# Stark Hole Burning of Aluminum Phthalocyanine Tetrasulfonate in Normal and Cancer Cells

**N. Milanovich, M. Rätsep, T. Reinot, J. M. Hayes,* and G. J. Small**

*Ames Laboratory—USDOE and Department of Chemistry, Iowa State University, Ames, Iowa 50011*

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The results of Stark hole burning of aluminum phthalocyanine tetrasulfonate (APT) in MCF-10F (normal) and MCF-7 (cancer) cells are reported. Hole broadening was observed for zero-phonon holes (ZPH) for both cell lines when laser polarization was parallel and perpendicular to an applied electric (Stark) field. Changes in dipole moment suggest that dye molecules are located near the cell membrane and, in normal cells, experience some degree of ordering not found in the cancer cells.

## Introduction

In a recent paper[1] (referred to hereafter as I) we demonstrated that nonphotochemical hole burning (NPHB) spectroscopy can be applied to dye molecules within cells. Our ultimate objective is to determine whether NPHB can be used to "image" anomalies in subcellular structures as a normal cell transforms into a cancer (diseased) cell. By analogy with magnetic resonance imaging (MRI), one can refer to our concept as hole burning imaging (HBI). With NPHB, however, several properties can be measured including the homogeneous width of the zero-phonon hole (ZPH), the electron−phonon coupling, and the response of the ZPH to pressure and external electric (Stark) fields. Earlier work on NPHB of dye molecules in polymers and glasses had shown that NPHB is sensitive to the microenvironment around the dye.[2]

In I, the dye molecule aluminum phthalocyanine tetrasulfonate (APT) in (normal) MCF-10F, human breast epithelial cells was studied in vitro. APT was chosen as the test molecule because it is membrane-permeable (having been used in photodynamic cancer therapy) and because its NPHB properties in hyperquenched and annealed glassy films of water,[3−5] ethanol, and methanol[6] have been thoroughly studied. In I, results on the homogeneous width, electron−phonon coupling, dispersive hole growth kinetics, and pressure-induced linear shifting of APT's ZPH in normal cells were reported. It was concluded that APT in the cell is in an aqueous environment, one that is glassy rather than crystalline at low temperatures. However, it appears that the hydrogen bonding of water molecules with APT in pure glassy water is somewhat disrupted in the cell owing to APT's interactions with other cellular components. Although APT's hole burning efficiency in the cell is lower (7×) than in glassy water, it is still high (a burn fluence of 6.0 $\mu$J/cm$^2$ produces a fractional hole depth of 0.1). Subsequently, we performed the same experiments for APT in MCF-7 human breast adenocarcinoma cells (unpublished results). Briefly, no significant reproducible differences were observed. While the results for MCF-10F cells from different samples were highly reproduceable, those for MCF-7 cells were less so, perhaps reflecting the heterogeneity of this cell line.[7]

The inability of APT to distinguish between normal and cancer cells on the basis of the above types of experiments is probably mainly due to the fact that APT is not organelle-specific, vide infra. Ideally, one would like to use a probe molecule that is specific to a cellular component known to undergo significant structural changes as a normal cell evolves into a cancerous cell. Nevertheless, we present hole burning data which show that, with the Stark effect (external electric field), APT can distinguish between the two cell lines.

## Experimental Section

MCF-10F and MCF-7 cells were obtained from the American Type Culture Collection (ATCC). MCF-10F cells were cultured in a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium containing 5% horse serum, 100 ng/mL cholera enterotoxin, 10 $\mu$g/mL insulin, 0.5 $\mu$g/mL hydrocortisol, and 20 ng/mL EGF. MCF-7 cells were cultured in minimum essential medium (Eagle) with nonessential amino acids, Earle's BSS, 1 mM sodium pyruvate, 10 $\mu$g/mL bovine insulin, with 10% fetal bovine serum. All culture media components were purchased from Sigma-Aldrich Chemicals. Both cell lines were incubated at 37 °C under an air atmosphere containing 5% $CO_2$; cells were passed weekly. Staining of MCF-10F and MCF-7 cells were carried out as described previously for MCF-10F cells. MCF-7 cells were suspended in a freezing medium different from MCF-10F. The MCF-7 freezing media consisted of MCF-7 culture media with 5% DMSO. The reader is referred to I for a detailed discussion of overall protocol. Sample aliquots, in their respective freezing media, were diluted 1:1 (by volume) with glycerol and transferred to 5 mm o.d. gelatin capsules for facile formation of a glass at 4.5 K. The hole burning apparatus which utilizes the fluorescence excitation mode for recording of absorption and hole burned spectra is described in I. Line width of the laser used for burning and recording of hole burned spectra was $<$20 MHz.

The Stark cell used for this experiment consisted of two Teflon walls separated by a distance of 11 mm and two copper electrodes positioned perpendicular to the walls. A separation of 4.95 mm was maintained between the electrodes by placing them into grooves made on the inside of the Teflon walls. The experiments were done using a fluorescence excitation scheme described previously,[1,3] and, therefore, the electrodes had to be placed above and below the sample to avoid repositioning of the sample during the experiment. The laser beam was able to access the samples through a slit on one of the Teflon walls. Samples were placed lying on their sides at ∼45° relative to the Teflon walls and immediately plunged into a cryostat

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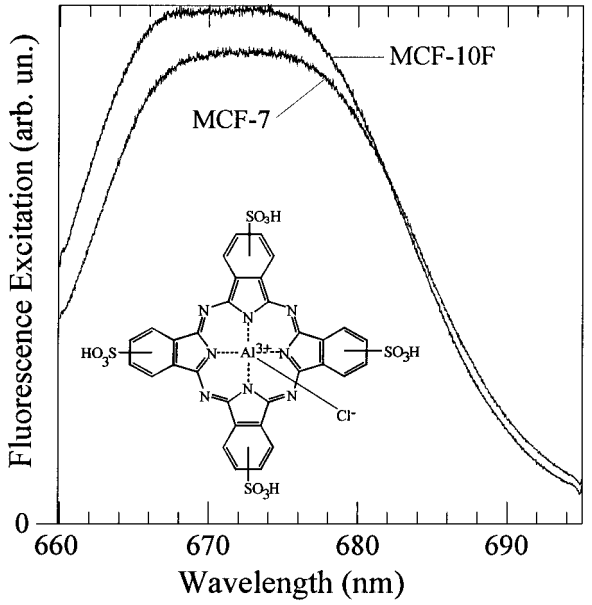


**Figure 1.** Fluorescence excitation spectra of APT in MCF-10F cells ($\lambda_{max}$ = 675.4 nm) and MCF-7 cells ($\lambda_{max}$ = 676.2 nm) at 1.8 K.

precooled to 4.2 K; subsequent cooling to 1.8 K was done by applying a vacuum to the cryostat's sample chamber. A polarizer placed in front of the Stark cell was used to orient the laser beam polarization either parallel or perpendicular to the applied Stark field. Electric field strengths up to 10.1 kV/cm were utilized.

## Results and Discussion

Fluorescence excitation spectra for APT-stained MCF-10F and MCF-7 cells at 1.8 K are given in Figure 1. Both spectra have nearly flat-topped peaks and are composed of two separate bands that are slightly resolved for the MCF-10F sample; the identities of these two bands have been discussed elsewhere.[1,3−6] Fluorescence excitation maxima of 675.4 and 676.2 nm correspond to the lower energy bands of MCF-10F and MCF-7, respectively, and were determined by fitting two Gaussian line shapes to each spectrum. In I, APT was shown to have considerable broadening of the fluorescence excitation band and a large red-shift in the band maximum (681.3 nm) in 1:1 MCF-10F freezing media/glycerol relative to APT in cells. A similar result was obtained for APT in 1:1 MCF-7 freezing media/glycerol (spectrum not shown) relative to MCF-7 cells stained with APT, thus verifying the presence of APT inside MCF-7 cells. The width of the bands for the two freezing media suggest that APT can be found in a variety of different environments within the freezing media while APT in the cell samples is in a less disordered environment.

The effects of a 10.1 kV/cm Stark field on the ZPH of APT in MCF-10F and MCF-7 cells are shown in Figure 2 for both parallel (spectra a and c) and perpendicular (spectra b and d) orientations of the burn laser polarization relative to the Stark field. For both cell lines, only Stark broadening was observed for parallel and perpendicular orientations. Broadening effects were found to be reversible when the electric field was turned off, thus returning the holes to their original widths while hole depths decreased slightly owing to spontaneous hole filling (spectra not shown).[8] No Stark splitting was observed for MCF-10F or MCF-7 cells for either polarization. Broadening of the ZPH for MCF-7 cells (Figure 2c,d) is comparable for both laser orientations, whereas the hole broadening for MCF-10F cells

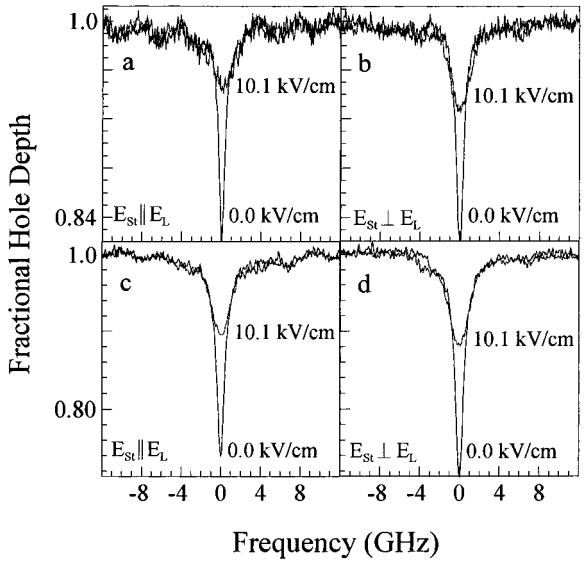


**Figure 2.** ZPH's at zero-field and with a 10.1 kV/cm external field for APT in MCF-10F cells (panels a and b) and in MCF-7 cells (panels c and d). Panels a and c correspond to burn laser polarizations parallel to the Stark field, and panels b and d represent a laser polarization perpendicular to the Stark field.

**TABLE 1: Electric Field-Induced Dipole Moment Changes for APT in MCF-10F and MCF-7 Cells**

| cell line | $f\cdot\Delta\mu$ (D)[a] | laser polarization | $\lambda_B$ (nm) |
|---|---|---|---|
| MCF-10F | 0.27 | ∥ | 677.5 |
| | 0.22 | ⊥ | 678.0 |
| MCF-7 | 0.20 | ∥ | 678.0 |
| | 0.21 | ⊥ | 677.5 |

[a] Uncertainty is ±0.01 D.

(Figure 2a,b) is greater for the parallel orientation than for the perpendicular.

A more quantitative comparison of the hole broadening for the four cases given in Figure 2 can be made by calculating the change in dipole moment, $\Delta\mu$, for each case as described by Kador et al.[9] This calculation gives the result for $f\cdot\Delta\mu$, where $f$ is the local field correction, which is unknown for the APT matrix. Results for these calculations are given in Table 1 and were determined by fitting a plot of hole width as a function of electric field strength. An example of this is given in Figure 3 for MCF-10F and MCF-7 cells for the parallel orientation. From the data in Table 1, one can see that, indeed, a difference in $\Delta\mu$ exists between parallel and perpendicular polarization for MCF-10F cells. What is also interesting is that $\Delta\mu$ values for the perpendicular orientation for the two cell lines are nearly the same whereas in the parallel case these values are different. Furthermore, the $\Delta\mu$ values are for holes burned at either 677.5 or 678.0 nm, but $\Delta\mu$ values for holes burned near 684 nm are nearly identical to those in Table 1 for a given cell line at a given laser orientation. Therefore, the changes in dipole moment are independent of burn wavelength.

To understand these results, we must consider that the change in dipole moment, $\Delta\mu$, is equal to the sum of the molecular dipole moment difference, $\Delta\mu_o$, and a matrix-induced component, $\Delta\mu_{ind}$. For the case where $\Delta\mu_o \gg \Delta\mu_{ind}$, one would expect to observe Stark splitting for one laser orientation and Stark broadening for the other orientation.[10] However, when Stark broadening occurs at both polarizations, where $\Delta\mu_{\parallel} \cong \Delta\mu_{\perp}$, $\Delta\mu_{ind}$ will dominate, thus indicating $\Delta\mu_{ind}$ is randomly oriented with respect to the transition dipole of APT and the laser polarization. This appears to be the case for APT in MCF-7

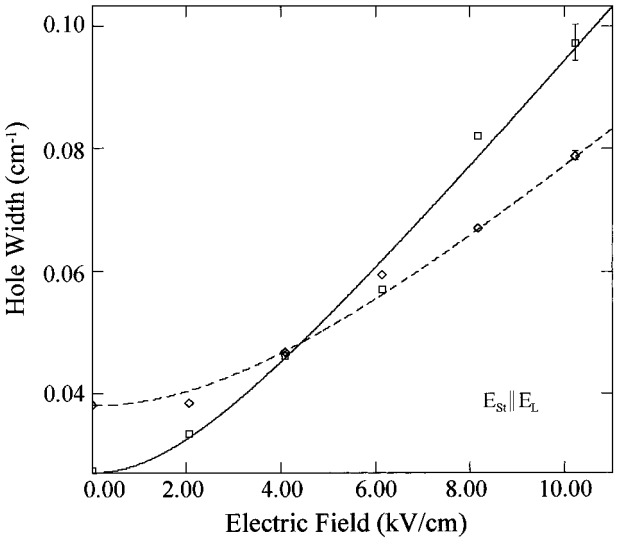


**Figure 3.** Dependence of hole width on Stark field for APT in MCF-10F cells (squares) and MCF-7 cells (diamonds) for a burn laser polarization parallel to the Stark field. Holes were burned at 677.5 and 678.0 nm for MCF-10F and MCF-7 cells, respectively, using a burn fluence of 139 $\mu$J/cm$^2$ for both. Theoretical fits are also given for MCF-10F cells (solid line) and MCF-7 cells (dashed line). Error bars are included at maximum Stark field which resulted in the largest hole width error.

cells but not for MCF-10F cells. MCF-10F data show that holes broaden in an electric field (Figure 2) for both polarizations but result in $\Delta\mu_{\parallel} \neq \Delta\mu_{\perp}$. For MCF-10F cells, $\Delta\mu_{\perp}$ is similar to the $\Delta\mu$ values for MCF-7, and, thus, we can conclude that $\Delta\mu_{\perp}$ for MCF-10F cells is also caused by a similar randomly distributed $\Delta\mu_{\text{ind}}$. However, the fact that $\Delta\mu_{\parallel} > \Delta\mu_{\perp}$ suggests that $\Delta\mu_{\text{ind}}$ in normal cells is not completely random. According to Vauthey et al.[11] we can consider $\Delta\mu_{\text{ind}}$ to be the sum of a random component, $\Delta\mu_{\text{ind}}$ (random), and a nonrandom component, $\Delta\mu_{\text{ind}}$ (nonrandom). This implies that some degree of ordering of APT molecules exists within the normal cells that does not exist within the cancer cells.

We are currently uncertain as to what causes this ordering of APT, but we propose that the ordering of dye molecules is caused by the presence of APT at the cell membrane. Various photodynamic therapy studies have set out to determine the intracellular location of APT, resulting in conflicting data suggesting that APT can be found within lysosomes[12] or mitochondria,[13] or near the cell membrane.[14] From the results in I, we have established that APT has a mainly aqueous local environment with a modified hydrogen-bonding network similar to that of glassy water. Moreover, since intracellular water is associated with the hydrophilic portion of lipid bilayer membranes,[15] e.g., the cell membrane, one can infer that perhaps APT molecules can be found within the aqueous region near the membrane lipids. A modification in the hydrogen-bonding network would arise from the presence of hydroxyl groups associated with the hydrophilic portion of membrane lipids, which are usually phospholipids or glycolipids.[16,17] This would also be consistent with our results in I where APT in cells has a fluorescence excitation maximum and mean-phonon frequency intermediate to that of glassy water and glassy ethanol. However, from the above argument alone we cannot say whether APT is associated with lysosomes or the cell membrane because both are composed of membrane lipids.[16,17] Müller et al.[14] have reported that photosensitization of APT in urinary bladder carcinoma cells resulted in blistering and rupture of cell membranes. The authors go on to conclude that APT is mainly associated with the cell membrane rather than with lysosomes or mitochondria. Previously, we have argued that the fluorescence excitation spectra of APT in cells is consistent with the dye being localized in an acidic medium such as lysosomes. The Stark measurements, however, would argue for at least some of the APT being located near the plasma membrane. This conclusion is based upon the attenuation that occurs within the cytoplasm when an external electric field is applied to a cell. This may be calculated by assuming that the cell is spherical and composed of a sphere of cytoplasm surrounded by a thin membrane. The resistivity of the membrane is much higher than that of the cytoplasm. The effective field within the cytoplasm sphere is then equal to the ratio of the product of cytoplasm resistance times its radius to the membrane resistance times its thickness.[18] For typical membrane and cytoplasm resistances, the field within the cytoplasm is calculated to be attenuated by a factor of $\sim 10^4$. For such a low effective field, a Stark effect would not be observable. Note that this does not exclude the possibility of some of the dye being in lysosomes. However, such molecules would not contribute to the Stark effect although they would contribute to the hole burned in zero field. That only dye molecules associated with the membrane contribute to the Stark broadening can also explain why Stark hole burning can distinguish between normal and cancerous cells while other hole burning properties do not. It is well-established that the cellular morphology, i.e., the plasma membrane and cytoskeleton, of cancer cells are distinctly different from normal cells. Thus dye in the membrane of the two cell types must experience distinctly different environments. The ordering of APT within MCF-10F cells, which we have said to be the result of $\Delta\mu_{\text{ind}}$ (nonrandom), would then be attributed to the influence of the cell membrane potential on APT. We must mention that further work is needed to verify that APT is, in fact, associated with the cell membrane of the two cell lines and that Stark hole-burning is able to distinguish between the cell membrane of normal and cancer cells. We are currently investigating the effect of staining time to determine whether differences in Stark effects can be enhanced by increased localization of dye molecules at the cell membrane.

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