Agència de Gestió d'Ajuts Universitaris i de Recerca

AGAUR

Memòria justificativa de recerca de les convocatòries BCC, BE, BP, CTP-AIRE, INEFC i PIV

La memòria justificativa consta de les dues parts que venen a continuació:
1.- Dades bàsiques i resums
2.- Memòria del treball (informe científic)

Tots els camps són obligatoris

1.- Dades bàsiques i resums

Nom de la convocatòria

Beatriu de Pinós

Llegenda per a les convocatòries:

BCC	Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior
BE	Beques per a estades per a la recerca fora de Catalunya
BP	Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós
CTP-AIRE	Ajuts per accions de cooperació en el marc de la comunitat de treball dels Pirineus. Ajuts de mobilitat de personal investigador.
INEFC	Beques predoctorals i de col·laboració, dins de l'àmbit de l'educació física i l'esport i les ciències aplicades a l'esport
PIV	Beques de recerca per a professors i investigadors visitants a Catalunya

Títol del projecte: ha de sintetitzar la temàtica científica del vostre document.
NANOMOTORS: NEW STRATEGIES OF (BIO)SENSING BASED ON MOTION.

Dades de l'investigador o beneficiari

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Paraules clau: cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.
Nanomotors, microengines, (bio)sensors, biomolecules, isolation, biotransport.

Data de presentació de la justificació
27-11-2012

Nom i cognoms i signatura del/de la investigador/a	Vist i plau del/de la responsable de la sol·licitud

Resum del projecte: cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

Resum en la llengua del projecte (màxim 300 paraules)

Resum en anglès (màxim 300 paraules)

Nanomotors are nanoscale devices capable of converting energy into movement and forces. Among them, self-propelled nanomotors offer considerable promise for developing new and novel bioanalytical and biosensing strategies based on the direct isolation of target biomolecules or changes in their movement in the presence of target analytes. The main achievements of this project consists on the development of receptor-functionalized nanomotors that offer direct and rapid target detection, isolation and transport from raw biological samples without preparatory and washing steps. For example, microtube engines functionalized with aptamer, antibody, lectin and enzymes receptors were used for the direct isolation of analytes of biomedical interest, including proteins and whole cells, among others. A target protein was also isolated from a complex sample by using an antigen-functionalized microengine navigating into the reservoirs of a lab-on-a-chip device. The new nanomotor-based target biomarkers detection strategy not only offers highly sensitive, rapid, simple and low cost alternative for the isolation and transport of target molecules, but also represents a new dimension of analytical information based on motion. The recognition events can be easily visualized by optical microscope (without any sophisticated analytical instrument) to reveal the target presence and concentration. The use of artificial nanomachines has shown not only to be useful for (bio)recognition and (bio)transport but also for detection of environmental contamination and remediation. In this context, micromotors modified with a superhydrophobic layer demonstrated that effectively interacted, captured, transported and removed oil droplets from oil contaminated samples. Finally, a unique micromotor-based strategy for water-quality testing, that mimics live-fish water-quality testing, based on changes in the propulsion behavior of artificial biocatalytic microswimmers in the presence of aquatic pollutants was also developed. The attractive features of the new micromachine-based target isolation and signal transduction protocols developed in this project offer numerous potential applications in biomedical diagnostics, environmental monitoring, and forensic analysis.

Resum en anglès (màxim 300 paraules) – continuació -.

2.- Memòria del treball (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

1. INTRODUCTION

Nanomotors are currently the subject of intense interest due to their great potential to perform (at the nanoscale) a broad range of complex tasks. Nanoscale assembly and fabrication, autonomous transport of cargo sensing and biosensing and environmental remediation have motivated the development of this project.

Recent efforts have demonstrated the ability of synthetic nanomotors to convert chemical energy into autonomous motion. In particular, the efficient electrochemical propulsion of catalytic microtube engines has been demonstrated in the presence of hydrogen peroxide fuel. Such microtube motors display high power and speed, along with a precise motion control and can be easily modified to impart new functionalities. These new capabilities have opened up new opportunities of using catalytic nanomotors for diverse and important applications. This research explores new methods to fabricate electrochemically propelled artificial nanomotors and apply them not only for the chemical sensing and biosensing of target analytes, but also for the direct isolation of target biomolecules and cells from complex biological samples. Efficient isolation of target biomaterials from a complex mixture represents one of the most challenging tasks in biology. Biological targets, ranging from nucleic acids to proteins and cancer cells, are typically isolated from raw biological samples by using laborious, time-consuming and/or expensive protocols. Receptor-functionalized nanomachines can provide a fundamentally new concept for addressing the challenge of isolating biological targets from complex body fluids and transporting them to a clean environment for downstream analysis.

Such nanomachine-enabled target isolation offers considerable promise for diverse biotechnological and bioanalytical applications, and for designing miniaturized lab-on-chip systems, integrating the capture, transport and release operations. The ability of electrochemically-propelled nanomotors to respond to their surrounding environment has opened the door for developing new motion-based detection platforms. Motion-based transduction relies on the use of an optical microscope for tracking changes in the speed of microtubular motors in the presence of the target analyte, hence obviating the need for sophisticated analytical instruments. Specific biorecognition events can thus be translated into useful speed/distance signals where the binding event leads to a change in the motion. The successful realization of these new motion-based sensing and isolation platforms require an optimal locomotive performance, large towing power in complex biological environments and judicious control of their surface chemistry. In this project was explored the fabrication and operation of electrochemically-propelled synthetic nanomotors for their use in motion-based signal transduction and target isolation. The first part of the project was devoted to fabricate both tubular microengines and multi-fuel driven janus micromotors. Once characterized, the second part is dedicated to explore different applications mainly of biomedical and environmental interest, which is summarized in the following sections.

2. FABRICATION AND CHARACTERIZATION

2.1. Highly Efficient Catalytic Microengines: Template Electrosynthesis of Polyaniline/Platinum Microtubes.

Highly efficient catalytic microtubular engines are synthesized rapidly and inexpensively using an electrochemical growth of bilayer polyaniline/platinum microtubes within the conically shaped pores of a polycarbonate template membrane. These mass-produced microtubular engines are only 8 μm long, are self-propelled at an ultrafast speed (of over 350 body lengths s^{-1}), and can operate in very low levels of the hydrogen peroxide fuel (down to 0.2%). The propulsion characteristics and optimization of these microtubular engines are described, along with their efficient operation in different biological environments which holds great promise for biomedical applications.

2.2. Multi-Fuel Driven Janus Micromotors.

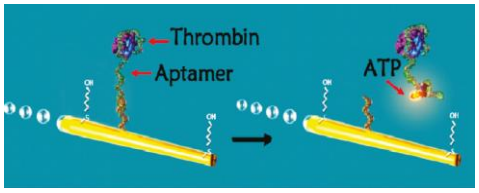
Here it is presented the first example of a chemically powered micromotor that harvests its energy from the reactions of three different fuels. The new Al/Pd Janus microspheres prepared by depositing a Pd layer on one side of Al microparticles, propelled efficiently by the thrust of hydrogen bubbles generated from different reactions of Al in strong acidic and alkaline environments, and by an oxygen bubble thrust produced at their partial Pd coating in hydrogen peroxide media. High speeds and long lifetimes of 200 μ m s^{-1} and 8 min are achieved in strong alkaline media and acidic media, respectively. The ability to autonomously adapt to the presence of a new fuel (surrounding environment), without compromising the propulsion behavior is illustrated. These data also represent the first example of a chemically powered

micromotor that propels autonomously and efficiently in alkaline environments (pH > 11) without additional fuels. The ability to use multiple fuel sources to power the same micromotor offers a broader scope of operation and considerable promise for diverse applications of micromotors in different chemical environments.

3. APPICATION OF THE DEVELOPED ANALYTICAL TOOL.

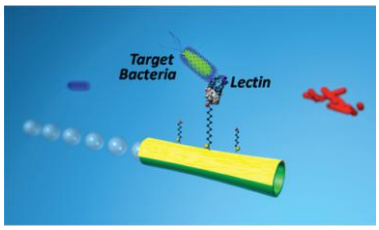
3.1. Biomedical applications.

3.1.1. Dynamic Isolation and Unloading of Target Proteins by Aptamer-Modified Microtransporters.



We describe here a new strategy for isolating target proteins from complex biological samples based on an aptamer-modified self-propelled microtube engine. For this purpose, a thiolated thrombin or a mixed thrombin-ATP aptamer (prehybridized with a thiolated short DNA) was coassembled with mercaptohexanol onto the gold surface of these microtube engines. The rapid movement of the aptamermodified microtransporter resulted in highly selective and rapid capture of the target thrombin, with an effective discrimination against a large excess of nontarget proteins. Release of the captured thrombin can be triggered by the addition of ATP that can bind and displace the immobilized mixed thrombin-ATP aptamer in 20 min. The rapid loading and unloading abilities demonstrated by these selective microtransporters are illustrated in complex matrixes such as human serum and plasma. The new motion-driven protein isolation platform represents a new approach in bioanalytical chemistry based on active transport of proteins and offers considerable promise for diverse diagnostic applications.

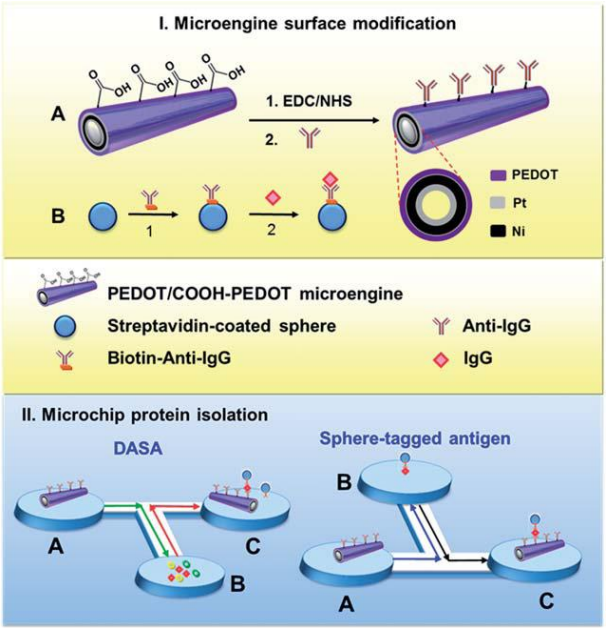
3.1.2 Bacterial Isolation by Lectin-Modified Microengines.



New template-based self-propelled gold/nickel/polyaniline/platinum (Au/Ni/PANI/Pt) microtubular engines, functionalized with the Concanavalin A (ConA) lectin bioreceptor, are shown to be extremely useful for the rapid, real-time isolation of *Escherichia coli (E. coli)* bacteria from fuel-enhanced environmental, food, and clinical samples. These multifunctional microtube engines combine the selective capture of E. coli with the uptake of polymeric drug-carrier particles to provide an attractive motion-based theranostics strategy. Triggered release of the captured bacteria is demonstrated by movement through a low-pH glycine-based dissociation solution. The smaller size of the new polymer-metal

microengines offers convenient, direct, and label-free optical visualization of the captured bacteria and discrimination against nontarget cells.

3.1.3. Micromotor-based lab-on-chip immunoassays.



Here we describe the first example of using self-propelled antibody-functionalized synthetic catalytic microengines for capturing and transporting target proteins between the different reservoirs of a labon-a-chip (LOC) device. A new catalytic polymer/Ni/Pt microtube engine, containing carboxy moieties on its mixed ply(3,4-ethylenedioxythiophene) (PEDOT)/COOH–PEDOT polymeric outermost layer, is further functionalized with the antibody receptor to selectively recognize and capture the target protein. The new motor-based microchip immunoassay operations are carried out without any bulk fluid flow, replacing the common washing steps in antibody-based protein bioassays with the active transport of the captured protein throughout the different reservoirs, where each step of the immunoassay takes place. A first microchip format involving an 'on-the-fly' double-antibody sandwich assay (DASA) is used for demonstrating the selective capture of the target protein, in the presence of excess of non-target proteins. A secondary antibody tagged with a polymeric-sphere tracer allows the direct visualization of the binding events. In a second approach the immuno-nanomotor captures and transports the microsphere-tagged antigen through a microchannel network. An anti-protein-A modified microengine is finally used to demonstrate the selective capture, transport and convenient label-free optical detection of a *Staphylococcus aureus* target bacteria (containing proteinA in its cell wall) in the presence of a large excess of non-target (*Saccharomyces cerevisiae*) cells. The resulting nanomotor-based microchip immunoasay offers considerable potential for diverse applications in clinical diagnostics, environmental and security monitoring fields.

3.1.4. Toward imaging inflammation with ultrasound based molecular imaging

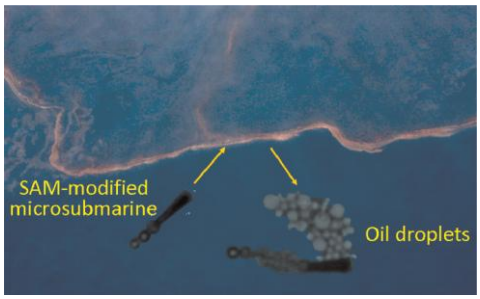
In this work we present a new class of a motor-based ultrasound molecular imaging agents that generate microbubbles in tissues containing elevated levels of hydrogen peroxide H_2O_2. Our approach extends upon micromotors that are designed to move rapidly through fluids by catalyzing H_2O_2 and propelling forward by the escaping

oxygen microbubbles. To detect motion by microscopy, micromotors must be exposed to 1000 times greater H_2O_2 concentration than the maximum expected level in vivo that is produced by activated neutrophils. We aimed to prove that ultrasound can detect the expelled oxygen microbubbles, determine the minimum H_2O_2 concentration needed for microbubble detection, and explore alternate designs to detect the H_2O_2 produced by activated neutrophils to justify proceeding in vivo. Oxygen microbubbles were detectable by ultrasound at 2.5 mM H_2O_2, which is 25 times less than what is needed to detect motion. The detection limit was further reduced by assembling an inner catalase coating. Our best results were achieved with a 400-500 nm spherical design with alternating surface coatings of catalase and PSS over a silica core. Detection limits varied with particle number and diluent, reaching 10-100 µM when assays were done in plasma. Most importantly, we were able to distinguish freshly isolated neutrophils induced to undergo a respiratory burst by phorbol myristate acetate from naïve neutrophils. Future work will continue to optimize particle design to further decrease the H_2O_2 detection limit, decrease particle size, and improve biocompatibility to detect pathological H_2O_2 levels in vivo.

3.2. Environmental applications

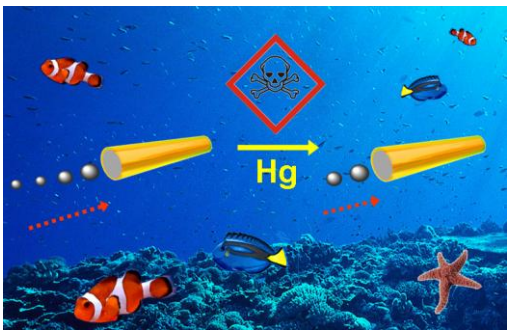
3.2.1. Superhydrophobic Alkanethiol-Coated Microsubmarines for Effective Removal of Oil.

We demonstrate the use of artificial nanomachines for effective interaction, capture, transport, and removal of oil droplets. The simple nanomachine-enabled oil collection method is based on modifying microtube engines with a superhydrophobic layer able to adsorb oil by means of its strong adhesion to a long chain of self-assembled monolayers (SAMs) of alkanethiols created on the rough gold outer surface of the device. The resultant SAM-coated Au/Ni/PEDOT/Pt microsubmarine displays continuous interaction with large oil droplets and is capable of loading and transporting multiple small oil droplets. The influence of the alkanethiol chain length, polarity, and head functional group and hence of the surface hydrophobicity upon the oil_nanomotor interaction and the propulsion is examined. No such oil-motor interactions were observed in control experiments involving both unmodified microengines and microengines coated with SAM layers containing a polar terminal group. These results demonstrate that such SAM-Au/Ni/PEDOT/Pt micromachines can be useful for a facile, rapid, and efficient collection of oils in water samples, which can be potentially exploited for other water-oil separation systems. The integration of oil-sorption properties into self-propelled microengines holds great promise for the remediation of oil-contaminated water samples and for the isolation of other hydrophobic targets, such as drugs.



3.6. Artificial nanofish for water-quality testing

Finally, we present a novel micromotor-based strategy for water-quality testing based on changes in the propulsion behavior of artificial biocatalytic microswimmers in the presence of aquatic pollutants. The new micromotor toxicity testing concept, mimics live-fish water-quality testing, relies on the toxin-induced inhibition of the enzyme catalase, responsible for the biocatalytic bubble propulsion of microtube engines. The locomotion and survival of the artificial nanofish are thus impaired by exposure to a broad range of contaminants, that leads to distinct time-dependent irreversible losses in the catalase activity, and hence of the propulsion behavior. Such use of catalase-powered nanofish offers highly-sensitive direct visualization of changes in the swimming behavior in the presence of common contaminants and hence to real-time assessment of the water quality. A biocompatible polymeric (PEDOT)/Au-catalase tubular microengine is used as the synthetic mimicking fish. The observed changes in the swimming behavior in the presence of model heavy metals, pesticides and herbicides correlate well with the inhibition behavior of these toxic substances. Quantitative data on the adverse effects of these toxins upon the swimming behavior of the enzyme-powered artificial swimmer are obtained by estimating common ecotoxicological parameters under chemical stress, including the EC 50 (exposure concentration causing 50% attenuation of nanofish locomotion) and the swimmer survival time (lifetime expectancy). Such novel use of artificial nanofish addresses major standardization and reproducibility problems and ethical concerns associated with live-fish toxicity assays and hence offers an attractive alternative to the common use of aquatic organisms for water-quality testing.



4. Conclusions

In this project we developed new sensing and biosensing concepts based on electrochemically-propelled nanomotors. In particular 2 different nanomotors, i.e. tubular and janus micromotors were fabricated. Microengines were used for the development of novel sensing protocols based on the direct isolation of target biomolecules from complex media or upon changes in the nanomotor speed induced by the target analyte. While chemically powered Janus micromotors propel autonomously and efficiently in different chemical environments without additional fuels. Such motion-based sensing and target isolation represent a fundamentally new paradigm in analytical chemistry, as it relies on the motility of artificial nanomotors for advanced biosensing applications. The nanomachine-based target-isolation strategy promises to greatly enhance medical diagnostics and biodetection by enabling and expediting the isolation, removal and transport of biological targets of different scales from unprocessed biological fluids. The unique features of the new motion-driven bio-isolation platform make it an extremely attractive alternative for current sample processing protocols.

New motion-driven sensing protocols, based on different biomolecular interactions and motion transduction principles, are expected in the near future. The motion-based signal transduction could thus be readily extended to the detection of a broad range of target biomolecules in connection to different biomolecular interactions and motion transduction principles. While the micromachine target isolation concept has been illustrated using DNA, proteins and whole cell targets, it can be further extended to additional biomaterials (e.g viruses) through the use of different surface-confined receptors (lectins, peptides). Recent advances in the collective behavior of multiple motors should also be of great interest for isolating biological (and not biological) targets and for multiplexing experiments such as environmental remediation. Progress in the field will likely come from integration of achievements of the latest few years into more complete and functional devices. The attractive analytical features of the new motion-driven bioassays along with the new capabilities of man-made nanomachines offer numerous opportunities in diverse areas, including biomedical diagnostics, forensic analysis, food safety or environmental monitoring. As future nanomachines become more functional and sophisticated they are expected to perform more diverse tasks and demanding activities. The exciting research area of nanomachines is expected to make important contributions to the field of analytical chemistry, and to the development of new sensing strategies.

5. LIST OF ARTICLES PUBLISHED IN THIS PROJECT.

10. **Jahir Orozco**, Victor García-Gradilla, Mattia D'Agostino, Wei Gao, Allan Cortés and Joseph Wang. Artificial Enzyme-Powered Nanofish for Water-Quality Testing. Accepted **ACS nano**.

9. ES Olson**, J. Orozco**, Z. Wu, B. Yi, W. Gao, M. Eghtedari, J. Wang, R. Mattrey. Toward imaging inflammation with ultrasound based molecular imaging. In preparation.

8. Miguel García, **Jahir Orozco**, Maria Guix, Wei Gao, Sirilak Sattayasamitsathit, Arben Merkoçi, Alberto Escarpa and Joseph Wang. *Micromotor-Based Lab-on-Chip Immunoassays*. **Nanoscale**. DOI: 10.1039/c2nr32400h.

7. **Jahir Orozco** and Linda K. Medlin. Review: advances in electrochemical genosensors-based methods for monitoring blooms of toxic algae. **Environmental Science and Pollution Research.** DOI 10.1007/s11356-012-1258-5

6. Wei Gao, Mattia D'Agostino, Victor Garcia-Gradilla, **Jahir Orozco**, Joseph Wang. *Multi-Fuel Driven Janus Micromotors*. **Small**. DOI: 10.1002/smll.201201864

5. Maria Guix, **Jahir Orozco**, Miguel García, Wei Gao, Sirilak Sattayasamitsathit, Arben Merkoçi, Alberto Escarpa and Joseph Wang. *Superhydrophobic alkane-thiol-coated microsubmarines for effective removal of oil.* **ACS nano** 6 (2012), 4445-4451.

4. Susana Campuzano, **Jahir Orozco**, Daniel Kagan, Maria Guix, Wei Gao, Sirilak Sattayasamitsathit, Jonathan C. Claussen, Arben Merkoçi, Joseph Wang. *Bacterial Isolation by Lectin-Modified Microengine*s. **Nanoletters** 12 (2012) 396-401.

3. **Jahir Orozco**, Susana Campuzano, Daniel Kagan, Ming Zhou, Wei Gao, Joseph Wang. *Dynamic Isolation and Unloading of Target Proteins by Aptamer-Modified Microtransporters*. **Analytical Chemistry** 83 (2011) 7962-7969.

2. Susana Campuzano, Daniel Kagan, **Jahir Orozco**, Joseph Wang. Motion-driven sensing and biosensing using electrochemically propelled nanomotors. **Analyst** 136 (2011) 4621-4630.

1. Wei Gao, Sirilak Sattayasamitsathit, **Jahir Orozco**, Joseph Wang. *Highly Efficient CatalyticMicroengines: Template Electro- synthesis of Polyaniline-Platinum Microtubes*. **Journal of The Americal Chemical Society** 133 (2011)11862–11864.