Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information:

Membrane mixing and dynamics in hybrid POPC/Poly(1,2-butadiene-*block*-ethylene oxide) (PBd-*b*-PEO) lipid/block co-polymer giant vesicles

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Synthesis and characterisation of fluorescent amphiphilic polymer

Materials

PBd_{22}-PEO_{14} (PDI 1.01) has a hydrophobic PBd block of 1200 $g.mol^{-1}$ (> 85% 1,2 addition) and a hydrophilic PEO block of 600 $g.mol^{-1}$ was purchased from Polymer Source (Dorval, Montreal, Canada). 6-dodecanoyl -2- dimethylaminonaphthalene (Laurdan) and anhydrous 4-dimethylaminopyridine (DMAP) were obtained from Sigma-Aldrich Co. (Gillingham, U.K.). NN'-Disuccinimidyl carbonate was purchased from ThermoFisher Scientific Ltd. (Loughborough, Leicestershire, U.K.). 5-(and-6-)- ((N- (5-aminopentyl) amino) carbonyl) tetra methylrhodamine (TMR) was purchased from Insight Biotechnology (Wembley, U.K.). Anhydrous triethylamine was purchased from Fluorochem Ltd. (Hadfield, Derbyshire, U.K.). Dimethylformamide (DMF) was obtained from VWR International Ltd. (Lutterworth, U.K.).

Methods

Amphiphilic block copolymer PBd_{22}-PEO_{14} has a hydroxy-terminal group that was used to form a carbamate bond with tetramethyl rhodamine cadaverine (TMR) *via* an amino-reactive carbonate intermediate to give the fluorescently labelled polymer PBd_{22}-PEO_{14}-TMR by the following procedure.

PBd_{22}-PEO_{14} was frozen at -20 °C and dried in a vacuum desiccator to remove water. A Schlenk line was established and the reaction vessel was placed under nitrogen flow. All solvents and reactant materials were anhydrous and kept in sealed containers under nitrogen. The dried PBd_{22}-PEO_{14} polymer (50 mg, 0.0277 mmol) was dissolved in anhydrous DMF (5 mL). N,N'- Disuccinimidyl carbonate (6.4 mg, 0.0249 mmol) was dissolved in DMF (5 mL) at 50 °C. This was then transferred *via* syringe to the polymer solution. Anhydrous DMAP (338.4 µg, 2.77 µmol) was dissolved in anhydrous triethylamine (11.58 mL) at 50 °C. This was added to the polymer solution dropwise over a few minutes. The reaction mixture was left stirring overnight at 60 °C under nitrogen flow. At this point the solution was a clear yellow colour. Tetramethylrhodamine cadaverine (14.3 mg, 0.0277 mmol) was dissolved in DMF at 50 °C and added *via* syringe to the reaction mixture. The reaction mixture was left under stirring for 72 hours until the solution consistently maintained an opaque brown colour. The reaction solution was then dried using a GeneVac EZ2-Elite, producing a dark pink wax, which was then stored, protected from light, at 4 °C until purification. The wax was dissolved in water and run on a Sephadex G50 column under gravity. The first eluent was collected and freeze-dried on a VirTis Benchtop Pro Lyophiliser overnight. The fluorescently tagged polymer solid (PBd_{22}-PEO_{14}-TMR) was stored at -20 °C until use. ^{1}H and 2D COSY NMR spectra were recorded on a Bruker AV4 NEO 11.75 T (500 MHz ^{1}H) NMR spectrometer (500-CP) Spectrometer and processed with MestReNova 14.2.1 ©2021 Mestrelab Research S.L. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane. The following abbreviations were used to explain the multiplicities: s = singlet, bs = broad singlet, t = triplet, q= quartet. Liquid Chromatography-Mass Spectrometry (LC-MS) analysis was performed using a Thermo Scientific Ultimate 3000 HPLC system, interfaced with a Bruker amaZon Speed mass spectrometer equipped with an electrospray-ionization source operated in the positive mode. The fluorescent polymer was further characterised using a Bruker Alpha Fourier Transform Infra-Red (FT-IR) spectrometer.

Characterisation Results

H-NMR Spectroscopy

The structure of PBd_{22}-PEO_{14}-TMR was confirmed by ^1H and 2D COSY NMR as shown in Figure 1a and b. The formation of the carbamate bond between the TMR substituent and the -OH group at the chain end of PBd_{22}-PEO_{14} polymer (indicated as $OC(O)NH_f$ in Figure 1a) is shown as a peak observed at $\delta = 6.50$ ppm. This conjugation is further confirmed by the appearance of the signal for -$CH_{2e'}$-$OC(O)NH_f$ at 4.35 ppm. The degree of conjugated polymer was calculated by comparing the integral of the -CH_c=$CH_dH_{d'}$ repeating units ($\delta = 6.50$ ppm, bs) with that of the -$C(O)NH_j$ ($\delta = 8.51$, s) in the TMR group, and was found to be ~32.5%. Relevant correlations between coupled signals are highlighted in the ^1H-^1H COSY NMR spectrum (Figure 1b). The isolated product still contained traces of minor residuals of other components (such as triethylamine: $\delta = 2.96$ ppm, q, $N(CH_2CH_3)_3$ and $\delta = 1.10$ ppm, t, $N(CH_2CH_3)_3$) which represent ~ 4% w/w of the final mass. No further purification was attempted.

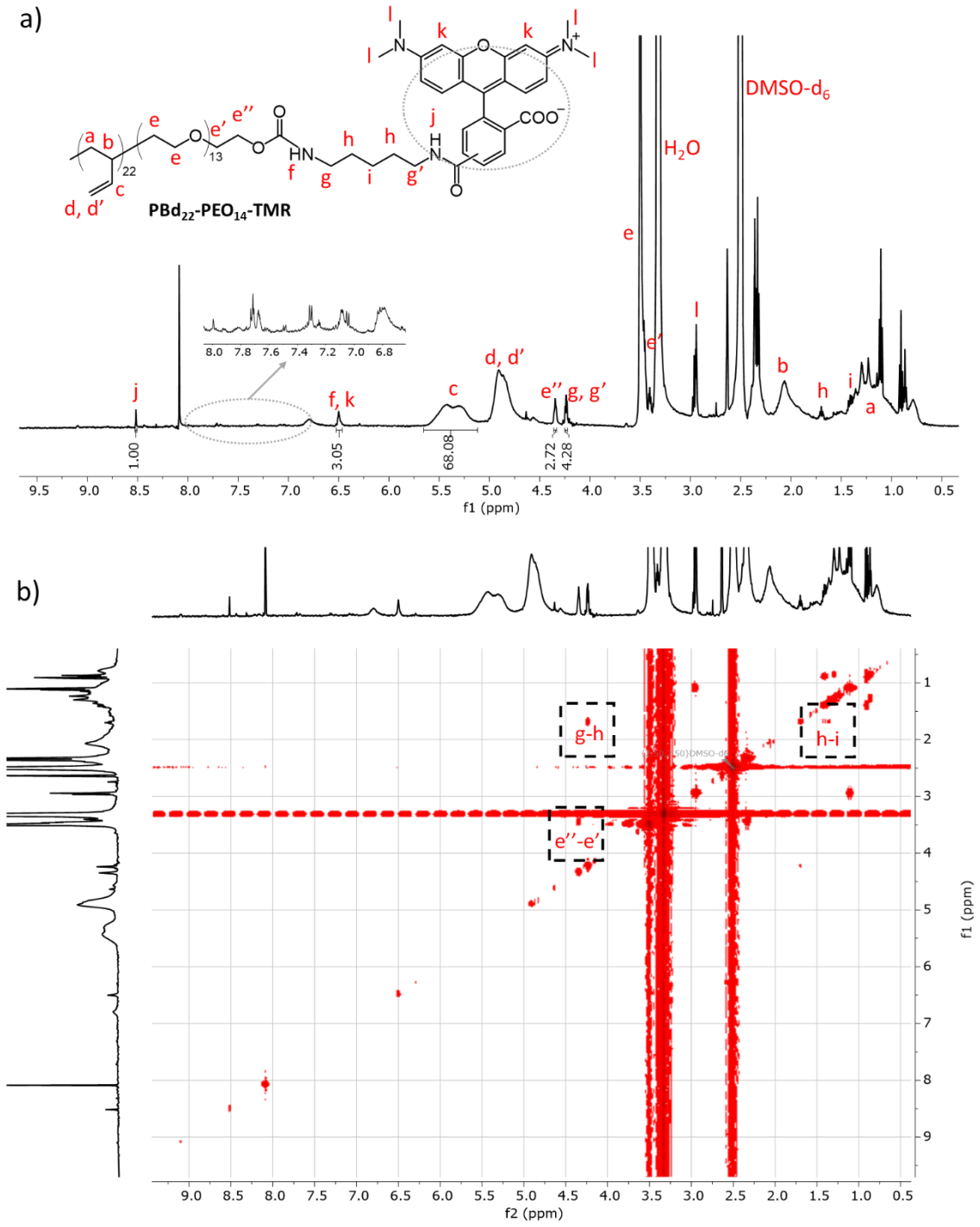

Figure 1: a) ^{1}H NMR spectrum and b) ^{1}H-^{1}H COSY of PBd_{22}-PEO_{14}-TMR in DMSO-d_6.

LC-MS

LC-MS chromatographic separations were performed using a Kinetex C18 (2.1×50 mm i.d., 2.6 µm particle size; Phenomenex, Torrance, CA, USA) at a column temperature of 40°C. The mobile phases were (A) 0.1 % Formic acid in water and (B) 0.1 % Formic acid in acetonitrile. A gradient was used starting at 98 % of A and 2 % of B over 1.2 minutes, ending with 2 % A and 98 % B at a flow rate of 1.3 mL/min. The UV spectrometer was set at a wavelength of 211 nm.

LC-MS spectrum of PBd_{22}-PEO_{14}-TMR was obtained as shown in **Error! Reference source not found.**. PBd_{22}-PEO_{14}-TMR is expected to have an average MW of 2358.64 $gmol^{-1}$. As shown in

Error! Reference source not found., the *m/z* values found for PBd_{22}-PEO_{14}-TMR cover a broad range, which would be expected from a polymeric compound. The main peak found is 979.63, which is in the expected range of MW distribution for *m/z* $[M-2Na]^{2+}$.

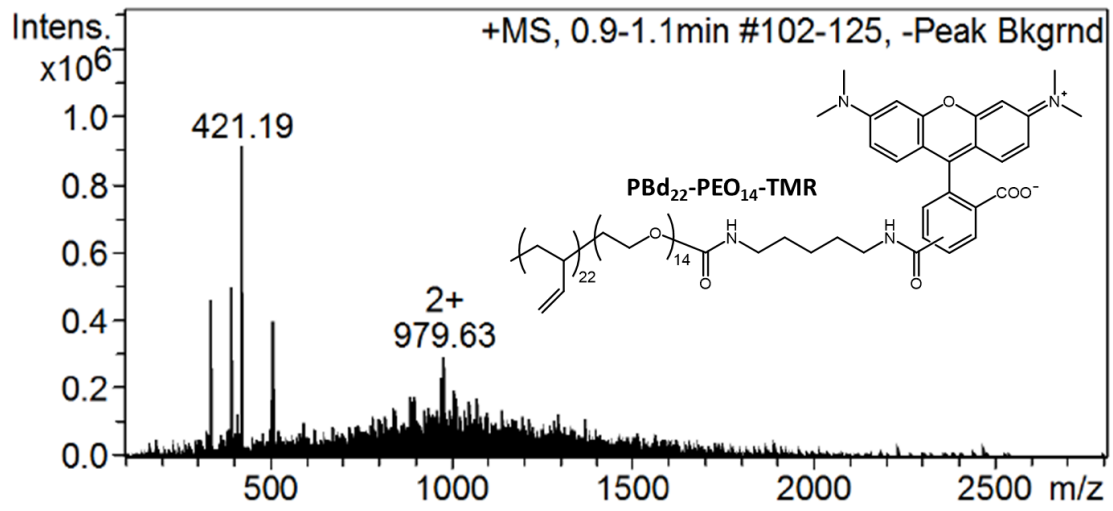

Figure 2: LC-MS spectrum of PBd_{22}-PEO_{14}-TMR, with structure of PBd_{22}-PEO_{14}-TMR inset.

FT-IR Spectroscopy

FT-IR analysis of starting material PBd_{22}-PEO_{14}: 3457, 3073, 2965, 1638 and 1101.26 cm^{-1}. FT- IR analysis of PBd_{22}-PEO_{14}-TMR: 3457, 3056, 2965, 1927, 1592, 1443 1215 and 1101 cm^{-1}.

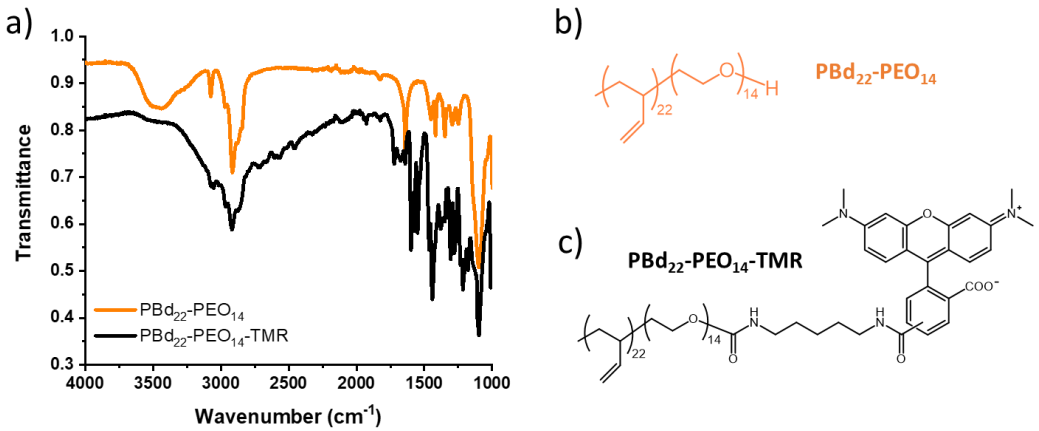

Figure 2: IR spectra of a) PBd_{22}-PEO_{14} and PBd_{22}-PEO_{14}-TMR alongside structures of b) PBd_{22}-PEO_{14} and c) PBd_{22}-PEO_{14}-TMR

Lipid and polymer mole fractions in GUVs

Hybrid GUVs were prepared using the electroformation method from 6.57 mM POPC and PBd-b-PEO solutions in various polymer-to-lipid ratios, with 2 mol% DiO and 10 mol% PBd_{22}-PEO_{14}-TMR for FRAP and lipid/polymer ratio experiments. With the addition of fluorophores, the POPC/PBd_{12}-PEO_{12} polymer solutions become ternary or quaternary mixtures. True mole fractions for each composition label are given in Table 1**Error! Reference source not found.**.

Table 1: True mole fractions of POPC, PBd-b-PEO, DiO and PBd_{22}-PEO_{14}-TMR in PBd_{22}-PEO_{14} and PBd_{12}-PEO_{11} GUV compositions. The addition of 2 mol% DiO and 10 mol% PBd_{22}-PEO_{14}-TMR to the hybrid vesicles alters their composition.

Sample label	POPC (mol%)	PBd-b-PEO (mol%)	DiO (mol%)	PBd_{22}-PEO_{14}-TMR (mol%)
0 mol%	98.0	0.00	2.0	0.0
25 mol%	66.5	22.5	1.8	9.2
50 mol%	43.6	45.4	1.8	9.2
75 mol%	22.7	66.5	1.8	9.0
100 mol%	0.00	90.0	0.0	10.0

DiO and PBd_{22}-PEO_{14}-TMR intensity images

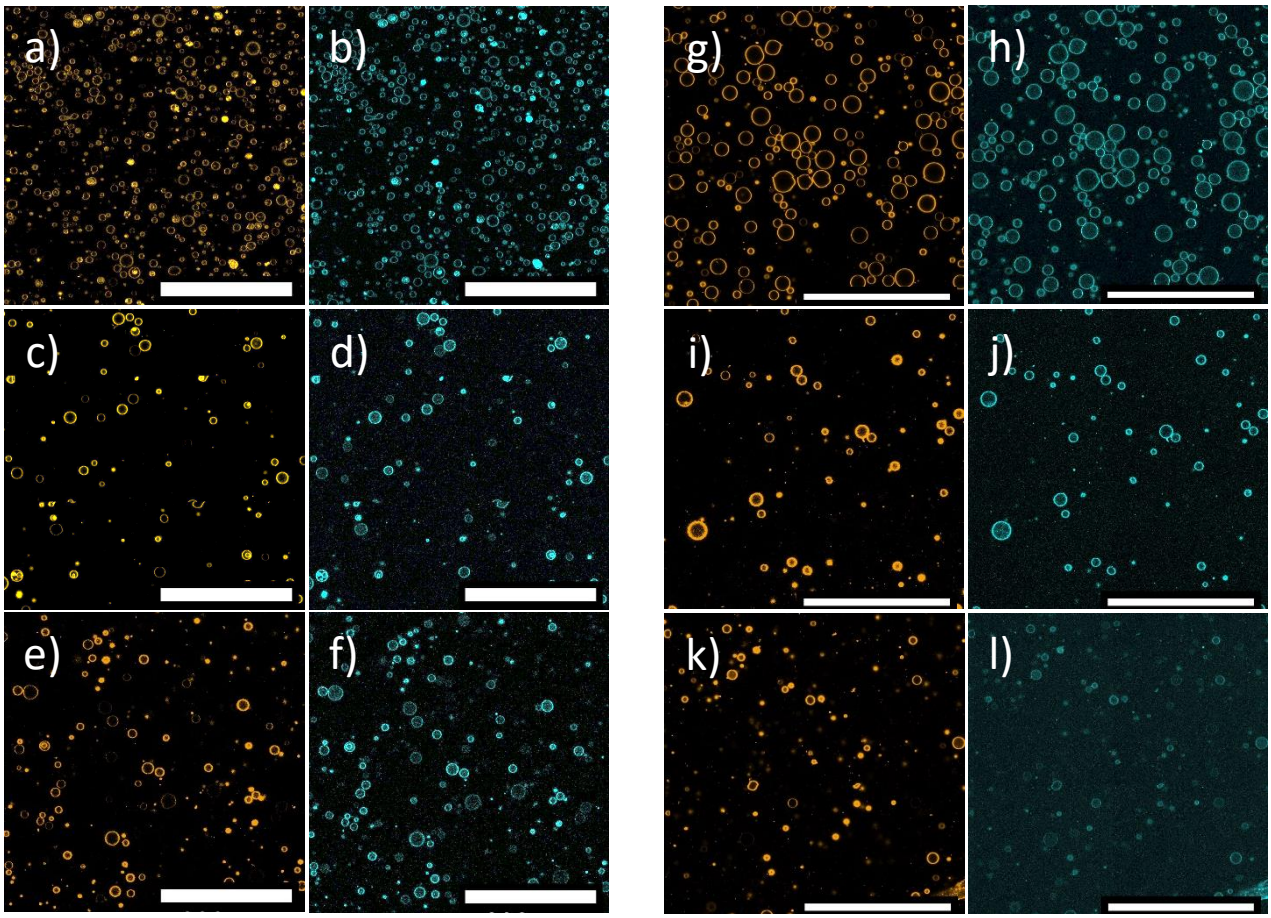

Figure 4: Tile scans showing intensity contributions from a) DiO and b) PBd_{22}-PEO_{14}-TMR in 25 mol% PBd_{22}-PEO_{14}; c) DiO and d) PBd_{22}-PEO_{14}-TMR in 50 mol% PBd_{22}-PEO_{14}; e) DiO and f) PBd_{22}-PEO_{14}-TMR in 75 mol% PBd_{22}-PEO_{14}; g) DiO and h) PBd_{12}-PEO_{14}-TMR in 25 mol% PBd_{12}-PEO_{11}; i) DiO and j) PBd_{12}-PEO_{14}-TMR in 50 mol% PBd_{12}-PEO_{11}; k) DiO and l) PBd_{12}-PEO_{14}-TMR in 75 mol% PBd_{12}-PEO_{11}. The scale bar is 200 µm.

Spectral imaging of Laurdan

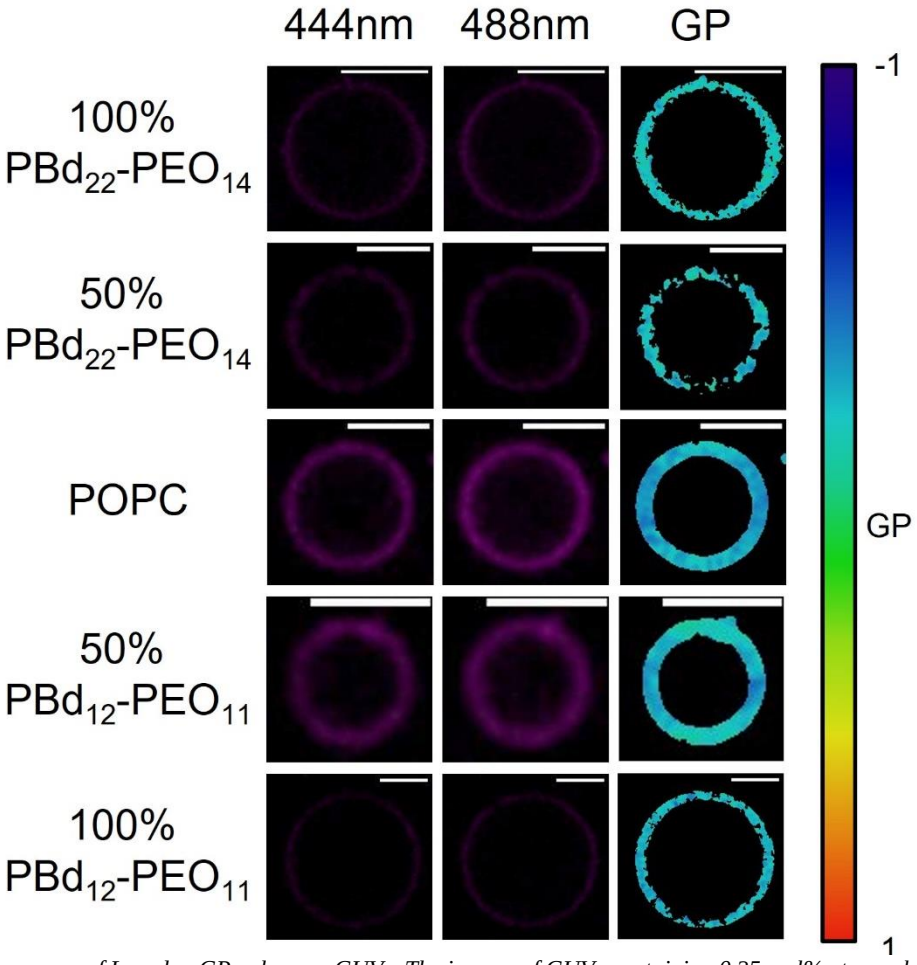

Figure 5: Colour maps of Laurdan GP values on GUVs. The images of GUVs containing 0.25 mol% at wavelengths 444 nm and 488 nm that contribute to the final GP. Scale bars indicate 10 µm.

All GUV compositions contained 0.25 mol% Laurdan. The brighter GUVs give a more complete GP image. Although the same fraction of each composition was Laurdan, the distribution of Laurdan in individual vesicles may cause the difference in brightness.