Journées du Campus d'Illkirch 2024

Talks

Ecological benefits associated with the exploitation of exogenous siderophores as an iron source by pathogenic bacteria

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Résumé

Infectious diseases remain one of the leading causes of death worldwide. Due to high microbial adaptation capacities, our solutions for disease control and prevention are continually challenged. One of the most important bacterial adaptations in infectious environment concerns **the supply of iron**. A common way for bacteria to acquire this essential nutrient is through the secretion of **siderophores**, small organic molecules that scavenge iron and deliver it to the bacteria via specific receptors. While many bacteria use their own siderophores to acquire iron, some also have the ability **to exploit** those produced by other microorganisms.
This capacity of siderophore exploitation is a common trait among many human bacterial pathogens. However, the actual significance of this siderophore exploitation *in vivo* conditions is not yet fully understood. Especially, **little is known about the environmental conditions favoring an ecological advantage of exogenous siderophore exploitation.** Here, we evaluate if fluctuating environmental conditions close to those found in intestinal tract may influence the exploitation of exogenous siderophores by bacteria.
In our bacterial model *Salmonella enterica,* our findings indicate that **under acidic growth conditions, the growth benefit associated with the exploitation of exogenous siderophores is significantly enhanced compared to neutral pH.** This more pronounced reliance on exogenous siderophores at acidic pH is explained by a reduction in the production and uptake of the endogenous siderophores of *Salmonella*. In addition, the affinity for iron of the endogenous siderophores decreases under acidic conditions. Preliminary experiments suggests that RpoS, the central regulator of the general stress response is involved in the repression of endogenous siderophore production and uptake at acidic pH.

Mots-Clés: Iron, siderophore exploitation, ecological advantage, gut, pH, Salmonella

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Development of Mechanochemical reaction conditions for Buchwald-Hartwig amination reaction

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Résumé

Solvents are acknowledged to hold significant environmental impact, accounting up to 90% of the mass utilization in a given chemical reaction. As one of the largest users of organic solvents, the pharmaceutic industry, have made it a priority these last 20 years to make their production greener by the minimization, replacement, recycling or removal of said solvents1.
In medicinal chemistry, transition metal catalysed coupling reactions play an important role by facilitating diverse bond formations2; such as the formation of an aromatic carbon-nitrogen bond through the Buchwald-Hartwig amination reaction3. However effective, these reactions necessitate the use of organic solvents, making them costly to the environment, and making the scale-up pharmaceutical manufacturing disadvantageous.

Mechanochemistry4, is the discipline based on the use of mechanical energy generated through milling or grinding for chemical transformation. It has experienced a significant comeback thanks to its applicability to green chemistry5. Through avoiding the use of bulk solvents, solvent-free mechanochemical reactions provide safer reaction conditions for the environment and profitable conditions for industrial applications.

Although the use of various metal-catalysed reactions under mechanochemical reaction conditions have been investigated6, few publications can be found for Buchwald-Hartwig aminations 7–9. In our lab, we developed alternative conditions for the C-N bond formation using (Pd(π-allyl)Cl)2 and Buchwald monophosphine ligands.
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Mots-Clés: Buchwald, Hartwig amination, Green chemistry, Mechanochemistry, Cross, coupling reaction

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Tailoring fluorescent polymeric nanoparticles with stealth peptides: bioimaging and pH sensing applications

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Résumé

The functionalization of nanoparticles (NPs) with peptides offers promising solutions to ensure their colloidal stability and facilitate their use in both in-vitro and in-vivo applications such as targeting, sensing, and imaging.1 The fluorescent dye-loaded polymeric NPs have shown exceptional brightness, due to efficient encapsulation and minimized aggregation-caused quenching of 100-10000 dyes with bulky counterions.2,3 Their functionalization with DNA yielded nanoprobes for amplified biosensing,4 whereas peptide-grafting has not been explored yet. Herein, we have developed 20-nm poly(ethyl-methacrylate-comethacrylic acid) PEMA-MA NPs functionalized with peptides via azide-alkyne click chemistry, achieving a high coverage density of 0.72 peptides/nm2. We used polysarcosine (N-methyl glycine) peptides, which can provide NPs with stealth properties,5 but, in contrast to PEG, are biodegradable, non-toxic, and non-immunogenic. We evaluated the stability and stealth properties of these NPs in physiological media by dynamic light scattering (DLS) and non-specific interaction with serum proteins via fluorescence correlation spectroscopy (FCS). Their interaction with living U87 cells was studied by epi-fluorescence microscopy and flow cytometry, revealing their stealth behavior. These stealth NPs were further functionalized with ratiometric fluorescent pH-sensitive probes for mapping both intracellular and extracellular pH of U87 cells, as well as, ligands-specific targeting and imaging of proteins of interest within cells. This approach allows biosensing and bioimaging in a native cellular environment with minimized non-specific interactions and a high signal-to-noise ratio.
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Mots-Clés: fluorescence, polymeric nanoparticles, peptides, stealth properties, pH sensor

Photoactivatable Plasma Membrane Probe through Self-Triggered Photooxidation Cascade for Live Super-Resolution Microscopy

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Résumé

Super-resolution microscopy has revolutionized bioimaging as it enables to overcome the limits of resolution imposed by conventional microscopy and thus visualize living biological structures up to a few nanometers. Photoactivatable fluorophores are known as adapted tools for Photoactivated Localization Microscopy (PALM) as they can be stochastically activated into a fluorescent state upon light irradiation.
Here we present a new concept called Self-Triggered Photooxidation Cascade based on the photooxidation of a plasma membrane targeted Leuco-rhodamine, a non-fluorescent reduced form of a rhodamine probe. Upon visible light irradiation, a small amount of already oxidized rhodamine acts as a mild photosensitizer to generate singlet oxygen. We showed that singlet oxygen is able to oxidize a neighboring leuco-rhodamine OFF state into its fluorescent ON state. This phenomenon has been proved to be kinetically favored by a high local concentration and propagates and amplifies quickly when the probe is embedded in the membrane. In addition, the close proximity of the dyes also favors the photobleaching.
Consequently, at the single-molecule level, the concomitant photoactivation and photobleaching phenomena allow reaching a blinking system suitable for live Single Molecule Localization Microscopy and enable reconstruction of the plasma membrane at the nanoscale.

Mots-Clés: Fluorescence, plasma membrane probe, live SMLM, photooxydation

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Pro-healing effect of ancient remedies from Arab Medieval Pharmacopoeia

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Résumé

The MANSA (MANuscrits Scientifiques Anciens) is a pluridisciplinary project whose one of the research areas is the exploration of ancient pharmacopoeias in written tradition for application in **anti-infectious therapy**. For this, we are finding past remedies to prevent and treat present-day illnesses by understanding remedies' methods of action. Among them is Al Kindī's pharmacopoeia **remedy for skin abscesses**, a mix of 4 plant extracts combined with a metal, copper acetat which is investigating in particular for its effect on the **inflammatory reaction and cutaneous healing**. *In vitro* studies of the plant extracts showed **antibacterial effects** on both Gram positive and negative bacteria, with **no pro-inflammatory effect** on macrophage murine cell lines. Preliminary results suggest **anti-inflammatory cytokine production** by the macrophages in presence of the various plant extracts. As anti-inflammatory cytokines are known to induce tissue regeneration and healing, in the present work, the skin healing capacity of the plant extracts is investigating. The plants extracts, without copper, were tested alone and in combination for skin healing capacity using a well-known wound-healing assay called 'gap closure' using a culture of human skin cells (HaCaT). First of all, we determined the optimal doses to be used to observe their **healing effect with no toxicity** on cells, and showed no or **low toxicity** for three distinct ingredients. Unfortunatly, the ammonia gum (*Ferula communis L.*) seems to be toxic at high doses for HaCat cells.

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Using the non-toxic doses of the 4 plant extracts, we are now investigating the **wound healing capacity of the extracts** by testing the impact of the remedy on **cell migration and/or proliferation** through two different ways: in direct contact of skin cells, or with the intermediary of macrophages which produced cytokines and growth factors involved in skin repair.

Mots-Clés: ancient pharmacopoeia, plant extract, wound healing, skin, inflammation

Synthesis of conjugates between siderophores and phospholipids: toward the preparation of sonobactericide targeted echogenic microbubbles

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Résumé

Antibiotic resistance emerges as a major health concern and represents an urgent challenge regarding antibiotic treatment ineffectiveness (**1**). The development of resistance mechanisms is faster than release of new antimicrobials on the market. More specifically new strategies are required to fight bacterial biofilms infecting implantable medical systems (catheters, prostheses, cardiac valves) (**2**). Amongst bacteria involved in such infection *Pseudomonas aeruginosa* is one of the more challenging. Beside the antibiotherapies, innovative antibacterial strategies should emerge to regain upper hands on this critical pathogen. The use of sound (sonobactericide) is one of the most promising option and an alternative to surgery for fragile patients. Cavitation of microbubbles (MBs) under ultrasound (US) induces shear stress able to destroy both bacteria and biofilm, increasing also the penetration of antibiotic in this poorly permeable bacterium (**3**). Shear stress induced by MBs cavitation could affect both the bacteria and the host cells. Therefore, the use of MBs having affinity for bacterial structures could at one and at the same time increase the US effect on bacteria and lower the impact on human cells. Such targeted MBs were only scarcely reported in the literature. *P. aeruginosa*, like almost all bacteria, secretes siderophores to acquire iron from the host. These metabolites chelate iron(III) and the complex is recognized by specific outer membrane transporters (OMT) at the interface between the extracellular medium and the outer membrane (**4**), with an affinity in the nM range (**5**). This tight interaction could be therefore used to address MBs closed to bacteria. New functionalized siderophore analogues useable by *P. aeruginosa* were synthesized and conjugated to the phospholipids used for MBs shell. While some analogues have been synthesized in entirety, others are derived through hemi-synthesis approaches that enable functionalization, particularly for conjugation to phospholipids enhancing their potential applications. Concurrently, to mitigate fluorescence loss resulting from iron(III) chelation, we have developed of a chemical spacer designed to incorporate a fluorophore whose fluorescence remains unaffected by iron. In our investigation, we engineered an advanced chemical platform with three crucial elements: Firstly, a moiety featuring a fluorophore and an azide functional group to enable conjugation of

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phospholipids through Strain-promoted Azide-Alkyne Cycloaddition (SPAAC). Secondly, a specialized linker spacer was incorporated to facilitate subsequent coupling reactions with siderophore analogues. Additionally, this crafted synthesis offers the capability for precise coupling reactions with amines, thus opening avenues for advanced investigations in this field especially regarding molecules detection or for biological tracking. The resulting siderophospholipids were used to generate echogenic MBs and were tested on *P. aeruginosa* biofilm.

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Mots-Clés: Bacterial resistance, Biofilm, Click Chemistry, Fluorophore, Pseudomonas aeruginosa, Siderophores, Siderophospholipids, Sonobactericide

Diastereoselective synthesis of C,C-glycosyl amino acids via iron-catalyzed MHAT coupling

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Résumé

Glycoproteins made up of a chain of amino acids linked to at least one sugar, represent a class of biomolecules involved in a wide variety of physiological processes (immune response, tissue differentiation) and diseases (cancer, autoimmune diseases). The design of *C*-glycosidic analogues of these entities should allow to obtain glycopeptide mimetics of therapeutic interest, resistant to enzymatic hydrolysis *in-vivo*. However, the synthesis of *C*-glycosyl amino acids remains poorly described and represents a real synthetic challenge. Here, a versatile method for synthesizing *C,C*-glycosyl amino acid building blocks has been developed based on the MHAT (metal catalyzed hydrogen atom transfer) methodology previously developed in SYBIO's team (1). In this methodology, a tertiary pseudo-anomeric radical is formed starting from an *exo*-glycal and is able to subsequently react with an engineered dehydroalanine used as a Michael-type acceptor.

Under these conditions, the challenge was to control the stereochemistry of the two asymmetric centers formed both at the level of the pseudo-anomeric position and at the level of the amino acid unit (2).

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Mots-Clés: exoglycals, glycoproteins, hydrogen atom transfer, catalysis

*Intervenant

A deep-red fluorogenic antimicrobial peptide for rapid wash-free staining and detection of bacteria

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Résumé

Among the various technics for bacterial detection fluorescent probes have developed quickly in the past years, as they offer the advantages of rapid analysis, high sensitivity, possibility of multiplexing and non-invasive character.(1,2) Here we report the design and synthesis of new bacteria targeting peptides based on the truncated version of ubiquicindin, an antimicrobial peptide. The peptide was either combined with Alared,(3) an environmentally sensitive fluorescent amino acid derived from Nile Red or tethered to a Nile Red derivative with a minimum flexible linker. The new peptide-based probes displayed deep red emission (λmax= 665 nm) and enabled staining of Gram-positive and Gram-negative bacteria. The probes were evaluated using flow cytometry and the best probe was shown to be compatible with confocal microscopy and super resolution microscopy. The solvatochromic properties of the peptide-based probe were used for probing the local microenvironment by measuring the generalized polarization (GP) of fluorescence which characterizes the shift of fluorescence emission spectra of our probe. The GP of the probe was used to explore the local environment of the labeling site and assess perturbations in the bacteria cell envelope caused by heat inactivation and exposure to antibiotics.

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Mots-Clés: fluorescent probe, environment, sensitive probe, bacterial detection, antimicrobial peptide, flow cytometry

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Analysis of a mechanism for reducing the synthesis of deleterious proteins by co-translational degradation of their messenger RNAs

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Résumé

A mechanism of co-translational degradation of messenger RNAs (mRNAs) encoding aberrant proteins and/or polypeptides present in excess has been identified but is still poorly understood. This mechanism contributes to disease and several cancers in human. It involves the degradation of the poly(A) tails of mRNAs encoding deleterious proteins (misfolding, aggregation of nascent peptides...) by the CCR4-NOT complex, considered as the main eukaryotic deadenylase. In *S. cerevisiae*, large scale data and biochemical results from the host team suggested an interaction between Caf130, a subunit of the CCR4-NOT complex, and Btt1, a subunit of the nascent polypeptide associated complex (NAC). The latter complex is involved in the folding of nascent polypeptides at the ribosome exit tunnel. Caf130 is known to regulate the level of mRNAs encoding the ribosomal protein Rpl4, which, when produced in excess, induces toxicity. Interestingly, in humans, the CCR4-NOT complex interact with SCAPER, which co-translationally regulates the level of mRNAs encoding tubulin; a protein that results in cellular defects when present in excess. To elucidate this particular mechanism involved, TAP purification followed by mass spectrometry analysis identified Cal4, Not1, the ribosome and the chaperones Btt1 and Egd2 as partners of Caf130. The interactions between these proteins were validated and mapped by two-hybrid assays and confirmed by expression of recombinant factors in *E. coli*. These data enabled a prediction of the structure of the complex using Alphafold2 that solidified previous results. In order to understand how all these factors operate, a structural analysis using crystallography is currently underway. Functional analyses to determine the role of the various factors in the mechanism, using a functional assay based on co-translational degradation of the mRNA encoding Rpl4 when it is in excess was developed. The completion of structural and functional studies will provide a global understanding of this quality control system.

Mots-Clés: CCR4, NOT complex, NAC complex, ribosome, RNA decay, translation, yeast

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Push-pull fluorescent dyes with trifluoroacetyl acceptor for sensing of lipid droplets

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Résumé

Imaging and sensing of lipid droplets (LDs) attracted significant attention, due to growing evidences for their important role in cell life. Solvatochromic dyes are promising tools to probe LDs local polarity, but this analysis is biased by their non-negligible emission from intracellular membranes and capacity to emit from both the apolar core and polar interface of LDs. Here, we developed four push-pull solvatochromic dyes based on naphthalene and fluorene core bearing an exceptionally strong electron acceptor, trifluoroacetyl group. The latter was found to boost the optical properties of the dyes by shifting their absorption and emission to the red, increasing their extinction coefficient, photostability, and sensitivity to solvent polarity (solvatochromism). In contrast to classical solvatochromic dyes, such as parent aldehydes and reference Nile Red, the new dyes exhibited strong fluorescence quenching by mM water concentrations in organic solvents. In live cells, two selected trifluoroacetyl dyes exhibited high specificity to LDs, whereas the parent aldehydes and Nile Red showed detectable background from intracellular membranes. Experiments in model lipid membranes and nanoemulsions droplets confirmed high selectivity of new probes to LDs in contrast to classical solvatochromic dyes. Moreover, the new probes were found to be selective to LDs oil core, where they can sense lipid unsaturation and chain length. Their ratiometric imaging in cells revealed strong heterogeneity in polarity within LDs, which covered the range of polarities of unsaturated triglyceride oils, whereas Nile Red failed to properly estimate local polarity of LDs. Finally, the probes revealed that LDs core polarity can be altered by fatty acid diets, which correlated with their chain length and unsaturation.

Mots-Clés: environment sensitive probes, fluorescent detection, lipid droplets

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(E, E)-Farnesol and myristic acid-loaded lipid nanoparticles overcome colistin resistance in Acinetobacter baumanii.

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Résumé

Acinetobacter baumannii has emerged as one of the most frequently isolated bacteria from respiratory samples of patients with ventilator-acquired pneumonia (VAP) worldwide, making it among priority number 1 pathogens for critical development of new antibiotics1. Antimicrobial therapy, including, colistin (CST), remains an option for treating pneumonia caused by *A. baumannii.* Nevertheless, renal toxicity associated with CST is a problematic. Combining drugs to enhance bacterial susceptibility to CST can lower the required dosage, thus reducing toxicity risks. Naturally occurring compounds like terpene alcohols and fatty acids hold promise as antibiotic adjuvants due to their ability to penetrate and disrupt bacterial membranes, making bacteria more susceptible to CST2,3. However, a major obstacle to using these promising adjuvants directly is their high lipophilicity, which makes them poorly soluble in water and therefore difficult to administer effectively. One strategy to address this issue is to encapsulate them in water-soluble lipid nanoparticles (LNPs).
In our study, various LNPs loaded with fatty acids and terpene alcohols, with two average sizes were formulated and screened. These LNPs were then tested *in vitro* with checkerboard and time kill curves (TKC) experiment against five clinical strains of *A. baumannii*, each with varying susceptibilities to colistin. Two particularly effective combinations with CST were identified: one containing (E,E)-farnesol and another containing myristic acid. These formulations significantly enhanced CST efficacy against several *A. baumannii* clinical isolates, demonstrating *in vitro* activity at least 16 times higher than CST alone.

In order to access the effect on bacterial membrane, the two particularly effective combinations were submitted to propidium iodide uptake and scanning electron microscopy (SEM). The apparent cell lysis by SEM imaging and the uptake of propidium iodide, which occurs 45-fold and 13-fold faster in the presence of 60 mg/L Myristic acid-LNP and (E, E)-farnesol-LNP respectively, compared to colistin alone, validated the impact of these combinations on bacterial membranes. Furthermore, their low toxicity was observed in either bone marrow macrophages or *Galleria mellonella* larvae and importantly, *in vivo* evaluation of larvae

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infection models revealed significantly improved larval survival with the combinations compared to CST alone, suggesting potential for further therapeutic development.
The synergy between colistin and adjuvants along with an approach using nanoparticles, demonstrates significant potential for treating bacterial infections of *A. Baumanii*.

Mots-Clés: Multidrug resistant bacteria, lipid nanoparticles

Quantitative imaging of the molecular dynamics at the origin of actin cytoskeleton reorganization driving cell shape changes in the early *C. elegans* embryo

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Every multi-cellular living organism is an ensemble of a diversity of cells organizing and synergizing into tissues and organs. Cells differ in their morphology and molecular content, thus specializing in a variety of physiological functions. However, they all originate from a single progenitor, called zygote, and then diverge and specialize through finely regulated processes including asymmetric cell divisions and fate determination events. Defects in the regulation of these processes result in a variety of embryonic lethality. How a zygote, by dividing into multiple cells, sets the bases for the origin of the cell type multitude constituting an adult organism, is still mostly unclear, and yet, it is also a prerequisite for thoroughly understanding its pathological dysregulation.
In order to shed light on the mechanisms underlying embryonic cell division and differentiation, I focus on the regulation of actin. Actin is a protein ubiquitously found in every cell of every living organism, as it is one of the main constituents of the cellular architecture (cytoskeleton). These actin networks are essential for the generation of the mechanical forces that are required for driving cell shape changes, for example when forming the contractile furrow which progressively divides one cell into two daughters. Actin architectures also participate to shape the intracellular space into various cellular sub-compartments. As such, actin is constantly under remodeling in the scale of seconds. This process of remodeling is finely regulated by several proteins which interact with actin at various levels and are collectively called actin-binding proteins.
Due to these crucial roles, actin and its associated machinery are highly conserved across all living organisms, allowing us to use simple model systems, such as C. elgans, for its study. C. elegans has numerous advantages: easy lab maintenance, well-understood genetics for manipulation, transparency facilitating optical access to intracellular mechanisms, and rapid, stereotyped development (a 6-cell embryo forms from a unicellular zygote in about 30 minutes). Taking advantage of all these aspects, I use a library of transgenic worms where various actin regulatory proteins are labelled with fluorescent reporters, and I image, live at the microscope, the formation of 6-cells embryos from single cell zygotes, through a series of successive cell divisions. I then use a combination of image processing and analysis approaches to quantify various aspects of the process, including proteins expression levels, topological distribution, and the relative sub-cellular localization of actin and associated proteins within filopodia and lamellipodia, which are specialized structures in the cortex (surface) of C. elegans embryo. My goal is to provide an accurate description of the regulatory mechanisms under normal conditions and semi-automated analysis pipelines, thus setting the ground for the formulation and testing of new working hypotheses and of their dysregulation under pathological conditions.