8-9 juin 2023

Pôle API - ESBS
Université de Strasbourg
Parc d'Innovation d'Illkirch

3èmes Journées du GDR ChemBio

cnrs GDR Groupement de recherche ChemBio Chémobiologie

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Journées Scientifiques du GDR ChemBio 8 et 9 Juin 2023 Strasbourg

Jeudi 8 Juin 2023

Accueil à partir de 9h00

9h30 - 9h50 : Présentation des journées (CoDir GDR ChemBio et Dominique Bonnet)

9h50 - 10h30 : Eva Jakab Toth (CBM, Orléans)
Metal complexes in MRI : from lanthanides to transition metal

10h30-11h Pause café

11h00-11h20 : DUO1 Alexia Kindler & Jessica Coulleray
Deciphering of retinoid storage and metabolic pathways using bioorthogonal chemistry-based strategies

11h20-11h40 : CO1 Emilie Lesur (Paris Saclay)
Acides nucléiques peptidiques pour le couplage protéine-protéine via des réactions bioorthogonales de ligation et de coupure

11h40-12h00 : CO2 Clémence Simon (Lille)
An organelle-specific photoactivation and dual-isotope labeling strategy reveals phosphatidylethanolamine metabolic flux

12h00-12H20 : CO3 Gaëtan Mislin (Strasbourg)
Conjugates between enterobactin analogues and antibiotics, against pathogenic bacteria: The Trojan war has begun

12h20-14h00 Pause déjeuner / Session Posters (nombre pair)

14h00-14h40 : Corentin Spriet (UGSF, PLBS, Lille) & Pierre Pauze (Faction.art)
xSublimatio : a digital drug experience as NFTs

14h40-15h00 : CO4 Patricia Busca (Paris)
Who is DLODP ? Design and synthesis of molecular tools to characterize this ophan enzyme involved Type I Congenital Disorders of Glycosylation (CDG-I)

15h00-15h20 : CO5 Arnaud Chevalier (Paris Saclay)
"CinNapht" as tunable Cinnoline/Naphthalimides fused hybrid dyes for fluorescence imaging in living cells

15h20-16h00 : *Flash talks session 1*
Flash 1 Floriane Eshak
Identification d'épitope en utilisant des approches in silico : une étude de cas nanocorps liant le récepteur mglu5
Flash 2 Luis Vigetti
Combining surface chemistry and live imaging to decipher the minimal requirements for high-speed motility of the eukaryote Toxoplasma gondii

Flash 3 Karen Plé
Development of bio-active fluorescent probes targeting the Rho-kinase ROCK : new tools for the screening of active ingredients
Flash 4 Lucille Weiss
Fluorescent Turn-ON Detection of Bacteria with Targeted Bioconjugates

16h00-16h30 Pause café

16h30-16h50 : CO6 Alain Burger (Nice)
Intermolecular Dark Resonance Energy Transfer (DRET): Applications to Fluorogenic detection of Nucleic Acids

16h50-17h50
Table ronde : L'enseignement de la chémobiologie en France
Animée par Dominique Guianvarc'h (ICMMO, Paris-Saclay)

Vendredi 9 Juin 2023

9h00-9h40 : Alain Wagner (LCAMB, Strasbourg)
Interfacing Chemistry and droplet µFluidics: Toward improved understanding of Single-Cell Biology

9h40-10h00 : CO7 Hélène Puja (Strasbourg)
Design d'analogues de la pyoverdine par ingénierie enzymatique : un vecteur pour la stratégie du Cheval de Troie

10h00-10h20 : CO8 Blaise Dumat (Paris)
Hybrid chemogenetic reporters based on small fluorogenic probes and circular permutations of HaloTag for imaging and sensing

10h20-10h50 Pause café

10h50-11h10 : CO9 Guillaume Clavé (Montpellier)
The synthesis of N-acylsulfonamide-linked nucleosides via the Sulfo-Click reaction discloses new applications in the field of nucleic acid chemistry

11h10-11h30 : *Flash talks session 2*
Flash5 Maxime Pascouau
Innovative NIR light responsive nanoparticles for enhanced antibacterial PDT and singlet oxygen mediated antibiotic release
Flash6 Carole Guimard
Design and synthesis of activity-based probes derived from schweinfurthins for target identification

11h30-12h10 : DUO2 Manon louis et Paul Monassa
Chimie click pour l'étude des cibles d'un agent infectieux induisant la dysenterie

DUO3 Francesco Calzaferri & Marie Lopez
Identification of the first SUV4-20 proteolysis targeting chimera for cancer treatment

12h10-14h00 Pause déjeuner / Session Posters (nombre impair)

14h00-14h20 : *Flash talks session 3*
Flash 7 Mélaine Balcon
Optical control of PIEZO1 channels
Flash 8 Dáire Gibbons
Molecular tools for the optimized targeting of reactive oxygen species (ROS) overproduction in the tumor microenvironment

14h20-14h40 : CO10 Yelisetty Venkata Suseela (Strasbourg)
Fluorescent amyloid-beta peptide for real time probing of reactive oxygen species generated by bound Cu

14h40-15h00 : CO11 Mayeul Collot (Strasbourg)
Photoconvertible and Photoswitchable Targeted Fluorescent Probes Based on Directed Photooxidation for Advanced Bioimaging

15h00-15h30 : *Flash talks session 4*
Flash 9 Alexandra Bristiel
Post-synthetic functionalization of an antisense oligonucleotide by Strain-Promoted Sydnone-Alkyne Cycloaddition : synthesis and application
Flash 10 Baptiste Dupouy
Synthesis of chemical tools to decipher the mode of action of antiparasitic redox-active 3-benzylmenadiones
Flash 11 Rania Benazza
Development of a SEC-MS method in denaturing conditions (dSEC-MS) for adapted and specific in-depth analysis of rebriged mAb-based formats

15h30-15h50 Conclusion

ABSTRACTS

CONFERENCES PLENIERES

Type de communication : communication orale ☒ flash ☐ duo ☐ affiche ☐

Metal complexes in MRI: from lanthanides to transition metals

Eva Jakab Toth

Centre de Biophysique Moléculaire, CNRS-UPR4301, Orléans

Correspondance : eva.jakabtoth@cnrs.fr

Metal complexes are widely used as imaging probes in various imaging modalities. One important field in molecular imaging involves the detection of physical-chemical parameters of tissues, concentration of ions, metabolites, etc. by applying smart, activatable imaging probes that are responsive to the specific parameter to detect. Magnetic Resonance Imaging is particularly well adapted to the design of responsive probes, involving Gd^{3+}-based or PARACEST (Paramagnetic Chemical Exchange Saturation Transfer) agents. The efficacy (relaxivity or CEST properties) of the probe has to be selectively influenced, based on coordination chemistry concepts, by the particular biomarker that we wish to detect. We have been developing potential smart contrast agents to detect cation or neurotransmitter concentration changes, or to monitor redox state and enzyme activity.[1] Following recent toxicity concerns related to the use of Gd^{3+} complexes in MRI, there is an active research for more biocompatible alternatives. Among these, Mn^{2+} chelates have great promise. However, the lower charge and the lack of ligand-field stabilization energy for Mn^{2+} are not favorable to achieve high thermodynamic stability, and the highly labile nature of Mn^{2+} sets an even more difficult challenge to meet. We have been exploring rigid and pre-organized ligand structures which are particularly interesting in this respect.[2] In this talk, some representative examples from these fields will be discussed.

[1] (a) C. S. Bonnet, É. Tóth, *Curr. Opin. Chem. Biol.* **2021**, *61*, 154. (b) F. Oukhatar, S. Même, W. Même, F. Szeremeta, N. K. Logothetis, G. Angelovski, and É. Tóth, *ACS Chem. Neuroscience*, **2015**, *6*, 219. (c) F. Oukhatar, H. Meudal, C. Landon, C. Platas-Iglesias, N. K. Logothetis, G. Angelovski, and É. Tóth, *Chem. Eur. J.* **2015**, *21*, 11226. (d) F. Oukhatar, S. V. Eliseeva, C. S. Bonnet, M. Placidi, N. K. Logothetis, S. Petoud, G. Angelovski and É. Tóth, *Inorg. Chem.* **2019**, *58*, 13619. (e) J. He, C. S. Bonnet, S. V. Eliseeva, S. Lacerda, T. Chauvin, P. Retailleau, F. Szeremeta, B. Badet, S. Petoud, É. Tóth and P. Durand, *J. Am. Chem. Soc.* **2016**, *138*, 2913−2916. (f) S. Laine, J.-F. Morfin, M. Galibert, V. Aucagne, C. S. Bonnet and É. Tóth, *Molecules*, **2021**, 26, 2176.
[2] (a) D. Ndiaye, M. Sy, A. Pallier, S. Même, I. de Silva, S. Lacerda, A. M. Nonat, L. J. Charbonnière and É. Tóth, *Angew. Chem. Int. Ed.* **2020**, *59*, 11958. (b) P. Cieslik, P. Comba, B. Dittmar, D. Ndiaye, É. Tóth, G. Velmurugan, H. Wadepohl, *Angew. Chem. Int. Ed.* **2022**, *61*, e202115580, (c) D. Ndiaye, P. Cieslik, H. Wadepohl, A. Pallier, S. Même, P. Comba, É. Tóth, Mn^{2+} bispidine complex combining exceptional stability, inertness and MRI efficiency, *J. Am. Chem. Soc.* **2022**, *144*, 22212.

Keywords: MRI, contrast agent, molecular imaging, lanthanides, manganese

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☐

xSublimatio : a digital drug experience as NFTs

Pierre Pauze[a], Corentin Spriet[b]

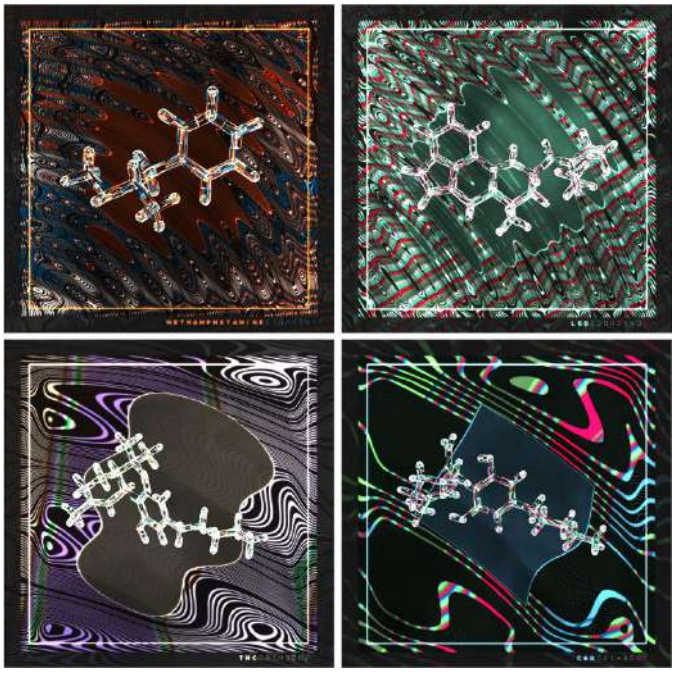
a. Faction.art - https://faction.art/project/xsublimatio

b. UAR2014 Plateformes Lilloises en Biologie et Santé (PLBS); UMR 8576 – Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), Université de Lille, Centre national de la recherche scientifique (CNRS), F-59000 Lille, France

Abtract :

xSublimatio is an interactive artistic experience that allows people to acquire digital molecules and create digital drugs on the blockchain using NFTs. xSublimatio is based on Pierre Pauze's 2019 film "Please Love Party". In this movie Pauze questions what love is from the viewpoint of chemistry and waves. After synthesizing a molecular love potion in the laboratory — in reality a powerful psychotropic drug made from chemical components and drugs found on the Darknet — Pauze transferred its essential information into water using a wave transmission device. According to the controversial scientific theory of "water memory", this process enables water to be loaded with information that alters its molecular composition in order to transmit data or to transform it into any substance. The drug thus becomes homeopathic and fictional.

In this context, xSublimatio is questioning of the aesthetic potential of web3, coding and artificial intelligence, in the era of mass digitalisation of all aspects of reality. While the consumption of images and data on the Internet have now, more than ever, become an addiction stimulating the release of feel-good hormones, this project uses modern NFT technology to stage the representations of molecules (symbolising our tangible reality) through the prism of drugs and water. Pierre Pauze's xSublimatio is an art project that allows anyone to collect, trade and combine digital molecules into virtual drugs, all in NFT form. Collectors can purchase these molecules which have been randomly selected from 64 different types generated by AI. To create their desired drug out of the 19 possible options, holders need to obtain the right combination of ingredients by trading or buying extra molecules. xSublimatio is thus an art project that allows anyone to collect, trade and combine digital molecules into virtual drugs, all in NFT form. Collectors can purchase these molecules which have been randomly selected from 64 different types generated by AI. To create their desired drug out of the 19 possible options, holders need to obtain the right combination of ingredients by trading or buying extra molecules.



Keywords: art-science, NFT, digital drugs, love.

Type de communication : communication orale ☐ flash ☐ affiche ☐

Interfacing Chemistry and droplet µFluidics: Toward improved understanding of Single-Cell Biology

Alain Wagner[a]

a. UMR 7179 CNRS-Université de Strasbourg, équipe Chimie Bio-Fonctionnelle, Faculté de Pharmacie, 67401 Illkirch Cedex

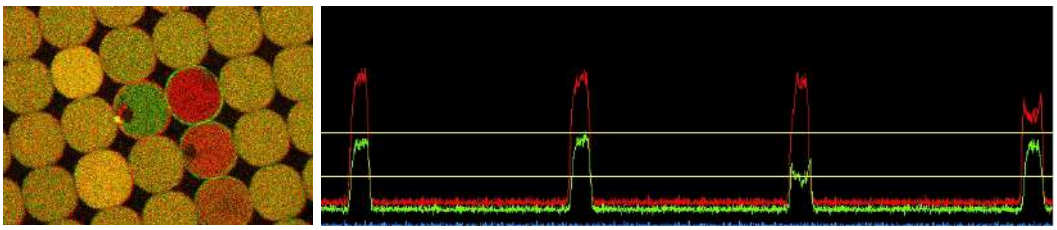
* Correspondance : alwag@unistra.fr *

The fast development of single cell technologies has made possible detailed investigations of mechanisms that could not be observed from bulk cells samples. For instance, fluorescence-activated cell sorting (FACS) allows the isolation of cell subpopulation from heterogeneous samples, on the basis of a selected panel of surface protein expression. More recent transcriptomic-based single cell enabled the simultaneous molecular analysis of hundreds or thousands of cells. It is specifically efficient for the analysis of transcriptome variations allowing the discovery of previously undetectable cell subtypes.

Today the development of advanced generation of technologies opening-up quantification of single cell at their proteome and metabolome level is highly desired. In particular, secreted molecules are main modulators of most adaptive and immunomodulatory processes. However technologies to analyze single cell secretion of large cell populations and to recover rare clones have not yet been reported.

To advance that field of research, we have used a biorthogonal chemistry approach to decorate the inner surface of microfluidic droplets with molecules that can serve as biomarker-specific capture hook. Each droplet is thus transformed into a functional pico-liter size compartment enabling to imprison all protein secreted by the inner cell and capture the proteins at the droplet surface. Those secreted and captured biomarkers are then analyzed via novel Droplet Surface Immunoassay by Relocation (D-SIRe).

Convenient screening of millions of cells as a function of secretion criteria can be performed by using this robust, highly sensitive and versatile technology. It is compatible with primary B-cell, hybridoma, HEK cells and was tailored for the discovery of antibodies against soluble proteins, peptides but most notable against native GPCR. New development focusing ofvapplication around exosome secretion and diagnostic applications are currently ongoing.



Multiplexed D-SIRE (Droplet Surface Immunoassay by Fluorescence Relocation)
HTS hybridoma screening for Antigen-specific mAb

ABSTRACTS

COMMUNICATIONS ORALES

Type de communication : communication orale ☒ flash ☒ affiche ☐

Acides nucléiques peptidiques pour le couplage protéine-protéine *via* des réactions bioorthogonales de ligation et de coupure

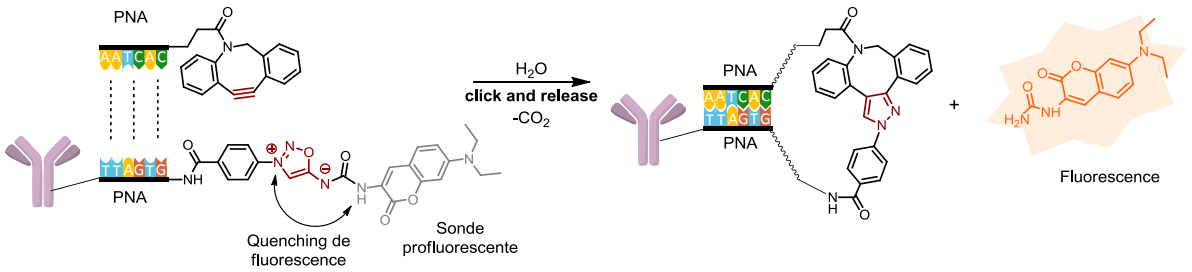
Lesur Emilie,[a] Baudet Judith,[a] Porte Karine,[a] Audisio Davide,[a] Gasser Gilles,[b] Taran Frédéric.[a*]

a. CEA-Saclay, Département Médicaments et Technologies pour la Santé, Service de Chimie Bioorganique et de Marquage
b. Chimie Paris-Tech, Université PSL, CNRS, Institute of Chemistry for Life and Health Sciences, Laboratory for inorganic Chemical Biology

* Correspondance : emilie.lesur@cea.fr

Résumé :

Ces dernières années, les réactions bioorthogonales de ligation et de coupure (click-and-release) sont devenues des réactions très importantes pour les études *in vivo*, trouvant de grandes applications dans le développement du diagnostic et de l'innovation thérapeutique.[1] La réaction entre les iminosydnones et les alcynes contraints conduit, dans des conditions physiologiques, à deux produits résultant d'une réaction de ligation formant un pyrazole et d'une coupure libérant une urée.[2] Cependant, cette réaction est relativement lente (k~10 $M^{-1}.s^{-1}$) ce qui limite son utilisation *in vivo*. Afin d'améliorer la cinétique de cette réaction de click-and-release, nous étudions la combinaison de la reconnaissance moléculaire entre des acides nucléiques peptidiques (PNA) complémentaires et de la réaction bioorthogonale de click-and-release. Plusieurs couples de PNAs, dont chaque partenaire possède un des deux motifs clickables, de différentes longueurs ont été synthétisés. La cinétique de la réaction de click-and-release entre les deux partenaires de chaque couple a ensuite été étudiée dans différents milieux et à différentes concentrations permettant ainsi d'évaluer l'influence de la taille et du milieu sur la cinétique de la réaction. Les réactions sont suivies grâce à la libération d'une sonde profluorescente. Les premiers résultats montrent que plus les PNAs sont longs plus la cinétique est rapide et plus l'effet du milieu est faible. La présence de ces PNAs permet d'augmenter de façon impressionnante la cinétique de la réaction qui devient de pseudo-ordre 1, et donc de cinétique constante quelle que soit la concentration. Grâce à ces résultats prometteurs, les meilleurs PNAs ont pu être couplés à un anticorps ou à une protéine modèle dans le but de les évaluer en tant que nouveaux espaceurs clivables bioorthogonaux. Les premiers résultats sont très encourageants et pourraient permettre de réaliser des couplages entre deux protéines ou de libérer un médicament à la place de la sonde fluorescente.



[1] Porte, K. et al. ; *ChemBioChem*, **2020**, *22*, 100-113. DOI : 10.1002/cbic.202000525.
[2] Bernard, S. et al., *Angew. Chem. Int. Ed.*, **2017**, *56*, 15612 –15616. DOI : 10.1002/anie.201708790.

Keywords: chimie bioorthogonale, click-and-release, sydnone, reconnaissance moléculaire

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☐

An organelle-specific photoactivation and dual-isotope labeling strategy reveals phosphatidylethanolamine metabolic flux

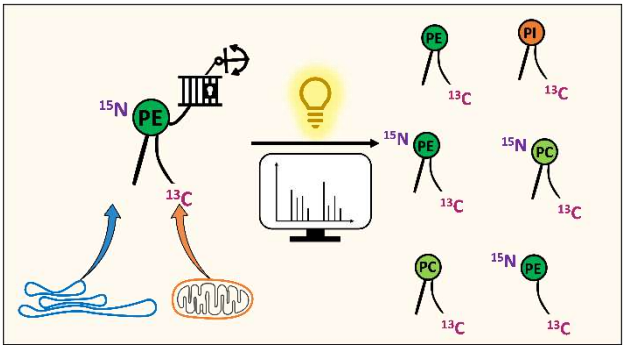
Clémence Simon[a*], Suihan Feng[b], Howard Riezman[c],

a. Unité de Glycobiologie Structurale et Fonctionnelle, UGSF-UMR 8576, University of Lille, 59000 Lille, France
b. Center for Microbes, Health and Development (CMDH), Institut Pasteur of Shanghai, China
c. NCCR Chemical Biology, University of Geneva, CH-1211 Geneva, Switzerland

* Correspondance : clemence.simon@univ-lille.fr

Abtract:

Phosphatidylethanolamine (PE) represents 25% of all phospholipids in mammals and is the second most abundant glycerophospholipids behind phosphatidylcholine. Its metabolism plays essential roles in eukaryotic cells but has not been completely investigated due to its complexity.[1] This is because lipid species, unlike proteins or nucleic acids, cannot be easily manipulated at the single molecule level or controlled with subcellular resolution, two of the key factors toward understanding their functions. Here, we use the organelle-targeting photoactivation method to study PE metabolism in living cells with a high spatiotemporal resolution. Containing predefined PE structures, probes which can be selectively introduced into the ER or mitochondria were designed to compare their metabolic products according to their subcellular localization. We combined photo-uncaging with dual stable isotopic labeling to track PE metabolism in living cells by mass spectrometry analysis. Our results reveal that both mitochondria- and ER-released PE participate in phospholipid remodeling, and that PE methylation can be detected only under particular conditions. Thus, our method provides a framework to study phospholipid metabolism at subcellular resolution.



(1) van der Veen, J. N.; Kennelly, J. P.; Wan, S.; Vance, J. E.; Vance, D. E.; Jacobs, R. L. The Critical Role of Phosphatidylcholine and Phosphatidylethanolamine Metabolism in Health and Disease. *Biochim Biophys Acta Biomembr* **2017**, *1859*, 1558–1572. https://doi.org/10.1016/j.bbamem.2017.04.006.

(2) Vance, D. E. Phospholipid Methylation in Mammals: From Biochemistry to Physiological Function. *Biochim Biophys Acta* **2014**, *1838* (6), 1477–1487. https://doi.org/10.1016/j.bbamem.2013.10.018.

(3) Simon, C.; Asaro, A.; Feng, S.; Riezman, H. An Organelle-Specific Photoactivation and Dual-Isotope Labeling Strategy Reveals Phosphatidylethanolamine Metabolic Flux. *Chem. Sci.* **2023**, *14* (7), 1687–1695. https://doi.org/10.1039/D2SC06069H.

Keywords: phosphatidylethanolamine metabolism**,** photoactivation, isotopic labeling

Type de communication : communication orale ☒ flash ☐ affiche ☐

Conjugates between enterobactin analogues and antibiotics, against pathogenic bacteria: The Trojan war has begun

Moynié Lucile,[a] Hoegy Françoise,[b,c] Milenkovic Stefan,[d] Munier Mathilde,[b,c] Paulen Aurélie,[b,c] Gasser Véronique,[b,c] Faucon Aline L., [b,c] Zill Nicolas,[b,c] Naismith James H.,[a] Ceccarelli Matteo,[d] Schalk Isabelle J.,[b,c] Mislin Gaëtan L.A. [b,c*]

a. The Rosalind Franklin Institute, Harwell Campus, Oxfordshire, UK OX11 0QS
b. CNRS, UMR7242 Biotechnologie et Signalisation Cellulaire, 300 Boulevard Sébastien Brant, F-67412 Illkirch, Strasbourg, France
c. Université de Strasbourg, Institut de Recherche de l'Ecole de Biotechnologie de Strasbourg (IREBS), 300 Boulevard Sébastien Brant, F-67412 Illkirch, Strasbourg, France.
d. Department of Physics, University of Cagliari, 09042 Monserrato, Italy.

* Correspondance : mislin@unistra.fr

Summary :

Iron is an essential nutrient for bacterial proliferation. This element is poorly available during infection therefore evolution endowed bacteria with efficient iron uptake systems. The most important uptake mechanism is based on siderophores, small secondary metabolites able to chelate Fe(III) in the extracellular medium and promote its translocation into bacterial inner space. Enterobactin is a tris-catechol siderophore used by multiple bacterial species. The enterobactin-dependent iron uptake is a potential gate in the bacterial envelope useable to shuttle antibiotics (Trojan horse strategy). Bis- and Tris-Catechol Vectors (BCV and TCV respectively), were shown using structural biology and molecular modeling, to mimic enterobactin binding to the outer membrane transporter PfeA in *P. aeruginosa.*[1] TCV appears to cross the outer membrane when linked to an antibiotic and is therefore a promising vector for Trojan horse strategies to transport antibiotics into bacteria (Figure 1).[2]

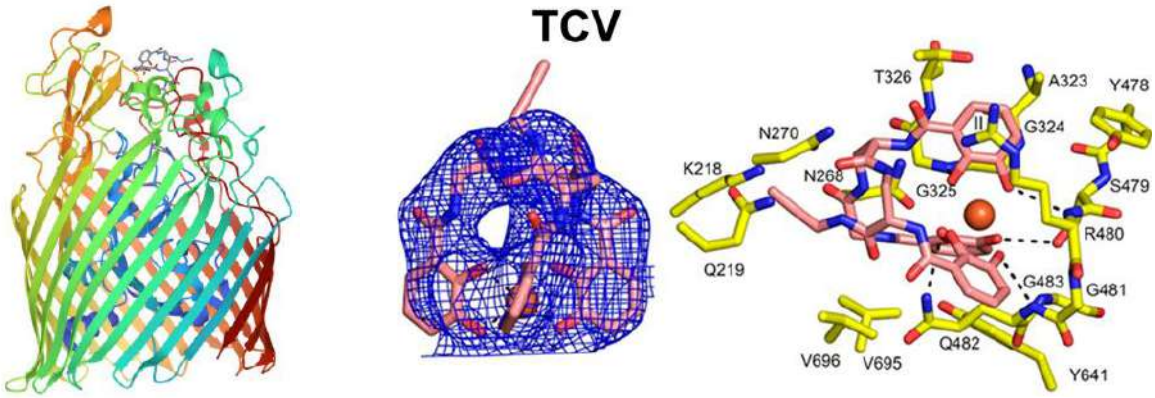


Figure 1 : Structures of enterobactin transporter PfeA (left) and of the ferric vector TCV in complex with PfeA (midle and right).

1. Moynié, L.; Milenkovic, S.; Mislin, G.L.A.; Gasser, V.; Malloci, G.; Baco, E.; McCaughan, R.P.; Page, M.G.P.; Schalk, I.J.; Ceccarelli, M.; Naismith, J.H. *Nat. Commun.* **2019**, 10, 3673. doi: 10.1038/s41467-019-11508-y.
2. Moynié, L.; Hoegy, F.; Milenkovic, S.; Munier, M.; Paulen, A. Gasser, V.; Faucon, A.L.; Zill, N.; Naismith, J.H.; Ceccarelli, M.; Schalk, I.J.; Mislin, G.L.A. ACS *Infect. Dis.* **2022**, *8*, 1894-1904. doi: 10.1021/acsinfecdis.2c00202.

Type de communication : communication orale ☒ flash ☐ affiche ☐

Who is DLODP ? Design and synthesis of molecular tools to characterize this ophan enzyme involved in Type I Congenital Disorders of Glycosylation (CDG-I)

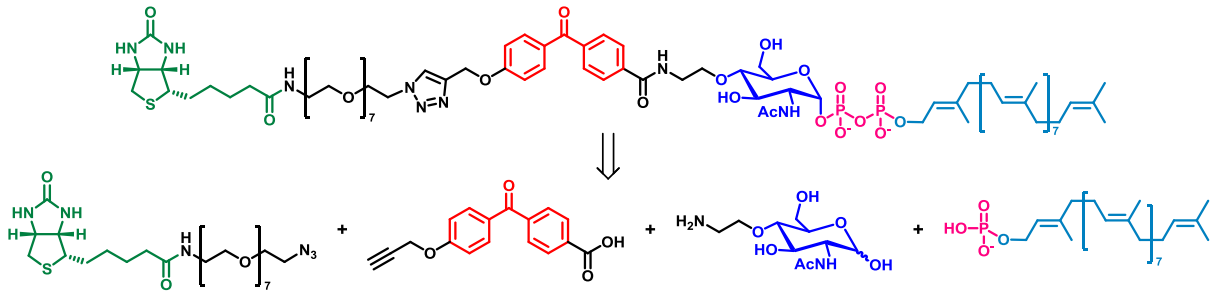
Bosco Michaël,[a] Paik Su-Jin,[b] Chantret Isabelle,[b] Moore Stuart E. H.,[b] Busca Patricia,[a*] Gravier-Pelletier Christine.[a]

a. Université Paris Cité, UMR 8601 CNRS, LCBPT, Paris, France
b. Université Paris Cité, Inserm U1149, Paris, France

* Correspondance : patricia.busca@u-paris.fr

Abstract :

N-glycosylation of proteins relies on the transfer of an oligosaccharide precursor carried by dolichol ($Glc_3Man_9GlcNAc_2$-PP-dolichol, DLO) onto nascent proteins in the lumen of the endoplasmic reticulum. This process is essential for life, and mutations in the genes necessary for the biosynthesis of DLO, or the transfer of its oligosaccharide onto the protein, provoke accumulations of truncated DLO intermediates and hypoglycosylated proteins. They are hallmarks for rare diseases called Type I Congenital Disorders of Glycosylation (CDG I) that can affect different organs.[1] DLO diphosphatase (DLODP) is an orphan enzyme that cleaves the diphosphate bridge of DLO intermediates produced under certain pathophysiological situations such as CDG-I. This enzyme could play a key role in destroying toxic truncated DLO intermediates that would otherwise cause cell pathology.[2] In order to test this hypothesis, our objective is to isolate and purify DLODP.[3] To reach this goal, complex biotinylated photoaffinity probes were designed as substrate analogs.[4]



In this communication, we will describe our recent results regarding the design, synthesis and activity of these molecular tools.

1. Haltiwanger, R.S. ; Lowe, J.B. *Annu. Rev. Biochem.* **2004**, *73*, 491.
2. a) Massarweh, A. ; Bosco, M. ; Iatmanen-Harbi, S. *et al. J. Lipid. Res.* **2016**, *57*, 1029. b) Massarweh, A. ; Bosco, M. ; Iatmanen-Harbi, S. *et al. J. Lipid. Res.* **2016**, *57*, 1477.
3. Project supported by ANR (ANR-18-CE44-0007).
4. Bosco, M. ; Massarweh, A. ; Iatmanen-Harbi, S. *et al. Eur. J. Med. Chem.* **2017**, *125*, 952.

Keywords: biotinylated probes; photoaffinity labelling; glycobiology and glycochemistry

Type de communication : communication orale ☒ flash ☐ duo ☐ affiche ☒

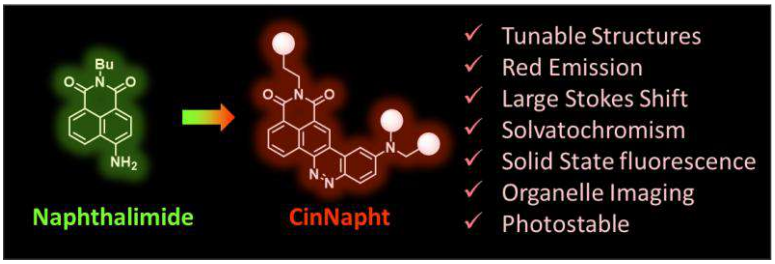
"CinNapht" as tunable Cinnoline/Naphthalimides fused hybrid dyes for fluorescence imaging in living cells

Arnaud CHEVALIER,[a] Minh-Duc HOANG,[a] Eléonore TACKE, [a] Jean-Baptiste BODIN, [d,] Farah SAVINA, [d,] Kevin TATOUEIX,[b] Benoit KEROMNES,[a] Elsa VAN ESLANDE,[a] Philippe DURAND,[a] Gregory PIETERS,[b] Gilles CLAVIER,[c] Rachel MEALLET-RENAULT,[d,]

a. Institut de Chimie des Substances Naturelles (CNRS UPR2301, 1 avenue de la Terrasse, 91198 Gif-sur-Yvette, France)
b. CEA Saclay (CEA Saclay, 91191 Gif-sur-Yvette, France)
c. Ecole Normale Supérieure Paris-Saclay (4 avenue des Sciences, 91190 Gif-sur-Yvette, France)
d. Institut des Sciences Moléculaires d'Orsay (CNRS UMR 8214, Rue André Rivière, Bt. 520, Université Paris-Saclay, 91405 Orsay)

* Correspondance : arnaud.chevalier@cnrs.fr

Abtract :



The development of new fluorescent structures that can be easily adapted is an important issue in the development of tools dedicated to fluorescence cell imaging. In this context, combining a stokes shift enhancement with a redshift of the absorption and emission wavelengths is one of the most addressed topics. The 4-amino-1,8-naphthalimide fluorophore (ANI) is a good example of tunable dye exhibiting a large stokes shift that can be used for many applications.[1] The easy functionalization of their imide nitrogen atom enables fine-tuning of the fluorophore properties such as solubility or organelle targeting.[2] However, their uses in imaging experiments are limited to the green region of the spectrum. Seeking to overcome this drawback, a six-membered diaza ring of Cinnoline has been fused on Naphthalimide dye to give a donor-acceptor system called CinNapht.[3] These red-shifted fluorophores were found to exhibit a large Stoke Shift and quantum yield up to 0.52. They are also characterized by a strong solvatochromic effect for green to red emission and can be used for living cell imaging. Moreover, the photophysical and/or organelle targeting properties of CinNapht fluorophores can be easily modulated.[4] Here we present how the modularity of the CinNapht scaffold provides access to a broad diversity of fluorophores in both photophysical behavior and in cell imaging purposes..

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Keywords: Fluorophores; Organic Chemistry; Modularity; Cell Imaging

Type de communication : communication orale ☒ flash ☐ affiche ☐

Intermolecular Dark Resonance Energy Transfer (DRET): Applications to Fluorogenic Detection of Nucleic Acids

Barnoin Guillaume,[a] Gosh Srijayee,[b] Richert Ludovic,[b] Mély Yves,[b] Benoît Michel,[a] Alain Burger, [a*]

a. Université Côte d'Azur, CNRS, Institut de Chimie de Nice, UMR 7272, Parc Valrose, 06108, Nice cedex 2, France

b. Laboratoire de Biophotonique et Pharmacologie, UMR 7213 CNRS, Université de Strasbourg, Faculté de Pharmacie, 74 Route du Rhin, 67401 Illkirch, France

* Correspondance : Alain.BURGER@univ-cotedazur.fr

Résumé :

The fact that RNAs play a central role in many biological functions (coding, decoding, regulation and expression of genes) and that they are expressed in a spatio-temporal manner underlines the importance of detecting and localizing them in time in the cell. The process of resonance energy transfer between two fluorescent molecules (FRET) has made a major contribution to meeting this challenge. However, problems related to spectral overlaps between the donor and acceptor limit its sensitivity, which generate a poor signal-to-noise ratio. To overcome this limitation, a quenched donor presenting a large Stokes shift can be combined with a bright acceptor to perform Dark Resonance Energy Transfer (DRET). To this end, we designed a push–pull probe that is strongly quenched in single stranded oligonucleotides and used it as a DRET donor with Cy5-labeled complementary sequences. Excitation of the dark donor in the double-labeled duplex switched on the far-red Cy5 emission (Figure). The consequent fluorogenic response from the acceptor considerably improves the signal-to-noise ratio.[1] The proof of concept was then performed *in cellulo* for the detection of the oskar mRNA. We further showed that DRET is amenable for super-resolution imaging.

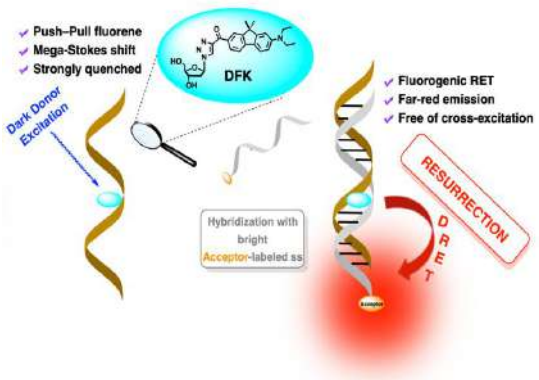

Figure : Principle of turned on emission from a dark donor to a bright acceptor

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Keywords: Fluorogenic probes; RNA imaging; DNA PAINT, super resolved images

Type de communication : communication orale ☒ flash ☐ affiche ☐

Design d'analogues de la pyoverdine par ingénierie enzymatique : un vecteur pour la stratégie du Cheval de Troie.

Puja Hélène[a], Bianchetti Laurent [b], Revol-Tissot Johan[a], Forster Anne [a], Shatalova Anastasiia [b], Stote Roland [b], Mislin Gaëtan[a], Dejaegere Annick [b], Potier Noëlle [c], Rigouin Coraline[a, *].

a. CNRS, UMR7242 Biotechnologie et Signalisation Cellulaire, ESBS - 300 Boulevard Sebastien Brant 67400 Illkirch-Graffenstaden.
b. Département de Biologie Structurale et Intégrative, IGBMC - 1 rue Laurent Fries, 67400 Illkirch-Graffenstaden.
c. CNRS, UMR 7140 Chimie de la Matière Complexe, Institut Le Bel – 4, rue Blaise Pascal 67081 Strasbourg Cedex.

* Correspondance : rigouin@unistra.fr

Résumé :

Pseudomonas aeruginosa est un pathogène opportuniste capable de développer une forte résistance aux antibiotiques et de causer des échecs thérapeutiques. Il est ainsi considéré comme une priorité critique pour le développement de nouvelles stratégies thérapeutiques. Pour répondre à cette problématique, notre équipe développe une stratégie du Cheval de Troie basée sur la pyoverdine, un sidérophore produit par *Pseudomonas,* afin de vectoriser des antibiotiques qui sont incapables de passer la membrane externe bactérienne[1,2]. Cependant, la structure de la pyoverdine de *P. aeruginosa* ne permet pas de greffer facilement un antibiotique dessus, par manque de groupement fonctionnel utilisable.

La pyoverdine est synthétisée par quatre enzymes multimodulaires appelées non-ribosomal peptide synthetases (NRPS), chaque module étant responsable de l'ajout d'un acide aminé spécifique dans la chaine peptidique. L'objectif de notre projet est d'utiliser l'ingénierie microbienne afin de modifier cette biosynthèse, et de produire un analogue fonctionnalisé qui pourra être conjugué à un antibiotique. Pour réaliser une conjugaison stéréospécifique, notre méthode consiste à incorporer dans la pyoverdine un acide aminé non naturel (nnAA) qui contient un groupement azoture (N_3), à la place de l'acide aminé naturel normalement incorporé lors de la synthèse, la thréonine. Ce groupement N_3 pourra alors réagir par chimie-click avec un antibiotique sur lequel a été ajouté un groupement dibenzocyclooctine (DBCO).

Grâce à la construction d'un modèle 3D du domaine A de l'enzyme NRPS responsable de l'ajout d'une thréonine, nous avons prédit les résidus clés permettant la sélection du substrat et les avons mutés par mutagenèse dirigée. Nous avons cultivé les mutants construits avec ou sans nnAA, et analysé les pyoverdines produites par spectrométrie de masse. L'un des mutants obtenus était capable d'incorporer un nnAA et a permis la détection d'une pyoverdine-N_3. Nous avons pu procéder à une réaction de chimie click entre la pyoverdine-N_3 et une biotine-DBCO, montrant ainsi que notre méthode permet la production d'un analogue clickable de la pyoverdine, que nous pourrons utiliser pour de nombreuses applications futures.

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Keywords: Trojan Horse, Pyoverdine, Pseudomonas aeruginosa, NRPS, engineering, Sideromycine

Type de communication : communication orale ☒ flash ☐ affiche ☐

Hybrid chemogenetic reporters based on small fluorogenic probes and circular permutations of HaloTag for imaging and sensing

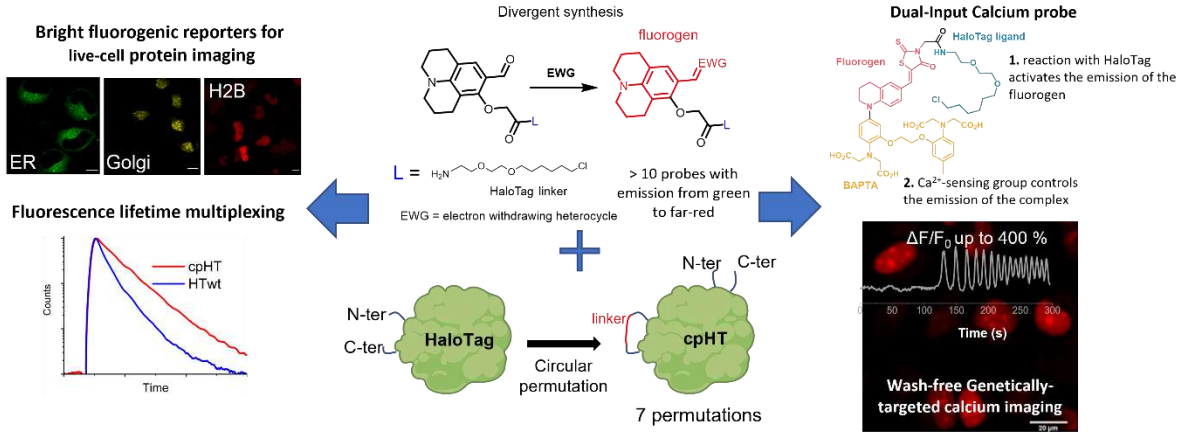
Sylvestre Bachollet,[a] Justine Coïs,[a] Kristina Mironova,[a] Vincent Vialou,[b] Nicolas Pietrancosta,[a,b] Jean-Maurice Mallet,[a] Arnaud Gautier,[a] Blaise Dumat*[a]

a. Laboratory of Biomolecules, UMR7203, CNRS/ENS/SU, Ecole Normale Supérieure, Chemistry Deprtment, Paris, France
b. Neurosciences Paris Seine, UMR8246, CNRS/INSERM/SU, Sorbonne Université, Paris, France.

* Correspondance : blaise.dumat@ens.psl.eu

Résumé :

Hybrid chemogenetic reporters that associate a self-labeling protein tag (SLP-tag) with a molecular probe bearing the cognate ligand have emerged as promising imaging tools benefiting from the targeting selectivity of recombinant proteins and from the versatility and diversity of small organic fluorophores.[1] We have previously developed small viscosity-sensitive fluorogenic probes (fluorogens) targeting the self-labeling protein tag HaloTag that ensures fast and specific labeling of genetically-encoded fusion protein in live cells.[2,3]



By combining the synthesis of new fluorogens and the production of circular permutations of the HaloTag (cpHT) proteins, we have obtained brighter reporters. Depending on the permutation, the probes display different photophysical properties opening new imaging possibilities such as lifetime multiplexing.

Finally we have also modified our fluorogens to incorporate sensing groups for calcium or pH in order to develop dual-input hybrid reporters for genetically-targeted imaging.[4]

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Keywords: Fluorogenic probes; HaloTag; Calcium imaging ; Fluorescence imaging

Type de communication : communication orale ☒ flash ☐ affiche ☐

The synthesis of *N*-acylsulfonamide-linked nucleosides *via* the Sulfo-Click reaction discloses new applications in the field of nucleic acid chemistry

Guillaume Clavé,* Romain Amador, Enes Dursun, Jean-Jacques Vasseur and Michael Smietana

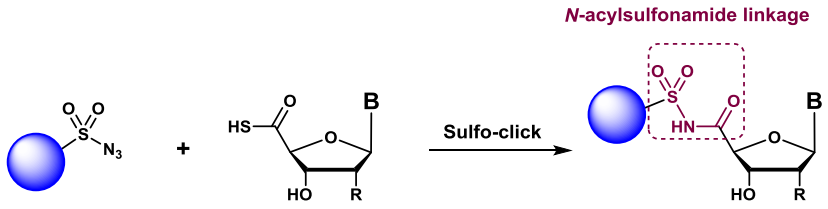
IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

* Correspondance : guillaume.clave@cnrs.fr

Résumé :

Click reactions are fast, chemoselective, and high-yielding covalent reactions between two reactive functions without generating armless byproducts.[1] Such ideal reactions have found a wide range of applications[2] in particular in the context of bioorthogonal chemistry.[3] Although the number of click reactions available has considerably grown over the past twenty years, the toolbox continues to rise and improvements are still required to achieve compatible reactions in a biological environment.[4]

The sulfo-click reaction is an emergent surrogate click reaction involving a thioacid that reacts specifically with a sulfonyl azide leading to the formation of a *N*-acylsulfonamide linkage. The sulfo-click reaction fulfills the criteria of click reactions, generates only sulfur and dinitrogen as byproducts and is compatible with aqueous conditions. These characteristics are of particular interest in the field of nucleic acid chemistry. We recently developed the synthesis of original 4'-thioacid nucleoside analogues that opened the way to new interesting applications of the sulfo-click reaction.



We will present our endeavor taking advantage of the biorthogonality of the sulfo-click reaction for bioconjugation.[5] Indeed, a variety of sulfonyl azide derivatives were successfully conjugated to 4'-thioacid nucleosides under aqueous biocompatible conditions. Then, the interesting properties of the *N*-acylsulfonamide linkage in the field of medicinal chemistry[6] were exploited to synthesize new cyclic dinucleotides for potential therapeutic applications by activating the native immune response.

Keywords: sulfo-click reaction; *N*-acylsulfonamide; bioconjugation.

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Type de communication : communication orale ☒ flash ☐ duo ☐ affiche ☐

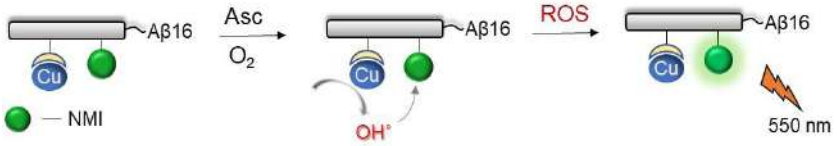
Fluorescent amyloid-beta peptide for real time probing of reactive oxygen species generated by bound Cu

Yelisetty Venkata Suseela,[1] Sabyasachi Mandal,[2] Denis Jacquemin,[3] Gilles Ulrich,[4] Thimmiah Govindaraju*[2] and Peter Faller*[1]

[1]Institute de Chimie (UMR 7177), University of Strasbourg – CNRS, 4 Rue Blaise Pascal, 67081 Strasbourg, France.[2] Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru 560064. [3]Laboratoire CEISAM, UMR CNRS 6230, Université de Nantes, 2 Rue de la Houssinière, BP 92208, 44322 Cedex 3 Nantes, France. [4]UMR7515: Institut de Chimie et Procedes pour l'Energie l'Environnement et la Sante, ICPEES, 25, rue de Becquerel, 67087 Strasbourg, France.

* Correspondance : *yelisetty@unistra.fr*

Abstract: In Alzheimer's disease Cu is found accumulated in amyloid plaques mostly bound to the main constituent of the peptide Amyloid-β (Aβ). In vitro, Cu bound to Aβ catalyzes the production of ROS (superoxide, H_2O_2, and $HO^{\bullet}$), which has been proposed to contribute to the oxidative stress and neuronal cell loss in Alzheimer's disease. However, the direct generation of ROS mediated by Cu and its contribution to the Aβ(Cu) toxicity *in vivo* has not been directly demonstrated. Among ROS, $HO^{\bullet}$ radicals are very short-lived, as they react fast with a lot of biomolecules and leads to their destruction. Here, we aim to establish a fluorescence tool to monitor the $HO^{\bullet}$ produced by the Cu bound to Aβ, but not from other potential sources. The strategy was to attach a $HO^{\bullet}$ sensor close enough to the Cu-site in Aβ, and to identify a suited fluorophore that has a turn-on response upon reacting with $HO^{\bullet}$ (among all the ROS). Here, we identify naphthalene monoimide (NMI) as a fluorescent tool that is capable of probing $HO^{\bullet}$ radical species selectively via a turn-on mechanism. We synthesized Aβ1-16 peptide sequence conjugated with NMI fluorophore to obtain $A\beta_{16}^{NMI}$ and studied the ROS production catalyzed by Cu^{II} bound to $A\beta_{16}^{NMI}$ ($Cu(A\beta_{16}^{NMI})$) in presence of ascorbate ($AscH^-$) and O_2. NMI showed several-fold increase in fluorescence response during the $AscH^-$ consumption and subsequent ROS production catalysed by $Cu(A\beta_{16}^{NMI})$. Further screening with radical scavengers like catalase enzyme established the selectivity of NMI to $HO^{\bullet}$ among other oxidant species (H_2O_2, superoxide, HOCl and singlet oxygen). Also, NMI in $A\beta_{16}^{NMI}$ showed selectivity towards the proximal produced $HO^{\bullet}$ via the Cu-$A\beta_{16}^{NMI}$, in comparison to a bulk source (Fe-EDTA) of $HO^{\bullet}$ generation. Further, NMI of $A\beta_{16}^{NMI}$ showed turn-on response to the ROS produced in PC12 cells treated with $Cu(A\beta_{16}^{NMI})$ and $AscH^-$. However, free NMI added to cells did not show any increase in fluorescence response to the ROS produced by $Cu(A\beta_{16})$ and $AscH^-$ highlighting the importance of probes' proximal availability for ROS species especially $OH^{\bullet}$ radicals (which are less diffusive and highly reactive). We also delineated the molecular mechanism for efficient turn-on response of NMI upon reaction with $HO^{\bullet}$ resulting in demethylated NMI with high quantum efficiency. In conclusion, we identify NMI as a potential probing tool to assess the ability of $Cu(A\beta_{16}^{NMI})$ to generate $HO^{\bullet}$ and further being investigated to measure the same in presence of full-length Aβ peptide.



Keywords: Copper, amyloid beta, fluorescence tool, reactive oxygen species, hydroxy radicals

Type de communication : communication orale ☒ flash ☐ duo ☐ affiche ☐

Dual-emissive photonconvertible fluorescent probes based on Directed Photooxidation Induced Conversion for advanced bioimaging applications

Lazare Saladin,[a] Victor Breton,[b] Andrey Klymchenko,[a] Pascal Didier,[a] Lydia Danglot,[b] Mayeul Collot*[a]

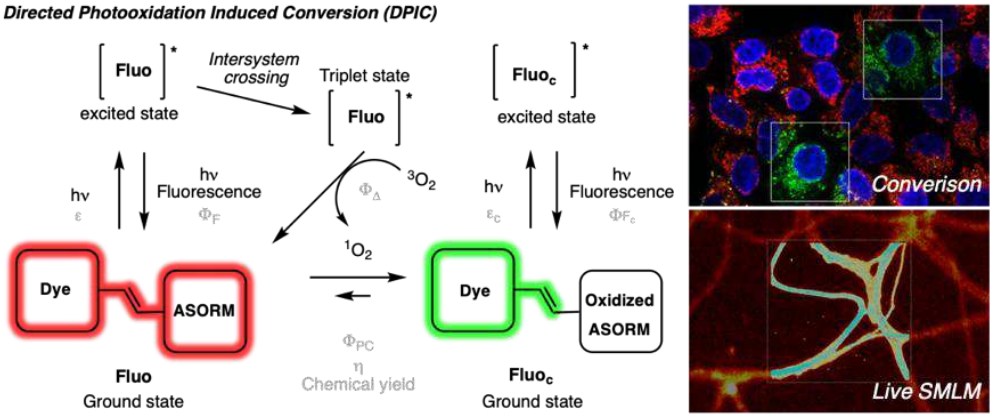
a. Laboratoire de Bioimagerie et Pathologies, UMR 7021, CNRS/Université de Strasbourg, 74 route du Rhin, 67401 Illkirch-Graffenstaden, France

b. Université de Paris, Institute of Psychiatry and Neuroscience of Paris, INSERM U1266, Membrane Traffic in Healthy & Diseased Brain, 75014, Paris, France

* Correspondance : mayeul.collot@unistra.fr

Abstract:

Bright Fluorescent dyes able to photoconvert into another bright form, also called dual emissive photoconvertible fluorophore remain extremely rare. Herein, we propose a rational approach to design such fluorescent probes, based on a new mechanism: Directed Photooxidation Induced Conversion (DPIC, Figure).[1-2] This mechanism involves the conjugation of a fluorophore to an Aromatic Singlet Oxygen Reactive Moiety (ASORM) that 1) leads to a red-shifted fluorophore (Fluo) and 2) directs the photooxidation reaction of singlet oxygen ($^1O_2$) that is generated upon irradiation toward the ASORM. The directed photooxidation thus provokes the disruption of the electronic conjugation and leads to a converted fluorophore ($Fluo_c$) with a hypsochromic shift in emission. This new mechanism was applied to develop bright photoconverters and photoswitchers that were further targeted to cell compartments like plasma membrane, mitochondria and lipid droplets for photoconversion application as well as for live super resolution Single Molecule Localization Microscopy (SMLM) in neurons (Figure).



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Keywords: Fluorescent probes, photoconversion, photoswitching, super resolution microscopy.

ABSTRACTS

COMMUNICATIONS DUO

Type de communication : communication orale en duo.

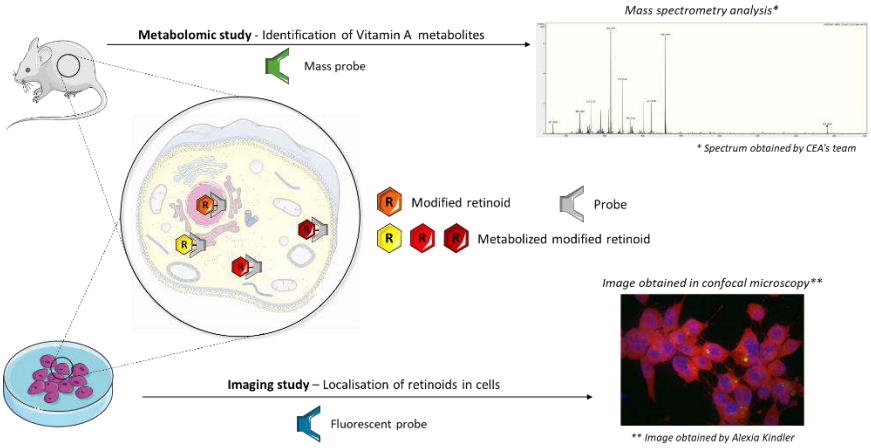
Deciphering of retinoid storage and metabolic pathways using bio-orthogonal chemistry-based strategies

Kindler Alexia[1,*], Coulleray Jessica[2,*], Naim Ahmad[3], Colsch Benoit[3], Fenaille François[3], Wagner Alain[2], Krezel Wojciech[1]

1. Institut de Génétique, Biologie Moléculaire et Cellulaire (IGBMC), CNRS UMR 7104 - Inserm U 1258, Université de Strasbourg, Strasbourg ; 2. Laboratoire de conception et application de molécules bioactives (CAMB), Chimie BioFonctionnelle (BFC), UMR 7199, Université de Strasbourg, Strasbourg ; 3. Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Paris Saclay

*, presenting: kindlera@igbmc.fr ; jcoulleray@unistra.fr

Retinoids are small lipophilic molecules of nutritional origin, essential for normal development and physiology in vertebrates.[1] Despite years of research, their storage, metabolism and functions in specific cell types throughout the body remain still not completely understood.[2] Indeed, some retinoids display low abundance[3] and are weakly ionizable making them difficult to detect with standard techniques. The aim of our project is to develop and assay molecular retinoids probes suitable to undergo biorthogonal reactions with mass tag or fluorescent probes. The synthesis of a panel of modified retinoids required optimization since their modification is poorly described in literature, especially with late-stage methods. Among the panel of biorthogonal reactions, Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC) was chosen to first study azido-retinaldehyde (N_3-RAL). Biological activity of N_3-RAL was first validated via assay of enzymatic conversion, transcriptional activity, and differentiation of embryonal carcinoma P19 cells into GABAergic neurons. Optical imaging of N_3-RAL was then tested in both living and fixed P19 cells by reaction with different fluorescent probes, previously chemically synthesized. On the other hand, reacting N_3-RAL with probes carrying mass-tags allowed retinoids' detection and quantification in biological matrix with high sensitivity. To conclude, we succeeded in the synthesis of a library of molecular retinoids probes to be used with functionalized probes. All of them have allowed their application in both living cells and biological matrices.



Keywords: retinoids; click chemistry; fluorescent probe; mass tag.

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Type de communication : communication orale ☒ flash ☐ affiche ☐

Chimie click pour l'étude des cibles d'un agent infectieux induisant la dysenterie

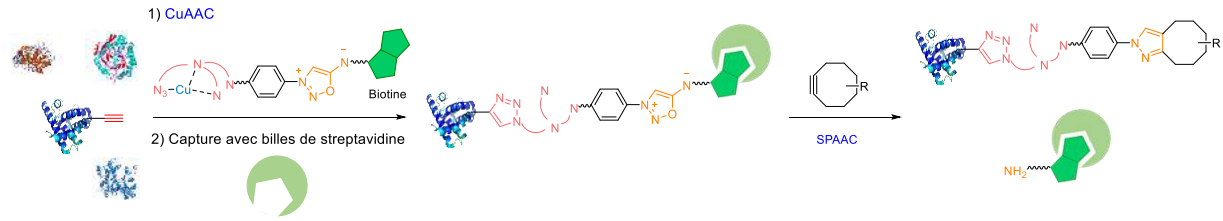
Louis Manon,[a]* Monassa Paul,[b]* Sallustrau Antoine,[a] Giglione Carmela,[b] Meinnel Thierry,[b] Taran Frédéric.[a]

a. CEA-Saclay, Département Médicaments et Technologies pour la Santé, Service de Chimie Bioorganique et de Marquage
b. Université Paris Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198 Gif-sur-Yvette cedex, France

* Correspondance : manon.louis@cea.fr, paul.monassa@i2bc.paris-saclay.fr

Résumé :

Le genre *Shigella* comprend 4 espèces pathogènes de bacilles à Gram négatif utilisant leur système de sécrétion protéique de type 3 engendrant la shigellose, une dysentérie bactérienne. Parmi les protéines excrétées, le facteur de virulence IpaJ est l'un des responsables de l'inhibition directe de la croissance des cellules eucaryotes, grâce à son action sur la myristoylation. IpaJ est la seule protéine connue capable de retirer la N-myristoylation d'une protéine[1]. La N-myristoylation - une modification essentielle considérée comme irréversible des eucaryotes - correspond à l'ajout d'un groupement myristate (lipide C14:0) sur la glycine N-terminale d'une protéine. La N-myristoylation est particulièrement difficile à mettre en évidence de par sa nature hydrophobe et la faible expression des protéines myristoylées. Il a été démontré qu'elle représente environ 2% du protéome - soit plus de 600 cibles chez l'homme - alors que moins d'une centaine de protéines myristoylées ont in-vivo chez l'homme[2], rendant difficile la caractérisation des effets de la demyristoylation médiée par IpaJ. Afin d'identifier l'ensemble des cibles d'IpaJ dans les cellules humaines il est nécessaire de développer de nouvelles réactions efficaces, telles que la réaction de CuAAC (Copper-catalysed Alkyne-Azide Cycloaddition). Cette réaction met en jeu une fonction azoture et une fonction alcyne en présence de cuivre(I), pour former un triazole. Cependant, la réaction de CuAAC est difficilement applicable *in-vivo* en raison de la toxicité du cuivre pour les cellules. Pour diminuer cette toxicité, notre laboratoire a développé des azotures chélatants permettant de complexer le cuivre au plus près du site de réaction (azoture) et ainsi de diminuer la quantité de cuivre lors de la CuAAC.[3] Par ailleurs, les iminosydnones sont des composés mésoioniques connus pour leur réactivité avec les cyclooctynes (SPAAC pour Strain-Promoted Azide-Alkyne Cycloaddition), ce qui en fait de potentiels linkers clivables.[4] En exploitant ces propriétés, nous avons synthétisé des sondes portant 1) un azoture chélatant permettant la réaction de CuAAC sur les protéines MYR à identifier et fonctionnalisées par un alcyne, 2) une iminosydnone permettant la réaction de SPAAC et le relargage des protéines enrichies. Après avoir enrichi les cellules avec un précurseur alkylé du myristate, les protéines myristoylées peuvent être progressivement enrichies et identifiées.



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Keywords: Target fishing, Chimie click, N-myristoylation, *S.flexneri*, IpaJ

Type de communication : communication orale ☒ flash ☐ affiche ☐

Identification of the first SUV4-20 proteolysis targeting chimera for cancer treatment

Calzaferri Francesco,[a,b,#,*] **Julien Eric**,[b] **Lopez Marie**[a*]

a. Institut des Biomolécules Max Mousseron (IBMM), CNRS-UM-ENSCM UMR5247, Montpellier
b. Institut de Recherche en Cancérologie de Montpellier (IRCM), INSERM, Montpellier

* Correspondance: marie.lopez@cnrs.fr / francesco.calzaferri@cnrs.fr

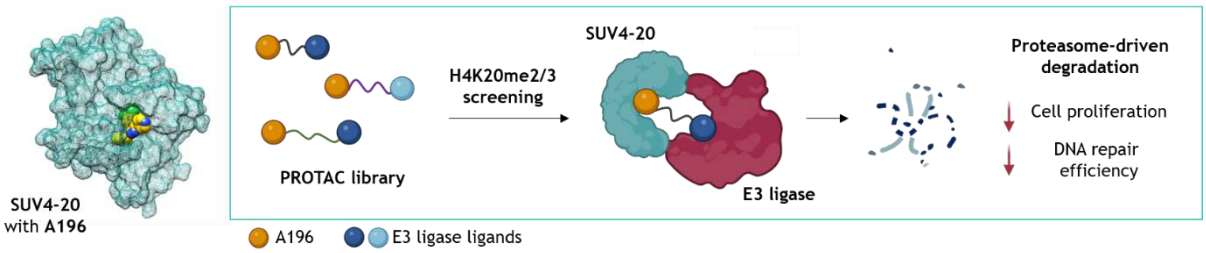
[#] laureate of the GDR mobility fellowship

Di- and trimethylation of lysine 20 on histone 4 (H4K20me2/3) is an epigenetic modification catalysed by SUV420H1 and SUV420H2 enzymes (collectively called SUV4-20). In agreement with their role in genome integrity and DNA repair, SUV4-20 loss or dysregulation results in low cell proliferation, defects in cell-cycle progression and sensitivity to DNA damage.[1] Thus, pharmacological modulation of SUV4-20 represents a novel strategy for cancer treatment, although much need to be still investigated.

The phthalazine-based A-196 is the only SUV4-20 inhibitor discovered so far. It showed to decrease H4K20me2/3 and DNA repair efficiency in osteosarcoma cells.[2] However, its low solubility hampers further *in vivo* evaluation and previous chemical modification attempts were unsuccessful.

Driven by the promising data obtained from SUV4-20 knock out cell lines,[3] we envisaged the development of the first SUV4-20 proteolysis targeting chimeras (PROTACs), as an alternative strategy to investigate the effects of SUV4-20 inhibition and degradation in cancer. We designed and synthesised a small library of PROTACs composed by A-196 and cereblon or Von Hippel-Lindau E3 ligase ligands, connected by alkyl and PEG linkers of different lengths. We then assessed H4K20me2/3 levels and SUV420H2 degradation by western blot in DU145 prostate cancer cell line treated with $PROTAC_{SUV4\text{-}20}$ at different time point.

By our research, we successfully synthesised and characterised the first SUV4-20-targeting PROTAC candidate, affecting H4K20 methylation and SUV4-20 levels in prostate cancer cells. Our data pave the way to the understanding of SUV4-20 functions in cells and how SUV4-20 degradation affects cell proliferation and death in cancer.



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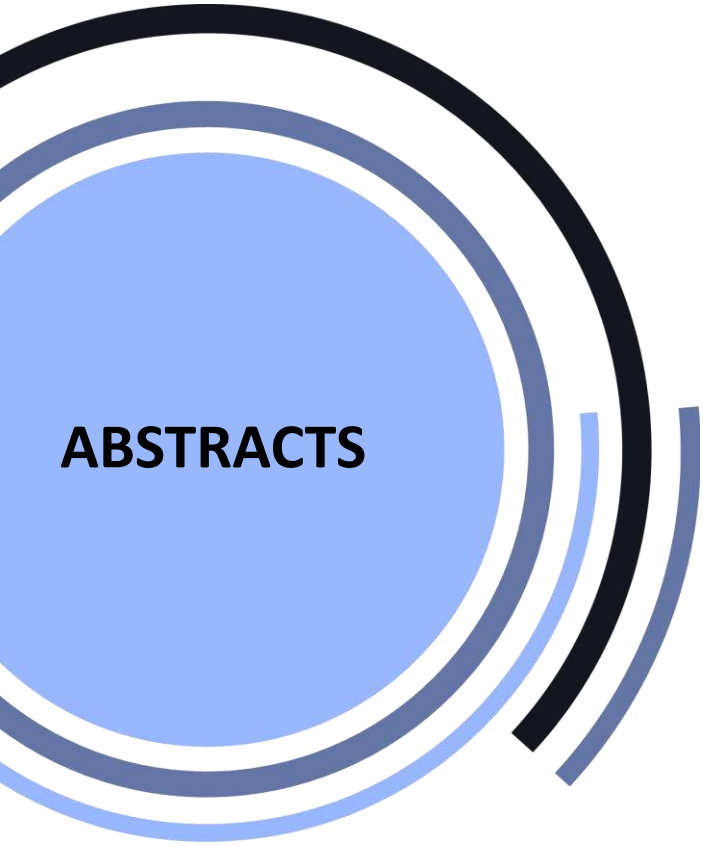
Keywords: SUV4-20, H4K20 methylation, PROTAC, Epigenetics, Prostate cancer



ABSTRACTS

COMMUNICATIONS FLASHS

Type de communication : communication orale ☒ flash ☐ affiche

L'IDENTIFICATION D'ÉPITOPE EN UTILISANT DES APPROCHES *IN SILICO* : UNE ÉTUDE DE CAS NANOCORPS LIANT LE RÉCEPTEUR MGLU5

Floriane Eshak[a], Anne Goupil-Lamy[b], Francine Acher[a]

[a] Université Paris Cité, SPPIN, Paris, France
[b] Biovia Science Council, Dassault Systèmes, Vélizy-Villacoublay, France

* Correspondance : floriane.eshak@parisdescrates.fr

Résumé :

La schizophrénie est un trouble mental qui affecte environ 1% de la population mondiale. Les scientifiques ont développé une hypothèse qui pourrait expliquer sa physiopathologie, qui serait liée à un déséquilibre du glutamate dans le cerveau. Parmi les cibles du glutamate, dans cette étude, nous nous sommes concentrés sur le récepteur métabotropique du glutamate 5 (mGlu5). Cependant, cibler sélectivement les récepteurs mGlu est un défi en raison de leur site de liaison orthostérique hautement conservé. Pour surmonter cela, une approche alternative a été développée, qui consiste à utiliser des nanocorps comme modulateurs allostériques[1]. Un nanocorps est défini comme le fragment variable de l'anticorps constitué uniquement de chaînes lourdes. Sa petite taille et sa stabilité dans des conditions physiques extrêmes en font un agent thérapeutique intéressant.

Cette étude est basée sur des données biologiques antérieures indiquant que le nanocorps Nb5A est un modulateur allostérique positif sélectif du récepteur mGlu5 de rat. L'objectif de l'étude est d'identifier l'épitope de Nb5A et son mode de liaison au récepteur mGlu5 de rat, suivi du ré-épitoping ou de la re-conception du nanocorps pour une liaison au récepteur humain. Des techniques de modélisation moléculaire et d'algorithmes d'intelligence artificielle tels que la modélisation par homologie[2], AlphaFold, IgFold et ImmuneBuilder ont été utilisées pour prédire les structures de Nb5A et du récepteur mGlu5 de rat. Cela a été suivi par un docking rigide-rigide[3] et flexible en aveugle pour générer des poses de docking où deux épitopes possibles ont été sélectionnés. Parmi ceux-ci, un seul épitope a été identifié en utilisant la dynamique moléculaire, et a été validé par des expériences biologiques.

Enfin, le ré-épitoping de ce nanocorps a été réalisé dans le but de cibler à la fois le récepteur mGlu5 de rat et mGlu5 humain à des fins thérapeutiques.

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Mots-clés: Modélisation Moléculaire; Nanocorps; mGluRs; Intelligence Artificielle

Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☒ (flash talk en priorité)

Combining surface chemistry and live imaging to decipher the minimal requirements for high-speed motility of the eukaryote *Toxoplasma gondii*

Luis Vigetti [a] *, Bastien Touquet [a], Galina Dubacheva [b], Isabelle Tardieux [a] *

a. Institute for Advanced Biosciences, Université Grenoble Alpes, 38700 Grenoble, France.
b. Département de Chimie Moléculaire, Université Grenoble Alpes, CNRS, 38000 Grenoble, France

* Correspondance : luis.vigetti@univ-grenoble-alpes.fr , isabelle.tardieux@inserm.fr

Abtract :

Toxoplasma gondii is a single-celled eukaryotic parasite, which has evolved a developmental morphotype called tachyzoite. The tachyzoite is a polarized cell which moves apex first within extracellular matrices (ECMs) by a high-speed helical trajectory called gliding. Gliding proceeds through a series of sub-membranous myosin motors with direct retrograde translocation of actin filaments (F-actin) along the parasite length. In this linear motor and continuous force scenario, the tachyzoite secretes adhesins from the front pole that couple and translocate with ECMs ligands and F-actin [1]. However, we uncovered an apical and periodic traction force that contradicts this continuous force model, showing that these actomyosin motors locally transfer a mechanical load onto a basket of spiraled microtubules which generate a spring force and drive helical motility [2]. To nail down the minimal requirement for helical gliding, we next investigated if the front and rear adhesions and their dynamics were sufficient to generate gliding forces. To this end, we tested the tachyzoite performance on substrates composed of alternating adhesive and non-adhesive high-precision areas. Using micrometer-resolution micropatterning, surface chemistry, and high-speed confocal video-microscopy, we confirmed that the tachyzoite undergoes typical helical gliding using only the two polar adhesion points and an apical traction force. To further identify and characterize the substrate molecules that would support building a productive force platform, we developed a bio-conjugated layer-by-layer assembly protocol in order to create surfaces with quantitatively controlled exposed moieties. These surfaces were also biophysically characterized using Quartz Crystal Microbalance with Dissipation (QCM-D). First data identify the pro-motile effect of specific substrate-parasite interaction. We will discuss how these data allow refining the *Toxoplasma* gliding motility model in a minimalist work-frame.

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Keywords: Toxoplasma gondii, cell-substrate interaction, bio-conjugated layers, live imaging, micropattern.

Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☒

Development of bio-active fluorescent probes targeting the Rho-kinase ROCK : new tools for the screening of active ingredients.

Karen Plé[a], S. Hammoud[a], J. Corret[b], Béatrice Vallée[b], Hélène Bénédetti[b], Pierre Daligaux[c], Christophe Chesné[c], Sylvain Routier[a*]

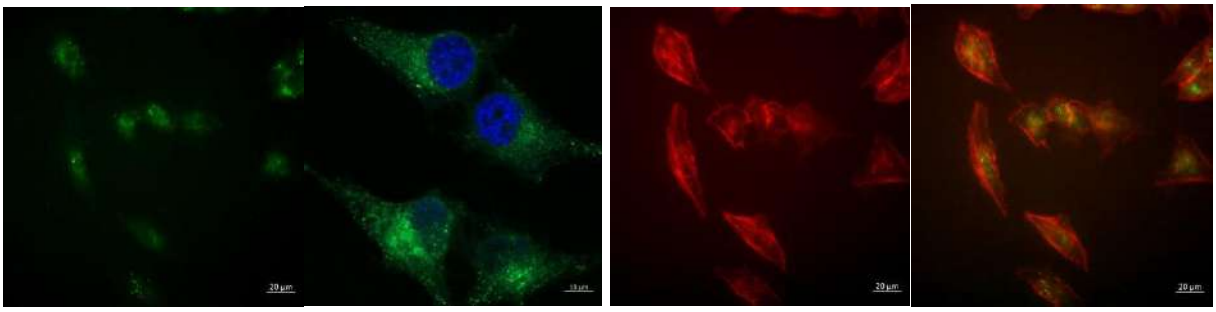
[a] *Institut de Chimie Organique et Analytique, Université d'Orléans, CNRS 7311, Orléans, France*

[b] *Centre de Biophysique Moléculaire, CNRS 4301, Orléans, France*

[c] *Starlight, 111 Boulevard Duhamel de Monceau, Olivet, France*

* Correspondance: sylvain.routier@univ-orleans.fr

Having bags or puffiness under the eyes, although not a serious medical condition, is common with age as the tissues around eyes weaken, due to fatigue and lifestyle choices. They are essentially formed by an excess of liquids that the circulation of the lymph in the lymphatic vessels of the eye contour (interstitial liquid), has failed to evacuate due to a deficiency. Unlike the arterial and venous network, lymph circulation results from contraction of the muscle cells of the lymphatic system. The inclusion of active ingredients which stimulate lymphatic circulation in cosmetics could allow a faster and more consistent deflation of these bags and thus improve the effectiveness of current methods, mainly based on the application of cold compresses and micro-massages. The Rho-associated protein kinase (ROCK) is one of the major players in endothelial and lymphatic muscle cell contraction regulation as it controls tone and contractility of lymphatic vessels and the integrity of their intercellular barriers. We have designed fluorescent probes to visualize the activity of this kinase. We have tested our probes on HeLa model cell line as well as on a more specialized HepaRG cell line, a unique and well-established hepatic cell system capable of producing early hepatic progenitor cells as well as fully mature human hepatocytes. Our probes enter the cell and is active as it affects cell cytoskeleton dynamics. Our fluorescent probes are new tools that can be used to easily monitor the effect of cosmetic actives on the ROCK protein and therefore on lymphatic tone. Because of the fluorescence reading mode, an application in cosmetic active ingredient screening could be developed and easily monitored.



Probe | Probe + Hoechst | Actine (Phalloidine) | Superposition

Keywords: Fluorescent probes, ROCK, lymphatic system

The authors would like to thank the Région Centre Val de Loire and Cosmetosciences Program for their financial support and Cosmetic Valley for their certification of this research project.

Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☒

Fluorescent turn-on detection of bacteria with targeted bioconjugates

Lucille Weiss[a], Patrick Wagner[a], Martijn Visser[a], Stéphanie Riché[a], Océane Flores[a], Dmytro Dziuba[b], Pierre Fechter[c], Dominique Bonnet[a]*, Julie Karpenko[a]*

a. Laboratoire d'Innovation Thérapeutique, UMR 7200 CNRS/Université de Strasbourg, Institut du Médicament de Strasbourg, 74 route du Rhin, 67401 Illkirch.2
b. Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS/Université de Strasbourg, 74 route du Rhin, 67401 Illkirch.
c. Biotechnologie et Signalisation Cellulaire, UMR 7272 CRNS/Université de Strasbourg, ESBS, 300 Bd Sébastien Brant, 67412 Illkirch.

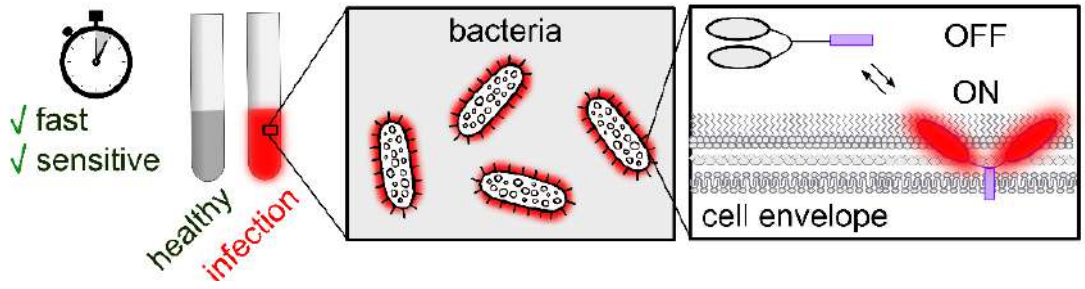
* julie.karpenko@unistra.fr, dominique.bonnet@unistra.fr

Abstract:

Rapid detection and identification of bacterial infections are among the key actions to prevent antibiotic misuse and limit the spread of resistant bacteria. An instantaneous mix-and-read assay for the detection of bacteria in human body fluids and identification of its antibiotic susceptibility would be of high value to public health and society. Fluorescent probes hold great promise in the field of biomedical express diagnostics.1 However, existing fluorescent bacterial probes are poorly suitable for direct and fast detection of bacterial infections in complex biological samples, such as blood or urine.2

Here we propose a new concept of targeted fluorescent turn-on probes for bacteria, based on aggregation-caused quenching (ACQ).3,4 The probes are composed of bacteria-targeting vectors (antibiotics, antimicrobial peptides) and covalent dimers of far-red aromatic dyes, which exist in water in the form of non-fluorescent π- stacked H-aggregates (the OFF state). In a less polar medium (such as organic solvents or components of the bacterial cell envelope), the H-aggregate is disturbed, and the fluorescence of the probes is restored (the ON state).

A set of Cy5.5 and squaraine dyes and dimers have been synthesized and coupled to bacteria-targeting molecular vectors. Fluorescence studies in a series of solvents demonstrated the ability of these probes to generate a strong fluorescence turn-on response when passing from an aqueous to a less polar medium. The most efficient probes were characterized by high selectivity for bacterial vs eukaryotic cells and enabled the detection of living bacteria in no-wash conditions by fluorescence spectroscopy and fluorescence microscopy.



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Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☐

Innovative NIR light responsive nanoparticles for enhanced antibacterial PDT and singlet oxygen mediated antibiotic release.

Maxime Pascouau [a], Anaïs Brion [a], Frédéric Bolze [a], Antoine Kichler [a], Gilles Prévost [b], Alexandre Specht [a]

a. Laboratoire de Conception et Application de Molécules Bioactives UMR 7199, Faculté de Pharmacie, Université de Strasbourg, 67401 Illkirch, France.
b. Virulence Bactérienne Précoce : fonctions cellulaires et contrôle de l'infection aiguë et subaiguë UR 7290, Faculté de Médecine, Université de Strasbourg, 67000 Strasbourg, France.

* Correspondance : m.pascouau@unistra.fr

Abtract :

Antibiotics overuses, misuses and environmental factors led to a global increase in antimicrobial resistance. This poses a serious threat over human life worldwide, mainly because it allows the spread of nosocomial infections that have severe human and material costs every year. Therefore, there is an urgent need for new and efficient methods to reduce infection-related damages. Photodynamic Therapy (PDT) is a powerful tool allowing the formation of singlet oxygen upon light irradiation of a photosensitizer. As such, it has been widely used to treat various diseases, including infections. In this latter case, it is called Antimicrobial Photodynamic Therapy, or aPDT[1]. Recently, there has been reports of the synergistic potential of combining aPDT with antimicrobials[2]. This project aims at exploiting this potential in a more controlled and practical way by developing nanocarriers that allows both aPDT and the release of an antibiotic through breakage of a self-immolative linker. These nanocarriers would be linked to polyurethane catheters in order to allow their application in a medical context.

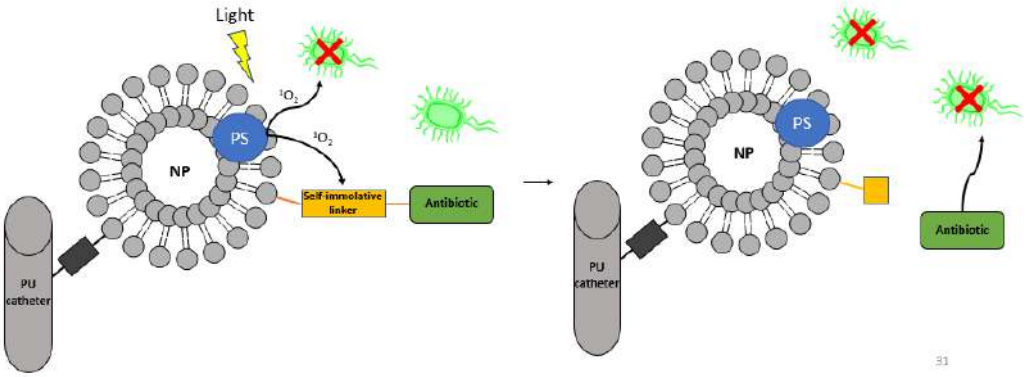

Figure 1. Schematic representation of the lipidic nanoparticle doped with a photosensitizer (blue) that, upon NIR light irradiation, would trigger the formation of singlet oxygen for a double effect: death of nearby bacteria (≈ 20 nm) and release of a caged antibiotic (green) through cleavage of a self-immolative linker (orange).

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Keywords: Antibacterial photodynamic therapy (aPDT), self-immolative linker, multi-step synthesis, antibiotic resistance.

Type de communication : communication orale ☒ flash ☐ duo ☐ affiche ☐

Design and synthesis of activity-based probes derived from schweinfurthins for target identification

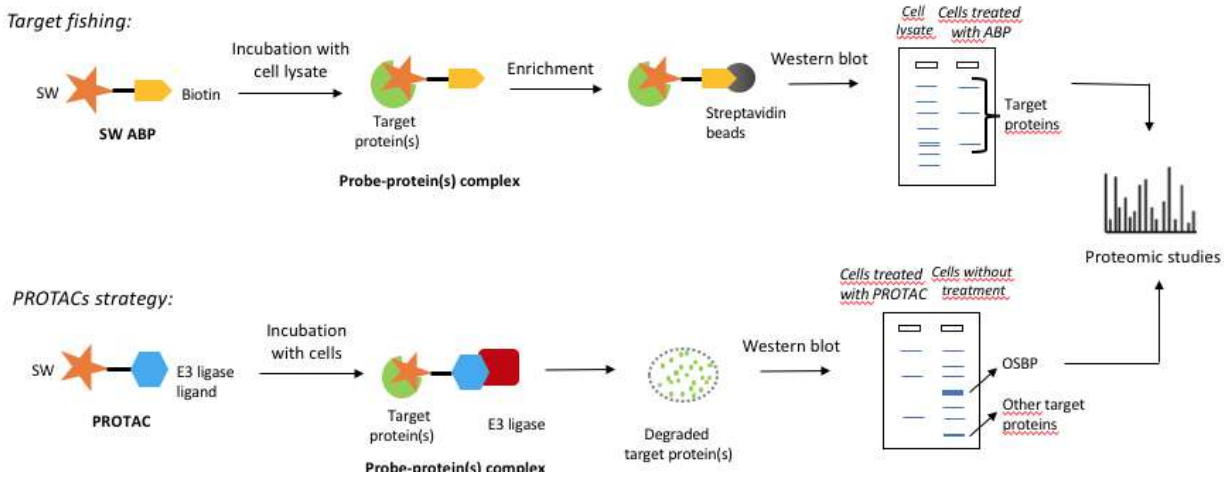
Carole Guimard[a], Gwenaëlle Jezequel[a], Emma Melotti[a], Laurie Askenatzis[a], Bruno Mesmin[b], Joëlle Bigay[b], Jérôme Bignon[a], Sandy Desrat[a], Fanny Roussi[a].

[a]Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France

[b]Université Côte d'Azur, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, UMR7275, 06 560 Valbonne, France

* Correspondance : carole.guimard@cnrs.fr

Abtract : Schweinfurthins (SW)[1] are a family of natural molecules of great interest for developing new therapies due to their strong cytotoxic potential on specific cancer cell lines. Their original mechanism of action involves an intracellular cholesterol transport protein: OSBP (OxySterol Binding Protein).[2] However, the mechanism of action (moa) of these molecules is still not very clear, involving other(s) target(s) responsible for the cell death. To go further in the development of new drugs, we wanted to better understand the moa of the SWs through the design of activity-based probes (APBs). For that purpose, we are focusing on the proteomic approach through the development of two different strategies: "target fishing" and the use of PROTACs (PROteolysis-TArgeting Chimeras)[3]. Concerning the target fishing strategy, we have synthesized active and inactive probes with a SW moiety and a biotin tag connected by a linker. The use of PROTACs is a less conventional strategy for target identification. These bifunctional molecules, which specifically induce the degradation of the target protein by hijacking the ubiquitin-proteasome system of the organism, can be used in proteomics to identify degraded proteins compared to untreated cells. We will describe herein the synthesis of these two types of probes, their cytotoxic activity on different cancer cell lines, and their validation (interaction with OSBP and OSBP fishing).



Keywords: Schweinfurthins, PROTACs, target fishing, OSBP

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3. M. J. Bond, C. M. Crews, *RSC Chem. Biol.*, 2021, *2*, 725.

Type de communication : communication orale ☐ flash ☒ affiche ☐

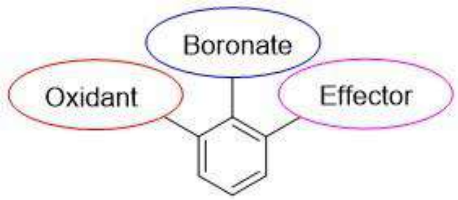
Molecular tools for the optimized targeting of reactive oxygen species (ROS) overproduction in the tumor microenvironment

Dáire J. Gibbons, Raphaël Labruère

Recently, prodrugs activated by the overproduction of reactive oxygen species (ROS) in tumor cells have been developed.[1–3] In particular, benzeneboronate moities have been coupled to drugs by a covalent bond in order to mask the activity of the latter. Oxidation of benzeneboronates by hydrogen peroxide or peroxynitrite results in the release of the drug in its active form.[3] Nonetheless, the concentration of ROS at the tumor site is quite low due to their short half-life time, which limits the yield of release and thus limits the pharmacological effect of the active drug (effector in figure below).[4] We wish to improve this strategy by designing a programmed molecular entity where the yield of liberation of the effector will be increased thanks to a system allowing the self-catalyzed activation of release. This technology is based on a boronate-type prodrug containing a self-immolative spacer capable of releasing an active ingredient concomitantly with a peroxynitrite generator, a powerful oxidant of boronates.

Affiliation: Equipe de Chimie Bioorganique et Bioinorganique, Institut de chimie moléculaire et des matériaux d'Orsay, Université Paris-Saclay, CNRS, 91405, Orsay, France.

* Correspondance : daire.gibbons@universite-paris-saclay.fr & raphael.labruere@universite-paris-saclay.fr



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Keywords: Fluorescent probe, Prodrug, Reactive oxygen species, Molecular tools, Auto/self amplification

Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☐

Optical control of PIEZO1 channels

Francisco Andrés Peralta, Mélaine Balcon[a], Adeline Martz, Deniza Biljali, Federico Cevoli, Benoit Arnould, Antoine Taly, Thierry Chataigneau & Thomas Grutter

a. Université de Strasbourg, UMR - 7199, Strasbourg, France

* Correspondance : melaine.balcon@etu.unistra.fr

Abtract :

Sensing mechanical forces in the environment is vital for organism's survival. In 2010, the discovery of Piezo ion channels in vertebrates showed that they are the molecular sensors of mechanical sensitivity, the least known sense. Activation of Piezo channels requires sophisticated and non-specific methods of mechanical stimulation of the cell. The development of alternative activation methods able to specifically and rapidly activate these channels in vivo is challenging and therefore needs new technologies. Using biomolecular engineering combined with patch-clamp electrophysiology, we have developed an opto-chemical technology that makes the mouse Piezo1 channel sensitive to light. By covalently tethering an azobenzene-based photoswitch to a cysteine introduced by site-directed mutagenesis, we showed that light irradiation at 365 nm rapidly opened the pore (Fig. 1). The reprogramming of this channel allows, in the absence of mechanical stimulus, to rapidly modulate its activity by light, without changing its mechanical sensitivity. Furthermore, this tool could provide a basis for understanding the mechanism of Piezo channels.

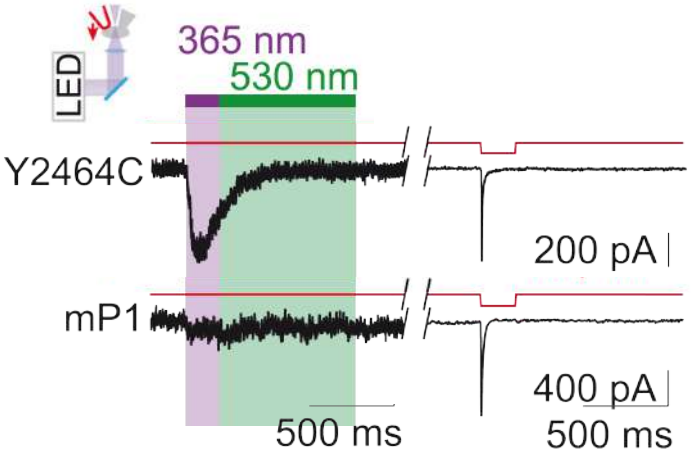

Figure 1. Light-gating of the engineered mouse Piezo1

Representative traces of inward currents in the whole-cell (WC) configuration in response to light irradiation (violet: 365 nm; green: 530 nm) and poking stimulation (P). Human Embryonic Kidney-Piezo1 KnockOut cells are expressing the cysteine mutant mouse OptoPiezo1 (mOP1) or the mouse Piezo1 (mP1).

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Keywords: Piezo channels ; Photoswitch ; Chemical optogenetic ; Azobenzene

Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☐

Post-synthetic functionalization of an antisense oligonucleotide by Strain-Promoted Sydnone-Alkyne Cycloaddition : synthesis and application

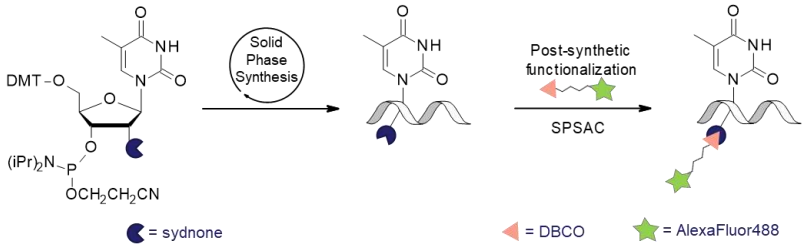
Alexandra Bristiel[a,b], Dominique Urban[a], Mathieu Pizzonero[b], Raphaël Guignard[b] , Dominique Guianvarc'h[a]*

a. Équipe Synthèse de Molécules et Macromolécules pour le Vivant et l'Environnement, ICMMO, Université Paris-Saclay, CNRS, 91400 Orsay
b. Unité Drug Design Small Molecules, Institut de Recherche et Développement Servier Paris-Saclay, 22 route 128, 91190 Gif-sur-Yvette

* Correspondance : dominique.guianvarch@universite-paris-saclay.fr

Abstract :

Of the many ways to target the expression of messenger RNA, antisense oligonucleotides (ASOs) have recently undergone considerable development[1]. These ASOs can be obtained by chemical synthesis and are used to recognize a specific messenger RNA by complementary base pairing and modulate the synthesis of the corresponding protein. By modifying their chemical structure, their stability in cells and their affinity for their target have been significantly improved in recent years. Although there have been important advances in the field, their cellular trafficking, location and their ability to penetrate cells still need to be studied. Site-selective post-synthetic modification of oligonucleotides provides a simple and effective method allowing the synthesis of a range of diverse labelled oligonucleotides from a common precursor. We present here a seven-step synthesis[2] of a 2'-sydnone-containing nucleoside. After its incorporation into an antisense oligonucleotide sequence, this modified nucleoside allows post-synthetic functionalization by different dyes to monitor the cellular penetration and the trafficking *via* a Strain Promoted Sydnone Alkyne Cycloaddition (SPSAC)[3].



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Keywords: Antisense oligonucleotide, modified nucleoside, sydnone, SPSAC, post-synthetic functionalization

Type de communication : communication orale ☐ flash ☒ affiche ☐

Synthesis of chemical tools to decipher the mode of action of antiparasitic redox-active 3-benzylmenadiones

Dupouy Baptiste,[a,*] Mourad Elhabiri,[a] Davioud-Charvet Elisabeth[a]

a. LIMA UMR 7042 (Strasbourg, ECPM)

* Correspondance : baptiste.dupouy@etu.unistra.fr

Abstract:

One of the main research topic of the Bioorganic and Medicinal Chemistry team is focused on the development of redox-active antiparasitic drug candidates based on a 3-benzylmenadione (bMD) core. This presentation will thus focus on two different strategies that are currently being developed for the identification/visualisation of the biological targets of bMD, a crucial step necessary to elucidate the mode of action and to synthesise more effective drugs in the future through rational drug design.[1] The first strategy is based on a bioorthogonal click reaction between novel bMD-derived alkyne probes and an azide fluorophore using the copper-catalysed azide-alkyne cycloaddition reaction (CuAAC) to visualize bMD-treated parasitized red blood cells. In addition to the preparation of these alkyne bMD probes, azide fluorophores based on a flavylium scaffold and able to react efficiently with these alkyne-bMDs to generate bright bMD-flavylium adducts are also being currently developed. This is justified by the fact that, compared to costly commercial fluorophores (e.g. rhodamine azide) that are difficult to modify or functionalise, home-made azide fluorophores accessible in a minimum of synthetic steps (simple and fast synthesis, scale-up, photostable, bright, water-soluble, tuneable emission, post-functionalisation) can be developed. For example, the length of the spacer separating the flavylium core from the azide function or the nature of the chromophore substitution offers several interesting perspectives. By exploiting the redox properties of the bMD moiety, an interesting fluorogenic response can be achieved within the bMD-flavylium adducts. These represent redox-sensitive fluorescent probes whose emission is closely related to the redox state of the bMD moiety, thus offering interesting prospects for parasite imaging. The second strategy still aims to develop other bMD-based probes for parasite imaging, but with the design of the first bMDs functionalised with an azido group, allowing copper-free cycloadditions (SPAAC) with constrained alkynes (e.g. DBCO functionalised with a flavylium fluorophore) to follow the transport and localisation of the pharmacophore within the parasite.

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Keywords: click reaction; fluorophore azide; medicinal chemistry; parasite imaging; redox.

Type de communication : communication orale ☐ flash ☐ affiche ☒

Development of a SEC-MS method in denaturing conditions (dSEC-MS) for adapted and specific in-depth analysis of rebriged mAb-based formats

Benazza Rania [a,b], Koutsopetras Ilias[c], Chaubet Guilhem[c], Hernandez-Alba Oscar [a,b], Cianférani Sarah[a,b]

a. Laboratoire de Spectrométrie de Masse BioOrganique, IPHC UMR 7178, Université de Strasbourg, CNRS, 67087 Strasbourg, France
b. Infrastructure Nationale de Protéomique ProFI—FR2048, 67087 Strasbourg, France
c. Bio-Functional Chemistry (UMR 7199), LabEx, Medalis, University of Strasbourg, 67400 Illkirch-Graffenstaden, France
* Correspondance : rania.benazza@etu.unistra.fr

Résumé :

Disulfide rebridging methods are emerging recently as new ways to specifically modify antibody-based entities and produce future conjugates. Briefly, the antibody or Fab fragment are reduced in controlled conditions and further covalently attached with a rebridging agent allowing the incorporation of one drug or more. Many examples of successful rebridging provide homogeneous conjugates[1]. However, partial rebridging leads to the development of heterogeneous products that cannot be identified by a simple SDS-page gel due to its lack of sensitivity, low resolution and low mass accuracy[2]. LC-MS approaches have already been demonstrated as highly promising alternatives for the characterization of newly developed mAb-based formats[3-4]. We report here the in-depth characterization of covalently rebriged antibodies and Fab fragments in-development, using size-exclusion chromatography hyphenated to mass spectrometry in denaturing conditions (dSEC-MS). DSEC-MS was used here to monitor closely the rebridging reaction of a conjugated Trastuzumab, in addition to conjugated Fab fragments.

For Fab fragments, the dSEC separation showed the presence of covalently rebriged Fab with presence of unmodified LC and Fd subunits, further confirmed by dMS detection. Upon integration of the chromatographic peak area of the fragment signals, the rebridging efficiency could be estimated to 78% and 51% for Fab #A and Fab #B, respectively. This highlighted the slight difference in rebridging efficiency of the two samples. The average drug-to-antibody ratio (avDAR) values were thus determined based on the dMS spectra (0.7 ± 0.1 and 0.6 ± 0.1. for Fab #A and Fab #B, respectively). The characterization of intact Trastuzumab using dSEC-MS was even more informative, as it allowed the observation of different rebridging products of different sizes: intact mAb, half-mAb, LC and HC species; which allowed the relative quantification of the rebridging efficiency (~43%), along with the determination of an avDAR value of 2.5 ± 0.1 for the covalently rebriged mAb**.**

Overall, this all-in-one approach allowed a straightforward analysis of the studied samples with precise mass measurement; critical quality attributes assessment along with rebridging efficiency determination.

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Keywords: SEC-MS; disulfide rebridging; mAb.

ABSTRACTS

POSTERS

Type de communication : communication orale ☐ flash ☒ affiche ☐

Fluorescent glycolipid microparticles to investigate lectin-induced phagocytosis

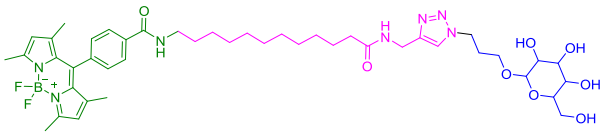
Sophie Michelis,[a*] Blaise Dumat,[a] Florence Niedergang,[c] Jacques Fattacioli,[b] Jean-Maurice Mallet.[a]

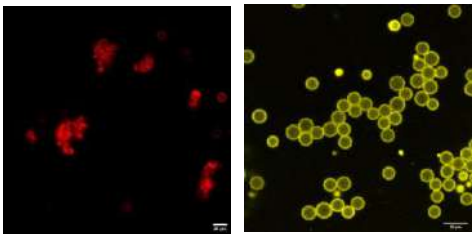
a. Laboratoire des Biomolécules, UMR 7203, CNRS/ENS/SU, Ecole Normale Supérieure, Département de chimie, 24 rue Lhomond 75005 Paris
b. Institut Pierre-Gilles de Gennes pour la microfluidique, 6 rue Jean Calvin, 75005 Paris
c. Université de Paris, Institut Cochin, INSERM, U1016, CNRS, UMR 8104, Paris

* sophie.michelis@ens.psl.eu

Résumé

In this work we report on the development of mannose-coated fluorescent lipid microparticles to study the role of C-type lectin membrane receptor in phagocytosis. Their role in phagocytosis and especially their ability to trigger phagocytosis or to cooperate with other receptors is not determined.[1,2] We have performed a bacterium simulation with the specific recognition of a microparticle by macrophages thanks to a specific pattern on its surface. We have developed lipid microparticles formulated. [3,4] These micrometer sized oil-in-water emulsion droplets were functionalized with synthetic fluorescent (bodipy fluorophores) amphiphilic glycolipids, exposing on their surface sugars. Droplets functionalization by glycolipids and interaction with concanavalin A, which was used as a model lectin, were monitored by fluorescence microscopy. We present here the synthesis and characterization of a series of fluorescent glycolipids and their use to detect the binding and the agglutination of lectin receptors but also to study the cellular environment during internalization. This work brings evidence that these glycolipid-functionalised lipid droplets specifically interact with lectin receptors and may provide insights into the comprehension of lectin-dependent phagocytosis.





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Keywords: oil-in-water microparticles; fluorescent glycolipids; fluorescence lifetime; phagocytosis; bodipy; lectin receptor

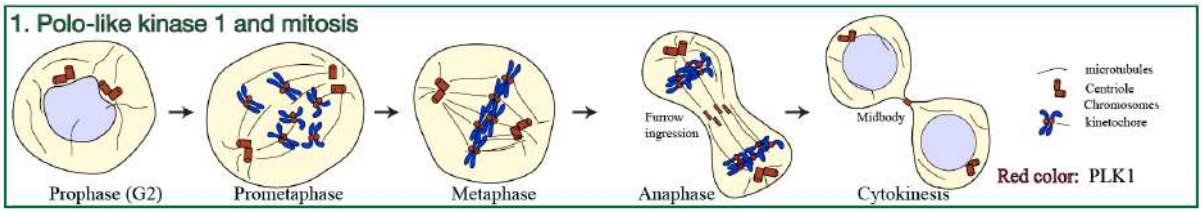
Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Targeting the polo-like kinase-1 with monoclonal antibodies inside living human cells for evaluating inter-proteins assembly in cell functioning

Manuela Chiper, Mariel Donzeau Guy Zuber [a,]

a. UMR 7242 Biotechnologie et Signalisation Cellulaire, 300 Bld S Brant 67400 Illkirch

* Correspondance : zuber@unistra.fr



Abstract :

The human polo-like kinase 1 (PLK-1) is a key regulator of the mitotic event and it intervenes at various steps by timely and rightly phosphorylating various proteins.[1] The PLK-1 is overexpressed in many types of human cancers. Selective silencing with siRNA of its expression in cancer cells consistently induce mitotic arrest and cell death in vitro but also in vivo.[2,3] Inhibition of the PLK-1 enzyme activity induces aw well apoptosis in many cancer cells and the BI 6727 (volasertib), a leading molecule binding to the ATP-binding pocket, demonstrated anti-leukemic activity in association low dose cytarabine in a phase III clinical trial. Unfortunately, the study did not meet the primary endpoint due to the severity of adverse side events.[4] If the two above-mentioned technologies allow demonstrating the indispensable presence of PLK1 during mitosis and the implication of its enzyme activity, they do not provide a detailed understanding on how PLK1 associates and phosphorylates multiple interacting proteins throughout the multiples mitotic stages. To expand further the understanding on how the proteins nearby PLK1 interfere with the function of PLK1, we electroporated monoclonal antibodies into living HeLa cells to target and append protein sub-domains of PLK1 with a bulky 150 kDa IgG. We will show that cytosolic delivery of antibodies into HeLa cell is efficient and that the immunofluorescence signatures of live-cell delivered anti-PLK1 antibodies were reminiscent but remarkably different than the classical immunofluorescences performed on PFA-fixed and permeabilized death cells. Moreover, we will show that delivered antibodies perturbed mitotic chromosome segregation in an antibody- and dose- depend manner.

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Keywords: anticorps, intracorps, polo-like kinase 1, interactome,

Type de communication : communication orale ☐ flash ☒ affiche ☐

Design and characterization of monoclonal antibody peptide mimics

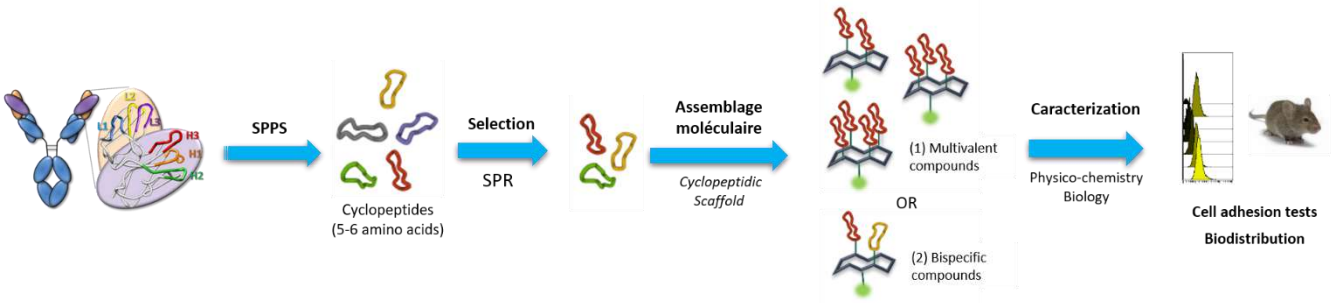
Océane Ricloux [a], Chanantida Jongwohan [a], Jordan Cossu [a], Liliane Guerente [a], Didier Boturyn [a]

a. Département de Chimie Moléculaire – Ingénierie et Interactions BioMoléculaires

Correspondence: oceane.ricloux@univ-grenoble-alpes.fr ; didier.boturyn@univ-grenoble-alpes.fr

Abtract:

Since the nineties, monoclonal antibodies (mAb) have emerged as a promising class of pharmaceuticals for the treatment of many human diseases. Monoclonal antibodies, such rituximab, were successfully used for cancer. However, several limitations related to nature of mAbs such as their low tissue penetration, immunogenicity and their production that requires a very expensive biotechnological procedure limits their extensive clinical use. Because of these limiting factors, new technological solutions have to be explored. One solution could be the use of smaller organic mAb mimics that endow mAb biological activities. In this context, we are interested in the development of mAb mimics that targets the CD20 antigen, a transmembrane protein, which is overexpressed on mature B lymphocytes. The CD20 antigen is a key target for immunotherapy: several mAbs (including rituximab, ibritumomab) were initially developed to treat B cell proliferative disorders but also autoimmune diseases. We propose to design macromolecular compounds comprising cyclopeptides selected from rituximab[1] Fab as recognition elements for CD20 in combination with a detection element and/or a cytotoxic unit for biological studies. mAb mimics will be designed *via* the assembling of several selected cyclopeptides on a well-known cyclodecapeptide scaffold to obtain different compounds.[2] For this purpose, several chemoselective ligations (click chemistry) will be used.[3] To study the interaction of the mAb mimics and CD20-displaying surface, we will exploit sensitive surface techniques such as surface plasmon resonance (SPR)[4]. Finally, we will evaluate the



binding of compounds to several commercially available cell lines and in mice.

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Communication type: talk ☑ or flash-talk ☐

Substituted oligosaccharides as protein mimics: deep learning free energy landscapes

Benjamin Bouvier[1]

[1]) benjamin.bouvier@u-picardie.fr
Génie Enzymatique et Cellulaire
UMR7025 CNRS / Université de Picardie Jules Verne, Amiens

Abstract:

Protein-protein complexes power the majority of cellular processes. Interfering with the formation of such complexes using well-designed mimics is a difficult, yet actively pursued, research endeavor. Due to the limited availability of results on the conformational preferences of oligosaccharides compared to polypeptides, the former have been much less explored than the latter as protein mimics, despite interesting ADMET characteristics.

In this work, the conformational landscapes of a series of 956 substituted glucopyranose oligomers of lengths 3 to 12 designed as protein interface mimics are revealed using microsecond-timescale, enhanced-sampling molecular dynamics simulations. Deep convolutional networks are trained on these large conformational ensembles, to predict the stability of longer oligosaccharide structures from those of their constituent trimer motifs. Deep generative adversarial networks are then designed to suggest plausible conformations for oligosaccharide mimics of arbitrary length and substituent sequences, that can subsequently be used as input to docking simulations. Analyzing the performance of the neural networks also yields insights into the intricate collective effects that dominate oligosaccharide conformational dynamics.

B. Bouvier, *J. Chem. Inf. Model.*, accepted, 2023.

Keywords: protein-protein interfaces; protein mimics; oligosaccharides; deep learning; molecular dynamics.

Type de communication : communication orale ☒ flash ☐ duo ☐ affiche ☐

Neolectins, towards new tools for selective sugar targeting

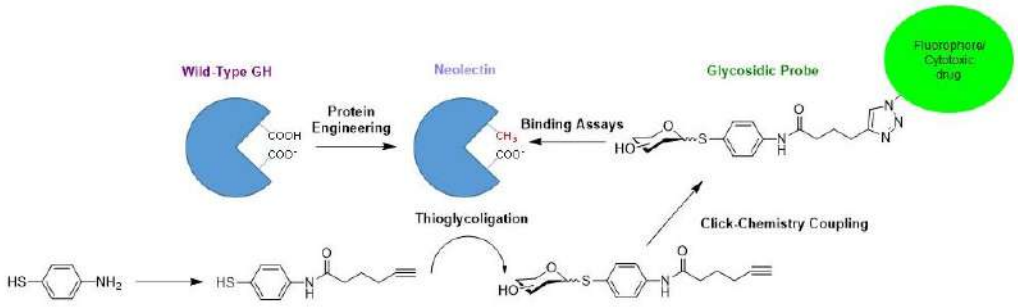
Burrini Nastassja [a], Daniellou Richard [b], Lafite Pierre [a]

a. Université d'Orléans, ICOA, CNRS, UMR7311, F-45067 Orléans, France
b. Chaire de Cosmétologie AgroParis Tech, 10, rue Léonard de Vinci, 45100 Orléans, France

* Correspondance : pierre.lafite@univ-orleans.fr

Abstract :

Lectins are carbohydrate-binding proteins specific for sugar molecules incorporated in glycosides or glycans at cell surface, playing a critical role in recognition processes at cellular and molecular levels. Engineering of lectins has been a challenge for many years. In our group, a synthetic lectin has recently been developed by engineering a galactofuranosidase, able to recognize and hydrolyse the galactofuranose entity, by removing its catalytic activity and retaining its sugar binding ability, resulting in a "neolectin".[1] Based on this result, we aim at developing a library of neolectins, by selecting glycoside hydrolase selective for targeted carbohydrate structures and turning them into corresponding "neolectin" by site-directed mutagenesis. However, to determine their binding affinity and sugar selectivity, a matching library of glycosides has to be synthesized to serve as chemical probes for *in-vitro* assays. In this context, an original biocatalytic approach has been chosen as the engineered neolectins are also thioglycoligase able to catalyze the formation of the corresponding *S*-glycosides.[2,3] Thus, the neolectins to be assayed as sugars receptors will also serve as biocatalysts to generate their own dedicated chemical probes. Several examples of chemoenzymatic synthesis of such *S*-glycosides bearing either a fluorescent moiety or biorthogonal functions for subsequent coupling will be presented.



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Keywords: neolectin, glycoside hydrolase, thioglycoligase

Type de communication : communication orale ☐ flash ☒ affiche ☒

An azo-based fluorogenic smart probe to visualize a mitochondrial azoreductase activity in live cells

MICHEL Laurane[a,*], AUVRAY Marie[b], BADET-DENISOT Marie-Ange[a], DURAND Philippe[a], MAHUTEAU-BETZER Florence[b], CHEVALIER Arnaud[a,*]

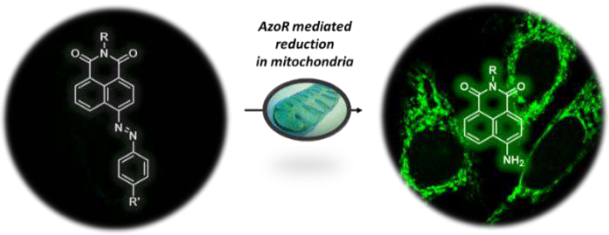
a. *Institut de Chimie des Substances Naturelles, CNRS UPR2301, 1 Avenue de la Terrasse, 91198 Gif sur Yvette, France*
b. *Institut Curie, CNRS UMR9187 - Inserm U1196, Bâtiment 110 Rue Henri Becquerel, 91401 Orsay, France*

* Correspondance : laurane.michel@cnrs.fr , arnaud.chevalier@cnrs.fr

Résumé :

Mitochondria is the center of energy metabolism in the cell. Dysfunctions of this organelle have been related to many diseases, such as cancer, diabetes, cardiovascular or neurodegenerative diseases, and more.[1] In this context, the study of biological phenomena at the intramitochondrial level is particularly relevant. Fluorescence imaging, and more specifically fluorogenic smart probes, are potent tools to observe chemical transformations at the subcellular level. This approach has allowed the identification of an intramitochondrial nitroreductase activity.[2] Recently, this enzymatic activity has been exploited for the activation of prodrugs selectively inside mitochondria.[3]

Here, we describe our works on the design and utilization of fluorogenic probes thought for the observation of an intramitochondrial azoreductase activity in live cells. We have designed and synthesized azo-based probes derivated from 4-amino-1,8-Naphthalimides fluorophores. The particularity of these probes lies in their non-emissive structure, making them interesting OFF-ON probes. Their azo N=N double bond can be reduced by an AzoR activity, restoring a brightly emitting naphtalimide fluorophore. These sensors have been studied *in vitro* with multiple enzymes and were found to be stable under biological conditions, as well as highly sensitive and selective to AzoR. Confocal microscopy experiments conducted on different living cell lines showed the presence of a mitochondrial AzoR, expressed at different levels depending on the cell line. This interdisciplinary work involving organic chemistry, photophysics, and cell biology has provided convincing results making AzoR a plausible and promising alternative to NTR for specific drug delivery into mitochondria.



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Keywords: mitochondria, fluorogenic probes, azoreductases, fluorophores

Type de communication : communication orale ☐ flash ☒ duo ☐ affiche ☐

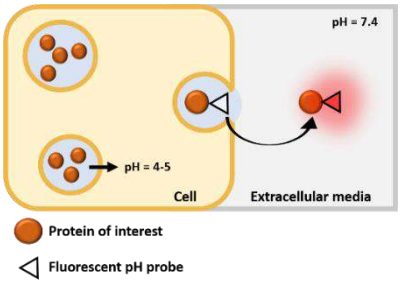
Hybrid chemogenetic fluorescent pH probes to study protein trafficking and secretion

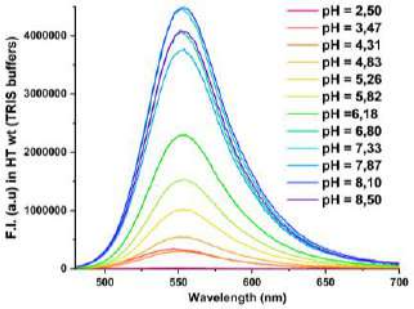
Justine Coïs [a,b], Vincent Vialou [b], Jean-Maurice Mallet[a] and Blaise Dumat[a]

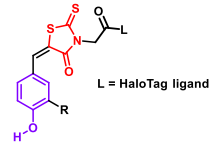
a. Laboratoire des Biomolécules, UMR7203, CNRS/ENS/SU, Ecole normale supérieure, Département de chimie, 24 rue Lhomond, 75005 Paris – Neurosciences Paris Seine, UMR8246, CNRS/INSERM/SU, 7 quai St-Bernard, 75005 Paris
b. Neurosciences Paris Seine, UMR8246, CNRS/INSERM/SU, 7 quai St-Bernard, 75005 Paris

* Correspondance : justine.cois@ens.psl.eu

Live imaging of exocytosis dynamics is crucial for a precise spatiotemporal understanding of secretion phenomena. Thus, biologists use approaches that rely on pH sensitive fluorescent proteins[1] (SEP, pHuji) with a pKa around 7 that enables the study of protein secretion from acidic vesicles to neutral extracellular media. As an alternative, we set out to develop molecular fluorescent reporters with suitable spectral properties and pKa range as a more versatile toolkit[2]. The idea is to use a hybrid chemogenetic fluorescent platform based on HaloTag labelling activation[3,4] enabling the development of "dual-input" probes with a pH sensitive emission. By combining protein engineering of HaloTag[5] mutant and organic synthesis of red-shifted molecular pH probes analogue to GFP chromophore, we obtained a series of "dual-input" pH indicators with a pKa around 6 and an interesting specific dynamic both to pH and HaloTag protein. These obtained probes have been tested and characterized in cells to study the secretion of the protein Hevin which is a matricellular protein that plays a role in neuronal plasticity. We will present then the characterizations and first biological results of these pH-probes during the flash communication.







$\lambda_{abs}(nm) = 470\ nm$
$\lambda_{em}(nm) = 553\ nm$
$\varepsilon(10^3 M^{-1}cm^{-1}) = 36$
$\Phi_F = 7.3\%$
$\varepsilon.\Phi_F = 2634$

Photophysical properties measured in PBS1X (pH = 7.4).

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Keywords: pH probes, HaloTag, fluorescence, protein

Type de communication : communication orale ☐ flash ☐ affiche ☒

Model surfaces based on streptavidin/biotin chemistry: development, physico-chemical characterization and use for mimicking biological interactions

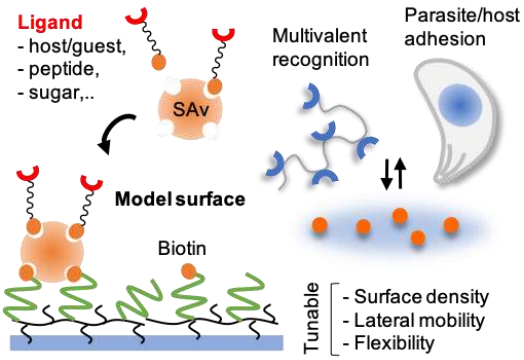
Darzhan Sadvokassova,[a] Luis Vigetti, [b] Hugues Bonnet, [a] Isabelle Tardieux,[b] Galina V. Dubacheva[a*]

a. Département de Chimie Moléculaire, University Grenoble Alpes, CNRS, 38000 Grenoble, France
b. Institute for Advanced Biosciences, University Grenoble Alpes, 38700 Grenoble, France

* Correspondance : galina.dubacheva@univ-grenoble-alpes.fr

Résumé :

Due to their high affinity and specificity, streptavidin (SAv)/biotin interactions are widely used in biotechnology for labeling, detection and purification. In particular, SAv-mediated assembly at interfaces (e.g. surface coatings, nanoparticles, membranes) provided the basis for various biochemical approaches intended for biosensing, biospecific targeting, drug delivery, bioactive coatings and biomimetic model systems. Self-assembled monolayers (SAMs) and supported lipid bilayers (SLBs) are among the most studied biotinylated interfaces.[1,2] In our previous work, we showed that surface chemistry plays an important role in SAv/biotin binding and can be used to control the residual valency and orientation of SAv on biotinylated SAMs and SLBs.[1] More recently, we studied SAv/biotin interactions at the interfaces composed of biotinylated poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) copolymers, which are widely used in material and biological sciences. Using a combination of optical and acoustic physico-chemical techniques, we screened for interfacial characteristics which govern SAv/biotin binding, among which the surface density and lateral mobility of biotin and the flexibility of the linker connecting biotin to the surface appear to be the most important (Fig. 1). The developed well-defined and tunable SAv/biotin model surfaces allow to mimic and study biological interactions, such as multivalent recognition at biological membranes.[3] Our preliminary results on the use of SAv/biotin model surfaces to study molecular interactions at the *Toxoplasma gondii*/host interface, with a focus on the promoted adhesion and motility functions,[4] will also be presented.



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Keywords: streptavidin/biotin binding, tunable model systems, physico-chemical characterization, biological interactions at interfaces

$Mn^{II/III}$ – Bispidine complexes for redox-activated MRI probes

Anli Mahamoud,[1,2] *Aline Nonat*[1] *and Eva Jakab Toth*[2]

1. Equipe de Synthèse Pour l'Analyse, Université de Strasbourg, CNRS, IPHC UMR 7178. (anli.mahamoud@etu.unistra.fr). https://iphc.cnrs.fr/
2. Centre de Biophysique Moléculaire, CNRS UPR 4301, Université d'Orléans. http://cbm.cnrs-orleans.fr/en/le-laboratoire-accueil/

Abstract:

Recent achievements in the synthesis and coordination chemistry of bispidines (3,7-diazabicyclo[3.3.1]nonane) have paved the way to a new field of application of bispidine coordination complexes: Magnetic Resonance Imaging (MRI). These derivatives are highly pre-organized ligands that can coordinate metal ions with cis-octahedral, square-pyramidal, or pentagonal geometries.[1] This pre-organization allows bispidine ligands to be a privileged scaffold for metal-based imaging agent. The search for more biocompatible alternatives to Gd^{3+}-based MRI contrast agent led us to $^{55}Mn(II)$ which is biogenic and less toxic. For 35 years now, Gd-complexes have been used in millions of human examinations and are considered among the safest diagnostic drugs. However, almost one third of the commercially available Gd^{3+}-based contrast agents have been suspended from the market since 2017. When Gd^{3+} is released into the body of patients with advanced kidney disease, Gd^{3+} retention is responsible of nephrogenic systemic fibrosis a sever, even lethal disease.[2] Herein, we propose new bispidine ligands for $Mn^{II/III}$. We have developed a series of $Mn^{II/III}$-bispidine complexes with exceptional kinetic inertness and competitive relaxivities to Gd-chelates.[3, 4] Herein we present new ligands for $Mn^{II/III}$ complexation to the use as redox MRI probes.

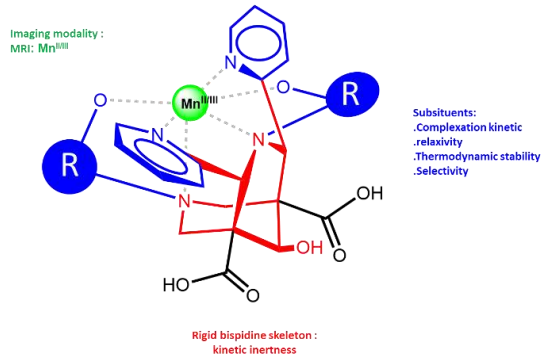

Figure 1 Kinetically inert bispidine complexes for MRI

Keywords: medical imaging, chelators, redox MRI, contrast agent

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Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

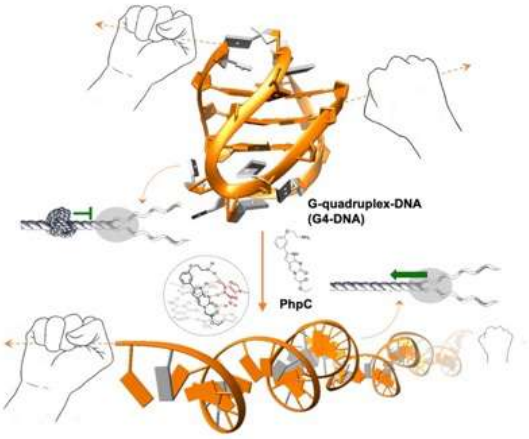
The small molecule PhpC modulates G-quadruplex landscapes *in cella*

Jérémie Mitteaux [a], Angélique Pipier [a], Robert H. E. Hudson [b], David Monchaud [a]*

a. Institut de Chimie Moléculaire, ICMUB CNRS UMR 6302, UBFC, 21078 Dijon, France
b. Dept. of Chemistry, University of Western Ontario, London, Ontario N6A 5B7, Canada

* Correspondance : david.monchaud@cnrs.fr

Abstract :



Massive efforts have been invested to discover new G-quadruplex (G4)-ligands, evaluate their G4-interacting properties *in vitro* and use them as chemical biology tools to interrogate cellular networks that might involve G4s.[1] In sharp contrast, only uncoordinated efforts aimed at developing small molecules that destabilize G4s have been invested to date, even though it is now recognized that such molecular tools would have tremendous applications in the field of genetic diseases, notably neurobiology as many age-related pathologies might be caused by an overrepresentation of G4s.[2] Our project aims at identifying G4-destabilizing small molecules and characterize their effects on cellular G4s. An *in vitro* workflow (comprising a panel of biophysical and biochemical techniques such as CD, UV-vis titrations, PAGE, fluorescence-based and qPCR stop assays) led to the identification of a phenylpyrrolocytosine named PhpC, which is the first fully validated G4-destabilizer reported to date.[3] In cells, we used optical imaging to show that the number of G4 *foci* significantly decreases upon PhpC pre-treatment (by 3.2-fold in cancer cells). We also implemented an affinity-precipitation method referred to as G4RP-RT-qPCR[4] to show that the abundance of two G4-containing RNAs, NRAS and VEGFA RNA, decreases upon PhpC pre-treatment (-53 and -33% for NRAS and VEGFA, respectively). The G4-destabilizing properties of PhpC were thus established both *in vitro* and *in cella*. Our results represent the very first demonstration that a small molecule can modulate G4 landscapes in cells: this makes PhpC a promising surrogate for G4-helicases,[2] which might be of utmost importance for treating genetic diseases originating in helicase deficiency that are currently suffering from severe drug attrition.

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Keywords: G-quadruplex, small molecule, screening, genetic diseases

Type de communication : communication orale ☐ flash ☐ affiche ☒

Caractérisation des agents ciblant les microtubules : un test cellulaire quantitatif faisant le lien entre les tests cellulaires et les tests sur tubuline purifiée

Bosc Lauriane[a], Laisne Marie-Catherine[a], Michallet Sophie[a] et Laurence Lafanechère [a]*

a. Institute for Advanced Biosciences, Team Cytoskeletal Dynamics and Nuclear Functions, INSERM U1209, CNRS UMR5309, Université Grenoble Alpes, 38000 Grenoble, France

* Correspondance : laurence.lafanechere@univ-grenoble-alpes.fr

Résumé :

Contexte : Les agents ciblant les microtubules et modifiant leur dynamique sont communément utilisés pour les traitements anti-cancéreux. Classiquement, on distingue les agents dépolymérisants des microtubules (MDAs) comme par exemple les alcaloïdes de la Vinca et les agents stabilisants (MSAs) comme les taxanes. Cependant, l'utilisation thérapeutique de ces inhibiteurs de la dynamique des microtubules est limitée par leur toxicité et les résistances qu'ils génèrent lors du traitement. Ainsi, il y a un effort continu pour développer de nouveaux agents ciblant les microtubules. Pendant la caractérisation de ces agents, la plupart du temps par analyses *in vitro* utilisant de la tubuline purifiée et des tests de cytotoxicité, les comparaisons quantitatives sont essentielles. Or, la relation entre l'effet des drogues sur la tubuline purifiée et sur la viabilité cellulaire n'est pas toujours directe.

Méthodes : Dans cette étude, nous avons conduit une analyse comparative systématique de l'effet de quatre MDAs largement caractérisés sur la cinétique de l'assemblage de la tubuline *in vitro*, sur le contenu en microtubules cellulaire, à l'aide d'un test cellulaire quantitatif que nous avons développé (1), et sur la viabilité cellulaire.

Conclusions : Ces trois tests donnent des résultats complémentaires. De plus, nous avons trouvé que les effets des drogues *in vitro* sur la polymérisation de la tubuline ne permet pas de prédire complètement leur toxicité sur les cellules. Par contre, leur effet sur le contenu en microtubules cellulaire est intimement lié à leur effet sur la viabilité cellulaire. En conclusion, le test que nous avons développé permet de faire le lien entre les tests de tubuline *in vitro* et les tests de viabilité cellulaire. Les conditions expérimentales de ce test cellulaire peuvent aussi être adaptées pour la quantification de l'effet stabilisant de MSAs (2).

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Keywords: cancer therapy; microtubules; cell-based assay; drug discovery

Type de communication : communication orale ☐ flash ☐ affiche ☒

Towards Multiple Myeloma in vivo PET imaging with bispidine antibody-conjugates

COURSEYRE Manon,[1*] NONAT Aline,[1] DETAPPE Alexandre,[2]

a. Institut Pluridisciplinaire Hubert Curien, CNRS UMR 7178, Strasbourg, France
b. Institut de Cancérologie Strasbourg-Europe, Strasbourg, France

* Correspondance : manoncourseyre@etu.unistra.fr

Abstract :

Cancer cells targeting is an essential challenge for an early detection of the complication and for the post-therapy following of the patients with multiple myeloma (MM). Current clinical imaging of MM patients is mostly based on Positron Emission Tomography (PET) with non-specific tracers like 18F-FDG which is employed to see the tumor activity. [1] However, in some case, this tracer is not able to fix the plasma cell population because of the low cellular proliferation and leads to false negative result. In our team, we aim to develop a novel specific PET agent labeled with 64Cu to improve the monitoring of these cells. Because of is β^+ decay with high energy and long half-life ($t_{1/2}$ = 12.7 h),[2] 64Cu is the perfect radioisotope for such imaging. The use of bispidine chelators as pre-organized ligands for the coordination of copper ions, has shown good results in term of thermodynamic stability and kinetic inertness in biological media.[3,4] These ligands are conjugated with biological vectors (monoclonal antibody (mAb)/ Single variable domain on a heavy chain (VHH)) characteristics for a specific detection of antigens sur expressed on the surface of abnormal myeloma cells. During these first months of PhD, I have achieved the synthesis of the ligands and the *in vitro* characterization of the first immunoconjugates.

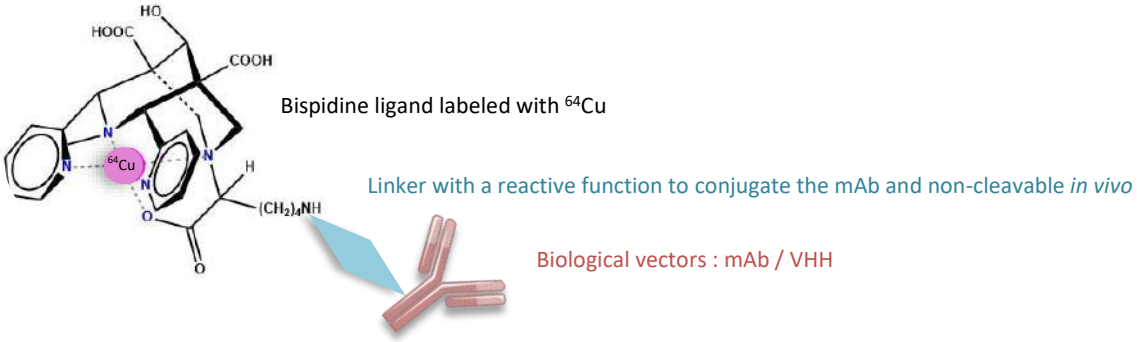

Figure 1 : Structure of the bispidine ligand radiolabeled and linked to a biological vector

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Keywords : bispidine; medical imaging; multiple myeloma; positron emission tomography

Type de communication : communication orale ☐ flash ☐ affiche ☒

Mouse models, analytical and technical support available in PCBIS platform

DAUBEUF François,[a*] LEHALLE Christine,[a] GIZZI Patrick,[a] VILLA Pascal,[a]

a. Plate-forme de Chimie Biologique Intégrative de Strasbourg (PCBIS), UAR 3286 CNRS / Université de Strasbourg, ESBS, Illkirch

* Correspondance : daubeuf@unistra.fr

Abtract:

The PCBIS platform provides services in mouse models, analytical and technical support including associated biologicals analysis. After selection of bioavailable compound combining the best in vitro activity and safety index, it remains necessary to confirm the in vivo activity in an animal model of the target pathology to highlight drug-candidates. To date, there is no alternative model sufficiently complex to reproduce all the biological and immune systems involved in pathologies and the mechanisms of degradation, elimination that can affect safety, biodegradability, pharmacokinetics and efficacy of drug candidates. Each study is customizable according to client's demand. The quality management of PCBIS has been recognized by international ISO9001 certification and animal studies are conducted according to in-house standard Operating Procedures with the approval of the local ethics committee that regulates animal research at the University of Strasbourg (CREMEAS).

In addition, PCBIS platform offers the analysis of biological samples (blood, tissue, cells) from cells or animal experiment. ELISA (cytokines, chemokines…), colorimetric (protein, mucus, collagen, HDL, LDL/VLDL…) and enzymatic (LDH, MPO, catalase, creatine…) tests are designed to be performed in 96 and 384 well plate to reduce the sample volume needed. Tissues may be fixed and embedded in paraffin for histopathology studies using general, specific and polychromatic colorations or immunostaining (IHC, IF). Isolated cells from blood, broncholaveolar lavage (BAL) or dissociated tissues may be analyzed by multi-color flow cytometry with a large panel of antibodies (3 to 12 colors panels are used routinely). Detection and quantification of ADN and mRNA may be performed on tissues or cells sampling by PCR, QPCR and RT-QPCR (96, 384 well plate and micro fluidic card using TaqMan™ assay).

Extensive on-demand work, including discussion of the objectives and tasks is available to customize all studies to guarantee useful data and results.

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Keywords: Mouse model; biological analysis; platform

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Affinity chemical probes based on bisubstrate analogues: identification of new GRP94 inhibitors in cancer cells

Dany Pechalrieu [a,b], Fanny Assémat [b], Ludovic Halby [b,c], Marlène Marcellin [d], PengRong Yan[e], Karima Chaoui[d], Sahil Sharma[e], Gabriela Chiosis[e], Odile Burlet-Schiltz[d], Paola B. Arimondo [b,c], Marie Lopez*[a,b]

a. IBMM, CNRS-UM-ENSCM UMR5247, Montpellier - France
b. ETaC, CNRS FRE3600, Toulouse, France
c. EpiCBio, Insitut Pasteur, CNRS UMR3523, Paris - France
d. IPBS, Univ. Toulouse, CNRS, UPS UMR5089, Toulouse - France
e. Program in Chemical Biology, Memorial Sloan Kettering Cancer Center, New York – USA
* Correspondance: marie.lopez@cnrs.fr

This work describes the use of bisusbtrate-based affinity probes in cancer cells and the subsequent identification of new GRP94 inhibitors. In order to understand the mode of action of bisubstrate-type derivatives previously identified,[1] we applied chemical biology experiment using well-designed chemical tools. We first synthetised affinity-based chemical probes of cytosine-adenosine analogues. We then use the affinity-based protein profiling (ABPP) strategy in different cancer cell lines and proteomic analysis enable us to identify several potential drug targets. Interestingly only one protein, the chaperone glucose-regulated protein 94 (GRP94), was significantly over-represented with our probe *vs* control in all cell lines. The validation of the proteomic analysis showed our chemical probe as a selective inhibitor of GRP94, a target of interest for several types of cancers.[2] Moreover, the identified new scaffold behaves as a GRP94 selective inhibitor over other HSP90 proteins. This is the first report of ABPP applied in a HSP90 context in living cells.

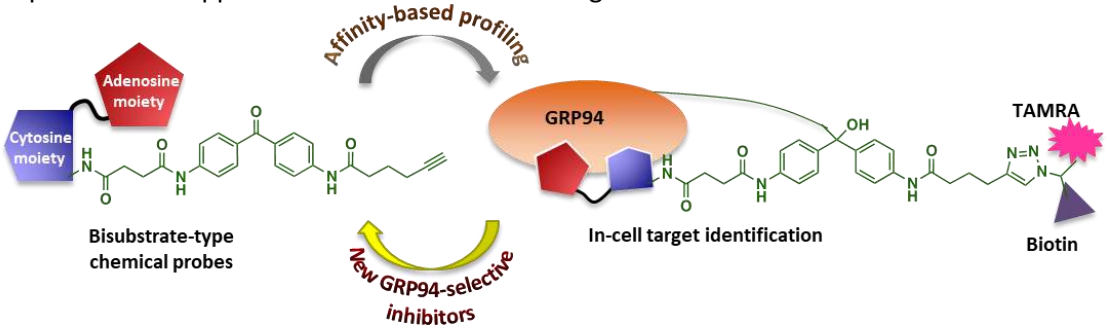

Figure1. Affinity-based profiling using bisubstrate analogues lead to the identification of new GRP94-specific inhibitors

This project is a multi-disciplinary project involving chemical probe design and synthesis, in cell fishing, proteomic analysis and enzyme inhibition assay for validation experiments. Therefore, as a result of the use of adenosine-substituted cytosine analogues to synthetise affinity probes and their application in a chemical-biology methodology, this work opens the way to the development of a new family of GRP94 inhibitors that could be of therapeutic interest.[3]

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Keywords: Affinity probes, Bisubstrat analogues, Enzyme Inhibitors, GRP94, Cancer

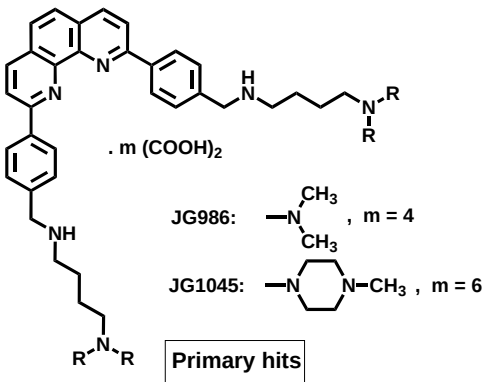
Synthesis, biophysical and biological evaluation of original heterocyclic ligands as anti-SARS-CoV-2 agents by targeting G-quadruplexes

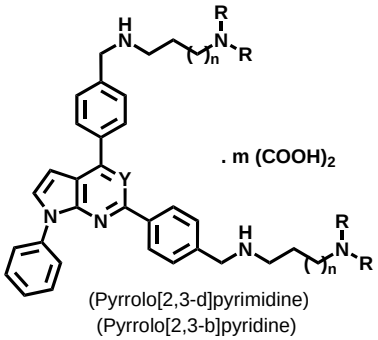
Guillon Jean[a,*], Savrimoutou Solène[a], Gouar Amina[a], Albenque-Rubio Sandra[a], Moreau Stéphane[a], Helynck Olivier[b], Chiaravalli Jeanne[b], Boudria-Souilah Rofia[c], Pinaud Noël[d], Ronga Louisa[e], Bernabeu de Maria Mikel[e], Mergny Jean-Louis[f,*], Lavigne Marc [c,*], Munier-Lehmann Hélène[b,*]

[a] *Univ. Bordeaux, Lab. ARNA, INSERM U1212, CNRS UMR5320, UFR des Sciences Pharmaceutiques, Bordeaux,*
[b] *Institut Pasteur, Université de Paris, CNRS UMR3523, PF-CCB, Paris, France*
[c] *Institut Pasteur, Université de Paris, Dept of Virology, Paris, France*
[d] *Univ. Bordeaux, ISM - CNRS UMR5255, Talence Cedex, France*
[e] *Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau, France*
[f] *Laboratoire d'optique et Biosciences, Ecole Polytechnique, Inserm U1182, CNRS UMR7645, Institut Polytechnique de Paris, Palaiseau, France*
* Correspondance : jean.guillon@u-bordeaux.fr; jean-louis.mergny@polytechnique.edu; marc.lavigne@pasteur.fr; helene.munier-lehmann@pasteur.fr

The Covid-19 pandemic caused by the emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the deadliest in human history[1]. There is an urge to develop new antiviral agents, in particular those targeting viral-host interactions. The multidomain non-structural protein 3 (Nsp3) is the largest protein encoded by coronavirus (CoV) genomes and several regions of this protein are essential for viral replication. Our work aims at interfering the interaction between Nsp3 and cellular partners.

Recently, we have shown that SARS-CoV-2 Nsp3 contains a SARS-Unique Domain (SUD), which can bind Guanine-rich non-canonical nucleic acid structures called G-quadruplexes (G4)[2]. These interactions can be disrupted by mutations that prevent oligonucleotides from folding into G4 structures and, interestingly, by molecules known as specific ligands of these G4s. By taking into account the various structural parameters required concerning the previously described G4 ligands, we are currently designing, and synthesizing new bioisoster compounds analogs of the first identified bioactive phenanthroline hits (JG986 and JG1045 as examples)[3].





In the present study, we report on the synthesis of these new compounds (such as Series A-B) and their efficient *in vitro* activities targeting the SUD/G4 interaction (by HTRF) and SARS-CoV-2 replication (in A549-Ace2 cells infected by different variant viruses). We also present promising data on the pharmacological properties of these molecules in cells and small animal models of viral infection.

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Type de communication : communication orale ☐ flash ☐ affiche ☒

Biological relevance and targeting of *i*-motif DNA

H. Bonnet,[a] D. Gomez,[b] J.-F. Riou,[c] A. Granhzam,[d] E. Defrancq,[a*] J. Dejeu[a,e*]

[a]Département de Chimie Moléculaire, CNRS, Université Grenoble Alpes, [b]Institut Pharmacologie et Biologie Structurale, CNRS, Université de Toulouse, [c]Structure et Instabilité des Génomes, MNHN, CNRS, INSERM, Paris, [d]CMBC (Institut Curie), CNRS, INSERM, Université Paris Saclay, [e]Institut FEMTO-ST, CNRS, Université de Franche-Comté, Besançon.

* Correspondance : eric.defrancq@univ-grenoble-alpes.fr, jerome.dejeu@univ-grenoble-alpes.fr

i-Motifs of DNA (hereafter, *i*-DNA), known *in vitro* for nearly three decades, are unusual, four-stranded structures, in which cytosines are intercalated *via* a stack of hemi-protonated C–C base pairs (CH^+:C) (Fig. 1A, B). Some of these structures have been well characterized *in vitro* and, because *i*-DNA may mirror other four-stranded G-rich structures (G-quadruplexes) present in gene promoters or at telomeres, their biological relevance is being investigated.

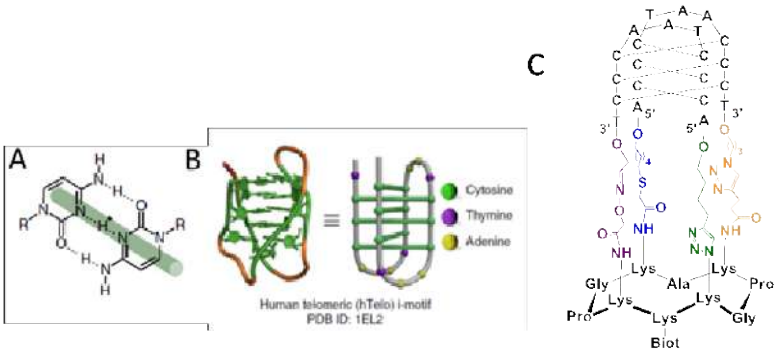

Figure 1. A/ Hemi-protonated C-C base pair. B/ Schematic structure of the telomeric i-DNA. C/ Structure of constrained i-motif

However, our knowledge about *i*-DNA biology is still limited: the main challenges in this regard being the strong pH dependency, flexibility, and polymorphism of *i*-DNA, that introduce potential bias into studies. In particular, low-pH conditions that are required for the formation of *i*-DNA can lead to the protonation of many ligands (including small molecules or proteins), strongly increasing their non-specific nucleic acid binding. In this context, we have developed a peptide-DNA conjugate (Fig. 1C) being able to fold into a stable *i*-motif at room temperature and, most importantly, at near-neutral pH.[1]

The stabilized mimic of the *i*-motif adopted by the telomeric sequence was used to study the interactions with already reported ligands (TMPyP4, mitoxantrone, IMC-48, berberine, *etc*) at physiologically relevant pH by Bio-Layer Interferometry (BLI) and CD. We demonstrated that none of the reported ligands were shown to discriminate between folded and unfolded *i*-motif structures.[2]

In conclusion, the constrained i-motif reveals to be a powerful tool for studying i-motif structure.

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Keywords: I-motifs DNA, ligands, Bio-Layer Interferometry

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Development of a new series of water-soluble NIR-II aza-BODIPYs for fluorescence imaging applications

Chazeau E.[1,2,3,*], Privat M.[1], Godard A.[1,4], Racoeur C.[2, 3], Bodio E.[1], Sancey L.[4], Paul C.[2,3], Goze C.[1]

[1]ICMUB, UMR 6302 CNRS, Université de Bourgogne, 21078 Dijon, France
[2]LIIC, EA7269, Université de Bourgogne, 21000 Dijon, France.
[3]Laboratoire d'Immunologie et Immunothérapie des Cancers, EPHE, PSL Research University, 75000 Paris.
[4]Université Grenoble Alpes, Institute for Advanced Biosciences, INSERM U1209, CNRS, UMR 5309, Grenoble, France.

*Correspondance: elisa_chazeau@etu.u-bourgogne.fr

The use of NIR-II (1000-1700 nm) emitting fluorophores for optical imaging can greatly improve the image resolution by limiting the autofluorescence of tissues. For this purpose, aza-BODIPYs are promising compounds thanks to their strong photostability and photophysical properties in the NIR-II regions[1]. However, due to their relative hydrophobicity, they are prone to aggregation phenomena. Therefore, we synthesized and bioconjugated three water-soluble aza-BODIPYs emitting in the NIR-II region and we evaluated their properties *in vitro* before their use in rodents. Three compounds derived from the same aza-BODIPY core were synthesized. Each of these differs according to the added water-solubilizing group. Site-specific bioconjugation of the three fluorophores was then achieved thanks to a reaction mediated by a microbial transglutaminase on previously deglycosylated trastuzumab. Optimizing conditions for the bioconjugation steps were investigated to obtain a DOL around 2 (measured by mass spectrometry) after FPLC purification. The localization of the bioconjugation site was confirmed by electrophoresis. Finally, the affinity of the bioconjugates for their target was measured by bio-layer interferometry. Cytometry and microscopy experiments were also carried out on cells (ovarian and breast cancer cells) expressing or not the HER2 receptor. To conclude, we developed a series of innovative water-soluble NIR-II aza-BODIPY fluorophores and we performed their site-specific bioconjugation on trastuzumab. They displayed a strong affinity for their target. *In vitro*, they bind specifically the cells expressing the HER2. Now, *in vivo* experiments are going to be performed to determine the best candidate for NIR-II optical imaging, and to evaluate the influence of solubilizing group on the biodistribution.

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Keywords: Optical Imaging; azaBODIPY ; NIRII ; bioconjugation

Targeting DNA junctions for devising a new anticancer strategy

Angélique Pipier[a], Titouan Chetot[b], Apollonia Kalamatianou[b], Anton Granzhan[b], David Monchaud[a]

a. Institut de Chimie Moléculaire, ICMUB CNRS UMR 6302, UBFC, 21078 Dijon, France
b. Institut Curie, CNRS UMR9187, Inserm U1196, 91405 Orsay, France

* Correspondance : david.monchaud@u-bourgogne.fr

Abstract:

Even if DNA is massively found under its double helix structure (duplex- or B-DNA) in cells, it can also adopt a variety of alternative, non-B-DNA secondary structures[1]. Some of these structures, notably G-quadruplexes (or G4), have been thoroughly investigated thanks to the use of small interacting molecules (or ligands), which allows for interrogating and manipulating biological circuitries where non-B-DNA structures are suspected to be involved in a controlled and reversible manner. These chemical biology approaches were instrumental to show that G4 ligands strongly interfere with DNA transactions, both in vitro[2,3] and in cells[3], thereby representing a new class of antiproliferative (anticancer) therapeutics. For several years now, we study another type of non-B-DNA structure, the three-way DNA junctions (or TWJ). These structures comprise three duplex arms, which converge to a junction point and create a central cavity, prone to accommodate ad hoc TWJ ligands. We have shown that our first prototype, TrisNP, induced DNA double-stranded breaks (DSBs) and had high antiproliferative activity against cancer (MCF7) cells[4]. Our new results, collected in HeLa cells, show a strong inhibition of both replication and of transcription, with a notable accumulation of DSBs in the G1 phase. However, TrisNP does not interact solely with TWJs and can also stabilize G4, which might cast a doubt about the actual origins of the cellular outcomes mechanistically interpreted. In order to find exquisitely selective TWJ ligands, we screened libraries of compounds and found that some fluorinated TrisNP analogues elicited similar TWJ stabilization properties but with a far better specificity for TWJs against G4 and B-DNA. With these new molecular tools in hands, we are currently investigating the localization of TWJs in our genome and how (and to which extent) these structures are involved in cellular metabolism, given that their natural occurrence is ca. 2-fold higher than that of G4.

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Keywords: three way junctions ; anticancer ; DNA damage ; replication

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Smart and multivalent ligands for DNA and RNA G-quadruplexes

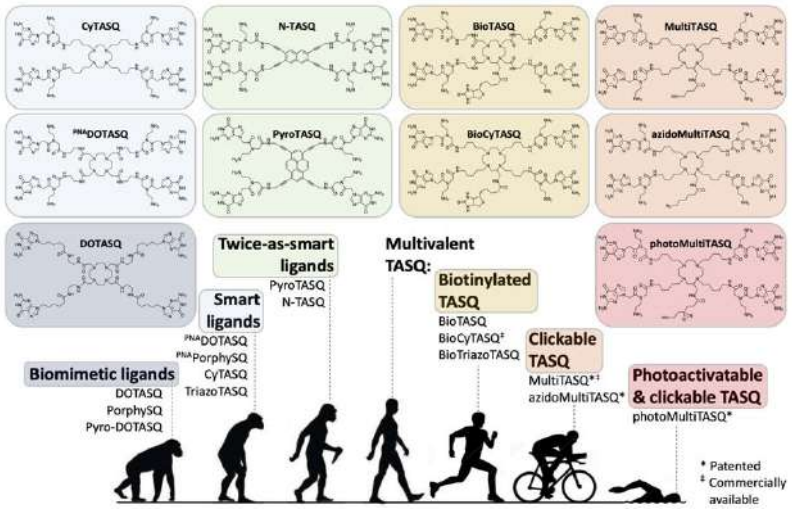
Sandy Raevens [a], Francesco Rota Sperti [a], Cécile Desingle[a], Marc Pirrotta[a], Ibai E. Valverde [a], David Monchaud [a]*

a. Institut de Chimie Moléculaire, ICMUB CNRS UMR 6302, UBFC, 21078 Dijon, France

* Correspondance : david.monchaud@cnrs.fr

Abstract :

G-quadruplexes (G4s) are four-stranded nucleic acid structures that fold from guanine (G)-rich DNA or RNA sequences. The prevalence of G4-forming sequences in the human genome and transcriptome was recently demonstrated by a series of sequencing-based methods (including G4-seq[1] and G4-ChIP-seq[2] for G4-DNA, and rG4-seq[3] and G4RP-seq[4] for G4-RNA). This very high prevalence prompted researchers to investigate the possible biological roles that G4s might play. To this end, the development of G4-specific molecular tools (or ligands)[5] was pivotal: indeed, G4 ligands were used to perturb cellular equilibria in which G4s are suspected to intervene, thus providing readouts amenable to mechanistic interpretations. Among these tools, TASQs (for template-assembled synthetic G-quartets)[6] were the first biomimetic ligands that interact with G4s according to a bioinspired, 'like-likes-like' approach. Several prototypes of TASQs were developed over the past years, including *i*- the smart G4 ligands (*e.g.*, ^{PNA}DOTASQ) that adopt their active conformation only in the presence of their G4 targets, *ii*- the twice-as-smart G4 ligands (*e.g.*, N-TASQ) that act as both smart ligands and smart fluorescent probes, and more recently *iii*- the multivalent G4 ligands that display additional functionalities enabling the detection and characterization of G4s *in vitro* and *in vivo*. Here, the latest TASQ prototypes will be presented, including the clickable MultiTASQ, azidoMultiTASQ and photoMultiTASQ (licensed by Merck KGaA, commercially available at Sigma-Aldrich). Their affinity and selectivity for DNA and RNA G4s will be presented along with their ability to be used as molecular bait to fish G4s out of human cell lysates.



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Synthesis and liposomal encapsulation of Au(III) 5,10,15,20-(tetraphenyl)porphyrin for their possible applicability as photosensitizers

Sofia Leo [1,2], Jean-Luc Coll [1], Lucie Sancey [1], Mans Broekgaarden [1], Benoit Habermeyer [2].

[1] Institute for Advanced Biosciences, Grenoble-Alpes University, INSERM U 1209, CNRS UMR 5309, Grenoble, France
[2]PorphyChem SAS, 390 Rue Charles de Freycinet, 21600 Longvic - France

Contact : Sofia.Leo@univ-grenoble-alpes.fr

Porphyrins are widely used for their phototherapeutic properties, in particular for photodynamic therapy of cancer, infections, and medical imaging[1]. Their properties as photosensitizers are due to their π-system, making them highly efficient to absorb photons, causing the excitation of the molecule leading to toxic photochemical reactions able to kill cancer cells. This process has demonstrated to be further catalyzed thanks to the presence of metal nanoparticles, such as gold[2] although the exact mechanisms remain unclear.
The aim of this project is to investigate the effect of the Au on the photochemical properties of porphyrins. To do so, we first improved the synthetic routes already present in literature[3] and tested the possible encapsulation inside liposomes of Au (III) 5,10,15,20-(tetraphenyl)porphyrin (AuTPP). We optimized the direct metalation process of the 5,10,15,20-(tetraphenyl)porphyrin (TPP) using $KAuCl_4$ as metal salt and we showed how the reaction time plays a fundamental role in the metalation process. The product was characterized by MALDI-TOF mass spectrometry, NMR spectroscopy and HPLC analysis. This molecule has no solubility in water and to test it under physiological conditions, we encapsulated the compound inside liposomes using lipid film hydration method. Liposomal encapsulation had no effect on the optical properties of AuTPP: The compound exhibited a strong absorption at 408,5 nm (Soret band), and a Q-bands around 519nm. The absorption spectrum differed from the free base TPP, which had a Soret band at 420 nm, and 4Q-bands between 500 and 600nm.
In conclusion, we optimized the synthesis of AuTPP and efficiently encapsulated it inside liposomes. Future test to determine the photodynamic activity of such system will be further investigated to prove the applicability of the molecule as photosensitizer.

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Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Towards the hijacking of OBSP by the use of chemical probes derived from schweinfurthins

Clément Grisel [a*], Ophélie Bento [a], Isabel Ramon Martinez [a], Laurie Askenatzis [a], Jérome Bignon [a], Bruno Mesmin [b], Sandy Desrat [a] and Fanny Roussi [a]

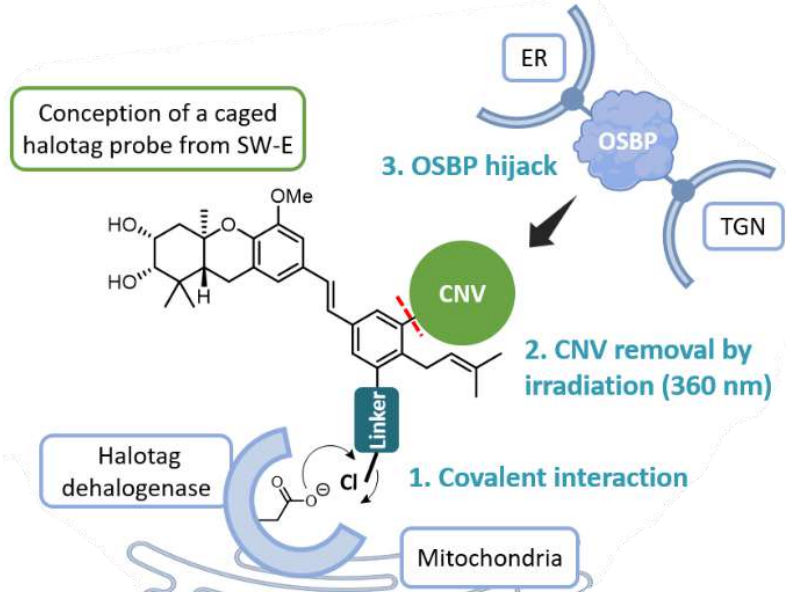
a. *Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198 Gif-sur-Yvette Cédex, France*

b. Université Côte d'Azur, Inserm, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France

* Correspondance : clement.grisel@cnrs.fr

Abstract :

Schweinfurthins (SWs) are natural fluorescent molecules isolated from plants of the genus *Macaranga spp* (Euphorbiaceae)[1] that are highly cytotoxic against specific cancer cell lines. Their original mechanism of action implies a new target in the field of cancer: OxySterol Binding Protein (OSBP). This protein, responsible for cholesterol transfer[2], is located at membrane contact sites between the endoplasmic reticulum (ER) and trans-Golgi network (TGN)[3,4]. However, the complete mechanism of action of SWs remains unclear. To better understand it, we designed various chemical probes and evaluated them *in cellulo*. One of the strategies explored by the team relies on hijacking OSBP from its normal localization to observe the consequences on lipids transfers, cell structure, and cell viability. To achieve this goal, we synthesized novel SW probes carrying a HaloTag substrate (*i.e.,* capable of interacting specifically with a halotag protein fused to a mitochondrial membrane protein) and a photoremovable protecting group (PPG), temporarily preventing their interaction with OSBP.



Keywords: Schweinfurthins, OSBP, Probes, HaloTAG

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Fluoroproline scanning.

A new approach describes PRM conformations using a proline-rich fragment from DNM2. Fluorine 19 NMR spectroscopy quantifies cis-trans conformers to reconstruct the conformational ensemble of the wild-type peptide using combinatorial analysis.

Mariam Sai [a,b], Vladimir Torbeev [b], Bruno Kieffer [a] *.

a. Integrative structural biology, IGBMC, Strasbourg, France
b. Laboratoire de Chimie des Protéines, ESBS, Illkirch, France

* Correspondance : kieffer@igbmc.fr

Abtract :

Fluorine NMR spectroscopy enables quantification of cis-trans conformers in Proline Rich Motifs (PRMs), which are commonly found in proteins involved in protein-protein interactions but present challenges for traditional structural analysis techniques due to their high flexibility and structural disorderliness resulting from proline cis-trans isomerization [(1)]. In this study, we present a novel method for describing PRM conformational ensembles using a proline-rich fragment from Dynamine 2, with each proline substituted with -(4R)-fluoroproline or -(4S)-fluoroproline to enable quantification of cis-trans conformers using 19F NMR spectroscopy [(2)]. Our method allowed us to reconstruct the conformational ensemble of the wild-type peptide using combinatorial analysis, providing a new tool for studying PRM conformational dynamics.

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Keywords: Fluorine NMR, Proline Rich Domains, Fluoroproline Scanning, Peptide Chemistry

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Management and quality control of the chemical compound collection of the Institut Pasteur Paris

Marine Ghazarian [a*], Agnès Zettor [a], Frédéric Bonhomme [b], Bruno Vitorge [c], Iñaki Guijarro [c], Fabrice Agou [a] & Jeanne Chiaravalli [a]

a. Institut Pasteur, CNRS UMR 3523, Chemogenomic and Biological Screening Core Facility, Center for Technological Resources and Research (C2RT), Paris, France
b. Institut Pasteur, CNRS UMR 3523, Epigenetic Chemical Biology, Department of Structural Biology and Chemistry, Paris, France
c. Institut Pasteur, CNRS UMR 3528, Biological NMR and HDX-MS Technological Platform, Center for Technological Resources and Research (C2RT), Paris, France

* Correspondence: marine.ghazarian@pasteur.fr

Summary:

High throughput screening (HTS) facilities are crucial for drug discovery, allowing researchers to test large numbers of compounds quickly and efficiently. However, the success of an HTS campaign is dependent on the quality of the chemical libraries used in the screening. The Chemogenomic and Biological Screening Platform (PF-CCB) from the Institut Pasteur Paris has a collection of nearly 62,000 compounds purchased from commercial suppliers or synthetized in-house. Upon delivery, compounds are cataloged and stored properly to ensure their integrity. A unique identifier is assigned to each compound, and its properties, such as molecular weight and solubility, are recorded in a database (CDD Vault). Regular analysis of the purity and identity of the chemical libraries, along with systematic screening of hits, are crucial to ensure a successful primary screening campaign and to isolate and validate true-positive compounds. High-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) is used to confirm the identity of the tested compounds and to detect any impurities or degradation products. Nuclear magnetic resonance (NMR) is also used to confirm the structural conformation and purity of the compound. Finally, the solubility of the compounds is determined by Dynamic Light scattering (DLS) experiments. These monitor the size distribution of particles and enable the exclusion of potential aggregators and small molecule aggregations. In conclusion, appropriate storage and periodical control quality can extend the life service of the compound libraries beyond the conventional 5 years announced by suppliers. Moreover, implementation of quality control measurements throughout the HTS process guarantee the reliability and reproducibility of the screening results, leading to the identification of potential drug candidates.

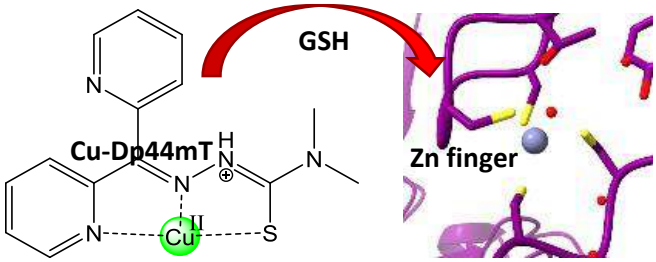
Keywords: Compound management; HTS; Quality-control; Chemical Library

Type de communication: communication orale ☐ flash ☐ affiche ☒

Cu^{II}-Dp44mT complex as an activator of HSP33 under anaerobic conditions

Lebrun Vincent, Faller Peter, le Mire Anne*
Institut Le Bel, 4 rue Blaise Pascal, 67000 Strasbourg
* Correspondance : anne.le-mire@etu.unistra.fr

Résumé:



In many diseases such as Alzheimer or cancer, aggregation of key proteins was observed and often accompanied by an excess of copper in the same area. Similarly, protein aggregation is a crucial mechanism in the antimicrobial effect of Cu. On the other hand, chaperone proteins such as the holdase HSP33, have been proven to protect bacterial cells from excess of Cu meaning that Cu has to activate these chaperones in a way they can stabilize or refold proteins[1]. The HSP33 holdase has so called Zn-finger. In this motif, Zn^{II} is coordinated by 4 cysteines which, under oxidative stress, will be oxidized to two disulfur bonds, that leads to Zn^{II}-release and a conformational change of HSP33[2] in order to activate it. Moreover, HSP33 activation by copper involves Cu^{I}. To better understand the mechanism of this activation, that could lead to design better antimicrobial compounds, in the presented study, the interaction of a Cu^{II} complex and a peptide model[3] of the HSP33 Zn finger is investigated. Cu^{II}-Dp44mT enters better cells than Cu salts which should increase its activity[4]. However, in contrast to many Cu-complexes it is keeping Cu^{II} coordinated even with an excess of glutathione (GSH), a biological reducing agent[5]. Hence, we were interested if Cu^{II}-Dp44mT is able to activate HSP33 which if it is the case should be by a different mechanism compared to Cu^{I} and could have a different selectivity due to the different coordination chemistry properties between Cu^{I} and Cu^{II}.

Here we show that, under low O2 conditions and 4mM of glutathione, Cu^{II}-Dp44mT is able to react with HSP33-model, releases Zn from the Zn finger and oxidizes the peptide leading to its activation. But, under anaerobic conditions, Cu^{II}-Dp44mT is also reduced slowly by GSH allowing a competition with a mechanism involving Cu^{I}.

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Identification et évaluation d'un nouveau motif antifongique pour traiter les Candidoses Invasives

Popczyk Pierre,[a,*] Spriet Corentin,[b] Bance Thomas,[b] Ghinet Alina,[c,d] Bortolus Clovis,[a] Sendid Boualem,[a] Dubar Faustine.[a]

a. INSERM U1285, Université de Lille, CHU de Lille, UMR CNRS 8576-UGSF—Unité de Glycobiologie Structurale et Fonctionnelle, 59000 Lille, France.

b. Plateforme d'imagerie cellulaire et tissulaire Biolmaging Center Lille (BICeL) Faculté des Sciences et Technologies, Bâtiment SN3, 59650 Villeneuve d'Ascq, France.

c. Sustainable Chemistry Team, Laboratory of Sustainable Chemistry and Health, Junia, Health & Environment Department, 59000, Lille, France.

d. Institut Pasteur Lille, U1167 - RID-AGE - Facteurs de Risque Et Déterminants Moléculaires Des Maladies Liées Au Vieillissement, University Lille, Inserm, CHU Lille, 59000, Lille, France.

*Correspondance : pierre.popczyk.etu@univ-lille.fr

Résumé :

Chaque année, les infections fongiques invasives (IFIs) engendrent 150 millions de cas graves, provoquant 1,7 millions de décès dans le monde.[1] Plus de 50% de ces infections sont causées par la levure opportuniste de *Candida*, qui atteint et colonise les organes profonds, ces infections sont alors qualifiées de candidoses invasives (CI). 5 espèces de *Candida* sont responsables de plus de 90% de ces infections, mais *Candida albicans* (*C. albicans*) reste l'espèce prépondérante avec un taux d'environ 57% des cas en Europe. L'actuel arsenal thérapeutique pour lutter contre les CI est constitué de 4 classes de molécules antifongiques, les échinocandines, les azolés, les polyènes et les dérivés pyrimidiques.[2] Cependant, des diagnostics tardifs et l'émergence de souches résistantes aux thérapeutiques actuelles telles que *Candida glabrata* (*C. glabrata*), *Candida krusei* (*C. krusei*) ou *Candida auris* (*C. auris*) entrainent des difficultés de prises en charge des patients et des impasses thérapeutiques dans le contexte hospitalier.[2] Cette situation inquiète l'Organisation Mondiale de la Santé (OMS), dont la lutte contre les résistances aux antimicrobiens est maintenant un axe majeur afin de préserver l'efficacité des antibiotiques et antifongiques.

Il devient urgent de développer de nouveaux traitements antifongiques spécifiques et sensibles pour lesquels les cibles thérapeutiques sont novatrices.

Dans ce contexte, le Dr Faustine Dubar a initié un projet de recherche afin de développer de nouvelles molécules ciblant la voie de biosynthèse du tréhalose chez *Candida*, qui est un dissaccharide non-réducteur important aux multiples rôles chez la levure, notamment de facteur de virulence et de protecteur en condition de stress. Une étude computationnelle de *de novo* design a permis d'identifier un motif aux propriétés antifongiques potentiellement intéressantes, le motif hydrazone.

Ainsi, la synthèse de divers dérivés possédant ce motif hydrazone, leur évaluation biologique et l'étude de leur impact sur différents facteurs biologiques ont permis d'étudier l'efficacité antifongique des composés sur divers paramètres du développement de *Candida*, notamment la filamentation, l'adhérence, mais aussi sur la formation du biofilm et leur action sur le biofilm mature.

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Keywords : Hydrazone ; Candida ; Antifongique

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Modification of the terminal functionality of desferrioxamine B for the synthesis of new Zr^{4+} chelators for applications in PET imaging

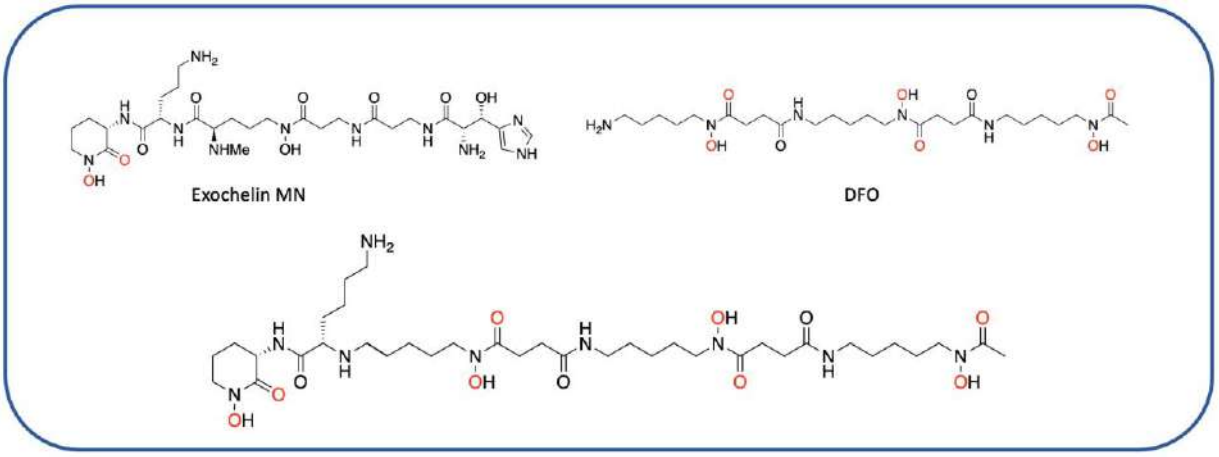
Laurie Zujew* [a,b], Laurent Raibaut[c], Jean-Claude Chambron[a], Alessio Imperiale[b]

a. Chimie des ligands à architecture contrôlée, Institut de chimie, UMR 7177, Strasbourg
b. Imagerie moléculaire, Institut pluridisciplinaire Hubert Curien, UMR 7178, Strasbourg
c. Biométaux et chimie biologique, Institut de chimie, UMR 7177, Strasbourg

* Correspondence: laurie.zujew@etu.unistra.fr

Abstract:

Positron emission tomography (PET) is an imaging technique used to detect and monitor the progression of cancer by administering a pharmaceutical compound containing a positron-emitting isotope. When this isotope is linked to an antibody that allows it to reach its target, this method is called immuno-PET.[1] ^{89}Zr is a radionuclide of interest for immuno-PET because its half-life time of 3 days is in accordance with the time required for the antibody to reach its target.[2] Nevertheless, the current chelators based mainly on desferrioxamine B (DFO) cause a release of ^{89}Zr which is found in the bones *in vivo*.[3] The synthesis of a new ligand composed of two siderophore chelating units from exochelin MN and DFO was considered but a modification of the terminal amine of the DFO was necessary to link these two parts. An enantiomerically pure chelator was synthesized from modified DFO bearing an aldehyde. This ligand is composed of 4 hydroxamate functions including a cyclic one allowing to complete the coordination number of Zr^{4+}.



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Keywords: Immuno-PET, Zirconium, Desferrioxamine B

Type de communication : communication orale☐flash ☐ affiche☒

Design and synthesis of protein complexes with non-classical topologies

Elodie Soehnlen,[a] Vladimir Torbeev[a]*

a. Équipe de Chimie des Biosystèmes, ESBS, UMR 7242 Biotechnologie et Signalisation Cellulaire
*Correspondance: torbeev@unistra.fr

Abstract:

Initially, proteins were described as “rigid” or “semi-rigid” assemblies, assumed to possess a unique and stable three-dimensional structure defining its biological function. Although a large number of proteins are part of this structure-function relationship, studies made by Dunker's team from the mid-1990s [1], by Wright and Dyson from the same epoch [2], and those of Uversky [3] revealed that many functional proteins (or functional protein regions) do not have well-defined three-dimensional structures: they are intrinsically disordered proteins (or regions) (IDPs).

IDPs play an important role in a wide range of biological functions. They exhibit a certain conformational flexibility and structural heterogeneity that allow them to target and interact with different interaction partners. In particular, IDPs are involved in the gene transcription machinery of the cell. This is the case for ACTR (p160) and p300/CBP which are transcriptional co-activators. These proteins are over expressed in many cancers and are involved in the formation of multiprotein complexes with nuclear receptors to regulate the transcription of many genes.

The interaction domains (the ACTR activation domain and the nuclear coactivator binding domain (NCBD) of p300/CBP) can be synthesized chemically. Indeed, different conformationally constrained variants of ACTR containing alpha-methylated amino acids were prepared [4]. Such modifications allowed to bias the conformational ensemble of the unstructured free activation domain of ACTR and to significantly increase its affinity for the nuclear coactivator binding domain (NCBD) of CREB-binding protein (CBP). In addition, the first X-ray structure of the modified ACTR domain in complex with NCBD could be solved.

In connection with this work, the objective is to deepen the study of the ACTR/NCBD complex as well as its influence on biological mechanisms. For this, [2]rotaxane protein complex will be designed and synthesized. Its synthesis will be carried out by cyclization of the ACTR activation domain when it is bound to NCBD. The cyclization will be carried out both by Native Chemical Ligation (NCL) [5] as well as by enzymatic ligation using the plant asparginyl endopeptidase, OaAEP1b [6]. We will also study the influence of the [2]rotaxane formation on the inhibition of protein-protein interactions in vitro. Ultimately, the objective is to study the formation of a protein complex with the topology of [2]rotaxane in the cells and the subsequent inhibition of native protein-protein interactions. This innovative study will pave the way for the development of new molecular tools to study the complexity of gene transcription regulation.

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Keywords: Intrinsically Disordered Proteins (IDPs); [2]rotaxane ; ACTR ; NCBD ; NCL reaction

Incorporation of fluoro-proline analogues into unstructured peptide sequences

Anastasiia Trostianetskaia [a,b], Bruno Kieffer [b], Vladimir Torbeev [a*]

a. *Équipe de Chimie des Biosystèmes, ESBS, UMR 7242 Biotechnologie et Signalisation Cellulaire*
b. *Structure et Dynamique des polymères biologiques par Résonance Magnétique Nucléaire, IGBMC*

*Correspondance: torbeev@unistra.fr

Abstract :

The internal organization of eukaryotic proteins can be divided into three types: 1) ordered proteins, 2) structured proteins with intrinsically disordered regions (IDPRs), and 3) intrinsically disordered proteins (IDPs). For a long time, it was believed that the role of IDPRs in protein sequences was to bind structured domains. However, recent studies have shown that they are also involved in various functions mediated by proteins [1]. For example, they are subjected to extensive posttranslational modifications, which increase the functional states in which the protein can exist in the cell.

Our work focuses on the study of intramolecular interactions between SH3 domain and proline rich motifs (PRMs) of amphiphysin 2 (BIN1) related to centronuclear myopathies (CNM), a family of genetic diseases leading to muscle weakness and atrophy [2]. These diseases are due to mutations within either BIN1 or dynamin 2 (DNM2). One of the IDPR sequence characteristics is the abundance of proline residues, either as part of a polyproline domain, or as part of a Xaa-Pro phosphorylation site. Proline is described as the most disorder-promoting residue when present as a single residue in a peptide chain [3].

While molecular disorder excludes the use of X-ray and cryo-EM approaches, the use of Nuclear Magnetic Resonance (NMR) faces the difficult issue of assignment of individual proline residues within a polyproline homopolymer environment due to overlapping proton signals. In our work, we replaced two proline protons with fluorines, which allowed us to use fluorine NMR not only as convenient tools for residue-specific assignment (achieved through spectral dispersion of the proton signals of a given fluorinated proline) but also as a powerful method to study protein structural and dynamic properties of prolines in biological context.

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Keywords: Fluoroproline; Intrinsically Disordered Protein (IDP); fluorine NMR; proline rich motifs (PRM); amphiphysin 2 (BIN1).

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Tittle : Chemical biology of intrinsically disordered protein domains

The abstract title should be short and informative, and must not exceed 200 characters.
It must be written in 'sentence case' – capitalise only the first word and any words or abbreviations that must be capitalised, e.g. Revisiting the Staphylococcus aureus SarA regulon by high-throughput screening.
Do not write the title in all capital letters.

Alexis Jouin [a]*, Vladimir Torbeev [a],

[a] Biotechnologie et signalisation cellulaire, Université de Strasbourg
* Correspondance : ajouin@unistra.fr

Abtract :

In this study, we propose an approach to target complex formation that involves IDPs that is based on conformational editing of the IDP domain by utilizing backbone conformational constraints (α-methylation) to facilitate binding and stabilize or destabilize helical structures. I am performing the synthesis of several new variants of Activation Domain 1 (AD1) of transcriptional corgulator ACTR and work on the characterization of their complexes with Nuclear Co-activator Binding Domain (NCBD) of CREB-binding protein (CBP) by different approaches (NMR, ITC, X-ray crystallography, Native MS, Ion mobility, Fluorescence anisotropy decay, Circular dichroïsm). I will also study different vectorization pathways for delivery into cells (electroporation,polymer, nanoparticles). Our next goal is to make the screening of small molecules to find some hits as potential inhibitors of ACTR/CBP interactions. In this poster, I will present the synthesis of three new variants of AD1-ACTR as well as the optimization of their synthesis in order to reduce the aspartimide formation occuring in precedent protocols applied. Moreover, I will introduce early results of Circular dichroïsm, ITC and Native MS.

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Bauer, V.; Schmidtgall, B.; Gógl, G.; Dolenc, J.; Osz, J.; Nominé, Y.; Kostmann, C.; Cousido-Siah, A.; Mitschler, A.; Rochel, N.; Travé, G.; Kieffer, B.; Torbeev, V. Conformational Editing of Intrinsically Disordered Protein by α-Methylation. *Chem. Sci.* **2021**, *12* (3), 1080–1089. https://doi.org/10.1039/D0SC04482B.

Keywords: IDP domains; α-methylation; AD1- ACTR, NCBD-CBP,

One page maximum

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☐

Synthesis of Glycolipids for the Development of New Vaccine Adjuvants with Modulation of the Immune Response.

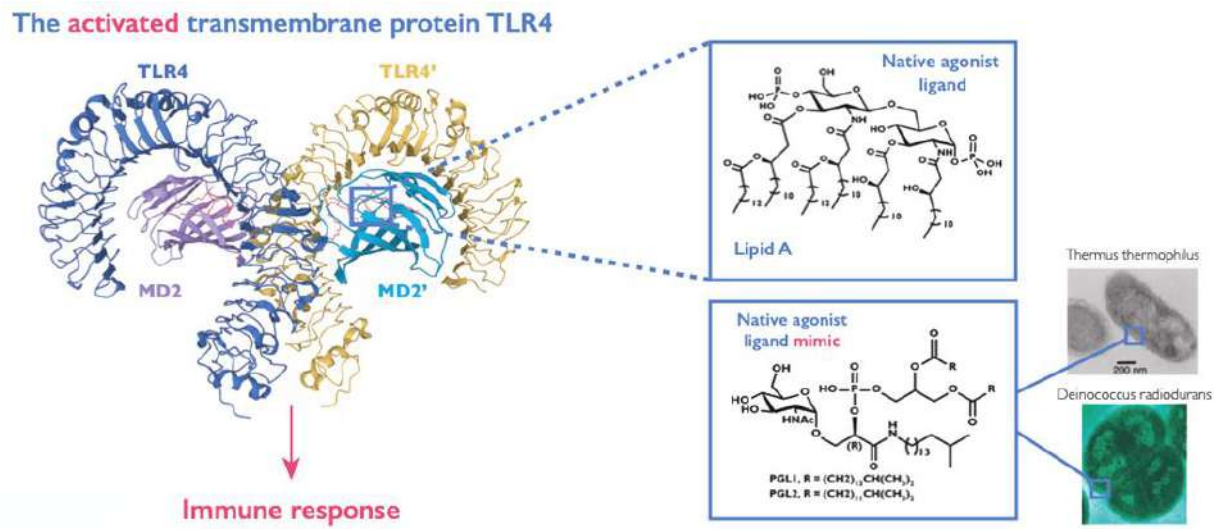
Jonathan Martins [a], José Kovensky [b], Sylvestre Toumieux [a], Sylvain Laclef [a*]

a. Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources (LG2A, UR7378), Amiens

* Correspondance : sylvain.laclef@u-picardie.fr

Abtract :

The development of vaccines has become crucial in the fight against influenza pandemics as well as other diseases such as tuberculosis and HIV. A major factor in their effectiveness is the adjuvant. This component can induce an innate immune response, which can then trigger the development of specific acquired immunity. The use of bioactive adjuvants, agonists of TLR4, is beneficial, as observed with lipid A.[1] The aim of this study is to develop new adjuvants from the structure of natural products isolated from membranes of extremophilic bacterias.[2] A new synthesis methodology developed in the laboratory will be used for a more efficient total synthesis of these molecules.[3] Molecular modeling will allow the synthesis of biomimetic analogues, obtained by chemical modification of the natural product with higher TLR4 activity. Their physical properties and their ability to form liposomes will be measured and the most promising molecule will be formulated as an adjuvant.



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Keywords: TLR4, Glycolipids, Vaccine, Extremophile, Biomimetic

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Octadentate bispidine chelators for Tb(III) complexation: pyridine carboxylate versus pyridine phosphonate donors.

Lucas Petitpoisso [a], Anli Mahamoud [a], Aline Nonat [a]

a : Equipe de Synthèse pour l'Analyse, Université de Strasbourg, CNRS, IPHC UMR 7178, F-67 037 Strasbourg, France.
Email: aline.nonat@unistra.fr

Abtract :

With their rigid and preorganized skeleton, bispidine chelators (3,7-diazabicyclo[3.3.1]nonane) are very appealing for the formation of metal complexes with high kinetic inertness. With the aim to develop new Tb(III)-based medical imaging probes, this study presents the synthesis and physico-chemical properties and luminescence properties of two novel terbium(III) complexes with octadentate bispidine ligands substituted with either pyridinephosphonate (H6L1) or picolinate (H4L2) units.

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Keywords: Bispidine, Terbium, PET

Type de communication : communication orale ☐ flash ☐ duo ☐ affiche ☒

Development of a new detection method for small analytes based on the fluorogenic supramolecular assembly of aptamers

Karkori Rim [a,b], **Nicolas Spinelli** [a], **Isabelle Baussanne** [b]

a. Département de Chimie Moléculaire, Université Grenoble Alpes, UMR 5250 CNRS, Grenoble, France
b. Département de Pharmacologie Moléculaire, Université Grenoble Alpes, UMR 5063 CNRS, Grenoble, France

* Correspondance : rim.karkori@univ-grenoble-alpes.fr

Abtract :

There is an increasing demand for the detection of small molecules in order to monitor human health (molecular markers of diseases, toxins, heavy metals, etc.) A notable limitation of aptasensors for small analytes is their low sensitivity due to non-specific and false positives signals. To circumvent this drawback, the split aptamer strategy was developed by Stojanovic et al [1], in which an aptamer is split into two fragments that can specifically form a ternary assembly in the presence of the ligand. Because of this dual recognition mechanism, the resulting specificity and sensitivity is significantly increased. Unfortunately, splitting decreases affinity, and the resulting ternary complex is quite unstable. To overcome this issue, Yang et al [2] introduced a host-guest signaling system into a split-aptasensor in which β-cyclodextrin and dansyl were respectively conjugated at the ends of the two fragments of an adenosine aptamer: the inclusion complex provided a supplementary interaction and induced stabilizing effects. In this project, both the stabilizing interaction and the transduction signal are due to the formation of a fluorescent indolizine occurring upon binding of the split-aptamers to their target, with each strand carrying a precursor of the fluorophore (figure A). In this construct, the [3+2] dipolar cycloaddition of an in situ generated pyridinium ylide with an activated alkyne is said to be DNA-templated as it is catalyzed by the binding of the aptamer strands to their ligand.

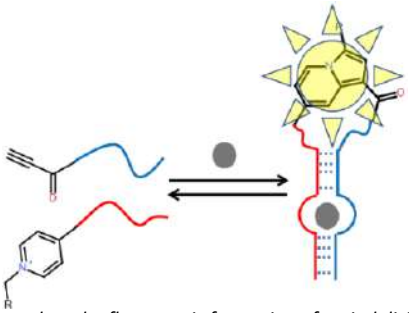

Figure A. Aptasensor model based on the fluoregenic formation of an indolizine via 1,3-dipolar cycloaddition

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Keywords: Aptamer, Fluorescence, Indolizine, Templated Chemistry, Turn-On

Acidic conditions increase the benefit of hydroxamate siderophore exploitation for the bacterial pathogen *Salmonella enterica*

Manon Ferry[1], Isabelle Schalk[1], Olivier Cunrath[1]
[1]Institut de Biotechnologie et Signalisation Cellulaire, UMR 7242, Illkirch

Infectious diseases remain one of the leading causes of death worldwide[1]. Due to high microbial adaptation capacities, our solutions for disease control and prevention are continually challenged[2]. One of the most important bacterial adaptations in infectious environment concerns **the supply of iron**[3]. This metal is a nutritional resource essential for almost all living organisms including pathogenic microorganisms. A common way for bacteria to acquire this 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,** present in their environment. This is the case of the pathogenic bacterium *Salmonella enterica*, which can exploit at least three exogenous siderophores (exo-siderophores) in addition to produce its own siderophores to acquire iron[4]. Although the nature of the three exo-siderophores that *S. enterica* can exploit to acquire iron has been identified, little is known about the environmental conditions favoring an ecological advantage of exo-siderophore exploitation. **In this study, the goal is to assess whether acidity of the intestinal environment may influence the exploitation of exo-siderophores in *S. enterica*.** In an interesting way, we found that acidic growth conditions increase the growth benefit associated to exo-siderophores exploitation more strongly than at neutral pH in *S. enterica*. Understanding how, when and where siderophores can be exploited by pathogens will give us important insights into pathogen biology and will be crucial for the design of pathogen control strategies.

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Preclinical evaluation of Lysyl Oxidase-like 2 as a target for the nuclear imaging of idiopathic pulmonary fibrosis

Romane Vizier [a], Anaïs-Rachel Garnier [b], Alexandre Dias [b], Mathieu Moreau [a], Michael Claron [a], Bertrand Collin[a,b], Franck Denat [a], Pierre-Simon Bellaye [b], Victor Goncalves [a]

a. Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR 6302, CNRS, Université de Bourgogne, 9 avenue Alain Savary, 21078 Dijon Cedex, France
b. Centre Georges François Leclerc, Service de Médecine Nucléaire, Plateforme d'Imagerie et de Radiothérapie Précliniques, 1 rue du Professeur Marion, 21079 Dijon Cedex, France

* Correspondance : romane.vizier@u-bourgogne.fr

Abstract :

Non-invasive imaging of idiopathic pulmonary fibrosis remains a challenge. IPF is a chronic and irreversible disease, characterized by an excessive and progressive deposition of extracellular matrix in the lung parenchyma, leading to the destruction of the lung architecture.[1] Lysyl Oxidase-like 2, an enzyme involved in the fibrogenesis process, contributes to collagen cross-linking in the ECM by catalyzing the oxidative deamination of lysine in collagen.[2] The aim of this study was to develop an antibody-based radiotracer targeting LOXL2, for *in vivo* SPECT/CT imaging of pulmonary fibrosis. AB0023 (Arresto Biosciences), a murine antibody targeting LOXL2, was selected as targeting agent.[3] Site-specific chemoenzymatic conjugation of DOTAGA-PEG_4-NH_2 to AB0023 was done using microbial transglutaminase. Biolayer interferometry (BLI) measurements were conducted in order to determine the affinity of the immunoconjugate for LOXL2. *In vivo* experiments were performed on a fibrotic mice model obtained after intratracheal administration of bleomycin (2 mg/kg). The immunoconjugate was radiolabeled with indium-111. [^{111}In]In-DOTAGA-AB0023 was injected in two groups of mice (control and fibrotic). SPECT/CT images were recorded after 24h, 48h, 72h and 96 hours p.i.. An *ex vivo* biodistribution study was performed by gamma counting. To conclude, we report the first tracer targeting LOXL2. It was produced efficiently through an enzymatic site-specific conjugation. The radioimmunoconjugate showed promising results in a preclinical model of bleomycin-induced pulmonary fibrosis, with high lung uptake in fibrotic areas. These findings encourage us to study this tracer in other types of fibrosis.

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Keywords: enzymatic bioconjugation; LOXL2; idiopathic pulmonary fibrosis; SPECT/CT imaging

Photoactivation of (pro-)activity-based proteome profiling probes to investigate the interactome of the antimalarial drug plasmodione

Ilaria Iacobucci,[1,2] Agnès Hovasse,[2] Christine Schaeffer-Reiss,[2] Sarah Cianferani,[2] Mourad Elhabiri[1] and **Elisabeth Davioud-Charvet**[1,*]

[1] *Chimie Bio(IN)organique et Médicinale (CBM), LIMA UMR 7042, 67087, Strasbourg,*
[2] *Laboratoire de Spectrométrie de Masse BioOrganique, Université Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg,*

Keywords: antimalarial, click, photoaffinity labeling, proteome, redox

email: elisabeth.davioud@unistra.fr

One of the main research topic of the CBM team is focused on the development of redox-active antiparasitic drug-candidates based on the 3-benzylmenadione core. Experimental data indicate that the early lead drug, called plasmodione (PD), could exhibit several modes of action (MoA) via its metabolite (PDO), depending on parasitic stages, and protein targets. Accordingly, recent studies in yeast identified Nde1 of the mitochondrial respiratory chain, as the main target of PD, and other flavoenzymes as minor targets. We expect that other flavoenzymes are likely to contribute to PD activity. To identify them and fully characterize PD MoA we propose to use an activity-based protein profiling (ABPP) strategy in *Plasmodium* parasites. For this purpose, we synthesized PD- and PDO-derived ABPP probes (Fig. 1) and used the PDO-based probes, which possess intrinsic benzophenone-like moiety, to crosslink to proteins upon photoirradiation.[1] Finally, we optimized the conditions of UV-crosslinking, click reaction and pull down of the probe-protein adducts in physiological media with minimum side reactions and protein precipitation, with the recombinant model glutathione reductase. Before starting with the investigation of the proteome from the malaria parasite, we started to set up the activity-based protein profiling (ABPP) conditions on model proteomes from *Saccharomyces cerevisiae* and *E. coli*.

A) Structures of the PD-and PDO-derived probes

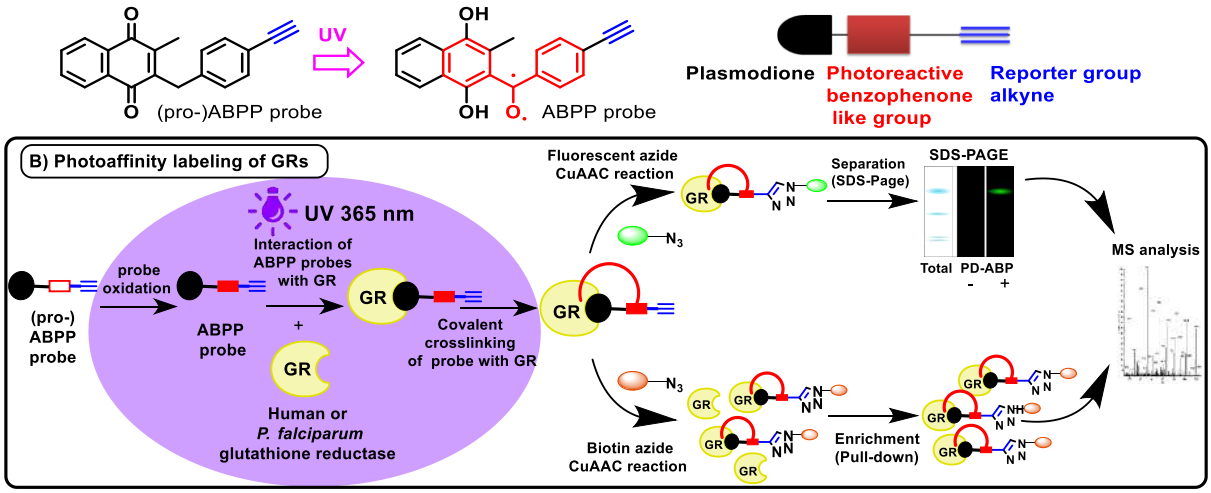

Fig.1. ABPP strategy used to photolabel proteins with designed (pro-)PD-ABPP probes.

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Type de communication : communication orale ☐ flash ☐ affiche ☒

Large scale synthesis of trypanosomatid cap 4 RNA upon use of an alkyl-chain-soluble support.

Sophie Vichier-Guerre[1], Lidia Watanabe Reolon [2,3], Bruno Moisés de Matos [2,3], Laurence Dugué[1], Tatiana Reichert da Silva Assunção [2], Nilson Ivo Tonin Zanchin [2], Sylvie Pochet[1], Beatriz Gomes Guimarães [2,3]

[1] Unité de Chimie Biologique Epigénétique, groupe Chimie et Biocatalyse, Institut Pasteur, CNRS UMR3523, Université Paris Cité, Paris, France

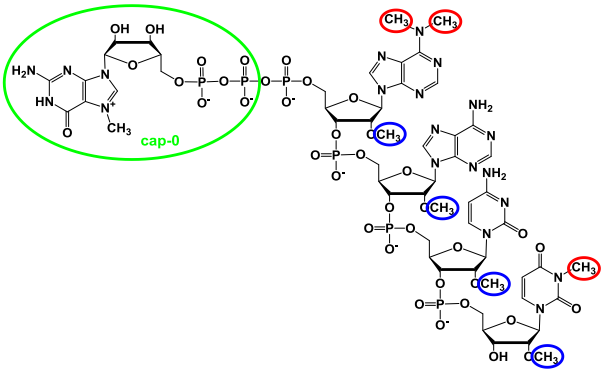
[2] Carlos Chagas Institute, Oswaldo Cruz Foundation, FIOCRUZ-PR,

[3] Biochemistry Postgraduate Program, Federal University of Parana, Parana, 81350-010, Brazil

* Correspondance : sophie.vichier-guerre@pasteur.fr

Summary:

The association of the initiation factor eIF4E with the mRNA cap structure is a key step for translation. Trypanosomatids present six eIF4E homologues, showing a low conservation and differing significantly from the IF4Es of multicellular eukaryotes. On the mRNA side, the trypanosomatid mRNA features a cap-4, which is formed by a cap-0 (7-methyl-GTP), followed by the AACU sequence containing 2'-O-ribose methylations and base methylations on nucleotides 1 and 4. Studies on eIF4E-cap-4 interaction have been hindered by the difficulty to synthesize this elaborated cap-4 sequence. To overcome this problem, we have developed a robust and efficient access to trypanosomatid cap-4 (tryp cap-4) based on a liquid-phase oligonucleotide methodology [1].



This approach significantly reduces the amount of phosphoramidite derivatives for RNA assembly (from 10 to 2.5 equivalents) and consequently the costs and time for the preparation of large quantity of trypa cap-4. All the synthetic steps have been validated affording tryp cap-4 up to 30 mg, allowing the first structural studies of the interaction of trypa cap-4 with *T. cruzi* EIF4E5 [2].

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Keywords: alkyl-chain-soluble support, cap 4, trypanosomatid, eIF4E

Type de communication : communication orale ☐ flash ☐ affiche ☒

Development of xanthene-based fluorogenic amides for bioimaging.

Carolina Chieffo,[a,b] Blaise Dumat,[a] Michele Fiore [b]

a. Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL Université, Sorbonne Université, CNRS, 75005 Paris, France
b. Université de Lyon, Claude Bernard Lyon 1, Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, ICBMS, UMR 5246, Bâtiment Lederer, 69622 Villeurbanne Cedex, France

* Correspondance: carolina.chieffo@ens.psl.eu

Résumé:

Fluorescence microscopy is a key method for cell study on molecular scale and scientific efforts have been spent in the development of specific probes for different applications, such as to monitoring the presence of an analyte or the change of environment. The goal of this research is to improve and diversify the tools, developing probes based on xanthenes. Xanthene-based dyes have pivotal role in this field thanks to their good biocompatibility, low toxicity, high quantum yields and availability in a wide range of emission wavelengths. The xanthene scaffold and the aromatic ring linked to it can both be subject of chemical alterations or modification. However, the amidation of the carboxylic moiety of the aromatic ring is particularly captivating. The fluorescence of the resulting probes can be controlled thanks to the presence of a dynamic equilibrium between two forms: the open fluorescent amide and the closed dark spirolactam. While the presence of an electron-withdrawing group (EWG) on the amide shifts the equilibrium towards the fluorescent form,[1,2] electron-donor (ED) bearing amides have tendency to stay in the spirolactam, and may have a turn-on effect selectively in presence of specific analytes.[3,4] We widened the scope to rhodamines, fluorescein and a class of less explored compounds, the rhodafluors (or rhodols) [5] to obtain probes exploitable in a wide range of wavelengths.

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Keywords: fluorescent probes, xanthenes, amides, spirolactam

Dual labeling of low-density lipoproteins to image and characterize atherosclerosis with elevated LDL uptake

Malorie Privat[1], Honorine Jardel[2, 3], Elisa Chazeau[1, 4, 5], Cindy Racoeur[4, 5], Adrien Dubois[1], Pierre-Simon Bellaye[6], Bertrand Collin[1,6], Alexandre Dias[6], Naig Le Guern[2, 3], Victor Goncalves[1], Mathieu Moreau[1], Catherine Paul[4, 5], Christine Goze[1], Franck Denat[1], Thomas Gautier[2, 3]

[1] Université de Bourgogne Franche-Comté, ICMUB, Dijon, France
[2] Université de Bourgogne Franche-Comté, LNC UMR1231 INSERM, Dijon, France
[3] LabEx, LipSTIC, Dijon, France
[4] Université de Bourgogne Franche-Comté, LIIC, Paris, France
[5] PSL Research University EPHE, Laboratoire d'Immunologie et Immunothérapie des Cancers, Dijon, France
[6] Centre Régional De Lutte Contre le Cancer Georges-François Leclerc, CGFL, Dijon, France

* Correspondance : malorie.privat@u-bourgogne.fr

Abstract :

Dysregulation of LDL uptake is critical in atherosclerosis onset and progression. The overall objective of our project is to develop a MOMIP (Monomolecular Multifunctional Imaging Probe) for the double labeling of LDL (radioactive/fluorescent) in a stable manner, to detect arterial areas that display high LDL uptake *in vivo*, and to precisely reveal the areas of interest in relation with atherosclerotic plaque phenotype. For the development of MOMIPs, we considered a fluorophores Cyanine-5, a DOTAGA for the chelation of 111In, and a squaramate as a covalent binding group. Bioconjugation of the probes was performed at 37°C in a pH 9.3 buffer. The physico-chemical properties of the bioconjugates were analyzed by electrophoresis and lipid extraction. The functionality of our labeled LDL was tested in vitro with CT26 cells expressing or not the LDL receptor. In vivo, after i.v. injection of labeled LDL in wild-type and LDLR-KO mice fed with a western-type diet, en-face, and histological fluorescence imaging was performed respectively on whole aortas and aortic sinuses. Our MOMIP allowed fast and efficient labeling and it specifically bound ApoB, the main protein of LDL. The 30-fold decrease in the uptake of these labeled LDL by CT26 cells after LDLR invalidation demonstrated that our selected MOMIP does not impair LDL recognition by their specific cellular receptor. En-face imaging of aortas from mice with atherosclerosis showed a high level of fluorescence in the aortic arch, whereas healthy animals displayed barely detectable signals. Finally, histological analyses showed fluorescence accumulation in specific areas of the atherosclerotic plaques. In this study, a MOMIP was selected for stable LDL labeling. This probe showed a minimal impact on the biological properties of LDL while providing efficient and specific fluorescent labeling *in vivo*. Our LDL conjugates appear promising for dual imaging, allowing the visualization and analysis of the atherosclerotic plaques.

Keywords: Atherosclerosis,LDL uptake,dual probe,fluorescence/PET imaging

Dual labelling of an anti-CEA Nanobody for PET and fluorescence imaging *via* a chemically synthesised trivalent platform

Jelena Saliën,[a,b] Romane Vizier,[a] Marcus C.M. Stroet,[b] Michael Claron,[a] Delphine Vivier,[a] Jessica Bridoux,[b] Sam Floru,[b] Sophie Hernot,[b] Victor Goncalves,[a] Franck Denat.[a*]

a. Université de Bourgogne, Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR CNRS 6302, Dijon, France
b. Vrije Universiteit Brussel, In Vivo Cellular and Molecular Imaging, Brussels, Belgium

* Correspondance : Franck.Denat@u-bourgogne.fr

Résumé:

The combination of PET/CT and near-infrared (NIR) fluorescence offers unique possibilities for image-guided surgery, since it allows surgical planning and real-time intra-operative guidance, respectively. We developed a bimodal tracer based on a chemically synthesised trivalent platform and a Nanobody® (Nb) as the targeting molecule because of its rapid pharmacokinetics and specific tumour uptake.[1] NODAGA was incorporated to allow ^{68}Ga-radiolabelling at room temperature, and the next generation NIR fluorophore s775z was selected for its improved properties compared to IRDye®800CW.[2]

Diethyl squarate was attached to a lysine-based trivalent platform *via* a PEG_3 spacer as a first step to synthesise the bimodal tracer. Subsequently, a NODAGA chelator and the NIR fluorophore s775z were coupled stepwise to the ε- and α-amino groups of the lysine, respectively. Tracer purification steps were performed *via* semi- preparative reverse-phase (RP) HPLC, and the purity of the products was confirmed by RP-HPLC-MS. The bimodal tracer was randomly conjugated to an anti-CEA Nb in carbonate buffer (pH 9.7, c = 0.1 M) at 37 °C to obtain the targeted tracer, and the degree of labelling (DOL) was determined by RP-HPLC-MS. Radiolabelling with 68Ga was performed at room temperature at pH 5, and its radiochemical purity was confirmed by radio-HPLC.

As a first step in the tracer development, optimisation steps including adaptations in protection/deprotection strategies and amide coupling reagents were performed. Ultimately, the imaging probe was synthesised from commercially available precursors in 7 steps with a 5% overall yield. A sufficient amount in the milligram range was obtained for preliminary testing. Afterwards, a bioconjugate with an average DOL of 1 was successfully obtained. Radiolabelling of the chemically synthesised probe with ^{68}Ga was performed, leading to a radiochemical purity > 99% after 10 minutes at room temperature.

In this work, we have successfully synthesised a bimodal tracer incorporating a nuclear and fluorescent label, and a targeted tracer was obtained by bioconjugation to an anti-CEA Nb. Furthermore, we obtained > 99% labelling of the probe with ^{68}Ga after 10 minutes. Next steps include upscaling of the synthesis procedure and further *in vitro* and *in vivo* characterisation of the imaging tracer.

This work was supported by UBFC Excellence Initiative Project ISITE-BFC. We gratefully acknowledge the PACSMUB platform (Université de Bourgogne, Dijon, France) for the analytical data.

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$Mn^{II/III}$ – Bispidine complexes for redox-activated MRI probes

Anli Mahamoud,[1,2] *Aline Nonat*[1] *and Eva Jakab Toth*[2]

1. Equipe de Synthèse Pour l'Analyse, Université de Strasbourg, CNRS, IPHC UMR 7178. (anli.mahamoud@etu.unistra.fr). https://iphc.cnrs.fr/
2. Centre de Biophysique Moléculaire, CNRS UPR 4301, Université d'Orléans. http://cbm.cnrs-orleans.fr/en/le-laboratoire-accueil/

Abstract:

Recent achievements in the synthesis and coordination chemistry of bispidines (3,7-diazabicyclo[3.3.1]nonane) have paved the way to a new field of application of bispidine coordination complexes: Magnetic Resonance Imaging (MRI). These derivatives are highly pre-organized ligands that can coordinate metal ions with cis-octahedral, square-pyramidal, or pentagonal geometries.[1] This pre-organization allows bispidine ligands to be a privileged scaffold for metal-based imaging agent. The search for more biocompatible alternatives to Gd^{3+}-based MRI contrast agent led us to $^{55}Mn(II)$ which is biogenic and less toxic. For 35 years now, Gd-complexes have been used in millions of human examinations and are considered among the safest diagnostic drugs. However, almost one third of the commercially available Gd^{3+}-based contrast agents have been suspended from the market since 2017. When Gd^{3+} is released into the body of patients with advanced kidney disease, Gd^{3+} retention is responsible of nephrogenic systemic fibrosis a sever, even lethal disease.[2] Herein, we propose new bispidine ligands for $Mn^{II/III}$. We have developed a series of $Mn^{II/III}$-bispidine complexes with exceptional kinetic inertness and competitive relaxivities to Gd-chelates.[3, 4] Herein we present new ligands for $Mn^{II/III}$ complexation to the use as redox MRI probes.

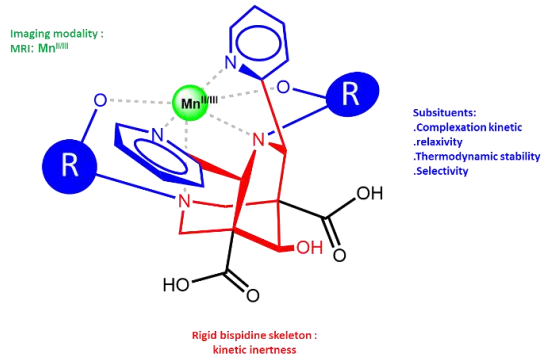

Figure 1 Kinetically inert bispidine complexes for MRI

Keywords: medical imaging, chelators, redox MRI, contrast agent

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Type de communication : communication orale ☐ flash ☐ affiche ☒

Synthèse de *N*-acétylchitooligosaccharides fluorescents : Applications à la caractérisation du transport lysosomal d'oligosaccharides (LOST)

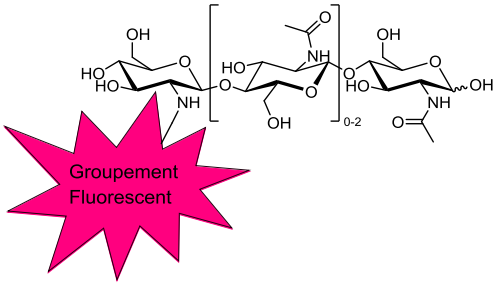
Bosco Michaël,[a*] Bouzidi Younès,[b] Gao Haïfei,[a] Pradeau Stéphanie,[c] Chantret Isabelle,[b] Busca Patricia,[a] Fort Sébastien,[c] Moore Stuart E. H. ,[b] Gravier-Pelletier Christine[a]

a. Université Paris Cité, CNRS UMR8601, 45 rue des Saints Pères, 75006 Paris, France.
b. Université Paris Cité, INSERM U1149, 16 rue Henri Huchard, 75018 Paris, France.
c. Université Grenoble Alpes, CNRS, CERMAV, 38000 Grenoble, France.

* Correspondance : michael.bosco@parisdescartes.fr

Résumé :

Les oligosaccharides libres de type polymannose (fOS) sont traités par des enzymes cytosoliques pour générer du $Man_5GlcNAc$ qui est transporté dans les lysosomes pour y être dégradé[1]. Le transport lysosomal d'oligosaccharides (LOST) a été démontré *in vitro* en utilisant des lysosomes de foie de rat, mais cette activité est mal caractérisée et n'a pas été identifiée au niveau moléculaire. Nous développons des outils chimiques pour étudier LOST *in vitro* et des sondes fluorescentes variées, à base de chitooligosaccharides (COS, oligomères linéaires de *N*-acétylglucosamine liés en β1,4), comme substrats potentiels de LOST.



Ces chitooligosaccharides sont préparés à partir de chitine par des procédés chimioenzymatiques[2]. Ils sont ensuite couplés à différents groupements fluorescents comme la rhodamine B, la coumarine ou le BODIPY.

Sur cette affiche, l'utilité de ces oligosaccharides modifiés à leur extrémité non réductrice sera abordée pour la caractérisation du transport lysosomal d'oligosaccharides.

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Keywords: oligosaccharide transport; fluorescent labels; biochemical applications.

Type de communication : communication orale ☐ flash ☐ affiche ☒

A Modular One-Pot Strategy for the Synthesis of Bivalent Tracers

Bailly Thibaud,[a] Prignon Aurélie,[b] Morgat Clément,[c] Victor Goncalves,[a] Franck Denat, [a] Ibai Valverde [a*]

a. Institut de Chimie Moléculaire de l'Université de Bourgogne, ICMUB UMR CNRS 6302, Université Bourgogne Franche-Comté, Dijon;
b. Laboratoire d'Imagerie Moléculaire Positonique, LIMP UMS28, Sorbonne Université, Paris
c. Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, INCIA UMR 5287, Université de Bordeaux, Bordeaux

* Correspondance : ibai.valverde@u-bourgogne.fr

Résumé :

Over the last years, there has been an increasing interest for multireceptor targeting justified by tumor heterogeneity and the concomitant overexpression of different membrane receptors by tumor cells[1]. In this context, monomolecular heterobivalent tracers are expected to offer higher affinity for tumor tissue as well as longer residence time on tumor cells compared to the corresponding monovalent tracers[2]. A striking example of multireceptor overexpression is prostate cancer where both PSMA and GRPR are overexpressed[3,4].

In this study we describe a modular and straightforward method that provides access to such heterobivalent tracers, based on a trifunctional pyridazine platform5. A series of bivalent ligands were successfully synthesized and the reported procedure was modified to be performed in a one pot approach, thus increasing the yields. A heterobivalent conjugate targeting PSMA and GRPR, NODAGA(KuE-pdz-JMV594), was successfully radiolabeled with ^{68}Ga and the pharmacological properties of the radiolabeled tracer (logD, KD, internalization, efflux) were evaluated on PC3 and LNCap cell lines. The heterobivalent conjugate was also radiolabeled with ^{68}Ga and evaluated in vivo on double-xenograft athymic nude mice. PET-CT images showed uptake of the tracer in both PSMA-positive and GRPR-positive tumors. These results were further confirmed with an ex vivo study.

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Keywords: Bioconjugation, Click chemistry, Drug delivery, Heterobivalent ligands, Radiopharmacy

Type de communication : communication orale ☐ flash ☐ affiche ☒

Mechanism-guided development of covalent prodrugs for targeted anticancer therapy

Margaux Bossuat,[a,b,c] Nadège Preuilh,[c] Stéphanie Ballereau,[a] Antonio Peixoto,[c] Frédéric Rodriguez,[a] Isabelle Fabing,[a] Vania Bernardes-Génisson,[b] Valérie Maraval,[b] Remi Chauvin,[b*] Sébastien Britton,[c*] Yves Génisson[a*]

a. SPCMIB, UMR 5068, CNRS-Université Toulouse III, Toulouse, France
b. LCC, UPR 8241, CNRS, Toulouse, France
c. Institut de Pharmacologie et de Biologie Structurale, IPBS, Université de Toulouse, CNRS, Toulouse, France

* Correspondance : yves.genisson@univ-tlse3.fr

Résumé :

Naturally occurring acetylenic lipids are a unique source of inspiration for the development of novel pharmacologically relevant compounds.[1] A series of anti-cancer chiral Lipidic AlkynylCarbinols (LACs) was devised by introducing an (hetero)aromatic ring between the aliphatic chain and the dialkynylcarbinol chiral warhead. The resulting Phenyl diAlkynylCarbinols (PACs) or heteroaromatic analogues were shown to exhibit enhanced intrinsic stability, while preserving the range of cytotoxic activity against HCT116 and U2OS cancer cells, with IC_{50} values down to 42 nM for a PAC eutomer.

A set of 30 racemic PACs with various substitution patterns and motifs were synthesized via three alternative routes. Enantioenriched samples were produced by chiral supercritical fluid chromatographic resolution of a racemic sample. Viability assays on HCT116 and U2OS cells allowed the generalization of the structure-activity relationships established for non-aromatic LACs.[2]

Thanks to fluorescent labelling, an alkyne-tagged clickable PAC probe confirmed that, similarly to other LACs presenting a dialkynylcarbinol warhead, PACs behave as cytotoxic pro-drugs: after enantiospecific bio-oxidation of the secondary carbinol center by the HSD17B11 Short-chain Dehydrogenase/Reductase, the resulting ynones covalently modify surrounding cellular proteins as Michael acceptors, leading to endoplasmic reticulum stress, ubiquitin-proteasome system inhibition and apoptotic cell death.[3] Finally, comparing the cellular bioactivation of a panel of PACs by HSD17B11 and its paralog HSD17B13 provided the first insights into the design of LAC prodrugs with enhanced selectivity.

Altogether this work provides evidence that the PAC series offers a promising direction to evolve synthetic LAC into bioinspired anticancer prodrugs with an original mechanism of action.

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Keywords: marine lipids, clickable probes, mechanism of action, covalent anticancer agents

Type de communication : communication orale ☐ flash ☐ affiche ☒

In-cell synthesis of cytotoxic phenanthridine through bioorthogonal cyclization: the "Cycl'in-Cell" strategy

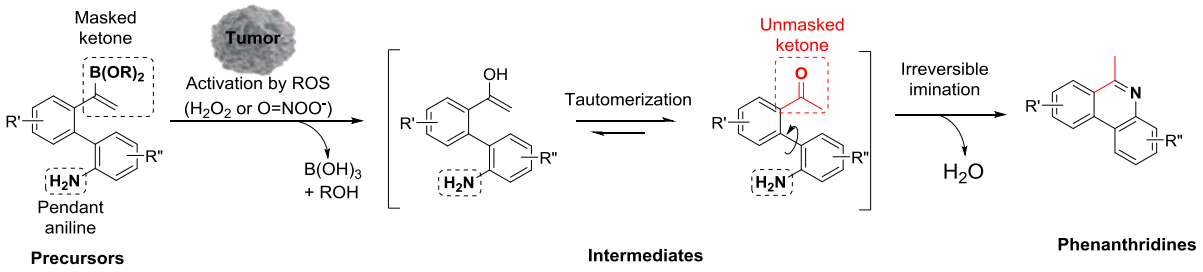
Maslah Hichem,[a] Skarbek Charles,[a] Jullien Ludovic,[b] Le Saux Thomas,[b] Labruère Raphaël.[a,*]

a. Institut de chimie moléculaire et des matériaux d'Orsay, CNRS, Université Paris-Saclay, 91405, Orsay, France.
b. PASTEUR, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 24, rue Lhomond, 75005 Paris, France.

* Correspondance : raphael.labruere@universite-paris-saclay.fr

Résumé :

Pharmacological inactivation of antitumor drugs toward healthy cells is a critical factor in prodrug development.[1] Typically, pharmaceutical chemists graft temporary moieties to existing antitumor drugs to reduce their pharmacological activity as much as possible.[2] Here, we report a platform where the structure of the prodrug excludes the preexisting antitumor drug motif and is based on an inactive synthetic precursor able to generate the cytotoxic agent by bioorthogonal cyclization within a tumor environment. Using phenanthridines as cytotoxic model compounds, we designed ring-opened biaryl precursors that generated the phenanthridines through bioorthogonal irreversible imination. This reaction was triggered by reactive oxygen species, commonly overproduced in cancer cells,[3] able to convert a vinyl boronate ester function into a ketone that subsequently reacted with a pendant aniline. An inactive precursor was shown to engender a cytotoxic phenanthridine against KB cancer cells. Moreover, the kinetic of cyclization of this prodrug was extremely rapid (< 10 ms) inside living cells of KB cancer spheroids so as to localize drug action.[4] The synthesis and in cellulo results of the phenanthridines and precursors will be described in this presentation.



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Keywords: Prodrug; Antitumor agents; ROS; Bioorthogonal cyclization

Type de communication : communication orale ☐ flash ☐ affiche ☒

Targeting A_{2A} receptor in Autism: Single domain antibodies for direct inhibition or antagonist vectorization

MOREIRA DA SILVA Louis[a], LABOUTE Thibaut[b], LE MERRER Julie[b], AJ BECKER Jérôme[b], WAGNER Alain[a], CHAUBET Guilhem[a],

a. Bio-Fonctional Chemistry (UMR 7199), University of Strasbourg, 74 Route du Rhin, 67400 Illkirch-Graffenstaden, France
b. IBrain (UMR 1253), University of Tours, Inserm, Parc de Grandmont, 3200 Tours, France

* Correspondance : l.moreiradasilva@unistra.fr, chaubet@unistra.fr

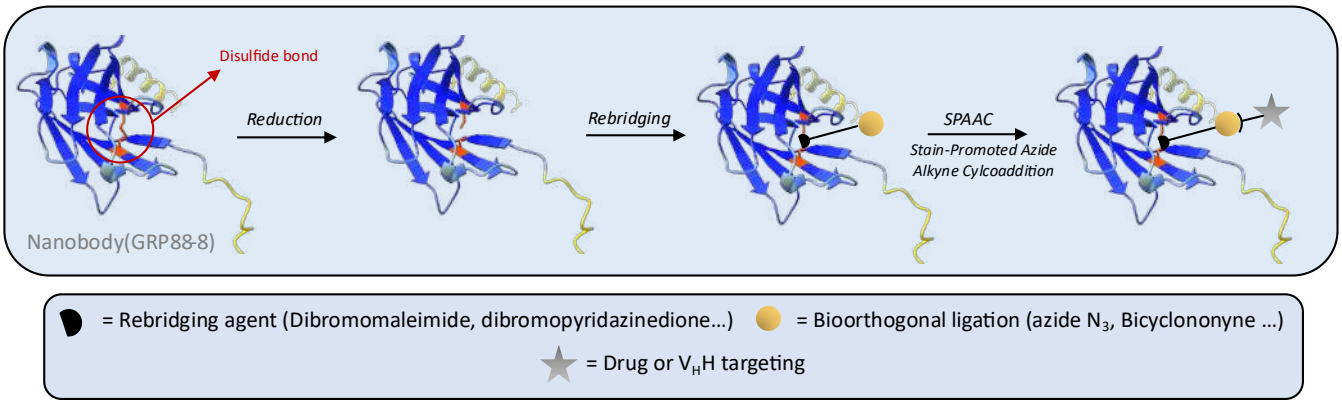
Summary

Adenosine A_{2A} receptor ($A_{2A}R$), a member of G-protein coupled receptor (GPCR), has recently been identified as a promising target for the treatment of autism spectrum disorder (ASD) and depression. Laboute *et al.* have recently demonstrated the positive therapeutic effects of $A_{2A}R$ antagonism in three genetic mouse models of ASD. [1]

The pharmacological utilization of $A_{2A}R$ antagonist for central nervous system (CNS) disorders is currently possible but remains hampered by numerous side effects such as problems of selectivity towards $A_{2A}R$ receptors subtypes (A1, A2AB…), and issues of diffusion in the cardiovascular and immune system (high expression of $A_{2A}R$).

A solution was recently proposed: the utilization of single-chain antibody containing the variable domain only of camelid $IgG_{2/3}$, also known as V_HH (nanobody). Thanks to their low molecular weight [~15 kDa], these small proteins are able to cross the blood-brain barrier and diffuse selectively on their target.[2] Additionally, Laboute *et al.* have identified nanobodies behaving as negative allosteric modulators (NAM) in mouse and displaying brain penetrance properties making them good candidate for selective vectors of drug conjugate.

The overall aim of my project is to chemically modify two nanobodies – i.e., Nb.DRA201, targeting $A_{2A}R$ and acting as a NAM in mice, and Nb.GPR88-8, targeting the GPR88 receptor located exclusively in the striatum– to equip them with bioorthogonal motifs that would allow their subsequent functionalization. Nanobodies having only one solvent-accessible disulfide bond, we chose a "rebridging" approach as our chemical conjugation, known to be both chemo- and region-selective.[3] One of the possible applications of our strategy would be the bio-production of protein-drug conjugates, employing istradefylline as a drug for its antagonist effect of $A_{2A}R$.[4] Additionally, such an approach would also allow us to form Nb.DRA201/Nb.GPR88-8 heterodimers with a pharmacological action on $A_{2A}R$ and striatum-targeting.



Keywords: Nanobody; Autism; Bioconjugation

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Type de communication : communication orale ☐ flash ☐ affiche ☒

PCBIS: Chemical libraries, biological models, technological tools and early ADMETox

Villa[1*]Pascal, Obrecht[1]Adeline, Hany[1]Romain, Bourban[1]Claire, Valencia[1]Christel, Calco[1]Valérie, Gioria[1]Sophie, Gizzi[1]Patrick, Didier[1,2]Bruno, Marsol[1,2]Claire, Daubeuf[1,2]François, Lehalle[1,2]Christine

1 : Plate-forme de chimie biologique intégrative de Strasbourg (PCBIS) UAR 3286 CNRS, Université de Strasbourg ; ESBS et Fac de Pharmacie Illkirch - France

2 : Laboratoire d'Innovation Thérapeutique UMR7200; CNRS, Université de Strasbourg
Correspondance : pascal.villa@unistra.fr

Résumé :

From assay development to lead characterization PCBIS proposes its expertise to contribute to the development of drug candidates:

- Assay development, miniaturization, automation and validation

- Screening of chemical libraries and extracts

- Early ADMETox evaluation to characterize compounds

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Last publications:

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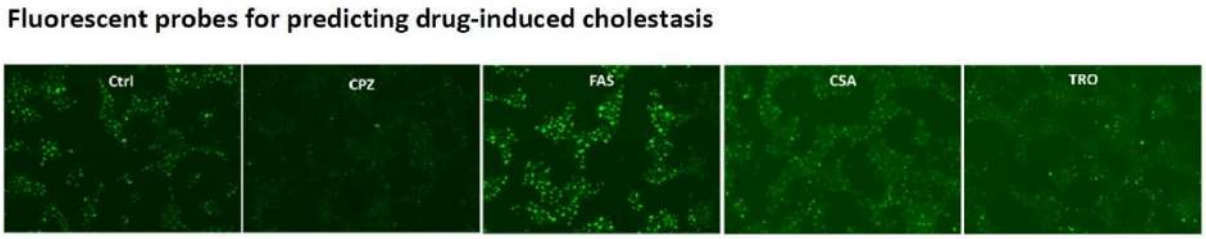
Keywords: Chemical Biology; screening; ADME

Fluorescent probes for predicting drug induced cholestasis

Marion Gay[1], Abdelkhalek Ben Jamaa[1], Françoise Bree[3], Christiane Guguen-Guillouzo[3], Pierre Daligaux[3*], Christophe Chesné[3], Karen Plé[1], Sylvain Routier[1*]
[1] *Institut de Chimie Organique et Analytique, Université d'Orléans, CNRS 7311, Orléans, France*
[3] *Starlight, 111 Boulevard Duhamel de Monceau, Olivet, France*
*Corresponding author e-mail: p.daligaux@wepredic.com, sylvain.routier@univ-orleans.fr

Many cosmetic ingredients pass through the skin and are subject to extensive toxicological studies under Reach if certain tonnages are attained. Although cosmetic ingredients are usually harmless for the consumer, it was found that the liver is potentially the most frequently targeted organ by these ingredients[1]. Intrahepatic cholestasis, which induces the most severe lesions, leads to bile excretion disorders and accumulation of bile acids, bilirubin and cholesterol. Current preclinical models and screening methods are not able to sufficiently predict cholestasis. The aim of this work is the synthesis of fluorescent probes derived from bile acids to be used as biomarkers for the development of such a test.

To develop these probes, various fluorophores were selected and attached via a linker to cholic acid and its derivatives. A study of the importance of the acid part was also studied and methyl ester, taurocholic and glycocholic compounds were synthesized. The HepaRG™ cell line was used as a model for the generation of cholestasis and to mimic cholestatic events. These cells were treated with various molecules known to induce cholestasis such as Chlorpromazine (CPZ), Fasudil (FSD) and Bosentan (BSN).



2D-differentiated HepaRG cells (HPR116 from Biopredic) were incubated with 5µM FluobileTM probe at 37°C for 30 min. After washing, cells were incubated with reference cholestatic compounds (CPZ: Chlorpromazine, FAS: Fasudil, CSA: Cyclosporine A, TRO: Troglitazone).

Our Fluobile™ probes accumulate in bile canaliculi and thus allows the visualization of bile acid transport during cholestasis. The rapid detection of cellular bile accumulation and the easy evaluation of the constriction/dilation state of the bile canaliculi allow our probes to be used to predict the cholestatic potential of chemical compounds including cosmetic ingredients.

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Liste des participants

- Agou Fabrice
- Bachollet Sylvestre
- Balcon Melaine
- Ballereau Stéphanie
- Becker Jérôme
- Benazza Rania
- Berthomé Yann
- Bianchino Gabriella
- Bihel Frédéric
- Biot Christophe
- Bonnefoy Zoé
- Bonnet Dominique
- Bosc Lauriane
- Bosco Michael
- Bourderioux Aurélie
- Bourel Line
- Bouvier Benjamin
- Bret Guillaume
- Bristiel Alexandra
- Burger Alain
- Buron Frédéric
- Burrini Nastassja
- Busca Patricia
- Calco Valérie
- Calzaferri Francesco

- Chaignon Philippe
- Chaubet Guilhem
- Chazeau Elisa
- Chevalier Arnaud
- Chiaravalli Jeanne
- Chieffo Carolina
- Cianferani Sarah
- Clavé Guillaume
- Clément Grisel
- Coïs Justine
- Collot Mayeul
- Coulleray Jessica
- Courseyre Manon
- Daubeuf François
- Davioud-Charvet Elisabeth
- Decroos Christophe
- Defrancq Eric
- Dejeu Jérôme
- Desingle Cécile
- Desrat Sandy
- Dubacheva Galina
- Dubar Faustine
- Dumat Blaise
- Dupouy Baptiste
- Dziuba Dmytro
- Eshak Floriane
- Ferry Manon
- Galzi Jean-Luc
- Genisson Yves
- Ghazarian Marine
- Gibbons Dáire
- Gioria Sophie

- Gomez Zamorano Dennis Carlos
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- Goossen Alexandre
- Goze Christine
- Grandjean Cyrille
- Guianvarch Dominique
- Guimard Carole
- Gulea Mihaela
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- Johannes Ludger
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- Kindler Alexia
- Klimezak Maxime
- Klymchenko Andrey
- Krezel Wojciech
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- Labruere Raphael
- Laclef Sylvain
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- Lantus Indréalie
- Latour Léa
- Le Merrer Julie
- Le Mire Anne
- Lecat Sandra
- Leo Sofia
- Lesur Emilie
- Lherbet Christian
- Lopez Marie
- Louis Manon

- Lugnier Claire
- Mahamoud Anli
- Marsol Claire
- Michel Laurane
- Michelis Sophie
- Mislin Gaëtan
- Mitteaux Jérémie
- Monassa Paul
- Mouftakhir Safae
- Moumné Roba
- Munier-Lehmann Hélène
- Nonat Aline
- Ouadfel Lydia
- Papot Sébastien
- Petitpoisson Lucas
- Pipier Angélique
- Pirrotta Marc
- Ple Karen
- Popczyk Pierre
- Pozhydaiev Valentyn
- Privat Malorie
- Puja Hélène
- Raevens Sandy
- Raibaut Laurent
- Renard Pierre-Yves
- Revol-Tissot Johan
- Ricloux Océane
- Rigouin Coraline
- Routier Sylvain
- Sabot Cyrille
- Sai Mariam
- Sainlos Matthieu

- Saliën Jelena
- Schmitt Martine
- Seemann Myriam
- Simon Clémence
- Simonin Frederic
- Sinou Véronique
- Soehnlen Elodie
- Spanedda Maria Vittoria
- Specht Alexandre
- Spriet Corentin
- Susan Victoria
- Taran Frédéric
- Tardieux Isabelle
- Tilly Marie-Julie
- Tlatlik Finn
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- Villa Pascal
- Villezcas Grisel
- Vizier Romane
- Wagner Alain
- Weiss Lucille
- Wong Yung-Sing
- Yelisetty Venkata Suseela
- Zujew Laurie