Supplemental Information

Supplemental Methods

Choice of 3.70 Å cutoff for hydrogen bond cutoff: The reported frequency of weak CH···O hydrogen bonds depends, in large part, on the choice of cutoff for CH···O hydrogen bond distance. 3.7 Å represents the van der Waals distance for a C-H bond and an approaching oxygen atom, and thus has been used in the past as a conservative cutoff, most notably by the highly influential studies of Taylor and Kennard as well as Derewenda and colleagues[1,2]. For especially weak hydrogen bonds such as methyl groups, this distance cutoff may be too short to give an accurate depiction of the extent of methyl group hydrogen bonding[3]. However, to take the most conservative approach possible, we chose to use the 3.70 Å cutoff, but remind readers that the results presented here therefore represent the minimum possible level of methyl CH···O hydrogen bonding in the analyzed neutron structures.

Quantum Mechanics energy calculations: Quantum Mechanical counterpoise-corrected energy calculations were performed using Gaussian 09[4], MP2 or wB97xD/6-31+2d,p, which has been used frequently in the past to calculate accurate CH···O bond energies[5,6]. All methyl CH···O hydrogen bond pairs in which neither the donor or acceptor groups were engaged in other hydrogen bonds were analyzed.

Explicit Setup of Molecular Dynamics Simulation: Use the following MMTSB commands to replicate our simulation protocol.

```
minCHARMM.pl –par dielec=rdie,epsilon=4,minsteps=50 2XQZ.pdb >
2XQZ.min.pdb
convpdb.pl –solvate –cutoff 9 2XQZ.min.pdb > 2XQZ.solvated.pdb
enerCHARMM.pl –charge 2XQZ.solvated.pdb
convpdb.pl –ions SOD:12 2XQZ.solvated.pdb > 2XQZ.solvated.ions.pdb
minCHARMM.pl –par minsteps=500,boxx=75.4,boxy=75.4,boxz=75.4
2XQZ.solvated.ions.pdb > 2XQZ.solvated.ions.min.pdb
mdCHARMM.pl –par
dynsteps=1000,boxx=75.4,boxy=75.4,boxz=75.4,dyntemp=100 –final
2XQZ.md.pdb 2XQZ.solvated.ions.min.pdb
mdCHARMM.pl –par
dynsteps=1000,boxx=75.4,boxy=75.4,boxz=75.4,dyntemp=200 –final
2XQZ.md.pdb 2XQZ.solvated.ions.min.pdb
mdCHARMM.pl –par
dynsteps=1000,boxx=75.4,boxy=75.4,boxz=75.4,dyntemp=250 –final
2XQZ.md.pdb 2XQZ.solvated.ions.min.pdb
mdCHARMM.pl –par
dynsteps=1000,boxx=75.4,boxy=75.4,boxz=75.4,dyntemp=300 –final
2XQZ.md.pdb 2XQZ.solvated.ions.min.pdb


mdCHARMM.pl –par
dynsteps=5000000,boxx=75.4,boxy=75.4,boxz=75.4,dyntemp=300,dynoutfrq=10
,dyntemp=300 –trajout 2xqz.dcd
```

Determination of Hydrogen Bonds by Distance/Angle Cutoffs: All analysis scripts are open source and freely available to download at https://github.com/jyesselm/omega. Each of the 2328 frames from the explicit water simulation of 2XQZ was processed with the OMEGA package. Each bond that existed for at least one frame was indexed and ranked based on the total number of frames that the bond was found in. This full list can be found on our github repository.

Parameter File Used for OMEGA: To allow for complete customization of hydrogen bond donors and acceptors, OMEGA requires a parameter file. Parameter files are composed of two parts: Declaration of types, both donors and acceptors, as well as the interactions to be measured between them. Types are declared by Type1 = AtomElement.NumofBonds with parenthesis denoting required bonds. For example, MethylCarbon = C.4(H.1)(H.1)(H.1) describes a carbon with 4 bonds that is bonded to 3 hydrogen atoms. The type name MethylCarbon is user defined, any name can be used and will be recognized later in the file. Each measurement can have two cutoffs declared, a max and min value. For example, finding an interaction distances between all Type1 and Type2 atoms is declared by Type1-Type2.MinDistance = X1 and Type1-Type2.MaxDistance = X2. Angles are declared between three types in a similar fashion to distance, Type1-Type2-Type3.MinAngle = X1 and Type1-Type2-Type3.MaxAngle = X2. The available interactions to be measured are distance, angle and plane angle. Another feature is the R wild card character. R matches to any atom connected to type. For example RType1-Type1-Type2.MaxAngle = 150, will record all angles between each atom bonded to type1, type and type2 below 150 degrees.

See parameter file below for full example.

```
#Parameters for OMEGA
####################
#First declaration of types, these are the atoms you want OMEGA
to identify. These are declared as molecule strings in the form
AtomElement.BondNumber with () denoting a bond.
#Next declaration of angles and distances to measure
#Distances are declared as
```

```
#Type1-Type2.MinDistance = X
#Type1-Type2.MaxDistance = X
#

#Declaration of Types
#Methyl Carbon: Carbon with 4 bonds with 3 bonds with hydrogens
MethylCarbon  = C.4(H.1)(H.1)(H.1)
#Methyl Hydrogen
MethylCarbonH = H.1(C.4(H.1)(H.1))

SP3Oxygen = O.2(H.1)
SP2Oxygen = O.1

#MethylCarbon - SP3Oxygen Cutoffs
#R before any name is the atom directly connected to it
#R
# \
#   O *** CH3
#
RSP3Oxygen-SP3Oxygen-MethylCarbon.MaxAngle = 160
RSP3Oxygen-SP3Oxygen-MethylCarbon.MinAngle = 75

# A number following an interaction allows for multiple possible
# Here there are two criteria that will yield an acceptable match
MethylCarbon-SP3Oxygen.0.MaxDistance = 3.25
#R
#  \
#   H3C *** O
#
RMethylCarbon-MethylCarbon-SP3Oxygen.0.MaxAngle = 160
RMethylCarbon-MethylCarbon-SP3Oxygen.0.MinAngle = 75

MethylCarbon-SP3Oxygen.1.MaxDistance = 3.69
RMethylCarbon-MethylCarbon-SP3Oxygen.1.MaxAngle = 140
RMethylCarbon-MethylCarbon-SP3Oxygen.1.MinAngle = 85

#MethylCarbon - SP2Oxygen Cutoffs
MethylCarbon-SP2Oxygen.0.MaxDistance = 3.25
RMethylCarbon-MethylCarbon-SP2Oxygen.0.MaxAngle = 160
RMethylCarbon-MethylCarbon-SP2Oxygen.0.MinAngle = 75

MethylCarbon-SP2Oxygen.1.MaxDistance = 3.69
RMethylCarbon-MethylCarbon-SP2Oxygen.1.MaxAngle = 140
RMethylCarbon-MethylCarbon-SP2Oxygen.1.MinAngle = 85

MethylCarbon-SP2Oxygen.MaxPlaneAngle = 50

#
# R == O *** CH3
#
```

```
RSP2Oxygen-SP2Oxygen-MethylCarbon.MinAngle = 90

#MethylCarbonH - SP3Oxygen Cutoffs
MethylCarbonH-SP3Oxygen.MaxDistance = 2.70
#  C
#   \
#    H *** O
RMethylCarbonH-MethylCarbonH-SP3Oxygen.MaxAngle = 220
RMethylCarbonH-MethylCarbonH-SP3Oxygen.MinAngle = 140

#  C     H - O
#   \  **
#    H
RMethylCarbonH-HSP3Oxygen-SP3Oxygen.0.MaxAngle = 150
RMethylCarbonH-HSP3Oxygen-SP3Oxygen.0.MinAngle = 0

RMethylCarbonH-HSP3Oxygen-SP3Oxygen.1.MaxAngle = 360
RMethylCarbonH-HSP3Oxygen-SP3Oxygen.1.MinAngle = 220

#MethylCarbonH - SP2Oxygen Cutoffs
MethylCarbonH-SP2Oxygen.MaxDistance = 2.70
RMethylCarbonH-MethylCarbonH-SP2Oxygen.MaxAngle = 280
RMethylCarbonH-MethylCarbonH-SP2Oxygen.MinAngle = 100
MethylCarbonH-SP2Oxygen.MaxPlaneAngle = 50
```

Determination of Hydrogen Bond Dihedral Distributions: For each frame in the simulation all dihedral angles in the form R''-R'-C-H were calculated for each methyl group where R' is the atom bonded to the methyl group and R'' is every atom bonded to R'. Also all acceptor dihedral angles were calculated for all Oxygen atom acceptors that had at least 1 hydrogen bond in 1 frame in the simulation in the form R''-R'-C-O. Control dihedrals were generated by considering the 8 methyl groups in the protein that did not have a single hydrogen bond throughout the simulation to any oxygen including waters. We considered this to have random distribution as their motion should not be correlated.

Each distribution was quantified using Origin 6 (OriginLab) to determine the number of peaks for each distribution, center of each peak, and the standard deviation of each peak (peaks were fit to a Gaussian). We used a custom-fitting algorithm, which is also available in the repository. The centers and standard deviations of the peaks of the hydrogen atoms were then compared to those of the oxygen peaks and the closest overlay was determined and the difference between peak centers and standard deviations were quantified. For each peak combination category (# of hydrogen peaks, # of oxygen peaks) the distribution of the difference in peak centers and standard deviation are plotted (Fig. S1)

Supplemental Figures

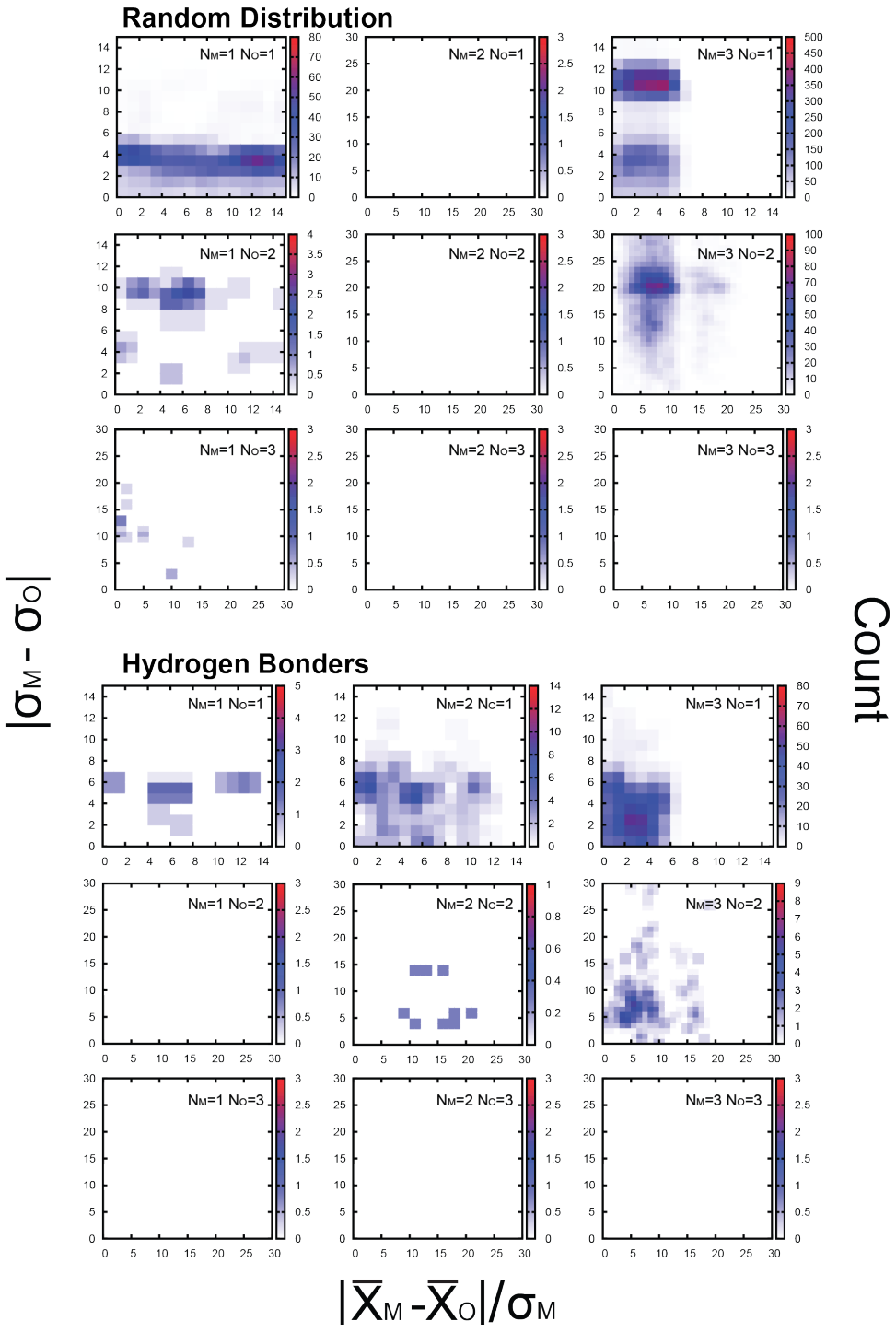

Figure S1: Complete aggregates of overlap traces for both random distributions (top) and hydrogen bonders (bottom) with all combinations of methyl and oxygen dihedrals. N_M=3 NO=1 shown in Fig 2C and subjected for JSD analysis (Fig 2D).

References

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