Capturing Metabolism-Dependent Solvent Dynamics in a Trafficking Lysosome

Filippo Begarani [a], Francesca D'Autilia [b], Giovanni Signore [a], Ambra Del Grosso [c], Marco Cecchini [c], Enrico Gratton [d], Fabio Beltram [a], Francesco Cardarelli [a]

[a] Laboratorio NEST - Scuola Normale Superiore, Pisa, 56127, Italy.
[b] Center for Nanotechnology Innovation@NEST (CNI@NEST), Pisa, 56127, Italy
[c] NEST, Istituto Nanoscienze-CNR and Scuola Normale Superiore, Pisa, 56127, Italy
[d] Laboratory for Fluorescence Dynamics, Department of Biomedical Engineering, University of California, Irvine, CA 92697-2715, USA

e-mail: francesco.cardarelli@sns.it

Keywords: (lysosome, ACDAN, RICS, generalized polarization, 3D orbital tracking, metabolism)

The eukaryotic cell contains distinct dynamic, membrane-enclosed sub-compartments spanning over a broad spatial scale, from tens of nanometers (e.g. synaptic vesicles, clathrin-coated pits) to hundreds of nanometers (e.g. endosomes, lysosomes, mitochondria) to several microns (e.g. Golgi apparatus, Endoplasmic Reticulum) that are designed to concentrate specific sets of components and functions within confined cell regions [1]. This spatial organization determines a discontinuous landscape of the physicochemical properties of the intracellular solvent, in terms of its intrinsic polarity, viscosity, dynamics, etc. These properties, in turn, play an active role in the regulation of the biochemical processes within each specific district. In spite of the amount of solvatochromic dyes developed as environment-sensitive probes, monitoring solvent physicochemical properties within sub-cellular compartments at the timescale relevant to molecular processes (i.e. micro-to-millisecond regime) is, at present, an unattained task. In fact, optical-microscopy-based analysis of sub-cellular structures is severely hindered by their continuous rapid movement in the 3D cellular environment. State-of-the-art microscopy tools for delivering sub-cellular information at molecular resolution fail to subtract the 3D evolution of the entire system while preserving the temporal resolution required to successfully probing the local environment. This experimental bottleneck is tackled here by focusing an excitation light-beam in two periodic orbits around the sub-cellular structure of interest. The recorded signal (fluorescence) is used as feedback to track the structure of interest (**Fig. 1**). Such strategy is able to subtract the natural movement of the subcellular reference system while preserving the required temporal resolution to study biological processes, i.e. microseconds along the orbits, milliseconds between the orbits [2-3]. The lysosome is selected as a paradigmatic sub-cellular target in light of its important role in metabolism, signalling, and cell-growth regulation. In order to probe the physicochemical properties of the lysosome lumen we combined the feedback-based orbital tracking of single lysosomes with the use of the 6-acetyl-2-dimethylaminonaphthalene (ACDAN) molecule. ACDAN shows peculiar properties that are ideal for its application to the lysosome case. Specifically, (i) it enters cells and partitions mostly into hydrophilic environments, while concomitantly becoming brighter within the most viscous/crowded ones, such as the lysosome lumen; (ii) it is not sensitive to pH changes in the range from 4 to 10; (iii) it shows exquisite sensitivity to solvent dipolar relaxation, displayed as red- or blue-shift in the emission spectrum. Such properties make ACDAN suitable for spontaneous labeling of lysosomes in live cells (**Fig. 2**) and, combined with feedback-based tracking, offer the opportunity to monitor the extent of solvent dipolar relaxation at a time resolution ranging from microseconds to milliseconds and to several seconds (total time of tracking of a single lysosome). Standard Generalized Polarization (GP) calculations are used based on the ACDAN emission shift at any time point. Then, Raster Image Correlation Spectroscopy (RICS) is performed to extract characteristic dissipation time of ACDAN GP fluctuations in the lysosome lumen (**Fig. 3**). It is observed that intra-lysosomal ACDAN GP fluctuates with characteristic dissipation times in the micro-to-millisecond range (classified as 'fast'). Control experiments demonstrate that such fluctuations are greatly slowed down by basification of lysosomal pH or deprivation of metabolic energy in the form of ATP. Also, GP fluctuations are observed in live cells, but disappear upon cell fixation. Based on these findings we argue that intra-lysosomal ACDAN GP fluctuates according to the ongoing lysosomal metabolism (**Fig. 4**). We finally show that ACDAN GP fluctuations are altered both in amplitude and timing in the lysosomes of a cellular model of Krabbe disease, a lysosomal storage disorder (LSD) in which the organelle metabolism is impaired by a specific enzymatic loss [4].

[1] Alberts, B. Garland, New York: 1998
[2] Levi, V.; Ruan, Q.; Gratton, E. *Biophys J* **2005,** *88*, 2919-2928.
[3] Cardarelli, F.; Lanzano, L.; Gratton, E. *Proc Natl Acad Sci U S A* **2012,** *109*, 9863-9868.
[4] Begarani, F. et al. *ACSNano* **2019,** *13*, 1670-1682

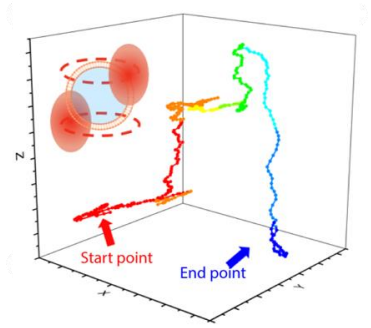

Fig. 1. Example of a single-lysosome trajectory originated from a 3D orbital tracking experiment. Ticks on axes: 50 nm. On the top-left, a representation of 3D-tracking configuration, with a lysosome at the center and the PSF creating two orbits separated along the vertical axis

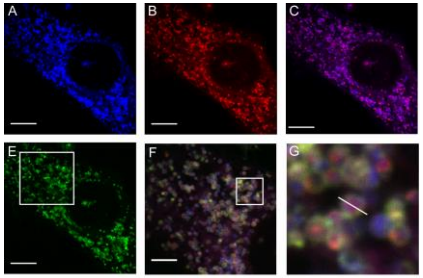

Fig. 2. ACDAN-based staining of the lysosomal lumen in living cells. (A) ACDAN; (B) Lysotracker; (C) Overlay of A and B; (E) GFP-labelled lysosome membrane protein; (F-G) Zoom and overlay of signals from A, B, and E

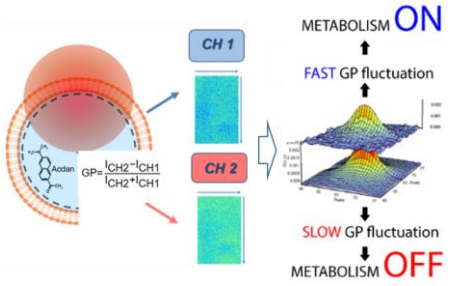

Fig. 3. GP calculations are performed to analyze the ACDAN emission shift at any time point. Then, RICS extracts the characteristic dissipation time and amplitude of ACDAN GP fluctuations in the lysosome lumen

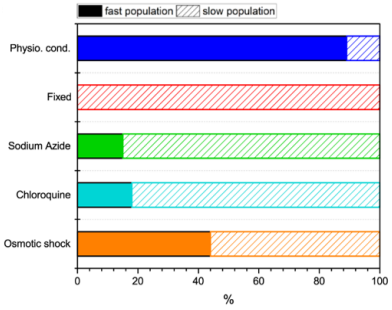

Fig. 4. Plot summarizing the fractions of GP fluctuations classified as 'fast' (filled histogram) or 'slow' (dashed histogram) for all the tested conditions. Fast ones are dominant in the physiological condition. Slow ones are appear in conditions of metabolism impairment (e.g. fixation)