

**THE CONTRIBUTION OF PHOSPHATIDYLSERINE PARTITION AND
TRANSMEMBRANE POTENTIAL TO THE INTERACTION OF
MERCOCYANINE 540 WITH LIPID BILAYERS**

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The anionic fluorescent dye merocyanin 540 (MC540) has a high affinity to phospholipid (PL) membranes. It was recently observed that the redistribution of PLs in the membranes of activated blood platelets leading to the exposure of the negatively charged PLs, mainly PS, results in the suppressed interaction of MC540 with membrane PLs. However, it has been suggested that MC540 binding also depends on membrane lipid fluidity and transmembrane potential (TMP). Our aim was to assess the contribution of each of the above factors to the governing of MC540 interaction(s) with the lipid bilayer. Liposomes with an increasing phosphatidylserine (PS): phosphatidylcholine (PC) molar ratio (1:99, 5:95, 10:90 and 20:80) were prepared using the injection method [Disalvo et al. *Chem. Phys. Lipids* **84** (1996) 35]. Flow cytometry was applied to monitor the effects of PS partitioning and TMP alterations on MC540 binding. A $[K^+]_{out}$ gradient was generated using valinomycin. 5-doxyloleic acid and 16-doxyloleic acid were employed to determine the dependence between PS content and membrane lipid fluidity using the ESR technique. First, we showed that with increased PS partitioning in the lipid bilayer of PS/PC vesicles, MC540 binding was attenuated. Secondly, membrane lipid fluidity was not altered along with the increased PS/PC ratio in the vesicle membranes, regardless of the depth of a given lipid bilayer. This suggests that the lipid fluidity of the bilayer was not a significant determinant of MC540 binding in the examined model system. Thirdly, a higher $[K^+]_{out}$ concentration makes PS/PC liposomes bind more MC540, which implies that more negative TMP favours the interactions of MC540 with the PL bilayer. However, we showed that the variability attributed to the binding of MC540 with PLs is explained only to a minor extent by the generated TMP (7.2%), and far more by the variations in PS content (by up to 60%). We conclude that the partitioning of negatively charged PLs is the major factor influencing the binding of MC540 to PL membranes.

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