

Supplementary Material

Supplementary Figure 1: Strategy for identifying single fluorescent molecules at the plasma membrane of live cells. Single fluorophores should have: **D**iffraction limited spot size, **I**ntensity that is similar to that of individual fluorophores in control specimens, **S**ingle-step photobleaching; **H**alf-life proportional to laser power (so-called DISH criteria).

A – Tracks of individual molecules are derived by computer analysis of video recordings. These data enable the intensity and spatial trajectories to be plotted as a function of time. Further analysis (shown in the main paper) gives rise to the MSD vs. δt plots.

B – Individual molecules have a diffraction limited point spread function (see graph inset) and an intensity that is typical of single fluorophores measured in control specimens. Note that the peak intensity measured here is around 140 counts and the average counts per pixel integrated over the diffraction limited spot size (5x5 pixels) is around 40 counts per pixel (see histogram described in D below).

C – The intensity of individual fluorophores shows characteristic single step photobleaching (e.g. disappearance of the spot within a single video frame). The average lifetime is directly proportional to laser power.

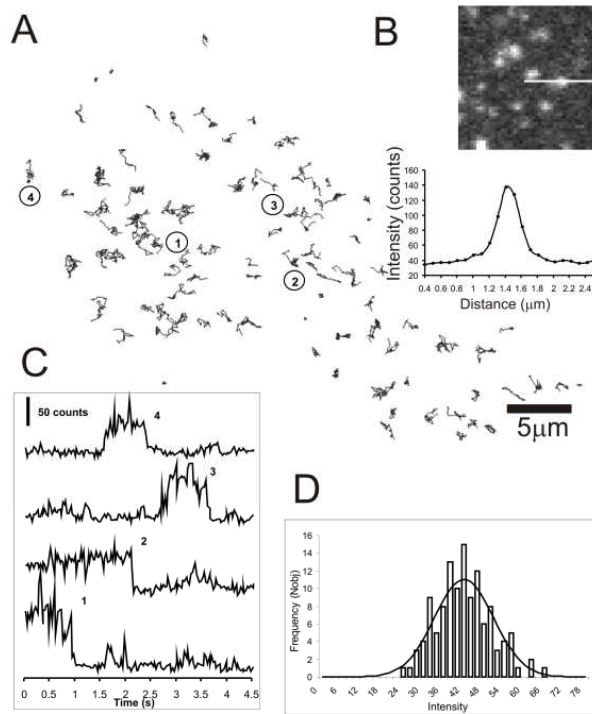
D – The intensity distribution of individual fluorophores is approximately Gaussian with a maximum corresponding to the average intensity of single fluorophores measured in control specimens.

Supplementary Figure 2.

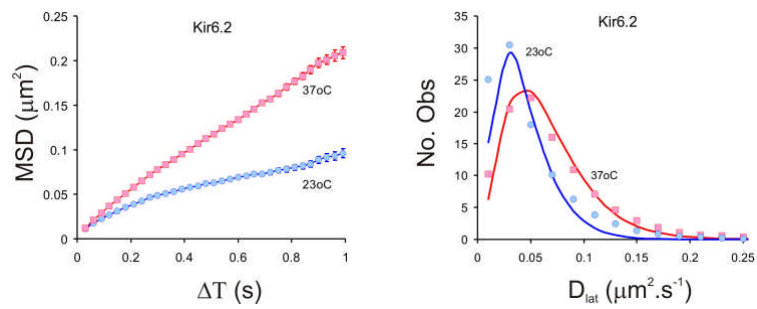
Graphs describing the diffusive motion of Kir6.2-GFP/SUR2A at the plasma membrane of HL-1 cells measured at 23°C and 37°C using TIRFM. A) Plots of the averaged MSD vs. ΔT are shown for Kir6.2-GFP/SUR2A at 23°C (blue graph) and 37°C (red graph). B) Distribution of estimates of D_{lat} for the population of molecules studied shown as a histogram for Kir6.2-GFP/SUR2A at 23°C (blue graph) and 37°C (red graph).

Supplementary Figure 3.

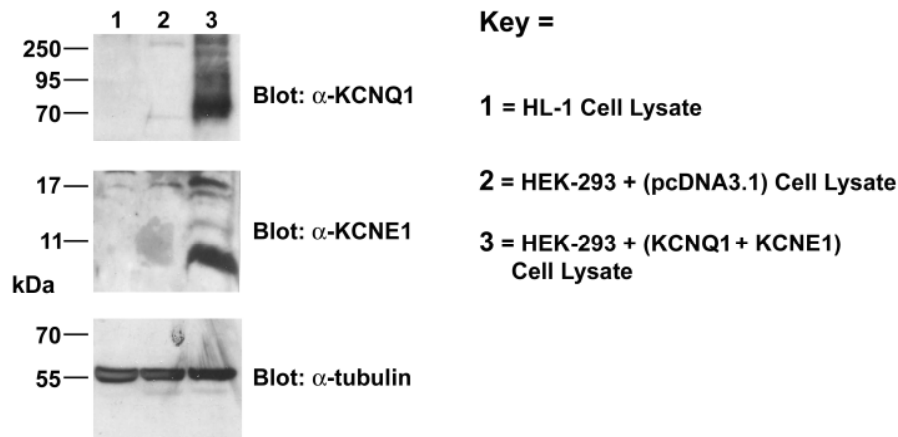
Western blot analysis of endogenous expression of KCNQ1 and KCNE1 in HEK-293 and HL-1 cells. For each lysate, equal amounts of protein were loaded. After separation, the proteins were transferred to stable membranes. The membranes were then probed with either; α -KCNQ1 (rabbit polyclonal (Alomone) (1:5000)), α -KCNE1 (rabbit polyclonal (Alomone) (APC-008) (1:500)) or α -tubulin, to determine protein loading, (mouse monoclonal (Sigma) (1:1000)). The primary antibody was then detected using either an anti-mouse-HRP conjugated antibody (Amersham) (1:3000) or an anti-rabbit-HRP conjugated antibody (Santa Cruz Biotechnology) (1:3000). HEK-293 cells that had been transiently transfected with KCNQ1 and KCNE1 (Lane 3) were used as a positive control.



Supplementary Figure 1:



Supplementary Figure 2:



Supplementary Figure 3:

Supplementary Table 1: Protein mobility in HL1 cells at 37°C. Analysis of the graphs shown in Figure 8 in the main paper. (*Modal = (shape-1) / scale)

Protein	Mean D_{lat} ($\mu\text{m}^2 \text{s}^{-1}$)	MSD-dT		Gamma Distribution		n_{obs} (n cells)
		gradient	r^2	*Modal	r^2	
KCNQ1	0.11	0.039	0.85	0.057	0.8	14423 (10)
KCNQ1(R518X)	0.14	0.092	0.91	0.08	0.99	6174 (4)
KIR6.2	0.07	0.053	0.99	0.043	0.98	15906 (13)
A1	0.16	0.115	0.98	0.108	0.97	5542 (6)

Supplementary Table 2: Student's t -test values calculated for pair-wise comparisons between different proteins in HL-1 cells (see Table 1 in the main paper).

Proteins	KCNQ1 WT vs (R518X)	KCNQ1 vs Kir6.2	KCNQ1 vs A1	Kir6.2 vs A1
Cell Type	HL-1	HL-1	HL-1	HL-1
t -value	-1.192	4.614	-3.857	-10.081
D.F.	12	12	14	17
P - value	N.S.	<0.1%	<0.5%	<<0.1%

Where:

$$t_s = \frac{(\bar{Y}_1 - \bar{Y}_2)}{\sqrt{\left[\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \right] \times \left(\frac{n_1 + n_2}{n_1 \times n_2} \right)}}$$

$$D.F. = n_1 + n_2 - 2$$

Where: n_1 and n_2 are number of cells; Y_1 and Y_2 are means; s_1 and s_2 are standard deviations.

Supplementary Table 3: Student's t -test values calculated for pair-wise comparisons between the same proteins in different cell types (HL-1 vs HEK) (see Table 1 in the main paper).

Proteins	KCNQ1	A1
Cell Types	HL1 vs HEK	HL1 vs HEK
t -Value	-6.379	-12.106
D.F.	17	12
P - value	<<0.1%	<<0.1%

Supplementary Movies

- Movie 1 – Movement of KCNQ1-GFP\KCNE1 containing vesicles in HL1 cell recorded at 37°C using TIRFM. Imaging rate 20 frames s^{-1} .
- Movie 2 – Movement of KCNQ1-GFP\KCNE1 containing vesicles (green palette) in HL1 cell co-transfected with β -tubulin-mRFP (red palette) recorded at 37°C using TIRFM. Imaging rate 20 frames s^{-1} .
- Movie 3 – Fusion of KCNQ1-GFP containing vesicles to plasma membrane of HEK293 cells recorded at 37°C using TIRFM. Imaging rate 10 frames s^{-1} .
- Movie 4 – Movement of KCNQ1-GFP\KCNE1 molecules on plasma membrane of HL1 cell recorded at 37°C using TIRFM. Imaging rate 33 frames s^{-1} .
- Movie 5 – Movement of Kir6.2-GFP\SUR2A molecules on plasma membrane of HL1 cell recorded at 37°C using TIRFM. Imaging rate 33 frames s^{-1} .
- Movie 6 – Movement A1-GFP molecules on plasma membrane of HL1 cell recorded at 37°C using TIRFM. Imaging rate 33 frames per second
- Movie 7 – Movement of KCNQ1-GFP\KCNE1 in HL-1 cell. This movie should be viewed alongside the analyzed data Movie 8. Conditions: 37°C, 33 frame per second.
- Movie 8 – KCNQ1-GFP\KCNE1 identified in Movie 7 using the ASPT algorithm (See main paper for details) were tracked and each object that persisted for at least 10 frames was plotted as a single dot on each video frame. The color coding of each dot represents the spot intensity measured for that object (average pixel counts over a 5x5 pixel region). The color key is shown as an inset on the movie. The histogram plotted on the top right shows the distribution of spot intensities measured for each frame. The fitted line (blue) is the sum of four Gaussian terms (see equation 1 below). Note how the spots are mainly blue at the start of the record but red towards the end. Many of the individual spots gradually change from Violet to Blue to Yellow/Green to Red during the course of the Movie. The colors correspond (approximately to intensities assigned to 4,3,2,1 fluorophores. However, note that inspection of individual intensity trajectories shows very complex behavior due to blinking and other phenomena (see main text for further details and discussion).

Equation 1:

$$I_{obs} = A_1 e^{-\left(\frac{I-I_1-I_0}{w}\right)^2} + A_2 e^{-\left(\frac{I-2I_1-I_0}{\sqrt{2}w}\right)^2} + A_3 e^{-\left(\frac{I-3I_1-I_0}{\sqrt{3}w}\right)^2} + A_4 e^{-\left(\frac{I-4I_1-I_0}{\sqrt{4}w}\right)^2}$$

Where:

I_{obs} = Number of objects with intensity level “I”

A_1, A_2, A_3, A_4 = Number of singly, doubly, triply, quadruply labeled species

I = Intensity value (independent variable)

I_0 = Background fluorescence

I_1 = Single fluorophore Intensity

W = Standard deviation of intensity noise.

Movie 9 – Movement of A1-GFP adenosine receptors in HL-1 which should be viewed alongside the analyzed data shown in Movie 10. All conditions as Movie 7.

Movie 10 – A1 receptors identified in Movie 9 using the ASPT algorithm (See main paper for details) were tracked and each object that persisted for at least 10 frames was plotted as a single dot on each video frame. The conditions (laser power, temperature etc were exactly the same as for Movies 7, 8). The histogram plotted on the top right hand side shows the distribution of spot intensities measured for each frame. The fitted line is the sum of two Gaussian terms (see equation below). Note how the spots are mainly Yellow/Green at the start of the record but red towards the end. Note that inspection of individual intensity trajectories shows very complex behavior due to blinking and other phenomena (see main text for further details and discussion).