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
```

```
[ bonds ]
;  ai   aj  funct   c0        c1
   1    2    2   0.1010   2.1076e+07
   2    3    2   0.1010   2.1076e+07
   2    4    2   0.1380   1.1000e+07
   4    5    2   0.1230   1.6600e+07
   4    6    2   0.1380   1.1000e+07
   6    7    2   0.1010   2.1076e+07
   6    8    2   0.1010   2.1076e+07
[ pairs ]
;  ai   aj  funct  ;  all 1-4 pairs but the ones excluded in GROMOS itp
   1    5    1
   1    6    1
   2    7    1
   2    8    1
   3    5    1
   3    6    1
   5    7    1
   5    8    1
[ angles ]
;  ai   aj   ak  funct   angle     fc
   1    2    3    2    113.00   545.00
   1    2    4    2    115.00   460.00
   3    2    4    2    115.00   460.00
   2    4    5    2    124.00   730.00
   2    4    6    2    120.00   560.00
   5    4    6    2    124.00   730.00
   4    6    7    2    115.00   460.00
   4    6    8    2    115.00   460.00
   7    6    8    2    113.00   545.00
[ dihedrals ]
; GROMOS improper dihedrals
;  ai   aj   ak   al  funct   angle     fc
   4    2    5    6    2      0.00   167.36
[ dihedrals ]
;  ai   aj   ak   al  funct    ph0      cp     mult
   2    4    6    7    1    180.00    33.50    2
   3    2    4    6    1    180.00    33.50    2
[ exclusions ]
;  ai   aj  funct  ;  GROMOS 1-4 exclusions
```

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    2    2   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
```

```
   7    5   11   12    1      0.00     1.05    3
  11    5    7    8    1      0.00     1.05    3
[ exclusions ]
;  ai   aj  funct  ;  GROMOS 1-4 exclusions
```

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.

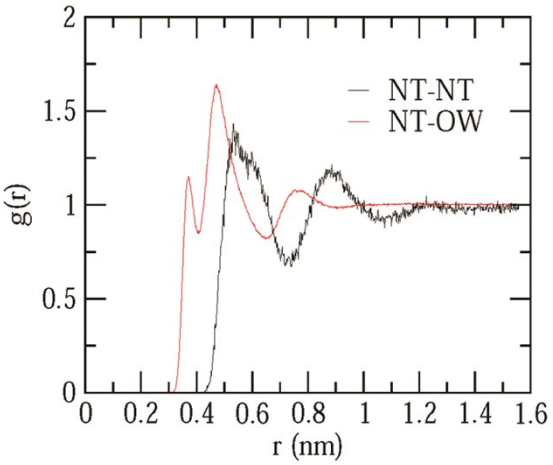
System	N_{water}	N_{urea}	N_{TMAO}
Apo_water	6418	-	-
Apo_U 4 M	5459	500	-
Apo_U 4 M_T 2 M	4756	500	250
Holo_water	6422	-	-
Holo_U 4 M	4760	500	-
Holo_U 4 M_T 2 M	4709	500	250

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)×10^{-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].

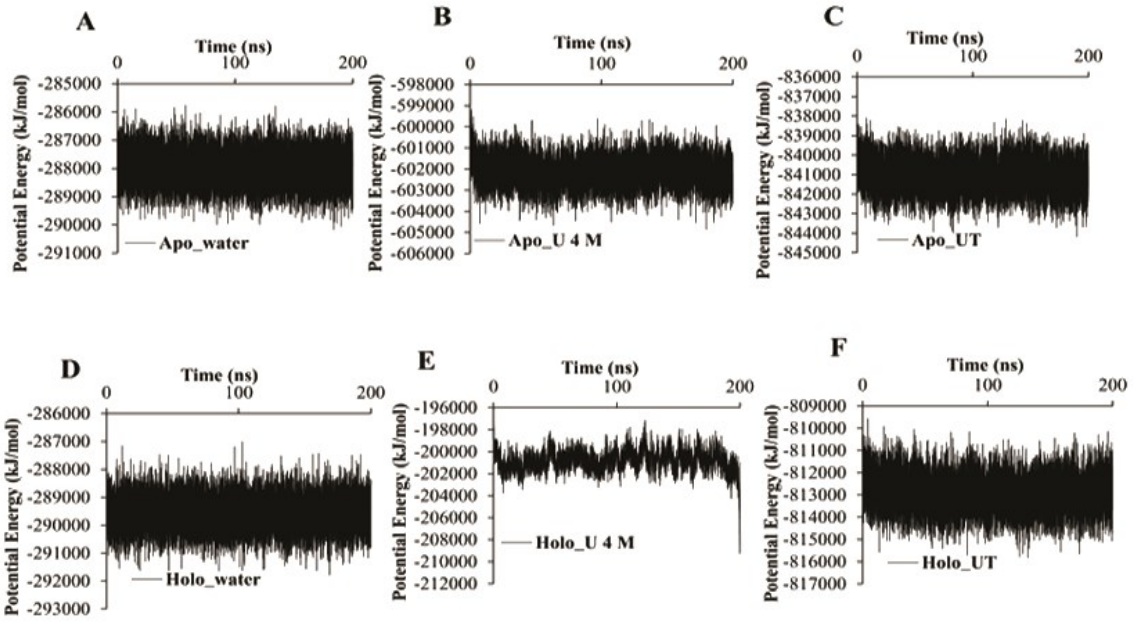


Figure S1: Potential energy of apo and holo SOD1 in (A & D) water, (B & E) urea and (C &F) urea-TMAO solution.

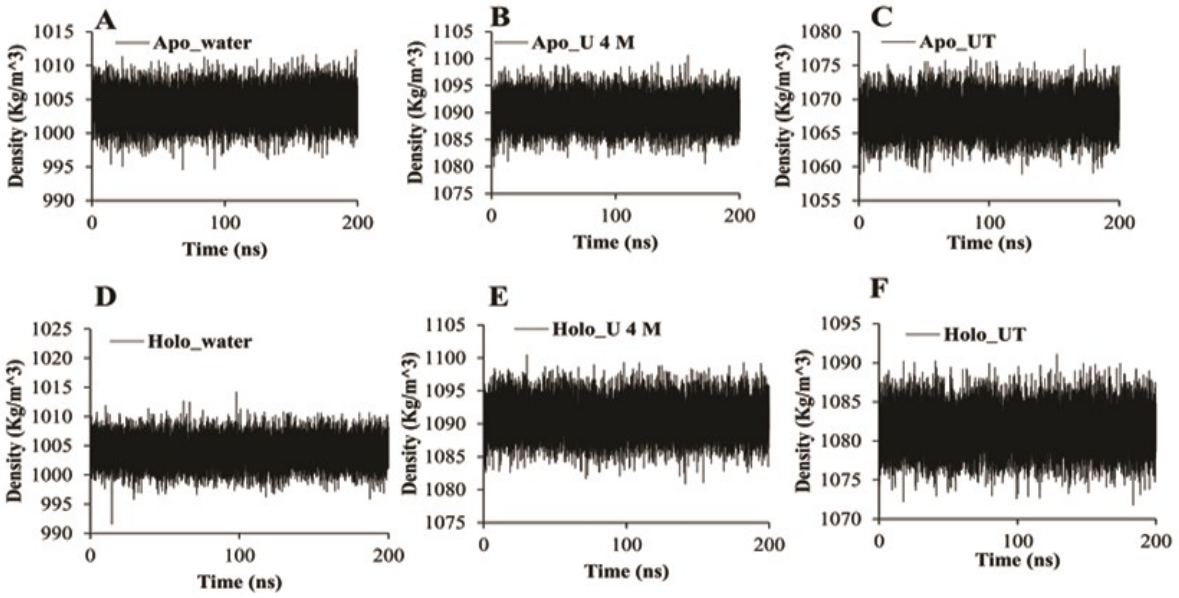


Figure S2: Density of apo and holo SOD1 in (A & D) water, (B & E) urea and (C & F) urea-TMAO solution.

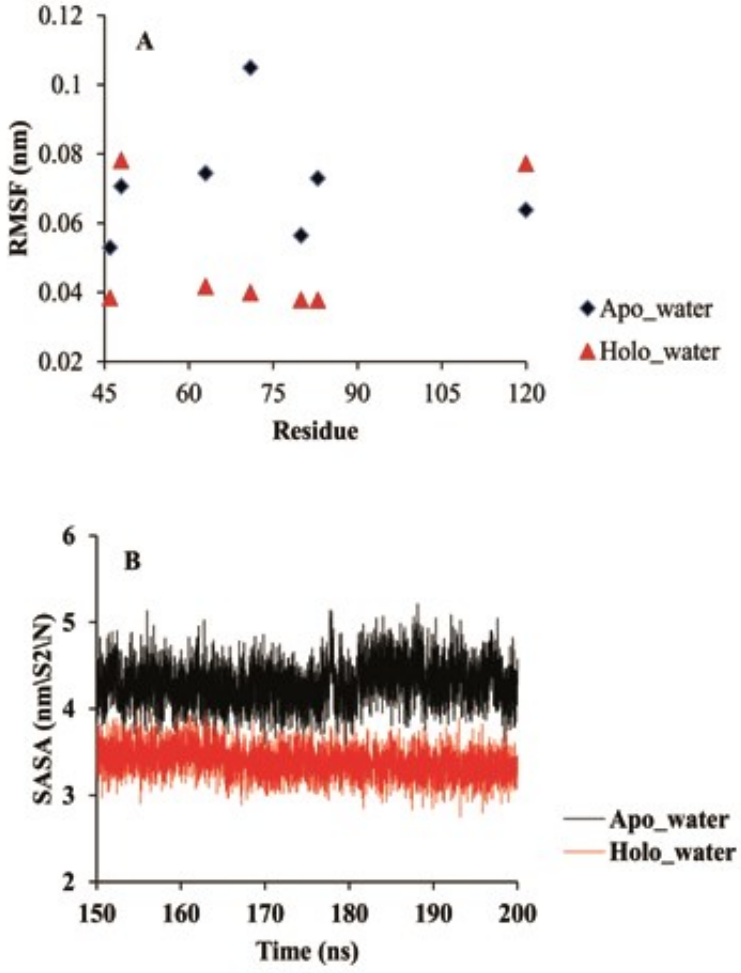


Figure S3: (A) The root mean square fluctuation (RMSF), (B) hydrophobic SASA of residues interacted with metal ions in apo and holo SOD1 in water.

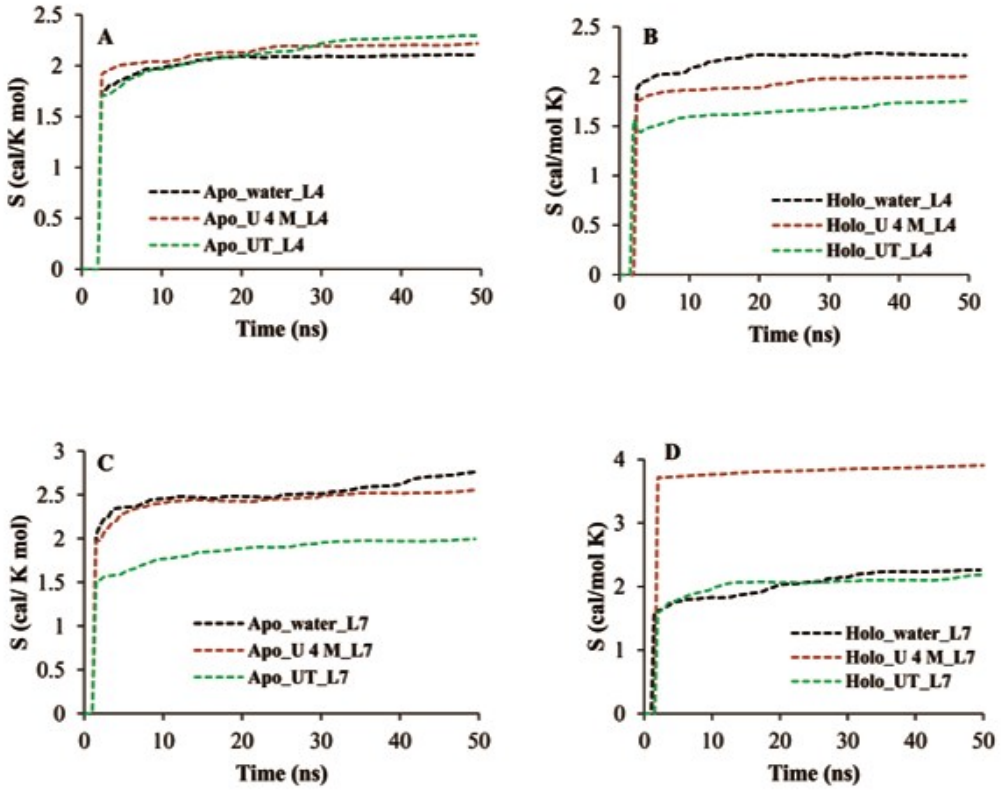


Figure S4: Configurational entropy of backbone (per atom) of loop Ⅳ & Ⅶ of apo and holo SOD1 in water, urea and urea-TMAO solution.

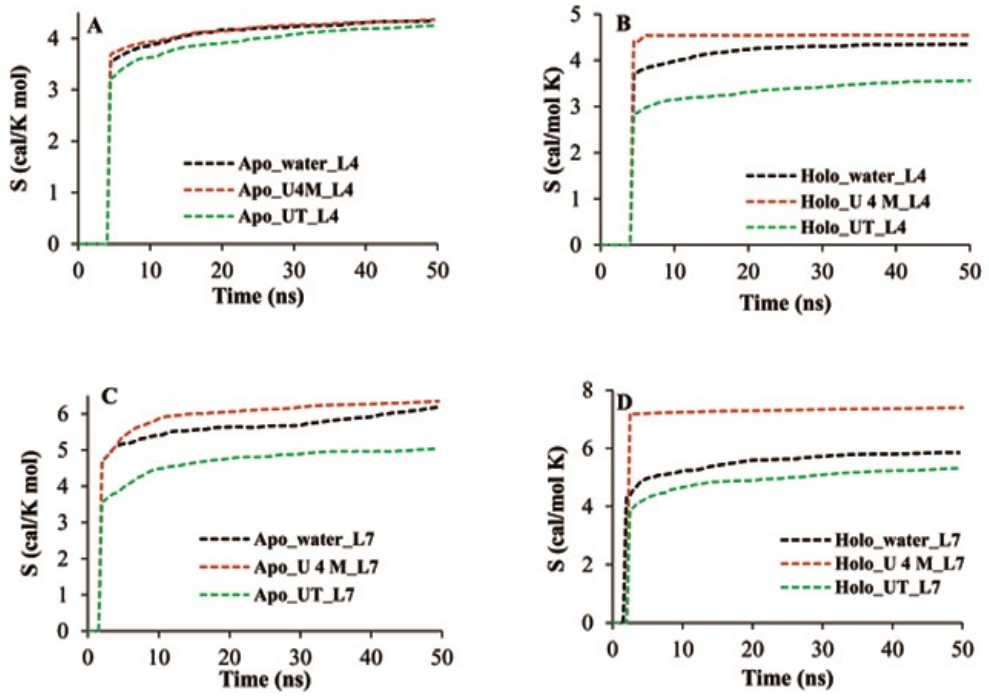


Figure S5: Configurational entropy of sidechain (per atom) of loop Ⅳ & Ⅶ of apo and holo SOD1 in water, urea and urea-TMAO solution.

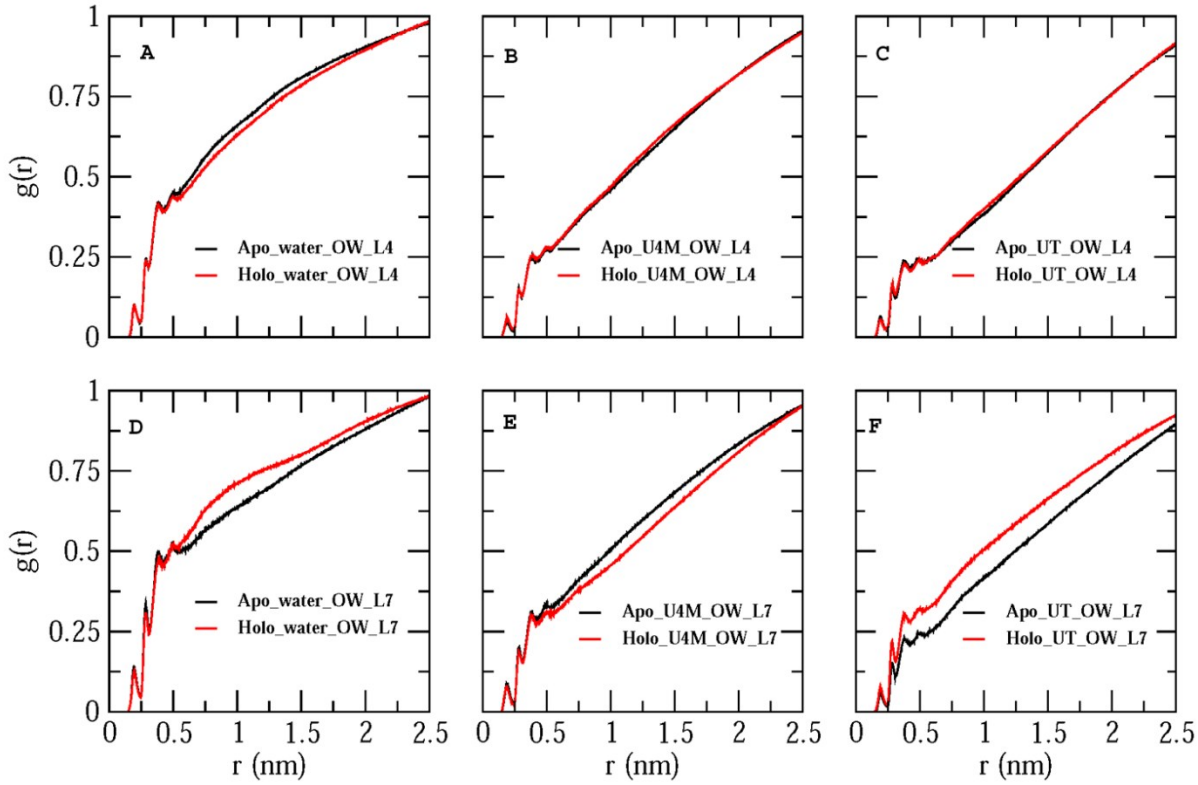


Figure S6: Radial distribution of oxygen of water (OW) molecules around loop Ⅳ & Ⅶ of apo and holo SOD1 in all the three system.

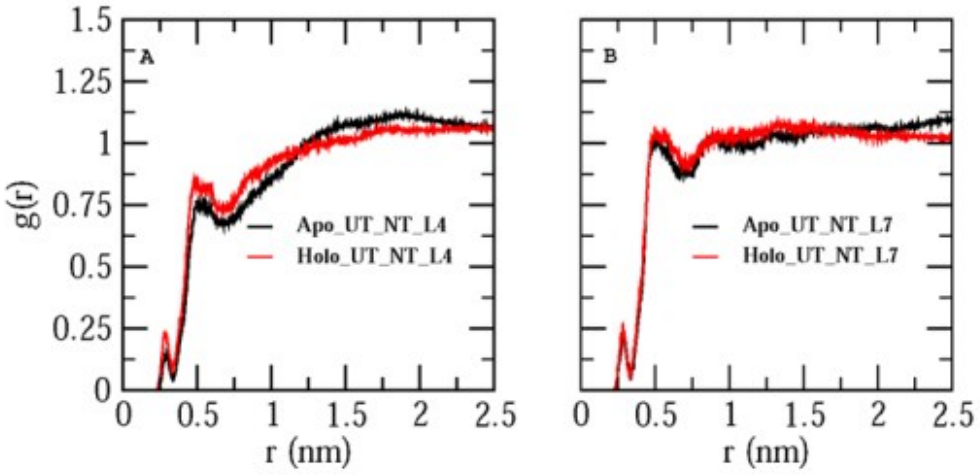


Figure S7: Radial distribution of nitrogen of TMAO (NT) molecules around loop Ⅳ & Ⅶ of apo and holo SOD1 in ternary system.

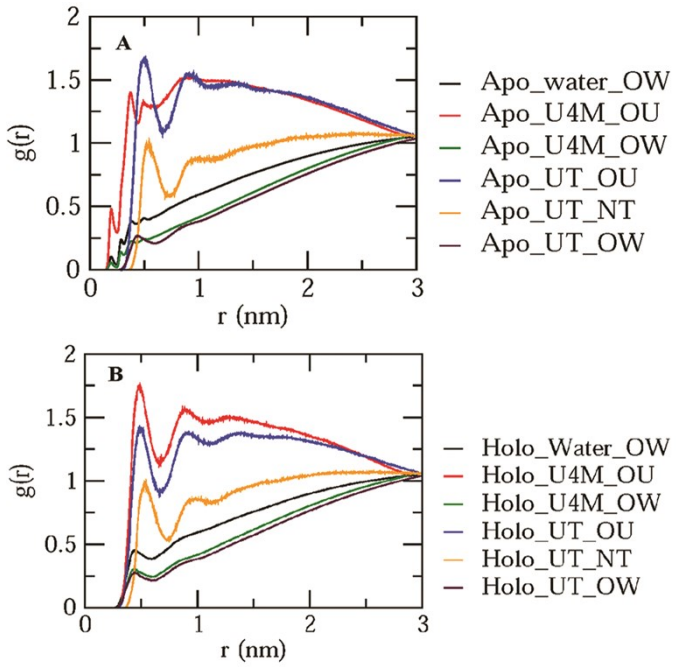


Figure S8: Radial distribution of oxygen of water (OW), oxygen of urea (OU) and nitrogen of TMAO (NT) molecules around apo and holo SOD1 protein in water, urea and ternary solution.

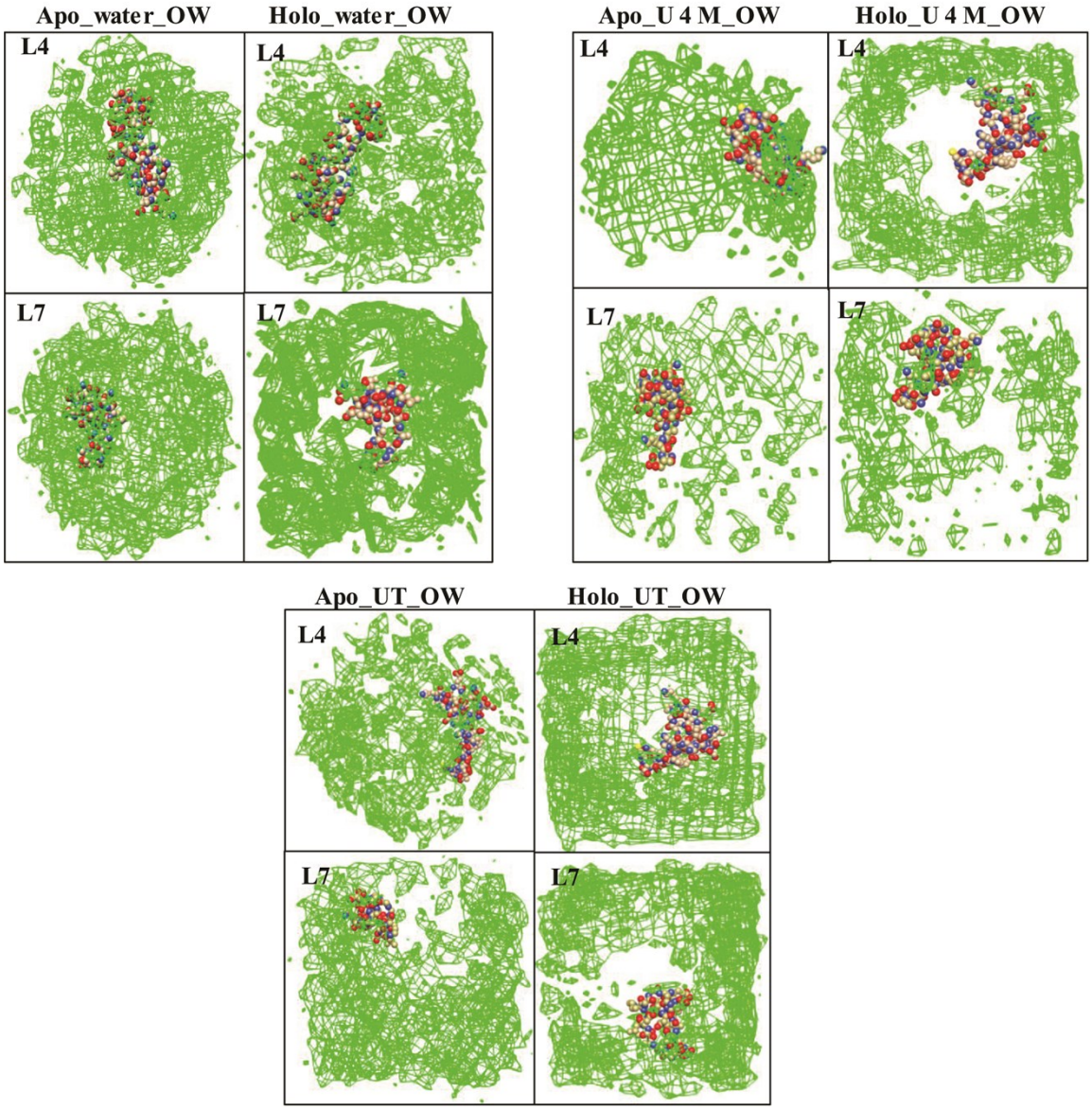


Figure S9: The SDF of oxygen of water (OW) molecules around loop Ⅳ & Ⅶ of apo and holo SOD1 in all the three system (loops are shown in VDW method and C, N, O in khaki, blue and red colour respectively) (SDF's are shown in iso-surface in green colour in the first solvation shell corresponding to rdf).

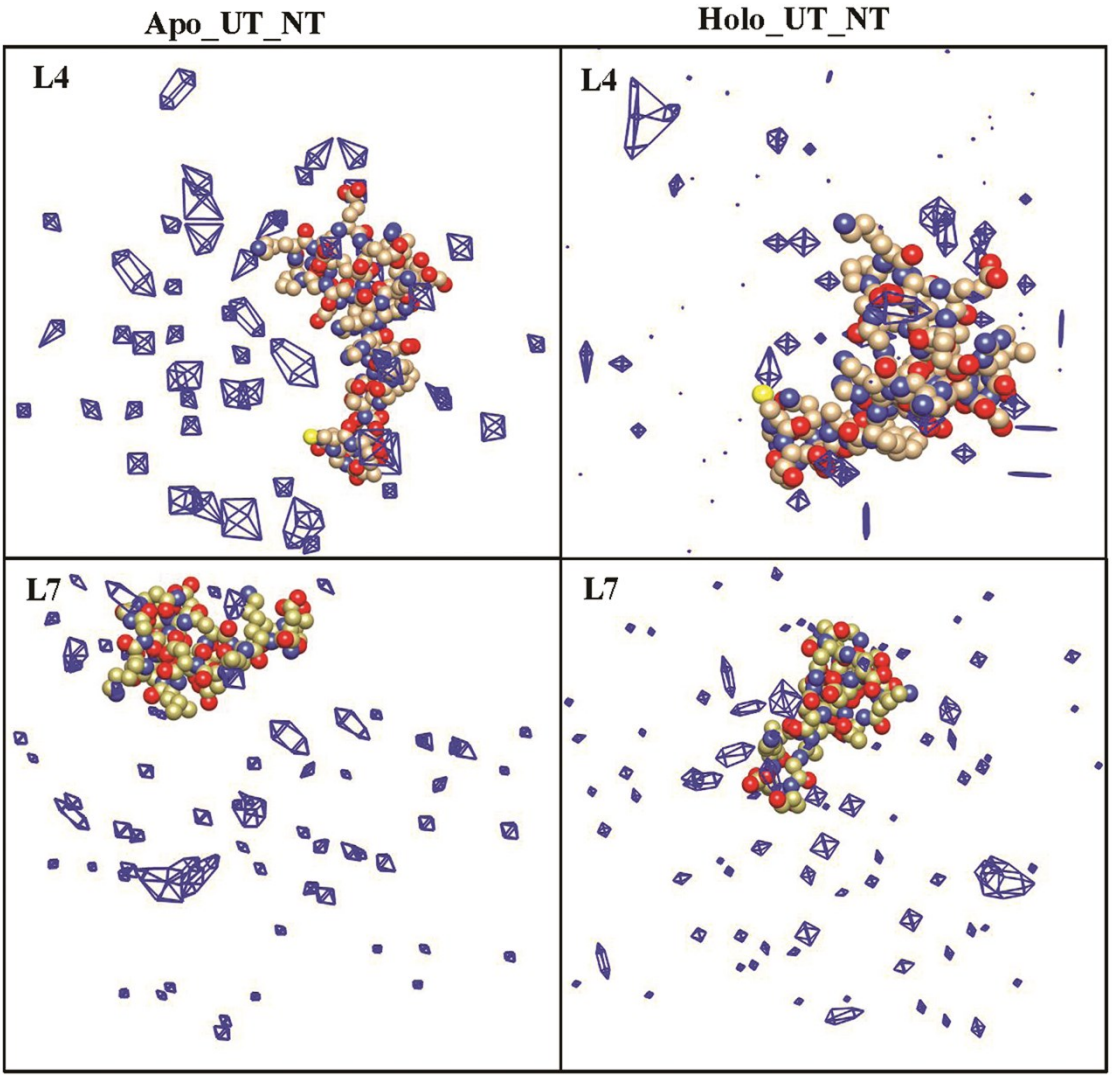


Figure S10: The SDF of nitrogen of TMAO (NT) molecules around loop Ⅳ & Ⅶ of apo and holo SOD1 in ternary system (loops are shown in VDW method and C, N, O in khaki, blue and red colour respectively) (SDF's are shown in iso-surface in blue colour in the first solvation shell corresponding to rdf).

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.