65e Congrès de l'Union des professeurs de physique et de chimie
Limoges, 28-31/10/2017

Les métaux et le vivant : un défi pour le chimiste

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Département de Chimie de l'ENS,

Laboratoire des BioMolécules (LBM), UMR7203

Les éléments du vivant

Chimie des corps organisés → Chimie organique

H																	He
Li	Be											B	**C**	**N**	**O**	F	Ne
Na	Mg											Al	Si	**P**	**S**	**Cl**	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Ni	Co	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	Lantha -nides	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Ti	Pb	Bi	Po	At	Rn
Fr	Ra	Acti- nides	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Uut	Fl	Uup	Lv	Uus	Uuo

Lanthanides	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Actinides	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Tableau périodique et métaux

Chimie des corps organisés → Chimie organique
Et les métaux ? Chimie inorganique

Μέταλλον (grec) ou metallum (latin) : mine, tranchée

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Ni	Co	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	Lantha-nides	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Ti	Pb	Bi	Po	At	Rn
Fr	Ra	Acti-nides	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Uut	Fl	Uup	Lv	Uus	Uuo

Lanthanides	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Actinides	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Galène

Copeaux métalliques

Cations métalliques en solution aqueuse

Améthyste

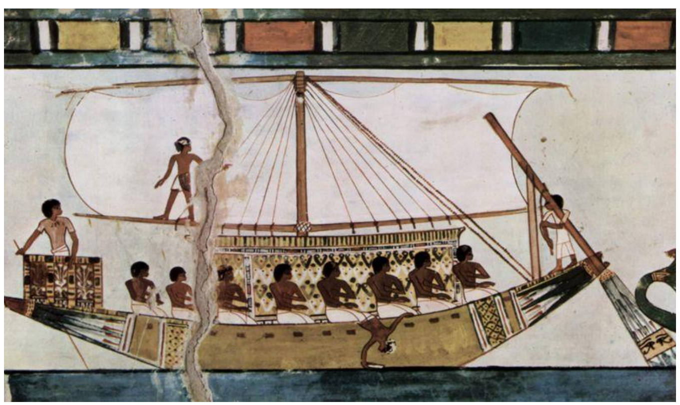
Galène: http://www.geoforum.fr/uploads/monthly_02_2009/post-2474-1235731181.jpg — Copeaux : http://www.dreamstime.com/royalty-free-stock-photos-metal-filings-image11289378 —Cations : photographie de wikipedia metaux de transition — Améthyste http://www.quizz.biz/quizz-409973.html#

Des dérivés métalliques dans la pharmacopée depuis des siècles

Pb
(Sb)

As

Un échantillon de Salvarsan (1936). Le salvarsan était utilisé pour traiter la syphilis jusqu'en 1940. Image de *Chemical Heritage Foundation Collections*



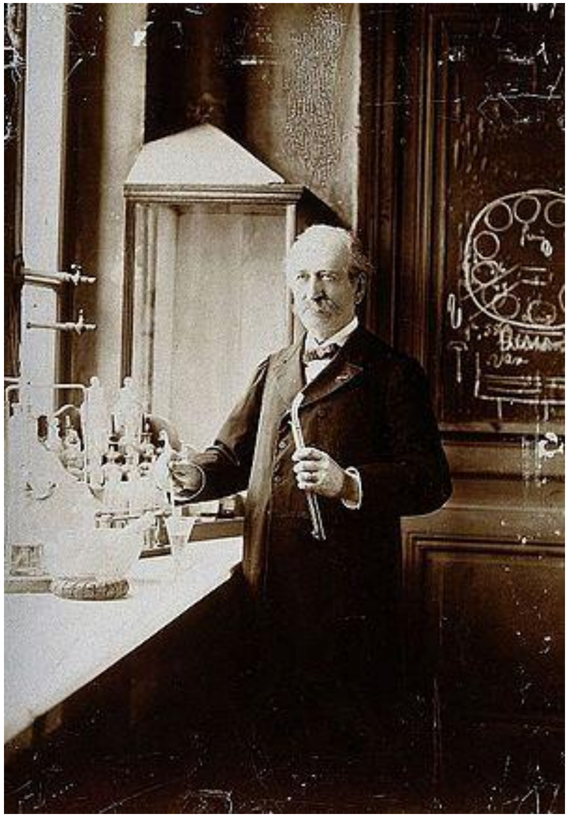
Pb

Pt

Autres métaux de la pharmocopée égyptienne : Fe, Zn, Cu

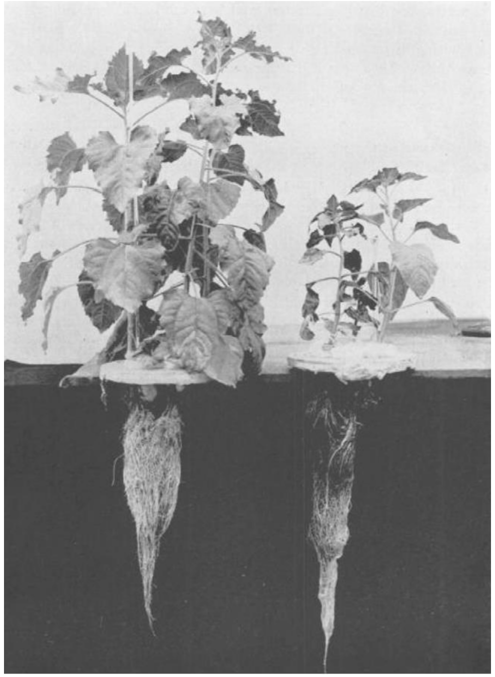
https://fr.wikipedia.org/wiki/Projet:Histoire_de_l%27art/100_articles_pour_l%27histoire_de_l%27art
http://club.quomodo.com/la-voile-dhier-a-aujourdhui/introduction/les_premiers_voiliers.html
https://www.chemheritage.org/historical-profile/paul-ehrlich
http://acouphenes.ca/page21/acouphenes-dommages-auditifs-medicaments.html

Les cations métalliques, des constituants endogènes



Jules Raulin, 1836-1896

Caractère indispensable de sels de zinc dans la croissance d'une moisissure (1869)



Sunflowers grown with (left) and without (right) zinc.

Sommer AL, Lipman CB. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. 1926;1:231–49.

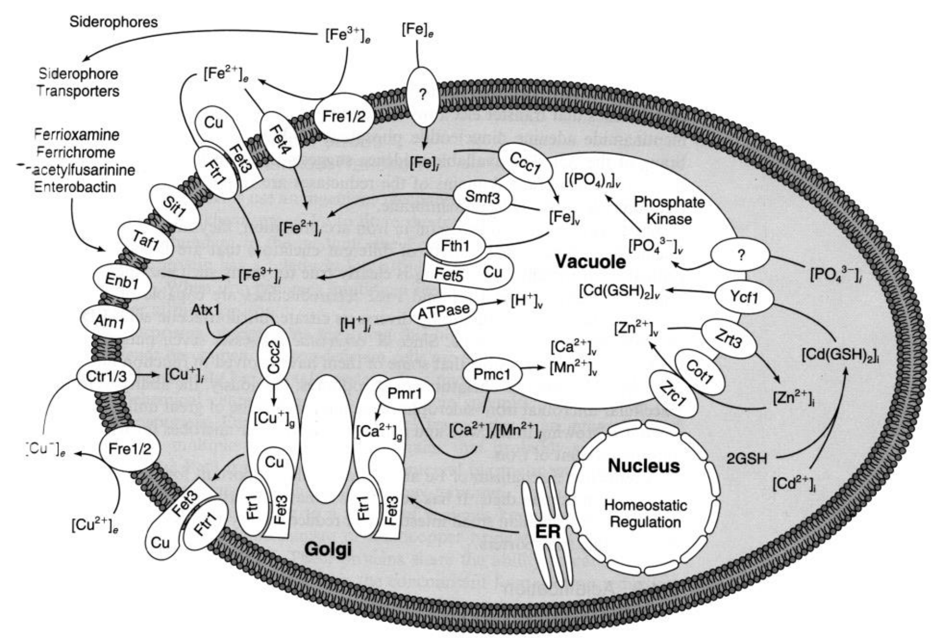
Abondance naturelle et dans le corps humain

Concentration (mM) dans l'eau de mer et le plasma sanguin

Table 1.2 Concentrations (mM) of some ions in seawater and blood plasma

	Eau de mer	Plasma
Ion	Seawater	Blood plasma
Cl^-	550	100
Na^+	470	140
Mg^{2+}	50	2
K^+	10	10
Ca^{2+}	10	3
MoO_4^{2-}	0.2 μM	2–20 μM
Cations métalliques: First row transition elements	1–10 nM	2–20 μM

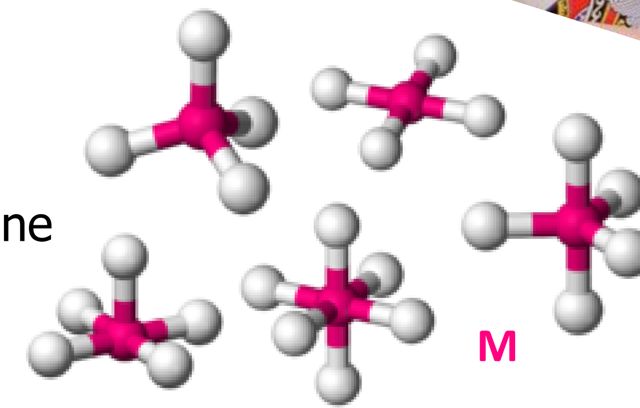
Table extraite de P.C. Wilkins, R.G. Wilkins, *Inorganic chemistry in biology*, Oxford, **1997**

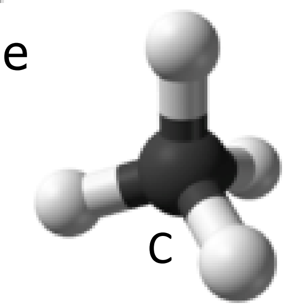


Overview of the Fe and Cu uptake system in *Saccharomyces cerevisae*

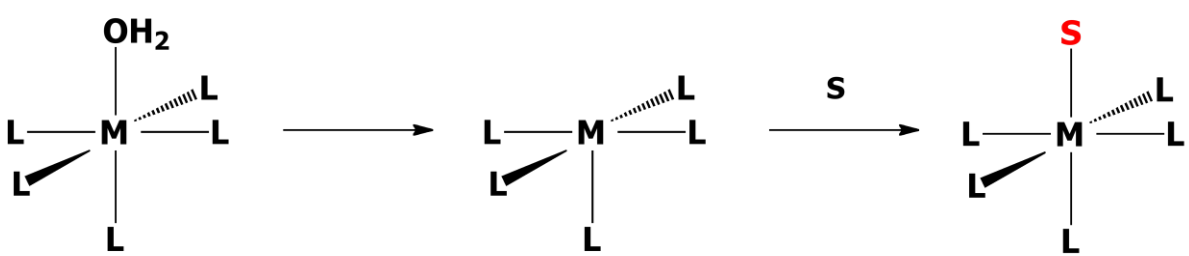
I. Bertini, H. Gray, E.I. Stiefel, J.S. Valentine (edited book) *Biological Inorganic Chemistry, Structure and Reactivity,* University Science Books, 2007

Les cations métalliques : quels atouts ?





- Charge ; échange d'électrons (potentiel redox)
- Paramagnétisme, lien avec activation du dioxygène
- Forme : une source de diversité structurale
- Complexes métalliques et force de liaison : dynamique et échange



Un exemple de séquence d'échange de ligands

Énergie

Usine : synthétiser, produire

Médecine

Soigner

Imager

La bioinspiration

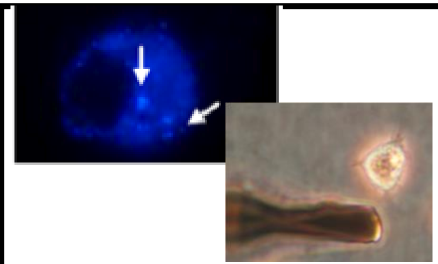
Énergie

Usine : synthétiser, produire

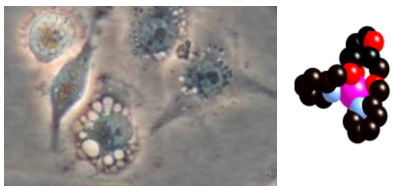
Médecine

Soigner

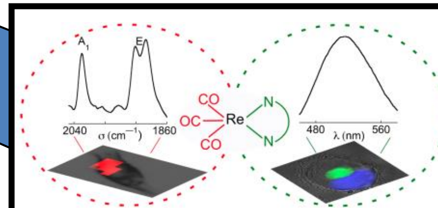
Imager



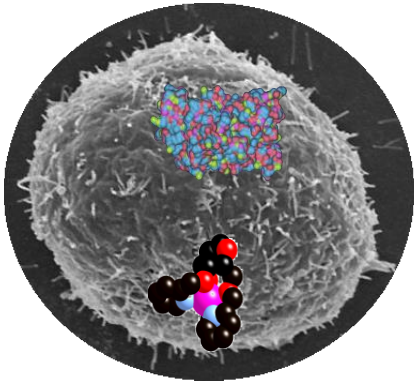
Biodetection: fluorescence, electrochemistry

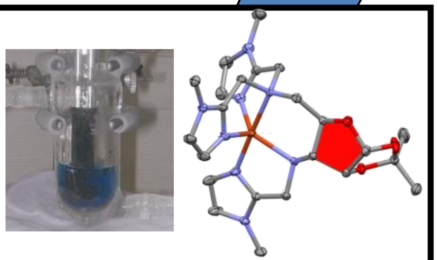


Cellular activity of antioxidant complexes



M-CO derivatives as Single Core Multimodal Probes





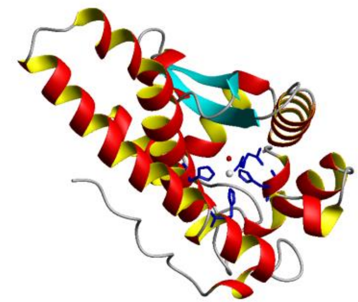
Enzyme mimics design and metal complexes with controlled properties

The research group in Inorganic Cellular Chemistry, September 2013

Les superoxyde-dismutases (SOD) : des métalloenzymes très efficaces

$$2O_2^{\bullet -} + 2H^+ \longrightarrow O_2 + H_2O_2$$

$$k_{spont.pH7} = 6{,}0 \times 10^5\ mol^{-1}.L.s^{-1}$$



Superoxyde dismutases : Mn, Fe, Cu, ou Ni pour une même fonction (échange d'électron avec le superoxyde)

$$O_2^{-} \qquad O_2$$

$$M^{n+} \qquad M^{(n-1)+}$$

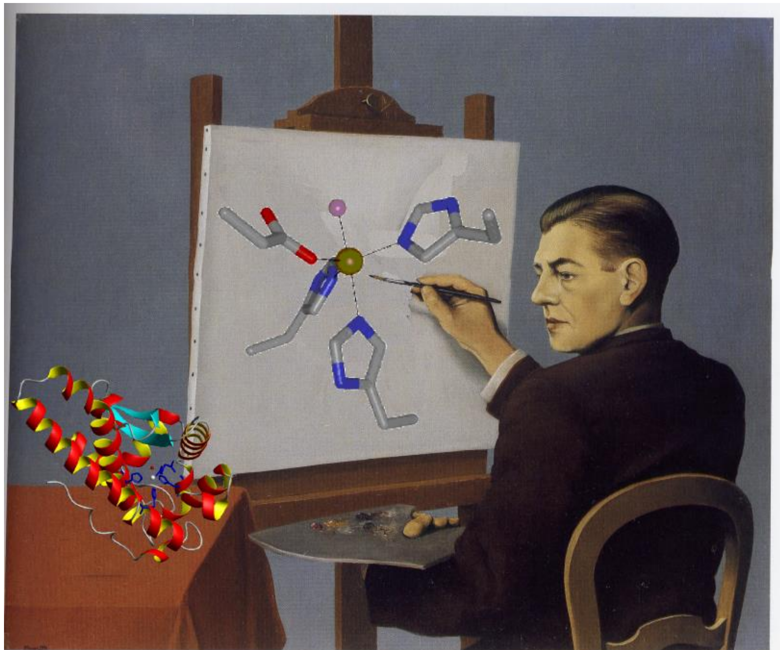
$$H_2O_2 \qquad O_2^{-} + 2H^+$$

M = Cu(Zn), Fe, Mn, Ni

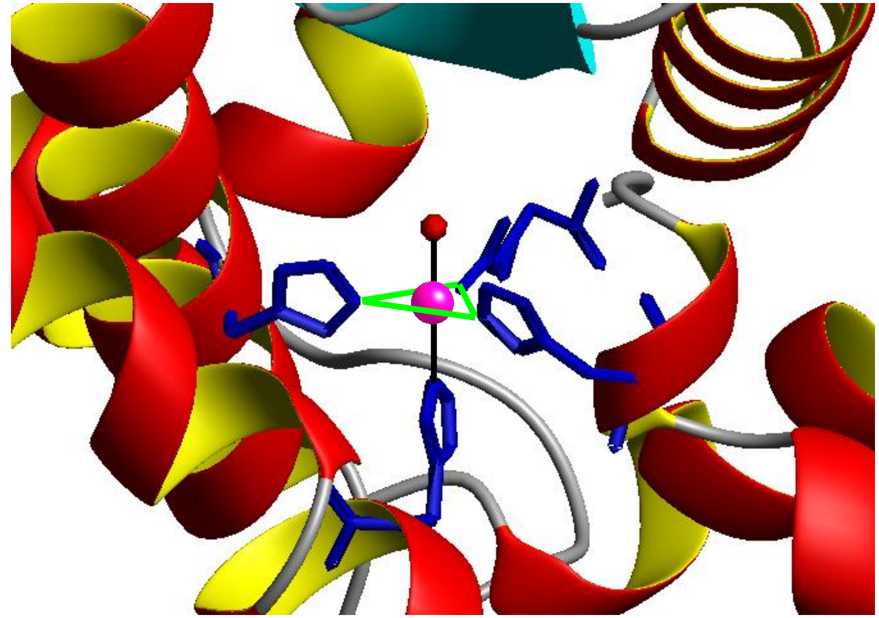
$k_{cat} = 10^9\ mol^{-1}.L.s^{-1}$

Mimer les SODs : un enjeu fondamental et thérapeutique…

Mécanisme simplifié de type « ping-pong »

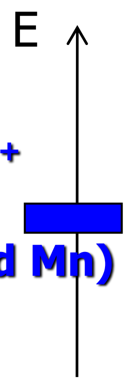


D'après Magritte, « La clairvoyance » 1936



MnSOD mitochondriale humaine

Borgstahl *et al., Cell,* 1992,171,120



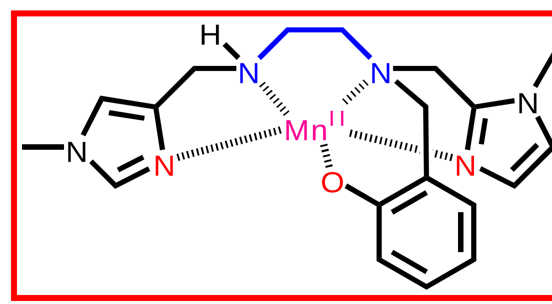
M^{n+} /$M^{(n-1)+}$

SODs

(Cu(Zn), Fe and Mn)

0.36V/NHE

0.12V/SCE



E=0.19V/SCE

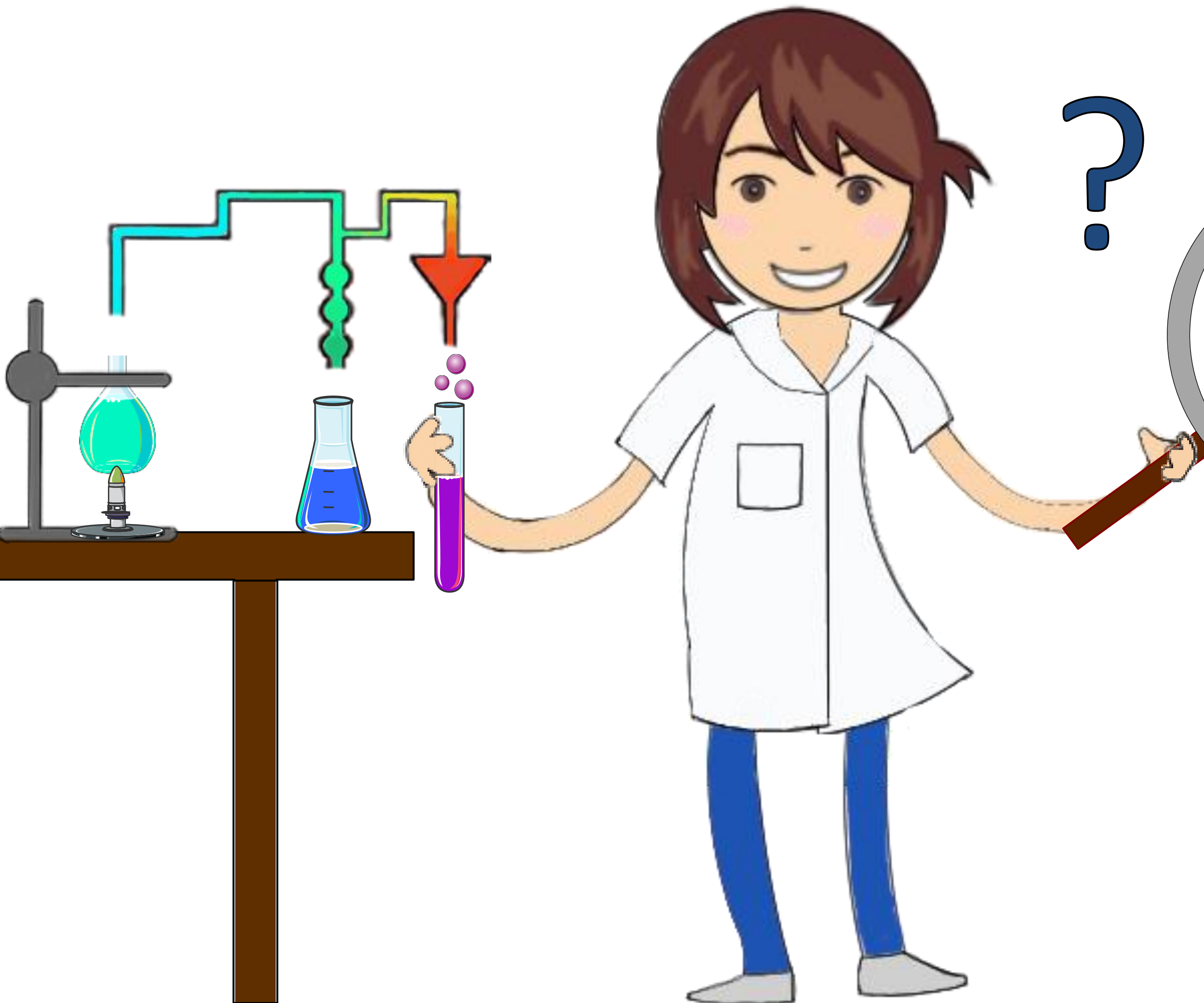
(Activité *ca* 1% de celle de l'enzyme

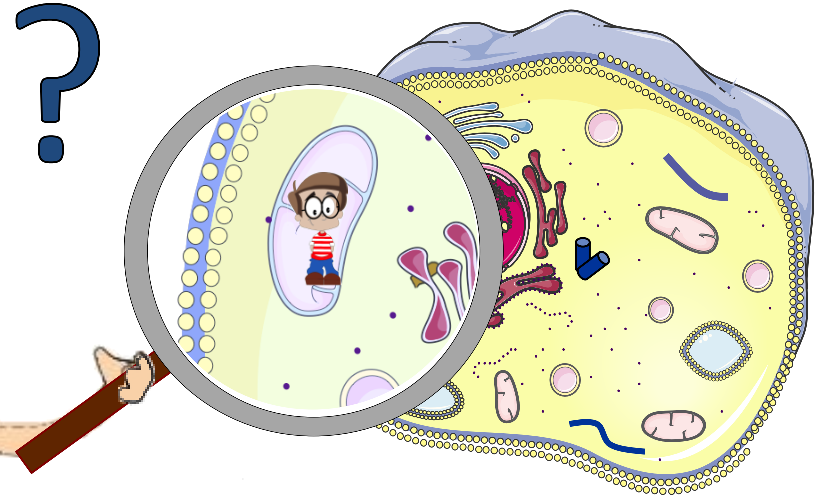
Synthèse aisée, pas de réaction immunitaire à craindre et structure modulable)

C. Policar, *Mimicking SODs, Why and How: Bio-Inspired Manganese Complexes as SOD Mimics*
in: J.S. Reboucas, I. Batinic-Haberle, I. Spasojevic, D.S. Warner, D. St. Clair, Redox Active Therapeutics, Springer, 2016
PDF disponible sur demande: clotilde.policar@ens.fr

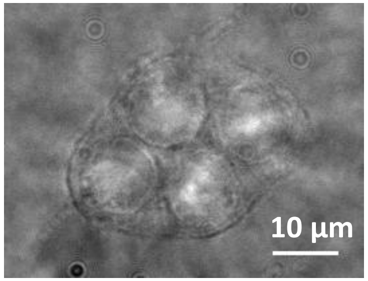
F. Cisnetti *et al.*, Eur. J. Inorg. Chem., 2007, 4472-4480

Des molécules mimes d'antioxydants pour lutter contre l'inflammation : une approche en chimie inorganique cellulaire et biologique





Diapositive d'Emilie Mathieu, doctorante au laboratoire (MT180)



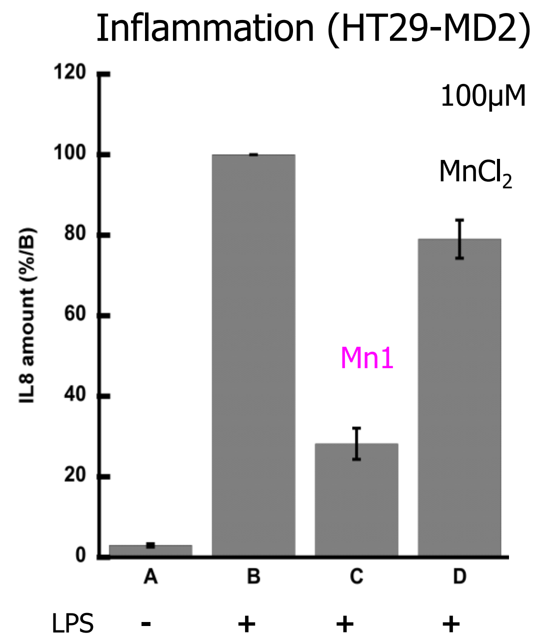
HT29 MD2 : lignée de cellules intestinales humaines

LPS → **ROS** → **Interleukine 8 (IL8)**

Activation | **Espèces oxydantes** | **Marqueurs de l'inflammation**

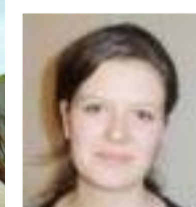
LPS complexe

Surnageant : IL8 (ELISA)



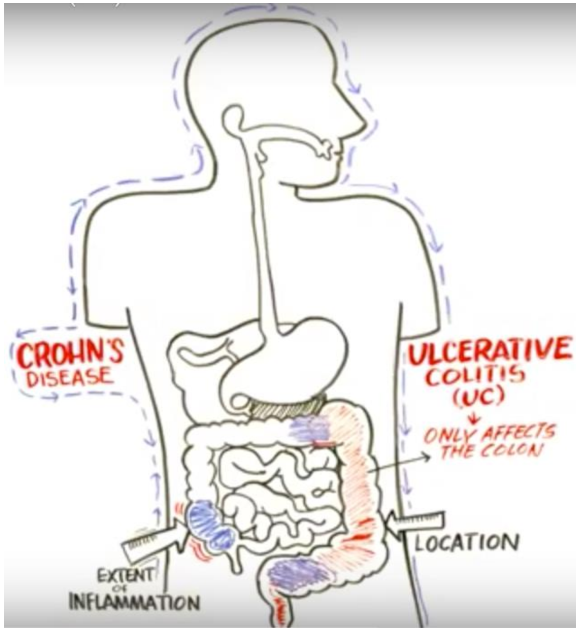

E. Quévrain


E. Mathieu

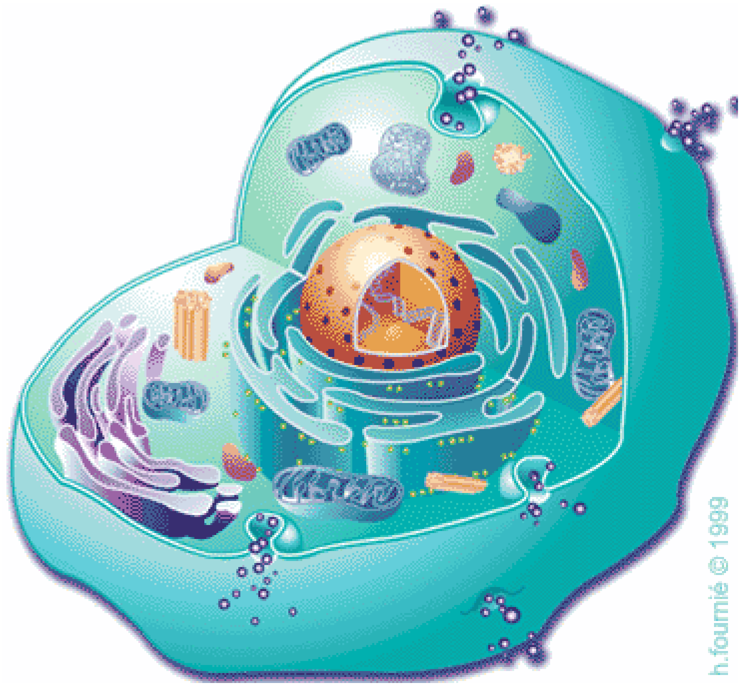

AS Bernard

E. Mathieu, A.-S. Bernard et al., Inorganic Chemistry **2017**, ***56***, 2545−2555

Imager



http://www.evanshealthlab.com/ibd/



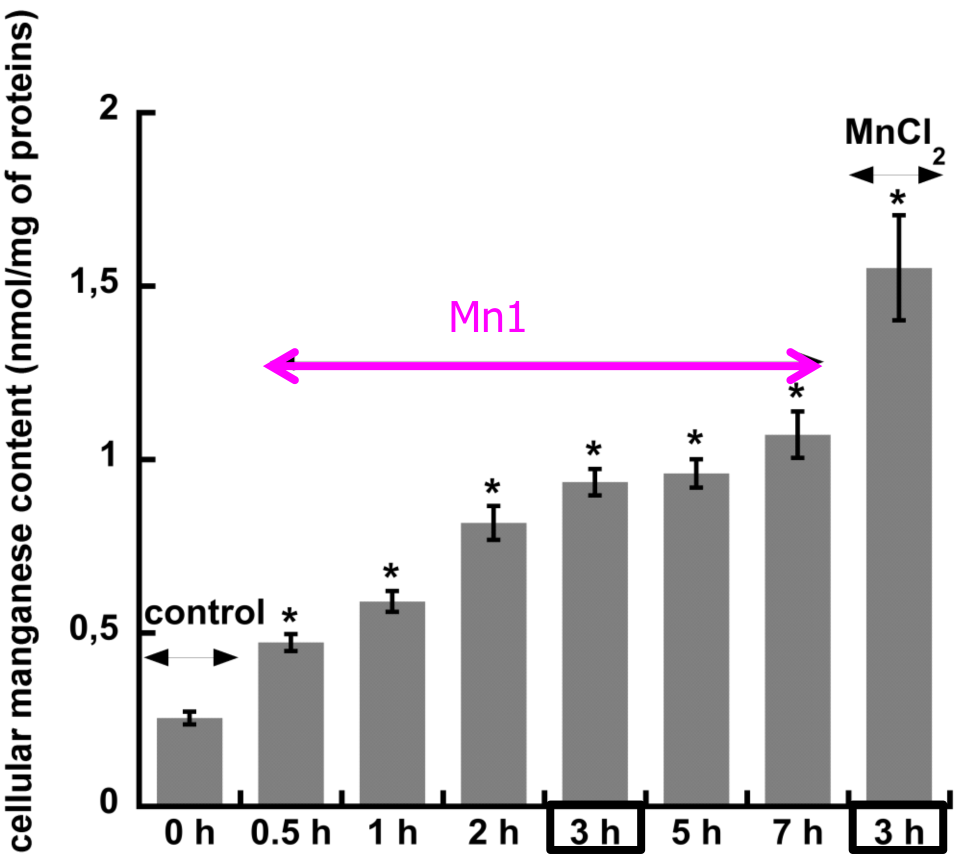
https://www.thinglink.com/scene/834549958584565762

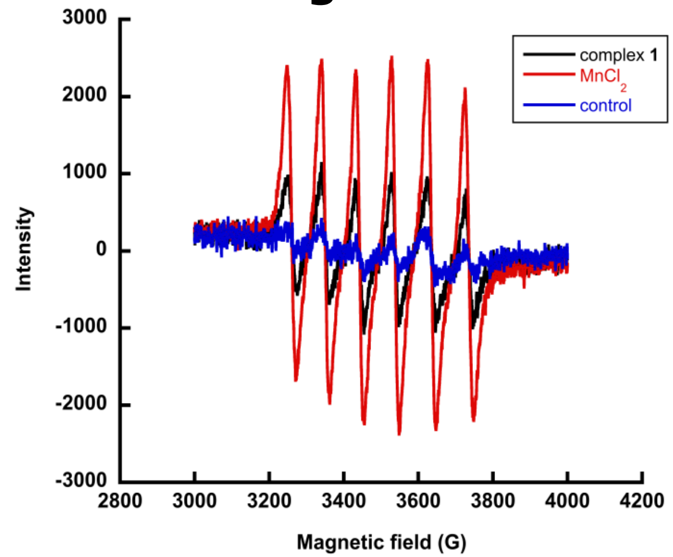
https://www.commeuncamion.com/2015/01/03/sac-a-dos-herschel-test-avis/

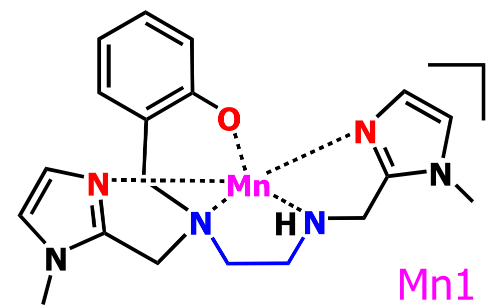
Quelle activité ?
Combien, qui et où ?

Pénétration dans la cellule : quantification du manganèse

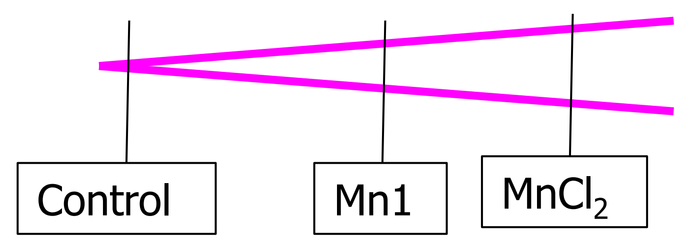
Quantification du Mn(II) dans des lysats acidifiés par résonance paramagnétique électronique







Mn1

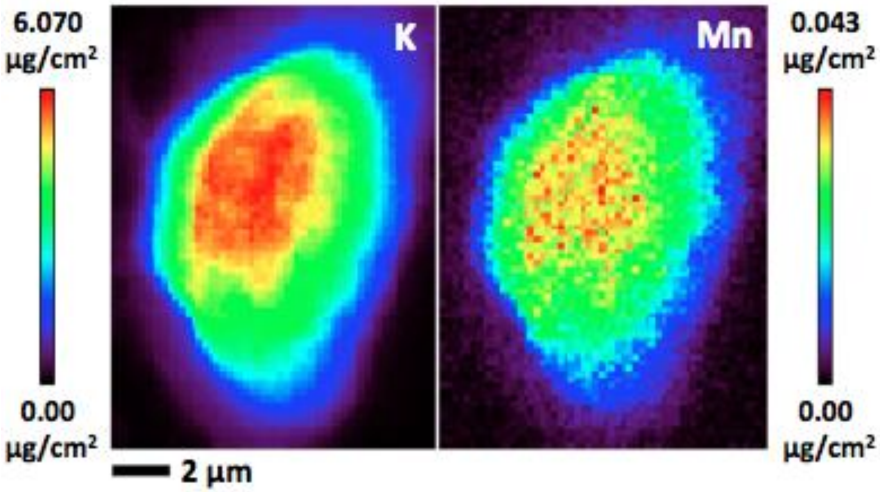


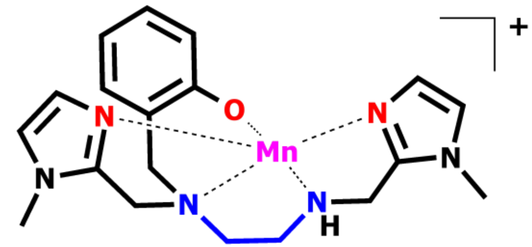
Qualitative scale

E. Mathieu, A.-S. Bernard et al., Inorg. Chem. **2017**, *56*, 2545–2555

Imagerie sub-cellulaire d'un mime de SOD : imager directement le Mn

Incubation avec Mn1, 100 µM, 2h, cryofixation et lyophilisation

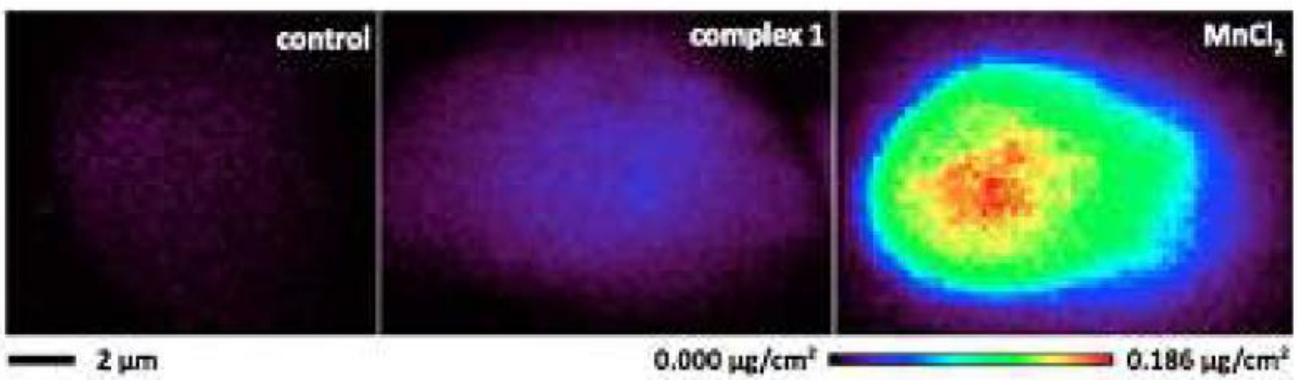




Imagerie de **fluorescence-X** : K et Mn
noir < rouge

Trois cellules différentes
Incubées avec :

— Mn1 $MnCl_2$



Une échelle qualitative

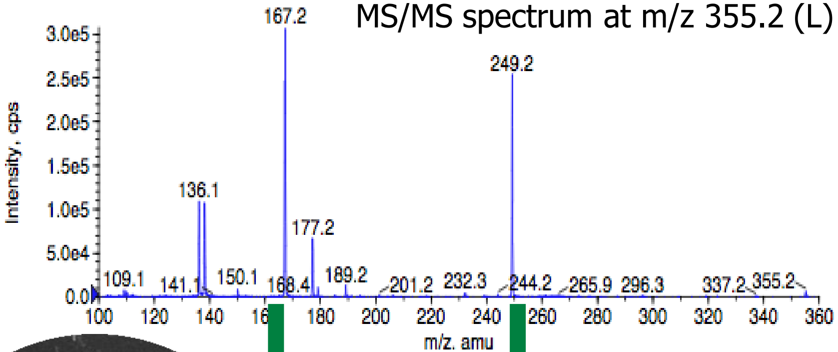
E. Mathieu, A.-S. Bernard et al., Inorganic Chemistry **2017**, ***56***, 2545–2555

Coll. B. Lay, APS, Argonne
2-ID-D/E beamline for micro-Xfluorescence

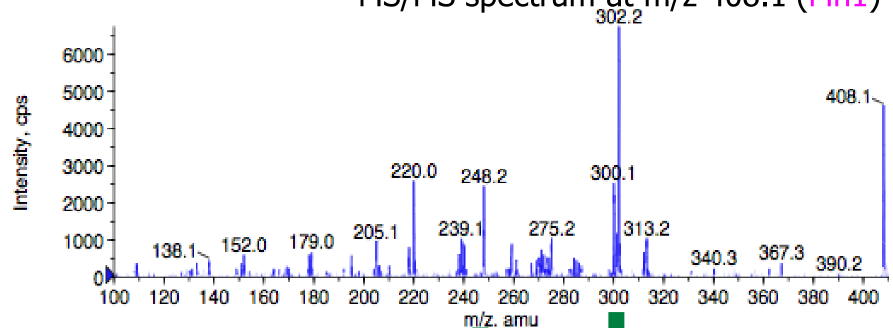
Cell Penetration: LC-MS/MS

Mn1 10 pM, water

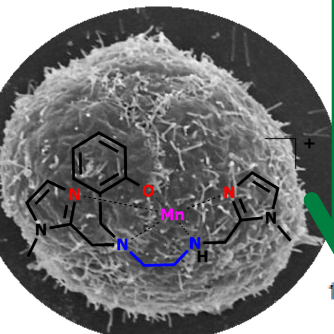
MS/MS spectrum at m/z 355.2 (L)



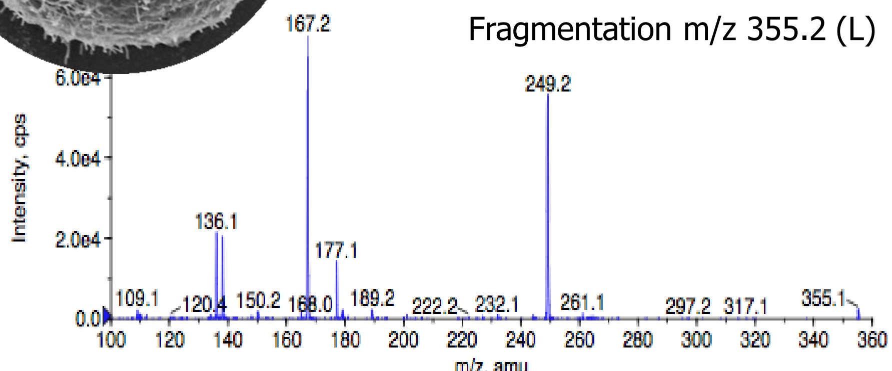
MS/MS spectrum at m/z 408.1 (Mn1)



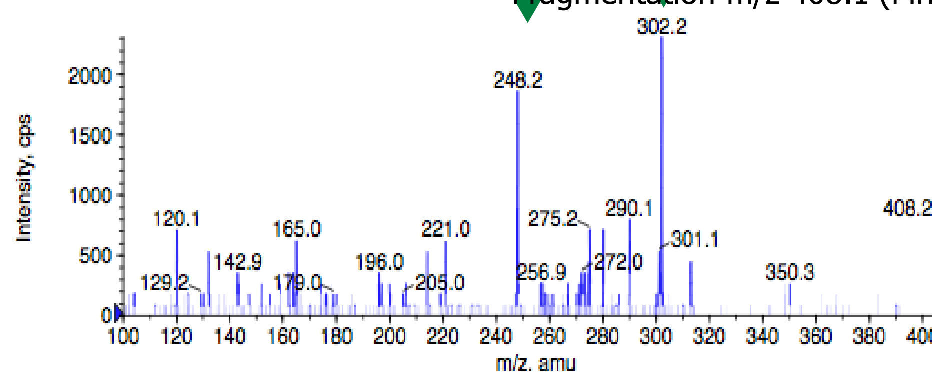
Cell lysates incubated with Mn1 (100µM, 3h), filtration (cutoff 3kDa)



Fragmentation m/z 355.2 (L)



Fragmentation m/z 408.1 (Mn1)



Mn1 is present in the cell lysates

Quantification still needed…

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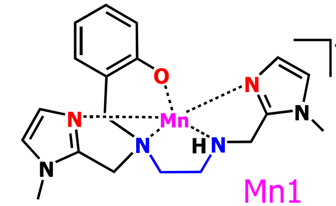
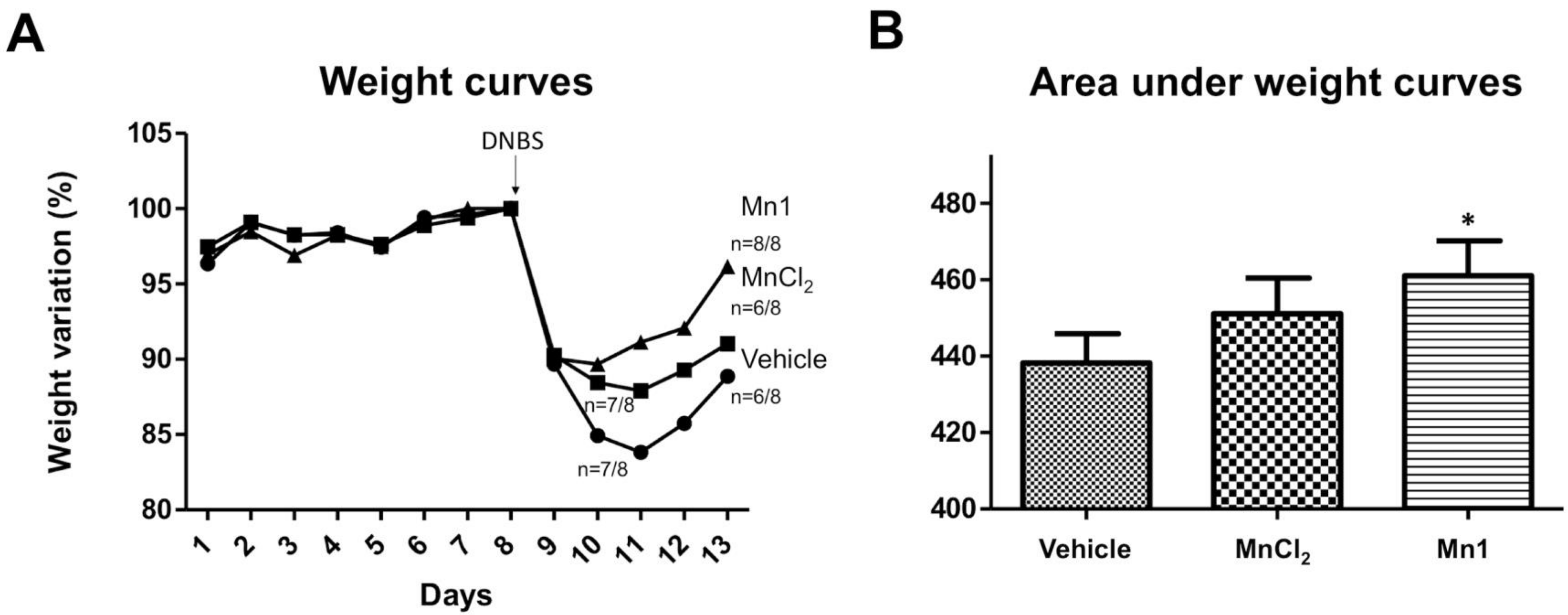
From cells in culture to mice…



Mn1



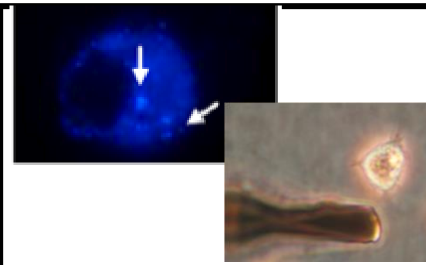
Mn1 reduces inflammation in a chimo-induced colitis

P. Langella

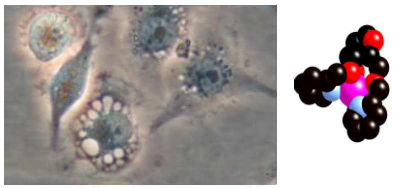
F. Chain

E. Mathieu, A.-S. Bernard et al., Inorg. Chem. **2017**, *56*, 2545–2555

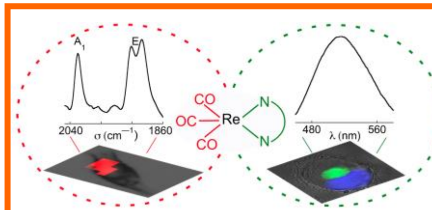
Hôpitaux Universitaires Est Parisien SAINT-ANTOINE



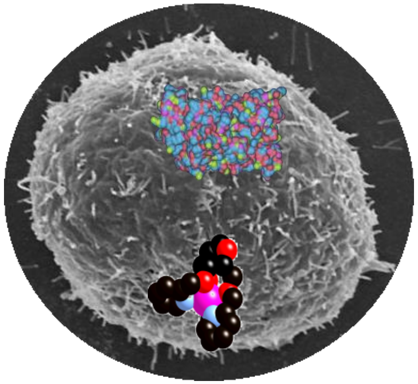
Biodetection: fluorescence, electrochemistry

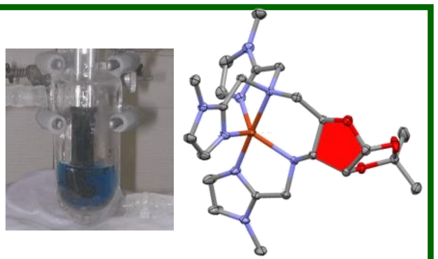


Cellular activity of antioxidant complexes



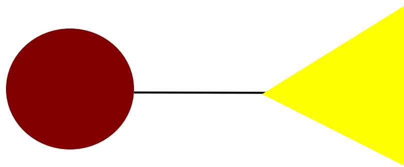
M-CO derivatives as Single Core Multimodal Probes



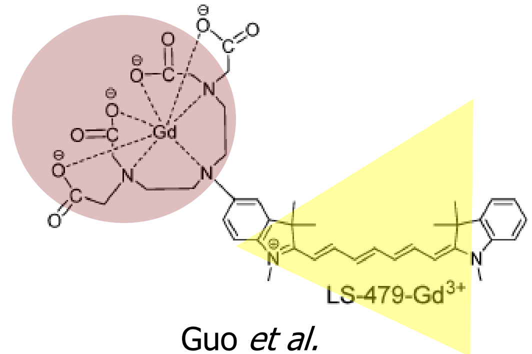


Enzyme mimics design and metal complexes with controlled properties

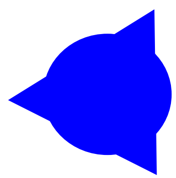
The research group in Inorganic Cellular Chemistry, September 2013



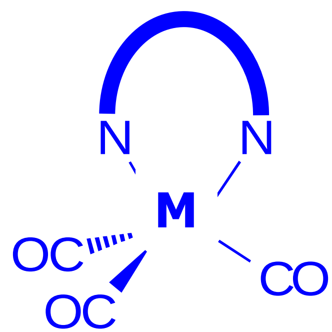
Conjugation
of two probes



Guo *et al.*
Chem. Commun., 2010, **46**, 3705–3707



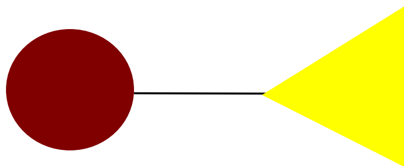
A single molecular
probe with two
modalities



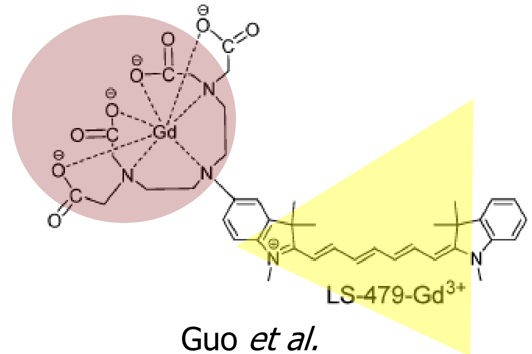
Multimodal Probe in a Nutshell:

Single Core Multimodal Probe for Imaging (SCoMPIs)

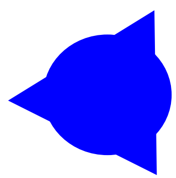
S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M.-A. Plamont, P. Dumas, A. Vessières, C. Policar, Chem. Commun., 2012, 48, 7729–31
Selected for Highlights SOLEIL 2012



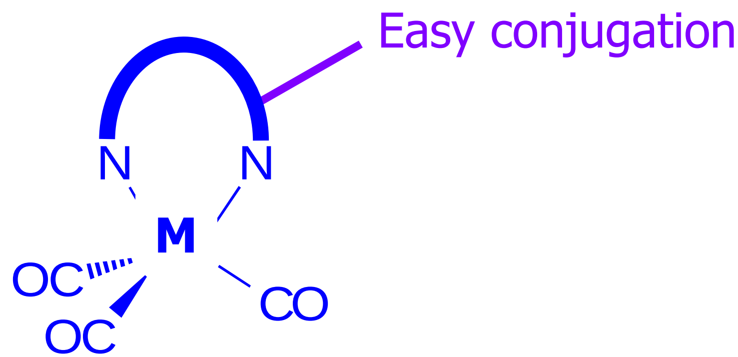
Conjugation
of two probes



Guo *et al.*
Chem. Commun., 2010, **46**, 3705–3707



A single molecular
probe with two
modalities



Multimodal Probe in a Nutshell:

Single Core Multimodal Probe for Imaging (SCoMPIs)

S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M.-A. Plamont, P. Dumas, A. Vessières, C. Policar, Chem. Commun., 2012, 48, 7729–31
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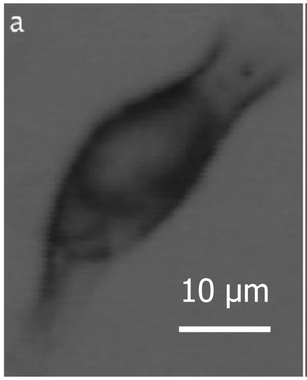
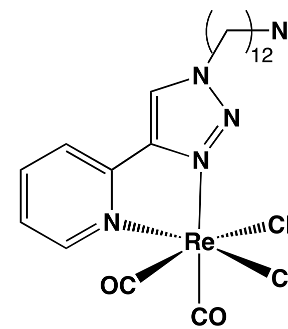
Synchrotron based-FTIR imaging (SMIS-beam line)



Bright field

MDA-MB-231 cell incubated with 1 (10 µM, 1 h, 37 °C, 5% CO_2, fixation 4% PFA)

1

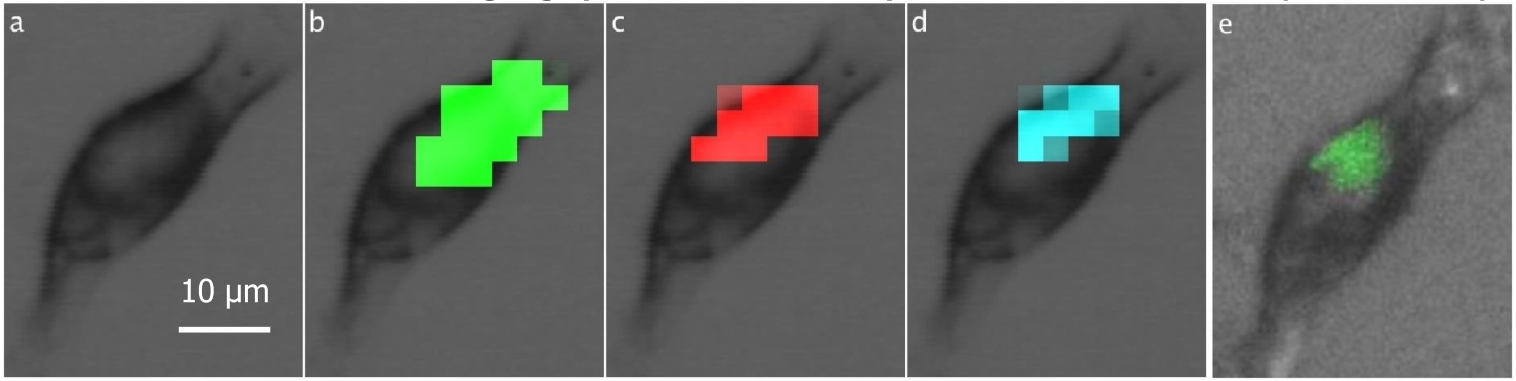


S. Clède

S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M.-A. Plamont, P. Dumas, A. Vessières, C. Policar, Chem. Commun., 2012, 48, 7729–31

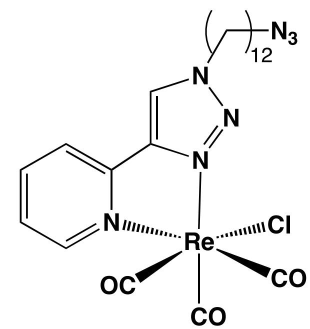
Selected for Highlights SOLEIL 2012

Synchrotron based-FTIR imaging (SMIS-beam line): Resolution ca. 3×3µm², hot-spots



Bright field | Amide I | E-band | A1-band | Fluorescence

MDA-MB-231 cell incubated with 1 (10 µM, 1 h, 37 °C, 5% CO_2, fixation 4% PFA)



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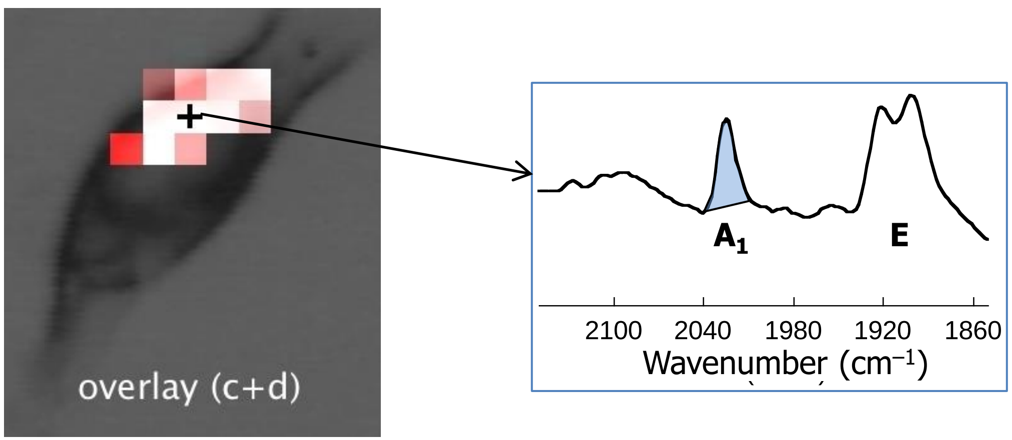
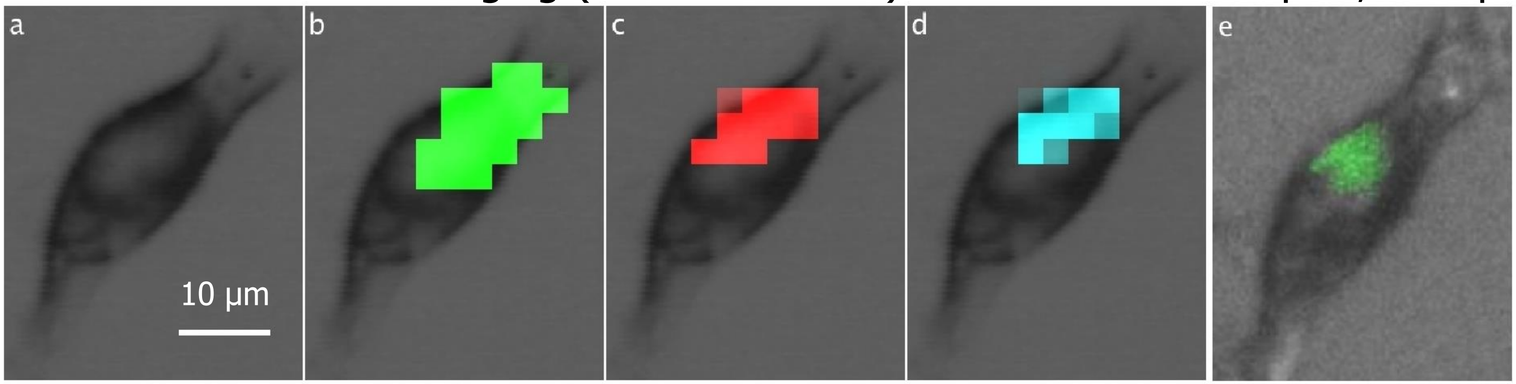


S. Clède

S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M.-A. Plamont, P. Dumas, A. Vessières, C. Policar, Chem. Commun., 2012, 48, 7729–31

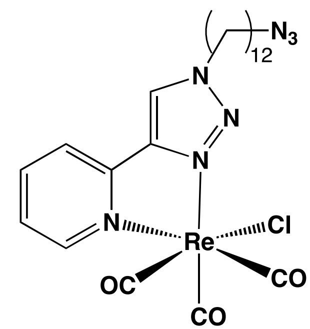
Selected for Highlights SOLEIL 2012

Synchrotron based-FTIR imaging (SMIS-beam line): Resolution ca. 3×3μm^2, hot-spots



Bright field   Amide I   E-band   A1-band   Fluorescence

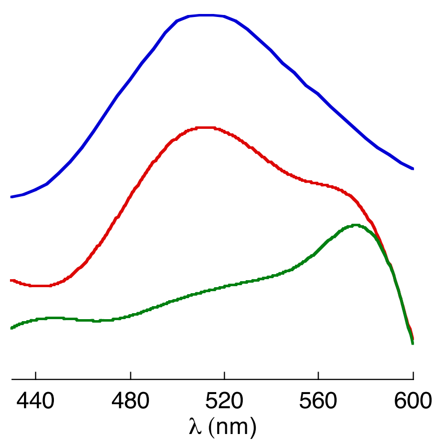
MDA-MB-231 cell incubated with 1 (10 μM, 1 h, 37 °C, 5% CO_2, fixation 4% PFA)



1

Microspectroscopy

Em. luminescence



Pure 1 , solution

Incubated single cell (10μM, 1h, PFA)

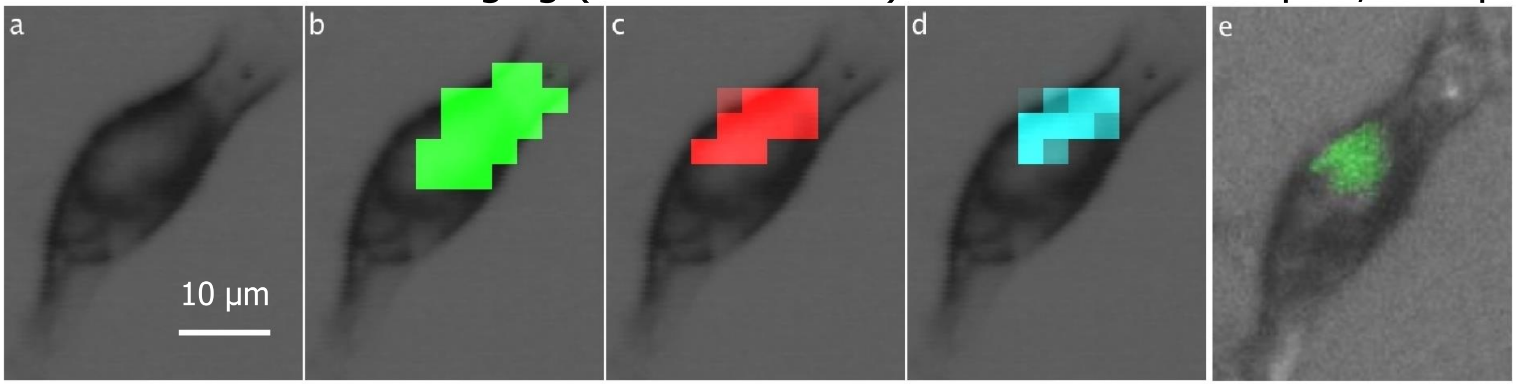
Control cell

S. Clède

S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M.-A. Plamont, P. Dumas, A. Vessières, C. Policar, Chem. Commun., 2012, 48, 7729–31

Selected for Highlights SOLEIL 2012

Synchrotron based-FTIR imaging (SMIS-beam line): Resolution ca. $3\times3 \mu m^2$, hot-spots



Bright field | Amide I | E-band | A1-band | Fluorescence

MDA-MB-231 cell incubated with 1 (10 µM, 1 h, 37 °C, 5% CO_2, fixation 4% PFA)

SOLEIL SYNCHROTRON

SMIS

Microspectroscopy

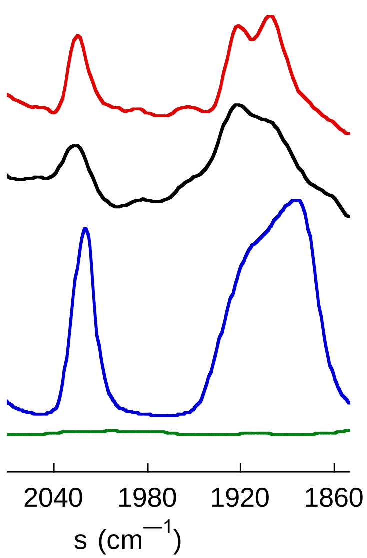
Abs. IR

Incubated single cell (10 µM, 1 h, PFA)

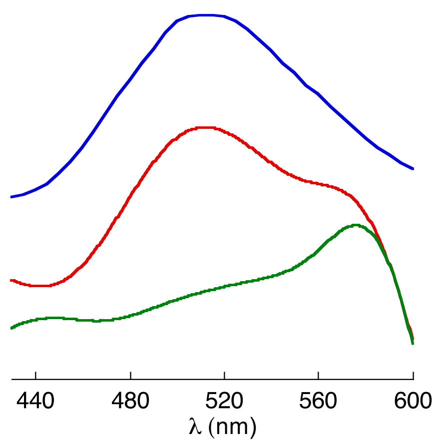
Cell population (10 µM, 1 h, 40000 cells)

Pure 1, solid

Control cell



Em. luminescence

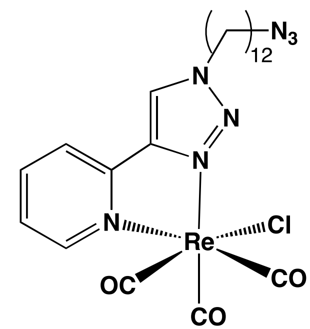


Pure 1 , solution

Incubated single cell (10 µM, 1 h, PFA)

Control cell

1



S. Clède

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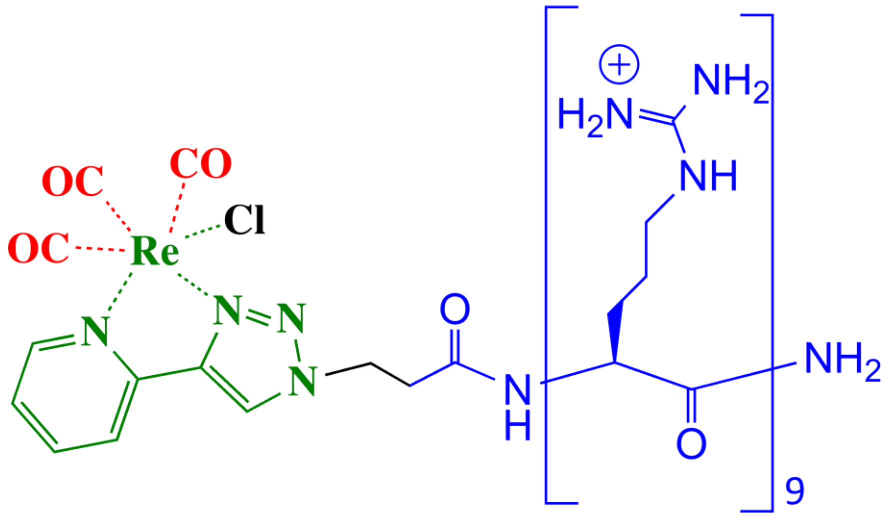
Tagged CPP

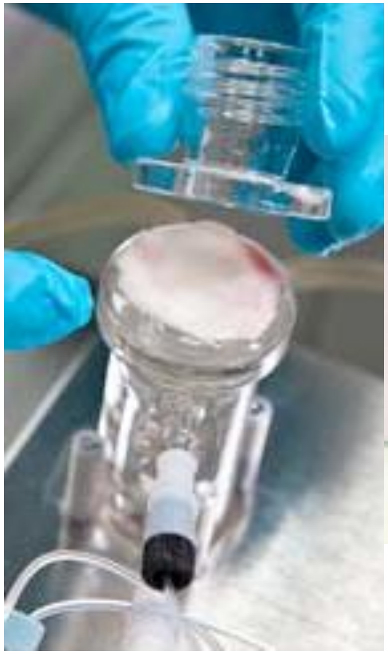


Vibrational and luminescence detection

Potentially good permeation properties

Tissue Imaging

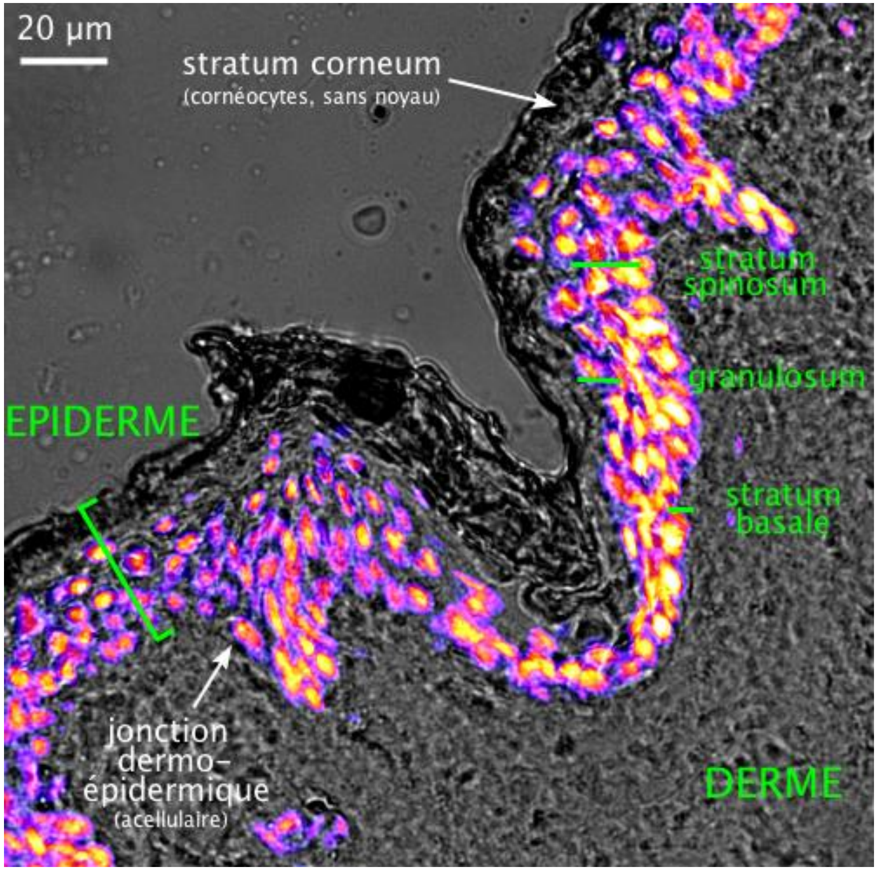
Cell-permeation through a Franz-cell



http://www.lagep.cpe.fr/wwwlagep7/viscosimetre/

http://kiosque.irsn.fr/archives/aktis/n_006.html

Skin Structure



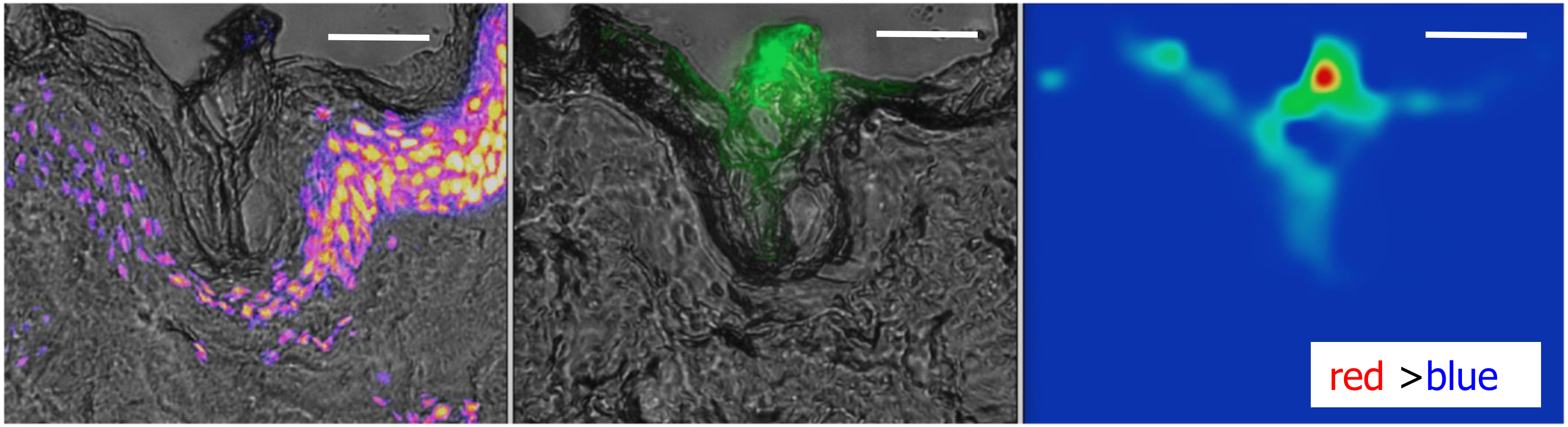
Ex-vivo studies of per-cutaneous permeation

Coll. A. Baillet, Univ. Paris Sud

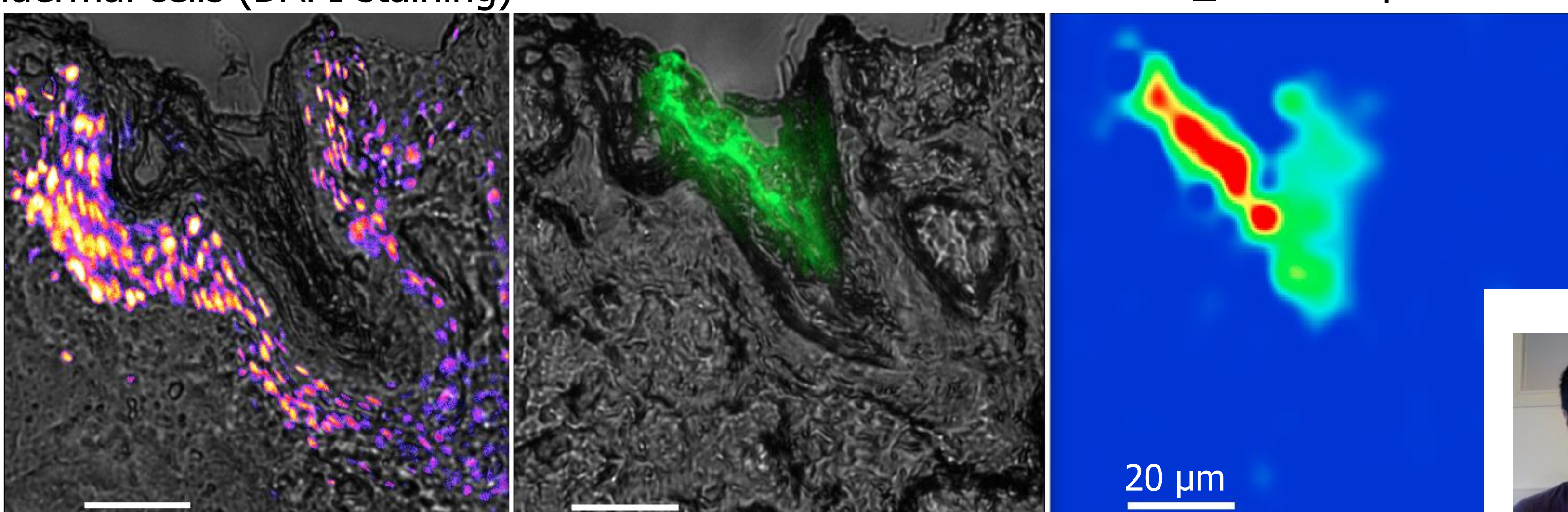
Skin-slice (10-µm-thick)

6-hour exposure

0.02 M water solution, 200 µL deposited for cell-permeation
10-µm-thick human skin slices



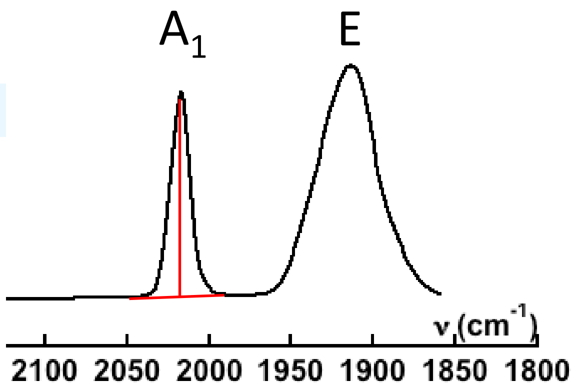
Epidermal cells (DAPI staining) | 1 luminescence | 1 IR absorption

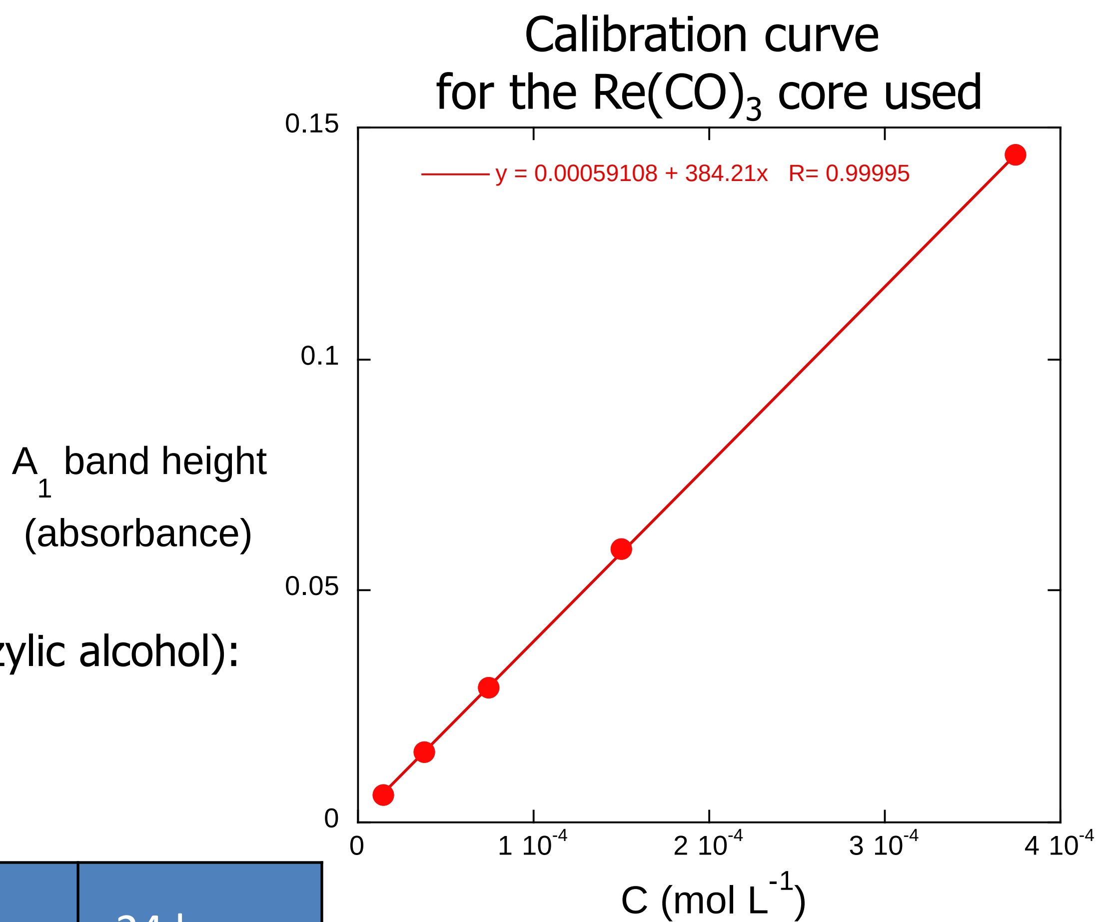


S. Clède

S. Clède, N. Delsuc, C. Laugel, F. Lambert, C. Sandt, A. Baillet-Guffroy, C. Policar, *Chem. Commun.* 2015, *51*, 2687-2689.

Quantification using FTIR





In three solvents (MeCN, EtOH, benzylic alcohol):
$\varepsilon = 365 \pm 5\%\ m^2\ mol^{-1}$

$S = 1$ pixel $= 10 \times 10\ \mu m^2$

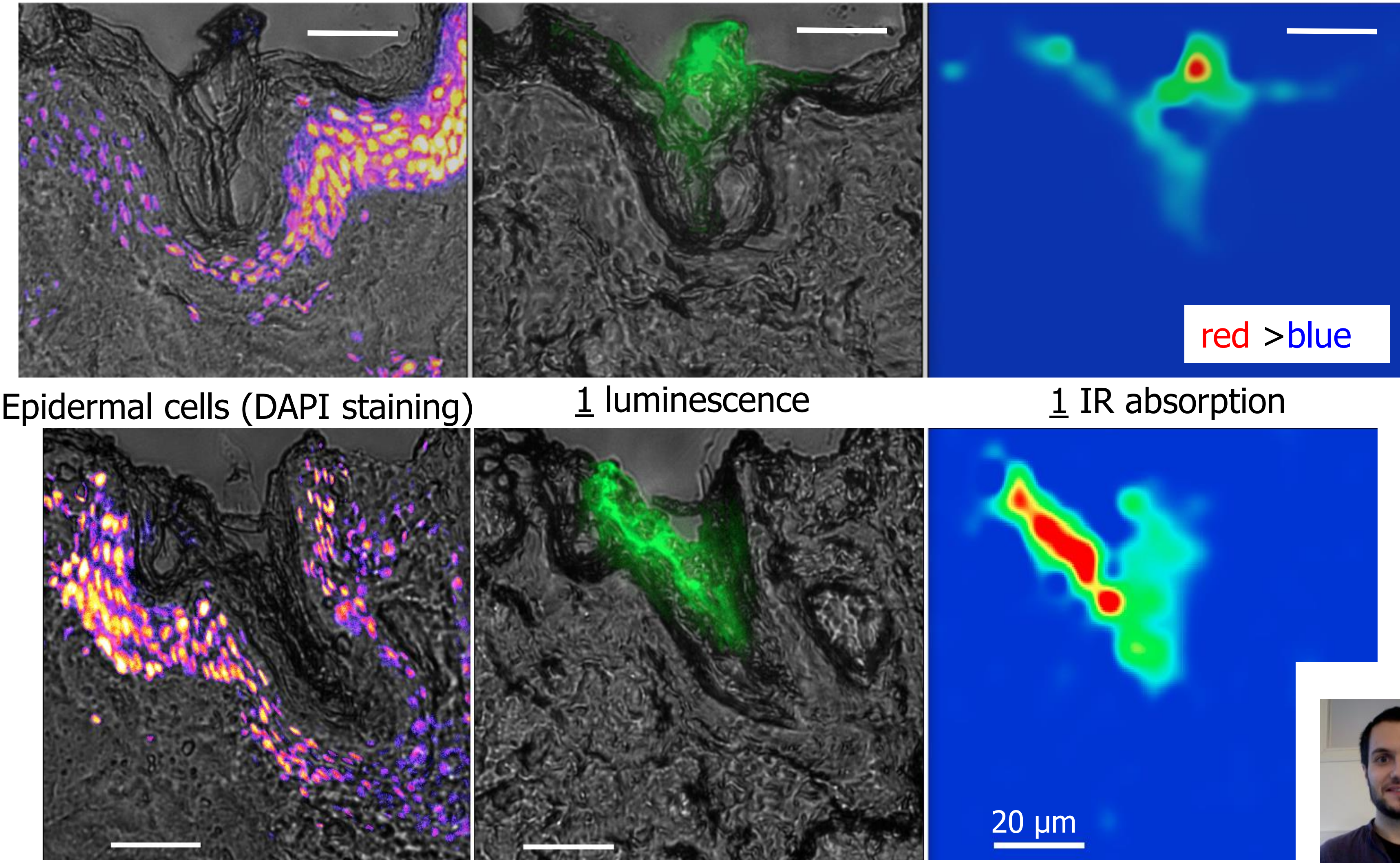
Exposure time / N (mole) (for 10x10 μm^2)	6 hours	24 hours
In the SC	$8.2\ 10^{-15}$	$1.3\ 10^{-15}$
In the epidermis	$5.7\ 10^{-16}$	$5.5\ 10^{-15}$

After 6 h, $N_{epi}/N_{SC} = 0.07$
After 24 h, $N_{epi}/N_{SC} = 4$

Skin-slice (10-µm-thick)

0.02 M water solution, 200 µL deposited for cell-permeation
10-µm-thick human skin slices

6-hour exposure



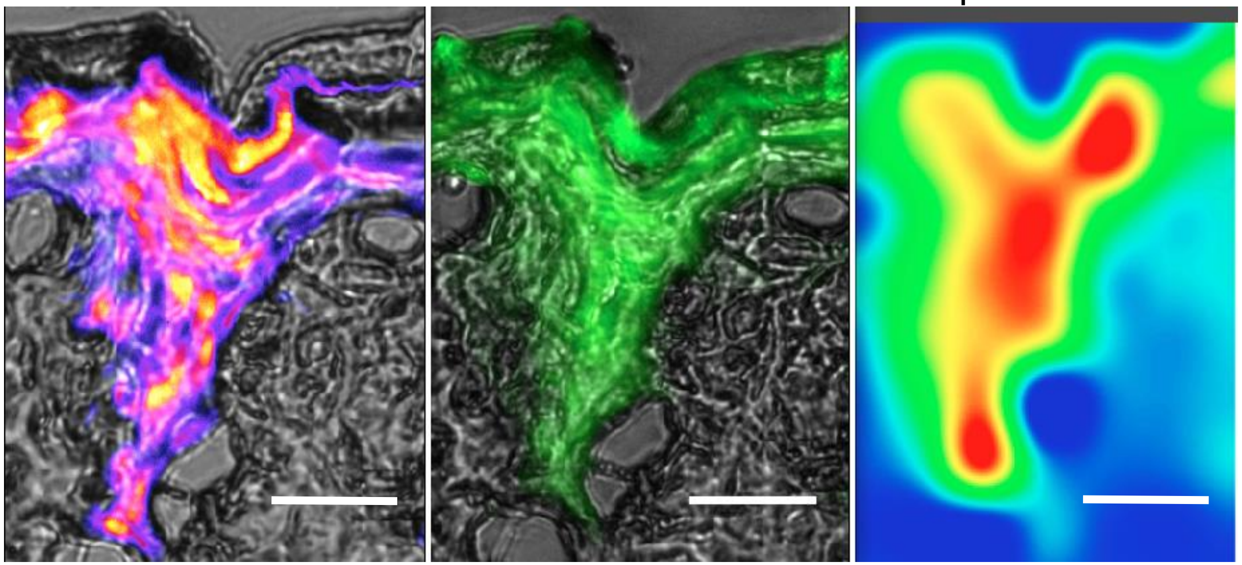
S. Clède

S. Clède, N. Delsuc, C. Laugel, F. Lambert, C. Sandt, A. Baillet-Guffroy, C. Policar, *Chem. Commun.* 2015, *51*, 2687-2689.

Skin-slice (10-µm-thick)

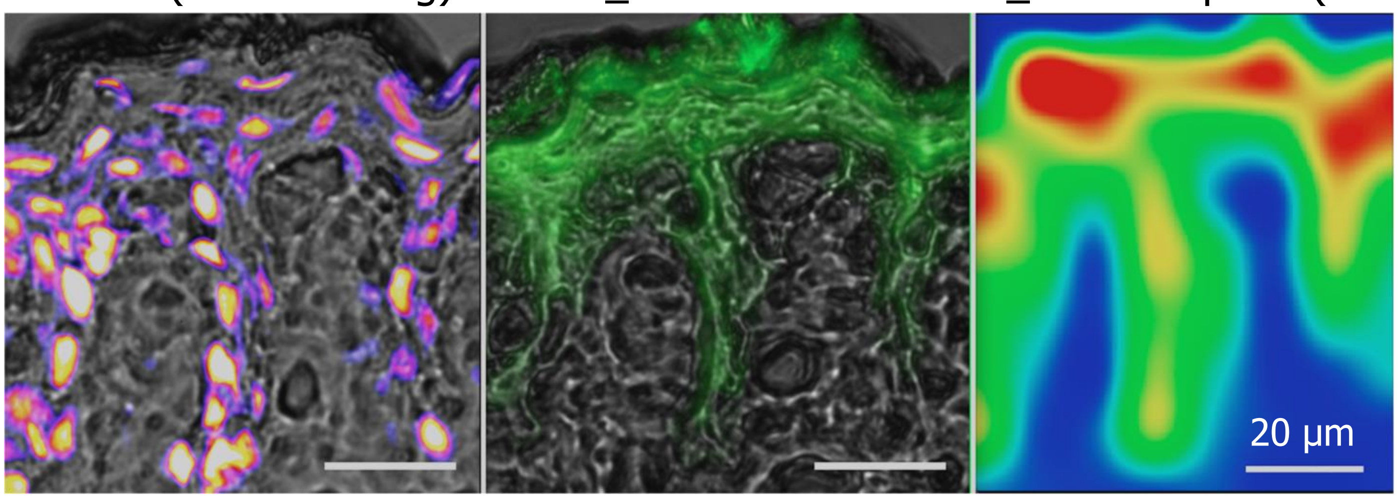
24-hour exposure

0.02 M water solution, 200 µL deposited for cell-permeation
10-µm-thick human skin slices



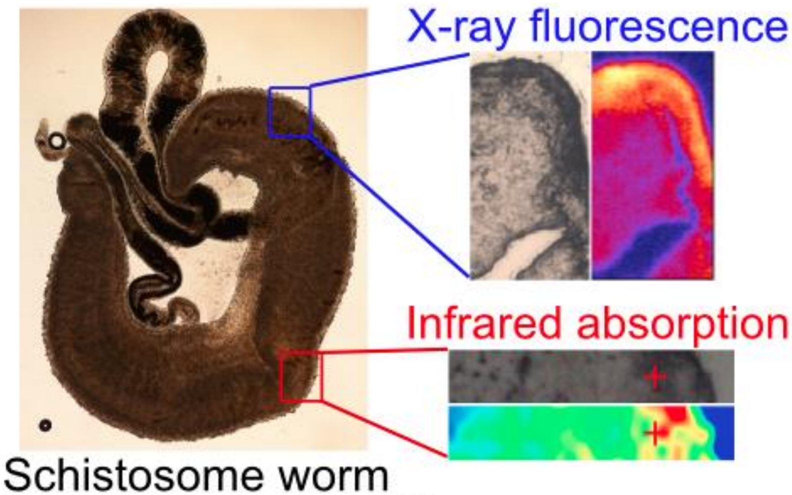
red > blue

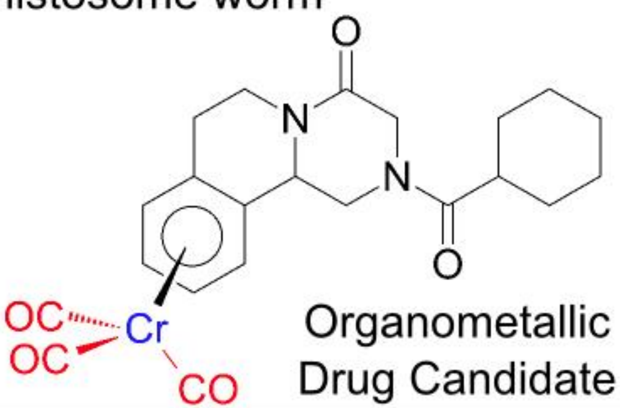
Epidermal cells (DAPI staining) — 1 luminescence — 1 IR absorption (red>blue)



S. Clède, N. Delsuc, C. Laugel, F. Lambert, C. Sandt, A. Baillet-Guffroy, C. Policar, *Chem. Commun.* 2015, *51*, 2687-2689.

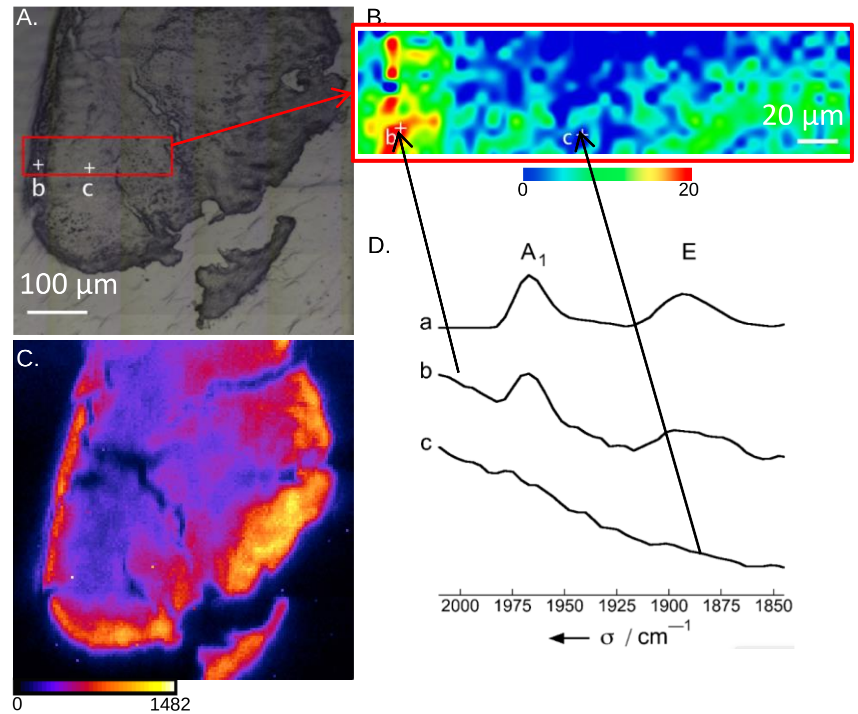
Detection of M(CO) in tissue section





Organometallic Drug Candidate

IR-map: integration in the 1980-1945 cm^{-1} energy range



Micro-XRF imaging (Cr, 6.15 keV)

12 µm thick cryo-section, deposited on ultralene

S. Clède, N. Cowan, F. Lambert, H.C. Bertrand, R. Rubbiani, M. Patra, J. hess, C. Sandt, N. Trcera, G. Gasser, J. Keiser, C. Policar, ChemBioChem, 2016, in press

SCoMPIs for biological studies

-Successful detection and quantification of an exogenous metal-carbonyl compound in whole cells by classical FTIR

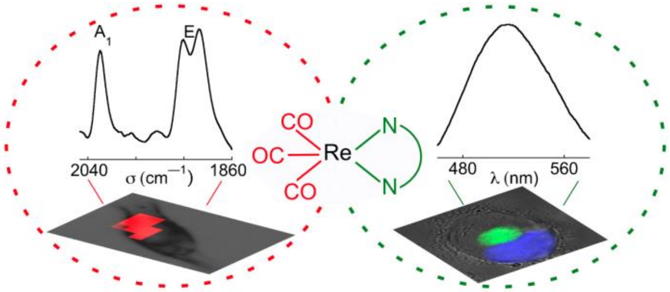
-Possible FTIR-imaging in fixed cells and in live cells

-Multimodal mappings metal-carbonyl tagged-derivative, after incubation in the µM range, at the sub-cellular and tissue levels using IR, luminescence, and X-fluorescence

Other aspects:

-Design of graftable multimodal probes

-Possible tagging of nano-systems



Merci à ...


H. Bertrand


N. Delsuc


F. Lambert


J. Masliah


E. Mathieu


A.-S. Bernard


E. Quévrain


S. Clède


P. Seksik,
Saint-Antoine


P. Langella


F. Chain

Micalis, INRA


Gilles Gasser
U. Zurich und
chimieParistech


Jennifer Keiser
U. Basel

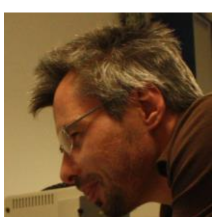
A. Baillet-Guffroy
C. Laugel

Univ.
Paris-Sud


B. Lai

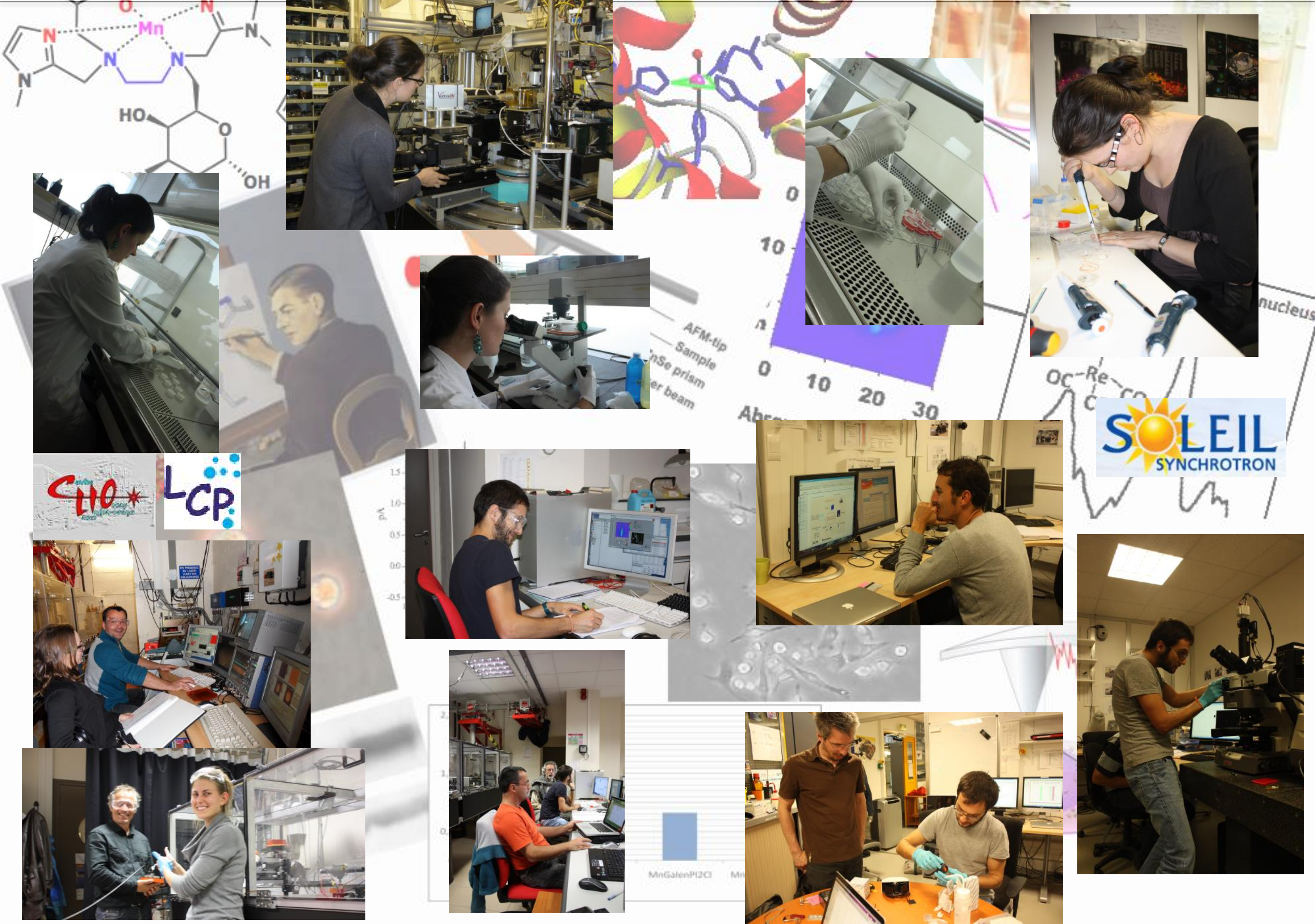
APS-Argonne


P. Dumas


C. Sandt

SOLEIL

Financements :
- ANR 2010, 2011, 2015
- ACI-JCJC (2004-2008)
- CNRS (*projets à risque,* PCV, 2009)
- Ministère de la recherche
- Contract franco-indien (CEFIPRA) (2007-10)
- ENS
- UPMC
- PROSFER program (GENS-ECNU)
- PSL structuration 2015-2017
- AFA bourse de recherche 2015
- FRM 2016-2019


Mn
HO
OH
AFM-tip
Sample
nucleus
LCP
SOLEIL
SYNCHROTRON
MnGalenPI2Cl


Merci de votre attention !