ABSTRACT: G protein-coupled receptors (GPCRs) are signaling hubs in cell membranes that regulate a wide range of physiological processes and are popular drug targets. Serotonin_{1A} receptors are important members of the GPCR family and are implicated in neuropsychiatric disorders. Cholesterol is a key constituent of higher eukaryotic membranes and is believed to contribute to the segregated distribution of membrane constituents into domains. To explore the role of cholesterol in lateral dynamics of GPCRs, we utilized single particle tracking (SPT) to monitor diffusion of serotonin_{1A} receptors under acute and chronic cholesterol-depleted conditions. Our results show that the short-term diffusion coefficient of the receptor decreases upon cholesterol depletion, irrespective of the method of cholesterol depletion. Analysis of SPT trajectories revealed that relative populations of receptors undergoing various modes of diffusion change upon cholesterol depletion. Notably, in cholesterol-depleted cells, we observed an increase in the confined population of the receptor accompanied by a reduction in diffusion coefficient for chronic cholesterol depletion. These results are supported by our recent work and present observations that show polymerization of G-actin in response to chronic cholesterol depletion. Taken together, our results bring out the interdependence of cholesterol and actin cytoskeleton in regulating diffusion of GPCRs in membranes.

INTRODUCTION

Plasma membranes are complex, quasi-two-dimensional, noncovalent, organized assemblies of various lipids and proteins that allow confinement of cellular contents and offer an appropriate environment to maintain normal functioning of membrane proteins. Besides acting as an interface between the extracellular and intracellular space, biomembranes facilitate communication between cellular exterior and interior via signal transduction. Our current understanding of biological membranes has progressed significantly starting from the “fluid mosaic model” proposed by Singer and Nicolson to a dynamic and complex macromolecular heterogeneous assembly of lipids and proteins often distributed nonrandomly in the membrane. In addition, cell membranes are often crowded, and the organization of membrane constituents in this nonhomogeneous milieu involves the concept of lateral heterogeneities, collectively called “membrane domains.” These specialized domains are thought to be enriched in specific membrane proteins and lipids (such as cholesterol and sphingolipids) which are further held together by the actin cytoskeleton underlying the membrane. These specialized areas on the membrane act as portals for processes such as vesicle trafficking, protein sorting, signal transduction, and entry of pathogens, which span a wide range of spatiotemporal scale.

Because membrane proteins are embedded in lipids, knowledge of the role of membrane lipids in regulating the dynamics of membrane proteins would help in a better understanding of membrane function.

Cholesterol is an essential lipid in higher eukaryotic cellular membranes and is crucial in the maintenance of membrane structure, dynamics, and function. Cholesterol is often nonrandomly distributed in specialized domains in biological and model membranes. Some of these domains (sometimes referred as “lipid rafts”) have been proposed to play an important role in the organization and function of plasma membranes. The concept of such specialized domains on the membrane assumes relevance in the physiology of cells because crucial membrane functions such as signal transduction processes, sorting and trafficking, and the entry of pathogens have been associated with these regions.

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Cholesterol-Dependent Dynamics of the Serotonin_{1A} Receptor Utilizing Single Particle Tracking: Analysis of Diffusion Modes

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SPT has emerged as a powerful approach to monitor lateral diffusion and organization of membrane lipids and proteins.24,25 Although single particle tracking has been utilized to measure dynamics of membrane proteins,26,27 the application of SPT to GPCRs is relatively limited.38,39,40 In this work, we explored the diffusion characteristics of the human serotonin1A receptor in live cells under acute and chronic cholesterol depleted conditions utilizing SPT. Analysis of GPCR diffusion in the membrane helps understand GPCR function and provides insight into cellular signaling.32 By analysis of diffusion modes, we show how acute and chronic cholesterol depletion could influence diffusion behavior of the serotonin1A receptor, which could have crucial implications in GPCR function.

**METHODS**

**Materials.** BSA, CaCl2, methyl-β-cyclodextrin (MβCD), DMSO, doxycycline, d-glucose, EDTA, gentamycin sulfate, MgCl2, MnCl2, NaHCO3, penicillin, poly(1-lysine), streptomycin, Triton X-100, and Tris were purchased from Sigma (St. Louis, MO). Qdot 655 streptavidin conjugate, Alexa Fluor 546 phallolidin, and Amplex Red cholesterol assay kit were obtained from Molecular Probes/Invitrogen (Eugene, OR). Antimyc tag antibody C-terminal (Biotin) was obtained from Abcam (Cambridge, UK). Antimyc antibody Alexa Fluor 488 conjugate was purchased from Millipore (Bedford, MA). Lovastatin was purchased from Calbiochem (San Diego, CA). DMEM/F-12 (Dulbecco’s modified Eagle medium) nutrient mixture F-12 (Ham) (1:1)), hygromycin, and fetal calf serum were obtained from Invitrogen/Life Technologies (Grand Island, NY). Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

**Cell Culture and Treatment.** HEK-293 cells stably expressing the N-terminal myc-tagged serotonin1A receptor (HEK-S-HT1AR) were generated as described previously.23 The stock solution of lovastatin was prepared as described previously.23 Cells grown for 24 h were incubated with 2.5 μM lovastatin in complete DMEM/F-12 medium for 48 h in a humidified atmosphere at 37 °C with 5% CO2. Control cells were grown without lovastatin under similar conditions. Acute cholesterol depletion was performed using MβCD as described previously.74 Briefly, after growing cells for 3 days in complete DMEM/F-12 medium, cells were incubated in serum-free DMEM/F-12 medium for 3 h in a humidified atmosphere at 37 °C with 5% CO2. Cholesterol depletion was performed by treating cells with 5 mM MβCD in serum-free DMEM/F-12 medium for 30 min in a humidified atmosphere at 37 °C with 5% CO2, followed by washing with PBS. See the Supporting Information (Section S1) for more details.

**Receptor Localization Using Confocal Microscopic Imaging.** HEK-S-HT1AR cells were seeded at a density of ~10^4 cells on 22 × 22 mm glass coverslips and grown for 3 days as described earlier.38 Serotonin1A receptors were labeled with the antimyc antibody Alexa Fluor 488 conjugate and imaged by an inverted Zeiss LSM 880 confocal microscope (Jena, Germany) with a 488 nm argon laser, and emission was collected from 500 to 560 nm. See the Supporting Information (Section S2) for more details.

**Estimation of Cellular Cholesterol Content.** An Amplex Red cholesterol assay kit was used to estimate cholesterol content from cell lysates obtained from control, 2.5 μM lovastatin-treated, and 5 mM MβCD-treated cells.76 Choles-
terol content values were normalized to total cellular protein estimated by using the BCA assay.\textsuperscript{77}

**Single Particle Tracking Experiments.** Serotonin\textsubscript{1A} receptors in HEK-5-HT\textsubscript{1A}R cells were labeled with antibody conjugated quantum dots (QDs) for SPT measurements. A Cascade II S12 EM-CCD camera (Roper Scientific, Tucson, AZ) operating at 25 Hz acquisition frequency on a Zeiss Axioobserver A1 microscope (Jena, Germany) at room temperature (∼22 °C) was used to track the trajectories of QDs at the cell surface. The Multiple Target Tracing program developed by Serge et al.\textsuperscript{56} was used to analyze the trajectories of all QDs in a video sequence. The diffusion coefficient inside the confined trajectory segments and the size of the domains (or radius of confinement (R)) were determined by fitting MSD(t) with its theoretical expression for confined diffusion.\textsuperscript{79} See the Supporting Information (Section S3) for more details.

**F-Actin Labeling of Cells.** F-actin labeling in HEK-5-HT\textsubscript{1A}R cells under control (without treatment), 2.5 μM lovastatin-treated, and 5 mM M\textsubscript{CD}-treated conditions was performed by using Alexa Fluor 546 phalloidin as described previously.\textsuperscript{32,80,81} See the Supporting Information (Section S4) for more details.

Quantitation of F-Actin. F-actin was imaged by using an inverted Zeiss LSM 510 Meta confocal microscope (Jena, Germany). F-actin quantitation in HEK-5-HT\textsubscript{1A}R cells under control (without treatment), 2.5 μM lovastatin-treated, and 5 mM M\textsubscript{CD}-treated conditions was performed as described previously.\textsuperscript{32,80,81} See the Supporting Information (Section S5) for more details.

### RESULTS AND DISCUSSION

In this work, we utilized HEK-293 cells stably expressing the N-terminal myc-tagged serotonin\textsubscript{1A} receptor (HEK-5-HT\textsubscript{1A}R, see Figure 1a) to explore the role of cholesterol on receptor dynamics (lateral diffusion). The serotonin\textsubscript{1A} receptor expressed in HEK-5-HT\textsubscript{1A}R cells is predominantly localized on the plasma membrane as shown in a representative confocal microscopic image at a midplane section of a cell (see Figure 1b). Notably, we previously demonstrated that serotonin\textsubscript{1A} receptors heterologously expressed in HEK-5-HT\textsubscript{1A}R cells could mimic the pharmacological and cellular function of the native receptors in terms of ligand binding, coupling to G proteins, cAMP signaling, and cellular trafficking.\textsuperscript{73,82} In addition, the N-terminal myc-tag allows tracking the trajectory of the serotonin\textsubscript{1A} receptor using SPT by fluorescently labeling the receptor in HEK-5-HT\textsubscript{1A}R cells with quantum dots (QD) conjugated to streptavidin which are precoupled to a biotinylated anti-c-myc antibody.

Signal transduction mediated by membrane-bound receptors has been proposed to involve differential lateral mobility of various interacting partners in the membrane.\textsuperscript{83–85} This constitutes the basis of the “mobile receptor” hypothesis which proposes that lateral diffusion determines the interaction between receptor and effector molecules and subsequent signaling at the plasma membrane.\textsuperscript{82} Because signaling via GPCRs involves the functional interaction of effector molecules in a heterogeneous membrane environment, the spatiotemporal dynamics of these components in membranes acts as a determining factor of the signaling output by these receptors.\textsuperscript{83,85} To explore the role of membrane cholesterol in regulating the diffusion of the serotonin\textsubscript{1A} receptor, we depleted cholesterol from HEK-5-HT\textsubscript{1A}R cells using both chronic and acute cholesterol depletion methods. The main difference between chronic and acute cholesterol depletion methods is the experimental time scale associated with the two processes.\textsuperscript{32,86} Acute cholesterol depletion involves physical removal of cholesterol using carriers such as M\textsubscript{CD}, a water-soluble carbohydrate polymer with a central nonpolar cavity, that efficiently and selectively extracts membrane cholesterol in a relatively short time (approximately minutes).\textsuperscript{87,88} On the other hand, chronic cholesterol depletion is performed over a longer time period (approximately days) by using cholesterol biosynthetic inhibitors that mimic physiological conditions.\textsuperscript{89} Cholesterol lowering agents such as statins are extensively used for chronic cholesterol depletion and represent some of the best-selling oral cholesterol-lowering drugs.\textsuperscript{76,27} Statins competitively inhibit the rate-limiting enzyme HMG-CoA reductase in the cellular cholesterol biosynthetic pathway.\textsuperscript{25} Figure 2 shows the cholesterol content in cell lysates from cholesterol-depleted HEK-5-HT\textsubscript{1A}R cells. The cellular cholesterol content exhibited reduction upon treatment with lovastatin and M\textsubscript{CD}. For example, cholesterol content was reduced to ~40% of control (without treatment) in lysates from HEK-5-HT\textsubscript{1A}R cells treated with 5 mM M\textsubscript{CD}. HEK-5-HT\textsubscript{1A}R cells treated with 2.5 μM lovastatin show ~7% reduction in cholesterol content relative to control cell lysates.

To ensure the reliability of the single-particle tracking measurements, we optimized the experimental conditions before acquiring the systematic video sequences. We first tested the specificity of QDs for labeling the N-terminally myc-tagged serotonin\textsubscript{1A} receptor. We observed fluorescence signal from ~20 QDs per cell when HEK-5-HT\textsubscript{1A}R cells were
incubated with streptavidin-conjugated QDs precoupled to biotinylated anti-c-myc antibody. These QDs remained bound even after washing the cells several times. In contrast, we found only 2–3 QDs on each cell in the case of labeling with QDs alone (without prior coupling to biotinylated anti-c-myc antibody). We recorded a series of 80 s video sequences for each cell with varied antibody/QD ratios to calculate the short-term diffusion coefficient (\(D_{1-2}\), where “1–2” refers to the first two points of the MSD). In accordance with previous literature,\(^\text{50}\) we chose the antibody/QD ratio of 1:10 for our SPT experiments to minimally perturb receptor diffusion (more detailed examination of labeling artifacts requires further investigation). We could obtain analyzable tracks from those QDs that were present on top of the cells. Data acquisition was performed in control (without treatment), 2.5 \(\mu M\) lovastatin-treated, and 5 mM MJ/CD-treated HEK-5-HT\(_{1A}\)R cells using these conditions. As a first step, we performed a global analysis of all trajectories collected for each condition. Figure 3 shows the distributions of the short-term diffusion coefficient \(D_{1-2}\) for control and cholesterol-depleted conditions. As shown in the figure, we observed a shift in the distribution of \(D_{1-2}\) toward lower values for lovastatin- and MJ/CD-treated cells relative to the control condition. The average \(D_{1-2}\) value for control cells was (4.4 ± 0.3) \(\times 10^{-2}\) \(\mu m^{2}\) s\(^{-1}\) (data represent means ± SE). The short-term diffusion coefficient of the serotonin\(_{1A}\) receptor obtained by using the SPT method was found to be similar to the diffusion coefficient obtained for other class A GPCRs such as the neurokinin-1 receptor\(^\text{72}\) and the \(\mu\)-opioid receptor.\(^\text{72}\) The values of average \(D_{1-2}\) for cells treated with lovastatin and MJ/CD were found to be (3.0 ± 0.3) \(\times 10^{-2}\) and (2.5 ± 0.2) \(\times 10^{-2}\) \(\mu m^{2}\) s\(^{-1}\), respectively. Such a reduction in diffusion coefficient of membrane proteins upon cholesterol depletion has been reported earlier\(^\text{2,7,9-13}\) and could be due to the further compartmentalization of the plasma membrane driven by enhanced actin polymerization under these conditions.\(^\text{2,7,9-13}\)

In addition, the reduction observed in diffusion coefficient upon acute cholesterol depletion could be due to the inherent regional microheterogeneity in the plasma membrane.\(^\text{9,24}\)

SPT provides specific information about lateral dynamics of single particles in a well-resolved spatiotemporal scale which is crucial for the study of complex biological membranes. A characteristic feature of SPT measurements is that it allows diffusion analysis of individual trajectories and therefore the distribution of these trajectories among different diffusion modes could be analyzed. In addition, SPT allows to distinguish subpopulations of molecules undergoing different modes of diffusion, which is very useful to understand the microheterogeneity (domains) in membranes. We identified four diffusion modes by analysis of video trajectories: random, confined, transiently confined, and directed diffusion (Figure 4). Interestingly, upon acute cholesterol depletion (using MJ/CD), the fraction of trajectories characterized by random diffusion mode exhibited reduction. In addition, both chronic and acute cholesterol depletion led to an increase in confined diffusion mode relative to control condition (see Figure 4a). Along with the change in various diffusion modes, we observed significant changes in the diffusion coefficient \(D_{1-2}\) of the serotonin\(_{1A}\) receptor among different diffusion modes. For example, we observed a significant decrease in \(D_{1-2}\) for random diffusion mode upon statin treatment (see Figure 4b). On the other hand, \(D_{1-2}\) for confined population remain invariant, whereas \(D_{1-2}\) for transiently confined mode exhibited a significant reduction upon acute and chronic cholesterol depletion (Figure 4b). These observations are supported by previous reports suggesting that cholesterol depletion results in the rearrangement of the actin cytoskeleton.\(^\text{32,31,32}\) The generation of these compartments (domains) on the cell membrane could be attributed to the polymerization of the actin cytoskeleton which induces dynamic constraints on receptor diffusion and therefore reduces the population of random diffusion along with a concomitant increase in confined (or transiently confined) diffusion modes.

The effect of the increase in dynamic constraints upon chronic cholesterol depletion gets further manifested in the serotonin\(_{1A}\) receptor diffusion, as evident from the overall reduction in the distribution of the radius of confinement (\(R\)) and an overall shift toward lower values of the confinement radius (see Table 1 and Figure 5). The average radius of confinement was ~213 nm for receptors undergoing confined diffusion in control cells (Table 1). The corresponding values for the average radius of confinement upon chronic cholesterol depletion showed a prominent reduction. The average radius of confinement undergoing confined diffusion values for receptors under chronic cholesterol depleted condition was ~176 nm, resulting in ~17% reduction relative to the control condition (Table 1). The average radius of confinement upon

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**Figure 2.** Cholesterol content of lysates from HEK-5-HT\(_{1A}\)R cells. Cholesterol content was estimated in lysates from HEK-5-HT\(_{1A}\)R cells treated with 2.5 \(\mu M\) lovastatin or 5 mM MJ/CD (** and *** correspond to significant \((p < 0.01\) and \(p < 0.001\) difference in cholesterol content in lovastatin- and MJ/CD-treated cells, respectively, relative to control cells). Values are normalized to cholesterol content in control (untreated) cells. Data represent means ± SE of three independent experiments.

**Figure 3.** Distribution of diffusion coefficient of serotonin\(_{1A}\) receptors determined by SPT. Distribution of short-term diffusion coefficient (\(log D_{1-2}\)) calculated from the trajectories of serotonin\(_{1A}\) receptors obtained by SPT in control (blue circles, \(n = 77\)), 2.5 \(\mu M\) lovastatin-treated (red triangles, \(n = 101\)), and 5 mM MJ/CD-treated (inverted green triangles, \(n = 102\)) HEK-5-HT\(_{1A}\)R cells. The time duration for the video recordings was set to 80 s, and other details are as described previously.\(^\text{58}\)
acute cholesterol depletion using MβCD displayed no significant decrease (for the confined population). Overall, these results suggest that dynamic constraints on receptor diffusion upon chronic cholesterol depletion increase, which could lead to the formation of small-sized confined zones induced by the actin cytoskeleton. Interestingly, the transiently confined population showed a significant reduction in radius of

Table 1. Diffusion Parameters of the Serotonin

<table>
<thead>
<tr>
<th>Diffusion Parameters</th>
<th>Diffusion Modes</th>
<th>Control</th>
<th>Lovastatin</th>
<th>MβCD</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>D_{1-2} (10^{-2} \mu m^2/s)</td>
<td>4.6 ± 0.5</td>
<td>3.4 ± 1.1</td>
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<tr>
<td></td>
<td></td>
<td>No. of trajectories</td>
<td>21</td>
<td>6</td>
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<td></td>
<td></td>
<td>R (nm)</td>
<td>213 ± 8</td>
<td>214 ± 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D_{1-2} (10^{-2} \mu m^2/s)</td>
<td>3.2 ± 0.3</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of trajectories</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R (nm)</td>
<td>176 ± 27</td>
<td>203 ± 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D_{1-2} (10^{-2} \mu m^2/s)</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of trajectories</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R (nm)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

"The concentration of lovastatin was 2.5 \mu M. The concentration of MβCD was 5 mM."
confinement upon acute cholesterol depletion (Table 1). This reinforces our previous observations using beam-radius-dependent FRAP that acute cholesterol depletion induces dynamic (transient) confinement of the receptor.\textsuperscript{78} 

To test whether the observed confinement upon cholesterol depletion was due to change in actin polymerization, we performed quantification of F-actin under acute and chronic cholesterol depleted conditions using a quantitative high-resolution confocal microscopy based approach previously developed by us that allows measurement of F-actin content using an image reconstruction method.\textsuperscript{81} To quantitatively estimate the extent of F-actin reorganization, we labeled F-actin with Alexa Fluor 546 conjugated phalloidin (a specific fluorescent probe for F-actin\textsuperscript{81}). Figure 6a–c shows representative confocal micrographs with maximum intensity projections (MIPs) of the actin cytoskeleton under various treatment conditions. The panels in Figure 6d–f represent iso-surface maps (contours obtained by joining voxels of equal fluorescence intensity across all $z$ sections) of cellular F-actin corresponding to the projected images shown in Figure 6a–c. To quantitatively estimate cellular F-actin content, we normalized the fluorescence volume enclosed by the isosurface in each case to the projected area of cells. This ratio (fluorescence volume/area of cells) is a measure of the F-actin content in cells and is shown in Figure 6g. As shown in the figure, HEK-5-HT\textsubscript{1A}R cells treated with lovastatin resulted in $\sim$11% increase in cellular F-actin levels, while M\textsubscript{J/CD} treatment show negligible change in F-actin levels. These results are in overall agreement with our recent observations in CHO-K1 cells where we showed that F-actin content significantly increases upon chronic cholesterol depletion but not in acute cholesterol depletion.\textsuperscript{25} In this overall backdrop, the effect of the crosstalk between cholesterol and actin cytoskeleton by quantitatively measuring changes in F-actin content as a consequence of acute and chronic cholesterol depletion on the dynamics of the serotonin\textsubscript{1A} receptor assumes relevance.

### CONCLUSION

We utilized SPT to measure diffusion behavior of the serotonin\textsubscript{1A} receptor under cholesterol-depleted conditions. The advantage of SPT measurement is its ability to distinguish subpopulations of receptors undergoing various modes of diffusion which yields useful information about complex microheterogeneous environments such as biological membranes. Our results show that the overall short-term diffusion coefficient of the serotonin\textsubscript{1A} receptor exhibits reduction upon both acute and chronic cholesterol depletion (Figure 3). Notably, we observed a significant increase in the confined receptor population under cholesterol-depleted conditions. Such a change in the subpopulations of the serotonin\textsubscript{1A} receptor undergoing various modes of diffusion upon cholesterol depletion could have implications on receptor function. These results provide one of the first systematic studies of diffusion behavior of the serotonin\textsubscript{1A} receptor by using two kinetically different methods of depletion of cholesterol and provides information about the subpopulations of the receptor undergoing various modes of diffusion. Interestingly, our results show that the nature of confinement varies depending on the method of cholesterol depletion. This is due to the fact that whereas chronic cholesterol depletion results in permanent confinement involving remodeling of the actin cytoskeleton, acute cholesterol depletion leads to transient confinement. In addition, this work highlights the role of cholesterol and actin cytoskeleton on the dynamics of the serotonin\textsubscript{1A} receptor which could be helpful in understanding the correlation between dynamics and function of the serotonin\textsubscript{1A} receptor. We conclude that measurements of receptor dynamics at varying spatiotemporal scales could provide novel insight toward developing a conceptual framework for cellular signaling.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.2c03941.

- Section S1: cell culture and treatment; Section S2: confocal microscopic imaging of receptor localization; Section S3: single particle tracking experiments; Section S4: F-actin labeling of cells; Section S5: fluorescence microscopy and F-actin quantitation (PDF)

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Notes

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article:

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Author Contributions

S.S. and P.S. contributed equally to this work.

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REFERENCES

(32) Sarkar, P.; Kumar, G. A.; Shrivastava, S.; Chattopadhyay, A. Chronic cholesterol depletion increases F-actin levels and induces cytoskeletal reorganization via a dual mechanism. J. Lipid Res. 2022, 63, 100206.


