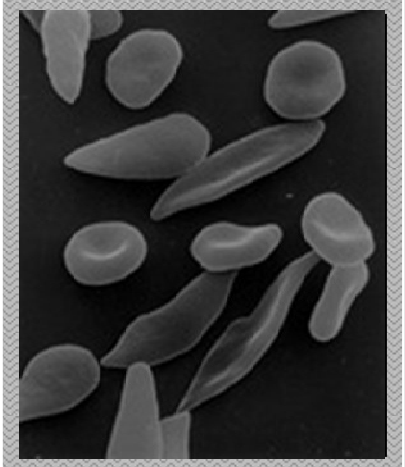

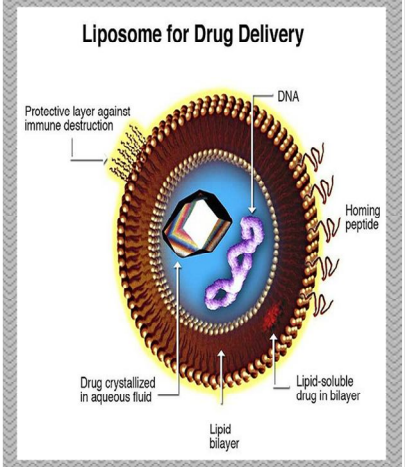


# EMGEN Newsletter

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

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# Article

## *Characterization of surface proteins of Cronobacter muytjensii using monoclonal antibodies and MALDI-TOF Mass spectrometry*

*The paper entitled: Characterization of surface proteins of Cronobacter muytjensii using monoclonal antibodies and MALDI-TOF Mass spectrometry, which is published in BMC Microbiology (Jaradat et al. BMC Microbiology 2011, 11:148). discribed the Cronobacter spp. It is newly emerging pathogen, which causes meningitis in infants and other diseases in elderly and immunocompromised individuals that is a newly emerging pathogen. Surface antigenic determinants in Cronobacter spp was investigated in this study. Using some monoclonal antibodies (MAbs) and MALDI-TOF Mass spectrometry. The study was carried out by Dr. Ziad W Jaradat from Jordan University of Science and Technology, Jordan.*

**Dr. Ziad W Jaradat**

*Cronobacter* spp., which is a major cause of meningitis in infants and other diseases in elderly and immunocompromised individuals is a newly emerging pathogen. Infants born prematurely with low birth weights and infants in neonatal intensive care units are highly sensitive to *Cronobacter* infections with the pathogen being carried primarily from contaminated environments to the infant formula during the preparation. In 2004, a joint FDA/WHO workshop announced an alert, warning of the presence of *Cronobacter* in powdered infant formula (PIF) and suggested applying higher microbiological standards during its manufacturing. This warning led to improved research efforts to study *Cronobacter* such as the development of improved isolation and identification methods, and understanding of the growth and survival features. This study was carried out to explore surface antigenic determinants using monoclonal antibodies (MAbs) and MALDI-TOF Mass in spectrometryin *Cronobacter* spp.

The study involved the production of monoclonal antibodies against Outer Membrane Proteins (OMPs) extracted using Sarkosyl which selectively solubilizes the cytoplasmic membrane and leaves only the outer membrane. The OMPs or LPS extracts were used for the immunization of Balb/c mice for the production of the MAbs against OMP or LPS separately.

Myeloma SP2 cells kept in RPMI media supplemented with 10% Fetal Calf Serum (FCS), and antibiotics were combined with spleen cells from immunized mice with OMP or LPS in separate fusions. The fusion was performed using 40% (w/v) polyethylene glycol 4000 as the fusing agent and at a ratio of 8:1 spleen to

myeloma. Actively growing hybrids were screened for the production of specific MAbs by ELISA and were cloned three times. Five favorable MAbs were selected which are A1, B5, 2C2, C5 and A4 . MAbs A4 was an IgM but A1, B5, 2C2 and C5 were of IgG2a isotype. Specificity of the MAbs was defined by using immunoblotting with outer membrane protein preparations (OMPs) drawn out from 12 *Cronobacter* and 6 non-*Cronobacter* bacteria. Upon characterization, it appeared that none of the Mabs identified LPS structure in *Cronobacter* while all produced MAbs identified the same OMP epitope as defined by an Additive Index ELISA their epitopes appeared to be conformational rather than sequential. The inability of the MAbs to recognize LPS could be due to the simplicity of the LPS structure which is a linear unbranched chain of repeating polysaccharide units as reported in the literature.

Gel Electrophoresis was used to identify the protein bands recognized by the MAbs. All MAbs identified proteins had a molecular weight varying from 36 to 49 kDa except for one isolate (44) in which no OMPs were detected. Furthermore, MAbs identified two bands (38-41 kDa) in four of the non-*Cronobacter* bacteria. Most of the proteins identified by the MAbs were determined by MALDI-TOF peptide sequencing and appeared to be heterogeneous with the identities of some of them still unknown. For instance, a 44 kDa protein that was recognized by all the monoclonal antibodies in *C. sakazakii* appeared to be a novel protein that did not match with any identified protein and thus was termed a hypothetical protein. This protein appears to contain a highly antigenic epitope that is capable of enhancing strong immune response in mice against the *Cronobacter* strain used in the immunization procedure. The feature of this protein was identified by using MALDI-TOF MS to be a hypothetical outer membrane protein ESA_03699 [*Enterobacter sakazakii* ATCC BAA-894]. This protein appeared to be dominant in this specific strain and is highly accessible to the host immune system because it is protruding to the surface. The function of this protein is unidentified but it would be of significant interest in future studies because it was not detected in other strains. Furthermore, the 35 kDa protein identified in the *Cronobacter* isolate $146_A$ also showed to be a novel protein termed a hypothetical protein that showing no similar to any known protein sequence deposited in the protein sequence bank. Two *Cronobacter* isolates ($160_A$ and C13) contained a protein (42 kDa) with an identity as a flagellar hook protein FlgE and an outer membrane porin protein in the two isolates respectively. Further, a 40 kDa protein was recognized in *Cronobacter* isolate 112, and appeared to be an outer membrane protein F which is similar to an outer membrane protein F in *E. coli*. another similar protein with a MW of 38 kDa was identified in

both *E. coli* and *Salmonella* as an outer membrane protein A. A dot blot assay was used for the study of the resilience of the antigens upon the treatment of acid or base and to gain antigens upon the treatment of acid or base and to gain insights about the nature of the binding between the MAbs and their target epitopes. ELISA and Dot-blot were done using different antigens (OMPs, heat killed bacterial cells, LPS) which were subjected to different treatments (acid, alkaline, denaturing agents and heat). Acid and base-treatments of whole cell antigens resulted in an increase in the binding affinity between the MAbs and those antigens and exposed these antigens. These results were confirmed by immunoelectron microscopy experiments. In conclusion, this study is the first study that indicates the production of monoclonal antibodies against whole cells of *C. muytjensii* by using MALDI-TOF spectrometry. As apparent from the MALDI-TOF protein identification, the final results showed that the major OMPs found in the *Enterobacteriaceae* are sufficiently conserved thereby promoting antigenic cross-reactivity between genera. Moreover, the single-banding pattern and the high titers obtained in immunoblotting and ELISA for the *Cronobacter* strains showed that the OMPs are better conserved in closely related strains compared with other genera evaluated. The results of this research can be of great help for possible vaccine production against this pathogen in infants and young children.

The original article was published in BMC Microbiology (Jaradat et al. BMC Microbiology 2011, 11:148) with the full article being found at the following link; http://www.biomedcentral.com/1471-2180/11/148

**Refrence:**

Ziad W Jaradat, A.M.R., Qotaiba O Ababneh, Saied A Jaradat and Arun K Bhunia, *Characterization of surface proteins of Cronobacter muytjensii using monoclonal antibodies and MALDI-TOF Mass spectrometry*. BMC Microbiology 2011. doi:10.1186/1471-2180-11-148.

# Interview

## Interview with Dr Ziad W Jaradat an associate professor in Microbiology and Biotechnology

**1. Please introduce yourself and explain your scientific discipline.**

I am Ziad W Jaradat, an Associate professor in Microbiology and Biotechnology. I have obtained B.Sc. in Public Health/Nutrition from Yarmouk University, Jordan, and an MSc and PhD in Microbiology and Biotechnology form the University of Manitoba, Canada. Upon finishing graduate studies, I have joined Purdue University, West Lafayette, Indiana, as a postdoctoral research associate for almost three years and then worked in a biotechnology firm in San Antonio, Texas for two years. After completing the North American education and training, I have decided to go back to Academia where I joined Jordan University of Science and Technology (JUST). At JUST, I taught a lot of biotechnology, microbiology and related courses. I have supervised 18 M.Sc. students as main advisor or co-advisor. Since the beginning of my academic career I have co-authored over 30 Journal papers, a book and authored 3 book chapters in the Toxicology and Microbiology field. Currently I am heading the Academic Affairs of Fatima College of Health Sciences and Al Ain Campus of the college in the United Arab Emirates.

**2. Could you please tell us what your main research area is?**

My main research area is molecular and epidemiological typing of *Staphylococcus, Cronobacter* and *Salmonella*. My research also focuses on the antibiotic resistance profiles and other virulent characteristics of these bacteria.

**3. Why did you choose this field of research?**

I have chosen this field of research since it is exploring bacteria that are closely associated with human beings and are considered dangerous. Therefore, studying it might help in revealing some of its pathogenic characteristics which will be reflected positively on the health and welfare of mankind.

**4. Do you use any biotechnology or genomics tools in your research?**

In fact most of my research comes under the umbrella of Biotechnology. Yes, I do use genomics in my research wether to ascertain the correct identification of the pathogen or to look for the presence of a gene encoding for a certain pathogenic character or a distinguishing marker.

**5. Are biotech scientists trained in your own country or abroad?**

At the beginning, the scientists were trained abroad, however, since over a decade the technology is no longer restricted to those who study and work abroad. The technology is available now in almost all research institutions where graduate students are using it as a tool in their research projects.

**6. What about quality of knowledge they gain? Which one is better?**

I do firmly believe that our graduates gain a compatible knowledge and hands-on experience in biotechnology fields in Jordan or neighboring countries to knowledge and experience gained abroad.

**7. Are there significant biotechnology centers in your country?**

Yes, there are a few biotechnology centers; however, they still need some time to have an impact on the biotechnology community in Jordan and the field itself globally.

**8. What kinds of difficulties do you face, in research and commercialization of medical Biotechnology in your country?**

The biggest difficulty is the team work, I want to be able to commercialize products, for which extensive team work is needed and also the promotion rules & regulations and some other intrinsic issues. However, to conduct research is relatively easy, but again due to certain circumstances, obtaining external funds is mainly subjected to whom you know in that granting agency.

**9. Do you have any governmental support for biotechnology in your country? And at what level?**

Definitely there is support for biotechnology at the university level and the country level, but due to t-

he issues that I mentioned, the support is limited.

**10. What about public perception of biotechnology in your country?**

The public perception of biotechnology is very good, and it is further increasing as biotechnology graduates start to work in many medical and industrial fields inside or in the neighboring countries and creating a positive impact in this field.

**11. Are there any biotechnology products that have been made in your country? (I.e. your native researchers involved in the project).**

Not that I know.

**12. Is there any journal that is published in your country and deals with biotech issues?**

Not Biotechnology per se, there are some journals that publish Biotechnology articles but the scope of these journals is not limited to this discipline.

**13. What is your opinion about the development of the biotechnology & genomics in your place?**

The development of this field is moving, but at a slow pace.

**14. Would you tell us about the differences of biotechnology and its applications between developed & developing countries? What should we do in this regard?**

One of the major differences between developed and developing countries is the lack of communication between academia and industry in developing countries as opposed to the excellent connections between these two entities in the well developed countries. In addition, as I said in my previous answers, the lack of extensive team work. To overcome these obstacles, I think the aim of the research should be modified *i.e.,* to move it from being exercised merely for promotion purposes and every effort should be exercised to bridge the gap between academia and industry. For that, I urge the industry to start by approaching the academics with their problems seeking solutions or their vision of new products rather than the other way around.

**15. Are you familiar with EMRO countries and EMGEN (Eastern Mediterranean Health Genomics and Biotechnology Network)? Would you please tell us how you know the EMGEN?**

Yes, I am familiar with EMRO and The EMGEN network from the emails that I receive from you and some information from the website.

**16. What are your suggestions for the EMGEN development?**

I have a few suggestions that I can summarize in the following points;

•To initiate a regional conference on biotechnology that is held each year on one of the member states.

•To have a vigorous advertising campaign for enrolling every biotechnologist in these countries in its data base. Further,

•Initiating a quality journal that publishes the research outcome in these countries and of course for the world. It would give it a momentum and will certainly maximize its impact not only in the Middle East but worldwide.

•Initiating a consortium of biotechnology journals that is available for members.

**17. At the end of interview is there anything special you want to mention?**

I would like to thank EMGEN for giving me the opportunity to express my views in the Biotechnology field and to publish a summary of one of my published biotechnology articles.

**Thank you Dr Ziad W Jaradat for sharing information and your opinion with us. Also we are grateful for your kind and useful cooperation.**

# Homology modeling or comparative modeling

Insight of the 3D structure of protein has aided us to understand how proteins function and interact with each other and also with other molecules such as DNA. Additionally it can be used for structure-based drug design, finger out antigenic behavior, rational design of site direct mutations in a protein and rational design of proteins.

3D structure of protein can be experimentally designated by NMR spectroscopy and X-ray crystallography. Protein size and inability to produce sufficient protein crystals suitable for testing are some of the major drawback of these procedures. These tests are often complicated procedures and time consuming.

UniProt/TrEMBL or Protein sequence databank contains more than 10 million protein sequences available in 2010, but at the same time, the Protein Data Bank (PDB) contains almost 64,100 structures, out of which only around 4300 are "unique" at chain level. There is obviously a huge gap within the world of known proteins structure and the universe of known protein sequences. This goes to show that structural genomic projects are unable to keep up with newly discovered genes.

Building a model based on the known 3d structure of a homologous protein (homology modeling) is the only way to tackle to the limitations of experimental techniques.

Homology modeling is based on two observations:

1) the structure of a protein is uniquely determined by its amino acid sequence. Knowing the sequence of protein is enough to obtain the structure.
2) studies indicate that the protein structure is more stable than the related sequence and the changes in structure level is slower during evolution, so that similar sequences tend to practically identical structures and distantly related sequences still fold into similar structures.

Homology modeling is a multi step process; and at each step a modeler should make a choice which he can't be completely sure of. Thus a main part of the homology modeling depends on series of thoughts about how to distinguish between choices that appear similar. The computer automatically is doing these critical choices nowadays and bioinformaticians are trying to develop new tools to make this step more accurate.

In addition, the success of homology modeling relays on some other points such as: (a) the availability of a known structure homologue, (b) our skill to find this homologue, and (c) the feature of the model building process after the homologue is detected.

In practice, homology modeling can be sorted into seven steps:

1. Template selection
2. Multiple sequence alignment and alignment correction
3. Backbone generation
4. Loop building
5. Side-Chain Modeling
6. Refinement of the final model
7. Model Validation

**Template selection:**

The first and most crucial step in modeling is template recognition and selection. As I discussed above, proteins, the sequences of which share high identity share similar structures as well. The threshold for identity within target and template is 30%, but if template shares more than 40% identity with target, the model is more reliable.

We know however that this is not always true. Roessler et al. recently reported the discovery of two native Cro proteins sharing 40% sequence identity but with different folds. Moreover, Alexander et al. were able to design two proteins with 88% sequence identity with different structure and function. Conversely, it is not uncommon for proteins, especially enzymes carrying the same function across the tree of life to share a somewhat low sequence identity and at the same time being structurally similar. All these results show that the template selection is far from being a trivial task and more caution should be considered.

The situation is more difficult if there is no suitable template for our target. This is one of the current challenges of the post-genomic era that fold-recognition methods is dealing with, i.e. to identify a suitable template for homology modeling.

In practice, just query sequence feeds to the BLAST servers on the web, PDB is selected as a database,

and obtains a list of hits—the modeling templates and corresponding alignments. Then we can choose the best template which has the highest identity with our target, to be precise and select the best template. We can consider some other parameters such as E-value and resolution of the hits. For retrieving our target sequence and also run BLAST we can use the NCBI server:

http://www.ncbi.nlm.nih.gov

**Multiple sequence alignment and alignment correction**

The accuracy of the sequence alignment is another critical step in the homology modeling process. Conservation level of your protein could be explore by a multiple sequence alignment of protein homologous to your protein with a known tertiary structure. It will help understand us which amino acid residues are conserved or invariant across all kingdoms of life; which ones are conserved only in bacteria or eukaryotes, what possible role they may play in the structure, etc. When large insertions and deletions are found, a homology modeling project must be carefully planned to create a correct alignment. Furthermore, a sequence alignment could be very useful in cases when you need to select within potential modeling templates. This information will help you in the later stages of the modeling project to decide on the feasibility of modeling and to correct them. There are some techniques for aligning sequences and scoring them for their accuracy, but homology modeling usually is based on: (a) standard pair wise sequence alignment using dynamic programming, (b) multiple sequence alignment when the target and template sequences belong to a large family for which many sequences and structures are known, and (c) direct alignment of the sequence on the structure of the template using a threading technique. There are many programs that try to improve the raw sequence alignment which come from one of the techniques discussed above. CLUSTALW is one of the best online programs for this purpose.

Sometimes it may be difficult to align two sequences in an area sharing very low sequence identity, so we can use another sequence from homologues proteins that aligns easily to both of them to solve the problem. This is what multiple sequence programs do for improving the quality of multiple sequences.

CLASTALW can be used from the EBI server:

http://www.ebi.ac.uk/Tools/msa/clustalw2

**Backbone generation**

When the alignment is ready, the actual modeling can start. Creating the backbone is trivial for most of the models. There are two ways for backbone generation: (I) simply copy the coordinates of those residues that show up in the alignment with the model sequence. Only the backbone coordinates can be copied in those cases where two aligned residues differ. If they are the same, side chains can also be included (e.g. SWISS MODEL). (ii) In another approach (e.g. Modeller) restrains are identified from the template and a model is built using their restraint.

**Loop building**

Another key component in homology modeling is the loop-building. Loops serve some function in a protein such as enzyme activity, ligand-receptor interactions, and antigen-antibody recognition. Predicting loop conformation is difficult because of the flexible nature of loops. There are two main approaches to tackling this problem:

1) Methods that use databases of loop conformations
2) Ab-initio methods.

A library of protein fragments which their size corresponds to the size of the loop to be modeled is analyze for fragments whose end-to-end distance matches the corresponding distance in the framework in the database approach. The library is derived from the known protein structures in the PDB. This method can be used when the loop is short (maximum of seven residues). It can be extended up to nine residues when the database method is mixed with a restrained energy minimization. If our residues extend from nine we can't use database search for loop modeling because the library provides a poor sampling of the conformational space accessible, thus Ab-initio method can be used. This technique is based on a conformational search guided by a scoring function. The accuracy of this method is low, especially when dealing with very long loops.

**Side-Chain Modeling**

We can copy conserved residue from template to the model because after comparing side chain

conformations of conserved residues which occur in proteins with similar structure, we find that they often have similar χ1-angles. By doing this, higher accuracy can be achieved compared to copying just the backbone and predicting the side chains. For this purpose we use libraries of common rotamers extracted from X-ray structures with high resolution. Since there is more than one conformation for each side chain, an enormous search is needed for finding a suitable rotamer. The key to make this tremendous search space tractable rests in the protein backbone. Certain backbone conformations strongly tend to have certain rotamers and thus greatly reduce the search space. We can use position-specific rotamer libraries. To prepare a library, at first high-resolution structures are identified collecting all stretches of three to seven residues with a given amino acid at the center.

**Refinement of the final model:**

Energy refinement, originally proposed by Levitt and Lifson is the basis of methodologies for protein structure refinement against experimental data. Without experimental restraints, refinement by energy minimization generally moves the protein structure away from its X-ray structure. Some recent studies have indicated that the negative trend can be traced by the inclusion of evolutionary derived distance constraints through the combination of sophisticated sampling techniques based on replica exchange molecular dynamics and statistical potentials, through the addition of a carefully designed, differentiable smooth statistical potential, or by careful consideration of the solvent effects. These studies are a source for hope.

Two ways to achieve that precision are currently being pursued:

1. Quantum force fields
2. Self-parameterizing force fields

The most direct approach to model optimization is to run a molecular dynamics simulation of the model. One thus hopes that the model will complete its folding and home into the true structure during the simulation.

Programs like Desmond, Macromodel and Gromacs are used for energy minimization**.**

**Model Validation**

After building a model by homology modeling some errors may occur. These depend on:

1. The percentage sequence identity within template and target: The accuracy of the model can be close to crystallographically determined structures, if it is more than 90%. If identity is from 50% to 90%, the rms error in the modeled coordinates can be as large as 1.5 A°, with considerably larger local errors. In models with an accuracy of less than 25%, the alignment leads to extreme errors.

2. The number of errors in the template: Location of errors is very important. Errors located in a place which doesn't affect protein function are acceptable. For eg., an incorrectly placed loop far away from an enzyme's active site is acceptable.

For estimating errors there are two ways:

(i) Calculating the model's energy based on a force field

(ii) Determination of normality indices that describe how well a given characteristic of the model resembles the same characteristic in real structures. Many aspects of a protein for normality analysis structures are well suited. The majority of them are based on the analysis of inter-atomic distances and contacts.

We can validate our model with WHATIF or PROCHECK structure validation online tools:

http://swift.cmbi.ru.nl/whatif/ and http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/

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# Plants are new factories for Vaccines

**Molecular farming:**

Pharmaceutical drugs are not new in the field of biotechnology, but they are limited to production within a close system using microbial and animal tissue cultures. This is achieved by inserting the desired gene into the production organism, which then expresses it. In contrast, molecular farming is a new technology that uses plants to produce pharmaceutical vaccines instead of using cell culture. In molecular farming, suitable genetically modified plants are used as production organisms, and pharmaceutical substances that are produced by these plants are extracted and purified. Some crops such as potato, maize, carrot and tobacco have been already used for production of the vaccines. Using plants for producing pharmaceuticals is adequate and easy compared to other production methods. This approach has some advantages, in contrast to other expression systems (prokaryotic and eukaryotic ) such as costs, safety and speed.

The technology used in producing pharmaceuticals derived from plants is based on GM crop production. A DNA molecule carries the genetic properties which are essential to produce the therapeutic substance. This DNA molecule is inserted into the plant by a process called transformation, where it becomes part of the plant genome. There are two types of transformation: The genes can be incorporated permanently, called Stable transformation. On the other hand, Transient transformation is when the gene transfer is for a short period. After transformation the plant is ready to produce the pharmaceutical along with other plant proteins. In this way the plant is used as a bioreactor to produce pharmaceutically active substances.

**Vaccines and molecular farming**

Vaccines are one of the most successful medical progresses till now. They were initially introduced as a cost effective methods for preventing acute infectious disease. Advances in biotechnology and the unraveling of immune response have provided a new opportunity for vaccine development, such as plant-derived vaccines. Now a range of immunogenic antigens can be produced in plants. It was proposed to induce B- and T-cell mediated immune responses using plants as a source of 'edible vaccines', where the vaccine antigens are eaten in a fruit or raw vegetable. The advantages of plant-derived vaccines are: they don't require cold storage or sophisticated expertise for their distribution, administering a vaccine via food eliminates the problems enabling participation of less developed countries in phar-maceutical production, with an emphasis on addressing local health issues, and food-borne vaccines will be **considerably less expensive** on the market because of minimal downstream processing which reduces the manufacturing costs up to 80%.

**Vector design for introduction of desired gene into the plant**

Design of vector is another key for molecular farming. Some vectors have been designed based on DNA- and RNA-based plant virus genomes. Viral vectors can be used both as single- and multi-component expression systems based on the protein of interest. Ease of manipulation, low cost speed, and high yield of proteins are the benefits of these systems. Furthermore, *Agrobacterium*-mediated expression also allows the production in plants of complex proteins assembled from subunits. Currently, the transient expression methods are preferred over any other transgenic system for the exploitation of large and unrestricted numbers of plants in a contained environment. By designing ideal constructs and related forms of delivery into plant cells, the technology plan mediates scenarios that envisage high yield of bio-products and ease in monitoring the whole spectrum of upstream production before entering good manufacturing practice facilities. By doing this, plant-derived bio-products indicate a high competitiveness against available eukaryotic cell factory systems.

**Some progresses**

Designing a vaccine for Cholera and Hepatitis C that would be administered through the seeds of a to-

mato is a good example of plant-derived vaccines which was done by researchers from the Universidad Católica in Santiago, Chile. They have combined genetic sequences of these two pathogens and then transferred them into the target plants. The tomato plants later produced key proteins of both pathogens. The similar key proteins found in conventionally created vaccines using cell cultures from animals or microbes. The benefits of the vaccine derived from tomato is that it can be easily stored in the seed of the tomatoes, according to lead researcher Patricio Arce. Historically, vaccines are built based on encouraging the body to develop antibodies by injecting small amounts of a given bacteria or virus into a patient's system. Recently, this has been done by introducing selected proteins into the system to generate the same effect. Plant derived vaccines will function using a similar principal by isolating the genetic codes that produce the proteins used today for vaccines. After isolating these codes, they will be introduced into the genetic material of the plant, which is stored in its seeds.

HIV with tobacco is not familiar to us and not something a doctor would normally recommend, but it's true and scientists have used it for the development of an HIV-neutralizing antibody to prevent virus transmission during intercourse. The plant-derived antibody, made into a gel, can be applied to the vagina to prevent HIV transmission and is a better choice compared to other methods of avoiding infection such as condom, since it does not affect fertility. For instance, in sub-Saharan Africa, it's very tough for a woman to persist on condom use, and in such cases, a HIV neutralizing agent comes in handy as it can be made available cheap and on a large scale, as production can be easily carried out in developing countries themselves.

**Drawbacks**

Plant derived vaccines however, have some disadvantages such as: Risk of contamination of food crop production as it could lead to desensitization if a vaccine were utilized negligently and also these plants could be eaten by animals or enter the active substances to the groundwater that could cause harmful effects. For example, a soya field was found to be contaminated with transgenic maize producing the pharmaceutically-active substance Trypsin, making the destruction of the complete harvest of 13,500 tons inevitable. Hence, research is moving towards inedible plants, such as tobacco. The best solution to reduce these effects would be to grow plant-derived vaccines in the glasshouse.

There are some hurdles restraining as to making these vaccines cheap and easily accessible, especially in developing countries as investments in these countries are so small that as Ma explains, “It would be a push to make pharmaceutical companies switch their production methods, because they’ve invested so much in existing systems”.

**Conclusion**

All in all, using plants as bioreactors for producing pharmaceutical drugs is an enormous leap that has been made in the field of molecular farming. Some progresses have been made such as production of H1N1 vaccines in tobacco. Also, clinical trials of vaccines which treat B-cell lymphomas are going on. But molecular farming had, and still has some problems which have been discussed, earlier that need to be solved, but molecular farming has overcome conventional method problems, breaking the limits of current standard technologies. It certainly has a long way to go, but promises to be the next generation panacea.

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## Seeking HIV Treatment Clues in the Neem Tree

**Village pharmacy is the name of Neem tree in India.**

An assistant professor at Kean University in New Jersey, Arora indicated that some active compounds in Neem extracts that effect a protein that is essential for HIV to replicate. Extract of Neem bark, flowers are used in India to fight against pathogenic fungi and bacteria. The villagers in India keep their teeth and gums healthy by use Neem tree branches as a toothpaste and toothbrushes and also they control the spread of malaria by using Neem extract. She was keen to know more about Neem extract and she started to read articles. During her course of reading she was faced with new reports about effects of Neem extract on HIV-AIDS patients, these patients showed a decrease in the amounts of HIV particles in their blood. She was motivated to try and find out if she could figure out what was in the Neem extract that seemed to fight off the virus. She has tried to use bioinformatics and structural biology and modeled some compound in the Neem extract and her colleague discovered that a majority of the Neem compounds attacked the HIV protease. She is hopeful to discover a new drug from Neem extract to fighting HIV virus.

Reference: http://www.sciencedaily.com/releases/2012/04/120422162215.htm

## Women With Heart Disease More Likely to Have Baby Girls

**Women with heart disease are more likely to give birth to female rather than male babies according to a new study at Tabriz University in Iran.**

Two hundred women diagnosed with cardiac disease were studied within the research period. These women delivered 216 babies out of which 75 per cent were female. The Chromosome of man's sperm is responsible for the gender of a child but this study proposes that there may be a connection between the cardiac diseases of the mother and gender of the babies. As the number of women with heart disease is meaningful around the world, this could prove to be a very interesting area for further research. One of the biggest causes of death among women is cardiovascular disease (CVD), especially in the third world countries compare to the industrialized countries.

Reference: http://www.sciencedaily.com/releases/2012/04/120420105730.htm

## Cognitive Biometrics: A Very Personal Login

**We always hear about some personal anatomical characteristics such as retina, iris scan and Pam as a personal password. But Revett, scientist of el-sherouz reveal an alternative approach to user authentication.**

Cognitive biometrics technique is built upon the response to stimulus such as a song, familiar photograph, a puzzle or even a Rorschach ink blot. This response to stimulus can be detected by some technique, consisting of: electroencephalogram (EEG), electrocardiogram (ECG), electrodermal response (EDR), blood pulse volume (BVP). Other techniques like electromyogram (EMG), eye trackers (pupilometry), near-infrared spectroscopy (NIR), hemoencephalography (HEG) and similar technologies may be used too. The user can be validated by matching their response to stimulus. The stimuli are created in such a way to obtain responses which are sensitive to the individual's genetic predisposition. This technique can be combined with some other technique like mouse dynamics and/or keystroke, to improve the level of security.

Reference: http://www.sciencedaily.com/releases/2012/04/120422162215.htm

## 'Housekeeping' Mechanism for Brain Stem Cells Discovered

**Researchers at Columbia University Medical Center (CUMC) have identified a molecular pathway that controls the retention and release of the brain's stem cells.**

This research is based on current studies, which indicated that stem cells after being detached from their niche, they lose their identity. Some proteins that regulate various stem cell properties are called proteins. These proteins were knocked down or silenced, in a genetically modified strain of mice and mice that died before 24 hours of birth. This experiment revealed that Id proteins directly modify the production of a protein called Rap1GAP, that in turn controls Rap1, one of the important regulators of cell adhesion. The scientist shows that the Id-Rap1GAP-Rap1 pathway is mandatory for the adhesion of NSCs to their niche and for NSC maintenance. Dr. Iavarone believes that if they could change the pathway in the future, they might be able to manage NSC properties for therapeutic purposes.

Reference: Francesco Niola, X.Z., Devendra Singh, Angelica Castano, RyanSullivan, Mario Lauria, Hyung-song Nam, Yuan Zhuang, Robert Benezra, Diego Di Bernardo, Antonio Iavarone, Anna Lasorella., *Id proteins ynchronize stemness and anchorage to the niche of neural stem cells.* Nature Cell Biology, 2012. DOI: 10.1038/ncb2490.

# Cancer Tumor Life:

# Biological and Physical Aspect

**ISBN:** 978-1-62081-411

**Pub. Date:** 2012 3rd Quarter

Mutual interaction of cancer and its host body have been explained in this book. The authors demonstrate that this interaction is mediated by human consciousness. Although the term cancer refers to uncontrollable and undisciplined cellular growth, a basal cell carcinoma of the skin is quite unequal in comparison to adenocarcinoma of the lung, breast, or prostate, lymphomas , brain tumors, leukemias, and other malignancies. These all vary markedly with respect to growth rates, metastatic tendencies, and sensitivity to neuroendocrine or immune system influences, especially those that might be modulated by stress.

(Imprint: Nova Biomedical)

**Table of Contents:**



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# Announcement


**http://www.icebb.or**


**http://www.icbem.org**


**http://selectbiosciencess.com**

# Announcement


**http://www.qatarhealth.info**


**http://www.omicsonline.org/biotechnology2012**


**http://www.ecb15.org**

## Title: Plant Transformation Using *Agrobacterium tumefaciens*

*Agrobacterium tumefaciens*, the gram negative and rod shape bacteria is the cause of crown gall disease in over 140 species of dicot, which is the soil bacterium and has the ability to induce plant cells. T-DNA is the genetic material which introduced to the plants cells and symptoms are caused by this insertion. Plant engineering has used *Agrobacterium*-mediated transformation to introduce new gens in to the plants. T-DNA include two types of genes: genes which are responsible for enzymes involved in the synthesis of cytokinins and auxins and those involved in synthesis of opines. These genes encode the enzymes, provide the carbon and nitrogen sources which are consumed by *Agrobacterium tumefaciens.*

http://en.wikipedia.org/wiki/Genetic_manipulation

## Title: Sickle-cell disease

Sickle-cell disease (SCD) or drepanocytosis or sickle-cell anemia (SCA) is characterized by a typical sickle shape, and is an autosomal recessive genetic blood disorder with over dominance. SCD is characterized by red blood cells. Mutation in hemoglobin gene causes this disease and life confidence is its consequence, which was 48 years in females and 45 years in males, these patients can live longer today because of better management of the disease. SCD appear in the people from areas where malaria is or was common, for example 30% of people in sub Saharan Africa carry the gene.

http://en.wikipedia.org/wiki/Sickle-cell_disease

## Title: Liposome for drug delivery

Liposomes are artificial vesicles made of lipid bilayers. These bilayers may carry some other molecules so that they can fuse with the cell membrane and deliver the liposome content. Molecules inside the liposome can be hydrophilic as well as hydrophobic, but mostly they are DNA or drugs which cannot diffuse through the membrane. Lipofection is a kind of liposome containing DNA. Liposomes can be used also to deliver anticancer drugs. In a healthy human being blood vessels have an endothelial wall that stops leaking of large particles out of blood. Blood vessels in tumors are not as tight as healthy vessels, because of the Permeability and Retention effect. Consequently, small liposomes can pas through tumor blood vessel walls that cannot pass through normal blood vessel walls. Using this kind of liposomes we can target cancer cells directly.

http://en.wikipedia.org/wiki/Liposome