Hitting moving RNA drug targets

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By accounting for the floppy, fickle nature of RNA, researchers at the University of Michigan and the University of California, Irvine have developed a new way to search for drugs that target this important molecule. Their work appears in the June 26 issue of *Nature Chemical Biology*.

Once thought to be a passive carrier of genetic information, RNA now is understood to perform a number of other vital roles in the cell, and its malfunction can lead to disease. The versatile molecule also is essential to retroviruses such as HIV, which have no DNA and instead rely on RNA to both transport and execute genetic instructions for everything the virus needs to invade and hijack its host. As more and more links to disease are discovered, the quest for drugs that target RNA is intensifying.

Searching for such drugs is not a simple matter, however. Most of today's drug-hunting tools are designed to find small molecules that bind to protein targets, but RNA is not a protein, and it differs from proteins in many key features. "So there's a growing need for high-throughput technologies that can identify compounds that bind RNA," said Hashim M. Al-Hashimi, the Robert L. Kuczkowski Professor of Chemistry and Professor of Biophysics at U-M.

Al-Hashimi and coworkers adapted an existing computational technique for virtually screening libraries of small molecules to determine their RNA-binding abilities. In this approach, the shape of a target molecule is first determined by X-ray crystallography or NMR spectroscopy; next,

researchers run computer simulations to compute how well various small molecules---potential drugs, for example---nestle into and bind to the target structure. RNA presents a major challenge to this methodology because it doesn't have just one configuration; it's a floppy molecule, and depending on which small molecule it binds, it can assume vastly different shapes.

It once was thought that encounters with drug molecules actually caused RNA's shape changes, and that it was impossible to predict what shape an RNA would adopt upon binding to a given small molecule. However, in earlier research, Al-Hashimi's team challenged this conventional "induced-fit" concept by showing that the RNA, on its own, can dance through the various shapes that it adopts when bound to different drugs. The team discovered that each drug molecule simply "waits" for the RNA to morph into its preferred shape and then latches onto it.

The researchers' previous work involved creating "nano-movies" of RNA that capture this dance of shape changes. In this new study, the researchers froze individual "frames" from the nano-movies, each showing the RNA in a different conformation, and subjected each of them to virtual screening. To test the method in the "real world," they first tried it on compounds already known to bind a particular RNA molecule from HIV called TAR.

"We showed that by virtually screening multiple snapshots of TAR, we could predict at a useful level of accuracy how tightly these different compounds bind to TAR," Al-Hashimi said. "But if we used the conventional method and virtually screened a single TAR structure determined by X-ray crystallography or NMR spectroscopy, we failed to predict binding of these drugs that we know can bind TAR."

Next, the researchers tried using the method to discover new TAR-targeting drugs. They screened about 51,000 compounds from the U-M

Life Sciences Institute's Center for Chemical Genomics. "From this relatively small compound library, we ended up identifying six new small molecules that bind TAR and block its interaction with other essential viral molecules," Al-Hashimi said.

What's more, one of the six compounds, netilmicin, showed a strong preference for TAR.

"Netilmicin specifically binds TAR but not other related RNAs," said former graduate student Andrew Stelzer. "We were very pleased with these results because one of the biggest challenges in RNA-targeted drug discovery is to be able to identify compounds that bind a specific RNA target without binding other RNAs. The ability of netilmicin to specifically bind TAR provides proof of concept for this new technology," said Stelzer.

Further experiments showed that, for the six potential drug molecules, the method not only successfully predicted that they would bind to TAR, it also showed---with atomic-level accuracy—where on the RNA molecule each drug would bind.

Al-Hashimi then turned the six drug candidates over to David Markovitz, a professor of infectious diseases at the U-M Medical School, who tested them in cultured human T cells infected with HIV. The point of this experiment was to see if the drugs would prevent HIV from making copies of itself, an essential step in the disease process.

"Netilmicin did in fact inhibit HIV replication," Markovitz said. "This result demonstrates that using an NMR spectrometer and some computers we can discover drugs that target RNA and are active in human cells."

In addition to testing compounds in existing molecular libraries, the

virtual screening technique can be used to explore the potential of new compounds that have not yet been synthesized, Al-Hashimi said. "This opens up a whole new frontier for exploring RNA as a drug target and finding new compounds that specifically target it."

More information: *Nature Chemical Biology*: www.nature.com/nchembio/index.html

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