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  $V(\overline{R_r}^2)$  values of 3 and 4 occur in the three cell lines, indicating a strong interaction of EGFR with actin corrals [21].

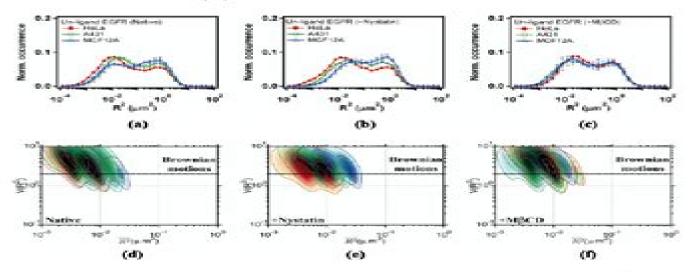
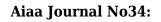


Fig. 4 a, b, c Histograms of mean-square displacements (MSD) and (d, e, f) $V(\overline{R}r^2)$ - $\overline{R}r^2$ plots of unliganded EGFR (Qdot585-Ab-EGFR) diffusing in the plasma membrane of (a, d) native cells, (b, e) nystatin-pretreated cells, and (c, f) M  $\beta$ CD-pretreated cells. Trajectories were sampled with a frame period of  $\tau$ =25 ms. Data are shown in red for HeLa cells, green for A431 cells, and bine for MCF12A cells

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-pretreated 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 choles-

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#### **Table of Contents Aiaa Journal No34**

- 1. Understanding the eBook Aiaa Journal No34
  - The Rise of Digital Reading Aiaa Journal No34
  - Advantages of eBooks Over Traditional Books
- 2. Identifying Aiaa Journal No34
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
- 3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Aiaa Journal No34
  - User-Friendly Interface
- 4. Exploring eBook Recommendations from Aiaa Journal No34
  - Personalized Recommendations
  - Aiaa Journal No34 User Reviews and Ratings
  - Aiaa Journal No34 and Bestseller Lists
- 5. Accessing Aiaa Journal No34 Free and Paid eBooks
  - o Aiaa Journal No34 Public Domain eBooks
  - Aiaa Journal No34 eBook Subscription Services
  - Aiaa Journal No34 Budget-Friendly Options
- 6. Navigating Aiaa Journal No34 eBook Formats

- o ePub, PDF, MOBI, and More
- Aiaa Journal No34 Compatibility with Devices
- Aiaa Journal No34 Enhanced eBook Features
- 7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Aiaa Journal No34
  - Highlighting and Note-Taking Aiaa Journal No34
  - Interactive Elements Aiaa Journal No34
- 8. Staying Engaged with Aiaa Journal No34
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Aiaa Journal No34
- 9. Balancing eBooks and Physical Books Aiaa Journal No34
  - Benefits of a Digital Library
  - o Creating a Diverse Reading Collection Aiaa Journal No34
- 10. Overcoming Reading Challenges
  - $\circ\,$  Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
- 11. Cultivating a Reading Routine Aiaa Journal No34
  - Setting Reading Goals Aiaa Journal No34
  - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Aiaa Journal No34
  - Fact-Checking eBook Content of Aiaa Journal No34
  - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
  - Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
- 14. Embracing eBook Trends
  - Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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