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The Charge of the Zinc Ion in ATCase and its Effect on Tertiary Structure

Michelle L. Dietrich
Union College - Schenectady, NY

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By

Michelle L. Dietrich

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ABSTRACT

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Metals present in enzymes serve a variety of functions. Often they are involved in enzyme catalysis or regulation of enzymatic activity. Another interesting role metals play in enzymes is a structural one. A few enzymes depend on metals for their structural stability, such as the protein ATCase. The B chain of the protein contains a zinc ion that does not have a catalytic role and is predicted to stabilize the protein structure. The goal of this research is to model the area around the zinc in the B chain and to determine what effect the zinc has on the stability of the protein. The area will be modeled using empirical energy calculations.

Empirical energy calculations require the partial charges for the zinc and the surrounding cysteine residues. Thus, before the modeling can begin, the partial charges must be determined. The partial charges are being found using MNDO in the program AMPAC. MNDO charges are found using a Mulliken population analysis. Since Mulliken charges are not considered to be accurate the MNDO charges will be converted to ab initio charges using a method describe by Merz. After the charges are found, molecular dynamics computations can be done.

Acknowledgment

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INTRODUCTION

As the structures of proteins are becoming available through crystallography and other methods, it is being discovered that many proteins contain metal ions. Metalloenzymes are interesting because the metals produce strong electrostatic interactions that not only affect the protein's catalytic mechanism but also are a major influence on the protein's tertiary structure.[1]

One metal ion that is frequently found in proteins is that of zinc. Zinc ion can have one of four roles in metalloenzymes. The categories are catalytic, structural, regulatory and noncatalytic.[2] A zinc ion that is necessary for and involved in enzymatic catalysis is considered catalytic. Removal of a catalytic zinc will result in an inactive apo-protein.

A zinc with a structural role in an enzyme is required for the protein to retain its tertiary structure and is not directly involved in any catalytic process. It is important to note that although a structural zinc is not directly needed for catalysis, the loss of structure in its apo-protein will probably affect the catalysis. Therefore structural zinc ions are responsible for the stability of the protein. Regulatory zinc ions can either regulate enzymatic activity or protein stability but are required for neither. A noncatalytic zinc is one which falls into none of the previous categories and whose purpose is as of yet unclear.

The focus of this research is to study the structural effects of the zinc ion in the protein aspartate transcarbamylase, ATCase. Another example of the ways in which zinc plays a structural role in

metalloenzymes are zinc fingers. Klug and Rhodes showed in 1987 that zinc will cause folds in the protein chain by tetrahedrally bonding to cystines and histidines.[3] The folds shown in Figure 1 will then interact with the DNA double helix.

The zinc in ATCase is required for the protein's structural stability. The protein ATCase consists of twelve chains, three each of the A, B, C, and D chains. In the protein the A chain is identical to the C chain and the B chain is identical to the D chain. This research focuses on the B chain (Figure 2), because the B chain contains the zinc tetrahedrally coordinated to the sulfurs of four cysteine residues. The goal of the research is to determine how the zinc affects the structure of the enzyme and its stability.

The zinc's affect on the stability of ATCase will be determined by comparing the energies of the native and apo-protein. The relative energies can be determined from empirical energy calculations. The energy calculations will be found using the force field AMBER in the program Macromodel. AMBER will use the atoms' partial charges and other factors to determine the energies. Therefore, before the energy calculations can be completed, the partial charges of the zinc and cystines must be determined.

Zinc ion has a formal charge of +2. However, Merz showed in 1991 that the zinc in Human Carbonic Anhydrase, HCA, did not have the expected formal charge of +2.[4] Instead, the +2 charge was spread out over the zinc's ligands. Therefore, it would be incorrect to assume the formal charge model when studying metalloenzymes. The zinc in HCA is surrounded by three histidines and a water, while

ATCase's zinc has four cysteine ligands, so it would also be incorrect to assume Merz's model for the zinc ion in ATCase.

The charge on the zinc will be found using MNDO. The program AMPAC will do the MNDO calculations to give the partial charge of the atoms. MNDO charges are found using Mulliken population analysis. A Mulliken population analysis finds the probability of finding the electron in a small volume. The charges are assigned based on that probability. However, the MNDO charge model is not considered to be very realistic. Merz used a combination of Mulliken charges and electrostatic potential charges to determine the ab initio charges for his model of Human Carbonic Anhydrase. Therefore the first object of the research will be to determine if a relationship exists between MNDO and ab initio charges. The relationship will allow us to convert our MNDO charges to the equivalent ab initio charges.

Once a relationship has been found for MNDO and ab initio charges, a series of models of the zinc pocket of ATCase will made taking into account various sizes of the zinc's adjoining cysteine residues. The charges of the atoms will be found using MNDO and converted to ab initio charges. Depending on the results, either the average charges over the models or the charges from the largest model will be taken as the accepted value.

After the partial charges are determined they will be used as parameters for the zinc ion and the cysteine residues of ATCase. Molecular dynamics calculations will then be done using Macromodel to minimize the energy of the zinc pocket. The energies will be

compared to the energies of the apo-protein to determine the effect of the zinc on the structural stability of ATCase.

Experimental:

The goal of the research was to determine the partial charges of the atoms in the zinc pocket of ATCase. The calculations were to be done using semi-empirical quantum mechanical methods found in the program AMPAC[5]. This program is be used calculates the charges using a Mulliken population analysis. The method, however, is not considered to give the most realistic charges. Therefore, the first part of the research involved finding a relationship between the semi-empirical charges calculated in AMPAC and the charges derived from ab initio methods.

Merz's paper[6] suggested that there is a linear relationship between the two types of charges. Electrostatic potential charges from ab initio calculations were given for four of the models that Merz had used to determine the charges on the zinc in Human Carbonic Anhydrase. These four models, shown in Figures 3-6, were drawn using Chem 3D, their structures minimized, and then their coordinates were made into a Cartesian coordinate file to be used in AMPAC. The AMPAC input files with the minimized geometries can be found in Appendix A. The MNDO Hamiltonian was used within the program AMPAC. AMPAC was set up to further minimize the geometry of the molecules and to determine the Mulliken partial charges of each of the atoms.

The MNDO charges for these four model compounds were then plotted against Merz's electrostatic potential charges (ESP). The charges for Merz's models and their corresponding MNDO charges are in Appendix B. Figures 7-10 show the relationship between the two

kinds of charges is linear. Although the R^2 value is not exactly one, the deviations from linearity can be partially explained because the MNDO method assumes a symmetrical geometry and charge distribution while the ab initio method does not. So, the vertical lining up of points can be ignored. The linear regression will be used to convert MNDO charges to ab initio charges.

Once the relationship between the MNDO and ab initio charges was known, the modeling of the zinc pocket in the protein ATCase could begin. Four models of the zinc pocket of ATCase were chosen. The models shown in Figures 11-14 (which will be referred to by the number of atoms they contain) are the molecules, $Zn[SHCH_3]^{+2}$ (Zn-25), $Zn[SHCH_2CH_3]^{+2}$ (Zn-37), $Zn[SHCH_2CH_3NH_2]^{+2}$ (Zn-45), and $Zn[SHCH_2CH_3COOH]^{+2}$ (Zn-49) .

The main structures of the four molecules were determined from the Protein Data Base file for ATCase[7]. The coordinates were placed in Chem 3D and the appropriate hydrogen atoms were added. The coordinates for the four models with hydrogens were taken from Chem 3D and placed in AMPAC. The AMPAC input files containing the PDB geometry are in Appendix C. The MNDO calculations were run and Mulliken charges were determined for each model.

The MNDO charges were changed to ab initio charges using the average slope from the graphs of the two large Merz models, the larger model assumed to be the most realistic. These ESP charges, however, could not be directly used in the research because the conversion of the charges from MNDO to ab initio charges changed the overall charge on the molecules. So, the molecules had to be forced back to a formal charge of +2. The charges were added

together and the amount needed to make their sum +2 was determined. The excess charge was then distributed over the whole molecule so that the formal charge of the models was +2.

RESULTS

The calculated charges of Merz's models using the MNDO method are in Appendix B. The equation for the conversion of MNDO charges was determined by averaging the slopes of Merz's two large models (Figures 5-6). The linear regressions of the the two plots (Figures 9-10) were respectively:

ESP Charge = 1.2898*(MNDO charge)

ESP Charge = 1.2171*(MNDO charge)

The y-intercept of the graphs was neglible and was therefore set equal to zero for the calculations. The average of the two linear regressions was used for the formula of charge conversions which was:

ESP Charge = 1.2535*(MNDO charge)

The MNDO charges for each of the ATCase models were then converted to ESP charges using the above formula. However, since the conversion of the charges from MNDO to ESP charges changes the overall charge on the molecule, the charges for each model had to be adjusted in order for them to retain a formal charge of +2. The difference between the +2 formal charge and the overall ESP charge on the model was calculated. That difference was divided by the total number of atoms in the molecule to give the excess charge per atom. This excess charge was then distributed over each atom, by addition, so that the formal charge of the molecule would remain equal to +2. The MNDO, ESP and adjusted ESP charges for each of the models (Figures 11-14) are Tables i-iv, Appendix D.

DISCUSSION

The charge of the zinc ion in ATCase was determined to be 0.673 by averaging the values from each of the models in Appendix D. The new charge for the zinc ion can now be be used in the force field AMBER to do empirical energy calculations on the protein. However, if the charge on the zinc is 0.678, there is still a charge of 1.32 which remains to be accounted for, because the formal charge of the zinc pocket must is +2.

Average Charge on the Atoms in the Zinc Pocket

Atom	Charge
Zn	0.673
S	-0.035
C_β	0.021
C_α	0.025

Assuming that the excess charge is distributed equally over each of the zinc's four adjacent cysteine residues, each residue should have a net charge of 0.327. The charge on the sulfur is slightly negative according to data therefore the excess charge should be placed on the α and β carbons. The charges that were determined for the C_α and C_β show that they should be about equal in partial charge.

Since it would be necessary to change the force field AMBER to reflect the new charge on the zinc ion for ATCase, it is also necessary to create a new set of parameters for cysteine. The parameters of cysteine must be changed to reflect the charge that is distributed over the residue by the zinc ion.

Partial Charge for Cysteine in ATCase	
S	-0.035
C_β	0.185
C_α	0.179

The charges for the alpha and the beta carbons are determined by taking the initial partial charge for each atom and adding half of the excess charge, 0.164, to C_β and C_α. The new partial charges for cysteine and the zinc are now ready to be entered into AMBER and to be used in empirical energy calculations of the protein.

The partial charges of the zinc ion and the cysteine residue were calculated using the MNDO method because of problems that arose while trying to use the preferable ab initio method. Originally the research's goal was to use the programs Gaussian 80[8] and CHELP[9] to determine the partial charges for the zinc pocket. Gaussian 80 did find the parameters that were needed for CHELP to determine the partial charges. CHELP, however, was unable to determine the partial charge of atoms for our models. After spending a great deal of time trying to make CHELP work, it was decided to use the relationship between ab initio charges and MNDO charges that was indicated in Merz's paper.[10] The relationship has given reasonable charges for the zinc pocket and can be used in setting up the force field AMBER for ATCase.

End Notes

[1]Merz, K., Hoops, S. and Anderson, K. *Journal of the American Chemical Society*, **1991**, 113, 8262.

[2]Vallee, B. *Zinc Enzymes*, Ed. Spiro, T.G., Krieger Publishing Company: Malabar, **1991**.

[3]Klug, A. and Rhodes,D. *Trends in Biochemical Science*, **1987**, *12*, 464.

[4]Merz, K., Hoops, S. and Anderson, K. *Journal of the American Chemical Society*, **1991**, 113, 8262.

[5]Liotard, D., Healy, J., Rulz, J., Dewer,M. *AMPAC*, Version 2.1, University of Texas at Austin, **1989**.

[6]Merz, K., Hoops, S. and Anderson, K. *Journal of the American Chemical Society*, **1991**, 113, 8262.

[7]Brookhaven National Laboratory, Associated Universities INC.,Long Island, New York.

[8]*Guassian 80 UCSF*, QCPE 446.

[9]*CHELP*, QCPE 524.

[10]Merz, K., Hoops, S. and Anderson, K. *Journal of the American Chemical Society*, **1991**, 113, 8262.

Figures

Figure 1

Interaction of Zinc fingers with the Double Helix

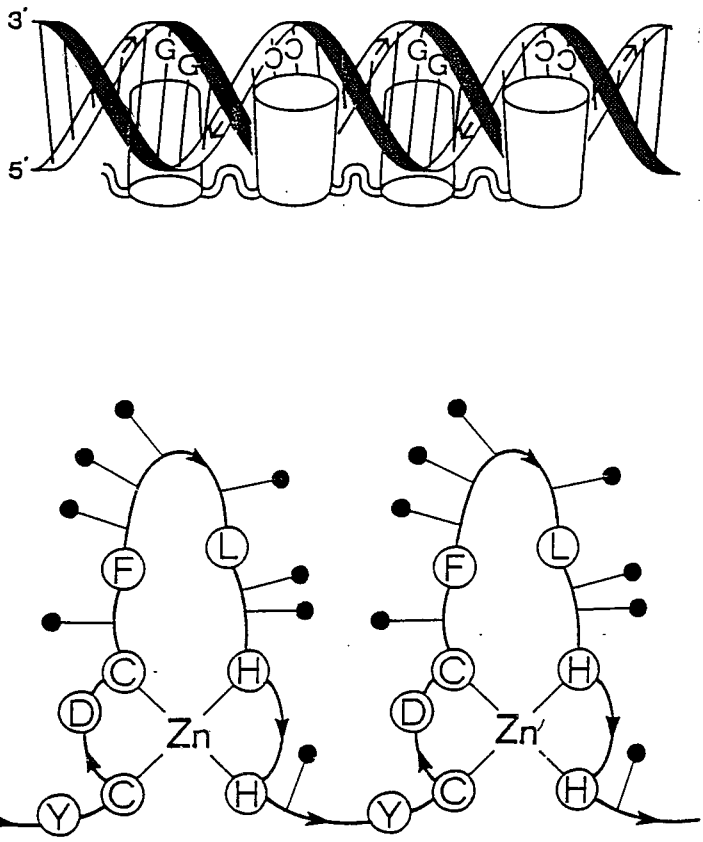


Fig. 2. Schematic folding scheme for a linear arrangement of repeated domains, each centred on a tetrahedral arrangement of Zn ligands. Ringed residues are the conserved amino acids which include the Cys and His Zn ligands, the negatively charged Asp11, and the three hydrophobic groups that may form a structural core. Black circles mark the most probable DNA-binding side chains. (Figure taken from Ref. 4.)

Klug, Aaron and Daniela Rhodes. "Trends in Biochemical Science" 12:464-469.

Figure 2

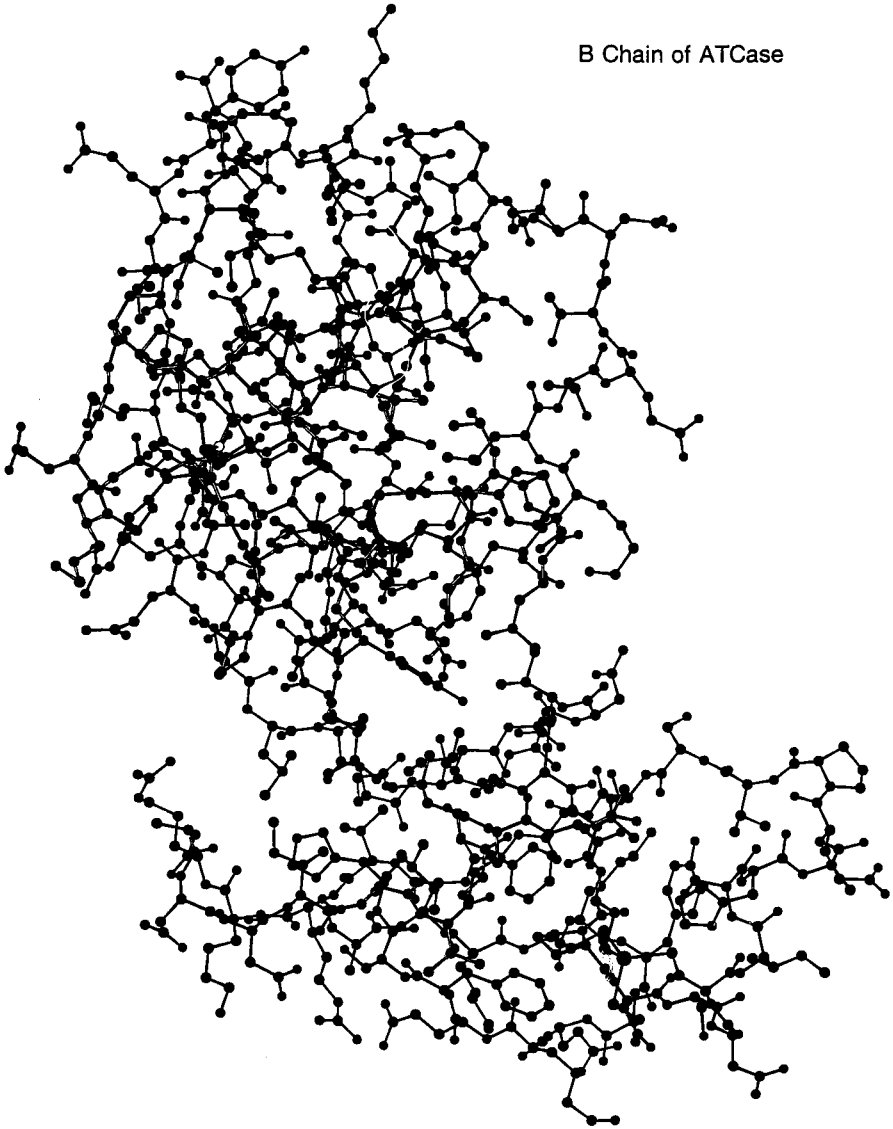


Figure 3

Merz's Small Molecule with H2O Ligand

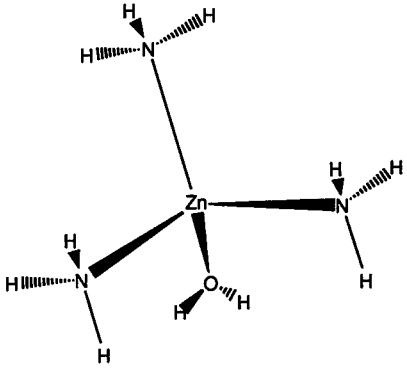


Figure 4

Merz's Small Molecule with OH Ligand

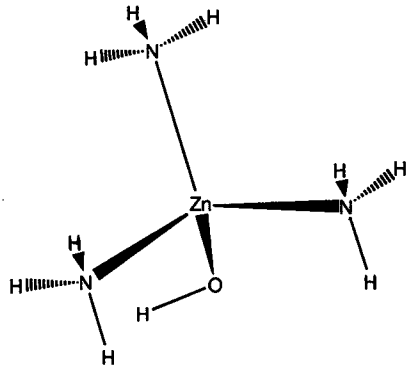


Figure 5

Merz's Large Molecule with H20 Ligand

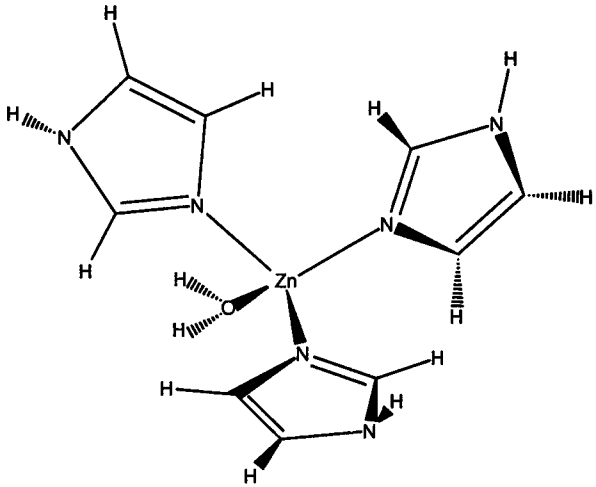


Figure 6

Merz's Large Molecule with OH Ligand

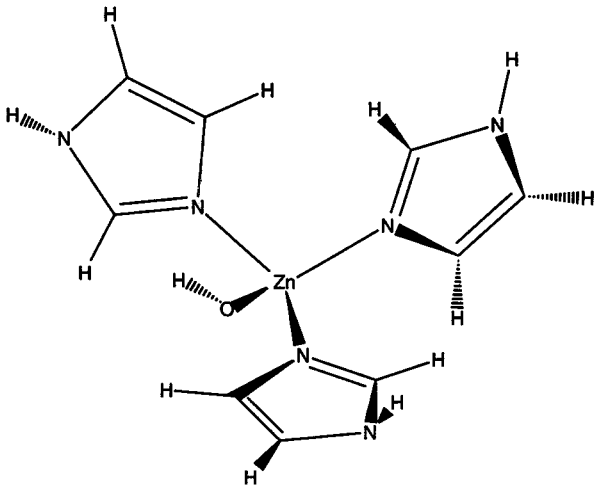


Figure 7

ESP vs. MNDO charges for Merz's Small Molecule with H20 Ligand

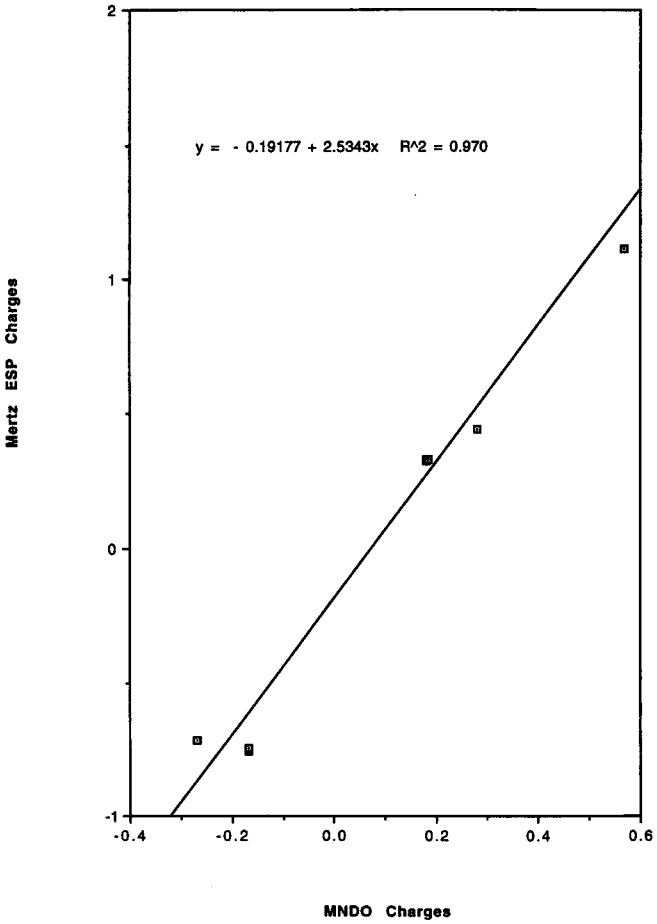


Figure 8

ESP vs. MNDO Charges for Merz's Small Molecule with OH Ligand

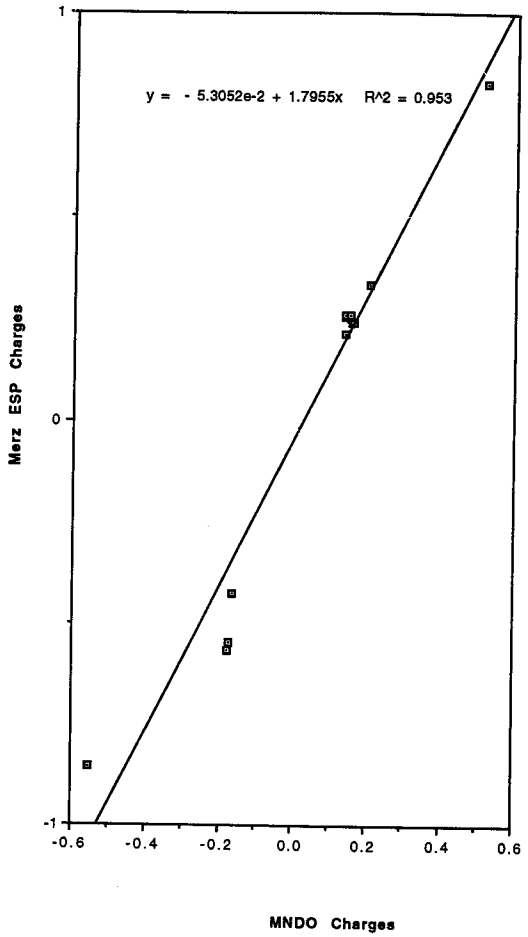


Figure 9

ESP vs. MNDO Charges for Merz Large Molecule with H2O Ligand

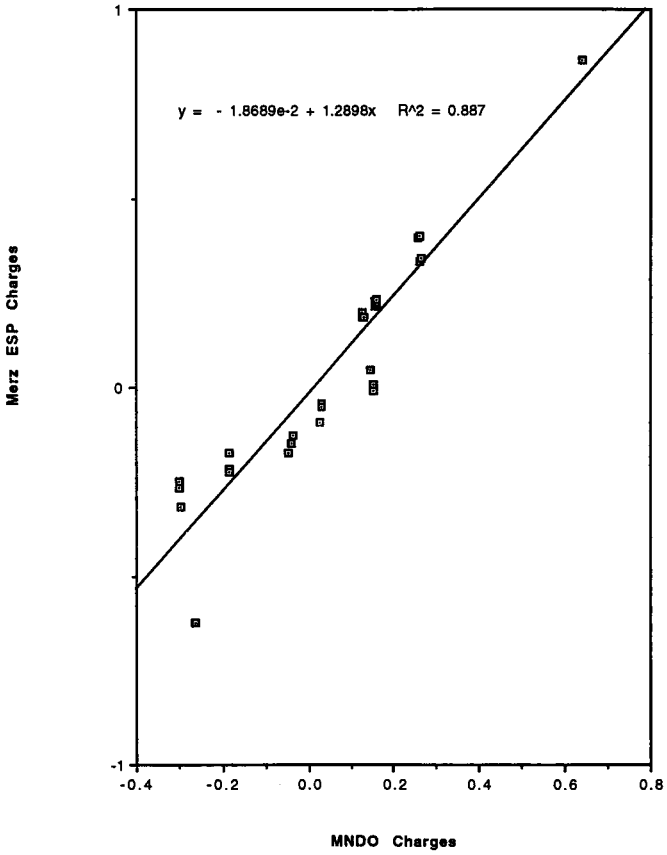


Figure 10

ESP vs. MNDO Charges for Merz Large Molecule with OH Ligand

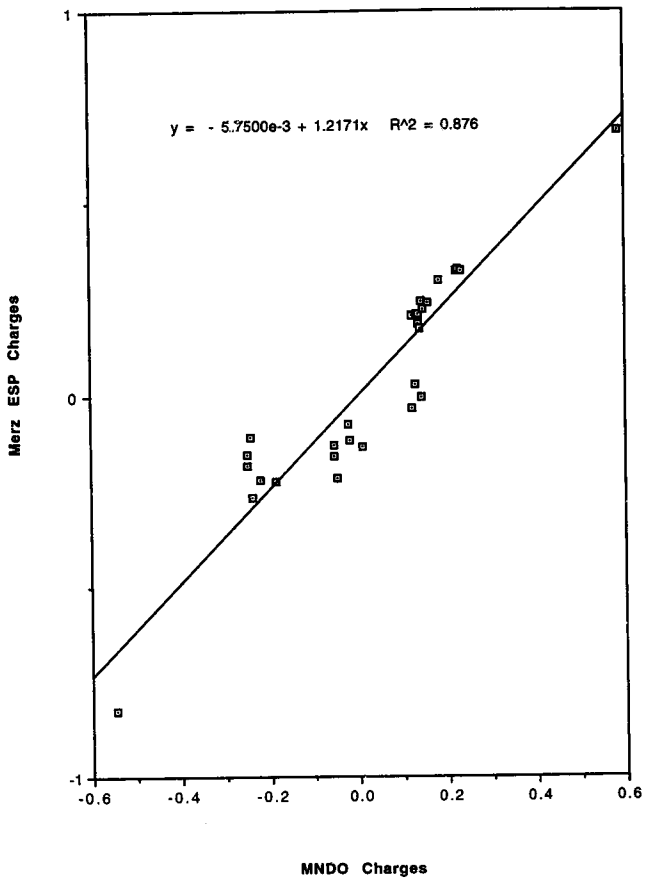
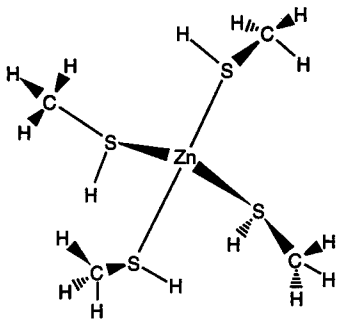


Figure 11

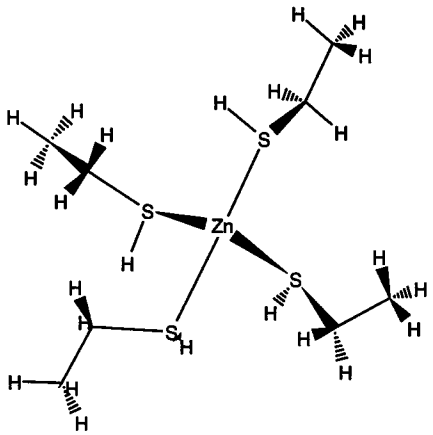
$Zn[SHCH_3]^{+2}$



Zn-25

Figure 12

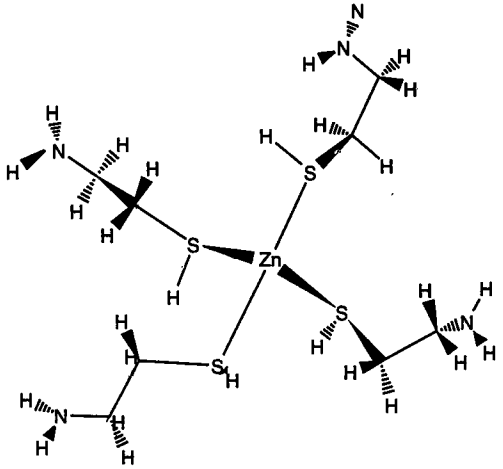
$Zn[SHCH_2CH_3]^{+2}$



Zn-37

Figure 13

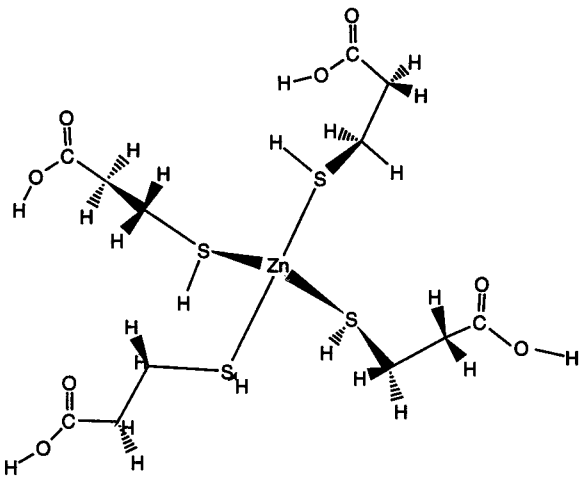
$Zn[SHCH_2CH_3NH_2]^{+2}$



Zn-45

Figure 14

$Zn[SHCH_2CH_3COOH]^{+2}$



Zn-49

Appendix A

TABLE 1
AMPAC INPUT FILE FOR FIGURE 3
MERZ SMALL MOLECULE WITH H_2O LIGAND

```
T=600M XYZ CHARGE=2
     GEOMETRY OPTIMIZATION AND CHARGE DETERMINATION
       FOR MERZ'S SMALL MOLECULE WITH H2O LIGAND
 30   -0.009521  1   0.066788  1   0.012543  1
  7    0.975952  1  -1.743210  1   0.024185  1
  7    1.366180  1   1.601425  1   0.009949  1
  7   -1.194321  1   0.214630  1   1.692383  1
  8   -1.163223  1   0.190674  1  -1.647064  1
  1    0.327881  1  -2.530746  1   0.025848  1
  1    1.571472  1  -1.849411  1   0.845474  1
  1    1.575821  1  -1.856812  1  -0.792908  1
  1    0.916931  1   2.517151  1   0.009598  1
  1    1.972626  1   1.570145  1  -0.809540  1
  1    1.973587  1   1.571487  1   0.828751  1
  1   -1.701416  1   1.098969  1   1.726059  1
  1   -0.647644  1   0.155700  1   2.551544  1
  1   -1.892426  1  -0.527725  1   1.734940  1
  1   -1.787384  1  -0.514847  1  -1.649185  1
  1   -0.635406  1   0.023163  1  -2.409225  1
```

TABLE 2
AMPAC INPUT FILE FOR FIGURE 4
MERZ SMALL MOLECULE WITH OH LIGAND

```
T=600M XYZ CHARGE=1
      GEOMETRY OPTIMIZATION AND CHARGE DETERMINATION
        FOR MERZ'S SMALL MOLECULE WITH OH LIGAND
 30     -0.196274 1 -0.328079 1 -0.042694 1
  7      1.716858 1 -0.332214 1  0.946213 1
  7     -0.794235 1  1.711777 1 -0.501282 1
  7     -1.698400 1 -1.294510 1  1.147827 1
  8     -0.011688 1 -1.347290 1 -1.950073 1
  1      2.042328 1 -1.311920 1  1.106247 1
  1      1.643097 1  0.140594 1  1.872726 1
  1      2.428604 1  0.161835 1  0.370483 1
  1     -1.602585 1  1.646561 1 -1.171539 1
  1     -0.009369 1  2.166824 1 -1.013931 1
  1     -1.053925 1  2.210693 1  0.336243 1
  1     -2.599945 1 -1.335968 1  0.642700 1
  1     -1.836365 1 -0.775711 1  2.040939 1
  1     -1.407547 1 -2.270248 1  1.392670 1
  1     -0.938766 1 -1.714706 1 -2.174591 1
```

TABLE 3
AMPAC INPUT FILE FOR FIGURE 5
MERZ LARGE MOLECULE WITH H_2O LIGAND

```
T=600M XYZ CHARGE=2
     GEOMETRY OPTIMIZATION AND CHARGE DETERMINATION
       FOR MERZ'S LARGE MOLECULE WITH H2O LIGAND
30              -0.018  1       -0.538  1       -0.066  1
7                1.140  1       -1.563  1       -1.056  1
8               -0.946  1       -1.600  1        1.051  1
7               -1.171  1        0.319  1       -1.210  1
7                0.905  1        0.716  1        0.906  1
6                1.043  1       -1.845  1       -2.325  1
7                2.142  1       -2.700  1       -2.767  1
1               -1.513  1       -2.222  1        0.525  1
6                2.960  1       -2.920  1       -1.583  1
6               -0.986  1        1.471  1       -1.790  1
7               -2.119  1        1.825  1       -2.642  1
6                1.287  1        0.625  1        2.149  1
7                1.980  1        1.835  1        2.583  1
6                1.981  1        2.718  1        1.424  1
6                1.356  1        2.067  1        0.440  1
6               -3.058  1        0.718  1       -2.517  1
6               -2.509  1       -0.164  1       -1.679  1
6                2.379  1       -2.258  1       -0.579  1
1               -1.521  1       -1.044  1        1.637  1
1                0.247  1       -1.506  1       -3.004  1
1                1.977  1       -3.470  1       -3.458  1
1                3.875  1       -3.532  1       -1.587  1
1               -0.103  1        2.117  1       -1.681  1
1               -2.457  1        2.815  1       -2.704  1
1                1.118  1       -0.232  1        2.817  1
1                1.841  1        2.217  1        3.549  1
1                2.431  1        3.722  1        1.429  1
1                1.178  1        2.443  1       -0.578  1
1               -4.022  1        0.683  1       -3.045  1
1               -2.950  1       -1.119  1       -1.360  1
1                2.735  1       -2.211  1        0.460  1
```

TABLE 4
AMPAC INPUT FILE FOR FIGURE 6
MERZ LARGE MOLECULE WITH OH LIGAND

T=600M XYZ CHARGE=1
GEOMETRY OPTIMIZATION AND CHARGE DETERMINATION
FOR MERZ'S LARGE MOLECULE WITH H_2O LIGAND

30	-0.331	1	-0.986	1	0.362	
7	0.835	1	-2.063	1	-0.562	1
8	-1.312	1	-1.992	1	1.487	1
7	-1.442	1	-0.153	1	-0.837	1
7	0.591	1	0.275	1	1.326	1
6	0.723	1	-2.500	1	-1.784	1
7	1.758	1	-3.313	1	-2.232	1
6	2.590	1	-3.358	1	-1.127	1
6	-1.245	1	0.985	1	-1.442	1
7	-2.353	1	1.311	1	-2.336	1
7	-1.442	1	-0.153	1	-0.837	1
7	0.591	1	0.275	1	1.326	1
6	0.723	1	-2.500	1	-1.784	1
7	1.758	1	-3.313	1	-2.232	1
6	2.590	1	-3.358	1	-1.127	1
6	-1.245	1	0.985	1	-1.442	1
7	-2.353	1	1.311	1	-2.336	1
6	0.970	1	0.187	1	2.570	1
7	1.668	1	1.395	1	3.000	1
6	1.677	1	2.273	1	1.838	1
6	1.049	1	1.622	1	0.856	1
6	-3.292	1	0.203	1	-2.210	1
6	-2.764	1	-0.654	1	-1.334	1
6	2.119	1	-2.649	1	-0.108	1
1	-1.655	1	-1.449	1	2.176	1
1	-0.146	1	-2.236	1	-2.405	1
1	2.240	1	-2.852	1	-3.003	1
1	3.534	1	-3.922	1	-1.107	1
1	-0.368	1	1.638	1	-1.325	1
1	-2.694	1	2.296	1	-2.435	1
1	0.797	1	-0.668	1	3.240	1
1	1.525	1	1.782	1	3.964	1
1	2.132	1	3.274	1	1.841	1
1	0.873	1	1.996	1	-0.163	1
1	-4.240	1	0.147	1	-2.765	1
1	-3.209	1	-1.604	1	-1.004	1
1	2.567	1	-2.505	1	0.886	1

Appendix B

TABLE 5
CHARGES FROM FIGURE 7
MERZ SMALL MOLECULE WITH H_2O LIGAND

ATOM	MNDO CHARGES	MERZ'S ESP CHARGES
Zn	0.5693	1.1133
N	-0.1676	-0.7567
N	-0.1675	-0.7450
N	-0.1678	-0.7446
O	-0.2695	-0.7176
H	0.1822	0.3336
H	0.1819	0.3256
H	0.1825	0.3329
H	0.1832	0.3304
H	0.1823	0.3235
H	0.1828	0.3315
H	0.1832	0.3316
H	0.1826	0.3234
H	0.1819	0.3301
H	0.2800	0.3440
H	0.2804	0.3439

TABLE 6
CHARGES FROM FIGURE 8
MERZ SMALL MOLECULE WITH OH LIGAND

ATOM	MNDO CHARGES	MERZ'S ESP CHARGES
Zn	0.5200	0.8288
N	-0.1638	-0.4267
N	-0.1725	-0.5494
N	-0.1752	-0.5677
O	-0.5519	-0.8570
H	0.1590	0.2394
H	0.1388	0.2128
H	0.1566	0.2414
H	0.1532	0.2582
H	0.1527	0.2523
H	0.1375	0.2548
H	0.1518	0.2590
H	0.2388	0.2586
H	0.2531	0.2606
H	0.2020	0.3348

TABLE 7
CHARGES FROM FIGURE 9
MERZ LARGE MOLECULE WITH H_2O LIGAND

ATOM	MNDO CHARGES	MERZ'S ESP CHARGES
Zn	0.6417	0.8663
N	-0.3007	-0.2633
O	-0.2624	-0.6197
N	-0.3012	-0.2445
N	-0.2963	-0.3138
C	0.1525	-0.0075
N	-0.1855	-0.1725
H	0.2601	0.4013
C	0.0285	-0.0900
C	0.1533	0.0105
N	-0.1862	-0.2107
C	0.1452	0.0485
C	-0.1834	-0.2215
C	0.0300	-0.0421
C	-0.0385	-0.1455
C	0.0317	-0.0430
H	-0.0455	-0.1703
H	-0.0371	-0.1230
H	0.2579	0.3995
H	0.1622	0.2341
H	0.2628	0.3300
H	0.1584	0.3000
H	0.1617	0.2263
H	0.2635	0.3432
H	0.1586	0.2207
H	0.2634	0.3446
H	0.1586	0.2173
H	0.1282	0.1885
H	0.1593	0.2229
H	0.1286	0.2016
H	0.1306	0.1857

TABLE 8
CHARGES FROM FIGURE 10
MERZ LARGE MOLECULE WITH OH LIGAND

ATOM	MNDO CHARGES	MERZ'S ESP CHARGES
Zn	0.5869	0.6886
N	-0.2100	-0.1853e
O	-0.5466	-0.8242
N	-0.2419	-0.1082
N	-0.2508	-0.1576
C	0.1430	-0.0048
N	-0.1871	-0.2252
C	-0.0544	-0.1308
C	0.1205	-0.0336
N	-0.2200	-0.2241
C	0.1300	0.0288
N	-0.2403	-0.2689
C	-0.2030	-0.0752
C	-0.0458	-0.2177
C	0.0095	-0.1346
C	-0.0541	-0.1596
C	-0.0170	-0.1190
H	0.1817	0.2975
H	0.1470	0.2215
H	0.2212	0.3202
H	0.1312	0.2092
H	0.1420	0.2427
H	0.2314	0.3215
H	0.1569	0.2396
H	0.2261	0.3276
H	0.1344	0.1976
H	0.1202	0.2073
H	0.1349	0.2097
H	0.1358	0.1848
H	0.1406	0.1730

Appendix C

TABLE 9
AMPAC INPUT FILE FOR ZN-25

```
SYMMETRY CHARGE=2 T=60M
          INTERNAL COORDINATE FILE FOR ZN-25
      MNDO CALCULATION OF SMALL ZN POCKET GEOMETRY AND CHARGES
   C     0.000000  0     0.000000  0     0.000000  0   0  0  0
   S     1.79747   1     0.000000  0     0.000000  0   1  0  0
  ZN     2.32448   1   101.5790    1     0.000000  0   2  1  0
   S     2.33763   1   112.3709    1    61.2370    1   3  2  1
   C     1.79146   1    96.9472    1    53.7742    1   4  3  2
   S     2.34679   1   112.1191    1   -62.498     1   3  2  1
   C     1.79953   1    92.4709    1   -68.3640    1   6  3  2
   S     2.31818   1   105.6146    1   179.48      1   3  2  1
   C     1.80774   1   110.6574    1  -151.589     1   8  3  2
   H     1.098     1   109.3       1   180.000     1   1  2  3
   H     1.098     1   109.3       1    60.000     1   1  2  3
   H     1.098     1   109.3       1   -60.000     1   1  2  3
   H     1.34442   1    18.7704    1   -13.0208    1   2  3  4
   H     1.098     1   109.3       1   180.000     1   5  4  3
   H     1.098     1   109.3       1    60.000     1   5  4  3
   H     1.098     1   109.3       1   -60.000     1   5  4  3
   H     1.3444    1    34.5792    1   -36.7269    1   4  3  2
   H     1.098     1   109.3       1   180.000     1   7  6  3
   H     1.098     1   109.3       1    60.000     1   7  6  3
   H     1.098     1   109.3       1   -60.000     1   7  6  3
   H     1.3444    1     3.8105    1    89.5513    1   6  3  2
   H     1.098     1   109.3       1   180.000     1   9  8  3
   H     1.098     1   109.3       1    60.000     1   9  8  3
   H     1.098     1   109.3       1   -60.000     1   9  8  3
   H     1.3444    1    81.4913    1   -58.3483    1   8  3  2
   0     0.000000  0     0.000000  0     0.000000  0   0  0  0
2,1,4,6,8
2,2,4,6,8
1,1,5,7,9
1,2,5,7,9
10,1,14,18,22
10,2,14,18,22
11,1,12,13,15,16,17,19,20,21,23,24,25
11,2,12,13,15,16,17,19,20,21,23,24,25
```

TABLE 10
AMPAC INPUT FILE FOR ZN-37

```
CHARGE=2 XYZ T=600M
        CARTESIAN COORDINATE FILE FOR ZN-37
    MNDO CALCULATION OF SMALL ZN POCKET GEOMETRY AND CHARGES
16       2.123184  1     0.317154  1     0.038712  1
 6       2.123184  1     2.107100  1     0.038712  1
30      -0.068359  1    -0.457657  1     0.038712  1
 6       3.487686  1     2.614761  1     0.485855  1
16      -0.125244  1    -0.439056  1    -2.650665  1
 6       0.998000  1     0.813049  1    -3.262604  1
 6       0.874863  1     0.908844  1    -4.777649  1
16      -1.236084  1     0.194214  1     1.967133  1
 6      -0.561737  1    -0.657822  1     3.389542  1
 6      -1.342896  1    -0.262207  1     4.635696  1
16       0.087524  1    -2.770523  1     0.018478  1
 6      -1.322784  1    -3.456070  1    -0.844711  1
 6      -2.383179  1    -3.872696  1     0.165955  1
 1       2.756943  1    -0.130981  1    -1.058975  1
 1       1.907593  1     2.478081  1    -0.988251  1
 1       1.341614  1     2.478088  1     0.738876  1
 1       3.487686  1     3.727768  1     0.485840  1
 1       4.269257  1     2.243759  1    -0.214294  1
 1       3.703278  1     2.243790  1     1.512833  1
 1      -1.977554  1    -0.033295  1    -3.274200  1
 1       2.042206  1     0.539000  1    -2.991867  1
 1       0.742233  1     1.795670  1    -2.806717  1
 1       1.573288  1     1.687424  1    -5.158157  1
 1      -0.169342  1     1.182877  1    -5.048386  1
 1       1.130630  1    -0.073746  1    -5.233536  1
 1      -1.111404  1     1.521683  1     2.139267  1
 1      -0.641006  1    -1.757248  1     3.235458  1
 1       0.507706  1    -0.377319  1     3.517441  1
 1      -0.923584  1    -0.792038  1     5.520142  1
 1      -2.412338  1    -0.542679  1     4.507797  1
 1      -1.263626  1     0.837219  1     4.789810  1
 1       1.219269  1    -3.138168  1    -0.608765  1
 1      -1.001633  1    -4.344559  1    -1.433044  1
 1      -1.745895  1    -2.689377  1    -1.531616  1
 1      -3.260086  1    -4.298950  1    -0.370789  1
 1      -2.704330  1    -2.984207  1     0.754333  1
 1      -1.960068  1    -4.639389  1     0.852859  1
```

TBALE 11
AMPAC INPUT FILE FOR ZN-45

```
CHARGE=2 T=600M XYZ
          CARTESIAN COORDINATE FILE FOR ZN-45
     MNDO CALCULATION OF SMALL ZN POCKET GEOMETRY AND CHARGES
  S     1.433426 1    0.700485  1 -0.011826   1
  C     1.433426 1    1.595520  1  1.538345   1
  C     2.869308 1    1.849365  1  1.977997   1
  N     2.871033 1    2.469070  1  3.288849   1
  H     1.693832 1    1.550659  1 -1.020157   1
  S    -1.540741 1    1.929184  1 -1.594528   1
  C    -0.663910 1    3.361771  1 -0.975861   1
  C    -1.252731 1    4.623184  1 -1.593506   1
  N    -0.179871 1    5.522003  1 -1.972397   1
  H    -3.495026 1    2.145523  1 -1.250916   1
  S    -1.158081 1   -1.854065  1  1.321121   1
  C    -0.074219 1   -3.268723  1  1.154007   1
  C    -0.428300 1   -4.307129  1  2.210266   1
  N     0.533035 1   -5.391602  1  2.163315   1
  H    -0.986008 1   -1.298386  1  2.533051   1
  S    -0.507156 1   -1.375839  1 -2.378357   1
  C    -2.116150 1   -1.388809  1 -3.162476   1
  C    -2.808900 1   -2.714890  1 -2.877930   1
  N    -3.139938 1   -2.661500  1 -1.467270   1
  H     0.372818 1   -0.738083  1 -3.170990   1
 Zn    -0.649078 1   -0.278076  1 -0.341537   1
  H    -1.447968 1   -4.706253  1  2.011032   1
  H    -0.405411 1   -3.834427  1  3.217575   1
  H     0.981827 1   -2.945221  1  1.291153   1
  H    -0.197495 1   -3.712936  1  0.141022   1
  H     3.366959 1    2.525375  1  1.247300   1
  H     3.419632 1    0.883087  1  2.023697   1
  H     0.908737 1    0.994064  1  2.313965   1
  H     0.908768 1    2.568008  1  1.405258   1
  H    -1.994293 1   -1.262161  1 -4.261475   1
  H    -2.732254 1   -0.554276  1 -2.759109   1
  H    -3.746826 1   -2.777512  1 -3.473755   1
  H    -2.095932 1   -3.550522  1 -3.057098   1
  H     0.412674 1    3.283508  1 -1.247100   1
  H    -0.764800 1    3.408966  1  0.131531   1
  H    -1.845932 1    4.352585  1 -2.495500   1
  H    -1.913391 1    5.125702  1 -0.852188   1
  H     3.832626 1    2.639100  1  3.583313   1
  H     2.505905 1    1.810440  1  3.976746   1
  H    -0.574173 1    6.366852  1 -2.386078   1
  H     0.367050 1    5.101135  1 -2.723480   1
  H     0.295929 1   -6.087036  1  2.870743   1
  H     1.450500 1   -5.044952  1  2.443329   1
  H    -3.612198 1   -3.523819  1 -1.195862   1
  H    -3.832626 1   -1.929900  1 -1.308014   1
```

TABLE 12
AMPAC INPUT FILE FOR ZN-49

```
CHARGE=2 T=600M XYZ
          CARTESIAN COORDINATE FILE FOR ZN-49
     MNDO CALCULATION OF SMALL ZN POCKET GEOMETRY AND CHARGES
 16    2.460373 1   1.555191 1   1.630783 1
  6    2.884644 1   3.294006 1   1.646164 1
  6    2.541916 1   3.886749 1   2.997971 1
  6    3.029861 1   2.991318 1   4.146332 1
  8    2.139893 1   2.778687 1   5.340027 1
  8    4.203278 1   2.430511 1   4.103012 1
 16    2.396072 1   0.930191 1  -2.291580 1
  6    4.079681 1   0.337143 1  -2.435028 1
  6    4.152481 1  -0.705017 1  -3.543259 1
  6    4.141235 1  -0.025482 1  -4.919876 1
  8    5.170197 1   1.001297 1  -5.283752 1
  8    3.218979 1  -0.345428 1  -5.792114 1
 16   -0.962463 1   1.776840 1  -0.360153 1
  6   -2.192169 1   1.537949 1   0.918518 1
  6   -3.443939 1   2.331543 1   0.571854 1
  6   -4.135513 1   1.686676 1  -0.644135 1
  8   -4.658417 1   0.284088 1  -0.547958 1
  8   -4.264084 1   2.359772 1  -1.748428 1
 16    0.804398 1  -1.737183 1   0.167419 1
  6    0.537567 1  -2.834671 1  -1.219467 1
  6   -0.943909 1  -2.939270 1  -1.518219 1
  6   -1.740021 1  -3.353958 1  -0.274048 1
  8   -1.489044 1  -4.673676 1   0.394150 1
  8   -2.659912 1  -2.557724 1   0.204865 1
 30    1.175888 1   0.636337 1  -0.211456 1
  1    2.848709 1   0.778473 1   2.613174 1
  1    2.301605 1   3.827591 1   0.847168 1
  1    3.970108 1   3.415405 1   1.452774 1
  1    1.437302 1   4.023117 1   3.085541 1
  1    3.028290 1   4.893494 1   3.097198 1
  1    2.596298 1   3.157425 1   6.167480 1
  1    1.825394 1   1.529877 1  -3.306931 1
  1    4.391998 1  -0.126968 1  -1.469757 1
  1    4.755630 1   1.183900 1  -2.675598 1
  1    3.262970 1  -1.380829 1  -3.463654 1
  1    5.076050 1  -1.313431 1  -3.425629 1
  1    4.904648 1   1.447433 1  -6.165649 1
  1   -1.213333 1   2.572739 1  -1.369934 1
  1   -1.793442 1   1.885406 1   1.895065 1
  1   -2.450851 1   0.454941 1   0.984146 1
  1   -3.169159 1   3.381210 1   0.335007 1
  1   -4.141327 1   2.318314 1   1.442000 1
  1   -5.169785 1   0.051727 1  -1.398956 1
  1    0.790909 1  -2.210571 1   1.389526 1
  1    0.940292 1  -3.845078 1  -0.974228 1
  1    1.072754 1  -2.432495 1  -2.119629 1
  1   -1.096695 1  -3.716949 1  -2.316177 1
  1   -1.324371 1  -1.966522 1  -1.904495 1
```

Appendix D

TABLE 13
Conversion of MNDO Charges to ab initio Charges for ZN-25

	CHARGES		
ATOM	**MNDO**	**ESP**	**Adjusted ESP**
Zn	0.5526	0.6927	0.6724
S-1	-0.0384	-0.0481	-0.0684
C-2 (S-1)	0.0545	0.0683	0.0480
S-3	0.0007	0.0009	-0.0194
C-4 (S-3)	0.0395	0.0495	0.0292
S-5	0.0432	0.0541	0.0339
C-6 (S-5)	0.0332	0.0416	0.0213
S-7	0.0327	0.0410	0.0207
C-8 (S-7)	0.0375	0.0470	0.0267
H (S-1)	0.1308	0.1640	0.1437
H (C-2)	0.0797	0.0999	0.0796
H (C-2)	0.0602	0.0755	0.0552
H (C-2)	0.0465	0.0583	0.0380
H (S-3)	0.1197	0.1500	0.1298
H (C-4)	0.0821	0.1029	0.0826
H (C-4)	0.0614	0.0770	0.0567
H (C-4)	0.0460	0.0577	0.0374
H (S-5)	0.1094	0.1371	0.1169
H (C-6)	0.0808	0.1013	0.0810
H (C-6)	0.0476	0.0597	0.0394
H (C-6)	0.0697	0.0874	0.0671
H (S-7)	0.1123	0.1408	0.1205
H (C-8)	0.0793	0.0994	0.0791
H (C-8)	0.0472	0.0592	0.0389
H (C-8)	0.0718	0.0900	0.0697
Total Charge	2.0000	2.5069	2.0000

Table 14
Conversion of MNDO Charges to ab initio Charges
for ZN-37

	CHARGES		
ATOM	MNDO	ESP	Adjusted ESP
Zn	0.5423	0.6797	0.6660
S-1	-0.0035	-0.0044	-0.0181
C-2 (S-1)	0.0118	0.0148	0.0011
C-3 (C-2)	-0.0033	-0.0041	-0.0178
S-4	-0.0552	-0.0692	-0.0829
C-5 (S-4)	0.0289	0.0362	0.0225
C-6 (C-5)	-0.0018	-0.0023	-0.0160
S-7	0.0502	0.0629	0.0492
C-8(S-7)	0.0010	0.0013	-0.0124
C -9(C-8)	-0.0001	-0.0001	-0.0138
S-10	0.0421	0.0528	0.0391
C-11 (S-10)	0.0020	0.0025	-0.0112
C-12 (C-11)	0.0084	0.0105	-0.0032
H (S-1)	0.1124	0.1409	0.1272
H (C-2)	0.0468	0.0587	0.0450
H (C-12)	0.0578	0.0724	0.0587
H (C-3)	0.0617	0.0773	0.0636
H (C-3)	0.0289	0.0362	0.0225
H (C-3)	0.0391	0.0490	0.0353
H (S-4)	0.1322	0.1657	0.1520
H (C-5)	0.0476	0.0597	0.0460
H (C-5)	0.0594	0.0745	0.0608
H (C-6)	0.0605	0.0758	0.0621
H (C-6)	0.0338	0.0424	0.0287
H (C-6)	0.0278	0.0348	0.0211
H (S-7)	0.0980	0.1228	0.1091
H (C-8)	0.0651	0.0816	0.0679
H (C-8)	0.0509	0.0638	0.0501
H (C-9)	0.0603	0.0756	0.0619
H (C-9)	0.0384	0.0481	0.0344
H (C-9)	0.0273	0.0342	0.0205
H (S-10)	0.0987	0.1237	0.1100
H (C-11)	0.0721	0.0904	0.0767
H (C-11)	0.0481	0.0603	0.0466
H (C-12)	0.0603	0.0756	0.0619
H (C-12)	0.0107	0.0134	-0.0003
H (C-12)	0.0394	0.0494	0.0357
Total Charge	2.0001	2.5070	2.0000

TABLE 15
Conversion of MNDO Charges to ab initio Charges
for ZN-45

ATOM	CHARGES MNDO	ESP	Adjusted ESP
Zn	0.5326	0.6676	0.6563
S-1	-0.0203	-0.0254	-0.0367
C-2 (S-1)	0.0172	0.0216	0.0103
C-3 (C-2)	0.0864	0.1083	0.0970
N-4 (C-3)	-0.2896	-0.3630	-0.3743
S-5	-0.0105	-0.0132	-0.0244
C-6 (S-5)	0.0159	0.0199	0.0087
C-7 (C-6)	0.0841	0.1054	0.0941
N-8 (C-7)	-0.2949	-0.3696	-0.3809
S-9	0.0868	0.1088	0.0975
C-10 (S-9)	-0.0073	-0.0092	-0.0204
C-11 (C-10)	0.0903	0.1132	0.1019
N-12 (C-11)	-0.2917	-0.3656	-0.3769
S-13	0.1016	0.1274	0.1161
C-14 (S-13)	-0.0067	-0.0084	-0.0197
C-15 (C-14)	0.0751	0.0941	0.0829
N-16 (C-25)	-0.3182	-0.3988	-0.4101
H (S-1)	0.1084	0.1359	0.1246
H (C-2)	0.0609	0.0763	0.0651
H (C-2)	0.0543	0.0681	0.0568
H (C-3)	0.0133	0.0167	0.0054
H (C-3)	0.0344	0.0431	0.0318
H (N-4)	0.1304	0.1634	0.1522
H (N-4)	0.1372	0.1720	0.1607
H (S-5)	0.1192	0.1494	0.1381
H (C-6)	0.0568	0.0712	0.0599
H (C-6)	0.0590	0.0740	0.0627
H (C-7)	0.0273	0.0342	0.0229
H (C-7)	0.0266	0.0333	0.0221
H (N-8)	0.1300	0.1629	0.1517
H (N-8)	0.1322	0.1657	0.1544
H (S-9)	0.0837	0.1049	0.0936
H (C-10)	0.0540	0.0677	0.0564
H (C-10)	0.0710	0.0890	0.0777
H(C-11)	0.0316	0.0396	0.0283
H (C-11)	0.0160	0.0201	0.0088
H (N-12)	0.1343	0.1683	0.1571
H (N-12)	0.1324	0.1660	0.1547
H (S-13)	0.0744	0.0933	0.0820
H (C-14)	0.0689	0.0864	0.0751
H (C-14)	0.0492	0.0617	0.0504
H (C-15)	0.0461	0.0578	0.0465
H (C-15)	0.0480	0.0602	0.0489
H (N-16)	0.1282	0.1607	0.1494
H (N-16)	0.1216	0.1524	0.1411
Total Charge	2.0002	2.5072	2.0000

TABLE 16
Conversion of MNDO Charges to ab initio Charges for ZN-49

	CHARGES		
ATOM	**MNDO**	**ESP**	**Adjusted ESP**
Zn	0.5642	0.7072	0.6968
S1	-0.0183	-0.0229	-0.0333
C-2 (S-1)	0.0274	0.0343	0.0240
C-3 (C-2)	-0.0118	-0.0148	-0.0251
C-4 (C-3)	0.3475	0.4356	0.4252
O-5 (C-4)	-0.2636	-0.3304	-0.3408
O-6 (C-4)	-0.3889	-0.4875	-0.4978
S-7	-0.0240	-0.0301	-0.0404
C-8 (S-7)	0.0341	0.0427	0.0324
C-9 (C-8)	-0.0136	-0.0170	-0.0274
C-10 (C-9)	0.3424	0.4292	0.4188
O-11 (C-10)	-0.2651	-0.3323	-0.3426
O-12 (C-10)	-0.3815	-0.4782	-0.4885
S-13	0.0354	0.0444	0.0340
C-14 (S-13)	0.0138	0.0173	0.0069
C-15 (C-14)	-0.0128	-0.0160	-0.0264
C-16 (C-15)	0.3382	0.4239	0.4136
O-17 (C-16)	-0.2716	-0.3404	-0.3508
0-18 (C-16)	-0.3615	-0.4531	-0.4635
S-19	0.0200	0.0251	0.0147
C-20 (S-19)	0.0180	0.0226	0.0122
C-21 (C-20)	-0.0245	-0.0307	-0.0411
C-22 (C-21)	0.3506	0.4395	0.4291
O-23 (C-22)	-0.2566	-0.3216	-0.3320
O-24 (C-22)	-0.4117	-0.5160	-0.5264
H (S-1)	0.1472	0.1845	0.1742
H (C-2)	0.0487	0.0610	0.0507
H (C-2)	0.0659	0.0826	0.0723
H (C-3)	0.0593	0.0743	0.0640
H (C-3)	0.0860	0.1078	0.0974
H (O-5)	0.2492	0.3124	0.3020
H (S-7)	0.1490	0.1868	0.1764
H (C-8)	0.0548	0.0687	0.0583
H (C-8)	0.0609	0.0763	0.0660
H (C-9)	0.0618	0.0775	0.0671
H (C-9)	0.0866	0.1085	0.0982
H (O-11)	0.2480	0.3109	0.3005
H (S-13)	0.1067	0.1337	0.1234
H (C-14)	0.0467	0.0585	0.0482
H (C-14)	0.0836	0.1048	0.0944
H (C-15)	0.0516	0.0647	0.0543
H (C-15)	0.0891	0.1117	0.1013
H (O-17)	0.2447	0.3067	0.2964
H (S-19)	0.1271	0.1593	0.1490
H (C-20)	0.0811	0.1017	0.0913
H (C-20)	0.0722	0.0905	0.0801
H (C-21)	0.0952	0.1193	0.1090
H (C-21)	0.0496	0.0622	0.0518
H (O-23)	0.2491	0.3122	0.3019
Total Charge	2.0002	2.5072	2.0000