P-2.56

Measuring Ph in Insulin Secretory Granules by Phasor-based Flim and Orbital Tracking

Samuele Ghignoli [1], Valentina De Lorenzi [1], Gianmarco Ferri [2], Ranieri Bizzarri [3], Francesco Cardarelli [1]

[1] Scuola Normale Superiore - NEST Laboratory, Pisa, Italy

[2] Fondazione Pisana per la Scienza ONLUS, Pisa, Italy

[3] NEST - Istituto Nanoscienze - CNR, Pisa, Italy

pH regulation is crucial during the maturation of secretory vesicles and through the conversion of proinsulin to mature insulin inside insulin secretory granules (ISG). However, a calibrated measure of ISG pH is still missing. In this work, we employed a genetically encoded E1GFP pH reporter inserted into the C-peptide of proinsulin and expressed in Insulinoma 1E cells as β-cell model to properly measure ISG pH by Phasor-based Fluorescence Lifetime Imaging Microscopy (FLIM). We confirmed the acid nature of ISG with an average pH of ~5.8, and we showed that acidity is actively maintained. Moreover, we highlighted that ISG inside cytoplasm were slightly less acid compared to those which are proximal to the plasma membrane even during the early phase of insulin secretion induced by glucose stimulation. We employed E1GFP combined with orbital tracking (OT) to measure ISG pH fluctuations on a millisecond timescale. We observed that 80% of the fluctuations ranged between 4.85 and 6.1 pH values. A robust alternative to classical ratiometric methods is offered by FLIM, while OT provides millisecond timescale sensitivity. E1GFP sensor with these techniques pave the way for measuring pH and its fluctuation inside granules (i.e. ISG) and other intracellular compartments in physiology and disease. [Funded by the European Research Council (ERC); grant agreement No 866127, project CAPTUR3D].