

# Investments in Proteomics Ready for Payback

## Second Swedish Proteomics Society Symposium Predicts Returns

Carina Schmidt

At the recent Swedish Proteomics Society (SPS) Symposium in Lund, over 200 scientists from industry and academy met to hear results and discuss thoughts about the future of proteomics. Among the speakers were scientists from Switzerland, the U.S., Germany, and Italy, with colleagues from Sweden and Denmark.

Lund has a strong proteomics profile. Internationally established powerhouses in the region include Lund University, the new medical mass spectrometry center at Uppsala University, the Human Proteome Resource project at KTH (Royal Institute of Technology) in Stockholm, and Odense University in Denmark.

Applied Biosystems (www.appliedbiosystems.com) actively supported the symposium, as well as SWEGENE (a consortium for functional genomics research established by the Chalmers University of Technology, Göteborg University, and Lund University/The Lund Institute of Technology), Agilent, Amersham Biosciences, Bruker, ThermoFinnigan, and Waters Micromass.

"Scandinavia already has a strong position internationally in proteomics, and in the future we expect that this will result in more start-up companies that could generate interesting, innovative technologies in proteomics, including improvements in bioinformatics, protein arrays, and nanotechnology solutions for protein analysis," said Bob Galvin, European business development manager at Applied Biosystems.

Pharma and biotech firms with a strong interest in proteomics were well represented by speakers and delegates from Active Biotech, Ace Biosciences, ALK-Abello, AstraZeneca, Cellzome, Ferring, NeuroNova, Novartis, and Novo Nordisk.

Jan van Oostrum from Novartis reported that the company's proteomics program has already resulted in two new drug candidates in clinical development. Additionally seven drug candidates are in the pipeline in preclinical development. Van Oostrum also revealed that in addition to the drug candidates, two biomarkers are now being evaluated in clinical studies.

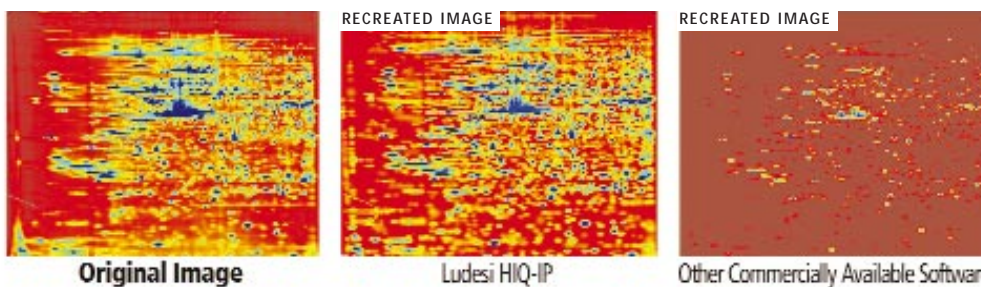
"I think the potential is there to see an increasing number of proteomics-derived candidates and biomarkers in clinical trials over the coming years. This is just the start," explained György Markovarga, responsible for the proteomics group of AstraZeneca and chairman of the Swedish Proteomics Society.

"Today we have a strong pro-

teomics profile to support our drug development programs. One example is the case-control safety study started in Japan this fall, where gene markers and biomarkers are being mapped in patients treated for lung cancer, in what is currently the largest safety study at AstraZeneca."

The speakers did reflect this anticipated development with a great focus on presenting results from disease-associated research. The aim of the studies is to interpret biology by mapping pathways and interference between pathways, to identify biomarkers, as

Ludesi's HiQ-iP technology was designed to improve 2-D-data interpretation, the company notes.



well as to study clinical response and outcomes in disease.

Although 2-D electrophoresis clearly remains one of the workhorse technologies in these studies, improvements in methods

using mass spectrometry (MS) detection were in great use to generate the data presented during the symposium. Many of the presented approaches included combining MS-detection with pretreat-

ment of the samples to reduce complexity, by removing abundant proteins and thereby increasing the potential for detecting concentration differences in less

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## TOOLS FOR GENE KNOCKDOWN



New & Exciting  
**siRNAs**

versus

Effective & Reliable  
**Morpholinos**

YES	More than 500 publications	YES
NO	Allows you to modify splicing	YES
NO	Effective & specific in embryos	YES
NO	Free of off-target effects	YES
NO	Free of interferon effects	YES
NO	Typical first-try targeting success	YES
NO	Functions in both cytosol & nucleus	YES
NO	Complete stability & long-term efficacy	YES
NO	Well understood mechanism	YES

### Key siRNA publications:

- Expression profiling reveals off-target gene regulation by RNAi. Jackson et al. Nat Biotechnol. 2003 Jun;21(6):635-637.
- Small RNAs with Imperfect Match to Endogenous mRNA Repress Translation: Implications for off-target activity of small inhibitory RNA in mammalian cells. Saxena S et al. J Biol Chem. 2003 Nov 7;278(45):44312-44319.
- Activation of the interferon system by short-interfering RNAs. Sledz CA et al. Nat Cell Biol. 2003 Sep;5(9):634-639.
- Study questions siRNA specificity; insiders say issue is par for the course. GenomeWeb staff. RNAi News 2003 Sep 8;1(1):1. GenomeWeb Intelligence Network.
- Trouble in Paradise: RNA Interference Beset with New Complexity. Reid B. 2003. Preclinica 1(5):223-224.

### Key Morpholino publications:

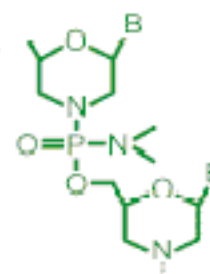
- Special Issue: Morpholino Gene Knockdowns. Genesis 2001 Jul;30(3). - all 27 papers.
- Inhibition of zebrafish fgf8 pre-mRNA splicing with Morpholino oligos: a quantifiable method for gene knockdown. Draper BW, Morcos PA, Kimmel CB. Genesis 2001 Jul;30(3):154-156.
- Evidence for a causal role of CD38 expression in granulocytic differentiation of human HL-60 cells. Munsch CB et al. J Biol Chem. 2002 Dec 20;277(51):49453-49456.
- Morpholino antisense oligomers: the case for an RNase H-independent structural type. Summerton J. Biochim Biophys Acta. 1999 Dec 10;1489(1):141-50.
- Morpholino oligos: making sense of antisense? Heasman J. Dev Biol. 2002 Mar 15;243(2):209-214.

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abundant proteins.

Improvements in managing the vast amounts of data generated by these approaches are the basis of a number of young start-up companies in the region. **Ludesi** ([www.ludesi.com](http://www.ludesi.com)) has improved 2-D data interpretation with its HiQ-iP™ technology. In a study recently presented with the CancerCentre Karolinska Institute (CCK; Stockholm), Ludesi could find almost 70% more information than CCK

had been able to detect with previously established methods.

"It is impressive that all the information we normally extract was found by Ludesi, plus almost 70% more. If only half of the proteins associated with this information can be identified, this would be a considerable achievement," commented professor Gert Auer, coordinator of the Swedish cancer proteomics project.

**Alphalyse** ([www.alphalyse.com](http://www.alphalyse.com)), spun off from Ace Bio-

science in 2002, has integrated cutting-edge 2-D and MS analysis into automated processing of large numbers of samples to generate data with extensive bioinformatics support. The service is offered to pharma and biotech companies on a CRO basis.

Another Odense company, **MDS Denmark**, is offering complex proteomics mapping services, with a focus on developing software for advanced data analysis.

### Protein Array Approaches

Protein array approaches were not discussed to any great extent. However, Leigh Anderson, Ph.D., one of the early pioneers in proteomics and founder and CEO of the Plasma Proteome Institute in Washington, DC, did point toward the gap between the technologies used in current proteomics mapping and the needs that must be

met to apply the knowledge from this science in clinical research.

To validate biomarkers in thousands of samples and eventually include their use in clinical practice, methods need to become less costly and suitable for repeated analysis of patient samples with high quality and safety in results.

One limitation discussed was that improvements in availability and quality of antibodies are needed before any great breakthrough is possible. Both **BioInvent International** ([www.bioinvent.com](http://www.bioinvent.com)) and **Affibody** ([www.affibody.com](http://www.affibody.com)) have libraries of monoclonal antibodies (n-CoDeR®) and designed affinity ligands, respectively, that could serve as sources of content for protein arrays.

Late in 2003, **Gyros** ([www.gyrosmicro.com](http://www.gyrosmicro.com)) launched its Gyrolab BioAffy system for nanoliter quantification of proteins, with the possibility to assay up to 104 samples in parallel, with either single or multiplex assay design.

**Biacore** ([www.biacore.com](http://www.biacore.com)) uses its Surface Plasmon Resonance

(SPR) technology as a base for its SPR-MS array platform, developed and commercialized in collaboration with Bruker, for biomolecule interaction studies.

**Zeptosens** ([www.zeptosens.com](http://www.zeptosens.com)) also has a platform for array analysis of biomolecules, based on a technology using thin film planar waveguides. When asked about the reason for the current bias toward MS, however, one of the speakers admitted that it could be that MS analysis simply is so much fun. Suppliers of protein array solutions might need to consider this in their development.

The next Swedish Proteomics Society Symposium will most likely be held in conjunction with the largest biotech conference in Scandinavia, "BioTech Forum," held in Copenhagen in October 2004.

"Sweden and Denmark have the tradition and power to be in the frontline in proteomics internationally, and one of the objectives with SPS is to strengthen and communicate this position," said Marko-Varga. GEN

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probes (microarrays) or gene-specific primer pairs (real-time PCR), which can only verify the presence of genes from a limited subset available on public genome databases, there is no prerequisite for sequence data with Tangerine™ profiling.

Therefore, Tangerine profiling can detect expressed genes, even at low expression levels, in any species, and identify new genes, all within a single experiment, the firm says, adding that changes in expression can be detected at two orders of magnitude below the detection threshold for microarrays.

Using total or messenger RNA as starting material, the RNA is reverse-transcribed to cDNA, selectively cleaved to generate specific transcript fragments for PCR

amplification, and finally separated by capillary electrophoresis.

Using proprietary software developed by scientists at Global Genomics, electropherograms are compared and gene-profile lists generated that contain identified and unidentified genes with expression levels and fold-changes.

"As this new approach to gene expression profiling identifies virtually all genes and can be used to study any animal model, it should provide valuable insights into processes taking place in healthy, diseased or drug-treated cells and thereby improve the decision-making process about which targets to pursue in drug discovery," says Ulf Boberg, Ph.D., CEO of Global Genomics. GEN

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manufacturers and contract manufacturing organizations provides information and insights on current capacity, utilization, projected future capacity needs, and reasons for production bottlenecks.

With as many as 125 new drugs reaching the market over the next

five to seven years and more than 370 biotech therapeutics currently in clinical trials, the shift toward outsourcing certain projects may come as a boon to contract manufacturing organizations.

### Maximizing Internal Capacity

Many larger biopharmaceutical companies are convinced that, despite the strategic advantages of bringing production capability in-house, overinvestment in capacity can be highly risky. Those that do invest in in-house production capabilities do so primarily to maximize their return on investment (ROI) in facilities and capital by operating at near full capacity.

Because of the unpredictability of the drug-development pipeline, however, sizing a plant to maintain maximum capacity and avoid idle capacity is challenging. To reduce risks, some drug developers plan to divert some of their capacity to contract manufacturing services to handle their excess internal demand.

Another factor in the shift toward contract manufacturing is that smaller biopharmaceutical developers, in particular, are finding that biologics manufacturing and process development is not part of their core competency.

"One factor driving the upward trend in the number of projects being outsourced is the recognition that biologics process development and manufacturing is often not part of the core competency within smaller companies," believes Geoff Hodge, vp of technology at **Xcellerex** (Marlborough, MA), a process developer and contract manufacturer.

"Manufacturing and process development in a GMP environment requires complex sets of skills. "A number of companies

are increasingly recognizing that their time and resources may be better spent in drug discovery and lead optimization

Hodge is hesitant to generalize, however, to or categorize companies. In his experience, the determination to outsource is dependent upon multiple variables. Smaller companies often do not have the resources or capital to invest in process development and manufacturing facilities. But even among the larger pharmaceutical companies, there are some that are more amenable to outsourcing, while others are more inclined to keep as much as possible in-house.

"Ultimately, it may be a function of a company's personal experience with outsourcing, control issues, or intellectual property concerns," says Hodge.

### What's Getting Outsourced?

Most biopharmaceutical developers would prefer to manufacture biopharmaceuticals in-house in order to build institutional knowledge and retain control over resources, time and production schedules, intellectual property, and quality issues. Such control issues are central to many of the common arguments for and against outsourcing.

On the other hand, smaller, less mature companies may do the opposite and send their more challenging projects out-of-house. Smaller companies often do not have the internal expertise to tackle the more challenging problems. As an example, a smaller biotech considering in-licensing a project with a poorly designed, commercially infeasible process; a questionable regulatory perspective; and challenging scalability factors, may likely consider bringing in

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ucts, including extended-release Newstatin, from the U.S. marketplace by virtue of a U.S. patent covering Newstatin. If the extended-release Newstatin becomes the preferred commercial embodiment, then Inventor A is free to sell extended-release Newstatin in the U.S. without being blocked by Inventor B, who has no U.S. patent on the extended-release formulation.

The situation in Europe is much different. There, Inventor A cannot sell extended-release Newstatin without infringing Inventor B's European patent; however, Inventor B cannot sell any Newstatin in

Europe, including extended-release Newstatin, without infringing Inventor A's European patent. The result is an effective standoff between Inventor A and Inventor B in Europe with respect to the extended-release Newstatin product.

It is thus necessary to pay close attention to the laws in both the U.S. and Europe to assure consistent results when trying to obtain patent protection in both places. Assumptions made with respect to one set of laws can result in very different results when applied to the other set of laws.

In general, it is good advice to

file patent applications on inventions before publishing, disclosing, using, or selling the inventions, and to get to the patent office as quickly as possible. With respect to dealing with collaborators and licensees, always be careful of what nonpublic information is disclosed, and assure that proper confidentiality agreements are in place that limit the usage of such disclosed information. GEN

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## New Standards Issued for Microplates

The Society for Biomolecular Screening (SBS) reports that its proposed four standards for the design of microplates used in drug discovery and other testing have been approved as universal industry standards by the American National Standards Institute (ANSI). Experts expect the new standards to result in more efficient, cost-effective early-stage drug discovery, notes SBS.

"Until now, if a scientist ran a screen, he or she had to program the instrument for every microplate. We might have, for example, 100 different types of 96-well microplates,

each slightly different from the other," explains Carol Ann Homon, co-chair of SBS' microplate standards development committee (MSDC).

"Now we can be sure that if plates meet the ANSI/SBS standards, results will be consistent across platforms, and costs to laboratories will be reduced."

### APPROVED STANDARDS

Standards are approved as follows: ANSI/SBS 1-2004: Footprint Dimensions; ANSI/SBS 2-2004: Height Dimensions; ANSI/SBS 3-2004: Bottom Outside Flange

Dimensions; and ANSI/SBS 4-2004: Well Positions (the fourth standard addresses 96-, 384-, and 1,536-well-density formats, which are currently considered to be the platform for 98% of the screening assays).

The four ANSI/SBS standards apply to all microplates, without regard to their plastic composition. The MSDC is now working on two additional standards, one that addresses microplate side-wall rigidity and another that addresses the "flatness" of well bottoms that are critical for use with imaging devices and other automated equipment.