## WATER'S HIDDEN DENSITY DANCE: FROM CHARGED INTERFACES TO PROTEIN DYNAMICS

## Luís D. Carlos

<sup>a</sup>Phantom-G, CICECO-Aveiro Institute of Materials, Physics Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal, Icarlos@ua.pt

Luminescence nanothermometry has emerged as a transformative technique, enabling precise submicrometer thermal flow measurement beyond conventional electrical methods. While diverse phosphors (e.g., polymers, quantum dots, and lanthanide-doped nanomaterials) have been explored, the field now focuses on robust theoretical foundations and standardized methodologies, including improved data acquisition and reproducibility.<sup>[1]</sup>

In this seminar, we demonstrate how luminescence nanothermometry can be applied to investigate both liquid water density fluctuations and protein dynamics. We first examine the intricate interplay between temperature, pH, and Brownian motion in differently sized upconversion nanoparticles.<sup>[2,3]</sup> Acting as nanorulers, these particles reveal the relative abundance of high-density (HDL) and low-density (LDL) water in their hydration shells, supporting the two-state model of liquid water and highlighting the critical role of nanoparticle surfaces in probing density fluctuations at charged interfaces.

We then discuss how protein unfolding is related to temperature-dependent density fluctuations in both light and heavy water. Although water's dynamic behavior at protein interfaces is fundamental to biomolecular function, it remains difficult to observe directly. By analyzing the Brownian velocity of enhanced green fluorescent protein (EGFP) colloidal suspensions,<sup>[4]</sup> we link the LDL–HDL crossover temperature to the onset of protein unfolding. This strategy enables the decoupling of water density dynamics from protein denaturation, providing insights into hydration-mediated protein stability and offering a versatile platform for bio-thermometry and the development of thermally robust protein-based technologies.

[1] C. D. S. Brites, R. Marin, M. Suta, A. N. Carneiro Neto, E. Ximendes, D. Jaque, L. D. Carlos, *Adv. Mater.* **35** (2023) 2302749.

[2] C. D. S. Brites, B. Zhuang, M. L. Debasu, D. Ding, X. Qin, F. E. Maturi, W. W. Y. Lim, D. W. Soh, J. Rocha, Z. Yi, X. Liu, L. D. Carlos, *J. Phys. Chem. Lett.* **11** (2020) 6704.

[3] F. Maturi, R. S. Raposo Filho, C. D. S. Brites, J. Fan, R. He, B. Zhuang, X. Liu, L. D. Carlos, J. Phys. Chem. Lett. 15 (2024) 2606.

[4] Y. Guo, F. Maturi, C. D. S. Brites, L. D. Carlos, Adv. Phys. Res. 3, (2024) 2400085.