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Interdiciplinary

Unknown Medical Condition

Suppresses Osteoblast Apoptosis

Highlights

Classical Approach to Security

Teaching Two Dimensional Motions

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 3 VERSION 1.0

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Interdisciplinary

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Market Dynamics: A Classical Approach to Security Price Movements

By Joshua F. Dayanim
Market Dynamix, United States

Abstract- The recently introduced Market Dynamics method demonstrates parallelisms between security price indicators and their physical counterparts. Specifically, the security price is viewed as a potential energy density, and events such as earnings releases as forces that affect security prices. This dynamic representation of security price movements is extended and applied towards developing an event driven approach for measuring security price movements and the associated price charts. A conservation of capital principal further underlines the central role of capital flow in the formation of support levels. This classical approach to security price movements enables access to a vast pool of existing scientific knowledge and opens new insights into analyzing security price movements with potential applications in the fields of finance and investment management.

Keywords: *security, price, dynamic, movement, capital, money flow, conservation, support, divergence.*

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Market Dynamics: A Classical Approach to Security Price Movements

Joshua F. Dayanim

Abstract- The recently introduced Market Dynamics method demonstrates parallelisms between security price indicators and their physical counterparts. Specifically, the security price is viewed as a potential energy density, and events such as earnings releases as forces that affect security prices. This dynamic representation of security price movements is extended and applied towards developing an event driven approach for measuring security price movements and the associated price charts. A conservation of capital principal further underlines the central role of capital flow in the formation of support levels. This classical approach to security price movements enables access to a vast pool of existing scientific knowledge and opens new insights into analyzing security price movements with potential applications in the fields of finance and investment management.

Keywords: security, price, dynamic, movement, capital, money flow, conservation, support, divergence.

I. Introduction

Historically, various hypothesis have been developed in rationalizing the observed behavior and movements of security prices. The Efficient Market Hypothesis introduced the notion that price fully reflects all available information about a security at its equilibrium state (Fama 1970). This suggests a demand and supply approach to the financial market. The Rational Expectations Hypothesis proposes that market participants act rationally with respect to the available information, possibly without fully realizing their role (Muth 1961). Behavioral finance further considers how various agents act based on social, emotional, and cognitive factors potentially creating market inefficiencies that can be exploited (Shefrin 2005, Kukacha and Barunik 2013).

At the same time, the premise that security prices follow repeating and identifiable patterns has long been a mainstay of technical practitioners in the investment field but this approach has suffered from the lack of a supporting theoretical framework (Edwards and Magee 2001). More recently, behavioral finance appears to signal that such human factors as herding, over-confidence, and market sentiment contribute to irrational behavior and inefficiencies in the financial markets, factors that may contribute to the observed price trends (Shefrin 2005).

It is apparent that a foundational theory describing the movement of security prices is desirable and can lead to a better understanding of factors influencing security price movements as well as support improved prediction of their anticipated future direction. Utilizing concepts from the classical science of motion, Market Dynamics applies a partial differential to the price equation and introduces a conservation of capital principal (Dayanim 2011). The approach incorporates tangible factors such as earnings along with behavioral aspects of various agents in their aggregate form as forces driving price movements. In a manner similar to the efficient market hypothesis the conservation of capital principal leads to an equilibrium condition that is achieved over a span of time as capital from investor trading activities flows into a security. Price uncertainty may be encountered during this transitional period resulting in temporary separation between the stable equilibrium price and the observed market prices, indicating potential market inefficiencies.

The theoretical framework for the dynamics of price movement is first introduced including the price equation, the conservation of capital principle, and several indicators useful in measuring price movements. The methodology is then applied to sample securities demonstrating close match to observed market pricing and highlighting its potential application in analyzing security price movements and identifying market price inefficiencies.

II. Dynamics of Price Movement

a) The Price Equation

Movements in security prices are often attributed to specific events, whether fundamentals based such as earnings surprises, or behavioral such as changes in investor sentiment (Thomsett 1998). The price of a security is defined as a product of it earnings per share (EPS) and Price to Earnings Ratio (PE) as follows:

$$P = EPS.PE \tag{1}$$

The impact of an external event can be measured in terms of changes in a security's Earnings Per Share and Price to Earnings ratio. The expected price change is obtained through a partial differentiation of the price equation with respect to time, that is:

$$\Delta P = \Delta EPS.PE_0 + EPS_0.\Delta PE \tag{2}$$

$$P_T = P_0 + \Delta P \tag{3}$$

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where ΔP is the expected price change resulting from changes in EPS or PE attributed to the event; P_0, EPS_0, and PE_0 are the initial stable values just before the event's onset; and P_T is the new target price.

b) Conservation of Capital

Previously, it has been demonstrated that parallels can be developed between security price movements and the classical science of motion (Dayanim 2011). The Conservation of Capital principal states that changes in market capitalization of a security must be matched by an equal flow of capital from investor trading activities. The change in market capitalization of a security can be written as:

$$\Delta MC = S.\Delta P \quad (4)$$

where ΔMC represents the change in market capitalization and S is the number of issued shares.

Separately, the investor trading activities can be measured as the accumulated flow of capital into a security as follows:

$$MF = \sum_{n=1}^{N}\{s_n.\Delta P_n\} \quad (5)$$

where Money Flow MF is the change in accumulated flow of capital attributed to investor trading activities, s_n is the number of shares exchanged in an individual trade transaction, ΔP_n is the difference between a buyer and a seller's per share cost for such transaction, and N is the number of completed transactions during the observation period. Contributions from individual trade transactions are added in order to obtain the accumulated change in investor capital. This analysis ignores any transaction related costs in the form of ask-bid spread or trade execution fees.

Assuming an initial stable price point, an equality is defined between the left hand sides of equations (4) and (5) framing the conservation principal for the event simply as:

$$\Delta MC = MF \quad (6)$$

The latter effectively states that a change in market valuation of a security should be supported by a corresponding level of investor trading activities or flow of investor capital that fully accounts for the observed price movement.

Of particular interest to this discussion is the nature of capital and its relationship to investor activities. As a security's price rises or falls, its market capitalization can fluctuate greatly in a relatively short span of time due to trading activities in a small portion of its shares. There appears to be an imbalance between the level of investment capital flow due to trading activities and the observed movement in the security's market valuation. This leads to the notion of an event's Time Horizon, or the elapsed time after an event's onset until such time when sufficient investor capital has been accumulated to account for the corresponding change in market capitalization. At that time a new equilibrium point is reached at the target price level.

c) Superposition of Events

It is not unusual to encounter multiple events with separated or overlapping time horizons such as consecutive earnings releases, or earnings releases combined with PE movements reflecting changes in investor behavior. As equation (2) demonstrates, the change in price is additive and proportional to the change in the underlying force such as earnings thus indicating a linear system. Using the Superposition principal of linear systems the individual contributions from multiple events can then be added to obtain the aggregate impact (Serway and Jewett 2013). The outcome from two independent events remains the same whether they are separated in time or partially overlapping. In this manner, contributions to price from each event is individually added over time to the original starting price in order to measure and track expected and target prices throughput the observation period.

d) Aggregate Securities

The approach can be extended to a group of securities such as an exchange or industry index by aggregating the individual results from each listed security in the group. Hence, market capitalization values and earnings of the underlying securities are grouped and added in order to obtain the price and earnings data for the aggregate security. For example, the aggregate money flow starting from a stable initial price point for the aggregate security is measured by summing the individual contributions as follows:

$$MF = \sum_{m=1}^{M} MF_m \quad (7)$$

where M is the list of underlying securities in the index.

e) Dynamic Indicators

Markets frequently over- or under-react to an event resulting in changes in market capitalization that materially diverge from the observed flow in investor capital. In order to analyze the price behavior, several indicators are defined that are useful in measuring the expected price and the accumulated flow of investor capital for an event over time.

The Support indicator is a ratio of the accumulated investor capital at time t over the observed change in market capitalization, and is measured as follows:

$$Support(t) = \frac{MF}{\Delta MC} \quad (8)$$

As the support ratio approaches 1 an equilibrium state is attained and a stable and fully supported price level is formed at the target price level. These points correspond to the observed price resistance or support levels in technical analysis which describe levels at which price is stabilized in the

aftermath of a decline or rise in a security's price (Murphy 1999).

The event's Time Horizon can be estimated by measuring the progress rate towards the equilibrium state by dividing the elapsed time t into the current support ratio, that is:

$$T(t) = \frac{t}{Support\ (t)} \quad (9)$$

This estimation assumes a linear change in the Support ratio over time, although alternative non-linear estimations may also be used. The event time horizon may vary widely in duration with shorter intervals indicative of a broader level of investor interest and higher trading volumes.

The Expected Price for a security can be similarly stated from equation (3) by using the Support ratio as a progress indicator, as follows:

$$P_E(t) = P_0 + \Delta P.Support(t) \quad (10)$$

The Expected Price can be viewed as a moving Support Line and represents the price level currently supported by the accumulated flow of investor capital. The support line touches the security's target price line at the equilibrium point where the change in market capitalization equals the accumulated investor capital flow.

Divergence represents the gap or spread between the target price and the current market price. In this manner, the Divergence indicator measures the remaining appreciation potential in price using the following equation;

$$Divergence(t) = \frac{P_T - P(t)}{P(t)} = \frac{\Delta P_T}{P} \quad (11)$$

As the price moves towards the target level, the divergence approaches 0. A negative divergence value indicates an expected price drop, while a positive value indicates an expected rise. Divergence may also be viewed as the current rate of price change representing the upward price pressure.

Conversely, Expectance is defined as the separation gap between the expected price and the current market price. The Expectance indicator can be measured as follows:

$$Expectance(t) = \frac{P_E - P(t)}{P(t)} = \frac{\Delta P_E}{P} \quad (12)$$

Expectance represents the potential price exposure due to a lack of sufficient investor capital flow. It often acts as a retarding price pressure and a counter-balance to the investor enthusiasm that is driving the change in market capitalization. In contrast, a positive Expectance value may indicate a cooling condition where the price has temporarily dropped below the support line.

After an event's onset the price is expected to move along the support line during the event's time horizon as investor capital flow continues to accumulate. Price then reaches the target price at the equilibrium point. However, investors may react to the event by pushing the price to near or past its target level in a short span of time. This can create a negative price pressure and result in price volatility caused by a lack of sufficient accumulated investor capital. For securities with a high level of trading activity and investor interest this period may be short in duration, while for others it may span many months.

III. Estimation Methods

a) Money Flow

The calculation of investor capital flow poses a clear challenge due to the complexity involved in gathering all historical trade transactions for a security, obtaining the buyer's original purchase cost, and summing up the individual capital flow contributions. However, this activity can be estimated by summing the product of daily trade volume and price change over the covered period, as follows:

$$MF = \sum_{t=1}^{T} V(t).\{P(t) - P_0\} = \sum_{t=1}^{T} V(t).\Delta P_0 \quad (13)$$

where V(t) is the daily trade volume, P(t) is the daily close price, P_0 is a stable starting price, and T is the number of elapsed days. This approach effectively uses the stable starting price as the cost basis for all trade transactions during an event's time horizon. The estimation relies on a capital flow transfer or leveling process where the accumulated capital flow from individual trade transactions are distributed across all issued shares of a security.

b) Price to Earnings Ratio

Historical PE values may be used in measuring expected price change by using averaged or weighted PE values for a set number of days, for example by using a 30 day running average. This approach while straight forward and reasonably adequate for most analysis suffers from a delayed response to changes in PE. While there is currently no method of modeling PE using its underlying behavioral factors, an alternative dynamic estimation is available by using equation (2) as follows:

$$\Delta PE = \frac{\Delta P - PE_0.\Delta EPS}{EPS_0} \quad (14)$$

$$\Delta PE = \frac{\Delta MC/S - PE_0.\Delta EPS}{EPS_0} \quad (15)$$

which uses the relationship between price and market capitalization where S represents the number of outstanding shares in the security. The conservation of capital principle states that the money flow attributed to an event must equal the change in market capitalization of the security once a stable and supported price level is established. As new investment accumulates over time

with each trade transaction, the expected change in PE at time t following an event may be restated using a time variant money flow allowing its direct measurement, as follows:

$$\Delta PE_E(t) = \frac{MF(t)/S - PE_0.\Delta EPS}{EPS_0} \quad (16)$$

c) Earnings Per Share

Earnings data is typically available for each financial reporting quarter for each traded security. Since earnings data may not be publicly available for some time after the end of the covered earnings period, it can impact or delay the earnings event's timing.

IV. Applied Market Dynamics

a) A Dynamic Model

The dynamic pricing method has been implemented using a computer system for securities listed on U.S. exchanges including New York Stock Exchange (NYSE), NASDAQ, and American Exchange (AMEX) for which historical price, volume, and earnings data was readily available. Figure 1 demonstrates the price movement for a sample security, Agilent Technologies, for a nine month period beginning on July 1, 2010 and ending June 1, 2011. Starting at a relatively stable price level, an upwards pattern emerges as corporate earnings increase starting around September 1, 2010. The PE ratio remains relatively consistent and hovers in the 18 to 21 range. The target price is regularly revised due to movements of EPS and PE values and appears as a stepped ladder, while the expected price forms a smooth line and is calculated using the accumulated investor capital flow and trends up until it finally reaches the target price and forms a stable point in the first quarter of 2011 as shown by the diamond marker.

Fig. 1: Agilent Technologies Price Channel

Figure 2 displays the price to earnings ratio for the same time period that is calculated monthly by averaging the prior 30 day values. Earnings values are also recorded at the end of each quarterly release period without consideration for when the data was actually reported.

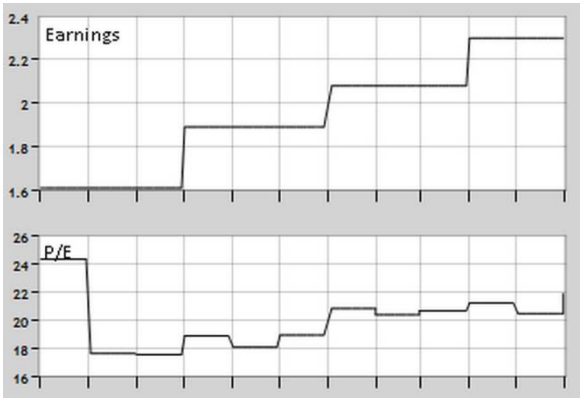


Fig. 2: Agilent Technologies Earnings and PE

As viewed in Figure 3, Expectance is negative during much of the price trajectory and reaches zero once the target price is reached. This negative pressure counter-balances the investor enthusiasm that is driving the change in market capitalization. A Price Channel is formed in the area between the Expected and Target prices wherein one would expect to observe the market price line. A rising price channel indicates a bullish pattern, while a dropping channel indicates a bearish sentiment. In a bearish pattern the Target and Expected price lines switch positions with the Target price forming the lower boundary of the price channel. In such case, the Expectance value tends to be positive and pointing towards the support line.

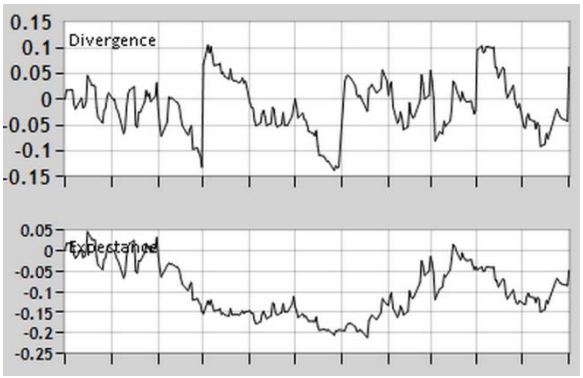


Fig. 3: Agilent Technologies Divergence and Expectance

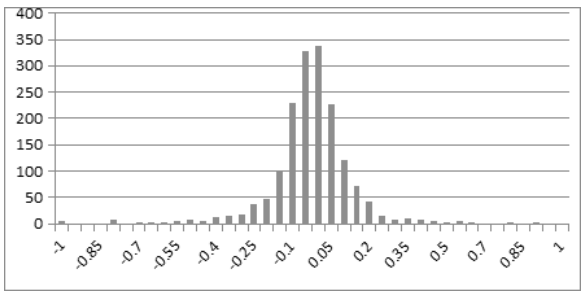
The model is extended by aggregating the results for the NYSE listed securities. In total around 1,650 securities were used for which earnings data was readily available. For this analysis market capitalization values and corporate earnings of the underlying securities are separately grouped and added in order to obtain the price and earnings data for the aggregate security. The investor capital flow is also estimated by using equation (13) and summing the product of daily trade volume and price change for each underlying security, as follows:

$$MF = \sum_{t=1}^{T} \sum_{m=1}^{M} V_m(t).\Delta P_{m0} \quad (17)$$

where M represents the number of underlying securities and t is the elapsed days from a stable initial price point for the aggregate security. Figure 4 displays the related price chart using a two year observation time period.

Fig. 4: NYSE 8/15/2012-9/20/2014

The price values are normalized for display purposes by dividing into the initial market capitalization value. The chart exhibits a similar price channel to that observed for an individual security. Figure 5 shows a histogram of Divergence values for the covered securities on NYSE with a possible Divergence range between -1 and +1. The simulation results indicate a mean value close to zero and a standard deviation of 0.153 for November 15, 2013. Divergence appears evenly split on both sides of the market price line forming an approximate bell curve distribution. A 10% average deviation is observed between the securities' market and target price points with over 95% falling within a 30% range of their target as prices continue along their individual movement paths. The aggregate market price line continues to stay reasonably close to the price channel after the iterative application of the model over a 2 year time period. As may be expected, the price movement chart is sensitive to the choice of the start date due to the differential nature of the dynamic pricing method and reliance on a stable starting price point.



Mean	Median	Standard Deviation	Average Deviation
-0.026	-0.022	0.153	0.099

Fig. 5: NYSE Divergence 11/15/2013

Observations of the individual and aggregate charts appear to confirm the effectiveness of the dynamic pricing method in modeling expected and target prices using historical price, volume, and earnings data. The method also identifies inefficiencies between expected and observed security prices with potential application in optimizing investment portfolios.

b) Forecasting Price Movements

The discussion thus far has focused on investigating historical prices based on known information about a security. However, the dynamic pricing method can also be applied towards forecasting future price movements by utilizing projections of quarterly earnings and PE values. Since such forecasts depend upon future estimates they are subject to a greater level of risk and uncertainty.

V. Conclusion

This study expands on recent classical approach to security price movements by presenting an event driven dynamic pricing method. The approach starts with the price equation and introduces a conservation of capital principle. This leads to a key finding that changes in market capitalization must be matched by an equal flow of investor capital which accumulates over time with successive investor trade transactions. Once the flow of investor capital matches the observed change in market capitalization an equilibrium condition is reached representing a stable and fully supported price level.

Several dynamic price indicators are developed for measuring the level of price support due to accumulated capital flow, the target and expected prices over time, and the separation gap from observed market prices. The time based aspect of the formulation results in a window of time where price can fluctuate between the expected and target price lines until it converges to a stable price at the equilibrium point. The event driven aspect of the formulation creates an opportunity to take into account the available data about the security such as price, volume, and earnings, as well as less tangible aspects such as investor sentiment and behavior. While a model for the latter behavioral factors was not presented here, their contribution was aggregated into a single factor that directly influences the price movement. The Market Dynamics method was further applied to actively traded securities and exchanges demonstrating it closely tracks the observed market price movements over a long span of time.

The method provides needed insight and understanding of the mechanisms responsible for security price movements. The classical approach enables access to a vast pool of existing scientific knowledge with its potential application to the fields of finance and investment management. The Market Dynamics method may be extended to any security or

market with an orderly clearance of trade transactions, where intrinsic price values can be associated with the underlying traded commodities and goods. The valuations should follow a linear price equation that factors in the underlying market and human elements.

Biography

Joshua Dayanim is the founder of Market Dynamix, a website dedicated to providing investor information and education on Market Dynamics. As an independent investor, he has studied various approaches to security pricing analysis and investment management. This eventually led to the development of Market Dynamics, providing a model for security pricing movements and formation of support and resistance levels. He holds Masters degrees in Business Administration and Electrical Engineering, with an undergraduate focus in Physics. He can be reached at jdayanim@mdynamix.com.

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Extreme Nonlocality Exhibited in an Unique Individual Born with an Unknown Medical Condition

By Elgin Ong

Introduction- I am from Singapore and alumni of the University of Tasmania and I am doing an independent study on the current evolutionary patterns of homo sapiens.

I have actually met a person with a mysterious medical condition but who refused to see a doctor and was why it is so undocumented. I am unable to find anything like this online and in research archives.

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Extreme Nonlocality Exhibited in an Unique Individual Born with an Unknown Medical Condition

Elgin Ong

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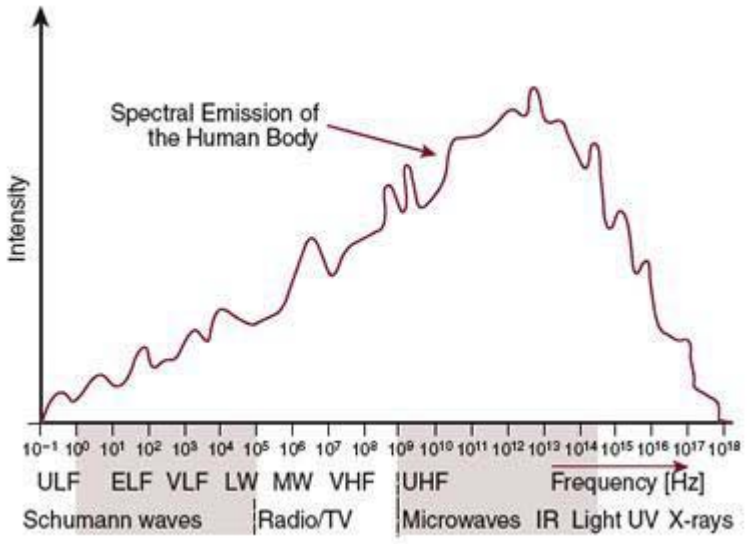

Figure 1: The power spectrum of the human body emission

I have actually met a person with a mysterious medical condition but who refused to see a doctor and was why it is so undocumented. I am unable to find anything like this online and in research archives.

He is able to detect human beings like an electric fish(not a good example), which is called 'passive electrolocation'. However, this person generates no electricity or light; everything is invisible. There are no electroreceptors in his body and I am trying to figure out how particles in his cells are able to be released over incredible distances from his body.

He is able to detect human beings just by detecting their body heat. I am studying infrared sensing in animals; Snakes, vampire bats, bed bugs and beetles. At night, the pit organs allow snakes to 'see' an image of their predator or prey — as an infrared camera does — giving them a unique extra sense, up to a metre away. Nerve cells in the pit organ contain an ion channel called TRPA1 — an infrared receptor. The vampire bat locate their 'warm-blooded' prey with the help of three heat-sensitive pits on their nose that are thermally insulated from the surrounding tissue. Analogous to snakes and vampire bats, the blood-sucking bed bugs employ this sensory modality to help locate their 'prey', evidently using a cave-like organ situated on the antennae.

Amongst the hymenopterans, a parasitoid braconid wasp possesses a peculiar type of antennal sensillum that is inferred to be a wave-guide for infrared perception and might play a role in finding a potential host.

Three species (Melanophila acuminata, Merimna atrata and Acanthocnemus nigricans) of beetles have been shown to use this capacity for the detection of forest fires, not to facilitate escape but to lay their eggs in newly burnt wood. Despite this shared

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function, their infrared-detecting organs are very different, with M. atrata showing interesting similarities to the boid snakes and A. nigricans converging on the crotaline snakes. Interestingly, it has recently been shown that the equally pyrophilous Australian flat bug Aradus albicornis has independently evolved infrared receptors that resemble those of Melanophila beetles.

In terms of distance, I am not sure which of the species can detect over the longest distance except for the snake which is only up to a metre away. However, through my observation, this person's range could be beyond the earth's atmosphere and if you imagine it as a vector with him as a centre, from Singapore to Canada.

There is more to just passive electrolocation and the incredible range of his properties. He could even encapsulate the body heat of his victim and do harm to their blood vessels using a complex function of his brain which I am still figuring out.The potential of this harm is so huge that it could be fatal to anyone.

Many male animals have evolved exaggerated traits that they use in combat with rival males to gain access to females and secure their reproductive success. Though some male animals invest in nuptial gifts that gains them access to females. For example, males in the dung beetle *Onthophagus nigriventris*, like those of many other species in this genus, fight over tunnels that have been excavated beneath dung by females. Males spar awkwardly with their spear-like thoracic horns as they battle over the entrances to these tunnels, and winners mate repeatedly with the female inside the tunnel. This species inhabits cool, moist pastures along mountainsides in East Africa such as the rim of the Ngorongoro Crater, and introduced populations thrive in high, cool pastures of Eastern Australia and Hawaii. The giant Japanese rhinoceros beetle (*Trypoxylus dichotomus*) flies at night throughout much of East Asia, converging on sap flows on the sides of Fraxinus and other host trees. Males fight over these feeding sites and mate with females as they feed. The stag beetle *Cyclommatus metallifer* also lives in East Asia and fights over sap flows on the sides of host trees, and many aspects of their mating system and diet are similar to those of *T. dichotomus* (yet the weapon, in this case, comprises a massively enlarged pair of mandibles rather than a rigid outgrowth horn).

"Every animal relies on a weapon of some kind- cats have claws, eagles have talons, even the dogs we keep as pets have a respectable set of teeth. In rare cases, we find species whose weapons have become stunningly outsized, some with tusks so massive that those who wield them look like they should collapse under the weight."-------Douglas Emlen.

This is a very brilliant design by nature as his weapon is hidden in his body and the nonlocality invisible and unfelt by bystanders.

External investigation of this phenomenon can only be done if we know the properties of this unknown particle(energy field) around his body. Perhaps, using serendipity in physics and construction of a detector. The medical examination could include utrasound of the brain and body, fMRI, DNA full genome sequencing or specific genes/genomes test, massive parallel sequencing, EEG, etc.

II. Analogy

A "weapon system" is actually evolved in his body. You must think of him as the centre of this massive gigantic infinite field (illustrated below). The victim's body heat came into this magnetic field and was detected by him. He is then able to encapsulate this heat and form a capsule around the "weapon system". It is like a camera that has snapped a 360 degree spherical image of the victim. Therefore, he can only encapsulate one person at a time.

Once you understand that you will know that he can feel the capsule's body heat including the heat from the victim's blood. I am still figuring out the exact use of this "weapon system" to pull/push the victim's blood vessels. However, the heat from the blood is able to let him know it must be the victim's blood vessels.

The victim could feel his blood vessels tearing from his body as if got someone inside him doing that. This is what I meant by a capsule which the perpetrator has around the "weapon system" inside his body. The victim is actually his capsule.

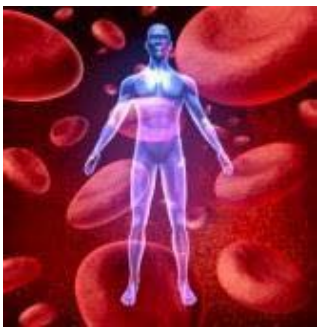


Figure 2

Take a look at this image. He is just like this person (the "weapon system" is actually an exact reproduction of this person in his body whose only function is to do harm to the victim; or what some scientists might call an indentical conjoined twin which are sometimes born inside the other twin's body) inside the victim's body which are all red blood cells. He can feel the heat from the red blood cells and manipulate his blood vessels.

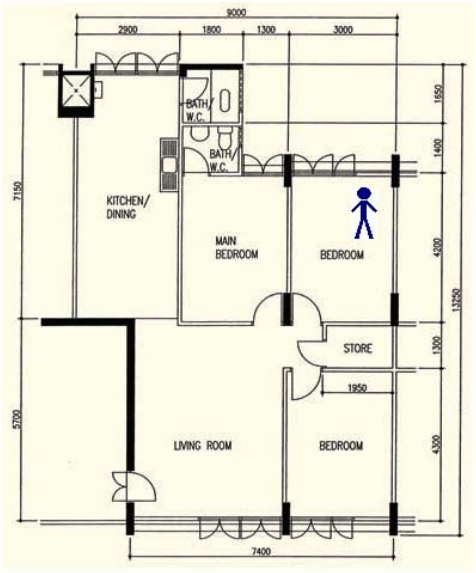
I am unable to find a comparative example for this part of his weapon system in the animal kingdom. This part of his "weapon system" exhibited extreme nonlocality in a human individual.

Nonlocality in the biological world can make reference to quantum psuedo-telepathy, remote

viewing, energy medicine, external qigong, the comparative animal examples here and also Figure 1. Figure 1 could also identify the sources of his unknown energy.

In physics, nonlocality or action at a distance is the direct interaction of two objects that are separated in space with no perceivable intermediate agency or mechanism. They can make reference to gravity and Einstein's "spooky action at a distance" of quantum entanglement.

Below is a rough illustration of this individual's range which can reach the far flung of the universe.



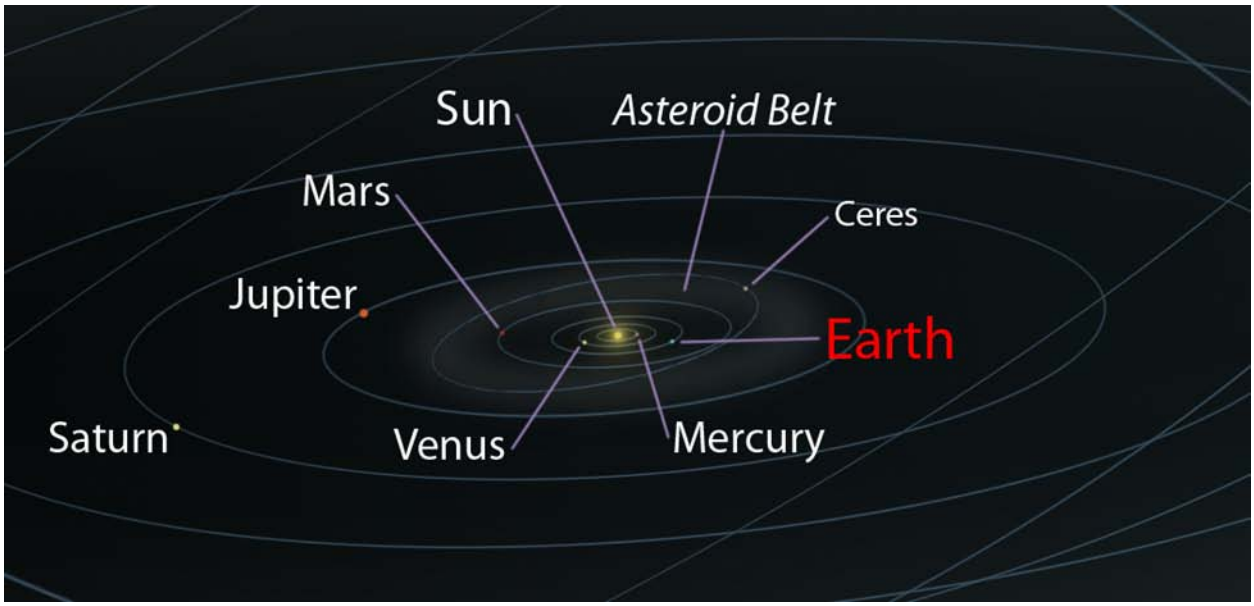


Figure 3

III. Method

The location is a HDB flat in Yishun, Singapore. I have gathered 5 volunteers. All are perfect strangers. 3 will be in the complementary or control group. One will be the human target and one to witness the entire experiment. All have never heard of such phenomenon. The human subject, target and the control group will be blindfolded and earplugged. The human subject will be asked to start his unique function of encapsulation only when he has set down in the room and to release the target when he is about to exit the room. The human subject and the control group will be sitting outside the room and will only take turns entering the room by random assignment.

IV. Test Procedure

Control subject 1 will the first to enter the room and sitting on the chair provided in front of the human target who is sitting down. The target will be asked whether he feels the sensation of any external energy in his body which he previously had not felt. This is the

process of encapsulation. Next, he will be asked whether he feel any pain or the sensation of someone pulling down his blood vessels. This is the unique extra function of the subject to pull/push his victim's blood vessels. The human subject will be 3rd in line to enter the room.

V. Results

In the presence of the human subject, the target could felt an external energy entering and remaining in his body. He could also felt the sensation of someone pulling down his blood vessels. Extreme pain could be felt sometimes. However, after the subject left the room, all pain and sensation all gone and he could feel the external energy leaving the body.

VI. Discussion

This experiment could provide us some insight into the extraordinary properties of this person and for the design of future experiments on this human subject.

a) Genomic Study

A whole genome sequencing was done on a hair sample from the subject in August 2016. They have found that about 46 variants were not assigned any RSIDs. They presume these variants to be novel and potentially rare. After variant calling and snpeff, two variants were predicted to have high impact. Interesting thing about these two variants was that, it was not assigned any RSIDs, hence they might be potentially novel. Out of the 14 moderate impact variants, some were not assigned any RSIDs and do not belong to any documented genes.

RSID - The rs number is an accession number used by researchers and databases to refer to specific SNPs. It stands for Reference SNP cluster ID.

Relevance in Neuroscience, Consciousness, Quantum Biology and Genetics

Ultraweak photon emissions (UPE) have been measured from cells, organs, and organisms, and the precise coupling between molecular pathways and specific wavelengths of these pervasive electromagnetic phenomena have recently been established.

I would like to cite photosynthesis and vertebrate vision in reverse as examples of the subject's extracellular emission. Energy from light is absorbed by proteins called reaction centres that contain green chlorophyll pigments. In plants, these proteins are held inside organelles called chloroplasts. If we think in reverse, there is a possibility that there are proteins and cells in his body that can emit particles that satisfy the conditions of quantum entanglement.

However, photosynthesis is a energy conversion process. Vertebrate vision is based on the absorption of light by photoreceptor cells in the eye. The human retina contains about 100 million such cells.

"Locality or local cause requires each physical event or change in the physical event to have a physical proximity which occupies the immediate space-time of the effect. The concept of nonlocality is implicitly coupled to the occurrence of excess correlations between the temporal variations in matter or electromagnetic patterns in loci separated by significant distances for which there is no obvious physical mediation or substrate for this mediation. Photons, the electromagnetic phenomena that display both particle and wave properties, appear to be central to the demonstration of non-locality. Calculations have been presented to suggest that the quantum of energy associated with the visible range of electromagnetic frequencies (or light) can be related to the "pressure" within the universe (the product of average mass density and the square of the velocity of light, c) from gravitational energies. The value for the upper limit of the rest mass of a photon and the estimated mass of the universe results in a photon density whose average intensities approximate the energies associated with frequencies associated with visible light (~10-19 J) and many of the processes associated with the action and resting membrane potentials of neurons (~10-20 J) that are strongly correlated with cognition.

The direct relevance to the study of consciousness has been apparent since cognition-coupled photon emissions were first measured by Dotta and his colleagues. If photons are strongly correlated with or actually are the physical processes that are identities with thinking and consciousness, then the understanding of the experimental conditions that create "excess correlations" between different loci of space may facilitate the representation of consciousness across these spaces. If consciousness is a field of photons as described by Bokkon (2005), then consciousness as a tensor or dynamic field would be more applicable to traditional models of holographic phenomena. The most unusual property of these phenomena is that the characteristics of the whole are represented within the unit and the characteristics of each unit are represented within the whole.

The technology to create excess correlations between processes separated by non-traditional distances has implications that would alter the future of human exploration of the unknown. If two spaces that are entangled and display properties of non-locality are established, then a change in one space would be associated "instantaneously" with the representation of that change in the other. Presumably the changes could occur, if the two loci were entangled, on the other side of the universe. If this condition were possible, then the delays involved with electromagnetic communications over vast distances would no longer be an impediment. The second implication is that consciousness, as a photonic field, might be represented in the second locus at any distance given the conditions within the two loci

satisfied the conditions for entanglement."-------Michael Persinger, PhD.

A project led by John Novembre of the University of California Los Angeles and Vincent Mooser of UK-based drug company GlaxoSmithKline, reports that more than 95% of variants found by sequencing 202 genes in 14,002 people were rare, and that 74% of the variants were carried by only one or two people in the study.

"Research carried out fifty years ago, showed that the mutant gene had only one man among a thousand, and now five people", explained John Novembre.

The scientists have made an unexpected and unsettling discovery - a large number of new and previously unseen mutations have been detected among humans.

As Prof Paul Davies like to put it, "Viruses are continually infecting organisms on Earth and uploading their DNA into the genomes of existing organisms, so there is a well understood pathway for getting information into DNA. We're littered with it. Our own genomes have got huge amounts of this junk that has climbed onboard from viruses over evolutionary history".

In causal adequacy principle, an object must contain at least as much reality as the object itself, whether formally or eminently. Descartes defends this principle by quoting Roman philosopher Lucretius: "Ex nihilo nihil fit", meaning "Nothing comes from nothing"

Descartes can offer two explanations of his own for this phenomenon:

“Heat cannot be produced in an object which was not previously hot, except by something of at least the same order of perfection as heat.”

To the skeptics, the pain and suffering experienced by the victim cannot be reproduced by any disease or illness (mental or physical).

"A stone, for example, which previously did not exist, cannot begin to exist unless it is produced by something which contains, either formally or eminently everything to be found in the stone."

This can refer to the DNA structure of this human subject, whether it is fetal mutation or some cryptic mutation in his family tree.

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Chi3l1 induction in response to LPS suppresses osteoblast apoptosis

By Huang Liying, Li Yishan & Weng Xiquan
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Abstract- Upregulation of Chitinase-3-like protein 1(Chi3l1), a member of glycohydrolase family 18, is frequently seen in diseases associated with inflammatory responses, such as atherosclerosis, meningitis and asthma. However, little is known about either its regulation or its functions in the physiological and pathological processes in bone and related cells. In the mouse model of osteomyelitis used in this study, Chi3l1 was induced in the infected area. In vitro stimulation of osteoblasts and mesenchymal stem cells (MSCs) by lipopolysaccharide (LPS) resulted in elevated Chi3l1 expression. Overexpression of Chi3l1 attenuated TNF**α**-induced osteoblast apoptosis and promoted cell survival. Furthermore, Chi3l1 induced phosphorylation of AKT in a time-dependent fashion, while an inhibitor of the AKT signaling pathway abolished both the pro-survival and the anti-apoptotic effects of Chi3l1. Therefore, Chi3l1 might play a protective role in infected or inflammatory bone tissues by suppressing osteoblast apoptosis via an AKT-dependent pathway.

*Keywords: AKT, apoptosis, chitinase-3-like protein 1, LPS, TNF**α**.*

GJSFR-I Classification: FOR Code: 060199

Strictly as per the compliance and regulations of :

Chi3l1 induction in response to LPS suppresses osteoblast apoptosis

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Abstract- **Upregulation of Chitinase-3-like protein 1(Chi3l1), a member of glycohydrolase family 18, is frequently seen in diseases associated with inflammatory responses, such as atherosclerosis, meningitis and asthma. However, little is known about either its regulation or its functions in the physiological and pathological processes in bone and related cells. In the mouse model of osteomyelitis used in this study, Chi3l1 was induced in the infected area. In vitro stimulation of osteoblasts and mesenchymal stem cells (MSCs) by lipopolysaccharide (LPS) resulted in elevated Chi3l1 expression. Overexpression of Chi3l1 attenuated TNFα-induced osteoblast apoptosis and promoted cell survival. Furthermore, Chi3l1 induced phosphorylation of AKT in a time-dependent fashion, while an inhibitor of the AKT signaling pathway abolished both the pro-survival and the anti-apoptotic effects of Chi3l1. Therefore, Chi3l1 might play a protective role in infected or inflammatory bone tissues by suppressing osteoblast apoptosis via an AKT-dependent pathway.**

Keywords: AKT, apoptosis, chitinase-3-like protein 1, LPS, TNFα.

I. Footnotes

The abbreviations used are: Chi3l1, chitinase 3-like protein 1; LPS, lipopolysaccharides; mesenchymal stem cells , MSCs; TNFα, tumor necrosis factor-α; DMEM, Dulbecco's modified Eagle's medium; α-MEM , alpha minimum essential medium; RT, reverse transcription; GAPDH, glyceraldehydes 3-phosphate dehydrogenase; PI3K, phosphatidylinositol 3-kinase.

Osteomyelitis, caused by pathogenic bacterial infection [1, 2], is primarily mediated by lipopolysaccharide (LPS) in a manner similar to that described for other infectious bone diseases [3, 4]. In addition to stimulating bone resorption, LPS also exerts adverse effects on bone formation through the induction of cytokine production (e.g., TNFα) [5]. Apoptosis of osteoblasts induced by cytokines is an important contributing factor to bone destruction [6, 7]; therefore, the identification of molecules that could prevent infection- or inflammation-induced osteoblast apoptosis might provide an effective potential anti-osteomyelitis therapy.

Chitinase 3-like protein 1 (Chi3l1), a member of the mammalian chitinase family [8], is induced in many inflammatory diseases, including arthritis, athero-sclerosis, and meningitis [9-11]. Since LPS can trigger inflammation in various tissues, it is reasonable to speculate that LPS might be responsible for the induction of Chi3l1 expression in infected bone and relevant cells.

Although some hypotheses about the functions of Chi3l1 have been proposed, its physiological or pathological role has not been elucidated. Some research indicated that Chi3l1 may have a protective function in response to some types of stress [12]. However, whether Chi3l1 could suppress cell apoptosis, or whether AKT activation mediates the anti-apoptotic effect of Chi3l1, is still unclear. Nonetheless, it has been widely accepted that the AKT pathway plays a critical role in the regulation of cell survival and apoptosis in various cells and tissues [13, 14]. In this study, therefore, we examined the expression patterns of Chi3l1 in bone tissues and related cells under infectious stimulation and tested the hypothesis that Chi3l1 may inhibit osteoblast apoptosis via the AKT pathway.

II. Materials and Methods

a) Generation of the mouse model of osteomyelitis

We generated osteomyelitis in mice using the method described by Yoshii, T. [15]. Briefly, a piece of cotton thread was immersed in an overnight culture of S. aureus for 1 h, then dried for 2 h. BALBc mice were anesthetized and incisions were made in the femurs to insert either this piece of cotton thread or a piece of sterile thread. The mice were sacrificed at the indicated post-infection time points. All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals at Tsinghua University, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals. The femurs were collected for real-time PCR and Western blotting analysis.

b) Cell culture

Primary mouse calvarial osteoblastic cells were isolated from 6-day old mice as described previously [16]. Briefly, calvaria was cleaned and finely minced and then digested by collagenase and trypsin. The released cells were allowed to grow for 48 h and were then subcultured in α-minimum Eagle's medium (α-MEM) supplemented with 10% FBS and replaced every 3 days. Mesenchymal stem cells (MSCs) were obtained from 4- to 6-week-old normal BALBc mice, sacrificed by cervical dislocation to isolate the femurs. The bone marrow was

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consequently harvested by flushing the bone with DMEM containing 10% FBS. The cells were seeded on a culture plate, after 3 days, the non-adherent cells were discarded by replacing the medium. Adherent cells were designated as the first generation MSCs. The medium was changed twice a week, after reaching 90% confluence, the MSCs were detached using trypsin to undergo the subculture process. The third generation cells were used in our experiments.

c) Quantitative Real-time PCR

Total RNA was isolated using Trizol (Invitrogen), and the first-strand cDNAs were synthesized using the SuperscriptTM First-Strand Synthesis System for RT-PCR kit (Invitrogen).

Real-time PCR was performed using the TaqMan Universal PCR Master Mix kit (?) and the MX-3000P Real-time PCR Instrument (Stratagen). PCR assays were performed using the primers shown in Table 1. The PCR conditions were 95 °C for 10 min (initial denaturation), followed by 40 cycles at 95 °C for 15 sec, and 58 °C for 1 min.

d) Expression of Chi3l1 protein

The full length coding region of mouse Chi3l1 was cloned into pRSET A and protein expression was induced by IPTG in E. coli. This protein was used to generate polyclonal antibodies against Chi3l1.

The coding region of the Chi3l1 gene was also cloned into pcDNA3.1(+). The expression vector or empty vector was transfected into cos7 cells using lipofectamine 2000 (Invitrogen). After 72 h, the medium was collected and centrifuged. The supernatants were stored at -80°C for later experiments.

e) Cell viability assay

Primary osteoblastic cells were seeded in a 96-well plate. 24 h later, the Chi3l1 expression supernatant or the control supernatant collected from cos7 cells were added into the culture medium and then the cells were exposed to TNFα (20 ng/ml, Peprotech). After another 48 h, the culture medium was discarded and 180 μl α-MEM and 20 μl MTT (5 mg/ml) were added. The incubation was continued for 4 h. Finally, the solution was removed and 150 μl DMSO was added. Cell viability was assessed by measuring the absorbance at 492 nm, with a reference wavelength at 650 nm.

f) Cell cycle analysis by flow cytometry

Primary osteoblastic cells were seeded in a 10-cm culture plate. The Chi3l1 expression supernatant or the control supernatant collected from cos7 cells was added into the culture medium. After 48 h, the cells were collected by trypsin treatment, rinsed in PBS, fixed with ice-cold 70% ethanol for 20 min. Fixed cells were incubated with 50 μg/ml propidium iodide (PI) for 15 min, analyzed using a BD Biosciences FACScan flow cytometer.

g) Analysis of apoptosis

Primary osteoblastic cells were incubated with Chi3l1 expression or the control supernatant. Apoptosis was stimulated by TNFα, then the cells were incubated with FITC-conjugated Annexin V and PI (BD Biosciences) for 15 min. Cell apoptosis was analyzed using a BD Biosciences FACScan flow cytometer.

To measure caspase-3 activity, a caspase-3 activity assay kit (Nanjing KeyGen) was used. Briefly, after treated with Chi3l1 expression supernatant or control supernatant, the cells were stimulated with TNFα for 8 h. Then, the cells were lysed and 100 μg of protein in 100 μl volume was mixed with caspase-3 substrate for 5 h at 37℃. The caspase-3 activity was evaluated by measuring the absorbance at 405 nm.

h) Detection of AKT activation by Western blotting

Primary osteoblastic cells were serum-starved overnight, subsequently treated with Chi3l1expression or the control supernatant collected from cos7 cells for 0～60 min. The cells were then rinsed with ice cold PBS and lysed with RIPA buffer. Total protein concentrations were determined by a Protein Assay kit (Biorad). This was followed by Western blot assays using antibodies against AKT, or Ser473-phosphorylated AKT (Cell Signaling Technology).

i) Statistical analysis

The results were expressed as means ± S.D. A student's t-test was used to determine statistical significance. $P < 0.05$ was considered significant.

III. Results

a) Chi3l1 was up-regulated in osteomyelitis

We examined Chi3l1 expression in a mouse model of osteomyelitis. Real-time PCR analysis revealed a significant increase in Chi3l1 expression the second day after infection, compared with the sham-operated and the contra-lateral control (Figure 1A). Western blot assay showed similar results: as early as the second day, there was an increase in Chi3l1 proteins levels; upon reaching the fifth day, the increase became even more pronounced (Figure 1B).

b) LPS induced Chi3l1 expression in osteoblasts and MSCs

Primary osteoblastic cells were stimulated with LPS. Real-time PCR and Western blotting showed an obvious promotion of Chi3l1 expression at both the RNA and protein levels (Figure 2A and B).

MSCs were isolated from the bone marrow of mice and they could be induced to differentiate into osteoblasts both in vivo and in vitro. The cells were characterized by fluorescence-activated cell sorting (FACS) analysis. MSCs were negative for CD34 and

CD31, but positive for CD29 and CD44 (Supplementary Figure 1). We stimulated MSCs with LPS and observed similar stimulating effects on Chi3l1 expression level as in osteoblasts (Figure 2A and C).

c) *Chi3l1 promoted osteoblast survival without affecting the cell cycle*

MTT assays demonstrated that treatment with TNFα resulted in decreased viability of primary osteoblastic cells. However, incubation with the Chi3l1 expression supernatant partially reversed this phenomenon (Figure 3A). Cell cycle analysis were performed to investigate whether this increase in survival was due to the increase of cell proliferation. After incubation with Chi3l1 for the indicated time period, cells were stained by PI and flow cytometry data showed that no obvious difference in cell growth was observed (Figure 3B, C). When the cells were stimulated by Chi3l1 in the absence of FBS, the percentage of cells in S phrase changed from 4.68%±1.7% to 5.3%±2.1%. And in the presence of FBS, the percentage changed from 20.2%±3.8% to 24.6%±5.1%.

In order to identify the concentration of Chi3l1 in the supernatant, Western blotting was performed and protein concentration was quantified by gray intensity analysis using software (Figure 3D). The estimated concentration of Chi3l1 in the supernatant was about 1000 ng/ml.

d) *Chi3l1 suppressed TNFα-induced osteoblast apoptosis*

Since Chi3l1 had no obvious effect on cell cycle, it probably possessed the activity of repressing apoptosis. We performed Annexin V and PI staining to scan cell apoptosis. The results showed that the percentage of apoptotic cells decreased from 58.2±4.6% to 40.1±3.2% (Figure 4A, B) after treatment with Chi3l1 supernatant. Moreover, Chi3l1 also suppressed the increase of caspase-3 activity stimulated by TNFα (Figure 4C). These data suggested that Chi3l1 had no effect on osteoblast growth, instead, it promoted cell survival at least partly mediated by preventing apoptosis

e) *AKT activation mediated the pro-survival and anti-apoptotic effects of Chi3l1*

The PI3K/AKT signaling pathway is considered to be a critical regulator of cell survival and apoptosis. An activated AKT is in the downstream region of PI3K. Our data showed that AKT phosphorylation could be observed in a time-dependent manner after the addition of Chi3l1 (Figure 5A). Furthermore, incubation with 1L-6-hydroxymethyl-chiro-inositol 2-(R)-2-O-methyl-3O-octadecylcarbonate (HIMO, Calbiochem), an inhibitor of the AKT signaling pathway, blocked both the phosphorylation of AKT and the pro-survival effect of Chi3l1 (Figure 5B, C). Meanwhile, the same concentration of HIMO had no obvious effect on cell survival. These data indicated that Chi3l1 played a protective role in osteoblasts via the activation of the AKT signaling pathway. Furthermore, Chi3l1 also showed anti-apoptotic effects on osteoblasts (Figure 5D, E). Therefore the pro-survival effect of Chi3l1 was at least partly mediated by the suppression of apoptosis.

IV. Discussion

Chi3l1, secreted by chondrocytes, macrophages and a number of other cells, is a member of the mammalian chitinase family [9, 17, 18]. In this research, we demonstrated that its expression was induced as a part of the disease symptoms of osteomyelitis. Previous studies have reported that the secretion of Chi3l1 is often elevated in serum or tissues of patients suffering from inflammatory stress, the results of our study supported these observations.

To identify the cells that may contribute to the Chi3l1 expression in osteomyelitis, we performed an in vitro study, which showed that primary osteoblastic cells and MSCs may be the sources of Chi3l1 elevation. The administration of LPS has been widely used to simulate the influence of bacterial infection on organisms. Our data showed that LPS treatment resulted in Chi3l1 up-regulation in both osteoblasts and MSCs.

A recent report has demonstrated that serum Chi3l1 concentration was elevated in multiple myeloma (MM) patients [19]. MM is associated with inflammatory stress and increased secretion of cytokines, which are essential for the progress of MM [20, 21], our results are in line with these observations. In osteomyelitis tissues, cytokines are induced shortly after infection [22]; we also found that TNFα could up-regulate Chi3l1 in MSCs and osteoblasts (our unpublished data). Thus, it is reasonable to propose that the induction of Chi3l1 is a response of bone tissue to an unfavorable environment, such as inflammation, both in MM and in osteomyelitis. However, whether the expression level of Chi3l1 in osteomyelitis reflects the severity of the disease, as is seen in the case of MM or rheumatoid arthritis, is still unknown. Clinical research with relevant patients may enable us to address this problem.

TNFα, as a primary inflammatory cytokine induced by LPS, has been shown to enhance osteoblast apoptosis [23]. Excessive apoptosis decreases the osteoblast population and has a negative impact on bone formation, indicating that osteoblast apoptosis induced by cytokines contributes to inflammatory bone loss [7, 24, 25]. Our findings have demonstrated that Chi3l1 promoted osteoblast survival and blocked TNFα-induced apoptosis. Meanwhile, it had no obvious effect on osteoblast proliferation. Therefore, the increase of cell viability is due to decrease of apoptosis rather than promotion of cell growth. In our opinion, promotion of cell survival by suppression of cytokine-induced apoptosis is an important mechanism by which this

protein protects inflammation-stressed bone. The Chi3l1 concentration we used falls into the pathological concentration range in human beings [11, 26, 27], Therefore, it is reasonable to speculate that, by down-regulating osteoblast apoptosis, Chi3l1 might reduce the risk of bone destruction associated with deficient bone formation in vivo.

Some studies have shown that the PI3K signaling pathway is involved in cell apoptosis regulation. PI3K/AKT mediates the anti-apoptotic effect of Wnt in MC3T3-E1 cells [28] and AKT signaling is essential for survival of acute lymphoblastic leukemia cells, osteoblasts and endothelial cells [29-31]. Thus, we hypothesized that Chi3l1 promoted osteoblast survival and reduced apoptosis by activation of the AKT signaling pathway. Our data illustrated that Chi3l1 had an obvious inducing effect on AKT phosphorylation. Moreover, an inhibitor of the AKT pathway abrogated the pro-survival and anti-apoptotic effects of Chi3l1.

The association of Chi3l1 expression with both normal and pathological tissue turnover has been reported [12, 32] and a protective role in these situations has been proposed. The skeleton is constantly resorbed by osteoclasts and replaced by osteoblasts in the tissue turnover process, so osteoblasts are essential for maintaining the integrity of bone. However, any decrease in osteoblast viability as a result of infectious or inflammatory skeletal diseases disrupts the balance between bone formation and resorption. The discovery of the enhancement of cell survival and the suppression of apoptosis by Chi3l1 thus suggests a potential therapeutic opportunity for controlling bone loss in relevant pathological processes.

V. Acknowledgements

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Figure legends

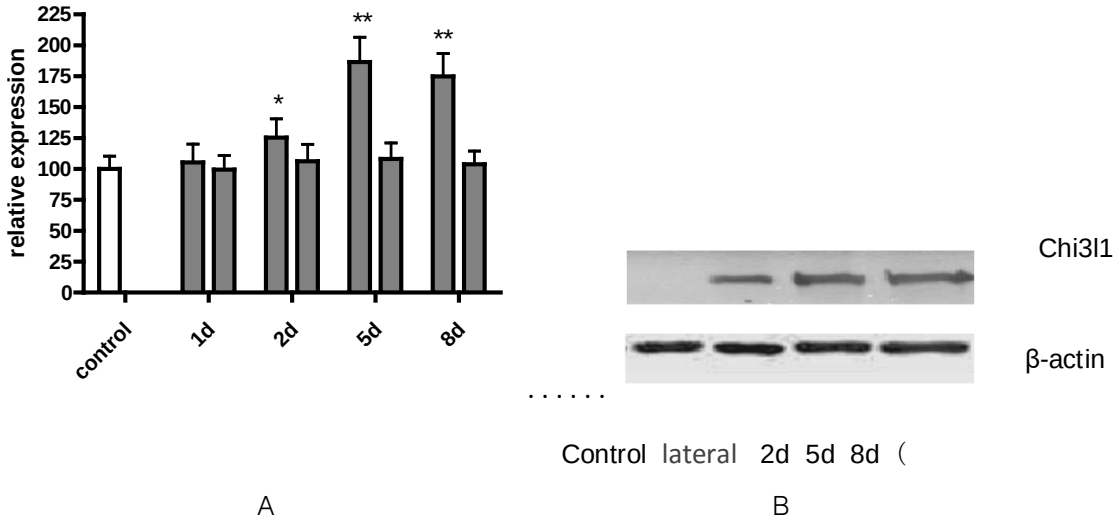
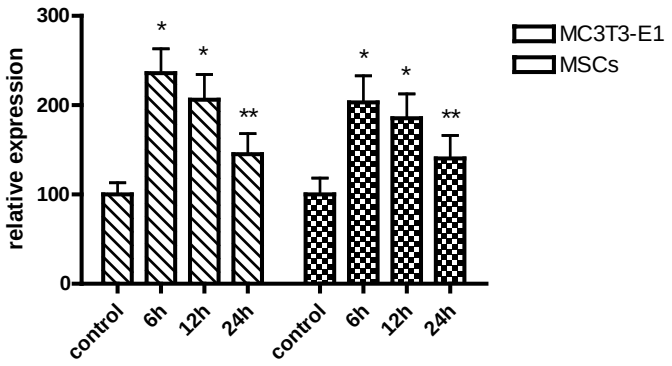


Fig. 1: Changes of Chi3l1 expression in osteomyelitis. (A) At different time points after infection, the femur tissues around the incisions (0.3cm upwards and downwards from the incisions) were collected and cleared of surrounding tissues. The contra-lateral femur and the one infected by bacteria was isolated at the same time. The infected and lateral femur were represented by the left bar and right bar in each group (grey bars) respectively. The bone tissues were immediately minced with shears, then ground in liquid nitrogen. Trizol was added to the tissue powder and the mixture was homogenized to ensure sufficient yield of RNA. Then real-time PCR assays were performed. The expression levels of Chi3l1 were normalized to GADPH levels. The results are expressed as the copy numbers relative to GADPH. *, $P < 0.05$; **, $P < 0.01$ vs control. (B) The femurs were isolated and homogenized as described above. After homogenization, the total proteins were extracted by Trizol from bone according to the protocol. Then Western blot assays were performed. β-actin was used as an internal control.



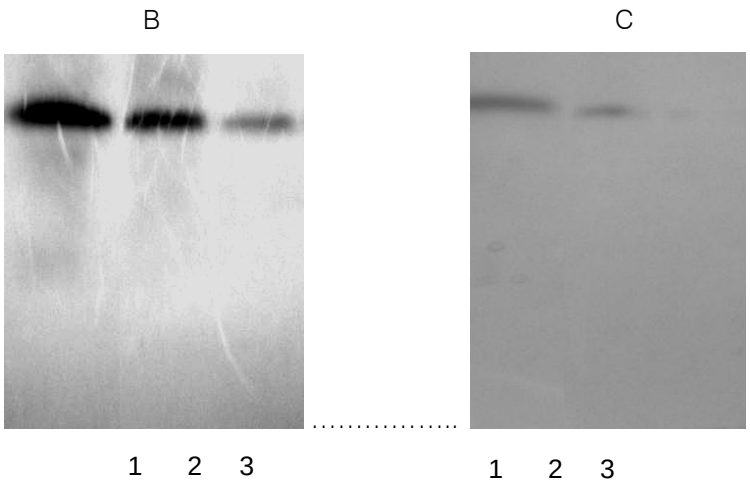
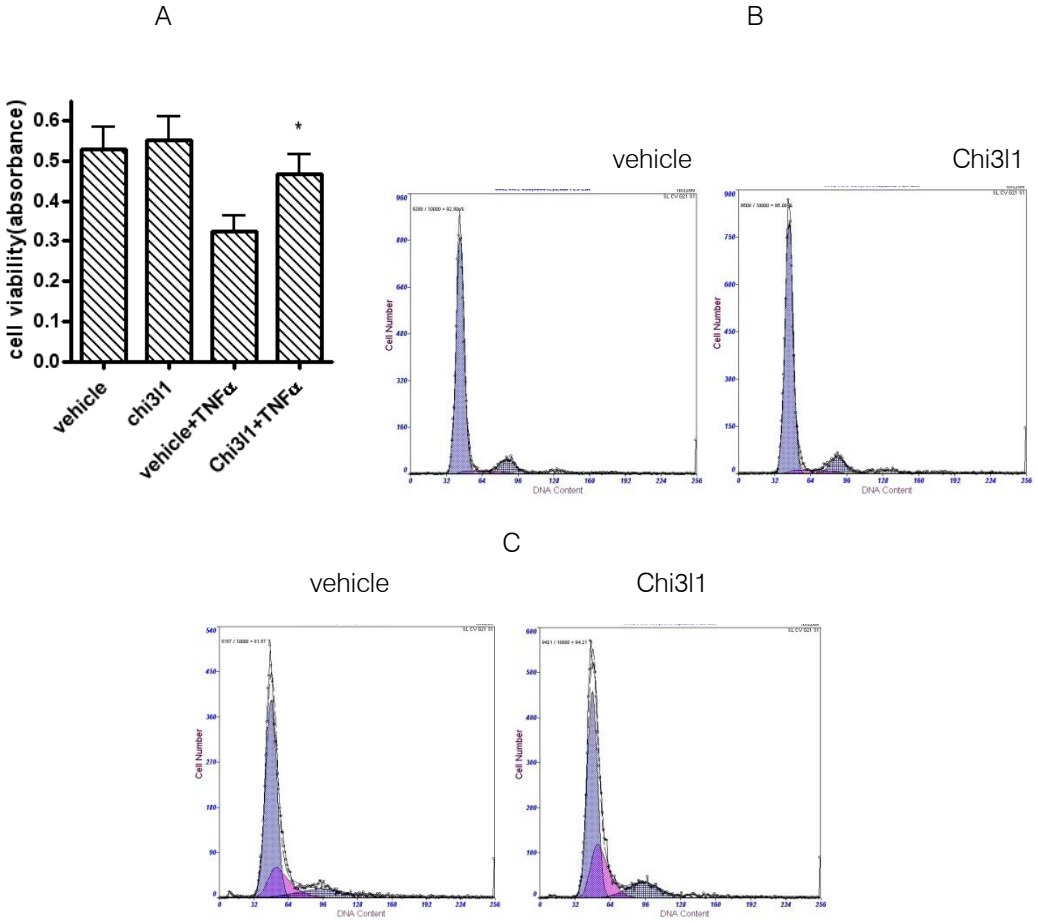


Fig. 2: LPS-stimulated Chi3l1 expression. (A) Primary osteoblastic cells were stimulated by LPS (1ug/ml) for 6 h, 12h or 24 h; then the mRNA expression levels were analyzed by real-time PCR. GADPH was used as an internal control. * , P <0.01;**, P < 0.05 vs control. (B) Primary osteoblastic cells were treated with LPS (1ug/ml); culture media were collected after 48 h and proteins were precipitated by the addition of 3 volumes of ice-cold ethanol. Western blotting was performed to confirm the results of real-time PCR. (C) MSCs cells were stimulated with LPS in the same way as primary osteoblastic cells. Western blot assays were performed to examine the changes of Chi3l1 expression at protein level. 1: The recombinant protein purified from E.coli. was used as a positive control . 2. The cells treated with PBS were used as a negative control. 3. The cells were treated with LPS



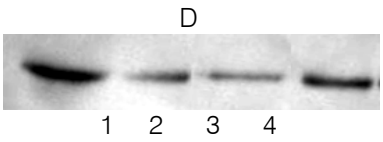
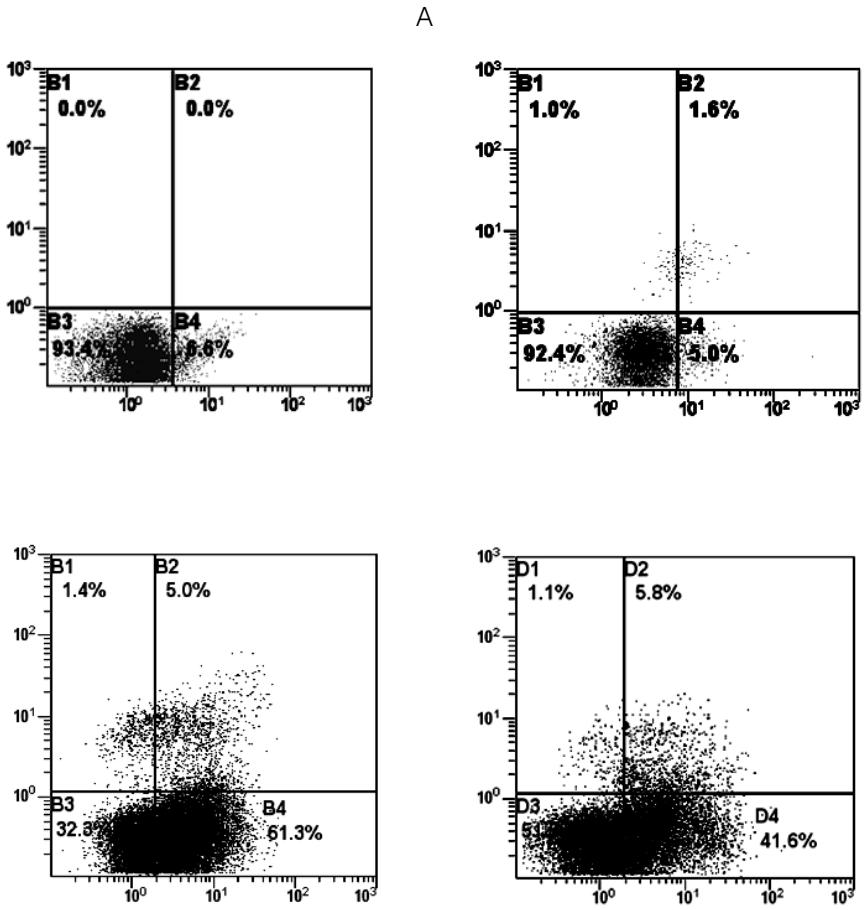


Fig. 3: Chi3l1 promoted survival and did not influence cell growth in osteoblasts. (A) Cos7 cells were transfected with the expression vector of Chi3l1 or the empty vector and the supernatant was collected after 72h. The primary osteoblastic cells were cultured with the expression supernatant or the control supernatant with or without TNFα (20ng/ml) for 48h. MTT assays were performed to evaluate cell viability. The results are expressed as the absolute absorbance values. *, P <0.05 vs the vehicle+TNFα group. (B) Primary osteoblastic cells were cultured in serum –free medium for 16h, then treated with Chi3l1 expression supernatant or the control supernatant for 48h. The cells were collected and stained by PI. Flow cytometry assays were performed to analyse the cell cycle. (C) The primary osteoblastic cells were cultured in medium containing 10% FBS, and then incubated with the expression supernatant or the control supernatant for 48h. Flow cytometry assays were performed to analyze cell cycle. (D) Cos7 cells were transfected with the expression vector of Chi3l1 and 72h later, supernatant was collected. Western blotting was performed with expression supernatant and a series of concentrations of Chi3l1 protein purified from E.coli. 1, 2, 3: The recombinant protein of Chi3l1 from E.coli. The concentrations of these were 400, 800, 3000ng/ml, respectively. 4: The expression supernatant collected from cos7 cells



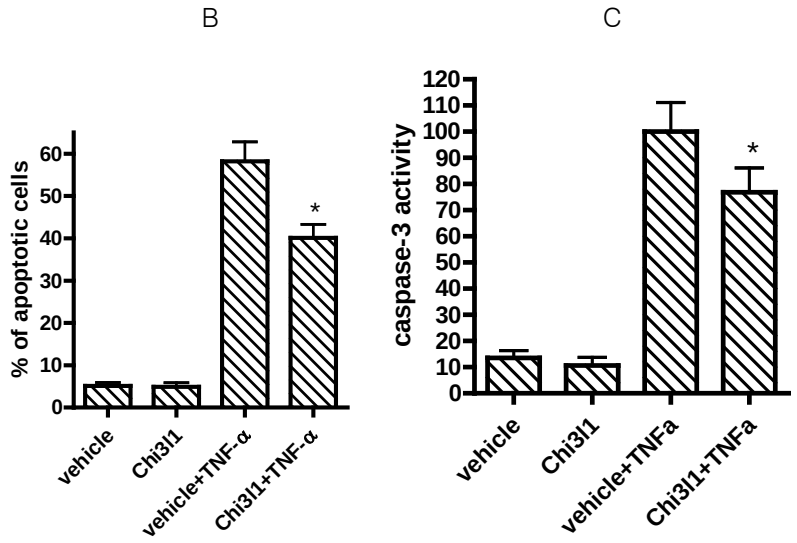
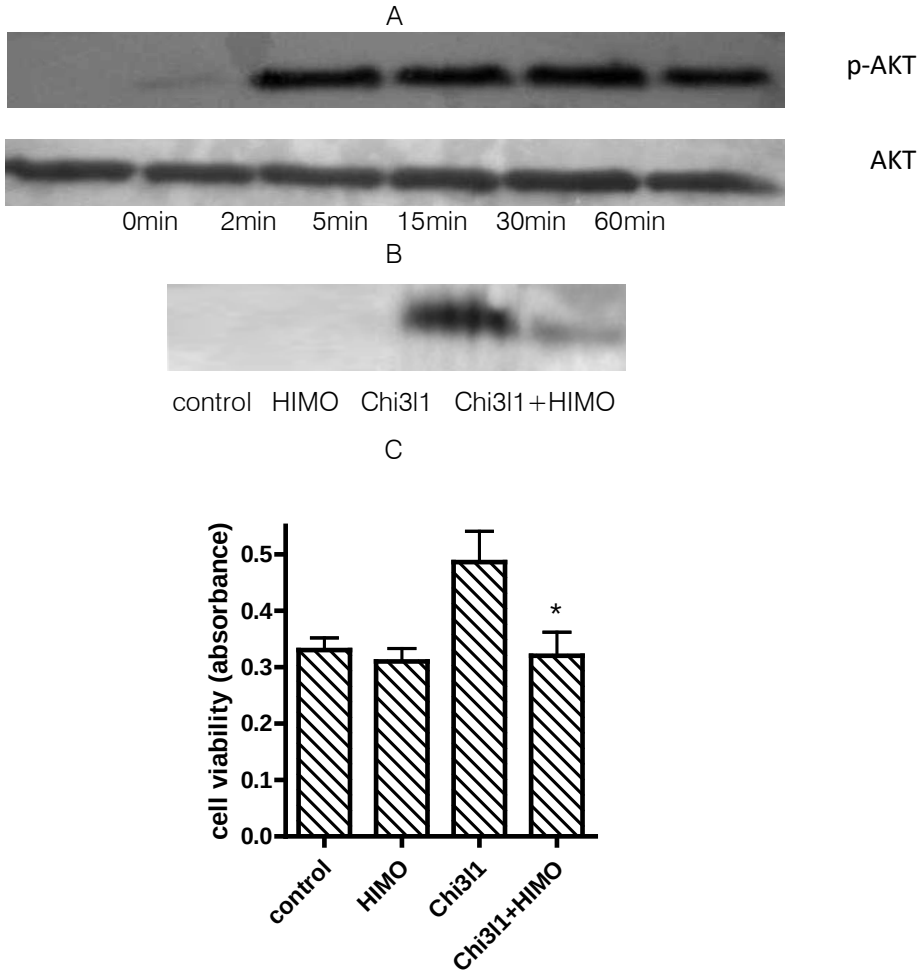


Fig. 4: Chi3l1 inhibited apoptosis in osteoblasts. (A) Primary osteoblastic cells were cultured with Chi3l1 expression supernatant or the control supernatant with or without TNFα (20ng/ml). 24h later, Annexin V and PI staining was performed to assess cell apoptosis. Representative figures of flow cytometry analysis were shown.The numbers in the figures indicate the percentage of cells within respective subpopulations. (B) The statistic analysis of the flow cytometry data. (C) Primary osteoblastic cells were cultured with the expression supernatant or the control supernatant with or without TNFα (20ng/ml) for 8h. Then cell lysates were prepared and relative caspase-3 activity was evaluated by a commercial available kit according to the protocol. *, $P < 0.05$; **, $P < 0.01$ vs the vehicle+TNFα group



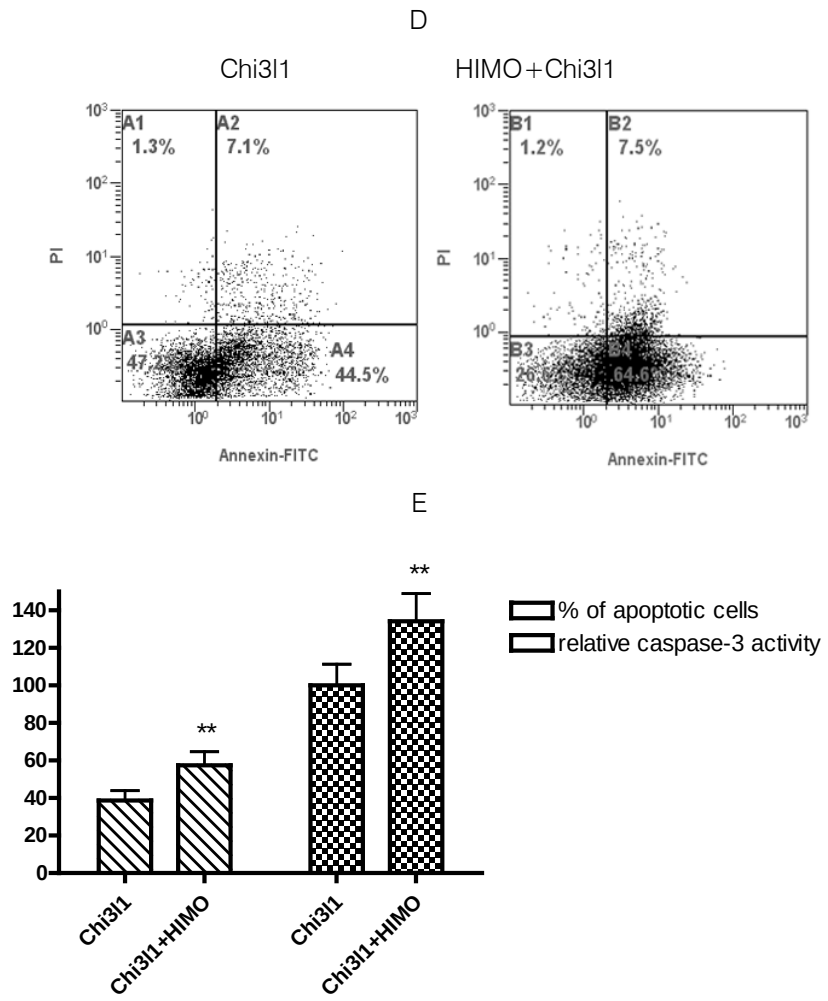


Fig. 5: The AKT pathway mediated the protective effect of Chi3l1. (A) Primary osteoblastic cells were cultured in serum-free medium overnight, then cells were incubated with Chi3l1 expression supernatant collected from cos7 cells for 0~60min (with a volume ratio of 1:1). At the end of the indicated period, cells lysates were prepared and the phosphorylated AKT was detected by Western blotting. (B) Effect of HIMO on AKT phosphorylation. Primary osteoblastic cells cells were incubated with control or Chi3l1 expression supernatant for 30min with or 10μM without HIMO. (C), (D) and (E) Primary osteoblastic cells cells were incubated with Chi3l1 expression supernatant collected from cos7 cells with or without 10μM HIMO. The cells were incubated with TNFα (20ng/ml) for 48, 24 or 8 h, respectively. MTT, Annexin V and PI staining, as well as relative caspase-3 activity assays, were performed. *, $P < 0.01$; **, $P < 0.05$ vs the Chi3l1+ TNFα group

Table 1: Oligo-deoxyribonucleotide primers and probes sequences used in real-time PCR

Gene	Primer(5'-3')	Probe(5'-3')
GADPH	F: CTGCCAAATATGATGACA	AGGTGGTGAAGCAGG
	R: CCCAGGATGCCCTTGA	CGTCG
Chi3l1	F: TCCAGCCAGGCAGAGAGAA	TCCTGCTCAGCGCAGC
	R: TGTCAATGGCCACCTTTCCT	TTTGTCA

The reporter dye of probe used on the 5' end was FAM and quencher dye on the 3' end was DABCYL.

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Excel Files for Teaching Two Dimensional Motions and their Curvature

By Pavlos MIhas
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Abstract- Spreadsheets are presented which can help students to understand the the vector representation of velocity, acceleration and force, and the understanding of the radius of curvature\. The examples include: sinusoidal curve, cardioid, Neile's parabola, motion of a charged body on which act a combination of magnetic and electric field, a body sliding on a rotating disk.

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Excel Files for Teaching Two Dimensional Motions and their Curvature

Pavlos MIhas

Abstract- Spreadsheets are presented which can help students to understand the the vector representation of velocity, acceleration and force, and the understanding of the radius of curvature\. The examples include: sinusoidal curve, cardioid, Neile's parabola, motion of a charged body on which act a combination of magnetic and electric field, a body sliding on a rotating disk.

I. Introduction

In physics education research, student understanding ontopics of introductory mechanics has been thoroughly studiedfor several decades (for example Clement 1982, Halloun and Hestenes 1985). This research, has demonstrated that many students retain fundamentalconceptual difficulties, even after instruction (Kim and Pak 2002).

Two dimensional motions are examined in secondary education (for example: Motion on a circle, parabolas, elliptic motions of planets, circular pendulums, conical pendulums).

Two dimensional motions is a subject which requires the understanding of the vector nature of velocity, acceleration and force (Mihas & Gemoysakakis 2002).

We need to present to the students general principles, which they will apply to any kind of two dimensional motions.

The principles that students need to learn are: A) the velocity as a vector tangent to the orbit. B) The acceleration (centripetal acceleration) in case of constant speed is perpendicular to the velocity vector. The size of the centripetal acceleration depends on the speed and the radius of curvature of the orbit. C) Newton's second law. D) In the case of a tangential component of the force they have to think of the total acceleration as composed of two components: tangential acceleration and centripetal acceleration. E) The direction of the force with the direction of velocity can have an acute, right or obtuse angle if the measure of the velocity increases, remains constant or decreases and so the kinetic energy increases, remains constant or decreases. F) The curve can be approximated at a certain point with a circle, which has radius the radius of curvature which is expressed as $\rho = \frac{\left(\left(\frac{dx}{ds}\right)^2 + \left(\frac{dy}{ds}\right)^2\right)^{3/2}}{\left(\frac{dx}{ds}\right)^3 \frac{d^2y}{dx^2}}$. The radius of curvature can have values from zero to infinity (in case of a rectilinear path o if $\frac{d^2y}{dx^2} = 0$. We will see examples of these cases in the following.

In this paper we examine several curves with their properties which can help as examples to elucidate the concepts, and we end up with the relative motion of a sliding body on a rotating disk which can gives us some unexpected results.

II. Sinusoidal Curve

One curve that can be used to introduce students to the ideas of radius of curvature and the relation between angle of velocity and acceleration is the sinusoidal curve.

The length s on the sinusoidal y =B·sinkx is expressed by use of elliptic integrals of the second kind:

$$s = \int \sqrt{1 + B^2 k^2 \cos^2 kx} \cdot dx = \sqrt{1 + B^2 k^2}\, E(\alpha, x)$$

where $\alpha = \frac{B \cdot k}{\sqrt{1 + B^2 k^2}}$

Since elliptic integrals are calculated very fast, it is easy to find solutions of the coordinates as a function of s. This permits to construct simulations. If we assume a speed that is increasing, with an increase of the speed which is proportional to the time, we can use this example to show the relation between angle of velocity and acceleration.

Usually the students pick up from the simulation some facts but they do notgenerally have a coherent picture. So they do not realize that the radius of curvature is infinit in the points where the inflection changes sides (in the sinusoidal curve at the points where the sign of y changes from plus to minus and vice versa).

For example they pick up the information that for a constant speed the acceleration should be perpendicular to the velocity, but they generalize it to the linear parts of the curve where radius of curvature is $\rho = \infty$ and so $a_{centripetal} = v^2/\rho = 0/$

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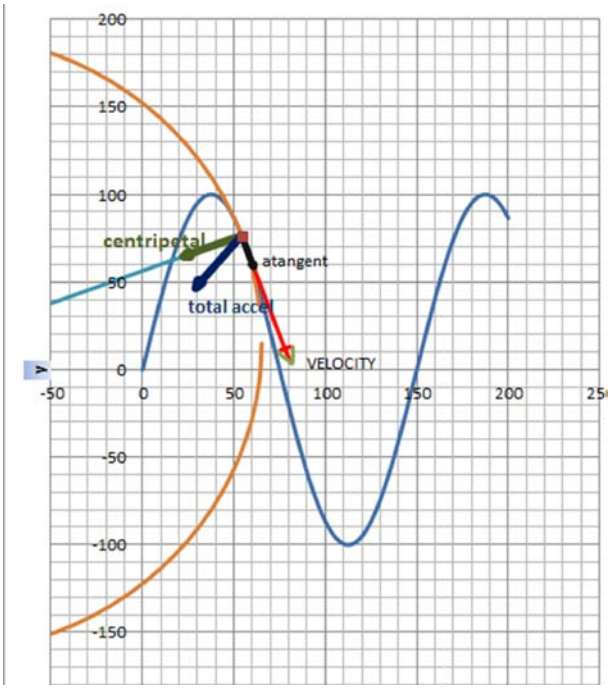

Figure 1: Sinusoidal with vectors of acceleration analyzed to entripetal and tangent

III. Tutorial Curve

This curve is used by McDermott etal in the Tutorials of introductory physics (in Greek p. 285 2011). This curve is a closed one and shows the change of direction of the centripetal acceleration but also the total acceleration the circle that corresponds to the curvature at the point where the moving body is located. The student can observe that when the body passes through points such as B and C the acceleration is almost tangent to the curve. This curve helps the student to understand that the angle of the acceleration to the velocity is acute when the speed increases and to see that the centripetal acceleration is greatest in the points where the radius of curvature is smallest. The worksheets comes in two forms (with macros, which permit a continuous change) and without, where the body moves by sliding a scroll bar (worksheet tutorial curve.xslx and tutorial curve macros). The points A,…E are points where the student should predict thedirection of the acceleration and also draw a circle with a radius equal to the radius of curvature.

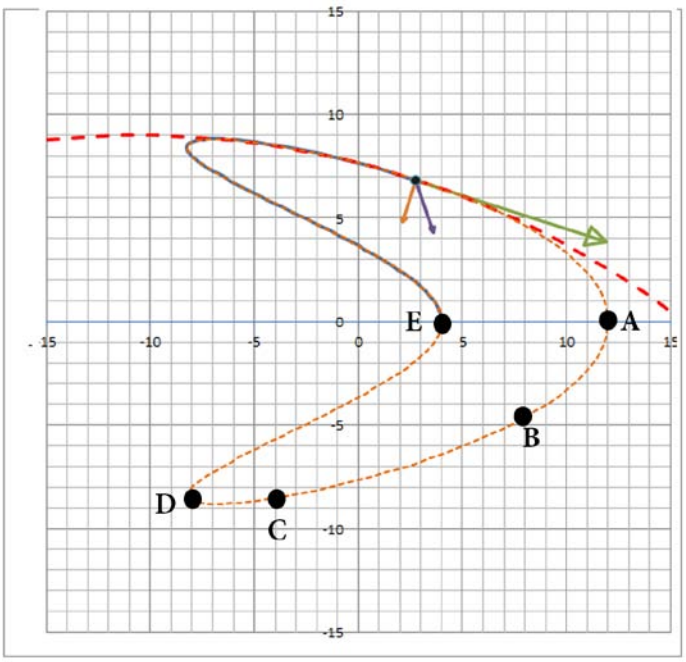

Figure 2: Closed curve used in Tutorials with constant tangential acceleration

IV. Neile Parabola

Aristotle was the first who was concerned with the composition of motions (Dugas p. 21). "Let a moving body be simultaneously be actuated by two motions that are such that the distances traveled in the same time are in a constant proportion. Then it will move into the diagonal of a parallelogram which has as sides two lines whose lengths are in this constant relation to each other" .Much later Galileo (Two New Sciences) analyzedthe motion of projectiles into two components and proved that the orbit described by the projectile will have the shape of a parabola.

Parabola was analyzed as performing on the same time an inertial motion and a motion under the influence of gravity. In Neile's parabola the motion is under the influence of two forces: A horizontal force arising from the reaction of the curve and a vertical force arising from the perpendicular component of the reaction and the weight. For a special value of the initial velocity, its vertical (perpendicular) component remains constant. In this case the total force has only a horizontal component. For larger speed the vertical motion will be an accelerated motion, and for lesser values of the speed it will be a decelerated motion. To find the curve for the special case of constant vertical component of the speed, letαbe the initial horizontal component of the velocity and β the value of the vertical componentof velocity, then the vertical component of the reaction is equal to the weight for the case of constant β.

$\frac{dy}{dt} = \beta :\quad \frac{dx}{dt_0} = a$ .Fromwork-energytheorem:

$$\frac{1}{2}m\cdot v^2 = \frac{1}{2}m\cdot\left(\frac{dx}{dt}\right)^2 + \frac{1}{2}m\cdot\beta^2 - m\cdot g\cdot y \text{ and so } \frac{dx}{dt} = \frac{dx}{dy}\cdot\frac{dy}{dt} = \frac{dx}{dy}\cdot\beta = -\sqrt{a^2 - 2\cdot g\cdot y}:$$

$$dx = -\frac{\sqrt{a^2 - 2\cdot g\cdot y}}{\beta}dy \text{ and by integration we get: } x = \frac{(a^2 - 2gy)^{\frac{3}{2}}}{3g\beta} \quad y<y_{max} \text{ where } y_{max}=a^2/(2\cdot g)$$

This curve was the first one to have its length calculated. This calculation is done easily by using the conservation of energy $\frac{ds}{dt} = \sqrt{v_x^2 + v_y^2} = \sqrt{v_0^2 - 2gy}$, then:

$$s = \int \sqrt{v_0^2 - 2gy}\cdot dt == \frac{1}{3g\beta}\left[v_0^3 - (v_0^2 - 2gy)^{3/2}\right]$$

It is interesting that the radius of curvature of this curve is zero for $y=y_{max}$. If the speed is different from zero then the centripetal acceleration will be infinite. The radius of curvature can be expressed as:
which for Neile's curve is expressed as:

$$\rho = \frac{\sqrt{\alpha^2 - 2gy}\cdot\left(\alpha^2 + \beta^2 - 2gy\right)^{3/2}}{\beta^2 g}.$$

We can see that for the highest point $\rho=0$. In case of finite velocity we get infinite centripetal acceleration. The student can study the Neile's parabola with the spreadsheet "Neile curve"

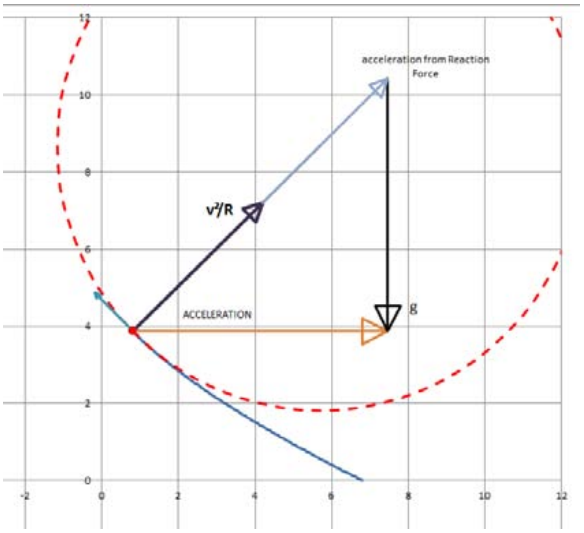

Figure 3: Neile's Parabola with circle of curvature and accelerations from spreadsheet "Neile Curve"

a) Cycloidalpendulum

In this pendulum the string is constantly in touch with a cycloid. For this pendulum the radius of curvature is studied easily, since it is equal to the length of the string that does not touch the upper curves. The bob of the pendulum describes cycloid. As the bob approaches the highest point the radius of curvature becomes smaller and eventually it becomes zero. In the case of vibration that starts at this point the velocity is zero and so the centripetal force is zero. This can be contrasted to what happens in Neile curve, At this point the total force (weight) is tangent to the curve.

The equations of the curve are: $x=R\cdot(\theta-\sin(\theta))$, $y=-R+R\cdot\cos(\theta)$ $(y<=0)$ where R is the radius of the circle that produces the cycloid. The length of the string for this pendulum is L=4R
We can find that the radius of curvature is:

$$\rho = 2\sqrt{-2yR}.$$

For y=0 (at the ends of the cycloid) the radius of curvature is zero. At the lowest point y=-2R and then $\rho=4R=L$ = string's length.

As it was found in tests and exams the students could easily arrange the points 1,2,3 of figure according to their radii of curvature but.

The cycloidal pendulum has the property of a constant period $T = 2\pi\cdot\sqrt{\frac{l}{g}}$ that does not depend on the amplitude of the oscillations. This can be contrasted with the simple pendulum for which the period is $T = 4\cdot K(a)\cdot\sqrt{\frac{l}{g}}$ where a =sin(φ/2) (φ = amplitude) and K(a) is the complete elliptic integral.

The student can use the spreadsheet "cycloid pendulum" (with macros or not) to study the motion. The circle of curvature at the highest point has a minimum value which is zero if the bob starts from the cycloid. The student can compare the movement with the simple pendulum and see that the period of the cycloid pendulum is independent of the amplitude of the oscillation.

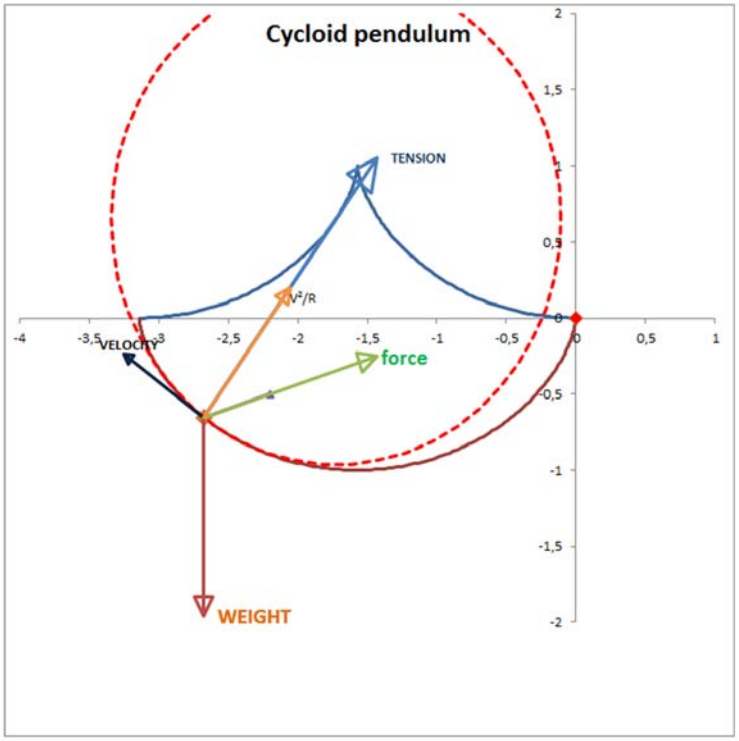

Figure 4: Cycloidal pendulum with curvatur circle and forces acting on the bob

b) *Motion of a charged body in the combination of a central force field and a magnetic field – or motion in a rotating frame, Foucault's pendulum*

In this case the magnetic force $\vec{F} = q\vec{v}\times\vec{B} = -q\vec{B}\times\vec{v}$ is the cause of a curving of the orbit. . With k/m = λ, ε =q.B/m, The equations of motion are

$$m\frac{d^2x}{dt^2} = k\cdot x + qBv_y \quad m\frac{d^2y}{dt^2} = k\cdot y - qBv_x$$

Here k can be either positive or negative.

We consider a charged body starting with an initial velocity $v=v_{x0}$ and located at $x_0=0$, $y_0=R$. With $\varepsilon=qB/m$ and $\lambda=k/m$ we have two cases: $-\varepsilon^2+4\lambda>0$ and $-\varepsilon^2+4\lambda<0$ (which holds for a small repulsive force and always for attractive central force)
In this case we have an oscillatory motion.

c) *Solution for the equations of the motion of a charged body in the combination of a central force field and a magnetic field*

The equations: $\frac{d^2x}{dt^2} = \lambda\cdot x + \varepsilon\cdot v_y$, $\frac{d^2y}{dt^2} = \lambda\cdot y - \varepsilon\cdot v_x$

can be solved with elementary functions.

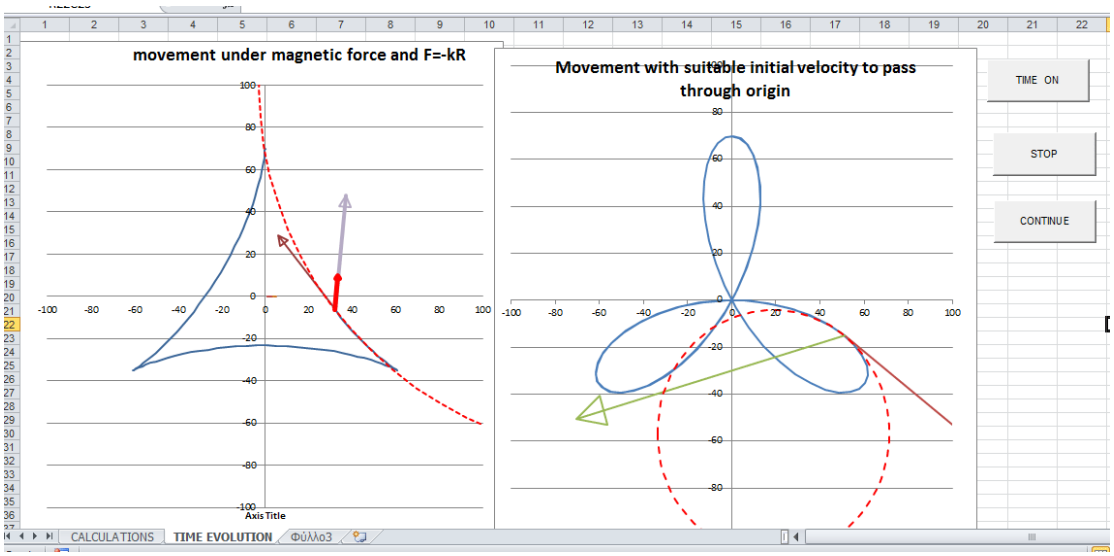

Figure 5: Movement in a combination of E and B, left with intial speed = zero, on the right with a sutibable initial horizontal speed so to pass through the origin (in the worksheet kappa= -50, B=5, mass=1)

By multiplying the second by $i=\sqrt{-1}$ and adding to the first we get the complex variable z=x+iy and the equation of motion: $\frac{d^2z}{dt^2}=\lambda\cdot z-i\varepsilon\frac{dz}{dt}$ With $z=A\cdot\exp(\mu\cdot t)$

$$\mu^2+i\varepsilon\mu-\lambda=0\ \ \mu_{1,2}=\frac{-i\varepsilon\pm A}{2}$$

If $-\varepsilon^2+4\lambda>0$ ($\lambda>0$ repulsiveforce) μ_1 and μ_2 are complex,
$z=f_1\exp((-ie+A)t/2)+f_2\exp((-i\varepsilon-A)t/2)$ where $f_1$and f_2 complex numbers. With initial conditions x=0, y=R and $(v_{x0}-\varepsilon R/2)2/A=\Delta$ we get:
$f_1=M\exp(i\varphi)$
$f_2=-M\exp(-i\varphi)$
where $M=\sqrt{\Delta^2+R^2}/2$ και $\phi=\arctan(R/\Delta)$ and $\Gamma=A/2$

$$z=M[\exp(i\phi-i\varepsilon t/2+\Gamma t)-\exp(-i\phi-i\varepsilon t/2-\Gamma t)]$$

$$x=M[\cos(\phi-\varepsilon t/2)\cdot\exp(\Gamma t)-\cos(\phi+\varepsilon t/2)\cdot\exp(-\Gamma t)]$$

$$y=M[\sin(\phi-\varepsilon t/2)\cdot\exp(\Gamma t)+\sin(\phi+\varepsilon t/2)\cdot\exp(-\Gamma t)]$$

If $-\varepsilon^2+4\lambda<0$ ($\lambda<0$ orsmall repulsive) we have $\sqrt{4\lambda-\varepsilon^2}=D$

we have two imaginary roots $\mu_1\mu_2$

$$\mu_1=-(\varepsilon+D)/2,\ \mu_2=-(\varepsilon-D)/2$$

$$z=-i\frac{(v_{x0}+\mu_2\cdot R)\exp(i\mu_1 t)-(v_{x0}+\mu_1\cdot R)\exp(i\mu_2 t)}{\mu_1-\mu_2}$$

$$x=\frac{(v_{x0}+\mu_2\cdot R)\sin(\mu_1 t)-(v_{x0}+\mu_1\cdot R)\sin(\mu_2 t)}{\mu_1-\mu_2}$$

$$y=-\frac{(v_{x0}+\mu_2\cdot R)\cos(\mu_1 t)-(v_{x0}+\mu_1\cdot R)\cos(\mu_2 t)}{\mu_1-\mu_2}$$

If $v_{x0}-\varepsilon\cdot R/2=0$,nd the body will pass through the origin
Or $x=R\cdot\sin(\varepsilon\cdot t)\cdot\cos(D\cdot t)$, $y=R\cdot\cos(\varepsilon\cdot t)\cdot\cos(D\cdot t)$.

We can see that the body will pass through the origin with a period $2\pi/D$.

With a suitable combination of E and B fields we can have the charge move with very small radius of curvature around a circle or much larger radius.

d) Similarity with Foucault's pendulum

This motion is similar to the motion of a Foucault's pendulum.

Actually the equations of motion are very similar. In Foucault's pendulum we have the Coriolis force $\vec{F}_{Coriolis}=-2m\,\vec{\omega}\times\vec{v}$ $\vec{\omega}$ *angular velocity* which has the same form as the magnetic force. This was mentioned in many papers (Semon & Schmieg 1981, Sivardiere 1983, Opat 1990) By comparing the two forces we can see that the Magnetic Force corresponds to an angular velocity $\omega=qB/(2m)=\varepsilon/2$

We propose the following question: for what combination of initial speed and magnetic field will the orbit pass through the origin? For finding the answer we can use the similarity with Foucault's pendulum. Here we describe a pendulum located on a turntable. The bob of the pendulum describes a rotating ellipse. In the laboratory frame the bob has an initial velocity ($\vec{v}=\vec{\omega}\times\vec{R}$).

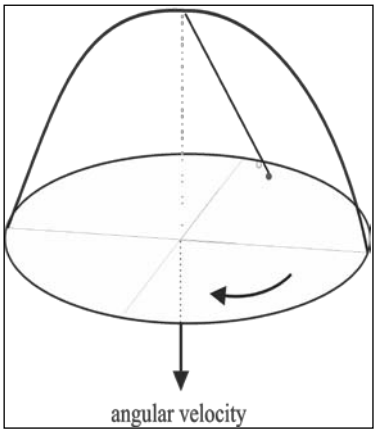

Figure 7: Foucault's pendulum

If on the other hand the pendulum is hanged from a basis in the laboratory frame then the motion starts with initial velocity equal to zero in the laboratory frame.

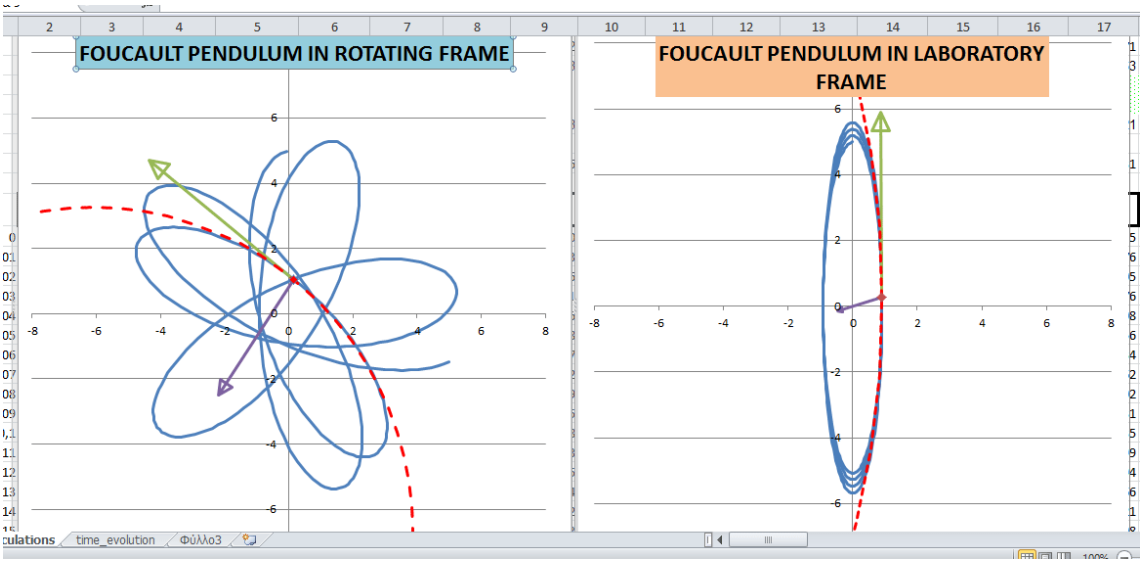

Figure 6: The Foucault pendulum in two frames of reference

The corresponding velocity in the rotating frame will be $\vec{v} = -\vec{\omega} \times \vec{R}$. The motion in the laboratory frame will be confined in a plane. In this case the pendulum will pass through the origin. The same thing can happen in a magnetic field if $v_{x0} = qB/(4m)$. Then the motion will be that of a pendulum starting with $v_{x\ initial}=0$ in the laboratory frame. This can help us to calculate the time needed for a charge to pass through the origin.. For a body moving under a central attractive force F=-k·x in the laboratory frame $T = 2\pi\sqrt{m/k}$. In the case of a rotating frame then we should take into account the centrifugal force $m\omega^2 r$. The constant of the oscillatory motion is $-k_{\ effective} = -k+m\cdot\omega^2$. This will be the effective constant. Then for the magnetic field $-k_{\ effective} = -k+m\cdot(qB/(2m))^2 = -k+\varepsilon^2/4$. With $\lambda = -k_{\ effective}/m$ and λ_0 the corresponding value for the laboratory frame, $\lambda_0 = \lambda - \varepsilon^2/4$ and so the period is:

$$T = 4\pi\sqrt{m/(4\lambda - \varepsilon^2)}$$

V. Two Dimensional Motion on a Rotating Frame

A rotating frame is very interesting from the point of didactics of physics. There is a need for introduction of frames of reference and "inertial" forces. As Galili & Kaplan (1997) pointed out, standard Introductory Physics Courses do not usually consider more than one observer. He examined the presentation of Energy and Momentum in different frames of references.

Another issue that is usually neglected is the mass of the base. Galili & Kaplan examined the case of a ball sliding on base of finite mass and considers the energy and momentum in the corresponding frame of reference.

These issues will be considered here for the case of a rotating frame of reference. First will be addressed the rotating frame of reference with constant angular speed ω. In this case the inertia of the base can be considered infinite. Then there will be a consideration of a base of finite mass.

a) Base with constant angular velocity

Usually textbooks deal with relative motion in rotating frames of reference in connection with the Centrifugal force and the Coriolis force.

b) Absence of friction

The current paper deals with a particle lying at a point with coordinates (0, A). The rotating frame has an angular velocityω.

One characteristic of the rotating frame is that while the angular velocity is the same for every point of the rotating frame, the velocity each point is different.

c) Considerations of friction

Considering the effects of friction we obtain some results, which are not anticipated by the students (or even from physics teachers) if they do not grasp the notion of relative velocity. For a given location of the sliding body, the relative velocity has to be composed from the velocity of the point of the turntable and the velocity of sliding body.

Now the frictional force can be expressed as:

$$F_x = -\mu \frac{V_{x,relative}}{|V_{relative}|} \cdot m.g, \quad F_x = -\mu \frac{V_{y,relative}}{|V_{relative}|} \cdot m.g$$

This expresses the fact that the frictional force is directed opposite to the direction of relative velocity of the sliding body to the base.

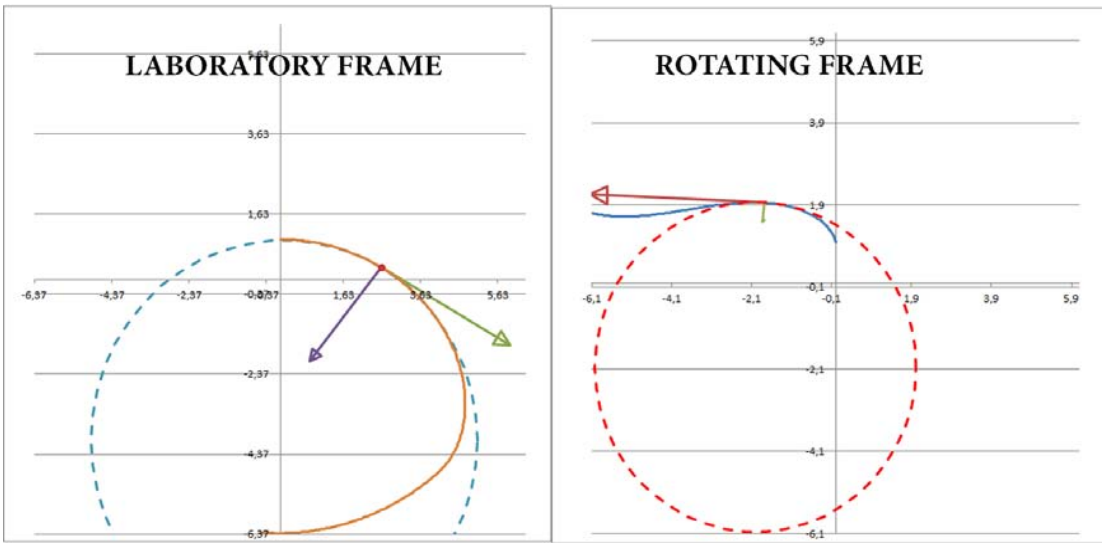

Figure 7: Otbits in different frames of reference

Since the direction of the frictional force is opposite to the direction of the relative velocity, the friction can act as an accelerating agent in the lab frame.

The path of a puck sliding on a turntable will become a curve. This can be explained by picturing the friction as dragging the sliding body from its rectilinear motion to a curved one. In this case the force vector and the velocity vector in the lab frame will have an acute angle. In the rotating frame the angle will be 180°.

Diagrams of the position of the body, its velocity and acceleration can be generating by using a spreadsheet or a Visual Basic program. In both cases are possible simulations of the motion.

The sliding body will get energy from the rotating frame. So the energy of the body will increase.

d) Energy considerations

The kinetic energy of the body in the laboratory frame will be constant in the case of no friction. The kinetic energy will increase in the case of frictional forces if the angular velocity of the base is constant.

A question that may be asked is: 'In the lab frame the friction can act as an accelerating force, what is its role in the rotating frame?' In the rotating frame the direction of the relative velocity is tangential to the path, so the direction of the friction is opposing the direction of velocity. So in the rotating frame the friction will decelerate the movement as it is anticipated from 'normal' cases. In the case of the observer on the rotating frame then a «centrifugal» potential can be used, to explain the energy increase as due to the action of the «centrifugal» force on the sliding body. This explanation holds true only if the angular velocity of the base is constant.

On the other hand if the rotating frame is of a finite mass it is expected that the energy

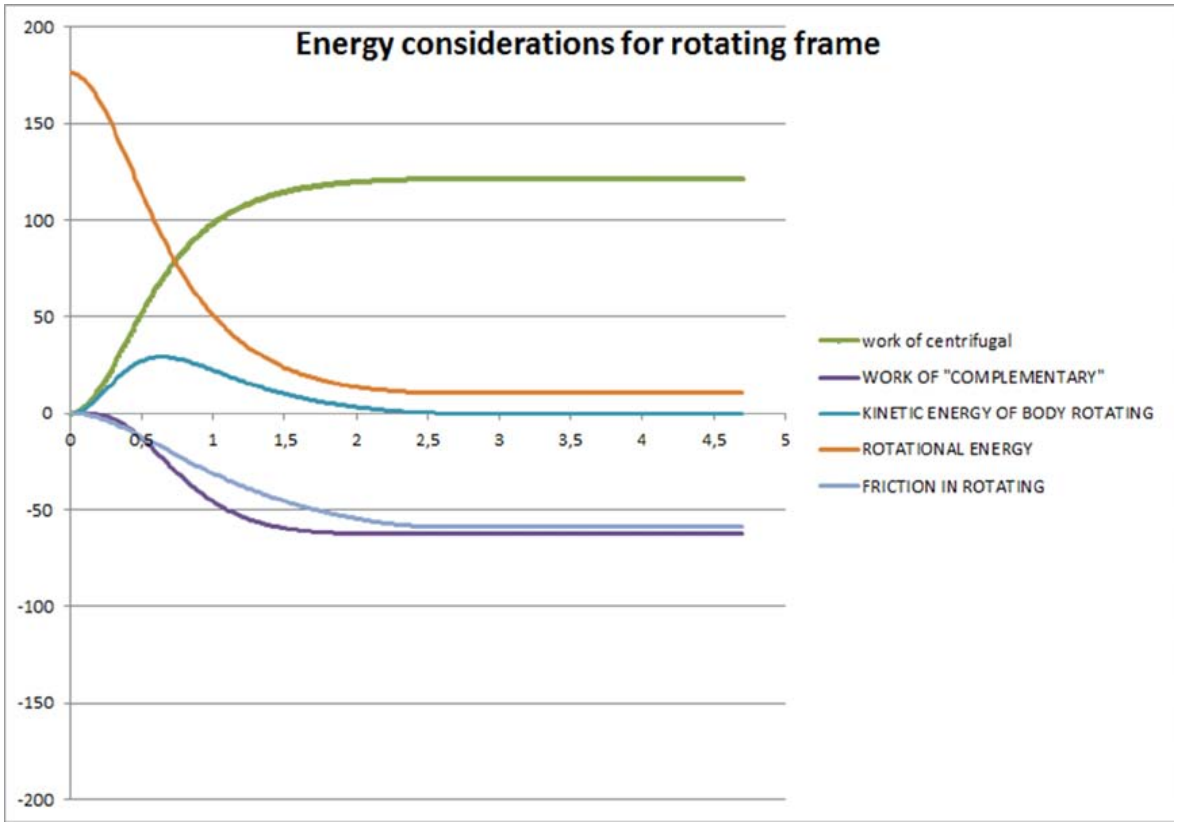

Figure 8: Energyin a rotating frame which is affected by the friction

of the rotating disk will decrease, due to the interaction of the sliding body (puck) with the rotating body. The sliding body acts through its frictional force (which is the one part of the action – reaction pair). The moment of this force decreases the angular velocity of the base. The decrease of the angular velocity can be calculated by the use of the conservation of angular momentum. The force vector and the velocity vector in the beginning of the motion will make a 90° angle in the laboratory, later on the angle becomes acute, and later it becomes obtuse. In this case the speed in the laboratory frame is reduced. When the body stops in the rotating frame, the angle becomes 90° in the lab frame.

In the case changing angular velocity the work of the friction plus the work of the centrifugal force in the rotating frame of reference will not in general be equal to the change of the kinetic energy. If the angular velocity of the base is affected because of its finite inertia, then the complementary acceleration must be taken care of, which gives an additional inertial force. The Coriolis force of course does not contribute to the change of the kinetic energy. The need to introduce an additional inertial force can be shown through a computer program, which shows to the user the sum of different terms if it is equal to the change of the kinetic energy in the rotating frame.

The final state of the body for the rotating observer will be that the sliding body will be motionless. For the observer in the lab frame the whole system will turn with a reduced angular speed. The body will be will describe a circle with the reduced angular speed of the base.

In case of rapid reduction of the angular speed, the graph of velocities in the rotating frame of reference ceases to have the periodic character, which has in the case where the base is not affected by the sliding body.

VI. Applications in Class

The teaching of two dimensional motions was done in the elementary education department of Democritus University (Mihas P. and Gemoysakakis T. 2007) and there was a special laboratory on friction in which except the basic ideas of friction the lesson was extended for the sliding friction on a rotating disk (Evangelopoulou and Mihas 2011). The results of this work were applied with less success to High School students (Evangelopoulou A and Mihas P.2012).

As was seen by Mihas & Gemoysakakis, the students who attended the classes could draw correctly the vectors of acceleration in different paths, and also draw the “circles of curvature”, while for students who did not attend the classes they did not have any success.

The use of the files on friction for a “puck” moving on a rotating disk can be: a) Explanation of the direction of Friction in a turntable. b) Elucidation of the meaning of relative velocity in the lab frame and in the

rotating frame of reference. c) The consideration of the direction of acceleration and the direction of the velocity vectors for the case of increasing, decreasing or constant speed. d) The application of the Work – Energy theorem in different cases. e) The need to introduce the complementary acceleration in the case of accelerating frame of reference.

A note on the software used in this paper

For each part of this paper there are Excel files used to draw the figures.

Section	Software
Sinusoidal Curve	sinusoidal MAKRO or sinusoidal (without a macro)
Tutorial Curve	tutorial curve
Neile Curve	Neile curve
Cycloidal pendulum	cycloid pendulum with macros
Motion of a charged body in a combination of electric field and magnetic field	Movement in combination of fields macros or without macros
Foucault's Pendulum	FOUCAULT PENDULUM MACROS
Two dimensional motion with friction	sliding on rotating frame small inertia plux mR2 sliding on rotating frame BIG INERTIA

All the files are found at http://kyriakosxolio.gr/w_dm_curv.html

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Towards A Cooler Singapore

By Ming Xu
Future Cities Laboratory

Abstract- Urban heat island, together with urban noise and urban air pollution, are the three major environmental challenges of future more livable cities. Urban heat island is defined as the phenomenon that the air temperature in urban area is consistently higher than its rural area (Oke, 1973). It has posed similar heat-related stress and health issues (Kovats and Hajat, 2008; Lo and Quattrochi, 2003; Oikonomou et al., 2012), higher energy costs (Kolokotroni et al. 2012) and downgraded urban living quality (Mavrogianni et al., 2011).

Earlier studies in Singapore has identified an urban heat island intensity of 4.5 °C (Wong and Chen, 2006). Another study of Chow and Roth (2006) has reported that the maximum urban heat island intensity occurs in central business districts, low-rise and high-rise residential area around six hours after sun sunsets. It is also found that stronger urban heat islands are observed in May to August during Southwest monsoon. The maximum urban heat island intensity could be as high as 7 °C observed at Orchard Road at 9pm.

While many causes of the urban heat island have been identified as in Gartland (2008), the contribution of each component strongly depends on the individual city and its geography. To understand the science behind urban heat island and propose possible countermeasures in Singapore, it is of critical importance to identity each type of heat sources and sinks and their respective contributions.

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I. Heat Sources

a) Solar radiation (93%)

Built-up urban area will absorb solar radiation during daytime and emit heat during nighttime, which is the major heat source of urban heat island. Table 1 shows the total amount solar energy reaches the ground surface of Singapore is around 4,325,871TJ. With an average absorptivity of 0.7, the total amount of absorbed solar energy is 3,028,109TJ. In 2013, the total amount energy demand is 460,452TJ, which is only 15% of the absorbed solar energy. If 50% of the energy used in Singapore finally dissipate into the environment in the form of heat, the absorbed solar energy should account for 93% of total heat of 3,258,335 TJ.

b) Anthropogenic activities (7%)

In 2013, industrial activities in Singapore have consumed 252,078 TJ, which accounts for 4% of the total heat. Traffic in Singapore has consumed 108,490 TJ in 2013, which contributes 2% of the total heat into the environment. Other anthropogenic activities such as household energy consumption will account for the last 1% of the total heat.

II. Heat Sinks

a) Greenery

Greenery in Singapore, which includes trees and grasses, is the major heat sink of the city. Trees have the effects of wind shielding, air cooling, air humidifying, dissipating urban noise and purify urban air. In terms of cooling effects of trees, evapotranspiration contributes most but the exact amount of heat it removes needs to be investigated with more details. Grasses also help to cool down the environment through evaporation.

b) Water bodies

Water bodies include rivers, coastal seas, swimming pools and other water features in the city help to balance the surrounding air temperature by avoiding it to be neither too cold nor too hot. In Singapore, water bodies mainly help to keep the city away from being too hot. More detailed work needs to be done to identify its contribution to mitigating urban heat island in Singapore.

Towards a cooler Singapore, an integrated system which includes solar radiation, buildings together with air conditioners, traffic, greenery and water bodies needs to be established. With this fully coupled system which ensures interactions between all components, we will be able to identify their respective contribution to urban heat island and evaluate proposed mitigation countermeasures.

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Table 1: Solar energy (TJ) reaches the ground surface of Singapore

Time	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
6:01- 7:00	0	0	0	0	0	0	0	0	0	3	3	0
7:01- 8:00	34	31	39	75	83	65	49	44	96	140	132	70
8:01- 9:00	342	360	391	479	481	414	404	378	469	595	541	430
9:01-10:00	777	872	846	973	968	810	857	826	880	1028	958	833
10:01-11:00	1180	1222	1294	1330	1390	1167	1188	1222	1276	1372	1279	1144
11:01-12:00	1504	1644	1563	1726	1662	1462	1582	1514	1558	1576	1494	1380
12:01-13:00	1695	1840	1719	1908	1750	1654	1739	1664	1558	1659	1561	1486
13:01-14:00	1721	1742	1791	1623	1649	1623	1569	1654	1667	1576	1483	1447
14:01-15:00	1582	1809	1631	1781	1519	1574	1636	1530	1462	1411	1343	1390
15:01-16:00	1310	1538	1369	1465	1240	1305	1354	1286	1382	1110	1074	1165
16:01-17:00	934	1038	1002	849	841	906	921	921	898	733	712	818
17:01-18:00	541	632	611	536	435	489	551	515	489	355	334	419
18:01-19:00	140	194	158	122	78	104	142	114	83	39	36	70
19:01-20:00	3	5	3	0	0	0	3	0	0	0	0	0
Daily total	11762	12926	12417	12867	12096	11573	11995	11669	11819	11596	10949	10651
Monthly days	31	28	31	30	31	30	31	31	30	31	30	31
Monthly total	364612	361941	384913	386008	374963	347182	371834	361724	354559	359477	328468	330189
Annual total	4325871											

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Mozingo Studies II. Similarity of the Planktonic and Deposited Diatom Assemblages

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Keywords: *plankton taphonomy; biocenosis; thanatocoenosis; paleoecology; paleolimnology.*

GJSFR-I Classification: *FOR Code: 040599*

Mozingo Studies II. Similarity of the Planktonic and Deposited Diatom Assemblages

Kurt A. Haberyan

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Mozingo Studies III: Fidelity of the deposited diatom assemblage with that of the plankton

I. Introduction

Paleolimnological reconstruction often relies on the faithful presence of certain plankton components in the sediments. It has long been known that, while many taxa may appear in the living plankton, only a small subset is represented in the sediments; thus cellular remains of 'soft-bodied' algae are generally not useful in paleolimnology. In contrast, some taxa are likely to be preserved in proportions that more closely approximate a long-term average of the actual plankton.

Despite the widespread acceptance, few studies have examined plankton-to-sediment fidelity. In smaller lakes, the deposited assemblage of diatoms may closely resemble the plankton (Battarbee 1978; Battarbee 1981; Haworth 1980); littoral taxa may be under-represented (Anderson 1989; Stewart and Lamoureux 2012), or over-represented (Rautio et al. 2000). In addition, the method of phytoplankton collection (e.g. nets) may not accurately represent the modern plankton (e.g. Battarbee 1979).

Diatom deposition in larger lakes is complicated by extended sinking time, unless diatoms are encased in fecal pellets from grazers. Fecal pellets account for at least 40% of deposited diatoms in southern Lake Tanganyika, but caused only minor distortions to the overall diatom record (Haberyan 1985). In other large lakes, however, deposited assemblages may be surprisingly distinct from the plankton. For example, in Lake Malawi the relative abundance of *Nitzschia* declined steadily with increasing sediment trap depth, but littoral taxa increased. In surface sediments from 92 m deep and about 1.3 km offshore, *Aulacoseira* was over-represented and *Nitzschia* was drastically under-represented (Haberyan 1990); some littoral taxa in these sediments were over-represented (*Fragilaria brevistriata, Rhopalodia*) while others were under-represented (*Nitzschia epiphyticoides, Surirella, Encyonema*) relative to the plankton (Haberyan 1988).

Given the widespread use of diatoms in paleolimnological analysis, it is important to validate the relationship between planktonic and deposited assemblages. The purpose of this study, therefore, is to investigate the fidelity of the diatom assemblage in the sediments by comparing it to regular samples of live phytoplankton from the lake.

a) Site description

Mozingo Lakeis a reservoir in northwestern Missouri (40.45° N, 94.78° W; Fig. 1). The maximum depth of the lake is 15 m, of which 2.6 m was the former stream channel that had been deeply incisedinto the floodplain. The watershed of the lake has an area of 5013 hectares, aside from the 400 ha of the lake itself. Physical, chemical, and planktonic characteristics of the lake have been characterized previously (Haberyan 2016 and submitted): since impoundment in 1994, the pH has averaged 7.99, nitrate-N averaged 0.60 mg/L, orthophosphate 0.28 mg/L, and silica 1.48 mg/L. Phytoplankton biovolume has averaged 5.9 x 10^6 um^3/mL, mostly represented by cyanobacteria (49%), cryptophytes (42%), and diatoms (6%).

II. Methods

Mozingo Lake was sampled from 1994 to 2014, generally from a mid-lake station over the former floodplain (Station 1), about 330m north of the dam and 30m east of the drowned stream channel (Fig. 1; 40.3514°N, 94.774°W). An alternate location, Station 2, was sampled during inclement conditions (e.g. storms and thin ice), using a floating dock where the lake was 3m deep (40.3566°N, 94.7765°W). For this analysis, 81

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quarterly samples were considered (samples from mid-January, -April, -July, and -October).

Phytoplankton samples were collected with wide-mouth glass jars (at least 600mL in volume and less than 118mm tall), submerged to a depth of approximately 30cm with the mouth downward, then slowly turned upright. Samples were preserved with Lugol's solution within two hours. After at least four days of settling, the supernatant was siphoned off; the remaining liquid was swirled and 0.035mL was placed on a slide, covered with a cover slip, and sealed with clear nail polish to delay drying. Community composition was characterized on an Olympus CH-2 microscope at 150x until at least 100 living algal units (unicells, colonies, or filaments) had been encountered. Cells without cytoplasm were considered to be resuspended and were ignored. Identifications were based on Krammer and Lange-Bertalot (1991). Taxa were sorted into 12 morphogroups due the similarity of some genera when mounted in water. *Navicula*+ herein includes *Neidium, Acnanthidium,* and similar pennate diatoms (however *Asterionella* and *Fragilaria* were distinct), while *Cyclostephanos*+includes *Cyclostephanos, Stephanodiscus, Cyclotella* and other discoid diatoms.

Sediments were collected from a depth of 11m at Station 1 in July 2014, using a Peterson dredge that had been modified to descend open and collect the uppermost sediments. The sample was mixed thoroughly and four subsamples were examined as smear slides at 100x. Another subsample was processed for diatoms at room-temperature in HNO_3 and H_2O_2 until the sediment was completely oxidized (Stoermer et al. 1995). After three rinses, an aliquot was dried on a coverslip, mounted in Naphrax®, and examined with an Olympus CH-2 microscope. Diatoms were identified at 1000x using Krammer and Lange-Bertalot (1991) and counted at 400x until at least 400 valves had been encountered. While taxa were identified to genus, data were compiled into the same morphogroups as the planktonic data.

For comparison with the sediment assemblage, diatoms in live phytoplankton samples were volumetrically normalized, sample by sample, to the equivalent number per milliliter of lake water. These were then totaled for each morphogroup and converted to a composite percentage. Percentage data were transformed with the square-root transformation prior to a chi-squared test ($p = 0.05$) in which the abundances in sediments (i.e. observed data) were compared to the planktonic abundances (i.e. expected values).

III. Results

The volumetrically-normalized phytoplankton samples were dominated by cryptophytes, cyanobacteria, and diatoms. Among the planktonic diatoms, the most common were*Aulacoseira* (48% of all diatoms)and *Cyclostephanos*+ (18%) (Table 1). On the smear slides the most common structures were cells of *Aulacoseira granulata*, followed by *Stephanodiscus, Cyclotella*, *Synedra,* and tests of testate amoebae. Rarely-encountered structures included other algae (*Encyonema, Cocconeis, Rhopalodia, Gyrosigma, Pinnularia, Schroderia*), sponge spicules, grass cuticles, post-abdominal claws, and *Bosmina* head shields. In the acid-processed sediment, 423 diatom valves were encounted; almost 47% belonged to *Cyclostephanos*+ (including *Cyclotella bodanica, C. compta,* and *C. ocellata*) and 34% belonged to *Aulacoseira granulata*.

Relative abundances of diatom morphogroups varied markedly between planktonic and sediment samples (Table 1). *Cyclostephanos*+ was over-represented in sediments (by a factor of 2.5), as was *Navicula*+ (3.9x); other taxa were rare in the plankton but strongly over-represented in sediments (up to 1000x). Taxa that were under-represented in the sediments included *Aulacoseira* (0.71x), *Fragilaria* (0.30x), and *Asterionella*+ (0.13). Despite these differences, the chi-squared test indicated no significant difference in the assemblages ($\chi^2 = 9.299$, df = 11, p = 0.594).

IV. Discussion

Sedimented diatoms represent those in the plankton, but proportionality is not necessarily preserved; even relative ranks may vary (Table 1). For the living phytoplankton samples, the most common taxa were, in order, *Aulacoseira, Cyclostephanos*+, *Asterionella,* and *Fragilaria*; together, these account for 97.4% of all diatoms. However, in the sediment sample the rank was different: *Cyclostephanos*+, *Aulacoseira, Cocconeis, Fragilaria, Navicula*+,and *Asterionella*, together accounting for 96.1% of all diatoms. Shannon-Weiner diversity was nearly identical (0.587 and 0.598), and the assemblages were not significantly different according to the chi-squared test (p = 0.594).

The differences between the modern phytoplankton and sediment assemblages does not seem to reflect diatom habitat: while some benthic taxa were over-represented in the sediments (*Cocconeis, Navicula, Encyonema, Gomphonema*), others were under-represented (*Gyrosigma, Rhopalodia, Epithemia*). Understandably, several taxa that were exceedingly rare in the plankton (< 0.04%) were absent from the sediments, suggesting that additional counts from the sediments may reveal their presence.

For paleolimnologists, it may be of some concern that *Aulacoseira* was the most common genus among diatom frustules in the plankton (48%), but it ranked second in the sediments (34%). This is similar to a study on Lake Saanajärvi, Finland (Rautio et al. 2000), where *Aulacoseira* was under-represented in sediments (6.4% in plankton and 0.3% in sediments) and *Cyclotella*

was over-represented (40.2% in plankton and 48.7% in sediments). In Mozingo, dissolution cannot account for these differences, because *Aulacoseira* frustules were less dissolved than was *Cyclostephanos+*; thus dissolution does not explain the over-representation of *Cyclostephanos+* in sediments.

It is also possible that the number of counted diatoms in the sediment (n = 423) is too small to accurately reflect the deposited assemblage, but the 95% confidence interval for this sample size is ±5% (Mosimann 1965); this may account for differences among the rare taxa, but not for taxa whose relative abundances differed by 10% or more: *Aulacoseira, Cyclostephanos+, Asterionella,* and *Fragilaria.*

Other studies have reported that littoral diatoms are over-represented in sediments (Anderson 1989; Rautio et al. 2000; Stewart and Lamoureux 2012). Data from Mozingo Lake confirms these: littoral diatoms comprise 1.0% of the planktonic diatoms but 10.8% of the sediment diatoms (Table 1). However, this distinction is largely due to *Encyonema*, *Cocconeis,* and *Gomphonema* (7.2% of sediment diatoms); other taxa are under-represented, including *Gyrosigma, Epithemia,* and *Rhopalodia*. Therefore, while littoral taxa in general seem to be over-represented in sediments of small lakes, certain littoral taxa do not follow this trend. This conclusion supports by Rautio et al. (2000), who found that some littoral taxa were over-represented in sediments (e.g. *Achnanthes, Cocconeis, Fragilaria*) while others were under-represened (e.g. *Cymbella, Denticula, Eunotia*). Within a genus, some species were under-represented while others were over-represented (e.g. *Navicula*).

In a core from large, deep lake Lake Tanganyika, *Aulacoseira* (then called *Melosira*) was more common inside fecal pellets than outside, while *Stephanodiscus* was more common outside of pellets; this suggested that copepod feeding preferences influenced differential diatom deposition (Haberyan 1985). In another deep lake, Lake Malaŵi, sediment trap collections differed progressively with depth compared to plankton, and the sediment assemblage magnified this trend. In that case, both *Stephanodiscus* and *Aulacoseira* (then called *Melosira*) were over-represented in sediments, in part because *Nitzschia* was strongly under-represented (Haberyan 1990). In both of these studies, *Aulacoseira* was over-represented in sediments, contrary to Mozingo Lake, where *Aulacoseira* is under-represented. The difference may, in part, reflect the shallow depth of Mozingo (15 m), compared to the previously-studied sample sites (> 90 m); in Mozingo, lateral transport and sinking distances are much shorter, and fecal pellets are likely to be less important in diatom deposition. Nonetheless, the rapid sinking of *Aulacoseira* is well known (e.g. Lund 1954), and *Aulacoseira* should be over-represented relative to slower-sinking taxa like *Stephanodiscus.*

Other factors that may contribute to the observed differences include sediment focussing, which transports sediments enriched in littoral taxa to offshore locations; such transport of dead frustules may outweigh outwash of live ones. In addition, differences may relate to sampling frequency (which may fail to capture important monthly variations) and to sampling depth; samples from 30 cm deep may not be representative of the entire water column.Finally, a variety of site-specific factors may influence differential diatom deposition, such as lake morphology and chemistry (Flower 1993).

While the differences between plankton and deposited assemblages may not be severe enough to alter qualitative interpretation of diatom stratigraphies, they may indeed affect quantitative interpretations, for example those based on transfer functions that relate diatom percentages to estimates of water chemistry. It is therefore important that we paleolimnologists be appropriately cautious when interpreting estimates of ancient lake conditions.

V. Acknowledgements

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Table 1: Relative abundances of diatom morphogroups in planktonic and acid-processed sediment samples, as percent of all diatom frustules. Sediment: plankton ratio compares relative abundances and is rounded to two significant figures. Abbreviation: est., estimated.

Morphogroup	Taxa included	Percent of diatoms in plankton	Percent of diatoms in sediment	Sediment: plankton ratio
Aulacoseira	*Aulacoseira* only	48.239	34.3	0.71
Cyclostephanos+	*Cyclostephanos, Cyclotella,Stephanodiscus,* and other discoid diatoms	18.831	46.8	2.5
Asterionella	*Asterionella formosa* only	16.085	2.1	0.13
Fragilaria+	*Fragilaria crotonensis* and others	14.296	4.3	0.30
Synedra	*Synedra ulna* only	1.546	1.9	1.2
Navicula+	*Navicula, Neidium, Acnanthidum,* and similar pennate diatoms	0.928	3.6	3.9
Encyonema	*Encyonema* only	0.028	1.2	43
Gyrosigma	*Gyrosigma* only	0.035	0.0	0
Cocconeis	*Cocconeis* only	0.005	5.0	1000
Gomphonema	*Gomphonema* only	0.003	1.0	330
Epithemia	*Epithemia* only	0.002	0.0	0
Rhopalodia	*Rhopalodia* only	0.002	0.0	0
	Number of frustules counted	16,194 (est.)	423	
	Shannon-Weiner Diversity	0.587	0.598	

Figure caption

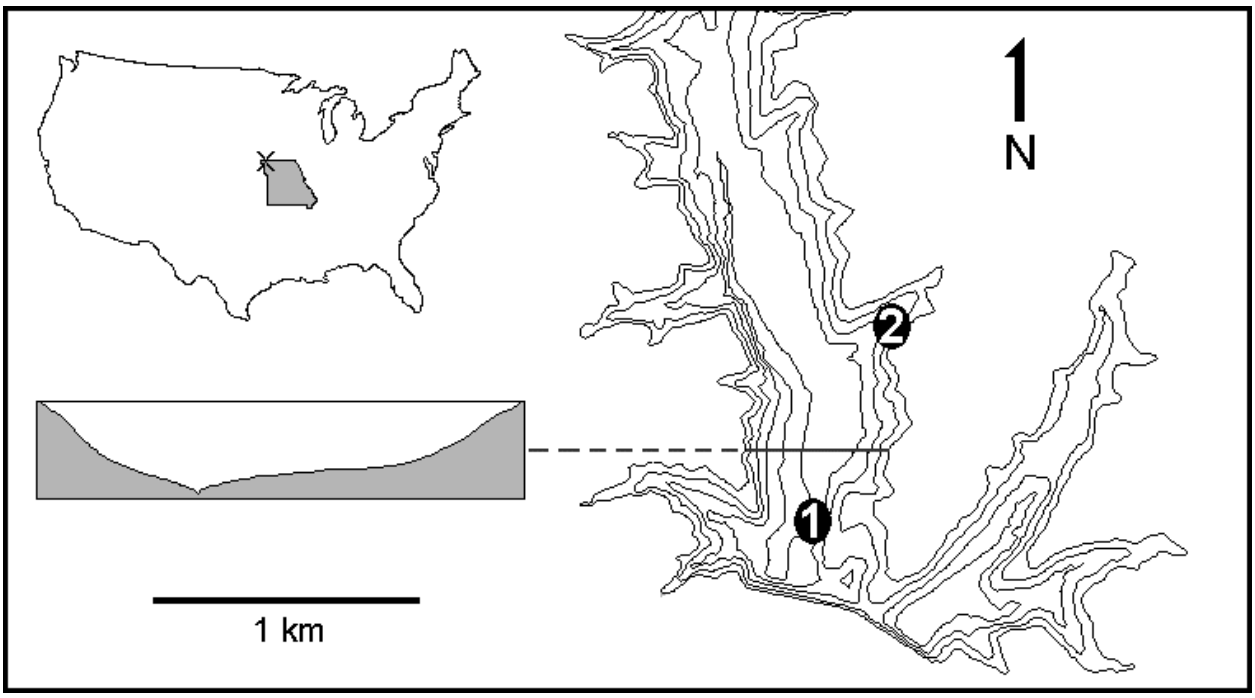

Figure 1: Bathymetric map of the southern third of Mozingo Lake; contour interval 3 m. Vertical exaggeration of transverse profile is 3.7. Inset shows Missouri and the lake's location ("x")

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Physiochemical and Functional Characterization of a Dominant Grain Endosperm Protein Called Glutelin in Rice (*Oryza Sativa* L.) using *in Silico* Methods

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Abstract- Glutelin protein is the most well-known abundant seed storage protein in rice seed endosperm. A total of 9 glutelin and glutelin type protein sequences from *Oryza* species available in uniport were evaluated by using bioinformatics tools to investigate physico-chemical properties, secondary structure prediction, putative phosphorylation sites and conserved motif search. Physicochemical analysis offers data such as pI, EC, AI, GRAVY and II about these sequences and the results showed that all glutelin protein sequences are basic, hydrophilic, thermo stable, having some extracellular portion. The secondary structure of the protein sequences were also predicted using SOPMA server. It was observed that alpha helix was predominant, followed by random coil, extended strand and least beta turn was found. Putative phosphorylation sites were also identified which are found to be conserved in plant species and the results showed that the most abundant phosphorylation site is serine residues in glutelin protein sequences.

Keywords: *glutelin protein, cupin family proteins, in silico, and homology modeling.*

GJSFR-I Classification: *FOR Code: 060799*

Strictly as per the compliance and regulations of:

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Keywords: glutelin protein, cupin family proteins, in silico, and homology modeling.

Abbreviations

pI	:	Isoelectric point
EC	:	Extinction coefficient
AI	:	Aliphatic index
GRAVY	:	Grand average of hydropathy
II	:	Instability index

I. Introduction

Rice (*Oryza sativa* L.), is one of the staple food crop for millions of people worldwide, provides 27 per cent of dietary energy supply and 20 per cent of dietary protein intake. Rice protein is superior in lysine content to wheat, corn and sorghum (Hegsted, 1969) and has a more balanced amino-acid profile. High-protein rice has the potential to enhance human nutrition in poor rural families where rice serves as the staple food (Li *et al.,* 2004). Therefore, in the improvement of rice storage protein, the main target has been to improve the quantity and nutritional quality of the protein in rice.

The major storage proteins found in rice are the glutelins, which according to previous studies, account for 80% or more of the total seed protein (Tecson *et al.,* 1971; Juliano, 1972; Villareal and Juliano, 1978). The remaining 20% is divided as follows: albumins, 1 to 5%; globulins, 4 to 15%; and prolamines, 2 to 8% (Houston *et al.,* 1968). Till date there are some little efforts have been made to characterize this rice glutelin protein. Earlier report on characterization of glutelin protein shows a remarkable similarity exists between the globulin storage protein fraction of oat (Brinegar and Peterson, 1982; Walburg and Larkins, 1983) and the 11S globulin or legumin fraction of pea (Derbyshire *et al.,* 1976) and soybean (Derbyshire *et al.,* 1976). Hence, the present study was undertaken and it was predicts some of the properties of rice glutelin protein such as physico-chemical properties, secondary structure prediction, putative phosphorylation sites, motifs searches etc. The study will be valuable to understand the structural features and molecular function of rice glutelin protein and will raise the prospects of its potential use in research. The obtained results could be used for further *in silico* analysis and homology modeling studies.

II. Material and Methods

a) Sequence retrieval

Expasy (uniprot KB) that provides protein sequences and annotation data (Jain *et al.,* 2009) was used to retrieve the chalcone synthase 1 protein sequences. These were downloaded in FASTA format to be used for further analysis (http://www.uniprot.org).

b) Physio-chemical characterization

For Physio-chemical characterization, theoretical Isoelectric Point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand

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average of hydropathy (GRAVY) were computed using the Expasy Protparm server (Gasteiger *et al.,* 2005) (http://us.expasy.org/tools/protparam.html).

c) Secondary structure prediction

SOPMA tool (Self-Optimized Prediction Method with Alignment) (Geourjon and Deleage, 1995) was applied to extract the information regarding the secondary structures that consist of Alpha helix, Extended strand, Beta turn and Random coil.

d) Putative phosphorylation sites and motif search

The amino acid sequences of the selected plants were analyzed for the putative phosphorylation sites at the NetPhos 2.0 Server (http://www.cbs.dtu.dk/services/NetPhos/) (Blom *et al.,* 1999). Motif search (www.genome.jp/tools/motif) was used to find the number of motifs, motif ID, description and position of the motif found. Analysis of domain and conserved protein motifs was performed using MEME (http://meme.sdsc.edu/meme/meme.html) (Timothy *et al.,* 1994).

III. Results and Discussion

a) Physiochemical characterization

Glutelin protein sequences of *Oryza sativa* (Table 1) were analyzed in this study and corresponding protein sequences were collected from Uniport (http://www.uniprot.org/). Physiochemical properties of these protein sequences computed using Expasy Protparm server and the analyzed results were presented in table 2. The isoelectronic point is the pH at which the protein does not migrate in an electric field. It plays an important role in protein purification. The computed pI value that was less than 7 (pI<7) indicates that proteins were considered as acidic or greater than 7 (pI>7) reveals that proteins were basic in character. The pI value of all the sequences under study having more than 7 (pI>7) reveals that these proteins were basic in nature. The computed isoelectric point will be useful for separating the protein on a polyacrylamide gel by isoelectric focusing. Total numbers of negatively charged residues are lower than the total number of positively charged residues implies that these proteins are having extracellular portion. The extinction coefficient of a protein as calculated by the program depends on the molar extinction coefficient of Tyrosine, Tryptophan and Cysteine residues. Difference in the extinction coefficient values these glutelin proteins as evident from Table 2 was due to the difference in concentration of these three residues. The extinction coefficient can be used to calculate the concentration of a protein in solution. Instability index relies upon the occurrence of certain dipeptides along the length of the protein to distinguish between the unstable and stable protein. If the index is less than 40, it is probably stable in the test tube. If the value is greater than 40, it is probably not stable (Guruprasad *et al.,* 1990). The value for instability index for glutelin proteins are more than 40, hence these proteins are probably not stable (Guruprasad *et al.,* 1990). The aliphatic index refers to the relative volume of a protein that is occupied by aliphatic side chains and contributes to the increased thermo stability of protein. The aliphatic index of a protein is a measure of the relative volume occupied by aliphatic side chain of the following amino acids *viz.,* alanine, valine, leucine and isoleucine. The aliphatic index values of glutelin protein sequences ranging from 74.26 to 80.95. The very high aliphatic index of all glutelin protein sequences supports the view that these may be stable for a wide range of temperatures. Grand average of hydropathicity (GRAVY) index indicates the solubility of proteins: a positive GRAVY value indicates that proteins are hydrophobic in nature whereas a negative GRAVY value indicates more surface accessibility of the protein to interact with water (hydrophilic in nature). GRAVY values of glutelin protein sequences were ranged from -0.456 to -0.568. The very low GRAVY index of glutelin protein sequences implies that these protein sequences could result in a better interaction with water (hydrophilic in nature).

b) Functional characterization

The secondary structure of the protein sequences were predicted using SOPMA server (Table 3). It was observed that alpha helix was predominant, followed by random coil, extended strand and least beta turn was found. The secondary structure was predicted by using default parameters (window width 17, similarity threshold: 8 and number of conformational states: 4). Using the NetPhos 2.0 Server the putative phosphorylation sites were identified for glutelin proteins (Table 4). The output score was given in 0.000-1.000 range and the score above the threshold (0.500) shows the confidence rate of true phosphorylation site by the server. Several putative phosphorylation sites are completely conserved in plant species and interestingly more phosphorylation sites were found in these protein sequences. Conserved protein motifs subjected to MEME to obtain the best possible matches (table 5). Other protein motifs found in the glutelin proteins are most of them belongs to cupin family proteins (fig 2).

c) Conclusion

The present *in silico* study describes some important physiochemical and functional properties of rice glutelin proteins. Physiochemical and functional analysis reveals that rice glutelins are a basic, hydrophilic, thermo stable, having some extracellular portion and which has many phosphorylation sites. Conserved protein motifs are observed in these proteins and other protein motifs found in the glutelin proteins are most of them belongs to cupin family proteins. The obtained results could be used for further *in silico* analysis and homology modeling studies of these glutelin proteins.

IV. Acknowledgements

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Table 1: Details of glutelin protein sequences from *Oryza sativa*

Entry	Entry name	Protein names	Gene names	Organism	Length (No. of A.A)
Q09151	GLUA3_ORYSJ	Glutelin type-A 3	GLUA3 GLUA-3 GT22 GT3 Os03g0427300 LOC_Os03g31360 OSJNBa0083F15.19	*Oryza sativa* subsp. *japonica* (Rice)	496
P07728	GLUA1_ORYSJ	Glutelin type-A 1	GLUA1 GLUA-1 Os01g0762500 LOC_Os01g55690 P0460E08.38 P0512C01.36	*Oryza sativa* subsp. *japonica* (Rice)	499
P07730	GLUA2_ORYSJ	Glutelin type-A 2	GLUA2 GLUA-2 GT1 Os10g0400200 LOC_Os10g26060 OSJNBa0050N08.16	*Oryza sativa* subsp. *japonica* (Rice)	499
P14323	GLUB1_ORYSJ	Glutelin type-B 1	GluB1-A GluB-1 Os02g0249800 LOC_Os02g15169 OJ1113_G05.6 OSJNBa0011N12.36; GLUB1-B GLUB-1 Os02g0249900 LOC_Os02g15178 OJ1113_G05.4 OSJNBa0011N12.34	*Oryza sativa* subsp. *japonica* (Rice)	499
Q6ERU3	GLUB5_ORYSJ	Glutelin type-B 5	GLUB5 GLUB-5 Os02g0268100 LOC_Os02g16820 P0693E08.14	*Oryza sativa* subsp. *japonica* (Rice)	500
P14614	GLUB4_ORYSJ	Glutelin type-B 4	GLUB4 GLUB-4 Os02g0268300 LOC_Os02g16830 P0693E08.16	*Oryza sativa* subsp. *japonica* (Rice)	500
Q02897	GLUB2_ORYSJ	Glutelin type-B 2	GLUB2 GLUB-2 GluB-7 GLUB7 Os02g0249600 LOC_Os02g15150 OSJNBa0011N12.30	*Oryza sativa* subsp. *japonica* (Rice)	495
Q0E261	Q0E261_ORYSJ	Glutelin	Os02g0268300 OsJ_06189	*Oryza sativa* subsp. *japonica* (Rice)	500
Q40689	Q40689_ORYSA	Glutelin	Gt2	*Oryza sativa* (Rice)	499

Table 2: Details of Physiochemical Properties of chalcone synthase 1 protein sequences from different species

Entry	Entry name	Length (No. of A.A)	M.wt	pI	-R	+R	EC	II	AI	GRAVY
Q09151	GLUA3_ORYSJ	496	56015.0	8.81	44	51	45435	46.23	80.95	-0.456
P07728	GLUA1_ORYSJ	499	56246.9	9.09	42	52	45435	51.36	76.77	-0.539
P07730	GLUA2_ORYSJ	499	56306.1	8.93	42	50	50935	50.18	76.37	-0.509
P14323	GLUB1_ORYSJ	499	56550.5	9.26	39	50	50685	52.11	76.19	-0.495
Q6ERU3	GLUB5_ORYSJ	500	56808.0	9.00	42	50	43820	47.94	78.22	-0.508
P14614	GLUB4_ORYSJ	500	56818.0	9.00	42	50	43820	47.81	78.22	-0.510
Q02897	GLUB2_ORYSJ	495	56046.8	9.11	39	48	50685	51.22	74.26	-0.498
Q0E261	Q0E261_ORYSJ	500	56818.0	9.00	42	50	43820	47.81	78.22	-0.510
Q40689	Q40689_ORYSA	499	56239.9	9.09	42	52	45435	50.14	75.19	-0.568

Table 3: Details of secondary structures of chalcone synthase 1 protein sequences from different species

Entry	Entry name	Alpha helix	Extended strand	Beta turn	Random coil
Q09151	GLUA3_ORYSJ	32.86%	21.98%	13.91%	31.25%
P07728	GLUA1_ORYSJ	32.67%	20.64%	14.83%	31.86%
P07730	GLUA2_ORYSJ	32.26%	21.84%	12.42%	33.47%
P14323	GLUB1_ORYSJ	33.87%	21.04%	12.63%	32.46%
Q6ERU3	GLUB5_ORYSJ	35.20%	22.00%	11.00%	31.80%
P14614	GLUB4_ORYSJ	35.20%	20.80%	11.00%	33.00%
Q02897	GLUB2_ORYSJ	36.36%	18.99%	13.74%	30.91%
Q0E261	Q0E261_ORYSJ	35.20%	20.80%	11.00%	33.00%
Q40689	Q40689_ORYSA	29.86%	21.44%	14.63%	34.07%

Table 4: Putative phosphorylation residues in chalcone synthase 1 protein sequences from different species

Entry	Entry name	Putative phosphorylation residues		
		Serine	Threonine	Tyrosine
Q09151	GLUA3_ORYSJ	15	2	3
P07728	GLUA1_ORYSJ	15	2	3
P07730	GLUA2_ORYSJ	21	3	4
P14323	GLUB1_ORYSJ	14	2	3
Q6ERU3	GLUB5_ORYSJ	20	3	4
P14614	GLUB4_ORYSJ	11	4	2
Q02897	GLUB2_ORYSJ	14	2	3
Q0E261	Q0E261_ORYSJ	14	2	3
Q40689	Q40689_ORYSA	15	2	3

Table 5: Different motifs commonly conserved in glutelin protein sequences with best possible match amino acid sequences

Motif	Width	Best Possible Match
1	50	SQSQKFRDEHQKIHRFRQGDIVALPAGVAHWCYNDGDAPVVAIYVTDLNN
2	50	HYVVLKKAEHEGCQYIAFKTNPNSMVSHMAGKNSIFRAMPVDVIANAYRI
3	50	ADTYNPRAGRITNLNSQKFPILNLVQMSATKVNLYQNAILSPFWNINAHS

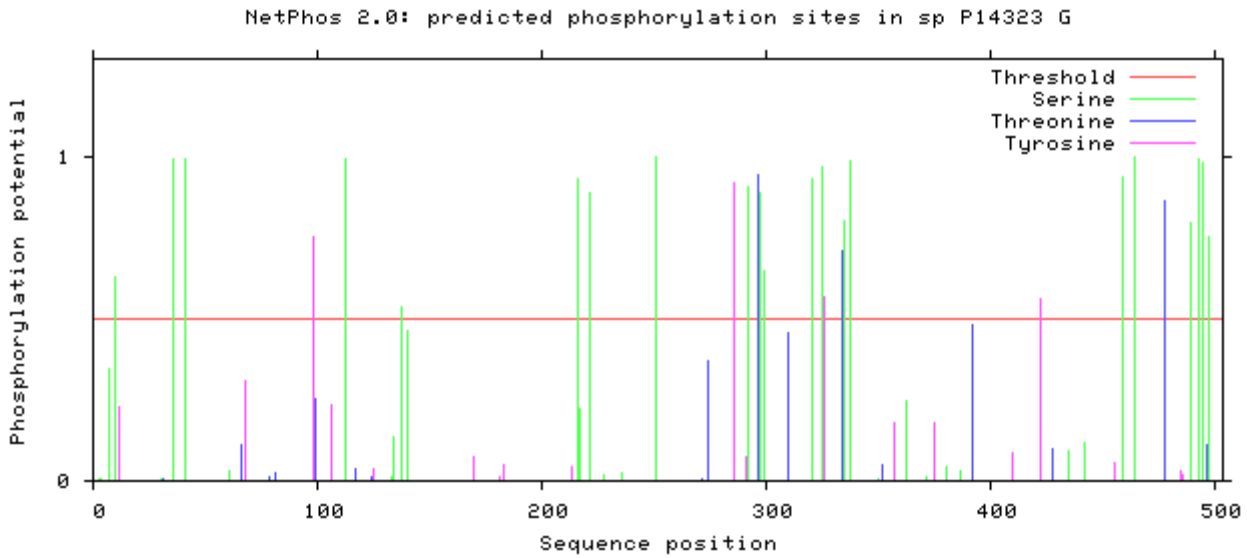

Fig.1: Putative phosphorylation residues in Glutelin type-B 1 protein sequences from *Oryza sativa* subsp. *japonica* (Rice)

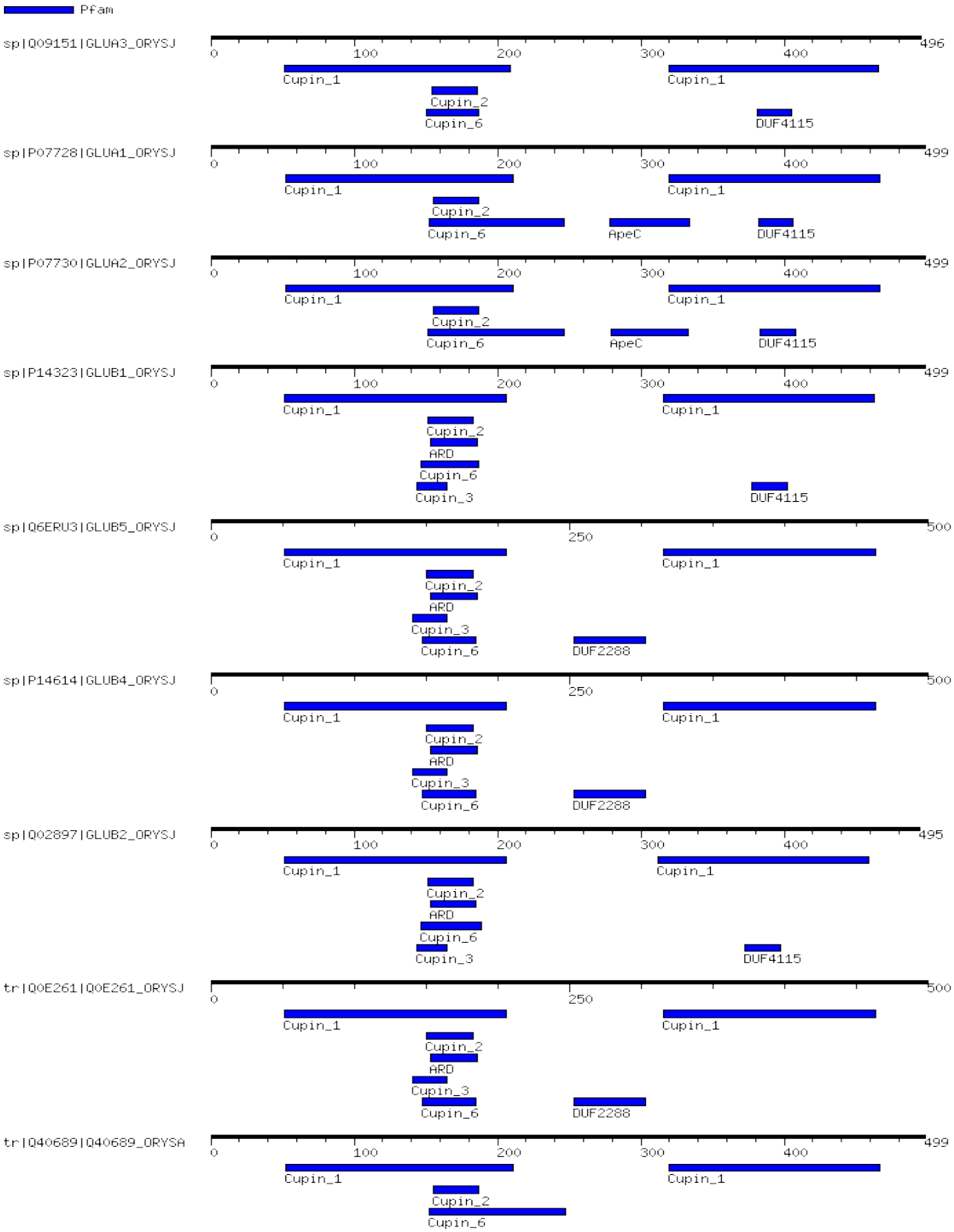

Fig. 2: Different other protein motifs found in glutelin proteins of *Oryza sativa*

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(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

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Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

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- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

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Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

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21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

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- Please note the criterion for grading the final paper by peer-reviewers.

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· Present your points in sound order

· Use present tense to report well accepted

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- Give details all of your remarks as much as possible, focus on mechanisms.
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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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