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Supplemental Information

The RNA-Binding Site of Poliovirus 3C Protein

Doubles as a Phosphoinositide-Binding Domain

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Supplemental Figures

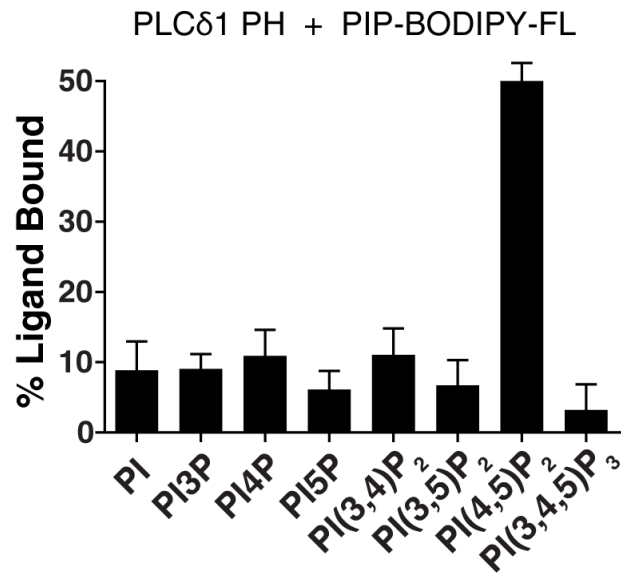


Figure S1. Validation of the fluorescence polarization-based phosphoinositide (PIP)-binding assay. Related to Figure 3. The well-characterized phospholipase C-delta1 pleckstrin homology (PLC- δ 1 PH) domain shows phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) specificity, as previously described (Garcia et al., 1995). The experiment was conducted at a fixed PLC δ 1 PH concentration (34 nM) using 0.4 nM of each PIP-probe in a solution containing 20 mM HEPES at pH 7.5 and 100 mM NaCl. Error bars represent the SEM (n = 3).

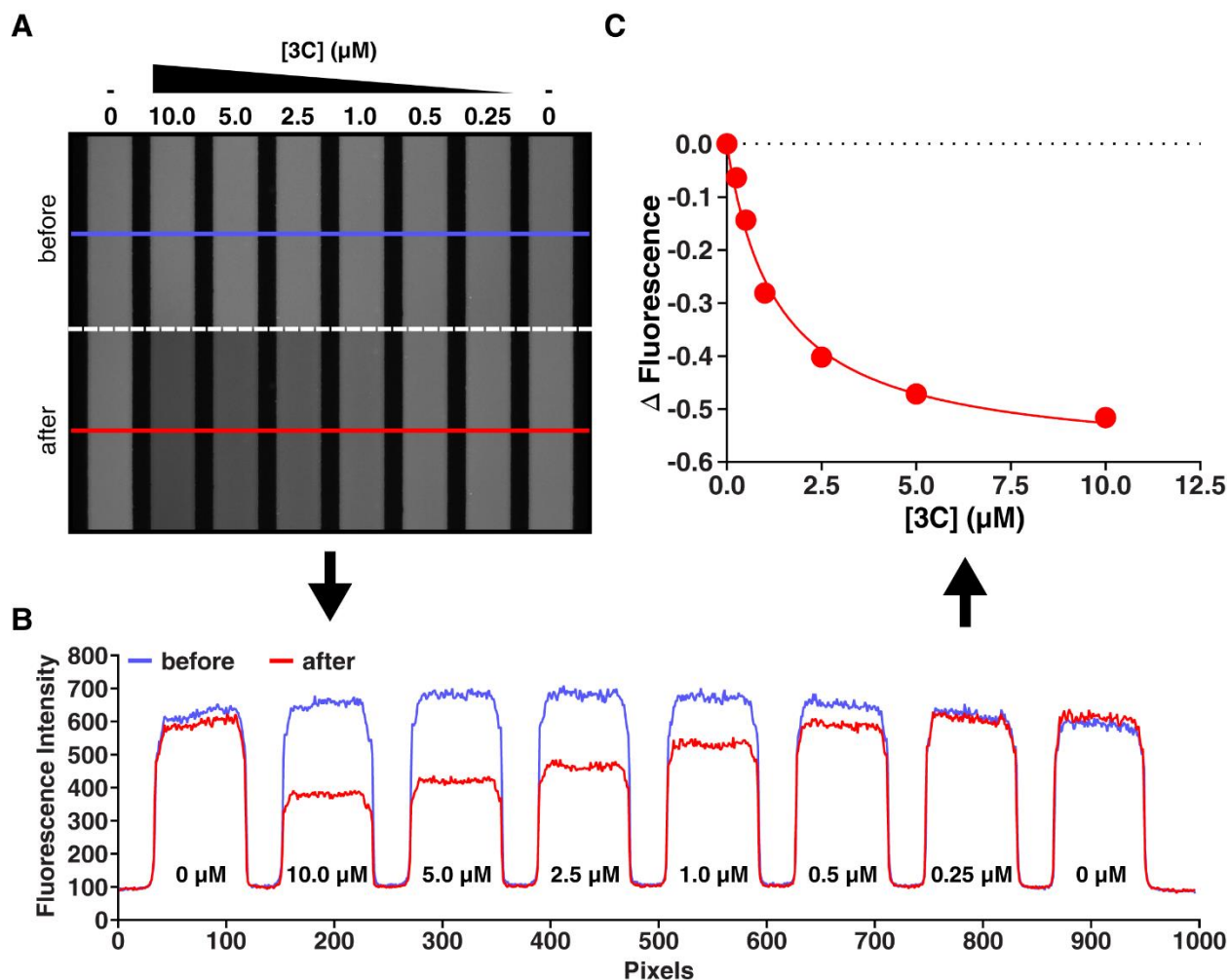


Figure S2. Data analysis for supported lipid bilayer binding experiments. Related to Figure 4. (A) The view of the microchannels before and after adding 3C at indicated concentrations. A 10X objective was used for imaging along with Alexa 568 filter set with an excitation and emission at 576 nm and 603 nm, respectively. Exposure time was set at 200 ms. (B) Data from the line scan across microchannels is plotted as a function of fluorescence intensity vs. pixels. Blue, before adding 3C; and red, after adding 3C. (C) Data is processed to calculate the change in fluorescence within each microchannel followed by normalization based on the reference channels using the following equation:

$$\frac{\left[\frac{\Delta F_{protein}}{\Delta F_{blank}} \right]_{post} - \left[\frac{\Delta F_{protein}}{\Delta F_{blank}} \right]_{pre}}{\alpha}$$

Here $\Delta F_{protein}$ represents the background subtracted fluorescence intensity of the protein channel, ΔF_{blank} represents the background subtracted fluorescence intensity of the reference channel, *pre* and *post* refer to before and after protein titration steps, and α represents the correction factor which is the ratio of the fluorescence intensities of the protein channel and the blank channel ($[F_{protein}/F_{blank}]_{pre}$) at a pre-protein titration step. Since the fluorescence intensity decreases as a function of 3C concentration, normalized data is negative in value. Shown is the hyperbolic fit, which is used to calculate an apparent dissociation constant ($K_{d,app}$).

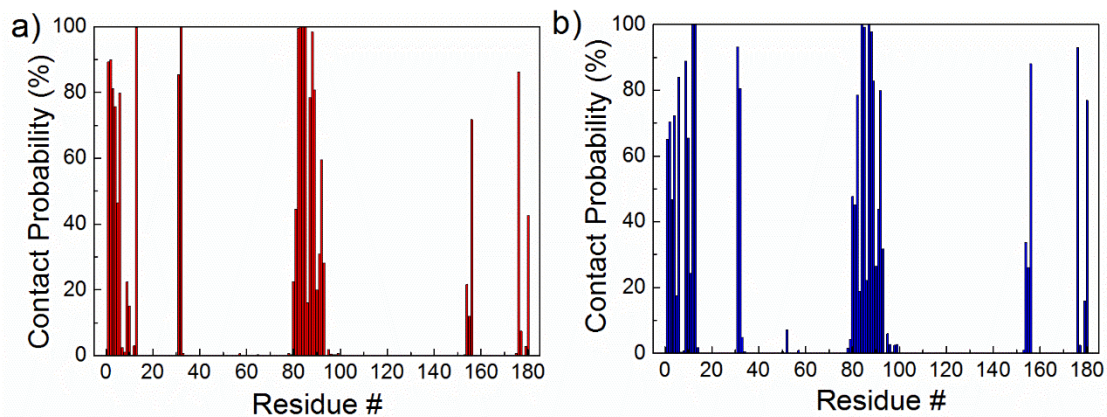


Figure S3. Contact frequency of residues of 3C with the membrane. Related to Figure 5. For this analysis, the last 300 ns of the trajectory was used. Residues within 5 Å of PI4P or PI(4,5)P₂ are counted. The 5 Å cutoff was selected because this distance captured interactions mediated by cations. Most interactions could have been captured by a cutoff less than or equal to 4 Å. a) for POPC/PI4P membrane; b) for POPC/PI(4,5)P₂ membrane.

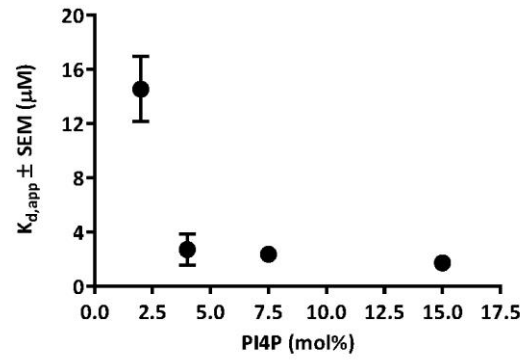


Figure S4. The apparent dissociation constant, $K_{d,app}$, is sensitive to the mole fraction of PI4P. **Related to Figure 5.** The apparent dissociation constants, $K_{d,app}$, determined for the 3C-PI4P interaction using the SLB binding assay at different mole fractions of PI4P (mol%).

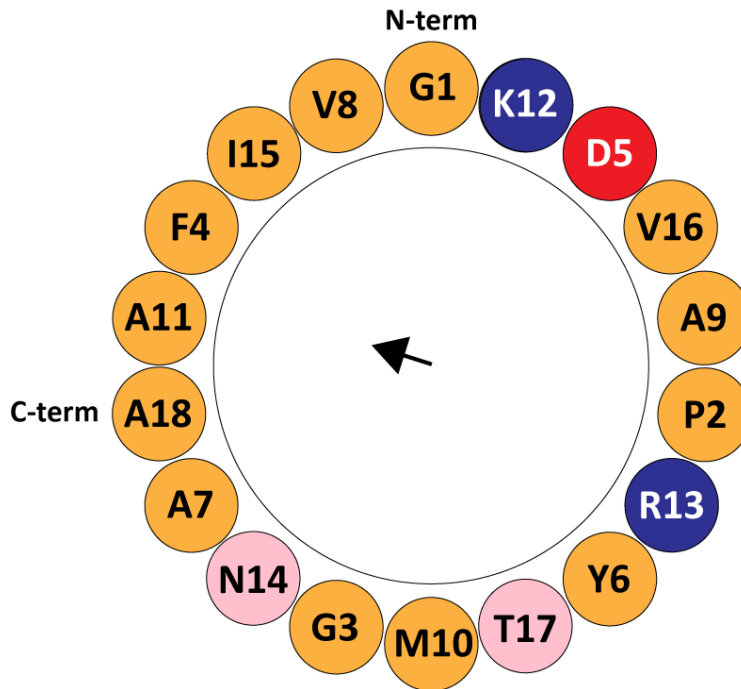


Figure S5. The N-terminal region of 3C forms an amphipathic helix. Related to Figure 6. The HeliQuest-Analysis web server was used to plot the N-terminal residues (1-18) of 3C into an alpha helix (<http://heliquet.ipmc.cnrs.fr/>). The arrow indicates the hydrophobic momentum. Hydrophobic residues, orange; basic residues, blue; acidic residues, red; polar uncharged residues, pink.