An Enumerated Outlook of Intracellular Micropolarity Using Solvatochromic Organic Fluorescent Probes

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ABSTRACT: The spatiotemporal distribution of intracellular physical parameters of a live cell is heterogeneous and complex. Measuring physical properties inside given cellular compartments (organelles) is challenging and important for therapy and diagnostics. The tiny volume of a single cell and even tinier organelles are not accessible by classical measuring devices. The microenvironment inside an organelle vastly controls the outcome of any biochemical and biophysical processes taking place inside it, which is crucial for the overall cellular health. Therefore, it is very important to understand the microenvironmental physical properties inside cellular organelles. Moreover, specific alterations of such microenvironmental properties were observed in the disease condition, making them a diagnostic hallmark. With this high demand, small-molecule organic fluorophores are emerging as the most successful tool due to their small relative size, bioavailability, and ease of functionalization. In this mini-review, the development of micropolarity-sensitive small organic fluorophore with the capability of targeting a specific cellular organelle has been discussed. Here, we have highlighted the strategies of targeting a specific organelle, the micropolarity, and the challenges and prospects of the field.

1. INTRODUCTION

A living cell is a well-organized and immensely synchronized combination of diverse biophysical and biochemical processes. A biological cell is not a homogeneous entity but rather consists of various membrane-enclosed compartments or organelles. For the proper functioning of a live cell, every component has its unique role to play. Organelles such as the nucleus, endoplasmic reticulum (ER), mitochondria, Golgi body, lysosome, lipid droplet, and plasma membrane are highly coordinated, where the essential biochemical and biophysical processes occur. The local physical environment, a.k.a. microenvironment, more specifically polarity, viscosity, temperature, and electrical potential across the membrane, plays a crucial role in determining the outcome of the surrounding biochemical processes. Impairment of these reactions causes malfunctioning of normal cellular performance and could lead to abnormal cellular function and ultimately to a particular disease. Deciphering the microenvironmental properties inside a cellular organelle is essential because it not only provides an in-depth mechanism of the cellular process but also serves as early disease markers.

Measuring the physical parameter inside cellular organelles is impractical with common equipment. A biological cell has a few picoliters of volume with spatial and temporal heterogeneity. Only the molecular size probes or those having a small relative size compared to the spatial heterogeneity, which can specifically localize into a particular organelle, are the typical choice. In recent years, many research groups, including us, have been working in this field with increasing interest, and new information is appearing rapidly. Several reviews have been published in recent years, highlighting the development of chemosensing probes to understand the local concentration of ions, small molecules, reactive species, enzymes, lipids, and other biologically relevant molecules. Currently, the amount of microenvironment-related research is vast, so considering the space limitation of a mini-review, we decided to focus on the recent development of intracellular micropolarity. Among various materials that have been employed in this regard, here we will review recent development of the understanding of the micropolarity of organelles using small and purely organic intramolecular charge transfer (ICT) fluorophore-based molecules that have an electron-rich donor part (D)
and electron-deficient acceptor part (A) connected by a π-conjugated linker (Figure 1). The ICT class of molecules shows solvatofluorochromic properties and the emission maxima shift from shorter to longer wavelength with increasing solvent polarity. Generally, these classes of fluorophores have higher dipole moments in the excited state as compared to their corresponding ground state due to electronic excitation. The high dipole moment of the fluorophore interacts strongly with the environment and results in either a significant shift in the emission maxima (larger Stokes shift) or a change in the emission intensity. The larger Stokes shift of ICT probes in a polar solvent can be understood by the Lippert–Mataga equation as follows

\[ \Delta \nu = \frac{2\Delta f}{h c a} (\mu_e - \mu_g)^2 + b, \Delta f = \left( \frac{e}{2c + 1} - \frac{n^2 - 1}{2n^2 + 1} \right) \]

where \( \Delta \nu = \nu_{abs} - \nu_{em} \) stands for Stokes shift; \( \nu_{abs} \) and \( \nu_{em} \) are absorption and emission frequency (cm\(^{-1}\)); \( h \) is the Planck’s constant; \( c \) is the velocity of light in vacuum; \( a \) is the Onsager radius; and \( b \) is a constant. \( \Delta f \) is the orientation polarizability; \( e \) is the dielectric constant; \( n \) is the refractive index; and \( \mu_e \) and \( \mu_g \) are the dipole moments of the emissive and ground states, respectively. \( (\mu_e - \mu_g)^2 \) is proportional to the slope of the Lippert–Mataga plot. Therefore, the polarity of a cellular organelle can be metered in various physiological conditions if such ICT dyes are specifically localized. To maintain continuous flow, we have unified all results in the same solvent polarity scale \( E_T(30) \), expressed in kcal mol\(^{-1}\). The \( E_T(30) \) solvent polarity scale is well accepted because of its high sensitivity to environmental polarity. The obtained values are empirical, and it is a descriptor of both hydrogen bonding and electrostatic interaction of the solvent. In the end, the challenges and future outlook of this field of research will be discussed.

2. ORGANELLE-SPECIFIC MICROPOLARITY STUDIES

2.1. Plasma Membrane Boundary. In the living system, an amphiphilic film is mainly assembled of lipid molecules, forming a bilayer that typically delimits the surrounding environment. Lipid molecules have a spontaneous propensity to self-aggregate, which makes the outside of the bilayer hydrophilic and inside hydrophobic. Other than the function being a separator, it also acts as a messenger in signal transduction, cellular transport, and various molecular recognition processes. Lipid molecules interact synergistically with other biomolecules such as protein and carbohydrate and form a complex assembly to function properly. The phase behavior of lipid layers depends on its molecular composition and has quite an important role in cellular health. There is increasing evidence that membrane lipids impart a tight regulation of numerous cellular functions. Therefore, membrane lipids have emerged as an alternative molecular target for various diseases such as cancer and several viral infections. The cellular transport and signaling processes are associated with the biophysical properties of cellular membranes.

2.1.2. Membrane Polarity. In the initial reports, the measurement of membrane polarity was performed using a Prodan (6-acyl-2-aminonaphthalene 1, Figure 2) based probe such as Laurdan (2-dimethylamino(6-lauroyl) naphthalene, 2), 7-Nitro-2-oxa-1,3-diazole-4-yl (NBD, 3) based lipid probes with modification of the sn-2 acyl chain of different phospholipids are also popular. From these classes of the probe, the polarity of the unilamellar vesicle was determined to be similar to methanol and ethanol. It is important to mention that any environmental properties of the membrane which are assessed by modifying the lipid molecule by a fluorescent probe can cause membrane perturbation and a change in the local native properties. Prodan-based two-photon active fluorescent probes with varying alky1 chain lengths are capable of displaying the lateral heterogeneity of the membrane in live cells. In 2017, Xu and his team utilized live-cell super-resolution microscopy using Nile Red (S) as a fluorescent marker to understand the compositional

![Figure 1](image1.png)

**Figure 1.** Concept of using intramolecular charge transfer (ICT) solvatofluorochromic dyes for measuring organelle micropolarity. ICT dye is comprised of a donor (D) and acceptor (A) connected with a π-conjugated linker (top left); it can be customized with an organelle-targeting group (TG) for specific intracellular localization. ICT dyes show solvatofluorochromic properties (bottom left, adapted with permission from Pal, K.; Kumar, P.; Koner, A. L. Photochem. Photobiol. B 2020, 206, 111848–111854). Copyright 2020. Elsevier) which can be implemented to understand the micropolarity inside a cellular organelle in live cells using spectral scanning techniques under a fluorescence microscope.

![Figure 2](image2.png)

**Figure 2.** Chemical structures of the probes used for studying polarity of the plasma membrane.
heterogeneity. The plasma membrane polarity is very much dependent on the cholesterol-rich lipid-raft-like nanodomains. So far, the micropolarity obtained for the plasma membrane ranges between 51.9 and 54.8 ($E_T(30) \text{ kcal mol}^{-1}$).

2.2.1. Mitochondria: The Powerhouse of the Cell. The mitochondrion is one of the most ubiquitous organelles in the eukaryotic cell. It is a double-membrane-enclosed semiautonomous organelle and plays an extremely crucial role by supplying energy and hosting many biochemical processes for the maintenance of proper functions of a living cell. It is essential to understand the micropolarity inside mitochondria as it reflects a healthy and impaired condition related to many diseases. Due to strong negative membrane potential, lipophilic and positively charged moieties (e.g., triphenylphosphonium or TPP, quinolinium, etc.) are evident to be localized inside mitochondria. A micropolarity-sensitive fluorophore either attached with a positively charged lipophilic vector or the fluorophore itself is modified in such a way that it is a suitable reporter of micropolarity inside mitochondria.

2.2.2. Mitochondrial Polarity. Mitochondrial polarity is an important characteristic as it governs cellular events and fate because it is a hub of many specific chemical and biochemical reactions. To determine local polarity, ICT probes are the primary choice. A coumarin-hemicyanine-based probe BOB (6, Figure 3) is used to determine the polarity inside mitochondria of healthy and cancerous cells. Later, a polarity-sensitive TPP-conjugated fluorescent probe (MITFPS, 7) with two-photon activity is used. Using FLIM they found heterogeneity in mitochondrial polarity even within the same cell. MCY-BF$_2$ (8), an ICT probe with emission in the NIR range, has been successfully used for the determination of mitochondrial polarity at different embryonic developmental stages of Caenorhabditis elegans. Pal et al. introduced a completely new and robust class of ICT probes “propellerocein” (9) and successfully measured the mitochondrial polarity in healthy and peroxide-induced apoptotic cells. They have found that the polarity inside mitochondria is similar to the mixture of acetonitrile and isopropanol, which becomes more polar during peroxide-induced apoptosis. Polarity-sensitive fluorescent small-molecule HXPI-P (10) and NRTP (11) have been successfully utilized for the polarity determination during mitophagy and photoinduced cell death. The experimental value of micropolarity of mitochondria in healthy conditions is 40–49 ($E_T(30) \text{ kcal mol}^{-1}$).

2.3.1. Nucleus: The Brain of the Cell. The nucleus is a double-membrane-enclosed organelle of eukaryotes, the central hub of the cell. It maintains the genetic integrity of a species and timely regulates gene expression. The main constituents of the nucleus are DNA, RNA, nucleic-acid-binding protein, etc. Micropolarity inside the nucleus is likely to be different from the cytoplasm. Looking at the components of the nucleus and nuclear pore at the entrance of the nucleus, many fluorescent small molecular probes have been developed. Alternatively, attaching a nuclear localizing peptide signal to an environment-sensitive fluorophore is also adopted as a strategy to investigate the microenvironment inside the nucleus.

2.3.2. Polarity Inside Nucleus. Like other cellular organelles, the nucleus hosts many important biochemical

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Figure 3. Chemical structures of the probes used for studying the polarity of mitochondria.
reactions; hence, a quest of knowing the micropolarity inside the nucleus is immense. A small-molecule fluorescent probe with nucleus-targeting ability and microenvironment sensitivity is rare in the literature. Mandal and his co-workers have introduced a class of ultrasmall solvatochromic fluorophores with molecular weight less than 200 Da.15 With these two different derivatives, namely, DEAB and DEAMB, they have found that the micropolarity of the perinuclear membrane is close to tetrahydrofuran or ethyl acetate (37.4−38.1 (Et(30) kcal mol^-1)).

2.4.1. Lysosome: The Heterogeneous Trash Bag.
Lysosomes are membrane-bound organelles found in all eukaryotic cells. The size and shape of lysosomes differ depending on the cell type and related to their function. It is considered to be one of the major digestive compartments of the cell. Lysosomes are enriched with various hydrolytic enzymes and specialized in the breakdown of various intracellular and exogenous proteins, lipids, and other complex biomacromolecules into their constituents’ building blocks. The distinctive property of lysosomes is the internal acidic pH (ranging between 4.0 and 5.5), which mainly takes care of the recycling of all sorts of macromolecules like proteins, polysaccharides, and nucleic acids, etc. Dysfunction of lysosomal hydrolytic enzymes and permeases, which exports the hydrolyzed products to different parts of the cell, leads to a group of disorders commonly known as lysosomal storage disorders (LSDs).16 The lysosomal structure, composition, and internal environment can vary considerably depending on its functional state. Therefore, the determination of the microenvironmental physical properties of lysosomes would be useful to understand its pathology, physiology, and overall health.

2.4.2. Lysosomal Polarity. The lysosomal micropolarity change has become the ultimate parameter for understanding the cellular apoptosis process and ultimately cell death in complex living organisms. Next, we need to develop a selective probe to lysosomes that is also sensitive to the environmental polarity, and the most common design choice is the ICT-type fluorophores. The first report on the qualitative determination of lysosomal polarity came from the Fan group in 2016. They have designed and synthesized a series of BODIPY-based fluorophores connected to a quaternary ammonium moiety.17 In this series, one of the probes (BP-2, 12, Figure 4) displays an “off−on” polarity-sensitive fluorescent property and selective localization to the lysosome. This probe can demonstrate that the local hydrophilicity changes inside the lysosome upon the treatment of 80 mM of sucrose. Subsequently, coumarin-based morpholine containing a solvatochromic probe (Lyso-OC, 13) has been developed for selective lysosome targeting and monitoring the change of its local polarity in live cells.3 According to their report, the micropolarity of lysosomes is quite nonpolar compared to the bulk polarity. Further, a naphthalene-based fluorescence probe (NOH, 14) was reported, which has dual emission peaks in less polar solvents such as dioxane and selectively localizes in lysosomes.7 By analyzing the fluorescence ratio of NOH at two different monitoring channels, the polarity value of the lysosomal microenvironment in MCF-7 cells was measured to be similar to dichloromethane. However, upon inducing the lysosomal storage disorder, the local polarity increases and displays a value similar to methyl isopropyl ketone. Alongside this, a phenalene-based ICT and two-photon active ratiometric fluorescent probe (DC, 15) has been reported, and the lysosomal micropolarity change was evaluated during the apoptosis process.5 Later in 2019, a naphthalimide-based, morpholine-appended probe (MND-Lys, 16) for selective targeting and quantitative determination of lysosomal polarity was reported.3 The micropolarity of the lysosome determined using this probe was similar to the toluene and dioxane
mixture. Recently, our group has designed and synthesized a propellerocein-based solvatochromic ICT dye (LyPol, 17), functionalized with a lysosome-directing morpholine group separated with a five-carbon alkyl spacer. LyPol shows a significant polarity-sensitive shift in the emission maxima and is well tolerant of the pH and viscosity of the medium. The distinct and important feature of LyPol compared to other ICT-based probes is the high fluorescence quantum yield in polar solvents. The polarity of lysosome determined using LyPol is much lower compared to bulk water, and it is similar to dimethyl sulfoxide. Our method indicated that the micropolarity inside the lysosome of cancer cells is higher compared to normal cells. Recently, a curcumin-based environment-sensitive fluorescent probe (KSLP1, 18) was found for the determination of micropolarity in cells and living C. elegans. This probe possesses excited-state ICT properties and emits at near-infrared regions. KSLP1 selectively localizes in the lysosome and has good biocompatibility. After confirming the polarity in the cellular system in healthy and lysosomal disordered conditions, the authors have also demonstrated that with aging in C. elegans the micropolarity of lysosomes increases. Accumulating the value reported so far in the literature, we found that the micropolarity inside the lysosome is in the range of 34–45 (E(30) kcal mol\(^{-1}\)).

2.5.1. Endoplasmic Reticulum and Golgi Apparatus: The Protein Synthesis and Transport Agents. The endoplasmic reticulum, being the largest membrane system of the eukaryotic cell, is an important organelle with a three-dimensional network of membrane tubules and cisternae where secretory and transmembrane proteins enter unfolded and exit as either folded or misfolded proteins, after which they are directed either toward another organelle or to degradation, respectively. Being a central intracellular organelle in the secretory pathway, ER is an important site for protein and lipid synthesis, calcium homeostasis, and detoxification of poisonous substances. The accumulation of large amounts of unfolded or misfolded proteins in the ER activates the ER stress response, which has been implicated in various human diseases such as diabetes and Alzheimer’s.

Golgi apparatus, found in most of the eukaryotic cells, plays a similar yet much more extensive role in terms of accumulation, modification of proteins, and transporting them to the vesicles. It acts as a carrier agent between ER and lysosomes as newly synthesized proteins and enzymes are transported from the ER through the Golgi apparatus to lysosomes. As there is a transport dynamics between the ER and Golgi apparatus, any change in micropolarity in ER would also affect the Golgi apparatus micropolarity. Being one of the main interconnected organelles, Golgi micropolarity remains a key indicator of cellular homeostasis.

2.5.2. ER Polarity. The micropolarity inside the ER is believed to be a fundamental physical parameter to understand cellular health. The unsettled homeostasis due to the accumulation of the misfolded and unfolded proteins in the ER in turn results in the ER stress, leading to several fatal symptoms and diseases like neurodegeneration, cardiovascular aberration, diabetes, and cancer. Accumulation of such proteins is also known to alter the ER micropolarity. Kim and co-workers reported an ER-targeting fluorescent bimodal probe capable of determining ER viscosity and polarity changes using FLIM and ratiometric fluorescence response. The designed probe contained a polarity-sensitive Nile red moiety connected to a BODIPY moiety with a six-
carbon-long alkyl spacer (19, see Figure 5). To measure the micropolarity, the probe was initially incubated with HeLa cells, and the solvent polarity value corresponding to the lifetime of the probe was found to be 41.3. However, upon treatment of HeLa cells with tunicamycin to induce ER stress, the solvent polarity parameter value became 42.2, which indicates a detectable ER polarity using this probe.

Tang and co-workers came up with an ICT-based NIR emitting fluorescent probe ER-P (20) to visualize the polarity in live cells, liver tissues, and diabetic mice using photoacoustic (PA) imaging.3 As ER-P gave rise to unlike PA signals in 700 and 800 nm wavelengths, their ratio, i.e., PA700/PA800, was measured in different polar media. In the lower polarity, the ratio was higher. Also, the probe itself showed weak fluorescence in a polar environment. Considering these observations, stressed HL-7702 cells suggested enhancement of polarity compared to in untreated conditions. They also performed flow cytometry experiments to compare polarity in normal and cancer cells and observed that the ER environment in HepG2 cells is more polar than HL-7702 cells. Finally, PA imaging in the liver tissue of normal mice revealed an $E_T(30)$ value of 41.3. A steep rise in polarity was observed in stage I and stage II diabetic mice with polarity similar to DMF and slightly more polar than DMSO, respectively.

A commercially available ER tracker dye (ER-DPX, 21) was used to measure polarity inside the ER and differentiate normal and cancer cells.1 ER-DPX is a solvatochromic dapoxyl derivative that binds with the thiol groups present in the ER membrane. The spectral scanning with this probe in the ER showed the emission maxima to be 10 nm red-shifted in A549 cells ($\lambda_{em}^{max} = 510$ nm) compared to WI38 cells ($\lambda_{em}^{max} = 500$ nm). As reported, the polarity is very similar to chloroform ($\lambda_{em}^{max} = 506$ nm) with an $E_T(30)$ value of 39.1 kcal mol$^{-1}$. A two-photon active, solvatochromic, ICT-based fluorophore 10b (22) was prepared by Koner and co-workers containing a sulfonamide moiety for ER-targeting purposes.6

Analyzing the spectral scanning results in MDA-MB-231 cells incubated with 10b and the emission profile of the probe in different solvents, it was concluded that ER polarity resembles a mixture of chloroform and dichloromethane ($E_T(30) \sim 40$ kcal mol$^{-1}$).

A recent report from Lin and co-workers discussed the distinguishability of normal and cancer cells based on ER polarity changes using a naphthalimide-based probe NSp (23).19 The probe contained a $p$-toluene sulfonamide moiety, helping it to localize in the ER. To get an idea regarding the polarity, two different sets of normal and cancer cells were treated with NSp in similar conditions. While the probe showed green fluorescence in all the cells, signals from the blue channel could be recorded in both the cancer cells. Interestingly, normal cells did not fluoresce in the blue channel. This observation indicated the difference in ER polarity. A similar intensity-based observation on polarity differences in normal and cancer tissues was made, and a more precise report with quantified values would have been useful in this case. Like other organelles, the micropolarity obtained for the ER is varied in the different reports and ranged from 39 to 55 ($E_T(30) = \text{kcal mol}^{-1}$).

Tang and co-workers developed a Golgi-apparatus-targeted NIR fluorescent probe Golgi-P in order to study the micropolarity in the mice brain in normal and depression-related conditions. The micropolarity range is $E_T(30) \sim 32–52$ kcal mol$^{-1}$.
like behavioral conditions. The probe contains merocyanine and benzoyl difluoroborate moieties as electron-rich and -deficient centers, causing the ICT process. As the colocalization studies confirmed the specific localization of the probe in the Golgi apparatus, the micropolarity of the normal cell SMMC-7721 and cancer cell HL-7702 was measured in terms of mean fluorescence signal intensity of the probe. Eventually, polarity in cancer cells was found to be less than normal cells. Furthermore, studies in mice brains revealed that increased Golgi polarity is an indication of decreased synthesis of the brain-derived neurotrophic factor which mainly regulates the nervous system functions. However, this study does not provide any quantitation of the Golgi micropolarity.

2.6.1. Lipid Droplets: The Storehouse of Lipids. Lipid droplets (LDs) are the universal intracellular storage organelles that store excess energy in the form of neutral lipids and are found in most cells, from yeast to human. The ultrastructure of LDs consists of a neutral core of lipids surrounded by the phospholipid monolayer which also has the presence of integral and peripheral proteins. Lipid droplets play an important role in maintaining cellular lipid metabolism. They are also involved in many cellular processes, such as membrane formation, trafficking, and many protein–protein interactions. Any misregulation in LD functions would lead to many diseases like obesity, diabetics, and atherosclerosis. A direct correlation can be established between LD homeostasis and local polarity. However, only the latter half of the past decade has seen exclusive efforts made to understand these properties and their effects.

2.6.2. LD Polarity. The microenvironment of lipid droplets use Coumarin 153 (C153, 24, for structure see Figure 6) as a marker. The emission spectrum of C153-stained LDs in MCF7 cells was recorded in the confocal microscope in a wavelength scanning mode and compared with the spectra recorded in different solvents in a spectrofluorometer. This showed the polarity of LD to be similar to n-butyl acetate ($E_T(30) = 38.5$ kcal mol$^{-1}$). Further experiments were performed to compare the micropolarity in noncancerous cell lines. In MCF10A cells, the polarity inside LDs remained in between n-butyl acetate and ethyl acetate, and the reported dielectric constant was 5.5 (equivalent to $E_T(30) \sim 38.3$ kcal mol$^{-1}$). These studies reveal that LD polarity is lower in cancer cells than that of noncancerous cells. Significantly, LDs in both cell lines were found to be quite nonpolar as compared to cytoplasm. The polarity of cytoplasm in MCF7 cells was similar to a mixture of 83% ethanol and 17% methanol, whereas in MCF10A it was like pure ethanol ($E_T(30) = 51.9$ kcal mol$^{-1}$). Even though significant progress could be made in terms of measuring the micropolarity using C153, water solubility remains a point of concern. Tang and co-workers have synthesized several molecules based on the AIE mechanism for LD-specific live-cell imaging. Among those, a two-photon active AIE-based probe was used to study the LD polarity in live HeLa cells. The probe, namely, TPA-BI (25), showed a large two-photon cross-section, Stokes shift up to 202 nm, and fast localization in LDs driven by its hydrophobicity. The emission maximum of TPA-BI inside LDs was at 495 nm, which is similar to its emissive nature in diethyl ether ($E_T(30) = 34.5$ kcal mol$^{-1}$). Upon comparison, the solvent polarity parameter of LDs in HeLa cells is equivalent to $E_T(30) = 34.5$ kcal mol$^{-1}$, which reflects the lower polarity than the cytoplasm. Useful contributions in this field have been made by Lin and co-workers, as they have developed a couple of donor-π-acceptor probes for sensing polarity.
CTPA (26) was based on coumarin, while in CBMC (27) a carbazole dye was connected to an acceptor group, developing ICT property. CTPA showed excellent solvatochromism due to its large conjugation and red-shifted emission, making it useful for fluorescence imaging. The polarity measurements in LDs were performed in U87 cancer cell lines and NHA normal cell lines. A similar observation came up, as the LD polarity in cancer cells was lower than normal cells verified by intracellular spectral scanning of CTPA. The emission maximum in U87 cells was 503 nm, 32 nm blue-shifted compared to NHA cells. The corresponding empirical parameter of solvent polarity \( E(30) \) is 32.35 in cancer cells U87 and 35.54 in NHA cells. Along with this, studies at the single-molecule level showed that a higher number of LDs are present in cancer cells. With CBMC, again, the discrimination between normal and cancer cells was demonstrated based on LD polarity using both one-photon and two-photon microscopy. In this study, the reported \( E(30) \) values were 32.21 and 35.32 in U87 and NHA cell lines, respectively. Klymenko and co-workers have developed DAF (28), a donor–π–acceptor system-based fluorophore, which was designed to be solvatochromic, fluorogenic, and eventually a potential molecular tool for sensing polarity in LDs. The emission maxima of DAF in LDs were at 440 nm, and hence the polarity is similar to toluene (\( E(30) = 33.9 \) kcal mol\(^{-1}\)). Interestingly, the difference in polarity between the surface and core of the LDs was investigated using ratiometric fluorescence imaging (Figure 7). The lipid core showed lower polarity compared to the surface, which is exposed to the cytoplasm, suggesting the heterogeneity in the lipid composition in the lipid droplets. This study was followed by the development of a dioxaborine-based push–pull NIR molecule DXB-NIR (29) for mapping the micropolarity changes of LDs upon induced stress. HeLa cells were treated with methyl-β-cyclodextrin to extract the cholesterol and thereby induce cellular stress. This increased LD polarity is indicative of the enhanced emission intensity of the probe in the NIR channel. Another recent report came from Chen and co-workers where they have developed a series of LDs targeting push–pull fluorophores to study the polarity heterogeneity. Among the four probes of LDP1–LDP4, only LDP4 (30) was eventually taken for differentiating the polarity between the cytoplasm and LDs using ratiometric fluorescence imaging in live U87 cells. The signal from LDs was in the green channel, while that from the cytoplasm is in red. The \( I_{red}/I_{green} \) values for LDs and cytoplasm were 0.57 and 2.6, respectively. This indicates the lower polarity of LDs compared with the cytoplasm.

3. CHALLENGES AND FUTURE OUTLOOK

In this mini-review, we have discussed the micropolarity, one of the most important microphysical properties, inside the cellular organelles in homeostatic and nonhomeostatic conditions; analyzed how cellular health is directly correlated with these physical properties; differentiated physical parameters between normal and unhealthy cells; and study the potential of these microphysical parameters to be considered as early disease markers. Each of the studies covered here was exclusively performed using small organic fluorescent molecules as the reporter probe. As the discussion progresses, it becomes evident that small fluorescent probes having organelle-specific targeting moieties hold several advantages over other commonly used molecular tools, e.g., polymers, nanoparticles, and quantum dots, in terms of better membrane permeability, functionalizability, less cytotoxicity, etc. The main strength of these probes lies in their environment-sensitive optical response and simple yet specific targeting groups. Going forward, it is understood that a significant number of studies have been performed starting from the development of new probes to quantify the physical parameters inside different cellular compartments. Nevertheless, the heterogeneity in the measured numbers or quantification of any physical parameter is due to intrinsic heterogeneity present in the cellular system, which is still an issue and requires more sophisticated multifaceted measurements and analysis to decrypt the exact interacting partner. Certain examples show that membrane permeability has often resulted in such heterogeneous outcomes. Only trends in certain conditions of some physical parameters have been reported, while absolute values are yet to be measured. This mini-review comes across a few instances where targeting probes are bimodal or multimodal; i.e., they can internalize in two or more organelles at the same time and can report more than one physical parameter. While such probes could be useful for studying the dynamics between cellular organelles, they are less preferable for microenvironmental studies in a particular organelle to provide more accurate results. Moreover, other than nonspecific localization, the optical responses coming from the reported dyes are also dependent on multiple microenvironmental properties and make the interpretation difficult. Finally, the past decade has seen significant efforts made toward the understanding of the microenvironment demonstrated in live cells, organoids, and animals using small organic fluorophores. A direct correlation between a measured parameter and cellular health could be established in some cases to comment on the health or stage of that particular cell or organs. However, the development of potential probes for quick and efficient microenvironmental studies can take this field of research a big leap forward eventually to be able to use these microphysical properties as early disease markers. The current challenges of nonspecific localization, cytotoxicity, etc. can be accomplished if a suitable polarity-sensitive probe can be introduced by genetic modification inside a particular organelle.

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Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c04920

https://dx.doi.org/10.1021/acsomega.0c04920

ACS Omega 2021, 6, 28–37
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Notes
The authors declare no competing financial interest.

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ACKNOWLEDGMENTS

We wish to acknowledge the financial support from the host institute IISER Bhopal. T.D. thanks IISER Bhopal for his doctoral fellowship.

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