Biophysical Journal, Volume *118*

Supplemental Information

To Hop or not to Hop: Exceptions in the FCS Diffusion Law

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SUPPLEMENTARY INFORMATION

To hop or not to hop: Exceptions in the FCS diffusion law

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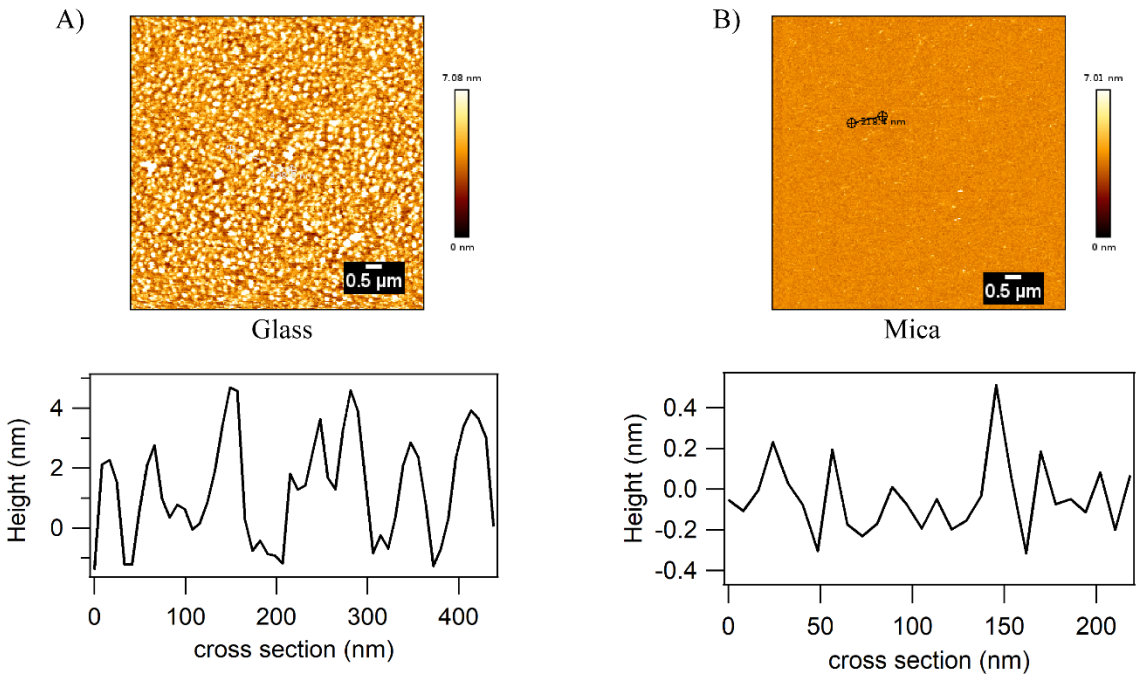

Figure S1: Roughness profile of (A) glass and (B) mica - the commonly used substrates for lipid bilayer studies.

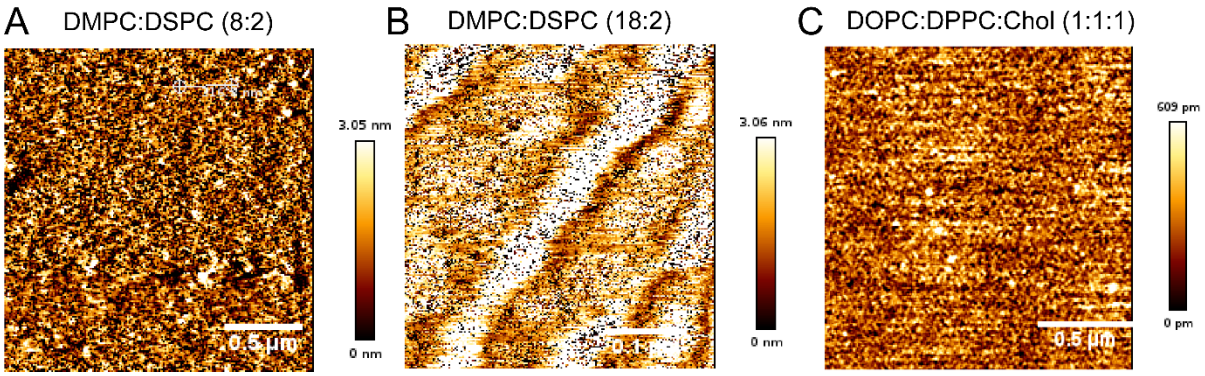

Figure S2: Magnified AFM images of lipid bilayers exhibiting negative FCS diffusion law intercepts. (A) DMPC:DSPC (8:2) on glass (B) DMPC:DSPC (18:2) on glass (C) DOPC:DPPC:Chol (1:1:1) on mica. The colour scales are adjusted according to the domain heights in the respective samples.

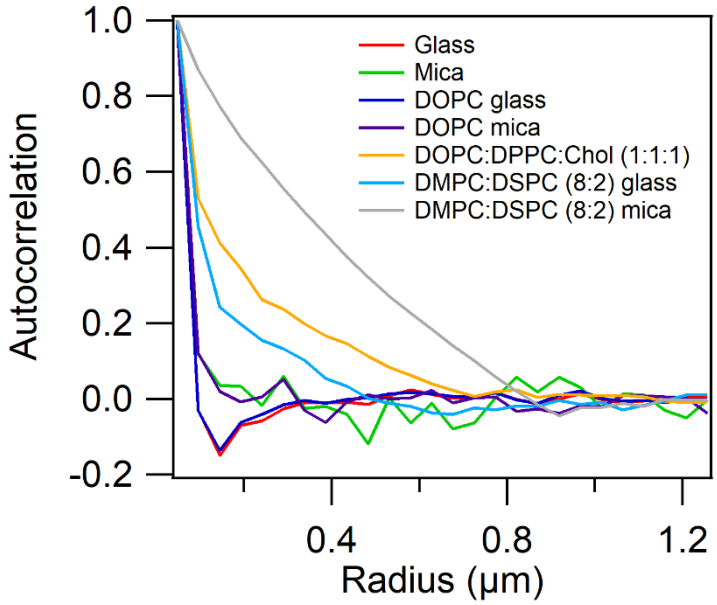

Figure S3: Normalized spatial autocorrelation calculated on AFM images of glass, mica, DOPC prepared on glass, DOPC prepared on mica, DOPC:DPPC:Chol (1:1:1) on mica, DMPC:DSPC (8:2) on glass and DMPC:DSPC (8:2) on mica.

Interdomain distances	FCS diffusion law intercept
42 nm	39.35 s
80 nm	3.89 s
190 nm	0.54 s

Table S1: The dependence of diffusion law intercept over the inter-domain distance analysed by simulations. The data shown here is simulated for a confinement strength of P_{in} /P_{out} =20, with P_{in} = 0.1 and P_{out} = 0.005. Here we performed FCS Diffusion law analysis at interdomain distances of 42 nm, 80 nm and 190 nm.

Sample	FCS diffusion law intercept	Domain Size (r) min	Interdomain distance (d) max	r/d min
DLPC:DSPC (1:1) on glass	2.67 ± 0.52	0.5 μm	5.0 μm	0.1
DLPC:DSPC (1:1) on mica	2.90 ± 1.07	1.0 μm	9.0 μm	0.1
DOPC:DPPC (1:1) on glass	0.43 ± 0.08	0.1 μm	0.7 μm	0.14
DOPC:DPPC (1:1) on mica	-	1.0 μm	9.0 μm	0.11
DMPC:DSPC (8:2) on glass	-0.43 ± 0.11	30 nm	58.0 nm	0.51
DMPC:DSPC (8:2) on mica	1.27 ± 0.04	0.2 μm	1.4 μm	0.14
DMPC:DSPC (18:2) on glass	-0.14 ± 0.20	10 nm	39.0 nm	0.25
DOPC:DPPC:Chol (1:1:1) on glass	-0.21 ± 0.04	-	-	-
DOPC:DPPC:Chol (1:1:1) on mica	-0.21 ± 0.11	30 nm	56.0 nm	0.53

Table S2: Summary of AFM and FCS diffusion law analysis results in phase separated lipid bilayers. Here we show the minimum domain size (r) and maximum interdomain distance (d) observed on AFM images of each of the samples. Further, we calculate the minimum (r/d) value that is observed in each of the samples. DOPC:DPPC (1:1) bilayers prepared on mica undergo constant reorganizations due to which the quality of FCS data obtained in this case was unreliable. Thus, we do not report it in the table above. Despite the negative intercepts obtained on DOPC:DPPC:Chol (1:1:1) bilayers prepared on glass, the AFM images for this sample do not show any lipid domains thus, we do not report domain characteristics in the table above.