Surrogate antibodies

By Michael J. Haas, Senior Writer

A study by researchers at **The Scripps Research Institute** and **Sea Lane Biotechnologies LLC** describes the generation of Surrobodies, or antibody-like molecules with high antigen-binding affinities, using phage display and combinatorial methods.[1] The authors think the unique structural features of Surrobodies could produce molecules that have greater diversity and more functionality—perhaps including intracellular targeting—than antibodies.

The research, led by Richard Lerner, president and professor of chemistry and immunochemistry at Scripps, and Ramesh Bhatt, VP of research at Sea Lane, was published in the *Proceedings of the National Academy of Sciences*.

"We've done proof-of-concept to show that we can generate libraries of Surrobodies—and select from the library those with high binding affinity for the antigen—just as you can with classical antibodies," Lerner told *SciBX*.

Companies contacted by *SciBX* agreed that the team's approach could have advantages over existing methods of antibody generation, but they said it remains to be seen whether Surrobodies are more effective therapeutics than antibodies.

It's unclear how long it will take for such comparative efficacy data to emerge, as Sea Lane is not disclosing its plans for the Surrobody technology. The company focuses on therapeutic discovery using biologic platforms and technologies.

When a body meets a body

The Lerner-Bhatt team's work followed a 2007 report on the crystal structure of the pre–B cell receptor (pre-BCR) complex, which was solved by researchers at **Stanford University School of Medicine**.[2] Surrobodies are modeled on the pre-BCR complex, which is an antibody precursor with a structure similar to that of an antibody.

Whereas an antibody contains a pair of identical variable light chains, the pre-BCR contains a pair of identical surrogate light chains that differ from antibody light chains in three ways:

First, whereas antibody light chains are composed of single,

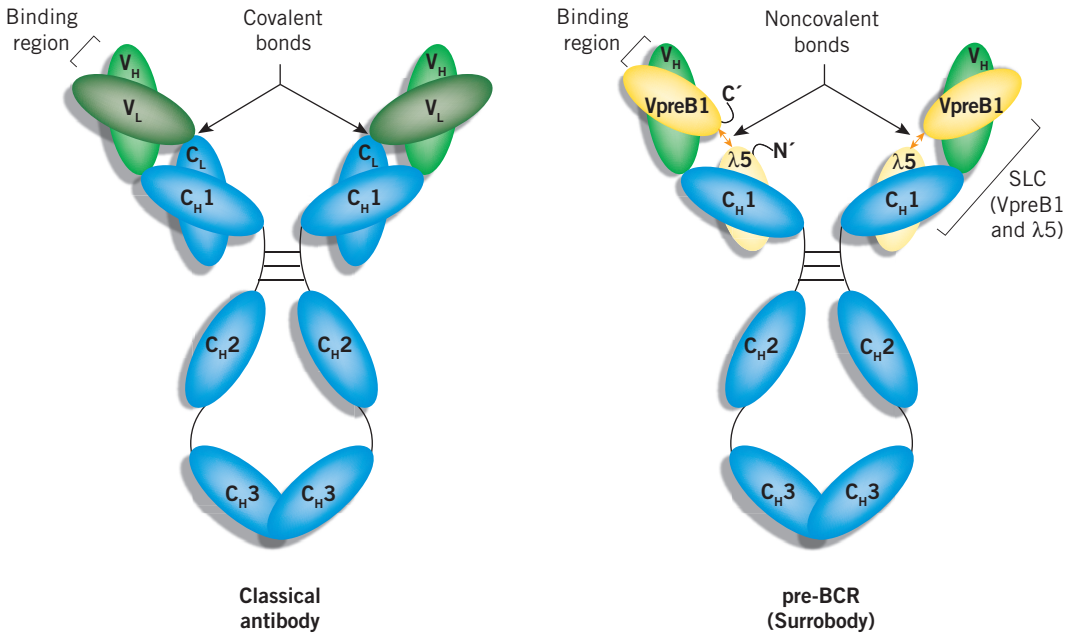

Figure 1. Classical antibody versus Surrobody. The classical antibody is composed of one pair of identical heavy chains (H) and one pair of identical light chains (L). Each chain is composed of constant (C) and variable (V) peptide sequences that are covalently bound. Each heavy-light chain pair is bound by disulfide linkages. The resulting antibody has two antigen-binding regions, formed by the V regions in each heavy-light chain pair.

The pre–B cell receptor (pre-BCR) complex, or Surrobody, contains one pair of identical heavy chains (H) of the same types found in classical antibodies and a single type of surrogate light chain (SLC).

The SLC is composed of two peptide sequences: immunoglobulin λ-5 polypeptide (CD179B; λ5) and VpreB1 (CD179A). These two sequences are noncovalently bound by hydrophobic interactions.

Additionally, each part of the naturally occurring SLC has a peptide sequence, or 'tail', that extends beyond the hydrophobic interaction. λ5 has a 50-amino-acid tail at its N terminus (N′). VpreB1 has a 21-amino-acid tail at its C terminus (C′).

In engineered Surrobodies, the N′ tail or C′ tail can be removed entirely—as the researchers did in the *PNAS* study[1]. Additionally, the researchers suggested the tails could be modified to alter the properties of the resulting Surrobody.

covalently bound peptide sequences, the surrogates are composed of two distinct peptide segments—VpreB1 (CD179A), which is coded by *prelymphocyte gene 1* (*VPREB1*), and immunoglobulin λ-5 polypeptide 1 (CD179B; λ5)—which are bound to one another noncovalently.

Second, the peptide sequences of VpreB1 and λ5 are invariant.

Third, both peptide segments have tails that extend beyond the noncovalent binding site between them (*see* **Figure 1, "Classical antibody versus Surrobody"**).

The Lerner-Bhatt team hypothesized that variations could be introduced into the sequences of both VpreB1 and λ5, thereby producing pre-BCR-like structures, the Surrobodies, with greater diversity than antibodies.

The team wrote in *PNAS* that Surrobody libraries could achieve greater size than antibody libraries, and therefore could provide greater diversity, because Surrobodies have three different components that can be varied: the heavy chain and each of the two peptide sequences—VpreB1 and λ5—of the surrogate light chain. By contrast, an antibody has only two variable components: the heavy chain and the light chain.

To test their hypothesis, the researchers set out to construct Surrobodies and test their binding affinities.

To start, they engineered five variations of the naturally occurring surrogate light chains. Then they constructed six different Surrobodies by pairing each of these five variations—plus the naturally occurring surrogate light chain—with heavy chains from a human anti-influenza antibody.

Next, the team showed that all six Surrobodies bound influenza antigen. Two Surrobodies had antigen-binding affinities comparable to that of an antibody containing the same heavy chains.

Lastly, the team generated two combinatorial Surrobody libraries from an antibody library previously generated at Sea Lane.[3] Phage display techniques selectively enriched the fractions of antigen-binding Surrobodies in each library to 95% and 99%, respectively.

The results showed that Surrobodies with high antigen-binding affinity can be generated in and then selected from combinatorial libraries in the same manner as antibodies.

Although the team found that Surrobodies retaining one or both of the VpreB1 and λ5 tails had poor binding affinities, they wrote that varying the peptide sequences of those tails might restore or improve binding performance.

The paper also suggested that varying the sequence of the tails—which antibodies do not have—might give Surrobodies functionalities that antibodies lack, such as the ability to reach intracellular targets.

Lerner told *SciBX* that the *PNAS* study did not attempt to add diversity or functionality to Surrobodies because its primary purpose was to establish proof-of-concept.

Diverse questions

Antibody companies contacted by *SciBX* agreed that the Surrobody scaffold was a potentially advantageous alternative to classical antibodies. But they said the technological and therapeutic potential of the approach would depend on whether adding diversity and/or functionality improved or compromised the antigen-binding properties of Surrobodies.

"The idea is great," said Linda Masat, director of antibody discovery and engineering at **Xoma Ltd.** "I see it as a new technology that might allow faster discovery of antibodies."

She noted that conventional antibody library construction involves isolating both heavy chain repertories and light chain repertories from human donors—a laborious process given the hundreds of thousands of possible variations for each chain.

"If you already have a library of light chains made—in the form of surrogate light chains—you can pair that premade library with any heavy chain repertoire, which could result in a shorter time to discovery" via faster library construction, Masat said.

She also suggested that diversifying the surrogate light chain, the VpreB1 tail or the λ5 tail, or any combination of these, might accelerate lead optimization.

"If the researchers show that they can get more diversity than conventional technology, you could use this technology for rapid affinity maturation," Masat said, because the larger and more diverse a given library, the greater the likelihood of finding molecules with high binding affinities for antigen.

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> ***—Linda Masat, Xoma Ltd.***

An important question, said Masat, is whether surrogate light chains confer—or can be modified to confer—functionalities that antibodies do not have.

"The Lerner team shows that there is an antigen-binding site in pre-BCR complex, so it is possible that there are other functions to the Surrobody as well," she said.

Masat said proper assessment of the therapeutic potential of Surrobodies would involve *in vivo* studies of their pharmacokinetics, pharmacodynamics and potential to form aggregates.

The potential immunogenicity of Surrobodies also would have to be tested in humans. "It's not likely to be a problem, since the surrogate light chain is already fully human," but the question will have to be addressed nonetheless, she said.

Xoma's Human Engineering technology is used to reduce the immunogenicity of nonhuman antibodies. Next month, the company will report data from two Phase I studies of XOMA 052, a mAb targeting IL-1β to treat type 2 diabetes. The company plans to take the antibody into Phase II studies to treat rheumatoid arthritis (RA), acute gout and systemic juvenile idiopathic arthritis (SJIA) this year.

Hans De Haard, senior director of discovery research at **Ablynx N.V.**, also thinks Surrobodies have potential. "The ingredients are there, since it is a fully human molecule with a high degree of structural and sequence homology to the most successful therapeutic biological—that is, the human antibody," he said.

"What needs to be studied in detail is if—and how—the surrogate light chain can contribute to antigen binding," De Haard added.

Although Lerner's team showed that the surrogate light chain can associate with antibody heavy chains, De Haard noted that the team also wrote in *PNAS* that a Surrobody's binding affinity might depend primarily on the heavy chain. He said this suggests the surrogate light chain does not contribute significantly to the pre-BCR's binding affinity and thus raises the question of whether diversifying the surrogate light chain would produce Surrobodies with better binding affinities than antibodies.

De Haard also questioned whether a Surrobody with a non-natural surrogate light chain would be stable. He noted that the Stanford team that solved the pre-BCR structure also found that the VpreB1 and λ5 tails, as well as the peptide loops in the rest of the surrogate light chain, may play an important role in stabilizing the pre-BCR complex.[2]

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Both the heavy and light chains of antibodies have peptide loops—called complementarity determining regions (CDRs)—that vary in sequence, thereby giving an antibody its ability to bind a specific antigen. The surrogate light chain also contains peptide loops, but because the sequence of the chain is ordinarily invariant, the antigen-binding capability of these CDRs is unclear, De Haard said.

"Their role in binding to antigen might be rather limited, since they are so important for stabilizing the complex between the surrogate light chain and the heavy chain region," suggesting that these CDRs cannot be completely randomized, he said.

These issues need to be addressed in experiments "clearly demonstrating that the Surrobody can be engineered to participate in antigen binding without altering its ability to interact with the heavy chain," De Haard said. If and when that is accomplished, he said, "then I sincerely believe the Surrobody can be applied as a therapeutic scaffold molecule."

Ablynx's lead compound, ALX-0081 is a nanobody targeting von Willebrand factor (vWF). Nanobodies are functional antibodies composed of only heavy chains and are smaller than antibodies. They are derived from species of the *Camelidae* family, such as camels and llamas, whose antibodies naturally lack light chains.

ALX-0081 is in a Phase I/II trial to treat acute coronary syndrome (ACS).

"The *PNAS* paper describes a very interesting approach that would be a way to come up with an alternative scaffold that still uses antibody heavy chains," said Bassil Dahiyat, president and CEO of **Xencor Inc.**

Although Dahiyat thinks the VpreB1 and λ5 tails on the surrogate light chain could provide avenues for incorporating greater diversity into Surrobodies than is possible with antibodies, "the paper stops way short of even giving a glimpse at that potential," he said. "All it shows is that you get stable complexes and that the extra peptide segments are likely detrimental to stability."

Nevertheless, Dahiyat said, "if they really do get extra diversity from those peptide segments, or even from the loops [CDRs] available in the pre-BCR domains, it would be a potentially exciting new scaffold."

Xencor uses its Protein Design Automation (PDA) technology to engineer antibodies for cancer and therapeutic proteins for inflammation and autoimmune disorders. The company's lead compound, XmAb 2513, is an anti-CD30 mAb in Phase I testing to treat Hodgkin's lymphoma and anaplastic large cell lymphoma.

Surrobodies surfacing

Lerner said the team's follow-on work has focused on diversifying the variable regions on the surrogate light chain and on the VpreB1 and λ5 tails. Results of those studies will be reported in future publications, he said.

"We now know that you can put a wide range of different tails on the surrogate light chain—any length, anything you want—though a tail that is too long might begin to interfere with binding," he said.

Lerner added: "We have a function for these Surrobodies—a function that goes beyond simple binding. The antibody companies aren't going to like this."

Lerner declined to disclose what new function or functions the team has incorporated into Surrobodies.

Sea Lane owns the Surrobody IP and has submitted a patent application for the technology, according to COO and General Counsel Michael Horowitz.

He would not disclose whether Sea Lane has any Surrobodies in active development.

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COMPANIES AND INSTITUTIONS MENTIONED

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