# A Parameterization of Cholesterol for Mixed Lipid Bilayer Simulation within the Amber Lipid14 Force Field

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PARAMETERIZATION

**Sterol Ring Parameters.** Because the majority of the cholesterol is based on the basic sterol unit and is largely composed of *sp3* carbon atoms, parameters of the sterol carbons and hydrogens are important to overall lipid bilayer structure. Within the time scales of an all-atom lipid bilayer and cholesterol MD simulation it is likely that the sterol portion of the molecule will mostly be inflexible due to the ring arrangement of the sterol.[1,2] However, the van der Waals of this region can influence important bilayer structural properties such as head group packing as well as volume of the bilayer.[1,2] Therefore, the van der Waals parameters of the carbons and hydrogens in this region were re-examined.

The atom types of most sterol carbons in cholesterol are basic *sp3* atom types adapted from GAFF.[3] The hydrogen atom types from GAFF are hydrogens bonded to an aliphatic carbon without an electron withdrawing group.[3] The initial parameters for carbon include a van der Waals radius of 1.908 Å and a well-depth of 0.1094 kcal/mol. Hydrogens have a radius 1.4870 Å and a well-depth of 0.0157 kcal/mol. In order to examine a similar system and isolate these parameters, a small ringed molecule was chosen: decalin also known as decahydronaphtalene. The relevant *trans* conformation of decalin was modeled and simulated with MD.
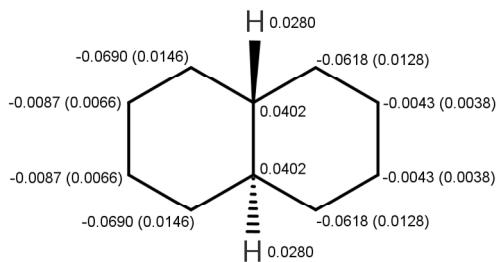


**Figure S1.** Decahydronaphtalene (*trans*-decalin) partial charges. Decalin was used as a sterol model for study of van der Waals parameters. All carbons are adapted from the GAFF *sp3* carbons (Lipid14 cA) and all hydrogens are adapted from the GAFF hydrogens bonded to an aliphatic carbon without an electron withdrawing group (Lipid14 hA).[3,4]

Figure S1 shows the structure and partial atomic charges of *trans*-decalin used for the gas and liquid phase simulations. GAFF parameters were applied to decalin, with the intent of evaluating the carbon and hydrogen parameters. Partial charges were derived for decalin using the RESP method described previously.[4] One structure was optimized and ESP was calculated at the HF/6-31G* level. Atomic partial charges were fit in two stages in the normal RESP method.[4] Topology and parameters were assembled with the Amber program *Leap*.[5]

**Table S1.** Partial atomic charges for hexadecane

| Atom name | Charge | Atom name | Charge |
|-----------|--------|-----------|--------|
| C | -0.0991 | H, H1, H2 | 0.0214 |
| C1 | 0.0220 | H3, H4 | 0.0059 |
| C2 | -0.0248 | H5, H6 | 0.0147 |
| C3 | -0.0336 | H7, H8 | 0.0130 |
| C4 | -0.0214 | H9, H10 | 0.0124 |
| C5 | -0.0192 | H11, H12 | 0.0093 |
| C6 | -0.0215 | H13, H14 | 0.0124 |
| C7 | -0.0244 | H15, H16 | 0.0136 |
| C8 | -0.0310 | H17, H18 | 0.0133 |
| C9 | -0.0315 | H19, H20 | 0.0148 |
| C10 | -0.0280 | H21, H22 | 0.0171 |
| C11 | -0.0395 | H23, H24 | 0.0176 |
| C12 | -0.0384 | H25, H26 | 0.0175 |
| C13 | -0.0341 | H27, H28 | 0.0195 |
| C14 | 0.0193 | H29, H30 | 0.0095 |
| C15 | -0.1210 | H31, H32, H33 | 0.0269 |

Hexadecane was also simulated in mixtures of *trans*-decalin. Parameters were taken directly from Lipid14 including the updated van der Waals and dihedrals terms. Partial charges were derived using the Lipid14 RESP methodology with multiple conformations.

VALIDATION

**Phospholipid and Cholesterol Area per Molecule.** Another experimental comparison is the lateral area per molecule for cholesterol and lipid bilayers. Usually the experimental area per molecule is derived from volume and thickness bilayer measurements.[6] While it is possible to calculate individual area per molecules for a binary bilayer,[7] the simple average area per molecule $A_M$ for both cholesterol and lipid is reported here, where $A_M = A_{XY}/(n_L + n_{CHL})$. The lateral area is denoted by $A_{XY}$ and the number of lipids and cholesterol is $n_L$ and $n_{CHL}$, respectively.
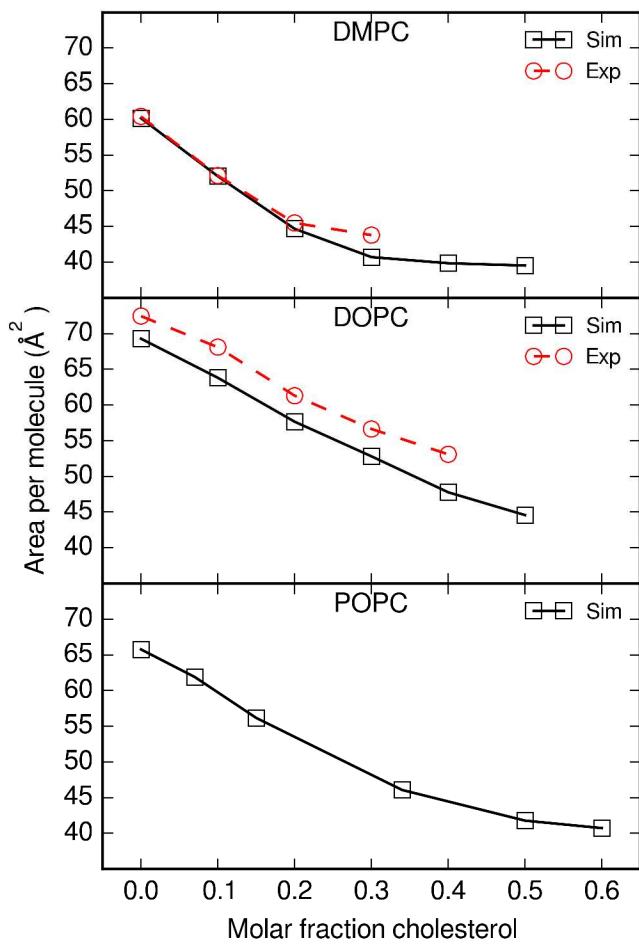
**Figure S2.** Average area per molecule for lipid and cholesterol bilayers. Boxes are the average simulation area per molecule. Circles are the experimental area per average molecule at 30 C from Pan et al.[6]

Figure S2 shows the average area per molecule for cholesterol and lipid bilayers. Increasing cholesterol content correlates to decreasing area per molecule in both simulation and experiment. This condensing effect is well known in bilayers with cholesterol.[8,9] Simulations with other cholesterol force fields result in bilayers with low area per molecule: Jambeck et al reports an area of $57 \pm 0.5$ Å$^2$ and $43 \pm 0.4$ Å$^2$ for 20% and 50% cholesterol in a DOPC bilayer, respectively.

Cholesterol also has an experimentally observed saturation point when mixed with lipid bilayers.[10,11] Beyond saturation, cholesterol precipitates from the bilayer as cholesterol monohydrate. Huang et al report a cholesterol saturation point in phosphatidylcholine bilayers to be 66%.[11] Hung et al report a lower saturation point: ~44% in DMPC bilayers and ~40% in DOPC bilayers.[10] More recently, Mainali et al show that cholesterol bilayer domains form in DMPC bilayers at 50% molar fraction cholesterol and form cholesterol crystals at 66% molar fraction cholesterol.[12] It is possible that the simulations presented here approach the saturation points of these bilayers.

PARAMETERS

**Lipid14 Cholesterol Parameters.** Please see http://ambermd.org for specific implementation details for AMBER. Please see http://ambermd.org/formats for the specification for the AMBER force field parameter and topology files.
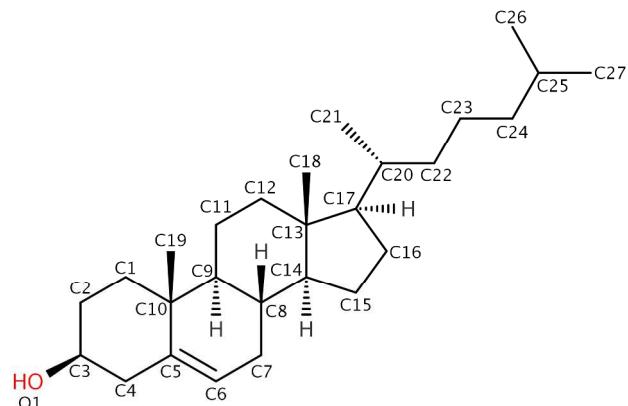


**Figure S3.** Atom names for cholesterol. The Lipid14 residue name is CHL. Hydrogens follow the Lipid11 naming convention. Namely, hydrogens follow the format "HXY" where X is the carbon number and Y is 1, 2, or 3 for each attached hydrogen. All atom names are also listed in table S2.

**Table S2.** Lipid14 cholesterol atom names, types, and charges. Bonded hydrogens are displayed in the same row as their corresponding heavy atom.

| Name | Type | Charge | Name | Type | Charge |
|------|------|--------|------|------|--------|
| C1 | cA | -0.0317 | H11, H12 | hA | 0.0094 |
| C2 | hA | -0.0881 | H21, H22 | hA | 0.0446 |
| C3 | hE | 0.2936 | H31 | hA | 0.0296 |
| C4 | cA | -0.1622 | H41, H42 | hA | 0.0900 |
| C5 | cB | -0.1395 | | | |
| C6 | cB | -0.2082 | H61 | hB | 0.1228 |
| C7 | cA | -0.0699 | H71 | hA | 0.0479 |
| C8 | cA | -0.0114 | H81 | hA | 0.0729 |
| C9 | cA | 0.0196 | H91 | hA | 0.0293 |
| C10 | cA | 0.0791 | | | |
| C11 | cA | -0.0664 | H111, H112 | hA | 0.0301 |
| C12 | cA | -0.0650 | H121, H122 | hA | 0.0104 |
| C13 | cA | 0.0574 | | | |
| C14 | cA | 0.0058 | H141 | hA | 0.0316 |
| C15 | cA | -0.1020 | H151, H152 | hA | 0.0265 |
| C16 | cA | -0.0921 | H161, H162 | hA | 0.0354 |
| C17 | cA | 0.0324 | H171 | hA | 0.0166 |
| C18 | cA | -0.1150 | H181, H182, H183 | hA | 0.0245 |
| C19 | cA | -0.1081 | H191, H192, H193 | hA | 0.0340 |
| C20 | cD | 0.0443 | H201 | hL | 0.0210 |
| C21 | cD | -0.1546 | H211, H212, H213 | hL | 0.0363 |
| C22 | cD | -0.0390 | H221, H222 | hL | 0.0084 |
| C23 | cD | -0.0285 | H231, H232 | hL | 0.0157 |
| C24 | cD | -0.1256 | H241, H242 | hL | 0.0401 |
| C25 | cD | 0.2124 | H251 | hL | -0.0025 |
| C26 | cD | -0.2578 | H261, H262, H263 | hL | 0.0580 |
| C27 | cD | -0.2578 | H271, H272, H273 | hL | 0.0580 |
| O1 | oH | -0.7030 | HO1 | hO | 0.4148 |

**Lipid14 Cholesterol Parameter File.** This is the cholesterol parameter file (*CHL.frcmod*) containing parameters compatible with

Lipid14 parameters included in AmberTools 14 (*leaprc.lipid14, lipid14.lib, lipid14.dat*).

```
CHL Lipid14 frcmod
MASS
hA 1.008                              H bonded to aliphatic carbon without electrwd. group (GAFF hc-)
hO 1.008                              H in Hydroxyl group (GAFF ho-)
oH 16.00                              sp3 oxygen in hydroxyl group (GAFF oh-)


BOND
cA-cB  328.3    1.5080        Lipid11 v1.0 (GAFF c3-c2)
cA-cD  303.1    1.5350        Lipid14 CHL  (GAFF c3-c3)
cA-hA  337.3    1.0920        Lipid11 v1.0 (GAFF c3-hc)
cA-oH  314.1    1.4260        Lipid11 v1.0 (GAFF c3-oh)
hO-oH  369.6    0.9740        Lipid11 v1.0 (GAFF ho-oh)


ANGLE
cA-cA-cB   63.530    111.440   Lipid11 v1.0 (GAFF c3-c3-c2)
cA-cA-cD   63.210    110.630   Lipid14 CHL  (GAFF c3-c3-c3)
cA-cA-hA   46.370    110.050   Lipid11 v1.0 (GAFF c3-c3-hc)
cA-cA-oH   67.720    109.430   Lipid11 v1.0 (GAFF c3-c3-oh)
cA-cB-cA   62.700    116.520   Lipid11 v1.0 (GAFF c3-c2-c3)
cA-cB-cB   64.330    123.420   Lipid11 v1.0 (GAFF c3-c2-c2)
cA-cB-hB   45.660    117.300   Lipid11 v1.0 (GAFF c3-c2-ha)
cA-cD-hL   46.370    110.050   Lipid14 CHL  (GAFF c3-c3-hc)
cA-cD-cD   63.210    110.630   Lipid14 CHL  (GAFF c3-c3-c3)
cA-oH-hO   47.090    108.160   Lipid11 v1.0 (GAFF c3-oh-ho)
cB-cA-hA   47.030    110.490   Lipid11 v1.0 (GAFF c2-c3-hc)
cD-cA-hA   46.370    110.050   Lipid14 CHL  (GAFF c3-c3-hc)
hA-cA-hA   39.430    108.350   Lipid11 v1.0 (GAFF hc-c3-hc)
hE-cA-oH   50.970    109.880   Lipid11 v1.0 (GAFF ha-c3-oh)


DIHE
cA-cA-cA-cA   1    0.18       0.0        -3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c3-c3-c3)
cA-cA-cA-cA   1    0.25     180.0        -2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c3-c3-c3)
cA-cA-cA-cA   1    0.20     180.0         1.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c3-c3-c3)
cA-cA-cA-cB   1    0.1556     0.0         3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c3-X )
cA-cA-cA-cD   1    0.3112   180.000       1.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cA-cD   1   -0.1233   180.000       2.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cA-cD   1    0.1149     0.000       3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cA-cD   1   -0.2199     0.000       4.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cA-cD   1    0.2170     0.000       5.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cA-hA   1    0.16       0.0         3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c3-c3-hc)
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```
cA-cA-cA-oH   1    0.1556       0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c3-X )
cA-cA-cB-cA   1    0.000        0.0            2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c2-X )
cA-cA-cB-cB   1    0.000        0.0            2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c2-X )
cA-cA-cB-hB   1    0.000        0.0            2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c2-X )
cA-cA-cD-cD   1    0.3112     180.000          1.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cD-cD   1   -0.1233     180.000          2.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cD-cD   1    0.1149       0.000          3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cD-cD   1   -0.2199       0.000          4.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cD-cD   1    0.2170       0.000          5.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cA-cA-cD-hL   1    0.16         0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (GAFF c3-c3-c3-hc)
cA-cA-oH-hO   1    0.16         0.0           -3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c3-oh-ho)
cA-cA-oH-hO   1    0.25         0.0            1.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c3-oh-ho)
cA-cB-cA-hA   1    0.000        0.0            2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c2-X )
cA-cB-cB-cA   1    6.65       180.0           -2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c2-c2-c3)
cA-cB-cB-cA   1    1.90       180.0            1.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c2-c2-c3)
cA-cB-cB-hB   1    6.650      180.000          2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c2-c2-X )
cB-cA-cA-hA   1    0.1556       0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c3-X )
cB-cA-cA-oH   1    0.1556       0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c3-X )
cB-cB-cA-hA   1    0.38       180.0           -3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF hc-c3-c2-c2)
cB-cB-cA-hA   1    1.15         0.0            1.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF hc-c3-c2-c2)
cB-cB-cA-hE   1    0.1556       0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c3-X )
cD-cD-cD-cA   1    0.3112     180.000          1.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cD-cD-cD-cA   1   -0.1233     180.000          2.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cD-cD-cD-cA   1    0.1149       0.000          3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cD-cD-cD-cA   1   -0.2199       0.000          4.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cD-cD-cD-cA   1    0.2170       0.000          5.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (Lipid14 cD-cD-cD-cD)
cD-cD-cA-hA   1    0.16         0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (GAFF c3-c3-c3-hc)
cD-cA-cA-hA   1    0.16         0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (GAFF c3-c3-c3-hc)
cA-cD-cD-hL   1    0.16         0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (GAFF c3-c3-c3-hc)
hA-cA-cA-hA   1    0.15         0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF hc-c3-c3-hc)
hA-cA-cA-hE   1    0.1556       0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c3-X )
hA-cA-cA-oH   1    0.00         0.0           -3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF hc-c3-c3-oh)
hA-cA-cA-oH   1    0.25         0.0            1.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF hc-c3-c3-oh)
hA-cA-cB-hB   1    0.000        0.0            2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-c2-X )
hA-cA-cD-hL   1    0.15         0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid14 CHL  (GAFF hc-c3-c3-hc)
hE-cA-oH-hO   1    0.500        0.0            3.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF X -c3-oh-X )


IMPROPER
cA-cB-cB-cA   1    6.65       180.0           -2.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c2-c2-c3)
cA-cB-cB-cA   1    1.90       180.0            1.000     SCEE=1.2 SCNB=2.0 Lipid11 v1.0 (GAFF c3-c2-c2-c3)

NONBON
  hA     1.4870  0.0157            OPLS
  hO     0.0000  0.0000            OPLS
  oH     1.7210  0.2104            OPLS
```

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