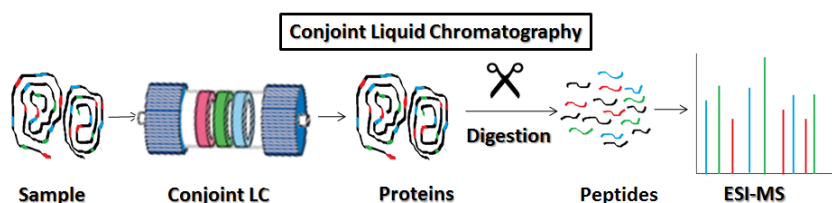
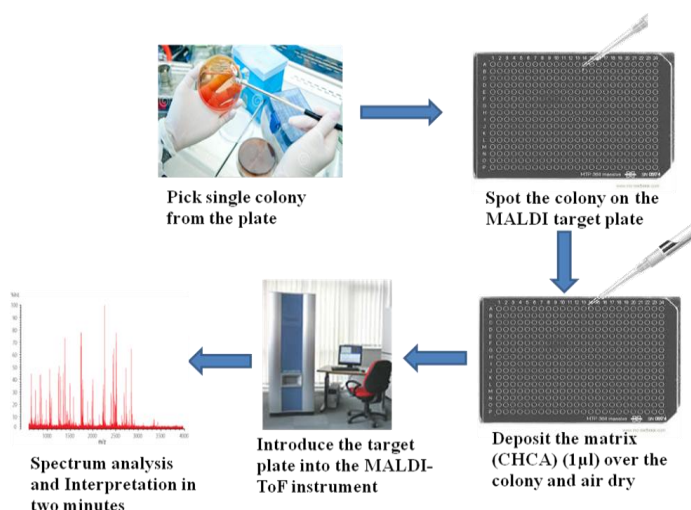




Proteomics Society, India (PSI)

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Detection of Microbes by MALDI-TOF analysis



Editors

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From the Editors

Dear Members,

As promised we are back with our second issue of the year.

This would not have been possible without the timely contributions from our members and backed by a sustained interest of the President, Dr. Zingde. Research highlights update you on some of the ongoing activities in their laboratories. Proteomics for detection of microbes and for profiling serum proteins from individuals infected with Plasmodium vivax and falciparum show the utility of the technology for diagnostics, prediction and therapeutic intervention respectively. The article on monoliths chromatography brings to attention modalities for reducing complexity of the sample for mass spectrometry.

The report by Dr. Subhra Chakraborty on her recent visit to the AOAPPO meeting in China gives us a glimpse of the meeting and a connectivity to our colleagues focusing on agricultural and plant proteomics.

In the two issues this year we have given you glimpses of research from different laboratories. If you would like a different approach such as a theme based issue, do write to us with suggestions. This would bring to attention our members who are focusing on a specific area and using Proteomics.

We look forward to hearing from you

With Best Wishes

Editors

PS: Please send your contributions for the News Letter to abhijit.chakrabarti@saha.ac.in



From the President, PSI

Dear Members,

Proteomics is developing at a fast pace to provide solutions for several different clinical conditions. Challenges in pathogen identification, understanding host-pathogen related biology, investigations on prognosis of disease, measures to control the disease, drug discovery etc are being addressed using Proteomics.

This Newsletter brings you articles addressing some of these issues. I am happy that contributions have come in from our members from different parts of India helping us to fulfill our mandate of providing an insight of their ongoing research.

The preparations for the 6th Annual PSI meeting to be held in Mumbai at IIT, Bombay are in full swing. PSI has also announced 10 travel awards for students/Post Docs. If you have as yet not registered for the meeting do consider attending so that you can meet the who's who in Proteomics in India and abroad.

Some of our members have come forward to organize Proteomics workshops/seminars in their Institutes/Centers/colleges for the benefit of the student and teacher community in their areas. These are in the offing in Mumbai, Madurai and Hyderabad. This is very encouraging. We will bring you updates on the programs in our Newsletters to follow. I would like PSI members from the North and East to come forward too with similar events.

At our recent Executive Council meeting in Hyderabad in July it was decided to celebrate Proteomics Day in the country on the 18th March every year. This is the day when PSI was formed in 2009. Members of PSI are requested to plan for activities which will promote Proteomics in their locales. Do keep us informed about your plans so that we can inform our members and inspire them to join in this effort.

I look forward to meeting you all at IIT Bombay in Dec 2014

With Best Wishes

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Identification of Microbes by MALDI – TOF Mass Spectrometry

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Background

Microorganisms have always attracted the interest of human either for their beneficial role or for causing diseases. Identification of microbes forms the very basis of Microbiology, which has undergone momentous transformation from culture and morphological identification to molecular identification adapting all the technologies available on time. Essentially in case of infectious diseases, the success of the therapeutic strategy heavily relies on the early and accurate identification of the causative pathogen. Significant delay in the identification or misidentification could result in therapeutic failure and even be fatal in case of acute infections caused by multidrug resistant pathogens. The scope for development in microbial identification ever grows to make the identification process simple but rapid, precise and cost effective.

Microbial identification through Mass Spectrometry

Though, DNA sequencing technologies have revolutionized the field of microbial identification, there are still a few pitfalls, like high cost and labour intensive procedures. The advent of mass spectrometry guided proteomics has shown promising results in microbial identification. Matrix assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometers (MS) were shown to be handy in rapid and effortless microbial identification. Mass spectrometers work on the principle of measuring the exact mass of enzyme digested peptides and subsequent protein identification by comparing the generated peptide mass fingerprints with the information available in databases. Identical to genomics tools where the ribosomal DNA has been the choice for microbial identification, ribosomal proteins are of prime interest in MS based microbial identifications.

Microbial identification work flow through MALDI-TOF MS

A classical workflow for microbial identification through MALDI-TOF MS starts with the isolation of a bacterium (or the microbe of interest) through culture methods. A single colony (representing a single species) is mixed with a MALDI matrix substance and plated on the MALDI plate. The matrices that are frequently used for microbial identification include 2, 5-dihydroxybenzoic acid, α -cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA) and ferulic acid (FA) [1]. There are methods which directly extract the proteins from the cells using acetonitrile and trifluoroacetic acid mixture to reduce the complexity of the samples [2]. Peptide mass fingerprints (PMF) are then generated which are compared to the protein databases. There are several tools publicly available which host reference spectra of microbes. The tools available for microbial identification using MALDI-TOF include Mascot, EasyProt (<http://easyprot.unige.ch>), SpectraBank (<http://www.spectrabank.org>), SPECLUST (<http://co.bmc.lu.se/speclust/>), SARAMIS (<http://www.anagnostec.eu/products-services/reference-databases.html>), MicroMass Data set (<http://archive.ics.uci.edu/ml/datasets/MicroMass>), Rapid Microorganism Identification Database (www.RMIDb.org), etc. There are other commercial tools like BiotyperTM, Axima-ID^{plus}, etc., that come with the machine and have their own data sets.

Merits and demerits of microbial identification using MALDI-TOF MS

Microbial identification using MALDI has several advantages like less time consuming, cost-effectiveness, rapid and accurate identification, analysis of large amount of samples at a time, etc. A study reports that the average time required for microbial identification is approximately about 6 minutes per sample which is far less than any other identification methods available [3]. Similarly the cost per sample is approximately 30% less than that of other microbial identification procedures, independent of the cost of the mass spectrometer. This explains the merit of MALDI or MS on the whole, for identification of microbes [3]. There are few reports which state that the method of microbial identification through MALDI has been already adapted in clinical setting as well for comparison and validation of results from other techniques [4, 5]. MALDI-TOF-MS has also been used for identification of bacterial toxin, antibiotic resistance study, identification of Archaea, protists, fungi and viruses [6] and Mycobacteria from clinical specimens [7].

Being a nascent technique with regard to microbial identification, MALDI mediated microbial identification finds few pitfalls. Limited availability of species specific spectra is a foremost concern which limits the utility of using MALDI-TOF MS in microbial identification especially in case of environmental samples, where novel or diverse microbes are expected to be present. Similarly, the success rate when compared

with other genomic tools ranges from 60 – 95% in various studies, which further restricts its use in clinical applications where unambiguous identification is essential.

Conclusion

As discussed above, the strategy of using MALDI TOF mass spectrometer for microbial identification is in an infant stage with immense scope for improvement and subsequent development in the near future. Cost-effectiveness and rapid identification are the key players that attract the attention of researchers and clinicians. Inclusion of spectra representing more genera and species of microbes from diverse habitats/environments will significantly increase the success rate and reproducibility. In the same way, development of new tools/software which could efficiently decipher the spectra obtained out of whole cells will bring about remarkable changes in MALDI – TOF MS mediated microbial identification and phylo-proteomic studies.

References

1. Toh-Boyo, G.M., S.S. Wulff, and F. Basile, Comparison of Sample Preparation Methods and Evaluation of Intra- and Intersample Reproducibility in Bacteria MALDI-MS Profiling. *Analytical Chemistry*, 2012. 84(22): p. 9971-9980.
2. Boïhme, K., et al., Species Differentiation of Seafood Spoilage and Pathogenic Gram-Negative Bacteria by MALDI-TOF Mass Fingerprinting. *Journal of Proteome Research*, 2010. 9(6): p. 3169-3183.
3. Seng, P., et al., Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. *Clinical Infectious Diseases*, 2009. 49(4): p. 543-551.
4. Schneiderhan, W., et al., Work flow analysis of around-the-clock processing of blood culture samples and integrated MALDI-TOF mass spectrometry analysis for the diagnosis of bloodstream infections. *Clin Chem*, 2013. 59(11): p. 1649-56.
5. Cobo, F., Application of maldi-tof mass spectrometry in clinical virology: a review. *Open Virol J*, 2013. 7: p. 84-90.
6. Seng, P., et al., MALDI-TOF-mass spectrometry applications in clinical microbiology. *Future Microbiol.*, 2010. 5(11), 1733–1754.
7. Khechine A.E., et al., Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Identification of Mycobacteria in Routine Clinical Practice. *PLOS ONE* September 13, 2011, DOI: 10.1371/journal.pone.0024720.

Proteomic Analysis of Serum in Vivax and Falciparum Malaria Patients to Investigate Disease Pathogenesis and Identify Surrogate Protein Markers of Infection



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Malaria is by far the world's most significant tropical infectious disease with estimated 207 million malaria cases worldwide in 2012, leading to 6,27,000 deaths [1]. According to the recent WHO statistics; about 100 countries in the world are considered malarious and 3.3 billion people i.e. nearly half of the world's population, are at risk of malaria. India significantly contributes to the global malaria burden and has the largest population in the world at risk of malaria [2]. Despite worldwide initiatives, emerging drug resistance in different species of *Plasmodium* and paucity of information about the exact underlying mechanism of the disease pathogenesis are hindering the management and eradication of malaria. *Plasmodium falciparum* (Pf) infection represents the major cause of malaria associated mortality worldwide. Although *Plasmodium vivax* (Pv) infection is historically regarded as benign, recently the severe and fatal incidents of *vivax* malaria reported from different regions of the world, rendered this clinical paradigm deceptive. Mechanisms that trigger the transition of uncomplicated malaria into severe-complicated manifestations are largely obscure, particularly in case of Pv infection [3].

The spectacular advancements, achieved in the preceding decade with the completion the genome sequence of different species of *Plasmodium* and its insect and vertebrate hosts [4], have propelled the growth of proteomics into different arenas of malaria research. Serum proteomics has attracted considerable interest for biomarker discovery since serum can be collected less invasively and it is an ideal biological sample that contains an archive of information due to the presence of variety of proteins released by diseased tissues. The rapid alterations in expression pattern of various serum proteins in response to external stimulus and correlation between the serum protein levels and pathogenic infections is extremely valuable and of particular interest from a clinical and diagnostic perspective. Over the last decade diagnostic applications of serum proteomics have steadily been growing [5].

Multifarious interaction between the human and malaria parasites provokes complex immune cascades in the host [6], whilst *Plasmodium* develops versatile mechanisms to escape their host defense machinery [7]. Ultimate clinical outcome of malaria is influenced by different host, parasite, environmental and social factors [8]. Plasmodial infections significantly affect different vital physiological processes and as a consequence modulate the host's proteome. Once infected, a continuous interaction between the pathogen and the host immune system initiates a complex immune response to prevent the pathogenic

infection and growth through multiple anti-parasitic effector functions, including inhibition of invasion and cytoadherence, antibody-dependent cytotoxicity and cellular inhibition. Blood biomarkers for pathogenic infections can be used for early diagnosis, discrimination between closely related infections with similar clinical manifestations as well as aid in scrutinizing disease progression, response to therapy, and predicting outcomes. Additionally, investigation of the pathogen induced alterations in host proteome under diseased conditions can provide valuable information regarding disease pathogenesis and host immune responses. Proteomic analysis of serum/plasma has been extensively performed for detection of surrogate proteins markers for cancers and different types of other human diseases. However, very few studies have been conducted to identify surrogate protein markers for malaria, particularly for *Pv* infection. A few studies have been conducted to identify serum/plasma biomarkers of malaria. In a recent study Armah et al., have demonstrated an co-relation between cerebral malaria (a deadly complication of *Pf* infection) associated mortality with elevated serum and CSF levels of apoptotic factors (IP-10, IL-1ra, sTNFR1, sTNFR2, sFas) as well as reduced serum and CSF levels of neuroprotective angiogenic growth factors (PDGFbb) [9], while in another study plasma superoxide dismutase (SOD-1) level has been identified as a surrogate marker of *Pv* malaria severity [10]. Kim and co-workers have performed serum proteome analysis of *vivax* malaria patients in Korean population and reported alteration in serum levels of haptoglobin and serum amyloid A [11]. In another recent interesting work, Kassa et al., have reported quite a few interesting inflammation-related serum biomarkers for malaria and demonstrated their interactions with malarial-related molecules is hemozoin (HZ), which is a dark-brown crystal formed by the parasite and released into the host during the burst of infected red blood cells [12]. Very recently, Bachmann et al., have reported elevated muscle proteins in plasma of children with cerebral malaria by affinity proteomics screening using antibody suspension bead arrays [13].

Our research group at Indian Institute of Technology Bombay, Mumbai is also performing comprehensive proteomic analysis of serum samples from patients suffering from *vivax* and *falciparum* malaria using different proteomic and immunological technologies to analyze alterations in the human serum proteome as a result of plasmodial infections to obtain mechanistic insights about disease pathogenesis, host immune response, and identification of protein markers of infection and disease severity [14,15]. This multi-centric study was conducted by involving malaria patients and controls from both the urban and the rural populations from three different malaria endemic regions of India; Mumbai, Kolkata and Bikaner. Recruitment of the subjects for this multidisciplinary study was carried out during 2010 to 2013 at multiple hospitals including Seth GS Medical College & KEM Hospital-Mumbai, Grant Government Medical College and Sir JJ Group of Hospitals-Mumbai, PD Hinduja National Hospital & Medical Research Centre- Mumbai, Medical College Hospital - Kolkata and Malaria Research Center- Bikaner, with the approval of the institutional ethics committee of each study site. Serum samples from *vivax* and *falciparum* malaria patients and healthy controls were analyzed using different gel-based (2-DE and 2D-DIGE) and MS-based quantitative proteomics (iTRAQ) approaches, and results were validated by employing immunoassay-based approaches (Figure 1). Functional pathway analysis involving the identified differentially expressed proteins revealed the modulation of different vital physiological pathways, including lipid metabolism and transport, acute phase response signaling, chemokine and cytokine signaling, complement cascades and blood coagulation in malaria [14,15]. Specificity of the identified serum markers for malaria was evaluated by analysis of two other infectious diseases resulting in fevers; dengue fever and leptospirosis [16,17]. A

panel of identified proteins consisting of six candidates; Serum amyloid A (SAA), Hemopexin (HPX), Apolipoprotein E (Apo E), Haptoglobin (HP), Retinol-binding protein 4 (RBP4) and Apolipoprotein A-I (Apo A-I) provided excellent discrimination of malaria from healthy community controls and other different infectious diseases [18]. Analysis of longitudinal cohorts (early febrile, defervescence and convalescent stages) indicated that majority of the proteins were maximally elevated in malaria patients during the early febrile phase of the infection. Identified proteins like SAA, Apo A-I and Apo E which exhibited a sequential alteration in their expression levels in different severity levels of malaria, could serve as potential predictive markers for disease severity. While candidates like HP, RBP4 and Ceruloplasmin, which exhibited cyclic alterations in their expression levels with the progression of the disease, can be utilized as possible markers for monitoring therapeutic interventions. The excellent discrimination among the malaria, controls and other infectious disease groups obtained by various prediction models on the basis of differentially expressed serum proteins, identified in this study testifies the potential of this analytical approach for the discrimination of *vivax* and *falciparum* malaria as well as other infectious and non-infectious human diseases on the basis of host proteomic alterations.

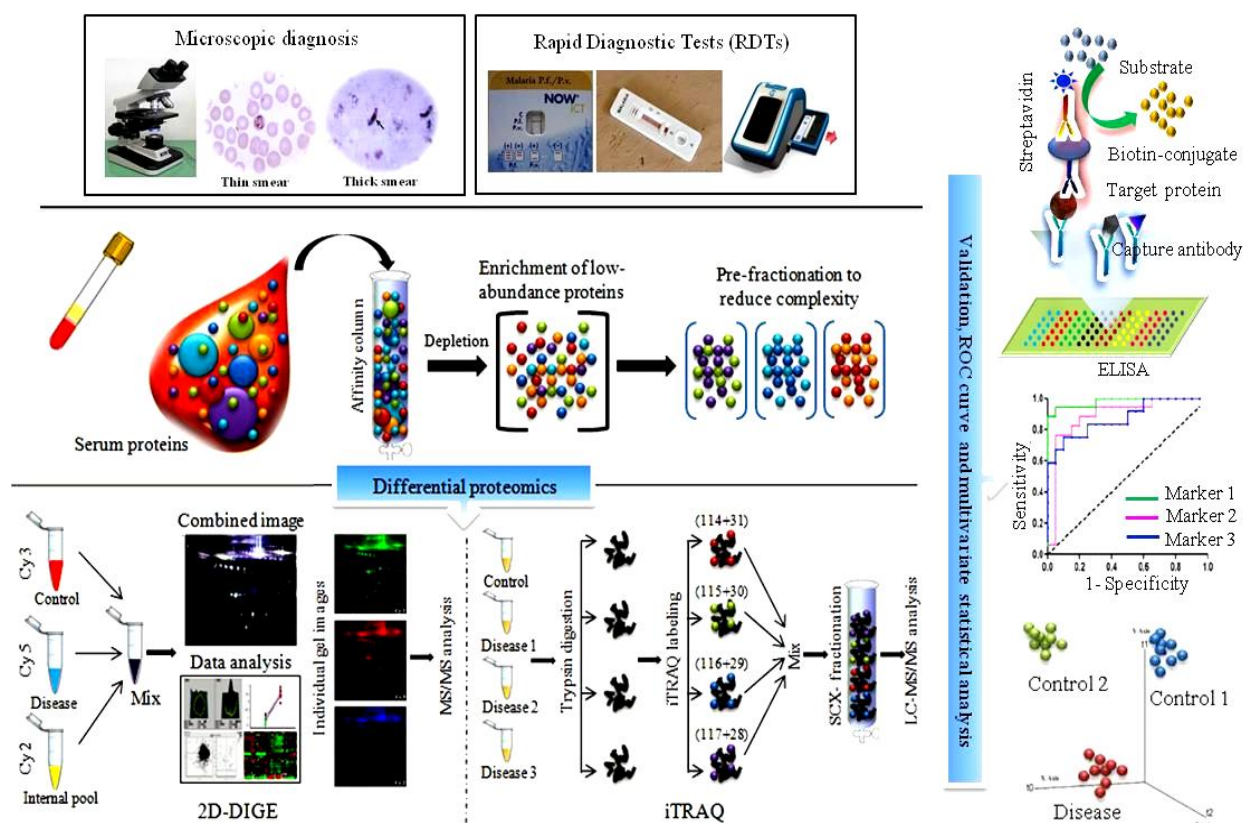


Figure 1. Schematic representation of experimental strategy for proteomic analysis of alterations in the human serum proteome in malaria. Comparative proteomic analysis of *falciparum* and *vivax* malaria, other infectious diseases (dengue fever and leptospirosis) and healthy controls was performed by using two complementary quantitative proteomic approaches; 2D-DIGE along with MALDI-TOF/TOF MS and iTRAQ-based quantitative proteomics in combination with ESI-Q-TOF LC/MS-MS; and results were validated by employing ELISA and western blotting. Individual performance of a few selected differentially expressed proteins was analyzed using receiver operating characteristic (ROC) curves. Identified

differentially expressed proteins in malaria were subjected to functional pathway analysis for understanding their biological contexts, involvement in various physiological pathways and association with disease pathophysiology (modified from Ref no. [18]).

References:

- [1] World Malaria Report, World Health Organization 2013. ISBN: 9 789241 56469 4 http://www.who.int/malaria/publications/world_malaria_report_2013/en/
- [2] Shah NK, Dhillon GPS, Dash AP, Arora U, et al. Antimalarial drug resistance of *Plasmodium falciparum* in India: changes over time and space. *Lancet Infect Dis*. 2011, 11, 57-64.
- [3] Rogerson SJ, Carter R. Severe *vivax* malaria: newly recognised or rediscovered. *PLoS Med*. 2008, 5, e136.
- [4] Gardner, M. J., Hall, N., Fung, E., White, O. et al., Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 2002, 419, 498-511.
- [5] Ray S, Reddy PJ, Jain R, Gollapalli K, et al. Proteomic technologies for the identification of disease biomarkers in serum: advances and challenges ahead. *Proteomics* 2011, 11, 2139-61.
- [6] Hiseida H. et al. Escape of malaria parasites from host immunity requires CD4+ CD25+ regulatory T cells. *Nat Med*. 2004, 10(1), 29-30
- [7] Olszewski KL, Morrissey JM, Wilinski D, Burns JM, et al. Host-parasite interactions revealed by *Plasmodium falciparum* metabolomics. *Cell Host Microbe*. 2009, 5, 191-9.
- [8] Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature* 2002, 415, 673-9.
- [9] Armah HB, Wilson NO, Sarfo BY, Powell MD, et al. Cerebrospinal fluid and serum biomarkers of cerebral malaria mortality in Ghanaian children. *Malaria J* 2007, 6, 147.
- [10] Andrade BB, Reis-Filho A, Souza-Neto SM, Raffaele-Netto I, et al. Plasma Superoxide Dismutase-1 as a surrogate marker of *vivax* malaria severity. *PLoS Negl Trop Dis*. 2010, 4, e650.
- [11] Bahk YY, Na B, Cho S, Kim J, et al. Proteomic analysis of Haptoglobin and Amyloid A protein levels in patients with *vivax* malaria. *Korean J Parasitol*. 2010, 48, 203-11.
- [12] Kassa FA, Shio MT, Bellemare M-J, Faye B, et al. New Inflammation-Related Biomarkers during Malaria Infection. *PLoS ONE* 2011, 6, e26495.
- [13] Bachmann J, Burté F, Pramana S, Conte I, et al. Affinity proteomics reveals elevated muscle proteins in plasma of children with cerebral malaria. *PLoS Pathog*. 2014, 17, 10, e1004038.
- [14] Ray S, Renu D, Srivastava R, Gollapalli K, et al. Proteomic investigation of *falciparum* and *vivax* malaria for identification of surrogate protein markers. *PLoS One* 2012, 7, e41751.
- [15] Ray S, Kamath KS, Srivastava R, Raghu D, et al. Serum proteome analysis of *vivax* malaria: An insight into the disease pathogenesis and host immune response. *J Proteomics*. 2012, 75, 3063-80.
- [16] Ray S, Srivastava R, Tripathi K, Vaibhav V, Patankar S, Srivastava S. Serum proteome changes in dengue virus infected patients from a dengue endemic area of India: Towards new molecular targets? *Omics* 2012, 16, 527-36.
- [17] Srivastava R*, Ray S*, Vaibhav V, Gollapalli K, Jhaveri T, et al., Serum profiling of leptospirosis patients to investigate proteomic alterations. *J Proteomics*. 2012, 76, 56-68. * Joint 1st Author.
- [18] Ray S, Patel S, Kumar V, Damahe J. Srivastava S. Differential expression of serum/plasma proteins in various infectious diseases: Specific or nonspecific signatures. *Proteomics Clin. Appl*. 2014, 8, 53-72.

Tailoring Methacrylate Monolithic Chromatography for Proteomics



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Proteomics has evolved as an exciting but challenging discipline. Proteomics attempts the complete analysis of proteins in complex biological samples. The invention of Mass Spectrometry (MS), an indispensable tool, has revolutionized the study of proteomics[1]. Continuous improvisation of MS instrumentation has enabled the researchers for better analysis of the proteome. The application of MS is not limited only for proteome analysis but for metabolites, lipids, carbohydrates, metals and more. One of the remarkable achievements in the proteomics was combining liquid chromatography systems with MS (Bottom Up approach), created a new dimension in proteomics, for enhanced analysis of the proteome and other macromolecules [2].

The ideal goal of proteomics is to identify and quantify as many proteins as possible from the proteome in the study. Despite advancements at both instrumentation and annotation levels, the major lacunae in proteomics is lack of complete analysis of complex biological samples, reproducibility, analysis time and cost effectiveness. To address a larger portion of a given proteome, an ideal requirement would be to develop a dedicated analytical system (integrated) for sample preparation, separation, and analysis combined with powerful data processing, analysis and information management technologies. By and large, it is noteworthy to say that liquid chromatography (LC) is an essential setup in proteomics.

Liquid Chromatography plays an important role both in the field of biotechnology and proteomics. Separation and purification of the analyte mainly depends on the properties of the stationary phase, the nature of ligand, the physio-chemical nature of the analyte and the mobile phase. At present Reverse Phase (RP) columns are predominately used in LC-MS/proteomics approach. These columns exhibit limitations like flow rate, diffusion, back pressure and are not applicable for all kind of samples. To overcome a few limitations Ultra High Pressure Liquid Chromatography (UPLC) columns were introduced and they have replaced the conventional RP columns[3].

In the past four decades, in the field of separation science and technology, the constant demand for efficient purification strategies has resulted in development of new matrices with better thermodynamic and hydrodynamic properties[4]. The conventional matrices used are natural polymers such as cellulose, agarose and dextran. Though these matrices are extensively used in biochromatography, they have

drawbacks like mechanical stability, pressure drop, clogging and column efficiency. Hence, their application is limited in the proteomics approaches. Taken together, the challenges exhibited by the conventional and silica supports, the use of ultra-fast rigid chromatographic supports called MONOLITHS hold a promising future in proteomics.

Monoliths are defined as continuous stationary phase that form a homogeneous column in a single piece and prepared in various dimension with agglomeration-type or fibrous microstructures[5]. The first monolith was synthesized by Hjerten *et al*, in 1989 by polymerization of N-N-methylene bisacrylamide and acrylic acid. This was followed by introduction of macroporous polymethacrylate monoliths by (Svec *et al*, 1992) and silica monoliths (Minakuchi *et al*, 1996)[6,7]. For biochromatographic applications, polymethacrylate monoliths are extensively used for purification of biomolecules like DNA, viruses, proteins, etc. The polymethacrylate based monoliths are commercially available as Convective Interaction Media (CIM®) manufactured by BIA Separations, Austria[5].

The advantage of CIM monolithic columns are fast separation and good resolution of analytes due to convective mediated transfer, no dead end pores, high mass transfer, high retention capacity and no column packing is required[8]. These monoliths are available in different geometries like high throughput system (96 well plates), disks (50 µL and 0.34mL), 1 mL tube and preparative columns ranging from 8 mL to 8 L. The monoliths are available in different chemistries such as ion exchange, affinity and hydrophobic similar to conventional soft gel matrices. Further, the monolithic columns facilitate for different immobilization techniques, wherein different ligands can be coupled onto their surface. The chromatographic conditions employed for purification with monolithic column is similar to that of conventional column except for injection of high sample volume and use of high flow rate (0.34 ml CIM disk can be used at a flow rate up till 8 mL/min)[9,10]. Thus efficient separation and resolution of analyte can be achieved in short time (reduction in process time) with this technology. The reproducibility, reusability and the cost-effectiveness of technology are added features for the application of this technology in biochromatography and proteomics[11].

Since the inception of the macroporous monolithic technology, researchers are exploiting the advantages of this technology for purification of various biomolecules. They can be vividly exploited in proteomics at different capacity. They can be used as capture/affinity capture systems, immobilized monolithic enzyme reactors (IMER), in multi-dimensional mode - conjoint liquid chromatography (CLC) and for high throughput analysis [11-13].

Immobilized Monolithic Enzyme Reactors : For analysis of complex proteome sample, the crucial step is efficient proteolysis. The in-solution approach is time consuming and sometimes not reproducible. This limitation can be overcome by use of IMER for rapid and efficient hydrolysis of proteins or carbohydrates in short time (seconds or minutes) and results in identification of more peptides/glycans. The efficiency of IMER can be ascertained to (i) right orientation of enzyme on the monolithic surface (ii) ease of immobilization with high concentration of enzyme (iii) high mass transfer and (iv) no autolysis. Due to the above merits IMER systems will give reproducible results. The added advantage of IMER systems is that it can be used in combination with affinity capture systems, so that the selected peptides are captured and identified by MS [14, 15].

Conjoint Liquid Chromatography (CLC): Given the miniature and disk type geometries of the CIM monolithic columns, they can be used in conjoint fashion, i.e. multi-dimensional mode. About three or four different functional group discs can be placed in a single housing and used for purification of different molecules. With such a set-up, large volume of complex biological sample can be processed in short time. This approach effectively minimizes the sample preparation and loss, and efficiently separates molecules for further analysis. The application of CLC in proteomics approach is advantageous in terms of reduction in complexity of samples due to selective capture of proteins/peptides. For example, complexity of the sample can be reduced in a sequential manner, with initial depletion of high abundant proteins, followed by capture of mid or low range proteins either by affinity or ion exchange column. The CLC can be used in on-line/off-line mode with MS for analysis of proteins/peptides [12].

Thus, the challenges and concerns in proteomics, in particular the LC, can be answered by use of the polymethacrylate monolithic technology. Taken together the advantages of monolith technology, application of this technology in proteomics is relatively easy, simple and straight-forward. This technology is encouraging and will open new pathways in the field of proteomics.

References

- [1] R. Aebersold, M. Mann, *Nature*, 422 (2003) 198-207.
- [2] J.R. Yates, C.I. Ruse, A. Nakorchevsky, *Annual review of biomedical engineering*, 11 (2009) 49-79.
- [3] M.E. Swartz, *Journal of Liquid Chromatography & Related Technologies*, 28 (2005) 1253-1263.
- [4] A. Jungbauer, *Journal of Chromatography A*, 1065 (2005) 3-12.
- [5] A. Podgornik, A. Štrancar, *Biotechnology annual review*, 11 (2005) 281-333.
- [6] K.K. Tetala, T.A. van Beek, *Journal of separation science*, 33 (2010) 422-438.
- [7] T.B. Tennikova, J. Reusch, *Journal of Chromatography A*, 1065 (2005) 13-17.
- [8] E. Vlakh, T. Tennikova, *Journal of Chromatography A*, 1216 (2009) 2637-2650.
- [9] M. Peterka, M. Jarc, M. Banjac, V. Frankovič, K. Benčina, M. Merhar, V. Gaberc-Porekar, V. Menart, A. Štrancar, A. Podgornik, *Journal of Chromatography A*, 1109 (2006) 80-85.
- [10] T. Barroso, A. Hussain, A.C. Roque, A. Aguiar-Ricardo, *Biotechnology journal*, 8 (2013) 671-681.
- [11] D. Josic, J.G. Clifton, *Journal of Chromatography A*, 1144 (2007) 2-13.
- [12] E.L. Pfau Miller, M.L. Paulemond, C.M. Dupper, D.S. Hage, *Analytical and bioanalytical chemistry*, 405 (2013) 2133-2145.
- [13] R.R. Prasanna, S. Sidhik, A.S. Kamalanathan, K. Bhagavatula, M.A. Vijayalakshmi, *Journal of Chromatography B*, 955 (2014) 42-49.
- [14] J. Ma, L. Zhang, Z. Liang, W. Zhang, Y. Zhang, *Journal of separation science*, 30 (2007) 3050-3059.
- [15] R. Nicoli, N. Gaud, C. Stella, S. Rudaz, J.-L. Veuthey, *Journal of pharmaceutical and biomedical analysis*, 48 (2008) 398-407.

Past Events

A Glimpse of the 1st AOAPO conference, the 6th International Symposium on Frontiers in Agricultural Proteome Research and the 5th Plant Proteomics Conference

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Ever since the term “Proteomics” was first coined in late 1990s followed by the term “Proteome” in 2004, few leading international proteomic organizations have been formed to represent and promote proteomics research through collaboration by fostering the development of newer technologies, training, and discussion on the progress in the respective fields during the annual meetings/conferences. Indeed, Asia Oceania Agricultural Proteomic Organization (AOAPO) is one such great initiative to bring together the Agricultural and plant proteomic community and encourage communication, cooperation, collaboration, discussion on the international trends in proteomics research and education among the fraternity. This year in the month of June from 23rd to 27th, the 1st AOAPO conference was held in the beautiful city Harbin, North-East China alongwith the 6th International Symposium on Frontiers in Agricultural Proteome Research and the 5th Plant Proteomics Conference in China. The conference was hosted by Heilongjiang University, Qiqihar University, and Northeast Forestry University in Hanlin Tianyue Hotel adjacent to the Heilongjiang University campus. Around 200 participants from various scientific disciplines, including agriculture, plant, food, micro-organisms, and biotechnology attended the conference. It was inaugurated by Professor Yuxian Zhu, Academician with his opening remarks followed by speeches from Professor Setsuko Komatsu, President AOAPO and Professor Ning Li representing the Chinese Society of Cell Biology. Welcome addresses were delivered by Vice Presidents and President of the three organizing Universities, all of whom emphasized the opportunities in Agricultural and plant proteomics, progress in the field in China, and also highlighted the contribution of AOAPO for strengthening proteomics research. The inaugural session concluded with a photography session.



The conference was attended by leading scientists in the field from across the globe. Their presentations showed how the fast advancing proteomics research and technologies are applied to solve important agricultural, plant and food biology problems. I am pleased and honored to serve as the Council member to AOAPO. In addition, it was my pleasure to deliver an invited talk during the conference. Every morning the conference started with a plenary lecture. Scientists invited from across the globe with diverse research interests in proteomics deliberated during the conference. The lectures delivered during the conference covered various areas, including Agriculture, Plant, Food and Micro-organismal proteomics and could be broadly categorized under Developmental and Organelle Proteomics; Agricultural, GMO and

Stress Proteomics; Hormone signaling and PTM Proteomic; and Stress Proteomics. Seven scientific sessions included two plenary talks, thirty one invited talks by leading national and international scientists in their respective field of research and three Industry talks on new technology developments in mass spectrometry and protein interaction studies. Besides the plenary lectures and invited talks, every day after dinner, there were informal poster sessions and exhibitions by sponsors for two and a half hours, which played a central role in vibrant discussions and exchange of scientific ideas amongst the students, researchers, and fellow colleagues. AOAPO council meeting was also held during the conference. Organizers provided ample opportunity for everyone especially for students and young investigators to participate in this congress. Couple of student presentations were selected from the submitted abstracts for talks. The attraction for the budding proteomics leaders was the “Best talk” and “Best Poster” Awards during the closing ceremony. The conference was a great success and it was brought to a close by vote of thanks to the organizers and participants, and the announcement of the 2015 meeting in Korea.

Overall, I was indeed very much impressed to observe the enthusiasm of young proteomics researchers and the positive attitude of the Agricultural and plant proteomics community. This is encouraging as it serves the purpose and goal of AOAPO. Nevertheless in comparison to human and animal proteomics, agriculture and plant proteomics has miles to go to usher in the benefits to the living world of plant, animal, and humankind. It is a great pleasure to express my sincere thanks to all student volunteers, participants, colleagues, and AOAPO members who made the event a great success.

The 2nd AOAPO conference will be held in Korea along with the 7th International Symposium on Frontiers in Agricultural Proteome Research in 2015. Get set ready, come forward to explore and enjoy the life and beauty of our evergreen world through PROTEOMICS.

Upcoming Events

Update on the 6th Annual Meeting of the Proteomics Society, India

Dear Members

You will be aware that the 6th Annual Meeting of the Proteomics Society is scheduled during 6th to 11th Dec 2014. Details are available at: <http://www.bio.iitb.ac.in/~sanjeeva/psi2014/>

On the **6th Dec 2014**: Education Day for College Teachers and Students

7th to 9th Dec 2014: 6th Annual meeting and International Conference on “Proteomics from Discovery to Function”


10th and 11th Dec 2014: Workshops on: Gel based proteomics, Mass spectrometry, Targeted proteomics Protein Microarrays and Surface Plasma Resonance.

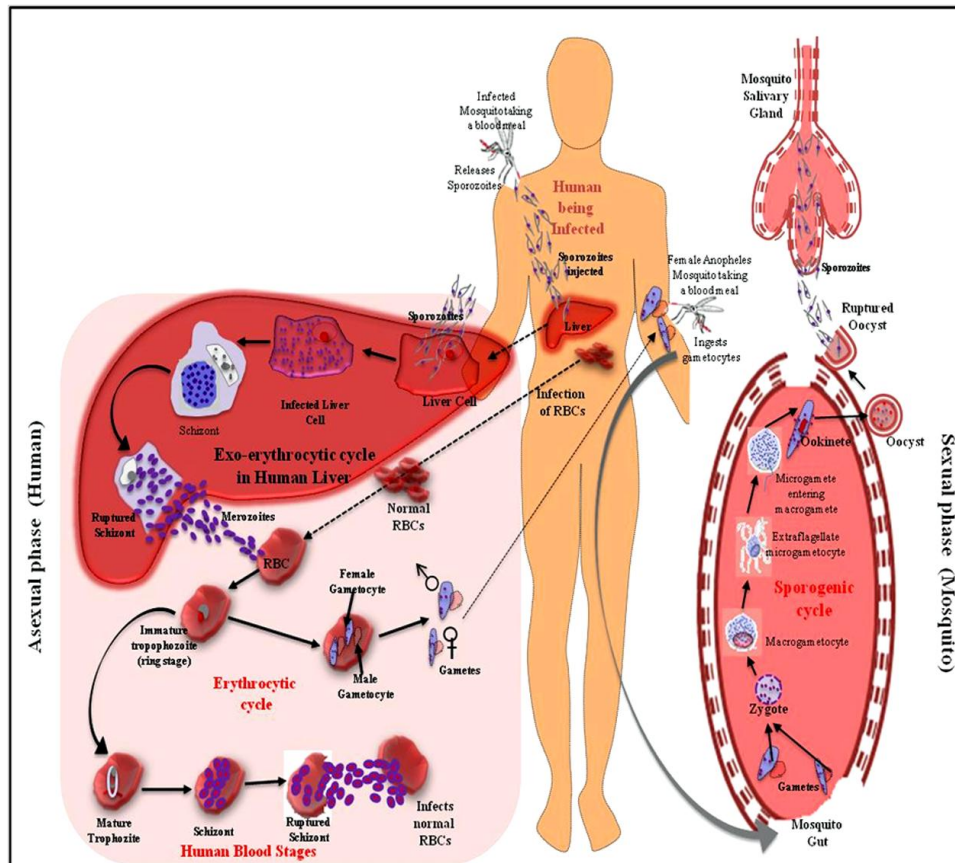
I hope you have already registered for the meeting and other events. Looking forward to meeting you in IIT Bombay

Sincerely

Sanjeeva Srivastava, Convenor, 6th Annual Meeting of PSI

Upcoming National and International Proteomics conferences / meeting / workshops

Conference/Meeting/Workshop	Date & Venue	Link
Targeted proteomics: experimental design and data analysis	September 28 th -October 3 rd , 2014, Spain	http://events.embo.org/14-targeted-proteomics/
HUPO 13th Annual World Congress "The proteome quest to understand biology and disease"	October 5 th to 8 th , 2014, Madrid, Spain	www.HUPO2014.com
 "Clinical Proteomics Workshop", supported by DBT and associated with Proteomics Society, India	October 10 th to 11 th , 2014, Proteomics Dept, Aravind Medical Research Foundation, Madurai, India	www.aravind.org/clinicalproteomics2014/index.htm
OurCon II 2014 Imaging Mass Spectrometry Conference and Congress of the Turkish Proteomics Society.	November 18 th to 21 st , 2014, Antalya, Turkey	http://www.ourcon2.org/
Proteomic Society, India Education Day for College Teachers and students: . Proteomics-principles and techniques	December 6 th , 2014, IIT Bombay, Mumbai, India	http://www.bio.iitb.ac.in/~sanjeeva/psi2014/
6th Annual Meeting of the Proteomics Society, India	December 7 th to 9 th , 2014, IIT, Bombay, Mumbai, India	http://www.bio.iitb.ac.in/~sanjeeva/psi2014/
Proteomic Society, India:Workshops on proteomic technologies	December 10 th to 11 th , 2014, IIT Bombay, Mumbai India	http://www.bio.iitb.ac.in/~sanjeeva/psi2014/
2 nd Brazilian Proteomics Society and 2nd PanAmerican HUPO Joint Meetings	December 7 th to 10 th ,2014, Brazil	http://www.brprot2014.com.br/
HUPO 14th Annual World Congress (HUOP 2015)	September 26 th to 30 th , 2015, Canada	http://www.hupo.org/events
5 th International Conference on Proteomics and Bioinformatics	November 3 rd to 5 th , 2015, Spain	http://www.proteomicsconference.com



Life cycle of the malarial parasite

Proteomics Society, India

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