# Supporting Information: Multiple Binding Poses in the Hydrophobic Cavity of Bee Odorant Binding Protein AmelOBP14

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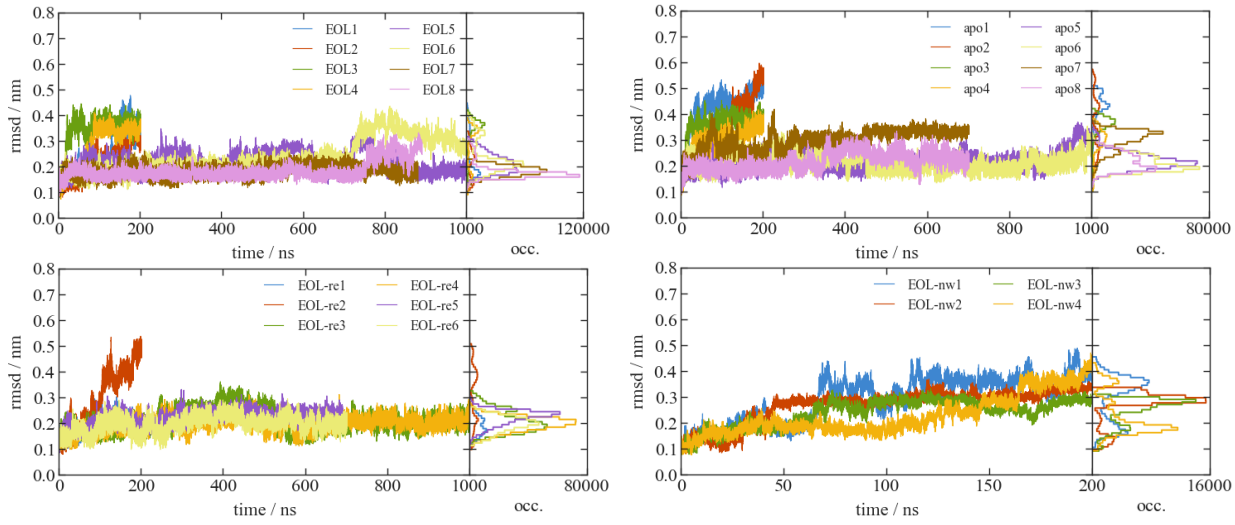


Figure S1: Backbone RMSDs.

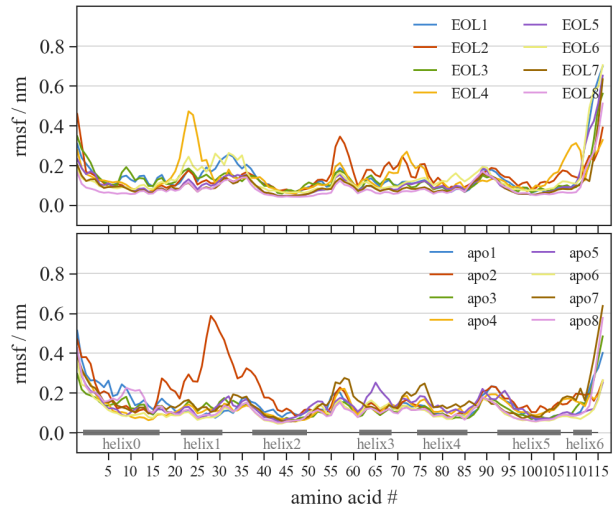


Figure S2: RMSF of backbone nitrogens.

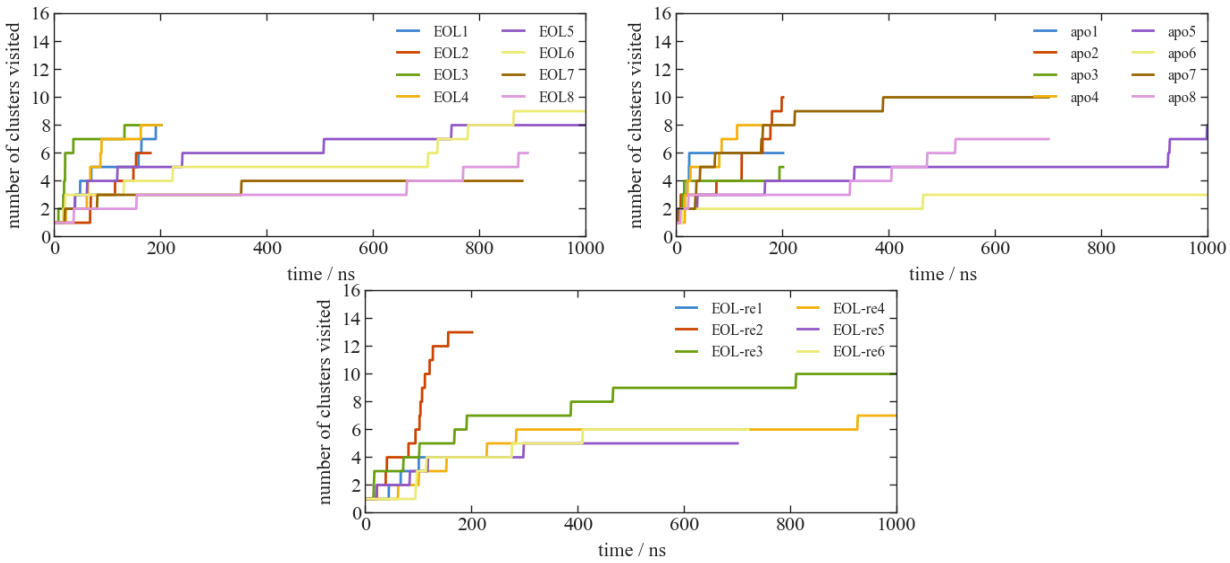


Figure S3: Timeseries of total number of distinct protein conformations for single simulations.

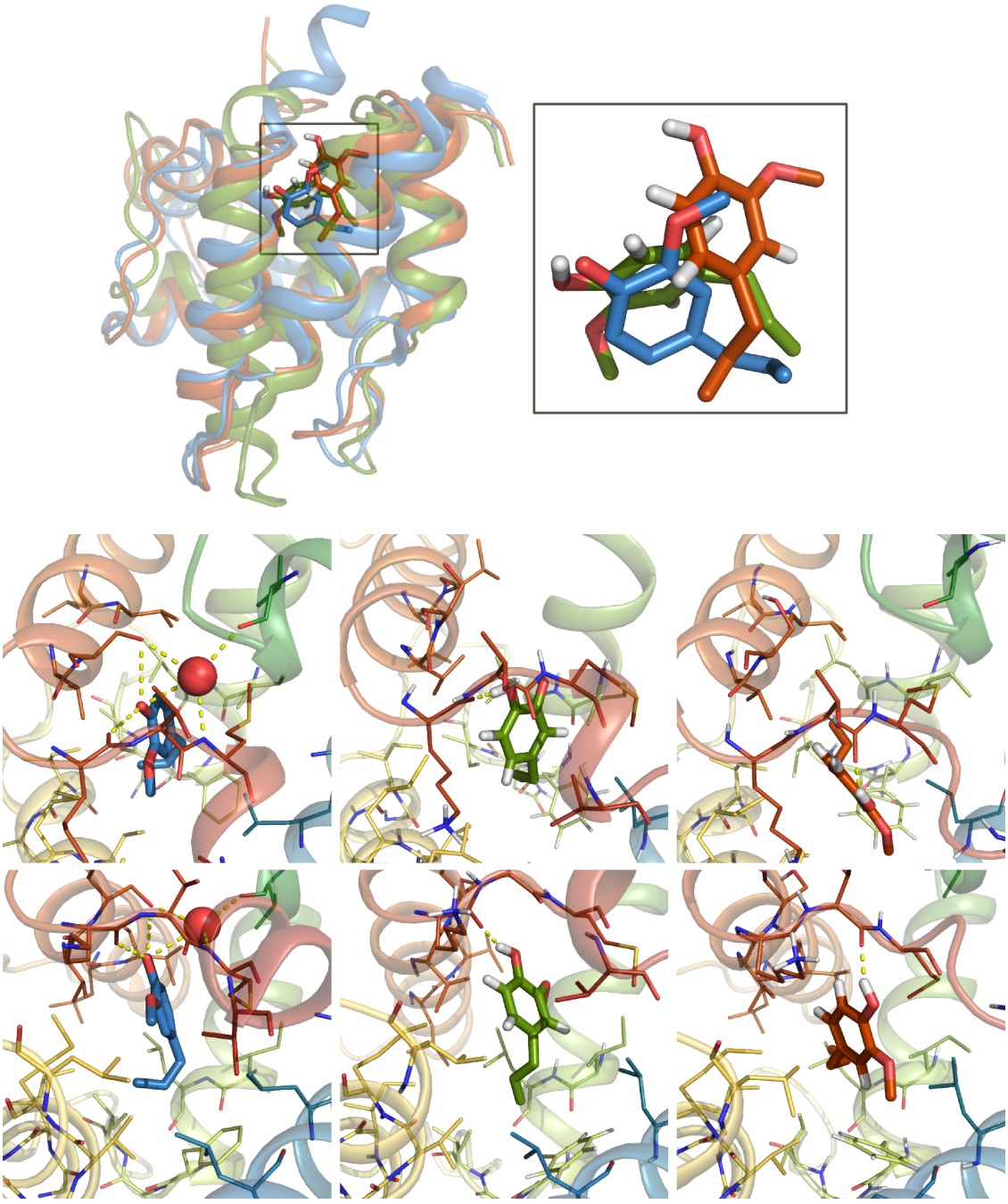

Figure S4: Top: Superposition of central member structures of clusters 0-2 (blue, red, green) from Figure 8 in the main article (clustering criterion ligand heavy atom rmsd $< 0.2$ nm). Bottom: Additional close-up views of EOL in the same structures (here the backbone is colored from blue to red as in Figure 1 of the main article). Amino acid residues that form part of the cavity wall in the crystal structures and those forming hydrogen bonds to the crystal water are shown as sticks.

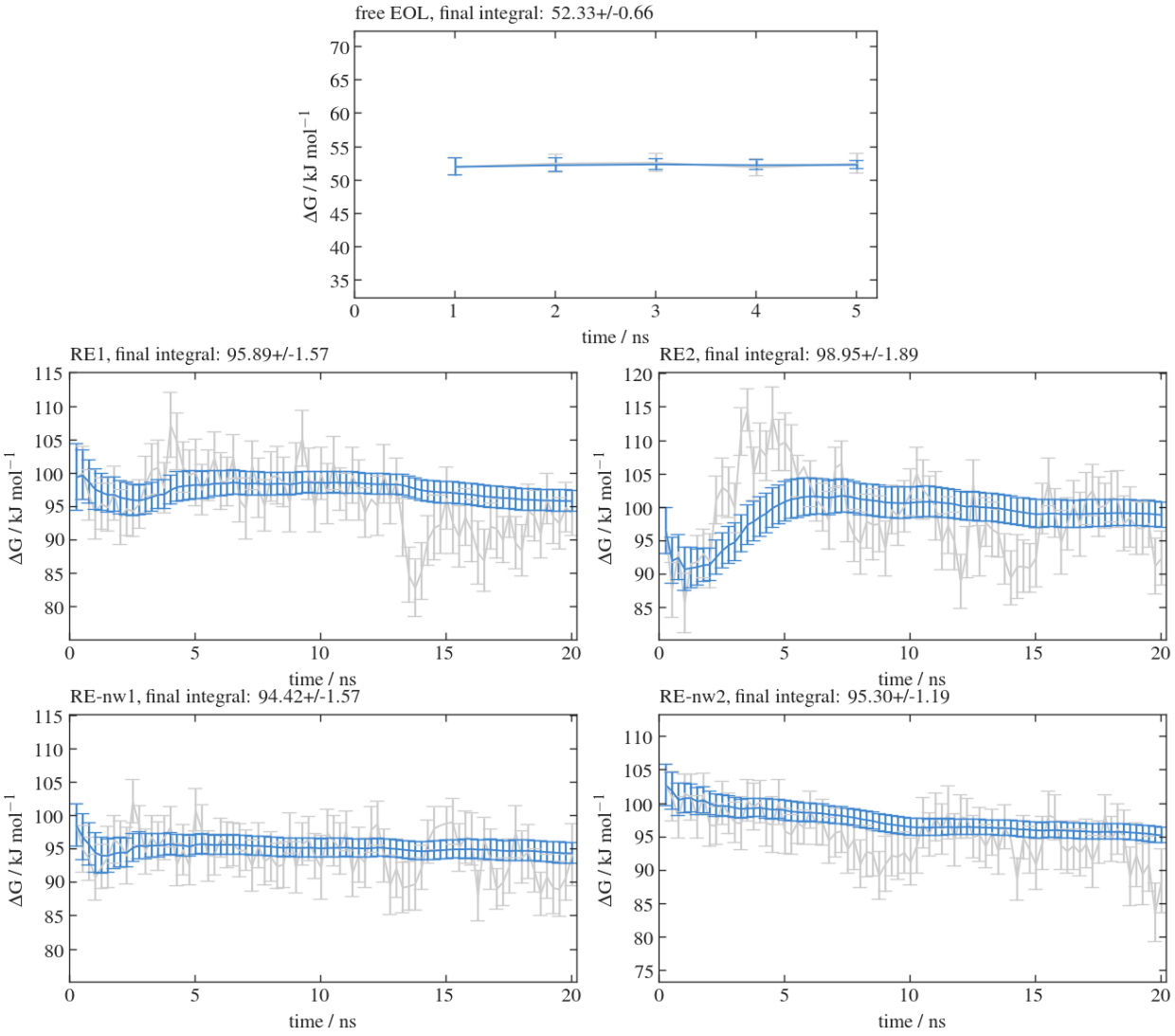


Figure S5: Cumulative (blue) and sequential (gray) time series of the decoupling free energy $\Delta G_{EOL \rightarrow DUM}$ in replica exchange simulations of the free (top) and the protein-bound ligand (four simulations below).

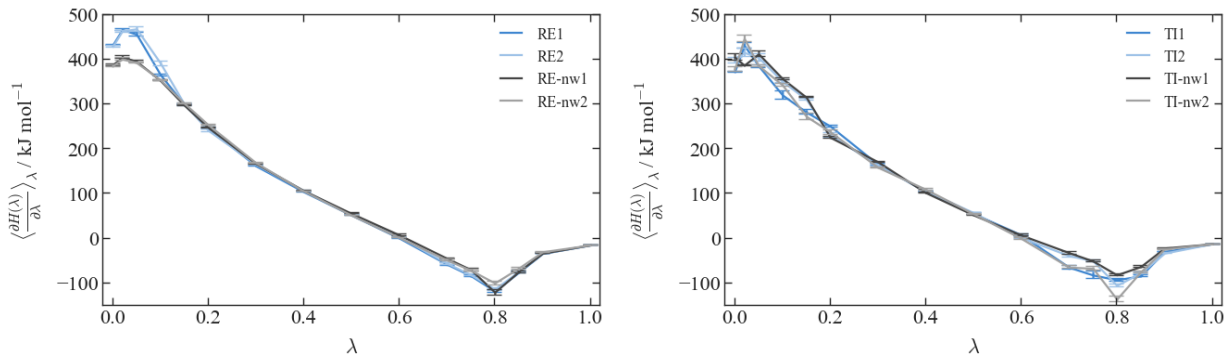


Figure S6: Final plots of $\langle \frac{\partial H(\lambda)}{\partial \lambda} \rangle_\lambda$ vs. $\lambda$ for thermodynamic integration after 20 ns of simulations with (left) and without (right) Hamiltonian replica exchange. Simulations 1 and 2 are identical except for the random seed, "nw" indicates simulations where cavity water was removed. Seemingly in contrast with the values in Table 2 (main article), in the left graph the differences between RE1 and RE-nw1/RE-nw2 seem larger than between RE1 and RE2. However, the striking difference observed between $\lambda$=0 and 0.1 is compensated by the differences at higher $\lambda$. In addition to the simulations at equally spaced $\lambda$ values (in steps of 0.1), we inserted more simulations at the apparent extrema close to $\lambda$=0 and $\lambda$=0.8.

Table S1: Binding free energies from thermodynamic integration of non-replica exchange simulations.

| | $\Delta G_{EOL \rightarrow DUM}$ | $\Delta G_{restraint}$ | $\Delta G_{bind}$ |
|---|---|---|---|
| TI1 | 91.2 ± 2.7 | -13.4 | -25.5 |
| TI2 | 95.8 ± 2.4 | -13.4 | -30.1 |
| TI-nw1 | 99.3 ± 2.0 | -13.4 | -33.6 |
| TI-nw2 | 89.8 ± 2.8 | -13.4 | -24.1 |

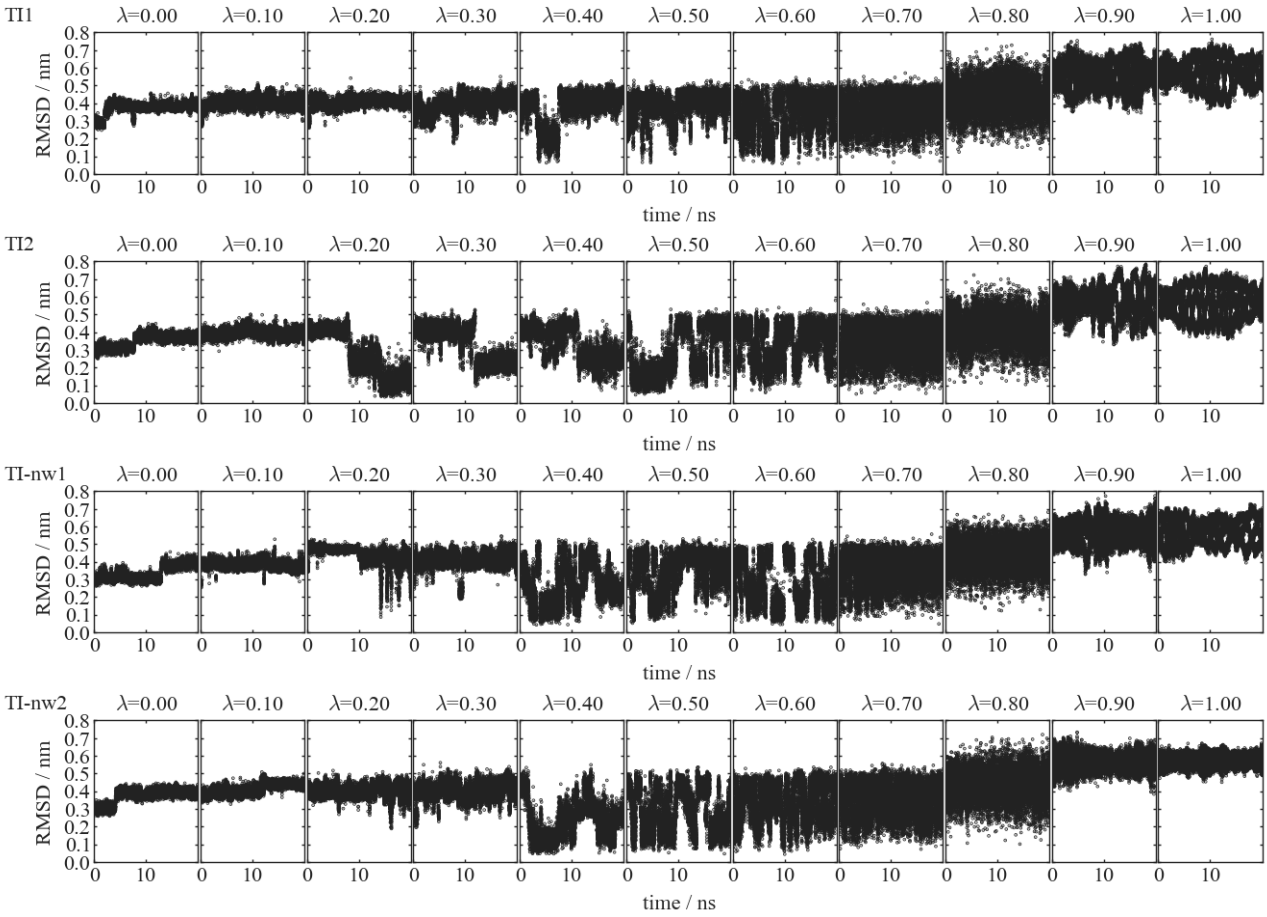


Figure S7: RMSD of the ligand with respect to the "RE"-pose (the dominant alternative ligand pose found in our RE simulations, see Figure 9B, cluster 2) in four sets of simulations performed without replica exchange between the individual simulations at different $\lambda$ values. Data for additional $\lambda$ points which were recorded around the extrema close to $\lambda$=0 and $\lambda$=0.8 are not shown.

## Molecular building block for Eugenol (EOL)

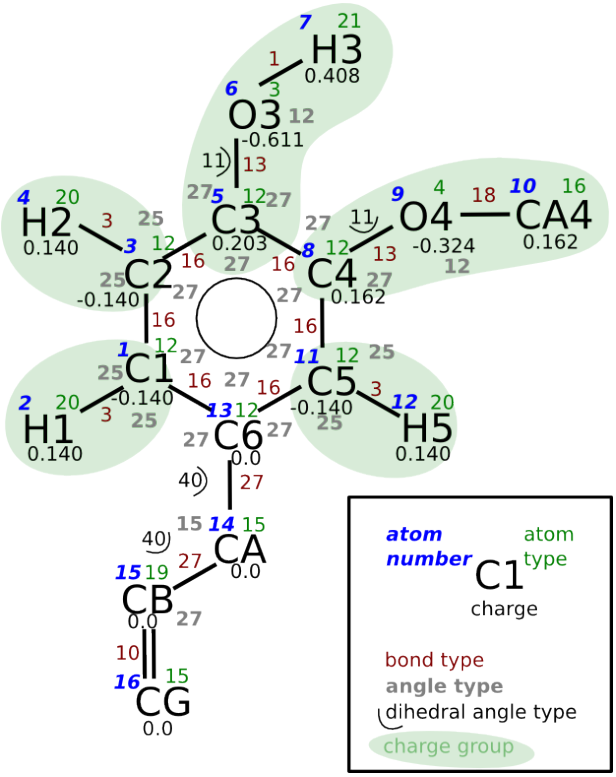


Figure S8: Eugenol bonded and non-bonded parameters for the GROMOS 54A8 set.

```
MTBUILDBLSOLUTE
# building block (residue, nucleotide, etc.)
# RNME
EOL
# number of atoms, number of preceding exclusions
# NMAT,NLIN
   16    0
# preceding exclusions
#ATOM                                MAE MSAE
# atoms
#ATOM ANM   IACM MASS         CGMICGM MAE MSAE
    1 C1     12   12    -0.14000   0  10    2    3    4    5    6    8
                                          11   12   13   14
    2 H1     20    1     0.14000   1   6    3    4    5   11   13   14
    3 C2     12   12    -0.14000   0   8    4    5    6    8    9   11
                                          13   14
    4 H2     20    1     0.14000   1   4    5    6    8   13
    5 C3     12   12     0.20300   0   7    6    7    8    9   11   12
                                          13
```

```
     6 O3       3   16   -0.61100   0   4    7    8    9   11
     7 H3      21    1    0.40800   1   1    9
     8 C4      12   12    0.16200   0   6    9   10   11   12   13   14
     9 O4       4   16   -0.32400   0   4   10   11   12   13
    10 CA4     16    5    0.16200   1   0
    11 C5      12   12   -0.14000   0   3   12   13   14
    12 H5      20    1    0.14000   1   2   13   14
    13 C6      12   12    0.00000   1   2   14   15
    14 CA      15    4    0.00000   1   2   15   16
    15 CB      19    3    0.00000   1   1   16
    16 CG      15    4    0.00000   1   0
# bonds
#  NB
    16
#  IB   JB  MCB
     1    2    3
     1    3   16
     1   13   16
     3    4    3
     3    5   16
     5    6   13
     5    8   16
     6    7    1
     8    9   13
     8   11   16
     9   10   18
    11   12    3
    11   13   16
    13   14   27
    14   15   27
    15   16   10
# bond angles
# NBA
    22
#  IB   JB   KB  MCB
     2    1    3   25
     2    1   13   25
     3    1   13   27
     1    3    4   25
     1    3    5   27
     4    3    5   25
     3    5    6   27
     3    5    8   27
     6    5    8   27
     5    6    7   12
```

```
    5    8    9   27
    5    8   11   27
    9    8   11   27
    8    9   10   12
    8   11   12   25
    8   11   13   27
   12   11   13   25
    1   13   11   27
    1   13   14   27
   11   13   14   27
   13   14   15   15
   14   15   16   27
# improper dihedrals
# NIDA
   12
#  IB   JB   KB   LB  MCB
    1    2    3   13    1
    1    3    5    8    1
    3    1    4    5    1
    3    1   13   11    1
    3    5    8   11    1
    5    3    6    8    1
    5    8   11   13    1
    8    5    9   11    1
    8   11   13    1    1
   11    8   12   13    1
   13    1    3    5    1
   13    1   11   14    1
# dihedrals
# NDA
    4
#  IB   JB   KB   LB  MCB
    3    5    6    7   11
    5    8    9   10   11
    1   13   14   15   40
   13   14   15   16   40
# LJ exceptions
# NEX
    0
#  IB   JB  MCB  NCO  IND  CON
END
```