

COMBINING ATOMIC-FORCE AND TWO-PHOTON FLUORESCENCE MICROSCOPY TO INVESTIGATE THE ARCHITECTURE OF THE PHOTOSYNTHETIC MEMBRANE

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In this work, we use two complementary scanning-probe techniques, namely atomic-force (AFM) and two-photon fluorescence microscopy, to investigate the arrangement of photosynthetic proteins in reconstituted membranes. Light-harvesting complexes I and II (LH1 and LH2) were isolated from the purple bacteria *Rps. acidophila* and separately mixed with lipids to form large two-dimensional arrays, as confirmed by the AFM topographs. These images show a uniform thickness of the sample adsorbed on a mica substrate, over a length scale of a few microns. The apparent height, 5-6 nm, points to the presence of a single lipid bilayer. The presence of the proteins in the lipid bilayer was proved by two-photon excited (TPE) fluorescence, a method by which the chromophores bound to the LH1 and LH2 are selectively sensitized. Despite the intrinsic differences between their spatial resolution, the AFM and the TPE images of the same sample correlate very well, thus indicating a successful insertion of the proteins into the reconstituted membranes. Furthermore, we also show preliminary results of a similar investigation approach on a more complex system, namely the thylakoid membrane isolated from green plants' chloroplasts.