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Conformational Dependence of Isotropic Polarizabilities

Pär Söderhjelm,[†] Jacob Kongsted,[‡] and Ulf Ryde*[,§]

[†]Department of Chemistry and Applied Biosciences, Computational Science, ETH Zürich, Via Giuseppe Buffi 13, CH-6900 Lugano, Switzerland

[‡]Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

[§]Department of Theoretical Chemistry, Lund University, Chemical Centre, P.O. Box 124, SE-221 00 Lund, Sweden

Supporting Information

ABSTRACT: We perform a statistical and energetic analysis of atomic polarizabilities obtained with the LoProp approach for all atoms in the avidin tetramer for 70 snapshots from molecular dynamics simulations with seven different biotin analogues, and from the crystal structure of the photosynthetic reaction center (in total 560 698 individual polarizabilities). Dynamic effects give a variation of the polarizabilities of 0.09 Å^3 on average. Atoms at different positions in the sequence show a variation of 0.14 Å^3 on average, caused by the conformational dependence of the polarizabilities. This variation gives errors of 2 and 1 kJ/mol for relative conformational and ligand-binding induction energies. Averaged elementwise or atom-type polarizabilities give larger errors, e.g., 9 and 7 kJ/mol, respectively, for the relative conformational energies. Therefore, we recommend that polarizabilities should be assigned atomwise (i.e., individual polarizabilities for each atom in all residues), in the same way as for charges. We provide such a set of extensively averaged polarizabilities (xAvPol) for all atoms in avidin and the photosynthetic reaction center, applicable at the B3LYP/aug-cc-pVTZ level, which is converged with respect to the basis-set limit.

INTRODUCTION

During the latest decades, molecular simulations have become a powerful alternative and complement to experiments to obtain information about the structure and function of macromolecules. Such simulations are mainly based on the molecular mechanics (MM) approach, employing empirical force fields.[1] One of the most crucial issues in these force fields is the treatment of electrostatics. The great majority of such MM force fields for macromolecules employ a simple Coulomb interaction between atom-centered fixed partial charges. The atomic charges are typically obtained from quantum mechanics (QM) calculations, by fitting them to reproduce either the QM electrostatic potential or intermolecular interaction energies.[2−5]

It has long been recognized that this provides a quite crude description of the electrostatics. In particular, induction effects are completely ignored or treated in an implicit average sense, although it is well-known that polarization typically constitutes 6−30% of the electrostatic interaction energy.[6−11] Consequently, there has been great interest in incorporating induction effects in the MM force field,[11−15] e.g., by using fluctuating charges,[16,17] induced dipoles,[18−20] or Drude oscillators.[21−23] The first polarizable force field appeared as early as in the mid-1970s,[19] and specialized and accurate force fields such as SIBFA, EFP, and NEMO also early employed polarizabilities (and higher-order multipoles).[13,24,25] During the past decade, polarized variants of the more widely used macromolecular force fields have started to appear, e.g., Amber02, PFF, and Amoeba,[11,26−28] all three of which are based on atomic isotropic dipole polarizabilities.

Naturally, the accuracy of polarizable force fields depends on the accuracy of the atomic polarizabilities employed. As for atomic partial charges,[2] atomic polarizabilities are not observables, meaning that there are no reference values that could be obtained from experiments or QM calculations.[11] Instead, atomic polarizabilities have to be determined by some (arbitrary) method that is optimized in a specific way. Several methods to obtain distributed polarizability from QM calculations have been suggested.[11] For example, the atomic polarizabilities can be obtained by partitioning molecular polarizabilities, either in real space (e.g., the atoms-in-molecules approach[29]) or in terms of the basis set.[30,31] Moreover, there are also several ways to apply the perturbing field.[32−34] Alternatively, the polarizabilities can be determined by fitting to a property calculated by QM methods, e.g., the molecular polarizabilities or induction energy.[18,35−42]

There are several sets of atomic polarizabilities available. Some of them are listed in Table 1.[18−20,26−28,35,43−45] Apparently, there is little agreement in the values used or how the polarizabilities should be assigned. Thole and van Duijnen have argued that good reproduction of molecular polarizabilities can be obtained by a single isotropic polarizability for each element,[20,46] and Warshel simply uses 0.5 Å^3 for hydrogen atoms and 1 Å^3 for all other atoms.[19] Other force fields use 8−15 atom types, with one to four different polarizabilities for each element for the normal amino acids. This is in sharp contrast to atomic charges, for which most general-purpose macromolecular force fields today employ individual charges on each distinct (by symmetry) atom in each amino acid. In fact, Woods and co-workers have shown that improved accuracy is obtained using specific atomic polarizabilities, rather than polarizabilities determined by the atom type.[42] They also tested the conformational dependence of the fitted polarizabilities and showed that it was quite small, ∼1%.

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Table 1. Comparison of 10 Different Sets of Atomic Polarizabilities (Å^3)

atom	Vogel[43]	Applequist[18]	Thole[20]	Dykstra[35]	Enzymix[19]	Charmm[45] [a]	Amber02[26] [b]	Amoeba[28] [c]	PFF[27] [d]	Amber09[11]
HC alkyl			0.514	0.00	0.5	0.044	0.135	0.496	0.25	0.443
HC aromatic	0.407	0.135	0.514	0.00	0.5	0.10	0.167	0.800	0.39	0.443
HO alcohol	0.405	0.135	0.514	0.00	0.5	0.044	0.135	0.496	0.22	0.443
HN amides		0.161	0.514	0.00	0.5	0.044	0.161	0.496	0.24	0.443
HN amines			0.514	0.00	0.5	0.044	0.135	0.496	0.24	0.443
HN in RNH_3^+			0.514	0.00	0.5	0.044	0.135	0.496	0.24	0.443
C alkyl	1.027	0.878	1.405	1.87	1.0	0.98	0.878	1.334	1.22	0.920
C aromatic			1.405	1.61	1.0	2.07	0.360	1.334	1.49	1.298
C amide	1.027	0.616	1.405	1.88	1.0	1.65	0.616	1.334	0.83	1.298
C in COO^-			1.405	1.88	1.0	1.65	0.616	1.334	0.82	1.298
N amine			1.105	1.64	1.0	1.10	0.530	1.073	1.33	0.934
N aromatic			1.105	1.29	1.0	1.10	0.530	1.073	1.42	0.934
N amide		0.530	1.105	1.29	1.0	1.10	0.530	1.073	1.15	0.934
OH aliphatic alcohol	0.604	0.465	0.862	0.75	1.0	0.84	0.465	0.834	0.77	0.606
OH aromatic alcohol			0.862	0.75	1.0	0.84	0.465	0.873	0.77	0.593
O backbone amide	0.841	0.434	0.862	0.25	1.0	0.84	0.434	0.837	0.91	0.593
O side-chain amide			0.862	0.25	1.0	0.84	0.434	0.834	0.91	0.593
O in COO^-			0.862	0.25	1.0	2.14	0.434	0.837	0.97	0.593
S					1.0	0.34	2.900	3.300	2.872	3.183

[a] Listed data for CHARMM are from an old but complete listing.[13] Newer developments for alcohols, alkanes, and amides[7−9] have used either slightly modified Applequist parameters[18] or the Thole parameters.[20] [b] Data from the parm99.dat file in the Amber10 distribution. [c] Data from the amoebapro.prm files in the Amber10 distribution. [d] Data from Table 8 in ref 27.

In this paper, we address these issues in a more systematic way. In previous investigations of the influence of the protein electrostatics on excitation and ligand-binding energies, we have calculated polarizabilities for all atoms in several proteins with QM calculations,[47,48] using the LoProp approach.[34] Here, we analyze those data, collecting statistics over the polarizabilities of each atom in the sequence. Thereby, we can address questions such as the following: How large is the conformational dependence of atomic polarizabilities? How are polarizabilities best assigned: by element, by atom type, or by atom? Can transferable polarizabilities be obtained by simply averaging over all calculated values?

METHODS

In this paper, we analyze polarizabilities calculated in two studies, viz., a study of the binding affinity of seven biotin analogues to the protein avidin[48] and new calculations for the photosynthetic reaction center (PRC) from *Rhodobacter sphaeroides*. Both these studies employed a multicenter−multipole expansion up to quadrupoles and anisotropic polarizabilities, obtained with the LoProp approach[34] using the Molcas software.[49] The LoProp method has been shown to be better than other related methods to calculate polarizabilities.[50] The calculations were performed at the density functional B3LYP[51] level, using either the 6-31G*,[52] aug-cc-pVDZ, aug-cc-pVTZ, or aug-cc-pVQZ basis sets.[53] These basis sets are of sizes smaller than, similar to, larger than, and much larger than, respectively, the popular Sadlej basis set designed for the calculation of polarizabilities.[54] Each basis set was turned into the atomic natural orbital form (as required by the LoProp procedure) by a linear transformation that does not affect the orbital optimization.

The properties were calculated for the whole protein by dividing it into the individual amino acid residues, which were capped with CH_3CO- and $-NHCH_3$ groups (dipeptides). The effects of the capping groups were removed by calculating the properties also of the overlapping $CH_3CONHCH_3$ fragments and subtracting them from the properties of the corresponding dipeptides—the molecular fractionation with conjugate caps approach,[55] which has been shown to give errors of 1 kJ/mol or less.[10] A separate calculation was performed on every residue in the structure, with the actual geometry obtained either from the crystal structure (PRC) or from 10 snapshots from a molecular dynamics (MD) simulation with the Amber02 force field (avidin[56]).

In the standard LoProp approach, anisotropic polarizabilities are obtained both for atoms and for bond isocenters. To facilitate the present comparison, we restricted this study to isotropic polarizabilities, because this is the form used in the Amber02, PFF, and Amoeba force fields. The isotropic polarizabilities were obtained as the average of the three diagonal elements of the anisotropic tensor. Moreover, only atomic polarizabilities were considered by partitioning the bond polarizabilities equally on the two bonded atoms.

Interaction energies were calculated with the Amber10 software,[57] using Amber exclusion rules, i.e., that polarization between atoms separated by one or two bonds is ignored, whereas for atoms separated by three bonds, the electric field was scaled by a factor of 1.2.[26] The induction energy was calculated iteratively until successive estimates of the induced dipoles agreed within 0.0001 D, using a second-order extrapolation scheme (indmeth=1).

The exclusion rules are important, because they influence the molecular polarizability resulting from a given set of atomic polarizabilities. Therefore, polarizabilities derived with a specific set of rules are in principle not comparable to those derived with other rules, and they cannot be directly transferred. Nevertheless, such transferability has sometimes been assumed, as in the development of the Amber 2002 force field,[26] in which Applequist polarizabilities, derived using coupling between all atoms, were adopted into the much more restricted coupling scheme of Amber. One can therefore expect that these polarizabilities are too small.

The same problem also occurs in this investigation, because the LoProp polarizabilities add up to the molecular polarizability

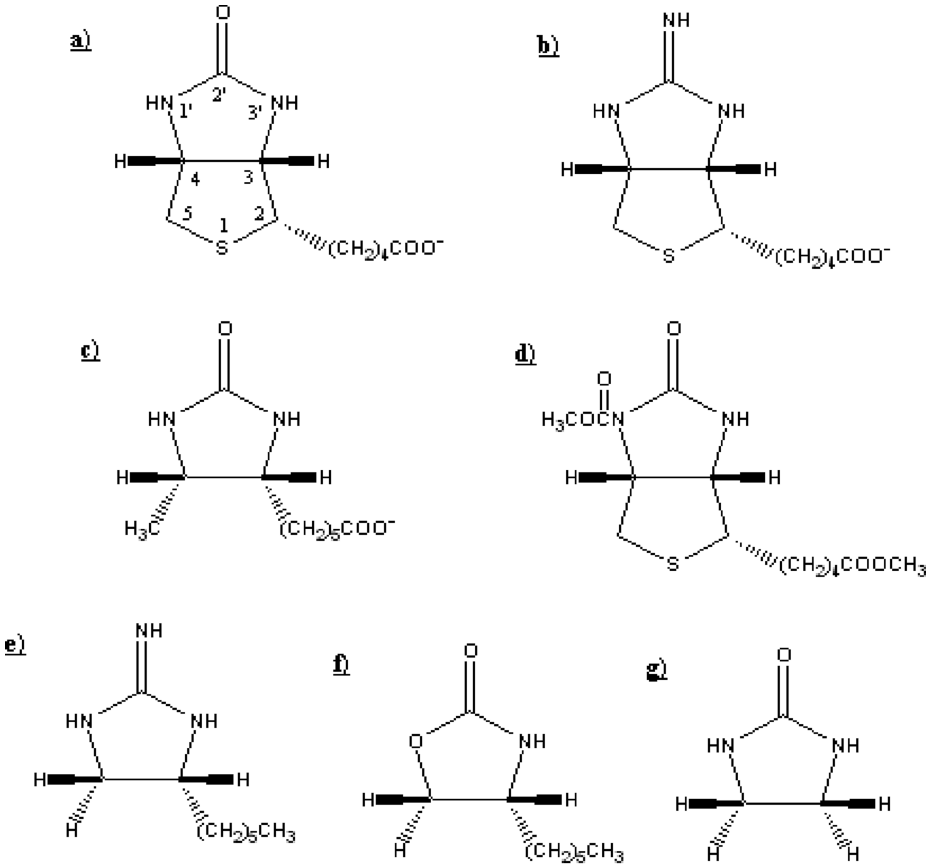

Figure 1. The seven biotin analogues used in this study. (a) Btn1 (biotin); (b−g) Btn2−Btn7.

and thus should not be coupled within the molecule used to calculate them, in our case a protein residue. Thus, when they are used with the Amber exclusion rules or numerically compared to Amber polarizabilities, they should in principle be scaled down to reproduce the (isotropic) molecular polarizability. To investigate the magnitude of this effect, we assumed a uniform scaling over all atoms in a molecule and calculated the required scale factor for each of the 991 molecules used to compute the LoProp polarizabilities for an avidin snapshot. On average, this factor was 0.987, with a standard deviation of 0.007. Because the influence of such scaling on the results would be negligible, we did not modify the polarizabilities. It should also be noted that the choice of exclusion rules also has an effect on the polarization caused by the static charges. However, in the Amber polarizable force field, the charges are derived by taking the statically induced dipoles into account so that the major part of this effect is canceled. Because of this connection, we did not specifically study this issue.

We studied the binding of the seven biotin analogues (Btn1−Btn7) in Figure 1 to avidin. The setup of the molecular dynamics simulations has been described before.[10,56] We used 10 snapshots (sampled every 20 ps) for each analogue taken from this investigation, performed by the polarizable Amber 2002 force field[11,26] (the 02ohp simulation in ref 56).

RESULT AND DISCUSSION

Polarizabilities. First, we studied the conformational dependence of the polarizabilities calculated with the LoProp approach[34] for all atoms in 10 snapshots from MD simulations of avidin bound to the seven different biotin analogues in Figure 1 using the B3LYP/6-31G* method. The LoProp polarizabilities range from 0.05 to 2.45 Å^3 (H in Phe-70 to SG in Cyx-452; Cyx denotes Cys in cystine linkages). For individual atoms, the range of the polarizability (i.e., the maximum minus the minimum value of the polarizability of the same atom) among the 70 snapshots varies from 0.008 to 0.35 Å^3 (for HH2 in Trp-219 and CD2 in Trp-68; average 0.09 Å^3). This illustrates the expected variation of the polarizabilities caused by dynamic effects. There is little similarity between the calculated polarizabilities and those in the Amber02 force field: In fact, for 6796 of the 7708 protein atoms

Table 2. Polarizabilities Calculated for Each Element in the 70 Snapshots of Avidin (Only Protein Atoms) (Å^3)[a]

	LoProp						Amber02	
element	no.	Aver	Stdev	Min	Max	Range	Min	Max
H	267 820	0.22	0.04	0.05	0.33	0.27	0.14	0.17
C	169 400	1.13	0.13	0.82	1.59	0.77	0.36	0.88
N	48 160	0.91	0.13	0.49	1.24	0.75	0.53	0.53
O	53 060	0.54	0.03	0.41	0.68	0.27	0.43	0.47
S	1 120	2.16	0.13	1.88	2.45	0.57	2.90	2.90

[a] no. is the number of individual polarizabilities obtained for each element. Aver, Stdev, Min, Max, and Range are the average, standard deviation, minimum, and maximum values for each element. Range is Max − Min. For comparison, the Min and Max values of the Amber02 polarizabilities are also included.

(88%), the Amber value is outside the range of the calculated polarizabilities in the various snapshots.

An interesting question is how polarizabilities are best assigned to atoms in a protein. Are they the same for each element, for each atom type, or should they be assigned atomwise, like point charges? Statistics for elemental polarizabilities are given in Table 2. It can be seen that the LoProp polarizabilities of all elements show a quite large variation, ranging from 0.27 Å^3 for H and O to ∼0.75 Å^3 for N and C. Thus, it does not seem to be a good idea to assign polarizabilities only on the basis of the element. For all elements, except sulfur, the averaged LoProp polarizabilities are higher than the corresponding Amber values. For H, N, and O, the Amber values are within the calculated range, but for C and S, at least some of the Amber values are outside the range of the LoProp values. The same applies to all the other sets of polarizabilities in Table 1, although with different elements.

The corresponding statistics for the Amber02 atom types are shown in Tables 3 and 4. Amber02 employs 27 atom types for a normal protein, which are all included and described in Table 4. However, most of the Amber02 atom types of the same element use the same polarizabilities. In fact, there are only 10 distinct polarizabilities in Amber (taken from Applequist;[18] three for C and H, two for O, and one for N and S). These are shown in Table 3. It can be seen that the LoProp polarizabilities still show large ranges, e.g., up to 0.77 Å^3 for carbon, and 0.57 and 0.75 Å^3 for S and N. Hydrogen has the lowest ranges (0.10−0.23 Å^3), followed by oxygen (0.23−0.27 Å^3). There is a fair correlation between the average calculated values and the Amber values (r^2 = 0.78).

The corresponding statistics for all the 27 Amber02 atom types are given in Table 4. It can be seen that the range is still large for most atom types, up to 0.77 Å^3 for CT (sp^3 carbon). In fact, the range is below 0.1 Å^3 only for three of the Amber atom types, H4, H5, and HP (explained in Table 3). For 20 of the 27 atom types, the Amber polarizabilities are outside the range of the calculated ones. In many cases, it is obvious that the Amber

Table 3. Statistics for LoProp Polarizabilities over the Amber02 Atom Types That Have Distinct Polarizabilities (Å^3)[a]

atom type	no.	Aver	Stdev	Min	Max	Range	Amber
C	41 020	1.15	0.06	0.94	1.39	0.45	0.62
CT	103 040	1.12	0.15	0.82	1.59	0.77	0.88
C other	25 340	1.17	0.09	0.88	1.52	0.64	0.36
H	59 920	0.17	0.02	0.05	0.23	0.18	0.16
HA, H4, H5	17 080	0.28	0.02	0.23	0.33	0.10	0.17
H other	190 820	0.24	0.03	0.09	0.32	0.23	0.14
N	48 160	0.91	0.13	0.49	1.24	0.75	0.53
O, O2	44 380	0.54	0.03	0.41	0.68	0.27	0.43
OH	8 680	0.53	0.03	0.45	0.68	0.23	0.47
S	1 120	2.16	0.13	1.88	2.45	0.57	2.90

[a] The columns have the same meaning as in Table 2. The atom types are explained in Table 4.

Table 4. Statistics for the LoProp Polarizabilities over All the Amber02 Atom Types for Proteins (Å^3)[a]

atom type	no.	Aver	Stdev	Min	Max	Range	Amber	description
C	41 020	1.15	0.06	0.94	1.39	0.45	0.62	sp^2 C in carbonyl groups
CA	20 020	1.15	0.08	0.98	1.51	0.53	0.36	aromatic C
CB	1 120	1.25	0.06	1.01	1.47	0.46	0.36	CD2 in Trp
CC	280	1.19	0.03	1.11	1.26	0.15	0.36	CG in His
CN	1 120	1.27	0.05	1.10	1.41	0.31	0.36	CE2 in Trp
CR	280	0.96	0.03	0.89	1.03	0.14	0.36	CE1 in His
CT	103 040	1.12	0.15	0.82	1.59	0.77	0.88	sp^3 aliphatic C
CV	280	0.97	0.04	0.88	1.07	0.19	0.36	CD2 in Hid
CW	1 120	1.15	0.04	1.02	1.25	0.23	0.36	CD2 in Hie and Hip, CD1 in Trp
C*	1 120	1.34	0.05	1.19	1.52	0.34	0.36	CG in Trp
H	59 920	0.17	0.02	0.05	0.23	0.18	0.16	H bound to N
H1	57 540	0.23	0.03	0.15	0.30	0.15	0.14	aliphatic H bound to C with one electron-withdrawing group
H4	1 400	0.28	0.02	0.23	0.30	0.07	0.17	HD1 in Trp, HD2 in Hid
H5	280	0.29	0.01	0.28	0.30	0.03	0.17	HE1 in Hid
HA	15 400	0.28	0.02	0.23	0.33	0.10	0.17	aromatic H
HC	119 840	0.25	0.02	0.18	0.32	0.13	0.14	aliphatic H bound to C without electron-withdrawing groups
HO	8 680	0.16	0.02	0.09	0.21	0.12	0.14	H in hydroxyl groups
HP	4 760	0.22	0.01	0.17	0.27	0.09	0.14	HE in Lys
N	37 660	0.96	0.09	0.64	1.24	0.61	0.53	sp^2 N in amide groups
N2	6 300	0.74	0.12	0.57	1.02	0.44	0.53	NE and NH in Arg
N3	2 520	0.64	0.02	0.49	0.70	0.21	0.53	NZ in Lys
NA	1 400	0.94	0.07	0.76	1.17	0.41	0.53	protonated N in aromatic rings
NB	280	0.87	0.04	0.79	0.97	0.18	0.53	nonprotonated N in aromatic rings
O	37 660	0.54	0.03	0.41	0.64	0.23	0.43	O in carbonyl groups
O2	6 720	0.58	0.04	0.42	0.68	0.26	0.43	O in carboxyl groups
OH	8 680	0.53	0.03	0.45	0.68	0.23	0.47	O in hydroxyl group
S	1 120	2.16	0.13	1.88	2.45	0.57	2.90	S

[a] The columns have the same meaning as in Table 2.

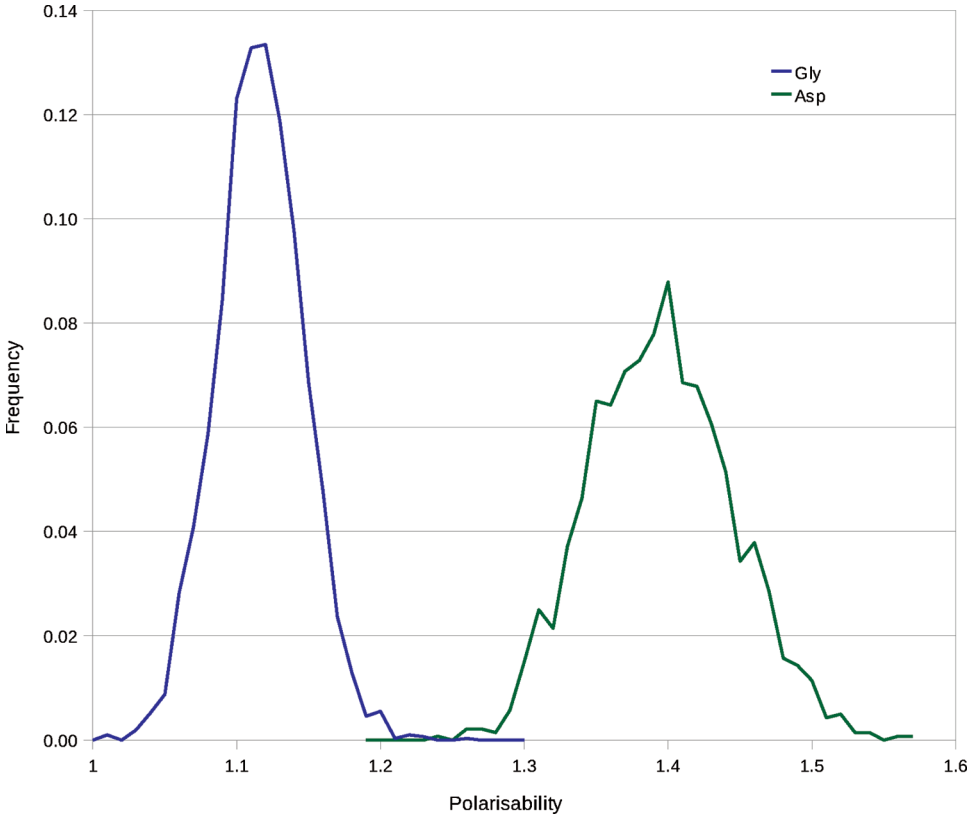

Figure 2. Frequency plot for the LoProp polarizabilities (Å^3) of the CA atom in Gly and Asp in avidin (3080 and 1400 individual polarizabilities, respectively).

atom types still are too crude to give accurate and transferable polarizabilities. This is clearly illustrated for CA atoms of Gly and Asp (which share the same Amber atom type), shown in Figure 2, where the frequencies of the LoProp polarizabilities are shown for the 70 snapshots and the 44 and 22 atoms of each type, respectively. It is obvious that the two distributions are distinct and essentially nonoverlapping, so that different polarizabilities are appropriate for the CA atom in these two amino acids.

Finally, we calculated the average of the polarizabilities for the same atom in the same residue anywhere in the sequence and over the 70 snapshots. This suppressed some of the variation. Now, the average range was 0.14 Å^3. 229 of the 388 distinct atoms (59%) showed a range of less than 0.15 Å^3 and only 28 atoms showed a range over 0.3 Å^3, with CD2 of Trp showing the largest range (0.46 Å^3). Other atoms with large ranges are always carbon and nitrogen atoms, as well as the two sulfur atoms. These are also the atoms with the highest polarizabilities. In fact, there is a good correlation between the size of the polarizabilities and the range ($r^2 = 0.76$), as is shown in Figure 3. This shows that there is a significant conformational dependence of the polarizabilities (23% on average), much larger than for small model compounds (1%).[42] In fact, 70% of the polarizabilities of all possible pairs of atoms from the same residue at different places in the sequence were statistically different at the 95% level according to a simple *t* test.

The largest polarizabilities are those of the two S atoms in Cys and Met (2.27 and 2.04 Å^3). Next largest are those of some carbon atoms, typically CA atoms in various residues, but also some CB and CG atoms (up to 1.39 Å^3 for CA in Asp). The smallest C polarizability is that of the CG atoms of Val (0.90 Å^3). The largest nitrogen polarizability is that of the backbone amide in Pro (1.14 Å^3), and the smallest one is that of the side-chain NZ of Lys (0.62 Å^3). The largest oxygen polarizability is that of the OH group in Tyr (0.64 Å^3). The smallest one is that of the amide backbone O of Cyx (0.43 Å^3). The hydrogen polarizabilities are well separated from those of the other elements. The largest one is that of HH2 in Trp (0.32 Å^3), and the smallest is that of the amide backbone H of Phe (0.16 Å^3).

There are several obvious groups of the calculated polarizabilities. For O, they are distinct and not overlapping: hydroxyl and backbone carbonyl groups (0.50−0.55 Å^3), side-chain carbonyl groups and all carboxyl groups (0.56−0.60 Å^3), and the hydroxyl group of Tyr (0.64 Å^3). The same applies to N atoms, although the ranges are larger: N in Lys side chains and in NH of Arg (0.62−0.69 Å^3), N in side-chain amides (0.71 Å^3), N in His and NE in Arg (0.86−0.91 Å^3), N in the backbone amides and NE in Trp (0.84−1.06 Å^3), and N in Pro (1.14 Å^3). However, for the hydrogen atoms, the ranges are large and overlapping: H in amide and NH_3^+ groups (0.14−0.18 Å^3), H in hydroxyl groups (0.16−0.17 Å^3), H in side-chain amide groups (0.18−0.22 Å^3), HC with electron-withdrawing neighbors (0.19−0.26 Å^3), H in aromatic groups (0.26−0.32 Å^3), and other HC (0.22−0.28 Å^3).

Finally, for carbon atoms, it becomes even harder to find natural groups: methyl groups, as well as CB and CD in Pro and CE1 in His have 0.90−0.96 Å^3, CD2 in His, CD in Arg, and CG and CD in Lys have 0.97−1.06 Å^3, C in side-chain carbonyl groups and all carboxyl groups have 1.04−1.13 Å^3, C in backbone carbonyl groups, as well as CD2 in Hie and Hip, and CD1 in Trp give 1.10−1.20 Å^3. However, the remaining aliphatic and aromatic C

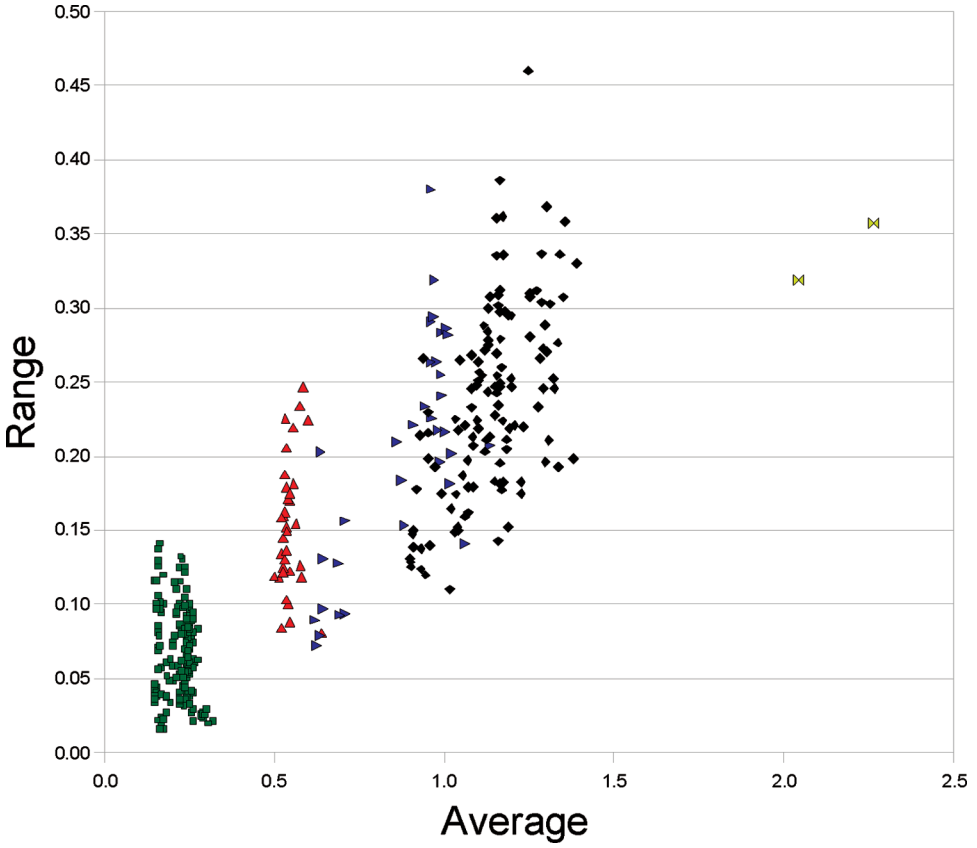

Figure 3. Correlation between the average size of the LoProp atomic polarizabilities and their range (both in units of Å^3). The points are coded according to the element: H, green squares; C, black diamonds; N, blue right-pointing triangles; O, red up triangles; S, yellow double triangles.

atoms still give large and overlapping ranges (1.03−1.39 and 1.07−1.34 Å^3, respectively), without any obvious grouping.

Figure 4 shows the correlation between the atomic polarizabilities and the Amber polarizabilities. It can be seen that there is some correlation ($r^2 = 0.72$), but there is room for significant improvement, in particular for the carbon, nitrogen, and sulfur atoms. Apparently, the polarizabilities of the atoms are very sensitive to their neighboring atoms in a way that is hard to describe without introducing very many atom types. Therefore, we suggest that, for accurate results, it is better to assign separate polarizabilities to each atom in every amino acid, rather than using atom types, in exactly the same way as done for the charges in most force fields, including Amber. In analogy with the extensively averaged electrostatic potential (xAvESP) charges obtained in a similar way,[58,59] we call these averaged LoProp atomic polarizabilities from avidin xAvPol1 in the following and they are provided in the Supporting Information, Table S1.

Basis-Set Dependence. It is well-known that calculated polarizabilities are sensitive to the specific electronic-structure method and the one-electron basis sets.[60] Owing to the presence of the electric-dipole operator in the second-order perturbation theory expression for the dipole−dipole polarizability, use of diffuse basis functions in accurate calculations of polarizabilities is usually of great importance. In the avidin calculations, we have used the B3LYP density functional combined with the middle-sized 6-31G* basis set. In order to check the reproducibility of these results, we need to ensure that polarizabilities calculated with other methods are not widely different. Fortunately, we have also polarizabilities calculated at the B3LYP/aug-cc-pVTZ level for one snapshot of two of the biotin analogues (Btn1 and Btn7; the results for the two ligands are very similar). Therefore, we can make a direct comparison of the polarizabilities obtained with this more accurate but much more expensive method. The polarizabilities calculated with the two methods differ by 0.12 Å^3 on average, with the larger basis set giving larger polarizabilities (only for ∼5% of the atoms does the calculation with the smaller basis set give larger polarizabilities, and only by up to 0.04 Å^3). As expected, the largest differences are obtained for the negatively charged carboxylate groups and for the sulfur atoms: The difference is 0.61 Å^3 for SD in Met, 0.44 Å^3 for SG in Cyx, 0.48−0.55 Å^3 for the carboxylate O atoms, and 0.42−0.51 Å^3 for the carboxylate C atoms (with slight differences between Asp, Glu, and the carboxy terminals). Other atoms with large differences are OE1 of Gln (0.31 Å^3), CE1 and NE2 of Hid (0.29 Å^3), OD1 of Asn, and CH2 and CZ3 of Trp (0.28 Å^3).

Again, there is a significant variation between the various atoms, which is impossible to describe elementwise and also hard to describe by atom types. Instead, it is best described by atomic polarizabilities. Then, the differences are highly reproducible: Only three atomic polarizabilities give differences over 0.01 Å^3 between the Btn1 and Btn7 simulations (SD in Met, OD1 in Asp, and C in the carboxy terminal, with differences of 0.04, 0.02, and 0.02 Å^3, respectively). Thus, the effect of the basis set is quite small and highly consistent and therefore the polarizabilities can quite easily be extrapolated to the larger basis set. This will increase the polarizabilities for all except five atoms (CA in Lys and Arg, CB in Ile and Val, and CG in Leu). Therefore, the difference toward the Amber polarizabilities will increase, except for the two S atoms,

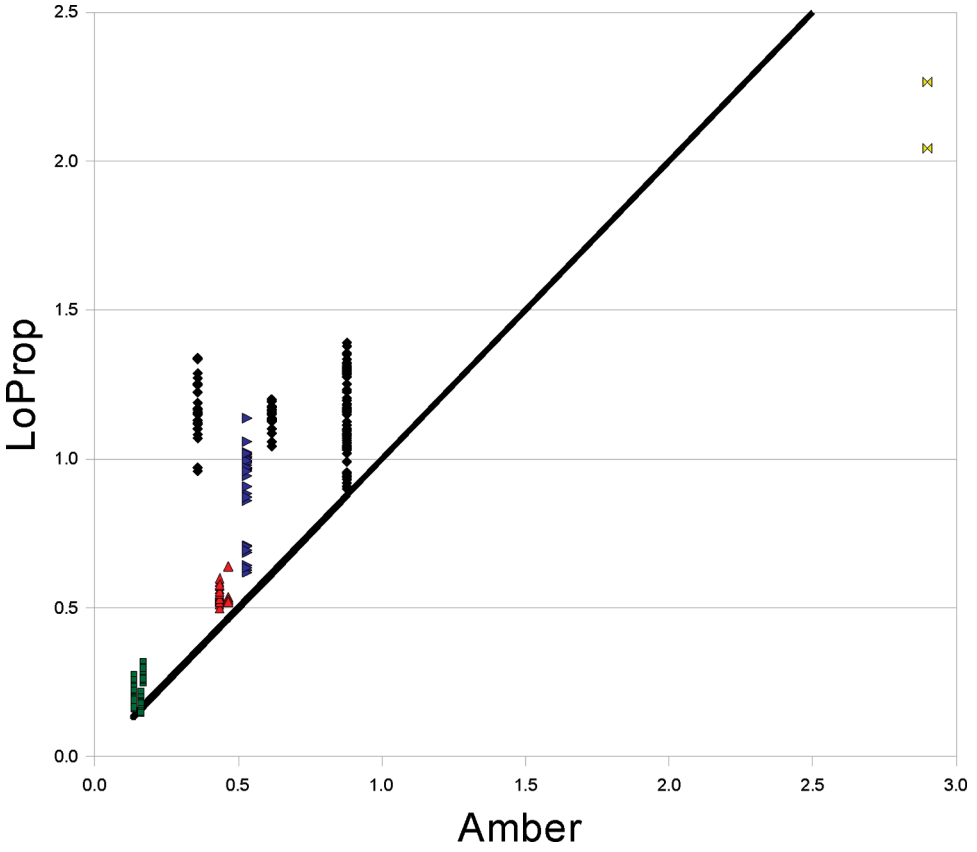

Figure 4. Comparison between the atomic LoProp and the Amber polarizabilities (both in units of Å^3). The points are coded according to the element: H, green squares; C, black diamonds; N, blue right-pointing triangles; O, red up triangles; S, yellow double triangles. The line is $x = y$.

which become similar with the larger basis set, 2.65 and 2.67 Å^3, for SD in Met and SG in Cyx, respectively (2.9 Å^3 in Amber).

To further study the basis-set dependence of the polarizabilities, we performed some additional calculations with the aug-cc-pVDZ, aug-cc-pVTZ, and aug-cc-pVQZ basis sets (still with the B3LYP method) for the groups that showed the largest dependence with respect to the basis set: Cys, Cyx, Met, Asp, and a carboxy terminal. The results show that the polarizabilities are reasonably converged at the aug-cc-pVTZ level: The polarizabilities calculated at the aug-cc-pVTZ and aug-cc-pVQZ differ by only 0.02 Å^3 on average, with a maximum difference of 0.09 Å^3 for SD in Met (the polarizability decreases when the basis set is increased). The SG atoms in Cys and Cyx also show rather large differences, 0.04−0.08 Å^3, whereas the polarizabilities of the carboxylate O atom change by only 0.03 Å^3 (but those of the carboxylate C atom change by 0.05 Å^3). Besides these atoms, the largest change is 0.04 Å^3 for some carbonyl O atoms. In fact, the polarizabilities are fairly converged already at the aug-cc-pVDZ level, with average and maximum differences of 0.03 and 0.15 Å^3 (again SD of Met gives the largest change) toward the aug-cc-pVQZ data. This shows that it is probably better to calculate the polarizabilities with the aug-cc-pVDZ or Sadlej basis set than with 6-31G*.

On the other hand, it is normally assumed that polarizabilities in the condensed phase are lower than those calculated in a vacuum,[27,61,62] e.g., by 7−9% for water. Therefore, the Friesner group uses a basis set without diffuse functions (cc-pVTZ-f[63]) for the calculation of polarizabilities, whereas MacKerell and co-workers scale down polarizabilities by a factor of 0.724.[62] However, the primary aim of this paper is not to establish a proper level to calculate polarizabilities, but rather to quantify the extent and effect of conformational dependence of polarizabilities in proteins.

Different Proteins. Next, we performed the same analysis for another protein, viz., the photosynthetic reaction center from *Rhodobacter sphaeroides*. We calculated the LoProp isotropic atom-centered polarizabilities for each atom (in total 12 818), but only for a single structure (crystal structure with added hydrogen atoms). From these, we calculated atomic polarizabilities by averaging over all residues of each type in the protein (xAvPol2; also included in Table S1 in the Supporting Information). For the 325 atoms that are common to avidin, the average difference between the two sets is only 0.02 Å^3, indicating that the LoProp atomic polarizabilities are remarkably transferable between different proteins. In particular, the largest differences (up to 0.13 Å^3) were observed for C and N atoms in Hid and Tyr residues, for which there is only one occurrence in the avidin monomer, showing that the deviation is mainly statistical in the nature (but it also indicates that there is a significant conformational dependence of the polarizabilities).

Finally, we constructed a set of atomic polarizabilities by averaging over the two proteins, weighting the average after the number of residues of each type in the monomer of each protein. For example, there are 79 Ala residues in PRC and four in the avidin monomer, so we summed the polarizability from PRC multiplied by 79 and that of avidin multiplied by 4 and divided the sum by 83. Note, however, that this weighting of the average has a maximum effect of 0.06 Å^3, so it is of little importance. This averaged set of atomic polarizabilities will be

Table 5. Description of the Various Sets of Polarizabilities Considered in the Work

			polarizabilities different for		
charge set	no. distinct polarizabilities	description	snapshots	same residue	based on protein
LoProp	547 880	LoProp atomic polarizabilities	yes	yes	avidin
Aver	7916	LoProp average over snapshots	no	yes	avidin
xAvPol1	459	Aver, averaged over residues	no	no	avidin
xAvPol2	309	like xAvPol1 but from PRC	no	no	PRC
xAvPol3	521	weighted average over xAvPol1 and xAvPol2	no	no	avidin, PRC
xAvPol4	395	xAvPol3 corrected to aug-cc-pVTZ basis	no	no	avidin, PRC
Element	5	LoProp averaged over elements (Table 2)	no	no	
Type	27	LoProp averaged over atom types (Table 4)	no	no	
Amber02	10	Amber FF02 polarizabilities[26]	no	no	
Amber09	7	new Amber polarizabilities[11]	no	no	
Charmm	9	CHARMM polarizabilities[45]	no	no	
Amoeba	8	Amoeba polarizabilities[28]	no	no	
Enzymix	2	Enzymix polarizabilities[19]	no	no	

called "xAvPol3" in the following. We also constructed a fourth set of polarizabilities by extrapolating the xAvPol3 polarizabilities to the aug-cc-pVTZ basis set with the atomic correction factors obtained in the previous section. The resulting set, xAvPol4, is also included in Table S1 in the Supporting Information.

Induction Energies. Up to now, we have only discussed the actual values of the polarizabilities. To put these into a more interesting perspective, we studied how these differences in the polarizabilities affect electrostatic interaction energies. Therefore, we have calculated three types of energies for avidin and its complexes with the seven biotin analogues in Figure 1. We tested 13 different sets of polarizabilities, viz., the original LoProp polarizabilities for avidin (LoProp), polarizabilities averaged over the 10 snapshots (Aver), xAvPol1, xAvPol2, xAvPol3, xAvPol4, the average elemental polarizabilities in Table 2 (Element), the averaged polarizabilities for the 27 Amber atom types in Table 4 (Type), and the Amber02, Amber09, Charmm, Amoeba, and Enzymix polarizabilities listed in Table 1. The polarizabilities are briefly described in Table 5. All the other MM parameters, including the atomic charges, were identical in the calculations. The calculations were performed with the Amber software[57] and the Amber02 charges.[26]

First, we studied the total induction energy within the whole avidin tetramer without any ligand and water molecules in the 70 snapshots. The absolute energies are not comparable, because different polarizabilities are used, but the fluctuations around the average value should be similar if the different force fields are to sample the same configurational space. Interestingly, all polarizabilities give fluctuations with a range (maximum minus minimum value among the 70 snapshots) of 1515−1651 kJ/mol. The force fields based on the LoProp B3LYP/6-31G* polarizabilities give a smaller range (1515−1533 kJ/mol) than the other polarizabilities (1553−1595 kJ/mol), and Enzymix gives the largest range (1651 kJ/mol).

Second, we compared these relative interaction energies for each snapshot, using the Aver polarizabilities as a reference (we cannot use the LoProp polarizabilities as a reference, because they change for each snapshot). Several conclusions can be drawn from the results presented in Table 6. First, the various force fields give mean absolute differences (MADs) of 2−65 kJ/mol in the order xAvPol1, xAvPol3, Type, Element, xAvPol2, Amber02, xAvPol4, Amber09, Charmm, Amoeba, and Enzymix. Thus, the polarizabilities are much less sensitive to the conformation than charges: The MAD between the Aver and xAvPol1, xAvPol2, or xAvPol3 sets is only 2 kJ/mol, and both the Type and Element polarizabilities give MADs less than 10 kJ/mol, which may be acceptable in many applications.

Third, the B3LYP/6-31G* polarizabilities are clearly not converged, because the B3LYP/aug-cc-pVTZ polarizabilities (xAvPol4) give induction energies that differ by 27 kJ/mol on the average. This shows that larger basis sets should be used for the calculation of the polarizabilities or they should be corrected in the same way as for xAvPol4.

Fourth, different standard force fields give widely differing results, differing from Aver by 26−65 kJ/mol, or up to 4% of the total variation. In most cases, the crude Enzymix polarizabilities give the largest difference. Of course, some of this difference may be caused by the fact that the Aver polarizabilities are based on calculations with a too small basis set. Therefore, we have added an extra row in Table 6 (MAD′) where we instead use the xAvPol4 results (which are close to the basis-set limit) as the reference. It can be seen that the MAD for Amber09, Amoeba, and Enzymix are reduced to 15, 35, and 42 kJ/mol, whereas the MAD for Charmm is not changed and that of Amber02 actually increases. This shows that there still are extensive differences between the polarizabilities of the various force fields, far beyond what is caused by the conformational dependence.

Finally, we note that the variation in the relative induction energies is appreciably smaller than the corresponding variation in relative electrostatic energies when the atomic charges were varied in a similar manner (up to 150 kJ/mol).[58] This is in accordance with the observation that induction energies typically are 6−30% of the electrostatic energies.[6−11] Still, differences of over 10 kJ/mol in relative energies may have a strong influence on the phase space visited during a MD simulation.

Ligand Binding Energies. Next, we studied the induction contribution to the binding energies of the seven biotin analogues in Figure 1 with 10 snapshots for each ligand and the same 13 sets of polarizabilities (and still with the same Amber02 charges). The energy was calculated as the difference between the interaction energies in the complex, the protein, and the ligand:

$$E(\mathrm{PL}) - E(\mathrm{P}) - E(\mathrm{L})$$

Table 6. Differences in Relative Polarization Energies Relative to Aver (kJ/mol)

	xAvPol1	xAvPol2	xAvPol3	xAvPol4	Element	Type	Amber02	Amber09	Charmm	Amoeba	Enzymix
MAD	2	2	2	27	9	7	26	35	36	58	65
Min	−5	−10	−9	−72	−36	−12	−69	−97	−133	−142	−151
Max	4	6	5	61	22	17	53	91	99	166	181
Range	9	15	14	134	57	29	122	188	232	308	333
MAD′ [a]							42	15	34	35	42

[a] Mean absolute deviation from the xAvPol4 results.

Table 7. Differences in Ligand-Interaction Polarization Energies, Compared to LoProp (kJ/mol)[a]

	Aver	xAvPol1	xAvPol2	xAvPol3	xAvPol4	Element	Type	Amber02	Amber09	Charmm	Amoeba	Enzymix
MAD	0.6	0.8	1.1	1.1	12.6	4.1	1.5	4.8	17.2	12.4	24.9	25.7
Max	2.6	3.6	4.8	4.6	30.5	10.8	5.4	11.9	39.3	36.3	54.2	61.1
MAD1−3	1.0	1.4	1.8	1.7	19.4	7.8	2.1	4.2	28.7	24.6	36.9	40.6
Max1−3	2.6	3.6	4.8	4.6	30.5	10.8	5.4	10.6	39.3	36.3	54.2	61.1
MAD4−7	0.3	0.4	0.6	0.6	5.8	1.3	1.0	5.2	8.6	3.4	15.9	14.5
Max4−7	0.9	1.4	2.2	2.0	12.9	4.6	3.2	11.9	23.1	8.1	37.1	33.2
MAD′ [b]								12.7	6.9	5.2	14.5	15.7
Max′ [b]								32.7	14.9	12.7	24.7	30.6

[a] Mean absolute (MAD) and maximum differences (Max) compared to those obtained with the LoProp polarizabilities are listed, calculated either over all seven ligands or over the charged (1−3) or neutral ligands (4−7). [b] Deviations from the xAvPol4 results (only for Btn1 and Btn7).

without any solvation. Only one of the biotin ligands in the tetramer (the fourth) was considered, whereas the other three were considered as a part of the protein. The results in Table 7 show that the Aver polarizabilities give induction contributions to the binding energies that are most similar to those obtained with the LoProp polarizabilities, with a MAD of 1 kJ/mol and a maximum error of 3 kJ/mol for the three charged ligands (Btn1−Btn3) and a MAD of 0.3 kJ/mol and a maximum error of 0.9 kJ/mol for the neutral ligands, respectively. The xAvPol1 polarizabilities also give excellent results with only slightly higher deviations. If the xAvPol2, xAvPol3, or even the atom-type polarizabilities are instead used, the MADs increase to 2 and 1 kJ/mol, respectively, and the maximum errors increase to 5 and 2−3 kJ/mol. On the other hand, the elemental polarizabilities give much worse results, with a MAD of up to 8 kJ/mol for the charged ligands (but only 1 kJ/mol for the neutral ligands). Recalculating the polarizabilities with a larger basis set (xAvPol4) has a major effect on the interaction energies, with MADs of 19 and 6 kJ/mol, respectively, again indicating that 6-31G* is a too small basis set for polarizabilities.

Among the various standard force fields, Amber02 polarizabilities give results that are closest to the LoProp results, with MADs of 4−5 kJ/mol and maximum errors of 11−12 kJ/mol. The other force fields give larger differences, e.g., MADs of 25−41 kJ/mol for the charged ligands and 3−16 kJ/mol for the neutral ligands. If we instead compare to the xAvPol4 results (available only for Btn1 and Btn7), the results for all force fields are improved (to 2−8 kJ/mol average deviation for Btn7 and 8−17 kJ/mol for Btn1), except for Amber02. This indicates that the Amber02 polarizabilities are not compatible with high-level QM calculations, presumably because the force field employs artificially restrictive exclusion rules, as discussed in the Methods section.

Previously, we have observed that effects of variations of the charges are strongly screened by solvation.[58] Therefore, we studied the effect of solvation also for the polarizabilities. Unfortunately, neither of the continuum-solvation models available in Amber is compatible with a polarizable force field. Therefore, we instead simply included all explicit solvent molecules in the calculation of the energy terms for the complex and the free protein. Of course, this is not a fully consistent method, but it at least gives an indication of how much solvation may screen the effect of differences in the polarizabilities. The results in Table 8 show that solvation has a small effect on the induction-energy part of the ligand-binding energies. In particular, no clear screening by solvation is seen. In fact, if different solvation models are used in the calculations (i.e., polarizabilities for the explicit water molecules that are consistent with the respective force field), the differences are typically increased, whereas if the same (LoProp) water polarizabilities are used in all calculations, the results are similar to those obtained without solvation.

CONCLUSIONS

In this paper, we have made a statistical and energetic analysis of isotropic atom-centered polarizabilities calculated individually for all atoms in two different proteins and for 70 snapshots from molecular dynamics simulations (in total 560 698 individual polarizabilities). As mentioned in the Introduction, atomic polarizabilities are not observables, so there are no true reference values of these. It is also well-known that polarizabilities strongly depend on the method and basis sets used for their calculation and that polarizabilities in the condensed phase are different from those in the gas phase.[27,60−62] Moreover, the polarizabilities are closely connected to the model used for the permanent electrostatics and exclusion rules used in the force field.[11] Therefore, it is not meaningful to discuss whether one set of polarizabilities is better than another without defining all the other components of the force field. Instead, this article is concerned with more general aspects of the polarizabilities, viz., their variation with conformation and chemical environment, and how polarizabilities are best assigned (by element, by atom type, or by individual atoms).

First, we show that dynamic effects induce a variation in the polarizabilities of individual atoms of 0.01−0.35 Å^3, with an

Table 8. Differences in Ligand-Interaction Polarization Energies, Compared to LoProp, with Explicit Solvent (kJ/mol)[a]

	Aver	xAvPol1	xAvPol2	xAvPol3	xAvPol4	Element	Type	Amber02	Amber09	Charmm	Amoeba	Enzymix
						Force Field Specific Water Polarizabilities						
MAD	0.6	0.9	1.0	0.9	23.9	4.2	1.5	5.3	38.5	17.8	23.1	47.9
Max	3.2	4.2	4.9	4.8	54.4	19.6	4.8	13.8	171.4	47.9	94.3	245.5
MAD1−3	1.0	1.5	1.5	1.5	41.2	7.0	1.9	7.4	59.2	36.6	35.7	77.6
Max1−3	3.2	4.2	4.9	4.8	54.4	19.6	4.8	13.8	171.4	47.9	94.3	245.5
MAD4−7	0.3	0.4	0.6	0.5	6.7	2.0	1.1	3.8	23.0	3.3	13.7	25.6
Max4−7	0.9	1.3	1.8	1.7	13.5	5.3	3.3	11.1	47.3	9.0	27.7	63.0
						LoProp Water Polarizabilities						
MAD	0.6	0.9	1.0	0.9	15.7	3.6	1.5	4.8	14.5	27.6	22.0	25.7
Max	3.2	4.2	4.9	4.8	32.8	8.3	4.8	13.5	36.6	82.5	55.3	60.3
MAD1−3	1.0	1.5	1.5	1.5	25.4	6.2	1.9	5.6	22.2	58.7	31.5	40.6
Max1−3	3.2	4.2	4.9	4.8	32.8	8.3	4.8	13.5	36.6	82.5	55.3	60.3
MAD4−7	0.3	0.4	0.6	0.5	6.0	1.6	1.1	4.2	8.7	3.6	14.9	14.6
Max4−7	0.9	1.3	1.8	1.7	12.8	4.8	3.3	11.4	23.2	9.0	36.2	33.1

[a] Mean absolute (MAD) and maximum differences (Max) compared to those obtained with the LoProp polarizabilities are listed, calculated either over all seven ligands or over the charged (1−3) or neutral ligands (4−7).

average of 0.09 Å^3 for the 7827 atoms in the avidin tetramer. The standard deviation ranges from 0.002 to 0.07 Å^3 (average 0.02 Å^3), indicating that up to 50 snapshots are needed to obtain a standard error of less than 0.01 Å^3 for all polarizabilities. This clearly shows that it is not enough to calculate polarizabilities for a single structure.

Second, we show that it is very hard to assign transferable polarizabilities by element or atom types. Elementwise polarizabilities would have an uncertainty of up to 0.77 Å^3, i.e., 50% of the magnitude of the polarizabilities themselves. This would induce errors of up to 36 kJ/mol in relative conformational induction energies and of up to 11 kJ/mol in ligand-binding energies. Likewise, polarizabilities assigned by the 27 Amber protein atom types would still have an uncertainty of up to 0.77 Å^3, and it would induce errors of up to 17 kJ/mol in relative energies and of up to 5 kJ/mol for ligand-binding energies (7 and 2 kJ/mol on average). We have also tried to design better groups of atom types, but this is very hard, in particular for aliphatic and aromatic carbon atoms, for which the range is up to 0.36 Å^3.

Therefore, we suggest that polarizabilities should be assigned the same way as for charges, i.e., atomwise. This suppressed the variation of the polarizabilities to 0.14 Å^3 on average, with a maximum of 0.46 Å^3. The average and maximum standard deviations are 0.01 and 0.07 Å^3. This remaining variation reflects the conformational dependence of the polarizabilities, and it cannot be further suppressed unless the conformational dependence is explicitly modeled. The variation is related to the size of the polarizabilities, with an average of 23%. The conformational dependence induces average and maximum errors of 2 and 5 kJ/mol for relative conformational energies, and of 1 and 4 kJ/mol for ligand-binding energies. Polarizabilities calculated in the same way for a different protein (the photosynthetic reaction center) give similar results: 2 and 9 kJ/mol average and maximum error for relative conformational energies and 1 and 5 kJ/mol for ligand-binding energies.

On the other hand, the polarizabilities strongly depend on the basis sets used in the QM calculations. Clearly, the 6-31G* basis set is too small to give converged polarizabilities. Instead, at least the aug-cc-pVDZ (and preferably, the aug-cc-pVTZ) basis set should be used in the calculations. Fortunately, the atomic correction factors between the 6-31G* and aug-cc-pVTZ basis sets are transferable, so the results can be easily extrapolated from bulk calculations with the 6-31G* basis set. In the Supporting Information, we present a set of such polarizabilities (xAvPol4), averaged over 70 molecular dynamics snapshots for avidin and over two different proteins, and finally extrapolated to the aug-cc-pVTZ basis set. These are the best atomic polarizabilities obtained in this paper.

■ ASSOCIATED CONTENT

Supporting Information. Table S1 showing the four sets of xAvPol polarizabilities (Å^3) for the various atoms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Ulf.Ryde@teokem.lu.se. Telephone: +46-46 2224502. Fax: +46-46 2228648.

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■ REFERENCES

(1) Ponder, J. W.; Case, D. A. *Adv. Protein Chem.* **2003**, *66*, 27–85.
(2) Bachrach, S. M. *Rev. Comput. Chem.* **1994**, *5*, 171–227.
(3) Sigfridsson, E.; Ryde, U. *J. Comput. Chem.* **1998**, *19*, 377–395.
(4) Bayly, C. I.; Cieplak, P.; Cornell, W. D.; Kollman, P. A. *J. Phys. Chem.* **1993**, *97*, 10269–10280.
(5) Brooks, B. R.; Brooks, C. L.; MacKerell, A. D.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archonitis, G.; Bartels, C.; Boersch, S.;

Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazardis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Veneable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. *J. Comput. Chem.* **2009**, *30*, 1545–1614.
(6) Gao, J.; Xia, X. *Science* **1992**, *258*, 631–635.
(7) Gao, J.; Habibollazadeh, D.; Shao, L. *J. Phys. Chem.* **1995**, *99*, 16460–16467.
(8) Gao, J.; Pavelites, J. J.; Habibollazadeh, D. *J. Phys. Chem.* **1996**, *100*, 2689–2697.
(9) Xie, W.; Pu, J.; MacKerell, A. D.; Gao, J. *J. Chem. Theory Comput.* **2007**, *3*, 1878–1889.
(10) Söderhjelm., P.; Ryde, U. *J. Phys. Chem. A* **2009**, *113*, 617–627.
(11) Cieplak, P.; Dupradeau, F.-Y.; Duan, Y.; Wang, J. *J. Phys.: Condens. Matter* **2009**, *21*, 333102.
(12) Halgren, R. A.; Damm, W. *Curr. Opin. Struct. Biol.* **2001**, *11*, 236–242.
(13) Gresh, N.; Cisneros, G. A.; Darden, T. D.; Piquemal, J.-P. *J. Chem. Theory Comput.* **2007**, *3*, 1960–1986.
(14) Warshel, A.; Kato, M.; Pisliakov, A. V. *J. Chem. Theory Comput.* **2007**, *3*, 2034–2045.
(15) Lopes, P. E. M.; Roux, B.; MacKerell, A. D. *Theor. Chem. Acc.* **2009**, *124*, 11–28.
(16) Rappé, A. K.; Goddard, W. A., III. *J. Phys. Chem.* **1991**, *95*, 3358–3363.
(17) Rick, S. W.; Stuart, S. J.; Berne, B. J. *J. Chem. Phys.* **1994**, *101*, 6141–6156.
(18) Applequist, J.; Carl, J. R.; Fung, K.-K. *J. Am. Chem. Soc.* **1972**, *94*, 2952–2960.
(19) Warshel, A.; Levitt, M. *J. Mol. Biol.* **1976**, *103*, 227–249.
(20) Thole, B. T. *Chem. Phys.* **1981**, *59*, 341–350.
(21) Dick, B. G.; Overhauser, A. W. *Phys. Rev.* **1958**, *112*, 90–103.
(22) Cao, J.; Berne, B. J. *J. Chem. Phys.* **1993**, *99*, 6998–7011.
(23) Lamoureux, G.; Roux, B. *J. Chem. Phys.* **2003**, *119*, 3025–3039.
(24) Day, P. N.; Jensen, J. H.; Gordon, M. S.; Webb, S. P.; Stevens, W. J.; Kraus, M.; Garmer, D.; Basch, H.; Cohen, D. *J. Chem. Phys.* **1996**, *105*, 1968–1986.
(25) Engkvist, O.; Åstrand, P.-O.; Karlström, G. *Chem. Rev.* **2000**, *100*, 4087–4108.
(26) Cieplak, P.; Caldwell, J.; Kollman, P. *J. Comput. Chem.* **2001**, *22*, 1048–1057.
(27) Maple, J. R.; Cao, Y.; Damm, W.; Halgren, T. A.; Kaminski, G. A.; Zhang, L. Y.; Friesner, R. A. *J. Chem. Theory Comput.* **2005**, *1*, 694–715.
(28) Ren, P.; Ponder, J. W. *J. Comput. Chem.* **2002**, *23*, 1497–1506.
(29) Ángyán, J. G.; Jansen, G.; Loos, M.; Hättig, C.; Hess, B. A. *Chem. Phys. Lett.* **1994**, *219*, 267–273.
(30) Stone, A. J. *Mol. Phys.* **1985**, *56*, 1065–1082.
(31) Le Sueur, C. R.; Stone, A. J. *Mol. Phys.* **1994**, *83*, 293–307.
(32) Dehez, F.; Chipot, C.; Millot, C.; Ángyán, J. G. *Chem. Phys. Lett.* **2001**, *338*, 180–188.
(33) Williams, G. J.; Stone, A. J. *J. Chem. Phys.* **2003**, *119*, 4620–4628.
(34) Gagliardi, L.; Lindh, R.; Karlström, G. *J. Chem. Phys.* **2004**, *121*, 4494–4500.
(35) Stout, J. M.; Dykstra, C. E. *J. Am. Chem. Soc.* **1995**, *117*, 5127–5132.
(36) Zhou, T.; Dykstra, C. E. *J. Phys. Chem. A* **2000**, *104*, 2204–2210.
(37) Nakagawa, S.; Kosugi, N. P. *Chem. Phys. Lett.* **1993**, *210*, 180–186.
(38) Alkorta, I.; Bachs, M.; Perez, J. J. *Chem. Phys. Lett.* **1994**, *224*, 160–165.
(39) Celebi, N.; Ángyán, J. G.; Dehez, F.; Millot, C.; Chipot, C. *J. Chem. Phys.* **2000**, *112*, 2709–2717.
(40) Dehez, F.; Soetens, J. C.; Chipot, C.; Ángyán, J. G.; Millot, C. *J. Phys. Chem. A* **2000**, *104*, 1293–1303.
(41) Soteras, I.; Curutchet, C.; Bidon-Chanal, A.; Dehez, F.; Ángyán, J. G.; Orozco, M.; Chipot, C.; Luque, F. J. *J. Chem. Theory Comput.* **2007**, *3*, 1901–1913.
(42) Elking, D.; Darden, T.; Woods, R. J. *J. Comput. Chem.* **2007**, *28*, 1261–1274.
(43) Vogel, A. I. *J. Chem. Soc.* **1948**, 1833–1847.
(44) Miller, K. J. *J. Am. Chem. Soc.* **1990**, *112*, 8533–8542.
(45) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, J. S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187–217.
(46) van Duijnen, P. T.; Swart, M. *J. Phys. Chem. A* **1998**, *102*, 2399–2407.
(47) Söderhjelm, P; Husberg, C.; Strambi, A.; Olivucci, M.; Ryde, U. *J. Chem. Theory Comput.* **2009**, *5*, 649–658.
(48) Söderhjelm, P.; Kongsted, J.; Ryde, U. *J. Chem. Theory Comput.* **2010**, *6*, 1726–1737.
(49) Karlström, G.; Lindh, R.; Malmqvist, P.-Å.; Roos, B. O.; Ryde, U.; Veryazov, V.; Widmark, P.-O.; Cossi, M.; Schimmelpfennig, B.; Neogrady, P.; Seijo, L. *Comput. Mater. Sci.* **2003**, *28*, 222–239.
(50) Söderhjelm, P.; Krogh, J. W.; Karlström, G.; Ryde, U.; Lindh, R. *J. Comput. Chem.* **2007**, *28*, 1083–1090.
(51) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372–1377.
(52) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213–222.
(53) Dunning, T. H. *J. Chem. Phys.* **1989**, *90*, 1007–1023.
(54) Sadlej, A. J. *Collect. Czech. Chem. Commun.* **1988**, *53*, 1995–2016.
(55) Zhang, D. W.; Zhang, J. Z. H. *J. Chem. Phys.* **2003**, *119*, 3599–3605.
(56) Weis, A.; Katebzadeh, K.; Söderhjelm, P.; Nilsson, I.; Ryde, U. *J. Med. Chem.* **2006**, *49*, 6596–6606.
(57) Case, D. A.; Darden, T. A.; Cheatham, T. E., III; Simmerling, C. L.; Wang, J.; Duke, R. E.; Luo, R.; Crowley, M.; Walker, R. C.; Zhang, W.; Merz, K. M.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossvary, I.; Wong, K. F.; Paesani, F.; Vanicek, J.; Wu, X.; Brozell, S. R.; Steinbrecher, T.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Mathews, D. H.; Seetin, M. G.; Sagui, C.; Babin, V.; Kollman, P. A. *Amber10*; University of California: San Francisco, 2008.
(58) Söderhjelm, P.; Ryde, U. *J. Comput. Chem.* **2009**, *30*, 750–760.
(59) Genheden, S.; Söderhjelm, P.; Ryde, U. *Int. J. Quantum Chem.* 2011, DOI: 10.1002/qua.22967.
(60) Giese, T. J.; York, D. M. *J. Chem. Phys.* **2004**, *120*, 9903–9906.
(61) Morita, A. *J. Comput. Chem.* **2002**, *23*, 1466–1471.
(62) Anisimov, V. M.; Lamoureux, G.; Vorobyov, I. V.; Huang, N.; Roux, B.; MacKerell, A. D. *J. Chem. Theory Comput.* **2005**, *1*, 153–168.
(63) Kaminski, G. A.; Stern, H. A.; Berne, B. J.; Friesner, R. A. *J. Phys. Chem. A* **2004**, *108*, 621–627.