JCI 2016

Communications orales / Talks

The optimized omega-3 EPA:DHA 6:1 formulation prevents endothelial dysfunction in monocrotaline-induced pulmonary arterial hypertension in rats

Amissi Said[1], Zahid Rasul Niazi[1], Burban Mélanie[1], Romain Kessler[2], Mathieu Canuet[3], Florence Toti[1], Laurent Monassier[4], Nelly Boehm[5], Cyril Auger[1], Ferhat Meziani[2] and Valérie B. Schini-Kerth[1]

[1]UMR CNRS 7213, Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie, Université de Strasbourg ; [2]EA 7293, Stress vasculaire et tissulaire en transplantation, Fédération de médecine translationnelle de Strasbourg, Faculté de Pharmacie, université de Strasbourg ; [3]Pneumologie-addictologie clinique, 5101 Nouvel Hôpital Civil, Hôpitaux Universitaires de Strasbourg ; [4]EA 7296, Laboratoire de Neurologie et Pharmacologie cardiovasculaire, Fédération de médecine translationnelle de Strasbourg, Faculté de Médecine, Université de Strasbourg; [5]Institut d'histologie, Fédération de médecine translationnelle de Strasbourg, Faculté de Médecine, Université de Strasbourg, France.

Pulmonary arterial hypertension (PAH) is characterized by a progressive pulmonary vascular resistance and elevated pulmonary arterial pressure leading to right heart failure. Omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA) have been shown to protect the cardiovascular system and reduce inflammation. The possibility that an omega-3 fatty acid formulation, EPA:DHA 6:1, prevents cardiopulmonary dysfunction and remodeling was assessed in an experimental model of PAH.

Male Wistar rats received 500 mg/kg/day of either EPA:DHA 6:1 or corn oil by daily gavage. After one week, PAH was induced by a single subcutaneous injection of monocrotaline (MCT, 60 mg/kg). After three weeks, cardiac function and morphology were assessed by echocardiography, pulmonary artery reactivity using organ chambers, vascular morphometry by histology, proteins level by immunofluorescence, and oxidative stress using dihydroethidium.

MCT treatment was associated in the pulmonary artery with a significant increased diameter, systolic PAP and blunted endothelium-dependent relaxations to acetylcholine, in pulmonary arterioles with increased wall thickness and oxidative stress, and in the heart with increased systolic RV pressure (SRVP), RV hypertrophy and a reduced cardiac output (CO). Compared to the MCT group, the EPA:DHA 6:1 treatment prevented the MCT-induced changes in the morphology and pressure in the pulmonary artery and the RV, and also prevented the decreased CO. EPA:DHA 6:1 treatment also reduced the MCT-induced pulmonary artery endothelial dysfunction, and the level of oxidative stress in pulmonary arterioles. The MCT-induced vascular oxidative stress was significantly reduced by N-acetylcysteine, VAS-2870, N^G-nitro-L-arginine and indomethacin. The protective effect of EPA:DHA 6:1 was associated with the prevention of the MCT-induced upregulation of eNOS, angiotensin type 1 receptors, endothelin A and B receptors, COX-1 and COX-2, and the NADPH oxidase subunits (p22phox and p47phox) in pulmonary arterioles, and a reduced pulmonary macrophages and lymphocytes infiltration.

The present findings indicate that the optimized EPA:DHA 6:1 formulation has a cardioprotective effect in PAH by preventing right ventricular failure, pulmonary artery and arterioles remodeling and endothelial dysfunction, and inflammation in lungs, most likely by preventing the NADPH oxidase-, the COX- and the uncoupled eNOS-mediated vascular oxidative stress.

The lipid transfer protein STARD3, a molecular link between endosomes and the endoplasmic reticulum, modulates intracellular cholesterol localization.

Léa WILHELM, Fabien ALPY and Catherine TOMASETTO

U964 INSERM UMR-7104 CNRS
I.G.B.M.C. département de Génomique Fonctionnelle et Cancer
1 rue Laurent Fries - BP 10142, F-67404 ILLKIRCH, France

STARD3 (also known as MLN64 or CAB1), is overexpressed in ~25% of human breast cancer. On cancer tissue sections, we noted that STARD3 labelled large vesicles often at a perinuclear position. Consistently, we found that STARD3 is a resident protein from late-endosomes (LE). Structurally, STARD3 is composed of two conserved regions. The N-terminal moiety called the MENTAL domain is anchored in the late endosome (LE) membrane and mediates oligomerization and cholesterol binding. The C-terminal part is a cytoplasmic START domain involved in cholesterol transfer. Among lipid transfer protein families (LTPs), STARD3 belongs to the START domain protein family, a group of 15 proteins in mammals. STARD3-overexpressing cells most often have a perinuclear accumulation of enlarged STARD3 positive LE and a general alteration of vesicles from the endocytic compartment. Using a correlative electron and light microscopy approach, we showed that STARD3 remarkably remodels the subcellular architecture and ties endosomes with the endoplasmic reticulum, creating membrane contact sites. We next addressed how STARD3 expression modulates cholesterol localization within the cell. Using cholesterol-specific fluorescent probes, we noted the presence of cholesterol-enriched endosomes at the expense of other cellular compartments in STARD3 positive cells. Moreover recent evidences indicate that this STARD3-dependent cellular cholesterol redistribution relies both on the activity of the START domain and on the presence of membrane contact sites.

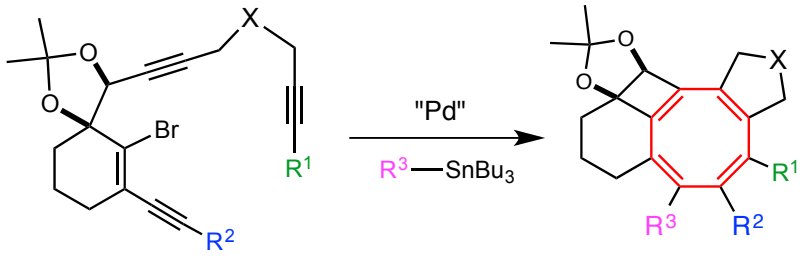
Synthesis of Cyclooctatetraenes through an Unprecedented Palladium-Catalyzed Domino Reaction

Sarah Blouin,[1] Vincent Gandon,[2,3] Gaëlle Blond,[1] and Jean Suffert[1]

[1]*Laboratoire d'Innovation Thérapeutique, UMR 7200 CNRS/Université de Strasbourg, Illkirch*
[2]*ICMMO, UMR 8182 CNRS/Université Paris-Sud, Orsay*
[3]*ICSN, UPR 2301 CNRS/Université Paris-Sud, Gif-sur-Yvette*

The quest for efficient and direct routes to build complex molecules or new scaffolds has stimulated innovative research for years. In this regard, domino reactions are some of the most powerful methods to easily access sophisticated polycyclic molecules. Several metal complexes have been used as catalysts to perform these transformations including palladium. Once the reaction has started from a simple precursor, it goes step by step, often in a regio and stereoselective manner to the final product.

Based on this method, we have developed a domino reaction leading to octasubstituted cyclooctatetraenes and proceeding through an unprecedented mechanistic pathway (see Scheme). The cyclooctatetraene framework exhibits a unique structure, very rare in Nature.[1] This motif displays very interesting properties and can be used as materials,[2] as ligands for metal catalysis[3] or as building blocks for organic synthesis.[4]



(1) Schwede, J. G.; Cardellina, J.H.; Grodf, S.H.; James, T. R.; Blackman, A. J. *Phytochemistry* **1987**, *26*, 155-158.
(2) P. Lu, H.; Hong, G.; Cai, P.; Djurovich, W. P.; Weber, M. E.; Thompson, M. E. *J. Am. Chem. Soc.* **2000**, *122*, 7480–7486.
(3) For a review on substituted COTs being used as ligands see: Roesky, P.W. *Eur. J. Inorg. Chem.* **2001**, 1653-1660.
(4) Glaeske, K. W.; Donaldson, W. A. *Mini. Rev. Org. Chem.* **2012**, *9*, 31–43.

Study of the molecular mechanism of P2X pore dilation

Mahboubi Harkat, Laurie Peverini, Adeline Martz, Alexandre Specht, Thierry Chataigneau and Thomas Grutter

CNRS, UMR7199, Laboratoire de Conception et Application de Molécules Bioactives, Equipe de chimie et neurobiologie moléculaire, Université de Strasbourg, F-67400 Illkirch, France

Abstract

P2X receptors are ligand-gated ion channels (LGICs) selectively permeable to cations. They are activated by extracellular ATP and are involved in many physiological processes such as neuromodulation, neuropathic pain or even platelet aggregation (1). P2X receptors are characterized by a trimeric structure. Following ATP binding, a rapid conformational change converts the ion-conducting pore from a closed to an open state, allowing sodium, potassium and calcium ions to flow through the open pore. Electrophysiological and fluorescence data have indicated that for some P2X receptors there is a second open state, which is permeable to larger cations, like *N*-methylglucamine (NMDG) (2). This mechanism, referred to as pore dilation, is thought to have a link with neuropathic pain (3). However, the molecular mechanism underlying this unique pore property remains ill-defined. We have used photoswitchable azobenzene-containing derivatives to investigate the dilation mechanism of P2X receptors with several engineered cysteine mutations. These molecules attached at specific sites within the pore domain can be switched between cis and trans configurations that subsequently induce helices displacement relevant to gating. Our data indicate that photoswitchable molecules are able to modulate the open state of the P2X2 receptor and possibly induce a dilated state.

1. Lemoine, D., et al., Ligand-gated ion channels: new insights into neurological disorders and ligand recognition.Chem Rev., 2012. 112 (12) : p. 6285-318.
2. Virginio, C., et al., Pore dilation of neuronal P2X receptor channels. Nat. Neurosci., 1999. 2 : p.315-321.
3. Sorge, R.E., et al., Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity Nat Med., 2013. 18(4) : p. 595-599.

Metal-mediated domino reactions to access sulfur heterocycles

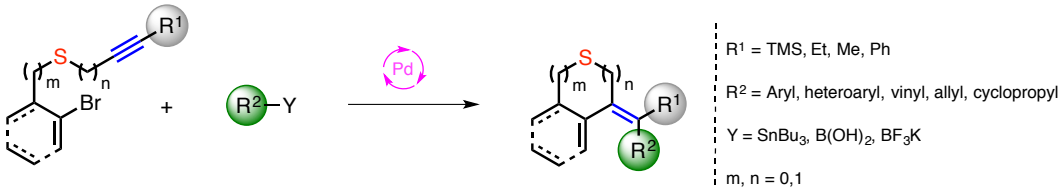
Thomas Castanheiro, Jean Suffert, Morgan Donnard and Mihaela Gulea

Laboratoire d'Innovation Thérapeutique, UMR 7200 CNRS/Université de Strasbourg, Faculté de Pharmacie, Illkirch.

Domino reactions involving transition metal catalysts or reagents represent a powerful tool in the synthesis of heterocyclic molecules. They provide a step-economic access to complex molecules from simple starting materials. On the other hand, despite the interest of sulfur heterocycles as constituents of a large number of drug structures,[1] their synthesis by using this synthetic approach remains limited. Therefore, our project consists in the development of new diversity-oriented syntheses to provide S-heterocyclic structures via metal-mediated domino reactions.

We have developed two types of metal-mediated domino processes for the synthesis of *S*- or mixed *N,S*-heterocycles from simple acyclic substrates containing a sulfur function. One involves a palladium-catalyzed domino reaction starting from propargyl sulfides or ynethioethers and the other uses copper cyanide and 2-amino-benzene disulfide in a three-component domino reaction.

Starting from appropriate precursors, original structures containing a 5- or 6-membered S-heterocycle and a stereodefined tetrasubstituted exocyclic double bond have been obtained via the cyclocarbopalladation/cross-coupling domino reaction (Scheme 1).[2]



Scheme 1 : Pallado-catalyzed domino reaction

Organic thiocyanates are important synthetic intermediates to access valuable sulfur-containing compounds.[3] We recently reported the aerobic copper-mediated cyanation of thiols/disulfides in order to obtain aromatic thiocyanates.[4] To extend the synthetic applicability of this method, we have envisioned that the S-cyanation could be integrated as the key-step in a domino three-component reaction involving aromatic thiols or disulfides bearing an amino group at the *ortho* position. Thus, we have developed a S-cyanation/cyclization/acylation domino sequence enabling a rapid and efficient synthesis of 2-aminobenzothiazole derivatives known to their pharmacological importance (Scheme 2).[5]

Scheme 2 : Copper-mediated domino reaction

[1] Ilardi, E. a; Vitaku, E.; Njardarson, J. T. *J. Med. Chem.* **2014**, *57* (7), 2832–2842.
[2] Castanheiro, T. ; Donnard, M. ; Gulea, M. ;Suffert, J. *Org. Lett.* **2014**, *16*, 3060-3063.
[3] Castanheiro, T. ; Suffert, J. ; Donnard, M. ; Gulea, M. *Chem. Soc. Rev.* **2016**, *45*, 494-505.
[4] Castanheiro, T. ; Gulea, M. ; Donnard, M. ; Suffert, J. *Eur. J. Org. Chem.* 7814-7817.
[5] *Manuscript in preparation*

Unravelling the regulation of human Poly(ADP-ribose) glycohydrolase and its isoforms

Eléa Heberle[1], @ , Giuditta Illuzzi[1], @ , Jean-Christophe Amé[1], @ , Barbara Camuzeaux[1], @ , Vincent Heyer[2], @ , Bernardo Reina San Martin[2], @ , Françoise Dantzer[1], @ , Valérie Schreiber[1], @

1 : Biotechnologie et signalisation cellulaire (BSC) - Site web, Université de Strasbourg - CNRS - UMR7242 ESBS 300, boulevard Sébastien Brant CS10413 67412 Illkirch Cedex - France
2 : Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) - Site web, CNRS - Université de Strasbourg : UMR7104, Inserm : U964, 1 Rue Laurent Fries - BP 10142 67404 Illkirch Cedex - France

---

Among post-translational modifications, poly(ADP-ribosyl)ation has emerged to be a crucial event in a wide range of processes from DNA damage signalling to regulation of chromatin structure and gene expression. While poly(ADP-ribosyl)ation of proteins occurs through the activity of a family of 17 proteins called the poly(ADP-ribose) polymerases (PARPs), the reversion of this modification is essentially driven by the degrading activity of poly(ADP-ribose) glycohydrolase (PARG). Although PARG is encoded by a single gene in the human genome, its regulation is finely tuned by several processes such as alternative splicing and translational re-initiation, generating at least five isoforms displaying various subcellular localisations and functions. Regulation of PARG function or activity by post-translational modifications has not been addressed so far.

The present work aims at deciphering the regulation of PARG function by phosphorylation. We have observed that a kinase activity co-purifies with PARG from cell extracts, PARG being itself a substrate for this phosphorylation activity. Proteomic studies identified several protein kinases involved in DNA damage response as PARG partners. For one candidate, in vitro phosphorylation assays using recombinant proteins validated that PARG is a specific target for phosphorylation. Our current work is to identify by mass spectrometry the phosphorylation site(s) within PARG, to subsequently generate unphosphorylable or phosphomimetic PARG mutants. Next, we will evaluate the influence of PARG phosphorylation status on its in vitro enzymatic activity. Through the generation of stable cell lines expressing these PARG mutants, we will evaluate the involvement of PARG phosphorylation on its function in DNA repair. We will also examine which PARG isoform is regulated by this phosphorylation. We expect that our results will shed light on the regulation of PARG that is needed to tightly control the level of PAR produced in response to DNA damage to avoid its detrimental accumulation

Nucleocapsid protein of HIV-1 – a potential target for future anti-HIV therapy

Lesia Kovalenko[1,2], Rajhans Sharma[1], Francesco Saladini[3], Alessia Giannini[3], Nicolas Humbert[1], Cristian Boudier[1], Mattia Mori[3], Savina Malancona[4], Steven Harper[4], Olga Zaporozhets[2], Maurizio Botta[3], Vincenzo Summa[4], Maurizio Zazzi[3], Yves Mély[1]

[1]*Laboratoire de Biophotonique et Pharmacologie, UMR 7213 CNRS, Université de Strasbourg, Illkirch,*
[2]*Analytical Chemistry Department, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine*
[3]*Department of Medical Biotechnologies, University of Siena, Siena, Italy*
[4]*IRBM Science Park, Department of Chemistry, Pomezia RM, Italy*

The actual requirement of new anti-HIV drugs is mainly due to the drug resistance for the current HIV-1 treatment. The highly conserved HIV-1 proteins are of particular interest for drug development. Among them, the nucleocapsid protein (NC) of HIV-1 is of particular interest, being involved in several key steps of viral life cycle and possessing highly conserved zinc fingers. Therefore, NC appears to be a favorable target for overcoming drug resistance [1]. Since currently no drug against NC is on the market and NC inhibitors discovered so far suffer from toxicity and limited specificity, further studies are required to find new potent and specific drugs.

Herein, we combined computational studies, organic synthesis, biophysical and antiviral assays to disclose new NC inhibitors. As an initial point, virtual screening was done based on known NC/DNA and NC/RNA structures. Further, selected hits were tested using an *in vitro* screening assay. This assay is based on the highly specific nucleic acid destabilization properties of NC [2]. In total, 650 compounds were screened with this assay. From the first round screening, 27 compounds out of 350 passed the threshold of 25% inhibition activity at 100 μM concentration. The selected compounds provided IC50 values in the range of 35 – 900 μM and represented 5 different chemical classes. An extensively hit-to-lead optimization was then performed for the most promising series. As a result, we identified compounds with low micromolar IC50 values. Furthermore, the most active compounds were tested in isothermal calorimetry to evaluate their affinity to NC. Their antiviral activity against the HIV-1 wild type strain and a panel of viruses with resistance mutations to approved HIV-1 drugs was examined. These compounds were found to be active against all tested viral strains at nanomolar to low micromolar concentration. Taken together our results provide a successful starting point with great potential for future anti-HIV drug.

[1] Mori M., Kovalenko L., Lyonnais S., Antaki D., Torbett B., Botta M., Mirambeau G., Mely Y., Current Topics in Microbiology and Immunology, 2015, 389, P. 53–92.
[2] Shvadchak V., Sanglier S., Roche S., Villa P., Haiech J., Hibert M., Van Dorsselaer A., Mely Y., de Rocquigny H., Biochimie, 2009, 91, P. 916–923.

Near-Infrared FRET imaging reveals the fate and integrity of lipid nanocarriers in healthy and tumor-bearing mice

Redouane Bouchaala[1,2], Luc Mercier [3] ,Bohdan Andreiuk [1], Ievgen Shulov [1] , Yves Mély [1], Thierry Vandamme [4], Jacky G. Goetz [3,], Nicolas Anton [4], Klymchenko Andrey [1].

1) CNRS UMR 7213, Laboratoire de Biophotonique et Pharmacologie, University of Strasbourg, 74 route du Rhin, 67401 Illkirch Cedex, France
2) Laboratory of Photonic Systems and Nonlinear Optics, Institute of optics and fine mechanics, University of Sétif 1, 19000 Algeria.
3) Inserm U1109, MN3T, unistra Strasbourg, F-67200, France,
4) CNRS UMR 7199, Laboratoire de Conception et Application de Molécules Bioactives, University of Strasbourg, 74 route du Rhin, 67401 Illkirch Cedex, France

Lipid nanocarriers emerged as promising candidates for drug delivery and cancer targeting because of their low toxicity, biodegradability and capacity to encapsulate a drug or a contrasting agent [(1)]. However, cause of poor understanding of their *in vivo* fate and integrity, their translation from laboratory to biomedical applications is limited. In this work, we exploited the Förster Resonance Energy Transfer (FRET) technique for real time investigation of their stability *in vivo*. Using our recently developed approach of hydrophobic counterion (TPB) [(2)], we encapsulated two NIR cyanine dyes (Cy 5.5/TPB and Cy 7.5/TPB) inside a lipid nanocarrier of 100 nm size [(3)]. After validation of our FRET nanocarriers in vitro, they were retro-orbitally injected into healthy and tumor bearing mice. Using two-color whole animal NIR imaging, we could quantify the content of nanoparticles in different compartments of the mice, observing that the particles remain stable in the blood circulation for at least 6h. The changes in the FRET signal, i.e. disintegration, was observed after ~4h and finished after 24h. Finally, we found a fast accumulation of nanocarriers inside tumors with the loss of the particle integrity after 1.30 h. In conclusion, we developed a FRET system that allows directly visualization and quantification of nanocarrier integrity in vivo.

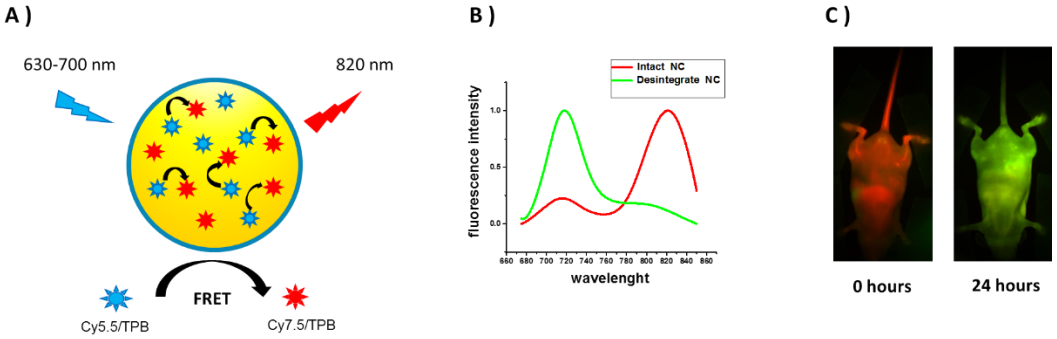


Figure. (A) Schematic presentation of FRET system inside lipid nanocarrier encapsulating NIR cyanine dyes. (B) Emission spectra of intact nanocarriers in water and after addition of dioxane. (C) NIR in vivo imaging in living mice using 100-nm FRET nanocarriers at 0h and 24 h.

1) Klymchenko, A.; Roger, E.; Anton, N.; Anton, H.; Shulov, I.; Vermot, J.; Mely, Y.; Vandamme, T. F. Highly Lipophilic Fluorescent Dyes in Nano-Emulsions: Towards Bright Nonleaking Nano-Droplets. RSC Adv. 2012, 2, 11876–11886.
2) Kilin VN, Anton H, Anton N, Steed E, Vermot J, Vandamme TF, Mely Y, Klymchenko AS. Counterion-enhanced cyanine dye loading into lipid nano-droplets for single-particle tracking in zebra fish Biomaterials, 6 (2014), pp. 4950–4957.
3) Bouchaala R. et al. in preparation.

Towards specific inhibition of histone deacetylases: the case of HDAC8 from *Schistosoma mansoni*

Tajith Shaik, @, Martin Marek, @, Sylvie Duclaud, @, Christophe Romier, @.
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) - Site web - IGBMC
Parc d'Innovation 1 Rue Laurent Fries - BP 10142 67404 Illkirch Cedex - France

---

Human neglected diseases (or Neglected Tropical Diseases - NTDs) are a major health concern with far reaching social and economic implications. Eukaryotic parasites that cause NTDs possess complex life cycles where epigenetic enzymes are seemingly playing crucial roles by driving parasites through their different morphological stages. To speed up the search for new drugs against pathogens, a piggyback strategy based on the modification of existing anti-epigenetic drug scaffolds can be used. However, selectivity and specificity represent bottlenecks of this strategy due to the high sequence conservation among epigenetic enzyme paralogues and orthologues.

We have used this piggyback strategy in the case of Histone Deacetylase 8 from *Schistosoma mansoni* (smHDAC8). *S. mansoni* causes schistosomiasis, a disease second after malaria in terms of deaths yearly. smHDAC8 plays an important role in parasite infectivity and cell homeostasis. Several anti-cancer drugs targeting human HDACs have been reported, some of them already FDA-approved. Our initial work has revealed unexpected active site structural differences between smHDAC8 and its human ortholog that helped identify scaffolds increasing specificity for smHDAC8 over human HDAC1 and HDAC3, but not HDAC6 and HDAC8.

Here we present collaborative work within the A-ParaDDisE consortium where the initial scaffolds identified have been modified to further increase specificity. Our crystallographic work shows that these new molecules make full use of the smHDAC8-specific structural features, enabling an increased specificity for smHDAC8 over human HDACs. These studies also highlight the importance for selectivity of loops at the rim of the active site.

Acknowledgements:

The A-ParaDDisE consortium is funded the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 602080

References:

- Marek, M. et al. (2013) Structural basis for the inhibition of Histone Deacetylase 8 (HDAC8), a key epigenetic player in the blood fluke *Schistosoma mansoni*. PLoS Pathog 9 (9):e1003645.
- Tino Heimburg et al. (2016) Structure-Based Design and Synthesis of Novel Inhibitors Targeting HDAC8 from *Schistosoma mansoni* for the Treatment of Schistosomiasis. J. Med. Chem., DOI: 10.1021/acs.jmedchem.5b01478.

Maleimido-dioxanes (MD): a serum-stable self-hydrolysable hydrophilic alternative to classical maleimide conjugation

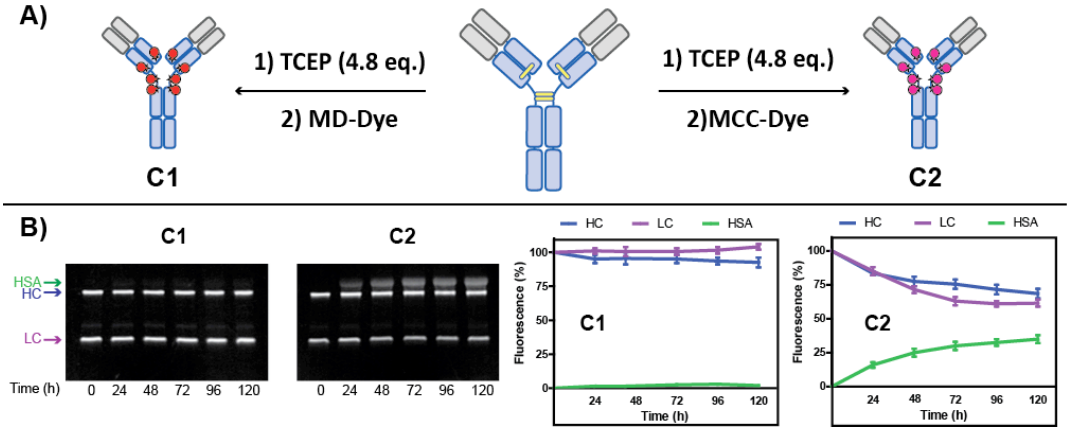
Igor Dovgan[1], Sergii Kolodych[2], Oleksandr Koniev[2], Alain Wagner[1]

[1]Laboratory of Functional ChemoSystems (UMR 7199), Labex Medalis, University of Strasbourg, France, [2]Syndivia SAS, 650 Boulevard Gonthier d'Andernach, 67400 Illkirch

Amine-to-thiol coupling is one of the most frequently used techniques in bioconjugate chemistry and an important tool for preparation of antibody-drug conjugates (ADCs) [1]. One of the two ADCs approved by FDA is produced through amine-to-thiol conjugation using SMCC reagent[2]. However, SMCC-based conjugates suffer from a limited stability in blood circulation and hydrophobic character of the MCC linker, which lead to major pharmacokinetic implications[3].

To address this issue, we have developed a heterobifunctional analogue of SMCC reagent, sodium 4-(maleimidomethyl)-1,3-dioxane-5-carbonyl)oxy)-2,3,5,6- tetrafluorobenzenesulfonate (MDTF) for amine-to-thiol conjugation. By replacing of the cyclohexyl ring in SMCC structure with the 1,3-dioxane we increased hydrophilicity of resulted MD linker.

Interestingly, the MD linker significantly increased the conjugate self-stabilization by thiosuccinimide ring opening into thiosuccinamic acid[4]. Our reagent was applied for preparation of antibody-dye conjugates. Comparison of their plasma stability with MCC-based conjugates showed that MD-based conjugates have substantially lower level of deconjugation and payload transfer to human serum albumin (HSA) (Scheme 1).



Scheme 1. A) Preparation of Trastuzumab-TAMRA conjugates **C1** and **C2** with Dye to Antobody Ratio (DAR) = 8 through maleimide-thiol reaction B) Fluorescent SDS-PAGE analysis of MD- and MC-based antibody−dye conjugates after incubation in human plasma. Two lanes correspond to the labeled heavy (HC) and light chains (LC) of the antibody, the third line corresponds to labelled human serum albumin (HSA) formed via thiol exchange reaction. Quantitative analysis of conjugate stability in human plasma demonstrated 38% of payload transfer to HSA over 120 h for MCC-based conjugate C2 in contrast with 3% for MD-based conjugate

[1] O. Koniev, A. Wagner, Chem. Soc. Rev. 44 (2015) 5495–5551.
[2] A. Ballantyne, S. Dhillon, Drugs 73 (2013) 755–65.
[3] A.D. Baldwin, K.L. Kiick, Bioconjug. Chem. 22 (2011) 1946–53.
[4] R.P. Lyon, J.R. Setter, T.D. Bovee, S.O. Doronina, J.H. Hunter, M.E. Anderson, C.L. Balasubramanian, S.M. Duniho, C.I. Leiske, F. Li, P.D. Senter, Nat. Biotechnol. 32 (2014) 1059–62.

INTAKE OF AN OPTIMIZED OMEGA 3 FORMULATION EPA:DHA 6:1 PREVENTS THE ANGIOTENSIN II-INDUCED HYPERTENSION AND ENDOTHELIAL DYSFUNCTION IN RATS

Zahid Niazi, Grazi Silva, Thais Porto Ribeiro, Faraj Zgheel, Cyril Auger, Valerie Schini-Kerth
UMR7213, CNRS - University of Strasbourg
Faculté de pharmacie 74, route du Rhin 67401 ILLKIRCH-FRANCE - France

---

Previous studies have shown that the optimized omega-3 formulation EPA:DHA 6:1 is a potent inducer of endothelium-dependent nitric oxide (NO)-mediated relaxations. The aim of the present study was to determine whether chronic intake of EPA:DHA 6:1 affects experimental hypertension and endothelial dysfunction induced by angiotensin II (Ang II).

Male Wister rats receiving 500 mg/kg/d of EPA:DHA 6:1 were implanted with mini-osmotic pumps infusing 0.4 mg/d of Ang II. Systolic blood pressure was measured by tail cuff sphingomanometry. Endothelial function was evaluated in second branch of mesenteric artery by wire myography. Target protein expression was evaluated by immunofluorescence.

Infusion of Ang II to rats induced a pronounced increase of systolic blood pressure. In second branch mesenteric artery rings, Ang II infusion induced an endothelial dysfunction characterized by reduced NO- and endothelium-dependent hyperpolarization (EDH)-mediated relaxations, and pronounced endothelium-dependent contractile responses to Ach. These effects were associated with a decreased expression of SKCa and Cx37, and an increased oxidative stress and expression of eNOS, COX-2, AT1R, NADPH oxidase subunits p47phox and p22phox in the vascular wall. Chronic intake of EPA:DHA 6:1 prevented the Ang II-induced hypertension and associated endothelial dysfunction by improving both the NO- and EDH-mediated relaxations and by reducing endothelium-dependent contractile responses. The expression of target proteins in the vascular wall was also significantly improved by EPA:DHA 6:1.

The present findings indicate that chronic intake of EPA:DHA 6:1 prevented the development of hypertension and endothelial dysfunction induced by the infusion of Ang II to rats, most likely by preventing oxidative stress.

Liposome-based vaccines for targeted cancer therapy

Célia Jacoberger-Foissac, Dr Benoit Frisch, Dr Béatrice Heurtault, et Pr Sylvie Fournel

UMR 7199 – Faculté de Pharmacie – Laboratoire CAMB – Equipe Biovectorologie

Despite being quite effective, conventional cancer therapies have the major drawback of triggering numerous side effects. Currently, a challenging goal in this area is the development of innovative targeted antitumoral immunotherapies with a long-term efficiency. In this context, my team took advantage of **liposomal nanoparticles** properties for the conception of **cancer vaccines.** In a previous study, the combination on liposomal surface of three elements (two peptide epitopes including one that is specific to tumor cells and an adjuvant, ligand of TLR2) crucial for immune response has demonstrated to induce complete regression of tumor growth after prophylactic or therapeutic treatments in mice grafted with the murine kidney carcinoma RENCA expression a human xenoantigen, the ErbB2 tumor specific antigen [1]. However, the therapeutic treatment efficiency quickly decreased with the increase of the time spent between tumor grafting and treatment start [2].

In order to optimize our treatment and show the universality of our nanoparticle approach, we proposed to validate our strategy in **another tumor mouse model** and with other adjuvants or combination of adjuvants. We had to **optimize the composition of our nanoparticles** by selecting new peptides specific for the new tumor cells and then to validate their immunogenic and antitumoral potential *in vivo.* Thereby, we observed an almost complete regression of tumor growth after vaccine injections on days 2 and 4 after tumor implantation with our classical adjuvant.

Thanks to the versatility of the lipid nanoparticles, it was possible to adapt the therapeutic treatment and make it effective even within another murine model without xenoantigen and therefore closer to spontaneous tumors. The next steps will be to test new adjuvants or adjuvant combinations to extend the time spent between tumor implantation and treatment. For example we plane to associate TLR-2 and NOD1 agonists as they already showed to have a synergistic effect in vitro [3].

[1] Thomann J.S., et al. Biomaterials 2011, 32: 4574-4583
[2] Roth A., et al. British Journal of Cancer 2005, 92: 1421-1429
[3] Fritz, J.H., et al. Immunity, 26: 445-459

Tip60 interacts with UHRF1 through its MYST domain in HeLa cells in S phase of cell cycle

Waseem Ashraf[1], Christian Bronner[2], Liliyana Zaayter[1], Tanveer Ahmad[1], Christian D. Muller[3], Ludovic Richert[1], Yves Mely[1], and Marc Mousli[1]

[1]CNRS UMR 7213 Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Illkirch.
[2]IGBMC, CNRS, INSERM, Université de Strasbourg, Parc d'innovation, Illkirch.
[3]CNRS UMR 7200 Laboratoire d'Innovation Thérapeutique, Université de Strasbourg, Illkirch.

Abstract:

UHRF1 is a well-known epigenetic regulator protein which is responsible for maintaining the epigenetic marks during the cell division [1]. However it is believed to have an oncogenic potential as it is highly expressed in the tumors and proliferating cells [2]. Tip60 is present in the same macromolecular epigenetic complex and is known to play important role in epigenetic regulation by its acetyl-transferase activity [3]. The aim of this study was to investigate the interaction mechanism between UHRF1 and Tip60.

Our results showed that Tip60 directly interacted with the UHRF1 through its MYST domain and the interaction of the two proteins occurred during the S phase of cell cycle when the DNA is being replicated. We also observed that the over expression of Tip60 in cancer cells led to down-regulation of UHRF1 and caused cell cycle arrest and apoptosis. This suggests that Tip60 is involved in the regulation of UHRF1 which can be further explored to target UHRF1 for anticancer therapy.

[1] Bronner C, Krifa M, Mousli M. Increasing role of UHRF1 in the reading and inheritance of the epigenetic code as well as in tumorogenesis. Biochemical pharmacology 2013;86:1643-9.
[2] Unoki M, Brunet J, Mousli M. Drug discovery targeting epigenetic codes: the great potential of UHRF1, which links DNA methylation and histone modifications, as a drug target in cancers and toxoplasmosis. Biochemical pharmacology 2009;78:1279-88.
[3] Du Z, Song J, Wang Y, Zhao Y, Guda K, Yang S, et al. DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. Science signaling 2010;3:ra80.

Therapeutic proof-of-concept in centronuclear myopathies

Hichem Tasfaout, @

Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) - Site web
CNRS - Université de Strasbourg : UMR7104, Inserm : U964,
Parc D'Innovation 1 Rue Laurent Fries - BP 10142 67404 ILLKIRCH CEDEX - France

---

Centronuclear myopathies (CNM) are severe muscle diseases characterized by muscle weakness and hypotrophic fibers with centralized nuclei. The most severe and neonatal X-linked form is caused by loss-of-function mutations in Myotubularin (*MTM1*), while the main Autosomal-dominant form is due to mutations in Dynamin 2 (*DNM2*). However, the physiopathological mechanisms are barely understood and more importantly no specific therapy is available. Therefore, there is an urgent need to validate therapeutic approaches for such devastating diseases.

Our team showed that genetic reduction of *Dnm2* in the myopathic *Mtm1* Knock out (*Mtm1*KO) mice restores a normal lifespan (from 8 weeks to 2 years) with improved muscle structure and function, validating the concept of “cross therapy” where downregulation of a CNM gene rescues the loss of another CNM gene. The aim of my project is to translate this proof-of-principle by reducing *Dnm2* expression using deliverable agents that could be ultimately used in CNM patients. Several shRNA that target specifically *Dnm2* mRNA were developed and screened *in vitro* on human embryonic Kidney (HEK) cells and mouse C2C12 myoblasts. The best candidates that knockdown *Dnm2* were selected for *in vivo* experiments on wild-type (WT) and *Mtm1* KO mice. For this purpose, Adeno-associated virus (AAV) that express the selected shRNA were generated and injected locally into tibialis anterior muscles of *Mtm1*KO mice. Among the tested candidates, two shRNA showed a knock-down of DNM2 protein level to about 50% which was accompanied with an improvement in muscle function and structure.

Altogether, we showed here *Dnm2* decrease rescues very efficiently the muscle defect in a mouse model for a severe myopathy. A validated and deliverable strategy to rescue centronuclear myopathy in a mammalian model will pave the way towards clinical trials in humans.

Keywords: myopathy, Dynamin, Myotubularin, therapy, Adeno-associated virus

Formation of C-N bonds in micellar aqueous solutions

Maud Bollenbach[1], Patrick Wagner[1], Pedro Aquino[1,2], Frédéric Bihel[1], Jean-Jacques Bourguignon[1], Christophe Salomé[1], Martine Schmitt[1]*

[1]*Laboratoire d'Innovation Thérapeutique, UMR7200, CNRS/Université de Strasbourg, Illkirch ;*
[2]*Laboratório de Pesquisa em Recursos Naturais, UFAL, Maceió, Brasil*

Before performing a chemical reaction, it is necessary to define 3 parameters: solvent, temperature and catalyst. Generally, reactions are realized in organic solvent at high temperature.[1] However, those solvents may be harmful for the manipulator and generate a lot of waste, dangerous for the environment. By taking that observation into account, the best solution would be to perform the organic reactions without any solvent. Unfortunately, solvent-free reaction can be applied only in few cases. Another attractive solution would be the use of water as solvent. Although some chemicals reactions are already successfully developed in water, in general manner, the poor solubility of organic reagents and catalysts is as a strong limitation of this approach. To overcome this, it is possible to add into the media a small amount of surfactant[2], because thanks to their amphiphilic properties, they spontaneously gathered in a micellar form, acting like a nanoreactor. In those nanoreactors, the reactants are more concentrated than in usual conditions, allowing working at lower temperature (25-50°C) and using less catalyst.

For instance, our work focused on the formation of C-N bonds. This type of reaction is important, because nitrogen-containing heterocyclic compounds are of considerable biological and chemical significance. Two major metallo-catalyzed reactions are usually used to form this bond using either palladium (via Buchwald-Hartwig reaction)[3,4] or copper (via Ullmann reaction)[5]. Herein, we described conditions and limitations for both reactions under micellar conditions.

1. D.S. Surry, S. L. Buchwald, *Chem. Sci.* **2011**, *2*, 27-50.
2. B. H. Lipshutz, S. Ghorai, A.R. Abela, R. Moser, T. Nashikata, C. Duplais, A. Krasovskiy, *J. Org. Chem.* **2011**, *76*, 4379-4391.
3. C. Salomé, P. Wagner, M. Bollenbach, F. Bihel, J.-J. Bourguignon, M. Schmitt, *Tetrahedron* **2014**, *70*, 3413-3421.
4. P. Wagner, M. Bollenbach, C. Doebelin, F. Bihel, J.-J. Bourguignon, C. Salomé, M. Schmitt, *Green Chemistry* **2014**, *16*, 4170-4178.
5. M. Bollenbach, P. Wagner, P. Aquino, J.-J. Bourguignon, F. Bihel, C. Salomé, M. Schmitt, *in redaction.*