Supplemental Materials

Molecular Biology of the Cell

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Possible effects of quantum dot labeling on membrane diffusion

The photostability and brightness of QDs allow them to be tracked at a single particle level for longer periods than conventional fluorophores, such as fluorescent proteins and organic dyes (Bannai et al., 2006, Alcor et al., 2009). Because QDs are bound to the extracellular part of the membrane molecules (ACHRs or GM1s), the diffusion coefficient of the bound dimmers can be written as $D_{\text{dim}} \simeq k_B T / (\xi_{\text{mem}} + \xi_{\text{QD}})$, where $k_B T$ is the thermal energy of the dimmer, $\xi_{\text{mem}}$ and $\xi_{\text{QD}}$ are, respectively, the frictional coefficients of the constituting membrane molecule and QD. This relation, which can be rewritten as $1/D_{\text{dim}} \simeq 1/D_{\text{mem}} + 1/D_{\text{QD}}$ with $D_{\text{mem}}$ and $D_{\text{QD}}$ being, respectively, the diffusion coefficients of the constituting membrane molecule and QD, suggests that the value of $D_{\text{dim}}$ is determined primarily by the smaller value of $D_{\text{mem}}$ and $D_{\text{QD}}$ (or larger value of $\xi_{\text{mem}}$ and $\xi_{\text{QD}}$). For the QDs used in the experiment, we have the QD radius $a = 11$ nm, viscosity of the culture medium (water) $\eta \simeq 10^{-3} Pa \cdot s$ and hence $D_{\text{QD}} \simeq \frac{k_B T}{6 \pi a \eta} \simeq 20 \mu m^2 / s$. This value of $D_{\text{QD}}$ is more than 300 times larger than the measured $D_{\text{dim}} \simeq 0.065 \mu m^2 / s$, suggesting that $D_{\text{dim}} \simeq D_{\text{mem}}$. The above estimate reveals that the dominant hydrodynamic drag, which determines the value of $D_{\text{dim}}$, comes from the effective membrane viscosity but not from the bound QD in contact with the culture medium. Thus, the relatively large size of QDs compared with AChRs or GM1s has negligibly small effect on the measured membrane diffusion. Similar finding was also reported by Mascalchi et al. (2012).

In fact, the effect of quantum dot labeling on membrane diffusion has been carefully examined in recent experiments (Triller and Choquet, 2008). In one study, Renner et al. (2009a) compared the diffusion coefficient of GM1s, $D_{GM1}$, labeled by a QD with that labeled by a single Cy5 fluorochrome on cultured hippocampal neurons. It was found that the median values of $D_{GM1}$ obtained by using the two complementary strategies are comparable. In another sets of experiments, Groc et al. (2004, 2007) reported their study of surface trafficking of a neurotransmitter receptor (AMPA receptor) on cultured hippocampal neurons. The AMPA receptors were labelled by three different single molecular complexes with a descending order of sizes: QD coupled to anti-GFP antibody, Cy5 fluorochrome coupled to anti-GFP antibody and Cy5 fluorochrome coupled to bungarotoxin. By comparing the diffusion of the three complexes on the non-confined (extra-synaptic) membrane region, the authors concluded that both the cumulative distributions of the instantaneous diffusion coefficient $D_{\text{AMPA}}$ and their median values show no significant difference among the three different labelling strategies.

Another possible effect of quantum dot labeling is to cause cross-link of multiple AChRs by a single QD because of its relatively large size. Such multivalence clustering will cause endocytosis of AChRs so that the resulting AChR clusters will move to the cell interior away
from the viewing plane on the cell membrane and be degraded at a later time (Lindstrom and Einarson, 1979, Lee et al., 2014). As a result, most QD-labeled AChRs on the plasma membrane remain monovalent. In this experiment, the QD labeling is performed at low tag densities to avoid AChR and/or QD clustering and to reduce tracking ambiguities between the consecutive images of the QDs. Typically, we have 110 QDs in a viewing area of 68×68 μm² or 0.024 QD per μm², so that a typical distance between two adjacent QDs is about 6.5 μm, which is approximately 600 times larger than the QD radius. At such low tag density, QD clustering is effectively removed. This is confirmed by the measured blinking rate of the QD trajectories. While blinking is an intrinsic property of individual QDs, QD clusters hardly blink because it is unlikely for all the QDs in a single cluster to blink at the same time. In a typical tracking of GM1 trajectories for 10 cells, we find that more than 96.5% of the trajectories blink at least once and their average dark time occupies 33% of the trajectory duration.

Because the concentration of the biotin-CTX (0.5 nM) applied to the cells is 5 times smaller than that for the streptavidin-conjugated QDs (2.5 nM), the probability for biotin-CTX clustering on a single QD is even smaller than that for AChR clustering. However, it is known that a single biotin-CTX can bind up to 5 GM1 molecules (Merritt et al., 1994). As a result, the GM1 trajectories discussed in this paper are actually trajectories of GM1 clusters; each contains 1-5 GM1 molecules. This level of polydispersity (or multivalence) in the GM1 cluster size, however, is not enough to produce an exponential distribution of δ, as shown in Fig. 7. This is because δ scales with the cluster size b as ln(1/b) (Saffman and Delbrück, 1975), which is essentially a constant for a moderately narrow distribution of cluster sizes. For example, the values of b for the GM1 clusters are in the range of 0.8–2.4 nm (Gerrow and Triller, 2010), and the corresponding percentage change of δ is given by Δδ/δ ≈ ε(Δb/b) ≈ 0.25 for Δb/b ≈ 1 where ε(≈ 0.25) is a numerical constant which depends on the membrane thickness and viscosity. This value of Δδ/δ represents a much narrower change of δ compared to that shown in Fig. 7. Therefore, we conclude that the small polydispersity of the GM1 clusters only affects the absolute value of δ slightly and has little effect on the observed dynamic heterogeneity and non-Gaussian statistics of membrane diffusion. The above argument also explains the small change in diffusion coefficient between GM1 and AChR, as shown in Fig. 5(a). Given the size ratio of GM1 to AChR, b_{GM1}/b_{AChR} ≈ 1.6/7, we expect the corresponding percentage change in diffusion coefficient to be (D_{GM1} − D_{AChR})/D_{GM1} ≈ ε ln(b_{AChR}/b_{GM1}) ≈ 0.37, which is very close to the measured value (D_{GM1} − D_{AChR})/D_{GM1} ≈ 0.37.

As shown in Fig. 5(a), the measured D_L has considerable cell-to-cell variations for both AChRs and GM1s. Similar variations were also observed in previous studies of membrane diffusion (Groc et al., 2004, Groc et al., 2007, Renner et al., 2009a, Gerrow and Triller, 2010, Kusumi et al., 2012, Barrantes, 2014, Fujiwara et al., 2016). The exact value of the diffusion coefficient D also depends on the sampling conditions of the molecule trajectories, such as the total length of the trajectories, the delay time τ sampled and the number of trajectories used in the statistical average. Thus, it is quite difficult to compare the absolute values of D among different experiments. For this reason, the measured PDFs, P(Δx′) and P(Δy′) in Fig. 6 and f(δ′) in Fig. 7, are all plotted as a function of a normalized variable so that their functional forms remain unchanged for different values of D.

Despite the experimental uncertainties in D, one can still observe some general trends of membrane diffusion when the data are analyzed and compared under the same sampling conditions. For example, Renner et al. (Renner et al., 2009b, Gerrow and Triller, 2010) found
that the diffusion coefficient of GM1s for hippocampal neurons \( (D_{GM1} \approx 0.15 \, \mu m^2 s^{-1}) \) is about 1.8 times larger than that of the AMPA receptors. Fujiwara et al. (Fujiwara et al., 2016) showed that the diffusion coefficient of a trans-membrane protein, transferrin receptor (TIR), is about the same as that of an unsaturated phospholipid, 1-\( \alpha \)-dioleylphosphatidylethanolamine (DOPE), in three mammalian cell lines (PtK2, HEPA-OVA, and HeLa). In other two mammalian cell lines [T24 and normal rat kidney (NRK)], the value of \( D_{DOPE} \) was found to be 1.4—2 times larger than \( D_{TIR} \). Freeman et al. (Freeman et al., 2018) showed that the diffusion coefficient of another transmembrane protein, \( Fc\gamma \) receptors, for bone-marrow-derived macrophages (BMDM) \( (D_{Fc\gamma} \approx 0.06 \pm 0.02 \, \mu m^2 s^{-1}) \) is close to (but slightly larger than) that of rigid glycopolymers, which are functionalized to insert into the outer leaflet of the BMDM membrane. These results are consistent with our findings.

As shown in Fig. 4(a), the diffusion coefficient \( D_L \) is defined at the long-time limit and thus is smaller than the short-time diffusion efficient, which is often used in previous studies (Groc et al., 2004, Groc et al., 2007, Renner et al., 2009a, Gerrow and Triller, 2010, Kusumi et al., 2012, Barrantes, 2014, Fujiwara et al., 2016). Because our QD imaging is taken on the bottom side of the membrane facing the substrate, which has a large flat area for better optical observation, it is necessary to check whether the lateral motion of the AChRs and GM1s is affected by the narrow cleft between the membrane and the substrate. Figure 12 shows a comparison of the measured \( D_L \) for AChRs in 10 cells under the two different imaging conditions. Although more immobile AChRs are found on the bottom side of the membrane, no systematic variation of \( D_L \) is observed for the mobile AChRs under the two imaging conditions. The average value of \( D_L \) over the 10 cells is \( \langle D_L \rangle = 0.022 \pm 0.005 \, \mu m^2 s^{-1} \) for the bottom side of the membrane, which is very close to the value of \( \langle D_L \rangle = 0.024 \pm 0.006 \, \mu m^2 s^{-1} \) for the apical side of the membrane. Furthermore, the measured PDFs \( P(\Delta x') \) and \( P(\Delta y') \) under the two imaging conditions are found to have the same exponential form, as shown in Fig. 6. The observation of the similar diffusion behaviors under the two different imaging conditions provides another strong evidence that the lateral mobility is not hindered by factors in the membrane exodomain. This conclusion was also supported by a recent study of the lateral motion of the QD-labeled IgE receptors on the bottom side of the membrane by total internal reflection fluorescence (TIRF) microscopy (Andrews et al., 2008).

Previous studies of the cell-substrate contacts by interference reaction (Bloch and Geiger, 1980) and transmission electron microscopy (Andrews et al., 2008) found that cells attach to the substrate at only a few discrete areas. A large fraction of the ventral surface of the cell remain unattached or only loosely associated to the substrate with a membrane-substrate distance over 100 nm. Where the cell is attached to the substrate, the most common contact is
that which was referred to as "broad, close contact" with a typical membrane-substrate distance of about 30 nm. This distance is 140% larger than the diameter of QDs used in the experiment. Much less frequently observed contacts are the focal contacts with a typical membrane-substrate distance of 10-15 nm. We believe that only at these focal contacts, the motion of QDs is severely hindered so that they become immobile.

The results discussed above thus suggest that the observed dynamic heterogeneity and non-Gaussian statistics for AChRs and GM1s are the intrinsic properties of membrane diffusion over a strongly coupled cortical actin network and are not caused by other effects in the membrane exodomain. Recent TIRF studies of the lateral motion of the QD-labeled Kv1.4 and Kv2.1 ion channels and IgE receptors on the ventral surface of the cells also concluded (Andrews et al., 2008, Sadegh et al., 2017) that the transient impedance in membrane diffusion is originated from the intracellular interaction with the cortical actin. This conclusion is further confirmed by the measurements with direct alteration of the underlying cortical actin network, as discussed in the main text.

Supplementary Movie S1

This video shows the moving trajectory of a ganglioside GM1, which is a glycosphingolipid residing on the outer leaflet of the membrane of a living Xenopus muscle cell dissected after two days. The GM1 is labeled by a fluorescent quantum dot. The movie was obtained using an inverted Leica microscope equipped with an Andor Ixon 897 EMCCD camera. The video is played in real time and the scale bar is 2.5 μm. The GM1 trajectory is digitally magnified by about 3 times for clearer display. In a period of 190 s, one sees the GM1 is transiently confined in three small domains of size 1-2 μm. Such confinement effect causes dynamic heterogeneity of the GM1s on live cell membrane.