Electronic Supplementary Material (ESI) for Faraday Discussions.

Supporting Information

Optically Sensing Phospholipid Induced Coil-Helix Transitions in the Phosphoinositide-Binding Motif of Gelsolin

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1. Supplementary Figures

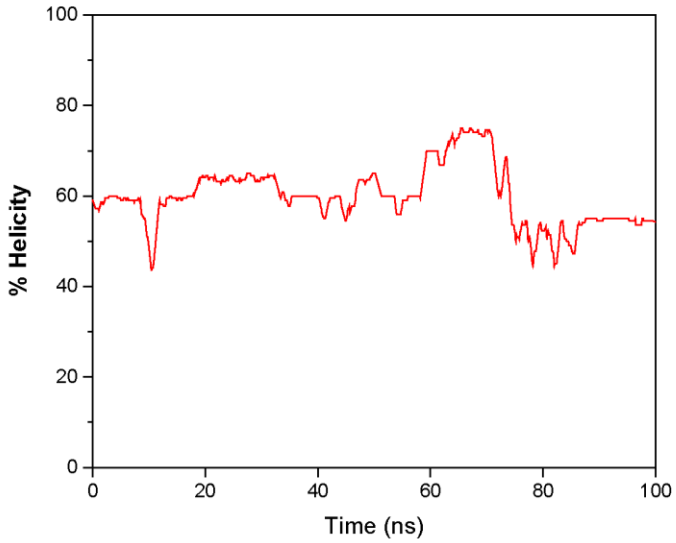


Figure S1: Helical character (%) of the Gel150-169 peptide monitored over 100 ns in the presence of PI(4,5)P2 containing membrane.

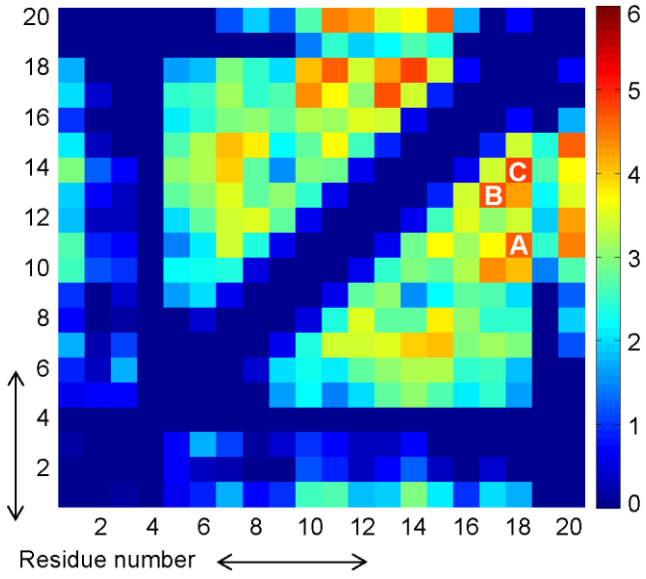


Figure S2. Distance difference map representing the inter residue distance changes between the alpha carbons within Gel150-169 sequence with amide as C-terminus upon going from the helix form to the coil form. The color scale represents the change in average distances between 0-6 Å. A, B and C indicate positions with maximum distance change.

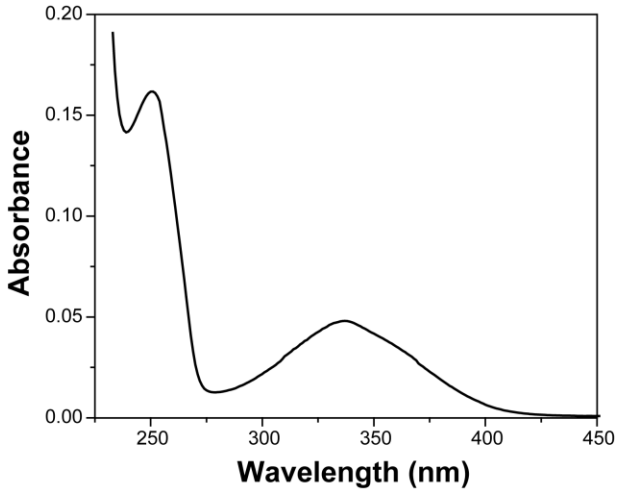


Figure S3. Absorption spectrum of 10 µM IAEDANS solution in aqueous buffer (20 mM HEPES, 100 mM NaCl, pH 7.4, 0.07 % DMSO). Molar absorption coefficient (ε) of IAEDANS at 287 nm is 1339 $M^{-1}cm^{-1}$. The concentration of IAEDANS was calculated based on the reported value of ε of IAEDANS which was 4500 $M^{-1}cm^{-1}$ at 337 nm. [(1)]

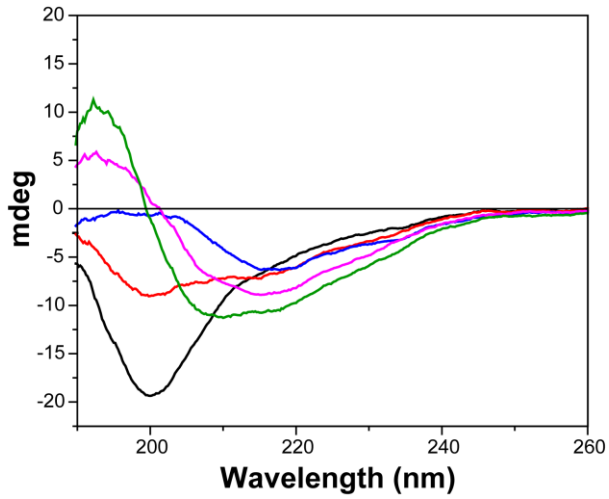


Figure S4. CD spectra of Gel-*Q11W-G18C* peptide solution (40 µM) in phosphate buffer (10 mM, pH 7.5) in presence of varying concentrations of PI(4,5)P2 micelles – 0 µM (black), 10 µM (red), 20 µM (blue), 40 µM (pink), 80 µM (green). CD spectra were recorded at 25 °C in a quartz cuvette (10 mm x 2 mm) in a JASCO J-1500 CD Spectrometer.

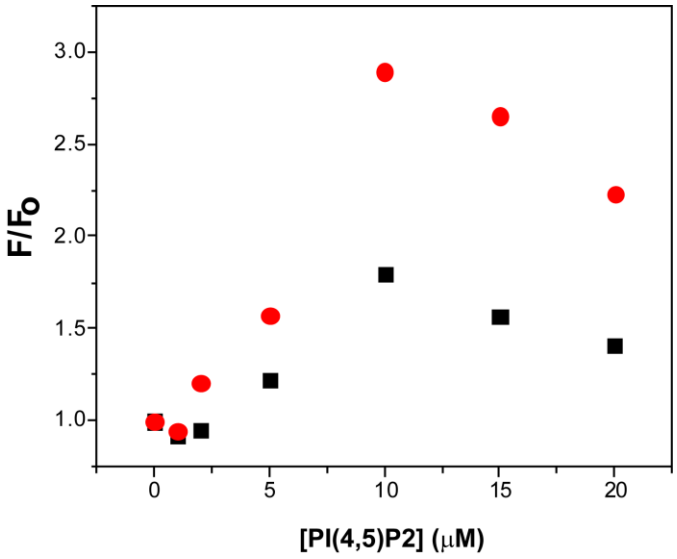


Figure S5. Normalized fluorescence emission at 480 nm for IAEDANS at λ_{ex} 340 nm (black) and at λ_{ex} 287 nm (red). Concentration of Gel-*Q11W-G18C*-IAEDANS was 10 μM.

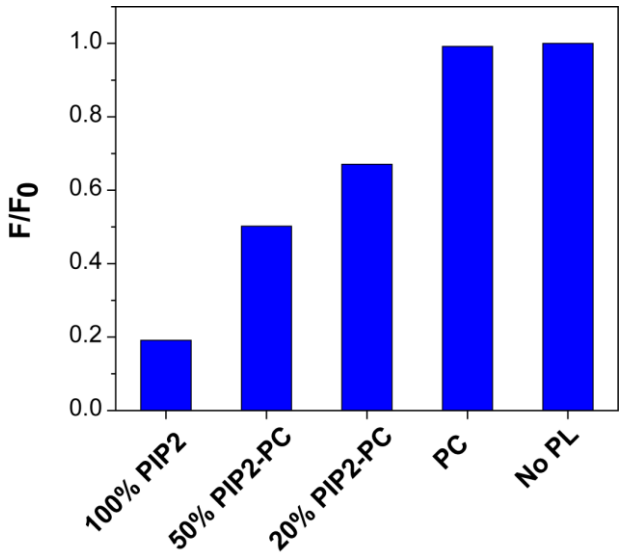


Figure S6. Normalized fluorescence intensity at 360 nm (λ_{ex} 287 nm) for Gel-*Q11W-G18C*-IAEDANS (10 μM) in the presence of 100% PI(4,5)P2 micelles, 50% PI(4,5)P2 vesicles, 20% PI(4,5)P2 vesicles, PC, and in the absence of phospholipids. All phospholipid concentrations were 10 μM.

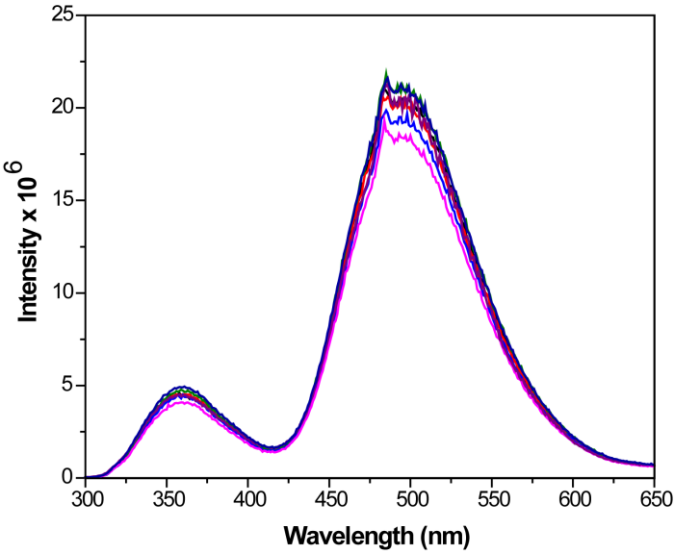


Figure S7. Fluorescence emission spectra for Gel-*Q11W-G18C*-IAEDANS (10 μM) with increasing concentrations of PC (0-20 μM), λ_{ex} 287 nm.

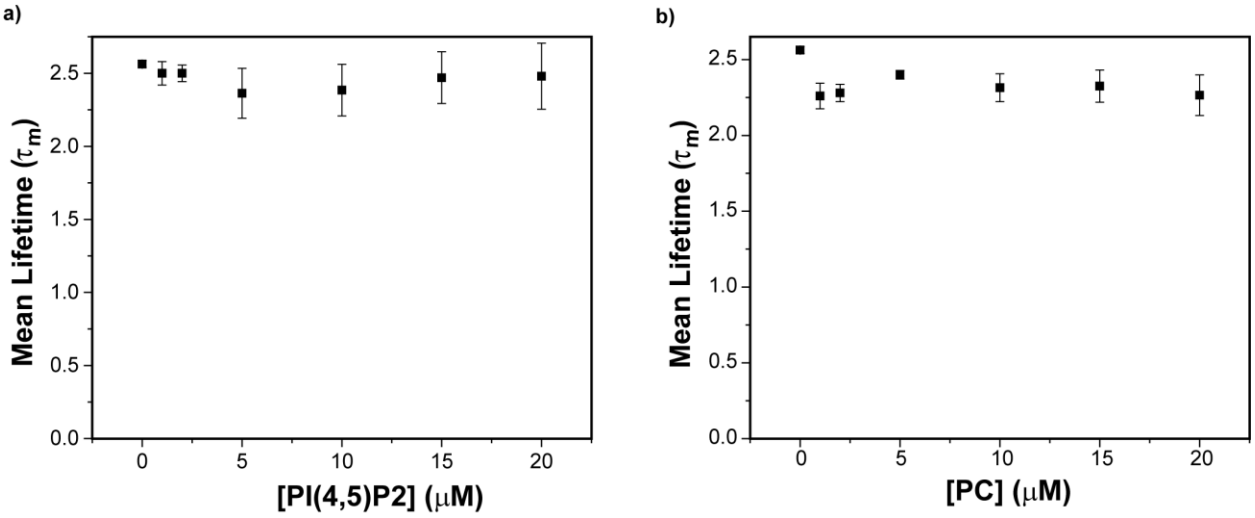


Figure S8. Average lifetime for tryptophan in Gel-*Q11W-G18C* (10 μM) in the presence of increasing concentrations of a) PI(4,5)P2-PC micelles (1-20 μM), and b) PC vesicles (1-20 μM), λ_{ex} 287 nm.

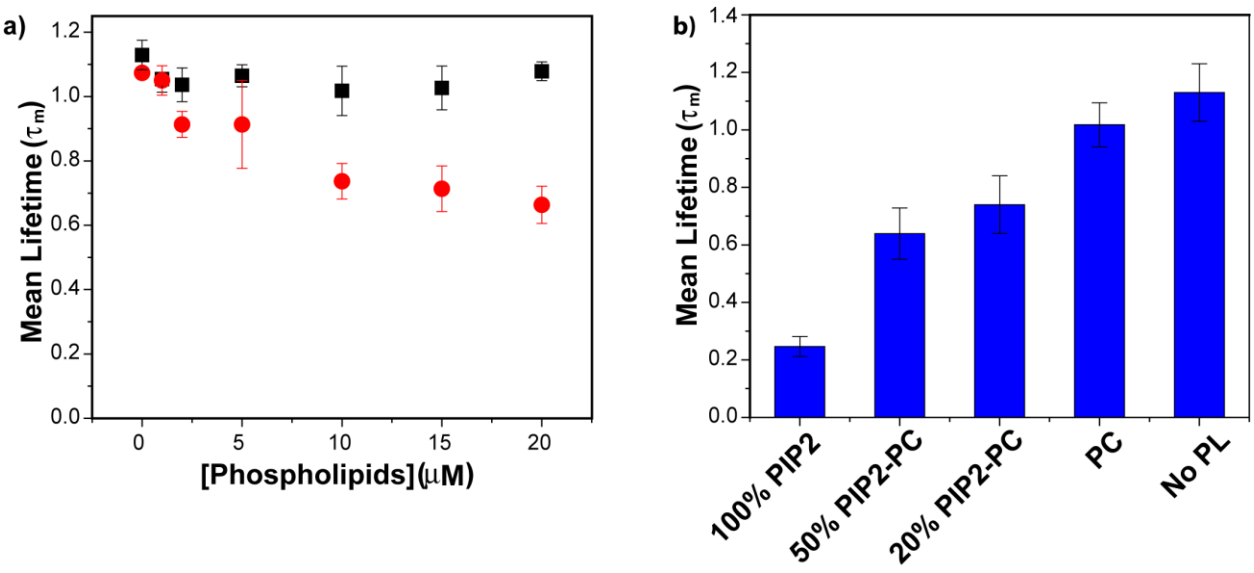


Figure S9. a) Average lifetime for tryptophan in Gel-*Q11W-G18C*-IAEDANS (10 μM) in the presence of increasing concentrations of PC (black squares) and increasing concentrations of 20% PI(4,5)P2-PC (red circles), λ_{ex} 287 nm. b) Average lifetime for tryptophan in Gel-*Q11W-G18C*-IAEDANS (10 μM) in the presence of 100% PI(4,5)P2 micelles, 50% PI(4,5)P2 vesicles, 20% PI(4,5)P2 vesicles, PC, and in the absence of phospholipids. All phospholipid concentrations were 10 μM.

2. Characterization and purity of peptides

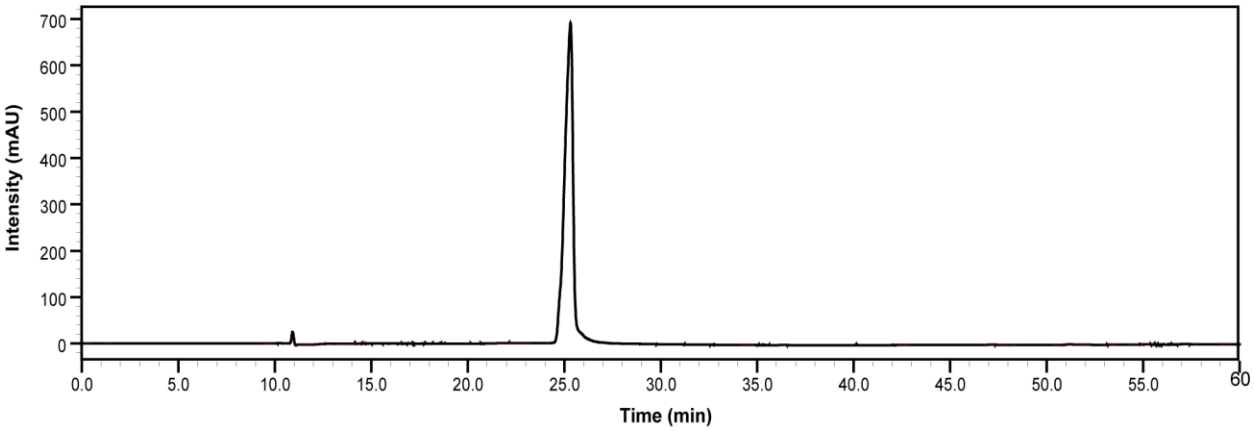
a) Gel-Q11W-G18C, KHVVPNEVVVWRLFQVKCRR-$CONH_2$

Molecular weight: 2492.01 (Calculated)

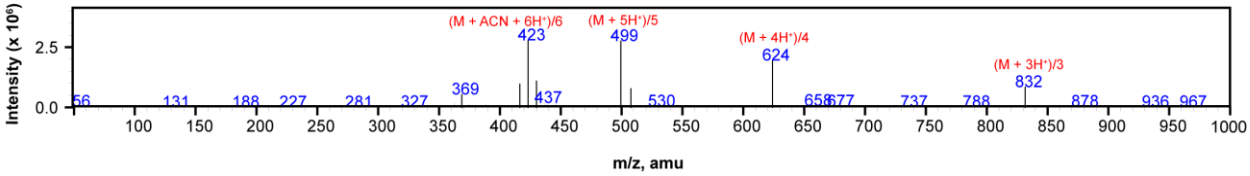
LR-ESI/MS, profile mode: 2492

MALDI-TOF-MS: m/z, 2492.90 ($M+H^+$)

LC elution trace at 280 nm:



ESI-MS (+ve mode) of peak (25 min) in LC:



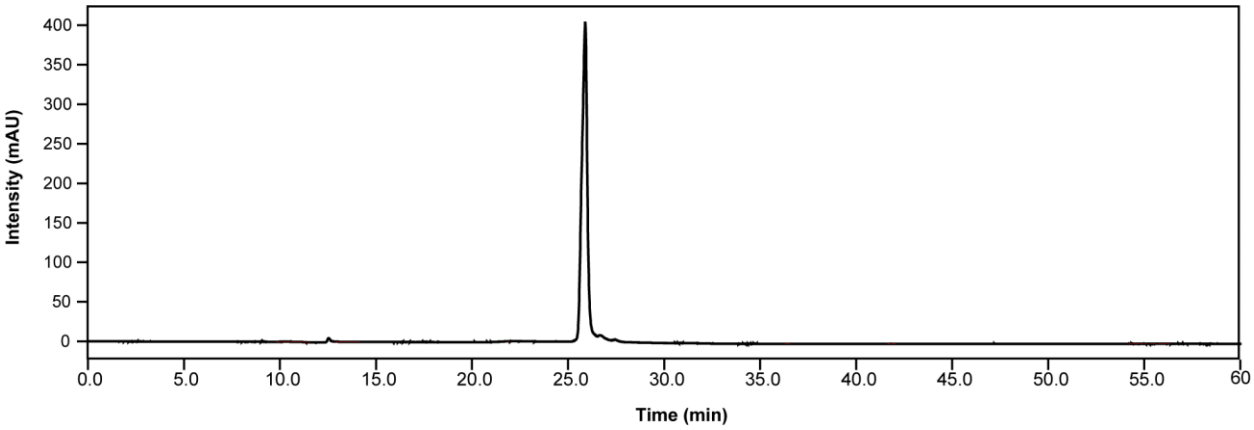
b) Gel-Q11W-G18C-IAEDANS: KHVVPNEVVVWRLFQVKC-(IAEDANS)RR

Molecular weight: 2798.35 (Calculated)

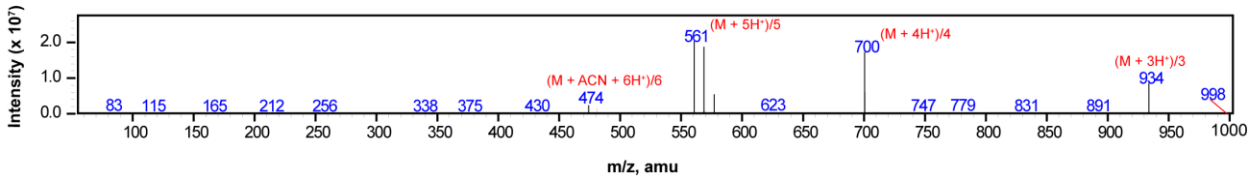
LR-ESI/MS, profile mode: 2798

MALDI-TOF-MS: m/z, 2799.07 ($M+H^+$)

LC elution trace at 340 nm:



ESI-MS (+ve mode) of peak (26 min) in LC:



3. Dynamic light scattering (DLS) data for phospholipid vesicles

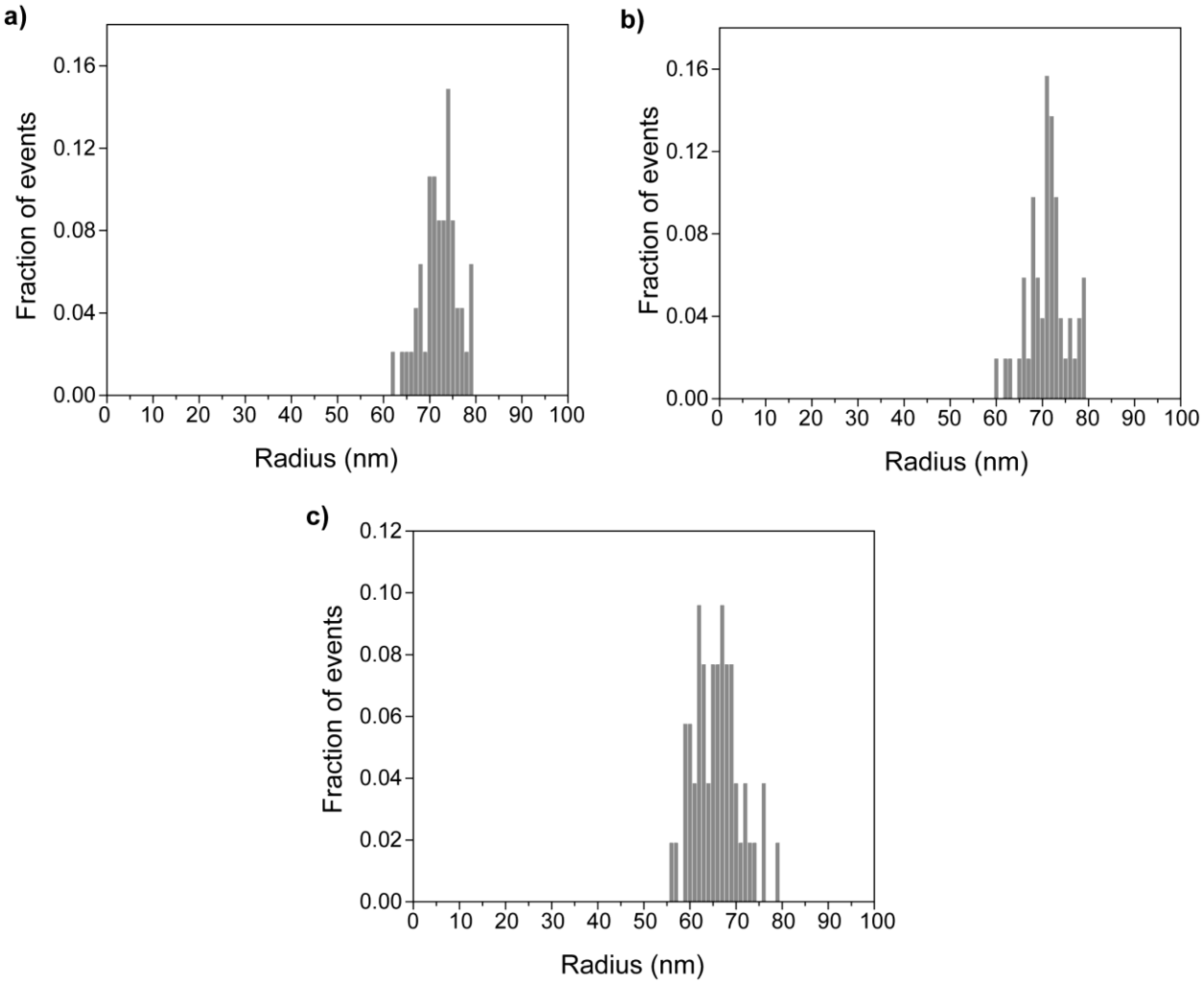


Figure S10. Size distribution (hydrodynamic radius) of a) PC vesicles, b) 20 % PI(4,5)P2-PC vesicles, and c) 50 % PI(4,5)P2-PC vesicles measured by DLS.

6. References

(1) Saxena, A. M.; Udgaonkar, J. B.; Krishnamoorthy, G. Characterization of Intra-molecular Distances and Site-specific Dynamics in Chemically Unfolded Barstar: Evidence for Denaturant-dependent Non-random Structure. *J. Mol. Biol.* **2006**, 359, 174-189.