

Protein Chromatography:

Tools for Protein Expression and Purification

Executive Summary

Biocompare Surveys and Reports
Published July 10, 2006

Table of Contents

I.	Report Introduction.....	1-2
II.	Market Overview.....	3-5
III.	Industry Insights.....	6
	i. Qiagen.....	6-7
	ii. Applied Biosystems.....	8-9
V.	Survey Introduction and Methodology.....	10
VIII.	Appendix I: Questionnaire.....	11-15
IX.	Appendix II: Presentation of Survey Data.....	16
	i. Demographic Survey Data.....	17-23

Report Introduction

The 2006 Protein Chromatography Report is composed of a market overview and an introduction to the 2006 Protein Chromatography Survey, which contains questions about chromatography systems and related applications that researchers are using. The report also includes a discussion of the survey results and conclusions and recommendations drawn from both a market analysis of protein chromatography and the survey data. A new feature, academic assessment, presents an interview with an expert in protein chromatography and protein expression systems that rounds out this comprehensive report.

Biocompare spoke with Irina Neverova, Ph.D., adjunct professor of physiology at Queen's University in Ontario, Canada, to discuss her perspective on proteomics and the advances in protein separation techniques. She describes the proteomics movement as “in a big transition period,” with the primary challenge being “pulling apart complex proteomes” in terms of the “different intrinsic properties” of the proteins and the span of their known concentrations reaching 10^{10} . She predicts that multidimensional fractionation will aid researchers in the separation of proteins, provided that the columns and samples are optimized and that conditions are controlled. When queried about automation, Dr. Neverova commented that the pharmaceutical industry has made significant progress in this area, while academia is less focused on automation in general. She remarks that high resolution and sensitivities are important benchmarks that have not yet been achieved, but are essential for molecular diagnostics, such as the detection of biomarkers in a complex protein mixture such as plasma. Dr. Neverova predicts that high-performance liquid chromatography (HPLC), in addition to reversed-phase, affinity, and ion-exchange chromatography methods, will continue to evolve in terms of selectivity and miniaturization advances as well.

The market overview supports the opinions of Dr. Neverova as the protein chromatography market has continued to grow, notably in clinical and biomarker applications. The diverse selection of chromatographic techniques, such as capillary electrophoresis, HPLC, and ion-exchange, affinity, and two-dimensional chromatography, allows for significant advances in the improvements and innovations that lead to higher sensitivities, specificities, and reproducibilities in protein separation. At present, protein chromatography products bring in revenues surpassing \$150 million and are expected to top \$237 million by 2010¹. The entire liquid chromatography market is predicted to exceed \$3 billion over the next 4 years².

Certain analytical systems are expected to outperform others, as the need for increased throughput and miniaturization increase, particularly in the pharmaceutical arena. HPLC and electrophoresis are expected to grow faster than other separation technologies. Niche separation products, such as chip-based microarrays and liquid chromatography/mass spectrometry (LC/MS) hybrid systems, are growing at over 15% annually. Such products are the result of technological innovations that drive improvements in resolution, speed, and sensitivity, of utmost importance in the growing clinical proteomics field.

The marketplace is crowded, however, and companies have had to strategize ways to stay one step ahead of their competition, including designing custom systems, offering value-based service and

Report Introduction (continued)

adequate training for sales personnel, increasing price-to-performance ratios, and continuing to innovate and differentiate their particular products and services. With the decreasing costs of instrumentation, profit margins are slim and the promise of huge profit margins in this product area will be difficult for vendors to realize.

1. Frost & Sullivan, "World Protein Liquid Chromatography Markets", March 8, 2004.
2. Ibid.

Market Overview

Proteomics has shifted the protein chromatography market into high gear. The main thrust stems from the goal to catalog the estimated 300,000 to one million proteins expressed in human cells. And while protein chromatography lies at one end of the process to characterize these proteins, mass spectrometry (MS) sits at the other end. “Central to most proteomics strategies” and “the technique of choice for identifying and probing the covalent structure of proteins”¹, MS is creating a vacuum for purified proteins and peptides. Easing the bottleneck of preparing proteins for MS will be a major step toward maintaining the workflow.

Researchers once looked to two-dimensional gel electrophoresis (2-DE) to separate proteins, first based on charge and then by molecular weight. The technique was sufficient when researchers were studying proteins expressed at high- or even mid-levels. But with most already identified, researchers now require the stronger resolving power of chromatography to probe the low abundance proteins that are relevant to disease.

“Clinical proteomics and biomarker studies continue to gain interest, boosting market opportunities,” said Sinead Igoe, an analyst with Frost & Sullivan.

Tackling proteomics means dissecting the proteome, or “the time- and cell-specific protein complement of the genome, encompassing all proteins expressed in a cell at any given time, including protein isoforms as well as co- and post-translational modified forms”², as defined in the December 2004 issue of the *Journal of Chromatography B*. That means constructing a picture of the complement of proteins present under various temperatures, nutritional statuses, biochemical influences and many other factors. Compounding such complexity is the diverse nature – size, charge, hydrophobicity, structure, post-translational modifications, for example -- of proteins within one proteome. The concentrations at which they are present, spanning a range of 10 to 12 orders of magnitude, also complicates study.

“Protein molecules have different structures and chemical properties and also vary with respect to their intracellular abundance in different cell types,” said Ravi Shankar, an analyst with Frost & Sullivan. “It is therefore virtually impossible to find an analytical technique that is capable of resolving and separating the thousands of protein forms present in a cell at a given time.”

What researchers do have is a selection of chromatography techniques. These include capillary electrophoresis, reversed-phase high performance liquid chromatography and cation exchange, affinity and two-dimensional chromatography. Researchers in both academia and industry have endeavored to take full advantage of these options. Through such efforts to improve and innovate, the current selection of chromatography products feature higher sensitivity, specificity and accuracy and better reproducibility.

Analysts indicate that these products will continue to gain a greater following. While worldwide sales of protein liquid chromatography products brought in revenues of \$154 million in 2003, they could bring in \$237 million by 2010³, according to Frost & Sullivan. Revenues for the entire

Market Overview (continued)

liquid chromatography market should reach \$3.383 billion by 2010⁴.

Among all analytical instruments, those for separation technology are expected to grow fastest⁵, Frost & Sullivan analysts suggest. While gas and ion chromatographs, liquid chromatography and electrophoresis equipment are expected to grow at a compound annual growth rate in the “high single” digits, growth for all analytical instruments is estimated to be 4.7% in 2006⁶. As a whole, technologies for analytical separation brought in an estimated \$7.8 billion in 2005⁷, according to analyst Kieran Lindsey of BCC Research. That figure is expected to reach more than \$11.8 billion in 2010 with an average annual growth rate (AAGR) of 8.6%.

Frost & Sullivan analysts indicate that high performance liquid chromatography and electrophoresis could grow faster than other separation technologies⁸. By Lindsey’s estimate, however, membrane separation products are growing at a rate of 14.1%, while the AAGR for all chromatography products is 11.3% and 8.3% for electrophoresis⁹. Growing at 15.2% are niche separation products, “which include chip-based microarrays, LC/MS hybrids, spinning systems, magnetic systems and supercritical fluids systems that recover product for sale”¹⁰.

In fact, Kalorama Information analysts expect healthy sales of analytical process microchips, such as those that perform electrophoretic and chromatographic separations. With revenues of \$5 million in 2004, the expected CAGR of 148% will lead to revenues of \$240 million by 2008¹¹. The CAGR of 27.4% during the period of 2009 to 2014 will lead to revenues approaching \$800 million.

With a crowded marketplace, companies have had to formulate strategies to stay ahead and attract and retain customers. According to Frost & Sullivan, successful companies will: 1.) design instruments according to customer requirements; 2.) offer value-based service; 3.) provide adequate training to sales personnel on cost benefits to clients from specific instruments; 4.) increase price-to-performance ratio; 5.) innovate and constantly differentiate products and services¹². The pressure to maximize value for every dollar, however, collides with the increasing cost of raw materials. Together with the decreasing price of instrumentation, “profit margins are expected to become so slim, that it is likely to become difficult for vendors to realize huge profit margins”¹³.

Researchers have benefited from the intense competition. Companies have introduced 24/7 helplines and online customer assistance. Customers can choose increasingly popular hyphenated instruments, which are offering higher sensitivity and speed. Also available are stand-alone instruments that readily integrate with downstream or upstream modules and that can be easily scaled according to throughput.

Competition and the needs of proteomics have also driven remarkable technological innovation. Waters Corporation (Milford, Mass.) has stood out with its “Acquity UPLC System”. Called ultra performance liquid chromatography, the system works with 1.7 micron particles (most other systems use beads of 5 microns). This simultaneously allows for greater resolution, speed and

Market Overview (continued)

sensitivity. Despite slowing sales, the company cites the popularity of its UPLC system for buoying revenues and profits¹⁴.

While the biopharma and medical research sectors have traditionally driven growth, other industries have begun to make an impact on sales. The military and civil defense sectors have appeared on the liquid chromatography scene as they pay more attention to biological warfare and the need for reliable sensors. Environmental monitoring and quality control in various industries have also helped sales.

1. Domon B and Aebersold R, "Mass Spectrometry and Protein Analysis", *Science* 312:212-217, April 14, 2006.
2. Neverova I and Van Eyk JE, "Role of Chromatographic Techniques in Proteomic Analysis", *Journal of Chromatography B*, 815(2005):51-63, December 2004.
3. Frost & Sullivan, "World Protein Liquid Chromatography Markets", March 8, 2004.
4. Ibid.
5. Frost & Sullivan, "World Laboratory Analytical Instrumentation Market", July 14, 2005.
6. Ibid.
7. Lindsey K, "Analytical Separations: Trends and Markets", BCC Research, December 2005.
8. Frost & Sullivan, "World Laboratory Analytical Instrumentation Market", July 14, 2005.
9. Lindsey K et al.
10. Ibid.
11. Kalorama Information, "US Markets in Analytical Chip Technology 2nd Ed", June 1, 2005.
12. Frost & Sullivan, "World Laboratory Analytical Instrumentation Market", July 14, 2005.
13. Ibid.
14. Waters Corp., Form 10-K for the fiscal year ended December 31, 2005.

Industry Insights - Qiagen

Biocompare spoke to Frank Schäfer, PhD, associate director of R&D at QIAGEN (Hilden, Germany), where he oversees products relevant to protein expression, purification, and downstream processing, recombinant proteins. Dr. Schäfer received his doctorate in biology from the Institute for Molecular Biology and Tumor Research, University of Marburg.

1. How is Qiagen involved in the chromatography business?

Qiagen is well-known for nucleic acid purification, which is mostly done by chromatography. We began offering protein chromatography in 1992 when we acquired the rights to Ni-NTA (nickel-nitrilotriacetic acid ligand) from Roche (Basel, Switzerland). It's still one of our most popular products for purifying proteins with the 6xHis (peptide tag of six histidine residues) affinity tag.

2. Has emphasis on proteomics affected the sale of these products?

Proteomics has had some influence on our business. But, it all ends up in the same workflow. In proteomics you might use mass spectrometry to identify a protein that could be a disease biomarker. But, if you really want to study the biomarker, you're going to use recombinant technology to produce sufficient amounts of the protein. This requires purification of both plasmids – or other forms of nucleic acids – and recombinant protein.

3. Are these customers from academia or industry?

Both. I'd say about half of our customers are from academic institutions and the other half is in the biopharmaceutical industry. Many use recombinant technology to insert the 6xHis tag to a target protein, which can then be purified with the affinity chromatography columns packed with Ni-NTA matrix. The researchers use the purified protein for functional assays or structure determination. And they also use the protein to generate antibodies. The difference with industry is that they do the same thing, but on a wider scale and higher throughput. For that, we have automated instruments. Our products can be used to scale up to manufacturing quantities, but that's a smaller customer base for Qiagen compared to the small-scale screening business.

4. Why is that?

Affinity chromatography is still not as accepted as completely mainstream, relative to older techniques like ion exchange. Affinity is a relatively new technology. And, some biopharmaceutical companies are concerned that the metal ligands could possibly leach into the sample. Although affinity chromatography is an extremely efficient purification step, you usually have to do a second round of purification for manufacturing processes. We're trying to convince the industry that affinity chromatography can actually be more economical. It's a calculation that individual companies have to perform themselves. Is it cheaper to do a two-step affinity chromatography? Or to do conventional chromatography, which requires many more purification steps?

5. What can you do about improving specificity?

Use affinity tags that are more specific, such as Strep, which is a nine-amino acid peptide. The binding ligand, Strep-Tactin, has a slightly higher binding specificity but lower binding affinity than Ni/NTA. If you want higher purity, Strep might be better. Use the 6xHis tag if you want

Qiagen (continued)

higher yield. Some people use both tags on the same protein. They first use the Ni/NTA column, and then use Strep-Tactin as a polishing step to increase purity. It all depends on what is most important to you.

6. Sounds like chromatography can still use some improvements. What are your customers asking for?

They usually ask for matrices that are specific for the molecule they want to purify. But this is the challenge that we face: Every protein behaves differently. Recombinant proteins have increased the diversity of proteins that people want to purify. So, it's difficult to make a product that's really robust so that it works in everyone's hands. I think this has been done well for Ni-NTA. We want to make it as easy as possible so people don't have to do their own optimizing. For that, we're looking for the one recipe that works for an entire class of proteins. This is certainly a challenge, but the protein business is growing. To grow it faster, new solutions will have to come.

6. What kinds of solutions?

One way to enrich for certain classes of proteins is to target specialized ligands. You can enrich for phosphorylated or glycosylated proteins by targeting the phosphate or sugar molecule. You can also fractionate samples if you know where the target protein is located in the cell. For example, Qiagen has enrichment kits for proteins in the mitochondria, nucleus and membrane.

7. Have automated devices proven to be a good solution for speeding up the pace of research?

Certainly. But you don't have to purchase machinery to improve efficiency. You can find affinity chromatography kits designed for 96-well plates in which ligands are immobilized in the wells. Or you can use the wells to hold Ni-NTA beads that have a magnetic core, which simplifies the task of separating out protein that's bound to the ligands. People looking for automation will find that the market offers instruments with a wide range of price tags. At the lowest end, there are devices that handle 12 to 15 samples and still need some hands-on intervention, such as lysing cells prior to using the device. Then there are the devices that can work with 96-well plates. The most expensive devices are fully automated and can lyse the cells and perform all the liquid handling. These are becoming increasingly popular because people are generally trying a number of constructs to express proteins. They use different tags, placed in different places on the construct to see which one works best. So, the tendency is to generate more constructs of proteins, which means you have more protein to purify.

Industry Insights - Applied Biosystems

Biocompare spoke to Paul Lynch, product manager for chromatography at Applied Biosystems Group (Foster City, Calif.). He joined the company as part of its acquisition of PerSeptive Biosystems, where he supported the chromatography product line as an applications scientist.

1. How is Applied Biosystems (AB) involved in the chromatography business?

AB offers chromatography media and columns for both manufacturing and research customers. We offer various types to make sure we can address the needs of our customers. These include products for reversed-phase, ion exchange, hydrophobic interaction and affinity chromatography products. We offer them in formats for small-scale purification and analytical applications, as well as for large scale production.

2. How are research products different from manufacturing products?

In general, the chemistries are similar but the requirements of the products can be vastly different. Researchers tend to need smaller pre-packed columns that use media with a smaller particle size. Manufacturing customers generally work with very large columns of 100 to 1000 liters packed with bulk media, typically with slightly larger particle size. This mitigates any backpressure issues associated with running such large columns and systems. Manufacturing customers also need products with a somewhat higher degree of quality, consistency and traceability because the products could be used for therapeutics. In fact, most of our manufacturing customers are producing FDA-approved monoclonal antibodies. Biologics are driving growth.

3. What kinds of products are your research customers looking for?

These days, customers are looking for generic solutions that are fast and easy to use. They want to spend time on their research, and not on developing or troubleshooting protocols or techniques.

4. Sounds as easy as “heat and serve”.

These days, chromatography instruments and kits have made chromatography much more intuitive. Ten to 15 years ago, researchers spent a lot of time on developing purification protocols, which took them away from focusing on more meaningful work. Now, they can use our cookbook strategy and spend most of their time on characterizing protein structure or studying their activities.

5. But can researchers really afford the luxury of kits and other conveniences?

When you purchase consumables, you're also purchasing consistent quality and standardization. You don't always get that when you make reagents yourself. Researchers see the investment as a more cost effective use of their time. They recognize that the new kits and instruments can make their work a lot easier, and allow them to move forward in their research in a more efficient manner.

6. What kinds of chromatography techniques do they seem to be favoring?

Right now, affinity chromatography is one of the major drivers of the chromatography market. People aren't using size exclusion as much anymore, especially on the large scale. It's too slow and

Applied Biosystems (continued)

requires more hands-on time to get it right. Other techniques such as ion exchange and hydrophobic interaction will continue to be popular, especially for preparative or large scale applications.

7. Why is affinity chromatography so popular?

It's a one-step purification protocol with great separation and selectivity. You can perform affinity chromatography in a manual mode. Or, you can automate it on an HPLC (high performance liquid chromatography) system. Years ago, researchers could spend weeks on complicated purification methods to obtain protein that was pure enough. These days, researchers can express their target protein in bacteria one day and get milligrams of purified protein. Affinity chromatography makes it so simple.

8. Does that mean that the sample is completely purified?

With metal chelate chromatography, in which metal chelates act as ligands in the column, you get purified protein nine times out of 10. So, sometimes you need to do another step, like size exclusion or ion exchange to remove the minor contaminants leftover in the sample. If you want avoid the second step, you can consider other affinity techniques. For example, with Protein A, which purifies IgG, you can get higher specificity and purity and not worry about a second purification step.

9. What do you need to do to increase purity?

Find a more specific tagging approach. Currently, metal chelate chromatography, which is the most popular for purifying recombinant proteins, are not 100 percent specific for the 6xHis tag (peptide tag of six histidine residues). There are some more specific tags, but most are larger and can affect protein conformation and activity. So, improving purity to greater than 95 percent will require us to develop a tag that's more specific but at a size that doesn't interfere with the target protein.

10. What are some other challenges that companies face?

The major challenge is developing products that provide effective solutions but are also easy and affordable. In protein chromatography, the challenge is developing a generic and simple workflow that is also powerful enough to purify proteins from very complex samples. But, the real challenge is with serum. One of the more popular applications these days is looking for biomarkers. With serum, you need to remove abundant proteins and isolate the proteins in very small minute quantities. That's a problem with all tissues. You always have to get rid of certain abundant proteins to find the low abundant proteins.

Survey Introduction and Methodology

The 2006 Protein Chromatography Survey is designed to provide vendors of protein chromatography systems with a better understanding of how their products are used in the research environment and how their company specifically rates among survey participants. Data were gathered from questions regarding how frequently chromatography techniques are used to purify proteins, which techniques are used currently or are planned to be used, what type of protein purification is performed, the primary applications of the purified protein, the products currently in use or are planned to be in use, the primary suppliers of those products currently in use and from whom those products are planned to be purchased, the specifics about the different products (e.g., types of media and important media attributes, expression systems, and automated chromatography systems), the types of biomolecules for which currently available media and techniques are insufficient, the types of affinity tags used, the key challenges faced when purifying proteins, the typical protein yields per preparation and the number of preps performed per month, whether custom services are outsourced and for which applications, the main criteria that affect the decision to purchase a chromatography system, and features not present on the researcher's current system that is desired on future models.

The 2006 Protein Chromatography Survey consisted of 32 questions. Of these, 18 included "other" as an answer choice and 2 were open-ended. Four questions were used for demographic information. The survey was administered on-line from June 5th–16th, 2006, and the data gathered, tabulated, and presented here.

Appendix I: Questionnaire

1. How would you characterize your use of chromatography techniques to purify proteins in your research or work?

- Frequent use – at least once a week
- Regular use – at least once a month
- Occasional use – at least once every 3 months
- I do not use chromatography techniques to purify proteins – exited from survey

2. Which of the following chromatography techniques do you use or plan to use? (Currently use, Plan to use, Do not use or plan to use)

- Affinity chromatography
- Gas chromatography
- Gas-liquid chromatography
- Gel filtration/size exclusion
- High performance liquid chromatography (HPLC)
- Ion exchange chromatography
- Reversed phase HPLC (RP-HPLC)
- Other (please specify)

3. What type of protein purification are you performing?

- Analytical separation
- Preparative separation
- Other (please specify)

4. What are the primary applications of your purified protein? (check all that apply)

- Antibody development/production
- Gel shift assays
- In vitro activity assay
- Mass spectrometric analysis
- N-terminal sequencing
- Probes for protein arrays/chips
- Vaccine development/production
- Cell-based assays
- Expression library screening
- In vivo activity assay
- Structural analyses (e.g. crystallography, NMR)
- Post-translational modification tests
- Protein:protein interaction assays
- Other (please specify)

5. Which of the following chromatography products do you use or plan to use? (Currently use, Plan to use, Do not use or plan to use)

- Automated chromatography system
- Empty columns
- Recombinant protein expression system
- Other (please specify)
- Chromatography media
- Pre-packed columns
- Spin columns

6. What type of chromatography media do you use? (check all that apply)

- Activated media
- Affinity media
- Hydrophobic interaction media
- Ion exchange media
- Other (please specify)
- Adsorbent chromatography media
- Ceramic Fluorapatite
- Hydroxyapatite media
- Size exclusion media

7. What company would you identify as your primary supplier of (pipe media type)?

- Bio-Rad
- Clontech
- IBA GmbH
- KPL
- Pierce Biotechnology
- Qiagen
- Stratagene
- Cayman Chemical
- GE Healthcare (formerly Amersham Biosciences)
- Invitrogen
- Novagen
- Promega
- Sigma-Aldrich
- Other (please specify)

8. What affinity tags do you use? (check all that apply)

- CBP
- GST
- HSV
- Strep-tag
- VSV-G
- c-MYC
- HA
- Protein A
- T7
- Other (please specify)
- FLAG
- His-6
- S Protein
- V5

9. What type(s) of recombinant protein expression system do you use? (check all that apply)

- Bacterial expression system
- Mammalian expression system
- Viral expression system
- In vitro translation
- Insect expression system
- Plant expression system
- Yeast expression system
- Other (please specify)

10. What key challenges do you face with purifying your recombinant protein? (check all that apply)

- Contaminating proteins
- Low activity of purified protein
- Low activity of purified protein
- Non-protein contaminants
- Other (please specify)
- High amount of insoluble protein
- High price of purification resins and/or columns
- Low yield of overall protein
- Protein degradation

11. What are the most important chromatographic media attributes for your research? Please rank the attributes below from 1 to 9. (1=high and 9=low)

- Resin capacity
- Resin pressure stability and flow rates
- Compatibility with detergents and reducing agents
- Compatibility with denaturing conditions
- Compatibility with non denaturing conditions
- Resin reusability (# of times reused)
- Low non-specific binding
- Type of resin chemistry (e.g. dextran, agarose, cellulose, fractogel, etc.)
- Compatibility with wide range of biomolecules

Please list the kind of biomolecules for which the current chromatographic media/techniques are not sufficient. (open-ended)

12. For the protein your work with most often, what is your typical yield for each prep?

- | | | |
|------------------|--------------------------|----------------|
| - Less than 10µg | - 100mg – 500mg | - 11 – 500 µg |
| - 500 mg – 1g | - 500 – 1mg | - More than 1g |
| - 1 mg – 10mg | - Other (please specify) | |

13. Please estimate how many protein preps you perform in a month.

- | | |
|---------------|----------------|
| - Less than 1 | - 1 – 2 |
| - 3 – 5 | - 6 – 7 |
| - 8 – 10 | - More than 10 |

14. Which of the following chromatography systems do you use?

- | | |
|--------------------------|------------------------------|
| - Capillary LC Systems | - GC Systems |
| - HPLC Systems | - Ion Chromatography Systems |
| - LC/MS Systems | - SPE Systems |
| - Other (please specify) | |

15. What brand of (pipe system type) automated chromatography system do you primarily use?

- | | |
|--------------------------|---|
| - Agilent | - Applied Biosystems |
| - Bio-Rad | - Caliper |
| - Cecil Instruments | - Dionex |
| - Gilson | - GE Healthcare (formerly Amersham Biosciences) |
| - PerkinElmer | - Shimadzu |
| - Thermo | - Varian |
| - Other (please specify) | |

16. Do you out-source protein custom services?

- Yes
- No

If yes, what are your primary needs?

- Protein expression and purification service
- Protein analysis service
- Production for clinical trials (non-cGMP)
- Other (please specify)

17. Which of the following do you plan to purchase and what is your purchasing timeframe? (check all that apply)

Within 3 months, 3 – 9 months, 9 – 12 months, More than 12 months, or Do not plan to purchase

- Capillary LC Systems
- GC Systems
- HPLC Systems
- Ion Chromatography Systems
- LC/MS Systems
- SPE Systems
- Other (please specify)

18. What are the three main criteria you would choose to make a purchasing decision of a chromatography system? (please choose three)

- | | |
|---------------------|---------------------------|
| - Advertisements | - After Sale Support |
| - Brand Recognition | - Breadth of Applications |
| - Budget | - Hardware Performance |
| - Past Use | - Personal Demonstration |
| - Recommendation | - Software performance |
| - Technical support | - Other (please specify) |

19. Are there any features not present on your current purification system that you would like to see available on future models? (open-ended)

Demographic Questions

1. In which type of institution do you work?

- Academic
- Pharmaceutical
- Private Research
- Other (please specify)
- Biotechnology
- Government
- Clinical Diagnostic Testing

2. Which title best applies?

- Professor/Instructor
- Business Development Director/Manager
- Department Head
- Account Manager
- Staff Scientist
- President/CEO/Owner/VP
- Postdoctoral Fellow
- Consultant
- Process Engineer
- Research Director/VP of Research
- Technician/Research Assistant
- Graduate Student
- Principal Investigator
- Lab Director/Chief Scientist
- Procurement Manager
- Other

3. Which of the following are your key areas of research or work?

- Bioinformatics
- Genomics/Genetics
- Drug Discovery
- Marketing/Sales
- Bioengineering
- Purchasing
- Microbiology/Virology
- Cell Biology
- Administration
- Pharmacology/Toxicology
- Neuroscience
- None of the Above
- Immunology
- Diagnostics/Pathology
- Biochemistry
- Molecular Biology
- Proteomics
- Other (please specify)

4. Which best describes your purchasing authority?

- Authorize
- Recommend
- Evaluate
- No Purchase Role

Appendix II: Presentation of Survey Data

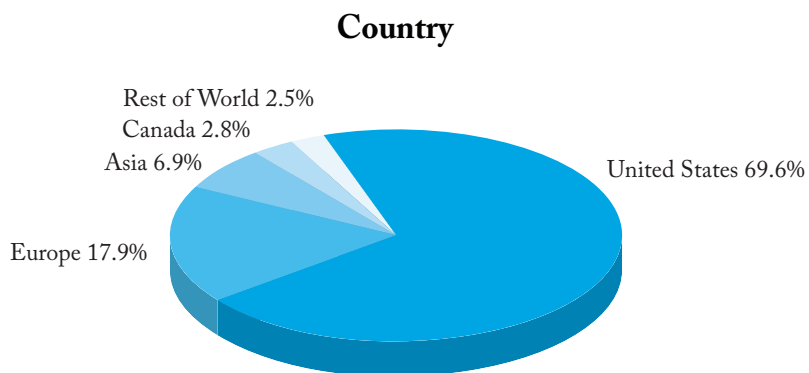
Demographic Survey Data

Country

73% of survey participants are from the United States and Canada; 18% are from Europe; 6% are from Asia.

N=319

Country	Frequency	%
United States	223	69.9%
Europe	57	17.9%
Asia	22	6.9%
Canada	9	2.8%
Rest of World	8	2.5%



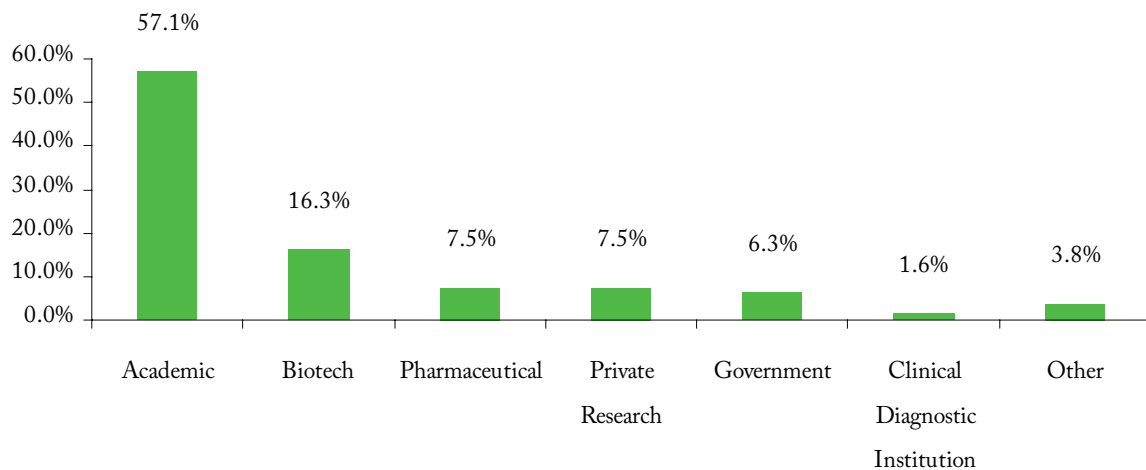
What is your Institution Type?

The majority of survey participants are from academic institutions and nearly one quarter are from Biotech and Pharmaceutical companies.

N=319

Institution Type	Frequency	%
Academic	182	57.1%
Biotech	52	16.3%
Pharmaceutical	24	7.5%
Private Research	24	7.5%
Government	20	6.3%
Clinical Diagnostic Institution	5	1.6%
Other	12	3.8%

Institution Type



Which title best applies?

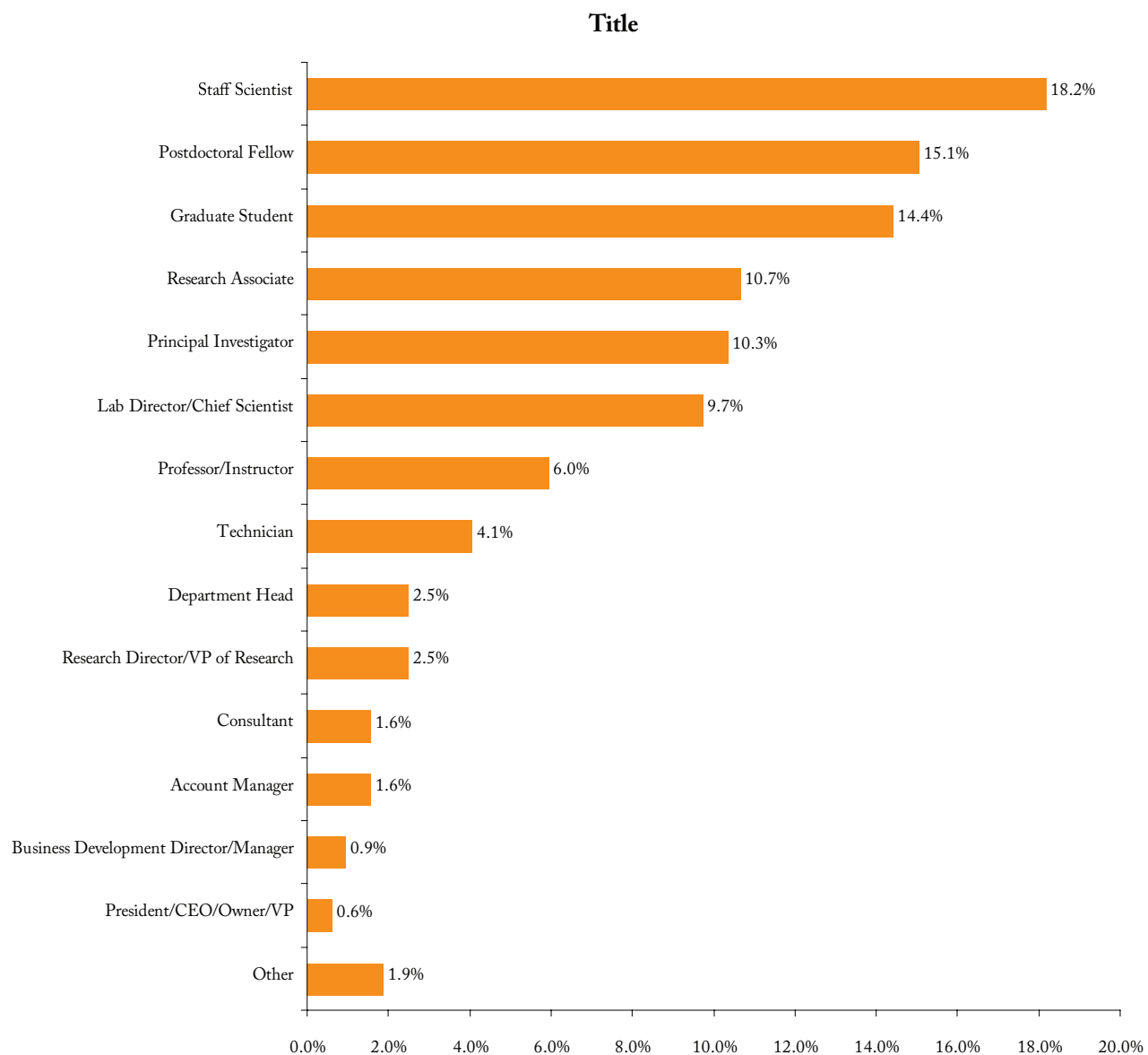
73% of survey takers work at the bench*.

N=319

Institution Type	Frequency	%
Staff Scientist	58	18.2%
Postdoctoral Fellow	48	15.1%
Graduate Student	46	14.4%
Research Associate	34	10.7%
Principal Investigator	33	10.3%
Lab Director/Chief Scientist	31	9.7%
Professor/Instructor	19	6.0%
Technician	13	4.1%
Department Head	8	2.5%
Research Director/VP of Research	8	2.5%
Consultant	5	1.6%
Account Manager	5	1.6%
Business Development Director/Manager	3	0.9%
President/CEO/Owner/VP	2	0.6%
Other	6	1.9%

*Includes: Postdoctoral Fellow, Staff Scientist, Graduate Student, Research Associate, Principal Investigator, Technician.

Which title best applies?



**Which of the following are your key areas of research or work?
(check all that apply)**

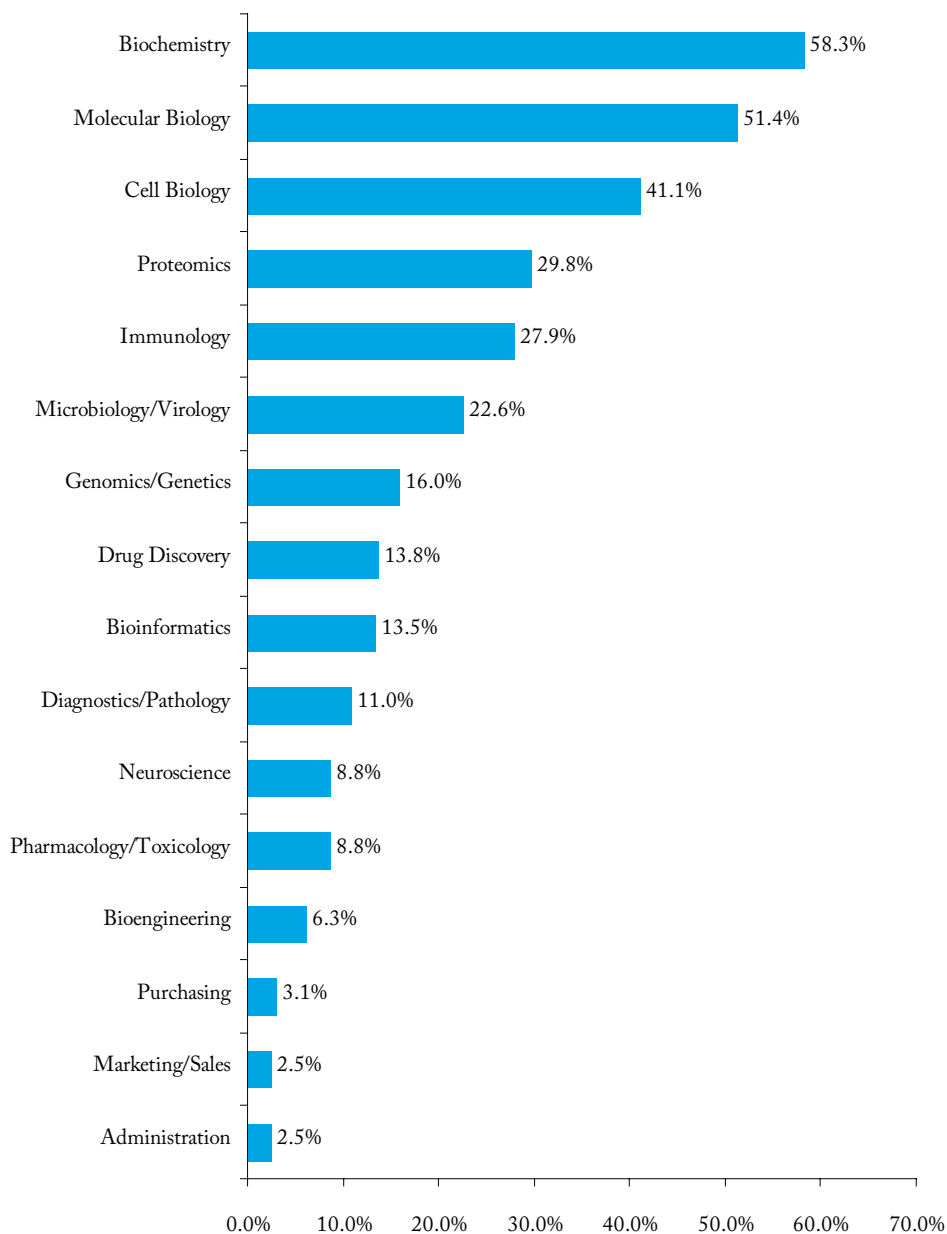
More than half of all survey participants identified Biochemistry and Molecular Biology as their key areas of research or work.

N=319

Research Area or Work	Frequency	%
Biochemistry	186	58.3%
Molecular Biology	164	51.4%
Cell Biology	131	41.1%
Proteomics	95	29.8%
Immunology	89	27.9%
Microbiology/Virology	72	22.6%
Genomics/Genetics	51	16.0%
Drug Discovery	44	13.8%
Bioinformatics	43	13.5%
Diagnostics/Pathology	35	11.0%
Neuroscience	28	8.8%
Pharmacology/Toxicology	28	8.8%
Bioengineering	20	6.3%
Purchasing	10	3.1%
Marketing/Sales	8	2.5%
Administration	8	2.5%
Other	16	5.0%

**Which of the following are your key areas of research or work?
(check all that apply)**

Area of Research or Work



Which best describes your purchasing authority?

89% of survey participants either authorize or recommend purchases.

N=303

Purchasing Authority	Frequency	%
Authorize	168	52.7%
Recommend	124	38.9%
Evaluate	19	6.0%
No purchase role	8	2.5%

