

微細構造解析プラットフォームにおける利用成果

Comprehensive structural analysis of multisubunit membrane protein complexes of photosynthetic membranes by 3D cryoEM: photosystem I and related supercomplexes

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【目 的】

In this project we aim at the structural characterization of photosynthetic complexes of the light and dark reactions by three dimensional cryo electron microscopy. We will strive for the improvement and development of sample preparation strategies for cryo-grids of fragile, light sensitive multisubunit membrane protein supercomplexes that are hard to crystallize suitable for high resolution structure determination. Single particle analysis will be the main approach to solve. We will further build upon the experiences made in the preparation of single particle cryo-EM compatible cryo-grids for the collection of more and better image data using the state-of-the-art Titan Krios and related facilities present at the Research Center for Ultra-High Voltage Electron Microscopy.

【成 果】

The equipment used was mainly the Titan Krios 300 kV FEI (now ThermoFisher) including the auto-grid clipping station that is needed to assemble the cryo-grids for insertion into the Titan Krios electron microscope. The workflow of screening cryo-grids for photosynthetic supercomplexes was successfully established now allowing the application of the GraDeR approach to this challenging type of membrane supercomplex under light sensitive conditions. Blotting conditions suitable for high resolution imaging were established and of several energy transforming supercomplexes accomplished.

(Gerle, C., Essay on Biomembrane Structure. The Journal of membrane biology, (2019) 252(2-3):115-30.)

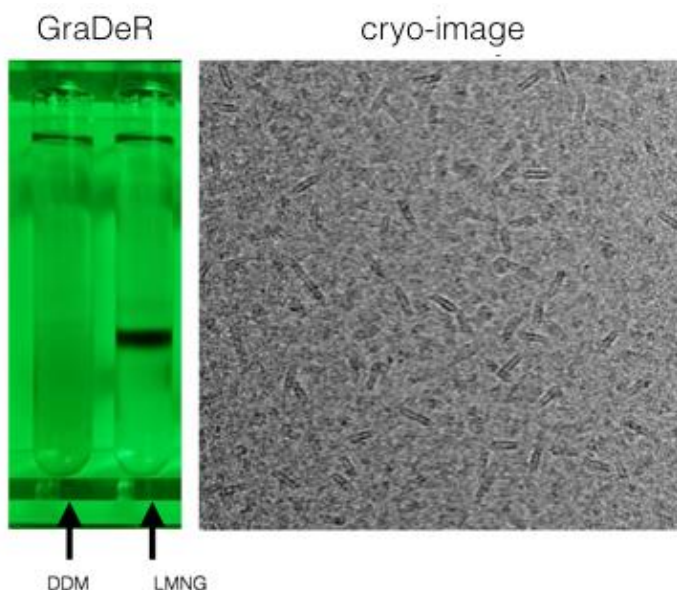


Fig. 1: Results in specimen preparation and cryo-image data collection of photosynthetic supercomplexes at the UHV-EM centre at Osaka University