## Molecular DNA Repair

Using a piece of molecular "Velcro" to attach a light-emitting probe to a protein molecule, University of Wollongong (UOW) researchers have unlocked the mystery of how an important protein goes about repairing damaged DNA in bacteria, with implications for understanding how antibiotic resistance develops. [21]

In new research, Hao Yan of Arizona State University and his colleagues describe an innovative DNA <u>walker</u>, capable of rapidly traversing a prepared track. [20]

Just like any long polymer chain, DNA tends to form knots. Using technology that allows them to stretch DNA molecules and image the behavior of these knots, MIT researchers have discovered, for the first time, the factors that determine whether a knot moves along the strand or "jams" in place. [19]

Researchers at Delft University of Technology, in collaboration with colleagues at the Autonomous University of Madrid, have created an artificial DNA blueprint for the replication of DNA in a cell-like structure. [18]

An LMU team now reveals the inner workings of a molecular motor made of proteins which packs and unpacks DNA. [17]

Chemist Ivan Huc finds the inspiration for his work in the molecular principles that underlie biological systems. [16]

What makes particles self-assemble into complex biological structures? [15]

Scientists from Moscow State University (MSU) working with an international team of researchers have identified the structure of one of the key regions of telomerase—a so-called "cellular immortality" ribonucleoprotein. [14]

Researchers from Tokyo Metropolitan University used a light-sensitive iridiumpalladium catalyst to make "sequential" polymers, using visible light to change how building blocks are combined into polymer chains. [13]

Researchers have fused living and non-living cells for the first time in a way that allows them to work together, paving the way for new applications. [12]

UZH researchers have discovered a previously unknown way in which proteins interact with one another and cells organize themselves. [11]

Dr Martin Sweatman from the University of Edinburgh's School of Engineering has discovered a simple physical principle that might explain how life started on Earth. [10]

Nearly 75 years ago, Nobel Prize-winning physicist Erwin Schrödinger wondered if the mysterious world of quantum mechanics played a role in biology. A recent finding by Northwestern University's Prem Kumar adds further evidence that the answer might be yes. [9]

A UNSW Australia-led team of researchers has discovered how algae that survive in very low levels of light are able to switch on and off a weird quantum phenomenon that occurs during photosynthesis. [8]

This paper contains the review of quantum entanglement investigations in living systems, and in the quantum mechanically modeled photoactive prebiotic kernel systems. [7]

The human body is a constant flux of thousands of chemical/biological interactions and processes connecting molecules, cells, organs, and fluids, throughout the brain, body, and nervous system. Up until recently it was thought that all these interactions operated in a linear sequence, passing on information much like a runner passing the baton to the next runner. However, the latest findings in quantum biology and biophysics have discovered that there is in fact a tremendous degree of coherence within all living systems.

The accelerating electrons explain not only the Maxwell Equations and the Special Relativity, but the Heisenberg Uncertainty Relation, the Wave-Particle Duality and the electron's spin also, building the Bridge between the Classical and Quantum Theories.

The Planck Distribution Law of the electromagnetic oscillators explains the electron/proton mass rate and the Weak and Strong Interactions by the diffraction patterns. The Weak Interaction changes the diffraction patterns by moving the electric charge from one side to the other side of the diffraction pattern, which violates the CP and Time reversal symmetry.

The diffraction patterns and the locality of the self-maintaining electromagnetic potential explains also the Quantum Entanglement, giving it as a natural part of the Relativistic Quantum Theory and making possible to understand the Quantum Biology.

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Author: George Rajna

## **Preface**

We define our modeled self-assembled supramolecular photoactive centers, composed of one or more sensitizer molecules, precursors of fatty acids and a number of water molecules, as a photoactive prebiotic kernel system. [7]

The human body is a constant flux of thousands of chemical/biological interactions and processes connecting molecules, cells, organs, and fluids, throughout the brain, body, and nervous system. Up until recently it was thought that all these interactions operated in a linear sequence, passing on information much like a runner passing the baton to the next runner. However, the latest

findings in quantum biology and biophysics have discovered that there is in fact a tremendous degree of coherence within all living systems. [5]

Quantum entanglement is a physical phenomenon that occurs when pairs or groups of particles are generated or interact in ways such that the quantum state of each particle cannot be described independently – instead, a quantum state may be given for the system as a whole. [4]

I think that we have a simple bridge between the classical and quantum mechanics by understanding the Heisenberg Uncertainty Relations. It makes clear that the particles are not point like but have a dx and dp uncertainty.

## Molecular Velcro helps illuminate DNA repair

Using a piece of molecular "Velcro" to attach a light-emitting probe to a protein molecule, University of Wollongong (UOW) researchers have unlocked the mystery of how an important protein goes about repairing damaged DNA in bacteria, with implications for understanding how antibiotic resistance develops.

Much like how an emergency services hub coordinates various emergency response teams, the protein RecA leaps into action when DNA is damaged. RecA sounds an SOS alarm, with more than 40 different genes responding to the call to action. It assesses the extent of the damage and what repairs are needed and coordinates the repair activity at the damage site.

However, the repair process can generate mutations, said Molecular Horizons Research Fellow and study lead author Dr. Harshad Ghodke. While RecA activates or switches on the various repair mechanisms that don't introduce errors, it also switches on mechanisms that introduce errors while replicating DNA as a last-ditch attempt to help cells survive. Unfortunately, this process can lead to critical changes in the DNA sequence.

"These changes or mutations are no longer recognised as errors, and the new sequence is replicated in new generations of cells. It doesn't revert back to its original form," Dr. Ghodke said.

This creates a problem for treating bacterial infections. While antibiotics go to work to kill the bacteria, RecA swoops in to help cells survive the antibiotic treatment, and the cells that survive now have potentially antibiotic resistant mutations that render drugs ineffective.

Time-lapse imaging of recA-gfp/pG353C-recA cells. Cells were imaged in the 488 channel every 5 min for 3 hr. In the presence of plasmid expressing excess untagged RecA, the RecA-GFP signal is sequestered in the form of an aggregate that is ...<u>more</u>

The difficulty for researchers thus far in understanding how RecA does its job, and potentially designing drugs that counter its repair work, is that no one has been able to see where exactly repair activities occur inside living cells.

"RecA surrounds a single strand of DNA to form a filament that then signals the SOS response," Dr. Ghodke said. "Typically, researchers would attach a bright fluorescent tag to RecA so they can take

images of it as it goes to work. But with the attached tag, the RecA doesn't do its job very well, and stops functioning as it would in the cellular environment."

The fluorescence signal from the tag also makes it difficult to distinguish RecA that is actively involved in repair work from that which is idle or stored away in the cell awaiting an emergency call out.

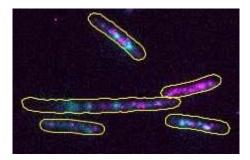
"Imagine taking an aerial photo of a city where you can only see fire trucks directly below. You can't tell if they are actively fighting fire or waiting for a call in the fire station. If we only wanted to see the fire trucks at the site of a burning building, you could attach lights to fire hydrants so that they turn on when fire trucks attach to them, and conclude that adjacent buildings are on fire.

Cells were irradiated at t = 0 with 20 Jm-2 of UV, and imaged every 5 min. In response to UV irradiation, the cells exhibit SOS induced cell filamentation, accompanied by expression from the recA promoter. Video is relayed at 15 fps. Credit: Ghodke et al / University of Wollongong"We did a similar thing with visualising the RecA filament. We used a viral protein that naturally interacts with the RecA filament so it wouldn't interfere with how it works, while lighting up the RecA filament as it takes part in the damage response."

To help visualise this radical new approach to illuminate this DNA <u>repair process</u> inside living Escherichia coli bacteria cells, the researchers turned to 3-D printing to create physical models of RecA to enable them to see its shape and form.

Molecular Horizons Director, Distinguished Professor Antoine van Oijen, said a key to <u>biological</u> <u>processes</u> is to think in structures and shapes.

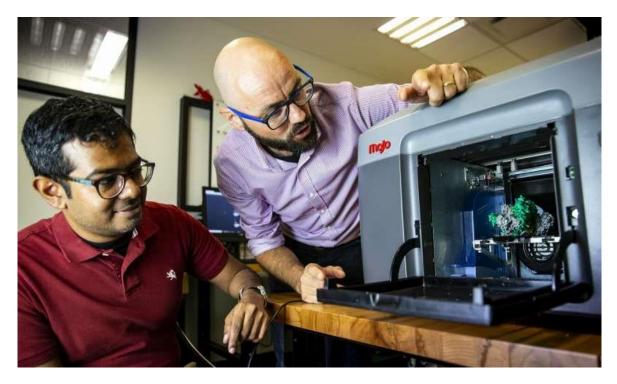
"We know from the imaging we do that proteins are dynamic objects. If we think of them as 3-D structures we can start to visualise how they change and what causes those changes, leading to a clearer understanding of how these proteins work.



Visualisation of RecA bundles in bacterial cells after DNA damage by UV exposure. Credit: Ghodke et al / University of Wollongong"With a <u>physical structure</u>, you can see the interfaces and design methods to attach other proteins. Then using sophisticated imaging tools we can take short films that for the first time, really show us how they work."

Professor van Oijen said the critical piece of the puzzle the team has solved paves the way for new drug treatments that overcome <u>antibiotic resistance</u>.

In some cases, mutated cells deactivate the drug or no longer have the target <u>protein</u> the drug molecules are searching for and a rogue cell is not destroyed.



Dr Dr Harshad Ghodke (left) and Professor Antoine van Oijen used 3D printing to visualise bacteria molecules and plan experiments."Antibiotic resistance is a hugely important global challenge. We don't want to get rid of antibiotics altogether because when they work they're incredibly effective. If we can visualise these processes we can then understand the physical connections between molecules and the structure of proteins and potentially design new drugs that will prevent bacterial cells from becoming resistant."

The research was published in the journal eLife. [21]

## Built for speed: DNA nanomachines take a (rapid) step forward

When it comes to matching simplicity with staggering creative potential, DNA may hold the prize. Built from an alphabet of just four nucleic acids, DNA provides the floorplan from which all earthly life is constructed.

But DNA's remarkable versatility doesn't end there. Researchers have managed to coax segments of DNA into performing a host of useful tricks. DNA sequences can form logical circuits for nanoelectronic applications. They have been used to perform sophisticated mathematical computations, like finding the optimal path between multiple cities. And DNA is the basis for a new breed of tiny robots and nanomachines. Measuring thousands of times smaller than a bacterium, such devices can carry out a multitude of tasks.

In new research, Hao Yan of Arizona State University and his colleagues describe an innovative DNA <u>walker</u>, capable of rapidly traversing a prepared track. Rather than slow, tentative steps across a surface, the DNA acrobat cartwheels head over heels, covering ground 10- to 100-fold faster than previous devices.

"It is exciting to see that DNA walkers can increase their speed significantly by optimizing DNA strand length and sequences, the collaborative effort really made this happen," Yan said.

Yan is the Milton D. Glick Distinguished Professor of Chemistry and Biochemistry at ASU and director of the Biodesign Center for Molecular Design and Biomimetics.

The study was led by Nils G. Walter, Francis S. Collins Collegiate Professor of Chemistry, Biophysics & Biological Chemistry, founding director of the Single Molecule Analysis in Real-Time (SMART) Center and founding co-director of the Center for RNA Biomedicine at the University of Michigan, and his team, along with collaborators from the Wyss Institute, the Dana Farber Cancer Institute and the Department of Biological Chemistry at Harvard (all in Boston, Massachusetts).

"The trick was to make the walker go head over heels, which is so much faster than the hopping used before—just as you would see in a kung fu action movie where the hero speeds up by cartwheeling to catch the villain," says Walter.

The improvements in speed and locomotion displayed by the new walker should encourage further innovations in the field of DNA nanotechnology.

The group's findings appear in the advanced online issue of the journal Nature Nanotechnology.

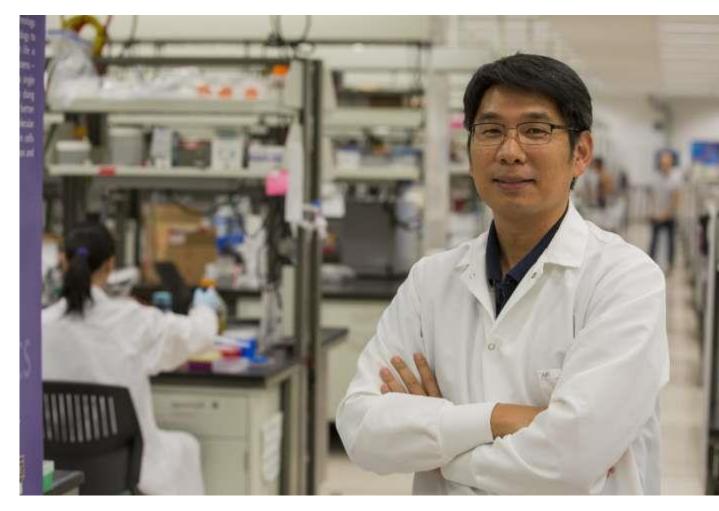
#### **Building with DNA**

Nanoarchitects build their DNA structures, motors and circuits using the same basic principle as Nature. The four nucleotides, labeled A,T,C and G, bind to each other according to a simple and predictable rule: Cs always pair with Gs and As always pair with Ts. Thus, varying lengths of DNA may be programmed to self-assemble, snapping together to form an unlimited variety of two- and 3dimensional nanostructures. With clever refinement, researchers have been able to outfit their oncestatic nano-creations with dynamical properties.

One of the more innovative applications of DNA nanotechnology has been the design of robotic walking devices composed of DNA strands that successively move in a stepwise fashion across a path. The method enabling DNA segments to stroll across a defined area is known as strand displacement.

The process works like this: One leg of the robotic device is DNA strand 1, which is bound to complementary strand 2, through normal base pairing. Strand 1 contains an additional, unpaired sequence dangling from its end, which is known as the toehold.

Next, DNA strand 3 is encountered. This strand is complementary to DNA strand 1 and includes a toehold sequence complementary to DNA strand 1. Once the toehold of strand 3 binds with the toehold of strand 1, it begins sequentially displacing each strand 2 nucleotide, one by one, until strand 2 has been is completely replaced by strand 3. Strand 2 then dissociates from strand 1 and the process can begin again. (See figure 1).



Hao Yan is the Milton D. Glick Distinguished Professor of Chemistry and Biochemistry at ASU and director of the Biodesign Center for Molecular Design and Biomimetics. Credit: Biodesign Institute at Arizona State University

Toehold-mediated strand displacement, which forms the basis of other DNA nanodevices, allows DNA structures to move from one complementary foothold on the walking surface to the next. As each DNA strand is displaced by a new strand, the nano-creature takes a step forward.

## **Race walking**

Successful DNA walkers of various kinds have been designed and have demonstrated the ability to ferry nano-sized cargo from place to place. Until now, however, the strand displacement reactions they rely on have been slow, generally requiring several minutes to move a short distance. This is much slower than naturally occurring processes in living systems like protein motors, which can perform feats of dissociation similar to strand displacement in much faster time frames.

While theoretical calculations suggest that individual operations by such nanodevices should occur in seconds or less, in practice, such operations typically require minutes or even hours. (A recently designed cargo-sorting walker for example required 5 minutes for each step, with foothold spacings just 6 nm apart. This speed was on a par with similar strand-displacement walkers.)

In the new study, researchers sought to optimize this process to see how quickly a walker designed with speed in mind could move. The limiting factor in terms of speed did not appear to be the strand displacement process itself, but rather the lack of fine-tuned optimization in the overall walker design.

The team redesigned their walker for maximum speed and used a fluorescent imaging technique known as smFRET (for single-molecule fluorescence resonance imaging transfer) to chart the DNA walker's progress and evaluate its subtle kinetic properties.

By altering the lengths of toehold sequences and branching migration points, the stepping rate could be keenly optimized, making for a briskly moving nanorobot that left competitors in the dust, boasting stepping rates a full order of magnitude faster than previous DNA walkers.

### **Freewheeling nanorobot**

Part of the robot's advantage over its competitors is due to its unusual technique of locomotion. Rather than simply stepping from one surface foothold to the next, the acrobatic walker moves head over heels in a cartwheel fashion, while remaining securely bound to at least one foothold at all times.

The stability of the double-stranded sequences anchoring the base of the robot to the track surface, while the free toehold searches out the next complementary sequence, may be one factor improving the walker's speed. The cartwheeling design also allows strand displacement to sequentially proceed in a direction away from the foothold surface, which improves efficiency.

Once the walker was optimized, super-resolved single particle tracking was used to observe the device's movement over a 2-D surface studded with footholds for the walker, covering a range of up to 2 microns. The best walker optimized in the study was able to search ~43 foothold sites per minute with a stepping distance of ~ 10nm. Strand displacement occurred at rates of about a tenth of a second. Analysis suggests the device can take hundreds of steps without dissociating.

#### **Future steps**

While still lagging behind naturally occurring protein reactions, the optimized cartwheeling walker offers a marked advancement in performance, representing an order of magnitude improvement over earlier versions, while not consuming any fuel. Borrowing further insights from natural systems may allow dynamical DNA devices like the walker to accelerate even more in the future by converting chemical energy into directed speed.

The study underlines the opportunities for optimization of a range of DNA nanostructures, considerably enhancing their speed and versatility. [20]

# Chemical engineers discover how to control knots that form in DNA molecules

Just like any long polymer chain, DNA tends to form knots. Using technology that allows them to stretch DNA molecules and image the behavior of these knots, MIT researchers have discovered, for the first time, the factors that determine whether a knot moves along the strand or "jams" in place.

"People who study polymer physics have suggested that knots might be able to jam, but there haven't been good model systems to test it," says Patrick Doyle, the Robert T. Haslam Professor of Chemical Engineering and the senior author of the study. "We showed the same knot could go from being jammed to being mobile along the same molecule. You change conditions and it suddenly stops, and then change them again and it suddenly moves."

The findings could help researchers develop ways to untie DNA knots, which would help improve the accuracy of some genome sequencing technologies, or to promote knot formation. Inducing knot formation could enhance some types of sequencing by slowing down the DNA <u>molecules</u>' passage through the system, the researchers say.

MIT postdoc Alexander Klotz is the first author of the paper, which appears in the May 3 issue of *Physical Review Letters*.

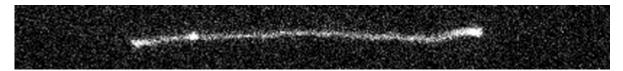
#### **Knots in motion**

Doyle and his students have been studying the physics of polymer knots such as DNA for many years. DNA is well-suited for such studies because it is a relatively large molecule, making it simple to image with a microscope, and it can be easily induced to form knots.

"We have a mechanism that causes DNA molecules to collapse into a tiny ball, which when we stretch out contains very big knots," Klotz says. "It's like sticking your headphones in your pocket and pulling them out full of knots."

Once the knots form, the researchers can study them using a special microfluidic system that they designed. The channel is shaped like a T, with an electric field that diverges at the top of the T. A DNA molecule located at the top of the T will be pulled equally toward each arm, forcing it to stay in place.

The MIT team found that they could manipulate knots in these pinned DNA molecules by varying the strength of the electric field. When the field is weak, knots tend to move along the molecule toward the closer end. When they reach the end, they unravel.



A knot near the end of a stretched DNA molecule is driven toward the end and unties, leaving an unknotted molecule. Credit: Alex Klotz

"When the tension isn't too strong, they look like they're moving around randomly. But if you watch them for long enough, they tend to move in one direction, toward the closer end of the molecule," Klotz says.

When the field is stronger, forcing the DNA to fully stretch out, the knots become jammed in place. This phenomenon is similar to what happens to a knot in a bead necklace as the necklace is pulled more tightly, the researchers say. When the necklace is slack, a knot can move along it, but when it is pulled taut, the beads of the necklace come closer together and the knot gets stuck.

"When you tighten the <u>knot</u> by stretching the DNA molecule more, it brings the strands closer to each other, and this ramps up the friction," Klotz says. "That can overwhelm the driving force caused by the electric field."

#### **Knot removal**

DNA knots also occur in living cells, but cells have specialized enzymes called topoisomerases that can untangle such knots. The MIT team's findings suggest a possible way to remove knots from DNA outside of cells relatively easily by applying an <u>electric field</u> until the knots travel all the way to the end of the molecule.

This could be useful for a type of DNA sequencing known as nanochannel mapping, which involves stretching DNA along a narrow tube and measuring the distance between two genetic sequences. This technique is used to reveal large-scale genome changes such as gene duplication or genes moving from one chromosome to another, but knots in the DNA can make it harder to get accurate data.

For another type of DNA sequencing known as nanopore sequencing, it could be beneficial to induce knots in DNA because the knots make the molecules slow down as they travel through the sequencer. This could help researchers get more accurate sequence information.

Using this approach to remove knots from other types of polymers such as those used to make plastics could also be useful, because knots can weaken materials.

The researchers are now studying other phenomena related to knots, including the process of untying more complex knots than those they studied in this paper, as well as the interactions between two knots in a molecule. [19]

## Researchers build DNA replication in a model synthetic cell

Researchers at Delft University of Technology, in collaboration with colleagues at the Autonomous University of Madrid, have created an artificial DNA blueprint for the replication of DNA in a cell-like structure. Creating such a complex biological module is an important step towards an even more ambitious goal: building a complete and functioning synthetic cell from the bottom up.

Copying DNA is an essential function of living <u>cells</u>. It allows for cell division and propagation of <u>genetic information</u> to the offspring. The mechanism underlying DNA <u>replication</u> consists of three important steps. First, DNA is transcribed into messenger RNA. Messenger RNA is then translated into proteins—the workhorses of the cell that carry out many of its vital functions. The job of some of

these proteins, finally, is to perform the last step in the cycle: the replication (or copying) of DNA. After a cell has replicated its DNA, it can divide into two <u>daughter cells</u>, each containing a copy of the original genetic material.

### **Closing the cycle**

Researchers had already realized all of the separate steps mentioned above. Japanese scientists, for instance, created a minimal, stand-alone system for messenger RNA and <u>protein</u> synthesis by taking the relevant components from *E. coli* and tweaking them. But no one had yet been able to combine this system with autonomous DNA replication. "We wanted to close the cycle and be the first to reconstruct the entire flow of genetic information inside a cell-like structure called a liposome," said group leader Christophe Danelon.

Combining the Japanese system with a module for DNA replication proved difficult. "We tried a few approaches, but none seemed to work convincingly," said Danelon. Then, Ph.D. student Pauline van Nies came up with the idea to use the DNA replication machinery of a virus called  $\Phi 29$ . "Viruses are very intriguing from a molecular biology point of view," said Van Nies. "They are extremely efficient in encoding proteins in a small genome and in robustly replicating their genetic information." In human cells, DNA replication is managed by hundreds of proteins.  $\Phi 29$  only needs four.

### **Composing DNA**

Many years ago, researchers working at the Autonomous University of Madrid discovered the DNA replication mechanism of the  $\Phi$ 29 virus and managed to isolate it. Van Nies and Danelon worked with these researchers to combine the genes that encode for the replication mechanism with the genetic code that is necessary to operate the Japanese module for transcription and translation.

Van Nies composed a unique DNA blueprint that took into account a number of different factors related to the flow of genetic information, such as a suitable binding site for the ribosome, an element that is essential for the production of proteins.

#### **Combining machinery**

A goal that now comes into view is combining the new module that regulates the flow of genetic information with other essential cellular functions such as growth and division. Last year, the Danelon group <u>created a way to synthesize the phospholipids</u> that make up liposomes, such as the ones the researchers used in this project. The yield of phospholipids was still too small to sustain growth, but Danelon is confident his group can optimize this process.

Cell division may be a tougher nut to crack. In modern cells, it requires a streamlined process in which copied DNA is neatly packed and then evenly distributed towards the poles of the cell. Concurrently, specialized proteins squeeze the mother cell into two daughter cells. Danelon thinks a simple 'budding' mechanism could also do the trick. "I think we can create liposomes that grow until they start budding. If enough DNA is being produced, hopefully enough of these primitive daughter cells will contain the new DNA to sustain a cell population." This may well be how the very first cells self-reproduced, before evolution equipped them with a more elegant and robust solution.

#### **Building a synthetic cell**

The mission that ties together all of the fundamental research described above is the construction of a synthetic cell that can grow, divide and sustain itself. Scientists at Delft University of Technology

play a leading role in this exciting new research direction that may ultimately lead to intimate understanding of the inner workings of a cell. Research supporting the initiative could lead to advances in biotechnology, health and energy. [18]

## Study reveals the inner workings of a molecular motor that packs and unpacks DNA

DNA is tightly packed into the nucleus of a cell. Nevertheless, the cellular machinery needs to constantly access the genomic information. An LMU team now reveals the inner workings of a molecular motor made of proteins which packs and unpacks DNA.

The genomic DNA of higher organisms is compacted in a highly condensed form known as chromatin. The DNA is tightly wound around a myriad of tiny histone spools called nucleosomes. A single human cell, for instance, accommodates in this manner about two meters of DNA. However, genes must be constantly transcribed into messenger RNAs to direct protein synthesis. Moreover, the entire DNA must be replicated before cell division and DNA damage needs to be repaired. Thus, there must be way to actively grant access to the genome.

This is when chromatin remodelers come into play. Chromatin remodelers have an essential role as they are molecular machines: they unpick and unpack segments of the DNA by sliding <u>nucleosome</u> spools back and forth, replacing individual histones, freeing up the DNA for transcription, and finally compacting it again, when the job is done. Since all of this happens in a highly dynamic fashion, chromatin remodelers enable <u>cells</u> to react rapidly to alterations in their environment – and this holds for brewer's yeast as well as for human cells. In mediating gene accessibility, chromatin remodelers are vital for development and cell differentiation; cell types are defined by the sets of genes they express, remodelers help to determine cell identity.

So far, however, very little is known about what remodeling proteins look like and how they go about doing what they do. In molecular terms, functional remodelers are often very large complexes comprising many different protein components, whose coordinated action makes them akin to molecular machines. These features also make it very difficult to determine their detailed structure. But a team led by Professor Karl-Peter Hopfner, who holds a Chair in Structural Molecular Biology at LMU's Gene Center, has now used cryo-electron microscopy to reconstruct the three-dimensional structure of the nucleosome-sliding remodeler INO80 (which itself consists of 15 subunits) bound to a single nucleosome. "Even with innovative approaches, the best available technology and intensive teamwork, we were always working at the cutting edge," says Dr. Sebastian Eustermann, who worked out the molecular structure of the complex on the basis of <u>electron micrographs</u> of thousands of individual complexes.

By analyzing images of randomly oriented views of the complex formed between INO80 and a nucleosome in the electron micrographs, Hopfner and his team have pieced together its structure at a resolution which has seldom been achieved for a chromatin complex of comparable size. This allowed the researchers to unravel the intricate interaction of the remodeler with its substrate DNA spooled around histones and dissect how the whole machinery works.

From a biochemical point of view, remodelers are responsible for heavy-duty reorganizational tasks. To perform these tasks, they must execute "large-scale conformational changes, which are carried out with astounding precision," says Eustermann. In order to alter the relative positions of nucleosomes, the INO80 complex must first weaken the contacts between the nucleosomal histones and the DNA. A molecular motor which is part of the INO80 complex segmentally detaches the double-stranded DNA from the nucleosome. In doing so, it progressively breaks the contacts that normally keep the DNA tightly wound around the histone particle.

The motor subunit feeds DNA it into the nucleosome. This results in the transient formation of a double-stranded DNA loop that is likely an important intermediate in complex remodeling reactions on the nucleosome. On one hand, the loop exposes some histone proteins that could be replaced by other histones to form a different type of nucleosome. On the other hand, the loop is eventually passed over another subunit and the machine then acts as a ratchet, allowing the nucleosome to "move" on the DNA. Throughout this unpacking process, other subunits in the complex serve to support and stabilize the partially 'denuded' nucleosome itself.

The structure of the complex revealed in the new study sheds new light on the function and mode of action of chromatin remodelers in general. These <u>molecular machines</u> play an essential part in the workings of the cell by maintaining the flexibility of the <u>chromatin</u>, thus enabling the genetic apparatus to respond dynamically to changing metabolic demands. "Our results provide the first well-founded picture of how they do that," says Hopfner. "Moreover, it has recently become clear that remodelers play a central role in tumorigenesis, because they often misregulated in tumor tissue. So structural and mechanistic insights into their functions will be vital for the future development of new therapies for cancer," he adds. [17]

## Biomimetic chemistry—DNA mimic outwits viral enzyme

Not only can synthetic molecules mimic the structures of their biological models, they can also take on their functions and may even successfully compete with them, as an artificial DNA sequence designed by Ludwig-Maximilians-Universitaet (LMU) in Munich chemist Ivan Huc now shows.

Chemist Ivan Huc finds the inspiration for his work in the molecular principles that underlie biological systems. As the leader of a research group devoted to biomimetic supramolecular chemistry, he creates 'unnatural' molecules with defined, predetermined shapes that closely resemble the major biological polymers, proteins and DNA found in cells. The backbones of these molecules are referred to as 'foldamers' because, like origami patterns, they adopt predictable shapes and can be easily modified. Having moved to LMU from his previous position at Bordeaux University last summer, Huc has synthesized a helical molecule that mimics surface features of the DNA double helix so closely that bona fide DNA-binding proteins interact with it.

This work is described in a paper published in *Nature Chemistry*. The new study shows that the synthetic compound is capable of inhibiting the activities of several DNA-processing enzymes, including the 'integrase' used by the <u>human immunodeficiency virus</u> (HIV) to insert its genome into

that of its host cell. The successful demonstration of the efficacy of the synthetic DNA mimic might lead to a new approach to the treatment of AIDS and other retroviral diseases.

The new paper builds on advances described in two previous publications in *Nature Chemistry* published earlier this year. In the first of these papers, Huc and his colleagues developed a pattern of binding interactions required to enable synthetic <u>molecules</u> to assume stable forms similar to the helical backbones of proteins. In the second, they worked out the conditions required to append their synthetic helix to natural proteins during synthesis by cellular ribosomes. "As always in biology, shape determines function," he explains. In the new study, he introduces a synthetic molecule that folds into a helical structure that mimics surface features of the DNA double helix, and whose precise shape can be altered in a modular fashion by the attachment of various substituents. This enables the experimenter to imitate in detail the shape of natural DNA double helix, in particular the position of negative charges. The imitation is so convincing that it acts as a decoy for two DNAbinding enzymes, including the HIV integrase, which readily bind to it and are essentially inactivated.

However, the crucial question is whether or not the foldamer can effectively compete for the enzymes in the presence of their normal DNA substrate. "If the enzymes still bind to the foldamer under competitive conditions, then the mimic must be a better binder than the natural DNA itself," Huc says. And indeed, the study demonstrates that the HIV integrase binds more strongly to the foldamer than to natural DNA. "Furthermore, although initially designed to resemble DNA, the foldamer owes its most useful and valuable properties to the features that differentiate it from DNA," Huc points out.

Thanks to the modular nature of foldamer design, the structures of these artificial DNA mimics can be readily altered, which enables a broad range of variants to be produced using the same basic platform. In the current study, Huc and his colleagues have focused on enzymes that are generically capable of binding to DNA, irrespective of its base sequence. However, it may also be possible to use the foldamer approach to develop DNA mimics that can block the action of the many important DNA-binding proteins whose functions depend on the recognition of specific nucleotide sequences. [16]

## Simulations document self-assembly of proteins and DNA

What makes particles self-assemble into complex biological structures? Often, this phenomenon is due to the competition between forces of attraction and repulsion, produced by electric charges in various sections of the particles. In nature, these phenomena often occur in particles that are suspended in a medium—referred to as colloidal particles—such as proteins, DNA and RNA. To facilitate self-assembly, it is possible to "decorate" various sites on the surface of such particles with different charges, called patches.

In a new study published in *EPJE*, physicists have developed an algorithm to simulate the molecular dynamics of these patchy <u>particles</u>. The findings published by Silvano Ferrari and colleagues from the TU Vienna and the Centre for Computational Materials Science (CMS), Austria, will improve our understanding of what makes self-assembly in biological systems possible.

In this study, the authors model charged patchy particles, which are made up of a rigid body with only two charged patches, located at opposite poles. They then develop the equations governing the dynamics of an ensemble of such colloidal patchy particles.

Based on an existing approach originally developed for molecular particles, their simulation includes additional constraints to guarantee that the electrical charge "decorations" are preserved over time. In this regard, they develop equations for describing the particles' motion; the solutions to these equations describe the trajectories of these colloidal particles. Such <u>molecular dynamics</u> simulations lend themselves to being run in parallel on a huge number of particles.

With these findings, the authors complement the lessons learned from experimental observations of similar particles recently synthesised in the lab. Recent experiments have demonstrated that <u>colloidal</u> <u>particles</u> decorated at two interaction sites display a remarkable propensity for self-organising into highly unusual structures that remain stable over a broad temperature range. [15]

## Scientists explore the structure of a key region of longevity protein telomerase

Scientists from Moscow State University (MSU) working with an international team of researchers have identified the structure of one of the key regions of telomerase—a so-called "cellular immortality" ribonucleoprotein. Structural and functional studies on this protein are important for the development of potential anticancer drugs. The results of the study have been published in *Nucleic Acids Research*.

Each cell goes through a DNA replication process before division. This is a precise, fine-tuned process controlled by the coordinated work of a sophisticated enzymatic machinery. However, due to the nature of the copying process, the termini of DNA molecules are left uncopied, and DNA becomes shorter with each replication. However, no important data is lost in the process, as the termini of DNA molecules (telomeres) consist of thousands of small, repeated regions that do not carry hereditary information. When the reserve of telomere repetitions is exhausted, the cell ceases to divide, and eventually, it can die. Scientists believe that this is the mechanism of cellular aging, which is necessary for the renewal of cells and tissues of the body.

But how do "immortal" strains and stem cells that give life to a huge number of offspring cope with this? This is where the enzyme <u>telomerase</u> comes into play. It can restore telomeric termini of chromosomes and therefore compensate for their shortening during mitosis. The telomerase protein catalytic subunit works together with the RNA molecule, and its short fragment is used as a template to synthesize telomeric repetitions. MSU-based scientists discovered the structure of the telomerase fragment that is in charge of this process.

"Our work is aimed at the structural characterization of the telomerase complex. In a living cell, it includes a catalytic subunit, an RNA molecule, a segment of telomeric DNA, and several auxiliary components. Anomalously low activity of telomerase caused by genetics can result in serious pathogenic conditions (telomeropathy), while its anomalous activation is the reason for the cellular

"immortality" of most known cancers. Information on the structure of telomerase and the relationships between its components is necessary for understanding the function and regulation of this enzyme, and in the future, for directed control of its activity," said Elena Rodina, assistant professor of the Department for the Chemistry of Natural Products, Faculty of Chemistry, MSU.

Working with thermotolerant yeast, a model eukaryotic organism, the researchers determined the structure of one of the major domains of the telomerase catalytic subunit (the so-called TEN-domain) and determined which parts of it are responsible for the interaction of the enzyme with the RNA molecule and the synthesized DNA. Based on the experimental data obtained, the scientists constructed a theoretical model of the catalytic core of telomerase.

The activity of the enzyme may be described in a simplified way: Telomerase can be represented as a molecular machine containing an RNA molecule. This machine, with the help of a template part of RNA, binds to the end of a long chain of DNA, and synthesizes a fragment of a new DNA chain along the remaining template fragment. After that, the telomerase machine has to move to the newly synthesized end of the DNA in order to continue to build up the chain. The scientists assume that the TEN-domain allows telomerase to synthesize DNA fragments of strictly defined length, after which the RNA template should be detached from the DNA strand to move closer to its edge. Thus, the TEN domain facilitates the movement of the enzyme to building up a new region, i.e. the next telomeric fragment, and this is how the synthesis cycle is repeated.

In addition, the researchers identified the structural core of the TEN domain that remained unchanged in a variety of organisms, despite all the evolutionary vicissitudes, which indicates the important role of this core in the function of the enzyme. The team also revealed the elements specific for different groups of organisms, which interact with own proteins of individual telomerase complex.

"The data obtained bring us closer to an understanding of the structure, function and regulation of telomerase. In the future, this knowledge can be used to create drugs aimed at regulating telomerase activity—either to increase it (for example, to increase the cell life span in biomaterials for transplantology) or to reduce (for instance, for immortal cancer cells to lose their immortality)," concludes Elena Rodina. [14]

#### Custom sequences for polymers using visible light

Researchers from Tokyo Metropolitan University used a light-sensitive iridium-palladium catalyst to make "sequential" polymers, using visible light to change how building blocks are combined into polymer chains. By simply switching the light on or off, they were able to realize different compositions along the polymer chain, allowing precise control over physical properties and material function. This may drastically simplify existing polymer production methods, and help overcome fundamental limits in creating new polymers.

The world is full of long, chain-like molecules known as polymers. Famous examples of "sequential" copolymers, i.e. polymers made of multiple <u>building blocks</u> (or "monomers") arranged in a specific

order, include DNA, RNA and proteins; their specific structure imparts the vast range of molecular functionality that underpins biological activity. However, making sequential polymers from scratch is a tricky business. We can design special monomers that assemble in different ways, but the complex syntheses that are required limit their availability, scope and functionality.

To overcome these limits, a team led by Associate Professor Akiko Inagaki from the Department of Chemistry, Tokyo Metropolitan University, applied a light-sensitive catalyst containing iridium and palladium. By switching a light on and off, they were able to control the speed at which two different monomers, styrene and vinyl ether, become part of a <u>polymer chain</u>. When exposed to light, the styrene monomer was found to be incorporated into the copolymer structure much more rapidly than in the dark, resulting in a single copolymer chain with different compositions along its length. Parts that are rich in styrene are more rigid than those rich in vinyl ether; by using different on/off <u>light</u> sequences, they could create polymers with a range of <u>physical properties</u> e.g. different "glass transition" temperatures, above which the <u>polymer</u> becomes softer.

The newly developed process is significantly simpler than existing methods. The team also found that both types of monomer were built into the polymer via a mechanism known as non-radical coordination-insertion; this is a generic mechanism, meaning that this new method might be applied to make polymers using a wide range of catalysts and monomers, with the potential to overcome the limited availability of <u>monomer</u> candidates. [13]

## Artificial and biological cells work together as mini chemical factories

Researchers have fused living and non-living cells for the first time in a way that allows them to work together, paving the way for new applications.

The system, created by a team from Imperial College London, encapsulates biological cells within an <u>artificial cell</u>. Using this, researchers can harness the natural ability of biological cells to process chemicals while protecting them from the environment.

This system could lead to applications such as cellular 'batteries' powered by photosynthesis, synthesis of drugs inside the body, and biological sensors that can withstand harsh conditions.

Previous artificial cell design has involved taking parts of biological cell 'machinery' - such as enzymes that support <u>chemical</u> reactions - and putting them into artificial casings. The new study, published today in *Scientific Reports*, goes one step further and encapsulates entire cells in artificial casings.

The artificial cells also contain enzymes that work in concert with the biological cell to produce new chemicals. In the proof-of-concept experiment, the artificial cell systems produced a fluorescent chemical that allowed the researchers to confirm all was working as expected.

Lead researcher Professor Oscar Ces, from the Department of Chemistry at Imperial, said: "Biological cells can perform extremely complex functions, but can be difficult to control when trying to harness one aspect. Artificial cells can be programmed more easily but we cannot yet build in much complexity.

"Our new system bridges the gap between these two approaches by fusing whole biological cells with artificial ones, so that the machinery of both works in concert to produce what we need. This is a paradigm shift in thinking about the way we design artificial cells, which will help accelerate research on applications in healthcare and beyond."

To create the system, the team used microfluidics: directing liquids through small channels. Using water and oil, which do not mix, they were able to make droplets of a defined size that contained the biological cells and enzymes. They then applied an artificial coating to the droplets to provide protection, creating an artificial cell environment.

They tested these artificial cells in a solution high in copper, which is usually highly toxic to biological cells. The team were still able to detect fluorescent chemicals in the majority of the artificial cells, meaning the biological cells were still alive and functioning inside. This ability would be useful in the human body, where the artificial cell casing would protect the foreign <u>biological cells</u> from attack by the body's immune system.

First author of the study Dr Yuval Elani, an EPSRC Research Fellow also from the Department of Chemistry, said: "The system we designed is controllable and customisable. You can create different sizes of artificial <u>cells</u> in a reproducible manner, and there is the potential to add in all kinds of cell machinery, such as chloroplasts for performing photosynthesis or engineered microbes that act as sensors."

To improve the functionality of these artificial cell systems, the next step is to engineer the artificial coating to act more like a biological membrane, but with special functions.

For example, if the membrane could be designed to open and release the chemicals produced within only in response to certain signals, they could be used to deliver drugs to specific areas of the body. This would be useful for example in cancer treatment to release targeted drugs only at the site of a tumour, reducing side effects.

While a system like that may be a way off yet, the team say this is a promising leap in the right direction. The work is the first example of fusing living and non-living components to emerge from Imperial and King's College's new FABRICELL centre for artificial cell science. [12]

## New interaction mechanism of proteins discovered

UZH researchers have discovered a previously unknown way in which proteins interact with one another and cells organize themselves. This new mechanism involves two fully unstructured proteins forming an ultra-high-affinity complex due to their opposite net charge. Proteins usually bind one another as a result of perfectly matching shapes in their three-dimensional structures.

Proteins are among the most important biomolecules and are the key mediators of molecular communication between and within cells. For two proteins to bind, specific regions of their <u>three-dimensional structures</u> have to match one another exactly, as a key fits into a lock. The structure of proteins is extremely important for their functioning and for triggering the required responses in cells.

Now, researchers at the University of Zurich, together with colleagues from Denmark and the U.S., have discovered that unstructured proteins can also have ultra-high-affinity interactions.

One of these proteins is histone H1, which, as a component of chromatin, is responsible for DNA packaging. Its binding partner, prothymosin  $\alpha$ , acts as a kind of shuttle that deposits and removes the histone from the DNA. This process determines whether or not genes in specific parts of the DNA can be read. Both proteins are involved in several regulatory processes in the body, such as cell division and proliferation, and therefore also play a role when it comes to a number of diseases, including cancer. Ben Schuler, professor at the Department of Biochemistry at UZH and head of the research project published in *Nature*, says, "The interesting thing about these proteins is that they're completely unstructured—like boiled noodles in water." How such disordered proteins should be able to interact according to the key/lock principle had puzzled the team of researchers.

Notably, the two proteins bind to one another much more strongly than the average <u>protein</u> partners. The research team used single-molecule fluorescence and <u>nuclear magnetic</u> <u>resonance</u> spectroscopy to determine the arrangement of the proteins. Observed in isolation, they show extended unstructured protein chains. The chains become more compact as soon as both binding partners come together and form a complex. The strong interaction is caused by the strong electrostatic attraction, since histone H1 is highly positively charged while prothymosin α is highly negatively charged. Even more surprising was the discovery that the <u>protein complex</u> was also fully unstructured, as several analyses confirmed.

To investigate the shape of the protein complex, the researchers labeled both proteins with fluorescent probes, which they then added to selected sites on the proteins. Together with computer simulations, this molecular map yielded the following results: Histone 1 interacts with prothymosin  $\alpha$  preferably in its central region, which is the region with the highest charge density. Moreover, it emerged that the complex is highly dynamic: The proteins' position in the complex changes extremely quickly—in a matter of approx. 100 nanoseconds.

The interaction behavior is likely to be fairly common. Cells have many proteins that contain highly charged sequences and may be able to form such protein complexes. There are hundreds of such proteins in the human body alone. "It's likely that the interaction between disordered, highly charged proteins is a basic mechanism for how <u>cells</u> function and organize themselves," concludes Ben Schuler. According to the biophysicist, textbooks will need revision to account for this new way of binding. The discovery is also relevant for developing new therapies, since unstructured proteins are largely unresponsive to traditional drugs, which bind to specific structures on the protein surface. [11]

#### Particles in charged solution form clusters that reproduce

Dr Martin Sweatman from the University of Edinburgh's School of Engineering has discovered a simple physical principle that might explain how life started on Earth.

He has shown that particles that become charged in solution, like many biological <u>molecules</u>, can form giant clusters that can reproduce. Reproduction is shown to be driven by simple physics—a

balance of forces between short-range attraction and long-range repulsion. Once cluster <u>reproduction</u> begins, he suggests chemical evolution of clusters could follow, leading eventually to life.

Many <u>biological molecules</u>, like DNA and proteins, might show this behaviour. Even the building blocks of life, amino acids and nucleobases, might show this behaviour. Reproduction in modern cells might even be driven by this simple physical mechanism, i.e. chemistry is not so important.

Dr Sweatman's research uses theoretical methods and computer simulations of simple particles. They clearly show giant clusters of molecules with the right balance of forces can reproduce. No chemistry is involved. However, these theoretical predictions have yet to be confirmed by experiment.

Dr Sweatman said, "Although it will be difficult to see this behaviour for solutions of small biomolecules, it should be possible to confirm this behaviour experimentally with much larger particles that can be seen under a microscope, like charged colloids.

"If this <u>behaviour</u> is confirmed, then we take another step towards Darwin's idea of life beginning in a warm little pond. A simple evaporation and condensation cycle in a pond might be sufficient to drive <u>cluster</u> reproduction initially. Survival of the fittest clusters of chemicals might then eventually lead to life."

The research has been published in the international journal *Molecular Physics*.

## Experiment demonstrates quantum mechanical effects from biological systems

Nearly 75 years ago, Nobel Prize-winning physicist Erwin Schrödinger wondered if the mysterious world of quantum mechanics played a role in biology. A recent finding by Northwestern University's Prem Kumar adds further evidence that the answer might be yes. Kumar and his team have, for the first time, created quantum entanglement from a biological system. This finding could advance scientists' fundamental understanding of biology and potentially open doors to exploit biological tools to enable new functions by harnessing <u>quantum</u> mechanics.

"Can we apply quantum tools to learn about biology?" said Kumar, professor of electrical engineering and computer science in Northwestern's McCormick School of Engineering and of physics and astronomy in the Weinberg College of Arts and Sciences. "People have asked this question for many, many years—dating back to the dawn of quantum mechanics. The reason we are interested in these new quantum states is because they allow applications that are otherwise impossible."

Partially supported by the Defense Advanced Research Projects Agency, the research was published Dec. 5 in *Nature Communications*.

Quantum entanglement is one of quantum mechanics' most mystifying phenomena. When two <u>particles</u>—such as atoms, photons, or electrons—are entangled, they experience an inexplicable link that is maintained even if the particles are on opposite sides of the universe. While entangled, the particles' behavior is tied one another. If one particle is found spinning in one direction, for example, then the other particle instantaneously changes its spin in a corresponding manner dictated by the entanglement. Researchers, including Kumar, have been interested in harnessing quantum entanglement for several applications, including quantum communications. Because the particles can communicate without wires or cables, they could be used to send secure messages or help build an extremely fast "quantum Internet."

"Researchers have been trying to entangle a larger and larger set of atoms or photons to develop substrates on which to design and build a quantum machine," Kumar said. "My laboratory is asking if we can build these machines on a biological substrate."

In the study, Kumar's team used green fluorescent proteins, which are responsible for bioluminescence and commonly used in biomedical research. The team attempted to entangle the photons generated from the fluorescing molecules within the algae's barrel-shaped protein structure by exposing them to spontaneous four-wave mixing, a process in which multiple wavelengths interact with one another to produce new wavelengths.

Through a series of these experiments, Kumar and his team successfully demonstrated a type of entanglement, called <u>polarization</u> entanglement, between photon pairs. The same feature used to make glasses for viewing 3D movies, polarization is the orientation of oscillations in light waves. A wave can oscillate vertically, horizontally, or at different angles. In Kumar's entangled pairs, the photons' polarizations are entangled, meaning that the oscillation directions of light waves are linked. Kumar also noticed that the barrel-shaped structure surrounding the fluorescing molecules protected the <u>entanglement</u> from being disrupted.

"When I measured the vertical polarization of one particle, we knew it would be the same in the other," he said. "If we measured the horizontal polarization of one particle, we could predict the horizontal polarization in the other particle. We created an entangled state that correlated in all possibilities simultaneously."

Now that they have demonstrated that it's possible to create <u>quantum entanglement</u> from biological particles, next Kumar and his team plan to make a biological substrate of <u>entangled</u> <u>particles</u>, which could be used to build a <u>quantum</u> machine. Then, they will seek to understand if a biological substrate works more efficiently than a synthetic one. [9]

## Quantum biology: Algae evolved to switch quantum coherence on and off

A UNSW Australia-led team of researchers has discovered how algae that survive in very low levels of light are able to switch on and off a weird quantum phenomenon that occurs during photosynthesis.

The function in the algae of this quantum effect, known as coherence, remains a mystery, but it is thought it could help them harvest energy from the sun much more efficiently. Working out its role in a living organism could lead to technological advances, such as better organic solar cells and quantum-based electronic devices.

The research is published in the journal Proceedings of the National Academy of Sciences.

It is part of an emerging field called quantum biology, in which evidence is growing that quantum phenomena are operating in nature, not just the laboratory, and may even account for how birds can navigate using the earth's magnetic field.

"We studied tiny single-celled algae called cryptophytes that thrive in the bottom of pools of water, or under thick ice, where very little light reaches them," says senior author, Professor Paul Curmi, of the UNSW School of Physics.

"Most cryptophytes have a light-harvesting system where quantum coherence is present. But we have found a class of cryptophytes where it is switched off because of a genetic mutation that alters the shape of a light-harvesting protein.

"This is a very exciting find. It means we will be able to uncover the role of quantum coherence in photosynthesis by comparing organisms with the two different types of proteins."

In the weird world of quantum physics, a system that is coherent – with all quantum waves in step with each other – can exist in many different states simultaneously, an effect known as superposition. This phenomenon is usually only observed under tightly controlled laboratory conditions.

So the team, which includes Professor Gregory Scholes from the University of Toronto in Canada, was surprised to discover in 2010 that the transfer of energy between molecules in the light harvesting systems from two different cryptophyte species was coherent.

The same effect has been found in green sulphur bacteria that also survive in very low light levels.

"The assumption is that this could increase the efficiency of photosynthesis, allowing the algae and bacteria to exist on almost no light," says Professor Curmi.

"Once a light-harvesting protein has captured sunlight, it needs to get that trapped energy to the reaction centre in the cell as quickly as possible, where the energy is converted into chemical energy for the organism.

"It was assumed the energy gets to the reaction centre in a random fashion, like a drunk staggering home. But quantum coherence would allow the energy to test every possible pathway simultaneously before travelling via the quickest route."

In the new study, the team used x-ray crystallography to work out the crystal structure of the lightharvesting complexes from three different species of cryptophytes.

They found that in two species a genetic mutation has led to the insertion of an extra amino acid that changes the structure of the protein complex, disrupting coherence.

"This shows cryptophytes have evolved an elegant but powerful genetic switch to control coherence and change the mechanisms used for light harvesting," says Professor Curmi.

The next step will be to compare the biology of different cryptophytes, such as whether they inhabit different environmental niches, to work out whether the quantum coherence effect is assisting their survival. [8]

## **Photoactive Prebiotic Systems**

We propose that life first emerged in the form of such minimal photoactive prebiotic kernel systems and later in the process of evolution these photoactive prebiotic kernel systems would have produced fatty acids and covered themselves with fatty acid envelopes to become the minimal cells of the Fatty Acid World. Specifically, we model self-assembling of photoactive prebiotic systems with observed quantum entanglement phenomena. We address the idea that quantum entanglement was important in the first stages of origins of life and evolution of the biospheres because simultaneously excite two prebiotic kernels in the system by appearance of two additional quantum entangled excited states, leading to faster growth and self-replication of minimal living cells. The quantum mechanically modeled possibility of synthesizing artificial selfreproducing quantum entangled prebiotic kernel systems and minimal cells also impacts the possibility of the most probable path of emergence of photocells on the Earth or elsewhere. We also examine the quantum entangled logic gates discovered in the modeled systems composed of two prebiotic kernels. Such logic gates may have application in the destruction of cancer cells or becoming building blocks of new forms of artificial cells including magnetically active ones.

#### **Significance Statement**

Our investigated self-assembly of molecules towards supramolecular bioorganic and minimal cellular systems depends on the quantum mechanics laws which induce hydrogen and Van der Waals bindings (Tamulis A, Grigalavicius, M, Orig Life Evol Biosph 41:51-71, 2011).

In the work presented here, quantum entanglement takes the form of a quantum superposition of the active components in synthesized self-assembling and self-replicating living systems. When a quantum calculation of an entangled system is made that causes one photoactive biomolecule of such a pair to take on a definite value (e.g., electron density transfer or electron spin density transfer), the other member of this entangled pair will be found to have taken the appropriately correlated value (e.g., electron density transfer or electron spin density transfer). In our simulations, the separation distance of supramolecular bio systems changes took place during geometry optimization procedures, which mimic real-world intermolecular interaction processes.

Our discovered phenomenon of the quantum entanglement in the prebiotic systems enhance the photosynthesis in the proposed systems because simultaneously excite two prebiotic kernels in the system by appearance of two additional quantum entangled excited states (Tamulis A, Grigalavicius M, Baltrusaitis J, Orig Life Evol Biosph 43:49-66, 2013; Tamulis A, Grigalavicius M, Krisciukaitis S (2014), J Comput Theor Nanos, 11, 1597-1608, 2014; Tamulis A, Grigalavicius M, 8:117-140, 2014.). We can propose that quantum entanglement enhanced the emergence of photosynthetic prebiotic kernels and accelerated the evolution of photosynthetic life because of additional absorbed light energy, leading to faster growth and self-replication of minimal living cells.

We can state that: Livings are self-assembled and self-replicating wet and warm stochastically moving supramolecular systems where quantum entanglement can be continuously generated and destroyed by non-equilibrium effects in an environment where no static entanglement exists; quantum entanglement involve the biomolecule inside one living or between other neighboring livings.

This warm quantum coherence is basic for the explanation of DNA stability and for the understanding of brain magnetic orientation during migration in more than 50 species of birds, fishes and insects. Exists experimental evidence for quantum-coherent is used for more efficient light-harvesting in plant photosynthesis. Quantum entanglement exists in supramolecules determining the sense of smell and in the brain neurons microtubules due to quantum vibrations.

In the work presented here, we started to design and quantum mechanical investigations of the molecular logical devices which are useful for construction of nano medicine biorobots against the molecular diseases such a cancer tumors, and against the new kinds of synthesized microorganisms and nano guns.



You can see in the enclosed figure the quantum entanglement phenomenon in the closely selfassembled two synthesized protocell system due to the photo excited electron charge transfer from one protocell to another that leads to closer self-assembly and exchange of energy and information.

Visualization of the electron charge tunneling associated with the 6th (467.3 nm) excited state. The transition is mainly from squarine molecule of the first protocell situated in the bottom of this bi cellular system to precursor of fatty acid (pFA) molecule of the second subsystem (in the top) and little from the 1,4-bis(N,N-dimethylamino)naphthalene molecule (in the top-right) to the same pFA molecule of the second subsystem (in the top). The electron cloud hole is indicated by the dark blue color while the transferred electron cloud location is designated by the gray color.

As a result, these nonlinear quantum interactions compressed the overall molecular system resulting in a smaller gap between the HOMO and LUMO electron energy levels which allows

enhanced tunneling of photo excited electrons from the sensitizer squarine and (1,4bis(N,Ndimethylamino)naphthalene) to the pFA molecule resulting in its cleavage. The new fatty acid joins the existing minimal cell thus increasing it in size. After reaching some critical size, the minimal cell should divide (i.e. self-replicate) into two separate smaller minimal cells. [7]

### **Quantum Biology**

Researchers have long suspected that something unusual is afoot in photosynthesis. Particles of light called photons, streaming down from the Sun; arrive randomly at the chlorophyll molecules and other light-absorbing 'antenna' pigments that cluster inside the cells of every leaf, and within every photosynthetic bacterium. But once the photons' energy is deposited, it doesn't stay random. Somehow, it gets channeled into a steady flow towards the cell's photosynthetic reaction centre, which can then use it at maximum efficiency to convert carbon dioxide into sugars. Quantum coherence in photosynthesis seems to be beneficial to the organisms using it. But did their ability to exploit quantum effects evolve through natural selection? Or is quantum coherence just an accidental side effect of the way certain molecules are structured? [6]

#### Quantum Consciousness

Extensive scientific investigation has found that a form of quantum coherence operates within living biological systems through what is known as biological excitations and biophoton emission. What this means is that metabolic energy is stored as a form of electromechanical and electromagnetic excitations. These coherent excitations are considered responsible for generating and maintaining long-range order via the transformation of energy and very weak electromagnetic signals. After nearly twenty years of experimental research, Fritz-Albert Popp put forward the hypothesis that biophotons are emitted from a coherent electrodynamics field within the living system.

What this means is that each living cell is giving off, or resonating, a biophoton field of coherent energy. If each cell is emitting this field, then the whole living system is, in effect, a resonating field-a ubiquitous nonlocal field. And since biophotons are the entities through which the living system communicates, there is near-instantaneous intercommunication throughout. And this, claims Popp, is the basis for coherent biological organization -- referred to as quantum coherence. This discovery led Popp to state that the capacity for evolution rests not on aggressive struggle and rivalry but on the capacity for communication and cooperation. In this sense the built-in capacity for species evolution is not based on the individual but rather living systems that are interlinked within a coherent whole: Living systems are thus neither the subjects alone, nor objects isolated, but both subjects and objects in a mutually communicating universe of meaning. . . . Just as the cells in an organism take on different tasks for the whole, different populations enfold information not only for themselves, but for all other organisms, expanding the consciousness of the whole, while at the same time becoming more and more aware of this collective consciousness. Biophysicist Mae-Wan Ho describes how the living organism, including the human body, is coordinated throughout and is "coherent beyond our wildest dreams." It appears that every part of our body is "in communication with every other part through a dynamic, tunable, responsive, liquid crystalline medium that pervades the whole body, from organs and tissues to the interior of every cell."

What this tells us is that the medium of our bodies is a form of liquid crystal, an ideal transmitter of communication, resonance, and coherence. These relatively new developments in biophysics have discovered that all biological organisms are constituted of a liquid crystalline medium. Further, DNA is a liquid-crystal, lattice-type structure (which some refer to as a liquid crystal gel), whereby body cells are involved in a holographic instantaneous communication via the emitting of biophotons (a source based on light). This implies that all living biological organisms continuously emit radiations of light that form a field of coherence and communication. Moreover, biophysics has discovered that living organisms are permeated by quantum wave forms. [5]

### **Creating quantum technology**

Another area of potential application is in quantum computing. The long-standing goal of the physicists and engineers working in this area is to manipulate data encoded in quantum bits (qubits) of information, such as the spin-up and spin-down states of an electron or of an atomic nucleus. Qubits can exist in both states at once, thus permitting the simultaneous exploration of all possible answers to the computation that they encode. In principle, this would give quantum computers the power to find the best solution far more quickly than today's computers can — but only if the qubits can maintain their coherence, without the noise of the surrounding environment, such as the jostling of neighboring atoms, destroying the synchrony of the waves. [6]

## **Quantum Entanglement**

Measurements of physical properties such as position, momentum, spin, polarization, etc. performed on entangled particles are found to be appropriately correlated. For example, if a pair of particles is generated in such a way that their total spin is known to be zero, and one particle is found to have clockwise spin on a certain axis, then the spin of the other particle, measured on the same axis, will be found to be counterclockwise. Because of the nature of quantum measurement, however, this behavior gives rise to effects that can appear paradoxical: any measurement of a property of a particle can be seen as acting on that particle (e.g. by collapsing a number of superimposed states); and in the case of entangled particles, such action must be on the entangled system as a whole. It thus appears that one particle of an entangled pair "knows" what measurement has been performed on the other, and with what outcome, even though there is no known means for such information to be communicated between the particles, which at the time of measurement may be separated by arbitrarily large distances. [4]

## **The Bridge**

The accelerating electrons explain not only the Maxwell Equations and the Special Relativity, but the Heisenberg Uncertainty Relation, the wave particle duality and the electron's spin also, building the bridge between the Classical and Quantum Theories. [1]

#### **Accelerating charges**

The moving charges are self maintain the electromagnetic field locally, causing their movement and this is the result of their acceleration under the force of this field. In the classical physics the charges will distributed along the electric current so that the electric potential lowering along the current, by linearly increasing the way they take every next time period because this accelerated motion. The same thing happens on the atomic scale giving a dp impulse difference and a dx way difference between the different part of the not point like particles.

#### **Relativistic effect**

Another bridge between the classical and quantum mechanics in the realm of relativity is that the charge distribution is lowering in the reference frame of the accelerating charges linearly: ds/dt = at (time coordinate), but in the reference frame of the current it is parabolic:  $s = a/2 t^2$  (geometric coordinate).

## **Heisenberg Uncertainty Relation**

In the atomic scale the Heisenberg uncertainty relation gives the same result, since the moving electron in the atom accelerating in the electric field of the proton, causing a charge distribution on delta x position difference and with a delta p momentum difference such a way that they product is about the half Planck reduced constant. For the proton this delta x much less in the nucleon, than in the orbit of the electron in the atom, the delta p is much higher because of the greater proton mass.

This means that the electron and proton are not point like particles, but has a real charge distribution.

## Wave - Particle Duality

The accelerating electrons explains the wave – particle duality of the electrons and photons, since the elementary charges are distributed on delta x position with delta p impulse and creating a wave packet of the electron. The photon gives the electromagnetic particle of the mediating force of the electrons electromagnetic field with the same distribution of wavelengths.

#### Atomic model

The constantly accelerating electron in the Hydrogen atom is moving on the equipotential line of the proton and it's kinetic and potential energy will be constant. Its energy will change only when it

is changing its way to another equipotential line with another value of potential energy or getting free with enough kinetic energy. This means that the Rutherford-Bohr atomic model is right and only that changing acceleration of the electric charge causes radiation, not the steady acceleration. The steady acceleration of the charges only creates a centric parabolic steady electric field around the charge, the magnetic field. This gives the magnetic moment of the atoms, summing up the proton and electron magnetic moments caused by their circular motions and spins.

## The Relativistic Bridge

Commonly accepted idea that the relativistic effect on the particle physics it is the fermions' spin another unresolved problem in the classical concepts. If the electric charges can move only with accelerated motions in the self maintaining electromagnetic field, once upon a time they would reach the velocity of the electromagnetic field. The resolution of this problem is the spinning particle, constantly accelerating and not reaching the velocity of light because the acceleration is radial. One origin of the Quantum Physics is the Planck Distribution Law of the electromagnetic oscillators, giving equal intensity for 2 different wavelengths on any temperature. Any of these two wavelengths will give equal intensity diffraction patterns, building different asymmetric constructions, for example proton - electron structures (atoms), molecules, etc. Since the particles are centers of diffraction patterns they also have particle – wave duality as the electromagnetic waves have. [2]

#### The weak interaction

The weak interaction transforms an electric charge in the diffraction pattern from one side to the other side, causing an electric dipole momentum change, which violates the CP and time reversal symmetry. The Electroweak Interaction shows that the Weak Interaction is basically electromagnetic in nature. The arrow of time shows the entropy grows by changing the temperature dependent diffraction patterns of the electromagnetic oscillators.

Another important issue of the quark model is when one quark changes its flavor such that a linear oscillation transforms into plane oscillation or vice versa, changing the charge value with 1 or -1. This kind of change in the oscillation mode requires not only parity change, but also charge and time changes (CPT symmetry) resulting a right handed anti-neutrino or a left handed neutrino.

The right handed anti-neutrino and the left handed neutrino exist only because changing back the quark flavor could happen only in reverse, because they are different geometrical constructions, the u is 2 dimensional and positively charged and the d is 1 dimensional and negatively charged. It needs also a time reversal, because anti particle (anti neutrino) is involved.

The neutrino is a 1/2spin creator particle to make equal the spins of the weak interaction, for example neutron decay to 2 fermions, every particle is fermions with ½ spin. The weak interaction changes the entropy since more or less particles will give more or less freedom of movement. The entropy change is a result of temperature change and breaks the equality of oscillator diffraction

intensity of the Maxwell–Boltzmann statistics. This way it changes the time coordinate measure and makes possible a different time dilation as of the special relativity.

The limit of the velocity of particles as the speed of light appropriate only for electrical charged particles, since the accelerated charges are self maintaining locally the accelerating electric force. The neutrinos are CP symmetry breaking particles compensated by time in the CPT symmetry, that is the time coordinate not works as in the electromagnetic interactions, consequently the speed of neutrinos is not limited by the speed of light.

The weak interaction T-asymmetry is in conjunction with the T-asymmetry of the second law of thermodynamics, meaning that locally lowering entropy (on extremely high temperature) causes the

weak interaction, for example the Hydrogen fusion.

Probably because it is a spin creating movement changing linear oscillation to 2 dimensional oscillation by changing d to u quark and creating anti neutrino going back in time relative to the proton and electron created from the neutron, it seems that the anti neutrino fastest then the velocity of the photons created also in this weak interaction?

A quark flavor changing shows that it is a reflection changes movement and the CP- and Tsymmetry breaking!!! This flavor changing oscillation could prove that it could be also on higher level such as atoms, molecules, probably big biological significant molecules and responsible on the aging of the life.

Important to mention that the weak interaction is always contains particles and antiparticles, where the neutrinos (antineutrinos) present the opposite side. It means by Feynman's interpretation that these particles present the backward time and probably because this they seem to move faster than the speed of light in the reference frame of the other side.

Finally since the weak interaction is an electric dipole change with ½ spin creating; it is limited by the velocity of the electromagnetic wave, so the neutrino's velocity cannot exceed the velocity of light.

#### **The General Weak Interaction**

The Weak Interactions T-asymmetry is in conjunction with the T-asymmetry of the Second Law of Thermodynamics, meaning that locally lowering entropy (on extremely high temperature) causes for example the Hydrogen fusion. The arrow of time by the Second Law of Thermodynamics shows the increasing entropy and decreasing information by the Weak Interaction, changing the temperature dependent diffraction patterns. A good example of this is the neutron decay, creating more particles with less known information about them.

The neutrino oscillation of the Weak Interaction shows that it is a general electric dipole change and it is possible to any other temperature dependent entropy and information changing diffraction pattern of atoms, molecules and even complicated biological living structures. We can generalize the weak interaction on all of the decaying matter constructions, even on the biological too. This gives the limited lifetime for the biological constructions also by the arrow of time. There should be a new research space of the Quantum Information Science the 'general neutrino oscillation' for the greater then subatomic matter structures as an electric dipole change.

There is also connection between statistical physics and evolutionary biology, since the arrow of time is working in the biological evolution also.

The Fluctuation Theorem says that there is a probability that entropy will flow in a direction opposite to that dictated by the Second Law of Thermodynamics. In this case the Information is growing that is the matter formulas are emerging from the chaos. So the Weak Interaction has two directions, samples for one direction is the Neutron decay, and Hydrogen fusion is the opposite direction.

## **Fermions and Bosons**

The fermions are the diffraction patterns of the bosons such a way that they are both sides of the same thing.

### Van Der Waals force

Named after the Dutch scientist Johannes Diderik van der Waals – who first proposed it in 1873 to explain the behaviour of gases – it is a very weak force that only becomes relevant when atoms and molecules are very close together. Fluctuations in the electronic cloud of an atom mean that it will have an instantaneous dipole moment. This can induce a dipole moment in a nearby atom, the result being an attractive dipole–dipole interaction.

## **Electromagnetic inertia and mass**

#### **Electromagnetic Induction**

Since the magnetic induction creates a negative electric field as a result of the changing acceleration, it works as an electromagnetic inertia, causing an electromagnetic mass. [1]

#### **Relativistic change of mass**

The increasing mass of the electric charges the result of the increasing inductive electric force acting against the accelerating force. The decreasing mass of the decreasing acceleration is the result of the inductive electric force acting against the decreasing force. This is the relativistic mass change explanation, especially importantly explaining the mass reduction in case of velocity decrease.

#### The frequency dependence of mass

Since E = hv and  $E = mc^2$ ,  $m = hv/c^2$  that is the *m* depends only on the *v* frequency. It means that the mass of the proton and electron are electromagnetic and the result of the electromagnetic induction, caused by the changing acceleration of the spinning and moving charge! It could be that the  $m_o$  inertial mass is the result of the spin, since this is the only accelerating motion of the electric charge. Since the accelerating motion has different frequency for the electron in the atom and the proton, they masses are different, also as the wavelengths on both sides of the diffraction pattern, giving equal intensity of radiation.

#### **Electron – Proton mass rate**

The Planck distribution law explains the different frequencies of the proton and electron, giving equal intensity to different lambda wavelengths! Also since the particles are diffraction patterns they have some closeness to each other – can be seen as a gravitational force. [2]

There is an asymmetry between the mass of the electric charges, for example proton and electron, can understood by the asymmetrical Planck Distribution Law. This temperature dependent energy distribution is asymmetric around the maximum intensity, where the annihilation of matter and antimatter is a high probability event. The asymmetric sides are creating different frequencies of electromagnetic radiations being in the same intensity level and compensating each other. One of these compensating ratios is the electron – proton mass ratio. The lower energy side has no compensating intensity level, it is the dark energy and the corresponding matter is the dark matter.

## Gravity from the point of view of quantum physics

### **The Gravitational force**

The gravitational attractive force is basically a magnetic force.

The same electric charges can attract one another by the magnetic force if they are moving parallel in the same direction. Since the electrically neutral matter is composed of negative and positive charges they need 2 photons to mediate this attractive force, one per charges. The Bing Bang caused parallel moving of the matter gives this magnetic force, experienced as gravitational force.

Since graviton is a tensor field, it has spin = 2, could be 2 photons with spin = 1 together.

You can think about photons as virtual electron – positron pairs, obtaining the necessary virtual mass for gravity.

The mass as seen before a result of the diffraction, for example the proton – electron mass rate Mp=1840 Me. In order to move one of these diffraction maximum (electron or proton) we need to intervene into the diffraction pattern with a force appropriate to the intensity of this diffraction maximum, means its intensity or mass.

The Big Bang caused acceleration created radial currents of the matter, and since the matter is composed of negative and positive charges, these currents are creating magnetic field and attracting forces between the parallel moving electric currents. This is the gravitational force experienced by the matter, and also the mass is result of the electromagnetic forces between the charged particles. The positive and negative charged currents attracts each other or by the magnetic forces or by the much stronger electrostatic forces!?

The gravitational force attracting the matter, causing concentration of the matter in a small space and leaving much space with low matter concentration: dark matter and energy. There is an asymmetry between the mass of the electric charges, for example proton and electron, can understood by the asymmetrical Planck Distribution Law. This temperature dependent energy distribution is asymmetric around the maximum intensity, where the annihilation of matter and antimatter is a high probability event. The asymmetric sides are creating different frequencies of electromagnetic radiations being in the same intensity level and compensating each other. One of these compensating ratios is the electron – proton mass ratio. The lower energy side has no compensating intensity level, it is the dark energy and the corresponding matter is the dark matter.

## The Higgs boson

By March 2013, the particle had been proven to behave, interact and decay in many of the expected ways predicted by the Standard Model, and was also tentatively confirmed to have + parity and zero spin, two fundamental criteria of a Higgs boson, making it also the first known scalar particle to be discovered in nature, although a number of other properties were not fully proven and some partial results do not yet precisely match those expected; in some cases data is also still awaited or being analyzed.

Since the Higgs boson is necessary to the W and Z bosons, the dipole change of the Weak interaction and the change in the magnetic effect caused gravitation must be conducted. The Wien law is also important to explain the Weak interaction, since it describes the  $T_{max}$  change and the diffraction patterns change. [2]

## **Higgs mechanism and Quantum Gravity**

The magnetic induction creates a negative electric field, causing an electromagnetic inertia. Probably it is the mysterious Higgs field giving mass to the charged particles? We can think about the photon as an electron-positron pair, they have mass. The neutral particles are built from negative and positive charges, for example the neutron, decaying to proton and electron. The wave – particle duality makes sure that the particles are oscillating and creating magnetic induction as an inertial mass, explaining also the relativistic mass change. Higher frequency creates stronger magnetic induction, smaller frequency results lesser magnetic induction. It seems to me that the magnetic induction is the secret of the Higgs field.

In particle physics, the Higgs mechanism is a kind of mass generation mechanism, a process that gives mass to elementary particles. According to this theory, particles gain mass by interacting with the Higgs field that permeates all space. More precisely, the Higgs mechanism endows gauge bosons in a gauge theory with mass through absorption of Nambu–Goldstone bosons arising in spontaneous symmetry breaking.

The simplest implementation of the mechanism adds an extra Higgs field to the gauge theory. The spontaneous symmetry breaking of the underlying local symmetry triggers conversion of components of this Higgs field to Goldstone bosons which interact with (at least some of) the other fields in the theory, so as to produce mass terms for (at least some of) the gauge bosons. This mechanism may also leave behind elementary scalar (spin-0) particles, known as Higgs bosons.

In the Standard Model, the phrase "Higgs mechanism" refers specifically to the generation of masses for the W<sup>±</sup>, and Z weak gauge bosons through electroweak symmetry breaking. The Large Hadron Collider at CERN announced results consistent with the Higgs particle on July 4, 2012 but stressed that further testing is needed to confirm the Standard Model.

#### What is the Spin?

So we know already that the new particle has spin zero or spin two and we could tell which one if we could detect the polarizations of the photons produced. Unfortunately this is difficult and neither ATLAS nor CMS are able to measure polarizations. The only direct and sure way to confirm that the particle is indeed a scalar is to plot the angular distribution of the photons in the rest frame of the centre of mass. A spin zero particles like the Higgs carries no directional information away from the original collision so the distribution will be even in all directions. This test will be possible when a much larger number of events have been observed. In the mean time we can settle for less certain indirect indicators.

#### **The Graviton**

In physics, the graviton is a hypothetical elementary particle that mediates the force of gravitation in the framework of quantum field theory. If it exists, the graviton is expected to be massless (because the gravitational force appears to have unlimited range) and must be a spin-2 boson. The spin follows from the fact that the source of gravitation is the stress-energy tensor, a second-rank tensor (compared to electromagnetism's spin-1 photon, the source of which is the four-current, a first-rank tensor). Additionally, it can be shown that any massless spin-2 field would give rise to a force indistinguishable from gravitation, because a massless spin-2 field must couple to (interact with) the stress-energy tensor in the same way that the gravitational field does. This result suggests that, if a massless spin-2 particle is discovered, it must be the graviton, so that the only experimental verification needed for the graviton may simply be the discovery of a massless spin-2 particle. [3]

### Conclusions

Exists experimental evidence for quantum-coherent is used for more efficient light-harvesting in plant photosynthesis. Quantum entanglement exists in supramolecules determining the sense of smell and in the brain neurons microtubules due to quantum vibrations.

In the work presented here, we started to design and quantum mechanical investigations of the molecular logical devices which are useful for construction of nano medicine biorobots against the molecular diseases such a cancer tumors, and against the new kinds of synthesized microorganisms and nano guns. [7]

One of the most important conclusions is that the electric charges are moving in an accelerated way and even if their velocity is constant, they have an intrinsic acceleration anyway, the so called spin, since they need at least an intrinsic acceleration to make possible they movement . The accelerated charges self-maintaining potential shows the locality of the relativity, working on the quantum level also. [1]

The bridge between the classical and quantum theory is based on this intrinsic acceleration of the spin, explaining also the Heisenberg Uncertainty Principle. The particle – wave duality of the electric charges and the photon makes certain that they are both sides of the same thing. The

Secret of Quantum Entanglement that the particles are diffraction patterns of the

electromagnetic waves and this way their quantum states every time is the result of the quantum state of the intermediate electromagnetic waves. [2]

These relatively new developments in biophysics have discovered that all biological organisms are constituted of a liquid crystalline medium. Further, DNA is a liquid-crystal, lattice-type structure (which some refer to as a liquid crystal gel), whereby body cells are involved in a holographic instantaneous communication via the emitting of biophotons (a source based on light). This implies that all living biological organisms continuously emit radiations of light that form a field of coherence and communication. Moreover, biophysics has discovered that living organisms are permeated by quantum wave forms. [5]

Basing the gravitational force on the accelerating Universe caused magnetic force and the Planck Distribution Law of the electromagnetic waves caused diffraction gives us the basis to build a Unified Theory of the physical interactions also.

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