TAILORING TWO-PHOTON FLUORESCENT PROBES FOR pH BIOIMAGING IN LIVING CELLS

Francisco Nájera,[a,c] Carlos Benítez-Martín,[a,c] Juan A. Guadix,[b,c] John R. Pearson,[c] José M. Pérez-Pomares,[b,c] Ezequiel Pérez-Inestrosa*,[a,c]

[a] *Universidad de Málaga-IBIMA, Departamento de Química Orgánica. Málaga, Spain.*

[b] *Departamento de Biología Animal. Universidad de Málaga-IBIMA, Málaga, Spain.*

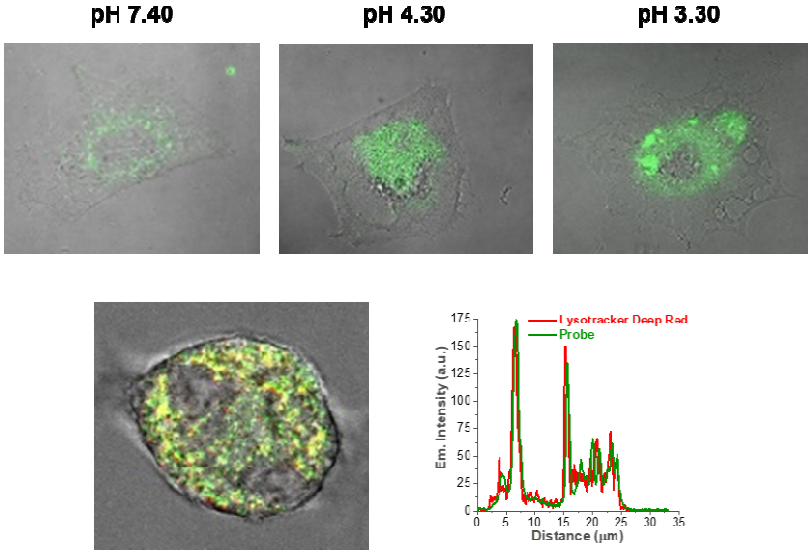
[c] *Centro Andaluz de Nanomedicina y Biotecnología-BIONAND. Parque Tecnológico de Andalucía, Málaga, Spain.*

e-mail: najera@uma.es

Keywords: *intracellular pH, pH sensor, two-photon absorption, fluorescent probe, biosensor*

Fluorescence biosensors are indispensable basic tools in modern biology. These type of molecules allow real-time visualization of biological events inside living cells. Especially important in many of these processes (proliferation, apoptosis or defense tasks) is the control of the cellular pH.[1] In consequence, a great variety of structural models have been developed for pH bioimaging in fluorescence microscopy.[2] Nonetheless, these efforts have been mainly focused on the development of one-photon (OP) probes.

Recently, we described a biosensor with excellent photophysical properties and appropriate two-photon absorption (TPA) behavior. This sensor allows selective and specific detection of hydroxyl radicals solely inside lysosomes.[3] Based on this scaffold, we have synthesized and characterized new TPA fluorescent probes. These molecules have an "off-on" response to different pH environments with a strong selectivity and sensitivity toward H^+. These naphthalene-indolenine derivatives have a high synthetic versatility through affordable and efficient synthesis. The synthetic modification of this model allows tuning subcellular targets through minor modifications and without affecting their emission properties. The effectiveness of these probes and their structural modifications for different pH-related applications has been probed in mouse embrionary fibroblast (MEF) cells.



References

[1] L. K.Truman, S. Comby, T. Gunnlaugsson *Angew. Chemie-Int. Ed.* **2012**, *51*, 9624.

[2] D. Wencel, T. Abel, C. McDonagh *Anal. Chem.* **2014,** *86*, 15.

[3] C. Benitez-Martin, J. A. Guadix, J. R. Pearson, F. Najera, J. M. Perez-Pomares, E. Perez-Inestrosa *Sens. Actuators B Chem.* **2019,** *284*, 744.