Design and Synthesis of Micellar Thermometric Systems based on Amphiphilic Block Copolymers-Lanthanide Complexes Conjugates

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Measuring intracellular temperature is critical to understand many cellular functions because it influences biochemical reactions, but still remains a challenge. Accurate and noninvasive monitoring of subcellular temperature changes in individual cells might help to clarify intricate cellular processes and led to development of novel diagnostic and therapeutic technologies. Most of the thermometric systems proposed for intracellular thermometry are noncontact luminescent nanothermometer (LNT) that use for detection optical properties such as emission intensity, band shape, bandwidth, lifetime or polarization anisotropy, which are capable of acquiring local temperatures with spatial and temporal resolution at the nanoscale. Magnetic induction heating of nanoparticles can be a powerful non-invasive technique for hyperthermia therapy of cancer and other diseases. The efficiency of such local heating systems requires of an adequate monitoring of the nanoheaters (MNHs) local temperature.[**1**] Single nanoparticles integrating both functions, magnetic heating and luminescent thermometry (MNT-LT), should therefore be the best option. LNT for intracellular mapping must operate at the physiological temperature range and show an outstanding performance in terms of temperature sensitivity uncertainty readout reproducibility and time and spatial resolution. However, other aspects as colloidal stability cell, toxicity, internalization capacity, functionalization with biomolecules or different vectors that can direct the LNT probes to specific organelles are also of great importance. Nevertheless, the main and most difficult requirement that LNT has to meet with is the stability in the cell culture medium and the intracellular environment. The intracellular environment is a complicated media with different local chemical compositions, wide range of pH, ionic strength and viscosity to which the thermometric response must be insensitive.

Amphiphilic block copolymers (ABCP) are formed by covalently bonded hydrophobic and hydrophilic blocks that can self-assemble into micelles able to encapsulate hydrophobic species improving their solubility and stability in aqueous environments. Combining controlled radical polymerization techniques with post-polymerization functionalization strategies has allowed the access to ABCPs with precisely controlled architectures, molecular masses and composition or different functionalities. By a rationally design of the ABCP composition, synthesized by reversible addition-fragmentation chain transfer polymerization (RAFT), and controlling the parameters of the self-assembly process, we have synthesized a new class of polymeric nanomicelles doped with Eu/Sm complexes that can work as ratiometric thermometry systems. Eu^{3+} and Sm^{3+} complexes have been covalently bonded to the hydrophobic block by coordination with an auxiliary ligand located at the polymer backbone. In this way, encapsulation efficiency, stability and emission of the lanthanide probes has been improved. The thermometric micelles show low toxicity, internalization capacity and high colloidal stability in aqueous media provided by a high dense PEG hydrophilic block. The adequate selection of the lanthanide complex combined with the protection provided by the hydrophobic block have made it possible to obtain Ln doped micelles that are a robust measurement system, insensitive to different environmental factors and retain their thermometric properties in the cell culture medium and inside intracellular environment.**[2]** In a proof of concept, 2D intracellular temperature mapping of MDA-MB468 culture cells with Ln^{3+} micelles has been recorded. Finally, a new dual MNH-LNT has been synthesized by changing composition of the hydrophobic block and the self-assembly method, and in combination with MNH. The MNH-LNT preserve their thermometric properties in the intracellular environment, this dual system constitutes an unique and promising alternative to measure local MNH temperature gradients in aqueous suspensions and in cell magnetic hyperthermia.

Seminar will be focused in the design and the methodology of preparation of the Luminescent Thermometric micelles.

[1]Piñol, R.; Brites, C. D. S.; Silva, N. J.; Carlos, L. D.; Millán, A., Chapter 6 - Nanoscale Thermometry for Hyperthermia Applications. In *Nanomaterials for Magnetic and Optical Hyperthermia Applications*, Fratila, R. M.; De La Fuente, J. M., Eds. Elsevier: 2019; pp 139-172.

[2] Piñol, R.; Zeler, J.; Brites, C. D. S.; Gu, Y.; Téllez, P.; Carneiro Neto, A. N.; da Silva, T. E.; Moreno-Loshuertos, R.; Fernandez-Silva, P.; Gallego, A. I.; Martinez-Lostao, L.; Martínez, A.; Carlos, L. D.; Millán, A., Real-Time Intracellular Temperature Imaging Using Lanthanide-Bearing Polymeric Micelles. *Nano Letters* **2020,** *20* (9), 6466-6472.