Dependence of Norfloxacin diffusion across bilayers on lipid composition

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Supplementary Information

1. Full data set of the vesicle tracking routine (Fig 2(c))

Using MATLAB the following properties are extracted for each identified vesicle. Each vesicle event is assigned a vesicle ID and the Movie associated with the vesicle is given a movie ID. The routine then tracks the vesicle from the starting frame where the vesicle appeared till the final frame when the vesicle left the field of view of the camera. In Figure2(c) frames 1763, 1765, 1766, 1768 and 1769 are shown. The table presents the details obtained from the MATLAB vesicle tracking routine. A sample vesicle whose intensity at the centre increases as Norfloxacin permeates over a period of time is shown in Fig S1.

Movie ID	Frame no	Vesicle ID	Border Touch	x-center	y-center	Major axis	Minor axis	Circularity	Intensity	Radius
1	1763	6	1	230.83	189.31	61.62	50.34	0.82	1684.87	27.99
1	1764	6	0	201.4	192.45	68.27	49.98	0.73	1720.22	29.5625
1	1765	6	0	170.82	195.16	68.57	50.12	0.73	1819.02	29.6725
1	1766	6	0	140.69	197.7	70.58	51.63	0.73	1869.16	30.5525
1	1767	6	0	110.57	200.37	69.79	51.28	0.73	1922.82	30.2675
1	1768	6	0	80.12	203.46	69.93	50.41	0.72	1976.45	30.085
1	1769	6	0	50.53	206.69	71	50.38	0.71	1978.39	30.345
1	1770	6	1	22.78	209.87	57.01	50.76	0.89	1876.9	26.9425
1	1771	6	1	8.04	213.67	43.08	23.08	0.53	1166.8	16.585

Only the Vesicles with circularity between 0.7 and 1 were used for analysis. Further frames in which they were touching the channel were ignored. Vesicles which were recognised to be of the sizes less than 17 µm

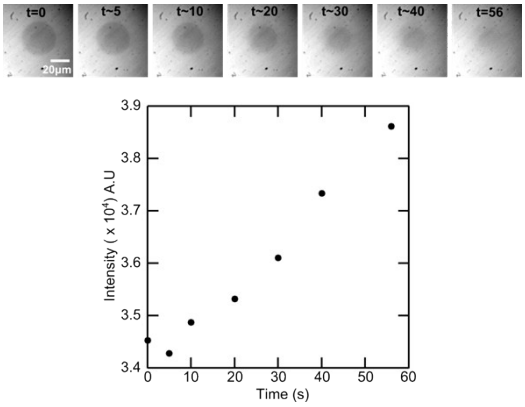

Fig S1**.** Intensity at the centre of the vesicle at various time points. As the Norfloxacin permeates the membrane, the vesicle becomes brighter.

or greater than 40µm in diameter were also ignored.

2. Post processing using LABVIEW

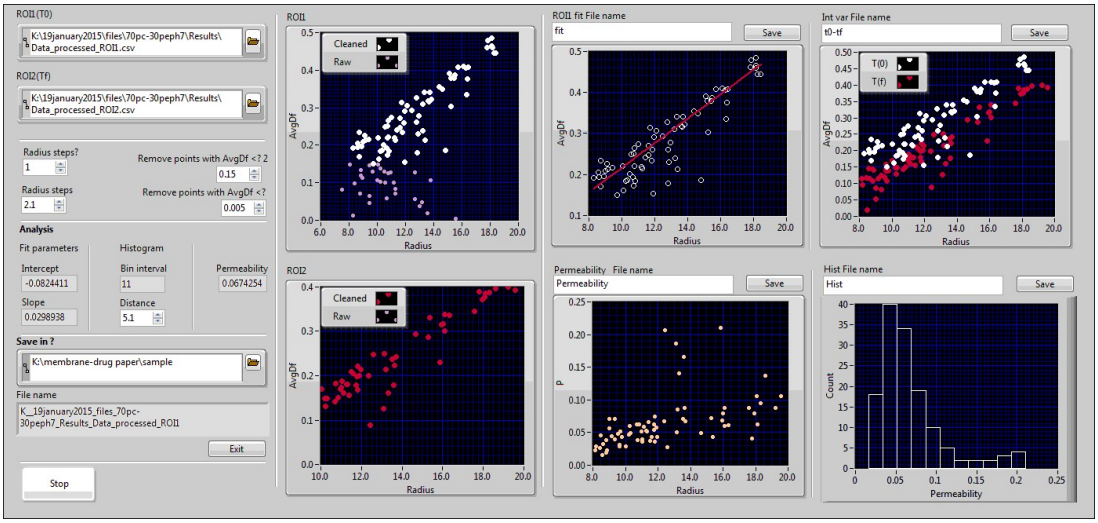

Fig S2 . Front panel of the Labview routine for post processing and calculation of permeability

Once the above mentioned details for each vesicle are collected, the average radius (from all the frames), average velocity from the information on frame rate of the Camera and the distance moved from the vesicle's initial appearance to the final frame where it moves out the field of view of the camera and the average normalised intensity differences were calculated by the same routine.

Once these details are logged, further post processing requires removal of the outliers. Labview routines were developed to do the post-processing of the images and calculate the permeability co-efficients Fig (S2). First the raw data obtained from MATLAB is plotted as a function of the radius and the corresponding intensity of the vesicle Fig (S3). The spread in the data can be seen for vesicle radii between 8 and 16µm. This could be due to various reasons such as the vesicle being not in focus, lipid aggregates that are falsely recognised or more than one vesicle stuck to each other etc.

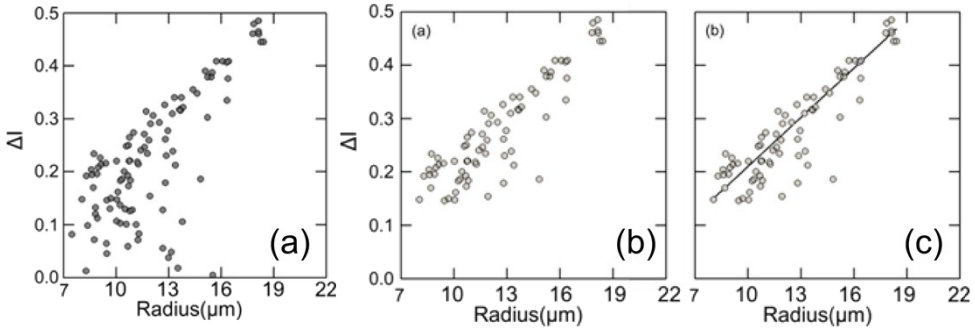

Fig S3. (a) Raw data obtained after MATLAB image processing. There is a spread in the ΔI values in vesicles of radii between 8 and 16 μm.(b) Cleaned data representing the vesicles detected at T_0 based on removing outliers whose value was above 1sigma from the average for all the intensities of vesicles whose radius was between r and r+1. The outliers can also be removed using other parameters such as maximum intensity, between lower and maximum intensity or by simply defining a manual threshold.(c) Linear fit to the cleaned data which is used for extracting permeability values. The slope and intercept values for calculation of intensities the vesicles detected at T_f. For the fit above, Intercept =-0.096, Slope = 0.0307, R^2=0.87.

A Labview routine gives the option of removing these outliers by either performing the averages of intensities of the vesicles between a radius range (1 μm steps in this case) and ignoring (1 sigma) signals beyond a user defined sigma or by looking at the maximum intensity for each vesicle and ignoring the points that are beyond a user defined sigma. Fig (S3 (b)) shows the data after removing outliers.
The routine then performs a linear fit. The slope and the intercept values are used to recalculate the intensity values corresponding to the vesicle radius detected in the ROI 2 (vesicles recognised at t_f) for performing the permeability calculation using the equation (eq1) .ESI Fig(S4,(c-d)).

Finally, a histogram is plotted to obtain the average permeability value for a given experimental run. Three runs for each experiment were performed.

3. Set up

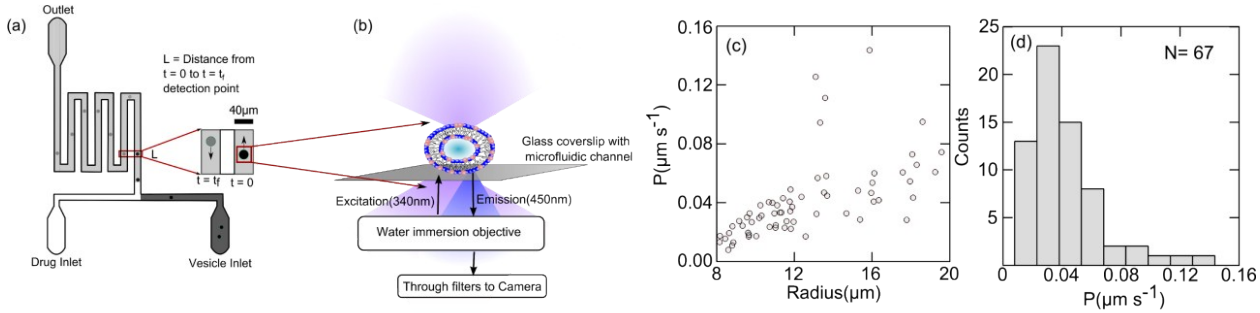

Figure S4 (a) Microfluidic chip used in the experiments. (b) Schematic of the setup. For details refer[1]. (c) Permeability values obtained for 70:30 DOPC: DOPE vesicles after processing using MATLAB and LABVIEW. (d) A histogram representation of permeabilities obtained for all vesicles. The permeability of these vesicles were 0.46 x10^{-5} cms^{-1}.

Additional datasets

List of other data sets obtained for the compositions mentioned in the article.

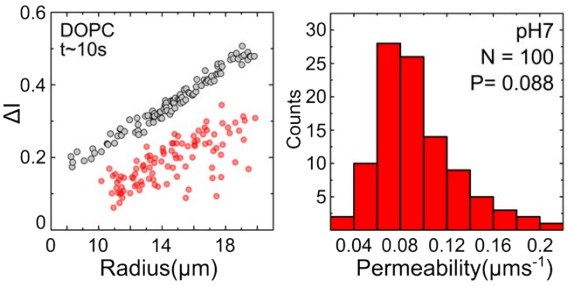

Fig S5. Permeability of pure DOPC vesicles. The permeability is 0.88×10^{-5} cms^{-1}.

Fig S6. Permeability of vesicles containing 70%DOPE. The permeability was 0.51×10^{-5} cms^{-1}

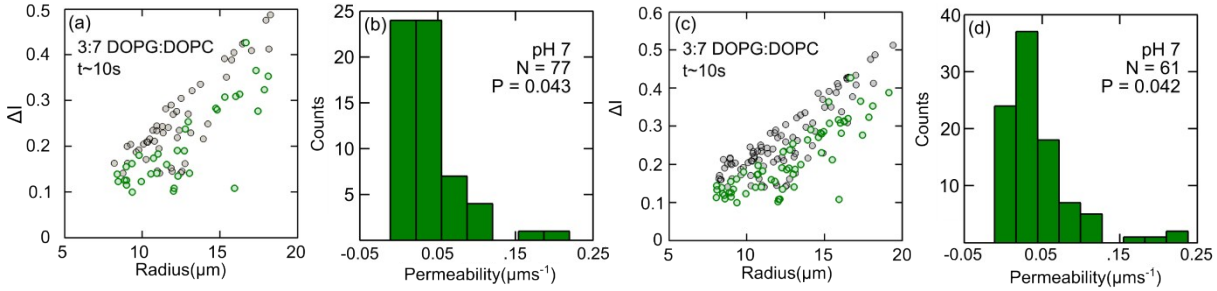

Fig S7. Data set for 30% DOPG in DOPC vesicles. (a) and (b) belong to one set of experiments whereas (c) and (d) belongs to the second run. The Permeability was 0.43×10^{-5} cms^{-1}.

References

1. Cama, J., Chimerel, C., Pagliara, S., Javer, A. & Keyser, U. F. A label-free microfluidic assay to quantitatively study antibiotic diffusion through lipid membranes. *Lab Chip* **14,** 2303–8 (2014).