Nanorobotics as a treatment for brain cancer

Newtoniña

Abstract

The aim of my Scientific Project is to propose a hypothetical design of nanorobots that can detect, target and bind to the surface [1, 4, 6, 8-13] of the astrocytoma cells. These functionalities are created with the scope of changing their microenvironment [3, 5-11, 13] and modify the chemical compounds [12] which they are made of. Additionally, they permit the endocytosis to get inside the core of the cell, destroy it and, in the end, execute the exocytosis and find a biomimetic system [3, 4, 8, 10, 11] in order to be excreted in a natural process. Moreover, the nanorobots show a broad range of applications such as: genes modifications [3, 11] within the DNA code, drug delivery into the cells and introduction of nanoparticles [1, 4-6, 8-13] stimulated by radio-frequency heat [2, 9, 12, 13] or other successful energy sources. It is important to note that nanobots could work in a decentralized way [6, 7] and we have considered this in the article design guideline. Furthermore, treating the brain cancer adds more complexity to the problem, because it requires the rupture of the BBB (Blood Brain Barrier) [5, 9] so that the nanorobots can access the brain. This may be resolved either by hyperthermia or by using natural degradations of the endothelial cells walls. The study is based on the investigations of various case studies, as well as current examples from multiple scientific journals, that lie their contribution on diverse approaches in order to solve the key challenges that this operation implies. By studying the physiology of the brain from a medical point of view, we have extracted the successful elements to design a hypothetical prototype of a brain cancer-cells killer. The use of enzyme-powered nanobots is proposed and we put forward new tracking analyzers that will be extremely useful to see how these robots could be administered. To achieve a more visual and disseminating insight, an online platform will be built and suggested for further understanding. The relevant data is analyzed and presented in a clear visual way.

Acknowledgm

ments

The research leading to this results has received several collaborations from diverse official entities within the Bioengineering field of Science. To mention all of them, we acknowledge the help welcomed from IBEC (Catalan Institute of Bioengineering), especially it's worth highlighting the work performed with Smart NanoBiodevices and SPECS' (groups) upon the last phases of the Scientific Project. To finish, as being part of the students selected for Argó Program, contribution from the Universitat Autònoma de Barcelona (foundation) resulted revealing along this long-way path of investigation. Likewise, not in the last place, regarding the teachers that have mentored me from the beginning of this ideal research, Institut Secretari Coloma needs to be alluded as for my high-school instruction.

State of the art
Hypothetical design
Experimentation and

21

60

results

79

Hypot

Hypothesis 1

Nanorobotics may be the most efficient, effective and minimally-invasive treatment pathway in order to cure brain cancers, such as the already known tumor called astrocytoma, more concretely, astrocytoma of first and second grade.

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Case Study 1

Visualization and imaging analysis of Janus spherical nanoparticles via Transmission Electron Microscope (TEM) instrument at the CCiTUB (Centros Científicos y Tecnológicos de la UB) [129].

Case Study 2

Analysis of the size and load of particles with Dynamic Light Scattering (DLS).

Case Study 3

Description and analysis of the movement of the particles with optical microscopy and posterior behavioral movement tracking.

Case Study 4

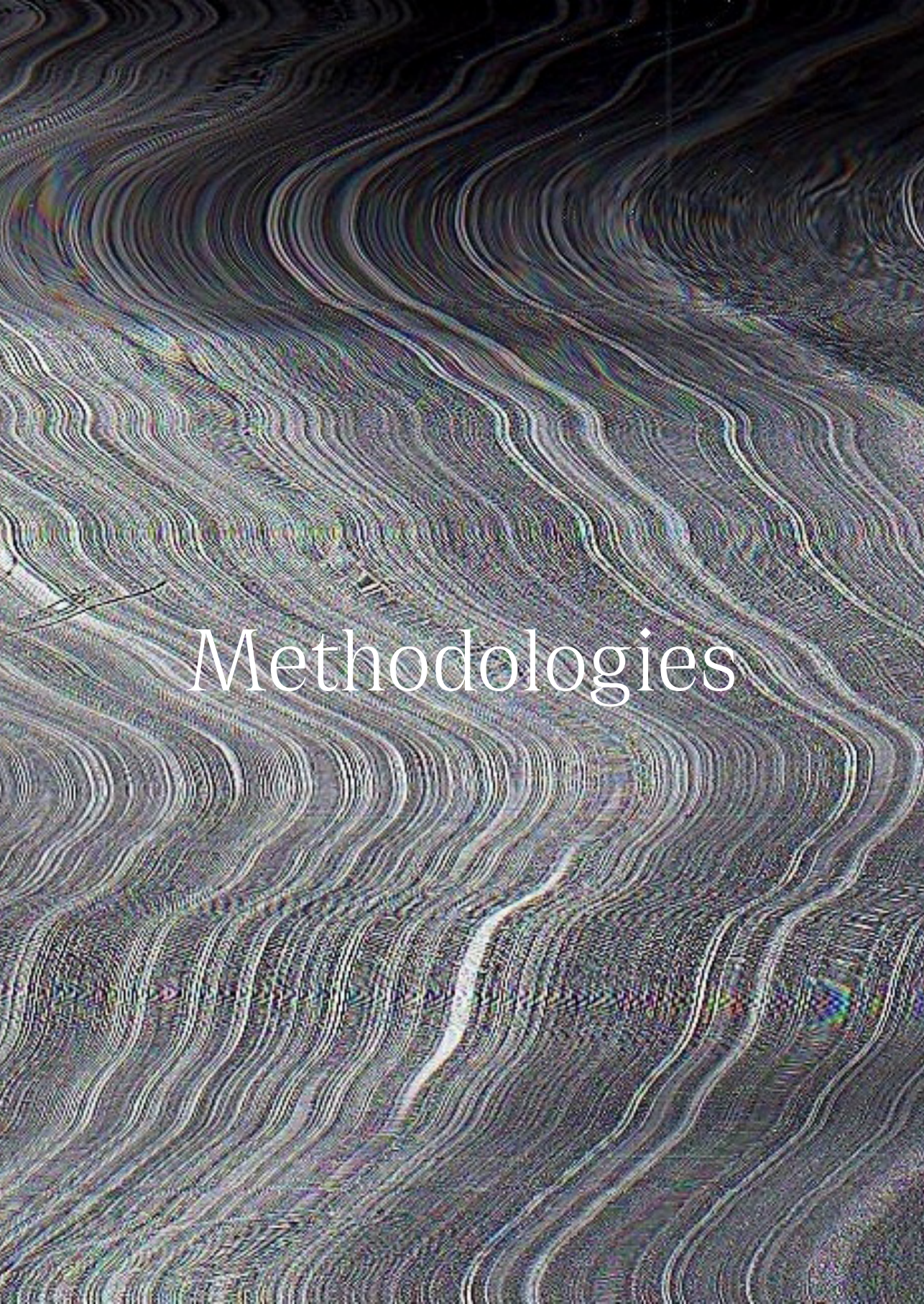
Incorporation of spherical nanobots into cells and the subsequent culture staining, in other words, internalization of nanomotors by cancerous (HeLa) cells.

5

Motivation

Since I was a child I have built miniature worlds. Although they were not related in specific to nanomedicine, all of them had a relevant importance to what it was making me me. They had all to do with the incognite and intrigue, which I find being the main characteristics that one may need to presume as an unstoppable motor. Likewise, the world of the untouchable has always been a locked door for me until recently, at least from my sight of view, as a scholar, through real world. Overall, this aspect made it more fascinating. It represented something intangible, untouchable, unseeable, insensible, unsensful. Moreover, I wasn't able to conceive the idea of such tiny things inside my mind. That's why, in the beginning, it consisted in an ambit from which I couldn't acquire knowledge, so I wouldn't comprehend the complete story. There were things I couldn't perceive with any of my senses. However, that scene changed as time passed and possibilities turned towards me. So, for this, I didn't know what it would be like to be a person compressed within 1 mm, which represented such a little scale, even though in literature everything in the universe, existing and unexisting, seemed possible. In fact, language was the only field trying to put it at the reach of humankind's imagination. Just that, putting the cherry on top (of the nanoscale) burst my inspiration, my stimulation and observation.

By the time I grew up, I started noticing a new breakthrough, a new revolution that would raise, and that was, indeed, the undergoing of the nanotechnology invading our comfort zone. A zone we already managed and we were already adapted to know and learn from. I was dreaming then but, now, I am quite convinced that one day, nanorobots will help us treat and win the battle with some of the diseases so many people suffer from, one of them being cancer. Cancer continues being one of the main causes of death worldwide. Brain tumor incidence, in particular, is high especially at children under 15. As a result, I am willing to dedicate all my efforts with one scope: trying to alleviate their suffering. Definitely, that is the force that has driven me towards this study, the work aimed to propose a new nanorobot prototype that might fight brain cancer.


Methodologies

1

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This initial phase consists of gathering as much knowledge [through online/ offline methodologies] and insights about the topic being researched. For this paper the following methods have been utilised.

Inspiration →

Figure 1
Brainstorm for the Scientific Project: nanorobotics as a treatment for brain cancer.

ROBOTICS

nanorobotics

destroy, eat, kill, cells
[collection of malignant cells]

nanomedical capsules, spheres, tubes
wireless communication, Physics fields, new laws …>
acoustic, optical and radiofrequency radiation
nanorobot's internalization

detectors …> natural, chemical or synthetic ones

MEDICINE

currently cancer …> brain cancer [glioma, operable and inoperable]

Blood Brain Barrier, how could we pass through that biological and innate immunological frontier?

BIOMIMETICS

BIOMIMICRY in animals: how do they survive in a cell microenvironment and how may we apply it in our project?

a swarm or colony that work without the central power and magnate orders: decentralized (com)mandments

materials: biocompatibility, efficiency, self-powering from human body sources

EMOTION

enjoyment of small things: what we are not capable to caption with our own sensible eyes?

neurosurgery as a field in need of a bridge between itself and bionegineering …> conveying with cutting edge technology and creating a new revolution!

nanorobotics: all in one, in the middle of a live breakthrough

?, INCOGNITAS

What if nanoparticles were recently, or in the near and exquisite future, used in order to perform the disintegration of malignant cells?
What is made the surface of the nanorobot of?
Does the nanorobot go intracellularly, that's to say, does it have a mechanism to target the cells' core by endocytosis?
What are, in a more specific and concrete signification, the nanoparticles and, consequently, the nanotubes? What are they used for? In which way are they essential?
How might we recognise the surface cells, in other words, the biomolecules composing them?
Why should we study the microenvironment? So that, perhaps, we, posteriorly, would make changes on it?

Desk Research

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Desk research is a type of research that involves collecting existing information, such as papers, information from newspapers, magazines, books... from online or offline sources. This methodology is very effective and crucial as a starting point since it enables a clear benchmarking overview.

The process
1 - Read, read and read
When analyzing previous work done in the multiple fields to find profound elemental compunds, several documental pieces of literature became the base of our fundamental acknowledgments. According to that, a list of scientific magazines, like for instance: Nature biotechnology, Elsevier, Science, Scientific Reports, Accounts of Chemical Research and National Geographic must be referenced and quoted. Moreover, a diverse multitude of webpages and scientific links, which drove to the allowance of electronic format, were taken into account. One may distinguish between the different typologies presented, but the most commonly known are PubMed, GOOGLE scholar/academics (from the same Google Company) and last, but not least, ResearchGate. Regarding the compilation of the global totality of scientific articles saved for the project itself, in order to assure and endure a reliable organization, the open-source software Mendeley was utilized. Nevertheless, graphic outlines to highlight and underly visual outcomes, including sketches and schematic drafts of the evolving conclusions and unknown concepts, were designed by specific tools provided by Mural and Sketch digital applications; whereas Bamboo Paper served as the most prominent translation as for the composition of the first ideas and, therefore, for what we originally call brainstorming.
2 - Detect the main focus areas which need to be research
3 - Keep digging
4 - Design a mental map in which the main ideas are organised

Expert interview

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Experts can get you up to speed quickly on a topic, giving you key insights into relevant history, context and innovations. They can often offer you a systematic overview, connecting your particular questions with the whole ecosystem.

The process
1 - Determine what kind of specialist you need
2 - Benchmark possible specialists you'd like to reach - keeping in mind diversity in their specialisation field

3 - Contact
4 - Plan interview: design the questions and topics you'd like to address
5 - Complete the interview
6 - Structure the knowledge in order to identify new leads

Creation of a project plan

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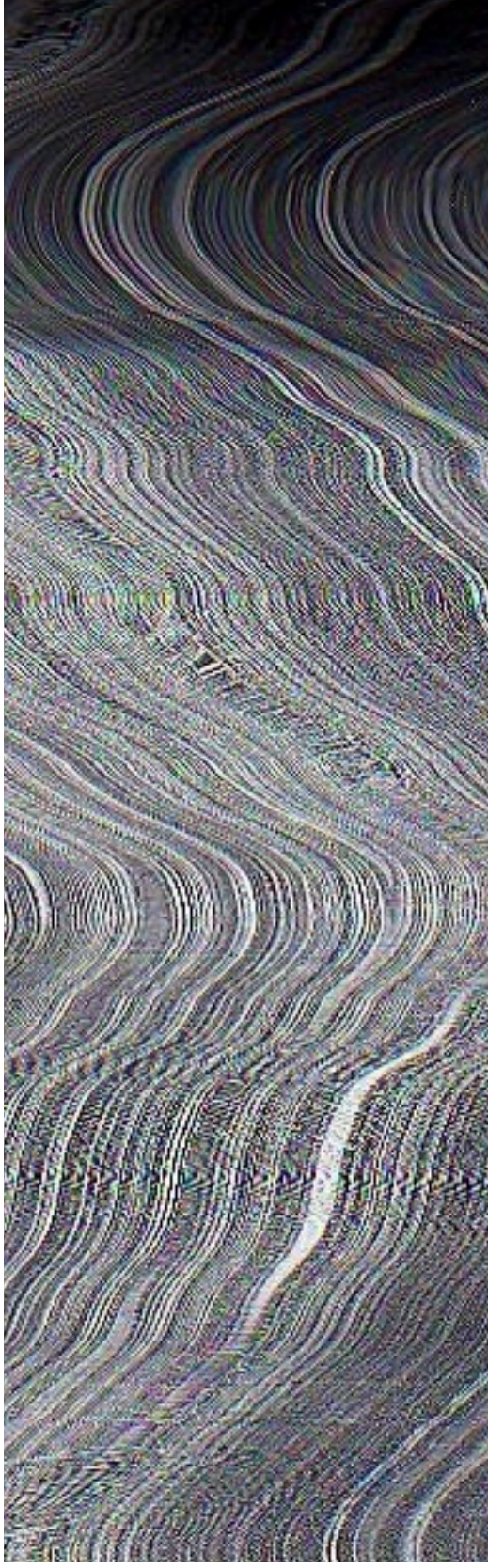
This bit consists in organising all the previous insights and designing a roadmap which will enable the successful delivery of this project. In consequence, this may be the perfect chance to think through all the logistics of my project, and even though they are bound to change as things progress, it will be for seeking if we can plan for what's ahead. Spending time reflecting on our timeline, our budget, the skills needed, the materials I'll need.

The process
1- An easy start for the overall organisation of the project is a paper calendar. In this medium, I can allocate timings for the general parts of the project.
2 - Trello/Mural board once the main parts are defined, more specific tasks can be listed on a trello board which enables tracking as well as the completion stage and last updates.



2

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Ideation is a creative process where ideas are generated. Participants of an ideation phase gather with open minds to produce as many ideas as they can to address a problem statement in a facilitated, judgment-free environment.

Ideation →

Create insight statement

A critical piece of the Ideation phase is plucking the insights that will drive our design out of the body of gathered information. This consists in composing sentences that will point the way forward, that's to say, that will help me frame HOW might we (down to three to five main insights) questions and give shape to subsequent Brainstorms.

We must identify the following central themes:

1. how might we create effective propellants for nanorobots?
2. how might the nanorobotics communication work?
3. how might we get nanorobots through the BBB?
4. how might we stimuli nanorobotics systems, that's to say,
using which kind of physics subdiscipline and biomimetics field will we achieve that scope?

These found themes should be rephrased as a short statement. It's merely transforming a theme into what feels like a core insight of the research and, consequently, it's a building block, not a resolved question.
Then, we have to look back to our design challenge, sift through the insight statements and discard the ones that do not directly relate to the objective. Another pass at refining the insights is needed, in which we have to make sure that they convey the sense of a new perspective.

Brainstorm

Drumming up a staggering amount of ideas gives energy to the project, that's why we use brainstorming (to behold in Figure 2) in order to tap into a broad body of knowledge and creativity. The specifics that make this experience fruitful are referred to Brainstorm rules and we should not supravalue immediate feasibility over other ideas. Being positive, optimistic and focused are the best characteristics atmosphere to generate as many ideas as possible.
Passing out pens and post-its to answer the posed question is a method to create ideas.

Create a concept

Movement from a handful of ideas and insights to a fully-fledged concept that refines and pushes forward the investigation. The refined things were performed when bundling the ideas, but now it is time to turn them into a sole concept. This starts to seem more like an answer to the How might we posed relevant questions, because this is the moment where we transition from problem to solution and boost up everything that comes next.
It is necessary to take all the ideas and create frameworks out of them, even more, to start to visualize where bundles/combinations are pointing,

but we have to think hard about inserting them into a system. This is not the final prospect, so we do not have to emphasize the details since we do not need a finely tuned funding strategy. The ideal is to get a robust, flexible concept that addresses the problem we're trying to solve. On the other hand, we must keep referring back to our how might we pose relevant questions in order to visualize which elements are missing or what are other variables and parameters that we could incorporate to come up with a better solution. The procedure includes a bit of trial and error, by that we mean that we may have to propose more that one concept, although at first didn't work out, but in the future confirmed the base of new concept definitions.

Determine a prototype

In this process, we learn which ways are the suitable ones to isolate what to test. Clearance about what is necessitated to learn and make decisions about the testable components that give guidance answers. To focus testing on the critical elements and to get optimized simple, scrappy prototypes may be a good option to keep in the sight of view. Just like in the case of learning about: How big should the nanorobot be?; which would be the characteristics wanted for further knowledge on the physics field?; which are the main components that compose the nanobot?; to be a perfect match, a biocompatible machine within the human body and, especially, inside the brain stem how should it be like?; which are the functions should we develop?; what do we hope for a nanobot to be able to do as future perspectives?, and finally, which programming or electronic source will be the nanorobot based on?
In addition, we must keep track of the progress and evolution by advancing, writing down the key elements of the ideas, thinking practically about the needs to be tested and reflecting on which would be the primary questions for each component. It is important to pick a few questions to answer and, interestingly, to test the prototype in the most sensible manner we consider to receive feedback and, furthermore, learn that succeeding in the first attempt is not very common, however, we can use the knowledge acquired in future attempts and improve.

3

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The process of putting a decision or
plan into effect; execution.

Implementation →

Live prototyping

It comprehends the best chance to run the solution in the real world, that's to say, in real-world conditions. Likewise, it condigures also, at the same time, the most powerful way to test the project in the marketplace. Until now, the prototypes consisted in rough, banal, superficial schemes, drawings and sketches and they had been enough to convey the idea we are proposing to test. Interestingly, this is the phase in which we need to learn the steps missing that we might not have taken into account or, on the other hand, how does it work as in a proper manner. Live prototyping is all about understanding the feasibility and viability of the idea. The first work to do is determine what do we want to test in Live prototyping, which will be the subject of observation. It could be the way people find out about the solution proposed and how the phenomenon will be distributed. Once we have given a thought at this background information, we can prepare the logistics of the Prototype Experience: develop and build a nanorobot, put in practice the theory learned and the knowledge acquired regarding the optimal design for drug-delivery in human body that has to find and destroy cancerous cells. After listing and sorting out the spaces, the people, and the extra stuff, and the capacity, we should start by applying in the reality. One should always remember to never stop iterating because replicas are a path that leads to the generation of plenty of quick ideas and new approaches. Moreover, it also

encapsulates the fundamental piece of the puzzle: reassurance of results when analyzing the collected data. Basically, it consists in pushing closer to the real appliance.

Measure & evaluate

My goal has already been mentioned numerous times, but, to be more precise, it consists in creating a hypothetical nanorobot that is hoped to develop diverse functionalities in order to arrive on the surface of the cells, and decide whether that is a malignant cell or a benign one. Further characterization of the nanorobot include decision-making regarding the tracing of chemical compounds on the surface, injection of nanoparticles intranasally or orally and performance of diverse internalizations, among others.
A first step is to create a design that will allow us to quantify the robot's performance, which will result in a successful tool in our solution. A second step is to be continuously informed about the state of the art and technological advancements while in parallel keep evaluating and improving the proposed solution. Finally, we can assess the impact we have in the outer world. The key of making a good measurement, above all, when trying to change a community's behaviour or adopt other technologies, is to pick the right laws that are being obeyed and pursue statistical calculations in order to make a nuanced approach. A few things as we strategize: we want to define what to demonstrate by utilizing them: to prove which are the good materials the nanocarriers have been constructed from?, to test the efficiency of the biomimicry regarding nanobots shape?, to experiment if it is viable in practice, long distance to theories? Key partners and stakeholders have already experience because they have been working and evaluating this theme for years so they may provide key insights. We might need external assessors and consultants to give us already organized processes. Moreover, we must try to have a balance between quantitative and qualitative measurements. If the solution doesn't lend itself to capturing hard numbers, stories from different research groups can be a pretty powerful tool. In the end, we can always tweak the based model on the information coming in order to maximize the success.

Figure 2
Brainstorm of the Scientific Project

0. ABSTRACT & INTRO

1. BACKGROUND INFORMATION

[ ] Nanorobotics in Medicine
[ ] What is Cancer?
[ ] Cancer treatment proposals -look for case studies...
[ ] Power generators
[ ] Coding simulation
[ ] Building methods
[ ] Metthodologies
[ ]BBB

Benchmarking is a collection of case studies = sucessfull cases. CS can work as complements of any of the previously listed topics.

[ ]case study 1
[ ]case study 2
[ ]case study 3

How might we questions: how might we create sustainable nanorobots to accelerate cancer treatement of bening tumor removal.

Conclude with Insights: all these curious findings which later on will help develop a prototype.

[ ]insight 1
[ ]insight 2
[ ]insight 3

Cancer Nanorobot

2. METHODS

Find at lesat 3 type of research methods [check IDEO - Design kit]

[ ]method 1
[ ]method 2
[ ]method 3

Add simple graphics to illustrate de methodology.

[ ]graph 1
[ ]graph 2
[ ]graph 3

3. RESULTS

[ ] Write intro about the results
+
[ ] Explain how the results are linked to the initial questions
+
[ ] Have we gathered any more insights?
+
[ ] Can we link these insights to any other first prototype?

5. CONCLUSION

[ ] Conclusion 1
[ ] Conclusion 2
[ ] Conclusion 3
[ ] Conclusion 4
[ ] Conclusion 5

Conclusions should come up after analysing all previous research.

6. ACKNOWLEDGEMENTS

[ ] Special thanks
[ ] Bibliography
[ ] Other references
[ ] Annex

4. RESULTS

[ ] Future vision perspectives
+
[ ] New hypothesis
+
[ ] Insights?
+
[ ] Reflections: how does this affect..?

*can we link this to any sustainability issues?

State of the art

1
Nanorobotic applications in medicine: current proposals and designs

Progress in technology, and subsequently, in other fields that base their acknowledgement on Physics and Chemistry, has increased exponentially. One factor that contributed to this advancement is the ability to manipulate nanomaterials in a very precise manner, complex and small scale. Nanotechnologies are of great interest for both medicine and pharmaceutical manufacturing. During the last years, a special term was introduced to define developments concerning both nanotechnology and medicine: nanomedicine. Basic humanity needs, such as surgery procedures, are implying a new area of studying the nanoscale world, and therefore, the idea of creating nanomachines that could interact within biological systems seems quite appealing.

This article provides with information about works in progress on neurorobotics and makes a complete review of the applications under research.

By definition, according to the National Nanotechnology Initiative (that we will call NNI - a US leadership to promote research in this subfield of classical science technology), the nanoscale ranges from 1 to 100 nanometers, even though developments' majority overgo the limits. Some sample sizes in the nanoscale domain are: a strand of DNA, that is approximately 2 nm, a molecule of albumin, having about 7 nm and a cell surface receptor, which is 40 nm wide. An example of what is the size representation ranging from a meter to a nanometer can be seen in Figure 3.

With the arrival of nanotechnology, many developments and procedures have been made possible. Nanotechnology not only has boosted up the techniques for higher sensitivity in detection of cancer and illness but it has also increased targeting of drug treatments, improved adverse effects of therapies (chemotherapy), enhanced effectiveness of antineoplastic therapies (cryotherapy, ultrasound) and drug delivery, to name just the most important achievements. There's been also another term designed for the mixture between medicine, biological sciences and the utilization of nanotechnology techniques and principles: nanobiotechnology.

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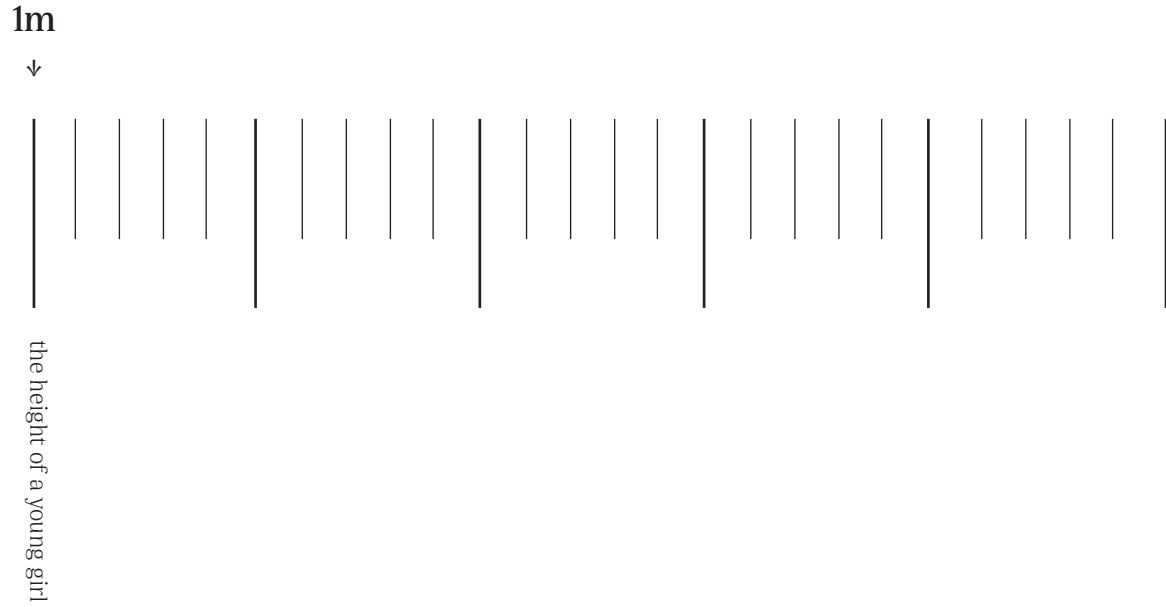

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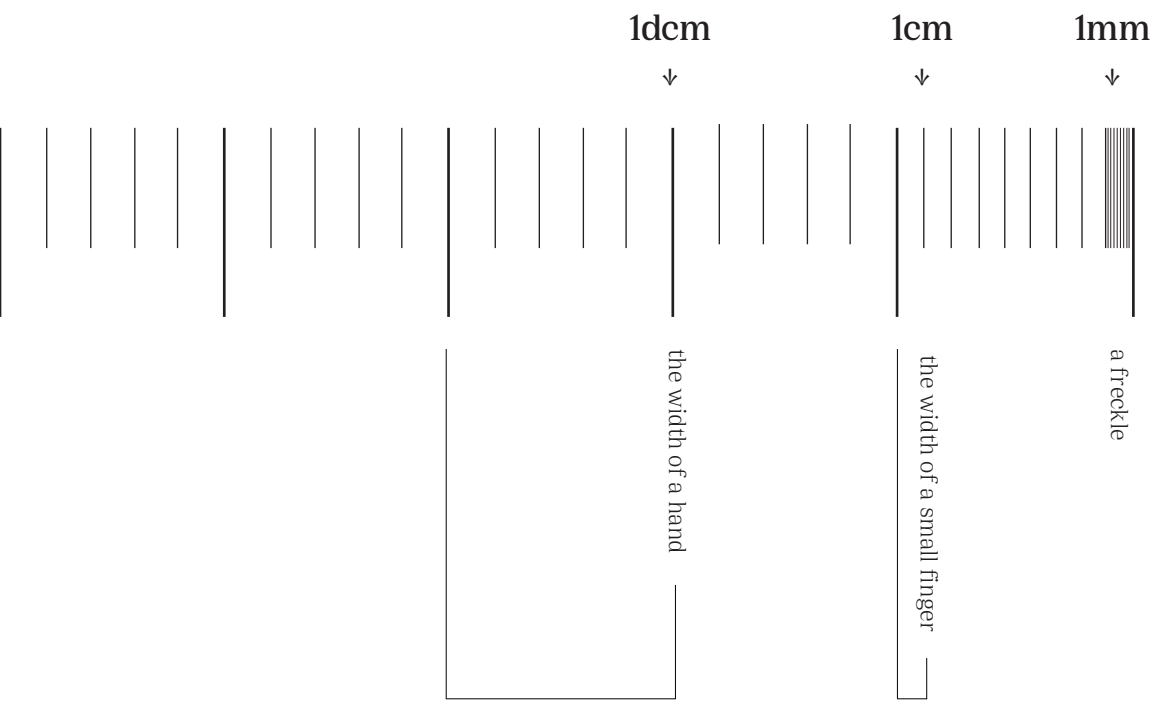
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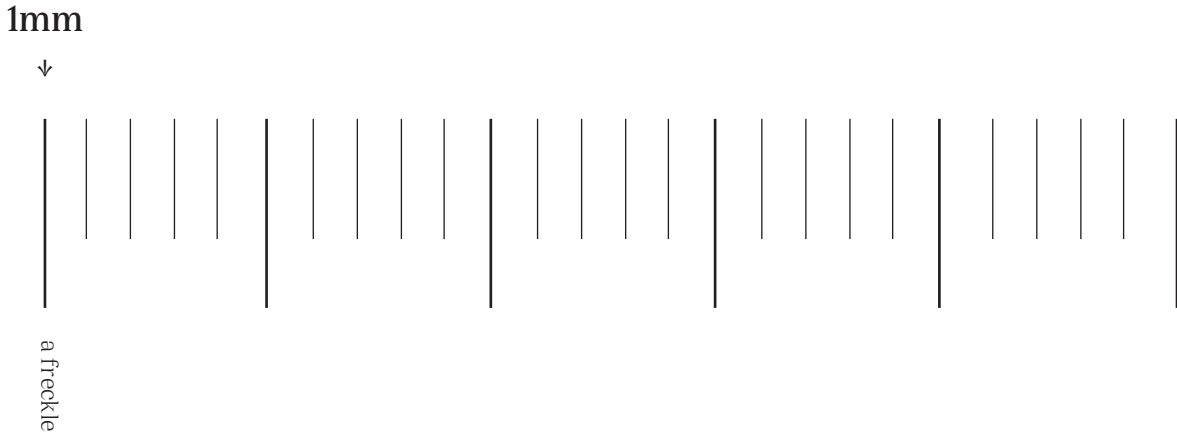

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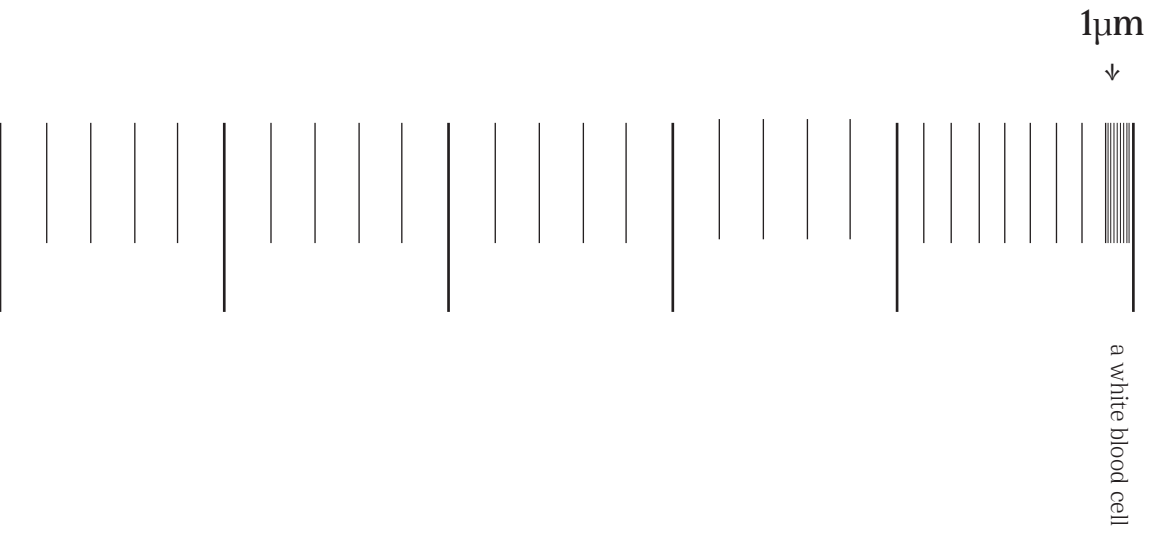

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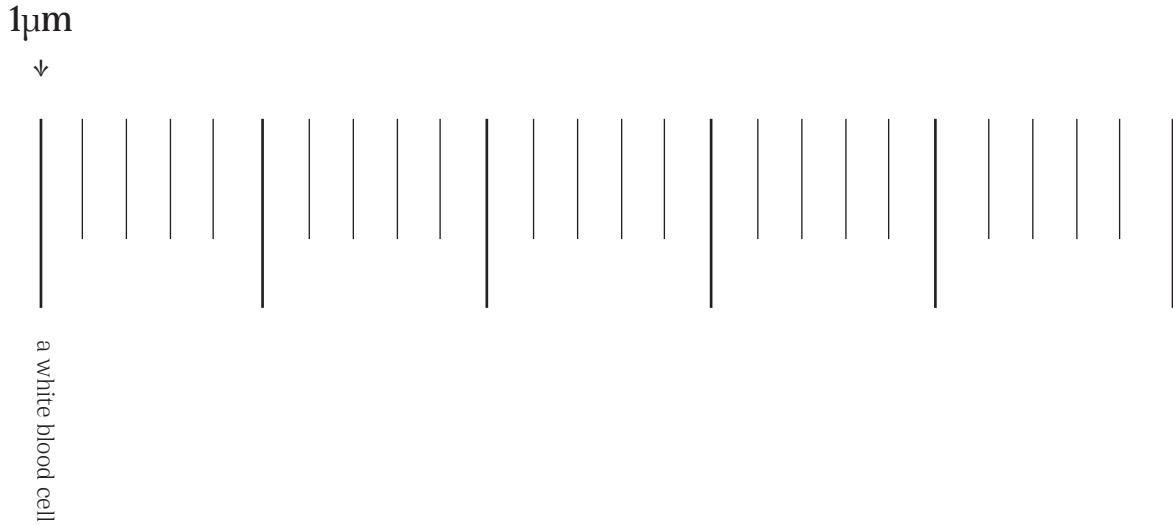

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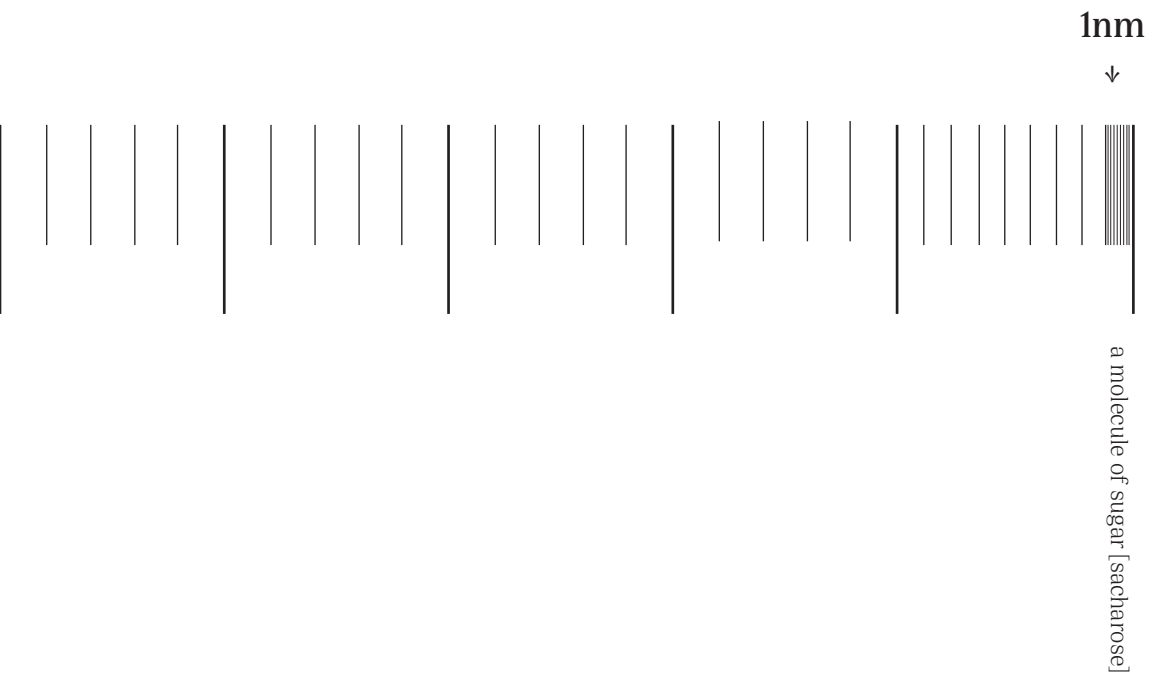


Brief introduction to nanotechnology

Take a step forward and enter into a wonderful dimension, where a drop of water seems larger than an ocean, where gold can be purple and man plays with atoms as if they were chess pieces, where the glasses are cleaned alone and the clothes are never stained, and a robot smaller than a hair repairs a blood vessel... Enter the nano dimension!

What happens at the nanoscale?

The concept of nano does not only imply a very, very small scale and size but also includes new behaviours. At the nanoscale, the particles from the same material can behave in a different manner than at larger scales. One of the most surprising discoveries in nanoscience has been the fact of studying and analysing the differences between the physical properties of the materials at big and small scale. These properties are the color, the forces they can produce, the conductivity, etc. and they vary tremendously when heading into a nanometric perspective.
Here are some examples of nanomaterials present in nature. First of all, there is a dragon (Basiliscus basiliscus) that has the ability of walking upside down, climbing trees backwards or running above wet surfaces without getting wet at all. How does it achieve that, without counting the magnificent properties of the polarity of water and other polar dissolvents? The answer asses the fact that its legs are covered with millions of special hairs, whose ends measure only 200 nanometers in width. Each of these ends interacts separately with surface molecules (stones, trees, etc.), thus providing impressive grip. The dragon mentioned before is the first example among other intriguing animals.
Stained glass artists of medieval times dominated the use of materials such as sand, wood ashes and powder metals, in order to obtain the many hues ("nuances") that defined their incredible masterpieces, many of them being preserved for almost a thousand years. However, these artists never understood how to form those tonalities. At the end of the 20th century, scientists determined that at the origin of those nuances was the existence of metal nanoparticles in the composition of the glasses.
In 1910, the company BF Goodrich added rubber carbonium compounds in order to create black tires that lasted ten times more than traditional tires, which were manufactured gently with rubber, and that they were white. However, it was not until a few decades later that scientists discovered the reason for this incredible increased durability of the material: nanoparticles of carbon regularly dispersed in the rubber network.

Nanomaterials

Nanomaterials are either natural materials or manufactured by humans. Their characteristics at nanoscale give them interesting properties like water repulsion, high electrical conductivity or huge mechanical force.

The wings of the butterfly contain nanomaterials that give them lightness and color.
The Lotus flower is an aquatic plant native to Asia. When coming into contact with water, its leaves are not wet. The existence of nanostructures on the surface of the leaves causes the water droplets to slip easily, in such a way that they drag dirt and leave the leaf clean and dry. With modern nanotechnology, we can build highly hydrophobic glasses or cloths, which instead of getting wet in contact with water, are self-cleaned and left dry. One interesting application of this is to avoid the freezing of rotor shovels of wind power plants.
Graphene is a natural material composed of a single layer of carbon. It is transparent, flexible, with high durability, waterproof, abundant, easy to extract and, above all, it drives electricity better than any other known material.
It is already incorporated into electronic devices, and could soon lead to a technological revolution in microchips, mobile phones, laptops, etc.

A carbon nanotube is a tube of 10 million times smaller than a straw. It is extracted exclusively from carbon atoms, such as graphene, graphite, coal and diamond. Its hexagonal stretching gives it special properties such as great electrical conductivity and high resistance and strength, as well as the possibility of mixing with other materials. The nanotubes are used to provide more strength and lightness to tennis racks, car parts, toys, etc.

Nanotechnology not only will allow us to improve existing products but also to create new ones. This will have a great impact on our lives, as happened with the introduction of electricity, automobiles and computers.

Nanofabrication: nanoscale manufacturing

Advances in microscopes and specialized equipment are allowing the construction of devices, such as gears or robots, at nano size.
Nanolithography is used along with a series of other techniques for scratching the surface at a nanometric scale, in the manufacture of chips.
Nanofabrication can be performed either from top to bottom - when a block of material is carved - or from bottom to top - when the different pieces are joined together as building blocks - or as a combination of both.
Nanotechnology is opening up many frontiers and is driving new research fields, such as photonics: the science of the generation, control and detection of photons, the particles of light.
This introductory information gives us a global picture of what is the current state of the art and we are now going to highlight some of the relevant applications that were done in medicine by using these acquisitions.

As we have mentioned initially, nanotechnology has a great impact in different branches of science such as Chemistry and Physics. But, interestingly, the idea of combining the nanotechnology field with

the medicine world seems to be pretty appealing in order to create nanorobotic systems that are able to interact with the cells and their environment. The aim is to impulse further and deeper answers to the questions we already have and the ones that will be asked by adding another dimension (nanodimension). On the other hand, one of the most important issues to discuss are diseases and, by that, we mean the treatments we administer as well. That's why as nanotechnology arises as a cutting-edge technology we propose to investigate robotic applications in medicine at the nanometer-size. Hence, we summarize hereafter the main achievements of nanorobotics in different branches of medicine in the recent years.

Microbiology

It was the perfect field where nanotechnology could start implementing robotic functions. In microbiology, the most important challenges are related to transportation and propulsion within the vascular system. Propulsion has been successful by coupling robots to magnetotactic bacteria (MTB), like Magnetococcus, Magnetospirillum magnetotacticum or Magnetospirillum magneticum. The smallest known in their species is the marine Magnetotacticum spirillum, which is 500 nm in size, little above the upper limit of the nanodomain given by the NNI. They are an efficient component because guidance via magnetic fields is possible. Problems with achieving high velocities for control in large vessels can be resolved by pursuing a two-component robotic system that is able to release small components into small vessels.

Hematology

There is a large array of applications ranging from emergency transfusions of non-blood oxygen to carrying compounds in order to restore primary hemostasis. A nanorobot under design is the so-called respirocyte, shown in Figure 4 (a, b), supplied with three functions as it travels through the bloodstream: collecting oxygen, collecting carbon dioxide from tissues that may release it into the lungs, and metabolizing circulating glucose to power itself. Due to the onboard nanocomputer and numerous chemical and pressure sensors (loading motors), the respirocyte's dimension needs to round thousand nanometres. The nanorobots' communication system it is remarked by the author Robert A. Freitas Jr. when explaining that "complex device behaviours can be remotely re-programmable by the doctor via externally applied acoustic signals". The attending physician can broadcast signals to molecular systems deployed in the human body. It is convenient to use modulated compressive pressure pulses received by mechanical transducers embedded in the surface of the respirocyte. Pressure fluctuations can be, then, converted into mechanical motions that can serve as input to a mechanical computer that requires transducers. These transducers function as pressure-driven actuators. Another

nanorobot was designed to carry out hemostasis (detention of hemorrhage via physiological mechanism) like it would be an artificial platelet. That's why it is named "clottocyte". Finally, another invention in this realm is Microbivores (phagocytic agents), robots designed to have binding sites on their surface for antigens or pathogens and are 80 times more efficacious than currently used phagocytic agents.

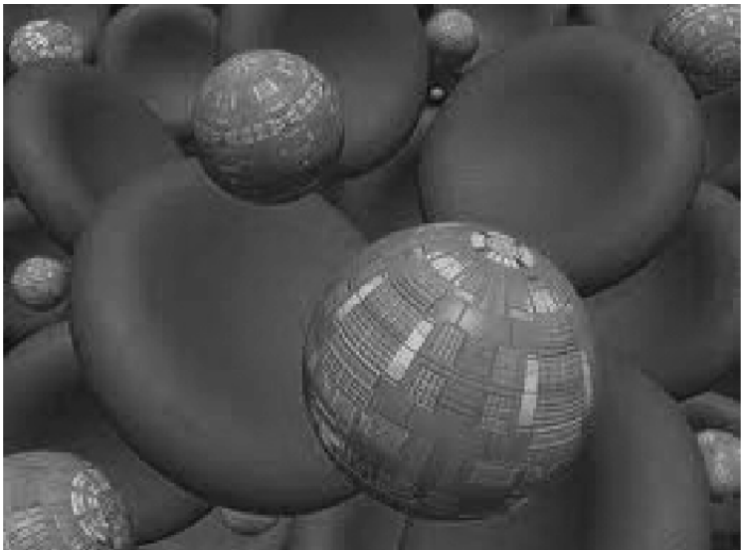


Figure 4

(a) The nanorobot so called respirocyte has been proposed in the field of hematology with the finality of supplementing and work in a symbiotic nature together with the already existing microorganisms that undergo the respiratory system functions. The reason for this ideation is the natural system, with which we were endowed since the beginning of life, failure. Extracted from: http://biomedical-4-research.blogspot.com/2012/08/respirocytes.html [62].

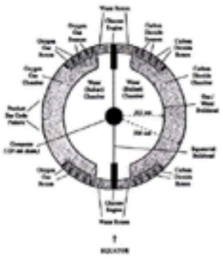


Figure 4

(b) Respirocyte Equatorial Cutaway. Equatorial cutaway view of respirocyte. The oxygen gas chamber is represented at left (south pole), the carbon dioxide gas chamber is at right (north pole), and the water (ballast) chamber occupies the center, surrounding the onboard computer system. The equatorial bulkhead separates the north and south hemispheres of the device. Taken from: A Mechanical Artificial Red Cell: Exploratory Design in Medical Nanotechnology by Robert A. Freitas Jr.

Dentistry

Dentistry could incorporate nanorobots to benefit from higher care level in all the elements of dental care, from routine cleaning, teeth whitening to orthodontics. Nanorobotics can potentially increase precision in root canals filling, or they could be dressed in accurately specific proteins to reduce guesswork and detect pathogens for the treatment of infections. The cause of hypersensitive teeth could also be resolved with nanorobots. Their intervention, in this case, would consists of a selective occlusion of tubules because that would suppress stimuli from provoking a pain response.

Neurosurgery

This area has benefited from improvements into detection of pathology, minimally-invasive intracranial monitoring, and pharmaceutical delivery and acceleration in manufacturing microelectromechanical systems. Amongst other methods, spinal cord injury and nerve damage is a significant life-changing event that may be treated controlling transaction axons and maneuvering them into position using dielectrophoresis and fusion between two ends by electrofusion, laser-induced cell fusion, and polyethylene glycol (PEG). An intravascular nanorobot with the capability of detecting aneurysm formation by detecting high levels of nitric oxide synthase protein in the affected vessel is proposed by Cavalcanti et al. [15,16,17,18]. Nanorobots can wirelessly communicate about proper vascular changes, decrease screening costs and frequent follow-up visits. Interestingly, this could enable other uses, such as tumor detection or ischemic (decreased blood circulation) changes.

Oncology

Nanotechnology shows promise in improving the management of cancer by improving imaging tools, overcoming drug resistance and increasing success rates in metastasis treatment. A nanorobot can be functionalised using engineered DNA to detect cancerous cells, release treatment agents at the site

of astrocytomas, to respond to cell surface receptors and release stored therapy. As an alternative to the DNA nanorobot mentioned, one can build a robot out of synthetic elements. The use of nanorobots in oncology will permit better detection of tumorous tissue margins and metastatic areas intraoperatively as well as resection of tumors.

Vascular

The use of nanorobotics in this field improves drastically the screening and monitoring for life-threatening conditions or chronic diseases. Intravascular nanorobots would constantly circulate and provide instant information to the doctors about the current patient state. This could be of great importance in cases of brain aneurysms or cancers with no screening protocols such as lung cancer. Nanorobots can also prevent aneurysm ruptures by using their navigational abilities, localize the drug delivery position and reduce the amount of bleeding. Summarizing, nanorobots can increase efficacy allowing delivery of an accurate dose treatment and, moreover, reducing the toxicity level.

In conclusion, nanotechnology will definitely bring a change in many paradigms we are postulating when reasoning about diagnosis, treatment, prevention and screening. Outside the bounds of medicine, this field can affect us in countless other ways through fields such as telecommunications and agriculture, to name just a few of them. Nanorobotics was meant to be a key point to start a new revolution into the nanometer size scale, but, in the meanwhile, researches and studies effectuated in the manufacture of DNA molecules impacted what we already know as nanomedicine. That's why, in this evolving process of surpassing the unknown and going beyond the limits has appeared a new series of chemical compounds that could lead to other advantages (to cite, for instance, nanoparticles (NPs)).
"In a famous Caltech lecture in 1959 recognised with the title "There's Plenty of Room at the Bottom", the Nobel laureate-to-be and American physicist Richard Feynman introduced the general idea of manipulating and manufacturing structures at the atomic level. Although, at the time, the applications he discussed were pure theoretical. In addition, his insights prophesied the discovery of many new properties at the nanometer scale that are not observed in materials at larger scales, paving the way for the ever expanding field of nanomedicine. These days, the use of nanosized materials, comparable in size to some proteins, DNA, RNA, and oligosaccharides, is making waves in diverse biomedical fields, including biosensing, imaging, drug delivery and even surgery procedures." This was explained by Guizhi Zhu, a postdoctoral associate at the health Cancer Center and in the department of Chemistry of the University of Florida, et al. in an online magazine named The Scientist [21].

Developments in nanomedicine in the coming years will probably save many lives: smart medications (nanosmart devices) that have fewer side effects and are more effective; regeneration or replacement of injured or diseased parts of the body or minimized implants to monitor and correct physiological problems. The new medical treatments, however, have to undergo clinical trials for many years, so, we may need to wait and have patience, "who is the mother of science."

Nanomedicines are thought to be administered systemically when travelling through the bloodstream. Their size is small enough in order to prevent clogging the vessels, although the nanomedicines are larger than many small-molecules drugs. As a result, they can stay for a prolonged interval of time, during which they can perform the biological process of endocytosis, that's to say, they even enter living cells after having penetrated leaky blood vessels in diseased tissues [22]. In the following paragraphs we will mention the novel nanocarriers that differ from the traditional ones as for their chemical structure, this is also shown in Figure 5.

Our study will focus on the application of nanorobots within the brain, more exactly, towards brain cancer treatment. The following chapter will present a brief introduction to the brain physiology and neuro-anatomy as well as the achievements of nanorobotics in this field.

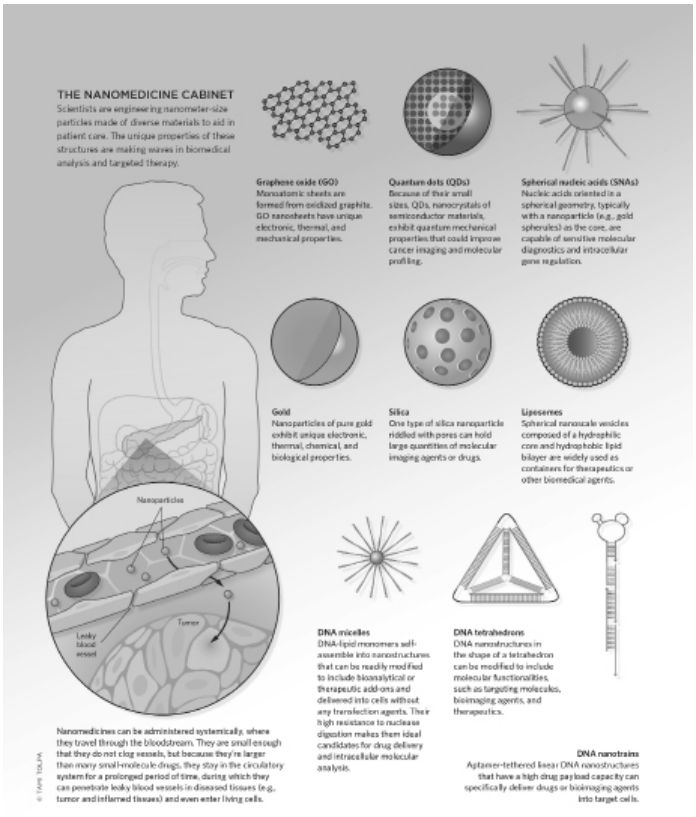
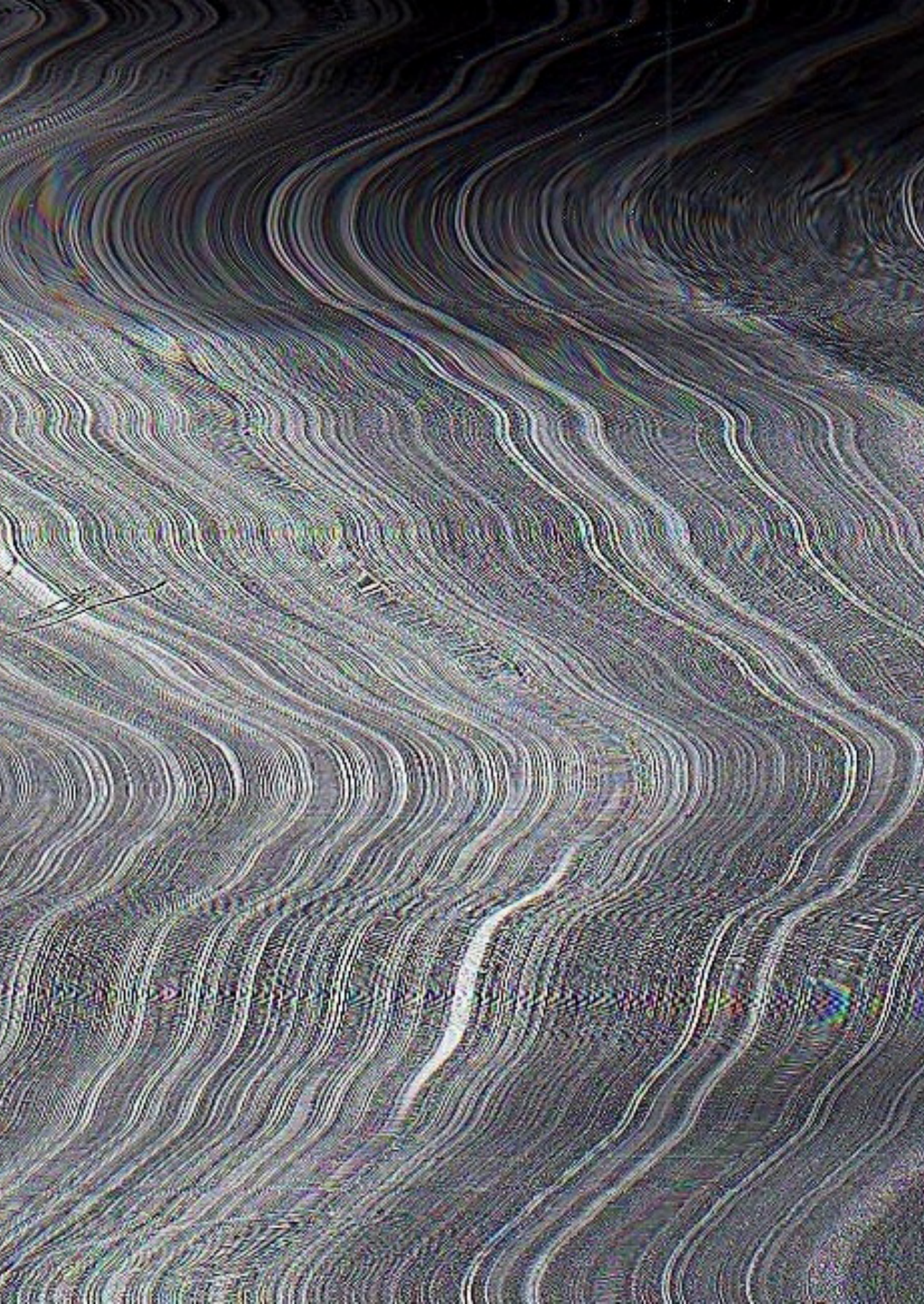


Figure 5

The nanomedicine cabinet shows which are the most advanced nanometric particles accomplished by the current scientists. These particles are made to aid in patient care. The unique properties of these emerging structures are making waves in biomedical analysis and targeted therapy. Copied from: [22].



2
Nanorobots in brain tumor

Brain physiology

Brain tissue consistency is soft and spongy and it is protected by the bones of the skull and three delicate membranes called meninges. Cerebrospinal fluid, a watery fluid, cushions the brain and can flow through spaces between the meninges and the ventricles. Within the brain and spinal cord that form the central nervous system (CNS) of a large variety of organisms, glial cells surround nerve cells and hold them tight. Our brains can control either voluntary and involuntary movements, and it takes charge of our senses, memory, emotions and personality as well. We can distinguish three grand parts that control different activities, presented in Figure 6:

1. CEREBRUM
It uses information from our senses to tell us what happens around us, in our medium, and how to react. It also directs reading, thinking, learning, speech and emotions.

2. CEREBELLUM
Its main control is on the balance and complex daily actions like walking and talking.

3. BRAIN STEM
It controls hunger, thirst, breathing, body temperature, blood pressure, and other basic bodily functions.

Blood Brain Barrier (BBB)

The BBB is a permeable membrane [120] that consists of endothelial cells united by tight junctions, astrocytic endfeet surrounding blood vessels, pericytes embedded in the vessel basement membranes (BMs), microglia and neurons, all of them playing essential roles in CNS homeostasis, as shown schematically in Figure 7.

One of the components that give constitution and identity to the BBB are astrocytes, which° contribute to the selection and exchange of molecules through the barrier. Although a hundred years have passed since the BBB was discovered by Goldmann, the mechanisms by which some drugs or parasites or pathogen organisms conquer the brain still remain unsolved. All together, the components important for BBB's integrity and maintenance, including

the extracellular matrix, are denoted as the neurovascular unit. This unit forms a highly coordinated system in which the cerebral microvascular permeability is dynamically regulated. Interestingly, the astrocytic coverage that may be conformed surrounding vessel endothelial cells and pericytes is discontinuous at only a few sites. Solely in these rare situations one can observe how microglial processes maintain neighbor contact with the basal lamina. Furthermore, control of BBB tightness is completed by astrocytes, which secrete soluble factors that influence endothelial cells. Moreover, astrocytes do not stop their functionalities at one single scope within the BBB, because rapid regulation of the BBB permeability is also regulated by the presence of numerous astrocyte endfeet close to the membrane. Why is it so relevant?

Velocity in BBB permeability is important due to the fact that this structures contain the secret of the innate immune system in the brain. As said before, astrocytes not only participate in the formation of the BBB by inducing tight junction formation in endothelial cells, but also modulating the expression and polarization of transporters, and by promoting specialized enzyme systems.

Many authors expressed the interaction between astrocytes, a member of the glial cells family, and endothelial cells as a “two-way-street”, due to the fact that their relation is beneficial one to each other, meaning that endothelial cells also complement the benefits received by the other cells, as they simultaneously boost astrocyte growth and differentiation. Clearly, astrocytes represent many features that are crucial to BBB physiology [119].

Vascularization of BBB

Vascularization could be defined as the formation process of blood vessels and capillaries in living tissue (see Fig.8). A recent study [5] used the molecular dynamics software (so called MD) to simulate the vascularization of a brain capillary with major detail. It was observed that the higher the coverage of the surface is achieved, the higher are the repulsions. Collectively, these simulations show that the targeted thrombin delivery system based on a reconfigurable DNA nanostructure exhibits promising in vivo antitumor efficacy, and the good news is that it can be applicable to tumors of diverse levels of vascularization. However, in our hypothetical design we do not include such properties, as the base of the nanorobot structure is configured with no particular strands made out of DNA.

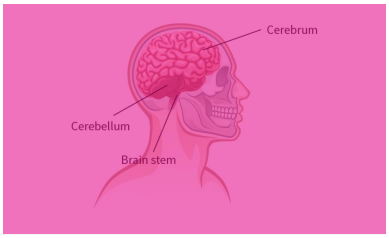


Figure 6

Cerebrum, cerebellum & brain steam anatomy and phisiology.
Source: https://www.pngkey.com/detail/u2w7q8y3w7u2y3q8_cerebellum-brain-stem-cerebrum-brain/medicine/brain [63].

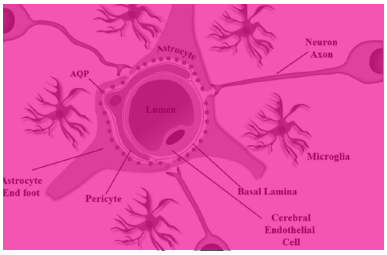


Figure 7

Schematic representation of the neurovascular unit at the capillary level. The BBB is composed of several cell types and molecules in close association, which include highly specialized and polarized endothelial cells, basal lamina, pericytes, and astrocyte endfeet, which by wrapping the microvessel walls, establish communication with neurons in the neurovascular unit. The neurovascular unit is important to maintain optimal brain function. Pericytes and astrocytes are important in barrier induction and maintenance. Microglia are CNS-resident immune cells. Adapted from Abbott (2013). Source: [120].

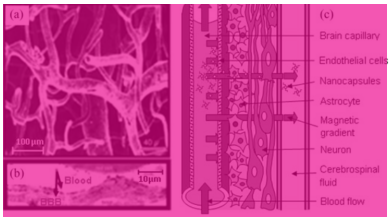


Figure 8

Nanocarriers invading the endothelial cells through the opened fenestrations. Copied from: https://www.researchgate.net/figure/a-Reconstructed-views-of-a-brain-capillary-provided-by-a-scanne r-imager-b-Electron_fig15_260519012 [60].

Brain tumors (cancer)

Cells are the most little alive structures within our body (however, we are technically prescinding from a simpler construction we comprise, which is conformed predominantly by bacterias), that's to say, they are like the "Lego blocks" our body is built with. When something goes wrong in their synthesization, cancer can be one of the main causes. Cells make up tissues, which make up organs, which make up systems, which finally form our pluricellular body in all its integrity. They take part in a regeneration process because when they die other cells are born. Sometimes these newly formed cells grow when the body doesn't need them, so that stimulates them to create a mass of tissue called a "growth" or a tumor. Brain tumors can be benign or malignant. In the first case they are not made from cancer cells and normally they are removed, and almost never grow back or spread to other parts of the body. They even do not attack nearby tissues. However, they can suppose life-threatening conditions because if they would press on sensitive areas, they could cause problems like blindness or seizures. Likewise, it is very rare that a benign tumor converts to a malignant one. Unlike them, cancer cells (malignant) are always more dangerous from the very first phases. In the worst case scenario they spread and develop metastasis. The tumors may be contained in a layer of tissue, the bones of the skull or other structures that limit them. These tumors are called encapsulated and are named after their morphological characteristics. Physicians grade brain tumors by grades, from the lowest (I) to the highest (IV) and the grade depends on the cells' properties seen under the microscope as well as on their reproducing velocity.

Cancer: a genetic disease

The word "cancer" (derives from the greek word karkinos, which means "crab" and later constituted καρκίνωμα, the term used to describe a state either of pain or ulcer) demonstrates an evil attack originated within the body. The disease denoted as cancer consists in the accelerated multiplication of certain genetically altered cells, which conglomerate in malignant tumors. Moreover, these cells have the ability of migrating to other corporal regions throughout the circulatory and lymphatic system. Furthermore, we may highlight that cancer cells differ from the healthy ones on high-speed reproducing velocity and specific membrane proteins, which act as antigens; however, when the immune response enabled is not appropriate, tumor growth increases continuously.

The field focused on antigen-expression in cancer cells and the triggered immune response mechanism receives the name of immunological therapy.

Mainly, the objective is to wait for the antibodies to destroy the tumorous cells. In order to increase the production of antibodies against the antigens of these cells, lymphocytes plus cells of myeloma (a tumor of the immune system) should be fused so that we finally obtain some hybrid cells (hybridomas). Hybridomas combine the function of producing antibodies with the proliferation of the tumor, the finality of which is obtaining a large amount of monoclonal antibodies. To note, they have given good results in leukemia and in lymphomas, although the same desirable results have not been obtained in solid tumors [95].

To increase efficiency again, monoclonal antibodies have been linked to radioactive, cytotoxic or antineoplastic molecules, which reduce solid tumors by up to 30%. By means of genetic engineering, scientists tried to make cancerous cells present a greater number of antigens, since they will be more easily attacked by the antibodies and more sensible to the cytotoxic drugs. It is from here that the idea of attacking cancer cells from astrocytoma is derived with the following antigens, already used and mentioned in the literature: anti-PD-1 and anti-PD-L1. As we have mentioned along the introduction, astrocytoma may derived into the most aggressive and most common brain tumor in adults: glioblastoma. Due to limited therapeutic efficacy of available treatments, it presents a very poor prognosis. The promising data descending from the use of immune checkpoint inhibitors (ICI) (see Fig. 9 below) in other cancers have prompted evaluation of its efficacy and possible use in patients with gliomas. However, data are not yet mature and preliminary studies do not expose a clear-cut advantage That's why we are far from excluding the concrete possibility of using ICI as potential treatment in patients with astrocytoma. Further analysis are needed to be done in order to clarify the immunological and proteomic aspects of this approach. Despite no potential benefit of immune checkpoint therapy found in astrocytoma, some practical approaches base their study on the reduction of PD-L1 expression in patients treated with temozolomide (TMZ). Moreover, the link between TMZ therapy and the control point target PD-L1 should be examined and analyzed collaterally by researchers. AutoPipe algorithm aimed to obtain a reliable RNA-sequencing data [96].

Likewise, in the following

section, thanks to the scientific explanation of Manel Esteller, we will understand the abysm that leads us from Genetics (DNA alterations) to Epigenetics.

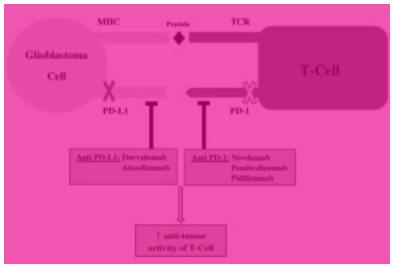


Figure 9

Interaction between immune checkpoints inhibitors that in a near future may be the evolving cutting-edge technology in regards to theranostics evaluation, apart from its currently use in immunotherapy. Definitely, the picture depicts how deep and profound immune responses carried out by lymphocytes may trigger specific antigens expressed on the glioblastoma cells, as well as in other gliomas, although its acknowledgment is forthwith premature. Adapted from: https://www.ncbi.nlm.nih.gov/pubmed/30709339 [96].

Epigenetics: the world beyond cancer expression

"We are not our DNA", postulates Manel Esteller, the professor of Genetics who imparts lectures at the Barcelona University. Furthermore, we are undergoing an enhanced revolution described as the Epigenetics revolution, because we are not only our genes expression, that's to say, our genes do not conform all we are. So, indeed, we should go beyond the path that guides ourselves into the Genetics affirmations in order to ascend to a higher level. Vital habits and multiple external factors are a complete additive above the DNA. Those tiny additions, found at the declining of molecular technology, are some chemical markers which regulate DNA's expression. Moreover, those markers are the ones that may take care of the epigenoma, the study of which opens up a whole new field of revolutionary treatments.

The revolution we were talking about has broken the determinant theories that adjudged to the DNA a sizable responsibility on our health. Science has demonstrated the fact that the most classical paradigm of biomolecular sciences has been unmounted. Therefore, DNA is not practically unalterable. During the last few decades the vision citizens had about Genetics has suffered radical changes and transitions. Currently, one might affirm that although the DNA sequence does not change, genes could be expressed in one way or the other depending on the amalgam and combination between modifiable factors, as, for example, the surrounding where we grew or the habits we had. When mentioning the phrase genetic expression we tend to make reference to genes actions, that is the moment when the genes talk to us: how

they activate themselves in order to agilitate the information processing and, most of all, its reading related to the generation of future proteins. Epigenetics' function is studying the information that may be transmitted without any kind of codification on the DNA. On one hand, the chemical markers have the capacity of regulating genes function and signal when they must express or deactivate, that's to say, silence.

In order to comprehend the distinction between Genetics and Epigenetics we can imagine a book, a vital book constituting each person, which contains all the information regarding our organism. But, we may distinguish both fields by adding the fact that they accomplish different and complementary nature labor. Genes would be the alphabet that makes us able to write the words, the sentences and, moreover, it makes us capable of the integration of intellectual tools, such as the abstraction and conceptual analysis. More concretely, DNA is written by using four simple letters, which are the followings: A, which stands for adenine; C, for cytosine; G, as for the guanine, and, lastly, T, which is the abbreviation for thymine. The diverse typologies of acid nucleic molecules are the lego boxes that may permit the design of new words and sentences in order to have a singular and unique book of life. On the other hand, Epigenetics is the style and the typography that compose the text. That's to say, the words we generated previously could be expressed in capital letters or in lowercase, in italic or bold font, in a bigger size or using a smaller one, highlighted by a chemical marker, etc. All that information not contained strictly on the DNA's alphabet is namely the Epigenetics, the one responsible for giving format and according and assigning meaning to all the words that the Genetics may write. Moreover, that code of signs and biochemical markers determines our genes. Mother Nature uses it with the finality of manipulating the data is localised on our DNA. We should note that is something trascendental because it's not the same to have written a head title to s photography note. One step further, Epigenetics is capable of making a gen express or, on the contrary, silence. Hence, its magnificent importance in Biology. As mentioned in the last paragraph, Epigenetics' occupation is monitoring changes that may occur on the uncodified information from the DNA. Those epigenetic modifications have been converted into one of the most fertiles fields of Medicine.

Epigenetic alterations can

be correlated to tumor development. That's why having the power and the knowledgeable techniques of how to actuate on those markers is the key of many doors. Doors that lead to new anticancer treatments, treatments that may defeat cancer. The first anticancer strategy begins with the idea of reverting an epigenetic marker in order to reactivate genes that protect us in front of the mass of growth. Researchers have demonstrated that in some occasions cancer occurs when making the DNA suffer a chemical transition. Firstly, this phenomenon has been related with the chemical process known as methylation, an epigenetic marker which has the ability of silencing genes trained to prevent cancer progression. In conclusion, empirical experiments on that topic and domain are undergone. These pharmacological treatments will permit to activate those genes, previously silenced by an erroneous methylation. Unlike what happens with our Genetics, the epigenetic changes are initially reversible. In the future we will be able to know which habits can help us undo unwanted epigenetic changes. At the same time, laboratories are investigating drugs that can modify the erroneous expression of genes. The opportunity to control genes. If there is an epigenetic alteration that causes a disease, this epigenetic mark could be modified to return it to the non-pathological state. Recently, it has been discovered new functionalized drugs that confront a wide typology of cancer cells, such as lymphatic ganglial tumors or leukemias. Through robotic techniques thousands of compounds are screened to analyze if they are capable of changing the epigenome. The objective is to obtain medicines that revert only the epigenetic alteration that we are interested in by modifying diagnostic tests. Second of all, one may believe that another solution to our biological complex calculus is avoiding the expression of precursor genes of malignant tumors. So, there are quantifiable genes that promote the initiation of the tumor development or metastasis. Medicine may be used in order to create a blockage on those biological pathways. At the IDIBELL centre, scientists have developed a test that analyzes the epigenome to detect colon and prostate cancer, and there are also specific epigenetic tests to predict the response of a brain tumor to chemotherapy. These are really useful when looking for a molecule that silences the gene that boosts, for instance, a type of astrocytoma. One the one hand, sleeping

cells (the ones that after waking up may produce cancer) could be controlled by stopping the epigenetic factor that propels them and produces their direction. Its success is encouraged and expected, knowing that it could permit the prevention of relapses and that it's quite possible that Epigenetics will be the new science, the science that resolves all type of pathologies. As for the third anticancer technique, researchers announce that analysis on epigenetic changes are the fundamental cases of prevention. In that case, we could detect and predict future transformations on epigenome throughout cancer. CRISPR technology is a genome-editing technique that is used to modify the expression of specific genes, and that could end up associating an epigenetic mark to the specific gene whose epigenetic defect we would like to correct.

It must be considered that membrane receptor proteins' alterations with mitogenical hormones may be related with their ceaseless division. The bridge from normal cell into cancerous cell, specified with the terminology of cancerous or neoplastic transformation, refers to different ambiental factors the majority of which actuates by altering the DNA chain. Once again, the existence of concrete genes, named protooncogenes, that with a small alteration produce the so called cancerous agents receive another definition: oncogenes, which are the ones responsible for the production of cancerous transformation. Moreover, there are other genes, the antioncogenes, or known at the present time as suppressors genes, that preserve intrinsically the capacitance of inhibiting cellular division and, consequently, establish a chemical equilibrium between the two fronts: the good ones and the bad ones, the villains. Newly, in humans have been more than one hundred oncogenes discovered and as for the antioncogenes, the future medicine, researchers have only exposed twelve antioncogenes in the literature. On the other hand, in clinical trials by using in vivo rats, it has been shown that cancerous cells are capable of provoking health tissues degeneration into malignant tumors, as if they were kidnapped and infected. This modification confirms the alteration that dominates cancer on DNA.

As we have centralised the aim of this Scientific Project on the nanorobotics advantages and possible developing treatments that may be considered in order to cure a very specifical brain cancer, astrocytoma of

both middle grades, grade II and III, the endothelial tight junctions membrane presents one of the most complexes body boundaries to cross through. Moreover, when designing nanodevices that have this determinant functionalization, the Blood Brain Barrier affects the calculus of all pharmacokinetics and physical trajectories. As a result, we may introduce in a profound manner the innate immune system of our brains, central organ, to discuss further biomedical applications of the hypothetical nanorobot confomed. Since Goldman's' discovery, the polycarbonate membrane represents one of the most appealing and glamorous challenges that one may need to overcome.

Brain tumors (cancer)

The Blood Brain Barrier (BBB) is one of the difficulties added to the treatment of brain cancer because its essential function is to protect, by developing chemistry mechanisms, the central nervous system (CNS). It consists of a capillary endothelial membrane that decides which molecules have the permission to pass through it, that's to say, it executes a pretty accurate selection between lipid-soluble molecules, pathogens and toxins, among others (see Figure 7). As for the first mentioned, the lipid-soluble molecules are allowed to cross the BBB (observe Fig.10) whereas the passage of pathogens or toxins is limited. However, this protection mechanism configures also a major obstacle during disease state since it tragically hinders the drug delivery. In recent years, interdisciplinary communities of a great variety of science branches have developed tactics that have been applied in order to assist drugs to cross the BBB, including osmotic disruption of the BBB and chemical modification of prodrugs. Moreover, nanoparticles (NPs)-mediated drug delivery conform a new approach and system for treating cerebral diseases as an effective and non-invasive technique. It is important to comprehend that NPs can cover either traditional nanocarriers or novel nanocarriers, which are emerging at the moment, such as DNA micelles. The traditional nanocarriers consist mostly of three different types of poly NPs: poly(butylcyanoacrylate), poly(lactic-co-glycolic acid) and poly(lactic acid). Additionally, these nanocarriers could be created from chemical compounds like liposomes and other inorganic systems. In the meanwhile, novel nanocarriers such as quantum dots and nanorbots will also be presented along with their recent applications within this topic: drug delivery. [20]

Hereafter we briefly specify which are the common symptoms that may indicate and alert to the patient that has a tumoral disease, and besides that, we explain the procedures the physician needs to realize in order to determine the presence of tumor growth in the CNS.

Cancer symptoms and diagnosis

The symptoms that accompany tumors mainly depend on the tumor's size, type and location. Examples could be swells or fluid building up within the skull, followed by headaches (potentiated during the morning), malfunctions and clear changes in speech, vomiting or nausea, hearing and vision difficulties, balancing or walking dysfunctions, changes in mood or personality (although they are not as worse as the ones presented in bipolar disorder), loss of the ability to concentrate, problems with memory, muscle jerking or twitching, seizures may be presented and tingling or numbness in the arm or legs.

Tumor diagnosis

In order to perform accurate tumor diagnosis three methodologies are mainly used:

Neurologic exams (checking for reflexes, muscle strength, coordination, alertness and response to pain)

CT scans (i.e. computerized tomography), that is, detailed computer generated pictures of the head based on X-ray images. The patient normally receives an injection of a special dye.

MRI images (i.e. magnetic resonance imaging), a powerful magnet and radio waves are used to take detailed pictures from inside the body; sometimes an intravenous injection with a special dye is administered to mark differences in tissues; it can be used to diagnose other diseases apart from tumors.

The biopsy procedure consists in removing a tissue to look for tumor cells, and afterwards, a pathologist should check for any abnormals (atypias) cells.

Typology of cancer brain cells

Knowledge regarding different kinds of malignant growth of cells led to a specific cataloging of their nature by respecting the origin where they start proliferating. As a result, their name is based on the organ where the uncontrollable production of cancerous cells is identified. In addition, scientists may distinguish between a few large categories depending on the

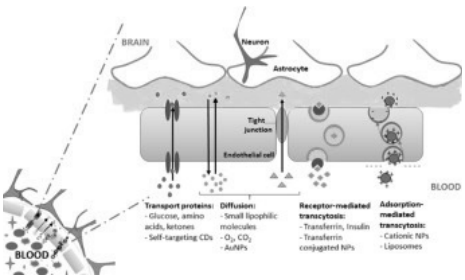


Figure 10

BBB: tight junctions of the endothelial cells. Taken from: https://ars.els-cdn.com/content/image/1-s2.0-S0168365917310829-fx1.jpg [27].

differentiate cell the cancer is made from, as we can see in the following and brief enumeration.

Cancers from brain tissue

There are different types of brain tumors, depending on the types of cells that make up their tissue, which appear in different parts of the brain. The most frequent are:

Medulloblastomas. Usually they occur in an area of the brain called later cast. This type of tumor can spread, that is, it can invade and colonize other parts of the body outside the brain.

Astrocytomas. According to their degree of gravity they are classified from I to IV. An astrocytoma of grade IV is also called glioblastoma multiforme (GBM). The lower the number, the smaller the possibility of the tumor being disseminated [121].

Ependymomas. They are classified, according to gravity, from II to IV. Grade IV is also called anaplastic ependymoma and it is the most malignant stage. They can appear anywhere in the brain and the spinal cord.

Primitive neuroectodermal tumors. They are highly malignant and behave aggressively. They can appear anywhere in the brain, but more frequently in the frontal, temporal or parietal lobes.

Hereby we will penetrate and dive into the knowledge of physiological and neurological tissue architecture revealed along multiple studies. Definitely, we will discuss scientific papers regarding one of the typologies underlined within brain cancer, which is named after the cells (astrocytes) affected: astrocytoma. In conclusion, the tumorous cells constituting the astrocytoma will be the main final localization attributed to the nanorobots postulated on this work. As mentioned before, that's only because the objective of the nanobots that will be later proposed will tend to achieve malignant astrocytes as for their target and, consequently, they will need to bind onto their surface thanks to antigen-antibody interactions, undergone at the proteomic molecular scale.

However, nothing remains still, as well as almost nothing resists in its benign stadium. That's why the situation becomes more complex

when these astrocytes are converted in malignant cells, like in astrocytoma. Around the region where the tumor is localised, intense neovascularization happens to occur, which easily contributes to the tumor growth. The vascularization [119] initiates in endothelial cells. Nevertheless, vessels will have new additional elements with the same functionality, because the cells constituting the tumor have enlarged their responsibilities. Gliomas comprise a group of tumors that originate in the brain. Although new avenues in treatments have been exposed, only a subtle progress about brain's physiology and biology has been effectuated. Astrocytes can undergo oncogenic transformation and give rise to gliomas. The mechanisms by which are fueled this kind of conversions are not fully understood [120]. Remember that group of tumors known as gliomas make reference to cancerous cells, that's to say, mass of malignant cells that may take advantage of the BBB characterizations to ensure survival and continuous growth.

The ideal suggestion was the similitude found in the morphological traits and molecular markers between macroglial cells and the rest of elemental cells composing the interior of the brain. Thus, astrocytes derive to astrocytoma; oligodendrocytes are referred to as oligodendrogliomas and ependymal cells, as ependymomas [119].

Gliomas account for 29% of all brain tumors in adults. The most attacked patients with malignant primary tumors are entering into their silver age: 64-84 years old. Nevertheless, gliomas also affect children, enumerating astrocytomas as for the most common pediatric histological types (52%). We might take into account other recurrent cases, such as:, primitive neuroectodermal tumors (PNETs), medulloblastomas (21%) and high-grade gliomas (19%). It must be mentioned that gliomas are highly heterogeneous, infiltrative and diffuse, plus they disguise different degrees of invasiveness. Definitely, they can penetrate through the brain, thus colonizing the entire organ far beyond the initial tumor mass. Despite this considerable invasive ability, gliomas rarely leave the nervous tissue in order to migrate toward other organs, remaining confined in the skull, yet was just evidenced systematic spread.

classification

The World Health Organization (WHO) has developed a system regarding the classification of brain tumors with the finality of intersubjectivity and universal community worldwide. The intention of this classification is a better resolution when administering treatment and effectuating accurate prognosis, thus a parallel improvement in healthcare of patients. Gliomas

classification has long been based solely on the histopathological features of the tumor tissues, obtained through neurosurgical resections, the first therapeutic intervention proposed. Different catalogations for posterior diagnostic criteria need to be collected, which include the morphology of the tumor cells, tissue architecture, and the immunohistological marker profile [119].
Another grading system should be included in the scientific project. Gliomas graduation is set and based on their progressive rate of aggressiveness and proliferation. There have been postulated distinctions between four grades of astrocytoma tumors, indicated by roman numbers as follows: I, II, III, IV. The lower two grades tend to be well differentiated and have only few cellular atypias. Ordinarily, they closely resemble their non-neoplastic cellular counterparts. Evidence has been provided for the existence of progressive accumulation of additional genetic alterations, which already reaffirm specific genetic mutations cited. The mechanism with which accumulation of aggressive traits regulate remains unknown, while the tumor evolves to higher malignancy grades and higher progression rates. Early stages of glioma development presents no apparent disruption of the BBB, despite what might happen in the next stages. They are not characterized by rapid endothelial cells formation (angiogenesis), which supports the fact of non-aggressiveness present, although it will aggravate as glioma progresses. The tumor itself is sustained by normal brain vessels. Nevertheless, gliomas can also give rise to slow growing tumors that alter the normal afflux of blood through the BBB, which is crucial. On the other hand, higher-grade tumors (starting with grade III) are anaplastic, demonstrating elevated vessel density, cellular atypias, high mitotic activity and signs of increased cell density [121].

As we arrive to the last acknowledged grade of astrocytoma, we can inform that tumors under this perspective receive the name of glioblastomas (multiforme). To continue with extensive precisions, two forms of GBM have been identified: de novo GBM, the most frequent, and secondary GBM. The first one results as the most frequent reported along the data, whereas the second one configures the recedivation of a preceding low-grade astrocytoma. In general, gliomas are underlined for being, aside from the end of glioma's evolution, an extremely aggressive tumor, formed by a multitude of cells type, including stem cells, which develop and migrate to healthy adjacents regions of the brain guided by the different prolongation of the existent blood vessels. The disruption of the chimeric and fragile vessels in the tumor mass resulting in peritumoral edema is also led by the uncontrolled and fast growth of cells.
By the time glioma progresses and rapidly grows, endothelial cells derived

from normal vessels are roughly separated from the main structure and form new angiogenic spots associated with the tumor site. Thus, the tumor will be nurtured. As these cells must undergo migration in order to arrive at the tumor site when forming new vessels, they disrupt normal vessel structure. Because tumors secrete many different substances that alter the normal microenvironment, one of the subsiting processes is damaged. Endothelial cell migration is impaired, which will be crystallized in the vascular architecture. As we may know, when suffering from glioblastoma, there are some morphological traits that induce alterations of blood vessels and involve the formation of fenestrations and tight junctions disruption. Besides, alteration may be visualised on the thickness of the basal lamina. To note, perivascular space is increased as well as the number of pericytes associated to the vessels. The disruption of the BBB can be detected through magnetic resonance imaging (MRI) thanks to a contrast medium (CM). The standard CM used is gadolinium, which under normal conditions is not able to cross the intact BBB, but since there is a disruption of the blood brain barrier, gadolinium can diffuse into the tissue and ring enhancing lesions are often perceived [119].

When this phenomenon occurs, novel nanocarriers should take action, as the complexity of the BBB is still unclear and we haven't knowledge of how the synergy in that cases will affect the nanobots behavior. Or at least, modifications during the reconfiguration and reevaluation phase of nanorobots' functionalization should be addressed. It might also influence their directionality, effectiveness and consecutive immunological responses, leading to a bit more than nano chaos. Additionally, we could experience that further control of the chemoflux of soluble substances within the disruptions already formed opens a new pathway. A verge where new possibilities of orchestrating the crossing of the BBB arise.

Treatment methods

Once the doctor knows the type and the stage of the tumor, he can decide which strategy to use from the existing ones: chemotherapy, radiation therapy or surgery. Some of the patients may be treated with a combination of them and whatever the stage is, the patient has access to drugs that control pain, side effects or ease emotional problems. This kind of treatment is called supportive or palliative care.

Surgery
The usual treatment for most brain tumors, when the skull is opened, is named craniotomy and is performed under general anesthesia. The patients that cannot have surgery (from several reasons, like age, for example) should address other treatments.

Radiation treatment
Uses high-energy rays like x-rays, gamma rays or protons, to kill tumor cells. The schedule on this treatment (usually takes only a few minutes) depends on the grade of the tumor and the age of the patient.

Chemotherapy
Consists of delivery of drugs to kill the cancerous cells. Unfortunately, it's a large duration treatment that takes over some weeks and, as a side effect, it also produces the death of healthy tissue.

To treat and find a solution to our problem statement these methods are not always effective because of their lack of sensibility and selectivity. That's why we introduce the new emerging field of nanorobotics above the typical ways to treat cancer, which would follow the order used to explain the section. That's to say, first of all, the first attempt of treating a cancer is by having an operation (speaking of an operable one, if not, we skip to the next phases) and the next step would be to follow a radiation or a chemotherapy treatment, depending on which is the extent of the mass of cancer cells. When cancer cells being focused and concentrated within a concrete space radiation treatment provides a precision that can narrow its focus to what it is intended to treat. On the other hand, if the astrocytoma (cancer to be treated, target) is distributed randomly and has already been metastasized, the technique most commonly used is chemotherapy. However, these approaches are not as effective as the nanorobotics we are willing to implement because researchers haven't found the correct path to only affect diseased cells, since healthy tissues degenerate parallely. Interestingly, when talking about the treatment of brain cancer, there are some challenges to go through. That's why it's important to create and propose a nanorobot that would present the following basic functionalities: detecting the abnormal cells (target), binding to the surface of the brain cancer tumor and, finally, achieving the drug delivery within the brain by crossing the Brain Blood Barrier.

Properties of nanomedical nanorobots

In most examples, nanorobots for medical applications have in common the properties we are going to postulate below. From the medical point of view, three microns is practically the upper limit for micro/nanorobots. If bigger they would block the capillary flow. The exterior cover of the nanorobots (usually made from diamond) has to be smooth and flawless as this prevents leukocytes' activities since the environment is chemically inert and has low bioactivity. Nanorobots can perform autonomously actions or, in some other cases, be remotely operated, hence the communication with the doctors. Communication between nanorobots and doctors may be performed mainly via acoustic signals at carrier wave frequencies of 1-100 MHz, and the doctor's orders could reach the nanorobot's acoustic sensors and then the robot should implement the orders/stimulies. They could also work in response to environmental stimuli and be programmed according to principles that produce macroscale results. Replication is a crucial capability for molecular manufacturing and it should be restricted to in vitro laboratory. Replication, however, can be dangerous because it could get out of control, as it happens with bacteria. The nanorobots should definitely leave the body after finishing their work in order to prevent malfunctioning.

Chemical sensors

A silicon-based chemical and motion sensor array with a two-level system architecture hierarchy has been successfully manufactured. Through the use of nanowires one can decrease up to 60% of the cost of the energy demand for data transfer and circuit operation. The array of nanowires can drastically reduce self-heating and thermal coupling for CMOS (Complementary metal–oxide–semiconductor, technology for constructing integrated circuits) functionality. Some of the advantages are the factor of low energy consumption and high-sensitivity. To further advance manufacturing techniques Silicon-On-Insulator (SOI) method has been used for assembling sub 90 nm circuits.

Energy supply

Remote inductive powering has been used both for RFID (Radio Frequency Identification Device) and biomedical implanted devices to supply power on the order

of milliwatts. Nanocircuits with resonant characteristics can operate as a chip providing electromagnetic energy supplying 1.7m at 3.3V, without any significant losses in transmission. Energy receiving works perfectly in inactive modes and they use RF signals to do so. As for communication and data transmission in liquid environments: acoustic, light, RF, and chemical signals may be considered as possible choices. The chemical signals are useful for nearby communication among nanorobots and for teamwork coordination. Acoustic communication is more appropriate for longer distance communication and energy detection rather than light communication. Although optical communication permits faster rates of data transmission, its energy demand makes it not ideal. Mobile phones could allow for continuous medical monitoring (follow-ups). They use electromagnetic radio waves to give the current status of nanorobots inside the patient that work thanks to CMOS sensors embedded in the nanorobot, which enables sending and receiving data through electromagnetic fields. Information can be sent back by wave resonance.

Implementation

Nanorobots' sensory capabilities allow them to identify the nearby possible obstacles in their environment as well as the biomedical target for their task, for instance, they could detect a very selective type of cells by markers.

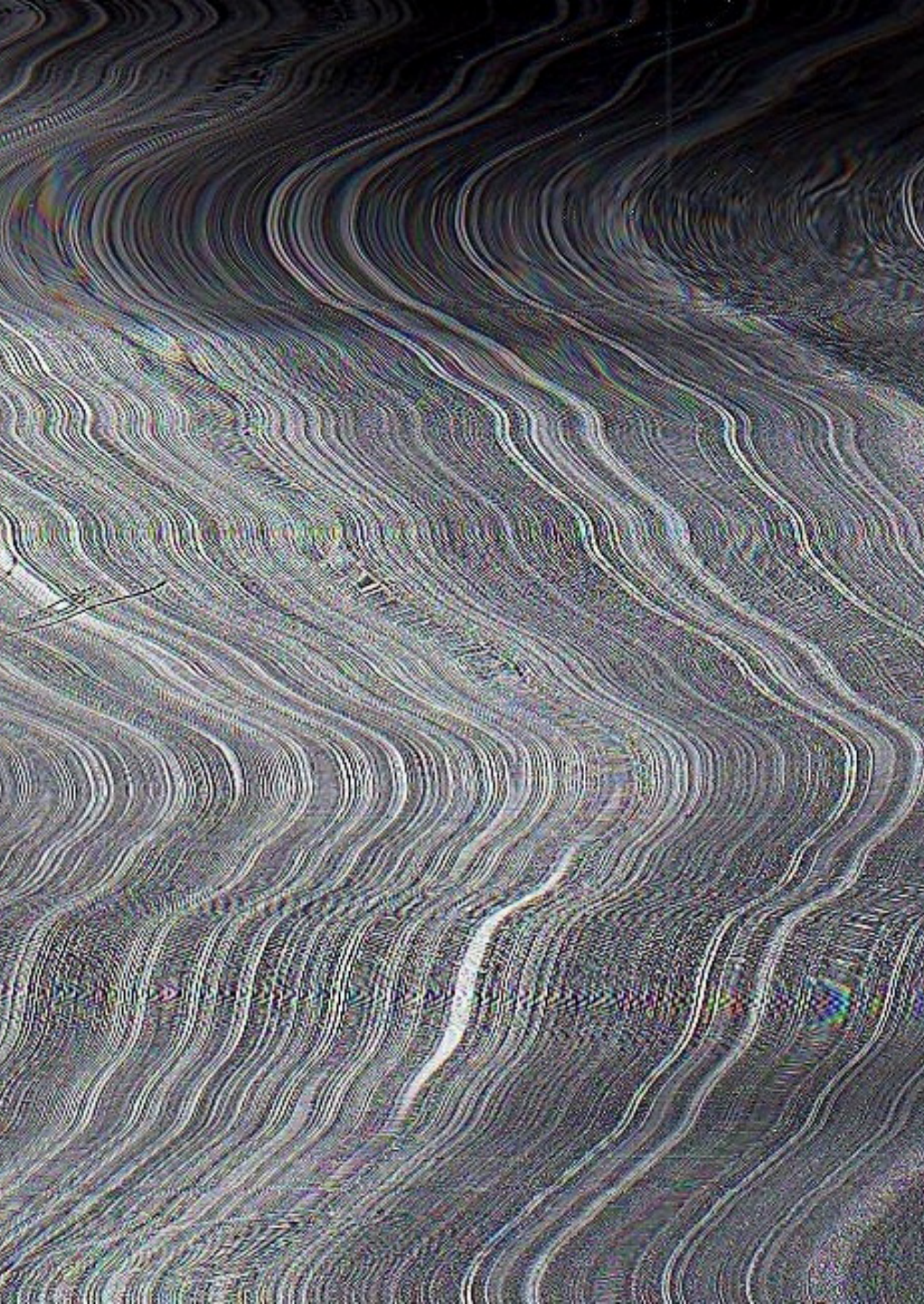
Delivery

Fully operational nanorobots for biomedical instrumentation should be achieved as a result of nanobioelectronics and proteomics integration (as shown by Adriano Cavalcanti from the Center for Automotion in Nanobiotech). Nanorobots take chemical and thermal changes as interaction choices for in vivo, and, as explained previously, the mobile phone controlling using RF is adopted because of its effectiveness, assuring both communication and energy supply. Nanorobots can be trained to distinguish between different cancer cells by their surface antigens, one percentage for each cell. Another approach would be to reach decentralized control for a distributed collective action in combat of cancer. Specifically, sensors can be programmed to detect different levels of *E-cadherin beta-catenin* (important role in maintaining epithelial integrity) whether they are in primary or metastasis stages. Thus, a higher gradient of signal intensity of E-cadherin is used as a chemical

parameter identification in guiding nanorobots to identify malignant tissues. The main goal when approaching cancer treatment is to target only the abnormal cells and not to destroy the healthy ones.

A recently proposed methodology consists in introducing the drug Photofrin into nanoparticles. Photofrin (also called sodium porfirmer) is mainly used for photodynamic therapy medication, which is utilised to treat some types of cancer. The process to perform such treatment needs light exposure as catalyst, that's to say, in order to activate and make the drug destroy cancer cells, Photofrin needs to be previously absorbed by the malignant cells and turned on with light. What happens? Photofrin is drawn through the bloodstream to tumors. A special laser is used to activate the drug, which collapses the blood vessels that feed the tumor. Without its blood supply, the tumor starves. An injection of 1 micron could hold 0.5 cm 3 nanodevices. Even more, virtually, such nanites are smart enough to show up at the correct group of cells that are targeted for destruction, so it seems 100% efficient. Onboard, levels of chemical agents can be checked to prevent overdose. Without any doubt, one of the biggest breakthroughs of nanotechnology would be tackling pain - the bitter side of cancer therapy.

Overall, we have presented the basic information and properties description one needs to acknowledge before proposing a new nanorobot design, as for instance the communication in order to implement and reprogram orders via acoustic signals ranging from 1 to 100 Hz, the scope of which is to transmit information as faster as physical laws permit by preventing huge energy demands in a such small decreased-scale device. The energy supply is generated by nanocircuits that are built based in a remote inductive powering of RF. Operational nanorobots can be remotely controlled using an electromagnetical field, and still comprise the fundamental energy supply. Moreover, the array of nanowires helps us decrease the self-heating and the amount of energy demand. Ideally, novel approaches propose the insertion of other manufactured drugs or chemical molecules into nanorobots that could be activated by using a special laser and, therefore clog the vessel by sprinkling the drug into small particles.



Hypothetical design

Our hypothetical design proposes a new concept of nanorobots. The key point of our design is the combination of the best and most compatible characteristics of nanorobots. We take advantage of previously discussed ideas that could be put in a new light as technology has advanced in the meantime. Advances in nanomaterial science let us imagine compositions that were not possible in the past. That's why, when the field of nanotechnology had amplified its background into nanorobotics we moved one step further from the nanoparticles and discussed whether we could implement it within the biocompatible and biomimetic nanorobots. Why nanorobots and yet not improving nanoparticles? This field didn't set aside the nanoparticles, but it is questioning the feasibility of some chemical engineered particles versus some nanomachines that could be remotely controlled and lead to higher precision. For this reason we have investigated theoretically which characteristics would be the more reliable when treating the brain cancer and how could we disrupt the BBB in order to deliver the drug into the targeted cells. The main idea is to use antibody interactions embedded on the surface of the nanorobot to recognize specific sites of tumors that express mostly one protein, but with a particularity added. This makes reference to the new part, where antibodies coated onto the surface of the nanorobot are their guides, that's to say, there is an equivalency between the indications and interactions of the antibody and microenvironmental stimuli, and the kinetic motion of the nanorbot. Imagine a red tail catfish (Phractocephalus hemioliopterus). It is a unique animal because we can distinguish it from others due to its nocturnal activity. Its movements and actions take place because it has some whiskers in its frontal part, which act like tactile sensors. Then, what if we could achieve the same efficiency as the catfish in our nanorobot by designing two different parts? On one hand, the correspondent of the fish body would be our nanometer size structure. On the other hand, the equivalent of its whiskers are the antibodies.

To conceive the whole nanorobot design it is important to take into account 5 phases which will be presented hereafter. Before adding anything else, we should specify the importance of having the finality of the robot crystal clear, which is targeting and destroying the cancer cells placed beyond the Blood Brain Barrier (astrocytoma). The endothelial and thin barrier is what makes the destruction of cancer masses more challenging, therefore the argument for creating nanoscale robots. Afterwards, the first grade we need to establish is the nanorobot's shape, where we could add the fact that it has to be dynamically effective with the microenvironment in which it navigates. Hence, the spherical shape. As a second step, we need to discuss the materials it has to be made out of in order to maintain its biocompatibility within the body. The best candidate for this seems to be, undoubtedly, the diamondoid structures (thanks to techniques such as DMS) that can be transformed into flawless surfaces. Another key factor is the cooperation between nanorobots, thus a swarm of nanobots is practically essential in combating cancer. The main reason for this is the shortening of the period of time the intervention needs, although, as the number of robots increases, the complexity of the control and dynamics augments considerably. Communication is a basic ingredient in order to equilibrate and keep in balance the interactions inside the nanorobots network. Acoustic signals are the best candidates for communications inside these systems, even though the rate of transmission is faster via optical waves communication. An analysis of the powering system of the nanorobot reveals that, ideally, magnetic components incrustated on the nanorobot's core would compose the optimal robot's motor. As a source of energy excitement (hyperthermia, drug-release) magnetic gradients are most promising, therefore the use of the MRI technique. Finally, each robot has a purpose. In this case, our hypothetical nanorobot's function is to detect, target and bind to the surface of the growth (astrocytoma grade I, II) and, most importantly, to cross the Brain Blood Barrier made of tight junctions of endothelial cells.

In the following sections we will consider into detail each component of the nanorobot. An analysis will be done for every main characteristic of the hypothetical proposal we have made. The characterization exposed is meant to clarify and dive "nanometrically" into the nanorobot's description we have pointed out before.

Nanorobot spherical form

This form is presented as a good option mainly because of its advantages for the dynamics function inside our body during the travel through the bloodstream. The spherical shape maximizes the volume for a given surface and assures low-contact surface when propagating through tumors and blood microenvironments. This shape comes as natural by observing existing blood cells: monocytes, basophil, neutrophil and lymphocytes, among others (observe the Fig. 11 below).

justification

A quite recent study [13] mentions a hypothetical red blood cell, called respirocyte, made out of 20 billion atoms, with a surface covered by diamondoid, presenting reversible selective pumps and the geometrical shape proposed was a bloodborne spherical one.

Colony of nanorobots

The use of colony has the advantage to prevent immunological attacks, like for example phagocytosis, which means the ingestion of bacteria or other material by phagocytes and amoeboid protozoans. If there is a swarm of nanorobots, as shown by Lewis et al. [7], simulations reveal that nanobots are behaving in a decentralized way. This makes us remember the same mechanism that bees present in a more deeper way.

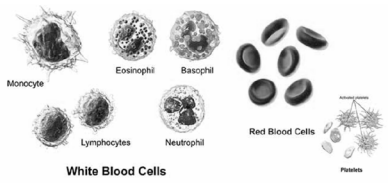


Figure 11

Blood cells types: monocyte, eosinophil, basophil, red blood cells, platelets, lymphocytes and neutrophil. Source: www.microbiologyinfo.com [64].

Once more, mother nature seems to have responses for all if we decide to listen to her. That can lead us to a pretty accurate designed-shape configuration for our robotics system. The strategy consists in having chemical substances that attract a swarm of nanobots, like a magnet would do, in a gradual process such to achieve total elimination of diseased cells. All that, plus their real aim: treating endogenous diseases of the brain, detecting the cancer cells, and finally elimination has to be adjusted with the cooperation between them like in a flock of birds.

In order to evaluate the technique globally, various configurations (on simulating softwares) of cancer are considered with:

- Cancer cells were in an aggregated form, that's to say, very condensed
- Cattered, when cells are equally distributed inside the body
- Isolated, that cells were independent from others and distributed randomly inside the body part

This cooperation makes reference to the release of acoustic waves to announce success through acoustic signal based communication.

Magnetic nanoparticles (MNPs): cargo carrying therapy

Medical constraints are compatible with the delicate and dedicated function that our robot must do: crossing the BBB by targeting antigen LRP-1 membrane receptors and traveling between endothelial cells junction. Our aim is to transport the nanorobots through local and active recruitment and via magnetic excitement of magnetonanoparticles on the (dysfunctional) endothelium. Afterwards, when the nanobot comes closer to the biomembrane cell surface and arrives at its target, it may perform site-selective delivery of the drug carried.

Magnetic field

Saadeh et al. [13] mention the current proposals and designs in a review paper regarding the nanorobotics applications in medicine. Their review represents the starting point from which we could infer the general characteristics a nanorobot

with medical applications may need. Many of them have already been explained in the previous parts: the diamondoid, smooth and flawless cover as biochemical prevention, and the transmission of the little carriers though liquid environments thanks to acoustic, optics or radiofrequency. Whether we choose one or the other, it is obvious that some physical parameters will change.

We will be using an electromagnetic field in order to energize magnetic nanoparticles (MNPs) embedded in nanocarriers having as a propelling force the magnetic 3D gradients superposed on high homogenous magnetic fields like Bo field [66] or MRI (Magnetic Resonance Imaging) scanner one. Inside a homogenous field, magnetic nanoparticles become saturated and steer towards a target anywhere in tissue using small shifts in gradient field [9].

To overcome the diffusion of the BBB we need to use MNPs because they allow the crossing phase with a therapeutic cargo. By using localized hyperthermia, i.e. if we focus heat induced by an AC (Alternating current) field, we won't compromise the navigation capabilities and we wouldn't step out of the compatibilities in medicine. In the presence of a gradient field of a modified MRI, the carriers (nanorobots inside of which molecules and aggregates of MNPs with high magnetization saturation ingrained inside the biocompatible and biodegradable polymer) would be identified as the mediator. These MNPs are designed to become magnetically excited and to release energy: they tend to release heat to the surrounding zone. Therefore, inside the brain's microvasculature, such increased temperature may disrupt the BBB and enable nanorbots to target astrocytoma cells. Overall, radiofrequency of electromagnetic waves should be used to stimulate the MNPs. The generation of heat by the MNPs has been previously studied and shown to be caused by the Neel and Brownian relaxations [24].

Moreover, Hamdi et al. [5] explain how the immersion of the nanoparticles or carbon nanotubes should be overcome in order to penetrate inside the cancer cell. The nanoparticle should be spontaneously adsorbed/embedded

onto the membrane, by deforming it. As a consequence, the endocytosis is an independent-mediated process, which includes a floating phase, a penetration of the lipid headgroup into the area and, finally, the rotating into the membrane core as a nanoneedle like process. When extrapolating such biomechanism to a living machine, we may take into account the explicited phases in order to contour the actions nanorobots should perform.

Theranostics

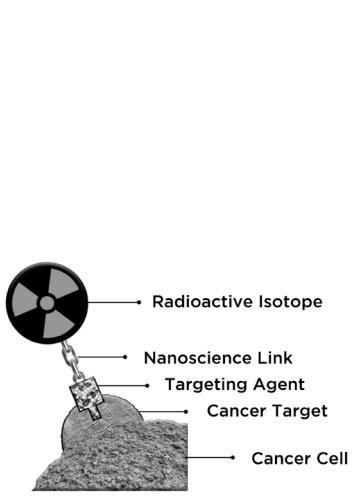
Combination of specific targeted therapy and diagnostic tests emerges as a new subdiscipline of nanomedicine, theranostics. Thus, it has been determined that the cascade of theranostics contributes to the transition bridge toward a much more personalized medicine approach. As a result, scientists may have now another paradigm to solve: the union between diagnostic and therapeutic applications with nanoscience apportations (see Figure 12). Altogether, it is meant to be an offer for the right treatment at the right time directioned to the appropriate patient. Its scope is to create a single actuator agent: allowing for diagnosis, drug delivery (efficient pharmacotherapy) and treatment response monitoring, at the same time. It's worth mentioning that this field [109, 112] uses the intrinsic and specific biological pathways in the human body. So, overall, theranostics is the equivalent of designing the amalgam between three key features: therapeutic, diagnostic and nanoscience [110]. Likewise, the hypothetical design of our nanorobot aims to combine the bifunctionality contributed via theranostics, plus the use of MRI methodologies and their intuitive guidance applications [111, 113].

Acoustic signals: ultrasound signals

As a general remark, we have observed that one can achieve very good communication with the nanorobots via acoustic signals. However, because it is something that will penetrate neurological zones, but not even that, just for the simple case of getting inside a tissue with a variety of biological functions, we should impose certain limitations. To begin with, a scale of 1 to 100 MHz.

Furthermore, this type of communication is more appropriate for longer distances than the optical one. Secondly, it is of greater use for energy detection. Some of its advantages are: low energy consumption and high-sensitivity. Combined with the using of nanowires once can decrease up to 60% the energy demand for data transfer and circuit operation. And besides that, it could also reduce self-heating and thermal coupling.

As we have already mentioned, the respirocytes [13] are thought to work by using acoustic sensors in the nanorobots hull, receiving ultrasound signals. Additionally, we have brought to the reader's attention a work explaining in much detail a technique born from a first rank observation, the observation of the nature itself and its biological systems [7]. The strategy used when making the logical calculations for the behavioral algorithm is based on the so called concept of Nanobees [7]. These nanocarriers interpret one of the most seen and represented insects in our culture and our living metaphor: to be a constant and persevering worker. Their coordination leads to perform some specifics tasks. One can make future improvements to the model presented, one of them being the reducing of both chemical and acoustic messages rate, as we should keep the acoustic contamination inside the body as low as possible.



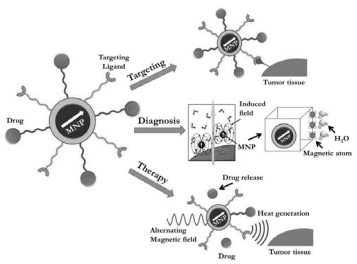


Figure 12

Little schematic that represents the amalgam drawn by a new pathway of medicine approaches, such as the combination of diagnostic and therapeutic approtations toward the nanoscopic world. The photography next to the left one is supposed to simplify such methodologies as for magneticonanoparticles (MNPs). Copied from: http://theranostics.com.au/what-is-theranostics/ & https://anuguleria.wordpress.com/research/multifunctional-magnetic-based-theranostic-nanoparticles/ [5].

Molecular mechanosynthesis technologies: diamondoid mechanosynthesis (DMS)

A smooth and flawless cover in nanoparticles [11,13] is a required feature because aerodynamics and kyneticodynamcs should be studied in a precise manner in order to prevent other molecules and cells from the outer environment to impede nanorobots propagation. The main purpose is to avoid leukocytes activities, and that's why researchers decided to use a base of diamond for the nanorobots manufacturing. To implement them the quantum ab initio and density-functional computational methods are used, showing once again that bioinformatics and technology have high impact on this project. Diamondoid mechanosynthesis builds models from diamond structures, atom by atom, this being the molecular feedstock, the skeleton of our nanorobots.

First and foremost, diamondoid materials include pure diamond. Diamondoid is the crystalline allotrope of carbon that is perhaps the strongest substance known. In addition, diamond materials also may include any stiff covalent solid that is similar to diamond in strength, chemical inertness and also presents a dense three-dimensional network of bonds.

In order to build complex mechanical diamondoid nanostructures in macroscale quantities at low cost the development of a new manufacturing technology - called positional diamondoid molecular manufacturing - was developed [23].

> *Consideration of mechanosynthesis and further analysis*
> Mechanosynthesis is defined as a term for hypothetical chemical syntheses. Chemical reactions outcomes are strictly studied with respect to technology and reactive molecules directioned to specific molecular sites. Currently, this technique is still not used in biological fields, as achieving this aim in their biological chemical syntheses hasn't been yet recorded. The pillar for this design construction begins with atomic placement in specific molecular sites, which is only featured with scanning tunnelling microscopes [101].

But how did we dive into such ideas? Which was the real motor of the perpetuated evolution? Regarding single atoms motion in a pure mechanical way, Eric Drexler was the scientist who proposed such biochemical models. To elaborate, this futuristic idea was presented in his pretty famous book: The Engines of Creation, 1986.

We usually, conventionally, comprehend chemosynthesis as stochastic phenomena, where reactive molecules encounter and bind to ligands or whatsoever through thermal variabilities. The theory of molecular kinetics may be of help when understanding the processes and molecules distribution in mother nature as well as physical states transitions. Hypothetically speaking, the process of mechanosynthesis means that reactive molecules would be attached to molecular mechanical systems, and their interactions would result from mechanical motions. Sequentially, molecules would be brought together in planned orientations, positions and sequences, like a minucius pattern. Keeping potential reactants apart would be the programmable solution as for unwanted reactions avoidance. In other cases, when we would seek for strongly desired reactions, we would confidently hold reactants together in optimal stable orientations for many molecular vibration cycles. The ribosome itself provides an example of a purely and synthetically mechanosynthetic device [101, 102].

This technique has opened new verges on the domain of futuristic models and hypothetical designs as for the implementation of nanodevices in nanomedicine. Whether we perform the action of removing or adding hydrogen atoms and, just after that, depositing carbon atoms, we are following the phases indicated in the theoretical work of diamond mechanosynthesis (abbreviated as DMS). This technique's claim is to enable diamondoid fabrication [103]. Freitas and Merkle et al. [105, 106] have contributed on numerous studies regarding this line of research, thanks to their persistent effort. They have reported that the most studied tooltip motif (DCB6Ge). Due to possible misplacement errors during the

performance of translational and rotational establishment, maximum acceptable limits were reported on several papers [107, 108].

Biological filters

From a biological point of view, it has been restricted that nanocarriers must be larger than the first tens of nanometers. The reason for this is because our natural biological and corporal system do not permit other passive pathways. That's to say, biological filters in our body remain primordial when designing a hypothetical nanorobot. In order to avoid this kind of processes that take place, for instance, in our kidneys, we should first approach this organ's structure and justify the argument planned. As it has been investigated, the glomeruli [98] is a wide web of tiny blood vessels that receive the name of capillaries, located within the Bowman's capsule [100]. These capillaries are coated by a thin layer of cells, the endothelium, the structure of which enables the filtration of the blood components and, furthermore, determines urine formation. In addition, it's worth noticing that those capillaries are fenestrated, meaning that they contain minute, let's say around 2-3 nanometres of diameter, fenestrations [99]. Hence, clear determination on using carriers for drug delivery ranging from one hundred or two hundred nanometers is revealed, because we would rather prefer for them not to be cleared by the biological system of the patient.

Hyperthermia

Experimental studies [5, 9, 59] show that morphological changes in the monolayer lining of microvessels could be used in hyperthermia. More concretely, in order to loose tight junctions between endothelial cells and let the nanorobots travel through them, producing possible fenestrations of degraded microvessels is of our own benefit. Furthermore, the methods currently used to microwave the body (as , for example, in trials with rats) affect the elements of the neural system, such as neurons, astrocytes, vessel wall cells as well as other glial cells. In addition, heat dissipated to ambient vessel cell walls can generate thermal stress, which is something wanted because it could be the perfect opportunity to

open the crossing tunnel from one side to another. This opening tunel can be a consequence of the thermal conduction generated by an electromagnetic field. On the other hand, it is a well known fact that the monolayer of the endothelial cells' lining are affected by the kind of stress they are exposed to. In order to assure the functionality of the nanorobots in these scenarios, the key parameters should be considered, as any disturbance of the BBB can cause: vasogenic edema, energy metabolism failure and subsequent structural brain damages. Moreover, the physiological changes in the vascular system are dependent both on the temperature and the heating duration. That's why dosage, in conclusion, has a crucial importance.

Quantum cellular effects

Pharmacytes [11,13] have been known as cutting-edge nanorobots because of their characteristics. In their hypothetical design quantum ab initio and density-functional computational methods were used there. Moreover, they would be produced by diamond mechanosynthesis.

The nanocarriers present overall stochastic-like characteristics as: Brownian forces [24,25,26], quantum cellular effects, and either steric and electrostatic repulsion mechanisms [67]. Quantum cellular effects appear for the simple reason of dealing with objects as small as a nanorobot. The quantum physics aims to give an answer to the questions from an atomic point of view. Otherwise, we would be intending to cross the brain blood barrier with magnetic nanobots without understanding the interactions between atoms at such a small scale. The nanometer scale implicitly rises the complexity of the problems in discussion. Therefore, something as small as a nanorobot may combine both characteristics, which are behaving at the same time as a corpuscle and a wave. Hence, we could extrapolate the fact that when talking about such decreasing scales, its point of mass becames then the perfect representation of the object responsible for transmitting energy along the wave.

Active targeting via antibody interactions: recognition specific sites tumors

Recent studies show that the nanoparticles could be attached to the cancer specific protein [1, 3, 4, 11], each cancer having its characteristic protein. There is a common effort to have a table cataloging each cancer to its most expressed antibody. The cancer antibody could be fixed with contrast agents to make it visible on the MR.

Nanoparticles and nanorobots can be coated with targeting ligands as, for example, peptides antibodies, aptamers, lecithin-carbohydrates, or antigen molecules. By doing it, endogenous antibodies are used to facilitate intracellular drug delivery. Antigen-antibody and ligand-receptor interactions are being exploited by molecular recognition tumor cells in order to efficiently target the nanoparticles, drug delivery vehicle, to the tumor core. This mechanism contributes to the biocompatibility of the nanorobot.

As the above mentioned approaches prove to be safe and immunologically inert, we intend to insert very precise type of ligands on the nanorobot surface, depending on the cancer cells we are treating. Being immunologically inert also means that there is no significant impact on cytokine levels and no observable cytotoxicity. On can check the ligands antibodies from astrocytoma cell surface by doing a liquid biopsy and distinguishing which are the most expressed chemicals. This could make reference to the personalized treatments arising nowadays.

BBB

Becauseof bloodglucosemaintenanceat4to7.8mM, thereisahighlevel of glucose substrate available within the blood. As we may know, our brain is glucose deficient. Additionally, brain metabolism requires high levels of glucose, and glucose transporters are well known to be overexpressed on the BBB. Hence, it is not farfetched to consider that blood glucose has a positive gradient toward the blood wall and a favorable distribution within the brain. Recently, researchers have demonstrated that conjugation with peptides that target the LRP-1 receptor is feasible. This receptor is overexpressed at the BBB, and it is associated with a transport mechanism known as transcytosis. By targeting this pathway, we can

deliver large macromolecules to CNS resident cells. Moreover, why couldn't we use this process in order to target our desired unhealthy and cancerous cells? In terms of biology, we must then acredit the fact that antibodies explicited in immunotherapy studies may be the next ones to take action so that the swarm of nanorobots could be able to continue their drug delivery trajectory until astrocytoma cells are captured. Definitely, last but not least, we note that our belief is that anti-LRP-1 may configure the protein characterized in order to functionalize the diamond surface of the colony of nanorobots. Thus, taking profit of their "gold" and primordial overexpression, the nanobots will bind onto the BBB for further biomembrane penetration [97].

justification

Finally, use of chemotaxis to augment biological barrier crossing has been enabled by minimal transformations in polymersomes and, therefore, a new enhanced carrier has been developed. This is proved by augmenting the delivery across the BBB, as it has been demonstrated an increase of almost fourfold in the amount of polymersomes gaining access to the brain parenchyma of rats compared to BBB-targeting facilitated by nonchemotactic polymersomes. This is a heavy finding [97]. That's why we envision next research approaches being a completely new trend in the design of drug delivery systems embracing the new advances proposed. In our hypothetical proposal, the nanodevice engineered is supposed to cross the membrane thanks to induced excitement of MNPs and subsequent release of thermal energy [so that the BBB may generate tiny (reversible) disruptions]. All of that, after binding to the LRP-1 receptors of the membrane.

Astrocytoma

As for the targeting immune checkpoints, we should attribute the fact that some molecules from our corporal system play a key role in checkpoint regulation. The most-widely investigated are

T-lymphocyte antigen 4 (CTL4) and the programmed death-1 (PD-1) protein, already denoted their bioavailability according to FDA's standards, as displayed in Figure 13.

Likewise, ipilimumab (anti-CTLA-4) and pembrolizumab (anti-PD-1) have been approved by the US Food and Drug Administration for the treatment of advanced melanoma. New emerging advances are currently pushing their contribution regarding malignant gliomas.

Furthermore, they developed a synergistic immunotherapy strategy (contemplate Figure 13) that locally targets the immunoinhibitory receptor PD1 for the treatment of melanoma through a microneedle-based transcutaneous delivery approach.

Chemotherapeutic medicament encapsulation

As for the drug that we desire to be releasing we will use the current gold standard of care for astrocytoma, which is Temozolomide (brand name: TMZ) (visualize Fig.14 beneath) [114], an alkylating agent used as a first-line treatment for astrocytoma, whereas

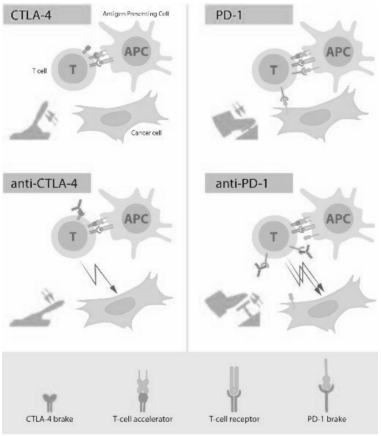


Figure 13

The principle for immune therapy. APC—antigen presenting cell; CTLA-4—cytotoxic T-lymphocyte antigen 4; PD-1—programmed death 1. Copied from: http://theranostics.com.au/what-is-theranostics/ [5].

the most common chemotherapy drug combination is PCV (procarbazine), lomustine and vincristine. It's given as an oral pathway drug. If therapeutic cargo drug delivery was about recurrent glioblastomas and other types of gliomas [115, 117, 118, 119], we should take into account the possibility of administering bevacizumab. According to some papers, bevacizumab focalizes on the signals that the cancerous cells may have send to the corporal system, the ones that provoke the posterior generation of sanguineus vessels in order to assure their nutrition. In conclusion, the chemotherapy medication decided to conform the nanobot will be placed at the device's nanocore.

justification

Hortelâo et al. [89] investigated the drug loading and releasing capabilities of nanoparticles in order to determine whether their enhanced diffusion, due to urea availability, relates with enhanced drug kinetics. Their model chemotherapeutic drug used was Dox (from the family of anthracyclines and antitumor antibiotics which prevents replications and thereby arrests the cells cycle) [116]. Their loading capacity and drug entrapment efficiency, that is, the drug content and the percentage of drug successfully encapsulated in the nanoparticles is, consecutively, as denoted:

$$\text{Drug Loading } (\%) = \frac{\text{Mass of Drug Loaded}}{\text{Mass of Nanobots}} \times 100$$

$$\text{Entrapment Efficiency } (\%) = \frac{\text{Mass of Drug Loaded}}{\text{Total Drug Mass}} \times 100$$

The same relating equations should be used when developing the drug encapsulation and the subsequent analysis for entrapment efficiency inside the nanobots diamondoid surface. One of the disadvantages we may found on these days is that our nanorobots are not enzyme-powered, which causes, sequentially, a leak of enhancement with respect to the release from cavities encountered. In the referenced group from above, the enhancement of the drug release from the mesoporous cavities was attributed to the increased diffusion of the nanobots in the presence of urea.

Our hypothetical proposal consists of a nanorobot with spherical form because this shape has been found as natural by observing the already existing blood cells in our circulatory system. To create a biocompatible and completely inert nanorobot we had to cover its surface by diamond performed with the DMS technique. Cooperation between the colony members would be taken place thanks to a decentralized way of behaviour. One should envision the fact that nanorobots will hide magnetic nanoparticles within its core, yet concerning its posterior consequence during kinetics movements and MRI guidance. The dimension of which should be ranged around of tens of nanometers. The scope of which is to allow the reversible disruption of the endothelium by letting

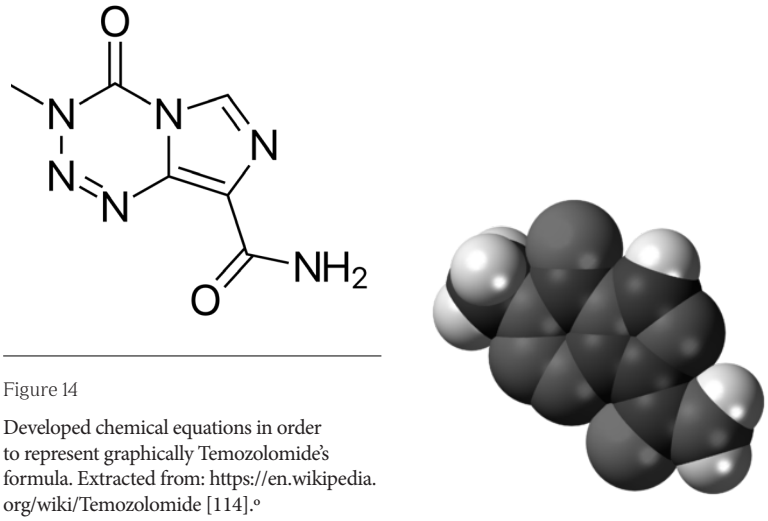


Figure 14

Developed chemical equations in order to represent graphically Temozolomide's formula. Extracted from: https://en.wikipedia.org/wiki/Temozolomide [114].º

themselves be biologically driven by magnetic gradients - hence, the importance of the magnetic field. Moreover, magnetic agent contrasts will be also used in order to excite the colony of the nanorbots when being locally recruited on the tumor sites with the finality of chemotherapeutic medication delivery. As a result, communication will be produced via acoustic signals. This last approach completes the cooperative system aimed to build biomimetic nanodevices related to the Nanobees proposed by Lewis et al. [7], who was totally inspired by the natural biomimetics. Additionally, the properties from ultrasound signals are the best ones regarding the longer distances needed to cover, the low energy-consumption, the unique high-sensitivity, the property of self-heating and thermal coupling. In order to go through the BBB studies have been studying how to induce hyperthermia, which is mainly caused by the thermal conduction produced by an electromagnetic field. The fact of entering the nanodimension introduces another fundamental component, which is the physical governing reign. So, interactions at such decreased scale take place differently than to the macro scale ones, hence the quantum laws. But in fact, the part that highlights more this unique nanorobot is the active targeting via antibody interactions that enable the recognition of specific tumor sites, which express mostly one protein. The nanorobot was inspired by the red tail catfish (Phractocephalus hemioliopterus). You can find an example of this fish in the Science Museum of Barcelona, more concretely, in the section called "Bosc Inundat". The image 15 of the red tail catfish [65] (Phractocephalus hemioliopterus) depicts its particular whiskers, the equivalency of which, in the nanorobot, is the antibodies coated onto the nanorobot diamondoid surface.

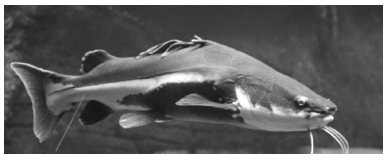


Figure 15

Red tail catfish, Phractocephalus hemioliopterus. Extracted from: https://upload.wikimedia.org/wikipedia/commons/4/49/Phractocephalus_hemioliopterus_-_1.jpg [65].

Experim

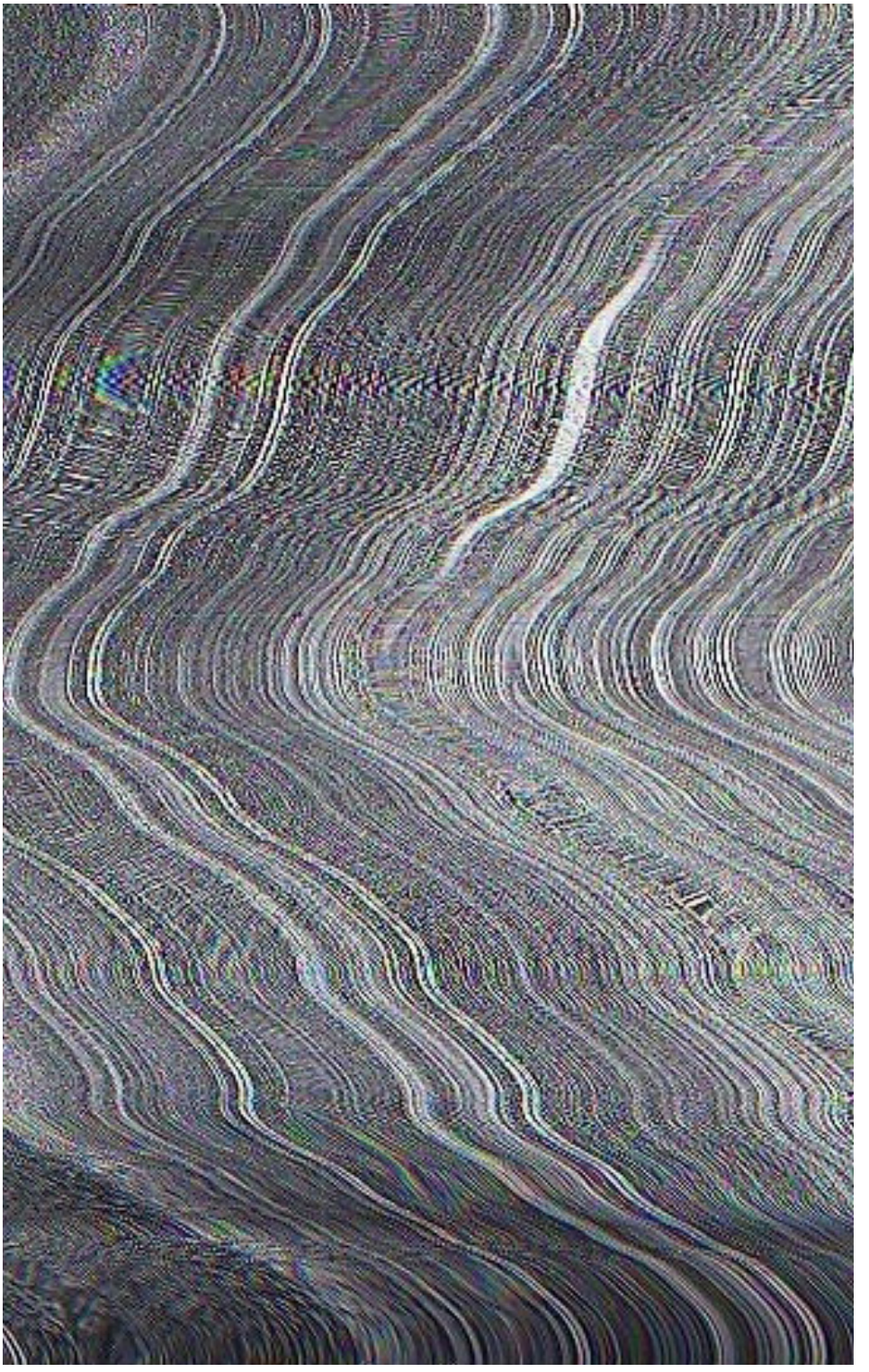
and results →

ientation

After creating a nanorobot prototype we shouldn't miss the other phases from the Scientific Method, in which experimentation is one of the central axis. That's why, the nanorobot should undergo a series of testing process and experimentals set-ups. Once the prototype has been analysed, specific programming on simulations will help making the decision of the nanorobot's feasibility.

Decision of implementing the nanorobot will come after evaluating the different phases that comprise the nanorbots' functionality and efficiency: first of all, the detection of brain cancer cells; then, the targeting phase of the device to the the abnormal mass; afterwards, the force produced by the binding of the nanorobot to the diseased cell; fourth of all, the period of time taken to release the drug within the cell; later, effectiveness related to the reprogrammable orders from the physician's control phone; moreover, the degree of reversibility when disrupting the BBB using as a main technique the phenomenon of hyperthermia and, finally, how do the interactions via acoustic signals affect the swarm colony behaviour. Statistically speaking, it may be interesting to add during this reiterative process replicas, in order to come to a set of conclusions. The replicas on the simulations have the objective to define the same parameters, variables and conditions, except the number of nanorobots. This way, results may be contrasted and analysed to determine which is the perfect number of nanorobots in order to create a balance between velocity and effectiveness.

Additionally, simulations could always be more advanced and developed from a simple environment to another one much more similar to the real model we know nowadays of the brain.



Introduction to the practical section _ _ _

Fundamental aspects of enzyme-powered nanoswimmers

Overcoming viscous forces and Brownian motion is a current need that presents a challenge, which is self-propulsion at the nanoscale. Observing the nature has brought the light of inspiration. Likewise, artificial micro- and nanomachines powered by catalytic reactions have been developed. As most commonly used fuels generate a great quantification of toxicity within the human body cells, enzyme catalysis has emerged accompanied by a versatile and biocompatible alternative for generating the machine itself the propulsion, the so-called self-propulsion. Different swimmer sizes and geometries have been explored. However, in this domain is still tangible the lack of understanding of the mechanisms underlying enzyme-mediated propulsion. Consequently, shape, size, distribution and quantity, as well as the intrinsic enzymatic properties, may play crucial roles in motion dynamics. Further efforts should be aimed to study their long-term stability, lifetime, and ability to navigate in complex environments [92].

Inspired by biological motors, artificial nanoswimmers are able to self-propel by harnessing free chemical energy from in situ chemical reactions and its conversion into mechanical work. Although a wide variety of systems, including rigidness and softness as for the materials have been carried out, most of them relied on the use of hydrogen peroxide as a power-supply, which have demonstrated potential applications in diverse areas such as in environmental remediation, tissue drilling, cargo transports and diagnostics. The incorporation of enzymes as catalytic engines is now the alternative power source, because it offers unique advantages, to cite: fuel biocompatibility, bioavailability and versatility. Either tethered to a particle or free in solution, enzymes have proven to generate active motion, which has already been reported as beneficial for enhanced drug-delivery and higher BBB penetration [97].

These encouraging results could open promising avenues toward the reinvention of therapeutic approaches in biomedicine.

At smaller lengths, it is difficult to differentiate between the diffusive motion arising from random fluctuations and the motion dynamics arising from enzyme activity. In these cases, the analysis of particle trajectories needs to be characterized by robust statistical approaches.

The first prototype of biocatalytic microswimmers was fabricated based on a carbon fiber (0.5–1 cm length and 7 μm diameter) able to self-propel at the water–air interface. Years later, Feringa et al. showed that the combination of glucose oxidase (GOx) and catalase enzymes resulted in bubble generation by aggregates of multiwalled carbon nanotubes. Afterward, tubes have emerged as successful structures for micro-nanojets. Sánchez et al. made use of rolling-up technology to fabricate, in a controllable manner, microtubes containing gold inner layers functionalized with thiol groups to bind catalase enzymes. Catalase-powered microjets were able to self-propel, showing about 10 times higher efficiency than the catalytic platinum-based microjets. Different techniques such as electrodeposition, electrospinning, layer- by-layer assembly, and reactive inkjet printing have been used to fabricate microjets with tunable size, shape, and enzyme localization. Depending on the enzyme position and nanojet length, different motion behaviours were observed. Despite the exciting

outcomes on the use of tubular shapes for biocatalytic self-propulsion, the limitations encountered by the fabrication procedures in terms of size tunability and material versatility have led to a more extensive development of spherical nanoswimmers. It's relevant to note that the diameter of the particle strongly determines the motion dynamics. While particles with sizes ranging from sub-100 to 800 nm in diameter have demonstrated enhanced diffusion, ballistic motion has been achieved when using 2 μm microspheres. Regardless of their size, an asymmetry is required to avoid null net forces. To induce asymmetric distribution of enzymes the most common approach has been the fabrication of spherical Janus particles, where only one half of the particle is coated with enzymes. Other asymmetric complex structures have been reported, such as the chemotactic synthetic vesicles [97], where the asymmetry is achieved by a heterogeneous copolymer composition, and the stomatocytes described by Wilson et al., where the asymmetry is achieved by the generation of a cavity from which the enzymatic products are released. Nonetheless, enzyme-mediated self-propulsion has also been observed for non-Janus (fully coated spherical particles). Furthermore, it has been reported along the scientific literature that coating made by enzymes displayed on polystyrene particles homogeneously presents enhanced diffusion and describes clear chemotactic behavior toward the fuel-rich areas [92].

To study the effect of enzyme distribution as for the threshold needed to generate self-propulsion, higher binding efficiency and number on the motion dynamics of micromotors, bioengineers have used stochastic optical reconstruction microscopy (STORM) combined with other different techniques such as optical tweezers.

The use of enzymes as power sources has been and keeps expanding the library regarding the nanoswimmers' energy supply. Over the last years, the predominant strategy consists in using individual enzymes, ascending catalase to the most widely used by the community since its first report by Sánchez et al.

Catalase may enhanced the propulsion of microjets because of the evolution of the chemical reaction that follows below: decomposition of hydrogen peroxide into water and oxygen (2H2O2 2H2O+ O2). Microjets are not the only structures that have been using catalase as power engine. We should puntualise the action of Janus Mesoporous Silica Nanoparticles (JMSNPs) of 90 and 389 nm in diameter as well as silica nanoclusters with sub-100 nm diameters. Several other groups have incorporated this approach in a varied range of spherical nanoswimmers. On the one hand, urease has also experimented a welcoming in the domain, as it has been utilized to power nanoswimmers by the hydrolyzation of urea into carbon dioxide and ammonia (NH2)2+H2O CO2+NH3). At the nanometer scale, urease has been identified as a particular component that permits the enhancement of the diffusion of JMSNP, mesoporous silica nanobots and polystyrene microparticles. In addition, it can induce self-propulsion in structures above 2μm as for the size [89].

On other occasions, for example, other enzymes including glucose oxide (GOx thanks to the degradation of β-D-glucose), acetylcholinesterase (AChE taking into account the consumption of acetylcholine) and trypsin have been used to propel nanoswimmers. Basic chemistry acknowledgments have shown to us that a specific compound can be at the same time product of a reaction and, sequentially, substrate of the cascade

unchained reaction. In order to increase the complexity of the system, some groups have conjugated more enzymes (observe Fig.16 underneath). The most prominent example is the combination of glucose oxide and catalase with the finality of a constructive attempt: first of all, converting glucose and oxygen into gluconic acid and hydrogen peroxide and, then, to decompose the product hydrogen peroxide by catalase. Moreover, it should be noted that this chained relation referred to a tandem motor, which powered the first biocatalytic nano swimmer. Since the discovery, the community has harnessed this power source for two main reasons: basically, because of the unsuitability of catalase alone in biomedical applications due to its toxicity and, secondly, owing to the fact that the low motion generation capabilities of GOx are due to its low turnover number (number of chemical conversions taken place in one second for each enzymatic site at a given substrate-concentration).

The mechanism reigning self-propulsion of enzyme-powered nanoswimmers still remains unclear in spite of the diversity of enzymes and combinations explored. Other experimental mechanisms observed need to be cited, like for instance: self-electrophoresis and bubble propulsion. As for the proposals taking into account single enzymes, mechanisms including thermal effect and the structural changes arising from catalysis or antigen-antibody interactions have been exposed in order to study their effect on self-propulsion [92].

In these situations, it may occur a binomial behaviour related to enzyme kinetics, which could be an indicator of influence on the motion dynamics of the swimmers. Experimentally, the Smart Nano Biodevices group conjugated catalase, GOx and urease with the finality of powering JMSNP and by uniting the conformation of urease, AChE (acetylcholinesterase), they propelled hollow silica microcapsules. Both times, researchers have analysed and, therefore, found a trend regarding their speed and active motion when being exposed to their optimal substrate concentration. Moreover, the key role of catalysis and the correlation between the conversion rate and active motion has been supported by the acquisition of data during substrate concentrations modifications. Depending on the regime of motion, substrate concentration-dependent improved diffusion and speed. De facto, growth of active motion follows the Michaelis-Menten kinetics fashion, which makes the nanoswimmer reach a plateau when the time needed for two consecutives collisions of enzyme-substrate happening is less than the time that it takes to generate the chemical reaction converting substrate into products. Hereby, when presenting a high number of substrate molecules that compete for active site binding there's a reason behind which anteriorly produced substrate inhibition due to high substrate concentrations. Definitely, decline in motion can be caused mainly for two motives: firstly, a high substrate concentration in the substrate molecules solution and, secondly, an increased viscosity of the media. In addition, it has been discovered by the same group that the use of inhibitors modulate the motion of enzymatic microswimmers, a phenomenon in which the inhibitors are interacting with allosteric sites of enzymes in a noncompetitive way, that's to say, without the seek for active sites.

Along the experimental section, diverse sizes of nanoparticles were exposed. In order to argument and base this decision, we simply denote the fact that in Bioengineering lots of successful trials

around biomedical applications have been proposed. Perhaps their dimensions encode a clear correlation with specific bindings and internalizations at tumorous cells. Smaller particles, capable enough to resist side effects coming from the possible immune responses and not to be trapped by the body filters, could demonstrate the opportunity to enter inside the cell core, that's to say, the nucleus. As a result, tumorous cells may be annihilated from their interior secrets, their analogous root, and might no longer need such big amounts of chemotherapeutic medicaments. Future perspectives should crystalized relations between the difference of cytosolic internalization and nuclei internalization, because it is said that generating apoptosis from the inner compounding area is more efficacious [92].

Although non replicas were effectuad, all experimental results and calculations were corroborated with the many articles cited along the Scientific Project. Real relevance is contained in the entire compilation of bibliography that has finally resulting from the evolution of this enlarged process. In practice, the final decision of taking part into IBEC's group was highlighted and supported by the fact that nanomotors powered by adding enzymes on their functionalized surface prove a higher penetration across the BBB [97]. So, this is one of the reasons why the subspecialization (enzyme catalysis to power nanomachines) has been explored.

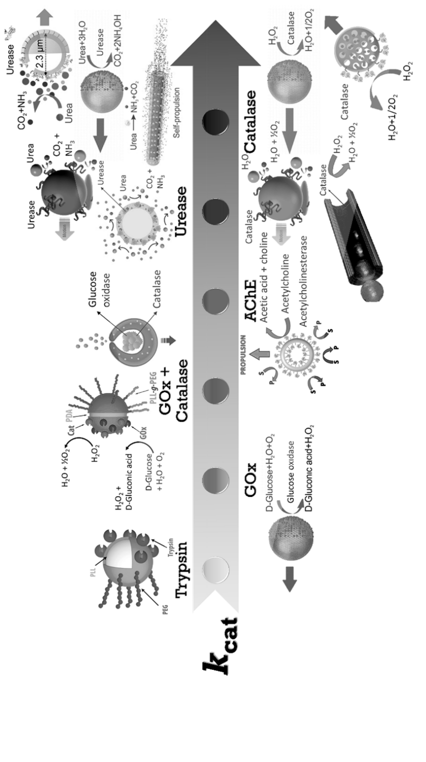


Figure 16

Micro- and nanoswimmers powered by enzyme mediated reactions (that have been reported along the description) categorised in ascending value of turnover number (kcat): trypsin, glucose oxidase (GOx), GOx coupled with catalase, acetylcholinesterase (AChE), urease and catalase. Adapted from reference: [92].

Case Study 1

Visualization and image analysis of Janus spherical nanoparticles via Transmission Electron Microscope (TEM)

Description: An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination.
As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects. Electron microscopes have electron optical lens systems that are analogous to the glass lenses of an optical light microscope.
When focusing within the giant knowledge regarding microscopes [85, 86], we should clarify the differences between the diverse type of electron microscopes, that's to say, we should mention that there isn't only one typology of electron microscopes, because it exists a great spectrum of variabilities, which leads to the following classification: Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM), Scanning Transmission Electron Microscope (STEM), Reflection Electron Microscope (REM), Serial-section Electron Microscope (ssEM).

Origins

At the time, electrons were understood as particles with charged matter until the De Broglie's hypothesis was proven to be true. In 1924 De Broglie published a formula where he depicted a physical association between wavelength and electrons. Afterwards, scientists realised (Ruska et al.) that the wavelength related with the electron was five times less than the wave associated with visible light, which really implied an increase in the microscope resolution. Basic optics of the first microscopes have been maintained until nowadays, the only difference in the most modern microscopes being the addition of more optical lenses in order to increment the augmental ambits and apport major versatility [127].

The transmission electron microscope (TEM) can be comprehended as a tool, artifact or instrument engineered specifically for the analysis and visualization of samples presented within ranges of an ever-decreasing scale, such as the micro- and nanometer dimensions. As highly complex levels of detail are inaccessible by conventional light microscope, scientists may use this kind of electron microscope which possesses this powerful and outrageous capability.

Theoretically [128], the following formula gives us the dependence of a microscope resolution (d) on the wavelength (λ), the refraction index (n) and the maximum half angle of the cone light that can enter the microscope lens (α):

$$d = \frac{\lambda}{2n\sin\alpha} \approx \frac{\lambda}{2\,\mathrm{NA}}$$

This formula shows us that the wavelength is the principal and primordial limitation in getting a better resolution on the microscope. The numerical aperture (N A = n sinα) is the other important factor that influences the resolution. The higher is the numerical aperture, the better resolution can be achieved.

Like all matter, electrons have both wave and particle properties. This means that a beam of electrons can be focused and diffracted much like light can. One can get the formula of the electrons wavelength from the De Broglie's formula, taking into account the relativistic effects:

$$\lambda_e \approx \frac{h}{\sqrt{2m_0E\left(1+\frac{E}{2m_0c^2}\right)}}$$

Where, h is Planck's constant, m0 is the rest mass of an electron and E is the energy of the accelerated electron. In the electron microscopes, the electrons can be accelerated either by thermionic emission - from a tungsten filament - or by field electron emission. Then, electrons are accelerated by an electric potential and, later, focused by electrostatic and electromagnetic lenses whereby the sample. Sequentially, the transmitted beam can be categorized in order to produce an image [128].

Components of TEM

From the top down, the TEM (contemplate Figure 17) consists of an emission source or cathode, which may be a tungsten filament or needle, or a lanthanum hexaboride (LaB6) single crystal source. One may heard the denomination of "electron gun", which stands for the tandem consisting of cathode and electrostatic lens elements. So, we may assure a high voltage on the gun, somewhere in the 100 - 300 kV range. When given sufficient current, the gun will initiate the emission of electrons in two pathways: either by thermionic or field electron emission into the vacuum. In the case of the first source (thermionic) cited, to provide preliminary focus of the emitted electrons into a beam, the electron source is typically mounted in a Wehnelt cylinder. In the meanwhile, it also stabilizes the current using a passive feedback circuit. Instead, a field emission source may need other material equipment, including electrostatic electrodes called extractor, suppressor, and gun lens, with different voltages on each, to control the electric field shape and intensity near the sharp tip. After the electron beam leaves the gun, the beam continues its projected trajectory in an accelerated way due to the action that a series of electrostatic plates generate on their wave convergence. When it reaches its final voltage, electrons enter the next part of the microscope: the condenser lens system. These upper lenses of the TEM further focus the electron beam at a desired size and target it on the sample point of interest [128].

Two physical effects are responsive for achieving manipulation of the electron beam. One of them is the interaction between the electrons and the magnetic field. Thus, it is accorded that given this interaction, the Fleming's rule (see

Figure 18) - so called left-hand rule - appears and dictates the following statement: “whenever a current carrying conductor is placed in a magnetic field, the conductor experiences a force which is perpendicular to both the magnetic field and the direction of current”. Hence, that phenomenon allows us to further manipulate the electron beam. So, one can achieve the creation of magnetic lens of variable focusing by changing the distribution of the magnetic flux.

Additionally, The second one is the interaction between the electrons and the electrostatic fields, which is used to deflect electrons at a constant angle. To allow for the formation of a shift in the beam path, allowing for beam shifting in TEM, coupling of two deflections in opposite directions with a small intermediate gap is sensed. Enough control over the beam path is possible for TEM operation, thanks to these two effects, as well as the use of electron imaging system. In conclusion, the optical configuration of a TEM can be changed, unlike that of an optical microscope, as lenses in the beam path can be more easily reshaped via the magnetic field flux.

Flexibility during operations modes, ability when focusing beams down to the atomic scale and magnification to create images on the screens of a TEM machine is also due to its lenses. Definitely, lenses are the important factors that may drive TEM equipments.

Nevertheless, apertures are equally important, so, they should not be underestimated. What are apertures? These are circular holes in thin strips of heavy metal, placed at well-chosen points in the column of lenses. Some of them are classified and fixed in size and position and play important roles when limiting x-ray generation and improving the vacuum performance, while they also prevent electrons from passing through the external parts of the magnetic lenses which, due to large lens aberrations, focus the electron beams extremely poorly. On the other hand, others can be freely switched among several different sizes and, moreover, have their positions adjusted. These “variable apertures” are used to quantify the beam current reaching the sample. Therefore, it also improves the ability to focus the beam.

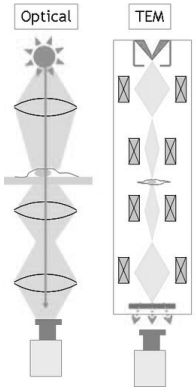


Figure 17

Comparison between the physical methodology used by an optical microscope and a transmission electron miscroscope. Adapted from: https://es.wikipedia.org/wiki/Microscopio_electr%C3%B3nico_de_transmisi%C3%Bn [128].

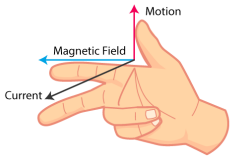
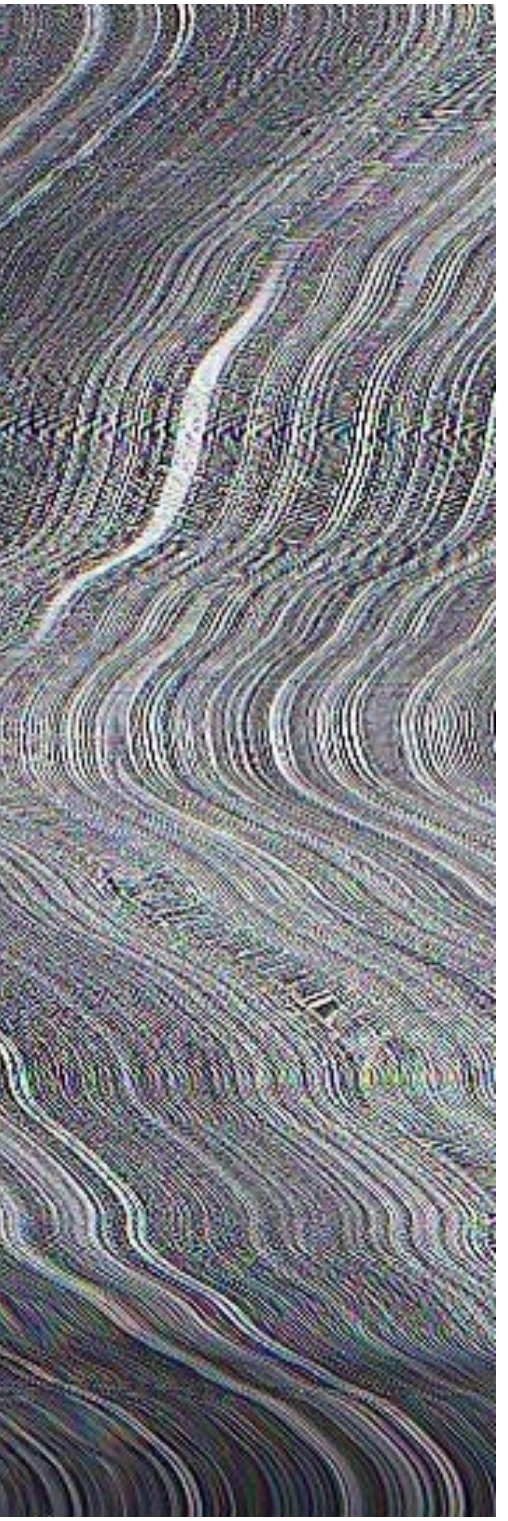


Figure 18

Simple picture denoting Fleming’s left hand rule. Adapted from: https://byjus.com/physics/flemings-left-hand-rule-and-right-hand-rule/ [124].



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TEM is known for being commonly consisting of either three phases or stages, which are: firstly, the bullets; next, the objective lenses, and, lastly, the projector lenses. To start with, the condenser lenses take care of primary beam formation. Meanwhile, objective lenses focalize the beam that comes though the sample itself (in STEM scanning mode, objective lenses above the sample make the incident electron beam convergent). Finally, the projector lenses are responsible for expanding the beam onto the phosphor screen or other imaging device. TEM magnification depends and correlates on the ratio of the distances between the specimen and the objective lens' image plane. Astigmatism is one of the most asymmetrical beam distortions of this techniques. To avoid it, additionals stigmators are allowed. It is needed to comprehend that TEM optical configurations differ significantly with implementation, meaning by that, manufacturers may be the consequent motive od spherical aberration as they use custom lens configurations. In addition, to correct electron chromatic aberration, TEM is provided with energy filtering.

Sample preparation for TEM is, indeed, a complex procedure. For a conventional TEM visualization, specimens should be less than 100 nanometers thick. Electrons in the beam interact more rapidly with the sample that particles seen in other procedures, counting neutron or X-Ray radiation. Quality of the sample should have a thickness comparable to the mean free path of the electrons that are transmitted, which are usually a few tens of nm. Relevance on the material under analysis and information to be obtained requires a specific mode of samples preparation.

Preparation may consist in a simple phase: deposition of object-dilution (containing the specimen) onto films or support grids. This case is only available for materials small enough to be electron transparent, as in the particular case of nanotubes and nanospheres. TEM is a complex procedure where diverse parameters should be adjusted, such as the withstand within the high vacuum in the sample chamber and the enabling cutting tissue into thin sections (electron transparent). To do so, biological specimens may be embedded in resin. On the other hand, biological samples can be also stained by using two different approaches: a negative staining material (uranyl acetate) for bacteria and viruses and to include embedded sections, heavy metals should be used so that staining is possible, including osmium tetroxide. Alternately, samples should be held at liquid nitrogen temperatures after embedding in vitreous ice (which we saw the technician doing).

Despite all we have acknowledged, science is supported by its paradigmatic knowledge, which is based on accumulation. As a result, change is a continuous variable. That's why we should emphasize various drawbacks of the TEM technique. Time consumption is one of them, because many materials, in order to produce a sample thin enough to be electron transparent, require, unlike resources, a large throughput of samples. Simultaneously, the structure of the sample may also be changed during the preparation process. Also, as the field of view is relatively small, the region analyzed might not be characteristic of the whole sample. There is potential evidence that the sample may be damaged by the electron beam. This is most likely to happen when analyzing biological materials [127].

My collaboration with Smart NanoBiodevices members' group aimed

the assimilation of the different types of characterization in the nanometric space. More concretely, as the spherical nanoparticles are the most relevant candidates for drug-delivery nano-robots, I decided to focus the experimental approaches on them. I had the opportunity of visiting a transmission electron microscope in CCITUB (Scientific and Technological Centres of Barcelona's University) [129]. Therefore, we analysed

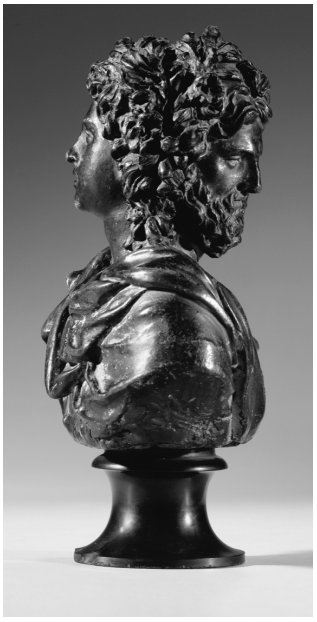


Figure 19

Bronze statue repsenting God Janus as a bifrontal work of art. Copied from: http://www.sothebys.com/en/auctions/ecatalogue/2014/arts-decoratifs-16-19eme-siecle-pf1411/lot.24.ht ml [125].

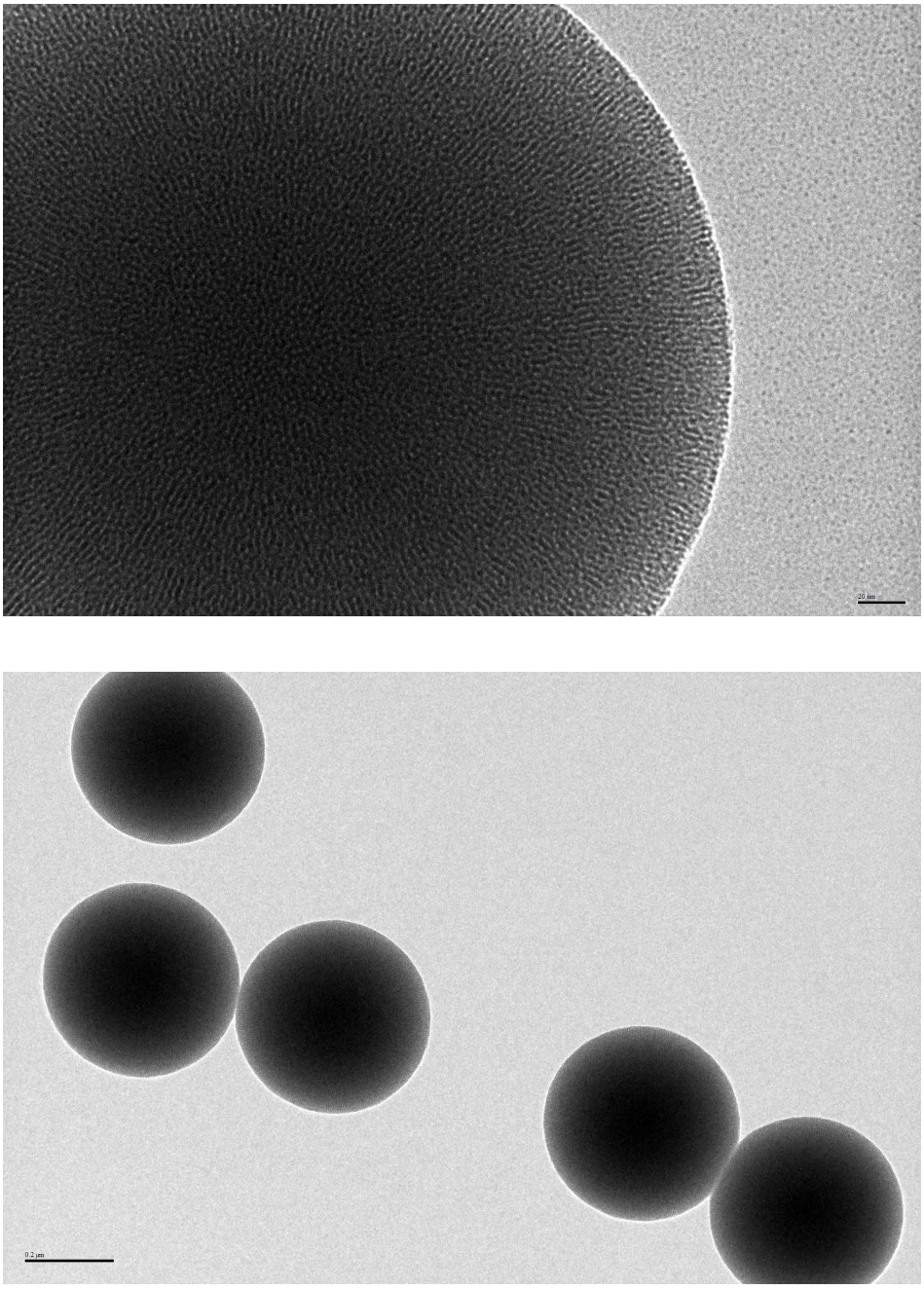
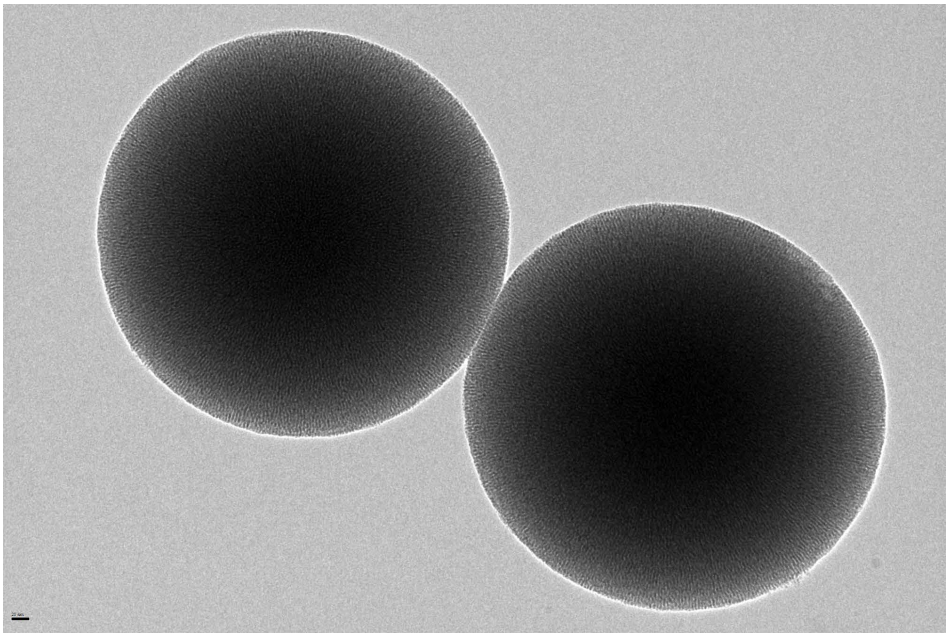
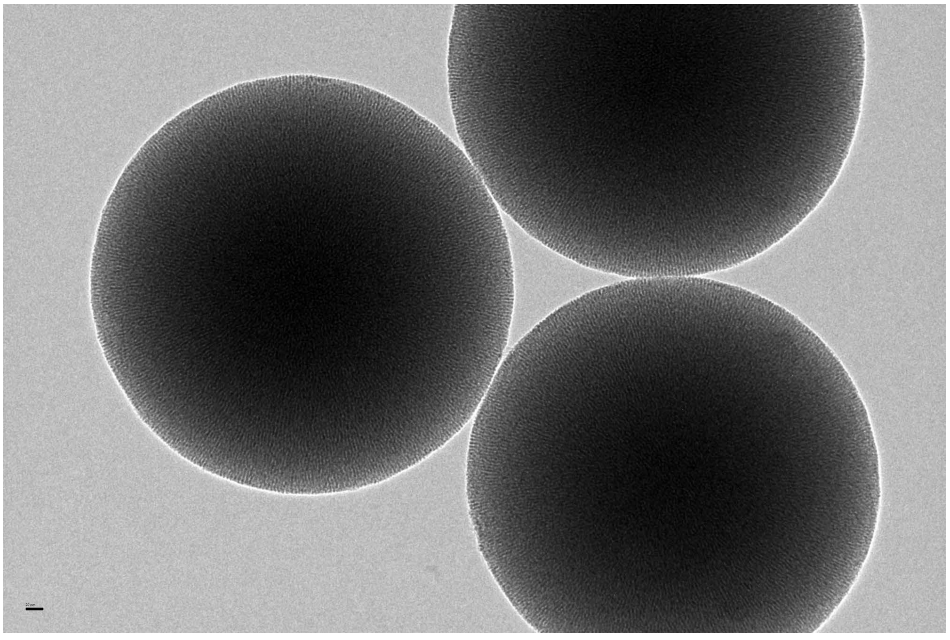


Figure 20

Visualization and subsequent analysis upon mesoporous silica nanoparticles (MSNPs), relevant because of their capacity of drug Dox entrapment into their surface cavities at TEM. a) TEM microscopy images of MSNPs, b) which emphasize the porosity characteristics of the nanoparticles, c) besides their spherical shape and d) symmetrical disposition and distribution of MSNPs characterization.





Case Study 2

Analysis of the size and load of particles with Dynamic Light Scattering (DLS)

Description: First of all, Dynamic Light Scattering (DLS), sometimes referred to as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering, is a technique classically used for measuring the size of particles typically in the sub-micron region, dispersed in a liquid. The sensitivity of some modern systems is such that it can also now be used to measure the size of macromolecules in solution.

The procedure is non-invasive, in other words, one could place its sample into a queue that the machine shines a laser into that sample, and the sample would not influenced at all by that laser. So, the sample extraction from the instrument will show us that it's exactly in the same state as when we introduced it. Also, it's capable of measuring the size of particles and molecules in suspension. And what we're specifically looking for is, again, Brownian motion. The characteristic of this motion is random fluctuations, that's to say, analyzing kinetics on these particles, previously mentioned, we will see that they are moving around randomly due to constant bombardment by the solvent molecules that surround them. What DLS does is to measure the speed at which these nanoparticles undergo Brownian motion. We should distinguish between two particular cases: small and large particles (observe Fig.21 below). Small particles diffuse rapidly whereas large particles diffuse slowly [135].

The velocity of the Brownian motion is defined by the translational diffusion coefficient (D). The majority of scientists would prefer working with particles' size instead of using the translational diffusion coefficient. That's the reason why the value of this coefficient can be converted into a particles' size via the Stokes-Einstein equation. The translational diffusion coefficient can be expressed in terms of the Boltzmann's constant (kb), the temperature (T), the viscosity (η) of the sample, the defined number of pi (π) and the particle radius (r) as:

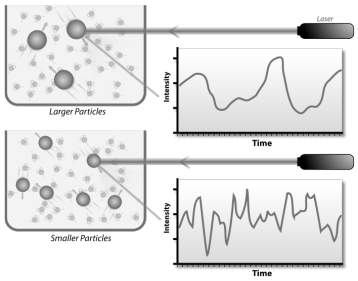


Figure 21

Description of a hypothetical dynamic light scattering. This schematic implies what we have explicit in the section from above, that's to say, DLS performed onto two different samples that include both extreme situations. On the top, larger particles (around hundreds of nanometers or micras) and on the half bottom of the figure, smaller particles. Copied from: https://en.wikipedia.org/wiki/Dynamic_light_scattering [135].

$$D = \frac{k_b T}{6\pi\eta r}$$

Therefore, from this measured diffusion coefficient, we can obtain our nanoparticles size as well as the hydrodynamic size [132, 134]. More concretely, the definition of Hydrodynamic Diameter (see Figure 22) is the diameter of a hard sphere that diffuses at the same speed as the particle or molecules being measured. This calculus is dependent mostly on the ionic strength, which influences the thickness of the cloud of ions that may exist around the particles. Additionally, in colloidal Chemistry, we find a concept known as the Debye length, that's to say, the thickness of the electrical double layer, the cloud of ions that could exist around the surface of the nanoparticle, which is inverse proportional to ionic strength. For this reason, as we increase the ionic strength, we compress the Debye length. Hence, the size we get from dynamic light scattering from any particle suspended in a salt solution is going to be smaller than the size we would get from the same particle if it was suspended in deionized water, for example.

Moreover, surface structure is influencing hydrodynamic radius, because if we had nodes or a polymer layer on that surface likely to conform their situation out into the medium, particles' diffusion speed would suffer transformations.

Contrary, shape is also important and opens new possibilities. Let's imagine we had irregular shaped particles, while they undergoes random diffusion during the measurement, depending on which orientation the particles were in, the different coefficients measured wouldn't be reliable as not being consistent. To remember, the size we obtain from DLS techniques corresponds to the diameter of a hard sphere which has the same average diffusion speed as the particle under examination [142].

Since hypothetical hard spheres are non-existent, the definition is somewhat problematic, as if it wouldn't suit visualization quite well [132].

Instrument components

With regard to the DLS components [137] that constitute the instrument (observe Figure 23 beneath), we need to take first a suitable cuvette that will posteriorly contain the sample object of the determinant study. The cuvette could be plastic disposable as well as glass or quartz, which have a low loading volume, like for example a content of 2 microliters. Later, we focus a laser into that sample and then the particles prepared on the

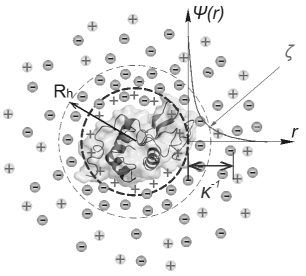


Figure 22

Lysozyme in a buffer solution (schematic): macro-ion, Debye-Hückel cloud and electrostatic double-layer (EDL). Where Rhequals to hydrodynamic radius; k 1 is the equivalent of saying Debye length; Ψ(r) represents mathematically the electrostatic potential, and, ς is the symbol that accords to zeta potential. Copied from: https://www.brookhaveninstruments.com/library/l/study-of-protein-hydrodynamics-with-light-scattering-siz e-and-charge-of-lysozyme [132].

sample will scatter light at all angles. The schematic (Fig. 23) below shows that detection occurs at 90 degrees with respect to the laser beam. However, in the vast majority of instruments sold, the measures are being held at 173 degrees to the beam, which is called backscatter detection. Note that the detector used is capable of counting individual photons. This detector is also in charge of the avalanche photodiodes, which makes it very sensitive. The signal the detector produces is basically the number of photons detected as a function of time. Referring back to the particular schematic, one may observe that the figure itself depicts a graphic related to intensity. In this case, intensity is fluctuating over time. Consequently, the time scales over which we're observing the scattering intensity is the key point, because they take place in very short intervals of time. That's the main reason why we are using nanoseconds or microseconds as time units. Continuously, if we kept the observations around larger time scales, of the order of seconds, the intensity would get averaged, hence, there wouldn't be perceived any kind of fluctuations. The measured signal is then transferred as data into a signal processor named correlator. Therefore, here is one of the reasons why one of the terminologies presented is photon correlation spectroscopy, because the machines is constantly correlating the detected photons. Ultimately, the digital signal processor, represented with a dark grey-box in the figure denotes the most important object of DLS. Thus, importance justified for insistence on this technique.

Afterwards, when observing the intensity fluctuations of both graphics (visualize Fig. 24), we may distinguish between a multitude of peaks and, on the other hand, at our right side, some pretty contour silhouettes. The first of which may be containing very small particles as low as nanoparticles, what leads to rapid intensity fluctuations. Conversely, if the sample would be containing much larger particles, of hundreds of nanometers, because the diffusion speed is much slower, the intensity fluctuates decreasingly slow. Size of the particles determine whether the rate of scattering intensity is quite high or low.

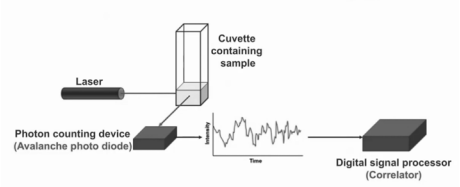


Figure 23

Description of the singular materials and components which configure the global Dynamic Light Scattering technique, including the laser focused onto the cuvette containing a dissolution, a photo counting device (avalanche photodiode) and the digital signal processor (namely, the correlator). Copied from: https://www.youtube.com/watch?v=FaQM7C4oTz0 [137].

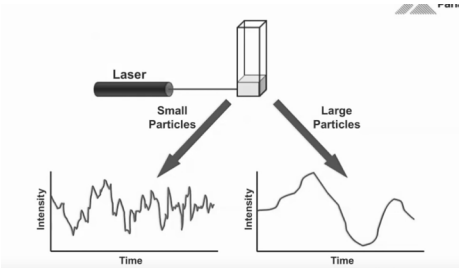


Figure 24

Analysis and schematic classification of graphics depending on the intensity fluctuations depicted on them, that's to say, it is exhibited how intensity silhouettes correlated with time may be one physical and visual attempt in order to comprehend the dimension of the particles being studied and characterized. Abstracted from: https://www.youtube.com/watch?v=FaQM7C4oTz0 [137].

To understand why intensity fluctuates over time we will use the next scheme (Figure 25), in which we localize two stationary particles. One of the possibles outcomes of this practical experiment is that they might scatter light. Remember that light scatters in all directions (Rayleigh scattering) as long as the size of the particles are lower than the one compared to the wavelength. At the top of the slide, we have got the maximum of the wavelengths drawn in one, opposite of the maximum in the other, or a minimum in one, opposite a minimum in the other. In that configuration, constructive interference is formed. So, when those scattered beams of light arrive in our detector, enhanced intensity is generated. However, if one of the particles moves with respect to the other, looking at the picture below, the configuration varies into another one as maximum in one is obviously a minimum in the other and so on. Here, we can observe what destructive interference is, predicted by the theory. Nevertheless, when they arrive at the detector, the resultant intensity is zero because of the cancellation produced by their interaction. Both examples presented are the extremes that we may consider, even though when measuring in a DLS machine, we are not talking about measuring two stationary nanoparticles, yet measuring billions of them overcoming Brownian motion. As a result of that, no such thing as zero may be experienced with the machine, because stability is hardly ever gained. It is usually all about an average intensity that fluctuates over ever-decreasing small intervals of time.

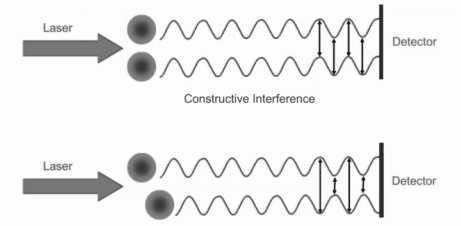


Figure 25

Detail and, at the same time, report of intensity fluctuations as two stationary particles are being analyzed and observed. Point made, we should acknowledge the fact that a particular bombardment with a beam of photons may generate the particles dispersion. In consequence, the relative distance between the nanoparticles under examination differs, apart from obeying the Physical Laws, which dictaminate that whatever particle at such ever-decreasing scale should undergo Brownian motion. Copied from: https://www.youtube.com/watch?v=FaQM7C4oTz0 [137].

Correlation in Dynamic Light Scattering is a technique for extracting the time dependence of a signal in the presence of noise. Thanks to that, time analysis must be carried out with a correlator. To continue our pursuable knowledge, we should construct the time autocorrelation function g(2)(τ) of the scattered light intensity according to the formula:

$$g^2(q;\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$

where τ is the correlation delay time. As we have mentioned, the dynamic information of the particles is derived from an autocorrelation of the intensity trace recorded. The second order autocorrelation curve (see Fig. 26) is generated as follows:

In many cases the intensity correlation function can be written in terms of the correlation function g (1) (τ) of the scattered light field through the so-called Siegert relation: g(2)(τ) = 1 + β [g(1) (τ)] 2 ,where β is the coherence factor,

determined largely by the ratio of the detector area to the coherence area of the scattered light; β is usually regarded as an unknown parameter to be fitted in the data analysis.

Sample preparation can be either by filtration or centrifugation in order to remove dust and artifacts from the solution.

**NOTE:* It's recommended to consult all links placed below the figures in order to visualize the data on which has been based the obtention of the graphics.

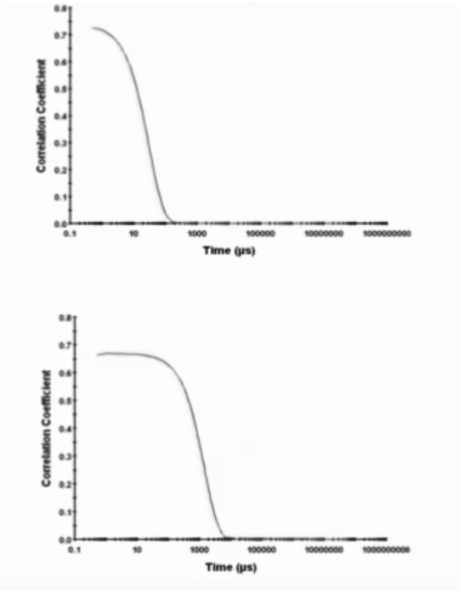
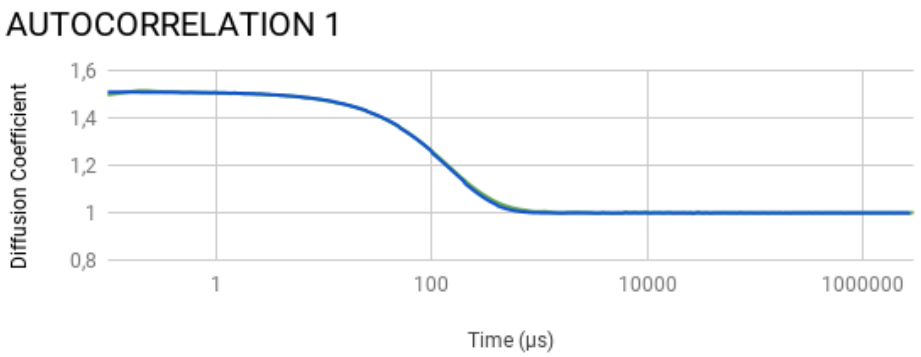
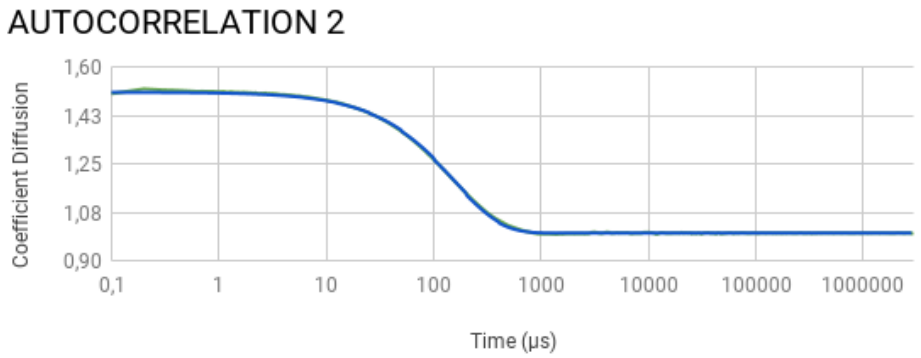
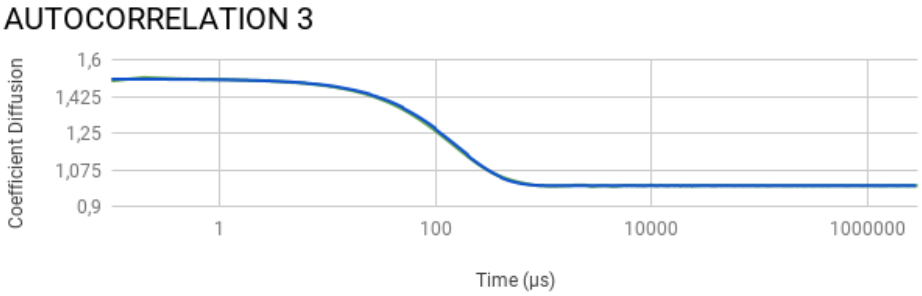


Figure 26

Here it is deployed the mathematical construction of correlation between time and scattered intensity according to the formula. So, the information extracted from this methodology is built upon the intensity trace recorded. In the end, all data is saved behind the secrets of the exact mathematical sciences. Moreover, the rate of decay depends on the particle size which makes larger particles diffuse slower than small particles and the correlation function of the first particles mentioned decreases at a slower rate. Adapted from: https://www.youtube.com/watch?v=FaQM7C4oTz0 [137].







Autocorrelation 1
https://docs.google.com/spreadsheets/d/1OmtGeiu7MAf-EIGp_uJCWc0NymcmyELxFUx7H_ KY8GY/edit?usp=sharing

Autocorrelation 2
https://docs.google.com/spreadsheets/d/12LOCTtT1pmde8SPPaHsmI1WRTXW3PB30iIw8S NUInKo/edit?usp=sharing

Autocorrelation 3
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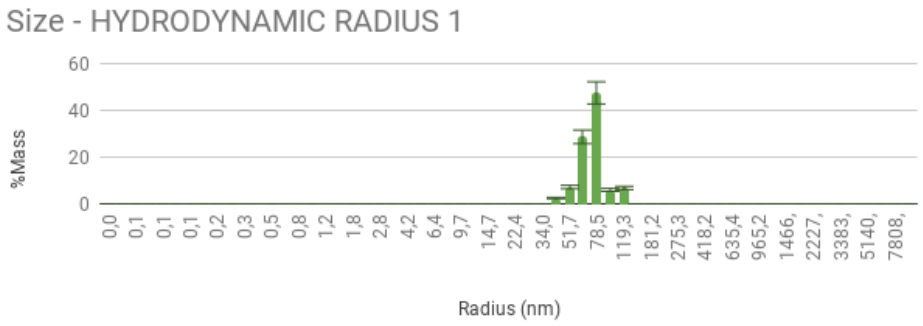
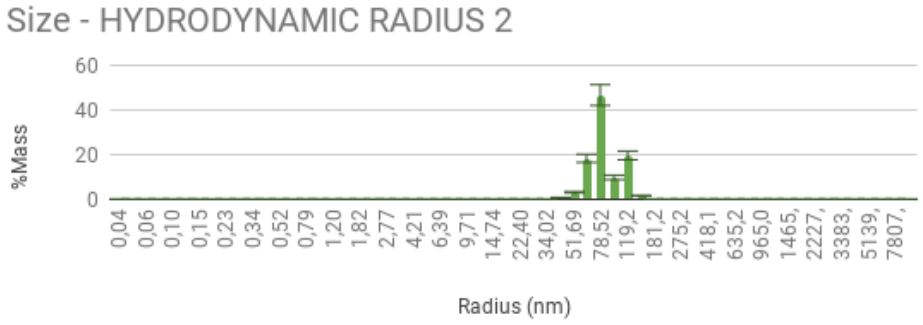
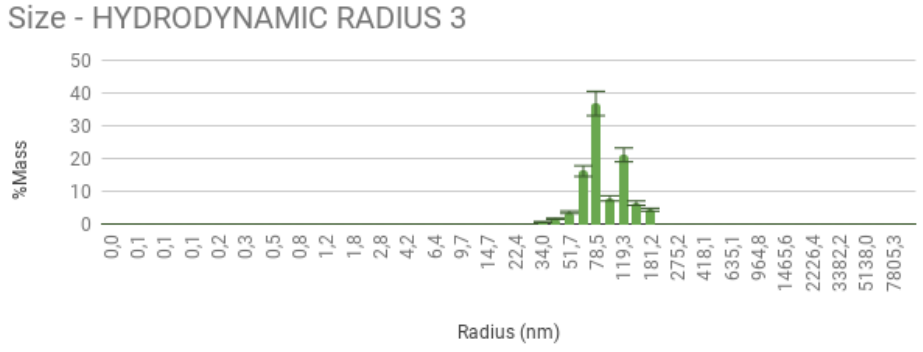






Radius 1
https://docs.google.com/spreadsheets/d/1WaJhiYedFwoMY8isgaeyfKnSwxseai_a2GSRJXwk KTc/edit?usp=sharing

Radius 2
https://docs.google.com/spreadsheets/d/1HZ__UycG9cnxX16KQlkkT3VoQow3Vn3bqTfCaN Olvhc/edit?usp=sharing

Radius 3
https://docs.google.com/spreadsheets/d/13Ta8RkijdzBcaoz3c-B5Oz-pMg1PfkyJOWXM-bvbr oQ/edit?usp=sharing

Characterization of the motion profiles and behaviours by Dynamic Light Scattering. The results obtained are fruit of experimental approaches assessed at the IBEC's laboratory during one of the four sessions of practical and nanoparticles physical characterizations. As for the autocorrelation time function of the scattered light intensity, we must agree on the fact that all three replicas present the decay time near to one thousand of microseconds, that's' to say, the valley created by the exponential functions may conchord altogether, confirming their viability and coherent information. Preparation of these nanoparticles was aimed to compose nanobots sized around the first hundred of nanometers, which is one of the conclusions drawn by the approximations effectuated owing to the size distribution of the nanoparticles radius (visualize Radius 1, 2, 3). The data shows that radius may have been varied (as they are organic and living matter), although the diverse measurements carried out corroborate their consistency, ranging from 100 to 78.5 nm. Globally, all six pictures are consecutively correlated by numbers, naming three groups, the number of which manifests the order in which the specimen's measurements have been contrived.

Theoretical argumentation / background

One of the many works that in our days researchers use come from the back day when Einstein assessed Brownian motion. Those days refer to the epoque of Einstein's juvenile concepts narrated in his PhD thesis. In Physics, he contributed specifically in the field of kinetic theory of gases by what we currently understand as the relation of Einstein-Smoluchowski [136, 138, 139], which determines the diffusion constant. The most general form of the equation is:

$$D = \mu k_b T$$

where D is the diffusion constant; μ is the "mobility", or the ratio of the particle's terminal drift velocity to an applied force, μ = v d / F ; kb is Boltzmann's constant; T is the absolute temperature.
In the second equation, the constant depends inversely proportional on the viscosity (η) of the determinant fluid and, moreover, on the circular radius (r) of the unique particle, object of the study under examination.

$$D = \frac{k_b T}{6\pi\eta r}$$

As for studying the diffusion of spherical particles through a liquid with low Reynolds number, the mobility μ is inversely proportional to the drag coefficient ζ. This is given by the damping ratio, normally used in order to investigate the relaxation time of dynamics, that's' to say, the minimal time for the inertial momentum to become insignificant when comparing to whatever momentum. Then, for spherical particles with r as ratio, Stokes law is as shown:

$$\zeta = 6\pi\eta r$$

Where η represents the medium viscosity so that the relation of Einstein-

Smoluchowski leads to the equation of Stokes-Einstein [136]:

$$D = \frac{k_b T}{6\pi\eta r}$$

Likewise, if the case would also consider rotational diffusion, the coefficient of friction within the medium results as: ζ=8πηr3, which implies that the constant diffusion will be given by:

$$D_r = \frac{k_b T}{8\pi\eta r^3}$$

Case Study 3

Description and analysis of the movement of the particles with optical microscopy and posterior behavioral movement tracking

Description: Optical Video Recording of Nanobots and MSD Analysis: an inverted optical microscope (Leica DMi8) with a 63× water objective was used for the observation and video recording of the nanobots movement. An aqueous solution of nanobots was placed on a glass slide and thoroughly mixed with the solutions of peroxide at the desired concentrations. Then, the mixture was covered using a cover slip to avoid artifacts caused by the drifting effect. Videos of 30 s were recorded up to the first 3 min after performing the mixture to ensure that the analysis is performed under the same conditions. The videos were obtained using a Hamamatsu camera at a frame rate of 50 fps, under bright field. Additionally, the videos were then analyzed using a Python code to obtain the tracking trajectories.

From the physical perspective [92], one can study and analyze the dynamics of self-propelled particles by calculating the mean squared displacement (MSD) of their positions over time. Therefore, we may assume a constant speed over time and randomize the particle's position and orientation owing to Brownian fluctuations. The formula seen below is giving us the MSD over time evolution:

$$\mathrm{MSD}(\Delta t) = \langle (\vec{r}(\Delta t) - \vec{r}(0))^2 \rangle = 4D_t \Delta t + \frac{v^2 {\tau_r}^2}{2}\left[\frac{2\Delta t}{\tau_r} + e^{-2\Delta t/\tau_r} - 1\right]$$

where r (0) is the position of the particle at the initial time, r (Δt) is the position of the particle after a time Δt, Dt is the translational diffusion coefficient, τ r is the rotational diffusion time, and v is the speed of the particle. When the particle is moving at constant speed and the particle experiences no torques, we can proceed by using this well-known equation. However, Physics tries to study and distinguish primordial and more intelligible cases from the general, universal and absolute equation. Therefore, two separate regimes, each one corresponding to a limit case, can be identified.

At longer time scales (Δt >> τ r), it can be shown that:

$$\mathrm{MSD}(\Delta t) = (4D_t + v^2 \tau_r)\Delta t = 4D_e \Delta t$$

which is analogous to the case of a passive Brownian particle and is referred to as enhanced diffusion regime. At shorter time scales (Δt << τ r), one gets:

$$\mathrm{MSD}(\Delta t) = 4D_t\Delta t + v^2\Delta t^2$$

So, in the first case, as well as in the second one, calculations of limits were performed using Taylor's series expansions.

Herein, we could introduce, by making a little parenthesis, what the Taylor's series [143] are. To note, a function can be approximated by using a finite number of terms of its Taylor series. Additionally, as bigger and reliable the compilation of data saved, equivalent to the number of terms in Taylor series, more exact is the result obtained. Taylor's theorem gives us an quantitative input about the error introduced by the use of such an approximation into the function. When taking some initial terms of the Taylor series, the polynomial formed is known under the terminology of Taylor polynomial.

$$f(a) + \frac{f'(a)}{1!}(x-a) + \frac{f''(a)}{2!}(x-a)^2 + \frac{f'''(a)}{3!}(x-a)^3 + \cdots,$$

where n ! denotes the factorial of n and f (n) (a) denotes the n th derivative of f evaluated at the point a. In the more compact sigma notation, this can be written as:

$$\sum_{n=0}^{\infty} \frac{f^{(n)}(a)}{n!}(x-a)^n.$$

From this formula we obtain the expansion of the exponential function ex (with base e) around x = 0:

$$e^x = \sum_{n=0}^{\infty} \frac{x^n}{n!} = 1 + x + \frac{x^2}{2!} + \frac{x^3}{3!} + \cdots$$

Since we should see an effective behavioral movement where the particle seems to continuously propel in a specific direction, the regime is called either propulsive or ballistic.

As the MSD expression proportionates statical averaged results, it is commonly used for the study of motion of catalytic and biocatalytic nanoswimmers. The rotational diffusion increases with the cube of their size (that's why the shape depicted by the MSD curve can change depending on the size). More concretely, for nanoparticles, when comparing both rotational diffusion time and time resolution of typical equipment, it is seen that the first one mentioned is quite smaller. Hence, only the enhanced diffusion regime can be observed. In those cases, an enhanced or effective diffusion may be obtained by fitting the experimental MSD to a linear function.

There are two basic methods in order to obtain the MSD, these are dynamic light scattering (DLS) and optical tracking. Many of the times, DLS results to be a

more efficient pathway in order to show comparable results, because it is less time-consuming than the other methodology. DLS can be utilized only when analyzing sub-micrometer active particles because larger particles exceed the resolution limit of common equipment. On the other hand, optical tracking can be used for micro- and nano swimmers. However, the optical resolution limit is around the 200 nm, but since that ranged limit is known, analysis made of particles below 200 nm might be unreliable. As a third way of observing the MSD, some authors suggest the nanoparticle-tracking analysis (NTA). Plus, although it's a similar method to DLS, it overcomes some disadvantages of DLS like for example aggregation of particles due to the fact that particles are not treated as an ensemble. Parallely, other effects such as fuel depletion or the presence of salts in the medium should always be considered. Note that the concentration of the particles for possible effects arising from interactions among them is relevant. The method employed is independent from the specific guidelines that one should follow in order to guarantee a correct and safe evaluation of the results. Real speed of Brownian particles measurements require very complex experiments. As it all depends on the tracking algorithm used, and, thus, it all depends on the biophysical model described, reporting the instantaneous speed of a particle cannot be accurate and might not be relevant. The models give us sensitivity. Hence, for nanoswimmers, the only proper metric parameter that could be used is the enhanced diffusion coefficient. Once again, it has been acknowledged that when the elapsed time increases, the variance of data increases because of the lack of data points. In order to minimize errors when fitting the MSD [89], it is recommended to have large time sensitivity, that's to say, it is needed to dispose of multiple frames per second at the tracking time and take only the initial part, approximately around the 10 percent. Since larger particles need a longer elapsed time to capture both diffusive and propulsive regimes, the size of the nanoswimmer should be taken into account. Finally, it is important to highlight that the well-known MSD formulas and its approximations only are valid for spherical particles, which navigate in Newtonian fluids at constant propulsive speeds. Each and every derivation of the mentioned nanoparticle profile, such as torque applications composed by dimer of particles or movements in blood fluid or mucus can be referred to a derivation of the original and singular formula. For instance, when studying the motion of non spherical enzyme powered swimmers, the value of the rotational diffusion coefficient may be conformed by length and radius. Particularly, the calculation of the velocity autocorrelation function may be one of the best ways to asses the directionality of the movement. The analysis shows that the slower the decay, the more directional the motion is.

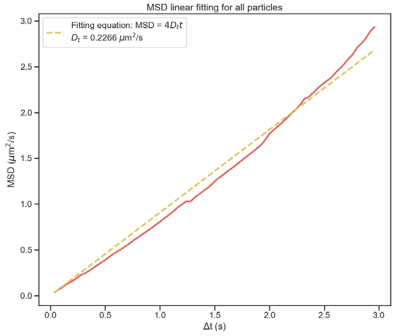
An apparent propulsive regime can be a consequence of drift in the solution, even though MSD can show clear linear and propulsive regime. Not noticing this effect may be the cause of misrepresentative results, especially for the case of sub-micrometer particles, which might show only short-timed propulsive regimes. Typically, drift is noticed when all particles move in the same direction with a very linear trajectory. Use of microfluidic channels to control the flow of the particles into the system is a path that lead to the avoidance of this problem. Moreover, it is recommended to record longer videos to confirm that both regimes appear, although one of the regimes may appear shorter than the other one.

Conversion of substrates into products [92], mediated by enzymes, has made possible biocatalytic nanoswimmers' propulsion. The use of enzymes gained force when looking for a response to the need of compatible fuels. The natural mechanism presented in enzymes, which categorizes them in a vast diversity, offers a unique versatility and the possibility of high specificity when designing nanoswimmers that become active on demand, just where and when the substrate is nearby. Although the field is still in its infancy, a great spectrum of applications has been proposed with functions in sensing, imaging, environmental remediation, nanosurgery, and drug delivery, and several milestones toward them have been achieved, such as facilitated drug transport to cells and tissues. Nonetheless, to fully comprehend and simulate the performance of enzymatic nanoswimmers, further experimental approaches into the fundamental aspects underlying their motion behavior are required. Herein, we discussed the role of particle size, shape, enzyme number and localization, as well as the key enzymatic properties that affect motion dynamics. However, most of the experimental approaches conducted so far have relied on the use of simple fluids such as water or PBS. A step forward into the development of enzymatic micro- and nanoswimmers as future tools in biomedicine will entail understanding how the different properties and components of physiologically relevant media can affect the motion capabilities of enzymatic swimmers and to what extent this will be dependent on their design and enzyme properties. Hereby, we introduce the idea of creating a fluid that could simulate with sufficient robustness and reliability the viscosity and particular texture of the enecephalorachidyc fluid. In order to study a great spectrum of options including enzyme–fuel configurations for on-demand applications, the fundamental understanding of enzyme catalysis, and motion in complex fluids, future direction will propose new guidance. Recently, enzyme encapsulation has indicated increased endurance, even though in some cases was reported poor long-term stability. Therefore, a multidisciplinary approach where the biochemistry, nanotechnology, and theoretical analysis evolve together is needed to guarantee successful progress in the field.

Hereafter, the nanoparticles observed by the optical microscope have been approximately ranged around one micrometre as for the diametre. First and foremost, the characterization carried out was focused on three principal groups: the control one, that's to say, the group in which no fuel of peroxide was added; the second group, which was successfully prepared in order to contain 2% of peroxide with respect to the power-supply needed to catalyze catalase nanorobots and, finally, the third group of samples was characterized by the fact that peroxide preparation was set at 2,6%. In fact, this description fits the physical theory, methodology and considerations taken into account, as the practical approach aimed to achieve the graphics of the Mean Square Displacement (MSD) by making either a linear or a quadratic fitting, depending on the regime that the nanoparticles exposed. In a second place, averages of all particles included, as the sample had to be significative, regarding the MSD were divided into two kind of visual graphics, the first of which record all the variance of data due to the increase of elapsed time and, on the other hand, the second schematic, ubicated at the right side of the page, reveals only the consistent data that one must insert into the algorithm. Thus, this is a method

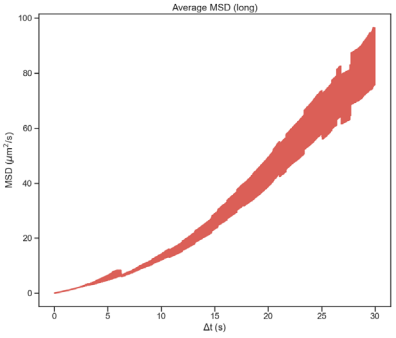
in order to decrease the amount of error created along the process. Below them, representations show exactly the trajectories followed by the nanoparticles indicated in our sample, which is, in fact, the initial phase from which data from above has been captured, that's to say, those pictures are the first matter used to obtain the MSD averages. Definitely, they contain all the trajectories configured from the total nanoparticles determined in that sample. Later, four random nanoparticles trajectories have been selected from the big multitude. The reason for this is because images depicting the regimes the NPs were describing were needed to be translated into tangible figures. On top of that, we filmed and also recorded movement effectuated by Janus silica nanoparticles, because posterior to that phase, undergone regimes by the nanoparticles would be studied, analized and tracked with a Python software code. Hence, we have attached this work, executed by an optical microscope performance (Leica DMi8), with three frames per group in order to see the important differences.

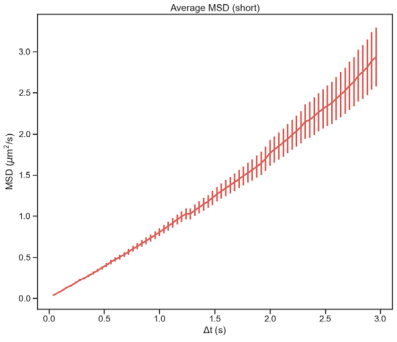
Note to remember, the adjective "large" denotes the fact that the entire duration of the film recorded was analyzed, that's to say, all data under the thirty seconds was exploited, which significantly changes with "short", which describes that only the first ten percent of the data points were used for further analysis and quantifications.

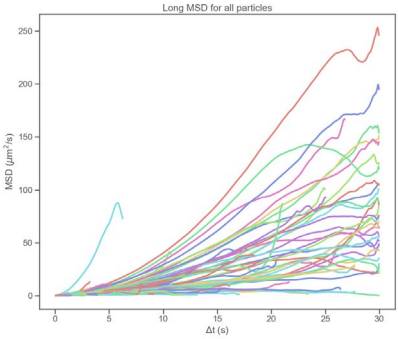


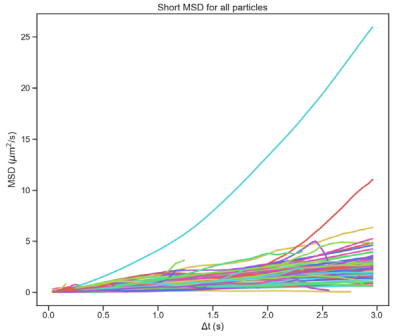
Control
Linear fittings

MSD Averages









trajectories

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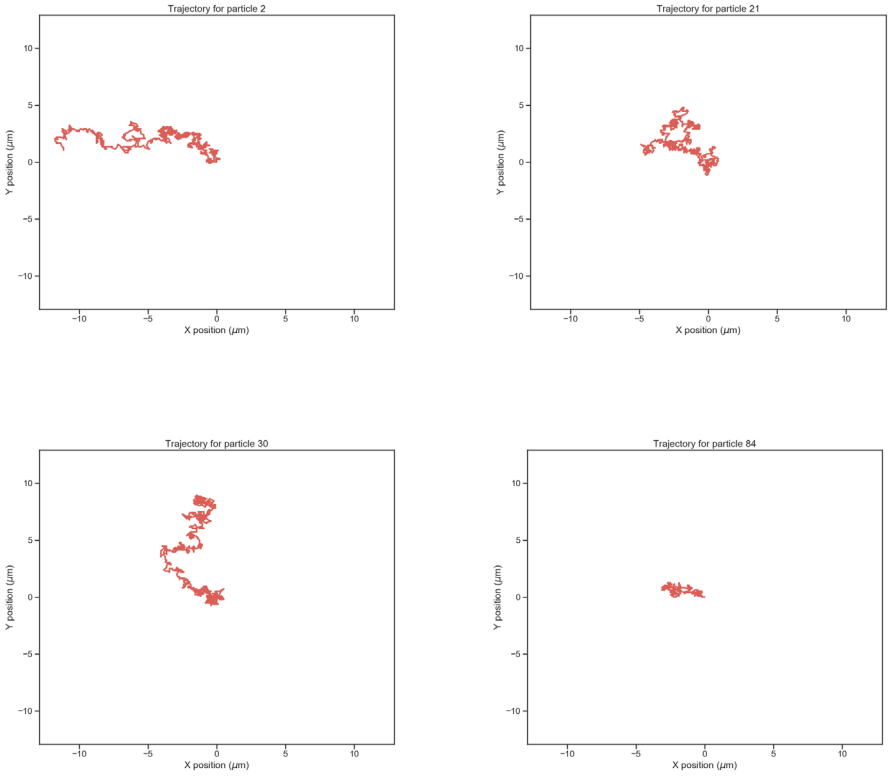

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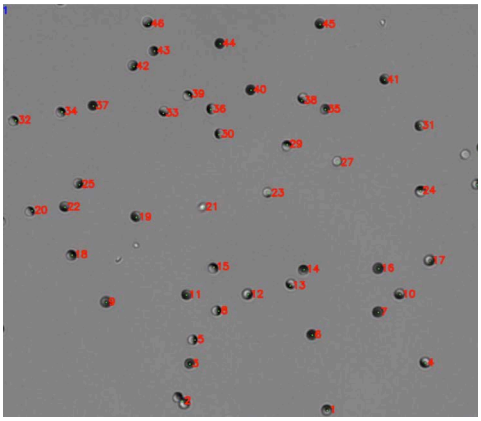
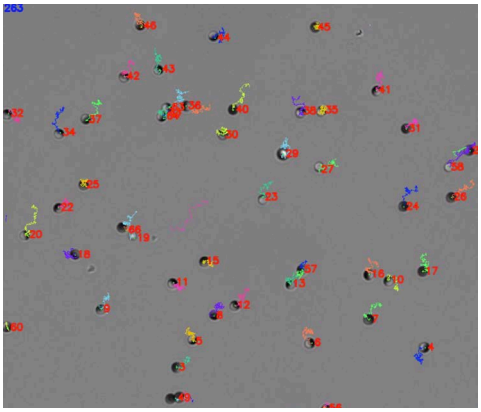
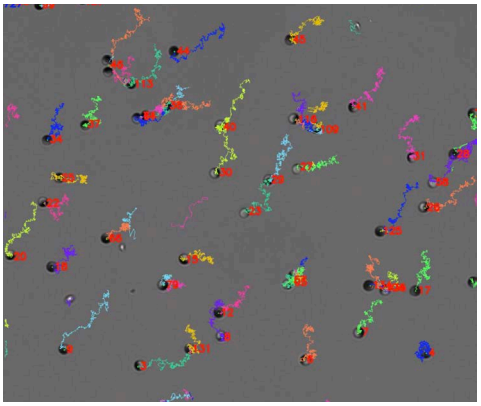
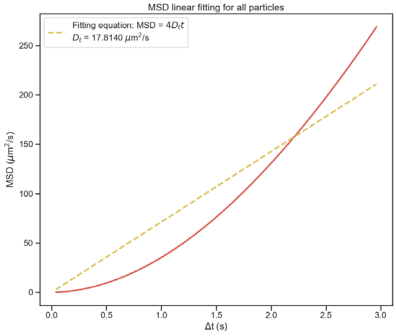
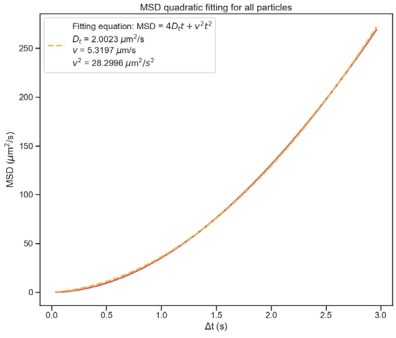






2% peroxide (fuel for catalase nanobots)
trajectories





MSD

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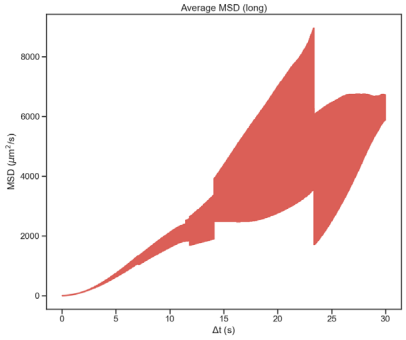
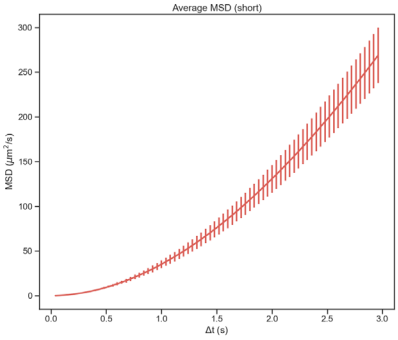
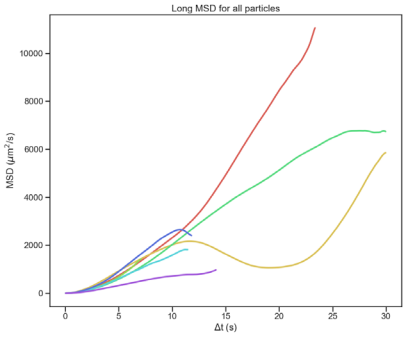
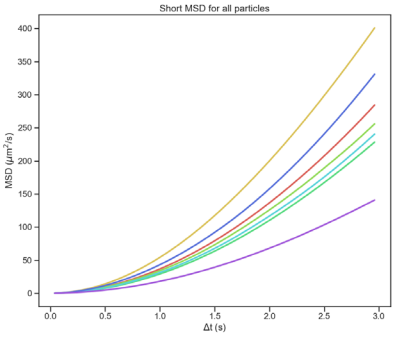

Average MSD (long)
MSD (μm²/s)
Δt (s)


Average MSD (short)
MSD (μm²/s)
Δt (s)


Long MSD for all particles
MSD (μm²/s)
Δt (s)


Short MSD for all particles
MSD (μm²/s)
Δt (s)

trajectories

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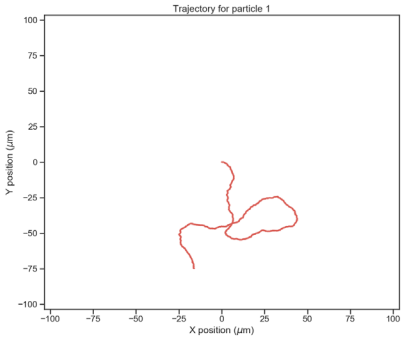

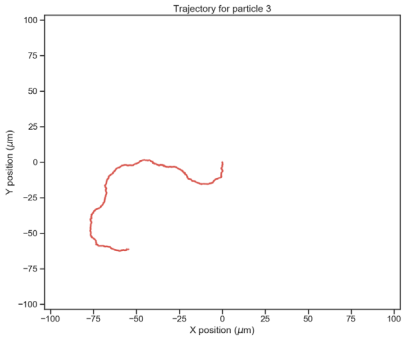

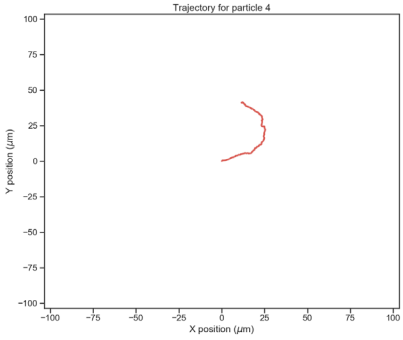

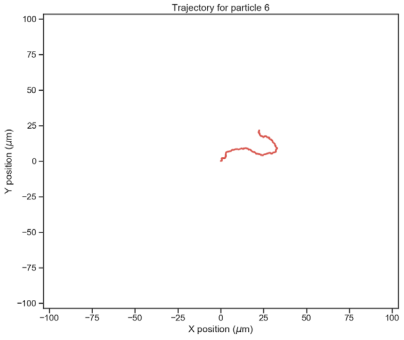

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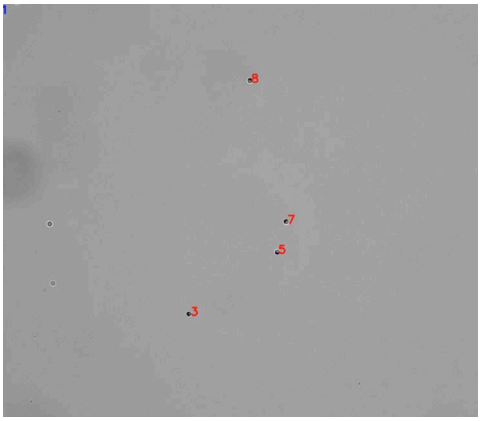
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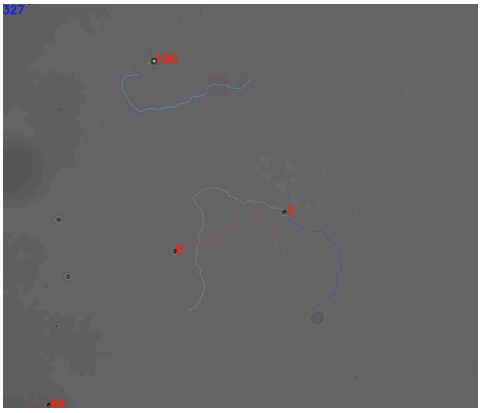
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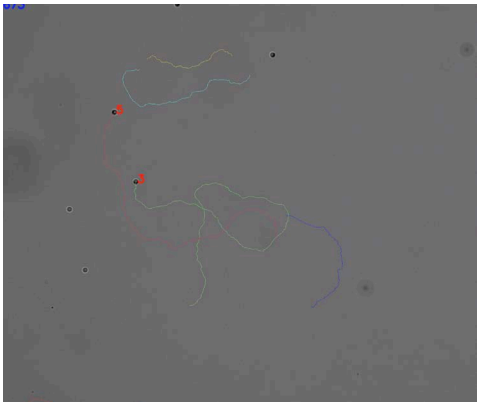
8
3

2,6% peroxide (fuel for enzymatic-powered nano-robots, concretely, with catalase nanorobots which decompose peroxide into water and oxygen)
trajectories

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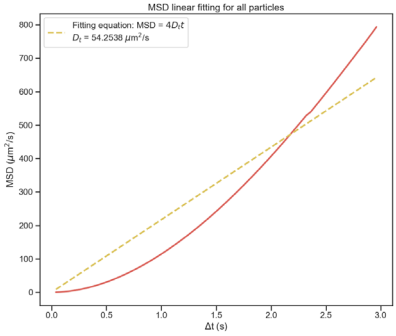

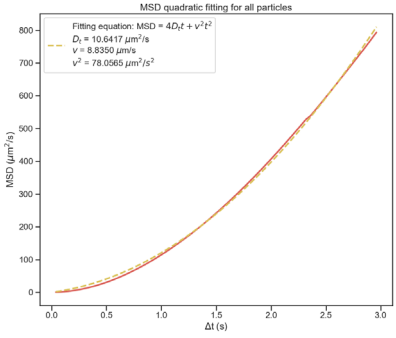

MSD

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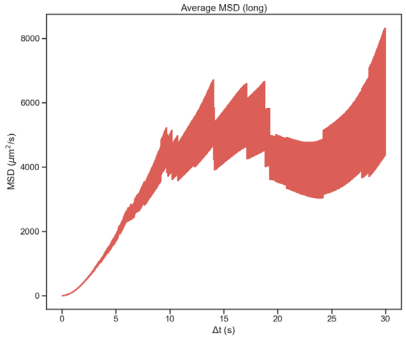
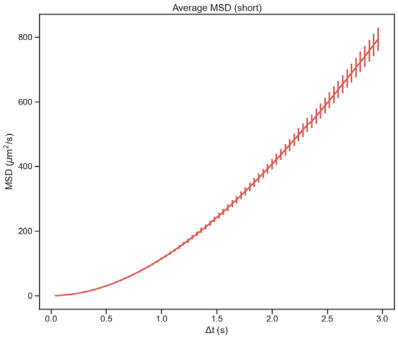
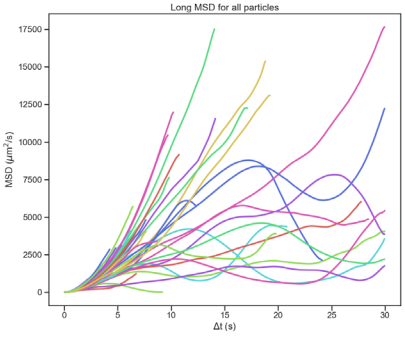
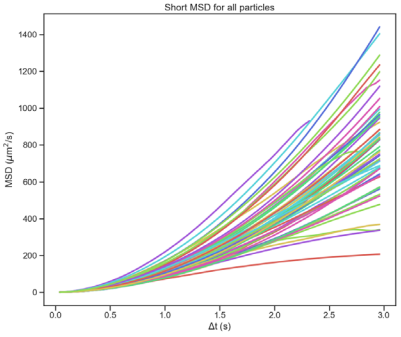

Average MSD (long)
MSD (μm²/s)
Δt (s)


Average MSD (short)
MSD (μm²/s)
Δt (s)


Long MSD for all particles
MSD (μm²/s)
Δt (s)


Short MSD for all particles
MSD (μm²/s)
Δt (s)

trajectories

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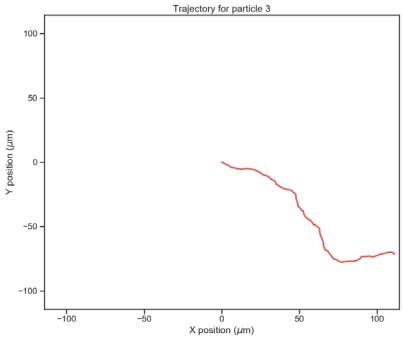

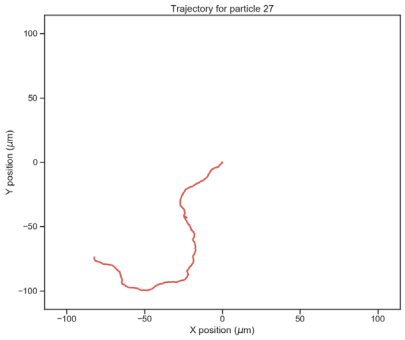

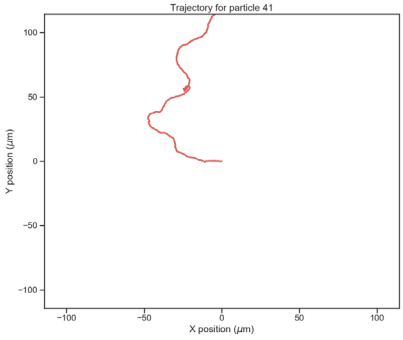

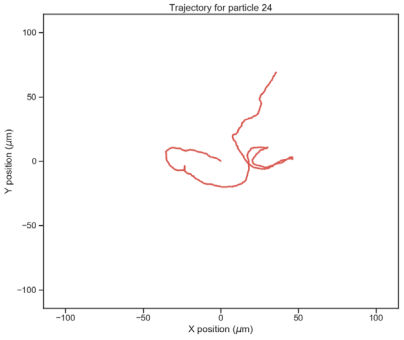

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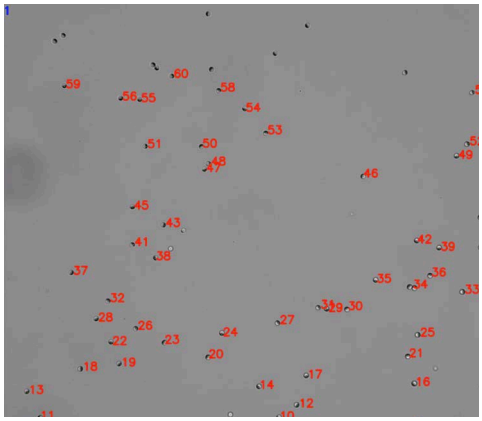
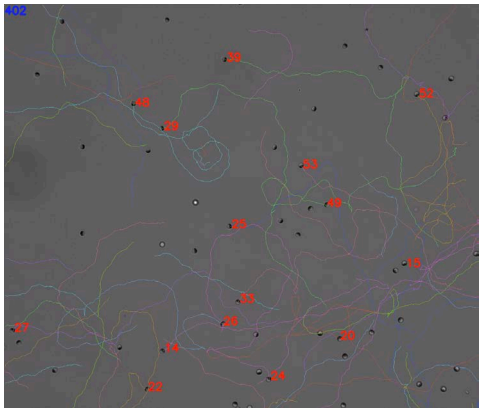
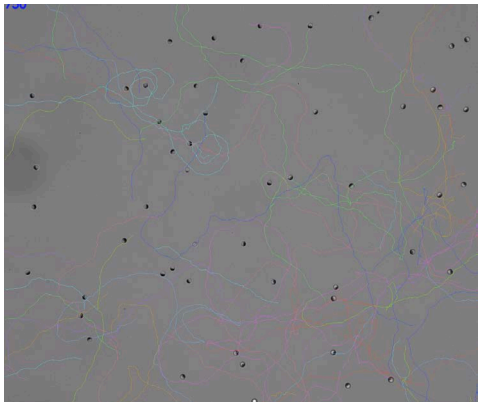






In order to comprehend the legible results and tracking analysis upon catalase nanorobots we should remember the chemical reaction of the enzyme mentioned before. So, we will obtain that this specific protein, at its active site, will undergo the following decomposition:

(2H2O2 → 2H2O+ O2)

in presence of peroxide, which actuates as the fuel so that the enzyme functionalized on the surface of the nanojets will catalyze and, parallely, the nanomotor will convert this chemical phenomena into kinetics.

Three groups have been discussed along the entire third case study, because different concentrations of fuel peroxide should aim conclusions to be drawn, due to the fact that the subsequent analysis, considerations, parameters and tracking methods will force us to establish a basic categorization of diverse regimes. Regimes which nanoparticles may effectuate correlating their size and concentration of fuel on the sample where they are placed in suspension. So, first of all, in the control group, one may define that, overall, when nanoparticles do not undergo other movements apart from the Brownian motion intrinsec within this particular molecules, the regime we are calculating and observing is passive Brownian motion, as vibratile, random and successive fluctuations are visualised upon the recopilation graphics. According to the Mean Square Displacement, the first representation fitted to the corresponding linear function is indeed a figure where no enhanced diffusion could be seen, as no parabolic or ballistic trait is predefined. To elaborate, their movement trajectories are purely Brownian and only obeying physical laws which reign Brownian passive motion. Thus, the formula used in that specific case was the mathematical equation derived from studying the casuistic at longer time scales. Furthermore, when analysing the next group, the equation is just the other way around, as we would like to observe how enhanced Brownian diffusion is overcome by the nanomotors, that's to say, here we will use smaller time intervals. Likewise, on the third case, the same formula is needed to be performed by the algorithm of which we dispose. On the one hand, the new regime appeared is opposed to the initial one, despite being the same particles, because one may notice the presence of directionality and no reverse things. Increasing the quantity of fuel dissolved in water only significates that, correlatively, the velocity of Janus nanoparticles will also be positively affected by the undergoing catalytic reactions at the active sites of the proteins. As a result, the tracking shows that the majority of nanoparticles included in the sample have localised directions, not to be confused with drift situations as the average movements do not coincide, that introduce a new factor quite interesting for biomedical applications, which is: biocatalytic nanoparticles that are capable of actively interact with molecular sites from our body parts.

Case Study 4

Internalization of Nanomotors by cancer cells

Description: The capability of self-propelled micro- and nanomotors for the conversion of chemical energy into motion has opened new and promising avenues for a high number of varied and innovative applications, including biomedicine. In this workshop, we will learn whether the HeLa (cervix adenocarcinoma) cells incorporate the nanorobots added into the same culture or, conversely, do not integrate the nanobots within the them. In addition, this internalization will be further analysed by using 3D images obtained via the fluorescence microscope (Leica DMi8).

Introduction and aim of the session

Enzyme-powered nanomotors hold a great potential towards biomedical applications due to their multiple characteristics. Their unique properties consist in biocompatibility, versatility and fuel bioavailability. [91] Hortelao's group has recently demonstrated that urease-powered nanomotors may be enhanced regarding their functionalities, such as the release and delivery of an anti-cancer drug to HeLa cells (see Figure 27). [89] During this session, the internalization of (urease-powered) nanomotors in human cervix adenocarcinoma epithelial cells (HeLa) was the primordial focus. Additionally, different types of nanoparticles and their intrinsic properties were observed along the session.

Equipment

In order to perform and obtain 3D images of fluorescently labelled cells exposed to urease-powered nanomotors, we employed an inverted wide field fluorescence microscope; more concretely, the model categorized as Leica DMi8. Prior to the imaging, it was needed the performance of a fluorescent labelling of the plasma membrane and the cell nucleus for a precise analysis of the intracellular location of nanomotors.

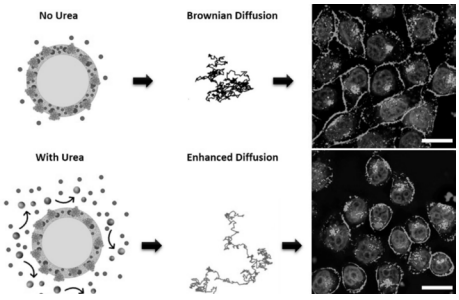


Figure 27

Due to their enhanced diffusion, which results in a higher drug delivery efficiency in HeLa cells, enzyme powered nanomotors exhibit, definitely, a higher drug release. The figures itself is adapted from: Hortelao et al., 2018 [89].

Materials and protocol

HeLa cells were seeded in a 35 mm glass bottom dish (observe Fig.28 below). After 24 hours, they were exposed to urease-nanomotors for 6 hours. Then, the nanomotor solution was washed away, cells were fixed in 4% paraformaldehyde and were ready for further staining (as shown in the figure in the last pictogram: step fourth) and subsequent analysis of the internalization of nanomotors.

Staining protocol

In order to label the plasma membrane and the nucleus, the following methodology was carried out:
Prepare a blocking solution: PBS-BSA 5% [phosphate-Buffered Saline - Bovine serum albumin]. To note, percentage is normally referred to g/100mL. Then, the calculations of the BSA necessary for 2 mL of blocking solution were needed to be made as follows:

$$2\ \text{mL}\ \frac{5g}{100\ mL} = 0{,}1\ \text{g BSA}$$

Incubation of the cells for 20 minutes was fulfilled. The incubation was implemented with 1 mL of blocking solution. During this step, BSA block nonspecific binding sites to avoid background information.

Continuously, the next step to accomplish was the preparation of the staining solution by adding 5 µL of Wheat Germ Agglutinin (WGA) and 1 µL of Hoechst 33342 to 1 mL of PBS-BSA 5%.

Later, we removed the PBS-BSA 5% solution from the culture dish and added 1 mL of staining solution.

Then, we incubated cells with staining solution for about 15 minutes at room temperature and Dark Conditions, as we used fluorescent agents that consume their potential at light exposure.

Afterwards, we had to wash twice, or even, thrice, the culture in 1,5 mL of PBS 1x. To finalize, it was required to add 500 µL of 70% Glycerol solution (pretty viscous texture), which has the function of increasing the fluorescence lifetime. Information about the staining markers used along the process:

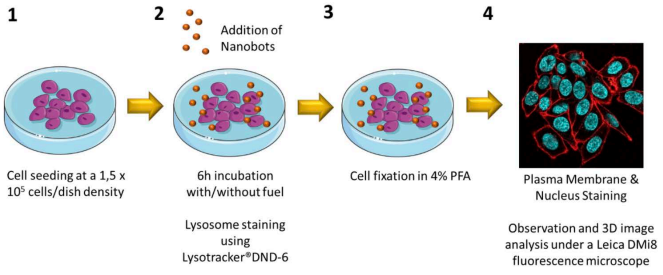


Figure 28

Schematic representation of the protocol needed to be followed for the analysis of nanomotors' internalization by cells.

WGA

WGA. Wheat germ agglutinin is a lectin widely used in cell biology which detects and binds specifically to the glycoconjugates present on cell membranes. WGA can be conjugated to a fluorescent dye (in this case we used Alexa Fluor 647) to allow their visualization under a fluorescent microscope. For more information on the possibilities for plasma membrane staining:

https://www.thermofisher.com/es/es/home/life-science/cell-analysis/cell-structure/plasma-m embrane.html [130]

- -

Hoescht 33342

It's a blue fluorescent dye that can cross the cell membrane, enter the nucleus and bind specifically to the adenin-timin regions of DNA.

However, one should take into account several SAFETY CONSIDERATIONS, even though Hoechst 33342 is considered as a non-hazardous product according to the European Commission and the Globally Harmonized System of Classification and Labelling Chemicals (GHS), managed by the United Nations. However, due to its ability to bind DNA, a special care must be considered while handling it. For this reason, in this session Hoechst 33342 will be already diluted to reduce any potential hazard and lab coat and gloves will be used while performing the experiment. Further information in Hoechst family dyes can be found here:

http://www.thermofisher.com/order/catalog/product/H1399 [131]

Finally, we proceeded to study the internalization of the nanobots within the cells by the biological process known as endocytosis. Posterior to that, observation and 3D image analysis under a Leica DMi8 was effectuated (Fig. 29, 30, 31). Jointly, we attach, to graphically envision the progress achieved, some pictures that ensamble different specimens prepared with HeLa cells and addition of nanobots into the culture. We paid special attention to the amount of nanorobots entrapped within the cells, which justifies the fact of analysing different point of views of the same slice taken with the microscope. This is what we describe as 3D image analysis, because we point out all the three axis (x, y, z) that contour our reality. As a result, we used the fluorescence microscope in order to exhibit that the nanoparticles (NPs) find themselves, indeed, inside the cervix cancerous cells, preferably siding next to the nuclei. To contribute to our recompilation of studies, the following serie of figures display the internalization of nanodevices carried out by cancerous cells.

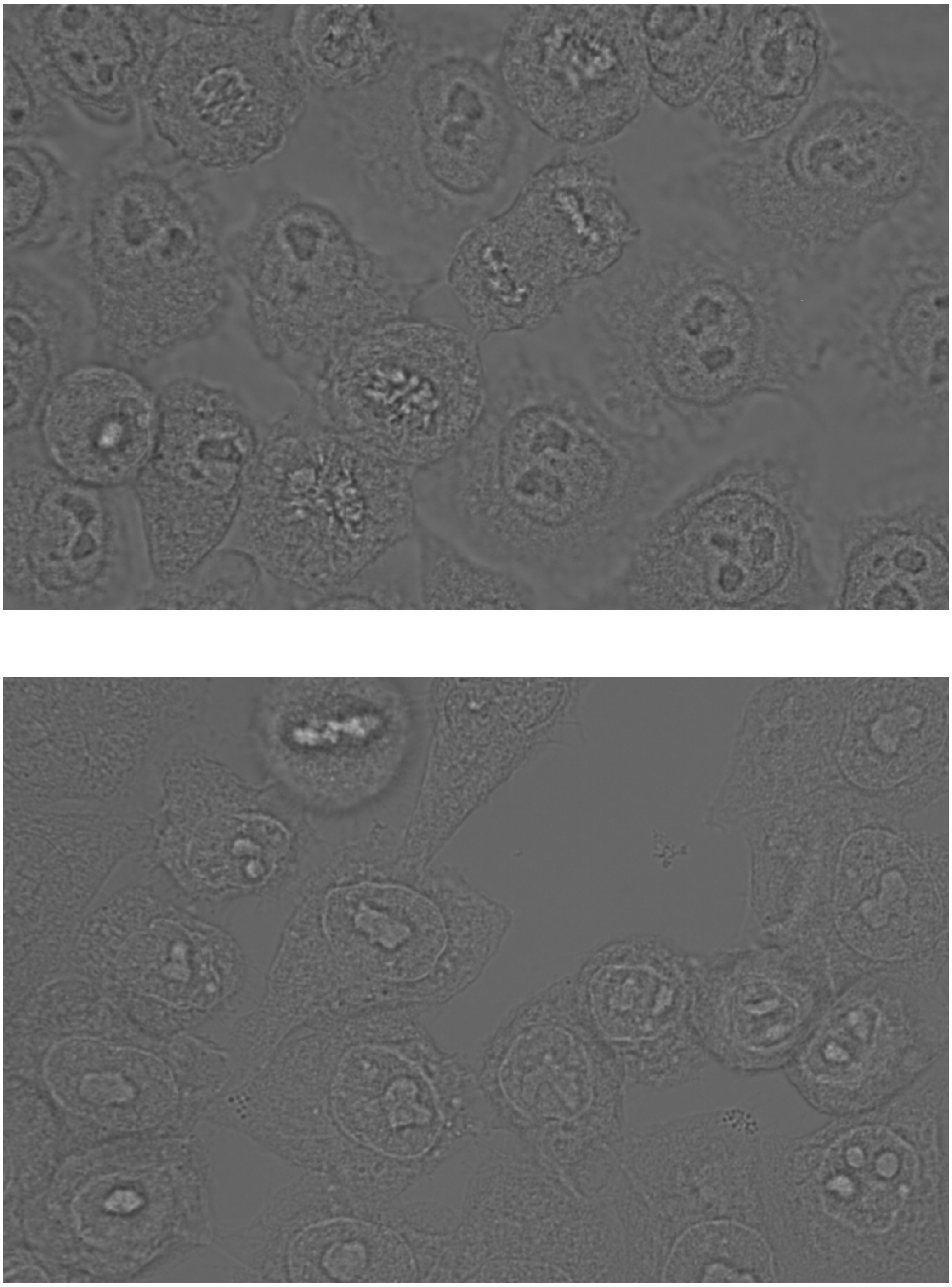
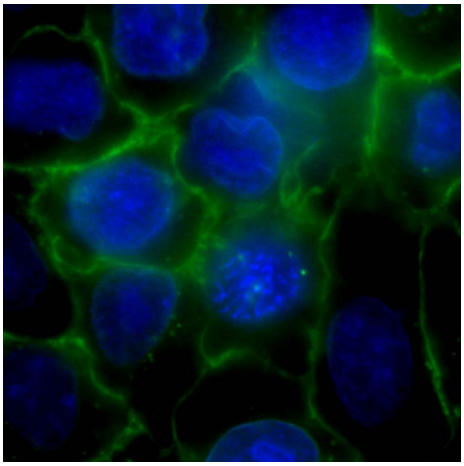
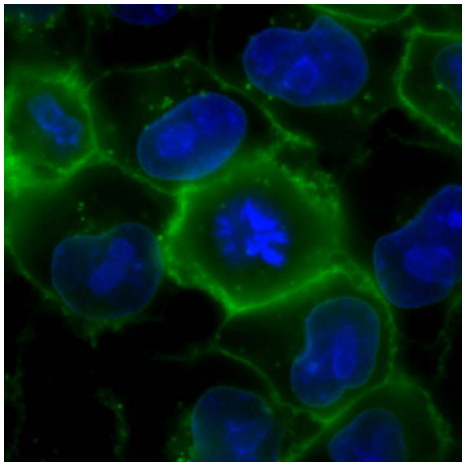


Figure 29

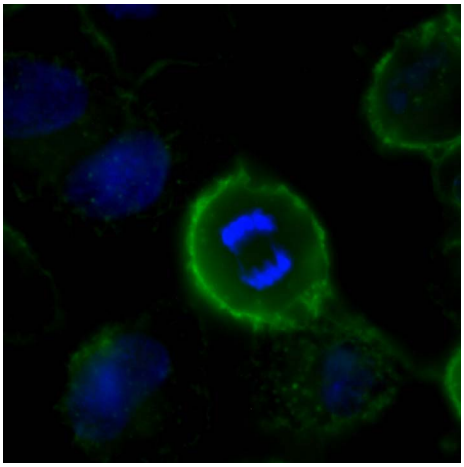
Schematic representing HeLa cells contained in the cultures prepared using the bright field of the fluorescence microscope. It looks resembling similar to what we would acknowledge via using an optical microscope resolution. Scale of grey permits the further analysis of the cell shapes and visualization.



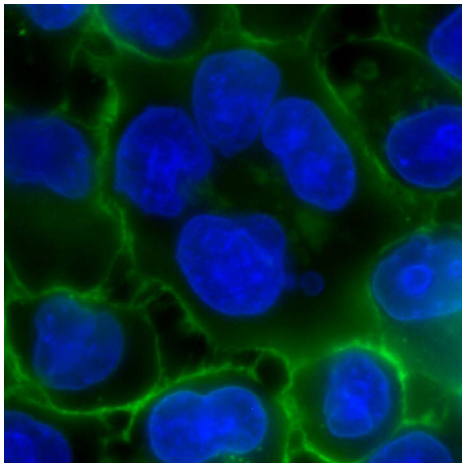
a)



b)



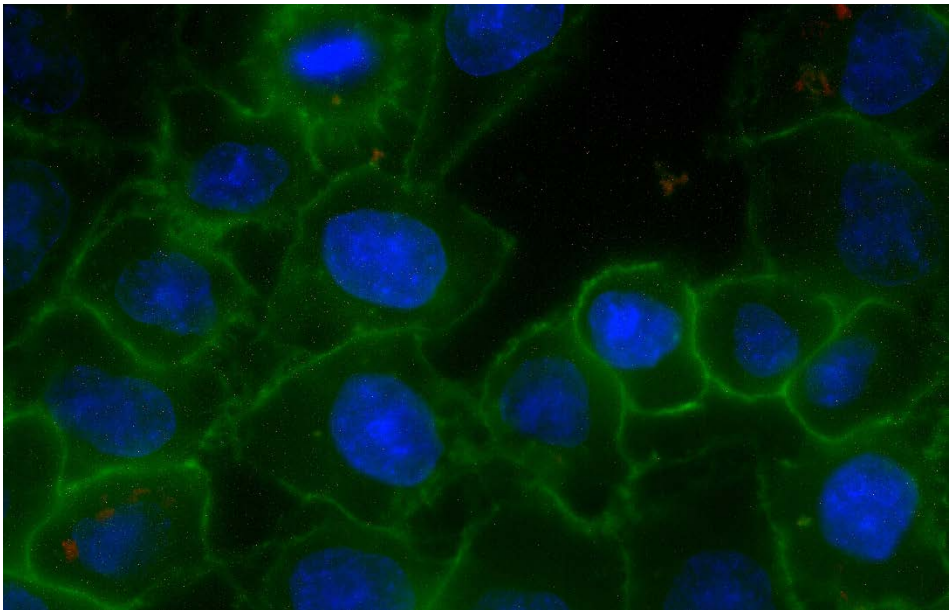
c)



d)

Figure 30

Visualizations of HeLa cells without nanomotors internalization because the photographs depict the control culture, that's to say, these pictures exhibit 3D images in which no expected nanorobots should be seen as the normal cellular culture is prepared without any kind of nanobots addition. The objective of this group is to have a reference when compared to the HeLa cells with nanobots. Likewise, note that it could be utilised in order to compare fluorescence agents from both cultures and quantify the background noise so that it could be subtracted in posterior analysis. Description of cell cycle in which the singular cells of the culture are found. a) central cell represents the prophase during division cell cycle, so we may distinguish how the nuclear core has been already destroyed, and chromatin fibers are condensed enough in order to be visualised as chromosomes via fluorescence microscope and they are also dispersed within the entire cell core (nucleus) b) we may assume that on the second figure (top right) is prometaphase shown because the chromosomes, led by their centromeres, migrate to the equatorial plane in the midline of the cell. This region of the mitotic spindle is known as the metaphase plate. The spindle fibres bind to a structure associated with the centromere of each chromosome called a kinetochore. Individual spindle fibres bind to a kinetochore[o] structure on each side of the centromere. Later on, the chromosomes will continue their condensation process. c) During telophase, the final stage of mitosis, the nuclear membrane reforms around the chromosomes grouped at either pole of the cell, the chromosomes uncoil and become diffuse, and the spindle fibres disappear. d) Not able to distinguish qualitatively the state of the particular cells enclosed.



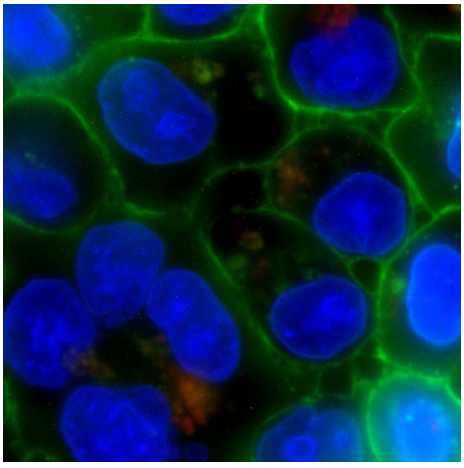
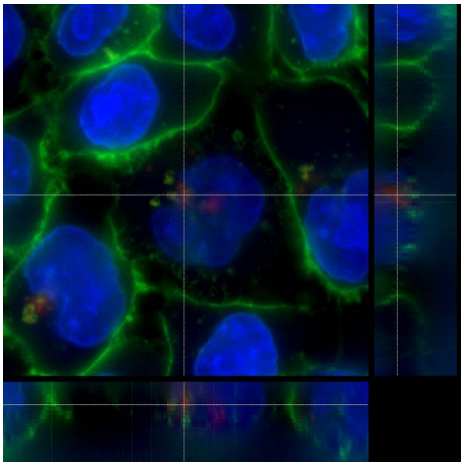
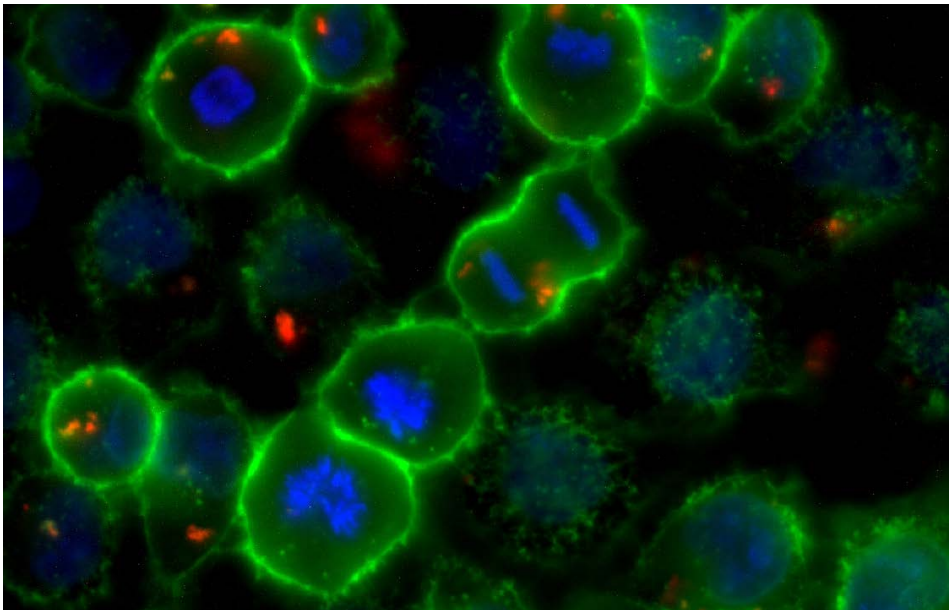


Figure 31

Internalization of spherical nanoparticles with HeLa cells. 3D images taken by a fluorescence microscope (Leica DMi8) showing the cell labeling and imaging acquisition; it can be distinguished how cell membranes were marked with wheat germ agglutinin (WGA, green), nuclei were labeled with Hoescht (blue) and, furthermore, red emission came from the nanoparticles encapsulated.





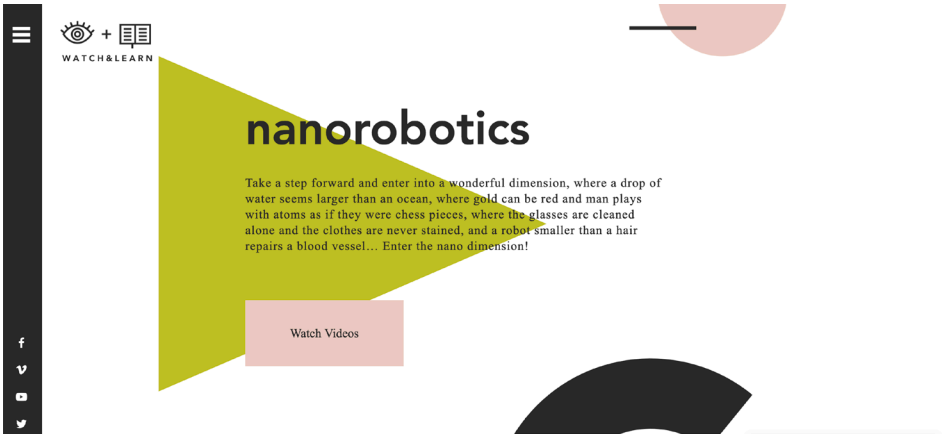
Dissemination: dev

platform →

In order to welcome you as my audience into the nanodimension, I have decided to develop a web page. This way, divulgation regarding the nanoscience, a science of such an ever-decreasing scale, will take place at a larger scale. So, the scope of explaining the most important aspects of this breakthrough on an online platform is thought to be a didactic (watch and learn) tool, where not only biomedical applications are discussed but also gadgets related to our daily-basis life. Briefly, the scrolling-page divides its content into four main groups, that's to say, we may distinguish between a first introductory paragraph where the topic is presented; secondly, a futuristic video upon which current novel carriers are assessed; thirdly, a panel with diverse themes such as nanoparticles, local probe microscopes and nanofabrication and, finally, the origin that details how scientists imagined the impact that nanomachines would have over human societies.
Click and enter the nanodimension:

https://filoteacrasovan.wixsite.com/nanorobotics!

velopment online



**Template inspiration was found thanks to wix.com numerous layouts.*

Eth
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There are important ethics issues related with nanotechnology and the presence of nano-robots inside the human body. On one hand, questions raised are related to the use of the nanotechnology in medicine. Huge amount of money should be invested in the nanotechnology research in order to reach a stage when the achieved results could be applied in hospitals. One could argue that all this money would be better used to provide medical care to people that live in poverty and cannot have access to the minimum level of health treatments and/or vaccinations. On the other hand, only rich people could afford the expensive treatments that will use nanotechnologies and this will lead, without any doubt, to an even larger gap between the social classes. Another interesting ethical problem that nanotechnology poses is: if there should be any limit in applying new techniques and devices in medicine with the only aim to improve the functionality of the human body. There are different views on this: a "purist conservative" (usually called "bioconservative") group of researchers state that one cannot touch the body just for the sake of improving some functionality in the case of a healthy person, and the only acceptable reason of an intervention would be to heal a disease or repair an imperfection, whereas the "modernist" (so-called "transhumanist") group accepts any type of intervention on the human body be it for improving or for treating a disease. The interventions that target improving of functionality can also bring disproportionalities inside society. The conservative group also states that one cannot actually improve the body functionality in case of a healthy person, because a healthy body operates at optimum parameters. This point of view resembles a christian (or theist) anthropology, according to which, the person is created on the resemblance of God, the body itself being in a perfect harmony. In any case, accidents could happen during time and the body could lose its initial "perfect" state.

Taking into account that the human person is a unity (mind and body or soul and body) any foreign device/organism that enters and stay for a shorter or longer time inside our body might affect its optimized functioning. There is a perfect equilibrium and harmony between all the organs that compose the human body. One can regard the human body as a "microcosmos". The development of the brain/mind is done in relation to the body that hosts it. There is an indestructible link between them and each person has its own specificities. More and more in medicine the concept of a customized treatment is present and physicians consider any patient is unique.

Any alteration in an organ of the body affect the other ones and, in consequence, any organ removal or foreign organ/device/robot that enters it will force the mind to make an effort to adapt itself to the new part that now resides inside the body. Of course, we expect that the less time a nanorobot stays inside our body, the less impact it will produce on the body as a whole.

Our body has its well developed protection mechanisms, one of them being the so-called Blood Brain Barrier (BBB) that restricts access of large or hydrophilic molecules into cerebrospinal fluid. Let's imagine the situation investigated in our work in which robots were injected into the brain itself, with the aim to stop a brain cancer. In these situations the foreign devices will become in facto parts of our brain as the BBB should be overcomed when injecting them in order to make their effectiveness high enough. Depending on the tumour in treatment,

the nanorobots should stay a longer or a shorter time inside the brain. We do not know how these nanorobots will affect the brain functionality or if a well recovery of its functionality is achievable. Little is known about how strange/foreign micro or nano-machines could interact with the human brain and the effects they could have on it. There are plenty of steps and clinical tests to be performed in order to assure that the interventions with nanorobots are safe or that the side-effects are not critical such to impede their use.

One should make sure that after the intervention all of them (as they conform a colony) have been taken out of the brain and that the brain goes back to its sane normal state. Increased efforts are done to achieve both BBB penetration and safe removal of the nanorobots after surgery.

Another ethical issue related to nanorobots use in medical surgery is the decision-making that a robot is doing every time it acts. Many voices in the ethics commissions and committees insist in having always a person supervising the robot's work. They claim that the final and critical decisions should be taken by a human being not by a machine. An ideal surgery team would be a remotely driven nanorobot or nanorobot colony assisted by a surgeon similar to the case of the da Vinci Surgical system [1] that successfully acts in a broad range of surgical cases spanning from urological till cardiologic ones.

The problem of decision making by robots, and the underneath artificial intelligence algorithms they are using, is heavily debated by ethical commissions [1] and we often underestimate their impact on our day-to-day life. More transparency is required with respect to the decisions the robots are taking, that are somehow hidden for the ones that use it, and the debates towards the establishment of a general accepted code of conduct should be encouraged [2].

Hypothesis Review

At the time of reevaluating the hypothesis affirmed in the beginning/introduction, we must identify that in no quantitative manner results should be shown, because there are not sufficient robust methods that could attribute the value of efficiency upon the drug delivery design applied by the nanocarriers previously postulated. So, in the end, the only effective way to exhibit a qualitative kind of final result, as for the nanorobotics possibility for astrocytoma treatment, is by demonstrating that we currently acknowledge their theoretical functionality, capability of carrying out their objective into the target and, furthermore, the efficiency of shape aerodynamics. However, no schematic trials have been published. Overall, no practical proof has been presented in order to dictate that it's the most effective treatment for brain cancer. Thus, here, the superlative may be bold.

Hypothesis 1: Nanorobotics may be the most efficient, effective and minimally-invasive treatment pathway in order to cure brain cancers, such as the already known tumor cells called astrocytomas of grade first and second.

While effectuating the globality of this Scientific Project, two more conclusive hypothesis were found to be interesting and attractive in order to be brought into light. Perhaps, next research phases will give us the opportunity of ascending the theoretical issues exposed into experimental results, already optimized as for the considerations needed to note and take into account. Hereafter, we show that there would be two evolving and possible redirections of the study carried out in order to prevent background noise, that's to say, two more fascinating possibilities to scrutinize the limits and secrets of the intrinsic.

Hypothesis 2: Allotropic shapes of carbon like monolayers of diamond could conform the base consistency of the nanorobotic structure, that's to say, resistance and other natural carbon characteristics should achieve an enhancement of the surface biocompatibility. Simultaneously, risk of further immune system reactions should be lowered as the nanorobots are not considered external pathogens original from the outside world.

Hypothesis 3: As many studies establish their fundamental pillars recalling how biomimetic systems work, reaching the optimized power of bees colonies (via a decentralised manner) may be an organisational structure interesting to investigate. Swarm of nanorobots and nanomachines could be copying already existing trophic chains with the finality of absorbing our mother nature knowledge.

Conclusions

Our problem statement is the non-effective pathways for treating astrocytoma (grade I and II). In conclusion, the study presents informations sectionised by four general phases, which are: the exhaustive state of the art, which, sequently, is divided in three grand parts, methodologies always used in every scientific and not scientific process, the nanorobotics applications in the field of nanomedicine and the anatomy and neurophysiology of the brain.

We have explained that administering a drug into the brain cavity is challenging because of the BBB, which is a membrane that creates a very selective tunnel between the CNS and the rest of the human body, that's to say, in the human organism we may distinguish two different microenvironments that hardly ever make contact. Hence, by knowing this kind of compounding structures there's a complexity added to the project. Overall, we have seen that nanorobotics may be the solution to perform minimally-invasive procedures, plus the precision when binding to selective parts of the tumor.

Secondly, the hypothetical design proposes a colony of nanorobots with the finality of detecting, targeting and binding to the diseased cells. Most importantly, it is needed to remember that after determining the nanobots functionalizations we may define the geometrical shape in order to make sure their action won't affect the circulatory system by side effects such as clogging vessels. That's why we decided to produce it as a spherical bloodborne shaped. To detect the cancer cells and, moreover, to prevent effects on the healthy tissues it is used a surface made out of diamondoid coated with an antibody, the one that may be more expressed in the tumor. (Currently, scientists are preparing a list with the chemical compound documentations we need to relate to each type of cancer. So, which are the most highlighted protein within their composition will be detailed in further and deeper investigations by interdisciplinary communities.) We note that the design proposed is inspired by the nocturnal animal red tail catfish (Phractocephalus hemioliopterus), the whiskers of which are the tactile sensor that equal to the function runned by the antibody's interactions. As a third step, talking about a device that drives under a micrometer size scale consequently means that the forces the nanorobot will be supposed to reign under the laws originated in the quantum mechanics model. Additionally, to achieve the crossing of the BBB hyperthermia is induced by an electromagnetic magnetic field. Finally, the nanorobot comprises in its core magnetic nanoparticles (MNPs, perhaps composed by iron) in order to make reference to the vascularization issue and the induced hyperthermia, that's to say, we need to activate the magnetonanoparticles to go through the endothelial cells junctions of the BBB, which need to be disrupted. Summarizing, shape, materials for manufacturing, motion, communication, and purpose are the main five grades that configure a nanorobot.

In order not to elude the appealing problem presented when sentencing brain blood barrier, we believe that nanobots may present two different types of antigens, one of which is cited below: anti-LRP-1 protein. Additionally, this particular coating may enhance the distribution of swarm of nanobots at the endothelial tight junctions' membrane, as low density lipoprotein receptor-related protein 1 is overexpressed at the outer surface of the brain. Thus, is a key signaling protein that takes part into various biological process such as cancerous ones. Hence, this

description stems from immunotherapy current methodologies and finishes with the reason why such phase is so relevant. The nanobots will then link and bind to the BBB, which after applying alternative current in order to stimuli the magnetic nanoparticles comprised (MNPs) in the nanomachines core and, hyperthermia finally induced, they may cross though the poor permeable membrane seeking to pursue their final objective: targeting the malignant cells that constitute astrocytomas. How? By using the other set of pair of proteins functionalized on the diamond surface, namely pembrolizumab. As a result, their new guidance formed by this chemical compounds, antigens, should transport the nanodevices to the brain area wherethere are more malignant in order to complete their mission: internalization by endocytosis and nanorobots disintegration by a concrete MRI agent. We expect for this new overcoming chassis to be fully embraced and it's good to mention that research should be deeper addressed and insisted for the development of smart self-propelled drug delivery vehicles and intelligent guidance for nanomotors.

Later, the blood clearance mechanisms of administered hard nanomaterials is elucidated in relation to blood flow dynamics, organ microarchitecture and cellular phenotype. Owing to the exemplified studies about elimination and excretion of the nanodevices, we have thought that they should be parallely larger than natural orifices and glomerular capillaries' fenestrations. Hence, it is needed for them to account for two-hundred nanometers. This way we also obviate the systemic inflammation or possible blockage of blood cells within the sanguineus vessels. The cross-talk between nanotechnology onto immunotherapy bases a new contemporary oncology treatment: the nanorobotics-based cancer immunotherapy. As seen, nanorobotics may be a field modulating for personalized and controllable functions, generating a more robust adaptive and durable antitumor technique. So, we can leave behind our confinement to traditional chemotherapeutic drugs, as nanoimmunotherapy becomes the mainstream of novel treatment approaches.

Upon this becoming process, the experimental section has comprised different types of characterizations as for the enzymatic-powered nanoparticles (especially fueled by peroxide due to their catalase addition) of which IBEC's group disposes. The majority of particles were ranged around the first hundred of nanometers, as it complements what has been postulated during the hypothetical design. Moreover, the practical methodologies have been studied under four groups cited as case studies. The serie of techniques used are various and will be enumerated as follows: firstly, imaging with transmission electron microscope at the CCiTUB in order to visualize mesoporous silica nanoparticles (MSNPs) of 100 μm radius was performed; next, to analyse the diverse physical features the 200 nm-spherical-nanoparticles may present, such as size composition, Dynamic Light Scattering (DLS) was used; furthermore, during the third independent case study, we focalized our study into the description and analysis of their behavioral movement to further track their trajectories, and eventually, we investigated whether spherical micromotors were internalized or not within the HeLa cells to further argument their efficiency as for drug carriers. Altogether, we focalized our study on the analysis and further visualization of nanoparticles because our aim was to comprehend the physical properties of

molecules at such ever-decreasing scale, as they describe Brownian fluctuations. Their trajectories, mean square displacement and graphical schematics representing their fittings (either linear or quadratic) enhances our conception regarding behavioral regimes, as well as introduces the future work we should base on self-propulsion (towards drug delivery). More discussion about issues like whether MRI guidance should be avoided or, adversely, achieved, should be assessed.

The next step is evaluating the characteristics that create a new conception and combination of features for the nanorobot. That's to say, in order to review the technique globally we could consider different systems of analysing the acoustic networking system that is produced by the colony of nanorobots, we also could define different configurations of astrocytomas when coding the parameters for the cerebral and brain tumor simulations. But most importantly, we seek to know if they achieve their objective: the crossing of the membrane formed by the tight junctions of the endothelial cells. In addition, it would be pretty interesting to analyse which are the navigation paths and actions that the nanorobot follows when it loses control or when the program depicts a non-frequently image such as not having diseased cells. In experimentation and results evaluation, statistical studies made from the simulation numbers will be the object of further studies.

As a final point, the ethical implications have different branches opened to deeper discussion. Ideally, the following questions summarize briefly which are the concepts that have been presented in the previous section. Do we agree with the basic thought of letting a nanodevice alter the balance found between soul and body? Do we want a nanorobot driving within our circulatory system before determining which are the side effects that make reference to a prolonged time presence of the nanorobot? Will it be only a therapeutic tool or, instead, they are meant to become permanent parts of the human body, which could permit a constant follow-up diagnosis information? How is it going to contribute to the society estates? Will it have a high impact by potentiating the gap that already exists, and goes bigger by minutes, between the developed, emerging and undeveloped countries? What do you think?

Personally, this paper has made me grow scientifically in a much more bigger scale than the highlighted along the document, the nanometers. That's to say, I believe that my acquired knowledge has shown a macro-meter impact. Interestingly, I have loved the opportunity of coming closer to the world of writing a Scientific Project, which may be the closest thing to writing a real article that could contribute the novel literature. As I have expressed in my motivation, my main scope is to go beyond the unknown legible, beyond frontiers, beyond the unseenable. Definitely, to scrutinize the profiles of the nano-worlds that are still emerging. As we know the human nature, achieving the impossible and better is the aim of science. Decreasing scales won't stagnate because of the undergoing quantum revolution. That's why perhaps in a few decades we will be welcomed to say: enter the pico-dimension!

Future Perspectives

During the evolution of this processing research, it has been noticed that further research in the discipline of nanorobotics must be administered and dosified ethically, because the multidisciplinary domain of Bioengineering is currently motivating nanoengines that present either chemical compounds, as the motor power is reserved to enzymes, or microorganisms, such as the motile cells [89] presented in the bio-hybrid approaches that require of magnetotactic bacterias [13] (in order to stimuli their mobile system ideal for biomedical applications).

Since other biochemical compounds and materials [91] have been popularized around the field towards diagnostics and treatment approaches, diamond remains an icognite for this purpose due to the lack of practical basis. It's important to mention that the majority of the researcher groups prefer the use and reutilization of liposomes (lipid conglomerates) [93, 112] and proteomic spherical nanoparticles owing to their agreability and affinity for further functionalization. However, that wouldn't resemble to a nanometer-machine. That's only because the background information of which we dispose nowadays with respect to robotic structure and architecture remains in hypotheticals. Nevrtheless, this shouldn't decline and, continuously, blockage the requirements of the hypothetical design proposed. When showing up that liposomes [90, 112] may be more suited at the time being, we should acknowledge that they have soft, deformable and biodegradable properties and, moreover, they functionally fulfill the co-delivery of multiple drugs. But due to their poor stability, engineering approaches are designed to overcomes issues of high cationic charge density and tendency to form clusters. For this, some liposomes have been modified with various specific ligands in order to enhance distribution and targeting to particular tissues sites. Yihai Liu et al. [112] shows that liposomes loaded with a long synthetic peptide should be a promising technique as a powerful cancer vaccine formulation.

It's demonstrated that nanoparticles should comprise porus [92, 93] in order to make internalization of the drug compatible. During this step, as the cancer under supervision has been astrocytoma of grade I (Pilocytic astrocytoma) and II (Diffuse astrocytoma), the drug that we should administer is temozolomide, known by the company that embraces its production as TMZ or temodar [114, 115, 117]. Due to this requisite, the diamond surface could be further enabled with super symmetrical little porus as mesoporous nanoparticles (MSNPs) shown by Samuel Sáncez et al. at IBEC's laboratory. MRI guidance is thought to excite consequent magnetonanoparticles in order to disable drug encapsulation and molecular tightness (as proposed in this project). This porus will have then the following binomial functionality: encapsulating the drug and releasing the chemotherapy medicament at the tumor sites, after targeting and binding effectively to the tumor. To be more precise, release will take place thanks to the application of an MRI contrast agent correlated with the magnetic nanoparticles (MNPs) kept inside the nanorobots' capsule. As a result, release will be enhanced because it is hypothetically expected to enlarge the cavities diameter (within the cell) via MRI. Hence, further investigation regarding the efficiency, reliableness and effectiveness as for the encapsulation of the drug and the subsequent release opens new venues of work, as well as enlightens the fact that our to do list gets longer as we might get closer to a culminating point.

Note to remember, fenestrations [5, 9, 119] of degraded microvessels are in our benefit when trying to have success in solving this challenge because those particular localisations are easier to cross. However, the BBB leakage may be uncontrollable as for the sanguineus flux circulation within the brain. That's why, as a consequence, reutilization and deeper concerns as well as examinations of the nanorobotic model proposed should be assessed in the near future. Then the paradigma will be focused on the multiform glioblastoma.

Other than synthetic coating materials, the source of biological elements in order to further compose the architecture of the nanorobot is a good choice that establishes biocompatibility and enhanced versatility. Moreover, it is well established that polyethylene glycol (PEG)-coated nanocarriers reduce immunogenicity and increase their affinity for angiogenic endothelial and tumor cells. To originate a facile camouflage mode, regardless the core compounds, coating nanorobots with amphiphilic polymer may assure water solubility and, respectively, biocompatibility, which should be taken into account to improve specific complications of the hypothetical design announced.

Alternatively, some novel literature has presented the idealistic methods of administering the nanoparticles intranasally, which is quite acute, as no blood brain barrier will appear on their pathway.

Future work on in situ guidance methods, such as the use of pH, thermal, or chemical gradients with the finality of attracting nanomotors in vitro and in vivo needs to be addressed. The reason for this is to "catalyse", again, ulterior development of smart and self-propelled drug delivery vehicles based on enzyme catalysis [89].

As a matter of fact, when describing the communication between the swarm of nanobots, we have showed that acoustic communication was one of the best suits in order to achieve a decentralized power, at the same time as this feature plays an important role for overcoming controversial fluctuations and time attenuations. Nevertheless, to come back at our scientific model and representation of physical and biochemical phenomena, which take place in reality, no such intelligence has been yet constructed. That's why, in a first place, chemical tracing may be the principal acquisition of the nanodevices leading to the completeness of their functionality. Hence, two kind of division regarding nanorobots were thought to be best,as capability of receiving the coded message to become the crucial part of the attacking process. Briefly, we would be exploiting two sides of the same action: first of all, the searching/navigating-nanorobots reporting either their findings or their status and, second of all, the nanorobots-actuators. Several simulations shall be carried out with the finality of investigating how Conway's Game of Life may guide us to the bottom of the room, where there's still plenty of room for unexpecting, surprising, innovative and futuristic breakthroughs. As Richard Feynman's urged at one of his famous lectures: tiny machines may be the new becoming surgeons, so we are the ones, the researchers after him, who will set the limit for the known and unknown. Philosophically speaking, we should alegate that the work carried out with this Scientific Project shows how humanity tends to comprehend the uncertainty of our world through one dynamic vision. Perhaps we may embrace a non-reductionist model of consulting reality in which the final scope is seeking the absolute truth. A world where all the existing subdisciplines of vigent Paradigmatic Knowledge converge in their unity, that is, their essence.

nanorobot device,
nanoscopic engine which may be able to overcome three different characterizations, including diagnostics, therapeutics plus inclusion of nanomedicine;

nanocarrier,
whichever device designed at the nanometric ever.decreasing nanoscale able to adminster drug either passively (passive nanocarrier) or actively (active nanocarrier);

synthetic micro/nanomachines,
man-made micro- and nano- scale devices capable of performing assigned tasks;

molecular machines,
molecular components assembled to produce mechanical work in response to specific stimuli;

biocatalytic energy,
energy obtained through biological conversion of chemically free energy; propulsion,

physical force that provokes motion;
enzymatic catalysis,
increase on the rate of a given reaction caused by the active site of a protein;

micro/nanomotors,
micro- and nanoscale devices capable of converting energy into active motion;

Vocabulary

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