

SUPPLEMENTARY MATERIALS

Bases of Bacterial Sodium Channel Selectivity among Organic Cations

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Optimization of parameters for hydration free energy calculations

The values of the hydration free-energies for the cations in TIP3P water are listed in Table S1 in comparison to previously reported data. The free-energies of hydration were calculated via the Free-Energy Perturbation (FEP) method using the staged protocol of Deng and Roux¹ with separate calculations to assess electrostatic (ΔG_{elec}^a), dispersive Lennard-Jones (LJ) (ΔG_{disp}^a) and repulsive LJ (ΔG_{repu}^a) potentials.¹ The non-electrostatic LJ potential was separated into purely repulsive and attractive parts using Weeks, Chandler, and Andersen's (WCA) decomposition scheme. The staging in FEP simulations, turning off and on the solute-water interactions, was achieved by using three coupling (or staging) parameters (s , ξ , and λ) implemented in the PERT module of the program CHARMM. The repulsive component of the hydration free-energy was obtained with the staging parameter s set to 0.0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0. The dispersive attraction was obtained with the coupling parameter ξ , which was varied from 0 to 1 by increments of 0.1. The electrostatic free-energy contribution was obtained using the coupling parameter λ , which was varied from 0 to 1 by increments of 0.05. FEP simulations corresponding to every value of the staging parameters were performed independently using protocols described previously.^{2,3} All FEP runs were performed for both forward and backward perturbation. The periodic boundary condition correction (PBC correction) due to the surface potential of TIP3P water⁴ in keeping with previously reported simulations by Lamoureux et al.^{5,6} Each of the FEP windows was run for 500 ps of equilibration, followed by 2.5 ns of forward and backward simulation time for every amino acid mimetic in both water and vacuum. Weighted Histogram Analysis Method (WHAM)^{7,8} was used to compute the components of the hydration free-energy shown in Table 1. All simulation systems used to calculate the hydration free-energy contained 216 TIP3P water molecules and one solute at the center of the simulation box. Positional constraints were applied

on the cation's center of mass. The electrostatic interactions were calculated using Particle-Mesh Ewald (PME) summation, with a coupling parameter of 0.34, 6th order spline for mesh interpolation, and cubic periodic boundaries. Non-bonded pair lists were maintained with a cutoff of 16 Å, and a real space cutoff of 14 Å was used for LJ interactions, which were truncated via an atom-based energy switch algorithm from 10 Å to 12 Å with LRCs applied.

Table S1: Absolute hydration free energies for cations.

Cation	ΔG_{exp} (kcal/mol)	ΔG_{calc} (kcal/mol)
Na ⁺	-98.8	-100.1
NH ₄ ⁺	-80 ^a	-81.5
MA ⁺	-73 ^{a,b} ; -74.9 ^c	-77.6
Gu ⁺	-77.3 ^b	-78.3
H ₂ N ⁺	-83 ^c	-81.5
TMA ⁺	-67 ^a	-69

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b, 108(41), 16264-16270; b. Kelly, C.P., Cramer, C.J. and Truhlar, D.G., 2005. SM6: A density functional theory continuum solvation model for calculating aqueous solvation free energies of neutrals, ions, and solute– water clusters. *Journal of chemical theory and computation*, 1(6), pp.1133-1152.

c, Housecroft, C.E. and Jenkins, H.D.B., 2017. Absolute ion hydration enthalpies and the role of volume within hydration thermodynamics. *RSC Advances*, 7(45), pp.27881-27894

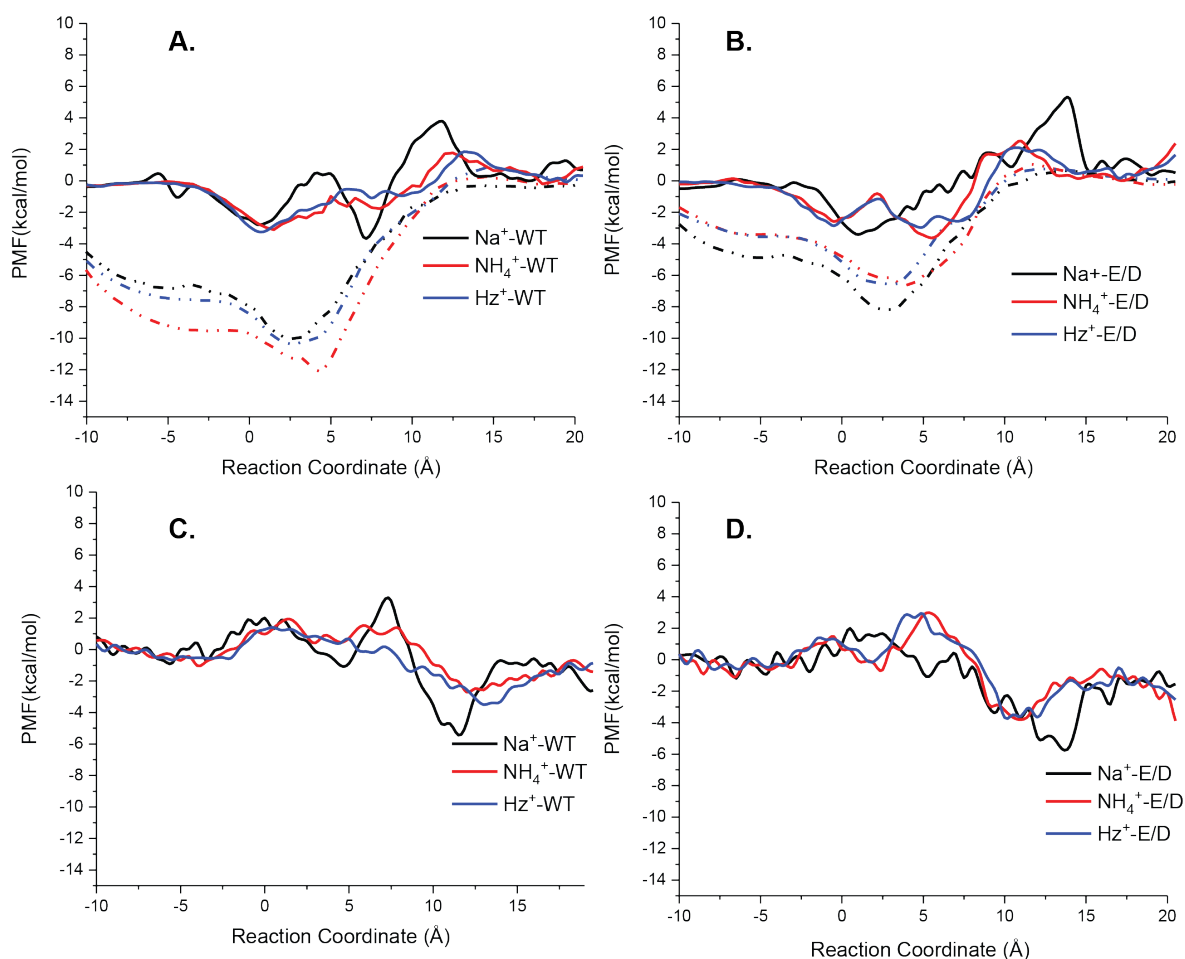


Figure S1. PMF Force Decomposition Analysis for Permeant Cations Contributions from Ion-Protein (A and B) and Ion-Water interactions (C and D) to the total Potential of Mean-Force for permeant cations (Na^+ , Hz^+ , and NH_4^+) in WT and E/D systems. The corresponding total PMFs are shown as dash-dotted lines on the Panels A. and B. for comparisons. The decomposition analysis was performed with the method detailed in Allen et al.⁹

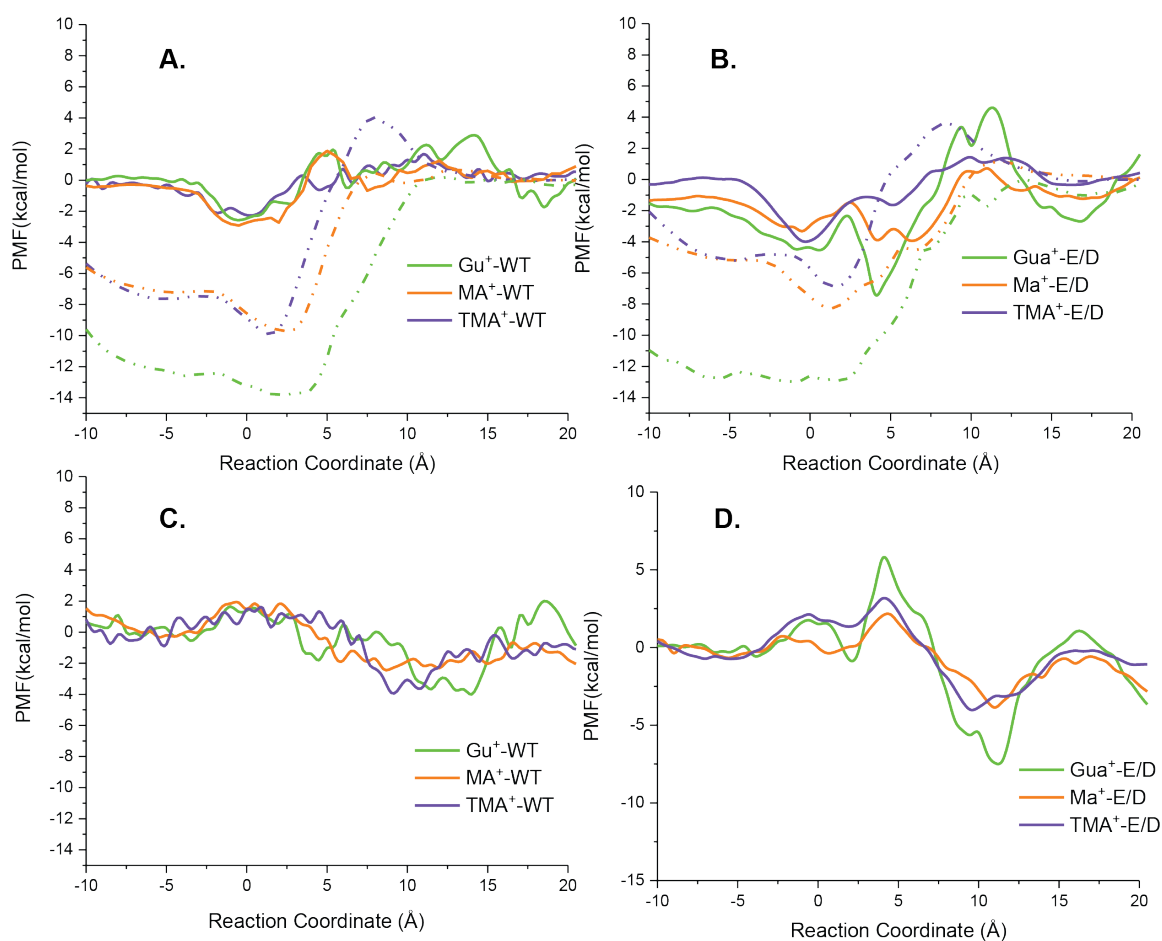


Figure S2. PMF Force Decomposition Analysis for Nonpermeant Cations
Contributions from Ion-Protein (A and B) and Ion-Water interactions (B and C) to the Potential of Mean-Force for nonpermeant cations (Gu⁺, MA⁺, TMA⁺) in WT and E/D systems, respectively. The PMF components and total PMFs are shown in solid and dotted lines, respectively.

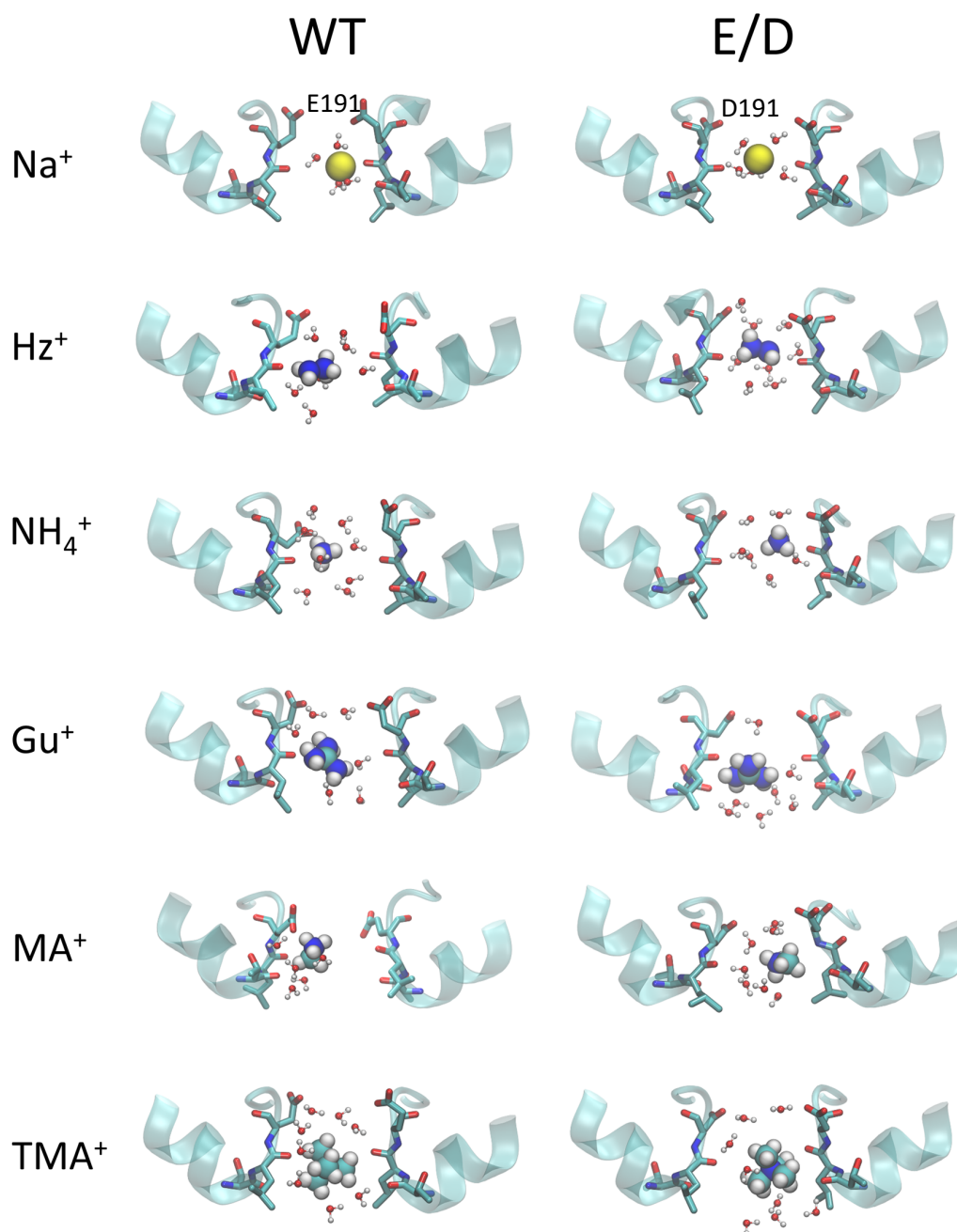


Figure S3.

The schematic illustration of the average structure of binding pocket corresponding to the minima in 1D PMF. Only two monomers are shown for clarity. The permeant ion is shown with a molecular sphere representation. The location of E191 or D191 is shown together with average number of water molecules within 3.5 Å within cation in the binding site.

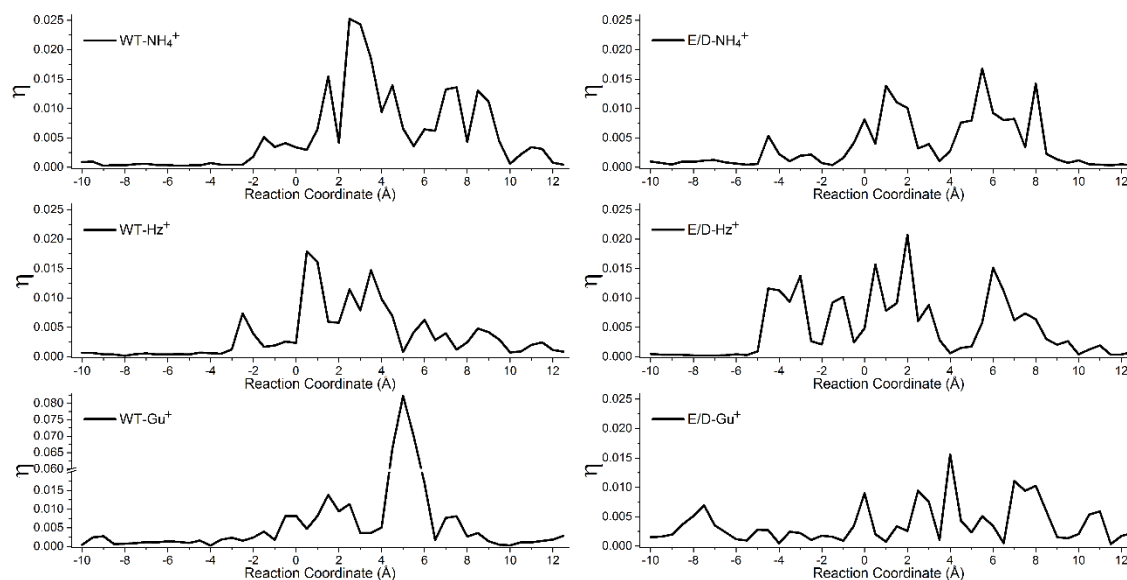


Figure S4

Relative entropy between the observed and WHAM histograms. The relative entropy values are all under 0.025 (except WT- Gu^+). We increased the simulation times for particular windows to keep the relative entropy at the same level for most of the organic cations. However, the value of relative entropy for Gu^+ in wild type posed particular difficulties. After 13 ns for window $z = 5$ and $5.5 \text{ \AA}$, the relative entropy only decreased by 0.01. Converged windows were employed to build H-REMD simulations.

Supporting References

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- 3 Zhao, C. F., Caplan, D. A. & Noskov, S. Y. Evaluations of the Absolute and Relative Free Energies for Antidepressant Binding to the Amino Acid Membrane Transporter LeuT with Free Energy Simulations. *Journal of Chemical Theory and Computation* **6**, 1900-1914 (2010).
- 4 Lamoureux, G., Harder, E., Vorobyov, I. V., Roux, B. & MacKerell Jr, A. D. A polarizable model of water for molecular dynamics simulations of biomolecules. *Chemical Physics Letters* **418**, 245-249, doi:10.1016/j.cplett.2005.10.135 (2006).
- 5 Lamoureux, G. & Roux, B. Absolute hydration free energy scale for alkali and halide ions established from simulations with a polarizable force field. *Journal of Physical Chemistry B* **110**, 3308-3322 (2006).
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- 7 Roux, B. The Calculation of the Potential of Mean Force Using Computer-Simulations. *Computer Physics Communications* **91**, 275-282 (1995).
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- 9 Allen, T. W., Andersen, O. S. & Roux, B. Molecular dynamics - potential of mean force calculations as a tool for understanding ion permeation and selectivity in narrow channels. *Biophys Chem* **124**, 251-267, doi:10.1016/j.bpc.2006.04.015 (2006).

Force field parameters for organic ions

Ammonium.str:

read rtf card

MASS 301 NAM 14.00700

MASS 302 HM 1.00800 AUTO ANGLES DIHE

RESI AMM 1.00

GROUP

ATOM NZ NAM -0.728

ATOM HZ1 HM 0.432

ATOM HZ2 HM 0.432

ATOM HZ3 HM 0.432

ATOM HZ4 HM 0.432

BOND NZ HZ1 NZ HZ2 NZ HZ3 NZ HZ4

ANGLE HZ1 NZ HZ2 HZ1 NZ HZ3 HZ1 NZ HZ4 HZ2 NZ HZ3 HZ2 NZ HZ4 HZ3 NZ HZ4

IC HZ2 HZ4 *NZ HZ1 1.0404 110.0200 120.2700 109.5000 1.0402

IC HZ3 HZ4 *NZ HZ1 1.0404 110.0200 -120.1300 109.4000 1.0401

IC HZ1 HZ2 *NZ HZ4 1.0402 109.4000 120.2700 110.0200 1.0402PATC FIRS NONE LAST
NONE

END

read param card

* MINI FORCE FIELD PARAMETER FILE.

*

BONDS

NAM HM 403.9 1.010

ANGLES

HM NAM HM 44.000 109.50

DIHEDRALS

IMPROPERS

NONBONDED E14FAC 1.000000

HM 0.000000 -0.100000 0.100000

NAM 0.000000 -0.170000 1.969900

END

Hydrazinium.str:

read rtf card

MASS 250 NZ2 14.00700

MASS 251 NZ3 14.00700

MASS 252 HZ 1.00800

AUTO ANGLES DIHE

RESI HYD 1.00

GROUP

ATOM N4 NZ3 -0.0784

ATOM H5 HZ 0.3182

ATOM H6 HZ 0.3182

ATOM H7 HZ 0.3182

ATOM N1 NZ2 -0.7142

ATOM H2 HZ 0.4190

ATOM H3 HZ 0.4190

BOND H5 N4 H6 N4 H7 N4

BOND N4 N1

BOND H2 N1 H3 N1

DONOR H5 N4

DONOR H6 N4

DONOR H7 N4

IMPR N4 H5 N1 H7

IMPR N1 H2 N4 H3

IC H6 N1 *N4 H5 0.0000 0.0000 120.0000 0.0000 0.0000

IC H7 N1 *N4 H6 0.0000 0.0000 120.0000 0.0000 0.0000

PATC FIRS NONE LAST NONE

END

read param card

* MINI FORCE FIELD PARAMETER FILE.

*

BONDS

NZ2 HZ 454.0 1.019

NZ3 HZ 421.0 1.028

NZ2 NZ3 324.0 1.452

ANGLES

HZ NZ2 NZ3 65.00 108.08

HZ NZ2 HZ 45.00 109.12

HZ NZ3 NZ2 50.00 110.96

HZ NZ3 HZ 45.00 107.84

DIHEDRALS

HZ NZ2 NZ3 HZ 0.1565 3 0.0

IMPROPERS

NZ2 X X HZ 20.0000 0 0.0000

NZ2 X X NZ3 20.0000 0 0.0000

NZ3 X X HZ 20.0000 0 0.0000

NONBONDED E14FAC 1.000000

NZ2 0.000000 -0.170000 1.890226

NZ3 0.000000 -0.170000 1.890226

HZ 0.000000 -0.015700 0.874959

END

Guanidinium.str:

read rtf card

MASS 267 HGP2 1.00800 ! polar H, +ve charge

MASS 291 CG2N1 12.01100 ! conjugated C in guanidine/guanidinium

MASS 344 NG2P1 14.00700 ! N for protonated imine/Schiff's base (C=N(+))H-R, acyclic amidinium, guanidinium)

MASS 345 NG2P_0 14.007000

MASS 346 HGP2_0 1.008000

MASS 347 HGP2_1 1.008000

MASS 348 HGP2_2 1.008000

MASS 349 NG2P_1 14.007000

AUTO ANGLES DIHE

RESI GUAN 1.000

ATOM C CG2N1 0.817

ATOM N1 NG2P_0 -0.813

ATOM H1 HGP2 0.437

ATOM H2 HGP2_2 0.437

ATOM N2 NG2P_1 -0.813

ATOM H3 HGP2_0 0.437

ATOM H4 HGP2 0.437

ATOM N3 NG2P1 -0.813

ATOM H5 HGP2 0.437

ATOM H6 HGP2_1 0.437

BOND H6 N3

BOND H5 N3

BOND N1 C

BOND N1 H2

BOND N1 H1

BOND H3 N2

BOND N2 C

BOND N2 H4

BOND C N3

IMPR C N1 N2 N3

PATC FIRS NONE LAST NONE

END

read param card

* MINI FORCE FIELD PARAMETER FILE.

*

BONDS

CG2N1 NG2P1 463.000 1.3650

NG2P1 HGP2 455.000 1.0000

CG2N1 NG2P_0 463.000 1.365

NG2P_0 HGP2 455.000 1.000

NG2P1 HGP2_0 455.000 1.000

NG2P1 HGP2_1 455.000 1.000

NG2P_0 HGP2_2 455.000 1.000

NG2P_1 HGP2_0 455.000 1.000

CG2N1 NG2P_1 463.000 1.365

NG2P_1 HGP2 455.000 1.000

ANGLES

NG2P1 CG2N1 NG2P1 52.000 120.0000 90.000 2.36420

CG2N1 NG2P1 HGP2 49.000 120.0000

HGP2 NG2P1 HGP2 25.000 120.0000

CG2N1 NG2P_0 HGP2 49.000 120.000

CG2N1 NG2P1 HGP2_0 49.000 120.000

NG2P_0 CG2N1 NG2P1 52.000 120.000 90.000 2.36420

HGP2 NG2P_0 HGP2 25.000 120.000

HGP2_0 NG2P1 HGP2 25.000 120.000

CG2N1 NG2P1 HGP2_1 49.000 120.000

HGP2 NG2P1 HGP2_1 25.000 120.000

CG2N1 NG2P_0 HGP2_2 49.000 120.000

CG2N1	NG2P_1	HGP2_0	49.000	120.000		
CG2N1	NG2P_1	HGP2	49.000	120.000		
NG2P_0	CG2N1	NG2P_1	52.000	120.000	90.000	2.36420
HGP2	NG2P_0	HGP2_2	25.000	120.000		
NG2P_1	CG2N1	NG2P1	52.000	120.000	90.000	2.36420
HGP2_0	NG2P_1	HGP2	25.000	120.000		

DIHEDRALS

NG2P1	CG2N1	NG2P1	HGP2	2.2500	2	180.0
NG2P_0	CG2N1	NG2P1	HGP2_0	0.163582980400664	1	180.0
NG2P_0	CG2N1	NG2P1	HGP2_0	1.870836659750243	2	180.0
NG2P_0	CG2N1	NG2P1	HGP2_0	0.187660256937964	3	0.0
NG2P_0	CG2N1	NG2P1	HGP2_0	0.307892748715624	4	0.0
NG2P_0	CG2N1	NG2P1	HGP2_0	0.051628313328628	6	180.0
NG2P_0	CG2N1	NG2P1	HGP2	2.250000000000000	2	180.0
HGP2	NG2P_0	CG2N1	NG2P1	2.250000000000000	2	180.0
HGP2_0	NG2P1	CG2N1	NG2P1	2.250000000000000	2	180.0
NG2P_0	CG2N1	NG2P1	HGP2_1	0.097285148889586	1	180.0
NG2P_0	CG2N1	NG2P1	HGP2_1	1.993571283530080	2	180.0
NG2P_0	CG2N1	NG2P1	HGP2_1	0.108867143148536	3	0.0
NG2P_0	CG2N1	NG2P1	HGP2_1	0.215264744238450	4	0.0
NG2P_0	CG2N1	NG2P1	HGP2_1	0.040105750865321	6	180.0
NG2P1	CG2N1	NG2P1	HGP2_1	2.250000000000000	2	180.0
NG2P_0	CG2N1	NG2P_1	HGP2_0	0.163582980400664	1	180.0
NG2P_0	CG2N1	NG2P_1	HGP2_0	1.870836659750243	2	180.0
NG2P_0	CG2N1	NG2P_1	HGP2_0	0.187660256937964	3	0.0
NG2P_0	CG2N1	NG2P_1	HGP2_0	0.307892748715624	4	0.0
NG2P_0	CG2N1	NG2P_1	HGP2_0	0.051628313328628	6	180.0
NG2P_0	CG2N1	NG2P_1	HGP2	2.250000000000000	2	180.0
HGP2	NG2P_0	CG2N1	NG2P_1	2.250000000000000	2	180.0
HGP2_2	NG2P_0	CG2N1	NG2P_1	0.024422263034803	1	0.0
HGP2_2	NG2P_0	CG2N1	NG2P_1	1.972971490233108	2	180.0
HGP2_2	NG2P_0	CG2N1	NG2P_1	0.241668160396296	4	0.0
HGP2_2	NG2P_0	CG2N1	NG2P_1	0.043010313701504	6	180.0
HGP2_2	NG2P_0	CG2N1	NG2P1	2.250000000000000	2	180.0
NG2P_1	CG2N1	NG2P1	HGP2_1	2.250000000000000	2	180.0

NG2P_1	CG2N1	NG2P1	HGP2	2.2500000000000000	2	180.0
HGP2_0	NG2P_1	CG2N1	NG2P1	2.2500000000000000	2	180.0
HGP2	NG2P_1	CG2N1	NG2P1	2.2500000000000000	2	180.0

IMPROPERS

CG2N1	NG2P1	NG2P1	NG2P1	40.0000	0	0.0
CG2N1	NG2P_0	NG2P1	NG2P1	40.000	0	0.0
CG2N1	NG2P_0	NG2P_1	NG2P1	40.000	0	0.0

NONBONDED E14FAC 1.000000

HGP2	0.00	-0.046000	0.224500
CG2N1	0.00	-0.110000	2.000000
NG2P1	0.00	-0.200000	1.850000

NG2P_0	0.00	-0.2000	1.8500
HGP2_0	0.00	-0.0460	0.2245
HGP2_1	0.00	-0.0460	0.2245
HGP2_2	0.00	-0.0460	0.2245
NG2P_1	0.00	-0.2000	1.8500

END

TMA.str

read rtf card

MASS	258	HGA3	1.00800	! aliphatic proton, CH3
MASS	267	HGP2	1.00800	! polar H, +ve charge
MASS	323	CG334	12.01100	! aliphatic C for methyl group (-CH3), adjacent to positive N (PROT NTER) (+)

MASS 364 NG3P3 14.00700 ! primary NH3+, phosphatidylethanolamine

AUTO ANGLES DIHE

RESI TMA 1.000

ATOM N	NG3P3	-0.357
ATOM C	CG334	-0.008
ATOM H1	HGP2	0.343
ATOM H2	HGP2	0.343
ATOM H3	HGP2	0.343
ATOM H4	HGA3	0.112
ATOM H5	HGA3	0.112
ATOM H6	HGA3	0.112

BOND H1	N	! 1
BOND H6	C	! 1
BOND H5	C	! 1
BOND N	C	! 1
BOND N	H3	! 1
BOND N	H2	! 1
BOND C	H4	! 1

END

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* MINI FORCE FIELD PARAMETER FILE.

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BONDS

CG334	NG3P3	200.000	1.4800
CG334	HGA3	322.000	1.1110

NG3P3 HGP2 403.000 1.0400

ANGLES

NG3P3	CG334	HGA3	45.000	107.5000	35.000	2.10100
HGA3	CG334	HGA3	35.500	108.4000	5.400	1.80200
CG334	NG3P3	HGP2	30.000	109.5000	20.000	2.07400
HGP2	NG3P3	HGP2	44.000	109.5000		

DIHEDRALS

HGA3	CG334	NG3P3	HGP2	0.0900	3	0.0
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IMPROPERS

NONBONDED E14FAC 1.000000

HGA3	0.00	-0.024000	1.340000			
HGP2	0.00	-0.046000	0.224500			
CG334	0.00	-0.077000	2.215000	0.00	-0.010000	1.900000
NG3P3	0.00	-0.200000	1.850000			

END

MA.str

read rtf card

MASS 258 HGA3 1.00800 ! aliphatic proton, CH3

MASS 267 HGP2 1.00800 ! polar H, +ve charge

MASS 323 CG334 12.01100 ! aliphatic C for methyl group (-CH3), adjacent to positive N (PROT NTER) (+)

MASS 364 NG3P3 14.00700 ! primary NH3+, phosphatidylethanolamine
AUTO ANGLES DIHE

RESI MTHA 1.000

ATOM N NG3P3 -0.357

ATOM C CG334 -0.008

ATOM H1 HGP2 0.343

ATOM H2 HGP2 0.343

ATOM H3 HGP2 0.343

ATOM H4 HGA3 0.112

ATOM H5 HGA3 0.112

ATOM H6 HGA3 0.112

BOND H1 N ! 1

BOND H6 C ! 1

BOND H5 C ! 1

BOND N C ! 1

BOND N H3 ! 1

BOND N H2 ! 1

BOND C H4 ! 1

PATC FIRS NONE LAST NONE

END

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* MINI FORCE FIELD PARAMETER FILE.

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BONDS

CG334 NG3P3 200.000 1.4800

CG334 HGA3 322.000 1.1110

NG3P3 HGP2 403.000 1.0400

ANGLES

NG3P3 CG334 HGA3 45.000 107.5000 35.000 2.10100

HGA3 CG334 HGA3 35.500 108.4000 5.400 1.80200

CG334 NG3P3 HGP2 30.000 109.5000 20.000 2.07400

HGP2 NG3P3 HGP2 44.000 109.5000

DIHEDRALS

HGA3	CG334	NG3P3	HGP2	0.0900	3	0.0
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IMPROPERS

NONBONDED E14FAC 1.000000

HGA3	0.00	-0.024000	1.340000
------	------	-----------	----------

HGP2	0.00	-0.046000	0.224500
------	------	-----------	----------

CG334	0.00	-0.077000	2.215000	0.00	-0.010000	1.900000
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NG3P3	0.00	-0.200000	1.850000
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END