SUPPLEMENTARY INFORMATION

Development of a Force Field for ATP – How Charge Scaling Controls Self Association

Tuan Minh Do,[*a,b] Nobuyuki Matubayasi,[b] and Dominik Horinek[*a]

[a] *Institute of Physical and Theoretical Chemistry, University of Regensburg, 93040 Regensburg, Germany. E-mail: dominik.horinek@ur.de*

[b] *Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, 560-8531 Toyonaka, Osaka, Japan. E-mail: do.minh@cheng.es.osaka-u.ac.jp*

Figure S1

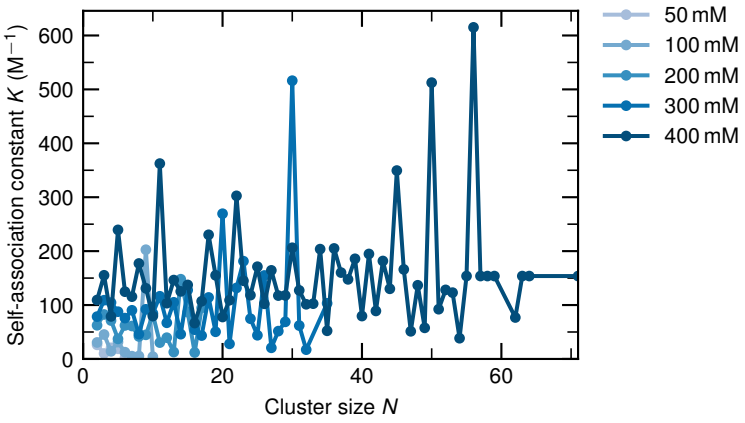

Figure 1: **Cluster-size dependent self-association constant $K(N)$.** $K(N)$ shows strong fluctuations as a function of N, making the direct application of Eq. (2) infeasible for comparison with experimental values. To address this, we employed an isodesmic model, which simplifies the analysis by assuming that K depends only on the total ATP concentration and the number of clusters.

System topology file `topol.top`

```
; GROMACS topology

; Include the force field
#include "amber03w.ff/forcefield.itp"

[ atomtypes ]
; name      at.num  mass     charge ptype  sigma      epsilon
O3           8      15.99940  0.0000  A   3.11690e-01  8.78640e-01 ; Steinbrecher et al., JChemTheory, 2012
O2           8      15.99940  0.0000  A   3.11690e-01  8.78640e-01 ; Steinbrecher et al., JChemTheory, 2012
OQ           8      15.99940  0.0000  A   3.22345e-01  8.78640e-01 ; Steinbrecher et al., JChemTheory, 2012
OR           8      15.99940  0.0000  A   3.15699e-01  7.11280e-01 ; Steinbrecher et al., JChemTheory, 2012
OWAT         8      15.99940  0.000   A   3.15890e-01  7.74903e-01 ; Tip4p/2005
HWAT         1      1.00800   0.5564  A   0.00000e+00  0.00000e+00 ; Tip4p/2005
MWAT         0      0.00000  -1.1128  D   0.00000e+00  0.00000e+00 ; Tip4p/2005

; Include ion topologies
#include "atp.itp"
#include "ions.itp"

; Include water topology
#include "tip4p2005.itp"

[ system ]
SYSTEMNAME

[ molecules ]
```

ATP topology file `atp.itp`

```
; ATP adenosin triphosphate

[ moleculetype ]
;name                nrexcl
ATP                  3

[ atoms ]
;nr    type  resnr res   atom  cgnr          charge          mass ; qtot
1      O3    1     atp   O1G   1          -0.666820     16.000000 ; qtot -0.666820
2      P     1     atp   PG    2           0.885500     30.970000 ; qtot  0.218680
3      O3    1     atp   O2G   3          -0.666820     16.000000 ; qtot -0.448140
4      O3    1     atp   O3G   4          -0.666820     16.000000 ; qtot -1.114960
5      OR    1     atp   O3B   5          -0.372540     16.000000 ; qtot -1.487500
6      P     1     atp   PB    6           0.969640     30.970000 ; qtot -0.517860
7      O2    1     atp   O1B   7          -0.622580     16.000000 ; qtot -1.140440
8      O2    1     atp   O2B   8          -0.622580     16.000000 ; qtot -1.763020
9      OR    1     atp   O3A   9          -0.398230     16.000000 ; qtot -2.161250
10     P     1     atp   PA    10          0.877240     30.970000 ; qtot -1.284010
11     O2    1     atp   O1A   11         -0.615930     16.000000 ; qtot -1.899940
12     O2    1     atp   O2A   12         -0.615930     16.000000 ; qtot -2.515870
13     OR    1     atp   O5*   13         -0.476930     16.000000 ; qtot -2.992800
14     CT    1     atp   C5*   14          0.055800     12.010000 ; qtot -2.937000
15     H1    1     atp   H50   15          0.067900      1.008000 ; qtot -2.869100
16     H1    1     atp   H51   16          0.067900      1.008000 ; qtot -2.801200
17     CT    1     atp   C4*   17          0.106500     12.010000 ; qtot -2.694700
18     H1    1     atp   H40   18          0.117400      1.008000 ; qtot -2.577300
19     OS    1     atp   O4*   19         -0.354800     16.000000 ; qtot -2.932100
20     CT    1     atp   C1*   20          0.039400     12.010000 ; qtot -2.892700
21     H2    1     atp   H10   21          0.200700      1.008000 ; qtot -2.692000
22     N*    1     atp   N9    22         -0.025100     14.010000 ; qtot -2.717100
23     CK    1     atp   C8    23          0.200600     12.010000 ; qtot -2.516500
24     H5    1     atp   H80   24          0.155300      1.008000 ; qtot -2.361200
25     NB    1     atp   N7    25         -0.607300     14.010000 ; qtot -2.968500
26     CB    1     atp   C5    26          0.051500     12.010000 ; qtot -2.917000
27     CA    1     atp   C6    27          0.700900     12.010000 ; qtot -2.216100
28     N2    1     atp   N6    28         -0.901900     14.010000 ; qtot -3.118000
29     H     1     atp   H60   29          0.411500      1.008000 ; qtot -2.706500
30     H     1     atp   H61   30          0.411500      1.008000 ; qtot -2.295000
31     NC    1     atp   N1    31         -0.761500     14.010000 ; qtot -3.056500
32     CQ    1     atp   C2    32          0.587500     12.010000 ; qtot -2.469000
33     H5    1     atp   H2    33          0.047300      1.008000 ; qtot -2.421700
34     NC    1     atp   N3    34         -0.699700     14.010000 ; qtot -3.121400
35     CB    1     atp   C4    35          0.305300     12.010000 ; qtot -2.816100
36     CT    1     atp   C3*   36          0.202200     12.010000 ; qtot -2.613900
37     H1    1     atp   H30   37          0.061500      1.008000 ; qtot -2.552400
38     OH    1     atp   O3*   38         -0.654100     16.000000 ; qtot -3.206500
39     HO    1     atp   H3'   39          0.437600      1.008000 ; qtot -2.768900
40     CT    1     atp   C2*   40          0.067000     12.010000 ; qtot -2.701900
41     H1    1     atp   H20   41          0.097200      1.008000 ; qtot -2.604700
42     OH    1     atp   O2*   42         -0.613900     16.000000 ; qtot -3.218600
43     HO    1     atp   H2'   43          0.418600      1.008000 ; qtot -2.800000

[ bonds ]
;ai     aj      funct                 r             k
42      43      1          9.600000e-02   4.627504e+05 ; O2*-H2'
40      41      1          1.090000e-01   2.845120e+05 ; C2*-H20
38      39      1          9.600000e-02   4.627504e+05 ; O3*-H3'
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36      37      1           1.090000e-01   2.845120e+05 ; C3*-H30
32      33      1           1.080000e-01   3.071056e+05 ; C2-H2
28      29      1           1.010000e-01   3.631712e+05 ; N6-H60
28      30      1           1.010000e-01   3.631712e+05 ; N6-H61
23      24      1           1.080000e-01   3.071056e+05 ; C8-H80
20      21      1           1.090000e-01   2.845120e+05 ; C1*-H10
17      18      1           1.090000e-01   2.845120e+05 ; C4*-H40
14      15      1           1.090000e-01   2.845120e+05 ; C5*-H50
14      16      1           1.090000e-01   2.845120e+05 ; C5*-H51
40      42      1           1.410000e-01   2.677760e+05 ; C2*-O2*
36      38      1           1.410000e-01   2.677760e+05 ; C3*-O3*
36      40      1           1.526000e-01   2.594080e+05 ; C3*-C2*
34      35      1           1.354000e-01   3.857648e+05 ; N3-C4
32      34      1           1.324000e-01   4.200736e+05 ; C2-N3
31      32      1           1.324000e-01   4.200736e+05 ; N1-C2
27      28      1           1.340000e-01   4.025008e+05 ; C6-N6
27      31      1           1.339000e-01   4.041744e+05 ; C6-N1
26      27      1           1.404000e-01   3.924592e+05 ; C5-C6
26      35      1           1.370000e-01   4.351360e+05 ; C5-C4
25      26      1           1.391000e-01   3.464352e+05 ; N7-C5
23      25      1           1.304000e-01   4.426672e+05 ; C8-N7
22      23      1           1.371000e-01   3.681920e+05 ; N9-C8
22      35      1           1.374000e-01   3.648448e+05 ; N9-C4
20      22      1           1.475000e-01   2.820016e+05 ; C1*-N9
20      40      1           1.526000e-01   2.594080e+05 ; C1*-C2*
19      20      1           1.410000e-01   2.677760e+05 ; O4*-C1*
17      19      1           1.410000e-01   2.677760e+05 ; C4*-O4*
17      36      1           1.526000e-01   2.594080e+05 ; C4*-C3*
14      17      1           1.526000e-01   2.594080e+05 ; C5*-C4*
13      14      1           1.410000e-01   2.677760e+05 ; O5*-C5*
10      11      1           1.480000e-01   4.393200e+05 ; PA-O1A
10      12      1           1.480000e-01   4.393200e+05 ; PA-O2A
10      13      1           1.610000e-01   1.924640e+05 ; PA-O5*
9       10      1           1.610000e-01   1.924640e+05 ; O3A-PA
6       7       1           1.480000e-01   4.393200e+05 ; PB-O1B
6       8       1           1.480000e-01   4.393200e+05 ; PB-O2B
6       9       1           1.610000e-01   1.924640e+05 ; PB-O3A
5       6       1           1.610000e-01   1.924640e+05 ; O3B-PB
2       3       1           1.480000e-01   4.393200e+05 ; PG-O2G
2       4       1           1.480000e-01   4.393200e+05 ; PG-O3G
2       5       1           1.610000e-01   1.924640e+05 ; PG-O3B
1       2       1           1.480000e-01   4.393200e+05 ; O1G-PG

[ pairs ]
;ai     aj      funct
41      43      1       ; H20-H2'
39      40      1       ; H3'-C2*
38      41      1       ; O3*-H20
37      39      1       ; H30-H3'
37      41      1       ; H30-H20
37      42      1       ; H30-O2*
36      43      1       ; C3*-H2'
33      35      1       ; H2-C4
30      31      1       ; H61-N1
29      31      1       ; H60-N1
27      33      1       ; C6-H2
26      29      1       ; C5-H60
26      30      1       ; C5-H61
24      35      1       ; H80-C4
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24      26      1       ; H80-C5
22      41      1       ; N9-H20
21      23      1       ; H10-C8
21      35      1       ; H10-C4
21      36      1       ; H10-C3*
21      41      1       ; H10-H20
21      42      1       ; H10-O2*
20      24      1       ; C1*-H80
20      37      1       ; C1*-H30
20      43      1       ; C1*-H2'
19      37      1       ; O4*-H30
19      41      1       ; O4*-H20
18      20      1       ; H40-C1*
18      37      1       ; H40-H30
18      38      1       ; H40-O3*
18      40      1       ; H40-C2*
17      21      1       ; C4*-H10
17      39      1       ; C4*-H3'
17      41      1       ; C4*-H20
16      18      1       ; H51-H40
16      19      1       ; H51-O4*
16      36      1       ; H51-C3*
15      18      1       ; H50-H40
15      19      1       ; H50-O4*
15      36      1       ; H50-C3*
14      37      1       ; C5*-H30
13      18      1       ; O5*-H40
10      15      1       ; PA-H50
10      16      1       ; PA-H51
38      42      1       ; O3*-O2*
35      40      1       ; C4-C2*
31      35      1       ; N1-C4
28      35      1       ; N6-C4
28      32      1       ; N6-C2
27      34      1       ; C6-N3
26      32      1       ; C5-C2
25      28      1       ; N7-N6
25      31      1       ; N7-N1
25      34      1       ; N7-N3
23      40      1       ; C8-C2*
23      34      1       ; C8-N3
23      27      1       ; C8-C6
22      36      1       ; N9-C3*
22      42      1       ; N9-O2*
22      32      1       ; N9-C2
22      27      1       ; N9-C6
20      25      1       ; C1*-N7
20      34      1       ; C1*-N3
20      26      1       ; C1*-C5
20      38      1       ; C1*-O3*
19      38      1       ; O4*-O3*
19      23      1       ; O4*-C8
19      35      1       ; O4*-C4
19      42      1       ; O4*-O2*
17      22      1       ; C4*-N9
17      42      1       ; C4*-O2*
14      20      1       ; C5*-C1*
14      38      1       ; C5*-O3*
14      40      1       ; C5*-C2*
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13       19       1         ; O5*-O4*
13       36       1         ; O5*-C3*
12       14       1         ; O2A-C5*
11       14       1         ; O1A-C5*
10       17       1         ; PA-C4*
9        14       1         ; O3A-C5*
8        10       1         ; O2B-PA
7        10       1         ; O1B-PA
6        11       1         ; PB-O1A
6        12       1         ; PB-O2A
6        13       1         ; PB-O5*
5        10       1         ; O3B-PA
4        6        1         ; O3G-PB
3        6        1         ; O2G-PB
2        7        1         ; PG-O1B
2        8        1         ; PG-O2B
2        9        1         ; PG-O3A
1        6        1         ; O1G-PB

[ angles ]
;ai     aj      ak      funct            theta             cth
41      40      42      1          1.095000e+02   4.184000e+02 ; H20-C2*-O2*
40      42      43      1          1.085000e+02   4.602400e+02 ; C2*-O2*-H2'
37      36      38      1          1.095000e+02   4.184000e+02 ; H30-C3*-O3*
37      36      40      1          1.095000e+02   4.184000e+02 ; H30-C3*-C2*
36      38      39      1          1.085000e+02   4.602400e+02 ; C3*-O3*-H3'
36      40      41      1          1.095000e+02   4.184000e+02 ; C3*-C2*-H20
33      32      34      1          1.154500e+02   4.184000e+02 ; H2-C2-N3
31      32      33      1          1.154500e+02   4.184000e+02 ; N1-C2-H2
29      28      30      1          1.200001e+02   2.928800e+02 ; H60-N6-H61
27      28      29      1          1.200001e+02   4.184000e+02 ; C6-N6-H60
27      28      30      1          1.200001e+02   4.184000e+02 ; C6-N6-H61
24      23      25      1          1.230501e+02   4.184000e+02 ; H80-C8-N7
22      23      24      1          1.230501e+02   4.184000e+02 ; N9-C8-H80
21      20      22      1          1.095000e+02   4.184000e+02 ; H10-C1*-N9
21      20      40      1          1.095000e+02   4.184000e+02 ; H10-C1*-C2*
20      40      41      1          1.095000e+02   4.184000e+02 ; C1*-C2*-H20
19      20      21      1          1.095000e+02   4.184000e+02 ; O4*-C1*-H10
18      17      19      1          1.095000e+02   4.184000e+02 ; H40-C4*-O4*
18      17      36      1          1.095000e+02   4.184000e+02 ; H40-C4*-C3*
17      36      37      1          1.095000e+02   4.184000e+02 ; C4*-C3*-H30
16      14      17      1          1.095000e+02   4.184000e+02 ; H51-C5*-C4*
15      14      16      1          1.095000e+02   2.928800e+02 ; H50-C5*-H51
15      14      17      1          1.095000e+02   4.184000e+02 ; H50-C5*-C4*
14      17      18      1          1.095000e+02   4.184000e+02 ; C5*-C4*-H40
13      14      15      1          1.095000e+02   4.184000e+02 ; O5*-C5*-H50
13      14      16      1          1.095000e+02   4.184000e+02 ; O5*-C5*-H51
38      36      40      1          1.095000e+02   4.184000e+02 ; O3*-C3*-C2*
36      40      42      1          1.095000e+02   4.184000e+02 ; C3*-C2*-O2*
32      34      35      1          1.110000e+02   5.857600e+02 ; C2-N3-C4
31      32      34      1          1.291001e+02   5.857600e+02 ; N1-C2-N3
28      27      31      1          1.193001e+02   5.857600e+02 ; N6-C6-N1
27      26      35      1          1.173001e+02   5.271840e+02 ; C6-C5-C4
27      31      32      1          1.186001e+02   5.857600e+02 ; C6-N1-C2
26      27      28      1          1.235001e+02   5.857600e+02 ; C5-C6-N6
26      27      31      1          1.173001e+02   5.857600e+02 ; C5-C6-N1
26      35      34      1          1.277001e+02   5.857600e+02 ; C5-C4-N3
25      26      27      1          1.324001e+02   5.857600e+02 ; N7-C5-C6
25      26      35      1          1.104000e+02   5.857600e+02 ; N7-C5-C4
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23      22      35      1          1.054000e+02   5.857600e+02 ; C8-N9-C4
23      25      26      1          1.038000e+02   5.857600e+02 ; C8-N7-C5
22      20      40      1          1.095000e+02   4.184000e+02 ; N9-C1*-C2*
22      23      25      1          1.139000e+02   5.857600e+02 ; N9-C8-N7
22      35      34      1          1.262001e+02   5.857600e+02 ; N9-C4-N3
22      35      26      1          1.062000e+02   5.857600e+02 ; N9-C4-C5
20      22      23      1          1.288001e+02   5.857600e+02 ; C1*-N9-C8
20      22      35      1          1.258001e+02   5.857600e+02 ; C1*-N9-C4
20      40      36      1          1.095000e+02   3.347200e+02 ; C1*-C2*-C3*
20      40      42      1          1.095000e+02   4.184000e+02 ; C1*-C2*-O2*
19      17      36      1          1.095000e+02   4.184000e+02 ; O4*-C4*-C3*
19      20      22      1          1.095000e+02   4.184000e+02 ; O4*-C1*-N9
19      20      40      1          1.095000e+02   4.184000e+02 ; O4*-C1*-C2*
17      19      20      1          1.095000e+02   5.020800e+02 ; C4*-O4*-C1*
17      36      38      1          1.095000e+02   4.184000e+02 ; C4*-C3*-O3*
17      36      40      1          1.095000e+02   3.347200e+02 ; C4*-C3*-C2*
14      17      19      1          1.095000e+02   4.184000e+02 ; C5*-C4*-O4*
14      17      36      1          1.095000e+02   3.347200e+02 ; C5*-C4*-C3*
13      14      17      1          1.095000e+02   4.184000e+02 ; O5*-C5*-C4*
12      10      13      1          1.082300e+02   8.368000e+02 ; O2A-PA-O5*
11      10      12      1          1.199001e+02   1.171520e+03 ; O1A-PA-O2A
11      10      13      1          1.082300e+02   8.368000e+02 ; O1A-PA-O5*
10      13      14      1          1.205001e+02   8.368000e+02 ; PA-O5*-C5*
9       10      11      1          1.082300e+02   8.368000e+02 ; O3A-PA-O1A
9       10      12      1          1.082300e+02   8.368000e+02 ; O3A-PA-O2A
9       10      13      1          1.026000e+02   3.765600e+02 ; O3A-PA-O5*
8       6       9       1          1.082300e+02   8.368000e+02 ; O2B-PB-O3A
7       6       8       1          1.199001e+02   1.171520e+03 ; O1B-PB-O2B
7       6       9       1          1.082300e+02   8.368000e+02 ; O1B-PB-O3A
6       9       10      1          1.500001e+02   1.061481e+02 ; PB-O3A-PA
5       6       7       1          1.082300e+02   8.368000e+02 ; O3B-PB-O1B
5       6       8       1          1.082300e+02   8.368000e+02 ; O3B-PB-O2B
5       6       9       1          1.026000e+02   3.765600e+02 ; O3B-PB-O3A
4       2       5       1          1.082300e+02   8.368000e+02 ; O3G-PG-O3B
3       2       4       1          1.199001e+02   1.171520e+03 ; O2G-PG-O3G
3       2       5       1          1.082300e+02   8.368000e+02 ; O2G-PG-O3B
2       5       6       1          1.500001e+02   1.061481e+02 ; PG-O3B-PB
1       2       3       1          1.199001e+02   1.171520e+03 ; O1G-PG-O2G
1       2       4       1          1.199001e+02   1.171520e+03 ; O1G-PG-O3G
1       2       5       1          1.082300e+02   8.368000e+02 ; O1G-PG-O3B

[ dihedrals ]
;ai    aj      ak      al      funct       phi_s         k_phi   mult
41      40      42      43      9           0.000      0.697333      3 ; H20-C2*-O2*-H2'
39      38      36      40      9           0.000      1.046000      1 ; H3'-O3*-C3*-C2*
39      38      36      40      9           0.000      0.669440      3 ; H3'-O3*-C3*-C2*
38      36      40      41      9           0.000      1.046000      1 ; O3*-C3*-C2*-H20
38      36      40      41      9           0.000      0.000000      3 ; O3*-C3*-C2*-H20
37      36      38      39      9           0.000      0.697333      3 ; H30-C3*-O3*-H3'
37      36      40      41      9           0.000      0.650844      3 ; H30-C3*-C2*-H20
37      36      40      42      9           0.000      1.046000      1 ; H30-C3*-C2*-O2*
37      36      40      42      9           0.000      0.000000      3 ; H30-C3*-C2*-O2*
36      40      42      43      9           0.000      1.046000      1 ; C3*-C2*-O2*-H2'
36      40      42      43      9           0.000      0.669440      3 ; C3*-C2*-O2*-H2'
33      32      34      35      9         180.000     28.451200      2 ; H2-C2-N3-C4
30      28      27      31      9         180.000     10.041600      2 ; H61-N6-C6-N1
29      28      27      31      9         180.000     10.041600      2 ; H60-N6-C6-N1
27      31      32      33      9         180.000     28.451200      2 ; C6-N1-C2-H2
26      27      28      29      9         180.000     10.041600      2 ; C5-C6-N6-H60
```

```
294 26    27    28    30    9        180.000    10.041600    2 ; C5-C6-N6-H61
295 24    23    22    35    9        180.000     7.112800    2 ; H80-C8-N9-C4
296 24    23    25    26    9        180.000    41.840000    2 ; H80-C8-N7-C5
297 22    20    40    41    9          0.000     0.650844    3 ; N9-C1*-C2*-H20
298 21    20    22    23    9          0.000     0.000000    2 ; H10-C1*-N9-C8
299 21    20    22    35    9          0.000     0.000000    2 ; H10-C1*-N9-C4
300 21    20    40    36    9          0.000     0.650844    3 ; H10-C1*-C2*-C3*
301 21    20    40    41    9          0.000     0.650844    3 ; H10-C1*-C2*-H20
302 21    20    40    42    9          0.000     0.650844    3 ; H10-C1*-C2*-O2*
303 20    22    23    24    9        180.000     7.112800    2 ; C1*-N9-C8-H80
304 20    40    36    37    9          0.000     0.650844    3 ; C1*-C2*-C3*-H30
305 20    40    42    43    9          0.000     1.046000    1 ; C1*-C2*-O2*-H2'
306 20    40    42    43    9          0.000     0.669440    3 ; C1*-C2*-O2*-H2'
307 19    17    36    37    9          0.000     1.046000    1 ; O4*-C4*-C3*-H30
308 19    17    36    37    9          0.000     0.000000    3 ; O4*-C4*-C3*-H30
309 19    20    40    41    9          0.000     1.046000    1 ; O4*-C1*-C2*-H20
310 19    20    40    41    9          0.000     0.000000    3 ; O4*-C1*-C2*-H20
311 18    17    19    20    9          0.000     1.603867    3 ; H40-C4*-O4*-C1*
312 18    17    36    37    9          0.000     0.650844    3 ; H40-C4*-C3*-H30
313 18    17    36    38    9          0.000     1.046000    1 ; H40-C4*-C3*-O3*
314 18    17    36    38    9          0.000     0.000000    3 ; H40-C4*-C3*-O3*
315 18    17    36    40    9          0.000     0.650844    3 ; H40-C4*-C3*-C2*
316 17    19    20    21    9          0.000     1.603867    3 ; C4*-O4*-C1*-H10
317 17    36    38    39    9          0.000     1.046000    1 ; C4*-C3*-O3*-H3'
318 17    36    38    39    9          0.000     0.669440    3 ; C4*-C3*-O3*-H3'
319 17    36    40    41    9          0.000     0.650844    3 ; C4*-C3*-C2*-H20
320 16    14    17    18    9          0.000     0.650844    3 ; H51-C5*-C4*-H40
321 16    14    17    19    9          0.000     1.046000    1 ; H51-C5*-C4*-O4*
322 16    14    17    19    9          0.000     0.000000    3 ; H51-C5*-C4*-O4*
323 16    14    17    36    9          0.000     0.650844    3 ; H51-C5*-C4*-C3*
324 15    14    17    18    9          0.000     0.650844    3 ; H50-C5*-C4*-H40
325 15    14    17    19    9          0.000     1.046000    1 ; H50-C5*-C4*-O4*
326 15    14    17    19    9          0.000     0.000000    3 ; H50-C5*-C4*-O4*
327 15    14    17    36    9          0.000     0.650844    3 ; H50-C5*-C4*-C3*
328 14    17    36    37    9          0.000     0.650844    3 ; C5*-C4*-C3*-H30
329 13    14    17    18    9          0.000     1.046000    1 ; O5*-C5*-C4*-H40
330 13    14    17    18    9          0.000     0.000000    3 ; O5*-C5*-C4*-H40
331 10    13    14    15    9          0.000     0.146440    3 ; PA-O5*-C5*-H50
332 10    13    14    16    9          0.000     0.146440    3 ; PA-O5*-C5*-H51
333 31    34    32    33    4        180.000     4.602400    2 ; N1-N3-C2-H2
334 27    29    28    30    4        180.000     4.184000    2 ; C6-H60-N6-H61
335 22    25    23    24    4        180.000     4.602400    2 ; N9-N7-C8-H80
336 38    36    40    42    9          0.000     4.916200    2 ; O3*-C3*-C2*-O2*
337 38    36    40    42    9          0.000     0.602496    3 ; O3*-C3*-C2*-O2*
338 35    22    20    40    9          0.000     0.000000    2 ; C4-N9-C1*-C2*
339 31    27    26    35    9        180.000    14.644000    2 ; N1-C6-C5-C4
340 31    32    34    35    9        180.000    28.451200    2 ; N1-C2-N3-C4
341 28    27    26    35    9        180.000    14.644000    2 ; N6-C6-C5-C4
342 28    27    31    32    9        180.000    20.083200    2 ; N6-C6-N1-C2
343 27    26    35    34    9        180.000    22.802800    2 ; C6-C5-C4-N3
344 27    31    32    34    9        180.000    28.451200    2 ; C6-N1-C2-N3
345 26    27    31    32    9        180.000    20.083200    2 ; C5-C6-N1-C2
346 26    35    34    32    9        180.000    17.363600    2 ; C5-C4-N3-C2
347 25    23    22    35    9        180.000     7.112800    2 ; N7-C8-N9-C4
348 25    26    27    28    9        180.000    14.644000    2 ; N7-C5-C6-N6
349 25    26    27    31    9        180.000    14.644000    2 ; N7-C5-C6-N1
350 25    26    35    34    9        180.000    22.802800    2 ; N7-C5-C4-N3
351 23    22    20    40    9          0.000     0.000000    2 ; C8-N9-C1*-C2*
352 23    22    35    34    9        180.000     6.903600    2 ; C8-N9-C4-N3
```

```
23     22     35     26     9          180.000      6.903600     2 ; C8-N9-C4-C5
23     25     26     27     9          180.000     10.669200     2 ; C8-N7-C5-C6
23     25     26     35     9          180.000     10.669200     2 ; C8-N7-C5-C4
22     20     40     36     9            0.000      0.650844     3 ; N9-C1*-C2*-C3*
22     20     40     42     9            0.000      0.650844     3 ; N9-C1*-C2*-O2*
22     23     25     26     9          180.000     41.840000     2 ; N9-C8-N7-C5
22     35     34     32     9          180.000     17.363600     2 ; N9-C4-N3-C2
22     35     26     25     9          180.000     22.802800     2 ; N9-C4-C5-N7
22     35     26     27     9          180.000     22.802800     2 ; N9-C4-C5-C6
20     19     17     36     9          180.000      0.418400     2 ; C1*-O4*-C4*-C3*
20     19     17     36     9            0.000      1.602472     3 ; C1*-O4*-C4*-C3*
20     22     23     25     9          180.000      7.112800     2 ; C1*-N9-C8-N7
20     22     35     34     9          180.000      6.903600     2 ; C1*-N9-C4-N3
20     22     35     26     9          180.000      6.903600     2 ; C1*-N9-C4-C5
20     40     36     38     9            0.000      0.650844     3 ; C1*-C2*-C3*-O3*
19     17     36     38     9            0.000      4.916200     2 ; O4*-C4*-C3*-O3*
19     17     36     38     9            0.000      0.602496     3 ; O4*-C4*-C3*-O3*
19     17     36     40     9            0.000      0.650844     3 ; O4*-C4*-C3*-C2*
19     20     22     23     9            0.000     10.460000     1 ; O4*-C1*-N9-C8
19     20     22     23     9            0.000      0.000000     2 ; O4*-C1*-N9-C8
19     20     22     35     9            0.000      0.000000     2 ; O4*-C1*-N9-C4
19     20     40     36     9            0.000      0.650844     3 ; O4*-C1*-C2*-C3*
19     20     40     42     9            0.000      4.916200     2 ; O4*-C1*-C2*-O2*
19     20     40     42     9            0.000      0.602496     3 ; O4*-C1*-C2*-O2*
17     19     20     22     9            0.000      2.719600     2 ; C4*-O4*-C1*-N9
17     19     20     22     9            0.000      1.602472     3 ; C4*-O4*-C1*-N9
17     19     20     40     9          180.000      0.418400     2 ; C4*-O4*-C1*-C2*
17     19     20     40     9            0.000      1.602472     3 ; C4*-O4*-C1*-C2*
17     36     40     42     9            0.000      0.650844     3 ; C4*-C3*-C2*-O2*
17     36     40     20     9          180.000      0.836800     1 ; C4*-C3*-C2*-C1*
17     36     40     20     9          180.000      1.046000     2 ; C4*-C3*-C2*-C1*
17     36     40     20     9            0.000      0.753120     3 ; C4*-C3*-C2*-C1*
14     17     19     20     9          180.000      0.418400     2 ; C5*-C4*-O4*-C1*
14     17     19     20     9            0.000      1.602472     3 ; C5*-C4*-O4*-C1*
14     17     36     38     9            0.000      0.650844     3 ; C5*-C4*-C3*-O3*
14     17     36     40     9          180.000      0.836800     1 ; C5*-C4*-C3*-C2*
14     17     36     40     9          180.000      1.046000     2 ; C5*-C4*-C3*-C2*
14     17     36     40     9            0.000      0.753120     3 ; C5*-C4*-C3*-C2*
13     14     17     19     9            0.000      4.916200     2 ; O5*-C5*-C4*-O4*
13     14     17     19     9            0.000      0.602496     3 ; O5*-C5*-C4*-O4*
13     14     17     36     9            0.000      0.650844     3 ; O5*-C5*-C4*-C3*
12     10     13     14     9            0.000     -1.698704     2 ; O2A-PA-O5*-C5*
12     10     13     14     9            0.000      2.466468     3 ; O2A-PA-O5*-C5*
11     10     13     14     9            0.000     -1.698704     2 ; O1A-PA-O5*-C5*
11     10     13     14     9            0.000      2.466468     3 ; O1A-PA-O5*-C5*
10     13     14     17     9            0.000      1.603867     3 ; PA-O5*-C5*-C4*
9      10     13     14     9            0.000     -6.527040     1 ; O3A-PA-O5*-C5*
8      6      9      10     9            0.000     -1.483228     2 ; O2B-PB-O3A-PA
7      6      9      10     9            0.000     -1.483228     2 ; O1B-PB-O3A-PA
6      9      10     11     9            0.000     -1.483228     2 ; PB-O3A-PA-O1A
6      9      10     12     9            0.000     -1.483228     2 ; PB-O3A-PA-O2A
6      9      10     13     9            0.000      3.753048     1 ; PB-O3A-PA-O5*
5      6      9      10     9            0.000      3.753048     1 ; O3B-PB-O3A-PA
4      2      5      6      9            0.000     -0.355640     3 ; O3G-PG-O3B-PB
3      2      5      6      9            0.000     -0.355640     3 ; O2G-PG-O3B-PB
2      5      6      7      9            0.000     -1.483228     2 ; PG-O3B-PB-O1B
2      5      6      8      9            0.000     -1.483228     2 ; PG-O3B-PB-O2B
2      5      6      9      9            0.000      3.753048     1 ; PG-O3B-PB-O3A
1      2      5      6      9            0.000     -0.355640     3 ; O1G-PG-O3B-PB
```

```
412 26     28     27     31     4          180.000      4.602400      2 ; C5-N6-C6-N1
413 35     23     22     20     4          180.000      4.184000      2 ; C4-C8-N9-C1*
```

Ions topology file `ions.itp`

```
[ moleculetype ]
; molname       nrexcl
CL              1

[ atoms ]
; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CL              CL       1      -0.70000


[ moleculetype ]
; molname       nrexcl
NA              1

[ atoms ]
; id    at type         res nr  residu name     at name  cg nr  charge
1       Na              1       NA              NA       1      0.70000
```

Water topology file tip4p2005.itp

```
;
; Note the strange order of atoms to make it faster in gromacs.
;
[ moleculetype ]
; molname nrexcl
SOL   2

[ atoms ]
; id  at type res nr  residu name at name cg nr charge
1       OWAT            1       SOL      OW     1       0.0
2       HWAT            1       SOL     HW1     1       0.5564
3       HWAT            1       SOL     HW2     1       0.5564
4       MWAT            1       SOL      MW     1      -1.1128

#ifndef FLEXIBLE
[ settles ]
; OW    funct   doh         dhh
1       1       0.09572     0.15139
#else
[ bonds ]
; i     j       funct   length  force.c.
1       2       1       0.09572 502416.0
1       3       1       0.09572 502416.0

[ angles ]
; i     j       k       funct   angle   force.c.
2       1       3       1       104.52  628.02  104.52  628.02
#endif

[ exclusions ]
1 2 3 4
2 1 3 4
3 1 2 4
4 1 2 3

; The position of the virtual site is computed as follows:
;
;   O
;
;       D
;
; H   H
;
; const = distance (OD) / [ cos (angle(DOH))  * distance (OH) ]
;   0.01546 nm  / [ cos (52.26 deg) * 0.09572 nm  ]

; Vsite pos x4 = x1 + a*(x2-x1) + b*(x3-x1)

[ virtual_sites3 ]
; Vsite from      funct a   b
4 1 2 3 1 0.13193828  0.13193828
```