



Supplementary Information for

**Unique dynamics and exocytosis properties of GABAergic
synaptic vesicles revealed by three-dimensional single vesicle
tracking**

Chungwon Park^a, Xingxiang Chen^a, Chong-Li Tian^{b, c}, Gyu Nam Park^{d, e}, Nicolas
Chenouard^f, Hunki Lee^{a, g}, Xin Yi Yeo^{h, i}, Sangyong Jung^{h, j}, Richard W. Tsien^k, Guo-
Qiang Bi^{b, c}, and Hyocheon Park^{a, l, m, 1}

¹Correspondence: Hyocheon Park
Email: hkpark@ust.hk

This PDF file includes:

Figures S1 to S3

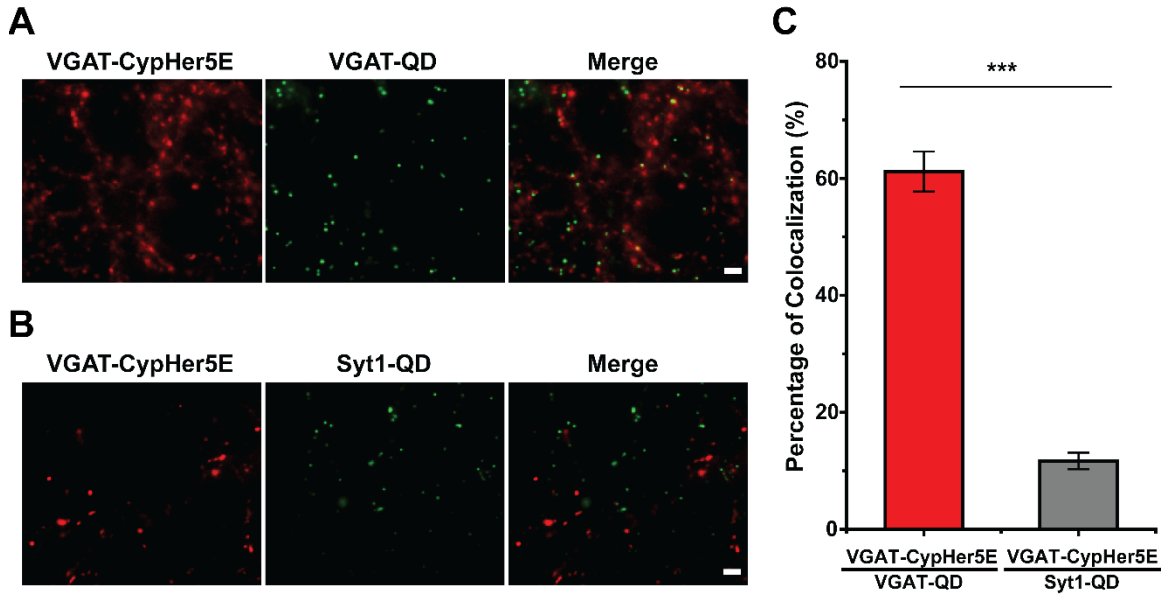


Fig. S1. VGAT-QD preferentially labels GABAergic synaptic vesicles in cultured hippocampal neurons. (A-B) Representative fluorescence images of GABAergic presynaptic terminals labeled with CypHer5E-conjugated antibodies against the luminal domain of VGAT (VGAT-CypHer5E, red) and synaptic vesicles loaded with QDs conjugated to antibodies against the luminal domain of VGAT (VGAT-QD, green; A) or antibodies against the luminal domain of Syt1 (Syt1-QD, green; B). Scale bars: 3 μ m. (C) Percentage of colocalization between VGAT-CypHer5E-labeled boutons and either VGAT-QD-loaded vesicles ($61 \pm 3.4\%$ (N = 13 images)) or Syt1-QD-loaded vesicles ($12 \pm 1.4\%$ (N = 13)). Error bars show SEM. *** $P < 0.001$ (Student's t -test).

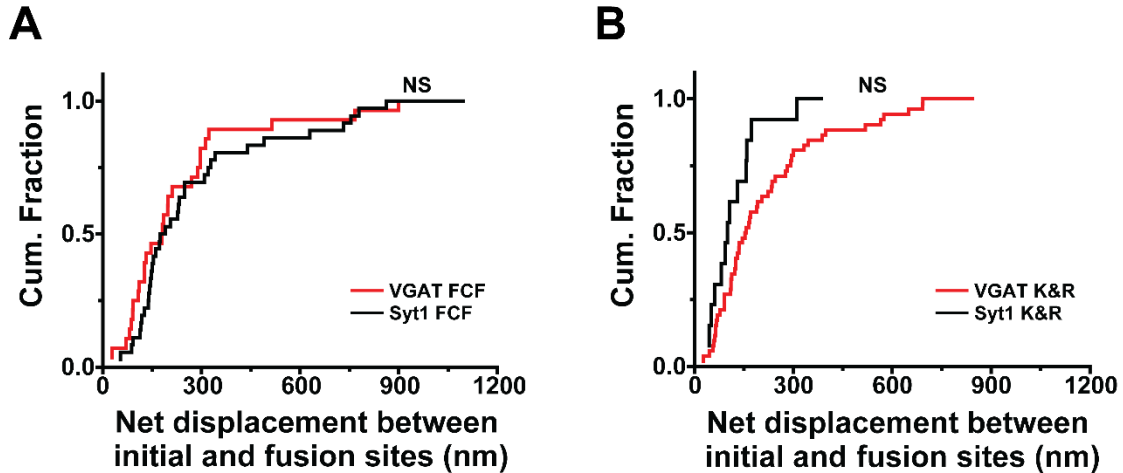


Fig. S2. Cumulative distribution of net displacement of VGAT-QD-loaded and Syt1-QD-loaded synaptic vesicles undergoing either full-collapse fusion or kiss-and-run fusion. (A) Cumulative distribution of net displacement of VGAT-QD-loaded vesicles and Syt1-QD-loaded vesicles undergoing full-collapse fusion. The net displacement of VGAT-labeled synaptic vesicles undergoing full-collapse fusion (VGAT FCF) was not significantly different from that of Syt1-labeled vesicles undergoing full-collapse fusion (Syt1 FCF) (260 ± 46 nm ($n = 28$ vesicles) vs. 302 ± 43 nm ($n = 36$), respectively; $P > 0.4$, K-S test). **(B)** Cumulative distribution of net displacement of VGAT-QD-loaded vesicles and Syt1-QD-loaded vesicles undergoing kiss-and-run fusion. The net displacement of VGAT-labeled synaptic vesicles undergoing kiss-and-run fusion (VGAT K&R) was not significantly different from that of Syt1-labeled vesicles undergoing kiss-and-run fusion (Syt1 K&R) (231 ± 27 nm ($n = 52$) vs. 143 ± 28 nm ($n = 13$), respectively; $P > 0.3$, K-S test). NS: not significant.

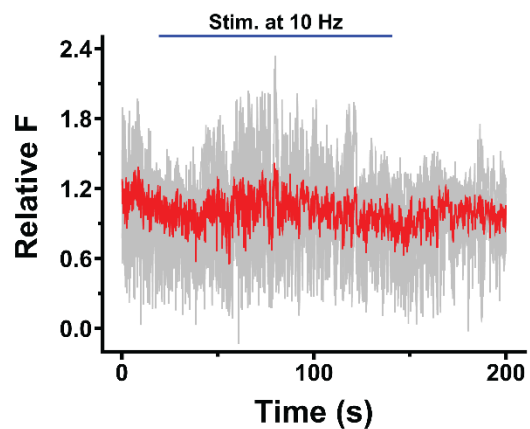


Fig. S3. Normalized fluorescence traces of non-releasing VGAT-QD-loaded vesicles. Five individual traces are shown in gray, and the average trace is shown with a red line. A blue horizontal bar represents electrical stimuli (10 Hz) for 120 s starting at 20 s.