UNIVERSITAT POLITÈCNICA DE CATALUNYA
BARCELONATECH
Escola Superior d'Enginyeries Industrial, Aeroespacial i Audiovisual de Terrassa

Motility study of shaped active colloids: Bacteria and Janus Particles

Treball realitzat per:
Giacomo Andreoni
University of Pisa

Dirigit per:
Tzanko Tzanov (director)
Julio Bastos-Arrieta (co-director)

Programa:
Erasmus+ for Study

Departament:
Departament d'Enginyeria Química
Grup de Biologia Molecular i Industrial (GBMI)

Terrassa, Juliol 2021

TREBALL FINAL DE MÀSTER

"Nun te reggae più! "

Rino Gaetano

RESUM

Durant l'última dècada s'han intentat desenvolupar noves tecnologies capaces d'interactuar amb sistemes a escales dimensionals molt baixes, des de nano fins a micròmetres, i realitzar tasques molt específiques. L'interès per aquest tipus d'investigacions sorgeix de la possibilitat de realitzar operacions de manera localitzada i controlada. Una de les tecnologies més prometedores és el concepte de Micronedador (*Microswimmer*), que pertany a la classe més àmplia de col·loides actius. En conseqüència, la manera d'arribar i operar en sistemes a escala micro / nano consisteix a utilitzar materials caracteritzats per una escala dimensional comparable a la dels sistemes amb els quals interactuaran. A la vista d'això, els micronedadors es poden definir com a objectes molt petits, funcionalitzats i optimitzats per realitzar accions específiques a través del seu moviment, com el lliurament de fàrmacs, la detecció d'analits o l'activitat antibacteriana d'esdeveniments.

El desenvolupament d'aquest Treball de Fi de Màster (TFM) és multidisciplinari, ja que requereix aspectes de moltes branques de la ciència: física, microbiologia, química i enginyeria de materials. En primer lloc, inclou el procés de construcció de micronedadors artificials (partícules Janus) i biològics (bacteris no patògens), definint un conjunt de metodologies per a la seva preparació. La caracterització d'aquests materials inclou tècniques instrumentals com la microscòpia òptica, la microscòpia electrònica i l'anàlisi de la càrrega superficial pel potencial Zeta. Posteriorment, es desenvolupa la caracterització de la mobilitat d'aquesta classe de col·loides actius, específicament per a bacteris i partícules de Janus, mitjançant el tractament d'imatges i vídeos, amb el programari gratuït *ImageJ*; a més de la creació de macros pròpis, per a l'anàlisi personalitzat dels materials. Finalment, es pretén explicar els diferents comportaments observats en diferents condicions d'estudi, amb perspectives futures per a aplicacions en camps com la nanomedicina, la detecció electroquímica o els agents antimicrobians. Aquest TFM s'ha dut a terme com a part de la Convocatòria 2020-2021 del programa de mobilitat internacional *Erasmus+ for Study.*

RESUMEN

Durante la última década se han realizado muchos intentos para desarrollar nuevas tecnologías capaces de interactuar con sistemas a escalas dimensionales muy bajas, desde nano hasta micrómetros, y realizar tareas muy específicas sobre ellos. El interés por este tipo de investigación surge de la posibilidad de realizar operaciones de forma localizada y controlada. Una de las más prometedoras entre estas tecnologías resulta ser el concepto de Micronadador (*Microswimmer)*, que pertenece a la clase más amplia de coloides activos. Según este nuevo paradigma, la forma de llegar y operar en sistemas en la micro/nano escala, es utilizar materiales caracterizados por una escala dimensional comparable a la de los sistemas con los que van a interactuar. A la luz de esto, los micronadadores pueden definirse como objetos muy pequeños, funcionalizados y optimizados para realizar una acciones específicas a través de su movimiento, como la administración de fármacos, detección de analitos o actividades antibacterianas.

El desarrollo de este Trabajo Final de Máster (TFM) es multidisciplinar, pues requiere de aspectos de muchas ramas de la ciencia: física, microbiología, química e ingeniería de materiales. En primer lugar se presenta el proceso de construcción de Micronadador Artificiales(Partículas Janus) y Biológicos(Bacterias no patógenas), definiendo un conjunto de metodologías para su preparación. La caracterización de estos materiales incluye técnicas instrumentales como microscopia óptica, electrónica y análisis de carga superficial por Potencial Zeta. Posteriormente, se desarrolla caracterización de la movilidad de esta clase de coloides activos, específicamente para Bacterias y Partículas Janus, mediante el tratamiento de imágenes y videos, con el software libre *ImageJ*; además de la creación macros propios, para el análisis personalizado de los materiales. Por último, intentaremos explicar los comportamiento diferentes observados en diferentes condiciones de estudio, con perspectivas futuras para aplicaciones en campos como la nanomedicina, detección electroquímica o agente antimicrobianos. Este TFM ha sido realizado como parte del programa de movilidad internacional de la Convocatoria 2020-2021 del Programa *Erasmus+ for Study*

SUMMARY

During the last decade many attempts have been made to develop new technologies capable of interacting with systems at very low dimensional scales, from nano to micrometers, and perform very specific tasks. Interest in this type of research arises from the possibility of conducting operations in a localized and controlled manner. One of the most promising among these technologies turns out to be the concept of Microswimmer, which belongs to the broadest class of active colloids. Accordingly, the way to arrive and operate in systems on the micro/nano scale is to use materials characterized by a dimensional scale comparable to that of the systems with which they will interact. In light of this, microswimmers can be defined as very small objects, functionalized and optimized to perform specific actions through their movement, such as drug delivery, analyte detection or event antibacterial activity.

The development of this Master's Degree Thesis (MDT) is multidisciplinary, since it requires aspects of many branches of science: physics, microbiology, chemistry and materials engineering. Firstly, it includes the process of construction of Artificial Microswimmers (Janus Particles) and Biological (Non-pathogenic Bacteria), defining a set of methodologies for their preparation. The characterization of these materials includes instrumental techniques such as optical microscopy, electron microscopy and analysis of surface charge by Zeta Potential. Subsequently, characterization of the mobility of this class of active colloids is developed, specifically for Bacteria and Janus Particles, through the treatment of images and videos, with the free ImageJ software; in addition to the creation of own macros, for the personalized analysis of the materials. Finally, it is aimed to explain the different behavior observed in different study conditions, with future perspectives for applications in fields such as nanomedicine, electrochemical detection or antimicrobial agents. This MDT has been carried out as part of the Call 2020-2021 of the international mobility program *Erasmus+ for Study*.

RINGRAZIAMENTI (AGRAÏMENTS)

Grazie al professor Tzanko Tzanov per avermi concesso di potermi mettere alla prova, accogliendomi presso il suo laboratorio.

Un immenso grazie ai miei compagni, a Julio, Antonio, Marina, Pol, Aleks e tutto il gruppo GBMI grazie al quale ho potuto sentire il sapore di casa, quando quella casa era lontana.

Alla mia famiglia, perchè se posso parlare di casa è grazie al loro affetto e supporto, che mai mi è stato fatto mancare.

A Nenè, grazie alla quale mi sono reso conto che casa non potrà mai essere troppo lontana, perchè tutto il mondo diventa casa se vissuto con le persone giuste.

Infine, a tutti quelli che hanno creduto in me.

Ed a quelli che ancora devono farlo.

El futuro es de los valientes.

TABLE OF CONTENTS

INDEX OF FIGURES

LIST OF ABBREVIATIONS

APTES	(3-Aminopropyl)triethoxysilane
FAD	Forward angle distribution
MDT	Master's Degree Thesis
MSD	Mean Square Displacement
MSw	Microswimmers
NPs	Nanoparticles
PDDA	Poly(diallyldimethylammonium chloride)
SD	Self-diffusiophoresis
SDA	Square Displacement Analysis
SE	Self-electrophoresis
TEOS	Tetraethyl orthosilicate
TFM	Treball de Fi de Màster
TFM	Trabajo Final de Máster
UAB	Universitat Autònoma de Barcelona
UPC	Universitat Politècnica de Catalunya
WHO	World Health Organization
ZP	Zeta Potential

1 INTRODUCTION

Modern medicine is in a state of upheaval. On the one hand, the technological progress of the past few years have brought a personalized medicine within reach. On the other hand, the Death rate as a result of longstanding defeated bacterial infections due to the increased occurrence (so-called. super bugs) multidrug-resistant germs dramatically. The spread of antibiotic resistance is today one of the greatest medical challenges, as recently reported in a global study provided by the World Health Organization (WHO)[1], [2].

Such tasks require, in particular, novel, innovative and often multidisciplinary approaches as the foundation for the development of new future technologies, based on nanomaterials and other active colloids. In the therapeutic range have the potential so-called. "Delivery on demand" approaches to overcome for example antibiotic resistance[3], [4]; as in the illustration in Figure 1. An active colloid is a suspension of particles capable of converting free energy from their environment into movement. Artificial (synthetic self-propelled particles) and Biological (Bacteria) microswimmers are well-known examples of active colloidal systems. Microswimmers(MSw) use the surrounding energy to carry out intrinsically non-equilibrium activities such as growth, replication and self-propelled motility[5]. The motility is achieved by the creation of field gradients localized around their bodies when they consume fuel or are heated by laser light. These gradients can change in orientation and thus thereby their propulsion direction; in contrast to passive particles which move along an externally set field gradient[6]. Different factors influence the phoretic activity of these systems, like particle shape and chemical composition.

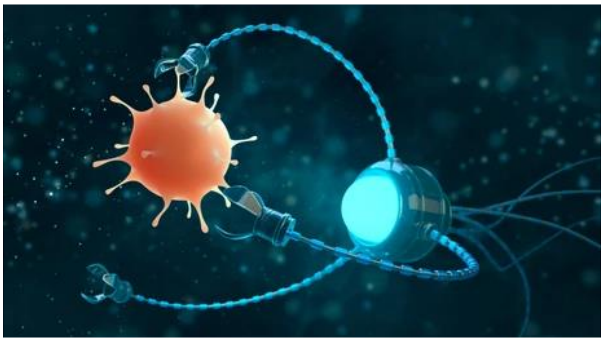


Figure 1: Schematic illustration of a Microswimmer interacting with a pathogenic agent.

Artificial MSw field has developed rapidly regarding preparation approaches, motion control, functionalization and propulsion strategies[7], [8]. Basically, three different mechanisms are attributed to catalytic generation the motion of catalytic micromotors: bubble-propulsion, **self-electrophoresis**(SE) and **self-diffusiophoresis**(SD)[9], [10] (See Figure 2). SE mechanism requires a self-generated electric field and a charged swimmer surface; which lead to an asymmetric distribution of the ions generated from electrochemical reactions of the fuel (oxidation and reduction) on the MSw surface. The most common fuel for electrophoretic motors is H_2O_2, thus, the oxidation reaction produces protons, while the reduction reaction consumes them. This produces a proton concentration gradient, establishing an asymmetric charge distribution and electrical dipole around the micromotor. The dipole generates a local electric field, inducing an electrical body force in the fluid and an electrosmotic slip around the MSw[11]. In SD mechanism, a colloidal particle composed by a non-conductive core material half covered by a catalyst moves in response to solute concentration gradient (propulsive force) caused by the chemical reactions that take place on the colloid's different surfaces. This concentration gradient induces a gradient driven fluid flows around the particle. For instance, Pt@SiO_2 Particles in H_2O_2 solutions are considered largely self-diffusiophoretic motors, as the decomposition of H_2O_2 takes place only on the Pt surface and therefore concentration gradients of solute that drive the movement are generated[10], [11]. Nevertheless, SD seems not to be the only operative mechanism in these micromotors, as some electrokinetic effects have been observed; for example an ionic current passing between the pole and equator of the Pt half-cap; that creates a local electric field and therefore SE[12], [13]. Therefore, it is important to further understand the locomotion driven forces interactions of different set of hybrid MSw particles in H_2O_2 solutions.

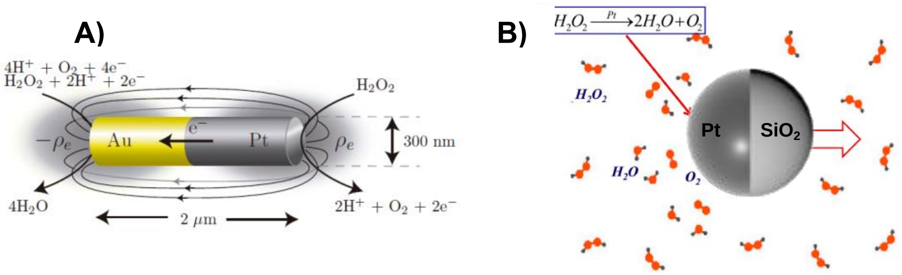


Figure 2:Schematic representation of the main movement mechanisms of artificial microswimmers: A) Self-Electrophoresis and B) Self-diffusiophoresis[14], [15].

The behavior of artificial microswimmers resembles strongly what we observe in natural motile microorganisms, like bacteria, molecular motors, algae or even sperm cells[16], [17]. A combination of them, in the so called biohybrid MSw systems the efficient swimming capabilities of natural systems and the versatility of artificial objects. **BacteriaBots** are a combination of motile bacteria as biological microswimmers coupled to inorganic functionalized particles[18] (See Figure 3). They offer a broad range of potential applications that come with the beneficial properties of both parts. The most critical point to create BacteriaBots are the coupling strategies, which need to be biocompatible, instantaneous, not oxidizing and strong enough to withstand the onset of motion. The features of the resulting hybrid bio-MSw on surface properties of the inorganic constituent as well as the outer bacterial layers and the impact on the behavior of the BacteriaBots in comparison with artificial microswimmers. The development of an efficient Bacteriabot as a biocompatible form of customized delivery for biotechnological and biomedical applications aims to exploit synergistic effects between bacteria, artificial microswimmers and materials science, to create an innovative mobile access to personalized medicine. [19]

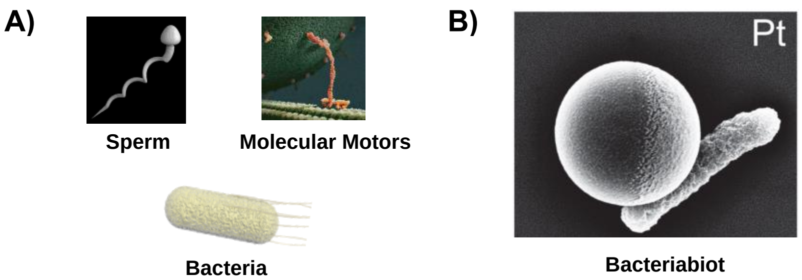
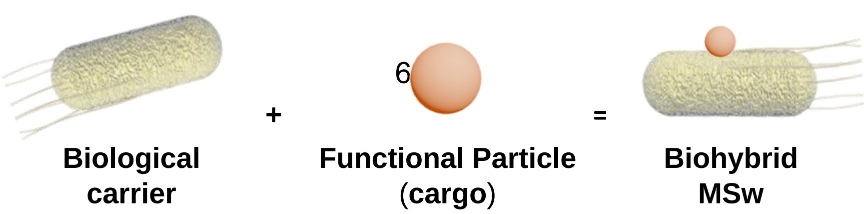


Figure 3: A) Biological microswimmers and b) biohybrid artificial – biological microswimmer[20]

Three basic building blocks are required for the preparation of a biohybrid microswimmer like bacteriabots. The first one is a **movement actuator or carrier**, by means of which the microswimmer is able to move around and reach the area of interest to perform a customized delivery[21]. Usually, for those kinds of systems the fuel is needed and used directly from the surrounding medium where the MSw operates; in this sense the main target is an environmentally friendly and biocompatible fuel. The second fundamental block is the **container – cargo entity**, as the desired molecule, drug or object to be transported (See Figure 4). Generally speaking, a cargo could be identified as every component belonging to the hybrid MSw structure which is not directly responsible for the movement generation. Examples are nanoparticles, drugs, or molecular dyes[22]. A third main component or element to be considered regard the control from an external stimulus, allowing them to be driven in a controlled manner in the system where they are operating.



So far, the microswimmers has been proposed for a variety of applications. Most relevant examples are the contaminated water treatment, drug carriage and antibacterial activities, even if the ability to engineer them allows them to be employed for very advanced and bizarre tasks, as could be the micro stirring[23]. Nevertheless, it is highlighted the use of microswimmers with antibacterial properties. In this context there are two basic applications [24], [25]. The first one regards the capture and eventual killing of bacteria. It must be stressed that not every type of microswimmers is able to accomplish every type of job. In fact, as we can often find in literature, some of them are able just to move, others just to attach to bacteria cells, and just a few of them can perform all the steps required to reach and kill the bacteria[26]. The second one instead is related to the destruction of the biofilm created by the bacteria as a defensive measure against threatening agents for themselves[27], [28].

1.1 Movement Actuation

There are mainly two lines of thought concerning the development of such systems. On one side the problem can be defined in a “biological framework”. A living being of microscopic dimension (i.e. bacteria) is used as a propulsion carrier. The natural ability to swim of bacteria is exploited in this context and, since the movement stems from a natural capability. For what concerns the cargo, different interaction (e.g. electrostatic, physical, chemical) which take advantage of the charge in bacteria surface are used. In this view, a biohybrid system is created[29].
Although many mechanisms have been proposed to take control over bacteria swimming trajectory, the more effective ones seem to be the ones related to the use of magnetic force[30]. For example, some bacteria present a magneto tactic behavior which allows them to align themselves to a magnetic field. Even more elaborated solutions can be found in literature, such as magnetic caps for instance, which represent the true state of art of this subject[31].

On the other hand, we can find a completely different approach to the problem, dealing with artificial MSw. Regarding the propulsion mechanism, for those last it's necessary to adopt a different strategy with respect to the first ones. The first condition of utmost importance is the laminar regime due to a low Reynold's number to which a system at this dimensional scale is subjected. At this stage, laminar flow is expected, in which viscoelastic interactions are the predominant, meanwhile at large scales (macroscopic world) turbulent interaction are prevalent (See Figure 5) [32].

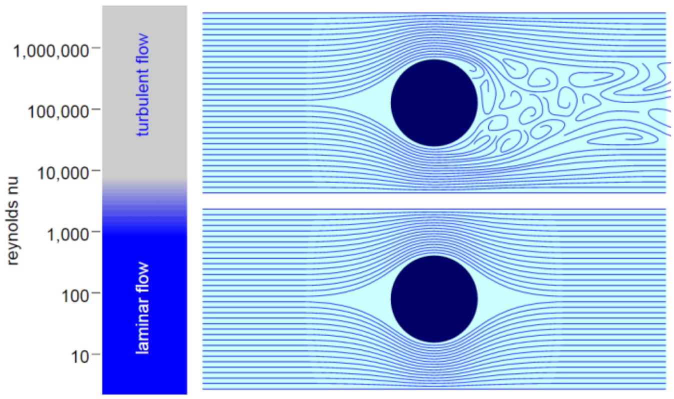


Figure 5:Representation of Reynold's Regime[32]

This has an enormous impact on the limitations concerning what mechanism can be effectively adopted, because. According to the Scallopp's theorem in fact, the movement cannot be based on the continuous repetition of an action which can be time-reversed, like a scallop does, and this fact rule out all those mechanisms that involve rotating or moving part with the aim to imitate the behavior of the "macro" counterpart (eg. boat helices) [11], [33]. To overcome those problems, the best solution appears to be the employment of Janus particles. The name of these particles recall the peculiar characteristic of their structure in analogy to the roman god Janus, who possessed two faces.
In fact, regardless of the shape (rods or sphere), Janus particles are constituted of two faces of different chemical composition (two materials) or physical properties (e.g. different hydrophobicity), as shown in Figure 6. This fact creates an asymmetry that in a final stage is exploited, jointly to various mechanisms ranging from SE, SD or bubble expulsion, to allow the system to move in a liquid medium at low Reynold number regime.[34]

As the propulsion mechanism is fundamentally different from that of the bacteria, able to swim, here a very big contribution to the particle flow is found to be given by the shape. For this reason, in the process of swimming optimization not only do we look for the best environmental conditions (i.e. swimming media, diffusion mechanism), but also for the best shape, which does not always turn out to be the most logical from a hydrodynamics point of view [35].

A) B)

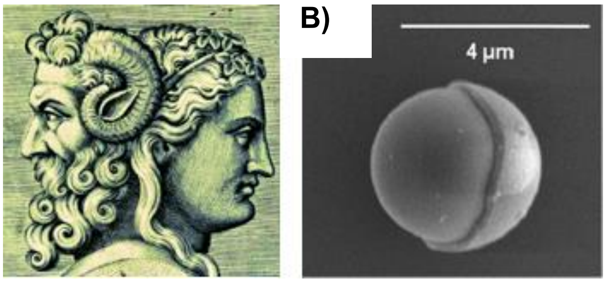


Figure 6:A) Double Face Janus Godness and B) Electron Microscopy image of a Janus particle [36].

1.2 Cargo attachment to a biohybrid microswimmer

Principles similar to the previous ones are used since even the particles have a surface charge(See Figure 7). In addition, thanks to the ability to functionalize the particle surface with various elements and composites, in the case of Janus particles there exists a wider range of methods employable to attain the cargo attachment. Even though science of micromotors synthesis has experienced a fast acceleration in recent years, defining a set of very sophisticated methods to build up virtually any kind of thinkable combination of shape/composition micro-objects, in this case again the introduction of magnetic component appear to be the most effective solution to drive Janus particles. [18], [37], [38]

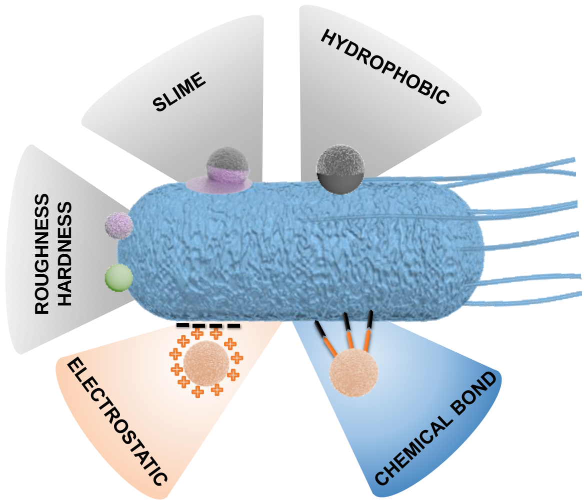


Figure 7:Attachment strategies for the preparation of Bacteriabots [18]

2 OBJECTIVES

Despite several examples the influence of shape on the features of a Biohybrid MSs has not been extensively analyzed and many issues remain to be understood; including the viscous forces exerted by the flow, which depend strongly on the body geometry[18], [37]. However, the lack of simple and reproducible preparation strategies for different shaped nano- or microparticles makes studies relatively difficult. **The general objective of this work is to address the shape influence (spherical or rod) on the movement of artificial or biological microswimmers.**

Accordingly, there has been stablished some specific objectives:

- Preparation and morphological characterization of shaped silica particles (Spheres and Rods)
- Chemical and physical functionalization of prepared particles to obtain different surfaces charges and Janus particle configuration.
- Morphological Characterization of rod shape biological microswimmers (bacteria)
- Evaluation of Brownian motion of the prepared artificial MSw by optical microscopy
- Evaluation of active movement of both types of MSw by optical microscopy
- Development of informatic tools towards a more efficient assessment of MSw movement behaviour.

3 METHODOLOGY

3.1 General Approaches

The very first step in MSw production is the synthesis and consequent functionalization of the particles. Due to the fundamental difference between bio-microswimmers and artificial-microswimmers, the set of procedures and protocols concerning the two types of active colloid are different. In this regard, the synthesis process for the microswimmers types will be treated separately.

3.1.1 Janus Particles

As one could expect, the main issue concerning the production of Janus particles is related to the ability to build up a unique particle well defined in shape and made up of two distinct materials. The proposed solutions toward this subtle problem have been developed greatly during the years. Despite the very broad variety of techniques adopted, it's possible to recognize at least two features which identify a macro-group to which practically all the synthesis methods belong and can be categorized.

3.1.1.1 Top-down method

Need to exceptionally high precision involves the usage of extremely advanced instruments. The particle dimension in fact should be in the range of nanometer up to the tenth of micrometers in order to be able to interact properly with systems in the same order of size. This requirement suggests a well-defined and established way to act, namely a template assisted technique.

The point of strength of these kinds of techniques usually is to take advantage of precise drawing techniques as well as highly controlled deposition methods. Among those techniques, like lithography it's by far the most used technique, either with light or e-beam.

To figure out what's the principle which stand behind this technique, let's imagine to draw a very simple shape(e.g. a sphere) on a paper sheet using a standard pencil. As far as one can try, even to the best of one's ability, neither Giotto itself would be able to obtain a figure with characteristic length scale smaller than that one of the pencil tip. This in such a way suggests to us that the tool "tip" dimension sets the lower scale limit for shapes drawable by the tool itself. Since in MSw production we deal with objects in the order of nano/micrometer, a "tip" featured by such dimension is required. Quantum mechanics give us insight on how to obtain this kind of instrument, stating that every particle is characterized by a wavelength which can be considered as our "tip" dimension.

Following this logic line, lithography is used to draw patterns up to the nanoscale. In the context of MSw design though, e-beam lithography is preferred due to its higher versatility. Even though the great majority of Janus particles produced by means of e-beam lithography result in a rod like shape, curious shapes such as helices are obtainable by means of this technique.

Figure 8:Electron micrograph of a micro helixes prepared by lithographic approach. [39]

In the class of deposition techniques on the other hand, the leading methods are Molecular Beam Epitaxy and Chemical Vapor Deposition. By means of these approaches not only is it possible to control with high accuracy the amount of material deposited (i.e. monolayer), but also we are able to vary its chemical composition. In change of high reliable and reproducible Janus particles, top-down techniques tend to be quite expensive due to the specific requirement needed (UHV, clean room, cumbersome apparatus).

3.1.1.2 Bottom-up method

With respect to the previous one technique, more than the ability to manipulate nanobjects, we concentrate on the capacity of self-assembling structure with defined chemistry composition. For this reason, most of the bottom-up approaches concerning microswimmers involve the usage of wet chemistry instead of a template assisted method. As dealing with Janus articles, it is required to exert precise control either on the shape or composition of the nanomaterial. The basic principle for particle shaping is to exploit the material's nanostructure characteristics (i.e. lattice structure) to encourage the growth in preferential directions. Even if on a first glance relying on the bare physical structure could appear as a limitation for the obtainable shape, a lot of different albeit complex Janus architecture are found in literature.

Chemical composition is adjusted by adding the desired component in successive steps or mixing them under specific conditions. A very good example are amphiphilic rods of micrometer dimension.

Layer-by-layer deposition (LbL) is an indispensable ingredient for materials deposition. The method is based on the presence of a surface charge of a well-defined overall sign. On top of this it's possible to lay another thin film of material with the opposite charge surface by means of electrostatic interaction; this new coating can be in turn the starting layer for iterating the LbL process. Beside the surface chemistry modification, such process lead to a surface charge modification, a very incisive feature to perform antimicrobial activity.

Nevertheless, despite no extremely complex and expensive instrumentation being required, many parameters must be accurately and constantly checked (stirring velocity, temperature, solvent..) in order to carry on the correct synthesis. The list of the main particles which have been produced by means of these methods is reported in table 1 in the annex.

3.1.2 Characterization Strategies

Many methods and experimental apparatus were used to characterize the Janus particles. As a matter of fact, not always the property under study can be inferred from a direct visual analysis (i.e. optical microscope), so it is necessary to exploit and combine a set of techniques in order to extract the information required to have a complete description of the particles.

Even though the principles of such techniques are well understood, it is worth at least having a smattering of the theory which stands behind those methods in order to be capable of better interpreting the results of the analysis. Here subsequently, the leading techniques are listed in the order they usually are performed in a characterization process.

3.1.2.1 Dynamic Light Scattering (DLS) and Zeta Potential

In the characterization based on Dynamic Light Scattering (DLS) apparatus is the first one from which fundamental features of the system can be gleaned. Despite many parameters that can be taken out from a DLS device, we dealt mainly with two of them: z-potential and dimension.

a) **Z-potential**

Let's recall at first what is the working principle of a zeta-potential (ZP) test, as well as the definition of ZP itself. We have to bear in mind that most of the particles we are dealing with have a surface charge due to a variety of effects ranging from Van der Waals forces to presence of different functionalizing surfactants.What is measured in a ZP experiment is indeed the amount of charge present in the nanoparticle surface. Through this parameter, the tendency of colloidal particles to aggregate or not is taken out. As rule of thumb, we can consider that ZP of an absolute value |Z|>30mV lead into stable particles in colloidal solution (i.e. do not agglomerate).

The process of measurement relies on the fact that due to electrostatic force, free ions from the surrounding medium are attracted to the particle surface, giving rise to a charge gradient which results in an electrostatic potential. Once considered this, it's straightforward to identify in the surrounding medium a parameter which bears an influence comparable to the one of the nanoparticle characteristics itself. For this reason, no sensible measure can be carried out without specifying the solution into which the colloidal particles are dispersed.

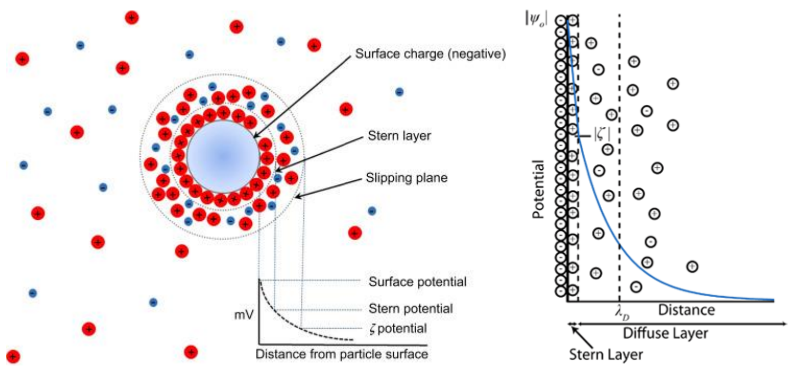


Figure 9:z-Schematic representaition of a charged colloid. A) Zeta Potential and B) Stern Layer.

Another effect that must be considered, is that in addition to generating an electrostatic field, charged particles undergo movement due to Lorenz force if subjected to an external electric field. This electrophoretic movement is described by means of the Henry equation, which state that the velocity is proportional to the intensity of the electric field through a parameter, said electrophoretic mobility, that take into account the effects of the charge distribution around the particle.

Summing up the aforementioned effects, the resulting equation which links the external electrostatic field to the ZP. Because of the principle used, which is strongly affected by the hydrodynamics of the particle, significant variation in the measure is expected for different shaped particles even though made of the very same material.

b) **Dimension/shape**

By means of DLS, a quantitative analysis on the particle dimension can be performed. This time the principle that the measure relies on is utterly different, even though much of the effects reported for the ZP measurement have to be considered influencing as well. The method is based on the scattering of the incident pulsed light by the particle under analysis. As one can expect, if the measure on a single particle of the scattered light intensity is repeated various times over the measuring period, it will result a difference in phase of the scattered light with respect to the phase measured at preceding time instants, due to the movement addressed to the thermal agitation of the particle itself. In fact at every instant the particles occupy a different position in space. So, even though the features of the scattered light are the same, the initial position from where it originated changes, leading to a change in the phase perceived from the analyzer.

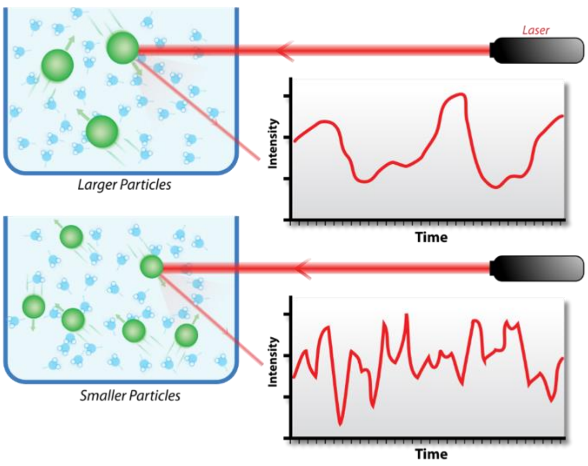


Figure 10:Schematic representation of DLS principle

All those effects are taken into account in the so-called autocorrelation function, which, in a nutshell, evaluates how fast the correlation between two consecutives scattered beams is lost. From statistical analysis (i.e. Cumulant analysis, non-negative least squares) it is possible to relate the autocorrelation function directly to the hydrodynamic dimension of the particle described by the Stokes-Einstein equation. Firstly, a marked trend between the autocorrelation function and the hydrodynamic dimension of a particle can be observed. In particular the smaller the particle, the smaller the decorrelation time. This trend can be easily explained considering the working principle of the technique. In fact small particles turn out in faster movement, which in turn result in a fast change in the scattering center position, thus leading to a faster decorrelation time. Also in this case, one should pay attention to the influence of the particle shape, which strongly affects the hydrodynamic behavior, and in final analysis the hydrodynamic dimension, of the particle.

3.1.2.2 Scanning electron Microscopy (SEM)

SEM is an instrument capable of offering a wide range of information from the surface of the sample. Its operation is based on sweeping a beam of electrons over an area of interest with a customized magnification while a monitor displays the information we have selected based on the detectors that are available.

Specifically, MERLIN FE-SEM overcomes the conflict between image resolution and analytical ability. The core of the MERLIN is the enhanced GEMINIS II column, which, with its dual capacitor system, achieves an image resolution of 0.8 nanometers. The equipment allows a current of up to 100 nanoamps to be obtained for analytical analyzes, such as X-ray dispersive energy spectroscopy (EDS) and backscattered electron diffraction (EBSD) analysis (See figure 11)

Figure 11: SEM instrument of high resolution, from Electron Microscopy facilities of Universitat Autònoma de Barcelona (UAB)

3.1.2.3 Optical Microscopy

Optical microscope is one of the most ancient instruments used for determining the properties of microscopic objects, so ancient that first rudimental microscopes are dated to Galileo's age. Optical microscopes have experienced a big evolution trail, being of fundamental importance in scientific research. To date, many techniques have been developed, which allow for a variety of complexes and sophisticated analysis, such as polarization microscopy, confocal microscopy, fluorescence microscopy and others.

The principle of these techniques stemmed from linear optics reasoning. To be more specific, the very basic components of a microscope are:

- Eyepiece lens
- Objective lens
- Light source

Whilst the first two components are subjected to limitation due to processing ability, which affect the lens qualities, the latter set a limit due to the intrinsic mechanism of the microscope. The main limiting factor is derived directly from the physical phenomena of diffraction, imposing the so-called diffraction limit. According to the physics which stand at the basis of such principle, the minimal distance observable (i.e. resolution) is inversely proportional to the wavelength of the light spot used to illuminate the sample, and no object having a dimension smaller than the one of the light's wavelength can be observed. As a result, all the improvements in the optical microscopy field have been done to pursue the overcoming of this limit. In the present work we dealt with particles in the order of hundreds of nanometers to micrometers, so no special precautions were necessary.

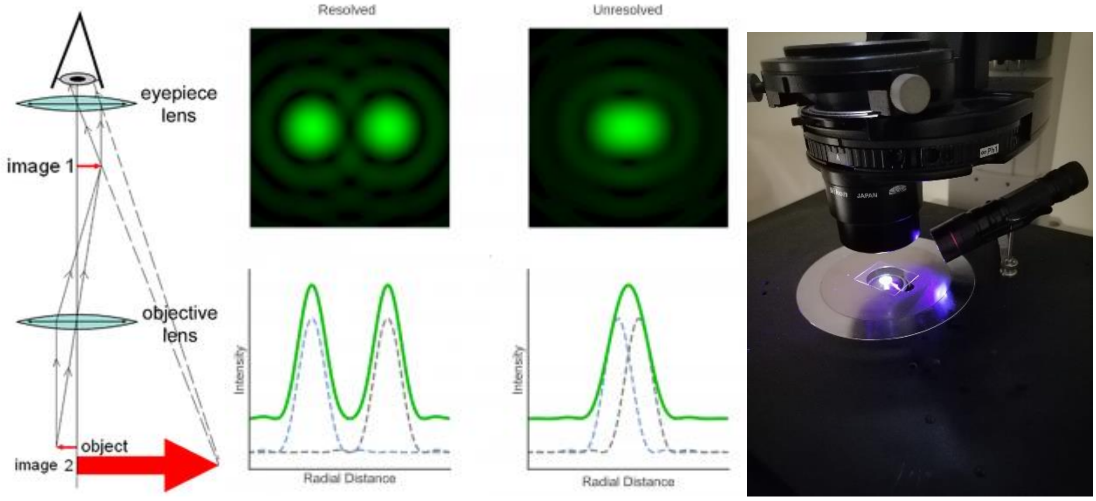


Figure 12:Schematic representation of optical microscope image analysis

Optical microscopy is of paramount importance to better visualize the processes under observation. For example, it gives out a clear inspection on either particle shape or dimension. Furthermore, it allows for real time observation of phenomena according to the time resolution of the instrument (tenth of milliseconds in this case). Regarding MSw analysis, the very big advantage brought to the use of microscope is the possibility to extract cheap and fast images of the systems, shedding light on their swimming properties. It should be underlined that in the case of bio-microswimmers, in-vivo observation is allowed.

3.1.2.3.1 Image Treatment and Movement evaluation by particle tracking

Although microscope observation is an extremely valuable analysis method, which in principle can reveal complex features of the system under observation even in a stand-alone usage - allowing to observe trajectory of particles in relation to real time environment modification- it gives out a very qualitative analysis. By the way, more than the microscope itself, we can regard the human being as the very indispensable part in the analysis process, in the sense that not really inspection on the system behavior came out from the bare magnified image in the microscope screen but that one bring about from the human brain ability to recognize complex pattern, as in the case of particles' trajectory analysis. In this respect, a set of software tools have been developed to accomplish a quantitative analysis, empowering objective parameters able to describe the experiment, and in the last to improve the analysis itself. Looking at the scientific domain, many tools of this type have already been developed and intensely used for the aforementioned purpose. Despite many of them being able to carry out a very precise analysis, implementing specific and complex algorithms, the majority undergo two non-negligible problems: the price and the high-demanding computation time.

Of course, both of the problems can be easily solved by allocating a considerable amount of money to obtain best optimized software or highly-efficient computational instruments; nevertheless, as in our, not always is suitable to spend such a substantial amount renouncing a fast and quite precise analysis. The very strength point of our home-made developed software tools indeed is the capability to make up either a low-cost instrument or fast analysis process. All the procedures are carried out by means of ImageJ open-source software, which allow for a plethora of investigation methods, ranging from solid state physics to biological environment.

3.2 Experimental Procedures

3.2.1 Particle Synthesis and functionalization

3.2.1.1 Synthesis of Spherical Silica Particles[40]

In a general procedure, 40mL of ethanol were mixed with 1 mL of Tetraethyl orthosilicate (TEOS) and 3mL of ammonia. The solution was stirred for 6h with the appearance of white tonality. Obtained particles were separated by centrifugation for 10 min at 6000 rpm and washed with ethanol three times. Finally, the SiO_2 particles were dried at 80°C overnight.

3.2.1.2 Synthesis of Spherical Silica Particles[41]

The preparation of silica Rods was carried out accordingly: 5 g of polyvinylpyrrolidone (40k) were dissolved in 50 mL of 1-penatnol. Thereafter, 5ml of ethanol, 1.7 mL of water and o.4mL of 18 mM sodium citrate were added while stirring for 5 min. Finally, 1.05mL of ammonia and 800 µL of TEOS were added and mixed with the previous solution. A short stirring stage (5min) was carried before leaving the solution in static conditions overnight. Obtained particles were separated by centrifugation for 10 min at 6000 rpm and washed with ethanol three times. Finally, the SiO_2 rods were dried at 80°C overnight.

3.2.1.3 Synthesis of Amphiphilic Silica Rods[41]

Rods were synthesized through consecutive hydrolysis of two silica precursors. In a typical synthesis of rods with a hydrophilic length of 1.8 µm and a hydrophobic length of 0.2 mm, the detailed synthetic procedure is as follows. In a 20 mL glass vial, 1 g of polyvinylpyrrolidone PVP, molecular weight 40 kgmo1) was dissolved in n-pentanol 10 mL) under sonication. Subsequently, deionized wáter (0.28 mL), anhydrous ethanol (1 mL), sodium citrate solution (0.1 mL, 18 mM in water), and ammonium hydroxide solution (0.17 mL, 28 wt%) were added to the above solution. The reaction mixture was vortexed for one minute to mix all the components. After the mixture was left standing for five minutes to release the gas bubbles, TEOS (0.06 mL) was added, and the solution was then gently shaken for 30 s. The hydrolysis of TEOS was allowed to proceed overnight at room temperature. After that, the hydrophobic monomer HDTMOS (hexadecyltrimethoxysilane; 0.03 mL) was added, and the mixture was again gently shaken. The hydrolysis of HDTMOS continued for another 12 h. The solution was then centrifuged at 6000 rpm for ten minutes, and the particles were washed five times with ethanol.

3.2.1.4 *Functionalization of Silica particles with (3-Aminopropyl)triethoxysilane (APTES)* [42]

The amination of silica particles with APTES was achieved following the next steps. 100 mag of particles were dispersed in 6mL of ethanol. Then, 60μL of APTES and 150μL of ammonia were added to the abovementioned solution, which was stirred for 12 hours. Obtained particles were separated by centrifugation for 10 min at 6000 rpm and washed with ethanol three times. Finally, the SiO_2 rods were dried at 80°C overnight. Positive values Zeta Potential measurements confirmed the effectiveness of the functionalization. [46]

3.2.1.5 *Functionalization of Silica particles with Poly(diallyldimethylammonium chloride)(PDDA)* [43]

This was performed by adding 320 μL of ammonium hydroxide to 5 mL of 2 wt % dispersions of colloidal silica (pH ≳11). Subsequently, the solution was placed in an ultrasonic ice bath at 4 °C, and then 5 mL of 1 wt % aqueous PDDA solution was added into the mixture. The resulting solution was left in the ultrasonic bath for 20 min. The tube containing the solution was then centrifuged at 3200 g for 10 min to remove unabsorbed polymer. Centrifugation and redispersion was repeated four times and transferred to a clean container. On the final rinse the silica was redispersed in 5 mL of water. Positive values Zeta Potential measurements confirmed the effectiveness of the functionalization.

3.2.1.6 *Preparation of Cu/Silica and Pt/ Silica Janus particles* [44]

First, monolayers of the particles were prepared by drop-casting the particles on a glass slide. Then, the particles were coated with a Cu layer (40 nm in thickness) and Pt (20nm) by thermal deposition.

3.2.1.7 *Preparation Bacteria strains*[45]

Bacteria Strains were grown overnight at 37 C in Mueller Hinton Broth (MHB) medium. For optical microscopy evaluation, bacteria were diluted to an Optical Density at 600nm (OD_{600}) of 0.01≈10^5–10^6 colony forming units per mL (CFU mL^{-1}).

3.2.2 Particle Characterization

3.2.2.1 Determination of Zeta Potential (ZP)

Diluted suspensions of particles in MiliQ water, were prepared to measure the Zeta Potential values in a Zetasizer Nano Z (Malvern Instruments Inc., U.K.).

3.2.2.2 Movement Evaluation by Optical Microscopy

Optical microscopy analysis of active colloids was performed in a NIkon optical microscope For the evaluation of Brownian motion of Janus particles 10 µL of particle suspension were placed on the glass slide. For active movement of bacteria cells OD_{600}= 0.01, 10 µL of bacteria suspension were placed on a glass slide. Similarly of active movement of silica particles, in which 10 µL of particle suspension were used, and hydrogen peroxide solution was added accordingly to obtain the appropriate concentration. Image and video analyses were carried out using 25 ms of exposure time and a frame rate of 40 frames per second (fps).

3.2.2.3 Scanning electron Microscopy

SEM characterization was performedon a Zeiss Merlin High resolution Microscope from the UAB. Metal coating of 2nm of AuPd was needed for biological samples (bacteria). [81]

4 RESULTS AND DISCUSSION

As mentioned in the previous section, the studies regarding microswimmers concern two types of those systems, which can be distinguished according to their motion mechanism. On one side, bacterias are used to make up the motor system, exploiting their natural ability to swim. On the other hand, the non-biological counterpart is represented by the usage of other man-made movement mechanisms, like the Janus particle in our case.

Because of the fundamentally different principle which stands behind those two last, the experiments to be carried out must follow a substantially different trial, though the analysis to be performed it's quite similar, or at least it takes advantage of the same set of tools. For this reason, the two MS2 types (biological and non-biological) are to be treated separately in the following section.

4.1 Shape and dimension

Firstly, it is needed to distinguish a specified type of particle according to its shape or dimension. This preliminary discerning turns out to be of fundamental importance when analyzing an ensemble of mixed particles. For example, after a process of synthesis one could want to visualize just the elements fitting in a specific dimension constraint. Concerning our work, this technique was used to perform an individual tracking on a mixture of spherical and rod-like particles. In the specific case in fact, the purpose of the study was the determination of the diffusion coefficient of those last under Brownian motion. As one can expect, because of the marked difference of the two types of particles in terms of hydrodynamic behaviour, the results cannot be compared among them, thus an individual treatment is imperative.

The same techniques could be used in every case in which objects described by different shape in a mixture are observed. The fact that elements of different nature are analyzed at the same time, bring the additional remarkable effect that non only the response of different entities can be inferred in the very same environment, but also the interaction behavior of those entities can be evaluated.[47]

Thanks to the large number of available parameters that can be chosen to properly recognize the particles we're working on, an accurate tailoring of the fitting parameters is allowed. For the specific case we used an ellipsoidal shape as a particle marker. What the ImageJ algorithm do, is to superimpose the smaller ellipsoid on the top of the area recognized as a particle. The recognition process relies on the fact that particles possess a net contrast with respect to the background, then it is possible to individuate them by measuring the intensity of the pixels, once set the background. An analogous process is performed using different shape descriptors. [48][49][50]

The measurement procedure gives out the parameters of the shape descriptors (major and minor axes in this case) as well as the coordinates of the identified particles. In addition, the overlay mask it's extracted, which shows just the selected objects. From this point on, all the analysis measurements can be carried out treating the overlay mask as base.(See figure 13)[51]

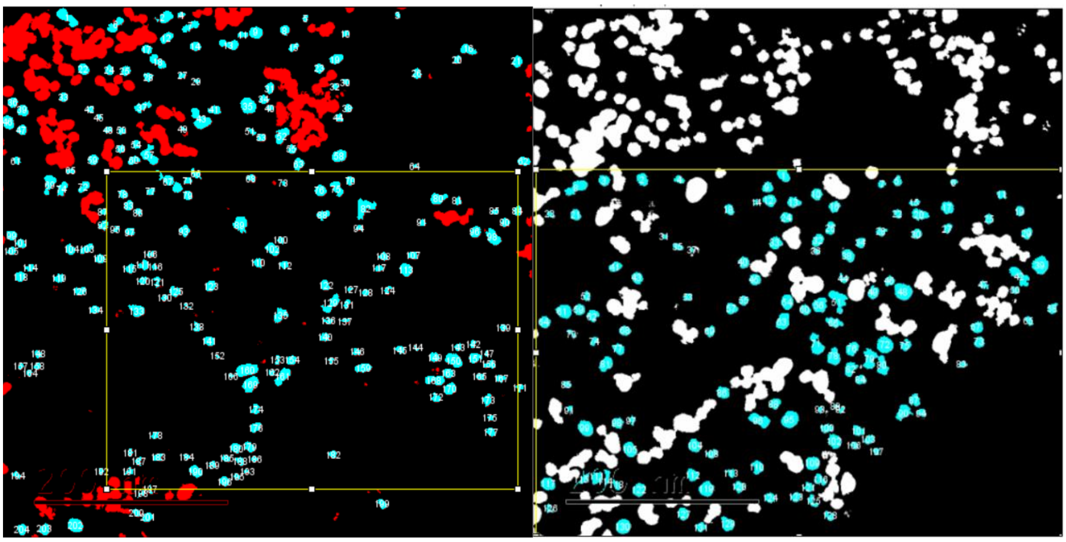


Figure 13:Examples of dimension analysis.

4.1.1.1 TrackMate Plugin

When performing analysis of movement for active colloids, it is not always easy to extract precise information due to the vast range of variables that can affect the observed behavior. Such variables are represented by local variation of the environment condition, like chemical species gradient or presence of flux and it's quite complex to take them into account in an analytical fashion. Nevertheless, all the effects related to the local environment have a very notable and non-quantifiable influence on the macroscopic behavior of the system we are going to analyze. For instance, a Janus particle moving in a medium that encounters a localized gradient can show a change in trajectory not expected, thus leading to misleading information. The problem is even more enhanced when dealing with living beings, which present a behavior not reproducible unless when considering a statistical behavior. To cope with the abovementioned problems is of utmost importance to understand in which limits the observed phenomena can be considered as relevant or must be treated as a random fluctuation of the system. The solution is represented by a statistical treatment of the data. Strictly speaking, the larger the amount of data we are able to process, the more reliable the measurement.[52]

In the specific case, values are represented by the microswimmers trajectories and their related features, like mean velocity or displacement. As said before, sometimes this kind of request can be effectively onerous from a computational point of view because of the amount of data that must be collected. Looking for a solution for such a problem we encountered the TrackMate plugin in the framework of ImageJ software for image processing. This reveals to be a very suitable tool for our purpose since it allows a quite accurate analysis in spite of his simplicity and velocity. By means of this software is it possible to track many particles at the same time, being able to obtain a considerable amount of results, from which statistical analysis can be carried out.

The tracking process expect the following steps:

- Parameter of the image to be process.
- Detector type: define algorithm used to particle individuation, as well as the parameter to fit the particles to analyzed.
- Visualization mode
- Tracking algorithm: define algorithm used to track the particle.
- Filters

In all the steps a wide range of parameters can be tuned to tailor the tracking process and can adjust to the required specific case. Despite the simplicity of this method, it is mandatory to perform a pre-treatment of the image in order to facilitate the task of the tracking algorithm, for example emphasizing the particle contrast with respect to the background in order to make them more easily identifiable.

The plugin includes some basic analysis tools from which it is possible to graph several parameters directly from the program interface. In addition, all the statistics can be exported to comma separated values (.csv) files to be further processed. In order to improve the automatization process, a simple Macro script has been implemented, allowing to perform all the steps in a faster fashion.

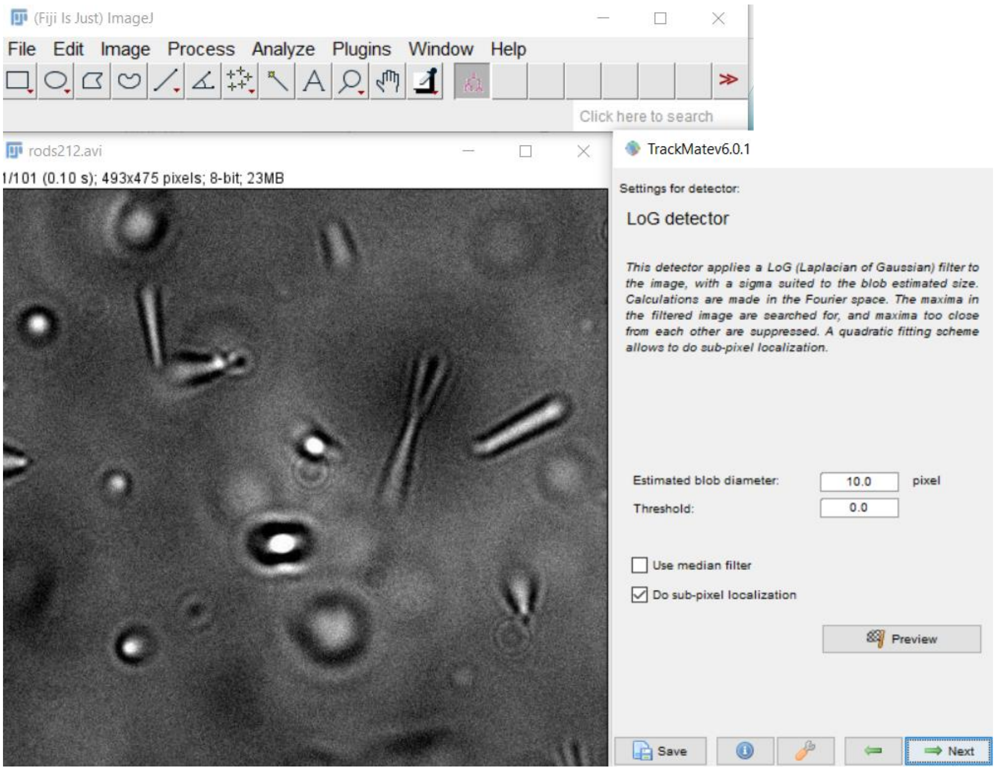


Figure 14:Visualization of TrackMate Plugin

Single particle tracking

Following a roadmap similar to the one defined by the use of the preceding described method, a Macro script to perform the analysis of a single particle was developed from scratch. Despite the simplicity of this last, the conjoint usage of other scripts leads to the determination of a set of representative parameters to describe the microswimmers movement. In the specific case the Macro script exploits some function of the ImageJ software which lets the measure of basic characteristics[53][54]. In particular we chiose to extract the following parameters:

- X,Y coordinate of the trajectory
- Shape descriptor of the particle: the very same procedure already used to the measure of the particle dimensions is employed.
- Orientation angle of the particle

Running the Macro scripts, all these results are obtained and exported to a .csv file, to be further processed by means of other software tools. Even in this case a pre-treatment of the image is required to obtain the cleanest as possible data. An action which is compulsory when using this script, in addition to all the others in common with the previous methods, is to crop the area of the image just around the particle under observation, that must be the only visible in the image 15.

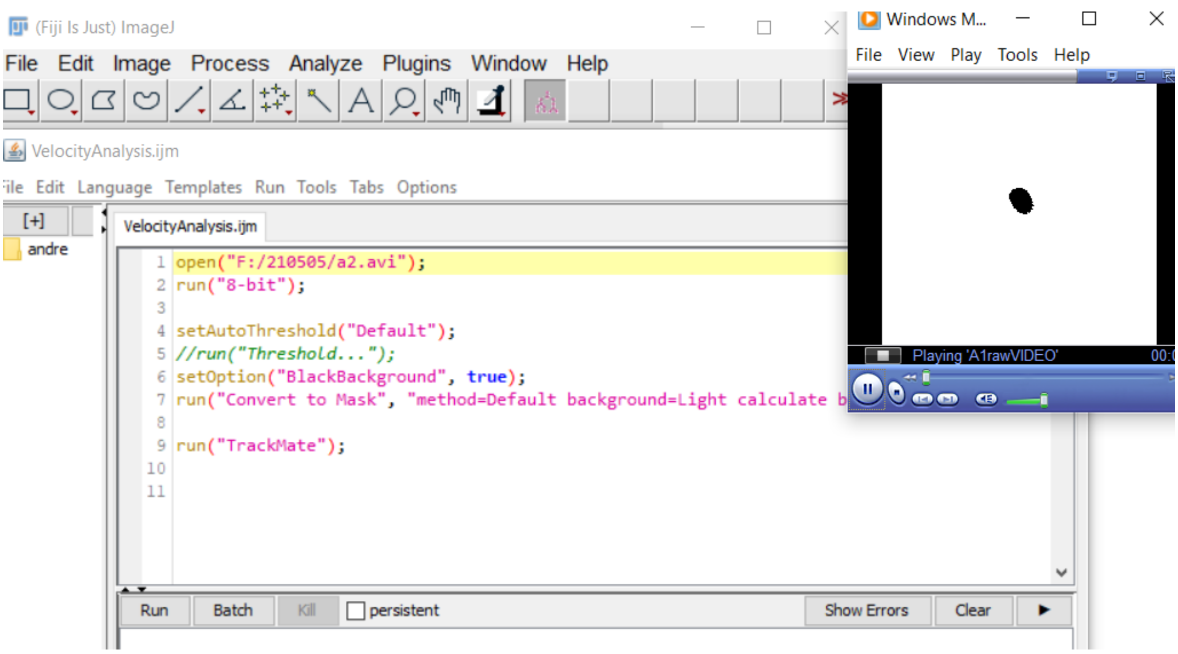


Figure 15:Single Particle tracking script.

Up to this point we limited ourselves to the bare description of the tools and instrumentation required to perform a quite complete analysis of the MSw behaviors, describing which are the parameters that one it's able to extract from them and specifying in which situation one or other techniques is used for. Nevertheless it would be appreciable to define a strategy to carry on the analysis, that is define a sequence under which the methods should be applied in order to obtain as much information as possible and eventually obtain a set of comparable parameters.

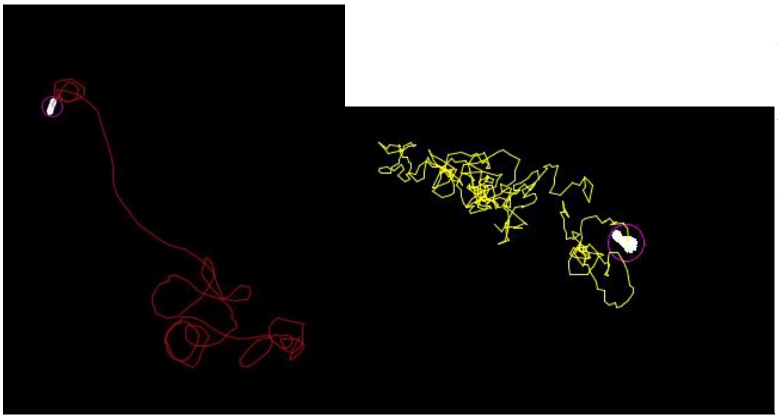
The objective now is then to determine a set of parameters by means of whom the system can be uniquely described.

For the present case, we design an experimental scheme making use of software tools we are in possession of, from which a collection of system's parameters can be deduced. It should be noted that even though this kind of software analysis is complementary to the "classic" analysis performed via microscope and other hardware instruments, via the former the description we are able to bring out is more refined since more quantitative data are extracted.

Our protocol involves 4 main steps. While the firsts three can be encountered quite commonly in the study of active colloid or ,generally speaking , every time is required to figure out the behavior of moving particles, the last step have been designed expressly for our case and it' s new in this kind of study , though it gives out very reliable results. It must be underlined that all the software tools (Macro and calculation scripts) have been developed from scratch on our own taking advantage of the basic functionalities of the imageJ program.

Trajectory Analysis

The first step in the procedure is to extract the coordinates of the trajectory. In order to do so, either the script for single particle analysis or the trackMate plugin can be used. Anyway the first one, despite its simplicity, turns out to be faster and more reliable than the other thanks to the fewer parameters to be chosen and to the less computational demanding algorithm. Once the coordinates are obtained, the trajectory of the particle can be plotted in an x,y graph. Through the visualization of the trajectory a preliminary visual inspection can be obtained, allowing for a fast though qualitative description of the particle's behaviour. In addition, errors and spurious events can be easily detected and eventually removed from the trajectory file.



A15007DATA - Notepad

File Edit Format View Help

	X	Y	Major	Minor	Angle
1	179.866	126.038	405.666	284.047	179.979
2	179.858	126.046	405.646	284.036	179.973
3	179.881	126.059	405.744	284.065	179.966
4	179.888	126.064	405.763	284.061	179.965
5	179.878	126.070	405.726	284.036	179.961
6	179.893	126.078	405.694	283.980	179.966
7	179.882	126.082	405.703	283.993	179.960
8	179.927	126.051	405.888	284.121	179.976
9	179.931	126.059	405.889	284.104	179.974
10	179.932	126.059	405.853	284.079	179.978
11	179.922	126.068	405.813	284.048	179.973
12	179.923	126.067	405.785	284.029	179.975
13	179.918	126.100	405.710	283.928	179.969
14	179.942	126.095	405.711	283.924	179.979
15	179.953	126.084	405.731	283.948	179.985
16	179.966	126.053	405.823	284.047	179.991
17	179.968	126.034	405.865	284.096	179.994
18	179.971	126.022	405.852	284.096	179.996
19	179.969	126.017	405.694	283.987	179.998
20	179.952	126.003	405.551	283.895	179.999
21	179.937	125.989	405.550	283.899	0.002
22	179.941	126.006	405.633	283.957	179.999

Figure 16:Visualization of trajectory analysis and output data file

Square displacement Analysis

From the coordinates of the trajectory, the square displacement (SD) is calculated according to the following formula.

$$SD = \sum [r(t_i) - r(0)]^2$$

Equation 1) Square displacement formula

The SDA parameter simply accounts for the vectoral distance of the particle from a defined point (i.e. starting point of trajectory) regardless of the actual distance travelled from the particle itself. Plotting the calculated parameter we can easily identify the behavior of the trajectory, more precisely evaluating whether the particle is moving in circular fashion or describing a linear trajectory.

The principle for such identification relies on the fact that every movement can be decomposed and considered as a linear superposition of two more fundamental trajectory patterns : the circular and the linear one. As a matter of fact, if we evaluate the SDA for a purely linear or circular trajectory, two easily distinguishable patterns are obtained, as shown in figure.

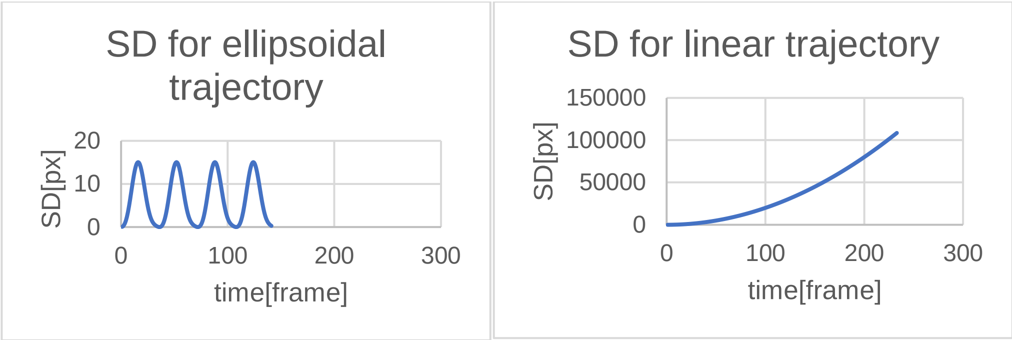


Figure 17:Different cases of SDA results

Following this line of logic, in the general pattern of a moving particle the specific aforementioned motives are readily recognized. The relative influence on the observed plot of the two preceding ones depends upon the extent of circular or linear motion the particle undergoes. For the sake of completeness it should be said that the measure of the plotted curve parameters (period length, line slope..), to which the trajectory is related, opens up the possibility of a more complete and quantitative characterization of the particle motion.

Mean Square displacement

Usually when analyzing moving particles, the mean square displacement (MSD) calculation is a must to do. In particular by means of this parameter it is possible to infer accurately whether the movement to which the body is subjected is Brownian or not, more specifically it reveals if we are dealing with an enhanced or a suppressed Brownian motion.[55][56][57]

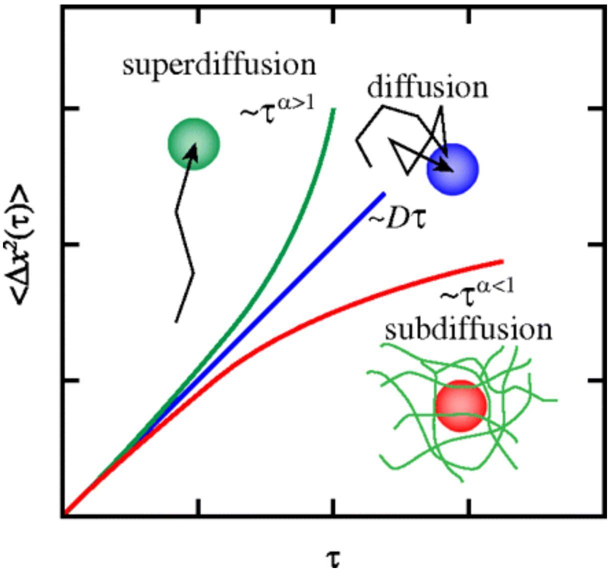


Figure 18:Schematic representation of MDS [46]

Brownian motion refers to the movement of a body driven by thermal fluctuations of the molecules belonging to the surrounding medium. From an analytical point of view, the equations of motion are usually figured out using the so-called Langevin equation, which basically is the classical Newtonian equation of motion where a term of random force is considered in addition to the usual external forces.

The solutions are treated thanks to Fourier analysis and other sophisticated mathematical tools, which in any case don't lead to a complete analytical solution but to a statistical answer. In this sense when working on MSD, we always avail computational tools to perform the calculations. The MSD for a discretized trajectory, as the ones we are concerned with, is defined by the following formula:

$$\text{MSD:}\quad \bar{\rho}_{A,n} = \frac{\sum_{i=1}^{N-n} l_{i,i+n}^2}{N-n}$$

$$\Downarrow$$

$$\bar{\rho}_{A,1} = (l_{1,2} + l_{2,3} \cdots + l_{12,13})/12$$

$$\bar{\rho}_{A,2} = (l_{1,3} + l_{2,4} \cdots + l_{11,13})/11$$

$$\bar{\rho}_{A,3} = (l_{1,4} + l_{2,5} \cdots + l_{10,13})/10$$

Equation 2)MSD Formula

As can be easily seen, the MSD calculation requires considerable computational effort. In the first instance, we tried to exploit the software Fiesta[58] since this software has been developed specifically for this kind of task. Nevertheless, in this fashion, we were able to determine the MSD just for the first 3 seconds (because of the program), at the expense of around one hour of calculation time. To overcome this problem, we developed a python script able to perform the calculation automatically once received the .csv trajectory file as input. Thanks to our program the calculation can be concluded in a few seconds, allowing for the determination of the MSD for the entire length of the trajectory. In addition, it is possible to specify a restricted zone in the trajectory file and extract the MSD value only for this last. This feature reveals to be of utmost importance when the particle undergoes different kinds of behaviors in the period of observation.

$$\Delta^2(m) = \frac{1}{N-m} \sum_{k=0}^{N-m-1} \left(\mathbf{r}(k+m) - \mathbf{r}(k)\right)^2$$

Equation 3)MSD COMPUTATIONAL FORMULA

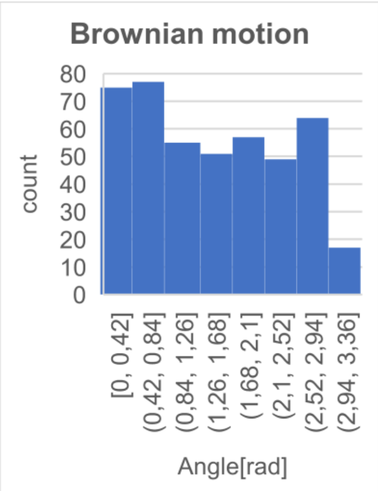
```
Created on Fri Jun  4 12:58:13 2021

@author: andreoni giacomo
"""
import pandas
import numpy as np
import matplotlib.pyplot as plt


disp=pandas.read_csv('F:/janus/Resume/A1rawDATA.csv',sep="\t")
dispFrame= pandas.DataFrame(disp)
xVec=[]
yVec=[]
SPSD=np.zeros((len(dispFrame),len(dispFrame)))
MSD=[]



for i in range((len(dispFrame)-1)): #define displacement vector
    xVec.append(dispFrame.iloc[i,1])
    yVec.append(dispFrame.iloc[i,2])

xVec=np.array(xVec)
yVec=np.array(yVec)


#len(dispFrame)

for j in range(1,len(dispFrame)-2):#modify here to obtain portion of trajectory
    pSum=0
    for i in range(0,len(dispFrame)-j-1):
       SPSD[j,i]=((xVec[i+j]-xVec[i])**2+(yVec[i+j]-yVec[i])**2)
       pSum=pSum+((xVec[i+j]-xVec[i])**2+(yVec[i+j]-yVec[i])**2)/(len(dispFrame)-j-1)

    MSD.append(pSum)


plt.plot(MSD,'.')
plt.show()
```

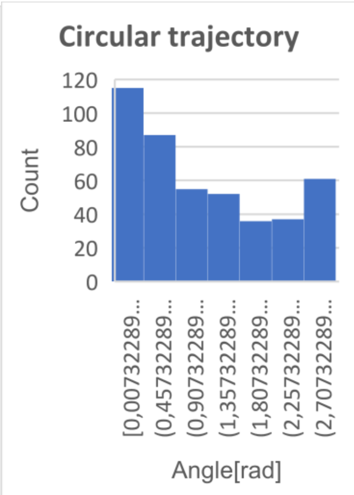
Figure 19:Visualization of MSD Script

Forward angle distribution (FAD)

The methods of analysis that have been listed until now are quite common in the analysis of the behavior of moving particles. However, despite them allowing for the determination of a set of valuable parameters, we observed that they do not entail a complete description of the movement of a certain body. That is, even though the movement of the particle can be inferred, it requires a perhaps expensive analysis and no parameter clearly underlines the behavior of the particle. For this reason, we have tried to develop a new analysis method, attempting to exploit some other feature of the system. For this case, we choose the angle between 2 consecutive tracts of the trajectory. In fact, regardless of the shape of path described by the particle, the trajectory turns out to be discretized. This means that every movement, being a curve or a line, can be approximated by a set of segments of arbitrary length whose inferior limit is set by the acquisition system.

By means of simple linear geometry reasoning, namely calculating the scalar product between 2 vectors whose inclinations are defined by the coordinates of the trajectory points, we manage to calculate the angles between two adjacent tracts. Then, summing up the angles obtained for an entire trajectory (defined by the segments) we succeed in the construction of an angle distribution histogram. What it was expected is the existence of a relationship between this distribution and the kind of movement the particle undergoes, from which one could be able to visualize the behavior of the body under observation at first glance.





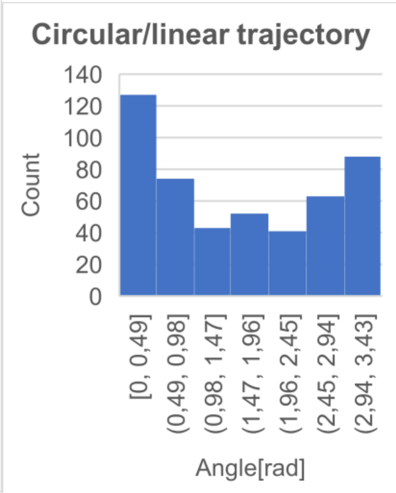


Figure 20:FAD circular/linear

Figure 21:FAD circular

Figure 22:FAD brownian

More specifically, in the presence of Brownian motion there should not exist a preferential direction of motion, resulting in an equiprobability that the particle, from a certain point along the trajectory, moves the next step in an angle in the 0°-360° range, eventually leading to a flat angle distribution. On the other hand, when the movement presents a preferential direction of motion (i.e. move forward), the angle distribution should be peaked around the angle specified by this last (i.e. 0° for forward motion).

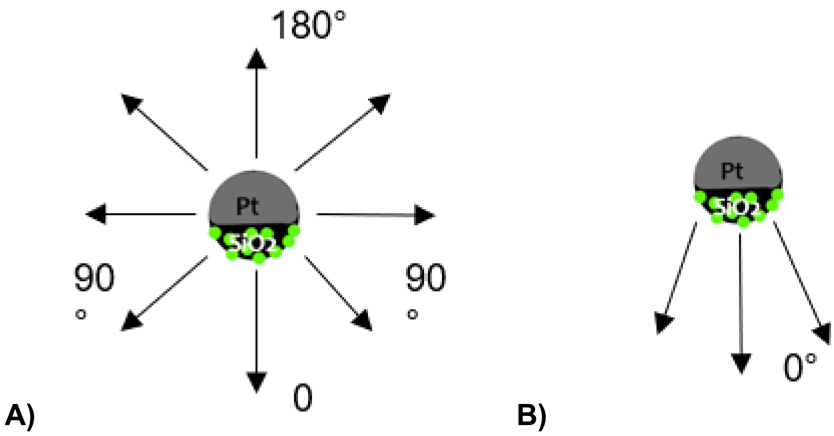


Figure 23:Brownian motion Particle FAD and b) Active motion Particle FAD representation

What we obtain experimentally indeed confirms markedly our suppositions, resulting in a very clear and reliable parameter to classify the motion under observation. Despite his reliability, this method has not been reported in literature to the best of our knowledge.

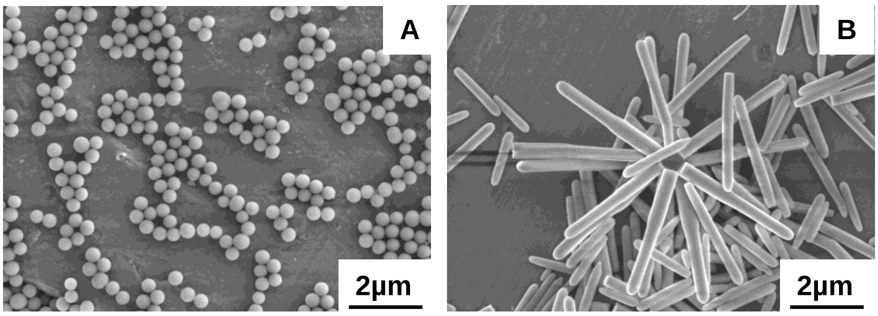


Figure 24:Scanning Electron Microscopy images of a) spherical, b) rod and particles.

4.2 Biological microswimmers

In the framework of biological microswimmers the first main issue is to track down a way to be able to influence the swimming behaviors of bacteria. In fact, since we are dealing with living beings, we are not allowed to modify intrinsically the mechanism responsible for the motion in the medium but interact externally with the system. Although a plethora of methods have been proposed, two main approaches can be recognized in broad terms. The first one relies on the ability to modify the shape of the swimming system, usually encapsulating the propulsion system (i.e. the bacteria) in an artificial container. According to this paradigm, the driving mechanism has to be examined and optimized mainly taking into account the hydrodynamics of the capsule.

The second avenue is to use the bacteria as-grown and change the medium in which they move, trying to tweak the swimming performance. In light of this, the only parameters we are able to affect belong to the environment where they move.

In this work, the study was carried on following the second approach. The other fundamental feature that a MSw should possess, is the ability to deliver some kind of cargo. To tackle this problem there exists two main approaches, analogously to the situation where dealing with swimming properties.

Even in this case, the first one exploits a man-made capsule, to which the bacteria is trapped. All the bonding properties of the MSw to a cargo are then addressed to the artificial functionalization of the encapsulating system whilst the movement is brought about exclusively by the bacteria. The second approach instead provides the direct exploitation of the surface properties of the outer membrane of the bacteria cell, for instance the presence of negative surface charge, to attach to the cargo.

In our experiment the bacteria species selected to be employed as a propulsion mechanism is the *E. coli*. This choice is dictated by the quite complete knowledge of this particular type of bacteria, which make them to be used frequently as case study when treating bacteria-related issues.

*E.co*li is a gram-negative bacteria able to swim thanks to the movement of flagella, propelled by a natural motor situated within the inner membrane of the prokaryotic cell. Depending on the movement of the flagella the microorganism can sustain various types of movement(See figure 25)

Figure 25: SEM micrograph of E. coli

4.2.1 Propulsion mechanism

In the experiment that has been carried on, a strain of E.coli was observed at microscope under various medium conditions. It is not straightforward to deduce how the medium affects the swimming behaviors of the body under analysis. By the way, there exist at least two leading factors which most of the influence can be attributed to.

The first effect that must be considered is the feeding of the bacteria. Those microorganisms extract their sustenance directly from the medium where they are, so there is a remarkable influence of the concentration of the dispersed nutritive substances in the media where bacteria live. The other factor is the cell stability, which is determined by the amount of anionic and cationic species present.

Though through observation we are not able to appreciate which of the two preceding parameters is specifically affected by the changing of media. In any case a marked influence resulting from an overall effect on the swimming behavior depending upon the kind of environment can be observed.[62]

Bacterias have been observed primarily in their grown media. A drop was deposited on the slide of the microscope and then a video recorded. This first operation is the basis in order to set a reference case (RAW). The comparison of all the other conditions have to be regarded considering this first case as a benchmark, for what concerns either the kind of movement the bacterias undergoes or their mean velocity. The swimming behavior of *E.coli* were then examined in response of the following medias:

- Water
- NaCl [0.1M]
- NaCl [0.01M]
- Phospate Buffer Solution

An Eppendorf containing bacteria in their growing media were centrifuged at 6000 rpm for 10 minutes, so that bacteria pellet is obtained. The pellet was successively dispersed in various Eppendorf containing the media above-listed, then left to incubate for around 30 minutes at 37°.

The experiment expects to deposit a drop of 10uL of the solution onto the slide. Then the observation is done at increasing concentration of medium. To do so, drops of different volume (2,4,6,8 uL) are added directly to the first one containing 10uL of bacteria dispersed in the media under analysis. The procedure is repeated for each environment. A couple of videos of about 30 seconds of the bacteria movement are recorded in each situation to be analyzed via software.

At this point, the swimming behavior has been characterized thanks to the TrackMate plugins of ImageJ, which allow the tracking of many particles at the same time. In particular we were able to extract the trajectory shape as well as the mean velocity along these trajectories. By writing a simple macro script, the entire process is automatized and requires a few minutes to be pulled off.[59]

It should be noted that more than 10,000 tracks were analyzed to acquire statistically trustworthy results, as seen in figure 26.

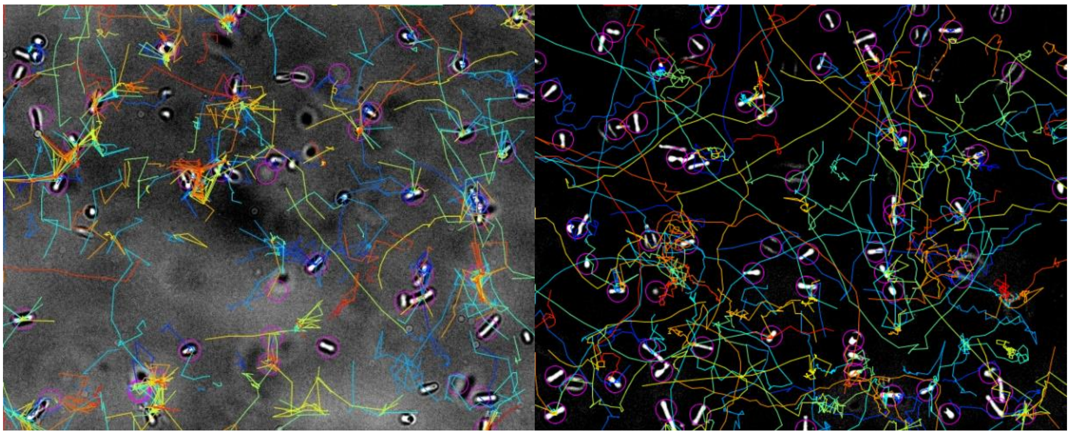


Figure 26:E. coli swimming tracking software visualization

Figure 27:E. coli swimming tracking microscope visualization

As we did expect, the bacteria velocity tends to increase as the medium concentration rises. This phenomenon is attributed to the presence of higher concentration of nourishment for the bacterias in the medium where they move.

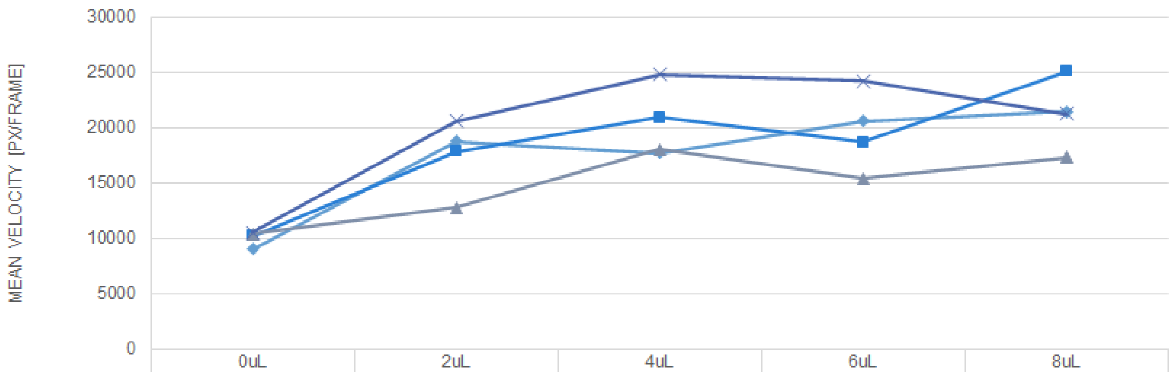
Also the cell stability, influenced by the amount of anionic and cationic species, turn out to be a very effective parameter for the velocity modification. In particular, the lowest velocities were observed for bacterias dispersed in solution of NaCl[0,1%].

Regardless of the type of media used for the experiments, the tendency of the velocity approaches a saturation limit for medium volume higher than 37% of the total volume, thus defining the conditions for which the velocity can be effectively influenced.

All the velocities are reported in [pixel/frame], but they can in principle be converted to different units of measurement simply by considering the scale factor between other quantities.

It should be noted that during the process of tracking, when performing the particle recognition, there is no distinction between live cells that actually produce movement and dead cells, which in theory could result in an overall lowering of the mean velocities[63]. Anyway, this problem is overcome by setting filters for the track that must be considered in the process of mean. This action is performed directly when using the TrackMate plugin; for this particular case the solution was represented by imposing a lower limit for the track displacement. All the trajectories whose distance between the initial and final point is lower than the decided threshold are discarded.

For what concern the shape of the trajectory, we did not observe any significant influence neither on the media nor the medium concentration. All the paths are comparable to those one characteristic of the as-grown bacteria.[60][61]



	0uL	2uL	4uL	6uL	8uL
H2O	9051,133	18740,62	17680,99	20614,88	21427,3
NaCl0,01	10198,13	17831,62	20893,01	18716,18	25063,27
NaCl0,1	10395,06	12757,92	18013,46	15376,72	17307,84
PBS	10555,21	20607,83	24785,47	24197,61	21275,89

Figure 28:Results of Bacteria velocity tracking and evaluation

4.3 Non-biological microswimmers

Dealing with non-biological microswimmers, the very first problem one has to face is the generation of motion. In fact, in contrast with the biological counterpart, for which the movement is guaranteed by the natural characteristic of bacteria, the artificial system does not present any kind of intrinsic characteristic capable of sustaining the movement. In the first instance it is then necessary to define a mechanism through which the particle can be propelled, and just in a second stage try to optimize and take control of this last. Because of the low control on the metal deposition mechanism, effects related to the asymmetry of the cap are easily observed and give rise to a variety of behaviors that have to be analyzed individually, although many of them show common characteristics. [69][70]

As mentioned in the previous section the behavior of this type of microswimmers, implemented by means of the Janus particle, is highly dependent on the morphology of the particle itself.[71][72] Due to the peculiar movement characteristic of each single body, it is not possible to perform a massive analysis of the system but it is compulsory to observe each case separately.

Despite that, one can figure out some common features, at least for what concerns the dependence of the Janus behavior upon specific conditions. For this reason just two case studies are reported on behalf of all the data collected. What we want to underline in doing so, is not the specific behaviors obtainable by means of these microsystems , which still remain of utmost importance, but the power and the versatility of the analysis techniques we used, that can be adapted for practically all the similar cases.

4.3.1 Propulsion mechanism

In the present case we will approach the problem by means of the Janus particle as reported in a previous section. Despite the great variety of mechanisms proposed for the propulsion of this kind of system, we uniquely focus on two of them, according to the features of the particles we build up.

The first one, concerning the Janus covered by a platinum cap (20nm), is a diffusiophoresis process. To the particle dispersed in water, an amount of peroxide is added. More specifically the platinum face acts as a catalyst for the following reaction.

The enhanced decomposition of peroxide close to just one of the particle faces gives rise to a concentration gradient of this compound along the Janus dimension. Then, the peroxide molecules coming from a zone with higher concentration (i.e. bulk concentration) try to reach the zones with lower concentration close to the surface in order to re-establish the species homogeneity. The flux of particles around the particle finally generates the movement. In other words, one could explain the phenomenon stating that the movement is not brought about by the particle itself but is due to the peroxide displacement behind the particle. The very same reaction is reported in literature for other mechanisms, like bubble generation for a rocket-like propulsion system.[73][74][75][77][80]

Hereafter is reported exclusively the procedure for the study of Janus rods SiO_2/Pt(C.1) and Janus sphere SiO_2/Pt@APTES(A.1), since they give out the best results in terms of motion ability. Nevertheless, the methodology for the study of the other types of particles is practically the same.

Evaluation of Silica Spheres and Rods.

Particles were suspended in water detaching them from the slide where the deposition took place. This was done by pull-and-pushing a drop of water (200uL) onto the slide by means of a micropipette. Performing this action, the top of particles is dispersed into the water drop. The obtained colloidal dispersion is diluted by pouring it in an Eppendorf filled with milli-Q water. This action serves to obtain an enough diluted dispersion to prevent the interaction among particles. A drop of this dispersion(10uL) is deposited on a microscope slide. Then a drop of peroxide at different concentrations (2%,5%,10%) of equal volume is added on top of it in order to supply the fuel for the Janus movement. For each condition, including the raw colloidal dispersion, a video is recorded to be further analyzed via software. It should be remarked that since no collective movement is expected, that is, every particle is characterized by a specific motion strongly dependent on his capping, it is not possible to infer a statistical property and every particle that undergoes movement must be treated individually. Once a satisfactory amount of data is obtained, the movies are analyzed using the single particle tracking method.

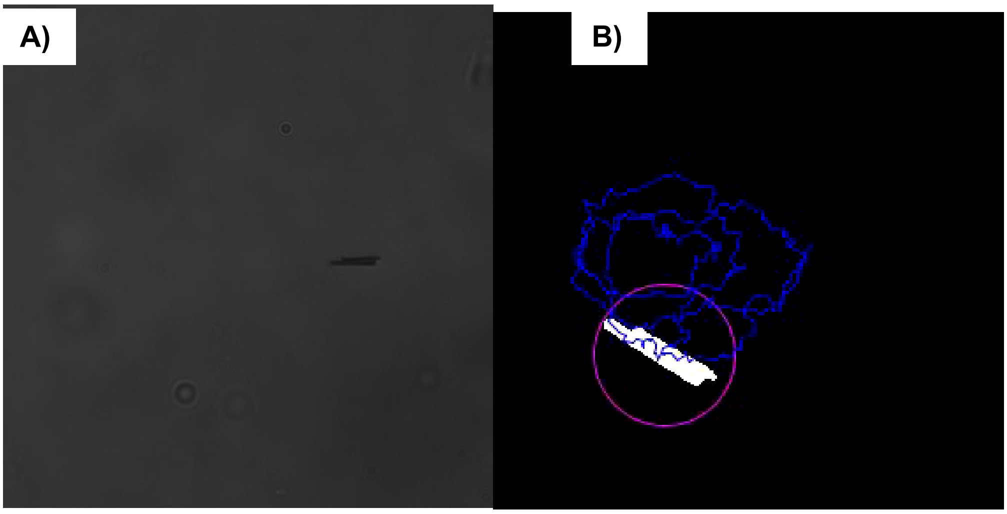


Figure 29:C1 A) Single Particle visualization in Microscope and B) Single particle track analysis

As we expected we can generally conclude that appreciable enhancement of the Brownian motion is achieved for concentration of peroxide above the 2.5% of the total volume, with a marked effect for concentration above 5% of the total volume. This confirms the diffusiophoresis proposed mechanism, defining the limit for the range required to propel the Janus particle[64][65]. Since the kind of movement vary enormously from a particle to a another, we can conclude that the deposition method used give rise to an high asymmetry of the cap; more than this, thanks to the observation of such movements, we are able to get a very precious insight on how the deposition effectively happen. A more controlled building procedure would lead to a less varied response of the particles.[66][67]

The characteristics of movement are well defined from the results of the 4-steps analysis. Hereafter a more specific comparison between a Janus rod which sustains a classical Brownian motion and a Janus rod under the influence of peroxide.

Trajectory Evaluation

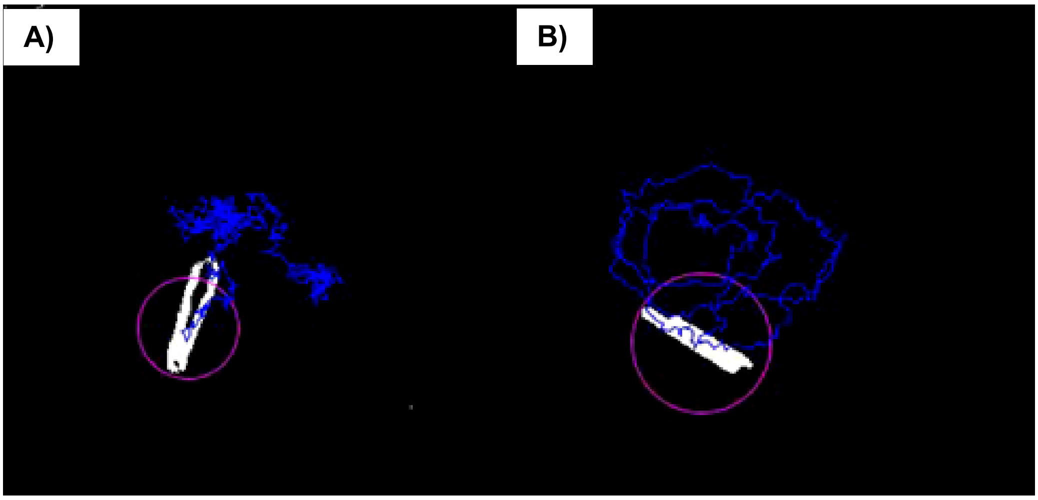


Figure 30:Silica rods tracking results, including A) Brownian motion and B) Enhanced Brownian Motion.

A visual comparison of the trajectory shape reveals a marked difference between the two cases. As we expect, the Brownian one do not show any identifiable pattern due to the randomness of the movement itself whilst when peroxide is added the movement is clearly not random.

Square Displacement

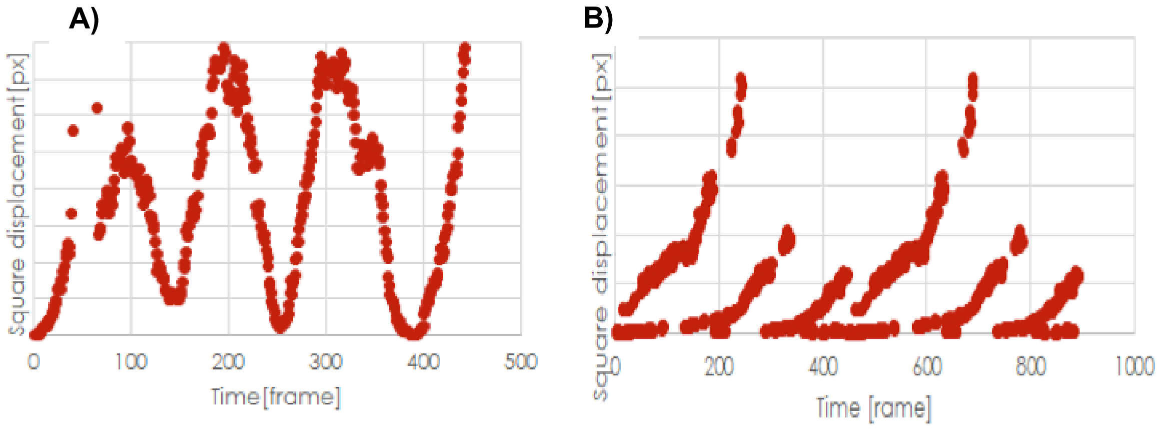


Figure 31:A) Brownian SDA and B) Enhanced Brownian Motion SDA for silica rods.

In relation to what is observed for the trajectory the difference between the two cases is net. In the Brownian case the SDA seems to be a sequence of linear motion (identified by a parabola arch) starting at different points in time. The interpretation is the following: when the SDA is 0 the particle reaches the starting point, to diffuse successively following a linear segment. This behavior is iterated various times as one expect for a Brownian motion. In this specific case, the particle starts turning when peroxide is added. The circumference defined by this motion results in the characteristic alternated trend of the SDA.

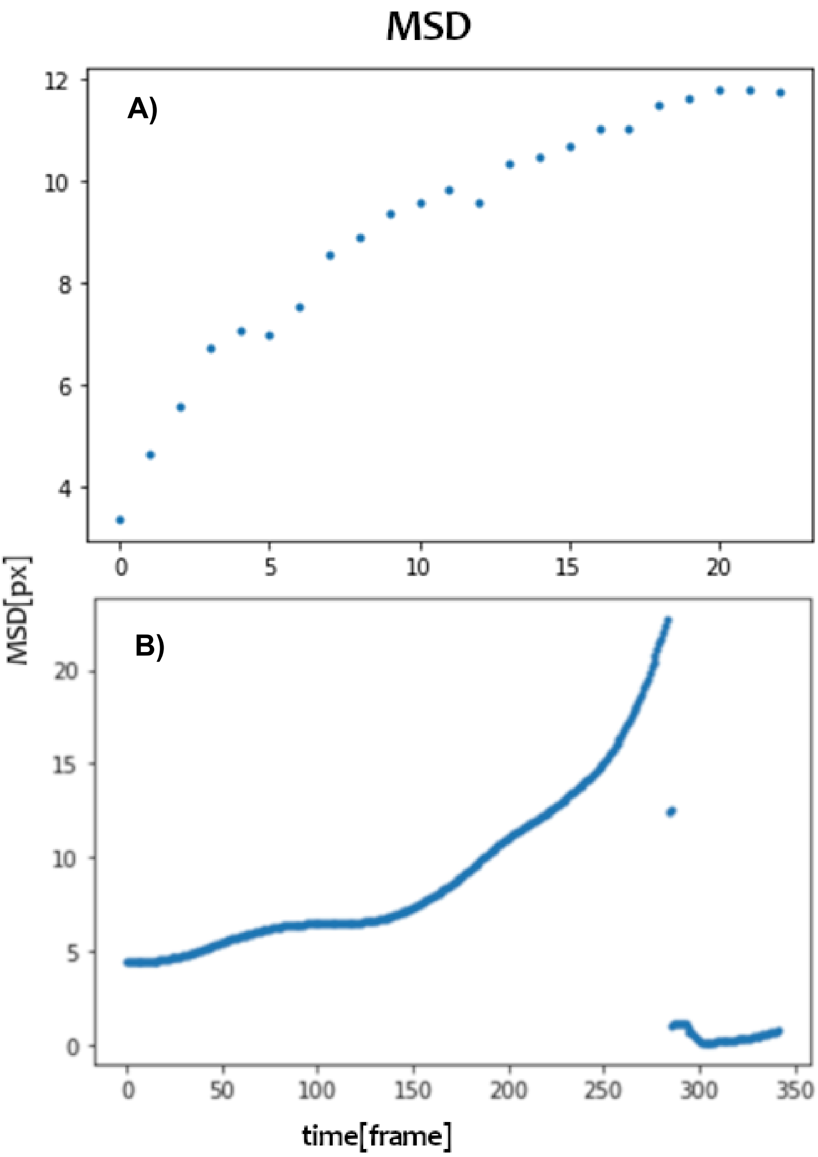
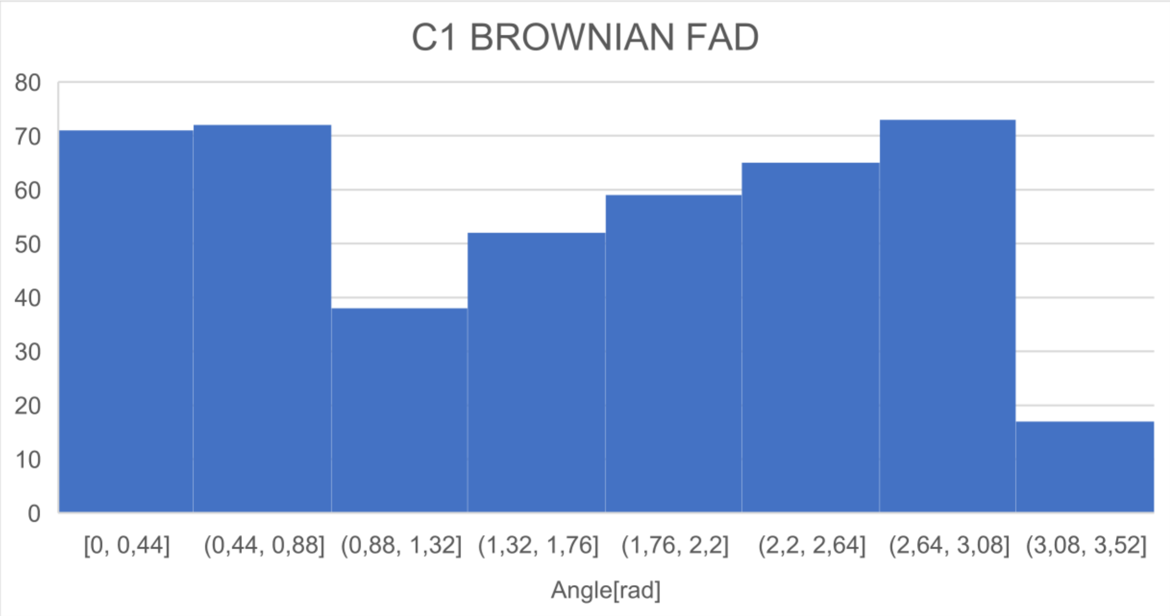


Figure 32: A) Brownian MSD and B) Enhanced Brownian Motion MSD for silica rods.

The MSD measurement gives results partially compatible with what is expected. In fact, if the particle under the influence of peroxide results in an evidently enhanced Brownian motion (i.e. circular movement), the one which should undergo pure Brownian motion shows a curve characteristic of a particle which sustains a random but suppressed motion. This effect could be addressed to the fact that the theory for Brownian motion takes into account exclusively the diffusion processes, without regarding the influence of the so called "rotational diffusion" through which the effect of the change in the direction of motion is considered.



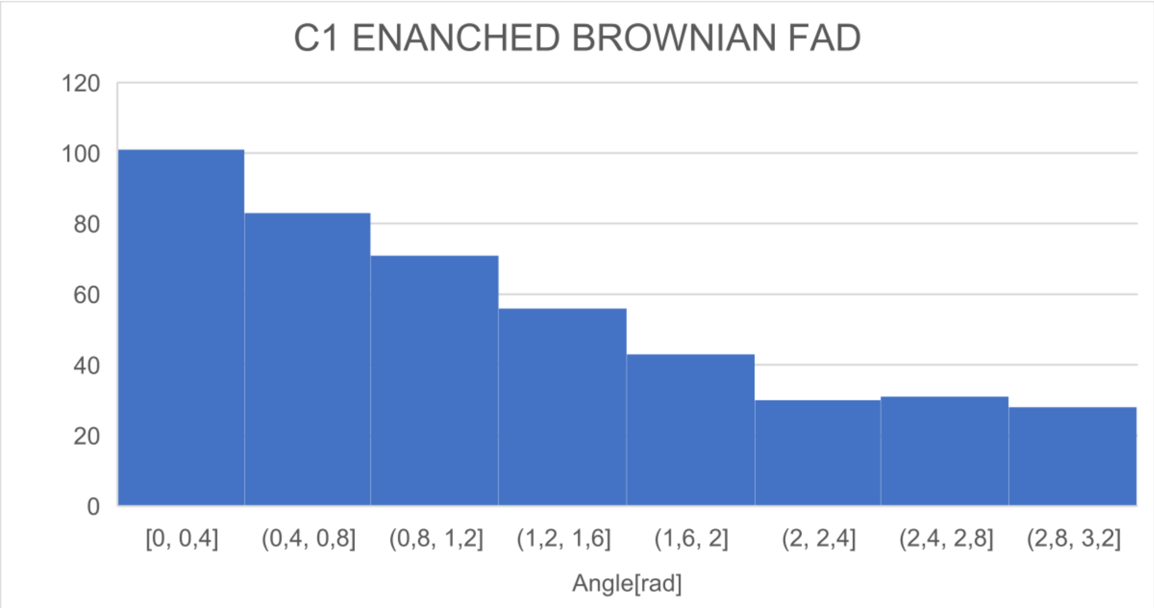


Figure 33:C1 FAD analysis for A) Brownian motion and B) enhanced Brownian Motion of Silica Rods

When analyzing the result of the Forward Angle Distribution(FAD) it appears clear at first glance the notable difference between the two motion regimes. As we expected the FAD for a pure Brownian motion turns out to be quite isotropic because of the absence of a preferential direction of motion. In net contrast, when the particle tends to move forward, the FAD is clearly peaked around the 0 rad value.

4.3.2 Cargo attachment

Since Janus particles are entirely man-made there is a lot of room for the choice of mechanism to obtain the cargo attachment. The limitation, in contrast to the biological microswimmers, is carried by the ability to functionalize a certain type of particle more than by intrinsic particle characteristics(i.e. Bacteria surface). In this vision, a good way to act is to functionalize the particle before performing the capping deposition. Thanks to the plethora of mechanisms available (ex. Chemical bonding, electrostatic force), it is possible to pool the mechanism to the specific situation.

However, in the experiment we didn't test the attachment properties directly on microorganisms, but we mimic those last by means of particles with comparable surface properties, or, generally speaking, with particles which can attach to the Janus in the same way a specific bacteria could. Since we are concerned not only in cargo attachment, but also in cargo transportation, to perform the experiment we did use just the particle we know they are moving.

The guideline of the experiment is then:

- Verification of attachment ability
- Verification of cargo transportation when particle is allowed to move

Due to the high mobility of Rod particles, we choose to use them as a test bench. For this kind of particle the attaching properties are brought about by the negatively charged surface, so we use particles with a positively charged surface as a prototype for cargoes[78][79]. Hereafter are reported the experimental procedures.

<u>SiO_2@PDDA microsphere (2 µm)</u>

5µL of Rod particles + 5µL of SiO2@PDDA(2um) were deposited on the slide in order to study the attachment properties of the rods to a particle whose surface is positively charged. The amount of the two types of particles were chosen to have a similar concentration of the species. It results that C.1 attaches to the particles.

10µL of H2O2[10%] was added to the previous mixture to obtain the enhanced brownian motion of C.1 observed in previous experiments. By the way when peroxide is deposited, all the SiO_2@PDDA particles fall and remain fixed on the slide. In this situation the rods which are glued to the SiO_2@PDDA particles turn around them because of the propulsion provided by the peroxide.

PolyStyrene (PS) @PDDA(PS@PDDA)

The procedure is very similar to the one proposed for SiO_2@PDDA particles.

1. 5µL PS@PDDA + 5µL C.1 + 10µL H_2O_2 : C.1 attaches the target, but no evident increase in motion can be observed.
2. 5µL PS@PDDA + 5µL C.1 + 20µL H_2O_2 : as in the previous condition C.1 attaches to the target, this time an improvement of the motion can be observed.

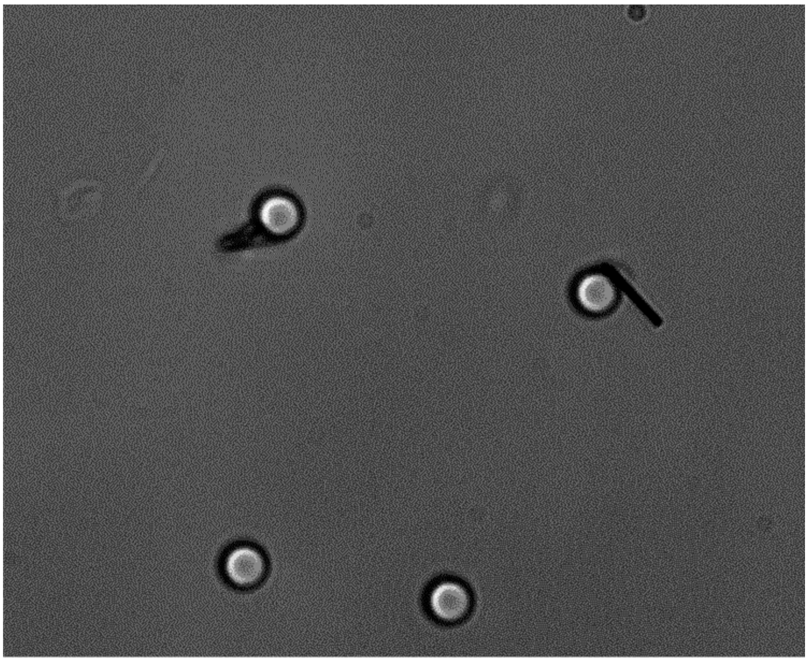


Figure 34:PS@PDDA attachment to silica rods

5 FUTURE PERSPECTIVES AND CONCLUSION

In this work we mainly focus on how to generate and study the movement in the micro-swimmers system, either in biological or non-biological form. We also partially treat the problem of cargo attachment for non-biological microswimmers. By the way, defining a roadmap for microswimmers employment in real cases, and eventually coming to their commercialization, a list of fundamental issues remains unsolved.[80]

Movement brought about by a mechanism of diffusiophoresis has been demonstrated for inorganic components. It would be ideal to be able to define the same methodology using bio-compatible compounds, to be able to introduce those systems in an organic framework. In this view we tried to use SiO_{2}/Pt@APTES incubated with Cerium, to generate a diffusiophoresis mechanism by beans of the reaction with Mannose. MSw should be controllable in order to carry out various tasks. This require the definition of some component belonging to the MSw to which an external agent (i.e. Human being) should be able to interact. The current state of art relies mainly on the usage of magnetic forces. Basically, a magnetic component is incorporated into the MSw body, allowing it to be driven by an external magnetic field. In this regard, an instrument to generate a controllable magnetic field has been specifically designed and built by a 3D printer to be used for observation in our microscope.

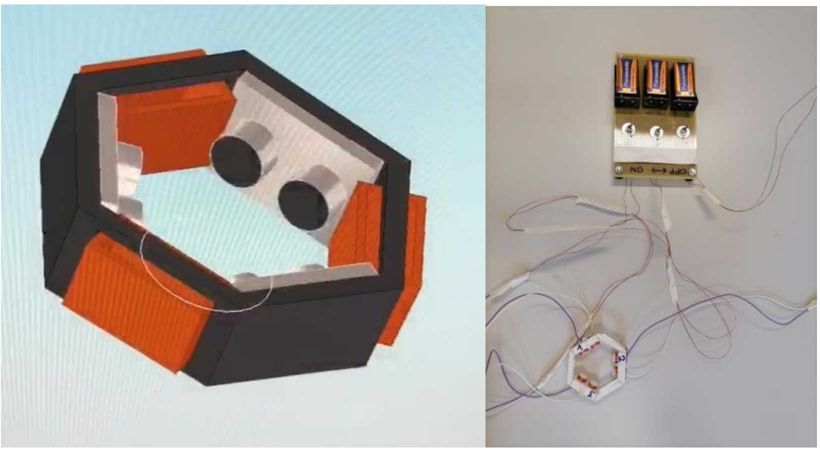


Figure 35:Magnetic field measurement tool 3D Design and actual device.

The attachment properties of a cargo must be better understood, in order to better engineer either the biological or non-biological system. In a second stage, depending on the type of cargo employed one should be able to define the actual occupation of these systems.

Regarding biological microswimmers, we deal with *E.coli* for a proof of concept. The next step would be to avail of non-pathogenic bacteria compatible with criteria of non-biohazard. In this sense, preliminary tests are being carried out with Bacillus Subtilis strain, a non-pathogenic Gram positive strain, part of human microbiome.

In final analysis, throughout this work we defined valuable instruments and methods to deal with microswimmers system.

- An hybrid bottom-up/top-down approach to build up Janus particle in a quite effective and cheap way have been established.
- Powerful software tools implemented on our own facilitate the characterization of those systems, allowing for a fast and efficient tracking.
- A well-defined procedure for Janus characterization was designed.
- We shed light on the mechanism that stand behind the cargo/propulsion system interaction when treating microswimmers.
- A roadmap for future development of microswimmers system have been traced.

7 References

[1] R. Nijhara and K. Balakrishnan, "Bringing nanomedicines to market: regulatory challenges, opportunities, and uncertainties.," *Nanomedicine*, vol. 2, no. 2, pp. 127–36, Jun. 2006.

[2] F. Caruso, T. Hyeon, and V. Rotello, "Nanomedicine themed issue Nanomedicine w," no. 7, 2012.

[3] a. K. Bajpai, S. K. Shukla, S. Bhanu, and S. Kankane, "Responsive polymers in controlled drug delivery," *Prog. Polym. Sci.*, vol. 33, no. 11, pp. 1088–1118, Nov. 2008.

[4] D. Patra, S. Sengupta, W. Duan, H. Zhang, R. Pavlick, and A. Sen, "Intelligent, self-powered, drug delivery systems," *Nanoscale*, vol. 5, no. 4, pp. 1273–1283, 2013.

[5] W. C. K. Poon, "From Clarkia to Escherichia and Janus: The physics of natural and synthetic active colloids," *Proc. Int. Sch. Phys. Enrico Fermi*, vol. 184, pp. 317–386, 2013.

[6] A. Zöttl and H. Stark, "Emergent behavior in active colloids," *J. Phys. Condens. Matter*, vol. 28, no. 25, p. 253001, 2016.

[7] M. Guix, C. C. Mayorga-Martinez, and A. Merkoçi, "Nano/Micromotors in (Bio)chemical Science Applications.," *Chem. Rev.*, May 2014.

[8] M. Safdar, J. Simmchen, and J. Jänis, "Light-driven micro- and nanomotors for environmental remediation," *Environ. Sci. Nano*, vol. 4, no. 8, pp. 1602–1616, 2017.

[9] H. Wang, G. Zhao, and M. Pumera, "Beyond platinum: Bubble-propelled micromotors based on Ag and MnO 2 catalysts," *J. Am. Chem. Soc.*, vol. 136, no. 7, pp. 2719–2722, 2014.

[10] S. Sanchez, L. Soler, and J. Katuri, "Chemically powered micro- and nanomotors," *Angewandte Chemie - International Edition*, vol. 54, no. 5. pp. 1414–1444, 2015.

[11] J. L. Moran and J. D. Posner, "Phoretic Self-Propulsion," *Annu. Rev. Fluid Mech.*, vol. 49, no. 1, pp. 511–540, 2017.

[12] A. Brown and W. Poon, "Ionic effects in self-propelled Pt-coated Janus swimmers," *Soft Matter*, vol. 10, no. 22, pp. 4016–4027, 2014.

[13] S. Ebbens *et al.*, "Electrokinetic effects in catalytic platinum-insulator Janus swimmers," *Europhys. Lett.*, vol. 106, no. 5, p. 58003, 2014.

[14] J. L. Moran and J. D. Posner, "Electrokinetic locomotion due to reaction-induced charge auto-electrophoresis," *J. Fluid Mech.*, vol. 680, pp. 31–66, 2011.

[15] J. Zhang, X. Zheng, H. Cui, and Z. Silber-Li, "The self-propulsion of the spherical Pt–SiO2 janus micro-motor," *Micromachines*, vol. 8, no. 4, p. 123, 2017.

[16] V. Magdanz, M. Guix, and O. G. Schmidt, "Tubular micromotors: from microjets to spermbots," *Robot. Biomimetics*, vol. 1, no. 1, p. 11, 2014.

[17] V. Magdanz and O. G. Schmidt, "Spermbots: potential impact for drug delivery and assisted reproductive technologies," *Expert Opin. Drug Deliv.*, vol. 11, no. 8, pp. 1125–1129, 2014.

[18] J. Bastos-Arrieta, A. Revilla-Guarinos, W. E. Uspal, and J. Simmchen, "Bacterial Biohybrid Microswimmers," *Front. Robot. AI*, vol. 5, p. 97, Aug. 2018.

[19] M. Medina-Sánchez, H. Xu, and O. G. Schmidt, "Micro-and nano-motors: the new generation of drug carriers," *Ther. Deliv.*, vol. 9, no. 4, pp. 303–316, 2018.

[20] M. M. M. M. M. Stanton *et al.*, "Biohybrid Janus Motors Driven by Escherichia coli," *Adv. Mater. Interfaces*, vol. 3, no. 2, pp. 1–8, Jan. 2016.

[21] S. Ashley, "Nanobot construction crews," *Sci. Am.*, vol. 285, no. 3, pp. 84–85, 2001.

[22] J. Fu and H. Yan, "Controlled drug release by a nanorobot," *Nat. Biotechnol.*, vol. 30, no. 5, pp. 407–408, 2012.

[23] W. H. Chong, Y. Huang, T. N. Wong, K. T. Ooi, and G. Zhu, "Magnetic nanorobots, generating vortexes inside nanoliter droplets for effective mixing," *Adv. Mater. Technol.*, vol. 3, no. 4, p. 1700312, 2018.

[24] J. Katuri, X. Ma, M. M. Stanton, and S. Sánchez, "Designing micro-and nanoswimmers for specific applications," *Acc. Chem. Res.*, vol. 50, no. 1, pp. 2–11, 2017.

[25] L. Schwarz, M. Medina-Sánchez, and O. G. Schmidt, "Hybrid BioMicromotors," *Appl. Phys. Rev.*, vol. 4, no. 3, 2017.

[26] S. Martel, "Flagellated bacterial nanorobots for medical interventions in the human body," in *Surgical Robotics*, Springer, 2011, pp. 397–416.

[27] M. M. Stanton *et al.*, "Magnetotactic Bacteria Powered Biohybrids Target E. coli Biofilms," *ACS Nano*, vol. 11, no. 10, pp. 9968–9978, 2017.

[28] Y. Dong *et al.*, "Magnetic Microswarm Composed of Porous Nanocatalysts for Targeted Elimination of Biofilm Occlusion," *ACS Nano*, vol. 15, no. 3, pp. 5056–5067, 2021.

[29] X. Ma and S. Sánchez, "Self-propelling micro-nanorobots: challenges and future perspectives in nanomedicine," *Nanomedicine*, vol. 12, no. 12, pp. 1363–1367, Jun. 2017.

[30] F. Qiu and B. J. Nelson, "Magnetic helical micro-and nanorobots: Toward their biomedical applications," *Engineering*, vol. 1, no. 1, pp. 21–26, 2015.

[31] A. Krichevsky *et al.*, "Trapping motile magnetotactic bacteria with a magnetic recording head," *J. Appl. Phys.*, vol. 101, no. 1, p. 14701, 2007.

[32] E. Lauga and T. R. Powers, "The hydrodynamics of swimming microorganisms," *Reports Prog. Phys.*, vol. 72, no. 9, 2009.

[33] S. Sánchez, L. Soler, and J. Katuri, "Chemically Powered Micro- and Nanomotors," *Angew. Chemie Int. Ed.*, vol. 54, no. 5, pp. 1414–1444, 2015.

[34] J. Hu, S. Zhou, Y. Sun, X. Fang, and L. Wu, "Fabrication, properties and applications of Janus particles," *Chem. Soc. Rev.*, vol. 41, no. 11, p. 4356, 2012.

[35] S. M. Mousavi *et al.*, "Clustering of Janus particles in an optical potential driven by hydrodynamic fluxes," *Soft Matter*, vol. 15, no. 28, pp. 5748–5759, 2019.

[36] O. Shchepelina, V. Kozlovskaya, S. Singamaneni, E. Kharlampieva, and V. V Tsukruk, "Replication of anisotropic dispersed particulates and complex continuous templates," *J. Mater. Chem.*, vol. 20, no. 32, pp. 6587–6603, 2010.

[37] A. Sahari, D. Headen, and B. Behkam, "Effect of body shape on the motile behavior of bacteria-powered swimming microrobots (BacteriaBots)," *Biomed. Microdevices*, vol. 14, no. 6, pp. 999–1007, 2012.

[38] B. Mostaghaci, O. Yasa, J. Zhuang, and M. Sitti, "Bioadhesive Bacterial Microswimmers for Targeted Drug Delivery in the Urinary and Gastrointestinal Tracts," *Adv. Sci.*, vol. 4, no. 6, pp. 1–9, 2017.

[39] R. Mhanna *et al.*, "Artificial bacterial flagella for remote-controlled targeted single-cell drug delivery," *Small*, vol. 10, no. 10, pp. 1953–1957, 2014.

[40] I. A. M. Ibrahim, A. A. F. Zikry, and M. A. Sharaf, "Preparation of spherical silica nanoparticles: Stober silica," *J. Am. Sci*, vol. 6, no. 11, pp. 985–989, 2010.

[41] J. He *et al.*, "Wet-chemical synthesis of amphiphilic rodlike silica particles and their molecular mimetic assembly in selective solvents," *Angew. Chemie - Int. Ed.*, vol. 51, no. 15, pp. 3628–3633, 2012.

[42] S. R. Karnati, D. Oldham, E. H. Fini, and L. Zhang, "Surface functionalization of silica nanoparticles to enhance aging resistance of asphalt binder," *Constr. Build. Mater.*, vol. 211, pp. 1065–1072, 2019.

[43] P. B. Landon *et al.*, "Designing hollow nano gold golf balls," *ACS Appl. Mater. Interfaces*, vol. 6, no. 13, pp. 9937–9941, 2014.

[44] J. Bastos-Arrieta, C. Bauer, A. Eychmüller, and J. Simmchen, "Galvanic replacement induced electromotive force to propel Janus micromotors," *J. Chem. Phys.*, vol. 150, no. 14, p. 144902, 2019.

[45] A. Ivanova, K. Ivanova, A. Tied, T. Heinze, and T. Tzanov, "Layer-By-Layer Coating of Aminocellulose and Quorum Quenching Acylase on Silver Nanoparticles Synergistically Eradicate Bacteria and Their Biofilms," *Adv. Funct. Mater.*, vol. 30, no. 24, p. 2001284, 2020.

[46] F. C. MacKintosh, "Active diffusion: the erratic dance of chromosomal loci,"*Proc. Natl. Acad. Sci.*, vol. 109, no. 19, pp. 7138–7139, 2012.

[47.] Meijering, E., Dzyubachyk, O., & Smal, I. (2012). Methods for cell and particle tracking. Methods in enzymology, 504, 183-200.

[48] Gal, N., Lechtman-Goldstein, D., & Weihs, D. (2013). Particle tracking in living cells: a review of the mean square displacement method and beyond. Rheologica Acta, 52(5), 425-443.

[49] Ohmi, K., & Li, H. Y. (2000). Particle-tracking velocimetry with new algorithms. Measurement Science and Technology, 11(6), 603.

[50] Rasband, W. S. (1997). ImageJ.

[51] Igathinathane, C., Pordesimo, L. O., Columbus, E. P., Batchelor, W. D., & Methuku, S. R. (2008). Shape identification and particles size distribution from basic shape parameters using ImageJ. Computers and electronics in agriculture, 63(2), 168-182.

[52] Tinevez, J. Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., ... & Eliceiri, K. W. (2017). TrackMate: An open and extensible platform for single-particle tracking. Methods, 115, 80-90.

[53] Michalet, X. (2010). Mean square displacement analysis of single-particle trajectories with localization error: Brownian motion in an isotropic medium. Physical Review E, 82(4), 041914.

[54] Kepten, E., Weron, A., Sikora, G., Burnecki, K., & Garini, Y. (2015). Guidelines for the fitting of anomalous diffusion mean square displacement graphs from single particle tracking experiments. PLoS One, 10(2), e0117722.

[55] Mahmoudi, M. R. (2021). A Computational Technique to Classify Several Fractional Brownian Motion Processes. Chaos, Solitons & Fractals, 111152.

[56] Andreanov, A., & Grebenkov, D. S. (2012). Time-averaged MSD of Brownian motion. Journal of Statistical Mechanics: Theory and Experiment, 2012(07), P07001.

[57] Volpe, G., Gigan, S., & Volpe, G. (2014). Simulation of the active Brownian motion of a microswimmer. American Journal of Physics, 82(7), 659-664.

[58] Waignier, G., Le Meur, A. F., & Duchien, L. (2007, September). Fiesta: A generic framework for integrating new functionalities into software architectures. In European Conference on Software Architecture (pp. 76-91). Springer, Berlin, Heidelberg.

[59] Berg, H. C. (2008). E. coli in Motion. Springer Science & Business Media.

[60] Hu, J., Yang, M., Gompper, G., & Winkler, R. G. (2015). Modelling the mechanics and hydrodynamics of swimming E. coli. Soft matter, 11(40), 7867-7876.

[61] Behkam, B., & Sitti, M. (2004, January). E. coli inspired propulsion for swimming microrobots. In ASME International Mechanical Engineering Congress and Exposition (Vol. 47063, pp. 1037-1041).

[62] Berry, R. M., & Armitage, J. P. (1999). The bacterial flagella motor. Advances in microbial physiology, 41, 291-337.

[63] Hamadi, F., Latrache, H., Elghmari, A., Zahir, H., Mabrrouki, M., & Elbouadili, A. E. (2005). Determination of Escherichia coli negative charge concentration from XPS data and its variation with pH. Journal of Surface Analysis, 12(3), 293.

[64] Dror-Ehre, A., Mamane, H., Belenkova, T., Markovich, G., & Adin, A. (2009). Silver nanoparticle–E. coli colloidal interaction in water and effect on E. coli survival. Journal of Colloid and Interface Science, 339(2), 521-526.

[65] Brown, A., & Poon, W. (2014). Ionic effects in self-propelled Pt-coated Janus swimmers. Soft matter, 10(22), 4016-4027.

[66] Ebbens, S., Gregory, D. A., Dunderdale, G., Howse, J. R., Ibrahim, Y., Liverpool, T. B., & Golestanian, R. (2014). Electrokinetic effects in catalytic platinum-insulator Janus swimmers. EPL (Europhysics Letters), 106(5), 58003.

[67] Wang, S., & Wu, N. (2014). Selecting the swimming mechanisms of colloidal particles: bubble propulsion versus self-diffusiophoresis. Langmuir, 30(12), 3477-3486.

[68] Jurado-Sánchez, B., Pacheco, M., Rojo, J., & Escarpa, A. (2017). Magnetocatalytic graphene quantum dots Janus micromotors for bacterial endotoxin detection. Angewandte Chemie International Edition, 56(24), 6957-6961.

[69] Sarkar, A., & Manthiram, A. (2010). Synthesis of Pt@ Cu core− shell nanoparticles by galvanic displacement of Cu by Pt4+ ions and their application as electrocatalysts for oxygen reduction reaction in fuel cells. The Journal of Physical Chemistry C, 114(1.

[70] Bastos-Arrieta, J., Bauer, C., Eychmüller, A., & Simmchen, J. (2019). Galvanic replacement induced electromotive force to propel Janus micromotors. The Journal of chemical physics, 150(14), 144902.

[71] Sridhar, V., Park, B. W., & Sitti, M. (2018). Light-driven Janus hollow mesoporous TiO2–Au microswimmers. Advanced Functional Materials, 28(25), 1704902.

[72] Wang, X., Baraban, L., Nguyen, A., Ge, J., Misko, V. R., Tempere, J., ... & Makarov, D. (2018). High-Motility Visible Light-Driven Ag/AgCl Janus Micromotors. Small, 14(48), 1803613.

[73] Ma, X., Hahn, K., & Sanchez, S. (2015). Catalytic mesoporous Janus nanomotors for active cargo delivery. Journal of the American Chemical Society, 137(15), 4976-4979.

[74] Llopis-Lorente, A., Díez, P., La Torre-Paredes, D., Sánchez, A., Sancenón Galarza, F., Aznar, E., ... & Villalonga, R. (2017). Enzyme-controlled nanodevice for acetylcholine-triggered cargo delivery based on Janus Au-mesoporous silica nanoparticles. Chemi.

[75] Xing, Y., Zhou, M., Du, X., Li, X., Li, J., Xu, T., & Zhang, X. (2019). Hollow mesoporous carbon@ Pt Janus nanomotors with dual response of H2O2 and near-infrared light for active cargo delivery. Applied Materials Today, 17, 85-91.

[76] Marschelke, C., Fery, A., & Synytska, A. (2020). Janus particles: From concepts to environmentally friendly materials and sustainable applications. Colloid and Polymer Science, 298(7), 841-865.

[77] Schattling, P., Thingholm, B., & Stadler, B. (2015). Enhanced diffusion of glucose-fueled Janus particles. Chemistry of Materials, 27(21), 7412-7418.

[78] Fu, J., & Yan, H. (2012). Controlled drug release by a nanorobot. Nature biotechnology, 30(5), 407-408.

[79] ASHLEY, Steven. Nanobot construction crews. Scientific American, 2001, 285.3: 84-85.

[80] Ma, X., & Sánchez, S. (2017). Self-propelling micro-nanorobots: challenges and future perspectives in nanomedicine.

[81] Fauzi, S. H. (1992). The principle of electron microscopy e SEM and TEM. Buletin Sains dan Teknologi Keadaan Pepejal Malaysia, 2(2), 55-60.

8 Annex

Table 1

La-bel	Composition	Description
A.1	SiO_2/APTES/Pt	Janus sphere SiO_2/Pt. SiO_2 face APTES functionalized.
A.2	SiO_2/APTES/Cu	Janus sphere SiO_2/Cu. SiO_2 face APTES functionalized.
B.1	SiO_2/Pt	Janus sphere SiO_2/Pt. SiO_2 face hydrophobic.
B.2	SiO_2/Cu	Janus sphere SiO_2/Cu. SiO_2 face hydrophobic.
C.1	SiO_2/Pt	Janus rods SiO_2/Pt.
C.2	SIO2/CU	JANUS RODS SIO2/CU.
D.1	ZNO/PT	JANUS SPHERE ZNO/PT.
D.2	ZNO/CU	JANUS SPHERE ZNO/CU.
E.1	ZNO/PDDA/PT	JANUS SPHERE ZNO/PT. ZNO FACE PDDAA FUNCTIONAL-IZED.
E.2	ZNO/PDDA/CU	JANUS SPHERE ZNO/CU. ZNO FACE PDDAA FUNCTIONAL-IZED.