

Biochimica et Biophysica Acta 1512 (2001) 375-380



Erratum

Corrigendum to: 'Giant vesicles as models to study the interactions between membranes and proteins' [Biochimica Biophysica Acta, 1467 (2000) 177–188]

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In this article Figs. 2–5 and 8 were poorly reproduced. The publisher sincerely apologizes for this error. They are repeated here together with their legends.

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[★] PII of original article S0005-2736(00)00217-0.

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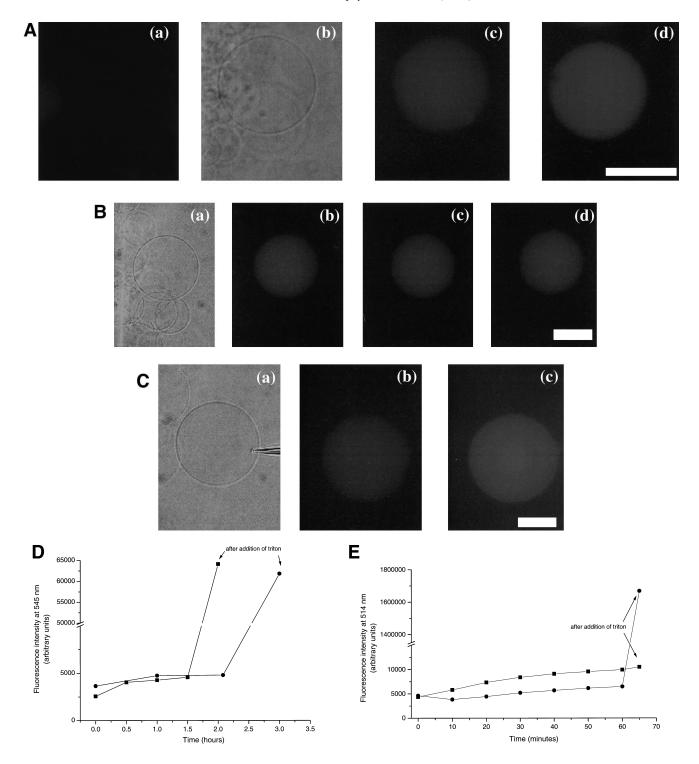


Fig. 2. The nucleic acid dye YO-PRO-1 and FDP permeate across the GV bilayers, but not across the LUV membranes. A: The vesicles were formed in water by the electroformation method, plasmid DNA (78 μg ml⁻¹) was injected and the GV was allowed to stand for 5–10 min. Then YO-PRO-1 was added externally to the vesicles at a final concentration of 1 μM. (a) Before DNA injection, (b, c) 15 min, (d) 50 min after YO-PRO-1 addition. (a, c, d) = fluorescence mode, (b) = DIC mode. Scale bar = 50 μm. B: Fluorescence intensity of DNA/YO-PRO-1 with time. After injection of the plasmid DNA, YO-PRO-1 was added externally to the vesicles. (a, b) 35 min, (c) 53 min, (d) 63 min after YO-PRO-1 addition. (a) = DIC mode, (b-d) = fluorescence mode. Scale bar = 50 μm. C: After injection of a 50 mU ml⁻¹ APase solution (in 12.5 mM NaCl, 6.25 mM Tris–HCl, 1.25 mM MgCl₂, 125 μM dithiothreitol), FDP was added externally to the vesicles at a final concentration of 5 μM. (a) After injection of APase, (b) 20 min, (c) 27 min after the addition of FDP. (a) = DIC mode, (b, c) = fluorescence mode. Scale bar = 50 μm. D: YO-PRO-1 is not permeable to LUV membranes. YO-PRO-1 (final concentration 1 μM) was added to purified LUVs containing encapsulated crude DNA and the fluorescence intensity was recorded as a function of time. Two typical results are shown. After 2 h (squares) and 3 h (circles), respectively, Triton X-114 was added (final concentration 2% w/v) and the total fluorescence of the solution was determined. E: FDP is not permeable to LUV membranes. FDP (final concentration 5 μM) was added to purified LUVs containing encapsulated APase and the fluorescence was recorded as a function of time (circles). The control experiment (squares) shows the fluorescence intensity without encapsulated APase. After 1 h, the vesicles were destroyed with Triton X-114 (final concentration 2% w/v) and the total fluorescence was measured.

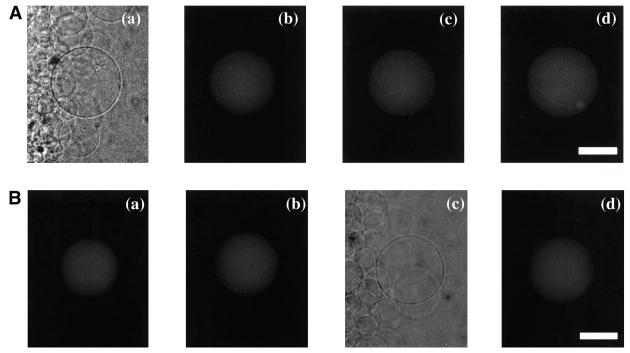


Fig. 3. Small nucleic acids and nucleotides do not leak out of a GV once microinjected into its water pool. A: GVs were formed in water. After injection of tRNA (20 mg ml⁻¹), YO-PRO-1 was added to the external medium. (a, b) 60 min, (c) 80 min, (d) 105 min after injection. (a) = DIC mode, (b-d) = fluorescence mode. Scale bar = 50 μ m. B: GVs were formed in water and fluorescein-12-UTP (2.5 mM) was injected. (a) 20 min, (b) 95 min, (c) 115 min, (d) 140 min after injection. (a, b, d) = fluorescence mode, (c) = DIC mode. Scale bar = 50 μ m.

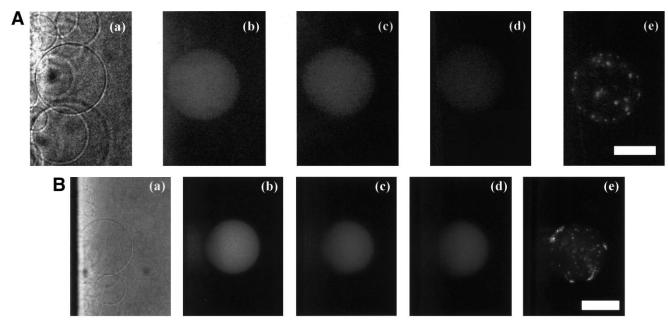


Fig. 4. DNase I and RNase A interact with the GV membrane and induce a decrease in the fluorescence of the nucleic acid/YO-PRO-1 complex. A: After injection of the plasmid DNA (78 μg ml⁻¹) and external addition of YO-PRO-1, DNase I was added externally to the vesicles (final concentration 200 μg ml⁻¹). (a, b) 16 min after DNA injection, (c) 1 min, (d) 14 min, (e) 41 min after DNase I addition. (a) = DIC mode, (b-e) = fluorescence mode. Scale bar = 50 μm. B: After injection of tRNA (20 mg ml⁻¹) and external addition of YO-PRO-1, RNase A was added externally to the vesicles (final concentration 70 μg ml⁻¹). (a, b) 1 min, (c) 16 min, (d) 33 min, (e) 79 min after RNase A addition. (a) = DIC mode, (b-e) = fluorescence mode. Scale bar = 50 μm.

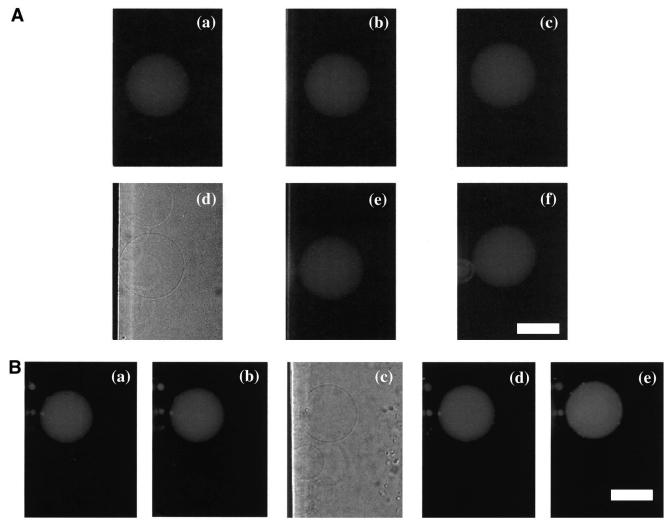


Fig. 5. Effect of non-specific enzymes on the entrapped nucleic acid. A: Proteinase K does not affect the stability of the DNA/YO-PRO-1 fluorescence intensity. After injection of DNA (78 μ g ml⁻¹) and external addition of YO-PRO-1, proteinase K was added externally to the GVs. (a) 20 min after DNA injection, (b) 6 min after the first addition of proteinase K (final concentration 160 μ g ml⁻¹), (c) 59 min after the first addition, (d, e) 15 min after the second addition of proteinase K (final concentration 320 μ g ml⁻¹), (f) 51 min after the second addition. Total observation time: 2 h. (a-c, e, f) = fluorescence mode, (d) = DIC mode. Scale bar = 50 μ m. B: DNase I does not affect the stability of the tRNA/YO-PRO-1 complex. DNase I (final concentration 200 μ g ml⁻¹) was added externally to the vesicles containing injected tRNA. (a) Before DNase I addition, (b) 19 min, (c, d) 33 min, (e) 46 min after DNase I addition. (a, b, d, e) = fluorescence mode, (c) = DIC mode. Scale bar = 50 μ m.

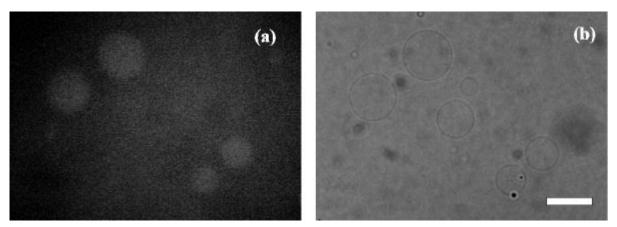


Fig. 8. YO-PRO-1 permeates across GV bilayers that were transferred into a new medium. The vesicles were formed by electroformation in an aqueous solution containing tRNA (4 μg ml $^{-1}$) and 5 mM sucrose. Then they were sucked into a large pipette and transferred into a medium containing 2.5 mM glucose and 5 μ M YO-PRO-1. (a, b) 1 h after transferring the GVs into the medium containing YO-PRO-1. (a) = DIC mode, (b) = fluorescence mode. Scale bar = 50 μ m.