

Application of RESP Charges To Calculate Conformational Energies, Hydrogen Bond Energies, and Free Energies of Solvation

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Abstract: We apply a new restrained electrostatic potential fit charge model (two-stage RESP) to conformational analysis and the calculation of intermolecular interactions. Specifically, we study conformational energies in butane, methyl ethyl thioether, three simple alcohols, three simple amines, and 1,2-ethanediol as a function of charge model (two-stage RESP vs standard ESP) and 1-4 electrostatic scale factor. We demonstrate that the two-stage RESP model with a 1-4 electrostatic scale factor of $\sim 1/1.2$ is a very good model, as evaluated by comparison with high-level *ab initio* calculations. For methanol and *N*-methylacetamide interactions with TIP3P water, the two-stage RESP model leads to hydrogen bonds only slightly weaker than found with the standard ESP charges. In tests on DNA base pairs, the two-stage RESP model leads to hydrogen bonds which are ~ 1 kcal/mol weaker than those calculated with the standard ESP charges but closer in magnitude to the best current available *ab initio* calculations. Furthermore, the two-stage RESP charges, unlike the standard ESP charges, reproduce the result that Hoogsteen hydrogen bonding is stronger than Watson-Crick hydrogen bonding for adenine-thymine base pairs. The free energies of solvation of both methanol and *trans-N*-methylacetamide were also calculated for the standard ESP and two-stage RESP models and both were in good agreement with experiment. We have combined the use of two-stage RESP charges with multiple conformational fitting—recently employed using standard ESP charges as described by Reynolds, *et al.* (*J. Am. Chem. Soc.* **1992**, *114*, 9075)—in studies of conformationally dependent dipole moments and energies of propylamine. We find that the combination of these approaches is synergistic in leading to useful charge distributions for molecular simulations. Two-stage RESP charges thus reproduce both intermolecular and intramolecular energies and structures quite well, making this charge model a critical advancement in the development of a general force field for modeling biological macromolecules and their ligands, both in the gas phase and in solution.

Introduction

It is hard to overestimate the importance of electrostatic effects in the energetics of most intermolecular interactions. The ability to simulate such intermolecular interactions accurately using empirical force fields requires great care in the development of the electrostatic model. The use of *ab initio* electrostatic potential derived (ESP) charges has been a promising start in this pursuit.¹⁻³ With a suitable basis set for the calculation that is balanced with effective two-body potential water models, e.g. 6-31G*, one expects a very good reproduction of experimental free energies of solvation. This is indeed the case.

One problem with electrostatic potential fit charges, however, is that they are conformationally dependent.⁴⁻⁶ Furthermore, the conformational energies which are calculated using standard ESP charges are not sufficiently in agreement with experimental results and high-level theoretical calculations and therefore require adjustment through the contribution of the torsional energy term. Because charges on common functional groups are not consistent between homologous molecules, one is unable to derive torsional parameters to adjust the conformational energies for certain classes of molecules.⁷ That is because any error in the confor-

mational energies resulting from the nonbonded electrostatic contribution is not systematic.

These problems have led to the development of a new charge model which restrains the magnitude of the partial atomic charges that are least well determined by the electrostatic potential—RESP charges. We show that this model reasonably meets the challenge to restrain the charges on nonpolar groups without greatly reducing the charges on polar groups and thereby having a deleterious effect on important intermolecular interactions such as hydrogen bonding and free energies of solvation. In addition, we address below the issue of whether to attenuate the electrostatic interaction between atoms separated by exactly three bonds (1-4 interactions). By comparison with high-level *ab initio* calculations on 1,2-ethanediol, we are able to suggest an optimum 1-4 electrostatic scale factor and evaluate the sensitivity of the model to the exact value of this scale factor.

Below we present the results of studies of conformational energies, hydrogen bonding energies, and free energies of solvation for a model which is a reasonable compromise between the need to have large charges on polar atoms to reproduce intermolecular interaction energies and small charges on nonpolar atoms to reproduce intramolecular conformational energies. The evolution of this two-stage model is described in detail in another paper.⁸ In both stages of the fit, restraints are used only on non-hydrogen atoms. In the first stage, the charges are optimized and any necessary molecular symmetry is imposed by constraining charges on equivalent atoms to have the same value. Two types of equivalent atoms are not constrained to be equivalent in the first stage, however. These are hydrogens within methyl and methylene

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Table I. Summary of Various Charge Models Examined in This Paper^{a,b}

model	hydrogen atoms	non-hydrogen atoms	methyl and methylene hydrogens	other equivalent atoms
un.ap	unrestrained	unrestrained	made equivalent <i>a posteriori</i> to fit	made equivalent <i>a posteriori</i> to fit
st.ap	unrestrained	strong restraint	made equivalent <i>a posteriori</i> to fit	constrained to be equivalent during fit
wk.ap	unrestrained	weak restraint	made equivalent <i>a posteriori</i> to fit	constrained to be equivalent during fit
st.eq	unrestrained	strong restraint	constrained to be equivalent during fit	constrained to be equivalent during fit
wk.eq	unrestrained	weak restraint	constrained to be equivalent during fit	constrained to be equivalent during fit
wk.fr/st.eq	unrestrained	stage 1, weak restraint; stage 2, strong restraint (only methyl and methylene groups, both C's and H's, refit in stage 2—other charges frozen)	stage 1, "free" (no constrained equiv); stage 2, constrained to be equivalent during fit	constrained to be equivalent during stage 1

^a Atoms made equivalent "a posteriori to fit" were made so by averaging their charges. ^b Only results for models employing a *hyperbolic* restraint are presented in this paper. The less satisfactory results obtained using a harmonic restraint are presented in a related paper.⁸

groups. The carbon and hydrogen atoms in those groups are reoptimized in the second stage of the fit in the presence of frozen charges from the first stage on the other atoms. This two-stage fit was found to be necessary because a one-stage fit which constrained methyl hydrogens to have equivalent charges adversely affected charges on nearby polar atoms.

Methods

A. Charge Models. The derivation of the final charge model is described in detail in another paper.⁸ The terminology and notation for describing the charge models are as follows. The term "RESP" is used to refer to any of the restrained ESP models. The models are distinguished by the strength of the restraint used (field 1) and the treatment of the methyl and methylene hydrogens (field 2). Standard ESP charges (un.ap) were calculated according to the method described by Singh and Kollman.³ The notation then refers to the fact that the charges were unrestrained (un) and that methyl and methylene hydrogen charges were averaged *a posteriori* (ap) to the fit. Even though all three methyl hydrogens are rarely equivalent by formal molecular symmetry, it is necessary for them to have equivalent charges because they will interchange under the conditions of molecular dynamic and should therefore be indistinguishable.

Five other models are examined in this paper. Four of the models resulted from one-stage optimization of the charges with the inclusion of hyperbolic restraints on non-hydrogen atoms. Both a strong restraint of 0.0010 au (st) and a weak restraint of 0.0005 au (wk) were tested. Methyl hydrogen atoms were either averaged *a posteriori* (ap) to the fit or constrained to be equivalent during the fit (eq). The four models arrived at were thus (st.ap), (st.eq), (wk.ap), and (wk.eq).

The fifth and preferred model (wk.fr/st.eq) resulted from a two-stage fitting process where the charges were optimized in the first stage with weak hyperbolic restraints of 0.0005 au on non-hydrogen atoms. In the second stage, charges were frozen on all atoms except those in methyl and methylene groups, and the charges on those atoms were then reoptimized in the presence of strong hyperbolic restraints on the non-hydrogen atoms (i.e. the methyl and methylene carbons). Methyl or methylene hydrogens were thus free (fr) in the first stage and not constrained to have equivalent charges within each group (eq) until the second stage. When charges on non-methyl or non-methylene atoms needed to be equivalent (such as those on an amino group's two hydrogens or the two oxygens in 1,2-ethanediol), they were constrained to be so in the first stage. This fifth model is also referred to as the "two-stage" model. The two-stage model presented here is not all-inclusive. Additional issues not addressed in this paper will be examined in a future paper presenting charges for the nucleic acids and amino acids.⁹ We summarize our notation in Table I.⁸

The quality of the fit of the classically generated (from the atomic charges) electrostatic potential to the quantum mechanically determined one is reported in terms of the relative rms (RRMS), given by

$$RRMS = \chi_{ESP}^2 / \sum V_i^2$$

B. Bonded and van der Waals Parameters. Bond, angle, and torsion parameters were taken from the Weiner *et al.*¹⁰ all-atom force field.

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Table II. Nonbonded Parameters Used in the Calculations^a

AMBER atom type	description	r* (Å)	ε (kcal/mol)
CT	sp ³ carbon	1.9080	0.1094
C	sp ² carbon	1.9080	0.0860
NT	sp ³ nitrogen	1.8240	0.1700
N	sp ² nitrogen	1.8240	0.1700
OH	alcohol oxygen	1.7210	0.2104
OS	ether oxygen	1.6387	0.1700
O	carbonyl oxygen	1.6612	0.2100
OW	TIP3P water oxygen	1.7683	0.1520
HC	hydrocarbon hydrogen	1.4870	0.0157
H1	hydrocarbon hydrogen with one electronegative neighbor	1.3870	0.0157
HA	aromatic hydrogen	1.4590	0.0150
H	hydrogen on nitrogen	0.6000	0.0157
HW	TIP3P water hydrogen	0.0000	0.0000
S	sulfur	1.9920	0.2500
LP	lone pairs on sulfur	0.0000	0.0000
P	phosphate phosphorous	2.1000	0.2000

^a CT, C, HC, and HA parameters from unpublished work by Spellmeyer and Kollman, ref 12. H1 parameters are from Veenstra and Ferguson, ref 13. P parameters are from the Weiner *et al.* force field, ref 10. TIP3P water is from Jorgensen, ref 14. All others are from the OPLS force field of Jorgensen and Tirado-Rives, ref 15.

Bonded parameters for the aliphatic amino group were adapted from existing parameters in the Weiner *et al.* force field. They are CT-NT, $r_0 = 1.471$ Å and $K_r = 367.0$; CT-CT-NT; $\theta_0 = 109.7^\circ$ and $K_\theta = 80.0$; HC-CT-NT, $\theta_0 = 109.5^\circ$ and $K_\theta = 35.0$; CT-NT-H2, $\theta_0 = 109.5^\circ$ and $K_\theta = 305$; and X-CT-NT-X, a 6-fold degenerate torsion with $V_3 = 1.0$ and a phase of 0° . Van der Waals parameters used are those adapted or developed for the new force field¹¹ and are presented in Table II.^{10,12-15} The conformational studies employed HC atom types on all of the aliphatic hydrogens.

C. Conformational Energy Analyses. Molecular mechanics minimizations were carried out using the AMBER program.¹⁶ Conformations corresponding to rotational barriers were examined using dihedral constraints imposed in the PARM module. For the 1,2-ethanediol minimizations, starting geometries were defined using canonical trans and gauche dihedral values with no constraints. Conformations which

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were not minima on the molecular mechanical potential energy surface were minimized with the necessary dihedral constrained to the 6-31G* quantum mechanically optimized value. Quantum mechanical calculations were carried out using the Gaussian 90 suite of programs.¹⁷

Models for 1,2-ethanediol using different 1–4 electrostatic scale factors were evaluated by three different measures and using three different sets of reference energies: MP3/6-31+G**//HF/6-31G* energies on the four lowest energy conformations¹⁸ and MP2/6-31G**//HF/6-31G* energies on the other six, MP2/6-31G**//HF/6-31G* energies for all conformations,¹⁹ and MM2^{19,20} minimized energies for all conformations. The first of the three measures was simply the sum of the absolute values of the difference between the relative reference energy and the relative molecular mechanics energy for each conformation. The second measure was a Boltzmann-weighted RMS of the difference between the molecular mechanics and reference relative energies. In this case the Boltzmann weight of each conformation was calculated from the reference energy. This procedure then penalized most heavily energy deviations in conformations which were "supposed to" be lower in energy. In the third measure, a Boltzmann-weighted RMS was again calculated, but in this case the molecular mechanics energy was used to assign the Boltzmann weight of each conformation. This procedure penalized for conformations which were not supposed to be low in energy but which had low calculated molecular mechanical energies.

One needs to consider both of the Boltzmann-weighted RMS values together—i.e. the one that uses the reference energy as a weight and the one that uses the calculated energy as a weight. This is because each one neglects the problem that the other measure is flagging. The Boltzmann-weighted RMS values serve best as a means of eliminating models and will not necessarily directly identify an optimal model.

D. Hydrogen Bond Energies. DNA base pairs were set up using the computer graphics program MIDAS.^{21,22} *N*-methylacetamide (NMA) homodimer and NMA–water dimer configurations were set up according to Jorgensen and Swensen.²³ Methanol homodimers and methanol–water dimers were set up according to Tse and Newton.²⁴ Each system was then minimized using the AMBER program with conjugate gradient minimization with a constant dielectric of 1 and 1–4 van der Waals and electrostatic scale factors of $1/2$.

E. Free Energy Perturbation Calculations. All simulations were run using the AMBER program with the all-atom type force field for the bond, angle, and dihedral parameters.¹⁰ Each system initially contained the solute *trans*-NMA with 259 TIP3P water molecules or methanol with 208 TIP3P water molecules. Each system was minimized using 1000 cycles of conjugate gradient minimization followed by 20 ps of molecular dynamics equilibration.

The perturbations of methanol to methane or ethane were carried out with over 202 ps of molecular dynamics simulation using the slow-growth approach.²⁵ Van der Waals parameters and charges were perturbed simultaneously. The potential of mean force (pmf) correction, necessary because of the manner in which AMBER defines the topologies of the perturbed groups of molecules, was calculated for perturbed bonds and added to the total free energy change.²⁶

The above protocol was carried out using the standard ESP charges. Results for free energy differences based on the other three charge sets were obtained by performing shorter perturbations for methanol, involving only changing the standard ESP charges into the new set. These

simulations were carried out using the windows approach²⁷ with 21 windows and 500 steps of molecular dynamics equilibration and 500 steps of data collection for a total of 42 ps.

The perturbation of NMA to methane was carried out with decoupling of the electrostatic and van der Waals components of the perturbation. NMA standard ESP charges were first perturbed to zero during 404 ps of molecular dynamics using the windows approach with 101 windows consisting of 1000 steps of equilibration and 1000 steps of data collection. The van der Waals perturbation was similarly carried out over 404 ps. For the second part of the electrostatic perturbation, that of perturbing methane's neutral atomic charges to standard ESP charges, results were taken from previously published calculations by Sun *et al.*²⁸

The solvation free energies reported for the three RESP models were obtained by carrying out electrostatic perturbations for NMA where only the standard ESP charges were perturbed to RESP charges. These simulations were carried out using the windows approach with 21 windows and 500 steps of molecular dynamics equilibration and 500 steps of data collection for a total of 42 ps. The results were then combined with those from the van der Waals and electrostatic perturbations described in the preceding paragraph. The necessary pmf correction was included as was the Born correction,²⁹ needed to account for the long-range electrostatic effects of perturbing a dipolar species into a nonpolar one.

All simulations were carried out at a constant pressure of 1 atm and a constant temperature of 300 K using a time step of 2 fs with SHAKE³⁰ applied to constrain bond lengths to equilibrium values. A constant dielectric of 1 was employed with an 8-Å cutoff for nonbonded interactions. Periodic boundary conditions were used. All perturbations were performed in the forward and reverse directions. The values of the free energies and errors reported are the mean values of and half of the difference between the free energies for the forward and reverse runs.

Results

A. Conformational Energies in Butane, Methyl Ethyl Thioether, and Simple Alcohols and Amines. Charges were derived for butane using the standard ESP model, the two one-stage restrained ESP models with *a posteriori* averaging on methyl hydrogens, and the two-stage restrained ESP model. The charge on each atom is 4–6 times greater in the standard ESP model compared to the two-stage restrained ESP model. The smaller restrained ESP charges are more consistent with the notion of a nonpolar alkane, but it is important to note that both sets of charges reproduce the molecular electrostatic potential quite well, with the standard ESP charges actually having a slightly smaller relative RMS value (RRMS = 0.89 vs RRMS = 0.90). This underscores the point that one should not rely too heavily on "chemical intuition" when evaluating charge models.

Table III^{31–33} presents the results of molecular mechanics minimization on butane, with the conformational energies and geometries presented as a function of charge model. We should note that the Weiner *et al.* force field used a 1–4 scale factor of $1/2$ for electrostatics. This scaling, however, as noted by Billeter *et al.*³⁴ and Smith and Karplus,³⁵ can lead to artifacts in the conformational energies if relatively large (e.g. 6-31G* electrostatic potential derived) charges are used.

As one can see from Table III, the conformational energies calculated with standard ESP charges are very sensitive to the

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Table III. Relative Molecular Mechanics Conformational Energies of Butane (kcal/mol)

model ^a	CCCC	exptl	standard ESP (un.ap) 1/1-4 electrostatic scale factor			one-stage RESP (wk.ap) 1/1-4 electrostatic scale factor			one-stage RESP (st.ap) 1/1-4 electrostatic scale factor			two-stage RESP (wk.fr/st.eq) 1/1-4 electrostatic scale factor			mm2 ³¹	mm3 ³¹
			1.0	1.2	2.0	1.0	1.2	2.0	1.0	1.2	2.0	1.0	1.2	2.0		
trans	trans	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	gauche	0.75 ³²	0.70	0.42	0.00	0.71	0.63	0.45	0.69	0.67	0.61	0.70	0.67	0.61	0.86	0.81
		(71°)	(68°)	(67°)	(65°)	(68°)	(68°)	(67°)	(68°)	(68°)	(68°)	(68°)	(68°)	(68°)	(65°)	(65°)
	ecl	4.56 ³³	5.06	4.69	4.11	5.20	5.08	4.84	5.19	5.16	5.07	5.20	5.16	5.08	4.73	4.83
gauche	skew		3.32	3.25	3.27	3.35	3.33	3.27	3.35	3.34	3.33	3.35	3.34	3.33	3.34	3.30
	trans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	gauche	0.75	0.66	0.57	0.40	0.68	0.65	0.59	0.68	0.67	0.64	0.66	0.65	0.61	0.86	0.81
		(71°)	(68°)	(68°)	(67°)	(68°)	(68°)	(68°)	(68°)	(68°)	(68°)	(68°)	(68°)	(68°)	(65°)	(65°)
	ecl	4.56	5.10	4.98	4.76	5.17	5.13	5.05	5.17	5.16	5.12	5.14	5.12	5.07	4.73	4.83
	skew		3.33	3.31	3.26	3.35	3.34	3.32	3.35	3.35	3.34	3.34	3.33	3.32	3.34	3.30

^a Conformation used to generate the charges.

Table IV. Relative Molecular Mechanics Conformational Energies of Methyl Ethyl Thioether (kcal/mol)

model ^a	CCSC	exptl H ^b	standard ESP (un.ap) 1/1-4 electrostatic scale factor			one-stage RESP (wk.ap) 1/1-4 electrostatic scale factor			one-stage RESP (st.ap) 1/1-4 electrostatic scale factor			two-stage RESP (wk.fr/st.eq) 1/1-4 electrostatic scale factor		
			1.0	1.2	2.0	1.0	1.2	2.0	1.0	1.2	2.0	1.0	1.2	2.0
trans	trans	0.05-0.20	0.00	0.00	0.63	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
	gauche	0.00	0.40	0.07	0.00	0.35	0.19	0.00	0.33	0.24	0.06	0.38	0.35	0.28
gauche	trans	0.05-0.20	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	gauche	0.00	0.30	0.17	0.00	0.29	0.19	0.03	0.26	0.21	0.08	0.26	0.23	0.19
		(66°)	(65°)	(65°)	(64°)	(65°)	(65°)	(64°)	(65°)	(65°)	(64°)	(65°)	(65°)	(65°)

^a Conformation used to generate the charges. ^b Experimental data given in ref 37.

scale factor and also somewhat sensitive to the conformation from which they were derived. Reynolds *et al.*^{6,36} have previously noted the problems inherent in the conformational dependence of electrostatic potential derived charges. The one-stage weakly restrained charge model (wk.ap) is less sensitive, and the two-stage restrained model (wk.fr/st.eq), the model of choice here, is rather insensitive to the 1-4 scale factor and has much less dependence on the conformation from which the charges were derived. In addition, the most important properties of butane, the relative energies of the trans and gauche conformations, the dihedral angle of the gauche conformation, and the relative energies of the skew and eclipsed conformations, are represented in quite good agreement with experiment using only 3-fold torsions on the C-C dihedrals. In principle, the relative energy of the eclipsed conformation of butane could be adjusted with an additional torsional potential. This is not the purpose of this study, however. Torsion parameters will be fine tuned at a later date.

Standard, one-stage restrained and two-stage restrained ESP charges were next calculated from the gauche and trans conformations of methyl ethyl thioether. In Table IV³⁷ we present the relative conformational energies calculated for the two conformers. Again, the two-stage restrained model has the least conformational and 1-4 scale factor dependence. Here, the wrong conformer is lower in energy, but this could easily be corrected with a small V_1 or V_2 torsional potential.

Standard and two-stage restrained ESP charges were calculated for methanol, ethanol, and propanol. In Table V³⁸⁻⁴¹ we present the conformational energies for these molecules. We use the

results of high-level *ab initio* calculations as an experimental reference for ethanol and propanol. The experimental energies for methanol are from microwave spectroscopy.³⁹ The methanol results are essentially insensitive to both the charge model used and the 1-4 electrostatic scale factor. The scale factor independence is a result of the fact that the 1-4 electrostatic energy is the same for both the staggered and eclipsed conformations. For ethanol, the two-stage model is superior in both its small dependence on 1-4 scale factor and (with 1-4 scale factor of 1/1.2) its excellent agreement with high-level *ab initio* theory.

The relative *ab initio* energies are very small for all conformations of propanol. Neither of the models agrees well with the highest level *ab initio* calculations either in the magnitudes of the relative energies found or in the identity of the global minimum conformation. However, while a different set of high-level *ab initio* calculations (MP2/6-31G**/HF/6-31G*) yields similarly small values for the relative conformational energies, yet another conformation is identified as the global minimum. Furthermore, the MM2 calculated energies also vary more in relative magnitude than the *ab initio* energies (although not as much as our molecular mechanics models) and also find the same global minimum conformation as do our models. Because of the disagreement seen with the higher levels of theory over the minimum energy conformation, we decided that we would be satisfied with a model that gave fairly small conformational energies and chose not to focus on the minimum energy conformation.

For propanol, the two-stage restrained model is both less dependent on the 1-4 scale factor and, with a scale factor of 1/1.2, in respectable agreement with the MM2 model. The fact that the two-stage RESP charges result in lower 1-4 electrostatic energies also means that minimized geometries are less sensitive to the choice of the 1-4 electrostatic scale factor. For example, with the standard ESP charges, the minimized value of the CCOH dihedral in the Gg conformation ranges from 67 to 49°, depending on whether a 1-4 electrostatic scale factor of 1 or 1/2 was used,

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Table V. Relative Molecular Mechanics Conformational Energies for Three Simple Alcohols as a Function of the Charge Model and 1–4 Electrostatic Scale Factor (kcal/mol)

molecule	-CCO ^a	-COH ^a	exptl	standard ESP (un.ap) 1/1–4 electrostatic scale factor			two-stage RESP (wk.fr/st.eq) 1/1–4 electrostatic scale factor			mm2	mm3 ³⁸
				1.0	1.2	2.0	1.0	1.2	2.0		
methanol		stage	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		ecl	1.07 ³⁹	1.01	1.00	1.00	1.02	1.02	1.02	0.87	0.78
ethanol	stag	trans	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	stag	g+	0.40 (54°) ⁴⁰	0.00 (55°)	0.22 (62°)	1.04 (73°)	0.36 (64°)	0.46 (65°)	0.65 (67°)	0.60 (65°)	0.40
	stag	ecl	1.66 ^b	0.65	1.19	2.65	1.50	1.67	2.00		
propanol	ecl	trans	3.08 ⁴¹	3.49	3.16	3.09	3.10	3.09	3.07	2.73	2.80
	trans	g+	0.19 ^b	1.41	1.14	3.92	0.99	0.94	1.74	0.62	
	g+	g-	0.33	1.78	0.22	0.00	1.32	0.60	0.00	0.97	
	g+	g+	0.17	3.01	1.54	1.55	2.36	1.64	1.06	1.03	
	trans	trans	0.18	0.00	0.00	3.40	0.00	0.00	0.90	0.00	
	g+	trans	0.00	1.78	0.67	1.55	1.56	0.93	0.50	0.34	

^a First dihedral atom refers to a carbon, when present. ^b MP3/6-31+G**//HF/6-31G* energies for ethanol CCOH barrier and propanol—unpublished results from T. A. Halgren.

Table VI. Relative Molecular Mechanics Conformational Energies for Three Simple Amines as a Function of the Charge Model and 1–4 Electrostatic Scale Factor (kcal/mol)

molecule	-CCN ^a	-CN(1p) ^a	exptl	standard ESP (un.ap) 1/1–4 electrostatic scale factor			two-stage RESP (wk.fr/st.eq) 1/1–4 electrostatic scale factor			mm2 ⁴²	mm3 ⁴³
				1.0	1.2	2.0	1.0	1.2	2.0		
methylamine		stag	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		ecl	1.98 ⁴⁴	1.97	1.98	1.99	2.01	2.01	2.01	1.90	1.45
ethylamine	stag	trans	0.00	0.00	0.00	0.38	0.00	0.00	0.00	0.13	0.10
	stag	g	0.34 ⁵	1.59	0.89	0.00	0.84	0.59	0.10	0.00	0.00
propylamine	trans	g+	0.30 ^b	0.01	0.61	3.16	0.20	0.20	0.94	0.00	(0.00) ^c
	g+	trans	0.32	0.55	0.00	0.00	0.53	0.11	0.00	1.00	0.42
	trans	trans	0.00	0.00	0.43	2.63	0.00	0.00	0.72	0.13	0.00
	g+	g-	0.25	1.32	1.01	1.50	1.35	0.96	0.90	0.65	(0.48)
	g+	g+	0.61	0.73	0.37	0.75	0.83	0.44	0.35	0.88	(0.88)

^a First dihedral atom refers to a carbon, when present. ^b MP3/6-31+G**//HF/6-31G* energies for propylamine—unpublished results from T. A. Halgren. ^c Schmitz and Allinger report two sets of relative energies for the five propylamine conformers. One set is relative to the ground state with the CCCN torsion in the trans conformation, and the other set is for a ground state with CCCN in the gauche conformation.

respectively. When two-stage RESP charges are used, this range is reduced to 63 to 60° and agrees well with the MM2 value of 62°.

Finally, we turn to the amines. Table VI^{42–45} presents the conformational energies as a function of the charge model and 1–4 electrostatic scale factor using standard and two-stage restrained ESP charges for methylamine, ethylamine, and propylamine. Methylamine is shown to be insensitive to the 1–4 scale factor, and its barrier to rotation is calculated as being in excellent agreement with the experimental value. For ethylamine, using the standard ESP charges, the minimum energy conformation and conformational energy difference are quite sensitive to the scale factor. This sensitivity is reduced with the restrained ESP charges, and using a scale factor of 1/1.2, the conformation having the lone pair gauche to the β -carbon is found to be 0.59 kcal/mol higher in energy than the trans conformation—about 0.3 kcal/mol too high. Again, this difference could possibly be adjusted with torsion parameters, depending on the error seen with this particular torsion in other contexts (such as propylamine). Our error is on the order of that given by MM3, which finds the trans conformation to be higher in energy by 0.1 kcal/mol.

The propylamine energies are evaluated against high-level *ab initio* calculations. As with propanol, the relative conformational energies are quite small. Here both the standard ESP and two-stage restrained ESP charges yield good results when a scale factor of 1/1.2 is used. All energies are about 1 kcal/mol or less, and the minimum energy conformation found by our molecular

mechanics models is in agreement with that found by the high-level *ab initio* calculations.

B. 1–4 Electrostatic Scale Factor Calculations on 1,2-Ethanediol. We chose 1,2-ethanediol as a particularly sensitive model system for examining conformational energies as a function of the charge model and 1–4 electrostatic scale factor. This sensitivity arises from the fact that 1,2-ethanediol has three significant dihedrals and two polar atoms in a 1–4 configuration. One can define 10 unique conformations where each of the three dihedrals is in either a trans or gauche conformation. Table VII presents the relative conformational energies calculated using only the two-stage fit charges (derived from the all-trans conformation) as a function of 1–4 scale factor. These energies are compared with two *ab initio* quantum mechanical models: the first is MP3/6-31+G**//HF/6-31G* energies for the four lowest energy conformations¹⁸ and MP2/6-31G**//HF/6-31G* energies for the rest; the second is MP2/6-31G**//HF/6-31G* energies for all 10 conformations. A third set of energies which is used for comparison was calculated using MM2.

These data show that, on the basis of the absolute errors, a scale factor of 1/1.1 performs best with the restrained ESP charges. However, the lowest energy conformations are arguably the most important, and the Boltzmann-weighted RMS values show that a slightly smaller scale factor results in better agreement for the four lowest energy conformations. We feel that a scale factor of 1/1.2 gives the best agreement with the *ab initio* energies and is significantly superior to a scale factor of 1 or 1/2 in this regard. This model performs even better than MM2 on this molecule, even though no reoptimization of torsional potentials has been carried out—only the standard 3-fold parameters from the Weiner *et al.*¹⁰ force field were used. The choice of a scale factor of 1/1.2 is supported by the conformational energies seen

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Table VII. Relative Molecular Mechanics Conformational Energies of 1,2-Ethanediol as a Function of Conformation and the 1–4 Electrostatic Scale Factor using Two-Stage RESP (wk.fr/st.eq) Charges (kcal/mol)^a

conformation ^b	MP3/MP2 ^c	MP2 ^d	MM2 ^e	1/1–4 electrostatic scale factor					
				1.0	1.1	1.2	1.3	1.5	2.0
tTt	2.69	3.37	1.51	0.00	1.16	2.58	3.91	6.24	10.53
tGt	4.45	4.45	2.19	3.75	4.51	5.16	5.81	7.03	9.40
tTg	3.41	3.41	2.52	1.50	2.53	3.83	5.04	7.18	11.13
gGt	4.65	4.65	3.21	5.96	5.94	6.37	6.82	7.69	9.45
gTg	3.49	3.49	3.75	3.32	4.17	5.31	6.39	8.30	11.81
gGg	3.56	3.56	2.63	4.16	3.57	3.47	3.46	3.57	4.01
gTg–	3.15	3.15	3.05	2.20	3.05	4.19	5.27	7.19	10.73
gGg–	0.65	0.24	1.11	1.86	1.30	1.13	0.99	0.75	0.40
tGg–	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00	0.00
gG–g	1.22	1.25	1.16	1.56	1.22	1.26	1.33	1.46	1.69
sum of absolute errors									
reference = MP3/MP2			7.58	10.24	5.20	6.43	11.95	22.14	42.38
reference = MP2			8.70	11.30	6.32	7.49	11.65	21.84	41.58
reference = MM2			0.00	11.16	7.02	12.17	18.13	29.00	49.44
Boltzmann-weighted RMS (wt from ref E)									
reference = MP3/MP2			0.25	0.70	0.34	0.25	0.28	0.49	0.99
reference = MP2			0.54	1.04	0.65	0.55	0.49	0.48	0.73
reference = MM2			0.00	0.63	0.37	0.54	0.84	1.45	2.61
Boltzmann-weighted RMS (wt from calcd E)									
reference = MP3/MP2			0.46	2.08	0.53	0.17	0.14	0.08	0.17
reference = MP2			0.62	2.58	0.77	0.32	0.29	0.24	0.13
reference = MM2			0.00	1.18	0.14	0.14	0.12	0.19	0.42

^a Charges derived from 6-31G* optimized all-trans conformation. ^b Conformations described using a capital letter for the central torsion (OCCO) and small letters for the CCOH torsions. ^c This set uses MP3/6-31+G**//HF/6-31G* energies for the tTt, gGg–, tGg–, and gG–g conformations (unpublished results from T. A. Halgren) and MP2/6-31G*//HF/6-31G* energies for remaining conformations. ^d MP2/6-31G*//HF/6-31G* energies for all conformations. ^e Fully relaxed MM2 energies for all conformations except gGt, which required constraints applied by using the dihedral driver on the gauche CCOH and OCCO torsions.

Table VIII. DNA Base Pairing Energies and Distances

base pair ^a	model ^b	–ΔE ^c (kcal/mol)	–ΔH ₂₉₈ ^d (kcal/mol)	R (H bonds) ^e (Å)	
A–T Watson–Crick	exptl			2.95	2.82
	QM	12.5	10.5	3.06	2.88
	standard ESP	14.0	11.9	2.93	2.89
	two-stage RESP	12.6	10.7	2.90	2.91
A–T Hoogsteen	exptl		13.0	2.86	2.93
	QM	13.5	11.3	3.09	2.85
	standard ESP	13.8	11.9	2.89	2.95
	two-stage RESP	13.0	11.2	2.91	2.90
G–C Watson–Crick	exptl		21.0	2.91	2.95
	QM	26.0	23.2	2.87	3.01
	standard ESP	28.1	25.6	2.88	2.92
	two-stage RESP	27.2	24.8	2.86	2.92

^a Base pair types—see Saenger, ref 22. ^b exptl = experimental enthalpies from ref 46. QM = *ab initio*, MP2/6-31G*//HF/6-31G* with basis set superposition error correction, as described in ref 47. “standard ESP” and “two-stage RESP” refer to (un.ap) and (wk.fr/st.eq) charge models. ^c Minimized energies. ^d After normal mode and thermal corrections for theoretical energies, see ref 47. ^e Experimental H bond distances from Saenger.

for butane, methyl ethyl thioether, and the simple alcohols and amines discussed above.

C. Hydrogen Bond Energies. In Table VIII^{22,46,47} we present the results of calculations on the hydrogen bond energies of 1-methylthymine:9-methyladenine in Watson–Crick and Hoogsteen geometries and 1-methylcytosine:9-methylguanine in the Watson–Crick geometry. The results obtained using the standard ESP charges are in fairly good agreement with the *ab initio* results, except that the Hoogsteen and Watson–Crick relative energies are reversed for the A–T base pairs. The use of the two-stage RESP charges leads to lower hydrogen bond energies which are in better agreement with experiment⁴⁶ for G–C, but poorer for

Table IX. Hydrogen Bonding Energies and Distances for NMA–Water and Methanol–Water Interactions with Different Charge Models^a

molecule	charge model	H ₂ O as proton acceptor ^{b,c}		H ₂ O as proton donor ^{b,c}	
		distance (Å)	ΔE (kcal/mol)	distance (Å)	ΔE (kcal/mol)
NMA	standard ESP	1.92	–7.1	1.70	–9.7
	(un.ap)				
	one-stage RESP (wk.eq)	1.95	–6.3	1.73	–8.8
methanol	two-stage RESP (wk.fr/st.eq)	1.94	–6.7	1.71	–9.4
	standard ESP	1.75	–7.0	1.80	–6.3
	(un.ap)				
	one-stage RESP (wk.eq)	1.78	–6.1	1.83	–5.8
	two-stage RESP (wk.fr/st.eq)	1.75	–7.0	1.81	–6.3

^a Water model used is TIP3P, ref 14. ^b Configuration definitions for NMA–water according to Jorgensen and Swensen, ref 23. ^c Configuration definitions for methanol–water according to Tse and Newton, ref 24.

A–T. Finally, the two-stage RESP charges restore the greater stability of A–T Hoogsteen over Watson–Crick hydrogen bonds found in the quantum mechanical results.

In Tables IX^{14,23,24} and X,²³ the hydrogen bond energies and distances are presented for methanol and *trans*-NMA complexes with water as well as their homodimers. Both standard ESP and two-stage RESP charges lead to nearly identical H-bond energies for methanol, whereas the hydrogen bond energies are about 0.3–0.5 kcal/mol weaker for NMA using the two-stage RESP model.

D. Solvation Free Energies. We have carried out free energy calculations on the aqueous solvation of methanol and *trans*-NMA to evaluate the effect of changing the charges from standard ESP (un.ap) to three RESP models: the one-stage weakly restrained (wk.ap) and (wk.eq) and the two-stage restrained (wk.fr/st.eq) models. The free energies reported for the RESP models were calculated by considering the effect of perturbing the standard ESP charges (un.ap) for methanol or NMA into the

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Table X. Hydrogen Bonding Energies and Distances for NMA and Methanol Homodimers with Different Charge Models

dimer	configuration ^a	charge model	ΔE (kcal/mol)	distance (Å)		
				r_{O-H}	r_{N-O}	r_{O-O}
NMA	parallel	standard ESP (un.ap)	-10.2	1.82	2.84	
		one-stage RESP (wk.eq)	-8.8	1.87	2.88	
		two-stage RESP (wk.fr/st.eq)	-9.8	1.88	2.87	
NMA	antiparallel	standard ESP (un.ap)	-10.4	1.84	2.85	
		one-stage RESP (wk.eq)	-8.9	1.88	2.89	
		two-stage RESP (wk.fr/st.eq)	-9.7	1.86	2.87	
NMA	stack	standard ESP (un.ap)	-8.6			
		one-stage RESP (wk.eq)	-7.9			
		two-stage RESP (wk.fr/st.eq)	-8.2			
methanol		standard ESP (un.ap)	-6.9	1.78		2.75
		one-stage RESP (wk.eq)	-5.6	1.83		2.80
		two-stage RESP (wk.fr/st.eq)	-6.8	1.76		2.76

^a Configuration definition according to Jorgensen and Swensen, ref 23.

Table XI. Relative Free Energies of Solvation for the Perturbations of Methanol \rightarrow Methane and Methanol \rightarrow Ethane with Different Charge Models

perturbation	charge model ^a	$\Delta\Delta G_{\text{solv}}$ (kcal/mol)
methanol \rightarrow methane	experiment ^b	7.0
	standard ESP (un.ap)	6.91 \pm 0.01
	one-stage RESP (wk.eq)	5.71 \pm 0.02
	one-stage RESP (wk.ap)	6.83 \pm 0.01
	two-stage RESP (wk.fr/st.eq)	6.86 \pm 0.01
methanol \rightarrow ethane	experiment ^b	6.8
	standard ESP (un.ap)	7.02 \pm 0.13
	one-stage RESP (wk.eq)	5.82 \pm 0.14
	one-stage RESP (wk.ap)	6.90 \pm 0.13
	two-stage RESP (wk.fr/st.eq)	6.93 \pm 0.13

^a Charges for methane are C = -0.464 and H = 0.116 and for ethane C = -0.027 and H = 0.009 with the standard ESP model. Using the two-stage RESP model, methane charges are C = -0.390 and H = 0.098 and ethane charges are C = 0.009 and H = -0.003. ^b Experimental numbers from Ben-Naim and Marcus, ref 48.

two-stage RESP (wk.fr/st.eq) charges. The results were then added to the results from the molecular perturbations using standard ESP charges. The effects of perturbing methane or ethane, which employed standard ESP charges (un.ap), into the two-stage RESP charges (wk.fr/st.eq) were within the noise of the calculations, so we did not carry out those calculations for the other two RESP models (wk.ap and wk.eq).

The free energies of solvation of methanol relative to both ethane and methane are presented in Table XI.⁴⁸ As one can see, the standard ESP (un.ap) charges as well as one-stage weakly restrained (wk.ap) and two-stage restrained ESP (wk.fr/st.eq) charges lead to relative solvation free energies very close to experiment.⁴⁸ Forcing equivalence on the methyl hydrogens during the fit in a one-stage restrained model (wk.eq) leads to a significantly less favorable solvation free energy. That is why we do not favor the use of this model over the more elaborate two-stage approach.

The relative free energies of solvation of *trans*-NMA and methane calculated with three charge models are presented in Table XII.^{48,49} The standard ESP (un.ap) and weak hyperbolic (wk.ap) models both lead to a solvation free energy ($\Delta G = -12.4$

Table XII. Relative Free Energies of Solvation for the Perturbation of NMA \rightarrow Methane with Different Charge Models

perturbation	charge model	$\Delta\Delta G_{\text{solv}}$ (kcal/mol)
(1) NMA electrostatic ($q \rightarrow 0$)	standard ESP (un.ap)	11.23 \pm 0.01
(2) methane electrostatic ($q \rightarrow 0$)	standard ESP (un.ap)	0.04 \pm 0.01
(3) NMA \rightarrow methane (VDW)		0.90 \pm 0.14
(4) Born correction ^a		0.3
NMA \rightarrow methane (1 + 3 + 4-2)	standard ESP (un.ap)	12.4 \pm 0.2
NMA \rightarrow methane ^b	one-stage RESP (wk.eq)	10.2 \pm 0.2
NMA \rightarrow methane ^b	one-stage RESP (wk.ap)	12.1 \pm 0.2
NMA \rightarrow methane ^b	two-stage RESP (wk.fr/st.eq)	11.6 \pm 0.2
NMA \rightarrow methane	experiment ^c	12.2

^a The Born correction (ref 29) accounts for the long-range electrostatic effects resulting from perturbing a dipolar species (NMA) to a nonpolar one. ^b Results with nonstandard ESP charge models obtained by adding $\Delta\Delta G_{\text{solv}}$ for perturbation of standard ESP charges to a given nonstandard model to $\Delta\Delta G_{\text{solv}}$ for NMA \rightarrow methane using standard ESP charges. ^c Experimental values from refs 48 and 49.

and $\Delta G = -12.1$ kcal/mol) in good agreement with experiment ($\Delta G = -12.2$ kcal/mol).^{48,49} The two-stage restrained model (wk.fr/st.eq) is less accurate but still good, resulting in $\Delta G = -11.6$ kcal/mol.

E. Conformational Dependence of ESP Charge Models: Intermolecular Effects. There are two issues which can be defined with respect to evaluating the conformational dependence of electrostatic potential fit charges. The first issue is how well the charges derived from one particular conformation of a molecule reproduce the electrostatic potential of another conformation of the molecule. Table XIII show the results of calculations on five low-energy conformations of propylamine, which examine the conformational dependence of the "intermolecular" properties of dipole moment and reproduction of the electrostatic potential. The standard ESP and two-stage restrained ESP models are compared. Table XIII gives the dipole moment calculated for each of the five conformations using the quantum mechanical wave function, unaveraged standard ESP charges (un.fr), and standard (un.ap) and two-stage restrained (wk.fr/st.eq) ESP charges calculated from each of the five conformers. The unaveraged standard ESP charges naturally give the best agreement, but cannot be used in simulations without averaging (vide infra).

The numbers shown in bold represent dipole moments calculated when charges were derived from and tested on the same conformation. Not surprisingly, they show the best agreement with the quantum mechanical dipole moments. The five dipole moments shown in bold which pertain to the standard ESP model show that in three of the five cases, the dipole moment is increased by *a posteriori* averaging, while in two of the cases it is decreased. These deviations result from *a posteriori* averaging and not conformational dependence. The dipole moments shown in standard type reflect the conformational dependence of the standard and two-stage restrained ESP models. Of the 20 nonbold dipole moments relating to the standard ESP model, in 18 of the cases the dipole moment given by the charge set overestimates the quantum mechanical dipole by up to 67%. This could result in the overstabilization in solution of conformations which were not used in the ESP charge derivation, due to their spuriously large dipole moments. It is encouraging that in 22 of the 25 combinations of model conformation/test conformation examined, the two-stage restrained ESP charges gave dipole moments which were closer to the quantum mechanical values than the standard ESP charges did.

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Table XIII. Effect of Conformational Dependence of Propylamine Standard and Two-Stage Restrained ESP Charges on Calculated Dipole Moments (D) and on the Relative rms (RRMS) of the Fit of the Classical Electrostatic Potential to the Quantum Mechanical Potential

Effect on Calculated Dipole Moments													
test conformation ^a	<i>E</i> (MP3) ^b	QM ^c	raw std ESP ^d	charge model ^{e,f}									
				Tt std ESP	Tt two-stage RESP	Tg std ESP	Tg two-stage RESP	Gg– std ESP	Gg– two-stage RESP	Gt std ESP	Gt two-stage RESP	Gg std ESP	Gg two-stage RESP
Tt	0.00	1.55	1.53	1.38^g	1.48	2.12	2.09	1.92	1.86	1.44	1.48	2.05	2.03
Tg	0.30	1.43	1.42	2.09	2.01	1.78	1.73	1.83	1.88	2.17	2.12	1.79	1.63
Gg–	0.25	1.49	1.47	2.48	2.44	2.48	2.39	1.79	1.55	2.23	2.11	1.79	1.91
Gt	0.32	1.56	1.50	1.44	1.52	2.13	2.09	1.93	1.92	1.43	1.48	2.05	1.97
Gg	0.61	1.41	1.40	2.16	2.09	1.86	1.80	1.84	1.92	2.18	2.13	1.80	1.59
sum of abs errors			0.12	2.69	2.32	2.93	2.66	1.87	1.69	2.49	2.18	2.04	1.69
range of dipoles		0.15	0.13	1.10	0.96	0.70	0.66	0.14	0.37	0.80	0.65	0.26	0.44
Effect on the RRMS													
test conformation ^a	<i>E</i> (MP3) ^b	raw std ESP ^h	Tt std ESP	charge model ^{e,i}									
				Tt two-stage RESP	Tg std ESP	Tg two-stage RESP	Gg– std ESP	Gg– two-stage RESP	Gt std ESP	Gt two-stage RESP	Gg std ESP	Gg two-stage RESP	
Tt	0.00	0.20	0.21^g	0.21	0.36	0.35	0.31	0.33	0.23	0.24	0.34	0.38	
Tg	0.30	0.17	0.42	0.38	0.27	0.26	0.32	0.37	0.45	0.43	0.31	0.29	
Gg–	0.25	0.20	0.57	0.53	0.59	0.54	0.31	0.27	0.45	0.40	0.33	0.35	
Gt	0.32	0.21	0.33	0.32	0.44	0.41	0.30	0.32	0.23	0.23	0.33	0.35	
Gg	0.61	0.20	0.51	0.47	0.42	0.39	0.31	0.35	0.46	0.43	0.30	0.26	
sum of RRMS's		0.98	2.04	1.91	2.08	1.95	1.55	1.64	1.82	1.73	1.61	1.63	

^a Conformations described by C-C-C-N and c-c-cn-(lp) torsions. ^b MP3/6-31+G**//HF/6-31G* energies in kcal/mol—unpublished results from T. A. Halgren. ^c Dipole moment calculated from a quantum mechanical wave function. ^d Dipole moment calculated from unaveraged ESP charges derived from and tested against the potential of the same conformation. ^e "std and ESP" and "two-stage RESP" refer to (un.ap) and (wk.fr/st.eq) models. ^f Model conformation is the one used to generate the charges. ^g Numbers in bold correspond to situations where charges were derived from and tested on the same conformation. ^h RRMS's calculated from unaveraged ESP charges derived from and tested against the potential of the same conformation. ⁱ Charge model conformation is the one used to generate the charges.

Table XIV. Effect of Conformational Dependence of Propylamine Standard and Two-Stage Restrained ESP Charges on Relative Conformational Energies (kcal/mol)^a

test conf ^b	<i>E</i> (MP3) ^c	charge model ^{d,e}									
		Tt std ESP	Tt two-stage RESP	Tg std ESP	Tg two-stage RESP	Gg- std ESP	Gg- two-stage RESP	Gt std ESP	Gt two-stage RESP	Gg std ESP	Gg two-stage RESP
Tt	0.00	0.43	0.00	1.36	0.51	0.05	0.00	0.00	0.00	0.19	0.43
Tg	0.30	0.61	0.20	0.60	0.00	0.00	0.15	0.55	0.54	0.00	0.00
Gg-	0.25	1.01	0.96	1.18	1.01	0.32	0.25	0.96	0.93	0.29	0.63
Gt	0.32	0.00	0.11	0.31	0.35	0.25	0.59	0.15	0.36	0.28	0.39
Gg	0.61	0.37	0.44	0.00	0.09	0.24	0.63	0.75	0.90	0.14	0.13
sum of abs errors		2.06	1.19	3.21	2.12	0.86	0.44	1.27	1.25	1.04	1.66

^a Energies calculated using a 1-4 electrostatic scale factor of 1/1.2. ^b Conformations described by C-C-C-N and c-c-cn-(lp) torsions. ^c MP3/6-31+G**//HF/6-31G* energies—unpublished results from T. A. Halgren. ^d Charge model conformation is the one used to generate the charges. ^e "std ESP" and "two-stage RESP" refer to (un.ap) and (wk.fr/st.eq) charge models.

Another way of evaluating the calculated dipole moments given by the different charge models is to calculate the range of dipole moments calculated for the five conformations. These data are also presented in Table XIII. The quantum mechanical dipole moments span a range of only 0.15 D. A higher sum of absolute errors might be acceptable if the range of numbers were appropriately small, since the different conformations would be more or less systematically overstabilized. By this measure neither the standard nor two-stage restrained ESP models is better in general. Interestingly, both the Gg- and Gg conformations provide charges which perform quite well.

F. Conformational Dependence of ESP Charge Models:

Intramolecular Effects. The second issue which can be defined with respect to conformational dependence is how well conformational energies calculated using charges derived from different conformations of a molecule compare with each other. Table XIV presents the conformational energies calculated for five conformers of propylamine as a function of charge model and the conformation used for the charge calculation. The MP3/6-31+G**//HF/6-31G* energies are shown as a reference. Since

the relative quantum mechanical energies for all five conformations are close to zero, it was thought unnecessary to Boltzmann weight the errors. We refer to the different conformations of propylamine with a capital letter to indicate the conformation of the C-C-C-N dihedral and a small letter to represent the C-C-N-(lp) dihedral. The Tt conformation yields standard ESP charges which do not reproduce the indicated global minimum energy conformation. In fact, only one of the five standard ESP charge sets yields the proper global minimum. The Tt two-stage restrained ESP charges do reproduce the proper global minimum conformation, as do two of the other four sets of two-stage restrained charges.

For all of the conformations except for Gg, the two-stage restrained ESP charges have better agreement with the quantum mechanical conformational energies than the standard ESP charges do. It is interesting to note that the Gg- conformation produces very good agreement with the quantum mechanical conformational energies using either the two-stage restrained or standard ESP models. Urban and Famini recently reported the results of a study on the conformational dependence of ESP

charges calculated for dopamine.⁵⁰ They found that, of the six conformations examined, the standard ESP charges calculated from the highest energy conformation (5.5 and 15.8 kcal/mol above the global minimum on the STO-3G and 6-31G* potential energy surfaces, respectively) did the best job of reproducing conformational energies. Both of these results are somewhat surprising and intriguing and merit further study. In particular, it would be of interest to examine the conformational behavior of two-stage restrained ESP charges calculated for dopamine to determine if the superior performance of the charges derived from the higher energy conformation was retained.

G. Multiple Conformation Fit Charges. Reynolds *et al.*³⁶ have shown that it is possible to derive ESP charges from more than one conformation of a molecule. Using such a procedure, they obtained a set of charges for propanol and also for threonine dipeptide which reproduced the dipole moments of different conformers better than any set of charges derived from a single conformation. Given recent advances in quantum chemistry software due to the implementation of direct SCF methods and more efficient integral routines,¹⁷ the computational burden associated with carrying out the requisite 6-31G* level SCF calculation on a molecule has been greatly reduced. It is therefore feasible to consider carrying out multiple conformation fitting in order to obtain the highest quality charges possible.

In this work we use propylamine as a test molecule for exploring multiple conformation fitting. The standard ESP multiple conformation charges were obtained by constraining corresponding heavy atoms to be equivalent between the different conformations, while all hydrogens were left free. Equivalent hydrogens were then averaged *a posteriori*. A second set of charges was derived where all equivalent atoms were constrained to have equivalent charges during the fit (i.e. methyl, α -methylene, β -methylene, and amino hydrogens were constrained to be equivalent within each group and between conformations). The second set of charges results in much better agreement between the calculated classical and quantum mechanical potentials and dipole moments for the five conformations, as shown in parentheses in Table XV. In three of the four multiple conformation models, however, this forced equivalence of hydrogens results in a significantly reduced charge on the nitrogen atom. The nitrogen charge changes from -1.046 to -0.914 (five-conformer model), from -1.080 to -0.950 (Tt/Tg model), and from -1.063 to -0.934 (Tg/Gt model). It is therefore likely that such charge sets would result in unacceptably low solvation free energies, as seen with methanol and NMA (*vide infra*).

The two-stage restrained ESP multiple conformation charges were obtained by constraining corresponding heavy atoms and the amino hydrogens to be equivalent between the different conformations in the first stage, while all methyl and methylene hydrogens were left free within and between conformations. The two amino hydrogens were also constrained to be equivalent within each conformation during the first stage. The corresponding methyl and methylene atoms were constrained to be equivalent between (for the carbons and hydrogens) and within (only for the hydrogens) conformations during the second stage optimization. The first stage optimization then resulted in three different charges for the carbons and 7 \times no. confs. different charges for the aliphatic hydrogens. After the second stage optimization, there were three new and different charges for the carbons and three new and different charges for the aliphatic hydrogens.

In addition, charges were also obtained by constraining only the amino nitrogen and hydrogens to be equivalent between conformations during the first stage with all methyl and methylene carbons and hydrogens allowed to optimize independently. The amino hydrogens were also constrained to have the same charges within each conformation. The second stage optimization then proceeded as described above. This second set of charges

performed almost exactly the same as the first set with respect to the RRMS of the fit, dipole moment errors, range of dipole moments, and conformational energy errors. The first model did perform slightly better, so those are the results we present.

Standard and two-stage restrained ESP charges are presented using all five conformers, the Tt and Gt conformers, the Tt and Tg conformers, and the Tg and Gt conformers. As found by Reynolds *et al.*³⁶ for propanol and threonine dipeptide, these multiple conformation fit charges consistently result in good agreement with the quantum mechanical dipole moments and overall potentials (Table XV). All of the single and multiple conformation charge sets result in a similar sum of RRMS values for the five conformers. The five-conformer standard ESP model is superior to all but one of the single conformation standard ESP charge sets, and the five-conformer two-stage restrained model is superior to all of the single conformation standard or two-stage restrained ESP charge sets.

The range of dipole moments calculated from each charge set is also presented in Table XV. By this measure the five-conformer models do particularly well, especially the two-stage restrained ESP model. Of the two-conformer models, the Tg/Gt models clearly exhibit the best behavior, performing nearly as well as the five-conformer models. Most importantly, for each of the three measures of intermolecular behavior—sum of dipole moment errors, range of dipole moments, and sum of RRMS values—the multiple conformation two-stage restrained ESP charges outperform the corresponding multiple conformation standard ESP charges in every case. Restraining the charges thus achieves improvement beyond that available through multiple conformation fitting.

The intramolecular behavior of propylamine multiple conformation fit charges is examined in Table XVI. Considering the standard and two-stage restrained ESP charges derived using all five conformers, both models result in very small relative conformational energies, in good agreement with the high-level quantum mechanical results. The two-stage restrained model has more success finding the proper global minimum conformation, and has an overall sum of absolute errors equal to 0.76 kcal/mol as compared to 1.46 kcal/mol for the five-conformation standard ESP charge model. The single conformation Tt standard and two-stage restrained ESP charges yielded conformational energies with absolute errors of 2.06 and 1.19 kcal/mol, respectively. Of the models examined in Table XVI, three of the two-stage RESP models identified the proper global minimum conformation and none of the standard ESP models did.

Of the two-conformer multiple conformation models studied, the standard and two-stage restrained ESP charge models which employed the Tt and Gt conformations performed best of all, with absolute errors of 0.69 and 1.50 kcal/mol, respectively. The Tg/Gt model also did quite well. The most important result is that, in all of the multiple conformation models examined, the two-stage restrained ESP models consistently outperformed the corresponding standard ESP models by from 29% to 54% in reproducing conformational energies.

Since the number of conformations of a molecule increases exponentially with the number of rotatable dihedrals, it will be important to identify the dihedral types which most affect electrostatic potential derived charges. In the case of propylamine multiple conformation restrained ESP charges, it appears that variation of the nitrogen position through the C-C-N dihedral is more important than variation of the lone pair position for obtaining the most robust set of charges.

Discussion

The study of conformational energies of butane, methyl ethyl thioether, methanol, ethanol, propanol, methylamine, ethylamine, propylamine, and 1,2-ethanediol makes clear that two-stage RESP charges exhibit less conformational and 1-4 electrostatic scale factor dependence than do the standard ESP charges. On the

(50) Urban, J. J.; Famini, G. R. *J. Comput. Chem.* 1993, 14, 353.

Table XV. Effect of RESP Model and Multiple Conformation Fitting of Propylamine Charges on Calculated Dipole Moments (D) and on the Relative rms (RRMS) of the Fit of the Classical Electrostatic Potential to the Quantum Mechanical Potential

Effect on Calculated Dipole Moments												
test conformation ^a	<i>E</i> (MP3) ^b	QM ^c	charge model ^{d,e}									
			Tt std ESP	Tt two-stage RESP	multi (5) std ESP	multi (5) two-stage RESP	multi (2) Tt,Gt std ESP	multi (2) Tt,Gt two-stage RESP	multi (2) Tt,Tg std ESP	multi (2) Tt,Tg two-stage RESP	multi (2) Tg,Gt std ESP	multi (2) Tg,Gt two-stage RESP
Tt	0.00	1.55	1.38 ^f	1.48	1.79	1.78	1.41	1.48	1.71	1.76	1.74	1.77
Tg	0.30	1.43	2.09	2.01	1.90	1.85	2.12	2.04	1.92	1.86	1.94	1.85
Gg-	0.25	1.49	2.48	2.44	2.11	1.92	2.35	2.13	2.45	2.40	2.29	2.09
Gt	0.32	1.56	1.44	1.52	1.78	1.77	1.41	1.47	1.75	1.78	1.72	1.73
Gg	0.61	1.41	2.16	2.09	1.92	1.85	2.15	2.05	2.00	1.94	1.96	1.85
sum of abs errors			2.69	2.32	2.06 (0.78) ^g	1.73	2.58 (1.73)	2.05	2.39 (1.47)	2.30	2.21 (1.00)	1.85
range of dipoles		0.15	1.10	0.96	0.33	0.15	0.94	0.66	0.74	0.64	0.57	0.36
Effect on the RRMS												
test conformation ^a	<i>E</i> (MP3) ^b	QM ^c	charge model ^{d,e}									
			Tt std ESP	Tt two-stage RESP	multi (5) std ESP	multi (5) two-stage RESP	multi (2) Tt,Gt std ESP	multi (2) Tt,Gt two-stage RESP	multi (2) Tt,Tg std ESP	multi (2) Tt,Tg two-stage RESP	multi (2) Tg,Gt std ESP	multi (2) Tg,Gt two-stage RESP
Tt	0.00	0.20	0.21 ^f	0.21	0.25	0.28	0.21	0.22	0.23	0.25	0.25	0.27
Tg	0.30	0.17	0.42	0.38	0.31	0.32	0.42	0.39	0.32	0.30	0.32	0.30
Gg-	0.25	0.20	0.57	0.53	0.40	0.32	0.50	0.40	0.55	0.51	0.47	0.38
Gt	0.32	0.21	0.33	0.32	0.27	0.27	0.26	0.23	0.35	0.34	0.28	0.27
Gg	0.61	0.20	0.51	0.47	0.35	0.32	0.47	0.41	0.45	0.41	0.39	0.33
sum of RRMS's		0.98	2.04	1.91	1.58 (1.37) ^g	1.51	1.86 (1.68)	1.65	1.90 (1.72)	1.81	1.71 (1.40)	1.55

^a Conformation described by C-C-C-N and c-c-n-(lp) torsions. ^b MP3/6-31+G**//HF/6-31G* energies—unpublished results from T. A. Halgren (kcal/mol). ^c Dipole moment from the 6-31G* wave function. ^d Charge model conformation is the one used to generate the charges. ^e "std" and "two-stage" refer to (un.ap) and (wk.fr/st.eq) models. ^f Numbers in bold refer to situations where the charges are being tested on (one of) the conformation(s) used to derive the charges. ^g Errors given in parentheses are those obtained when equivalencing of hydrogens is forced during fit. ^h Unaveraged charges derived from and tested against the potential of the same conformation.

Table XVI. Effect of RESP Model and Multiple Conformation Fitting of Propylamine Charges on Relative Conformational Energies (kcal/mol)^a

charge model ^{d,e}												
test conformation ^b	<i>E</i> (MP3) ^c											
		Tt std ESP	Tt two-stage RESP	multi (5) std ESP	multi (5) two-stage RESP	multi (2) Tt,Gt std ESP	multi (2) Tt,Gt two-stage RESP	multi (2) Tt,Tg std ESP	multi (2) Tt,Tg two-stage RESP	multi (2) Tg,Gt std ESP	multi (2) Tg,Gt two-stage RESP	
Tt	0.00	0.43	0.00	0.17	0.00	0.16	0.00	0.68	0.11	0.40	0.03	
Tg	0.30	0.61	0.20	0.12	0.00	0.52	0.35	0.46	0.00	0.34	0.00	
Gg-	0.25	1.01	0.96	0.51	0.44	0.93	0.80	0.97	0.87	0.85	0.61	
Gt	0.32	0.00	0.11	0.00	0.32	0.00	0.33	0.00	0.09	0.00	0.20	
Gg	0.61	0.37	0.44	0.08	0.34	0.49	0.69	0.08	0.16	0.17	0.26	
sum of abs errors		2.06	1.19	1.46	0.76	1.50	0.69	2.41	1.71	1.80	1.16	
				(1.05) ^f		(0.71)		(1.50)		(1.45)		

^a Energies calculated using a 1-4 electrostatic scale factor of 1/1.2. ^b Conformations described by C-C-C-N and c-c-n-(lp) torsions. ^c MP3/6-31+G**//6-31G* energies in kcal/mol—unpublished results from T. A. Halgren. ^d Charge model conformation is the one used to generate the charges. ^e "std ESP" and "two-stage RESP" refer to (un.ap) and (wk.fr/st.eq) models. ^f Errors given in parentheses are those obtained when equivalencing of hydrogens is forced during fit.

basis of these calculations, we suggest the two-stage RESP fit charges with an electrostatic scale factor of 1/1.2 to be a particularly promising model.

The choice of a 1-4 scale factor (<1) for van der Waals interactions has had considerable justification given the known overestimate of short-range repulsion by a 6-12 form of potential. Thus, the choice of a van der Waals scale factor of 1/2 for 1-4 interactions only, as used in the Weiner *et al.*¹⁰ force field, seems reasonable and justifiable here as well. Weiner *et al.* justified the 1-4 electrostatic scale factor of 1/2 mainly on empirical results with the alanine dipeptide. Billeter *et al.*³⁴ and Smith and Karplus³⁵ have shown that such scaling can cause artifacts in conformational energies. We suggest that scaling 1-4 electrostatic interactions by 1/2 and leaving 1-5 interactions intact can unbalance the electrostatics of the system, leading to results such

as the gauche conformation of butane being more stable than the trans. On the other hand, none of the valence force fields includes 1-3 interactions at all, but these interactions should remain fairly constant with rotation about a dihedral angle and thus should roughly cancel out between different conformations.

As Table VII shows, using an electrostatic scale factor of 1/2 for 1,2-ethanediol and simple 3-fold torsions results in conformational energies which are up to 8.3 kcal/mol in error for the 10 minimum energy conformations. One could, in theory, fix those energies with a contribution from the torsional energy, but the torsional parameters would have to be so large as to be physically quite unreasonable. Such torsional parameters would also not likely be transferable to that torsion in other molecules. Our study of 1,2-ethanediol suggests that a 1-4 electrostatic scale factor of 1/1.2 performs optimally and is a good compromise

between full inclusion of 1–4 electrostatics and the overly severe scale factor of $1/2$. The suitability of this scale factor is supported by the results given for the simple alcohols and amines.

Another difficulty associated with full inclusion of 1–4 electrostatic interactions using 6-31G* derived standard ESP charges is that it leads to very large angle distortion energies in the exocyclic amino groups in the nucleic acid bases. This behavior may result from the fact that this basis set consistently overestimates molecular polarity. This angle distortion energy can be as great as 8 kcal/mol in the case of adenine! Using two-stage restrained ESP charges and the 1–4 electrostatic scale factor $1/1.2$ significantly reduces the problem, such that the angle distortion energy is ~ 1 –2 kcal/mol. This is still larger than the ~ 0.5 kcal/mol angle distortion energy found with the Weiner *et al.* force field and a 1–4 electrostatic scale factor of $1/2$, but further work on these systems will be needed to find the best approach to reduce the angle distortion energy.

The conformational dependence of ESP charges as revealed in Tables XIII and XIV is an issue which will be studied in more depth as we are developing our new force field. The intermolecular problem, that of reproducing dipole moments accurately for more than one conformation of a molecule, is one which is likely to be present in any effective two-body force field which does not allow for polarization. It is encouraging that the two-stage restrained ESP charges exhibit less conformational dependence with regards to inter- and intramolecular properties than do the standard ESP charges. It is clear from the results presented above that both equivalencing and restraining the charges is beneficial for the derivation of an optimum charge model. Furthermore, the use of multiple conformations of a molecule in deriving RESP charges allows for further refinement of the model.

Why do we employ the two-stage RESP fit, rather than just using a one-stage calculation? As described in more detail in Bayly *et al.*,⁸ there are two issues: first, the need to reduce spuriously large charges which are statistically poorly determined, and second, the desire to make equivalent those charges on atoms which are not necessarily equivalent during the SCF calculation, but which can interconvert during a molecular dynamics simulation (e.g. the methyl hydrogens in methanol). Also of relevance is the use of the 6-31G* basis set for determining the electrostatic potential fit charges. The motivation for using this basis set has been that it consistently overestimates the dipole moment by an amount (5–20%) consistent with the TIP3P/SPC “effective two-body” models for water. Thus, it fortuitously contains approximately the amount of “polarization” contained in such water models and should therefore be “balanced” with respect to those water models. The 6-31G* basis set is then expected to enhance the solute dipole moment over the actual gas-phase value to about the same extent as seen in water models.

How does this work in practice? Methanol is a good example. The 6-31G* calculation on this molecule gives a dipole moment of 1.92 D, compared to the experimental gas-phase moment of 1.66 D. The “raw” unrestrained and restrained ESP fit charges lead to a dipole moment of ~ 1.9 D, but when one averages the methyl hydrogen charges after a standard ESP fit (un.ap), the dipole moment increases to 2.15 D. The two-stage RESP (wk.fr/st.eq) fitting only reduces the dipole moment to 2.14 D. If one forces equivalent charges on the methyl hydrogens during a one-stage fit (wk.eq), one obtains a dipole moment of 1.99 D. The rule of thumb that says solvation free energy should scale with the square of the dipole moment would predict that the one-stage RESP charges (wk.eq) should result in $\Delta\Delta G = 5.92$ kcal/mol for methanol to methane and $\Delta\Delta G = 6.01$ kcal/mol for methanol to ethane (relative to the standard ESP values). The free energy perturbation calculations actually yielded values of $\Delta\Delta G = 5.71$ kcal/mol for methanol to methane and $\Delta\Delta G = 5.82$ kcal/mol for methanol to ethane. Since this charge model (wk.eq) resulted in charges on the oxygen and hydroxyl hydrogen that were about

10% smaller than seen with the other models, we conclude that the magnitude of the heteroatom charges is as important as the overall dipole moment in determining intermolecular interactions.

Trans-NMA provides another example of the effects of making methyl hydrogens equivalent by a *posteriori* averaging *vs* constrained equivalence during the fit. Whereas the 6-31G* quantum mechanical calculation gives a dipole moment of 4.17 D compared to the gas-phase experimental value of 3.68 D, a *posteriori* averaging of each of the two methyl group hydrogens in a standard ESP fit (un.ap) increases this to 4.57 D. A two-stage RESP fit decreases the dipole moment to 4.42 D. Again, the simple relationship between dipole moment and free energy of solvation fails here. That relationship would predict relative free energies of solvation for NMA and methane of 10.47, 12.51, and 11.60 kcal/mol for the (wk.eq), (wk.ap), and (wk.fr/st.eq) charge models, respectively (relative to the standard ESP values). The free energy perturbation calculations yielded relative free energies of 10.2, 12.1, and 11.6 kcal/mol for those models. This serves as evidence that higher moments play an important role in the calculation of intermolecular interactions.

It is therefore clear that the two-stage fit, by keeping the heteroatoms and hydrogen bonding charges fixed, may lead to a dipole moment for the molecule which is larger than the dipole moment determined by the quantum mechanical wave function. In the case of methanol and NMA, it increases the enhancement in dipole moment to 10–20% over the gas-phase value, more in line with the $\sim 20\%$ enhancement of TIP3P water over gas-phase water. In the case of the nucleic acid bases, this enhancement is modest in percentage (increase of ~ 0.2 D for adenine, cytosine, and thymine and no change for guanine) and these lead to essentially no change in hydrogen bond energies for the base pairs. Obviously, as noted before, the dipole moment is a useful first estimate for what the hydrogen bonding or solvation free energy of a model will be, but the larger the molecule, the larger role higher moments must play.

Why bother with electrostatic potential fit charges at all—why not just use the empirical approaches embodied in TIP3P/OPLS models? In our opinion, the use of electrostatic potential fit charges allows us a general, unbiased, and more accurate representation of electrostatic charge distributions. This method is less subject to arbitrariness than empirically derived charges⁵¹ and can easily be generalized to any molecule or functional group. Given current and ever increasing computer power, 6-31G* electrostatic potential charges can be derived for virtually any molecule, possibly in multiple conformations. Electrostatic potential fit charges or those based on distributed multipole analyses will be even more critical if one hopes to go beyond the atom-centered monopole or empirical bond dipole models for charge distribution, where even more degrees of freedom are being fit.

Conclusion

In this paper and a related one,⁸ we have presented some new approaches to deriving electrostatic potential fit charges and have used these new approaches to study conformational energies, conformational dependencies, hydrogen bonding, and solvation free energies. It is clear that restraining these electrostatic potential charges has rather little effect on the quality of the fit to the potential and the calculated molecular properties and provides a better representation of conformational properties of molecules compared to the standard ESP model. The set of two-stage RESP charges thus derived gives an excellent fit to the solvation free energy of methanol and an adequate fit to the solvation free energy of NMA. We have also further evaluated the multiple conformation ESP fitting studied by Reynolds *et al.*³⁶ We have confirmed and extended their findings that such an approach is useful and suggest that the use of restraints and

(51) Cieplak, P.; Kollman, P. A. *J. Comput. Chem.* **1991**, *12*, 1232.

multiple conformation fitting will lead to an optimal set of charges for the broadest range of molecular systems.

The value of electrostatic potential derived charges in modeling the important electrostatic interactions in biological systems has been known for some time. We have shown that two-stage RESP charges retain this excellent intermolecular behavior while exhibiting intramolecular behavior which makes them suitable for conformational analysis. Two-stage RESP charges thus reproduce both intermolecular and intramolecular energies and structures quite well, making this charge model a critical advancement in the development of a general force field for modeling biological macromolecules and their ligands, both in the gas phase and in solution.

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Supplementary Material Available: Tables of the charges used in this work along with RRMS's to the quantum mechanical electrostatic potentials and resulting dipole moments (10 pages). Ordering information is given on any current masthead page.