Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

For

The protein-stabilizing effects of TMAO in aqueous and non-aqueous conditions

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Experimental section

Materials and Equipment

Lipase (Novozym 435) was purchased from MIK Pharm Co., Ltd. (Japan). *rac*-1-Phenylethanol, *rac*-1-phenylethyl acetate and vinyl acetate were purchased from Nacalai Tesque, Inc. Vinyl acetate was freshly distillated and dried over molsieve 4Å before used for reactions. Anhydrous TMAO were purchased from the Sigma-Aldrich Corp. and stored under vacuum desiccator.

GC analysis were performed on a Shimadzu GC-2010 Plus equipped with FID detector and a CP-Chirasil DEX CB column (Varian, 25 m × 0.32 mm, 0.25 μm film thickness) using He carrier gas (5 ml/min, head pressure: 274 kPa, injector: 180 °C, detector: 180 °C,). GC profile for *rac*-1-Phenylethanol and *rac*-1-phenylethyl acetate: t_r (40 °C (2min), 10 °C/min, 105 °C (10 min)), min: 13.5, (*R*)- 1-phenylethanol; 14.4, (*S*)- 1-phenylethanol; 11.3, (*R*)- 1-phenylethyl acetate; 10.3, (*S*)- 1-phenylethyl acetate; GC molar response factor of acetate/alcohol: 0.801.

Transesterification Reaction in Hexane

Lipase (10 mg), vinyl acetate (0.83 mmol), *rac*-1-phenylethanol (0.4 mmol) and hexane (10 mL) were added to a 20 mL round-bottom flask. The reaction mixture was vigorously stirred with a magnetic bar at 20 °C. Samples (200 μL) were withdrawn, filtered through EXtrelut® with 1 mL diethyl ether before being analyzed by GC. Conversion (c) was calculated as $c = ee_{substrate}/(ee_{product} + ee_{substrate})$ and confirmed by GC molar response factor of acetate/alcohol.

Hydrolysis Reaction in Water

Lipase (10 mg), *rac*-1-phenylethyl acetate (0.4 mmol), water (10 mL), and a magnetic bar were added to a 20 mL round-bottom flask. The reaction mixture was magnetically stirred at 20 °C. An aliquot (200 μL) was filtered through EXtrelut® with 1 mL diethyl ether before being analyzed by GC. Conversion (c)

was calculated as $c = ee_{substrate}/(ee_{product} + ee_{substrate})$ and confirmed by GC molar response factor of acetate/alcohol.

Pretreatment Conditions of Enzyme in Hexane

To a 5 mL capped vial, lipase (10 mg) was shaken with TMAO (0.1 mmol) or without TMAO (as the control) in hexane (2 mL) for 4h at 20 ^{o}C. Hexane was then evaporated under vacuum before water (4 mL) was added to the vial. The vial was shaken for another 4h. Then the pretreated enzyme was filtered off and carefully washed with water. The washed enzyme was dried under reduced pressure overnight before used for the reaction.

Molecular Dynamics Simulation

MD simulations were carried out using the GROMACS package (v. 2018) with a GROMOS 43a1 force field.[1] The crystal structure of Lipase B from *Candida Antarctica* (CALB), which is used in all of MD simulations, was taken from the Protein Data Bank (PDB) with PDB identifier 1TCA.[2] The SPC/E[3] model was used for water molecules. A united atom model has chosen for hexane molecules based on GROMOS54a7[4] force filed using automated topology builder (ATB).[5] TMAO molecules were parameterized based on the popular[6] Kast[7] model, which has achieved a good balance between solute-solute and solute-solvent interactions among different models.[8] It also reproduces key experimental quantities such as hydration properties, surface tensions, and transfer free energy from water to 1 M TMAO aqueous solutions.[9] As it is a more conventional concentration in MD simulations and to obtain more obvious results, 1 M TMAO was used in all simulations. In the first stage of simulation, we constructed periodic simulation boxes and added TMAO and solvent molecules to reach the desired concentration, both in water and in hexane. After energy minimization of the boxes, each system was equilibrated for 100 ps in an NVT ensemble at 300 K. A 100 ps MD simulation was carried out in the NPT ensemble at the same temperature and at constant (1bar) pressure. Next, each box was simulated for 10 ns to obtain pre-equilibrated solutions. Afterward, CALB was solvated by different pre-

equilibrated solutions or pure solvents in a cubic box in which the box size was chosen so that the minimal distance of protein atoms from the wall was greater than 1 nm. The dimensions and number of molecules in each system are presented in Table S1. One microsecond long equilibration MD simulation was performed at our experimental temperatures and pressures for each system. The Particle Mesh Ewald (PME) summation method was used for calculating the total electrostatic energy in a periodic box.[10] The other non-bonded interactions were calculated by L-J model with a cutoff distance of 10 Å. Steepest-descent energy minimization was used to relax the solvent molecules. The LINCS algorithm[11] was employed to fix chemical bonds between atoms in the protein and the SETTLE algorithm[12] was analogously used for solvent molecules. After minimization, system heating, equilibration and data sampling were carried out, respectively. To maintain a constant temperature and pressure for each system during simulations, the Berendsen coupling algorithm was used.[13] A weak-coupling algorithm was used for the temperature and pressure regulation with a coupling time of 1.0 ps. All simulations were repeated three times to test the convergence of the results.

Cluster analysis

The Daura algorithm[14] was used for cluster analyses of surfactant-like interactions (Fig. 8) and the active site residues (Fig. 9). Root mean square deviations (rmsd) were calculated using heavy atoms of each residue and the interacting TMAO molecules. A rmsd cut-off of 0.2 nm was used to group similar structures. The number of clusters were 3 (Fig. 8 (a)), 11 (Fig. 8 (b)), 7 (Fig. 8(c)), 10 (Fig. 8 (d)), 1 (Fig. 9 (a)), and 4 (Fig. 9 (b)). Moreover, the population of the major cluster are above 50% in all cases.

Graphics

PyMOL[15] software was used to construct the graphics.

Table S1: Number of solvent and solute molecules and volume of the different systems including CALB enzyme

System	N_{water}	N_{Hexane}	N_{TMAO}	Volume(nm^3)
Water	11435	0	0	348.30
Hexane	0	1749	0	339.94
1 M TMAO in Water	10338	0	202	338.21
1 M TMAO in Hexane	0	1532	202	341.11
1 M TMAO + 1 M water in Hexane	202	1498	202	340.66

Simulation of Burkholderia Cepacia lipase (BCL)

The lipase from *Burkholderia cepacia (BCL)*, formerly known as *Pseudomonas cepacia* lipase, widely known for its thermal resistance and tolerance against organic solvents and short-chain alcohols.[16] Similar to CALB, BCL[17] enzyme is known as active and stable enzyme in the different anhydrous conditions such as hexane.[18] We have performed simulations of BCL similar to CALB in different systems including pure hexane, pure water, 1 M TMAO in hexane, and 1 M TMAO in water to have comparable results. We have also used Netz model[19] as another popular force field[8] for TMAO molecules to judge about the results of Kast2016[7b] model in CALB simulations. Crystal structure of BCL was taken from protein data bank (PDB code: 3LIP[17]).[17] Simulations were carried out by GROMACS package (v. 2018) and GROMOS 43a1 force field.[1] Water molecules were modeled based on SPC/E^3. Other simulation details for each system are similar to CALB simulations (see above section). The time of the equilibration MD simulation for BCL also one microsecond. Number of molecules in each system are presented in Table S2.

Table S2: Number of solvent and solute molecules and volume of the different systems including BCL enzyme

System	N_{water}	N_{Hexane}	N_{TMAO}	Volume (nm^3)
Water	11908	0	0	395.76
Hexane	0	1824	0	388.36
1 M TMAO in Water	10763	0	230	387.12
1 M TMAO in Hexane	0	1614	230	380.26
1 M TMAO + 1 M water in Hexane	230	1584	230	379.67

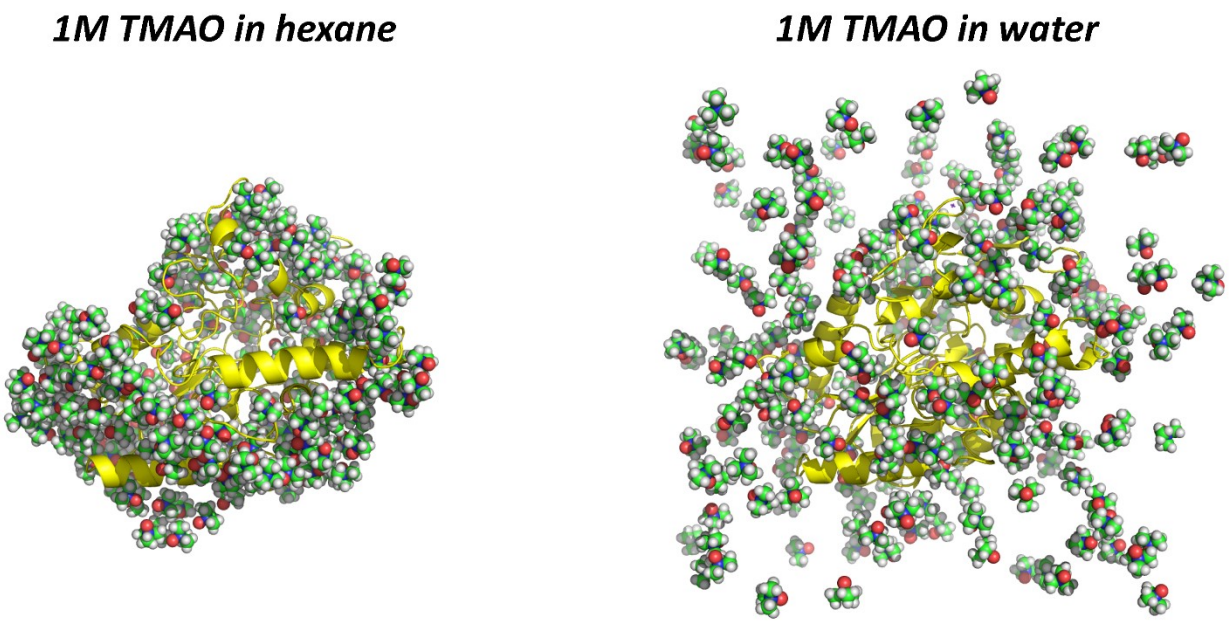

Figure S1. Distribution of TMAO about CALB enzyme (yellow carton) in hexane and in water (solvent molecules were removed for clarity)

Table S3: Average number of TMAO-BCL H bonds calculated from 50 ns final MD simulations

System	Hexane	Water
Total	203.22	73.53
TMAO-Side chains	115.58	66.35
TMAO-Main chains	87.23	6.91

Table S4: Average number of intra main chain H bonds of BCL enzyme calculated from 50 ns final MD simulations

System	H bond number
Water	153.90
Hexane	150.72
1 M TMAO in Water	151.03
1 M TMAO in Hexane	79.12
1 M TMAO + 1 M water in Hexane	119.78

Table S5: Average number of water-BCL H bonds calculated from 50 ns final MD simulations

System	H bond number
Water	537.67
1 M TMAO	443.35

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