Prof. Eléna ISHOW
Nantes Université
Faculté des Sciences et Techniques
CEISAM-UMR CNRS 6230
2 rue de la Houssinière - BP 92 208
44 322 Nantes cedex 3, France
Phone: +33-2-5112-5375
E-mail : elena.ishow@univ-nantes.fr

Statement on the Doctoral dissertation of Ms Mathilde PONCHELLE for an oral defense to confer the joint grade of Doctor in Chemistry (Cotutelle) from the Institute of Nuclear Chemistry and Technology (Warsaw – Poland) and University Paris-Saclay (Saclay – France)

Title of the PhD thesis

« Design and synthesis of micellar nanocarrier for internal radiotherapy" »

The PhD work reported by Ms Mathilde PONCHELLE relies on the buoyant field of nanomedicine in cancer treatment, allowing for multimodality, improved efficiency, and personalized treatment thanks to the manufacturing and use of nanometer-sized medicines that display preferential accumulation at the vicinity of tumorous tissues and can combine various imaging labels, drugs and targeting agents within a same nano-object. The interdisciplinary studies reported in the manuscript address in particular cancer treatment by radiotherapy using nano-constructs based on perfluorinated micelles, functionalized with the β^- and γ emitter ^{177}Lu radioisotope and encapsulating gold nanoparticles as radioamplifiers. The PhD thesis is organized along three chapters including a short introduction (27 pages), a conclusion and an experimental section completed with appendices mainly on biological studies.

The introductory chapter successively presents the general stakes in cancer development and associated treatments, as well as the main imaging techniques to detect and monitor the treatment evolution, with a specific focus on radioimaging. This is followed by a comparative presentation of the three technologies (nuclear reactor, cyclotron and generator) to generate the main radionuclides currently used, and a brief description of the two nuclear imaging techniques (PET and SPECT) and radiotherapy approaches, adopted in the clinics. In the context of radiotherapy, special attention is paid on the aftermaths of ionizing radiations (γ or X-rays) on organic matter, in terms of chemical damages caused by reactive oxidative species (ROS) whose generation is disfavored in hypoxic conditions, as commonly encountered in O_2-depleted surroundings of tumors. In order to overcome the shortcoming of dioxygen to make radiotherapy efficient, a smart therapeutic strategy is then presented, involving the administration of perfluorinated micelles, efficiently entrapping O_2 and carrying promoters of ROS production, namely $^{177}Lu^{3+}$ and gold nanoparticles serving as radio-amplifiers by photoelectric and Auger effects. This is thus the occasion to position nanoparticles in the field of nanomedicine and explicit their specific advantages with regard to the use of molecular agents, before presenting the systems and objectives of the conducted researches.

The first chapter is devoted to the first aspect of the studies, namely the synthesis of self-assembling perfluorinated and pegylated oligomers, the synthesis of gold nanoparticles and their encapsulation in micelles, and finally *in cellulo* studies in the absence and in the presence of X-ray irradiation. It starts with the general presentation of micelles through their constituents, especially those incorporating perfluorinated entities, and gold nanoparticles, in the context of cancer therapy. After reporting the synthesis of the perfluorinated species and characterizations of the resulting micelles, three strategies for the manufacturing of gold nanoparticles (Brust-Schiffrin, Turkevich and Stellacci) are described. Once encapsulated in micelles, only the nanoparticles issued from the last method provided nano-objects with adequate colloidal stability and little cytotoxicity when incubated with SKBR3 human breast cancer cells. External irradiation studies were carried out at three distinct energy dose (1, 2 and 4 Gy), by adapting the exposure time to an electron beam (9 MV, 10 $Gy.min^{-1}$). Two cell lines, SKBR3 breast cancer cells and B16F10 melanoma cancer cells were incubated with micelles alone and gold-trapped micelles (AuNP@micelles) and their cell viability studied 24, 48 and 72 h after exposure to the electron beam. Two operating conditions were adopted: i) cells were incubated with micelles for 24 h and directly exposed to radiation (chronic toxicity), ii) cells were first incubated with micelles for 24 h, the excess of micelles was washed out, and the stained cells were then exposed to radiation (acute toxicity). Only the more radiosensitive SKBR3 cell line, treated with gold-loaded micelles, showed a neat decrease in cell viability compared to that of non-treated cells or cells treated with micelles only. This effect is even more pronounced for acute toxicity experiments, validating the adopted approach of radiosensitization by gold nanoparticles.

The second chapter extends the biological strategy exposed in the first chapter to gold-encapsulated micelles (AuNP@micelles) incorporating at their surface $^{177}Lu^{3+}$. It starts with the description of the production of this radioisotope and its radioactivity characteristics in terms of half-life and energy emission. The pursued scientific objectives lead to the implementation of a seven-step synthesis to generate a perfluorinated amphiphilic unit, comprising the chelating DOTA ligand, amenable to strongly interact with lanthanide ions (like gadolinium). After optimization of the radiolabeling process using $^{177}Lu^{3+}$ ions, colloidal stability of the resulting micelles was demonstrated in various media of physiological interest. Both cell lines underwent a decrease in cell viability with increasing amounts of radiolabeled ligands. Detectable effects of gold nanoparticles as potential radioamplifiers could be observed also on both cell lines, especially with the less radiosensitive B16F10 cell lines for which chronic and acute toxicity experiments were conducted. The fact that these two series of experiments led to the same decrease in cell viability lets suggest than radioisotope internalization is required to effectively cause cell damages. Finally, *in vivo* experiments to test the efficacy of the radiolabeled micelles and establish their biodistribution were performed on mice, after systemic administration through the tail vein or direct injection in subcutaneous tumors. For the first kind of injection, the micelles were found to be quickly cleared from blood circulation and mainly accumulate in the lungs, liver and especially the spleen, before being excreted by the kidneys. Contrary to the first experiment where radioactivity mostly vanished after 168 h (except in the spleen), the second experiment showed permanent radioactivity in the tumor after the same duration, although part of the micelles was again excreted by the kidneys, in a 30 % lesser amount for radiolabeled gold-containing micelles compared to radiolabeled micelles alone. Assessment

of the tumor volume over 168 h showed visible effects of radiolabeled gold-containing micelles, leading to slight regression of the tumor with respect to mice treated with radiolabeled micelles only.

Overall, the PhD studies of Ms Mathilde PONCHELLE, spanning a large range of fields (organic synthesis, nanoscience, radiochemistry, radiotherapy, biological studies) display promising results. The thesis by itself is particularly concise, making its reading sometimes quite difficult since very little details are given. It would have deserved a longer description of the state-of-the-art in the field of radiotherapy using micelles to better quantify the advances brought by the current studies. The parts devoted to the general presentation to gold nanoparticles in chapter 1 and lutetium 177 in chapter 2 should rather have been placed in the introductory chapter, to avoid useless redundancy. The description of the micellar systems and gold nanoparticles would also gain clarity if each part, at the end, would specify the kinds of entities that would be retained during the PhD studies, in terms of geometry, structure, and properties. Several characterizations of the gold nanoparticles would have been appreciated since their TEM images or absorption spectra are missing. We can also regret that no experiments have been performed to assess the content of generated ROS upon exposure AuNPs@micelles to irradiation, in solution or after incubation with cells. In the same way, control experiments with gold nanoparticles alone would have been worth doing to assess the advantages of working with gold-encapsulated micelles. Finally, I would recommend to number and sort out the references cited at the end of the thesis in such a way that they correspond to a logic order (alphabetic order or chronological citation order).

In conclusion, despite the reported observations, I consider that the academic requirements (quality of research, originality of the conducted work, scientific advances for the community) are met to authorize Ms Mathilde PONCHELLE for a defense of her Cotutelle PhD work to obtain the joint grade of Doctor from ICHTJ (Poland) and University Paris Saclay (France).

Nantes, November 2025, 12th.

Eléna ISHOW
Full Professor of Chemistry