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Magnus Lundborg, and Erik Lindahl

J. Phys. Chem. B, **Just Accepted Manuscript** • DOI: 10.1021/jp505332p • Publication Date (Web): 24 Oct 2014

Automatic GROMACS Topology Generation and Comparisons of Force Fields for Solvation Free Energy Calculations

Magnus Lundborg[†] and Erik Lindahl[*,†,‡]

Department of Theoretical Physics and Swedish e-Science Research Center, Royal Institute of Technology, Science for Life Laboratory, Box 1031, SE-171 21 Solna, Sweden, and Department of Biochemistry and Biophysics, Center for Biomembrane Research, Stockholm University, 106 91, Stockholm, Sweden

E-mail: erik@kth.se

KEYWORDS: Molecular Dynamics Simulations, Hydration Free Energy, Topology Generation, Polarization Costs, High Throughput

[*]To whom correspondence should be addressed
[†]Royal Institute of Technology
[‡]Stockholm University

Abstract

Free energy calculation has long been an important goal for molecular dynamics simulation and force field development, but historically it has been challenged both by limited performance, accuracy, and creation of topologies for arbitrary small molecules. This has made it difficult to systematically compare different sets of parameters to improve existing force fields, but in the last few years several authors have developed increasingly automated procedures to generate parameters for force fields such as Amber, CHARMM, and OPLS. Here, we present a new framework that enables fully automated generation of GROMACS topologies for any of these force fields and an automated setup for parallel adaptive optimization of high-throughput free energy calculation by adjusting lambda point placement on the fly. As a small example of this automated pipeline, we have calculated solvation free energies of 50 different small molecules using the GAFF, OPLS-AA and CGenFF force fields and four different water models, and by including the often neglected polarization costs we show that the common charge models are somewhat underpolarized.

1 Introduction

Free energy is of paramount importance in chemistry. Almost all the experimental properties traditionally interpreted e.g. in terms of concentration, reaction rates, stability, folding, complex formation, binding catalysis, or solubility can equally well be described with free energy concepts, in particular on the molecular level. If we could rapidly calculate free energies for arbitrary complex reactions (such as protein folding or an antibody binding an antigen) it would not only be possible to make much more accurate predictions of experimental results from simulations, but it would enable an entirely new level of computational molecular design.

While the most complex systems are still limited by computational performance, the calculation of solvation free energies (i.e., the change in Gibbs free energy upon transfer from

gas phase to solvent) has matured rapidly. It is already used in pharmaceutical applications since only a small fraction of commercially available compounds have had their solvation free energy determined experimentally.[1,2] This makes computational predictions tractable, if they are proven to be reliable, and likely to pave the way to more complex applications. For a long time, the calculation of hydration free energies has been a critical performance test of biomolecular force fields used in molecular dynamics (MD) simulations.[3] There have also been a number of blind challenges to predict hydration free energies of provided compounds, with experimental data that is difficult to find, in order to assess the state of the art and to improve current methodology.[1,2,4–6]

A central concern for solvation free energy computations has been whether their accuracy is sufficient for practical use.[7] The biomolecular force fields currently used in molecular dynamics simulations were originally parameterized with amino acids and nucleic acids in mind. Over a number of years they have been extended to cover generic organic molecules, but some parameters still need to be improved in order to yield satisfying results. While it is certainly open to debate, we would argue the community should not assume that the free energy accuracy we get for 20 amino acid residues (for which we have spent almost 40 years improving parameters) is somehow typical for the general force field parameter quality when running simulations with organic molecules. One example is that the GROMOS 53A6 force field[8], unlike most other force fields, has been parameterized to reproduce hydration free energies, but still only based on amino acid analogs.

Likewise, the commonly used water models are good at reproducing properties of pure liquid water, but they are not quite as reliable for modeling hydration free energies.[9] Lately there have been efforts to amend this, by tweaking the water model parameters to improve the interaction energies without sacrificing the water properties.[9,10] It can be argued, though, that it would be better to use a good water model, such as TIP4P-Ew[11] or SPC/E[12] and modify the force field parameters to improve solvation free energies.[13] The polarization cost when using a fixed charge force field is also often overlooked; studies have suggested that the

partial charges commonly used in force fields are somewhat underpolarized.[14,15] This means that the force fields should be re-parameterized using more accurate charges, followed by re-calibration of the van der Waals parameters.[16,17] With these improvements it might be possible to further improve the accuracy of free energy calculations of current fixed charge force fields, rather than switching to polarizable force fields that are both computationally expensive and difficult to parameterize.[15] An alternative to accounting for polarization costs directly would be to include the cost when calculating the fixed partial charges, such as the IPolQ ("implicitly polarized charges") method, in which the partial charges are the average of the fully polarized state, in a reaction field, and the unpolarized state, in vacuum.[17]

These advances have been made possible both by faster computers, and because methods for free energy calculations have improved to the level where the *precision* (but not necessarily accuracy) of calculated solvation free energies now rivals experimental measurements.[9,18] In particular for small systems, this finally makes it possible to separate the classical simulation challenges of sampling efficiency vs. parameter quality and systematically improve both of them.

Calculating the solvation free energy of a small molecule is an important first step to predict its free energy of binding to a protein, which in turn is of interest when studying its effects in a biological system. However, doing this with MD simulations (or Monte Carlo, which is occasionally used as an alternative sampling technique[19]) requires molecular force field topologies describing the molecules to be studied, which is a particularly difficult hurdle in the early phase of a project when thousands of molecules need to be screened rapidly. For these applications, the question is not how accurately we in theory could parameterize a molecule with manual tuning (cf. the amino acids above), but how efficient automatic methods can be with only a couple of hours of computer time.

To facilitate these types of studies with the GROMACS molecular dynamics package[20,21], we have developed a new tool that enables automatic generation of topologies for generic small molecules: STaGE (Small molecule Topology GEnerator). The name is also meant to

describe its usage as a large-scale staging/preprocessing tool ahead of the actual simulations. GROMACS comes with a number of widely used force fields, and an important goal for this development was to enable automatic topology generation for usage both with AMBER[22], OPLS[23,24], and CHARMM[25] force fields to facilitate comparisons. STaGE uses both internal and several external tools, but they have been selected with the criteria that they must be possible to install locally (no web-service-only components) and preferably free open source, or at least completely free for academic research. Our scientific aim is to significantly increase the deployment of free energy calculations by enabling critical assessment of their scientific merits, and avoid issues whether confidential compound information can be sent outside the organization, or whether specific programs justify high licensing costs.

Some functions of STaGE are specific to one or two (optional) external programs, but in general the external components are exchangable, and it is possible to choose e.g. alternative charge generation algorithms. In particular, this means it is possible to completely avoid the few tools that are not freely available even in a commercial setting. The input to STaGE can be almost any molecular file format, including SMILES. A flexible plug-in system makes it easy to add other force fields or modify the provided generation protocols.

In order to illustrate the usability of the program it has been employed to generate GROMACS topologies of an evaluation set of 50 small neutral molecules chosen from the selection of 504 compounds used by Mobley *et al.*[26,27] We utilized four different explicit solvent models and three force fields in order to evaluate their performance. The free energy cost to induce the change in polarization upon transferring a molecule from vacuum to water was also taken into account to get a more correct free energy estimation.[14,15] This is something that is often overlooked or ignored when calculating hydration free energies with fixed charge models. Bear in mind that the results here illustrate the automated procedure facilitated by STaGE and Copernicus. It is not our intention to draw conclusions, about the quality of the compared force fields and water models or whether polarization costs should be explicitly included or not, based on this relatively limited set of data.

The purpose of the STaGE program is to quickly generate topologies of many molecules with as little intervention as possible and with a low error rate. Just as with other automatic, or semi-automatic, topology generation approaches there is no guarantee that the generated topologies are perfect. In order to achieve that they would have to be verified manually. STaGE is equally useful for generation topologies for binding free energy studies, since it can easily combine the topology file of the macromolecule with those of different ligands.

2 Application overview

It is important to point out that STaGE is meant to be used for quickly generating topologies of a large number of molecules. When generating a topology of a single molecule it is highly advisable to invest more time and manually inspect all parameters. However, this is simply not realistic for high-throughput projects using hundreds or thousands of compounds, so in order to create a level playing field that is representative of high-throughput usage we have not touched the automatically generated topologies here.

2.1 Installation

STaGE itself is written in Python and does not need any installation as such. There is, however, a CMake setup that makes it easier to download and compile (if required) external tools, although some of them require accepting license agreements and must be downloaded and installed manually. See Fig. 1 for more information about the external tools used by STaGE.

2.2 Molecule input

Any molecular format that can be converted to the mol2 format by Open Babel[35] can be used as input. A specific pH can be specified to set the approximate protonation state accordingly, using Open Babel, and when using mol2 as input it is possible to retain the partial charges.

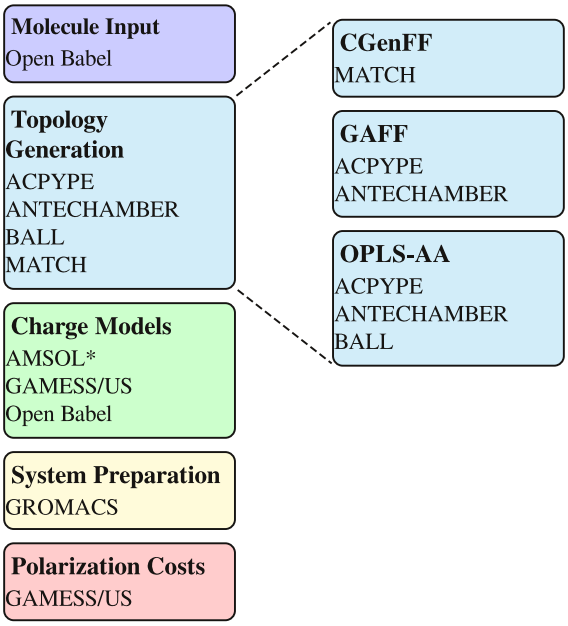


Figure 1: External applications used by STaGE. All applications (ACPYPE[28], AMSOL[29], ANTECHAMBER[30,31], BALL[32], GAMESS/US[33], GROMACS[20,21], MATCH[34], Open Babel[35]) are free for academic use and most of them are released under open source licenses. Only AMSOL[29] requires a license fee for commercial use, and this is an entirely optional component of STaGE. MATCH is a freely downloadable alternative to the CGenFF program[36,37] for generating parameters. It is possible that parameters assigned using MATCH are not completely correct, which means that erroneous parameters might not be due to CGenFF itself. The right column lists the external applications required for generating topologies of each currently supported force field. The applications in the Charge Models field can be used to assign charges based on alternative charge models - the default force field ones are always available , using ANTECHAMBER to assign AM1-BCC[38,39] charges for GAFF and MATCH to assign CGenFF charges. OPLS-AA partial charges are assigned based on the atom types, which might not work for all combinations of functional groups. Most functions of STaGE only require a subset of the external applications.

In this study, SMILES strings have consistently been used as input. Tautomers are not generated by STaGE. If topologies for different tautomers are required it is recommended to use pre-generated coordinate files as input.

2.3 Topology generation

Topologies are created using plugins for each force field. STaGE comes with plugins for GAFF[30], OPLS-AA[24] and CGenFF (CHARMM General Force Field)[40]. The GAFF plugin uses ACPYPE[28] and ANTECHAMBER[30,31] to generate topologies. The OPLS-AA plugin starts from the OPLS-AA output from ACPYPE, but since ACPYPE does not assign OPLS-AA atom types based on the chemical surroundings of the atoms STaGE instead assigns the atom types by its own set of SMARTS patterns using BALL[32]. If there are bonds, angles or dihedrals for which there are no OPLS-AA parameters the user is warned and the corresponding GAFF parameters (from the ANTECHAMBER assignment) are used instead. The CGenFF plugin uses MATCH[34] to generate the topology, which in turn is converted to GROMACS format using the charmm2gromacs-pvm.py script, included in the STaGE package and also available at www.gromacs.org (uploaded 2012-10-15). It will be easy to add future plugins for other force fields as well. Fig. 1 illustrates the applications required for generating topologies for the different force fields.

By default, the GAFF partial charges are assigned according to AM1-BCC[38,39], as generated by ANTECHAMBER[30,31]. For OPLS-AA the default charges are based on the GROMACS OPLS-AA force field atom types and often not suited for assigning charges to molecules with combinations of several functional groups, since there are not enough OPLS-AA atom types to correctly describe all possible combinations of functional groups. This can result in a non-integer net charge, in which case the user is alerted - the easiest solution for this is simply to use one of the alternative partial charge models available in STaGE. However, it is important to keep in mind that non-integer charges indicate that the atom types of some atoms in the molecule have not been correctly assigned. The OPLS-AA

topology should be carefully checked to avoid any errors. Jorgensen and Schyman have suggested to use the CM1A charge model, scaled by a factor of 1.14 for neutral molecules, to avoid the problem that partial charges are not available for all molecules in this force field.[41] The CGenFF partial charges are generated by MATCH using a bond charge increment (BCI) approach.[34]

2.3.1 Alternative Charge Models

While it is possible to use a specific charge model for all force fields, it is important to keep in mind that molecular force fields are parameterized using a specific method for applying partial charges. By definition, not all of the different charge models available in STaGE can correspond to the optimal charges for a specific force field, but we have made it easy for the user to employ different methods for assigning partial charges. On the other hand, some of these charge models may reproduce actual charge distributions (dipole moments and electronic surface potentials) better than others, which can be a good reason to use one of them. Many of them have also been successfully used when calculating solvation and binding free energies.[42,43]

AM1-BCC is the default charge model when using GAFF and it is based on Austin Model 1 (AM1) charges[44] with an applied bond charge correction to reproduce the HF/6-31G* electrostatic potential.[38,39] The charges are assigned using ANTECHAMBER.[30,31]

CM1A is a class IV charge model based on AM1[44] wave functions, parameterized to reproduce experimental properties.[45] AMSOL[29] is used for the calculations.

CM3A is similar to CM1A, but developed using a larger training set and more robust[46] and the charges are also assigned using AMSOL[29].

SM5.4/AM1 is the aqueous solvation model SM5 with charges derived from AM1 wave functions.[47] These charges are polarized, as opposed to CM1A and CM3A charges. The calculations are performed using AMSOL[29].

MMFF94 are the charges used in the force field with the same name[48] and assigned using Open Babel[35].

EEM (the Electronegativity Equalization Method) is a quick method to calculate charges similar to B3LYP/6-31G*[49,50]. The charges are calculated using Open Babel.[35]

B3LYP/PCM are charges reproducing the electrostatic potential from quantum mechanics chemistry (QM) using the B3LYP[51,52] functional method, a polarizable water model (c-PCM)[53–56] and a cc-pV(T+d)Z basis set.[57–60]. The QM calculations is peformed using GAMESS/US[33]. RESP (restrained electrostatic potential)[61] charges are applied using gmstoresp.sh (by Sarnoff Corporation, Princeton, NJ, USA), which in turn uses the respgen and resp programs in the ANTECHAMBER[30,31] program suite. It is important to keep in mind that these QM based charges can take a long time to calculate.

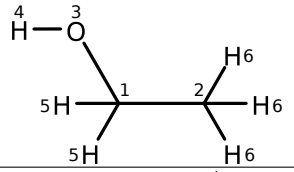
Tables 1 and 2 illustrate the different charge models applied to a few example molecules. In this first version of STaGE, B3LYP/PCM was chosen as the only included ab initio method, mainly to follow the protocol of Swope *et al*[14,15] for accounting for polarization costs. This does not mean that B3LYP/PCM is the single best ab initio method and more alternatives, such as MP2/cc-pV(T+d)Z with e.g. c-PCM, might be added in the future.[62,63]

As mentioned above, it is also possible to retain previously calculated atom charges, for instance when using a mol2 file as input. Charges can be multiplied by a constant factor in order to polarize the molecular charges in case the charge model does not take polarization into account.[42,64,65]

2.4 Solvation

If requested, a rhombic dodecahedron (the periodic unit cell most similar to a sphere, which minimizes the number of water molecules required) solvent box will be generated using the GROMACS editconf and genbox commands with the default minimum distance from the molecule to the edge of the solvent box set to 1.1 nm. In addition to the standard

Table 1: Atomic partial charges (in units of electron charge, e) of ethanol using the available charge models and the two force field-specific alternatives for CGenFF and OPLS-AA. The atoms are numbered as in the figure. Equivalent hydrogens share index, but the charges are not indentical for all models. GAFF does not have a charge model of its own, but uses AM1-BCC by default. The difference in partial charge on the hydroxyl group (atoms 3 and 4) can make a large difference in hydration free energy.

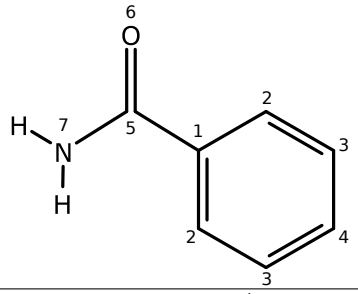


Charge model	Atoms					
	1	2	3	4	5	6
CGenFF	0.050	-0.270	-0.650	0.420	0.090	0.090
OPLS-AA	0.145	-0.180	-0.683	0.418	0.060	0.060
AM1-BCC	0.126	-0.136	-0.600	0.396	0.043	0.042
CM1A	0.000	-0.254	-0.510	0.352	0.063, 0.103	0.073, 0.085, 0.088
CM3A	0.010	-0.227	-0.493	0.340	0.055, 0.096	0.064, 0.076, 0.079
SM5.4/AM1	-0.003	-0.252	-0.561	0.392	0.078, 0.085	0.077, 0.091, 0.093
MMFF94	0.280	0.000	-0.680	0.400	0.000	0.000
EEM	-0.016	-0.430	-0.582	0.276	0.122, 0.134	0.157, 0.164, 0.175
B3LYP/PCM	0.411	-0.270	-0.711	0.419	-0.021	0.065

solvent models contained in the GROMACS installation, TIP3P-MOD[10] and TIP3P-M25[9] are also available in STaGE. If the system net charge is not zero it will automatically have ions added to make it neutral, unless the user explicitly asks for charged systems in this case. In this context it should be emphasized that the solvation process only refers to the generation of the solvent box around the molecule - it does not alter the atomic charges of the solute. Please note that STaGE only generates topologies for running GROMACS simulations. When calculating e.g. solvation free energies of charged molecules, corrections must be applied.[66] STaGE gives a warning that corrections must be applied for hydration free energy calculations if the molecule is charged.

The water model can be a suprisingly difficult choice; by default we recommend the the TIP4P-Ew[11] and SPC/E[12] water models since they have been parameterized with the polarization cost of water taken into account, and they reproduce water properties well[13], but as evident from the results below this does not automatically mean they provide the

Table 2: Atomic partial charges (in e) of benzamide using the available charge models and the two force field-specific alternatives for CGenFF and OPLS-AA. The atoms are numbered as in the figure. Only the partial charges of the heavy atoms are shown in the table and for space reasons nonpolar hydrogens are not shown. Equivalent atoms share index, but the charges are not identical for all models. GAFF does not have a charge model of its own, but uses AM1-BCC by default.



Charge model	Atoms						
	1	2	3	4	5	6	7
CGenFF	-0.020	-0.115	-0.115	-0.115	0.530	-0.510	-0.680
OPLS-AA	-0.115	-0.115	-0.115	-0.115	0.615	-0.500	-0.760
AM1-BCC	-0.142	-0.091	-0.139	-0.109	0.671	-0.610	-0.674
CM1A	-0.116	-0.076, -0.103	-0.138, -0.139	-0.108	0.590	-0.400	-1.132
CM3A	-0.118	-0.068, -0.095	-0.129, -0.131	-0.100	0.512	-0.483	-0.861
SM5.4/AM1	-0.143	-0.105, -0.109	-0.149, -0.150	-0.118	0.559	-0.519	-0.990
MMFF94	0.086	-0.150	-0.150	-0.150	0.544	-0.570	-0.800
EEM	-0.057	-0.084, -0.097	-0.101, -0.102	-0.100	0.555	-0.522	-0.873
B3LYP/PCM	-0.088	-0.108	-0.118	-0.117	0.707	-0.636	-0.870

most accurate results in all cases.

2.5 Polarization costs

Along with STaGE there is also a Python script for calculating the free energy cost of changing the polarization of a molecule in vacuum to what would be suitable in a solvent when using a force field with fixed partial charges.[14,15] The calculations are performed as described by Swope *et al.*[15] Only the dipolar component of the polarization cost is calculated. The user can either provide the output of a GAMESS/US[33] calculation, with dipole polarizability and dipole moment, or supply a mol2 file to start a gas phase structure optimization (B3LYP with a cc-pV(T+d)Z basis set[51,52,57–60]), followed by calculations to generate the dipole moment and polarizability (B3LYP calculations with an aug-cc-pV(T+d)Z basis set[51,52,60]). The dipole moment of the polarized molecule is calculated from the partial charges in a GROMACS topology file and the coordinates from the optimized structure in gas phase from the previous GAMESS/US calculations. The molecule center point for the dipole moment calculations is take from the GAMESS/US output of the gas phase dipole calculations. The polarization cost depends on the dipole polarizability and the difference in molecular dipole moment between gas phase and solvent phase as[15]

$$W_{\mathrm{pol}}^{\mathrm{D}} = \frac{1}{2}(\mu - \mu^0)^{\mathrm{T}}(\alpha^{-1})^{\mathrm{T}}(\mu - \mu^0), \tag{1}$$

where μ and μ^0 are the dipole moments when polarized and in gas phase, respectively, and α is the dipole–dipole polarizability tensor. The superscript T indicates that the expression should be transposed. The included STaGE script makes it straightforward to account for this polarizability for all solvation free energy calculations, either before or after the actual MD simulations. It is trivial to compare the polarization costs of different charge models since the time consuming QM calculations do not have to be re-executed.

3 Methods

3.1 System preparation

50 molecules were selected from the test set used by Mobley *et al.*[26,27] to obtain a good coverage of different functional groups and low to high solvation free energies. 40 of the molecules were manually selected to cover important functional groups, molecular sizes and a large span of hydration free energies. The last 10 molecules were randomly picked from the remaining set of 464 compounds. The functional groups of the selected molecules include aldehyde, alkenyl, alkyl, alkynyl, amide, amine, bromo, carbonyl, chloro, ester, ether, fluoro, hydroxyl, iodo, nitrile, nitro, phenyl, pyridyl, sulfide and thiol and the experimental hydration free energies range from -46.1 to 13.2 kJ/mol. The small molecule topologies and the solvated systems were generated from SMILES, using STaGE to obtain GAFF (General Amber Force Field)[30], OPLS-AA[24] and CGenFF (CHARMM General Force Field)[40] topologies. Unless otherwise stated, the suggested charge model was used for each force field, i.e. the MATCH bond charge increment method for CGenFF, AM1-BCC for GAFF, and atom type-based partial charges for OPLS-AA. Rhombic dodecahedron solvent boxes were generated with a minimum distance of 1.1 nm between the small molecule and the nearest edge of the box, as illustrated in Figure 2. Since all molecules were net neutral no counter ions were added. The versions of the programs used for system preparation were: ACPYPE 2013-01-02 (rev 7268), BALL 1.4.2, GAMESS/US 2013-05-01, GROMACS 4.6.4, MATCH 10/10/2011, AmberTools (including ANTECHAMBER) 13, STaGE 0.9 (corresponding to git rev 4fd65c6818) and Open Babel 2.3.2.

The evaluation set was prepared using four different water models, viz. TIP3P[67], TIP3P-M25[9], TIP4P-Ew[11] and SPC/E[12]. The total number of systems to simulate was 600 (50 molecules, three force fields and four water models). In addition, the B3LYP/PCM charge model was used in combination with GAFF in SPC/E water in order to evaluate the effect of using a more polarized charge model.

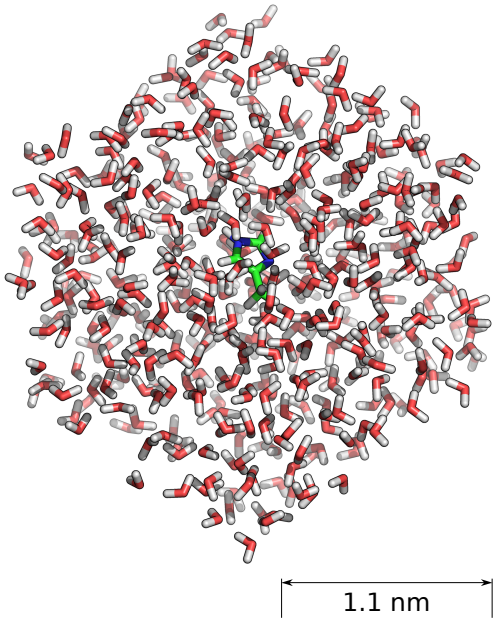


Figure 2: 4-methyl-1h-imidazole in a rhombic dodecahedron water box. 461 water molecules are included to keep the distance between the solute molecule and the nearest box edge above 1.1 nm.

3.2 Simulation setup

The simulations were performed using GROMACS[20,21] version 4.6.4. The simulation protocol started with steepest-descent energy minimizations, first 1500 steps with flexible bonds, followed by 1500 additional steps with all bonds constrained using the P-LINCS[68,69] algorithm (fourth order expansion), except for water molecules, which were kept rigid using the analytical settle constraints[70]. The same bond constraints were also used in all subsequent stages. The minimizations and simulations were run using smooth Particle-Mesh Ewald (SPME) electrostatics,[71] using a cubic interpolation order and with a fourier spacing of 0.12 nm and ewald-rtol set to 10^{-5}. The temperature during the simulations was 298 K, coupled using a velocity rescaling thermostat[72] and the pressure (when running NPT) was 1 bar, controlled using a Parrinello-Rahman barostat.[73] Equilibration was performed in three stages, the first stage in the NVT ensemble and the subsequent stages, as well as the actual production phase, in the isothermal-isobaric ensemble (NPT). During the first two equilibration

stages the atoms of the solute were restrained. The simulation time step was 2.0 fs, employing a leap-frog stochastic dynamics integrator and the group cut-off scheme. The simulation length was 50 ps in each equilibration stage. A cut-off set to 1.0 nm was used for van der Waals interactions and the same radius was used for the short-range PME component. The cut-off distance of the short-range neighbor list was 1.0 nm and the list was updated every 10 steps. A long-range dispersion correction was applied for energy and pressure. The van der Waals interaction cut-off was shorter than what is recommended for CGenFF (1.2 nm with a force-switch)[40], although dispersion corrections used in both cases means the difference should be small. This difference could theoretically influence the results, but it was decided to use the same settings for all force fields — it is also a common choice for simulations where performance matters. In order to ensure this assumption was correct a comparison of the recommended CGenFF parameters and the ones used for the production simulations was performed, for 10 molecules (every fifth from Table 3). The results are presented in Table S1. There was no significant difference between the two settings.

The solvation free energy calculations were performed using GROMACS and the Copernicus[74] parallel adaptive simulation toolkit version 2.0 (git rev 4d6504f0d2, which includes some modifications to the free energy module not shipped in release 2.0). The lambda point distribution is optimized fully automatically in Copernicus, by starting a number of shorter trial simulation, then calculating the sampling overlap between points based on the provisional lambda point distribution, and finally adjusting the location and spacing of lambda points. This is followed by automatic execution of the production simulations on all hardware clients available to the Copernicus server, after which the server uses the Bennett Acceptance Ratio (BAR) method[75] to calculate the change in free energy upon turning off interaction with the environment using lambda points. Coulomb and van der Waals interactions were decoupled independently. A soft-core transformation was used when decoupling Lennard-Jones interactions, with sc-alpha=0.5, sc-r-power=6, sc-power=1.0, sc-sigma=0.3 nm. The lambda point optimization scheme also developed by Sander Pronk, Szilárd Páll and Berk

Hess will be detailed in a separate publication, but consists of placing the lambda points so that the estimated expected per-sample standard deviation (from g_bar) gets close to 1 kT (by default). A brief summary of the calculation procedure is given in Figure 3. The target uncertainty of 0.35 kJ/mol is the combined estimated error from Lennard-Jones and Coulomb decouplings.

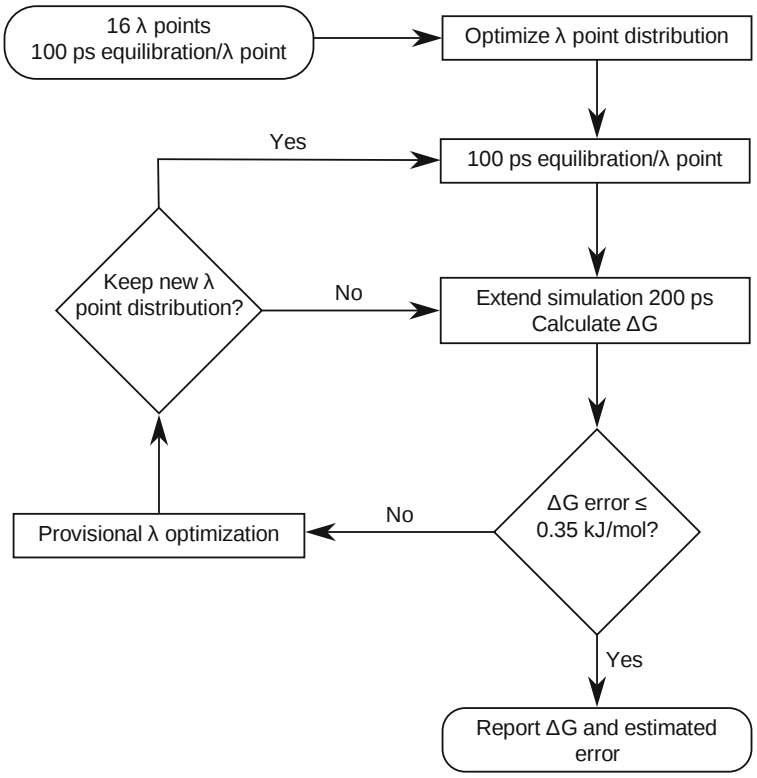


Figure 3: Iterative procedure for calculating $\Delta G_{solvation}$. When optimizing lambda point distributions the target for standard deviation per sample in each lambda interval is 1 $k_B T$. The provisional lambda point distribution was retained if the number of lambda points changed or if any lambda interval changed by more than 20%, otherwise the lambda distribution was not changed from what was previously used. ΔG and the estimated ΔG error are calculated using the Bennett Acceptance Ratio (BAR) (using the g_bar GROMACS tool). For error estimation g_bar split the data into 5 blocks and the error was determined from the average variance over those blocks.

4 Results and Discussion

OPLS-AA and GAFF topologies generated by STaGE were compared to corresponding entries in the GROMACS molecule & liquid database[76,77] to verify that the assigned atom types agreed. 1,3-dichloropropane, 1-chlorohexane (compared to 1-chlorobutane), 2-methylpropane, 2-nitropropane, benzaldehyde, ethanamide, ethanol, methanol, methyl benzoate, oct-1-yne (compared to prop-1-yne), octan-1-ol, p-cresol, pyridine and toluene had the same GAFF and OPLS-AA atom types when the topologies were generated by STaGE as in the database. For 2-iodopropane and bromoethane the GAFF atom types were the same, but the Lennard-Jones parameters of the halogens were different. For cyanobenzene the GAFF LJ parameters of the sp hybridized carbon (atom type cg) were different. These modifications are specified in the ANTECHAMBER manual as part of the developments of the GAFF force field. All parameters in the STaGE topologies of these three molecules were consistent with the output from ACPYPE and ANTECHAMBER. The OPLS-AA topology of thiophene was different when generated by STaGE compared to the GROMACS molecule & liquid database. STaGE had correctly assigned the sulfur as opls_633 (like S in thiazole, i.e. aromatic), whereas it was assigned as opls_202 (like S in a sulfide) in the database. This issue was already known and the database will be updated in the future. The other 32 compounds were not found in the database. For verifying the correctness of the CGenFF topologies the output from STaGE, which converts the output from MATCH using the charmm2gromacs-pvm.py script, the MATCH and STaGE topologies were compared. The comparison was limited to the topologies of every 10th molecule, i.e. 1-chlorohexane, 4-acetylpyridine, benzaldehyde, ethane and oct-1-yne. They were all consistent and the conversions were correct.

When generating the OPLS-AA topologies the SMARTS matching of atoms of 1-methyl-imidazole did not correctly assign some atom types, resulting in a non-zero net charge (-0.306) of the molecule. The version of the OPLS-AA force field in the GROMACS distribution does not contain the 1-methyl-imidazole atom types (opls_657–opls_666), which needs to be corrected before STaGE can be expected to produce a correct topology of that molecule.

The molecules 3-acetylpyridine, 3-methyl-1h-indole, 4-acetylpyridine, 4-methyl-1h-imidazole and thiophene seemed to have their atom types set correctly, but their net charges were still not correct (-0.040, -0.057, 0.050, 0.080 and 0.160 respectively). This is a consequence of the approach taken by OPLS-AA, to assign partial charges based on their atom types, since combinations of functional groups might lead to difficulties assigning atom types with compatible charges. As mentioned in section 2.3 STaGE gives a warning when there is a non-integer net charge of a molecule so that the user is alerted that something is probably wrong with the topology. These molecules with incorrect charges were still retained in the OPLS-AA simulations, to show the results using the output that was produced by STaGE without any intervention, which we argue is the most interesting aspect for an end user. But it should be kept in mind that the OPLS-AA statistics might be unfair to the force field because of this. To avoid this problem another charge model could be chosen when using the OPLS-AA force field, as suggested by Jorgensen and Schyman[41].

The reference hydration free energy values originate from Rizzo *et al.*[78] (original experimental data from Abraham *et al*[79], Chambers *et al*[47] and Gerber[80]) except for 3-methyl-1h-indole[81] and 4-methyl-1h-imidazole[81].

In almost all cases no experimental uncertainties were available and then they were estimated to 0.8 kJ/mol (approximately 0.2 kcal/mol), which has been appraised a typical uncertainty in experimental data for neutral molecules.[82] The target uncertainty for the computed hydration free energies was 0.35 kJ/mol and in many cases the estimated error was lower than that. Uncertainties in the calculated as well as experimental values were propagated to the RMSEs and mean differences. Since the experimental uncertainties were equal for all water models and force fields and the uncertainty of the calculated data had the same target in all cases, the resulting uncertainties were almost constant and were dominated by the approximated experimental inaccuracies. It is important to note that the estimated errors in the computed values only include errors due to limited sampling. The sampling error is relatively small, but the quality of the force field parameters is the limiting factor

for how well the calculations can correctly predict the hydration free energies.

The optimized lambda values for each compound, using the GAFF force field and AM1-BCC partial charges, in SPC/E water are presented in Table S2.

The production simulation lengths were on average 1.7 ns per molecule, including both Lennard-Jones and Coulomb decoupling simulations, but ranged from 0.6 ns to 3.6 ns (determined automatically by Copernicus). The simulation time (per molecule) for acquiring the reported hydration free energies in SPC/E water was typically in the range of 3 to 20 core hours on Intel Xeon E5-2660 2.20GHz CPUs, depending on the input molecule. One molecule used as much as 64 core hours. Since the simulations run in parallel the wallclock time is often less than a single hour per compound, which makes it straightforward to use e.g. cloud resources rather than supercomputers for this type of calculations. After this study it was noted that the simulation times in TIP4P-Ew were generally no slower than SPC/E, which could make that water model a better choice. However, there are many arguments for choosing a specific water model and this study does not propose one over the other.

4.1 SPC/E

A plot of the calculated vs. experimental hydration free energies is presented in Figure 4a and more detailed results are available in Table 3. The calculated results from CGenFF had a root-mean-square error (RMSE) of 7.94 ±0.12 kJ/mol (mean error 4.83±0.12 kJ/mol) compared to the experimental data, whereas GAFF had an RMSE of 5.95±0.12 kJ/mol (mean error 4.69±0.12 kJ/mol) and OPLS-AA had 8.97±0.12 (mean error 5.52±0.12 kJ/mol). If excluding the six compounds with non-zero net charges in OPLS-AA, the RMSE was 8.31±0.13 kJ/mol (mean error 5.76±0.13 kJ/mol). Notably, when including the polarization costs the RMSE increased to 11.61±0.12, 6.89±0.12 and 11.85±0.12 kJ/mol. The hydration free energies are overestimated, i.e. solvation is predicted to be less favorable, in SPC/E for all the studied force fields. The OPLS-AA outliers are mainly the molecules with highly negative hydration free energies, four of which have too high calculated ΔG and one too low. The four

obvious CGenFF outliers all have an overestimated ΔG (underestimated hydrophilicity). It seems like the chloro and bromo compounds get too high hydration free energy (too low predicted hydrophilicity), whereas fluoro and iodo compounds perform better in CGenFF. GAFF has a few outliers from the linear correlation, but those are closer to the experimental ΔG, which makes it difficult to draw any conclusions from them. The CGenFF fit also has a fairly good slope, but there are some clear outliers negatively affecting the predictability for an individual compound.

The results from using the more polarized charge model B3LYP/PCM are presented in Table S3. In this case only GAFF was used and the RMSE was 6.30±0.12 kJ/mol (mean error -0.08±0.12 kJ/mol). Including polarization costs reduced the RMSE to 5.05±0.12 kJ/mol as expected from a properly polarized charge model. The overall agreement with experimental data is much better using this charge model. The two main outliers are 2-ethoxyethanol and especially trimethylamine.

4.2 TIP3P

The results of the calculations using the TIP3P water model are illustrated in Figure 4b and Table S4. The calculated results from CGenFF had an RMSE of 7.03±0.12 kJ/mol (mean error 3.59±0.12 kJ/mol), whereas GAFF had an RMSE of 4.93±0.12 kJ/mol (mean error 3.29±0.12 kJ/mol) and OPLS-AA had 8.12±0.12 kJ/mol (mean error 4.76±0.12 kJ/mol). When excluding the six compounds with non-zero net charges in OPLS-AA, the RMSE was 7.43±0.13 kJ/mol (mean error 5.06±0.13 kJ/mol). When including the polarization costs the RMSE increased to 10.65±0.12, 5.88±0.12 and 11.10±0.12 kJ/mol, respectively. Compared to SPC/E it is clear that TIP3P performs better, with overall results closer to the experimental values. The outliers of the respective force fields are the same as when using SPC/E. GAFF performs better than the two other force fields, but the very hydrophilic compounds still have too high solvation free energies, which is a trend visible in the other two force fields as well.

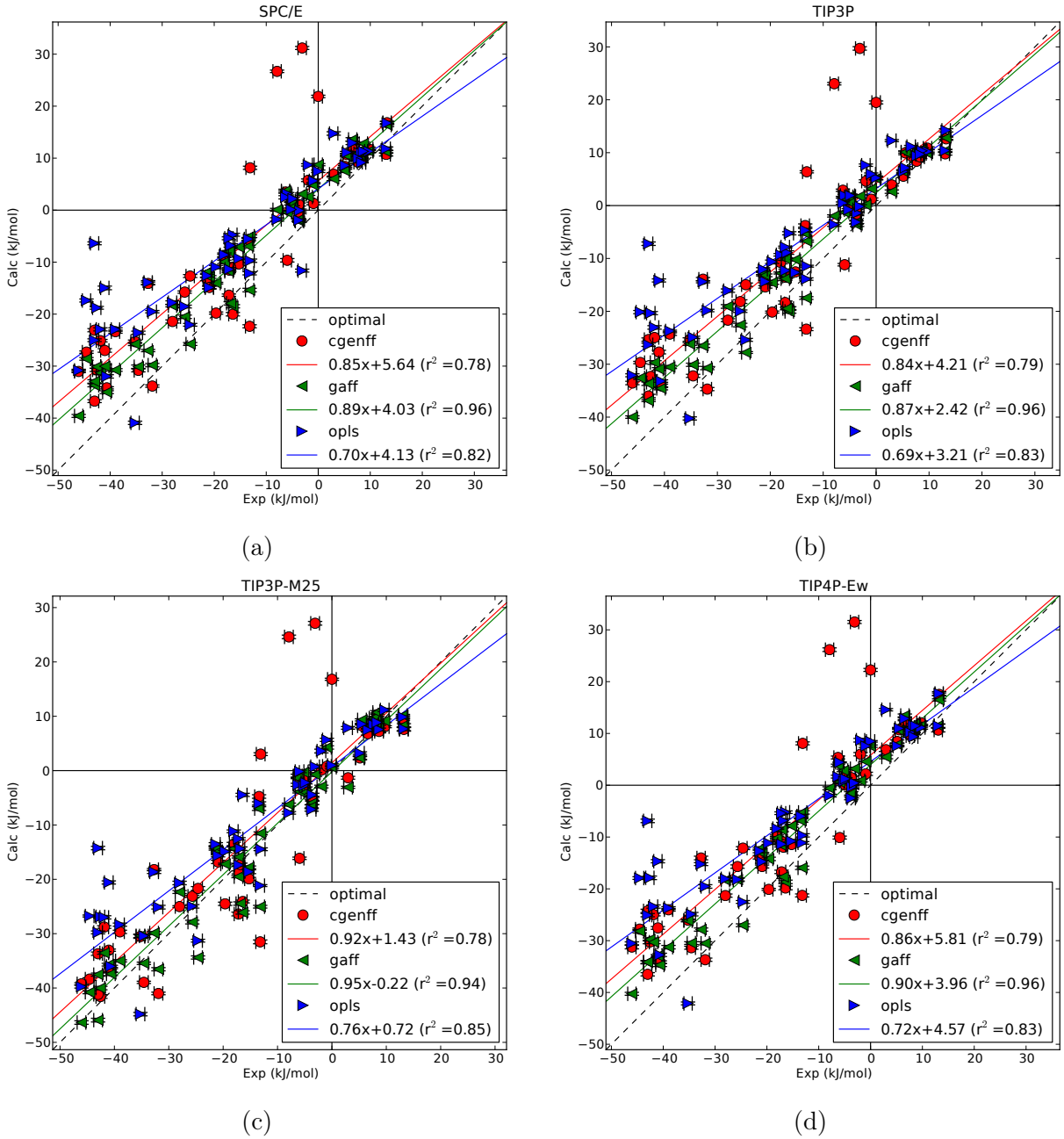


Figure 4: Calculated solvation free energies of 50 compounds in SPC/E (a), TIP3P (b), TIP3P-M25 (c) and TIP4P-Ew (d) water models using three different force fields. Polarization costs are applied to the calculated values. Error bars denote both experimental and calculated uncertainties, but the estimated calculated errors are small, making the error bars hard to see.

Table 3: Solvation free energies in SPC/E water using the CGenFF, GAFF and OPLS-AA force fields. All values in kJ/mol. The standard deviations of the mean unsigned errors (UE and UE_{corr}) were 0.12 kJ/mol for all three force fields.

Molecule	Exp.	CGenFF				GAFF				OPLS-AA			
	ΔG	ΔG	UE	ΔG_{corr}	UE_{corr}	ΔG	UE	ΔG_{corr}	UE_{corr}	ΔG	UE	ΔG_{corr}	UE_{corr}
1-chlorohexane	0.00	16.83	16.83	21.87	21.87	8.63	8.63	8.75	8.75	6.46	6.46	7.47	7.47
1-methyl-imidazole	−35.21	−31.41	3.80	−25.30	9.91	−25.92	9.29	−25.75	9.46	−57.65	22.44	−40.91	5.70
1,3-dichloropropane	−7.91	19.53	27.44	26.70	34.61	0.03	7.94	0.07	7.98	−1.83	6.08	−1.79	6.12
2-ethoxyethanol	−28.01	−24.38	3.63	−21.41	6.60	−18.48	9.53	−18.36	9.65	−19.95	8.06	−17.79	10.22
2-iodopropane	−1.93	2.26	4.19	5.75	7.68	0.18	2.11	2.54	4.47	−0.31	1.62	8.70	10.63
2-methylpropane	9.71	11.69	1.98	11.77	2.06	11.28	1.57	11.35	1.64	11.25	1.54	11.33	1.62
2-nitropropane	−13.10	−0.38	12.72	8.15	21.25	−5.67	7.43	−5.02	8.08	−10.07	3.03	−9.72	3.38
2,2,2-trifluoroethanol	−18.05	−11.69	6.36	−8.81	9.24	−14.19	3.86	−9.96	8.09	−15.49	2.56	−8.59	9.46
3-acetylpyridine	−34.58	−31.97	2.61	−30.82	3.76	−30.29	4.29	−30.12	4.46	−25.04	9.54	−23.58	11.00
3-methyl-1h-indole	−24.62	−18.72	5.90	−12.67	11.95	−25.87	1.25	−25.74	1.12	−22.41	2.21	−22.05	2.57
4-acetylpyridine	−31.90	−35.62	3.72	−33.83	1.93	−30.95	0.95	−29.81	2.09	−23.14	8.76	−19.53	12.37
4-cyanophenol	−42.58	−36.66	5.92	−30.71	11.87	−32.12	10.46	−30.12	12.46	−30.91	11.67	−18.78	23.80
4-methyl-1h-imidazole	−43.00	−36.79	6.21	−36.73	6.27	−33.32	9.68	−32.95	10.05	−27.81	15.19	−25.02	17.98
4-nitroaniline	−43.00	−31.24	11.76	−23.09	19.91	−35.75	7.25	−33.69	9.31	−28.73	14.27	−6.38	36.62
4-nitrophenol	−44.55	−32.29	12.26	−27.31	17.24	−31.80	12.75	−28.60	15.95	−29.73	14.82	−17.38	27.17
acenaphthene	−13.19	−24.41	11.22	−22.34	9.15	−15.70	2.51	−15.37	2.18	−12.19	1.00	−12.15	1.04
benzaldehyde	−16.83	−12.33	4.50	−11.00	5.83	−19.37	2.54	−17.92	1.09	−12.12	4.71	−6.79	10.04
benzamide	−46.05	−33.05	13.00	−31.03	15.02	−42.51	3.54	−39.58	6.47	−36.96	9.09	−30.82	15.23
benzene	−3.60	−0.38	3.22	−0.38	3.22	−2.12	1.48	−2.12	1.48	−0.08	3.52	−0.08	3.52
bromoethane	−3.09	−1.66	1.43	31.19	34.28	3.07	6.16	3.08	6.17	−17.38	14.29	−11.63	8.54
butane	8.67	10.93	2.26	10.93	2.26	12.81	4.14	12.81	4.14	11.31	2.64	11.31	2.64
cyanobenzene	−17.17	−17.50	0.33	−16.36	0.81	−11.83	5.34	−11.78	5.39	−8.93	8.24	−5.41	11.76
cyclohexane	5.15	8.39	3.24	8.39	3.24	7.55	2.40	7.55	2.40	8.60	3.45	8.60	3.45
decan-1-ol	−15.24	−10.82	4.42	−10.34	4.90	−7.32	7.92	−7.11	8.13	−9.86	5.38	−9.29	5.95
di-n-propylether	−4.86	−1.54	3.32	0.57	5.43	−0.55	4.31	0.60	5.46	1.38	6.24	2.21	7.07
ethanamide	−40.65	−35.41	5.24	−34.18	6.47	−37.60	3.05	−35.01	5.64	−33.73	6.92	−31.98	8.67
ethane	7.66	9.22	1.56	9.22	1.56	10.89	3.23	10.89	3.23	9.95	2.29	9.95	2.29
ethanol	−20.93	−18.37	2.56	−14.87	6.06	−14.08	6.85	−12.85	8.08	−18.94	1.99	−14.88	6.05
fluoromethane	−0.92	1.07	1.99	1.29	2.21	4.79	5.71	4.81	5.73	3.06	3.98	5.64	6.56
hex-1-ene	6.62	9.25	2.63	11.70	5.08	13.25	6.63	13.60	6.98	11.74	5.12	12.95	6.33
methane	8.33	9.75	1.42	9.75	1.42	10.39	2.06	10.39	2.06	9.16	0.83	9.16	0.83
methanethiol	−5.19	0.38	5.57	1.75	6.94	−1.03	4.16	1.31	6.50	−1.23	3.96	0.13	5.32
methanol	−21.35	−18.88	2.47	−13.27	8.08	−13.56	7.79	−11.92	9.43	−17.94	3.41	−12.61	8.74
methyl benzoate	−16.41	−22.73	6.32	−20.01	3.60	−20.29	3.88	−18.49	2.08	−9.40	7.01	−4.74	11.67
methyl ethyl sulfide	−6.28	3.18	9.46	3.49	9.77	3.06	9.34	3.70	9.98	0.63	6.91	2.47	8.75
n-acetylpyrrolidine	−41.03	−27.31	13.72	−26.98	14.05	−31.88	9.15	−30.04	10.99	−17.84	23.19	−14.96	26.07
n-butylacetamide	−38.98	−28.78	10.20	−23.38	15.60	−32.39	6.59	−30.75	8.23	−23.31	15.67	−22.96	16.02
n-decane	13.23	16.81	3.58	16.82	3.59	16.37	3.14	16.37	3.14	16.76	3.53	16.76	3.53
n-methylacetamide	−41.87	−25.15	16.72	−25.10	16.77	−32.50	9.37	−31.32	10.55	−23.06	18.81	−22.93	18.94
n,n-dimethylformamide	−32.70	−21.96	10.74	−14.10	18.60	−29.76	2.94	−27.01	5.69	−16.46	16.24	−13.90	18.80
oct-1-yne	2.97	6.38	3.41	6.89	3.92	5.86	2.89	6.02	3.05	10.84	7.87	14.75	11.78
octan-1-ol	−17.12	−13.05	4.07	−11.53	5.59	−9.26	7.86	−7.99	9.13	−13.09	4.03	−11.38	5.74
p-cresol	−25.67	−16.50	9.17	−15.76	9.91	−21.32	4.35	−20.51	5.16	−21.74	3.93	−18.54	7.13
propane	8.21	9.76	1.55	9.80	1.59	11.54	3.33	11.58	3.37	10.90	2.69	10.94	2.73
propene	5.53	6.74	1.21	10.69	5.16	10.00	4.47	10.44	4.91	9.12	3.59	11.10	5.57
pyridine	−19.64	−20.08	0.44	−19.80	0.16	−14.51	5.13	−14.04	5.60	−11.02	8.62	−10.95	8.69
tetrafluoromethane	13.06	10.72	2.34	10.72	2.34	11.24	1.82	11.24	1.82	11.81	1.25	11.81	1.25
thiophene	−5.95	−9.75	3.80	−9.64	3.69	−0.57	5.38	−0.41	5.54	3.14	9.09	3.15	9.10
toluene	−3.73	0.67	4.40	1.08	4.81	−1.91	1.82	−1.82	1.91	−2.05	1.68	−1.95	1.78
trimethylamine	−13.40	−12.50	0.90	−5.64	7.76	−13.23	0.17	−6.95	6.45	−11.00	2.40	−5.55	7.85
Mean			5.95		8.70		5.17		6.03		7.04		9.31

4.3 TIP3P-M25

Results of the calculations using the TIP3P-M25 water model are presented in Figure 4c and Table S5. The calculated results from CGenFF had an RMSE of 6.79±0.12 kJ/mol (mean error -0.37±0.12 kJ/mol), whereas GAFF had an RMSE of 4.36±0.12 kJ/mol (mean error -0.40±0.12 kJ/mol) and OPLS-AA had 6.46 ±0.12 kJ/mol (mean error 1.23±0.12 kJ/mol). If excluding the six compounds with non-zero net charges in OPLS-AA the RMSE was 5.19±0.13 kJ/mol (mean error 1.65±0.13 kJ/mol). When including the polarization costs the RMSE for CGenFF increased to 9.30±0.12 kJ/mol, GAFF to 4.54±0.12 kJ/mol and OPLS-AA to 8.39±0.12 kJ/mol. The fitted lines of both the GAFF and CGenFF force fields have a slope close to unity, but the accuracy of GAFF is clearly better here. TIP3P-M25 outperforms TIP3P at predicting hydration free energies. This is not surprising since it was developed to improve exactly that, but at the expense of general water properties, as the self-diffusion constant increased from 5.56 10^{-9} m^2/s to 6.88 10^{-9} m^2/s (experimental 2.30 10^{-9} m^2/s), ΔH_{vap} dropped from 42.249 kJ/mol to 41.567 kJ/mol (experimental 43.99 kJ/mol), but the density improved by rising from 0.9859 g/cm^3 to 0.9969 g/cm^3 (experimental 0.9972 g/cm^3).[9,83,84]

4.4 TIP4P-Ew

The results of the calculations using the TIP4P-Ew water model are presented in Figure 4d and Table S6. The calculated results from CGenFF had an RMSE of 7.86±0.12 kJ/mol (mean error 4.81±0.12 kJ/mol), whereas GAFF had an RMSE of 5.85±0.12 kJ/mol (mean error 4.53±0.12 kJ/mol) and OPLS-AA had 8.71±0.12 kJ/mol (mean error 5.67±0.12 kJ/mol). If excluding the six compounds with non-zero net charges in OPLS-AA the RMSE was 7.91±0.13 (mean error 6.08±0.13). When including the polarization costs the RMSE increased to 11.53±0.12, 6.76±0.12 and 11.72±0.12 kJ/mol respectively.

4.5 Outliers

In order to improve topology generation and force fields it is important to understand why certain molecules have a large error in the calculated hydration free energy, especially if only one force field suffers from it. We have limited this analysis to the SPC/E water model and summarize some of the molecules with relatively high mean unsigned error in Tables S7 through S15. Note that the CGenFF parameters were assigned using MATCH[34] and that there might be differences to parameters assigned using the CGenFF program[36,37]. In general it is difficult to compare parameters between force fields since other factors also differ, such as combination rules. The most clear comparison can be done between GAFF with AM1-BCC charges and with B3LYP/PCM charges, since only the partial charges differ. Anyhow, chlorohexane and dichloropropane, in Tables S7 and S8, indicate that it might be useful to investigate the chlorine parameters in CGenFF further. The B3LYP/PCM charges of 2-ethoxyethanol (Table S9) increase the polarization cost without any large change in ΔG_{solv} (Tables 3 and S3), making the predicted solubility too unfavorable. The results from 4-cyanophenol, 4-nitroaniline, 4-nitrophenol, n-acetylpyrrolidine, in Tables S10, S11, S12 and S14, can provide hints for improving AM1-BCC charges (if including polarization costs), whereas trimethylamine (Table S15) reflect that B3LYP/PCM charges do not always give better results than AM1-BCC charges. The bromoethane results using CGenFF are good before applying the polarization costs, but the changes in the dipole moment, compared to gas phase, make the final results unfavorable. In the end, there are many parameters that could be optimized. While simulations like these are useful to provide clues for parameters to investigate, much larger data sets are needed before deducing whether, and how, to alter specific parameters.

It has been shown that introducing additional point charges to compounds containing halogens (at least Cl, Br and I) can improve the electrostatic potential and also the free energy of hydration.[41,85–87] This has not been studied in this paper, but might be good to do before trying to improve halogen parameters further.

4.6 Force Field Parameter Modifications

Automated free energy calculations for entire sets of compounds make it easier to apply systematic changes in order to improve the parameterization, sometimes with quite modest means. As an example of such an attempt we focused on the data obtained for the SPC/E water model above. Since GAFF was the force field that agreed best with experimental data that was used as the starting point. The B3LYP/PCM charge model outperformed AM1-BCC, but it is not as computationally efficient, so the bond charge corrections for three functional groups were modified (AM1-BCC-pol) to more closely resemble the B3LYP/PCM charges. See Table 4 for more details on the changes. In order to test these modifications, the set of 50 compounds were divided into a training set and a test set of 25 compounds each. The training set contained every second molecule, starting with 1-chlorohexane (see Table 3). In order to have a compound with a cyano group in both sets 4-cyanophenol and 4-acetylpyridine were switched between the sets. Using the modified charges the RMSE dropped from 7.04±0.17 to 5.47±0.16 kJ/mol and the average error from 5.66±0.17 to 3.74±0.16 kJ/mol (see Figure 5b).

Table 4: Modifications to bond charge corrections used in AM1-BCC. Atom 1, atom 2 and bond order correspond to the BCC atom types and bond orders[39]. The BCC column lists the correction used in AM1-BCC and BCC-pol the modified parameters (used in the AM1-BCC-pol charge model).

Atom 1	Atom 2	Bond order	Examples	BCC	BCC-pol
11	31	1	Alcohol, ether	0.0718	0.1218
15	16	1	Cyanobenzene	0.0040	−0.0200
16	23	1	Nitrobenzene	−0.0452	0.0552
31	91	1	Alcohol	−0.2010	−0.2210
15	25	3	Cyano	0.3258	0.4300
23	31	9	Nitro	−0.1500	0.0300

The Lennard-Jones interactions were scaled in a fashion similar to how TIP3P-M25 was developed.[9] The same factors were used as for TIP3P-M25, but applied to all non-water atoms instead of water, i.e., f_σ=0.99 and f_ϵ=1.64. This was first used on the training set in

combination with the modified bond charge corrections, whereby the RMSE was reduced to 3.90±0.17 kJ/mol and the average error 0.34±0.17 kJ/mol (see Figure 5c). Applying the same parameters to the test set the RMSE was 4.34±0.17 kJ/mol with an average error or -0.24±0.17 kJ/mol (see Figure 5d).

While there is certainly still room for improvement, this brings the accuracy of calculated free energies of solvation close to a single kcal/mol even for a very trivial reparameterization of this varied set of small compounds. These modified parameters are mainly intended to illustrate that automating topology generation and MD simulations for calculating free energies of hydration can make it easier to test the effects of force field parameter modifications. The set of compounds used in this study is still limited and no other properties than the hydration free energy have been studied, so we do not suggest using these modified parameters without verifying that they perform better in general.

5 Conclusions

STaGE can be used to generate GROMACS topologies for multiple force fields using common molecular file formats as input. It can generate partial charges using a number of different charge models and also provides basic functionality for scaling or adjusting force field parameters, if required. There is no automatic parameter calibration, but it would be easy to implement a scheme to improve e.g. solvation free energies by modifying the van der Waals parameters, in approaches similar to those used by Nerenberg *et al.*[16] and Cerutti *et al.*[17] (the latter work first calibrated the partial charges). The generated system can be solvated and/or combined with previously generated macromolecular topologies. Most operations done by STaGE depend on external tools, all of which are freely available for academic research and all important programs are also free for commercial use.

While STaGE will continue to evolve as a program (in particular with new functionality and force fields), it is fully ready for production use and an important addition to the

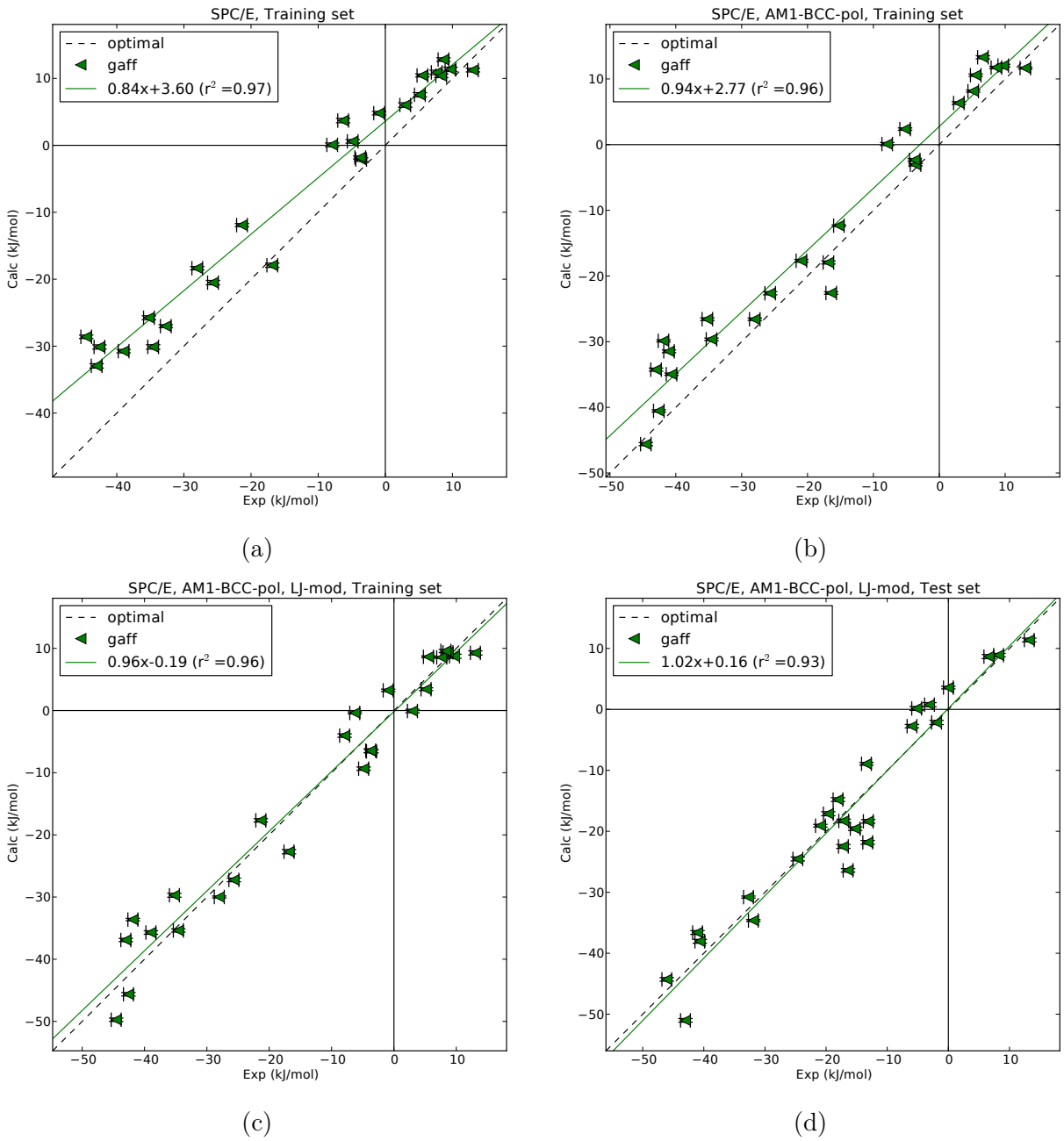


Figure 5: Calculated solvation free energies of 50 compounds in SPC/E water using the GAFF force field. The plots show the training set without any parameter modications (a), with the modified, more polarized, AM1-BCC charge model (b) and with modified Lennard-Jones interactions and the modified AM1-BCC charge model (c) and finally the test set with all parameter modifications (d). Polarization costs are applied to the calculated values. As can be seen from (b) the slope is improved when using the modified charges and when also including the scaled Lennard-Jones parameters (c) and (d) the agreement with experimental values gets very good. Error bars denote both experimental and calculated uncertainties, but the estimated calculated errors are small, making the error bars small.

GROMACS free energy calculation pipeline - small molecule topologies no longer require deep expertise in force field atom type selection, experience of quantum mechanics chemistry (QM) for partial charge calculation, or manual topology assembly in a text editor. Similarly, the fully automated optimization of free energy calculations, execution of dozens of independent simulations and BAR analysis made possible with Copernicus (e.g. in the cloud) means free energy calculations are more accessible than ever.

At the time of writing (spring 2014) the spot price for one core hour, on hardware comparable to what was used in this project, at a major cloud vendor was approximately $0.01, meaning the total cost for calculating the hydration free energies, not including QM calculations for polarization costs, of these 50 compounds for one of the figures would be approximately $10, based on the estimated calculation time per compound. This is an interesting alternative to maintaining hardware, and it emphasizes that free energy MD simulations do not necessarily require major hardware investments.

This should be useful for many applications, but one of the most important aspects is that it enables systematic critical assessment and comparisons both of force fields and methods to perform free energy calculations. There are huge efforts behind all modern force fields, and it is remarkable how much they have improved the last two decades, but they only way to further improve free energies is to find discrepancies and shortcomings.

The solvation free energy calculations of the 50 compounds included in this study show that all tested force fields reproduce the experimental results fairly well, but there is certainly room for improvements, with a mean unsigned error under 1.5 kcal/mol in almost all cases (except for the combination of OPLS-AA with SPC/E or TIP4P-Ew). Unfortunately, the force fields give worse results with the more correct water models, SPC/E and TIP4P-Ew, for which they have not been parameterized. When accounting for polarization costs it is clear that the charge models recommended for use with the three force fields employed in this study are underpolarized, since the errors increase when correctly applying the polarization cost. When taking the polarization cost into account, our results show a better

agreement with experimental values when using QM-based partial charges (B3LYP/PCM), as reported previously[62,63], instead of standard AM1-BCC charges for GAFF. However, this set of molecules is small and more extensive benchmarks are required to accurately compare the charge models. Nevertheless, this indicates that the force fields might profit from being reparameterized taking polarization costs into account. If using an underpolarized charge model, e.g. AM1-BCC, it is probably good not to include polarization costs and assume that the charges implicitly account for the polarization costs.[17]

By modifying the AM1-BCC bond charge corrections for a handful of groups to better resemble B3LYP/PCM charges, and slight modifications of the GAFF LJ parameters, it was possible to achieve a clear improvement of the solvation free energies for the present test set - the final setup has an RMSE close to a kcal/mol for a diverse set of arbitrary compounds with both topology generation and free energy calculations being fully automated.

This might be a starting point for re-parameterizing force fields to properly take polarization costs into account. It is important to keep in mind that the modifications herein have just been a proof of concept that small changes can make a large difference for the solvation free energies. The parameters need to be verified for other properties and further modifications for other functional groups would certainly be good, but that will be covered in a future publication.

Importantly, we do not suggest using one force field over any other based on this limited study. Many things need to be taken into account when selecting a force field, for instance whether the small molecule should be used as part of a larger system that has already been simulated with one of the force fields. Ultimately, STaGE leaves the force field decision to the user, and we hope it will lead to more direct comparisons even for complex systems. STaGE is open source and freely available from `https://gerrit.gromacs.org/#/admin/projects/STaGE`. The topologies for the 50 molecules for the CGenFF, GAFF and OPLS-AA force fields are available for download from `ftp://ftp.gromacs.org/pub/stage_topologies/stage_50_topologies_spce.tgz`. For GAFF there are

also different alternative charge models included, namely B3LYP/PCM and MMFF94, as well as the modified charges and Lennard-Jones parameters (see section 4.6), in addition to the standard AM1-BCC charges. These systems are pre-solvated in an SPC/E water box, but can be used with any water model.

Supporting Information Available

This information contains a comparison of free energies of hydration using the CGenFF force field with recommended cut-off parameters and the parameters used in this study (Table S1), a list of optimized lambda values using GAFF in SPC/E (Table S2) as well as results from calculations using GAFF with B3LYP/PCM charges in SPC/E (Table S3) and using all force fields (with standard charges) in TIP3P, TIP3P-M25 and TIP4P-Ew (Tables S4–S6). There are also tables of force field parameters of molecules with results with large deviations from experimental data (Tables S7 to S15). This material is available free of charge via the Internet at `http://pubs.acs.org/`.

Acknowledgement

We would like to thank Sander Pronk, Szilárd Páll and Berk Hess for their work on the free energy module of Copernicus, including the lambda point optimization scheme. We are also grateful to Paul van Maaren and David van der Spoel for feedback on the SMARTS patterns for setting OPLS-AA atom types.

Contract grant sponsor: EU FP7 project CRESTA; Contract grant number: 287703. Contract grant sponsor: Swedish e-Science Research Center; Contract grant sponsor: Swedish research council; Contract grant number: 2010-491, 2013-5901. Computational resources were provided by the Swedish National Infrastructure for Computing (2013/26-24).

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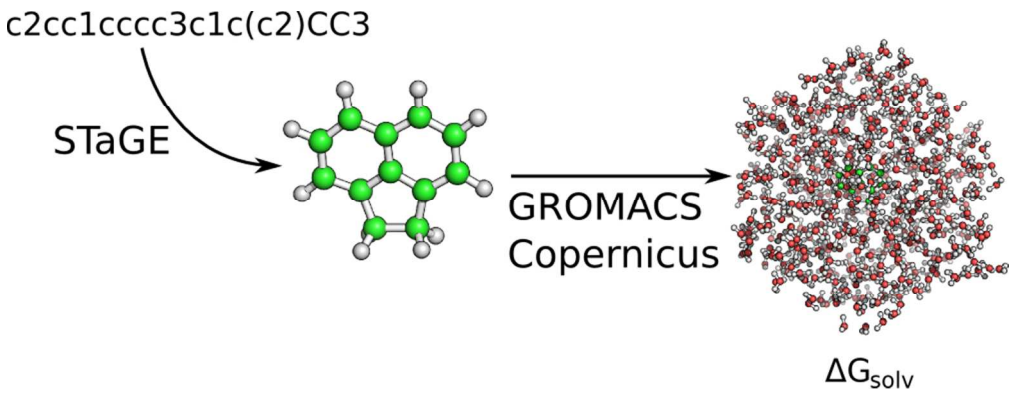

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