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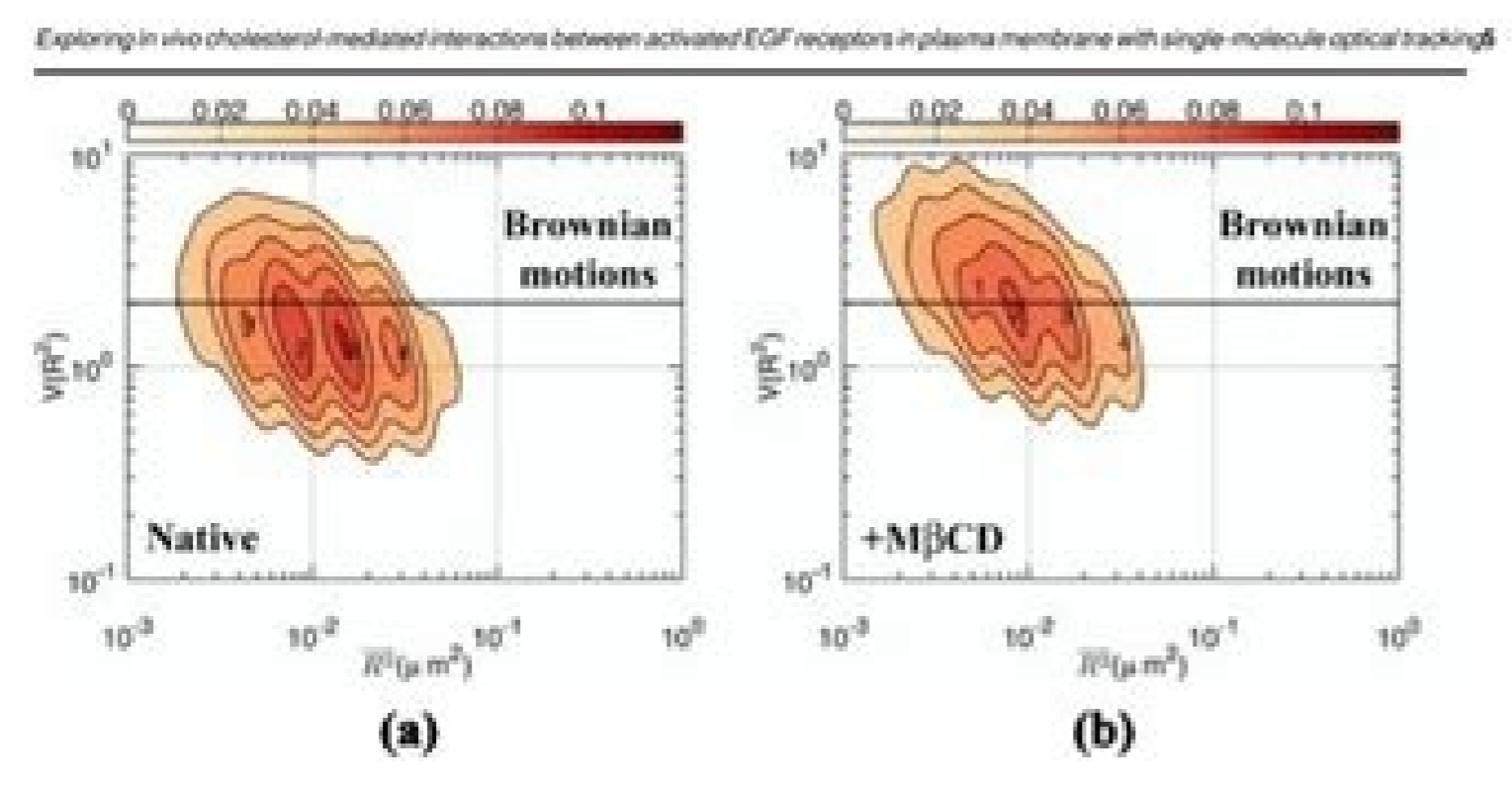


Fig. 3. Three-dimensional contour plot on the  $V(\overline{R^2})-\overline{R^2}$  plane for Qdot568-EGF-EGFR diffusing in the plasma membrane of (a) native HeLa cells and (b) M $\beta$ CD-treated HeLa cells.

embedded in lipid raft domains. When membrane cholesterol is depleted by M $\beta$ CD, CD59s are released from lipid raft domains, allowing them to diffuse freely.

**3.2 EGF binding causes EGFRs to translocate into cholesterol-rich lipid domains.**

Equipped with the result of CD59, we moved on to measure and analyze the trajectories of unliganded EGFRs in the three cell lines. By grouping the MSDs to an appropriate number of bins, a histogram of MSD can be prepared (see Figure 4a). Note that the diffusion coefficient is related to MSD by  $D = \overline{MSD(t)}/t$  with a time period of  $t=25$  ms. We can identify two groups of diffusing species in the histogram Tikhonov et al. (2013). The faster group displays a peak at about  $0.8 \mu m^2$ , attributable to free-confined diffusion. The slower group has a peak near  $0.012 \mu m^2$ , which can be ascribed to confined diffusion. Unliganded EGFRs of the two cancerous cell lines (HeLa and A431) prefer to diffuse in the confined diffusion state. The reverse is apparent in the normal epithelial MCF-12A cells. The corresponding  $V(\overline{R^2})-\overline{R^2}$  plots of unliganded EGFRs in the confined diffusion state are illustrated in Figure 4d for HeLa (Red), A431 (Green), and MCF-12A (Blue), respectively. The regions colored in black indicate the similarity of the confined diffusion state in the three cell lines. Two peaks with  $V(\overline{R^2})$  values of 3 and 4 occur in the three cell lines, indicating a strong interaction of EGFR with actin corral (Gómez-Lobregat et al. (2013)).

When different drug effects on membrane cholesterol are applied with nystatin and M $\beta$ CD, cholesterol-mediated interaction of EGFRs can be investigated. The results are shown in Figure 4b and c. For the nystatin-treated cells, the MSD profile of EGFR is similar to that in native cells. Remarkably in native MCF-12A cells, the population of unliganded EGFR diffusing in the fast state is larger, whereas that in the M $\beta$ CD-treated cells it becomes smaller. After M $\beta$ CD treatment the cell line dependent variations in MSD profiles become smaller (see Figure 4c), revealing that the lipid environments of unliganded EGFRs in the three cell lines are similar after membrane cholesterol is depleted. All these observations clearly indicate that the diffusion of unliganded EGFR is relevant to the amount of membrane cholesterol. Figure 4e and f display the corresponding  $V(\overline{R^2})-\overline{R^2}$  plots of unliganded EGFR in the cell

lines subjected to different drug treatments. Similar  $V(\overline{R^2})-\overline{R^2}$  values as those observed in native cells were observed, which is opposite to that of CD59 presented in Figure 3. The results suggest that unliganded EGFR may locate outside of the cholesterol-rich lipid domains.

Figure 5a depicts the diffusion behavior of liganded EGFR in the three cell lines. Comparing to the result of unliganded EGFR shown in Figure 4a, we found that receptor activation with EGF appears to promote the liganded EGFR (Qdot568-EGF-EGFR) to diffuse in the fast state. Figure 5b and c show the effects of nystatin and M $\beta$ CD on liganded EGFR. Among the three cell lines, the diffusion behavior of liganded EGFRs in A431 cells is least sensitive to changes in membrane cholesterol. The MSD profile of liganded EGFR in native HeLa cells is similar to that in A431, however, it becomes more similar to that in MCF-12A after M $\beta$ CD treatment (c).

Figure 5d presents the corresponding  $V(\overline{R^2})-\overline{R^2}$  plots of native cells. Compared to the result of unliganded EGFR shown in Figure 4d, EGF binding decreases  $V(\overline{R^2})$  to below the free diffusion limit, suggesting that liganded EGFR may encounter with different environments. The  $V(\overline{R^2})$  value of liganded EGFR increases from that in the native cells (Figure 5d) to approximately at the free diffusion limit in the nystatin-treated (Figure 5e) and M $\beta$ CD-treated cells (Figure 5f). By contrast, uniformly depleting membrane cholesterol does not change the  $V(\overline{R^2})$  value of unliganded EGFR. This finding suggests that liganded EGFRs may colocalize with membrane cholesterol, but unliganded EGFRs do not. The  $V(\overline{R^2})-\overline{R^2}$  plots of liganded EGFRs in the three cell lines are similar to that of CD59, further supporting that these two types of proteins are located in similar environments. These experimental results may be effectively explained by the concept that unliganded EGFRs are located outside the cholesterol-rich lipid domains and EGF binding causes the receptors to move into the cholesterol-rich lipid domains. Tikhonov et al. (2013).

**3.3 Cholesterol-mediated interaction between liganded EGF Receptors**

When a receptor protein passes a nearby receptor, it may experience an attractive force that can result in a correlated motion between the two molecules Huang and Lin (2015). To quantitatively display the correlation between two trajectories, we expressed the position vectors as a phase



These continued investments in functionality, and a growing acceptance of cloud-based solutions servicing the commodities markets, has provided Eka with a significant number of new clients for the product in 2016 with 11 announced new deals, spanning a number of commodities and industries, but including agricultural commodities and products, energies, and metals. Additionally, ComTech has observed growing interest in a comprehensive risk overlay as an architectural CTRM component across many parts of the industry. Eka's timing has been very good, introducing a product into an area of growing interest and demand and without significant competition.

**InSight CM**

Eka's 2016 performance was solid especially when taking into consideration the general economic conditions across the commodity sector. It announced a number of new CTRM license sales in the agricultural space in calendar year 2016, including Canadian ag concerns Broadgrain and Providence Grain, CJ International in Asia, Cremer in Germany, and others at a time when demand in ags & softs trailed expectations' under difficult market conditions in the sector. Eka does appear to continue to compete well across the agricultural space (and particularly in the grains area), with company management noting that "four out of the top five agricultural traders are Eka customers, including Bunge, Cargill, Glencore, and Louis Dreyfus." Additionally, its strong market presence in the agricultural space has allowed it to leverage a large client base in which to cross sell the Commodity Analytics Cloud solution.

Other notable new license sales in 2016 included Progress Energy, a Canadian based energy producer. Following the acquisition of EnCompass in April 2013, Eka has made obvious investments in the product to expand its commodity scope and market reach. According to the company, Eka's InSight CM® platform for energy has seen growing momentum, with additional sales in the US and Canada. Although Eka notes they have added European functionality since the acquisition, the energy solution has not yet been licensed outside of North America.

**Corporate Developments**

Despite a difficult year for technology sales in the commodity space, Eka's continues to exhibit growth opening three new offices to accommodate its rapidly expanding business, adding locations in Canada, the United States, and Europe. Headcount grew over 19 percent globally and will continue to grow with another office opening in the Americas in the first quarter.

Importantly, staff additions included four new executive level positions, with Colin Cooper named Eka's vice president of EMEA to manage Eka's expansion in the EMEA; Sudhir Anandarao was appointed chief operating officer, analytics to oversee development of Eka's analytics solutions; Sanjay Singla became Eka's general manager, CTRM to manage sales and support of Eka's commodity trading and risk management solution; and long term industry veteran, Sebastian Esposito joined the company in early 2017 to oversee sales for their products in North America. The company continues to strengthen its management team bringing in experienced and knowledgeable staff to support its plans for growth.

**Outlook and Analysis**

While the global markets for CTRM and CM products have been difficult for more than 2 years, with persistent low commodity prices negatively impacting a broad spectrum of market participants, Eka's results in 2016 suggest that the company has momentum across the space. During a period of slower growth generally, Eka's results are quite impressive and contrast sharply with those of competitors such as Triple Point, for example. With its InSight CM platform selling well and the rapid market adoption of the Commodity Analytics Cloud product, Eka does appear to be closing new business at a rate that places them in the top tier of the market for CTRM/CM vendors. Eka's ability to innovate, as evidenced with its adoption of cloud technologies and models for deploying enterprise-level analytics suites (including "big data" capabilities) and CM solutions, has positioned Eka strongly for continued success.

With the cloud in mind, ComTech continues to see growing acceptance of cloud delivered product and services across all market regions and segments. While the US markets have traditionally lagged Europe in terms of cloud adoption in CTRM and CM, recent ComTech research indicates this is changing rapidly and we expect the growth in demand for cloud solutions to significantly outstrip that of on-premises installations. Eka is one of the vendors in the space that has truly cloud-enabled products and it will benefit from this trend accordingly. There may well be the beginnings of a major technology shift occurring related to cloud deployment in which, should it occur, Eka will be strongly positioned. In fact, Eka recently announced that "75% of its new customers are choosing the cloud based solution."



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