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Supplemental Information

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Supporting Material

Regulation of PI3K by PKC and MARCKS: Single-Molecule Analysis of a Reconstituted Signaling Pathway

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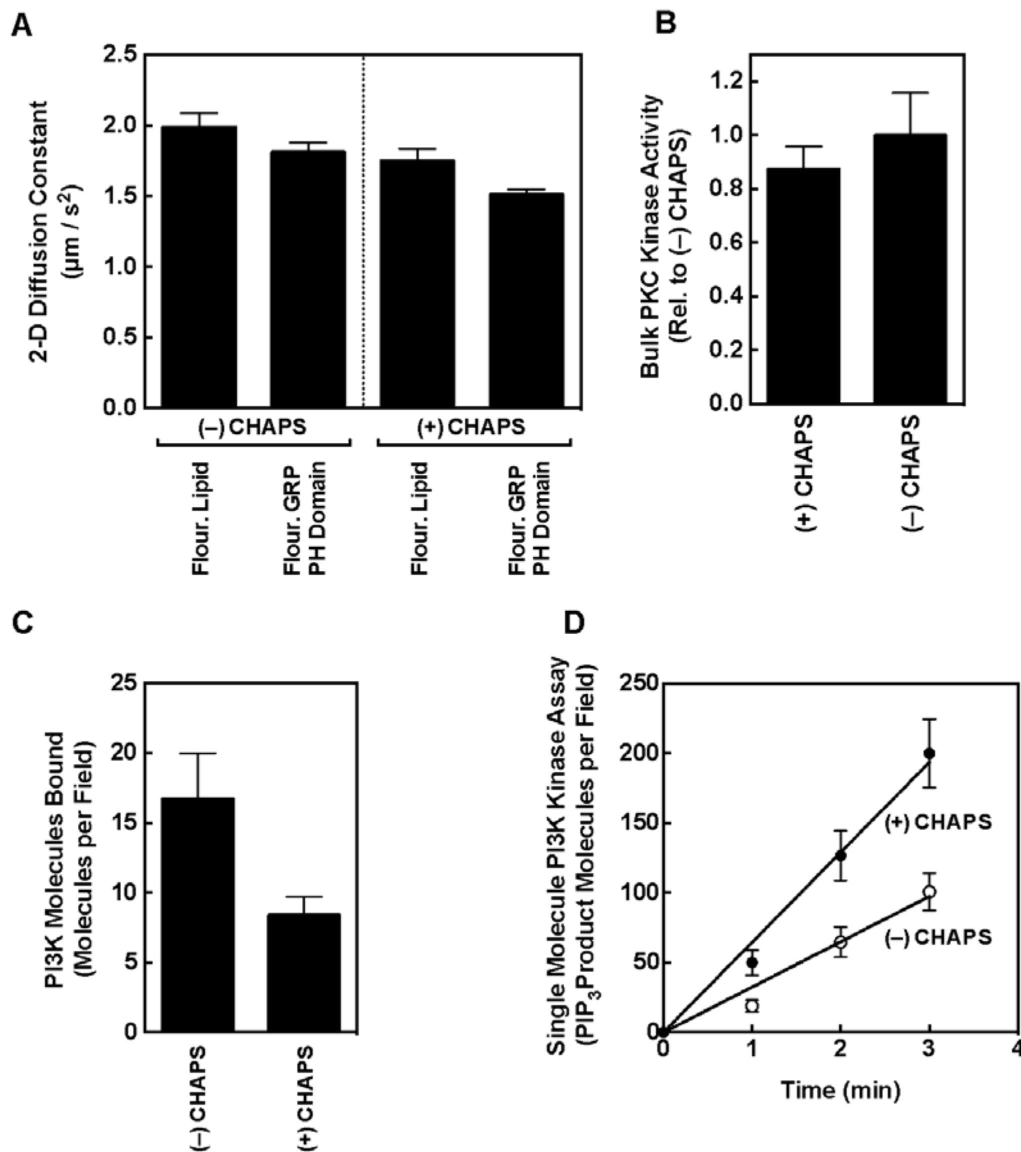
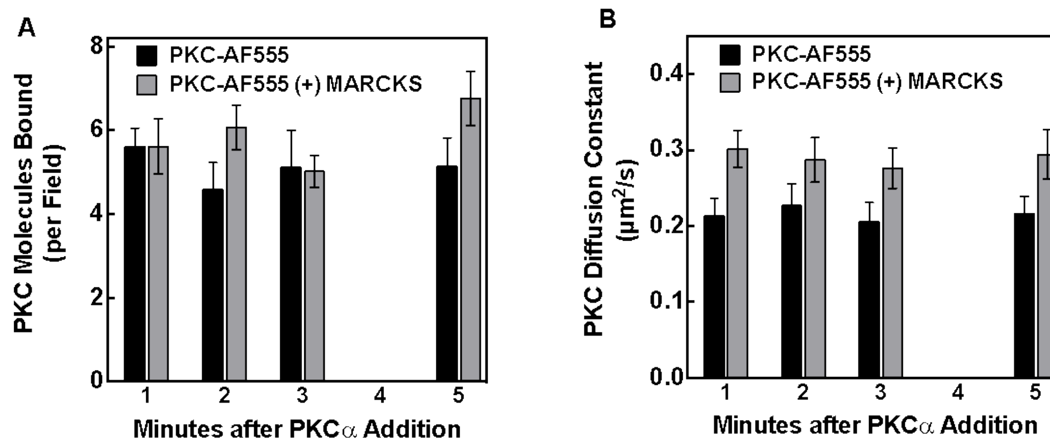
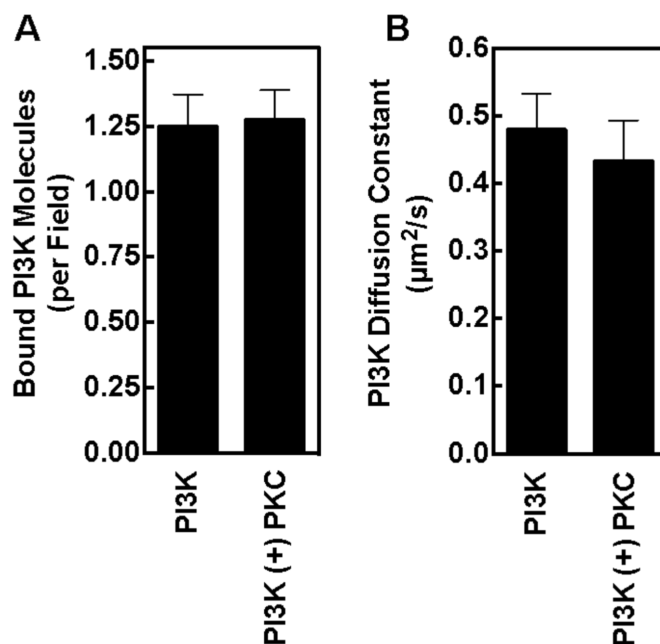


(**Supporting Material, Figure S1**) Control experiments to test effect of CHAPS on fluorescent lipid and protein diffusion and kinase activity. **(A)** CHAPS has no significant effect on the diffusion of the fluorescent lipid LRB-PE, but causes a small (<15%), reproducible ($p < 0.05$) diffusional slowing of fluorescent GRP1 PH domain bound to PIP_3. **(B)** CHAPS detergent has no significant effect on PKCα kinase activity. **(C)** CHAPS significantly decreases binding of PI3Kα to the target membrane ($p < 0.05$). **(D)** CHAPS significantly increases PI3Kα lipid kinase activity ($p < 0.01$). Single molecule TIRF was carried out at 21.5 $\pm$ 0.5 °C on PE/PS/DAG/PIP_3 ± 200 ppb LRB-PE (**A**) or standard PE/PS/DAG/PIP_2 (**B-D**) supported bilayers as detailed in Fig. 4.



(**Supporting Material, Figure S2)** Control experiments testing the effect of $MARCKS_p$ on fluorescent Ca^{2+}-PKCα binding and diffusion. **(A)** $MARCKS_p$ does not significantly affect Ca^{2+}-PKCα binding to bilayers consistent with its high affinity for PS even in the absence of PIP_2 (1). **(B)** $MARCKS_p$ generates a small (<25%), reproducible ($p < 0.005$) increase in the 2-D diffusion speed of membrane-bound Ca^{2+}-PKCα, likely due to PIP_2 ligand sequestration which prevents C2 domain binding to PIP_2 as well as preventing the resulting diffusional slowing (2). Single molecule TIRF assay at 21.5 $\pm$ 0.5 ºC on standard PE/PS/DAG/PIP_2 supported bilayers as detailed in Fig. 4. In these experiments PKCα was employed at the standard total concentration (Table 1) used in other experiments, but at this concentration the density of membrane bound PKCα is too high to resolve single particles for counting and tracking. Thus, a mixture of fluorescent (0.005%) and dark, unlabeled PKCα (99.995%) was employed yielding the density of bound fluorescent PKCα indicated in (A).



(**Supporting Material, Figure S3)** Control experiment testing for direct interaction between PKCα and PI3K on the membrane surface. PKCα is observed to have no significant effect on **(A)** the membrane binding or **(B)** the 2-D diffusion constant of fluorescent PI3K lecule TIRF assay at 21.5 ± 0.5 °C, indicating little or no direct inter on standard PE/PS/DAG/PIP_2 supported bilayers as detailed in Fig. 4.

REFERENCES

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