



## Evaluation Report of the PhD thesis

Title: **Neuroprotective lipid nanoparticles for regeneration from post-COVID-19 neuronal damage**

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Mrs. Thelma AKANCHISE prepared the thesis under the supervision of Mrs. Angelina ANGELOVA, within the research group Institut Galien Paris-Saclay and the doctoral school Therapeutic innovation: from basic to applied. I evaluated the thesis with respect to the criteria listed below.

### Thesis quality, style & illustrations

The PhD thesis of Mrs. T. Akanchise is a well-composed dossier consisting of five chapters completed with purpose and objectives of the thesis, list of abbreviations, and general introduction written in French and English. In the last part of the thesis, conclusions, and perspectives, the author briefly summarizes results in the context of the topic and outlines her view for the next research. Together, the text comprises 265 pages. Individual chapters are addressed to: bibliographic study compiled as a review paper (52 pages); experimental methodology (20 pages); and three chapters with experimental results and discussion. The results and discussion are 137 pages and take the reader through biophysical characterization of the nanoparticles, *in vitro* experiments on selected cell lines, and *in vivo* study. This thesis is not a collection of already published papers, however, each chapter specifies publication output and coauthors. The scientific results summarized in the thesis resulted in 3 published articles, two submitted manuscripts, and 1 manuscript under preparation. Mrs. T. Akanchise is the first author of published articles and manuscripts.

The text is written clearly, graphics and illustrations are of good quality, fulfilling requirements for the thesis. I found only a few typos there.

### Background, state-of-the-art, collaborations

The purpose of the thesis was to develop and characterize lyotropic lipid nanoparticles designed for the delivery of *Ginko biloba* bioactive phytochemicals for the treatment of neurodegeneration associated with oxidative stress, focusing on post-COVID neurological complications. The high vulnerability of neurons to inflammatory and oxidative stress manifested by the overproduction of reactive oxygen species (ROS) has been suggested as a crucial pathophysiological mechanism of the long COVID syndrom. The author published two review articles (Antioxidants, 2023, vol. 12, 393 and Pharmaceutics, 2023, vol.15, 1562) addressing neurological disorders and the potential benefit of nanomedicine. The studies gained more than 50 citations that validate the topicality and importance of the problem. The bibliographic study related to the benefits of phytochemicals extracted from *Ginko biloba* as potential antioxidants and the need for suitable nanocarriers to improve their bioavailability is discussed in the first chapter. Lyotropic lipid nanoparticles (cubosomes, hexosomes, and vesicles) were designed as carriers for antioxidants from *Ginko biloba*. Without a doubt, the originality of the



thesis lies in the rational engineering of nanocarriers for selected antioxidants that profit from the self-assembly of lipids and additives, forming intelligent stimuli-responsive systems for targeted delivery. The subject and the extent of the study are challenging in terms of orientation in the polymorphism of lipids combined with a deep knowledge of the pathophysiology of neurodegenerative diseases. The high quality and extensiveness of the study demonstrate a good coordination of the research team involved. As a reviewer, I would appreciate a brief comment specifying the contribution of the student in respect to the list of authors reported in individual chapters.

### **Scientific quality, methodology, experiments, validation**

Three chapters related to the experimental results constitute the heart of the thesis and fulfill the objectives of the project. The design and structural properties of the phytochemical loaded nanoparticles are introduced in Chapter 3. The structural polymorphism of monoolein and dimyristoylphosphatidylcholine (DMPC) was modulated by additives such as ionizable helper lipid, PUFA, or plasmalogen to obtain a pH- responsive system and show antioxidant activity when enriched with phyto-antioxidants. The nanoparticles were stabilized with Pluronic F127, a PEGylated lipid, or by polypeptide to improve targeting efficiency. The structural polymorphism of the nanoparticles was examined by using synchrotron SAXS (static and time resolved), the size was assessed by DLS, and the morphology was evaluated by cryo-TEM. The antioxidant properties were tested by the free radical scavenging activity of DPPH, antioxidant power, lipid peroxidation, and antioxidant release. Extensive *in vitro* studies (summarized in the fourth chapter) were performed on SH-SY5Y human neuroblastoma cells with induced oxidative and inflammation stress modeling post-COVID neuropathology. The developed nanoparticles were evaluated for their ability to restore physiological conditions, namely: glutathione peroxidase activity, suppress ROS accumulation, modulate key signaling cascades, and inhibit inflammatory pathways. I found 18 different assessments to quantify the effect of designed LPN on cells reported in the experimental methodology (Chapter 2). The experimental results obtained from the *in vitro* studies are summarized in 2 published articles (J. Med. Virol., 2024; ACS Biomater. Sci. Eng., 2025), one submitted, and one manuscript under preparation. The last chapter (Chapter 5) is devoted to *in vivo* studies using the Parkinson's disease model (a MitoPark mouse) to test nose-to-brain drug delivery by intranasal administration of phytotherapeutics- enhanced lipid nanoparticle formulations modified with the PACAP peptide. The findings confirmed excellent biocompatibility and significant neuroprotective transcriptomic changes, including up-regulation of mitochondrial, antioxidant and neurotrophic markers in combination with suppression of pro-apoptotic genes. The findings were summarized in the manuscript submitted for publication.

### **Personal contributions, originality, valorization, prospects**

The thesis of Mrs. Thelma Akanchise is original and brings novelty in findings related to enhanced therapeutic potential of poorly soluble bioactive compounds (ginkgolide B/C, quercetin, and kaempferol) extracted from *Ginkgo biloba* when encapsulated in a carrier formed by lyotropic liquid-crystalline nonlamellar mesophases of lipids. The goals set were fulfilled to the full extent. The candidate briefly outlined her perspectives on future research. The study revealed an important role for natural antioxidants in the treatment of serious neurological problems and offered findings that open the door to the "green pharmacy" and non-invasive prophylactic approach.

**Reviewer's conclusions**

The findings obtained by Mrs. Thelma Akanchise under her PhD project prove her systematic wide-ranging rigorous work that resulted in this thesis and five scientific papers. The results obtained are original, and the findings contribute to knowledge in the field of lipid-based pharmaceutical formulations and nanomedicine.

**Consequently, I give a very favorable opinion to the defense of this thesis by Mrs. Thelma Akanchise to get a PhD degree from the University of Paris-Saclay in the field of Pharmacotechnie et Biopharmacie.**

Bratislava, November 26, 2025



prof. RNDr. Daniela Uhríková, PhD.

## Rapport de thèse

Rapporteur : Monsieur **Mustapha Cherkaoui Malki**, malki@u-bourgogne.fr  
Qualité : Professeur des universités, Université Bourgogne Europe  
Mémoire : présenté par **Mme Thelma AKANCHISE**  
Diplôme : pour l'obtention du grade de Docteur de **l'université Paris-Saclay**.  
Spécialité de doctorat: Pharmacotechnie et Biopharmacie  
École doctorale n°569: innovation thérapeutique : du fondamental à l'appliqué (ITFA).

Soutenance prévue le : **11 décembre 2025**

Mme Thelma AKANCHISE a préparé sa thèse dans l'unité de recherche Institut Galien Paris-Saclay (Université Paris-Saclay, CNRS), sous la direction de Docteur Angelina ANGELOVA, Directrice de Recherche CNRS, Université Paris Saclay - UMR CNRS 8612. Le titre de la thèse est « *Neuroprotective lipid nanoparticles for regeneration from post-COVID-19 neuronal damage* ».

Compte tenu que le manuscrit de thèse est rédigé entièrement en anglais, à l'exception d'un résumé introductif de 12 pages en français, et dans le but de rendre ce rapport facilement accessible pour la candidate, le rapport est rédigé en anglais. A la fin de ce document, mon avis en tant que rapporteur sera rédigé à la fois en anglais et en français.

The thesis work was focused on the lyotropic lipid liquid crystalline nanoparticles as neuroprotective carriers targeting mitochondrial dysfunction. These PUFA- and Ginkgo biloba-based nanostructures were engineered to modulate oxidative stress, apoptosis, neurotrophic signaling, and neuroinflammation.

The manuscript, comprising 258 pages, is organized into five main chapters and includes lists of abbreviations, figures, and tables. It begins with a twelve-page introduction providing a concise presentation of the thesis in French, followed by a bibliographic study reviewing the relevant literature, an experimental methodology chapter, three chapters devoted to the results, a final section on conclusions and perspectives, and a supporting information appendix.

In the first chapter of "bibliographic study", Thelma AKANCHISE presented a concise bibliographic review of the literature (published in *Pharmaceutics* in 2023). This part reviewed relevant knowledge on the triggered brain mechanisms following the SARS-CoV-2 infection, particularly mitochondrial dysfunction and oxidative stress, and paralleled mechanisms involved in Parkinson's disease. This review emphasizes the potential role of Ginkgo biloba phytochemicals in the remedy of such neurological dysfunctions.

The second chapter appears to be central, presenting the experimental methodology used for multiple designs of lipid crystalline nanoparticles (LNPs), including pH-responsive Dlin-MC3-DMA-based nanocarriers as ionizable helper lipids, monoolein-DMPC phosphocholine derivatives, and PUFA-based nanoparticles as well as peptide-functionalized LNPs. One of the main objectives was to encapsulate, phyto-antioxidants such as ginkgolide B/C, quercetin, and kaempferol. The characterization of the size, shape, and structural organization of these macromolecules in solution was accomplished using high-brilliance synchrotron Small-Angle X-ray Scattering (SAXS),

complemented by Dynamic Light Scattering (DLS) to determine particle size and polydispersity. This chapter appears to be a compilation of the Materials and Methods sections derived from the different articles presented in Chapters Three to Five. As a result, the reader is led into an iterative back-and-forth reading between the various articles integrated throughout the chapters.

The third chapter was devoted to the physicochemical characterization of the designed lipid nanoparticles (LNPs), by analyzing their biophysical properties. The study revealed that the internal liquid-crystalline structure, interfacial properties, and release dynamics of LNPs are modulated by their composition—whether loaded with ginkgolide B and quercetin or not—, the pH, and peptide functionalization.

The *in vitro* evaluation of the LNPs was presented in the fourth chapter. In the part I, oxidative stress and inflammation were induced in the SH-SY5Y neuronal cell line—a model of neuroinflammation—by cotreatment with LPS and potassium persulfate. The findings showed that LNPs, particularly those encapsulating ginkgolide B  $\pm$  quercetin in cubosomal monoolein or vesicular 1,2-dimyristoyl-sn-glycero-3-phosphocholine, exhibited a higher free-radical scavenging activity, attenuation of ROS accumulation, and reduction of apoptosis in SH-SY5Y cells.

In part II of chapter four, Thelma AKANCHISE investigated the neuroprotective activity of designed PUFA-LNPs and functionalized with bioactive cell-penetrating pituitary adenylate cyclase-activating polypeptide on differentiated SH-SY5Y cells stressed with potassium persulfate. Obtained data showed that PUFA-LNPs mitigate cell oxidative stress triggered by potassium persulfate treatment, enhanced the expression of the mitochondrial synthase, and modulate neuron signaling pathway. Such cellular effect seems to be pH-dependent, as it influences the intracellular release of PUFA-LNPs. Part III presented results on encapsulated phytochemicals in PUFA-LNPs, which showed synergistic effects suppressing phytochemical inflammatory mediators and activating the NRF2 antioxidant defense pathway.

The last chapter reports the *in vivo* investigation on the activity of LNPs using the Parkinson' disease model, a MitoPark mouse. Due to mitochondrial dysfunction resulting from the knockout of mitochondrial transcription factor A (Tfam) in dopaminergic neurons, the mice developed a Parkinsonian phenotype. Intranasal administration of functionalized LNPs to these mice showed an attenuation of neurodegeneration with a partial restoration of ATP5A1 and tyrosine hydroxylase expressions in the substantia nigra. This was accompanied by widespread gene expression reprogramming particularly those involved in mitochondrial biogenesis, oxidative phosphorylation, and Parkinson'disease-related pathways. Of note, a mechanistic study was conducted in differentiated SH-SY5Y cells, revealing that PUFA-enriched LNPs promoted dose-dependent viability and activated key neuroprotective signaling pathways, including AKT, ERK, and STAT3 phosphorylation.

The manuscript presents very brief conclusions and perspectives on the thesis work. Nonetheless, the results obtained deserve a more extensive discussion and should be better contextualized within studies related to LNPs composition, structure, and potential neuroprotective effects.

**Reviewer recommendations:**

Miss Thelma AKANCHISE show in this manuscript its capacity and accomplishment in mastering several tools and methodologies related to lipid nanoparticles, formulation and physicochemical characterization and analysis, as well as their evaluation *in vitro* in a cell model and *in vivo* in MitoPark mouse model using several biochemical and molecular biology methods. Experiments are well conducted and support the drawn conclusions.

Remarkably, the manuscript is structured around five independent articles, four of which have already been published in peer-reviewed journals, one is under review, and one is currently in preparation. The document constitutes a comprehensive and well-written report, supported by clear and appropriate illustrations. The thorough and consistent review of the literature enhances the readability of the manuscript and facilitates the reader's understanding of the work. Collectively, the findings are notably original and contribute meaningful advances to the field of LNP-based nanomedicine.

I recommend the defense of this thesis by Miss Thelma AKANCHISE to get a PhD degree of the University of Paris-Saclay in the field of *Pharmacotechnie et Biopharmacie*.

**Avis du rapporteur :**

Madame Thelma AKANCHISE démontre dans ce travail de remarquables capacités à maîtriser un ensemble d'outils et de méthodologies liés aux nanoparticules lipidiques, à leur formulation et caractérisation physicochimique, ainsi qu'à leur évaluation *in vitro* dans un modèle cellulaire et *in vivo* dans le modèle murin MitoPark en utilisant de nombreuses méthodes biochimiques et de biologie moléculaire. Les expériences ont été bien menées et appuient les conclusions élaborées.

Fait remarquable, le manuscrit s'articule autour de cinq articles indépendants, dont quatre ont déjà été publiés dans des revues internationales à comité de lecture, un est en cours d'évaluation, et un dernier est actuellement en préparation. Le document constitue un rapport complet et bien rédigé, soutenu par des illustrations claires et pertinentes. L'examen approfondi et cohérent de la littérature renforce la lisibilité du manuscrit et facilite la compréhension du travail par le lecteur. Collectivement, les résultats présentent un caractère particulièrement original et apportent des avancées significatives dans le domaine de la nanomédecine basée sur les nanoparticules lipidiques.

En conclusion, je donne un avis favorable à la soutenance de la thèse de Madame Thelma AKANCHISE pour obtenir le grade de Docteur de l'Université de Paris-Saclay en *Pharmacotechnie et Biopharmacie*.

À Dijon le, 17 novembre 2025.  
Professeur Mustapha CHERKAOUI MALKI  
Université Bourgogne Europe.

