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Targeting RNA

Unique challenges face developers of drugs that hit disease-related RNAs rather than disease-related proteins

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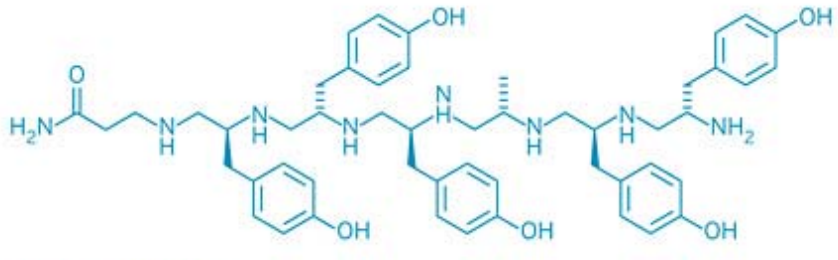
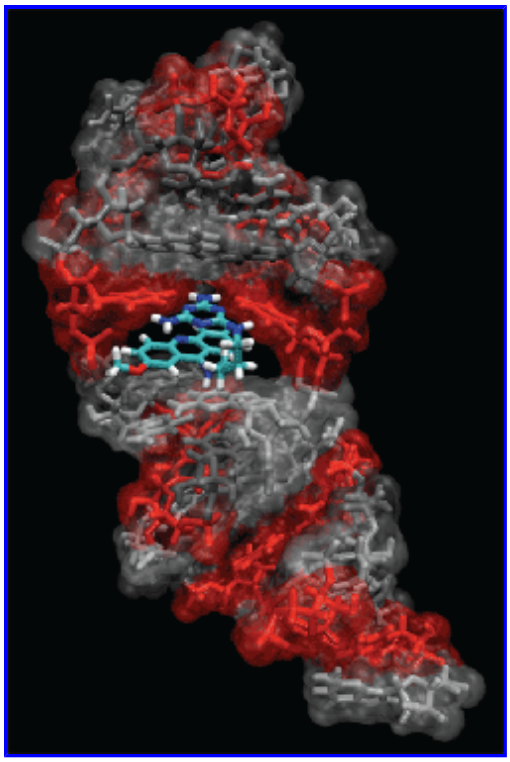


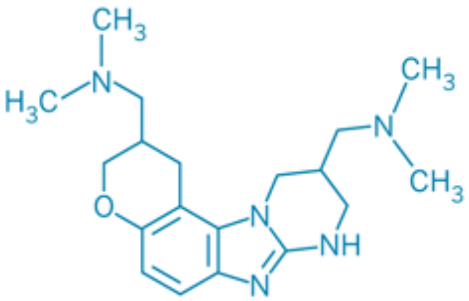
HIV INHIBITOR This multivalent-binding oligomer interferes with Tat-TAR binding, which boosts replication of the AIDS virus.



Courtesy of Anne Baranger and Steven Zimmerman

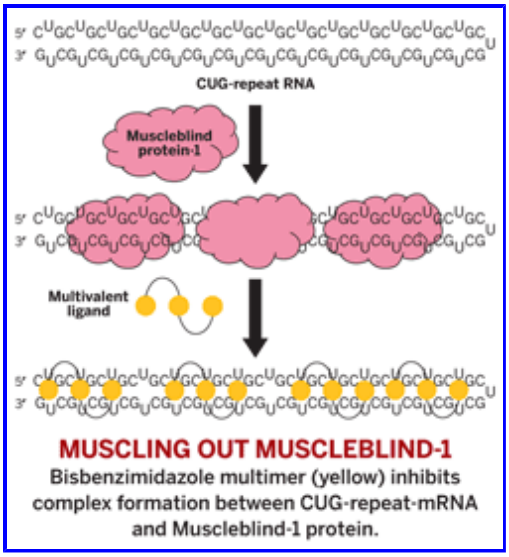
View Enlarged Image

IT'S A WRAP Model shows how triaminotriazine-acridine conjugate (cyan, red, blue, and white) may bind to mismatched U-U base pairs (red bands) in CUG-repeat mRNA (mostly gray).



HCV ENEMY

Benzimidazoles like this one inhibit translation of hepatitis C virus genes.



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Developing drugs that target RNA continues to gain traction. Short RNA sequences that interfere with translation of messenger RNA are making their way through clinical trials. Now, drug discoverers are increasingly testing the idea that nonnucleotide small molecules that selectively target RNA sites might be therapeutically useful. The effort to discover such molecules with druglike properties is intensifying. But it isn't easy.

"In medicinal chemistry and pharmaceuticals, the traditional targets are proteins, most commonly active sites of enzymes," says Daniel H. Appella of the synthetic bioactive molecules group at the National Institute of Diabetes & Digestive & Kidney Diseases, in Bethesda, Md. "That's the tried-and-true method. We have amassed a lot of molecular scaffolds and ideas on how to target protein active sites."

Switching to RNA targets means "we're living in another world," Appella says. "We have to start almost from scratch to create new libraries and rethink strategies for finding small-molecule drugs that target RNA."

Targeting RNA with small molecules isn't new. Some antibiotics, such as aminoglycosides and tetracyclines, target RNA structures in the bacterial ribosome. But researchers are now investigating a new generation of small molecules aimed at other RNA structures, both in pathogens and in human cells. Evidence of interest in the field was a symposium at the American Chemical Society national meeting last month coorganized by Appella and Mu--thiah Manoharan, vice president of drug discovery at Alnylam Pharmaceuticals.

In hitting RNA with small molecules, affinity is easy to achieve, Appella notes. "Getting inhibitors with nanomolar or picomolar binding affinities for an RNA target is no problem; all you have to do is make something with a lot of positive charges," he says. "But it won't have a lot of selectivity. So as chemists, we have to figure out how to create small molecules with the right hydrophobicity, aromaticity, and other properties to make them selective for specific RNAs. We really don't know how to do that, and that's the kind of problem a lot of individuals are trying to tease apart today."

Researchers have begun exploring various approaches to find small molecules to target RNA. A prime example involves efforts to find small-molecule medications for myotonic dystrophy type 1 (DM1). No effective treatment exists for DM1, which is characterized by weakness and slow relaxation of skeletal and smooth muscles, heart abnormalities, and cataracts.

The genetic defect in DM1 is a mutated gene that is transcribed to mRNA with hundreds to thousands of CUG (cytosine-uridine-guanine) repeats, which form hairpin structures. CUG-repeat mRNA accumulates in nuclei, where it binds the protein muscleblind-1 (MBNL1). The interaction prevents MBNL1 from performing its normal function, RNA splicing.

CUG-repeat mRNAs are a promising target for drug discovery because their repeat sequence is unique in the body. In July, neurologists Thurman M. Wheeler, Charles A. Thornton, and coworkers at the University of Rochester

Medical Center reported having exploited this advantage by developing a short string of nucleotides that recognizes and binds CUG-repeat mRNA and consequently reduces DM1 symptoms in mice (C&EN, July 20, page 15; *Science* **2009,** *325,* 336). Other groups are discovering nonnucleotide small molecules that can target DM1's mRNA. For example, bioorganic chemist Benjamin L. Miller of the University of Rochester Medical Center and coworkers recently used resin-bound dynamic combinatorial chemistry to identify potential DM1 drug leads. In this technique developed by Miller's group, high-affinity ligands from equilibrating combinatorial libraries are selected by competitive binding and then isolated by retention on a solid support.

With this approach, Miller and coworkers identified four peptide/small-molecule hybrids that bind CUG-repeat mRNA and inhibit its interaction with MBNL1 with low micromolar levels of activity (*J. Am. Chem. Soc.* **2008,** *130,* 16254). They are now trying to improve compound affinity and selectivity, in part by replacing the peptide components with peptidomimetics.

At the University of Illinois, Urbana-Champaign, chemistry professors Anne M. Baranger and Steven C. Zimmerman and coworkers are using rational drug design to find DM1 agents. Using structural information about CUG-repeat mRNA, they combined a sequence-selective binder, a nucleic acid-intercalating compound, and a linking group into a hybrid small molecule that they predicted would interact with the target (*Proc. Natl. Acad. Sci. USA,* DOI: 10.1073/pnas.0901824106). The resulting triaminotriazine-acridine conjugate binds selectively to CUG-repeat mRNA and inhibits its ability to interact with MBNL1 with low micromolar activity. "We have shown that our compound is actually quite selective" for CUG-repeat mRNA, Zimmerman says.

Also taking the rational drug design route to small-molecule DM1 agents is assistant professor of chemistry Matthew D. Disney of the State University of New York, Buffalo, and coworkers. They compiled a database of RNA-binding ligands and the RNA structures or motifs to which the ligands bind. From that database, they identified the bisbenzimidazole Hoechst 33258 as a ligand for CUG-repeat mRNA. They then combined multiple copies to form a modularly assembled construct with improved affinity and specificity for the target (*J. Am. Chem. Soc.* **2009,** *131,* 9767).

A bisbenzimidazole pentamer they designed by this route inhibits with low nanomolar potency the formation not only of the CUG-repeat-mRNA/MBNL1 complex, but also of a repeat-mRNA/MBNL1 complex involved in spino-cerebellar ataxia type 3, which causes lack of coordination of muscle movements. Disney and coworkers recently used the same database and modular assembly to find a lead compound for a closely related form of myotonic dystrophy (*ACS Chem. Biol.* **2009,** *4,* 345).

Researchers are also trying to find RNA-targeted medications for viral infections. For instance, at the University of California, San Diego, a team is studying how small molecules target the RNA genome of hepatitis C virus (HCV). HCV infection causes chronic hepatitis and hepatocellular carcinoma. New medications are urgently needed because current ones are weak and have serious side effects.

Benzimidazole derivatives were already known to bind to a subdomain of the internal ribosome entry site (IRES), the part of the HCV genome that causes host cells to translate viral RNA into proteins. Using fluorescence resonance energy transfer (FRET) and an IRES crystal structure they obtained earlier, the UC San Diego team determined the mechanism by which the benzimidazoles inhibit translation (*Nat. Chem. Biol.*, DOI: 10.1038/nchembio.217).

Assistant professor of chemistry and biochemistry Thomas Hermann and coworkers carried out the work. They found that binding of one of the benzimidazoles causes a conformational change in the subdomain's hairpin-loop structure that prevents the IRES from interacting correctly with the ribosome. This, in turn, shuts off IRES-driven translation of viral genes in HCV-infected cells, short-circuiting the virus's life cycle.

Similar mechanisms have been found before for ribosome-targeting antibiotics, but "to our knowledge, this is the first example of such a conformational mechanism proven for a biologically active small molecule that targets an RNA structure outside the bacterial ribosome," Hermann and coworkers write.

"We don't have plans to develop the benzimidazoles further because they are fairly difficult to make, not very druglike, and previously patented," Hermann tells C&EN. "We are now screening biased libraries of RNA-friendly compounds against the IRES target. The aim is to find new, synthetically more accessible and druglike classes of inhibitors that act by the same mechanism."

Hermann believes the way forward for RNA targeting is for researchers to refine the current understanding of what makes a ligand RNA-friendly while keeping druglikeness in mind. “Small molecules, not-so-small ones, and libraries thereof will have to be designed and synthesized specifically for RNA targets,” Hermann says. “This will require some compromises with regard to druglikeness, just as we see such compromises in natural antibiotics that target ribosomal RNA.” The goal is to increase “the success rate against RNA targets.”

Another viral RNA genome being targeted by small molecules is that of human immunodeficiency virus (HIV), the cause of AIDS. For instance, Appella and coworkers are trying to hit the transactivation response element (TAR), a component of HIV’s RNA genome that helps the virus replicate. Disabling TAR would prevent HIV replication.

Host-cell translation of HIV’s genome is boosted greatly when the HIV protein Tat binds to TAR, which has a hairpin shape. To find small molecules that bind to TAR and inhibit this interaction, Appella and coworkers synthesized and screened a library of functionalized polyamines, which are polypeptides with the backbone amides replaced by amino groups. These compounds, which they call multivalent-binding oligomers (MBOs), have nonionic side chains designed to hydrogen bond or stack with RNA bases, and their backbone amines tend to interact with the anionic RNA backbone.

The best MBOs they identified inhibit Tat-TAR binding at low micromolar levels of activity. The agents have good selectivity for TAR, enter cells easily, and have low toxicity. Structural information about the MBO-TAR complex “would be a dream come true for us so we could see how to chemically modify MBOs into more-active conformations,” Appella says.

At Clemson University, meanwhile, associate professor of bioorganic and medicinal chemistry Dev P. Arya and coworkers are also trying to develop small molecules—aminosugars, in their case—that inhibit HIV’s TAR domain. In these studies, “we are violating every possible rule of Lipinski,” Arya says, referring to Lipinski’s Rule of Five, a set of parameters for assessing a compound’s druglikeness and predicting its prospects for being orally active. Lipinski’s rule was developed for conventional drugs, which typically bind proteins, not RNAs.

Using a structure-guided approach, Arya’s group screened a library and identified modified aminosugars that bind TAR with nanomolar to sub-micromolar affinities. Some of the compounds inhibit the Tat-TAR interaction at nanomolar concentrations and protect cells from HIV infection at low toxicity levels. The compounds aren’t druglike by Lipinski’s criteria, but “our preliminary data indicate we have some promise here,” Arya said.

For inspiration in the field of small-molecule RNA targeting, Appella looks to studies in which RNA evolution experiments are used to identify RNAs with new functional properties. In those experiments, he says, “you start with enormous numbers of different RNAs and then slowly cull out, select, and amplify the ones you want.” They show that RNAs can be evolved to bind selectively to compounds such as caffeine or protein targets.

“I sense that there is selectivity in there somewhere, and we should be able to make small molecules that target RNA, too,” Appella says. “But we’re coming at it from the reverse direction”—identifying small molecules that bind RNA, not RNAs that bind small molecules—“and we don’t have the benefit of in vitro evolution, so it’s really a challenge. We have to use our ingenuity as chemists and think, ‘What are the physical properties of RNA, and how do these affect the way it likes to bind things?’ Hopefully we’ll converge on a basic idea of how to target RNA molecules.”

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