

Generalized Born Model: Analysis, Refinement, and Applications to Proteins

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A Generalized Born (GB) model, describing the mean-field electrostatic energy of a molecular system in a continuous, polarizable solvent environment, is compared with Kirkwood and Poisson–Boltzmann (PB) standard approaches. GB energy data for amino acids, a dodecapeptide, and three protein molecules are validated against the corresponding PB numerical results. A new approximation for the Born radii, dependent on the geometry of the system, as well as on the ratio of the solute and solvent dielectric constants, is proposed. The refined GB approach also takes into account the ionic strength. The most important discrepancies between the GB and PB results for proteins are identified and interpreted. A more precise procedure for computing the GB energy is proposed.

1. Introduction

Mesoscopic models and theories play a more and more important role in studies of large molecular and biomolecular systems and processes. This, in particular, refers to structure formation phenomena, virtual titration of biopolymers, specific molecular recognition phenomena, and models for mean-field forces and dynamics.^{1–6} Continuum models typically treat a solvent as a homogeneous environment with a high dielectric constant, and the solute is represented as a set of atomic point charges embedded in a low dielectric constant. Counterions are represented as charge densities in thermodynamic equilibrium with the environment, described by the Boltzmann distribution.

The most popular model is based on a differential Poisson–Boltzmann (PB) equation, which is solved numerically on a grid. Simplified models have become more and more popular and are applied to large biomolecular systems.⁷ In particular, a generalized Born (GB) model refers to an original study by Born of spherical ions in a solvent, with charges in their centers.⁸ A more advanced model, presented by Kirkwood,⁹ solves the PB equation for spherical objects with a number of point charges located inside the sphere, not necessarily in its center, and with solvent and counterions outside the sphere.

Whereas solving the PB equation for a macromolecule can take several minutes, the computation based on the GB model takes seconds. However, the gain in computing time results in a decrease in the accuracy of the electrostatic energy. This study will present a critical analysis of the GB model. GB calculations are validated against the PB and Kirkwood results, with varied parameter values of the solute/solvent dielectric constants and the ionic strength. We also test a fast algorithm for computing the electrostatic contribution to the free energy of solvation and propose an improvement of the GB model, which should result in better accuracy and extended applicability.

2. Methods

2.1. A Brief Overview of the Poisson–Boltzmann and Kirkwood Models. Continuum models deal with molecular objects embedded in a continuous environment characterized by a dielectric constant, ionic strength, and temperature. The models are described by the linear PB equation

$$\nabla[\epsilon(\mathbf{r})\epsilon_0\nabla\varphi(\mathbf{r})] = -\rho_{\text{ext}}(\mathbf{r}) + \lambda(\mathbf{r})\bar{\kappa}^2\varphi(\mathbf{r}) \quad (1)$$

where ϵ is a scalar dielectric function, $\bar{\kappa}^2 = \epsilon(r)\kappa^2$, $\kappa^2 = 2e^2I/\epsilon_0\epsilon RT$ is the Debye–Hückel screening parameter, and I is the ionic strength. $\epsilon(r)$ determines the solvent and solute areas, and $\lambda(r)$ describes the ion accessible area ($\lambda = 0$ for a domain inaccessible to ions, $\lambda = 1$ otherwise). For an overview of the PB equation and applications, see ref 5.

In the Kirkwood model, the molecule is described as a low-dielectric sphere with radius a , embedded in a high-dielectric solvent, and with counterions outside the radius b ($b > a$). Kirkwood proposed the analytical solution of the linear PB equation for a charge placed at any \mathbf{r}_0 inside the low-dielectric sphere. The sphere center is located in the origin of the coordinate system.

$$\varphi(\mathbf{r}) = \frac{q_0}{4\pi\epsilon_0\epsilon_{\text{in}}|\mathbf{r} - \mathbf{r}_0|} + \varphi_{\text{cs}}(\mathbf{r}) + \varphi_{\text{ci}}(\mathbf{r}) \quad (2)$$

The first term is the well-known Coulomb potential of a point charge in uniform space, with dielectric constant ϵ_{in} . The second and third terms are corrections to this potential, due to embedding of the sphere in an environment with a high dielectric constant, $\varphi_{\text{cs}}(\mathbf{r})$, and to the presence of counterions, $\varphi_{\text{ci}}(\mathbf{r})$. Because $\varphi_{\text{ci}}(\mathbf{r})$ introduces only a small perturbation to the potential (see numerical results to follow), we will mostly focus on the reaction field generated by the solvent, which is expressed as

$$\varphi_{\text{cs}}(\mathbf{r}) = \frac{q_0}{4\pi\epsilon_0\epsilon_{\text{in}}a} \sum_{l=0}^{\infty} \frac{(l+1)(\epsilon_{\text{in}} - \epsilon_{\text{ex}})}{(l+1)\epsilon_{\text{ex}} + l\epsilon_{\text{in}}} \left(\frac{r r_0}{a^2}\right)^l P_l(\cos \theta) \quad (3)$$

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P_l 's are Legendre polynomials, and θ is the angle between vectors \mathbf{r} and \mathbf{r}_0 . The classical Born solvation energy can be obtained from the Kirkwood solution by placing the atomic charge in the center of the sphere and neglecting the contribution derived from the ionic strength. The Kirkwood model provides the exact solution, however, only for spherical objects. It is sufficient to account for several hundred terms in eq 3 to get reliable results. The PB model gives solutions for any geometry, but some errors emerge from numerical calculations on the grid. The Kirkwood, as well as the PB, model provides reference solutions to the GB model.

2.2. The Generalized Born Model. The GB model is based on the linear PB equation. In the GB model, the electrostatic contribution to the free energy of solvation is given by the following formula^{1,10–14}

$$\Delta G = -\frac{1}{8\pi\epsilon_0} \sum_{i,j} \left(\frac{1}{\epsilon_{\text{in}}} - \frac{\exp(-\kappa f_{\text{GB}}^{ij})}{\epsilon_{\text{ex}}} \right) \frac{q_i q_j}{f_{\text{GB}}^{ij}} \quad (4)$$

where summation is carried out for each pair of atoms, i and j , including $i = j$. The ϵ_{in} and ϵ_{ex} are the dielectric constants of solute and solvent, respectively, κ is the Debye–Hückel screening parameter, q_i and q_j are the partial charges of atoms i and j , and the f_{GB}^{ij} function depends on the Born radii and the distance between the atoms i and j . The ionic strength of the solvent is included in the κ parameter. For $i = j$, the energy contribution specifies the solvation energy of the atom i in the molecule, which is immersed in solvent. When $i \neq j$, the energy contribution describes the change in electrostatic interactions between the two atoms upon solvation. The f_{GB} function is defined¹⁰ as

$$f_{\text{GB}}^{ij} = \sqrt{r_{ij}^2 + R_i R_j \exp(-r_{ij}^2/4R_i R_j)} \quad (5)$$

where r_{ij} is distance between atoms i and j , and R_i and R_j are the Born radii of the atoms i and j . Such construction of this function gives the proper asymptotic behavior. At large distances, it is close to r_{ij} , and at short distances, it is close to $\sqrt{R_i R_j}$. The parameter of 4 in the exponent has been chosen to link these asymptotic limits in a smooth way. Each atom is characterized by its own Born radius, which depends on the size and shape of the molecule, as well as on its location in the molecule. One should note that, for $i = j$, f_{GB}^{ii} is R_i . Using several crude approximations (e.g., a Coulomb field approximation) the Born radius formula was proposed as

$$\frac{1}{R_i} = \frac{1}{4\pi} \int_{\text{ex}} \frac{1}{r^4} dV \quad (6)$$

The integration is carried out in the space outside the molecular object, which is denoted as f_{ex} . This can be rewritten as

$$\frac{1}{R_i} = \frac{1}{a_i} - \frac{1}{4\pi} \int_{\text{in}, r > a_i} \frac{1}{r^4} dV \quad (7)$$

Here, integration is carried out over the space occupied by the molecule, excluding the sphere of radius a_i , and with the atom i located in the origin of the coordinate system. Typically, a_i is the atom's Van der Waals (VdW) radius, sometimes modified by a scaling parameter. This approximation is valid for spherically symmetric cases (e.g., a spherical ion immersed in a dielectric medium), and its generalization to more complex shapes may be too crude. It has been shown recently¹⁵ that, in general, the Coulomb field approximation is not valid. It gives

proper values for charges placed in the center of the sphere, but a shift in the position of the charge introduces a systematic error. The largest deviation is observed for charges close to the surface of the sphere, and it approaches 50% of the true solvation energy. The appropriate equation¹⁵ is

$$\frac{1}{R_i} = \left(\frac{3}{4\pi} \int_{\text{ex}} \frac{1}{r^6} dV \right)^{1/3} \quad (8)$$

and is based on the Kirkwood model. For charges at the center of the sphere, this equation has the same solution as eq 6. It also retains its accuracy when the charge is moved away from the center.

In both cases, the Born radii are equal to or greater than the VdW radii. In the simplest case (a spherical ion) the Born radius is equal to its VdW radius. These radii can be computed by numerical integration, but the computational cost of this procedure is high. The most popular method for fast evaluation of the integral is an overlapping spheres approach. The whole integral is divided into a sum of contributions arising from each atom. The value of the integral over the space occupied by one atom can easily be evaluated analytically. This method is very fast but introduces additional approximations and errors. Especially, it overestimates the value of the integral, because atoms in molecules overlap. It can be expressed by the formula

$$\frac{1}{R_i} = \frac{1}{a_i} - \sum_j V(r_{ij}, a_j, s_j) \quad (9)$$

for the conventional approximation, or

$$\frac{1}{R_i} = \left[\frac{1}{a_i^3} - \sum_j V(r_{ij}, a_j, s_j) \right]^{1/3} \quad (10)$$

in the refined approach. The Born radius of the atom i is R_i . The a_i is the radius of the i^{th} atom, usually equal to the VdW radius ($a_i = R_{\text{VdW},i}$). Similarly, $a_j = R_{\text{VdW},j}$. The r_{ij} value is the distance between the atoms i and j . Additionally, a scaling factor is used to account for systematic errors. The scaling factor can be introduced in a number of ways (e.g., the atom's VdW radius can be adjusted or the value of the integral can be rescaled). The scaling factor is denoted as s_j .

The function of V (or V') is quite complex but has an analytical form. It results from analytical evaluation of the integral given by eq 6 (or eq 8) over the space occupied by one atom, j . The exact formula of the V' function, without any scaling factor, can be expressed as

$$V(r_{ij}, a_j) = (n-3) \left[\frac{1}{4r_{ij}(n-4)x^{(n-4)}} - \frac{1}{2(n-3)x^{(n-3)}} + \frac{r_{ij}^2 - R_{\text{VdW},j}^2}{4r_{ij}(n-2)x^{(n-2)}} \right]_{x_{\min}}^{x_{\max}} \quad (11)$$

where x_{\min} and x_{\max} are the integral limits. Usually, when atoms i and j are separated, $x_{\min} = r_{ij} - R_{\text{VdW},j}$ and $x_{\max} = r_{ij} + R_{\text{VdW},j}$. For overlapping atoms, the limits change, because the lower limit should be greater than or equal to the VdW radius of the atom i ($x_{\min} \geq R_{\text{VdW},i}$). The value of n is determined from the formula of the Born radii, f_1/r^n ($n = 6$). One should note, however, that the function is also valid for different exponents n 's, $n > 3$ and $n \neq 4$.

The scaling factor is optimized either with respect to the experimental data or numerical PB results. This factor arises

TABLE 1: Molecular Systems Used in the Calculations

molecule	no. of atoms	PDB code (reference)
phosphoric diester hydrolase	1708	2PLE ^a
ovomucoid third domain	814	1OMU ^b
lysozyme	1961	1E8L ^c

^a Pascal, S. M.; Singer, A. U.; Gish, G.; Yamazaki, T.; Shoelson, S. E.; Pawson, T.; Kay, L. E.; Forman-Kay, J. D. *Cell* **1994**, *77*, 461–472. ^b Hoogstraten, C. G.; Choe, S.; Westler, W. M.; Markley, J. L. *Protein Sci.* **1995**, *4*, 2289–2299. ^c Schwalbe, H.; Grimshaw, S. B.; Spencer, A.; Buck, M.; Boyd, J.; Dobson, C. M.; Redfield, C.; Smith, L. J. *Protein Sci.* **2001**, *10*, 677–688.

from the fact that atoms in a molecule overlap, and the pairwise summation cannot account for this effect. The overlapping effect is unique for each molecule, and therefore, the scaling factors are also unique. They can be averaged using a representative ensemble of molecules. This will be discussed in more detail.

Results obtained with the GB model can be validated against the PB results. However, one should take into account differences in physical and mathematical assumptions, as well as in their numerical implementations. Regarding mathematical assumptions, both models define two separate areas, the high dielectric constant area and the low dielectric constant area, occupied by the solute. Note, however, that definitions of the areas are different. Typically, the PB model defines the space of the high dielectric constant as the solvent accessible area (SA), whereas the GB model defines it as the area outside the VdW spheres of the solute atoms. Moreover, the PB model can define the space available for counterions independently of the solvent accessible area. Because ions have different sizes than solvent molecules, the solvent and ion accessible areas can be different. In the GB model, both areas are the same. Therefore, the space of low dielectric constant in the GB model is a little smaller than in the PB model. Additional complications appear for large molecules, because a number of empty spaces, inaccessible to solvent, exist inside such molecules. In the GB model, all holes are assigned to solvent. In such situations, the solute atoms close to these holes may exhibit solvation energies that are too high. In the PB model, the holes belong to the solute space. Limitations of the PB model are related mostly to longer computing times and errors arising from numerical calculations on the grids. For a large system, a single computation can take minutes, whereas a GB calculation for the same system can take seconds.

2.3. Reference Molecules. In this study, we tested and refined the GB model using a set of reference biomolecules. These were 3 proteins, listed in Table 1, 1 dodecapeptide, and 21 single amino acids. The dodecapeptide with sequence Asp-Asn-Asp-pTyr-Ile-Ile-Pro-Leu-Pro-Asp-Pro-Lys is a substrate for 2PLE phospholipase. These molecular systems were used in our previous studies dealing with the optimization of a titration procedure based on the PB equation.^{3,4} In this study, a number of conformations were considered for each system. Conformations of small molecules were generated using Langevin Dynamics simulations. Regarding proteins, multiple NMR structures available in the protein database (PDB) were taken into account. Diversity of structures should allow extracting and indicating weaker elements of the GB model.

2.4. The Generalized Born and the Poisson–Boltzmann Calculations. The PB computations were carried out using the UHBD software package¹⁶ to solve the linear PB equation with a finite difference method (FDPB). Unless otherwise stated, we used standard parameters: a solute dielectric constant of 1.0, a solvent dielectric constant of 80.0, a solvent probe radius of 1.4 Å, ionic strength of 150.0 mM, an ion exclusion radius of

2.0 Å, at temperature of 300 K. The boundary of the molecular volume is defined as the solvent accessible surface (SAS), which is typical for continuum dielectric models. We will be comparing all GB calculations with these PB calculations to identify weak points of the GB approach.

Regarding the GB model, we implemented the overlapping spheres approach, and also modified the classical GB method for a regular grid of spheres. The overlapping spheres approach is described by eqs 9 and 10.

Using the PB calculations, we computed the one- and two-atom contributions to the solvation energy. The one-atom solvation energy, ΔG_{ii} , describes an effective interaction of the given atom with solvent

$$\Delta G_{ii} = -\frac{1}{8\pi\epsilon_0} \left(\frac{1}{\epsilon_{in}} - \frac{e^{-\kappa R_i}}{\epsilon_{ex}} \right) \frac{q_i^2}{R_i} \quad (12)$$

The total two-body solvation energy, ΔG_{two} , as computed using the PB method, consists of three terms. Two of them describe the one-atom solvation energies, and the third one, ΔG_{ij} , describes the change in the interaction energy between the atoms resulting from the presence of solvent (e.g., interaction between the first atom and the reaction field of the second one). ΔG_{ij} will be called the two-atom solvation energy

$$\Delta G_{two} = \Delta G_{ii} + \Delta G_{jj} + \Delta G_{ij} = \Delta G_{ii} + \Delta G_{jj} - \frac{1}{8\pi\epsilon_0} \left(\frac{1}{\epsilon_{in}} - \frac{\exp(-\kappa f_{GB}^{ij})}{\epsilon_{ex}} \right) \frac{q_i q_j}{f_{GB}^{ij}} \quad (13)$$

To obtain ΔG_{ij} from the PB calculations, one should subtract the one-atom solvation energy of each atom (ΔG_{ii} and ΔG_{jj}) from the whole two-body solvation energy (ΔG_{two}). All atomic charges, in both calculations, are set to 0.0, except for the selected single atom or pair of atoms i and j , which are assigned the normalized charge of 1.0. The same charge value for all atoms, regardless of the atom types, makes further analysis simple and clear for interpretation. Because we used the linearized PB equation, $\varphi(q) = q \cdot \varphi(q_0 = 1)$, and in order to obtain the real solvation energy, one should multiply the obtained energy values by proper atomic charges, both for the PB as well as the GB models. In the case of one-atom solvation energies, the larger values appear for atoms close to the surface of the molecule. For the solvation energies of atom pairs, larger values appear either for atoms close to the surface or for atoms close to each other.

3. Results

The subject of our study is the model defined by eq 4. In this approach, the Born radii are independent of the other parameters of the model, except the VdW radii and the positions of the atoms (see eq 8). In particular, the Born radii are independent of the solute and solvent dielectric constants, as well as the ionic strength.

The solvation energy of each atom in the molecule can be computed using the PB method, with other atomic charges and the ionic strength both set to 0. Then, the Born radii can be calculated using the formula

$$\Delta G_{ii} = -\frac{1}{8\pi\epsilon_0} \left(\frac{1}{\epsilon_{in}} - \frac{1}{\epsilon_{ex}} \right) \frac{q_i^2}{R_i} \quad (14)$$

The radii, obtained in this manner, are used for further calculations and will be called the effective Born radii.

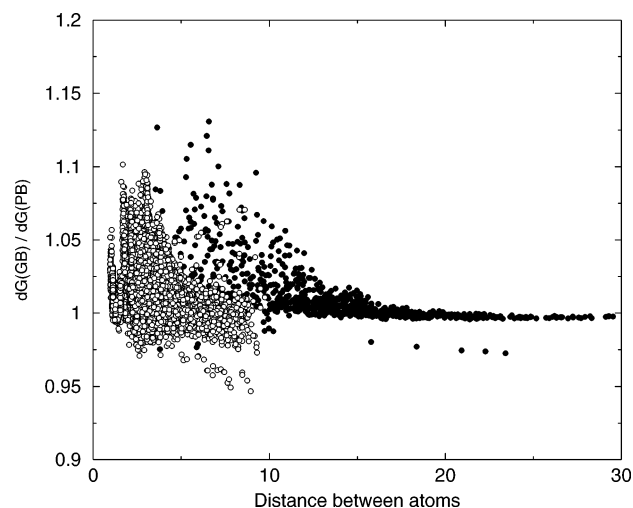


Figure 1. Ratio of the GB two-body and PB two-body contributions, presenting the accuracy of the f_{GB} function. About 1000 atom pairs from the IOMU protein (black points), and about 14 000 atom pairs from amino acids (gray points) are presented.

3.1. Validation of the GB Formula. To check the accuracy of the f_{GB} function, the two-atom solvation energies were computed using the PB model with standard environment parameters, except the ionic strength of 0. Then, using eq 4 and the effective Born radii, the respective GB solvation energies were computed along with about 15 000 energy values for atom pairs from proteins and amino acids. These energies match the PB values almost perfectly. The average ratio of the PB and GB energy is 1.017, with a standard deviation of 0.018. This shows that the form of the f_{GB} function works well for all molecules, regardless of their shape and size. At larger distances, the f_{GB} function behaves like r_{ij} , and for neighbor atoms, the correction to the distance becomes more important. A more detailed analysis shows that most of the badly computed energies are for neighbor atoms (see Figure 1). Note that the f_{GB} function depends only on the Born radii (R_i). Therefore, precision of computing the R_i determines the accuracy in computing the two-atom solvation energies. However, with the effective Born radii, we can expect errors up to 10% of f_{GB} .

It has been shown¹⁵ that, in the case of simple spherical molecular objects, the proper form of f_{GB} is $f_{GB}^{ij} = (r_{ij}^2 + R_i R_j)^{1/2}$. In the opposite case, for example, for two ions in water, with their radii much smaller than the distance between them, the following relationship $f_{GB}^{ij} = r_{ij}$ is valid. In the case of molecules of any shape, such a simple function does not exist. Atoms sometimes act like separate ions, and sometimes like the point charges in one common sphere. It seems to be adequate to assume an intermediate solution. We tested several other forms of f_{GB} (e.g., removing the exponent or changing the parameter of 4 in the denominator). The classical form of f_{GB} has appeared to be the most optimal one.

To check the dependence of the solvation energies on the ionic strength, a number of calculations were carried out with standard parameters, changing only the ionic strength. The solvation energy depends weakly on the ionic strength. The magnitude of the whole solvation energy is on the order of hundreds of kilocalories per mole, and the counterions' contribution is close to the one-tenth of a kilocalorie per mole. The PB energy contribution due to presence of counterions does not exceed 0.8% of the total solvation energy. Therefore, the two-atom energies are burdened with errors larger than the expected values of the ion solvation energies, so we will be comparing only the change of the one-atom solvation energies between a

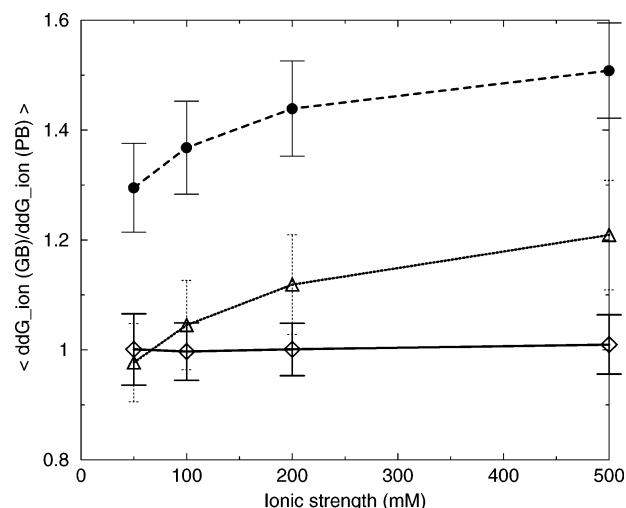


Figure 2. The average ratio of the GB and the PB ion solvation energies against the ionic strength. The dashed line (---) and circles (●) represent the ratio of the ion solvation energies computed with the original Born formula and the PB method. The dotted line (···) and triangles (Δ) represent the ratio of the ion solvation energies with changes proposed by Srinivasan. The continuous line (—) represents the ratio of the ion solvation energies with our modified Born formula, $\exp(-\kappa f_{GB}^{ij}) \rightarrow \exp[-0.53 \cdot (\kappa f_{GB}^{ij})^{0.77}]$. Additionally, the standard RMSD errors are presented for each method ($\sigma_x^2 = \frac{N}{N-1} \sum_{i=1}^N \frac{(x_i - \langle x \rangle)^2}{N-1}$, $x = \Delta\Delta G(\text{ion})_{GB} / \Delta\Delta G(\text{ion})_{PB}$). The calculations were carried out for 837 atoms of the ovomucoid (IOMU).

solvent with a given ionic strength and a solvent with no counterions, giving the ion solvation energy, $\Delta\Delta G(\text{ion}) = \Delta G(\text{ion}) - \Delta G(0)$. Figure 2 presents the dependence of the average ratio of $\Delta\Delta G(\text{ion})_{GB} / \Delta\Delta G(\text{ion})_{PB}$ on the ionic strength. The ion solvation energy is overestimated in the GB model, and this effect grows up with the increase of the ionic strength.

The exact form of the counterions' contribution is given by the Kirkwood model, and for the single charge at the center of the sphere with radius R_a , it becomes

$$\Delta\Delta G_{ii} = -\frac{1}{8\pi\epsilon_0\epsilon_{\text{ex}}R_a} \left(\frac{\kappa R_a}{1 + \kappa R_a} \right) \quad (15)$$

The same energy can be computed using the GB model

$$\Delta\Delta G_{ii} = -\frac{1}{8\pi\epsilon_0\epsilon_{\text{ex}}R} (1 - e^{-\kappa R}) \quad (16)$$

Note that the R_a radius is larger than R , because the solvent accessible area is larger than the space accessible by the counterions. The expression $(1 - e^{-\kappa R})$ should approximate the function

$$\frac{\kappa R_a}{1 + \kappa R_a}$$

Because values of R and R_a differ, a correction should be introduced. To compensate overestimation of the ion solvation energy, one could change this form. First, a correction related to the ionic strength was proposed by Srinivasan et al.¹⁴ ($\kappa \rightarrow 0.73\kappa$). We propose a little more complicated solution. The expression of κf_{GB}^{ij} is dimensionless; therefore, one can change

$$\exp(-\kappa f_{GB}^{ij}) \rightarrow \exp[-A(\kappa f_{GB}^{ij})^B] \quad (17)$$

The calculations give the best agreement for the PB energy with

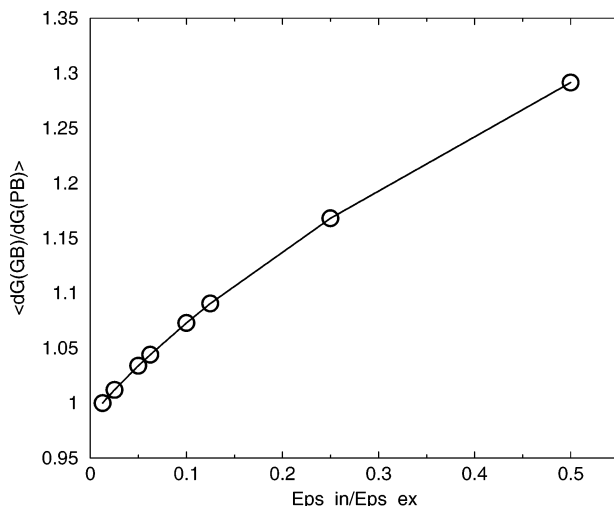


Figure 3. The average ratio of the Born/PB solvation energies vs the $\epsilon_{\text{in}}/\epsilon_{\text{ex}}$ ratio of the dielectric constants. The smallest value is for the solute dielectric constant of 1 and the solvent dielectric constant of 80 (the ratio being 1/80). The largest value is for $\epsilon_{\text{in}}/\epsilon_{\text{ex}} = 10/20$. Each point presents an average over 2000 solvation energies.

$A = 0.53$ and $B = 0.77$. The accuracy of the ion solvation energy does not depend on the ionic strength (see Figure 2). One should note that the largest values of the ionic strength in our calculations exceed the limits allowed for the linear PB equation. The applied procedure helps only to propose the proper function for the ionic strength dependence. It is advised not to exceed the ionic strength limits allowed by the linear PB.

To check the accuracy of the GB formula with respect to the dielectric constants, a number of calculations were carried out. The solute dielectric constant was set to 1.0, 5.0, and 10.0, and the solvent dielectric constant was set to 10.0, 20.0, 40.0, and 80.0. About 2000 one-atom solvation energies were computed for each pair of parameters. The effective Born radii were calculated for the dielectric constants of $\epsilon_{\text{in}} = 1$ and $\epsilon_{\text{ex}} = 80$. The accuracy of the Born solvation energy decreases with the increase of the ratio of the dielectric constants. The average ratio of the Born and PB solvation energies systematically grows up with the increase of the solute/solvent dielectric constant (see Figure 3). The average value depends on the ratio of the dielectric constants. For example, it is the same for the $\epsilon_{\text{in}}/\epsilon_{\text{ex}}$ being 5/20 or 10/40. This shows that there are no common Born radii, and the calculations should be carried out separately for each value of the solute/solvent dielectric constant ratio. Overestimation of the solvation energy reaches 30%. Typically, the solvent dielectric constant is 78 (for water) and varies in the range from 2 to 15 for molecules, which gives the ratio between 0.02 and 0.2. Therefore, one should expect up to a 15% overestimation of the solvation energy. To maintain the accuracy of the Born model, the solvation energies should additionally be adjusted. There is no straight correlation between the Born and PB solvation energies; therefore, a simple correction to compute the Born energies is not satisfactory. The definition of the Born radii had to be changed. Grycuk¹⁵ showed that a more proper form of the Born radii definition is $(f_{\text{ex}}/r^6)^{1/3}$. We propose to change the 6 and 1/3 exponents into n and $1/(n-3)$, respectively, making n dependent on the ratio of solvent and solute dielectric constants

$$\frac{1}{R_i} = \left(\frac{n-3}{4\pi} \int_{\text{ex}} \frac{1}{r^n} dV \right)^{1/(n-3)} \quad (18)$$

In conclusion, the GB formula given by eq 4 can be used to describe the electrostatic properties of solute–solvent systems.

However, this formula should be properly adjusted. The energy change resulting from the presence of ions is small compared to the total solvation energy. Using the conventional definition of the Born radii, given by eq 8, one introduces an error of several percent, while using the definition dependent on the ratio of the dielectric constants, one could reduce the error. We present a more detailed analysis in the text to follow. Computing proper Born radii is still the major task.

3.2. Validation of the New Born Radius Formula. Accuracy of the GB formula is linked with the precision of the Born radii. In studies to follow, we will focus on procedures for the Born radii computation and their analysis. As mentioned, the Born radii, given by eq 8, is approximate and is exact only for $\epsilon_{\text{ex}}/(\epsilon_{\text{in}} + \epsilon_{\text{ex}}) \rightarrow 1$. Here, we analyze the solvation energies computed using the GB model with its exact analytical values proposed by Kirkwood⁹ for a spherical molecule of radius R and the charges located inside the sphere and placed along the radius. The exact potential is expressed as an infinite series (see eq 3) and converges very well. The sum of 500 components gives the solvation energy with an accuracy of 10^{-10} . In this study, we accounted for 1000 components. We compare the solvation energies computed using the GB methodology and several Born radii definitions proposed recently. Namely, we compare the classical (f/r^4) method, the modification proposed by Grycuk,¹⁵ the modification proposed by Feig et al.,¹⁷ and our formula $[(f/r^n)^{1/(n-3)}]$. All methods, except that of Feig et al, make use of one integral specific to the given method. They give the same solutions for the charge placed in the center of the sphere (e.g., the Born radius is equal to the radius of the sphere, $R_B = R$). The solution proposed by Feig et al.¹⁷ is

$$R_i = \frac{1}{C_0 A_4 + C_1 \left(\frac{3\epsilon_{\text{ex}}}{3\epsilon_{\text{ex}} + 2\epsilon_{\text{in}}} \right) A_7} + D + \frac{E}{\epsilon_{\text{ex}} + 1} \quad (19)$$

where $C_0 = 0.3255$, $C_1 = 1.085$, $D = -0.14$, and $E = -0.15$ are optimized parameters. A_x represents appropriate integrals, $A_4 = 1/R_{\text{vdw}} - 1/4\pi \int_{\text{in}} 1/r^4 dV$ (classical formulation), and $A_7 = (1/4R_{\text{vdw}}^4 - 1/4\pi \int_{\text{in}} 1/r^7 dV)^{1/4}$. For the charge placed in the center of the sphere, the values of A_x are $A_4 = 1/R$ and $A_7 = (1/4R^4)^{1/4} = 0.25^{0.25}/R$. For dielectric constants $\epsilon_{\text{in}} = 10$ and $\epsilon_{\text{ex}} = 80$, we get the Born radius

$$R_i = R \frac{1}{C_0 + C_1 (0.25^{0.25}) \left(\frac{3\epsilon_{\text{ex}}}{3\epsilon_{\text{ex}} + 2\epsilon_{\text{in}}} \right)} + D + \frac{E}{\epsilon_{\text{ex}} + 1} = 0.97 \cdot R - 0.14 - 0.15/81.$$

The Born radius (R_i) computed in this way is smaller than the exact Born radius (R), and the decrease depends on the size of the sphere (R). The same behavior should be observed for the off-center ions. Furthermore, in the PB formalism without the ionic strength, one can show that the Born radii should be exactly the same for an environment with different dielectric constants satisfying the condition $\epsilon_{\text{in}}/\epsilon_{\text{ex}} = \text{constant}$ (e.g., 1/8 and 10/80).

The detailed comparison of all these methods is presented in Figure 4. The conventional GB solution was the worst one. Using the f/r^6 formula, we observed an increase in the error with the increase of $(\epsilon_{\text{in}}/\epsilon_{\text{ex}})$ up to about 15%. The errors grow as the charges approaching the surface of the sphere and, in almost the whole range, do not exceed 5%. The Feig et al.¹⁷ solution overestimated the solvation energies in the range 1–10% for almost all charges, and for surface charges, the error

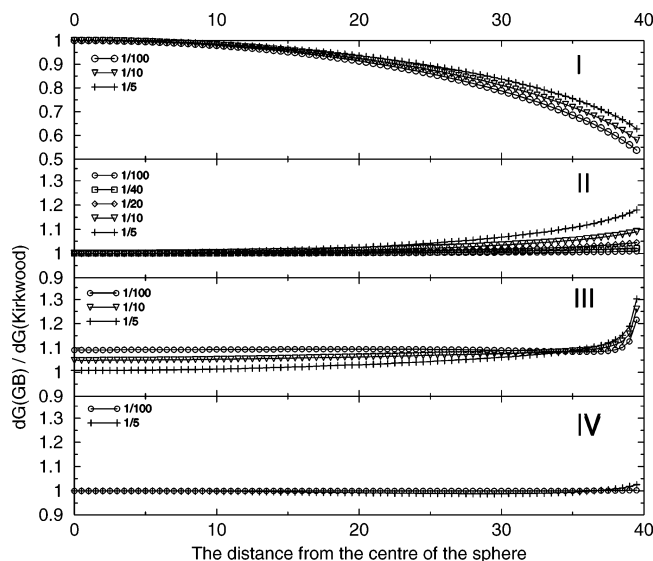


Figure 4. Ratio of the GB solvation energy with respect to the Kirkwood solvation energy for charges placed within the large sphere along the radius: (part I) classical Born approximation ($f1/r^4$), (part II) solution proposed by Grycuk¹⁵ [$(f1/r^6)^{1/3}$], (part III) solution proposed by Feig et al.,¹⁷ (part IV) our solution [$(f1/r^n)^{1/(n-3)}$]. The sphere has a radius of 40 Å. The solute dielectric constant is set to 1, and the solvent dielectric constant is varied ($1/\epsilon_{\text{ex}}$). Some graphs are omitted in a particular method to maintain transparency (the appropriate graphs are located between the neighboring graphs with the closest dielectric constants). Note that graphs have different y-axis ranges.

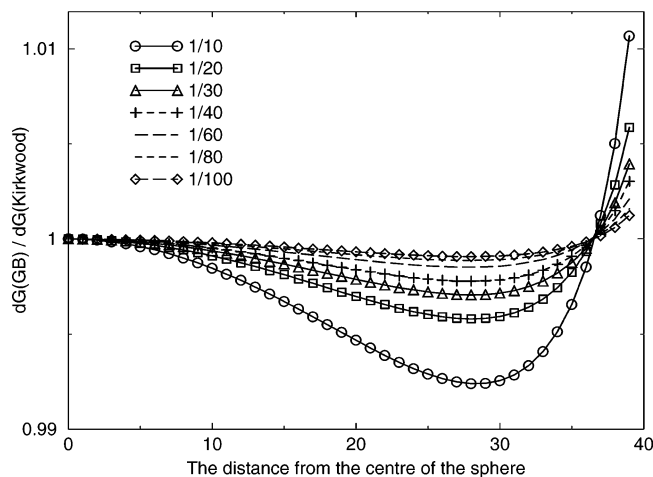


Figure 5. Ratio of the GB solvation energy with respect to the Kirkwood solvation energy for charges placed within the large sphere along the radius. Graph represents our solution [$(f1/r^n)^{1/(n-3)}$] in detail. The sphere has a radius of 40 Å. The solute dielectric constant is set to 1, and the solvent dielectric constant is varied ($1/\epsilon_{\text{ex}}$).

grows up to 30%. The solution proposed in this study gives the best agreement, remaining within 2% error (Figure 5). The accurate values of n are presented in Figure 6. The proposed dependence is

$$n = \frac{4.32}{(x + 0.33)^{0.3}} \quad (20)$$

where $x = \epsilon_{\text{in}}/\epsilon_{\text{ex}}$.

The new definition of the Born radii was used to compute the one-atom solvation energies of lysozyme (1E8L) for several different dielectric constants. The average ratio of the GB/PB solvation energies with its standard deviation is presented in Table 2. Note that the method of Feig et al.¹⁷ has been scaled

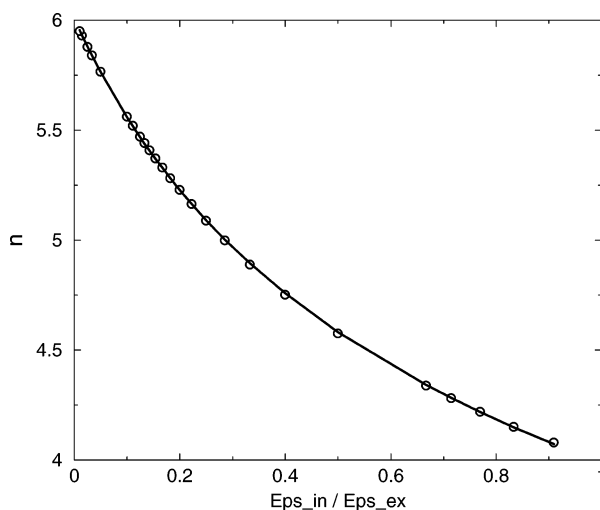


Figure 6. The optimal value of the exponent n in the formula $(f1/r^n)^{1/(n-3)}$, against the ratio $x = \epsilon_{\text{in}}/\epsilon_{\text{ex}}$. The computed values are represented as circles (\circ); the black line represents the fitted function with $n = 4.32/(x + 0.33)^{0.3}$.

TABLE 2: Average Value of the Ratio GB/PB Solvation Energies, Computed Using the Three Following Methods: Grycuk,¹⁵ Feig et al.,¹⁷ and This Study, with Standard Deviations ($\sigma_x^2 = \sum_{i=1}^N (x_i - \langle x \rangle)^2 / (N - 1)$, $x = \Delta G_{\text{GB}}/\Delta G_{\text{PB}}$)^a

ϵ_{in}	ϵ_{ex}	method					
		Grycuk ¹⁵		Feig et al. ¹⁷		this study	
		$\langle x \rangle$	σ_x^2	$\langle x \rangle$	σ_x^2	$\langle x \rangle$	σ_x^2
1	10	1.0131	0.0901	1.0881	0.1038	0.9607	0.0766
1	20	0.9758	0.0838	1.0732	0.1006	0.9497	0.0782
1	40	0.9549	0.0814	1.0630	0.0997	0.9423	0.0792
1	80	0.9436	0.0806	1.0568	0.0995	0.9374	0.0795
5	10	1.2270	0.1521	1.1050	0.1300	0.9947	0.0624
5	20	1.1056	0.1133	1.1027	0.1132	0.9812	0.0719
5	40	1.0302	0.0937	1.0890	0.1037	0.9651	0.0759
5	80	0.9857	0.0852	1.0750	0.1001	0.9534	0.0780
10	20	1.2270	0.1522	1.1031	0.1293	0.9947	0.0625
10	40	1.1056	0.1133	1.1015	0.1128	0.9812	0.0719
10	80	1.0302	0.0937	1.0884	0.1035	0.9652	0.0759

^a Calculations were carried out for lysozyme (1E8L) containing 1961 atoms.

for selected sets of molecules. Our approach exhibits similar or better performance without such scaling. One observes a slight underestimation of the GB energy in comparison to the PB one. There are two sources of the observed differences: The first results from a generalization of the ideal-sphere solutions to real molecules, which includes differences in shapes as well as differences in distributions of atoms inside the molecular domains. The second class of differences results from implementation of the algorithm. In particular, the SA surface is slightly different from that used in the reference PB calculations. Furthermore, the differences could also partially be caused by discretization of the atomic positions. Note that the atoms were virtually moved to the closest grid nodes (see Appendix A).

Our method could further be adjusted to correct these errors. This would require, however, more systematic and detailed studies. This article is mainly dedicated to the new, more optimal way of defining and computing the Born radii, and the observed small differences in the GB energy will be the subject of our further studies. Additional scaling could further improve the results.

3.3. Comparing Different Methods for Computing the Born Radii. The validity of the Born formula and the model

of space occupied by a molecule used in the overlapping spheres approach were tested. First, we computed the Born radii in three different ways to test the influence of the approximations made in the GB model. We calculated solvation energies for dielectric constants $\epsilon_{\text{in}} = 1$ and $\epsilon_{\text{ex}} = 80$. For these values, the modification proposed by Grycuk and our modification are almost the same (the exponent is $n = 5.94$). The Born radii were computed using the formula $(f_{\text{ex}} 1/r^6)^{1/3}$. The template Born radii were computed using the *UHBD* program, solving the linear PB equation.

The first method used a numeric integration procedure that was written to compute exact values of the above integral. The integration was carried on a cubic, rectangular lattice with a 0.1-Å spacing. This method of computing is slow, but it overcomes the overlapping spheres problem and is precise. The volume of the molecule is described by the volume occupied by atoms. This calculation can identify errors arising from the simplified definition of the molecular volume.

The second model was based on the fast overlapping spheres approach. The integral presented already is the sum of integrals on the VdW atomic spheres. The main problem is overestimation of the integral, caused by multiple counting of contributions from the overlap regions. This effect can be minimized by using scaling factors for either the VdW radii or the integral value. There are two scaling factors: The first has a constant value, common to each protein, and depends on the average density of atoms in molecules. It is applied to the whole molecular space. The second factor depends on local fluctuations from the average density. This is applied when computing the integral for the closest atoms, where deviations can be large, and the value of the integral is also large. The second scaling factor is proportional to $x \approx (S - C)/S$, where S is the sum of volumes of all nearest-neighbor atoms, and C is the sum of their overlapping regions.

The last, third model of computing the integral uses the regular grid of spheres, described in detail in Appendix A. The grid spheres radius was 0.1 Å. The two-scaling factors approach was applied. The space occupied by the molecule was computed using the SAS model. Then, this space is filled with small, identical spheres forming the regular grid. This algorithm is not scaled in any manner during the calculations for real molecules. The scaling factors are set up during the calculation of the Born radius for the large, spherical molecule and for the charge placed in its center.

These algorithms were applied for several molecules. The calculations were carried out for 3 proteins (Table 1), all amino acids (taking into account 10 conformations of each residue), and dodecapeptide (5 conformations), with a total of 9080 atoms.

Results are presented in Figure 7. The direct numerical calculation of the integral on a regular cubic grid reflects the major deficiency of such models, in particular, an improper representation of the molecular volume. It is clearly seen in calculations for large proteins that most of the atoms have the ratio of the calculated Born radii and the effective Born radii smaller than 1. For small molecules, the majority of atoms are located near the surface, and their Born radii are close to the VdW radii. For large molecules, buried atoms appear with Born radii much larger than their VdW radii, as presented in Figure 7 in the bottom graph. For them, calculations in the simplest molecular volume model introduce major error (e.g., some buried atoms act like surface atoms). In that model, if small bulk solvent domains exist in the molecule, buried atoms located near such domains behave like atoms near the molecular surface. The errors increase with the increase in size of the molecule.

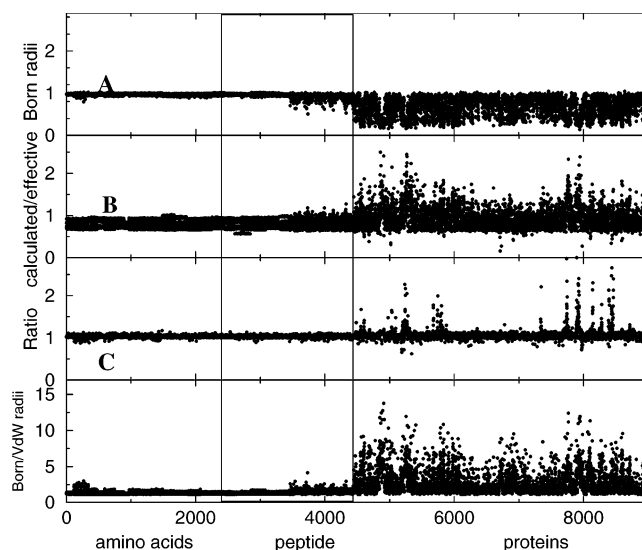


Figure 7. Numerical values of the Born radii, divided by the effective Born radii computed using the reference Poisson-Boltzmann model. The upper part represents calculations carried out using three different methods: Part A is the direct computation of the integral over the regular cubic grid of 0.1 Å; part B is the fast overlapping spheres approach; part C is a computation using the regular grid that consists of the 0.1-Å spheres. The bottom graph represents the ratio of the effective Born radii and the VdW radii. It reflects positions of atoms in molecules. A value of 1 means that the atoms are located on the surface, and a value of 10 is for buried atoms in large molecules (proteins). The calculations were carried out for all amino acids, dodecapeptide, and three proteins, a total of 9080 atoms.

The same problem occurs for the second, overlapping spheres approach. Deficiencies in definition of the molecular volume along with irregularities in distribution of the atoms could not be compensated with additional scaling factors. These obstacles can be partially overcome with a new method which is capable of precisely computing the overlap region's contribution.

In the third method, in which the calculation is carried out over the regular grid of spheres, these errors seem to vanish. The accuracy of the calculations depends on two factors: the radius of the small spheres (with the decrease of the sphere's radius, precision increases, but computing time increases too) and the way of describing SAS. Isolated peaks for proteins in the graph of the third method should not be treated as errors, but they rather reflect a slightly different volume definition.

4. Discussion

The generalized Born model is the approximate method for computing the electrostatic solvation energy. Its simple equation, based on the continuum dielectric model, describes the energy well but has to be used in a proper way. The Born radii description (eq 7) is not sufficiently exact. Better solutions were proposed by Grycuk¹⁵ (eq 8) and by Feig et al.¹⁷ (eq 19). But, in order to get even better results, one can use our approximation (eq 18) along with the variable definition of the Born radii, depending on the ratio of the solute/solvent dielectric constants. The interaction energy described with the use of the f_{GB} function introduces errors close to 10%. The ionic strength contribution to the solvation energy is very small (about 1%), and this effect is beyond errors resulting from other approximations. Nevertheless, improvement in the most popular way of introducing the ionic strength ($\exp(-\kappa f_{\text{GB}}^i)$ in eq 4) has been proposed (eq 17).

The most important step in the method is the precise computing of the Born radii. The overlapping spheres approximations introduce major errors, because of the improper

definition of the space occupied by the molecule. This, in particular, results in the enlarging of the solvation energy of selected buried atoms, because they act like surface atoms. This problem can be reduced by introducing a more complex way of describing the molecular region. It could also be overcome in the present models by enlarging the VdW radii of the atoms, in such a way that the empty spaces can be filled. The overlapping spheres method deals with another major challenge: the exact computation of the overlapping spaces contribution. Because the Born radii (eq 10) are very sensitive to errors in calculating the integral, more accurate methods are required. Present methods introduce parameters related only to the average density of the atoms, which are not satisfactory.

The Born radii calculation method, which is based on the regular grid of spheres, is very accurate. It is, however, slower than conventional methods. In this method, the space occupied by a molecule, defined by the SAS model, is filled with the regular grid of small spheres. It overcomes two major errors, the irregular distribution of the atoms and the improper definition of the molecular volume. The definition of the Born radii should account for the dependence of the n exponent on the $(\epsilon_{\text{in}}/\epsilon_{\text{ex}})$ ratio; see eq 20. Application of the extended, two-parameter scaling method is also recommended.

In regard to future developments, it is advisable to use the definition of the Born radii proposed in this study and the molecular volume defined by SAS.

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Appendix A: Computing the Born Radii Using the Regular Grid of Small Spheres

The main challenge is a fast and precise method for computing the Born radii. In the overlapping spheres approach, the value of the integral over the space occupied by the molecule (eq 7) was approximated by the sum over the atoms. That approximation is burdened with errors arising from irregularity in the distribution of the atoms as well as the wrong definition of the molecular volume. It can be overcome using a grid of regularly distributed small spheres. Space can be almost perfectly filled with identical, small spheres of radius a with the double cubic lattice sphere packing.^{18,19} The spheres are placed in nodes of a regular cubic lattice, with spacing of $2a$. To better fill out the space with the spheres, a second identical grid is used and shifted by a vector $[a, a, a]$. Now, for the given molecule, a simple SAS algorithm is used to cut off the spheres that belong to the solvent and keep the spheres that form the molecular volume.

The method of computing the Born radii was parametrized using a test system, a sphere of radius of 40 Å. It is a large sphere with a low dielectric constant embedded in solvent with a high dielectric constant. The sphere was filled with a grid of regularly distributed spheres, and the Born radius was calculated for the charge placed in its center. The Born radius should be equal to the radius of the sphere, $R_B = R$. This parametrization is required, because the grid of spheres does not fill the whole space perfectly. The grid of spheres has both gaps (free space not occupied by the spheres) and overlaps (space occupied by a number of spheres). The ratio of the total volume of the spheres and the volume of the whole space is $\pi/3$, and so additionally, a scaling factor for the radii of the spheres is required to compensate for this difference. The value of the integral is very sensitive to the scaling factor, so its value should

TABLE 3: Selected Sets of the Scaling Factors s_1 and s_2 ^a

n	$\epsilon_{\text{in}}/\epsilon_{\text{ex}}$	s_1	s_2
6.00		1.034 646 000 0	0.984 262 694 6
5.76	4/78	1.032 730 599 6	0.984 312 000 0
5.71	5/78	1.032 329 683 7	0.984 312 000 0
5.65	6/78	1.031 847 000 0	0.984 313 278 0
5.61	7/78	1.031 501 305 0	0.984 436 034 0
5.55	8/78	1.031 010 000 0	0.984 462 735 0
5.51	9/78	1.030 689 214 0	0.984 442 790 0
5.46	10/78	1.030 300 000 0	0.984 361 600 0
5.41	11/78	1.029 865 000 0	0.984 486 580 0
5.37	12/78	1.029 560 000 0	0.984 392 610 0
5.33	13/78	1.029 235 000 0	0.984 385 760 0
5.29	14/78	1.028 878 000 0	0.984 505 080 0
5.22	16/78	1.028 400 000 0	0.984 141 600 0
5.18	17/78	1.028 000 000 0	0.984 410 220 0
5.07	20/78	1.027 086 100 0	0.984 441 970 0
5.00	22/78	1.026 500 000 0	0.984 770 000 0

^a n is the optimal exponent in the Born radii definition $((f_1/r^n)^{1/(n-3)})$. Examples of $\epsilon_{\text{in}}/\epsilon_{\text{ex}}$ are presented for the optimal n . The $n = n(\epsilon_{\text{in}}/\epsilon_{\text{ex}})$ fit is given in eq 20.

be optimized carefully. Grycuk¹⁵ proposed to increase the radii of the spheres by a factor of 1.021 517 76. This could seem strange: The sum of the volumes of all spheres is larger than the occupied space (the ratio is $\pi/3$), so the radii should rather be decreased. Furthermore, as we tested, the scaling factor is dependent on the large sphere's dimensions. This results from the distribution of the gaps and overlaps in the grid of the spheres. Careful analysis of the integral shows that its value is overestimated by $\pi/3$ in almost the whole range of integral calculation; however, in the closest distance, the integral value is underestimated. Overestimation results from the average packing density of the spheres ($\pi/3$). Underestimation at the closest distance results from an unbalanced distribution of gaps and overlaps. Because the contribution to the integral, f_1/r^6 , is largest for the neighboring regions, the effect of underestimation dominates over the overestimation of the rest. Finally, it seems that the integral should be enlarged, which could be done by multiplying the radii of the spheres. Thus, the scaling factor accounts for both effects, but its value is not constant. Introducing one common scaling factor to all spheres makes it dependent on the molecular size. As the size of the molecule increases, the proportion between the neighbor regions (underestimation of the integral) and the rest of the molecule (overestimation of the integral) changes.

Therefore, it seems reasonable to introduce two separate scaling factors for the close region and for the rest. The size of the close region should be connected with the grid spacing. The whole integral is divided into two parts: $\int_{\text{in}, r > a_i} 1/r^n = \int_{\text{in}, 2a_i \geq r > a_i} 1/r^n + \int_{\text{in}, r > 2a_i} 1/r^n$. In the close region, the radii of the spheres should be multiplied by s_1 , and for the remote one, they should be multiplied by s_2 . The exact values of the scaling factors are $s_1 = 1.034 646$ and $s_2 = 0.984 262 694 6$, respectively. The s_2 value is very close to $(3/\pi)^{1/3} = 0.984745$, which results from the inverse density of the double cubic lattice sphere packing. These scaling factors are determined for $n = 6$ in the definition of the Born radii. Moreover, the scaling factors also depend on the definition of the Born radii, especially on the exponent in the integral. For different functions, the balance between the integral over the close region and the integral over the remote region changes. So, for each exponent in the definition of the Born radii, there is a set of two scaling factors that gives the best results. Table 3 presents the scaling factors for selected values of n . In conclusion, it is recommended to use the two-

scaling factor method, because it is neither more complicated nor time-consuming, and it is more precise than former approaches.

The described method was used to compute the Born radii for atoms placed along the radius of the 40-Å sphere every 0.1 Å. The radius of the small spheres was set to 0.8 Å. The resulting Born radii were compared with the effective Born radii computed using the exact, analytical Kirkwood solution. The solute and solvent dielectric constants were set to 1 and 80, respectively. The ionic strength was set to 0. The Born radii were computed using eq 10. The exponent should be changed in the definition of the Born radii, but the change $6.00 \rightarrow 5.94$ could improve the results by only 1%. The Born radii are properly computed only for atoms placed in the nodes of the grid. Distribution of the gaps and overlaps is periodic, with the period of $2 \cdot 0.8$ Å. In between, the local atom's environment changes, so one has to also change the scaling factor. In principle, one could make the scaling factor dependent on the relative position of the atom with respect to the grid. For now, it is sufficient to shift a given atom to the closest grid's node.

One should stress that the calculations of the Born radii are very sensitive to the scaling factors of the regular grid built of the small spheres. This also shows that, when using an irregular, real atomic set of VdW spheres, a very precise method of calculating the overlap effect is required to get the proper Born radii.

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