EuroSciCon

DAY 1

Scientific Tracks & Abstracts

EuroSciCon Congress on

Enzymology & Molecular Biology

August 13-14, 2018 Paris, France

DAY 1

Aug 13-14, 2018

Sessions

Enzymology & Proteomics | Enzymology in Drug Discovery | Molecular Enzymology | Cell Signaling | Enzymes And Green Solvents | Molecular Biology Techniques | Enzymology & Thermodynamics

Session Chair
Huseyin Bekir Yildiz
KTO Karatay University, Turkey

Session Introduction

Title: Molecular recognition as a crucial step in enzymatic reactions: 3D-RISM/KH study
F Hirata, Toyota Physical and Chemical Research Institute, Riken

Title: Making novel enzymatic biosensors by using DTP type conducting polymers
Huseyin Bekir, KTO Karatay University, Turkey

Title: Role of Mg2+ ions in DNA hydrolysis by EcoRV, studied by 3D-RISM and MD
Masayuki Irisa, Kyushu Institute of Technology, Japan

Title: Recombinant class-I and class-II collagenases: new formulations in cells extraction
Giulio Ghersi, University of Palermo, Italy

Title: Enzymatic ethyl lactate synthesis in a green reaction medium
Ayse Ezgi Unlu, Ankara University, Turkey

Title: Exosomes derived from human neural stem cells mediate cellular stress ability and promote neurological function recovery of cerebral ischemic rats
Guilong Zhang, Zhongda Hospital, Southeast University, China

Title: The extraction of biophenolics from olive leaf using green solvents
Ayse Ezgi Unlu, Ankara University, Turkey

Title: A novel 7D-QSAR approach, combining QM based grid and solvation models to predict hotspots and kinetic properties of mutated enzymes: An Enzyme engineering perspective
Pravin Kumar, Kcat LLP, India

Title: Combining stochastic deformation/relaxation and intermolecular contacts analysis as a novel approach for pharmacophore modeling based on X-Ray or homology-modelled ligand-receptor complexes
Mamon Hatmal, The Hashemite University, Jordan

Title: Differentiation of mesenchymal stem cells into nucleus pulposus like cells induced by co-culture system and hypoxia
Arjun Sinkemani, Zhongda Hospital, Southeast University, China

Title: Unfolded protein response exerts cytoprotection and promotes the proliferation of nucleus pulposus cells in TNF-α stimulation by activating NF-κB
Lu Chen, Zhongda Hospital, Southeast University, China

Title: Preimaginal exposure to azadirachtin affects digestive enzymes in adults of *Drosophila melanogaster*(Diptera: Drosophilidae)
Maroua Ferdenache, Badji Mokhtar University of Annaba, Algeria

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F Hirata, Insights Enzyme Res 2018, Volume 2
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MOLECULAR RECOGNITION AS A CRUCIAL STEP IN ENZYMATIC REACTIONS: 3D-RISM/KH STUDY

F Hirata

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Molecular Recognition plays a crucial role in an enzymatic reaction, as is implied in the Michaelis-Menten equation for the reaction rate. In order for the reaction to take place, all the players, substrates, ions, and/or co-enzymes, should be bound at proper positions in the active site of protein. Such a process is governed essentially by two physicochemical properties, the free energy change associated with binding of the ligands to active sites, and the structural fluctuation of protein. It is now widely appreciated that water plays essential roles in the both properties, as is represented by the *desolvation free energy*. A great progress has been made in theories of the molecular recognition during the past decade that features use of statistical mechanics of liquids, referred to as 3D-RISM-KH. The theory enables us to find small ligands, such as water molecules, ions, and drug compounds, located at an active site of protein. It also provides the solvation thermodynamics at molecular level, such as the desolvation free energy, that is crucial for the evaluation of binding affinity of a ligand to protein. The theory has been applied successfully to variety processes of molecular recognition, including molecular channels, drug screening, and so on. Recently, we have applied the method to a restriction enzyme, *EcoRV*, which catalyzes the hydrolysis reaction of DNA. The method has been employed to locate the position of water molecules and magnesium ions at the active site, which play crucial roles in the enzymatic reaction.

Biography

F Hirata has completed his PhD in 1977 from Hokkaido University, and did his Postdoctoral studies at SUNY, UT and Rutgers in USA. He is a Professor Emeritus of IMS in Japan. He has published more than 250 papers in reputed journals.

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MAKING NOVEL ENZYMATIC BIOSENSORS BY USING DTP TYPE CONDUCTING POLYMERS

Huseyin Bekir Yildiz[1], Hacer Azak[2], Emre Cevik[3], Buket Carbas[2] and Mehmet Senel[4]

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[2]Karamanoglu Mehmetbey University, Turkey
[3]IRMC-Imam Abdulrahman Bin Faisal University, Saudi Arabia
[4]SANKARA Brain & Biotechnology Research Center, Istanbul Biotechnology Inc., Turkey

Scientists have been trying to develop biosensors with great efforts for the wide fields of application including medical diagnostics, environmental monitoring, and food analysis for many years. The development of biosensors has become increasingly important in molecular diagnostics. They need new methods continuously to get higher sensitivity, lower cost, and better reproducibility. Because of their high sensitivity property, fluorescence and chemiluminescence are widely used in clinical diagnostic laboratories. However, the high instrument cost is a major disadvantage for these methods. Compared to optical detection methods, electrochemical methods are much simpler and not expensive. Among the various electrochemistry-based biosensors developed so far, the amperometric glucose sensing approach has attracted a great deal of attention. Conducting polymers, CPs, are used as suitable matrices supplying common properties for biomolecule immobilization. Their compatibility with biological molecules, easy preparation, high reproducibility and electrochemical properties make them fascinating in biosensor design. The dithieno [3, 2-b:2',3'-d] pyrrole (DTP) based conjugated organic materials have been precursor materials because their chemical structure with strong electron donating ability promises easily functionalization for polymers. Different unit substitution from pyrrole unit in excellent molecular DTP structures has been bringing a breath of fresh air to the field of conjugated polymer since thiophene-pyrrole-thiophene co-monomer structure has a fused ring system and good planar structure, which make an extended conjugated polymer during electropolymerization step. For that reason, a remarkable amount of research effort has been committed to synthesize and create applications of novel DTP based conjugated polymers. This presentation reports amperometric biosensors constructed by using DTP type conducting polymers and important enzymes in food technology which are glucose oxidase, alcohol oxidase, xantine oxidase. The biosensors showed outstanding analytical properties of high sensitivity, selectivity, and reliability being applied to food samples.

Biography

Huseyin Bekir Yildiz has completed his PhD in Chemistry from the Middle East Technical University and Postdoctoral studies from Institute of Chemistry at the Hebrew University of Jerusalem, Department of Chemistry at University of California Berkeley, Center for Molecular Protein Science at Lund University and Institute for Applied Biosciences at Technical University of Applied Sciences Wildau. He is affiliated to Department of Metallurgical and Materials Engineering, KTO Karatay University, where he is currently working as Professor. He is also the Director of Graduate School of Engineering and Natural Sciences at the same university. He has published more than 50 papers in reputed journals and has been serving as an Editorial Board Member of repute.

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ROLE OF MG^{2+} IONS IN DNA HYDROLYSIS BY *ECO* RV, STUDIED BY 3D-RISM AND MD

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[2]Kyushu University, Fukuoka, Japan
[3]Toyota RIKEN (TPCRI), Japan
[4]Institute for Molecular Science, Okazaki, Japan

The role of Mg^{2+} ions in DNA hydrolysis by homodimeric restriction enzyme *Eco*RV was elucidated based on the three-dimensional reference interaction site model (3D-RISM-KH) theory and the molecular dynamics (MD) simulation. From an analysis of the spatial distribution of Mg^{2+} in an active site using 3D-RISM-KH, we identified a new position for Mg^{2+} in the X-ray *Eco*RV-DNA complex structure (1RVB), which turns out to play a crucial role in the reaction. We refer to the position as site IV†. Site IV† is almost the same position as that of a Ca2+ ion in the superimposed active-site structure of X-ray PvuII-DNA complex (1F0O). The 3D-RSIM-KH was also used to locate the position of water molecules including the water nucleophile at the active site. MD simulations were carried out with the initial structure having two Mg2 + ions at site IV† and at site I*, experimentally identified by Horton et al., to find a stable complex structure in which rearrangement of the DNA fragment occurred to orient the scissile bond direction toward the water nucleophile. The equilibrium active-site structure of *Eco*RV-DNA complex obtained in MD simulation was similar to the superimposed structure of X-ray *Bam*HI-DNA complex (2BAM). In the active-site structure, two metal ions have the same position as that of 2BAM, and the scissile phosphate is twisted to orient the scissile bond toward the water nucleophile as is the case in 2BAM. We propose the equilibrium active-site structure obtained in this study as a precursor of the hydrolysis reaction of *Eco*RV.

Biography

Masayuki Irisa has completed a Doctoral program from Kyoto University without degree and got his PhD by the way of dissertation from Osaka University. He is an Associate Professor in Department of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, which belongs to a national university corporation, Japan. He has been serving as a Teacher and a Researcher in the Kyushu Institute of Technology for 20 years.

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RECOMBINANT CLASS-I AND CLASS-II COLLAGENASES: NEW FORMULATIONS IN CELLS EXTRACTION

Giulio Ghersi

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The major component of extracellular matrix is the collagen and collagenase enzymes are used to extract cells from biological tissues, which the challenging goal is to obtain a high number of healthy and living cells. The current collagenases utilized for regenerative medicine and cell therapy are extracted from the culture of *Clostridium Histolyticum* and the subsequent purification thereof of the bacterial proteins produced. The result of this process leads to a blend containing different percentage ratios of two main collagenase isoforms (class I and class II) plus a number of other lytic enzymes (clostripain, trypsin like, caseinase activity, etc.). Such blends present many limitations in terms of lot-to-lot consistency, variable enzymatic activity and purity. In order to better control the extraction processes and the enzymes formulation, we have generated recombinant collagenases of class-I (COL G) and class-II (COL H), which allows efficient, customized and standardized cell extraction procedures. These recombinant enzymes were used together with thermolysin (as a generic proteolytic enzyme) in the extraction processes of different cell types, for which the quantities of the two classes of collagenases plus the neutral protease were precisely defined. The current extraction procedure, with collagenases from *C. histolyticum*, is based on a formulation that use a weight collagenase ratio, due to the impossibility in determines the exact enzymes activity for each class of collagenases. Our study performed with COL G and COL H to extract *Langerhans* islets from rat pancreas highlighted how this formulation lead to variable results, while the formulation based on the enzymatic activity ratio (COL G : COL H, determined with the Grassmann method) allows a standardized and reproducible cell extraction. Based on this results, several extraction protocols have been improved, such as: cardiomyocytes from rat heart, chondrocytes from nose or cow's hoof cartilage, hepatocytes from rat liver, osteoblasts from rat skull cap and mesenchymal stem cells from rat adipose tissue. Each protocol was optimized, using as parameters the phenotype and the number of extracted cells, but also performing functional and /or differentiation assays.

Biography

Giulio Ghersi is Professor in Biochemistry and Applied Biochemistry in Biotechnology, Element of Biochemistry and Cellular Biology in Medical Engineering. Vice-Director Advanced Technology Network Center (ATeN Center) University of Palermo.CEO of ABIEL s.r.l. (www.abielbiotech.com) a spin-off of the University of Palermo and of the Council National of Research (C.N.R.) IAMC.PI of Mediterranean Center for Human Health Advanced Biotechnology (PONa3_00273 23 M€)PI of "SIB: Advanced solutions using biomaterial by composite matrix in repair and regeneration of articular cartilage using non invasive techniques (PON01_01287 1,6 M€).Unit ABIEL PI for Horizon 2020 project "Diabetes Reversing Implants with enhanced Viability and long-term Efficiency – DRIVE" (0,9 M€).The research activities of greatest interest are currently directed to the optimization of the extraction processes, of cells for applications in the field of regenerative medicine and tissue engineering, through the use of specific proteolytic enzymes. As well as their use in nanostructured systems for greater penetration into solid tumor masses, and the controlled release of drugs and / or biomolecules with antitumor activity.- Salamone M. et al (2016) "Proteolytic enzymes clustered in specialized plasma-membrane domains drive endothelia cell migration". PLOS ONE, vol.11. Dispenza C. et al.(2012) Minimal in Radiation Synthesis of Biomedical Functional Nanogels. Biomacromolecules, vol 13, p.1805-1817.

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ENZYMATIC ETHYL LACTATE SYNTHESIS IN A GREEN REACTION MEDIUM

Ayse Ezgi Unlu, Arıkaya A and Takac S
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Ethyl lactate is a commonly used biodegradable solvent and it is widely used in food additives, pharmaceutical preparations, and also in fragrances. It is naturally found in alcoholic beverages, various foods, such as cabbage, vinegar, butter, chicken and some fruits. In the literature, searches on ethyl lactate synthesis by the esterification reaction of lactic acid are mainly focused on i) the enhancement of the product yield by removing water employing various procedures ii) the facilitation of down-stream processing by using heterogeneous catalysis, and iii) the environmentally friendly processes by using enzymes. In this study, we investigated the synthesis of ethyl lactate by esterification reaction of lactic acid using lipase enzyme in a choline chloride-based deep eutectic solvent as green reaction medium. The synthesis of ethyl lactate was carried out in a batch system under different reaction conditions. According to the results, the initial reaction rate increased with the increase in initial lactic acid concentration at constant ethanol concentration. The molar ratio of the substrates strongly affected the rate of this reversible reaction. The temperature of the synthesis was found to have a significant effect on the initial reaction rate. Bell-shaped curve was obtained for the initial reaction rate as the temperature increased. High agitation rates increased the reaction rate by decreasing the mass transfer limitation in the medium. The overall results showed that deep eutectic solvent was successfully used in the esterification reaction of lactic acid.

Biography

Ayse Ezgi Unlu graduated from Ankara University, Faculty of Engineering, Department of Chemical Engineering in 2002. She completed her master degree in 2005 at Ankara University in Turkey. The synthesis of Naproxen, a member of NSAIDs, was the subject of the master thesis using commercial lipase subjected to various pre-treatment strategies that enhanced the activity. Investigation of different parameters on the production of lipase by *Candida rugosa* and also proteomic analysis of the isoenzymes was another subject of interest. Ayse Ezgi Unlu completed her Ph.D. in 2012 at Ankara University in Turkey. Two important antioxidant enzymes, catalase and superoxide dismutase production by *Rhodotorula glutinis* was studied comprehensively during PhD thesis. She received a postdoctoral grant from TUBITAK, with a project about the synthesis of flavonoid polymers using green solvents, at the Institute of Technical Biocatalysis, Technical University of Hamburg, Harburg in Germany, between 2014-2015. She is currently working at Biotechnological Research Group in the Department of Chemical Engineering, Ankara University. The research area includes enzymes, enzymatic reactions, fermentation, protein synthesis, proteomics, experimental design, enzymatic biopolymers and green solvents.

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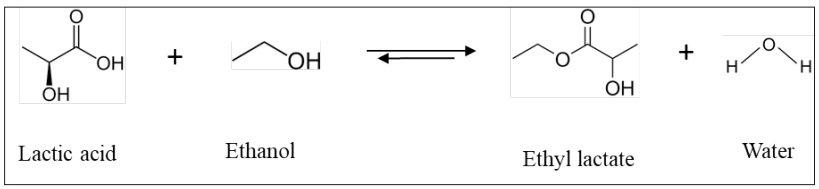

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EXOSOMES DERIVED FROM HUMAN NEURAL STEM CELLS MEDIATE CELLULAR STRESS ABILITY AND PROMOTE NEUROLOGICAL FUNCTION RECOVERY OF CEREBRAL ISCHEMIC RATS

Guilong Zhang, Lukui Chen, Hong Wang, Bingqian Li, Wanghao Chen, Yongbo Yu and ZhiHan Zhu

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Ischemic stroke recovery is associated with neural stem cells (NSCs) development and neurovascular unit reconstruction. Exosomes, as important intercellular players in cellular communication, mediate neuro-restorative events, however, their effects / mechanisms in the injured brain are unknown. The objective of this study is to determine the effect of human NSCs-derived exosomes on the potential abilities of cells, and whether human NSCs-derived exosomes can repair the functions of ischemic stroke rats. This study finds that IFN-γ stimulating managed the abilities of human NSCs-derived exosomes (hNSCs-Exo), including alleviated the level of oxidative stress of NSCs following H2O2 stimulating, augmented the NSC survival, and promoted the neuronal differentiation of NSCs. Furthermore, in rats ischemic stroke model, IFN-γ-hNSCs-Exo further facilitated the neurological functional recovery (such as modified Neurological Severity Score, Rotarod test, etc.) compared to hNSCs-Exo group, enhanced nerve cell survival and promoted neovascularization (microvessel density, MVD). Importantly, exosomes can be internalized or endocytosed by cells, after labeled with PKH67, it showed that exosomes migrated to the infarct regions together with cells, as interestingly, many exosomes entry into the nucleus. Next generation sequencing (NGS) revealed significant enrichment of hsa-miR-206 and hsa-miR-133a-3p in IFN-γ-hNSCs exosomes compared with hNSCs exosomes. The mechanisms or effects of exosomes were to deliver specific exosomal microRNAs to cells for increasing cell survival and proliferation. In summary, hNSCs-derived exosomes possess the ability of neural regeneration and modulate neurological functional recovery, and play more positive roles by stimulating with IFN-γ (IFN-γ-hNSCs Exo). Exosomes provide a novel and promising strategy in modulating disease treatment and tissue regeneration, avoiding the risk of teratomas associated with cells.

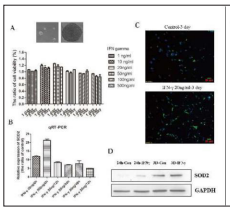

Figure: 1
The role of IFN-γ on NSCs

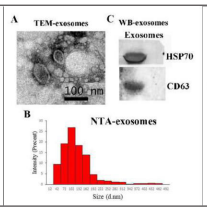

Figure: 2
The identity of exosomes

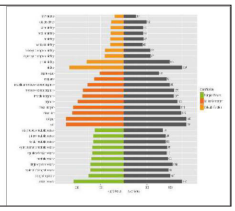

Figure: 3
The GO categories of NGS

Biography

Guilong Zhang is currently pursuing his PhD at the School of Medicine, Southeast University, Nanjing, China. His major is Neurosurgery, and will graduate at 2018/2019. His research field is Stem Cells Therapy and Clinical Translational Research. He has published about 10 papers in molecular and biomedical journals.

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THE EXTRACTION OF BIOPHENOLICS FROM OLIVE LEAF USING GREEN SOLVENTS

Ayse Ezgi Unlu, Arıkaya A and Takac S
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All aerobic organisms generate reactive oxygen species, which are highly reactive against most of the molecules present in the cell. Organisms produce antioxidant molecules in order to eliminate the harmful effects of reactive oxygen species. Antioxidant molecules are diverse type of compounds, such as enzymes, minerals, vitamins, carotenoids, polyphenols, etc. Biophenols, which are secondary metabolites, also have a significant role as antioxidant molecules. Olive leaf is a valuable natural source regarding biophenolic compounds. These compounds include oleuropein, verbascoside, rutin, apigenin-7-glucoside, hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, vanillin, etc. Many researches have been made for the extraction of biophenolics from olive leaf. They are commonly extracted by using organic solvents, mainly ethanol and methanol and/or with water. Green solvents gain increasing attention in many fields of research due to the increasing conscious of protection of the environment. Reports show that green solvents are good candidates to replace organic solvents in many biotechnological processes and are under extensive research for the ones that it is not yet. In this study, we investigated the effect of different types of deep eutectic solvents as green solvents on the extraction of biophenolics from olive leaf and compared the extraction yield and the type of extracted biophenols with conventional solvents. It was found that most of deep eutectic solvents tested provided promising results also depending on the extraction conditions.

Biography

Ayse Ezgi Unlu graduated from Ankara University, Faculty of Engineering, Department of Chemical Engineering in 2002. She completed her master degree in 2005 at Ankara University in Turkey. The synthesis of Naproxen, a member of NSAIDs, was the subject of the master thesis using commercial lipase subjected to various pre-treatment strategies that enhanced the activity. Investigation of different parameters on the production of lipase by Candida rugosa and also proteomic analysis of the isoenzymes was another subject of interest. Ayse Ezgi Unlu completed her Ph.D. in 2012 at Ankara University in Turkey. Two important antioxidant enzymes, catalase and superoxide dismutase production by Rhodotorula glutinis was studied comprehensively during PhD thesis. She received a postdoctoral grant from TUBITAK, with a project about the synthesis of flavonoid polymers using green solvents, at the Institute of Technical Biocatalysis, Technical University of Hamburg, Harburg in Germany, between 2014-2015. She is currently working at Biotechnological Research Group in the Department of Chemical Engineering, Ankara University. The research area includes enzymes, enzymatic reactions, fermentation, protein synthesis, proteomics, experimental design, enzymatic biopolymers and green solvents.

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A NOVEL 7D-QSAR APPROACH, COMBINING QM BASED GRID AND SOLVATION MODELS TO PREDICT HOTSPOTS AND KINETIC PROPERTIES OF MUTATED ENZYMES: AN ENZYME ENGINEERING PERSPECTIVE

Pravin Kumar R[1] and Roopa L[2]

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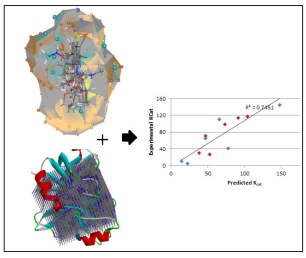
[2]Mount Carmel College, Bengaluru, India

The globalenzyme market was estimated at $7,082 million as of 2017 and is expected to reach $10,519 million in 2024. At a CAGR of 5.7% from 2018 to 2024, enzymes like transaminases are going to contribute the maximum for this growth. Enzymes are molecular machineries used in various industries such as pharmaceuticals, cosmetics; food and animal feed, paper and leather processing, biofuel etc. Nevertheless, this has been possible only by the breath-taking efforts of the chemists and biologists to evolve/engineer these mysterious biomolecules to work the needful. The methodologies for this research include the well-established directed evolution, rational redesign and relatively less established yet much faster and accurate insilico methods. Main agenda of an enzyme engineering project is to derive screening and selection tools to obtain focused libraries of enzyme variants with desired qualities. As a proof of concept, for the first time, receptor dependent 4D Quantitative Structure Activity Relationship (RD-7D-QSAR) to predict kinetic properties of enzymes has been demonstrated by *Pravin Kumar et al*. The methodology was extended to study transaminase. Induced-fit scenarios were explored using QM/MM simulations which were then placed in a grid that stores interactions energies derived from QM parameters (QM grid). The novelty of this study is that the mutated enzymes were immersed completely inside the QM grid and this was combined with solvation models to predict descriptors. After statistical screening of descriptors, QSAR models showed >90% specificity and >85% sensitivity towards the experimental activity. Mapping descriptors on the enzyme structure revealed hotspots important to enhance the enantioselectivity of the enzyme.



7D-QSAR protocol/paradigm to predict enzyme kinetic properties

Biography

Pravin Kumar R has completed his Doctorate in Computational Biology from Bharathiar University, Tamil Nadu, India. He has 15 years of Industrial Experience on different projects pertaining to target deconvolution and enzyme engineering studies. He has 25 international publications, most of it on techniques such as Protein Modelling, Molecular Dynamics, Quantum Mechanics Hybridised with Molecular Dynamics (QM/MM), 4D QSAR, etc. He has developed the Enzyme Engineering Framework which is composed of algorithms and screening protocols of core quantum mechanics, QM/MM and QSAR techniques. The framework can predict hotspots and enzyme variants with better activity (K_{cat}, K_m). This framework was used to engineer transaminase to expand its substrate scope towards bulky ketones. He has participated and given oral presentation in Enzyme Engineering conferences: BIOSIG 2014, Toyama, Japan, BIOSIG 2015 Boston, USA and BIOSIG 2015, Toulouse, France. He holds several positions such as, Bioinformatician in VittalMallya Scientific ResearchFoundation, Bangalore, India Aug ('2004 to Aug' 2007); Team Head of Research in Bioinformaticsat Jigsaw Bio Solutions Pvt Ltd., Bangalore, India (Sep'2007 to Dec'2008); Project head for Computational Biology at Prescient Biosciences Pvt. Ltd, Peenya, Bangalore, India (Jan'2009 to Aug'2010); Team lead and Senior Scientist, in silico, Polyclone Bioservices Pvt Ltd, Jayanagar, Bangalore, India (Oct' 2010 to Aug' 2016) and Director, Quantum Zyme, Bangalore, India from Sep' 2016 to May' 2018. He is the Reviewer of *Journals J. Biomolecular Structure and Dynamics, J. Molecular Catalysis, J. Computational Biology and Chemistry*.

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COMBINING STOCHASTIC DEFORMATION/RELAXATION AND INTERMOLECULAR CONTACTS ANALYSIS AS A NOVEL APPROACH FOR PHARMACOPHORE MODELING BASED ON X-RAY OR HOMOLOGY-MODELLED LIGAND-RECEPTOR COMPLEXES

Mamon Hatmal
The Hashemite University, Jordan

We previously combined molecular dynamics (classical or simulated annealing) with ligand-receptor contacts analysis as means to extract valid pharmacophore model(s) from single ligand-receptor complexes. However, molecular dynamics methods are computationally expensive and time consuming. Here we describe a novel method for extracting valid pharmacophore model(s) from a single crystallographic structure or form homology modelled structure within reasonable time scale. The new method is based on ligand-receptor contacts analysis following energy relaxation of predetermined set of randomly deformed complexes generated from the targeted crystallographic structure. Ligand-receptor contacts maintained across many deformed/relaxed structures are assumed to be critical and used to guide pharmacophore development. This methodology was implemented to develop valid pharmacophore models for different enzymes (i.e., PI3K-γ, and Akt3). The resulting pharmacophore models were validated by receiver operating characteristic (ROC) analysis against inhibitors extracted from CHEMBL database. Additionally, we implemented pharmacophores extracted from PI3K-γ to search for new inhibitors from the national cancer institute list of compounds. The process culminated in new PI3K-γ/mTOR inhibitory leads of low micromolar IC50s.

Biography

Dr. Ma'mon Hatmal has a PhD in Philosophy of Biochemistry and Molecular Biology with Honors from the University of Southern California (USC), USA (2012). He is now an assistant professor and a researcher at the Hashemite University/ Jordan. He was a Fulbright post-doctoral researcher at USC, USA (2017). He received many awards for his performance and research (i.e., Philadelphia University International award for best scientific software). His current research interests focus mainly on bioinformatics, in particular computer-aided molecular design and discovery towards new bioactive compounds, and computational prediction of 3D structures of biological macromolecules. He published couple of novel approaches of combining molecular dynamics (classical, simulated annealing, and stochastic deformation/relaxation) with contact analysis to extract valid pharmacophore model(s) from a single crystallographic structure within a reasonable time scale, these approaches culminated in new inhibitory leads (against enzymes involved in cancer and other diseases) of low micromolar and sub-micromolar IC50s.

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DIFFERENTIATION OF MESENCHYMAL STEM CELLS INTO NUCLEUS PULPOSUS LIKE CELLS INDUCED BY CO-CULTURE SYSTEM AND HYPOXIA

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Introduction: Intervertebral disc (IVD) is closely related to low back pain, is the major cause of disability worldwide, with more than 84% population experiencing pain in their life time. Biological and cell based therapies are on progress as an optional treatment for IVD degeneration. Many studies have also revealed that mesenchymal stem cells (MSCs) can also be differentiated into nucleus pulposus (NP) like cells phenotype. This study aimed to determine the newly defined healthy NP cells markers; HIF-1α, HIF-2α, GLUT-1, Shh, Brachyury, Aggrecan, Collagen II, Carbonic anhydrase 3, Carbonic anhydrase 12, CD24, Cytokeratin 8, Cytokeratin 18, and Cytokeratin 19 of Sprague-Dawley rat, whether these markers can be expressed in MSCs under co-culture condition and could be identified the differentiation of MSCs into NP-like cells.

Methods: NP cells and bone marrow derived MSCs from Sprague-Dawley rats were cultured under normoxic medium at 21% O_2 and 5% CO_2 at 37˚C and hypoxic medium at 2% O_2, 5% CO_2, 93% N_2 at 37˚C and MSCs were co-cultured with NP cells supernatant with the concentration of 50% and 100% for 7 days under both normoxic and hypoxic medium. Differentiation of MSCs and expression of recommended newly defined young healthy NP cells phenotypes were evaluated by quantitative real-time PCR (qPCR), Western blotting and immunofluorescence staining microscopy. The results were determined among the groups using unpaired Student's t-test. p-values<0.05 considered significant.

Results: MSCs co-cultured with the concentration of 50% NPcs supernatant; only collagen II showed the increased expression while with the 100% NPcs supernatant; brachyury, collagen II, Glut-1, KRT18 and KRT19 showed higher expressions under normoxic condition compared to MSC control. Under the hypoxic condition, MSCs co-cultured with 50% NPcs supernatant, HIF-2α, Glut-1, aggrecan, collagen II, shh, KRT8, KRT19, CA3, CA12 and CD24 showed increased expression compared to MSC control. More importantly, MSCs co-cultured with 100% NPcs supernatant under hypoxic condition, HIF-1α, HIF-2α, Glut-1, aggrecan, collagen II, shh, brachyury, KRT8, KRT19, CA3, CA12 and CD24 showed upregulated increased expressions compared to the MSC control, which showed that NP cells can stimulate MSCs differentiation to NP-like cells with paracrine interaction between MSCs and NPcs under co-culture condition.

Conclusion: This study suggested that MSCs were successfully differentiated into NP-like cells, which may be used as an ultimate cell-based therapy for IVD regeneration.

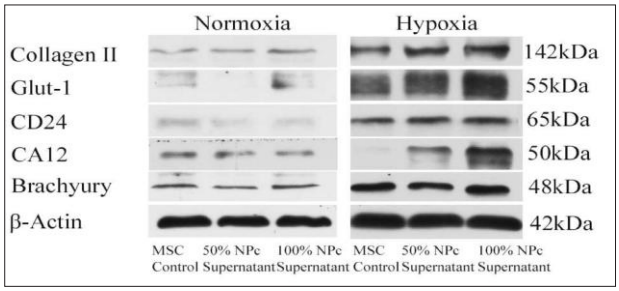

Fig. 1: Relative protein expression by Western blot in MSCs co-cultured with NPcs supernatant (50% and 100% concentration) under normoxia and hypoxia conditions for 7 days. Protein expression for each samples were normalized with housekeeping gene β-actin

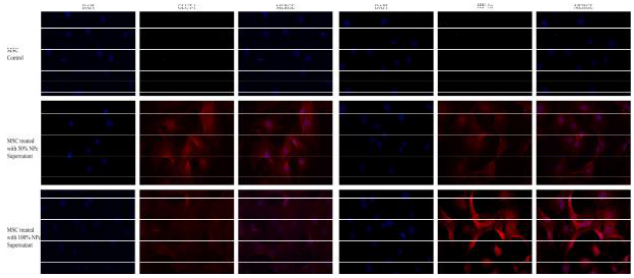

Fig. 1: Relative protein expression by immunofluorescence staining in MSCs co-cultured with NPcs supernatant (50% and 100% concentration) under hypoxia conditions for 7 days

Biography

Arjun sinkeman, has completed his Master's degree in Orthopaedic Surgery from Southeast University and now pursuing his PhD study in the same university. He has more than 5 scientific publications. He has participated in the 20th Asia Pacific Orthopaedic Association Congress, Turkey- Oral Presentation; the 12th International Congress of Chinese Orthopaedic Association, China- E-Poster Presentation; A O Spine Advanced Symposium, China- Controversial Case Discussion Forum; Orthopaedic Research Society 47th International Musculoskeletal Biology Workshop, USA- Poster Presentation; International Symposium on Life Science & Biological Engineering, Hong Kong- Oral Presentation; International Symposium on Life Science & Biological Engineering, Japan- Oral Presentation and the 10th International Congress of Chinese Orthopaedic Association, China. He holds the active Memberships of A O Spine, Orthopaedic Research Society and North American Spine Society. He is awarded with the Chinese Government Scholarship for outstanding international students; Nanjing Municipal Government International Students Scholarship; 1st Category Southeast University Scholarship and Chinese Government Scholarship.

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UNFOLDED PROTEIN RESPONSE EXERTS CYTOPROTECTION AND PROMOTES THE PROLIFERATION OF NUCLEUS PULPOSUS CELLS IN TNF-α STIMULATION BY ACTIVATING NF-αB

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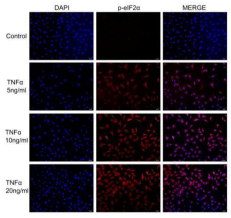
Background: Intervertebral disc (IVD) degeneration is a degenerative disease closely related to inflammation of nucleus pulposus (NP) cells. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine which induces NP cells (NPCs) apoptosis and accelerate IVD degeneration (IDD). The endoplasmic reticulum (ER) serves several important cell functions, which are essential for normal cell function and survival. Nuclear factor-kappa B (NF-κB) is important for genes involved in cell survival, adhesion, and proliferation. However, the roles of ER stress and NF-κB in IDD remain to be elucidated. This study aims to clarify the roles of NF-κB and ER stress related unfolded protein response (UPR) in TNF-α-induced biological changes in rat NP cells and IVD degeneration.

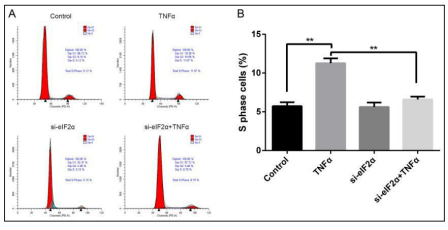
Methods: We cultured rat NPCs with different concentrations of TNF-α, with or without UPR and NF-κB pathway small interfering RNA (siRNA). The protein levels of UPR markers (XBP1s and p-eIF2α) and p-p65 were measured by immunofluorescence staining and Western blot analysis and were used to monitor UPR and NF-κB, respectively. Cell proliferation was evaluated by CCK-8 assay, cell-cycle analysis and cyclin proteins expression. Apoptosis was detected by flowcytometry, TUNEL staining and Western blot analyses. All the data were expressed as mean±SD and from multiple independent experiments. The results were compared among different groups by using unpaired student's test. P-values<0.05 were considered significant.

Results: TNF-α induced the apoptosis of some NPCs in the early stage and then accelerated the proliferation of surviving cells. In addition, TNF-α stimulus up-regulated XBP1s, p-eIF2α and p-p65 at the protein level, which indicated that TNF-α activated UPR and NF-κB signals in rat NP cells. However, these effects could be reversed by UPR and NF-κB siRNA, and UPR interference decreased the expression of p-p65 notably. In parallel, both UPR and NF-κB interference reduced cell proliferation and enhanced apoptosis.

Conclusions: Our study demonstrated that UPR reinforces the survival and proliferation of NPCs in TNF-α stimulus by activating NF-κB signalling, which could be an important therapeutic target in the future.



After treatment with different concentrations of TNF-α for 24h, the expression of UPR marker p-eIF2α was significantly up regulated by Immunofluorescence staining



The effect of UPR on NPCs proliferation. (A) The percentage of S phase population cells was measured by flowcytometry after eIF2α silencing under TNF-α stimulus. (B) eIF2α interference reduced cell proliferation in TNF-α significantly. (*p < 0.05)

Biography

Lu Chen has completed his Bachelor's and Master's degree in the School of Medicine, Southeast University and worked in Orthopaedic department as a Surgeon for 3 years. Now he is further pursuing his PhD in Southeast University, and doing the basic research work in the field of Degenerative Disc Disease. He has published more than 5 papers in reputed journals. He has participated in the 12th International Congress of Chinese Orthopedic Association (COA), China E-Poster Presentation; International Symposium on Life Science and Biological Engineering (ISLSBE), Hong Kong- Oral Presentation.

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PREIMAGINAL EXPOSURE TO AZADIRACHTIN AFFECTS DIGESTIVE ENZYMES IN ADULTS OF *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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Among the plant derived product, azadirachtin, a neem-based insecticide, is exceptional in having a broad range of bioactivity including toxicity, growth, development and reproductions effects, repellency and antifeedancy. If considerable progress on the physiological and biological activities and agricultural application of azadirachtin had been achieved, its exact mechanism of action remains uncertain. In this study, we evaluated lethal and sublethal effects of azadirachtin on digestive enzymes of *Drosophila melanogaster* as biological model. The bio-insecticide was applied topically on early third instar larvae (L3) at two doses, DL_{25} (0, 28 µg) and DL_{50} (0, 67 µg). Results showed a clear disruption of digestive enzymes activities responsible for the broken down of dietary components before its absorption by the intestinal epithelium. Indeed, an inhibition of α-amylase, chitinase and protease activities and an increase of lipasic activity were noted. These results may reflect interference of azadirachtin with regulation of metabolism, and provide some evidence of a long term antifeedancy and delayed effects through developmental stage which may reinforce the insecticidal activity of this bioinsecticide.

Biography

Maroua Ferdenache was awarded a Master's Degree in Biology by the University of Annaba (Algeria), graduated with first class honors and the winner of the doctoral competition. Last year, she got a scholarship to Paris-Saclay University. Now she is a first year PhD student at the French National Center for Scientific Research (CNRS) in Paris (France). She participated in 4 conferences and till now she have 2 papers in reputed journals and preparing another one.

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