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Talks
Bright light-harvesting nanoantenna with hydrophobic ion pairs

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Résumé

Bright light-harvesting nanoantenna with hydrophobic ion pairs
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Light Harvesting is one of the most fundamental and common natural phenomenon and display a wide range of applications in various fields1,2 including Light Harvesting Antenna (LHA). Among the synthetic LHA systems that can be used to harvest the energy of light, fluorescent dyes assembled within nanoparticles displaying high brightness, appeared as promising candidates. However, their fluorescence quantum yield (QY) is limited by aggregation-caused quenching (ACQ) upon incorporation into LHA3,4. In this respect, a simple platform to build LHA is dye-loaded polymeric nanoparticles5. Cationic dyes, such as rhodamines and cyanines can be effectively encapsulated inside polymer NPs with minimized ACQ using bulky hydrophobic counterions (e.g., tetrakis (pentafluorophenyl) borate, F5-TPB)5–7. In the present work, to further increase the dye loading while minimizing ACQ and therefore enhancing the antenna effect, we developed an approach of "hydrophobic" ion pairs (HIP), which decreases the presence of small hydrophobic counterions inside NPs. We synthesized a number of hydrophobic cations, composed of the same bulky hydrophobic counterion (F5-TPB) paired with optically-inactive hydrophobic cation. This allowed preparation of NPs with extremely high dye loading, yielding NPs with unprecedented brightness per volume (10400±100 M-1.cm-1.nm-3). The obtained nanoparticles of ~ 70 nm in size loaded with 40000 donor dyes undergo efficient FRET to a single acceptor, giving rise to antenna effect of 4800±300, which is by far the highest value achieved to date for LHA.

References:

*Intervenant
Translation of GGC repeat expansions into a toxic polyglycine protein in NIID defines a novel class of human genetic disorders: The polyG diseases

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Résumé

Microsatellites are repeated DNA sequences of 3-6 nucleotides highly variable in length and sequence and that have important roles in genomes regulation and evolution. However, expansion of a subset of these microsatellites over a threshold size is responsible of more than 50 human genetic diseases.

Among these diseases, neuronal intranuclear inclusion disease (NIID) is a rare neurodegenerative disease caused by an expansion of CGG repeats in the 5 UTR of the NOTCH2NLC (N2C) gene. Individuals with NIID are characterized by dementia, parkinsonism and cerebellar ataxia. Furthermore, NIID patients are characterized by the presence of intranuclear inclusions of unknown origin. To identify the pathogenic mechanisms underlying NIID and how this CGG repeat expansion causes neuronal cell death, we cloned these repeats and through cell transfection, immunoprecipitation and mass spectrometry analyzes, we found that these repeats are embedded in a previously unidentified small upstream open reading frame (uORF) (uN2C), resulting in translation of the CGG expansion in a novel polyglycine-containing protein, uN2CpolyG. We developed antibody against this protein and found that the typical intranuclear inclusions in tissue samples of individuals with NIID stain positive for uN2CpolyG. Furthermore, expression of this protein in neuronal cell cultures leads to formation of the typical NIID intranuclear inclusions. Finally, expression of uN2CpolyG in mice leads to locomotor alterations, neuronal cell loss, and premature death of these animals. These results suggest that translation of expanded CGG repeats into a novel and pathogenic polyglycine-containing protein underlies the presence of intranuclear inclusions and neurodegeneration in NIID.

Importantly, these data are reminiscent of another neurodegenerative disease, fragile X associated tremor ataxia syndrome (FXTAS), caused by expanded CGG repeats that are, alike in NIID, translated into a toxic polyglycine-containing protein. Overall, these data suggest the existence of a novel class of human genetic disorders, the polyG diseases, questioning whether a similar mechanism may exist in other genetic diseases caused by expanded CGG repeats, notably oculopharyngodistal myopathy (OPDM) and oculo-pharyngeal myopathy with leukoencephalopathy (OPML)

*Intervenant
PARP3: a promising therapeutic target in glioblastoma stem-like cells.

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Poly(ADP-ribose) polymerases, of which PARP1 is the founder member, are key enzymes of the DNA repair machinery also involved in tumor progression. PARP1 inhibitors (PARPi) have thus been proposed alone or as combination therapies for the treatment of namely homologous recombination deficient cancers. Nevertheless, because of the relative lack of selectivity of PARPi currently in clinical use and the fact that cancer cells may develop resistance mechanisms against these compounds, it is necessary to explore the role of other PARP family members. In this context, we recently uncovered a major and specific contribution of PARP3 in tumor aggressiveness and plasticity in breast cancer. On the basis of these results, we are currently investigating the contribution of PARP3 in other highly aggressive cancers, including glioblastoma, the most frequent brain malignancy for which no curative treatment is available. Failure to cure glioblastoma is in part due to the high heterogeneity of this tumor type and to the presence of glioblastoma stem-like cells (GSCs). In addition to their tumor-initiation ability, GSCs are characterized by the expression of stem cell markers, long-term self-renewal potential and multipotency. These cells have also developed strategies to resist to current chemo- and radio-therapeutic protocols. These include increased DNA-repair ability and enhanced pro-survival signaling. New therapeutic strategies are thus required to target GSCs.

Our recent data reveal that disruption of the PARP3 gene impairs GSC clonal and self-renewal properties as well as the expression of several stem cell markers, including CDH5 (VE-Cadherin). CDH5+ GSCs have been reported for their ability to form blood vessels de novo through a hypoxia driven transdifferentiation process, associated to poor prognosis, known as vasculogenic mimicry (VM). We are thus currently focusing our study on the ability of PARP3 deficient GSCs to form vasculogenic tubes both in vitro and in vivo as well as on the deregulation of signaling pathways involved in this process in the absence of the PARP3 protein.
BrightSwitch®: A New Family of Dual Emissive Photoconvertible Fluorescent Probes for Bioimaging

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Photoconversion is a powerful tool in bioimaging for tracking organelles and biomolecules in cells. Although photoconvertible proteins are available, small molecular photoconvertible probes remains rare. [1-2] Herein we present BrightSwitch®: a new family of dual emissive photoconvertible fluorescent probes. The BrightSwitch® probes are based on bright fluorophores like coumarins and BODIPYs conjugated to an ASORM (Aromatic singlet oxygen reactive moiety). Upon excitation, the fluorophore generates singlet oxygen, which oxidizes the ASORM and leads to an hypsochromic shifts in absorbance and emission spectra. This new mechanism is called DPIC (Directed PhotoInduced Conversion). These probes were designed to fit with typical microscope channels and are adapted to excitation sources like 405, 488, 560 and 640 nm. Owning to their functionalization site, the BrightSwitch® can be targeted to various organelles (plasma membrane, mitochondria, lipid droplets, etc) and can be tagged to proteins (SNAP, Halo, etc) offering a universal tool for organelles and biomolecules tracking in microscopy.

Figure. Properties of BrightSwitch® dyes and their applications in fluorescence imaging

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Distinct conformational dynamics of the human Cohesin SMC1A and SMC3 ATPase heads

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Résumé

The Cohesin complex is part of the family of Structural Maintenance of Chromosomes (SMC) protein complexes that associate with chromatin and have vital roles in 3D genome organization and in the maintenance of its stability and integrity. Cohesin is notably a key factor involved in major genome regulation processes such as sister chromatid cohesion, chromosome segregation, transcription regulation, and chromatin structure organization. In humans, the core mitotic Cohesin complex is composed of the SMC1A and SMC3 proteins that form a heterodimeric ATPase by interacting through their hinge domains and, upon ATP binding, through their ATPase head domains. The RAD21 kleisin subunit binds to the SMC3 and SMC1A ATPase heads via its N- and C-termini, respectively, to form the tripartite ring structure required to capture and entrap DNA. ATP binding and hydrolysis by SMC1A and SMC3 are known to drive important structural changes in the Cohesin complex, that dynamically enable its translocation on DNA and its association with chromatin. The ATPase activity of Cohesin plays therefore a major role in the functional roles of this complex. However, the molecular basis of ATP binding and hydrolysis by SMC1A and SMC3 and their associated structural changes within Cohesin remain poorly understood. Moreover, mutations in subunits of the Cohesin complex and its regulators are involved in various types of solid and hematologic cancers and in developmental disorders called cohesinopathies. Characterization of the human Cohesin ATPase is thus essential for the fundamental understanding of Cohesin functions and should provide the structural basis needed to understand the dysfunctions related to disease. We have investigated the ATP binding properties of the Cohesin ATPase domains of human SMC1A and SMC3 by biochemical, biophysical and structural studies. We reveal specific structural changes in SMC1A and SMC3 ATPase heads upon ATP binding and its hydrolysis into ADP. Our results notably highlight the differences in ATP binding by SMC1A and SMC3 and their distinct conformational dynamics, and reveal a so far unidentified conformation of the SMC3 ATPase domain. We thus provide novel molecular insights on the ATP binding, hydrolysis and release cycle of this essential biological motor.
Vectorization of Iridium(III) Complexes using Siderophore Surrogates: a Trojan Horse Strategy against Gram-Negative Pathogenic Bacteria

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Bacterial resistance to antibiotics is a major threat towards humanity, and requires innovative solutions to fight infections caused by Gram-negative bacteria, such as Pseudomonas aeruginosa. Antibacterial photodynamic therapy can be used as treatment against infections of skin or tissues. Photosensitizers (PS) are excited with visible light, leading to the production of reactive oxygen species, toxic to cells. However, the penetration of these PS is a challenge, especially in Gram-negative bacteria due to their low membrane permeability. It is nonetheless possible to hijack the iron uptake systems in order to promote PS uptake, using a Trojan horse strategy.¹

Bacteria acquire iron, a nutrient essential for their growth, using siderophores, which are small chelating molecules that can form a complex with iron(III).² The conjugation of a PS to a siderophore analogue could therefore increase the penetration of a drug in the bacterial inner space. The PS used in this project are iridium(III) complexes, having intrinsic and photo-induced antibacterial activities.³

This project is a collaboration with Pr. Jean-Luc RENAUD and Dr. Sylvain GAILLARD’s team (LCMT, EnsCaen), as well as Centre National de Référence de la Résistance aux Antibiotiques directed by Pr. Patrick PLÉSIAT (CHU Jean-Minjoz, Besançon).

References

Protein-like Particles through Nanoprecipitation of Mixtures of Polymers of Opposite Charge

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Résumé

Protein-like Particles through Nanoprecipitation of Mixtures of Polymers of Opposite Charge
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Polymeric nanoparticles (NPs) have various applications in biomedical field because of their capacity to encapsulate compounds such as drugs1 or contrast agents2. However, in vivo conditions generate many challenges as NPs stability, specific interactions or toxicity3. Proteins, in contrast, were designed by nature for this purpose and are a great model to design nano-sized objects for biomedical usage. In this work we used nanoprecipitation of polymers of opposite charge to obtain NPs with both charges present on their surfaces, as it is the case for proteins.

Two series of copolymers of ethyl methacrylate with 1 to 25 mol% of either methacrylic acid or a trimethylammonium bearing methacrylate are synthesized. These polymers are then mixed in different ratios and nanoprecipitated. The influence of the charge fraction, mixing ratio, and precipitation conditions on NP size and surface charge is studied. Using this approach, NPs of less than 25 nm with tunable surface charge are assembled. Encapsulation of fluorescent dyes yields very bright fluorescent polymer NPs, whose interactions with cells could be studied through fluorescence microscopy.

The resulting NPs are sensitive to pH and certain NP formulations have an isoelectric point and allow repeated charge reversal. These results show the potential of the concept of combination of oppositely charged polymers in NPs through nanoprecipitation for the design of NPs with precisely tuned surface properties. In fact, a small set of simple polymers allowed to precisely adjust size, surface charge, and interactions of nanoparticles simply through adjusting the assembly parameters, while maintaining a high capacity for encapsulation. Such a kinetically controlled approach could be a valuable tool to assemble precision NPs notably for biomedical applications.

References
Does HIV-1 Gag protein adopt a compact conformation in cells?

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During HIV-1 viral cycle, Gag protein is necessary and sufficient for the assembly and the budding of new viral particles. These steps require the specific selection of two strands of viral genomic RNA by Gag among many other RNAs present in the infected cell and its interaction with the cell plasma membrane to the detriment of intracellular membranes, both still remain a subject of discussion. One of the models explaining those specificities is based on in vitro studies and suggest that Gag is able to adopt a compact conformation, allowing the simultaneous interaction of the RNAs with both the matrix (MA) and the nucleocapsid (NC) domains of Gag.

My project consists in the study of the existence of this Gag compact conformation in cells and its importance in the viral cycle of HIV-1. For this study, we used the bimolecular fluorescence complementation (BiFC) technique, more precisely the bipartite split GFP. In this system, the GFP is split in two parts. In our system, we express in HeLa cells a Gag protein in which the 11th β strand of GFP was placed between the MA and capsid (CA) domains and the 10 other GFP strands are fused at the C-terminus of the protein. Alone, those two fragments are not fluorescent and the reconstitution of a complete GFP is necessary for the observation of fluorescence. This reconstitution is only possible if Gag adopts a compact conformation, bringing the two GFP fragments close to each other. By the use of the WT Gag and different mutants of Gag, we were able to confirm the presence of a compact form of Gag in cells and the implication of the NC domain and different residues in the MA and CA domains. Currently, several analyses are performed in order to understand its implication in the viral cycle of HIV-1 by studying the capacity of Gag defective mutants to produce infectious pseudoviral particles and to interact properly with RNA.

To resume, it is the first time that this specific conformation of HIV Gag is demonstrated ex vivo and we hope that it could help the design of new drugs specifically targeting this shape completing the therapeutic arsenal.
Multi-tissue transcriptomic profiling of X-Linked Centronuclear Myopathy

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X-Linked Centronuclear Myopathy (XLCNM) is a progressive muscle disorder affecting children and represents a significant burden for the patients and families. XLCNM is characterized by general muscle weakness and abnormal nuclear positioning in muscle fibers. However, the pathomechanism of the disease is not well understood and no curative treatment is available. Transcriptomic and proteomic analyses in tibialis anterior identified numerous dysregulated genes and pathways, but only focused on a single tissue and single muscle.

In order to verify the validity of these results in other tissues to provide the disease signature and identify therapeutic targets, I investigated the transcriptome in several skeletal muscles, the liver, and the heart of a murine XLCNM model (Mtm1-/y). In tibialis anterior, gastrocnemius, and diaphragm, 82 shared dysregulated genes linked to muscle development and function, inflammation and calcium transport were identified. Complementary Gene Set Enrichment Analysis (GSEA) revealed inhibited (fatty acid metabolism, bile acid metabolism, oxidative phosphorylation) and over-activated (muscle development and contraction, inflammation) molecular pathways. This work confirmed previous results and uncovered a disease signature common to several muscles.

In contrast, fewer dysregulated genes and affected pathways were identified in the liver and the heart, and a third of the affected pathways found in the heart showed opposite dysregulation compared to skeletal muscles. This may indicate compensatory mechanisms which could explain why there is no cardiac phenotype in XLCNM patients and mice.

Overall, our data indicates that there is a tissue-specific dysregulation of various signaling pathways, and in the perspective of in vitro confirmation, these results may uncover novel pathomechanisms underlying X-Linked Centronuclear Myopathy.
Membrane contact sites between organelles are organized by protein bridges. Among the components of these contacts, the VAP family comprises ER–anchored proteins, such as MOSPD2, that function as major ER–organelle tethers. MOSPD2 distinguishes itself from the other members of the VAP family by the presence of a CRAL-TRIO domain. We showed that MOSPD2 forms ER–lipid droplet (LD) contacts, thanks to its CRAL-TRIO domain. MOSPD2 ensures the attachment of the ER to LDs through a direct protein–membrane interaction. The attachment mechanism involves an amphipathic helix that has an affinity for lipid packing defects present at the surface of LDs. Remarkably, the absence of MOSPD2 markedly disturbs the assembly of lipid droplets.

These data show that MOSPD2, in addition to being a general ER receptor for inter-organelle contacts, possesses an additional tethering activity and is specifically implicated in the biology of LDs via its CRAL-TRIO domain.
Photoactivatable Spexin derivatives to study the implication of GALR2 in non-opioid pain pathways

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Chronic pain is a major public health issue with a huge impact on society. About 30-40% adults worldwide suffer from chronic pain and its total cost has been estimated at 560-635 billion dollars per year in the United States (a). Despite the progress in understanding pain, therapeutic strategies are still based on analgesics targeting the µ opioid receptor. However, opioid analgesics are known for their various side effects, such as respiratory depression, constipation, opioid–induced hyperalgesia, and are prone to induce tolerance and addiction. There is therefore an urgent need to identify and validate novel targets for pain treatment.

Recently, the neuropeptide Spexin (SPX), discovered using bioinformatics (b), was shown to activate GALR2 G protein-coupled receptor involved in pain pathways at the central (brain and spinal cord) and peripheral levels (Dorsal Root Ganglia) (c,d). In order to study the implication of GALR2 in non-opioid pain pathways and to validate SPX/GALR2 system as a novel target for pain treatment, one would require chemical biology tools which allow for precise spatio-temporal activation of the receptors in cellulo and in vivo.

In this context, we aimed at developing first light-activable (photochromic) SPX derivatives. On one hand, reversibly photoswitchable SPX analogues (Fig 1. left) were designed and synthesized by insertion of an azobenzene photoswitch motif as amino acid side chain or directly into the peptidic backbone of SPX. On the other hand, the photocaged analogues (Fig 1. right) of SPX were designed by caging Tyr9 amino acid, known for its importance to activate the GalR2.

Bibliographic references:
(c) Kim D-K et al. Endocrinology. 2014, 155, 1864.

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Figure 1. Photoswitch (left) and Photocage (right) Spexin derivatives
Gold Nanocluster Loaded Polymer Nanoparticles for SWIR Imaging

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Résumé

Fluorescence is widely used today for biomedical imaging from the subcellular to the organism level and for biosensing, but its performance still largely depends on the used fluorescent probes. Various probes emitting in the visible region are available, however, high autofluorescence and low depth of tissue penetration limit in vivo imaging in this wavelength region. A solution to bypass these problems is shifting the window of observation to 1000 – 1700 nm, which is known as Short Wave InfraRed (SWIR, or NIR II). Light at this wavelength can penetrate deeper into tissues due to reduced scattering and absorption, and autofluorescence is negligible, making it possible to achieve high spatial and temporal imaging resolution. However, probes emitting in this region are rare and creating bright contrast agents is today a crucial challenge to enable the development of SWIR imaging. Gold Nanoclusters (AuNC) are photostable, exhibit low toxicity and can be tuned to emit in the SWIR. However, they display a limited brightness and their fluorescence emission decreases strongly in aqueous media. Here, we therefore used nanoprecipitation to encapsulate high amounts of AuNCs in polymer nanoparticles (NPs) and so create objects with a very high brightness. In this way we obtained NPs with AuNC contents up to 50 wt% and sizes from 15 to 70 nm. Protection from water by the polymer shell allowed to achieve a very high intensity of emission. At the same time, we observed that increasing the amount of AuNC encapsulated in NPs induced a bathochromic shift associated with an enhanced brightness. These NPs were tested for imaging artificial vessel and quantify their sensitivity and resolution. Analysis of the signal to noise ratio and the overestimation of vessel size showed good sensitivity and resolution up to a depth of 5 mm.
Inactivating the lipid kinase activity of PI3K-C2β is sufficient to rescue X-linked myotubular myopathy in mice

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Centronuclear myopathies (CNM) are rare congenital disorders characterized by severe muscle weakness and by structural myofibers anomalies [1]. X-linked CNM (myotubular myopathy) is the most frequent form in humans and results from mutations in MTM1, encoding the phosphatidylinositol 3-phosphate (PtdIns3P) phosphatase myotubularin [2]. No therapy is currently available. As myotubular myopathy is linked to loss of myotubularin and an increase in PtdIns3P levels [3], previous studies showed that loss of the PtdIns-3-kinase PI3K-C2β improved the phenotype of a mouse model [4][5]. However, it remains unclear if the PI3K-C2β kinase activity is necessary or sufficient for the rescue. To determine the role of the kinase activity, we crossed a kinase-dead mouse (Pik3c2bD1212A) with Mtm1 KO mice (Mtm1+/y), which faithfully recapitulate the disease, to obtain double mutant mice. The Mtm1+/yPik3c2bD1212A/D1212A offspring underwent thorough phenotyping of muscle function and structure at 5 weeks.

Mtm1+/y mice showed a reduced lifespan of 8w, muscle atrophy and weakness, and histological and ultrastructural defects of mitochondria, fiber size and sarcomere organization. In contrast, Mtm1+/yPik3c2bD1212A/D1212A survived up to the end of the study at 16w, and showed restored muscle force and motor function with a significant improvement in hanging ability and a 7.8-fold increase in absolute force. The fiber size was normalized as well as the percentage of fibers with abnormal mitochondrial accumulation, from 32.4% to 2.3%. Moreover, myofibers showed a normal ultrastructure with an increased number of recognizable triads per sarcomere. Overall, PI3K-C2β inactivation fully rescues the muscle atrophy and weakness of Mtm1 KO mice, as well as ultrastructural and histological defects. This rescue correlates with normalization of PtdIns3P level in muscle. Furthermore, the heterozygous mutation of PI3K-C2β provided only a partial rescue, highlighting a dose-response effect. Finally, we provided a mechanistic explanation by showing the overactivation of the mTOR pathway in the Mtm1 KO mice was normalized upon PI3K-C2β inactivation.

To conclude, this study identified the activity of PI3K-C2β as a modifier of MTM1-related myopathy and supports the development of specific PI3K-C2β kinase inhibitors to cure X-linked CNM.