EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Ex vivo imaging of injected terpenoid-based nanoassemblies by X-ray fluorescence microscopy to understand their in vivo biodistribution.	**Experiment number**: **SC-3551**
Beamline:	**Date of experiment**: from: 29.11.12 to: 02.12.13	**Date of report**: 26.02.13
Shifts:	**Local contact(s)**: Cedric Montero (email: cedric.montero@esrf.fr)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Jean Doucet* (Laboratoire de Physique des Solides, univ. Paris-Sud 11)

Pr Patrick Couvreur (Institut Galien Paris-Sud)

Dr Barbara Fayard* (Laboratoire de Physique des Solides, univ. Paris-Sud 11

PhD Candidate Céline Epaule* (Laboratoire de Physique des Solides, univ. Paris-Sud 11)

Report :

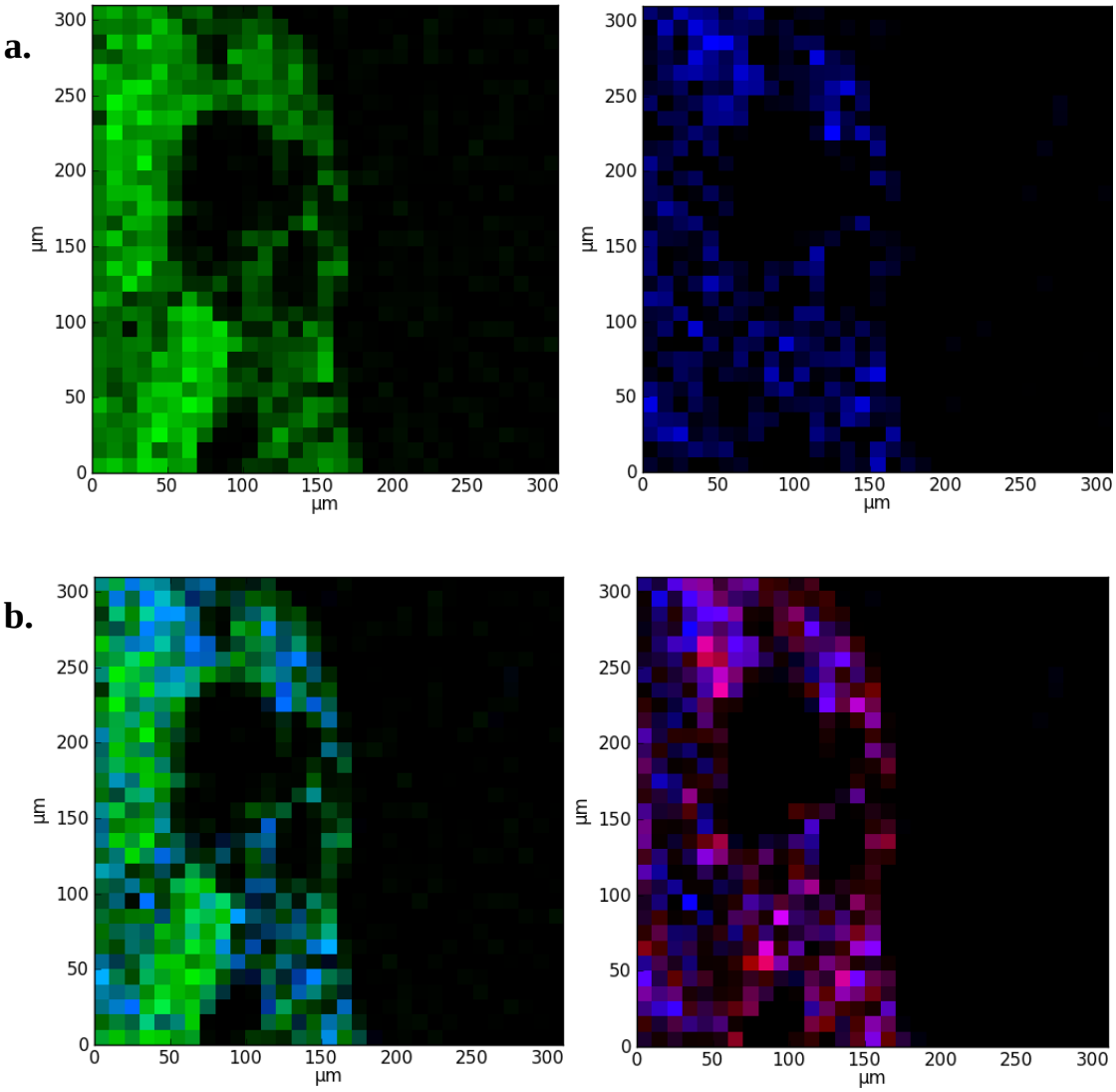
In this study our objective was to better understand how squalene-based nanoparticles, containing Cisplatin (CisPt), are distributed within various mouse organs, using X-ray fluorescence (μXRF) of platin atoms. Besides, we wanted to compare two types of nanoparticles to the commercial treatment without the drug incorporated into nanoparticles.

In this respect, we prepared different tissue sections (10 to 20 μm thick), from ten kinds of organs: brain, tumor, fat, kidney, muscle, stomach, spleen, hearth, lungs, intestine, collected after an in vivo experimentation and embedded into paraffin. The samples have been obtained under various conditions: number of terpenoid-chains bioconjugated to CisPt (1 or 2 chains are linked to the CisPt) administrated in mice, delay after an intravenous injection in mice (2, 8 or 24 hours), type of administration (oral administration or intravenously). The same samples from untreated mouse and the commercial drug (CisPt) were used as control samples. The different tissue sections were inserted between two 4 μm thick ultralene foils.

Data collection was carried out at beamline ID13 with an incident energy of 14 keV and a beam size 2*2 μm^2. The location of platinum (Pt), the special marker of our treatments in the tissues was visualized by following the intensity of the Lα-edge emission lines of Pt, mainly that at 9.45 keV which is not completely superimposed to more intense signals arising from the tissue. This emssion lines is closed to the Kβ-edge emission lines of zinc element.

Data analysis with PyMCA software is still under progress for mapping all tissues and quantify the platinum. Despite the rather low concentration of CisPt (a few ppm to tens of ppm), we manage to detect and map it in the tissues (Cf. Figure 1a. et 1b.). We are able to distinguish the contributions of platinum and zinc. The size of the mapped zones was about 300*300 μm^2, which is compatible with the visualization of histological details. We were able to analyze 15 samples, with one or 2 maps per sample. We analyzed one part of tissues above-cited:

- tumor section after 2h and 24h and injection of the three treatments, plus the control sample
- kidney section after 2h and injection of the of the three treatments, plus the control sample
- liver section after 2h and injection of the the two new treatments plus the control sample
- colon tumor after oral administration of one new treatment, the commercial treatement and the control tissue



<u>Figure 1:</u> *Distribution of CisPt in the edge of intradermal pancreatic tumor, 2 hours after intravenous injection of 2 mg/kg eq Pt (Maximum Tolerated Dose), mapping by X-ray fluorescence:* ***a.*** *mapping of platinum (blue), sulphur (green)* ***and*** *b. correlation of platinum with sulphur (green), iron (red).*

The sulphur repartition brings information about protein repartition, so, mapping of the sulphur element indicates the tissue zones. We notice that platinum is distributed everywhere in the tissue, as showed by the correlation between sulphur and platinum. There are some parts more intense of platinum correlated with the iron, indicating the tumor vascularisation.

In conclusion, we consider that this part of experiment was successfull. We can already conclude to differences of platinum concentration in the same tissues treated differently by the two types of NPs or the commercial treatment non encapsulated. We also noticed differences between tissues: more platinum is observed in kidney, compared with tumor and liver and according to the elimination of the drug. There is less platinum in liver section but more than in tumor section.
We need to analyzed the other conditions above-mentioned to understand the distribution mechanisms of our new drug delivery system. With the complete experiment, our objectif is to find the other organs where platinum is distributed and compare the different treatments.