

*Full Length Research Paper*

# Computational approach of protein analysis using affinity chromatography: application to proteomics

Allam Appa Rao

International Center for Bioinformatics, Department of Computer Science and Systems Engineering, Andhra University, Visakhapatnam-530003, India. E-mail: [principal@aucevizag.ac.in](mailto:principal@aucevizag.ac.in).

Accepted 11 January, 2017

Computerization is necessary to build the database of chromatography coupled mass spectrometric analytical data. On the basis of this proteomic data we can identify the proteins as biomarkers which are expressing dissimilarity between healthy and disease condition. Affinity chromatography is one of the fastest liquid chromatographic methods for the separation and purification of biomolecules due to its high molecular specificity. Proteomics and the tools used to identify and understand the biochemistry of proteins and pathways are in a new stage of development and evaluation. This article briefly reviews the computation of chromatography coupled mass spectrometric data and highlighting the role of affinity chromatography

**Key words:** Affinity chromatography, proteomics, computational aspects.

## Human genome project and proteomics

The draft version of the human genome (approximately 95% complete) was announced as a joint statement in June 2000 from both the publicly funded Human Genome Project (HGP) and a commercial venture led by Celera genomics. The popular press would have us believe that the HGP was a slow lumbering giant and that Celera, led by the 'charismatic' Craig Venter was the efficient private company that nearly beat HGP to finishing the project in a fraction of the time due to its innovative approach to genome sequencing. Recently Andrade et al. (2007) reviewed the recent progress in studies of neuroproteomics, and highlights the strengths and limitations of current proteomic profiling technologies used in studies of neuronal protein expression.

Genomics is the study of organisms genome and the use of genes. However, the post genomic era of molecular medicine is rapidly moving beyond "transcriptomics" gene lists and functional genomics to the new era of proteomics. "Proteomics" is sometimes defined as the analysis of part or all of the protein complement of a complex biological system at any given moment. With the elucidation of the human genome sequence, attention has focused on gaining an understanding of the gene products themselves, the proteins. However, the understanding of the diverse structural characteristics and

interactions of proteins represents a significantly greater analytical challenge than that posed by nucleic acid sequence analysis required for acquisition of the genome sequence. Proteomic technologies will play an important role in drug discovery, diagnostics and molecular medicine because it is the link between genes, proteins and disease. To understand characterize disease type, status, progression and response to therapy, there is need to discover biological markers have the proteins to be used as diagnostic and prognostic measure in clinical medicine.

## Computation of protein analytical data

Computational tools were becoming wide spread, and the improvement in performance, in speed, and in reduction in the cost of computers made ubiquitous the "desktop" PC. Communication changed dramatically as email became the primary method of communication in scientific circles. The World Wide Web provided initially, widespread access to collection of information and data, and soon thereafter, access to many tools and programs. Search engines for protein identification could be accessed on the web, with early site including protein prospec-

tor, profound, mascot and others.

Virtually every field of biology has recently undergone truly revolutionary changes in the way biological data is acquired, processed, stored, and accessed. The use of information technologies has become indispensable. However, the increased volume and complexity of biological information has been only slowly translated into a detailed understanding of biological systems and processes, an understanding that can be expressed as comprehensive, experimentally verified mathematical and computational models with substantial predictive power.

Integrative computational modeling poses an additional challenge, this one related to how complex models are managed and analyzed. In dealing with complex computational problems there is still a need for better algorithm development. For example detailed stochastic simulation of lysis-lysogeny decision in infection of bacterial cells by  $\phi$ -phase (Arkin and Ross, 1998; Gibson and Bruck, 2000) the power of super computer has to be analyzed.

### Chromatography in proteomics

It is well known that the basic analytical requirements in proteome analysis are high sensitivity, high resolution, and high throughput along with high-confidence protein identification. Proteins must also be quantified and post-translational modifications identified (James, 2001). To reduce sample complexity prior to mass spectrometry, one of two approaches is usually taken prior to protein identification: (1) proteins are first separated, then digested (this may be characterized as “top-down” proteomics (Kettman, 2001) (2) in “shotgun” proteomics, a complex protein mixture is first digested. Peptides are then chromatographically resolved (characterized as “bottom-up” proteomics Wolters, 2001). In both cases, separation technologies play a critical role in protein identification and analysis. With due respect to sample preparation, data analysis is rapidly becoming a major obstacle to the conversion of experimental knowledge into valid conclusions. It is interesting to note that many problems related to chromatographic techniques can be solved using techniques from computer science.

Over the last century, chromatographic techniques have become a powerful tool for understanding various aspects of chemical entities. It has helped us to enrich our understanding of complicated life processes, comprehension of which presents the greatest challenges to contemporary bioscience. It is important to stress that many problems associated with mass spectrometry can be solved using techniques derived from computer sciences, graph theory and discrete mathematics. In principle, the exploitation of informatics can be regarded as an advanced data analysis step which allows more information to be obtained than could otherwise be deduced from traditional data inspection. Not surprisingly then, mathematics is often called “the modern microscope of biology”.

High performance liquid chromatography (HPLC) using protein-based stationary phases have traditionally been proposed and successfully applied to chiral resolution of compounds (Milot, 2003). In the last years, capillary electrophoresis has become in a powerful analytical technique for enantiomeric separations due to its intrinsic characteristics: high separation efficiencies, short analysis times, low reagent consumption and small sample requirements, compared to HPLC (Rogan and Altria, 1993; Haginaka, 2000). In capillary electrophoresis proteins can be used as additives to the running buffer (affinity electrokinetic, chromatography, AEKC) or as immobilized selectors (affinity capillary electrochromatography, ACEC). AEKC is the most frequently methodology used because it has indeed several advantages over ACEC such as: (i) higher peak efficiency, (ii) easier methodological developments since protein immobilization is not necessary and

(iii) the structure and protein binding properties are not altered. In AEKC technique, the analyte and the analyte-protein complex have different mobility, the interaction of the compound with the protein results in a change in the net mobility of the analyte (Tanaka and Terabe, 1995). Novel affinity-based purification strategies are under consideration for genetically engineered antibodies and related molecules (Roque, 2004). The Areas of affinity chromatography and affinity electrophoresis, aptamers have been used in mobile phases, stationary phases and microbeads in applications such as analyte detection (German, 1998; Pavski and Le, 2001; Buchanan, 2003; Deng, 2003; Cho, 2004) purification (Romig, 1999; Chung, 2005; Deng, 2001) chemical and chiral separations (Michaud, 2003; Michaud, 2004; Berezovski, 2003) and studies of binding interactions (Huang, 2004; Cuatrecasas, 1968). Affinity chromatography can safely guide protein manufactures to successfully cope with the aforementioned challenges. Important technological advances related to affinity ligand design and selection lead affinity chromatography to maturity. Now the technique stands a realistic chance of dominating the protein pharmaceutical industry.

### Affinity chromatography

Affinity chromatography (AC) is one of the most selective and rapid tools for the separation and purification of biomolecules such as antibodies, enzymes, antigens, hormones, receptors, etc (Porath, 1975; Wilchek, 1974; Clonis, 1987; Vijayalakshmi, 1989). The success of affinity chromatography is essentially due to its high efficiency, which originates from the “specific” or “pseudo-specific” recognition between a solid phase-immobilized ligand and the molecule to be purified.

Affinity purification technology can be separated into two categories depending on whether the interaction mechanism of specific protein/ligand recognition is either biospecific or pseudo-biospecific (Scopes, 1978). Gene-

rally, proteins bind to biospecific ligands with high affinity in the range of  $10^{-7}$  to  $10^{-15}$  M. Consequently, protein desorption in the case of biospecific ligand affinity chromatography needs more drastic elution conditions which can elution cause ligand and/or protein denaturation. On the other hand, pseudo-biospecific ligand affinity chromatography leads to medium affinity interaction (Riggs, 2001) and methods have been devised to provide a fairly high selectivity allowing gentle methods for the purification of a large number of therapeutic proteins (Vijayalakshmi, 2002). A large amount of coupling chemistries have been proposed and used in affinity chromatography for linking protein affinity ligands to solid supports. The coupling reaction is known to affect the orientation of the polypeptide at the surface and hence, can influence the interactions with the target molecule. Despite this, the utilization of biospecific ligand for affinity separations can be counted as the most preferred way, considering the high cost of natural biomolecules, such as monoclonal antibodies, requiring purification. Recently Zhang et al. (2007) reported the phenylboronate affinity chromatography in a two-step enrichment scheme to selectively isolate first glycosylated proteins and then glycosylated tryptic peptides from human serum glycosylated *in vitro*. Enriched peptides were subsequently analyzed by alternating electron-transfer dissociation (ETD) and collision induced dissociation (CID) tandem mass spectrometry. ETD fragmentation mode permitted identification of a significantly higher number of glycosylated peptides (87.6% of all identified peptides) versus CID mode (17.0% of all identified peptides), when utilizing enrichment on first the protein and then the peptide level. This study illustrates that phenylboronate affinity chromatography coupled with LC-MS/MS and using ETD as the fragmentation mode is an efficient approach for analysis of glycosylated proteins and may have broad application in studies of diabetes mellitus.

IMAC is based on the interaction between molecules in solution and metal ions immobilized on a solid support. The molecules are separated according to their affinity for chelated metal ions, which depends on the coordination between the chelated metal ion and electron donor groups forming the ligand. Two characteristics of the metal-ligand bonds can be used for the successful separation of different ligands. Firstly, the strength of the metal-ligand bond varies from ligand to ligand and secondly, binding between the immobilized metal ions and the ligand is reversible. Hence, elution can be carried out by changing the conditions thereby breaking the metal-ligand bonds. Three different elution principles are used in IMAC, such as a competitive elution, stripping information to be obtained than elution and pH adjustment. Low cost of stationary phase, high binding capacity and relative high specificity of protein and metal interaction are the key advantages of IMAC. IMAC has been widely used in the purification of peptides (and the application of IMAC has been extended to the purification of viral vectors for use

in gene therapy application (Kenig, 2006; Ye, 2004) Recently Jiang (2004) applied this technique for the purification of herpes simplex virus type 1 (HSV-1) gene therapy vectors.

### **Computational aspects of chromatography coupled mass spectrometric data**

Liquid chromatographic mass spectral identification of the amino acid sequences of peptides and proteins is one of the most important and common issues encountered in biological and medical research. The most common approaches rely on available databases to match experimental MS/MS data. On the other hand, the *de novo* peptide sequencing problem is to derive the sequences of the peptides directly from experimental measurements of fragment ion masses. Over the years, various algorithms have been developed to address the *de novo* sequencing problem. One naïve approach is to list all possible candidate peptides that match the observed molecular ion (exhaustive listing), and then compare all of the candidate peptides with the experimental tandem mass spectrum to find out the best match. The computing complexity of such approach grows exponentially with the peptide length ( $20^n$ ), and was impractical for long time until the recent development of computationally efficient branching techniques made it possible to tackle the heavy computational time required (Sakurai, 1984). Recently Srinubabu et al. (2007) outlined the different chromatographic strategies that have been employed for analysis of complex mixtures of proteins/peptides, highlighting the role of liquid chromatography coupled to mass spectrometry.

To identify these peptides, spectra are scanned against protein sequence databases using a search algorithm. Several algorithms have been developed for this purpose (Bruni, 2005). The most commonly used algorithms are Sequest (Nesvizhskii and Aebersold, 2004) Mascot (Eng, 1994) and MS-Tag (Perkins, 1999) Sequest adopts a cross-correlation approach, in which peptide amino acid sequences from a protein database are used to construct theoretical mass spectra, and the degree of overlap, or cross-correlation, between the theoretical and experimental mass spectra determines the best match.

The Mascot method employs probability based matching: the MS/MS fragment masses calculated from peptide sequences in the database are compared with the experimentally observed peaks, and a score is calculated that reflects the statistical significance of the match between the experimental and theoretical spectra. The MS-Tag approach involves extracting a short, unambiguous section of the amino acid sequence of the peptide from the experimental data, which is used along with the measured mass of the peptide to determine the protein of origin.

Currently available *de novo* sequencing algorithms are computationally intensive and require high-quality data (Bruni, 2005). Therefore, for high-throughput proteomics

studies, protein identifications are restricted to those proteins whose sequences appear in the searched database; many post-translationally modified or mutated proteins will be overlooked.

However, different researchers often use their own preferred filtering criteria, making it difficult to compare results between (or even within) research groups. Software tools such as DTASelect (Clauser, 1999), INTERACT (Tabb, 2002) and CHOMPER (Eddes, 2002), compatible with Sequest and Mascot, are available to facilitate the filtering of data. Yet to allow the comparison of proteomic data between groups, peptide assignments should be validated using statistical programs developed to be compatible with existing database search tools (Bruni, 2005). The development and application of robust, transparent tools for the statistical analysis of proteomic data is essential. Several computational methods have recently become available (Eddes, 2002; Nesvizhskii, 2002; Keller, 2002; MacCoss, 2002)

Only when these programs have become standardized and widely used can one of the long-term aims of proteomics begin to be realized: the combined results from many different research groups could then be merged and applied to the whole genome, validating expressed genes at the protein level, and enabling the elucidation of patterns of protein expression that would be missed in individual experiments.

Information at the level of the proteome is critical for understanding a cellular phenotype and its implications for normal and disease conditions. Proteome alterations in disease processes occur in many different ways and some of these are not predictable from genomic analysis. It is clear that a better understanding of these alterations and their consequences within the biological context will have a substantial impact in medicine. The identification and development of biomarkers for diagnostics and early detection of disease is of high interest for the pharmaceutical industry. Moreover, the understanding of biological networks increasingly provides the rational basis for (preliminary) decisions on drug targets and target suitability (Kislinger, 2003)

### Nano proteomics

Unlike DNA samples, proteins cannot be amplified easily and low concentration protein samples are very hard to detect from their original concentrations. Especially on microchip systems where the sample volumes are very low, protein concentration is essential. Many efforts have been made to improve the sensitivity of detection on microchips. To improve the detection limit, one may concentrate the protein sample in the microchannel before separation or just before detection. This technique is known as preconcentration, and recent research has applied newly developed nanodevices to protein preconcentration on microchips (Song et al., 2004). Hanash (2003) intro-

duced a laser-patterned nanoporous membrane in microchips for protein concentration. At the junction of a cross channel in a microchip, nanoporous membranes with a molecular weight cutoff of >5.7 kDa were used to achieve protein concentration. New applications will demonstrate the abilities and limitations of nanodevices and will lead engineers to adopt such nanodevices for the analysis of real samples in the near future.

On-chip separations are mostly performed under an electrical field (Song et al., 2004); electro-based pumping systems are easier to implement on a microchip format since no valve is required. Nonetheless, these latter imply the addition of salts in the solution, which are detrimental for the analysis in ESI-MS (Kutter, 2000). Only a few compositions of the mobile phase are acceptable for both the electrochromatography and the nanoelectrospray ionization, leading to a non-satisfying compromise for real analytical work (Wilm and Mann, 1996; Vrouwe, 2000; Varesio E, 2002; Ivonov, 2003). Here, the flow relies on a hydraulic pumping system, which excludes any electro-driven separation. Recently Yue et al. (2006) reported an integrated glass microdevice for proteomics, which directly couples proteolysis with affinity selection.

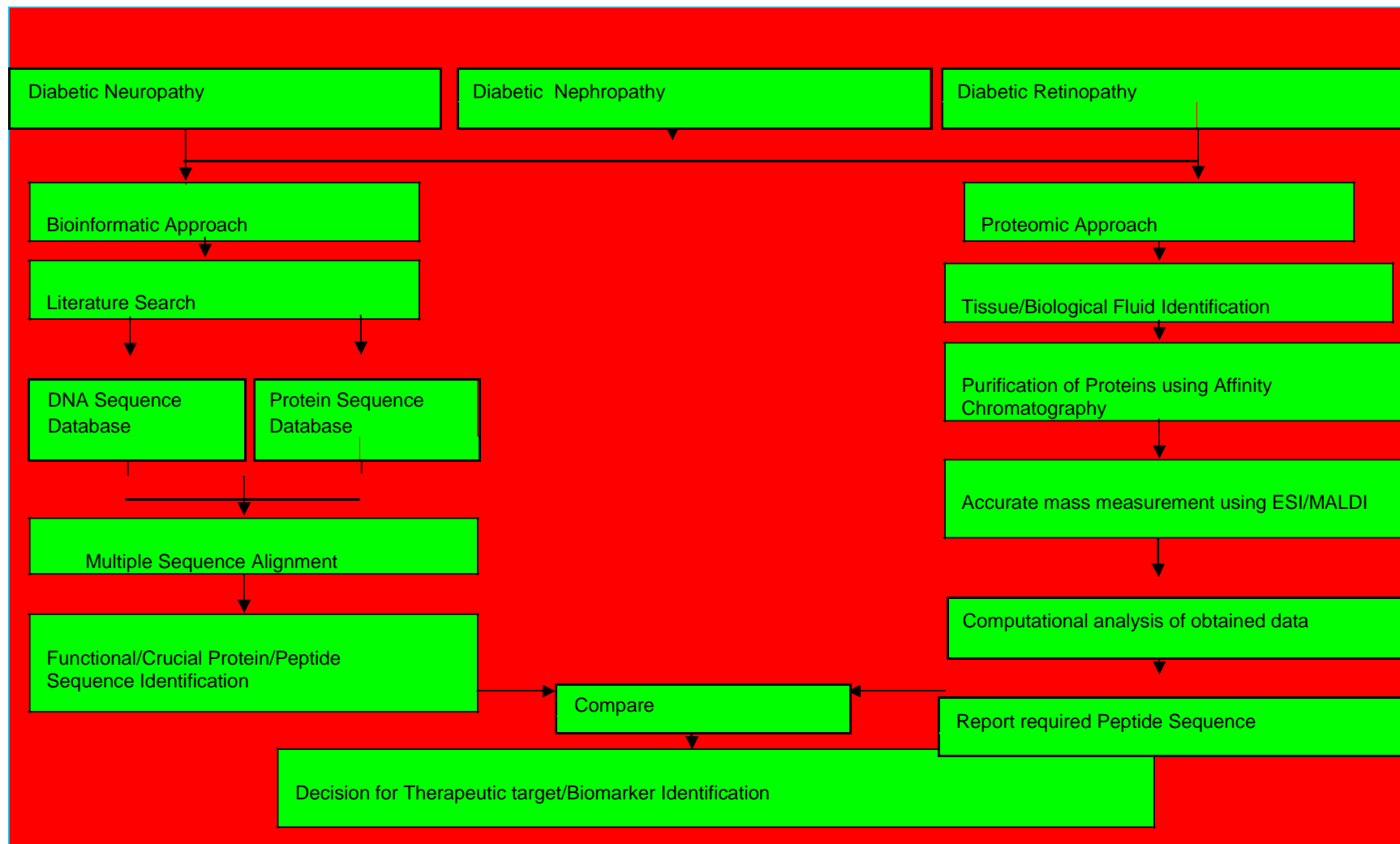
In our previous theoretical studies using bioinformatic tools for diabetes and its complications we have reported some of the proteins for diabetic nephropathy (Rao et al., 2007), diabetic retinopathy (Divakar et al., 2007), serum butyrylcholinesterase in type 2 diabetes (Sridhar et al., 2005) and cholinesterase connection between type 2 diabetes and alzimers disease (Rao et al., 2006). The schematic representation of the diabetic micro vascular complications with reference to bioinformatic and proteomic approaches for therapeutic drug target identification and / or biomarker identification is presented in Figure 1. Reported proteins using bioinformatic are expected to be useful as biomarkers and further experimental studies for the use of the above chromatographic techniques will be useful.

### Conclusion

This paper has reviewed recent development of affinity chromatography coupled with tandem mass spectrometry and pertinent techniques in proteome research. In its near future it is highly probable that affinity chromatography coupled with mass spectrometric data will become accepted as diagnostic and prognostic tool in the future of clinical medicine. As the technologies of proteomics develop and more studies are carried out, new proteins and protein associations will inevitably identified but this should not exclude us from moving forward. Applying a rigorous computational approach will ensure the validity of our interpretations and the result is likely to be a step forward in our scientific understanding.

### ACKNOWLEDGMENT

Author is so thankful to Srinubabu Gedela, who is helped



**Figure 1.** Schematic representation of the diabetic micro vascular complications with reference to bioinformatic and proteomic approaches for therapeutic drug target identification and/or biomarker identification

during the manuscript preparation particularly in chromatography aspects of proteomics.

## REFERENCES

- Andrade EC, Krueger DD, Nairn AC (2007). Recent advances in neuroproteomics; *Curr. Opin. Mol. Ther.* 9: 270-281.
- Arkin A, Ross J, McAdams HH (1998). Stochastic Kinetic Analysis of Developmental Pathway Bifurcation in Phage  $\lambda$ -Infected *Escherichia coli* Cells. *Genetics* 149: 1633-1638.
- Berezovski M, Nutiu R, Li Y, Krylov SN (2003). Nonequilibrium Capillary Electrophoresis of Equilibrium Mixtures *Anal. Chem.* 75: 1382-1387.
- Bruni R, Gianfranceschi G, Koch G (2005). Short acidic peptides isolated from wheat sprout chromatin and involved in the control of cell proliferation Characterization by infrared spectroscopy and mass spectrometry. *J. Peptide Sci.* 11: 225-229.
- Buchanan DD, Jameson EE, Perlette J, Malik A, Kennedy RT (2003). Multiple-injection affinity capillary electrophoresis to examine binding constants between glycopeptide antibiotics and peptides *Electrophoresis* 24: 1375-1378.
- Cho, Lee SSH, Chung WJ, Kim YK, Lee YS, Kim BG (2004). Electrophoresis Aptamer stationary phase for protein capture in affinity capillary chromatography *Anal. Chem.* 76: 3730-3736.
- Chung WJ, Kim MS, Cho S, Park SS, Kim JH, Kim YK, Kim BG, Lee YS (2005). Detection of the inferred interaction network in hepatocellular carcinoma from EHC0 (*Encyclopedia of Hepato-cellular Carcinoma genes Online*) *Electrophoresis* 26: 694-698.
- Clauser KR, Baker P, Burlingame AL (1999). Protein Identification by Mass Spectrometry *Anal. Chem.* 71: 2871-2875.
- Clonis YD (1987). Large-Scale Affinity Chromatography *Biotechnol.* 5: 1290-1296.
- Deng Q, German I, Buchanan D, Kennedy RT (2001). Chiral resolution of histidine using an anti-d-histidine I-RNA aptamer microbore column *Anal. Chem.* 73: 5415-5419.
- Deng Q, Watson CJ, Kennedy RT (2003). Highly sensitive assay for the measurement of serotonin in microdialysates using capillary high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. A* 1005: 123-132.
- Divakar CH, Allam AR, Raghu B, Korrapati (2007). *Proceedings of the Academy of Information Sciences and Management.* 11: 23-28.
- Eddes JS, Kapp EA, Frecklington DF, Connolly LM, Layton MJ, Moritz RL Simpson RJ (2002). Biomarkers discovery from the plasma Proteome multidimensional fractionation proteomics *Proteomics* 2: 1097-1103.
- Eng JK, McCormack AL, Yates JR (1994). Direct Analysis of Protein Mixtures by Tandem Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* 5: 976-982.
- German I, Buchanan DD, Kennedy RT (1998). Aptamer stationary for protein capture in affinity capillary chromatography *Anal. Chem.* 70: 4540-4548.
- Gibson MA, Bruck J (2000). A method for estimating stochastic noise in large genetic regulatory networks. *J. Phys. Chem. A* 104: 1876-1882.
- Gibson MA, Bruck J (2000). Computational cell biology in the post-genomic era. *J. Phys. Chem. A* 104: 1876-1889.
- Haginaka J (2000). A Comparative study of the enantioselective separation of several antiulcer drugs by high-performance liquid chromatography and supercritical fluid chromatography. *J. Chromatogr.* 875: 235-239.
- Han DK, Eng J, Zhou H, Aebersold R (2001). Method for characterizing biomolecules utilizing a result driven strategy. *Nat. Biotechnol.* 19: 946-951.
- Hanash S (2003). Proteomic technology for biomarker profiling in cancer: an update *Disease proteomics Nature.* 422: 226-232.
- Huang CC, Cao Z, Chang HT, Tan W (2004). Ultrasensitive Densitometry Detection of Cytokines with Nanoparticle-Modified Aptamers *Anal. Chem.* 76: 6973-6978.
- James P (2001). *Proteome Research: Mass Spectrometry*, Springer, Berlin, Heidelberg, New York pp. 53-59.
- Jiang C, Glorioso JC, Ataai M (2006). Presence of imidazole in loading buffer prevents formation of free radical in immobilized metal affinity chromatography and dramatically improves the recovery of herpes simplex virus type 1 gene therapy vectors. *J. Chromatogr. A* 1121:40-48.
- Jiang J, Wechuck JB, Coins WF, Krisky DM, Wolge D, Ataai MM, Glorioso JC (2004). Immobilized Cobalt Affinity Chromatography Provides a Novel, Efficient Method for Herpes Simplex Virus Type 1 Gene Vector Purification. *J. Virol.* 78: 8994-8998.
- Keller A, Nesvizhskii AI, Kolker E, Aebersold R (2002). The Application of New Software Tools to Quantitative Protein Profiling Via Isotope-coded Affinity Tag (ICAT) and Tandem Mass Spectrometry. *Anal. Chem.* 74: 5383-5392.
- Kenig M, Peternel S, Porekar VG, Menart V (2006). Influence of the protein oligomericity on final yield after affinity tag removal in purification of recombinant proteins. *J. Chromatogr. A* 1101:293-298.
- Kettman JR, Frey JR, Lefkovits I (2001). Leveraging protein purification strategies in proteomics *Eng. Biomol.* 18: 207.
- Kislinger T, Rahman K, Radulovic D, Cox B, Rossant J, Emili A (2003). Integrated proteomic and transcriptomic profiling of mouse lung development and Nmyc target genes. *Mol. Cell Proteomics* 2: 96-106.
- Kutter JP, Tr AC (2000). Ring-down absorption spectroscopy for analytical microdevices, *Trends Anal. Chem.* 19:352-357.
- lchek M (1974). *Methods Enzymol Affinity chromatography: Methods Protoc.* 34: 182-186.
- MacCoss MJ, Wu CC, Yates JR III (2002). A Method for comprehensive proteomic analysis of membrane proteins, *Anal. Chem.* 74: 5593-5599.
- Michaud ME, Jourdan A, Villet A, Ravel C, Grosset, Peyrin E (2003). Adaptive Recognition of Small Molecules by Nucleic Acid Aptamers through a Label-Free Approach. *J. Am. Chem. Soc.* 125: 8672-8678.
- Michaud ME, Jourdan C, Ravelet A, Villet A, Ravel C, Grosset, Peyrin E (2004). Aptamers as Ligands for Affinity Chromatography and Capillary Electrophoresis Applications, *Anal. Chem.* 76: 1015-1019.
- Millot C (2003). Enantioseparation of 2-O- $\beta$ -D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one and its 7-chloro-derivative by capillary zone electrophoresis using native and substituted  $\gamma$ -cyclodextrins as chiral additives *J. Chromatogr. B* 797: 131-136.
- Nesvizhskii AI, Keller A, Kolker E, Aebersold R (2003). A Dataset of Human Liver Proteins Identified by Protein Profiling Via Isotope-coded Affinity Tag (ICAT) and Tandem Mass Spectrometry. *Anal. Chem.* 75: 4646-4658.
- Nesvizhskii AI, Aebersold R (2004). The analysis of tandem mass spectrometric datasets: high-throughput investigations require high-quality validation *Drug Discov. Today* 9: 173-179.
- Pavski V, Le XC (2001). Aptamer stationary phase for protein capture in affinity capillary chromatography, *Anal. Chem.* 73:6070-6074.
- Perkins DN, Pappin DJ, Creasy DM, Cottrell JS (1999). Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* 20: 3551-3557.
- Porath J, Carlsson J, Olsson I, Belfrage G (1975). Metal-Affinity Separations: A New Dimension in Protein Processing *Nature* 258: 598-603.
- Rao AA, Hanuman T, Ramachandra SG, Annapurna A, Suresh BC, Siva RC, Tejaswi R, Siva PA, Divakar CH, Srinivas K (2007). Bioinformatics analysis of diabetic retinopathy using functional protein sequences-Med. Hypo. In press
- Rao AA, Sridhar GR, Hanuman T, Babu CS, Prasad AS, Divakar Ch (2006). *Lipid Health Dis.* 5: 28.
- Riggs L, Sioma C, Regnier FE (2001). The effects of temperature on the kinetics and stability of mesophilic and thermophilic 3-phosphoglycerate kinases. *J. Chromatogr. A* 924: 359-364.
- Rogan MM, Altria KD (1993). *Introduction to the Theory and Applications of Chiral Capillary Electrophoresis.* Beckman Primer, Fullerton, CA. (4): 56-62.
- Romig TS, Bell C, Drolet DW (1999). *J. Chromatogr. B Photoaptamer Technology: Development of Multiplexed Microarray Protein Assays* *Biomed. Sci. Appl.* 731:275-279.
- Roque ACA, Lowe CR, Taipa MA (2004). Production technologies for monoclonal antibodies and their fragments. *Biotechnol. Progress* 20: 639-645.

- Sakurai T, Matsuo T, Matsuda H, Katakuse I (1984). Mathematical tools in analytical mass spectrometry. *Biomed. Mass Spectrom.* 11:396-402.
- Scopes RK (1978). The effects of temperature on the kinetics and stability of mesophilic and thermophilic 3-phosphoglycerate kinases. *Eur. J. Biochem.* 91: 119-125.
- Song S, Sing AK, Kirby BJ, (2004). Electrophoretic concentration of proteins at laser-patterened nanoporous membranes in microchips. *Anal. Chem.* 76: 4589.
- Sridhar RG, Nirmala G, Allam Appa Rao, Madhavi S, Sreelatha S, Sudha Rani J, Vijayalakshmi P (2005). *Lipid. Health. Dis.* 4: 18.
- Srinubabu G, Narasimha Rao M (2007). Chromatographic Techniques for the separation of Peptides: Application to proteomics; *Chromatographia* 65: 511-518.
- Tabb DL, McDonald WH, Yates JR (2002). The Yeast Resource Center Public Data Repository III. *J. Proteome Res.* 1: 21–26.
- Tanaka Y, Terabe S (1995). Estimation of binding constants by capillary electrophoresis. *J. Chromatogr. A* 694: 277-284.
- Varesio E, Rudaz S, Krause K-H, Veuthey J-L (2002). Evaluation of nano-liquid chromatography–tandem mass spectrometry in a column switching setup for the absolute quantification of peptides in the picomolar range, *J. Chromatogr. A* 974: 135-141.
- Vijayalakshmi MA (1989). Metal-Affinity Separations: A New Dimension in Protein Processing *Trends in Biotechnol.* 3:71-78.
- Vijayalakshmi MA (1996). Affinity Purification of Secreted Alkaline Phosphatase Produced by Baculovirus Expression Vector System *Mol. Biotechnol.* 6:347-348.
- Vijayalakshmi MA (2002). *Biochromatography: theory and practice.* Taylor & Francis, London. pp. 526-534.
- Vrouwe EXJ, Gysler UR, Tjaden J, Van der Greef (2000). Chip-based capillary electrophoresis with an electrodeless nanospray interface, *Rapid Commun. Mass Spectrom.* 14:1682-1687.
- Wilm M, Mann MM (1996). The yeast protein Arc1p binds to tRNA and functions as a cofactor for the methionyl- and glutamyl-tRNA synthetases. *Anal. Chem.* 68: 1-8.
- Wolters DA, Washburn MP, Yates JR (2001). An Automated Multidimensional Protein Identification Technology for Shotgun Proteomics *Anal. Chem.* 73: 5683-5689.
- Ye K, Jin S, Ataai MM, Schultz JS, Ibeh J (2004). Tagging Retrovirus Vectors with a Metal Binding Peptide and One-Step Purification by Immobilized Metal Affinity Chromatography. *J. Virol.* 78: 9820-9826.
- Yue GE, Roper MG, Balchunas C, Pulsipher A, Coon JJ, Shabanowitz J, Hunt DF, Landers JP, Ferrance JP (2006). Protein digestion and phosphopeptide enrichment on a glass microchip. *Anal. Chim. Acta.* 30(564): 116-122.
- Zhang Q, Tang N, Brock JW, Mottaz HM, Ames JM, Baynes JW, Smith RD, Metz TO (2007). Enrichment and analysis of nonenzymatically glycosylated peptides: boronate affinity chromatography coupled with electron-transfer dissociation mass spectrometry. *J. Proteome Res.* 6:2323-2330.