Biophysical Journal, Volume 99

Supporting Material

Movements of individual BK_{Ca} channels in live cell membrane monitored by site-specific labeling using quantum dots

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Supporting Material

Cell culture

COS-7 cells were maintained in DMEM (Thermo, Waltham, MA) supplemented with 10% fetal bovine serum (Thermo, Waltham, MA) and neurobasal medium (Gibco-RRL, Carlsbad, CA) containing B-27 supplement (Gibco-RRL) and 200 mM Glutamax (Gibco-RRL), and incubated in 5% CO_2 at 37°C. Primary hippocampal neurons were cultured from the hippocampi of embryonic Sprague Dawley rats at day 18 (Orientbio Inc., Korea), following the modified protocol of Goslin and Banker. Hippocampi were dissociated by enzyme digestion with papain at 37°C for 30 min and triturated by two different sized Pasteur pipettes. For live imaging, neurons were plated onto 18-mm coverslips coated with 0.05 mg/ml poly-D-lysine (Sigma-Aldrich, St. Louis, MO) at a density of 4×10^4 cells per coverslip. Neurons were incubated in neurobasal medium (Gibco-RRL) containing B-27 supplement (Gibco-RRL), 200 mM Glutamax (Gibco-RRL) and 25 mM L-glutamate (Sigma-Aldrich). One hour after plating, the culture medium was changed to neurobasal medium (Gibco-RRL) containing B-27 supplement (Gibco-RRL) and 200 mM Glutamax (Gibco-RRL). Cells were incubated in 5% CO_2 at 37°C. At 7 days *in vitro* (DIV7), half of the medium was exchanged with new medium to support nutrition.

Confocal imaging and co-immunoprecipitation

For confocal imaging, COS-7 cells were plated onto 18-mm coverslips (Marienfeld, Lauda-Königshofen, Germany) coated with 0.05 mg/ml poly-D-lysine (Sigma-Aldrich). Cells were transfected with AP-rSlo-RFP and rβ4-GFP plasmids. At seventy-two hours after transfection, cells were fixed with 4% paraformaldehyde. Then the cells were imaged with confocal microscope (Olympus, Fluoview, FV1000).

For immunoprecipitation, COS-7 cells transiently expressing AP-rSlo-RFP together with GFP or rβ4-GFP were lysed with lysis buffer [1% NP-40, 40mM Tris-Cl pH7.5, 150mM NaCl, 10mM EDTA, 5mM EGTA, 5 % glycerol, 1mM PMSF, protease inhibitor cocktail (Calbiochem)] for 1 hr at 4°C. Cell lysates were centrifuged at 12,500 rpm for 10 min at 4°C for removing any insoluble matrix. Immunoprecipitation was performed using 2 μg anti-hSlo antibody (BD Biosciences) overnight. The immune complexes were captured using Protein G-Sepahrose (GE Healthcare) followed by washing with lysis buffer three times. The immunoprecipitated samples or 5 % of the input lysates were used for immunoblotting.

Supplementary Figures and Movies

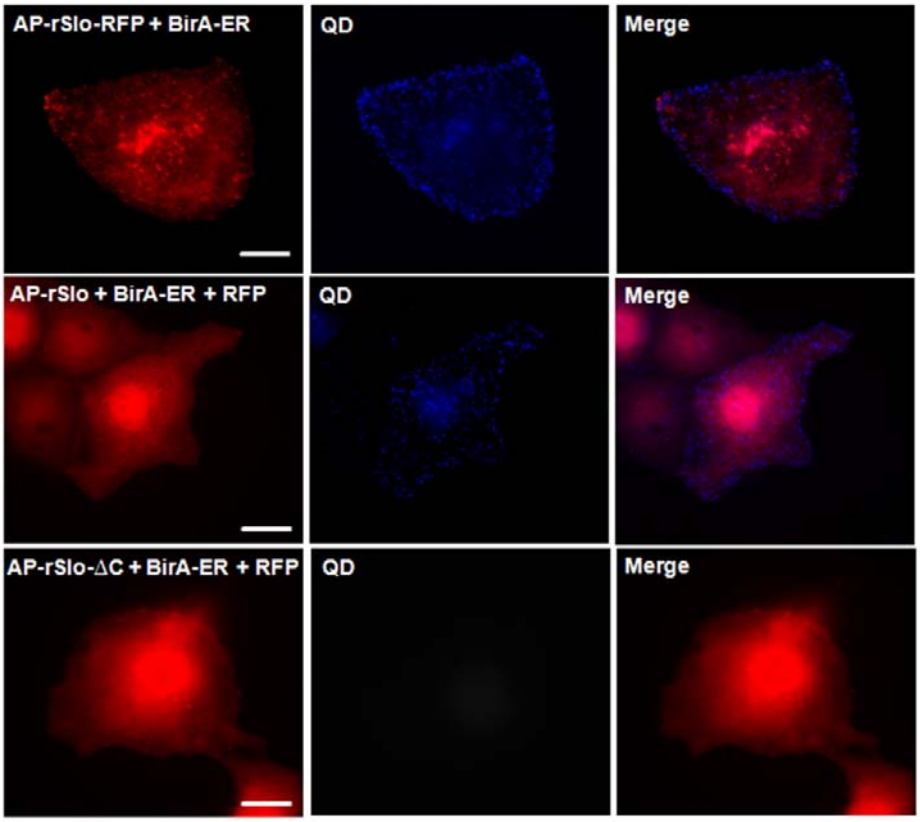


Figure S1

Extracellular QD labeling of three different BK_{Ca} α subunit constructs. COS-7 cells were co-transfected with AP-rSlo-RFP and BirA-ER (*1st* row), AP-rSlo and BirA-ER (*2nd* row), or AP-rSlo-ΔC and BirA-ER (*3rd* row). In the cases of AP-rSlo (*2nd* row) and AP-rSlo-ΔC (*3rd* row), a plasmid harboring RFP was co-transfected in order to visualize the transfectants. Cells were then labeled with streptavidin-conjugated QD605. Cells expressing RFP or AP-rSlo-RFP are *red* (*1st* column) and QD605 is *blue* (*2nd* column). Merged images are also shown (*3rd* column). Scale bar, 10 μm.

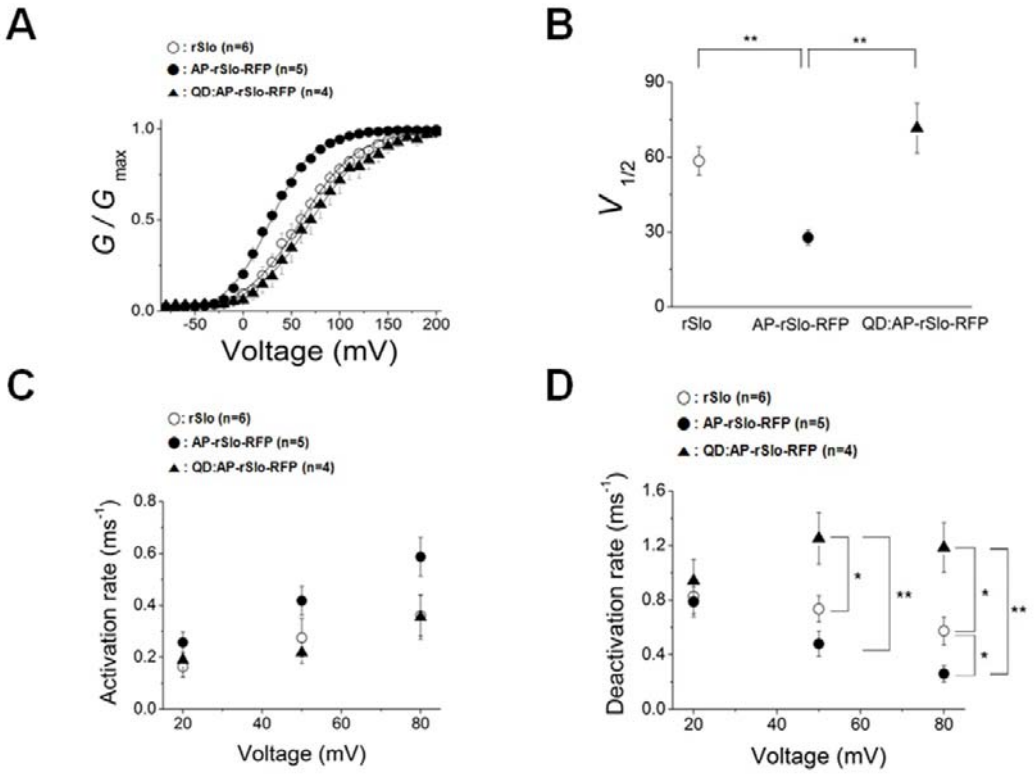


Figure S2

A. Conductance-voltage (*G-V*) relationship of the rSlo, AP-rSlo-RFP and QD labeled AP-rSlo-RFP (QD:AP-rSlo-RFP) at 10 μM $[Ca^{2+}]_i$. The membrane was held at -100 mV and then stepped from -80 mV to 200 mV in 10-mV increments. Conductance values were obtained from peak tail currents and normalized to the maximum conductance (G/G_{max}). Data points were fitted using the Boltzmann function. Throughout the figure, the symbols represent rSlo (*empty circle*), AP-rSlo-RFP (*filled circle*) and QD:AP-rSlo-RFP (*filled triangle*), respectively. **B.** Half-activation voltage ($V_{1/2}$) values of rSlo, AP-rSlo-RFP and QD:AP-rSlo-RFP channels obtained at 10 μM $[Ca^{2+}]_i$. **C** and **D.** Activation and deactivation kinetics of rSlo, AP-rSlo-RFP and QD:AP-rSlo-RFP channels. The activation and deactivation rate of the macroscopic current were plotted at 20, 50, and 80 mV in the presence of 10 μM $[Ca^{2+}]_i$. Time-constants of activation and deactivation were obtained by fitting the individual current traces to a single exponential function. Each data point represents the mean ± S.E.M., and pairs of data points found to significantly differ from each other by paired Student's t-test at $p < 0.05$ (*) or $p < 0.01$ (**) are indicated.

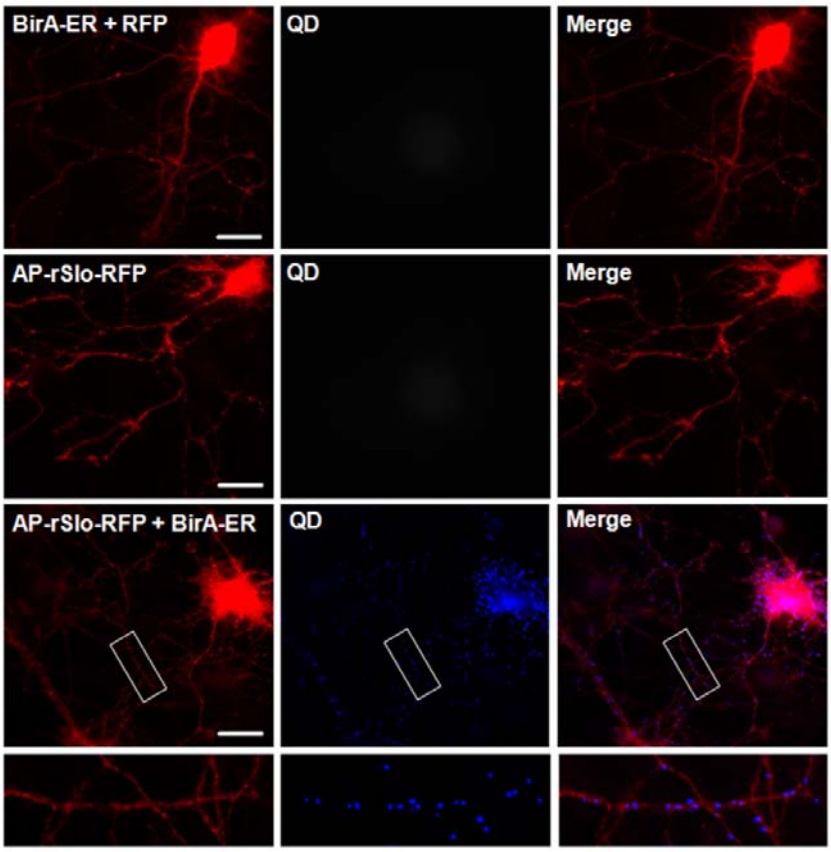


Figure S3

Specific labeling of AP-rSlo-RFP channels using QDs. Cultured rat hippocampal pyramidal neurons (DIV5) were transfected with BirA-ER and RFP (*1st* row), AP-rSlo-RFP (*2nd* row), or AP-rSlo-RFP and BirA-ER (*3rd* row). A magnified view (3×) of the highlighted regions in the *3rd* row is shown in the *4th* row. In the case of Bir-ER (*1st* row), a plasmid harboring RFP was co-transfected in order to visualize the transfectants. Cells were labeled with streptavidin-conjugated QD605 (Invitrogen). Cells expressing RFP or AP-rSlo-RFP are *red* (*1st* column) and QD605 is *blue* (*2nd* column). Merged images are also shown (*3rd* column). Scale bar, 10 μm.

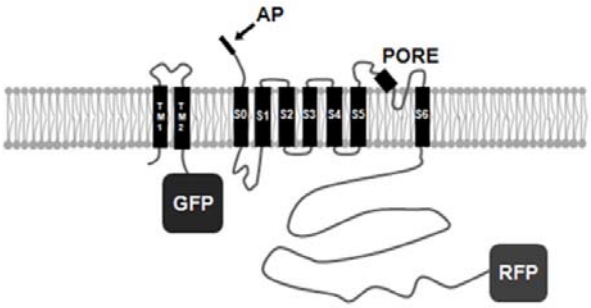
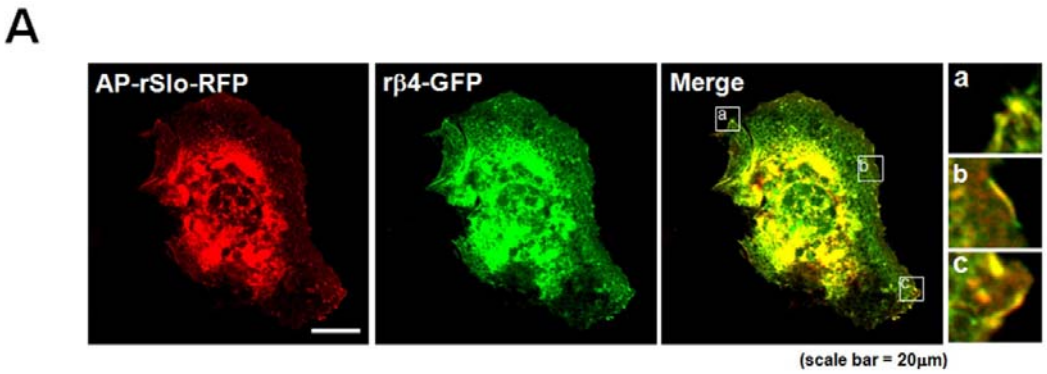


Figure S4

Schematic illustration of the α and β4 subunits of BK_{Ca} channel used in this experiment. Rat BK_{Ca} channel α subunit, rSlo, was tagged with an acceptor peptide (AP) at its N-terminus and a red fluorescent protein (RFP) at its C-terminus. Rat β4 subunit, rβ4, was tagged with green fluorescent protein (GFP) at its C-terminus.



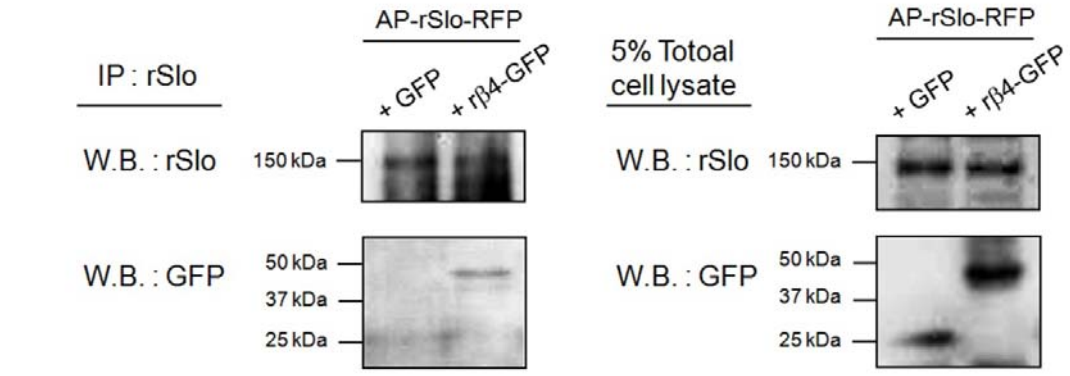


Figure S5

Interaction between AP-rSlo-RFP and rβ4-GFP. **A.** Confocal images of COS-7 cells co-expressing AP-rSlo-RFP and rβ4-GFP. Magnified views (4×) of the highlighted

regions were shown (**a-c**). **B**. Co-immunoprecipitation analysis of AP-rSlo-RFP and rβ4-GFP. COS-7 cells transfected with AP-rSlo-RFP together with GFP or rβ4-GFP were lysed. Cell lysates was subjected to immunoprecipitation using anti-hSlo antibody and the immune complexes were analyzed by western blot using anti-hSlo antibody or anti-GFP antibody.

Movie S1
QD-labeled rSlo channels in COS-7 cell.

Movie S2
QD-labeled rSlo channels in hippocampal neuron.

Movie S3
Zoom-in view: QD-labeled rSlo channels in soma of neuron.

Movie S4
Zoom-in view: QD-labeled rSlo channels in axodendrite of neuron.

Movie S5
QD-labeled rSlo/rβ4 channels in COS-7 cell

Movie S6
Zoom-in view: QD-labeled rSlo/rβ4 channels in soma of neuron

Movie S7
Zoom-in view: QD-labeled rSlo/rβ4 channels in axodendrite of neuron.