structure and function and relevance to human health, RNA remains an underexploited area of drug discovery. A major bottleneck toward this goal has been the identification of probes and drug leads that are specific for select RNAs and methods that will facilitate such discovery efforts. Our laboratory has recently developed an innovative approach for assaying RNA-small molecule interactions, catalytic enzyme-linked click chemistry assay or cat-ELCCA, which is a functional assay that takes advantage of the power of catalytic signal amplification combined with the selectivity and bioorthogonality of click chemistry. Importantly, through application of this platform assay technology to the challenging problem of identifying selective inhibitors of pre-microRNA maturation, we identified natural products as a potential source of such compounds. Herein we describe this methodology in addition to the downstream pipeline toward the discovery of natural product ligands for pre-microRNAs. Through cat-ELCCA, our goal is to discover novel ligands to facilitate our investigation of RNA recognition by small molecules.

1. Introduction

RNA-targeted probe and drug discovery is an exciting, yet challenging area of medicinal chemistry (Connelly, Moon, & Schneekloth, 2016; Garner, 2018b; Thomas & Hergenrother, 2008). A major bottleneck toward promoting this field is the discovery of new chemical space for targeting RNAs. Key examples from the discovery of ribocil, a synthetic ligand for the FMN riboswitch (Howe et al., 2015), and RNA-binding SMN-C and NVS-SM1 SMN2 splicing modulators (Naryshkin et al., 2014; Palacino et al., 2015; Sivaramakrishnan et al., 2017), have provided recent evidence for the potential druggability of RNA. Yet, these molecules were identified from phenotypic screening approaches, and the direct targeting of RNA with small molecules remains difficult, particularly as most efforts have centered solely on rational design strategies using a limited number of potentially promiscuous chemotypes, such as Hoechst dyes and aminoglycosides (Lorenz & Garner, 2018).

*Corresponding author: algarner@umich.edu.

Some of the earliest examples of RNA-binding molecules have come from natural products (NPs), and aminoglycoside, tetracycline and macrolide antibiotics function by binding to ribosomal RNA (rRNA) within the bacterial ribosome (Wilson, 2014). Historically, NPs have been invaluable for developing front-line drugs against cancer, infectious diseases, and parasites, and as tools for furthering our understanding of cellular biology (Newman & Cragg, 2012). In fact, a survey of all 246 anti-tumor agents approved by the FDA between the late 1940s and 2014 showed that >65% were either NPs or NP analogues, indicating the overwhelming success of NPs in the human pharmacopeia (Newman & Cragg, 2012). Despite this fact, the role of NPs in probe and drug discovery has diminished in recent years, in part due to the technical challenges of screening NP extract (NPE) libraries using traditional targeted assay formats, such as fluorescence polarization (FP) and fluorescence resonance energy transfer (FRET) assays (Harvey, Edrada-Ebel, & Quinn, 2015).

To facilitate the discovery of new chemical entities for targeting RNAs, we have developed high-throughput screening (HTS) technology termed catalytic enzyme-linked click chemistry assay, or cat-ELCCA, which is a robust screening platform that does not suffer from compound interference (Garner, 2018a; Lorenz & Garner, 2016, 2018; Lorenz, Song, & Garner, 2015; Lorenz, Vander Roest, Larsen, & Garner, 2018). Key advantages of cat-ELCCA include its increased sensitivity due to catalytic signal amplification, negligible compound interference in comparison to traditional fluorescence-based assays due to added washing steps, and HTS applicability with Z' factors >0.6 using automated liquid handling (Garner, 2018a; Garner & Janda, 2010, 2011; Lorenz & Garner, 2016, 2018; Lorenz, Kaur, et al., 2018; Lorenz et al., 2015; Lorenz, Vander Roest, et al., 2018; Song, Menon, Mitchell, Johnson, & Garner, 2017; Zhang, Chung, & Oldenburg, 1999). As proof of concept for RNA-targeted probe discovery, we applied cat-ELCCA toward the goal of identifying selective and RNA-binding inhibitors of Dicer-mediated microRNA (miRNA or miR) maturation (Fig. 1) (Garner, 2018a; Lorenz & Garner, 2016, 2018; Lorenz et al., 2015; Lorenz, Vander Roest, et al., 2018). Using this approach, we have recently completed a HTS of commercial small molecules and NPEs to identify compounds and areas of chemical space capable of selectively inhibiting the processing of oncogenic pre-miR-21 (Lorenz, Vander Roest, et al., 2018). From this screening campaign, we excitingly found that NPs may be a potential source of such molecules, indicating that more highly functionalized molecular scaffolds, as represented by NPs, may be necessary for selectively disrupting pre-miRNA-Dicer interactions and recognizing simple hairpin structures like pre-miRNAs. Herein we describe our cat-ELCCA methodology and the subsequent downstream assays that can be used to identify natural product ligands for targeting pre-miRNAs.

2. cat-ELCCA for dicer-mediated pre-miRNA maturation

In order to be active, miRNAs undergo two maturation steps, one in the nucleus (pri-miRNA to pre-miRNA) and one in the cytoplasm (pre-miRNA to mature miRNA), mediated by the RNase III enzymes Drosha and Dicer, respectively (Fig. 1) (Ha & Kim, 2014). Thus, as a biological basis for the targeting of miRNAs, we hypothesized that compounds able to productively bind to a pre-miRNA would inhibit miRNA maturation, and thereby, activity (Fig. 1). The targeting of pre-miRNAs was chosen due to their favorable size for assay

development, screening and chemical synthesis, in addition to cytoplasmic localization for intracellular targeting with a small molecule.

The scheme for cat-ELCCA for Dicer-mediated pre-miRNA maturation is shown in Fig. 2 (Garner, 2018a; Lorenz et al., 2015; Lorenz & Garner, 2016, 2018). In brief, a chemically synthesized, *trans*-cyclooctene (TCO)-labeled pre-miRNA substrate is first immobilized into the wells of a 384-well streptavidin-coated microtiter plate. In the presence of Dicer, the terminal loop is cleaved, and following reaction with a methyltetrazine (mTet) modified-horseradish peroxidase (HRP), no signal is observed. In the presence of an inhibitor, such as an active NPE or purified pre-miRNA ligand (as shown), the loop is retained, resulting in catalytic signal amplification from the covalently click chemistry-linked HRP.

An overview of the work flow toward the goal of identifying selective natural product ligands for a pre-miRNA is shown in Fig. 3. Following HTS of NPEs, hit validation is first performed by re-testing positive NPEs in cat-ELCCA (step 1). Reproducible hit extracts are subsequently analyzed for selectivity by examining inhibitory activity against Dicer-mediated processing of a second pre-miRNA substrate using cat-ELCCA (step 2). The activity of validated hits showing selectivity for the pre-miRNA-of-interest are then tested in a gel-based secondary assay of Dicer activity to eliminate false hits due to the assay format (step 3). Iterative testing of further fractionated samples (step 4) in cat-ELCCA and the gel-based assay is performed until a purified, active natural product is identified (step 5). IC₅₀ values in cat-ELCCA are then measured against the pre-miRNA target-of-interest, in addition to other substrates to assess selectivity (step 6). Direct binding to the pre-miRNA is finally measured via surface plasmon resonance (SPR) spectroscopy to determine binding kinetics and affinity (step 7).

3. Preparation of assay reagents

3.1 Equipment

- Orbital shaker
- Refrigerated centrifuge
- Nanodrop spectrophotometer
- Centrifugal filter (10,000MW cut-off)
- Mass spectrometer

3.2 Buffers and reagents

- Chemically synthesized pre-miRNA substrate (Dharmacon)
- Dimethyl sulfoxide (DMSO)
- (TCO)-PEG4-NHS (Click Chemistry Tools, A137)
- HRP (Pierce 31491)
- mTet-NHS (Click Chemistry Tools, 1128)
- Phosphate buffer (100mM, pH 8.0)

- Sodium acetate buffer (3.0M, pH 5.2)
- Cold ethanol
- Phosphate-buffer saline (PBS) (100mM phosphate, 150mM NaCl, pH 7.0)
- Bradford assay (Pierce)

3.3 Procedure

3.3.1 Preparation of pre-miRNA substrate

1. Design a pre-miRNA substrate (Fig. 4) to contain the following modifications:
(1) An 18-atom polyethylene glycol (PEG)-based biotin linker at the 5' phosphate terminus. Of note, modification is made at the 5' end to maintain the 3' 2-nucleotide overhang that is recognized by Dicer (Ha & Kim, 2014). (2) An aminoallyl uridine in place of a uridine or other nucleotide in the terminal loop that is cleaved by Dicer. Prior to use, the RNA must be deprotected, desalted and HPLC purified, which can be done by the manufacturer.
2. Dissolve the pre-miRNA substrate in phosphate buffer to a concentration of 1.0mM.
3. Prepare a stock solution of (TCO)-PEG4-NHS in DMSO to a concentration of 10mM.
4. Transfer 5.0 μ L of pre-miRNA substrate solution and 5.0 μ L of TCO solution to a 1.5mL microcentrifuge tube. Shake the reaction mixture at 25 °C for 1h.
5. Transfer the tube to an ice bucket, and precipitate the RNA by addition of $0.11 \times$ volume sodium acetate (1.1 μ L) and $4 \times$ volume cold ethanol (40 μ L). Centrifuge at $20,000 \times g$ for 40min at 4 °C.
6. Resuspend the RNA pellet in phosphate buffer at a concentration of 1.0mM based on the amount of RNA originally added to the tube. RNA concentration can be measured using a Nanodrop spectrophotometer. Store at -80 °C for long-term use.

3.3.2 Preparation of labeled HRP

1. Prepare a stock solution of mTet-NHS in DMSO to a concentration of 100mM.
2. Add 2.5mg HRP to a microcentrifuge tube and dissolve in 186 μ L of PBS. To the same tube, transfer 14 μ L of mTet solution (Fig. 5). Shake the reaction mixture at 25 °C for 3h.
3. Transfer the reaction mixture to a microcentrifuge concentrator (0.5mL), concentrate to 50–100 μ L and exchange the labeled HRP into PBS (5×400 μ L) to remove unreacted mTet-NHS and DMSO.
4. Determine protein concentration via the Bradford assay using BSA standards.
5. Store at 4 °C for long-term use.

4. High-throughput screening of natural product extract libraries

Note: This protocol can also be performed using a multi-channel pipette.

4.1 Equipment

- Black, standard-capacity streptavidin-coated 384-well plates(Pierce 15,407)
- Plate washer (Biotek 405 ELX or equivalent)
- Plate centrifuge
- Plate tape (Pierce)
- Multidrop Combi or comparable reagent dispenser
- Refrigerator or cold room
- Sciclone liquid handler with V&P pintool (or analogous liquid handling set-up)
- Chemiluminescence plate reader (PHERAstar or equivalent)

4.2 Buffers and reagents

- Pre-miRNA substrate from Section 3.3.1
- Phosphate buffer (100mM, pH 7.0)
- Dicer buffer (20mM Tris (pH 7.5), 12mM NaCl, 2.5mM MgCl₂, 1mM fresh DTT, 4.5% DMSO)
- NPE library (75 µg/mL) or purified NPs
- DMSO
- Human Dicer (108 µg/mL) (Lorenz & Garner, 2016; MacRae, Ma,Zhou, Robinson, & Doudna, 2008)
- mTet-HRP from Section 3.3.2
- Wash buffer (2mM imidazole, 260mM NaCl, 0.5mM EDTA, 0.1% Tween-20, pH 7.0)
- SuperSignal West Pico (Pierce)

4.3 Procedure

4.3.1 Immobilization of pre-miRNA substrate

1. Wash the wells of the streptavidin plate with 3×50 µL of phosphate buffer using a plate washer.
2. Transfer 5 µL of pre-miRNA substrate (500nM final) to the wells using a reagent dispenser.
3. Centrifuge the plates at $223 \times g$ for 1min.
4. Seal the plate with plate tape and incubate overnight at 4 °C.

5. Wash the wells with 3 × 50 µL of phosphate buffer using a plate washer.

4.3.2 Dicer-mediated pre-miRNA maturation

1. Transfer 5 µL of Dicer buffer to each of the wells using a reagent dispenser and centrifuge the plate at 223 × *g* for 1 min.
2. Add the NPEs to each well (50 nL; 75 µg/mL final) using a liquid handler. Of note, the same volume of DMSO should be added to the control wells. A representative plate map for the assay can be found in Fig. 6.
3. Incubate the plates at 25 °C for 15 min.
4. Transfer 5 µL of Dicer buffer containing 217 µg/nL Dicer (108 µg/mL Dicer, 5% glycerol, 0.01% Triton X-100 final; excess with respect to the concentration of immobilized pre-miRNA) using a reagent dispenser. An equivalent volume of Dicer buffer without enzyme should be added to the positive control wells (Fig. 6).
5. Centrifuge the plates at 223 × *g* for 1 min.
6. Seal the plate with plate tape and incubate for 5 h at 37 °C.
7. Wash the wells with 3 × 50 µL of phosphate buffer using a plate washer.

4.3.3 Click chemistry with mTet-HRP

1. Transfer 10 µL of mTet-HRP (750 nM final) to the wells using a reagent dispenser.
2. Centrifuge the plates at 223 × *g* for 1 min.
3. Seal the plate with plate tape and incubate for 2 h at 25 °C.
4. Wash the wells with 3 × 50 µL of wash buffer and 3 × 50 µL of phosphate buffer using a plate washer.

4.3.4 Chemiluminescence detection

1. Transfer 25 µL of SuperSignal West Pico solution, prepared according to the manufacturer's instructions, to the wells using a reagent dispenser.
2. Incubate for 5 min at 25 °C.
3. Measure chemiluminescence signal using a PHERAstar plate reader using the LUM plus module on autogain set to one of the positive control wells.

4.3.5 NPE hit validation and selectivity assessment

1. Following HTS, all NPEs showing 10% inhibition by plate based on the negative controls should be retested in triplicate for activity validation using cat-ELCCA as described above. NPEs showing inhibition at 3 standard deviations (SD) by plate from the negative controls can be considered confirmed hits.

2. Preliminary selectivity can be assessed by measuring inhibitory activity in cat-ELCCA using a second pre-miRNA substrate (prepared as in Section 3.3.1) and repeating procedures 4.3.1–4.3.5.
3. Dose-dependence of inhibition can be measured by performing cat-ELCCA across varying concentrations of NPEs or NPs to calculate IC₅₀ values.

5. Gel-based assay of dicer activity

5.1 Equipment

- SDS PAGE equipment
- Fluorescence gel imager (Protein Simple FluorChem M or equivalent)

5.2 Buffers and reagents

- Pre-miRNA substrates from Section 3.3.1 (can be labeled or unlabeled) Human Dicer (108 µg/mL) (Lorenz & Garner, 2016; MacRae et al., 2008)
- Dicer buffer (20mM Tris (pH 7.5), 12mM NaCl, 2.5mM MgCl₂, 1mM fresh DTT, 40U/mL RNaseOUT RNase inhibitor (Life Technologies)) NPEs (75 µg/mL) or purified NPs
- DMSO
- 12.5% TBE-urea gels (Life Technologies)
- SYBR Gold nucleic acid stain (Life Technologies)
- miRNA marker (New England Biolabs)

5.3 Procedure

1. Prepare a solution of pre-miRNA (0.5 µL, 50 µM stock) in Dicer buffer (8.5 µL) in a microcentrifuge tube.
2. Add the NPE/NP or DMSO (0.5 µL) and incubate at 25 °C for 15min.
3. Add Dicer in Dicer buffer (0.5 µL) and incubate at 37 °C for 30min.
4. Analyze the Dicer digestion reaction (10-µL total volume) by running the sample on a 12.5% TBE-Urea gel. Load a miRNA marker for visualization of 17, 21 and 25 residue small RNAs as a control. Electrophorese to obtain separation of the unreacted pre-miRNA and mature miRNA strands.
5. Stain the gel using SYBR Gold as according to the manufacturer's instructions.
6. Image on a fluorescence gel imager. If the NPE/NP is able to inhibit Dicer maturation, some retention of the pre-miRNA band will be observed as shown in step 3 of Fig. 3. If Dicer is still able to process the pre-miRNA, smaller miRNA bands will be observed.

6. SPR assay for pre-miRNAs

6.1 Equipment

- SPR (SensiQ Pioneer or equivalent)
- QDAT software

6.2 Buffers and reagents

- BioCap SPR chips
- Pre-miRNA substrate from Section 3.3.1
- Purified natural products
- Running buffer (50mM Tris (pH 7.6), 150mM NaCl, 5% glycerol, 0.05% Tween-20, 1mM magnesium chloride, 5% DMSO)
- Conditioning buffer (1M NaCl, 50mM NaOH)
- 0.5% SDS solution
- High salt running buffer (50mM Tris (pH 7.6), 850mM NaCl, 5% glycerol, 0.05% Tween-20, 1mM magnesium chloride, 5% DMSO)
- Regeneration buffer (50mM Tris (pH 7), 260mM NaCl, 0.5mMEDTA, and 0.1% Tween-20)

6.3 Procedure

1. Dock a BioCap chip into the SPR.
2. Prime the instrument $5 \times$ with running buffer.
3. Condition the chip with two 30 μ L injections of conditioning buffer, one 30 μ L injection of 0.5% SDS, a third 30 μ L injection of conditioning buffer, and one 30 μ L injection of high salt running buffer.
4. Prepare the NPs to be tested by dissolving in 800 μ L running buffer. Serially dilute the stock solution to make working stock solutions at 5 different concentrations (400 μ L total volume). Typical starting concentrations for analysis are 3–50 μ M.
5. Immobilize the pre-miRNA on channel 1 with a 100 μ L injection of 750nM RNA in high salt running buffer at a flow rate of 10 μ L/min to a final RU of about 2600 to saturate the chip. Channels 2 and 3 should be used as identical reference channels.
6. Test the compounds in random order by injecting 50 μ L of each compound at 50 μ L/min with a dissociation time of 60s.
7. Every four injections, inject 50 μ L of running buffer at a flow rate of 50 μ L/min and a dissociation time of 60s as a blank run.

8. After each compound or blank injection, regenerate the chip with a 10 μ L injection of regeneration buffer at a flow rate of 10 μ L/min.
9. Analyze the data using the manufacturer provided QDAT software.

7. Outlook

This chapter describes the use of cat-ELCCA, a biochemical assay that uses click chemistry-mediated catalytic signal amplification, for the discovery of new chemical space for targeting miRNAs. Although we have described its application toward natural products discovery, the pipeline outlined could also be used for the identification of small molecule pre-miRNA ligands from commercial or industrial compound collections. As the discovery of direct-binding, functional inhibitors of RNA biology is becoming a high priority in RNA-targeted drug discovery campaigns, assays like cat-ELCCA will play an important role in these early stage efforts. Further, as we have also demonstrated the applicability of cat-ELCCA to RNA-protein interactions (Lorenz, Kaur, et al., 2018), another important area of nucleic acid biology with therapeutic potential, we foresee that many important tools for interrogating this new frontier in drug discovery will arise from this platform assay technology.

Acknowledgments

This work was seedorted by a pilot grant from the University of Michigan Center for the Discovery of New Medicines and the NIH (R01 GM118329 to A.L.G. and T32 GM007767 to E.E.G.).

References

- Connelly CM, Moon MH, & Schneekloth JS Jr. (2016). The emerging role of RNA as a therapeutic target for small molecules. *Cell Chemical Biology*, 23, 1077–1090. [PubMed: 27593111]
- Garner AL (2018a). cat-ELCCA: Catalyzing drug discovery through click chemistry. *Chemical Communications*, 54, 6531–6539. [PubMed: 29781014]
- Garner AL (2018b). RNA Therapeutics; Topics in Medicinal Chemistry. vol. 27 Springer.
- Garner AL, & Janda KD (2010). cat-ELCCA: A robust method to monitor the fatty acid acyltransferase activity of ghrelin O-acyltransferase (GOAT). *Angewandte Chemie, International Edition*, 49, 9630–9634. [PubMed: 20845345]
- Garner AL, & Janda KD (2011). A small molecule antagonist of ghrelin O-acyltransferase (GOAT). *Chemical Communications*, 47, 7512–7514. [PubMed: 21594284]
- Ha M, & Kim VN (2014). Regulation of microRNA biogenesis. *Nature Reviews. Molecular Cell Biology*, 15, 509–524. [PubMed: 25027649]
- Harvey AL, Edrada-Ebel R, & Quinn RJ (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews. Drug Discovery*, 14, 111–129. [PubMed: 25614221]
- Howe JA, Wang H, Fischmann TO, Balibar CJ, Xiao L, Galgoci AM, et al. (2015). Selective small-molecule inhibition of an RNA structural element. *Nature*, 526, 672–677. [PubMed: 26416753]
- Lorenz DA, & Garner AL (2016). A click chemistry-based microRNA maturation assay optimized for high-throughput screening. *Chemical Communications*, 52, 8267–8270. [PubMed: 27284591]
- Lorenz DA, & Garner AL (2018). Approaches for the discovery of small molecule ligands targeting microRNAs. *Topics in Medicinal Chemistry*, 27, 79–110.
- Lorenz DA, Kaur T, Kerk SA, Gallagher EE, Sandoval J, & Garner AL (2018). Expansion of cat-ELCCA for the discovery of small molecule inhibitors of the prelet-7-Lin28 RNA-protein interaction. *ACS Medicinal Chemistry Letters*, 9, 517–521. [PubMed: 29937975]

- Lorenz DA, Song JM, & Garner AL (2015). High-throughput platform assay technology for the discovery of pre-microRNA-selective small molecule probes. *Bioconjugate Chemistry*, 26, 19–23. [PubMed: 25506628]
- Lorenz DA, Vander Roest S, Larsen MJ, & Garner AL (2018). Development and implementation of an HTS-compatible assay for the discovery of selective small-molecule ligands for pre-microRNAs. *SLAS Discovery: Advancing Life Sciences R & D*, 23, 47–54. [PubMed: 28686847]
- MacRae IJ, Ma E, Zhou M, Robinson CV, & Doudna JA (2008). In vitro reconstitution of the human RISC-loading complex. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 512–517. [PubMed: 18178619]
- Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z, et al. (2014). Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science*, 345, 688–693. [PubMed: 25104390]
- Newman DJ, & Cragg GM (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products*, 75, 311–335. [PubMed: 22316239]
- Palacino J, Swalley SE, Song C, Cheung AK, Shu L, Zhang X, et al. (2015). SMN2 splice modulators enhance U1-pre-mRNA association and rescue SMA mice. *Nature Chemical Biology*, 11, 511–517. [PubMed: 26030728]
- Sivaramakrishnan M, McCarthy KD, Campagne S, Huber S, Meier S, Augustin A, et al. (2017). Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule splicing modifiers. *Nature Communications*, 8, 1476.
- Song JM, Menon A, Mitchell DC, Johnson OT, & Garner AL (2017). High-throughput chemical probing of full-length protein-protein interactions. *ACS Combinatorial Science*, 19, 763–769. [PubMed: 29112379]
- Thomas JR, & Hergenrother PJ (2008). Targeting RNA with small molecules. *Chemical Reviews*, 108, 1171–1224. [PubMed: 18361529]
- Wilson DN (2014). Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nature Reviews. Microbiology*, 12, 35–48. [PubMed: 24336183]
- Zhang J-H, Chung TDY, & Oldenburg KR (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *Journal of Biomolecular Screening*, 4, 67–73. [PubMed: 10838414]

Fig. 1. miRNA biogenesis and activity, and the impact of targeting miRNAs with a natural product inhibitor of Dicer-mediated maturation. Ago2=argonaute 2. DGCR8=DiGeorge syndrome chromosomal region 8. miRISC=miRNA-loaded RNA-induced silencing complex. TRBP=TAR RNA binding protein.

Fig. 2.
cat-ELCCA for Dicer-mediated pre-miRNA maturation.

Fig. 3.
Overview of the pipeline for the discovery of natural product ligands for premiRNAs.

Fig. 4.
Synthesis of TCO-labeled pre-miRNA substrate for cat-ELCCA.

Fig. 5.
Synthesis of mTet-labeled HRP for cat-ELCCA.

Fig. 6.
Representative plate map for cat-ELCCA.