'Chain gang' delivers for hard labor

Antibodies remain a favorite tool for both basic and clinical research, but investigators are always on the lookout for new and faster ways to obtain more effective reagents. Michael Eisenstein takes a look at current strategies for building a better antibody.

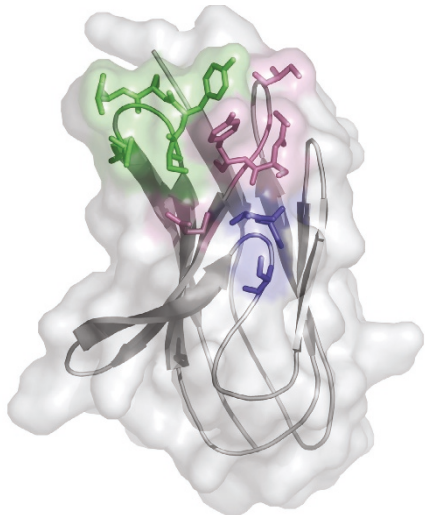
The consensus is in—antibodies are not going anywhere, at least not anytime soon. If anything, the demand has only grown, and company catalogs continue to swell to meet the needs of biologists: Rockland Immunochemicals' catalog now features 2,300 antibodies, Sigma-Aldrich offers 3,400 choices, and Abcam boasts nearly 19,000 different antibodies in their collection. But even this is not enough, and as understanding of the scale of the proteome grows, scientists are still likely to find themselves frustrated to discover that nobody has yet made an antibody to their protein of choice, or that available antibodies may not work for the assay they have in mind (**Box 1**). "If you go to almost any field of biology, they'll say that the antibodies are the rate-limiting step," says Stephen Johnston, head of the Center for Innovations in Medicine at Arizona State University. "Again and again, that seems to be the case." At the same time, the use of antibodies as human therapeutics is becoming more widespread, with 18 monoclonal-based compounds presently approved by the US Food and Drug Administration (FDA) and more than 150 now under development. The traditional strategy for monoclonal preparation—protein vaccination followed by hybridoma preparation—remains powerful and well validated, but nevertheless, there is considerable interest in finding new and effective ways to develop high-affinity antibodies for both basic research and clinical applications.

Plasmid power

It is no secret that for some antibodies the hardest step in preparation can be the first one—producing bulk quantities of antigen. Large-scale protein production can be a time-consuming process, and some proteins are particularly difficult to generate in cell culture for reasons that include toxicity or a tendency to misfold when overproduced.

Thus, it was a pleasant surprise to Johnston when he and his colleagues at the University of Texas found they could skip this step altogether by simply inoculating animals with DNA encoding a protein of interest. Using a 'gene gun' invented by Johnston and John Sanford, they delivered plasmid-coated microprojectiles into the ears of mice and found that more than half of the animals were producing high-titer antibodies within a month of injection[1]. 'Genetic immunization' also had other unexpected advantages, beside speed and efficiency. "We could pool genes," says Johnston. "We could put ten genes into one animal, take the spleens out and get ten kinds of hybridomas out of there." This strategy also has a surprising capacity for breaking host tolerance, according to Johnston: "Out of the 2,000 or so antibodies that we've made now, maybe a third to a half have been for mice, and we just made them right in mice."

The technique faced initial skepticism—the inaugural article was seen by six reviewers over the course of a year—but caught on quickly with many researchers and several companies. GENOVAC, now a subsidiary of Aldevron, was among the first to offer genetic immunization as an option for antibody production, and they adapted the system with specialized plasmids that enhance immunization and allow screening without the need for purified recombinant protein. They now tout a success rate of 84% for antibody generation, including antibodies against some difficult targets, such as G protein–coupled receptors. "Our focus is on native proteins, and we tend to focus at a minimum on a protein domain," says GENOVAC managing director John Thompson. "It's easy to express these and manipulate them on the DNA level as far as they can be identified as individual domains."



Schematic of a domain antibody. (Courtesy of Domantis, Ltd.)

Hans Herweijer, director of preclinical research at Mirus Bio, and his colleagues recently described an even simpler approach to genetic immunization, based on an existing gene-therapy approach. Using hydrodynamic intravenous delivery—the rapid injection of large volumes of DNA-containing solution—into a limb vein, Herweijer's group was able to transfect muscle fibers with plasmid in a manner that led to a rapid and strong immune response[2]. "Two deliveries are sufficient," says Herweijer, "[and] we see really good antibody responses after five weeks." Best of all, the procedure relies only on a simple injection, bypassing the need for a

gene gun altogether. Mirus is also exploring the therapeutic potential of this gene-delivery technique.

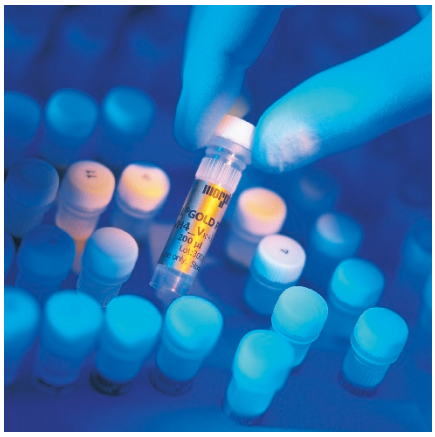
Johnston is now involved in more high-throughput proteomics applications and is presently exploring methods for antibody development that bypass animal use entirely, but for conventional antibody preparations, he still considers genetic immunization an ideal method. "If you're going to go through animals," he says, "this is the way to do it."

Options on display

Of course there are legitimate reasons for wishing to avoid the use of animals—successful hybridoma generation can be expensive and time-consuming—and molecular approaches offer speed advantages as well as additional process control. "The animal is basically a 'black box' once the antigen is injected," says Achim Knappik, head of research at AbD Serotec, a subdivision of MorphoSys. "With an *in vitro* methodology, the panning process can be set up to drive selection towards wanted properties."

By combining the cloned variable regions from one heavy chain and one light chain with a short connecting linker, one can generate single-chain variable fragments (scFvs) that offer a useful compromise for antibody development, combining high affinity with reduced size and ease of manipulation that is highly desirable (**Box 2**). They have also proven to be a perfect fit for *in vitro* selection, as Sir Gregory Winter's group at the MRC Laboratory of Molecular Biology demonstrated by performing several phage-display screens with libraries of antibody fragments, either naturally derived or synthetically generated[3], to isolate high-specificity, high-affinity binders against target antigens.

Winter cofounded Cambridge Antibody Technology (CAT), a company that has continued to explore the use of phage-display libraries for the selection of antibody therapeutics. CAT touts the use of purely natural libraries, obtained from rearranged immunoglobulin G (IgG) sequences in a variety of immune tissues from essentially healthy patients. The library preparation process is then optimized to expand the libraries' therapeutic utility. "We amplify both the variable heavy repertoire and the variable light repertoire separately, and then recombine them—and what that does is break



The HuCAL GOLD antibody library. (Courtesy of Morphosys AG.)

BOX 1 EVERYBODY INTO THE POOL

For many investigators, the growth of the field of proteomics has called attention to a fundamental unfulfilled need—access to a full range of specific protein-binding reagents. "Binding molecules... are a very widespread resource," says Michael Taussig, of the Babraham Institute, "but [people] generally tend to make them in a hypothesis-driven way: I need an antibody, I make or buy an antibody. What has been missing so far is a systematic global approach, and that has only been possible since we've understood what the proteome is, and how large it is, and what we need."

In response to this, the European Commission (EC) 6th Framework Programme is funding the ProteomeBinders project, headed by Taussig, which will bring together international researchers from academia and industry with the aim of making publicly available a collection of affinity reagents targeted against the full human proteome. These will not be strictly limited to antibodies, and alternative affinity reagents based on such scaffolds as ankyrin repeats or protein A domains, as well as nucleic acid aptamers, are also under consideration. "If they do the job, there's no reason why they shouldn't be widely accepted," says Taussig. "The question is, do they function as well as antibodies?" As such, one of the early steps of the ProteomeBinders will be establishing benchmark standards to identify reagents best suited for inclusion in this shared resource.

Two other recently launched consortia are strictly focused on antibody development. One, directed by the US National Cancer Institute (NCI), will emphasize the production of antibodies against cancer-associated proteins, whereas the other, the Human Proteome Organization's (HUPO) Human Antibody Initiative, is taking aim at the entire human proteome. HUPO's initiative is currently chaired by Uhlén, who is also involved in the NCI and EC projects. As with the other programs, many details—the number of antibodies per target, the standards for quality control—have yet to be worked out and are still under discussion, but the organizers are clear on the importance of bringing together academia and industry to maximize efficiency and access. "You can see this as a marriage of the commercial interest and the academic interest," says Uhlén, "and we tried to do this in a way that creates a win-win situation, so that all the commercial providers can participate in this and provide antibodies through this." By comparison, the EC hopes to establish more of a centralized resource, along the lines of the American Type Culture Collection.

These programs will operate independently, but representatives will come together annually at HUPO meetings, and the participants intend to link the data from these efforts. "The different databases need to work with each other," says Uhlén, "and this is going to be reinforced." Taussig hopes such a network will lead to improved antibody quality control. "You would have a database [that] would have all the properties of each antibody or each binder, and you'd be able to see at a glance whether it fulfilled [certain] criteria," he says, "and we think that, at the end of the day, it will be hard for a company not to have its antibodies [in] a database like that."

up any tolerance in the system," explains Alex Duncan, CAT's senior vice president for discovery. "The importance of that is that you can isolate antibodies to human proteins without too much difficulty." CAT currently boasts a 'naive' library comprising about 125 billion clones, as well as libraries from patients with pre-existing maladies, which can be useful for more specialized screens. Humira, the only phage display–derived antibody currently approved by the FDA, was derived from a CAT library.

MorphoSys has taken a more synthetic approach with their HuCAL GOLD phage library, which is based on Fab antibody fragments. HuCAL GOLD currently contains 2×10^{10} clones, which consist of 49 different antibody gene frameworks that were designed via computational analysis of the human germline repertoire, combined with synthetic complementarity determining regions (CDRs) generated from sequence-diversified oligonucleotide cassettes. Morphosys is focused on therapeutic-compound development through licensing arrangements—"From the top 20 pharmaceutical companies, we have deals with 12 at the moment, and most of them also have the library in house for targeted research," says Knappik—but is also one of the only companies to offer their library screening services to the research community.

Both scFv and Fab formats are an option with BioInvent's n-CoDeR library, which was first developed at the University of Lund by cofounder Carl Borrebaeck. n-CoDeR comprises a diverse range of natural CDR sequences in a human germline framework. "We took B cells from 40 different donors from peripheral blood monocytes, lymph nodes and tonsils, and then we biased the cDNA to get out the IgG and IgA in order to get as much mutation and variation in the CDRs as possible," says Roland Carlsson, vice president of preclinical research. The resulting library features 2×10^{10} clones, which can be rapidly screened by BioInvent's proprietary Robo-CoDeR automated screening platform.

Phage display is a well-established platform, but other display formats have shown promise as well, such as ribosome display, which was first adapted for use in antibody generation by Andreas Plückthun. The premise is simple: PCR-amplified scFv libraries are subject to *in vitro* transcription and translation under conditions in which the ribosome can not dissociate from the peptide. The nascent scFv chains can still fold, however, allowing affinity purification of ribosome-associated transcripts for amplification and additional screening. This offers several advantages, according to Taussig, who has worked extensively with ribosome display. "You can use the variability of PCR…to generate mutations and create this sort of evolutionary approach," he says. "And ribosome display, being based on PCR, can generate libraries of 10^{12} to 10^{13} clones without much difficulty." Most companies actively engaged in phage screening have yet to adopt ribosome display, although CAT recently began working with the Plückthun laboratory's version of this technology and now routinely use it in their development process. "Originally, we thought that it would be important

BOX 2 THE INCREDIBLE SHRINKING CHAIN

The size of antibodies prevents their use in some applications, and the idea of generating smaller molecules that retain the properties of full antibodies is quite appealing. In 1989, the Winter lab made important progress on this front, demonstrating that variable domain fragments from mouse antibodies could be expressed in bacteria to produce 'single-domain antibodies' that retain much of their original target specificity[7]. Unfortunately, these antibodies also exhibited some traits that discouraged further study. "These things were very sticky and hydrophobic," says Winter. "You'd purify them and they'd stick to the walls of tubes and aggregate."

Inspiration subsequently came from work at the Free University of Brussels, where Raymond Hamers' group fortuitously identified naturally occurring, homodimeric heavy chain–only antibodies in the blood serum of dromedary camels[8]. These antibodies benefit from extended variable-region surface loops that enhance diversity and confer the ability to bind epitopes inaccessible to standard antibodies, such as enzyme active sites. Serge Muyldermans, a member of Hamers' original team, went on to cofound a company, Ablynx, which is developing these camelid antibodies—now known as nanobodies—for therapeutic applications, and he continues to explore their potential as a reagent. "They are soluble; they are well expressed; they are functional even inside the cytoplasm because you don't need a disulfide bond; and they are strictly monomeric," he says. "It's a very clean and easy-to-prepare material." Similar natural single-chain antibodies, known as IgNARs, have also been found in certain shark species, although these are less well characterized.

The behavior of these camelid antibodies encouraged Winter's group to pour new effort into developing human-derived domain antibodies (dAbs), and subsequent successes by Winter and colleague Ian Tomlinson led to the launch of Domantis, a company focused on the production of therapeutic dAbs. The present generation of molecules are small—around 13 kDa—and show considerably improved stability, and several dAbs are undergoing preclinical assessment. Domantis is also using linked dAbs to generate therapeutic molecules that show specific targeting for cells expressing two different antigens. "We've got several bi-specific leads based on single domains," says Winter, "and we've had huge interest in that particular format."

A similar concept has been pursued by Micromet in the development of their bispecific T-cell engager (BiTE) molecules, which consist of two scFvs connected by a linker domain. Half of every BiTE is a standard scFv that recognizes CD3 on T cells, whereas the other half is customized to target a tumor antigen of interest; the resulting molecule effectively mediates T-cell killing of BiTE-labeled tumors. "They typically work at low pico- to femtomolar concentration, so you just need a handful of molecules to trigger an event," says Micromet CSO Patrick Baeuerle, "and the T cells get very potently activated by BiTE molecules but only when target cells are present."

for optimization," says Duncan, "[but] its utility has gone well beyond our original expectations."

Almost human

Even with these advantages, many in the field of therapeutic-antibody development find themselves turning back to the humble mouse and the powerful processes that it has naturally evolved for producing high-affinity antibodies. "If you've got very small quantities of antigen or it's in a huge mixture with other things... that's really where you have a huge advantage by immunization," says Winter. The use of animal antibodies in humans is problematic owing to the high risk of triggering a host immune response, but Winter's group found an effective solution to this problem when they showed that the affinity-determining CDRs from human and mouse antibodies could be swapped[4], demonstrating the potential for using CDR grafting to generate 'humanized' antibodies consisting of a human framework with mouse CDRs.

Several companies have since developed their own humanization strategies. One of the first was PDL BioPharma (formerly Protein Design Labs), which performs computerized modeling of the variable domains and structurally relevant framework residues in mouse antibodies, and then identifies strategies for transplanting these regions into a human antibody scaffold. Early application of this process yielded Zenapax (daclizumab), an anti–interleukin-2 receptor therapeutic that became the first humanized antibody to be approved by the FDA, and PDL now has a total of three humanized antibody products in clinical trials. "I would estimate that our success rate is on the order of 95% in retaining both binding affinity and biological activity over the many humanized antibodies that we have generated here at PDL," says staff scientist Paul Hinton.

XOMA was another early entrant into the field, with Human Engineering technology, a proprietary process that relies on cross-species comparison to identify antibody residues that can be changed to achieve a more human-like antibody without adversely affecting antigen binding or antibody structure. The resulting antibodies are 93–95% human, but retain the binding properties of the source antibody. "We have a perfect track record to date," says Mary Haak-Frendscho, XOMA's vice president for preclinical research and development. "Because the methodology is so robust and it doesn't require modeling, XOMA is able to provide 5 mg each of four variants within 12 weeks." XOMA recently began offering their process as a contract service, and is presently developing a clinical candidate for AVEO Pharmaceuticals; they also have a proprietary anti-inflammatory humanized antibody scheduled to enter the clinic in 2007.

Humanized antibodies have proven a powerful boon to therapeutic development, and offer the added asset of allowing drug researchers to revisit existing reagents. "A number of antibodies are already in existence that have therapeutic potential and need humanization in order to realize that potential," says Haak-Frendscho. In contrast, companies such as Medarex and Abgenix (recently acquired by Amgen) have gone one step further, generating transgenic mouse lines whose germline heavy and light chain sequences have been entirely replaced with counterpart human sequences, resulting in mice that produce fully human antibodies in response to an immune challenge.

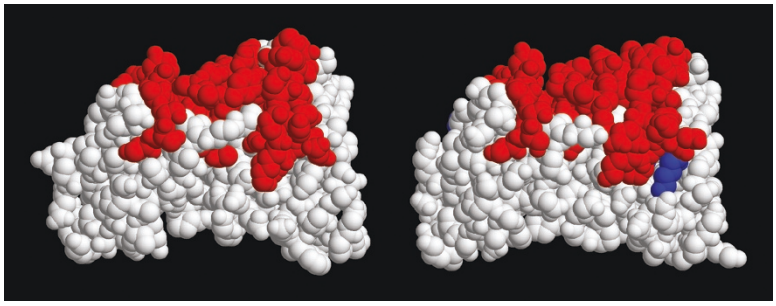
Medarex's UltiMAb platform features several such mouse lines, including one that contains a chromosome fragment comprising nearly the entire human antibody repertoire. Nils Lonberg, senior vice president and scientific director at Medarex, believes that their mice offer clear advantages for drug development by providing a fully natural platform for the selection of stable B-cell receptors, and adds that their mice have shown a surprising breadth in their immune response that makes them particularly useful. "We're able to get antibodies to [human] antigens, and that is fairly trivial in a case where there is a divergence between the mouse and human sequence," he says. "But it turns out that in our mice we've also been very successful at getting antibodies that are cross-reactive and high-affinity for the mouse homolog."

Dressed to kill

Until recently, therapeutic antibody development has largely come down to identifying safe, high-affinity molecules, but there are also other ways in which function can be optimized. "The antigen-binding site, in my view, has been pretty much sorted

out," says Winter. "I think it's the rest of the antibody, what you link to it, that has to be sorted out..., [and] engineering the effector side has got huge potential."

Hinton agrees and adds, "I think we've seen in the industry over the last five years a lot of efforts at improving serum half-life and various effector functions, such as [antibody-dependent cell-mediated cytotoxicity]." PDL is among the companies exploring the modulation of antibody function via changes to the antibody constant (Fc) region, which can bind to a variety of receptors to mediate downstream immune functions. Xencor has also made considerable progress at antibody optimization using their Protein Design Automation algorithms, and in recent work, Xencor scientists described the generation of monoclonal antibodies with engineered Fc regions that exhibit enhanced effector function *in vitro* and improved cell-killing capabilities *in vivo*[5]. These fall under the umbrella of Xencor's XmAb technology platform, which encompasses various Protein Design Automation–engineered enhancements to the antibody scaffold. "We've got other variants in the Fc region that have optimized binding for FcRn, the affinity of which has been correlated with half-life, [and] we've got variants that alter different types of effector functions," says lead author Greg Lazar. "We've also optimized the solubility and stability of the variable region, and we're getting higher levels of expression."



Three-dimensional model of the variable domains of a mouse antibody (left) and a humanized antibody (right). The framework regions are shown in white, and the CDRs are indicated in red. In the humanized antibody, CDRs from the mouse antibody—along with key framework amino acids (blue)—were transferred into a human framework. (Courtesy of Shankar Kumar, PDL BioPharma).

The presence or absence of different glycans can greatly affect the Fc region's binding affinity for different receptors, and GlycoFi, which was recently acquired by Merck, is now exploring the potential of engineering the glycosylation of

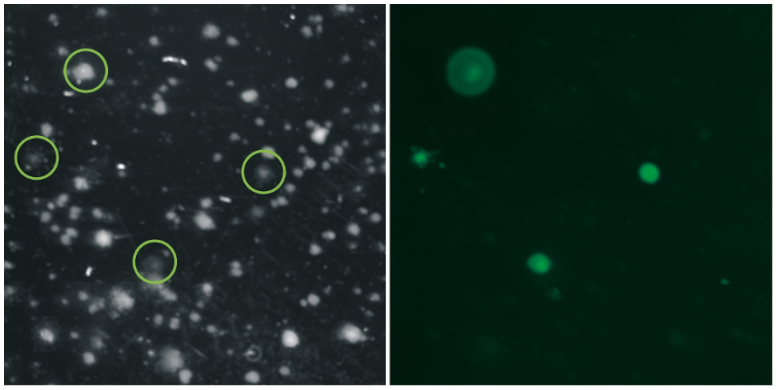
antibodies to modulate their therapeutic properties. Scientists at GlycoFi engineered *Pichia pastoris* yeast strains to perform specific *N*-glycosylation reactions; by expressing a given antibody in a particular strain, it becomes possible to control the nature of the specific glycoform that will be produced. Recent work with Rituxan (rituximab), a therapeutic antibody against CD20, showed that yeast-mediated addition of specific glycoforms enhances binding to stimulatory Fc receptors, leading to a dramatic increase in antibody-dependent cell-mediated cytotoxicity in patient blood samples at doses lower than those required for the unmodified antibody[6]. GlycoFi now has the capability to produce the full range of naturally occurring human glycoforms, according to cofounder and chief scientific officer Tillman Gerngross, and the hope now is to begin moving forward with the development of therapeutics. "I think we are very focused on... applying the technology to targets where we understand the biology well, where we know glycosylation is important, and where we know that we can make a difference," he says.

High-speed screening

With such techniques available, one could argue that the biggest obstacle to antibody generation is now in the screening process, which has traditionally been managed by performing large numbers of manual assays. Although the old-fashioned methods have proven valuable, they are also labor-intensive, and several manufacturers have responded by developing platforms that attempt to automate and accelerate hybridoma and monoclonal screening.

Applied Biosystems' 8200 Cellular Detection System uses a fluorescence-based approach for the high-throughput analysis of hybridomas in a variety of multiwell formats. Small amounts—typically five microliters—of clonal supernatant are added to wells containing cells expressing a surface antigen of interest or beads conjugated to soluble targets; detection of antibody binding is achieved with fluorophore-conjugated secondary antibodies. The macro-confocal imaging format limits the depth of focus to a range in which only cell- or bead-conjugated fluorescence is imaged, and the 8200 is designed for optimal detection of far-red emission wavelengths, dramatically reducing the background from autofluorescence or compound interference. The image data is processed in real time, after which it can be subjected to further analysis. "It saves everything that it sees in the well," says Carol Khodier, senior scientist at Applied Biosystems, "so you can reanalyze as often as you want [and] change the gates based on various parameters such as fluorescent intensity or color—and we have some two-color assays where you're looking at fluorescence ratios."

Fluorescence is also the basis of the ClonePix^{FL}, an instrument from Genetix that allows users to detect and pick hybridoma clones producing an antibody of interest. Diluted populations of hybridoma clones are plated in a semi-solid matrix containing fluorescently tagged antigen, and the concentration of fluorescence that results from active antibody production allows the system to actively identify and pick clonal colonies that not only produce a particular antibody, but also produce it at particularly elevated



Suspension CHO cells imaged by white light (left) and fluorescence (right) on the ClonePix^{FL} indicating the antibody-secreting colonies. (Courtesy of Genetix.)

levels—an especially useful feature for the production phase. "For the resulting cell line, we know it's a high producer, it's got high specificity, and it's clonal," explains Genetix chief scientific officer Julian Burke. Genetix also offers the QPDisplay, a unique platform for the automation of phage-display screens, which several leading companies have integrated into their workflow for the display-based isolation of single-chain human antibodies.

Biacore offers multiple platforms for the analysis of protein-protein interactions, two of which—the T100 and A100—are particularly well suited for antibody applications. The A100 takes advantage of an array format for high-throughput hybridoma clone screening and the analysis of antibody binding kinetics, and can screen approximately 3,800 interactions in twenty-four hours. The T100 is specialized more for antibody selection and characterization, and can be used for performing detailed thermodynamic, mechanistic and kinetic analyses for candidate antibodies. Both platforms perform their interaction analyses based on surface plasmon resonance, which allows investigators to perform label-free, real-time assays with considerable sensitivity. These systems have also proven valuable for characterizing serum-antibody responses in clinical immunogenicity studies—a major issue for therapeutic antibody development. "The key thing is that we can detect low- to medium-affinity antibody responses with very rapid kinetics, and those can be clinically significant because you can't necessarily tie the neutralization capacity of those reponses to the affinity," says Gary Franklin, industrial sector specialist for Biacore.

Beyond accelerating the rate of screening and discovery for traditional antibodies, such platforms also offer a useful tool for the exploration of other, non-immunoglobulin–based binding agents—of which more and more are emerging with each passing year. Mathias Uhlén of the Royal Institute of Technology in Stockholm, who has done considerable work with the combinatorial development of such alternative scaffolds, sees great promise in such tools, but also feels that this promise has yet to be fully realized. "The time-consuming part is not the selection, but it is the validation," he says. "Certainly it's going slower than I thought, and I do think that ten years from now, the dominant affinity reagent will still be the antibody." But, he adds, "there could also be a revolution."

1. Tang, D.-C. *et al. Nature* **356**, 152–154 (1992).
2. Bates, M.K. *et al. BioTechniques* **40**, 199–208 (2006).
3. Winter, G. *et al. Annu. Rev. Immunol.* **12**, 433–455 (1994).
4. Jones, P.T. *et al. Nature* **321**, 522–525 (1986).
5. Lazar, G.A. *et al. Proc. Natl. Acad. Sci. USA* **103**, 4005–4010 (2006).
6. Li, H. *et al. Nat. Biotechnol.* **24**, 210–215 (2006).
7. Ward, E.S. *et al. Nature* **341**, 544–546 (1989).
8. Hamers-Casterman, C. *et al. Nature* **363**, 446–448 (1993).

Michael Eisenstein is technology editor for *Nature* and *Nature Methods*.

SUPPLIERS GUIDE: COMPANIES OFFERING ANTIBODY PRODUCTS OR SERVICES

Company	Web address
21st Century Biochemicals	http://www.21stcenturybio.com
A&G Pharmaceutical	http://www.agrx.net
Abcam	http://www.abcam.com
AbD Serotec (Morphosys)	http://www.serotec.com
Abgent	http://www.abgent.com
Abnova	http://www2.abnova.com.tw
Ablynx	http://www.ablynx.com
Alpha Diagnostics International	http://www.4adi.com
Amgen	http://www.amgen.com
Applied Biosciences	http://www.appliedbiosystems.com
BD Pharmingen	http://www.bdbiosciences.com/pharmingen
Beckman Coulter	http://www.beckmancoulter.com
Biacore	http://www.biacore.com
BioInvent	http://www.bioinvent.com
Biosite	http://www.biosite.com
Cambridge Antibody Technology (AstraZeneca)	http://www.cambridgeantibody.com
Cambridge Research Biochemicals	http://www.crb.gb.com
Cell Essentials	http://www.cell-essentials.com
Covance Research Products	http://www.crpinc.com
Discerna	http://www.discerna.co.uk
Diversa	http://www.diversa.com
Domantis	http://www.domantis.com
Dragonfly Sciences	http://www.dragonflysciences.com
Dyax	http://www.dyax.com
Enzon Pharmaceuticals	http://www.enzon.com
Epitomics	http://www.epitomics.com
Eurogentec	http://www.eurogentec.com
Evogenix	http://www.evogenix.com
Fusion Antibodies	http://www.fusionantibodies.com
GeneService	http://www.geneservice.co.uk
Genetix	http://www.genetix.com
GENOVAC (Aldevron)	http://www.genovac.com
GlycoFi (Merck)	http://www.glycofi.com
Harlan BioProducts	http://www.hbps.com
Immuno-Precise	http://www.immuno-precise.com
Invitrogen	http://www.invitrogen.com
KPL	http://www.kpl.com
LAE Biotech International	http://www.laebio.com
Lampire Biological	http://www.lampire.com
Leinco Technologies	http://www.leinco.com
Mabtech	http://www.mabtech.com
Medarex	http://www.medarex.com
Micromet	http://www.micromet.de
MilleGen	http://www.millegen.com
Mirus Bio	http://www.mirusbio.com
NeoClone	http://www.neoclone.com
New England Peptide	http://www.newenglandpeptide.com
Open Biosystems	http://www.openbiosystems.com
Origen Therapeutics	http://www.origentherapeutics.com
OriGene	http://www.origene.com
PDL BioPharma	http://www.pdl.com
Pepscan Systems	http://www.pepscan.nl
Proteogenix	http://antibody.proteogenix.fr
ProtoPROBE	http://www.protoprobe.com
QED Bioscience Inc.	http://www.qedbio.com
Rockland	http://www.rockland-inc.com
Southern Biotech	http://www.southernbiotech.com
Strategic Biosolutions	http://www.strategicbiosolutions.com
Upstate	http://www.upstate.com
Xencor	http://www.xencor.com
XOMA	http://www.xoma.com
Yorkshire Bioscience	http://www.yorkbio.com