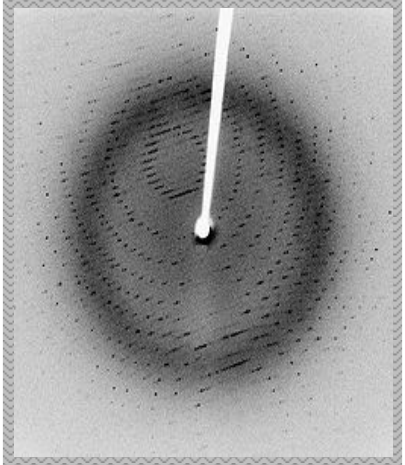


EMGEN Newsletter

Vol. 4, Issue 1, Nov-Dec., 2010

INSIDE THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMHGBN) was created in 2004 with collaboration of representatives of selected center of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO.

Address:
Biotechnology building, #69, Pasteur Ave., Pasteur Institute of Iran
Tehran, Iran, 13164
Tel: +98-21-66954324
Fax: +98-21-66465132
E-mail: emhgbn@gmail.com, secretariat@emhgbn.net
Website: www.emhgbn.net

Prepared by: Shiva Shajari
Page design: Shiva Shajari
Editor: Dr. Soroush Sardari

Article

Proteomic identification of human serum biomarkers in diabetes mellitus type 2

Dr. Samreen Riaz

The paper entitled: Genetic studies of Proteomic identification of human serum biomarkers in diabetes mellitus type 2., which is published in J Pharm Biomed Anal. 2010 Apr 6;51(5):1103-7, describes the levels of protein biomarkers specific to diabetes mellitus type 2 and effect of high dose thiamine on these levels. The study was carried out by Samreen Riaz , Saadia Shahzad Alam , and M. Waheed Akhtar . Corresponding Author of this paper is Dr. Samreen Riaz from School of Biological Sciences, University of the Punjab, Lahore. Pakistan and Department of Biochemistry and Molecular Biology, Institute of Structural and Molecular Biology, Bioscience Division, University College London, UK.

Diabetes mellitus is a chronic disease due to inherited and/or acquired deficiency in production of insulin by the pancreas, or by ineffectiveness of the insulin produced. Protein biomarkers are practical for diagnosis and prognosis of many disease states like diabetes mellitus, different type of cancer and other diseases. Certain proteins can be up- or down-regulated during disease progression. Detection of these differences in protein expression levels, as a function of disease progression is essential for prognosis.

During the proteomic era, one of the most rapidly growing areas in biomedical research is biomarker discovery, particularly using proteomic technologies. The proteomics is known to be a valuable field of study and has become one of the most attractive sub-disciplines in

clinical proteomics for human diseases. In the present research work, the levels of protein biomarkers specific to diabetes mellitus type 2 in the Pakistani population using proteomic technology have been identified and characterized and effect of high dose thiamine has been seen on the levels of these marker proteins.

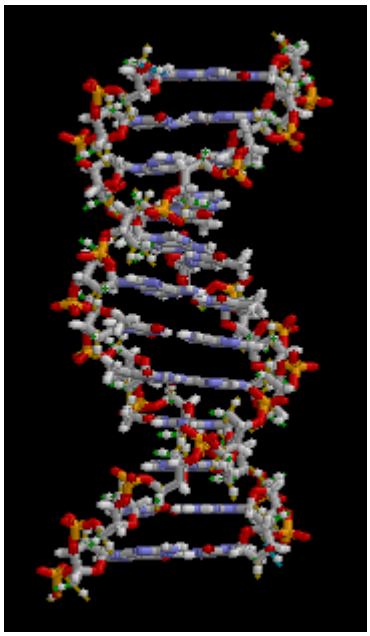
Above 125 type 2 diabetic patients, and 50 same age and sex-matched normal healthy controls were recruited from the Sheikh Zayed Hospital, Lahore, Pakistan. Total serum/plasma and urinary proteins were estimated and analyzed initially by different protein assays and 1-D SDS polyacrylamide gel electrophoresis. The samples were purified further by passing through the high abundance protein removal and desalting columns. These serum and urine samples from control and diabetic groups before or after thiamine therapy were further analysed by 2-D liquid chromatographic system in which samples were initially fractionated by chromatofocusing and the selected fractions were further analysed by reverse-phase high performance liquid chromatography. The proteins which showed variation between test and control samples were identified by mass spectrometry MALDI TOF/TOF and LC MS analysis.

All the samples belonging to the control and diabetic groups were then analyzed by ELISA and estimated the levels of some proteins which were found to vary. In the serum samples, the levels of apolipoprotein A-I was found to decrease by 6.4 % while apolipoproetin-E, leptin and C reactive protein (CRP) were increased by 802, 842 and 872 %, respectively in the diabetic patients as compared to the controls.

Elevated levels of CRP, apolipoprotein and leptin were found to be a powerful independent risk determinant. Apolipoprotein A-I levels also was down regulated among diabetics. Our data further consolidates the evidence that CRP, apolipoproteins and leptin as protein biomarkers for diabetes mellitus type 2. The discovery of these marker proteins might provide an adjunctive method for early detection of risk for this disease.

Gene Therapy

Gene therapy is the insertion of genes into an individual's cell and biological tissues to treat disease, such as cancer. In this method faulty mutant alleles are replaced with functional ones.



Gene therapy may be classified into the two following types:

- Germ line gene therapy

In this method, germ cells, i.e., sperm or eggs are modified by the insertion of functional genes. Therefore, the change due to therapy would be heritable and would be passed on to later generations. This new approach, theoretically, should be highly effective in treatment of hereditary diseases. However, many jurisdictions restrain this for application in human beings, at least for the present, for a variety of technical and ethical reasons.

- Somatic gene therapy

In the case of somatic gene therapy, the therapeutic genes are inserted into the somatic cells of a patient. The treatment will be restricted to the individual patient only, and modifications will not be inherited by the patient's offspring or later generations.

- The abnormal gene could be repaired by selective reverse mutation to get its normal function.

The approaches for correcting faulty genes:

- A normal gene may be transferred into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common.
- An abnormal gene could be replaced by a normal gene through homologous recombination.
- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

How does gene therapy work?

In most gene therapy studies, a "normal" gene is transferred into the genome to replace an "abnormal," disease-causing gene. Vector is a carrier molecule which, must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically modified to carry normal human DNA. Encapsulating and delivering genes to human cells is the most basic functions of viruses. Scientists have tried to use this capability and manipulate the virus genome to replace disease-causing genes by therapeutic ones.

Patient's target cells are infected with the viral vector. The vector then unloads its genetic material involving the therapeutic human gene into the target cell. The generation of a functional protein product from the therapeutic gene changes the target cell to a normal state.

Some of the different types of viruses used as gene therapy vectors:

- Retroviruses
- Adenoviruses
- Adeno-associated viruses
- Herpes simplex viruses

Non-viral methods

The most important advantages non-viral methods are simple large scale production and low host immunogenicity. Several methods of non-viral gene therapy are presented below:

Naked DNA

This is the simplest method of non-viral insertion. naked DNA plasmid is transferred to the target cells; however, the expression has been very low in comparison to other methods.

Oligonucleotides

The synthetic oligonucleotides are used to inactivate the faulty genes. There are several approaches by this method. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Second strategy uses small molecules of RNA called siRNA to signal the cell to adhere specific unique sequences in the mRNA transcript of the faulty gene, obstructing translation of the faulty mRNA, and therefore expression of the gene. Another strategy uses double stranded oligodeoxynucleotides as a decoy for the transcription factors that are required to activate the transcription of the target gene. The transcription factors bind to the decoys instead of the promoter of the faulty gene, which reduces the transcription of the target gene, lowering expression.

Lipoplexes and polyplexes

lipoplexes and polyplexes are used to protect the DNA from undesirable degradation during the transfection process.

Plasmid DNA can be surrounded with lipids in an organized structure like a micelle or a liposome. When the organized structure is combined by DNA it is called a lipoplex. There are three types of lipids, anionic (negatively charged), neutral, or cationic (positively charged). Complexes of polymers with DNA are called polyplexes.

Hybrid methods

Hybrid methods combine two or more techniques. Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone. Other methods involve combination of other viral vectors with cationic lipids or hybridising viruses.

Dendrimers

A dendrimer is a highly branched macromolecule with a spherical shape. The surface of the particle may be formulated in many ways and many of the properties of the resulting construct are determined by its surface. A new dendrimer called "Priostar" can be specifically functionalized to carry a DNA or RNA payload that transfects cells at a high efficiency with little or no toxicity.

Problems and ethics

Some of the problems of gene therapy include:

- Short-lived nature of gene therapy –Problems with inserting therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term treatments.
- Immune response –The risk of stimulating the immune system is always a possibility. Furthermore, the immune system's enhanced response to invaders makes it difficult for gene therapy to be repeated in patients.
- Problems with viral vectors –There are some potential problems with viral vectores, for example toxicity, immune and inflammatory responses, gene control and targeting issues. In addition, the viral vector, once inside the patient, may recover its ability to cause disease.

- Multigene disorders –Unfortunately, some disorders, like heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes.
- Chance of inducing a tumor (insertional mutagenesis) - If the DNA is inserted in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor.

Deaths have occurred due to gene therapy, including that of Jesse Gelsinger**.**

A few common examples for gene therapy:

Disease	Genetic Defect
hemophilia A	absence of clotting factor VIII
cystic fibrosis	defective chloride channel protein
muscular dystrophy	defective muscle protein (dystrophin)
sickle-cell disease	defective beta globin
hemophilia B	absence of clotting factor IX
severe combined immunodeficiency (SCID)	any one of several genes fail to make a protein essential for T and B cell function

Sources:

www.wikipedia.com

www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml

http://en.wikipedia.org/wiki/File:ADN_animation.gif

Mass spectrometry

Mass spectrometry (MS) is an analytical technique that used for determining of the elemental composition of a sample and its chemical structures of molecules, such as peptides and other chemical compounds. The MS principle contains ionizing chemical compounds to produce charged molecules or molecule fragments and evaluation of their mass-to-charge ratios.

How a mass spectrometer works

If something is moving and you subject it to a sideways force, instead of moving in a straight line, it will move in a curve - deflected out of its original path by the sideways force.

Imagine you wanted to deflect a cannonball as it went by you. All you've got is a jet of water from a hose-pipe that you can squirt at it. Frankly, it will hardly be deflected from its original course because the cannonball is so heavy.

But suppose instead, you tried to deflect a table tennis ball moving at the same speed as the cannonball using the same jet of water. Because this ball is so light, you will get a huge deflection.

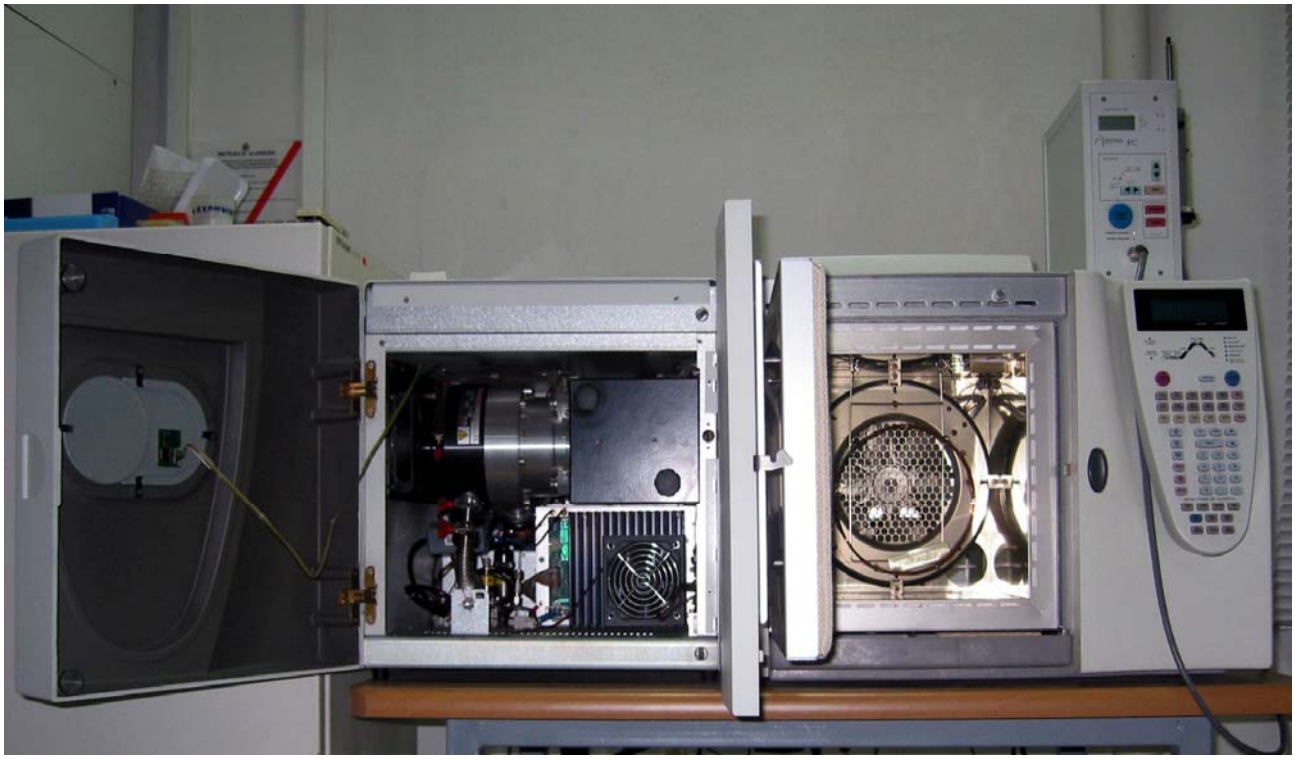
Picture of electrospray needle

The amount of deflection you will get for a given sideways force depends on the mass of the ball. If you knew the speed of the ball and the size of the force, you could calculate the mass of the ball if you knew what sort of curved path it was deflected

through. You can apply exactly the same principle to atomic sized particles.

In a typical MS procedure:

1. A sample is injected into the MS instrument, and vaporized.
2. The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam) to produce charged ions.
3. The ions are divided according to their mass-to-charge ratio in an analyzer by electromagnetic fields.
4. The ions are detected, usually by a quantitative method.
5. The ion signal is processed into mass spectra.



The insides of the GC-MS, with the column of the gas chromatograph in the oven on the right.

MS instruments consist of three modules:

- An *ion source*, which can transform gas phase sample molecules into ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase)
- A *mass analyzer*, which sorts the ions by their masses by applying electromagnetic fields
- A *detector*, which measures the value of a signal quantity and thus provides data for calculating the abundances of each ion present

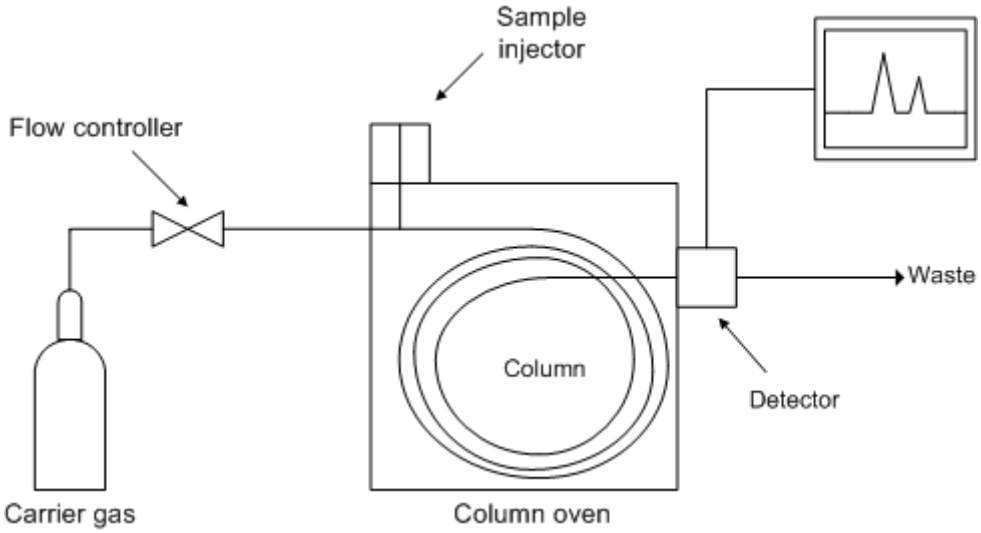


Diagram of a gas chromatograph.

The technique has both qualitative and quantitative uses. These contain identifying unknown compounds, determining the isotopic composition of elements in a molecule, estimating the structure of a compound by observing its fragmentation and assessing the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). Today MS is very common in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

Chromatographic techniques combined with mass spectrometry

An important achievement to the mass determining capabilities of mass spectrometry is using it in tandem with chromatographic separation techniques.

Gas chromatography

A common combination is gas chromatography-mass spectrometry (GC/MS or GC-MS). In this technique, a gas chromatograph is used to split different compounds. A metallic filament radiates electrons which ionize the compounds into the ion source. The ions can then further fragment, yielding predictable patterns. Intact ions and fragments pass into the mass spectrometer's analyzer and are detected.

Liquid chromatography

Similar to gas chromatography MS (GC/MS), liquid chromatography mass spectrometry (LC/MS or LC-MS) splits compounds chromatographically before they are introduced to the ion source and mass spectrometer. In this technique the mobile phase is liquid, usually a mixture of water and organic solvents, instead of gas. Most regularly, an electrospray ionization source is used in LC/MS.Also some newly developed ionization techniques like laser spray are used in LC/MS.

Ion mobility

In Ion mobility spectrometry/mass spectrometry (IMS/MS or IMMS) ions are first divided by drift time through some neutral gas under an applied electrical potential gradient before being introduced into a mass spectrometer. The IMS is lesstime consuming rather than liquid chromatography or gas chromatography separations and thus it can be coupled to these techniques

and producing triple modalities such as LC/IMS/MS.

Applications

Isotope ratio MS: isotope dating and tracking- Mass spectrometry is used to determine the isotopic composition of elements in a sample.

Trace gas analysis- Mass spectrometry could be used for trace gas analysis of air, breath or liquid headspace.

Atom probe- An atom probe is an instrument that used to map the location of individual atoms. It combines time-of-flight mass spectrometry and field ion microscopy (FIM).

Pharmacokinetics- Mass spectrometry is often used in pharmacokinetics researches because of its high sensitivity for microdosing studies.

Protein characterization- Mass spectrometry is a useful method for the characterization of proteins.

Glycan Analysis- Mass spectrometry (MS) is used in glycobiology for characterization and clarification of glycan structures.

Space exploration- As a standard method for analysis, mass spectrometers are used in space exploration to analyzed atmospheric samples.

Respired gas monitor- Mass spectrometers were used in hospitals for respiratory gas analysis in 70s, 80s and 90s. Some are maybe still in use but none are currently being manufactured.

Source:

http://www. Wikipedia.com
http://en.wikibooks.org/
http://www.chemguide.co.uk/analysis/masspec/howitworks.html
http://www.nelabgear.co.uk/used_lab_equipment/gc_mass_spec.asp

Scientists have discovered the signals that control size of the nucleus.

Nuclear size varies in different species, different types of cells in the same species and at different times during development. Also cancer cell's nucleus becomes larger as they become more malignant.

"Pathologists look at nuclear size in cancer cells for staging different cancers, but nobody knows what is behind this," said Rebecca Heald, professor of molecular and cell biology at the University of California, Berkeley.

As a result, she and post-doctoral fellow Daniel L. Levy discovered why the nuclei of two species of African clawed frog are so different in size and recognized that the large nucleus of *Xenopus laevis* sucks in more material while growing than does the small nucleus of *Xenopus tropicalis*.

They tracked down the proteins streaming into the nucleus and found that they were importing structural material used to build the web of lamin proteins that shores up the inside of the nuclear shell.

They found that another protein sits like a plug at the entrances to the nuclear envelope -- the nuclear pore complexes -- to slow the importation of large proteins. Together, these two different proteins -- the importing protein, importin-alpha, and the gatekeeper, ntf2 -- account for the difference in size between the nuclei of the two frogs.

"The different levels of these two factors are sufficient to account for our nuclear size differences," Levy said.

"There was a lot more importin-alpha and a lot less ntf2 in Xenopus laevis, and we

found out that we can convert *Xenopus tropicalis* into laevis just by adding excess importin and partially getting rid of ntf2 in egg extracts," Heald said. "We thought this could be really complicated, but it isn't.

"Now that we understand some of the mechanisms that regulate nuclear size, we can try to decrease nuclear size in cancer cells and ask, does the cancer cell care? Maybe it will and maybe it won't."

She and Levy used test tubes filled with the guts of hundreds of frog eggs. The guts contain cell-free cytoplasm derived from the cellular envelopes by centrifuging. By adding new proteins or antibodies that block existing proteins, many pathways inside the cell can be discovered.

They also applied the technique from *X. laevis* to the smaller *X. tropicalis*, whose eggs have about one-fifth the volume. Difference in size is due to the fact that *X. laevis* is tetraploid -- it has four copies of nearly every chromosome -- however *X. tropicalis* is diploid, with two copies of each chromosome. Working with extracts allowed the researchers to find essential proteins which are varied in concentration between the two species and thereby track down the mechanism of nuclear inflation.

"We can now ask physiologically what happens when you change nuclear size," Heald said. "If you make the nucleus bigger, does it become more cancer-like? How related are these two phenomena, cancer and nuclear size, which are associated but with no real causal effect."

Levy and Heald report their findings in the Oct. 15 issue of the journal *Cell*.

Source:

www.sciencedaily.com, posted on *Oct. 18, 2010*

Insect Virology

Publisher: Caister Academic Press

Editors: Sassan Asgari and Karyn N. Johnson

School of Biological Sciences, The University of Queensland, St Lucia QLD 4072, Australia

Publication date: September 2010

ISBN: 978-1-904455-71-4

Price: GB £180 or US $350 (hardback).

Pages: xii + 436 (plus colour plates)

Insect Virology

Written by internationally well known insect virologists, chapters focus on the current molecular biology of all the major groups of insect pathogenic viruses and present future directions for research. The book is divided into three sections: 1) DNA viruses 2) RNA viruses and 3) current hot-topics in insect virology. Virus groups considered include: Ascoviruses, Baculoviruses, Densoviruses, Entomopoxviruses, Hytrosaviruses, Iridoviruses, Nudiviruses, Polydnaviruses, Dicistroviruses, Iflaviruses, Nodaviruses, Tetraviruses and Cypoviruses. The special topics chapters review recent developments in insect virology including RNAi, insect antiviral responses, structural comparison of insect RNA viruses, and viral ecology. The book is recommended for every insect virologist and other virologists, particularly those interested in virus evolution, virus structure, viral vectors, biological control of insects and insect immunity.

Book Alert

Vaccine Design: Innovative Approaches and Novel Strategies

Publisher: Caister Academic Press

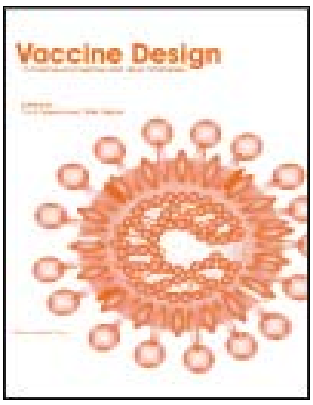
Editor: Rino Rappuoli and Fabio Bagnoli *Novartis Vaccines and Diagnostics, Research, 53100 Siena, Italy*

Publication date: February 2011

ISBN: 978-1-904455-74-5

Price: GB £180 or US $350 (hardback).

Pages: xii + 380 (plus colour plates)



Vaccine Design: Innovative Approaches and Novel Strategies

In this book, expert international authors critically review the current cutting-edge research in vaccine design and development. Particular emphasis is given to new approaches and technologies. The book has been divided into two parts. The first part reviews the technologies and approaches used to identify, generate and test new vaccines. Topics include: new strategies to identify protective antigens, generation of improved adjuvants, use of alternative immunization routes, improving vaccine safety, and finding and establishing the correlates of protection. The second part of the book focuses on the development of new vaccines to replace or complement currently available products or for diseases against which prophylactic strategies are missing. Examples include vaccines against nosocomial infections, streptococci, emerging viral diseases, P. aeruginosa, and bovine mastitis. Essential reading for everyone with an interest in vaccine R & D.

Announcement

Eastern Mediterranean Health Genomics and Biotechnology Network (EMGEN)

The Second EMGEN Steering Committee Election

We are pleased to announce you that the Second EMGEN Steering Committee Election will be held during 4 December 2010 to 18 December 2010 in electronic mode. This election will lead to establish a steering committee comprising of senior health scientists, researchers, and bio-technology industrial sector in EMRO region.

In this regard, if you are a researcher or academic member with valid institutional email address, or a graduate student (M.Sc. or Ph.D. with valid student ID) and consider to participate in current election to vote, please kindly visit WWW.EMHGBN.NET and WWW.EMGEN.NET or log in with below link during 4 December 2010 till 18 December 2010 (2 pm Iran local time=Greenwich Mean Time (GMT) +3).

http://www.emhgbn.net/en/vot_register.php

Cover Picture

Title: X-ray scattering technique

This is an X-ray diffraction pattern created when X-rays are hit on a crystalline material, in this case a protein. Each dot, named a reflection, forms from the coherent interference of scattered X-rays passing through the crystal.

X-ray scattering techniques are a group of non-destructive analytical techniques which make information about the crystallographic structure, chemical composition, and physical properties of materials and thin films.

Source: http://en.wikipedia.org/wiki/X-ray_scattering

Title: Scanning electron micrograph of HIV-1

Scanning electron micrograph of HIV-1, colored green, growing in a cultured lymphocyte. The scanning electron microscope (SEM) is a type of electron microscope that scans the sample surface with a high-energy beam of electrons in a raster scan pattern.

Source: http://en.wikipedia.org/wiki/Scanning_electron_microscope, http://en.wikipedia.org/wiki/AIDS

Title: Crick and Watson DNA model

The original model constructed in 1953, was rebuilt largely from its original pieces in 1973 and donated to the National Science Museum in London.

Source: http://en.wikipedia.org/wiki/James_D._Watson