PROPOSITION DE SUJET DE THESE
Campagne 2019/2020

Cible : étudiants Chinois à des thèses à l'ENS de Lyon
Diffusion : en Chine, via la plateforme du CSC
A remplir en français ou en anglais en fonction de la langue qui sera utilisée pour la thèse

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ECOLE DOCTORALE de Chimie

TITLE OF RESEARCH SUBJECT /TITRE DU SUJET DE RECHERCHE :

Design of NIR fluorophores for two-photon fluorescence in the second biological transparency window and for the in-vivo monitoring of oxygen pressure

Research team/Equipe de recherche : Functional Materials and Photonics (team Chemistry for Optics)
http://www.ens-lyon.fr/CHIMIE/recherche/Teams/Materiaux_Fonctionnels_Et_Photonique/chemistry-for-optics

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Lab Language/ Langue de travail: English or French

Abstract/Présentation du sujet :
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Research Proposal

Design of NIR fluorophores for two-photon fluorescence in the second biological transparency window and for the in-vivo monitoring of oxygen pressure

Angiogenesis, the growth of new blood-vessels from existing ones, is fundamental in tissue development, growth, and repair [1] after e.g. trauma and surgical interventions.[2] *In vivo* imaging at the preference on a microscopic scale is urgently needed to detect and analyse new functional micro-vessels after the onset of angiogenesis. Different physiological and morphological parameters of the functional microvasculature should be measured, such as: transport and velocity of red blood cells (RBC),[3] possible formation of RBC aggregates or thrombosis,[4] abnormality in vessel endothelial cells (or vascular permeability), interactions with cells of the immune system.[5] Among all the physiological parameters, the oxygenation status and the monitoring of the level of oxygenation (pO_2) are of paramount importance.

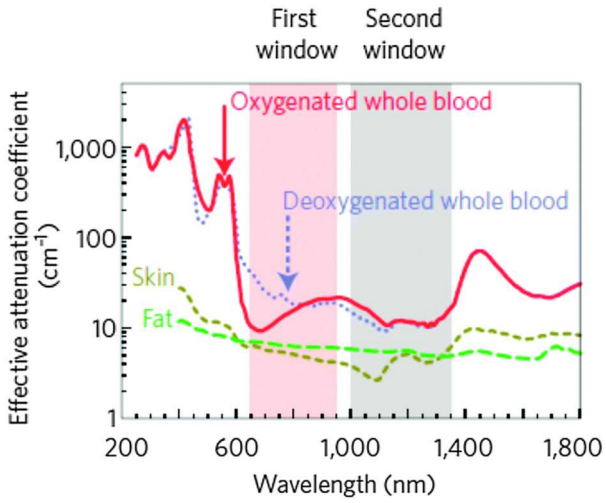

Figure 1.Absorption spectrum of human skin, blood and fat showing the first (NIR-I), second (NIR-II) and third (NIR-III) biological windows.

Two-Photon Near-infrared (NIR) fluorescence imaging using fluorescent dyes with absorption and emission in the NIR/IR region (700–1300 nm) is a very promising technique for obtaining such information *in vivo*.

The NIR region corresponds to the optical transparency windows of tissues in which light photon absorption and scattering by endogenous molecules and tissues is minimal for maximum penetration depth.[6]. If the NIR I window (650-950 nm) has been extensively exploited, it is only very recently that studies using the "second window" (NIR II, **Figure 1**) corresponding to wavelength range between 1000 and 1350 nm, have started to emerge offering huge perspectives in term of depth of penetration and minimization of diffusion. Fluorescent probes having both excitation and emission in the NIR window are therefore of high interest, probes having excitation in the second NIR window (>1000 nm) combined with emission in the first NIR window, in particular. The aim of this proposal is to further shift the absorption and the emission wavelength to the near-infrared so that NIR II / NIR I and even NIR II/NIR II imaging become possible, i.e. multiphoton excitation in the NIR II window and detection of fluorescence in the NIR I zone; or multiphoton excitation and fluorescence detection in the NIR II.

First part: new A-A heptamethine cyanine dyes

One of the research theme developed in our group is the design of functional heptamethine cyanine dyes. Such compounds owing to their large conjugated bridge, their ease of synthesis and functionalization, are particularly attractive for NIR two-photon fluorescence imaging. In that field, our group have recently highlighted the use of ketocyanine for deep in-vivo imaging.[7] and the possibility to modulate the absorption and the emission properties of heptamethine cyanine dyes by simple changes of the central atom of the bridge or of the end functional groups.[8-9] If cyanine bearing strong electron-donor groups at the extremity, such as in the compounds represented on **Figure 2** (D-D cyanine), are well known in the literature, heptamethine cyanine displaying strong electron-acceptor group instead (A-A cyanine), have been seldom described. These compounds, however, show strong red-shifted absorption and emission properties compared to the D-D fluorophores of similar length.

The first part of the PhD will therefore be dedicated to the design of new A-A heptamethine cyanine dyes, incorporating strong electron-accepting groups that have recently been developed in our group (**Figure 2**),[10] and to the study of their spectroscopic properties.

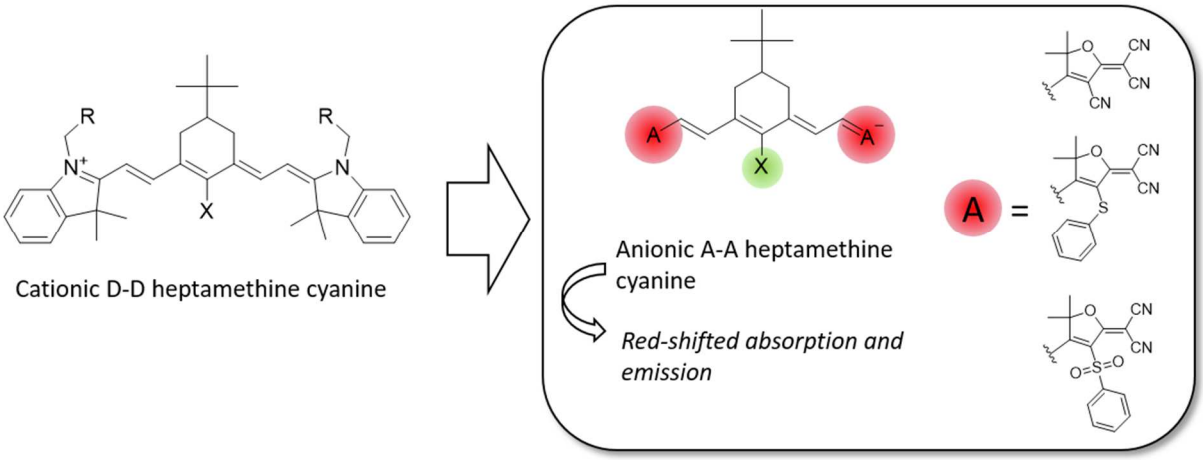

Figure 2. D-D heptamethine cyanine, targeted A-A heptamethine cyanine with the new electron-acceptor groups.

Then, work will focus on the design of water-soluble probes and also on maintaining the emission properties in aqueous media, which is indispensable for practical application in bio-imaging. We have gained considerable experience in this field during our previous researches,[7-11-12] and we are now able to encapsulate dyes in organic nanoparticles (NPs) of controlled size made of polymer shell and silica core, or cross-linked polymer shell only. In the NPs the fluorescence efficiency of the dye is preserved, giving access to highly fluorescent objects.[12] The NPs prove to be efficient blood-poll tracers for deep in-vivo two-photon fluorescence microscopy. We are now planning to change the polymer shell in order to have possibility of functionalization and *in-vivo* targeting. Biodegradable polymers are also in view. To that end, we have initiated a close collaboration with a team specialized in glycol-polymer chemistry (IMP@INSA in Lyon, France), who will help in the synthesis of these new NPs.

Second part: design of oxygen sensitive phosphorescent probes

In parallel to that work, we aim to develop new two-photon fluorophores for the measure of dissolved oxygen (pO_2) by two-photon optical fluorescence imaging. Currently very there are few techniques available to measure local parameters related to the development and evolution of tumors such as oxygen pressure (pO_2blood) in the blood and tissues ($pO_2tissue$) or the quantification of micro-vasculature. The most interesting one used palladium complexes of porphyrins. Such compounds are known to display characteristic phosphorescence in the NIR, in the biological transparency window, even at room temperature, in the absence of oxygen. This long live phosphorescence was shown to be highly sensitive to the concentration of dissolved oxygen but not upon the nature of the environment. However, the two-photon absorption cross-section associated with meso-tetraphenylporphyrins is weak (of the order of 10 to 50 GM only) and therefore an optimization of this parameter is needed for a practical application. Vinogradov and Prasad have developed an original approach consisting in grafting efficient two-photon chromophores on the central porphyrin complexes to efficiently enhance the cross-section via an antenna effect.[13-14]

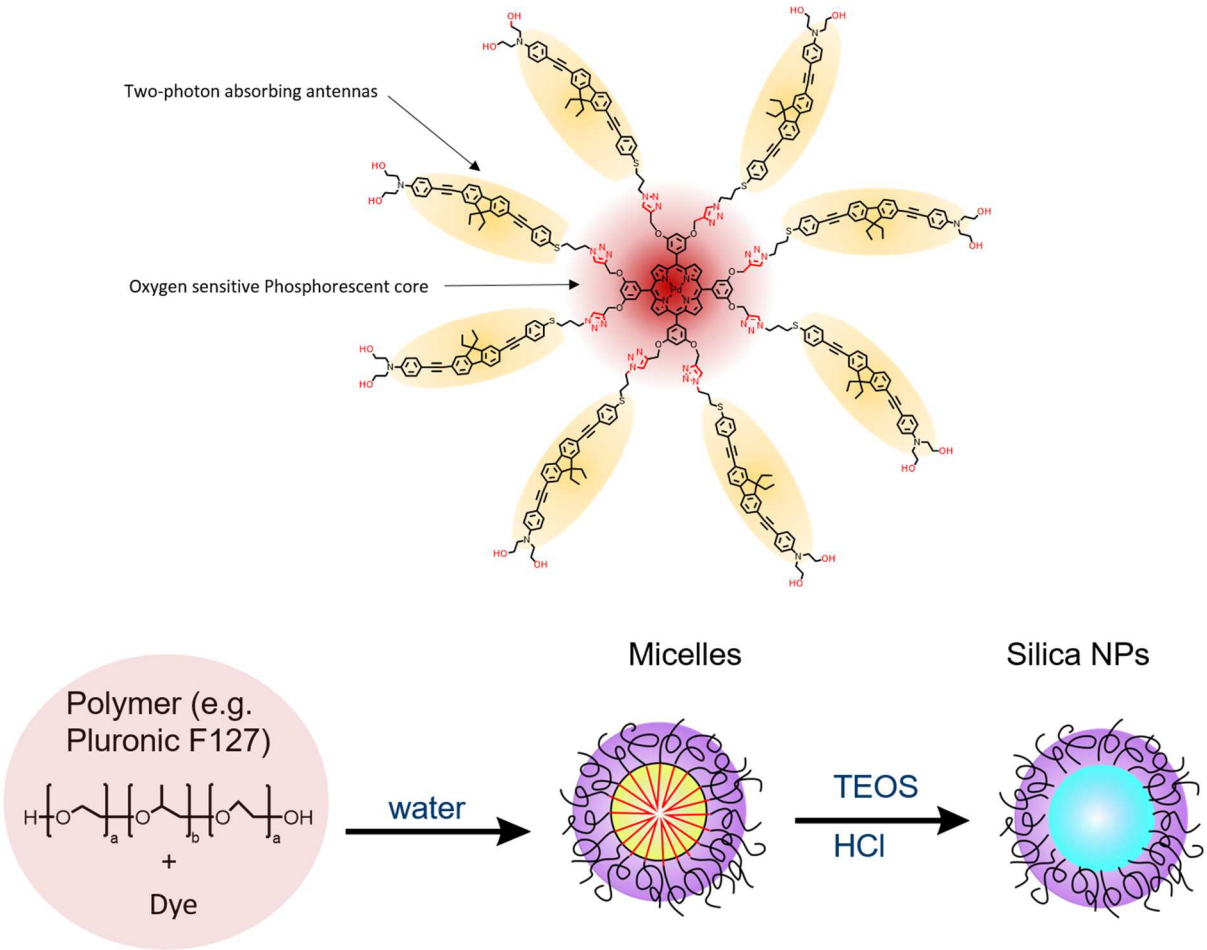

Figure 3. oxygen-sensitive two-photon phosphorescent probes based on FRET and polymer-silica NPs.

We have recently developed new two-photon antennas with which we have obtained oxygen-sensitive phosphorescent palladium-porphyrin complexes that can be efficiently excited by two-photon absorption at 800 nm (Figure).[15] We now want to expend this work to water soluble system and also to new palladium and platinum phosphorescent complexes, based on extended porphyrin or phthalocyanine ligands, whose

emission is further red-shifted, above 1000 nm. To that end, we will use the same approach developed in the first part, consisted in encapsulation in functionalized NPs.

A highly favorable research environment

This project is highly interdisciplinary and involves two partners in the field of organic chemistry, physical characterization and *in-vivo* imaging. It is a collaborating work between two laboratories (our group in Laboratoire de Chimie ENS de Lyon and the group of **B. van der Sanden** Université Grenoble Alpes) closed geographically, situated in Lyon and Grenoble (two cities 100 km apart). The complementary expertise of the laboratories involved both in synthesis and in spectroscopy should rapidly give access to efficient probes. The candidate will not only realize the synthesis of the designed probes, but also characterize all the optical properties including the two-photon absorption properties and the photo-acoustic properties. Active collaboration with the group in charge of the bio-imaging will be required to fulfill the project; a strong motivation towards interdisciplinary work is required.

This PhD project is planned for 36 months in the group of Chantal Andraud, that is hosted in a French top-ranked university (Ecole Normale Supérieure de Lyon). This group (around 20 people, 5 permanent staff) is recognized for its work in molecular engineering for applications of optics and nonlinear optics. The group merges together skills in design and synthesis of organic chromophores, advanced spectroscopy characterizations, proposing all the facilities for stationary, dynamic spectroscopic measurements in the visible up to the NIR range. The work will be co-supervised by Yann Bretonnière, one of the permanent member of the group. This PhD subject is highly interdisciplinary. Work in collaboration with the team of B. van der Sanden in Grenoble will be done for the biology/biophysic aspect with an emphasis on intravital microscopy and *in vivo* microvascular imaging. This team has a long experience in *in vivo* two-photon microscopy and more recently in photo-acoustic imaging of the normal vasculature and vascular diseases in animal models. The intravital microscopy platform is labelled by France Life Imaging and connected to small animal imaging platform for MRI, PET, SPECT and CT at the medical faculty of the Grenoble-Alps University

The broad impact of the project

Angiogenesis and inflammation processes are necessary for tissue repair damaged by injury in the acute phase before tissue replacement and remodelling. In that phase, the neovascular remodelling or angiogenesis supply the new cells and tissues with nutriments and oxygen.(16) This has further applications in organ transplantation, functional analysis of the vasculature of frozen or burn patients.(17) Today, there are no medical imaging technics available for microscopic analyses of these new micro-vessels necessary for tissue repair and remodelling. This is why this project, by providing new NIR/IR dyes designed for two-photon fluorescence and/or photo-acoustic, is an important step for deep micro-vessel imaging in vivo. NIR fluorescence imaging and in-depth photo-acoustic imaging are expected to have a major impact in biotechnology and medicine because of their high sensitivity, the fact that no radiation is necessary for

acquisition, and the versatility of the different reporter probes. This is proved by the recent development of tomographic imaging systems developed to detect NIR fluorescence in deep tissues including patients.(18) This research proposal aims at providing an issue of the current trial-and-errors determination of valuable chromophores to allow better targeting of the good candidate for *in vivo* imaging. In this context, new strategies aiming at a better imaging of these diseases will not only provide strong social and economic impacts for the diagnosis and treatment, when attaining the market, they will also allow manufactory development, production and sales of surgery tools (dedicated light sources, optical fibers, cameras, small ultrasound transducers…)

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