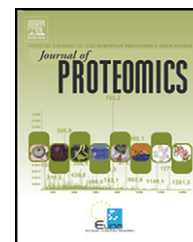


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Tutorial

Virtual Labs in proteomics: New E-learning tools[☆]

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ARTICLE INFO

Article history:

Received 17 December 2011

Accepted 7 March 2012

Available online 23 March 2012

Keywords:

E-learning

Gel-based proteomics

Mass spectrometry

Proteomics

Tutorial

Virtual proteomics labs

ABSTRACT

Web-based educational resources have gained enormous popularity recently and are increasingly becoming a part of modern educational systems. Virtual Labs are E-learning platforms where learners can gain the experience of practical experimentation without any direct physical involvement on real bench work. They use computerized simulations, models, videos, animations and other instructional technologies to create interactive content. Proteomics being one of the most rapidly growing fields of the biological sciences is now an important part of college and university curriculums. Consequently, many E-learning programs have started incorporating the theoretical and practical aspects of different proteomic techniques as an element of their course work in the form of Video Lectures and Virtual Labs. To this end, recently we have developed a Virtual Proteomics Lab at the Indian Institute of Technology Bombay, which demonstrates different proteomics techniques, including basic and advanced gel and MS-based protein separation and identification techniques, bioinformatics tools and molecular docking methods, and their applications in different biological samples. This Tutorial will discuss the prominent Virtual Labs featuring proteomics content, including the Virtual Proteomics Lab of IIT-Bombay, and E-resources available for proteomics study that are striving to make proteomic techniques and concepts available and accessible to the student and research community. This Tutorial is part of the International Proteomics Tutorial Programme (IPTP 14). Details can be found at: <http://www.proteomicstutorials.org/>.

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1. Introduction

1.1. Proteomics — an emerging platform for basic and applied research

Genomics is the study of an organism's entire genetic information to determine its sequence and function to unravel cell physiology, metabolic pathways and developmental pro-

cesses. Proteomics likewise, is the systematic study of proteins encoded by a genome for their expression, localization, interaction and post-translational modifications. Establishment and expansion of proteomics in the post-genomic era were possible due to the earlier achievements in genomics research, especially the successful completion of human genome project [1]. Generally three levels of analysis are performed with equal importance for understanding life processes at the

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molecular level, genomics, transcriptomics and proteomics i.e. the DNA, RNA and protein level, respectively (Fig. 1). Nonetheless, the plethora of modifications at the post-transcriptional and/or post-translational levels, the presence of protein isoforms, the dynamicity of protein content with time and condition, and above all, the close correlation with physiological functions, health and disease, make proteome level study more intensive, informative and effective than genome or transcriptome level analysis.

The diversity of protein content across the living organisms and dynamic changes in protein expression levels with time, and in response to the external stimulus or environmental conditions, make proteome analysis highly complex and challenging [2]. Proteins being the ultimate effector molecules in living

systems, have a closer correlation with biological functions and disorders as compared to genes; and provide better insight into the pathobiological status of diseased conditions, which has led to the enormous growth of clinical proteomics for “bed-side” applications [3]. Advancement in proteomics has been prodigious during the last decade with several promising technologies emerging to the forefront for complex proteome level analysis [4]. While the initial combination of two dimensional gel-based proteomics and mass spectrometry opened the door for global and differential proteome profiling of any organism, the advancement of mass spectrometry, successful integration of real-time, high-throughput array-based techniques, [5] and ultra-sensitive nanotechnological approaches [6] have paved the way for the entry of proteomics into basic and applied research.

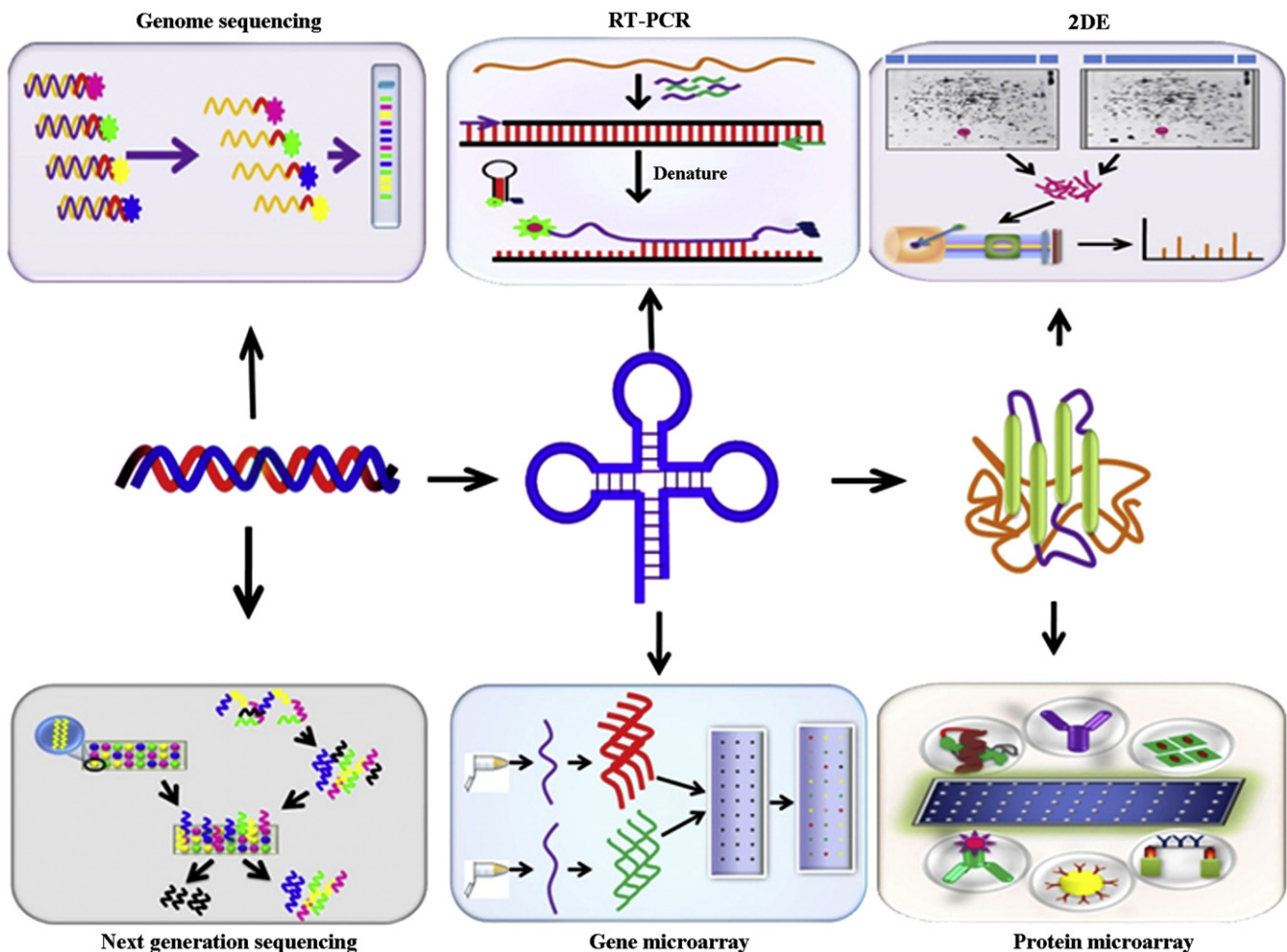


Fig. 1 – Transition from genomics to proteomics-advancements in ‘omic’ approaches for studying life processes at the molecular level. DNA, RNA and protein level analysis are commonly performed to study life processes at the molecular level. Molecular biology has experienced incredible advancements and versatile promising applications have been developed over the last decade, which have introduced new frontiers in genomics, transcriptomics and proteomics research. While long ago Sanger developed the DNA sequencing method using dideoxynucleotide triphosphates (ddNTPs), with the passage of time the high-throughput chip based next-generation sequencing technology has emerged and replaced the conventional approach. Similar technological revolution has been observed in transcriptome level research where gene microarray technologies have been emerged as high-throughput methods to study the whole transcriptome of an organism using the genome chip. Remarkable success in genome sequencing and advancement of gene microarray technology collectively initiated the route of proteomics in functional level investigation in a high-throughput manner. Two dimensional electrophoresis and mass spectrometry have played the prime role in proteome level studies, while high-density protein and antibody microarrays have recently emerged as high-throughput platforms for multiplex analysis.

1.2. Virtual Labs as web-based educational platform

E-learning has started making an impact in education across the world. It has been observed frequently that in traditional course curriculum, which involves the learning of theoretical concepts from textbooks without being able to see its practical application simultaneously, students find it difficult to understand the theory and also take longer to learn practical techniques in the lab. Furthermore, extended lab training is most often not feasible, due to the high level of expenditure associated with the establishment and maintenance of well-equipped labs. To overcome this situation, several academic institutions and research organizations alike, have started using E-learning resources in different disciplines including the biological sciences, as teaching materials. The end result has been the development of Web Courses, Video Lectures, Remote Triggered Labs and Virtual Labs [7]. Among these

versatile approaches, the Virtual Labs are found to be most effective, since they provide students an opportunity to learn practical techniques at their own pace in simulated environments designed to mimic actual lab conditions [8].

Various web courses in biology and biotechnology are already available and quite a few Virtual Labs have also been set up over the last few years to expand the E-learning platform in the biological sciences. Distance education programs have also begun using such resources as a part of their curriculum along with web lectures to make their course curriculum more interactive. Virtual Labs also prepare students for what they can expect in real labs without handling any hazardous materials, and are available round-the-clock for learning [9]. Since such facilities are easy to maintain after establishment, they help institutes to save valuable resources and make learning affordable even for those who cannot afford the expenses of higher education. Therefore, Virtual Labs hold the

Box 1

Definition of important proteomics related terminologies and terminologies.

Genome	The entire complement of hereditary material of an organism, which can transform information responsible for the identity, function and reproduction of organisms from generation to generations.
Proteome	The complete set of proteins in an organism complement to the whole genome and transcriptome at a given time and condition.
Genomics	The sub-branch of genetics to study the whole genome of an organism using high-throughput techniques such as sequencing.
Transcriptomics	The study of entire of RNA complement expressed at a particular time, including mRNA, tRNA, rRNA as well as the non-coding RNA of an organism.
Proteomics	The systematic study of the properties of all proteins expressed under a specific condition using high-throughput techniques.
Virtual Laboratory	A web-based educational platform where learners can obtain the experience of practical experimental procedures, devoid of any direct physical involvement in real bench works, using computerized models, videos, digital simulations and other instructional technologies.
Gel-based proteomics	High-throughput separation techniques for separation of proteins from complex mixtures using polyacrylamide gels. SDS-PAGE, 2DE and DIGE are the most commonly used gel-based techniques
2DE	A protein profiling technique that carries out separation using isoelectric focusing in first dimension, which separates proteins based on their isoelectric points (pI), followed by SDS-PAGE in the second dimension, which brings about further separation based on their relative molecular mass.
2D-DIGE	An advanced form of 2DE that allows simultaneous analysis of test and control samples on a single gel by carrying out differential labeling of each sample. This minimizes gel-to-gel variations and enables easy processing of large number of samples.
MALDI-TOF	MALDI is an efficient process for generating gas-phase ions of peptides and proteins for mass spectrometric detection. Accurate determination of the mass of the protein species present in the sample is achieved by the use of a time-of-flight (TOF) mass analyzer, which resolves ions based on their m/z ratios. The first TOF separates the ions generated by laser beam and the second one resolves the fragmented species generated by a collision chamber, which is present between the two TOFs.
Gene microarray	Gene microarray is the new revolution in genetics to study the whole expression profile of an organism using glass slides with immobilized cDNA constructs.
Protein microarrays	A valuable platform for functional proteome analysis that makes use of glass or polyacrylamide gel pad surfaces, onto which small quantities of thousands of proteins are simultaneously immobilized and analyzed.
Next-generation gene sequencing	New generation high-fidelity and high-throughput genome sequencing method used for a wide range of biological applications.
Real time-PCR	Method for simultaneous amplification and quantification of targeted gene expression using RNA or cDNA as a template.
SPR	Surface Plasmon Resonance (SPR) is a widely studied label-free technique for real time interaction studies by using immobilized target proteins on gold surface.
Molecular docking	A bioinformatics tool used to predict the active or binding site in a receptor and determine the orientation of a ligand in the binding site.
Homology modeling	Construction of the 3D structure of a protein with the help of primary amino acid chain by using the 3D structure of homologous protein as a backbone or template.
Sequence alignment	A method to determine the similarity in sequences of DNA, RNA or proteins to deduce their structural, functional and evolutionary relationship among diverse organisms.
MASCOT	Search engine to identify proteins based on the m/z values obtained from mass spectrometry techniques (http://www.matrixscience.com/search_form_select.html).

promise of making good quality practical coursework available to a much larger base of people than conventional on-campus education can hope to reach. Though practical e-learning approach cannot replace hands-on training in the biological sciences just yet, it can definitely make it easier for students to form an association between the textbook theory and practical lab experiments [10].

1.3. Virtual Labs for proteomics and related courses

Many modern educational systems have started integrating basic and applied proteomics as a part of their course curriculums since this emerging discipline has had an immense

impact on clinical and industrial research. To keep up with the pace of current trends and advancements in education system, academic institutions need to develop the necessary infrastructure that can provide practical training facilities to learners. Without adequate practical experience in the lab or a means to visualize the experiment and related instrumentation, it is extremely difficult for students to grasp the complicated concepts and working principles of advanced proteomic techniques like two dimensional gel electrophoresis (2DE), 2D-fluorescence difference gel electrophoresis (2D-DIGE), matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI TOF-MS), protein or antibody microarray, surface plasmon resonance (SPR), etc. (Box 1 and Fig. 2). In

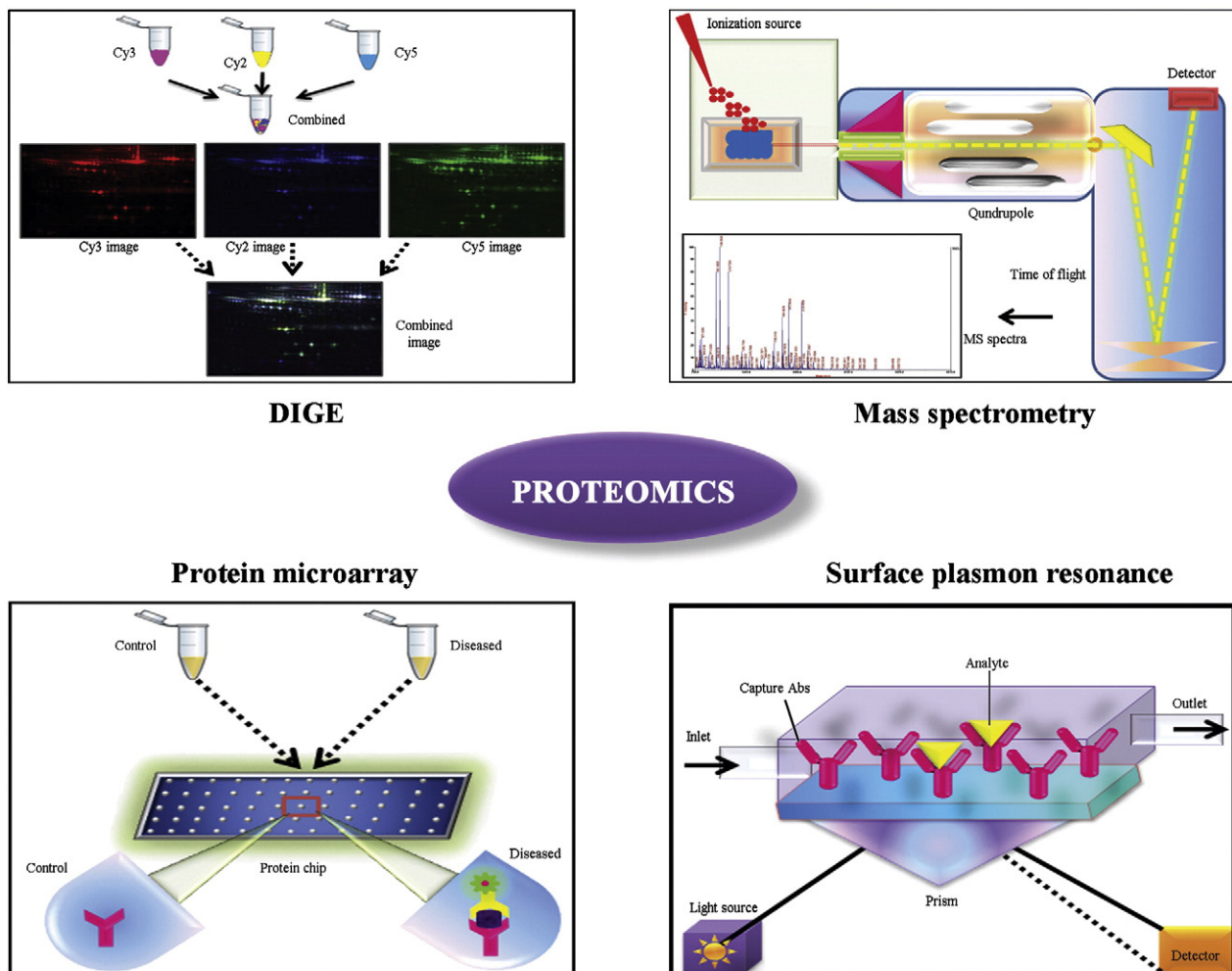


Fig. 2 – Advanced technologies for proteomics research: (A) 2D-DIGE: — an advanced form of 2DE that allows simultaneous profiling of test and control samples on a single gel by carrying out differential labeling of each sample using three different cyanine dyes (Cy2, Cy3 and Cy5). This minimizes gel-to-gel variations and enables easy processing of large number of samples. **(B) ESI-Q-TOF-MS/MS:** — MS/MS configuration that generates peptide ions using electrospray ionization (ESI), wherein ions are formed by spraying analyte solution at atmospheric pressure from the tip of a fine metal capillary generating a fine mist of droplets that become highly charged in the electric field. These ions are first separated using an oscillating electrical field in a quadrupole analyzer followed by further fragmentation in the collision cell and separation based on flight time in the second TOF analyzer. **(C) Protein microarray:** — a high-throughput approach where antigens or antibodies are printed at high density as capture agents for selective detection of target proteins from complex protein mixtures. **(D) SPR:** — a label-free, real-time detection platform commonly used to study protein–protein interactions by measuring changes in the refractive index of the medium directly in contact with sensor surface.

many countries, governments as well as private organizations have come forward to provide financial support to establish and maintain Virtual Labs and other E-learning tools for “omics-related” subjects. E-resources currently available for proteomics are spread out across different subject streams and not many in number. While, quite a few initiatives are in different stages of development, the Virtual Mass Spectrometry Laboratory of Carnegie Mellon and University of Pittsburgh [11], Annenberg Learner [12], Analytical Sciences Digital Library funded by the National Science Foundation [13] have started gaining popularity as virtual platforms that provide proteomics related E-learning contents (Table 1).

Similar initiatives have also been started in developing countries like India, to establish web-based educational platforms for protein chemistry and proteomics. As a part of the National Mission for Education through Information and Communication Technology (NME-ICT) — Sakshat Virtual Labs program, we have developed a Virtual Proteomics Lab at the Indian Institute of Technology Bombay (IITB). The Sakshat Labs [14] are one of the many open source web-based educational resources being set up by India’s Ministry of Human Resource and Development (MHRD) in an effort to take higher learning to the masses and make it available in every corner of the country. It is one of the foremost initiatives in developing countries for the establishment of web-based learning platforms for highly sophisticated, rapidly emerging disciplines of biological research. In this Tutorial, we will focus on the current status of different Virtual Labs and web resources available for proteomics study

globally, with a special emphasis on the Virtual Proteomics Lab recently developed by IIT Bombay. Prospects, merits and challenges for the development of such E-learning tools will also be discussed.

2. Basic concepts of Virtual Laboratory

A Virtual Lab in essence is the simulated model of a real laboratory. This virtual environment has none of the limitations faced in real classrooms or laboratories, due to safety protocols, limited time or access to lab resources and equipments brought on by budget constraints. This leaves the designers of these labs free to design dynamic course contents that combine important theoretical concepts with practical experimental protocols, which users can study and assimilate at their own pace. Furthermore, these labs are more economical to set up and maintain than traditional laboratories and classrooms.

Like all open source E-learning programs, VLabs are courses designed to explain practical concepts using interactive visual demonstrations supplemented with the corresponding theoretical basics. While this definition addresses the conceptual side of the design, there are a host of applications required to generate this content and make it digitally available and accessible. Simulators are required to create the interactive tutorials that give students a visual demonstration of techniques. Storage and application servers store lab contents such as videos, simulations, text files, assignments and additional reading materials and

Table 1 – Virtual Labs and E-learning sources for proteomics and related subjects.

Virtual Lab/E-learning resource	Associated institute(s)	Technologies discussed	URL
1. HHMI Biomedical Interactive Virtual Labs	Howard Hughes Medical Institute	ELISA	http://www.hhmi.org/biointeractive/vlabs/immunology/index.html
2. Virtual Mass Spectrometry Laboratory	Carnegie Mellon and University of Pittsburgh	Different types of mass spectroscopy	http://svmsl.chem.cmu.edu/vmsl/default.htm
3. MIT Open Course Ware	Massachusetts Institute of Technology	Bioinformatics and proteomics, antibiotics, toxins, protein engineering and protein folding	http://ocw.mit.edu/index.htm
4. Annenberg Learner	Annenberg Foundation	2DE, mass spectrometry, protein interaction analysis and microarrays	http://www.learner.org/courses/biology/textbook/proteo/index.html
5. Center for Cardiovascular Research — Molecular Biology	John A Burns School of Medicine, University of Hawaii — Center for Cardiovascular Research	SDS-PAGE, Western blotting, ELISA, protein microarrays	http://ccrhawaii.org/index.php/protein-techniques
6. Analytical Sciences Digital Library	University of Kansas, University of California — Riverside, University of Illinois Urbana-Champaign	Mass spectroscopy, ELISA, chromatographic techniques, NMR	http://www.asdlib.org/
7. J.P. Hornak NMR Tutorial	Rochester Institute of Technology	NMR	http://www.cis.rit.edu/htbooks/nmr/inside.htm
8. National Program for Technology Enhanced Learning	Joint Collaboration of several Indian Universities	Basic theory of proteomic technologies	http://nptel.iitm.ac.in/courses.php?disciplineId=102
9. Sakshat Virtual Biotechnology Engineering Labs (Virtual Proteomics Lab)	IIT-Bombay	2DE, MALDI-TOF MS, sequence alignment, homology modeling, protein function annotation, molecular Docking	http://iitb.vlab.co.in/?sub=41&brch=118
10. Solid State NMR Service	Durham University	Solid state NMR	http://www.dur.ac.uk/solid.service/information/

make these available to the user through access devices such as laptops and tablets. Lab management Software are required to organize the content into modules and experiments in the form of web pages with embedded media elements and applets [15] that have a structured flow which is user friendly and easy to understand.

Modern technology has made it easy to offer web based learning materials in the form of live web lectures and animations, simulations and video courses. Once set up, a Virtual Lab needs only minimal maintenance which saves money and time. The course material can be easily upgraded as and when necessary using the same lab platform. A static Virtual Lab has a simple architecture. Its development takes place in the following stages.

- Software programs for the preliminary input of the data.
- Software programs for the storage, processing, visualization and retrieval of the stored data.
- Incorporation of various external software environments for the advance facilities of video conferencing and simulation visualization.

The experts perform the experiments and make the videos and simulations of the protocols they follow for each experiment. Online content for each experiment consists of these results along with the theoretical basics, assignments and additional reading materials. Users and lab administrators are given different sets of access controls so that the lab can be protected from external tampering. The input data is stored on the server which can be accessed by the labs involved in the Virtual Lab construction. Users can access the information using a LAN/WAN connection through a standard web browser. The dialog part of the laboratory contains the different tabs that can be seen through this interface.

Virtual Biology Labs allow students to get acquainted with wet lab techniques while simultaneously understanding the theory associated with it. This adds a new dimension to regular teaching courses by making them more dynamic and interactive. However, in order to efficiently disseminate information to the user, the lab must have a proper pedagogical structure that makes learning easy and preferably adaptive. Courseware should be individualized so that it can provide material that fits the learner's learning style and current knowledge base [16]. While certain pre-requisite theoretical background is necessary to understand advanced concepts, the coursework should nonetheless, have a scheme that makes the content understandable to people from all specializations and not just limited to students of the field in question. Modules explaining different concepts should be organized so that students can easily correlate multiple concepts and apply these to assignments. All the material in a Virtual Lab should follow a predefined style that is easy to follow.

A majority of the existing Virtual Proteomics Labs are aiming at creating modules that cover the technical details and experimental simulations of high-resolution protein separation, identification and characterization technologies, commonly used in proteomics research (Box 1). Complex experiments and subsequent data analysis of results for some of these proteomics techniques, including two-dimensional gel electrophoresis (2DE), electrospray ionization, MS/MS analysis etc. have been

illustrated with examples in previously published Tutorials [17–19].

3. Current status of Virtual Labs and E-learning resources in spreading web-based proteomics education

Virtual Labs were initially created for the engineering and computer sciences; however, with passage of time, the growth of E-learning process has propelled them into many other disciplines. In consideration of their growing need, in recent years, significant attention has been dedicated for establishing similar web-based learning resources in different areas of the life sciences [20]. Even though Virtual Labs presently cannot replace hands-on training in proteomics and other biological fields, there is hope that as computer technologies progress, in the future Virtual Labs will be realistic enough to serve as substitutes for actual wet lab facilities [21]. Biotechnology Virtual Labs have been around for the last decade starting with the creation of the Howard Hughes Medical Institute (HHMI) BioInteractive labs, one of the first Virtual projects solely dedicated to biology [22]. Proteins are the most important biomolecules in living organisms and therefore, proteomics is now an integral part of biomedical research; for that reason, several online programs have started course-work related to proteins. Commonly used gel and MS-based proteomics techniques are generally included as a part of academic courses where they address specific problems such as drug discovery, biomarker detection or sample screening for toxins, enzyme inhibitors, etc. As discussed earlier, since these resources are spread out across different subject streams, it was not possible to include all the Virtual Labs related to the subject but the prominent initiatives in the field have been discussed in this section (Table 1).

3.1. Virtual Mass Spectrometry Laboratory — Carnegie Mellon and University of Pittsburgh

Virtual Mass Spectrometry Laboratory (VMSL) is an Internet based educational tool that teaches students four different methods of mass spectroscopic analysis using case studies. Students are allowed to learn the overall procedure and solve the assignments to complete the modules. The four tutorials cover matrix-assisted laser desorption ionization time of flight (MALDI-TOF), electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), gas chromatography-electron ionization/chemical ionization and electron impact/chemical ionization (EI-CI) magnetic sector mass spectrometry. The experiments require students to collect MS data and analyze it to solve the given problems [11]. Since, MS is one of most fundamental technical platform for proteomic research; this E-learning platform is very attractive for community interested to gain experience in chromatographic and MS analysis.

3.2. Annenberg learner

Created by the Annenberg Foundation, the aim of this program is professional development of K-12 teachers in American schools. The foundation uses media and telecommunications

to take this program to the public. The online database consists of a host of educational videos and written programs, with a section devoted to biology. This section contains a chapter on proteomics that covers all major proteomics techniques such as 2DE, mass spectrometry, protein interactions and microarrays among others [12].

3.3. John A Burns School of Medicine, University of Hawaii — Center for Cardiovascular Research

This E-learning resource provides instructional programs for prospective individuals who want to become molecular biology researchers. It focuses on DNA, protein and cell culture laboratory techniques. The protein module has sections covering SDS-PAGE, western blot, ELISA and protein microarrays [23].

3.4. Analytical Sciences Digital Library

This digital library funded by the National Science Foundation is a collection of cataloged and annotated web based materials that can be useful in curricular development and provide technical resources for the analytical sciences. The site offers links to many Virtual Labs and E-learning resources available on the web, for a variety of topics such as immunology, mass spectrometry, ELISA, chromatography and NMR, among others. Each web resource in the main collection of ASDL includes a detailed annotation describing the site and its useful attributes. It is a joint project between the University of Kansas, the University of California — Riverside, and the University of Illinois Urbana-Champaign, and is one of several collections funded by NSF's National Science Digital Library [13].

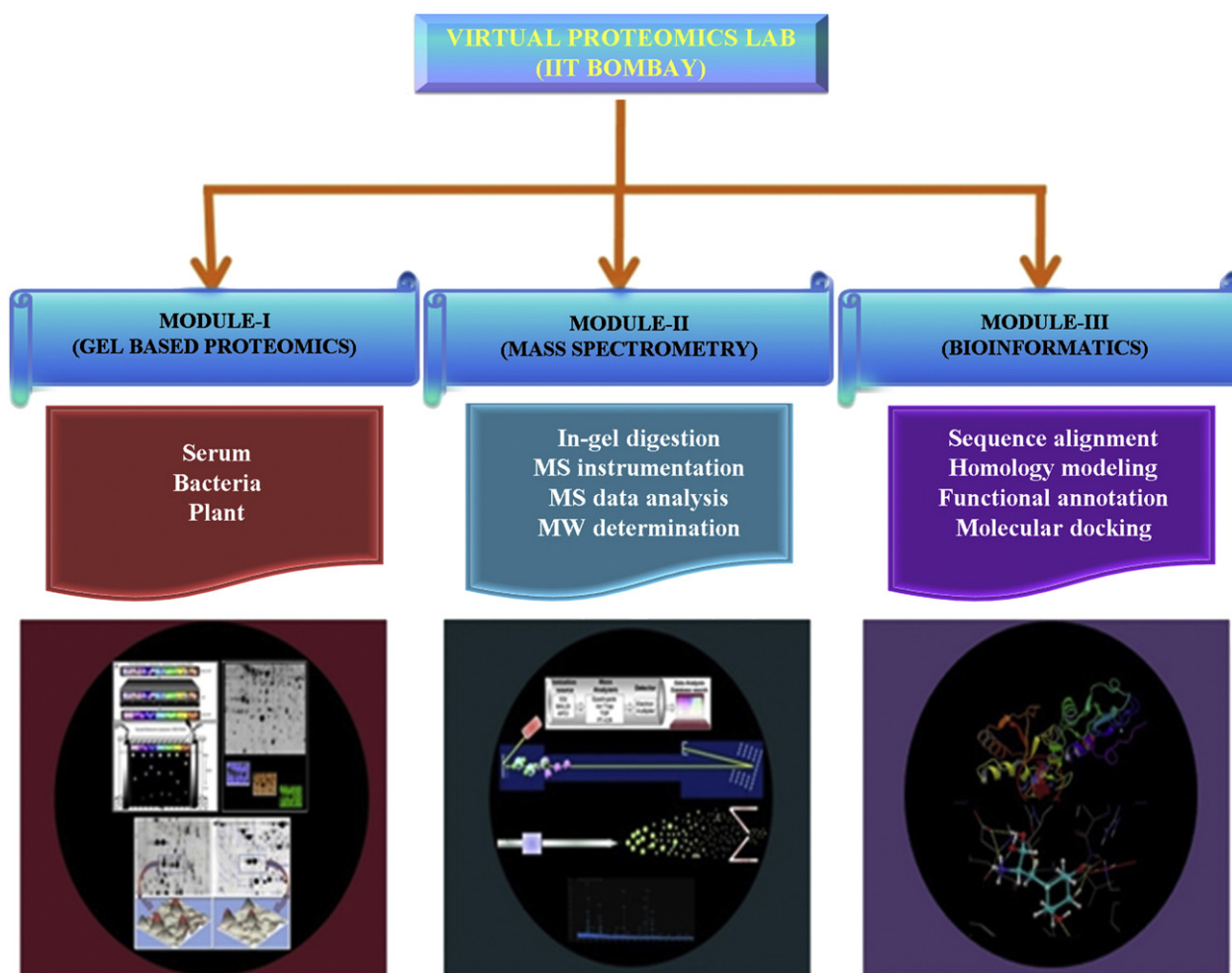


Fig. 3 – Virtual Proteomics Laboratory (VPL) set-up of IIT-Bombay. A static lab completely dedicated to proteomic technologies, comprised of 12 experiments organized into three modules (<http://iitb.vlab.co.in/?sub=41&brch=118&sim=785&cnt=1372>). Module I (An overview of Gel-based Proteomics) consists of three experiments: Gel-based proteomics to analyze human serum, bacterial, and plant proteome. Module II (An overview of MALDI-TOF MS) is dedicated to MS-based proteomics and consists of five experiments: In-gel digestion of proteins for MS analysis, Sample preparation for the MALDI-TOF MS analysis, MALDI-TOF instrumentation and analysis of serum proteins, MS data analysis — peptide mass fingerprinting (PMF), and Molecular weight determination of intact proteins. Module III (An overview of Bioinformatics) covers the bioinformatics tools which are commonly used in proteomics. This module includes four experiments: Sequence alignment, Homology modeling, Functional annotation and Molecular docking.

3.5. The ELISA BioInteractive Virtual Lab — Howard Hughes Medical Institute (HHMI)

The E-learning series at HHMI consist of lectures, videos, animations and Virtual Labs in a number of biology subjects. The ELISA lab is a part of HHMI's Virtual Lab repository and discusses the basics of humoral immunity, rationale behind ELISA and the specifics of the ELISA test [24]. Since, this immunoassay-based approach is often applied to validate the findings of proteomic studies; it is important for the learners to gain experience in ELISA and other immunological assays.

3.6. Rochester Institute of Technology — J.P. Hornak NMR Tutorial

This NMR E-learning module explains the basic principles of NMR, the components of NMR hardware, practical considerations necessary during operations and advanced spectroscopic techniques along with practice problems to solve at the end of the module [25]. This is one of the most attractive E-learning platforms for learning NMR, which is considered as an important technology for structural proteomics research.

3.7. MIT — Open Course Ware (OCW)

This comprehensive set of E-courses is the online version of all courses currently being taught at the Massachusetts Institute of Technology (MIT). Related courses on proteins including bioinformatics and proteomics, protein engineering and the protein folding are part of this repository [26].

3.8. Sakshat Virtual Biotechnology Engineering Labs

A joint collaboration of several universities in India, the Sakshat Labs are India's first comprehensive set of VLabs under the NME-ICT (National Mission for Education through Information and Communication Technology) initiative of the Ministry of Human Resource Development (MHRD). Set to be deployed in 2012, the project is a mixture of static and remotely triggered labs in the engineering and science faculties that allow students to perform experiments in video simulations and using remotely triggered instruments, respectively. The labs have a streamlined appearance and follow a step by step method of explanation. There are several Virtual Labs in this project dealing with proteomics, protein chemistry and other related disciplines of biological science [14].

Virtual Labs for proteomics are currently in the early stages of developments, and are often developed as a small part of E-learning modules dealing with other disciplines of biological research. As far our knowledge goes, hitherto there is no existing Virtual Lab dedicated solely to online proteomics education as yet, except ours. Therefore, we are anticipating that more initiatives will be taken in near future to develop independent Virtual Labs for proteomics and related disciplines due to their promising applications in many areas, especially in human health and economic improvement.

4. Worked example — The Virtual Proteomics Laboratory at IIT Bombay

As a part of Sakshat Virtual Labs program funded by the Ministry of Human Resource Development (MHRD) under the NME-ICT initiative, we have developed a Virtual Proteomics Laboratory at the IIT Bombay [14,27]. It is the first initiative in India for the establishment of a web-based learning platform in the area of proteomics. This is one of the few comprehensive proteomics labs, where all the experiments focus on different proteomic techniques associated with protein separation, identification and analysis such as 2DE, MALDI TOF-MS and molecular docking among others [14]. It is comprised of 12 experiments organized into 3 modules (Fig. 3; Figs. S1, S2 and S3), where every experiment is explained in a step-by-step manner that follows the order of the tabs: theory, experimental procedure, simulator, video, download, assignment, quiz, references and feedback. The modules for proteomics experiments are organized such that the experiments take students from theory to procedure to self-evaluation, in a step-wise manner.

Our overall Virtual Proteomics Lab is divided into three modules; an overview of Gel-based Proteomics (Module I), MALDI-TOF MS (Module II) and Bioinformatics (Module III), each with their own set of experiments (Fig. 3). The first module mainly deals with proteome separation and profiling using classical 2DE technology. The rationale behind 2DE is provided to make the learners familiar with basic concept and working principle of this proteomic technology. The experimental section comprehensively explains the details of protein extraction, sample preparation and the subsequent 2DE separation procedure for three most commonly studied samples: Human serum proteome to study host response under diseased condition, *Escherichia coli* proteome to investigate the mechanism of drug action and plant leaf proteome to test the effect of stress conditions on plant physiology (Fig. S1). The second module describes the identification of in-gel digested or intact proteins by MALDI-TOF mass spectrometry (Fig. S2). Students first learn how to perform in-gel digestion and extract proteins from the polyacrylamide gels and then process them prior to the MS analysis. Learners are then taken through the details of MALDI-TOF MS instrumentation by using interactive animations, videos and data analysis procedures. The third module explains various bioinformatics methods that can be used for protein chemistry and proteomic analysis. The module covers four important concepts: sequence alignment, homology modeling, protein function annotation and molecular docking (Fig. S3). Sequence alignment covers both pair-wise and multiple alignment. The experiment provides an introduction to the types of alignment, the criteria used to generate alignment, the file formats of sequences required for alignment and the types of results obtained. The simulation is split into two parts: protein sequence retrieval from Uniprot and sequence alignment. Homology modeling is the process of predicting a protein's three-dimensional structure, using other known proteins as templates. The docking methods and the procedure for the same are explained in this experiment.

In each module, the interactive simulation and live experimental videos follow a similar pattern. The downloads

section is a compilation of .pdf files of all the written material, jpg files of the gel pictures/MS spectra and the procedure, power point presentations for the assignments and flash videos of the video tutorials. The assignments are based on individual experiments and the quizzes to evaluate students. Simulations and power point presentations are provided for each experiment that explain the procedure and the results obtained. Individual quizzes and assignments are also provided for each experiment. Additional reference materials including research publications and text books are also mentioned as further reading resources. Presently, this Virtual Lab is available at <http://iitb.vlab.co.in/?sub=41&brch=118> [28], and it will be deployed via the Sakshat Labs website in 2012. This project is part of the second wave of open source E-learning resources being created by the MHRD, India. Currently our group is also developing a Remote-triggered Clinical Proteomics Lab funded by the MHRD project, where the learners will be allowed to use sophisticated equipments associated with proteomics directly from remote places through dedicated software and operating systems. These web-based educational platforms for advanced scientific disciplines such as proteomics will be beneficial worldwide and certainly be very cost-effective in developing countries like India.

5. Current limitations and working limits

Incorporation of proteomics and other related topics in course curriculums of educational institutions has propelled the growth of this rapidly flourishing discipline in web-based educational approaches. By virtue of several worldwide initiatives over the last decade, several Virtual Labs and other E-learning resources have been generated to support proteomics learning among various research groups as well as in graduate and undergraduate curriculum. Even so, it should be emphasized that as compared to other disciplines, development of Virtual Labs for proteomics is more challenging, since many of the experimental procedures in this field are very complex and difficult to explain precisely, with only use of simulations.

Establishment of good quality Virtual Labs requires the use of simulation software that are capable of creating highly realistic virtual environments that can give learners an idea of how experiments would be carried out in actual labs. Such programs could strengthen a student's concepts through repeated practice but nonetheless, there are many intricacies that can only be comprehended through practical experience. This is especially true in the case of the biological and chemical sciences, because the handling of chemicals and biological samples cannot be completely taught through simulations, as each person has a different level of understanding in such situations. Additionally, even with simulation exercises, the knowledge gained by the student is limited by the constraints and capabilities of the simulation software used [7]. Furthermore, very often the experimental procedures in real proteomics laboratories are beyond the easy step-by-step protocols, and the overall outcome of the experiment is dependent on the technical skill of performer, which cannot be gained

online learning alone. For instance, running a good quality two-dimensional gel, involves multiple steps and a high level of technical skill, which can be achieved only through continual hands-on practices. Nonetheless, Virtual Labs do allow students to perform experiments without the risk of exposure to any hazardous materials or direct handling of sensitive, sophisticated instruments. But students should also be made precisely aware of the required precautions, so that they can execute the experiments in real scenario in future without violating safety issues.

6. Future developments

Worldwide success in basic and applied proteomics research over the last two decades has attracted the attention of industries and academia. To make students and professionals familiar with the basic working principles of different proteomic techniques, as well as the current status and technical advancements in this promising field, various educational institutions have begun introducing proteomics into their course curriculums. Consequently, in recent years, considerable attention has been devoted globally to develop open-source proteomics E-learning resources so that at least the principles and practical concepts associated with different proteomic techniques will be available to the students and professionals who do not have access to laboratory set-ups for the same. But a consolidated approach is necessary to make these materials easily available to the scientific community. The Annenberg learner is a good resource for basic proteomics and the ADSL library contains modules from several different resources that deal with many proteomics techniques. The Virtual Proteomics Laboratory of IIT Bombay, on the other hand is completely devoted to proteomics techniques. Despite this, web-based education for proteomics is still in the nascent stages of development. Although, a number of initiatives have been undertaken to establish Virtual Labs for commonly used techniques like 2DE and MS-based quantitative proteomics (Table 1), nonetheless, a majority of the advanced proteomic techniques like 2D-DIGE, gel-free quantitative proteomic approaches like stable isotope labeling with amino acids in cell culture (SILAC), Isobaric tags for relative and absolute quantitation (iTRAQ), microarrays, label-free techniques and ultra-sensitive nanoproteomic technique-based Virtual Labs remain untouched. Presently, nearly all of the existing Biology Virtual Labs include proteomics only as a part of the overall course content rather than devoting complete courses to it. Therefore, there may be a significant wait before ideal, comprehensive Proteomics Virtual Labs are established. This is one of the reasons why the Virtual Proteomics Lab at IIT Bombay has been developed. And even though it doesn't feature all the important proteomics techniques presently, there are ongoing efforts to expand this endeavor to include other advanced proteomic techniques in the near future. We anticipate that these Virtual Labs including ours, will serve as a blueprint for future Virtual proteomics Lab endeavors at the global level.

Supplementary data to this article can be found online at [doi:10.1016/j.jprot.2012.03.014](https://doi.org/10.1016/j.jprot.2012.03.014).

Acknowledgments

The financial support from the Ministry of Human Resources and Development (MHRD) for the establishment of Virtual Proteomics Laboratory 10MHRD005 and Clinical Proteomics Remote Triggering Virtual Laboratory 11MHRD005 is gratefully acknowledged. Contributions of Karthik Kamath, Harini Chandra, Khaja Shaik, Seshu Mallipudi, Rajneesh Srivastava, Renisa D'Souza, Rekha Jain, Subhamoy Das and Meeti Soni in content development; and technical support rendered by Prof. Bipin Nair, Shyam Diwakar (Amrita University, Kerala) and Santosh Noronha (IIT Bombay, Mumbai) in development of this Virtual Proteomics Laboratory is gratefully acknowledged.

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² *Key major reviews.

³ ##Entry-level guides into the subject.



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