Conformational dynamics of superoxide dismutase (SOD1) in osmolytes: a molecular dynamics simulation study

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The force field parameter files i.e. urea.itp file generated using ATB server is given below.

[ moleculetype ]

; Name nrexcl
UREA 3

[ atoms ]
; nr type resnr resid atom cgnr charge mass
1  H  1  UREA   H22U  1   0.426  1.0080
2  NT  1  UREA   N2U  2  -0.978  14.0067
3  H  1  UREA   H21U  3   0.426  1.0080
4  C  1  UREA    CU   4   0.908  12.0110
5  O  1  UREA    OU   5  -0.656  15.9994
6  NT  1  UREA    N1U   6  -0.978  14.0067
7  H  1  UREA   H11U  7   0.426  1.0080
8  H  1  UREA   H12U  8   0.426  1.0080
; total charge of the molecule:  0.000
The Lennard-Jones parameters i.e. C6 and C12 for the GROMOS force field for urea are given below.

| Atom type | C6 (kJ mol\(^{-1}\) nm\(^6\)) | C12 (kJ mol\(^{-1}\) nm\(^{12}\)) |
|-----------|-------------------------------|----------------------------------|
| H         | 0                             | 0                                |
| NT        | 0.0024364096                  | 5.0625e-06                       |
| C         | 0.0023406244                  | 4.937284e-06                     |
| O         | 0.0022619536                  | 1e-06                            |
The force field parameter files i.e. tmao.itp file generated using ATB server is provided below.

```
[ moleculetype ]
; Name   nrexcl
TMAO  3
[ atoms ]
; nr  type  resnr  resid  atom  cgnr  charge    mass
1    HC    1    TMAO     H9    1    0.167   1.0080
2     C    1    TMAO     C3    2   -0.411  12.0110
3    HC    1    TMAO     H7    3    0.167   1.0080
4    HC    1    TMAO     H8    4    0.167   1.0080
5    NL    1    TMAO     N1    5    0.435  14.0067
6    OE    1    TMAO     O1    6   -0.705  15.9994
7     C    1    TMAO     C1    7   -0.411  12.0110
8    HC    1    TMAO     H1    8    0.167   1.0080
9    HC    1    TMAO     H2    9    0.167   1.0080
10   HC    1    TMAO     H3   10    0.167   1.0080
11   C    1    TMAO     C2   11   -0.411  12.0110
12   HC    1    TMAO     H4   12    0.167   1.0080
13   HC    1    TMAO     H5   13    0.167   1.0080
14   HC    1    TMAO     H6   14    0.167   1.0080
; total charge of the molecule:  -0.000
[ bonds ]
; ai   aj   funct   c0         c1
1    6    1   0.1090   1.2300e+07
2    3    2   0.1090   1.2300e+07
2    4    2   0.1090   1.2300e+07
2    5    2   0.1500   3.5556e+06
5    6    2   0.1380   4.4633e+06
5    7    2   0.1500   3.5556e+06
5    11   2   0.1500   3.5556e+06
7    8    2   0.1090   1.2300e+07
7    9    2   0.1090   1.2300e+07
7    10   2   0.1090   1.2300e+07
11   12   2   0.1090   1.2300e+07
11   13   2   0.1090   1.2300e+07
11   14   2   0.1090   1.2300e+07
[ pairs ]
; ai   aj   funct ; all 1-4 pairs but the ones excluded in GROMOS itp
1    6    1
1    7    1
1    11   1
2    8    1
2    9    1
2    10   1
2    12   1
2    13   1
2    14   1
```
3 6 1
3 7 1
3 11 1
4 6 1
4 7 1
4 11 1
6 8 1
6 9 1
6 10 1
6 12 1
6 13 1
6 14 1
7 12 1
7 13 1
7 14 1
8 11 1
9 11 1
10 11 1

[ angles ]
; ai aj ak funct angle fc
1 2 3 2 111.00 530.00
1 2 4 2 111.00 530.00
1 2 5 2 111.40 532.00
3 2 4 2 111.00 530.00
3 2 5 2 111.40 532.00
4 2 5 2 111.40 532.00
2 5 6 2 109.00 1680.51
2 5 7 2 109.00 1680.51
2 5 11 2 109.00 1680.51
6 5 7 2 109.00 1680.51
6 5 11 2 109.00 1680.51
7 5 11 2 109.00 1680.51
5 7 8 2 111.40 532.00
5 7 9 2 111.40 532.00
5 7 10 2 111.40 532.00
8 7 9 2 111.00 530.00
8 7 10 2 111.00 530.00
9 7 10 2 111.00 530.00
5 11 12 2 111.40 532.00
5 11 13 2 111.40 532.00
5 11 14 2 111.40 532.00
12 11 13 2 111.00 530.00
12 11 14 2 111.00 530.00
13 11 14 2 111.00 530.00

[ dihedrals ]
; GROMOS improper dihedrals
; ai aj ak al funct angle fc
[ dihedrals ]
; ai aj ak al funct ph0 cp mult
3 2 5 7 1 0.00 1.05 3
The Lennard-Jones parameters i.e. C6 and C12 for the GROMOS force field for TMAO are given below.

| Atom type | C6 (kJ mol\(^{-1}\) nm\(^6\)) | C12 (kJ mol\(^{-1}\) nm\(^{12}\)) |
|-----------|---------------------------------|-----------------------------------|
| HC        | 8.464e-05                       | 1.5129e-08                        |
| C         | 0.0023406244                    | 4.937284e-06                      |
| NL        | 0.0024364096                    | 2.319529e-06                      |
| OE        | 0.0022619536                    | 1.21e-06                          |

Table S1: Number of osmolytes and water molecules used during the simulations.

| System     | N\(_{\text{water}}\) | N\(_{\text{urea}}\) | N\(_{\text{TMAO}}\) |
|------------|-----------------------|---------------------|---------------------|
| Apo_water  | 6418                  | -                   | -                   |
| Apo_U 4 M  | 5459                  | 500                 | -                   |
| Apo_U 4 M_T 2 M | 4756              | 500                 | 250                 |
| Holo_water | 6422                  | -                   | -                   |
| Holo_U 4 M | 4760                  | 500                 | -                   |
| Holo_U 4 M_T 2 M | 4709              | 500                 | 250                 |

Validation of force field generated from ATB

In order to validate the force field generated from ATB, we have calculated the thermodynamic property (i.e. Density), transport property (self-diffusion coefficient) and structural property (RDF and # of hydrogen bonds) of 1.94 m TMAO box and compared our result with the Shea force field\(^1\) and United atom (UA) ff (generated from ATB) of TMAO\(^2\). We found that the density of TMAO solution in our case at 1.94 m TMAO is 978 kg/m\(^3\), for UA model it is 980 kg/m\(^3\) for solution of concentration 1.94 m and in case of Shea model it is 990 kg/m\(^3\) for TMAO solution of molality 1.94 m\(^2\). The ratio of self-diffusion coefficient of water in TMAO water solution to the self-diffusion coefficient of pure water in our case is found to be 0.71, in UA model it is 0.7 and for Shea model it is 0.7 (1.94 m)\times10^{-9} m^2/s. The number of hydrogen bond per TMAO molecule is 2.8 (1.94 m TMAO), for UA model it is <
2.5 and for Shea model it is < 3 (~2.8) for 1.94 mol/kg solution. The thermodynamic property obtained in our case is found to be approx. similar to the reported values which validates the force field used in the simulations. RDF of NT-NT is also calculated and the plot obtained in our case shows similar pattern as shown by UA model\(^2\).

**The RDF plot of NT-NT and NT-OW**

The thermodynamic properties of UA model shows similarity with the Shea model. However the deviation from the experimental data could be explained in terms of weak solute-water interactions, a consequence of non-optimal assignment of interaction parameter\(^2\).

**Figure S1:** Potential energy of apo and holo SOD1 in (A & D) water, (B & E) urea and (C &F) urea-TMAO solution.
Figure S2: Density of apo and holo SOD1 in (A & D) water, (B & E) urea and (C & F) urea-TMAO solution.
**Figure S3:** (A) The root mean square fluctuation (RMSF), (B) hydrophobic SASA of residues interacted with metal ions in apo and holo SOD1 in water.

**Figure S4:** Configurational entropy of backbone (per atom) of loop IV & VII of apo and holo SOD1 in water, urea and urea-TMAO solution.
Figure S5: Configurational entropy of sidechain (per atom) of loop IV & VII of apo and holo SOD1 in water, urea and urea-TMAO solution.
**Figure S6:** Radial distribution of oxygen of water (OW) molecules around loop IV & VII of apo and holo SOD1 in all the three system.

**Figure S7:** Radial distribution of nitrogen of TMAO (NT) molecules around loop IV & VII of apo and holo SOD1 in ternary system.
**Figure S8:** Radial distribution of oxygen of water (OW), oxygen of urea (OU) and nitrogen of TMAO (NT) molecules around apo and holo SOD1 protein in water, urea and ternary solution.
**Figure S9**: The SDF of oxygen of water (OW) molecules around loop IV & VII of apo and holo SOD1 in all the three system (loops are shown in VDW method and C, N, O in khaki, blue and red colour respectively) (SDF’s are shown in iso-surface in green colour in the first solvation shell corresponding to rdf).
Figure S10: The SDF of nitrogen of TMAO (NT) molecules around loop IV & VII of apo and holo SOD1 in ternary system (loops are shown in VDW method and C, N, O in khaki, blue and red colour respectively) (SDF’s are shown in iso-surface in blue colour in the first solvation shell corresponding to rdf).

References:

1. Larini, L.; Shea, J. E., Double resolution model for studying TMAO/water effective interactions. *J Phys Chem B* 2013, 117, (42), 13268-77.

2. Markthaler, D.; Zeman, J.; Baz, J.; Smiatek, J.; Hansen, N., Validation of Trimethylamine-N-oxide (TMAO) Force Fields Based on Thermophysical Properties of Aqueous TMAO Solutions. *J Phys Chem B* 2017, 121, (47), 10674-10688.