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Fab-ricating RNA crystals

By Steve Edelson, Executive Editor

Researchers at **Howard Hughes Medical Institute** and colleagues have developed a phage display technique that produces antibodies that bind RNA. Companies working in the RNA field think the immediate application of the technology—using antigen-binding fragments from antibodies to help stabilize and obtain crystal structures of RNA—will make it a useful tool for interrogating RNA biology. But the consensus is that the potential use of these fragments as RNA-binding therapeutics has too many roadblocks to become a reality.

In the paper in the *Proceedings of the National Academy of Sciences*, researchers used a phage display library to obtain antigen-binding fragments (Fabs) that bind the ΔC209 P4-P6 domain of the *Tetrahymena* group I intron.[1]

They picked the domain because its 3D structure is well known and has already been crystalized twice by other groups.[2,3] "We thought the domain was a good choice because it's well folded and well understood," said Joseph Piccirilli, associate professor in the Department of Biochemistry and Molecular Biology at the **University of Chicago** and an author on the paper. "Thus, we could focus on whether the technique works."

The researchers identified Fabs that bound the target with nanomolar affinity. Moreover, the structure of the ΔC209 P4-P6 domain was solved at 1.95-Å resolution, which Piccirilli said was better than either of the two previous crystal structures.

The technique appears to be applicable to other RNAs. Piccirilli told *SciBX* that his group has made adjustments to the library and obtained Fabs against a panel of 10 diverse RNAs.

In general, said Piccirilli, RNA crystals are very hard to obtain because RNA has "negative charges everywhere. Electrostatic surfaces are packed onto electrostatic surfaces." Moreover, RNA usually lacks immunogenicity and therefore is not recognized—or bound—by antibodies.

Thus, according to Alan Sachs, a VP at Merck & Co. Inc.'s **Merck Research Labs** unit, the Fab approach "should be useful when RNA can't otherwise be crystalized. With access to the libraries, this could really have an impact on RNA structure determination."

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Merck made a significant investment in RNA biology and therapeutics last year when it completed its acquisition of small interfering RNA company Sirna Therapeutics Inc. for $1.1 billion in cash. The deal was announced in October 2006.[4]

Sachs did note that use of the phage display technique would likely be limited to RNA structure studies. For other areas of RNA research, including detection and quantification, "you already have [polymerase chain reaction]-based methods. Any tool that's protein based would be at a disadvantage because you don't have the ability to amplify," he said.

On the other hand, Piccirilli noted that existing RNA detection tools, such as northern blots, have a hard time detecting methylation or other RNA modifications. "An antibody could be used to predict that," he said. "It could be challenging, but it's one of the things we want to do with this technology."

He noted that any company "with antibody or Fab libraries and the ability to work with RNA" could use the published technique. The researchers have not applied for patents, according to Piccirilli.

Markus Enzelberger, senior director of R&D at phage display specialist company **MorphoSys AG**, told *SciBX* that the approach published in *PNAS* "is similar to what some of our customers use to crystalize membrane proteins. Our HuCAL library is very large so we shouldn't need to make any modifications" to obtain compounds that bind RNA.

Nevertheless, he said, "the therapeutic use of anti-RNA antibodies is a long way off. The most critical obstacle is getting antibodies inside the cell. And if it does get in there, you'd have to compete with siRNA therapeutics."

Both Merck's Sachs and John Maraganore, CEO of siRNA therapeutic developer **Alnylam Pharmaceuticals Inc.**, agreed that antibodies against RNA are unlikely to have therapeutic applications. Instead, said Maraganore, "the more interesting application is the cocrystals of RNA with the antibody fragments" to show 3D pictures of RNA.

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COMPANIES AND RESEARCH INSTITUTIONS MENTIONED

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