**Supporting information**

**The transbilayer distribution of polyunsaturated phospholipids determines their facilitating effect on membrane deformation**

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This file contains:
one supplementary table (Table S1)
three supplementary figures (Fig S1, S2 and S3)

**Table S1.** Lipid composition of the simulated bilayers. For each system, the number of free lipids is given in the first two columns and the number of lipids (e.g. PU<u>X</u>C) that are submitted to the pulling force is given in the last two columns.

**PC membrane (Fig 2 and 3)**

| | | | |
|---|---|---|---|
| PUPC leaflet1 | 8964 | PUXC leaflet1 | 36 |
| POPC leaflet2 | 9522 | POXC leaflet2 | 45 |

**Dose-response 25% (Fig 3)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 6719 | POXC leaflet1 | 31 |
| PUPC leaflet1 | 2238 | PUXC leaflet1 | 12 |
| POPC leaflet2 | 9096 | POXC leaflet2 | 39 |

**Dose-response 50% (Fig 3)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 4478 | POXC leaflet1 | 22 |
| PUPC leaflet1 | 4482 | PUXC leaflet1 | 18 |
| POPC leaflet2 | 9249 | POXC leaflet2 | 39 |

**Dose-response 75% (Fig 3)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 2245 | POXC leaflet1 | 5 |
| PUPC leaflet1 | 6714 | PUXC leaflet1 | 36 |
| POPC leaflet2 | 9387 | POXC leaflet2 | 36 |

**Polar Head effect (Fig 4)**
**System 1 (polyunsaturated PS)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 4480 | POXC leaflet1 | 20 |
| PUPS leaflet1 | 4481 | PUXS leaflet1 | 19 |
| POPC leaflet2 | 8995 | POXC leaflet2 | 41 |

**System 2 (polyunsaturated PE)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 4478 | POXC leaflet1 | 22 |
| PUPE leaflet1 | 4479 | PUXE leaflet1 | 21 |
| POPC leaflet2 | 8994 | POXC leaflet2 | 42 |

**System 3 (monounsaturated PS)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 4725 | POXC leaflet1 | 18 |
| POPS leaflet1 | 4721 | POXS leaflet1 | 22 |
| POPC leaflet2 | 8954 | POXC leaflet2 | 46 |

**System 4 (monounsaturated PE)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 4806 | POXC leaflet1 | 27 |
| POPE leaflet1 | 4823 | POXE leaflet1 | 19 |
| POPC leaflet2 | 8960 | POXC leaflet2 | 40 |

**System PC:PS:PE (Fig S3)**

| | | | |
|---|---|---|---|
| POPC leaflet1 | 3326 | POXC leaflet1 | 22 |
| POPS leaflet1 | 3337 | POXS leaflet1 | 11 |
| POPE leaflet1 | 3334 | POXE leaflet1 | 14 |
| PUPC leafle2 | 2984 | PUXC leaflet2 | 13 |
| PUPS leaflet2 | 2982 | PUXS leaflet2 | 15 |
| PUPE leaflet2 | 2986 | PUXE leaflet2 | 11 |

**Neuron PM-like (Fig 5)**

| | | | |
|---|---|---|---|
| DPPC leaflet1 | 584 | DPXC leaflet1 | 1 |
| POPC leaflet1 | 936 | POXC leaflet1 | 9 |
| DOPC leaflet1 | 251 | DOXC leaflet1 | 1 |
| PAPC leaflet1 | 511 | PAXC leaflet1 | 2 |
| PUPC leaflet1 | 242 | PUXC leaflet1 | 1 |
| POPE leaflet1 | 467 | POXE leaflet1 | 1 |
| PAPE leaflet1 | 1361 | PAXE leaflet1 | 7 |
| PUPE leaflet1 | 2046 | PUXE leaflet1 | 6 |
| DPPS leaflet1 | 89 | DPXS leaflet1 | 1 |
| POPS leaflet1 | 358 | POXS leaflet1 | 2 |
| DOPS leaflet1 | 403 | DOXS leaflet1 | 2 |
| PAPS leaflet1 | 402 | PAXS leaflet1 | 3 |
| PUPS leaflet1 | 598 | PUXS leaflet1 | 5 |
| POPI leaflet1 | 269 | POXI leaflet1 | 1 |
| PAPI leaflet1 | 270 | PUXI leaflet1 | 2 |
| PUPI leaflet1 | 421 | POXC leaflet2 | 41 |
| POPC leaflet2 | 8959 | | |

**Control PM (Fig 5)**

| | | | |
|---|---|---|---|
| DPPC leaflet1 | 636 | DPXC leaflet1 | 3 |
| POPC leaflet1 | 1811 | POXC leaflet1 | 7 |
| DOPC leaflet1 | 261 | POXE leaflet1 | 22 |
| POPE leaflet1 | 4037 | POXS leaflet1 | 7 |
| DPPS leaflet1 | 81 | DOXS leaflet1 | 1 |
| POPS leaflet1 | 1469 | POXI leaflet1 | 6 |
| DOPS leaflet1 | 440 | POXC leaflet2 | 41 |
| POPI leaflet1 | 993 | | |
| POPC leaflet2 | 8977 | | |

**Large PC membrane 110*110 (Fig S2)**

| | | | |
|---|---|---|---|
| PUPC leaflet1 | 15960 | PUXC leaflet1 | 40 |
| POPC leaflet2 | 16968 | POXC leaflet2 | 40 |

**Large PC membrane 160*160 (Fig S2)**

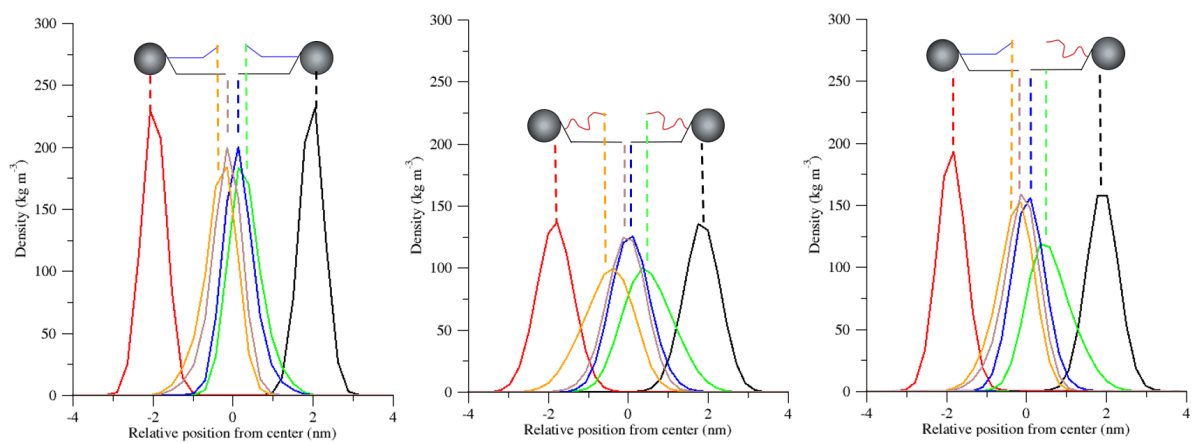| | | | |
|---|---|---|---|
| PUPC leaflet1 | 35381 | PUXC leaflet1 | 619 |
| POPC leaflet2 | 37617 | POXC leaflet2 | 651 |

**Fig S1.** Density plot across the bilayer of the polar head groups and of the terminal methyl of the acyl chains of symmetric and asymmetric PC bilayers. Left: symmetrical 18:0-18:1 PC; middle: symmetrical 18:0-22:6 PC; right: asymmetrical 18:0-18:1 PC/18:0-22:6 PC.
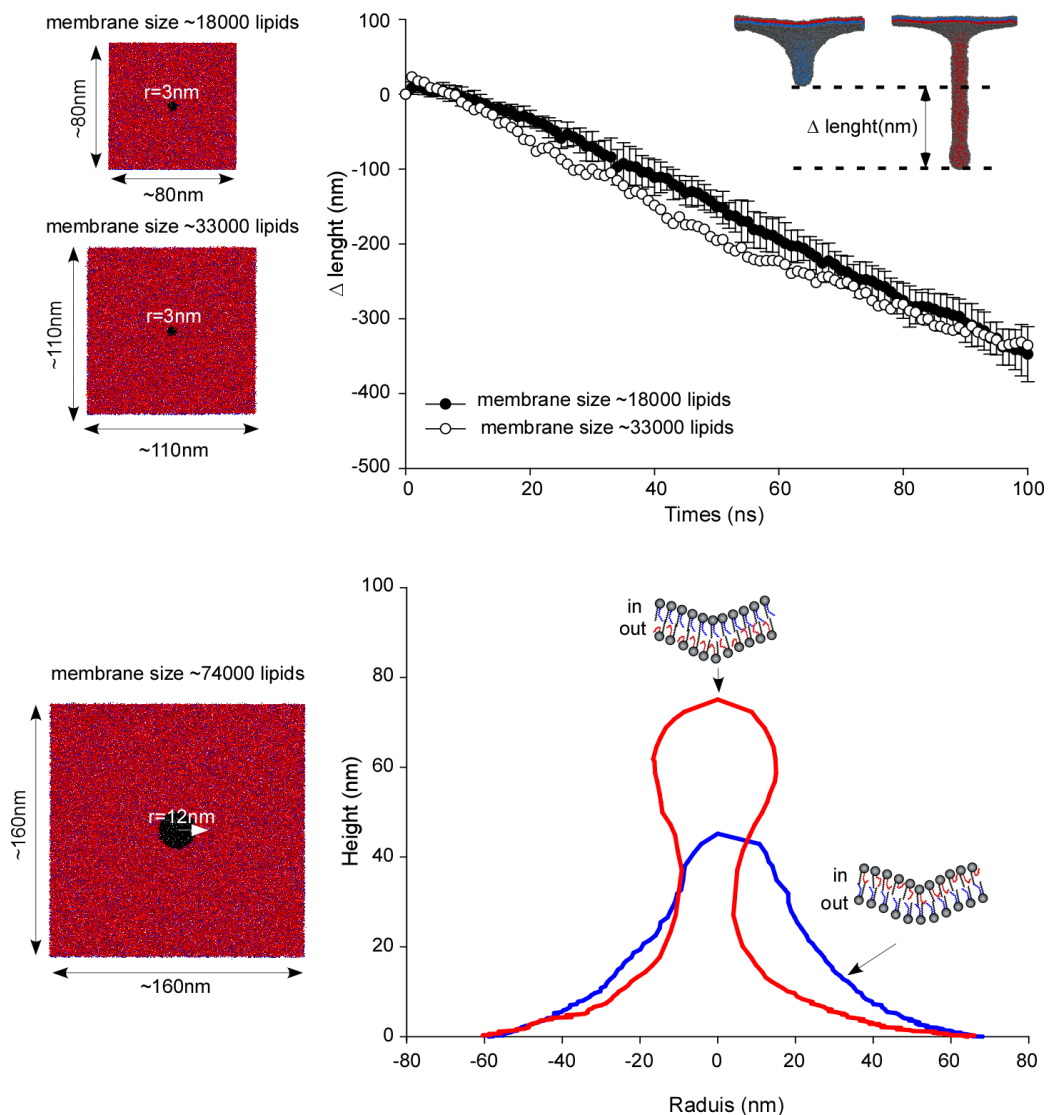
**Fig S2.** Top: membrane size does not influence the impact of the transbilayer distribution of polyunsaturated lipids. The pictures on the left illustrate the two systems used for the pulling simulations. As in Fig 2, we pulled a tube from a r = 3 nm patch either on the direction of the monounsaturated leaflet of on the direction the polyunsaturated leaflet. The graph on the right shows the difference in length between the two pulling configurations and between the two lipid systems. Bottom: the patch size does not influence the impact of the transbilayer distribution of polyunsaturated lipids. Here, we used a giant membrane surface and performed the pulling simulation from a very large (r = 12 nm) membrane patch. The red and blue lines show the contour of the membrane deformation obtained at t = 200 ns.
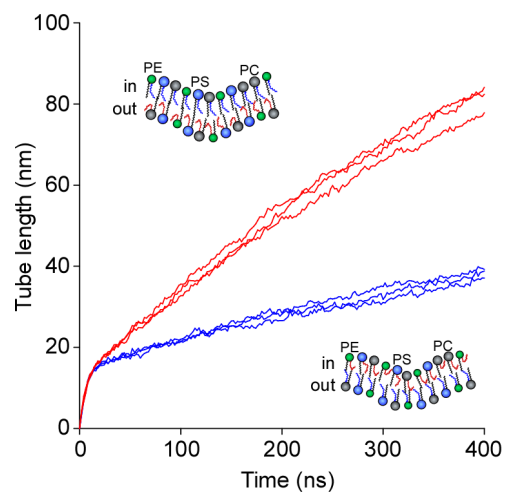
**Fig S3.** Kinetics of tube formation from asymmetrical PC:PE:PS bilayers. The two leaflets contained the same amount of PC, PE and PS. One leaflet contained 18:0-18:1 lipids, whereas the other leaflet contained 18:0-22:6 lipids. The pulling force was directed either on the side of the monounsaturated lipids (blue lines) or on the side of the polyunsaturated lipids (red lines). Three replica for each condition are shown.