Supporting information

Developing new 4-PIOL and 4-PHP analogues for photo-inactivation of γ-aminobutyric acid type A receptors

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S1

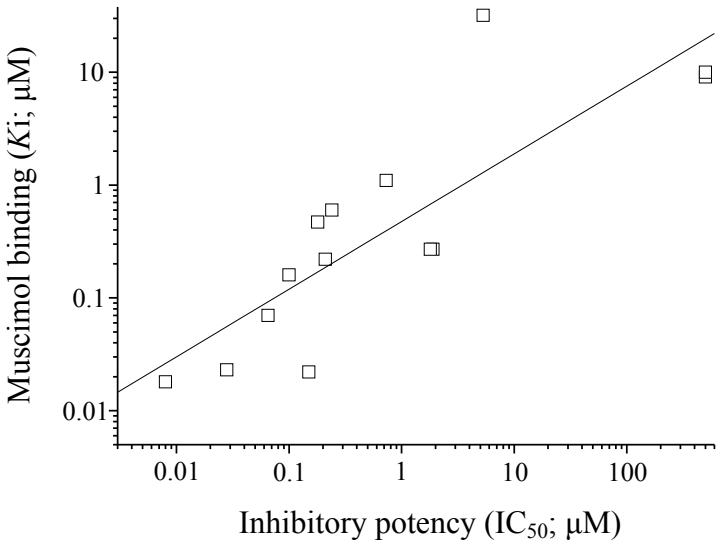

Figure S1. Correlation plot of ligand Ki, determined from ligand binding studies of 4-PIOL, 4-PHP and derivatives, and IC50, deduced from electrophysiological analysis and inhibition concentration relationships for the same compound range. Data are taken from Table 1. The line shows a best-fit correlation of R = 0.83773, n = 14.

S2

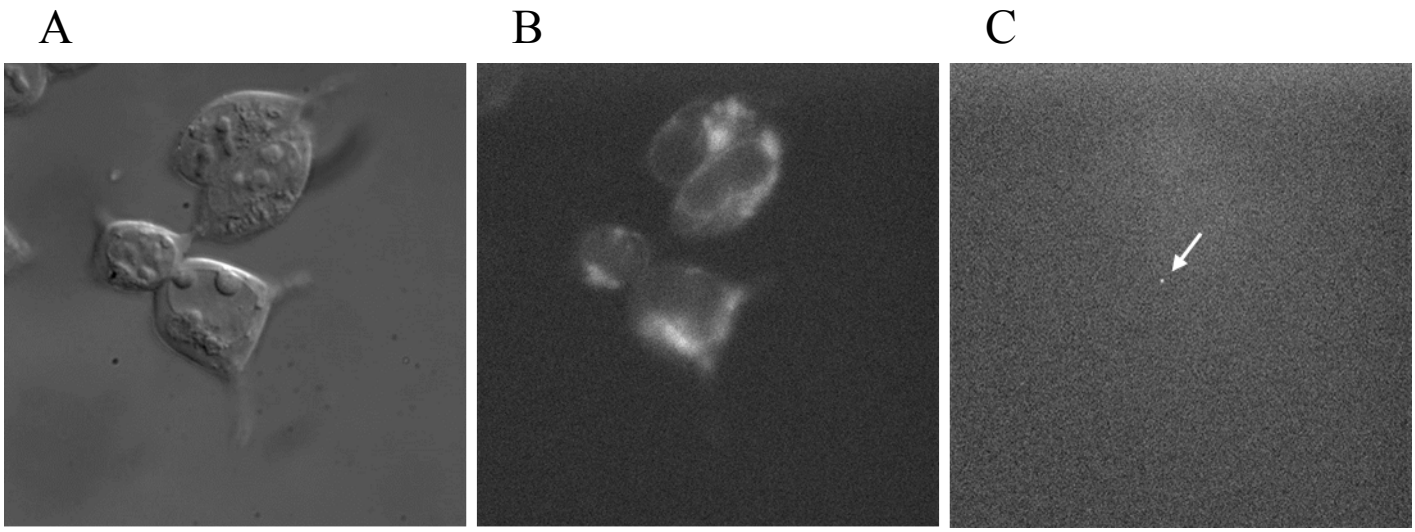

Figure S2. Example of an experiment on HEK293 cells expressing α1β2γ2L $GABA_A$Rs with **3b**. A cluster of HEK cells are shown in DIC image (A), and successful transfection was verified by GFP-reporter fluorescence (B). The final image (C) is a single frame obtained from Supplementary Video 1; this image shows the same cells (as in A, B), but with only a single QD_{655}-streptavidin specifically bound after preparation with **3b** (arrow head) according to Method 1: In short, the α1β2γ2L-transfected cells had been washed with krebs solution before incubation with 1 mM **3b** (2 min), followed by UV exposure for 10 sec, before replacement of solution, and incubation with 50 pM QD_{655}-streptavidin (2 min); finally cells were washed thoroughly to get rid of non-bound/non-specific QDs before imaging with 655nm filter cube. This unsatisfactory level of specific binding could not be improved on despite many variations in concentrations, incubation times and UV intensities and exposure times.

Video 1. Example of an experiment on HEK293 cells expressing α1β2γ2L $GABA_A$Rs treated with **3b** (Biotin analogue) and QD_{655}-streptavidin. Associated with Supplementary Figure S2, panel C. 15fps video; 200 frame recording; 480 x 360 pixel size. Conditions: $UV_{40\% \text{ of max}}$, fully open diaphragm.

S3

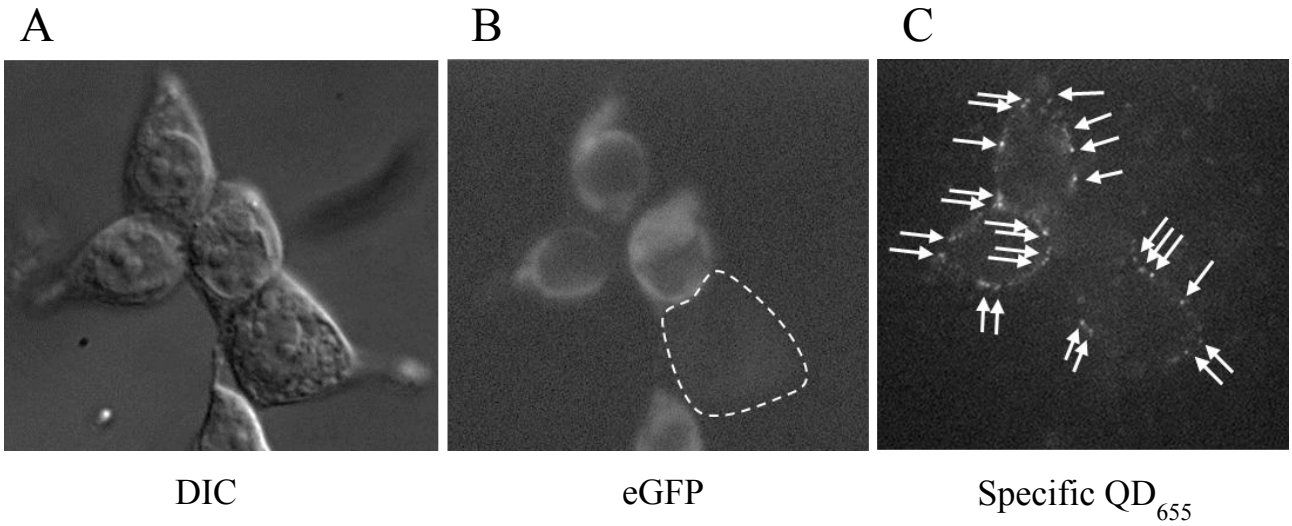

Figure S3. Example of a control experiment on HEK293 cells expressing $\alpha1_{BBS}\beta2\gamma2L$ $GABA_A$Rs with bungarotoxin-biotin. HEK cells are shown in DIC image (A), and successful transfection was verified by GFP-reporter fluorescence (B; the gfp signal of the outlined cell is weak but present). The final image (C) is a single frame obtained from Supplementary Video 2; cells were imaged with 655 nm filter cube, and clear specific binding of QDs were observed (arrow heads; note that not all QDs have been marked). The method preparation for this experiment were, in short, as follows: $\alpha1_{BBS}\beta2\gamma2L$-transfected cells had been washed with krebs solution before incubation with 0.44 μM bungarotoxin-biotin conjugate (2 min), washed several times with krebs, incubated with 50 pM QD_{655}-streptavidin (1 min); thoroughly washed in krebs to get rid of non-bound/non-specific QDs before imaging. This satisfactory level of specific binding would be suitable for QD tracking of trajectories by processing with ImageJ plug-in, SpotTracker 2D/3D and MatLab.

Video 2. Example of an experiment on HEK293 cells expressing $\alpha1_{BBS}\beta2\gamma2L$ $GABA_A$Rs treated with bungarotoxin-biotin and QD_{655}-streptavidin. Associated with Supplementary Figure S3, panel C. 15fps video; 200 frame recording; 480 x 360 pixel size. Conditions: $UV_{40\% \text{ of max}}$, fully open diaphragm.
Note that many non-bound QDs can be observed in fast random movements in solution.