Supporting Information: Pu, M., Orr, A., Redfield, A. G., and Roberts, M. F., "Defining specific lipid binding sites for a peripheral membrane protein in situ using sub-Tesla field cycling NMR"

Effect of PI-PLC concentration on PRE:

To show that the PRE measured for this ^{31}P reflects protein interacting specifically with the phospholipid, we examined the PRE for spin-labeled H82C PI-PLC interacting with PMe in 5mM PMe/5 mM PC vesicles (*Figure S.1.*). This spin-labeled protein was chosen because it is proximal to the active site and should definitely have an effect on the PMe. With 0.5 mg/ml protein, there was a significant increase in the R_1 at very low field consistent with proximity of the spin-label and bound PMe. Doubling the amount of protein roughly doubled the R_1 for PMe. For comparison, the $R_v(0)$ value for PMe in the presence of unlabeled protein is 4.4±0.4 s^{-1}. From this comparison, we chose to use 0.5 mg/ml of the different spin-labeled proteins since R_1>10 s^{-1} is difficult to measure accurately, and the lower protein concentration allows us to measure R_1 at lower fields, improving the accuracy of the data analysis for $r_{P\text{-}e}$.

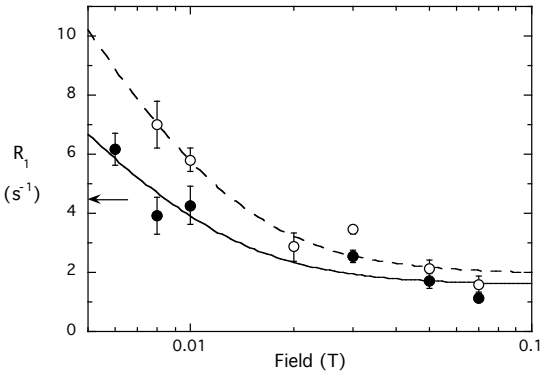

Fig. S.1. Effect of spin-labeled H82C PI-PLC concentration (●, 0.5 mg/ml; ❍, 1.0 mg/ml) on the low field relaxation rate for PMe in vesicles with PC. The arrow indicates $R_v(0)$ for these vesicles with unlabeled protein added.

Analysis of spin-labeled PI-PLC effects on short-chain phosphatidylcholines:

The PRE for diC_6PC caused by spin-labeled PI-PLC (either D205C and H82C) was initially fit with a single correlation time as shown by the dashed line in Fig. 3B. The optimum fit yielded $\tau_{P\text{-}e}$ and $\Delta R_{P\text{-}e}(0)$ as 70±8.8 ns and 0.95±0.07 s^{-1}, respectively, for spin-labeled D205C; for spin-labeled H82C, $\tau_{P\text{-}e}$ = 37.7±4.5 ns and $\Delta R_{P\text{-}e}(0)$ = 0.17±0.01 s^{-1}. However, fitting the data with a single correlation time, clearly underestimates the spin-label-enhanced relaxation in the region above 0.5 T. This systematic deviation suggested that there are two discrete dispersions in this profile, one with a slower correlation time (τ_s) that is dominant below 0.1 T, and one on a faster time scale (τ_f). Therefore, these data were fit with the following expression:

$$\Delta R_1 = \Delta R_s(0)/(1 + \omega^2\tau_s^2) + \Delta R_f(0)/(1 + \omega^2\tau_f^2) .$$

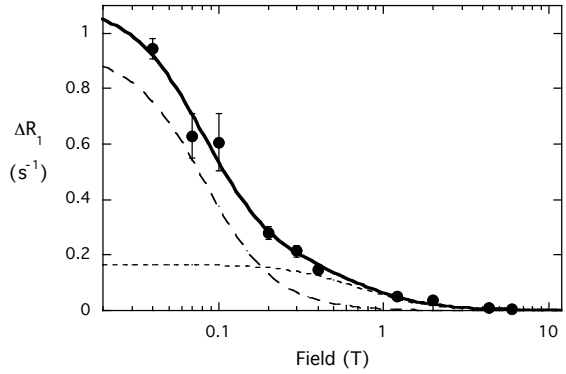

Fig. S.2. Contribution of the slow (- - -) and fast (· · ·) correlation times to ΔR_1, the PRE for diC_6PC (●) bound to spin-labeled 0.5 mg/ml D205C PI-PLC.

In analogy to the motions contributing to phospholipid ^{31}P relaxation in vesicles (1), the two time-scales likely represent a molecular wobble (τ_f) and overall micelle rotation (τ_s). *Figure S.2.* shows the contribution of each dipolar dispersion to the PRE curve for spin-labeled D205C. Comparing values extracted using a single correlation time versus two (Table 2) shows that while the $\Delta R_{P\text{-}e}(0)$ term is essentially the same, the τ value is 60% as much as τ_s. This would reduce the estimated P-e^- distance by $(0.6)^{-6}$ or 8-9%. τ_s, rather than τ_f, is used to estimate the P-e^- distance since it is more selective for a discrete complex (which has to have a lifetime at least as long as τ_s, 112 ns).

While neither spin-labeled protein had much of an effect on 5 mM (or 10 mM) diC_4PC relaxation rates, a small amount of additional relaxation was observed with spin-labeled D205C (see blow-up of the field dependence for this short-chain PC with both labeled D205C and H82C in *Figure S.3*). The residual relaxation

due to the spin-labeled D205C is 0.042 s^{-1} (spin-labeled H82C has no effect and its profile is essentially equivalent to diC_4PC alone) and the correlation time describing this is in the ns range (much like τ_f for the diC_6PC PRE). If we assume that diC_4PC binds to the same site as diC_6PC, we can use the ratio of $\Delta R_{P\text{-}e}(0)$ for 5 mM diC_4PC compared to the value for 5 mM diC_6PC (where we assume, at least to a first approximation, that the PC site is completely occupied) to estimate the fraction of diC_4PC bound to protein (0.25). This then provides a rough estimate of the K_d as 15 mM for diC_4PC binding to PI-PLC. The poor binding to PI-PLC of diC_4PC compared to diC_6PC that is monitored by these NMR experiments is consistent with its lack of kinetic activation of this bacterial PI-PLC (2).

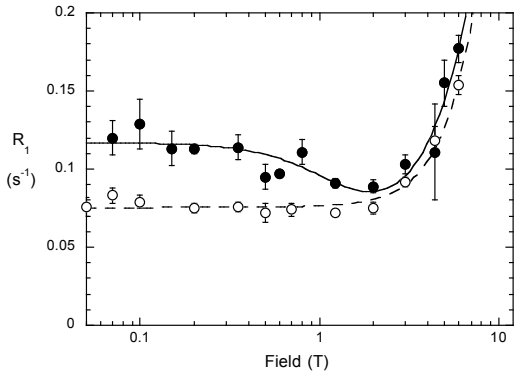

Fig. S.3. Field dependence of the R_1 for diC_4PC (5 mM) in the presence of 14.4 μM spin-labeled D205C (■) or H82C (❒).

Modeling of butylphosphocholine binding to the W47A/W242A dimer using Autodock4:

Binding of the butylphosphocholine was also evaluated to the structure of a mutated PI-PLC, W47A/W242A, that crystallized as a dimer (PDB code 2OR2 (3)). This structure is unlikely to be identical to a membrane bound dimer, should a PI-PLC dimer be formed on membranes, because the two tryptophan residues necessary for binding to PC vesicles (4) have been replaced by alanine. Nonetheless, the butylphosphocholine was docked to this dimeric structure to see if a site for PC could be predicted and whether or not the same region suggested with docking to the monomer would be identified. As shown in *Figure S.4*, the same region of the protein was highlighted by AUTODOCK as interacting with a phosphocholine headgroup, although individual interactions of protein molecules with the phosphocholine moiety were slightly different than when this ligand was docked to the monomer PI-PLC. In the dimer complex, energy minimization yielded structures where the phosphocholine phosphate moiety was significantly closer to the Asp205 carboxylate (~5 Å) than in monomer structure (~8 Å).

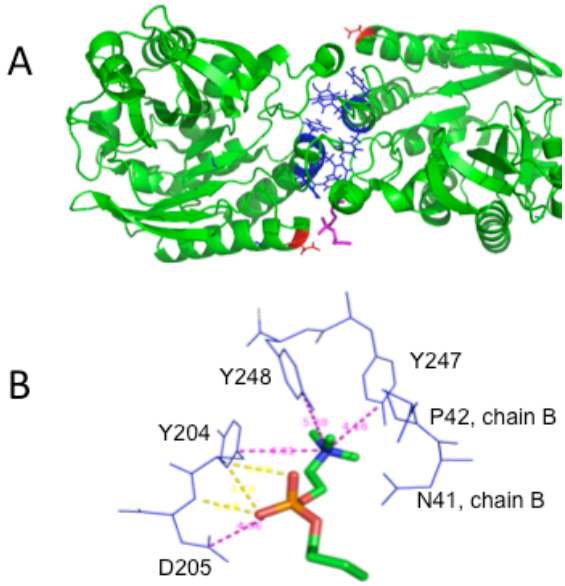

Fig. S.4. (A) Representative energy minimized structure of butylphosphocholine (pink) bound to the PI-PLC dimer structure. The tyrosine residues forming the dimer interface are blue. The red residue is Asp205. (B) Ligands forming the binding picket for butylphosphocholine; yellow dotted lines represent hydrogen bonds while the pink dotted lines indicate distances of atoms in different residues to the phosphocholine atoms.

References:

1. Klauda, J. B., Roberts, M. F., Redfield, A. G., Brooks, B. R., and Pastor, R. W. (2008) Rotation of lipids in membranes: molecular dynamics simulation, ^{31}P spin lattice relaxation, and rigid-body dynamics. *Biophys. J.* **94**, 3074-3083
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3. Shao, C., Shi, X., Wehbi, H., Zambonelli, C., Head, J. F., Seaton, B. A., and Roberts, M. F. (2007) Dimer structure of an interfacially impaired phosphatidylinositol-specific phospholipase C. *J. Biol. Chem.* **282**, 9228-9235
4. Feng, J., Wehbi, H., and Roberts, M. F. (2002) Role of tryptophan residues in interfacial binding of phosphatidylinositol-specific phospholipase C. *J. Biol. Chem.* **277**, 19867–19875