Sujet thèse IRTELIS campagne 2014

Stratégies innovantes pour le radiomarquage de peptides au fluor-18 pour des applications en imagerie moléculaire

Les peptides sont une classe de molécules au potentiel pharmacologique et pharmaceutique important. Naturels ou synthétiques, ils sont souvent dotés d'une plus grande activité biologique, d'une meilleure sélectivité et d'une toxicité moindre en comparaison avec des molécules bioactives de faible poids moléculaire. Bien que principes actifs de nombreux médicaments, ils sont encore largement sous-exploités en imagerie, notamment en Tomographie par Emission de Positons (TEP). Cette technique d'imagerie moléculaire et nucléaire est d'une incontestable utilité en diagnostic médical et d'une importance décisionnelle dans la R&D de nouveaux médicaments. L'usage de peptides en TEP nécessite un radiomarquage avec un émetteur de postions et les techniques actuelles sont complexes et trop souvent défaillantes, limitant leur pleine utilisation en tant que nouveaux outils d'imagerie moléculaire et candidat-médicaments de demain. Les objectifs de ce travail de thèse seront d'élaborer des méthodes innovantes et robustes de radiomarquage de peptides au fluor-18 pour des applications en imagerie TEP, notamment dans le domaine de l'oncologie. Ce sujet interdisciplinaire, caractéristique de l'imagerie moléculaire, sera à dominante chimie organique (chimie hétérocyclique, réactions clic) et radiochimie, complété par un versant automatisation et procédé avec une possible ouverture sur la chimie microfluidique. Le travail se déroulera au sein du Laboratoire de Chimie et Radiochimie du CEA-SHFJ dont l'expertise dans le domaine est reconnue par de nombreuses publications scientifiques.

Innovative strategies for peptide radiolabeling with fluorine-18 for molecular imaging

Peptides are widely recognized as fully "druggable" entities but their potential as tools in molecular imaging remains limited due to the lack, today, of reliable radiolabeling strategies. Positron Emission Tomography (PET) is a nuclear and molecular imaging modality with important applications in clinical diagnostic as well as in the R&D process of new drugs. The objectives of this PhD proposal are to elaborate innovative and robust radiolabeling strategies of peptides with fluorine-18 to create a powerful synergy between peptides and molecular imaging that will offer new tools in molecular imaging and the tomorrow's drug-candidates. This interdisciplinary project combines organic chemistry (heterocyclic chemistry/click reactions) and radiochemistry but also automation aspects and a potential opening to microfluidics. The project will be carried out in the Chemistry and Radiochemistry Laboratory at the CEA-SHFJ, which has a strong expertise in this field as demonstrated by numerous scientific publications.

Mots clés.
Peptides, chimie organique, radiochimie, fluor-18, émetteurs de positons, automatisation.

Keywords.
Peptides, organic chemistry, radiochemistry, fluorine-18, positron emitters, automation.

Innovative strategies for peptide radiolabeling with fluorine-18 for molecular imaging

Context and objectives.

Drug development has entered a turbulence area. Increasing economic pressure combined with a decrease of pharmaceutical R&D productivity have led to consider alternatives to the classical paradigm of drug development, essentially based on enzyme-substrate recognition, which is mediated by low-molecular-weight compounds. Entities that were not initially considered as "druggable" are now seen with the eyes of molecular biology, which has made understandable many protein interactions playing a crucial physiological and pathological role. Peptides are the most symbolic example of these bioactive entities that are today fully recognized as viable options for diagnostic and therapy. Compared to small molecule-based drugs, peptides often have the advantage of a higher biological activity, stronger affinity and selectivity and last but not least minimized toxicity [1-4]. Progress in peptide synthesis, functionalization and purification led to a revival of peptides in drug development. In addition to chemistry, biotechnologies (phage display) and natural peptide-based products (marine substances, venoms…) provide an ocean of potential candidates if their pharmacological properties can be rapidly and properly evaluated.

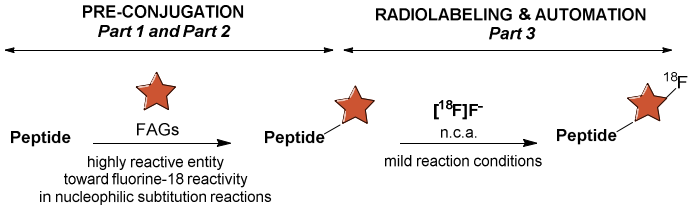
In parallel to this evolution (revolution?) in drug development, molecular imaging, and particularly PET, has changed the deal in biomedical sciences [5]. As a radioactivity-based imaging technique, PET relies on the use of a radiotracer, also called radiopharmaceutical if injected to humans, which is a chemical or biological entity labeled with a positron emitter. PET is thus a powerful non-invasive imaging technique that gives access to the pharmacodynamics and pharmacokinetics of the radiotracer as well as to some "ADMET" parameters (Adsorption, Distribution, Metabolism, Excretion and Toxicity). But PET is also a functional imaging technique with which physiological processes and parameters can be observed and measured using an adequate radiotracer [6].

The radiohalogen fluorine-18 ($T_{1/2}$: 109.8 min) belongs to the short-lived positron emitters family and is a particularly attractive radioisotope. It can be produced in large amounts using biomedical cyclotrons and its half-life is long enough to undertake multi-step syntheses followed by imaging protocols that can be extended over a few hours. Moreover, radiotracers labeled with fluorine-18 can be easily delivered outside the site of production and used in laboratories or nuclear medicine departments lacking of cyclotron/radiochemistry facilities.

The preparation of radiopeptides labeled with fluorine-18 has already been described in the literature [7] but the existing methods are often limited by their complexity regarding the half-life of fluorine-18 and automation possibilities or by their destructive character and lack of regioselectivity regarding the chemical structures of the peptides. If the anchoring of the radiolabel is not controlled, the peptide structure could be modified leading to a loss of the biological targeting. In spite of an indisputable renewed interest for peptides [3] and boom of molecular imaging, these limitations largely explain why the numbers of peptide-based radiotracers remain limited to only few examples.

The objectives of this proposal are to unlock the today existing limitations in developing innovative, universal and reliable strategies to radiolabel peptides with fluorine-18 for *in vivo* applications in molecular imaging with Positron Emission Tomography (PET) and more particularly in oncology. The research project proposes to disrupt the prosthetic paradigm, which consists in the sequential preparation of a fluorine-18-radiolabeled low-molecular-weight tag followed by its conjugation with the peptide. The disjunction of the two phases has advantages regarding the chemical structure of the peptide but is dramatically limiting due to its complexity regarding the half-life of fluorine-18 and automation possibilities. Note that a direct

introduction of the fluorine-18 atom into a non-modified peptide is totally prohibited due to the harsh reaction conditions required for such a chemical reaction. An innovative approach will be developed. A fluorine-18-accepting-group (FAG) will be pre-conjugated with the peptide followed by the reaction with fluorine-18 to obtain the desired radiopeptides. The FAGs will be tuned to let react fluorine-18 in reaction conditions that are mild enough to preserve the chemical structure of the peptide. This will provide a simplified and shorter radiochemical scheme that will be advantageous regarding the half-life of fluorine-18 and automation possibilities. It will moreover allow for a control of the position of the conjugation of the FAGs with the peptide.



Working plan.

The project will be divided into three parts:

Part 1: bibliographic analysis and organic synthesis (without radioactivity). Expected reactive heteroaromatic entities toward nucleophilic substitution (pyridines [8] but also others, displaying both an electron withdrawing group and a leaving one) will be selected, designed and synthesized. The FAGs will be then conjugated with amino-acids and peptides in a regioselective manner using click reactions or heterocycle formation. As models, unique amino-acids (Cys, Ser and Lys) and RDG-motive containing-peptides will be used. The synthesized FAGs as well as the construction with amino-acids and peptides will be fully characterized (NMR and MS).

Part 2: purification and quality control. In this second part, purification methods (HPLC) and quality control conditions will be determined to purify and characterize the future radiopeptides.

Part 3: Radiochemistry and automation. Finally, the radiofluorination reaction of the FAGs-amino-acids and FAGs-peptide conjugates will be studied in terms of temperature, base influence of the media and kinetics. The main challenge of this third part is to reach the best compromise between high yielding radiofluorination and stability of the peptide structure. The success of this approach would dramatically simplify the preparation of radiopeptides and facilitate the implementation of the corresponding processes on any type of synthesizer. Automation will be an important part of the project. In this third phase, a translation to microfluidics of part of the process will be envisaged.

The project will be carried out in the Chemistry and Radiochemistry Laboratory at the CEA-SHFJ, which has a strong expertise in this field [7-12]. The candidate will have in charge the non-radioactive synthesis of all compounds as well as the corresponding physicochemical analyses (NMR, MS) (Part 1). The candidate will have in charge the set-up of all purification and analytical HPLC methods required prior to radiolabelling (Part 2). For the third part, the candidate will participate to all radiosyntheses under the direct supervision of a team-member. Depending on the success of the third part, the opportunity to follow the preclinical characterization in a xenograft cancer model will be given to the candidate.

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