

Together, biological membranes prevail

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Researchers at the University of Illinois at Urbana-Champaign have developed a novel method to visualize the fusion of biological membranes at the single-event resolution. Observing the individual fusion events revealed an unprecedented detailed picture of membrane fusion, which was chronicled in one of the cover stories in the December 2006 issue of the journal *Proceedings of National Academy of Sciences*.

"Undoubtedly, understanding the mechanisms of this basic life phenomenon is of great biological interest, and there have been extensive studies at the theoretical and experimental level to this end," stated Taekjip Ha, a professor of physics at Illinois and a Howard Hughes Medical Institute investigator. "Previous studies suggested various physical intermediates for the membrane fusion, but the limitations of the approaches at hand did not allow a solid consensus to be reached."

Compartmentalization in cells is achieved by lipid membranes. Membrane fusion is an elementary biological event which comes in various forms. From cellular trafficking to neuro-transmitter release, merging of two membranes is essential for carrying out vital processes of life. This important task is executed by membrane proteins called SNAREs. Complementary SNARE proteins residing within the incoming and target membranes form a bundle, and bring the two membranes close enough for the merging to occur. The fusion is thought to proceed through highly fleeting intermediates that are very difficult to study when many membrane fusion events are averaged over as has been done in most of previous studies.

Alternatively, the Illinois team, led by Ha, developed an elegant method which used fluorescence resonance energy transfer (FRET) technique to detect SNARE mediated membrane fusion. In FRET, a pair of green and red dyes is used where only the green dye can directly be excited by a laser. The red dye lights up if some of the energy from the green dye hops over to the red dye which becomes increasingly efficient at short distances. Their team prepared two different batches of SNARE-decorated vesicles, one with the green dyes and the other red dyes, and observed them using highly sensitive fluorescence microscopy.

The vesicles with the red dyes were then specifically immobilized on a polymer-passivated glass surface. The polymer coating acted as a cushion and prevented the non-specific binding of vesicles on the surface. As the green labeled vesicles were injected into the flow chamber, the vesicles could fuse upon SNARE-SNARE interaction. The merging of the two membranes result in mixing of the dyes and the level of mixing is reflected as changes in the FRET signal. Using this approach, the researchers could obtain real-time movies of fusion events single vesicles and uncovered various intermediates and pathways during the fusion reaction. The different fates of liposomes subsequent to fusion were also dissected at the level of single-fusion events. The results of the study was highlighted by a commentary in the same issue of the journal PNAS and by a "News and Views" article in the January issue of Nature Structural and Molecular Biology.

The new single-vesicle approach to study membrane fusion, developed by postdoctoral associate Tae-Young Yoon and graduate student Burak Okumus, opens not only a new avenue for the SNARE field, but also provides a native-like environment for the single molecule studies of membrane proteins which would fill a gap in the researchers' toolkit. The work was done in collaboration with Fan Zhang and Professor Yeon-Kyun Shin of Iowa State University and was funded by grants from the National Institute of General Medical Sciences. The research team is

currently working on the neuronal SNAREs hoping to shed new light on the membrane fusion events essential for brain functioning.

Source: University of Illinois at Urbana-Champaign

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