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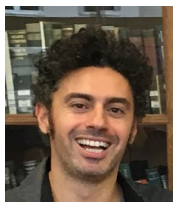

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CV/ biography (10 lines max.)

Zoher Gueroui is a CNRS researcher who joined the Ecole Normale Supérieure in 2010. Z.G. interest concerns the development of methods to control cellular functions by integrating tools from biophysics, nanoscience, and synthetic biology. He received a PhD in biophysics in 2002 at ENS-Lyon for his work on single molecule study of DNA transcription. He joined in 2002 the Rockefeller University as a postdoc to develop single molecule technics and cell biophysics. In 2005, he was appointed CNRS Researcher in Rennes (IPR), where he has studied the biophysics of cytoskeleton. In 2010, Z.G. has joined the Department of Chemistry of ENS to work on interdisciplinary approaches to study living matter.

Genetically encoded nanoparticles for the spatial manipulation of biomolecules

Abstract

The spatial manipulation of biomolecules using magnetic forces can provide a new dimension of control of biological processes to understand fundamental mechanisms *in vitro* and in living cells. However, despite numerous progresses achieved in the synthesis and functionalization of nanoparticles, as well as in the development of biophysical manipulation of magnetic objects, numerous technical challenges still need to be solved. In this context, we have engineered genetically encoded nanoparticles which have magnetic properties and versatile functionalization. This versatile platform that could target virtually any recombinant proteins can be monitored and manipulated by magnetic forces. During this talk, I will describe few proof-of-concepts experiments for the multi-scale control of biological assemblies.

Keywords: magnetic nanoparticles, biophysical manipulation, protein engineering, bio-assembly, biomineralization

11, 12 & 13 septembre
Session Nanobiology/Biotechnology
Keywords: emulsions;nanodroplets;drug delivery;ultrasound;imaging.

Fluorinated nanodroplets for therapeutic approaches using focused ultrasound

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MRI-guided focused ultrasound ablation is a recent technique clinically used in the treatment of fibroids and in ongoing clinical trials for the treatment of breast, liver, prostate, and brain cancer. The application of this technique beyond ablation requires the use of injectable, sonosensitive and MRI compatible agents. In this regard, fluorinated nanodroplets represent an ideal candidate as they can be detected by ^{19}F MRI and fluorinated liquids are compatible with the use of medical ultrasound. Thus, we have investigated nanodroplets made of perfluorooctyl bromide stabilized by fluorinated surfactants. These nanodroplets can be partitioned into a large fluorinated space and a small oily space in order to encapsulate hydrophobic drugs or dyes. The ultrasound-triggered delivery of encapsulated drugs is usually associated with the appearance of cavitation or the vaporization of the droplet. Both mechanisms induce unwanted damages to biological tissues. We used high intensity focused ultrasound at a frequency of 1MHz to induce the delivery of an encapsulated fluorescence dye and measured the percentage of delivered dyes as a function of the ultrasonic parameters. Our results show that a significant release can still be achieved without triggering cavitation or core vaporization, thus using small acoustic pressures less prone to cellular damages. We have also investigated the ultrasonic ablation mechanism in the presence of fluorinated droplets. The acoustic pressure required to reach elevated temperature is drastically reduced in the presence of these droplets. The lower acoustic pressures should lead to less unwanted bone burns.

Congrès 2018, Toulon

11, 12 & 13 septembre
Session Nanobiology, Biotechnology
Keywords: quantum dots, fluorescence, time-gated, in vivo imaging, sensing

Time-gated fluorescence *in vivo* imaging and sensing using long lifetime near infrared quantum dots

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The sensitivity of *in vivo* fluorescence imaging is limited by (i) absorption and diffusion of light by tissues (ii) auto-fluorescence from intrinsic fluorophores. The former can be addressed by the use of emitters that can be excited and re-emit in the near infrared (NIR) range. To address the latter, time-gated fluorescence imaging (TG-FI) uses a controllable delay between a pulsed excitation and fluorescence emission detection. This enables collecting photons from longer lifetime probes and rejecting those from shorter lifetime (ns) intrinsic fluorophores.

Here we present time-gated imaging and sensing using CuInSe2-based quantum dots (QDs), which emit in the NIR with a lifetime of 150-300 ns.

First, ZnCuInSe/ZnS QDs were coated with a multidentate imidazole-zwitterionic block copolymer and incorporated into live lymphoma cells. These QDs remain stable for long periods (=several days) in the cytoplasm of live cells. These cells were then injected intraveneously in a rat model. We show that QD-based TG-FI enables a 99% elimination of autofluorescence background and strongly enhances the imaging sensitivity. We demonstrate efficient detection of rare, fast and isolated cells circulating in the bloodstream with mm/s velocities.

In a second application, these QDs were used as a sensing platform. Their fluorescence lifetime could be controllably modulated through energy transfer with proximal acceptors. When these acceptors are conjugated to the QDs via an enzymatic substrate, the QD lifetime is shortened. Enzymatic activity cleaves the substrate, which translates into changes of the QD fluorescence lifetime. This enables NIR, autofluorescence-free, ratiometric imaging of enzymatic activity.

11, 12 & 13 septembre
Session Nanobiology Biotechnology
Keywords: polymer nanoparticles, ultrasmall, ultrabright, fluorescence, intracellular imaging

Protein-sized and Ultrabright Dye-loaded Polymer Nanoparticles for Intracellular Imaging

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Dye-loaded polymer nanoparticles (NPs) have become powerful tools for fluorescence imaging.[1] Their exceptional brightness makes them promising tools for tracking single biomolecules inside cells. But what are the size requirements needed for intracellular imaging? In this work we assembled a series of fluorescent polymer NPs with different sizes to study this question. For this we synthetized methyl methacrylate copolymers containing different amounts of positive or negative charged groups such as carboxylate, sulfonate and ammonium. The introduction of a few charged groups per polymer chain can strongly reduce the particle diameter through nanoprecipitation.[2] Furthermore, we obtained a finer size modulation by adding salt in the aqueous phase during nanoprecipitation. With these different features, the diameter of polymer NPs could be tuned from 50 to 7 nm.[3] The encapsulation of a high amount of fluorescent cationic dyes associated to a bulky hydrophobic counterion in NPs make them tenfold brighter than quantum dots,[4] and allows their tracking at the single-particle level. In order to study their behavior in cells, these NPs were microinjected in the cytoplasm. Observing their spreading and diffusion showed that only NPs smaller than a critical size of about 23 nm reach easily the whole cytosol. These ultrasmall polymer dye-loaded NPs have a great potential for diverse applications including high-speed tracking of single biomolecules with high localization precision.

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11, 12 & 13 septembre
Session : Nanobiology, biotechnology
Keywords: dendronized iron oxide nanoparticles, MRI contrast agent, magnetic hyperthermia, dendron effect, in vivo investigations, in vivo fate.

Mastering active targeting, theranostic properties, alleviating unspecific cellular uptake and tailoring *in vivo* fate of USPIOs through a dendritic coating

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Designing nanoparticles for targeted cancer diagnosis and therapy is currently one challenge in nanomedecine. To improve tumour targeting efficacy and to obtain better *in vivo* imaging properties, our studies explored the multivalency effect of a dendritic surface functionalization of SPIONs. Grafting of dendritic molecules on the surface of 10 nm spherical SPIONs using a phosphonate group as coupling agent has led to a new generation of contrast agents for *in vivo* MRI. The appeal of such strategy is due to the unique properties of the dendritic structures which can be chemically tuned to reach ideal biodistribution or highly and efficient targeting efficacies. They display good colloidal stability in isoosmolar media and MRI contrast enhancement properties higher than those of commercial products. No evident adverse effect was observed in rat after injection, even at high concentrations and a long time after injection. Their biodistribution study showed a fast hepatobiliary with a low urinary eliminations without RES uptake. Besides, we showed that, after i.v. injection in melanoma mice model, NPs coupled with a melanin targeting ligand were specifically uptaken by melanoma tumor cells with very favorable biodistribution and biokinetic properties. A start-up is under construction.
Therapeutic functions were added by designing the magnetic core and tuning its shape and compositions. By taking advantage of an INTERREG consortium NANOTRANSMED, the same investigations and in particular their *in vivo* biodistribution as well as their protein corona study and *in vivo* magnetic hyperthermia experiments, demonstrated a clear dendron effect and that these dendronized NPs are promising theranostic agents.

Congrès 2018, Toulon

11, 12 & 13 septembre
Session: Nanobiology - Biotechnology
Keywords: Dithiolene complexes ; Controlled drug delivery ; Photothermal therapy ; Theranostics

Photothermal properties of Metal-bis(dithiolene) complexes: a multipotent tool for photothermal therapy, photocontrolled drug delivery and photoacoustic imaging

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Abstract:

Metal-bis(dithiolene) complexes show large absorption in the NIR region with a high extinction coefficient (30 000 $M^{-1}.cm^{-1}$) [1,2]. These compounds being non-luminescent, it undergoes non-radiative deexcitation of the absorbed energy, converting light into heat with yields around 40%[3].

Moreover, the high versatility of the ligands carried by the metallic center allowed us to synthetize and encapsulate hydrophobic analogues in highly performant nanovectors such as thermosensitive liposomes or polymer nanoparticles [4,5]. A fluorescent marker (carboxyfluoresceine) or a common anticancer molecule (Doxorubicin) can also be co-incorporated inside the organic nanoparticles. Without laser irradiation a passive release of only 5% is observed over 3 month in storage condition at 4°C, whereas rapid and high release contents can be achieved with precise spatio-temporal control under short laser irradiation.

In addition, these vectors containing photothermally active complexes show no toxicity on different tumor cell lines for concentrations up to 100 $\mu g.mL^{-1}$. Nevertheless, local hyperthermia caused by laser irradiation in presence of complex led to a drop of cell viability by 80%, demonstrating the high potential of those nanovectors for photothermal therapy coupled to targeted chemotherapy.

More recently, we have also demonstrated that ditholene complexes embed in organic nanocargos can be tracked by photoacoustic imaging under pulsed irradiation, allowing for the monitoring of their localization, and showing their great potential for bioimaging.

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Congrès 2018, Toulon

11, 12 & 13 septembre
Session (Biotechnology)
Keywords: Rotation probe, Polarized luminescence, Lanthanide, Nanorods

Anisotropic nanocrystals shed light on the complex dynamics of micro-organisms and fluids

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Luminescent probes are universally used to observe the dynamics of matter and life in small scale. For positional tracking, the ultimate spatiotemporal resolution near 1 nm and 1 ns is already achieved. However, most dynamic processes from molecular to cellular level still remain secret, because they involve non-translational motions such as rotation, twisting, and bending that are difficult to observe. We design novel fluorescent probes emitting richly informative light that can signal the 3-dimensional orientation of their own and the tagged objects. We utilize the peculiarly polarized luminescence of lanthanides doped in nanocrystal hosts, which exhibits multiple emission dipoles with different polarization natures without problems of bleaching or blinking. We deliberately tune the host nanocrystal structure in order to maximize the luminescence intensity and the degree of polarization, thereby achieving the unprecedented orientation sensitivity [1]. Furthermore, by studying the rotational dynamics of the nanocrystals in fluids, we established a tomographic imaging of shear stress profile in microfluidic systems that are useful in biomedical analyses [2].

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[2] Nature Nanotechnology. 12, 914-919 (2017)

11, 12 & 13 décembre
Nanochemistry, synthesis & assembly
Keywords: self-assembly; nanocapsules; nanoparticles; multifunctional platform

Hybridosomes®: Innovative Multifunctional Nanocapsules

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We report on a new method to generate hollow capsules with a hybrid shell made of nanoparticles and polymers, which were coined "Hybridosomes" (Figure 1)[1]. The process is based on the formation of droplets in macroscopically miscible mixtures of organic solvent and water containing an hydrophobic solute, the so-called "ouzo effect". Our hypothesis is that nanoparticles stabilize such submicronic droplets by adsorbing at the liquid/liquid interface, similarly to Pickering emulsions. After addition of a crosslinking polymer and removal of the solvent core, hollow and porous capsules of diameter ~100 nm are obtained. These nanocapsules were prepared from Quantum Dots (QD), gold nanoparticles (AuNP), superparamagnetic iron oxide nanoparticles (SPION) and mixtures of different types of particles. The mechanical properties of the capsules were investigated at the single hybridosome level (via AFM nanoindentation) as well as at the ensemble level (via an osmotic pressure technique) [2]. The entrapment and the release of a fluorescent dye was also demonstrated. Thus, nanocapsules with dual properties (e.g. magnetic and fluorescent) are easily obtained. Interestingly, the magnetic/fluorescent nanocapsules enable Magnetic Resonance Imaging contrast enhancement of tumors in vivo and fluorescence imaging [1, 2]. Hybridosomes® therefore present a real potential for biomedical applications such as imaging and/or drug encapsulation, delivery and release. A further development will be the conjugation of the polymer shell to achieve active targeting.

(a) 

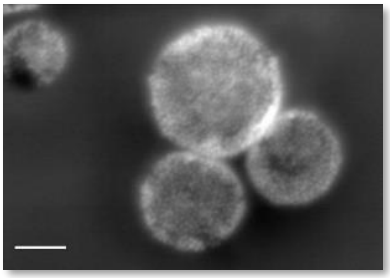
(b) 

Figure 1 – (a) Schematic representation of hybridosomes (b) SEM image of QD@PAA 450k hybridosomes (bar = 100 nm)

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*[2] F. Sciortino et al. Soft Matter **2017**, 13, 4393-4400. DOI : 10.1039/C7SM00705A*

11, 12 & 13 décembre
Session Nanobiology/Biotechnology
Keywords: nanocarrier; NP radiosensitization; imaging; therapy; cancer

Titanate nanotubes nanohybrids: radiosensitizers for cancer treatment

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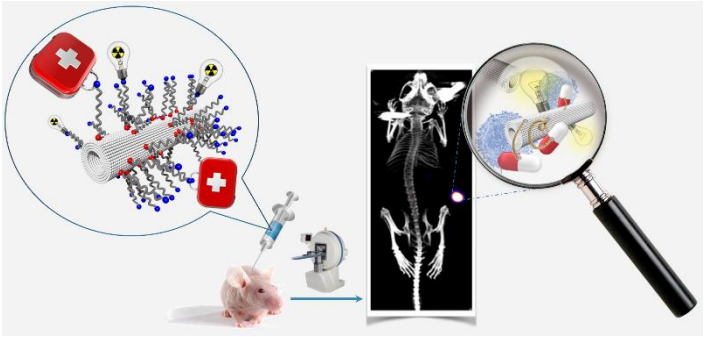
Systemic drug injections reach target sites too weakly; side effects are a critical issue and therefore limit the injectable drug doses, but recent developments of nanotechnology offered new strategies for carrying drugs directly into diseased cells. In this context, our team focused on one of the most diagnosed cancers in men: prostate cancer. Furthermore, we develop titanate nanotubes (TiONts) as nanocarriers and obtain them by hydrothermal synthesis; they can be internalized by cells without inducing cytotoxicity and have a radiosensitizing effect.
In this study, two nanohybrids are developed to fight prostate cancer cells by intratumoral administration of nanovectors.



First, TiONts are combined with a therapeutic agent against prostate tumor cells (docetaxel, DTX), and a chelating agent (DOTA) to evaluate the biodistribution of these nanovectors in mice by nuclear imaging. *In vitro* results on human prostate cancer cell lines (PC-3 and 22Rv1), *in vivo* SPECT-CT images and the first irradiation tests on prostate tumors will be detailed. The mice injected with our nanohybrid in the presence of radiotherapy (RT) showed a significant tumor growth delay compared to the mice that received DTX alone and RT.
Then, to improve the radiosensitizing effect of this first association, gold nanoparticles (AuNPs) functionalized with carboxyl-dithiolate derivatives were coupled with TiONts and grafted by DTX. This new combination confers not only nanovectorization *via* TiONts, but also the therapy and radiosensitization by DTX and AuNPs, while being detectable by X-ray, MRI and SPECT imaging. Thus, the functionalized TiONts appear as versatile nanocarriers and of great interest.

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11, 12 & 13 septembre
Session Nanobiology
Keywords: magnetic nanocomposites, theranostics, drug delivery, mesoporous silica

Hybrid magnetic protein nanocomposites for drug delivery and imaging

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Currently, there are very few methods allowing the efficient synthesis of particles made of proteins for drug delivery and imaging. The challenges are to develop simplified approaches with improved properties compared with existing methods in terms of biodegradability, toxicity and processing. These last years, we pioneered an original approach using isobutyramide (IBAM) grafts to assemble non-covalently, protein-based hollow capsules and particles without the need of an additional cross-linking or other adjuvant. The process consists in a single adsorption of proteins onto silica templates prealably grafted with IBAM groups followed by template removal[1]. The driving force is attributed to strong H-bonds between the IBAM interface and the polypeptide chains of the proteins. We applied this method to design bioresponsive hollow capsules and particles made of a range of proteins, including enzymes, insulin and human serum albumin.[2] Furthermore, such carriers were shown to release chemotherapeutic drugs upon biological stimuli e.g. through protease degradation or reductive mimetic cytosolic conditions.[3] This approach was also demonstrated for the design of ca. 100 nm size multifunctional protein-based NPs displaying simultaneously delivery of silencing RNA (siRNA) to cancer cells and magnetic resonance imaging (MRI) by grafting gadolinium complexes.[4]

In recent works, we translated this innovative protein nanocoating approach for the design of novel hybrid nanoplatforms made of magnetic cores covered with a mesoporous silica shell. Our aim was the design of new systems for imaging [5], magnetic hyperthermia and drug delivery ensured by alternating magnetic field [6]. The nanoplatforms were loaded with antitumoral agents (doxorubicin), and covered by a tight HSA shell to ensure biocompatibility, stealthiness, biodegradability and efficient encapsulation of DOX. The efficient drug release of such HSA-coated core-shell NPs theranostic NPs was shown in 3D cancer models.[7] Besides the functionalization of such composites with quantum dots allowed to develop a new generation of bimodal MRI/fluorescente cell probes evaluated in vitro with cells and in vivo [8,9].

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