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Supplemental Information

Multivalent Cation-Bridged $PI(4,5)P_2$ Clusters Form at Very Low Concentrations

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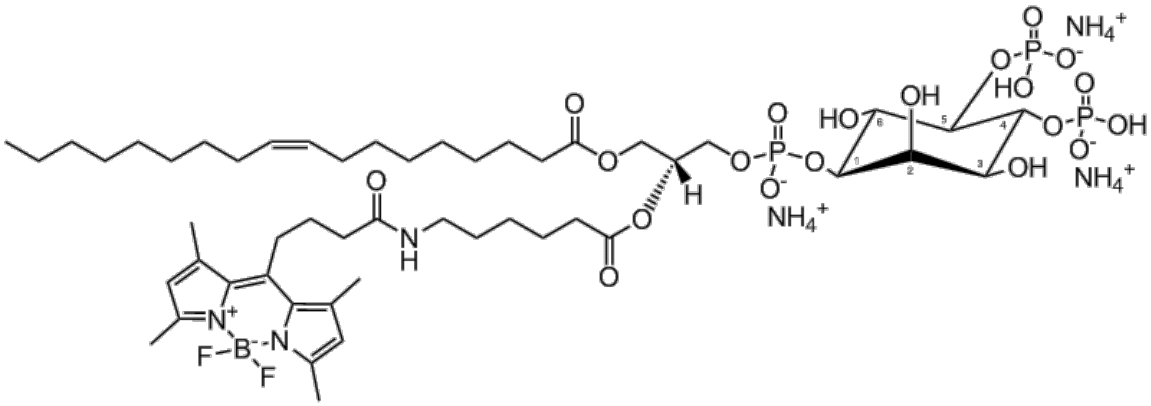
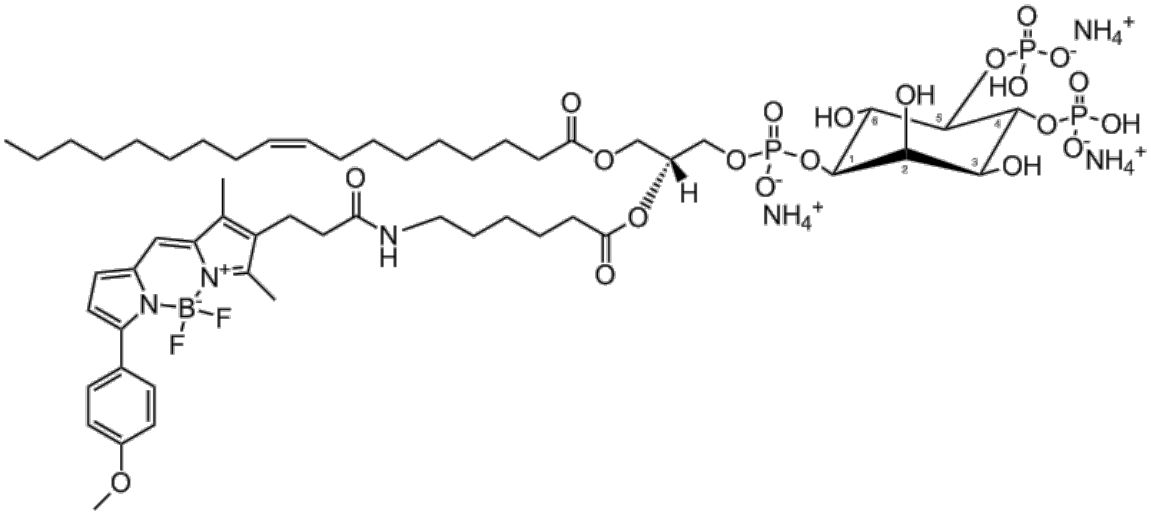


TF-PIP2

1-oleoyl-2-{6-[4-(dipyrromethene boron difluoride)butanoyl]amino}hexanoyl-*sn*-glycero-3-phosphoinositol-4,5-bisphosphate (ammonium salt)



TMR-PIP2

1-oleoyl-2-(6-((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene-2-propionyl)amino)hexanoyl)-*sn*-glycero-3-phosphoinositol-4.5-bisphosphate (ammonium salt)

Fig. S1. Chemical structures of TF-PIP2 (top) and TMR-PIP2 (bottom)

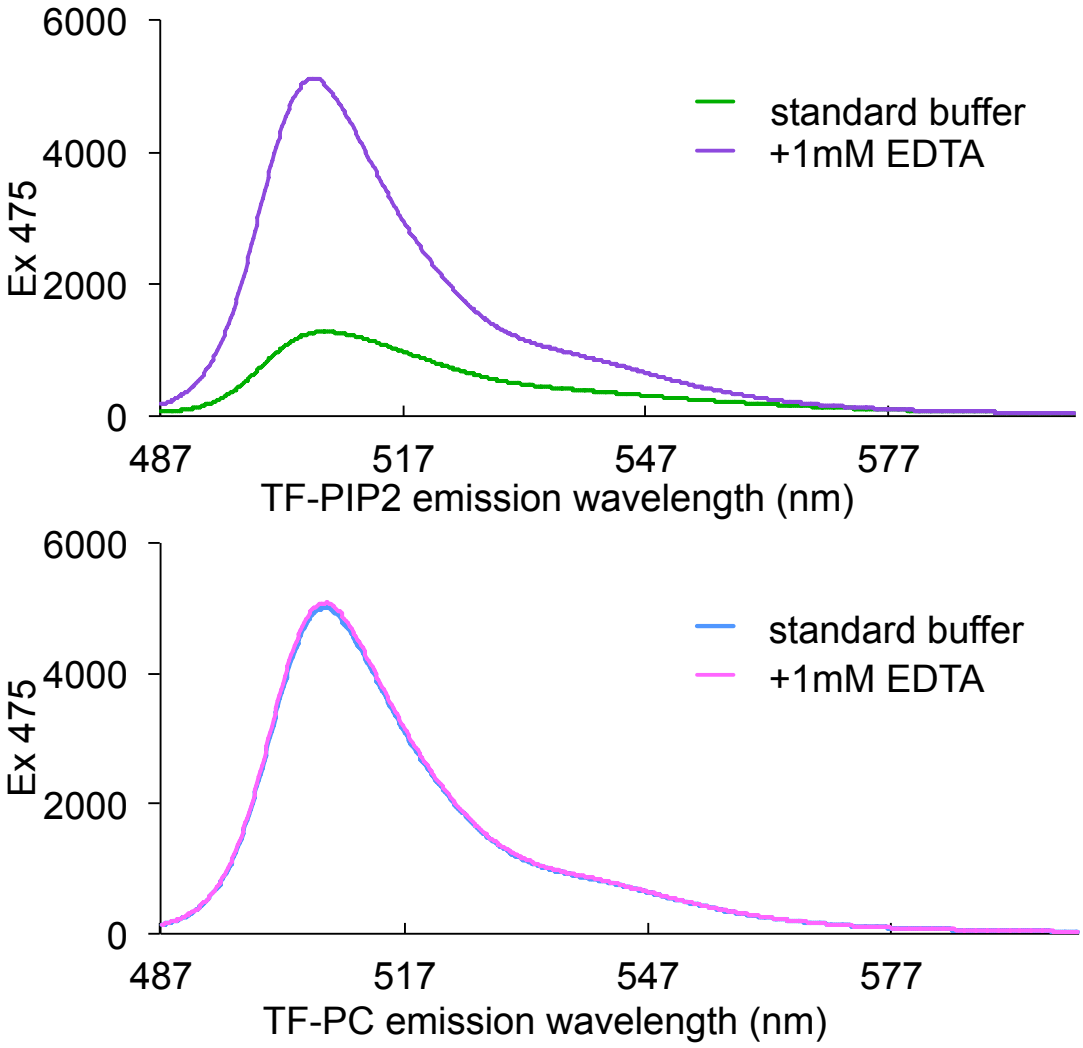


Fig. S2. EDTA eliminates self quenching of TF-PIP2, but has no effect on TF-PC in POPE/POPS/CHol (34/30/36). The emission spectrum of 0.3 mol% TF-PIP2 (top) and TF-PC (bottom) in inner leaflet model membranes was collected in standard buffer and in 1 mM EDTA.

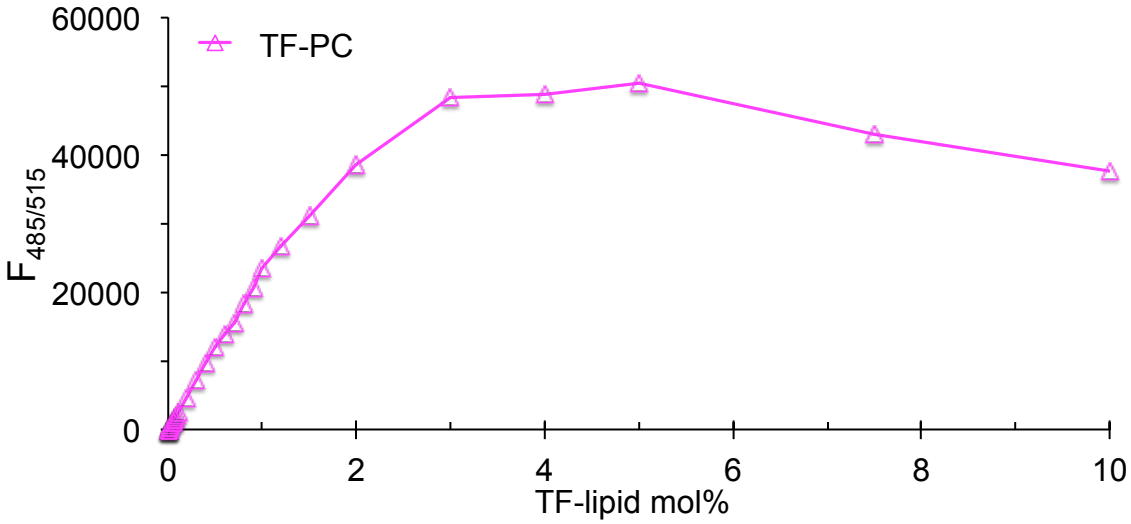


Fig. S3. Self-quenching of TF-PC occurs only above ~2% of total lipids, at least 50-fold higher than for TF-PIP2. Assays were performed as in Fig. 1.

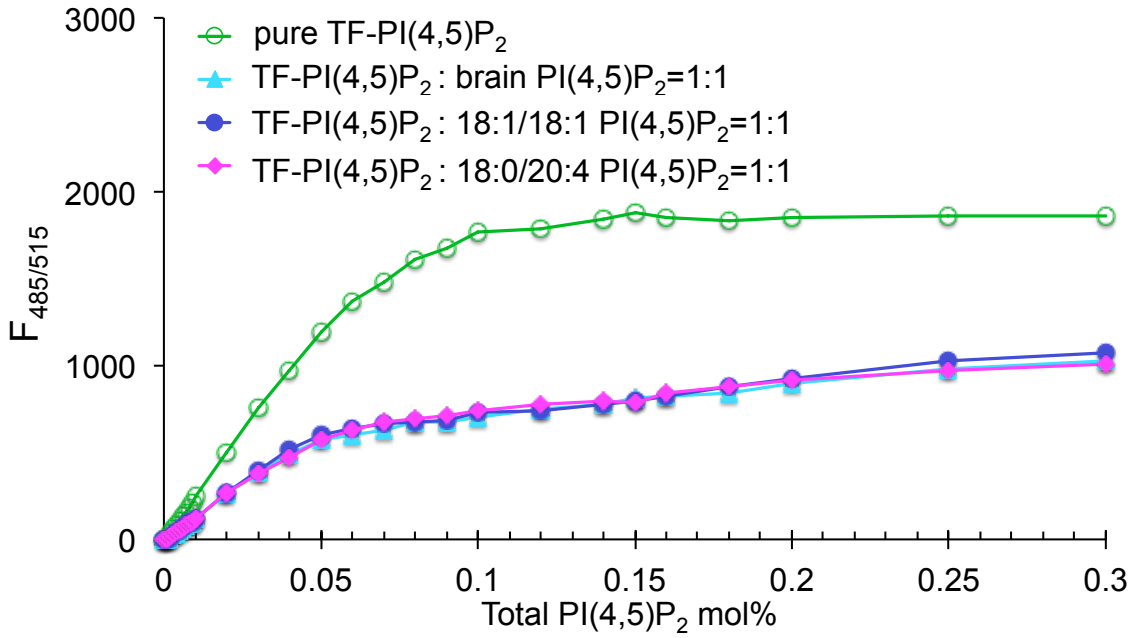


Fig. S4. Unlabeled PIP2 incorporates into TF-PIP2 clusters. Self-quenching assays were carried out in POPE/POPS/Chol (34/30/36) as in Fig. 1. Pure TF-labeled PIP2 (green) is diluted 1:1 with unlabeled PIP2, either brain-PIP2 (cyan), 18:1/18:1-PIP2 (blue), or 18:0/20:4-PIP2 (magenta).

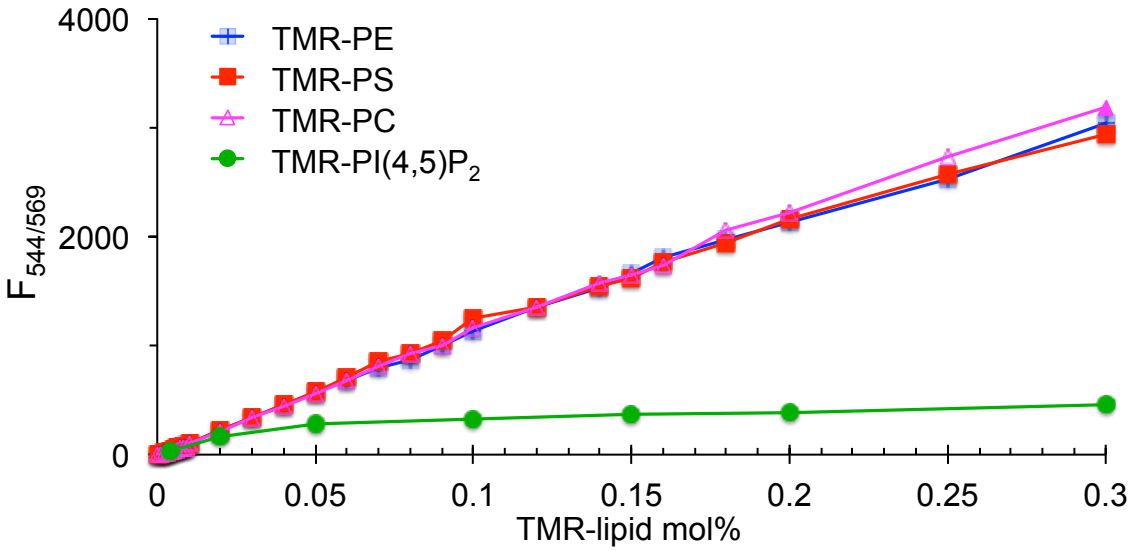


Fig. S5. TMR-PIP2 forms cation-bridged clusters, but other TMR-labeled phospholipids do not. Self-quenching measured as in Fig. 1 with results similar to those with TF-PIP2 shown in Fig. 1. TMR-PE (blue), TMR-PS (red), TMR-PC (magenta) and TMR-PIP2 (green).

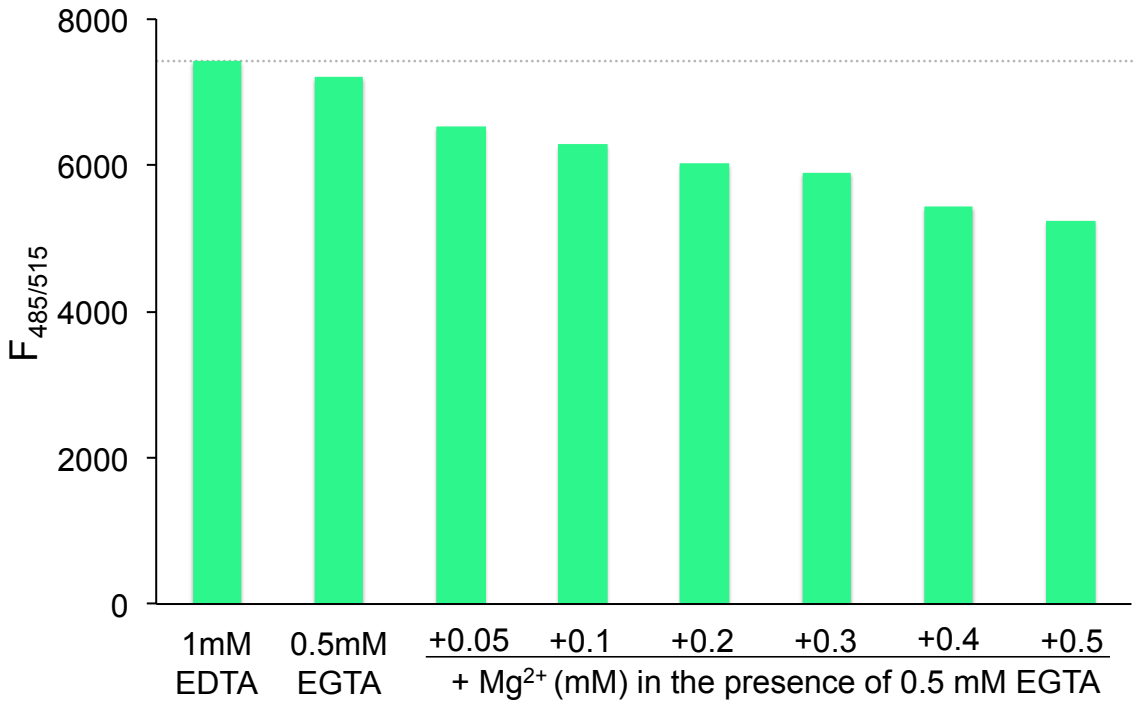


Fig. S6. Mg^{2+} drives PIP2 clustering. Bars represent the fluorescence from a fixed 0.3 mol% TF-PIP2 in POPE/POPS/Chol (34/30/36) with the following additions: 1mM EDTA, 0.5 mM EGTA, or increasing concentrations of Mg^{2+} from 0.05 mM up to 0.5 mM in the presence of 0.5mM EGTA.

Table S1

Multivalent cation analysis by ICP-OES					
Metal ion [µM]	**Al^{3+}**	**Ca^{2+}**	**Fe^{3+}**	**Mg^{2+}**	**Zn^{2+}**
MQ H_2O	-	-	-	-	-
5µM TF-PI(4,5)P_2 (in MQ H_2O)	0.4	0.2	-	-	0.1
5µM TMR-PI(4,5)P_2 (in MQ H_2O)	0.2	0.5	-	-	0.1
10µM Brain-PI(4,5)P_2 (in MQ H_2O)	0.2	0.5	-	0.2	0.4
6.3µM POPS (in MQ H_2O)	0.2	0.2	-	-	-
63µM POPS (in MQ H_2O)	0.3	0.4	-	-	-

Note: "-" means undetectable (< 0.1 µM).