2017 - Talks

Augmenting Human Haptic Perceptions through Knowledge Modeling for Industrial Quality Control

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Human perceptions are the result of a complex process which involves different phenomena at physical and psychological levels. Regarding the sense of touch, although the way each of us senses haptic stimuli through sensory receptors is relatively similar [1], perceptions can be very different from one person to another considering our own experience or knowledge (culture, education, memories, etc.). In the field of quality control, operators' subjectivity is a real problem that can lead to a high variability in the acceptability decision of products, and hence to economical cost and poor customer satisfaction. Thus, products manually controlled require the construction of strict protocols which aim at reducing this subjectivity. Several previous studies have aimed at reducing the variability of visual quality controls [2], but very few studies focused on improving haptic quality control processes. In addition, existing methods usually involve a high cognitive charge for operators (controllers) that have to mentally perform several complex tasks at the same time.

With the perspective of simplifying and standardizing perceived quality control, this study aims at providing a smart system based on knowledge modelling methods. This system is intended to guide manufacturers in the process of building control protocols related to haptic quality, but also to enhance controllers' touch capabilities by reducing the required cognitive charge and adapting their perception to expected haptic sensations. In order to solve current issues associated with quality control using the sense of touch, three main contributions are presented here. First, the proposed formalization of haptic sensations [3] paves the way for a simplification of the description of products' haptic quality, and verbalizes generic haptic anomalies. Nine categories, called elementary sensations, were extracted from a semantic analysis. They provide a precise, yet simple, description of tactile (perceived through the skin) and kinesthetic (perceived through the joints and muscles) sensations. Secondly, studying the influence of touch exploration on perception has already enabled the proposition of a method that transfers the cognitive charge from the controller to the system by adapting his level of perception to the expected quality and reducing ambiguities, hence using the controller as a (very smart) sensor. Thirdly, a knowledge model of the field of sensory perception was built [4] in order to automatize knowledge access and provide context-relevant control protocols.

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Counterion-enhanced encapsulation and emission of cationic dyes inside nanoparticles for multicolour long-term tracking of living cells

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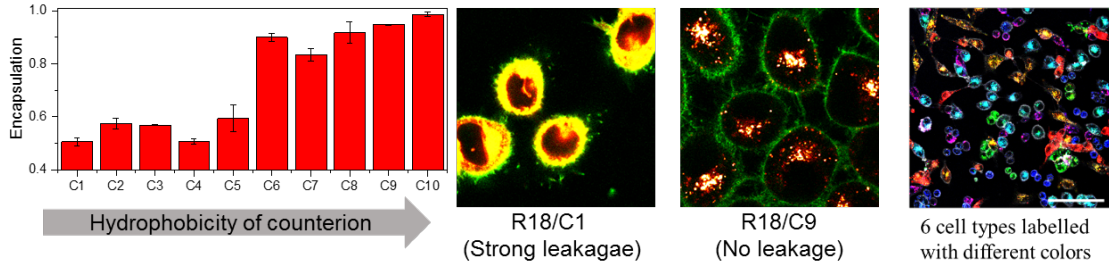
Fluorescent organic nanoparticles are of particular interest for bioimaging due to their high brightness and potential biodegradability, which is their key advantage over quantum dots, that are made of toxic elements and are not biodegradable.[1] The key challenges in the field of fluorescent organic nanoparticles are aggregation-caused quenching (ACQ) and poor encapsulation of loaded dyes. Our group recently reported,[2,3] that combining a cationic dye, rhodamine B octadecyl ester (R18), with a bulky hydrophobic counterion can strongly decrease ACQ and increase encapsulation efficiency of the dyes inside nanoparticles. Moreover, bulky counterions not only prevented quenching, but also induced interfluorophore communication, forcing whole particles to behave like single fluorophores – turning on and off (blinking) as a whole particle. Herein, we tested ten different counterions with different hydrophobicities, the least hydrophobic being perchlorate (C1), and the most hydrophobic being a fluorinated derivative of tetraphenylborate (C10). Their ion pairs with R18 dye were encapsulated inside the biodegradable polymer PLGA (poly(lactide-*co*-glycolide)).The results showed that increasing size and hydrophobicity of counterions decreases ACQ and size of nanoparticles and increases encapsulation efficiency of the dye. The most performant counterion (C10) was used to develop ultra-bright cyanine-loaded fluorescent nanoparticles of different emission colors. To this end, three cyanine dyes of distinct absorption and emission bands (DiO, DiI and DiD) were encapsulated with the C10 counterion into PLGA nanoparticles of the same size (~40 nm) and surface properties. All of them can spontaneously enter cells through endocytosis and show no leakage inside the cells, being a good tool for cell labeling. Moreover, it was found, that due to nearly identical surface properties and no difference in cellular uptake, mixing these nanoparticles of three colors in different ratios can generate at least 13 distinguishable color codes for cell labeling. This method was applied to different cell lines (HeLa, KB, 293T, CHO, RBL, U97 and D2A1) and it can be used for simultaneous tracking of co-cultured color-coded cell populations for more than 2 weeks. Applications for cell tracking in culture and in live zebrafish have been validated.



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Characterization and functional analyses of the basal transcription machinery during development and cellular differentiation.

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Cell fate and patterning during development are controlled by gene expression. Pre-initiation complex (PIC) formation at promoters is crucial to recruit the transcriptional machinery. The PIC has mainly been studied in vitro such as cancer cell lines, and little is known about its composition and function during development in physiological conditions.

Several evidences from the literature suggest that the transcriptional machinery has a certain variability, with some components that are found in some restricted cell types, and thus represents an additional level of gene regulation. TAF10, an architectural and ubiquitous protein, is a subunit of TFIID and of the transcriptional coactivator SAGA, and has been showed to be differentially required during development and depending on the cellular context. The differential requirement for TAF10 during development represents a good paradigm for studying the importance of the variability of the transcriptional machinery. In order to investigate the role of such variability in a physiological model, we characterized TFIID and SAGA's composition during mouse development.

Our data indicate that TAF10 is required for TFIID and SAGA assembly in the embryo, confirmed by results obtained in mouse embryonic stem cells. To analyze TAF10-dependent gene regulation during development, we conditionally deleted *Taf10* in the mesoderm. Our results provide evidence for a time window during which Taf10 loss in the presomitic mesoderm (PSM) does not prevent cyclic gene transcription or PSM patterning while lateral plate mesoderm (LPM) differentiation is profoundly altered. During this period, global steady state mRNA levels are not significantly affected in the PSM although the expression of a subset of specific genes is dysregulated. Together, our data support a differential sensibility of mesodermal tissues to the lack of TAF10 suggesting a differential requirement for TAF10 by the general transcriptional machinery during different developmental processes.

In order to investigate this differential sensibility, we aim to characterize TFIID and SAGA in the PSM and the LPM, and the functional consequences of *Taf10* deletion at the transcription level in those tissues.

Semi-Lagrangian and Particle Methods for Solving the Vlasov-Poisson System: Efficient Hybrid Parallel and Vectorized Code

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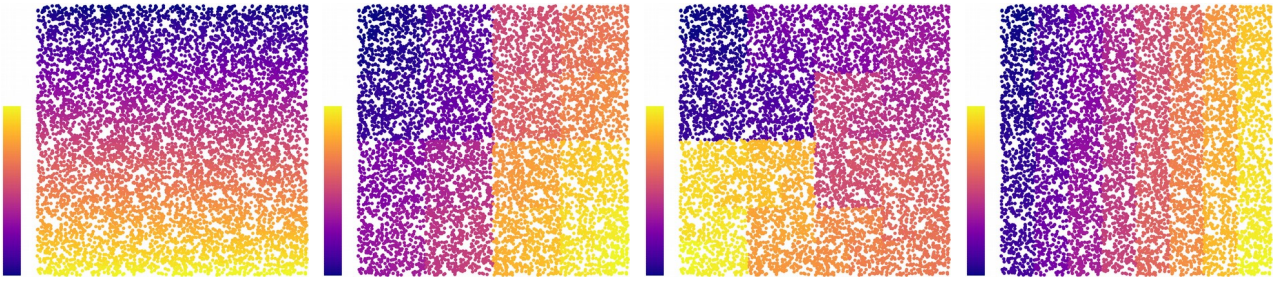
Precise simulations of plasma (gas at very high temperature) are needed in order to master controlled thermonuclear fusion, for example in the ITER tokamak (Cadarache, France).

Under some assumptions, we simulate the following Vlasov-Poisson system for one species. The unknown is the particle density f: the probability of existence of particles (electrons or ions) at time t, around position x and speed v.

$$\left\{ \begin{array}{l} \partial_t f + v \cdot \nabla_x f + \frac{q}{m} E \cdot \nabla_v f = 0 \\ -\Delta \phi = \frac{\rho}{\epsilon_0} \end{array} \right. \text{with} \left\{ \begin{array}{l} E = -\nabla_x \phi \\ \rho = q \int f \, dv \end{array} \right.$$

Two types of numerical methods are investigated: the semi-Lagrangian method and the particle method. Here, we focus on the particle method and on two-dimensional (2d) issues. Our code is written in C and is optimizing a previous code [2] written in Fortran, in the library SeLaLib [1].

One of our objectives is to provide efficient code for those numerical simulations. We thus present an innovative and judicious combination of several optimization techniques for achieving high performance when using automatic vectorization and hybrid MPI/OpenMP parallelism. Overall, our code processes 75 million particles/second per core on Intel Haswell (with hyper-threading) and achieves a good weak scaling up to 0.4 trillion particles on 8,192 cores. The optimizations mainly consist in using space-filling curves (see the pictures below) and appropriate code for automatic vectorization. The optimizations bring an overall gain in the execution time of 42% with respect to a standard code.



Row-major | Morton [5] | Hilbert [4] | L4D [3]

Another objective is to prove that our simulations follow the model. We thus tested the code on cases for which we know analytic approximations [6]. We also performed first and second-order dispersion analysis to verify a new 2d testcase, that has true 2d effects coming from the sole second order dispersion analysis.

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Biochemical and structural characterization of ncRNA mediated transcription anti-termination.

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Over the past decade, noncoding RNAs (ncRNA) have been shown to play important biological roles in all domains of life, from viruses to humans (1). ncRNAs have been implicated in translation regulation and more recently also in transcription (2). We are interested in two ncRNA elements called *putL* and *putR* (*p*olymerase *ut*ilization) found in the *E. coli* phage HK022 and described in (3, 4). Both of these ncRNA elements have been shown to directly affect their own transcription and stimulate anti-termination of *E. coli* RNA polymerase (RNAP), thereby promoting expression of genes downstream of transcription terminators. Of all the different ways in which ncRNAs regulate transcription, the role of these *cis*-acting ncRNAs in transcription anti-termination, a process so far known to be controlled exclusively by protein factors, is intriguing with implications for the RNA world hypothesis.

We have first biochemically characterized both ncRNA elements in terms of size, stability and anti-termination effect *in vivo* and *in vitro*. We will now use structural analysis to study the precise mechanism by which *putL* and *putR* modulate an RNAP elongation complex using cryo-electron microscopy in order to understand how ncRNAs alter the processivity of RNAP. As an alternative approach, we will also perform crosslinking analysis combined with mass spectrometry to map the interactions of these ncRNAs with *E. coli* RNAP.

Our work will contribute to a better understanding of the anti-termination mechanisms and will address a novel aspect of transcription regulation: the role played by ncRNAs.

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Dual acting opioid – neuropeptide FF ligands: screening and pharmacological characterization

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Opioid analgesics, such as morphine and fentanyl, continue to be the cornerstones for treating moderate to severe pain. However, upon chronic administration, their efficiency is limited because of prominent side effects such as tolerance and dependence.

One hypothesis for the occurrence of these side effects is that the chronic stimulation of the opioid system may trigger its endogenous counterparts, anti-opioid systems, producing hyperalgesia (opioid-induced hyperalgesia, OIH) and analgesic tolerance. Previous data from our lab and others have shown that RF9, an antagonist of neuropeptide FF receptors (NPFF1R and NPFF2R), efficiently blocks opioid-induced hyperalgesia and tolerance when co-administered with fentanyl or morphine in rodents. In this study multi-target molecules, that display mu-opioid receptor (MOR) agonist activity, as well as NPFF receptor antagonist properties, were designed. To this purpose a set of peptidic ligands was generated, which combines an already known high affinity mu-opioid receptor agonist together with the carboxyl-terminal RF-amide signature of NPFF. First, the affinity and activity of these molecules were determined for MOR. We identified high affinity ligands (<1nM) in binding competition studies with membranes from CHO cells stably expressing MOR. The MOR-agonist activity was confirmed in cAMP assay (Glo-sensor) in HEK cells stably expressing this receptor and furthermore the β-arrestin recruitment was investigated by BRET in HEK cells stably expressing MOR-luciferase. We then examined the NPFF receptor component in binding competition studies with membranes from CHO cells stably expressing NPFF1R or NPFF2R. Molecules that displayed good affinity for both NPFF1 and NPFF2 receptors were further evaluated in GTPγS and cAMP functional assays. This study allowed to identify one hybrid molecule that displays high affinity for and full agonist activity at MOR, NPFF1R and NPFF2R (compound 1) and a second hybrid molecule that shows full agonist activity at MOR as well as antagonist properties for both NPFF1 and NPFF2 receptors (compound 2). In accordance with *in vitro* results, we observed that acute s.c. administration of compounds 1 and 2 at low doses produced strong and long-lasting (> 5 hrs) antinociceptive effects in mice. After 7 days of chronic s.c. administration of compound 1, mice developed hyperalgesia and analgesic tolerance, two effects that were not observed upon chronic treatment with compound 2.

Further *in vivo* evaluation of analgesic properties in an inflammatory pain model of these two compounds and additional adverse side effects are currently in progress.

Role of sodium glucose co-transporter 2 in high glucose-induced endothelial senescence: Preventif effect of Empagliflozin

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The high glucose (HG)-induced endothelial dysfunction has been suggested to promote cardiovascular diseases in patients with type 2 diabetes by inducing premature endothelial senescence. Endothelial senescence is characterized by the down-regulation of the endothelial nitric oxide synthase (eNOS)-mediated formation of NO, a potent vasoprotective factor. Empagliflozin, a selective sodium glucose co-transporter 2 inhibitor, reduced cardiovascular mortality in type 2 diabetic patients.

This study investigated the possibility that empagliflozin prevents endothelial senescence. Porcine coronary artery endothelial cells were exposed to HG (25 mmol/L) for 96 h before the determination of the senescence level using the senescence-associated beta-galactosidase (SA-beta-Gal) activity, and the expression of target proteins by Western blot analysis. HG significantly increased the level of SA-beta-Gal activity by about 3-fold, such an effect was prevented by empagliflozin, and also by the antioxidant N-acetylcysteine, the NADPH oxidase inhibitor VAS-2870, and the cyclooxygenase (COX) inhibitor indomethacin. The HG-induced senescence was associated with an increased expression of senescence markers p21 and p16, p22phox and p47phox NADPH oxidase subunits, COX-2 but not COX-1, VCAM-1 and tissue factor, and the down-regulation of eNOS; all these effects were prevented by empagliflozin. Thus, empagliflozin prevents the HG-induced endothelial senescence most likely by inhibiting the expression of pro-oxidant enzymes such as NADPH oxidase and COX-2. As a consequence, empagliflozin retards premature vascular ageing as indicated by the persisted eNOS expression level, which, in turn, helps to counteract the induction of pro-atherosclerotic and pro-coagulant factors. Such an effect may contribute to explain the protective effect of empagliflozin on the cardiovascular system.

Next generation sequencing reveals recessive myopalladin mutations cause congenital cap myopathy

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Congenital myopathies are genetic disorders characterized by distinctive morphological abnormalities in skeletal muscle fibers. They define a class of severe muscle diseases with a strong impact on patient survival and quality of life. Several genes have already been linked to congenital myopathies. However, about half of the patients do not have a genetic diagnosis supporting the implication of a large number of yet unidentified genes.

To accelerate the identification and characterization of the genetic basis of congenital myopathies, we coordinate a French consortium called Myocapture with the aim to better characterize the clinical, histological and genetic data of patients. The strategy was to sequence 1000 exomes of patients and their family, previously excluded for mutations in genes known to be involved in the related myopathies. Within this project, we studied two unrelated families with a cap myopathy characterized by the presence of peripherally-placed, well-delimited structures resembling a cap in muscle fibres. In both families, we identified recessive truncating mutations in *MYPN. MYPN* encodes the Z-line protein myopalladin implicated in sarcomere integrity. Functional experiments demonstrate that the mutations lead to mRNA defects and to a strong reduction in full-length protein expression. Myopalladin signals accumulate in the caps together with alpha-actinin in patient muscles. Dominant *MYPN* mutations were previously reported in cardiomyopathies. Our data link for the first time *MYPN* mutations to a myopathy and implicate that defects in myopalladin cause either a cardiac or a skeletal muscle disorder through different modes of inheritance.

The identification, validation and characterization of novel implicated genes in congenital myopathies such as *MYPN* allows the development of novel diagnosis protocols to improve genetic counseling, including eventual prenatal or pre-implantation diagnosis. Moreover, novel myopathy genes represent new therapeutic targets.

Development of a pharmacophoric deconvolution method to accelerate the discovery of antiplasmodial molecules from Rhodophyta

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In natural-product research, the bioassay-guided isolation is usually used to discover new bioactive compounds. However, this strategy is time-consuming, onerous and sometimes leads to well-known molecules[1]. We are developing a new method based on the Differential Analysis of 2D-NMR Spectra (DANS) and the use of the hyphenated method HPLC-SPE-NMR to solve these inconveniences[2]. This method aims to accelerate the discovery process of new bioactive products from complex natural extracts, by avoiding their systematic isolation. The technological lock is the DANS step which enables the obtention of the chemical fingerprint of the bioactive molecule. To solve this problem, a software is under development to process 2D-NMR data (bucketing, spectral cleaning …). In this way, the chemical fingerprint of bio-active molecules from algal extracts enriched with artemisinin or chloroquine, two known anti-malarial compounds, was obtained.

This analytical strategy will be used to identify new anti-malarial molecules from active red algae extracts. Previously, it was shown that red algae are a source of antiplasmodial products[3]. The parasite *Plasmodium* possesses a relict organelle, the apicoplast, which is a plastid from a secondary endosymbiosis of a red alga[4]. Because of this particular evolutionary past, we hypothesized that red algae molecules could interfere with apicoplastic biosynthesis pathways in *Plasmodium* and inhibit its development.

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Do P2X receptors really dilate?

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P2X receptors are ligand-gated ion channels (LGICs) found in almost all cell subtypes, involved in many physiological and pathological processes such as synaptic transmission **[1]**, taste sensation **[2]**, inflammation **[3]** and neuropathic pain **[4]**. After the application of extracellular adenosine 5′-triphosphate (ATP) their activation allows the rapid passage of small cations (Na^+, K^+, $Ca^{2+)}$ through the cell membrane, a process known as "gating". Since 1999, it has been observed that a second opening state can be achieved for these receptors, known as "pore dilation" state and apparently results from an increase of the pore diameter allowing the passage of larger organic cations **[5]**. In order to elucidate the molecular motions underlying pore dilation of P2X receptors, we devised a new strategy based on our recent "opto-tweezers" method **[6]**. The strategy is based on the use of photoisomerizable tools containing an azobenzene moiety to control selectively processes of activation and deactivation of P2X receptor and then observe resulting molecular motions.

Azobenzenes are molecules that can be switched reversibly with light at an appropriate wavelength between two stereoisomers: *trans* and *cis*. These two isomers show a significant difference in their end-to-end distances **[8]**. We have designed photoswitchable crosslinkers with spacing arms of increasing length and two maleimides for efficient cross-linking at a couple of engineered cysteine-substituted P2X receptors. When covalently incorporated in the receptor they could induce movements relevant to conformational changes. To visualize the effect of light on channel activity we combined these tools with the patch-clamp electrophysiology technique coupled to light illumination that allows the detection of flow of ions through the membrane. With this strategy we were able to probe several molecular motions between transmembrane domains of the P2X receptor that allow the permeation of large cations. Our data, therefore, bring into question the existence of this controversial dilated state.

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PARP3 in continuous and stress-induced neurogenesis in brain

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Poly (ADP-ribosyl) ation is a post-translational modification of proteins mediated by Poly (ADP-ribose) polymerases (PARPs, 17 members). Most of the studies developed over the last 4 decades have essentially focused on the biochemical, physiological and pathological properties of the founding members of the PARP family, PARP1 and PARP2. Less is known on PARP3. Initial studies identified PARP3 as a key player in cell response to double-strand breaks (DSB) and mitosis (Boehler *et al*. PNAS 2011, Beck *et al*. NAR 2014). However the *in vivo* cellular functions and physiological settings in which PARP3 are involved remain to be determined. PARP3 was also shown to modulate transcription in the early stages of zebrafish development and to participate in epithelial to mesenchymal transition and stemness in breast cancer cells (Rouleau *et al*. Plos One 2011; Karicheva *et al*. Oncotarget 2016). Recent results in the lab reveal enhanced expression of PARP3 in response to H202 exposure and increased sensitivity of $PARP3^{-/-}$ mouse embryonic fibroblast to oxidative damage. Reactive Oxygen Species (ROS) play important roles in cell signaling and homeostasis during neurogenesis and in hippocampal synaptic plasticity. Furthermore, oxidative stress is a hallmark of stress-induced brain injury.

Together, these observations prompted us to investigate a possible role of PARP3 in ROS protection and neurogenesis in brain in both normal situations and upon ischemia.

Our current results reveal that PARP3 serves in astrocyte differentiation likely involving a response to ROS, and promotes neurogenesis induced by hypoxia-ischemia.

Etude de l'hydroformylation désymétrisante de l'acide dihydromuconique et son application à la synthèse d'alcaloïdes

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L'étude de l'hydroformylation désymétrisante de dérivés de l'acide dihydromuconique appliquée à la synthèse du (±)-Vindéburnol[i], un alcaloïde synthétique actuellement en développement clinique pour ses propriétés vasodilatatrice, a été effectuée. Dans un premier temps, la réactivité vis-à-vis d'amines primaires simples de l'aldéhyde obtenu par cette hydroformylation a été étudiée. Puis, l'utilisation de la tryptamine comme tri-nucléophile a permis d'obtenir le squelette pentacyclique du (±)-Vindéburnol par le biais d'une réaction en cascade dans laquelle quatre nouvelles liaisons sont formées. Une dernière étape de réduction a permis l'obtention de la molécule cible.[ii]

Nous souhaitons maintenant développer cette méthodologie pour accéder à d'autres alcaloïdes des familles des éburnamines et des aspidospermines. A terme, ces études visent la synthèse totale d'alcaloïdes bis-indoliques plus complexes[iii]. A cette fin, nous travaillons à la mise au point d'une stratégie de synthèse convergente à partir d'aldéhydes issus de l'hydroformylation de diesters symétriques.

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[ii] Girard & al., Journal of Organic Chemistry, **2017**, *82*, 2257-2262
[iii] Morita & al., Bioorganic & Medicinal Chemistry Letters **2010**, *20*, 2021–2024

Preparation of an injectable iodinated polymeric nanoparticulate contrast agent by nanoprecipitation for *in vivo* preclinical XR imaging

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Nowadays, formulations of contrast agent (CA) for X-ray imaging rely mainly on small hydrosoluble iodinated molecules capable of attenuating X-Ray and providing contrast enhancement in tissues where they are accumulated. However, these CAs suffer from a lack of long-time retention and fast excretion leading to use high doses for a poor efficiency and toxicity issues [1]. Literature shows that involving iodinated nanoparticles with improved pharmacokinetic thanks to tuned surface and good contrast property is one the best alternative to overcome those limitations [2]. Colloidal polymeric nanoparticles (PNPs), and more precisely iodinated ones, have been reported as very suitable to fulfill those needs because of they are stable nanocarrier capable of being loaded with high iodine content and delivered to site of interest [3, 4].

Here, we report the development of a new intravenously injectable radiopaque suspension of PNPs. The iodinated polymer was first produced by radical polymerization and then subjected to nanoprecipitation in presence of pegylated surfactant to yield nano-sized hydrophilic iodinated PNPs. Impacts of polymer and surfactant weight ratio were studied to find best compromise between suitable size for *in vivo* and iodine content for radiopacity property. Best candidate was thus selected for *in vivo* experiments. Size distribution and morphology investigations, respectively performed by dynamic light scattering and scanning electron microscopy, indicated the selected formulation was based on 160 nm spherical PNPs. Preliminary *in vitro* stability study in serum showed satisfying results leading us to pursue with *in vivo* assays. Once administrated to mice, PNPs were spontaneously accumulated in liver and spleen for which significant enhanced contrast was visible by X-ray scanner without any adverse effects. The production of non-toxic CA was though achieved and allowed clear delineation of soft tissues through X-ray imager.

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Synthesis and bioactivity evaluation of structural analogues of anti-*Leishmania* anthranoïds from *Psorospermum genus* (Hypericaceae) used in traditional medicine

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Leishmaniasis is a vector-borne disease caused by the protozoa *Leishmania*, which are spread by a variety of sandfly species1. It is mostly occurring in East-African and Asian countries and it is directly linked to poverty. With 1.3 million new cases and 20 000 to 30 000 deaths by year, leishmaniasis is a major cause of morbidity and mortality.

As available drugs show important adverse effects and become less and less efficient due to emerging drug-resistance, it is urgent to find new active compounds with original mechanism of action. Natural products through their huge and biologically-designed diversity of chemical structures have traditionally played an important role in drug discovery. Based on *Psorospermum* species used in traditional medicine for their antiparasitic activity, several anthranoid compounds have been isolated and characterized and their bioactivity against *Leishmania donovani* has been evaluated.

In order to understand the mechanism of action and improve the activity and selectivity of the molecules, we synthesized several analogues of natural anthranoid compounds and evaluated their antileishmanial activity.