

# Shortcut to protein redesign

By Michael J. Haas, Senior Writer

Proteins are desirable therapeutics because of the specificity of their interactions, but wild-type proteins often make suboptimal drugs because of poor bioavailability, pharmacokinetics or other properties. Companies have two main routes to improve these properties: genetic engineering or chemical modification. The latter technique can produce a more diverse range of modifications, but total synthesis and purification of a redesigned protein is lengthy and laborious.

Investigators at the **Institute for Research in Biomedicine** have been working for the last few years to develop a shortcut to redesigning proteins via chemical modification. In 2002, the researchers reported the development of a combinatorial synthesis technique that could produce redesigned peptides of high purity right on a bead substrate.<sup>1</sup> Now, the group has added another time-saving component to the technique: on-bead, high-throughput screening of the modified peptides.

Rather than wanting to adopt the entire technique, biotechs in the protein redesign space told *SciBX* that the screening method currently has more potential than the synthesis technique, although both components have limitations.

In the *Journal of the American Chemical Society*, a group led by Ernest Giralt reported the design of eight analogs of the hydrophobic core of the B domain of staphylococcal protein A.<sup>2</sup> Giralt is program head at the institute and professor of chemistry at the **University of Barcelona**.

Giralt's group chose the B domain because its structure and binding properties are well known. Thus, the researchers knew that to alter the B domain's binding to antibodies, modifications needed to be made to 4 key amino acids found within the 22-amino-acid hydrophobic core that direct the formation of the domain's surface. At the same time, the entire domain needed to retain its correct folding properties.

Instead of synthesizing the entire B domain and modifying the amino acids, the researchers divided the B domain into two parts—the hydrophobic core and the rest. The group synthesized 300 versions of the hydrophobic core, each analog on a single bead. The beads were

then screened against the remaining portion of the B domain and fluorescently tagged IgG.

Eight analogs of the core appeared to replicate the folding properties of the full wild-type domain because they bound IgG. Because the study was proof of concept, the researchers did not seek to optimize the binding. Rather, their goal was to show that altered peptide sequences could yield a domain with similar binding to the original.

Giralt told *SciBX* that the technique is well suited to optimizing a protein's interactions with other proteins because it offers two advantages over existing approaches.

One is that it is capable of a higher throughput than total synthesis or *in vivo* methods. "The synthesis of the 300-peptide library, including quality-control analysis, took five days," Giralt told *SciBX*. "The covalent [total] synthesis of one entire B domain would take about one week, from automatic assembling and cleavage, to purification and final characterization."

"The other advantage is maybe not so evident," he said. "The detection of proteins of interest is based directly on molecular recognition. You are screening directly for the interaction you are trying to modify or opti-

mize," without first having to purify the peptides or cleave them from the beads.

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—Ernest Giralt,  
*Institute for Research in Biomedicine*

## Screening potential

Although some protein-therapeutics companies contacted by *SciBX* saw potential for the synthesis technique, they were more interested in the screening technology.

"There could be complementarity between our computational peptide design and Giralt's screening method," said Bassil Dahiyat, president and CEO of **Xencor Inc.** "We could use computational design methods, like our own PDA technology, to prefilter the possible combinations and decide which peptide variations to synthesize. Giralt's approach would provide the experimental screening step."

Xencor uses its Protein Design Automation (PDA) technology to engineer antibodies for cancer and therapeutic proteins for both inflammation and autoimmune disorders. In January, the company began a Phase I trial of XmAb 2513, which targets CD30, for lymphoma and Hodgkin's disease. Xencor has other mAbs targeting CD19 and CD40 in preclinical development for B and T cell lymphomas, leukemia and cancer.

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The compound is doxorubicin encapsulated in a heat-activated liposome. Unlike the nanogel approach, ThermoDox relies on radio frequency ablation for the tumor-specific elevation of temperature that triggers release of doxorubicin from heat-sensitive liposomes.

## REFERENCES

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## COMPANIES AND RESEARCH INSTITUTIONS MENTIONED

**Celsion Corp.** (AMEX:CLN), Columbia, Md.  
**Cytimmune Sciences Inc.**, Rockville, Md.  
**Emory University**, Atlanta, Ga.  
**University of Utah**, Salt Lake City, Utah