|  |
| --- |
| Beyond confocal resolution: differentiating the lumen from the periphery of thin, elongated vesicles in endothelial cells.  **Panagiotis Lentzaris** 1,2#, **Evangeli Goula** 1,2, **Alexandra Papafotika** 1,2, **Michael Redd** 3, **Virginia Silio** 3, **Christopher Thrasivoulou** 3, **Vasiliki Lazani** 1,2, **Michalis Aivaliotis** 4,5 and **Savvas Christoforidis**1,2\*  1 Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece  2 Laboratory of Biological Chemistry, Department of Medicine, School of Health Sciences, University of Ioannina, Ioannina, Greece  3 UCL Confocal Imaging Facility, University College London, London, England  4 Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece  5 Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.  # Presenting author: Panagiotis Lentzaris, email: pglentzaris@bri.forth.gr  \* Corresponding author: Savvas Christoforidis, email: savvas\_christoforidis@bri.forth.gr |

abstract

In our study, we have identified Galectin -1 protein (Gal-1) as a novel cargo of Weibel-Palade bodies (WPBs), the primary secretory vesicles of endothelial cells1. Through proteomic analysis of the secretome profile in activated HUVECs (Human Umbilical Vein Endothelial Cells), and then, using confocal microscopy, we have found that Gal-1 is localized at WPBs. Interestingly, Gal-1 is only present in a subpopulation of cells and a sub-group of WPBs vesicles.

Given that Gal-1 is a cytoplasmic protein, the WPB-localized pool of this protein could be either present at the membrane facing the cytosolic side of WPBs, or in their lumen. As WPBs are elongated and extremely thin vesicles, having a diameter of only 100-300nm, confocal resolution (500nm in z axis2) is not sufficient to distinguish the lumen from the periphery of the vesicles. To overcome this limitation, we employed three independent approaches: (1) alteration of vesicle morphology by manipulating intra-vesicular pH, to increase their diameter and so improving the resolution limit of these vesicles; (2) super-resolution microscopy, comparing the capabilities of four different systems: ZEISS Elyra 7, Nikon AXR NSPARC, ZEISS Airyscan II 980 and Leica SP8 STED and (3) isotropic expansion of the entire cell using the powerful technique of ultrastructure expansion microscopy (U-ExM). Collectively, these approaches revealed that Gal-1 is localized within the lumen of WPBs, establishing it as a novel lumenal cargo of these vesicles.

Beyond the identification of Gal-1 as a lumenal cargo, our work highlights methodological strategies for overcoming confocal resolution limitations, offering a framework applicable to diverse cell types and vesicle systems.

**REFERENCES**

[1] Rondaij, M. G., Bierings, R., Kragt, A., van Mourik, J. A., & Voorberg, J. (2006). Dynamics and plasticity of Weibel-Palade bodies in endothelial cells. Arteriosclerosis, thrombosis, and vascular biology, 26(5), 1002–1007.

[2] Fouquet, C., Gilles, J.-F., Heck, N., Dos Santos, M., Schwartzmann, R., Cannaya, V., Morel, M.-P., Davidson, R. S., Trembleau, A., & Bolte, S. (2015). Improving Axial Resolution in Confocal Microscopy with New High Refractive Index Mounting Media. PLOS ONE, 10(3).