Evaluation of ab Initio Charge Determination Methods for Use in Continuum Solvation Calculations

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Received: April 23, 2003; In Final Form: July 2, 2003

Application of continuum electrostatic calculations to molecular modeling requires an accurate description of molecular charge distributions, typically as partial atomic charges. While for some systems, such as biological macromolecules, sets of charges have been parametrized on the basis of experimental data, for many other cases, ab initio methods of charge determination may be preferred. Presented here is a comprehensive evaluation of the performance of numerous methods for the ab initio determination of partial atomic charges in continuum electrostatic calculations. Charges were computed using several methods based both on fitting electrostatic potentials and on population analysis, and using various levels of theory ranging from semiempirical quantum mechanical methods through relatively high level ab initio quantum mechanical methods. All charge distributions were evaluated in terms of their ability to reproduce experimental free energies of solvation in the context of a continuum solvation model. Two sets of test molecules were used, one derived from the groups seen in proteins, and the other a more diverse set of neutral organic molecules. The results indicate that there are clearly preferred methods for determining charges and, conversely, that there are highly unsuitable methods. The agreement with experiment does not increase monotonically with increasingly accurate levels of theory, although the lowest level methods do perform particularly poorly. None of the methods performed uniformly well across all molecule types; the top performing methods tended to give charge magnitudes in the middle of the observed range, but both the under- and overpolarized charge distributions perform better for certain systems. The frequently used HF/6-31G* level of quantum mechanics did very well, ranking among the top methods, particularly when coupled with the Merz−Singh−Kollman charge fitting scheme or a restrained fit based on this scheme. For methods at a relatively high level of theory, the charges derived from B3LYP/6-311G*$^{+}$ potentials performed the best, while the computationally inexpensive B3LYP/4-31G derived charges provided the best performance for a fairly low level method.

1. Introduction

Over the past two decades, continuum solvation models have been shown to be very useful in gaining important insights into biomolecular processes, and they have become an important approach for the study of biological molecules. Continuum models allow the solvation energetics of biological macromolecules in an aqueous, moderately ionic environment (the milieu for much of biology) to be calculated relatively quickly and accurately.[1−6] This approach treats the solute in atomic detail, most often with a set of atomic radii used to construct the low-dielectric region and partial atomic charges located at atom centers, while solvent is treated by a continuum model, using a dielectric constant (high for aqueous solvent) to describe the response of solvent to the electric field of the solute, and a Debye−Hückel-like model of ionic screening. Continuum electrostatic calculations have been used to analyze in detail the role that electrostatic interactions play in the stability of proteins,[7−11] and to further our understanding of the binding energetics of proteins with other proteins, with nucleic acids, and with small molecules.[12−18] In addition, theoretical and methodological advances have made it possible to use continuum electrostatics as a tool in designing more tightly and specifically associating molecular complexes.[19−22]

An essential requirement for the successful application of continuum electrostatics is an appropriate description of the molecular charge distribution, which is most commonly represented as a set of atom-centered point charges for the molecules of interest. Whereas there are extensive parameter sets including charges for biological macromolecules readily available,[23−32] equally accurate charge models for the small molecules that bind to them are frequently lacking. A great deal of success has been found in fitting chemical parameters to physical data, and most parameter sets for use in molecular mechanics force fields have been determined at least partially in this manner. Where physical data are unavailable, results from ab initio quantum mechanical calculations are often used for parametrization. However, it is not obvious that the same set of charges will give the best performance both in molecular mechanics and in continuum electrostatic calculations, and in fact there is significant variation among the charges found in diverse empirical force fields. A recent study also showed that the parameters from several major empirical force fields reproduce experimental hydration free energies quite poorly when used to compute solvation free energies using a continuum electrostatic model.[33] Sitkoff et al. were successful in parametrizing

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10.1021/jp0350971 CCC: $25.00
Published on Web 08/26/2003

the charges on a small set of functional groups found in proteins to give good agreement with experiment in the context of a continuum solvation model.[28] This parameter set, PARSE, is extremely useful for proteins but does not include a sufficient range of functional groups to describe many small molecules, and thus alternative methods for determining partial atomic charges for small molecules have been sought. Correspondence and consistency between PARSE and alternative parametrizations is particularly desirable to allow both to be used together (e.g., PARSE for the protein and the alternative for the ligand candidate).

Ab initio charge determination methods are of particular interest, since detailed experimental data are not available for many potential ligands. In addition, in the context of *de novo* ligand design, the molecule of interest may have no experimental information available at all and, in fact, may have never been synthesized. Several ab initio methods for the determination of the partial atomic charges of small molecules exist, based both on analysis of the electron density[34] and on fitting point charges to reproduce the electrostatic potential around the molecule.[35−43] However, the best choice of charge determination method, as well as the most appropriate quantum mechanical level of theory and size of basis set, is not clear. While the performance of ab initio charge determination methods in molecular mechanics applications has been analyzed[30,43,44] and the performance of various parametrized charges in continuum electrostatic calculations has also been considered,[28,33] there has been little consideration of the performance of ab initio methods in continuum electrostatic applications. Some work has been done to validate methods of charge determination in the context of applying continuum methods to quantum mechanical calculations,[45,46] and the performance of a small number of ab initio methods has been compared with that of various force fields using a generalized Born solvation model,[47] but no systematic comparison of many methods has been done, particularly within a Poisson−Boltzmann model of solvation. The goal of this work is to analyze in detail the performance of ab initio charge determination methods in a continuum solvation model. We computed partial charges for a large number of small organic molecules using a range of different ab initio approaches, and each charge determination method was evaluated by comparison of experimental free energies of hydration to the free energy of hydration calculated using a continuum solvation model with the computed partial atomic charges.

2. Methods

Small Molecule Geometries. The structures of all molecules were energy minimized using the quantum chemistry program JAGUAR[48] or GAUSSIAN98[49] for all ab initio methods and using the program MOPAC[50] for all semiempirical methods, starting from an extended conformation with standard bond lengths and angles. Unless otherwise noted, the geometries were determined using the same quantum method as was used for the charge determination.

Small Molecule Partial Atomic Charges. Partial atomic charges were determined in numerous ways from the wave function calculated by a single-point calculation using the quantum chemistry program GAUSSIAN98.[49] Two levels of theory—Hartree−Fock (HF) and B3LYP—and a variety of basis sets—STO-3G, 3-21G, 4-31G, 6-31G, 6-31G(**)($^{++}$), and 6-311G(**)($^{++}$)—were used. B3LYP (a hybrid functional method) replaces the exchange energy of the Hartree−Fock formulation with an exchange-correlation functional. This incorporates some treatment of both local and nonlocal electron correlation along with an exchange functional which is a linear combination of the Hartree−Fock exchange energy and the Becke88 exchange functional. Charges were obtained by Mulliken population analysis,[34] as well as by fitting the electrostatic potential (ESP) using the Chelp procedure,[38] the ChelpG procedure,[39] and the Merz−Singh−Kollman (MK) method.[36,37] All these methods compute the charges that best reproduce the quantum mechanical electrostatic potential using a least-squares formulation based on Lagrange multipliers, but each method uses a different scheme to select the set of points at which the ESP is computed. Additionally, an enhanced Merz−Singh−Kollman procedure was performed with an increased size and density of the grid used for the determination of the electrostatic potential. As well as the standard ESP fit, a restrained fit to the potential was performed using the program RESP,[40,41] in three ways: (1) a single fit with weak restraints toward zero on all heavy atoms; (2) a two-stage fit with weak restraints on all heavy atoms in the first stage, followed by a second fit with aliphatic carbons more highly restrained and all polar atoms fixed at the values obtained in the first stage; (3) a single fit with aliphatic hydrogens fixed at zero and all heavy atoms weakly restrained. The addition of charge restraints during the ESP has been shown to reduce the variation in the computed charges for "buried" atoms—those atoms removed from the molecular surface and thus with charges poorly determined by the potential outside the molecule. Restrained-fitting methods have also been shown to produce charges less dependent on molecular conformation than those produced by unrestrained methods.[40] The restraints used were those suggested by Bayly et al.[40] Charge values were also fit from the semiempirical wave function calculated by the MOPAC computer program[50] using population analysis, as well as with the Merz−Singh−Kollman ESP fitting scheme.

Solvation Free Energy Calculations. Solvation free energies were calculated using a two-component Poisson−Boltzmann/surface area (PB/SA) procedure previously described.[28] The electrostatic component was computed by finite-difference solution of the Poisson equation (since the ionic strength is zero), using a locally modified version of the computer program DELPHI.[51−54] A 65 × 65 × 65 grid was used, with focusing boundary conditions in which the longest dimension of the molecule occupied first 23%, then 46%, and finally 92% of one edge of the grid. This resulted in a final grid spacing of at most 0.33 Å for all molecules. The boundary potentials for each calculation were taken from the previous resolution calculation, and Coulombic potentials were used at the boundary of the lowest resolution box. An internal dielectric constant of 2 was used, and a dielectric constant of 80 was used for the solvent, in accordance with the methodology outlined in the development of the PARSE parameter set.[28] The ionic strength was set to zero for consistency with the experimental conditions. The nonpolar (cavity and van der Waals) term was calculated from the solvent accessible surface area (calculated using the program MSMS[55]) using the relation $\Delta G = 5.4A + 920$ (ΔG is in cal/mol and A is the solvent accessible surface area in Å^2).[28] A probe radius of 1.4 Å was used for the generation of both the molecular surface (used to define the dielectric boundary) and the solvent accessible surface.

3. Results and Discussion

Because partial atomic charge is not a quantum mechanical observable, charges must be fit to results of quantum mechanical calculations; several different procedures have been developed to this end. One class of methods involves partitioning the

electron density between atoms and combining the assigned electron density with the nuclear charge to give a partial charge for each atom. A second class of methods involves computing the electrostatic potential (ESP) for the molecular wave function (which is an observable) and then fitting a set of partial charges to best reproduce this potential. Both procedures can be done in multiple different ways, with the most commonly used partitioning scheme being Mulliken population analysis[34] and with several methods, including Merz−Singh−Kollman,[36,37] RESP,[40,41] Chelp,[38] and ChelpG,[39] regularly used for fitting charges to the electrostatic potential.

Models based on partial atomic charges seek both to adopt a set of charge parameters that is physically reasonable and also to reproduce experimental results for the quantities of interest. With continuum electrostatics, one of the most important values to compute accurately is the free energy of solvation, since interactions in solvent often have strong solvation contributions. The ability to reproduce solvation free energies with a continuum model has been used in the parametrization of the PARSE charge and radii set for proteins[28] and to evaluate the accuracy of various other parameter sets in continuum electrostatic calculations.[33]

The free energy of solvation is not purely electrostatic in nature, and thus a continuum electrostatic model alone should not fully reproduce solvation free energies. In particular, the hydrophobic effect, which contributes to the unfavorable free energy of solvation of aliphatic molecules, requires a separate treatment. For a series of hydrocarbons, good agreement with experiment is attained using a linear relation to the solvent accessible surface area of the molecule.[28,56,57] This may be reasonable, considering that the larger the exposed surface area of a molecule, the larger the number of water molecules that will be involved in restructuring around the molecule,[58−60] although it is certainly an approximation.[61−68] In the model used here, this hydrophobic term is equated with the cost of forming a cavity of given surface area in the solvent, plus the van der Waals interactions between solvent and a hydrophobic molecule fully occupying the cavity, and is applied equally to all molecules, nonpolar and polar. The exact form of the relation is obtained by fitting to the solvation free energies of a series of hydrocarbons, given a set of radii. While this could be done for every set of charges used, since the electrostatic contribution to the solvation free energy for hydrocarbons is small for all charge determination methods, the relation determined in the PARSE parameter development[28] (using completely hydrophobic hydrocarbons) was used in all cases. This provides the added benefit of consistency with the PARSE parameter set. An alternative approach would be to fit the surface area term to the complete set of molecules for each charge determination method, which would improve the overall performance of all methods. This procedure, however, would add considerable complexity, and would make the comparison of different methods more difficult. In addition, it is unlikely that adding this variation would make a large difference in the results.

Molecules Representative of Protein Groups. An initial extensive set of calculations was performed on a set of molecules corresponding to the side chains of the 20 common amino acids with the exception of proline and glycine, as well as a small molecule representation of the peptide backbone. This set contains a reasonable number of functionalities, including both charged and neutral states for all ionizable groups, and contains both positively and negatively charged molecules. Charges and geometries were obtained for a variety of basis sets and theoretical methods, and these charges were subsequently used in the calculation of solvation free energies using a Poisson−Boltzmann/surface area model. The radii from the PARSE parameter set[28] were used for all computations; these radii have the advantage of being quite simple—they are an extension of the Pauling van der Waals radii[69] with the radius of hydrogen atoms set to 1.0 Å rather than 1.2 Å.

Charges Derived Directly from Theory Can Perform Nearly as Well as Those Fit to Experiment. The average absolute error in calculated hydration free energies in comparison to experiment was determined for each charge set (see Table 1). The range of results is significant, with average unsigned errors as low as 0.86 kcal/mol and as high as 5.47 kcal/mol, indicating that choosing an appropriate method for determining charges is essential for accurate continuum electrostatic calculations. This can be compared with a computed average error of 0.74 kcal/mol when PARSE charges, the de facto standard for continuum electrostatic calculations in protein systems, are used. This error is dominated by charged lysine, for which the solvation free energy is computed to be 8.10 kcal/mol more favorable than experiment—excluding this value yields an average error of 0.35 kcal/mol. While Sitkoff et al.[28] report an average error of 0.10 kcal/mol for the same set of molecules, a different procedure for obtaining geometries was used here, and these molecules were part of the data set used in fitting the PARSE charges. Sitkoff et al.[28] report an average error of 0.54 kcal/mol for a set of test molecules using the PARSE parameters, and Dixit et al.[33] report an average error of 0.39 kcal/mol for the solvation energies of the set of amino acid side chains in their standard states (again using PARSE parameters). Thus, the best performing method of ab initio derived charges, with an average error of 0.86 kcal/mol, performs nearly as well as a charge set parametrized to give optimal results for this data set. Dixit et al.[33] also computed solvation energies for the amino acid side chains using charges and radii from several molecular mechanics force fields, and they found average errors compared to experiment of between 1.89 and 2.97 kcal/mol. Thus, the top performing ab initio methods seem to provide charges better suited for continuum electrostatic calculations than are those from most molecular mechanics force fields.

Considering the results, several observations can be made. Charges determined by Mulliken population analysis performed uniformly worse than those determined by fitting to the electrostatic potential, with very poor performance at almost all levels of theory—average errors were below 2.0 kcal/mol in only a few cases, and in no case was the average error below 1.5 kcal/mol. This may be expected, since the solvation free energies are directly related to the electrostatic potential projected by the molecule into the solvent, and thus matching the potential well should lead to reasonable reproduction of solvation free energies. Of course, there remains the question of which method produces an electrostatic potential most compatible with the continuum model. Methods based on population analysis, however, make no deliberate attempts to accurately reproduce the electrostatic potential and, as a result, failed to accurately represent quantities dependent on the potential.

Ab Initio Methods Outperform Semiempirical Methods. Charges derived from semiempirical methods performed worse than those from all ab initio basis sets with the exception of STO-3G. The semiempirical population analysis charges all gave average errors of above 3.0 kcal/mol; semiempirical electrostatic potential fit charges performed only somewhat better. Only two methods gave errors below 3.0 kcal/mol—AM1 and MNDO. Similarly, the minimal ab initio STO-3G basis set, at both HF

TABLE 1: Errors in Calculated Hydration Free Energies of Molecules in the Protein Data Set[a]

Method	Basis	Mulliken	Chelp	ChelpG	MK ESP	MK ESP‡	RESP 1X	RESP 2X	RESP 2X‡	RESP PH
B3LYP	STO-3G	5.47	4.30	4.09	3.97	3.97	4.15	4.16	3.98	4.22
B3LYP	3-21G	1.91	1.84	1.55	1.49	1.53	1.56	1.56	1.53	1.66
B3LYP	4-31G	1.79	1.58	1.09	0.95	0.95	0.99	1.00	0.94	1.08
B3LYP	6-31G	1.92	1.48	1.25	1.14	1.13	1.08	1.08	1.12	1.17
B3LYP	6-31G*	1.87	2.10	1.56	1.39	1.37	1.58	1.59	1.37	1.69
B3LYP	6-31G*$^{+}$	3.17	1.63	1.26	1.06	1.03	0.99	0.99	1.02	1.11
B3LYP	6-31G**	3.17	2.18	1.62	1.44	1.43	1.66	1.67	1.43	1.78
B3LYP	6-31G**$^{+}$	1.96	1.72	1.28	1.07	1.04	1.02	1.03	1.04	1.15
B3LYP	6-31G**$^{++}$	3.00	1.76	1.28	1.06	1.04	1.02	1.02	1.03	1.16
B3LYP	6-311G	2.14	1.39	1.30	1.21	1.23	1.12	1.12	1.22	1.15
B3LYP	6-311G*	2.21	1.98	1.48	1.25	1.26	1.36	1.36	1.26	1.47
B3LYP	6-311G*$^{+}$	3.62	1.65	1.20	1.02	1.02	0.93	0.93	1.02	1.05
B3LYP	6-311G**	3.99	2.19	1.62	1.39	1.38	1.59	1.60	1.37	1.71
B3LYP	6-311G**$^{+}$	3.36	1.83	1.26	1.03	1.00	1.08	1.08	1.00	1.20
B3LYP	6-311G**$^{++}$	4.24	1.84	1.27	1.04	1.01	1.09	1.09	1.00	1.22
HF	STO-3G	5.22	3.67	3.52	3.42	3.42	3.62	3.63	3.43	3.67
HF	3-21G	2.95	1.18	1.10	1.26	1.30	1.06	1.06	1.30	1.13
HF	4-31G	2.52	1.66	1.76	1.66	1.68	1.47	1.47	1.67	1.44
HF	6-31G	2.57	1.75	1.87	1.78	1.79	1.58	1.58	1.78	1.54
HF	6-31G*	1.67	1.29	1.14	1.01	1.02	0.86	0.86	1.01	0.99
HF	6-31G*$^{+}$	4.19	1.41	1.47	1.41	1.46	1.17	1.18	1.45	1.32
HF	6-31G**	1.49	1.30	1.17	1.01	1.02	0.88	0.89	1.01	1.01
HF	6-31G**$^{+}$	2.69	1.46	1.48	1.37	1.42	1.16	1.17	1.40	1.31
HF	6-31G**$^{++}$	2.91	1.47	1.48	1.35	1.42	1.17	1.17	1.41	1.30
HF	6-311G	1.80	1.68	1.88	1.80	1.84	1.59	1.59	1.83	1.56
HF	6-311G*	1.83	1.30	1.14	1.13	1.16	0.92	0.92	1.15	1.04
HF	6-311G*$^{+}$	5.11	1.40	1.43	1.46	1.51	1.20	1.21	1.50	1.32
HF	6-311G**	3.11	1.50	1.21	1.03	1.06	0.93	0.93	1.05	1.06
HF	6-311G**$^{+}$	3.30	1.52	1.46	1.32	1.36	1.13	1.14	1.36	1.25
HF	6-311G**$^{++}$	5.54	1.62	1.42	1.28	1.32	1.09	1.09	1.31	1.21
S.E.	AM1	3.18			2.57					
S.E.	PM3	3.89			3.04					
S.E.	MNDO	4.50			2.59					
S.E.	MINDO3	4.54			3.99					

[a] Average absolute errors (in kcal/mol) in calculated hydration free energies for all charge determination methods are shown computed over a set of molecules representative of protein groups. Methods marked with ‡ used an extended and more dense grid for the calculation of the electrostatic potential.

and B3LYP levels of theory, gave very poor performance in all charge fitting methods, with average errors of over 3.0 kcal/mol in every case. For semiempirical methods, only population analysis and Merz−Singh−Kollman ESP fit charges were obtained. However, considering the relatively small variation among ESP fit charges from different procedures in the ab initio data, it is unlikely that different ESP fitting schemes would drastically improve the performance of these semiempirical methods. Charges fit to the electrostatic potential by any procedure with potentials computed with any ab initio method above the STO-3G level, on the other hand, did reasonably well, with average errors below 2.0 kcal/mol for all but two methods. Thus, it seems that the ab initio quantum mechanical electrostatic potential, as long as some minimum level of theoretical completeness is reached, is quite realistic and adequately reproduces the solvation energies.

Polarization and Diffuse Functions Have Significant Effects on Fit Charges. At the B3LYP level of theory, several basis sets performed worse than the others across all fitting methods based on the electrostatic potential (ESP). These included 3-21G, as well as 6-31G and 6-311G with polarization functions either on heavy atoms or on all atoms but with no diffuse functions. The 6-31G and 6-311G basis sets with neither polarization nor diffuse functions performed somewhat better. The best performance was given by the 4-31G basis set and by the 6-31G and 6-311G basis sets with both polarization and diffuse functions on heavy atoms (and optionally on hydrogens).

The results at the HF level of theory are almost exactly opposite. The worst performance was seen in the 4-31G basis set and in the 6-31G and 6-311G basis sets with no polarization or diffuse functions. The 6-31G and 6-311G basis sets with both polarization and diffuse functions on heavy atoms (or on all atoms) performed moderately well. The 6-31G and 6-311G basis sets with polarization functions on heavy atoms and optionally on hydrogens, but with no diffuse functions, performed best, closely followed by the 3-21G basis set. The good performance of the HF/6-31G* ESP-fit charges is consistent with the observations others have made in the case of simulations using explicit solvent.[44] The generally accepted explanation for the particularly good performance of charges derived from this method is that the charges are overpolarized by 10−20% (based on comparison to gas-phase dipole moments), and this overpolarization compensates for the lack of polarization effects assumed with a fixed-charge model.[37,44,70]

Choice of ESP Fitting Method Affects the Performance of the Resulting Charges. The Chelp method of fitting to the electrostatic potential gave charges which performed somewhat poorly at all levels of theory; the ChelpG method gave a slightly better average performance across basis sets. The Merz−Singh−Kollman method outperformed both in all but one case (HF/3-21G), for which all ESP fitting methods performed quite well and for which the Chelp and ChelpG methods performed better than those with any other theoretical level. Restrained ESP charge fitting was also carried out with the charges of aliphatic hydrogen atoms constrained to zero and with these hydrogens assigned a radius of zero. This gives a charge set consistent with a polar hydrogen/united nonpolar atom model. In all but one case (HF/4-31G), this polar hydrogen model gave average errors slightly worse than those of the analogous all-atom set, and in the case of HF/4-31G, the average errors of both models

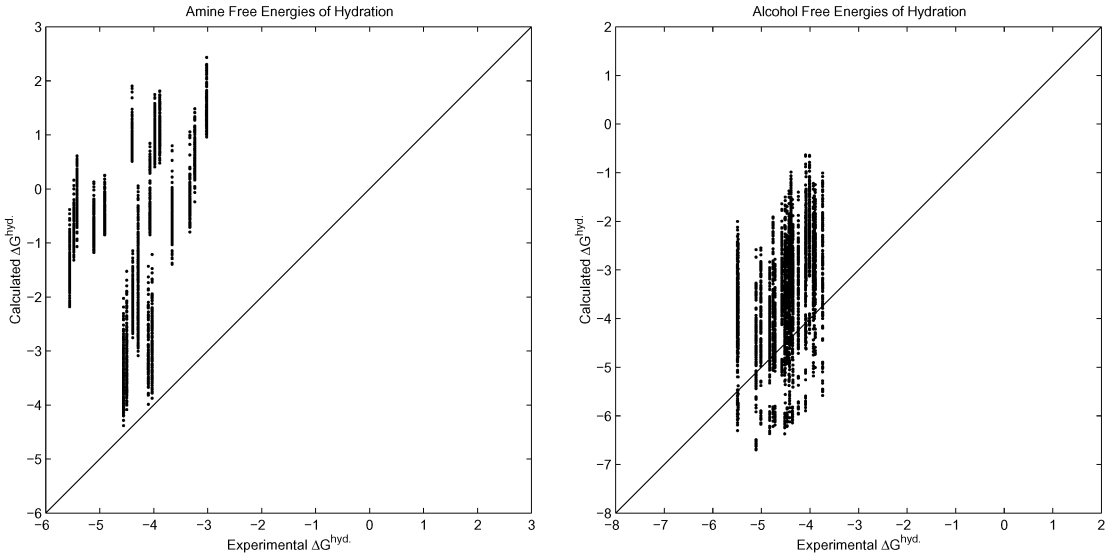

Figure 1. Variation in computed hydration free energies for amines and alcohols. For both the sets of all amines and of all alcohols, the calculated hydration free energy (in kcal/mol) is plotted relative to the experimental value. While a similar range is seen in the computed values for both sets, the results for alcohols span the line $y = x$, while the computed energies for amines are uniformly higher than the experimental values.

were virtually identical. Similar results were seen in calculations on a more extensive set of molecules, and these effects are discussed in more detail below.

The Merz−Singh−Kollman method was performed with two levels of detail, with additional layers and finer sampling of grid points added in the second case. However, this extension of the Merz−Singh−Kollman grid did not result in better performance of the fit charges. For unrestrained ESP fit charges, the greatest deviation in average error between the standard grid and the more extensive grid was 0.07 kcal/mol, and it was less than 0.05 kcal/mol for the majority of the methods. For charges obtained from restrained ESP fits, the more extensive grid yielded charges that performed more poorly on average. This difference is likely a result of the implementation of restraints in the RESP method, which become relatively weaker as the number of points at which the potential is calculated increases.[42]

Method for Geometry Determination Has Little Effect on Charges from Most Fitting Methods. In addition to the geometry at which the single-point calculation was performed, charges were determined from single-point calculations at all levels of theory using the geometries computed at the HF/3-21G, the HF/6-31G*, the B3LYP/6-31G*$^{+}$, and the B3LYP/6-311G**$^{++}$ levels of theory. These choices cover the range of methods used. The charges obtained from the different geometries were compared in detail (Figure 2). The charge determination method was seen to play a more significant role in the variation of calculated charges with geometry than did the differences between the basis sets used to calculate the geometry and to calculate the charges. In particular, the Chelp procedure produced the largest variations in charges obtained from different geometries, with rms deviations of the charges of 0.05*e* and a maximum deviation of over 1*e*! This sensitivity to geometry was noted by Breneman and Wiberg[39] as being a result of the method by which the fitting points are determined. The ChelpG procedure, with an ESP grid selection scheme specifically designed to overcome this drawback, produced charges that varied much less with differences in geometry. The Mulliken procedure also produced deviations in charge of up to 0.6*e*, and the united nonpolar atom charges showed variations of up to 0.5*e*. The ChelpG procedure, the unrestrained Merz−Singh−Kollman procedure, and the RESP procedure all gave maximum deviations of between 0.1 and 0.2*e* and rms deviations of between 0.01 and 0.02*e*. These minor variations suggest that using a relatively inexpensive ab initio method for geometry optimization, while performing the charge determination with a larger basis set, is a reasonable approach to minimize computational expense with minimal effects on the results.

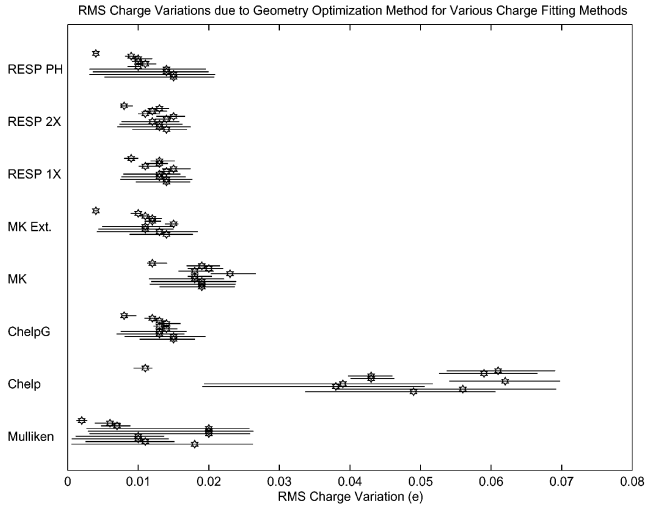

Figure 2. Variation in computed partial atomic charges with geometry. The variation of partial atomic charges computed from geometries determined at various levels of theory are plotted. For each charge fitting method, the rms deviation between the charges determined using the geometry at one of several levels of theory and those determined using the geometry determined at another level of theory was computed for every molecule in the protein data set. Each line represents the variation in the results for each molecule in the protein data set, spanning one standard deviation in the rms variations observed. Each star represents the rms charge variation over the protein data set as a whole. By far the greatest variation is seen for the Chelp ESP fitting methodology.

Extended Set of Small Organic Molecules. A similar evaluation of the performance of the various charge fitting methods was performed using a more extensive set of small molecules. This set included 324 small molecules of diverse functionalities—228 with a single functionality and 96 polyfunctional molecules.[71] The semiempirical methods and the ab initio STO-3G basis set both at the Hartree−Fock and B3LYP levels of theory were excluded due to their very poor performance on the protein-like data set. Similarly, the Mulliken

TABLE 2: Errors in Calculated Hydration Free Energies of Diverse Small Organic Molecules[a]

Method	Basis	MK ESP	RESP 1X	RESP 2X	RESP Polar H	ChelpG
B3LYP	3-21G	1.25	1.30	1.32	1.48	1.34
B3LYP	4-31G	1.25	1.28	1.29	1.37	1.37
B3LYP	6-31G	1.26	1.28	1.29	1.36	1.38
B3LYP	6-31G*	1.41	1.54	1.55	1.69	1.63
B3LYP	6-31G*$^{+}$	1.25	1.31	1.32	1.45	1.47
B3LYP	6-31G**	1.46	1.58	1.60	1.74	1.67
B3LYP	6-31G**$^{+}$	1.27	1.34	1.34	1.47	1.50
B3LYP	6-31G**$^{++}$	1.30	1.37	1.38	1.48	1.53
B3LYP	6-311G	1.30	1.30	1.31	1.36	1.42
B3LYP	6-311G*	1.30	1.39	1.40	1.53	1.54
B3LYP	6-311G*$^{+}$	1.24	1.29	1.30	1.44	1.46
B3LYP	6-311G**	1.40	1.50	1.51	1.64	1.65
B3LYP	6-311G**$^{+}$	1.30	1.37	1.38	1.50	1.54
B3LYP	6-311G**$^{++}$	1.30	1.37	1.38	1.50	1.54
HF	3-21G	1.61	1.52	1.53	1.54	1.42
HF	4-31G	1.87	1.78	1.78	1.72	1.91
HF	6-31G	1.96	1.86	1.86	1.78	1.98
HF	6-31G*	1.28	1.31	1.31	1.42	1.43
HF	6-31G*$^{+}$	1.41	1.39	1.39	1.50	1.52
HF	6-31G**	1.29	1.32	1.32	1.44	1.45
HF	6-31G**$^{+}$	1.41	1.40	1.40	1.51	1.54
HF	6-31G**$^{++}$	1.42	1.41	1.41	1.51	1.55
HF	6-311G	1.92	1.82	1.82	1.76	1.95
HF	6-311G*	1.39	1.38	1.38	1.50	1.47
HF	6-311G*$^{+}$	1.48	1.43	1.43	1.54	1.54
HF	6-311G**	1.38	1.39	1.39	1.50	1.53
HF	6-311G**$^{+}$	1.44	1.42	1.43	1.53	1.57
HF	6-311G**$^{++}$	1.45	1.43	1.44	1.53	1.58

[a] Average absolute errors (in kcal/mol) in calculated hydration free energies relative to experiment for all charge determination methods are shown computed over a large set of small organic molecules.

charge fitting scheme was excluded, again due to poor performance in the first set of calculations. While the Chelp method did not perform nearly as poorly as these in the initial calculations, it did not perform particularly well either. In addition, the Chelp methodology gave such large variations in charges when different geometries were used that it seems a poor choice for a general method. Therefore, the Chelp procedure was also excluded from this set of calculations.

Results from the Protein Data Set Are Confirmed in a Larger Set of Molecules. The results of the broad survey of methods (Table 2) were qualitatively similar to the results from the protein set. At the Hartree−Fock level, the charges obtained using the 6-31G* and 6-31G** basis sets reproduced the experimental solvation free energies the best, with charges from an unrestrained Merz−Singh−Kollman fit to the electrostatic potential performing slightly better than those obtained from restrained fits with all atoms. The solvation free energies calculated with charges obtained from both the restrained fit with nonpolar hydrogens fixed at zero and the ChelpG method reproduced the experimental values more poorly. At the B3LYP level of theory, charges from fitting the ESP obtained with several basis sets all reproduced the experimental solvation free energies quite well. The 6-31G and 6-311G basis sets, either with no diffuse or polarization functions or with both diffuse and polarization functions on heavy atoms only, as well as both the smaller 3-21G and 4-31G basis sets, all performed well, and again the Merz−Singh−Kollman ESP fitting scheme produced charge values that reproduced the experimental results best, with restrained ESP fitting with all atoms included producing charges that did only slightly worse. Both the ChelpG charges and those from a restrained fit with nonpolar hydrogens excluded did significantly worse with all these basis sets. All these top methods gave average errors relative to experiment below 1.35 kcal/mol, with the lowest average error being 1.24 kcal/mol for charges obtained by unrestrained Merz−Singh−Kollman fitting to the B3LYP/6-311G*$^{+}$ electrostatic potential. Several other methods based on the B3LYP/6-311G basis set, with variation of diffuse and polarization functions, produced charges that reproduced experiment with average errors of 1.30 kcal/mol, but the restrained fits at these levels of theory did worse.

In general, the charges determined by restrained fitting performed similarly to those obtained by an unrestrained fit to the same potential. In some cases slightly better results were seen with unrestrained charges, while in other cases the reverse was found to be true. Again this tends simply to highlight the point that a relatively broad range of hydrocarbon charges gives equivalent electrostatic potential fields—the small magnitude of the potential requires large changes in charge to make significant changes in the energetics. For example, three methods of fitting charges for propanol (MK, RESP, and ChelpG, all at HF/6-31G*) give computed solvation energies within 0.25 kcal/mol of each other and have similar hydroxyl charges (H, +0.43 to +0.44*e*; O, −0.71 to −0.76*e*), while the carbon charges vary much more (C_1, +0.23 to +0.37*e*; C_2, +0.04 to +0.18*e*; C_3, −0.16 to −0.41*e*). However, the polar hydrogen model charges, with aliphatic hydrogen atoms constrained to have no charge, in general did more poorly, except on pure hydrocarbons. When polar atoms are present, the higher electronegativity of the heteroatoms can lead to substantial charges on aliphatic groups. In a united-atom model for aliphatic groups, this forces the entirety of the charge onto the carbon, whereas in an all-atom model the charge can be distributed across hydrogens as well. This leads to a much better fit for the all-atom models, as a single point charge cannot adequately describe a polarized aliphatic group. This can also be seen in the reproduction of the quantum mechanical electrostatic potential—in general, the standard error of the fit for the united-aliphatic-atom model was found to be greater for polar molecules than for pure hydrocarbons (data not shown). For pure hydrocarbons, however, no aliphatic group is particularly polarized, and thus the united-atom model performs well.

Best Methods Vary for Different Functional Groups. The monofunctional compounds can be classified into molecular classes, and the performance of the charge determination methods can be evaluated by class. The number of molecules included for each class ranged from as few as one to as many as twenty eight. With the exception of the amines, the best performing method for every class gave an average error of less than 0.60 kcal/mol. However, the method for charge determination that was best for each class varied significantly, both in the potential fitting method and in the quantum mechanical level of theory used to generate the ESP. Over all monofunctional compounds, the best method was the same as that for the full set (B3LYP/6-311G*$^{+}$ electrostatic potentials with Merz−Singh−Kollman charge fitting), with an average error of 1.15 kcal/mol. However, the average error taking the best method for each class was 0.41 kcal/mol when each molecule was weighted equally and 0.59 kcal/mol when each class was weighted equally.

The set of amines did very poorly, with the best method giving an average error of 2.89 kcal/mol. Looking at how the methods performed as a whole (Figure 1), it is clear that all methods underestimated the favorable free energy of solvation; the calculated free energy of hydration was greater than the experimental value for every method and for every molecule. In addition, the range of performance of each molecule differed

significantly, with some molecules having some methods that reproduced experiment well, while others did poorly with all methods. Comparatively, the alcohol set (whose best performing method gave an average error of 0.44 kcal/mol) behaved in a qualitatively different manner. For this set, every molecule had some methods that did well, and both positive and negative errors were seen for every molecule as well. Similar difficulties in reproducing amine solvation free energies have been seen using a variety of computational methods, including free energy perturbation with molecular mechanics force fields[72,73] and ab initio quantum mechanics with continuum solvent.[45] The primary source of the inconsistency is thought to be due to the difference in strength of hydrogen bonds in which an amine is the donor or the acceptor. This is supported by the observation that adding nonlinear polarizability effects to the potentials used in free energy perturbation methods does not fully correct the anomalous amine solvation free energies,[46,72,74] while adding a hydrogen bond correction to ab initio solvation free energy calculations does significantly improve the agreement of theory with experiment.[46] More recently, it has been found that reasonable agreement between free energy perturbation methods and experimental results can be attained when hydrogen bond interaction energies are explicitly considered in the force field parametrization.[75] Thus, it seems likely that, in order to improve the performance of the amines, an appropriate correction term to ab initio charge may be necessary. However, the exact nature of such a correction would be dependent on the charge fitting method being used and is beyond the scope of the work described here.

The polyfunctional molecules, which all include at least two functional groups, but possibly two of the same type, can loosely be grouped into aliphatic, unsaturated, and aromatic compounds, with any molecule containing an aromatic group considered aromatic (even if it also contains other carbon types as well) and a molecule containing any number of nonaromatic double or triple bonds being classified as unsaturated. The best performing methods (Table 3) for the three groups of polyfunctional molecules reproduced experimental values with average errors of 1.50 kcal/mol for the aliphatic group, 0.84 kcal/mol for the unsaturated group, and 0.96 kcal/mol for the aromatic group, with the methods producing the best results for each set again being quite different. The best performing method for the full set of polyfunctional compounds was single-stage restrained fitting to the B3LYP/3-21G potential, one of the highly performing methods on the full set of molecules, with an average error of 1.32 kcal/mol relative to experimental values. The average error using the best method for each class was 1.24 kcal/mol with molecule based weighting and 1.10 kcal/mol with group based weighting.

The amount of data to consider in looking at the performance of all methods for every molecule class is too large to be feasible. However, the performance of a select set of methods over all classes is displayed in Table 4. The methods all used two-stage RESP fit charges and are based on the Hartree−Fock potentials with a 3-21G, 6-31G*, or 6-31G*$^{+}$ basis set or the B3LYP potentials with a 4-31G, 6-31G*, or 6-31G*$^{+}$ basis set. These include methods that performed well and that performed poorly at both the HF and the B3LYP levels and include both small and moderately sized basis sets. For several molecule classes (alkanes, fluorocarbons, and thiols) all the methods gave average errors below 1.0 kcal/mol, although the fluorocarbon and thiol sets contained only one and two molecules, respectively. For three additional classes (ketones, nitriles, and chlorocarbons), only one method gave an average error above 1.0 kcal/mol, with B3LYP/6-31G* performing badly on ketones, HF/6-31G*$^{+}$ performing badly on nitriles, and HF/3-21G performing badly on chlorocarbons. Charges from both the 3-21G and 6-31G*$^{+}$ basis sets at the Hartree−Fock level performed poorly on alkenes, while charges from both HF/6-31G*$^{+}$ and B3LYP/6-31G* potentials performed poorly on both aldehydes and the single amide in the set. Charges from the Hartree−Fock level of theory with both the 3-21G and the 6-31G*$^{+}$ basis sets, as well as from the B3LYP level with the 4-31G basis set, performed well on alcohols, while charges from B3LYP with the 6-31G* basis set did particularly poorly. For aromatic molecules, only two methods, Hartree−Fock with either the 6-31G* or the 6-31G*$^{+}$ basis set, gave average errors below 1.0 kcal/mol, and five additional molecule classes had only one of the methods that performed well. For esters, nitro compounds, and carboxylic acids, B3LYP/6-31G* charges were the only ones that did well, while, for alkynes, the only method that produced adequate charges was that using B3LYP with the 4-31G basis set. Only charges from HF/3-21G potentials did reasonably well for the two thioethers, and even this method gave errors of 0.99 kcal/mol. None of the selected methods produced charges that did well for three sets of molecules—ethers, pyridines, and amines. However, while for amines none of the entire set of methods did well, for both ethers and pyridines some of the other methods gave errors as low as 0.5 kcal/mol. Overall, with averages taken either over each molecule or over each class of molecules, three of the methods were seen to do well with the protein set; HF/6-31G*, B3LYP/4-31G, and B3LYP/6-31G*$^{+}$ outperform the other methods, with B3LYP/6-31G* charges being particularly disfavored. For the poly-

TABLE 3: Best Performing Charge Determination Methods by Molecule Class[a]

Class	*N*	Best method: Quantum mechanics	Best method: ESP fitting	Error
		Monofunctional		
alkanes	25	RHF/4-31G	RESP-2X	0.44
alkenes	22	B3LYP/6-31G**	ChelpG	0.52
alkynes	8	B3LYP/4-31G	ChelpG	0.36
aromatics	27	RHF/6-311G	MK	0.39
alcohols	25	RHF/6-311G	RESP-PH	0.44
ethers	12	RHF/4-31G	MK	0.50
aldehydes	8	B3LYP/6-311G	RESP-2X	0.11
ketones	15	RHF/6-311G**	ChelpG	0.28
carboxylic acids	3	B3LYP/6-31G*	MK	0.06
esters	28	B3LYP/6-31G**	MK	0.31
amines	20	RHF/6-31G*$^{+}$	MK	2.87
pyridines	15	RHF/6-311G	MK	0.45
nitriles	3	B3LYP/6-311G*	RESP-2X	0.10
amides	1	B3LYP/6-311G**$^{++}$	ChelpG	0.01
nitro	3	B3LYP/6-31G**	RESP-2X	0.09
fluorocarbons	1	B3LYP/6-311G*$^{+}$	MK	0.00
chlorocarbons	8	RHF/6-31G**$^{++}$	RESP-1X	0.15
thiols	2	RHF/4-31G	RESP-2X	0.05
thioethers	2	RHF/6-311G	MK	0.59
Overall	228	B3LYP/6-311G*$^{+}$	MK	1.15
		Polyfunctional		
aliphatic	52	RHF/6-31G**	MK	1.50
unsaturated	14	B3LYP/4-31G	ChelpG	0.84
aromatic	30	B3LYP/3-21G	RESP-1X	0.96
Overall	96	B3LYP/3-21G	RESP-1X	1.32
All molecules	324	B3LYP/6-311G*$^{+}$	MK	1.24

[a] The charge determination method producing the smallest average absolute error (in kcal/mol) in calculated solvation free energies is listed for each molecular class in the extended set of small organic molecules.

TABLE 4: Performance of Select Charge Determination Methods by Molecule Class[a]

			RHF			B3LYP		
Class	*N*	Best	3-21G	6-31G*	6-31G*$^{+}$	4-31G	6-31G*	6-31G*$^{+}$
				Monofunctional				
fluorocarbons	1	0.00	0.48	0.15	0.22	0.18	0.61	0.04
thiols	2	0.05	0.26	0.60	0.47	0.32	0.72	0.68
alkanes	25	0.44	0.44	0.54	0.54	0.54	0.50	0.54
nitriles	3	0.10	0.24	0.78	1.30	0.15	0.15	0.71
chlorocarbons	8	0.15	1.40	0.25	0.26	0.16	0.64	0.42
ketones	15	0.28	0.45	0.47	0.51	0.73	1.68	0.54
aldehydes	8	0.11	0.16	0.20	1.06	0.39	1.30	0.16
amides	1	0.01	0.03	0.05	1.52	0.54	1.80	0.09
alkenes	22	0.52	1.11	0.88	1.14	0.67	0.70	0.89
alcohols	25	0.44	0.64	1.33	0.61	0.70	2.23	1.08
aromatics	27	0.39	1.20	0.64	0.57	1.49	1.54	1.16
nitro	3	0.09	2.70	1.97	2.61	1.21	0.09	1.16
esters	28	0.31	2.54	1.55	2.28	1.50	0.32	1.23
carboxylic acids	3	0.06	3.15	1.72	2.66	1.88	0.36	1.12
alkynes	8	0.36	1.67	1.67	2.03	0.83	1.15	1.53
thioethers	2	0.59	0.99	1.53	1.44	1.57	1.71	1.71
ethers	12	0.50	1.18	2.09	1.88	1.43	2.61	2.07
pyridines	15	0.45	1.35	2.16	1.64	2.46	3.16	2.40
amines	20	2.87	3.18	3.37	3.04	3.47	3.72	3.26
Overall	228	1.15	1.36	1.29	1.32	1.26	1.52	1.27
				Polyfunctional				
aliphatic	52	1.50	1.90	1.57	1.57	1.64	1.85	1.63
unsaturated	14	0.84	1.82	0.97	1.50	0.91	0.99	1.01
aromatic	30	0.96	2.04	1.23	1.56	1.09	1.51	1.25
Overall	96	1.32	1.93	1.38	1.56	1.36	1.62	1.42
All molecules	324	1.24	1.53	1.31	1.39	1.29	1.55	1.32

[a] The average absolute errors in calculated free energies of hydration (in kcal/mol) are displayed for each molecular class for a select set of charge determination methods. The results are roughly grouped according to the average performance across the set of methods. All numbers are based on the RESP-2X charge fitting method.

functional molecules, HF/3-21G charges do poorly for all classes, while again HF/6-31G*, B3LYP/4-21G, and B3LYP/6-31G*$^{+}$ do relatively well across all sets. HF/6-31G*$^{+}$ charges do not perform any worse than others on the aliphatic set, but they do less well on the unsaturated and aromatic sets. The B3LYP/6-31G* charges, on the other hand, do poorly with both the aliphatic set and the aromatic set, but they do reasonably well on the unsaturated molecules. These results strengthen the overall observations from the monofunctional groups that the HF/6-31G*, B3LYP/4-31G, and B3LYP/6-31G*$^{+}$ charges give the best overall performance of these select methods, all giving average errors within 0.10 kcal/mol of the overall best performing method.

These results also explain one notable exception to the similarities in performance of the two sets of molecules—the charges fit from potentials computed at the HF/3-21G level of theory. While these methods gave a good performance on the initial set of protein functionalities, they did much more poorly on the larger set, but looking at the results broken down by molecular class, it becomes readily apparent why this so. The charges from HF/3-21G potentials did particularly well on those functionalities over-represented in the protein set—amides, alcohols, and alkanes make up 38% of the protein set but only 22% of the monofunctional compounds in the larger set—and did worst on more under-represented, or completely absent, functionalities—no nitro compounds or esters are present in the protein set, and two carboxylic acids comprise only 8% of the protein set, but these functionalities make up 15% of the larger set of monofunctional compounds. For the other methods, the differences in performance between the molecule types were less biased toward those found in the protein set, and thus the performance of these methods between the two sets of molecules showed similar trends. In most cases, however, the performance was worse for the larger set of molecules. This is not surprising, due to the necessity to balance the performance of each method over a much larger set of functionalities. In addition, several molecule types included only in the larger set, such as ethers and pyridines, showed relatively poor performance in all top methods, and this contributed to the increased average error given by all these methods.

Single Set of Charges (As Opposed to Method) Performs Best for Each Functional Group. Looking at the charges on several functional groups (see Figure 3), a number of observations can be readily made. First of all, the use of electrostatic potentials produced by Hartree−Fock quantum mechanics led to higher magnitude partial atomic charges than when potentials from B3LYP quantum mechanics were used. While there were overlapping regions, where certain HF and B3LYP based methods yielded similar charges, even when there was substantial overlap, the bias toward charges of higher magnitude for the HF method is clear. Second, in all cases considered there was some relationship between the value of the partial atomic charges and the quality of the reproduction of experimental free energies of hydration. For aldehydes, all methods resulting in an oxygen charge of approximately $-0.5e$ gave computed solvation free energies close to the experimental value, while all methods resulting in an oxygen charge that deviated from $-0.5e$ gave poorer computed solvation free energies. Similarly, for nitro compounds, computed solvation energies were close to experimental values for all methods, resulting in oxygen charges just below $-0.4e$, while the agreement with experiment became worse as the oxygen charge deviated from this value. A trend toward an alcohol hydrogen charge of about $+0.45e$ was also seen, although in this case the variation around the general trend was greater. In all three cases, there were similar trends for the more buried atoms of the functional group

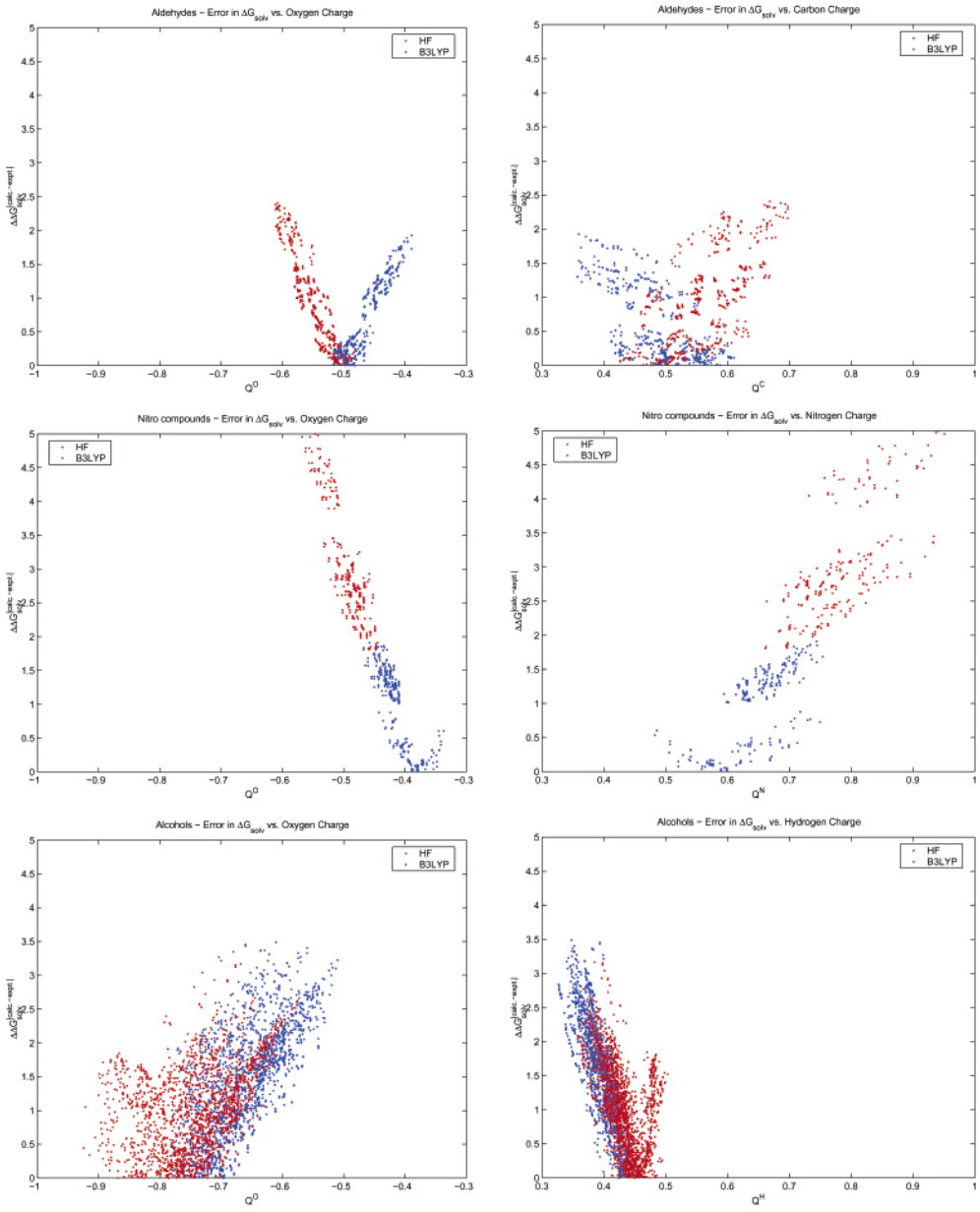

Figure 3. Relation of computed hydration free energies and partial atomic charges on select functional groups. The errors in computed free energies of hydration (in kcal/mol) are plotted in relation to the partial atomic charge on the functional group for aldehydes, nitro compounds, and alcohols. Red: Methods using HF quantum mechanical results generally produced more highly polar functional groups. Blue: Methods based on B3LYP wave functions generally yielded smaller magnitude charges. In all cases some relation between the charge and the error in computed energies is seen, although it is more pronounced in certain instances.

(aldehyde C, nitro N, and alcohol O), although in all these cases much larger deviations were seen.

Charges of Intermediate Polarity Perform Best Overall. The variation in the charges obtained by different methods explains one of the key observations from the survey of the performance of the various methods across the full set of molecules. With the 6-31G and 6-311G basis sets at the HF level of theory, the best performance was obtained with one or two polarization functions, and the addition of diffuse functions reduced the agreement with experiment. Conversely, at the B3LYP level of theory, the same basis sets performed better with both polarization and diffuse functions than with polarization functions alone. Looking at the charges obtained by the different methods, the B3LYP based methods generally gave lower magnitude charges than the HF based methods. In a similar fashion, the addition of diffuse functions tended to produce larger magnitude charges. As a result, some of the largest magnitude charges were found for HF methods including diffuse functions, while some of the lowest magnitude charges were obtained by using B3LYP methods without diffuse functions. Between were the charges obtained from HF methods with no diffuse functions and B3LYP methods with such functions. While for some systems (such as nitro compounds, esters, and carboxylic acids) the underpolarized charges gave the best results, for other systems (such as ketones, alcohols, and aromatics), the underpolarized charges did particularly poorly. Similar results were seen for the overpolarized charges, with very good performance seen for some molecules and very bad performance seen for others. The more intermediate charges performed optimally for some systems, aldehydes being a particularly clear example, but they rarely were seen to perform at the extreme end of poor reproduction of experimental values; when underpolarized charges were optimal, it was the overpolarized charges that did worst, and vice versa. Thus, when

TABLE 5: Performance of Select Charge Determination Methods on Hydrocarbons[a]

	MK ESP	RESP 1X	RESP 2X	RESP Polar H	ChelpG
			Alkanes		
HF/6-31G*	0.60	0.54	0.54	0.45	0.56
HF/6-31G*$^+$	0.60	0.54	0.54	0.45	0.57
B3LYP/6-31G*	0.56	0.51	0.50	0.44	0.52
B3LYP/6-31G*$^+$	0.58	0.54	0.54	0.45	0.56
			Aromatics		
HF/6-31G*	0.50	0.63	0.64	0.62	1.80
HF/6-31G*$^+$	0.54	0.56	0.57	0.56	1.89
B3LYP/6-31G*	1.27	1.53	1.54	1.53	2.57
B3LYP/6-31G*$^+$	0.86	1.15	1.16	1.14	2.67
			Alkenes		
HF/6-31G*	1.00	0.88	0.88	0.89	0.67
HF/6-31G*$^+$	1.26	1.14	1.14	1.16	0.72
B3LYP/6-31G*	0.76	0.70	0.70	0.72	0.56
B3LYP/6-31G*$^+$	1.01	0.89	0.89	0.91	0.58
			Alkynes		
HF/6-31G*	1.80	1.67	1.67	1.67	1.05
HF/6-31G*$^+$	2.17	2.03	2.03	2.03	1.01
B3LYP/6-31G*	1.28	1.16	1.15	1.15	0.61
B3LYP/6-31G*$^+$	1.66	1.53	1.53	1.54	0.54

[a] Even on pure hydrocarbons, there is significant variation in the performance of different methods. ChelpG ESP fitting does uniformly worse on aromatic molecules but uniformly better on alkenes and alkynes, than do the MK based methods. Average absolute errors are given in kcal/mol.

the results are taken as a whole, the methods that produced intermediate polarity charge distributions did the best. It has been noted that, due to the lack of polarization effects assumed with a fixed-charge model, methods that slightly overestimate (by about 10−20%) the gas phase charges generally perform better.[37,44,70] The charges derived from HF/6-31G* electrostatic potentials satisfy this criterion and do well both in this work and in free energy perturbation simulations.[44] The results described here are consistent with this view—the top performing methods all have similar charges on the most polar atoms, and the HF/6-31G* derived charges are among this set—but it is important to note that these charges do not perform optimally in all cases. For some molecule types, a charge distribution less polarized than that obtained from HF/6-31G* potentials is preferred, and in other cases a more polarized method does better. It is not clear, however, if the HF/6-31G* derived charges are equally overpolarized in all these cases.

ChelpG and Merz−Kollman Methods Differ Primarily in Hydrocarbon Charges. For many levels of theory, and for all of the top performing levels, charges fit to the electrostatic potential by the ChelpG procedure generally performed worse than those fitted by the Merz−Singh−Kollman scheme. The largest difference in the charges derived by these two methods was in the hydrocarbon charge distributions (Table 5). For all nonaliphatic hydrocarbons, ChelpG yielded smaller magnitude charges on the CH dipole, and this difference was greater than that seen by varying the level of theory at which the potentials were generated. In the case of aromatic residues, these smaller charges resulted in a much poorer performance for ChelpG relative to MK based schema, whereas a slight benefit was seen for ChelpG in the performance on alkenes, and a larger benefit was seen in the performance on alkynes. The poor performance on aromatic residues, coupled with the relatively large number of aromatic molecules in the data set, led to a slightly poorer performance by ChelpG overall. For alkanes, there were significant differences in charges between the ChelpG and MK methods, with ChelpG again producing smaller charges (although not as small as were obtained by restrained fits), but these changes had little effect on the energetics of solvation, as can be seen by the similar performance of the restrained and unrestrained MK based methods. As the same procedure is used to determine the charges from the ESP in both the ChelpG and the Merz−Singh−Kollman methods, these differences must result from differences in selection scheme for the points at which the ESP is calculated.

That such large variations in charge were seen between different electrostatic potential fitting procedures suggests that the weak electrostatic potential produced by hydrocarbons, even in the slightly polar unsaturated systems, poorly defines a point-charge distribution. In the polar functionalities, on the other hand, the potential is much stronger and thus clearly defines the fit partial atomic charges. For these groups, the ChelpG and MK fitting procedure gave nearly identical charges, with much smaller variation between the different fitting methods than was seen between different levels of theory for the calculation of the electrostatic potential.

It should be noted that all the electrostatic potential fitting procedures have several parameters that may be varied. For all methods, the density and expansiveness of the ESP grid can be changed, and the RESP method could be applied to the Chelp and ChelpG grids. The strength of the restraints used in the RESP method can also be varied. Furthermore, singular-value decomposition (SVD) may be used to precondition the (usually rank deficient) least-squares matrix in the Lagrange-multiplier methods,[76] and this preconditioning can be implemented in several ways. It is possible that variation of these parameters could improve the performance of the charges obtained through these procedures.

Another consideration is that the choices of radii and charges for continuum electrostatic calculations are not independent. Similar solvation energies may be computed if the radii of the atoms in a molecule increase along with the polarity. All the calculations here used a fixed set of radii—identical to those found in the PARSE parameter set for C, N, O, S, and H—based on Pauling van der Waals radii.[69] This provides for interoperability with the PARSE parameter set. However, a different choice of radii may favor the performance of different methods. Larger radii would likely make methods that resulted in more polarized charge give better agreement to experiment, as would smaller radii for methods giving less polarized charges. Pauling radii, however, give a very simple set of radii, based only on atom type and hybridization, and they thus are easily extendable to any molecule. In addition, a simple set of radii, based not on parametrization but on detailed computation or experimental observation (the Pauling radii are derived from crystal packing data),[69] is more consistent with the approach we have taken to evaluate the performance of existing charge determination methods, as opposed to fitting a new set of parameters. One valuable approach to select an alternative radius set is to simultaneously fit solvation and interaction free energies, since the latter should be less sensitive to radii than the former but equally sensitive to charge magnitude.

Other charge determination methods have been developed in addition to those included in this study, including several new methods that have been developed in recent years. These include (1) a modification of the SVD based method used in the Chelp/ChelpG procedures, in which a projection of the Mulliken charges into the null space replaces the null vectors generally used in SVD,[77] (2) a restrained ESP fitting procedure, based on RESP but modified to be less dependent on the ESP grid density,[42] and (3) a method based on combining AM1

population charges with a bond charge correction fit to reproduce HF/6-31G* ESP derived charges.[78] It would be interesting to see how these methods perform under a similar analysis, although, given the relatively small differences between various ESP fitting schemes at the same level of theory, it is unlikely that the first two methods would offer significant improvements. However, the third method—based on the semiempirical AM1 method—is much faster than ab initio methods at the levels of theory required for good performance. Since the correction procedure is designed to produce HF/6-31G*-like charges, which did particularly well in this study, it is possible that this method would provide a useful means for fast evaluation of charge distributions to use in continuum electrostatic calculations. The results described here may provide a basis of comparison for the performance of additional charge determination methods within a continuum solvation model, providing a detailed set of data against which any charge parameter set or charge determination method may be compared.

4. Conclusions

A detailed analysis of the performance of charges determined by a large number of ab initio methods in continuum solvation calculations was performed using two sets of molecules, one based on amino acid side chains, and a second representative of a broad range of organic functionalities. The results clearly demonstrate that particular basis sets and levels of quantum mechanical theory yield charges that give much closer agreement to experiment than others. Rather than larger basis sets and higher levels of theory giving better results, the best results for the data set based on protein groups are obtained with the modestly sized 6-31G* basis set at the Hartree−Fock level of theory. In addition, on the same data set, the charges determined at the HF/3-21G and B3LYP/4-21G levels of theory perform surprisingly well, surpassing the performance of many higher levels of theory. With a more extensive set of molecules, these theoretical methods, with the exception of HF/3-21G, continue to produce the charges that most accurately reproduce experimental values. Although the best method for the larger set is based on potentials from a relatively costly B3LYP/6-311G*$^{+}$ quantum mechanical calculation, the charges from certain lower levels of theory perform with almost identical accuracy. Among these are the HF/6-31G* derived charges, upholding the widely held view that the degree of polarization inherent in this level of theory is particularly well suited for the derivation of fixed-charge models. In addition, charges derived from the computationally inexpensive B3LYP/4-31G potentials perform similarly well.

Semiempirical methods and the minimal ab initio basis set STO-3G were both found to produce charges that did a very poor job of reproducing experimental solvation free energies, as was the case for charges at any theoretical level from Mulliken population analysis. Interestingly, the Merz−Singh−Kollman electrostatic potential fitting method and the associated RESP restrained ESP fitting procedure in general produced charges that are more suited for the solvation calculations done here than are the charges produced by the Chelp or ChelpG ESP fitting methods, although this may be a result of uneven sampling of certain chemical functionalities.

No method does well on all types of molecules, with some methods producing partial charges too large in magnitude, and others producing charges that are too small. The top performing methods attain a good balance for many molecules but still err on both sides for some functionalities. For molecules containing amines, none of the methods produced charges that can adequately reproduce experiment, a result observed in numerous previous studies using both explicit and continuum solvent and with both empirical force fields and quantum mechanics.[45,72,73] However, Rizzo and Jorgensen[75] have shown that reasonable agreement with experiment is attainable within a molecular mechanics framework using explicit solvent by explicitly considering hydrogen bond interaction strengths in the parametrization. Thus, with the appropriate correction terms, better performance for amines may be possible. In general, the same charges on functional group atoms give the best results in all molecules. This suggests that a functional group based parameter set may give the best results for a large number of molecules. While parameter set based methods always have the drawback of being not directly extensible to new functionalities, if the development was done using a clear protocol of combining charges derived from quantum mechanical electrostatic potentials and any available experimental data, this may be applicable to a large subset of the molecules of interest. The development of such a parameter set is beyond the scope of this work, but it could significantly enhance the accuracy of continuum solvation calculations on small molecules. In addition, the data described here provide a wide sampling of the performance of various charge determination methods, and thus, they may provide a basis for evaluating the performance of additional charge determination methods or parameter sets in terms of their applicability in continuum electrostatic calculations.

Acknowledgment. This work is dedicated to the memory of Professor Peter A. Kollman, who made great contributions to the computational study of biological systems, both through the development of new methodologies and by continuously pushing at the boundaries of computational experiments. Special thanks are extended to B. Woody Sherman and Michael D. Altman for numerous helpful discussions. This work was partially supported by a Merck/MIT Graduate Fellowship (to D.F.G.), by the National Institutes of Health (GM-066524 and GM-055758), and by the National Science Foundation (MCB-0091001).

Supporting Information Available: A list of all molecules included in each data set of this study. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Warwicker, J.; Watson, H. C. Calculation of the electric potential in the active site cleft due to α-helix dipoles. *J. Mol. Biol.* **1982**, *157*, 671.

(2) Gilson, M. K.; Honig, B. H. Calculation of electrostatic potentials in an enzyme active site. *Nature (London)* **1987**, *330*, 84.

(3) Davis, M. E.; McCammon, J. A. Electrostatics in biomolecular structure and dynamics. *Chem. Rev.* **1990**, *90*, 509.

(4) Bashford, D.; Karplus, M. pK_a's of ionizable groups in proteins: Atomic detail from a continuum electrostatic model. *Biochemistry* **1990**, *29*, 10219.

(5) Honig, B.; Sharp, K.; Yang, A.-S. Macroscopic models of aqueous solutions: Biological and chemical applications. *J. Phys. Chem.* **1993**, *97*, 1101.

(6) Sheinerman, F. B.; Norel, R.; Honig, B. Electrostatic aspects of protein−protein interactions. *Curr. Opin. Struct. Biol.* **2000**, *10*, 153.

(7) Gilson, M. K.; Honig, B. Destabilization of an alpha-helix-bundle protein by helix dipoles. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1524.

(8) Hendsch, Z. S.; Tidor, B. Do salt bridges stabilize proteins? A continuum electrostatics analysis. *Protein Sci.* **1994**, *3*, 211.

(9) Hendsch, Z. S.; Jonsson, T.; Sauer, R. T.; Tidor, B. Protein stabilization by removal of unsatisfied polar groups: Computational approaches and experimental tests. *Biochemistry* **1996**, *35*, 7621.

(10) Xiao, L.; Honig, B. Electrostatic contributions to the stability of hyperthermophilic proteins. *J. Mol. Biol.* **1999**, *289*, 1435.

(11) Spector, S.; Wang, M. H.; Carp, S. A.; Robblee, J.; Hendsch, Z. S.; Fairman, R.; Tidor, B.; Raleigh, D. P. Rational modification of protein stability by the mutation of charged surface residues. *Biochemistry* **2000**, *39*, 872.

(12) Zacharias, M.; Luty, B. A.; Davis, M. E.; McCammon, J. A. Poisson−Boltzmann analysis of the lambda-repressor-operator interaction. *Biophys. J.* **1992**, *63*, 1280.

(13) Misra, V. K.; Sharp, K. A.; Friedman, R. A.; Honig, B. Salt effects on ligand-DNA binding: Minor groove binding antibiotics. *J. Mol. Biol.* **1994**, *238*, 245.

(14) Froloff, N.; Windemuth, A.; Honig, B. On the calculation of binding free energies using continuum methods: Application to MHC class I protein-peptide interactions. *Protein Sci.* **1997**, *6*, 1293.

(15) Misra, V. K.; Hecht, J. L.; Yang, A.-S.; Honig, B. Electrostatic contributions to the binding free energy of the λ cI repressor to DNA. *Biophys. J.* **1998**, *75*, 2262.

(16) Hendsch, Z. S.; Tidor, B. Electrostatic interactions in the GCN4 leucine zipper: Substantial contributions arise from intramolecular interactions enhanced on binding. *Protein Sci.* **1999**, *8*, 1381.

(17) Nohaile, M. J.; Hendsch, Z. S.; Tidor, B.; Sauer, R. T. Altering dimerization specificity by changes in surface electrostatics. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3109.

(18) Archontis, G.; Simonson, T.; Karplus, M. Binding free energies and free energy components from molecular dynamics and Poisson−Boltzmann calculations. Application to amino acid recognition by aspartyl-tRNA synthetase. *J. Mol. Biol.* **2001**, *306*, 307.

(19) Lee, L.-P.; Tidor, B. Optimization of electrostatic binding free energy. *J. Chem. Phys.* **1997**, *106*, 8681.

(20) Kangas, E.; Tidor, B. Electrostatic complementarity at ligand binding sites: Application to chorismate mutase. *J. Phys. Chem. B* **2001**, *105*, 880.

(21) Sulea, T.; Purisima, E. O. Optimizing ligand charges for maximum binding affinity. A solvated interaction energy approach. *J. Phys. Chem. B* **2001**, *105*, 889.

(22) Sarkar, C. A.; Lowenhaupt, K.; Horan, T.; Boone, T. C.; Tidor, B.; Lauffenbuger, D. A. Rational cytokine design for increased lifetime and enhanced potency using pH-activated "histidine switching". *Nat. Biotechnol.* **2002**, *20*, 908.

(23) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983**, *4*, 187.

(24) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc* **1984**, *106*, 765.

(25) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. An all atom force field for simulations of proteins and nucleic acids. *J. Comput. Chem.* **1986**, *7*, 230.

(26) Jorgensen, W. L.; Tirado-Rives, J. The OPLS potential function for proteins. Energy minimizations for crystals of cyclic peptides and crambin. *J. Am. Chem. Soc* **1988**, *110*, 1657.

(27) van Gunsteren, W. F.; Berendsen, H. J. C. Computer simulation of molecular dynamics: Methodology, applications and perspectives in chemistry. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 992.

(28) Sitkoff, D.; Sharp, K. A.; Honig, B. Accurate calculation of hydration free energies using macroscopic solvent models. *J. Phys. Chem.* **1994**, *98*, 1978.

(29) Cornell, W. D.; Cieplek, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. A 2nd generation force-field for the simulation of proteins, nucleic acids and organic-molecules. *J. Am. Chem. Soc.* **1995**, *117*, 5179.

(30) Nina, M.; Beglov, D.; Roux, B. Atomic radii for continuum electrostatics calculations based on molecular dynamics free energy simulations. *J. Phys. Chem. B* **1997**, *101*, 5239.

(31) MacKerell, A. D.; Bashford, D.; Bellott, M.; Dunbrack, R. L.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B* **1998**, *102*, 3586.

(32) Banavali, N. K.; Roux, B. Atomic radii for continuum electrostatics calculations on nucleic acids. *J. Phys. Chem. B* **2002**, *106*, 11026.

(33) Dixit, S. B.; Bhasin, R.; Rajasekaran, E.; Jayaram, B. Solvation thermodynamics of amino acids. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 1105.

(34) Mulliken, R. S. Electronic population analysis on LCAO-MO molecular wave functions I. *J. Chem. Phys.* **1955**, *23*, 1833.

(35) Momany, F. A. Determination of partial atomic charges from ab initio molecular electrostatic potentials. Application to formamide, methanol, and formic acid. *J. Phys. Chem.* **1978**, *83*, 592.

(36) Singh, U. C.; Kollman, P. A. An approach to computing electrostatic charges for molecules. *J. Comput. Chem.* **1984**, *5*, 129.

(37) Besler, B. H.; Merz, K. M.; Kollman, P. A. Atomic charges derived from semiempirical methods. *J. Comput. Chem.* **1990**, *11*, 431.

(38) Chirlain, L. E.; Francl, M. M. Atomic charges determined from electrostatic potentials: A detailed study. *J. Comput. Chem.* **1987**, *8*, 894.

(39) Breneman, C. M.; Wiberg, K. B. Determining atom-centered monopoles from molecular electrostatic potentials. The need for high sampling density in formamide conformation analysis. *J. Comput. Chem.* **1990**, *11*, 361.

(40) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. A well-behaved electrostatic potential based method using charge restraints for determining atom-centered charges: The RESP model. *J. Phys. Chem.* **1993**, *97*, 10269.

(41) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Kollman, P. A. Application of RESP charges to calculate conformational energies, hydrogen bond energies, and free energies of solvation. *J. Am. Chem. Soc* **1993**, *115*, 9620.

(42) Henchman, R. H.; Essex, J. W. Generation of OPLS-like charges from molecular electrostatic potential using restraints. *J. Comput. Chem.* **1999**, *20*, 483.

(43) Henchman, R. H.; Essex, J. W. Free energies of hydration using restratined electrostatic potential derived charges via free energy pertubations and linear response. *J. Comput. Chem.* **1999**, *20*, 499.

(44) Carlson, H. A.; Nguyen, T. B.; Orozco, M.; Jorgensen, W. L. Accuracy of free energies of hydration for organic molecules from 6-31G*-derived partial charges. *J. Comput. Chem.* **1993**, *14*, 1240.

(45) Tannor, D. J.; Marten, B.; Murphy, R.; Friesner, R. A.; Sitkoff, D.; Nicholls, A.; Honig, B.; Ringnalda, M.; Goddard, W. A., III. Accurate first principles calculation of molecular charge distributions and solvation energies from ab initio quantum mechanics and continuum dielectric theory. *J. Am. Chem. Soc* **1994**, *116*, 11875.

(46) Marten, B.; Kim, K.; Cortis, C.; Friesner, R. A.; Murphy, R. B.; Ringnalda, M. N.; Sitkoff, D.; Honig, B. New model for calculation of solvation free energies: Correction of self-consistent reaction field continuum dielectric theory for short-range hydrogen-bonding effects. *J. Phys. Chem.* **1996**, *100*, 11775.

(47) Reddy, M. R.; Erion, M. D.; Agarwal, A.; Viswanadhan, V. N.; McDonald, D. Q.; Still, W. C. Solvation free energies calculated using the GB/SA model: Sensitivity of results on charge sets, protocols, and force fields. *J. Comput. Chem.* **1998**, *19*, 769.

(48) *Jaguar*, version 3.5; Schrödinger, Inc.: Portland, OR, 1998.

(49) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzerwski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, J.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1995.

(50) Stewart, J. J. P. *MOPAC 7.0*—Public Domain Version.

(51) Gilson, M. K.; Sharp, K. A.; Honig, B. H. Calculating the electrostatic potential of molecules in solution: Method and error assessment. *J. Comput. Chem.* **1987**, *9*, 327.

(52) Gilson, M. K.; Honig, B. Calculation of the total electrostatic energy of a macromolecular system: Solvation energies, binding energies, and conformational analysis. *Proteins* **1988**, *4*, 7.

(53) Sharp, K. A.; Honig, B. Electrostatic interactions in macromolecules: Theory and applications. *Annu. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 301.

(54) Sharp, K. A.; Honig, B. Calculating total electrostatic energies with the nonlinear Poisson−Boltzmann Equation. *J. Phys. Chem.* **1990**, *94*, 7684.

(55) Sanner, M. MSMS v.2.5.3. http://www.scripps.edu/pub/olson-web/people/sanner/html/msms_ho me.html.

(56) Chothia, C. Nature of accessible and buried surfaces in proteins. *J. Mol. Biol.* **1976**, *105*, 1.

(57) Giesen, D. J.; Cramer, C. J.; Truhlar, D. G. Entropic contributions to free energy of solvation. *J. Phys. Chem.* **1994**, *98*, 4141.

(58) Chothia, C. Hydrophobic bonding and accessible surface area in proteins. *Nature (London)* **1974**, *248*, 338.

(59) Chothia, C.; Janin, J. Principles of protein−protein recognition. *Nature (London)* **1975**, *256*, 705.

(60) Sharp, K. A.; Nicholls, A.; Fine, R. F.; Honig, B. Reconciling the magnitude of the microscopic and macroscopic hydrophobic effects. *Science (Washington, D. C.)* **1991**, *252*, 106.

(61) De Young, L. R.; Dill, K. A. Paritioning nonpolar solutes into bilayers and amorphous *n*-alkanes. *J. Phys. Chem.* **1990**, *94*, 801.

(62) Ben-Naim, A. Solvation of large molecules: Some exact results on the dependence on volume and surface area of the solute. *Biophys. Chem.* **1994**, *51*, 203.

(63) Chan, H. S.; Dill, K. A. Solvation: Effects of molecular size and shape. *J. Chem. Phys.* **1994**, *101*, 7007.

(64) Wallqvist, A.; Berne, B. J. Molecular dynamics study of the dependence of water solvation free energy on solute curvature and surface area. *J. Phys. Chem.* **1995**, *99*, 2885.

(65) Hermann, R. B. Modeling hydrophobic solvation of nonsperical systems: Comparison of use of molecular surface area with accessible surface area. *J. Comput. Chem.* **1997**, *18*, 115.

(66) Matubayashi, N.; Gallicchio, E.; Levy, R. M. On the local and nonlocal components of solvation thermodynamics and their relation to solvation shell models. *J. Chem. Phys.* **1998**, *109*, 4864.

(67) Shimizu, S.; Ikeguchi, M.; Nakamura, S.; Shimizu, K. Molecular volume, surface area, and curvature dependence of the configurational entropy change upon solvation: Effects of molecular bonding. *Chem. Phys. Lett.* **1998**, *284*, 235.

(68) Southall, N. T.; Dill, K. A. The mechanism of hydrophobic solvation depends on solute radius. *J. Phys. Chem. B* **2000**, *104*, 1326.

(69) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960.

(70) Watanabe, K.; Klein, M. L. Effective pair potentials and the properties of water. *Chem. Phys.* **1989**, *131*, 157.

(71) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. Group contributions to the thermodynamic properties of nonionic organic solutes in dilute aqueous solution. *J. Solution Chem.* **1981**, *10*, 563.

(72) Ding, Y.; Bernardo, D. N.; Krogh-Jespersen, K.; Levy, R. M. Solvation free energies of small amides and amines from molecular dynamics/free energy perturbation simulations using pairwise additive and many-body polarizable potentials. *J. Phys. Chem.* **1995**, *99*, 11575.

(73) Morgantini, P.-Y.; Kollman, P. A. Solvation free energies of amides and amines: Disagreement between free energy calculations and experiment. *J. Am. Chem. Soc.* **1995**, *117*, 6057.

(74) Meng, E. C.; Caldwell, J. W.; Kollman, P. A. Investigating the anomalous solvation free energies of amines with a polarizable potential. *J. Phys. Chem.* **1996**, *100*, 2367.

(75) Rizzo, R. C.; Jorgensen, W. L. OPLS all-atom model for amines: Resolution of the amine hydration problem. *J. Am. Chem. Soc.* **1999**, *121*, 4827.

(76) Francl, M. M.; Carey, C.; Chirlian, L. E.; Gange, D. M. Charges fit to electrostatic potentials. II. Can atomic charges be unambiguously fit to electrostatic potentials? *J. Comput. Chem.* **1996**, *17*, 367.

(77) Ridard, J.; Lévy, B. Effective atomic charges in alanine dipeptide. *J. Comput. Chem.* **1999**, *20*, 473.

(78) Jakalian, A.; Bush, B. L.; Jack, D. B.; Bayly, C. I. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: I. Method. *J. Comput. Chem.* **2000**, *21*, 132.