Bio-imaging: Imidazolone platform to multimodal fluorogenic and TEP bioprobes

I. Context and Ph-D research project untitled 'ImagIm'

1.1. Context and general objective :

Small fluorescent molecules are now well-established organic materials to track biochemical or biological phenomena [**1**]. To date, only a modest collection of dyes are available for fluorogenic bio-probes including most popular and commercially available families such as BODIPY, flurorescein, coumarins, rhodamines and cyanines (figure 1). They display excellent spectral characteristic and are highly chemically stable.

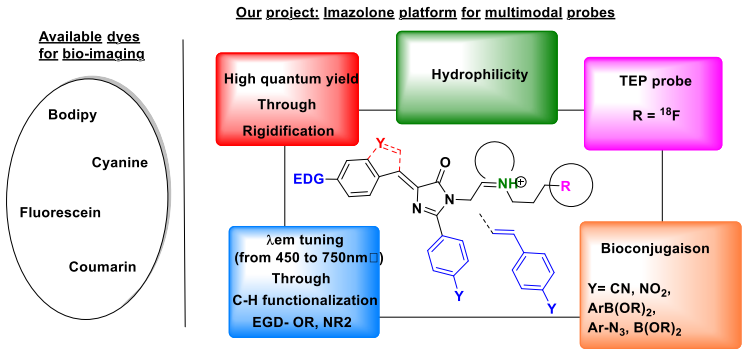

Figure 1. *Standard current fluorophores for bioimaging and our novel family*

A bottleneck of these common organic fluorophores concerns with the poor modularity in optical properties in UV/vis absorption and emission (ranging from 250 to 650nm) and poor diversity in tagging-group, mainly amine and acid moieties being available at high cost, thus limiting the amounts involved in the experiments. **In the current need to enhance the small fluorogenic 'core' for bio-imaging, the project aims at developing a first generation of highly modular small fluorescent 4-arylidene imidazolone platform that offers a great flexibility in optical properties, in group-tagging, biological probes (fluorogenic and TEP imaging) and in synthesis to achieve cost-effective production.**

1.2. State of art :

Imidazolone is a naturally-occurring fluorophore found in green fluorescent protein (GFP) [**2**] and latter in protein Kaede [**3**].This discovery awarded by a Nobel prize has paved the way for the active development of novel classes of imidazolone-based organic fluorophores. Biotechnology allows producing a broad range of GFP-type fluorescent mutant proteins including BFPs, CFPs, YFPs and DsRed that display a broad range of fluorescent shifts from blue to red (420<λ<610nm). **This first study pointed that the rare small 4-arylidene imidazolone fluorescent heterocyclic scaffold is of high interest to design original fluorogenic tag for biological and medicinal studies**. In this context, a first library of GFP and Kaede analogs bearing great variety of 'push-pull' conjugated system has been investigated demonstrating the unique flexibility of this fluorogenic scaffold to modulate fluorescent emission properties (420nm to 642nm).[**4**] Efforts have been then directed to the enhancement of quantum yields. Burgess and Yampolsky have proposed two GFP-type fluorophores embedding with a BF_2 group that displays high quantum yields, near to current commercially available dyes.[**5**] **However, their preparation remains highly fastidious and they are fraught with the difficulty of 'hula-twist' phenomena under irradiation.[6]** More recently *ortho*-hydroxylated GFP analogs have been proposed but the quantum yield is only slightly improved.[**7**] **To date, the designing of highly synthetically available 4-arylidene imidazolone-based fluorophore exhibiting a wide range of optical**

properties along with high quantum yields remains a great and competitive scientific challenge. This is the objective of the present project based upon the first designing of innovative 4-arylidene imidazolone-based fluorophores that offer a broad spectrum of modularity as depicted in figure 1 for: (1) ***Optical properties***: A full control is envisaged by modulation of the substitution at C-2 of imidazolone ring; (ii) ***Quantum yield***: It may be maximized through internal rigidification of the arylidene unit; (iii) ***Bio-conjugation***: Tagging-group will be introduced on the south aryl unit; (iv) ***Hydrophilicity***: It will be increased by quaternarization of the *N*-containing appendice; (v) ***Bimodality***: *TEP probes* may be also envisaged by introduction of a sultone group, reactive enough to allow displacement with radioactive fluoride ^{18}F.

1.3- Methods and expertises :

The current expertise of our laboratory in synthesis and direct C-H functionalization of 4-arylidene imidazolone will provide rational rules to develop the first fluorogenic multimodal imidazolone platform (figure 1) following four main phases of development depicted in figure 2: ***(1)- Building-block preparation***: We have recently developed a full innovative azide-free and convergent synthetic route towards 2-free 4-arylidene imidazolone building-block produced in multi-gram scale [**4c**]; ***(2)- Internal covalent rigidification***: The first goal of the project will target an internal linker onto the arylidene unit. Highly innovative step-economical tandem reactions using alkyne or full direct C-H bonds functionalization will be envisaged from hydroxylated-model (Y=OH) under palladium catalysis and from naked model (Y=H) (fig. 2). The expected catalysis for rigidification is depicted in figure 3; ***(3)- Designing of fluorophores***: The third part will be focused on the preparation of a library of fluorogenic platforms by using direct C-H arylation and alkenylation methodology recently developed [**4**] Moreover, novel cross-coupling partners bearing additional specific functions for bio-conjugation will be examined. As a final step of synthesis, the introduction of additional hydrophilic groups that also allow TEP experiments will be investigated.; ***(4)-Bio-imaging:*** The last part will be dedicated to the radiolabeling with fluorine-18 of the contrastophores and to their evaluation (stability, biodistribution) for in vitro and in vivo imaging investigations into the laboratory of Dr Cécile Perrio (Cycéron, Caen).[**9,10**] Application to the development of biopolymer-based probes will also be included for validation of the contrastophores from both chemical and imaging point of view.

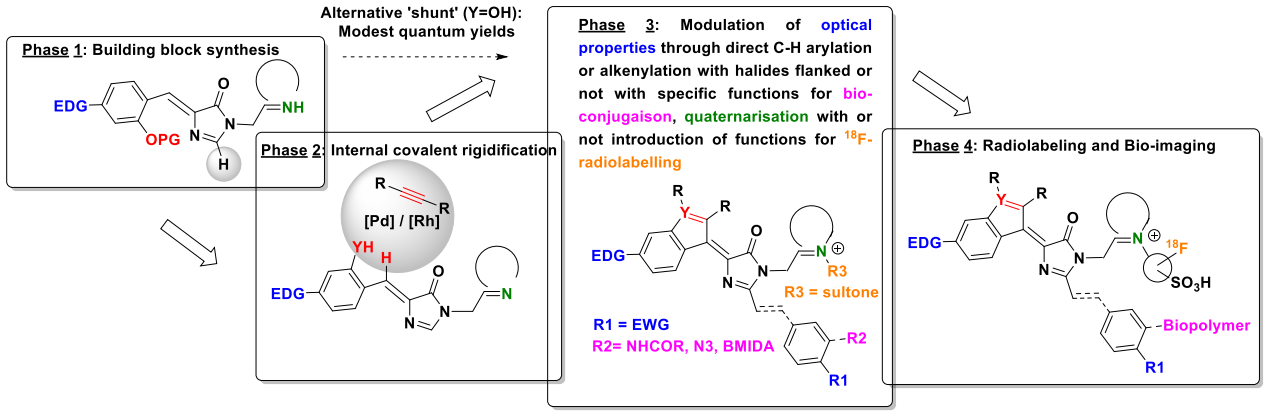

Figure 2. Expected Ph-D working plan: Designing of novel fluorescent *platforms and multi-modal bio-imaging*

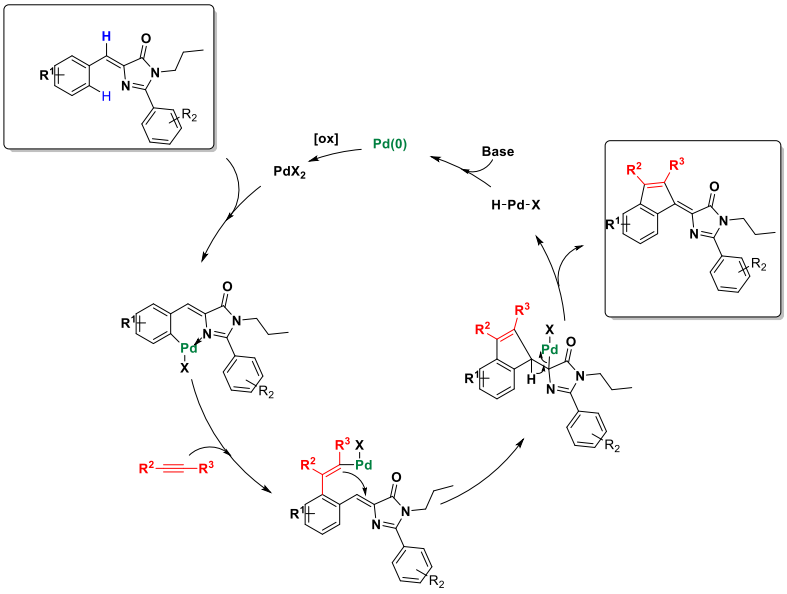

Figure 3. Expecting catalysis for rigidification of GFP-type fluorophore through double direct CH functionalization

II. Expected scientific impact of the project

The project represents an upstream work aiming to design and evaluate novel generation of one simple fluorescent heterocyclic platform displaying optimal optical and physical properties (broad range of fluorescence, high quantum yield, and hydrophilicity) effective for dual bio-imaging probes including highly attractive mono- and bi-photonic fluorescence or TEP modality. We are convinced that the envisaged synthetic strategies as well as the development of such unparalleled innovative tools for multi-modal bio-imaging probes will push the frontiers of knowledge in both active fields of research:

1) *In direct C-H functionalization of molecules*: In the current context to 'do better with less', the transition-metal catalyzed direct C-H functionalization of molecules is highly attractive. Mainly applied to standard classes of heterocycles, this field of research has now to enter in second phase of maturing to encounter a strong echo with main applicative fields of chemistry and notably the design of functional materials is strongly demanding. The present project deals with this challenge by developing unprecedented chemoselective dehydrogenative cross-coupling reactions on highly valuable GFP-like fluorescent platforms.

2) In bio-imaging fluorescent PET probes: Although the considerable advances in molecular imaging these last years, none of the modalities allows accurate identification of pathological hallmarks alone. The multimodality concept in molecular imaging became an essential approach leading to reconsider the design of imaging agents. The development of small bimodal probes remains a field of research poorly explored but highly demanding to achieve the understanding of the physiopathogical phenomena. The present project dealing to develop easily chemically accessible versatile platforms for the conception of multimodal probes joins perfectly in this objective.

As a consequence, the innovative synthetic methodologies will be delivered to scientific publication with high audience to a broad community of chemists in fields of synthetic methodologies and materials. The most important innovations in bio-imaging fluorescence probes may be patented.

III. Expertise and CV

The project involves two well-recognized scientific teams in transition-metal catalyzed direct C-H functionalization of molecules (Rouen) and in-vitro and in vivo bio-imaging experiments including fluorescence and TEP probes (Caen):

Christophe Hoarau (Prof. Dr, INSA of Rouen) develops its research in the team untitled 'Heterocycles' of Vincent Levacher (DR) inside the department untitled 'Laboratoire de Chimie Organique et Analytique' (UMR COBRA 6014, Rouen) directed by Xavier Pannecoucke that brings together 5 research teams in organic and bioorganic chemistry. He has obtained his Ph-D from Lille University under the direction of Dr Axel Couture and joined Oxford University under supervision of Dr Jeremy Robertson as a postdoctoral fellow. In 2002, he was appointed as 'Maître de conferences' at University of Rouen working as associate Professor in the laboratory of Pr Francis Marsais and Pr G. Quéguiner. He was promoted full Professor in 2012 at INSA Rouen. The group is involved in areas of heterocycles chemistry and transition metal catalysis decarboxylative and deshydrogenative (C-CO_2H and C-H bonds) cross-couplings. The group has developed innovative methodologies in several catalytic CH functionalization fields: (a) The control of selectivity by selection of catalytic metalation mode [ref. 8a,c,d and *J. Org. Chem*. **2008**, *73*, 7383]; (b) The functional diversity growing by designing of some original coupling partners in CH alkylation, benzylation, alkenylation and heteroalkenylation [**8b**,**8e** and *Chem. Eur. J.* ***2014***, *20*, 3610; *Chem. Eur. J.* **2014**, 20, 15000]; The structural diversity **by exploring original** aromatic and proaromatic heterocycles for material and pharmaceutical applications [**9**] and *J. Org. Chem.* **2015**, *80*, 5919; *Org. Lett.* ***2012***, *14*, 6012) and material ([**4**] and *Org. Biomol. Chem*. **2011**, *9*, 6215). He is coauthor of 54 articles in peer reviewed scientific journal and member of editorial board of ARKIVOC and the international scientific committee of European Colloquium of Heteocyclic Chemistry (ECHC). Since January 2018, he held the post of director of chemistry department of INSA of Rouen.

Cécile Perrio (Dr, DR at CNRS) develops its research in the group of Louisa Barré (DR CEA) untitled "Laboratoire de Développement Méthodologique en TEP" (LDM-TEP). This group is located at Cyceron, the imaging center of Caen, and is expert in radiochemistry and in development of PET radiotracers for pre-clinical and clinical PET imaging. Cécile Perrio got the PhD in 1990 at Caen in sulfoxide chemistry. She spent 2.5 years at Warwick University for a post-doctoral position in enzymatic synthesis in the group of Dr D. W. Hutchinson. She obtained the position of CR CNRS in January 1993 and joined the group of Prof M.C. Lasne in Caen. In 2004, she moved to the team of Louisa Barré and she was promoted DR in 2014. She is developing research work in the development of both new methodologies for synthesis and radiosynthesis of novel probes for PET imaging. She is involved in specific areas such as organometallic and heterocyclic chemistry, diastereoselective synthesis and radiochemistry using carbon-11 and fluorine-18. She has developed the radiochemistry of sultones. She is coauthor of 43 articles in peer reviewed scientific journals in both organic synthesis and radiochemistry for PET imaging, and she has given several oral communications in international meetings. She is working in a technological and scientific environment recognized in the field of radiopharmaceuticals for PET imaging, including biologist collaborators for both *in vitro* and *in vivo* evaluation of the tracers.

IV. Bibliographie

[**1**] For recent reviews, see: (a) Ha, Y.; Choi, H.-K.; *Chemico-Biological Interactions* **2016**, *248*, 36–51. (b) Xu, W.; Zeng, Z.; Jiang, J.-H.; Chang, Y.-T.; Yuan, L. *Angew. Chem. Int. Ed*. **2016**, *55*, 2-44. (c) Cotruvo, J. A.; Aron, A. T.; Ramos-Torres, K. M.; Chang, C. *J. Chem. Soc. Rev*. **2015**, *44*, 4400–4414. (d) Qian, X.; Xu, Z. *Chem. Soc. Rev*. **2015**, *44*, 4487–4493.(e) Teruya, K.; Tonissen, K. F.; Poulsen, S.-A. *Med. Chem. Commun*. **2016**, DOI: 10.1039/c6md00296j. (f) Lavis, L. D.; Raines, R. T.; *ACS Chem. Biol*. **2008**, *3*, 142–155.

[**2**] For a review on the GFP, see: Zimmer, M. *Chem. Rev*. **2002**, *102*, 759–781.

[**3**] (a) Ryoko, A.; Hiroshi, H.; Miki, Y.-H.; Hideaki, M.; Atsushi, M. *Proc. Natl Acad. Sci. USA*, **2002**, *99*, 12651–12656. (b) Hayashi, I.; Mizuno, H.; Tong, K. I.; Furuta, T.; Tanaka, F.; Yoshimura, M.; Miyawaki, A.; Ikura, M. *J. Mol. Biol*. **2007**, *372*, 918–926. (c) Yampolsky, I. V.; Kislukhin, A. A.; Amatov, T. T.; Shcherbo, D.; Potapov, V. K.; Lukyanov, S.; Lukyanov, K. A. *Bioorg. Chem.* **2008**, *36*, 96–104.

[**4**] (a) Muselli, M.; Baudequin, C. ; Perrio, C. ; Hoarau, C. ; Bischoff, L. *Chem. Eur. J*. **2016**, *22*,5520 –5524. (b) M. Muselli, C. Baudequin, C. Hoarau, L. Bischoff, *Chem. Commun*. **2015**, *51*, 745 –748. (c) Musseli, M.; Colombeau, L., Hedouin, J. Hoarau, C.; Bischoff, L. *Synlett* **2016**, *27*, 2819-2825. (d) Bischoff, L.; Baudequin, C.; Hoarau, C. Urriolabeitia, E. P. Adv. Organomet. Chem., vol. 69, 73; Frippiat, S.; Sarre, C.; Baudequin, C.; Hoarau, C.; Bischoff, L. *J. Org. Chem*. **2022**, *87*, 7464-7473

[**5**] (a) Wu, L.; Burgess, K. *J. Am. Chem. Soc*. **2008**, *130*, 4089-4096; (b) Baranov, M. S. ; Lukyanov, K. A. ; Borissova, A. O. ; Shamir, J. ; Kosenkov, D. ; Slipchenko, L. V. ; Tolbert, L. M.; Yampolsky, I. V. ; Solntsev, K. M. *J. Am. Chem. Soc*. **2012**, 134, 6025–6032; (c) Baranov, M. S. ; Solntsev, K. M. ; Baleeva, N. S. ; Mishin, A. S. ; Lukyanov, S. A. ; Lukyanov, K. A.; Yampolsky, I. V. *Chem. Eur. J*. **2014**, *20*, 13234–13241; (d) Baleeva, N. S. ; Myannik, K. A. ; Yampolsky, I. V.; Baranov, M. S. *Eur. J. Org. Chem*. **2015**, 5716–5721.

[**6**] (a) Chen, M. C.; Lambert, C. R.; Urgitis, J. D.; Zimmer, M. *Chem. Phys*. **2001**, *270*, 157–164. (b) Kummer, A. D.; Kompa, C.; Niwa, H.; Hirano, T.; Kojima, S.; Michel-Beyerle, M. E. *J. Phys. Chem. B* **2002**, *106*, 7554-7559. (c) Usman, A.; Mohammed, O. F.; Nibbering, E. T. J.; Dong, J.; Solntsev, K. M.; Tolbert, L. M. *J. Am. Chem. Soc*. **2005**, *127*, 11214–11215. (d) Gepshtein, R.; Huppert, D.; Agmen, N. *J. Phys. Chem. B* **2006**, *110*, 4434–4442. (e) Tolbert, L. M.; Baldridge, A.; Kowalik, J.; Solntsev, K. M. *Accounts Chem. Res*. **2012**, *45*, 171–181. (f) Rafiq, S.; Rajbongshi, B. K.; Nair, N. N. N.; Sen, P.; Ramanathan, G. *J. Phys. Chem. A* **2011**, *115*, 13733–13742.

[**7**] (a) Hsu, Y.-H.; Chen, Y.-A.; Tseng, H.-W.; Zhang, Z.; Shen, J.-Y.; Chuang, W.-T.; Lin, T.-C.; Lee, C.-S.; Hung, W.-Y.; Hong, B.-C.; Liu, S.-H.; Chou, P.-T. *J. Am. Chem. Soc*. **2014**, *136*, 11805 – 11812. (b) Baranov, M. S.; Lukyanov, K. A.; Borissova, A. O.; Shamir, J.; Kosenkov, D.; Slipchenko, L. V.; Tolbert, L. M.; Yampolsky, I. V.; Solntsev, K. M. *J. Am. Chem. Soc*. **2012**, *134*, 6025 –6032. (c) Hsieh, C.-C.; Chou, P.-T.; Shih, C.-W.; Chuang, W.-T.; Chung, M.-W.; Lee, J.; Joo, T. *J. Am. Chem. Soc*. **2011**, *133*, 2932– 2943. (d) Chuang, W.-T.; Hsieh, C.-C.; Lai, C.-H.; Lai, C.-H.; Shih, C.-W.; Chen, K.-Y.; Hung, W.-Y.; Hsu, Y.-H.; Chou, P.-T. *J. Org. Chem*. **2011**, *76*, 8189– 8202. (e) Chen, K.-Y.; Cheng, Y.-M.; Lai, C.-H.; Hsu, C.-C.; Ho, M.-L.; Lee, G.-H.; Chou, P.-T. *J. Am. Chem. Soc*. **2007**, *129*, 4534 – 4535.

[**8**] As five selected publication: (a) Martin, T. ; Verrier, C. ; Hoarau, C. ; Marsais F. *Org. Lett.* **2008**, *10*, 2909-2912 ; (b) Verrier, C. ; Hoarau, C. ; Marsais F. *Org. Biomol. Chem*. **2009**, *7*, 647-650; (c) Théveau ; C. ; Verrier ; C. ; Lassalas ; P. ; Fiol-Petit ; C. ; Hoarau, C. ; Marsais F. *Chem. Eur. J*. **2011,** *17***,** 14450-14463 ; (d) Théveau, L. ; Querolle, O. ; Dupas, G. ; Hoarau C. ***Tetrahedron*** **2013,** *69***,** 4375-4380 ; (e) Schneider, C. ; Masi, D. ; Couve-Bonnaire, S. ;Pannecoucke, X. ; Hoarau C. *Angew. Chem. Int. Ed*. **2013**, *52*, 3246-3249.

[**9**] Schmitt, S; Bouteiller, C; Barré, L; Perrio, C. *Chem Commun* **2011**, *47*, 11465-11467.

[**10**] (a) Dhilly, M; Becerril-Ortega, J; Colloc'h, N; MacKenzie, ET; Barré, L; Buisson, A; Nicole, O; Perrio, C. *ChemBioChem* **2013**, *14*, 759-769. (b) Marchand, P; Becerril-Ortega, J; Mony, L; Bouteiller, C; Paoletti, P; Nicole, O; Barré, L; Buisson, A; Perrio, C. *Bioconjugate Chem* **2012**, *23*, 21-26.