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Poisson-Boltzmann calculations versus molecular dynamics simulations for calculating the electrostatic potential of a solvated peptide*

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Abstract. We performed a very long molecular dynamics simulation of a peptide in explicit water molecules and ions and averaged the electrostatic potential caused by peptide, water and ions at eight points in the vicinity of the peptide. These electrostatic potential values were directly compared to the potential calculated by solving the non-linear Poisson-Boltzmann equation for the system, which describes the solvent using continuum electrostatics. We analyze the contribution of dielectric constant, conformational flexibility and solvation effects on the electrostatic potential at these eight points.

Key words: Protein – Solvation model – Electrostatic potential – Molecular dynamics simulation – Continuum electrostatics – Poisson-Boltzmann equation

1 Introduction

Two fundamentally different ways of treating solvent in molecular mechanics (MM) calculations of biological macromolecules are in widespread use today: (1) explicit treatment of the solvent molecules at atomic resolution, and (2) modeling of the solvent as an electrostatic continuum using the equations of classical continuum electrostatics. Both approaches can, with varying success and efficiency, be applied to similar problems, such as the calculation of the electrostatic contribution to the free energy of solvation of a solute [1], the calculation of pK_as of ionizable residues of a solute [2–4], or the calculation of electrostatic forces [5, 6] or simply the electrostatic potential [7] in the vicinity and possibly in the interior of a solute. The calculation of the electrostatic potential (short: potential) is in many ways the most generic of these problems, because it is at the heart of all the other problems as well. This article compares the potential in the neighborhood of a solute molecule, calculated on the basis of explicit and continuum representations of the solvent, respectively.

Specifically, we calculated the potential at several discrete points in space around a zinc finger peptide from (1) a molecular dynamics (MD) simulation employing an explicit (atomic resolution) water model, and (2) several calculations using the finite-difference Poisson-Boltzmann (FDPB) approach as implemented in the DelPhi package [4].

It is beyond the scope of this article to review exhaustively the characteristics of these two rivaling approaches to treating solvent in MM calculations. Instead, we briefly list those distinguishing features that are assumed to be important for the task of calculating potentials around a macromolecule:

1. *Sampling of solvent conformations:* Continuum electrostatics has no concept of solvent conformations and hence solving the equations of continuum electrostatics for one solute conformation results in a potential that is implicitly averaged over all possible solvent conformations. In contrast, the accessible solvent conformations have to be laboriously sampled in the case of an explicit solvent representation, and the potential must be averaged over these conformations. The sampling can be based on Monte Carlo techniques but is usually done using MD. In the latter case, the biological macromolecule is usually free to move as well, which leads us to the next point. The necessity to sample solvent conformations when using explicit solvent models makes this approach slow and prone to convergence problems.

2. *Sampling of solute conformations:* MD simulations sample solute conformations by default and hence incorporate the correct picture of a macromolecule as a dynamic object. Continuum electrostatics calculations are usually performed on a single solute conformation, although the importance of conformational flexibility has been recognized and there are now methods [8–10] to take care of this aspect – at the cost of a considerable increase in compute-time compared to single-conformation continuum electrostatics calculations.

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3. *Boundary conditions:* To avoid the dominance of boundary effects in MD simulations, it is common to apply periodic boundary conditions which lead to quasi-infinite systems. Furthermore, to tame the long-range nature of electrostatic interactions, it has become increasingly popular to employ lattice summation techniques such as Ewald summation [11–13]. Taken together, this amounts to the fact that MD typically does not simulate an infinitely diluted biological macromolecule but rather a lattice of identical macromolecules in solution. Continuum electrostatics offers the choice between several kinds of boundary conditions, including periodic boundary conditions and the equivalent of infinite dilution.

4. *Solvent polarization:* This means the reorientation of solvent molecules and atoms (and ultimately electrons) in response to electrostatic fields acting inside the solvent. Continuum electrostatics was in a sense developed to describe this situation and consequently should be able to describe this phenomenon where the continuum approximation holds, which we expect to be at most sites within the solvent that are not to close to the solute (see next points). Explicit solvent models have been shown (and sometimes calibrated) to be able to reproduce this characteristic as well [14].

5. *Solvation shells:* The phenomena that occur in the first (and possibly second) solvation shell around a solute molecule are by definition microscopic: hydrogen-bonding, salt bridges, restricted motion of solvent molecules. Naturally, continuum electrostatics cannot model the individual behavior of solvent molecules in these solvation shells, whereas explicit solvent models can.

6. *Solute polarization:* This is the relaxation of the solute in response to electrostatic fields at positions occupied by solute atoms and is closely connected to the question of how solute conformations are sampled. Hence techniques that treat the solute as flexible (such as MD) should excel here over continuum electrostatic techniques that keep the solute fixed and model solute polarization only via the very coarse-grained mechanism of a solute dielectric constant [15]. However, this dielectric constant can in principle describe electronic polarization, which is – for reasons of efficiency and force-field parametrization – usually only included implicitly in current MD techniques.

7. *Modeling of ions in the solvent:* MD calculations treat ions explicitly, whereas FDPB calculations incorporate the effect of ions through the Boltzmann equation. The redistribution of ions around the solute is usually seen as a form of solvent polarization. The explicit treatment of ions in MD simulations leads to the problem of sampling the ion distribution through long enough simulations – a problem that is avoided if the Boltzmann equation is used to model the ion distribution.

The ultimate aim of this project is to explain the influence of all the above factors on the potential values at discrete points in space in and around biological macromolecules. Here we present some preliminary results that mainly address the question of conformational flexibility and solvation shells in the context of the potential outside a peptide and at the peptide-water interface.

2 Results and discussion

We performed a 13.1 ns (excluding 0.8 ns equilibration time) MD simulation of a system consisting of an electrically neutral 18-residue zinc finger peptide [16], a Zn^{2+} ion as a ligand to this peptide, two Cl^- ions as counter-ions to the Zn^{2+} ion, and 2872 SPC/E water molecules in a periodic box of the size $(4.5\ \mathrm{nm})^3$. Thus the concentration of the protein is $18.2\ \mathrm{mmol \cdot l^{-1}}$. Approximately half the linear dimension of the system is occupied by the peptide; the rest is available to the solvent. The united-atom CHARMM19 force-field [17] was used for non-water-water interactions. The simulation was performed using a home-grown MD program [18, 19].

Electrostatic interactions were calculated with a conventional – albeit highly parallel – implementation of Ewald summation using 399 k-vectors, twin-range cut-off of 0.9 nm and 1.2 nm, respectively, and an Ewald decay parameter $\eta = 1.8\ \mathrm{nm^{-1}}$.

The peptide comprises the first zinc finger domain from the gag protein p55 from HIV1 [16]. This peptide contains a CCHC zinc finger motif, where a Zn^{2+} ion is co-ordinated to three Cys and one His residue. Due to this strong co-ordination, the peptide has a well-defined structure, which was solved by NMR and served as the starting structure ($t = 0$ ns) for this simulation. No unfolding or other major structural reorganizations of the peptide occurred during the simulation.

The peptide itself has no net charge; however the Zn^{2+} ion that is coordinated to it leads to net charge of $2+$ that had to be compensated in the MD simulation by two Cl^- ions.

We selected 2498 conformations of the protein (and its solvent) at intervals of 5 ps from this MD simulation for further analysis. The 2498 peptide conformations were fitted to some arbitrary reference conformation (taken at simulation time $t = 7$ ns) to remove translational and rotational movement of the peptide.

Eight points at a distance of 0.4 nm from each other and lying on a line that cuts approximately through the Zn^{2+} ion at the center of the peptide were selected. The location of these points was defined relative to the ensemble of superimposed peptide conformations. One can imagine that if an object of interest (such as an interaction partner of the peptide) is located at one of these points, then peptide and solvent assume several conformations as time elapses, and the object of interest "feels" an average electrostatic potential. These points will be referred to as potential points, because we calculated the electrostatic potential at these points from the MD simulation and several FDPB calculations. Figure 1 shows some of the conformations of the peptide and the location of the potential points.

2.1 Electrostatic potential from MD simulation

The heart of this paper is the calculation of the electrostatic potential at the potential points from the MD simulation. For each of the 2498 conformations we calculated the Ewald potential caused by the peptide, the

ions and the water molecules at the location of each potential point considering periodic boundary conditions. Thus the potential in this case is exactly the electrostatic part of the potential as it was used to carry out the MD simulation. Then the potential at each potential point is averaged over the 2498 conformations. The process of averaging includes both solvent and solute conformations. The convergence of the averaging is illustrated in Fig. 2 by plotting the running average of the potential against the number of conformations used to calculate the average. Figure 3 shows the potential at the eight potential points calculated in this way from the MD simulation. These potential values reflect (1) the usage of an explicit solvent model that should be able to describe solvation shell phenomena as well as continuum electrostatic phenomena, (2) the employment of periodic boundary conditions and Ewald summation to simulate a lattice of identical peptides in solution, (3) the taking into account of many peptide and solvent conformations, and (4) the explicit handling of the two Cl^- ions.

Looking at Fig. 3 we see that as we approach the peptide from the left, the potential rises from an initially

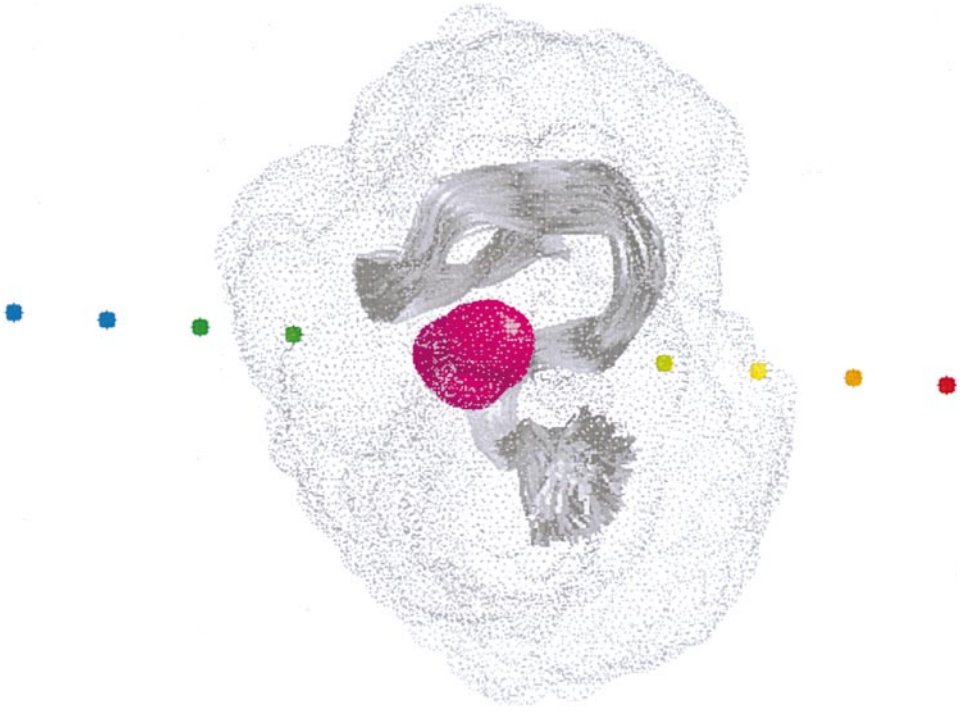

Fig. 1. A subset of the ensemble of conformations of the peptide taken from the molecular dynamics (MD) simulation, and the eight potential points at which the electrostatic potential was calculated with several methods. Potential points are color-coded from blue (1st point) to red (8th point). Also shown is the position of the Zn^{2+} ion (in purple) for each peptide conformation. Peptide conformations are visualized using a ribbon representation for each conformation and a dot surface for the combined surface for all conformations

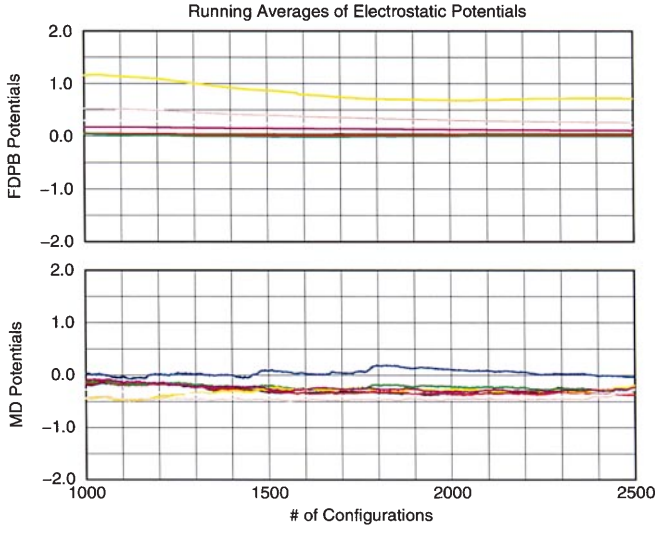

Fig. 2. Illustration of the convergence of the averaging of the electrostatic potential values at the 8 potential points for the two cases, MD simulation and FDPB calculation

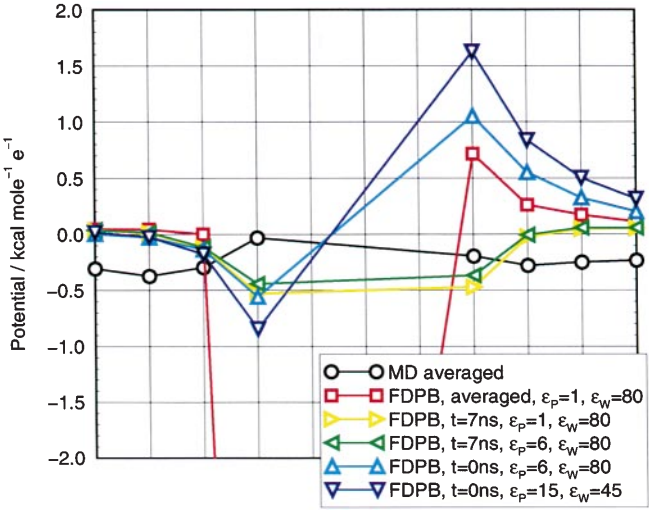

Fig. 3. The value of the electrostatic potential calculated by several methods at the location of the eight potential points in the vicinity of the peptide. The leftmost (1st) point in this figure corresponds to the blue point in Fig. 1, and the rightmost (8th) point in this figure corresponds to the red point in Fig. 1

negative value. The 4th point is in a region of space that is inside the peptide in some conformations, and inside the solvent in others. At the right side of the peptide, the potential values drop to approximately the same negative value as on the left side. The identity of the potential values at the extreme left (1st) and extreme right (8th) potential point is actually required since the MD simulation was done using periodic boundary conditions, and these two points lie close to the boundary of the MD system.

Why is the potential not zero at the outermost (1st and 8th) potential points? Obviously, the MD system is not large enough – although the peptide takes up only approx. 50% of the linear extension of the system! With an electrically neutral peptide that has a Zn^{2+} ion at its center, one would expect the potential to approach zero from a positive value as one increases the distance to the Zn^{2+} ion. That this is not the case can be explained by the presence of negatively charged Cl^- ions in the solvent and by the local partial charges of the residues at the surface of the peptide. This will be investigated in more detail below.

2.2 Electrostatic potential from FDPB calculations on single conformations

The most straightforward way of calculating the potential at the potential points on the basis of a continuum model for the solvent is by taking the experimental NMR structure of the peptide, which is the starting structure of the MD simulation ($t = 0$ ns), and using DelPhi to solve the non-linear Poisson-Boltzmann equation for this conformation via a finite-difference approach. This was done using the same atomic charges as were used in the MD simulation. Atomic radii were the default DelPhi radii. Firstly, we chose 6 for the dielectric constant of the protein and 80 for that of the solvent. This slightly higher-than-usual dielectric constant of the peptide should take into account the enhanced conformational mobility of this molecule compared to larger proteins. It would have been most consistent with the comparison to the MD simulation if the FDPB calculations had been performed with periodic boundary conditions. However, DelPhi did not converge in this case, so we were forced to use so-called coulombic boundary conditions in all the FDPB calculations reported in this work. The percentage of the linear system dimension taken up by the peptide in the FDPB calculations was taken to be as close to the MD calculation as possible (48.2%). Similarly, the ionic strength of 0.03644 $mol \cdot l^{-1}$ used in the FDPB calculations was chosen after the Cl^- ion concentration in the MD simulation. A grid size of 101^3 corresponding to a linear grid resolution of 0.048 nm was chosen. The actual size of the FDPB system was such that the outermost (1st and 8th) potential points are very close to

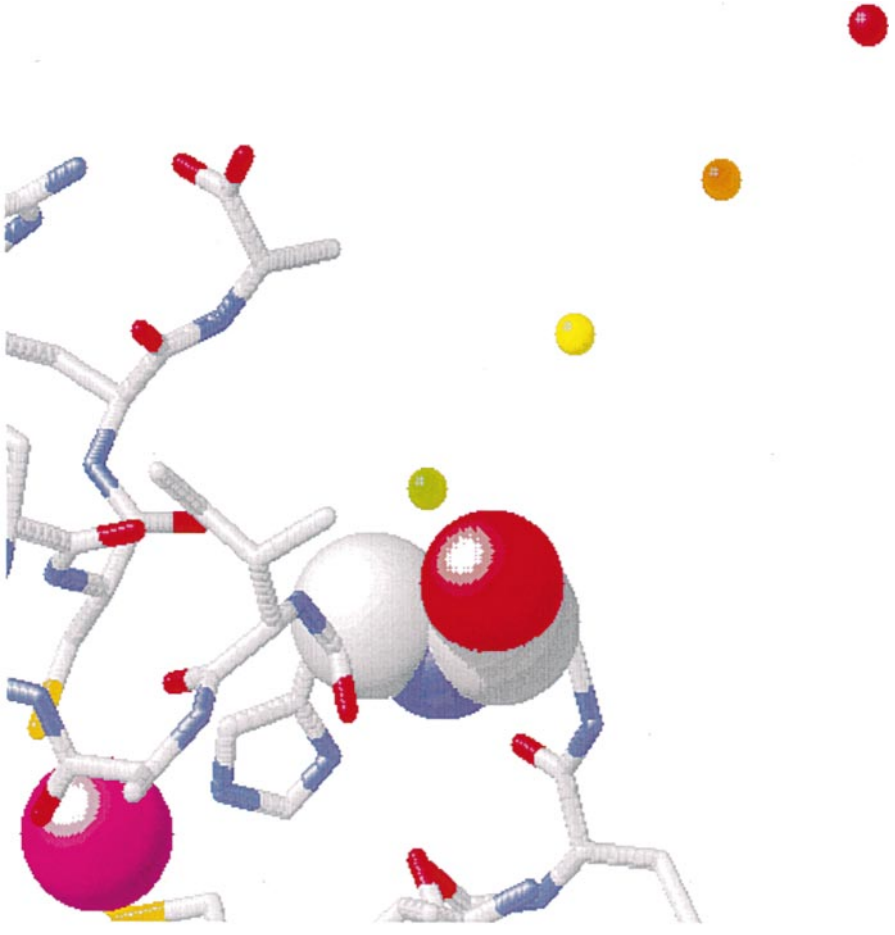

Fig. 4. Peptide conformation at $t = 7$ ns. The 5th to 8th potential points (green to red) are shown together with a wireframe drawing of part of the peptide. The nearest neighbors of the 5th potential point are shown as spheres, where the red sphere is the carbonyl oxygen of Gly10. The Zn^{2+} ion is the large purple sphere

the boundary of the system. Figure 3 shows the electrostatic potential at the potential points resulting from this FDPB calculation.

Also included in Fig. 3 are results from three other simple FDPB calculations. One other calculation was done on the NMR structure of the peptide ($t = 0$ ns) using a dielectric constant of 15 for the peptide and 45 for the solvent, respectively. These values were one result of a recent publication [20] and reflect the high polarizability of the peptide and the restricted polarizability of the water in the first few hydration shells around the peptide. We also performed two FDPB calculations on another arbitrarily chosen peptide conformation ($t = 7$ ns), one with the usual set of dielectric constants (6/80), the other with a dielectric constant of only 1 for the peptide. A dielectric constant of 1 means that the peptide is not able to undergo polarization – not even electronic polarization, which is usually modeled by a dielectric constant of 2. A dielectric constant of 1 for the peptide describes the situation in MM calculations that operate on a single conformation of the biological macromolecule. This calculation was done to provide a link to the averaging of the electrostatic potential calculated by FDPB calculations on all 2498 peptide conformations, which we will describe further below.

All electrostatic potentials derived from FDPB calculations on a single peptide conformation reflect (1) the usage of a continuum solvent model that can only describe macroscopic solvent behavior, (2) the solution of the Poisson-Boltzmann equation with boundary conditions that model the peptide in infinite dilution, (3) the restriction to just one peptide conformation, and (4) the modeling of ions in the solvent through the Boltzmann equation.

Figure 3 shows that the difference in potential values for different peptide conformations (0 ns and 7 ns, respectively) is usually greater than the differences caused by different dielectric constants for the same conformation – at least on the right side of the peptide!

Approaching the peptide from the right side, the potential moves towards positive values for the conformation at $t = 0$ ns, and towards negative values for the conformation at $t = 7$ ns. This is easily explained by the fact that in the conformation at $t = 7$ ns the carbonyl oxygen of Gly10 comes close to the 5th potential point and hence causes a negative potential at this point and also slightly at the 6th and 7th potential points (see Fig. 4). Thus, taking arbitrary conformations of the peptide as the basis of FDPB calculations can result in noteworthy different potentials.

On the left side of the peptide, the two different peptide conformations lead to very similar potential values that change from zero to negative as one approaches the peptide. This is in contrast to the MD potential values, which go from negative to zero as one gets closer to the peptide. Figure 5 shows the high concentration of negatively charged atoms on the left side of the peptide close to the 4th potential point for the con-

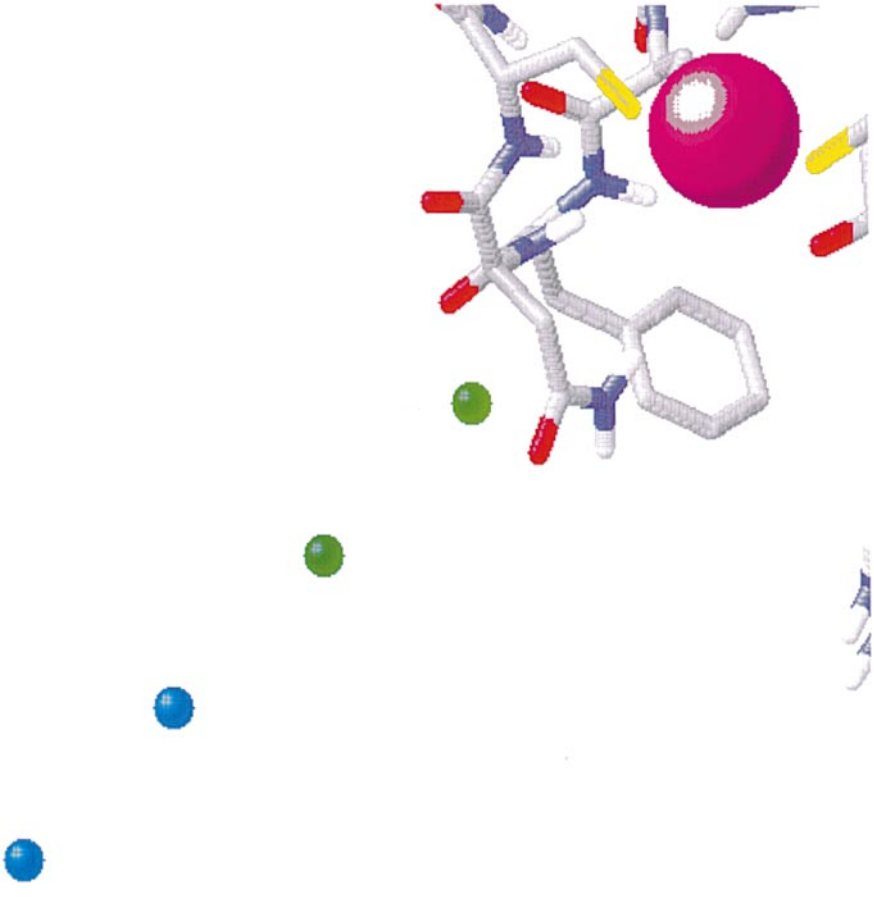

Fig. 5. Peptide conformation at $t = 7$ ns. The 1st to 4th potential points (blue to green) are shown together with a wireframe drawing of part of the peptide. The Zn^{2+} ion is the large purple sphere. The three (negatively charged) oxygen atoms close to the 4th potential point are from Phe4 and Asp5, respectively

formation at $t = 7$ ns. A very similar constellation can be observed at $t = 0$ ns. These negatively charged atoms explain the potential values resulting from the FDPB calculations. The MD calculation treats the water molecules explicitly and so we expect that the high concentration of negatively charged atoms on the surface of the peptide leads to a pronounced orientation of the water molecules in that region such that the water hydrogen atoms point towards the peptide oxygen atoms. This situation can indeed be observed and is shown in Fig. 6 for a snapshot at $t = 7$ ns. Comparing Fig. 6 and 5 shows that at least at the location of the 4th potential point the number of (positively charged) water hydrogen atoms is sufficiently high to give a significant positive contribution to the potential value at that point! Thus, taking into account solvation effects at the peptide surface alters the behavior of the potential close to the peptide.

2.3 Electrostatic potential from averaging FDPB calculations over many conformations

Finally, we solved the Poisson-Boltzmann equation in the same way as described above for all 2498 peptide conformations taken from the MD simulation. These calculations were done with a dielectric constant of 1 for the peptide to take into account that solute polarization is handled explicitly in this case through the presence of several solute conformations. We neglected electronic polarization (dielectric constant of 2) in order to mirror more closely the assumptions from the MD simulation. Using DelPhi, we calculated the potential at each potential point for each peptide conformation and averaged over all calculations for each potential point. The convergence of the average is demonstrated in Fig. 2, and the resulting averaged potential value at each potential point is shown in Fig. 3.

The averaged FDPB potential values reflect (1) the usage of a continuum solvent model, (2) the solution of the Poisson-Boltzmann equation with boundary conditions that model one peptide in infinite dilution, (3) the taking into account of many peptide conformations, and (4) the modeling of ions in the solvent through the Boltzmann equation.

Figure 3 shows that on the left side of the peptide the average potential is similar to the potential values derived from FDPB calculations on just one conformation. The noteworthy difference is the 4th potential point, where the averaged FDPB potential is extremely negative due to the fact that this potential point comes very close to the negative oxygen atoms of residues Phe4 and Asp5 in some of the peptide conformations. While the same thing happens when calculating the potential at

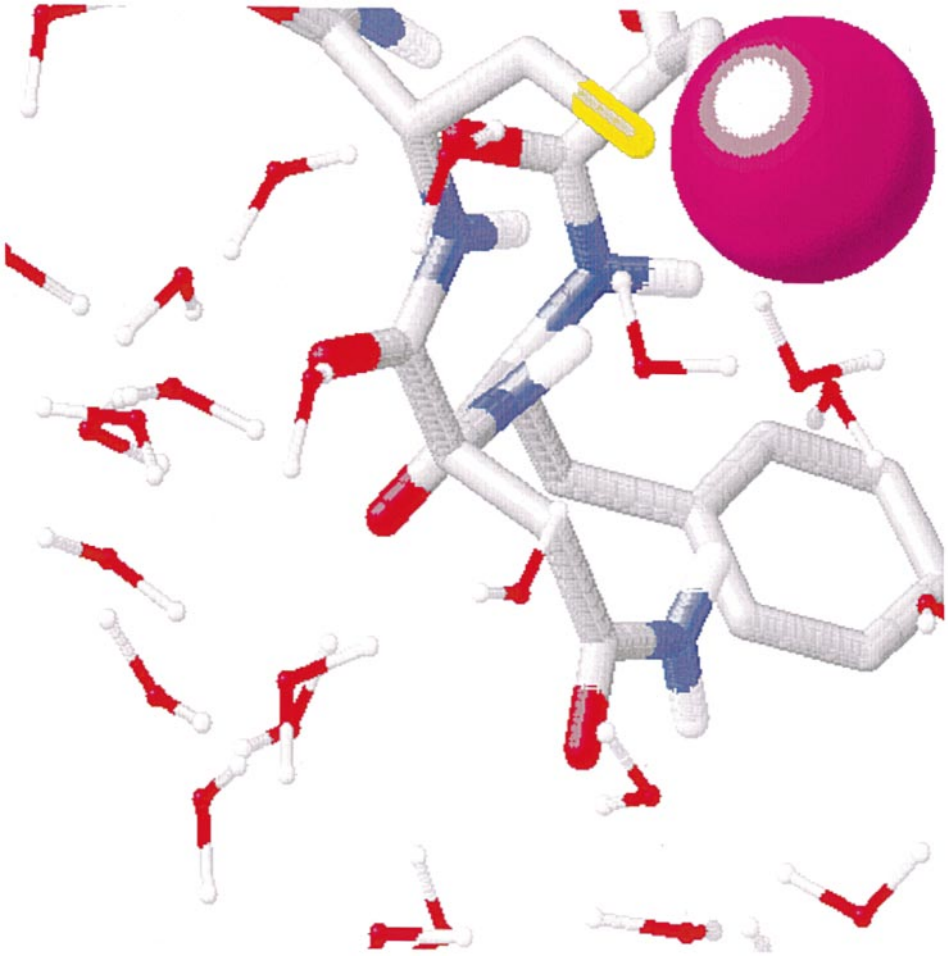

Fig. 6. Peptide and water conformation at $t = 7$ ns. This figure shows the peptide in the same orientation as in Fig. 5 and also shows the water molecules that are within 0.5 nm of the oxygen atoms from Phe4 or Asp5

this point from the MD simulation, the presence of positive water hydrogen atoms in the latter case keeps the potential in balance.

On the right side of the peptide, the averaged FDPB potential lies between the potential derived from the two conformations at $t = 0$ ns and $t = 7$ ns, respectively. This shows that neither of these single conformations constitutes an exceptionally extreme case, but both must be considered typical for the behavior of the peptide.

The differences between the averaged FDPB potential and the MD potential are at least approx. 0.4 kcal$\cdot$mol$^{-1}\cdot$e^{-1}. These differences exist regardless of the identical treatment of peptide conformations in these two cases and must hence be attributed to the differences in (1) treating the solvent (as a continuum or as explicit molecules), and/or (2) handling boundary conditions (infinite dilution or periodic boundary conditions), and/or (3) modeling the ions (through the Boltzmann equation or as explicit particles), respectively! Future work will analyze these individual contributions.

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