Design of Quantum Dot-Conjugated Lipids for Long-Term, High-Speed Tracking Experiments on Cell Surfaces

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Supplemental Experimental Methods

Confocal Fluorescence Correlation Spectroscopy. To evaluate the colloidal stability of QDs with hydrophilic surface coatings in aqueous solution, fluorescence correlation spectroscopy (FCS) experiments were conducted using a Confocor 2 two-channel fluorescence correlation spectrometer (Zeiss, Germany). In particular, QDs were dispersed in 0.1M PBS buffer and analyzed after $t = 10\,\text{min}$ and $t = 1 week$ using FCS. The Ar ion laser of the FCS system was employed to excite the QD samples with a wavelength of 488nm. The laser light was focused to a diffraction-limited spot by a water immersion objective (C-Apochromat, 40x, 1.2Corr) and, after passing through a long pass filter (LP530 nm), the fluorescence signal was detected using one of the Confocor's two avalanche photodiodes. All FCS studies were conducted using QDs of 570nm emission, which have a size distribution of ~10%, as previously verified by TEM.[S1] Because the confocal volume is well approximated by a 3D Gaussian volume, the fluorescence fluctuations can be described by an autocorrelation function G(τ) of the form

$$G(\tau) = 1 + \frac{1}{N}\left(1 + \frac{\tau}{\tau_D}\right)^{-1}\left(1 + S^2 \bullet \frac{\tau}{\tau_D}\right)^{-1/2} \qquad (1)$$

where N is the average number of fluorophores in the confocal volume, τ is the time, τ_D is the average diffusion time molecules as they pass through the confocal volume, and S is a structure parameter for the confocal volume describing its width/height ratio. The parameter τ_D can be determined from the inflection point of the autocorrelation curve. First, τ_D of the organic dye Rho6G, whose diffusion coefficient D in ethanol is known, was measured to determine the radius of the confocal width ω. Next, the various QD samples were investigated and analyzed using *Eq.* 1 to obtain τ_D. Using τ_D and the previously determined ω, the Stokes-Einstein relationship

$$r = \frac{2\tau_D kT}{3\pi\eta\omega^2} \qquad (2)$$

can be applied to determine the hydrodynamic radii of QDs with hydrophilic surface coatings. In *Eq.* 2, r is the hydrodynamic radius, k is the Boltzmann constant, T is temperature, and η is the viscosity of the solution.

Wide Field Single Molecule Fluorescence Microscopy. Single molecule imaging was employed to study nano-scale organization and dynamics of the model systems. Tracking experiments on solid-supported bilayers were conducted as described recently.[S2] In short, a 100 mW frequency doubled Nd:YAG laser (532nm) was used as the excitation source, whose intensity was regulated by a continuously variable neutral density filter. The excitation beam was delivered to the EPI port of an inverted microscope (Zeiss Axiovert S100TV) equipped with the dichroic mirror DM (Omega XF1051) a Raman edge filter (Omega XR3002 540AELP) and focused by a high aperture objective (Zeiss, oil immersion, 100x NA=1.3). The fluorescent light was detected by an intensified CCD camera (iPentaMAX 512EFT, Princeton Instruments). This filter set is optimized for the excitation and passage of fluorescent light from individual TRITC fluorophores centered at 566nm. A Uniblitz shutter (VMM-D1) of 3 mm open aperture was used to control the exposure time and lag time between images, and was synchronized with the electronic shutter within the CCD camera. The exposure time and the time lag between exposures were set to 10 and 38ms, respectively. Shutter control, image acquisition and image analysis was performed with ISee Imaging software (ISee Imaging) installed on a Dell PC running the Fedora core 4 Linux platform. To maximize tracking accuracy, the optical setup was mounted to a vibration isolation table, resulting in nanometer tracking resolution as verified by tracking immobile fluorescent molecules stuck to a glass slide.

Oblique Angle Fluorescence Microscopy. For continuous tracking of QD-lipids in the plasma membrane of live cells oblique angle fluorescence imaging was used to excite fluorescence in the apical cell surface. The excitation laser (HeNe, 543nm emission, 0.4 W/mm^2 in the focal plane, Newport) was expanded, filtered (543/10 nm line width bandpass filter, Chroma) and directed towards the microscope objective (100x, NA 1.4 oil immersion, Olympus) parallel but off the optical axis through a dichroic mirror (~560 nm Cutoff, Chroma). The excitation beam was set such that it was just outside of the condition for total internal reflection, thus allowing for a deeper excitation while still reducing background due to excess fluorescent matter in solution. The resultant fluorescence image was projected through the dichroic mirror and an emission filter (600/75 nm bandpass, Chroma) and collected on a dual MCP intensified, cooled CCD camera (XR/Turbo-120z, Stanford Photonics, Inc.).

Single Molecule Tracking Analysis. Analysis of single molecule tracking data on model systems closely follows a previously reported procedure.[S2] For both quantum dot and dye-labeled lipid tracking, the x, y coordinates of individual, diffraction-limited, fluorescent spots were recorded as a function of time, thereby generating two-dimensional trajectories. From these trajectories, the square displacement as a function of the lag time between successive frames was calculated using Eq. 3.3.

$$r_n^2(\tau_n) = \left[(x_n - x_0)^2 + (y_n - y_o)^2\right] \qquad (3)$$

The square displacements were calculated for each step along a molecule's trajectory and averaged to determine the mean square displacement, $\langle r^2 \rangle$ for a given t_{lag}. For these experiments, the lag time was set to t_{lag} = 50ms. To achieve statistical significance, each sample was analyzed using a minimum of 150 time steps of the same t_{lag} (50ms). In the case of QD-labeled lipid tracking, 150 steps are easily achieved by tracking a single, stable fluorophore, but a

sampling error can occur by observing only a single molecule. As a result, at least 30 molecules were tracked for either the QD or dye-labeled lipid systems. On-off blinking assured that single molecules were tracked.

The experimental error in tracking analysis was determined by repeating the measurements on identical samples, as described in the work reported by Deverall et al..[S2] These repeating studies revealed an average uncertainty in $\langle r^2 \rangle$ of ~5%. Due to their high photostability, QD-lipids were also analyzed using $\langle r^2 \rangle$-time analysis. In this case, the square displacement r^2 from the molecule's point of origin is calculated after each step in the molecule's trajectory. An r^2 value is averaged at each successive t_{lag} in the observation time with the r^2 values of at least 150 other trajectories to ensure statistical significance, yielding $\langle r^2 \rangle$ values for the entire duration of observations at t_{lag} intervals, with each data point being an average of 150 points. The $\langle r^2 \rangle$-time analysis was mainly pursued to determine the type of diffusion (Brownian, two component, or anomalous) with high accuracy because any deviation from linearity in a $\langle r^2 \rangle$ vs. time plot represents a deviation from Brownian diffusion. This method is more accurate than the CDF-approach because the diffusion is analyzed over a longer time range.

Analysis of QD-lipid Trajectories on Live Cells. The apparent position of the particle from video was determined as in Gelles et al. (1988).[S3] Briefly, a kernel image of the diffusion probe was created with a 2-D Gaussian profile with standard deviation 1.6 pixels. The kernel is cross correlated with each video frame near the particle of interest. For each frame, the resulting cross-correlation function is thresholded and the particle position found as the center-of-mass of the thresholded correlation intensity.

Analysis of the motion was carried out based on the $\langle r^2 \rangle$ methods described previously.[S4,S5] The $\langle r^2 \rangle$ vs. time plots ($\langle r^2 \rangle$-t plots) are classified into describing simple Brownian, confined or hop diffusion as described in Fujiwara et al..[S6] The short-term diffusion coefficient was determined from a linear fit to the $\langle r^2 \rangle$-t plot at 2, 3, and 4 frames of elapsed time (as defined in Kusumi et al. (1993)).[S4] The trajectories determined to represent hop diffusion are fit with the result expected for diffusion through an infinite array of partially permeable barriers,[S7] which yields an estimate of the confining compartment size.

(S1) Murcia, M. J.; Shaw, D. L.; Woodruff, H.; Naumann, C. A.; Young, B. Y.; Long, E. L. *Chem. Mater.* **2006**, 18, 2219-2225.

(S2) Deverall, M. A.; Gindl, E.; Sinner, E.-K.; Besir, H.; Ruehe, J.; Saxton, M. J.; Naumann, C. A. *Biophys. J.* **2005**, 88, 1875-1886.

(S3) Gelles, J.; Schnapp, B. J.; Sheetz, M. P. *Nature* **1988**, 331, 450-453.

(S4) Kusumi, A.; Sako, Y.; Yamamoto, M. *Biophys. J.* **1993**, 65, 2021.

(S5) Tomishige, M.; Sako, Y.; Kusumi, A. *J. Cell Biol.* **1998**, 142, 989-1000.

(S6) Fujiwara, T.; Ritchie, K.; Murakoshi, H.; Jacobson, K.; Kusumi, A. *J. Cell Biol.* **2002**, 157 1071-1081.

(S7) Powles, J. G.; Mallett, M. J. D.; Rickayzen, G.; Evans, W. A. B. *Proc. R. Soc. Lond.* A 1992, 391-403.