Fate of self-assembled squalene non-lamellar liquid crystalline nanoparticles acting as painkillers in a biomimetic medium assessed by scattering techniques

Hugo Roncin [1], Sinda Lepetre-Mouelhi [2], Gaspard Huber [3], Javier Perez [4], Anne Martel [5], Lorenzo Metilli [5], Emeline Cournède [1], Frédéric Gobeaux [1], Fabienne Testard [1]

[1] Université Paris-Saclay, CEA Saclay, CNRS, NIMBE, UMR 3685, LIONS, 91191 Gif-Sur-Yvette Cedex, France
[2] Institut Galien Paris-Saclay, UMR CNRS 8612, Université Paris-Saclay, 91400 Orsay, France
[3] Université Paris-Saclay, CEA Saclay, CNRS, NIMBE, UMR 3685, LSDRM, 91191 Gif-Sur-Yvette Cedex, France
[4] Synchrotron SOLEIL, L'Orme des Merisiers, 91190 Saint-Aubin, France
[5] Institut Laue Langevin, Grenoble, France

hugo.roncin@cea.fr

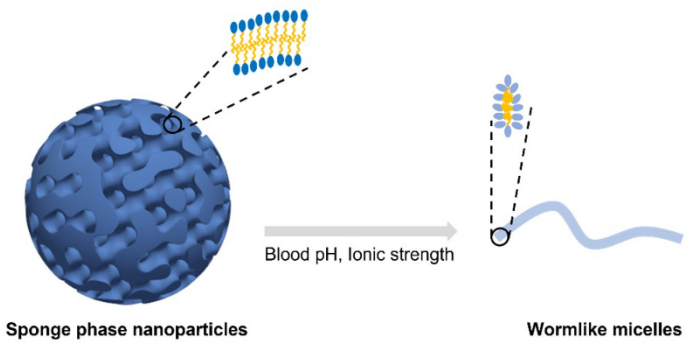
Recently, the Leucine-enkephalin-squalene (LENK-SQ) prodrug, when formulated as nanoparticles (NPs), has been shown to be an efficient formulation for pain treatment after intravenous administration, offering a promising alternative to conventional opioid therapies [1]. These LENK-SQ NPs belong to the general class of non-lamellar lyotropic liquid crystalline lipid NPs whose unique supramolecular organization enables enhanced targeting, reduced toxicity, and improved pharmacokinetic profiles [2]. Despite these advantages, their physicochemical behavior *in vivo* remains incompletely understood, particularly regarding NP morphology, colloidal stability, and interactions with biological components.

Using the specific case of LENK-SQ bioconjugate, which bears an ionizable group on its LENK peptide moiety, we explored how the pH and ionic strength influence its supramolecular organization and stability, an area that remains largely unstudied despite its likely strong impact on therapeutic efficacy.

We elucidated the behavior of LENK-SQ NPs in biomimetic environments using complementary physicochemical techniques, with particular emphasis on small- and wide-angle X-ray scattering (SAXS/WAXS), small-angle neutron scattering (SANS) and cryogenic electron microscopy (cryo-TEM). In this respect, the NPs were studied in buffers simulating physiological pH and ionic strength.

Our results revealed a dynamic equilibrium between free LENK-SQ bioconjugates, sponge phase NPs, and elongated micelles, strongly influenced by buffer composition and concentration. The relative proportions of these species were found to be primarily governed by pH, highlighting its critical role in controlling the supramolecular morphology of self-assembled bioconjugates, which may influence their biological performance [3].

Furthermore, ongoing investigations into the behavior of these NPs in the presence of plasma proteins, recently initiated [4,5], also suggest a transition of the NPs towards micelles in equilibrium with the proteins. These findings provide important insights into how the physicochemical environment modulates peptide amphiphile NPs and, potentially, biological performance. Moreover, they offer rational tools for designing NPs with tunable morphologies and controlled release [5].



Nanoparticles/micelles transition triggered by blood pH and ionic strength.

References:

[1] J. Feng, et al. Sci Adv 5 (2019) eaau5148.
[2] J. Zhai, et al. ACS Nano 13 (2019) 6178–6206.
[3] H. Roncin, et al. Submitted.
[4] S. Lepetre-Mouelhi, et al. Chem. Mater. 36 (2024) 694–707.
[5] F. Gobeaux, et al. Nanoscale 12 (2020) 2793–2809.