

## Fluorescence study of protein–lipid complexes with a new symmetric squarylium probe

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### Abstract

The novel symmetric squarylium derivative SQ-1 has been synthesized and tested for its sensitivity to the formation of protein–lipid complexes. SQ-1 binding to the model membranes composed of zwitterionic lipid phosphatidylcholine (PC) and its mixtures with anionic lipid cardiolipin (CL) in different molar ratios was found to be controlled mainly by hydrophobic interactions. Lysozyme (Lz) and ribonuclease A (RNase) exerted an influence on the probe association with lipid vesicles resulting presumably from the competition between SQ-1 and the proteins for bilayer free volume and modification of its properties. The magnitude of this effect was much higher for lysozyme which may stem from the amphipathy of protein  $\alpha$ -helix involved in the membrane binding. Varying membrane composition provides evidence for the dye sensitivity to both hydrophobic and electrostatic protein–lipid interactions. Fluorescence anisotropy studies uncovered the restriction of SQ-1 rotational mobility in lipid environment in the presence of Lz and RNase being indicative of the incorporation of the proteins into bilayer interior. The results of binding, fluorescence quenching and kinetic experiments suggested lysozyme-induced local lipid demixing upon protein association with negatively charged membranes with threshold concentration of CL for the lipid demixing being 10 mol%.

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