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Posters
Site-selective faithful monitoring at the single molecule level
of local changes in nucleic acids

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Numerous cell mechanisms and pathways rely on dynamic interactions of RNAs or DNAs with proteins that induce local and transient changes in their secondary and tertiary structures. Information on the structure of the protein/nucleic acid complexes is already very well documented thanks to techniques such as X-ray diffraction, NMR or electron microscopy. But their limits are quickly reached when it comes to monitor the dynamics of these interactions. In this case, Fluorescence-based techniques represent the best solution, and given that nucleobases are almost not fluorescent, the introduction of thienoguanosine (thG), a truly faithful emissive and responsive surrogate for Guanosine, represents a breakthrough in our work.

Currently we are building a setup which will allow us to perform single molecules experiments with thG labelled oligonucleotides and take advantages of its environment sensitive fluorescence properties. The aim is to report and document thG fluorescence changes during DNA methylation and more precisely to faithfully monitor the base flipping process occurring during this methylation.

Complementary to the setup, several steps need to be completed in order to achieve these observations, starting by the functionalization of our quartz slides, to specifically attach thG labelled ODNs to our surface and avoid parasite fluorescence due to the thG excitation wavelength located in the UV range (between 300 and 400 nm). Another important part of our work will be to perform a clear and reproducible activity test on all the proteins involved in the base flipping process (DNMT1, SRA, UHFR1) and a potential production on site;

The future work includes a collaboration with the Université de Technologie de Troyes and aiming to achieve a higher brightness and photostability of thG. The objective will be to generate a surface plasmon resonance (SPR) on Al nanoparticles that match with the thG absorption spectrum. This SPR generation require nevertheless an extreme precision both in the size of the nanoparticles and the distance between the dye and the metallic surface, but represents a determining factor leading to a better signal collection and thus significantly increase the quality of our acquisitions.
Optimizing size of dye-loaded polymer nanoparticles for penetration into fixed cells

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Fluorescent polymer nanoparticles (NPs) encapsulating large quantity of dyes, so-called dye-loaded polymer NPs have attracted growing interest in bioimaging over the past years thanks to their high brightness, biocompatibility, flexibility in terms of cargo loading\(^1\). Previously, our team developed a concept of encapsulation of cationic dyes with bulky counterions into polymer NPs, that allows obtaining ultrabright nanoparticles. Moreover, we showed that small size of polymer NPs (from 10 to 100 nm, depending on the polymer\(^2,3\)) can be obtained through nanoprecipitation of specially designed charged polymers. More recently, we found that NPs below a critical size of 23 nm is a requirement for spreading throughout the cytosol of living cells\(^4\). However, variety of cell imaging applications, such as immunostaining, requires cell fixation. Here, we asked a question: what is the critical size to reach the cytosol of fixed cells? To answer this question, we studied by fluorescent microscopy ultrabright NPs of varied size (12 to 38 nm) coated with polyethylene glycol (PEG) after incubation with fixed Hela cells. We showed that NPs with a size inferior to 25 nm are able to reach and move freely in the cytosol and in the nucleus. Then above 32 nm, these NPs cannot reach the nucleus anymore, but they are still able to pass though the membrane and move inside the cytosol. Subsequently, we tested if the same NPs functionalized with nucleic acid can enter cytosol of fixed cells. We found that DNA coated NPs with a size of 65 nm are blocked on the membrane of fixed cells whereas DNA coated NPs with a size of 28 nm can pass through the membrane and spread in the cytosol. In brief, small size of NPs (<30 nm) is required to reach the cytosol of fixed cells, which will allow diverse applications such as detection of single molecules inside fixed cells.

References
Evaluation of intracellular fate of peptide-coated nanocluster containing 102 gold atoms after transduction in living HeLa cells

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Mercaptobenzoic acid (MBA) monolayer-protected nanoclusters of average formula Au102MBA44 are attractive nanometric materials because of their precise formula, small sizes and ease of functionalization via exchange of the MBA ligands with thiols but not with disulfide.

The small size renders the materials compatible for diffusion in water and dense and filament-rich tissue and cytosol. Moreover, the exclusive sensitivity of the monolayer coverage to thiolated molecules can be exploited for selective release of the cluster’s coverage inside the cytosol since this compartment contains large amounts of reduced glutathione. To evaluate the diffusion ability and stability of the Au-thiol bond into the cell, we prepared and characterized various peptide-coated gold nanoclusters by exchanging some MBAs to thiolated peptides selective to intracellular subcellular organelles. The peptide-covered gold nanoclusters were then electroporated inside living HeLa cells and their fates are determined by detecting the gold clusters. Our data show that these nanoparticles diffuse into the dense and filament-rich cytosol without impacting the cell viability. We also provide data on the stability of the peptide-coated gold nanoclusters within living cells.

Altogether, our results will help to conceive novel nanometric materials with biomedical application.
The accumulation of protein aggregates is the hallmark of numerous amyloid diseases (amyloidoses), including for instance Parkinson's, Alzheimer's and polyglutamine (polyQ) diseases. One therapeutic strategy against amyloidoses consists in preventing the formation of amyloid aggregates. Reliable high-throughput (HT) techniques are thus needed to discover aggregation modulator compounds. In this regard, we recently reported the development of a novel in vitro HT assay, SynAggreg, which overcomes some limitations of existing HT assays (1). SynAggreg was validated by performing a pilot screening of the Prestwick Chemical Library®, a collection of 1,280 molecules including 95 % FDA approved drugs, to identify modulators of aggregation of a well characterized 41-glutamine (Q41) model polyQ amyloid.

Here, we studied a fragment of the main amyloid peptide that accumulates in Huntington's disease (HD), the most prevalent polyQ disorder. This fragment contains two regions of the Huntingtin protein, which both contribute to the aggregation through distinct mechanisms: the first 17 aminoacids (N17) and a 46-glutamine (Q46) stretch. At equivalent concentrations, N17Q46 aggregates much faster and displays sensibly more kinetics variability than Q41 (1). Our goal in the present study was (i) to show that the SynAggreg technology is reliable to screen for aggregation modulators of N17Q46, (ii) to increase the throughput and scale-up our assay by performing a larger screen and (iii) to identify novel aggregation modulators of N17Q46.

To ensure the highest reproducibility at HT and facilitate the comparison of independent experiments, we first produced and purified single batches of all needed proteins (SynAggreg uses a mixture of unlabelled N17Q46 that drives the aggregation, and traces of N17Q46 coupled with fluorophores for quantification). We then optimized our HT processes and demonstrated that our assay is suitable for HT screening (HTS). Next, we developed strategies to screen up to ~2,500 compounds per day. We performed a primary screening of 7,360 compounds, validated our primary hits by "cherry-picking" and finally confirmed the validated hits with new batches of compounds.

Our most efficient hits mainly belong to two chemical families, which paves the way to structure–activity relationship (SAR) studies to find the best analogue compounds. Moreover, as SynAggreg was specifically designed to identify synergistic combinations of aggregation modulators, we will now perform a combinatorial screening using our newly discovered confirmed hits. Furthermore, we intend to study the effect of our most promising hits or combinations of hits in in vivo models of HD.

Taken together, our results demonstrate the robustness of SynAggreg for the study of amyloid aggregation and for the discovery of aggregation modulator compounds.


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Layer-by-Layer coating of liposomes to tune their physico-chemical properties toward biological applications

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Among a variety of drug delivery systems, liposomes are promising candidates considering their biocompatibility, biodegradability and their drug loading capacity for both hydrophobic and hydrophilic molecules (1). However, their major drawback is their instability in biological environments (2). One approach to increase their resistance is the layer-by-layer (LbL) coating with oppositely charged polymers to obtain structures called 'layersomes' (3).

Based on a previous work, the poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) combination was chosen by our group for layersomes formulation. Indeed, PLL and PGA coating was shown to increase the robustness of liposomes and to be resistant to hydrolysis. Moreover, the polyelectrolytes side chains can be crosslinked (4).

Small unilamellar liposomes (Lp) composed of phosphatidylcholine, phosphatidylglycerol and cholesterol were used as starting materials. Alternate deposition of positively and negatively charged polymers was achieved by slowly dropping the liposomes into a PLL solution and then by slowly dropping the obtained Lp-PLL suspension into a PGA solution. Excess of polymer was removed after each coating step.

We first demonstrated that the alternate deposition of positively and negatively charged PLL and PGA, respectively leads to an increase of the average diameter and a variation of the zeta potential values compared to primarily liposomes, which is consistent with a layer-by-layer coating. In parallel to formulation, we performed the synthesis of PLL, and PGA covalently coupled to rhodamine B and fluorescein, respectively, to demonstrate the alternate coating of liposomes. A light Fluorescence Resonance Energy Transfer was observed \( n = 3 \) and strongly confirmed the alternate deposition on liposomes.

The formulation procedure described above was optimized and we succeeded in developing stable and monodisperse layersome formulations with up to 8 polyelectrolyte layers of about 400 nm average diameter (half-value width: 50 nm). Preliminary stability experiments in simulated media, in the presence of detergents, bile salts and enzymes were conducted and are currently being tuned. They suggest an increased resistance for layersomes with 4 layers and more in the presence of low concentrations of detergents. In addition, we recently performed the synthesis of an ester activated form of PGA to allow a covalent locking of the peptide side chains via amide bonds and to strengthen the polymeric coat. Stable formulations with PLL and this modified PGA were obtained, and their resistance in biological media is currently being investigated.

TLC-DB-MS to accelerate the discovery of new antimicrobial compound from mushrooms

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The discovery of new antibiotic compounds becomes one of the major priorities for medical and pharmaceutical research\textsuperscript{1}. Mushrooms are under-explored natural resources with few metabolites described\textsuperscript{2} that grow in a competitive environment from an ecological point of view. Therefore, they might produce new potent molecules against microorganisms.

In order to accelerate the discovery of new antibiotics from macromycetes, we applied a thin-layer chromatography- direct bioautography ((TLC-DB) test hyphenated with mass spectrometry (MS) detection\textsuperscript{3}. This strategy allows the rapid targeted isolation of active molecules from crude extracts.

Several methanolic extracts of mushrooms from Alsatian forests were screened by TLC-DB against a strain of \textit{Staphylococcus epidermidis}. Some of them showed various inhibition zones revealing the presence of molecules with antibiotic properties. A common molecule to all mushroom extracts was particularly active against \textit{S. epidermidis}. With the help of TLC-MS interface, this compound was desorbed and analysed by MS directly from the eluted plate without additional process on the crude extract. By combining TLC-DB with MS, the active compound was faster identified. To conclude, the combination of TLC with biological and chemical detections offers great potential for the new bioactive natural products research.

Structural and functional studies of Human type II Topoisomerases and their post-translational modifications

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Type II DNA Topoisomerases (Top2) are essential enzymes involved in DNA replication, DNA condensation and chromosome segregation. They remove topological stress that appear in the DNA duplex by releasing catenates, relaxing supercoils and removing knots through transient double strand breaks. This mechanism is currently targeted by compounds used in cancer chemotherapy, that prevent religation of DNA breaks and lead to cell death (1).

The implication of these enzymes in the cell cycle raises the question of their regulation notably by post-translational modifications (PTM). Anti-cancer drugs such as doxorubicin and etoposide were shown to induce hyperphosphorylation or hypophosphorylation in cancer cells (2, 3). However the role of PTM in the catalytic regulation of Top2 is not clearly understood.

In this work, we have focused on the post-translational modifications of the human Top2. We have systematically identified the phosphorylation and acetylation sites in the Top2 isoforms, and observed a distinct distribution of both modifications. This identification allowed us to understand the role of a catalytic residue subject to an acetylation and the potential impact of this modification (4). We currently trying to evaluate the impact of the multiple phosphorylations on the structure and the catalytic activities of the Top2.

Algerian thermal water incorporated in innovative topical formulations

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Thermo-mineral waters (TW) are unlimited natural sources widely used in balneotherapy to treat dermal pathologies such as atopic dermatitis [1]. Their therapeutic effects are related to their rich composition of chemical elements [2]. In the skin, TW enrich the cells with minerals and trace elements that enter in enzymatic and immune biological reactions and intracellular exchanges [3]. They can also be used as an active ingredient in dermal formulations that preserve their therapeutic benefits and improve their bioavailability in different skin types [4].

In the first part of our work, we therefore aim to optimize a hydrogel based on a highly mineralized Algerian thermal water (3162 mg / L). A factorial design approach was used to study the effect of the gelling agent and the humectant amount solubilized in a highly mineralized TW, on the rheological and sensory properties of the hydrogel. In a comparative preclinical study on rabbits, the optimized hydrogel-TW proved a healing and an anti-inflammatory effect in less than 10 days by comparing with a placebo hydrogel based on demineralized water. The hydrogel has demonstrated favorable clinical efficacy against atopic dermatitis symptoms in 67% of patients in a comparative clinical trial with the placebo hydrogel.

In order to improve the bioavailability of minerals in the deepest layers of the skin even after the repair of stratum corneum which limits the absorption of hydrophilic molecules [5], we are currently working on the encapsulation of TW in liposomal nanovectors. TW-loaded liposomes are characterized for their size, zeta potential, encapsulation efficiency, stability and cytotoxicity as a function of mineral concentrations. The anti-inflammatory effect of TW-loaded liposomes on LPS-activated RAW macrophages will be assessed by nitric oxide (NO) assay in the cultured medium determined by the Griess reaction. In the following work, the flow rheological properties of the TW-hydrogel, containing TW-loaded liposomes will be investigated.

Impact of the zinc finger motifs in HIV-1 Gag on the specific selection of genomic RNA and its trafficking to the plasma membrane.

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Assembly of human immune deficiency virus-1 (HIV-1) is a highly regulated process which includes the selection and packaging of host cellular and viral components to produce infectious virion. The viral protein Gag orchestrates the assembly process1. Indeed, Gag selects the gRNA from the bulk of cellular and spliced/unspliced HIV-1 RNAs by specifically interacting with the packaging signal (Psi) which consists of four stem loops (SL1-4) located in the 5’ end of genomic RNA. As the cellular trafficking of the viral Ribonucleoprotein remains yet to be precisely defined, we investigated the role of the zinc fingers in NC domain in cells to better understand the Gag mediated-specific selection of gRNA, and its trafficking to the PM.

More in detail, we compared the interactions of the wild-type Gag precursor, several NC mutants and a non-myristoyled version of Gag, that cannot interact with the PM (GagG2A) with gRNA by combining bio-imaging approaches based on fluorescence such as confocal microscopy, PALM-Storm (Photo-Activated Localization Microscopy and Stochastic Optical Reconstruction Microscopy), microinjection assays, FRET-FLIM (Fluorescence Resonance Energy Transfer - Fluorescence Lifetime Imaging Microscopy), and RISC (Raster Image Correlation Spectroscopy).

Our data showed that Gag interacts with viral RNAs in the cytoplasm, and at the PM as well, where they co-localize. Importantly we found that this interaction is driven by ZF motifs in the NC domain, and one ZF is sufficient to ensure the gRNA recruitment in the cytosol and its trafficking to the assembly sites at PM. Besides, the two ZF motifs displayed a similar role. Finally, the substitution of the Glycine at the N-terminus prevented as expected the co-localization with the gRNA at the PM, but did not impair the specific recruitment of gRNA.

Taken together, our data clearly show that the discriminants for the specific Gag-gRNA selection exclusively reside in the ZF motifs in the NC domain.

Fluorocarbon-peptide conjugates (FPC): new concept to increase the metabolic stability of peptides for therapeutic applications.

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Over the past decade, peptides have shown an increasing interest for therapeutic applications as they are selective and efficacious signaling molecules. To date, 60 therapeutic peptides have been already approved by the FDA. However, they are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short in vivo half-life due to rapid enzymatic degradation.

To address the peptides instability issue for therapeutic applications, we propose an unprecedented strategy based on the grafting of fluorocarbon chains (F-chains) onto peptides. Thereby, the hypothesis was to induce the self-organization of fluoropeptides in aqueous solution, resulting in the protection of the native peptide from enzymatic degradation.

To demonstrate the efficacy of our approach the apelin-17 peptide, a neuro-vasoactive peptide which presents a short plasma half-life, was selected as model. Different F-chains were then grafted onto apelin-17 following a solid-phase approach. The highest plasma stability fluoroapelin was then evaluated in rat model demonstrating the positive impact of F-chain to greatly improve the in vivo efficacy of apelin-17. In this communication, we will present some preliminary results to gain insight into the mechanism leading to the increase of human plasma stability of fluoroapelin.

Altogether, these promising results should open the route to a convenient, safe and general approach to greatly increase the metabolic stability of numerous peptides for their in vivo use as pharmacological tools and/or therapeutic agents.

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A novel approach for the development of surfactant free polymeric nanoemulsions

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Nano-emulsions (NE) are fine, stable, and in general optically clear and translucent dispersions of two immiscible liquids (oil-in-water or water-in-oil) stabilized by an amphiphilic surfactant. They are stable and very powerful systems when it concerns the encapsulation of lipophilic compounds and their dispersion in aqueous medium. On the other hand, when the properties of the nano-emulsions aim to be modified, e.g. for changing their surface properties, decorating the droplets with targeting ligands, or modifying the surface charge, the dynamic liquid / liquid interfaces make it relatively challenging. Generally, NE are prepared in the presence of surfactants to reduce interfacial tension and enhance the stability of NE over time by stabilizing adsorption at the oil/water interface through electrostatic and/or stearic repulsions. But there are some limitations of using surfactants in the formulations like high cost, questionable biocompatibility, interaction with continuous and dispersed phases, formation of interconnected network with the polymer at the interface and alteration of nanoparticle properties.

In this study, we have explored the development of nano-emulsions which were not anymore stabilized with a classical low-molecular weight surfactant, but instead, with an amphiphilic polymer based on poly(maleic anhydride-alt-1-octadecene) (PMAO) and Jeffamine®, a hydrophilic amino-terminated PPG/PEG copolymer. Using a polymer as stabilizer is a potential solution for the nano-emulsion functionalization, ensuring the droplet stabilization as well as being a platform for the droplet decoration with ligands (for instance after addition of function groups in the terminations of the chains). The main idea of the present work was to understand if the spontaneous emulsification – commonly performed with nonionic surfactants – can be transposed with amphiphilic polymers, and a secondary objective was to identify the main parameters impacting on the process. PMAO was modified with two different Jeffamine®, additionally different oils and different formulation conditions were evaluated. As a control, the parent monomer, Octadecyl succinic anhydride (OSA) was also modified and studied in the similar way as that of polymer. The generated nano-emulsions were mainly studied by dynamic light scattering and electron microscopy, which allowed identifying the crucial parameters in the spontaneous process, originally conducted with polymers as only stabilizer.
Designing 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 introduced in the cytoplasm through microinjection and electroporation. 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.

New strategies to overcome Temozolomide resistance in glioblastoma

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Purpose: Glioblastoma is a malignant, fast growing and the most aggressive brain tumour, characterized with poor prognosis and an overall median survival of less than 15 months after diagnosis. Currently there is no effective long-term treatment for this killer disease and the standard of care is surgical resection, followed by radiotherapy and chemotherapy. One of the major challenges in glioblastoma therapy is the development of therapy resistance leading to recurrence of the disease. In the laboratory it has been shown that expression of α5β1 integrin participate to therapy resistance by interfering with the p53 signaling pathway. This study explores novel strategy to overcome acquired chemotherapy resistance in U87 MG glioma cells in particular in Temozolomide (TMZ - the standard of care chemotherapy) resistant cells.

Experimental Design: TMZ resistant cells were generated by increasing the drug concentration over a period of three months and resistance to temozolomide was confirmed with a live-cell analysis system (Incucyte). Cells were treated with a p53 activator (Nutlin3a), an α5β1 integrin antagonist (K34C) or both in combination. In addition, the effects of compound 9 able to activate p53 and concomitantly inhibit the integrin were explored. Signaling pathways were studied using western blot, qRT-PCR and Flow cytometry.

Results: Long-term exposure to TMZ induced resistance in U87 MG glioma cells. In resistant cells we noted an increase in α5β1 integrin protein expression. Treatment with Nutlin3a or K34c alone significantly inhibited proliferation but combination of both drugs was more effective. In addition, compound 9 alone has similar effects than combination of Nutlin 3a and K34c. This effect was decreased when α5 integrin subunit was deleted by the Crispr/Cas9 technology. Preliminary experiments suggest that growth inhibition is associated with apoptosis.

Conclusion: These data suggest that activation of p53 associated with the inhibition of α5β1 integrin may be used as a salvage therapy after acquired resistance against TMZ and may prevent glioma recurrence.
Synthesis and characterization of new organic nanoantennas for FRET-based detection of nucleic acids by RGB camera

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Dye-loaded polymeric nanoparticles have proved themselves as a powerful bioimaging tool due to their high brightness and capacity to bear multiple functional groups[1]. However when the nanoparticles are loaded with high concentration of fluorophores, aggregation caused quenching (ACQ) limits the brightness of these systems. This problem was solved in our group by employing cationic dyes functionalized with hydrophobic groups paired with bulky hydrophobic counterions. In addition to an effective spacing of the fluorophores inside the nanoparticles, counterions generate a supramolecular organization, which features a cooperative behavior of the dyes[2], [3]. Such collective behavior of our system was employed for preparing light-harvesting nanoantenna particles that enable Fluorescence Resonance Energy Transfer (FRET) from thousands of rhodamine B derivative dyes to few cyanine 5 derivative dyes; leading to >1000-fold amplification of the acceptor intensity[4].

The aim of the current work is to obtain super-bright nanoantenna particles operating in a spectral region compatible with a RGB camera. To this end, DNA-functionalized nanoparticles based on the yellow-emitting octadecyl rhodamine 6G ester as a FRET donor and the red-emitting ATTO647N dye as acceptor were developed. The photophysics of the as-prepared system was studied: absorption and emission spectra, quantum yield, FRET efficiency and antenna effect were investigated. Finally, new nanoantenna systems were tested in a FRET assays for detection of nucleic acids via both spectroscopy and microscopy experiments. It was found that the ratio between the red and green channel intensities changes significantly in response to the target DNA sequence, which enables biosensing using a RGB camera, similar to those used in conventional photography.

Water, a green solvent for copper-catalyzed cyanation of aryl iodide

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Nitriles is a chemical function present in more than 30 marketed drugs and 20 are currently under clinical development. This functional group exhibits unusual geometry due to its short polarized triple bond, allowing hydrogen bonding in a specific spatial region. While being able to bind protein pocket, nitriles is also considered as a bioisoster of carbonyl, hydroxyl and carboxyl functions. This group is also an interesting precursor in organic chemistry and can be derivatized into many other functions including, carboxylic acids, alcohols or amines.

Common cyanation reactions are done using expensive palladium catalyst under harsh conditions and highly toxic cyanide sources such as KCN.

In the past years, our laboratory worked on improving the environmental impact of metal-catalyzed reactions by using abundant metals such as copper and by substituting organic solvents by water.

We succeeded to develop a new strategy for the cyanation of aryl iodide in water, using copper iodide as a catalyst and sodium nitroprusside as a non-toxic source of cyanide, affording Ar-CN with good yields (up to 81%). A crucial point was the use of D-Glucose as a biosourced reducing agent allowing to transform Cu(II) into an active Cu(I) catalyst in water at only 50°C.
Caracterisation of the interaction of coreceptor CD44v6 and MET after ligand-induced activation

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The receptor tyrosine kinase (RTK) MET and its ligand Hepatocyte Growth Factor (HGF) are key regulators of cell signaling (Fig. 1). They control cell survival, proliferation, differentiation and mobility [1], [2]. An uncontrolled upregulation of MET/HGF pathway may promote tumoral progression and metastasis [3], [4]. We have shown that HGF binding to MET causes its dimerisation by molecular biology and quantitative microscopy (FRET-FLIM and FCS) [5].

For MET/HGF pathway, the team of Prof Orian-Rousseau (KIT, Karlsruhe) has shown that the transmembrane glycoprotein CD44v6, isoform of CD44 family, acts as a co-receptor for signaling. It also promotes the formation of a ternary complex with MET and HGF (Fig. 1) [6]. The coreceptor functions are two-fold: the extracellular part of CD44v6 is involved in MET phosphorylation, while the cytoplasmic tail of CD44v6 connects to the cytoskeleton through ERM proteins (ezrin, radixin, moesin), thereby inducing MET-dependent signaling [7].

To characterize the mechanisms of interaction of CD44v6 with MET promoted by HGF, quantitative microscopy techniques are carried out in live HEK-293T cells. With FRET followed by FLIM, we showed how HGF can modify the oligomerization of MET (Fig. 2) and the formation of CD44v6/MET complexes. In parallel, analysis by different approaches of Fluorescence Fluctuation Spectroscopy (FFS) are used to determine the behavior of CD44v6 and MET in non-induced cells and in the presence of HGF. These results allow us to identify the diffusion range of MET and its coreceptor CD44v6 in the plasma membrane before and after binding with HGF. Hence, we can track a possible relocalisation of activated proteins in the membrane, allowing a better understanding of MET pathways.
Innovative HTRF binding assay to screen the apelin receptor using Tag-lite® technology

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Tag-lite® technology is an original and efficient method that combines the use of suicide enzyme as tags fused to targets of interest with Homogeneous Time Resolved Fluorescence (HTRF) as detection readout. Non-radioactive, homogeneous and very sensitive, this method has found successful applications for high-throughput screening but also the study of GPCR oligomerization.

To extend the Tag-lite® range to the apelin receptor, a series of fluorescent ligands was designed, synthetized and evaluated on the apelin receptor expressed in a transient cell line. The resulting dissociation constants determined for all fluorescent probes were found in the nanomolar range for the receptor.

The screening assay was further validated by competition experiments with reference competitors.
High Throughput Screening (HTS) is the technology which best facilitates the search of new molecules with the potential of becoming the drugs of tomorrow. Until recently this expensive technology was only available in pharmaceutical companies.

Starting in 1999, the "Plate-forme de Chimie Biologique Integrative de Strasbourg" (PCBIS) developed the expertise in this field in order to be able to offer this technology in an academic context. One of our main goals is to offer our expertise to laboratories aiming to find new drugs to cure rare and/or neglected diseases. Our commitment to quality drove us to set up a quality management system granted by ISO 9001 and NF X50-900 certifications.

The PCBIS's expertise and equipment necessary for new drug discovery is now proposed to academic laboratories, start-ups and industries interested in a fast paced approach to screening.

We also propose to train people and give an access to our technologies to interested laboratories.

We will show some of the tools that PCBIS can propose to the scientific community.
Development of innovative antitumoral platinum (II) compounds to induce immunogenic cell death

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Some cancer treatments like chemotherapeutic agents (anthracyclines, platinum derivatives,...) are able to activate the antitumor immune response by inducing a particular cell death: the immunogenic cell death (ICD). This process is characterized by the exposition of the endoplasmic reticulum chaperone calreticulin at the cell surface as well as the release of ATP and non-histone chromatin-binding protein high mobility group box 1 (HMGB1) which serve as immunostimulatory damage-associated molecular patterns (DAMPs) and increase the antitumor immune response. We focused on N-heterocyclic carbene platinum complexes associated with polyethyleneimine, a transfection agent, to create multivalent cationic platinum compounds (NHC-Pt(II)-PEI) that induce apoptosis in vitro and in vivo in xenograft immunodeficient mouse model [1].

To evaluate the potential implication of the immune response on the NHC-Pt(II)-PEI in vivo effect, immunocompetent mice bearing tumors were treated with platinum particles and the results revealed an antitumor effect of our conjugates, in the same range than the clinical used platinum drug oxaliplatin, but with less side effects. We evaluated if NHC-Pt(II)-PEI were able to induce ICD. First results showed expression of calreticulin upon NHC-Pt(II)-PEI treatment. We are then evaluating if their association with immune danger signals could enhance this effect.

Altogether our results reveal the possibility of creating Pt(II) derivatives that can be used as chemotherapeutic agents by killing tumor cells and as immunotherapeutic agents by triggering the antitumor immune response.