The shape of things

Advances in crystallographic methods, the rise of nuclear magnetic resonance, and the blossoming of global structural genomics initiatives have helped to fuel a renaissance in the development of methods for high-resolution protein structural biology. Michael Eisenstein reports.

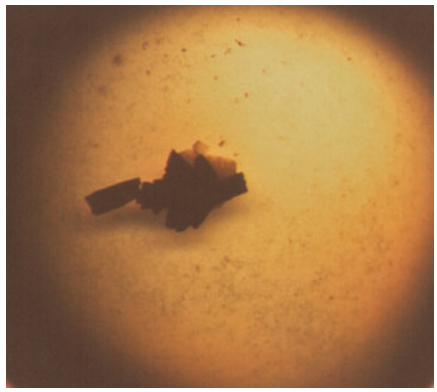
It might seem surprising that an established, half-century-old scientific technique relies greatly on 'black art'—but according to Naomi Chayen of Imperial College in the UK, this is an apt description of the way many scientists approach protein crystallization. "Many X-ray crystallographers aren't interested in 'why'; they just want to get a crystal and get a structure," she says.

Many pitfalls await the crystallographer, as each target protein requires the testing of numerous reaction conditions, consisting of varied concentrations of precipitating reagents at different temperature and pH set-points. The earliest stages of screening tend to be run in a high-throughput, trial-and-error manner, with investigators bludgeoning protein preparations with as many conditions as possible. However, the need for broad screening does not obviate the value of introducing a touch of the scientific method into the process.

Crystallization is mostly achieved by the diffusion of solvent from a small droplet of protein combined with precipitating reagents. Evaporation increases the concentration of the precipitating reagent and protein, until the solution reaches a state amenable to crystal nucleation. However, precipitation conditions arise if evaporation continues, and the process must be monitored to encourage the growth of a few ordered crystals rather than many small, poorly ordered crystals. Chayen advocates planning experiments with 'phase diagrams', which methodically chart the crystallization process so that investigators can zero in on ideal nucleation and growth conditions. "You do it by slowing down or controlling the process, by really nurturing it," she explains. "You go into the nucleation zone just enough to get a few nuclei, and then somehow back off into the area that is ideal for growth." Phase diagram–based planning has also proven helpful in microfluidic screening platforms, which represent a promi-sing new approach for faster and more efficient protein crystallization (see **Box 1**).

Predesigned 'screens' for testing crystallization conditions first became commercially available from Hampton Research in the early 1990s, based on the work of investigators who had empirically identified 50 crystallization conditions that had proven suitable for use with numerous different test proteins[1]. Since then, Hampton and other companies have launched a wide variety of specialized screening kits, and screen design has become increasingly rationally grounded, thanks in part to information from data repositories like the Protein Data Bank (PDB) and from high-throughput structural genomics efforts, such as the consortia backed by the US National Institutes of Health (NIH) through the Protein Structure Initiative (PSI).

Jena Bioscience is careful to proceed in the footsteps of earlier structural biology work in designing screens. "We go to the databases and try to extract the most successful conditions for proteins in general, or more specific protein classes, like membrane proteins," explains chief operating officer Thomas Billert. "We've recently introduced a screen just for protein kinases, because they are involved in many processes of medical interest." Emerald BioSystems also offers a wide range of optimized screens, many based on conditions identified by academic and institutional groups, such as the PrecipitantSynergy kit, originally developed at the NIH[2]. "The hit rate that you get, the number of crystals, is still the same, but the crystals produced in the first round are much more likely to be useful for structure determination," explains Emerald director Peter Nollert. Emerald also offers a database application, Crystal Miner, that is capable of designing screens for new targets based on previously recorded experimental data.



Light microscopy image of α-crystacyanin from lobster shell, grown on bioactive gel-glass nucleant particles. Reprinted from ref. 3 with permission.

Of course, access to data—especially negative data—remains a limiting factor. Lance Stewart, vice president of deCODE biostructures, credits the protein expression purification and crystallization database (PepcDB), a joint venture of PSI and PDB, as a valuable resource for crystallization data, but also points out an important shortcoming. "People tend to only report positive crystallization information," he says, "and 95% of possible crystallization data—for things that don't crystallize—gets left out." Chayen agrees, adding, "People are recognizing that they also have to realize what does not work."

The development of nucleating agents, which could act as a generic scaffold to drive the early stages of crystal formation, has also been seen as a promising means for taking the guesswork out of crystal production. Although progress in this area has been relatively limited, Chayen and her colleagues recently achieved notable success with the use of a mesoporous bioactive gel-glass as a nucleating reagent[3], which is now available commercially through Nanonucleant.

BOX 1 PAIN IN THE MEMBRANE

For many structural biologists, membrane proteins are the metaphorical elephant in the room: targets that are too challenging to solve and so get ignored in favor of simpler proteins—so-called 'low-hanging fruit'—even though this protein class includes many valuable drug targets. "30% of human proteins are membrane proteins," explains So Iwata. "[But] we only have up to 100 independent membrane protein structures in the PDB," and only a few of these are human.

Membrane proteins tend to be large and are often partially disordered, and their hydrophobic transmembrane domains lose their structural integrity outside the membrane context, making crystallization a challenge. G protein–coupled receptors (GPCRs) have proven particularly difficult to express and purify, and the only GPCR with a successfully solved crystal structure is bovine rhodopsin—a landmark achievement in structural biology[7].

This is clearly an untenable state of affairs, and several initiatives have been launched to remedy the situation. Iwata was a founder of the European Membrane Protein consortium (E-MeP), which brought together crystallographers and protein experts from across Europe to confront these intransigent targets, although so far success has been limited. "We established the consortium two years ago," says Iwata, "and we have only delivered three structures!" Similar programs are underway elsewhere, including the PSI-funded Specialized Centers at the University of California at San Francisco and the New York Structural Biology Center, as well as a Japanese initiative scheduled to launch in 2007. Iwata has also received funding from the Japanese government to launch the ERATO Human Receptor Crystallography Project, which will pursue new methods for membrane protein crystallization.

Several strategies are now under investigation, such as the co-crystallization of membrane proteins embedded in detergent micelles with antibody fragments, which improve crystallization by increasing the hydrophilic surface area, although success has still been limited. Bacterial overexpression is generally not a viable solution for generating eukaryotic membrane proteins, and this has made production a problem, forcing investigators to look to alternative organisms, such as the yeast *Pichia pastoris*.

The German company m-phasys has made the wily membrane protein its primary quarry, using a process called M-FOLD to generate inclusion bodies that are gradually refolded for structural analysis. "We've developed a rational method for finding initial starting conditions for refolding," explains CEO and founder Hans Kiefer. "We've done a large-scale program, and the result was that for 75% of the targets tested we could get refolded protein." Although crystals have been produced in several cases, these are typically not of diffraction quality. Nonetheless, progress is being made, and the company has received funding from the German government and—as part of a collaboration with ProSpect Pharma and University of California at San Diego investigator Stan Opella—from the NIH for long-term structural biology projects. m-phasys is also working with solid-state NMR, and has encountered initial success with two-dimensional GPCR structural solution[8]. Kiefer indicates that this method, which enables retention of proteins in a lipid bilayer, may prove ideal for tackling these targets. "I think that solid-state will be the method to use to study membrane proteins," he says.

Crystal clear?

Good crystals require good protein, which can also pose a challenge. Bacterial expression systems remain the method of choice for many investigators, because of their speed and robustness, but some proteins require processing or post-translational modifications that only take place in eukaryotic systems, whereas others—such as membrane proteins—are particularly intransigent to overexpression and purification (see **Box 2**).

The use of baculovirus in insect cells is a popular option for eukaryotic expression, although this can be expensive and labor-intensive to set up, and some yeast-based expression systems are also currently under investigation. Jena Bioscience offers a unique alternative with its LExSy platform, which relies on protein expression in the reptile parasite *Leishmania tarentolae*. "It's not pathogenic to humans, and you can cultivate it in cheap media with high growth rate relative to other eukaryotic organisms," explains Billert. LExSy has demonstrated some unexpected perks as well, including mammalian-style protein glycosylation. "Glycosylated proteins coming out of LExSy are very homogeneous, which is very interesting if you want to crystallize and determine the structure of glycoproteins," says Billert.

Some proteins may require a bit of tinkering on the part of the investigator. "We can engineer a number of proteins so that we can express and characterize functional fragments, as opposed to full-length proteins that might be beyond the range of crystallography," explains Zygmunt Derewenda, of the University of Virginia. On the other hand, working with full-length protein is often preferable, and in such cases direct sequence engineering may prove necessary. Derewenda and his colleagues have developed a system for introducing substitutions to individual surface residues that have the potential to interfere with crystal formation, and they have demonstrated the utility of this method for crystallizing some particularly difficult targets[4]. "In most cases, it is enough to generate two or three mutants, with each differing from the native sequence by no more than about three mutations concentrated at a particular site," he says, adding that these changes seldom affect protein function. Jena Bioscience also offers a surface engineering kit, which methylates surface lysine residues—an alteration that may improve the quality of crystallization.

In the pipeline

Although the broadly directed, content-centric aims of the structural genomics community have been an area of some debate among structural biologists, many agree on at least one important benefit that the consortia mentioned above have yielded—the development of efficient, automated processes for protein preparation and processing. "With automated crystallization, we can try many crystallization conditions and we don't need as much sample," says So Iwata of Imperial College. "We can scale down by at least 10- or 20-fold—and with the latest techniques, even 1,000-fold."

Automated solutions can range from compact benchtop robots such as Douglas Instruments' Oryx fluid-handling units, all the way up to massive systems such as the CrystalMation system, constructed by Rigaku for The Scripps Research Institute. "There's basically complete vertical integration, going from purified protein all the way to a final

The CrystalMaker provides start-to-finish automation for protein crystallography. (Courtesy of Rigaku.)

structure that gets deposited in the PDB," says Rigaku chief scientific officer Joseph Ferrara. Automation is also central in Iwata's laboratory at the new Diamond Light Source, which is slated to begin operation in 2007. "We want to have a high-throughput crystallization laboratory where we'll combine the automated crystallization facility with the beamline," he says. "As soon as we get the crystals, we can directly mount them in the beamline and screen them."

Most academic groups opt for simpler systems that expedite individual experimental stages but can also be incorporated into a larger workflow. Rigaku offers several such solutions, including the Alchemist II, a fluid-handling system for screen preparation that takes advantage of an extensive database of commercial screens and crystallization conditions to simplify screen design. Emerald offers a similar platform with its Matrix Maker, which works in conjunction with Crystal Miner to accelerate experiment development. "The Matrix Maker can produce an optimization screen within less than 35 minutes," says Nollert.

Each sample must then be monitored for crystallization—a process that grows increasingly tedious with each additional 96-well plate. Several systems are now available that can automatically image crystallization plates on a user-designed schedule. Bruxer AXS and Discovery Partners have designed their range of CRYSTAL FARM units for this purpose, with units that can house up to 150—or up to 5,000—plates at once. Rigaku offers the Minstrel HT, which combines automated incubation with a proprietary imaging system that illuminates plates with a controllable LED pattern that makes crystals stand out more clearly in the well. The Rhombix product family, available from Thermo Fisher Scientific, is also a popular option, combining high plate capacity and a variety of imaging modalities. "We have bright-field, which is basic; we have dark-field, which enhances the edges... and we have true polarized imaging,

where you can take advantage of a property of crystals called birefringence," explains product manager Paige Vinson. Rhombix units also maintain a database of automation settings and crystallization conditions—including positive and negative results—as a resource for planning experiments.

What happens next, however, typically falls to human judgment—and eyes. "Image analysis is still a sore point," says Nollert. "Although there's some technology available that tries to rank images, crystallographers tend to be very skeptical." Oxford Diffraction has recently launched a standalone system for X-ray diffraction, the PX Scanner, which the company claims can dramatically improve automated crystal identification and even distinguish protein crystals from salt crystals. For the most part, however, scientists need to make the final call. "Most people use screening software for negative conditions, to screen out wells that are perfectly clear," says Bruker AXS chief technology officer Roger Durst, after which "the user can manually go through the wells and decide where he sees the best crystals."

BOX 2 THE CRYSTAL CHIP

Looking at some of the imposing automated workstations that have been assembled to accelerate the processes of crystal production and analysis, it may seem hard to believe that these operations could also be shrunk down to a couple of microchips. Nonetheless, a handful of investigators and companies are actively—and successfully—pursuing this goal, doing their best to make a growing field considerably smaller.

Stephen Quake of Stanford University and James Berger of the University of California at Berkeley were the first to combine microfluidics and crystallography. In 2002, Berger, Quake and colleagues published an article describing a microfluidic chip that could perform 144 parallel crystallization screens using a process known as free-interface diffusion (FID). FID had long been regarded as a promising solution for crystallization screens, but the interference of gravity with the process limited its use; however, FID proved quite suitable for generating diffraction-quality crystals in a microfluidic environment[9]. These FID chips would ultimately be commercialized by Fluidigm, which currently incorporates this technology into their integrated TOPAZ system for automated crystallization screening.

In subsequent work, Quake has continued to develop and refine these microfluidic platforms, producing chips that allow tighter control—and therefore optimization—of the crystal formation process and that enable in-chip data collection from the resulting crystals. Combined, these devices have given his team the ability to solve novel protein structures using microfluidics from start to finish. "We have a new paradigm in which we use a device to screen the phase behavior of the protein and design a rational crystallization screen, then we do the crystallization in the FID chip, and then we scale up and collect the data in the diffraction chip," says Quake. "I'm hoping to turn my lab into a mini-production facility."

Rustem Ismagilov of the University of Chicago has developed an alternative solution, using nanoliter-sized 'plugs' of solution to dramatically shrink crystallization reactions. These plugs of protein and precipitant solution are transported within a fluorocarbon carrier fluid through a soft lithographic fluidic system that controls their mixture. In the platform's most recent incarnation, Ismagilov described a system that allows users to independently control nucleation and growth stages to optimize crystal growth[10]. "The big advantage is that the principle is conceptually very simple," says Ismagilov. "You can use Teflon tubing, which is much cheaper than fabricating real microfluidic chips." The group also has developed a 'hybrid' microfluidic chip, which handles both screening and optimization processes, for the successful on-chip crystallization of membrane proteins, a challenging class of target[11]. Ismagilov's group is developing the technology in collaboration with academic and corporate partners at the ATCG3D, and hopes that such microfluidic platforms will soon be making their way into the hands of structural biologists who crave high throughput but lack access to automation. "There are a lot of very important structures coming out of individual labs," he says, "and enabling this small-scale, inexpensive and rapid screening and crystallization would have a lot of impact for these people."

The MICROSTAR rotating-anode generator provides high X-ray intensity for the home laboratory. (Courtesy of Bruker AXS.)

Going straight to the source

The growing number of dedicated, 'third-generation' synchrotron X-ray sources has made beamline access more practical and affordable, and improved the resolution of structures that can be obtained. Adding to this the advent of 'FedEx crystallography' services, which allow users to mail crystals to facilities such as Brookhaven National Laboratory or the European Synchrotron Research Facility for analysis, and one could argue that crystallographers have never had it so good.

However, many researchers would welcome the chance to keep their work even more 'in-house'. "If a time-critical problem comes up, where you need a structure that week, it is very convenient to be able to get a structure in the home lab," says Durst. "And for some private-sector projects, there are security issues." For such cases, the new generation of rotating-anode X-ray generators offers a useful alternative. These have benefited from the rise of single-wavelength anomalous diffraction (SAD) techniques, an alternative to multiwavelength anomalous diffraction (MAD) that eliminates the need for tunable X-ray wavelengths, which are only available at the synchrotron.

With their MICROSTAR generator, Bruker AXS claims to have achieved the highest X-ray intensity to date in a rotating-anode home-lab source—greater than 8×10^{10} photons per square millimeter per second. "It's comparable to many second-generation synchrotron beamlines, in terms of available intensity," says Durst. Bruker's X8 PROTEUM combines the MICROSTAR with high-end digital detection, improving signal-to-noise ratios and accelerating data collection. "A synchrotron beamline will almost always provide higher-resolution data," says Durst, "but in terms of getting primary structural information, it's becoming possible to get almost any protein structure, albeit at a slower data acquisition rate."

Ferrara points out that home-lab data collection can be a valuable first step. "If you have the structure solved when you get to the beamline, the trip then becomes about simply collecting high-resolution data, which is a much lower-stress scenario," he says. Rigaku's FR-E DW SuperBright generator, which provides the highest brilliance of any home laboratory source, offers a unique feature among rotating-anode systems—a chromium radiation source, in addition to the standard copper source. This can double the signal obtained in a SAD experiment from atoms such as sulfur, potentially eliminating the need for heavy-atom derivatization for many targets. "Something like 97% of human proteins are expected to have enough sulfur to use chromium SAD," he says. "So this allows you to skip steps like selenomethionine derivatization and use the native protein."

Several groups are now developing 'portable' synchrotrons, which could potentially be installed in an institutional core facility. For example, Japan's Photon Production Laboratory manufactures the MIRRORCLE-20ST for protein crystallography applications, and Lyncean Technology is developing its Compact Light Source (CLS) as part of the PSI's Accelerated Technologies Center for Gene to 3D Structure (ATCG3D). The CLS will provide a room-sized, tunable hard X-ray source for performing MAD experiments outside the synchrotron. Although Iwata doubts these sources will provide a universal solution for resolving extremely difficult targets, he adds that "for most structural genomics people, this is really good—and it could replace full-scale synchrotrons."

The 'nuclear' option

Nuclear magnetic resonance (NMR) spectrometry is hardly new, but its application to the solution of protein structure is still a relative novelty—although many laboratories both inside and outside the structural genomics community are coming to recognize its power and distinctive benefits as a valuable counterpart to crystallography. "There are many examples in our work where we were not able to get crystals, but could solve the structure by NMR," says Gaetano Montelione, whose lab at Rutgers University is active in the Northeast Structural Genomics (NESG) Consortium.

Protein NMR spectrometry involves subjecting concentrated solutions of proteins that have been isotopically labeled (typically with ^{15}N and ^{13}C) to a strong magnetic field, then bombarding them with radiofrequency (RF) energy. A variety of experimental methods are available that allow researchers to assign peaks from the resulting spectral profile to individual atoms, and eventually assemble a structure. However, this becomes considerably more difficult as targets increase in size. "As proteins get larger, they tumble more slowly... and their relaxation processes become more efficient, and the signal decays away rapidly," explains Montelione. "Higher

The ECA NMR system from JEOL can currently be configured with field strength of up to 920 megahertz. (Courtesy of JEOL USA.)

magnetic field strength has been especially valuable for dealing with larger or slowly tumbling systems."

"We're always competing for the highest magnetic field," explains Douglas Meinhart, analytical instruments director for JEOL USA, "and we've been supplying 920-megahertz magnets for a while now." Most leading manufacturers have now pushed their highest-end instruments beyond the 900-megahertz range, and Bruker Biospin recently announced the production of an industry-leading 950-megahertz version of their Avance II instrument for the David H. Murdock Research Institute in North Carolina. Such high-end instruments confer considerably improved sensitivity and resolution, but also have price tags to match—well into millions of dollars—and many users are benefiting from technologies that extend the effectiveness of instruments with intermediate fields of 600–700 megahertz.

Cryogenically cooled probes have proven to be a critical advance in this regard. "The electronic circuitry is cooled to a very low temperature," explains Clemens Anklin, vice president of NMR applications and training at Bruker Biospin. "The cold RF-coil on one hand increases the signal while a cold pre-amplifier reduces the noise, so you have increased signal-to-noise." The probes can confer up to a fourfold increase in sensitivity, and Bruker and Varian, Inc. offer a variety of specialized options. Recently, Varian expanded its line of probes with the Salt Tolerant Cold Probe, designed for improved sensitivity in high-salt samples. "We've invested significantly in improving sensitivity for biologically relevant systems, where you have to have a certain level of salt and buffer to mimic physiological conditions," explains product marketing manager Judit Losonczi. Bruker has also launched a series of 'microprobes', which allow users to analyze far smaller samples, alleviating the strain of protein production and labeling. "Instead of 300 microliters, which is a typical sample volume, we can get a spectrum from as little as 5–7 microliters," says Montelione, who is now exploring the use of such probes for the analysis of limited quantities of small proteins.

Gains have also been made in data recording and processing. Some of these have come from the academic side, such as the G-matrix Fourier Transform method developed by Thomas Szyperski of the State University of New York at Buffalo and the NESG[5], which simplifies the recording of multidimensional protein spectra in a manner that accelerates data collection and structural analysis. Other gains have come at the instrument end, through improvements in spectrometer technology. For example, Losonczi touts the flexibility and speed of the Varian NMR System. "It features very fast and accurate switching of RF phase, amplitude and frequency," she says. JEOL's Meinhart also points out the importance of an effective analytical framework. "When you add it all up, you have three or four simultaneous RF channels running independently and synchronized, and you have hundreds of events in these channels," he says. "What we've brought out are quite sophisticated spectrometer technologies for doing these multiple-channel experiments."

The bigger they are...

As indicated above, size can present a limitation for NMR spectrometry structural analysis. Several important innovations, such as the protein deuteration techniques and the transverse relaxation–optimized spectroscopy (TROSY) method desired by Kurt Wüthrich of the Eidgenössiche Technische Hochschule Zürich, have pushed the size limit farther, but most investigators still limit their analyses to proteins with a maximum size of 25–40 kilodaltons.

Of course, these limits are not iron-clad, and several investigators, such as Lewis Kay of the University of Toronto, have developed techniques for analyzing far larger targets. "We exploit a labeling scheme whereby we prepare highly deuterated proteins that are methyl-protonated," explains Kay. "Methyls have three protons and they're also dynamic, so there's a lot of averaging going on, and even if you're in the context of a big protein, the dynamics mean that you have something that looks smaller. We can record high-quality spectra even for molecules as large as the proteasome." Another promising technique for targeting larger proteins is stereo-array isotope labeling (SAIL), developed by Masatsune Kainosho of the Tokyo Metropolitan University, in which stereospecific and regiospecific isotopic labeling and cell-free protein synthesis are combined to make fast, high-resolution NMR structure determination possible for moderately large proteins[6].

These advances aside, however, even strong advocates of NMR spectrometry agree that the greatest benefits from the technique are likely to come through its ability to monitor protein dynamics. "NMR is very sensitive to protein motions [and] it can characterize rates of conformational interconversion on multiple timescales," says Montelione. "I think perhaps the more exciting thing about NMR is not just getting atomic coordinates, but the ability to get dynamic information about flexibility and its role in determining protein function." Meinhart agrees: "If you put NMR against crystallography, head to head, crystallography wins the vast majority of the time. What NMR brings to the table that other techniques don't is the ability to do dynamic, time-resolved studies—to get a handle on actions happening in the proteins."

The Varian NMR System can be equipped with probes designed for increased sensitivity in high-salt biological solutions. (Courtesy of Varian, Inc.)

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SUPPLIERS GUIDE: COMPANIES OFFERING PRODUCTS FOR CRYSTALLOGRAPHY OR NMR SPECTROMETRY

Company	Web address
Accelrys	http://www.accelrys.com
Acorn NMR, Inc.	http://www.acornnmr.com
Advanced Research Systems, Inc.	http://www.arscryo.com
Area Detector Systems Corporation	http://www.adsc-xray.com
Art Robbins Instruments	http://www.artrobbinsinstruments.com
Astex Therapeutics	http://www.astex-therapeutics.com
Axygen Biosciences	http://www.axygenbio.com
Biotom	http://www.biotom.net
Bio-Xtal	http://www.bioxtal.com
Blake Industries, Inc.	http://www.pittdifsoc.org/blake.htm
Bruker AXS	http://www.bruker-axs.de
Bruker BioSpin	http://www.bruker-biospin.de
BSI Proteomics	http://www.bsiproteomics.com
Caliper Life Sciences	http://www.caliperls.com
Carna Biosciences	http://www.carnabio.com/english
Corning Life Sciences	http://www.corning.com/lifesciences
Cryo Industries of America	http://www.cryoindustries.com
CrystalGenomics	http://www.crystalgenomics.com
Crystal Impact	http://www.crystalimpact.com
deCODE Chemistry and Biostructures	http://www.decodebiostructures.com
Douglas Instruments, Ltd.	http://www.douglas.co.uk
Emerald BioSystems	http://www.emeraldbiosystems.com
Fluidigm	http://www.fluidigm.com
Formulatrix	http://www.formulatrix.com
Genevac	http://www.genevac.com
Genomic Solutions	http://www.genomicsolutions.com
Gilson	http://www.gilson.com
Greiner Bio One	http://greinerbioone.com/en/start
Hamilton Life Sciences	http://www.hamiltoncompany.com
Hampton Research	http://www.hamptonresearch.com
Hecus Graz X-Ray Systems	http://www.hecus.at
HUBER Diffraktionstechnik GmbH	http://www.xhuber.com
Imperial Innovations	http://www.imperialinnovations.co.uk
Incoatec GmbH	http://www.incoatec.de/incoatec/index.php
Innovadyne Technologies, Inc.	http://www.innovadyne.com
Invitrogen	http://www.invitrogen.com
Jena Bioscience	http://www.jenabioscience.com
JEOL USA	http://www.jeolusa.com
Korima, Inc.	http://www.korimainc.com
L.A. Systems, Inc.	http://www.las.jp
Lyncean Technologies, Inc.	http://www.lynceantech.com
mar research	http://www.marresearch.com
Micronics	http://www.micronics.net
MiTeGen	http://www.mitegen.com
Molecular Dimensions, Ltd.	http://www.moleculardimensions.com
m-phasys	http://www.m-phasys.com
Nanonucleant	http://www.nanonucleant.co.uk
Neuro Probe	http://www.neuroprobe.com
Nexus Biosystems	http://www.nexusbio.com
Norgen Biotek Corp.	http://www.norgenbiotek.com
One Moon Scientific, Inc.	http://www.onemoonscientific.com
Oxford Cryosystems	http://www.oxfordcryosystems.com
Oxford Diffraction	http://www.oxford-diffraction.com
PANalytical	http://www.panalytical.com
Photon Production Laboratory, Ltd.	http://www.photon-production.co.jp
Princeton Instruments/Acton	http://www.piacton.com
Proteros Biostructures	http://www.proteros.com
Qiagen	http://www.qiagen.com
Rigaku	http://www.rigaku.com
Shamrock Structures, LLC	http://www.shamrockstructures.com
Sigma-Aldrich	http://www.sigmaaldrich.com
Takara Bio, Inc.	http://www.takara-bio.co.jp/english
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Thermo Fisher Scientific	http://www.thermo.com
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TTP LabTech	http://www.ttplabtech.com
Varian, Inc.	http://www.varianinc.com
Xenocs	http://www.xenocs.com