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A comment on the localization of cyanine dye binding to brush-border membranes by the fluorescence quenching of *n*-(9-anthroyloxy) fatty acid probes

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This work comments on the location and orientation of 3,3′-dipropylthiodicarbocyanine (diS-C_3-(5)) in renal brush-border membrane vesicles (RBBMV) (Cabrini, G. and Verkman, A.S. (1986) Biochim. Biophys. Acta 862, 285–293) evaluated from collisional quenching of *n*-(9-anthroyloxy)stearic acid (*n*-AS) fluorescence. At variance with these authors, it is concluded that the quenching is due to resonance energy transfer. It is also shown that the fluorescence data are not clear evidence for the reported monomer and dimer locations.

Cabrini and Verkman [1] reported that the fluorescence quenching of *n*-AS by diS-C_3-(5) in RBBMV is described by a collisional mechanism and concluded that the cyanine dimer locates deep in the membrane, as it is a more efficient quencher for the inner probes, e g 12-AS and 16-AP

We have recently used *n*-AS probes in resonance (long-range) energy transfer experiments, in order to study locations both in micelles [2] and model systems of membranes [3], and we were aware that this mechanism of interaction could be a feasible one regarding the quenching of *n*-AS by diS-C_3-(5) Furthermore, from the reported Stern-Volmer quenching rate constant for 16-AP by the dimer [1], we worked out the dimer diffusion coefficient via the Smoluchowski equation [3] For this purpose, the molar volume of the lipid was determined, considering 77 Å^2 for the lipid head-group area and 40 Å for the bilayer thickness [4] The diffusion coefficient for 16-AP, was assumed identical to the one reported for a similar nitroxide probe in a fluid membrane $D \approx 2.5 \cdot 10^{-7}$ cm^2 s^{-1} [3], and τ_0 (16-AP) ≈ 10 ns [1] The value obtained, $D \approx 10^{-5}$ cm^2 s^{-1} seems too high for the diffusion of this species in a membrane These facts prompted us to reexamine the reported study [1], and carry out the described experiments

Small unilamellar vesicles (SUV) of dipalmitoylphosphatidylcholine (DPPC) were obtained, and the absorption and fluorescence experiments were carried out as described elsewhere, namely the spectral correction and the determination of Forster radius, R_0 [3] Energy transfer efficiencies were determined in solutions 10^{-3} M in lipid and 10^{-5} M in donor

Previous to the energy transfer study, the cyanine monomer-dimer equilibrium constant ($K = 3(\pm 2) \cdot 10^{-5}$ M) in the presence of lipid (10^{-3} M), was determined by absorption spectroscopy [5], as well as the monomer and dimer absorption spectra

From the reported donor's quantum yields [6], we obtained moderately high R_0 values, (see Fig 1), in the range 22–35 Å

In Fig 1 are presented the relative fluorescence quantum yields of 2-AS and 9-AS vs. the acceptor surface concentration σ, calculated for the limiting situations of the dye only as a monomer or totally dimerized For the purpose of σ evaluation, 50 Å^2 (at 25 °C) and 70 Å^2 (at 50 °C) were considered as the phospholipid head-group areas, 2/3 of the lipid being assigned to the outer vesicle half-bilayer [4] In Fig 1 are also depicted the theoretical variation of ϕ/ϕ_0 as a function of σ, for the relevant R_0 values, considering both donor and acceptor on the same plane, obtained after integration of Eqn A2b from Ref 2

We can safely conclude that no collisional mechanism is operative in the *n*-AS–cyanine interaction given

Abbreviations RBBMV, renal brush-border membrane vesicles, *n*-AS, *n*-(9-anthroyloxy)stearic acid, *n*-AP, *n*-(9-anthroyloxy)palmitic acid, SUV, small unilamellar vesicles, diS-C_3-(5), 3,3′-dipropylthiodicarbocyanine, DPPC, dipalmitoylphosphatidylcholine

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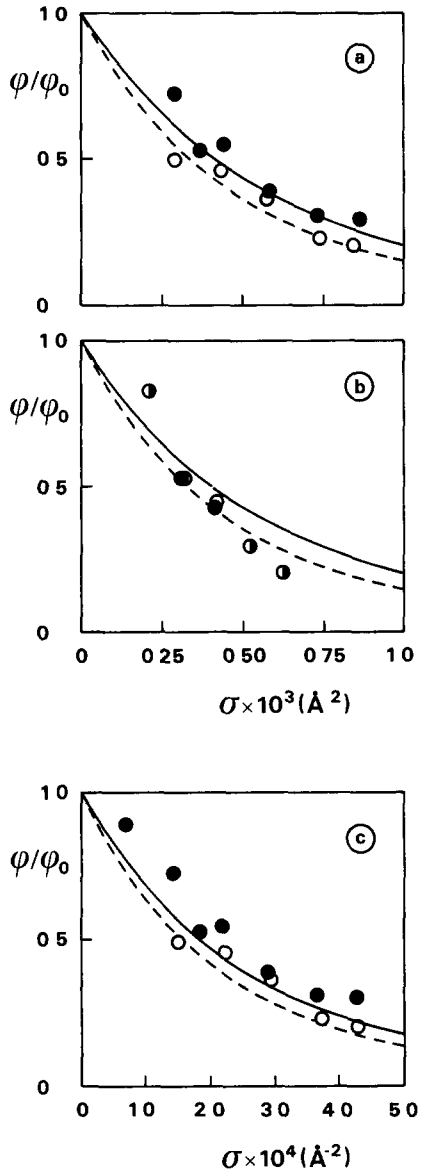

Fig 1 Relative quantum yields of fluorescence ϕ/ϕ_0 and theoretical dependence of energy transfer efficiency on surface concentration of acceptor σ (monomer or dimer of diS-C_3-(5)) for the systems (a) 9-AS(●, $R_0 = 22$ Å (———)), 2-AS (○, $R_0 = 24$ Å (– – –)) to monomer at 25 °C (b) 9-AS (●, $R_0 = 22$ Å (———)), 2-AS (○, $R_0 = 25$ Å (– – –)) to monomer at 50 °C (c) 9-AS (●, $R_0 = 32$ Å (———)), 2-AS (○, $R_0 = 35$ Å (– – –)) to dimer at 25 °C

the significant R_0 values obtained The n-AS fluorescence quenching is therefore of a long-range nature, being qualitatively explained (Fig 1), both in gel and liquid-crystal phases, on the basis of dipolar energy transfer, with insignificant diffusional contribution ($R_0 > (2D\tau_0)^{1/2}$ [2], $\tau_0 < 10$ ns [1])

Although energy transfer allows in principle the determination of absolute locations in the membrane (e g , Eqn A2a, Ref [2]), the complexity of this system (n-AS time dependent emission [7], simultaneous transfer to monomer and dimer), prevents the precise determination of cyanine monomer and dimer locations

In addition we would like to stress that in our experiments with SUV of DPPC (for this purpose a good model for RBBMV [8]), no linear Stern-Volmer relationship (ϕ_0/ϕ vs [cyanine]) was in general obtained, the quenching efficiency in our work being greater for 2-AS ('a surface probe') relative to 9-AS (an 'inner probe'), at variance with results in Fig 1 of Ref 1

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