Fluorescent Rotors for Imaging Viscosity In Cells

Introduction and Research Results

Measuring viscosity in biological systems is of paramount importance for the understanding of biophysical processes governing both the normal cell function and the cell demise. More specifically, membrane viscosity have been linked with alterations in physiological processes, such as carrier-mediated transport, activities of membrane-bound enzymes and receptor binding, which, in turn, are associated with aging and disease pathogenesis. Three most widespread methods based on fluorescence have been developed to probe the viscosity on a microscopic scale: fluorescence recovery after photobleaching (FRAP), fluorescence anisotropy and the use of molecular rotors.

Molecular rotors refer to small synthetic fluorophores in which fluorescence emission is sensitive to the viscosity of the surrounding environment. This sensitivity, based on the non-radiative decay of the excited state, allows a precise calibration of fluorescence parameters with viscosity. Fluorescence lifetime imaging (FLIM) is an excellent technique to probe the viscosity in biological systems as it is concentration independent and a single excitation wavelength/emission interval is required.

The aim of this project is to study the sensitivity to viscosity of new BODIPY (Boron DIPYrromethene) derivatives. The design and synthesis of the latest involve two different collaborations. In the first one with Dr Rachel Meallet-Renault and Dr Gilles Clavier, from the Supramolecular and Macromolecular Photophysics and Photochemistry (PPSM) laboratory, the fluorophores were designed to be emitting in the near-infrared of the electromagnetic spectrum, presenting a better match with the tissue therapeutic window. I synthesised compounds **1** and **2** (**figure 1**) and carried out their purification at Imperial College London as well as spectroscopic experiments to explore their properties as molecular rotors. In the second collaboration with Dr Ismael Lopez-Duarte and Dr Maria A. Izquierdo-Arcusa, the BODIPY derivatives containing a double positive charge on the aliphatic chain were synthesised, which was aimed to stain specifically the plasma membrane of live cells.

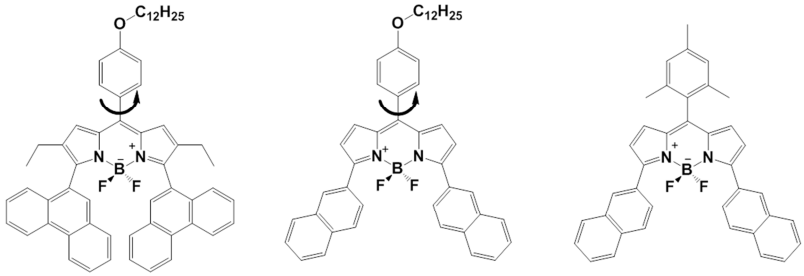

Figure 1. *Molecular structures of new red-emitting BODIPY derivatives.*

The free rotation through the C – C bond on the meso position (indicated by the arrow in **figure 1**) favours the non-radiative deactivation pathway which had been shown to be the key mechanism in the molecular rotor properties (1). However, based on the results obtained so far, we can conclude that compounds **1** and **2** don't show any molecular rotor properties but seemingly prove to be sensitive to the temperature instead, with probably a contribution of the polarity sensitivity as well. The free rotation through C – C and the substitution by conjugated groups on the BODIPY core are very likely to be responsible for the new observed properties as a control BODIPY (compound **3**) showed no change in the fluorescence quantum yield and lifetime in solutions of different viscosities and in a temperature range from 10°C to 90°C. Moreover, the key mechanism of non-radiative deactivation can be assessed to be the same as for the molecular rotor without conjugated substituents. These results will be reported in a paper which is currently in preparation and will be submitted to journals such as J Phys Chem B, PCCP or Chem. –Eur. J.

In the second collaboration, two positively charged BODIPY (compounds **4** and **5**, figure **2**) were successfully synthesised by Dr Ismaël Lopez-Duarte and Nicolas Duchemin (undergraduate student). I was in charge of supervision of Nicolas in the analytical lab and of their spectroscopic study in solution and in cells. Compounds **4** and **5** are shown to be effective molecular rotors, with a better brightness for compound **4** compared to **5** and on contrary a better sensitivity to viscosity for compound **5**. It has also been shown that both compounds selectively stain the cell plasma membrane (**figure 3**). A very first measurement of the viscosity of the cell plasma membrane has been done with compound **4** and these results have been published in the journal of Chemical Communications where I am a joint first author, along with Dr Lopez Duarte (2). A first application based on the molecular rotor properties of compound **4** have also been explored, in a collaboration with Dr Ulrike Eggert and Dr Eleonora Muro, from King's college. In staining experiment on Hela cells in the process of cell division and at different stages, compound **4** has shown that the viscosity in the plasma membrane doesn't change significantly when the cells undergo the division process. This unexpected result might be the beginning of a new understanding of this biologic process. The differences and complementarity between the properties of compounds **4** and **5** as viscosity probes will be reported in a paper which is in preparation and to be submitted to journals such as Chem Commun or Chem- Eur. J.

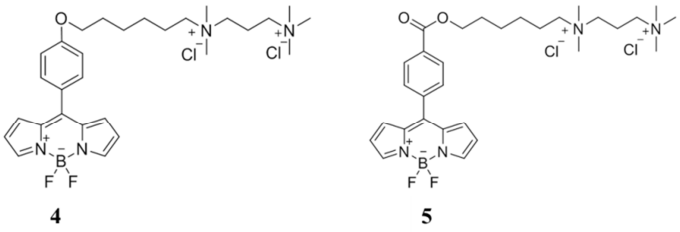

Figure 2. *Molecular structures of new charged BODIPY derivatives.*

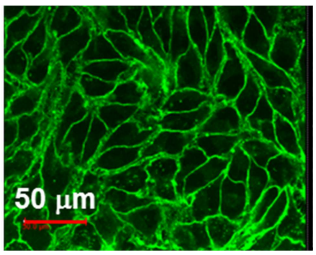

Figure 3. *Fluorescence image of Hela cells stained with compound* **4**

Personal Development

The excellent environment provided by Dr Marina K. Kuimova´s research group in a world-renowned research institution as Imperial College London has been a stimulating and enriching experience for my development and career prospects. This research project has allowed me to gain a greater appreciation of the biochemical sciences and to broaden my knowledge from physical chemistry into biochemistry as well as into computational chemistry

During the two-year period of my fellowship, I have carried out three types of experimental work:

a) Spectroscopic study in solution using different viscous solutions such as mixtures of methanol/glycerol and different types of oils (silicon oils, castor oil). I learned how to prepare the solutions in a reproducible way, how to have a constant control on their composition and how to handle them in presence of the dyes of interest in order to obtain precise and reliable results.

b) Cell culture, staining experiments and toxicity assays. I have learned how to culture different types of cells (SKOV-3, Hela cells) and how to optimize the staining conditions in order to get FLIM image with great contrast and without damaging the cells. In order to address this last issue, I have learned how to check the cytotoxicity of a dye (viability assays, tests using Sytox Green for instance). I also carried out for the first time FLIM technique on biologic systems and how to analyse the data, using new software such as Matlab (phasor approach) or LAF AS Lite and SPC to extract lifetimes from the images.

c) Computational chemistry. I have learned, under Dr Alexandra Simperler's supervision, how to perform calculations on a simple BODIPY derivative and molecular rotor, in the ground and excited states, in order to get a better understanding of the mechanism behind the molecular rotor properties.

Finally, my interpersonal communication skills have been polished working as member of a team, as well as through the guidance of students, involving continual supervision and leadership on my part. My intellectual education has been complemented attending several courses on techniques to write and to review papers and on how to plan my professional career. I also had opportunities to take part in outreach events (Imperial Festival, Science Uncovered), to attend schientific conferences and to extend my professional network.

References

(1) M. Kuimova, *Phys. Chem. Chem. Phys*., **2012**, *14*, 12671-12686
(2) T.-T. VU, *Chem. Commun.*, **2014**, *50*, 5282-5284