Supporting Information

Development of a direct and continuous phospholipase D assay based on the chelation-enhanced fluorescence property of 8-hydroxyquinoline

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Development of the method

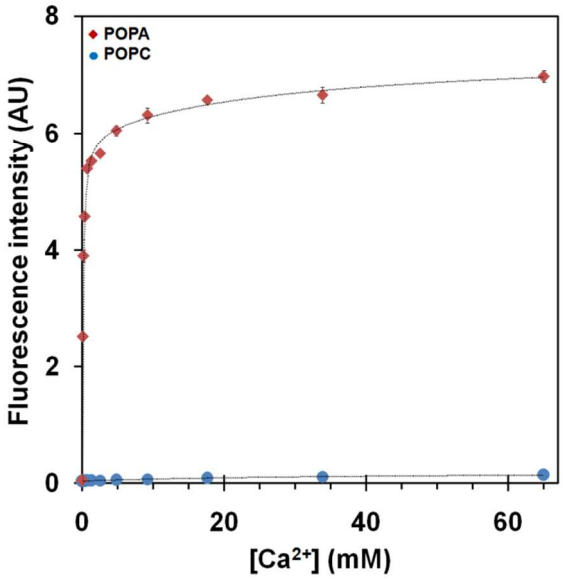

Figure S1. Fluorescence emission intensity of 8HQ as function of increasing amounts of Ca^{2+} and in the presence of mixed SDS/Triton X-100 micelles containing 0.26 mM of either POPC (●) or POPA (◆). Values presented are the means ± SD obtained from three independent experiments and fitted (dashed curves) using a non-linear regression. Bandwidths were set at 9 nm for excitation and 20 nm for emission. (AU: Arbitrary Units).

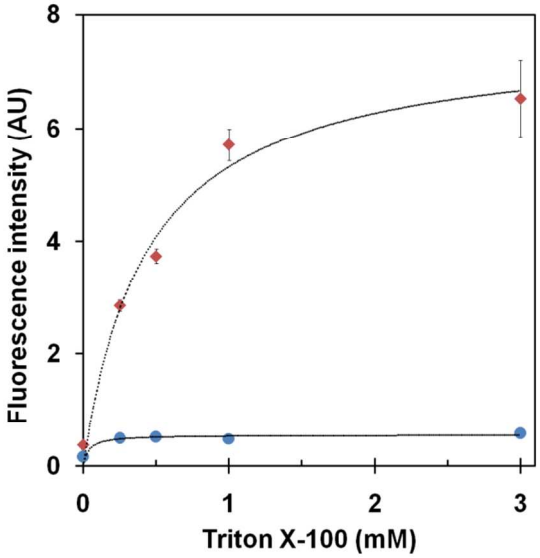

Figure S2. Fluorescence emission intensity of 8HQ in the presence of 0.26 mM of either POPC (●) or POPA (◆) as function of increasing amounts of Triton X-100. Values presented are the means ± SD obtained from three independent experiments and fitted (dashed curves) using a non-linear regression. Bandwidths were set at 9 nm for excitation and 20 nm for emission. (AU: Arbitrary Units).

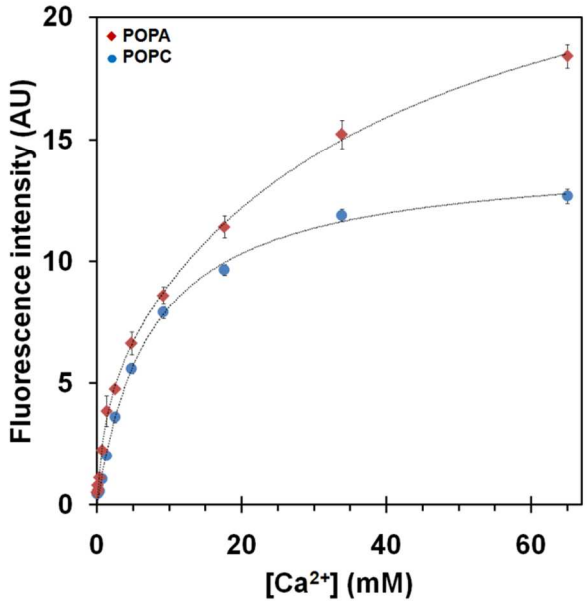

Figure S3. Fluorescence emission intensity of 8HQ5S as function of increasing amounts of Ca^{2+} and in the presence of mixed SDS/Triton X-100 micelles containing 0.26 mM of either POPC (●) or POPA (◆). Values presented are the means ± SD obtained from three independent experiments and fitted (dashed curves) using a non-linear regression. Bandwidths were set at 9 nm for excitation and 20 nm for emission. (AU: Arbitrary Units).

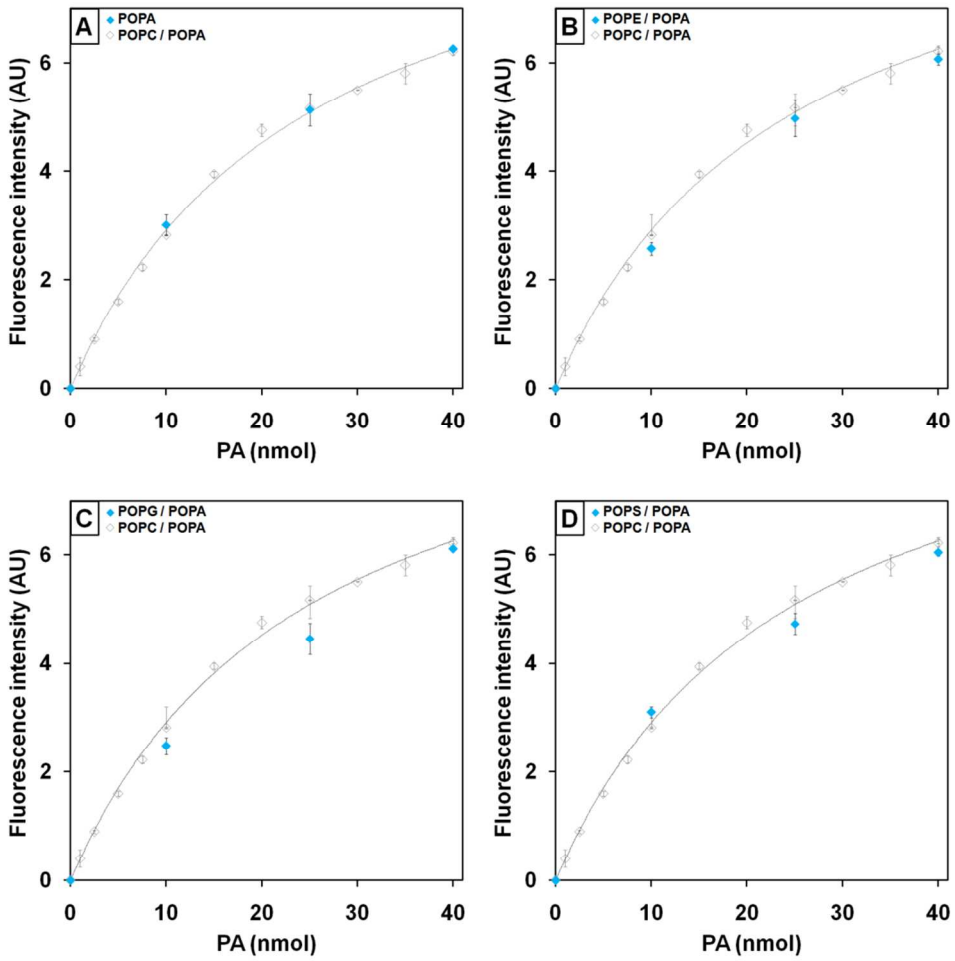

Figure S4. Calibration curve for the quantification of PA using 8HQ fluorescence at 490 nm (λ_{ex} 366 nm). 8HQ was incubated with SDS/Triton X-100 mixtures containing variable amounts of (A) POPA, (B) POPE/POPA, (C) POPG/POPA and (D) POPS/POPA at pH 8, in the presence of 20 mM Ca^{2+}. Values were compared with the calibration curve obtained with POPC/POPA mixtures and fitted (dashed curve) using a non-linear regression. Except for POPA, each value was obtained by increasing the proportions of POPA in line with decreasing the proportions of the selected phospholipid to maintain a constant final concentration of phospholipids (0.26 mM, final concentration). Values presented are the means ± SD obtained from three independent experiments. Bandwidths were set at 9 nm for excitation and 20 nm for emission. (AU: Arbitrary Units).

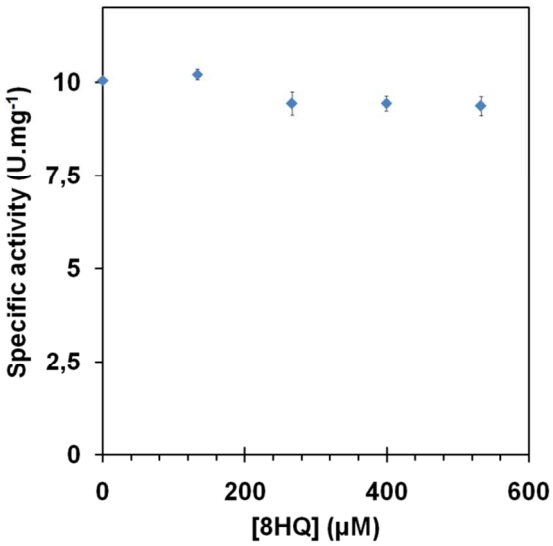

Figure S5. Specific activity of the purified recombinant VuPLD toward mixed SDS/Triton X-100 micelles containing POPC (0.26 mM, final concentration) as the substrate and in the presence of increasing amount of 8HQ. Free choline was quantified using the enzyme-coupled choline oxidase / peroxidase assay described in the Experimental section. One unit of PLD activity was defined as the amount of enzyme releasing 1 µmol of choline per minute under the experimental conditions. Values presented are the means ± SD obtained from three independent experiments.

Kinetic recordings of the PLD-catalyzed formation of PA.

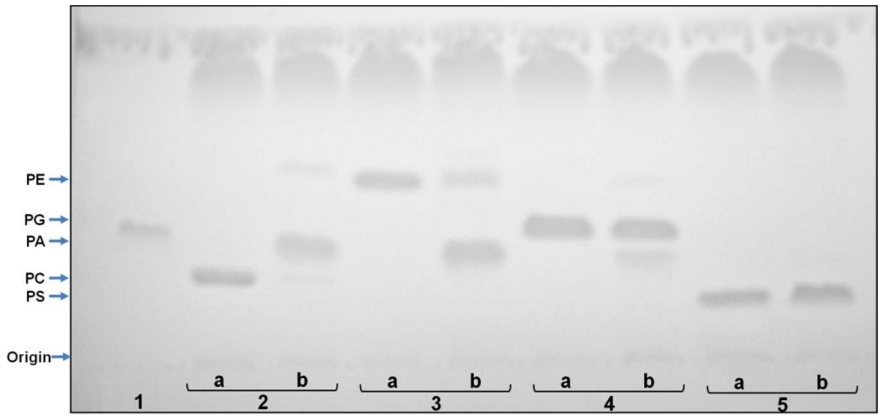

Figure S6. TLC analysis of the VuPLD-catalyzed hydrolysis of mixed SDS/Triton X-100 micelles containing 0.26 mM of POPC, POPE, POPG or POPS. Lipids were extracted either immediately after (a) starting the reaction or after 30 min (b) of reaction, developed as described in the Experimental section and revealed using iodine vapor. Arrows point to POPA, POPC, POPE, POPG, POPS and origin of application. Lane 1: POPA, lane 2: POPC, lane 3: POPE, lane 4: POPG, lane 5: POPS.

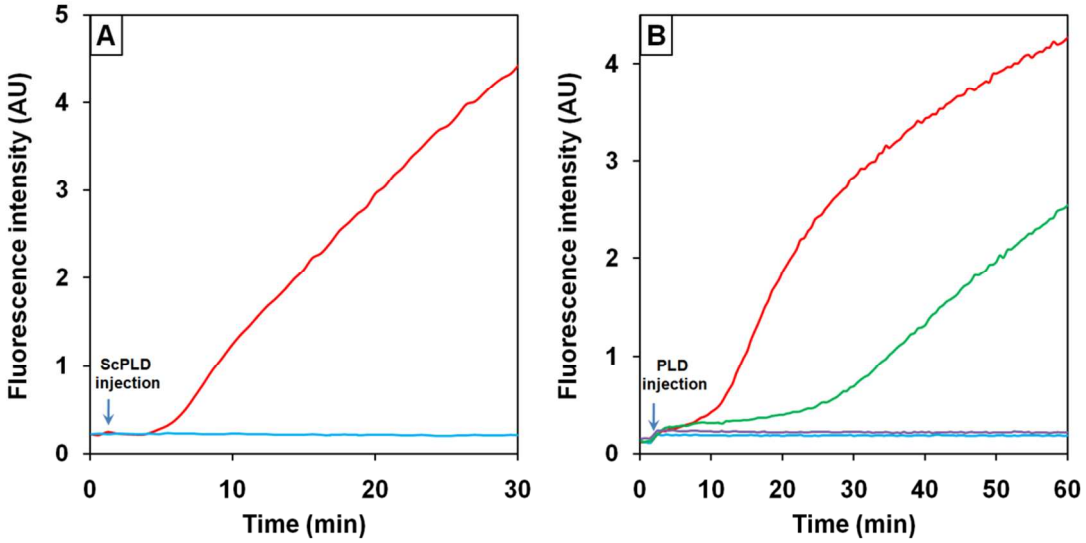

Figure S7. Kinetic recording of POPA formation using 8HQ fluorescence at 490 nm (λ_{ex} 366 nm) and mixed SDS/Triton X-100 micelles containing POPC (0.26 mM, final concentration) as the substrate. (A) Kinetic recording of POPA formation after an injection (arrow) of 0.15 µg of ScPLD (red curve) or buffer alone (blue curve). (B) Kinetic recording of POPA formation after an injection (arrow) of 0.06 µg of BoPLD (red curve) or heat-inactivated BoPLD (blue curve) and 0.04 µg of AhPLD (green curve) or heat-inactivated AhPLD (purple curve).