Construction of Artificial Lipid-Containing Vesicular Compartments in Living Cells Using Tryptophan-Rich Peptides

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Lipid membrane-bound compartments, i.e. organelles, are important in the cell since they provide a closed environment with selective membrane permeability for specific biological reactions. Up to the state, scientists have been trying to manipulate the intracellular membranes by changing their locations and compositions[1]. However, there is no report in constructing new membrane-bound structures as controlling the genesis and assembly of lipids is thought to be challenging. Herein, we introduce the first successful construction of artificial phospholipid-containing compartments in living cells using genetically encoded peptides. We demonstrate that peptide sequence with tryptophan repeats (W) spontaneously forms vesicular structures, and Raman imaging confirms the presence of lipids within these assemblies.

Tryptophan is well known for interaction with phospholipids through hydrogen bonding and hydrophobic interaction, thus we designed a peptide containing 9 tryptophan repeats (W9) fused with super-folder green fluorescent protein (sfGFP) for imaging. Using an approach we previously reported[2], plasmid DNA encoding W9-sfGFP was prepared and delivered to COS-7 cells by lipofection. We hypothesize that W9-sfGFP bind to intracellular membranes first, then formation of W9-domains caused membrane disturbance and bending, which eventually caused independent vesicle formation. Our investigations showed that these vesicles contain lipids and are independent from intracellular organelles. The mechanism of vesicle formation was also studied in in vitro models. This work opens new avenues for constructing and engineering lipid-containing compartments in cellular environments.

1) I. Takanari, *Nat. methods* **2020,** *17* (9), 928-936. 2) T. Miki, *Nat. Commun.* **2021**, *12*, 3412.