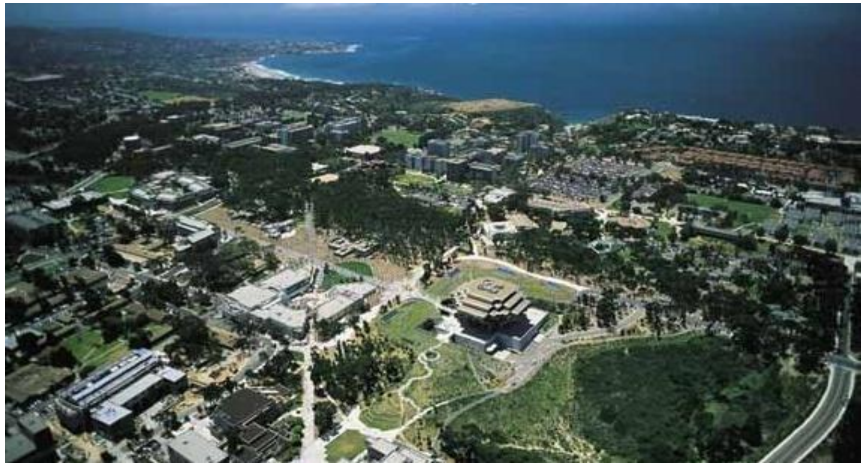
Nanomotor-based Active Intracellular Delivery

Joe Wang
Dept. of Nanoengineering,
San Diego



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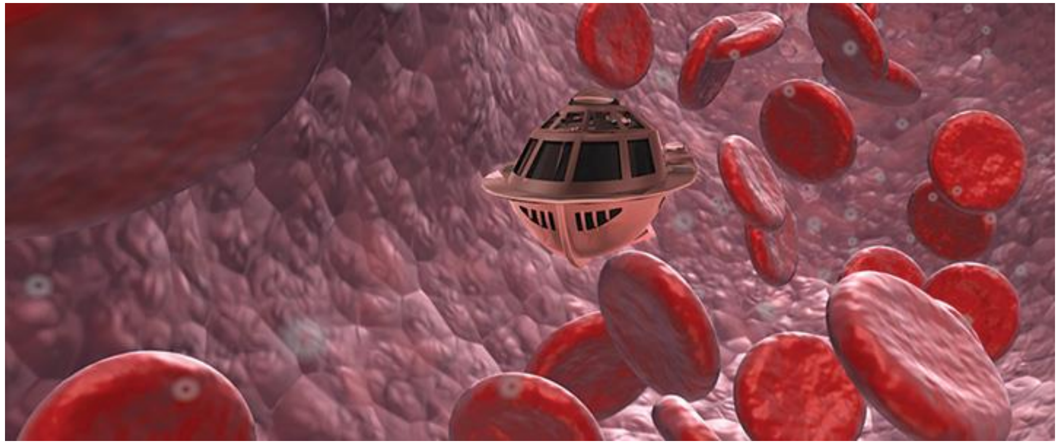
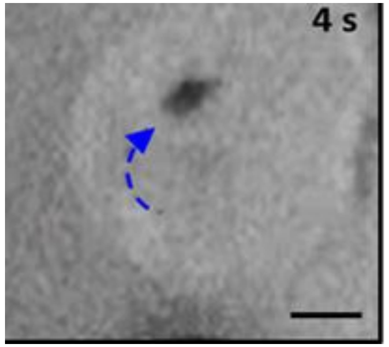


The 15th U.S.-Korea Forum on Nanotechnology: Nanomedicine Focusing on Single Cell Level

Developing Dynamic Nanoscale Vehicles Overcoming Cellular Barriers

Background: The internalization of therapeutic and imaging agents, such as nucleic acids, peptides, proteins, or molecular probes, into cells represents a crucial step in many of the next generation strategies for the diagnosis and treatment of different diseases

Goal: Nanomotor-based Active Intracellular Delivery



Being so small they can operate within cells.

SYNTHETIC NANOMACHINES

Nanomotors are nanoscale devices capable of converting energy into movement and forces.

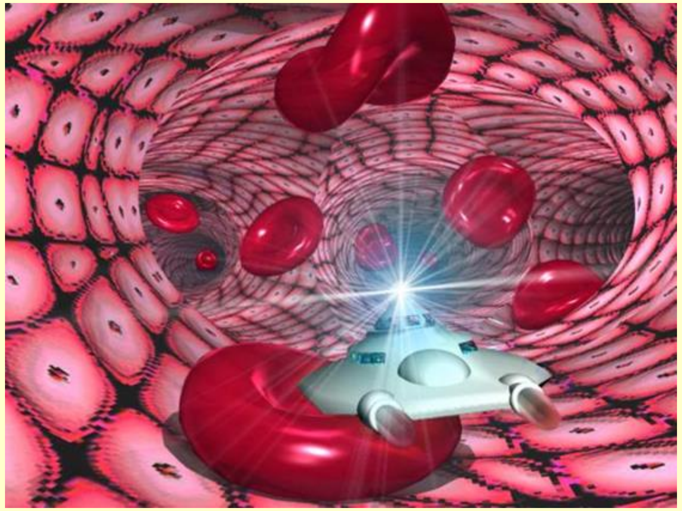
Nanomotors are subject of intense interest due to their great potential to perform broad range of complex tasks at the nano scale.

Motivation: Nanoscale assembly and fabrication and manufacturing, autonomous transport of cargo, actuation of nanorobots, directed drug delivery, nano-surgery, biosensing, intracellular tasks, environmental remediation, defense applications.

MAN-MADE MACHINES

Convert chemical energy to mechanical motion and perform tasks

Shrinking 1 million times

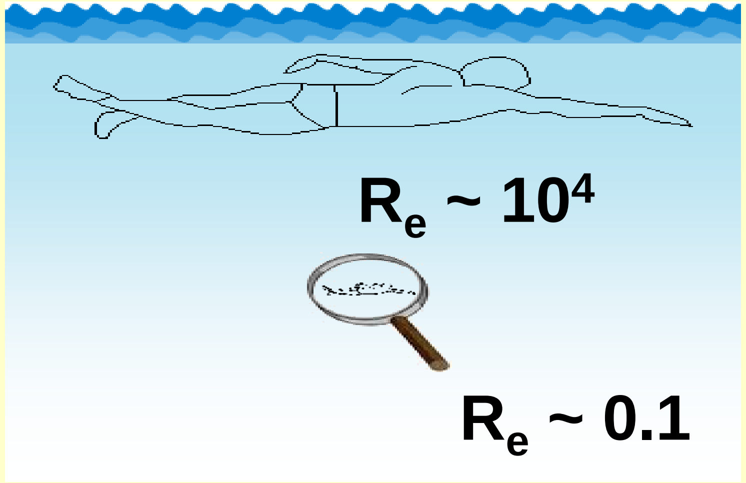


1966 *Fantastic Voyage*

Challenge: How to power and navigate (*steer*) this nanovehicle?

CHALLENGES TO NANOSCALE MOTION

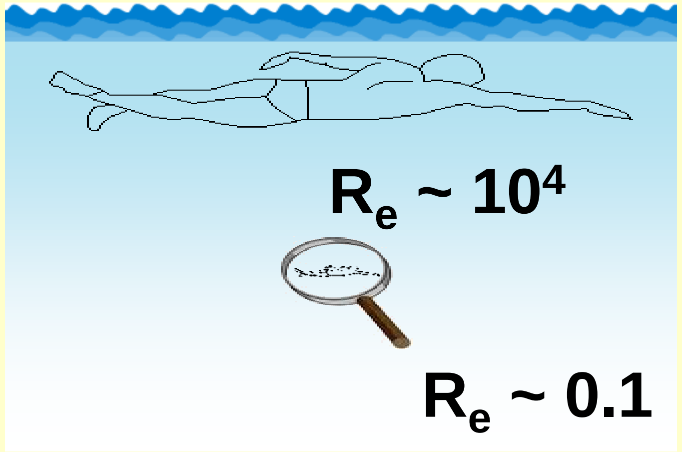
Physics works differently at the nanoscale.
The propulsion of nanoscale objects is challenging because of the combination of
Brownian motion and low Reynolds numbers (where viscosity dominates).



Hence, design principles used in the macro-scale world may not applicable in the nanoscale.

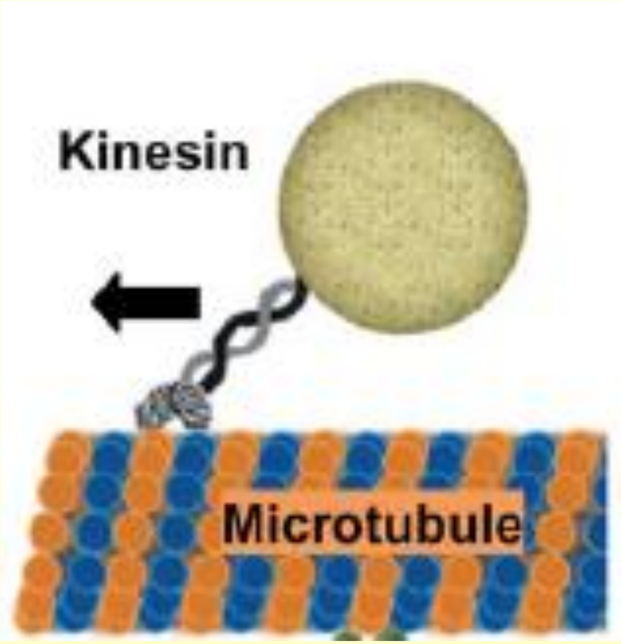
Nanoscale Motion Requires
Sophisticated Propulsion Mechanisms and Advanced Motion Control:

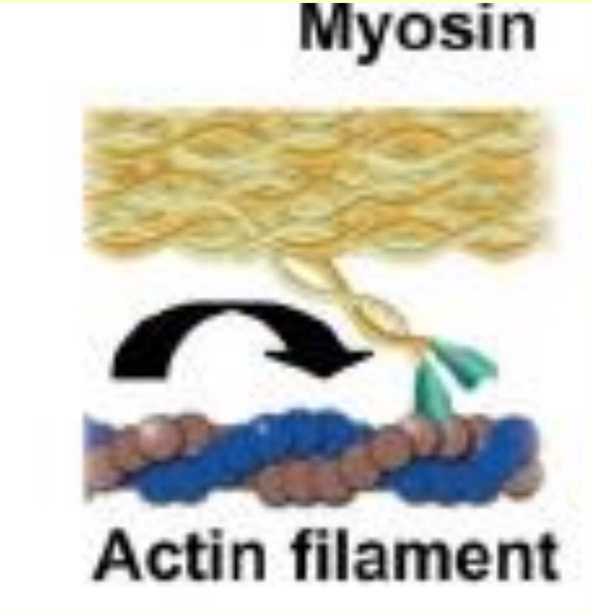
- ‘Swimming’ strategy that works in low Reynolds numbers environments.
- A strategy for steering and directing the motion that can overcome the Brownian motion.



How Nature Regulates the Motion of its Biomotors?

To overcome viscosity forces and Brownian motion the muscle **myosin** and walking protein **kinesin** move along actin or microtubule protein filaments, respectively, which act as **motor 'tracks'** or 'highways'.

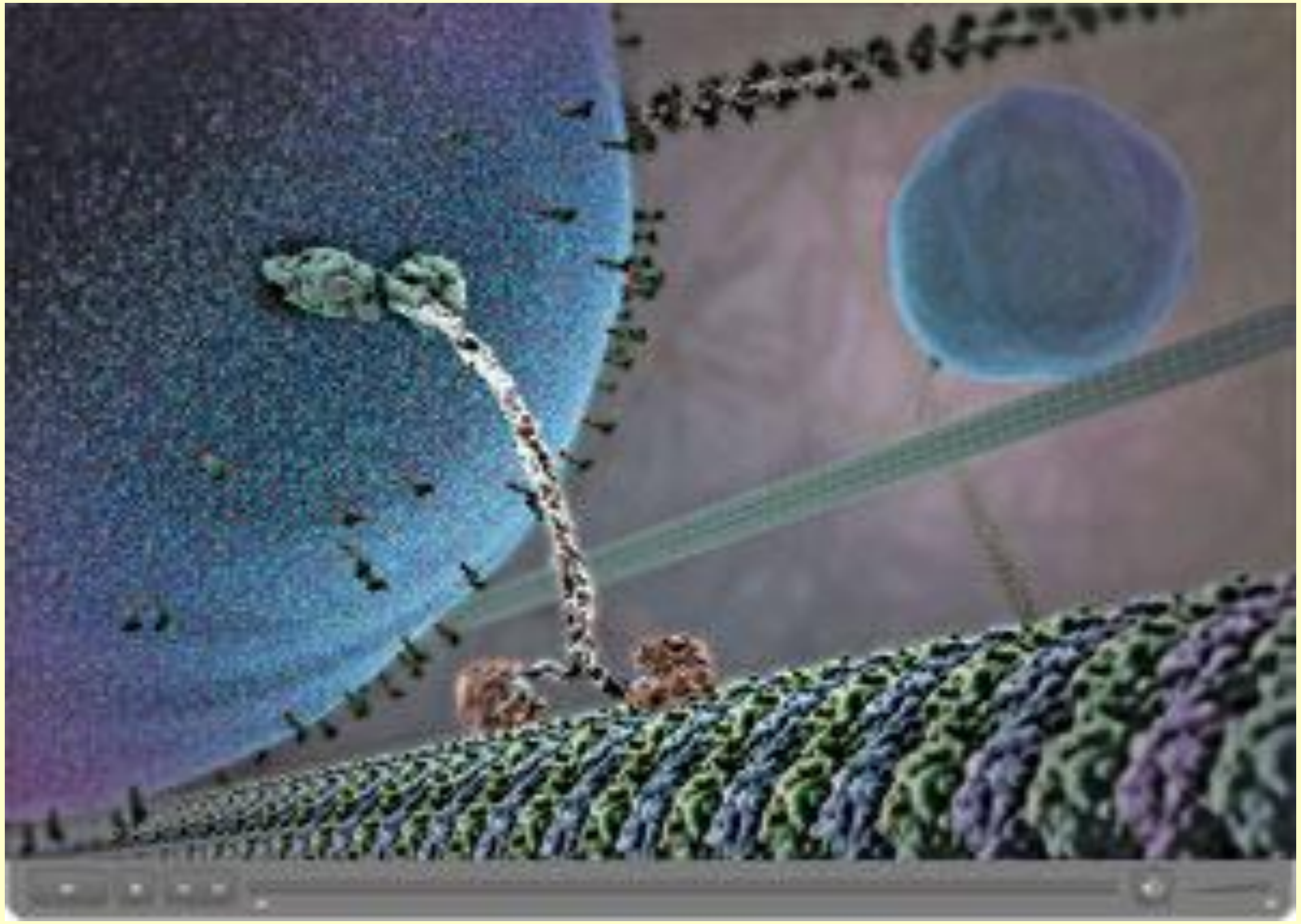




'Track'-Guided Movement

Intracellular Transport System:

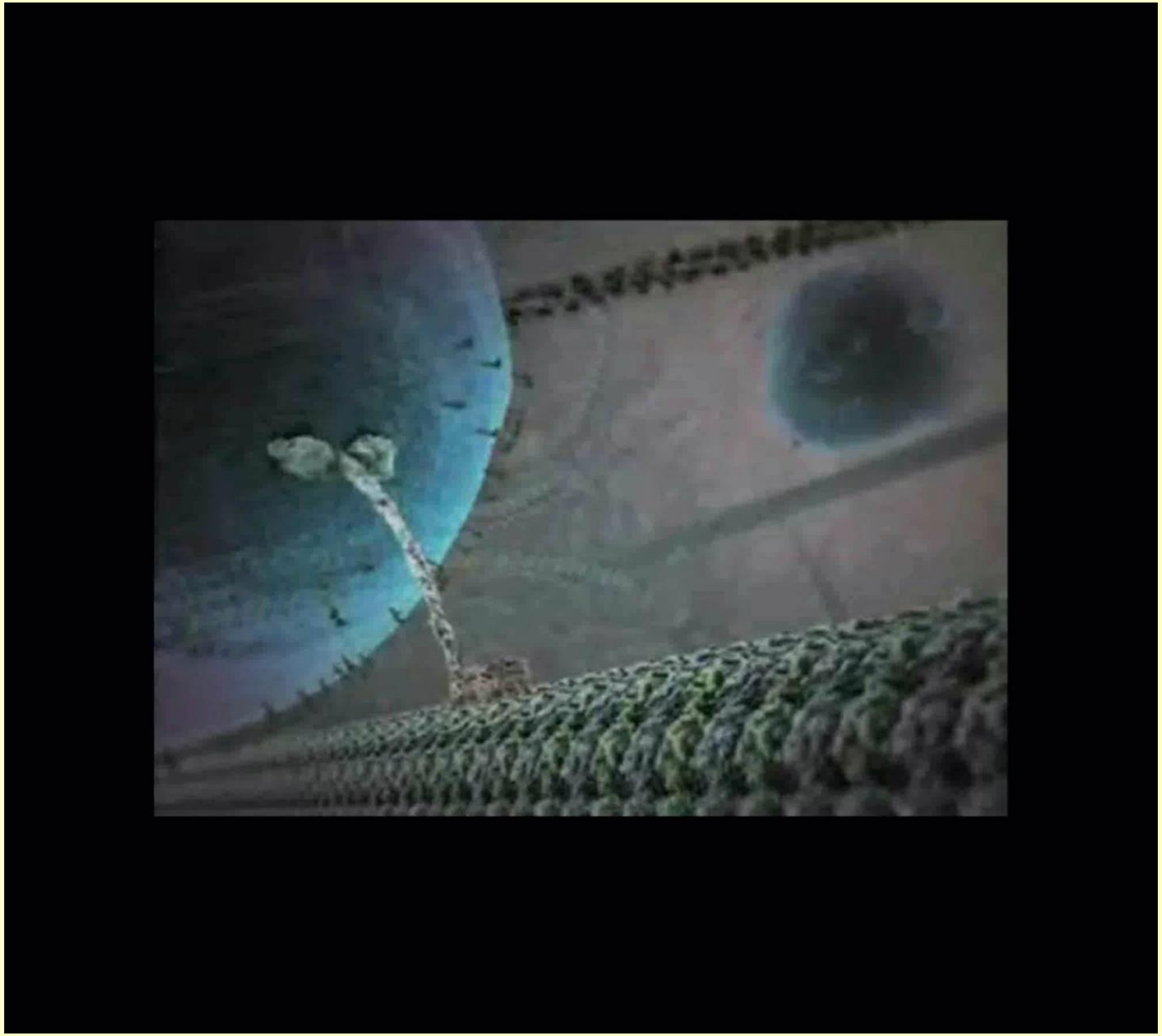
Kinesin moves various cargos along tracks of microtubules (‘cellular highways’) inside cells.



Meeting the demands for cargo delivery within the cell.

Intracellular Transport System:

Kinesin moves various cargos along tracks of microtubules ('cellular highways') inside cells.



TOWARDS SYNTHETIC NANOMACHINES

The remarkable motion capabilities of biomotors provides an inspiration for the development of artificial **nanomachines**, operated on locally supplied fuels and performing various tasks.

Learning From Nature:

Key Questions **(in developing synthetic nanomotors)**:

- How nature addresses the challenges of nanoscale motion?
- Can we use similar chemistry (fuels) to drive synthetic nanomotors? Can we mimic the remarkable cargo towing?
- Can we compete with biological motors?
- Can we mimic the advanced navigation and speed regulation of biomotors?
- Can synthetic microparticles 'communicate' and swarm?
- What tasks can they perform?
- Can we integrate our nanoengines with complex microchip systems?
- What environments they can function?

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Capabilities and Sophistication of Synthetic Nanomachines

The greatly improved capabilities and versatility of modern nanomachines, including their speed and power, advanced motion control, cargo-towing force, collective action and multi-functionality, offer great promise for perform complex tasks at the nanoscale.

These capabilities of synthetic nanomotors make them extremely promising vehicles for variety of operations at the cellular level.

Biomedical Applications

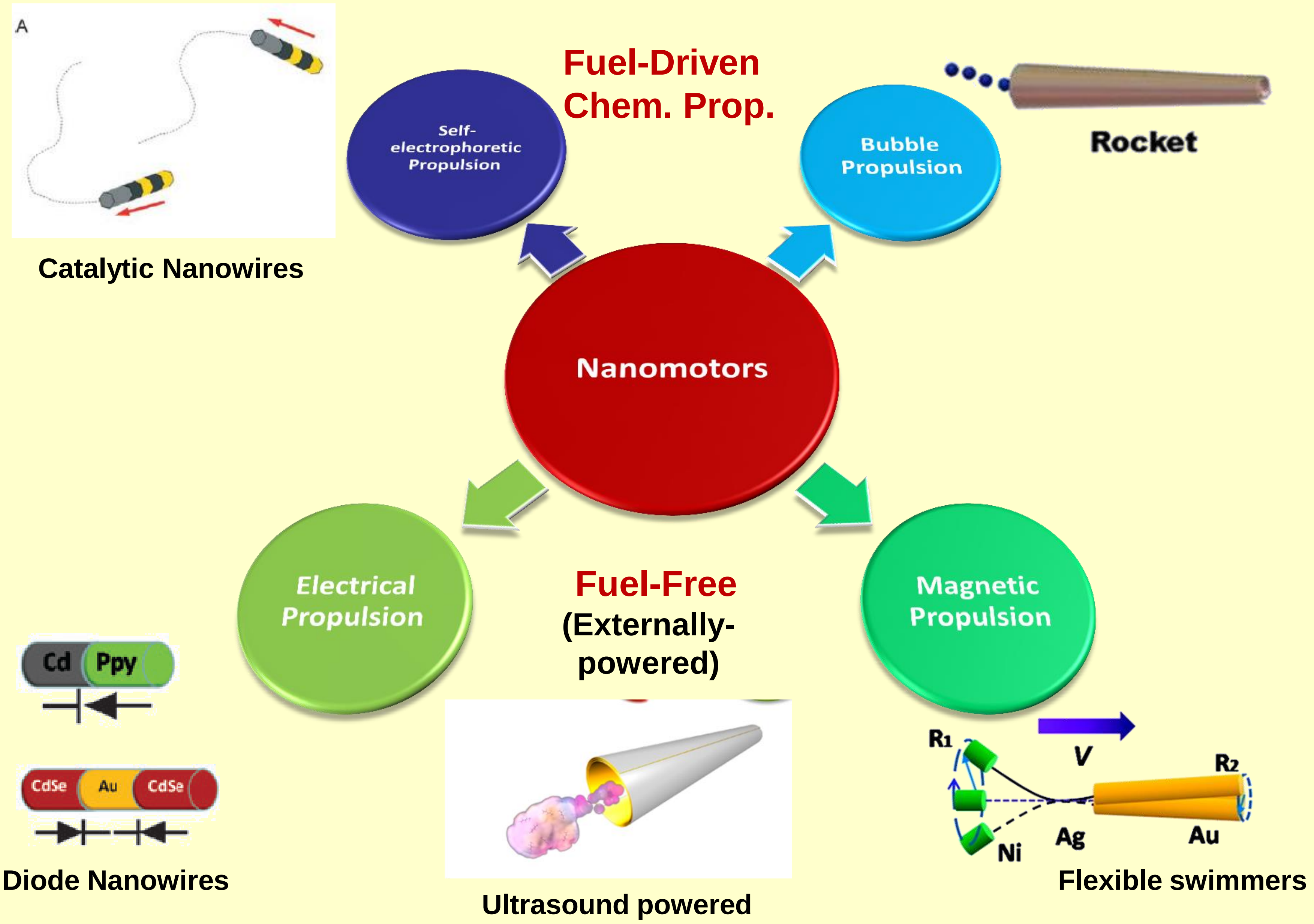
Possibilities for the not-so-distant future

- Directed drug delivery
- Taking biopsy
- Microchip bioassays
- Nanosurgery
- Cleaning clogged arteries.
- Intracellular operation (delivery and sensing)

Wang, Science Robotics, 2017

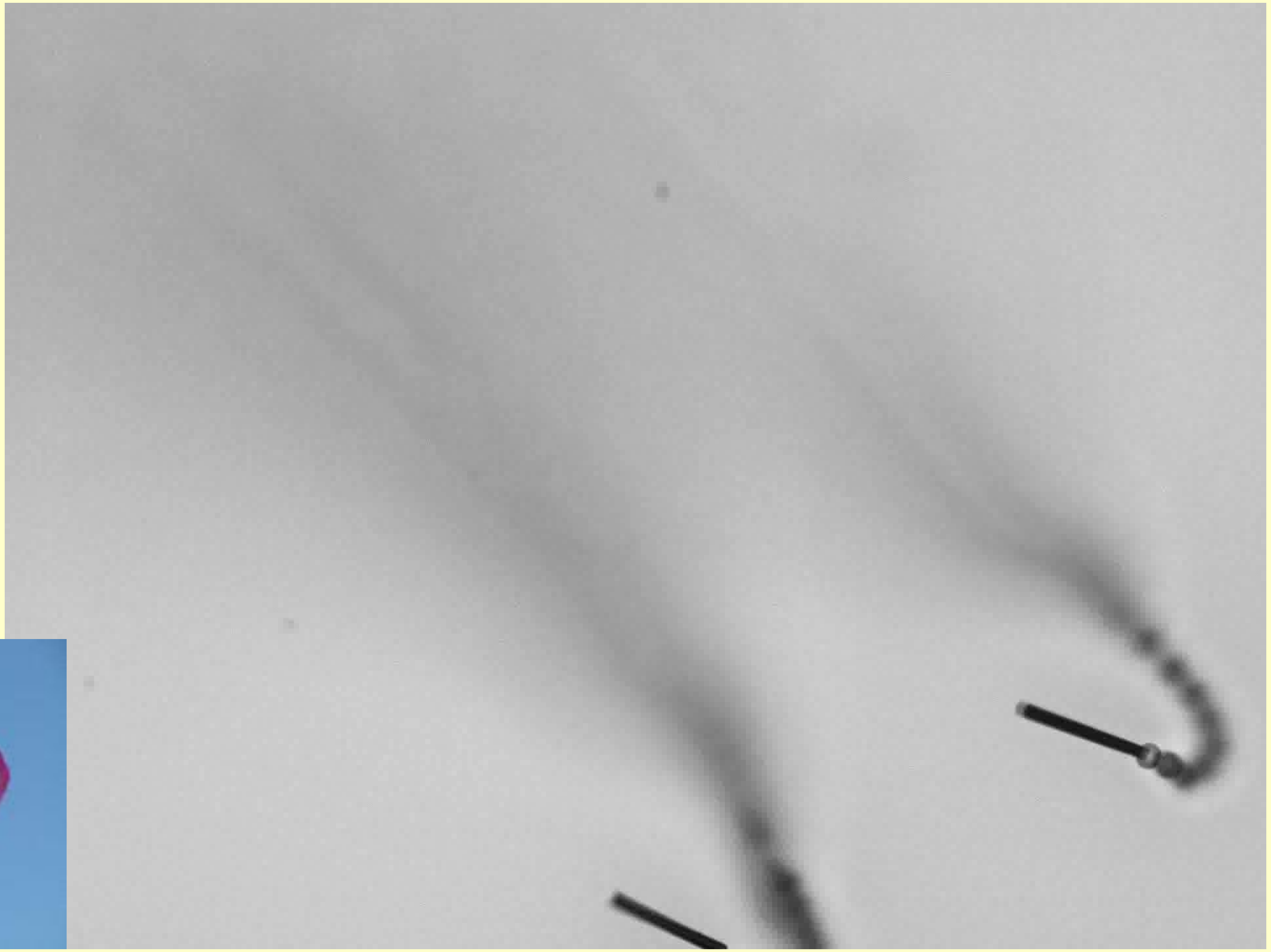
Different Propulsion Mechanisms

Types of Nanomotor Designs and Movement Principles

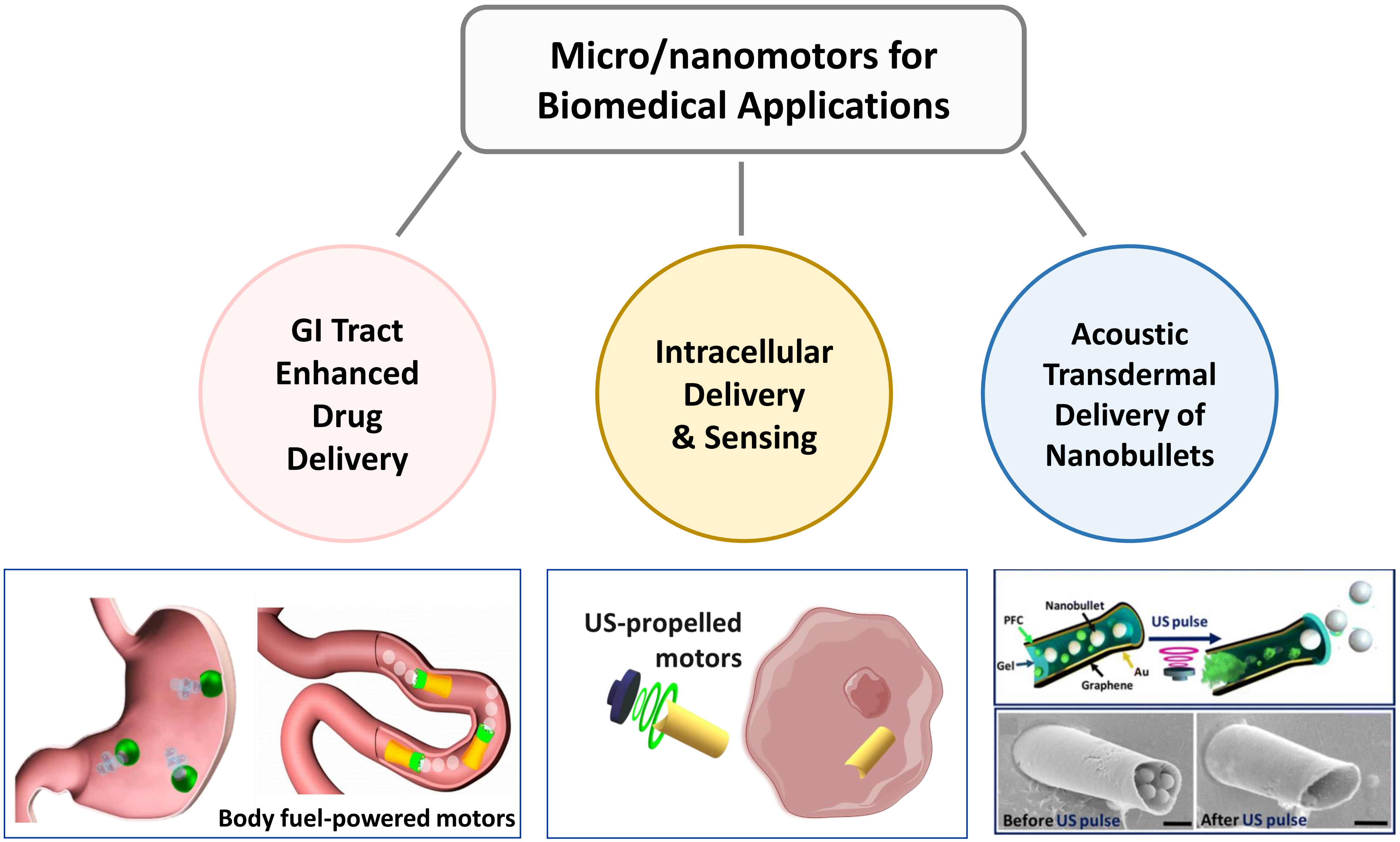


Controlled Coordinated Motion of Microtube Engines:

MAGNETIC GUIDANCE



Coordinated Motion


Micro/nanomotors for Biomedical Applications
GI Tract Enhanced Drug Delivery
Intracellular Delivery & Sensing
Acoustic Transdermal Delivery of Nanobullets
Body fuel-powered motors
US-propelled motors
Nanobullet
PFC
Gel
Graphene
Au
US pulse
Before US pulse
After US pulse

Dual Action and Multi-Tasking

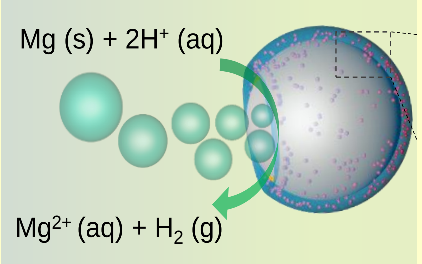
Micromotors spontaneously neutralize gastric acid for pH-responsive payload release

Water-powered Mg microsphere motors

Dual-Function in 2 Steps:

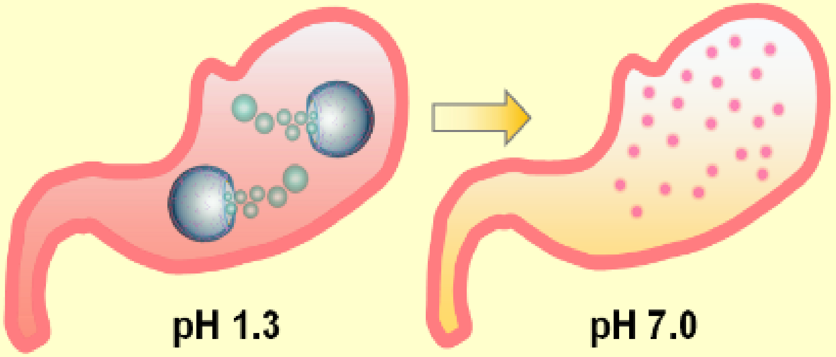
1. *In vivo* acid neutralization.

The propulsion reaction of the motor's magnesium core with the gastric fluid leads to **rapid proton depletion**:



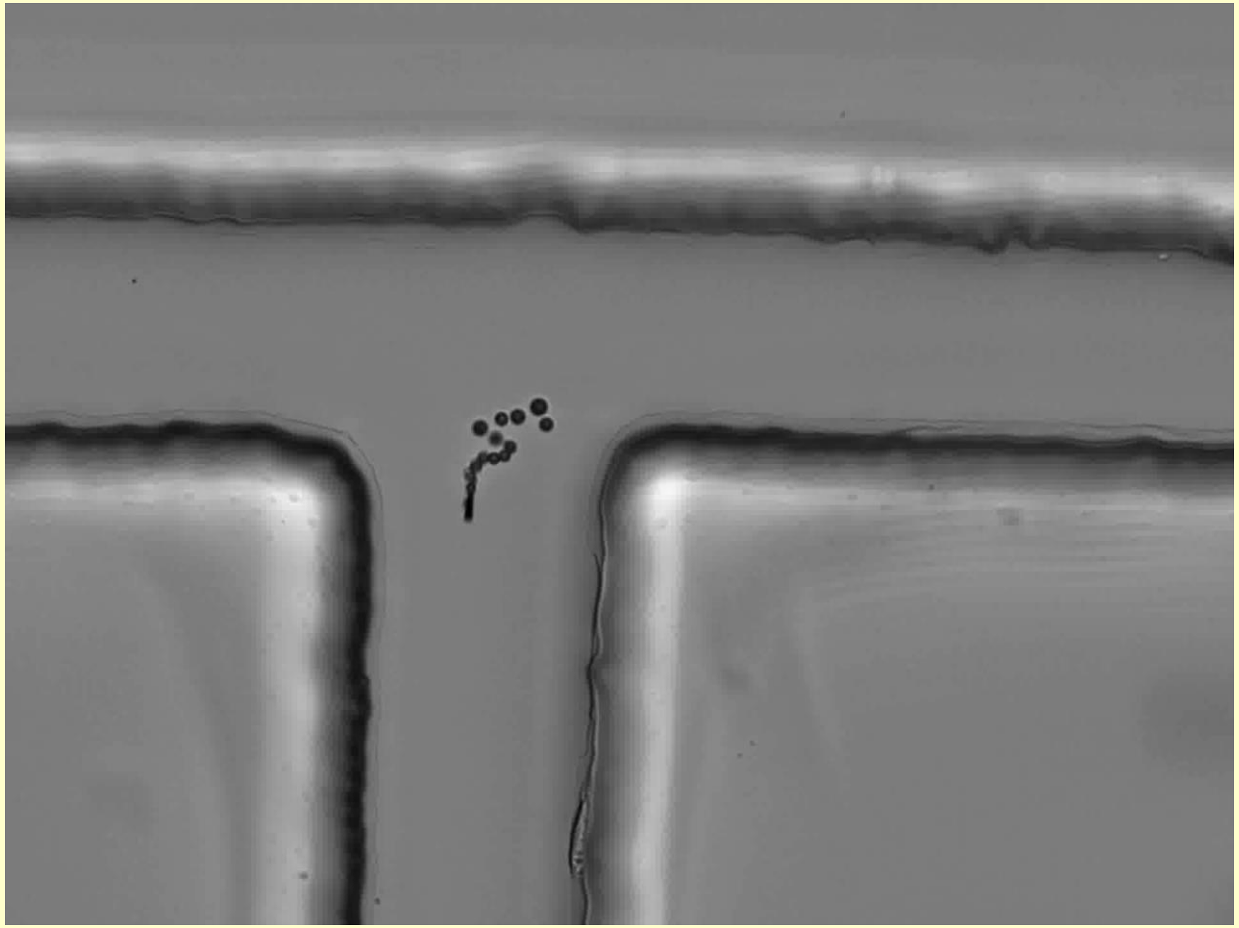
2. pH-triggered payload release.

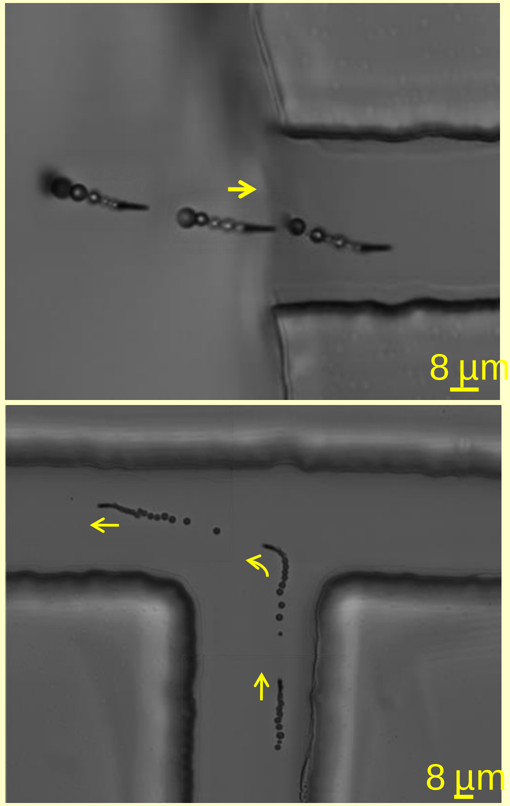
Coupled to a pH-sensitive payload-containing enteric polymer coating, this pH change can lead to autonomous release of the encapsulated cargo.



A pH-responsive enteric coating shield the motors from acidic gastric fluid (pH 1~3), but dissolves in the GI fluid (pH 6~7)

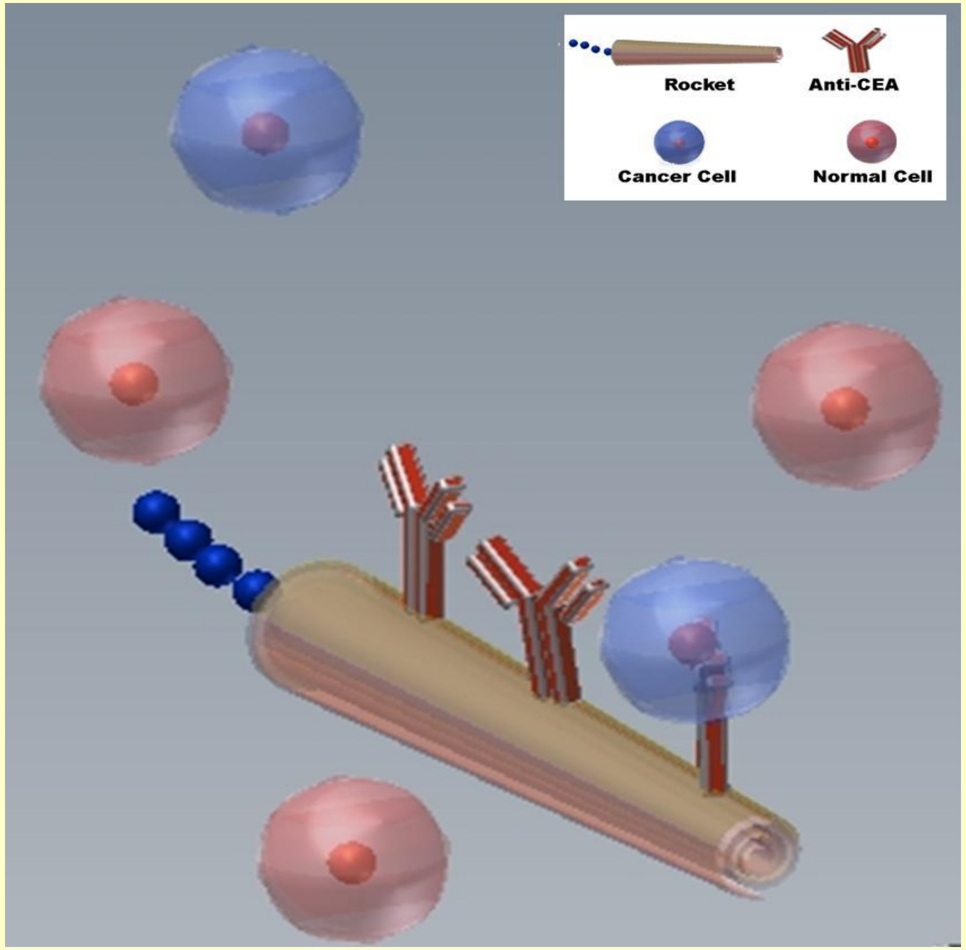
Motion-based Biosensing: Moving the Receptor in Microchip Channels





Movement of antibody-functionalized synthetic microrocket within microchannels

Selection and Isolation of Cancer Cells in Biological Fluids



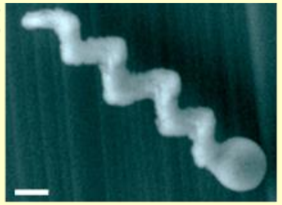
Immuno-micromachine for isolating CTCs

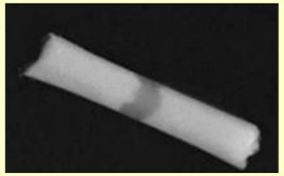
Capture and transport of a CEA+ pancreatic cancer cell by an anti-CEA mAb modified rocket.

Angew Chemie 2011

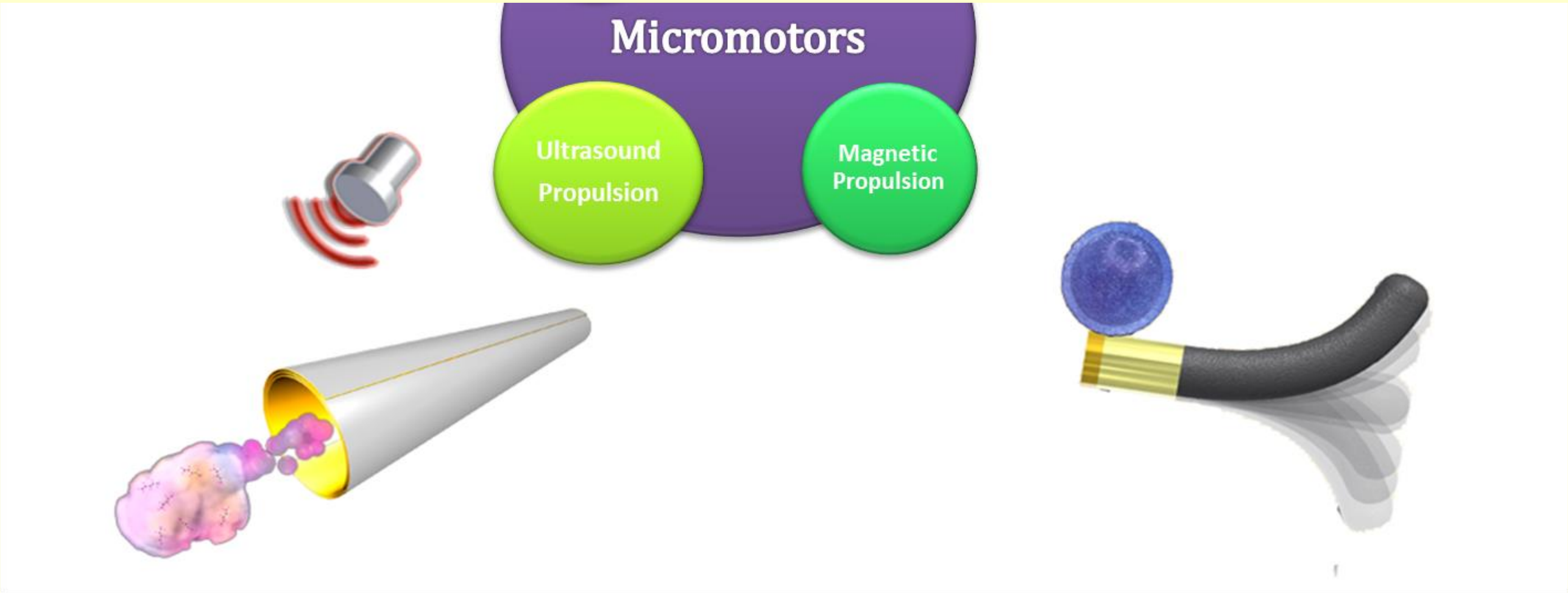
Fuel-Free (Externally-Powered) Nanomotors

Biocompatible energy transduction
mechanisms for powering micromotors.





- Magnetically-Driven Swimmers
- Ultrasound-Driven Micromotors

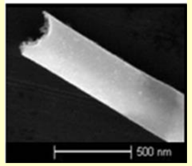


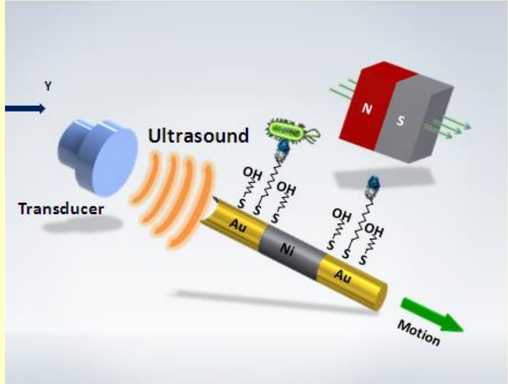
Ultrasound and Magnetically-Driven Micromotors

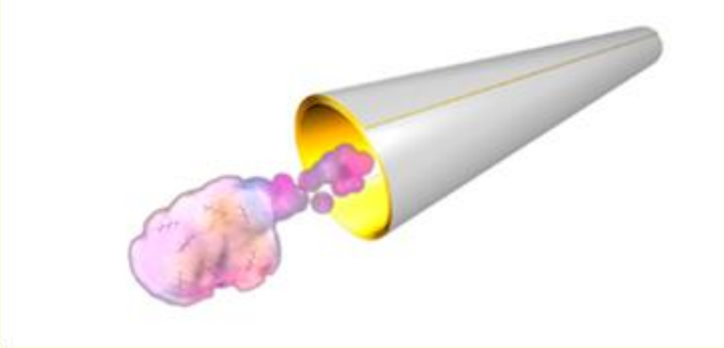
Ultrasound-Actuated Micromotors

Ultrasound is a biocompatible energy transduction mechanism, widely used in medicine . We demonstrated the attractive possibilities for using ultrasound for driving and controlling micromachines in biologically environments.

Ultrasound (US)-powered nanowire motors represent an attractive platform to overcome biological barriers



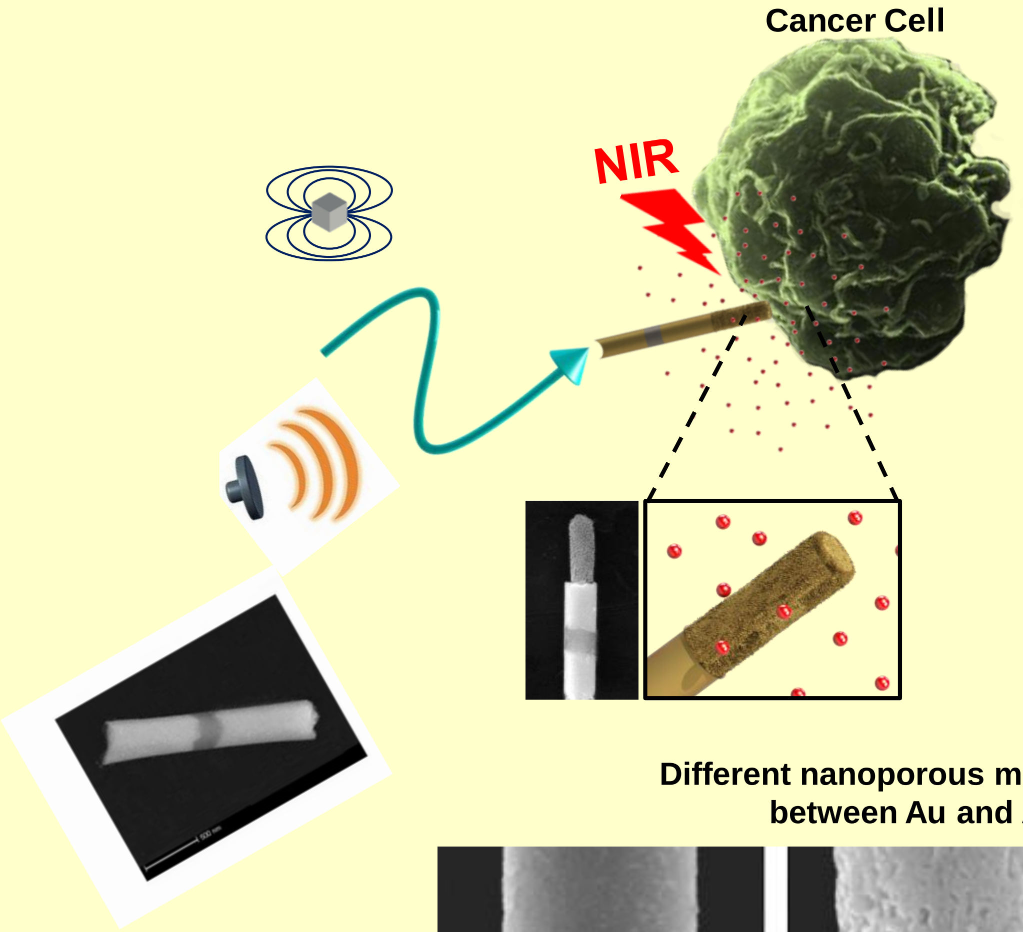


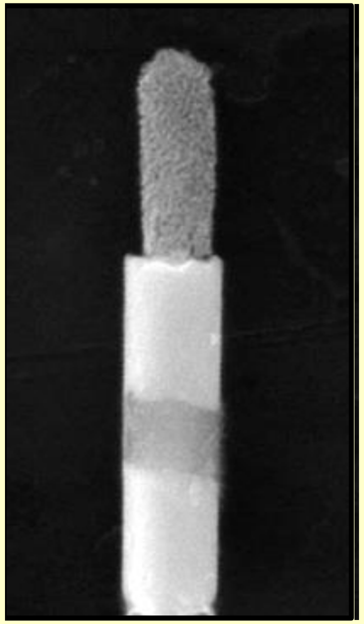


Concave Nanowires motors (ACS Nano 2012)

Tubular Microbullets (Angew Chemie 2012)

NIR light-Triggered Drug Delivery Ultrasound-Propelled Nanoporous Gold Micromotors

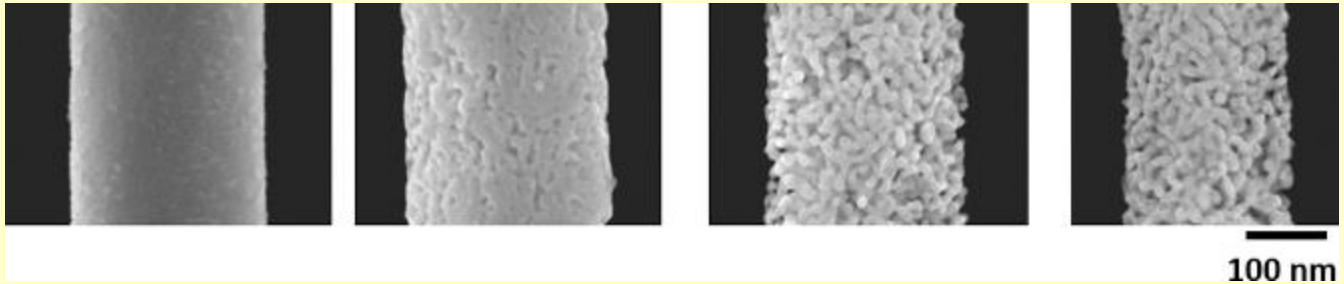




Adding a **nanoporous** Au segment for increasing the **loading capacity** of the drug.

Nanoporous Au segment by depositing a Ag-Au alloy and dealloying the Ag.

Different nanoporous morphologies by changing the ratio between Au and Ag ranging from 6:4 to 9:1.



Small 2014

Fast cellular internalization and rapid intracellular movement

The miniaturization advantages of small nanomotors have been exploited also for overcoming cellular barriers and internalizing into cells.

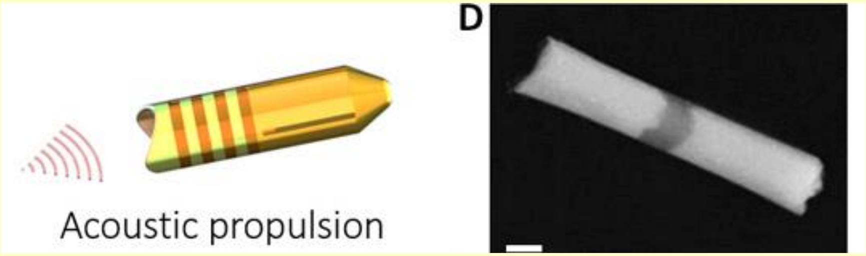
The ultrasound (US)-powered propulsion leads to fast internalization and rapid intracellular movement and delivery of functional proteins and RNAs.

The resulting intracellular delivery vehicles offer significantly higher efficiency and speed.

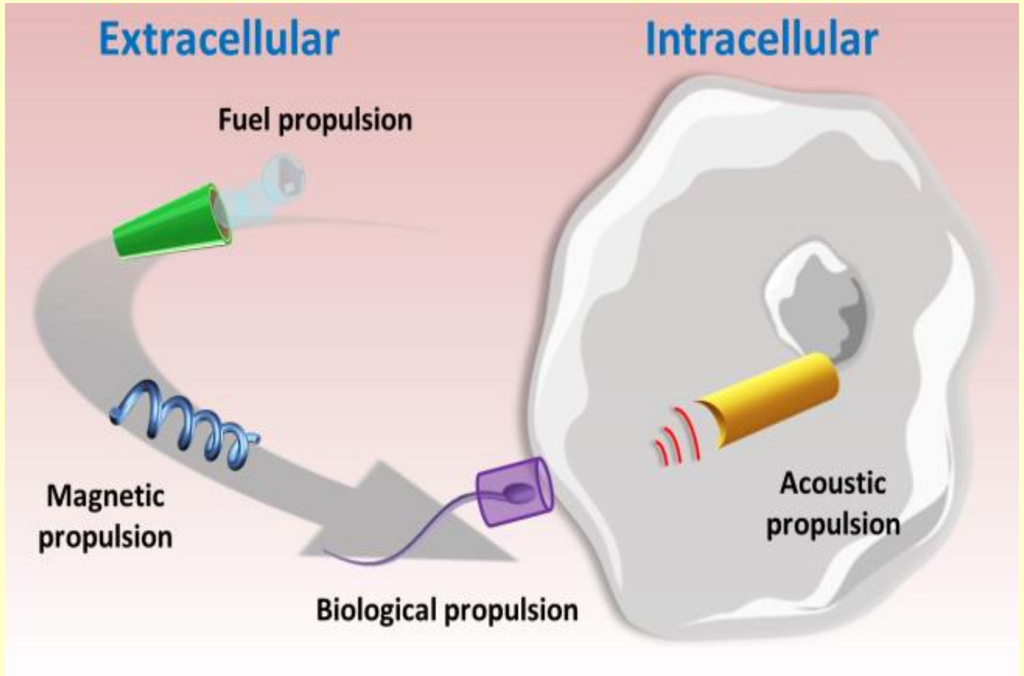
Efficient intracellular delivery vehicle

(e.g., siRNA, Caspase)

Delivery at the intra-cellular level



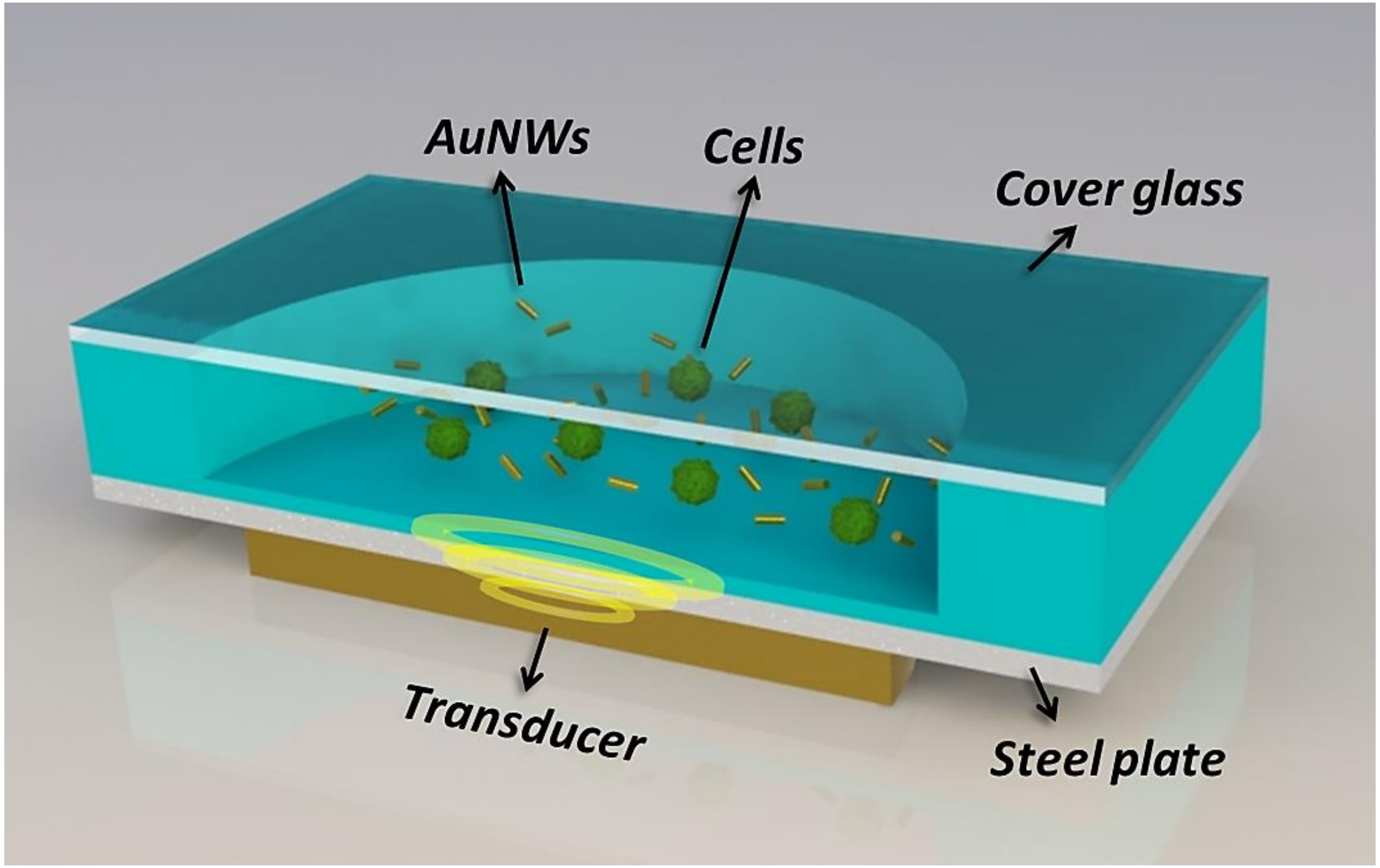
Ultrasound (US)-powered nanowire motors represent an attractive platform to overcome biological barriers.



While catalytically and magnetically-propelled vehicles have been preferably used for extracellular applications, US-propelled nanomotors are the preferred choice for the intracellular operations.

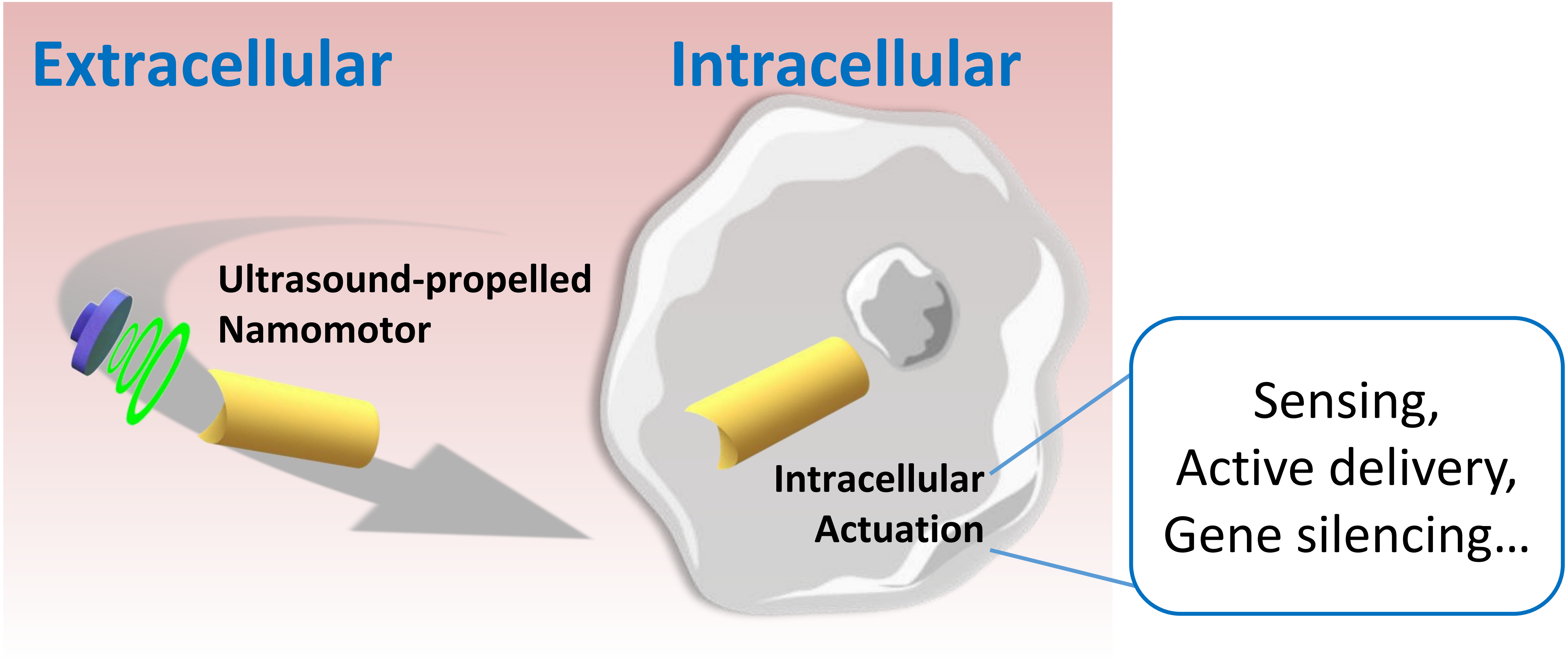
Being so small they can operate within cells.

Cellular Applications Using US-Propelled Nanomotors



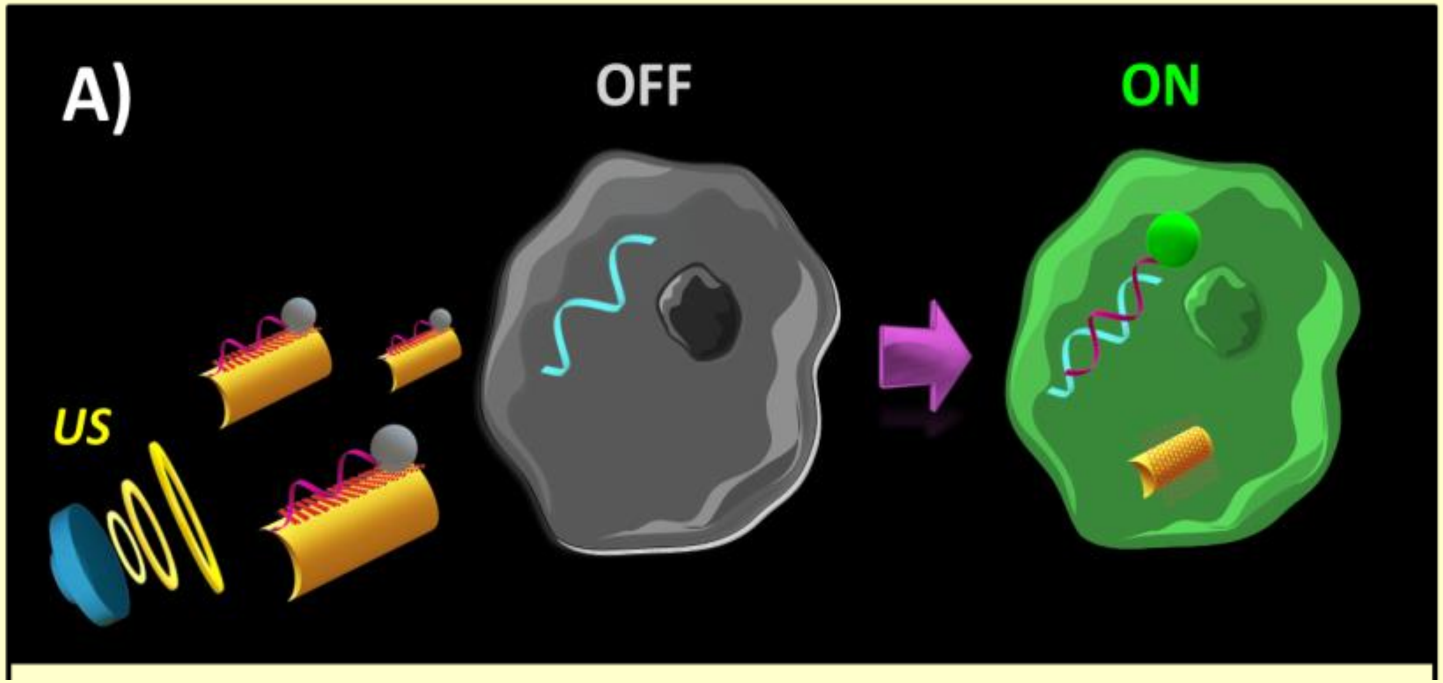
ACS Nano 2015, 9, 6756.
ACS Nano, 2016, 10, 4997.

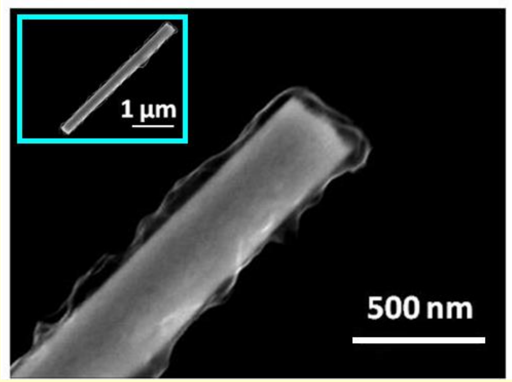
Nano/micromotors: Intracellular Applications



Real time biosensing of relevant targets and
processes at the single cell level

Nanomotor-based Single Cell Real-Time ‘Off-On’ miRNAs Sensing





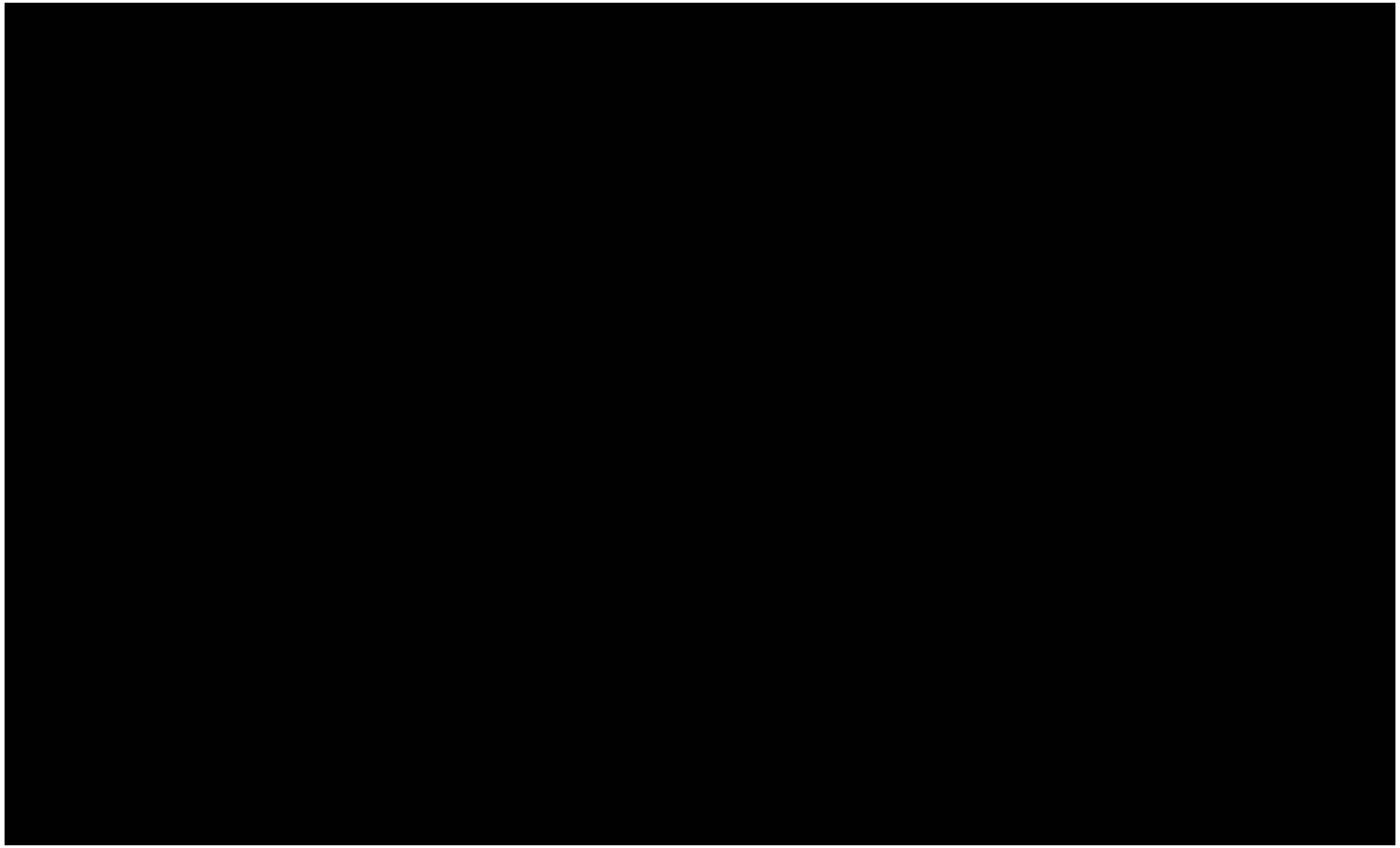
ssDNA@graphene oxide-functionalized gold nanowire motors

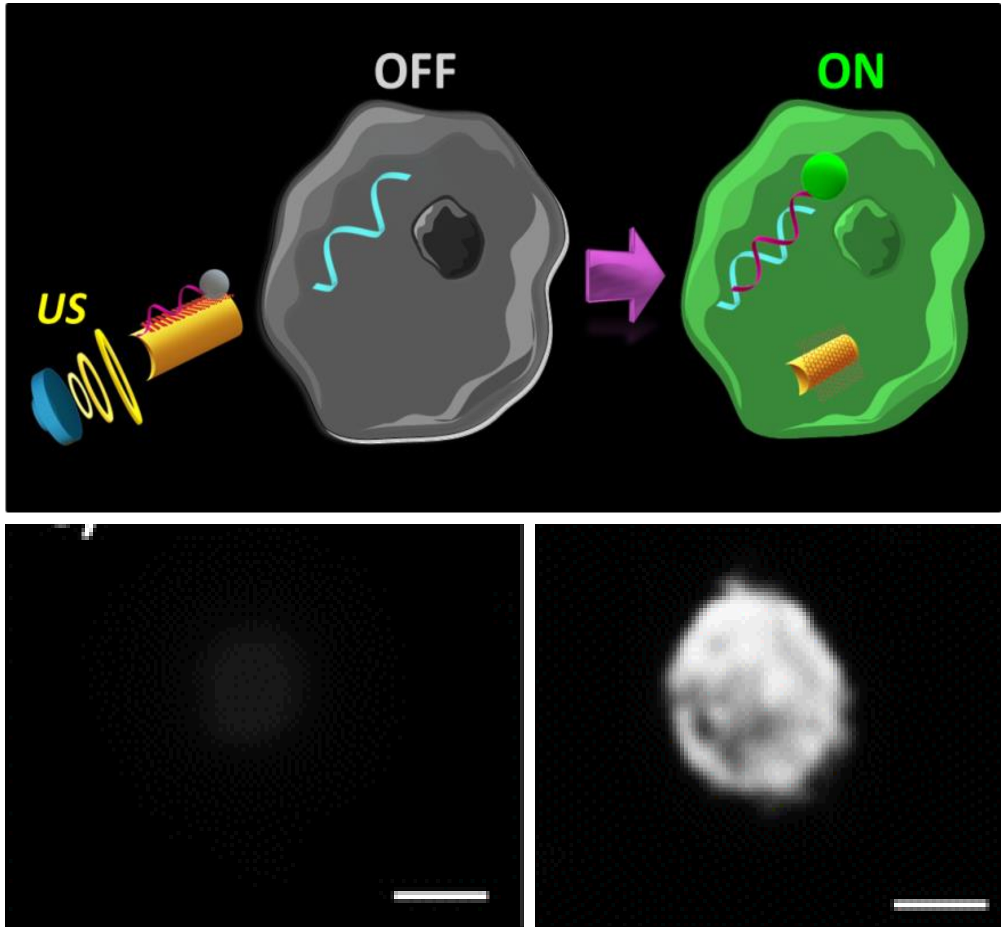
Intracellular detection of miRNAs by DNA -functionalized gold nanomotors.

Monitoring of miRNA-21 expression in individual intact cancer cells.

ACS Nano 2015

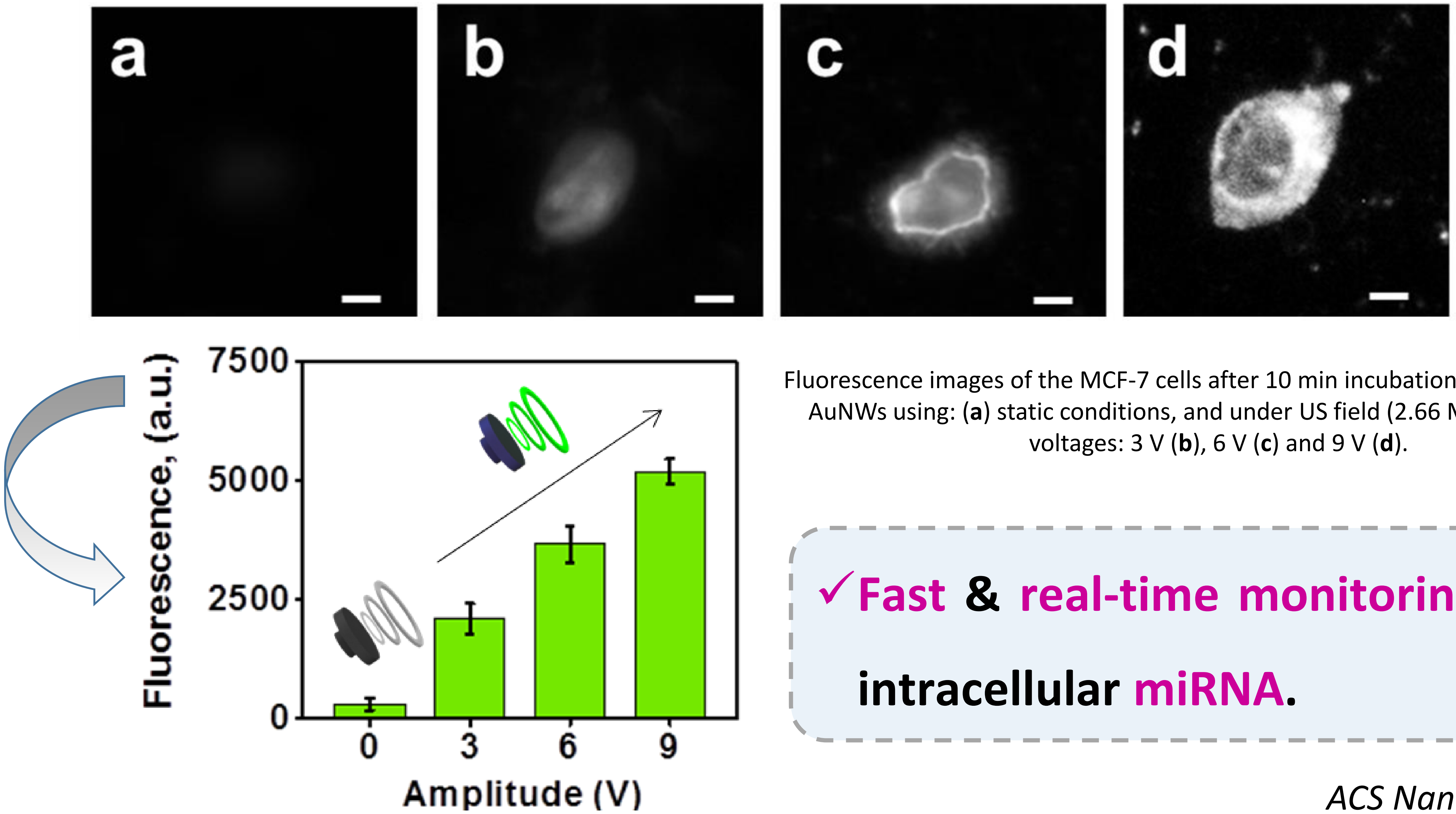
miRNAs Intracellular Sensing





ACS Nano **2015**, 9, 6756.

Intracellular miRNA Sensing

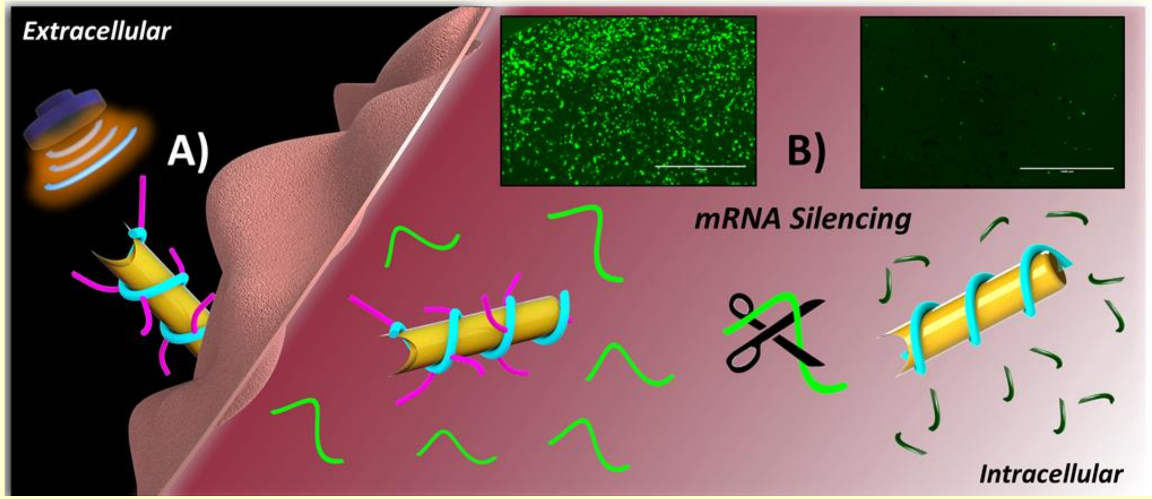


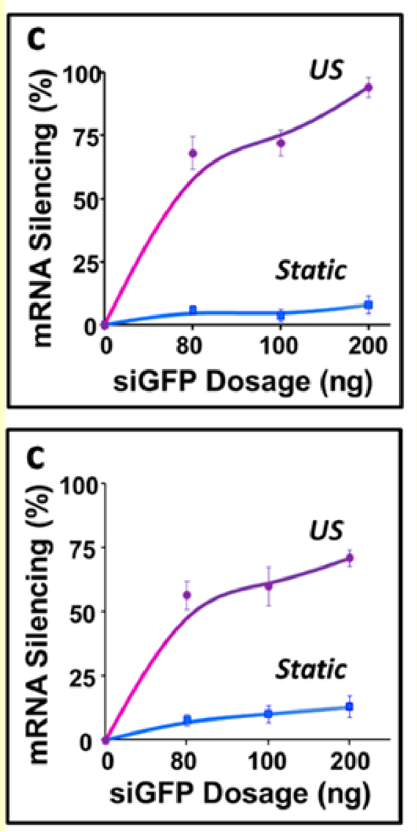
Fluorescence images of the MCF-7 cells after 10 min incubation with the modified AuNWs using: (**a**) static conditions, and under US field (2.66 MHz) at different voltages: 3 V (**b**), 6 V (**c**) and 9 V (**d**).

✓**Fast & real-time monitoring of intracellular miRNA.**

ACS Nano **2015**, 9, 6756.

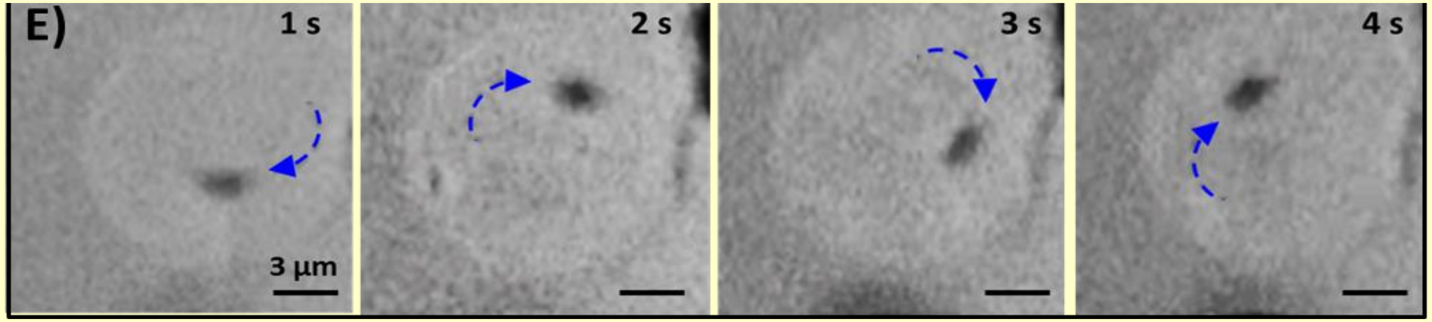
Acoustically-Propelled Nanomotors for Intracellular siRNA Delivery





The gold nanowire motors are wrapped with a Rolling Circle Amplification (RCA) DNA strand, serving to anchor the siRNA therapy.

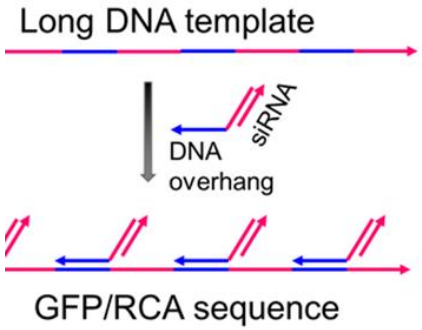
Accelerated siRNA delivery and silencing response of different cell lines (HEK-293 and MCF-7)

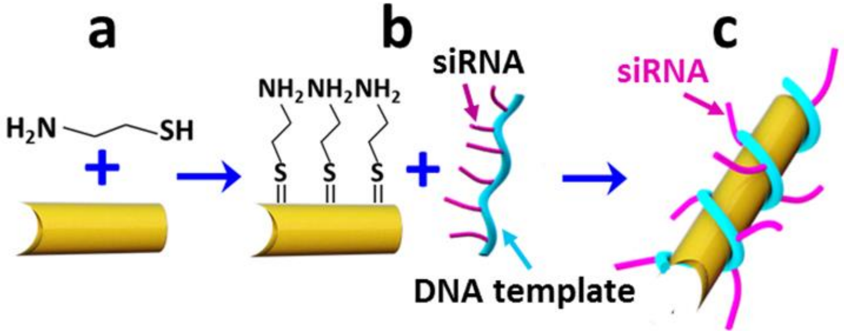


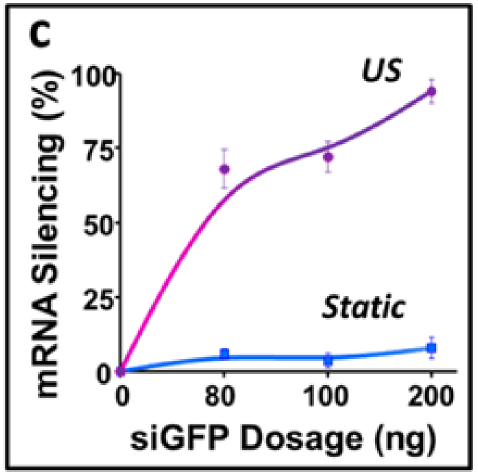
The acoustic propulsion leads to fast internalization and rapid intracellular movement, that leads to accelerated siRNA delivery and silencing response.

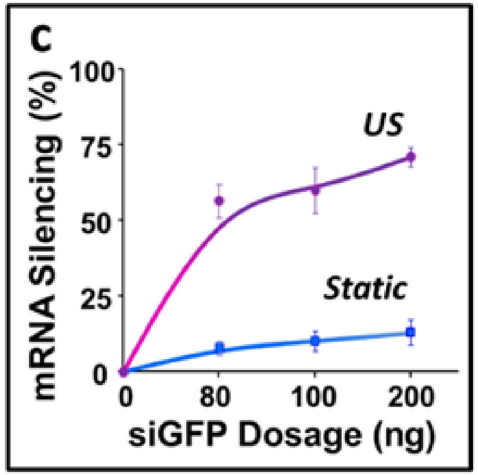
ACS Nano 2016

siRNAs Delivery for Gene silencing





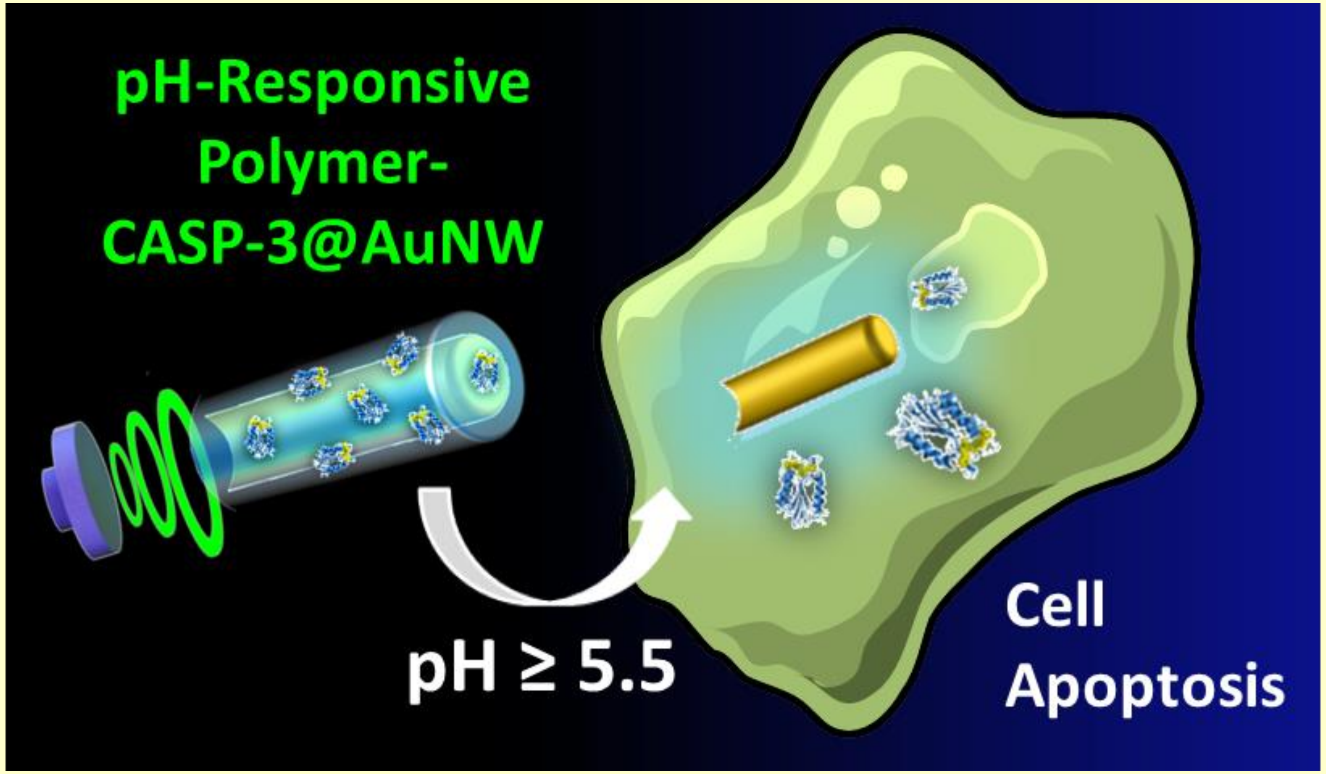




Accelerated siRNA delivery and silencing response of different cell lines (HEK-293 and MCF-7)

ACS Nano, 2016, 10 (5), 4997.

Nanomotor-based apoptotic strategy

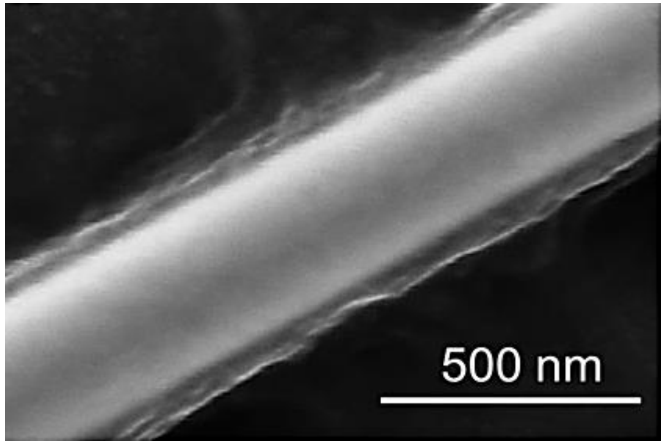


Use of caspase-3 (CASP-3)-modified nanowire motors for rapid and efficient cell apoptosis.

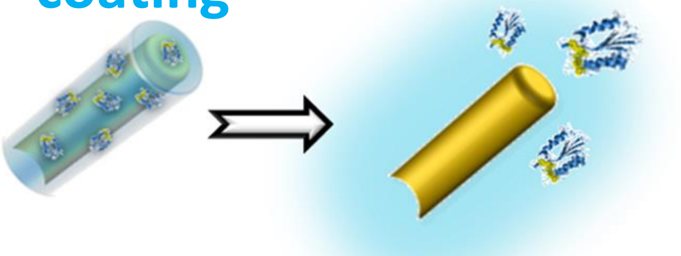
ACS Nano 2017

Getting Proteins into Cells is Challenging due to their Large Size and Polarity.

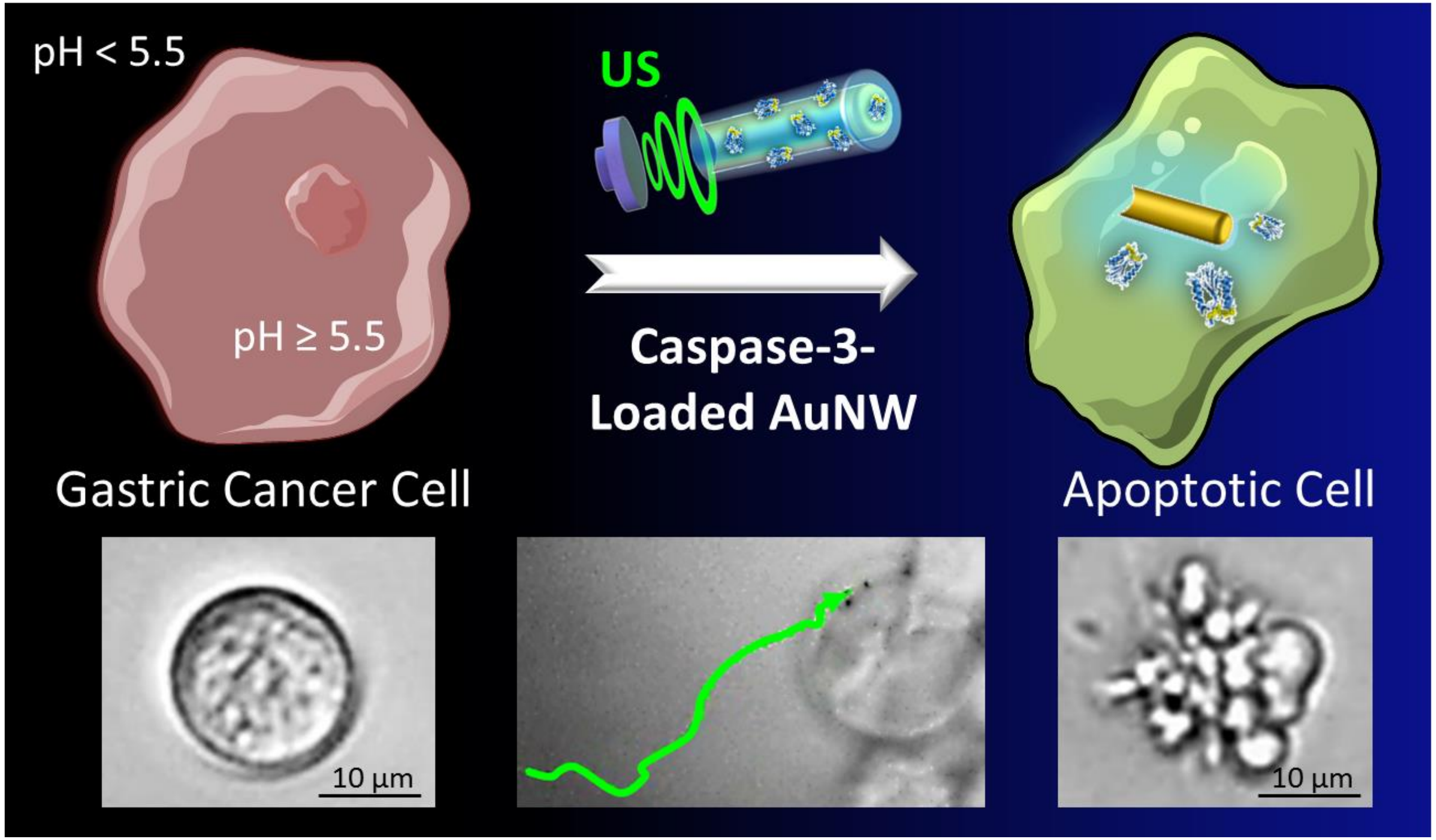
Caspase-3 Delivery to induce Cell Apoptosis



pH responsive coating

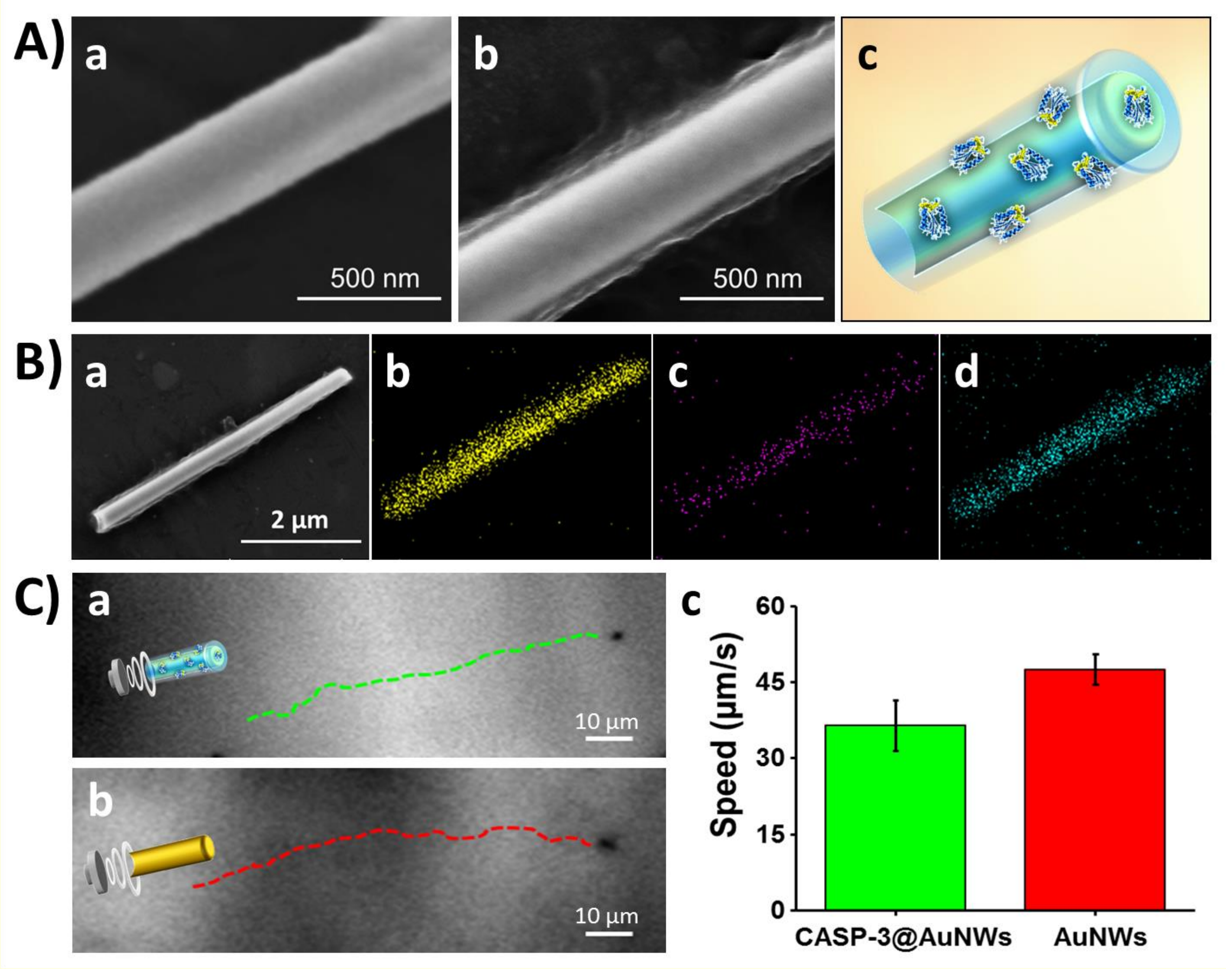


The high pH-responsive CASP-3 delivery strategy was selected taking into account the gastric tumor microenvironment and the pH values found in malignant tumor cells, characterized by an acidic (~6) extracellular space and neutral (~7.5) intracellular cytosol.



ACS Nano, 2017, 11 (6), 5367.

Characterization of the polymer/CASP-3@AuNWs



Characterization of the polymer/CASP-3@AuNWs. (A) Comparison of scanning electron microscopy (SEM) images of an uncoated AuNW (a) and a polymer/CASP-3@AuNW (b), along with a schematic image of the polymer/CASP-3@AuNW motor (c).

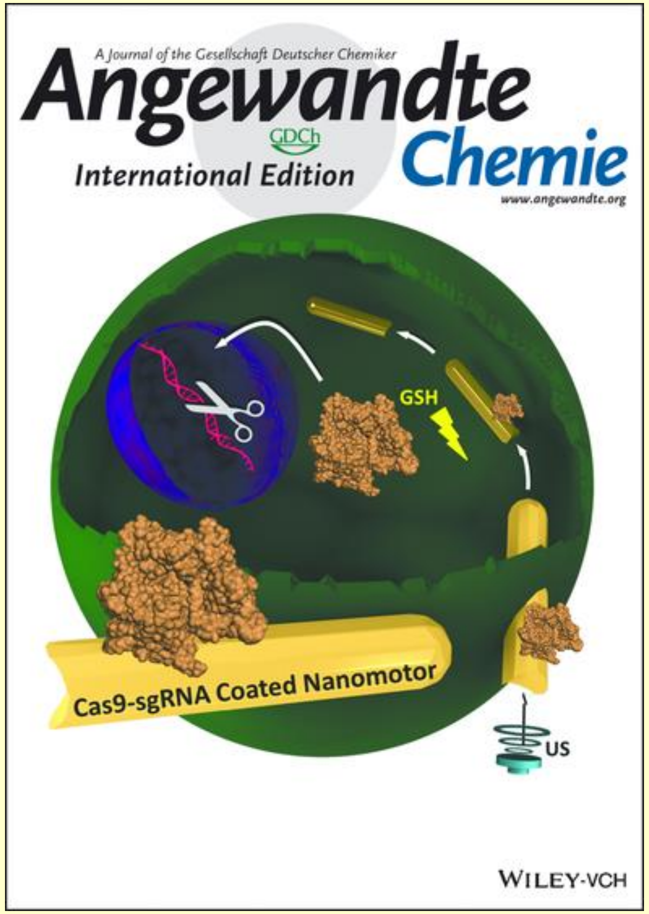
SEM image of a polymer/CASP-3@AuNW motor (a), and corresponding energy-dispersive X-ray spectroscopy (EDX) images showing the distribution of gold (b), carbon (c), and nitrogen (d). (C) Time-lapse optical images, taken from Supporting Video S2, showing the propulsion behavior of a polymer/CASP-3@AuNW motor (a) and an uncoated AuNW motor (b);

Comparison of the speed of the US-powered polymer/CASP-3@AuNW motors (green bar) and uncoated AuNW motors (red bar). US field: 6 V, 2.56 MHz.

Active and direct intracellular delivery of Cas9-sgRNA complex

The Cas9-sgRNA@AuNW motors rapidly and effectively internalize the Cas9-sgRNA complex to cells and induce rapid and efficient gene knockout. The Cas9-sgRNA payload is released autonomously from the nanomotor surface, mediated by the high intracellular concentration of glutathione (GSH) in tumour cells.

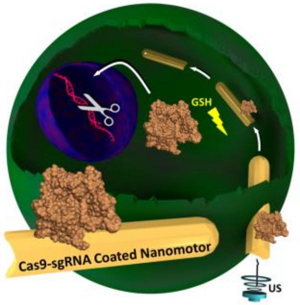
The Cas9-sgRNA@AuNW motors displayed improved cell transfection and faster GFP knockout compared with common cell transfection agent.



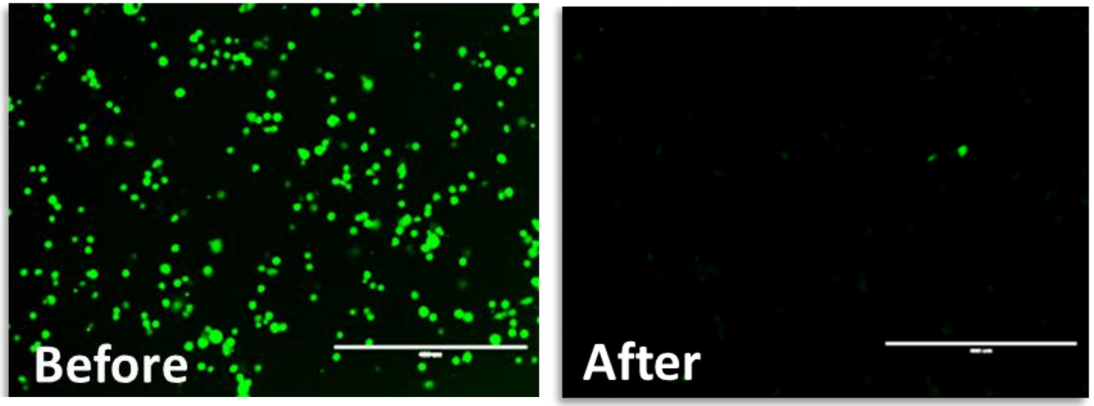
Rapid cell penetration of Cas9-sgRNA loaded nanomotors and markedly **improved the efficiency and speed of the GFP gene knockout** process.

Angew Chemie
VIP paper 2018

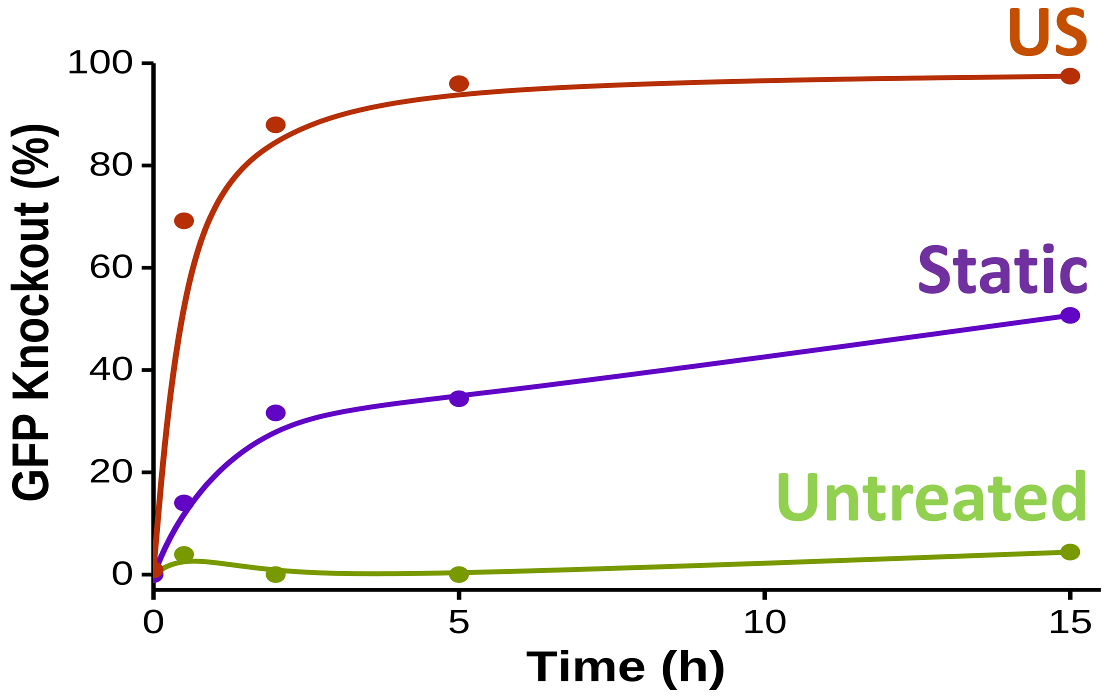
Active Delivery of Cas9-sgRNA Complex



GFP Knockout using GFP expressing B16F10 cells



Fluorescent images of the GFP-B16F10 cells before and after treatment with US-propelled Cas9-sgRNA@AuNWs.



Effect of treatment time on the knockout efficiency for the GFP-B16F10 cells treated at different conditions.

Mean GFP knockout percentage of the GFP-B16F10 cells treated with the different conditions

Conclusions

The growing sophistication and capabilities of current nanomachines have made them extremely attractive for performing complex biomedical tasks at the nanoscale.

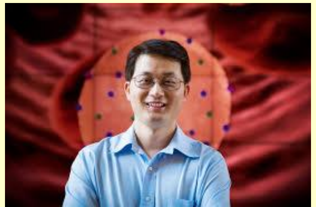
Nanoscale vehicles offer considerable promise for monitoring complex intracellular processes and for modulating such cellular processes.

The resulting intracellular delivery vehicles offer rapid cell penetration and significantly higher efficiency and speed.

Future efforts will aimed at Manipulating nanomotors within the cellular interior for delivering payloads to specific locations within the cytoplasm.

Nanomotors are expected to play an important role in future treatment and diagnostics strategies.

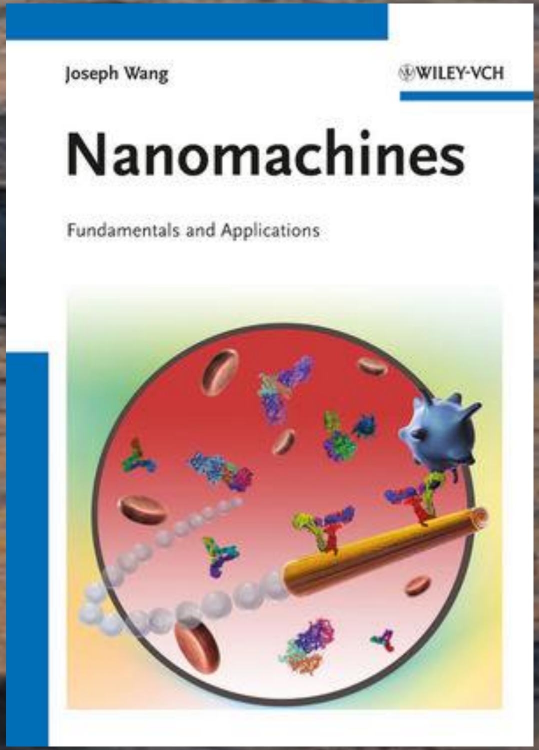
$$$: NSF, DTRA, ONR, DOE



Collaborators:
Liangfang Zhang, Yi Chen

THANK YOU!


UCSD
NANOENGINEERING

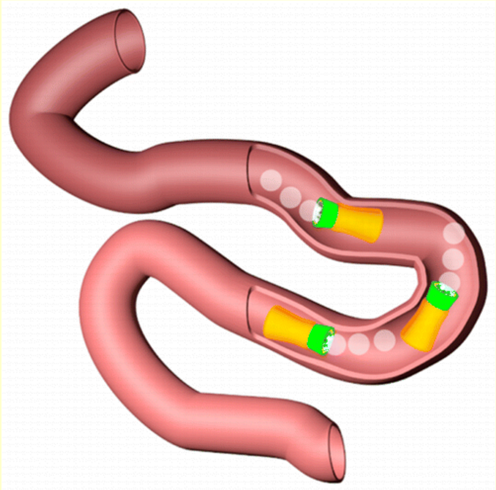

Joseph Wang
WILEY-VCH
Nanomachines
Fundamentals and Applications

alternative intracellular transport mechanism, and the practical benefits of the cytoplasmic delivery together with the retention of high cell viabilities

The membrane however reseals almost immediately upon removal of the excitation such that the viability of the cell is highly preserved.

OUTLINE

- Nanomotor Design and Performance
- Biomedical Opportunities and Challenges
- Motion-based Sensing, Isolating, Writing, Imaging, Delivering, Repairing, and Cleaning.



Medical Microrobots

Challenges to Biomedical Applications?

A) Locomotion under physiological
conditions - Biofouling

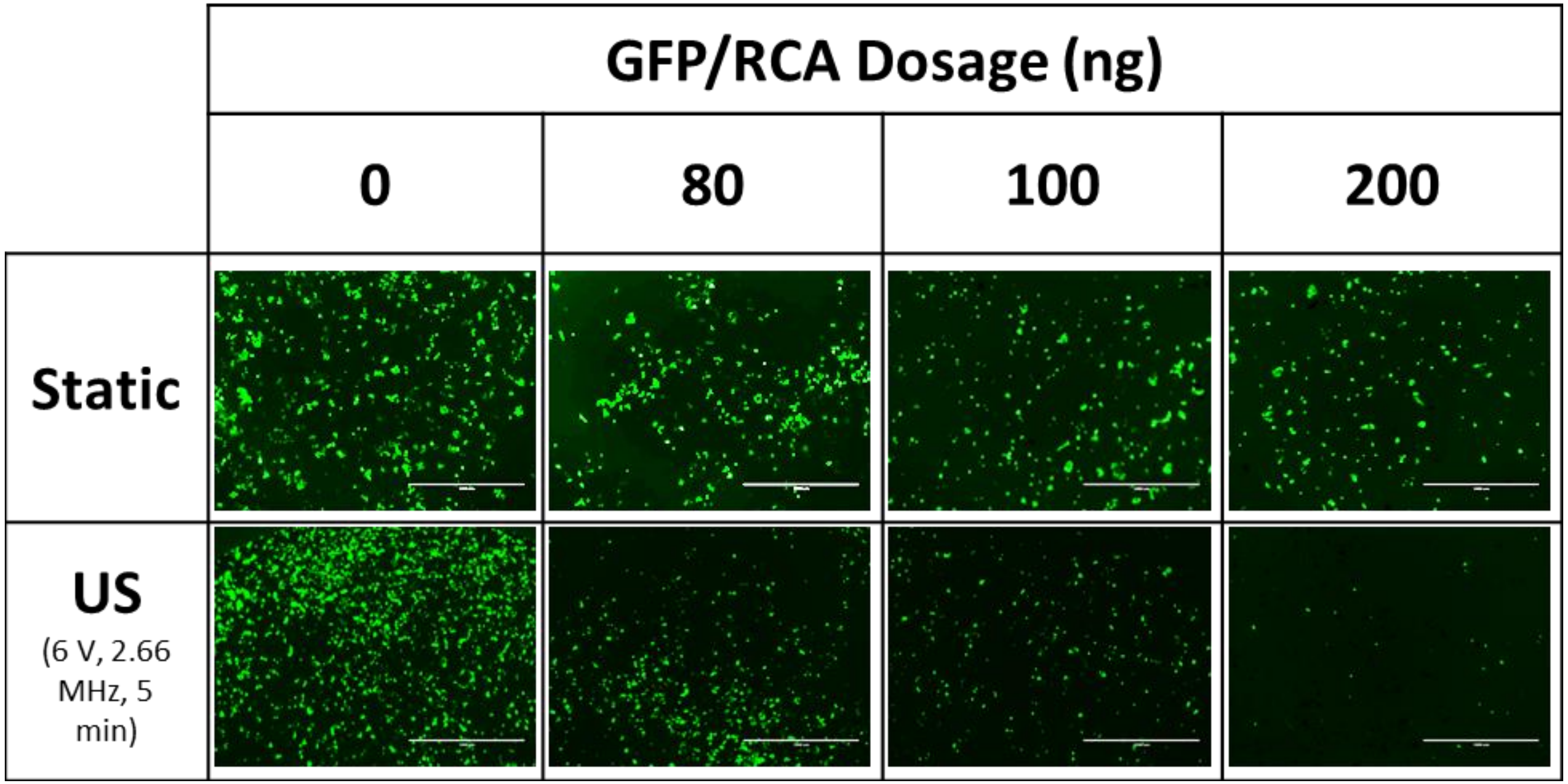
B) Internal fuel supply

C) Demanding complex tasks

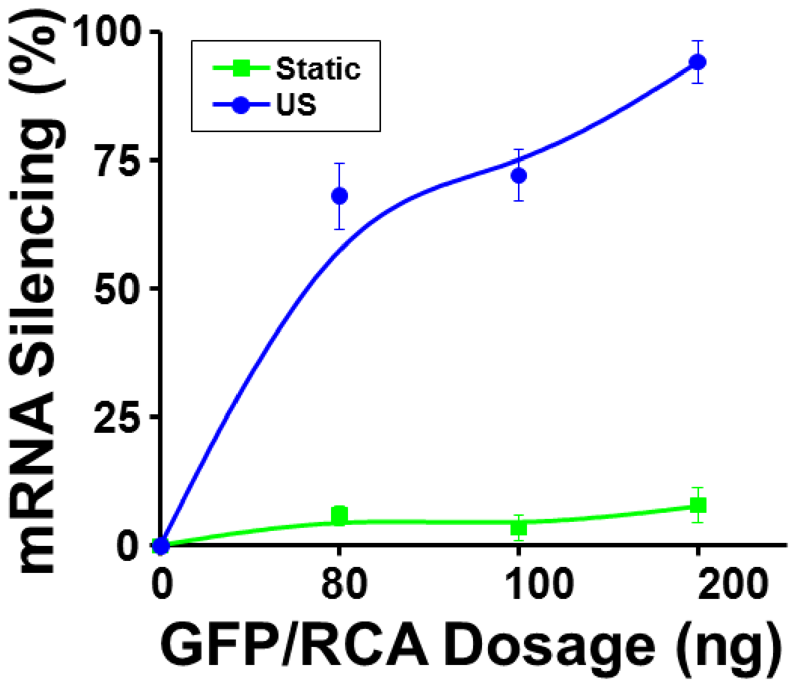
D) Body Response- Toxicity/Biocompatibility

siRNAs Delivery for Gene silencing

Biocompatible option for siRNA delivery which greatly enhances intracellular gene-silencing by dramatically decreasing cell uptake time and increasing siRNA payloads.

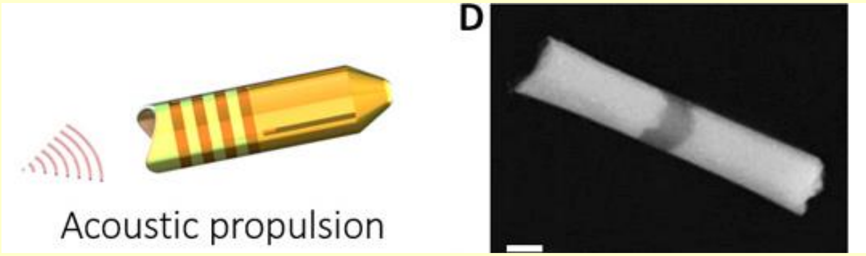


HEK293-GFP cells after treatment with siRNA-Au Nanomotors



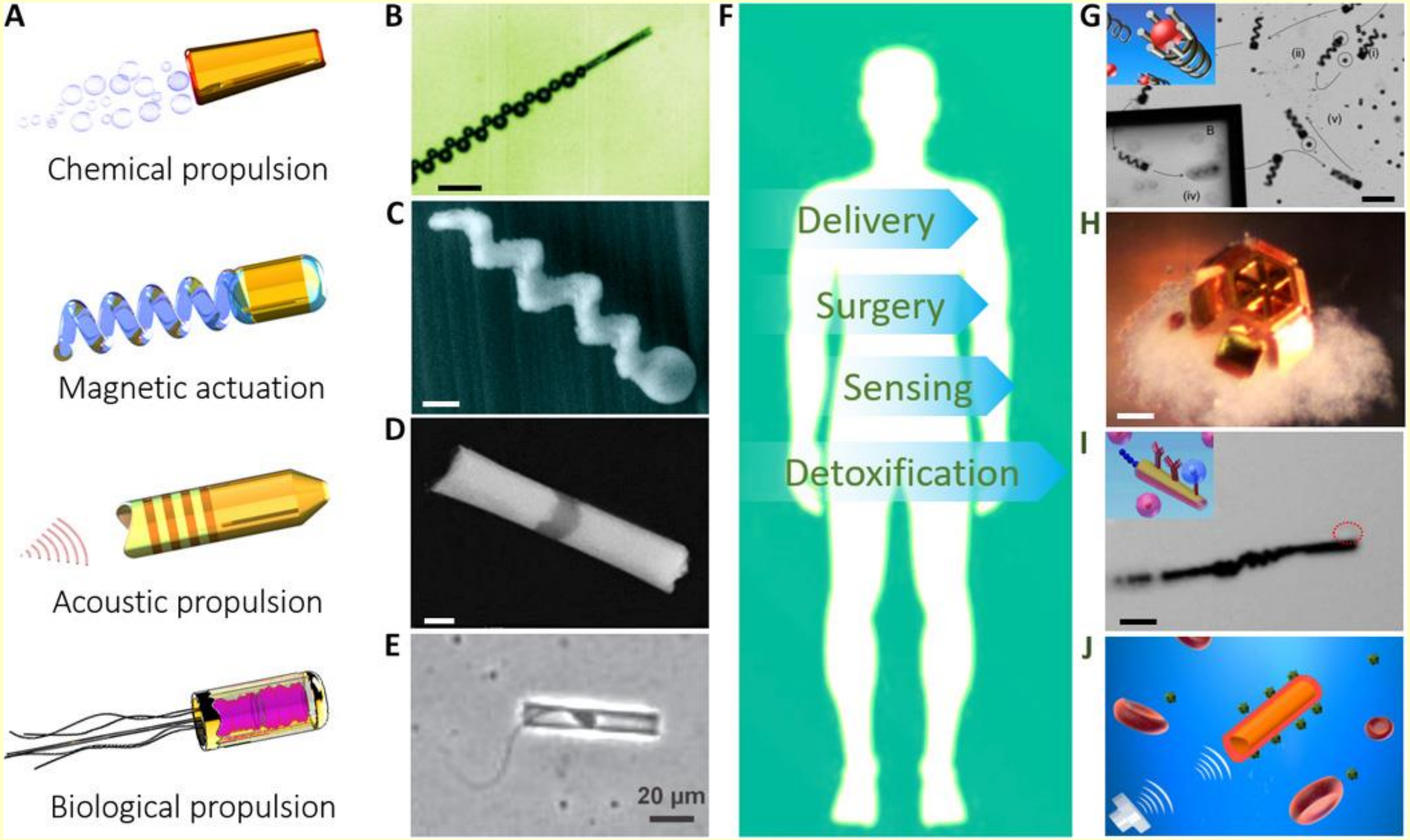
ACS Nano, 2016, 10 (5), 4997.

Micro/nanorobots for Biomedicine: Delivery, Surgery, Sensing, and Detoxification



REVIEW MEDICAL ROBOTS

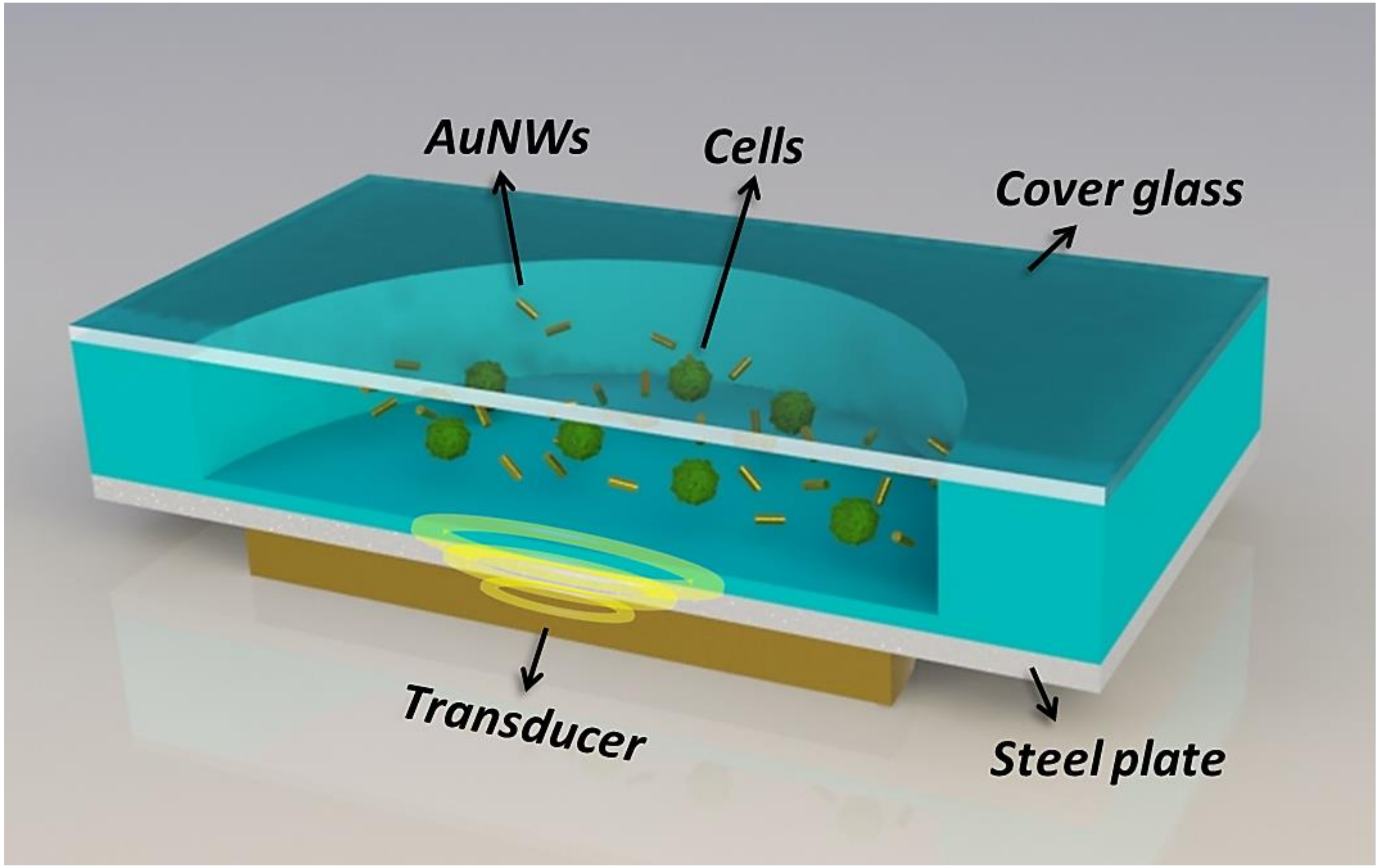
Micro/nanorobots for Biomedicine: Delivery, Surgery, Sensing, and Detoxification



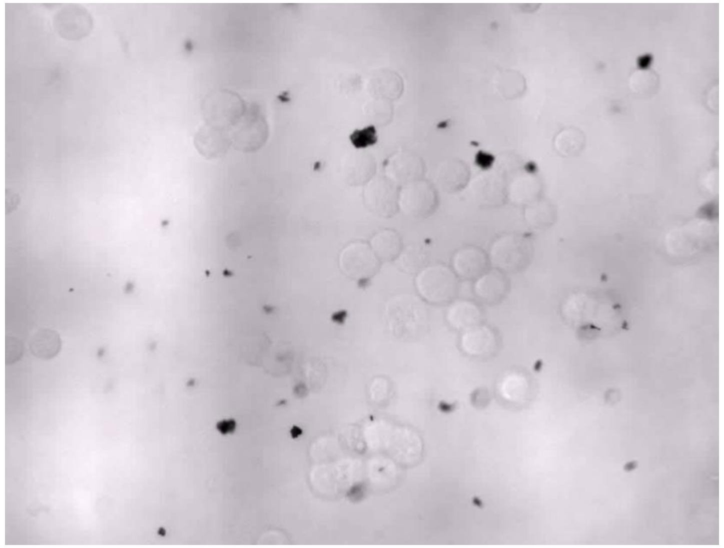
Science Robotics

Science Robotics 2017.

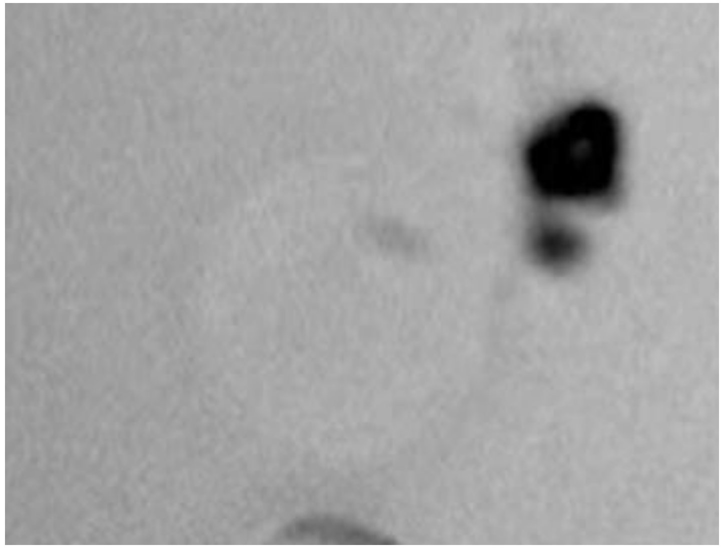
Cellular Applications Using US-Propelled Nanomotors



Cell & Motor Concentration



Spinning motion



ACS Nano 2015, 9, 6756.
ACS Nano, 2016, 10, 4997.

- Ultrasound (US)-powered nanowire motors represent an attractive platform to overcome biological barriers and physical limitations associated to conventional methods for functional protein internalization,[27] in order to develop a simple and efficient strategy for intracellular delivery of CASP-3. The present nanomotor-based apoptosis strategy relies on the accelerated intracellular delivery of active CASP-3, encapsulated within a biocompatible pH-responsive polymeric coating on US-propelled gold nanowire (AuNW) motors. The commercial pH-responsive polymer Eudragit® L30 D-55 was selected for encapsulating CASP-3 enzyme, due to its rapid dissolution above pH 5.5,[43] thus ensuring safe arrival of the active enzyme to the intracellular environment while preventing the CASP-3 release in the extracellular space. The high pH-responsive CASP-3 delivery strategy was selected taking into account the gastric tumor microenvironment and the pH values found in malignant tumor cells, characterized by an acidic (5.5-6.8) extracellular space and neutral (7.3-7.8) intracellular cytosol.[44,45] It should be pointed out that while the proof-of-concept has been demonstrated here for intracellular delivery in gastric cancer cells, a major goal of this study is to demonstrate the ability of the US-powered motors for intracellular delivery of therapeutic proteins.

Ingestible Capsules

- The development of ingestible sensors offers significant opportunities in medical diagnostics and the monitoring of the human body[1]. In contrast to wearable sensors, which are mostly limited to contact with the skin, ingestible sensors can be immersed in the gut, an environment in which the concentrations of chemicals exchanged by our body are high[1,2]. However, the field of ingestible sensors remains a relatively underdeveloped area of technology, though advances in electronics and sensors now provides a base for creating reliable, safe, low-cost and durable ingestible chemical sensors. Commercial ingestible sensors are currently mainly limited to pH and pressure profilers, medication monitoring tools, and esophageal optical coherence tomography monitoring using tethered capsules[1,3,4]. In order to develop powerful ingestible capsules that can sense the chemical components of the gut, a first step is to identify the right target analytes of the gut, as certain gas constituents have been identified as efficient biomarkers that contain a plethora of information about the health of our bodies[5,6,7,8].

- A visit to a physician typically begins with the measurement of your body temperature, blood pressure, heart rate and respiratory rate. These vital sign measurements are a powerful indicator of general health and are quick, inexpensive and easy to acquire. Future routine medical care could soon be complemented by an additional layer of measurements thanks to the development of ingestible electronic capsules (Fig. 1). Ingestible electronics, which are also known as swallowables, have distinct advantages over the wearables currently used to obtain traditional vitals. In particular, they are not limited by the skin barrier and can be immersed in the environment of the gut, the site of chemical exchange with the body. Swallowables are especially useful for measuring vitals when traditional measurement techniques are not feasible or practical, such as during intense physical activity[1]. Moreover, swallowables have access to the ordinarily hidden gut microbiome, whose connection to both health and disease is just beginning to be understood[2].