Supporting Information

Structure-based screening and molecular dynamics simulation studies for the identification of potential Acetylcholinesterase inhibitors

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Figures

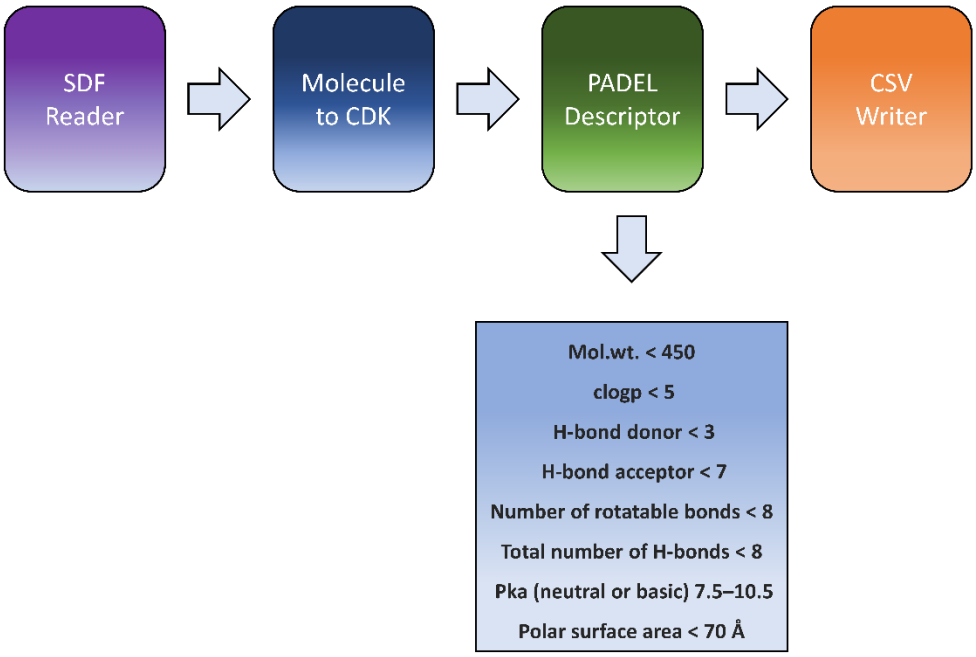


Figure S1. Schematic representation of Knime workflow utilized for the removal of compounds having undesirable properties for CNS activity.

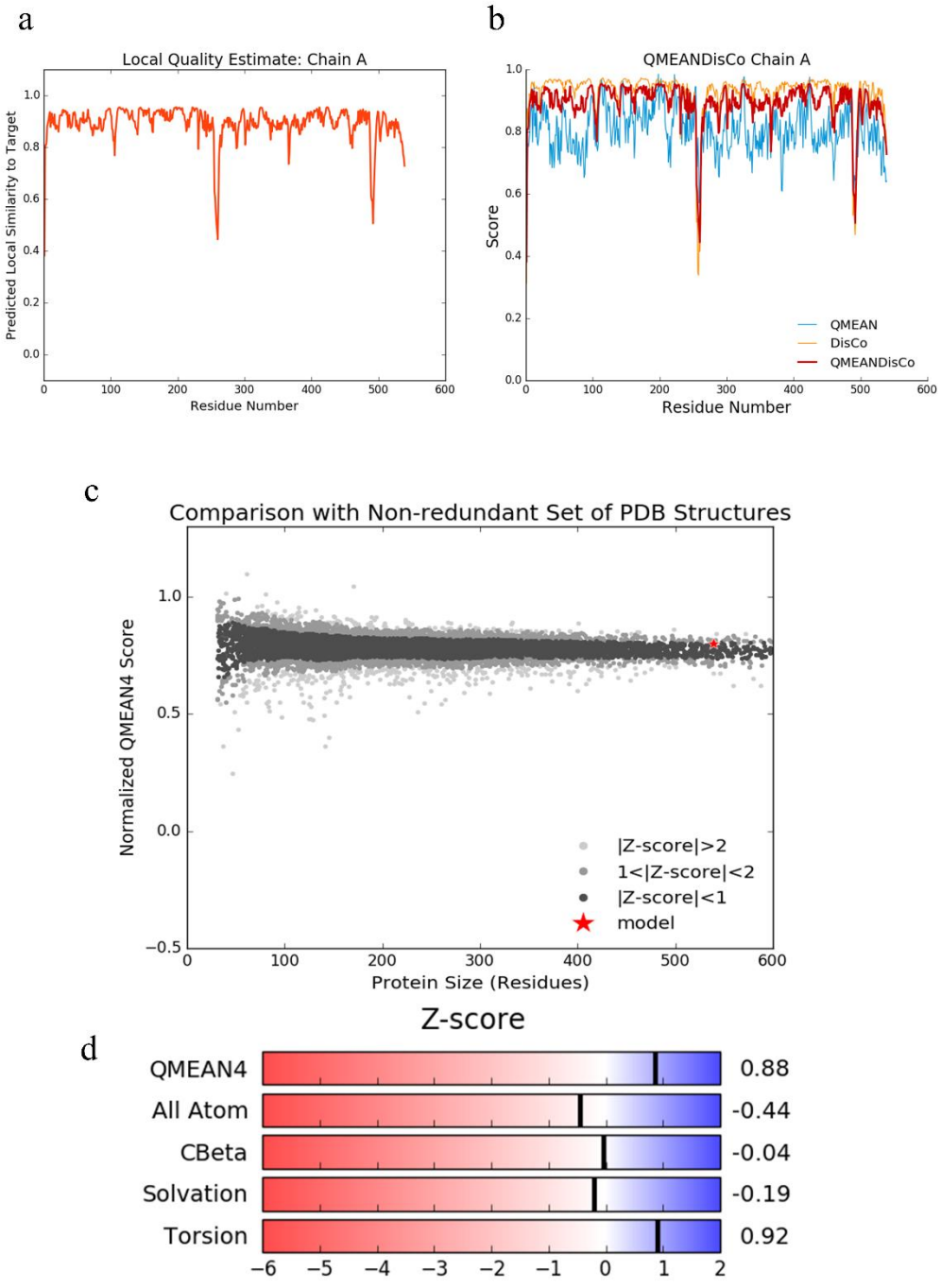


Figure S2. (**a)** Local quality estimation of the developed model, (**b)** Graphical representation of QmeanDISCO, (**c)** Comparison of the model with set of PDB structures, (**d)** Representation of QMWAN4 and its constitutive elements of the developed model.

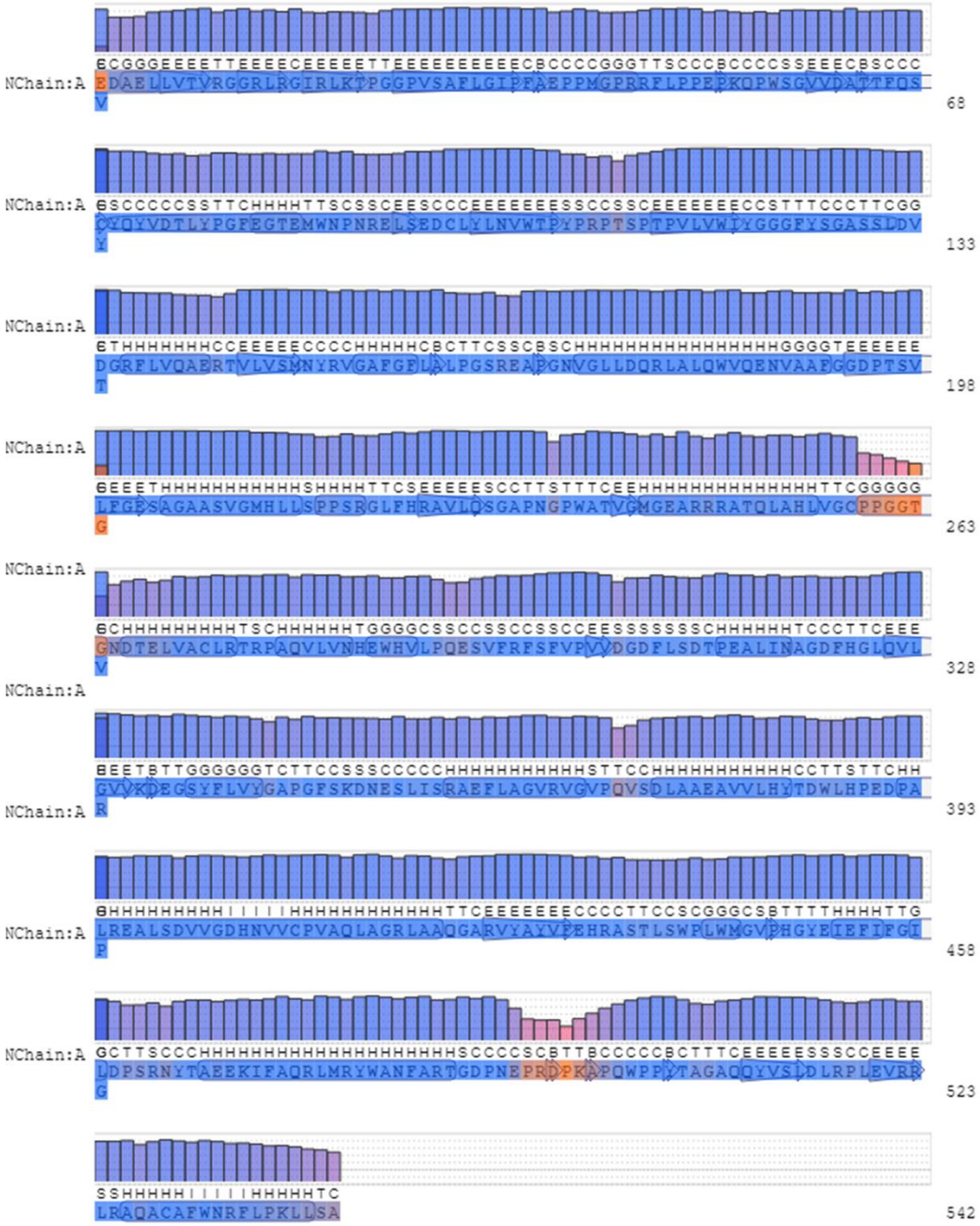


Figure S3. Residue-wise representation of QMEAN score for the complete protein.

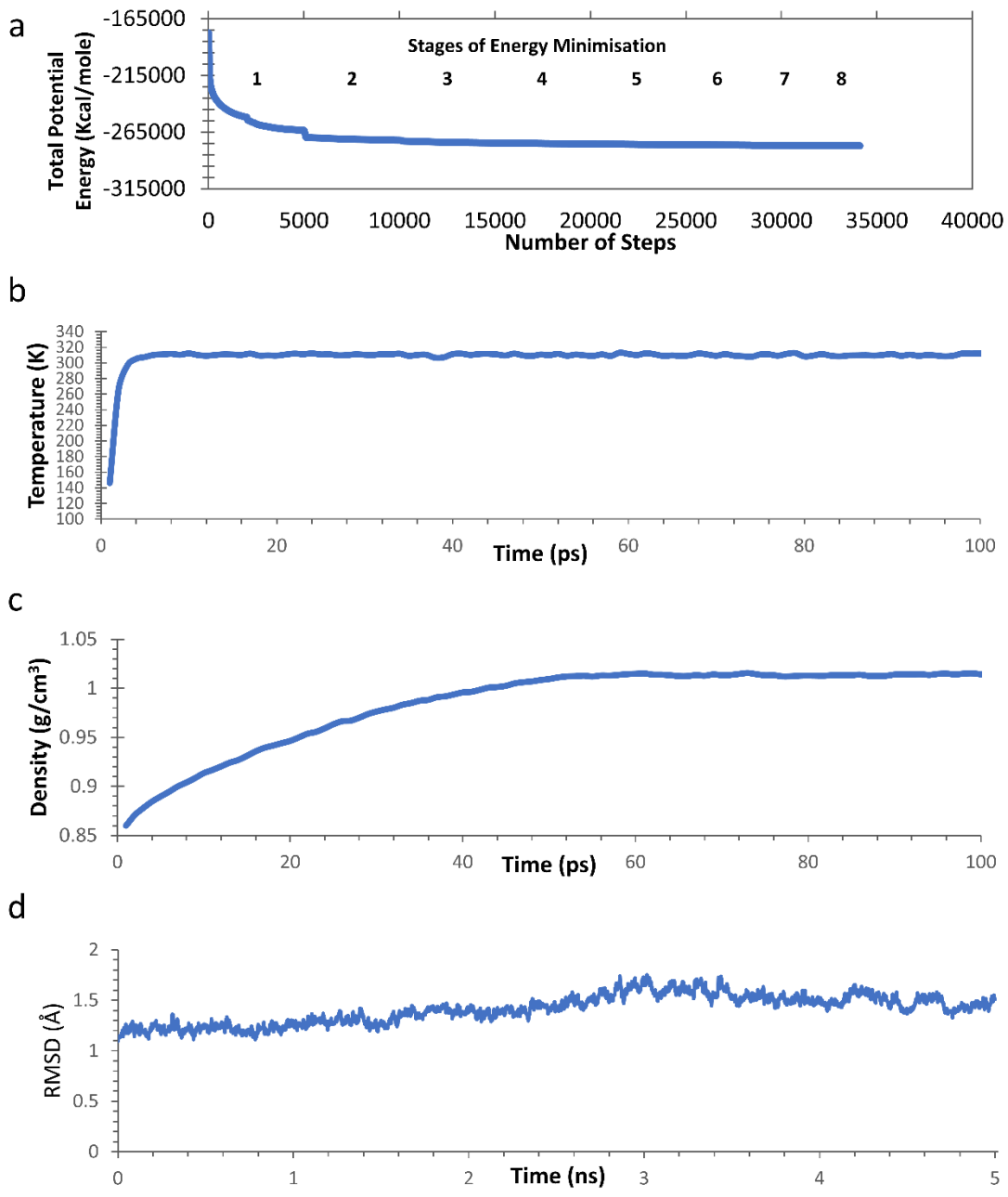


Figure S4 Plots depicting. (a) stages of energy minimisation (b) Heating (c) Density equilibration (d) MD run.

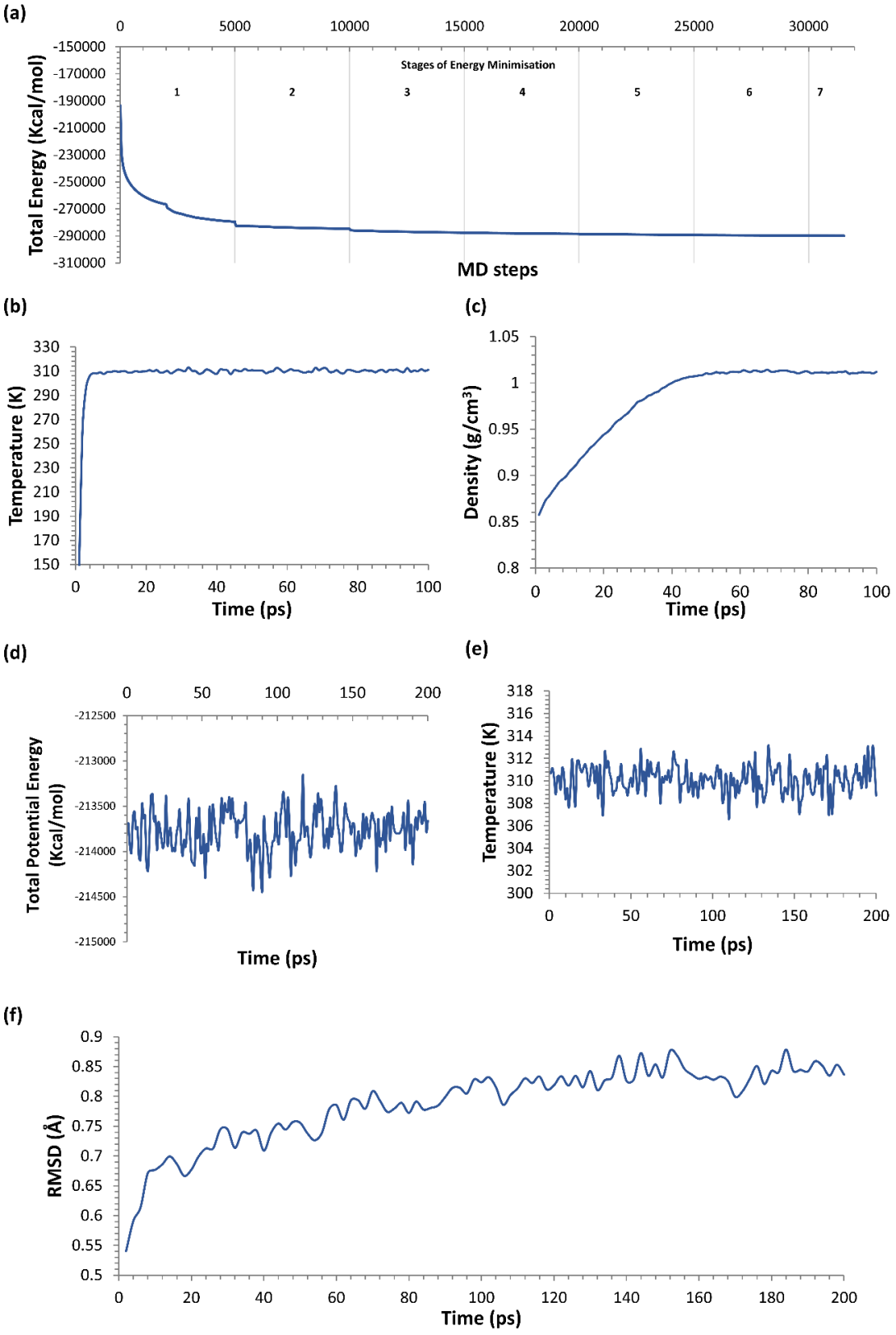
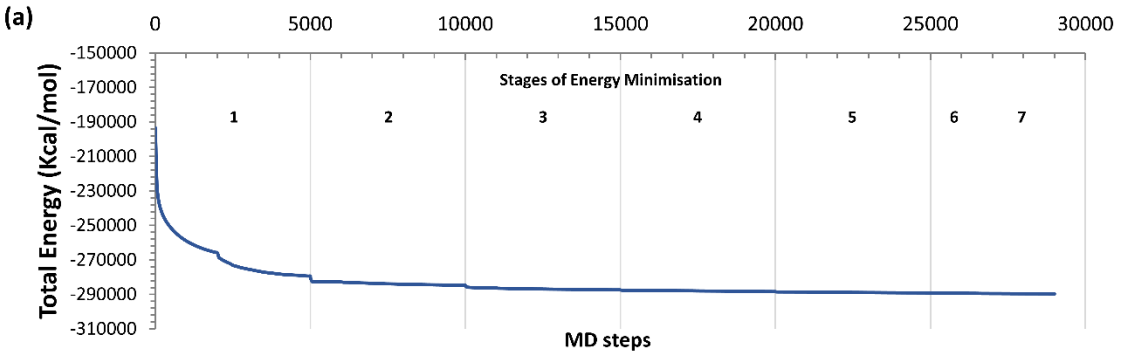

(a)
Total Energy (Kcal/mol)
MD steps
Stages of Energy Minimisation
1
2
3
4
5
6
7
(b)
Temperature (K)
Time (ps)
(c)
Density (g/cm³)
Time (ps)
(d)
Total Potential Energy (Kcal/mol)
Time (ps)
(e)
Temperature (K)
Time (ps)
(f)
RMSD (Å)
Time (ps)

Figure S5. Plot depicting (a) energy minimisation (b) heating (c) density equilibration (d) total potential energy of short NPT equilibration run (e) temperature of short NPT equilibration run (f) RMSD of short NPT equilibration run of protein-ZINC000013719534 complex


(a)
Stages of Energy Minimisation
1
2
3
4
5
6
7
Total Energy (Kcal/mol)
MD steps

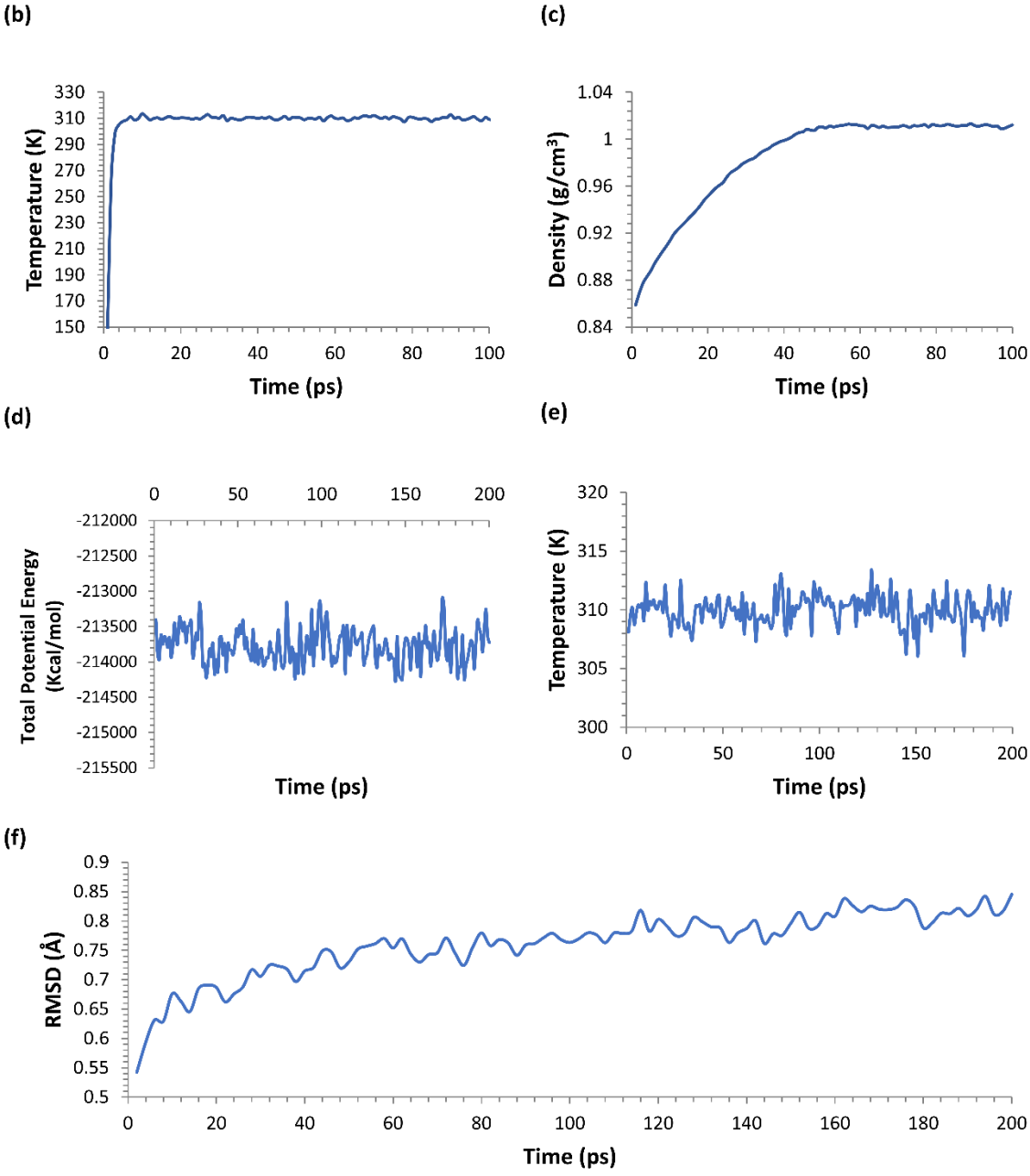
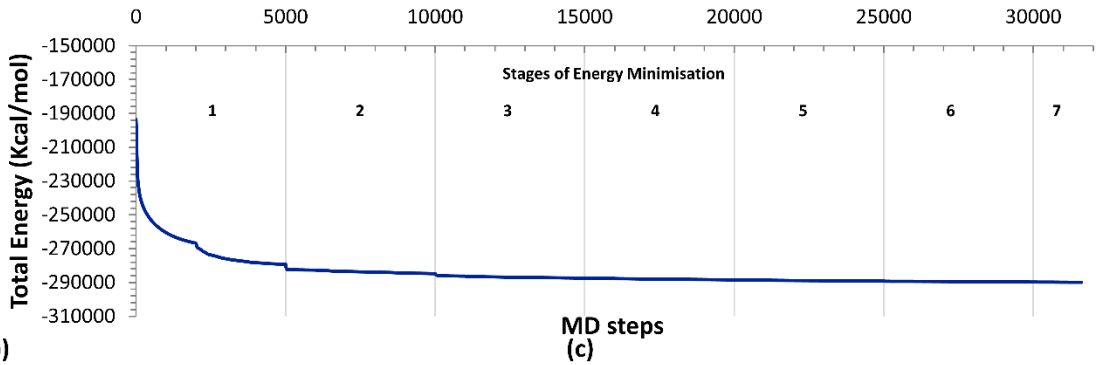
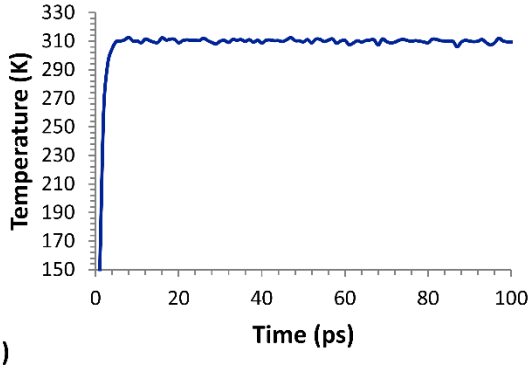

(b)
Temperature (K)
Time (ps)
(c)
Density (g/cm³)
Time (ps)
(d)
Total Potential Energy (Kcal/mol)
Time (ps)
(e)
Temperature (K)
Time (ps)
(f)
RMSD (Å)
Time (ps)

Figure S5. Plot depicting (a) energy minimisation (b) heating (c) density equilibration (d) total potential energy of short NPT equilibration run (e) temperature of short NPT equilibration run (f) RMSD of short NPT equilibration run of protein-ZINC000035551243 complex

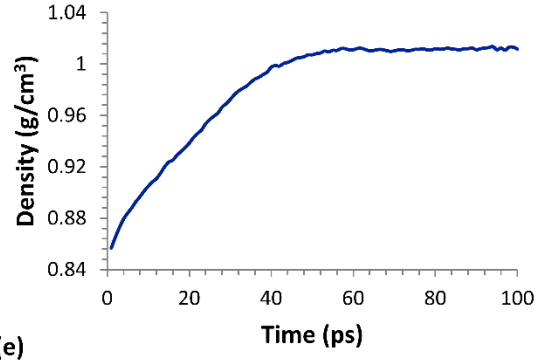
(a)


Total Energy (Kcal/mol)
-150000
-170000
-190000
-210000
-230000
-250000
-270000
-290000
-310000
0
5000
10000
15000
20000
25000
30000
Stages of Energy Minimisation
1
2
3
4
5
6
7
MD steps

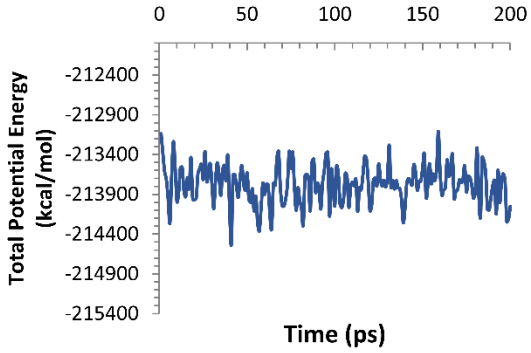
(b)


Temperature (K)
330
310
290
270
250
230
210
190
170
150
0
20
40
60
80
100
Time (ps)

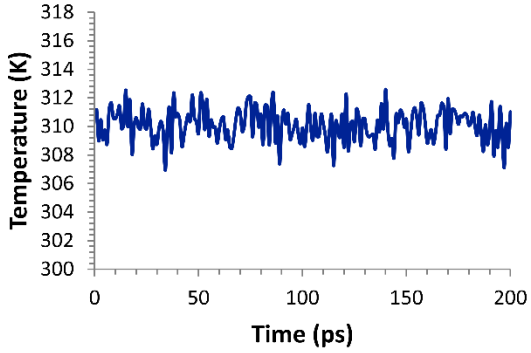
(c)


Density (g/cm³)
1.04
1
0.96
0.92
0.88
0.84
0
20
40
60
80
100
Time (ps)

(d)


Total Potential Energy (kcal/mol)
-212400
-212900
-213400
-213900
-214400
-214900
-215400
0
50
100
150
200
Time (ps)

(e)


Temperature (K)
318
316
314
312
310
308
306
304
302
300
0
50
100
150
200
Time (ps)

(f)

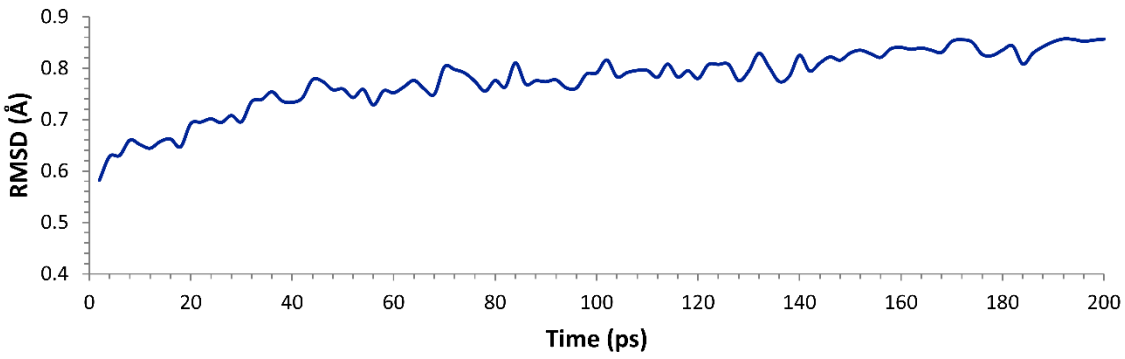

RMSD (Å)
0.9
0.8
0.7
0.6
0.5
0.4
0
20
40
60
80
100
120
140
160
180
200
Time (ps)

Figure S5. Plot depicting (a) energy minimisation (b) heating (c) density equilibration (d) total potential energy of short NPT equilibration run (e) temperature of short NPT equilibration run (f) RMSD of short NPT equilibration run of protein-ZINC000035596918 complex

Tables

Table T1. Protocol for energy minimisation carried out before molecular dynamic simulation in AMBER2018.

Stage	Maximum number of steps (steepest descent)	Maximum number of steps (conjugate gradient)	Weight for the positional restraints. (in kcal/mol-Å^2)	Restrained elements
1	2000	3000	500	Protein and ligand
2	1000	4000	500	Protein and ligand except for their hydrogen
3	1000	4000	500	The backbone of protein and ligand
4	1000	4000	50	Protein and ligand except for their hydrogen
5	1000	4000	50	The backbone of protein and ligand
6	1000	4000	5	Protein and ligand excep for their hydrogen
7	1000	4000	5	The backbone of protein and ligand

Table T2. PLIP report displaying various interactions between 4EY7 and Donepezil

(a) Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	86A	TRP	3.84	8403	649
2	337A	TYR	3.66	8400	2530
3	338A	PHE	3.77	8394	2543
4	338A	PHE	3.83	8400	2545
5	341A	TYR	3.77	8387	2568
6	341A	TYR	3.95	8389	2566
7	341A	TYR	3.60	8394	2569

(b) Hydrogen bonding

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Donor Atom	Acceptor Atom
1	295A	PHE	1.99	2.94	159.54	2197 [Nam]	8408 [O2]

(c) π-π Stacking

Index	Residue	AA	Distance	Angle	Offset	Type	Ligand Atoms
1	86A	TRP	3.89	14.31	0.72	P	8402, 8403, 8404, 8405, 8406, 8407
2	286A	TRP	3.82	4.29	1.37	P	8385, 8386, 8387, 8388, 8389, 8390
3	341A	TYR	4.23	27.95	1.41	P	8388, 8389, 8391, 8392, 8393

Table T3. LGA parameters for virtual screening and precision docking.

Docking	**Number of GA run**	**Population size**	**Maximum number of generations**	**Maximum number of evaluations**	**Rate of Gene mutation**	**Rate of crossing over**
Virtual screening	10	150	27000	250000	0.02	0.8
Precision docking	100	150	27000	2500000	0.02	0.8

Table T4. Protocol for energy minimisation carried out before MM-GBSA, MM-PBSA, and Alanine scanning in AMBER18.

Stage	**Maximum number of steps (steepest descent)**	**Maximum number of steps (conjugate gradient)**	**Weight for the positional restraints. (in kcal/mol-Å^2)**	**Restrained elements**
1	1000	1000	500	Protein and ligand
2	1000	9000	5	Protein and ligand except their hydrogen

Table T5. Detail molecular dynamics protocol for MM-GBSA, MM-PBSA, and Alanine scanning.

(a)				**Heating**	
Number of steps	**Intial temperature(K)**	**Final temperature(K)**	**Temperature regulation**	**collision frequency (in ps^{-1})**	**Ensemble**
25000	100.0	310.15	Langevin dynamics	2	NVT
(b)				**Equilibration**	
Number of steps	**Temperature(K)**	**Temperature regulation**	**Pressure regulation**	**SHAKE bond length constraints**	**Ensemble**
50000	310.15	Langevin dynamics	Berendsen	bonds involving hydrogen	NPT
(c) MD run					
Number of steps	**Temperature(K)**	**Temperature regulation**	**Pressure regulation**	**SHAKE bond length constraints**	**Ensemble**
500000	310.15	Langevin dynamics	Berendsen	bonds involving hydrogen	NPT

Table T6. Detailed molecular dynamics protocol for Molecular dyanmics.

(a)				**Heating**	
Number of steps	**Intial temperature(K)**	**Final temperature(K)**	**Temperature regulation**	**collision frequency (in ps^{-1})**	**Ensemble**

50000	100.0	310.15	Langevin dynamics	2	NVT
(b) equilibration				**Density**	
Number of steps	**Temperature(K)**	**Temperature regulation**	**Pressure regulation**	**SHAKE bond length constraints**	**Ensemble**
50000	310.15	Langevin dynamics	Berendsen	bonds involving hydrogen	NPT
(c) Equilibration					
Number of steps	**Temperature(K)**	**Temperature regulation**	**Pressure regulation**	**SHAKE bond length constraints**	**Ensemble**
100000	310.15	Langevin dynamics	Berendsen	bonds involving hydrogen	NPT