
Modulation of Phospholipid Bilayers by Cholesterol and a Fluorescent Probe

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Abstract

Electroporation is a method of introducing a target chemical into biological cells and synthetic vesicles (liposomes). This method involves exposing the cell or vesicle to an electric field pulse, which will distort the shape of the membrane shell and cause pores to open. This process often kills any living cells exposed to the pulse. In order to gain a better understanding of how the electroporation process works, the process must be broken down into its basic steps and studied. The current experiments aim to develop and test a method for introducing different compounds (in particular cholesterol) into the phospholipid bilayer of synthetic vesicles, so that the effects of the additive on electroporation may then be studied.

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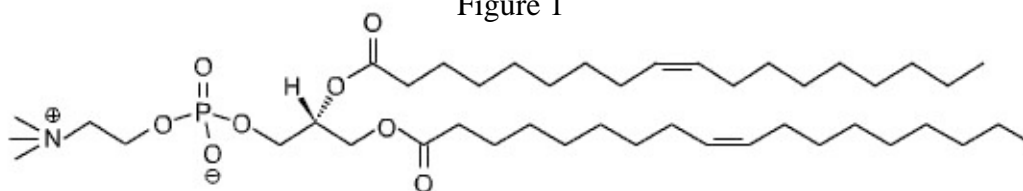
Introduction

In order to study the effects of a chemical compound on a living cell, the chemical must be introduced into the cell's interior. The semi-permeable membrane of the cell is usually enough to keep these chemicals from naturally traveling from the outside of the cell to the inside. It is possible to inject the chemical directly into the cell, but that method is not practical for large-scale tests where many cells need to be introduced to the chemical being tested. A common large-scale method of introducing chemicals into a living cell is electroporation. In this process the cell is exposed to a sudden and sustained high-voltage electric field. This field causes the cell to elongate into an ellipsoid. While in this ellipsoidal state, the cells develop pores in the polar cap regions. Due to the reduced volume of these ellipsoids, some material from inside the cell is ejected into the surrounding solution. Once the field is turned off, the cells return from ellipsoid shape into their natural spherical state. This increase in volume allows the surrounding solution to enter through the pores. As the pores slowly close, the cell regains normality with the exception of the introduced chemical.

One major concern in this process is the amount of time during which the electric field acts upon the cells. Too little exposure to the electric field will result in the pores not opening sufficiently to allow the chemical to enter the cell. Too much exposure and the pores will open so large that they rupture the cell membrane and destroy the cell. The time of exposure required to properly develop pores in the cell is based upon the rigidity (elasticity modulus) of the cell membrane. The more rigid the membrane, the longer exposure the cell will require for electroporation to occur.

Rigidity is based upon the nature of phospholipids, organic complexes, ion channels, and proteins that make up the cell membrane. In order to better understand the process of electroporation, we must discover what effect each of these materials has upon the process of electroporation. To measure the effects of various structures found in cell membranes, the compound 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC, Figure 1) was formed into a bilayer vesicle (Figure 2) which mimics the phospholipid bilayer in a biological cell. To this bilayer, other compounds could be added to change the rigidity of the membrane and the effects of that particular additive on electroporation. For this experiment, we wished to discover if cholesterol (Figure 3) would incorporate into the DOPC bilayer. Once cholesterol has been successfully incorporated into the bilayer, investigation of the effects of cholesterol on the electroporation of the bilayer can begin.

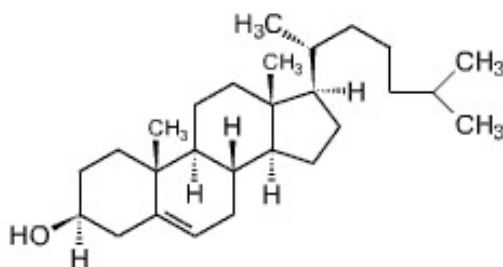
Figure 1



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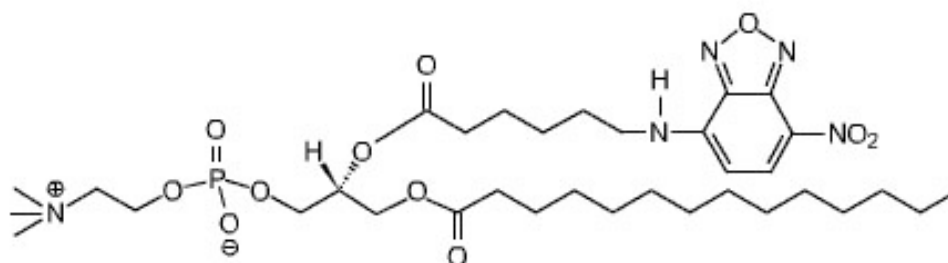
Figure 2



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Figure 3

In addition to adding the cholesterol, the fluorescent lipid 1-Myristoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-sn-Glycero-3-Phosphocholine (NBD, Figure 4) was added to test the effect the cholesterol would have on the fluorescence of the indicator lipid.



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Figure 4

Procedure

The procedure begins by withdrawing 0.2 mL of 20 mg/mL DOPC with chloroform solvent and placing into a round-bottom flask. After that, any other experimental compounds dissolved in chloroform solvent are added. The resulting mixture is then

placed in a rotary evaporator. Using an aspirator as a source of vacuum, the solvent is let evaporate for 30 minutes. After that, the residual film is let dry a further 60 minutes using a vacuum pump as a source of vacuum.

After the drying process is complete, 10 mL of distilled water is added directly to the phospholipid film. The entire solution is then mixed by placing the flask on a Vortex machine and allowing it to mix for 5 minutes. During this hydration process, multilamellar vesicles (MLV) are formed. To convert the MLV to unilamellar vesicles, the solution is placed inside a nearly airtight cylinder (an extruder) and forced—using pressurized nitrogen gas—to pass through two polycarbonate membrane filters with a pore-size of 0.2 μm . This process is repeated four more times to ensure that the vesicles are single-bilayer vesicles and not multiple-bilayer vesicles.

This extruded solution was then tested in a UV-vis spectrophotometer and a fluorescence spectrophotometer.

Discussion and Conclusion

I. UV-Visible Spectrophotometer Results

The UV-vis spectral results for the DOPC lipid bilayer integrated with cholesterol were different from the results without the cholesterol in the region from about 250 nm to 300 nm (Appendix A-B). The slight absorption peaks in this region correspond to the peaks obtained by running cholesterol dissolved in decane versus pure decane (Appendix C). Because cholesterol does not dissolve in water, the presence of these peaks in the results for the DOPC integrated with cholesterol shows that the cholesterol is in fact integrating within the phospholipid bilayer. Additionally, when this test was performed at a higher concentration of cholesterol the peaks rose in absorbance in the same region. This leads me to conclude that even at levels up to 50 mole percent in a DOPC/cholesterol bilayer, the cholesterol is integrating within the lipid bilayer.

II. Fluorescence Spectrophotometer Results

The fluorescence spectral results (Appendix D-F) for the DOPC lipid bilayer with the indicator lipid added were as follows:

Bilayer Contents	Emission Peak Wavelength (nm)	Emission Peak Intensity (cps)
DOPC with NBD only	544.031	12E+05
DOPC with NBD and cholesterol (30 mole%)	543.458	15E+05
DOPC with NBD and cholesterol (50 mole%)	542.849	16E+05

These results tell us that the florescence profile of the lipid bilayer incorporated with the NBD changes with the incorporation of the cholesterol. The wavelength at which the emission peak is greatest changes slightly depending upon the amount of cholesterol added, but the intensity changes dramatically with increased amounts of cholesterol.

These results indicate that the way the cholesterol changes the bilayer allows the environment-dependent NBD to create greater fluorescence.

The purpose of this experiment was to determine if the cholesterol would successfully incorporate into the lipid bilayer, and the experimental data indicate that it does indeed incorporate into the bilayer as expected. Armed with this information, further research can be placed into the study of what effect the cholesterol will have upon the electroporation of the bilayer vesicle. This question is particularly important for utilizing electroporation for the preparation of subnanometer size quantum dots—a novel method developed in this laboratory.

Acknowledgments

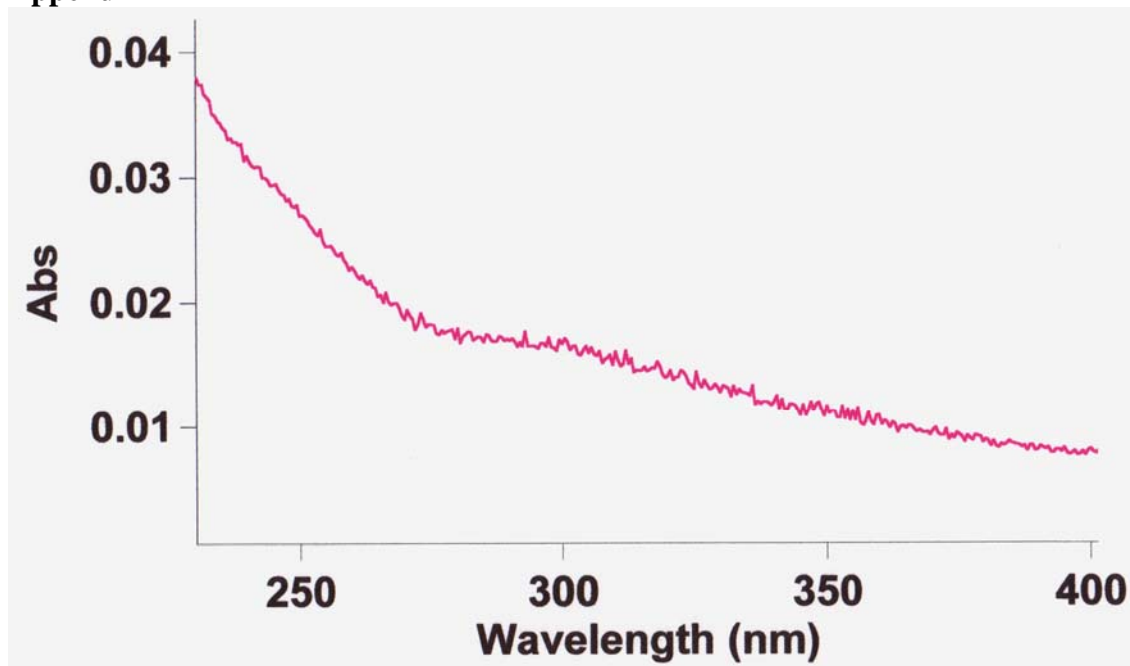
I would first and foremost like to thank the Robert A. Welch Foundation for making this experience possible. I would also really like to thank the University of Texas at Arlington for hosting us here. The first person I would like to thank is Helen for teaching me how to use every single piece of equipment I would need to use, as well as doing her best to explain what any of this data meant. I would also like to thank Dr. Schelly for taking me into his lab and coming by early every once in a while to check up on me.

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I would like to thank my parents, for always supporting me. I would like to thank my sister for always offering me a quick way to get off campus if I felt I needed a break from all the chemistry. Finally, I would like to thank my high school chemistry teachers, Mrs. LeGrand and Mr. TenEyck for instilling in me a love for chemistry, as well as pointing me towards this wonderful program. Without any of the people mentioned above, this experience wouldn't have been nearly as good, if at all possible. Thank you.

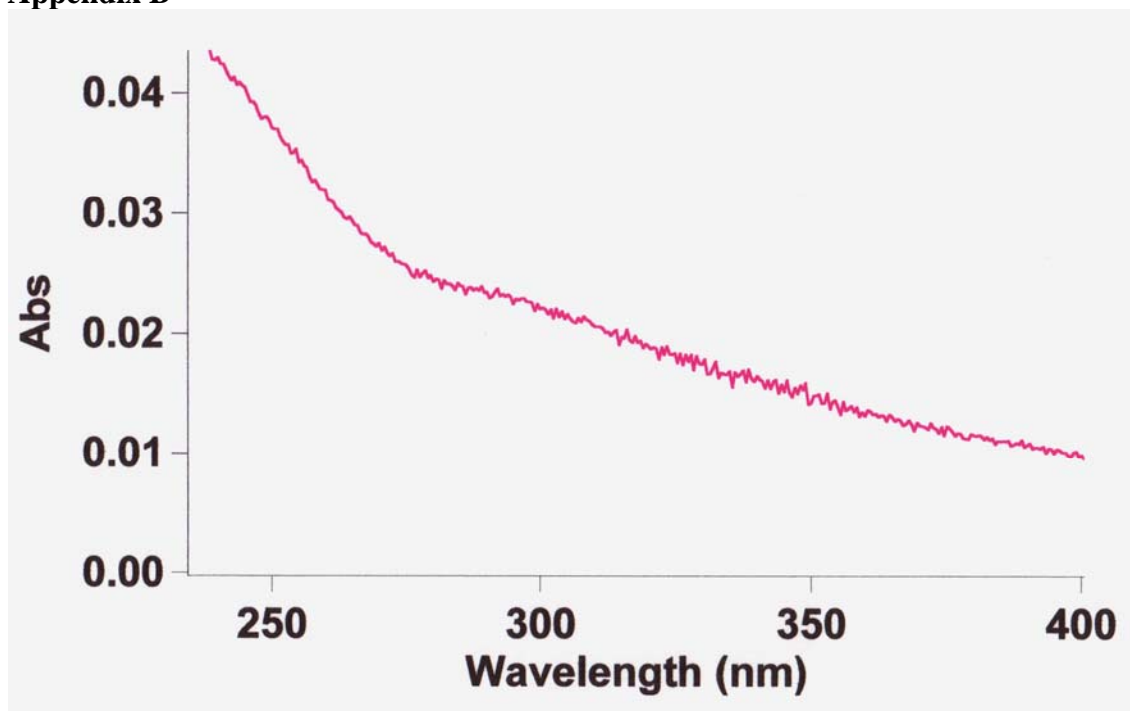
Appendices

Appendix A



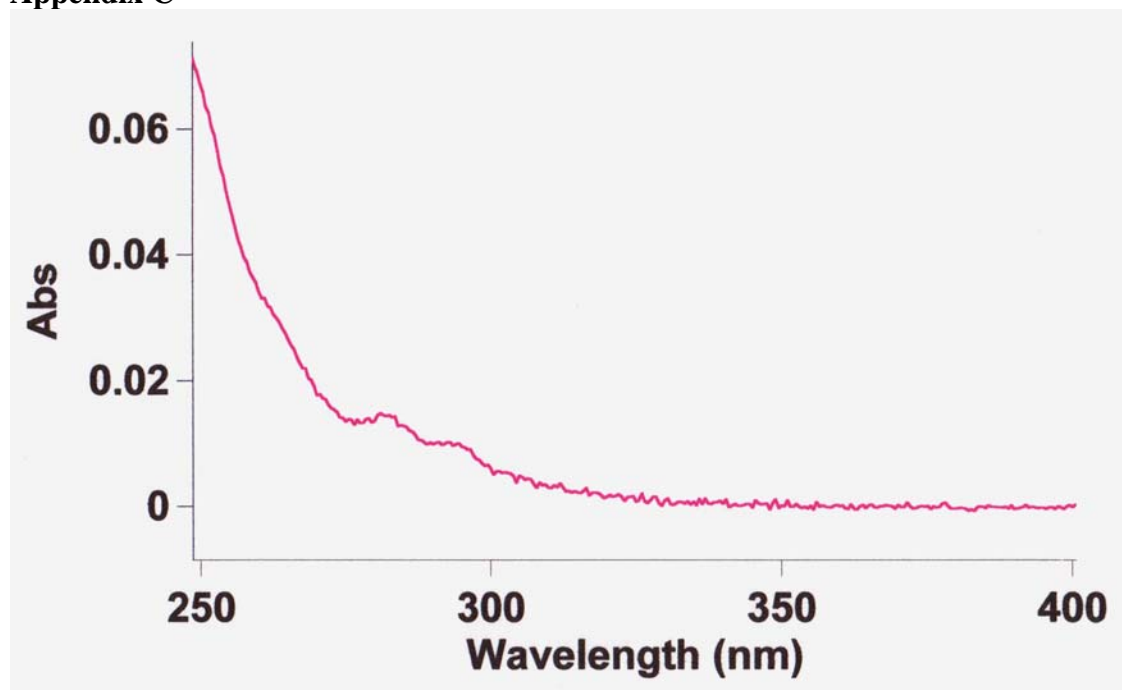
Absorption spectrum of 0.04 mg/mL DOPC vesicle with cholesterol (30 mole%) versus 0.04 mg/mL DOPC vesicle only

Appendix B



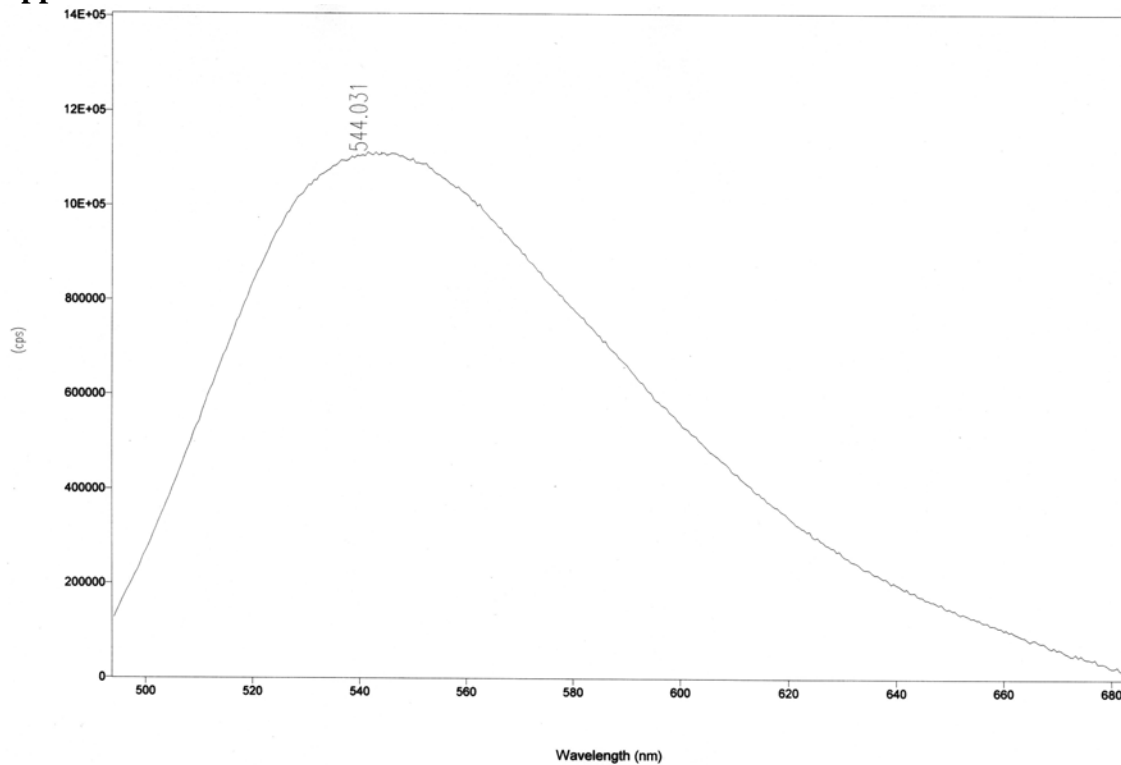
Absorption spectrum of 0.04 mg/mL DOPC vesicle with cholesterol (50 mole%) versus 0.04 mg/mL DOPC vesicle only

Appendix C



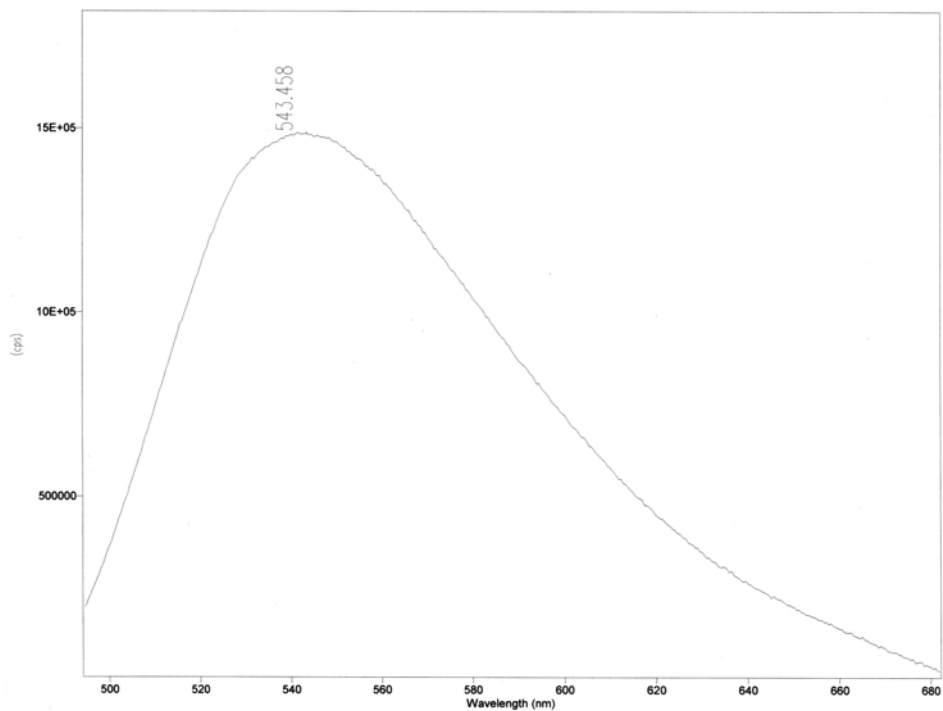
Absorption spectrum of cholesterol dissolved in decane versus decane only

Appendix D



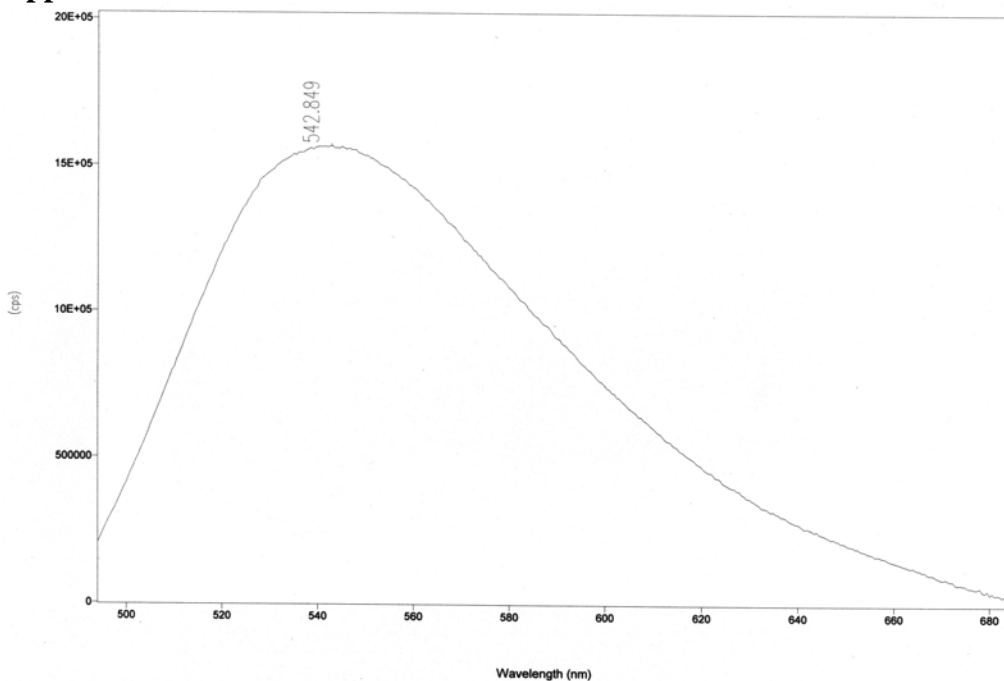
Fluorescence spectrum of 0.4 mg/mL DOPC vesicle with NBD only. Excitation wavelength: 478 nm.

Appendix E



Fluorescence spectrum of 0.4 mg/mL DOPC vesicle with NBD and cholesterol (30 mole%). Excitation wavelength: 478 nm.

Appendix F



Fluorescence spectrum of 0.4 mg/mL DOPC vesicle with NBD and cholesterol (50 mole%). Excitation wavelength: 480 nm.