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Linking Phospholipase Mobility to Activity by Single-Molecule Wide-Field Microscopy

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Supporting Information

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Journal name:

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Title:

Linking phospholipase mobility to activity by single molecule wide-field microscopy

Authors:

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Supporting Figures

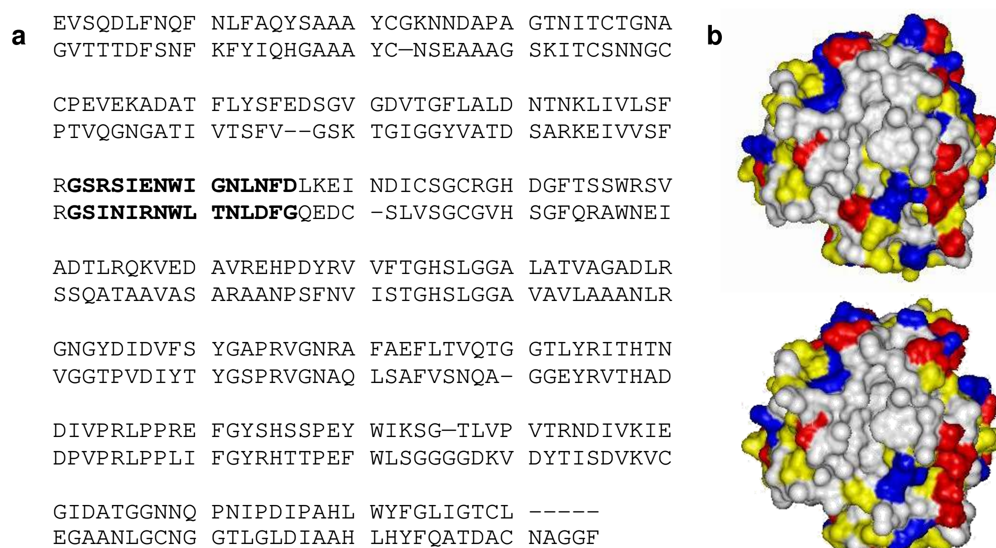

Figure S1. Sequence and structural differences between TLL and aPLA1. (**a**) amino acid sequence of TLL (*first line*) and aPLA1 (*second line*). The residues located on the lid are in bold. (**b**) Open structure model of TLL (*top*) and aPLA1 (*bottom*). The model of the open structure of aPLA1 is constructed from the X-ray structure of the aPLA1 backbone except at the open part of the lid region, where it is modeled on the open part of the TLL structure. The properties of the surface residues are indicated by colour coding. The hydrophobic residues are shown in white, hydrophilic residues in yellow, positively-charged residues in blue and negatively-charged residues in red.

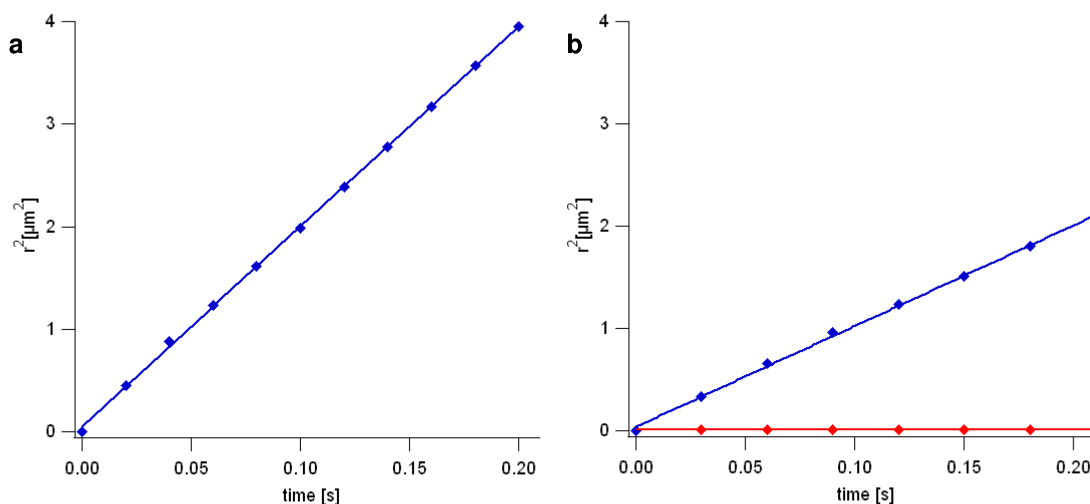

Figure S2. Phospholipid diffusion constants. Diffusion constant of the lipid probe DiI while diffusing on POPC multilayers, D = (4.88 ±0.03) × 10^{-8} $cm^2.s^{-1}$ (a) and on POPC bilayers, D ~ 0 and D = (2.46 ±0.03) × 10^{-8} $cm^2.s^{-1}$.

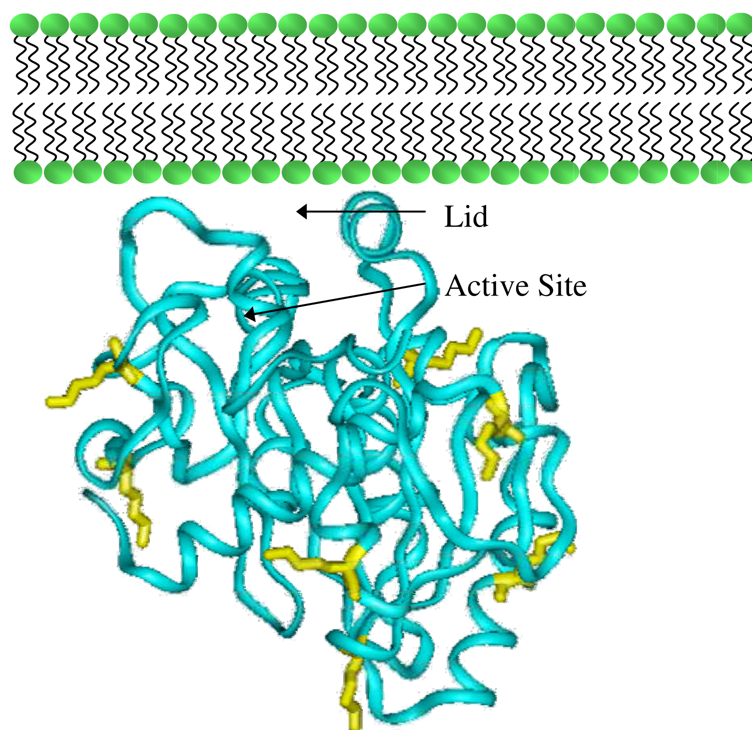

Figure S3. PLA1 model backbound in cyan and the 7 Lysines in yellow. The active site and lid are indicated by black arrows. The phospholipid surface contact is represented on the top. The enzyme will have an open lid and be penetrated slightly into the phospholipid layer.

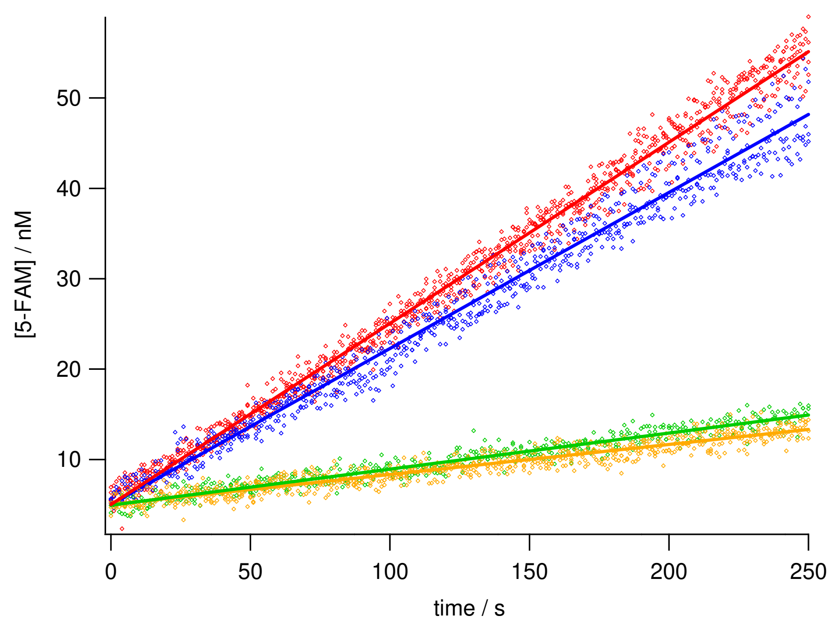

Figure S4. Fluorescence intensity of 5-FAM as function of time. For the activation of the surface enzyme in solution (e.g, in order to open the lid protecting the active site), Triton X-100 was added to the solution. 5-CDFA: 0.72mM; Triton X-100: 0.5mM *blue* non-labeled PLA1 (3.7×10^{-8} M), 0.1721 ± 0.0009 nM 5-FAM.s^{-1} *red* PLA1 labeled with PDI (3.7×10^{-8} M), 0.2005 ± 0.0006 nM 5-FAM.s^{-1} *green* inactive PLA1 (3.7×10^{-8} M), 0.03990 ± 0.00049 nM 5-FAM.s^{-1} *yellow* 5-CFDA autohydrolysis 0.03333 ± 0.00042 5-FAM.s^{-1}. From the figure it shows that the labelling of the enzyme with NHS-PDI has no affect on its activity towards 5-CFDA.

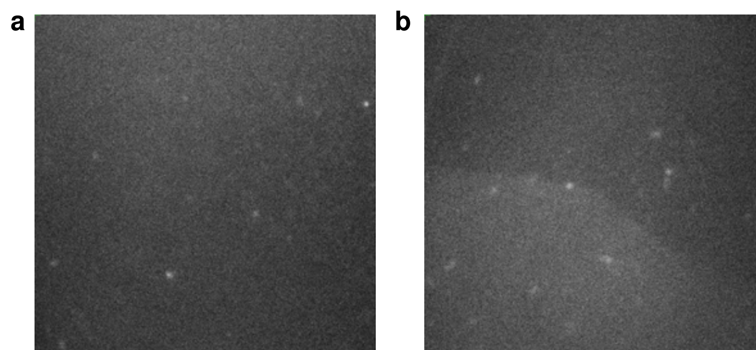

Figure S5. Fluorescence images of labelled aPLA1 and labelled POPC bilayers (**a**) Using one color excitation (**b**) Using dual color excitation.

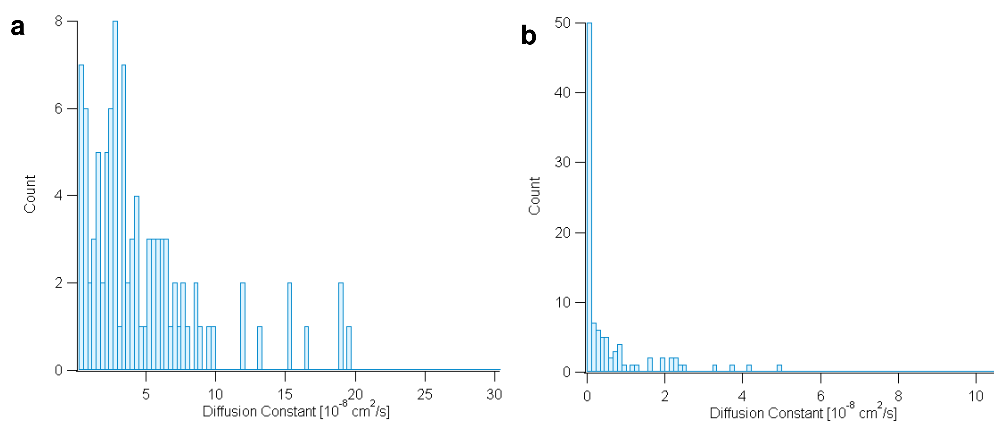

Figure S6. Distribution of the diffusion coefficients calculated for each individual track. **(a)** enzyme molecules diffusing on the layer **(b)** and on the edge

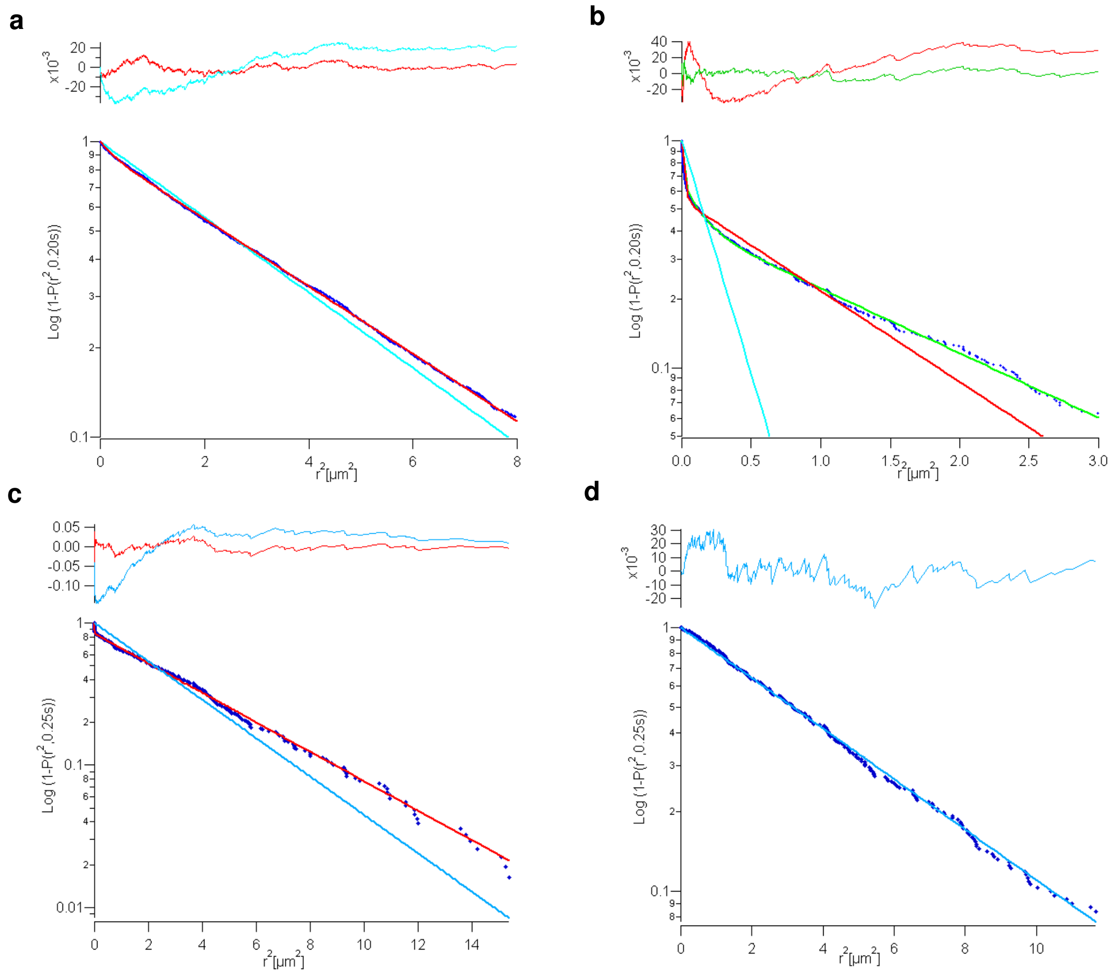

Figure S7 Fits of the CDFs to the experimental data for aPLA1. *Dark blue* experimental points, *light blue* CDF with one motion, *red* CDF with two motions, *green* CDF with three motions (**a**) active enzyme diffusing on the layer (**b**) active enzyme diffusing at the layer edge (**c**) inactive enzyme diffusing on the layer and on the edge (**d**) inactive enzyme diffusing on the layer only.

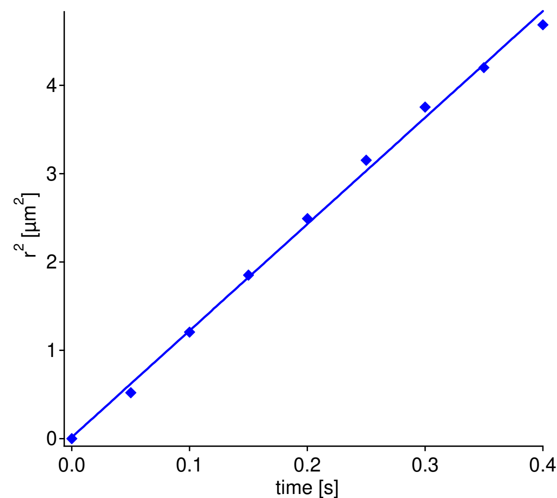

Figure S8. TLL diffusion coefficient on bilayers. The TLL molecules diffuse on the layer with D = 3.0 x 10^{-8} cm^2**.s^{-1}**.

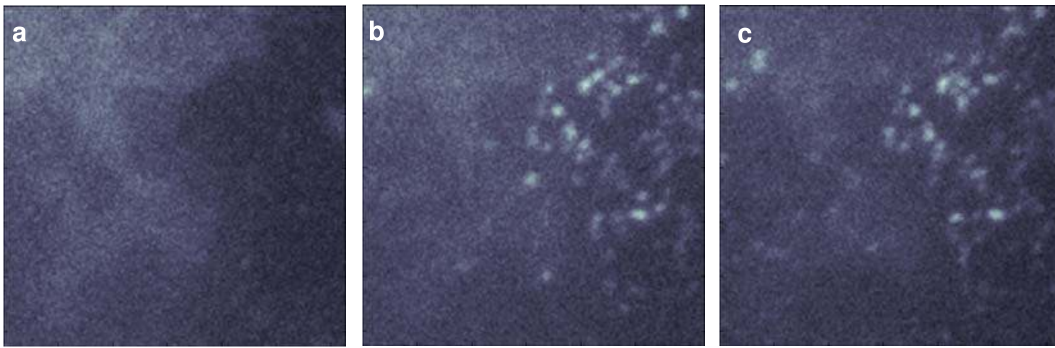

Figure S9. Interaction between the enzyme molecules and the mica support. (**a**) Fluorescence image of POPC labelled multilayers on the mica support before enzyme addition. (**b-c**) The added enzyme shows a strong affinity by the mica support, where it remains immobilized (time lapse between images: 200ms)

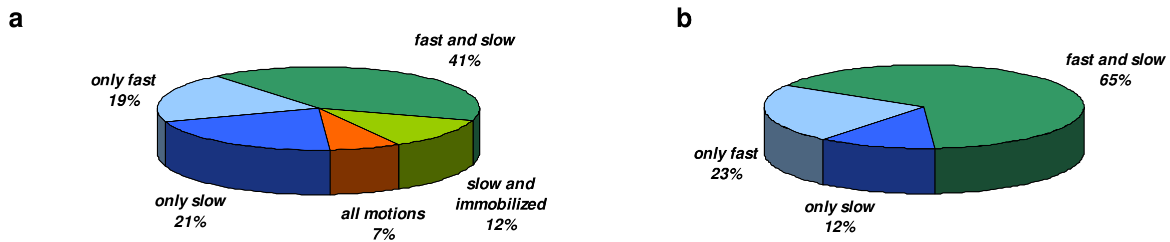

Figure S10. Heterogeneity of single aPLA1 trajectories. Percentages of trajectories with single and multiple modes of motion (**a**) on the edge of the layer (**b**) on top of the layer.

Supporting Table

Supporting Table Diffusion coefficients for supported bilayers.

Lipid composition (support)	Dye	Method	Diffusion Constant (10^{-8} $cm^2.s^{-1}$)
POPC (glass)	TMR-POPC	SPT	1.42 (1)
POPE:POPC 7:3 (glass)	Cy5-DHPE	SPT	4.6 (2)
DOPC (mica)	C8-BODIPY 500/510 C5-HPC	FCS	3.1 (3)
	BODIPY FL DHPE		3.5
	DiD		3.8

(1) Schmidt, T., Schütz, G.J., Baumgartner, Gruber, H.J., Schindler, H. Characterization of photophysics and mobility of single molecules in a fluid lipid membrane, *J. Phys. Chem.* **99**, 17662-17668 (1995).

(2) Sonnleitner, A., Schütz, G.J., Schmidt, T. Free brownian motion of individual lipid molecules in biomembranes, *Biophys. J.*, **77**, 2638-2642 (1999).

(3) Przybylo, M., *et al*. Lipid diffusion in giant unilamellar vesicles is more than 2 times faster than in supported phospholipid bilayers under identical conditions. *Langmuir*, **22**, 9096-9099 (2006).

Supporting Video Legends

Supporting Video 1

This movie shows the hydrolysis of labelled phospholipid multilayers by non-labelled aPLA1 over an area of 41 x 41 μm^2. When in presence of multilayers the hydrolysis leads to collapse of the first bilayer in a wave-front-like pattern. The movie runs at 5 times normal speed.

Supporting Video 2

This movie shows the hydrolysis of a labelled phospholipid bilayer on mica by non-labelled aPLA1 at different enzyme concentrations. For high phospholipase concentrations, desorption of the hydrolyzed phospholipids occurs with a wave-front-like pattern. With an enzyme concentration of ~ 2 x 10^{-7} M, the desorption of the phospholipid molecules creates channel-like patterns. At lower aPLA1 concentration, collapse of the bilayer proceeds through nibbling of the layer edges. The movie runs at 5 times normal speed.

Supporting Video 3

This movie was obtained with a high concentration (~10^{-7} M) of labelled aPLA1 on labelled POPC bilayers. These images show that hydrolysis occurs mainly at the edges of the layer and that areas that recede fastest coincide with areas of higher local enzyme concentration. The movie runs at normal speed.

Supporting Video 4

After addition of a lower concentration (~10^{-9} M) of labelled aPLA1 to labelled POPC bilayers, it is possible to follow simultaneously enzyme diffusion and hydrolysis of the substrate. On top of the layer only fast diffusing enzymes are observed, while at the layer edge region single enzyme docking events are also detected. The movie runs at 0.5 times normal speed.

Supporting Video 5

Even at low aPLA1 concentration (~10^{-9} M) it is still possible to visualize the retraction of the top layer of a labelled POPC multilayer. In this movie images are accumulated over 20 frames, and shown every 100 frames. The movie runs at 50 times normal speed.

Supporting Video 6

The behaviour of iPLA1 and TLL differs from aPLA1. When iPLA1 is added to labelled POPC, no layer retraction is observed. In contrast to the freely diffusing enzymes on top of the layer, the inactive enzyme molecules at the layer edge remain immobilized until the label photobleaches. To visualize TLL a concentration 100 times higher is required. Even then, the enzyme diffuses with no binding. The movie runs at 0.5 times normal speed.