Supporting Information 1 – Modifications to MOIL for the N_ε-H Tautomer of His64

The MOIL software package[1] that we employ in the MD simulations of MbCO defines force fields for two histidine structures: a neutral structure with the histidine protonated at N_δ and a singly charged structure with both N_ε and N_δ protonated. Simulating the N_ε-H tautomer with MOIL required defining the connectivity of the new structure and the associated force field parameters. MOIL derives atomic partial charges and Lennard-Jones parameters from the OPLS force field[2], it obtains parameters describing bond lengths, bond angles, and torsional angles from the AMBER force field[3], and parameters describing improper torsional interactions from the CHARMM force field[4]. We obtained force field parameters for the N_ε-H tautomer from the OPLS and AMBER force fields, for consistency with MOIL. An additional approximation was introduced in the case of the improper torsion interaction of C_β, C_γ, C_δ, and the unprotonated N_δ. This interaction was approximated using the parameters for the improper torsion interaction of C_β, C_γ, C_δ, and the protonated N_δ of the doubly protonated histidine residue defined in MOIL.

Our modifications of the MOIL force field files are shown below. The first section is introduced into the MOIL file ALL.MONO, which defines the amino acids recognized by MOIL in terms of the atom types and bonding. The second section is introduced into the MOIL file ALL.PROP, which specifies all potential parameters.

```
~ ------------------------------------
~ NEW AA: HIE           wgn 8/19
~ ------------------------------------
~ HISTIDINE (eps N protonated)
~
~     H      O
~     |      |
~     N - CA - C ... N
~         |
~         CB
~         |
~         CG - CD2 - NE2 - HE2
~           |           |
~         ND1 ------ CE1
~
MONO=(HIE)  #prt=13           chrg=-0.57
~ backbone
UNIQ=(N)    PRTC=(NH)
UNIQ=(H)    PRTC=(HN)
UNIQ=(CA)   PRTC=(CAH)
UNIQ=(C)    PRTC=(CO)
UNIQ=(O)    PRTC=(OC)
~ epsilon protonated histidine
UNIQ=(CB)   PRTC=(CH2)
UNIQ=(CG)   PRTC=(CGHE)
UNIQ=(ND1)  PRTC=(NDHE)
UNIQ=(CE1)  PRTC=(CHEE)
UNIQ=(CD2)  PRTC=(CHDE)
UNIQ=(NE2)  PRTC=(NEHE)
UNIQ=(HE2)  PRTC=(HENE)
UNIQ=(N)    PRTC=(NH)   NEXT
DONE
BOND
N-H N-CA CA-CB
CB-CG CG-CD2 CD2-NE2 CG-ND1 ND1-CE1 CE1-NE2 NE2-HE2
CA-C C-O C-N*
DONE
~
~ ------------------------------------
~ END OF NEW AA: HIE          wgn 8/19
~ ------------------------------------
```

The parameters defining the interactions of the epsilon protonated histidine are as follows:

```
~ ------------------------------------------------
~ residues of new AA: HIE           wgn 8/20/01
~ ------------------------------------------------
~   OPLS     MOIL-NAME     MOIL NO.   AMBER
~    44        CGHE          wgn1       CC
~    42        NDHE          wgn2       NB
~    43        CHEE          wgn3       CP
~    40        NEHE          wgn4       NA
~    45        CHDE          wgn5       CG
~    41        HENE          wgn6       H
~
```

```
~ ------------------------------------------------
~ particles of new AA: HIE          wgn 8/20/01
~ ------------------------------------------------
~ eps protonated histidine (HIE)
PNAM=(CGHE)   PMAS=12.   PCHG=0.10   PEPS=0.145   PSGM=3.750   (wgn1)
PNAM=(NDHE)   PMAS=14.   PCHG=-0.49  PEPS=0.170   PSGM=3.250   (wgn2)
PNAM=(CHEE)   PMAS=13.   PCHG=0.41   PEPS=0.145   PSGM=3.750   (wgn3)
PNAM=(NEHE)   PMAS=14.   PCHG=-0.57  PEPS=0.170   PSGM=3.250   (wgn4)
PNAM=(CHDE)   PMAS=13.   PCHG=0.13   PEPS=0.145   PSGM=3.750   (wgn5)
PNAM=(HENE)   PMAS=1.    PCHG=0.42   PEPS=0.0498  PSGM=0.300   (wgn6)
~ ------------------------------------------------
~ END new AA: HIE             wgn 8/20/01
~ ------------------------------------------------


~ ------------------------------------------------
~ BOND of new AA: HIE               wgn 8/20/01
~ ------------------------------------------------
~ eps protonated histidine (HIE)
CH2   CGHE  317.0 1.504
CGHE  NDHE  410.0 1.394
CHDE  NEHE  427.0 1.381
CGHE  CHDE  518.0 1.371
HENE  NEHE  434.0 1.010
~ C N double bond
CHEE  NDHE  488.0 1.335
CHEE NEHE   477.0 1.343
~ ------------------------------------------------
~ END NEW AA: HIE             wgn 8/20/01
~ ------------------------------------------------


~ ------------------------------------------------
~ ANGLE of new AA: HIE              wgn 8/20/01
~ ------------------------------------------------
~ eps protonated histidine (HIE)
~    CH2     CAH      NH    80.0    109.7 already present for HIS
CAH   CH2   CGHE  63.0  113.1
CH2   CGHE  NDHE  70.0  121.05
CH2   CGHE  CHDE  70.0  129.05
CHDE NEHE   HENE  35.0  126.35
CGHE  NDHE  CHEE  70.0  105.3
CHEE  NEHE  HENE  35.0  126.35
CHDE  CGHE  NDHE  70.0  109.9
CGHE  CHDE  NEHE  70.0  105.9
CHDE  NEHE  CHEE  70.0  107.3
NDHE  CHEE  NEHE  70.0  111.6
~ ------------------------------------------------
~ END NEW AA: HIE             wgn 8/20/01
~ ------------------------------------------------
```

```
~ ------------------------------------------------
~ TORSION of new AA: HIE            wgn 8/20/01
~ ------------------------------------------------
~ eps protonated histidine (HIE)
NH    CAH   CH2   CGHE  0.0   0.0   1.0   3     1.0
CO    CAH   CH2   CGHE  0.0   0.0   1.0   3     1.0
CAH   CH2   CGHE  NDHE  0.0   0.0   0.0   2     0.0
CAH   CH2   CGHE  CHDE  0.0   0.0   0.0   2     0.0
CH2   CGHE  NDHE  CHEE  0.0   2.4   0.0   2      -1.0
CHDE  CGHE  NDHE  CHEE  0.0   2.4   0.0   2      -1.0
CH2   CGHE  CHDE  NEHE  0.0   7.95  0.0   2      -1.0
NDHE  CGHE  CHDE  NEHE  0.0   7.95  0.0   2      -1.0
CGHE  NDHE  CHEE  NEHE  0.0  10.0   0.0   2      -1.0
CGHE  CHDE  NEHE  HENE  0.0   3.0   0.0   2      -1.0
CGHE  CHDE  NEHE  CHEE  0.0   3.0   0.0   2      -1.0
NDHE  CHEE  NEHE  CHDE  0.0   4.65  0.0   2      -1.0
NDHE  CHEE  NEHE  HENE  0.0   4.65  0.0   2      -1.0
~ ------------------------------------------------
~ END NEW AA: HIE             wgn 8/20/01
~ ------------------------------------------------


~ ------------------------------------------------
~ IMPROPER of new AA: HIE           wgn 8/20/01
~ ------------------------------------------------
~ eps protonated histidine (HIE)
CGHE  CH2   CHDE  NDHE  90.0  0.0
NEHE  CHDE  CHEE  HENE  45.0  0.0
~ ------------------------------------------------
~ END NEW AA: HIE                   wgn 8/20/01
~ ------------------------------------------------
```

References

(1) Elber, R.; Roitberg, A.; Simmerling, C.; Goldstein, R.; Li, H.; Verkhivker, G.; Keasar, C.; Zhang, J.; Ulitsky, A. *Comput. Phys. Commun.* 1994, *91*, 159-189.

(2) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc* 1988, *110*, 1666-1671.

(3) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc* 1984, *106*, 765-784.

(4) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* 1983, *4*, 187-217.

Supporting Information 2 – Time-Frequency Contour Plots of Multidimensional Vibrational Echo Data for Various T_w

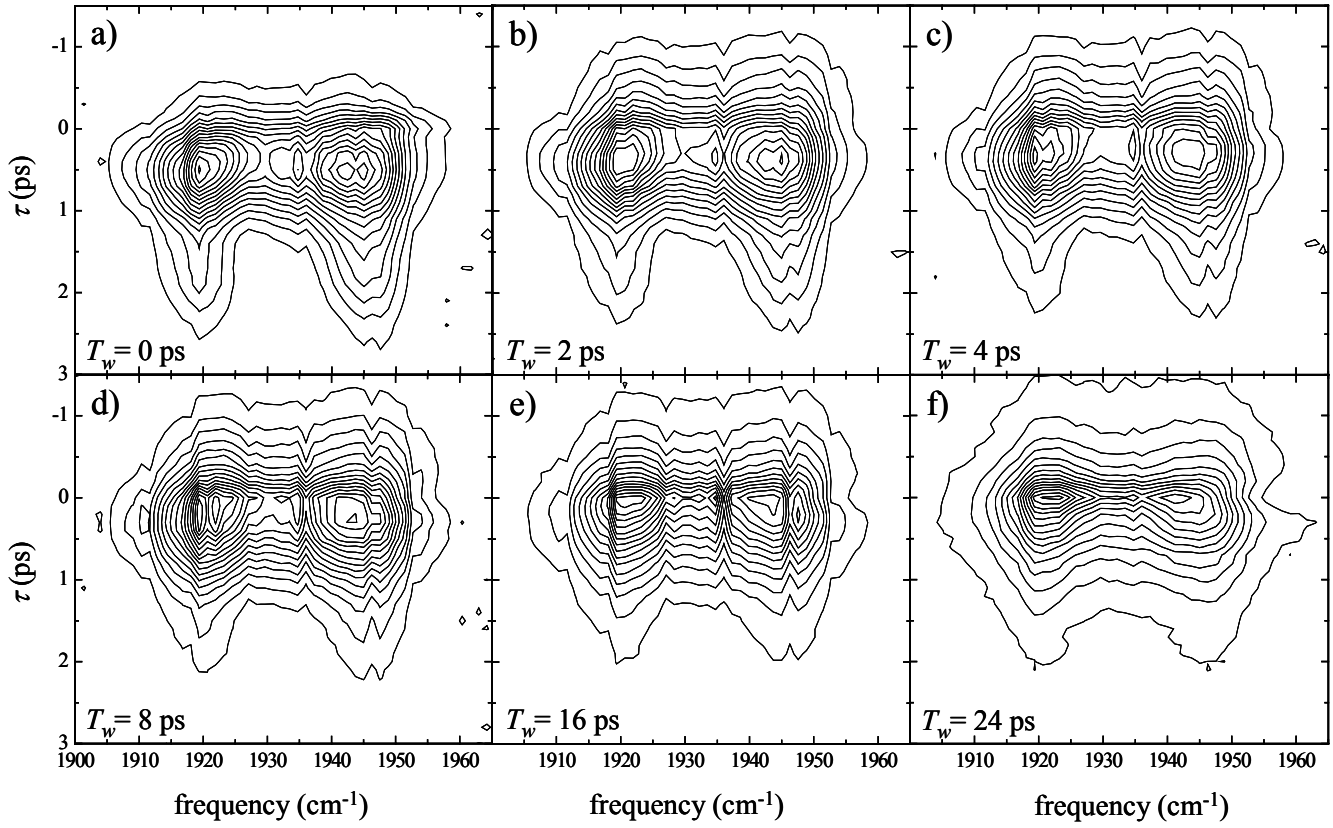

Figure S1. Contour plots of spectrally resolved stimulated vibrational echo data from MbCO at 300K for a) T_w = 0 ps. b) T_w= 2 ps. c) T_w = 4 ps. d) T_w = 8 ps. e) T_w = 16 ps. f) T_w = 24 ps. The vibrational echo decays are highly frequency dependent. As T_w is increased, the vibrational echo decays become faster because of the influence of slower and slower processes on the time evolution of the signal. The vibrational echo decays become more uniform as a function of frequency for longer values of T_w.