



## PINNING DOWN RNA

Aside from being a big step toward new drugs for HCV treatment, this structure is a notable advance, say the researchers, for structure-based, computer-aided drug design (CADD) in general. Having played a role in developing Viagra, HIV-inhibitors and other therapeutic drugs, structure-based CADD has within the past decade gained a significant role in pharmaceutical research — reducing lengthy and costly trial-and-error laboratory work. Nearly all of the CADD effort, however, has focused on proteins, virtually none on nucleic acids such as RNA.

In many pathogens, RNA molecules present an inviting target for therapeutic drugs, but they have not been conducive to CADD. “By its inherent nature,” says Cheatham, “RNA is much harder to precisely define structurally than a protein. It’s flexible, very sensitive to surroundings, and binding of small molecules often radically changes the structure. As a result there’s not been a lot of effort in CADD with RNA or other nucleic acids.”

To overcome these challenges, the Utah team applied advanced techniques of NMR (nuclear magnetic resonance), a widely used method of obtaining structural information from crystallized molecules — basically it’s like doing MRI on a molecule and using the information gained to deduce its three-dimensional structure. Along with distances between hydrogen atoms, usually available from NMR, Davis obtained “residual dipolar couplings” (RDC) — NMR information that defines directional alignment of one part of the molecule with another.

The next step is to refine the structure — a process for which Davis and Cheatham relied on molecular dynamics (MD), a computational method that models the forces and distances between atoms. With MD computations, the scientists could test potential structures — among many that meet the NMR constraints — to find which have the lowest energy expenditure, the native state of a molecule, and align best with the NMR information, including RDC.

One of the major problems with RNA structure refinement has been the applicable MD “force fields” — expressions that describe the atom-to-atom interactions, and a goal of Cheatham’s HCV work is to improve these force fields. “RNA can adopt many different conformations,” says Cheatham. “A loop can fall apart because it’s moving. We know there’s systematic problems, and we can test the force fields so that we know we’ve got the correct one.”

## STRAIGHTENING THE BEND

The Utah scientists focused on a particular stretch of HCV’s RNA, its internal ribosomal entry site (IRES), a loop-like structure near one end that is, in effect, a marker for where the RNA-copying process begins. Prior research showed that there’s a helical bend — a more than 90° turn — in this loop of RNA when it’s not bound with another molecule, resulting in an overall L-shape for the IRES region.

Working with the NMR data, Davis first used an MD program called Xplor to refine the HCV IRES and small molecule bound together by themselves — without surrounding water molecules and ions as in the natural cellular environment. Cheatham then used TeraGrid systems to run the more exacting AMBER (Assisted Model Building with Energy Refinement) MD package, which he has helped to develop. With an extensive series of runs, he corrected problems with the RNA force fields and, taking as input the results from Davis’s work, further refined the structure of the IRES-drug complex, this time including surrounding water and ions, to arrive at a more accurate set of final structures.

The refined structure shows that binding of the small molecule straightens the bend of the IRES loop, a dramatic structural shift that explains the biological effect of inhibiting replication. With this success as a starting place and demonstration of method, the Utah team is working to narrow the candidates of similar small-molecule drug compounds that have ability to stop the virus, which can then be synthesized and laboratory tested in virus cultures to find the most potent possible molecules to develop as drugs.

“This gives us a starting place,” says Cheatham, “to further optimize this class of compounds, to increase the affinity with the IRES binding site and the specificity of its binding to only the RNA target we’re aiming at, ultimately leading to a new class of highly potent HCV therapeutics.”

**“ THIS STRUCTURE PRESENTS A NEW CHEMICAL PARADIGM FOR SMALL MOLECULE INHIBITORS OF STRUCTURED RNAs. ”**

A number of other viral RNAs — including yellow fever, dengue and swine fever — are known to have an IRES region like that of HCV, suggesting a structure-based CADD program to develop RNA drugs for a range of viral diseases. The Utah scientists have mapped a path that suggests this Mt. Everest will be climbed. “This structure,” says Davis, “presents a new chemical paradigm for small molecule inhibitors of structured RNAs.”

### MORE INFORMATION

[www.psc.edu/science/2009/hcv/](http://www.psc.edu/science/2009/hcv/)

## PAINKILLER SNAILS

Along with their work on hepatitis C, Thomas Cheatham and his colleagues at Utah are investigating potent snail venoms that show promise of treating chronic pain without addiction. The same cone-shaped shells that populate beaches are home to cone snails, carnivorous hunters that can paralyze small fish almost instantly with a neurotoxic venom.

These venoms, known as conotoxins, have shown significant potential to treat “neuropathic pain” — chronic long-term pain, usually involving nerve damage. Unlike morphine, conotoxins don’t affect opioid receptors and therefore offer the tantalizing prospect of suppressing pain without addictive side effects.

“This snail produces thousands of different types of these toxins,” says Cheatham, “and they can lead to total paralysis. If you break down the separate groups, each affects a different part of your nervous transmission. We might be able to design these to treat a particular pain or disease state in selective ways, with minimal side effects.”

In 2008, Cheatham and grad student Pawel Gruszczynski from the University of Gdansk in Poland used Pople, PSC’s shared-memory SGI Altix, to run long-timescale molecular dynamics on several

versions of conotoxins. As they reported in *ChemMedChem* (March 2009), their analogue conotoxin model retained the ability to produce analgesia, and suggests one model for new painkiller therapeutics.

In another study, they modeled the binding site of two different “pain channels” and demonstrated conotoxin selectivity. “One conotoxin binds well to one channel and doesn’t bind well to the other and vice-versa,” says Cheatham. “Additionally, we think there are multiple binding modes — which not only helps explain the experimental data but also helps to explain the diversity of activity of these compounds.” Their results from both studies, analyzed in collaboration with University of Utah experimentalists Greg Bulaj and Toto Olivera, who lead the field of conotoxin research, are steps toward mapping the molecular features responsible for the drug’s biological effect.

A conotoxin average structure from 200 nanoseconds of MD simulations by Cheatham and colleagues. The side chains show the heavy atoms — carbon (gray), nitrogen (blue), oxygen (red). The backbones, a different color (red, blue and yellow) for each structure, show motion, and the side chains show differential localization.

