

## Supporting Information

### Phase Separation Behavior of Mixed Lipid Systems at Neutral and Low pH:

#### Coarse-grained simulations with DMD/LIME

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To obtain the partial charges of the DPPC and DSPS (at neutral and low pH) for our GROMACS simulations, we performed quantum mechanical (QM) calculations using the GAUSSIAN09 software package.<sup>1</sup> Since QM calculations can easily get trapped in a local energy minima for large molecules, such as DPPC and DSPS, we decided to consider the head groups and tail groups of these large lipid molecules separately. The DPPC and DSPS molecules share the same tail group, but have different head groups. **Figures S1, S2 and S3** show the head groups (capped by a methyl group) of DPPC, DSPS (at low pH) and DSPS (at neutral pH), respectively. We capped the head groups of each lipid (DPPC, DSPS at neutral pH and DSPS at low pH) to calculate the partial charges of each molecule. The tail group of each lipid molecule is composed of two long hydrophobic alkyl chains. We calculated the partial charges for the first three alkyl groups on each lipid tail (from the head end) and neglected the remaining charges, which are usually set to zero. The tails are capped by an  $\text{NH}_3\text{PO}_4$  group, as shown in **Figure S4**.

It is worth to noting that the total charge on each molecule in Figures 1, 2 and 4 is neutral, but the total charge on the molecule in Figure 3 is -1.000 since a proton atom in the head group of the DSPS is absent.

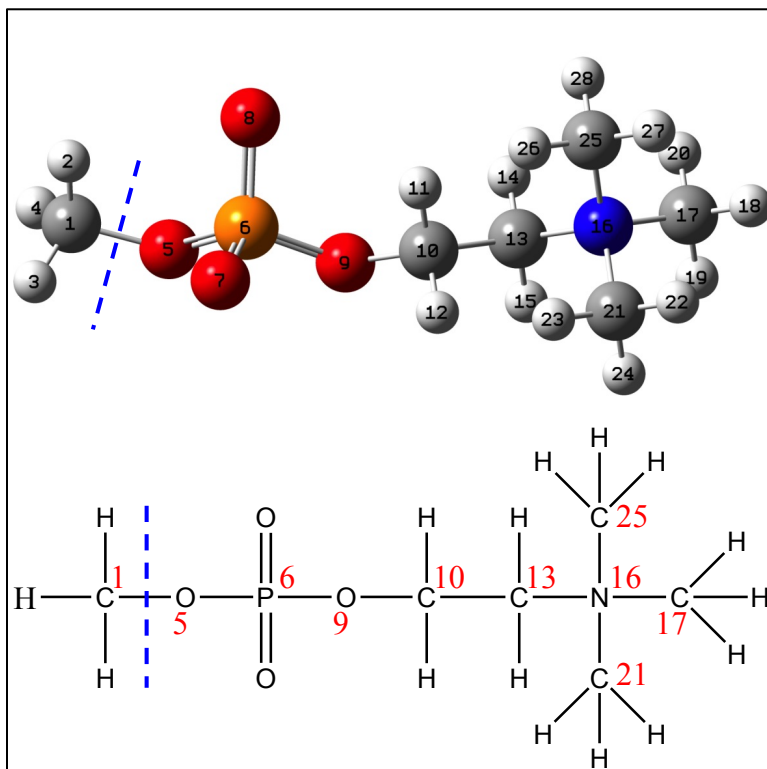


Figure S1: Secondary and chemical structures of the DPPC head group with a methyl group cap. The total charge of the molecule is neutral. The numbers on each atom correspond to the numbers listed for each DPPC head group partial charge in Table S1.

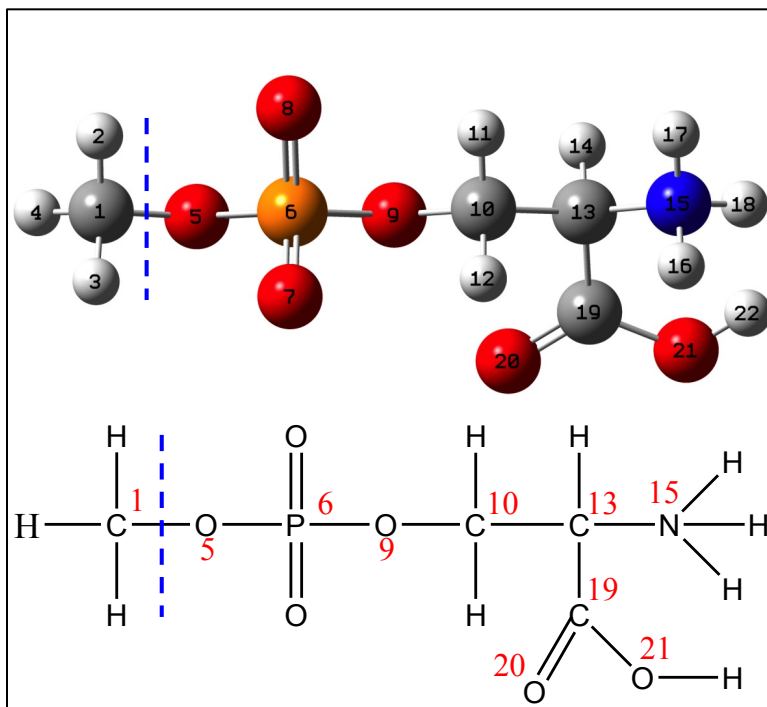


Figure S2: Secondary and chemical structures of the DSPPS head group at low pH with a methyl group cap. The total charge of the molecule is neutral. The numbers on each atom correspond to the numbers listed for each DSPPS (low pH) head group partial charge in Table S2.

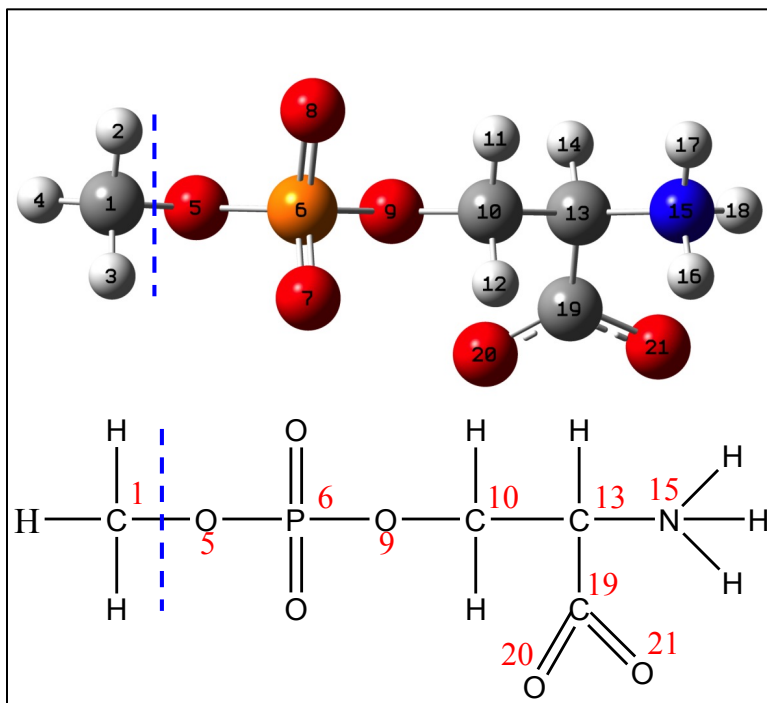


Figure S3: Secondary and chemical structures of the DSPS head group at neutral pH with a methyl group cap. The total charge of the molecule is -1.00. The numbers on each atom correspond to the numbers listed for each DSPS (neutral pH) head group partial charge in Table S3.

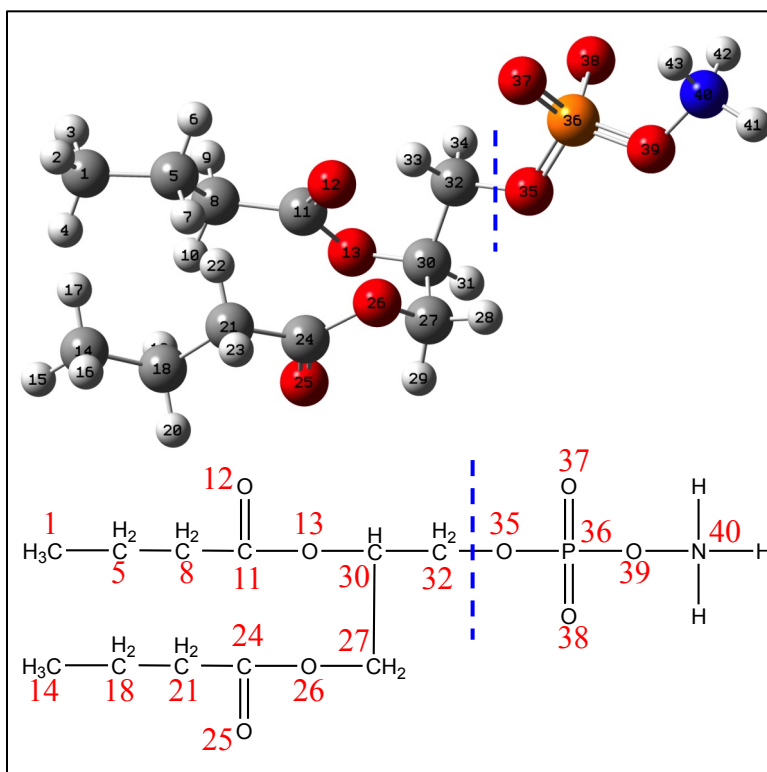


Figure S4: Secondary and chemical structures of the DPPC, DSPS (low pH) and DSPS (neutral pH) tail group with a common cap of  $\text{NH}_3\text{PO}_4$ . The total charge of the molecule is neutral. The numbers on each atom correspond to the numbers listed for tail group partial charge in Table S4.

The two-stage restrained electrostatic potential (RESP) charge-fitting protocol was performed to calculate the partial atomic charges.<sup>2,3,4</sup> The geometry of each molecule was optimized at the HF/6-31G\* level and the electrostatic potential for each optimized molecule was calculated using the basis set of HF/6-31G\*. In the first stage of the RESP charge-fitting protocol a least-squares fit is used to set the charges on each atom to the charges in the electrostatic potential. In the second stage of this procedure all the charges

on all of the degenerate hydrogen atoms on the methyl or methylene groups are recalculated so that they become equivalent. Since in united atom models, the hydrogen and carbon atoms on methyl and methylene groups are grouped together into one atom, the united atom partial charges were found by setting the partial charge of all the degenerate hydrogen atoms on methyl or methylene groups (beside those in the capping group) to zero and combining the total charges on each methyl or methylene group into single charges on each central carbon atom in the first-stage RESP fit. To find the charges of the hydrogen atoms in the capping group we use the regular two-stage RESP fit charge protocol.

The charge distributions of the DPPC and DSPS molecules were calculated following these steps: (1) We computed the partial charges of the DPPC and DSPS (low pH) head groups. The charge of the hydrogen atoms in the capping methyl group was calculated; however, the charge of the remaining hydrogen atoms in the methyl and methylene groups of the head group was neglected. The total charge of the capping methyl group is 0.152, and the remaining head group has a charge of -0.152. Therefore, the overall charge of the capped head group is 0. (2) We computed the partial charges of the DSPS (neutral pH) head group. The charges of the capping methyl group were set equal to those calculated for the capping methyl group of DSPS (low pH). The DSPS lipid (at neutral pH) has a net charge of -1.00. Therefore, the charge of the DSPS (neutral pH) head group (excluding the capping methyl group) is -1.152. (3) We computed the partial charges of the tail groups of DPPC, DSPS (low PH) and DSPS (neutral pH) capped by an  $\text{NH}_3\text{PO}_4$  group. The charge of the tail groups was restrained to 0.152 and the charge of the capping  $\text{NH}_3\text{PO}_4$  group was set to -0.152. **Tables S1, S2, and S3** list the atomic

charges of the DPPC, DSPS (low pH) and DSPS (neutral pH) head groups and the capping methyl groups. **Table S4** lists the atomic charges calculated for the tail groups and the capping  $\text{NH}_3\text{PO}_4$  group.

Index of atom	Partial charge	Index of atom	Partial charge	Index of atom	Partial charge
1	-0.241	11	0.000	21	0.116
2	0.131	12	0.000	22	0.000
3	0.131	13	0.118	23	0.000
4	0.131	14	0.000	24	0.000
5	-0.387	15	0.000	25	0.116
6	1.081	16	0.320	26	0.000
7	-0.691	17	0.116	27	0.000
8	-0.691	18	0.000	28	0.000
9	-0.444	19	0.000		
10	0.194	20	0.000		

Table S1: Partial charges of the DPPC head group and the capping methyl group. The

index of atom is shown in Figure S1

Index of atom	Partial charge	Index of atom	Partial charge	Index of atom	Partial charge
1	-0.337	9	-0.530	17	0.395
2	0.163	10	0.244	18	0.395
3	0.163	11	0.000	19	0.598

4	0.163	12	0.000	20	-0.593
5	-0.340	13	0.459	21	-0.530
6	1.263	14	0.000	22	0.416
7	-0.780	15	-0.764		
8	-0.780	16	0.395		

Table S2: Partial charges of the DSPPS (low pH) head group and the capping methyl group. The index of atom is shown in Figure S2.

Index of atom	Partial charge	Index of atom	Partial charge	Index of atom	Partial charge
1	-0.337	9	-0.530	17	0.395
2	0.163	10	0.244	18	0.395
3	0.163	11	0.000	19	0.598
4	0.163	12	0.000	20	-0.593
5	-0.340	13	0.459	21	-0.530
6	1.263	14	0.000	22	0.416
7	-0.780	15	-0.764		
8	-0.780	16	0.395		

Table S3: Partial charges of the DSPPS (neutral pH) head group and the capping methyl group. The index of atom is shown in Figure S3.

Index of atom	Partial charge	Index of atom	Partial charge	Index of atom	Partial charge



1	-0.057	16	-0.020	31	0.000
2	0.019	17	-0.020	32	0.028
3	0.019	18	-0.014	33	0.000
4	0.019	19	0.000	34	0.000
5	-0.021	20	0.000	35	-0.367
6	0.000	21	0.151	36	1.171
7	0.000	22	0.000	37	-0.750
8	0.027	23	0.000	38	-0.750
9	0.000	24	0.341	39	-0.520
10	0.000	25	-0.471	40	1.484
11	0.643	26	-0.196	41	-0.140
12	-0.399	27	0.287	42	-0.140
13	-0.378	28	0.000	43	-0.140
14	0.060	29	0.000		
15	-0.020	30	0.154		

Table S4: Partial charges of the DPPC, DSPS (low pH) and DSPS (neutral pH) tail group and the capping  $\text{NH}_3\text{PO}_4$  group. The index of atom is shown in Figure S4.

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