

Perform pure salt simulation (NH₄Cl)

1. Parameterisation methods
2. Measurements

Testing parameters

System settings: 0.15M 4-4-4 nm boxes (6 NH₄, 6 Cl, 2022 H₂O)

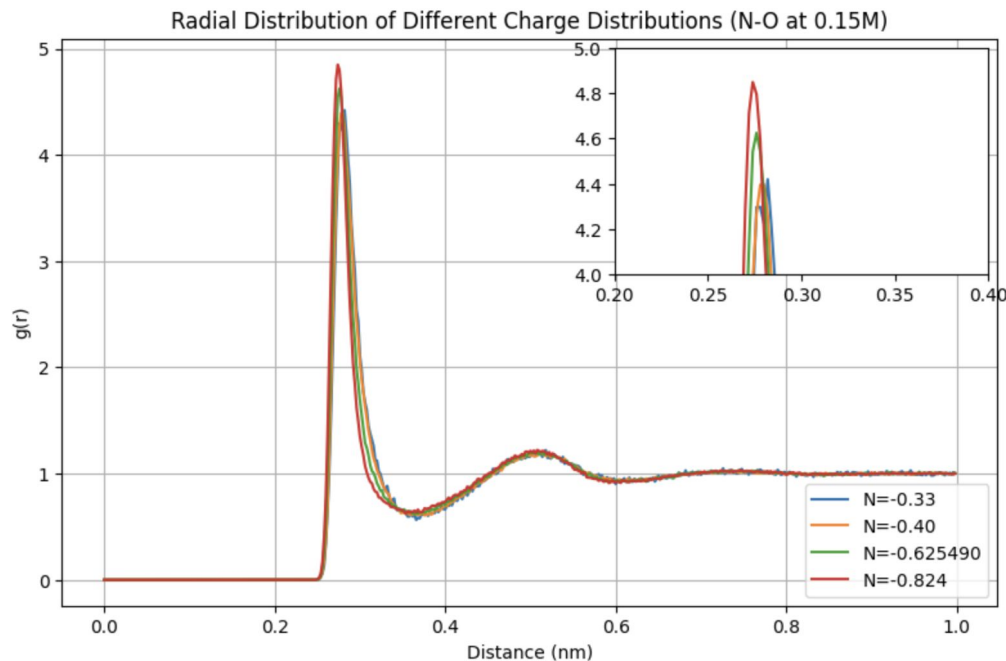
		Source
charge0	N: -0.33 H: +0.33	Original CHARMM parameters
charge1	N: -0.40 H: +0.35	NH ₄ ⁺ partial charges using OPLS charges, as OPLS and CHARMM partial charges of NH ₃ ⁺ in Lysine are nearly identical [b]
charge2	N: -0.625490 H: +0.406372	Partial charges were derived using electrostatic potential fitting. Energy constants were taken from the CHARMM, version 22, forcefield for primary amines.[h]
charge3	N: -0.824 H: +0.456	NH ₄ charges by restrained ESP-fit method using ChelpG approach [a]

RDF results (N-O)

200ns, 4-4-4	r_max	CN	500ns, 4-4-4	r_max	CN	200ns, 7-7-7	r_max	CN
0.15M, q=-0.33	0.282	6.98	0.15M, q=-0.33	0.28	7.0	0.15M, q=-0.33	0.28	6.98
0.15M, q=-0.40	0.28	7.353	0.15M, q=-0.40	0.28	6.759	0.15M, q=-0.40	0.28	6.975
0.15M, q=-0.62	0.278	6.599	0.15M, q=-0.62	0.276	6.19	0.15M, q=-0.62	0.278	6.528
0.15M, q=-0.824	0.276	6.25	0.15M, q=-0.824	0.276	6.163	0.15M, q=-0.824	0.276	5.968
1M, q=-0.33	0.282	6.377						

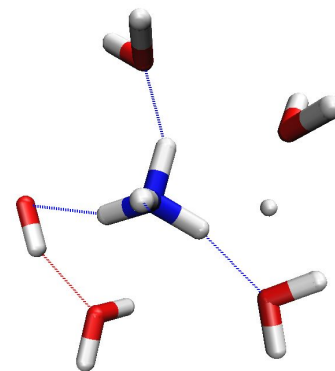
RDF results (N-O)

500ns, 7-7-7	r_max	CN
0.15M, q=-0.33	0.282	6.98
0.15M, q=-0.40	0.28	6.975
0.15M, q=-0.62	0.278	6.528
0.15M, q=-0.824	0.276	5.968

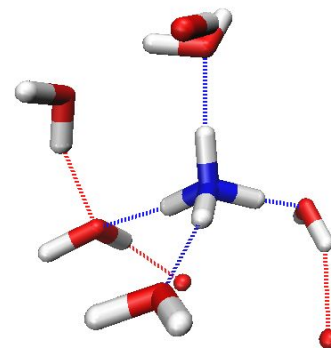


Number of hydrogen bond

500ns, 4-4-4	r_min	CN	Avg. no. of H-bond (4-4-4)	Avg. no. of H-bond (7-7-7)
0.15M, q=-0.33	0.368	7.0	2.15	2.25
0.15M, q=-0.40	0.364	6.759	2.25	2.34
0.15M, q=-0.62	0.354	6.19	2.52	2.61
0.15M, q=-0.824	0.36	6.163	2.13	2.82
1M, q=-0.33	0.362	6.377	2.48	



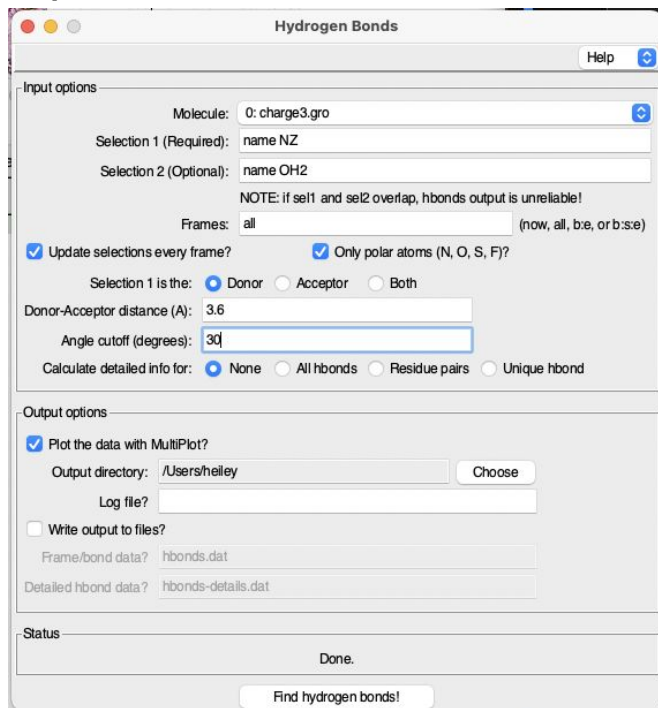
3 H-bonds



4 H-bonds

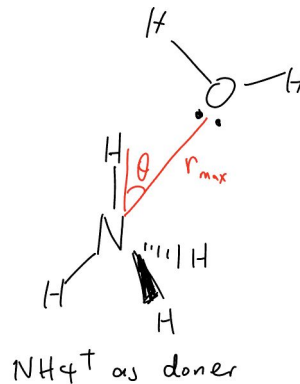
Hbond vmd plugin

Input



The screenshot shows the 'Hydrogen Bonds' dialog box in VMD. The 'Input options' section includes a 'Molecule' field set to '0: charge3.gro', 'Selection 1 (Required)' set to 'name NZ', and 'Selection 2 (Optional)' set to 'name OH2'. A note states: 'NOTE: if sel1 and sel2 overlap, hbonds output is unreliable!'. The 'Frames' field is set to 'all'. Checkboxes for 'Update selections every frame?' and 'Only polar atoms (N, O, S, F)?' are both checked. Radio buttons for 'Selection 1 is the:' are set to 'Donor'. The 'Donor-Acceptor distance (A)' is 3.6, and the 'Angle cutoff (degrees)' is 30. The 'Calculate detailed info for:' section has 'None' selected. The 'Output options' section has 'Plot the data with MultiPlot?' checked, 'Output directory' set to '/Users/heiley', and 'Log file' set to an empty field. 'Write output to files?' is unchecked. At the bottom, the 'Status' bar shows 'Done.' and a 'Find hydrogen bonds!' button.

A hydrogen bond is formed between an atom with a hydrogen bonded to it (the donor, D) and another atom (the acceptor, A) provided that the distance D-A is less than the cut-off distance and the angle D-H-A is less than the cut-off angle



Parameters used in the calculation of hydrogen bonds:

- Atomselection 1: (name NZ) and (name "N.*" "O.*" "S.*" FA F1 F2 F3)
- Atomselection 2: (name OH2) and (name "N.*" "O.*" "S.*" FA F1 F2 F3)
- Update selections every frame: yes
- Initial frame: 0
- Frame step: 1
- Final frame: 2001
- Donor-Acceptor distance: 3.54
- Angle cutoff: 30
- Type: unique
- Write a file with H bond/frame data: yes
- Filename: /Users/heiley/Desktop/hbonds3.dat
- Details output file: /Users/heiley/Desktop/hbonds3-details.dat

Hbond vmd plugin

Input

Hydrogen Bonds

Help

Input options

Molecule: 0: charge3.gro

Selection 1 (Required): name NZ

Selection 2 (Optional): name OH2

NOTE: if sel1 and sel2 overlap, hbonds output is unreliable!

Frames: all (now, all, bse, or b:se)

☒ Update selections every frame? ☒ Only polar atoms (N, O, S, F)?

Selection 1 is the: ☒ Donor ☐ Acceptor ☐ Both

Donor-Acceptor distance (A): 3.6

Angle cutoff (degrees): 30

Calculate detailed info for: ☒ None ☐ All hbonds ☐ Residue pairs ☐ Unique hbond

Output options

☒ Plot the data with MultiPlot?

Output directory: /Users/helley Choose

Log file?

☐ Write output to files?

Frame/bond data? hbonds.dat

Detailed hbond data? hbonds-details.dat

Status

Done.

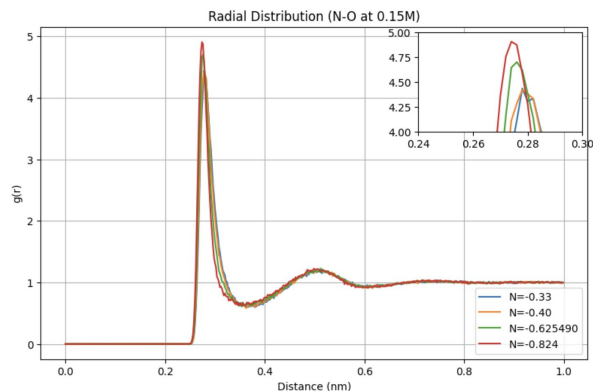
Find hydrogen bonds!

Donor vs acceptor

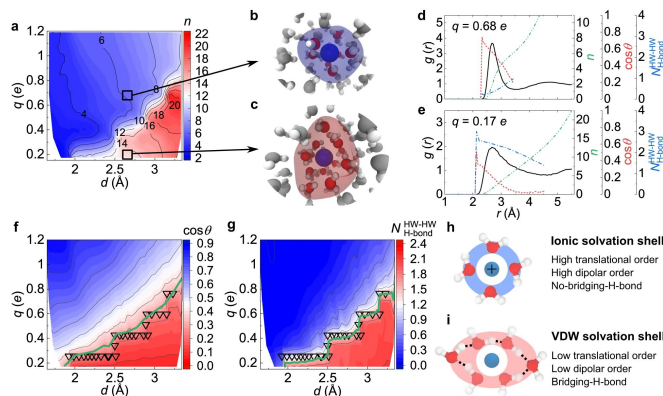
```
if {$DA == "D" || $DA == "both"} {  
    set hbondsingleframe1 [measure hbonds $dist $ang $sel1 $sel2]  
    set count1 [llength [lindex $hbondsingleframe1 0]]  
}  
if {$DA == "A" || $DA == "both"} {  
    set hbondsingleframe2 [measure hbonds $dist $ang $sel2 $sel1]  
    set count2 [llength [lindex $hbondsingleframe2 0]]  
}  
  
lappend framecount $f  
  
lappend numHbonds [expr $count1 + $count2]
```

Explanation of lower CN as $|q_N|$ increases

500ns, 4-4-4	r_max	CN
0.15M, $q=-0.33$	0.282	6.98
0.15M, $q=-0.40$	0.28	6.975
0.15M, $q=-0.62$	0.278	6.528
0.15M, $q=-0.824$	0.276	5.968



- The coordination number n drops sharply from $n \geq 12$ to $n = 4 - 8$ at $qc(d)$ as q increases (Fig. [1a](#)). This result indicates that an ion with low charge density, behaving like an electrically neutral particle, forms a thick solvation shell, whereas an ion with high charge density develops a thin, well-developed solvation shell (Fig. [1a-c](#)). We call the former the “VDW solvation shell” and the latter the “ionic solvation shell” according to their dominant interactions to form the shell.
- In the VDW solvation shell, a water molecule in the original second shell location penetrates the open space between the first and the second shell while stabilised through its H-bonding with two molecules in the first shell (Fig. [1d, e](#)).
- As q increases, the ion-induced water dipole reorientation destroys these bridging H-bonds, kicking the penetrating water out from the first shell. We have confirmed this mechanism in Supplementary Fig. [3](#) by directly monitoring the water dipole ordering and H-bonding in a non-equilibrium simulation.



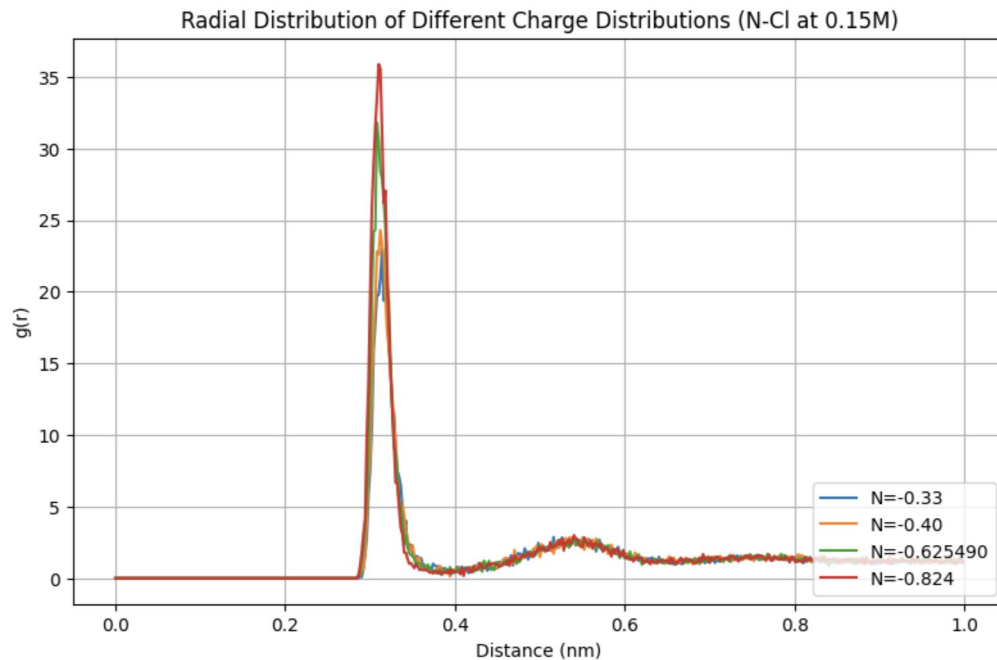
Shi, R., Cooper, A.J. & Tanaka, H. Impact of hierarchical water dipole orderings on the dynamics of aqueous salt solutions. *Nat Commun* 14, 4616 (2023). <https://doi.org/10.1038/s41467-023-40278-x>

RDF results (N-CI)

200ns, 4-4-4	r_max	CN	500ns, 4-4-4	r_max	CN	200ns, 7-7-7	r_max	CN
0.15M, q=-0.33	0.314	0.078	0.15M, q=-0.33	0.316	0.08	0.15M, q=-0.33	0.314	0.082
0.15M, q=-0.40	0.314	0.08	0.15M, q=-0.40	0.314	0.084	0.15M, q=-0.40	0.314	0.084
0.15M, q=-0.62	0.312	0.091	0.15M, q=-0.62	0.312	0.097	0.15M, q=-0.62	0.314	0.097
0.15M, q=-0.824	0.31	0.102	0.15M, q=-0.824	0.31	0.107	0.15M, q=-0.824	0.31	0.107
1M, q=-0.33	0.314	0.429						

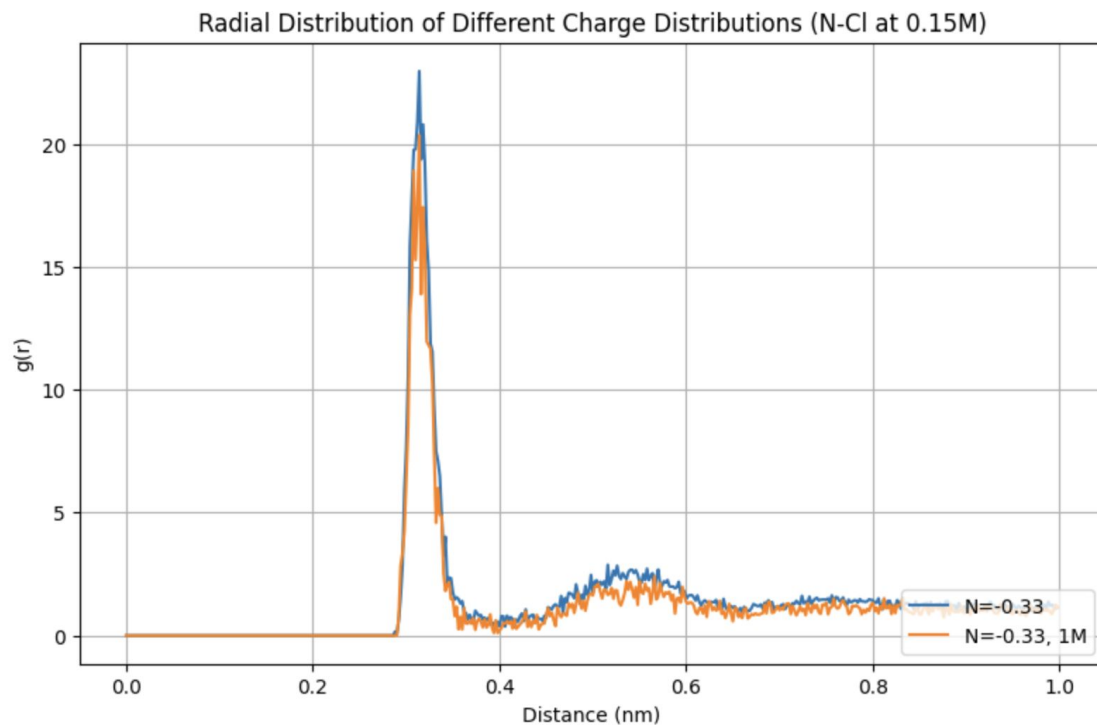
RDF results (N-Cl)

500ns	r_max	CN
0.15M, q=-0.33	0.314	0.082
0.15M, q=-0.40	0.314	0.084
0.15M, q=-0.62	0.314	0.097
0.15M, q=-0.824	0.31	0.107



RDF results (N-Cl)

200ns	r_max	CN
0.15M, q=-0.33	0.314	0.082
1M, q=-0.33	0.314	0.429



Research on NH₄ conduction in ion channel

[a] Detailed Mechanism for AmtB Conducting NH₄ /NH₃: Molecular Dynamics Simulations

- Optimised charges by restrained ESP-fit method using ChelpG approach
 - N: -0.824; H: +0.456

[b] Ammonium Recruitment and Ammonia Transport by E. coli Ammonia Channel AmtB

- NH₄⁺ partial charges using OPLS charges
- NH₄⁺ bond and bond angle using CHARMM27 parameters, same as NH₃ moiety of Lysine

[c] Molecular Dynamics Simulations on the Escherichia coli Ammonia Channel Protein AmtB: Mechanism of Ammonia/ Ammonium Transport

- Apply Mulliken charges at the HF/6-31G(d)
- LJ parameters same as amide groups

[d] Ammonium Transporters Achieve Charge Transfer by Fragmenting Their Substrate

- NH₄⁺ and surrounding water molecules and protein side chains are described by a polarizable force field based on the classical Drude oscillator, (62-65) and parametrized to reproduce both the free energy of hydration and the ion–protein interactions
- Pair-specific LJ parameters between the ions and atoms of the ligands are adjusted to reproduce the ab initio interaction energies
- Full geometry optimizations of the complexes between these ligands and the three cations Na⁺, K⁺, and NH₄⁺ are performed at the MP2(FC)/6-311++G(d,p) level using Gaussian 09 program

[g] Ammonium Transport Proteins with Changes in One of the Conserved Pore Histidines Have Different Performance in Ammonia and Methylamine Conduction

- The force field parameters of ammonia and methylamine were obtained from server SwissParam. [52]
- In order to validate the force field parameters, the salvation free energy of ammonia and methylamine in water was calculated using free energy perturbation (detailed protocol shown in Text S1).
- The calculated solvation free energies for ammonia and methylamine are -5.00 ± 0.24 and -4.49 ± 0.18 kcal/mol (Figure S1) respectively, very similar to the experiment values (-4.31 kcal/mol for ammonia and -4.57 kcal/mol for methylamine [53]).
- Therefore the parameters used here should be suitable for simulations.

Research on NH₄ conduction in ion channel

	Method	Force field, water	Charges
Default	Parameters same as methylammonium MAMM N from primary NH ₃ ⁺ , phosphatidylethanolamine	CHARMM36, TIP3P	N: -0.33 H: +0.33
[a]	NH ₄ charges by restrained ESP-fit method using ChelpG approach	GROMOS87, SPC	N: -0.824 H: +0.456
[b]	NH ₄ ⁺ partial charges using OPLS charges, as OPLS and CHARMM partial charges of NH ₃ ⁺ in Lysine are nearly identical	CHARMM27, H atoms were added to crystal waters using PSFGEN in NAMD	N: -0.40 H: +0.35
[c]	N, H charges using Mulliken charges at the HF/6-31G(d)	CHARMM, TIP3P	Not specified
[d]	<ul style="list-style-type: none"> - NH₄⁺ and surrounding water molecules and protein side chains are described by a polarizable force field based on the classical Drude oscillator, and parametrised to reproduce both the free energy of hydration and the ion–protein interactions. - Pair-specific LJ parameters between the ions and atoms of the ligands are adjusted to reproduce the ab initio interaction energies. - Full geometry optimizations of the complexes between these ligands and the three cations Na⁺, K⁺, and NH₄⁺ are performed at the MP2(FC)/6-311++G(d,p) level using Gaussian 09 program. 	CHARMM27, TIP3P	/
[e]		CHARMM36, TIP3P	Adapted from [a]

Research on NH4 conduction in ion channel (2)

	Method	Force field, water	Charges
Default	Parameters same as methylammonium MAMM N from primary NH ₃ ⁺ , phosphatidylethanolamine	CHARMM36, TIP3P	N: -0.33 H: +0.33
[g]	<ul style="list-style-type: none"> Parameters of ammonia from server SwissParam Validated with salvation free energy of ammonia in water using free energy perturbation 	CHARMM27, TIP3P	
[h]	<ul style="list-style-type: none"> Parameters were obtained using the Extensible Computational Chemistry Environment (ECCE)49 after performing quantum mechanics calculations using NWChem Geometrically optimised at RHF level with 6-31111G(3df,3pd)basis set with Pople(3df,3pd) as a polarization function,and with Pople-style as a diffusion function Partial charges were derived using electrostatic potential fitting. Energy constants were taken from the CHARMM, version 22, forcefield for primary amines. 	CHARMM, TIP3P	N: -0.625490 H: +0.406372

	Elements	Energy constant (K_{θ}) ^a [kcal/(mol Å ²)]	Bond length (Å)
Stretching	N—H	403.0	1.003
		Energy constant (K_{θ}) ^a [kcal/(mol rad ²)]	Bond angle (°)
Bending	H—N—H	44.0	109.471
Electrostatic	N	Partial charge -0.625490	
	H	+0.406372	

Other NH₄ parameterisations

	Method	Force field, water	Charges
Default	N from primary NH ₃ ⁺ , phosphatidylethanolamine H from polar H ⁺	CHARMM36, TIP3P	N: -0.33 H: +0.33
Madrid [5]	N, H charges using OPLS-based model, scaled by 0.85	Madrid-2019, TIP4P/2005	N: -0.3400 H: +0.2975

RDF data (Na-O)

	No. of OH ₂ in first hydration shell	First peak	First minimum
Simulation [1]	5.702	2.36	3.18
Literature [2]	4.0-8.0	2.36	
MC in TIP4P [3]	6.2	2.50	
Madrid [6]	5.4	2.33	

RDF data (N-O)

	No. of OH ₂ in first hydration shell	First peak	First minimum
Simulation [1]	6.581	2.82	3.66
Literature [2]	8.1-10.0	2.6-3.05	
MC in TIP4P [3]	7.3	2.90	
MC in TIP3P [4]	7.0-7.1	2.63-2.70	
Madrid [5]	5.5	2.66	
NH ₃ MD [16]		3.25	
NH ₃ Ab Initio [16]		2.75	

RDF plots of $\text{N}_{\text{NH}_4^+}-\text{O}$

[4]

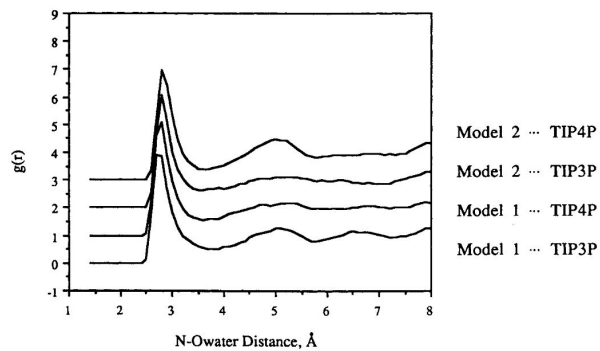
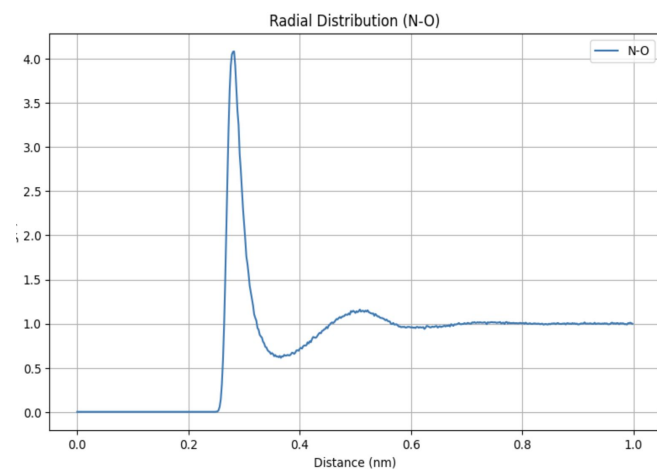
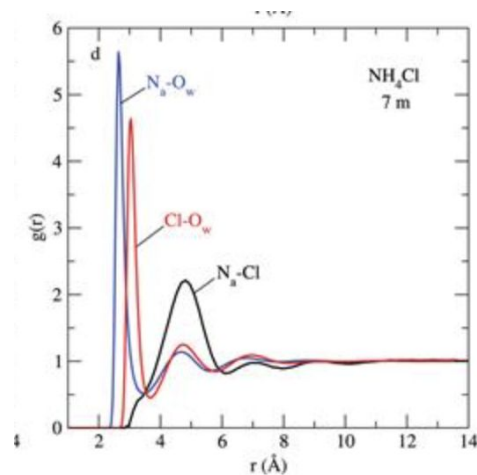


Figure 2. $\text{N}-\text{O}_{\text{water}}$ radial distribution functions for model 1 and 2 in TIP3P and TIP4P.

[5]



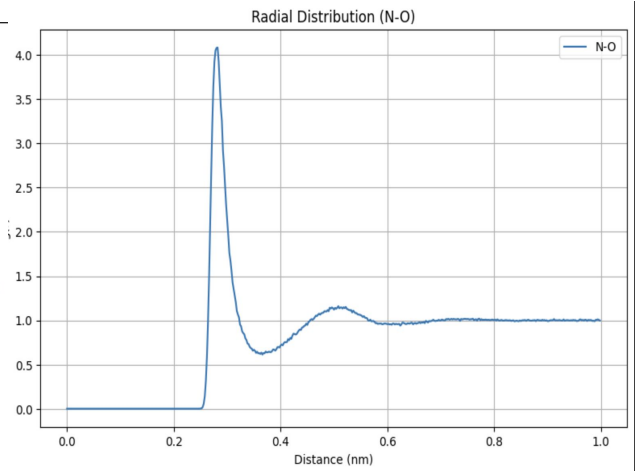
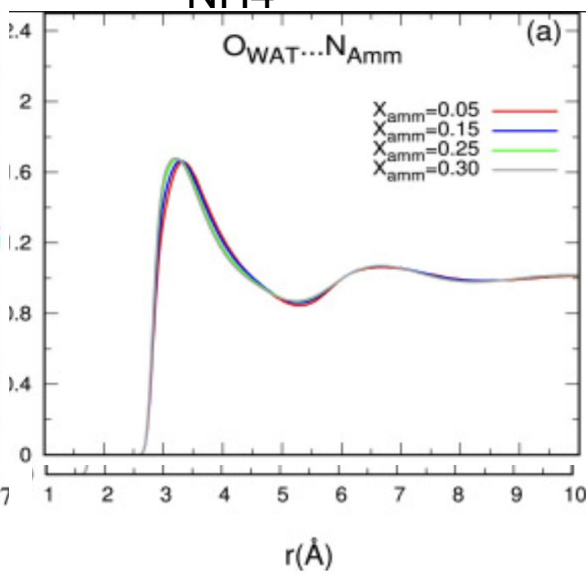
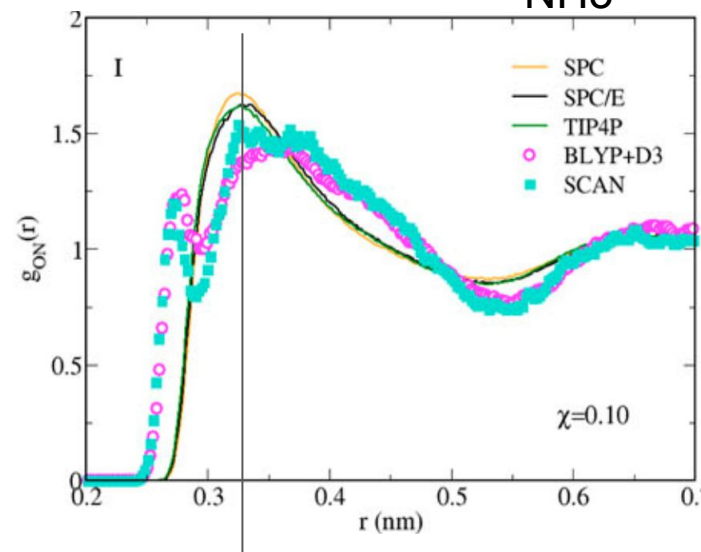
NH_4 :

First maximum found at $r = 0.282\text{nm}$

Coordination number = 6.581

$\chi = 0.02$

RDF plots of N_{NH3}-O and N_{NH4}-O

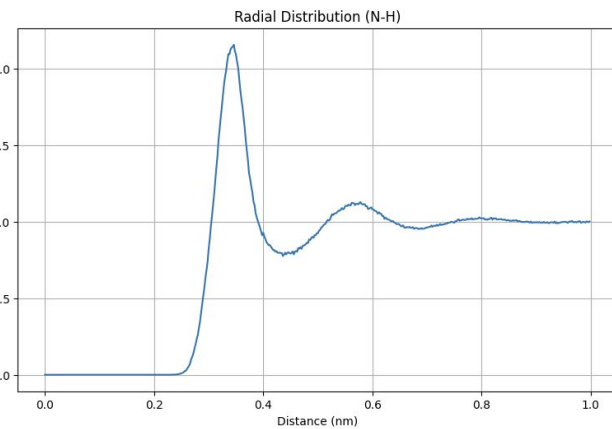
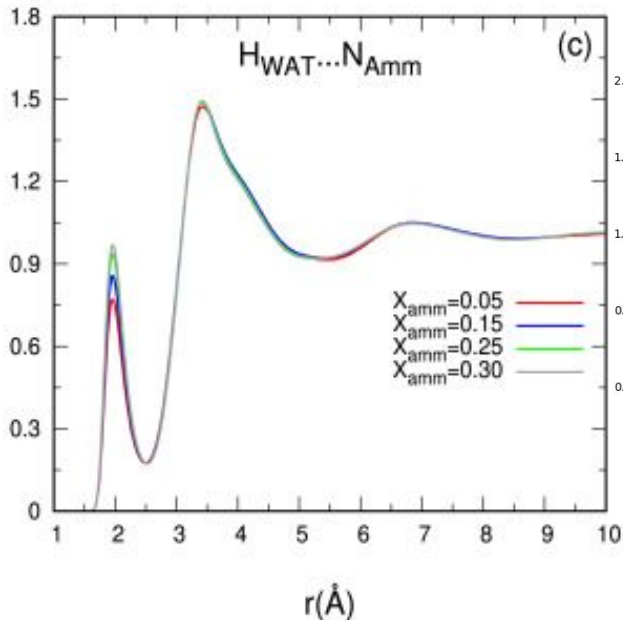
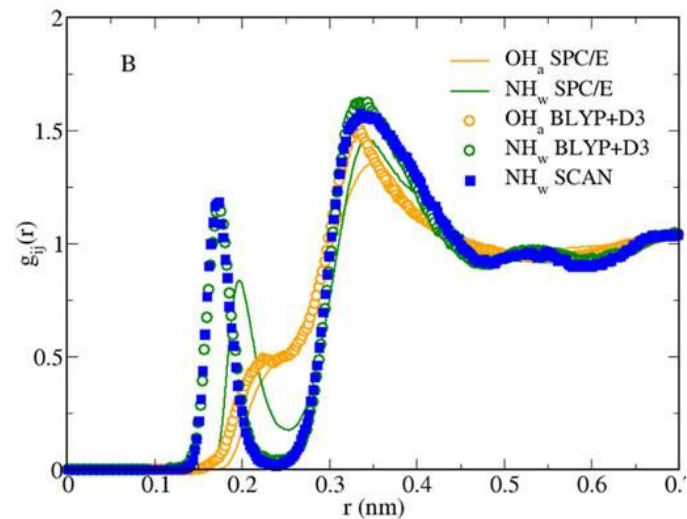


NH₃:
MD (lines) and AIMD (symbols) at $\chi = 0.1$ (NH₃:H₂O). The three different force fields used for water are represented by orange (SPC), black (SPC/E), and green (TIP4P) lines, while ab initio BLYP+D3 and SCAN correlations are indicated as magenta circles and cyan squares, respectively. [16]

NH₃:
MD simulation at $\chi = 0.05-0.30$ [17]

NH₄:
First maximum found at $r = 0.282$ nm
Coordination number = 6.581
 $\chi = 0.02$

RDF plots of $N_{NH_3}-H$ and $N_{NH_4}-H$



NH3:
Comparison between SPC/E (lines), BLYP+D3 (circles), and SCAN (squares) radial distributions functions involving hydrogen atoms for $T = 295$ K and $\chi = 0.10$. Correlations between atoms of the same molecule type are reported in (a), whereas cross-correlations are shown in (b). [16]

NH3:
MD simulation at $\chi = 0.05-0.30$ [17]

NH4 from original simulation:
First maximum found at $r = 0.344$ nm
Coordination number = 20.891
 $\chi = 0.02$

RDF data (Cl-O)

	No. of OH ₂ in first hydration shell	First peak	First minimum
Simulation, NaCl [1]	7.911	3.16	3.84
Simulation, NH ₄ Cl [1]	7.227	3.16	3.80
Literature [2]	5.9-8.5	3.1-3.3	
MC in TIP4P [3]	7.6	3.25	
Madrid [6]	5.9	3.05	

Free energy calculations

	Absolute hydration energy of NH ₄	Absolute hydration energy of Na	Relative hydration energies between NH ₄ ⁺ and Na ⁺
Experiment [7]	-78.6		-21.7 kcal/mol
Free energy perturbation [4]	-79.55 (+ Born) -98.85		-28.87 kcal/mol
Empirical model [8]	-68.1	-87.2	-19.1 kcal/mol
MC simulation [3]	-73.1	-92.3	-19 kcal/mol

Osmotic coefficient at 1M/1m

	NH ₄ Cl	NaCl
Isopiestic vapour pressure method [9]	0.897	
MC simulations [10]		0.91-0.92
Experiment [11]		0.94
MD simulations [12]		0.93
Experiment [13]	0.927	
Hückel equation calculation [14]	0.902	
Experiment [15]	0.9671	

References

- [1] <https://github.com/Heiley-W/project/blob/main/RDF-analysis.ipynb>
- [2] Ionic radii in aqueous solutions <https://doi.org/10.1021/cr00090a003>
- [3] Halide, Ammonium, and Alkali Metal Ion Parameters for Modeling Aqueous Solutions <https://doi.org/10.1021/ct600252r>
- [4] Free Energy Calculations Involving NH_4^+ in Water <https://doi.org/10.1002/jcc.540120106>
- [5] Further extension of the Madrid-2019 force field: Parametrization of nitrate and ammonium ion <https://doi.org/10.1063/5.0177363>
- [6] A force field of Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , and in aqueous solution based on the TIP4P/2005 water model and scaled charges for the ions <https://doi.org/10.1063/1.5121392>
- [7] Ref 33 and 22 of [4] respectively
- [8] A simple empirical model describing the thermodynamics of hydration of ions of widely varying charges, sizes, and shapes [https://doi.org/10.1016/0301-4622\(94\)00051-4](https://doi.org/10.1016/0301-4622(94)00051-4)
- [9] The osmotic and activity coefficients of aqueous solutions of ammonium chloride and ammonium nitrate at 25° <https://doi.org/10.1039/TF9534900027>
- [10] Osmotic and activity coefficients from effective potentials for hydrated ions <https://doi.org/10.1103/PhysRevE.55.5689>
- [11] Ref 20 of [10]
- [12] Osmotic coefficients of atomistic NaCl (aq) force fields <https://doi.org/10.1063/1.2185105>
- [13] The Osmotic and Activity Coefficients of Some Salts Having Relatively Large Molar Volumes <https://doi.org/10.1021/je60071a020>
- [14] Mean Activity Coefficients and Osmotic Coefficients in Aqueous Solutions of Salts of Ammonium Ions with Univalent Anions at 25 °C <https://doi.org/10.1021/je300474k>
- [15] The Vapor Pressures and Activity Coefficients of Aqueous Solutions of Ammonium Chloride at 25° <https://doi.org/10.1021/ja01286a019>
- [16] The structure of water-ammonia mixtures from classical and ab initio molecular dynamics <https://doi.org/10.1063/5.0220328>
- [17] Effects of concentration and pressure on the aqueous solvation structure of ammonia and composition dependent ion solvation scenario in water-ammonia mixtures <https://doi.org/10.1016/j.fluid.2020.112507>

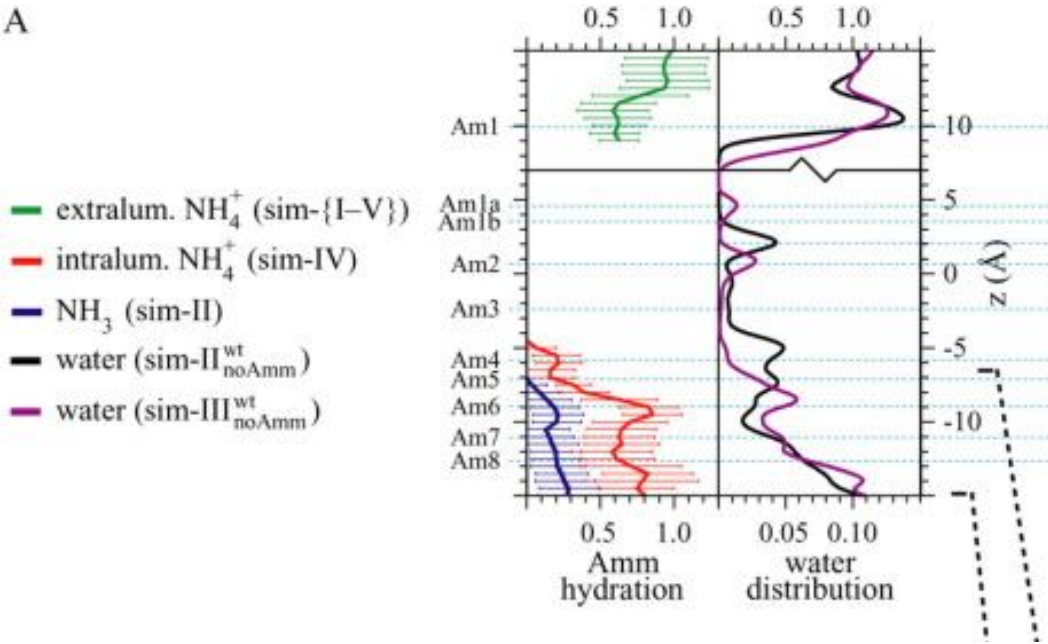
References

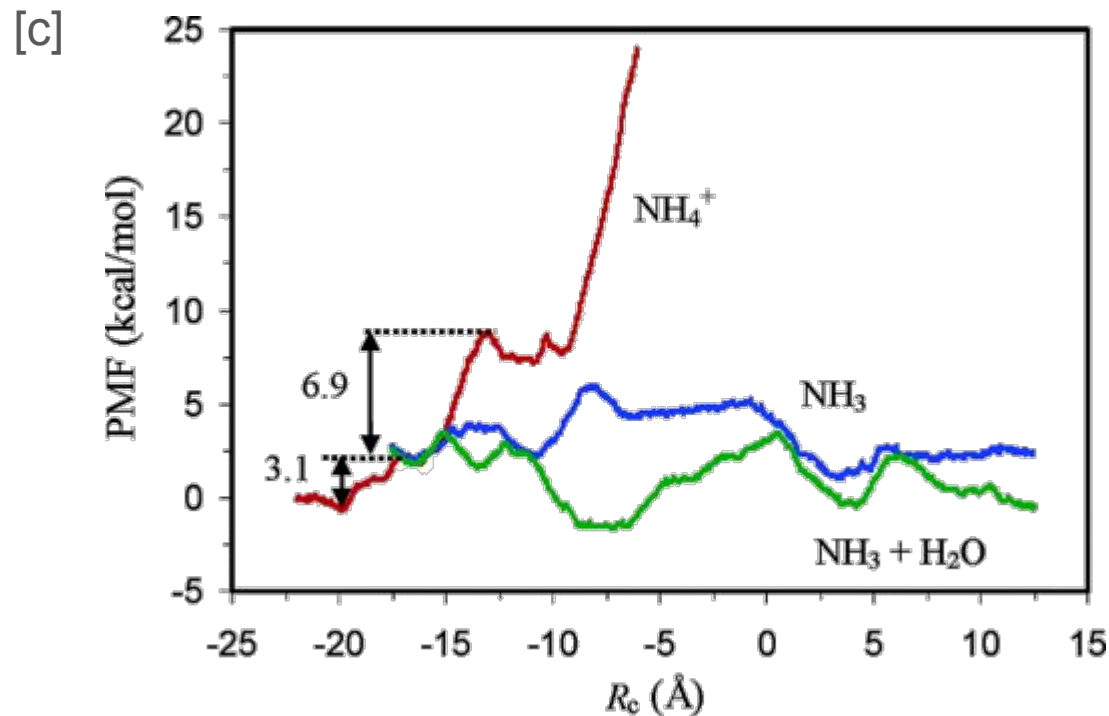
- [a] Detailed Mechanism for AmtB Conducting $\text{NH}_4^+/\text{NH}_3$: Molecular Dynamics Simulations <https://doi.org/10.1529/biophysj.106.090191>
- [b] Ammonium Recruitment and Ammonia Transport by E. coli Ammonia Channel AmtB <https://doi.org/10.1529/biophysj.106.089714>
- [c] Molecular Dynamics Simulations on the Escherichiacoli Ammonia Channel Protein AmtB: Mechanism of Ammonia/Ammonium Transport <https://doi.org/10.1021/ja0631549>
- [d] Ammonium Transporters Achieve Charge Transfer by Fragmenting Their Substrate <https://doi.org/10.1021/ja300129x>
- [e] A two-lane mechanism for selective biological ammonium transport <https://doi.org/10.1101/849562>
- [f] The mechanism of ammonia transport based on the crystal structure of AmtB of Escherichia coli <https://doi.org/10.1073/pnas.0406475101>
- [g] Ammonium Transport Proteins with Changes in One of the Conserved Pore Histidines Have Different Performance in Ammonia and Methylamine Conduction <https://doi.org/10.1371/journal.pone.0062745>
- [h] Free-energy profiles for ions in the influenza M2-TMD channel <https://doi.org/10.1002/prot.22376>

Research on NH4 conduction in ion channel

[b]

A



Research on NH_4 conduction in ion channel

Research on NH4 conduction in ion channel

[f] Experiment

