Tracking tonoplast protein behaviors in intact vacuoles

Subject Code: C02

With the support by the National Natural Science Foundation of China, the research team led by Prof. Lin Jinxing(**林金星**)at the College of Biological Sciences & Biotechnology, Beijing Forestry University, overcame the limitations of existing techniques and expanded the study of protein characteristics from the plasma membrane to the vacuole membranes. This study was published in *Molecular Plant* (2016, DOI: 10.1016/ j.molp).

Membrane proteins perform a vast array of functions in vital cellular activities, and the real-time behaviors of proteins can affect their activity and functions. Consequently, the tracking and analysis of the behaviors of membrane proteins in living cells can further our understanding of the mechanisms regulating the biological functions of proteins. In mammalian cells, TIRFM has become a powerful tool to observe fluorescently labeled plasma membrane (PM) proteins at high temporal and spatial resolution and excellent sensitivity. The application of TIRFM in plant cell research has been limited by the fact that plant cells contain cell walls, thick peripheral layers surrounding the PM, which restrict the penetration of the evanescent field and therefore affect the imaging. To circumvent this problem, Lin's group developed a variable-angle TIRFM by setting up a device that can vary the angle of incidence for TIRFM (VA-TIRFM) to image and observe the behaviors of fluorescent-protein tagged proteins in the plant PM. The related researches, also supported by the National Natural Science Foundation of China, were published in the prestigious international journal *PNAS* and *Plant Cell*.

However, because of the confining excitation of the fluorophores to a depth of ~200 nm in the specimen, VA-TIRFM is almost impossible to unambiguously determine the behavior and characteristics of the proteins in the membranes of organelles. Jinxing Lin's group used cell wall enzymatic lysis to produce mesophyll protoplasts and obtained intact vacuoles from these protoplasts. Then, they adhered vacuoles to a coverslip by polylysine-mediated adhesion and monitored the behavior of individual protein particle at the tonoplast using VA-TIRFM and an automated tracking program. In addition, via comparing the dynamics of the same protein in different membrane systems (plasma membrane and tonoplast) and the different proteins in the same membrane system (tonoplast), they found that the lateral mobility of proteins was affected by the cellular components or protein subcellular locations and the different behaviors of tonoplast proteins are regulated by proteins themselves. In summary, Jinxing Lin's group established an approach for monitoring and kinetic analysis of protein behaviors in the tonoplast of intact vacuoles. This method can track and quantitatively analyze tonoplast proteins, thus enabling new approaches to help in deciphering the functions of plant vacuoles.

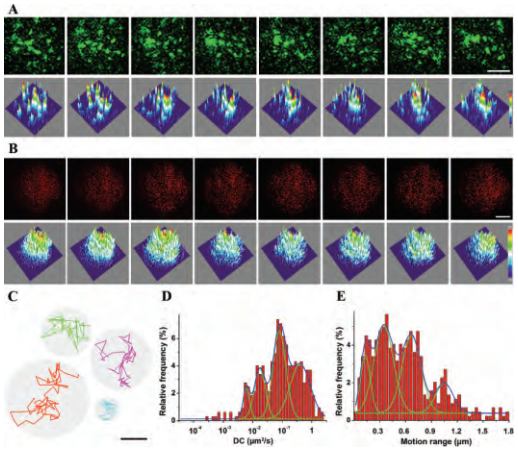

Figure Dynamic distribution (A, B) and behavior characteristics (C, D, E) of protein particles at the tonoplast.