Biophysical Journal, Volume *111*

Supplemental Information

The Charge Properties of Phospholipid Nanodiscs

Cheng Her, Dana I. Filoti, Mark A. McLean, Stephen G. Sligar, J.B. Alexander Ross, Harmen Steele, and Thomas M. Laue

THE CHARGE PROPERTIES OF PHOSPHOLIPID NANODISCS

Cheng Her,* Dana I. Filoti,* Mark A. McLean,† Stephen G. Sligar,†, J.B. Alexander Ross‡, Harmen Steele‡ and Thomas M. Laue*

*Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham NH, USA; †Department of Biochemistry, University of Illinois at Urbana-Champaign, Champaign IL, US; ‡Department of Chemistry and Biochemistry, University of Montana, Missoula MT, USA

Supporting Materials

Table S1
Hydrodynamic properties of lipid Nanodiscs

Nanodisc	M_b (kDa)	$S_{20,w}$ [a]	R_s (nm)	$\bar{\upsilon}$ (cm^3/g)
MSP1D1 POPC	14.3 ± 0.4	$3.0_{52} \pm 5.0 \times 10^{-3}$	$4.7 \pm 5.0 \times 10^{-2}$	$0.88_8 \pm 3.0 \times 10^{-3}$
MSP1D1 10% POPS	15.5 ± 0.4	$3.1_{27} \pm 5.0 \times 10^{-3}$	$4.9 \pm 3.0 \times 10^{-2}$	$0.89_2 \pm 4.0 \times 10^{-3}$
MSP1D1 30% POPS	17.8 ± 0.6	$3.5_{09} \pm 3.0 \times 10^{-3}$	$5.0 \pm 5.0 \times 10^{-2}$	$0.87_9 \pm 4.0 \times 10^{-3}$
MSP1D1 70% POPS	21.6 ± 0.7	$4.4_{27} \pm 5.0 \times 10^{-3}$	$4.8 \pm 4.0 \times 10^{-2}$	$0.88_6 \pm 3.0 \times 10^{-3}$
MSP1E3D1 POPC	16.8 ± 1.2	$2.9_{37} \pm 4.0 \times 10^{-3}$	$5.7 \pm 8.0 \times 10^{-2}$	$0.89_9 \pm 3.0 \times 10^{-3}$
MSP1E3D1 10% POPS	19.0 ± 0.6	$3.2_{59} \pm 2.0 \times 10^{-3}$	$5.8 \pm 1.0 \times 10^{-1}$	$0.88_9 \pm 2.0 \times 10^{-2}$
MSP1E3D1 30% POPS	20.6 ± 0.6	$3.6_{21} \pm 4.0 \times 10^{-3}$	$5.7 \pm 6.0 \times 10^{-2}$	$0.89_1 \pm 4.0 \times 10^{-3}$
MSP1E3D1 70% POPS	26.7 ± 1.1	$4.6_{41} \pm 3.0 \times 10^{-3}$	$5.7 \pm 1.0 \times 10^{-1}$	$0.88_9 \pm 3.0 \times 10^{-3}$
MSP1D1 10% POPA	14.6 ± 0.8	$3.0_{22} \pm 3.0 \times 10^{-3}$	$4.8 \pm 6.0 \times 10^{-2}$	$0.89_1 \pm 4.0 \times 10^{-3}$
MSP1D1 30% POPA	15.7 ± 0.8	$3.1_{76} \pm 6.0 \times 10^{-3}$	$4.9 \pm 6.0 \times 10^{-2}$	$0.88_9 \pm 4.0 \times 10^{-3}$
MSP1D1 70% POPA	18.5 ± 1.0	$3.5_{95} \pm 8.0 \times 10^{-3}$	$5.1 \pm 1.1 \times 10^{-1}$	$0.88_9 \pm 3.0 \times 10^{-3}$
MSP1D1 10% POPE	14.6 ± 0.6	$3.0_{31} \pm 4.0 \times 10^{-3}$	$4.8 \pm 4.0 \times 10^{-2}$	$0.88_9 \pm 2.0 \times 10^{-3}$
MSP1D1 10% PIP_2	-	-	-	-
MSP1D1 Cardiolipin	-	-	-	-
MSP1D1 *E. coli* [b]	-	$3.2_{87} \pm 5.0 \times 10^{-3}$	-	-
MSP1E3D1 *E. coli* [b]	-	$5.0_{63} \pm 9.0 \times 10^{-3}$	-	-

Entries without values (-) indicates no quantities were obtained. R_s values of PIP_2, Cardiolipin and *E. coli* Nanodiscs were obtained from the averages of other MSP1D1 and MSP1E3D1 Nanodiscs and used for the calculation of Z_{DHH} from the electrophoretic mobilities.

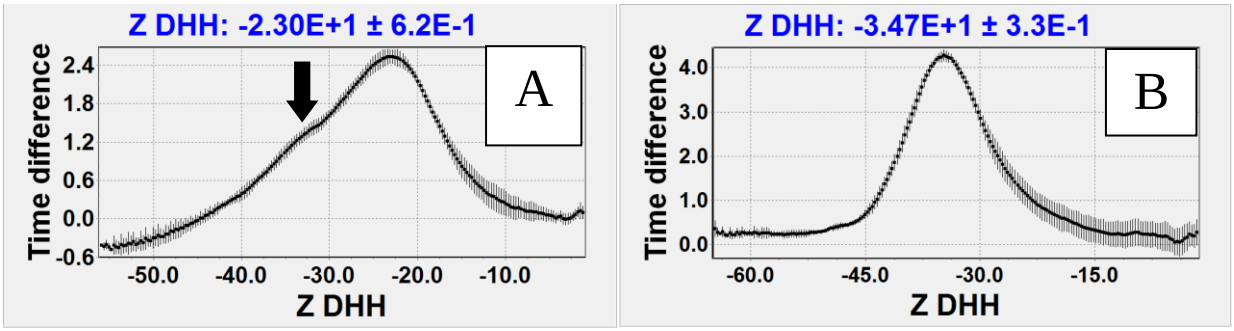
[a] Subscripted numbers are the values used in subsequent calculations, even though they are beyond the precision of the measurements

[b] Lipid content estimated by Avanti Polar Lipids

Table S2
Solvent properties at 20 ^{o}C

Buffer	Conductivity (mS)	Viscosity (cp)
100 mMNaCl, 50 mM Tris pH 7.4	12.0-12.4	1.02_{67}
34 mM NaCl, 17 mM Tris pH 7.4	3.9 - 4.1	1.01_{02}
50 mM NaCl, 25 mM Tris pH 7.4	5.6 - 6.0	1.01_{42}
68 mM NaCl, 34 mM Tris pH 7.4	8.1 - 8.3	1.01_{87}
84 mM NaCl, 42 mM Tris pH 7.4	10.1 - 10.4	1.02_{27}
150 mM NaCl, 75 mM Tris pH 7.4	17.5 - 18.0	1.03_{90}
100 mM NaCl, 50mM Tris, 3 mM $CaCl_2$ pH 7.4	10.0 - 10.5	1.02_{72}
100 mM NaCl, 50mM Tris, 3 mM MgCl pH 7.4	11.8 - 12.3	1.02_{80}
100 mM KCl, 50 mM Tris pH 7.4	14.3 - 14.7	1.01_{60}
100 mM LiCl, 50 mM Tris pH 7.4	9.8 - 10.4	1.04_{21}
1X PBS	13.5 - 13.8	1.02_{00}
100 mM KCl, 50 mM Tris pH 7.4[a]	12.8-13.2	1.10_{90}
100 mM KCl, 50 mM Tris pH 7.4[b]	19.8-21.1	0.71_{90}

Solvent properties of different buffers used to determine the electrophoretic mobility and Z_{DHH} of Nanodiscs by MCE. Experiments, unless denoted, were performed at 20^{o}C.
[a]Conductivity and viscosity values of standard buffer at 25^{o}C
[b]Conductivity and viscosity values of standard buffer at 35^{o}C



Figures S1 (A) and **S1 (B)** MCE data for MSP1D1 30POPA Nanodiscs in the presence of 3 mM Ca2+ (a) and 3 mM Mg2+ (b). Unlike the distribution data seen in **Figure 1B**, there is a pronounced shoulder (indicated by the arrow) in this data in accord with the aggregation characterized by sedimentation velocity. Similar Z_{DHH} distributions were also observed with 10 and 70POPA Nanodiscs in the presence of Ca^{2+}.

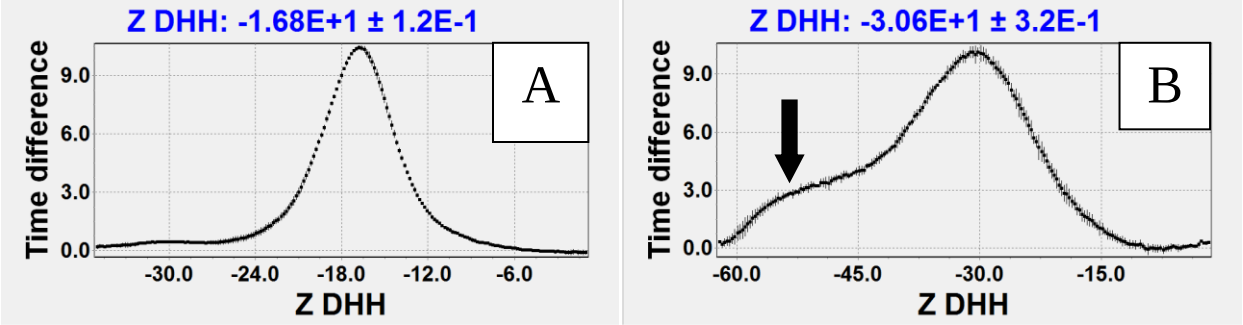


Figure S2 MCE data for MSP1D1 10PIP$_2$ Nanodiscs in the presence of 3 mM Ca^{2+} (**A**) and 3 mM Mg^{2+} (**B**). Unlike the distribution data seen in **Figure 1B**, there is a pronounced shoulder (indicated by the arrow) in the presence of Mg^{2+}. This observation is opposite to that of POPA Nanodiscs, in which POPA Nanodiscs aggregated in Ca^{2+}, but not Mg^{2+}.

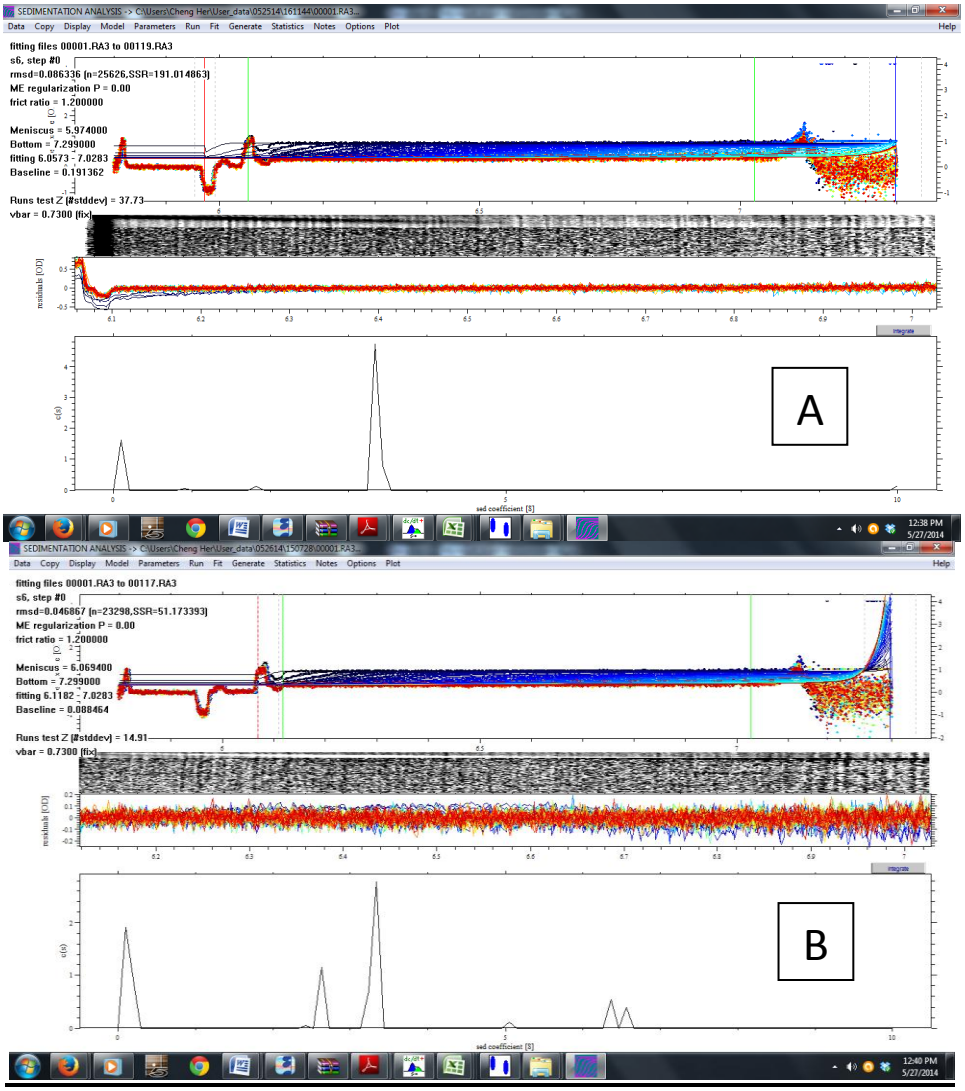


Figure S3 Sedimentation velocity data of MSP1D1 10POPA in the absence (**A**) and presence (**B**) of Ca^{2+}.

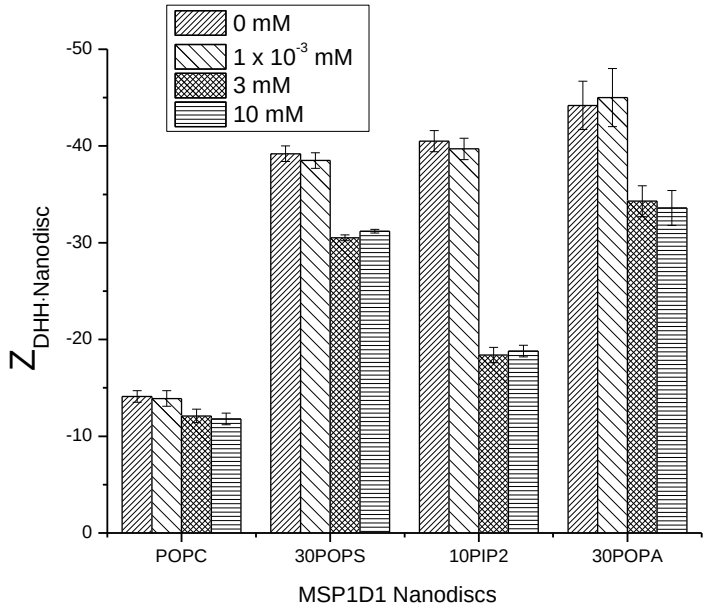


Figure S4 The Z_{DHH} of MSP1D1 POPC Nanodiscs in at varying concentrations of Ca^{2+} (POPC, 30POPS and 10PIP2) and Mg^{2+} (30POPA).

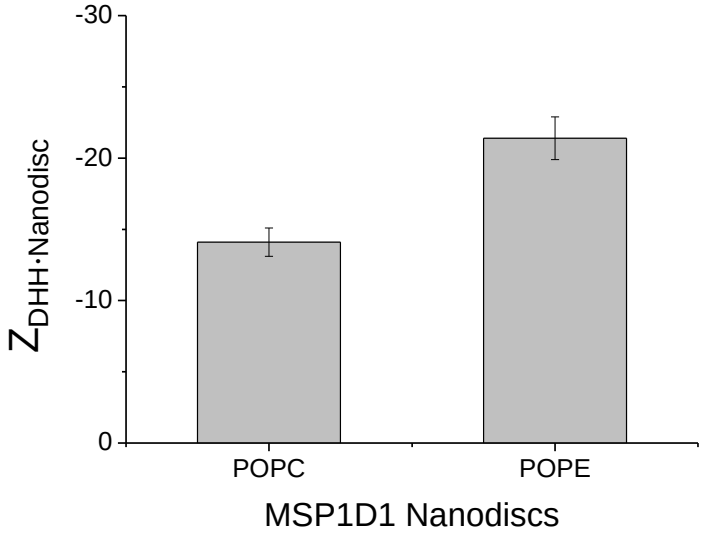


Figure S5 Comparison of Z_{DHH} for MSP1D1 POPC and 10% POPE Nanodiscs.

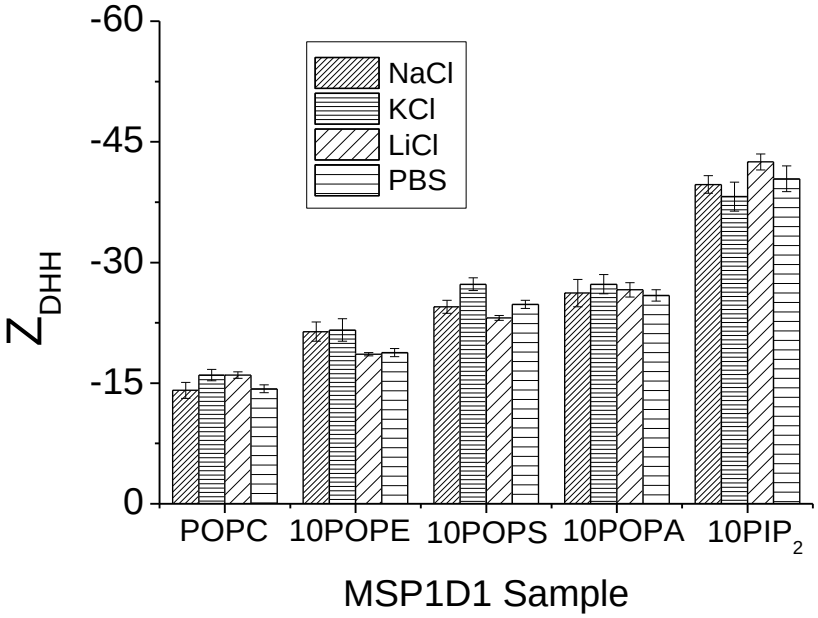


Figure S6 Z_{DHH} of MSP1D1 Nanodiscs in the presence of different monovalent alkali cations.

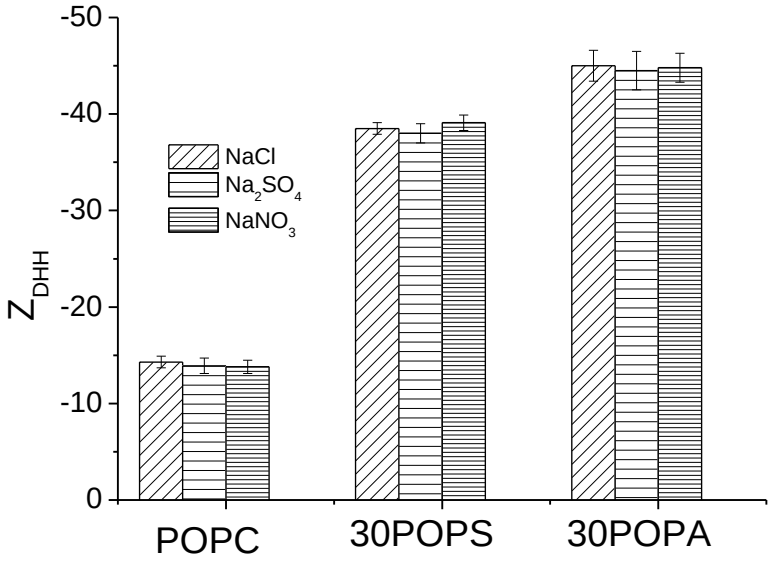


Figure S7 Z_{DHH} of MSP1D1 POPC, MSP1D1 30POPS and MSP1D1 30POPA Nanodiscs in the presence of anions.

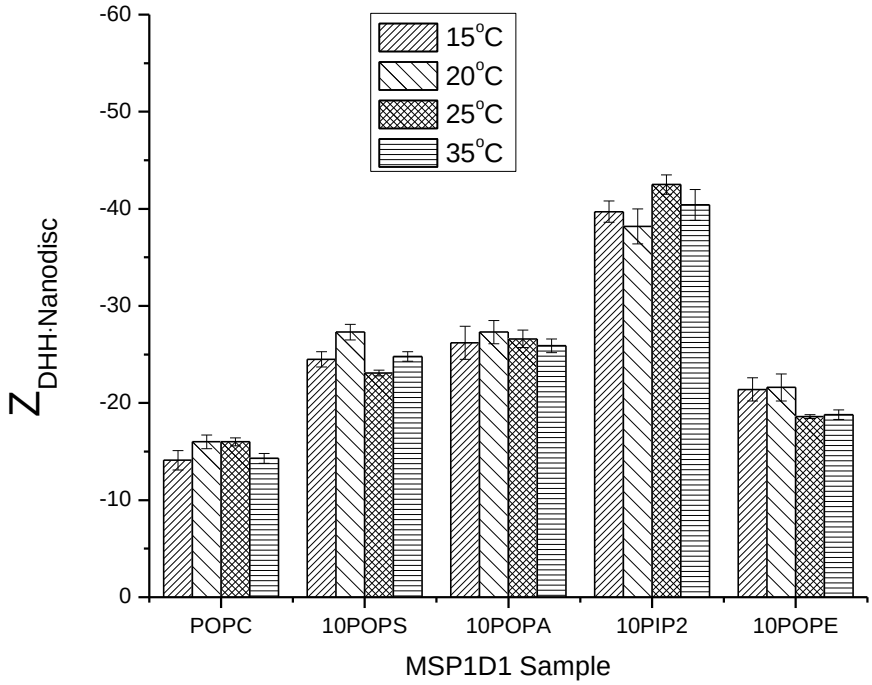


Figure S8 Temperature dependence of Z_{DHH} for POPC, POPS, POPA, PIP_2 and POPE Nanodiscs.

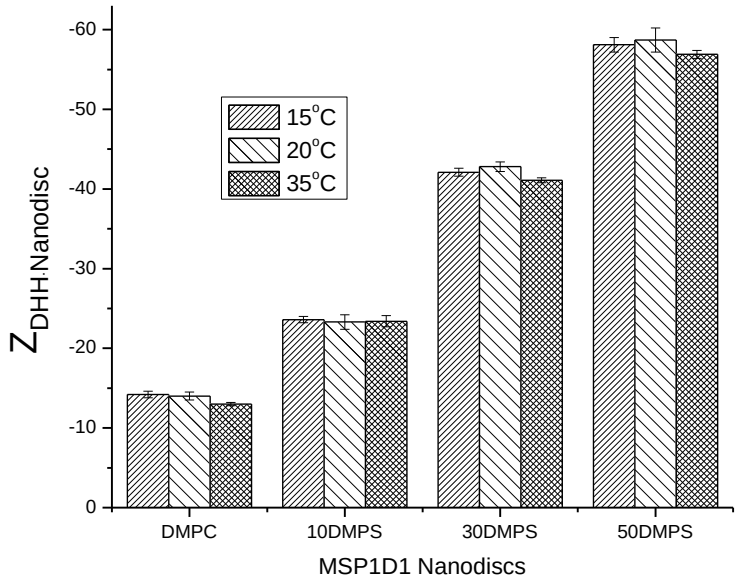


Figure S9 Temperature dependence of Z_{DHH} for DMPC and DMPS Nanodiscs.

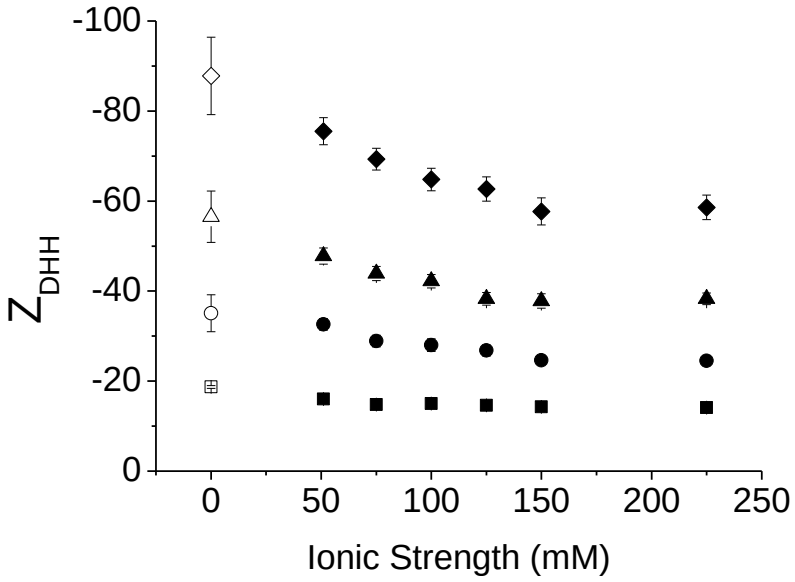


Figure S10 Z_{DHH} as a function of Na^+ concentration for MSP1D1 POPC (■), 10POPS (●), 30POPS (▲)and 70POPS (♦) Nanodiscs. In this figure, the closed data points are the experimental Z_{DHH} values and the open data points are the extrapolated intercept value at zero salt. The data were fit to a 3rd order polynomial in order to generate the extrapolated value at zero salt. The 3rd order provided a significantly better fit (F-test) than a 2nd order polynomial, whereas a 4th order fit provided no improvement over the 3rd order and yielded ill-determined coefficients. Similar observations were made for POPA nand PIP_2 Nanodiscs.

Table S3
Z_{DHH} extrapolated to zero salt

Nanodisc	$Z_{DHH \cdot Nanodisc}$ at zero salt	$Z_{calculated}$[a]
POPC	-18.7 ± 0.3	-16
10POPE	-32.7 ± 2.7	-29
10POPS	-35.1 ± 4.1	-29
30POPS	-56.5 ± 5.7	-54
70POPS	-87.8 ± 8.6	-104
10POPA	-35.9 ± 4.8	-32
30POPA	-69.8 ± 2.0	-63
70POPA	-83.5 ± 5.0	-125
$10PIP_2$	-52.6 ± 2.0	-54

[a] Charge calculated includes the charge calculated for two MSP1D1 MSPs (Z_{DHH} = -16).

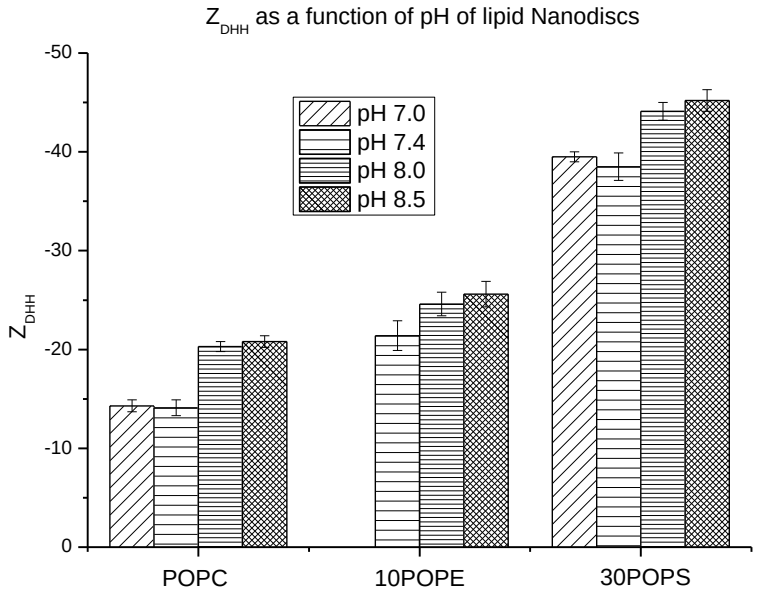


Figure S11 pH dependence of Z_{DHH} for MSP1D1 POPC, MSP1D1 10POPE and MSP1D1 30POPS Nanodiscs. Electrophoretic mobility measurements on 10POPE Nanodiscs at pH 7.0 could not be made. The reason for this is unclear, as sedimentation velocity data on 10POPE Nanodiscs at pH 7.0 show a monodisperse size population. Please note therefore, that in this figure, 10POPE Nanodiscs do not have a Z_{DHH} plotted at pH 7.0.

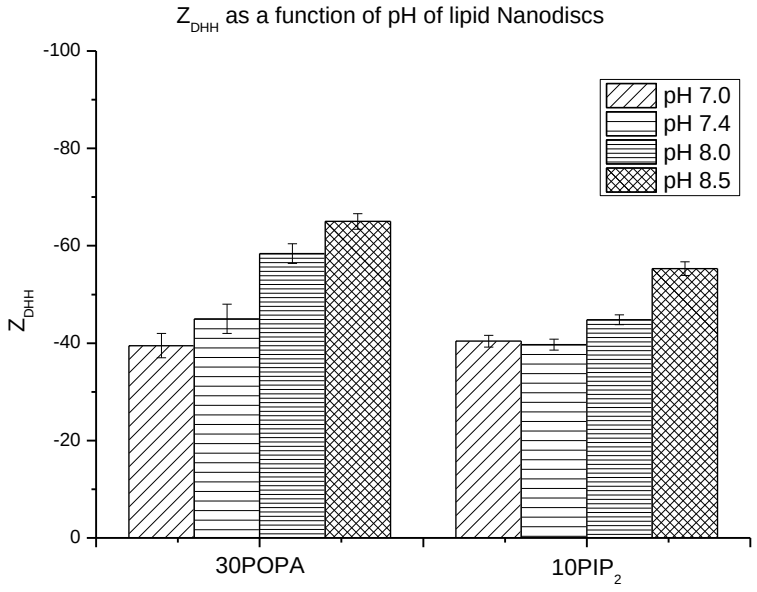


Figure S12 pH dependence of Z_{DHH} for MSP1D1 30POPA and MSP1D1 10PIP_2 Nanodiscs.

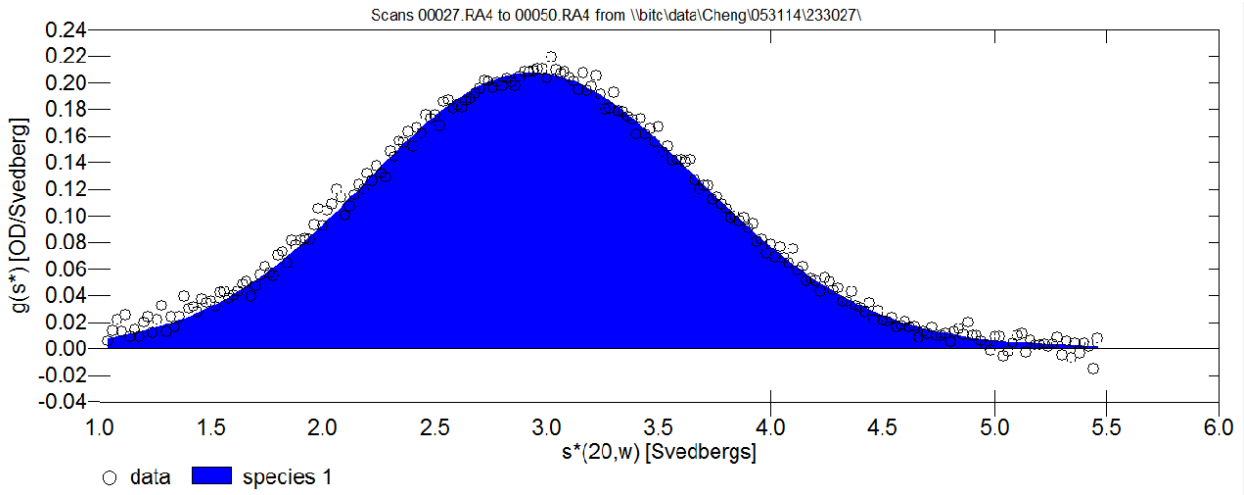


Figure S13 Sedimentation velocity data of 10POPE Nanodiscs in standard buffer at pH 7.4 using DC/DT+ [Philo, 2000].

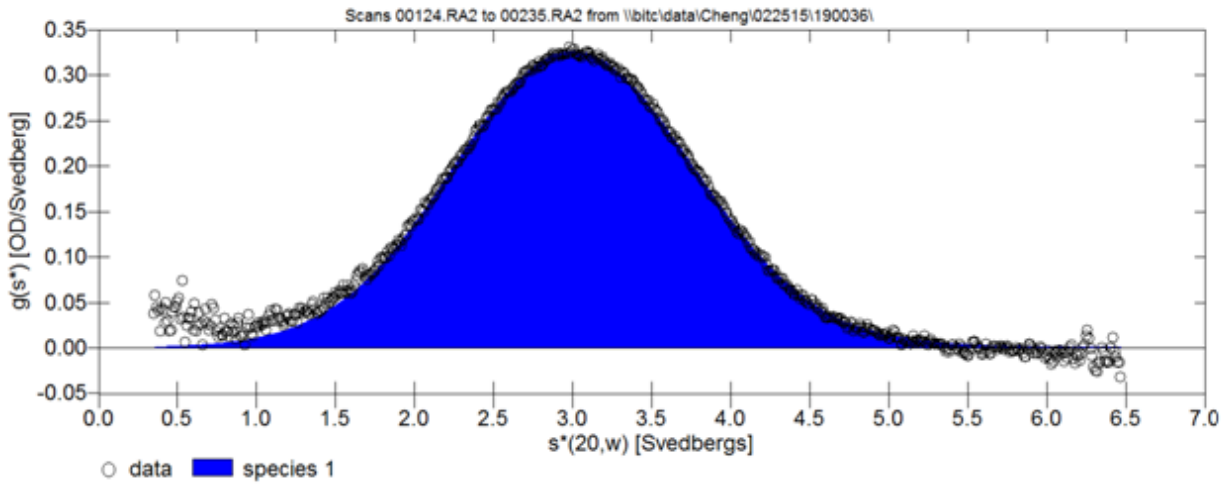


Figure S14 Sedimentation velocity data of 10POPE Nanodiscs in standard buffer at pH 7.0 using DC/DT+ [Philo, 2000].